

Concentrations of Heavy Metals (Cu, Cd, Zn and Ni) and PAHs in *Perna viridis* Collected from Seaport and Non-seaport Waters in the Straits of Johore

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Received: 15 January 2012 / Accepted: 20 September 2012 / Published online: 2 October 2012
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Abstract In this study, the ranges of pollutants found in the soft tissues of *Perna viridis* collected from Kg. Masai and Kg. Sg. Melayu, both located in the Straits of Johore, were 0.85–1.58 µg/g dry weight (dw) for Cd, 5.52–12.2 µg/g dw for Cu, 5.66–8.93 µg/g dw for Ni and 63.4–72.3 µg/g dw for Zn, and 36.4–244 ng/g dry weight for ΣPAHs. Significantly ($p < 0.05$) higher concentrations of Cd, Cu, Ni, Zn and ΣPAHs in the mussels were found in the water of a seaport site at Kg. Masai than a non-seaport site at Kg. Sg. Melayu population. The ratios of low molecular weight/high molecular weight hydrocarbons (2.94–3.42) and fluoranthene/pyrene (0.43–0.45) in mussels from both sites indicated the origin of the PAHs to be mainly petrogenic. This study has demonstrated the utility of using the soft tissues of *P. viridis* as a biomonitor of PAH contamination and bioavailability in the coastal waters of Peninsular Malaysia.

Keywords Heavy metals · Polycyclic aromatic hydrocarbons · *Perna viridis*

The Straits of Malacca, including the Straits of Johore, receive a broad range of anthropogenic micropollutants, both inorganic and organic, from various local land- and marine-based sources (Law 1994; Amin et al. 2009).

Organic micropollutants have also been transported from other regions via the atmosphere and global ocean transport systems (Iwata et al. 1993). Therefore, environmental monitoring studies are important in order to know the pollutant levels in the aquatic ecosystem.

Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants of the marine environment. PAHs have carcinogenic and mutagenic properties (Lehr and Jerina 1977), and are generated by three main processes, namely: (1) combustion of organic matter at very high temperatures; (2) release of petroleum; or (3) diagenetic processes (degradation of organic matter) (Neff 1979). The mixture of PAHs that is generated by each process can serve as a fingerprint. Therefore, by studying the composition of the PAH mixture, it is possible to determine the process that generated the PAHs. PAHs in general are characterized by the occurrence of organic compounds having a wide range of molecular weights, while petroleum hydrocarbons are dominated by PAHs with lower molecular weights (Neff 1979).

Marine mussels have been proposed as being useful for biomonitoring trace levels of toxic contaminants in coastal waters due to their wide distribution, sessile lifestyle, ease of sampling, tolerance to a considerable range of salinities, resistance to stress and high accumulation of a wide range of chemicals (Goldberg et al. 1978). In particular, the green-lipped mussel, *Perna viridis* has been utilized as a biomonitor throughout the Indo-Pacific region under the Asia-Pacific Mussel Watch Program (Tanabe et al. 2000).

Several studies on heavy metals (Yap et al. 2003a; 2004) and PAHs (Zakaria et al. 2000; Tsutsumi et al. 2002; Isobe et al. 2007) have been conducted on *P. viridis* in Malaysia. However, a comparison of inorganic and organic pollutants between seaport and non-seaport coastal waters has not yet been reported in the literature. Therefore, the objectives of this study were to analyze and compare the concentrations

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of heavy metals and PAHs in seaport and non-seaport waters in the Straits of Johore.

Materials and Methods

Mussels with shell lengths of about 110 mm were collected from Kg. Masai (seaport site) and Kg. Sg. Melayu (non-seaport site) (Fig. 1, Table 1) on 11 August 2004. The samples were kept in clean plastic bags and put into an ice compartment at $<10^{\circ}\text{C}$ until transported to Universiti Putra Malaysia (UPM). After measuring the shell of each mussel, the soft tissues were dissected by removing the byssus and the shell.

For metal analysis, 20 individuals from each population were selected and analyzed individually. After reaching constant dry weight in an oven (105°C), the dried tissues were digested in 10 mL concentrated HNO_3 (Analar grade, BDH 69 % purity). They were initially placed in a hot-block digester first at a low temperature for 1 h (h) and then were fully digested at a high temperature (140°C) for at least 3 h (Yap et al. 2003b). The digested samples were diluted to 40 mL with double-distilled water (DDW). After filtration, the heavy metal levels were determined by using an air-acetylene flame atomic absorption spectrophotometer (Perkin-Elmer Model Analyst 800, Bridgeport Avenue Shelton, USA). The data are presented in $\mu\text{g/g}$ dry weight.

