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Assessment of Heavy Metal Pollution in the Straits of Johore by Using Transplanted Caged Mussel, *Perna viridis*

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ABSTRACT

In this study, a polluted site at Kg. Pasir Puteh was assessed for heavy metal pollution by using transplanted caged mussel (*Perna viridis*) from a relatively clean population, Sg. Melayu; both are located in the Strait of Johore. For control purposes, the *P. viridis* from Kg. Pasir Puteh were also simultaneously transplanted in Sg. Melayu at the same time. It was found that Zn was the metal which got accumulated fastest in the transplanted mussel while Cd was the slowest. This study indicated that the byssus of *Perna viridis* was most effective for biomonitoring of Cd, Ni, Pb and Zn, while the shell could be used for the biomonitoring of Cu, Ni and Pb and the total soft tissue for the biomonitoring of Ni since they were able to accumulate and eliminate the respective metals well. By using mussel as a biomonitor, the present study found that Kg. Pasir Puteh, which is located in the eastern part of the Strait of Johore, had significantly higher contamination and bioavailabilities of Cd, Cu, Fe, Ni, Pb and Zn. Therefore, the use of the transplanted caged mussels is very useful for heavy metal assessment purposes since it can increase the validity of data interpretation by minimizing ecological factors.

Keywords: Heavy metal, *Perna viridis*, Strait of Johore, transplant

INTRODUCTION

Anthropogenic activities have been increasing as the population of humans grows rapidly. The increase of anthropogenic activities has always caused heavy metal pollution in coastal waters. Monitoring of coastal waters which are exposed to chemical pollution due to industrial production and high urbanization

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(Giarratano *et al*., 2010) by using mussels has been widely reported in the literature. However, a more efficient and practical approach for assessing heavy metal pollution is needed. Recently, to define the areas of metal pollution, caged or transplanted mussels have been employed in many coastal waters such as Ushuaia Bay, Argentina (Giarratano *et al*., 2010), North West of Mediterranean Sea (Faverney *et al*. 2010), Boston Harbour, United States (Hunt & Slone, 2010) and New Caledonia Lagoon in the South Pacific of France (Hedouin *et al*., 2011).

The Johore Strait is a narrow strait that separates the Malaysian state of Johore from Singapore and it is separated into two distinct portions by a causeway which connects Peninsular Malaysia and Singapore. It is an important area for fishing and aquaculture activities (Yap *et al*., 2006; Zulkifli *et al*., 2010). Furthermore, the existence of mangrove, sea grass, coral and mudflat ecosystems also make the Johor Straits an important strait (Zulkifli *et al*., 2010). Oil pollution has been identified as the major contributor to the pollution of the water in the Straits of Johor (DOE, 1994; Moradi, 2001; Shahbazi *et al*., 2010). Shipping activities involving tankers and other vessels can easily be found in the Strait of Malacca, while land-based industrial and urban sources have been recognised as the sources of pollutants for the strait (Abdullah *et al*. 1996). The eastern part of the straits is more polluted than the western part since marina, petrochemical plants and port activities such as Pasir Gudang Port (ranked 82nd in 2007 global TEU), Tanjung Pelepas Port (ranked 18th in 2007 global TEU) and Tanjung Langsat Port (a new petrochemical port) are located on the eastern coast (Bayen *et al*., 2003; Yap *et al*., 2004, 2006). Kg. Pasir Puteh, which is located in the eastern part, is reported as a highly polluted site, where large shipyard repair and construction facilities, fossil fuel fired electrical power plants and shipping dock activities can be found (Yap *et al*., 2003d, 2004; Zulkifli *et al*., 2010). On the other hand, Kg. Sg. Melayu in the western part is considered as a site with low human activities and the only activities which can be found there are fish and mussel aquaculture (Yap *et al*., 2006). Owing to its strategic location and ecological importance, the Johore Strait has become a hotspot for pollution studies (DOE, 2007; Shazili *et al*., 2006; Wood *et al*., 1997).

Francesco and Enzo (1994) noted that the accumulations of Pb, Fe and Mn in the mussels, which were transplanted from a clean site to a heavy metal polluted environment, would reach a steady state only after 2 weeks. The results showed that mussels could easily equilibrate with polluted coastal waters. However, the time-integration capacity of mussels might vary for different metals. In fact, many important biological processes associated with metal exposures have generally been studied under laboratory conditions but not in field experiments (Regoli, 1992; Yap *et al*., 2003a). In this respect, mussel transplantation is a more practical approach to be used in monitoring the metal accumulation of mussels at fixed sites.

Marine mussels are suitable for transplantation experiments because they are cost effective and reliable (Farverney *et al*., 2010). In particular, *P. viridis* fulfils the necessary criteria which are sedentary lifestyle, enough tissues for metal analysis, suspension feeder, tolerant of high heavy metal concentrations (Yap *et al*. 2003a) and prone to bioaccumulate and magnify such metals (Yap *et al*. 2004) and of relatively low genetic differentiation (Yap *et al*., 2002a,b).

The advantages of adopting transplantation are mainly due: (1) to the fact that the monitoring sites may be chosen independently of the presence of natural populations (Hedouin *et al*., 2011), (2) to access the status of bioaccumulation in hard-to-reach areas such as in different strata of the water column (Hunt & Slone, 2010; Giarratano *et al*., 2010), (3) it can

be site-specific, (4) control exposure times, depth of transplantation, age, size, stage of sexual maturity of mussels, which can interfere with and affect the accumulation rate (Alfonso *et al*., 2010), and (5) usually with low genetic variation and in the same phase of the reproductive cycle since they are abundant and easily available from commercial markets (Gorbi *et al*., 2007). The use of transplanted mussels has been proven to be a useful strategy for biomonitoring marine pollution (Nasci *et al*., 2002; Romeo *et al*., 2003; Regoli *et al*., 2004; Nigro *et al*., 2006).

By using transplanted mussels, the effects of external and internal factors such as seasonal variations, size or age, which can cause bias in data comparison, are minimized (Regoli & Orlando, 1994; Hedouin *et al*., 2011). Since there has been no study which reported on heavy metals in transplanted mussels in Malaysia, the objective of this study was to assess the heavy metal pollution in the Strait of Johore by using transplanted mussels.

MATERIALS AND METHODS

Transplantation of Mussel Populations

About 200 individuals of *P. viridis* were collected from a polluted site at Kg. Pasir Puteh and transplanted to a relatively unpolluted site at Sg. Melayu, in the Strait of Johore on 28 November 2009. On the same day, the same amount of mussels were also collected from Sg. Melayu and transplanted to Kg Pasir Puteh. After the mussels had been collected, the whole cluster was rinsed 3 times using seawater to get rid of any visible sediment on the mussel shells. The mussels were then divided randomly into sub-groups of 40 individuals and each sub-group was placed in a polyethylene cage of 20 x 15 x 18 cm which permitted water circulation through it. Four cages were used per site and left suspended in the water column at an average depth of 1.5 m using a rope which was modified from Faverney *et al*. (2010). The samples of mussels were taken at the beginning of the experiment (t=0), at 2 (t=2), 6 (t=6) and 10 (t=10) weeks time of the exposure. The collected mussels had been rinsed with seawater before they were transported back to the laboratory in an ice compartment. However, at week 10, all the mussel samples died and no soft tissues were found. Thus, only byssus and shells were analysed at week 10.

