Chapter 4

Results and Discussion

CHAPTER IV: RESULTS AND DISCUSSION

4.1 Precision and Accuracy

In the present study, hydrocarbons were extracted using hexane, which would extract not only hydrocarbons of petrogenic origin, but also those of biological origins. The quantitative determination using fluorescence spectrophotometry is a widely accepted procedure for the estimation of petrogenic hydrocarbons (Parsons et al, 1984). Because of the insensitivity of fluorescence spectrophotometry to non-aromatic hydrocarbons such as saturated aliphatic compounds, this fraction is quantified using gas chromatography with mass spectrophotometer as the detector.

The aromatic content of petroleum oil varies both in terms of its total content and composition, which means that the accuracy of quantification is dependent on the standard used. Ideally, the standard should be identical in molecular composition to the samples. However, when the sources of pollution are unknown, measurement can be expressed as equivalent crude oil concentrations. According to Law (1981), chrysene can also be used as a reliable standard when the measurements involve only PAHs. This method has been used by Integrated Global Ocean Station System of IOC/WMO monitoring programmes. Although it is a relatively simple technique and subjected to some limitations, fluorescence spectrometry is a widely accepted analytical procedure for the determinations of petrogenic hydrocarbons.

To facilitate analysis, hydrocarbons are fractionated into various classes; aliphatic and aromatic hydrocarbons and PAHs. Fractionation also served to eliminate lipoidal materials as well as non-hydrocarbon components such as azoarenes, phenols and sterols.

In the analysis of environmental samples, where the concentration of the pollutant of interest is usually very low, the determination of the accuracy and precision of the chosen method of analysis is of fundamental importance to ensure a specified degree of confidence in the data generated. Hence, the precision of the method employed was established by replicate analysis of samples of the appropriate matrix, while the accuracy of the method was determined by analysis of samples spiked with known amount of hydrocarbons.

The recoveries of cockle tissues fortified with Seligi crude oil ranged from 71% to 105%, while the coefficient of variation ranged from 9.7% – 15.1% as determined by fluorescence spectrometry (Table 4.1). The recoveries of artificial seawater fortified with Seligi crude oil ranged from 85% - 100% with coefficient of variation ranging from 7.9% - 9.9% (Table 4.2). Similarly, a sediment sample spiked with a known amount of Seligi crude oil in five replicates showed a recovery of 69% - 80% with an average coefficient of variation of 12.2%.

The use of internal standards introduced into the environmental sample at the beginning of the procedure also reflected the ability of the procedure to recover the analyte to a sufficiently high degree of accuracy. The recoveries of the internal standards, octadecene and dotriacontane spiked in samples are shown in Table 4.3. The percentage recovery was calculated as follows:

$$\% \text{ Recovery} = \frac{\text{quantity of IS recovered}}{\text{quantity of IS spiked}} \times 100$$

$$= \frac{A_{IS}}{A_{IST}} \times \frac{C_{IST}}{C_{IS}} \times \frac{V_S}{V_{IS}} \times 100$$

where:

A_{IS} = area of IS peak in sample chromatogram

A_{IST} = area of IS peak in standard chromatogram

C_{IS} = concentration of IS spiked

C_{IST} = concentration of IS in the standard solution

V_{IS} = volume of IS spiked

V_S = volume of sample prior to the injection

Table 4.1 **Recovery and precision of the adopted methodology for Seligi crude oil added to cockle tissue as determined by fluorescence**

Amount added # (µg/g)	Recovery (%)	Coefficient of Variation (%)
0.01	71 – 105	11.5
0.10	78 – 95	15.1
1.00	85 – 97	9.7
10.0	79 – 95	9.7

n = 5

Table 4.2 **Recovery and precision of the adopted methodology for Seligi crude oil added to artificial seawater as determined by fluorescence**

Amount added # (µg/g)	Recovery (%)	Coefficient of Variation (%)
0.01	85 – 99	9.9
0.10	89 – 95	7.9
10.0	90 – 100	8.5

n = 5

Table 4.3 **Percentage recovery of internal standards**

Sample	Sample type	Internal Standard	Recovery (%)
KS1(A)	Water	Octadecene	81.1
KS2(A)	Water	Octadecene	88.0
KS5(A)	Water	Octadecene	93.2
T3(A)	Water	Octadecene	77.2
T4(A)	Water	Octadecene	60.8
T5(A)	Water	Octadecene	76.7
KS2(A)	Sediments	Dotriacontane	97.4
KS5(A)	Sediments	Dotriacontane	72.9
T5(A)	Sediments	Dotriacontane	80.7
T3(A)	Tissues	Dotriacontane	83.8

4.2 Oil pollution in the mudflats of Kuala Selangor and Kuala Sepetang

The objective of the present study is to determine the level of hydrocarbon pollution in the coastal environment at the selected sites by means of measuring the levels of hydrocarbons in water, surficial sediments and cockles tissues. The analytical methods involved are UV-fluorescence spectrometry (UVF) and gas chromatography-mass spectrometry (GCMS). Eventhough the application of UVF has received widespread acceptance for the quantitative analysis of hydrocarbons in environment as described by Ehrhardt (1983), it is imperative to realise that, this method is not specific for only petrogenic hydrocarbons, but is sensitive to any fluorescing compounds in the sample. Therefore, it is wise to adopt the UVF techniques in combination with other methods such IR spectrometry, gas and liquid chromatography as well as mass spectrometry as proposed by several monitoring bodies such as the US Coast Guard and R&D Centre, Institute of Petroleum, UK and German Association of Petroleum Science and Coal Chemistry. In the present study, a combination of liquid-solid chromatography (LSC), UVF and GCMS was used.

