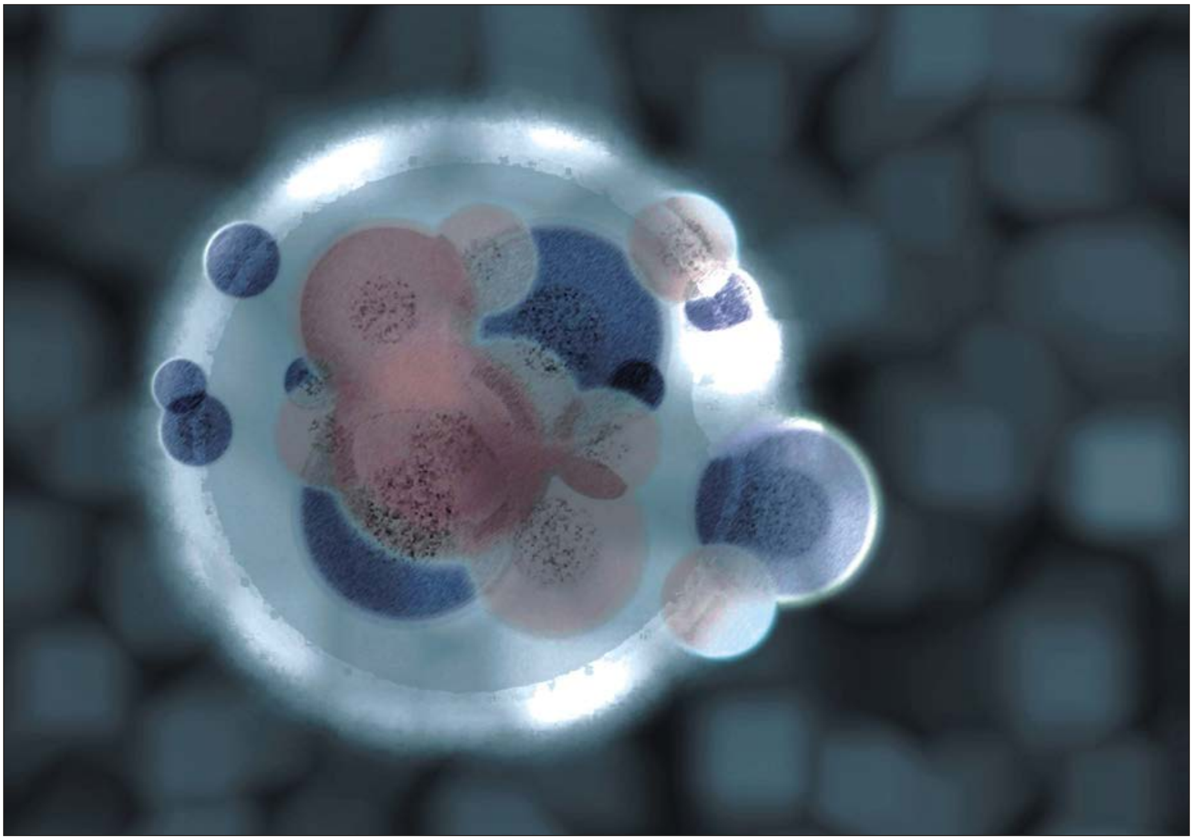

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Cd, Cu and Pb Concentration Levels in Horseshoe Crab Nesting Grounds of Pahang Coast, Malaysia

[1]K. Zaleha, [2]B.Y. Kamaruzzaman, [2]B. Akbar John and [3]M.C. Ong
[1]Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
[2]Institute of Oceanography and Maritime Studies, International Islamic University Malaysia, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia
[3]Department of Marine Science, University Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

Abstract: Balok, Pekan and Penor have been observed to be nesting grounds of the horseshoe crab. Present study was conducted to assess the metal concentration in sediment of horseshoe crab the nesting ground along the east coast of peninsular Malaysia. Acid Digestion was performed using (HF, HNO_3, HCl, EDTA and H_3BO_4) before determining the actual concentration of heavy metals using ICPMS. 2-way ANOVA was used to determine the significance of the results. In all stations, Pb was in highest concentration (40.36 $\mu g\ g^{-1}$) followed by Cu (13.44 $\mu g\ g^{-1}$) and the least concentrated metal in the nesting ground was Cd with the high concentration of (0.26 $\mu g\ g^{-1}$). EF values showed that Pb is of anthropogenic source and Cd is of minimum enrichment. Although the levels of Pb are low and regarded harmless to the horseshoe crab, the fear of it affecting those who consume the eggs as well as the crab is still in consideration.

