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ANALYSIS OF TRACE METALS (Ni, Cu, and Zn) IN WATER, MUD AND VARIOUS TISSUES OF MUD CRAB, *Scylla olivacea* FROM SETIU WETLANDS, TERENGGANU, MALAYSIA

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Abstract: Mud crabs, belonging to the genus *Scylla* from the family of Portunidae are important crustaceans of the mangrove ecosystem including Setiu Wetlands, Terengganu. *Scylla olivacea* is one of the three species of mud crabs that are indigenous to Setiu Wetlands, and are commonly found in the nypa palm at the lagoon areas. The mud crabs samplings were done during low tides at three locations designated as A, B, and C alongside Setiu Wetlands for trace metal analysis (Ni, Cu, and Zn). Samples of water and mud at the designated sampling points were analysed for trace metals by using an Inductive Couple Plasma-Mass Spectrometry (ICP-MS) gauge. As for the mud crabs, various parts of their body (gills, hepatopancreas, and claws) were being separated prior to trace metal analysis in each of the tissue. The average concentration for Ni, Cu, and Zn in water was 6.920±0.24 µg/L, 81.280±28.70 µg/L, and 13.400±7.50 µg/L respectively. It was found that, in the mud samples, the average values of Ni, Cu, and Zn were 5.833±0.73 ug/g, 4.782±0.77 ug/g, and 17.926±2.29 ug/g. The crab tissues analysis shows that, the average values of these trace metals in gills were 4.811±1.32 ug/g (Ni), 78.482±35.37 ug/g (Cu) and 96.803±22.28 ug/g (Zn) whereas in hepatopancreas, the average values were 4.131±2.50 ug/g (Ni), 90.903±25.56 ug/g (Cu) and 166.96±30.19 ug/g (Zn). In the claws tissues, the average value of Ni (3.550±0.955 ug/g), Cu (66.235±52.30 ug/g), and Zn (170.607±12.22 ug/g). The presence of Ni, Zn, and Cu in claws of mud crabs was significantly correlated with the metal content in the mud (r in range between 0. 975 and 0.999). Zinc content in the mud was also correlated significantly with Zn content in hepatopancreas (r = 0.9205) implying that the main source for metals accumulation in tissues of mud crabs comes from the mud surrounding their burrows. The data obtained provide the current status of the trace metal contents in wild mud crabs in relation to the environmental conditions of the habitat.

KEYWORDS: *Scylla olivacea,* trace metals, Setiu Wetlands, inductive couple plasma-mass spectrometry, mud crab

Introduction

Setiu Wetlands is located in the north-eastern part of the Peninsular Malaysia, facing the southern part of South China Sea. This area has diverse ecosystems and comprises of vast biodiversity of flora and fauna. Among these, the abundance of wild mud crabs become utilisable natural resources for commercial fisheries and aquaculture industries in this area. To date, three species of these mud crabs, namely *S. olivacea, S. paramamosain,* and *S. tranquebarica* are indigenous to Setiu Wetlands and are commonly found in the nypa palm areas at the lagoon of Setiu Wetlands. Recently, the number of wild mud crab caught had gradually decreased, and the maturity size of adult mud crabs had also decreased (9-11cm carapace width) (Ikhwanuddin *et al.,* 2010a). Several factors might contribute to these decrements; among them are large scales of aquaculture activities within and nearby the wetlands which could threaten the ecosystems of this area. Aquaculture activities such as brackish water cage culture, pond culture, pen culture and oyster farming are the major and the fastest growing economic activities in this locale. The rapid growth of aquaculture farming activities leading to the loss of mangrove forest, dwindling fish and crustaceans stock due to disease outbreaks and discharge of particulate organic materials (Tovar *et al.*, 2000). These anthropogenic activities might as well directly contributed to the contamination of trace metals in the ecosystem and therefore, affect the physiological and biochemical of mud crabs (Kamaruzzaman *et al.*, 2012).