Seawater

The physico-chemical readings of the seawater from Sg. Melayu and Kg. Pasir Puteh at around 1.5 m depth were recorded *in situ* at the beginning of the experiment (t=0), at weeks 2 (t=2), 6 (t=6) and 10 (t=10) for temperature (°C), specific conductivity (ms/cm), salinity (ppt) and dissolved oxygen (mg/L) during the transplantation experiment using YSI brand Physico-chemical meter (PCM) model no. 556 MPS.

Pre-treatment of the Samples

Sediment samples at each site were also collected by using an Ekmen Grab (16 cm x 16 cm) and these samples were stored in polyethylene bags and transported in an ice compartment back to the laboratory. The samples were dried until constant weights at 105°C for at least 16 hours (Tanner *et al*., 2000). The samples were then sieved through 63µm stainless steel aperture

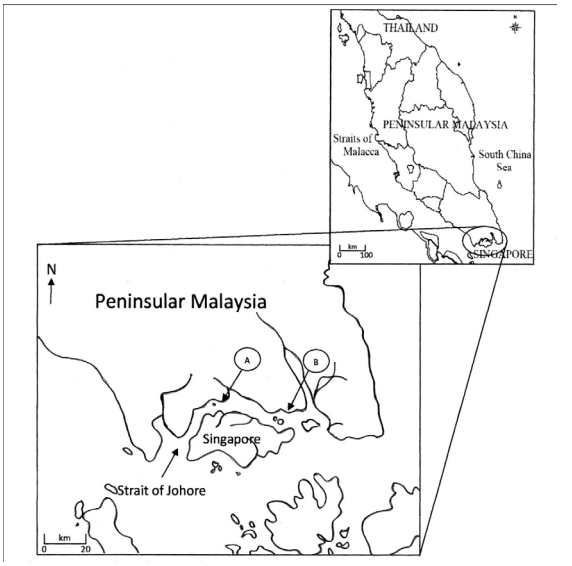

Fig.1: Map showing the transplantation sites for *Perna viridis*
Note: A- Sungai Melayu B- Kampung Pasir Puteh

and vigorously shaken to produce homogeneity. All individuals of *P. viridis* were dissected into byssus, total soft tissue and shell. Half gram of the samples was digested in concentrated nitric acid (AnalaR grade, BDH 69%) in a hot-block digestor first at a low temperature (40°C) for 1 hour before the temperature was increased to 140°C for 3 hours. The digested samples were later diluted in 40ml double distilled water (DDW). The sample was then filtered through Whatman No. 1 filter paper and the filtrate was stored in an acid-washed pill box until metal determination. For the analyses of the total heavy metal concentrations in sediment samples, the open digestion method was used. About 1g of each dried sample was digested in a combination of concentrated nitric acid (AnalaR grade, BDH 69%) and perchloric acid (60%) in the ratio of 4:1. On top of that, the geochemical fractions of Cd, Cu, Fe, Ni, Pb and Zn in the sediments were obtained by using the modified Sequential extraction technique (SET), as described by Yap *et al*. (2002). The first fraction was EFLE (Easily, freely, leacheable or exchangeable). About 10g of the sample was continuously shaken for 3 hours with 50ml 1.0M ammonium acetate (NH_4CH_3COO) at pH 7.0 at room temperature for this fraction. The second fraction was 'Acid-reducible'. The residue from fraction 1 was continuously shaken for 3 hours with 50ml 0.25M hydroxylammonium chloride ($NH_2OH.HCL$) acidified to pH 2 with HCL at room temperature, and this was followed by the third fraction which was 'Oxidisable-organic'. The residue from fraction 2 was first oxidized with 30% H_2O_2 in a water bath at 90–95°C. After cooling, the metal released from the organic complexes was continuously shaken for 3 hours with 1.0M ammonium acetate (NH_4CH_3COO) acidified to pH 2.0 with HCL at room temperature. 'Resistant' was the last fraction where the residue from fraction 3 was digested in a combination of concentrated nitric acid (AnalaR grade, BDH 69%) and perchloric acid (AnalaR grade, BDH 60%), as performed in the open digestion method.

TABLE 1: Size of mussels in average (mean ± SE, mm), condition index (CI) and physico-chemical characteristics of the water samples at different time intervals at Kg. Pasir Puteh

Sg. Melayu – Kg. Pasir Puteh

	Sizes of mussel (mean±SE, mm)										PCM				
Week	Length			Width			Height			CI	Temp (°C)	SC (ms/cm)	Sal (ppt)	DO (mg/L)	pH
0	30.45	±	2.64	9.07	±	1.04	14.64	±	1.54	21.53	29.26	45.17	29.11	4.57	7.52
2	56.67	±	3.68	16.77	±	3.67	23.94	±	3.19	23.54	30.01	38.61	24.42	3.14	7.15
6	68.88	±	4.51	20.67	±	3.92	29.22	±	2.06	21.19	30.61	44.47	28.56	7.59	7.68
10	79.94	±	6.37	24.38	±	2.83	35.17	±	3.17	20.61	29.96	39.90	25.33	13.82	7.03

TABLE 2: Size of mussels in average (mean ± SE, mm), condition index (CI) and physico-chemical characteristics of the water samples at different time intervals at Sungai Melayu

Kg. Pasir Puteh – Sg. Melayu

	Sizes of mussel (mean±SE, mm)										PCM				
Week	Length			Width			Height			CI	Temp (°C)	SC (ms/cm)	Sal (ppt)	DO (mg/L)	pH
0	34.46	±	1.33	10.22	±	1.26	15.66	±	2.37	26.18	30.04	41.54	26.49	3.88	7.15
2	53.17	±	3.14	16.96	±	3.11	22.39	±	3.88	24.22	29.06	46.31	29.94	5.85	7.16
6	66.15	±	4.57	19.37	±	4.31	29.61	±	4.32	21.39	29.06	46.31	29.94	5.74	7.16
10	78.74	±	5.39	23.41	±	3.94	35.10	±	4.16	17.45	29.97	41.59	26.53	4.22	7.15

TABLE 3: A comparison of heavy metal concentrations (µg/g dry weight) between measured values and certified values in the Certified Reference Materials (CRM) for dogfish liver DOLT-3