Key words: Acid digestion, bioaccumulation, enrichment factor, horseshoe crab, nesting ground

INTRODUCTION

Horseshoe crabs are at the epicentre of one of the most interesting marine resource Management issues along the coast. They are marine chelicerate arthropod. Despite their name, they are more closely related to spiders, ticks and scorpions than to crabs. There are four extant species of horseshoe crabs, *Tachypleus tridentatus*, *Tachypleus gigas*, *Carcinoscorpius rotundicauda* and *Limulus polyphemus*. The first three species inhabit the Southeast Asian coast and the *Limulus polyphemus* inhabits in the east coast of North America. Their spawning season varies according to latitude, but it peaks in the month of May and June in the east coast of peninsular Malaysia (John *et al*., 2010). The peak of spawning will usually occur on evening high tides during the full and new moons (Jackson *et al*., 2005; Nordstrom *et al*., 2006). These horseshoe crabs will seek beaches that are at least partially protected from surf, within bays and coves. The preference characteristics associated with spawning locations are the presence of large intertidal sand flats near the spawning beach, a reducing depth of around 30 cm or more from the surface and sediment types (Brockmann *et al*., 2000; Jackson *et al*., 2006). Since horseshoe crabs dig pits to bury their eggs, the exposure of metals in sediments to its eggs may cause an effect. The interpretation of metal concentration among invertebrates is difficult because toxicity only occurs when the rate of uptake exceeds the rates of detoxification and excretion, rather than solely metal concentrations in the body of an individual (Selck and Forbes, 2003; Croteau and Luoma, 2009; Casado-Martinez *et al*., 2010). Therefore, the establishment of whether heavy metals or other parameters are the limiting factors contributing towards changes in horseshoe crab populations should be investigated.

Intensive agriculture and coastal engineering due to human impacts to coastal areas have been observed to threaten marine life in the past two centuries (His *et al*., 1999). The property of metals which are non biodegradable in the environment and are able to bio-accumulate differentiate them from other pollutants. According to some scientists, metals are the most dangerous because of its non-biodegradable property and its innate ability to remain within the ecosystem, especially among sediments (Kamaruzzaman *et al*., 2011; Liaghati *et al*., 2003; Barosso *et al*., 2010).

It is observed that metal pollutions and the effects of anthropogenic events in the environment can be monitored using sediment particles. It can be assumed

Corresponding Author: B.Y. Kamaruzzaman, Institute of Oceanography and Maritime Studies, International Islamic University Malaysia, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia

that sediment particles act as a sink for metal deposition in the aquatic environment (Kamaruzzaman *et al*., 2011a; Fernandes, 1997; Soares *et al*., 1999; Niu *et al*., 2009). Many coastal areas were targets of aquaculture activities, specifically in prawn farming along the mangrove areas (Primavera, 2006). This is with regard to the locals who depend on waters of the sampling site for food and source of income.

This study was conducted to assess the concentrations of metals in sediments of the horseshoe crab nesting ground. To predict sources of metals, the Enrichment Factor (EF) is used. It is important to predict effects of metal accumulation towards its eggs and larvae survival. By such an assessment, the degree of contamination (if present) might be predicted and thus would ultimately help in proper monitoring and to implement various mitigation measures.

MATERIALS AND METHODS

Sampling sites and samples collection: The sampling was conducted in three stations in Pahang state during July 2009 (Fig. 1), the three sampling areas were identified as Pantai Balok (Balok: Lat3°56.194' N, Long103°22.608' E), Cherok Paloh (Penor: Lat3° 38' 60 N, Long103° 22' 0 E) and Tanjung Gosong (Pekan: Lat3°36.181' N, Long103°23.946' E). These three areas were observed to be a nesting ground by mating pairs of horseshoe crabs. Furthermore, the sediments of the sampling location were observed to be soft which may contribute to the ease of laying eggs and egg burial by the female horseshoe crab. The tide table of Tanjung Gelang is used to predict tide levels and date of sampling. The sediment is obtained from the highest tidal mark using a plastic scoop. The samples are placed into an oven (Memmert 40050-IP20) for 2 days at 60°C to ensure the sample is completely dry. Sediments in the Petri dish meant for heavy metal analysis are then placed into a mechanical shaker (Retsch-Germany). Only sediments in the 63 μm^{-1} sieve were kept in a plastic container (pre-washed with 5% HNO_3) and used for the acid digestion.

