Pertanika J. Trop. Agric. Sci. **35 (2)**: 199 - 207 (2012)

ISSN: 1511-3701
© Universiti Putra Malaysia Press

Is a Mussel Processing Site a Point Source of Zn Contamination? Evidence of Zn Remobilization from Boiled Mussel, *Perna viridis*

Yap, C. K.*, Rashiq, M. and Edward, F. B.

Department of Biology, Faculty of Science,
Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia
**E-mail: yapckong@hotmail.com*

ABSTRACT

Sediment sampling in the Straits of Johore revealed that the surface sediments collected at a jetty near a mussel processing factory in Kg. Sg. Melayu had elevated Zn concentration in its first geochemical fraction; namely, easily, freely, leachable or exchangeable (EFLE) and its total concentration. This total Zn level in the sediment was comparable to the polluted sites on the west coast of Peninsular Malaysia. It was assumed that the tap water, in which mussels had been boiled, might have contained high levels of Zn which would then be released to the drainage system and finally emptied into the coastal waters where the jetty is located. In order to confirm this point source of Zn contamination, a laboratory study was designed to ascertain if the boiled mussels contained higher concentrations of metals compared to a control group. The laboratory results showed that distilled water, in which fresh mussel tissues had been boiled for 15 minutes, possessed significantly ($P < 0.05$) higher levels of dissolved Zn. In addition, Zn concentrations in the total boiled soft tissues and boiled shells of fresh mussel *Perna viridis* were significantly ($P < 0.05$) lower than the Zn levels before boiling, and this finding evidently showed that Zn in the mussel tissues was remobilized and thus released to the water. Therefore, these results supported the conclusion that the mussel processing factory at Kg. Sg. Melayu, which used tap water to boil the mussels before shucking, was a point source of Zn contamination in this area in the Straits of Johore.

Keywords: *Perna viridis*, Zn contamination, boiled mussels

INTRODUCTION

Before visiting a mussel (*Perna viridis*) processing factory, the authors did not have any idea that the factory, which is situated at a jetty in Kg. Sg. Melayu, could be point source of pollution for heavy metals into the nearby coastal environment in the Straits of Johore. An earlier study of the heavy metal concentrations in the sediment at the jetty at this site had revealed an elevated concentration of Zn (Yap *et al*., 2006a) which was comparable to the polluted sites on the west coast of Peninsular Malaysia (Yap *et al*., 2003a). This raised the question whether the tap water in which the mussels had been processed by boiling in a metal tank contained a high level of Zn (*Fig. 1A*). It was assumed that this mussel-boiled water would then be released into the drainage and finally emptied into the coastal waters where the jetty is located. The boiling of the fresh mussels is necessary to facilitate the shucking of the soft tissues out of the mussel shells before packaging (*Fig. 1B*). To confirm the hypothesis, the present study was designed to ascertain if the boiled mussels contained higher concentrations of metals. The objective of this study was to provide evidence for the above by

*Corresponding Author

comparing the concentrations of Cd, Cu and Zn between the boiled and unboiled soft tissues and the shells of *P. viridis*, including the water used to boil the mussels.

MATERIALS AND METHODS

Mussels and surface sediment samples were collected from the jetty of the mussel processing factory at Kg. Sg. Melayu which is located in the Straits of Johore. They were stored in a cool box upon transportation to laboratory in Universiti Putra Malaysia (UPM).

For the laboratory experiment, fresh total soft tissues were dissected from the shells of *P. viridis*. The mussels were separated by sex, according to the colour of gonadal tissues as described by Yap *et al*. (2006b). Total soft issues and shells were placed in a beaker of distilled water (500 mL) and heated in a water bath. The temperature was maintained between 95-97°C for 15 minutes. A control group was analyzed for metal concentrations without further processing. The dissolved water used to boil the mussel samples was collected before and after the 15

Fig. 1: Containers with tap water (A) used to boil the mussel Perna viridis *before they were shucked from their shells (B) for packaging. Photos were taken at the mussel processing factory at Kg. Sg. Melayu in the Straits of Johore*

TABLE 1
Comparisons of dissolved concentrations (mg/L) of Cd, Cu and Zn in the water used to boil the mussels between boiled and unboiled mussels using *t*-test in STATISTICA.

