Pertanika J. Trop. Agric. Sci. **35 (1)**: 183 - 190 (2012)
ISSN: 1511-3701

Bioaccumulation of Heavy Metals (Cd, Pb, Cu and Zn) in *Scylla serrata* (Forsskal 1775) Collected from Sungai Penor, Pahang, Malaysia

Kamaruzzaman, B. Y.*, Akbar John, B., Maryam, B. Z., Jalal, K. C. A. and Shahbuddin, S.

Department of Biotechnology, Kulliyyah of Science,
International Islamic University Malaysia,
Jalan Sultan Ahmad Shah, Bandar Indera Mahkota,
25200, Kuantan Pahang, Malaysia
**E-mail: kama@iium.edu.my*

ABSTRACT

A study was conducted to assess the bioaccumulation levels of heavy metals (copper, zinc, cadmium and lead) in different body parts (carapace, claw, walking legs and intestinal tract) of the common mud crab *Scylla serrata* collected from Sungai Penor, Pahang, on January 2009. Accumulation of metal was determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Average Cu concentrations in carapace, claws, walking legs and gut were 43.83 ± 16.43μgg^{-1}, 21.54 ± 7.14μgg^{-1}, 28.2 ± 12.76μgg^{-1} and 57.06 ± 13.47μgg^{-1}, respectively. Meanwhile, average Zn concentrations in carapace, claws, walking legs and gut were 387.38 ± 17.89μgg^{-1}, 376.62 ± 21.91μgg^{-1}, 361.92 ± 26.68μgg^{-1} and 496.31 ± 20.59μgg^{-1}, respectively. Since Zn and Cu are the precursors of most enzymatic activities, all the body parts have significantly higher tendency to accumulate Zn and Cu. It was apparent that Zn concentration was higher in crab body parts, followed by Cu. It was also observed that intestinal track had higher levels of metals than other body parts. Since high calcium content inhibits lead uptake into the gut, a large portion of the lead burden was sequestered in the carapace. Average Pb concentrations in carapace, claws, walking legs and gut were observed to be 7.17 ± 0.46μgg^{-1}, 6.27 ± 0.75μgg^{-1}, 6.52 ± 0.37μgg^{-1} and 2.27 ± 0.82μgg^{-1}, respectively. Among the analyzed heavy metals, Cd concentration was low in all the body parts of the crab. Average Cd concentrations in carapace, claws, walking legs and gut were 0.68 ± 0.05μgg^{-1}, 0.42 ± 0.05μgg^{-1}, 0.35 ± 0.04μgg^{-1} and 0.13 ± 0.05μgg^{-1}, respectively. Exoskeleton absorbs higher level of Cd than internal gut region, but the accumulation of Pb was higher in gut region than the exoskeleton of the crab. It was evident from this study that all the heavy metal (Cd, Pb, Cu and Zn) accumulations in *Scylla serrata* were higher than the international standard Maximum Permissible Level (MPL). Hence, a detailed investigation needs to be addressed on this issue to determine the pollution status in crabs inhabiting along the Sungai Penor waters.

Key words: Bioaccumulation, Heavy metal, ICP-MS, *Scylla serrata,* Sungai Penor

INTRODUCTION

The natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other man-made activities (Velez & Montoro, 1998; Conacher *et al.,* 1993). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraj, 2005; Vosyliene & Jankaite, 2006; Farombi *et al.,* 2007). Thus, determination of harmful and toxic substances

Received: 17 January 2011
Accepted: 18 April 2011
*Corresponding Author

in water sediments and biota will give direct information on the significance of pollution in the aquatic environment (Hugget *et al.,* 1973). Among other, estuaries and coastal zones receive pollutant input from both specific and non-specific sources, especially such ecosystems as seaports, cities or other industrialized coastal areas that receive chronic input of metals. Since many species of crustaceans inhabit estuaries, numerous studies have aimed at examining the bioaccumulation and effects of various toxicants in these animals (Bryan, 1971; Rainbow, 1990).

