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Contamination of Heavy Metals and other Organic Pollutants in *Perna viridis* from the Coastal Waters of Malaysia: A Review Based on 1998 Data

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Author's contribution

The author personally had done all the samplings conducted in 1998. All the heavy metal analysis and drafting/writing of this whole paper was solely done by the author himself.

Review Article

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ABSTRACT

Since 1990s until today, the Asia-Pacific Mussel Watch approach has been widely used for biomonitoring purpose in Malaysia by using the green-lipped mussel *Perna viridis* in particular. This paper reviewed the concentrations of heavy metals (Cd, Cu, Pb and Zn), Linear Alkylbenzenes (LABs), Polycyclic Aromatic Hydrocarbons (PAHs), phenolic Endocrine Disrupting Chemicals (EDCs) [nonylphenol (NP), octylphenol (OP), and bisphenol A (BPA)] and organochlorine (OC) compounds (PCBs, DDTs, CHLs, HCHs and HCB) in nine mussel populations collected in 1998 (because I involved in the sampling field trip when I was a trainee researcher) from the coastal waters of Malaysia. In fact, all of these data were published separately in five different peer-reviewed journals in the literature (see Yap et al. [6,7]; Tsutsumi et al. [14]; Isobe et al. [15]; Monirith et al. [16]). Since they discussed only based on the group of contaminants which they focused upon, this review paper aimed to see a holistic picture and understanding of the impacts of the different chemical contaminants in relation to the description of the sampling sites. Based on seven mussel populations with complete 11 chemicals (ranging from heavy metals, LABs, PAHs, phenolic EDCs and OCs), a dendrogram was established using single linkage cluster analysis. A dendrogram was established using single linkage cluster analysis showing two major subclusters. The first subcluster comprised Tanjung Rhu,

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Trayong, Kuala Penyu and Pasir Panjang populations, indicating relatively uncontaminated conditions while the other subcluster consists of Penang, Kg. Pasir Puteh and Anjung Batu which indicated contaminated conditions as it is well supported by the elevated levels of some chemicals. The subcluster combining Penang and Kg. Pasir Puteh populations were mainly due to the elevated levels of LABs and PAHs in both sites while Kg. Pasir Puteh also had elevated levels of Cu, Pb, PCBs and CHLs. Anjung Batu, which is also clustering together with Penang and Kg. Pasir Puteh population can be explained by its elevated levels of three OC compounds namely DDTs, CHLs and HCHs.

Keywords: Mussel watch programme; Perna viridis; Malaysia.

1. INTRODUCTION

Nowadays, Mussel Watch approach has been widely employed for biomonitoring of chemical contaminants in the coastal waters. Until 11 June 2013, a total of 2,557 articles related to Mussel Watch program or approach can be easily found under the Sciencedirect website alone, indicating there is even more numbers of such studies can be found in the literature with other scientific indexing websites. The latest paper on Mussel Watch is that by Maruya et al. [1] published in *Marine Pollution Bulletin* while the latest one on *Perna viridis* was that on the transplanted caged mussels in the Straits of Johore by Eugene Ng et al. [2] published in *Pertanika Journal of Science and Technology*.

According to Widdows and Donkin [3], the main reasons for assessing the levels of different groups of chemical contaminants in coastal waters were 1) to protect human health by estimating exposure via dietary route back to man and 2) to protect valuable living natural resources. Hence, the Mussel Watch approach initially proposed by Professor E. D. Goldberg in 1975 for the assessment of chemical contaminants in the coastal waters is of very much contributive to biomonitoring studies [4].

Basically, application of green-lipped mussel *Perna viridis* as a biomonitor for organic and inorganic pollution in the Malaysian coastal waters was initiated by several researchers who conducted biomonitoring studies using blue mussel *Mytilus edulis* [4,5]. This is due to the ecological characteristics of marine mussels such as being wide geographical distribution, sedentary lifestyle, stable population, easy sampling, bioaccumulative of and correlative properties with the average pollutants of the environment, tolerance to salinity, resistance to stress due to high accumulation of wide range of pollutants and providing an assessment of bioavailability. Ever since, there is an increasing numbers of such biomonitoring studies utilizing mussels in Malaysia being reported in the literature [6-13].

The heavy metal data reviewed and cited in this paper was that by Yap et al. [6] who reported the four popular heavy metal (Cd, Cu, Pb and Zn) concentrations in the total soft tissues of *P. viridis* from Peninsular Malaysian coastal waters while the data from East Malaysia (Sabah) was cited from Yap et al. [7].

For Linear Alkylbenzenes (LABs), the data were both reported by Tsutsumi et al. [14] and Isobe et al. [15]. In particular, Tsutsumi et al. [14] applied the LABs to be molecular markers for sewage input. In addition, the data on Polycyclic Aromatic Hydrocarbons (PAHs), phenolic Endocrine Disrupting Chemicals (EDCs) were also reported from Isobe et al. [15]. The phenolic EDCs reported included Nonylphenol (NP) and Octylphenol (OP), and

Bisphenol A (BPA) in which they are collectively termed as phenolic EDCs because of their phenolic structures in the molecules. The Alkylphenols such as nonylphenol (NP) and octylphenol (OP) are degradation products of alkylphenolpolyethoxylates (APnEO), which are commercially important nonionic surfactants with industrial, agricultural, and domestic applications.

