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Evaluation of the potential bioaccumulation ability of the blood cockle (*Anadara granosa* L.) for assessment of environmental matrices of mudflats

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HIGHLIGHTS

- The concentration of PAHs in sediment, water and blood cockle of Malaysian mudflats was studied.
- T-PAHs, individual profile of PAH level, BAF and BSAF results showed the ability of blood cockle in the bioaccumulation.
- These findings showed that the blood cockle is a good biomonitor of PAHs in mudflats.

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ABSTRACT

The spatial distribution of 19 polycyclic aromatic hydrocarbons (tPAHs) was quantified in aquacultures located in intertidal mudflats of the west coast of Peninsular Malaysia in order to investigate bioaccumulation of PAH in blood cockles, *Anadara granosa* (*A. granosa*). Fifty-four samples from environmental matrices and *A. granosa* were collected. The sampling locations were representative of a remote area as well as PAH-polluted areas. The relationship of increased background levels of PAH to anthropogenic PAH sources in the environment and their effects on bioaccumulation levels of *A. granosa* are investigated in this study. The levels of PAH in the most polluted station were found to be up to ten-fold higher than in remote areas in blood cockle. These high concentrations of PAHs reflected background contamination, which originates from distant airborne and water-borne transportation of contaminated particles. The fraction and source identification of PAHs, based on fate and transport considerations, showed a mix of petrogenic and pyrogenic sources. The relative biota–sediment accumulation factors (RBSAF), relative bioaccumulation factors from filtered water ($RBAF_w$), and from suspended particulate matter (SPM) ($RBAF_{SP}$) showed higher bioaccumulations of the lower molecular weight of PAHs (LMWs) in all stations, except Kuala Juru, which showed higher bioaccumulation of the higher molecular weight of PAHs (HMWs). Calculations of bioaccumulation factors showed that blood cockle can accumulate PAHs from sediment as well as water samples, based on the physico-chemical characteristics of habitat and behaviour of blood cockles. Correlations among concentrations of PAHs in water, SPM, sediment and *A. granosa* at the same sites were also found. Identification of PAH levels in different matrices showed that *A. granosa* can be used as a good biomonitor for LMW of PAHs and tPAHs in mudflats. Considering the toxicity and carcinogenicity of PAHs, the bioaccumulation by blood cockles are a potential hazard for both blood cockles and their consumers.

Abbreviations: A. granosa, *Anadara granosa*; c-PAHs, carcinogenic PAHs including Chry, BaAnt, BkFluo, BaPy, DBahAnt; HMWs, higher molecular weight of PAHs, sum of 1-MPy, Pyr, Chry, BaAnt, BkFluo, BeAce, BePy, BaPy, DBahAnt; LMWs, lower molecular weight of PAHs, sum of Nap, DbT, Phe, Anth, 9-MPhe, 3-MPhe, 2-MPhe, 1-MPhe, 2-MAnt, Fluo); MP/P, ratio of methyl phenantherene to phenantheren; $RBAF_w$, relative bioaccumulation factors from filtered water; $RBAF_{SP}$, relative bioaccumulation factors from suspended particulate matter; SPM, suspended particulate matter; RBSAF, relative biota–sediment accumulation factor; S, sediment; Sg., Sungai, the local word for river; tPAHs, sum of 19 individual PAHs (Nap (naphthalene), DbT (dibenzothiophene), 2-MAnt (2-methylanthracene), 1-MPhe (1-methylphenantherene), 2-MPhe (2-methylphenantherene), 3-MPhe (3-methylphenantherene), 9-MPhe (9-methylphenantherene), 1-MPy (1-methylpyrene), Phe (phenantherene), Ant (anthracene), Fluo (fluoranthene), Pyr (pyrene), BaAnt (benzo(a)anthracene), BaPy (benzo(a)pyrene), BePy (benzo(e)pyrene), BeAce (benzo(e)acephenantherene), BkFluo (benzo(k) fluoranthene), Chry (chrysene), DBahAnt (dibenzo(a,h)anthracene); W, water.

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1. Introduction

The Straits of Malacca, one of the busiest and narrowest waterways in the world, is the most important source of seafood for Malaysians and accounts for half of marine-landed catches (Abdullah, 1997; Ismail, 2006; Tan and Yap, 2006). If oil contaminants from ship traffic in this area reach the coast and spread into estuaries, they accumulate in sediments over time. Furthermore, if these contaminants are bioavailable to epibenthic organisms in the coastal and estuarine areas, they could pose a risk to both wildlife and humans. Intertidal mudflats are shallow-sloped shorelines with expanses of fine sediment that are flooded at each high tide. They are often components of estuaries and are revealed when the tide goes out. Because there is very little space between the fine particles, the fine sediments in the mud have high organic content and anoxic conditions (Capital Regional District, 2010). One important class of the oil contaminants associated with these mudflats consists of polycyclic aromatic hydrocarbons (PAHs) (Cailleaud et al., 2009; Shukla et al., 2007; Zakaria et al., 2002). Mudflat aquacultures thrive in Malaysia and represent a major component of the economy in coastal zones, yet the environmental quality of many of these areas has never been studied. Most of the studies in the Asia-Pacific region such as International Mussel Watch have been using bivalves like mussels and oysters as bioindicators to ascertain the quality of coastal marine waters (Tanabe, 1994). In Malaysian coastal waters distribution of petroleum pollutions have been studied in some areas using green-lipped mussel, *Perna viridis* as a biomonitor (Shahbazi et al., 2010a,b) but, they have limited distribution in Malaysia and nearby countries compare to cockles. The marine blood cockle, *Anadara granosa* (*A. granosa*), is cultured commercially in the tidal mudflats along the west coast of Peninsular Malaysia (Ibrahim, 1995). In previous studies, cockles were commonly used as organisms to biomonitor several contaminants (Lobo et al., 2010; Ong, 1989; Peake et al., 2006; Price and Pearce, 1997; Yap et al., 2008) because they are sessile, filter/deposit feeders that have a high filtration rate, are limited in ability to biotransform contaminants, and have a high tolerance to many types of pollution (Chan et al., 2002; Fleming et al., 2008; Floch et al., 2003; Yap et al., 2008). Although some studies have focussed on bioaccumulation abilities of heavy metals by *A. granosa* (Alkarkhi et al., 2008; Chan et al., 2002; Ibrahim, 1995; Yap et al., 2008), there is little information on organic compound accumulation, such as persistent organic pollutants and hydrocarbons (Ang et al., 2005; Cheevaporn and Menasveta, 2003; Consumers' Association of Penang, 2005; Holmgren, 1994; Patel and Eapen, 1989), some of which are potentially carcinogenic for human (Mirsadeghi et al., 2011). *A. granosa* presents tidally oriented vertical distribution and is characterized by full vertical and horizontal distribution over the entire estuary. Therefore, it is exposed to sediment as well as to overlying suspended particulate matter (SPM) and dissolved phase (Broom, 1982; Department of Fisheries Malaysia, 2007). Therefore, the exposure of blood cockle to complex mixtures of PAHs at PAH-contaminated sites can increase, which has the potential to influence the bioavailability—and hence toxicity—of PAH contaminants on direct-contact exposure routes or sediment ingestion. Thus, determining the distribution of contamination is an important step in mapping possible exposure pathways to aquatic organisms, since contaminants in sedimentary areas are bioavailable to sediment dwelling organisms. Previous studies have observed the acute toxicity of the lower molecular weight of PAHs (LMW) and the chronic toxicity of the higher molecular weight of PAHs (HMW) (Bakhtiari et al., 2009; Mirsadeghi et al., 2011; Neff, 1979). Most studies have taken into consideration the toxicity of petroleum by using biomonitor organisms enhanced with water accommodated fractions as contamination solutions. However, these exposure solutions do not take into account most of the particulate oil formed by particulate matter (Shahbazi et al., 2010a).

Bioaccumulation reflects the transfer of a substance from external media to tissues within an organism (U.S. Army Corps of Engineers, 1994). Compared to the concentration of a substance in water, this phenomenon causes increased chemical concentration in an aquatic organism as a result of chemical uptake through all possible routes of chemical exposure (Gobas and Morrison, 2000; Mackay and Fraser, 2000). To formulate comparisons between the different stations, the relative bioaccumulation factor (RBAF) and the biota–sediment bioaccumulation factor (RBSAF) were calculated (Baumard et al., 1999).

In this study, the sum of 19 PAH (tPAHs) concentrations in blood cockle tissue is related to their concentration in sediments as well as in water and suspended particulate matter (SPM) in order to estimate the bioaccumulation factor (BAF) and biota–sediment accumulation factor (BSAF), which is defined as the ratio between the PAH concentrations in tissues, water and sediments. The relative abundance of PAH in sediment (RBSAF), water ($RBAF_w$) and SPM ($RBAF_{sp}$) is used to investigate probable PAH sources and the relationship between sediment, water and blood cockle tissue PAH concentrations. The utility of blood cockle as a biomonitor of PAH contaminations is also evaluated. In addition, the source identification of PAHs in blood cockle is discussed.

2. Material and methods

2.1. Study area

Six extended mudflat aquaculture plots in the Straits of Malacca located on the west coast of Peninsular Malaysia were studied. The location of these stations, which include Sungai Buloh (A), Pantai Remis (B), Sungai Haji Durani (C), Kuala Gula (D), Kuala Juru (E) and Kuala Perlis (F) are shown in Fig. 1. Designs of three replicate samples within 1/2 km^2 of the sampling stations were collected for studies of PAH distribution.

