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Heavy metal concentrations in the different tissues of horseshoe crabs collected from intertidal sites of the polluted Juru River and the relatively unpolluted Sepang Besar River, Peninsular Malaysia

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ABSTRACT Horseshoe crabs (*Tachypleus gigas*) were collected from intertidal sites of the polluted Juru River (Penang) and the relatively unpolluted Sepang Besar River (Selangor) in September and December 2007. They were dissected into six parts namely carapace, muscle, telson, leg, operculum and gills. For each site, different tissues from the horseshoe crabs were determined for the concentrations of Cd, Cu, Ni, Fe, Pb and Zn in them. Gills accumulated the highest concentrations of Cu, Cd, Fe, Ni and Pb. Muscles were found to have the highest concentrations of Zn. Most distinctively, the concentrations of Cu, Ni and Zn in the muscles of the Juru River population were significantly ($P < 0.05$) higher than those from the Sepang Besar River, indicating that the Juru River had higher contaminations and bioavailabilities of these metals than the Sepang Besar River. This conclusion was also well supported by the sediment data. These results suggest that *T. gigas* (especially the muscle) is a potential biomonitor of Cu, Ni and Zn contaminations and bioavailabilities in tropical intertidal areas.
(**Keywords**: Horseshoe crab, *Tachypleus gigas*, Heavy metals, Peninsular Malaysia)

INTRODUCTION

Horseshoe crab, *Tachypleus gigas* (Family: Limulidae) is a marine chelicerate arthropod. The animal has a body divided into two main subdivisions, the prosoma (head) and the opisthosoma. There are four species of living horseshoe crabs in the world which are similar in terms of ecology, morphology, and serology [1]. Three of the world's four species are found in Asia while *Limulus polyphemus* occurs on the east coast of the United States of America [2], especially at Delaware Bay.

All the four horseshoe crab species are now considered as being under threat. In Asia, *T. gigas*, *T. tridentatus* and *Carcinoscorpius rotundicauda* are consumed. Their eggs are regarded as a delicacy, for example in Singapore, Malaysia, Borneo [3], Thailand [4], Hong Kong and China [5].

Horseshoe crabs are benthic or bottom dwelling arthropods that use both estuarine and continental shelf habitats. They inhabit shallow marine waters, generally on sandy bottoms where they move about or burrow just beneath the surface, preying on other animals. Since horseshoe crabs deposit and fertilize eggs in the sand, their eggs serve as a food source for shorebirds. Therefore, their ecological characteristics are of much concern from the ecotoxicological point of view.

Elsewhere in the world, horseshoe crab populations have been reported to be in decline, with the major causal factors being loss of spawning and nursery habitats and over harvesting for food, fertilizers, and fish baits in locations such as Thailand [6].

Besides, horseshoe crabs are diets of shorebirds that feed on them during their migration [7]. Therefore, their consumption by humans and migratory birds are two major concerns why their metal concentrations need to be determined. Many studies were done to determine the metal levels in horseshoe crabs and their effects on the development of the embryos of horseshoe crabs [8-13]. However, similar studies from Malaysia are still very limited in the literature.

The objective of this study was to determine the heavy metal concentrations (Cd, Cu, Fe, Ni, Pb and Zn) in the different tissues of *T. gigas* collected from polluted and relatively unpolluted intertidal sites in Peninsular Malaysia.

MATERIALS AND METHODS

Samplings of horseshoe crabs and sediments were conducted in the intertidal areas of the Juru River and the Sepang Besar River of Peninsular Malaysia between September and December 2007 (Figure 1). The sampling conditions and site descriptions are given in Table 2. The samples were then stored in an ice container at 10°C until transportation to the UPM laboratory. In the laboratory, the horseshoe crabs were measured for total wet weight, length, width and height and these measurements are shown in Table 1.

Then, the males and the females were carefully dissected into different parts namely carapace, muscle, leg, operculum, gill, and telson. Eggs of the individual females were also dissected for metal analysis. For each site, the dissected individual parts were pooled and placed in aluminum foils and then were dried in the oven for 72 hours at 105˚C to constant dry weights [14]. The analytical procedures for the sediment followed those described by Yap et al. [15].