		Minimum	Maximum	Mean	Std error	Significance level
Female soft tissues	N= 5					
Cd	Boiled	0.001	0.024	0.014	0.004	P> 0.05
	Unboiled	0.005	0.025	0.020	0.004	
Cu	Boiled	0.001	0.016	0.006	0.003	P> 0.05
	Unboiled	0.001	0.025	0.006	0.005	
Zn	Boiled	0.014	0.160	0.093	0.025	P< 0.05
	Unboiled	0.020	0.036	0.027	0.003	
Female shells	N= 5					
Cd	Boiled	0.010	0.023	0.018	0.002	P> 0.05
	Unboiled	0.011	0.023	0.016	0.003	
Cu	Boiled	0.001	0.005	0.003	0.001	P> 0.05
	Unboiled	0.001	0.005	0.002	0.001	
Zn	Boiled	0.019	0.065	0.036	0.009	P> 0.05
	Unboiled	0.014	0.044	0.026	0.006	
Male soft tissues	N= 6					
Cd	Boiled	0.013	0.029	0.018	0.002	P> 0.05
	Unboiled	0.010	0.025	0.016	0.002	
Cu	Boiled	0.001	0.023	0.006	0.004	P> 0.05
	Unboiled	0.001	0.002	0.001	0.000	
Zn	Boiled	0.015	0.181	0.083	0.024	P< 0.05
	Unboiled	0.022	0.029	0.026	0.001	
Male shells	N= 6.					
Cd	Boiled	0.015	0.027	0.019	0.002	P> 0.05
	Unboiled	0.014	0.028	0.020	0.002	
Cu	Boiled	0.001	0.010	0.003	0.002	P> 0.05
	Unboiled	0.000	0.001	0.001	0.000	
Zn	Boiled	0.017	0.073	0.045	0.009	P< 0.05
	Unboiled	0.011	0.032	0.024	0.003	

Note: Values in bold indicate significant (P < 0.05) differences

minutes' boiling (i.e. as approximate time to boil the mussels at the factory). This treated water was further analyzed. Unboiled distilled water was used as a control. All the experimental treatments were conducted in triplicates. For this laboratory experiment, distilled water obtained from our laboratory was used instead of tap water. This was because distilled water possessed no metal contamination and this could be a scientific basis of valid interpretation, should there be any differences in the metal levels between the unboiled and boiled distilled waters.

All of the tissues of the mussels and sediment samples were dried at 105°C for 72 hrs until constant dry weight. Three replicates

TABLE 2
Comparison of the concentrations (µg/g dry weight) of Cd, Cu and Zn between the boiled and unboiled mussels in their total soft tissues and shells by gender using *t*-test in STATISTICA.