2.2. Sampling

Surface sediment, water and cockle samples were collected between January 20 and March 25, 2008. To avoid sampling during the spawning season, blood cockle samples were taken only once from all six sites. Forty individual blood cockles of approximately the same size (4–6 cm in length) and age were randomly collected from the aqua culture areas. Surface sediment samples were taken from a depth of 20 cm using a Van Veen grab (area 0.04 m^2). Organisms and shell debris were removed by hand, and the finer particles from the top sediment layer were analysed (Chouksey et al., 2004; Mirsadeghi et al., 2011; Syracuse Research Corporation, 2002). Surface water was sampled using a 5 l Niskin bottle (Mirsadeghi et al., 2011; Tong et al., 1999). Samples were collected from the surface water column systematically in a fixed location during one tidal cycle (Mirsadeghi et al., 2011). Environmental matrix samples and blood cockles were stored in a cooler box with dried ice until their transfer to the laboratory. The water samples were filtered through GF/F Whatman (0.45 μm Millipore) filter paper, and then the filtered water extracted immediately, and the filter papers and the other samples were frozen at −10 °C prior to further analysis (Chouksey et al., 2004; Mirsadeghi et al., 2011). If water and sediment samples were not analysed within 2 h of collection, they were acidified to a pH < 2 with HCl or H_2SO_4 (Mirsadeghi et al., 2011; Tong et al., 1999) to stop the microbial degradation of the target compounds (McGroddy and Farrington, 1995).

2.3. Extraction and purification—sediment and cockle

Sediment, filter papers and cockle were extracted according to the method explained in Mirsadeghi et al. (2011). In summary, samples were spiked with 50 μl of 10 μg/g mixture of surrogates (naphthalene-d_8, anthracene-d_{10}, benzo(a)anthracene-d_{12} and perylene-d_{12}) and 50 μl of a 10 μg/g internal standard (*p*-terphenyl-d_{14}) (Siddiqi et al., 2009). The

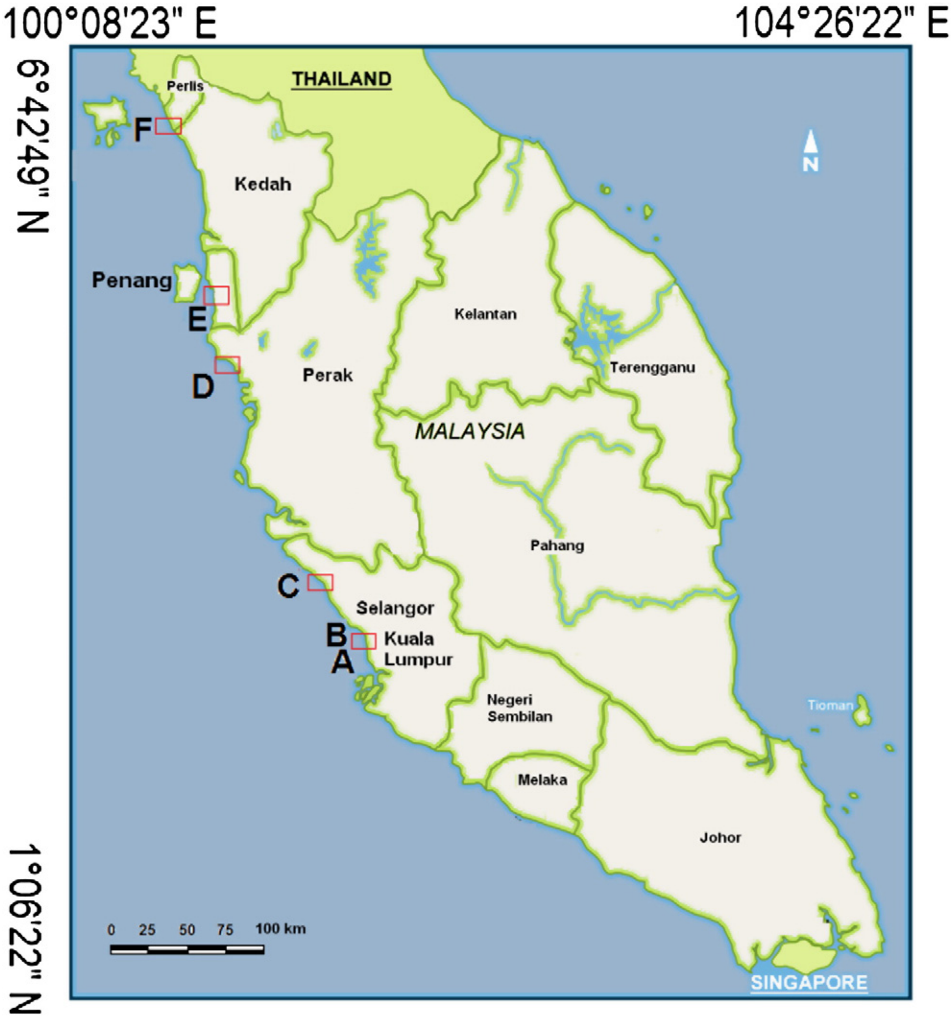

Fig. 1. Locations of sampling areas from the mudflats of west coast of Peninsular Malaysia (A. Sungai Buloh, B. Pantai Remis; C. Sungai Haji Durani; D. Kuala Gula; E. Kuala Juru; F. Kuala Perlis).

samples were then extracted by soxhlet dichloromethane (Anyakora et al., 2005; Culotta et al., 2006; Nikolaou et al., 2009). The extracted samples were purified and fractionated into an aliphatic and an aromatic fraction through two-step silica gel column chromatography (Boonyatumanond et al., 2006; Mirsadeghi et al., 2011; Yim et al., 2005; Zakaria et al., 2001). Samples were collected after column chromatography and concentrated to a final volume to preserve them until analysis with gas chromatography–mass spectrometry (GC–MS).

2.4. Extraction and purification—water

Filtered water was extracted by the solid-phase extraction (SPE) method, which is based on hydrophobic retention of the sorbent for the interfering components. The SPE cartridge type SiliaPrep C18 nec (23%) was used for the PAH study (Mirsadeghi et al., 2011). For the preparation of the sample, 5–25 ml of methanol and five surrogate PAHs were added to 200 ml of filtered water samples. The column of cartridge was conditioned with methanol followed by sample pre-treatment solvent (water: methanol 85:15 v/v) with a 2–4 ml/min flow rate. The water sample was loaded directly on top of the cartridge with the same flow rate as the cartridge volume. A small layer of sodium sulfate anhydrous was then added to the top of the cartridge. Next, the sample was washed with sample pre-treatment solvent with 1–2 ml min^{-1} flow rate. At the PAH elution step, 1 ml multiple two of dichloromethane with 1 ml min^{-1} flow rate were introduced to the column of cartridge (Mirsadeghi et al., 2011; SiliCycle, 2007; Simpson et al., 2002). Finally, the samples were concentrated to a final volume and preserved until they were analysed by GC–MS.

The PAHs quantified in this study were naphthalene (Nap), dibenzothiophene (DbT), Phe, Ant, 3-methylphenantherene (3-MPhe), 2-methylphenantherene (2-MPhe), 2-methylanthracene (2-MAnt), 9-methylphenantherene (9-MPhe), 1-methylphenantherene (1-MPhe), Fluo, Pyr, 1-methylpyrene (1-MPy), chrysene (Chry), benzo(a)anthracene (BaAnt), benzo(k)fluoranthene (BkFluo), benzo(e) acephenantherene (BeAce), benzo(e)pyrene (BePy), benzo(a)pyrene (BaPy), and dibenzo(a,h)anthracene (DBahAnt). The first ten PAHs listed (Nap through Fluo) are considered LMW PAHs and the final nine PAHs listed (Pyr through Da,hAnth) are considered HMW PAHs (Neff, 1979; Sanger et al., 1999).

2.5. Instrumental analysis

Two μl of extracted samples were injected manually into a 6890N Agilent gas chromatograph coupled to a 5973N Agilent mass spectrometer (Agilent Technologies, Avondale, PA, USA). The identification and quantification of the PAH compounds were achieved using ChemStation® software, based on matching their retention time with a mixture of PAH standards. The instrumental details were explained in Mirsadeghi et al. (2011).

2.6. Quality control and quality assurance

The quality control (QC) and quality assurance (QA) considered in this study include the following measures: blanks were prepared in the same manner and processed together with samples; limits of detection (LDs) were estimated as the average signal of the blanks ($n = 3$) plus three times the standard deviation of the signals of the blanks (Abdullah et al., 1996; Chouksey et al., 2004; Cortazar et al., 2008); and each individual PAH compound was compared by the retention time and the abundance of quantification ions/confirmation ions with respect to reliable PAH standards (Arias et al., 2009). Quantification was performed by the internal standard method using a 16 PAH reference material mixture (PAH_{16}, paratherphenyl D14 as internal injection standard [IIS] and surrogates) (Siddiqi et al., 2009); three-point internal calibration curves were built in the range of 2.0–10.0 $\mu g\ g^{-1}$ for PAHs with correlation coefficients for calibration curves all higher than 0.993; the recovery of surrogates generally ranged from 60% to 110% of the spiked concentration. PAH concentrations were corrected for recovery according to the nature of the compound, which is volatile and easily diffused (Cortazar et al., 2008; Omar et al., 2006). The GC–MS were auto tuned every day to check the condition of the instrument; GC–MS columns were cleaned frequently by a column purge programme.