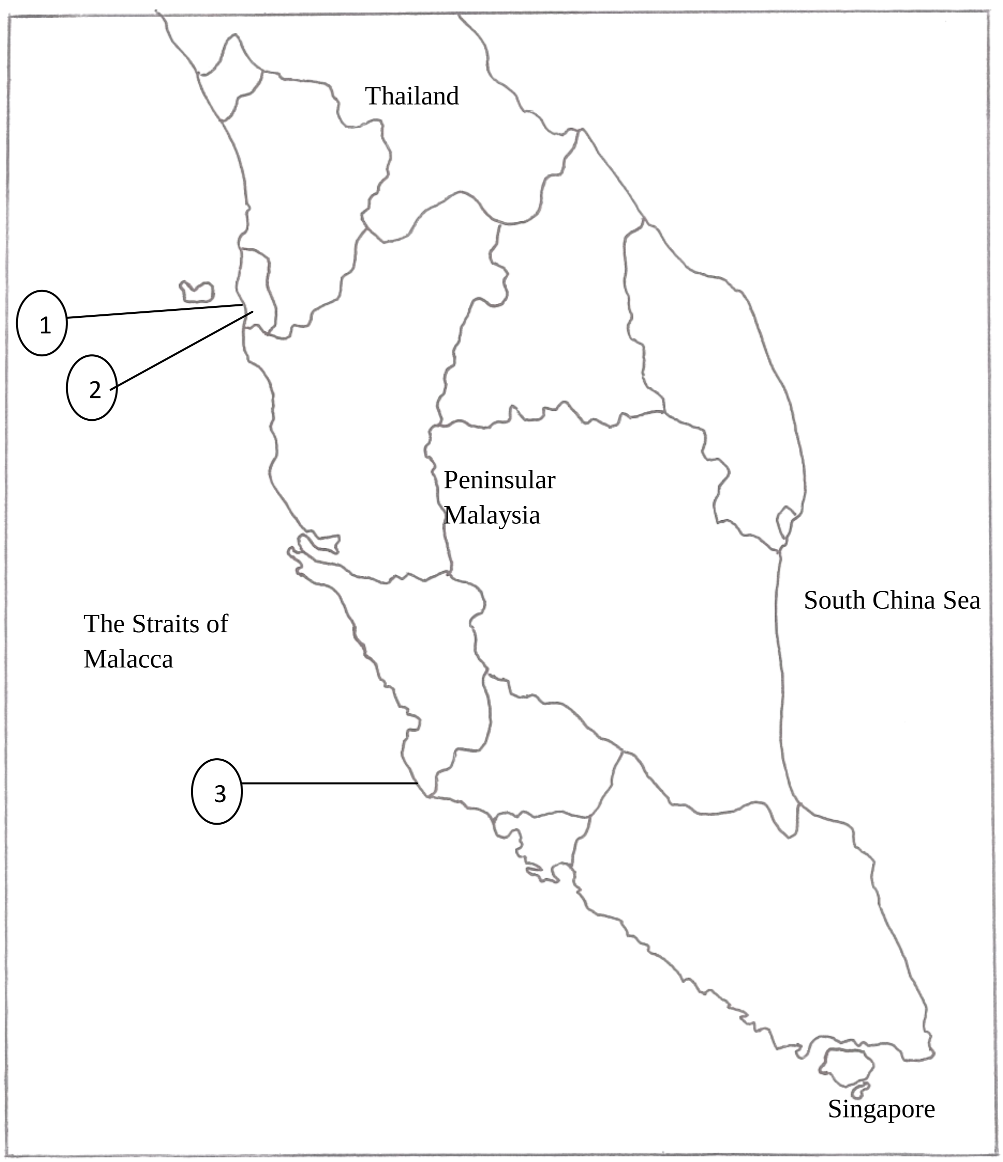

Figure 1: Map showing sampling sites in the Juru River and the Sepang Besar River, Peninsular Malaysia [Note: (1) Juru River 1; (2) Juru River 2; (3) Sepang Besar River]

The dried tissues of the horseshoe crabs were digested in concentrated nitric acid (AnalaR grade, BDH 69%) by placing them in a hot-block digester, 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 the Cd, Cu, Fe, Ni, 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.

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 analytical procedures for the horseshoe crabs were checked with the Certified Reference Material (CRM) for dogfish liver (DOLT-3, National Research Council Canada) and the recoveries of all the metals were satisfactory (Table 3).

RESULTS AND DICUSSION

From Tables 4-9 it is noteworthy to see that the concentrations of Cd (Table 4), Cu (Table 5), Fe (Table 6), Ni (Table 7), Pb (Table 8) and Zn (Table 9) were higher in the muscles of the Juru population (in either Juru-1 or Juru-2) than in those from the Sepang Besar River. This finding is well supported by the sediment data (Table 10) in which the concentrations of Cu, Ni and Zn in the surface sediments were significantly higher ($P< 0.05$) in the Juru River than in the Sepang Besar River.

The muscle is a more reliable tissue for biomonitoring of heavy metal bioavailability and contamination when compared to the gills, carapace, legs, telson and operculum in that these tissues are more exposed to the external ambient environment. Also, the muscle is less affected by physiological conditions such as fatty acid levels and merging with eggs. The muscle is a solid tissue with a high protein content which can better reflect the accumulation of heavy metals by the horseshoe crabs.

From Tables 4-9 again, it is found that the higher metal levels are usually found in the gills when compared to the other parts. For Cu and Pb, the concentrations in the gill ranged from 93.81-240.29 µg/g dw and 10.36-17.44 µg/g dw, respectively. Kannan *et al*. [8] reported that the concentrations of Fe and Pb were highest in the gill.

Fe in the operculum also showed a similar pattern of accumulation as the gill, with the highest accumulation at all the sites. Fe accumulation in the operculum ranged from 244.47- 435.57 µg/g dw. James [16] reported that Fe is an essential metal for oxygen transport in the blood of some invertebrates.

Muscles were highly accumulative of Zn and Cu. Muscles from male individuals in Juru River 2 showed the highest accumulation of Cu (137.86 µg/g dw) whereas the horseshoe crab in the Sepang Besar River showed the lowest Cu concentration (38.23 µg/g dw). These differences indicated that the Juru River was more polluted than the Sepang Besar River and this is in agreement with the most recent Malaysian Department of Environment report [17].

