Chapter III

Results & Discussion

Hydrochemistry of Water

The quality of water in the studied areas is shown in Annex II.

III.1. SUEZ CANAL

III.1.1. Oil Content
III.1.1.1. Water samples

Visual assessment throughout the Suez Canal showed the absence of floating oils (oil slicks). This means that the collected samples obtained were good representatives of the surface waters and give a real state of pollution. Gravimetric quantification of the oil content contaminating Suez Canal water samples is shown in Table (5), concentrations are ranging between 33.6 and 65.6 mg/l. The obtained data reveal that all locations are highly contaminated. The observed high values are considered to cause lethal effect which is reviewed to occur in the range of 1-10 mg/l **(Thourya, 1986).**

III.1.1.2. Sediment samples

Petroleum input on sediments cause a massive kill to benthic organisms or bottom community in the water environment. Knowledge of the hydrocarbon types and concentrations in sediment samples can be used to detect possible effects in aquatic life.

Quantitative determinations of oil content in sediment samples give more information about the level of oil pollution in the studied sites. The results of oil content in Suez Canal sediment samples are listed in Table (5), ranging between 110.5- 587.5mg/kg of dry weight. It was noticed that marine sediments are classified into three groups according to their total petroleum hydrocarbon(TPH) level :1 to 4 µg/kg in unpolluted sediments, 4 to 100 µg/kg have low to moderate chronic effect and 100 up to 12000 µg/kg in highly polluted sediments **(El-Tokhi and Moustafa 2001).**

According to this classification, the investigated samples are considered to be in the last group (highly polluted sediments). It is observed from Table

(5) and Fig.(12) that the values of TPH in sediment samples are high compared to their corresponding water samples. This difference may be attributed to the ability of sediments to accumulate and retain oils while water is mobile and tends to migrate down through sediments (**Barakat et al., 1996**). Pollutants concentrations in water are also liable to be decreases by dilution (**Moustafa[a], 2004**).

At the end, the TPH content in Suez Canal for water and sediment samples are high enough to affect the aquatic life in one way or another.

Table (5): Oil content in water, sediment samples of Suez Canal

Sites Number	Type of samples	
	Water(mg/l)	Sediment(mg/kg)
I-12	59.0	305
I-13	65.6	587.5
I-14	48.0	233.6
I-15	33.6	160.97
I-16	44.3	110.5
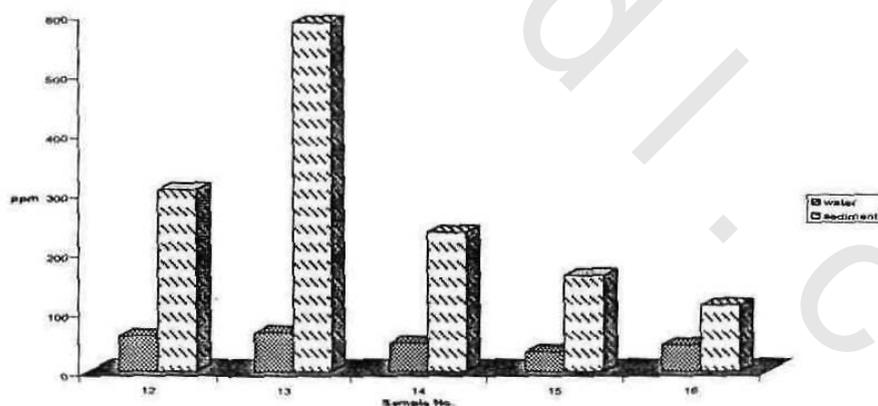
Fig.(12): Oil content in water and sediment samples of Suez Canal

III.1.2. Gas Chromatographic Analysis

Characterization and differentiation of hydrocarbons from different sources represent an essential part of any objective oil spill study. After oil spill, oil hydrocarbons often mix with other background hydrocarbon sources of hydrocarbons contributing to the petrognic source is the biogenic hydrocarbons. The backbone detection of the origin of hydrocarbon pollutants mainly depends on the gas chromatographic profile.

III.1.2.1. Water samples

The gas chromatograms of the extracted oil are shown in Fig.(13). Studying the general characteristic features of their chromatograms according to the previous concepts, it is clear that all samples are contaminated by mixed petrogenic (the presence of UCM hump) the UCM indicating that the source of contamination is mainly petroleum hydrocarbons **(Readman et al., 2002 & De Oteyza and Grimalt , 2006)** with biogenic hydrocarbons (the presence of nC25 predominant peak). The odd carbon numbers are thought to be produced by phytoplankton zooplankton, benthic algae and other biosynthesis which contribute to the hydrocarbon load of the aquatic environment **(El-Tokhi and Moustafa, 2001& Medeiros et al., 2005 & Moustafa et al., 2005).** Since many odd n-alkanes are biosynthesized by marine algae. Samples can be classified according to the type of pollutants into two groups. The first group includes samples W12 and W13, which are characterized by bimodal pattern reflecting mixed crude oil pollutant type. A bimodal alkane distribution has also been reported in sediments by **Glober and Bullin, 1989 & Méndez et al., 2001** and it has been proposed as originated by the combination of terrestrial plant waxes and petroleum sources. Bimodal distribution also reported in sediments by **Jeng and Huch, 2006** and attributed to at least two hydrocarbon sources aquatic and higher plants.

The second group include samples (W14, W15 and W16) which are characterized by severe weathering profile (all normal and iso-alkanes are

either depleted or present as very minor peaks) reflecting old petroleum inputs.

No diagnostic parameters can be calculated for chromatograms affected by a sever weathering effect. The initial carbon number is nC16 for all samples, except sample W15 is nC22. On the other hand, they differ in the final carbon number; it is nC42 for sample W12, nC37 for sample W13 and nC35 for sample W14, W15 and W16.
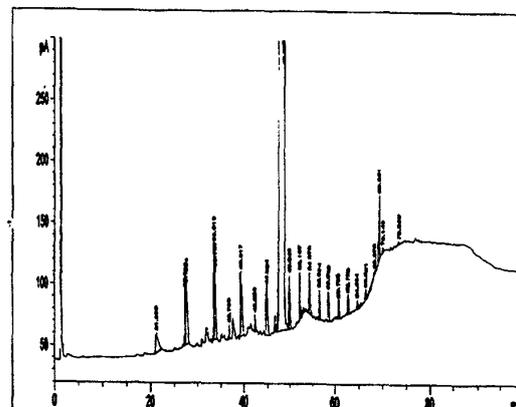
In conclusion, the presence of both weathered and severly weathering profile indicates continuous petroleum inputs in the studied area.

W12
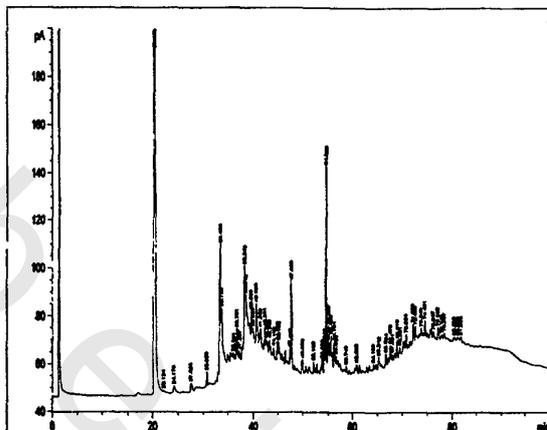
W13

W14

W15
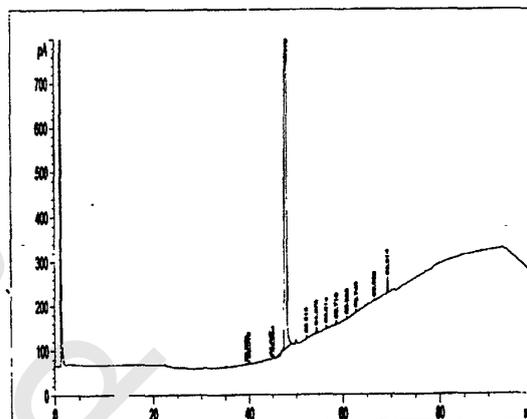
W16
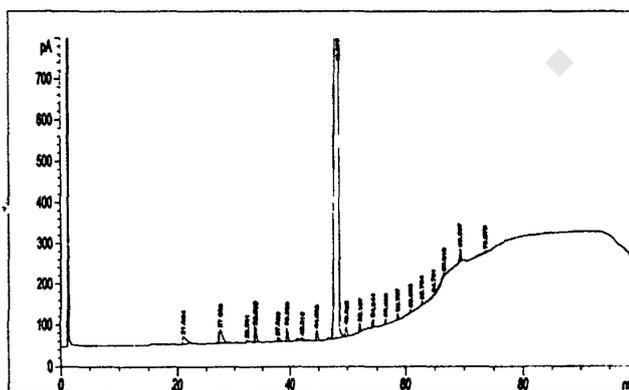
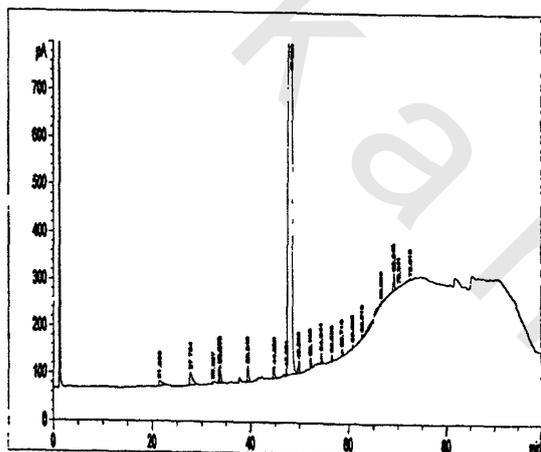**Fig.(13):** Gas chromatographic profiles of extracted oil from water samples of Suez Canal

III.1.2.2. Sediment samples

The gas chromatographic spatial pattern distribution of the extracted oil as shown in Fig.(14) reveal that the origin of contamination is mainly petrogenic. Based on the concept that each type of hydrocarbon pollutants has its own feature (fingerprint) gas chromatographic fingerprint of the studied samples reveal that the type of contamination can be attributed mainly to lubricating oil. According to the weathering degree, samples can be classified to either weathered sample S13 or very highly weathered samples S12, S14, S15 and S16. No GC parameters can be calculated from such a very highly weathered patterns.

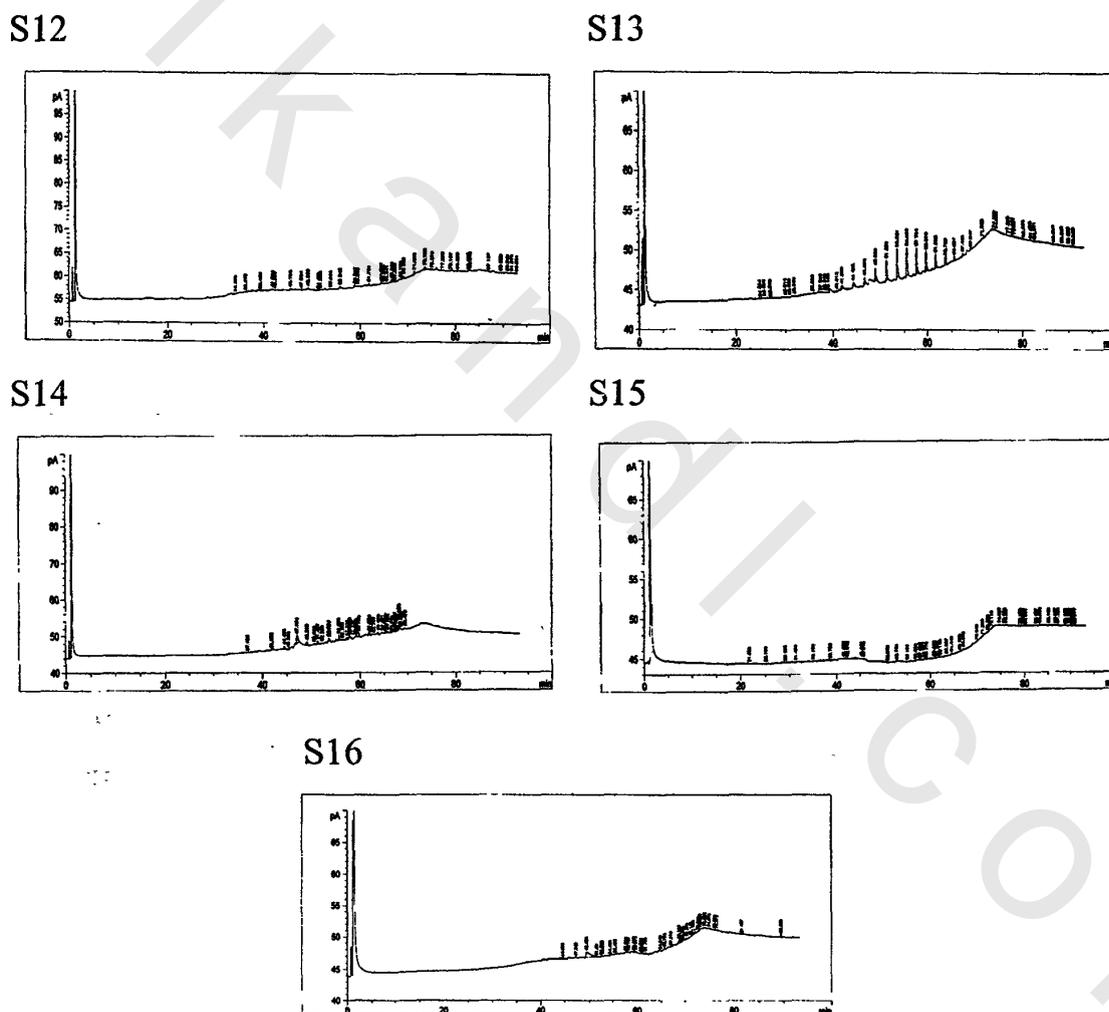S12   S13

S14   S15

S16

**Fig.(14):** Gas chromatographic profiles of extracted oil from sediment samples of Suez Canal

III.1.3. Ultraviolet Spectroscopic study:

The gas chromatographic information provides strong evidence that the hydrocarbons contaminating the Red Sea environment are mainly due to petroleum inputs. However, it is generally advisable to support gas chromatographic evidence by data from alternative methods of analysis. This confirmation is provided by the UV absorption technique.

UV spectra of petroleum and its products are very similar, showing a small number (one or two) of broad, superimposed bands at almost the same absorption wavelengths. No marked structural details are present on UV spectra of petroleum. It was reported that at least 95% of the components in the water-soluble fraction of petroleum are aromatic components having 6-13 carbons **(Coleman et al., 1984)**.

The absorption spectra mainly reflect the presence of aromatic compounds. Their detection is a good criterion of oil pollution. Careful examination of the spectra obtained show that all samples of both water and sediment samples have generally the same spectral profiles. All have the same basic features of aromatic compounds, confirming petroleum inputs. A representative model for each water and sediment samples are shown in Fig.(15). The presence of absorption maximum at 230nm is probably indicative for high concentration of alkylbenzens derived from lighter hydrocarbons originally present in crude oil and/or due to the photo-oxidation and breakdown products of higher aromatics. Small peaks are detected lower than 230nm, indicates low molecular weight PAHs.
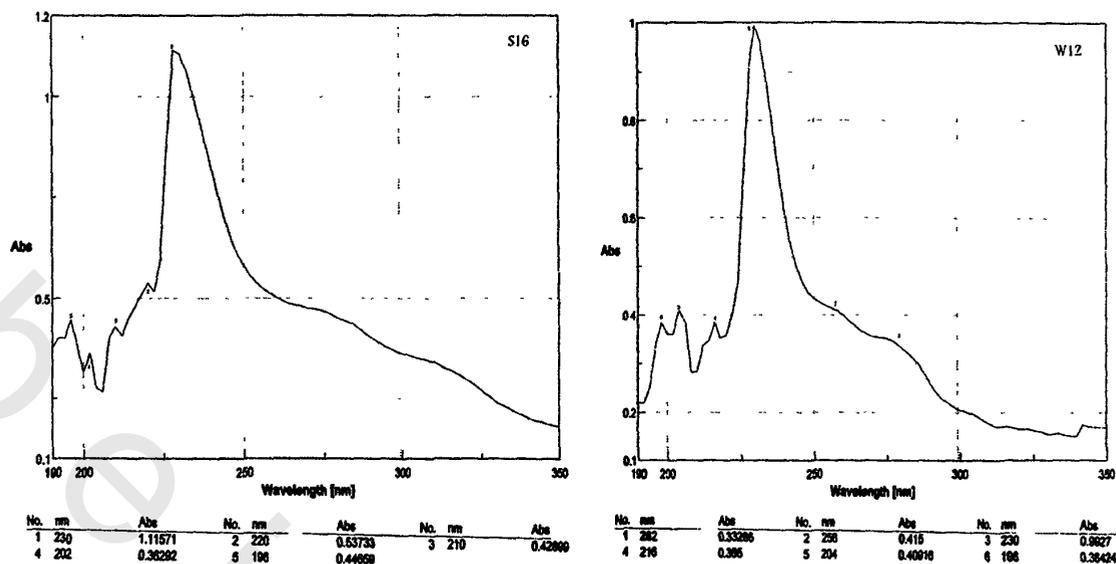
Fig.(15): Representative models for UV spectra of oil extracted from water and sediment samples of Suez Canal.

III.1.4. High Performance Liquid Chromatography

It is a very useful technique in the field of analysis of polynuclear aromatic hydrocarbons (PAHs) in oil pollution studies **(Pilli et al., 2005)**. The distribution and separation of individual PAHs of the reference 16 PAHs listed by the US EPA **(Ke et al., 2002)** using HPLC is shown in Fig.(16).
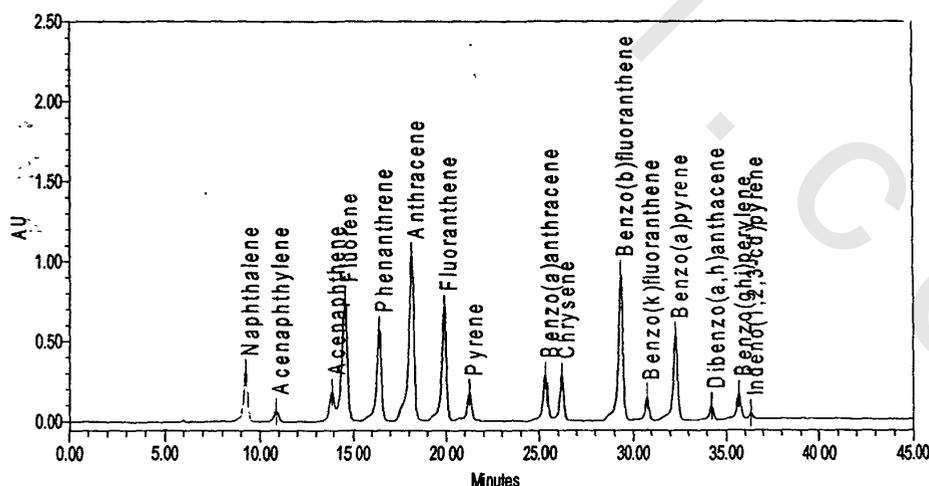
Fig.(16): The distribution of individual PAHs of the reference 16 PAHs as listed by the US-EPA using HPLC

III.1.4.1. Water samples

Investigation of individual PAHs in the extracted oil from water samples is a very important part of environmental quality assessment and hence their impact on the ecosystem (**Samanta et al., 2002& Maskaui et al., 2002 & Moustafa, 2005**). Qualitative identification of individual PAHs using HPLC technique was done. Representative model for all the studied samples is shown in Fig.(17). The total PAHs concentrations in water ranged from 212.34 to 348.33 µg/l as shown in Table (6), It can be observed also that water samples are generally characterized by low ratios of Σ6LPAHs: Σ10HPAHs. It rages between 1:6 and 1:27 as shown in Table (6-a). This might be presumably because of the relatively higher volatility of these LPAHs compounds which restrict their residence in water, especially in warm weather (**Ahmed et al., 1998 & Zakaria et al., 2001**).