Lastly, the most comprehensive data on monitoring organochlorine compounds (OCs) and polychlorinated biphenyls (PCBs) pollution in the Asia-Pacific region including Malaysia, was that by Monirith et al. [16]. OCs represents ‘‘persistent organic pollutants (POPs)’’ and are of great concern due to their bioaccumulative nature and toxic biological effects on wildlife and humans [17]. The undesirable effects of some of these chemicals are linked to the occurrence of immunologic and teratogenic dysfunction, reproductive impairments and endocrine disruption in lower and higher trophic levels [18]. In this paper, besides PCBs data were reviewed, other OCs data included were DDT and its metabolites (DDTs), hexachlorocyclohexane isomers (HCHs), chlordane compounds (CHLs), and hexachlorobenzene (HCB).

Most interestingly, all the mussels samples from Malaysia from the above papers reviewed and reported, came from a series of sampling trips in which I involved in sampling field trip of Peninsular Malaysia when I was a trainee researcher. The sampling trip was in fact, one of the series for Asia-Pacific Mussel Watch program in 1998. As a research student, I focused on heavy metal analysis in the mussel samples collected in the same year in order to establish background data for Malaysian coastal waters. The aim of this paper was to review and compile all the reported data for heavy metals, linear alkylbenzenes, polycyclic aromatic hydrocarbons, phenolic endocrine disrupting chemicals and organochlorine compounds in *Perna viridis* collected in 1998, from the coastal waters of Malaysia, in order to discuss them in a more holistic understanding on the different groups of chemical contaminants focusing on Malaysia’s scenario.

2. MATERIALS AND METHODS

Nine sampling sites for mussels were conducted in 1998. Sampling sites are shown in Figure 1 while descriptions for each of the sampling sites are given in Table 1.

Briefly, for metal analysis, the samples were digested in concentrated HNO_3 (AnalaR grade, BDH 69%). The digested samples were then diluted to a 40mL with double distilled water (DDW). After filtration, the prepared samples were determined for heavy metals by an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model 4100 (Yap et al. [6,7]).

Table 1. Information of *Perna viridis* samples collected from Malaysian coastal waters in 1998

No.	Sampling sites	GPS	Sampling dates	N	Moisture (%)	Lipid (mg/g dry)	Shell length (mm)	Site description
1.	Sangkar Ikan (SIkan), Langkawi	Lat: 6º16´ Long: 99º45´	20 Sep 1998	25	83.8	44	69.8 (61.9-91.0)	A fish aquaculture site.
2.	Tanjung Rhu (TRhu), Langkawi	Lat: 6º25´ Long: 99º44´	20 Sep 1998	25	84.9	73	70.6 (67.0-74.7)	A recreational beach and fish aquaculture site.
3.	Penang Bridge (Penang), Penang-1	Lat: 5º20´ Long: 100º20´	21 Sep 1998	25	81.2	66	73.4 (47.4-100.0)	Shipping lane, industry and urban area.
4.	Pasir Panjang (PPjg), Negeri Sembilan	Lat: 2º25´ Long: 101º56´	22 Sep 1998	25	81.3	86	88.6 (68.1-109.1)	A recreational beach and mussel aquaculture site.
5.	Anjung Batu (ABatu), Malacca	Lat: 2º14´ Long: 102º08´	22 Sep 1998	25	83.7	60	92.2 (84.3-106.1)	An agriculture and fish aquaculture area.
6.	Pantai Lido (PLido), Johore	Lat: 1º27´ Long: 103º41´	1) 30 May 1998 2) 23 Sep 1998	25	83.1	71	59.4 (46.8-72.2)	City area.
7.	Kg. Pasir Puteh (KPPuteh), Johore	Lat: 1º26´ Long: 103º55´	23 Sep 1998	25	81.0	112	64.4 (41.9-92.9)	Shipping, near a port, industry and urban area.
8.	Trayong, Sabah.	Lat: 5º12´ Long: 115º43´	29 Sep 1998	25	87.1	54	109.8 (89.8-120.6)	Agriculture and aquaculture area.
9.	Kuala Penyu (KPenyu), Sabah.	Lat: 5º53´ Long: 115º52´	29 Sep 1998	21-25	88.1	59	66.9 (57.1-76.6)	Agriculture and aquaculture area.

Note: Data for moisture and lipids were cited from Isobe et al. *[15]*
Long= Longitude; Lat= Latitude.

For LABs analysis, approximately 15 g of homogenized wet samples were macerated with dichloromethane (DCM) and anhydrous sodium sulfate using a Polytron RT2000 (Kinematica, Switzerland). The extracts were then subjected to purification, fractionation, and instrumental analysis. Briefly, the extracts were purified and fractionated using two-step silica gel column chromatography and the alkylbenzene fraction was determined by gas chromatography–mass spectrometry (GC–MS) in selected ion monitoring mode at m=z ¼ 91, 92 and 105 [14].