Meanwhile, heavy metals from natural and anthropogenic sources are continually released into aquatic ecosystem and they cause serious threats because of their toxicity, long persistence, as well as bioaccumulation and biomagnifications in the food chain (Kamaruzzaman *et al*., 2007). The fate of heavy metals introduced by human activities into the aquatic ecosystems has recently become the subject of wide spread concern, since they become toxic beyond the tolerable limits (Rainbow, 1995). However, metals can be excreted from the tissues of aquatic animals. It was reported that there are several factors influencing the elimination of metals from the tissues of aquatic animals (Skejelkvale *et al.,* 2001). These include period of exposure, surrounding temperature, interacting agents, age and the metabolic activity of the animal, and biological half life of metals. Metal elimination routes are more numerous than uptake routes; however, metal accumulation is more rapid than its elimination, and this is probably due to the presence of metal binding proteins in the tissues (Soegianto *et al.,* 1999).

Studies carried out on various fish species have shown that heavy metals may alter the physiological activities and biochemical parameters both in the tissues and in the blood (Soegianto *et al.,* 1999). The toxic effects of heavy metals have been reviewed, including their bioaccumulation (Tinsley, 1979). The organisms developed a protective defence against the deleterious effects of essential and inessential heavy metals and other xenobiotics that produce degenerative changes like oxidative stress in the body (Bahadorani & Sepehr, 2008).

It has been documented that the industrial and domestic dumping of sewage mostly contain plastic wastes contribute to the pollution at mangrove forests and their surrounding water body (Kamaruzzaman *et al*., 2009). In addition, the decomposition of litter gives harmful effects on marine life by increasing level of phytoplankton which in turn deoxygenates the water and adversely affects water quality. Furthermore, the lack of proper planning and management leads to drastic increase in pollutant level in this area.

Mud crab (*Scylla serrata*) is one of the important fishery commodities in Southeast Asian countries. This species is a very popular seafood and has a high commercial value, and it is extensively used as a candidate species in aquaculture practices. Their distribution was recorded in the present sampling site (Sungai Penor) which is situated along the east coast of Malaysia, facing the South China Sea in the state of Pahang. Their feeding behaviour will lead to a higher accumulation of toxic metals in their body parts which are biomagnified through food chain. It was also proposed as a biomonitor species for the current RWQPP Marine Monitoring Programme because of its capacity to bioaccumulate a range of contaminants, and its significance as a target species for subsistence, commercial and recreational fisheries. They have limited territorial ranges and are large enough to provide ample tissue for chemical analysis (Ryan, 2003). Various studies have been carried out on heavy metal accumulation in mud crabs but similar studies from Malaysian waters on this very species are still scanty. Hence, the present research was conducted to examine the bioaccumulation level of selected heavy metals in various parts of common mud crab (*S. serrata*) sampled from Sungai Penor, Pahang, Malaysia.

MATERIALS AND METHODS

Sampling Site, Sample Collection and Preparation

Sungai Penor (3^0 39’ 10.13’’ N, $103^0$21’ 15.77’’ E), which is located in Kuantan, Pahang,

