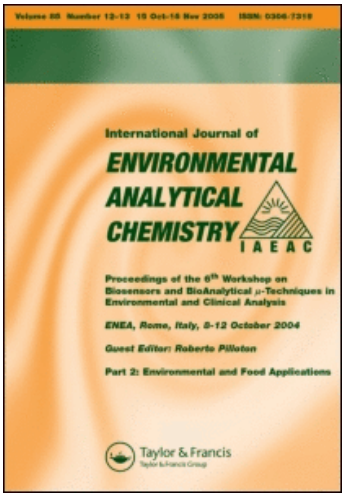

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Spatial distribution and sources of polycyclic aromatic hydrocarbons (PAHs) in green mussels (*Perna viridis*) from coastal areas of Peninsular Malaysia: implications for source identification of perylene

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Spatial distribution and sources of polycyclic aromatic hydrocarbons (PAHs) in green mussels (*Perna viridis*) from coastal areas of Peninsular Malaysia: implications for source identification of perylene

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Distribution of polycyclic aromatic hydrocarbons (PAHs) was determined in green mussels (*Perna viridis*) from various sites in coastal waters of Peninsular Malaysia between August 2004 and January 2007, in order to assess contamination by petroleum hydrocarbons. The range of $\sum$PAHs detected in mussels was from 766 to 110500 (ng/g lipid wt.). High concentrations of PAHs were found in mussel tissues collected near Penang Bridge. The ratios of methyl phenanthrenes to phenanthrene ($\sum$MP/P ratio) for Penang, Kg. Pasir Puteh and Tebing Runtuh (Johore Straits) were greater than 2, indicating extensive input of petrogenic PAHs. The results indicated that male individuals elevated more considerable concentrations of PAHs in their soft tissues in comparison to female individuals. The results of independent sample T-test showed that there were no significant differences ($p > 0.05$) between male and female mussels analysed in the Pasir Panjang station. Negative significant correlations ($r = -0.890$, $p < 0.01$) and ($r = -0.0655$, $p < 0.05$), were found between weight and total of PAHs in female and male species, respectively. This indicated that body weight of each individual was not affected by the PAHs concentrations. The present study proposes the use of soft tissue of *Perna viridis* as a biomonitor of perylene bioavailability and contamination in coastal waters of Peninsular Malaysia.

Keywords: distribution; PAHs; *Perna viridis*; soft tissue; mussel watch; Peninsular Malaysia

1. Introduction

Malaysia is surrounded by one of the busiest waterways in the world, which is recognised as a heavy shipping traffic lane in Southeast Asia. The coastal zone of west and south

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Malaysia are heavily populated and lined by urban, industrial, agricultural areas and shipping ports. As a result, the coastal waters of this region receive a broad range of anthropogenic organic micropollutants from various land-based and marine-based sources. Organic micropollutants are also transported from other regions via atmospheric and global ocean transport systems [1]. In Malaysia, most of the human activities are concentrated within the coastal areas. The west coast of Peninsular Malaysia accommodates over 20 rivers which flow into the Straits of Malacca. These rivers flow through heavily populated areas, and some of the most congested industrial estates in the country, carrying with them multiple diffuse pollution inputs. The three main ports are also situated in the west coast of Peninsular Malaysia, namely Penang, Klang and Johore. This fact may result in a wide range of pollution especially the anthropogenic organic micropollutants from various sources such as industrial and municipal effluents, agricultural effluent and oil spills into the marine system. Therefore, one of the primary aims of environmental quality studies is to understand the impacts of anthropogenic compounds such as organic micropollutants on the ecosystem, in order to minimise or prevent adverse effects.

Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants throughout the environment. PAHs can be generated by three main processes: (1) combustion at a very high temperature of organic matter; (2) release of petroleum; or (3) digenetic processes (degradation of the organic matter) [2]. Pyrogenic aromatic hydrocarbons are characterised by the occurrence of PAHs of a wide range of molecular weights, while petroleum hydrocarbons are dominated by the lowest molecular weight of PAHs [2]. PAHs have carcinogenic and mutagenic properties and due to their hydrophobic characteristic, these organic compounds tend to rapidly absorbed to particles and fat tissue of filtrating organisms such as oysters and mussels. Therefore, it is very important to assess PAHs pollutant levels and their temporal changes in a given coastal marine zone, and to compare these levels and changes among different zones including their sources.

Mussels have been proposed as an excellent biomonitor for monitoring trace toxic contaminant levels in coastal waters due to their wide distribution, sedentary lifestyle, easy sampling, tolerance to a considerable range of salinity and pollutants, resistance to stress and high accumulation of a wide range of chemicals. In particular, the green-lipped mussel, *Perna viridis* has been utilised as a biomonitor throughout the Indo-Pacific region and has been successfully used in the Mussel Watch Programme. The Asia-Pacific Mussel Watch Programme (APMW) started in 1994, under the umbrella of the International Mussel Watch-Asia Pacific Phase, a project that mainly involves coastal monitoring using sentinel organisms such as mussels as biomonitor in ascertaining the quality of coastal waters in the Asia-Pacific region [3]. The 'Mussel Watch' approach focused on the use of total soft tissues (STs) of mussels after removing the byssus (BYS) and the shell as a quantitative indicator to reflect the organic contaminations in the coastal areas [4]. Several works have been conducted on the biomonitoring of organic pollutants and heavy metals using *Perna viridis* in Malaysia [5–13].

The first objective of this present study is to determine the PAHs distribution and bioavailability of perylene, in the total soft tissues of *P. viridis* collected from different geographical populations in coastal areas of Peninsular Malaysia. The second objective is to study levels of target pollutants in female and male tissues and to identify the sources of PAHs. It is expected that the results can provide a basis of comparison for future studies to elucidate temporal changes in pollution levels. Evaluation of the significance of the PAHs levels observed in this study will provide an assessment of risks to human consumption of the green mussels in Southeast Asia in general and in Malaysia in particular.