For PAH analysis, the soft tissues of 25 individuals from each population were homogenized and stored at -10°C until analysis. The procedures for homogenization, extraction and column chromatography followed those described

by Takada et al. (1994) and Tsutsumi et al. (2002). The identification and quantification of 18 PAH compounds were achieved using the ChemStation software (Texas, USA) based on matching their retention times with those of a mixture of PAH standards. The 18 individual PAH compounds identified in this study were: dibenzothiophene (Dibenz), phenanthrene (Phen), anthracene (Anth), 3-methylphenanthrene (3-MP), 2-methylphenanthrene (2-MP), 2-methylanthracene (2-MA), 9-methylphenanthrene (9-MP), 1-methylphenanthrene (1-MP), fluoranthene (Fluo), pyrene (Pyr), 1-methylpyrene (1-MePy), chrysene (Chy), benzo[a]anthracene B(a)A, benzo[k]fluoranthene B(k)F, benzo[e]acephenanthrylene B(e)A, benzo[e]pyrene B(e)P, benzo[a]pyrene B(a)P and dibenzo[a,h]anthracene D(a,h)A.

An aliquot of 1 g of each of the homogenized soft tissue categories was weighed on a pre-weighed aluminum pan and dried for at least 24 h at 65°C to a constant dry weight. The percentage of dry weight was determined by calculating the ratio of tissue weights after and before placement in the oven.

For lipid determination, the method described by Bligh and Dyer (1959) was used whereby the lipid content was gravimetrically determined. A total of 25 individuals of mussels from each population was used to determine the lipid content. In short, 5 g of sample were mixed with 15 mL of chloroform:methanol (1:2, v/v). The mixture was homogenized for 2 min in a Sorvall Omnimixer homogenizer, centrifuged (10 min, 3000 rpm) and filtered. The residue was rehomogenized with 5 mL of chloroform, centrifuged (10 min, 3000 rpm), filtered and collected together with the previous filtrate. This filtrate was mixed

Fig. 1 Map showing the sampling sites of *P. viridis* in the Straits of Johore

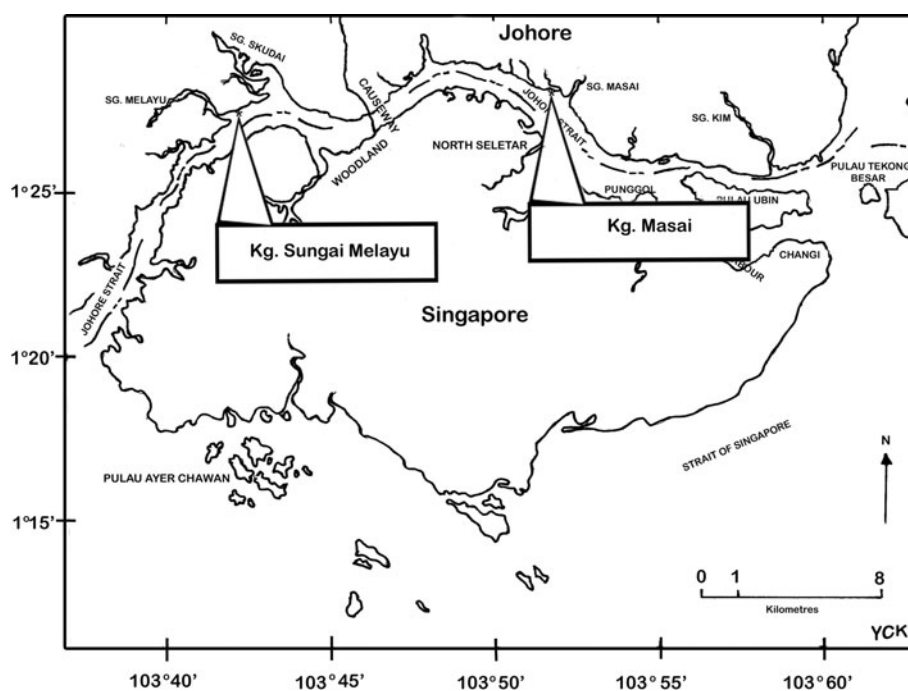


Table 1 The site descriptions of mussel populations in Kg. Masai and Kg. Sg. Melayu