Samples can be arranged according to their ascending values as follows: W13, W15, W16, W14 and W12. The detected concentrations of PAHs in water ranged between 0–0.45µg/l for 2-ring PAHs (naphthalene), 10.99–40.61µg/l for the 3-ring PAHs (acenaphthylene, acenaphthene, fluorine, phenanthrene and anthracene), 2.17- 18.2 µg/l for the 4-ring PAHs, 0- 4.72 µg/l for the 5-ring PAHs and 158.2- 313.3µg/l for the 6-ring PAHs. All samples are characterized by the absence of the two membered rings PAHs (naphthalene) except sample W16 which has value of 0.45µg/l. This may be due to naphthalene have higher vapor pressure and Henry's law constant (0.085 mmHg at 25°C, **Ambrose et al., 1975**; 0.00044 atm-m^3/mole, **Shiu and Mackay, 1997**) which means that it is preferred to transport by atmosphere-water interaction (**Shi et al., 2005**). It is clear that HMW PAHs are more predominant than LPAHs due to the small-sizeof LPAHs which are more labile, and are expected to degrade faster than the lager ones and also due to the higher vapor pressure and water solubilities (**Mackay et al., 1992**). **Maskaoui et al., 2002** stated that from an ecotoxicological point of view, a majority of water samples have high enough concentrations of PAHs to induce immediate mortality in exposed organisms

The ratio of Phe over Ant. was extensively used to identify the source of PAHs contaminating water. Phe is thermodynamically more stable and its prevalence over Ant supports petrogenesis of PAHs. Indeed, petroleum usually exhibits a quite high Phe/Ant ratio. Sample W12 has phe/Ant ratio close to zero indicating pyrogenic origin which is confirmed by Flu/Pyr which has value of infinity (due to absence of pyrene) indicating pyrogenic origin. Sample W13 have Phe/Ant ratio >1 indicating petrogenic origin which is confirmed by Flu/Pyr which has zero value (due to absence of fluoranthene). Samples W14, W15 and W16 regarding to Phe/Ant ratio have values of infinity due to the absence of anthracene indicating purely petrogenic origin In contrary to Flu/Pyr which have also infinity value due to absence of pyrene indicating purely pyrogenic origin.this contradiction between the two parameters may be due to the weathering effects which act on such four hydrocarbon with different rates. The gas chromatographic profiles of samples W14, W15 and W16 show clearly that they are highly weathered. These two parameters go well giving the same results mainly in fresh or slightly weathered samples.

PAHs are considered environmental pollutants that can have a detrimental effect on the flora and fauna of the plants resulting in the uptake and accumulation in food chain. These compounds have also toxic, mutagenic and/or carcinogenic properties **(Wu et al., 2003&Moustafa, 2005 and Tfouni et al., 2007)**.

Table (6): Individual PAHs distribution and their concentrations (µg/l) in water samples of Suez Canal

Ring No.	PAHs	water samples of Suez Canal				
		W12	W13	W14	W15	W16
2	Naph	0	0	0	0	0.45
	Total	0	0	0	0	0.45
3	A	22.4	0	9.4	9.4	34.6
	Ace	3	3.3	0	2.3	4
	F	0.69	19.9	1.5	1.2	1.8
	Phe	0.24	7.3	0.09	0.12	0.21
	Ant	1.8	0.72	0	0	0
	Total	28.13	31.22	10.99	13.02	40.61
4	Flu	0.8	0	0.38	0.47	3.4
	Pyr	0	15.2	0	0	0
	BaA	2.4	3	0.83	1.7	3.5
	Chr	3.7	0	1.2	0	6
	Total	6.9	18.2	2.41	2.17	12.9
5	BbF	0	4.7	0	0	0
	BkF	0	0.016	0	0.025	0
	BaP	Nil	Nil	Nil	Nil	Nil
	DahA	0	0	0	0	0
	Total	0	4.716	0	0.025	0
6	BP	4.9	0	0	0	0
	IP	308.4	158.2	298.7	218.7	237.5
	Total	313.3	158.2	298.7	218.7	237.5
	Total ΣPAHs	348.33	212.336	312.1	233.915	291.46

Table (6-a): PAHs parameters used to distinguish petrogenic from pyrogenic origin of PAHs of Suez Canal in water samples

Parameter	W12	W13	W14	W15	W16
phe/Ant	0.13	10	∞	∞	∞
flu/pyr	∞	0	∞	∞	∞
ΣLPAHs:ΣHPAHs	1:11	1:6	1:27	1:17	1:6

Phen/Ant<1 indicates Pyrolytic origin and Phen/Ant >1 indicates petrogenic origin

Flu/Pyr<1 indicates petrogenic origin and Flu/Pyr>1 indicates Pyrolytic origin

III.1.4.2. Sediment samples

Qualitative identification of the individual EPA 16 PAHs for the extracted oils from sediment samples were done and a representative model is shown in Fig.(17). Careful examination of their HPLC chromatograms show that, each sample has its own distribution pattern and all have most of the target compounds. The brief identification of the present PAHs as shown in Table (7) reveals the presence of both LPAHs and HPAHs. The concentrations of the total 16 PAHs ($\Sigma 16$ PAHs) are ranging between between 231.76 and 3099.82μg/kg. As weathering increases the summation of the 6LPAHs decreased which makes the $\Sigma 6$LPAHs: $\Sigma 10$HPAHs parameter not useful to detect the origin. On the other hand it could be useful in the comparison of the weathering effects on the samples **(Zakaria et al., 2001).**

Data obtained show low ratios of $\Sigma 6$LPAHs: $\Sigma 10$HPAHs it ranges between 1:4 and 1:700 as listed in Table (7-a). From this quantitative data, it is clear that only sample S12 has low value while the other samples are characterized by relatively high values. Generally the highest value is observed in case of sample S15 (3099.82μg/kg). The high concentrations of PAHs in sediment samples compared to the corresponding values of PAHs in water samples is attributed to continuous PAHs inputs and their low water solubilities and high partition coefficients, these compounds are strongly sorbed onto the surface of particles associated with the organic compounds of solid phase matrix and can be deposited to the underlying sediments **(Doong and Lin, 2004).** Samples can be arranged according to their ascending values as follows: S12, S16, S13, S14 and S15. All samples are characterized by the absence of two membered rings compounds (naphthalenes) except sample S16 which has value of 2.6μg/kg. For the 3-rings PAHs the values are 1.82–50.38μg/kg (acenaphthylene, acenaphthene, fluorine, phenanthrene and anthracene), 1.3- 17.08 μg/kg for the 4-rings PAHs, 0- 10.6μg/kg for the 5-rings PAHs and164.3- 3083.5 μg/kg for the 6-rings PAHs.

The absence of some PAHs as shown in Table (7) may come to the fact that such PAHs in sediments resulting from the release of petroleum were

thought to be readily available for microbial degradation under aerobic conditions.

As mentioned before, PAHs distribution is the most useful tool in distinguishing petrogenic from pyrogenic origin of the contaminants. All samples as shown in Table (7-a) are characterized by the predominance of HPAHs over the LPAHs. In contrast, petrogenic PAHs are normally abundant in low molecular weight compounds which are readily modified by weathering. Due to evaporation, dissolution and biodegradation, low molecular weights PAHs are thought to be selectively disappeared.

Its previously mentioned, petroleum often contains more thermodynamically stable compounds such as phenanthrene, while fluoranthene and Anthracene are usually most abundant compounds in pyrolytic PAHs **(Doong and Lin, 2004)**. Regarding to the Phe/Ant ratios samples S12 and S15 have values of infinity due to absence of anthracene indicating purely petrogenic origin.in contrary to Flu/Pyr which have also infinity value (due to absence of pyrene) indicating pyrogenic origin.this contradiction may be due to the effect of weathering as previously discussed. Sample S13 show also contradicting values but in a reverse manner.

For sample S14, Phe/Ant have value of infinity indicating petrogenic origin confirmed by Flu/Pyr which have zero value indicating also purely petrogenic

Sample S16, has Phe/Ant value close to zero indicating pyrogenic origin which is confirmed by Flu/Pyr which have infinity value indicating also purely pyrogenic origin.

No definite correlation could be observed between the values (Σ16PAHs) obtained from extracted oil from water and sediment samples indicating that the origin of PAHs play an important role in the results obtained.

It is generally observed that water and sediment samples of Suez Canal are characterized by high concentration of PAHs which have negative impacts to both human health and to the environment.
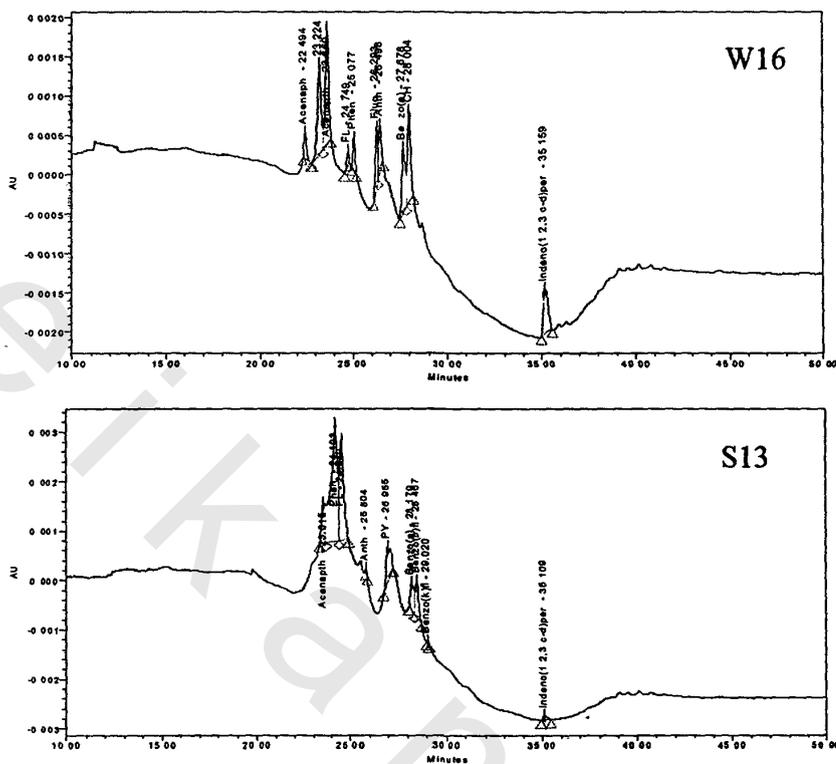
Fig.(17): Representative HPLC chromatograms of the PAHs in oil extracted from; water and sediment samples of Suez Canal

Table (7): Individual PAHs distribution and their concentrations (µg/kg) in sediment samples of Suez Canal

Ring No.	PAHs	sediment samples of Suez Canal				
		S12	S13	S14	S15	S16
2	Naph	0	0	0	2.6	0
	Total	0	0	0	2.6	0
3	A	20.7	0	26.8	0	11.8
	Ace	0	0	2.32	0	5.6
	F	29.25	3.9	0.7	1	0.86
	Phe	0.43	0	0.09	0.82	0.47
	Ant	0	7.2	0	0	8.9
	Total	50.38	11.1	29.91	1.82	27.63
4	Flu	3.4	0	0	0.57	3.67
	Pyr	0	0	0	0	0
	BaA	4.68	5.4	2	0.73	4.55
	Chr	9	0	0.15	0	8
	Total	17.08	5.4	2.15	1.3	16.22
5	BbF	0	0	0	3.7	0
	BkF	0	0	0.77	0.0021	0
	BaP	Nil	Nil	Nil	Nil	Nil
	DahA	0	0	0	6.9	0
	Total	0	0	0.77	10.6021	0
6	BP	0	0	0	0	0
	IP	164.3	1309.6	2495.4	3083.5	1195.8
	Total	164.3	1309.6	2495.4	3083.5	1195.8
	Total ΣPAHs	231.76	1326.1	2528.23	3099.8	1239.65

Table (7-a): PAHs parameters used to distinguish petrogenic from pyrogenic origin of PAHs of Suez Canal

Parameter	S12	S13	S14	S15	S16
phe/Ant	∞	0	∞	∞	0.05
flu/pyr	∞	0	0	∞	∞
ΣLPAHs:ΣHPAHs	1:4	1:118	1:84	1:700	1:44

Phen/Ant<1 indicates Pyrolytic origin and Phen/Ant >1 indicates petrogenic origin

Flu/Pyr<1 indicates petrogenic origin and Flu/Pyr>1 indicates Pyrolytic origin

III.2. TEMSAH LAKE

III.2.1. Oil content

III.2.1.1. Water samples

Visual assessment of all the studied sites except sites W6, W9 and W11 shows the absence of either oil slicks or thick patches of chocolate-brown emulsion "mousse" on the surface of the water, which means that the collected water samples were generally good representative samples that give real state of pollution. The first step in assessing the seriousness of hydrocarbons pollution involves determining the magnitude of its concentration.

Gravimetric determination of oil content for water samples, as shown in Table (8), gives ranges between 2.80 and 103.3mg/l. Based on the biological responses values that might accompany or follow oil pollution, lethal effects occur in the range of 1 to 10mg/L, while sub-lethal effects range is as low as 1mg/l **(Egaas et al., 1982)**. According to the obtained values, the studied areas except sites W1 and W10; have values high enough to cause lethal toxicity where the components of the oil, especially aromatics interfere with cellular or sub-cellular processes in the living organisms causing carcinogenesis in some cases .

III.2.1.2. Sediment samples

Surface sediments are popular matrices for the analysis of hydrocarbon levels in the environment. Sediments are also commonly used for the assessment of petroleum pollution in coastal aquatic systems. This approach arises from the concept that the sediments act as pollutant sinks and provide an integrated picture of the events taking place in the water column. Studies have shown that hydrocarbons from spilled petroleum persist in the sedimentary environment for substantial period of time may be years.

As previously mentioned **Metwalley et al., 1997**, noticed that marine sediments are classified into three groups according to their TPH levels: 1 to

4mg/Kg in unpolluted ocean sediments, <100mg/Kg in moderately polluted coastal sediments and up to 12000mg/Kg in highly polluted sediments. Based on this classification, the obtained values listed in Table (8) ranging between 135.73 and 634.61mg/kg dry weight, are considered to be highly polluted sediments. This high alarming concentration levels poses potential threats to marine life and to their consumers. These high TPH concentrations may be attributed to the disposed of oil that may be originated from shipping activities, ballasting water, and maintenance and maritime in the several docks around (Temsah Lake map, Fig.10). This high contamination level is the cause behind the decline in the lake biodiversity and decrease in fish quality harvested from the lake. As previously mentioned the high values of TPH in case of sediment samples compared to that of water samples may be attributed to the persistence and bioaccumulation of oil in sediment (El-Tokhi and Moustafa, 2001). Same observation was reported by Moustafa[a] (2004) and attributed to the ability of sediments to accumulate and retain oils while water is mobile and tends to migrate down through sediments. Pollutants concentrations in water are also liable to be decreased by dilution. This can be also attributed to the nature of oil which exhibit hydrophobic associations with surface of suspended particulate, or interact with natural dissolved materials to form micelle aggregations. Also materials of petroleum origin are generally non-polar and very poorly soluble in water (Moustafa et al., 2005). Colombo et al., 2005 reported that due to the hydrophobic nature of TPH, the environmental distribution of hydrocarbons reflects the increasing affinity sequence:water<<sediments<biota<soils. The iso-contour map for oil content of water and sediment samples are shown in Fig (18).

Table (8): Oil content in water, sediment and fish samples of Temsah Lake

Sites Number	Type of samples		
	Water (mg/l)	Sediment (mg/Kg)	Fish (mg/Kg)
II-1	2.8	634.61	138.60
II-2	34.33	Rock area	Not represented
II-3	32.40	295.60	101.90
II-4	13.25	180	Not represented
II-5	40.25	Rock area	Not represented
II-6	55.00	Rock area	Not represented
II-7	32.66	174.40	Not represented
II-8	21.75	460	Not represented
II-9	103.30	135.73	133
II-10	5.25	136.29	Not represented
II-11	70.76	296	128.80
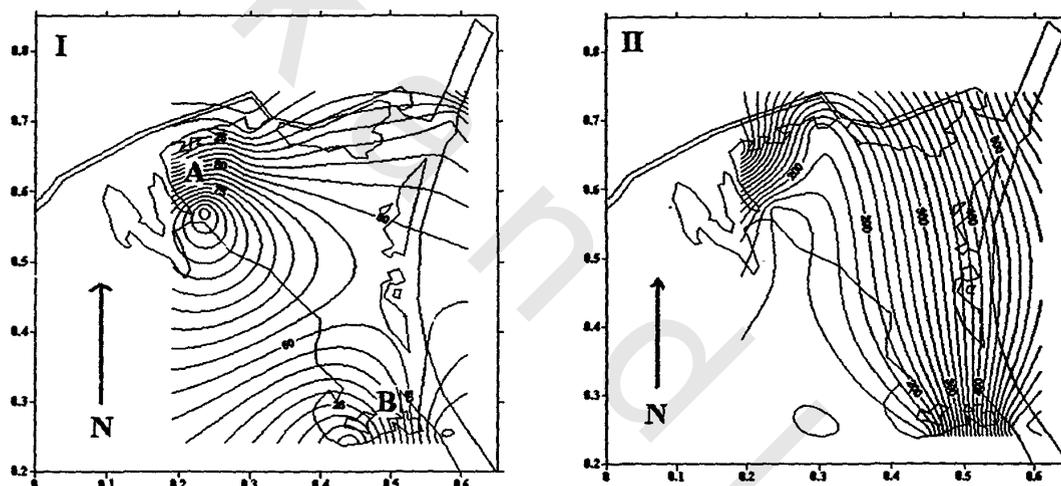
Fig.(18): Iso-contour map for oil content (I) for water and (II) for sediment samples of Temsah Lake.

The iso-contour map for oil content of water samples has two areas of relatively high oil concentration; (A) North West and (B) South East they are almost on a straight line. The lowest value is recorded at the extreme North East part of the lake. Concentration of petroleum hydrocarbons in marine water is a subject of various forces that control the horizontal and vertical distribution of oil such as ambient temperature, water current, wind direction and speed **(El-Samra and El-Deeb 1998)**. Such factors are likely to be involved in the distribution of petroleum load in studied sites.