2. Experimental

2.1 *Sampling and sampling preparation*

More than 20 individuals of *P. viridis*, ranging in size from 42 to 110 mm, were collected from each sampling site between August 2004 and January 2007. The detailed description of the locations and sampling sites are shown in Table 1 and Figure 1. At each sampling site, the shell surface of *P. viridis* was cleaned of encrusting organisms such as barnacles and bryzoa. Samples were kept in clean Zipped-Locked® bags and placed in dry ice, transported to the laboratory and stored frozen until further analysis. Briefly, the mussel samples were thawed at room temperature on a clean tissue paper with the posterior margins downwards to drain away the excess water. After measuring the shell length and weight, the soft tissues from each mussel were dissected using clean scalpel and the byssus and the shell removed. The soft tissues were homogenised and stored at −10°C until analysis.

2.2 *Chemical and reagent*

The organic solvents namely hexane and dichloromethane (Merck, Germany) were purified by distillation to remove interfering isomers before use. HPLC grade methanol (MeOH) and acetone were used for rinsing glassware while distilled isooctane was used for the make-up solution in gas chromatography mass spectrometry (GC-MS) analysis. Standard solution of PAHs were purchased from (Sigma Chemical Company, St. Louis, Missouri, USA) and spiked as internal standards. The deuterated PAHs consist of naphthalene-d_8, anthracene-d_{10}, benzo (a) anthracene-d_{12}, perylene-d_{12} and p-terphenyl-d_{14} were used as Surrogate Internal Standard mixture (SIS). PAHs concentrations were recovery corrected using the spiked surrogates. P-terphenyl-d_{14} was used as Internal Injection Standard (IIS) and spiked to the sample extract prior to GC-MS analysis for

Table 1. Sampling dates, number of samples analysed (*n*), shell length (mm) of mussels and descriptions of sampling sites of *P. viridis* collected from the west and south coast of Peninsular Malaysia.

Locations	Sampling date	*n*	Shell length (mm)	Latitude (°N)	Longitude (°E)	Site description
1. Tanjung Dawai, Kedah	250706	26	55–95	5°43′	100°19′	Fishing village and aquacultural areas
2. Penang Bridge, Penang	280106	29	54–96	5°20′	100°21′	Industrial and vehicular areas
3. Pasir Panjang, Port Dikson	90107	90	47–110	2°31′	101°43′	Recreational and agricultural areas
4. Tebing Runtuh, Johore straits	110804	25	55–78	2°10′	102°13′	Industrial and vehicular areas
5. Sebatu, Malacca	140904	25	44–89	2°7′	102°23′	Agricultural and urban areas
6. Kampong Masai, Johore Bahru	110804	25	42–88	1°25′	103°50′	Industrial and vehicular areas
7. Kg. Pasir Puteh, Johore Bahru	270806	44	44–90	1°22′	104°1′	Industrial and vehicular areas

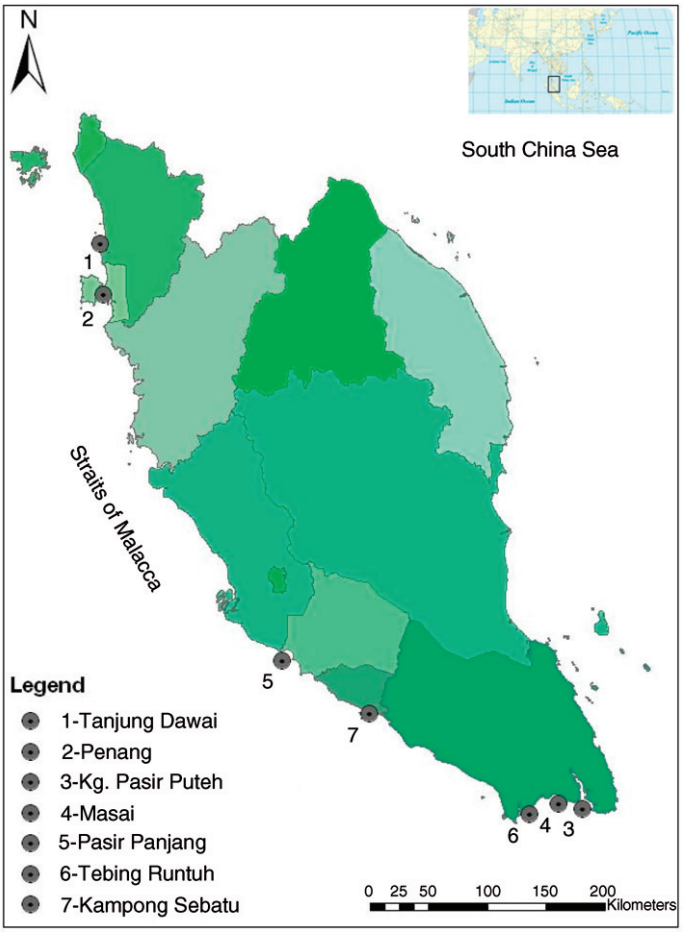

Figure 1. Map of Peninsular Malaysia showing sampling sites of (*P. viridis*) from the west coast of Malaysia.

quantitation of all PAHs analysed in this study and recognised the error of injection. Silica gel (mesh size 60–200 with particle size 0.063–0.2 mm) (Merck, Darmstadt, Germany) for column chromatography was purchased from Aldrich Chemical Company and activated by baking at 380°C for 3 hours to remove organic contaminants and then baked at 200°C for additional 12 hours to fully activate the silica gel.