Metals	Certified values (C)			Measured value (M)			Percentage of recovery [(M/C) x 100]
Cu	31.20	±	1.00	26.81	±	0.25	85.93
Cd	19.40	±	0.60	14.68	±	0.34	75.67
Fe	1484.00	±	57.00	1213.42	±	10.67	81.77
Ni	2.72	±	0.35	3.37	±	0.20	123.90
Pb	37.38	±	12.81	29.37	±	0.33	78.56
Zn	86.60	±	2.40	76.12	±	0.81	99.90

TABLE 4: A comparison of heavy metal concentrations (µg/g dry weight) between measured values and certified values in the Certified Reference Materials (CRM) for Soil China (NSC DC73319)

Metals	Certified values (C)			Measured value (M)			Percentage of recovery [(M/C) x 100]
Cu	21.00	±	2.00	18.28	±	0.74	87.05
Cd	4.30	±	0.40	4.64	±	0.14	107.91
Ni	20.40	±	1.80	17.16	±	0.51	84.12
Pb	98.00	±	6.00	110.8	±	9.39	113.06
Zn	680.00	±	25.00	566.76	±	6.39	83.35

Note: CRM for Fe was not available.

Metal Analysis

After filtration, the samples were analysed for Cd, Cu, Fe, Ni, Pb and Zn using an air-acetylene flame atomic absorption spectrophotometer (AAS), Perkin-Elmer Model AAnalyst 800. The data are presented in $\mu g.g^{-1}$ dry weight. To avoid possible contamination, all the glassware and equipment used were acid-washed and the accuracy of the analysis was checked against blanks. For data validation, Certified Reference Material (CRM) was checked with the samples for dogfish liver (DOLT-3, National Research Council Canada) and Soil China (NSC DC73319, China National Analysis Centre), as shown in Tables 3 and 4.

Condition Index

To examine the individual Condition Index (CI) of mussels, 5 specimens were taken at each exposure time to obtain an estimate of the ratio of weight of the soft parts to shell volume. Soft tissues were separated from the shell and dried at 60°C until constant weight was achieved. Condition index (g/cm^3) of each mussel was calculated according to Lares and Orians (1997):

$$\text{Condition index} = \frac{\text{dry tissue weight (g)}}{\text{shell length (cm) x shell width (cm) x shell height (cm)}} \text{x1000}$$

Data Analysis

The concentration factor (CF) was calculated according to Yap *et al*. (2003a).

$$CF = \frac{\text{Metal level}_{\text{end of metal accumulation}}}{\text{Metal level}_{\text{initial}}}$$

The elimination factor (EF) was also calculated according to Yap *et al*. (2003a).

$$EF = \frac{\text{Metal level}_{\text{end of metal elimination}}}{\text{Metal level}_{\text{initial}}}$$

The rate of metal accumulation was calculated according to a formula proposed by Yap *et al*. (2003a), as follows:

$$\text{Rate of metals accumulation} = \frac{\text{Metal level}_{\text{exposed}} - \text{Metal level}_{\text{initial}}}{\text{Day(s) of metal exposure}}$$

Meanwhile, the rate of metal elimination was calculated according to the following formula (Yap *et al*., 2003a):

$$\text{Rate of metals elimination} = \frac{\text{Metal level}_{\text{exposed}} - \text{Metal level}_{\text{initial}}}{\text{Day(s) of metal elimination}}$$

Note: $\text{Metal level}_{\text{end of metal accumulation}}$ = heavy metals value at week 6 or week 10.

$\text{Metal level}_{\text{end of metal elimination}}$ = heavy metals value at week 6 or week 10.

$\text{Metal level}_{\text{initial}}$ = heavy metals value at week 0.

$\text{Metal level}_{\text{exposed}}$ = heavy metals value at weeks 2, 6 or 10.

Statistical Analysis

The statistical analyses were done by using STATISTICA version 8.0 for Windows. In addition, analysis of variance (ANOVA) was applied to find the differences between the means of heavy metal concentrations in the different parts of the mussels during the transplantation. Students-Newman-Kuel was applied to compare the mean values of heavy metal concentrations between the different parts of the mussels. T-test was carried out to test between the means of the end of the metal exposures and the initial metal exposures. The mean differences between Kg. Pasir Puteh and Sg. Melayu were tested using the Mann-Whitney test (Zar, 1996).

RESULTS AND DISCUSSION

Physico-chemical Parameters and Sediment Data

The physico-chemical parameters from Kg. Pasir Puteh and Sg. Melayu were found to be similar during the 10-week transplantation (see Tables 1 and 2), with ranges of 29.06-30.61°C for temperature, 38.61-46.78 ms/cm for specific conductivity, 24.42-30.24 ppt for salinity, 3.14-13.82 mg/L for dissolved oxygen and 7.03-7.68 for pH. As for sediment, heavy metal concentrations in both the studies sites are presented in Table 5. For Kg Pasir Puteh, the concentrations (µg/g dry weight) for the sediment data ranged from Cd (2.73-3.24), Cu (105.71-112.71), Fe (1.83-2.00%), Ni (45.20-52.46), Pb (61.90-73.47) and Zn (225.83-289.61). On the other hand, the concentration ranges obtained from Sg. Melayu, (µg/g) for the sediment data were Cd (1.42-1.80), Cu (31.39-60.20), Fe (1.74-1.80%), Ni (26.27-37.19), Pb (31.97-42.21) and Zn (83.21-95.18).

The sediment data obtained from Kg. Pasir Puteh in this study were generally higher, although not significantly, as compared to the sediment data reported by Yap *et al*. (2002) for Cd (1.45 µg/g), Cu (104 µg/g), Pb (70 µg/g) and Zn (170 µg/g) from the same sampling site of the sediments collected in 2002. All the metal concentrations (except for Fe) in Kg Pasir Puteh were significantly (Mann-Whitney Test, $p < 0.05$) higher than those of Sg. Melayu. This indicated that Kg. Pasir Puteh was more contaminated by metals than Sg. Melayu. The cause of the high availability of metals, especially Cu, Pb, Zn at Kg. Pasir Puteh, was probably due to the presence of shipyards which used biofouling paints (Bayen *et al*., 2003).