Muscle was studied because it is important to know the metal levels in this tissues since horseshoe crabs are consumed by the local human populations [18]. From the literature, Burger [19] reported that the level of Pb was significantly lower in the muscle while Kannan *et al*. [8] reported that the Zn concentration was higher in the muscle.

In particular, the heavy metal concentrations in the muscles and eggs of horseshoe crabs are of much interest for three reasons: 1) as a biomonitor of metal levels in intertidal areas, 2) as an indicator of potential problems for developing horseshoe crabs, and 3) as food sources of migratory shorebirds [20].

According to Peakall [21] and recent literature, increasingly reports are using marine organisms as indicators (whether bioindicators or biomonitors) of environmental contamination. According to Burger [19], the levels of heavy metals in horseshoe crab eggs should be relatively low because the crabs are low in the food chain. This is in agreement with the present findings.

The metal levels accumulated in the horseshoe crabs can come from two sources: 1) sequestration by the female during egg formation, and 2) from the surrounding water. Since in birds, heavy metals are sequestered in eggs [22,7], it is not surprising that they would be sequestered in the eggs of invertebrates as well. Burger ‘s [19] finding that the mean concentrations of Cd and Pb in the muscles of female *Limulus polyphemus* collected from the Delaware Bay were all below those of the eggs, was indicative of some possible adverse effects.

Table 1: Allometric data for *Tachypleus gigas* collected from the Juru River and the Sepang Besar River.

Site	Sex	N	Total wet weight (g)	Length (cm)	Width (cm)	Height (cm)
Juru River-1	Male	4	71.42 ± 12.26	24.70 ± 0.64	10.89 ± 0.51	3.18 ± 0.11
			(46.56-103.86)	(23.26-26.36)	(10.12-12.41)	(2.94-3.48)
	Female	1	149.35	35.84	13.26	4.71
Juru River-2	Male	3	106.73 ±12.80	29.16 ± 0.48	11.71 ± 0.18	3.31 ± 0.42
			(82.96-126.84)	(28.24-29.85)	(11.52-12.07)	(2.68-4.12)
	Female	2	187.75 ±16.39	37.42 ± 2.96	13.61 ± 0.36	4.80 ± 0.34
			(171.36-204.14)	(34.46-40.38)	(13.25-13.97)	(4.46-5.14)
Sepang Besar River	Female	2	687.80 ± 60.33	44.68 ± 1.07	16.28 ± 0.09	7.44 ± 0.09
			(627.47-748.14)	(43.61-45.75)	(16.19-16.37)	(7.35-7.53)

Note:
Length was measured from anterior carapace (prosoma) to the posterior telson;
Width was measured from tip of right carapace (prosoma) to the tip of left carapace (prosoma); Height was measured from top carapace to the bottom carapace.

Table 2: Site description and sampling conditions for the horseshoe crabs.

	Sepang Besar River	Juru River 2	Juru River 1
GPS reading	02˚ 36.042'N 101˚ 42.461' E	5º 35.096' N 100º 37.307' E	5º 35.096' N100º 37.307' E
Date of sampling	1 Dec 2007	8 Dec 2007	10 Sep 2007
Time of sampling	11.15 - 11.30am	10.30am – 11.05am	NA
Weather	Hot and a little bit windy	sunny and bright day	NA
Site description	An ex-pig farming area	The well-known Juru Industrial Area in the upstream	The well-known Juru Industrial Area in the upstream
Water temperature	27.87	NA	NA
Conductivity (µs/cm)	36677.2	NA	NA
TDS (g/L)	22.6	NA	NA
Water salinity (ppt)	21.79	NA	NA

NA: Data are not available.

Table 3: Comparison of metal concentrations (µg/g dry weight) between measured and their certified values of the certified reference materials (CRMs)-DOLT-3 Dogfish-liver.

Metals	CRM values (C)	Measures values (M)	Percentage % of recovery (M/C)
Cd	19.4 ± 0.600	20.5 ± 0.439	106 ± 2.26
Cu	31.2 ± 1.00	26.5 ± 2.58	85.0 ± 8.28
Fe	1484 ± 57.0	1070	72.1
Ni	2.72 ± 0.350	2.77 ± 0.741	102 ± 27.2
Zn	86.6 ± 2.40	80.9 ± 1.94	93.4 ± 2.24

Note: CRM for Pb is not available

However, the present findings that all the metal levels in the horseshoe crabs eggs were higher than those in the muscles might indicate the possibility of much more adverse effects. Therefore, further studies are needed to investigate this problem. There are very limited published data on heavy metals in horseshoe crabs.

For example, Burger [19] reported that mean concentrations of Cd and Pb in the muscles of female *Limulus polyphemus* collected from the Delaware Bay were 0.021 µg/g wet weight for Pb and 0.013 µg/g wet weight for Cd.

However, for comparative purposes, the metal concentrations (µg/g dry weight) in the muscle of the green-lipped mussel *Perna viridis* collected from the Straits of Johore [23] averaged 89.8 for Fe, 16.1 for Ni, 1.43 for Cd, 6.98 for Cu, 10.2 for Pb and 77.3 for Zn.

