

**Copper and Zinc in commonly consumed bivalves from Pantai Remis Jeram,
Selangor, Malaysia**
(Kandungan Kuprum dan Zink di dalam dwi-cengkerang yang biasa dimakan di Pantai Remis Jeram,
Selangor, Malaysia)

Bede Emeka Udechukwu, Ahmad Ismail

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia.

Received 6 July 2013; accepted 5 September 2013

ABSTRACT

Four commonly consumed bivalves in Pantai Remis Jeram, Selangor, were evaluated for heavy metals concentration. Bivalve species were included cockle (*Anadara granosa*), clams (*Pholas orientalis*, *Fragum unedo* and *Donax faba*), which are locally known as kerang, mentarang, kepah and lala respectively. The purpose of this study was to provide basis information on the concentrations of potentially harmful heavy metals (Cu and Zn) in commercialized seafood samples from Pantai Remis Jeram, Selangor. Metals concentration were determined using air-acetylene Perkin Elmer™ flame atomic absorption spectrophotometer (AAS), model A Analyst 800. Data was analyzed using SPSS 17 software for ANOVA test, and Post Hoc Test (Turkey HSD, $p < 0.05$). The maximum Cu and Zn concentrations in these bivalve samples were ($20.42 \pm 0.73 \mu\text{g/g}$ dry weight) (mentarang) and ($906.57 \pm 0.65 \mu\text{g/g}$ dry weight) (kerang) respectively. A significant ($P < 0.05$) high level of zinc was found in cockle, though not exceeding the maximum permissible limit set by Malaysia Food Regulation.

Keywords: Pantai Remis, Cu and Zn, Mentarang, Lala, Bivalves

ABSTRAK

Kepekatan logam berat dari empat jenis dwi-cengkerang yang biasanya dimakan di Pantai Remis Jeram, Selangor telah dikenalpasti. Spesies dwi-cengkerang yang dimasukkan ialah kerang (*Anadara granosa*), kerang (*Pholas orientalis*, *Fragum unedo* dan *Donax faba*) yang dikenali dengan nama tempatan sebagai kerang, mentarang, kepah dan lala. Tujuan kajian ini adalah untuk memberi maklumat asas mengenai kepekatan logam berat berbahaya (Cu dan Zn) dalam sampel makanan laut komersial dari Pantai Remis Jeram, Selangor. Kepekatan logam ditentukan menggunakan air-asetilena Perkin Elmer™ spektrofotometer penyerapan atom nyala (AAS), Model A Analyst 800. Data dianalisis dengan menggunakan perisian SPSS 17 untuk ujian ANOVA dan Ujian Post Hoc (Turki HSD, $p < 0.05$). Kepekatan maksimum Cu dan Zn dalam sampel-sampel ini adalah ($20.42 \pm 0.73 \mu\text{g/g}$ berat kering) (mentarang) dan ($906.57 \pm 0.65 \mu\text{g/g}$ berat kering) (Kerang). Kepekatan zink yang tinggi ($P < 0.05$) ditemui pada kerang, walaupun tidak melebihi had maksimum yang dibenarkan yang ditetapkan oleh Peraturan Makanan Malaysia.

Katakunci: Pantai Remis, Cu dan Zn, Mentarang, Lala, Dwi-cengkerang

INTRODUCTION

Intense anthropogenic activities have recently led to the contamination of marine ecosystems (Galloway et al., 2002). Water pollution leads to sea food contaminations with toxic metals from many sources e.g. industrial and domestic waste water, natural runoffs and contributory rivers (Marcursen et al., 2007). A wide range of metals and metallic compounds found in the marine environment pose risks to human health through the consumption of seafood where the contaminant content and exposure are significant (Han et al., 1998, Osfar et al., 1998, Chan et al., 1999). Heavy metal contamination in marine ecosystem arises from urban and agricultural runoff, industrial effluents, boating and recreational use of water bodies, chemical spills, sewage treatment plants, leaching from domestic garbage dumps and mining operations (Peters et al., 1997). Unfortunately, Malaysia is one of the fast growing developing countries in the world and as a result, continuous chemical input resulting from intense and heavy human activities is expected.

Asian cultures have traditionally employed sea foods as natural source of food. Though sea food consumption is very common in Malaysia, relatively few studies have reported on the quantities of heavy metals that some indigenous Malaysia sea foods contain and or their potential health effect on the population. This study focuses on the concentrations of two common heavy metals (Cu and Zn) in four species of Malaysian indigenous sea foods, (*Anadara granosa*, *Pholas Orientalis*, *Fragum unedo* and *Donax faba*) which were collected from Pantai Remis Jeram, Selangor. The *A. granosa*, known as blood cockles, are consumed fresh and are also converted into processed products (Haslaniza et al., 2010).