TABLE 5: Heavy metal concentrations (µg/g dry weight) in the surface sediments collected from Sg. Melayu and Kg. Pasir Puteh

		Cd			Cu			Fe			Ni			Pb			Zn		
Weeks		P.Puteh	Sg Melayu	M-W Test	P.Puteh	Sg Melayu	M-W Test	P.Puteh	Sg Melayu	M-W Test	P.Puteh	Sg Melayu	M-W Test	P.Puteh	Sg Melayu	M-W Test	P.Puteh	Sg Melayu	M-W Test
0	Aqua-regia	2.90	1.62	$p>0.05$	105.71	60.20	$p<0.05$	1829.57	1775.22	$p>0.05$	46.62	27.57	$p<0.05$	61.90	31.97	$p<0.05$	225.83	83.21	$p< 0.05$
	F1	0.68	0.23	$p>0.05$	1.07	0.42	$p>0.05$	1.49	2.43	$p>0.05$	1.42	1.18	$p>0.05$	1.76	1.97	$p>0.05$	20.56	2.26	$p< 0.05$
	F2	0.72	0.57	$p>0.05$	0.18	0.33	$p>0.05$	0.08	0.13	$p>0.05$	1.36	2.02	$p>0.05$	2.33	2.98	$p>0.05$	78.90	2.12	$p< 0.05$
	F3	0.84	0.50	$p>0.05$	71.20	9.67	$p<0.05$	601.50	669.41	$p>0.05$	27.27	13.87	$p<0.05$	31.17	15.13	$p<0.05$	109.07	58.78	$p< 0.05$
	F4	0.16	0.13	$p>0.05$	15.49	6.62	$p<0.05$	494.81	657.76	$p<0.05$	4.63	3.62	$p>0.05$	15.44	10.02	$p>0.05$	35.32	15.73	$p< 0.05$
4	Aqua-regia	NC	1.42		NC	31.39		NC	1735.29		NC	37.19		NC	42.21		NC	95.18	
	F1	NC	0.25		NC	0.97		NC	13.88		NC	2.30		NC	2.64		NC	2.80	
	F2	NC	0.64		NC	0.52		NC	10.49		NC	4.25		NC	2.49		NC	2.35	
	F3	NC	0.57		NC	15.29		NC	561.66		NC	16.58		NC	16.54		NC	62.18	
	F4	NC	0.07		NC	9.68		NC	524.25		NC	6.15		NC	11.43		NC	17.61	
6	Aqua-regia	3.24	1.80	$p<0.05$	112.71	36.83	$p<0.05$	1944.11	1795.78	$p>0.05$	52.46	26.27	$p<0.05$	73.47	39.61	$p<0.05$	253.37	94.80	$p< 0.05$
	F1	0.73	0.17	$p<0.05$	2.20	0.99	$p>0.05$	18.29	2.50	$p<0.05$	4.09	1.43	$p<0.05$	3.64	3.07	$p>0.05$	23.08	2.66	$p< 0.05$
	F2	0.63	0.50	$p>0.05$	0.40	0.47	$p>0.05$	0.88	0.42	$p>0.05$	2.89	2.66	$p>0.05$	3.58	4.14	$p>0.05$	84.63	2.18	$p< 0.05$
	F3	0.99	0.47	$p>0.05$	91.87	20.24	$p<0.05$	884.20	727.54	$p>0.05$	34.11	21.16	$p<0.05$	43.91	20.54	$p<0.05$	116.00	55.15	$p< 0.05$
	F4	0.28	0.33	$p>0.05$	19.79	12.19	$p<0.05$	699.10	744.04	$p>0.05$	6.87	6.63	$p>0.05$	23.19	13.00	$p<0.05$	51.73	15.38	$p< 0.05$
10	Aqua-regia	2.73	NC		109.85	NC		2008.96	NC		45.20	NC		63.27	NC		289.61	NC	
	F1	0.70	NC		1.53	NC		3.15	NC		1.82	NC		3.06	NC		22.29	NC	
	F2	0.82	NC		0.18	NC		1.46	NC		1.63	NC		3.98	NC		84.79	NC	
	F3	0.93	NC		94.47	NC		868.70	NC		38.93	NC		44.09	NC		130.00	NC	
	F4	0.18	NC		18.09	NC		729.17	NC		5.57	NC		23.18	NC		38.37	NC	

Note: F1: easily, freely, leacheable or exchangeable; F2: Acid-redicible; F3: Oxidisable-organic; F4: Resistant; NC: sample were not collected; M-W Test; Mann-Whitney test.

Metal Accumulation in the Mussels

During the 10-week duration of transplantation from Sg. Melayu to the sampling site at Kg. Pasir Puteh, all the metal concentrations were generally found to have increased from 1.49 to 6.93 µg/g for Cd, 7.31 to 25.63 µg/g for Cu, 29.27 to 3150.06 µg/g for Fe, 17.13 to 33.98 µg/g for Ni, 6.13 to 43.59 µg/g for Pb and 8.19 to 184.73 µg/g for Zn (see Fig.2 to Fig.7). In specific, the heavy metal concentrations for all the metals in Kg. Pasir Puteh were significantly higher than those in Sg. Melayu (independent t-test, $p< 0.05$). Meanwhile, Fe accumulated the highest in the byssus and the total soft tissue while Pb accumulated the highest in the shells of the mussels transplanted from Sg. Melayu to Kg. Pasir Puteh.

As for Cd, significant differences (one-way ANOVA, SNK, $p< 0.05$) were registered between the tissues for the mussels transplanted from Sg. Melayu to Kg. Pasir Puteh for T_0 and T_2. However, starting from T_6, the total soft tissue and byssus were significantly different from the shell. For Cu, the shell and byssus were significantly different from the total soft tissues during the transplant period, i.e. from T_0 to T_{10}. Meanwhile, significant differences were found between byssus, shell and total soft tissues for Fe concentrations. The Ni concentrations did not register any significant difference between TST, byssus and shell. For both sites, significant differences (one-way ANOVA, SNK, $p< 0.05$) were registered between the shell and byssus from the total soft tissues for Pb concentrations from T_0 to T_{10}. On the other hand, the Zn levels of byssus and total soft tissue were significantly different (one-way ANOVA, SNK, $p< 0.05$) from the shell in both the transplanted mussels.

Table 6 shows the rate of accumulation for all the metals in the different parts of the mussels transplanted from Sg. Melayu to Kg. Pasir Puteh according to weeks. The rates of the metal accumulation were fastest in all the mussel parts in week 2 (T=2) but these became slower in the following weeks and up to week 10 (T=10). During the first 6 weeks of transplantation, the total soft tissues showed the highest concentration factors (CF) in Cd (2.06), Cu (1.81) and Fe (2.04) but the lowest CF in Ni (1.11). Meanwhile, byssus showed the highest CF in Pb (2.28) and Zn (2.28), and the shell showed the highest CF in Ni (1.80). For the period of 10-week transplantation, the total soft tissues showed the highest concentration factorss (CF) in Cd (2.06), Cu (1.81) and Fe (2.22). Byssus showed the highest CF in Pb (3.17) and Zn (3.30), while the shell showed the highest CF in Ni (1.98). These indicated that TST accumulated the highest Cd and Cu concentrations, while byssus accumulated the highest Pb and Zn concentrations and the shell accumulated the highest Ni concentration.