LSC was used to separate the aliphatic and aromatic fractions by means of elution through chromatographic column of alumina and silica as stationery phases prior to the UVF measurement and injection into the GCMS. The fluorescence of hydrocarbons in each fraction for water, sediments and tissues were measured against Seligi crude oil and chrysene as standards. Since UVF measurement is only sensitive to the aromatic but not the aliphatic and olefinic hydrocarbons, it is therefore regarded as representative of petrogenic hydrocarbons. This assumption has been widely accepted because PAHs are generally absent in biogenic hydrocarbons (Ehrhardt, 1983).

The results of the water analysis, reported as the average of triplicate determinations are given in Table 4.4 and Figure 4.1. Complete results of the measurements are given in appendix B.

Table 4.4 **Petroleum hydrocarbons in estuarine water**

Sampling Sites	Stations	Concentration (μg/L) Seligi Crude Oil Equivalent			Total HCs	Concentration (μg/L) Chrysene Equivalent			Total HCs
		F1	F2	F3		F1	F2	F3	
Kuala Sepetang	T1	0.750	55.50	15.78	72.03	0.005	1.420	0.430	1.855
	T2	0.385	85.30	6.905	92.59	0.010	1.620	1.445	3.075
	T3	1.490	32.52	4.230	38.24	0.080	0.645	0.855	1.580
	T4	0.485	25.97	4.360	30.82	0.005	0.555	0.655	1.215
	T5	4.840	25.26	2.950	33.05	0.100	0.580	0.515	1.195
Kuala Selangor	KS1	0.170	50.13	6.370	56.67	0.000	2.750	0.265	3.015
	KS2	0.155	57.88	6.780	64.82	0.005	2.010	0.260	2.275
	KS3	0.250	63.15	5.090	68.49	0.000	2.225	0.195	2.420
	KS4	0.095	80.45	5.805	86.35	0.000	2.790	0.215	3.005
	KS5	0.270	60.20	3.385	63.86	0.005	2.260	0.125	2.390

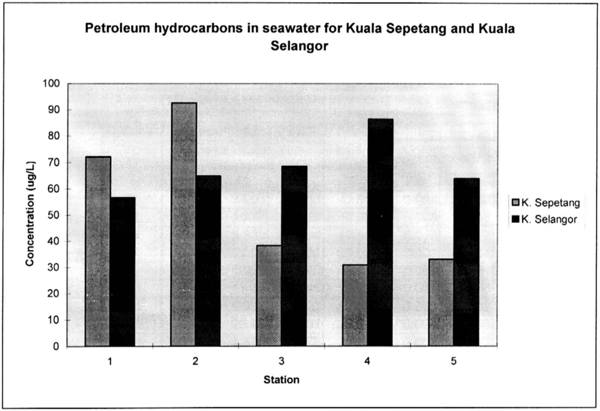


Figure 4.1 : Petroleum hydrocarbons in seawater for both sites

The results of UVF measurements show that the level of hydrocarbons in water at both sites ranged from 30.82 μg/L to 92.59 μg/L Seligi crude oil equivalents (1.195 μg/L - 3.075 μg/L chrysene equivalents). The highest level of hydrocarbons in Kuala Sepetang was recorded at Station 2: 92.59 μg/L and 3.075 μg/L expressed as ESSO Seligi crude oil and chrysene equivalents respectively. The hydrocarbon levels at Stations 1 and 2 were also noted to be very much higher compared to Stations 3, 4 and 5, the latter three stations were relatively low and uniform (30.82-38.24 μg/L Seligi crude oil equivalents). This observation was attributed to the fact that Stations 1 and 2 were located nearer to the river mouth while dilution of land-based oils contributed to the observed lower levels in Stations 3, 4 and 5. In contrast, the levels of hydrocarbon in sea water at Kuala Selangor were more uniformly distributed, except for Station 4 which were observed to be significantly higher (86.35 μg/L and 3.005 μg/L expressed as Seligi crude oil and chrysene equivalents respectively).

The results of the sediment analysis, reported as the average of triplicate determinations are given in Table 4.5. Complete results of the measurements are given in appendix B.

Table 4.5 : **Petroleum hydrocarbons in surficial sediments**

Sampling Sites	Stations	Concentration (μg/g) Seligi Crude Oil Equivalent			Total HCs	Concentration (μg/g) Chrysene Equivalent			Total HCs
		F1	F2	F3		F1	F2	F3	
Kuala Sepetang	T1	0.165	34.95	7.695	42.81	0.004	1.139	0.249	1.392
	T2	0.300	30.47	14.73	45.50	0.008	1.098	0.508	1.614
	T3	0.179	8.330	11.30	19.81	0.003	0.297	0.416	0.716
	T4	0.182	21.04	2.632	23.85	0.003	0.764	0.135	0.902
	T5	0.335	17.39	2.140	19.87	0.001	0.561	0.093	0.655
Kuala Selangor	KS1	0.448	17.44	1.343	19.23	0.038	0.580	0.291	0.909
	KS2	0.093	9.933	1.322	11.35	0.008	0.632	0.288	0.928
	KS3	0.130	25.48	0.701	26.31	0.004	1.061	0.108	1.173
	KS4	0.171	1.370	0.875	2.416	0.004	0.045	0.043	0.092
	KS5	0.057	0.753	2.364	3.174	0.001	0.071	0.022	0.094

Levels of hydrocarbons in seawater are subjected to significant fluctuations which are influenced by the rate of discharge of oil from both land and sea-based sources and the rate of weathering processes on the oil, in addition to seasonal variations such as the monsoon season which in turn affects the tidal currents. Sediments of fine particles tend to be good accumulators of organic pollutant including petrogenic hydrocarbons due to their greater effective area (Law, 1981; Burns et. al., 1982).