In general, these metal concentrations are comparable and similar to the concentrations of three non-essential metals, Cd, Ni and Pb in the muscle of *T. gigas* while the essential metals, Cu, Fe and Zn are found at comparatively higher levels in the muscles of *T. gigas* when compared to the muscles of *P. viridis*.

The relatively high concentrations of the same metals in horseshoe crab eggs suggest that shorebirds are acquiring metals from these or other invertebrates, and may be storing them in their bodies for later mobilization and sequestration in their feathers. Overall, the levels of heavy metals in the muscles of horseshoe crab females were comparatively low.

Carapace was also examined in this study since there were reports in some organisms that metals are sequestered in the skin or eggshells as a method of metal excretion [24-25]. Generally, the burden of heavy metals such as Cd, Pb and Ni were considerably higher in the shell or hard tissue. Yap *et al*. [26] found that shell of *P. viridis* can be a potential biomonitoring material for Cd, Pb and Zn.

However in this study carapace shows the highest accumulation of Fe, ranging from 43.46- 75.17 µg/g dw. Meanwhile for Cd, accumulation in the carapace ranged from 0.63- 0.79 µg/g dw. This may be due to the composition of the carapace being not similar to that of bivalve shells. The other hard tissues namely the telson and leg showed similar patterns as the carapace.

The Fe concentrations in the leg ranged from 158.40- 209.30 µg/g dw whereas for telson, the concentrations of Fe ranged from 29.87- 86.39 µg/g dw. In contrast, concentrations of Cd in leg and telson ranged from 0.61- 0.93 µg/g dw and 0.76- 1.19 µg/g dw respectively. Itow *et al*. [10] reported that elevated levels of Cd could inhibit the regeneration of walking legs in horseshoe crabs.

Besides the muscles, the Pb and Ni concentrations in eggs of horseshoe crabs from the Juru River are again higher than those from the Sepang Besar River. The present finding proved that the Juru River and its vicinity is contaminated by high levels of heavy metals and this could due to its proximity to the Prai Industrial Estate [27]. Many studies in horseshoe crabs focused on the heavy metals in the eggs.

This was due to two reasons: (1) the development of the horseshoe crab themselves might be affected, and (2) vulnerable populations of shorebirds might receive a high dose of heavy metals during a critical migratory period when they must double their weight [28-30].

Itow *et al*. [10] stated that horseshoe crab eggs can accumulate heavy metals, with Hg, organotin, and Cd being the most toxic.

Biota-sediment accumulation factors (BSAF) have been proposed as a simple model for predicting the bioaccumulation of sediment-associated contaminants by infaunal invertebrates [8]. BSAF can be estimated based on the metal concentrations in the horseshoe crabs divided by the metal concentrations in the sediment, basically using the formula BSAF = horseshoe crab dw/Sediment dw.

In this study, BSAF is based on metal concentrations in the muscle tissue with the total metal concentrations of the sediment. The BSAF values from this study are presented in Table 11. The BSAF values are in the range of 0.40-3.73 for Cd, 1.36-2.50 for Cu, 0.00-0.01 for Fe, 0.20-0.32 for Ni, 0.13-0.40 for Pb and 0.89-2.68 for Zn. The greater bioaccumulation potential of Cd, Cu and Zn by *T. gigas* should be interpreted carefully and should prompt more future studies.

These results imply the specific bioaccumulation of Cd, Cu and Zn in *T. gigas*. On the other hand, lower BSAF values of Fe, Ni and Pb are found.

Table 4: The mean concentrations (µg/g dry weight) of Cd in different parts; carapace, leg, telson, muscle, gill, operculum and egg of *Tachypleus gigas* from Juru River 1, Juru River 2 and Sepang River.

Location	Sex	N	Part	Mean	SE	Min	Max
Juru River 1	Male	4	Carapace	0.79	0.08	0.68	0.95
		4	Leg	0.88	0.13	0.72	1.16
		4	Telson	0.76	0.16	0.52	1.07
		4	Muscle	0.96	0.03	0.91	1.03
		4	Gill	1.31	0.18	1.07	1.67
		4	Operculum	1.29	0.13	1.15	1.43
	Female	1	Carapace	0.73	0.12	0.52	0.95
		1	Leg	0.93	0.06	0.87	1.00
		1	Telson	0.83	0.08	0.75	0.92
		1	Muscle	1.05	0.02	1.03	1.07
		1	Gill	1.15	0.02	1.12	1.20
		1	Operculum	1.15	0.08	1.08	1.24
		1	Egg	1.34	0.06	1.27	1.47
Juru River 2	Male	3	Carapace	0.63	0.06	0.56	0.75
		3	Leg	0.74	0.04	0.68	0.84
		3	Telson	0.92	0.05	0.83	1.03
		3	Muscle	1.12	0.10	0.96	1.31
		3	Gill	1.65	0.08	1.50	1.78
		3	Operculum	1.41	0.01	1.39	1.43
	Female	2	Carapace	0.65	0.06	0.59	0.71
		2	Leg	0.91	0.04	0.84	0.99
		2	Telson	1.19	0.03	1.15	1.23
		2	Muscle	1.53	0.08	1.43	1.71
		2	Gill	1.65	0.13	1.51	1.79
		2	Operculum	1.81	0.01	1.40	1.43
		2	Egg	1.93	0.11	1.75	2.15
Sepang Besar River	Female	2	Carapace	0.68	0.10	0.51	0.87
		2	Leg	0.61	0.16	0.36	0.92
		2	Telson	0.99	0.08	0.83	1.11
		2	Muscle	1.46	0.24	1.15	1.94
		2	Gill	1.57	0.13	1.35	1.83
		2	Operculum	1.60	0.10	1.39	1.74
		2	Egg	1.22	0.26	1.91	2.74

Note: N represent the number of individuals analyzed.