		Minimum	Maximum	Mean	Std error	Significance level
Female shells	N= 12					
Cd	Boiled	3.40	4.83	4.14	0.10	P> 0.05
	Unboiled	0.37	5.39	4.14	0.37	
Cu	Boiled	3.45	6.66	5.63	0.33	P> 0.05
	Unboiled	4.60	8.38	5.88	0.31	
Zn	Boiled	6.14	9.25	7.57	0.26	P< 0.05
	Unboiled	7.03	10.74	8.62	0.38	
Female soft tissues	N= 12					
Cd	Boiled	1.52	2.72	2.15	0.12	P> 0.05
	Unboiled	1.63	2.72	2.21	0.09	
Cu	Boiled	3.18	11.92	8.52	0.90	P> 0.05
	Unboiled	4.14	14.11	8.34	0.97	
Zn	Boiled	88.33	177.73	139.17	7.65	P< 0.01
	Unboiled	120.52	215.22	168.33	7.24	
Male shells	N= 15					
Cd	Boiled	2.74	4.76	4.06	0.13	P> 0.05
	Unboiled	0.39	5.30	4.24	0.30	
Cu	Boiled	3.50	6.66	5.53	0.24	P> 0.05
	Unboiled	4.14	6.34	4.97	0.17	
Zn	Boiled	5.49	9.81	8.18	0.34	P< 0.05
	Unboiled	7.48	14.23	9.68	0.47	
Male soft tissues	N= 15					
Cd	Boiled	1.40	2.89	2.14	0.12	P> 0.05
	Unboiled	1.25	2.59	1.96	0.11	
Cu	Boiled	3.10	12.43	8.59	0.83	P> 0.05
	Unboiled	4.01	14.35	8.24	0.81	
Zn	Boiled	98.68	170.02	134.60	6.61	P< 0.01
	Unboiled	132.18	220.12	166.67	8.15	

Note: Values in bold indicate significant (P < 0.05) differences

of each mussel treatment were then digested in concentrated nitric acid (AnalaR grade; BDH: 69%). The dried sediment samples were crushed using a mortar and pestle and sieved through a 63 µm aperture stainless steel sieve and shaken vigorously to produce homogeneity. For the analyses of the total Cd, Cu and Zn concentrations in the sediment samples, three replicates were analyzed by using direct aqua-regia method. About 1g of each dried sample was digested in a combination of concentrated HNO_3 (AnalaR grade; BDH 69%) and $HClO_4$ (AnalaR grade; BDH 60%) in the ratio of 4:1. The mussel and sediment samples were put into a hot-block digester first at low temperature (40°C) for 1 hour and they were then fully digested at 140°C for at least 3 hours. Later, the digested samples were diluted to a certain volume (40 mL) with double distilled water. After filtration, the prepared samples were determined for Cd, Cu and Zn, using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. The data were presented in µg/g dry weight basis.

For the analytical procedures of the four geochemical fractions of the surface sediments, sequential extraction technique (SET), described by Badri and Aston (1983) with a slight modification by Yap *et al.* (2002a), was used in this study. These four fractions were: 1), easily, freely, leacheable or exchangeable (EFLE), 2) acid-reducible, 3) oxidisable-organic, and 4) resistant.

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 of standard solutions. The percentages of recoveries for the heavy metal analyses were 90-110%.

The dissolved concentrations of the metals in the water used to boil the mussels, between the boiled and unboiled water/mussel samples, were statistically analyzed using the *t*-test in STATISTICA (99' Edition).

TABLE 3
Comparisons of concentrations (µg/g dry weight) of Cd, Cu and Zn between the boiled and unboiled mussels in their total soft tissues and shells by combining male and female samples using *t*-test in STATISTICA; N= 27.

		Minimum	Maximum	Mean	Std error	Significance level
Shells						
Cd	Boiled	2.74	4.83	4.09	0.08	P> 0.05
	Unboiled	0.37	5.39	4.20	0.23	
Cu	Boiled	3.45	6.66	5.57	0.19	P> 0.05
	Unboiled	4.14	8.38	5.38	0.19	
Zn	Boiled	5.49	9.81	7.91	0.22	P< 0.05
	Unboiled	7.03	14.23	9.21	0.32	
Soft tissues						
Cd	Boiled	1.40	2.89	2.15	0.08	P> 0.05
	Unboiled	1.25	2.72	2.07	0.08	
Cu	Boiled	3.10	12.43	8.56	0.60	P> 0.05
	Unboiled	4.01	14.35	8.28	0.61	
Zn	Boiled	88.33	177.73	136.63	4.93	P< 0.01
	Unboiled	120.52	220.12	167.41	5.45	

Note: Values in bold indicate significant (P < 0.05) differences

RESULTS AND DISCUSSION

To compare between the boiled and unboiled control water samples in the female and male soft tissues of *P. viridis* shown in Table 1, the concentrations of Cd and Cu in both water types were found to be not significantly ($P > 0.05$) different. However, both the male and female soft tissues showed significantly ($P < 0.05$) higher levels of dissolved Zn in the boiled distilled water than in the unboiled water. The male shells also had significantly ($P < 0.05$) higher level of dissolved Zn in the boiled water than in the unboiled one. Similarly, the metal levels in the mussel samples from both the boiled and unboiled treatments were also analyzed to further understand this finding.