Trace metals plays an important role in the biochemical process in living organisms which contribute to growth, development and physiological activities (Shanker, 2008). Their continuous exposure to the environment may cause an adverse effect on living organisms and ecosystem if the concentration is over permissible limits in a long term. It can cause degradation in biochemical mechanism and gives health impact on aquatic organisms (Mudgal *et al.*, 2010) including wild mud crabs. Furthermore, these mud crabs were directly exposed to these trace metals during their moulting process. During this process, mud crabs will uptake 85% of water in the environment before the hardening of exoskeleton takes place (Nguyen *et al.*, 2014). This paper reports the current finding on the concentration of trace metals (Ni, Cu, and Zn) in water, mud and in the tissues of the wild mud crabs *(S. olivacea)* at Setiu Wetlands; the content of trace metals accumulated in the crabs tissues can be used as a guidance for safe consumption of the crabs.

Materials and Methods

Sampling Site

Wild mud crabs, water, and mud were collected at three locations along Setiu Wetlands during low tides (post monsoon seasons) as shown in Figure 1. Location A is located near to sea opening (N 05° 39.466 E 102° 44.412), location B is about 3.3 km from location A (N 05° 38.543 E 102° 45.711) and location C is located 3 km from location B and near to the river mouth of Sungai Penarik (N 05° 38.089 E 102° 46.495). Water quality parameters such as temperature, salinity, pH were measured *in situ* by using a Salinity Conductivity Temperature (SCT) meter.

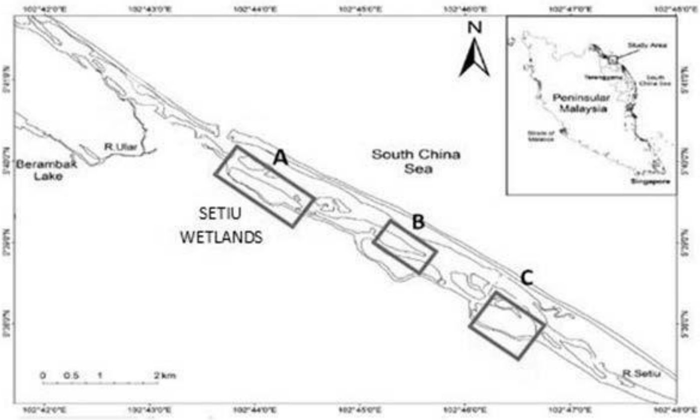

Figure 1: Map of Setiu Wetlands (modified from Suratman *et al.*, 2014) showing three different sampling locations of wild mud crabs, water and mud samples carried out in this study.

Mud Crab and Mud Sampling

Wild mud crabs at each location were caught and labeled using a crab trap (20 units per location, and placed at random, they were left overnight before collection). Mud samples from the same site were collected by using scoops and were placed into clean, sterile polyvinyl bottles.

Both crabs and mud samples were transported back to the laboratory for metal analysis. In the laboratory, the assorted mud crabs were classified according to their species, sex, and maturity. Body weight (BW) of mud crabs were weighed by using an electronic balance and the carapace width (CW) was measured by using calipers. The matured male of mud crabs (CW > 8.97 cm) (Ikhwanuddin *et al.*, 2010b) was used in this study for the trace metals analysis. The crabs sample were kept at -80$_o$C and the mud was placed at 4$_o$C prior to trace metal analysis.

Acid Digestion for Water Samples

Water samples were collected (triplicate) at each location by using water sampler at 1m depth and stored in 1 litre polypropylene bottles. Water samples were preserved with 10% of nitric acid (HNO $_3$) at room temperature prior analysis. Then, water was filtered through a 125 mm size filter paper (Whatman No 2) and before analysed for trace metals by using an Inductive-coupled plasma-mass spectrophotometer (ICP-MS Perkin-Elmer Elan 9000) (Voica *et al.*, 2011).

Acid Digestion for Mud and Tissues Samples

Analysis of heavy metals in mud crabs were done according to the method ensued by Azlisham *et al.* (2009) for oysters with slight modifications. Mud crab extraction was modified to use less concentrated HNO3 (20%) for digestion. Tissues of mud crabs (claws, gills, and hepatopancreas) were dissected before being freeze-dried. The mud samples were set to dry in a furnace at a temperature of 500°C for a duration of 5 hours. After the drying process, each samples (mud and tissues) were being ground with a mortar and pestle at room temperature. Each sample (0.2g) was weighed and added with 2 mL of concentrated hydrochloric acid (HCl). These mixtures were then being heated on a hot plate until it dried. The samples were allowed to cool prior to addition of 10 mL of 20% HNO3. The mixtures (both for mud and tissues) were heated in a water bath for 1 hour. Afterwards, the mixtures were made up to 50 mL using Milli Q water. The mixtures were then filtered through a filter paper Whatman No 2 (125mm) before being analysed for trace metal content by using an ICP-MS.