The concentrations of Cd in the shell and Ni in TST in the transplanted *P. viridis* at week 10 reached the values similar to those measured in the initial *P. viridis* (T_0). Similar findings had previously been reported for Cu and Zn in the soft tissues of the mussel *Mytilus edulis* transplanted to a temperate polluted bay (Roesijadi *et al*., 1984) and Cr and Cu in *Isognomon isognomon* and Co, Ni and Zn in *Gafrarium tumidum* transplanted from a polluted site to a clean site in a New Caledonia lagoon (Hedouin *et al*., 2011). As for Ni in byssus, Fe in TST, Pb in byssus and shell and Zn in TST from the transplanted mussels (T_{10}), the levels significantly increased during the transplantation period but did not reach the values measured in the initial *P. viridis* (T_0). This could be due to the transplantation period being not long enough for the transplanted mussels to accumulate the metals completely. Comparable results had been

TABLE 6: Concentration factor (CF), rates of accumulation (µg/g per day) of Cd, Cu, Fe, Ni, Pb and Zn in byssus, total soft tissue and shell of transplanted *P. viridis* from Sg. Melayu to Kg. Pasir Puteh

		Week				
		CF		Rate of accumulation		
	Parts	6	10	2	6	10
Cd	Byssus	1.114	1.134	0.010	0.009	0.006
	TST	1.815	2.060	0.033	0.029	0.023
	Shell	1.061	1.098	0.011	0.009	0.009
Cu	Byssus	1.443	1.498	0.161	0.090	0.061
	TST	1.666	1.807	0.316	0.225	0.164
	Shell	1.490	1.720	0.145	0.085	0.075
Fe	Byssus	1.742	1.898	48.662	29.330	21.286
	TST	2.041	2.218	17.309	13.826	9.706
	Shell	1.295	1.535	0.379	0.205	0.224
Ni	Byssus	1.322	1.460	0.378	0.157	0.134
	TST	1.081	1.109	0.149	0.059	0.048
	Shell	1.802	1.984	0.774	0.327	0.241
Pb	Byssus	2.279	3.172	0.231	0.419	0.426
	TST	1.736	1.862	0.232	0.108	0.076
	Shell	1.187	1.345	0.196	0.120	0.133
Zn	Byssus	2.756	3.299	2.642	2.342	1.839
	TST	1.918	2.270	2.653	1.388	1.153
	Shell	1.518	1.867	0.134	0.101	0.102

previously reported for the oyster *Crassostrea rhizophorae* (Wallner-Kersanach *et al*., 2000), as well as oyster *Isognomon isognomon* and clam *Gafrarium tumidum* from the New Caledonia lagoon (Hedouin *et al*., 2011). However, Cd in byssus did not show any significant increase during the transplantation from Sg. Melayu to Kg. Pasir Puteh. This corresponded with similar observations for the Cd and Zn concentrations in *Crenomytilus grayanus* after two months of transplantation (Shulkin *et al*., 2003). The lack of Cd accumulation in the transplanted *P. viridis*, as observed in the current study, suggested that this particular element was rather poorly bioavailable for them or that *P. viridis* had efficient regulation mechanisms preventing these metals from being accumulated (Hedouin *et al*., 2011).

For the mussels transplanted from Sg. Melayu to Kg. Pasir Puteh, the metal levels in all

the parts had decreased. The high CF values in the metals (Table 6) showed that *P. viridis* was capable of accumulating these metals. In particular, TST was highly capable of accumulating Cd and Cu while the byssus was capable of accumulating Pb and Zn and the shell accumulated the highest Ni concentration. The total soft tissues of *P. viridis* showed the highest CF values for most of the metals, and this indicated that it is useful for monitoring metal contamination in coastal waters. The results obtained in the present study are also comparable to those of Yap *et al*. (2003a, 2004) which showed that *P. viridis* is a good accumulator for Cd, Pb and Zn. This test revealed the ability of *P. viridis* to accumulate and eliminate metals in short periods of time. Among the CF values for week 6 and week 10, Zn was the one which was accumulated the fastest while Cd was the slowest.

Metal Elimination in Mussels

Fig.2 to 7 show that all the metal concentrations in the mussels transplanted from Kg. Pasir Puteh to Sg. Melayu had decreased starting from T_0 to $T_{10.}$ The Cd level decreased from 6.55 to 2.3 µg/g, Cu from 37.81 to 2.84 µg/g, Fe from 2909.69 to 14.98 µg/g, Ni from 55.06 to 13.64, Pb from 46.43 to 16.01 µg/g and Zn from 176.24 to 7.05 µg/g. Similar findings were also reported by Gabr and Gab-Alla (2008) for clams (*Ruditapes decussatus* and *Venerupis pullastra*) transplanted from a polluted site to a clean site.

As for the mussels transplanted from Kg. Pasir Puteh to Sg. Melayu, there was no significant difference (one-way ANOVA, SNK, $p< 0.05$) observed for all the tissues in the Cd concentrations for T_0, starting from T_2, however, the shell was found to be significantly different from the total soft tissue and byssus. As for Cu, the total soft tissue and byssus were significantly different from the shell from T_0 to T_{10}. On the other hand, the total soft tissue and byssus were significantly different from the shell for the Fe concentration from T_0 to T_{10}.

The rate of elimination (Table 7) for all the metals in the different parts of the transplanted mussels from Kg. Pasir Puteh to Sg. Melayu was the fastest in all the mussel parts in week 2 (T=2) and this became slower in the subsequent weeks until week 10 (T=10). For byssus, the rate of elimination was 0.23(T=2), 0.084 (T=6) and 0.033 (T=10) in Cd. During the first 6 weeks of transplantation, the elimination factor (EF) in byssus was the fastest for Cd (0.42) and Ni (0.49). Meanwhile, the EF in shell was the fastest in Cu (0.22) and Pb (0.35) and slowest in Cd (0.80). The EF in TST was the fastest for Cu (0.69), Fe (0.65), Pb (0.72) and Zn (0.74). During the 10-week transplantation, the elimination factor (EF) in byssus was the fastest for Cd (0.38), Ni (0.25) and Zn (0.37). Meanwhile, the EF in shell was fastest for Cu (0.19), Fe (0.42) and Pb (0.34) and the slowest for Cd (0.80). These results indicated that byssus eliminated Cd, Ni concentrations the fastest while shell eliminated Cu and Pb the fastest.