2.7. Bioaccumulation calculation

The extent of chemical bioaccumulation is usually expressed in the form of the BAF, which is the ratio of the chemical concentration in the organism (C_m) to that in water. Defining BAF according to the extent of pollutants, which are freely dissolved in the water column (C_{wd}), (Eq. (1)) or adsorbed to SPM (C_{sp}) (Eq. (2)), makes it universally applicable from site to site (Gobas and Morrison, 2000).

$$\mathrm{BAFw} = \frac{C_m}{C_{wd}} \tag{1}$$

$$\mathrm{BAFsp} = \frac{C_m}{C_{sp}} \tag{2}$$

In sediment dwelling organisms, the BSAF describes bioaccumulation. BSAF is the concentration of contaminations in benthic organisms (C_m) relative to that in sediment (C_s) (g organism/g sediment) (Baumard et al., 1999; Cortazar et al., 2008; Gobas and Morrison, 2000):

$$\mathrm{BSAF} = \frac{C_m}{C_s}. \tag{3}$$

To formulate comparisons between the different stations, relative BAFs and BSAFs were calculated as shown in Eqs. (4) and (5) (Baumard et al., 1999), in which RBSAF and RBAF are relative bioconcentration factors (%); BAF is the bioaccumulation factor for each individual PAH; BSAF is the biota–sediment accumulation factor for each individual PAH; $\sum$BAF is the sum of 19 individual BAFs from DbT to DBahAnt; and $\sum$BSAF is the sum of 19 individual BSAFs from DbT to DBahAnt.

$$\mathrm{RBAF} = \frac{\mathrm{BAF}}{\sum \mathrm{BAF}} \times 100 \tag{4}$$

$$\mathrm{RBSAF} = \frac{\mathrm{BSAF}}{\sum \mathrm{BSAF}} \times 100 \tag{5}$$

2.8. Statistical analysis

Statistical data analysis was carried out using the Statistical Package for the Social Sciences (SPSS) 13.0. Since concentration values were normally distributed and homogeneous, an analysis of variance (ANOVA) with the parametric Tukey test was used for comparing more than two groups of data. The differences among data sets were considered statistically significant when the p-value was lower than 0.05 (probability of 95%). The purpose of the statistical analysis was to determine significant differences between sediment, SPM, water and blood cockle tissue. Multivariate analysis of variance (MANOVA) was used to differentiate concentrations of PAH in blood cockle, sediment, filtered water, and SPM among the stations. The application of multivariate methods has increased in analysing environmental data. Because these methods are useful when several dependant variables are measured on each sampling unit, MANOVA can be used to test for significant differences (Alkarkhi et al., 2008). Principal component analysis (PCA) was employed to derive a few new components (principle components) as a linear combination of the original variables, which provide a description of the data structure with a minimum loss of information. PCA has been applied successfully by some researchers to group the samples or to reveal the proximity between the sources of these compounds (Augosto et al., 2009; Stella et al., 2002; Zhao et al., 2006). The application of PCA techniques is used to analyse data from different matrices, transform data into percent composition, and detect and distinguish as independent factors the role of the matrices and the source types. PCA was performed to attempt to identify factors responsible for PAH distribution. Sample PAH fingerprints of the data set were divided between waterborne particulate matter and blood cockle on the first principal component, the most important difference being the physicochemical properties that govern the partitioning among the fractions that adsorb on particulate matter and sediment samples, dissolve in water, and adsorb or sink into the tissue and the routes of contamination (Stella et al., 2002). PCA was also performed to discriminate patterns of variation in the PAHs for PAH profiles of different environmental matrices and blood cockle using the entire PAH concentrations as input variables from different environmental matrices. In this study, PCA was performed using XLSTAT2010 software after Varimax rotation based on Eigen value (>1).

3. Results

3.1. Sediment, water and cockle PAHs concentration

Non-polar organic contaminants, PAHs, are bound by particles in the water and then settle and accumulate in sediments (Kukkonen and Landrum, 1995). PAHs attached to heavy particles settle downward in the estuary where the freshwater meets the salty water of the sea and then are deposited in the bottom sediment and intertidal mudflats. tPAH levels were not normalized by TOC and reported based on dry weight (d.w.) because there was no significant correlation between TOC and tPAHs in the sediments of the study area ($p = 0.05$). The present finding is in agreement with others that did not find any relationship between PAHs and TOC (Cailleaud et al., 2009; Ouyang et al., 2006; Phelps, 2000). A possible explanation is that tPAHs are introduced into the environment from industrial and shipping ports and routes, whereas TOC, which are natural, are consistent with land uses (Ouyang et al., 2006). This inconsistency occurs because of the differences in TOC and hydrocarbon sources. The tPAH concentrations in sediments were between 19.83 ± 2.55 and 481.17 ± 15.55 $ng\ g^{-1}$ d.w. (Fig. 2). The remote area of Pantai Remis had the lowest concentration, whereas Sungai Buloh showed the highest concentration, in which the differences between the levels of PAHs were significant ($p < 0.05$).

The tPAH concentrations ranged from 230.88 ± 9.81 to 1449.67 ± 249.67 $ng\ l^{-1}$ in SPM and from 28.09 ± 3.79 to 143.61 ± 9.27 $ng\ l^{-1}$ in surface water (Fig. 2). The highest concentration of surface water was observed at Kuala Juru station. As shown in Fig. 2, tPAH concentrations in total soft tissues were from 30.80 ± 4.79 to 243.54 ± 21.34 $ng\ g^{-1}$

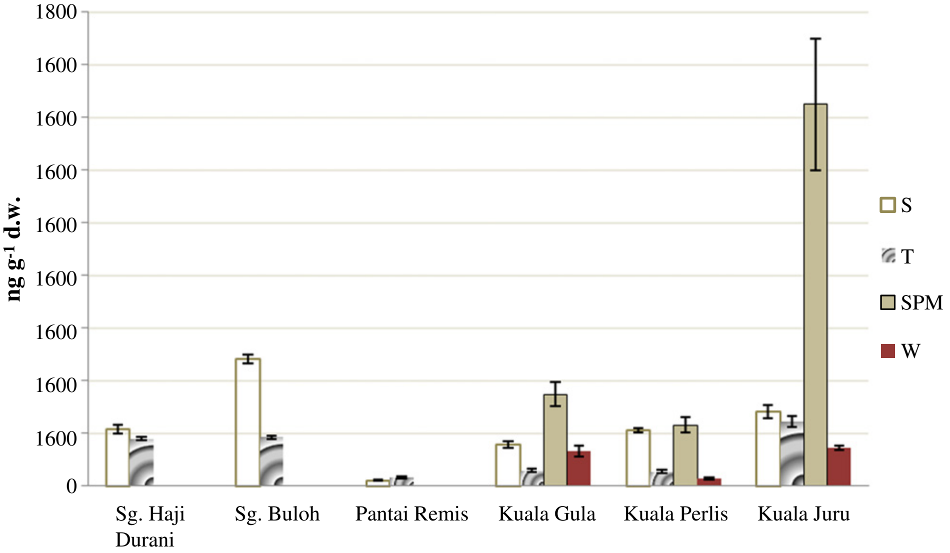

Fig. 2. Mean tPAHs concentrations in sediment (S), blood cockle soft tissue (T) (ng g^{-1}), water (W) and suspended particulate matter (SPM) samples (ng l^{-1}) at different locations (mean ± STD of three replicates, $p < 0.05$).

d.w. The lowest and the highest concentrations were found in samples from Pantai Remis and Kuala Juru, respectively.

The fraction identification of PAHs is described in more detail in Malaysian environmental matrices of extended mudflats, which consider the most toxic compounds. As shown in Fig. 3, dominant tPAHs were three-ring PAHs (sum of Ant, Phen, 9-MPhe, 3-MPhe, 2-MPhe, 1-MPhe, 2-MAnt, and Fluo), followed by four-ring PAHs (sum of 1-MPy, Chry, BeAce, BaAnt, and BkFluo) in the sediment samples of all stations. Three-ring PAHs were in the range of 9.52 ± 2.85 to 223.87 ± 16.87 ng g^{-1} and four rings ranged from 6.08 ± 1.64 to 216.86 ± 27.81 ng g^{-1}. Pantai Remis showed the lowest and Sungai Buloh showed the highest three- and four-ring PAHs. Two-ring PAHs (sum of Naph and DBT) had the smallest portion of tPAHs in the sediment samples of all stations, ranging from 0.00 ± 0.00 ng g^{-1} in Pantai Remis and Kuala Perlis to 11.10 ± 0.56 ng g^{-1} in Sungai Buloh. In surface water on average, two- and three-ring compounds occupied 82.6 to 100% of tPAHs, and in SPM, 63.4 and 87.5% of tPAHs. In addition, four- and five-ring PAHs on average occupied 0.0 to 17.4% of tPAHs in surface water and 12.5% to 36.6% of tPAHs in SPM. As shown in Fig. 3, in total soft tissues, the compositional pattern of PAHs displayed the dominance of tri- and tetra-ring PAHs. The most abundant PAHs, tri-aromatics, on average occupied 49.5% to 69.8% of tPAHs in total soft tissues. In addition, tetra- and penta-ring PAHs on average occupied 21.7% to 39.2% of tPAHs.

3.2. Bioaccumulation factor and biota–sediment accumulation factor

Despite the high concentration of parent PAHs in the soft tissue of *A. granosa*, the calculated BSAF values of tPAHs were low, ranging from 0.17 ± 0.06 to 0.84 ± 0.27 at several sites, except for Pantai Remis with values of 1.55 ± 0.54 ($p < 0.05$) (Table 1). The BAF$_W$ values were from 0.41 ± 0.11 to 1.96 ± 0.36, whereas the BAF$_{SP}$ values were from 0.17 ± 0.05 to 0.23 ± 0.09, ($p < 0.05$).

The results in Fig. 4 clearly show that blood cockle from Sungai Buloh, Pantai Remis, Sungai Haji Durani and Kuala Gula stations have larger BSAF values for three-ring PAHs, which presented higher accumulations of the LMW compounds from sediment. In contrast, Kuala Juru and Kuala Perlis showed higher BSAF values for HMW from sediment, which explains the ability of blood cockle to accumulate compounds of higher molecular weight.

As described above, BAF$_w$ and BAF$_{sp}$ were also calculated for contaminants accumulated in blood cockle tissue from both water and SPM. Relative BAF$_W$ and BAF$_{SP}$ shown in Fig. 5 indicates that in Kuala Juru, the BAF$_{SP}$ of four and five rings was 69.8% of total BAF$_{SP,}$ whereas the 75.7% total BAF$_W$ was four and five rings. In Kuala Gula, 62.2% of BAF$_{SP}$ was five rings, but 54.4% of total BAF$_W$ was three- and four-ring PAHs. In addition, 90.9% BAF$_{SP}$ of Kula Perlis was three- and four-rings, but the dominant BAF$_{SP}$ of PAHs was five rings at 81.2%.

3.3. Isomer ratios, biomarkers and source identification

The results of source identification studies showed that the source of PAHs in different environmental matrices of Malaysian mudflats were mostly petrogenic and slightly pyrogenic. Exceptions were found in the water samples of two stations, Kuala Perlis and Kuala Juru, which were dominated by oil pollution from pyrogenic sources (Table 2). Normally, petrogenic PAHs are from mainly the leakage of crude oil spills and refined products, such as gasoline, discarded used crankcase oil, discharge from municipal and industrial wastewater and runoff, which is found in water columns. On the other hand, long-term petroleum combustion and prevailing contamination sources are from mainly the combustion of diesel fuel and fuel oil from urban and aquatic vehicular exhaust emissions, which are deposited into the water and then attach mostly to particulate matter and sediments.