The iso-contour map for oil content of sediment samples shows that the oil content increases gradually towards East getting its maximum towards North East this may be resulted from the presence of some potential polluters such as ships and tankers passing through the Suez Canal. The overall high concentration of TPH in these studied areas may be due to the presence of dockyards and ship-maintenance works for example S.C.A. workshop yard Lake Temsah, El-Temsah Ship yard (private workshop) and Arab Cont. ship yard (Temsah Lake map, Fig.1).

III.2.1.3. Fish samples

The concept of using essential organisms for monitoring the concentration of selected pollutants in the aquatic environment and as an indicator of their bioavailability has gained a wide acceptance and programs have been established on a national and international basis (Choiseul et al., 1998). Oil content values for the collected fish samples F1, F3, F9 and F11 are 138.6, 101.9,133 and 128.8mg/Kg, respectively which are considered to be very alarmingly highly affected by oil pollution in these studied locations. This confirms the reason for decline in the lake biodiversity damage of the marine environment of lake. It was reported that fish can be tainted very rapidly on exposure, within a few hours at concentrations above 1 mg/l hydrocarbons in the ambient water (Moller et al., 1989). Humans can taste petroleum hydrocarbons in fishes at concentrations between 5-20ppm (Shriadah, 1998); in fact, directly or indirectly the presence of the pollutants in fish tissues may be a risk for potential bioaccumulation in the food (Méndez et al., 2001).

III.2.2. Gas chromatographic analysis

The GC/FID approach is used as a quick screening characterization of the estimated oil samples. The GC/FID chromatogram gives a descriptive "picture" of the distribution of the dominating hydrocarbons in a sample e.g. individual resolved n-alkanes and major isoprenoids; as shown in Fig.(19,21,23). They also provide good information on the weathering extent of the spilled oil, and also if any "characteristic features" or possible "contaminating" components are present (Daling and Faksness, 2002).

III.2.2.1. Water sample

The gas chromatographic profile of the extracted oil from water samples Fig.(19) show regularly spaced n-alkane peaks with different initial and final carbon number as shown in Table (9), protruded on a prominent unresolved envelope (hump); indicating that the source of contamination is mainly petroleum hydrocarbons **(Readman et al., 2002 & De Oteyza and Grimalt , 2006)**. From the GC peak area calculations, it is clear that, the percentage sum of both aromatics and cycloalkanes (hump) are representing values between 23.58% and 80.57% of the contaminating oil while the sum of n- and iso-paraffins (resolved peaks) together represent values between 19.43% and 76.42%, as listed in Table(9). The mentioned data regarding the amount and nature of hydrocarbon contaminants in the area under study show a chronic case of pollution in which the most toxic and persistent hydrocarbons are abundant. **Atlas, 1981 & McNally et al., 1998** came to the same conclusion for their studied areas. The chromatograms of Samples W2, W3 and W10 show a UCM signal in the high molecular weight region suggesting that the samples were exposed to the most chronic oil pollution and/or reveal that the source of contaminations by lubricating oils **(El-Tokhi and Moustafa, 2001 & Moustafa[b], 2004)**. This may result from the presence of some potential polluters such as dry docks, ships and tankers passing through the Suez Canal, also they may be due to the presence of dockyards and ship-maintenance works for example S.C.A. workshop yard Lake Temsah, El-Temsah Ship yard (private workshop) and Arab Cont. ship yard (Temsah Lake map, Fig.,10). Sample W10 shows a well defined and broad UCM with a predominant peak at C_{26} this might indicate contamination with mid range distillates which might be attributed to the nearby of this site to ARAB Cont. Ship Yard. Mid range distillates; kerosene, jet fuel and diesel products are typical products in a relative broad carbon range (C_6-C_{26}) and the UCM is well defined **(Wang and Fingas, 2003)**.

The components up to nC_{17} for samples; W5 and W10 and up to nC_{14} for all other samples were lost due to the natural weathering processes; vaporization, dissolution and/or photo-oxidation **(Kaplan and Kitts, 2004 & De Oteyza and Grimalt , 2006)**, confirming a slow weathering rate of the spilled oil.

Fig.(19): Gas chromatographic profile of extracted oil from water samples of Temsah Lake

Table (9): Diagnostic parameters in water samples of Temsah Lake

Sample Number	Initial nC_n	Final nC_n	UCM%	TRP%	UCM/TRP	Pr/Ph	CPI
W1	C_{14}	C_{31}	49.10	50.90	0.96	0.67	2
W2	C_{14}	C_{34}	53.20	46.80	1.14	0.9	1.6
W3	C_{14}	C_{31}	67.75	32.25	2.1	0.92	2
W4	C_{14}	C_{33}	23.58	76.42	0.31	1.29	1.7
W5	C_{17}	C_{33}	51.55	48.45	1.06	0	1.76
W6	C_{14}	C_{33}	32.80	67.2	0.49	1.9	2
W7	C_{14}	C_{33}	46.44	53.56	0.87	0	1.8
W8	C_{14}	C_{33}	54.12	45.88	1.18	0	1.75
W9	C_{14}	C_{33}	43.01	56.99	0.75	2	2.5
W10	C_{17}	C_{30}	80.57	19.43	4.15	0.7	1.75
W11	C_{14}	C_{33}	56.30	43.70	1.28	1.1	1.47

Weathering causes considerable changes in the chemical and physical properties of the spilled oils (Tolosa et al., 2005). The extent and rate of weathering is different for each spill and is controlled by a number of spill conditions and natural processes such as, type of the spilled oil, environmental conditions and microbiological activities, accordingly water samples can be classified into three groups. The first group includes samples W1, W4, W6, W7 and W9 which are characterized by a pattern of low weathered crude oil. The second group includes samples W2, W5, W8 and W11 which are characterized by a moderately weathered crude oil. The third group is characterized by a highly weathered crude oil profile including samples W3 and W10 the presence of the three groups gives an indication of continuous petroleum inputs.

Pristane/Phytane ratio; all the investigated samples are characterized by the presence of both Pr and/or Ph with different concentrations; indicating petroleum origin (Medeiros and Bicego, 2004). Pr is not found in three samples W5, W7 and W8, similar observation was reported by Medeiros and Bicego, 2004. Wang and Fingas, 2003 reported that pristane and phytane can be lost due to high weathering. Pr/Ph ratios are listed in table (9); most water samples have values lower than 1, indicating mainly a petroleum products source and/or slight biogenic contribution. But Pr/Ph ratio is greater than 1 is observed in samples W4, W6, W9 and W11 which indicates

contribution from biogenic hydrocarbons biosynthesized by zooplankton and marine algae **(Moustafa[b], 2004)**.

Carbon preference index (CPI); All the investigatedwater samples are characterized by CPI >1, they have values ranging between; 1.47 and 2.5, indicating biogenic addition **(Tolosa et al., 2005)**.

Generally the gas chromatographic patterns and the histograms of n-parrafin distributions of water samples (Figs.19 and 20, respectively) show the abundance of odd carbon numbers. The predominant peak in samples; W1, W4, W6, W7, W8, W9 and W11 is nC_{25} ,samples W2 and W3 is nC_{15}, while the predominant peak in sample W5 is nC_{21}. The short-chain odd carbon number (C 25) are thought to be produced by phytoplankton zooplankton, benthic algae and other biosynthesis which contribute to the hydrocarbon load of the aquatic environment **(El-Tokhi and Moustafa, 2001& Medeiros et al., 2005 & Moustafa et al., 2005)**. Since many odd n-alkanes are biosynthesized by marine algae, fluctuations in this source of input or their own biosynthesis of such compounds can influence their concentration **(Méndez et al., 2001)**. Various reports have illustrated the ability of marine organisms to produce aliphatic hydrocarbons **(Ahmed et al., 1998)**.

W1
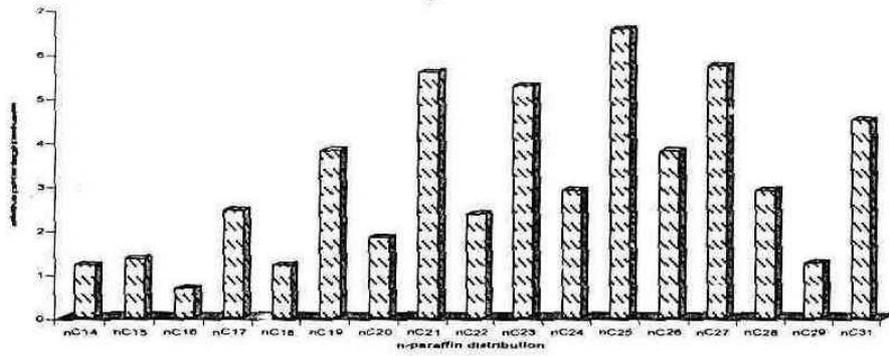
W2
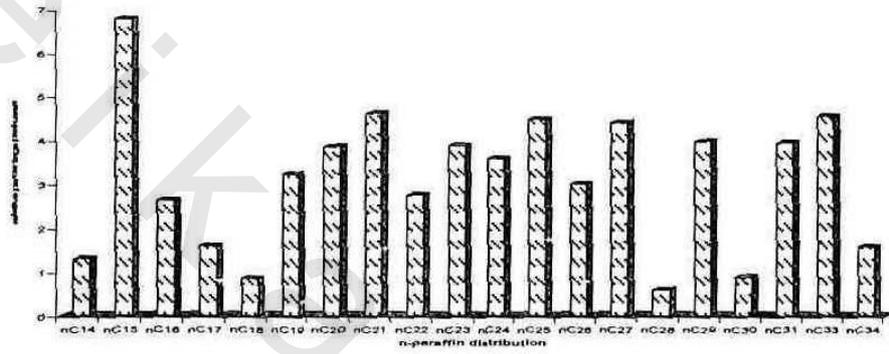
W3

W4

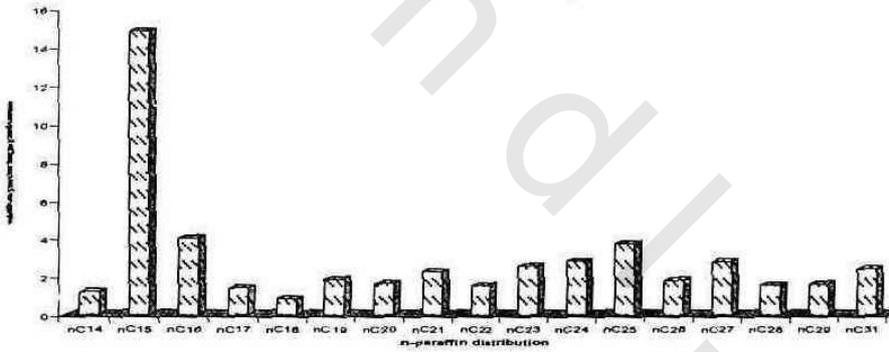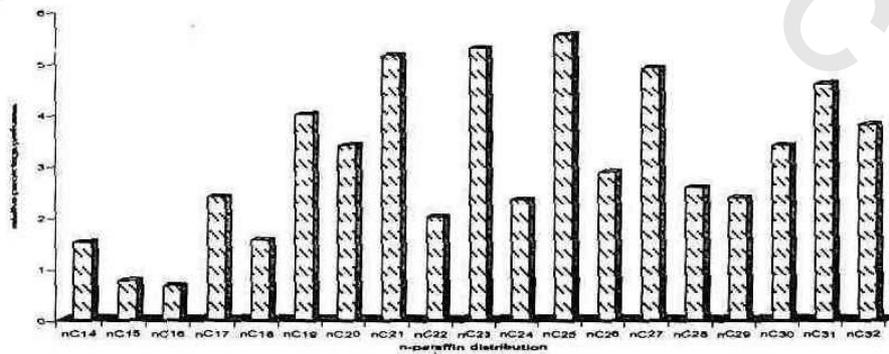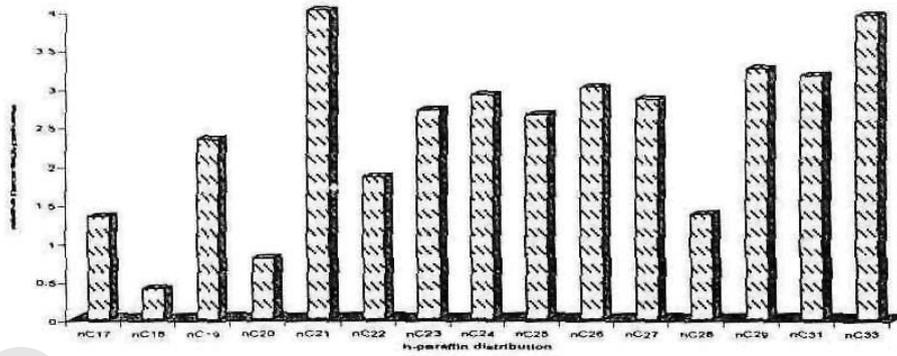**Fig.(20):** Histogram for n-paraffin distribution of extracted oil from water samples of Temsah Lake

W5

W6
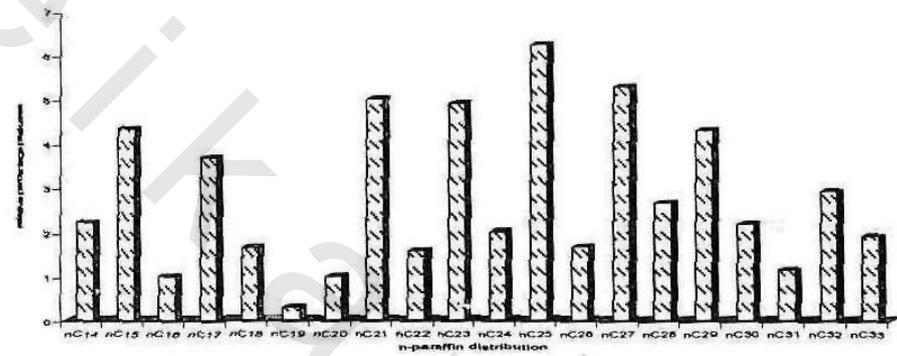
W7
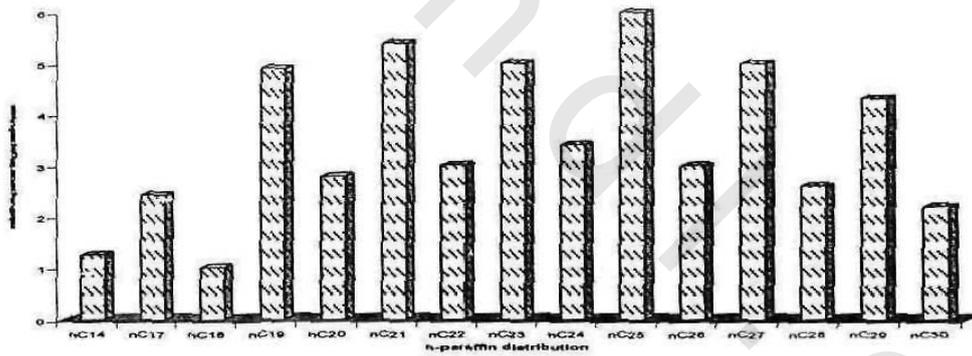
W8
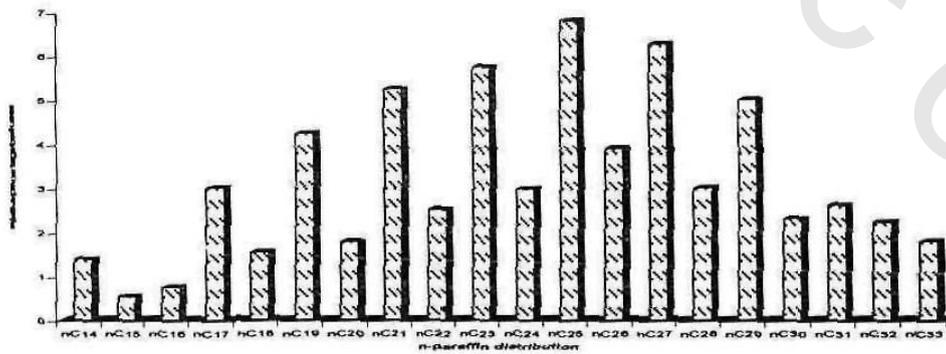
Con. Fig.(20): Histogram for n-paraffin distribution of extracted oil from water samples of Temsah Lake

W9
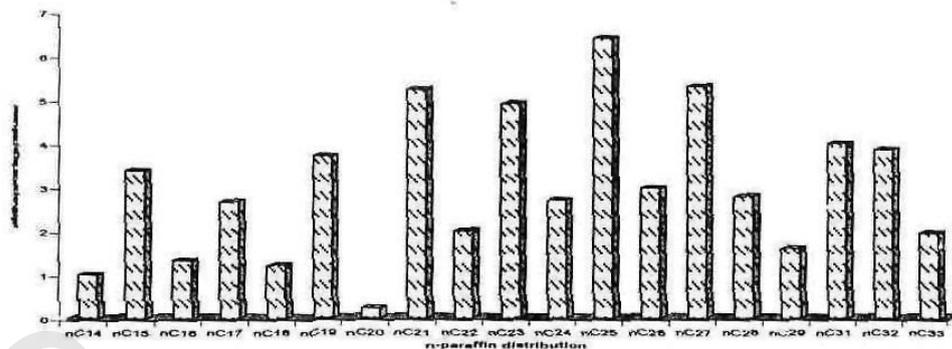
W10
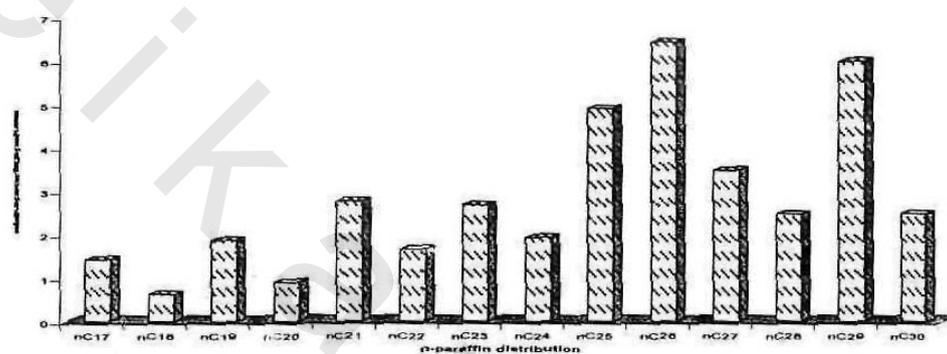
W11
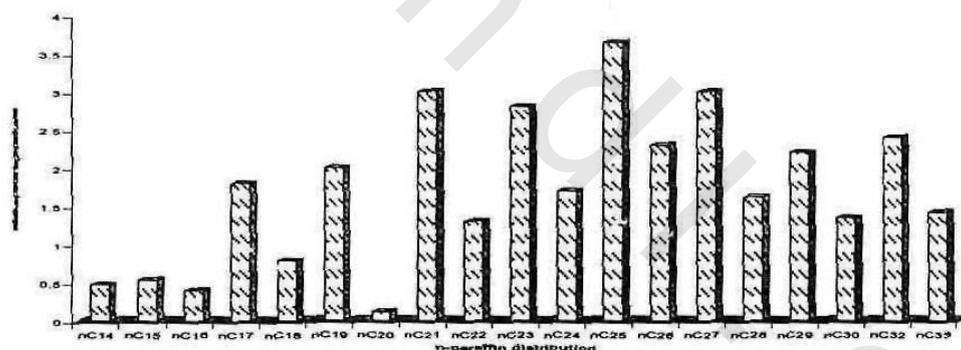
Con. Fig.(20): Histogram for n-paraffin distribution of extracted oil from water samples of Temsah Lake

Generally there is an obvious elevation in the long-chain odd carbon number (C 25) with variable concentrations, except for sample W3. This pattern is characteristic of higher plant waxes and thus reveals the occurrence of prominent terrestrial inputs to these sampling sites. The percentage of the most abundant normal alkanes related to biogenic terrestrial sources (C_{27}, C_{29}, C_{31} and C_{33}) and referred as alk_{terr} (**Medeiros et al., 2005**).