Results and Discussion *Physico-chemical Parameters of Water at Setiu Wetlands Estuaries*

The measurement of physico-chemicals of water at three sampling locations is presented in Figure 2. The water quality values, especially salinity and conductivity, are highest at location A, followed by C and B ($p<0.05$). Location A is situated at the sea opening, hence this area receives direct input of seawater. Interestingly for location B, which was located in between locations A and C, both water salinity and conductivity measured are significantly low compared with other two locations ($p<0.05$). The topography of this location (Figure 1) and the location of aquaculture activity nearby might contribute to the quality of water measured in this area. Solid wastes, chemicals, and therapeutics from aquaculture activities can cause the changes of nutrients and organic matters, such as ammonia and nitrite that will influence the salinity and electrical conductivity of water (Cao *et al.*, 2007). Previous studies had obsereved that, some of aquaculture activities prefer to use low salinity environments by modification of the rearing medium with potassium and magnesium fertilizers to enhance the growth of shrimps (Roy *et al.*, 2010). Although location C is located near to the river mouth, the water physico-chemical parameters measured at this location are similar to location A (at the sea opening), indicating the homogeneity of water quality at Setiu Wetlands, except at location B. For temperature (range from 27-29 °C) and pH (ranged from 7-8) readings, the results gave no significant difference ($p>0.05$) in both water quality parameters measured at the three sampling locations.

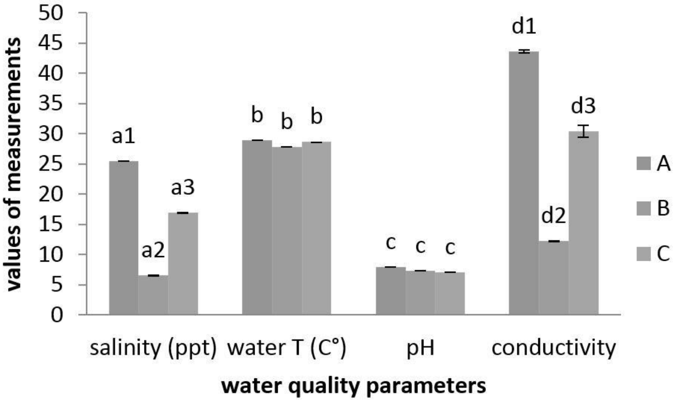

Figure 2: Physico-chemical parameters of water at Setiu Wetlands at where the mud crabs were collected. Different superscript number shows significant difference ($p<0.05$) by using One-way ANOVA followed by Duncan test.

Trace Metals in Water Samples

The concentrations of trace metals in water samples are presented in Table 1. Ni and Zn were found highest in location B compared to other locations which values are 7.154±0.03 and 22.027±0.00 µg/L, respectively ($p<0.05$). Boating activity, aquaculture, and agriculture activities at location B are potential factors responsible for the presence of contaminants of trace metals in water. Another study shows that these two elements were high in aquaculture areas, they were probably being deposited from commercial fish feeds, anti-fouling, antibiotics, maintenance works, fecal and waste products (Tovar *et al.*, 2000; Farmaki *et al.*, 2014). Furthermore, these metals may form dissolving metals through dissolution process. This condition happens when water interact with metallic objects which causes it to break apart and become freely ions in water thus contributes to the elevation of Zn and Ni in water (Lim *et al.*, 2012). Moreover, estuary ecosystems are easily contaminated with heavy metals that derived from land-based anthropogenic activities (Vijayavel *et al.*, 2009). Rainfall of acids will wash the pollutants in lands and carried them into the river, estuary and next to the sea (Vosyliene & Jankaite, 2006).