TABLE 4
Concentrations (mean ± standard error, µg/g dry weight) of Cu, Cd and Zn in the sediments collected from the jetty of the mussel processing factory at Kg. Sg. Melayu.

Sampling site	Mussel processing factory		
GPS reading:	N 01˚ 27.626’ E 103˚ 42.335’		
Date of sampling:	11 August 2004		
Time of sampling:	9.26 am		
Weather:	Sunny, humid and hazy		
Location description:	1.Orang Asli housing area 2. The place where soft tissues are separated from the shells for packaging.		
Geochemical fractions	Cu	Cd	Zn
F1: EFLE	0.668 ± 0.011	0.186 ± 0.018	19.2 ± 0.215
F2: Acid-reducible	0.401 ± 0.011	0.228 ± 0.037	78.7 ± 0.715
F3: Oxidisable-organic	30.0 ± 1.38	0.864 ± 0.112	96.0 ± 0.203
F4: Resistant	35.7 ± 0.996	0.925 ± 0.342	88.3 ± 2.38
Summation (F1+F2+F3+4)= *SUM*	66.7	2.20	282.2
Non-resistant fractions (F1+F2+F3)= *NR*	31.1	1.28	193.9
$\frac{NR}{SUM} \times 100\%$	46.5%	58.0%	68.7%
Total metal concentration based on aqua-regia method	46.6 ± 2.38	2.83 ± 0.469	310 ± 14.9
Biological effects within ranges of metal concentrations in marine and estuarine sediments: Effects range low (ERL) (Long *et al.*, 1995)	34	1.2	150
Biological effects within ranges of metal concentrations in marine and estuarine sediments: Effects range median (ERM) (Long *et al.*, 1995)	270	9.6	410
Sediment Quality Values (SQV) for Hong Kong-Low (Chapman *et al.*, 1999)	65	1.5	200
Sediment Quality Values (SQV) for Hong Kong-High (Chapman *et al.*, 1999)	270	9.6	410

Note: F1= easily, freely, leachable or exchangeable (EFLE)

The boiled and unboiled control mussel samples [soft tissues and shells, Table 2] did not show any significant difference ($P > 0.05$) in the concentrations of Cd and Cu between both sexes. Interestingly for Zn, both the total soft tissues and shells of *P. viridis* [both male and female] had significantly ($P < 0.05$) lower levels of Zn after 15 minutes of boiling. To further strengthen the conclusion, when the male and female data are combined, similar results were found in which only the Zn levels in both the total soft tissues and shells were significantly ($P < 0.05$) decreased compared to the levels of Cd and Cu (Table 3). These results were inversely related to those observed in the water samples used to boil the mussels. This suggested that the losses of Zn in the soft tissues and the shells of *P. viridis* after 15 minutes boiling were due to the release into the water since significantly higher Zn level was detected in the treated water samples.

Heat treatment by either steam-blanching or baking of polluted fish leading to a decrease of the heavy metal content in all the tested fish parts was also reported by Atta *et al.* (1997). Similarly, Howarth and Sprague (1978) also reported that the cooking process decreased the protein content of the fish parts; hence, since heavy metals are bound to protein, this would be followed by the decreased concentrations of metals. Therefore, the decreased Zn concentration found in the soft tissues of *P. viridis* after 15 minutes of boiling is not surprising.