Polycylic Aromatic Hydrocarbons (PAHs) and phenolic endocrine disrupting chemicals done by Isobe et al. [19] covered a wide range of compounds from non-polar hydrocarbons (e.g. n-alkanes) to polar compounds (e.g., bisphenol A). They include various EDCs (i.e., PCBs, DDTs, PAHs, NP, OP, phthalates, BPA, and natural estrogens) and molecular markers (e.g., alkylbenzenes and hopanes). This method relied upon maceration/homogenization, extraction, gel permeation chromatography (GPC), two-step silica gel chromatography, and gas chromatography-mass spectrometry (GC-MS). Detailed analytical conditions and quantification procedures are described by Isobe et al. [15,19]. LABs and PAHs were determined by a Hewlett Packard 5973 or 5972 quadrupole mass spectrometer equipped with HP6890 or HP5890 gas chromatograph, respectively.

Polychlorinated biphenyls (PCBs) and organochlrorine (OC) insecticides such as DDT and its metabolites (DDTs: p; p0-DDT, p; p0-DDD, and p; p0-DDE), chlordane compounds (CHLs: trans-chlordane, cischlordane, cis-nonachlor and oxychlordane), hexachlorohexane isomers (HCHs: alpha-HCH, beta-HCH, and gamma-HCH) and hexachlorobenzene (HCB) were analyzed following the method described by Tanabe et al. [20], as mentioned by Monirith et al. [16]. Chromatographic separation was performed on a Hewlett-Packard 5890 Series II gas chromatograph with a 30 m × 0:25 mm (i.d.) DB-1 capillary column coated with 0.25 µm film. PCBs were quantified by a capillary gas chromatography with a 63Ni electron capture detector (GC-ECD) equipped with a fused silica capillary column coated with SE-54 (Supelco, Inc., PA, USA).

For the statistical analysis, the data were $\log_{10}(× + 1)$ transformed before cluster analyses, in order to reduce the variance. The cluster analysis (Single Lingkage Euclidean distance) was performed in the STATISTICA software, version 5.5A.

3. RESULTS AND DISCUSSION

3.1 Heavy Metal Contamination

The mean concentrations (µg/g dry weight) of Cd, Cu, Pb and Zn in the total soft tissues of *P. viridis* are given in Table 2. The four metals ranged from 0.25-1.18 for Cd, 6.39-14.92 for Cu, 1.27-5.23 for Pb, 65.1-121.8 for Zn [7]. Most obviously, the highest concentrations of Cu (14.92) and Pb (5.23) were found in Kg. Pasir Puteh while the highest Cd (1.18) and Zn (121.8) level was found in Trayong. However, the Cd and Zn levels were lower than those at Kuala Linggi (1.25) for Cd and Kg. Pasir Puteh (128.90) for Zn, both collected in 2000 [6]. The ranges of Cd, Cu, Pb and Zn in *P. viridis* reviewed from Yap et al. [6] were close to other previously reported studies from the west coast of Peninsular Malaysia. In Asia, the highest levels of Cd (19.1 µg/g), Cu (279 µg/g), Pb (259 µg/g) and Zn (213 µg/g) in the soft tissue of *P. viridis* were reported from Hong Kong [21], the Gulf of Thailand [22] and the Chao Phraya Estuary of Thailand [23], respectively. From the above comparison, the levels of Cd, Cu, Pb and Zn in *P. viridis* found in Peninsular Malaysia were therefore much lower than the highest values of these metals reported in the literature. Nevertheless, it should be noted that the higher levels of Cu and Pb at Kg. Pasir Puteh population were also found based on samples collected in 2000 [6]. The higher metals at Kg. Pasir Puteh could be related to the discharge of effluents from the nearby domestic and industrial inputs. In addition, Kg. Pasir Puteh is a marina site which potentially receiving petro-chemicals and port activities wastes. Cu leachate from the antifouling paints of boats and the areas semi-enclosed topography may aggravate the pollution problem [6,9].

Table 2. Mean concentrations (μg/g dry weight) of Cd, Cu, Pb and Zn in the total soft tissues of *Perna viridis* collected from Malaysian coastal waters. (See Yap et al. [6, 7])

	Location	Cd	Cu	Pb	Zn
1.	SIkan	0.37	6.80	4.02	100.28
2.	TRhu	0.25	9.83	4.38	94.52
3.	Penang	0.67	10.05	1.27	89.85
4.	PPjg	0.47	6.39	4.28	65.09
5.	ABatu	0.51	9.19	1.80	75.33
6.	PLido	0.65	9.80	3.02	80.49
7.	KPPuteh	0.53	14.92	5.23	97.16
8.	Trayong	1.18	10.74	2.51	121.79
9.	KPenyu	0.51	7.29	3.46	110.59