The solubility of trace metals in water vary depending on the type of elements and were being influenced by tidal and rainfall (Coban *et al.*, 2003). In this study, the highest concentration of metals in water was Cu followed by Zn, and Ni ($p<0.05$). Sources of Cu in the aquatic ecosystem are generally from the industry, agriculture, mining activities and boatyard activities (Bidone *et al.*, 2001). Zn is widely used in paints of boats, fish pellets, fertilizers and pesticides (Gerhard, 2000). Contamination of Ni in water may originate from the domestic wastewater effluents (Cempel & Nikel, 2006). The concentration of Cu in water measured in the present study exceeds the permissible limit authorised by Malaysian Marine Water Quality Criteria and Standard (MMWQCS) (0.0029 mg/L or 2.9 µg/L) for estuary area (Class 4). The concentrations of Cu (0.41 µg/L) in Setiu Wetlands measured in 2009 by Azlisham *et al.*, (2009) was under the permissible limit by MMWQS, the results suggest that the accumulation of this trace metal in water in this area has increased significantly due to the increasing human activities. The condition worsens during the dry season as higher concentration of trace metals present in water compared with the monsoon seasons (Azlisham *et al.*, 2009). In another study carried out at Tanjung Bin, Johor, Malaysia, the concentration of Cu measured (0.05 mg/L) was also found to be exceeding the permissible limit by MMWQCS. The high usage of Cu as a biocide for protection of marine vessels (Sabri *et al.*, 2012), contributes to the high concentration

Cu in that area. The concentration of Cu in Langat River is 0.15 mg/L (Yusoff *et al.*, 2009) was observed due to the industrial activities. Therefore, the concentration measure of Cu in water at Setiu Wetlands (0.08 mg/L) well considered low.

Table 1: Concentrations of trace metals (µg/L) in water at Setiu Wetlands.

Sampling site	**Concentrations (µg/L)**		
	Ni	Cu	Zn
A	6.932±0.00$_{a1}$	94.407±0.00$_{b1}$	8.462±0.00$_{d1}$
B	7.154±0.03$_{a2}$	48.369±0.01$_{b2}$	22.027±0.00$_{d2}$
C	6.678±0.00$_{a3}$	101.066±0.00$_{b3}$	9.712±0.00$_{d3}$
Average	6.920±0.24	81.280±28.70$_{*}$	13.400±7.50
***MMWQCS**	NA	2.9	50

*MMWQS: Malaysia Marine Water Quality Criteria and Standard 2010
NA: not available, Different superscript number shows significant difference ($p<0.05$) by using One-way ANOVA followed by Duncan test.

Trace metals in mud samples

The concentration of trace metals of Ni, Cu, and Zn in mud samples are presented in Table 2. The concentration of these metals, in general, is higher at location A compared with other locations. However, the concentration of these trace metals in mud samples did not exceed the permissible limits by Interim-Sediment Quality Guideline (ISQG). In the present study, the concentration of Zn was the highest in the mud samples followed by Cu and Ni (Table 3). The highest concentration of Zn was also reported in sediments at Setiu Wetlands (Azlisham *et al.*, 2009). Zn was the highest trace metals (ranging from 0.05 µg/g to 0.1 µg/g dry weights of sediment) deposited in the sediments of Paka estuary, Terengganu (Kamaruzzaman *et al.*, 2006). This element is believed to be deposited in the sediments from the natural origin that derived from weathering of rocks in that area. Vosyliene and Jankaite (2006) forwarded that trace metals will be diluted with various surface water components in the water bodies, and some of the trace metals will sink and accumulate into sediments and take a longer period to be eliminated from the environment. However, when acid raining occurs, the water pH will be declined, and trace metals may be mobilised to enter the water bodies and hence give negative effects to aquatic organisms.

Table 2: The Concentrations of trace metals in mud samples (µg/g) taken at three sampling locations.

Sampling site	**Concentrations (µg/g)**		
	Ni	Cu	Zn
A	6.673±0.22$_{a1}$	5.677±0.06$_{b1}$	20.362±0.37$_{d1}$
B	5.350±0.10$_{a2}$	4.364±0.30$_{b2}$	15.814±0.08$_{d2}$
C	5.474±0.22$_{a2}$	4.304±0.17$_{b2}$	17.603±0.14$_{d3}$
Average	5.833±0.73	4.782±0.77	17.926±2.29$_{*}$
ISQG-low	21	65	200
ISQG-high	520	270	210

ISQG-low: Interim Sediment Quality Guidelines (ISQG) for a particular contaminant that is not excessive, the element is unlikely to cause any biological effect on organisms inhabiting the sediments. ISQG-high: ISQG for which the biological impact is likely to be high (Aris *et al.*, 2014).
*shows highest concentration with significantly ($p<0.05$), Different number shows significant difference $p<0.05$ by using One-way ANOVA followed by Duncan test.