UCM/TRP; W3 and W10 showed the highest value UCM/TRP ratio; 2.1 and 4.15, respectively providing evidence of losses in TRP and/or n-alkanes and increase in UCM ratios, which are indicative of high degrees of weathering and chronic oil pollution **(Tolosa et al., 2005)**. Samples W2, W5, W8 and W11 have UCM/TRP ratio values range between 1.06 and 1.28 which indicates moderate weathering. However, samples W1, W4, W6, W7 and W9 have UCM/TRP ratio lower than 1, representing low degree of weathering and/or indicative of more recent inputs of oil **(Tolosa et al., 2005)**.

III.2.2.2. Sediment samples

Surface sediments are popular matrices for the analysis of hydrocarbon levels in the environment. The gas chromatographic profiles; Fig.(21) show resolvable peaks with different initial and final carbon numbers as illustrated in Table (10) superimposed on a prominent UCM. The sources of contamination are either petrogenic or mixed petrogenic with biogenic hydrocarbons. From the GC peaks areas calculations, it is clear that, the percentages of UCM range between 71.32% and 98.28% of the contaminating oil while the percentages of resolved peaks range between 2.14% and 28.68%, as listed in Table (10). The histograms of n-paraffin distribution of oil extracted from sediment samples illustrated in Fig.(22) show the absence of some n-alkanes. The high UCM values and the decrease and/or absence of n-alkanes are indicative of chronic, degraded and highly weathered petroleum contaminations **(Tolosa et al., 2005)**. Sample S10 as shown in Fig.(21) shows a well defined UCM signal in the high molecular weight region with nearly no resolved alkanes being present suggesting that the samples were exposed to the most chronic oil pollution and/or reveal that the source of contamination is by lubricating oils. This might be attributed to the nearby of this site to ARAB Cont. Ship Yard (Temsah Lake map, Fig.10). The chromatograms of S1 and S11 as shown in Fig.(21) displayed an unusual bimodal distribution centered on nC_{17}-nC25 and nC26-nC36. A bimodal alkane distribution has also been reported in sediments by **Glober and Bullin, 1989 & Mendez et al., 2001** and it has been proposed as originated by the combination of terrestrial plant waxes and petroleum sources. Bimodal

distribution also reported in sediments by **Jeng and Huch, 2006** and it is attributed to at least two hydrocarbon sources aquatic and higher plants.

It is obvious from Fig.(22) that the components up to nC_{14} for S7 and up to nC_{17} for S1, S10 and S11 were lost due to the natural weathering processes; vaporization, dissolution and/or photo-oxidation **(Kaplan and Kitts, 2004 & De Oteyza and Grimalt , 2006)**, confirming relatively recent oil input and /or a slow weathering rate of the spilled oil. The higher components up to nC_{18} for S3 and S9 and up to nC_{19} for S4 and S8 were lost due to the above mentioned reason in addition to photo-oxidation and biodegradation indicating chronic pollution and sever weathering **(Wang et al., 2001)**.

Concerning weathering effect; sediment samples can be classified into three groups. The first group includes samples S3 and S7 which are characterized by a pattern of weathered crude oil. The second group includes samples S10 and S11 which are characterized by highly weathered crude oil. The third group includes samples S1, S4, S8 and S9 which are characterized by a pattern of very highly weathered crude oil.

Pristane / Phytane ratio; the two isoprenoid hydrocarbons; Pr and Ph are absent in samples S3, S4, S8 and S10; similar observation was reported by **Medeiros and Bicego (2004)**. For lightly weathered oils, significant loss occur in the LMWT n- alkanes however in heavily weathered oils, the n-alkanes and even the isoprenoids in some cases may be completely lost **(Wang and Fingas, 2003)**.

Pr/Ph ratios are listed in Table (10); samples S7 and S11 have values higher than 1; which indicates biogenic hydrocarbons biosynthesized by zooplankton and marine algae **(Moustafa[b], 2004)**. While samples S1 and S9 have values of Pr/Ph ratios lower than 1; indicating petroleum products source of contamination. The petrogenic sources for S1 may be resulted from the presence of potential polluters such as ships and oil tankers passing through the Suez Canal while in case of S9 it may be due to the presence of dockyards and ship-maintenance works for example S.C.A. workshop yard

Lake Temsah, El-Temsah Ship yard (private workshop) and Arab Cont. ship yard (Temsah Lake map, Fig.10).

Carbon preference index (CPI); for sediment samples S1,S9,S10 and S11 CPI value ranges between 1.5-1.9 which show a weak predominance of odd to even hydrocarbons with the presence of UCM is also an indication of petrogenic input characteristic of crude petroleum **(Méndez et al., 2001)**. High CPI value in most of beach and sub-tidal sediments is a common feature indicates biological hydrocarbons input from a marine biological source; planktonic or bacterial origin **(Wang et al., 1999)**. The low value of CPI for sediment samples S3, S4, S7 and S8 ranges between 0.5-0.8 with the presence of the UCM, indicate the petrogenic contamination of these samples **(Méndez et al., 2001)**. The predominant peak in samples; S3, S8, S9 and S10 is nC_{25}, while for sample S11 is nC_{29}, for sample S4 is nC_{31}, while, it is nC_{33} for sample S1. For S7 peaks; nC_{27}, nC_{31}, nC_{35} and nC_{37} are the major alkanes, indicating an input from terrestrial higher plants **(Medeiros and Bicego, 2004)**.

UCM/TRP; S1, S4, S8 and S9 show the highest value UCM/TRP ratio ranges between 21.73 and 57.14 providing evidence of losses of TRP and/or n-alkanes and increase in UCM, which are indicative of very high degree of weathering and chronic oil pollution **(Tolosa et al., 2005)**. Samples S10 and S11 have UCM/TRP values of 12.7 and 10.14, respectively which indicates high degree of weathering. However, samples S3 and S7 have the lowest UCM/TRP values; 3.77 and 2.49, respectively representing slow rate of weathering and/or indicative of more recent input of oil **(Tolosa et al., 2005)**. The higher molecular weight UCM may be persistently incorporated into tar balls and sediments, while Ph with other more soluble lower weight components may partition preferentially into the water column **(Anderlini et al., 1981)**.

Table (10): Diagnostic parameters in sediment samples of Temsah Lake

Sample Number	Initial nC_n	Final nC_n	UCM%	TRP%	UCM/TRP	Pr/Ph	CPI
S1	C_{17}	C_{36}	97.86	2.14	45.73	0.57	1.8
S3	C_{18}	C_{33}	79.03	20.97	3.77	----	0.5
S4	C_{19}	C_{36}	95.6	4.4	21.73	----	0.8
S7	C_{14}	C_{37}	71.32	28.68	2.49	1.12	0.6
S8	C_{18}	C_{37}	96.82	3.18	30.45	----	0.7
S9	C_{18}	C_{35}	98.28	1.72	57.14	0	1.9
S10	C_{18}	C_{38}	92.70	7.30	12.70	----	1.7
S11	C_{14}	C_{36}	91.02	8.98	10.14	2.78	1.5
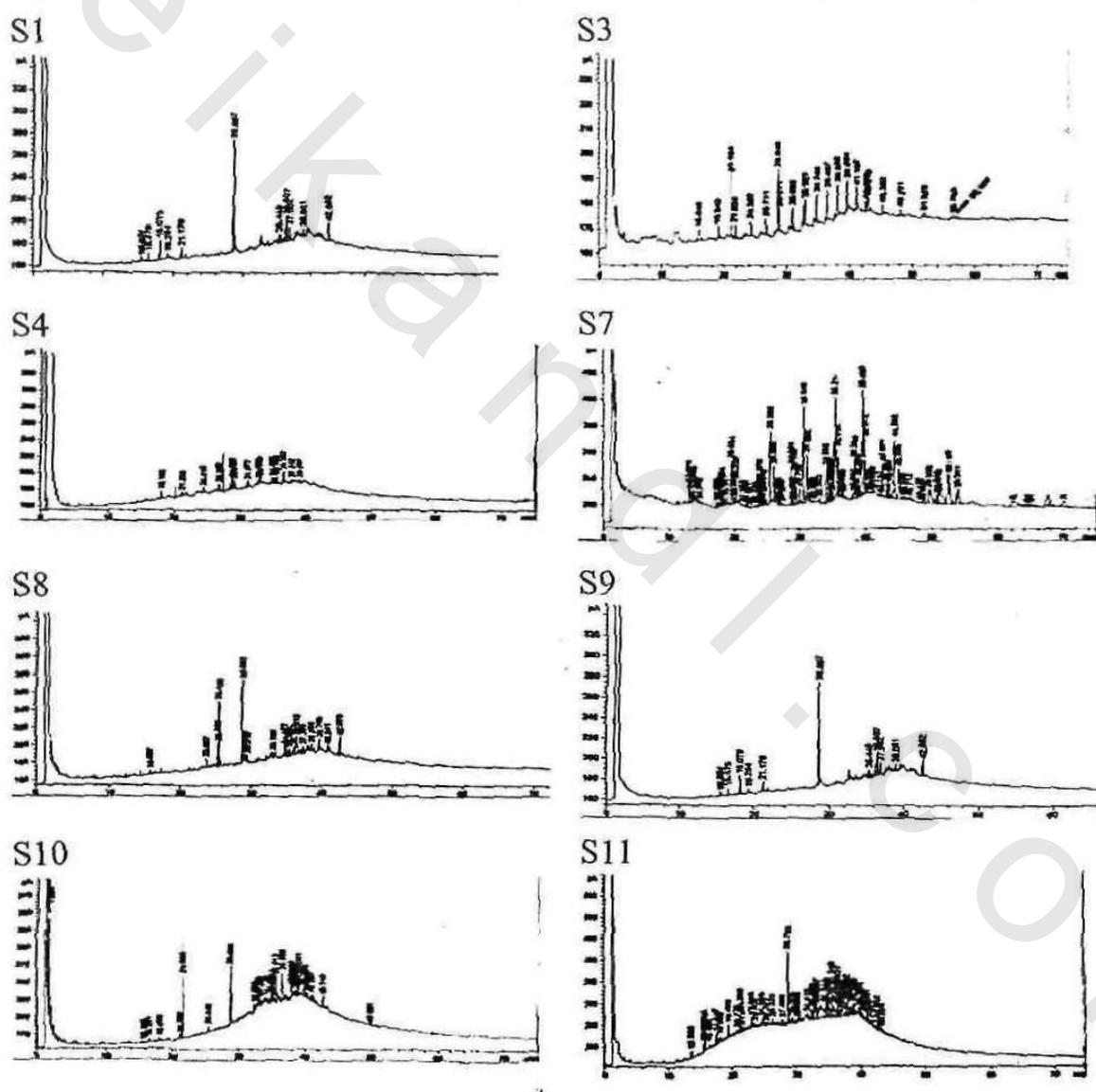
Fig.(21): Gas chromatographic profiles of extracted oils from sediment samples of Temsah Lake.

S1
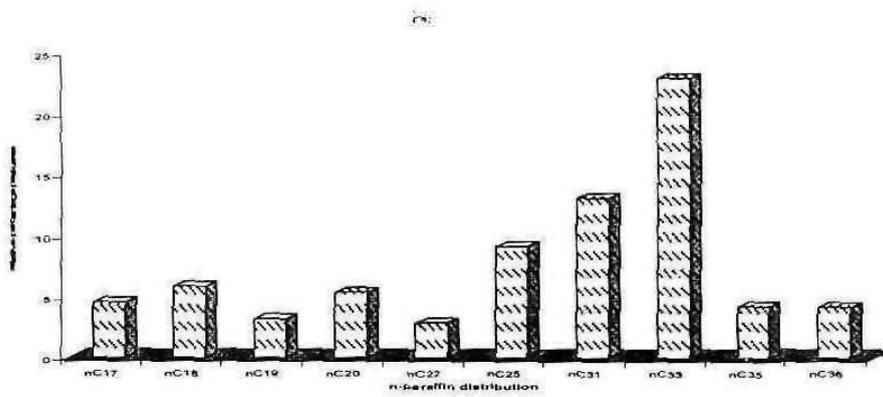
S3
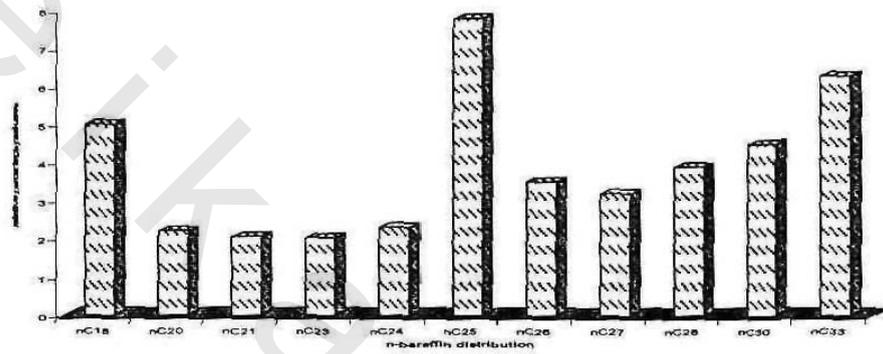
S4
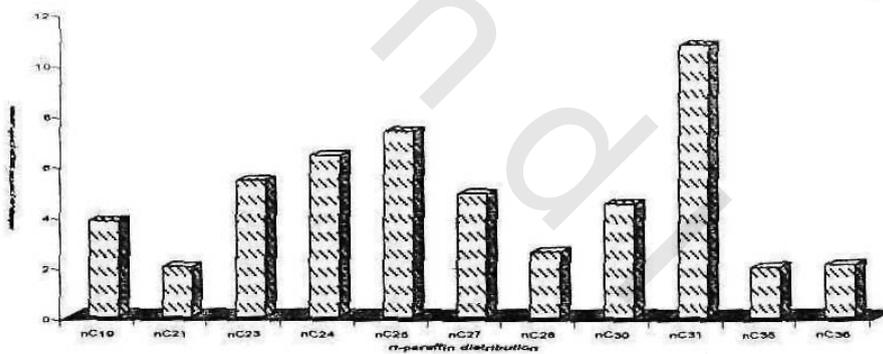
S7

Fig.(22): Histogram for n-paraffin distribution of extracted oil from sediment samples Temsah Lake

S8
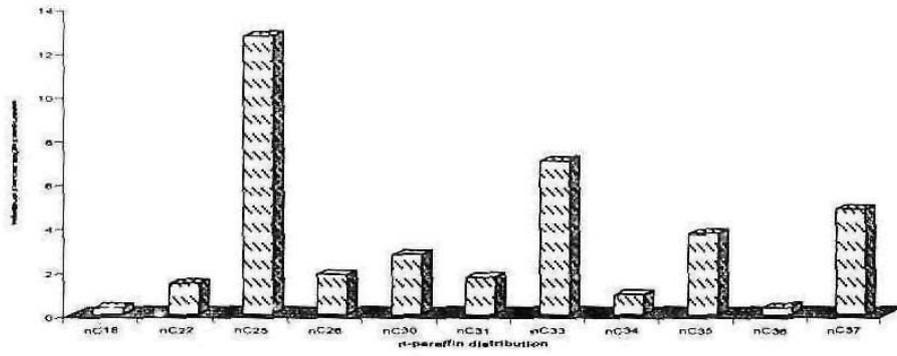
S9
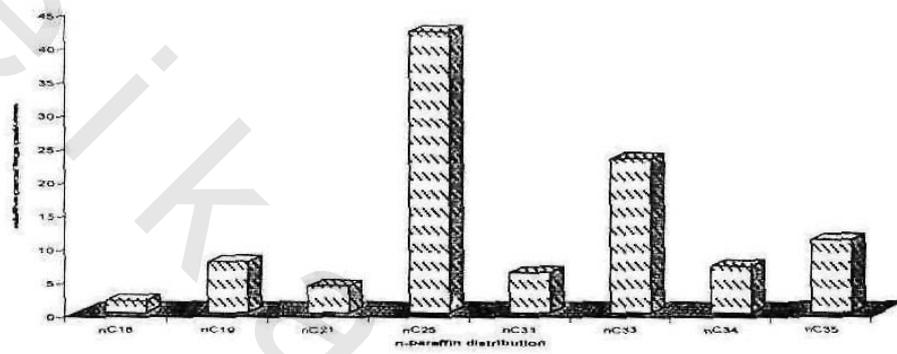
S10
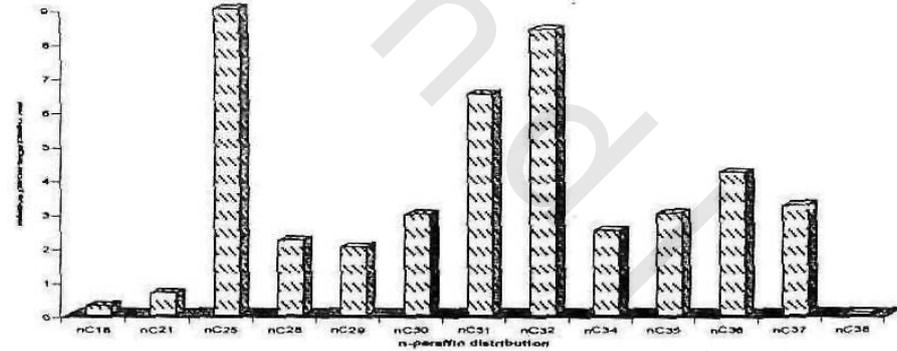
S11
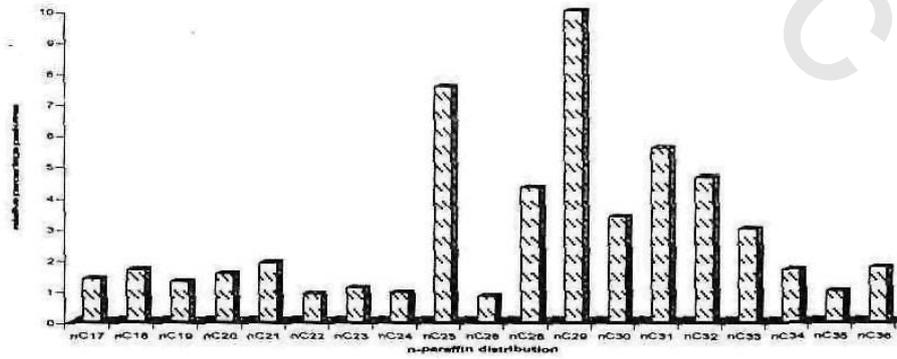
Con. Fig.(22): Histogram for n-paraffin distribution of extracted oil from sediment samples Temsah Lake

III.2.2.3.Fish samples

Aliphatic hydrocarbons of extracted oil from fish samples collected from different locations of Temsah Lake were also monitored by GC and their chromatographic profiles are illustrated in Fig. (23). Gas chromatographic profiles of extracted oil from fish samples shows regularly spaced n-alkane peaks which are either represented as minor peaks or depleted due to the effect of sever conditions superimposed on a prominent UCM.