3.2 Linear Alkylbenzenes Contamination

The concentrations of LABs (sewage input) are given in Table 3. Concentrations of LABs in mussel samples collected from the Malaysian coastal waters ranged from 11 to 807 ng/g dry wt. [14]. High levels (ng/g dry wt.) of LABs observed in Penang Bridge (764) and Kg. Pasir Puteh (807) and these levels were comparable to those at Tokyo Harbor (1011; see Tsutsumi et al. [14]) in Tokyo Bay, which is one the most polluted coastal zones of the world. This suggests the intensive input of sewage into coastal waters of these populated areas. In comparison with other Asian countries, the levels (ng/g dry wt.) found at Penang Bridge and Kg. Pasir Puteh are lower than those at Madras (1638) in Tamil Nadu, Malabon (1478) in Metro Manila and Kawasaki (1898) in Tokyo Bay (see Tsutsumi et al. [14]) However, relatively low concentrations of LABs were observed in other sampling sites in Malaysia may be due to lower human activities and/or less usage of synthetic detergents in the surrounding areas.

Table 3. Linear alkylbenzenes (LABs) in the total soft tissues of *Perna viridis* collected from Malaysian coastal waters. (Data were cited from Tsutsumi et al. [14])

No.	Site	Sampling dates	SL (MM)	LABs (ng/g dry wt.)[a]	$\sum C_{12}$-C_{14} (ng/g dry wt.)[b]
1.	SIkan	NA	NA	NA	NA
2.	TRhu	20 Sep 1998	85	29	22
3.	Penang	21 Sep 1998	91	764	560
4.	PPjg	21 Sep 1998	93	21	17
5.	ABatu	22 Sep 1998	85	198	147
6.	PLido	30 May 1998	69	430	315
7.	KPPuteh	23 Sep 1998	89	807	565
8.	Trayong	29 Aug 1998	70	11	8
9.	KPenyu	29 Aug 1998	79	11	8

Note: NA= data not available.

[a]Sum of the 26 LAB congeners; n.c.: no reliable values calculated due to being overlapped by TAB peaks.

[b]Sum of 17 LABs congeners with alkyl carbons ranging from C_{12} to C_{14}.

[c]A ratio of (6-C_{12}AB + 5-C_{12}AB) relative to (4-C_{12}AB + 3-C_{12}AB + 2-C_{12}AB);

LABs consist of isomers with different phenyl substitutional positions. External isomers are preferentially biodegraded as compared to internal isomers [24]. To quantitatively express the isomer composition, a ratio of internal to external isomers (I/E ratio: a ratio of sum of 6-C12 AB and 5-C12 AB relative to sum of 4-C12 AB, 3-C12 AB and 2-C12 AB) has been proposed as an index of the degree of LAB degradation [25]. In synthetic detergents and untreated sewage I/E ratios are around 0.7, whereas secondary (biological) sewage treatment increases the ratio to as much as 7 [26]. A higher value of this ratio means a greater depletion of external isomers and, hence, greater degradation, whereas a lower I/E ratio means less degradation. I/E ratios for mussels collected from Malaysian coastal waters are given in Table 4 and they ranged from NC (no reliable values calculated due to low concentrations) to 2.83. These values are clearly much lower than those from Tokyo Bay (3.00–8.43), and indicate that LABs in the Malaysian coastal zones are less degraded. This can be explained by the difference in the type of wastewater being discharged. Primary effluent as well as raw sewage has a low I/E ratio (0.5–0.9; [26]) because it is essentially a physical settlement of sewage particles that allows for the limited opportunity of aerobic degradation. On the other hand, due to facilitated microbial degradation, secondary effluent shows a much higher I/E ratio, ranging from 2 to 7 [26]. The considerably lower I/E ratios (NC-2.83) found in all the sampling sites indicated that the Malaysian coastal zones have been receiving sewage effluents with limited (primary effluent) or no (raw) treatment, and is probably due to a lack of sewage treatment facilities or experience frequent heavy rainfall with subsequent overflow of sewage treatment plants, thereby flushing the untreated sewage into the coastal zones. In summary, LABs determination demonstrated extensive inputs of poorly treated wastewater to aquatic environments in Malaysia.

Table 4. Concentrations (ng/g dry tissues) of linear alkylbenzenes (LABs), octylphenol (OP), nonylphenol (NP) and bisphenol A (BPA) in the total soft tissues of *Perna viridis* collected from Malaysian coastal waters (Data cited from Isobe et al. [15])

Site	Concentrations (e)				
	LABs[a]	OP	NP	BPA	I/E ratio[b]
SIkan	NA	1	18	(0.56)	NA
TRhu	29	NA	NA	NA	NC
Penang	764	NA	NA	NA	1.79
PPjg	21	NA	NA	NA	NC
ABatu	198	9	290	(0.51)	2.83
PLido	430*	16	663	4.2	2.21*
KPPuteh	807	NA	NA	NA	1.99
Trayong	11	NA	NA	NA	NC
KPenyu	11	NA	NA	NA	NC

*Note: * data cited from Tsutsumi et al. [14]*
[a]Sum of the concentrations of the 26 LAB congeners.
[b]A ratio of (6-C12AB+5-C12AB) relative to (4-C12AB+3-C12AB+2-C12AB).
NA= not analyzed
Values in parenthesis are not significant in comparison to the procedural blanks.
NC= no reliable values calculated due to low concentrations.