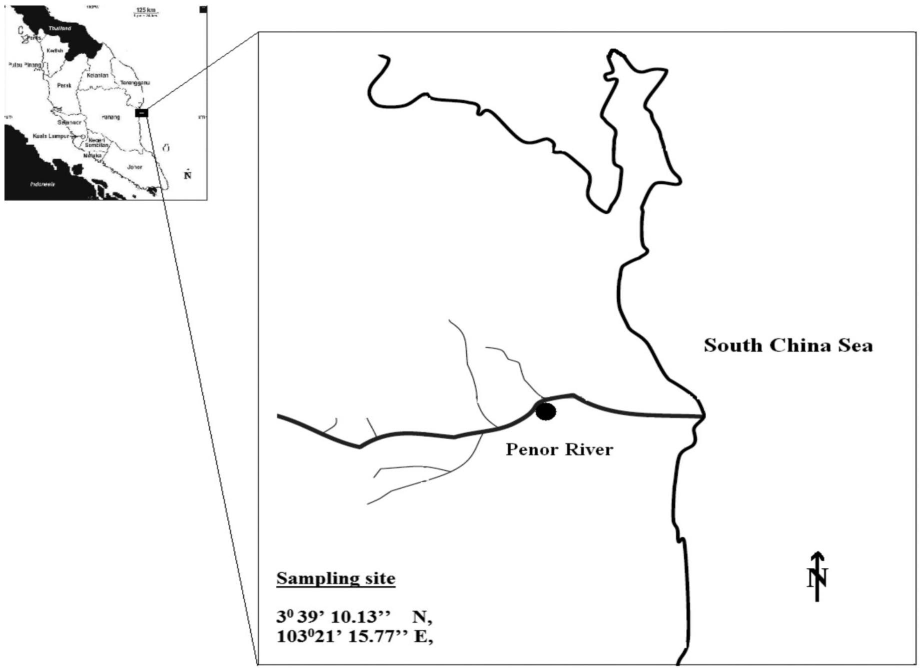

Fig. 1: Location of the sampling site at Sungai Penor, Kuantan, Pahang, Malaysia

Malaysia (*Fig. 1*), is threatened by a series of pollutants, such as industrial discharges and domestic sewage, discharged from nearby fishermen villages. A total of 65 crab traps were set (at every 50 metres) along Penor River for overnight during January 2009. Samples were collected on next day and identified using a standard reference (Keenan *et al.,* 1998) prior to storage at -20^0C. A total of 15 crabs were analyzed based on their weight and size according to carapace width (CW) which varied between 100 – 540g and 8.5 – 14.4 cm, respectively. Prior to the analysis, the crabs were cleaned with running tape water and thawed at room temperature. The carapace, claws, walking legs and gut region (after removing the gut contents) of each crab were dissected by using a pair of sterile stainless steel scissors. All the parts were replicated and transferred to Petri dishes. The tissues were dried in an oven for three days at 60 ^{0}C. The desired constant dry weight (0.5g) of each sample was obtained after three days of drying process.

Acid digestion and ICP-MS Analysis

The acid digestion method was performed to digest the samples; this involved heating 0.5g of dried tissues of crabs in a Teflon beaker with mixed concentrated acids (Hydrogen Peroxide (H2O2), Nitric acid (HNO3), hydrochloric acid (HCl) and sulphuric acid (H2SO4) in the ratio of 1:1 (Kamaruzzaman et al., 2007). After the digestion process, hundred times of dilution were performed using Mili-Q water before the samples were analyzed using Inductively Coupled Plasma Spectrophotometer (ICP-MS). The values of the heavy metal concentrations in the tissues were calculated based on their dry weights as this discounts the variability due to the inner part differences in the moisture content of organisms. The international certified standards (DORM-2) by the National Research Council of Canada and a blank in replicates were used to control the accurateness of this procedure, and the percentage of recovery was between 95 – 105%. Analysis of Variance (ANOVA) statistical test was performed to check

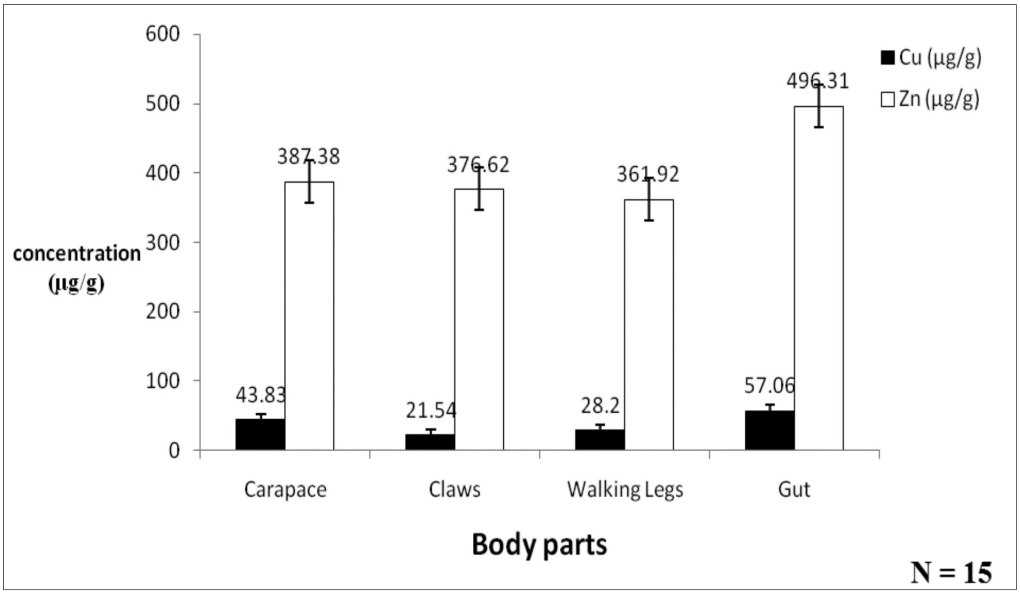

Fig. 2: The average concentrations of Zn and Cu (μgg^{-1} dry weight) in the different body parts of Scylla serrata.

the significance in the bioaccumulation of metals in the different body parts.