Sampling site	Kg. Masai	Kg. Sg. Melayu
GPS reading	N01°27.910' E103°51.772'	N01°27.264' E103°42.125'
Time of sampling	2.52 p.m.	9.45 a.m.
Weather	Sunny, humid and hazy	Sunny, humid and hazy
Location description	Mussels culturing using buoys. Adjacent to seaport (Pasir Gudang Port)	Mussels culturing using buoys
Water physico chemical parameters		
Temperature (°C)	30.1	29.3
Water salinity (PSU)	26.8	23.4
Conductivity (µs/cm)	45085	37039
pH	8.22	7.54

with 5 mL of distilled water and shaken vigorously. The final biphasic system was allowed to separate by centrifugation (10 min, 3000 rpm). The upper aqueous phase was eliminated. The lower chloroformic phase was filtered through anhydrous sodium sulphate and collected. Lipid content was then determined gravimetrically after chloroform was evaporated using a rotary evaporator under vacuum followed by further drying under nitrogen.

For PAHs, quality control samples made previously from the standard solution of five deuterated PAH compounds were used to avoid possible contamination. Surrogate standards were used to examine the recovery of each sample and for quantifying the analytes. The range of the surrogate standard recovery was between 40 % and 120 %. P-Terphenyl- d_{14} acted as the Internal Injection Standard (IIS) for quantifying and recognizing errors of injection. In addition, a procedural blank was performed for every batch of samples as a contamination control.

For the quality control of heavy metal analysis, all glassware and equipment used were acid-washed. Procedural blanks and quality control samples made from standard solutions of Cd, Cu, Ni, and Zn were analyzed after every 5–10 samples in order to check for sample accuracy. The percentages of recoveries for the heavy metal analyses were acceptable at 80 %–110 %. In addition, the quality of the analytical method was checked with the Certified Reference Material for Dogfish (DOLT-3, National Research Council Canada) and the metal recoveries were satisfactory (80 %–100 %). The detection limits for Cd,

Cu, Ni, and Zn were 0.009, 0.01, 0.01, and 0.007 mg/L, respectively.

The data obtained were analyzed statistically by using the Statistical Package for the Social Sciences (SPSS for Windows, Release 10.0.5, Standard Version, Copyright© SPSS Inc., 1989–1999). The independent sample *t* test was applied to determine the significance of the difference at $p < 0.05$ between the two sampling sites.

Results and Discussion

The concentrations of heavy metals (Cd, Cu, Ni and Zn) in *P. viridis* from Kg. Masai were higher than those from Kg. Sg. Melayu (Table 2). Heavy metal concentrations in the total soft tissues of *P. viridis* from this study were compared with data reported in the literature. The present findings are within the range of or lower than those reported for polluted mussels from Kennedy Bay of Hong Kong (Nicholson and Szefer 2003), Thailand (Sukasem and Tabucanon 1993) and south east coastal waters of India (Senthilnathan et al. 1998), and comparable to the concentrations of Cu, Cd and Zn reported for *P. viridis* collected from throughout Peninsular Malaysia (Yap et al. 2003a).

The lipid content was significantly lower (0.22 %) in Kg. Masai than Kg. Sg. Melayu (Table 2). The large difference of lipid contents between Kg. Masai and Kg. Sg. Melayu could be attributable to multiple factors such as lifecycle, sex, variation of plankton in different seasons and temperature, which could influence physiological activities and metabolism (Li et al. 2007). The difference could be due to higher values of temperature, water salinity, conductivity and pH at Kg. Masai when compared to Kg. Sg. Melayu (Table 1). However, only further studies can confirm the direct relationship between lower lipid contents and differences of the above water parameters between the two sites.

Mussels from the Kg. Masai site had significantly ($p < 0.05$) higher concentrations of total PAHs (summation of 18 PAHs) than the mussels from the Kg. Sg. Melayu site (Table 2). It is unlikely that the large difference in lipid content be the main reason for the observed differences in PAHs levels between the two sites because a significant positive relationship (0.79, $p < 0.01$) between PAHs and lipid contents was reported for *P. viridis* (Yap et al. 2010). Since a low lipid content (0.22 %) was found in Kg. Masai with elevated PAHs concentrations, the PAHs was most possibly related the closer proximity of the Kg. Masai mussels to local sources of pollution. For a better characterization of the PAH mixture and possible sources, several PAH ratios were calculated, including LMW/HMW, MP/P, Fluo/Py and Phen/Anth, as presented in Table 2. Since the ratios of LMW/HMW are higher than 1.0, the PAHs identified in the populations of Kg. Sg. Melayu and Kg.