2.3 *PAHs analysis*

The procedures for chemical analysis were carried out using the methods previously described in Zakaria *et al*. [6] and Nakada *et al*. [14]. Briefly, the soft tissues were thawed, pooled and homogenised with 8000–10000 rpm for 12 min using the homogeniser (*NISSEI AM*-7, Japan, (0–200) *rpm* × 100). The soft tissues were dried with anhydrous sodium sulphate and 50 µL of 10 ppm deuterated surrogates were directly spiked onto the sample. The sample (~15 g) was Soxhlet extracted with dichloromethane for 8 h, sample extracts were passed through a 5% H_2O deactivated silica gel column (0.9 cm i.d × 9 cm height) to

remove polar components and then fractionated using a fully activated silica gel (0.47 cm i.d × 18 cm height) to obtain three fractions. PAHs with (3–7) benzene rings were eluted with hexane/dichloromethane (3 : 1, v/v). The target compound was concentrated to a few milliliters using rotary evaporator followed by gentle stream of pure N_2 gas. PAHs fraction was analysed by gas chromatography coupled to mass spectrometry. An HP 6890 series II GC coupled with a Hewlett-Packard 5973, a quadrupole mass selective detector (MSD) was used for PAHs analysis and operated under the Selected Ion Monitoring mode (SIM). The capillary column stationary phase used was an HP-5 (30 m fused silica, 0.25 mm i.d., 0.25 μm film thickness). The carrier gas was helium on a constant pressure at 100 kg/cm^2. GC-MS operating condition was 70 ev ionisation potential with the source at 200°C and electron multiplier voltage at ~2000 ev. The injection port was maintained at 300°C and the sample was injected in splitless mode followed by purge 1 min after the column temperature was set at 70°C for 2 min, then programmed at 30° C/min to 150°C, 4°C/min to 310°C and held for 10 min. Identification and quantification of 18 PAHs compounds were achieved using ChemStation® software based on matching their retention time with a mixture of PAHs standards. The 18 PAHs 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 (Py), 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, dibenzo[a, h]anthracene D(a, h)A.

2.4 *Dry weight and lipid determination*

An aliquot 1 g of each category of homogenate soft tissues were placed into pre-weighed aluminum pans and dried for at least 24 hours at 65°C to a constant dry weight. The per cent dry weight was determined with calculating the ratio of tissue weights after and before placement in the oven. Lipid content of each species was gravimetrically determined which reported as previously by Bligh and Dyer [15], in order to give the results on lipid weight basis.

2.5 *Quality control*

Quality control of samples were processed to avoid possible contamination and made from standard solution of five deuterated PAH compounds that previously mentioned. The range of surrogate standard recovery was between 40 to 120%. Surrogate standards were used for examining the recovery of each sample and quantifying the analytes. P-terphenyl-d_{14} as Internal Injection Standard (IIS) was used for quantitation and recognising error of injection. In addition, procedural blank was also performed in every batch of samples for pollution control.

3. Statistical analysis

The data obtained was statistically analysed by using the Statistical Package for the Social Sciences (SPSS). Independent samples T-test was applied to determine significant difference between male and female individual analysed.

4. Results and discussion

4.1 *Distribution and sources of PAHs*

Petroleum hydrocarbons were detected in all homogenised soft tissue of mussel samples from the coastal waters of Peninsular Malaysia and their concentrations ranged from 766 to 110500 (ng/g lipid wt.) (Table 2). Concentrations of PAHs in green mussels (*P. viridis*) were normalised to lipid weight basis although wet and dry weights were also measured for comparison purposes. As expected, high pollution levels of PAHs were found in mussels near Penang Bridge (110500 ng/g lipid wt.) which indicating a wide range of anthropogenic PAHs from various sources such as industrial and municipal effluent, agriculture effluent and oil spills into this polluted marine system. Firstly, Penang is located in west Peninsular Malaysia that is considered as one of the most populous and industrious urban locations in Malaysia. The area is surrounded by huge population from both the Penang Island and the mainland area of Seberang Prai which are linked by the Penang Bridge. Consequently the high value of PAHs could have originated from urbanisation, motorisation, heavy industrial activities and international shipping via port activities. Secondly, the coastal area of Penang Bridge located in the west coast is facing the Straits of Malacca where it has heavy supertankers traffic that carry crude oil from the Middle East *en route* to Northeast Asian countries. In addition, there is no oil refinery in the vicinity of Penang Bridge and thus it can be concluded that the oil pollution sources may have originated from shipping activities and industrial activities in the catchment although further studies has to be conducted to confirm this suggestion. Lubricating oil signature was found around Penang Bridge mussels indicating Middle East origins and the finding is consistent with that mentioned in Zakaria *et al*. [16]. The compounds of lubricating oil residues may have reached into the marine environment through street run-off and accumulated in soft tissues of the green mussels collected near the Penang Bridge. This finding was also further supported by results from alkanes and hopanes analysis reported by Awang Jambi *et al*. [17], which clearly demonstrated the utility of hopanes (triterpanes) specifically the C_{29}/C_{30} and $\sum C_{31}–C_{35}/C_{30}$ ratios. The ratios were utilised as molecular tools in green mussels of Penang Bridge that showed the oil contamination in green mussels has originated from Middle East Crude Oil (MECO).

The mussels data in Table 2 clearly showed that concentrations of total PAHs were highest near Penang Bridge followed by Kg. Pasir Puteh (105000 ng/g lipid wt.). Kg. Pasir Puteh is a major industrial area in Malaysia. Moreover, Kg. Pasir Puteh, is located less than one kilometer away from Johor Port, one of the largest ports in Malaysia. Therefore, the observed elevated levels of PAHs at this site could have been due to anthropogenic activities including industrialisation, urban run-offs and multiple diffuse sources from a neighbouring country, Singapore. More data have to be collected in the area to support this hypothesis in the future.