Table 5: The mean concentrations (μg/g dry weight) of Cu in different parts; carapace, leg, telson, muscle, gill, operculum and egg of *Tachypleus gigas* from Juru River 1, Juru River 2 and Sepang River.

Location	Sex	N	Part	Mean	SE	Min	Max
Juru River 1	Male	4	Carapace	4.35	0.56	3.61	5.47
		4	Leg	71.82	5.22	63.40	81.40
		4	Telson	15.53	0.52	14.49	16.08
		4	Muscle	91.63	7.17	77.87	102.04
		4	Gill	193.22	10.20	178.43	212.79
		4	Operculum	125.50	2.86	122.64	128.38
	Female	1	Carapace	2.65	0.166	2.34	2.90
		1	Leg	49.20	0.06	19.14	19.27
		1	Telson	11.74	0.14	11.60	11.88
		1	Muscle	128.65	7.66	120.99	136.31
		1	Gill	93.81	4.46	86.21	101.66
		1	Operculum	94.99	1.85	93.14	96.85
		1	Egg	199.82	4.78	192.13	208.60
Juru River 2	Male	3	Carapace	8.56	1.15	6.83	10.75
		3	Leg	74.63	2.01	70.88	77.79
		3	Telson	24.31	0.08	24.22	24.48
		3	Muscle	137.86	9.72	118.46	148.69
		3	Gill	240.29	13.43	216.62	263.12
		3	Operculum	133.95	0.98	132.96	134.94
	Female	2	Carapace	3.04	0.18	2.86	3.24
		2	Leg	49.19	4.60	42.24	57.91
		2	Telson	13.71	1.16	12.54	14.88
		2	Muscle	86.87	3.18	82.68	93.13
		2	Gill	139.52	3.61	135.91	143.14
		2	Operculum	66.93	6.58	60.35	73.52
		2	Egg	117.27	0.80	116.19	118.84
Sepang Besar River	Female	2	Carapace	1.97	0.18	1.71	2.34
		2	Leg	42.33	2.76	39.09	47.83
		2	Telson	10.23	2.07	6.12	12.73
		2	Muscle	38.23	2.31	33.80	41.60
		2	Gill	102.85	7.29	88.78	113.22
		2	Operculum	68.25	2.69	65.32	73.63
		2	Egg	111.71	0.53	111.09	112.77

Note: N represent the number of individuals analyzed.

Table 6: The mean concentrations (µg/g dry weight) of Fe in different parts; carapace, leg, telson, muscle, gill, operculum and egg of *Tachypleus gigas* from Juru River 1, Juru River 2 and Sepang River.

Location	Sex	N	Part	Mean	SE	Min	Max
Juru River 1	Male	4	Carapace	73.17	6.74	59.68	80.11
		4	Leg	209.30	8.08	193.39	219.70
		4	Telson	20.45	0.36	19.74	20.90
		4	Muscle	238.67	1.77	236.13	242.10
		4	Gill	915.08	17.14	892.77	948.79
		4	Operculum	435.57	4.27	431.29	439.85
	Female	1	Carapace	43.46	1.38	41.12	45.93
		1	Leg	172.53	6.64	165.89	179.18
		1	Telson	36.96	4.51	32.45	41.48
		1	Muscle	130.19	4.89	125.30	135.08
		1	Gill	795.60	3.03	789.86	800.16
		1	Operculum	323.51	5.74	317.77	329.27
		1	Egg	69.23	2.55	64.37	73.05
Juru River 2	Male	3	Carapace	65.35	4.72	58.45	74.40
		3	Leg	158.40	11.64	138.22	178.55
		3	Telson	43.04	3.33	36.75	48.13
		3	Muscle	132.99	5.56	122.03	140.16
		3	Gill	786.75	7.45	772.12	796.53
		3	Operculum	244.47	7.01	237.46	251.49
	Female	2	Carapace	75.17	2.01	73.16	77.19
		2	Leg	170.65	12.84	147.56	191.97
		2	Telson	85.78	10.43	75.35	96.22
		2	Muscle	159.19	5.19	149.32	166.91
		2	Gill	961.19	14.13	947.05	975.33
		2	Operculum	358.47	6.53	351.94	365.02
		2	Egg	74.02	5.12	64.50	82.06
Sepang Besar River	Female	2	Carapace	63.69	3.88	55.93	67.95
		2	Leg	205.29	14.28	186.10	233.21
		2	Telson	29.87	4.04	24.89	37.89
		2	Muscle	111.38	4.48	163.87	179.37
		2	Gill	845.91	13.87	819.09	865.45
		2	Operculum	386.82	5.67	376.86	396.51
		2	Egg	51.37	1.86	48.53	54.89

Note: N represent the number of individuals analyzed.