Concentration of Trace Metals in Mud Crab's Tissues and Correlation to Their Surrounding

Generally, there is no significant difference of heavy metals (Ni, Cu, and Zn) measured for gills, hepatopancreas and claws of the sampled mud crabs (Tables 3) ($p>0.05$) between the three locations. Accumulation of trace metals in the crabs' tissues showed that Zn was the highest trace metal measured (in gills, 96.803±22.28 µg/g, hepatopancreas, 166.96±30.19 µg/g, and claws, 170.607±12.22 µg/g). This result is supported by Ong *et al.* (2017) where the concentration of Zn (112±7.2 µg/g) was the highest content of trace metals in edible tissues of mud crabs. Zn was also detected as the highest concentration in oyster's tissue collected from Setiu lagoon (Najiah *et al.*, 2008; Azlisham *et al.*, 2009) which indicate that most aquatic animals in that area tend to accumulate Zn in their tissues from the environment. In another marine organisms such as shrimps, other metal elements, Fe was detected at the highest concentration in their tissues (Heidareih *et al.*, 2013) suggesting that different organisms have a different mode of tolerance to accumulate the different type of trace metals from their surrounding into their body.

Table 3: Concentrations of trace metals in each tissue of mud crabs.

Mud crabs tissues	Sampling site	Concentrations of trace metals (µg/g)		
		Ni	Cu	Zn
Gills	A	6.158±1.44$_{a1}$	82.900±6.84$_{b1}$	121.773±69.31$_{c1}$
	B	4.753±0.68$_{a1}$	111.432±24.10$_{b1}$	89.685±23.51$_{c1}$
	C	3.521±1.12$_{a1}$	41.113±14.94$_{b2}$	78.951±51.57$_{c1}$
	Mean (A, B and C)	4.811±1.32	78.482±35.37	96.803±22.28*
Hepatopancreas	A	6.175±2.50$_{a1}$	75.330±29.27$_{b1}$	208.910±92.56$_{c1}$
	B	1.339±0.08$_{a1}$	120.400±97.70$_{b1}$	108.390±96.92$_{c1}$
	C	4.879±1.57$_{a1}$	76.978±8.02b$_{1}$	183.590±56.67$_{c1}$
	Mean (A, B and C)	4.131±2.50	90.903±25.56	166.96±30.19*
Claws	A	4.643±1.26$_{a1}$	126.226±18.98$_{b1}$	183.650±54.75$_{c1}$
	B	3.132±1.34$_{a1}$	30.192±6.20$_{b2}$	159.420±8.06$_{c1}$
	C	2.875±0.41$_{a1}$	42.288±18.68$_{b2}$	168.750±37.33$_{c1}$
	Mean (A, B and C)	3.550±0.955	66.235±52.30	170.607±12.22*
FAO (1983)		70-80	30	30

FAO: Food and Agriculture Organization (1983) (Anim *et al.*, 2010).
*shows highest concentration with significantly ($p<0.05$), Different number shows significant difference $p<0.05$ by using One-way ANOVA followed by Duncan test.

Cu and Zn are essentials elements which are essential in biochemical and physiological activity of aquatic organisms (Tchounwou *et al.*, 2012). As an example, Cu acts as cofactors for several enzymes such as catalase, superoxide dismutase, monoamine oxidase. Ni on the other hand, is one of the non-essential elements and is not involved in the biological process in animals (Tchounwou *et al.*, 2012). High concentration of trace metals whether they are essential or not, will cause toxicity in aquatic organisms. The concentrations of Cu and Zn obtained in this study exceed the permissible limit by Food Act Organization 1983, therefore, high contaminated mud crabs with these metals may pose health risk to humans who consumed these animals.