Results from Table 2 also indicated that Kg. Masai and Pasir Panjang with total PAHs concentrations of (76800 ng/g lipid wt.) and (42800 ng/g lipid wt.) respectively, were relatively contaminated by PAHs while Sebatu was less contaminated by the PAHs (766 ng/g lipid wt.). These differences are due to that Kg. Masai is located near an industrial site and is very near to the city of Johore Bahru (southern Peninsular Malaysia). High PAHs levels might have been discharged from the effluents from the nearby domestic and industrial inputs. On the other hand, Pasir Panjang has an intense agricultural activity and there is a thriving fish mariculture in the area. As a result, relatively high concentrations of PAHs in mussels could be caused by a few possible sources such as soil

Table 2. Total PAH concentrations in green-lipped mussels (*P. viridis*) in different locations of Peninsular Malaysia.

	Wet weight (g)		Total PAHs concentrations[a] (ng/g)			Ratios			
Stations	Mean ± SD	Lipid content (%)	Wet weight	Dry weight	Lipid weight	LMW/HMW[b]	∑MP/P[c]	Fluo/Py[d]	Phen/Anth[e]
1. Tanjung Dawai	15.0 ± 1.31	0.96	121	163	12600	2.19	1.73	0.55	10.41
2. Penang Bridge	15.0 ± 1.52	1.53	1690	2060	110500	3.91	8.07	0.22	0.21
3. Pasir Panjang	15.0 ± 1.52	2.00	856	2054	42800	3.89	1.86	1.12	0.01
4. Tebing Runtuh	15.0 ± 0.42	2.40	20	36	850	3.42	2.80	0.45	0.42
5. Sebatu	18.8 ± 0.57	1.63	12	17	766	1.09	1.36	0.48	5.96
6. Kampong Masai	17.6 ± 0.57	0.22	169	244	76800	2.94	1.74	0.43	6.33
7. Kg. Pasir Puteh	15.0 ± 1.26	0.92	966	1200	105000	1.30	2.69	0.38	4.42

Notes: [a]Total PAHs concentrations: Sum of concentrations 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.
[b]LMW/HMW: Sum of concentrations of dibenzothiophene to fluoranthene relative to sum of concentrations of pyrene to dibenzo[a,h]anthracene.
[c]∑MP/P: Ratio of sum of concentrations of 3,2,9,1 methyl-phenanthrenes relative to phenanthrene concentration.
[d]Fluo/Py: Ratio of fluoranthene to pyrene.
[e]Phen/Anth: Ratio of phenanthrene to anthracene.

run-off, sewage discharged from this area into sea water and long-range transport. Further studies need to be conducted to afford explanation of high levels of PAHs in Pasir Panjang area since visual inspection of the area seems to be of remote and clean area. This phenomenon is further deliberated in this paper under source identification of PAHs in the next few sections.

It is interesting to note that there was a depletion of PAHs concentration for mussels collected in Sebatu station. A possible reason for low contamination of PAHs in this area is that Sebatu is a rural fishing village. However, in the hinterland areas, agricultural activities and pockets of urbanisation can also be found. Since those activities are located beyond the perimeters of Sebatu, the influence of PAHs pollution may be minimised.

Coastal waters of Tanjung Dawai have direct linked to Kuala Perlis as a PAHs contaminated site [18]. Even though this area was not so industrialised but is near to the ferry station to Langkawi (a very popular tourist destination) in a small enclosed bay with a narrow mouth to the open sea. As such, the water exchange with open waters was relatively slow and limited, and oil pollution caused by the ferry and ship traffic could have been one of the factors contributing to the elevated levels of PAHs in this station.

PAHs fingerprints showed dominance of lower molecular weight hydrocarbons in all samples especially in mussels collected near the Penang Bridge. Therefore, it can be suggested mussels receive more petrogenic PAHs as compared to that of pyrogenic origin. Also, mussel PAHs burden was characterised by the negligible occurrence of high molecular weight (HMW) compounds. The explanation could be that low molecular weight (LMW) PAHs are more susceptible to microbial degradation and volatilisation and dissolution into the water column [16]. Mussels are enriched in LMW PAHs compared to the HMW PAHs in relation to the sediment. This fact describes the mechanism that mussels as filter-feeder organism which can filter large volume of water and absorb *xenobiotics* from two pathways: the direct one is the absorption of compounds present in the water phase through the gills, and the indirect one is the absorption of *xenobiotics* adsorbed on the small grain size fraction of particles through the digestive system [19]. They are exposed to both dissolved and particulate forms of hydrocarbons present in the water column. On the other hand, PAHs are hydrophobic compounds with very low water solubilities and their concentrations in the water column is very low (100–200 ng/g) [20]. Besides, the partitioning of PAHs into either dissolved or particulate forms is due to their specific water solubility. Thus, mussels can directly absorb lower weight PAHs through interstitial filtrated water, while HMW hydrocarbons (four or more rings) are mainly ingested in particle form through the digestive system [19,21].

In this study, to better characterise PAH distribution, some diagnostic parent PAH ratios such as LMW/HMW, $\sum$MP/P, Fluo/Py and Phen/Anth was used as previously described by Budzinski *et al*. [22]. Some characteristic values of these indices are given in Table 2. Among tri-aromatic isomers, phenanthrene (Phen) is more thermodynamically stable than anthracene (Anth). Pyrolysis of organic matter at very high temperature generates PAHs, characterised by a low Phen/Anth ratio (<10), while the slow maturation of petroleum at lower temperatures leads to much larger values of the Phen/Anth ratio (>25) [23,24]. In the same way, the index Fluo/Py is also discriminating. Fluoranthene (Fluo) is less thermodynamically stable than pyrene (Py) and a predominance of fluoranthene over pyrene is characteristic of pyrogenic products, while in petroleum-derived PAHs, pyrene is more abundant than fluoranthene [22]. Therefore, for the Fluo/Py

ratios, values greater than 1 are attributed to pyrogenic origin whilst values less than 1 are related to petrogenic sources [25].