At week 6, the concentrations of Cd in TST, Cu in byssus and TST, Ni in byssus and shell, Fe in byssus and TST, Pb in byssus and TST and Zn in byssus and TST were far from reaching the concentrations measured in the initial population (T_0). At week 10, only Cu, Fe, Pb and Zn in byssus and Ni in shell were far from reaching the concentrations measured in the initial population (T_0). Such findings had also been reported by several authors when organisms from polluted areas were transplanted to clean areas such as Zn in the mussel *M. edulis* (Roseijadi *et al*., 1984; Simpson, 1979), Cd and Cu in the oyster *Crassostrea gigas* (Geffard *et al*., 2002), Cr,

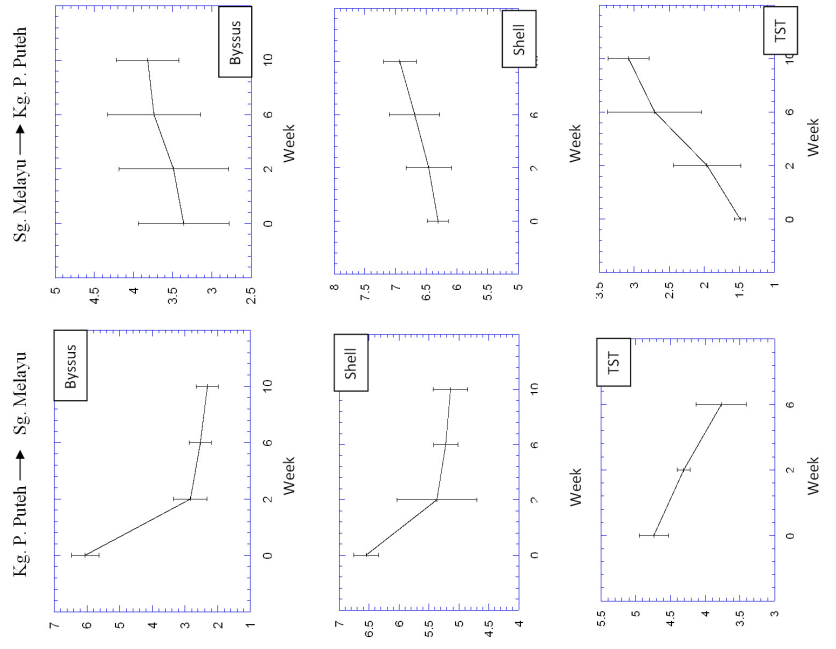

Fig.2: Comparison of Cd concentrations (mean± SE, µg/g dw; N=3) for *Perna viridis* transplanted from Kg. Pasir Puteh to Sg. Melayu and from Sg. Melayu to Kg. Pasir Puteh

*0= Initial, 2=2 weeks, 6= 6 weeks, 10= 10 weeks.
Note: TST= Total soft tissue

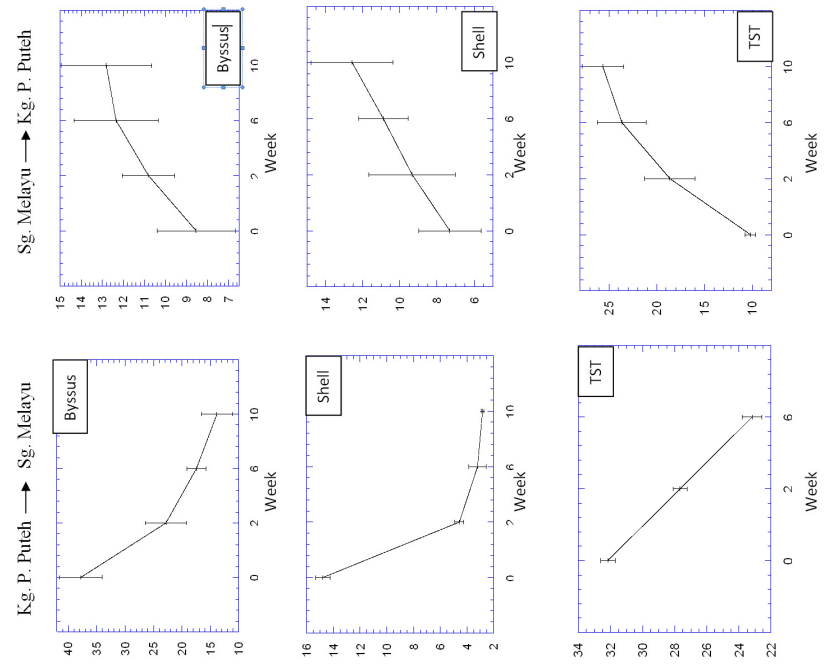

Fig.3: Comparison of Cu concentrations (mean± SE, µg/g dw; N=3) for *Perna viridis* transplanted from Kg. Pasir Puteh to Sg. Melayu and from Sg. Melayu to Kg. Pasir Puteh

*0= Initial, 2=2 weeks, 6= 6 weeks, 10= 10 weeks.
Note: TST= Total soft tissue

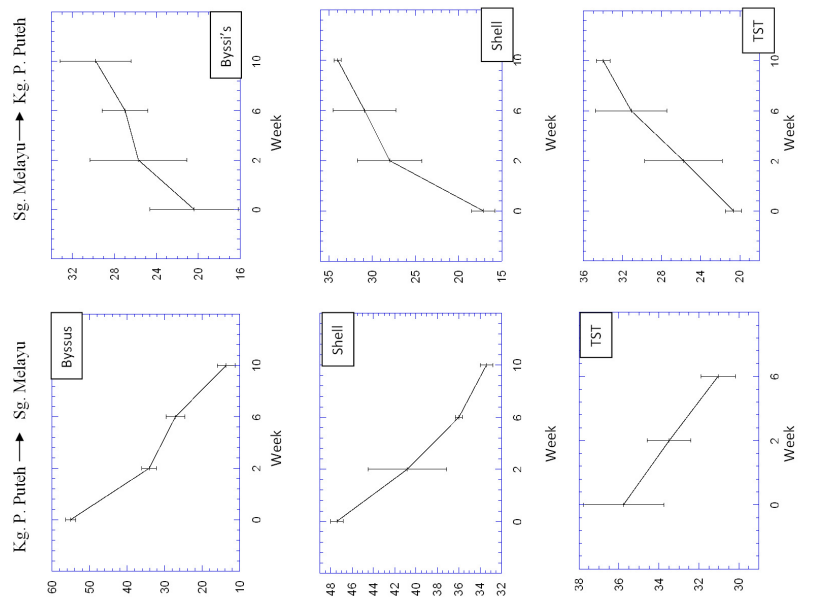

Fig.4: Comparison of Ni concentrations (mean± SE, µg/g dw; N=3) for *Perna viridis* transplanted from Kg. Pasir Puteh to Sg. Melayu and from Sg. Melayu to Kg. Pasir Puteh