Table 2 Concentrations of heavy metals and PAHs in the green-lipped mussel *P. viridis* collected from Kg. Masai and Kg. Sg. Melayu, the Straits of Johore

	Unit	Kg. Masai	Kg. Sg. Melayu	<i>t</i> test
Cd	µg/g dry weight	1.58 ± 0.26	0.85 ± 0.24	<i>p</i> < 0.05
Cu	µg/g dry weight	12.2 ± 1.36	5.52 ± 0.55	<i>p</i> < 0.05
Ni	µg/g dry weight	8.93 ± 0.87	5.66 ± 0.48	<i>p</i> < 0.05
Zn	µg/g dry weight	72.3 ± 10.6	63.4 ± 9.69	<i>p</i> < 0.05
Wet weight	G	17.6 ± 0.57	15.0 ± 0.42	<i>p</i> > 0.05
Lipid content	%	0.22 ± 0.05	2.40 ± 0.80	<i>p</i> < 0.05
ΣPAHs	ng/g dry weight	244.1 ± 20.1	36.4 ± 11.9	<i>p</i> < 0.05
ΣPAHs	ng/g wet weight	169 ± 13.5	20.4 ± 3.22	<i>p</i> < 0.05
ΣPAHs	ng/g lipid weight	13810 ± 256	850 ± 65	<i>p</i> < 0.05
LMW/HMW	Ratio (unitless)	2.94	3.42	<i>p</i> < 0.05
MP/P	Ratio (unitless)	1.74	2.80	<i>p</i> < 0.05
Fluo/Py	Ratio (unitless)	0.43	0.45	<i>p</i> > 0.05
Phen/Ant	Ratio (unitless)	6.33	0.42	<i>p</i> < 0.05

Ratios of concentrations: LMW/HMW = Sum of the lower molecular weight PAHs versus Sum of the higher molecular weight PAHs; MP/P = Sum of the 3,2,9,1 methyl-phenanthrenes versus phenanthrene; Fluo/Py = fluoranthene versus pyrene; Phen/Ant = phenanthrene versus anthracene

ΣPAHs = Sum of concentration of dibenzothiophene, phenanthrene, anthracene, 3-methylphenanthrene, 2-methylphenanthrene, 2-methylanthracene, 9-methylphenanthrene, 1-methylphenanthrene, fluoranthene, pyrene, 1-methylpyrene, chrysene, benzo[a]anthracene, benzo[k]fluoranthene, benzo[e]acephenanthrylene, benzo[e]pyrene, benzo[a]pyrene, dibenzo[a,h]anthracene

Masai were dominated by the presence of LMW PAHs, suggesting petrogenic and petroleum related compounds as the main PAH sources (Kennish 1977). These results are consistent with those of Tsutsumi et al. (2002) who reported that the sources of PAHs in the sediments in Malaysia were mainly petrogenic. Therefore, although the Kg. Masai population had significantly (*p* < 0.05) higher ΣPAH levels than the Kg. Sg. Melayu population, in both populations the PAHs originated mainly from petrogenic sources.

The presence of LMW PAHs in both mussel populations could be due to the higher bioavailability of LMW PAHs in the coastal waters of the two sampling sites. This could be due to the LMW PAHs in the coastal waters were being more susceptible to microbial degradation and volatilization and dissolution into the water column (Zakaria et al. 2000), thus, becoming more bioavailable to mussels. Consequently, mussels were more enriched with LMW PAHs in relation to their environments. The present finding agreed with those

reported by Isobe et al. (2007) in which they reported >1.0 ratios of LMW/HMW in 33 out of 34 mussel populations from seven Asian countries. Skarpheðinsdottir et al. (2007) also reported that blue mussels collected close to harbours had relatively higher levels of LMW PAHs. Baumard et al. (1999) found that the blue mussels sampled in the western Baltic Sea were enriched in LMW PAHs. Thus, the presence of higher LMW PAHs in marine mussels is due to the marine mussels are known to be selectively uptake LMW PAHs (Farrington et al. 1983; O'Connor 1991).