3.3 Phenolic Endocrine Disrupting Chemicals

For Phenolic EDCs in mussels, the concentrations of alkylphenols namely nonylphenol (NP) and octylphenol (OP), in mussels from Malaysian coastal waters are given in Table 4 (data cited from Isobe et al. [15]. The NP concentrations ranged from 18 to 663 ng/g dry tissue. Elevated concentration (ng/g dry tissues) of NP was observed in Pantai Lido population (663) which was comparable to those from Indonesia (72-643), Singapore (605) and and Philippines (21-578), although lower than those in Tokyo Bay (47-1347). The NP levels (ng/g dry tissues) in the Malaysian samples were higher than those from India (72-202), Thailand (81-228), Cambodia (27-92), Vietnam (71-121) [15]. Therefore, the monitoring NP results suggested that the status of NP pollution in some locations in Malaysia had already as severe as those in industrialized countries such as Japan (47-1347 ng/g dry tissues). The OP concentrations (1-16 ng/g dry tissues) in the Malaysian mussels were one to two orders of magnitude lower than those of NP. This is probably due to a combination of less production and usage of octylphenol ethoxylate surfactants and lower hydrophobicity of OP than NP [15].

Another phenolic EDC, bisphenol A (BPA), which ranged from 0.51-4.2 ng/g dry tissues in the Malaysian mussels but the relatively high concentrations of BPA was found in the Pantai Lido population (4.2 ng/g dry tissues). The Pantai Lido level (ng/g dry tissues) was within but lower those in Tokyo Bay (0.54-13.4) and India (1.1-13.7) [15]. The BPA levels (0.51-4.2 ng/g dry tissues) in the Malaysian mussels are actually comparable to those levels (ng/g dry tissue) from Indonesia (0.32-6.3), Singapore (3.3), Thailand (0.96-4.94) and Philippines (1.1-4.7) [15]. The fact that BPA was significantly detected in these marine mussels warranted the necessity for a broader and regular biomonitoring studies on BPA in Asian marine coastal environment.

For source estimation of the phenolic EDCs, LABs concentrations in the mussels were compared with those of phenolic EDCs. Elevated NP concentrations were detected for locations where low LABs were found at Pantai Lido. This result indicates that there are some other sources of phenolic EDCs other than from urban sewage, although urban sewage is one of the major sources of phenolic EDCs.

3.4 Polycyclic Aromatic Hydrocarbons (PAHs)

Based on Isobe et al. [15], they quantified PAHs with three to six rings. Total concentrations of PAHs in mussels based on eight mussel populations of Malaysian coastal waters ranged from 11 to 309 ng/g dry tissue (Table 5). The Malaysian range levels (ng/g dry tissue) were lower when compared to those from India (63-1133) and Tokyo Bay (42-1269) in Japan, comparable to those from Indonesia (58-549), Thailand (84-211), Philippines (75-266), and higher than those from Cambodia (21-32), Vietnam (24-110) [15].

Table 5. Compositional parameters of PAHs in the total soft tissues of *Perna viridis* collected from Malaysian coastal waters (Data cited from Isobe et al. [15])

Code	Total PAH[a] (ng/g dry Wt.)	Compositional parameters				
		H/L[b]	MP/P[c]	CPP/MP[d]	Py/Flu[e]	Phe/An[f]
SIkan	NA	NA	NA	NA	NA	NA
TRhu	18	0.37	1.13	0.065	0.74	8.92
Penang	265	0.20	5.15	0.012	1.06	12.21
PPjg	41	1.19	2.67	0.115	0.70	5.55
ABatu	57	0.58	1.78	0.067	1.01	7.36
PLido*	157	0.16	2.39	0.047	5.12	7.46
KPPuteh	309	0.44	5.69	0.035	2.50	2.70
Trayong	38	0.16	1.79	0.066	1.04	8.43
KPenyu	11	0.11	1.90	0.061	1.05	14.74

Note: Data for PLido were based on samples collected on 30 May 1998.*

[a]Sum of concentrations of phenanthrene, anthracene, 1-methylphenanthrene, 2-methylphenanthrene, 3-methylphenanthrene, 9-methylphenanthrene , fluoranthene, pyrene, benz [a] anthracene, chrysene, benzo [b] fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo [e] pyrene, benzo[a] pyrene, perylene, indeno [1,2,3-cd] pyrene, benzo [ghi] perylene, and coronene.

[b] H/L ratio: a ratio of the sum of benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[1,2,3-cd]pyrene, benzo[ghi]perylene and coronene to sum of phenanthrene, anthracene, methylphenanthrenes, fluoranthene, pyrene.