The levels of petrogenic hydrocarbons in surficial sediments were higher than those in the water column by three orders of magnitude. However, relatively low levels of petrogenic hydrocarbons were observed in surficial sediments for both sites ranging from 2.42-45.50 μg/g Seligi crude oil equivalents (0.092-1.614 μg/g chrysene equivalents). Relatively higher levels of petrogenic hydrocarbons were found in Kuala Sepetang (average: 30.37 μg/g Seligi crude oil equivalents) compared to Kuala Selangor (average: 12.49 μg/g Seligi crude oil equivalents). The highest levels found in Kuala Sepetang were observed at Station 2 although very similar levels were also noted at Station 1, while the remaining stations were significantly lower. Very low levels were also noted at Stations 4 and 5 in Kuala Selangor.

The results of the cockle tissues analysis, reported as the average of triplicate determinations are given in Table 4.6. Complete results of the measurements are given in appendix B.

Table 4.6 **Petroleum hydrocarbons in cockle tissues**

Sampling Sites	Stations	Concentration (μg/g) Seligi Crude Oil Equivalent			Total HCs	Concentration (μg/g) Chrysene Equivalent			Total HCs
		F1	F2	F3		F1	F2	F3	
Kuala Sepetang	T1	0.861	13.18	6.326	20.37	0.041	1.223	0.250	1.514
	T2	0.371	7.039	2.708	10.12	0.011	0.402	0.101	0.514
	T3	0.285	10.23	3.551	14.06	0.004	0.089	0.083	0.176
	T4	0.337	2.902	2.482	5.721	0.001	0.174	0.084	0.259
	T5	1.282	27.72	9.474	38.48	0.006	0.285	0.141	0.432
Kuala Selangor	KS1	0.378	22.20	8.238	30.82	0.011	0.414	0.250	0.675
	KS2	0.121	3.442	0.864	4.427	0.000	0.092	0.098	0.190
	KS3	0.242	12.05	3.579	15.87	0.004	0.426	0.130	0.560
	KS4	0.359	14.40	2.873	17.63	0.008	0.530	0.122	0.660
	KS5	0.425	10.09	3.010	13.52	0.006	0.367	0.079	0.452

Concentrations of petrogenic hydrocarbons in cockle tissues were noted to be in the same order of magnitude to those found in the sediments. Petrogenic hydrocarbons in cockle tissues ranged from 4.427 to 38.48 μg/g Seligi crude oil equivalents (0.176-1.514 μg/g chrysene equivalents) for the two sites. However, unlike the levels in the sediments there were no significant differences between the average concentration of petrogenic hydrocarbons in Kuala Sepetang (average: 17.75 μg/g Seligi crude oil equivalents) and that found in Kuala Selangor (16.45 μg/g Seligi crude oil equivalents). The highest concentration of hydrocarbons in Kuala Sepetang were found in cockles collected from Station 5 (36.48 μg/g Seligi crude oil equivalents) while in Kuala Selangor the highest concentration were observed in Station 1 (30.82 μg/g Seligi crude oil equivalents).

Figure 4.2 shows the comparative levels of petrogenic hydrocarbons in sediments and tissues in Kuala Sepetang. There does not appear to be a correlation between hydrocarbon levels in sediments and those in the cockles. Hence, higher concentrations of hydrocarbons in sediments were not reflected by higher concentrations in the tissues as exemplified by the observed concentrations in the two media in Station 1 and Station 5. Samples in Kuala Selangor were also consistent with this observation. There was also an absence of correlation between hydrocarbon levels in tissues and those in the water column as exemplified by Station 1 and Station 5 in both Kuala Selangor and Kuala Sepetang.

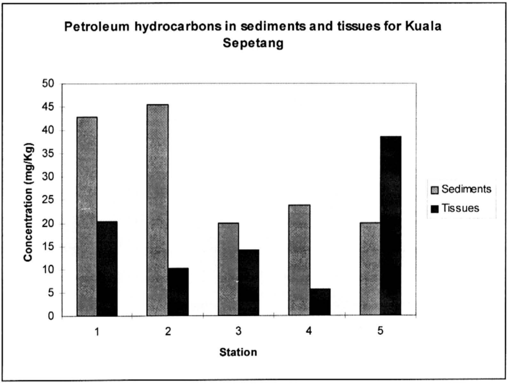


Figure 4.2 : Petroleum hydrocarbons in sediments and tissue for Kuala Sepetang

In general the levels of petrogenic hydrocarbons in water at the two sites reflect some pollution in the coastal environment presumably arising from both sea-based as well as land-based sources of pollution. However, all the observed measurements in water were well below the level of 2.5 mg/L and hence are classified as relatively unpolluted (Marchand et. al., 1982). It has been suggested that unpolluted coastal sediments contain less than 100μg/g hydrocarbons (FAO, 1982). Based on this guideline, as all the sediment samples contain less than 50 μg/g, it would appear the sediments from the two sites were relatively unpolluted.

Levels of oil pollution have been determined in Malaysian waters in previous surveys. In a survey conducted in the coastal waters of the Straits of Malacca, the range of petrogenic hydrocarbons found in water and sediments were found to be 5 - 386 μg/L and 52.81-733.74 μg/g Seligi crude oil equivalents respectively (Abdullah *et al*, 1994). A subsequent study to the latter survey conducted in coastal waters throughout Peninsular Malaysia and East Malaysia showed levels of petrogenic hydrocarbons ranging from 1.5 - 186.40 μg/L and 5.5-703.89 μg/g Seligi crude oil equivalents in water and sediments respectively (Abdullah and Samah, 1996). In general the levels of oil pollution observed in the present study were within the range of concentrations recorded in these surveys. The surveys mentioned above were chosen for comparison with the present study based on similar method of analysis as well as the use of Seligi crude oil as the standard.