The presence of the well known UCM is one of the more convincing indications of the petrogenic pollution of these fishes by weathered or biodegraded petroleum and/or its derivatives **(Méndez et al., 2001)**.

GC chromatographic profile of oil extracted from fish sample F11 shows a UCM signal in the high molecular weight region suggesting that the samples were exposed to the most chronic oil pollution and/or reveal that the source of contamination is by lubricating oils **(Wang and Fingas, 2003)**.

All samples are characterized by the presence of predominant peaks nC_{17}, nC_{23}, nC_{25} and nC_{27} for sample F1, nC_{23}, nC_{25} and $nC_{27}$ for sample F3 nC_{23} and nC_{25} for sample F9 and nC_{17}, nC_{23} and nC_{25}, while nC_{27} for sample F11. These odd carbon numbers of normal paraffin are confirmatory signs for biogenic addition which could be derived from zooplankton, phytoplankton and benthic algae **(Wang et al., 1995 and Wang et al., 1999)**. The predominance of such peaks disturbs the distribution pattern. Various reports have illustrated the ability of marine organisms to produce aliphatic hydrocarbons **(Quintero and Diaz, 1994 & Ahmed et al., 1998)**. Residues of aliphatic hydrocarbons that reside in fish tissues are the product of some intrinsic and extrinsic factors. These reveal that the main source of pollution is mainly biogenic hydrocarbons mixed with very highly weathered crude oil. n-alkanes ranged from nC_{12}-nC_{27} for sample F1, nC_{17}-nC_{27} for sample F3, nC_{17}-nC_{30} for sample F9 and nC_{17} - nC_{32} for sample F11.

It is also probable that the high temperature prevailing in August would affect oil slicks through photodecomposition or evaporation. Such assumption is particularly true with the loss of volatile low range hydrocarbons. Alternatively, such high temperature could also activate various degradation reactions, marine biota undertake in the course of oil metabolism **(Laws 1993 & Al-Yakoob et al., 1993).**
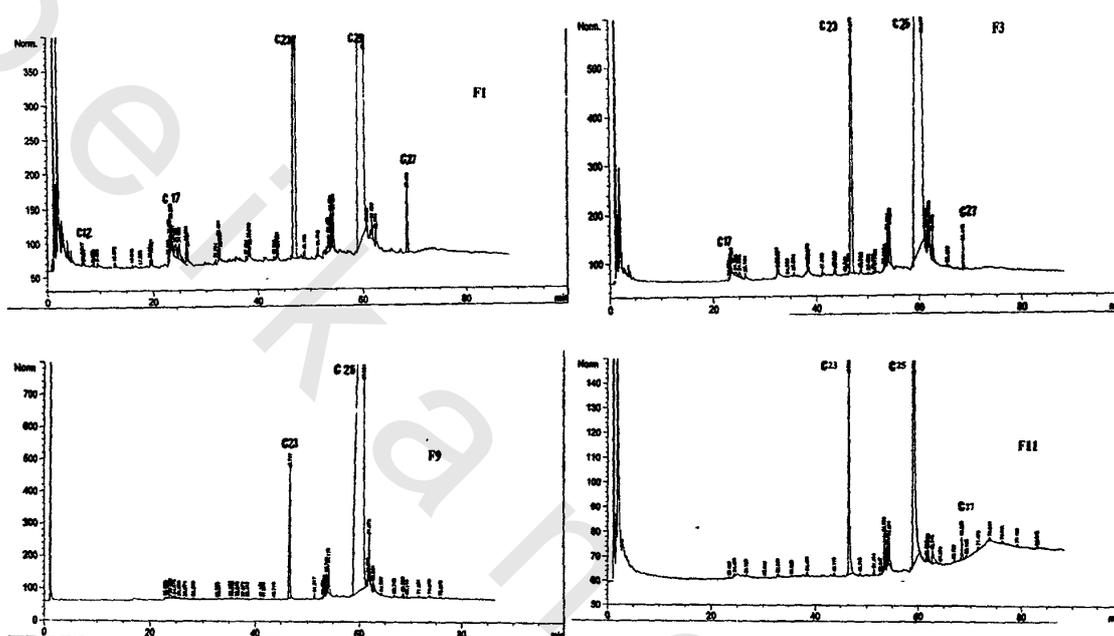
Fig.(23): Gas chromatographic profile of extracted oil from fish samples of Temsah Lake

III.2.3. Ultraviolet spectroscopic study

III.2.3.1. Water and sediment samples

Careful examination of the spectra obtained show that all the investigated water and sediment oil samples have generally the same spectral profiles indicating that they have the same basic features for aromatic compounds. All the spectra indicate petroleum inputs. Representative models for the spectra obtained for the oil extracted from water and sediment samples are shown in Fig (25). They mainly have very strong absorption band at about 228nm and moderate absorption maximum at about 260nm-265nm. The presence of the 228nm absorbance band is probably indicative

for high concentrations of alkyl-benzenes derived from somewhat lighter hydrocarbons originally present in crude oils and /or due to the photo-oxidation and break-down products of higher aromatics **(Neff et al., 1975 & Moustafa et al., 2005)**. This gives an indication that water and sediment samples are incorporated by these aromatic compounds which have both lethal and sub-lethal effects on marine organisms and also explain the decline of fish quality of Temsah Lake. Polycyclic aromatic hydrocarbons larger than the alkyl-naphthalene also have strong absorption maxima at wavelength longer than 230 nm **(Neff et al., 1975 & Moustafa et al., 2005)**, consequently the absorption at 230-270nm region, for all the studied spectra probably indicates the presence of such carcinogenic compounds. These compounds are more resistant to the different environmental weathering process **(Hwang and Foster, 2006)**. The presence of such compounds is also very harmful to marine life. Also the wavelength of about 265nm could be described to the phenols of crude oil **(Rodríguez and Padr n, 2000)**. Phenols have large toxic effects on the environment and have great danger to human health **(Ojumu et al., 2005)**.

III.2.3.2. Fish samples:

Careful examination of the spectra of hydrocarbons extracted from fish samples Fig.(24), shows that these samples have profile slightly different from that of water and sediment samples, but generally they have the same basic features for aromatic compounds spectra. Sample F9 is highly polluted with PAHs this indicates also the contamination of these organisms with petroleum hydrocarbons. All the studied spectra of fish samples are characterized by the presence of a strong band at 228nm and have a shoulder at 285nm. As previously mentioned the presence of strong band at 228nm is indicative for high concentrations of alkyl-benzenes derived from lighter hydrocarbons originally present in crude oils and /or due to the photo-oxidation and break-down products of higher aromatics. This confirms that fish samples are incorporated by these aromatic compounds which have both lethal and sub-lethal effects this also explain the decline of fish quality of Temsah Lake. Alkyl-naphthalenes band is little bit shifted towards 229nm-230nm this observation was also detected by **(El-Bassiouny, 1998)**.

Generally petroleum naphthalenes have characteristic band at 230nm **(Rodríguez and Padr n, 2000).**

The characteristic band of PAHs at 256nm is very well detected in sample F3 and the R-value ratio (228/256nm) which is a well known parameter for oil pollution studies, independent on oil concentration and reflects the high molecular weight aromatic components in the oil. For this sample R-value ratio is ≈ 2.73 which can be taken as a strong indication of relatively higher concentrations of low aromatic hydrocarbons. This observed relatively high value might be attributed to the breakdown products of higher aromatics. These data confirmed the GC results Fig.(23) where the sample is polluted with weathered crude oil. For sample F9; there are three sharp and fused bands at 230nm, 233nm and 236nm which are indicating the high contamination levels of this sample with high concentration of petroleum naphthalenes and diaromatic ring compounds. Bicyclic aromatics have a characteristic band at 236nm **(Moustafa et al., 2005).**

Again, this may be taken as a strong proof that the pollutants extracted from Temsah Lake and its fish population are of petrogenic origin. Finally, one can conclude that both the two types of analytical techniques (GC mainly based on the aliphatic hydrocarbons and the UV on the aromatic compounds), leave little doubt that the extracted hydrocarbons were indeed of mainly petrogenic hydrocarbons. UV spectroscopic technique fail to give more information regarding the individual PAHs present, so the distribution of individual PAHs will further investigated using high performance liquid chromatography (HPLC).
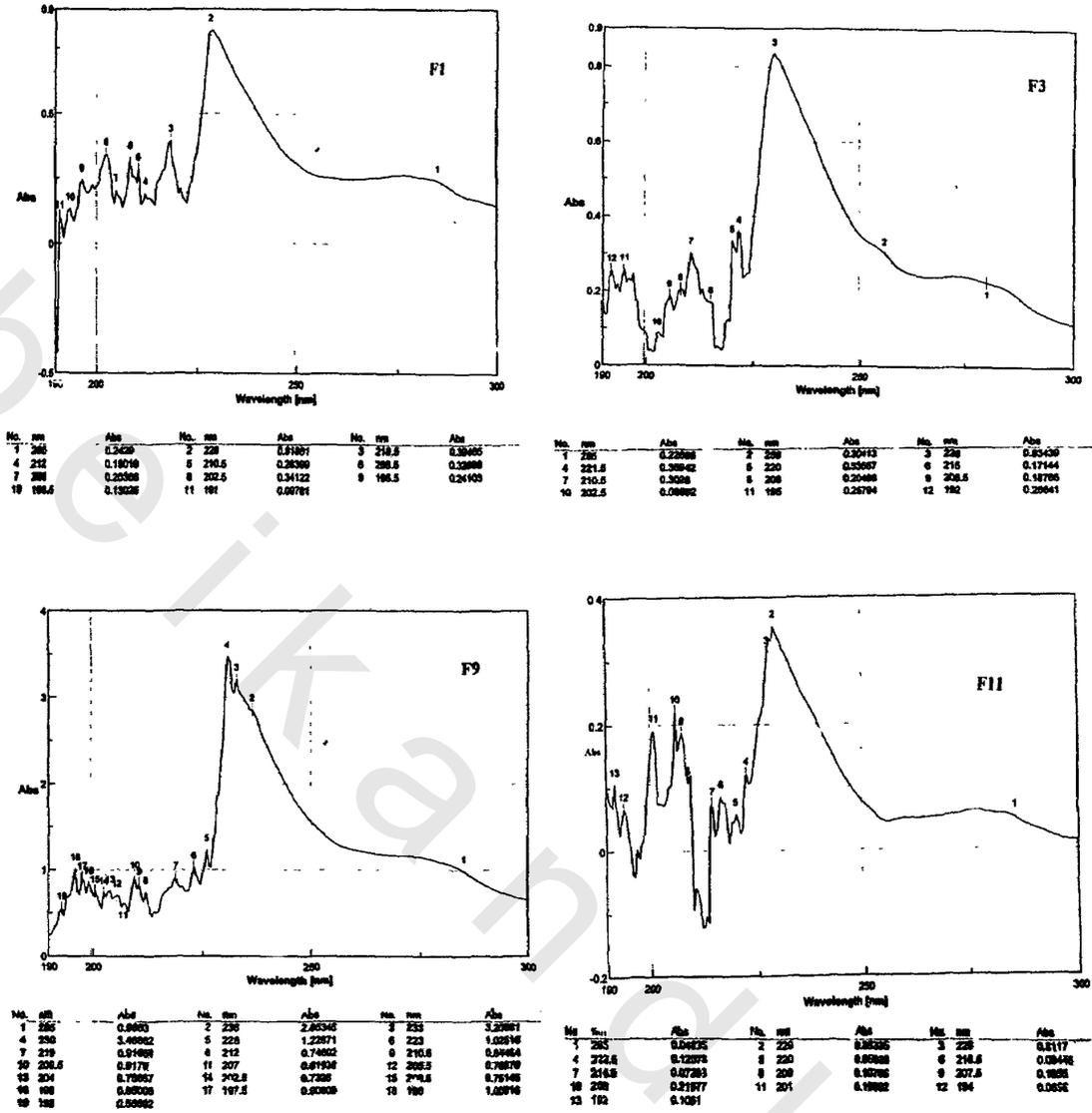
Fig.(24): UV spectra of oil extracted from fish samples of Temsah Lake
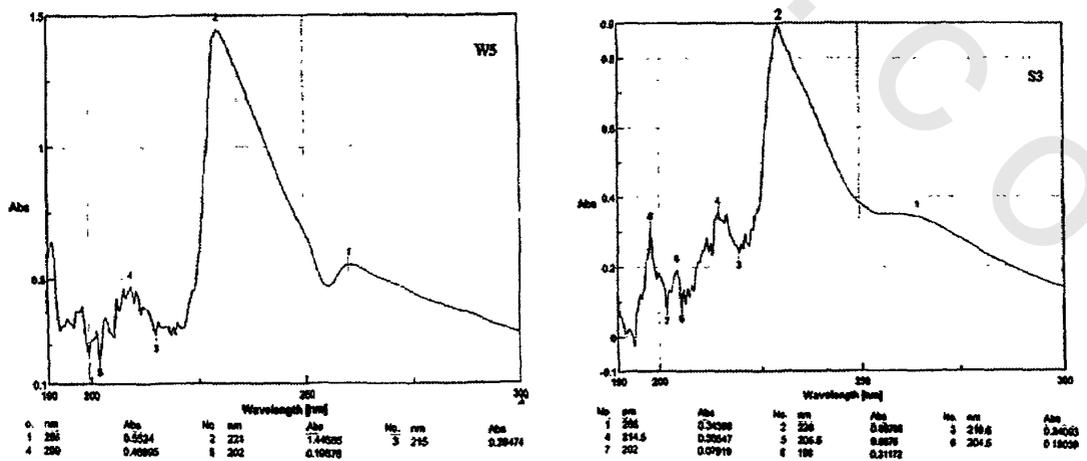
Fig.(25): Representative models for UV spectra of oil extracted from water and sediment samples of Temsah Lake

III.2.4. High Performance Liquid Chromatography Determination of PAHs by HPLC:

Qualitative and quantitative estimation of the individual PAHs for the extracted oils from water, sediment and fish samples of Temsah Lake were done and representative model samples are shown in Fig.(26).

Careful examination of the HPLC chromatograms show that, each sample has its own distribution pattern and all have most of the target compounds. The brief identification of the present PAHs as shown in Tables (11, 12 and 13) reveal the presence of both LPAHs and HPAHs. Generally in all the water, sediment and fish samples there is a higher concentration of HPAHs compared to that of LPAHs. It is well known that the most important anthropogenic sources of PAHs are petrogenic and Pyrolytic, the latter type is usually prevalent in aquatic environments **(Zakaria et al., 2002 & Stout et al., 2004).**

As previously mentioned, some molecular indexes play a major role in establishing the origin of PAHs, in particular; the ratios between low and high molecular weight PAHs **(Magi et al., 2002)**, or the ratio of some isomers; e.g. phenanthrene/anthracene and flourantherene/Pyrene. They were chosen according to their thermodynamic stability **(Tolosa et al., 2004)**, among the three-ring isomers; Phe is thermodynamically more stable and its prevalence over Ant. supports petrogenesis. Indeed, petroleum usually exhibits a quite high Phe/Ant ratio, among the four-ring isomers; Flu. is thermodynamically less stable than Pyr. Flu/Pyr>1; indicates Pyrolytic and Flu/Pyr<1 indicates petrogenic genesis of PAHs. **Baumard et al., 1998** suggested that only the evaluation of both ratios Phen/Ant and Flu/Pyr could prevent a misleading estimate of PAH sources. According to **De Luca et al., 2005**; petrogenic contamination is characterized by the predominance of the LPAHs (with three and four fused rings), while the HPAHs prevail in Pyrolytic PAHs. But ratio of LPAHs : HPAHs is usually used as indices of weathering **(Bernard et al., 1996& Żakaria et al., 2001& Moustafa[b], 2004 & Hwang and Foster, 2006).**

III.2.4.1. Water samples

Qualitative and quantitative distribution of PAHs detected in water samples have followed no particular pattern, but were rather irregular. Their levels are high, ranging between 52.46-3393µg/l which are alarmingly high. Generally the samples are characterized by high concentrations of the six membered ring; Indeno(1,2,3-cd)Pyrene, ranging between 501-3308.9µg/l except for sample W6 which is characterized by low concentration of IP of 15.22µg/l. It can be observed also that water samples are generally characterized by low ratios of Σ6LPAHs: Σ10HPAHs, as shown in Table (11) which might be presumably because of the relatively higher volatility of these LPAHs compounds which restrict their residence in water, especially in warm weather **(Ahmed et al., 1998 & Zakaria et al., 2001)**. This may also indicates high pyrogenic genesis of PAHs in Temsah Lake **(Magi et al., 2002)**. Regarding to Phe/Ant ratio, samples W4, W7, W10 and W11 have values of infinity due to the absence of anthracene indicating purely petrogenic origin, In contrary; regarding to Flu/Pyr ratio of these four samples have the value of infinity which is due to the absence of Pyrene indicating purely pyrogenic origin. This misleading in results may be due to weathering or the presence of both pyrogenic and petrogenic genesis of PAHs due to the different outlets of effluents discharged into the lake and the accessibility of Temsah Lake to different sources of potential contaminates and polluters. For samples W5 and W6 Phe/Ant ratio is zero due to the absence of Phe indicating purely pyrogenic origin, which is confirmed by the ratio of Flu/Pyr; which are infinity and 169 for W5 and W6, respectively. For sample W1; Phe/Ant value is <1 and Flu/Pyr>1, indicating pyrogenic genesis of PAHs. For samples W2 and W9; Phe/Ant value >1 and Flu/Pyr<1, indicating petrogenic genesis of PAHs. For sample W3; Phe/Ant and Flu/Pyr values are close to one, indicating a mixture of petrogenic and pyrogenic origin of PAHs. On the other hand, sample W8 has a zero value for Flu/Pyr ratio and Phe/Ant value is close to zero, indicating also a mixture of petrogenic and pyrogenic genesis of PAHs. In conclusion, there are some discrepancies between the results obtained using the different parameters. The petrogenic genesis in Temsah Lake may result from the presence of some potential polluters such as dry docks and ships and tankers passing

through the Suez Canal, also they may be due to the presence of dockyards and ship-maintenance works for example S.C.A. workshop yard Lake Temsah, El-Temsah Ship yard (private workshop) and Arab Cont. ship yard (Temsah Lake map, Fig.,10). The high pyrogenic genesis is probably the result of the massive discharge of industrial effluents into the lake **(Tundo et al., 2004)** and the surrounding Forsain drain out fall, covered drainage and El-Mahmasa drainage, which is an indirect main point for sanitary waste water (Temsah Lake map, Fig.,10) **(Tundo et al., 2004)**.