[c] MP/P ratio: a ratio of the sum of 1-methylphenanthrene, 2-methylphenanthrene, 3-methylphenanthrene, 9-methylphenanthrene to phenanthrene

[d] CPP/MP ratio: 4H-cyclopenta [def] phenanthrene to sum of 1-methylphenanthrene, 2-methylphenanthrene, 3-methylphenanthrene, and 9-methylphenanthrene.

[e] Py/Flu ratio: a ratio of pyrene to fluoranthene.

[f] Phe/An ratio: a ratio of phenanthrene to anthracene.

NA= not analyzed.

Based on categorization of PAH concentrations in mussels proposed by Baumard et al. [27], PAHs concentrations in Malaysia can be classified as “low (0–100 ng/g dry) to moderate (100–1,000 ng/g dry)”. A comprehensive review on the source identification ratios were also done by Isobe et al. [15]. The mussel populations collected from Penang Bridge and Kg. Pasir Puteh showed a considerably higher methylphenanthrenes/phenathrene MP/P ratio

(5.15-5.69) than those observed in mussels from Tokyo Bay (0.8–3.1), suggesting a higher contribution of petrogenic inputs to coastal waters in these areas [28].

A ratio of CPP (cyclopenta(def) pyrene) to the sum of methylphenanthrenes (CPP/MP ratio) has been proposed as another index to distinguish petrogenic and pyrogenic PAHs [29]. The CPP/MP ratios from this study ranged from 0.012 to 0.115 and therefore they were of between petrogenic sources (<0.07) to petrogenic plus minor contributions from pyrogenic sources (0.07–0.2). The CPP/MP ratios were also less than the mean values observed in Tokyo Bay mussels (0.15). The other ratios of PAH species (pyrene to fluoranthene: Py/Flu ratio, phenanthrene to anthrancene: Phe/An) were also examined for source-identification. All populations except for Pasir Panjang and Tanjung Rhu, their Py/Flu ratios were >1, supporting that these samples had petrogenic contribution more from pyrogenic inputs. For some populations, the pyrogenic signature was not consistent with the petrogenic signature suggested from the MP/P ratio and CPP/MP ratio. This can be explained by the fact that the contribution from petrogenic PAHs varies among PAH species. Actually lower Py/Flu ratios have been observed in some crude oil samples [30]. Phe/An ratios higher than 10 at Penang Bridge and Kuala Penyu supported petrogenic contributions to these sites whereas if conflicted with the estimation from the alkylated PAHs for some other locations (such Kg. Pasir Puteh). Lower Phe/An ratios ("pyrogenic" signature) were again observed in some crude oil and petroleum products [31]. In general, sampling sites at Penang Bridge and Kuala Penyu, all compositional parameters (MP/P, CPP/MP, Py/Fluo, Phe/An ratios) consistently exhibited petrogenic signatures. In conclusion, these sites in Malaysian coastal waters were heavily affected by petrogenic PAHs, although inputs from pyrogenic sources were detected.

In Kuala Penyu (Sabah), which is close to an oil platform, where the contribution of crude oil may be occurring. A comprehensive study reported by Zakaria et al. [32] identified the used crankcase oil as the major source of petrogenic PAHs in Malaysian aquatic environments. Of course, there are some other potential sources including accidental oil spills, routine tanker operations, spillage from oil fields and oil refineries, spillage from fishing boats, leakage from land-based storage, gasoline and diesel fuel from automobiles, street dust, and others.

To conclude, at several populations collected from Penang Bridge, Kuala Penyu, all compositional parameters (MP/P, CPP/MP, Py/Fluo, Phe/An ratios) consistently exhibited petrogenic signatures.

3.5 Organochlorine Contamination

Five compounds of OC in *P. viridis* collected Malaysian coastal waters are given in Table 6. In general, lower DDTs levels (16-130 ng/g lipid wt.) were observed in mussels from Malaysia. This finding indicated less usage of DDTs in Malaysia the Malaysian DDTs ranges were much lower than those from China (830–54,000 ng/g lipid wt.), Hong Kong (640–61,000 ng/g lipid wt.) and Vietnam (220–34,000 ng/g lipid wt.) [16].

In general, low concentrations PCBs (5.1-250 ng/g lipid wt.) were found in mussels from Malaysia, which indicated fewer local sources. The PCBs levels (ng/g lipid wt.) were much lower than some urban/industrialized cities in Asian countries which showed relatively higher concentrations, such as Bombay (600) and Cochin (420) in India, Chong Ming Dao, Shanghai (600) in Eastern China and Manila (640) in Philippines [16].