Bivalves provide a cheap source of protein for human consumption (Yap et al., 2004). However, the consumption of metal -contaminated seafood in high doses may cause toxicity to human beings, since heavy metals are inorganic chemicals that are non-biodegradable, cannot be metabolized and will break down into harmful forms (Kromhout et al., 1995). Levels of heavy metals above the permissible limit would certainly create a notorious image from the public point of view (Yap et al., 2004). Thus, people who eat large amounts of seafood from estuarine or coastal areas that are associated with chemical industry are at a risk of heavy metal poisoning (Nawal, 2008). Therefore the aim of this work is to provide a basis information on the concentration of potentially harmful heavy metals (Cu and Zn) present in commercialized seafood samples from Pantai Remis Jeram, Selangor, and for the fact that Cu and Zn are widely distributed in the coastal environment, both from natural geological process and anthropogenic activities, this study is of much interest to public health.

MATERIALS AND METHODS

Sampling, storage and sample preparation

Samples were bought from shops located onshore of Pantai Remis Jeram, Selangor, Malaysia on August 2010. Samples were still alive, and in their provided natural habitat during collection. The bivalve samples were brought back to the laboratory in plastic bags, and kept frozen at -10°C. Twenty pooled individuals of each species were dissected to separate the whole body from the shells. The total soft tissues were dried using air-circulating oven to a constant dry weight at 80°C (Ismail, 1993) and homogenized prior to analysis.

Sample digestion

The direct aqua regia method as described by Ismail (1993), and Yap et al. (2002) was employed for the heavy metal analysis. About 1g of each dried sample species was weighed into a digesting tube and digested in a mixture of concentrated nitric acid (AnalaR grade, BDH 69%) and perchloric acid (AnalaR grade 60%) in a ratio 4:1. The samples were then placed in the digesting block, and the temperature was first set at a low temperature of 40°C for 1hr and later increased to 140°C for at least 3hrs. After cooling, the content of each digesting tube was diluted to 40ml with double distilled water, and then filtered using Whatman No.1 filter paper into acid washed pill bottles.

Determination of Cu and Zn

The digested materials were then determined for Cu and Zn using an air-acetylene Perkin ElmerTM flame atomic absorption spectrophotometer (AAS), model A Analyst 800. The data were presented in µg/g dry weight (dw) basis. A conversion factor was devised for these four species of bivalves from dry weight to wet weight (WW) according to their sizes and dry/wet weight ratio (Mo and Neilson, 1994). The dw was converted into ww by using a conversion factor of 0.09 for *Pholas orientalis* and 0.13 for *Anadara granosa*, *Fragum unedo* and *Donax faba* (Table 3). Calibration curves were generated by analyzing Multiple-level Calibration standards. Standard solution of Cu and Zn were prepared from 1,000mg/l (BDH Spectrosol[®]) stock solution.

Quality control

In order to avoid contamination, all glass wares were acid soaked in 10% HCl for 24hrs and later rinsed with double distilled water. Certified Reference Material (CRM) for dogfish liver (DOLT-3, National Research Council Canada) was used to check the quality of this method. The analytical results for reference material and its certified values showed satisfactory agreement with the recoveries (Table 1). A quality control sample was analyzed in every eight samples during AAS metal analysis.

Table 1. Analytical results for the reference material and the certified value for each metal ($\mu\text{g/g}$ dry weight)

Metal	Sample	Certified Reference Material (CRM)	Measured value	Percentage of Recovery (%)
Cu	DOLT-3 Dogfish-liver	31.2 \pm 1.00	32.0	103
Zn	DOLT-3 Dogfish-liver	86.6 \pm 2.4	100	116

Data analysis

Data was analyzed using SPSS 17 software for ANOVA test and Post Hoc Test (Turkey HSD, $p < 0.05$). Microsoft excell was employed for the graphics.