RESULTS AND DISCUSSION

The concentrations of metals in all the 15 crabs were analyzed using ICP-MS with the dilution factor of 10. Significant differences in metal accumulation were observed in different body parts of the crabs ($P<0.05$). The concentrations of Zn and Cu were observed to be higher in the gut region of the crab (469.31 and 57.06 μgg^{-1}, respectively), followed by carapace (387.38 and 43.83 μgg^{-1}, respectively).

The average Zn accumulation was higher in the claws (376.62 μgg^{-1}) as compared to the walking legs (361.92 μgg^{-1}), but the Cu accumulation was comparatively higher in the walking legs than the claws of *S. serrata*. It was apparent that Zn concentration was higher in the crab's body parts, followed by Cu. It was interesting to note that intestinal track had a higher level of metal concentration than the other body parts (*Fig. 2*). In the present study, heavy metal accumulation in med crab flowed in Zn > Cu > Pb > Cd order. This pattern of accumulation is also in agreement with that of the previous studies (see Krishnamurti, 1998; Srinivsa, 2007). The high Zn concentration observed in the crab's body parts than Cu concentration was probably due to major role played by these metals as a precursor in most enzymatic activities and hence, all the body parts have a high tendency to accumulate higher levels of these metals (Kamaruzzaman *et al.,* 2009).

It is worth highlighting that copper and zinc are essential trace metals in crabs but they may be toxic at high concentrations (Phillips & Rainbow, 1993). The haemolymph of crabs, in particular, contains high concentrations of copper which is involved in oxygen transport. These essential trace metals (copper and zinc) are highly regulated within crabs over a wide range of bio-availabilities, while non-essential metals such as cadmium are usually accumulated in proportion to availability. Mud crabs may therefore be useful biomonitors of non-essential metals, such as arsenic, mercury, cadmium and selenium, but more research is still needed in combination with direct water and/or sediment samples to confirm this. It was also observed that the accumulations of Cu and Zn were higher in the gut region of the crab compared to other body parts and the accumulations of Pb and Cd were lesser in the gut region compared to other organs in the case of *S. serrata*. It was also evident from this study that heavy metal accumulation in *S. serrata* was higher than the international standard references (Table 1).

TABLE 1
Maximum Permissible Level of heavy metal concentrations in aquatic crustacean species enforced by Food and Drug Administration (FDA, 2001), Singapore Food Regulations (1990), Food Standards Australia (2002) and European Union Commission Regulation (2001)

	Heavy metal concentration (in $\mu g g^{-1}$)			
	Vital elements		Toxic elements	
	Cu	Zn	Pb	Cd
FDA (2001)	100	150	1.5	0.2
SFR (1990)	-	-	2	1
FSA (2002)	-	-	2	2
EUCR (2001)	-	-	1	1
	Comparison with the present study			
Present Study	6.38-79.38	325.39-523.21	1.49-7.84	0.05-0.78

Ololade *et al.* (2008) suggested that the crabs are the bottom feeders and generally expected to concentrate more heavy metals than the mobile fish. The variation in metal uptake is also an indication of the degree to which the species pick up particulate matter from the surrounding water and sediment while feeding. A higher level of metal concentration was observed in the gut region and this was probably due to the feeding behaviour of the crabs. A sex specific difference in the metal uptake by the crabs was also observed (Sastre *et al.,* 1999). The male crabs, which are the common residents of low saline area, are expected to have higher metal concentrations in the body compared to the female crabs which generally exhibit seasonal migratory behaviour, especially for reproductive purposes that might help them in exchange of metallic ions into the clean environment. Therefore, the male crabs are more likely to accumulate higher amounts of heavy metals than the female crabs (Ololade *et al.,* 2008). Due to the small sampling size, the sex and size related heavy metal concentrations in crabs were not established in this study. However, Poovachiranon (1991) observed the high levels of Cu and Zn in young crabs were probably due to the faster growth rate of young crabs and the feeding behaviour. It was also due to the food preference by the young and adult crabs, whereby young crabs prey more on aquatic benthic invertebrates, while the larger crabs prefer plant materials (Poovachiranon, 1991). Meanwhile, various studies have shown that aquatic invertebrates tend to accumulate high level of heavy metals (Janczur *et al*., 2000). Thus, determination of harmful and toxic substances in water sediments and biota gives direct information on the significance of pollution in the aquatic environment (Hugget *et al.,* 1973). It is believed that the Cu accumulated in the crabs is due to their feeding habits, even though there is no clear evidence indicating the Cu dietary transfer, and many studies have demonstrated that diet is the most important route of Cu accumulation in aquatic animals (Sindaigaya *et al*., 1994). Decapode crustaceans, including crabs, maintain relatively low and constant body level of Zn when exposed to a range of dissolved Zn concentrations in seawater because of the low integument permeability of the metals. Hence, a detailed study is needed to determine the sex and size related metal accumulations in the med crabs inhabiting Sungai Penor, Kuantan.