Heavy metal analysis: The sediment samples were digested according to the published methods with some modifications (Kamaruzzaman *et al*., 2011; Sen Gupta and Bertrand, 1995; Yuan *et al*., 2004; Defew *et al*., 2005) with some modifications. An inductively-Coupled Plasma Mass Spectrometer (ICP-MS) was used for the quick and precise determinations of Cd, Cu and Pb digested marine sediment. Briefly, the digestion method involved the heating of 50 mg of a < 63 μm^{-1} size sample in a sealed teflon vessel with a mixed concentrated acids of HF, HNO_3 and HCl in the ratio of 2.5: 3.5: 3.5. The teflon vessels were kept at 150°C for 3-5 h. After cooling, a mixed solution of boric acid and EDTA was added and the vessel was again heated at 150°C for at least 5 h. After cooling to room temperature, the content of the vessel was thoroughly transferred into a 10 mL^{-1} polypropylene test tube and was diluted to 10 mL^{-1} with deionized water. A clear solution with no residue should be obtained at this stage. The precision assessed by replicate analyses was within 3%. The accuracy was also examined by analyzing, in duplicate a Canadian Certified Reference Materials Project standard (DL-1a) and the results coincided with the

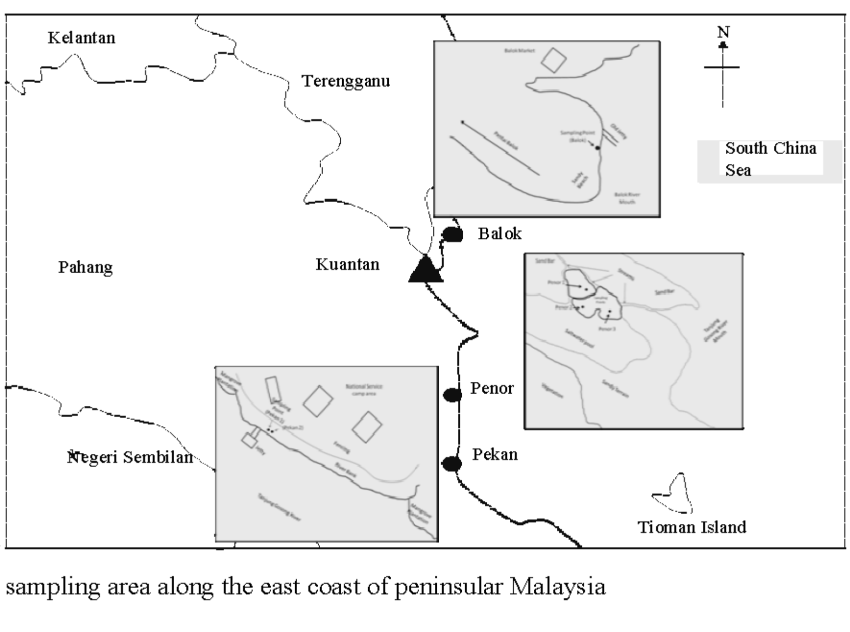

Fig. 1: Location of 3 sampling area along the east coast of peninsular Malaysia

certified values within a difference of±3%. An Enrichment Factors (EF) was calculated to determine the source of heavy metals at the nesting ground of horseshoe crabs. Two way ANOVA analyses were conducted to find the significance of the metals concentration at different sampling sites.

RESULTS AND DISCUSSION

Table 1 shows the results of Cd, Cu and Pb concentrations in the dried samples analysed. From the results obtained, the range of Cd concentration was (0.08 to 0.26)±0.19 $\mu g\ g^{-1}$ with the mean concentration of (0.192± 0.06 $\mu g\ g^{-1}$ dw) and the highest concentration was observed at Penor 3. The highest concentration of Cu on the other hand was 13.44±8.64 $\mu g\ g^{-1}$ observed in Pekan 1. The mean concentration range of Cu for all station was (3.45 to 13.44) ±8.64 $\mu g\ g^{-1}$ dw. The highest concentration of Pb was observed at Penor 3 (40.36±26.31 $\mu g\ g^{-1}$). In contrast, the concentration range of Pb was 16.01 to 40.36 $\mu g\ g^{-1}$ with the mean concentration of (26.31±9.54 $\mu g\ g^{-1}$ dw) at all the stations. Al showed the highest concentration in Penor 3 (2.52±1.63 %) as well. As a comparison among all sampling locations, Pb showed the highest value for all sampling locations, followed by Cu and Cd. Therefore, the concentration of metals follow the ascending order of Cd < Cu < Pb in sediment. There was no significant difference between the sampling locations and the metals analyzed ($p>0.05$).

Present study revealed that the concentration of Cd at the nesting ground of horseshoe crab was lower than the concentration observed in the Kelantan River sediments (Ahmad *et al*., 2009). The concentration of Cu on the other hand showed lower values when compared with Balok, Penor and Pekan samples. The concentration of Pb was also higher than Balok but lower than Penor and Pekan. In comparison to the coastal environment, Ong and Kamaruzzaman (2009) reported that the concentration of Cu obtained from the bottom sediment of South China Sea coastal waters was much higher than Balok, Penor and Pekan. The concentration of Pb on the other hand was lower than Penor and Pekan but higher than Balok (Table 2).