As Fig. 6 shows, PCA was conducted on the tPAH compositional data of blood cockle tissues and mudflat sediments, water and SPM collected on the west coast of Peninsular Malaysia. It is informative to consider the relative positions of the sampling stations of blood cockle together with the suspected sources of PAH pollution from different environmental matrices (sediments, water and SPM) (Fig. 6). This non-overlapping feature in samples from different environmental matrices was studied by a model describing the relation between the loading vectors of the experimental matrix and the PAH descriptive molecular parameters (Stella et al., 2002). The results showed that the sources of PAHs in sediment and blood cockle soft tissues of Pantai Remis were similar as they strongly correlated with D1 from factor loading as shown in Fig. 6. The source of PAHs in water, sediment and blood cockle soft tissues of Kuala Gula also showed a

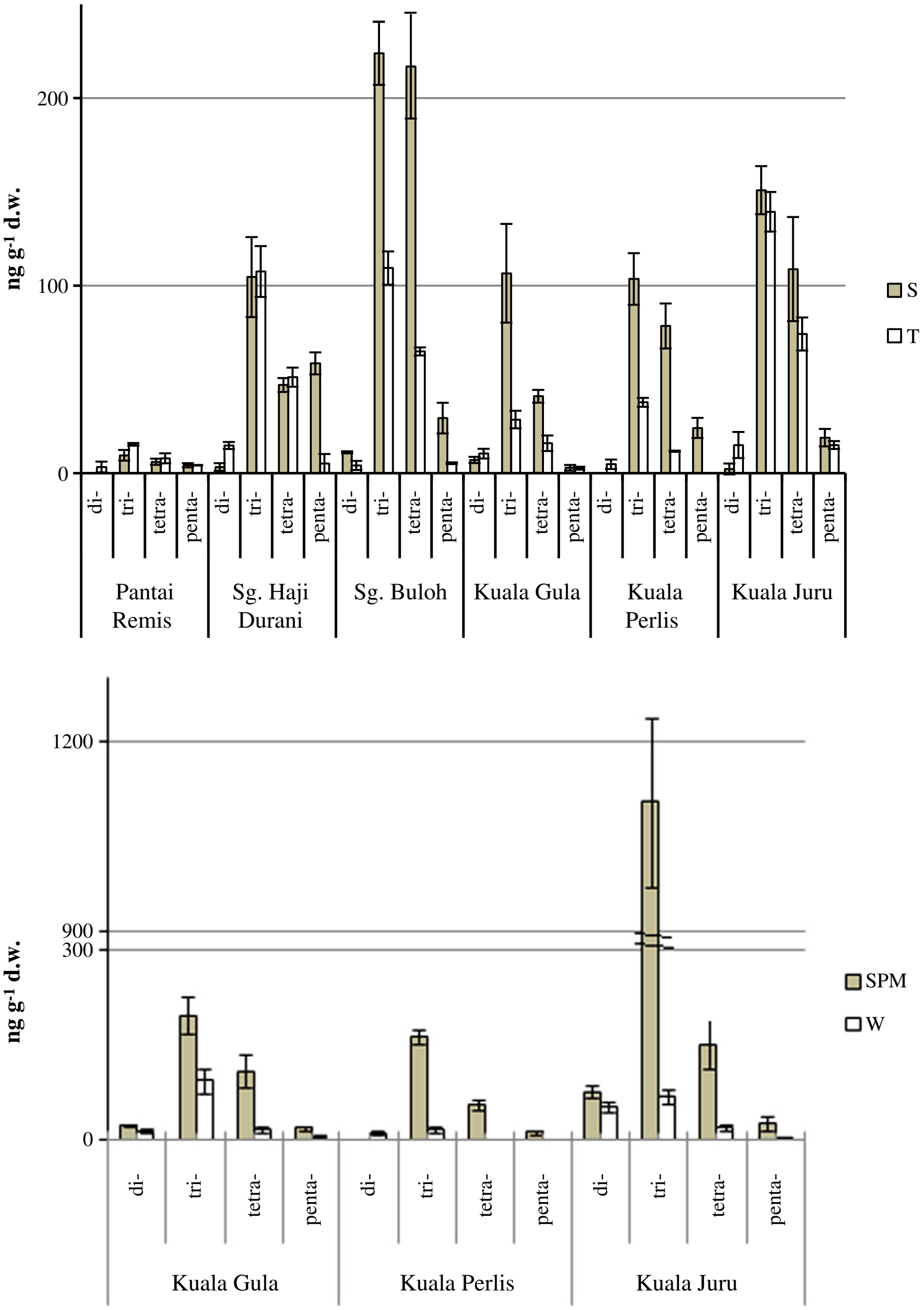

Fig. 3. Concentrations of PAHs by group of aromaticity (di-, tri-, tetra-, and penta-aromatics) sediment (S), blood cockle soft tissue (T) (ng g^{-1}), water (W) and suspended particulate matter (SPM) samples (ng l^{-1}) based on ring numbers (mean ± STD of three replicates, $p < 0.05$).

strong correlation with D1. A correlation with D1 was also found in water and soft tissues of Kuala Perlis (Fig. 6). On the contrary, sediment and soft tissues of Sungai Buloh showed a strong correlation with D2, which indicates a lower correlation between the sources of PAHs in sediment and soft tissues. On the other hand, water, sediment and tissues of Kuala Juru showed no correlation with D1 and D2; therefore, there was not a strong correlation. In addition, no correlation was found between sediment and soft tissues of Sungai Haji Durrani. The results of the present study showed that under environmental conditions, the sedimentary load of hydrocarbons appears to be one of the factors controlling their bioavailability.

4. Discussion

4.1. PAH concentration in mudflat areas

The concentration of tPAHs in the sediment of Malaysian mudflats can be classified as low to moderate pollution, which is in agreement with other findings from the South and Southeast Asia region (Isobe et al., 2007). Moreover, the results were the same magnitude as the reported values of estuarine and river sediments reported for sediments from polluted rivers along the west and east coasts of Peninsular Malaysia as well as some other developing countries (Bakhtiari et al.,

Table 1
Bioaccumulation factor (BAF) and biota–sediment accumulation factor (BSAF) of blood cockle based on rings of PAHs in different matrices (mean ± STD of three replicates, $p < 0.05$).

Criteria	Ring no. Station	Σtwo	Σthree	Σfour	Σfive	c-PAH[a]	tPAH
BSAF	Pantai Remis	3.97 ± 1.70	1.67 ± 0.44	1.28 ± 0.30	1.00 ± 0.14	3.75 ± 1.05	1.55 ± 0.54
	Sg Haji Durani	3.60 ± 0.95	1.09 ± 0.24	1.02 ± 0.19	0.09 ± 0.02	0.70 ± 0.11	0.84 ± 0.27
	Sungai Buloh	0.31 ± 0.10	0.48 ± 0.14	0.33 ± 0.10	0.18 ± 0.02	0.28 ± 0.03	0.39 ± 0.15
	Kuala Gula	0.43 ± 0.10	0.14 ± 0.05	0.12 ± 0.06	0.00 ± 0.00	0.32 ± 0.09	0.17 ± 0.06
	Kuala Perlis	5.84 ± 1.39	0.21 ± 0.23	0.24 ± 0.22	0.00 ± 0.00	0.11 ± 0.09	0.23 ± 0.06
	Kuala Juru	0.20 ± 0.09	0.12 ± 0.05	0.31 ± 0.04	0.43 ± 0.04	0.84 ± 0.20	0.17 ± 0.07
BAF_{SP}[b]	Kuala Gula	0.43 ± 0.10	0.14 ± 0.02	0.12 ± 0.05	0.00 ± 0.00	0.12 ± 0.02	0.17 ± 0.05
	Kuala Perlis	2.36 ± 1.26	0.23 ± 0.03	0.20 ± 0.02	0.00 ± 0.00	0.24 ± 0.08	0.23 ± 0.09
	Kuala Juru	0.20 ± 0.09	0.11 ± 0.04	0.29 ± 0.05	0.43 ± 0.03	0.65 ± 0.30	0.17 ± 0.08
BAF_W[c]	Kuala Gula	0.88 ± 0.61	0.26 ± 0.07	0.75 ± 0.41	0.00 ± 0.00	1.07 ± 0.24	0.41 ± 0.11
	Kuala Perlis	0.37 ± 0.19	1.82 ± 0.38	1.67 ± 0.04	0.00 ± 0.00	1.11 ± 0.07	1.96 ± 0.36
	Kuala Juru	0.29 ± 0.12	1.67 ± 0.12	3.90 ± 1.06	2.36 ± 0.78	2.43 ± 0.09	1.69 ± 0.07

[a] c-PAHs: carcinogenic PAHs including Chry, BaAnt, BkFluo, BaPy, DBahAnt.
[b] BAF_{SP}: bioaccumulation factor of adsorbed contaminants into suspended particular matter in water.
[c] BAF_W: bioaccumulation factor of freely dissolved contaminants in water.