Mussels are known to be filter-feeding organisms which they can filter large volumes of water (Yap et al. 2003c) and absorb xenobiotics by two pathways: direct absorption of compounds present in the water phase through the gills, and; indirect absorption of compounds adsorbed onto small-grained particles passing through the digestive system (Baumard et al. 1998). Mussels are exposed to both dissolved and particulate forms of hydrocarbons present in the water column. The partitioning of PAHs into either dissolved or particulate forms is due to their specific water solubilities. Hence, the mussels can directly absorb lower weight PAHs from the water that they filter. Heavier weight hydrocarbons (four or more rings) are mainly obtained by ingestion of particulate matter into the digestive system (Broman et al. 1990, 1999; Widdows and Donkin 1992; Piccardo et al. 2001).

The concentrations of the sum of four methyl-phenanthrene compounds to phenanthrene (ΣMP/P) was greater than 2 for petroleum while it was lower than 1 for pyrolytic sources of PAHs in individual mussels (Garrigues et al. 1987). Based on the guideline for MP/P, the MP/P ratio of 1.74 for the Kg. Masai population fell between petrogenic and pyrolytic origins. The Kg. Sg. Melayu population had a MP/P ratio of 2.8, suggesting petrogenic origins. Indeed, the ratio of fluoranthene to pyrene (Fluo/Py) <1 for both mussel populations, indicated petrogenic sources too (Table 2). However, individuals which exhibited phenanthrene/anthracene (Phen/Ant) ratios <10 for both mussel populations indicated a pyrogenic origin. It should be noted that pyrolytic PAHs are mainly anthropogenic, which could be due to industrial activities. Since there was no petroleum industry observed here, with the only industrial activities being mussel processing, PAH transport via atmospheric aerosols and particles could be considered as one of the pyrogenic inputs.

The PAH levels in the total soft tissues of *P. viridis* from this study are comparable with those of earlier studies on polluted and clean sites using other marine mussel species. For example, Broman et al. (1990) reported the mean level of a total of 19 PAHs in the tissues of *Mytilus edulis* sampled from the Swedish coast of the Baltic Sea to be 443 ng/g dry wt. (40–1330 ng/g dry wt.). Baumard et al. (1998) analyzed blue mussels sampled near the German

coast in the southwest Baltic Sea near point sources of pollution. The sum of 18 PAHs ranged between 90 and 3,900 ng/g dry wt., a much wider range than found in our study (36.4–244 ng/g dry wt.). This indicates that the PAH contamination in the Straits of Johore is not as great as in those sites from the Baltic Sea. Skarpheðinsdóttir et al. (2007) reported total 32 PAH levels as 10–11,670 ng/g dry wt. in *Mytilus* spp. from Nordic coastal waters. Baumard et al. (1999) studied levels of 14 PAHs in *M. galloprovincialis* from 10 remote and polluted sites of the Mediterranean coasts of Spain and France, and found them to range from 25 to 337 ng/g dry wt. Hence, in general, our PAH levels (20.4–169 ng/g) seem to correspond to the less polluted areas of Europe. However, it should be noted that a comparison of the concentrations of heavy metals and PAHs in *P. viridis* between studies should be done with caution. Many factors could affect the tissue pollution levels, such as intertidal impacts, seasonal variations, spawning conditions and lipid levels (Gosling 1992).

The higher concentrations of heavy metals and PAHs that were found in Kg. Masai than in Kg. Sg. Melayu could be related to its vicinity to a port, Pasir Gudang, and hence, industrial effluents (Yap et al. 2006). Besides, the high levels of pollutants found in the mussels from Kg. Masai could also be due to domestic wastes through riverine inputs such as from the Tebrau, Skudai and Segget rivers, which all empty into the narrow Straits of Johore (Yap et al. 2006). These inputs would carry heavy metals and PAHs, which could put the local marine ecosystem at risk.

Based on ratios of LMW/HMW and Fluo/Pyr, it was found that the PAH in the Kg. Masai and Kg. Sg. Melayu mussel populations mainly originated from a petrogenic source. However, there were significantly ($p < 0.05$) higher concentrations of heavy metals and \sum PAHs in the total soft tissues of *P. viridis* collected from the coastal water of Kg. Masai than from Kg. Sg. Melayu. In general, the results also indicated that the soft tissues of *P. viridis* were useful for biomonitoring heavy metal and PAH contamination and bioavailability in the coastal waters of Peninsular Malaysia.

Acknowledgments The authors wish to acknowledge the financial support provided through the Research University Grant Scheme (RUGS) [vote no.: 9316800], provided by Universiti Putra Malaysia.

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