Table 7: The mean concentrations (µg/g dry weight) of Ni in different parts; carapace, leg, telson, muscle, gill, operculum and egg of *Tachypleus gigas* from Juru River 1, Juru River 2 and Sepang River.

Location	Sex	N	Part	Mean	SE	Min	Max
Juru River 1	Male	4	Carapace	1.90	0.28	1.35	2.30
		4	Leg	2.89	0.29	2.55	3.47
		4	Telson	5.74	0.21	1.39	2.13
		4	Muscle	8.84	0.12	2.66	3.07
		4	Gill	6.10	0.25	5.70	6.57
		4	Operculum	3.89	0.27	3.62	4.17
	Female	1	Carapace	4.73	0.25	4.24	5.08
		1	Leg	8.72	0.94	7.78	9.68
		1	Telson	6.52	0.82	5.70	7.35
		1	Muscle	9.11	0.44	8.67	9.56
		1	Gill	11.12	0.63	9.86	11.89
		1	Operculum	9.46	0.82	8.65	10.29
		1	Egg	7.48	0.58	6.62	8.60
Juru River 2	Male	3	Carapace	5.21	0.36	4.60	5.88
		3	Leg	6.95	0.28	6.43	7.43
		3	Telson	6.57	0.51	5.57	7.30
		3	Muscle	8.24	0.33	7.60	8.75
		3	Gill	8.74	0.76	7.36	9.99
		3	Operculum	8.03	0.19	7.84	8.23
	Female	2	Carapace	2.77	0.52	2.25	3.29
		2	Leg	6.15	0.17	5.97	6.50
		2	Telson	5.12	0.23	4.89	5.36
		2	Muscle	6.49	0.27	6.21	7.05
		2	Gill	9.09	0.02	9.07	9.12
		2	Operculum	7.37	0.12	7.24	7.50
		2	Egg	7.59	0.39	6.80	8.09
Sepang Besar River	Female	2	Carapace	2.99	0.10	2.86	3.21
		2	Leg	6.86	0.37	6.12	7.37
		2	Telson	5.94	0.18	5.71	6.32
		2	Muscle	5.22	0.08	8.07	8.36
		2	Gill	8.79	0.39	8.15	9.51
		2	Operculum	8.57	0.27	8.12	9.07
		2	Egg	6.72	0.51	5.73	7.47

Note: N represent the number of individuals analyzed.

Table 8: The mean concentrations (µg/g dry weight) of Pb in different parts; carapace, leg, telson, muscle, gill, operculum and egg of *Tachypleus gigas* from Juru River 1, Juru River 2 and Sepang River.

Location	Sex	N	Part	Mean	SE	Min	Max
Juru River 1	Male	4	Carapace	5.57	0.46	4.67	6.08
		4	Leg	6.40	0.60	5.42	7.49
		4	Telson	7.67	0.37	6.95	8.23
		4	Muscle	12.97	0.73	11.85	14.35
		4	Gill	14.17	0.06	14.09	14.31
		4	Operculum	10.80	0.08	10.72	10.90
	Female	1	Carapace	7.01	0.38	6.27	7.55
		1	Leg	5.42	0.01	5.42	5.44
		1	Telson	5.57	0.18	5.38	5.76
		1	Muscle	7.96	0.73	7.23	8.71
		1	Gill	11.56	0.42	10.87	12.33
		1	Operculum	9.52	0.67	8.85	10.20
		1	Egg	11.26	0.22	10.82	11.57
Juru River 2	Male	3	Carapace	10.18	1.04	8.70	12.21
		3	Leg	10.98	0.97	9.06	12.23
		3	Telson	14.03	0.89	12.26	15.14
		3	Muscle	15.67	0.05	15.60	15.79
		3	Gill	17.44	0.66	16.16	18.35
		3	Operculum	15.43	0.90	14.52	16.34
	Female	2	Carapace	4.15	0.10	4.05	4.26
		2	Leg	6.48	0.58	5.73	7.65
		2	Telson	6.27	0.88	5.40	7.16
		2	Muscle	8.69	0.03	8.64	8.77
		2	Gill	10.68	0.42	10.27	11.11
		2	Operculum	9.22	0.68	8.54	9.91
		2	Egg	9.44	0.49	8.72	10.40
Sepang Besar River	Female	2	Carapace	3.19	0.26	2.69	3.58
		2	Leg	5.02	0.75	3.81	6.41
		2	Telson	3.32	0.24	2.86	3.67
		2	Muscle	5.39	0.88	4.12	7.09
		2	Gill	10.36	0.39	9.43	11.01
		2	Operculum	7.28	0.31	6.69	7.76
		2	Egg	7.96	0.47	7.03	8.59

Note: N represent the number of individuals analyzed.

Table 9: The mean concentrations (µg/g dry weight) of Zn in different parts; carapace, leg, telson, muscle, gill, operculum and egg of *Tachypleus gigas* from Juru River 1, Juru River 2 and Sepang River.