Table (11): Individual PAHs distribution and their concentrations (µg/l) in water samples of Temsah Lake

Ring No.	PAHs	Water samples										
		W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11
2	Naph	1.3	1.1	0.66	3.3	11	5.12	7	0.34	29.7	93	123
	Total	1.3	1.1	0.66	3.3	11	5.12	7	0.34	29.7	93	123
3	A	0.22	0.76	4	26.7	1.02	5.1	7.7	2	1.1	0	Nil
	Ace	0.084	3.9	16.8	0	0	0.21	0	0	2.9	0.08	0
	F	3.9	18.6	46.2	3.1	0	0	0	0	14	0	0
	Phe	2	9.3	23.8	2.2	0	0	0.402	0.34	4.9	0.41	0.42
	Ant	57	8	14.5	0	0.44	3.3	0	2.6	4.2	0	0
	Total	63.204	40.56	105.3	32	1.46	8.61	8.102	2.94	27.1	0.49	0.42
4	Flu	3.4	5.4	19.1	1.07	2.9	2.7	3.3	0	2.7	2.7	2
	Pyr	0.36	19.5	15.2	0	0	0.016	0	0	8.9	0	0
	BaA	5.1	0	2.8	2.2	8	0.011	6.6	5.7	2	4.1	2.4
	Chr	0.018	6.2	0	5	0	0	0	0	0.53	0	0
	Total	8.878	31.1	37.1	8.27	10.9	2.727	9.9	5.7	14.13	6.8	4.4
5	BbF	8.7	8.7	5.1	4.6	0	4.5	0	0	4.6	0	0
	BkF	3.9	1.5	1.8	0	0	4.5	0	0	1.02	0	0
	BaP	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	DahA	15.8	1.3	0	0.59	0	0.037	0	1.9	0.54	0.187	0.109
	Total	28.4	11.5	6.9	5.19	0	9.03	0	1.9	6.16	0.187	0.109
6	BP	93.11	0	0	0	1430.3	11.75	404.3	0	77.29	50.85	1.58
	IP	2960.8	3308.9	501	3083.5	584.6	15.22	1064	764.87	1493.6	388.4	909.5
	Total	3053.9	3308.9	501	3083.5	2015	26.97	1468.3	764.87	1570.9	439.3	911.12
	Total PAHs	3155.7	3393	650.98	3132.3	2038.4	52.46	1493.3	777.75	1648	539.77	1039

Table (11-a): PAHs parameters used to distinguish petrogenic from pyrogenic origin of PAHs Temsah Lake

Parameters	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11
Phe/Ant	0.035	1.163	1.64	∞	0	0	∞	0.13	1.16	∞	∞
Flu/Pyr	9.44	0.28	1.26	∞	∞	168.8	∞	0	0.3	∞	∞
ΣLPAHs:ΣHPAHs	1:48	1:80	1:5	1:88	1:163	1:3	1:98	1:236	1:28	1:5	1:7

Phen/Ant<1 indicates Pyrolytic origin and Phen/Ant >1 indicates petrogenic origin
Flu/Pyr<1 indicates petrogenic origin and Flu/Pyr>1 indicates Pyrolytic origin
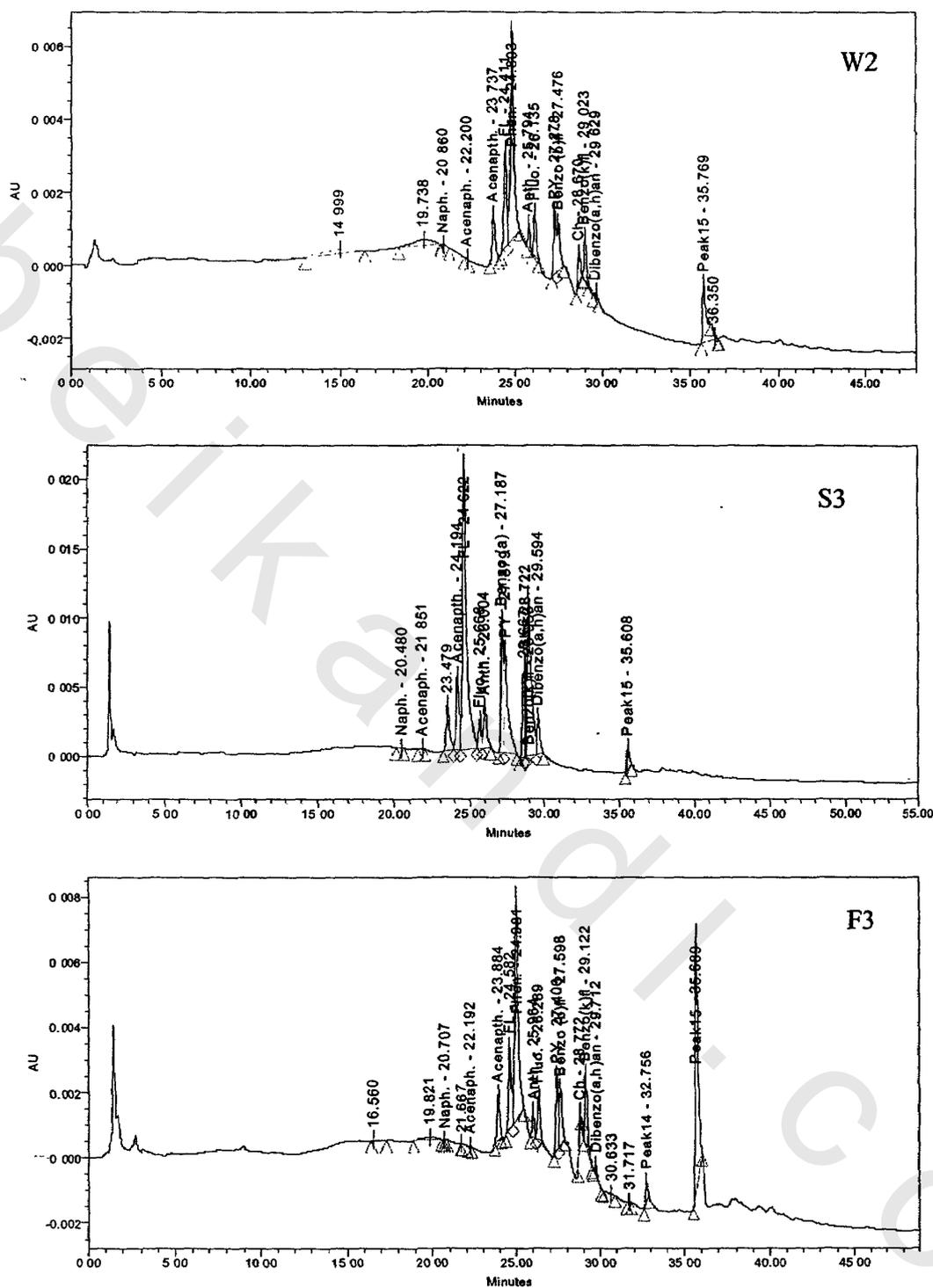
Fig.(26): Representative HPLC chromatograms of the PAHs in oil extracted from; water, sediment and fish samples of Temsah Lake.

III.2.4.2. Sediment samples

Qualitative and quantitative distribution of PAHs detected in sediment samples have followed no particular pattern, but were rather irregular and their level are alarmingly high, ranging between 585.9-8592.8μg/kg, the presence of such high concentrations are considered to be toxic **(Moustafa[b], 2004)**. PAHs are contaminants of marine coastal sediments because of their hydrophobic character (water solubility between 10-10 and 10-13 mol/l) they are easily sorbed onto suspended particulate **(Raoux et al., 1999 & De Luca et al., 2005)**. In this form they are more persistent to biodegradation in comparison to dissolved PAHs **(Chiou et al., 1998& Soclo et al., 2000 & De Luca et al., 2005)**. This explains why their concentration in sediments could be higher than that in the overlaying water column as in samples of S3, S8, S9 and S10, generally all the collected sediment samples are characterized by high concentrations of the six membered ring Table (12); Indeno(1,2,3-cd)Pyrene, ranging between 408.5-8373.3μg/kg. Sample S3 has high concentration of Fluorene and Pyrene; 230μg/kg and 158μg/kg, respectively. Samples S8 and S9 are characterized by high concentration of Benzo[ghi]perylene; 700μg/kg and 185.99μg/kg, respectively. Generally all the collected sediment samples are characterized by low concentrations of diaromatic ring compound; naphthalenes except for sample S11 of concentration 137μg/kg and also they are characterized by low concentrations of triaromatic ring compounds except for samples S3 and S10 of concentration; 321.5μg/kg and 130.3μg/kg, respectively. Weathering causes considerable changes in the chemical and physical properties of spilled oils as well as the PAHs **(Zakaria et al., 2001)**. Due to evaporation, dissolution and biodegradation, low molecular weights PAHs are thought to be selectively disappear. As weathering increases the summation of the 6LPAHs decrease which makes the Σ6LPAHs: Σ10HPAHs parameter not useful to detect the origin. On the other hand it could be useful in the comparison of the weathering effects on the samples **(Zakaria et al., 2001)**. Data obtained show low ratios of Σ6LPAHs: Σ10HPAHs as listed in Table (12-a) indicating that all the sediment samples were subjected to weathering in different degrees. Based on this; weathering could explain the decrease of naphthalenes concentration in all samples. Sample S11 can be considered as

the least affected by weathering. Considering the different origins of PAHs; these sources induce a different chemical behavior in marine sediments; e.g. the interaction with sediments and resistance to biodegradation is higher for pyrolytic than for petrogenic PAHs **(Gustafsson et al., 1997 & Wang et al., 2006)**, this could explain the high concentrations of the HPAHs in all the collected sediment samples compared to that of the LPAHs. Regarding to Phe/Ant ratio; samples S1 and S4 are < 1 with Flu/Pyr >1, indicating pyrogenic genesis of PAHs. But for samples; S3 and S9 Phe/Ant ratio is < 1 with Flu/Pyr < 1, indicating a mixture of petrogenic and pyrogenic genesis of PAHs. For samples S7, S8 and S11; regarding to Phe/Ant ratio they have values of infinity due to the absence of anthracene indicating purely petrogenic origin, but in contrary; regarding to Flu/Pyr ratio of these samples S8 and S11 they also have the value of infinity which is due to the absence of Pyrene and for sample S7 Flu/Pyr ratio is 490; indicating purely pyrogenic origin. This misleading in results may be due to weathering or the presence of both pyrogenic and petrogenic genesis of PAHs due to the different outlets of effluents discharged into the lake as previously mentioned.

Table (12): Individual PAHs distribution and their concentrations (µg/Kg) in sediment samples of Temsah Lake

Ring No.	PAHs	Sediment samples							
		S1	S3	S4	S7	S8	S9	S10	S11
2	Naph	0.15	0.68	0.07	0.17	39	21	0	137
	Total	0.15	0.68	0.07	0.17	39	21	0	137
3	A	0.4	3.2	0.09	0.04	0	0.023	59	0
	Ace	0.01	21.3	0.03	0.08	0	0	30	0
	F	0.17	230	0.03	0	0.32	0	23	0
	Phe	0.02	0	0	0.4	0.18	0.37	9.3	0.81
	Ant	0.28	67	3.6	0	0	6.1	9	0
	Total	0.88	321.5	4.04	0.52	0.5	6.493	110.3	0.81
4	Flu	0.74	12	0.18	0.49	2.3	0	0	0.9
	Pyr	0.23	158	0.05	0	0	0	29	0
	BaA	0.06	41	0	0.02	6.3	6	26	6
	Chr	0.06	35	0.03	0.14	0	0	0	0
	Total	1.09	246	0.25	0.65	8.6	6	55	6.9
5	BbF	0	0	2.8	0	0	0	16	0
	BkF	0	30	0.02	0.02	0	0	4.9	0
	BaP	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	DahA	0.02	36	0	0.01	0.01	0	0	0.18
	Total	0.02	66	2.8	0.02	0.01	0	20.9	0.18
6	BP	4.06	0	0	0	700	185.9	0	32.5
	IP	790.	2109	927	792	2054	8373	4027	408
	Total	794	2109	927	792	2754	8559	4027	441
	Total ΣPAHs	796	2744	933	793	2802	8592	4233	585

Table (12-a): PAHs parameters used to distinguish petrogenic from pyrogenic origin of PAHs of Temsah Lake

Parameters	S1	S3	S4	S7	S8	S9	S10	S11
Phe/Ant	0.07	0	0	∞	∞	0.06	1.03	∞
Flu/Pyr	3.22	0.1	3.91	490	∞	0	0	∞
ΣLPAHs:ΣHPAHs	1:77	1:8	1:23	1:115	1:70	1:31	1:37	1:3

Phen/Ant<1 indicates Pyrolytic origin and Phen/Ant >1 indicates petrogenic origin

Flu/Pyr<1 indicates petrogenic origin and Flu/Pyr>1 indicates Pyrolytic origin

III.2.4.3. Fish samples

Qualitative and quantitative distribution of PAHs detected in fish samples have also followed no particular pattern, but were rather irregular. Various reports have pointed to such irregularity in PAHs detected in fish (**Cocchieri et al., 1990 & Al-Yakoob et al., 1993 & Ahmed et al., 1998**). **Varanasi et al., 1989** explained these irregularities in view of PAHs uptake by fish as well as levels and routes of exposure, environmental factors, salinity and differences in species, age and sex. Moreover, laboratory studies have been shown that the extraction and analytical methods used can overestimate or underestimate the concentrations in fish samples (**May et al., 1982 & Ahmed et al., 1998**). Generally, the concentrations of PAHs in fish samples involved in this study is high, which could explain the decline of fish population in Temsah Lake. Similar observations were listed by **Ahmed et al., 2001** and reported that high contamination level is the main cause behind the decline in the lake biodiversity and fish quality harvested from the lake. All of the collected fish samples are characterized by the presence of high concentrations of the six membered ring compound Table (13); Indeno(1,2,3-cd)Pyrene; ranging between 1013.9µg/kg and 4122.2µg/kg. Samples F1 and F9 are characterized by the presence of high concentrations of the six membered ring compounds Benzo[ghi]perylene; 1224.2µg/kg and 600.0µg/kg, respectively, but it is not found in samples F3 and F11. Regarding to Phen/Ant ratio, samples F1 and F3 have value close to 1 indicating a mixture of petrogenic and pyrogenic origin of the PAHs. The Flu/Pyr<1 indicates that the petrogenic genesis of PAHs are higher than that of pyrogenic one. But for samples F9 and F11; Phen/Ant ratio is zero due to the absence of Phen, indicating purely pyrogenic origin this is contradicted by the Flu/Pyr ratio which is <1 this may be due to their proximity to a dockyard and a ship-maintenance works (Temsah Lake map, Fig., 10) where oil and other contaminants spills are likely could be the reason for such high concentration of PAHs (**Tundo et al., 2004**).

The concentrations of LPAHs in the fish samples ranges between 35.95-131µg/kg. Low-boiling point aromatic hydrocarbons have been found to be the most toxic fractions and to be the primary cause of fish mortality.

It can be observed also that fish samples are generally characterized by low ratios of $\Sigma 6LPAHs$: $\Sigma 10HPAHs$, as shown in Table (13-a) which is considered as a very serious alarm for the sever toxicity of these ten HPAHs. Several PAHs are known to be potential human carcinogen; these include benz[a]anthracene, chrysene, benzo[b] flouranthene, benzo[a]pyrene and benzo[ghi] perylene **(Guillen et al.,2000 & Anyakora,2005)**. The health hazards posed by these compounds have been studied extensively by several authors **(Perera et al., 1988 & Schoket et al., 1993)**. The presence of these compounds in the environmental samples (sediment, water and fish) has also been studied by numerous authors **(Xu and Fang 1988 & Speer et al., 1990)**.

Table (13): Individual PAHs distribution and their concentrations (µg/Kg) in Fish samples of Temsah Lake

Ring No.	PAHs	Fish samples			
		F1	**F3**	**F9**	**F11**
2	Naph	0.04	1.1	1.5	1.5
	Total	0.04	1.1	1.5	1.5
3	A	0.63	2.8	5.1	0.65
	Ace	4.4	17	2.9	5.1
	F	18	53	18	19
	Phe	11.9	27	0	0
	Ant	10	31.2	13	11.2
	Total	44.93	131	39	35.9
4	Flu	6.5	17	2.4	2.1
	Pyr	27	36	9.3	11
	BaA	0	12.9	3.8	3.8
	Chr	3.5	11.8	2.8	0
	Total	37	77.7	18.3	16.9
5	BbF	10	12.3	0	10
	BkF	3.49	32	1.8	0.75
	BaP	Nil	Nil	Nil	Nil
	DahA	2.9	2	1.98	0
	Total	16.39	46.3	3.78	10.7
6	BP	1224	0	600	0
	IP	1013	2577	4122	1631
	Total	2238	2577	4723	1631
	Total ΣPAHs	2336	2834	4785	1696

Table (13-a): PAHs parameters used to distinguish petrogenic from pyrogenic origin of PAHs of Temsah Lake

Parameters	F1	F3	F9	F11
Phe/Ant	1.19	0.86	0	0
Flu/Pyr	0.24	0.47	0.26	0.19
ΣLPAHs:ΣHPAHs	1:51	1:20	1:12	1:44

Phen/Ant<1 indicates Pyrolytic origin and Phen/Ant >1 indicates petrogenic origin

Flu/Pyr<1 indicates petrogenic origin and Flu/Pyr>1 indicates Pyrolytic origin

III.3.GULF OF SUEZ

III.3.1.Oil Content

III.3.1.1.Water samples

Visual assessment of all the studied sites shows the absence of oil slicks on the surface of the water except samples W22 and W23 which are characterized by visible film floating on the water surface. It is clear from the quantitative determination of the oil extracted from all of the studied samples Table (14) that they have varying values. Ranging between 36-230 mg/L such values may cause lethal or sublethal oil pollution problems on marine life. The obtained data reveal that samples W22 and W23 are characterized by relatively high values due to the petroleum activities in these two sites (Ras Gharib and Shukier respectively).

III.3.1.2.Sediment samples

Quantitative data for extractable oils in the studied samples as shown in Table (14) varied from 160 to 360 mg/kg of dry weight ,except samples S22 and S23 which are characterized by relatively high values, 901 and 1791 mg/kg dry weight respectively as in case of water samples. This relatively high value may be attributed to the petroleum activities in these both areas (Ras Gharib and Shukier respectively). All the obtained values are considered to be highly alarming values and have lethal effect.

Generally it is observed that the oil content of sediment samples is much higher than its corresponding water samples Fig.(27). As previously mentioned this difference may be attributed to the ability of sediments to accumulate and retain oils while water is mobile and tends to migrate down through sediments (Barakat et al., 1996). Pollutants concentrations in water are also liable to be decreased by dilution (Moustafa, 2004).

Generally it is noticed that the oil content of water and sediment samples increases towards the south of the Suez Gulf where most of petroleum activities are concentrated.

The relatively high concentrations in our samples give indication that the studied areas are subjected to massive petroleum activities (and hence pollution).