The concentrations of petrogenic hydrocarbons in the water of Kuala Selangor determined in the latter survey were found to be in the range of 15.6 - 21.5 μg/L (average: 18.8 μg/L) and 32.88-161.89 μg/g (average: 87.6 μg/g) Seligi crude oil equivalents in water and sediments respectively (Abdullah and Samah, 1996). These concentrations were of the same order of magnitude to those found in the present study. It should also be noted that while the levels in water were lower than those observed in the present study (average: 68.04 μg/L Seligi crude oil equivalent), the level in sediments were found to be significantly higher compared to the present study which averaged 12.49 μg/g Seligi crude oil equivalent).

As best as can be ascertained, there has not been any report of oil pollution levels in Kuala Sepetang from previous studies. As a comparison, waters of nearby Lumut were reported to be polluted with petrogenic hydrocarbons to the extent of 20.5 - 186.4 μg/L and 9.11-191.44 μg/g Seligi crude oil equivalents in water and sediment respectively (Abdullah and Samah, 1996). It should be noted however that the sources of pollution in Lumut may be quite different to those to be found in Kuala Sepetang.

One of the highest levels of hydrocarbons in coastal waters in Peninsular Malaysia was reported by Abdullah et. al. (1994) in Kukup ranging from 1999 to 2795 μg/L and 91.76-174.71 μg/g Seligi crude oil equivalents in water and sediment respectively. The area has been declared unsafe for fish breeding by the appropriate authority due to severe oil pollution caused by bilge discharges and tanks cleaning by ships before repairing process in Singapore (The Star, 28th August, 1999).

It would also be useful to compare the results of the present study to those found elsewhere in the world. For instance, the range of petroleum hydrocarbons level in water from Manila Bay and Indonesian waters were reported as 1250 - 6980 μg/L and 300 – 11500 μg/L respectively (Bilal. 1980). Connel et.al. (1998), found that the range of hydrocarbon background level in marine sediment from coastal area of Hong Kong was 40 - 60 μg/g. The level of hydrocarbons in water and sediments from English Channel, known for its extensive shipping activities, were 3 - 14 μg/L and 0.3 - 56 μg/g dry weight respectively (Fileman and Law, 1988). Baumard et. al. (1997), reported that the total PAHs concentrations ranged from 1 - 2 μg/g in the sediment collected from western Mediterranean Sea, while the contamination levels in the range of 1.6 - 33 μg/L in water and 3.1 - 40.35 μg/g dry weight in sediments were reported in the Omani coastal waters (Badawy & Al-Harhty, 1991).

While the hydrocarbon levels in seawater and sediments for many regions in the world are available for comparison, the corresponding data on concentrations in marine biota is generally lacking, and in the Malaysian context little work has been done. This concern arises particularly from the human health aspect as aquatic organisms such as fish, molluscs and bivalves are potential bioindicator or bioaccumulator for environmental contaminants including hydrocarbons, and these marine biota are also consumed as seafood.. Serrazanetti et.al (1997) reported that the total aliphatic hydrocarbons in four species of molluscs collected from Adriatic Sea, Italy ranged from 35.0 to 68.1 mg/g dry weight, while Baumard et.al. (1998), determined PAHs concentrations in mussels collected near a harbour in western Mediterranean Sea using mass spectrometry and found that the levels were up to 8.5 μg/g.

Organisms collected near oil refineries can have a petroleum-like taint. Ogata and Miyake (1973) measured the hydrocarbon level in eel and mullet from an oil-polluted harbour in Japan and found that the ratio of concentration of toluene in the fish to that in the surrounding water was 2.4:1 with levels in the mullet was as high as 5 μg/g. Mullets collected near oil refineries in Australia were also observed to contain series of hydrocarbons similar to that found in kerosene, including several alkylated naphthalene (Shipton et. al., 1970).

4.2.1 Gas chromatographic analysis

Qualitative identification of specific hydrocarbons in the environmental samples were conducted by means of retention time comparison with standard hydrocarbons in addition to the use of GCMS Data System library. Sample chromatograms of the aliphatic hydrocarbon standards and those of water, sediment and tissue extracts are provided in Figures 4.3, 4.4, 4.5 and 4.6 respectively.

Hydrocarbons detected in the aliphatic fractions of every sample were found to correspond with the standards series, making identification relatively easy and more reliable. However, for the alkenic and aromatic fraction, many of the retention times of the available standards did not correspond to the observed peaks. This is attributed to the fact that the variety of aromatic compounds is higher than the straight-chained aliphatic hydrocarbons, particularly for the higher carbon numbered hydrocarbons due to the chemical stability gained through aromaticity. Hence, quantification was only carried out for the aliphatic hydrocarbon fractions.

All the aliphatic hydrocarbons detected in the samples are given in Tables 4.7, 4.8 and 4.9 for water, sediments and tissues respectively. It should be emphasized that the compounds listed in Tables 4.7, 4.8 and 4.9 are not the only hydrocarbons contained in the samples, but only those which can be identified with the present method. Therefore, the sum of their concentrations for each sample would not represent total hydrocarbons in the sample.

Water samples were found to contain straight-chained hydrocarbons from C_{16} up to as high as C_{28} based on the available standards. Sediment samples were observed to contain straight-chained hydrocarbons as low as C_{14} and as high as C_{30} while cockle samples were also observed to contain straight-chained hydrocarbons as low as C_{14}. The total concentrations of the detected straight-chained hydrocarbons in water, sediment and cockle observed to be significantly higher than the corresponding fluorometric measurements, indicating the contribution from biogenic sources as well as the larger aliphatic hydrocarbons contribution from petrogenic oils.