*0= Initial, 2=2 weeks, 6= 6 weeks, 10= 10 weeks.
Note: TST= Total soft tissue

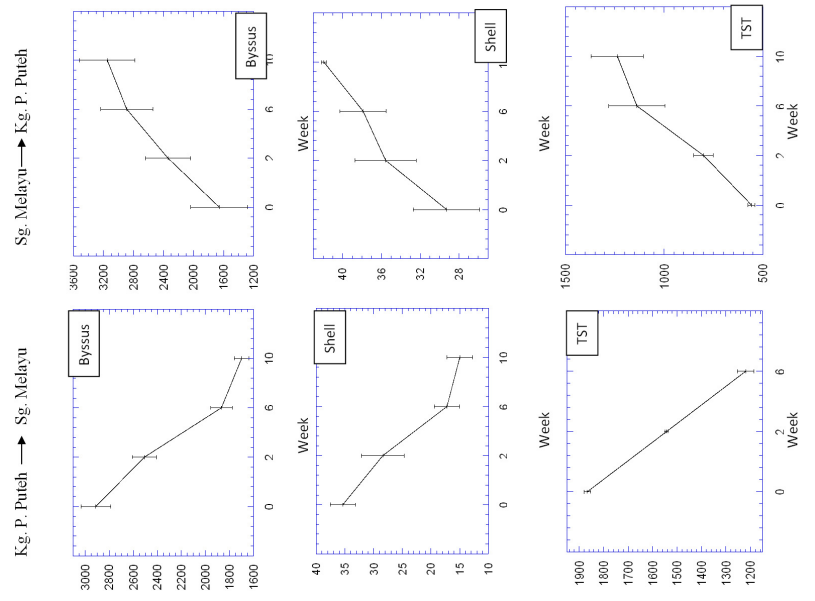

Fig.5: Comparison of Fe concentrations (mean± SE, µg/g dw; N=3) for *Perna viridis* transplanted from Kg. Pasir Puteh to Sg. Melayu and from Sg. Melayu to Kg. Pasir Puteh

*0= Initial, 2=2 weeks, 6= 6 weeks, 10= 10 weeks.
Note: TST= Total soft tissue

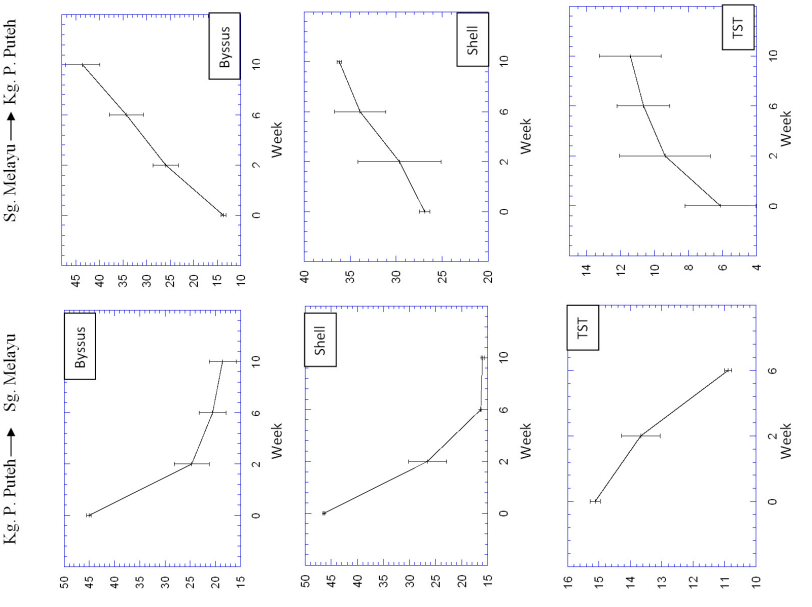

Fig. 6: Comparison of Pb concentrations (mean± SE, µg/g dw; N=3) for *Perna viridis* transplanted from Kg. Pasir Puteh to Sg. Melayu and from Sg. Melayu to Kg. Pasir Puteh

*0= Initial, 2=2 weeks, 6= 6 weeks, 10= 10 weeks.
Note: TST= Total soft tissue

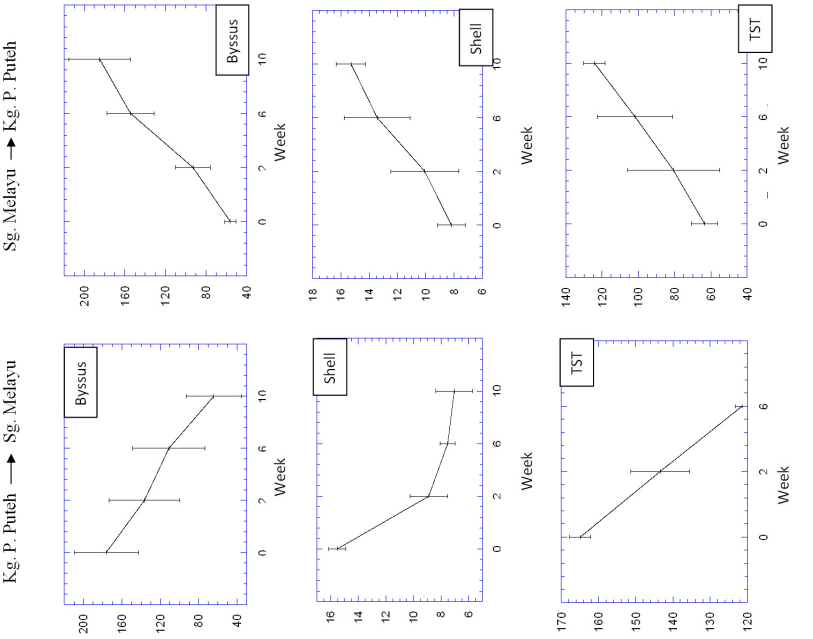

Fig.7: Comparison of Zn concentrations (mean± SE, µg/g dw; N=3) for Perna viridis transplanted from Kg. Pasir Puteh to Sg. Melayu and from Sg. Melayu to Kg. Pasir Puteh

*0= Initial, 2=2 weeks, 6= 6 weeks, 10= 10 weeks.
Note: TST= Total soft tissue

TABLE 7: Elimination factor (EF), rates of elimination (µg/g per day) of Cd, Cu, Fe, Ni, Pb and Zn in byssus, total soft tissue and shell of transplanted *P. viridis* from Kg. Pasir Puteh to Sg. Melayu

		Week				
		EF		Rate of elimination		
	Parts	6	10	2	6	10
Cd	Byssus	0.4177	0.383	0.2298	0.084	0.0331
	TST	0.7954	NC	0.0307	0.0231	NC
	Shell	0.7969	0.8	0.0843	0.0317	0.0749
Cu	Byssus	0.4612	0.3663	1.0724	0.4851	0.1979
	TST	0.69	NC	0.1779	0.2374	NC
	Shell	0.217	0.192	0.7303	0.2758	0.0406
Fe	Byssus	0.6417	0.5844	28.6902	24.8251	24.2906
	TST	0.6531	NC	23.1779	15.4255	NC
	Shell	0.4868	0.4235	0.4982	0.4323	0.214
Ni	Byssus	0.4931	0.2477	1.4939	0.6646	0.1949
	TST	0.868	NC	0.1629	0.1124	NC
	Shell	0.9275	0.7044	0.1141	0.0818	0.4771
Pb	Byssus	0.4558	0.4118	1.4579	0.5843	0.2653
	TST	0.7202	NC	0.1036	0.1007	NC
	Shell	0.3519	0.3448	1.4182	0.7165	0.2287
Zn	Byssus	0.6309	0.3653	2.8325	1.5489	0.9196
	TST	0.736	NC	1.5321	1.0367	NC
	Shell	0.4846	0.4543	0.4734	0.1904	0.1007

Note: TST = total soft tissue, NA = reading is not available because mussels samples had died.