RESULTS AND DISCUSSION

The metal concentration in soft tissues of the four species of seafood ranged from 5.17 to 20.42 $\mu\text{g/g}$ dw (0.47 - 2.65 $\mu\text{g/g}$ ww) for Cu and 562.32 to 906.57 $\mu\text{g/g}$ dw (50.61- 81.59 $\mu\text{g/g}$ ww) for Zn (Table 2). When compared with the permissible limits set by the Malaysia Food Regulation, (1985) for Cu (30.0 $\mu\text{g/g}$ ww), and Zn (100 $\mu\text{g/g}$ ww), all mean values of Cu from all samples were lower than the limits. In the case of Zn, the concentrations in all samples were also lower than the Malaysia set limits. The Cu levels were also lower than the recommended guide lines set by Ministry of public Health Thailand (MPHT, 1986) and Australian Legal Requirement for food safety (NHMRC, 1997), while that of Zn levels were higher respectively (Table 3). Yap et al., (2004) reported metal concentrations of *P.viridis* ranging from 7.76 to 20.1 $\mu\text{g/g}$ dw (1.32 – 3.42 $\mu\text{g/g}$ ww) for Cu, which is similar when compared to this study with metal concentration of 5.17 – 20.42 $\mu\text{g/g}$ dw (0.47-2.65 $\mu\text{g/g}$ ww) for Cu. However, when the zinc levels of this study (Zn: 562.32 to 906.95 $\mu\text{g/g}$ dw) (50.61-81.59 $\mu\text{g/g}$ ww) was compared to that reported by Yap et al., (2004) (Zn: 75.1 to 129 $\mu\text{g/g}$ dw) (12.8 – 21.9 $\mu\text{g/g}$ ww), a remarkable difference was observed. This wide variation could possibly be due to the difference in biological sample, site or area of collection, increased development and continuous human activities, bioavailability and capacity of Zn accumulation by the organisms. The accumulation of trace elements in living organisms also depends on physiological conditions of the organisms as well as lipid dynamics of the tissue (Farkas et al., 2003) and on the rate metabolism of the organisms. The comparison of mean levels of Cu and Zn in four bivalve species is shown in figures 1 and 2 below:

Table 2: Mean concentrations of Cu and Zn in whole body of bivalves. Values presented in $\mu\text{g/g}$ (dw) & (ww)

	N	Cu		Zn	
		dw	ww	dw	ww
<i>Anadara granosa</i>	20	5.17 \pm 0.04	0.47	906.57 \pm 0.65	81.59
<i>Fragum unedo</i>	20	7.91 \pm 0.14	0.71	562.32 \pm 0.49	50.61
<i>Pholas orientalis</i>	20	20.42 \pm 0.73	2.65	582.57 \pm 0.7	75.73
<i>Donax faba</i>	20	6.35 \pm 0.03	0.57	581.95 \pm 0.87	52.38

dw = dry weight, ww = wet weight

Table 3: Comparison of this study with bivalve study in Malaysia and heavy metal guidelines for food safety set by some regional countries

Location	WB	Cu($\mu\text{g/g}$)	Zn($\mu\text{g/g}$)
Permissible limit set by Malaysia Food Regulation (1985)	ww	30.00	100
Permissible Limits set by Ministry of Public Health, Thailand (MPHT, 1986)	dw	133	667
Australian Legal Requirements (NHMRC, 1987)	dw	350	750
Study on <i>P. viridis</i> , Malaysia (Yap et al., 2004)	ww	1.32 – 3.42	12.8 – 21.9
	dw	7.76 -20.1	75.1 - 129
Metal levels of some commercialized seafood <i>Anadara granosa</i> , <i>Pholas orientalis</i> , <i>Fragum unedo</i> and <i>Donax faba</i> from Pantai Remis (this study)	ww	0.47-2.65	50.61-81.59
	dw	5.17-20.42	562.32-906.57

WB = weight basis

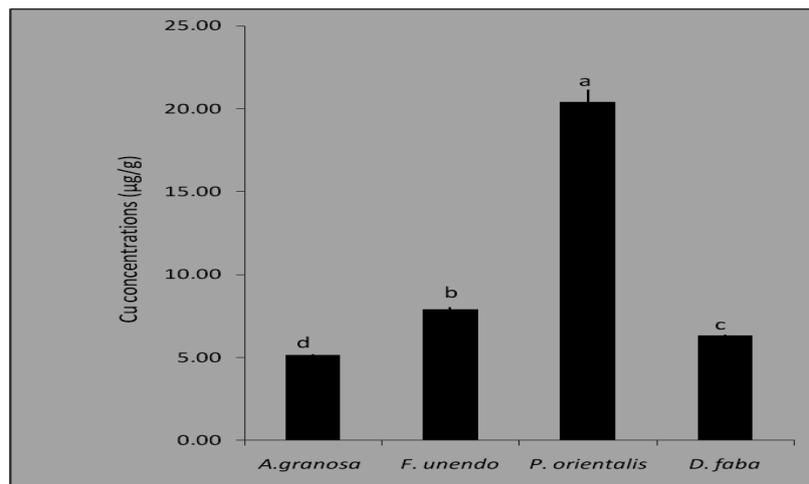


Figure 1. comparison of mean Cu levels in the bivalves. a-d: means with different letter indicates significant difference ($p < 0.05$).