The observation of Zn release from the boiled mussel shells is interesting from a biomonitoring point of view. The results of the present study imply the possibility of Zn release from the mussel shells inhabiting natural environment if the mussels are submerged near surface water at a relatively higher water temperature for a long time. Therefore, although *P. viridis* shells have been suggested as a potential biomonitoring material for Zn (Yap *et al.*, 2003b, 2004), the seawater temperature could also influence the accuracy of the Zn interpretation. Thus, this abiotic factor should be taken into account when interpreting data. However, it can still be argued that the coastal waters would hardly reach up to 40°C and therefore the Zn bound to crystalline lattices of the shell structures could hardly be released (Yap *et al.,* 2003b).

The use of *P. viridis* as a biomonitor of heavy metals in the Malaysian coastal waters has been reported in some earlier studies (e.g. Yap *et al.,* 2003c, 2003d, 2004, 2006a). However, the phenomenon of Zn partial regulation that is thought to reduce the efficiency for the biomonitoring purpose is still very much debated. Therefore, the findings of the present study support the easy remobilization of Zn in the soft tissues of the *P. viridis* (Phillips, 1985; Yap *et al.,* 2002a, 2003c) which could possibly explain the partial regulation of Zn in this mussel species (Phillips, 1985).

Another interesting finding was the high concentration of Zn found in the surface sediment samples at the jetty of the mussel processing factory (Table 4). Based on the levels of Cu and Cd, the area could be considered as unpolluted compared to those reported by Yap *et al.* (2002b, 2003a), but the concentrations of Zn (310 µg/g dry weight) were comparable to those reported for the polluted sites, such as at Kuala Juru (306 µg/g dry weight) in Penang (Yap *et al.*, 2003a) and at a drainage (344.4 µg/g dry weight) in Serdang Metal Industrial site (Yap *et al.*, 2007). Besides the total Zn concentration, am elevated level was also evidenced in the first geochemical fraction of Zn; namely, easily, freely, leachable or exchangeable (EFLE) (Table 4).

In order to estimate the possible environmental consequences of Cd, Cu and Zn at the studied sites, the metals were compared to the Sediment Quality Guidelines of Effects Range Low (ERL) and Effects Range Median (ERM), as proposed by Long *et al.* (1995) (Table 4). The results showed that the concentrations of the three metals in Kg. Sg. Melayu were between the values for ERL and ERM. Similarly, compared to the Sediment Quality Values (SQV) of Cd, Cu and Zn for Hong Kong (Chapman *et al.*, 1999), the present Cd, Cu and Zn ranges fell between SQV-low and SQV-high for the three metals, and thus, indicating 'moderately polluted' status. Based on the percentage of non-resistant fraction to the total summation of four geochemical fractions, however, the percentage for Zn is the highest (68.7%) among the three metals, indicating that most of the total Zn was related/contributed by anthropogenic sources (Badri & Aston, 1983). Zn remobilization in *P. viridis* soft tissues, due to boiling process, had caused the release of Zn into the boiling water which was finally drained into the coastal water nearby.

An environmental concern could arise from the release of the mussel boiled water into the coastal waters. This could contaminate the surrounding waters, as well as increase the Zn bioavailability to the nearby coastal waters.

CONCLUSION

The presence of Cd, Cu and Zn was detected in both the shells and soft tissues of *P. viridis* which were sampled from a jetty situated at the Straits of Johore. Boiling the shells and soft tissues for 15 minutes hardly reduced the concentrations of Cd and Cu in both parts of the mussels. However, the concentrations of Zn significantly decreased in the shells and soft tissues after the boiling process. One plausible explanation is that the Zn was released into the boiled processing water and then discharged into the coastal waters. Hence, the findings of the present study showed that the mussel processing factory at Kg. Sg. Melayu was a point source of Zn contamination in the Straits of Johore. However, it should be noted that the Zn release could depend on the boiling conditions (time, temperature and medium of cooking) which should merit further studies.