Table 6. Concentrations of organochlorines (ng/g lipid wt.) in the total soft tissues of *Perna viridis* collected from Malaysian coastal waters (data cited from [16])

Location of sample	Lipid (%)	PCBs	DDTs	CHLs	HCHs	HCB
SIkan	0.92	6.0	95	41	9.4	<1.1
TRhu	1.1	5.1	16	2.5	4.9	<0.90
Penang	1.0	60	71	180	<0.10	2.4
PPjg	1.6	11	93	60	3.5	<0.60
ABatu	1.3	22	100	610	12	<0.80
PLido	NA	NA	NA	NA	NA	NA
KPPuteh	2.1	250	130	470	5.2	<0.50
Trayong	0.65	8.3	32	4.1	3.1	<1.5
KPenyu	0.73	7.5	100	8.7	<1.4	<1.4

Note: NA: No data available.
DDTs: p, p'-DDE + p, p'-DDD + p, p'-DDT.
CHLs: trans-chlordane + cis-chlordane + trans-nonachlor + cis-nonachlor + oxychlordane.
HCHs: α-HCH + β-HCH + γ-HCH.

The residue levels (ng/g lipid wt.) of CHLs in mussels collected from Malaysia coastal waters are also given in Table 6. Our levels (17–630) of CHLs from Malaysia were considered 'lower' than those observed in mussels from Japan (150–1800) and China (40–870), and comparable to those from Hong Kong (18–750) and Singapore (520) (see [16]). Malaysia showed higher concentrations of CHLs at sites in proximity to harbor, aquaculture, urban and densely populated areas such as ABatu and KKPuteh. Higher percentages of trans-nonachlor were found in mussels from Malaysia and again it is comparable to those found from Japan, Singapore, India, Philippines and China. Monirith et al. [16] so found that the presence of trans-nonachlor in the Malaysian mussels might suggest the recent usage of technical CHLs. The concentrations and compositions of CHLs Malaysian mussels might reflect the input of CHLs into coastal environment in 1998 or before.

The HCHs levels (ng/g lipid weight) (summation of α-HCH + β-HCH + γ-HCH) in the Malaysian mussels ranged from <0.10- 12 (Table 6). Our levels were again lower than those mussels from Japan (13-50), Hong Kong (2.1–30), South Korea (1.9-80), Thailand (0.16-27), China (11-110) and India (20-590) [16]. According to Monirith et al. [16], mussels from Singapore and Malaysia contained higher percentages of γ-HCH (up to 90%). Previous reports had also indicated the use of lindane (purified γ-HCH) in Malaysia based on studies by Tan and Vijayaletchumy [33] and Kannan et al. [34].

Lastly, the HCB levels (ng/g lipid weight) ranged from <0.50- 2.4 (Table 6). Our range was again lower than those from Japan (<0.60–29), China (<0.90–540) and India (<0.40–63).

3.6 General Discussion

Cautions should be exercised when interpreting all the above data since they can be influenced by biological and environmental factors. Biological factors such as gender [11], sizes [8] and spawning conditions [35] can mask all major anthropogenic impacts while environmental factors such as salinity, temperature, tidal levels and stress due to desiccation can also cause the data interpretation misleading. We can argue that since the present samplings were conducted between 20-29 September 1998 (which was a relatively short interval) except for the first sampling at Pantai Lido conducted in May 1998, the spawning factor thus is greatly reduced to a minimal. Other biological factor which is the mussel sizes investigated indicated by shell lengths could influence the data reviewed in this paper. For example, the mean shell lengths for Pantai Lido population was 64.4 cm, comparing to 109.8 cm for Trayong population. This indicates Trayong population is bigger or older when compared to Pantai Lido population. However, it should be noted that the growth rate of mussels are subjected to food availability and population structures. For the environmental factors such as temperature and salinity, since those water parameters varied within a range of tolerance for the mussel mussels, it can be assumed that these factors can little influence the present data reviewed. Certainly, this is still debatable whether the present data reviewed can accurately reflect the anthropogenic impact on the accumulation of the different contaminant groups in *P. viridis*. Nevertheless, it is argued that since 1) the clustering pattern are very much related to the site description (although it is not always true), and 2) there is no other background data reported in the literature such as the data reviewed in this paper, the present data reviewed can serve as important future reference which had covered the important contaminant groups such as heavy metals, LABs, PAHs, EDCs and OCs.

To conclude the overall chemical contamination, a dendrogram established using single linkage cluster analysis based on seven sampling sites covering eleven chemicals namely Cd, Cu, Pb, Zn, LABs, PAHs, PCBs, DDTs, CHLs, HCHs and HCB, is shown in Fig. 2, since only seven sampling sites had a complete data for the eleven chemicals. The clustering pattern shows two major subclusters. The first one comprising Tg. Rhu, Trayong, KPenyu and PPjg population, indicating relatively uncontaminated conditions while the other subcluster consists of Penang, Kg. Pasir Puteh and ABatu which indicated contaminated conditions as it is well supported by the absolute values of some chemicals. The subcluster combining Penang and Kg. Pasir Puteh populations could be mainly due to the elevated levels of LABs and PAHs in both sites (Table 3) while ABatu is within the major clusters with Penang and Kg. Pasir Puteh is due to the elevated levels of DDTs, CHLs and HCHs. Kg. Pasir Puteh also had elevated levels of Cu, Pb, PCBs and CHLs.