It can be concluded that these two Phen/Anth and Fluo/Py ratios in mussel samples have been developed in order to distinguish and provide a good estimate between PAHs of diverse origins [23,24,26]. In this case, ratios of Phen/Anth <10 (except on station Tanjung Dawai) suggesting significant PAHs inputs from pyrogenic sources. On the other hand, for the Fluo/Py ratios, values less than 1, tends to indicate that the PAHs contamination is from petroleum sources [27]. In this case, for several samples (e.g. Tanjung Dawai, Penang Bridge, Kg. Masai, Sebatu, Tebing Runtuh, and Kg. Pasir Puteh), the petrogenic signature has been suggested from the Fluo/Py ratios <1.

Hence, it can be estimated that all stations, in addition to the petrogenic origin for the major fraction of the PAHs, a slight dominance of pyrogenic PAHs can be observed. This contribution of pyrogenic PAHs in STs of mussels analysed from Pasir Panjang station were indicated by Fluo/Py $= 1.12$ (the largest among all the seven sites) and Phen/Anth $= 0.01$ (the smallest among all the seven sites) and consistently exhibited pyrogenic signature (Table 2).

To discriminate between these possible PAH sources, the value of the ratio $\sum$MP/P (ratio of the sum of the concentrations of the methyl-phenanthrenes *vs* the concentration of phenanthrene) was introduced [28]. $\sum$MP/P ratios for pyrogenic PAHs are around 0.5 whereas the petrogenic PAHs have the $\sum$MP/P ratio above 2. The $\sum$MP/P ratios, an indicator for the origins of PAHs were found to be high in green mussels for all the sites indicating that the PAHs signature was of petrogenic origin. Also, the $\sum$MP/P ratios above 2 were recorded in Penang Bridge, Tebing Runtuh and Kg. Pasir Puteh PAHs pollution was mainly controlled by petrogenic inputs (Figure 2).

4.2 *Source identification of perylene*

Perylene could be introduced to the aquatic environments by various processes including: the incorporation of atmospheric particles (pyrogenic origin); of fossil fuels such as petroleum (petrogenic origin) or by *in situ* production by degradation of biogenic

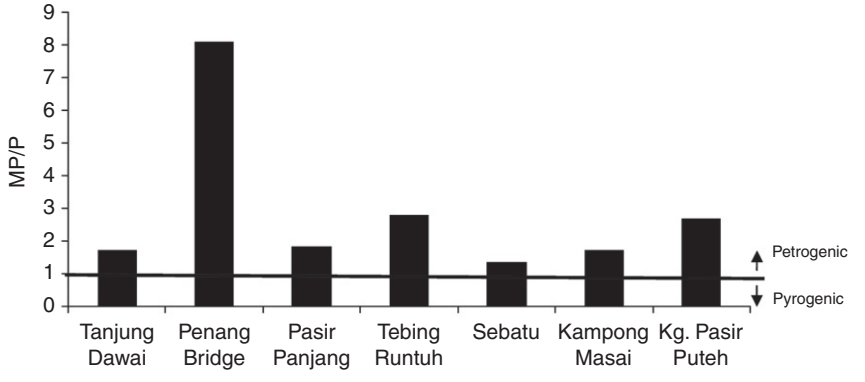

Figure 2. Ratio of $\sum$MP/P in total soft tissues of green mussels (*P. viridis*) collected from coastal waters of Peninsular Malaysia. Characterisation of the origins of PAHs pollution in green mussels.

Table 3. Concentrations of perylene (ng/g) dry weight in green mussels (*P. viridis*) from coastal waters of Peninsular Malaysia.

Stations	Dry weight (g)	Pyrene Con.	Perylene Con.	∑Penta Aromatic Isomer ∑PAI[a]	∑PAHs[b]	Pyrene/ Perylene	Perylene/ ∑PAHs (%)	Perylne/ PAI
1. Tanjung Dawai	11	25	0.28	17	163	89	0.17	0.016
2. Penang Bridge	12	110	1.88	76	2060	58	0.09	0.025
3. Pasir Panjang	6	22	ND	ND	2054	ND	ND	ND
4. Tebing Runtuh	12	15	ND	ND	36	ND	ND	ND
5. Sebatu	13	3	ND	0.66	17	ND	ND	ND
6. Kampong Masai	12	37	0.27	9.30	244	137	0.11	0.029
7. Kg. Pasir Puteh	12	166	0.33	129	1200	503	0.03	0.0025

Notes: [a]∑PAI: Sum of the Penta Aromatic Isomer concentrations (benzo[a]anthracene, benzo[k]-fluoranthene, benzo[e]acephenanthrylene, benzo[e]pyrene, benzo[a]pyrene and perylene).
[b]See the legend of Table 2 for definition of ∑PAHs.
ND: not detected.

precursors (diagenetic origin) [29]. Due to these processes, perylene are readily available for uptake by green mussels (*P. viridis*). Typically, for pyrogenic PAHs, perylene represents about 1–4% of the ∑PAH concentrations [30]. It is also interesting to note that, perylene concentrations taken from Malaysian waters ranged from 1 to 884 ng/g dry sediment [16]. Concentrations of perylene >10% of the total penta-aromatic isomers indicate a probable diagenetic input, whereas those <10% indicate a probable pyrolytic origin of the compound [19]. Perylene concentrations in mussel samples ranged from $0.27 < 1$ to 1.88 ng/g dry wt. In order to accurately access the perylene origin in green mussel samples, the relative concentrations of perylene (concentration of perylene versus sum of penta-aromatic concentrations) were calculated in Table 3. Conclusively, the results obtained revealed the same trend with that of perylene concentration versus sum of PAHs concentrations and we can deduce that perylene in the mussel samples had their origins from pyrogenic sources.