Table (14): Oil Content of water and sediment samples of Gulf of Suez

Sites Number	Type of samples	
	Water(mg/l)	Sediment(mg/kg)
III-17	50	230
III-18	47	160
III-19	51	232
III-20	48	360
III-21	36	322
III-22	70	901
III-23	230	1791
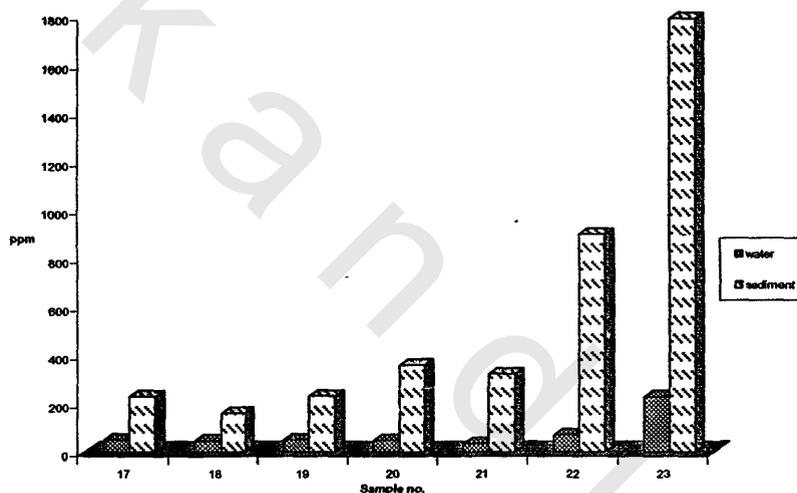
Fig.(27): Histogram shows the oil content of water and sediment samples of Gulf of Suez

III.3.2.Gas chromatographic analysis

III.3.2.1.Water samples

The gas chromatographic profile, Fig.(28) shows regularly spaced n-alkane peaks with different initial and final carbon number superimposed over UCM as shown in Table (15), their gas chromatographic profiles reveal that the source of contamination is either petrogenic (samples W21, W22 and W23) or mixed petrogenic with biogenic (samples W17, W18, W19 and W20) which are characterized by the predominance of nC_{25}. From the GC

peak area calculations, it is clear that, the percentage sum of both aromatics and cycloalkanes (hump) are representing values between 21.85% and 92% of the contaminating oil while the sum of n- and iso- paraffins (resolved peaks) represent values between 11.18% and 78.15%, as listed in Table(15).

Based on their gas chromatographic profile, the type of pollutants contaminating this area can be classified into two main categories, the first category, include sample W17 which has characteristic feature corresponding to lubricating oil mixed with biogenic hydrocarbons represented in the chromatogram as predominance of nC_{25}. Since many odd n-alkanes are biosynthesized by marine algae, fluctuations in this source of input or their own biosynthesis of such compounds can influence their concentration (**Méndez et al., 2001**). While the second category include the other samples having characteristic patterns of crude oil. The second category can be also classified according to their weathering degrees which causes considerable changes in the chemical and physical properties of the spilled oils (**Tolosa et al., 2005**). The extent and rate of weathering is different for each spill and is controlled by a number of spill conditions and natural processes such as, type of the spilled oil, environmental conditions and microbiological activities, accordingly water samples can be classified into two groups. The first group include (samples W18, W19 and W20) which have UCM/TRP ratio lower than 1, representing low degree of weathering and/or indicative of more recent input of oil. The second group (samples W21, W22 andW23) showed higher values UCM/TRP ratio; 11.56, 7.27and 7.9, respectively providing evidence of losses of TRP therefore increase in UCM indicating high degree of weathering and chronic oil pollution. W17 can not be correlated to the other samples due to the nature of lubricating oil. According to Pristane/Phytane ratio listed in Table (15); most of the investigated samples are characterized by the presence of both Pr and Ph with different concentrations; having values lower than 1, indicating mainly a petroleum products source. Pr is not found in two samples W20 and W21, similar observation was reported by **Medeiros and Bicego, 2004. Wang and Fingas, 2003** reported that pristane and phytane can be lost due to high weathering. According to Carbon preference index (CPI) for water samples W18, W19 and W20 CPI values are 9, 15.8 and 40.6, respectively indicating biogenic addition. The low value of CPI for water samples W21, W22 and W23 is 1.1, 0.89 and 0.97, respectively with the presence of the high UCM, indicate petrogenic and/or slightly biogenic contamination.

Table (15): Diagnostic parameters in water samples of Gulf of Suez

Sample Number	Initial nC_n	Final nC_n	UCM%	TRP%	UCM/TRP	Pr/Ph	CPI
W17	C_{16}	C_{35}	81.9	18	4.5	0.17	4.6
W18	C_{13}	C_{37}	21.85	78.15	0.3	0.85	9
W19	C_{16}	C_{38}	32.99	67	0.5	0.73	15.8
W20	C_{18}	C_{30}	28.66	71.3	0.4	---	40.6
W21	C_{16}	C_{37}	92	7.95	11.56	---	1.1
W22	C_{16}	C_{37}	87.9	12	7.27	0.39	0.89
W23	C_{17}	C_{44}	88.8	11.18	7.9	0.1	0.97
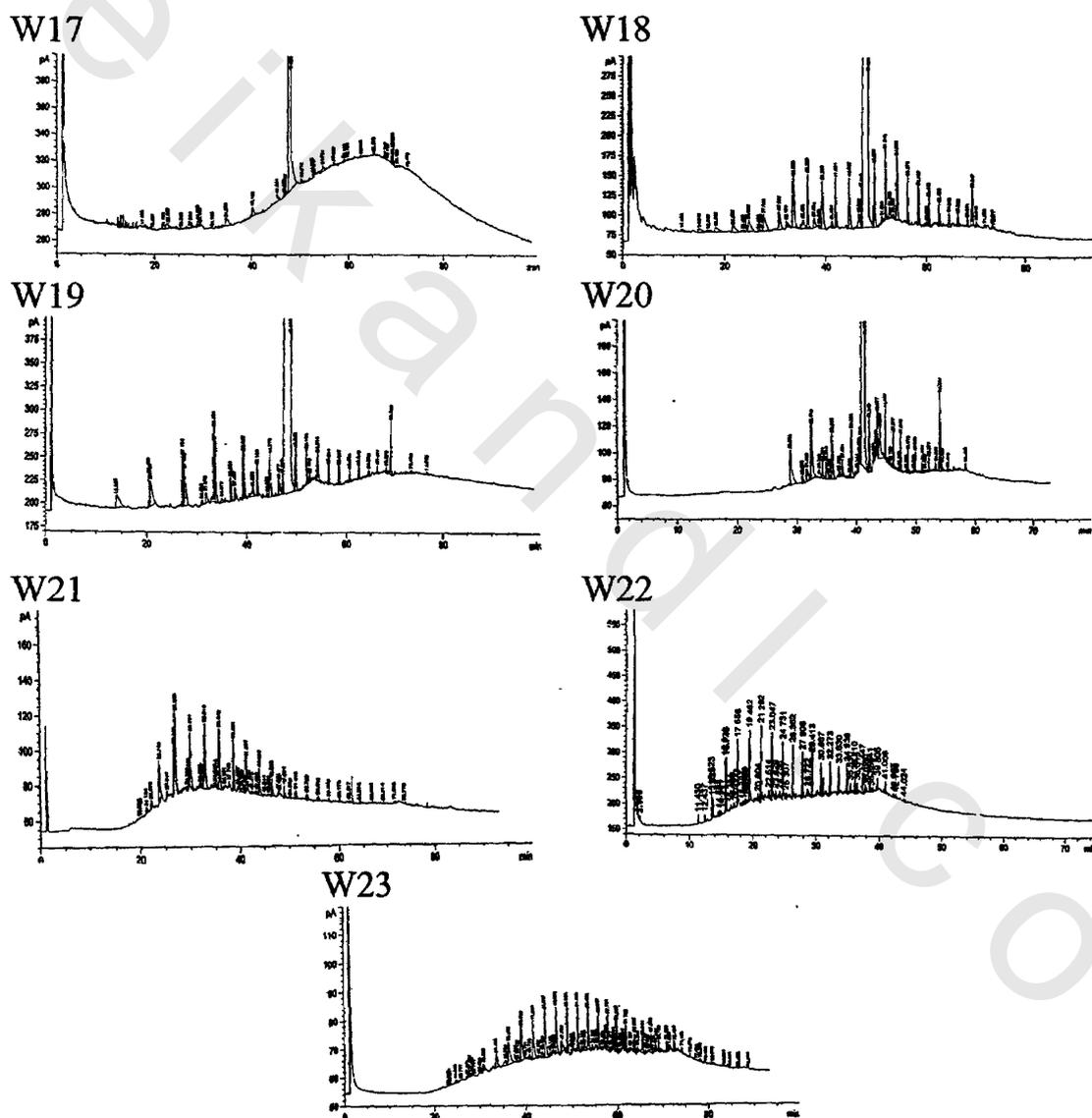
Fig.(28): Gas chromatographic profile of extracted oil from water samples of Gulf of Suez

III.3.2.2. Sediment samples

Surface sediments are popular matrices for the analysis of hydrocarbon levels in the environment. The GC chromatographic profiles; Fig.(29) show resolvable peaks with different initial and final carbon numbers as illustrated in Table (16). Due to the severe weathering the n-alkanes peaks are depleted and the initial and final carbon number can not be marked for sample S20. The profiles reveal that the sources of contamination are either petrogenic (S18, S19, S20 and S23) or mixed petrogenic with biogenic hydrocarbons (S17, S21 and S22) (represented as predominance in nC_{25} peak). From the GC peak areas calculations, it is clear that, the percentages of UCM range between 84.76% and 97.85% of the contaminating oil, the high UCM values and the decrease and/or absence of n-alkanes are indicative of chronic, degraded and highly weathered petroleum contamination. While the percentages of resolved peaks ranges between 2.14% and 14.24%, as listed in Table (16). Samples can be classified into five groups according to their chromatographic profiles. The first group includes samples S18 and S19 show a well defined UCM signal in the high molecular weight region with nearly no resolved alkanes suggesting that the samples were exposed to the most chronic oil pollution and/or reveal that the source of contamination is lubricating oils. Data confirmed by UCM/TRP values which are 26 and 33.7, respectively. The second is characterized by a pattern of very highly weathered crude oil including sample S20. Its n-paraffin peaks are present as very minor peaks. The third group includes sample S21 displaying an unusual bimodal distribution (two UCM humps) indicates that oil pollution originates from diverse sources. A bimodal alkane distribution has also been reported in sediments by **Glober and Bullin, 1989& Méndez et al., 2001** and it has been proposed to be originated by the combination of terrestrial plant waxes and petroleum sources. Bimodal distribution were also reported in sediments by **Jeng and Huch, 2006** and it was attributed to at least two hydrocarbon sources aquatic and higher plants. The fourth includes sample S17 which is characterized by very high CPI (7.7) due to the predominance of peaks nC_{15} and nC_{25} which disturb the chromatographic profile. The last group includes samples S22 and S23 which are characterized by a pattern similar to weathered crude oil; Low UCM values in comparison to that of the other

sediment samples (84.76 and 88.25 %) with low UCM/TRP values (5.54 and 7.5, respectively). Pr/Ph ratios are listed in Table (16); sample S21 have value higher than 1; which indicates biogenic hydrocarbons. While samples S22 and S23 have values of Pr/Ph ratios 1.0and 0.8, respectively; indicating petroleum products source of contamination. Concerning Carbon preference index (CPI) for sediment samples S17, S21 and S22 CPI values are 7.7, 1.25 and 1.34, respectively which indicate biogenic addition. High CPI value in most of beach and sub-tidal sediments is a common feature indicates biological hydrocarbons inputs from a marine biological source; planktonic or bacterial origin (**Wang et al., 1999**). The low value of CPI for sediment sample S23 with the presence of the UCM, indicates the petrogenic contamination of this sample.

All the sediment samples were subjected to weathering in different degrees. The presence of crude oil pollutants of different weathering degrees indicate the continuous petroleum inputs in the studied area specially as the Suez Gulf contains the largest offshore Egyptian oil fields and hence the major activities of exploration and production.

Table (16) Diagnostic parameters in sediment samples of Gulf of Suez

Sample	Initial nC_n	Final nC_n	UCM%	TRP%	UCM/TRP	Pr/Ph	CPI
S17	C_{19}	C_{35}	93	6.9	13.4	-----	7.7
S18	C_{26}	C_{46}	96.3	3.7	26	-----	-----
S19	C_{13}	C_{44}	97	2.87	33.7	-----	-----
S20	-----	-----	97.85	2.14	45.6	-----	-----
S21	C_{14}	C_{41}	89.58	10.4	8.6	33.23	1.25
S22	C_{17}	C_{41}	84.76	14.24	5.54	1.0	1.34
S23	C_{17}	C_{44}	88.25	11.75	7.5	0.8	0.89
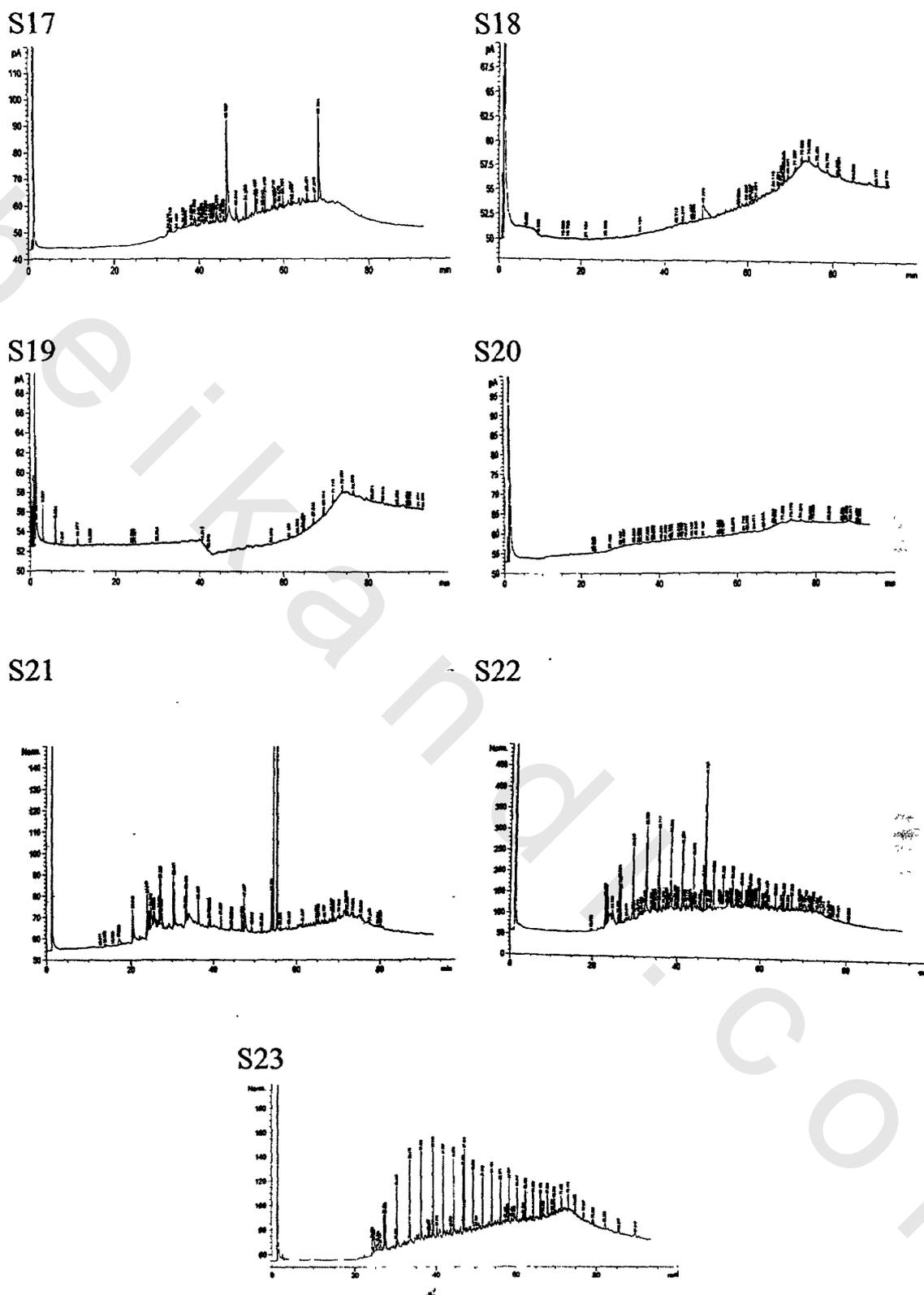
Fig.(29): Gas chromatographic profile of extracted oil from sediment samples of Gulf of Suez

III.3.3. Ultraviolet spectroscopic study:

The previous discussion about gas chromatographic pattern revealed that most of the studied pollutants were subjected to weathering. Since aromatics in general persist biodegradation compared to saturated and unsaturated chain hydrocarbons, so the residues of oils are mainly aromatics and naphthenes.

Careful examination of the spectra for the studied water and sediment samples, reveal that all samples have nearly the same basic profiles. A representative model for each type is shown in Fig.(30).they show strong broad absorption band at 230 which is probably indicative for alkylbezenes, derived from lighter hydrocarbons originally present in crude oils and /or due to the photo-oxidation and break-down products of higher aromatics (**Neff et al., 1975 & Moustafa et al., 2005**). This gives an indication that water and sediment samples are incorporated by these aromatic compounds which have both lethal and sub-lethal effects on marine organism. Polycyclic aromatic hydrocarbons larger than the alkyl-naphthalene also have strong absorption maxima at wavelength longer than 230 nm similar observations were reported by (**Neff et al., 1975 & Moustafa et al., 2005**); consequently the absorption at 230-270nm region, for all the studied spectra probably indicates the presence of such carcinogenic compounds. These compounds are more resistant to the different environmental weathering process (**Hwang and Foster, 2006**). Water samples are characterized by an extra band at 280 nm which are corresponding to polycyclic aromatic hydrocarbons larger than alkyl naphthalene.
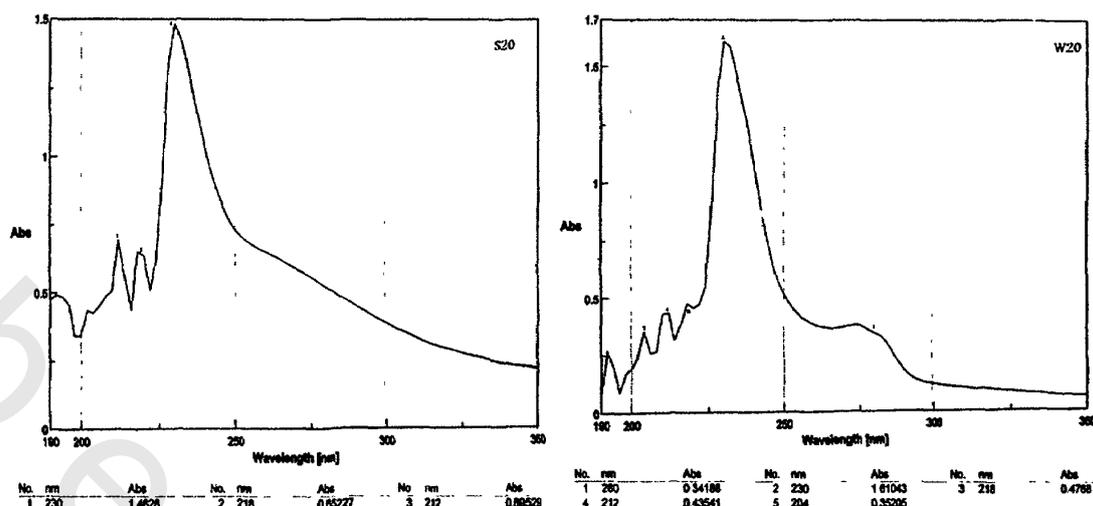
Fig.(30): Representative models for UV spectra of oil extracted from water and sediment samples of Gulf of Suez.