Location	Sex	N	Part	Mean	SE	Min	Max
Juru River 1	Male	4	Carapace	25.52	0.66	24.19	26.25
		4	Leg	137.16	8.26	127.32	153.58
		4	Telson	20.45	0.36	19.74	20.90
		4	Muscle	238.67	1.77	236.13	242.10
		4	Gill	141.48	7.01	133.19	155.41
		4	Operculum	103.41	15.21	88.20	118.63
	Female	1	Carapace	17.76	0.73	16.70	19.18
		1	Leg	136.77	16.52	120.25	153.29
		1	Telson	26.16	2.43	23.73	28.60
		1	Muscle	254.85	6.99	247.86	261.86
		1	Gill	113.79	0.91	112.49	115.56
		1	Operculum	107.41	2.18	105.22	109.60
		1	Egg	144.35	3.31	137.89	148.85
Juru River 2	Male	3	Carapace	19.55	2.47	15.90	24.28
		3	Leg	70.11	3.56	64.97	76.96
		3	Telson	15.02	0.35	14.35	15.57
		3	Muscle	255.61	9.66	236.54	267.86
		3	Gill	90.84	3.11	84.78	95.15
		3	Operculum	82.49	0.04	82.45	82.54
	Female 2	2	Carapace	19.08	1.99	17.09	21.08
		2	Leg	106.03	6.04	98.59	117.99
		2	Telson	21.63	2.38	19.25	24.02
		2	Muscle	240.80	3.78	236.37	248.33
		2	Gill	102.60	1.05	101.55	103.66
		2	Operculum	102.47	10.88	91.59	113.36
		2	Egg	134.27	1.37	131.57	136.00
Sepang Besar River	Female	2	Carapace	10.16	1.47	8.23	13.06
		2	Leg	80.72	4.74	72.76	89.19
		2	Telson	7.25	0.56	6.12	7.85
		2	Muscle	236.42	4.17	231.58	244.73
		2	Gill	103.03	2.53	98.21	106.77
		2	Operculum	93.49	4.31	88.85	102.13
		2	Egg	122.84	0.38	122.12	123.41

Note: N represent the number of individuals analyzed.

Table 10: Concentrations (µg/g dry weight ± SE) of Cd, Cu, Fe, Pb, Ni and Zn in the surface sediments collected from the Juru River and the Sepang Besar River (SBR).

Digestions/ Heavy Metals	Sites	Cd	Cu	Fe	Ni	Pb	Zn
Total concentration	Juru	0.41± 0.06	63.81±0.56	27906± 450	32.74 ± 0.29	38.83± 0.43	269 ± 0.41
	SBR	3.68 ±0.12	15.28 ± 0.19	32489 ± 2753	16.10 ± 0.63	41.25± 0.84	88.3 ± 4.91
Sum of SET	Juru	1.02± 0.14	74.59± 0.62	34535± 410	40.46 ± 0.38	35.09± 0.56	270 ± 3.34
	SBR	3.07 ±0.00	16.12 ± 0.65	29071 ± 1081	15.39 ± 0.40	37.68± 0.04	106.15 ± 2.45
EFLE	Juru	0.20± 0.12	2.38± 0.02	202 ± 15.0	2.44 ± 0.26	0.62± 0.16	51.84 ± 0.48
	SBR	0.43 ±0.02	0.05 ± 0.03	816 ± 27.0	0.53 ± 0.13	1.72± 0.12	1.55 ± 0.08
Acid-reducible	Juru	0.15± 0.03	0.10± 0.03	600 ± 52.0	3.42 ± 0.12	0.53± 0.12	61.88 ± 0.79
	SBR	0.03 ±0.01	0.13 ± 0.02	1451 ± 251	0.28 ± 0.03	1.39± 0.20	19.08 ± 2.00
Oxidisable-organic	Juru	0.05± 0.00	16.89± 0.55	4581± 285	19.72 ± 0.36	22.83± 0.29	84.98 ± 1.45
	SBR	0.01 ±0.00	4.47 ± 0.00	6380 ± 140	6.06 ± 0.48	11.12± 0.19	43.83 ± 2.61
Resistant	Juru	0.62± 0.06	55.22± 0.03	29152± 629	14.87 ± 0.36	11.12± 0.80	71.34 ± 0.61
	SBR	2.59 ±0.00	11.46 ± 0.70	20424 ± 1166	8.54 ± 0.18	23.45± 0.17	41.68 ± 1.76

Note: Sum of SET= Summation of EFLE, Acid-reducible, Oxidisable-organic and resistant fractions.

Table 11: Biota-sediment accumulation factors (BSAF) based on the muscles of *Tachypleus gigas*.

Location	Sex	Cd	Cu	Fe	Ni	Pb	Zn
Juru River 1	Male	2.34	1.44	0.01	0.27	0.33	0.89
	Female	2.56	2.02	0.00	0.28	0.20	0.95
Juru River 2	Male	2.73	2.16	0.00	0.25	0.40	0.95
	Female	3.73	1.36	0.01	0.20	0.22	0.90
Sepang Besar River	Female	0.40	2.50	0.00	0.32	0.13	2.68

CONCLUSIONS

The most significant finding of present study is that the muscle of *T. gigas* is a potential biomonitoring organ of Cu, Ni and Zn.This is due to higher levels of these metals being found in horseshoe crabs from the polluted Juru River than in those from the relatively unpolluted Sepang Besar River.