4.3 *Bioavailability of PAHs in female and male individuals*

4.3.1 *Length–weight relationship*

The result of the power function regression analysis between shell length and soft tissue expressed in dry weight for female ($y = 1.613e^{0.018x}$, $r^2 = 0.826$) and male mussels ($y = 0.656e^{0.028x}$, $r^2 = 0.833$) showed a significant positive relationship. The results showed strong positive relationship that can be used to monitor biomass production in populations of *P. viridis* in the south and west of Peninsular Malaysia.

4.3.2 *Effect of body size on PAHs concentrations*

According to Wang and Fisher [31], the correlation of PAHs in shellfish is related to body weight as a power function and can be expressed in the following equation:

$$Me = aW^b$$

This relationship is often linearised through a logarithmic transformation which yields an equation stated below:

$$Log_{10}[Me] = \log a + b\log_{10} W,$$

where [Me] is the total organic micropollutant concentrations (PAHs), W is body weight, a is equal to the logarithm of the multiplicative coefficient of the power function and b is the slope of the linear function. Studies have shown that the regression slopes relating tissue concentration to body size are not constant but may vary significantly depending on locations, habitats or years [32].

The levels of most abundant (tri aromatics) and heavier (tetra and penta aromatics) of PAH compounds and total PAHs concentrations for both female and male individuals are summarised in Tables 4 and 5. Levels of total petroleum hydrocarbons ranged from 5845 to 24600 (ng/g lipid wt.) and 4400 to 61130 (ng/g lipid wt.) in female and male tissues, respectively. The results showed marked variations in the concentrations of compound-specific PAHs between individuals as well as between males and females. The results also revealed that a higher uptake and storage of PAHs were observed in male green-lipped mussels compared to females. In a study conducted by Mashinchian [18], the kinetics of uptake and release of PAHs for male and female mussels showed that in short-term exposed mussels, there were no significant differences ($p > 0.05$) between the release rates of PAHs for male and female mussels. Furthermore, the T-test had indicated that in long-term exposed mussels similar trends of release rate occurred between two sexes. This difference between sexes was also observed in the uptake phase where females had higher rate of uptake than males. In summary, the results of Mashinchian [18] are consistent with this study. Unfortunately, sound scientific evidence is unavailable as to why male mussels had higher uptake or storage than the female counterparts. We postulate that, female mussels may have different hormone levels or physiological difference than that of male mussels which can influence the uptake or release of pollutants such as PAHs. Furthermore, female mussels undergo a period spawning phase which may influence the uptake and release of pollutants in their tissues. Three phase compartment model in oyster *Crassosttrea virginica* suggested by Stegeman and Teal [33] explained that there were no obvious differences between male and female oysters. Since the test was conducted in temperate waters, the kinetics of uptake and release of chemicals by green mussels that live in tropical regions could be different. This affords further investigations in order to resolve these answers.

In this study, independent sample T-test ($p > 0.05$) showed there were no significant differences in male and female mussels. Total PAHs concentrations decreased with increase in body weight in all female and male gender (Table 4). The negative correlation between concentrations of total PAHs and body weight in female ($r = -0.890, p < 0.01$) and male ($r = -0.0655, p < 0.05$) confirmed negative relationship of PAHs concentrations and body weights in the range of sizes analysed in this study (Figure 3).

It is commonly thought that decreasing PAHs concentrations in bigger individuals is due to larger surface area to volume ratio [34]. On the other hand, larger individuals tend

Table 4. Total PAH concentrations in soft tissue of female and male individual green mussels (*P. viridis*) from Pasir Panjang station.

Individuals						
				Total PAHs concentrations[a] (ng/g)		
No. Gender	Dry weight (g) Mean ± SD	Shell length (mm) Mean ± SD	Lipid content (%)	Wet weight	Dry weight	Lipid weight
1. Female	5.76 ± 0.57	75 ± 0.56	1.26	310	390	24600
2. Female	6.04 ± 0.57	77 ± 0.41	1.28	258	320	20150
3. Female	8.85 ± 0.58	85 ± 0.65	1.86	220	268	11850
4. Female	9.02 ± 1.52	95 ± 1.86	1.89	202	245	10616
5. Female	9.43 ± 1.53	95 ± 1.85	1.88	201	244	10610
6. Female	9.56 ± 1.52	95 ± 1.86	1.89	200	243	10540
7. Female	9.61 ± 0.57	100 ± 1.31	1.89	199	242	10530
8. Female	10.91 ± 0.25	110 ± 1.33	1.95	198	257	10153
9. Female	11.05 ± 0.26	110 ± 1.33	1.96	196	252	9950
10. Female	11.49 ± 0.25	110 ± 1.33	1.96	195	253	9946
11. Female	11.85 ± 1.60	105 ± 1.56	1.97	171	222	8705
12. Female	11.98 ± 0.30	110 ± 0.61	1.98	157	204	7915
13. Female	17.75 ± 0.25	100 ± 0.65	2.14	125	169	5845
1. Male	3.87 ± 0.63	70 ± 0.42	0.78	477	542	61130
2. Male	4.77 ± 0.74	70 ± 0.45	0.98	497	571	50656
3. Male	5.01 ± 0.62	70 ± 0.56	0.99	342	393	34530
4. Male	5.46 ± 1.39	70 ± 0.41	0.99	128	149	12906
5. Male	6.26 ± 1.62	80 ± 0.54	1.24	124	144	9962
6. Male	6.40 ± 0.54	78 ± 0.50	1.24	122	142	9822
7. Male	7.15 ± 1.59	80 ± 0.44	1.55	103	119	6609
8. Male	8.49 ± 1.86	81 ± 0.44	1.86	95	109	5118
9. Male	8.95 ± 1.31	98 ± 1.32	1.88	91	104	4814
10. Male	12.98 ± 1.87	100 ± 1.86	2.02	88	114	4400

Note: [a]See the notes of Table 2 for definition of total PAHs concentrations.

to pump less water through their body per unit body weight; therefore the uptake is lower than in small individuals.