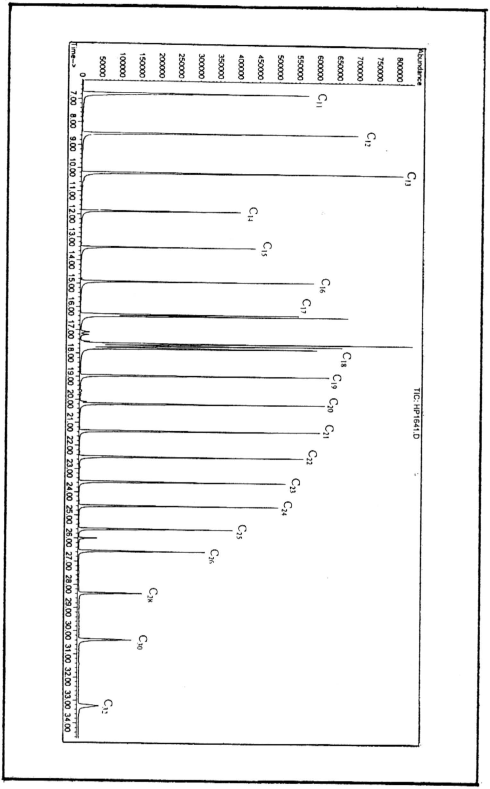


Figure 4.3: Chromatogram of the aliphatic hydrocarbons standards

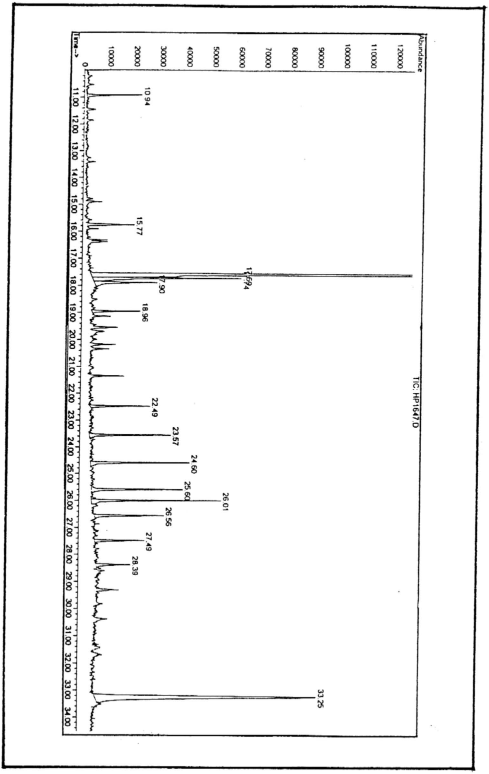


Figure 4.4: Representative chromatogram for water samples

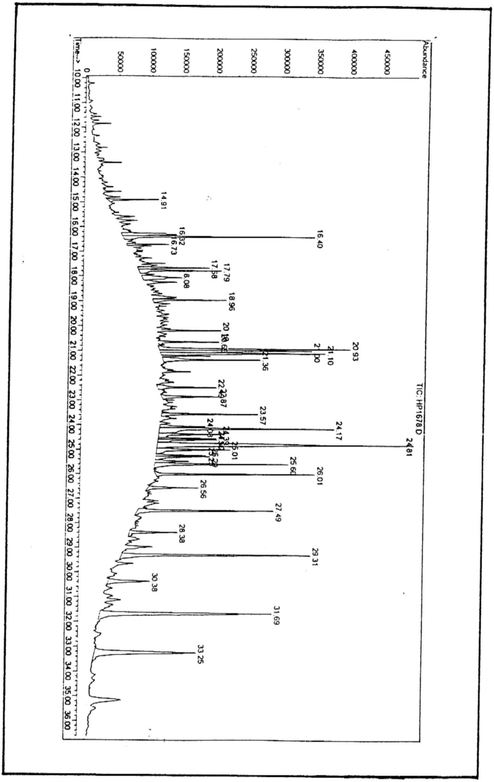


Figure 4.5: Representative chromatogram for sediment samples

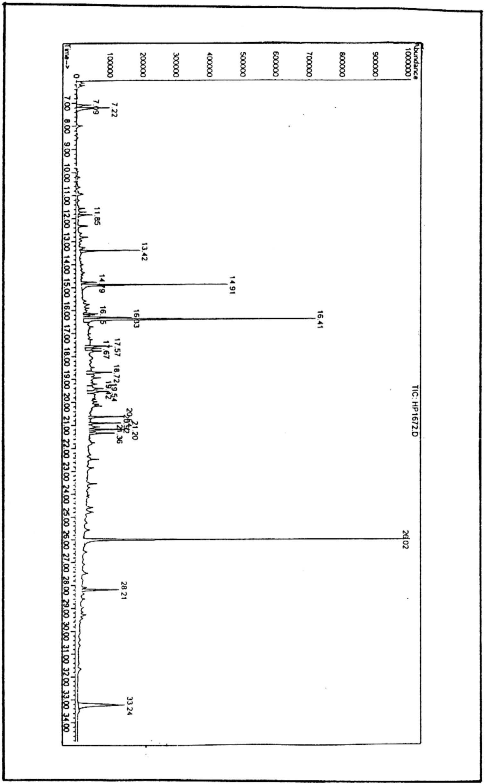


Figure 4.6: Representative chromatogram for tissue samples

Table 4.7 **Concentrations of selective aliphatic hydrocarbons identified from water samples in μg/L**