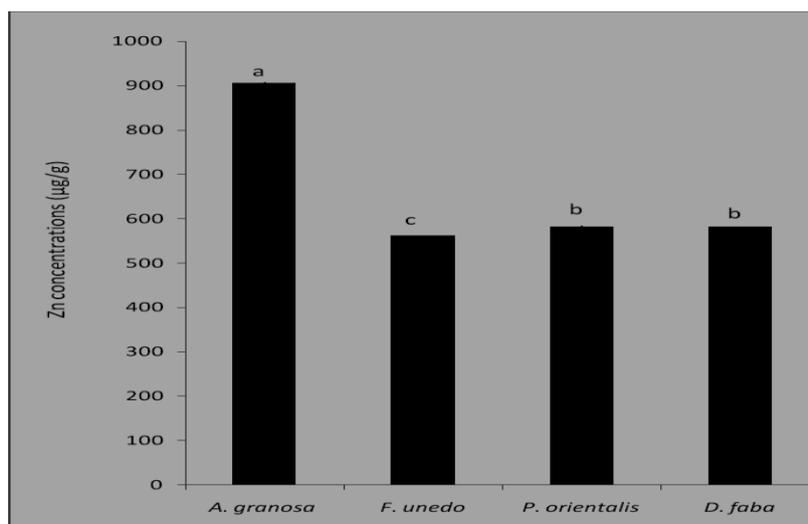


Figure 2. comparison of mean Zn levels in the bivalves. a-c: means with different letter indicates significant difference ($p < 0.05$).

Among these species *P. orientalis* (white shell fish) is found to accumulate the highest concentrations of Cu ($20.42\mu\text{g/g dw}$) while the *A. granosa* accumulates the least concentration of Cu ($5.17\mu\text{g/g dw}$) and the highest concentrations of Zn ($906.57\mu\text{g/g dw}$). *Fragum unedo* accumulates the least concentration of Zn ($562.32\pm 0.49\mu\text{g/g dw}$). The mean Cu levels in clams (*P. orientalis*, *Fragum unedo* and *Donax faba*) showed a significant difference ($p < 0.05$) (Figure 1) when compared to that of cockle, however, the mean Zn levels in cockle (*A. granosa*) showed a significant difference ($p < 0.05$) when compared to clams. It may possibly be an interesting thing to note that cockles accumulate more concentration of Zn than clams and clams in turn accumulate more concentrations of Cu than cockles, in this particular habitat, but this may be an underestimate of the specie's ability to accumulate the metal in question under sediment conditions where metals exhibit greater availability (MacFarlane et al., 2007). The ability of heavy metals to accumulate in these macro-invertebrate depends heavily on the bioavailability of these metals within the sediment, which refers to the state of the trace metal in the form that is readily available for uptake by biota in the surrounding environment. The bioavailability of these metals from the sediment is affected by such factor as sediment characteristics. Several authors (Harbison, 1986; Bendell-Young and Harvey, 1991; Janssen et al., 1997; Bryan and Langston, 1992) have emphasized that low bioavailability could result when heavy metals are absorbed on ion exchange sites of fine silt/clays or within iron, aluminum, and manganese colloidal compound. They further reported that incorporation of metals into lattice structure of clay can cause low bioavailability. Although humans can handle proportionally large concentrations of zinc, high levels of zinc can still cause eminent health problems, such as stomach cramps, skin irritations, vomiting nausea and anemia. Very high levels of zinc can damage the pancreas and disturb the protein metabolism, and cause arteriosclerosis.

CONCLUSION

The result of this study, revealed no indication of possible occurrence of acute toxicity of Cu and Zn to the consumers of seafood from Pantai Remis, yet the considerable high levels of zinc in cockles may pose a concern for human health. A closer monitoring of heavy metal levels in seafood from Pantai Remis is recommended in view of the possible risk to the consumers' health while adequate measures should be taken by appropriate quarters to curb the contamination of this marine ecosystem