2009; Burgess et al., 2009; Feng et al., 2007; Liu et al., 2001; Ma et al., 2007; Sakari et al., 2008a,b; Sanger et al., 1999; Zakaria et al., 2002). Among other Southeast Asian countries, Malaysia is undergoing rapid industrial development, urbanization, motorization and industrialization. Therefore, toxic industrial pollution has affected most of the environmental matrices, including sediment. The term of development usually coincides with the use of oil as an energy source. The most polluted station is Sungai Buloh, which is near the area where an oil spill occurred (Zakaria et al., 2002). The comparison between present PAH concentrations in Sungai Buloh and Sungai Haji Durani (Fig. 2) with those found by a previous study on Kuala Selangor (Zakaria et al., 2002) confirms increased PAH levels. The most likely reason is that the estuaries in this region are affected by development, industrialization and urbanization. The second area in the present study, Kuala Juru, with concentrations of pollution (see Fig. 2) is in Penang which is an industrialized and urbanized state on the northwest coast of Peninsular Malaysia which is a main oil shipping route. One of the main commercial activities on the mudflats in this area is a port where petroleum is unloaded from ships (Alkarkhi et al., 2008; Sakari et al., 2008a). The present study (Fig. 2) found less than the reported amount of tPAHs from nearby offshore and coastal sampling sites (Shafiee et al., 2008; Zakaria et al., 2002), which is because of the unique characteristics of the mudflats. First, the proportions of particles less than 53 μm in diameter in the sediments of the study areas containing soft intertidal mud (silt and clay) were from

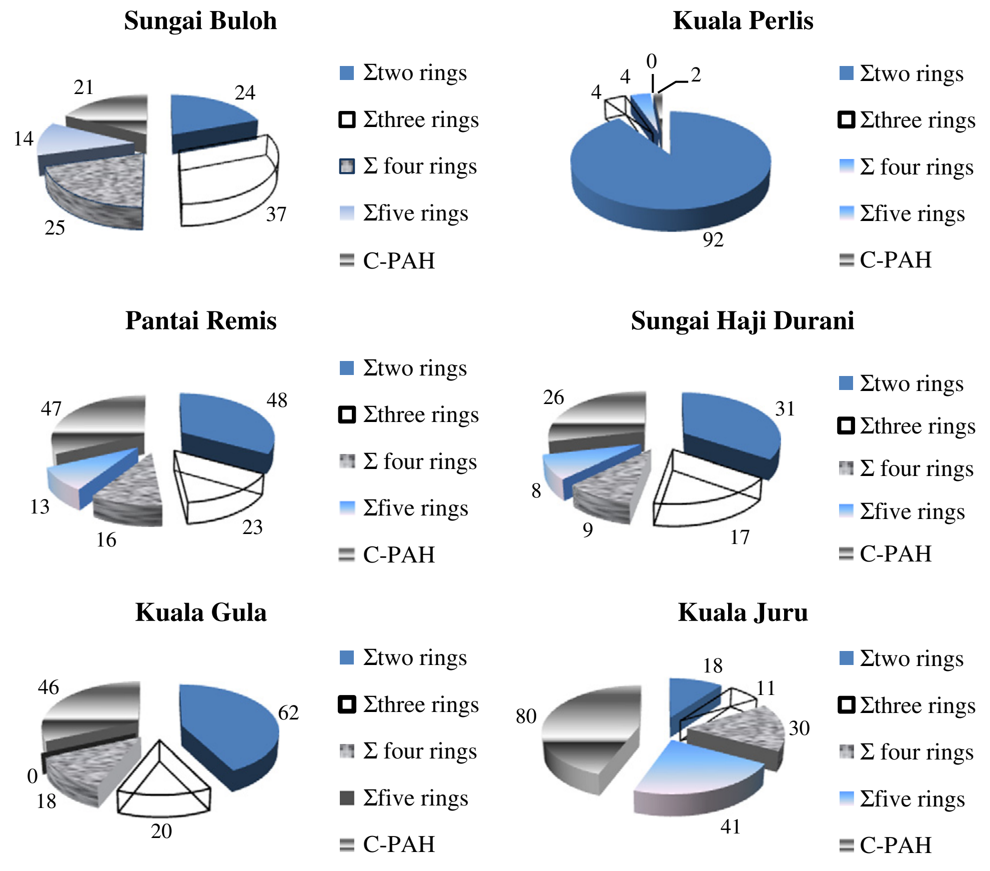

Fig. 4. Relative percentages of biota–sediment accumulation factors of blood cockle soft tissue (mean of three replicates, $p < 0.05$).

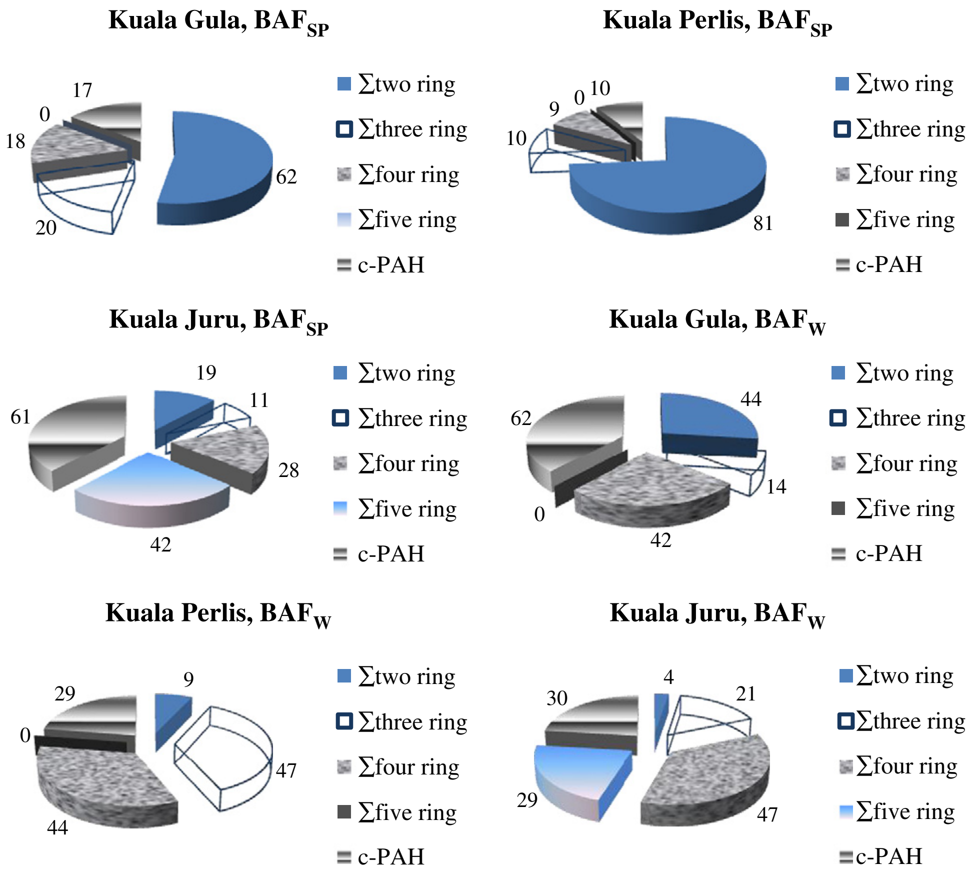

Fig. 5. Relative percentages of bioaccumulation factor of water (BAF_W) and bioaccumulation factor of SPM (BAF_{SP}) of blood cockle soft tissue (mean of three replicates, $p < 0.05$).

46% to 90%, and 90% of the particles were less than 124 μm in diameter (Broom, 1982, 1985). The results showed the lowest concentration of PAHs associated with smaller sized fractions (less than 124 μm) as well as silt and clay fractions (Wang et al., 2001). Second, bacteria are very prevalent because of the high organic content of the mud. In the mudflats, the plentiful bacteria help break down contaminants from urban runoff, such as hydrocarbons (Capital Regional District, 2010). Cammen (1991) reported that bacterial biomass and bacterial production, were four to five times higher in the mudflats than in the sandflats (Cammen, 1991). Third, although heavy rain and particle sedimentations transferred land-based pollutants into aquatic environments (Zakaria et al., 2002), the effect of rain could deplete PAHs and large amounts of river-born contaminants. Because the sampling was conducted at the end of the rainy season, depletion might have occurred in the sediments.

The tPAH concentrations in SPMs found in the study area were three to ten orders of magnitude higher than those found in filtered

Table 2
Different diagnostic tracers in mudflat sediment samples (mean ± STD of three replicates, $p < 0.05$).

Matrix	Ratio Station	LMW/HMW[a]	Phen/Ant[a]	Fluo/Pyr[a]	MP/P[a]	BaAnt/Chry[a]	Source
S[a]	Pantai Remis	0.9 ± 0.1	0.4 ± 0.2	0.0 ± 0.0	4.0 ± 1.6	0.7 ± 0.3	Mostly Pet
	Sg. Haji Durani	1.0 ± 0.5	0.2 ± 0.1	0.6 ± 0.1	1.9 ± 0.2	0.8 ± 0.3	Mostly Pet
	Sungai Buloh	1.0 ± 0.2	1.0 ± 0.3	0.7 ± 0.1	1.0 ± 0.0	0.6 ± 0.1	Pet and Pyr
	Kuala Gula	2.6 ± 0.3	0.2 ± 0.0	0.6 ± 0.1	8.2 ± 3.4	0.0 ± 0.0	Mostly Pet
	Kuala Perlis	1.0 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	11.3 ± 2.9	0.0 ± 0.0	Mostly Pet
	Kuala Juru	1.2 ± 0.3	0.2 ± 0.1	0.6 ± 0.3	8.2 ± 2.0	0.0 ± 0.0	Mostly Pet
W[a]	Kuala Gula	4.8 ± 0.9	0.5 ± 0.1	0.0 ± 0.0	2.1 ± 0.7	1.0 ± 0.3	Mostly Pet
	Kuala Juru	5.5 ± 1.8	0.5 ± 0.1	1.3 ± 1.1	0.3 ± 0.3	1.0 ± 0.2	Mostly Pyr
	Kuala Perlis	30.0 ± 5.0	0.5 ± 0.2	2.0 ± 0.5	0.0 ± 0.0	1.0 ± 0.4	Mostly Pyr
SPM	Kuala Gula	1.7 ± 0.3	0.7 ± 0.5	0.8 ± 0.3	3.4 ± 0.6	0.0 ± 0.0	Mostly Pet
	Kuala Juru	4.4 ± 0.3	0.4 ± 0.1	0.5 ± 0.2	2.4 ± 0.3	0.0 ± 0.0	Mostly Pet
	Kuala Perlis	2.5 ± 0.5	0.6 ± 0.2	0.0 ± 0.0	4.7 ± 0.4	0.0 ± 0.0	Mostly Pet
T[a]	Pantai Remis	1.6 ± 0.2	0.51 ± 0.12	0.00 ± 0.00	2.24 ± 0.50	0.00 ± 0.00	Mostly Pet
	Sg. Haji Durani	2.2 ± 0.6	0.2 ± 0.1	0.7 ± 0.2	3.2 ± 1.1	0.4 ± 0.4	Mostly Pet
	Sungai Buloh	1.6 ± 0.3	0.6 ± 0.1	0.9 ± 0.2	2.0 ± 0.5	1.0 ± 0.3	Mostly Pet
	Kuala Gula	2.2 ± 0.5	0.2 ± 0.1	0.6 ± 0.2	11.8 ± 1.5	0.3 ± 0.2	Mostly Pet
	Kuala Perlis	3.6 ± 0.3	7.4 ± 1.2	0.0 ± 0.0	0.6 ± 0.2	0.0 ± 0.0	Mostly Pet
	Kuala Juru	1.7 ± 0.7	0.4 ± 0.2	0.6 ± 0.3	2.3 ± 0.4	0.0 ± 0.0	Mostly Pet

[a] LMW/HMW: a ratio of total lower molecular (sum of Nap, DbT, Phe, Ant, 9-MPhe, 3-MPhe, 2-MPhe, 1-MPhe, 2-MAnt, Fluo) to total higher molecular (sum of 1-MPy, Pyr, Chry, BaAnt, BkFluo, BeAce, BePy, BaPy, DahAnt); Phe/Ant: the ratio of phenantherene to anthracene; Fluo/Pyr: the ratio of fluoranthene to pyrene; BaAnt/Chry: the ratio of benzo(a)anthracene to chrysene.