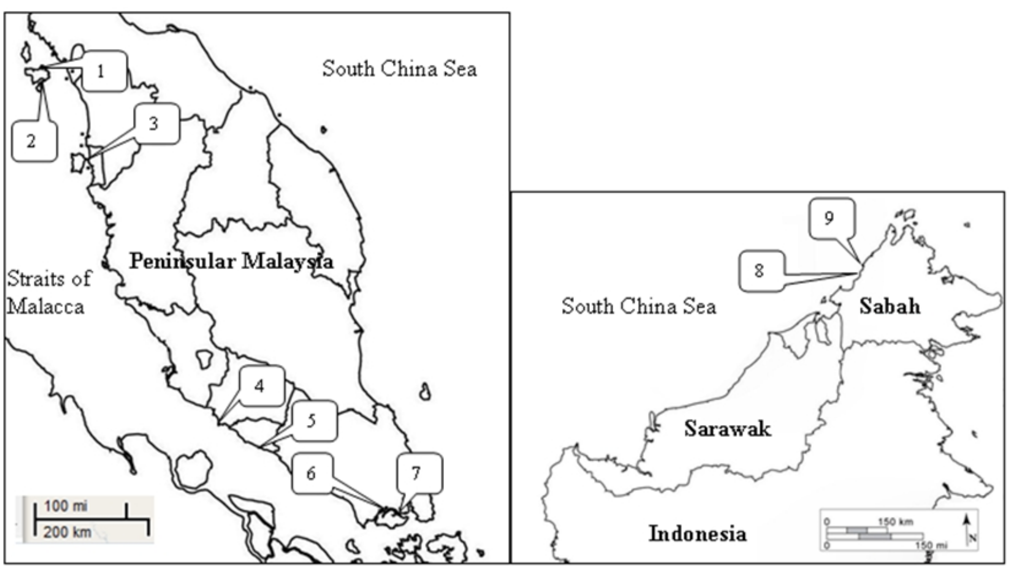

Fig. 1. Map showing sampling sites of *Perna viridis* from Malaysian coastal waters.

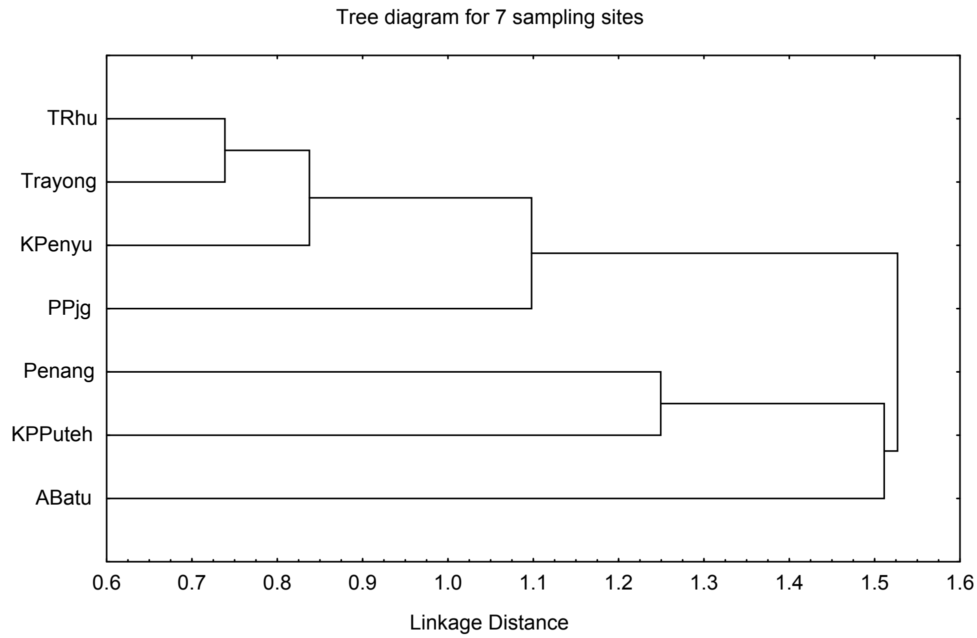

Fig. 2. Cluster analysis based on Single Linkage Euclidean distances, on the eleven chemicals (PCB, DDT, PAH, LAB, CHL, HCH, HCB, Cd, Cu, Pb and Zn) in the seven mussel populations, based on $\log_{10}(\times + 1)$ transformed data.

4. CONCLUSION

Kg. Pasir Puteh received higher concentrations of Cu, Pb, LABs, PAHs, PCBs and CHLs while Penang population received higher concentrations of LABs and PAHs. ABatu population particularly received anthropogenic OCs including DDTs, CHLs and HCHs. There is always need and concern as we may ask 'What are these chemical pollutant status in the Malaysian coastal waters in 2018 or after 20 years?' This benchmark review based on 1998 samples covering heavy metals, LABs, PAHs, phenolic EDCs and OCs can always be used for future comparison and reference since more extensive and systematic monitoring of all these chemical pollution in Malaysian coastal waters using mussels or other molluscs as biomonitors are always encouraged and welcome in future.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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