Cu and Zn in the clam *Mercenaria mercenaria* (Behrens & Duedall, 1981), Ag, Co and Ni in the oyster *Isognomon isognomon* (Hedouin *et al*., 2011). When a comparison was made between weeks 6 and 10, more metals in week 6 were far from reaching the concentrations measured in the initial population (T_0) due to the accumulation being dependent on the transplantation period (Hedouin *et al*., 2011). This indicated that the eliminations of the metals were not complete throughout the 10-week period of transplantation for *P. viridis*. Other studies (e.g., Andres *et al*., 1999; Hickey *et al*., 1995; Martincic *et al*., 1992; Wallner-Kersanach *et al*., 2000) found that the equilibration of the trace metals in bivalves with their environment could range between 30 days to 77 days and even longer for some other species. Such a wide range in the equilibration times among the species could be due to previous exposure histories, different

life stages of bivalve, associated metabolic activities with adjustments to a new environment, temperature changes, and food availability (Burt *et al*., 2007).

On the other hand, for the mussels transplanted from Kg. Pasir Puteh to Sg. Melayu, the metal levels in all the parts showed decreases in heavy metal levels. During the transplantation, the accumulation rate and the elimination rate were the highest at T_2, which later decreased from T_2 to T_{10}. According to Yap *et al*. (2003a), the rates of accumulation and elimination were higher during the initial period due to detoxification process. After that, the mussels had slower rates of accumulation and elimination after 2 weeks, which could be caused by some tightly bound compartments, such as metallothionein and lysosomes for Pb. According to Amiard *et al*. (2006), Marigómez *et al*. (2002) and Viarengo *et al*. (2003), metallothionein plays an important role in the elimination of metals in mussels. The high rates of accumulation and elimination were also found in byssus at both sites, indicating that this organ could act as an excretion route for Pb, as reported by Yap *et al*. (2003b,c). The low EF value in the metals (Table 7) showed that *P. viridis* was capable of eliminating these metals. The EF for 6 weeks and 10 weeks showed that byssus was good at eliminating Cd and Ni, while shell was capable of eliminating Cu and Pb in the shortest time. The EF value indicated that Ni was the metal which was eliminated the fastest at week 6, while Cd was eliminated the fastest in week 10. However, Cu was eliminated the slowest in both week 6 and week 10.

Levels of Metals in the Different Parts of P. viridis *after the Transplantation*

Similar patterns of accumulation and elimination of metals found in both total soft tissue and byssus suggested that the metal levels in these parts of *P. viridis* were mainly due to metabolic pathways rather than the direct contact with the surrounding seawater. This finding is on par with the results reported by Ikuta (1986a), Szefer (1999) and Yap *et al*. (2003b) for the byssus of *M. edulis* and *P. viridis,* especially the high EF values in Fe and Zn for byssus. When the heavy metal levels in the soft tissue were high, these metals would be transferred metabolically to the byssus (Ikuta, 1986b; Yap *et al*., 2003b) due to the metal level regulation in the soft tissue. Regulation and sequestration are important mechanisms (Phillips & Rainbow, 1989; Rainbow, 1997; Phillips, 1995) to minimize the harmful effects of high levels of these metals. The high metal accumulation found in the byssus could also be due to the material for byssus formation. The byssus was secreted from a byssal gland in the foot and is composed of a protein component, collagen. These protein components contain some potential metal binding sites, largely composed of glycine and proline amino acid residues (Szefer *et al*., 1999; Yap *et al*., 2003b).

The highest level of Pb was accumulated in the transplanted mussel shell. This result is comparable with those of Yap *et al*. (2003a) and it could be due to the chemical composition of the shells. The present study revealed that the shells of bivalves were accumulative of non-essential metals such as Cd, Ni and Pb. The results obtained by Yap *et al*. (2004) showed that the metals found in the shells of gastropod could be due to the substitution of the calcium ions in the crystalline phase of the shell or were associated with the organic matrix of the shell. In particular, shells accumulated higher concentrations of metals initially due to surface adsorption. According to Wang (2010), uptake of perfluorinated compounds by shell starts with adsorption

or passive deposition of the target chemicals to the shell organic matrix, and this is followed by a biomineralisation process. Therefore, the contaminants bound to the organic matrix in the shell microstructure were sequestrated and hard to release. Meanwhile, the high levels of metals accumulated in the total soft tissue could also be due to the synthesis of metallothionein (Yap *et al*., 2004). This is the reason why the mussels eaten could be a source of metals and why mussels can survive and accumulate high level of metals in their soft tissues in highly contaminate sites.

High levels of Cu and Zn accumulations were found in the TST of the transplanted mussels. These results are similar with those of Yap *et al*. (2011) for *Anadara granosa*. The soft tissues of bivalves were found to be able to induce metallothionein-like protein production at high metal concentrations (Chan *et al*., 2002). The secretion of metallothionein was to counteract metal toxicity at high metal concentrations (Phillips & Rainbow, 1993). MT is generally accepted to be involved in the regulation of essential metals such as Cu and Zn for cell growth and development, although their functions are still not fully understood (Mackay *et al*., 1993). On the other hand, Cu and Zn are also known as essential metals for metabolic functions (Mackay *et al*., 1993) and they can be regulated in bivalves (Yap *et al*., 2003a). This could explain why the concentrations of Cu and Zn were high in the TST.

CONCLUSION

The data reported in this paper clearly demonstrated that Kg. Pasir Puteh was a more polluted site than Sg. Melayu based on the sediment and *P. viridi*s samples for all the six metals. Among the six metals, Zn was the one which was accumulated at the fastest rate, while Cd was the slowest. As for elimination, Ni was the fastest at week 6, while Cd was the fastest at week 10 and Cu was the one with the slowest elimination rate at both weeks 6 and 10. The byssus of *P. viridi*s could be used as a good monitor for Cd, Ni, Pb and Zn, while shell could be used to monitor Cu, Ni and Pb. However, to get more accurate levels of heavy metal pollutions in coastal waters, studies with longer transplantation periods should be carried out since some of the metals needed longer time periods for elimination and to reach equilibrium levels.

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