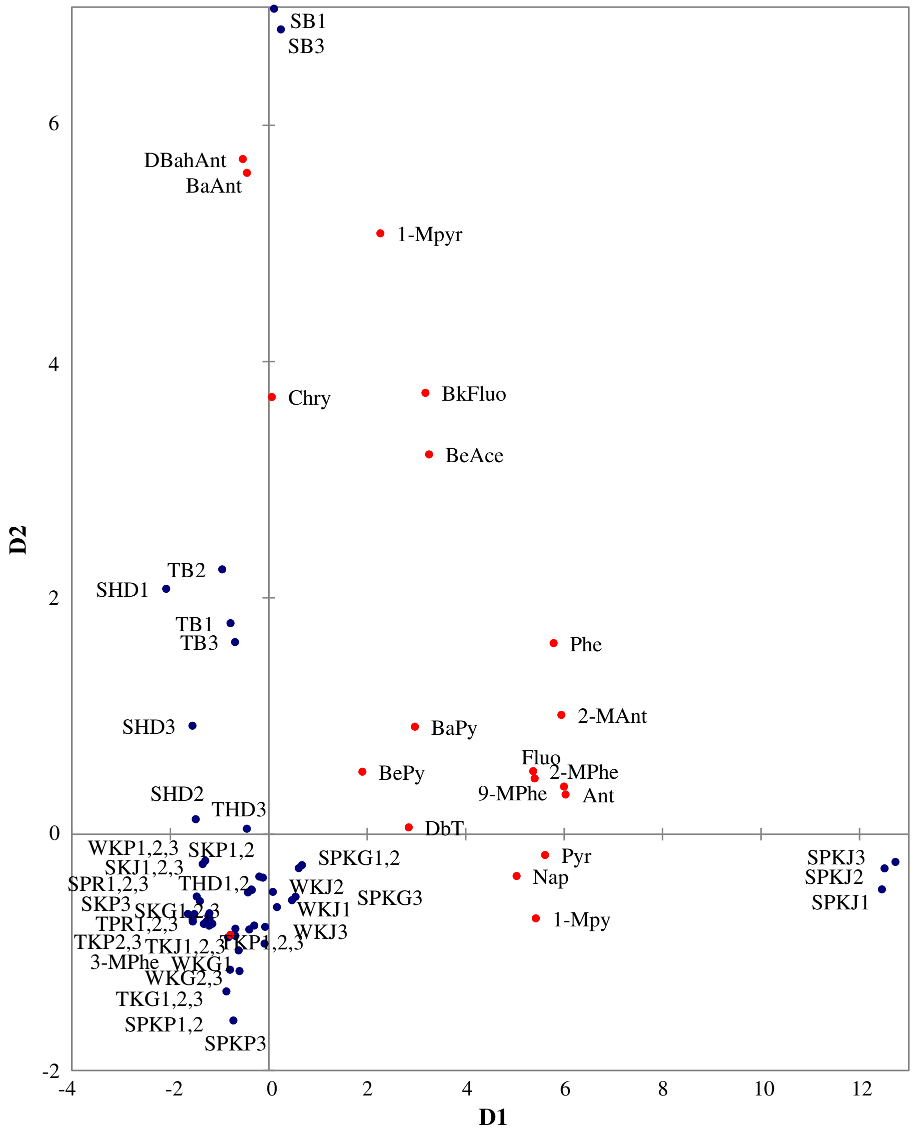

Fig. 6. Biplot of scores and loadings onto the first two principal components (D1 = 46.53%, D2 = 18.83% of the explained variance) of sediment (S), water (W), suspended particles (SP) and tissue (T) data (HD: Sungai Haji Durani; B, Sungai Buloh; PR: Pantai Remis; KP: Kuala Perlis; KG: Kuala Gula; KJ: Kuala Juru).

water, as shown in Fig. 2. Possible reasons for this high concentration of PAHs in SPM include the following. First, the partition of PAHs between the particulate and dissolved phase should be in favour of the particulate phase, as PAHs are hydrophobic and prefer to associate with SPM, colloids, or dissolved organic matter in water, before depositing in sediment (Arias et al., 2009; Guo et al., 2009). Second, higher concentrations of PAHs in SPM are attributable mainly to the tidal currents associated with the transport of sediments. During the rainy season, re-suspension increases owing to the high velocity and flow quantity of the river (Guo et al., 2009). Because the rainfall is well distributed throughout the year, and it rains as many as 150 to 200 days throughout the year almost everywhere in Malaysia, the turbidity of Malaysian rivers is high. On the other hand, it has already been shown that organism activity in sediments increase PAH concentrations in the overlying waters as a result of the bioturbation of sediments (Arias et al., 2009). Third, PAHs are reported to degrade rapidly in sea water, whereas in the highly turbid waters of tidal estuaries, adsorption of PAHs by suspended solids has been found to reduce photolysis, volatilization and microbial degradation (Patel and Eapen, 1989).

The majority of worldwide PAH studies in water focused mainly on seawater (coastal or offshore) and estuarine water. Most surveys of PAH contamination in water bodies were conducted in North America and Europe, such as the USA, England and France (Arias et al., 2009; Cailleaud et al., 2009; Valavanidis et al., 2008; Zhang et al., 2004). Some information concerning water pollution with PAHs in Asia has been reported in China, India and Malaysia. Comparison between results showed water columns of developed countries contained more PAHs than did mudflat areas in Malaysia. Although PAHs in the water of Malaysian mudflats were in the same magnitude as China, they are higher than in Indian rivers and in studied areas of Malaysian coastline waters (Cao et al., 2005; Chouksey et al., 2004; Consumers' Association of Penang, 2005; Feng et al., 2007). As mentioned above, the Juru area in Penang is an established industrial zone that allows different industries to be active there. However, blood cockle farmers reported that the discharge of industrial effluent from the nearby Prai Industrial Estate is affecting the growth of their blood cockles, and those very close to the discharge point are dying (Din and Ahamad, 1995). Higher levels of PAHs in green mussels were also reported in the Penang area (Shahbazi et al., 2010b). The results of tPAHs were the same magnitude as the values reported in studies of other bivalves in Malaysia and some other developing countries (Cheevaporn and Menasveta, 2003; Fang et al., 2009; Francioni et al., 2007; Froun et al., 2007; Isobe et al., 2007; Oros et al., 2007; Siddiqi et al., 2009; Valavanidis et al., 2008; Vinas et al., 2009). Based on the categorization of PAH concentrations in mussels, PAH concentration in blood cockles in Malaysian mudflats can be classified as low (0–100 ng g^{-1} d.w.) to moderate (100–1000 ng g^{-1} d.w.) (Isobe et al., 2007).

The fraction identification of PAHs in the extended mudflats sampled in the present study showed that di-aromatic PAHs have very low concentrations in sediments. The most likely reason is that di-aromatic PAHs are sensitive to light and degrade rapidly. These findings are in agreement with others that showed the PAH contaminations in sediment are dominated mainly by three- four- and five-ring (sum of BePy, BaPy, DBahAnt) PAHs (Feng et al., 2007; Guo et al., 2009; Luo et al., 2006). The profiles of tPAHs in surface water were dominated by lower molecular weights; in particular, two- and three-ring compounds were the most abundant tPAHs. High concentrations of two- and three-ring compounds in water samples are in agreement with other studies (Arias et al., 2009; Cailleaud et al., 2009; Chen et al., 2007; Luo et al., 2006). Low levels of two-ring compounds and relatively high levels of four and five-ring compounds were observed in SPM samples. Nap was one of the most frequently detected compounds in almost all surface water samples (except the SPM of Kuala Perlis). The results showed di-aromatic PAHs, such as Nap, which are usually associated with the dissolved fraction, and penta-aromatic PAHs, such as BaPy, whose occurrence in water primarily adsorbs into the particulate fraction. Due to the high hydrophobicity and thereby low water solubility, the HMW of PAHs have a higher affinity for organic particulate matter (Bellas et al., 2007). The results showed that HMW of PAHs are quite abundant in the soft tissues of Malaysian blood cockles. Other researchers found that faunal organisms accumulate major groups of PAHs differentially. For instance, tri- and penta-ring PAHs have a low uptake rate when compared with four-ring compounds in commercial shellfishes (Vinas et al., 2009). In mussels, *Mytilus galloprovincialis*, oysters, *Crassostrea* sp., crabs, *Carcinus maenas* and mullets, *Mugil cephalusis*, the accumulation pattern of PAHs showed a predominance of tetra- and penta-ring PAHs (Orbea et al., 2002). The differences in bioconcentration patterns among species could be related to the route of uptake of xenobiotics, duration of exposure, seasonal variation, and species biochemistry and physiology among other chemical and/or biological factors (Orbea et al., 2002). High levels of three-ring PAHs, as toxic PAHs, threaten the ecology and biology of ecosystems, aquaculture beds, human health, and traditional ways of residents' lives (Capital Regional District, 2010).