Stations	KS1	KS2	KS3	KS4	KS5	T1	T2	T3	T4	T5
Undecane	X	X	x	X	X	X	X	X	X	X
Dodecane	X	X	x	X	X	X	X	X	X	X
Tridecane	X	X	x	X	X	X	X	X	X	X
Tetradecane	X	X	x	X	X	X	X	X	X	X
Pentadecane	X	X	x	X	X	X	X	X	X	X
Hexadecane	X	X	x	X	0.0130	X	X	X	X	X
Heptadecane	X	X	x	X	X	0.0059	X	0.0043	X	0.0070
Pristane	X	X	x	X	X	0.0028	X	X	X	0.0035
Octadecane	0.0234	0.0445	0.0606	0.0256	0.0461	0.0460	0.0011	0.0437	0.0320	0.0453
Phytane	0.0093	0.0097	0.0158	0.0124	0.0090	0.0093	X	0.0096	0.0073	0.0106
Nonadecane	0.0126	0.0116	0.0229	0.0378	0.0140	0.0056	X	X	X	0.0113
Eicosane	0.0119	X	0.0096	0.0103	X	X	X	X	X	X
Heneicosane	0.0214	X	0.0129	0.0151	X	0.0076	0.0041	0.0057	0.0048	0.0072
Docosane	0.0237	0.0165	0.0265	0.0317	0.0169	0.0155	0.0110	0.0117	0.0089	0.0141
Tricosane	0.0343	0.0267	0.0440	0.0455	0.0287	0.0257	0.0195	0.0173	0.0174	0.0332
Tetracosane	0.0500	0.0339	0.0601	0.0631	0.0427	0.0362	0.0264	0.0265	0.0252	0.0336
Pentacosane	0.0597	0.0385	0.0760	0.0687	0.0480	0.0427	0.0300	0.0275	0.0296	0.0477
Hexacosane	0.0720	0.0363	0.0877	0.0791	0.0529	0.0395	0.0331	0.0344	0.0357	0.0470
Heptacosane	□	□	□	□	□	□	□	□	□	□
Octacosane	0.0586	0.0352	0.0644	0.0667	0.0432	0.0306	0.0245	0.0213	0.0331	0.057
Nonacosane	□	□	x	□	X	□	□	X	□	□
Triacontane	X	X	x	X	X	X	X	X	X	X

x : undetected

□ : unquantified

O : unidentified

Table 4.8 **Concentrations of selective aliphatic hydrocarbons identified from sediment samples in μg/g**

Stations	KS1	KS2	KS3	KS4	KS5	T1	T2	T3	T4	T5
Undecane	X	X	x	X	X	X	x	X	X	X
Dodecane	X	X	x	X	X	X	x	X	X	X
Tridecane	X	X	x	X	X	X	x	X	X	X
Tetradecane	X	X	x	X	0.0350	X	x	X	X	X
Pentadecane	0.0183	X	x	X	X	X	x	X	X	0.1007
Hexadecane	0.0208	0.0177	x	X	0.0394	X	0.0537	X	X	0.0360
Heptadecane	0.0327	0.0206	0.1138	X	0.0152	0.0181	0.1073	0.0384	0.0144	0.1081
Pristane	0.0550	0.0243	x	X	X	0.0177	0.0970	0.0186	0.0149	0.1066
Octadecane	0.0228	0.0185	x	X	0.0368	X	0.0738	0.0387	X	0.0583
Phytane	0.0178	0.0102	x	X	0.0246	0.0064	0.0462	0.0149	0.0045	0.0480
Nonadecane	0.0336	0.0153	x	X	X	0.0170	0.0749	0.0532	0.0159	0.1328
Eicosane	0.0265	0.0178	x	X	0.0504	X	0.0543	0.0520	X	0.0567
Heneicosane	0.0542	0.0284	x	X	X	0.0179	0.1250	0.1284	0.0209	0.1191
Docosane	0.0372	0.0222	x	X	0.0461	X	0.0830	0.0844	X	0.0565
Tricosane	0.1018	0.0255	0.1897	X	X	0.0233	0.1111	0.2373	0.0242	0.1391
Tetracosane	0.0646	0.0277	x	X	X	0.0468	0.1079	0.1351	0.0353	0.0897
Pentacosane	0.0323	0.0373	0.6587	X	X	0.0461	0.2619	0.3807	0.0359	0.3318
Hexacosane	0.0883	0.0392	x	X	X	0.0198	0.1162	0.1174	X	0.1154
Heptacosane	□	□	□	X	X	□	□	□	□	□
Octacosane	0.1091	X	x	X	X	X	0.2390	0.1543	X	0.2009
Nonacosane	□	X	□	X	X	□	□	□	□	□
Triacontane	0.1601	X	x	X	X	X	0.5013	0.4292	X	0.4834

x : undetected

□ : unquantified

O : unidentified

Table 4.9 **Concentrations of selective aliphatic hydrocarbons identified from tissue samples in μg/g**

Stations	KS1	KS2	KS3	KS4	KS5	T1	T2	T3	T4	T5
Undecane	X	X	x	X	X	X	x	X	X	X
Dodecane	X	X	x	X	X	X	x	X	X	X
Tridecane	X	X	x	X	X	X	x	X	X	X
Tetradecane	X	X	x	X	0.0305	X	0.0294	0.0307	0.0075	X
Pentadecane	0.4215	X	0.0261	0.2196	0.0720	0.0932	0.1036	0.1846	0.0168	0.4184
Hexadecane	0.3430	0.0048	0.0336	0.1749	0.0992	0.1513	0.1581	0.1809	0.0122	0.3722
Heptadecane	0.8101	0.0044	0.0157	0.8809	0.0506	0.0598	0.0519	0.0947	0.0074	0.2520
Pristane	0.6519	0.0049	0.0635	0.5005	0.1547	0.0764	0.1139	0.2226	0.0109	0.3120
Octadecane	X	0.0026	x	X	0.0132	0.2250	x	0.0208	X	X
Phytane	X	X	x	X	0.1088	X	x	0.0050	X	X
Nonadecane	0.1625	0.0042	x	0.5554	0.0228	X	x	X	X	X
Eicosane	X	X	x	X	X	X	x	X	X	X
Heneicosane	1.288	0.0391	0.0167	0.3167	0.0729	X	0.0257	0.0507	0.0061	X
Docosane	X	0.0058	x	X	0.0189	X	x	0.0273	X	X
Tricosane	X	0.0058	x	X	X	X	x	0.0459	X	X
Teteracosane	X	0.0081	x	X	X	X	x	0.0743	X	X
Pentacosane	X	0.0079	x	X	0.0278	X	x	0.0987	X	X
Hexacosane	X	0.0082	x	X	X	X	x	0.0973	X	X
Heptacosane	X	□	x	X	X	X	x	□	X	X
Octacosane	X	0.0074	x	X	0.0893	X	x	0.0736	X	X
Nonacosane	X	X	x	X	X	X	x	X	X	X
Triacontne	X	X	x	X	X	X	x	X	X	X