In comparison to heavy metals, similarly; the dependence of metal concentration on body size has been reported for all metals except for Zn [35]. Generally, these results were consistent with literature concerning *Mytilus edulis* [31,32], and *Perna viridis* [10], that reviewed relationship between body size and tissue concentrations of different metals in different molluscs such as related bivalve *Crassostrea gigas* [36] and in marine gastropods [37]. It was shown that metal concentrations in mollusks can either be positively, negatively correlated or independent body size.

All of these studies showed, rates of absorption will be influenced by factors such as season, mussel age, size and location [38]. Besides this, as previously mentioned, physiologically condition is considered one of the main factors with potential to control distribution and retention of contaminations in mussels.

As stated earlier, in order to differentiate pyrogenic and petrogenic sources of PAHs in green mussel samples from Pasir Panjang station, the ratio of lower molecular weight to

Table 5. Concentrations of PAHs per group of aromacity (tri-, tetra- and penta aromatics) and characteristic values of molecular indices for the sources of PAHs according to specific ratios in total soft tissues of female and male (*P. viridis*) from Pasir Panjang station.

	Concentration of PAHs (ng/g dry wt)			Compositional Parameters					
Gender	∑Tri[a]	∑Tetra[b]	∑Penta[c]	LMW/HMW[d]	∑MP/P[e]	Fluo/Py[f]	Origin	Phen/Anth[g]	Origin
1. Female	224	132	34	1.94	8.46	0.46	Petrogenic	0.88	Pyrogenic
2. Female	148	114	58	1.15	2.00	0.42	Petrogenic	1.96	Pyrogenic
3. Female	152	75	41	1.66	1.16	0.31	Petrogenic	3.38	Pyrogenic
4. Female	116	41	88	1.08	1.49	0.58	Petrogenic	1.15	Pyrogenic
5. Female	123	44	77	1.34	1.31	0.93	Petrogenic	2.50	Pyrogenic
6. Female	119	108	16	1.24	1.25	0.33	Petrogenic	2.64	Pyrogenic
7. Female	168	41	33	3.07	0.95	0.86	Petrogenic	3.69	Pyrogenic
8. Female	211	30	16	5.95	0.99	0.52	Petrogenic	37.7	Petrogenic
9. Female	170	47	35	2.90	0.92	0.69	Petrogenic	2.43	Pyrogenic
10. Female	135	47	71	1.43	0.76	0.65	Petrogenic	5.38	Pyrogenic
11. Female	123	60	39	1.80	0.86	1.00	Petrogenic	11.9	Pyrogenic
12. Female	84	58	62	0.89	0.05	0.34	Petrogenic	27	Petrogenic
13. Female	110	39	20	2.90	8.70	0.80	Petrogenic	0.68	Pyrogenic
1. Male	287	91	164	1.28	0.90	0.30	Petrogenic	21	Petro and Pyro
2. Male	260	139	172	1.03	1.07	030	Petrogenic	1.61	Pyrogenic
3. Male	301	79	13	4.94	1.39	0.63	Petrogenic	3.70	Pyrogenic
4. Male	90	40	19	2.32	1.51	0.60	Petrogenic	1.17	Pyrogenic
5. Male	77	67	ND	1.93	0.89	0.36	Petrogenic	2.31	Pyrogenic
6. Male	108	34	ND	5.07	4.06	0.44	Petrogenic	0.66	Pyrogenic
7. Male	78	31	10	6.34	0.19	6.03	Petrogenic	16	Petro and Pyro
8. Male	49	36	24	1.30	6.28	0.79	Petrogenic	0.33	Pyrogenic
9. Male	75	29	ND	5.82	3.30	0.88	Petrogenic	1.88	Pyrogenic
10. Male	53	50	11	1.66	1.74	0.81	Petrogenic	1.23	Pyrogenic
						<1[h]		<10[j]	
						>1[i]		>25[k]	

Notes: [a]Tri: Sum of the tri aromatic concentrations (dibenzothiophene, phenanthrene, anthracene, 3-methylphenanthrene, 2-methylphenanthrene, 2-methylanthracene, 9-methylphenanthrene, 1-methylphenanthrene).
[b]Tetra: Sum of the tetra aromatic concentrations (fluoranthene, pyrene, 1-methylpyrene, chrysene, benzo[a]anthracene).
[c]Penta: Sum of the penta aromatic concentrations (benzo[k]fluoranthene, benzo[e]acephenathrylene, benzo[e]pyrene, benzo[a]pyrene, dibenzo[a,h]anthracene).
[d]See the legend of Table 2 for definition of LMW/HMW.
[e]See the legend of Table 2 for definition of ∑MP/P.
[f]See the legend of Table 2 for definition of Fluo/Py.
[g]See the legend of Table 2 for definition of Phen/Anth.
[h,i]Budzinski *et al*. [5].
[j,k]Scolo [24].