The high concentrations of toxic PAHs in sediment causes the bioaccumulation of HMW of PAHs by the deposit feeder bivalve, *A. granosa*, in Malaysian mudflats because excess amounts of PAHs can accumulate in the bodies of invertebrates, such as bivalves (Capital Regional District, 2010). In this study, the calculated BAF and BSAF values for tPAHs were similar to previously reported values for bivalves and clams (Bender et al., 1988; Cortazar et al., 2008; Mitra et al., 2000; Qian et al., 2001; Viganò et al., 2007). The findings showed notable responses of blood cockle tissue to different level of PAHs in samples from the different stations. The least contaminated station, Pantai Remis, had higher BSAF values but the Kuala Juru station (the second most contaminated station) generally had the lowest BSAF. As stated in Lobo et al. (2010), the time of exposure is known to be a critical factor for the bioaccumulation of contamination. As previously mentioned, the re-suspension of PAHs because of heavy rain, high turbidity, and probable fresh input of pyrogenic contaminates in Kuala Juru station might create a complex situation so that blood cockles may need adaptation prior to reaching the limit of accumulation in relation to the concentration in the sediment under a steady-state condition (Lobo et al., 2010). Higher BAFs in the least contaminated sediment were also observed in the common cockle (*Cerastoderma edule* L.), which suggested that they were a good biomonitor organism (Lobo et al., 2010). Blood cockle tissue from Sungai Haji Durani, Kuala Perlis, Kuala Gula, Kuala Juru, and Sungai Buloh had low BSAF values largely due to the high concentrations of parent PAHs in the sediments at these sites. The calculated BSAF and BAF values for the LMW of PAHs showed different trends in different stations, indicating that the bioavailability of PAHs may be different for different sources of PAHs, which is probably due to the differences in chemical and physical properties of these compounds and their origins (Hellou et al., 2005; Qian et al., 2001). Moreover, the low BSAF values of PAHs might be the results of the restricted bioavailability of PAHs in sinking particles and in sediment. The bioavailability of a neutral hydrophobic compound depends mainly on the physicochemical properties of the compounds (e.g., lypophylic characteristics and water solubility) and on the physicochemical properties of the sediment (e.g., grain size distribution) (Qian et al., 2001). Furthermore, BSAF values may be less than one if the mussel metabolizes the chemical or the system has not reached steady-state (chemicals may not be fully available to the mussels because of very slow desorption or very strong binding) (Thorsen, 2003). In this study, relative bioaccumulations for LMWs are higher than HMWs, but the BAFs or BSAFs were higher for HMWs in samples from the Kuala Juru station. The possible reason is occurrence of heavy rain during the sampling of re-suspended seabed sediments, thereby increasing the total load of organic contaminates in blood cockle tissue. These events modified ingested PAHs enriched by HMW of PAHs, which are bound to sedimentary particles (Stella et al., 2002). It has been established that higher BSAF of HMWs can be seen in high turbidity areas (Baumard et al., 1999). It was found that in water columns and low turbid areas, bivalves can accumulate more LMWs, whereas in high turbid areas, HMWs are slightly higher (Baumard et al., 1999). Hence, heavy rain, which re-suspends sedimentary PAHs in tropical areas (Zakaria et al., 2002), is a main reason for higher levels of HMW in samples taken from the Kuala Juru station. In addition, smaller particles might be partially depleted in the LMW compounds relative to larger fractions of sediments (Baumard et al., 1999). The present results suggest that blood cockle can be used as a good biomonitor of PAH contaminations in mudflats. The profiles of tPAHs between the samples of water and blood cockles from Kuala Perlis and Kuala Gula stations were similar, but in Kuala Juru they were different ($p < 0.05$). Blood cockle tissue showed BAF_W values higher than those in the active bioaccumulation of PAHs by *A. granosa*. It seems that other physico-chemical factors control the bioaccumulation of PAHs at Kuala Juru station. On the other hand, the statistical analysis showed high correlations between sediment and blood cockle in most of the study areas, except Sungai Haji Durani and Kuala Juru. The same profile of individual PAHs together with high correlations between PAHs of sediment and *A. granosa* ($p < 0.05$) showed the high ability of blood cockle in the bioaccumulation of PAHs. This finding is in agreement with previous results that showed the ability of blood cockle to bioaccumulate persistent toxic pollutants in Malaysian offshore areas (Ang et al., 2005; Consumers' Association of Penang, 2005; United Nations Environment Programme, 2002). The results of PCA of tPAHs (Fig. 6) in the present study showed that under environmental conditions, the sedimentary load of hydrocarbons appears to be one of the factors controlling their bioavailability. Other studies observed the accumulation of sediment associated contaminants through either the aqueous phase or ingestion of contaminated sediment particles (Boscolo et al., 2007; Menon and Menon, 1999). Accumulation of sediment associated contaminants may occur through the ingestion of contaminated sediment particles. The relative importance of these routes depends on the ecology and feeding behaviour of the organism and characteristics of the sediment and chemicals (Boscolo et al., 2007; Kukkonen and Landrum, 1995; Menon and Menon, 1999). In addition, the rate of absorption is influenced by other factors, such as the season, age of mussels, and location (Shahbazi et al., 2010a). Although the mechanism of bioaccumulation of sedimentary PAHs by blood cockle, *A. granosa*, is not clear, other sediment dwelling species feed copiously on the suspended sediment as observed during the course of experiments. Powerful lateral cilia located on either side of the gill filaments are responsible for the generation of the main feeding currents, which result in the increased availability of sediment-derived PAH. Some studies found that suspension filter/deposit feeding benthic bivalves, such as *Tapes philippinarum* and *Sunetta scripta* L., appear suitable indicators in sedimentary contamination monitoring (Boscolo et al., 2007; Menon and Menon, 1999).

4.2. Blood cockle as a biomonitor

Because the profiles of tPAHs between environmental matrices and blood cockle were similar, these findings show that the blood cockle can be used as a good biomonitor of PAH contaminations in mudflats. Multiple comparisons of tPAHs by MANOVA showed no significant differences ($p > 0.05$) between tPAHs and LMW of SPM and sediment samples in all six stations. On the other hand, tPAHs and LMWs were significantly higher in SPM compared with the soft tissues of blood cockle and the water samples ($p < 0.05$). MANOVA analysis of HMW showed no significant differences ($p > 0.05$) between tPAH of blood cockle tissue and other environmental matrices. It is well established that only a fraction of tPAHs in the sediment is available for equilibrium partitioning to the aqueous phase, which depends mainly on the sediment–water partitioning qualities of PAH. The partitioning of the hydrophobic organic contaminants between particulate and dissolved phases controls their environmental fate and availability to aquatic organisms (Menon and Menon, 1999). By the action of tidal currents or storms, less dense sediment particles are brought into suspension. Thus, the ultimate fate of hydrophobic organic contaminants in estuaries is linked closely to their associations with particles and to the transport, degradation, and burial of these particles (Guo et al., 2009; Menon and Menon, 1999). Malaysian estuaries and mudflats have a very high quantity of suspended solids, which is comprised of phytoplankton debris, lipids, proteins and so on. These contribute to the rich organic load of the suspended particulate matter (Broom, 1985; Menon and Menon, 1999) and high concentration of PAH contamination. The extremely high concentration of PAHs in the SPM samples indicated the existence of potential pollution sources (Guo et al., 2009; Menon and Menon, 1999). Due to low aqueous solubility, PAHs in aquatic systems generally adsorb to organic and inorganic particulate materials that become deposited in bottom sediment and accumulate in the tissues of aquatic organisms (Roper et al., 1997). Our study supports this scenario as tPAH concentrations in the water column were low but were elevated in the sediment. The statistical analysis showed that the concentration of sedimentary PAHs is higher than that of dissolved PAHs at all stations because of their persistence in the sediment compared to the water phase. tPAH concentrations in the blood cockle tissues were less than those in the sediment. PAH content in the *A. granosa*, irrespective of its ecological niche, maintained tissue levels in equilibrium with the sediment (Patel and Eapen, 1989). In general, the bioaccumulation of organic compounds present in solution has been directly related to the octanol/water partition coefficient. The bioavailability of these compounds could also be modified by the presence of humic materials in estuarine and coastal waters (Patel and Eapen, 1989).

Table 3
Comparison of tPAHs, LMW and HMW levels between the soft tissues of *A. granosa* and the environmental matrices in different stations.

	Matrix order	Tissue	Sediment	Water	SPM
tPAHs	1	Kuala Juru	Sungai Buloh	Kuala Juru	Kuala Juru
	2	Sungai Buloh	Kuala Juru	na [a]	na
	3	Haji Durani	Haji Durani	na	na
	4	Kuala Gula	Kuala Perlis	Kuala Gula	Kuala Perlis
	5	Kuala Perlis	Kuala Gula	Kuala Perlis	Kuala Gula
	6	Pantai Remis	Pantai Remis	na	na
LMW	1	Kuala Juru	Sungai Buloh	Kuala Juru	Kuala Juru
	2	Haji Durani	Kuala Juru	na [a]	na
	3	Sungai Buloh	Kuala Gula	na	na
	4	Kuala Perlis	Haji Durani	Kuala Gula	Kuala Gula
	5	Kuala Gula	Kuala Perlis	Kuala Perlis	Kuala Perlis
	6	Pantai Remis	Pantai Remis	na	na
HMW	1	Sungai Buloh	Kuala Juru	Kuala Juru	Kuala Juru
	2	Kuala Juru	Sungai Buloh	na [a]	na
	3	Kuala Perlis	Haji Durani	na	na
	4	Haji Durani	Kuala Gula	Kuala Gula	Kuala Gula
	5	Kuala Gula	Kuala Perlis	Kuala Perlis	Kuala Perlis
	6	Pantai Remis	Pantai Remis	na	na

[a] na: data is not available.