x : undetected

□ : unquantified

O : unidentified

4.2.2 Biogenic versus petrogenic hydrocarbons: Carbon Preference Index

As a result of the biosynthetic pathway in the formation of hydrocarbons in biota, the chain lengths of biogenic hydrocarbons tend to have odd numbers of carbon atoms. Petrogenic hydrocarbons, as a result of long term diagenesis have both odd and even numbers of carbon atoms. Therefore the ratio of odd and even numbers of carbon atoms as observed in a gas chromatogram of an environmental sample allow for the estimation of petrogenic contribution. The closer the ratio of odd : even the greater the petrogenic contribution. In the present study the carbon preference index (CPI) is used to estimate the input from petrogenic sources. Hence, Albaiges (1980) concluded that oil pollution has occurred when he observed a CPI of approximately 1 in the range of C_{21} - C_{33}. Lower CPIs are characteristic of polluted sites.

In the present study, higher ranges of n-alkanes were selected to estimate CPIs, as they are less susceptible to evaporation. The CPI for the straight-chained alkanes in fraction 1 of each sample are shown in Table 4.10, 4.11 and 4.12 for water, sediments and tissues respectively.

Table 4.10 **CPI values for water sample extracts**

Sample	Sampling site	Station	Carbon range	CPI
Water	Kuala Selangor	1	C_{19} - C_{26}	0.93
		2	C_{22} - C_{27}	1.01
		3	C_{19} - C_{28}	0.96
		4	C_{18} - C_{29}	1.01
		5	C_{22} - C_{27}	0.96
	Kuala Sepetang	1	C_{21} - C_{28}	0.98
		2	C_{21} - C_{28}	0.91
		3	C_{21} - C_{28}	0.91
		4	C_{21} - C_{28}	0.90
		5	C_{21} - C_{28}	1.05

Table 4.11 **CPI values for sediment sample extracts**

Sample	Sampling site	Station	Carbon range	CPI
Sediments	Kuala Selangor	1	$C_{15} - C_{30}$	2.37
		2	$C_{16} - C_{27}$	1.10
		3	$C_{15} - C_{29}$	Odd*
		4	$C_{18} - C_{29}$	Odd*
		5	$C_{22} - C_{27}$	Odd*
	Kuala Sepetang	1	$C_{23} - C_{26}$	1.06
		2	$C_{16} - C_{29}$	2.18
		3	$C_{17} - C_{30}$	2.32
		4	$C_{23} - C_{24}$	6.34
		5	$C_{15} - C_{30}$	2.59

*Predominantly odd-numbered

Table 4.12 **CPI values for tissue sample extracts**

Sample	Sampling site	Station	Carbon range	CPI
Tissues	Kuala Selangor	1	C_{15} - C_{22}	7.93
		2	C_{21} - C_{28}	2.89
		3	C_{15} - C_{18}	0.92
		4	C_{15} - C_{22}	30.1
		5	C_{14} - C_{19}	0.91
	Kuala Sepetang	1	C_{14} - C_{17}	7.93
		2	C_{14} - C_{17}	3.67
		3	C_{21} - C_{28}	1.25
		4	C_{14} - C_{17}	1.06
		5	C_{14} - C_{17}	30.1

For water samples from both sites, the CPIs were found to be close to unity, indicative of petrogenic hydrocarbon pollution. As polluted sites tend to have CPI values considerably lower than unpolluted sites it was interesting to note that the CPI values for sediment samples all exceeded 1, in many cases substantially so. This observation is attributed to the nature of the mud being highly biological and productive in nature, characteristic of the mudflat ecosystem. CPI values in cockle tissues were also found to exceed 1 indicating the predominance of biogenic hydrocarbons in the tissues. Hence, it would appear that the aliphatic hydrocarbon fractions were dominated by hydrocarbons with odd numbered carbon atoms indicative of the predominance of biogenic hydrocarbons particularly in the sediment and cockle tissues. The presence of PAHs as seen by the fluorescence measurements which are generally absent in biogenic hydrocarbons, is at the same time indicative of petrogenic hydrocarbon input. Furthermore, the presence of unimodal UCM in the range of nC_{18} - nC_{28} as seen in the sample chromatograms in Figure 4.5 is well-known to be linked to the degraded and weathered petroleum residues (Farrington and Tripp, 1975). The UCM which arise from a mixture of many structurally complex isomers and homologous branched and cyclic hydrocarbons and cannot be resolved by GC capillary column is quite prominent in sediment samples due to accumulation via sedimentation process. The fact that it occurs in the higher range of alkanes series indicates that it is attributed to the stable and undegraded petroleum products, rather than to bacterial degradation of natural organic inputs.

Pristane which is normally ubiquitous in the environment is surprisingly absent in most of the water samples, but can be observed in almost all of sediment and tissue samples. However, the absence or presence of this compound is not indicative of environmental contamination.