Table 1: Concentration of selected heavy metals in the horseshoe crab nesting ground expressed in $\mu g\ g^{-1}$ dry wt.

Location	Cd	Cu ($\mu g\ g^{-1}$ dw)	Pb
Balok	0.20	8.38	16.01
Penor 1	0.18	6.23	26.27
Penor 2	0.08	3.45	18.21
Penor 3	0.26	11.70	40.36
Pekan 1	0.18	13.44	22.29
Pekan 2	0.25	8.65	34.72

The Enrichment Factors (EF) is used to indicate the source of metals in sediment. The EF is usually used for the assessment of aerosols to understand composition in terms of isolated and anthropogenic elemental sources (Hung and Hsu, 2004; Mil-Homens *et al*., 2007). The Eq. below was used to calculate EFs of the analyzed elements:

$$EF = (E/Al)_{sediment} / (E/Al)_{crust}$$

where, $(E/Al)_{sediment}$ and $(E/Al)_{crust}$ are the relative concentration of the respective elements, E and Al in the sediment and as well as the crustal material, respectively (Khalik *et al*., 1997; Prudencioa *et al*., 2007; Zhang *et al*., 2007; Kamaruzzaman *et al*., 2011).

An EF close to 1 would indicate a crustal origin while factors greater than 10 are considered to have a non-crustal source. Based on the results of the EF (Table 3), only Penor 2 showed moderate enrichment of Cd whereas other sampling sites showed significant enrichment. All sampling locations showed significant enrichment of Pb. In addition, only Pekan 1 showed moderate enrichment of Cu whereas other sampling locations showed deficiency to minimal enrichment. Similar observation was reported by El-Nemr*et al.* (2007) along the Mediterranean coast of Egypt where minimal enrichment of Cu and Significant enrichment of Cd and Pb was detected. The spatial distribution of cumulative heavy metals in the sediments suggested that the Cu mainly originated from point sources, while the Pb and Cd probably came from non-point sources in the estuary (Liu *et al*., 2003).

Table 2: Comparison of heavy metal concentration ($\mu g\ g^{-1}$ dw) observed in present study with other published data

Author	Location	Cd ($\mu g\ g^{-1}$ dw)	Cu ($\mu g\ g^{-1}$ dw)	Pb ($\mu g\ g^{-1}$ dw)
Ahmad *et al.* (2009)	Kelantan River	1.82	6.74	20.82
Ong and Kamaruzzaman (2009)	South China sea pahang coast	-	19.70	24.49
	Balok	0.20	8.38	16.10
Present study	Penor	0.18	7.13	28.28
	Pekan	0.22	11.05	28.50

Table 3: Enrichment Factor value of 3 sampling stations (Balok, Pekan and Penor)

Stations	Metals	EF	Criteria
	Cd	11.71	Significant enrichment
Balok	Cu	1.96	Deficiency to minimal enrichment
	Pb	6.33	Significant enrichment
	Cd	9.37	Significant enrichment
Penor 1	Cu	1.28	Deficiency to minimal enrichment
	Pb	9.12	Significant enrichment
	Cd	4.79	Moderate enrichment
Penor 2	Cu	0.81	Deficiency to minimal enrichment
	Pb	7.20	Significant enrichment
	Cd	8.26	Significant enrichment
Penor 3	Cu	1.48	Deficiency to minimal enrichment
	Pb	8.61	Significant enrichment
	Cd	12.26	Significant enrichment
Pekan 1	Cu	3.63	Moderate enrichment
	Pb	10.16	Significant enrichment
	Cd	10.91	Significant enrichment
Pekan 2	Cu	1.51	Deficiency to minimal enrichment
	Pb	10.26	Significant enrichment

CONCLUSION

This study has shown that among all three metals analyzed, the most persistent heavy metal was Pb in all sampling sites. EF value of Pb showed significant enrichment and increasing from uncontaminated to moderately contaminated level. The concentration of Pb might not pose a threat to the horseshoe crabs as well as its eggs but might cause physiological changes to the organisms that consume horseshoe crab and their eggs. EF values also showed that Cu concentrations were of natural occurrence. The concentration of Cd on the other hand might be influenced by a slight introduction of anthropogenic activity because of the discharges of various sources of Cd along the sampling locations. Therefore, the concentrations of Cd and Cu do not pose any threat (Cole and Volpe, 1983; Segura *et al.*, 2006).

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