Cadmium is widely distributed at low level in the environment and most food has an inherently low level of Cd which has been shown to bind to the protein and accumulate significantly in higher level (FDA, 2001). The present study revealed that the average concentrations of Pb and Cd were higher

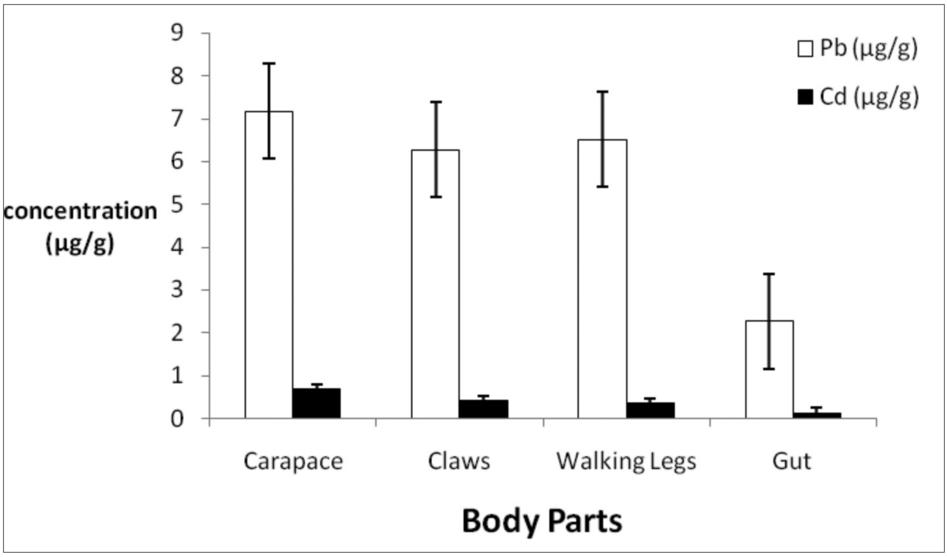

Fig. 3: The average concentrations of Pb and Cd (µgg^{-1} dry weight) in the different body parts of Scylla serrata.

($p<0.05$) in carapace (7.17 and 0.68 µgg^{-1}, respectively) but lower in the gut region (2.27 and 0.13 µgg^{-1}, respectively), indicating that the carapace has higher exposure and tendency to accumulate more concentrations of both metals than the gut region. Slight changes in the average Pb accumulation was observed and it was higher in the walking legs (6.52 µgg^{-1}) compared to the claws (6.27 µgg^{-1}). Nevertheless, the Cd accumulation was comparatively higher in the claws than in the walking legs of *S. serrata*. It is also evident from the study that the carapace showed a high tendency to accumulate more Pb and Cd concentrations compared to other body parts (*Fig. 3*).

Ploetz *et al*. (2007) reported that Cd level is almost 10 times higher in shell fish than in finfish. In this study, the high levels of Pb and Cd were observed in the carapace compared to the other parts of the crab, and these were probably due to the calcium content having a high affinity towards these heavy metals (Du Preez *et al*., 1993; Reinecke *et al.,* 2003). Nevertheless, periodic molting could help in eliminating Pb and Cd levels in the exoskeleton.

The observed levels of selected heavy metals in the mud crabs from the present study were higher than the maximum permissible levels (MPL) set by various international consortiums. Although these food materials are processed (heating, cooking) before consumption, the effects of processing could be minimal, since the heavy metals are non-degradable. Thus, consumption of these heavy metal contaminated crabs may result in bioaccumulation of toxic metals in the human system and may lead to adverse health effects (Bergback *et al.,* 1992; Koller *et al.,* 2004).