The concentration of trace metals of Ni, Zn, and Cu in the claws of the mud crabs were significantly correlated with the concentration of these metals in mud (0.975< r >1.000) (Table 4). The concentration of Zn in mud samples was also significantly correlated with the metals in the hepatopancreas of the crabs (r = 0.920). There are however, moderate correlations were being observed between the concentration of Ni in mud samples (r = 0.842) and in hepatopancreas (r = 0.765), and also the concentration of Zn in the mud and in gills (r = 0.800). There were nill or weak correlations observed between the concentration of metals in water and in all tissues of the mud crabs, and similar results also were found on the concentration of Cu in the mud and in gills and in the hepatopancreas of the mud crabs. Normally, the aquatic organisms are exposed to pollutants through direct intake from the water bodies, and with indirect intake from the food chains. In the present study, such correlation between metals in water and in the mud crabs tissues was not observed. Thus, the metals in the surrounding water do not act as sources for the metal accumulation in the crabs' tissues. The content of trace metals in water fluctuates due to the continuous flow of river water and rainfall that cause the elimination of these metals at the crabs' habitat. Therefore, different concentration of trace metals measured in water at the three sampling locations does not affect the metal content in tissues of the mud crabs. As observed in this study, the trace metals present in the mud especially Ni and Zn are potentially transferred and accumulated in tissues of the mud crabs (in claws and hepatopancreas) through the feeding and burrowing behavior of mud crabs (Kamaruzzaman *et al*, 2012). The mud crabs, *S. olivacea* in their natural habitat feed on fishes, detritus, mud, and sand (Viswanathan & Raffi, 2015). These mud crabs are bottom feeders and their burrowing behavior influences the accumulation of trace metals in tissues of mud crabs from the surrounding muds (Kamaruzzaman *et al.,* 2012; Chainy and Paital, 2012). Thus, trace metals that are present in the mud can be easily accumulated in the crabs' tissues through bioaccumulation and biomagnification. Besides that, crustaceans have a close relationship with the sediments as it is their habitat and feeding site (Sultana *et al.,* 2015). This is the first study to relate sources of trace metals in the environment and accumulation of the metals in tissues of the mud crabs.

Table 4: Correlation of trace metals (a) Ni (b), Cu (c) and Zn with water, mud and tissues samples. Values of *r* for correlation among water, mud and tissues studied.

(a)

	Water	Mud	Gills	Hepatopancrease	Claws
Water	1				
Mud	-0.046	1			
Gill	0.500	0.842*	1		
Hepatopancrease	-0.679	0.765*	0.295	1	
Claw	0.173	0.976*	0.939*	0.606	1

(b)

	Water	Mud	Gills	Hepatopancrease	Claws
Water	1				
Mud	0.295	1			
Gill	-0.870	0.214	1		
Hepatopancrease	-0.989	-0.434	0.787*	1	
Claw	0.500	0.975*	-0.007	-0.622	1

(c)

	Water	Mud	Gills	Hepatopancrease	Claws
Water	1				
Mud	-0.846	1			
Gill	-0.356	0.799*	1		
Hepatopancrease	-0.987	0.920*	0.501	1	
Claw	-0.841	1*	0.805*	0.917*	1

Based on the results of the present study, the concentrations of metals in mud have a significant correlation with metal accumulation in mud crab's tissues. The measurement of trace metals in water on the other hand, does not indicate the actual end product or damage imposed to the ecosystem by anthropogenic activities. The animals at the contaminated areas are therefore, the best indicators of any pollutants present in the ecosystems.

Conclusions

This study presents the current data on the concentration of trace metals (Ni, Cu, and Zn) in water, mud, and its accumulation in the tissues of mud crabs, *S. olivacea* at Setiu Wetlands, Terengganu. The concentrations of Ni and Cu measured in water at Setiu Wetlands exceeded the permissible limits authorised by MWQCS, however there is no observed correlation with metals in water and their accumulation in the crab's tissues. The concentrations of Cu and Zn in claws and hepatopancreas of the mud crabs at Setiu Wetlands were found to exceed the approved limits of FAO, the accumulation in tissues of the mud crabs comes from the mud surrounding their burrows as the crabs are bottom feeders with burrowing activities in their muddy habitats. This unique behavior of the wild mud crabs benefits them to become a better candidate for assessment of trace metal pollutants present in the aquatic ecosystems.

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