Since this finding is well supported by the sediment data, the use of the muscle of *T. gigas* as a biomonitor of Cu, Ni and Zn contaminations and bioavailabilities of the sampling sites, is therefore proposed for future biomonitoring studies.

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REFERENCES

1. Shuster C. N. (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. (eds. Chase V.) Baltimore, MD, pp. 34-38.
2. Sekiguchi K. and Nakamura K. (1979). Ecology of the extant horseshoe crabs. Progress in Clinical and Biological Research 29: 37-45.
3. Chatterji A., Mishra J.K. and Parulekar A.H. (1992). Feeding behaviour and food selection in the horseshoe crab, *Tachypleus gigas* (Muller). Hydrobiologia 246: 41-18.
4. Kungsuwan A., Nagashima Y., Noguchi T., Shida Y., Suvapeepan S., Suwansakornkul P. and Hashimoto K. (1981). Tetrodotoxin in the eggs of horseshoe crab, Carcinoscorpius rotundicauda inhabiting Thailand**.** Progress in Venom Research and Toxin Research, pp 470-474.
5. Chiu H.M.C. and Morton B. (1999). The distribution of horseshoe crabs (*Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*) in Hong Kong**.** Asian Marine Biology 16: 185-194.
6. Sekiguchi K., Nakamura K., Sen T.K. and 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.
7. Burger J. and Gochfeld M. (1995). Heavy metal and selenium concentrations in eggs of herring gulls (*Larus argentatus*): temporal differences from 1989 to 1994. Archive of Environment Contamination and Toxicology 29: 192–197.
8. Kannan, K., Yasunaga, Y., Iwata, H., Ichihashi, H., Tanabe, S. and R. Tatsukawa. (1995). Concentrations of heavy metals, organochlorines, and organotins in horseshoe

crab, *Tachypleus tridentatus*, from Japanase Coastal Waters. *Environmental Contamination and Toxicology*. **28**: 40-47.

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

10. Itow, T., Loveland, R.E. and M.L. Botton. (1998). Developmental abnormalities in horseshoe crab embryos caused by exposure to heavy metals. *Environmental Contamination and Toxicology*. **35**: 33-40.

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

12. Burger J., Dixon C., Shukla T., Tsipoura N. and Gochfeld M. (2002). Metal levels in horseshoe crabs (*Limulus polyphemus*) from Maine to Florida**.** Environmental Research 90 (3): 227-236

13. Burger J., Dixon C., Shukla T., Tsipoura N., Jensen H., Fitzgerald M., Ramos R. and Gochfeld M. (2003). Metals in horseshoe crabs from Delaware Bay. Archives of Environmental Contamination and Toxicology 44 (1):36-42.

14. Mo C. and Neilson B. (1994). Standardization of oyster soft dry weight measurements. Water Research 28: 243 – 246.

15. Yap C.K., Ismail A., Tan S.G. and Omar H. (2002). Correlation between speciation of Cd, Cu, Pb and Zn in sediment and their concentrations in total soft tissue of green-lipped mussel *Perna viridis* from the west coast of Peninsular Malaysia. Environment International 28:117-126.

16. James, W.M. (1991). Inorganic contaminants of surface water- Research and monitoring priorities. Springer- Verlag. New York: USA. p. 64-321.

17. Department of Environment (2007). Final report on development of water quality and standards for Malaysia. Minisry of Natural Resources and Environment, Malaysia.

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

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

20. Burger J., Dixon C., Shukla T., Tsipoura N., Jensen H., Fitzgerald M., Ramos R. and Gochfeld M. (2003). Metals in Horseshoe Crabs from Delaware Bay. Archives of Environmental Contamination and Toxicology 44: 36–42.

21. Peakall D. B. (1992). Animal markers as pollution indicators, Chapman and Hall, London, England.

22. Fimreite N., Brevik F. M. and Torp R. (1982). Mercury and organochlorines in eggs from a Norwegian gannet colony. Bulletin Environment Contamination and Toxicology 28: 58–60.

23. Yap C. K., Ismail A., Edward F.B., Tan S. G. and 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.

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

25. Burger J. (1994). Heavy metals in avian eggshells: another excretion method. Journal Toxicology Environmental Health 41**:** 207–220.

26. Yap C.K., Ismail A., Tan S.G. and 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, Coastal and Shelf Science 57: 623-630.

27. Yap, C.K. Ismail, A. and Tan, S.G. (2004). Heavy metal (Cd, Cu, Pb and Zn) concentrations in the green-lipped mussel *Perna viridis* (L.) collected from some wild and aquaculture sites in the west coast of Peninsular Malaysia. *Food Chemistry* 84: 569-575.

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

29. Castro G. and Myers J.P. (1993). Shorebird predation on eggs of horseshoe crabs during spring stopover on Delaware Bay. Auk 110: 927–930.

30. Tsipoura N. and Burger J. (1999). Shorebird diet during spring migration stop-over on Delaware Bay. Condor 101**:** 35–6