CONCLUSION

The accumulation of heavy metals (Cu, Zn, Pb and Cd) in crabs is to a considerable extent, and it is relatively more than that reported from other regions in the literature. The high bioaccumulation of these metals is believed to be occurring due to the rigorous anthropogenic input of bioaccumulative contaminants into the aquatic environment. It was evident from this study that heavy metal accumulations in *S. serrata* were higher than almost all the international standard levels. Detailed and extensive studies should address this issue to determine the bio-accumulative heavy metal concentrations in edible parts of mud crab and find out the suitability of it flesh for human consumption.

ACKNOWLEDGMENT

Authors would like to express their sincere gratitude to the International Islamic University Malaysia for providing the infrastructure facilities.

REFERENCES

Ashraj, W. (2005). Accumulation of heavy metals in kidney and heart tissues of Epinephelus microdon fish from the Arabian Gulf. *Environmental Monitoring Assessment, 101*(1-3), 311-316.

Bahadorani, S. (2008). *The effects of heavy metal toxicity and oxidative stress on genetically-compromised models of* Drosophila melanogaster. (Doctoral dissertation) York University, Toronto, Canada.

Bergback, B., Anderberg, S., & Lohm, U. (1992). Lead load: Historic pattern of lead use in Sweden. *Ambio, 21*(2), 159-165.

Bryan, G. W. (1971). The effects of heavy metals (other than mercury) on marine and estuarine organisms. *Proceedings of Royal Society of London, Series B, Biological Science* (1934-1990), *177*, 389-410.

Conacher, H. B., Page, B. D., & Ryan, J. J. (1993). Industrial chemical contamination of foods. *Food Addit Contam, 10*(1), 129-143.

Du Preez, H. H., Steenkamp, V. E., & Schoonbee, H. J. (1993). Bioaccumulation of Zinc and lead in selected tissues and organs of the freshwater crab, *Potamonautes warreni*. *Science of the Total Environment, Supplement, 134*, 469-478.

European Union Commission Regulation (EUEC), (2001). No 466/2001 of 8 March 2001.

Farombi, E. O., Adelowo, O. A., & Ajimoko. Y. R. (2007). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat fish (*Clarias gariepinus*) from Nigeria Ogun river. *International Journal of Environmental Research and Public Health, 4*(2), 158-165.

FDA. (2001). *Fish and Fisheries Products Hazards and Controls Guidance* (3rd ed). Centre for Food Safety and Applied Nutrition, US Food and Drug Administration.

Food Standards Australia, (2002). *Issue 53*, Standard 1.4.1 and 1.4.2.

Hugget, R. J., Bencher, M. E., & Slone, H. D. (1973). Utilizing metal concentration relationships in the eastern oyster (Craostrae virginica) to detect heavy metal pollution. *Water Research, 7*, 451-460.

Janczur, M., Kozlowski, J., & Laskowski, R. (2000). Optimal allocation, Life history and heavy metal accumulation: Dynamic programming model. In Kamenga and R. Laskowski (Eds.). Demography in Ecotoxicology (pp. 179 – 197). UK: Wiley Chichester.

Kamaruzzaman, B. Y., Ong, M. C., Jalal, K. C. A., Shahbudin, S., & Mohd Nor. O. (2009). Accumulation of Lead and Copper in *Rhizophora apiculata* from Setiu mangrove forest, Terengganu. *Malaysian Journal of Environmental Biology*, *30*(5), 821-824.

Kamaruzzaman, B. Y., Zaleha, K., Ong, M. C., & Wilson, K. Y. S. (2007). *Copper and Zinc in Three Dominant Brackish Water Fish Species from Paka Estuary, Terengganu, Malaysia*. *Malaysian Journal of Science, 26*(2), 65-70.

Keenan, C. P., Davie, P. J. F., & Mann, D. L. (1998). A revision of the genus *Scylla*de Haan, 1833 (Crustacea: Decapoda: Brachyura: Portunidae). *The Raffles Bulletin of Zoology*, *46*(1), 217-245.

Koller, K., Brown, T., Spurgeon, A., & Levy, L. (2004). Recent development in low level exposure and intellectual impairment in children. *Environmental Health Perspective, 112*(9), 987-994.