To support the use of blood cockle as a biomonitor of PAH pollution in the intertidal mudflats, a comparison of PAH concentrations among the sediments, water, SPM and blood cockle was conducted (Table 3 and Fig. 2). Table 3 shows that the highest levels of tPAHs were found in the surface sediments of Sungai Buloh and Kuala Juru, which was in agreement with the high levels of tPAHs found in the soft tissues of blood cockle from these sites. Similar to the distribution patterns of PAHs in sediment samples, the concentrations of PAHs in water and SPM samples from Kuala Juru were also significantly higher than those from other stations ($p < 0.05$). Based on the results of ANOVA analysis among different stations, there were no significant differences ($p < 0.05$) between levels of tPAHs in Kuala Juru and Sungai Buloh or between Kuala Perlis and Kuala Gula. Therefore, disorders in patterns of PAH levels in blood cockle and other environmental matrices were not statistically significant. Based on the results, *A. granosa* is suggested to be a good biomonitor of tPAHs. This is in agreement with the findings of other studies, which reported that bivalves and cockles can be used as sentinel organisms to monitor PAH contamination. These organisms concentrate PAHs from the surrounding water media, therefore making chemical analysis simpler and less prone to error than PAHs in water. In surface seawater, possible photo-degradation and weathering can reduce PAH concentrations (Valavanidis et al., 2008).

ANOVA analysis clearly showed that LMW levels were significantly different ($p < 0.05$) only between Kuala Juru and Pantai Remis as well as Sungai Buloh and Pantai Remis. Any significant differences between other stations were not found. Table 3 and Fig. 7(a) show high levels of LMWs found in the blood cockle, water, and SPM of Kuala Juru. Although some disorders were clear in the pattern of LMW in different matrices, the statistical results did not show any significant differences between stations ($p < 0.05$). Therefore, the analysis showed that blood cockle can be used as a biomonitor of LMW with some considerations.

ANOVA analysis found that levels of HMWs were significantly different ($p < 0.05$) between Sungai Buloh and other stations. In addition, the HMW of Sungai Haji Durani was significantly different from that of Pantai Remis. Table 3 and Fig. 7(b) show that HMWs were not in the same order in the sediment and blood cockles of Sungai Buloh ($p > 0.05$). Unfortunately, there were not enough data to compare water and SPM of Sungai Buloh. As available data from other stations did not show any statistical differences ($p > 0.05$), further studies are necessary to confirm the utility of *A. granosa* as a biomonitor of HMWs.

The results of this study prove that the bioavailability of PAH to suspension feeding bivalves is enhanced because of their inhabited environment (Menon and Menon, 1999). In addition, our findings show that there is a high correlation between water and the soft tissues of blood cockle ($p < 0.05$). This finding is in agreement with other studies reporting that hydrocarbons dissolved in sea water reflect the body burden more than those associated with the particulate form (Boscolo et al., 2007; Menon and Menon, 1999; Patel et al., 1985; Patel and Eapen, 1989). When blood cockles live in low turbidity areas and/or are located near the air–water interface (0–2 m from

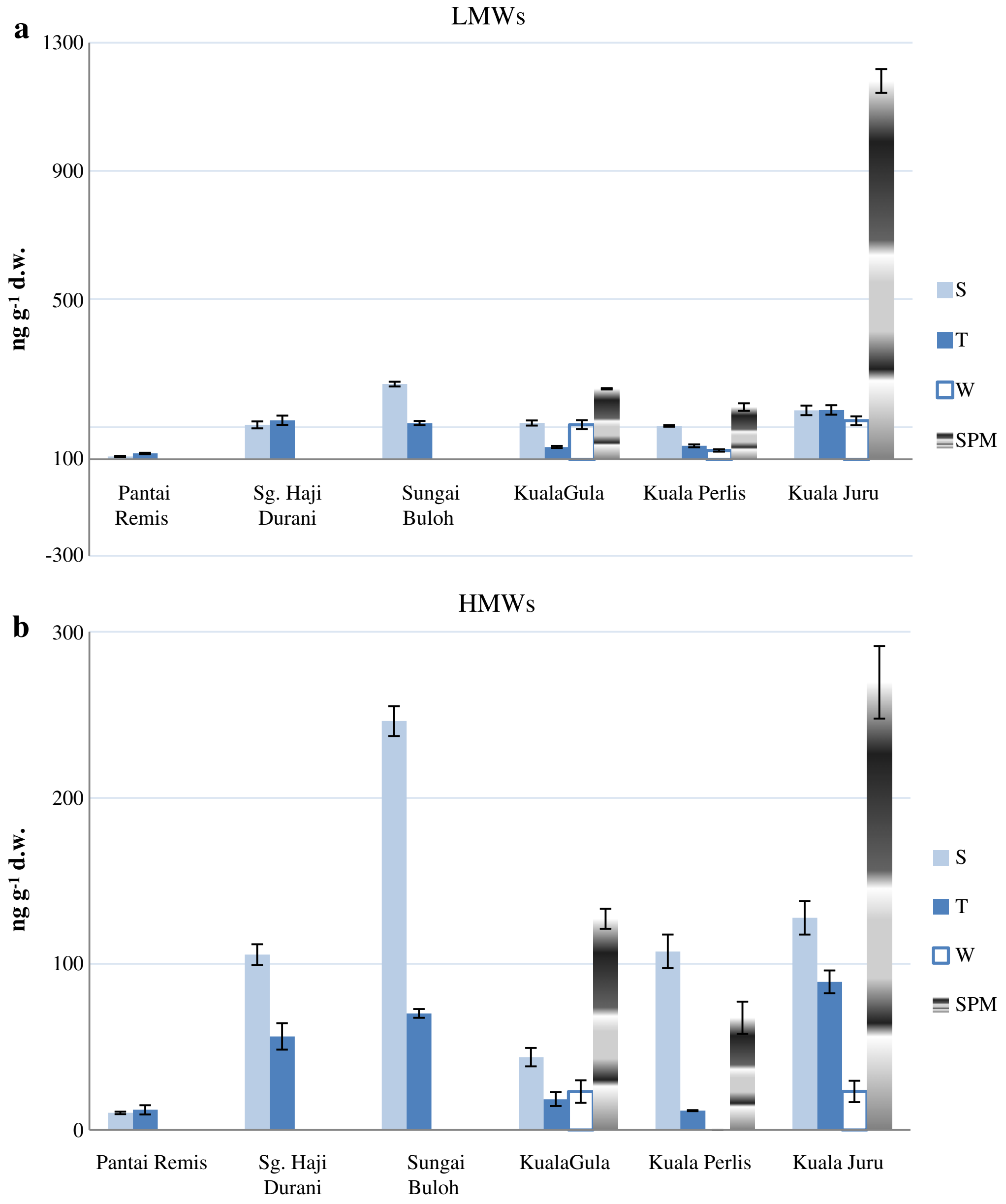

Fig. 7. LMW (a) and HMW (b) of PAH (2–4 rings) distribution patterns in the water (W), sediment (S), suspended particulate matter (SPM) and blood cockle tissue (T) at different locations (mean ± STD of three replicates, $p < 0.05$).

surface), they are exposed to a water soluble fraction of contamination. The blood cockle samples in high turbidity areas accumulate HMW compounds to a slightly higher degree than do LMWs. Blood cockles close to sediment but not in turbid areas accumulate more LMWs than HMWs do. Moreover, blood cockles can absorb contamination from re-suspended sediment as well as the dissolved fraction of PAHs (Baumard et al., 1999).

4.3. Isomer ratios, biomarkers

According to the source identification in this study, PAH compositional patterns and diagnostic ratios reflected a mixture of both petrogenic and pyrolytic sources. Petrogenic sources were predominant in most of the samples of sediments in stations along the Malaysian mudflats. Researchers found similar results in Malaysian urban riverine sediments that were heavily affected by petrogenic PAHs (Bakhtiari et al., 2009; Sakari et al., 2008b; Zakaria et al., 2001, 2002). However, these results contrast other findings that showed pyrogenic PAHs are unavailable in the water phase, whereas PAHs may associate more strongly with particles (McGroddy and Farrington, 1995). The exceptions among the studied areas were found in the water samples from Kuala Perlis and Kuala Juru, which were dominated by pyrogenic sources of PAHs (Table 2). The possible explanation for this observation is that the PAH inputs were very fresh and did not have enough time to reach equilibrium (the sorption experiments suggest a time scale from 24 h to 48 h) (McGroddy and Farrington, 1995). In other industrialized countries, researchers found a mixture of petrogenic and pyrogenic sources with a dominance of pyrogenic sources in the estuarine sediments of urban rivers (Cho et al., 2008; Feng et al., 2007; Holland et al., 1993; Huntley et al., 1995; Liu et al., 2008; Ma et al., 2007; Sanger et al., 1999; Shafiee et al., 2008; Zakaria and Mahat, 2006; Zakaria et al., 2002).

5. Conclusion

Low levels of tPAHs were found in dissolved water, moderate levels of tPAHs were found in *A. granosa*, and high levels of tRAHs were found in sediment and SPM. The source of PAHs in different environmental matrices was mostly petrogenic, except for water from Kuala Perlis and Kuala Juru, in which dominant pyrogenic sources were observed. In water and SPM samples, two- and three-ring PAHs were dominant, whereas in sediment and blood cockle tissue samples, three- and four-ring PAHs were dominant. PCA and MANOVA analyses found that blood cockles can accumulate PAHs from water as well as sedimentary PAHs ($p < 0.05$), based on the physico-chemical properties of their niche. The BAFw values were higher than 1.0 in all stations except Kuala Juru, while BSAF and BAFsp values were less than 1.0. RBAF and RBSAF showed higher bioaccumulation of PAHs from LMWs in all stations, except for Kuala Juru where HMWs showed higher bioaccumulation in blood cockle samples. In conclusion, *A. granosa* showed a high response to sediment bond PAHs as well as freely dissolved PAHs in water column ($p < 0.05$). Therefore, blood cockle is suggested as a suitable biomonitor organism in PAH studies on extended mudflats. Considering the acute toxicity of LMWs in addition to the potential mutagenicity and carcinogenicity of HMWs, the bioaccumulation of PAHs are a potential hazard for both blood cockles and their consumers.

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