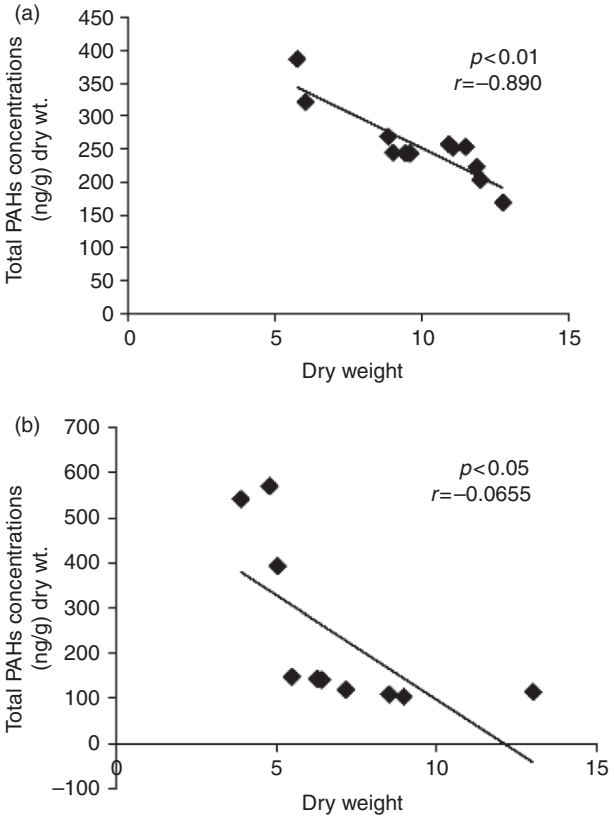

Figure 3. Negative correlation between PAH concentrations and body weights in female green mussels (a). Negative correlation between PAH concentrations and body weights in male green mussels (b). Collected from Pasir Panjang station.

higher molecular weight (LMW/HMW) PAHs were calculated (Table 5). Source identification of PAHs in female and male mussels collected in Pasir Panjang was dominated by presence of LMW PAHs suggesting mostly petrogenic and petroleum related compounds. The finding was consistent with studies reported in Neff [2]; Zakaria *et al*. [16].

As already stated, pyrogenic PAHs are characterised by a high abundance of parent PAHs, whereas petroleum hydrocarbons are characterised by a predominance of alkylated PAHs over parent compounds [39,40]. Therefore, the ratio of the sum of the concentrations of the methyl-phenanthrane to the phenanthrene concentration ($\sum$MP/P) has been shown to be greater than 2 for petroleum and lower than 1 for pyrogenic source of PAHs in female and male individuals. However, the pyrogenic PAHs are mainly anthropogenic, and thereby can be sourced to industrial activities, but this area is mostly dominated by agricultural activities. Therefore, we conclude that the contribution of PAHs pollution may be attributed to long range transport via atmospheric aerosols and particles can be considered as one of the pyrogenic inputs mixed with petrogenic inputs. The possibility of PAHs being trans-boundary transported can be explained by the fact that Pasir Panjang is located near the Island of Sumatra, Indonesia. There are various

petro-chemical industries on the East Coast of Sumatra which produced combustion of fossil fuels. Furthermore, the Island experienced extensive biomass burning which transported pyrogenic particles across the Straits of Malacca and may reach Pasir Panjang via Monsoon winds. Wet and dry deposition of the PAHs laden particles may then become the background PAHs that have been revealed in this study. Further investigations using specific fingerprinting studies using radioisotopic anlaysis of PAHs must be conducted to prove this interesting hypothesis.

Finally, female and male individuals exhibited phenanthrene/anthracene (Phen/Anth) ratios <10 further confirming the pyrogenic origin. Indeed, the ratio of fluoranthene to pyrene (Fluo/Py) <1, were probably of less than unit indicated petrogenic sources (Table 5).

5. Conclusion

The results of monitoring the coastal waters of west and south Malaysia using green mussels (*P. viridis*) as a biomonitor showed clearly the status of contamination by PAHs in this region which suggested serious contamination by mostly petrogenic origin for most of the locations. This could be potentially related to major industrial, agricultural areas and shipping ports in west and south Peninsular Malaysia which receive organic micropollutants including PAHs from atmospheric and global ocean transport systems. It was concluded that among the seven stations that were sampled, Penang Bridge with the total PAHs concentrations of 110500 (ng/g lipid wt.) can be categorised as the 'hot-spot'; while Sebatu with the lowest total PAHs concentration 766 (ng/g lipid wt.) can be classified as the 'clean site'. Molecular indices calculated to assess the pyrogenic and petrogenic sources of PAHs showed that female and male mussels analysed in Pasir Panjang also, can provide mostly a mixture of pyrogenic and petrogenic origins. Differences between sexes (female and male individuals) was also indicated male species were capable accumulate more concentrations of PAHs in comparison to female species. This could be explained that female mussels had a slightly higher rate of uptake and release than males. Besides, lipid content and composition of male and female may be different and these differences may affect PAHs accumulation by each sex. Mussels tend to absorb lower molecular weight of PAHs when compared to the higher molecular weight PAHs that, confirmed that the more water soluble compounds are preferentially accumulated in the water-dissolved form through the gills while the heavier molecular weight compounds are preferentially absorbed from filtered particles as they pass through the digestive system.

Generally, the result indicated the soft tissue of *Perna viridis* was a good biomonitor of perylene bioavailability and PAHs contamination in coastal waters of Peninsular Malaysia. Perylene in high concentrations originated from biological sources which are attributed to activities of termites in pristine areas. On the other hand, perylene in low concentrations may have originated from pyrogenic sources that could of occurred in developed areas where termite nests are rarely found (e.g. in some locations such as Tanjung Dawai, Penang Bridge, and Kg. Masai).

Results obtained in this study reflect the extent of PAHs contamination along the western coast of Peninsular Malaysia. Through demonstrating the significant contribution of petrogenic PAHs in the mussels collected in urban locations, this study has afforded an important benchmark for future studies in the Southeast Asian region. More studies

should be done to cover a whole range of the Southeast Asian region and to understand the sociobiogeochemistry of PAHs in mussels and their impacts to human health.

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