REFERENCES

- Bendell-Young, L., Harvey, H.H., 1991. Metal concentrations chromids in relation to the geochemical characteristics of surficial sediments. *Archives of Environmental Contamination and Toxicology* **21**, 202-211.
- Bryan, G.W., Langton, W.J., 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special refrecne to United Kingdom estuaries: a review. *Environmental Pollution* **76**, 89-131.
- Chan, H. M., Trifonopoulos, M. Ing, A., Johnson, E., 1999. Consumption of fresh water fish in Kahnawake: Risk and benefits. *Environ. Res.* **80**, 213-222.
- Farkas, A. Salánki, J., Speziár, A., 2003. Age and size-specific patterns of heavy metals in organs of fresh water fish *Abranis brama* L. populating a low-contaminated site. *Water Res.* **37**, 959-964.
- Galloway, T.S., Sanger, R.C., Smith, K.L., Fillmann, G., Readman, J.W., Ford, T.E., Depldge, M.H., 2002. Rapid assessment of marine pollution using multiple biomarkers and chemical immunoassay. *Environmental Science & Technology*, **36**, 2219-2226.
- Han, C.C., Jeng, G.T. F., Hung, T.C., Tseng, R.J., 1998. Estimation of target hazard quotients and potential health risks foe metals by consumption of seafood in Taiwan. *Arch. Environ. Contam. Toxicol.* **35**, 711-720.
- Harbison, P., 1986. Mangrove muds: a sink or source for trace metals. *Marine Pollution Bulletin* **17**, 246e 250.
- Haslaniza, H., Maskat, M. Y., Wan Aida, W. M., Mamot, S., 2010. The effects of enzyme concentration, temperature and incubation time on nitrogen content and degree of hydrolysis of protein precipitate from cockle (*Anadara granosa*) meat wash water. *International Food Research Journal* **17**, 147-152.
- Ismail, A., 1993. Heavy metals Cd, Cu, Pb, and Zn in green mussel (*Pernia Viridis* L.) From west Coast of peninsular Malaysia. IRPA/UKM workshop and Seminar, Allson Kelana, Seremban Malaysia. 9- 12 December, 1993.
- Janssen, R.P.T., Peijnenburg, W.J.G.m., Posthuma, L., van der Hoop, M.A.G.T., 1997. Equilibrium partitioning of heavy metals in Dutch soils. I. Relationship between metal partition coefficients and soil characteristics. *Environmental and Toxicology Chemistry* **16**, 2470-2478.
- Kromhout, D., Bosschiester E. B., Lezenne, C.C. 1995. The inverse relationship between fish consumption and 20-year mortality from coronary heart disease. *New England Journal of Medicine* **312**, 1205-1209.

- MacFarlane, G. R, Koller, C. E., Blomberg, S. P. 2007. Accumulation and partitioning of heavy metals in mangroves: a synthesis of field-based studies. *Chemosphere* **69**, 1454-1464.
- Malaysian Food Regulation. 1985. Malaysian law on food and drugs Malaysia Law publishers.
- Marcursen, H., Holm, P.E., Ha, L.T., Dalgard, A. 2007. Food aspects of toxic elements in fish from well water fed pond in Hanoi-Vietnam. *Tropical medicine and international health* **12**, 34-39.
- Mo, C., Neilson, B., 1994. Standardization of Oyster soft dry wet measurements. *Water Research* **28**, 234-246.
- MPHT (Ministry of Public Health, Thailand) 1986. Residues in foods. 1123-1124, Part 23 Vol. 103, Special issues 16th February 1986. The Government Gazette, Bangkok, Thailand.
- Nawal Al-Bader, 2008. Heavy Metal Levels in Most Common Available Fish Species in Saudi' Market. *Journal of Food Technology* **6** , 173-177.
- NHMRC (National Health and Medical Research Council 1987. National food standard A 12: metal and contaminants in food. Canberra, Australia: Australian Government Publishing Services.
- Osfor, M. M., El-Dessouky, S.A., El-sayed A., Higazy, R.A. 1998. Relationship between environmental pollution in ManzaDonax fabake and health profile of fishermen. *Nahrung* **42**, 42-45.
- Peters, E.C., Gassmen, N.J., Firman, J.C., Richmond, R.H., Power, E.A., 1997. Ecotoxicology of tropical marine ecosystem. *Environ. Toxicol. Chem.* **16**, 12 – 40
- Yap, C.K., Ismail, A., Tan, S.G., Omar, H., 2002. Correlations between speciation of Cd, Cu, Pb and Zn in the 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.
- Yap, C.K., Ismail, A., Tan, S.G. 2004. Heavy metal (Cd, Cu, Pb and Zn) concentrations in green-lipped mussel *Perna Viridis* (Linnaeus) collected from some wild and aquacultural sites in the West Coast of peninsular Malaysia. *Food Chemistry* **84**, 569-575.