4.3 Uptake and Depuration of Petrogenic Hydrocarbons by *Anadara granosa*

Based on a preliminary experiment, the initial WSFs of the two oils were used directly without further dilutions. Analysis of aliquots of the WSFs gave initial concentrations of 1.32 µg/L and 1.71 µg/L for the Seligi crude oil and marine engine oil, (a refined oil) respectively. Table 4.13 shows the uptake and depuration of cockles exposed to the crude oil and refined oil. Concentrations in the tissues were averaged over triplicate pooled samples of 5 animals per replicate.

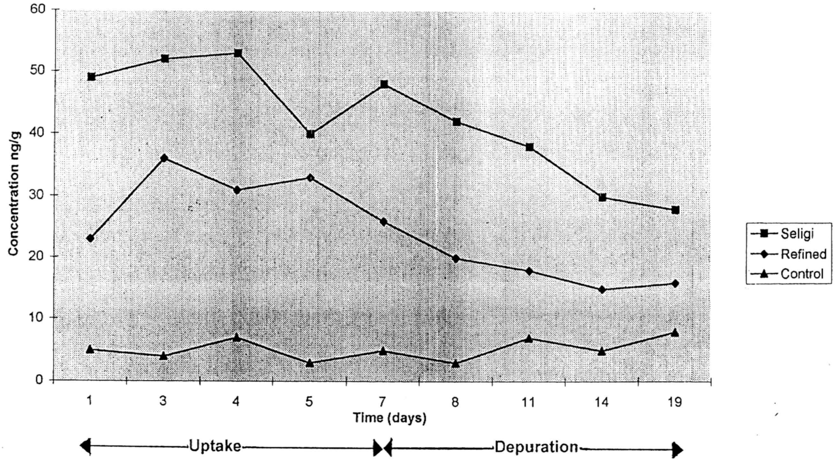
Table 4.13 **Uptake and depuration of aromatic hydrocarbons by *Anadara granosa* exposed to Seligi crude oil (1.32 μg/L) and refined oil (1.71 μg/L)**

	Concentration of hydrocarbons in animals'tissue (ng/g) (Seligi Crude Oil equivalent)								
	Uptake					Depuration			
Exposure (days)	1	3	4	5	7	8	11	14	19
Seligi	49	52	53	40	48	42	38	30	28
Refined	23	36	31	33	26	20	18	15	16
Control	5	4	7	3	5	3	7	5	8

The uptake of aromatic hydrocarbons as measured by fluorescence spectroscopy are clearly evident from the 1st day of exposure when compared to control animals. Cockles appear to take up greater amounts of crude oil compared to refined oil even though the initial concentration of the crude oil was lower than the refined oil. At the end of the 7 days exposure period, the concentrations of petrogenic hydrocarbons in the cockles were 48 ng/g and 26 ng/g Seligi crude oil equivalents from crude oil and refined oil respectively.

When removed into clean water, depuration was observed to take place. At the end of 12 days in clean water, the levels of aromatic hydrocarbons in the tissues were still significantly higher than those in the control animals. Crude oil-exposed animals had 38 ng/g Seligi crude oil equivalents of petrogenic hydrocarbons (77.6 % of the concentration following 1 day exposure) while refined oil-exposed animals had 18 ng/g Seligi crude oil equivalents (78.3 % of the concentration following 1 day exposure). Further evidence of uptake can be seen in Figures 4.7, 4.8 and 4.9 from fraction 1 of the extracts from control and exposed tanks. The chromatogram of exposed cockles were characteristic of petrogenic inputs evidenced by the UCM present in fraction 1 of the extract.

Figure 4.7 : Uptake and Depuration of Aromatic Hydrocarbons in Anadara Granosa Exposed to Seligi Crude Oil (1.32 ug/L) and Marine Engine Oil (1.71 ug/L)



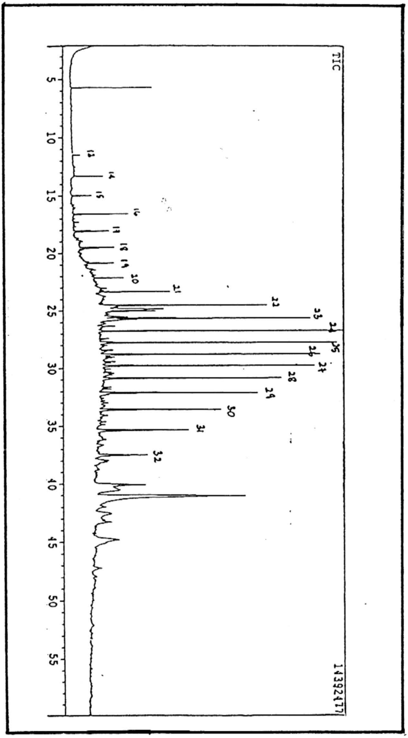


Figure 4.8 : **Gas chromatogram of cockle tissue extract from test tank (crude oil exposed, 1.32 μg/L, 4 days)**

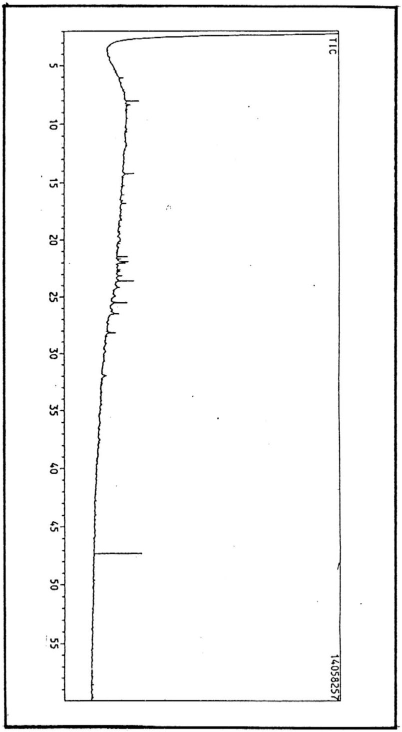


Figure 4.9 : Gas chromatogram of cockle tissue extract from control tank