Krishnamurti, A. J., & Vijayalakshmi, R. N. (1998). Concentration of metals in shrimps and crabs from Thane-Bassein creek system, Maharastra. *Indian Journal of Marine Science,* 28, 92-95.

Ololade, I. A., Lajide, L., Amoo, I. A., & Oladoja, N. A. (2008). Investigation of heavy metals contamination of edible marine seafood. *African Journal of Pure and Applied Chemistry*. *2*(12), 121-131.

Phillips, D. H., & Rainbow, P. S. (1993). *Biomonitoring of trace aquatic contaminants.* London: Chapman and Hall.

Ploetz, D. M., Fitts, B. E., & Rice, T. M. (2007). Differential Accumulation of Heavy Metals in Muscle and Liver of a Marine Fish, (King Mackerel, *Scomberomorus cavalla* Cuvier) from the Northern Gulf of Mexico, USA. *Bulletin of Environmental Contamination and Toxicology, 78*, 134-137.

Poovachiranon, S. (1991). Biological studies on the mud crab (Scylla serrata) of the mangrove ecosystem in the Andaman Sea. *Report of the Seminar on the Mud Crab Culture and Trade.* pp. 49-57.

Rainbow, P. S. (1990). Heavy metal levels in marine invertebrates In R. W. Furness, and P.S. Rainbow (Eds.). *Heavy metals in the marine environment*. Boca Raton, Florida: RC Press, Inc.

Rainbow, P. S. (1995). Biomonitoring of heavy metal availability in the marine environment. *Marine Pollution Bulletin, 31*, 183-192.

Reinecke, A. J., Snyman, R. G., & Nel, J. A. J. (2003). Uptake and distribution of Lead (Pb) and Cadmium (Cd) in the freshwater crab, *Potamonautes perlatus* (Crustacea) in the Eerste river, South Africa. *Water Air and Soil Pollution, 145*, 395-408.

Ryan, S. (2003). *Queensland mud crab fishery: ecological assessment*. In Report Series: Queensland Department of Primary Industries, pp. 55.

Sastre, M. P., Reyes, P., Ramos, H., Romero, R., & Rivera, J. (1999). Heavy metals bioaccumulation in Puertorichen blue crab (*Callinetes sapidus*). *Bulletin of Marine Science, 64*, 209-217.

Sindaigaya, E., Cauwenbergh, R. V., Robberecht, H., & Deelstra, H. (1994). Copper, Zinc, Manganese, Iron, Lead, cadmium, mercury and Arsenic in fish from Lake Tanganyika, Burundi. *Science of the Total Environment, 144,* 103-115.

Singapore Food Regulations, (1990). Cap. 283, 1st Ed., Singapore.

Skejelkvale, B. L., Andersen, T., Fjeld, E., Mannio, J., Wilander, A., Johansoon, K., Jensen, J. P., & Moiseenko, T. (2001). Heavy metal surveys in Nordic Lakes: Concentrations, geographic patterns and relation to critical limits. *Ambio, 30*(1), 2-10.

Soegianto, A., Charmantier-Daures, M., Trilles, J. P., & Charmantier, G. (1999). Impact of cadmium on the structure of gills and epipodites of the shrimp *Penaeus japonicus*. *Aquatic Living Recourses, 12*(1), 57–70.

Srinivasa, R., Bhavesh, M., Sunil, D., Manish, J., Leena, K., Venkatrama, K., Susarla, S., Shaik, B., Gadde, R., & Prashant, B. (2007). Bioaccumulation of heavy metals in some commercial fishes and crabs of Gulf of Cambay, India. *Current Science, 92*(11), 1489-1491.

Tinsley, I. J. (1979). *Chemical concepts in pollutants behaviour*. J. Willey and Sons Inc. NY. Niger Delta Environmental Survey (NDES), Phase I Report, Vol. 1. Environmental and Socio-economic characteristic (revised edition).

Velez, D., & Montoro, R. (1998). Arsenic speciation in manufactured seafood products: a review. *Journal of Food Protection, 61*(9), 1240-1245.

Vosyliene, M. Z., & Jankaite. A. (2006). Effect of heavy metal model mixture on biological parameters of rainbow trout *Oncorhynchus mykiss*. *Ekologija, 4*, 12-17.