ACKNOWLEDGEMENTS

This study was supported by *Skim Penyelidikan Lantikan Baharu* [vot no: 53135], and Research University Grant Scheme (RUGS), [Vote no: 91986], both provided by Universiti Putra Malaysia.

REFERENCES

Atta, M. B., El-Sebaie, L. A., Noaman, M. A., & Kassab, H. E. (1997). The effect of cooking on the content of heavy metals in fish (*Tilapia nilotica*). *Food Chemistry, 58*(1), 1-4.

Badri, M. A., & Aston, S. R. (1983). Observation on heavy metal geochemical associations in polluted and non-polluted estuarine sediments. *Environment Pollution Series B*, *6*, 181-193.

Chapman, P. M., Allard, P. J., & Vigers, G. (1999). Development of sediment quality values for Hong Kong special administrative region: A possible model for other jurisdictions. *Marine Pollution Bulletin*, *38,* 161-169.

Howarth, R. S., & Sprague, J. B. (1978). Copper lethality to rainbow trout in waters of various hardness and pH. *Water Research, 12*, 455–462.

Long, E. R., MacDonald, D. D., Smith, S. C., & Calder, F. D. (1995). Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environmental Management*, *19*(1), 81–97.

Phillips, D. J. H. (1985). Organochlorines and trace metals in green-lipped mussels *Perna viridis* from Hong Kong waters: a test of indicator ability. *Marine Ecology Progress Series*, *21*, 251– 8.

Yap, C. K., Ismail, A., Tan, S. G., & Omar, H. (2002a). Correlations 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*(1-2): 117-126.

Yap, C. K., Ismail, A., Tan, S. G., & Omar, H. (2002b). Concentrations of Cu and Pb in the offshore and intertidal sediments of the west coast of Peninsular Malaysia. *Environment International*, *28***,** 467-479.

Yap, C. K., Ismail, A., & Tan, S. G. (2003a). Cd and Zn in the straits of Malacca and intertidal sediments of the west coast of Peninsular Malaysia. *Marine Pollution Bulletin, 46*, 1348-1353.

Yap, C. K., Ismail, A., Tan, S. G., & Abdul Rahim, I. (2003b). Can the shell of the green-lipped mussel *Perna viridis* (Linnaeus) 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.

Yap, C. K., Ismail, A., Omar, H., & Tan, S. G. (2003c). Accumulation, depuration and distribution of cadmium and zinc in the green-lipped mussel *Perna viridis* (Linnaeus) under laboratory conditions. *Hydrobiologia, 498*, 151-160.

Yap, C. K., Ismail, A., & Tan, S. G. (2003d). Background concentrations of Cd, Cu, Pb and Zn in the green-lipped mussel *Perna viridis* (Linnaeus) from Peninsular Malaysia. *Marine Pollution Bulletin, 46*, 1043-1048.

Yap, C. K, Ismail, A., & Tan, S. G. (2004). The shell of the green-lipped mussel *Perna viridis* as a biomonitoring material for Zn: Correlations of shells and geochemical fractions of surface sediments. *Malaysian Applied Biology, 33*(1), 79-88.

Yap, C. K., Ismail, A., Edward, F. B., Tan, S. G., & Siraj, S. S. (2006a). 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. *Toxicology and Environmental Chemistry*, *88*(4), 683-695.

Yap, C. K., Ismail, A, Tan, S. G., & Rahim Ismail, A. (2006b). Is gender a factor contributing to the natural variations in the accumulation of heavy metals (Cd, Cu, Pb and Zn) by the green-lipped mussel *Perna viridis* ? *Indian Journal of Marine Sciences, 35*(1), 29-3.

Yap, C. K., Pang, B. H., Fairuz, M. S., Hoo, Y. I., & Tan, S. G. (2007). Heavy metal (Cd, Cu, Ni, Pb and Zn) pollution in surface sediments collected from drainages receiving different anthropogenic sources from Peninsular Malaysia. *Wetland Science, 5*(2), 97-104.