III.3.3. High Performance Liquid Chromatography

III.3.3.1.Water samples

Based on the 16 PAHs listed by the US-Environmental Protection Agency (EPA) as priority pollutants. The concentrations of individual parent (non-substituted) PAH and the total parent PAHs are listed in Table (17), All samples are characterized by the absence of the two membered rings compounds (naphthalenes). Naphthalene prefers to transport by atmosphere-water interaction **(Shi et al., 2005)**. Due to evaporation, dissolution and biodegradation, low molecular weights PAHs are thought to be selectively disappeared. It can be seen that total PAHs concentrations ranges from 81.15-4441.88 µg/l the highest PAHs concentrations are observed for samples W21, W22 and W23. According to the number of aromatic rings, the 16 PAH compounds were divided into three groups, representing two-, and three-, four-, and five-, and six-rings PAHs **(Qiao et al.,2006)**, the detected concentrations of PAHs in water ranged between 0–85.71µg/l for 3-ring PAHs (acenaphthylene, acenaphthene, fluorine, phenanthrene and anthracene), 0.73- 41.8µg/l for 4-ring PAHs, 0- 27.56µg/l for 5-ring PAHs and 77.99- 4384.7 µg/l for 6-ring PAHs. Data obtained show low ratios of Σ6LPAHs: Σ10HPAHs Table (17-a) indicating that all the water samples were subjected to weathering in different degrees. As weathering increases

the summation of the 6 LPAHs decrease. This makes the $\Sigma 6LPAHs: \Sigma 10HPAHs$ parameter not useful to detect the origin. On the other hand it could be useful in the comparison of the weathering effects on the samples **(Zakaria et al., 2001)**.Several studies showed that there are a high concentrations of HPAHs compared to that of LPAHs , due to the small-size of LPAHs they are more labile, and are expected to degrade faster than the lager ones and also due to the higher vapor pressure and water solubilities **(Mackay et al., 1992)**. From an ecotoxicological point of view, a majority of water samples had high enough concentrations to induce immediate mortality in exposed organisms. Regarding to Phe/Ant ratios samples W22, W17 and W19 have zero value indicating pyrogenic origin which is confirmed by Flu/Pyr which have infinity value indicating also purely pyrogenic origin. For sample W18, Phe/Ant ratio has infinity value indicating petrogenic origin in contrary to Flu/Pyr having infinity value indicating purely pyrogenic origin. For sample W20 and W21 Phe/Ant ratio<1 indicating pyrogenic origin incontrary to Flu/Pyr which have zero value indicating petrogenic origin. For sample W23 have Phe/Ant ratio>1 indicating petrogenic origin in contradiction with Flu/Pyr which have infinity value indicating pyrogenic origin. The reason for this contradiction and the sources of contamination were previously discussed.

Table (17): Individual PAHs distribution and their concentrations (µg/l) in water samples of Suez Gulf

Ring No.	PAHs	water samples of Suez Gulf						
		W17	W18	W19	W20	W21	W22	W23
2	Naph	0	0	0	0	0	0	0
	Total	0	0	0	0	0	0	0
3	A	11.3	10.12	0	0	5.7	0	33.6
	Ace	7	3	0	1.2	1.1	0	0
	F	15	1.6	0	0.1	0.44	0	5.7
	Phe	0	0.19	0.01	0.09	0.32	0	6.3
	Ant	11.8	0	85.7	1	0.4	0	3.4
	Total	45.1	14.91	85.71	2.39	7.96	0	49
4	Flu	2.3	1.3	37	0.26	0.81	1.7	3.7
	Pyr	19.6	0	4.8	0	11.5	0	0
	BaA	0	2.7	0	0.47	3.2	5.9	0.69
	Chr	9.6	0.36	0	0	0	0	0
	Total	31.5	4.36	41.8	0.73	15.51	7.6	4.39
5	BbF	0	0	12	0	0	0	3.3
	BkF	1.8	0	1.36	0.037	7.2	0	0.49
	BaP	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	DahA	0	0	14.2	0	0	0	0
	Total	1.8	0	27.56	0.037	7.2	0	3.79
6	BP	0	0	0	0	159.7	0	0
	IP	158	98	98.6	77.99	830.7	1553.8	4384.7
	Total	158	98	98.6	77.99	990.4	1553.8	4384.7
	Total ΣPAHs	236.4	117.27	253.67	81.147	1021.07	1561.4	4441.88

Table (17-a): PAHs parameters used to distinguish petrogenic from pyrogenic origin of PAHs

parameter	W17	W18	W19	W20	W21	W22	W23
phe/Ant	0	∞	0.00012	0.09	0.8	0	1.85
flu/pyr	∞	∞	∞	0	0.07	∞	∞
ΣLPAHs:ΣHPAHs	1:4	1:7	1:2	1:33	1:127	∞	1:99

Phen/Ant<1 indicates Pyrolytic origin and Phen/Ant >1 indicates petrogenic origin
Flu/Pyr<1 indicates petrogenic origin and Flu/Pyr>1 indicates Pyrolytic origin

III.3.3.2. Sediment samples

Qualitative and quantitative distribution of PAHs detected in sediment samples have followed no particular pattern, but were rather irregular and their levels are alarmingly high, ranging between 763.5 and 6741 µg/kg, the presence of such high concentrations are considered to be toxic (**Moustafa**[b], **2004**), representative samples for the distribution patterns of the individual PAHs from the extracted oil are presented in Fig.(31). Analysis of the individual PAHs composition is helpful to track the contaminant source, and illustrate the fate and transport of PAHs in multimedia environment (**Gigliotti et al., 2002 & Shi et al., 2005**).The concentrations of the 16 PAHs detected in the extracted oil from sediments are listed in Table (18).

Generally all the collected sediment samples are characterized by the absence of diaromatic ring compounds except samples S21 and S23 which have values (17 and 0.3, respectively). As previously mentioned naphthalenes have relatively higher vapor pressure and Henry's law constant (0.085 mmHg at 25°C, **Ambrose et al., 1975**; 0.00044 atm-m^3/mole, **Shiu and Mackay, 1997**). therefore naphthalenes can be easily transported by atmosphere-water interaction (**Shi et al., 2005**).The detected concentrations of PAHs in sediments ranged 31.5 –224.7µg/kg for the 3-ring PAHs (acenaphthylene, acenaphthene, fluorine, phenanthrene and anthracene), 4.18- 270.1 µg/kg for the 4-ring PAHs, 0-267.8 µg/kg for the 5-ring PAHs and 724.6- 6460 µg/kg for the 6-ring PAHs. It has been mentioned that small-size PAHs are more labile, and are expected to degrade faster than larger ones (**Fernandes et al., 1997 & Shi et al., 2005**), while high molecular weight PAHs are more resistant to degradation processes occurred in sediments (**Cerniglia, 1992 & Luo et al., 2006**). Data obtained show low ratios of Σ6LPAHs:Σ10HPAHs as listed in Table (18-a). Indicating that all the sediment samples were subjected to weathering in different degrees.

It was noticed that according to our study Phe/Ant ratio for sampleS18 and S20 have value of infinity indicating petrogenic origin in contrary to Flu/Pyr ratio have value of infinity indicating pyrogenic origin while samples

S17, S19, S21, S22 and S23 have Phe/Ant ratio close to zero indicating pyrogenic origin in contrary to Flu/Pyr have ratio close to zero also indicating petrogenic origin.

Major sources of these contaminants include accidental release and leakage from ship traffic, barge activity, fuel and oil transportation by supertankers, offshore oil exploratory activities, and natural seepage of crude oil similar observation were reported by **(Barakat et al., 2002)**. In addition, to oil leakage there were a lot of boats and ships traveling constantly in the area; many of them were discharging black smokes throughout their movement; hence there were many non-point source in the area, contributing to the very high concentrations of PAHs detected **(Maskaoui et al., 2002)**.

Discrepancies in the results obtained as discussed before show that the different parameters (given by some authors) reflecting the degree of biodegradation weathering leads sometimes to different results.

Table (18): Individual PAHs distribution and their concentrations (µg/kg) in sediment samples of Suez Gulf

Ring No.	PAHs	Sediment samples of Suez Gulf						
		S17	S18	S19	S20	S21	S22	S23
2	Naph	0	0	0	0	17	Nil	0.3
	Total	0	0	0	0	17	Nil	0.3
3	A	32.9	35.26	12.3	27.6	Nil	Nil	Nil
	Ace	3	4.76	48.7	2.7	1.2	14	29
	F	0.99	1.29	20.6	1.1	Nil	9.3	61
	Phe	0.23	0.21	0	0.1	5.8	1.4	7
	Ant	3.7	0	31.56	0	140	200	100
	Total	40.82	41.52	113.16	31.5	147	224.7	197
4	Flu	1	1	3.5	0.8	Nil	Nil	5.5
	Pyr	9.28	0	37.5	0	36	100	54
	BaA	2.95	3	0	2.5	6.7	4.1	149
	Chr	0.44	0.18	28.5	4.1	26	166	7.8
	Total	13.67	4.18	69.5	7.4	68.7	270.1	208.5
5	BbF	0	0	0	0	15.6	95	250
	BkF	0	0	0	0	5.7	5.7	2
	BaP	Nil	Nil	Nil	Nil	Nil	Nil	10.46
	DahA	0	0	0	0	27	137	5.4
	Total	0	0	0	0	48.3	237.7	267.7
6	BP	0	0	0	5580	11700	1160	
	IP	1194.8	2914.4	1309.6	724.6	880	10190	500
	Total	1194.8	2914.4	1309.6	724.6	6460	21890	1660
	Total PAHs	1249.29	2960.1	1492.26	763.5	6741	22623	2333.2

Table (18-a): PAHs parameters used to distinguish petrogenic from pyrogenic origin of PAHs

Parameter	S17	S18	S19	S20	S21	S22	S23
phe/Ant	0.06	∞	0	∞	0.04	0.007	0.07
flu/pyr	0.1	∞	0.09	∞	0	0.053	0.1
ΣLPAHs:ΣHPAHs	1:30	1:70	1:12	1:23	1:40	1:100	1:11

Phen/Ant<1 indicates Pyrolytic origin and Phen/Ant >1 indicates petrogenic origin
Flu/Pyr<1 indicates petrogenic origin and Flu/Pyr>1 indicates Pyrolytic origin
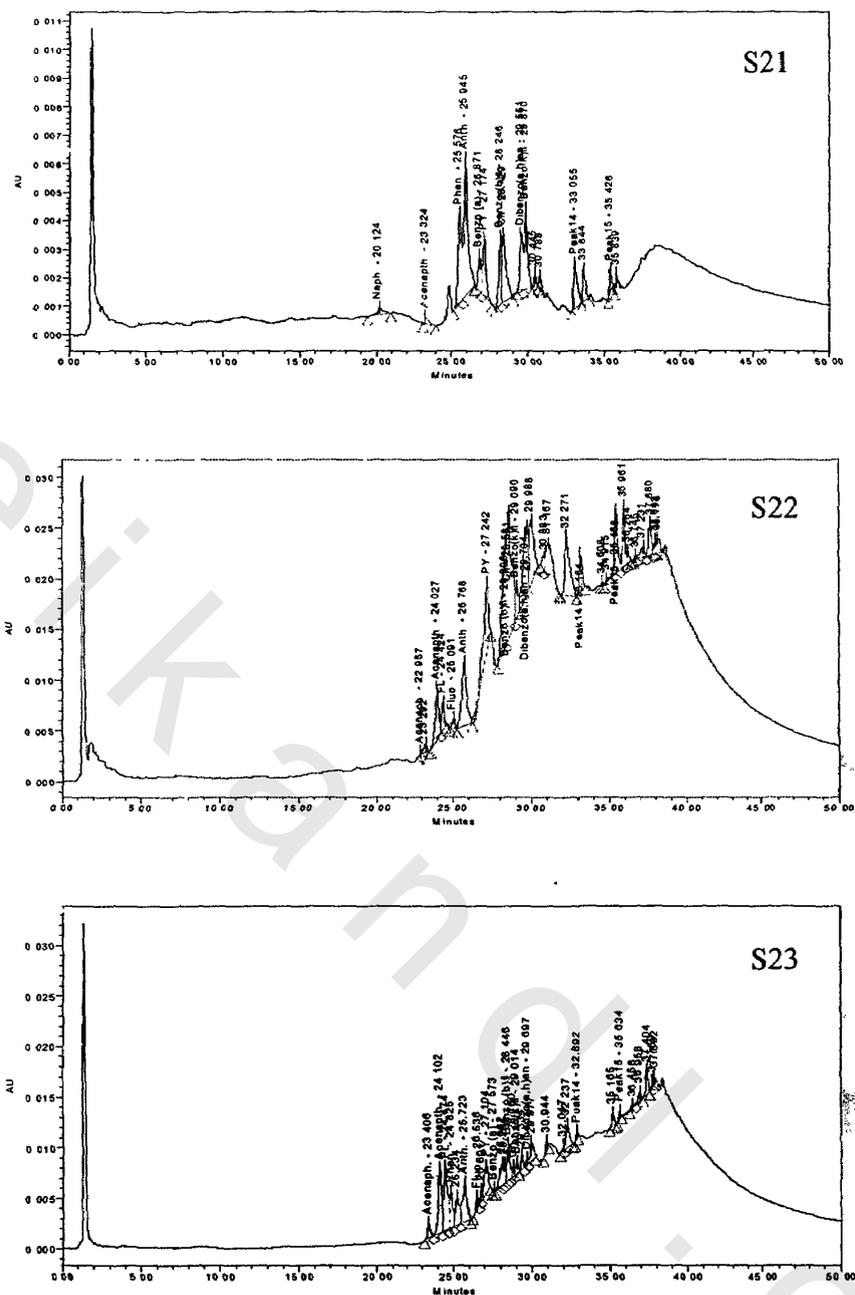
Fig.(31): Representative HPLC chromatograms of the PAHs in oil extracted from; sediment samples of Gulf of Suez

III.4. Biotreatment of oil contaminated sediments

The extensive use of petroleum products leads to the contamination of almost all components of the environment. Biodegradation of hydrocarbons and its mechanism by natural populations of microorganisms is the main process acting in the depuration of hydrocarbon-polluted environments and have been extensively studied and reviewed **(Atlas, 1994& Leahy and Colwell, 1990 &Van Hamme, 2003&Chaillan et al., 2004& De Oteyza and Grimalt , 2006).**

Traditional chemical analysis and microbial counts are used in the assessment of contaminated sediments and soils, and in monitoring the effectiveness of remediation processes, chemical analyses alone may not be sufficient **(Abbondanzi et al., 2006).**

Monitoring the survival of the introduced microbial degraders and/or the indigenous microbial population at the contaminated sites is an essential attribute in the reduction of contamination levels by the microbial population **(Macnaughton et al., 1999& Mishra et al., 2004).** Effective bioremediation necessitates the survival of the microorganism at the treated sites and the expression of their catabolic potential. Conventional methods to monitor survival of microorganisms for example most-probable-number (MPN) method and direct counting methods to measure the total viable count (TCFU/ml) are associated with number of anomalies **(Mishra, et al., 2004).** For effective bioremediation it is essential that the augmented microorganisms survive over a long period of time in the contaminated sites for complete reclamation **(Mishra et al., 2004).**

Total viable count of indigenous and augmented bacteria is monitored throughout the incubation period for the three treated sediment samples and recorded in Table (19).

In this study; the total viable count (TCFU) of biostimulation and bioaugmentaion flasks at zero time decreased as the initial TPH concentration increased in the treated samples; S21 (322ppm), S22 (901ppm) and S23 (1791ppm). It is well known that high levels hydrocarbon contamination might cause inhibitory or toxic effects on the indigenous microbial communities **(Trindad et al., 2005)** due to oxygen and/or nutrient limitation, not only this but the terrestrial oil spills are characterized primarily by vertical movement of the oil into the soil rather than the horizontal spreading

associated with slick formation. Infiltration of oil into the soil might prevent evaporative losses of volatile hydrocarbons, which are toxic to microorganisms **(Atlas, 1981& Leahy and Colwell, 1990 & El-Tokhi and Moustafa, 2001)**. In addition, the low microbial community in oil polluted sediment samples might be attributed to the environmental habitat of the site from where they were collected, as the soil at that sites are coastal dune sand and generally, coastal dune sand is characterized by negligible carbon, nitrogen and phosphorus content which are essential for microbial maintenance **(Kaplan and Kitts, 2004)**.

From results listed in Table (19) it is obvious that there is no apparent lag phase as there is a significant increase in TCFU observed in all treatments cultures after one week of incubation. Similar observation was reported by **Trindad et al., 2005** and explained by the weathering of the studied area, where the pervious exposure of the native microorganisms to the contaminant for long time, leading to a selection of the oil-degrading and/or tolerant ones. As reported also by **Alexander (1999)**, once the indigenous communities of microorganisms have been acclimated to the degradation of a chemical and the activity becomes marked, the potential for this activity may continue to remain higher than in comparable soils that have not acquired this capacity. In addition, it is well known for the bioaugmentation with previously isolated microorganisms from hydrocarbon polluted environment is extremely important in eliminating the adaptation phase **(Alexander, 1999 &Trindad et al., 2005)**. Microbial growth seemed to have reached a plateau before the end of the experiment as maximal values were often observed after two weeks of incubation except for treatment of S22 and S23 with NSh37 and bioaugmentation treatment of S22 where maximum growth occurred after four weeks of incubation.

In biostimulation flasks regardless of the initial TPH concentration in the treated samples TCFU showed a regular increment during the first two weeks with a sharp increase within the first week then decrease there after. These results suggest the presence of active and adapted hydrocarbon degrading population in the treated samples. Similar observation was reported by **Rahman et al., 2003 and Chaîneau et al., 2005**.

Treatment with *Staphylococcus gallinarum* NSh37 showed different trends in growth with the three treated samples; for the low polluted sediment sample (S21) there is a sharp increase in the TCFU within the first week which remained nearly sustained up to the fourth week of incubation, then a sharp decrease occurred there after within the sixth week. For the moderately polluted sediment sample (S22) the TCFU increased during the first week then remained nearly sustained up to the second week then sharply increased throughout the fourth week of incubation which then decreased within the sixth week. But for the heavily polluted sediment sample (S23) the TCFU showed a regular increase up to the fourth week with a sharp increase within the first week then declined with the sixth week of incubation.

In bioaugmentation flasks; for the low polluted sediment sample (S21) the TCFU increased gradually within the first two weeks then starts to decrease from the fourth week of incubation with a sharp decrease throughout the sixth week of incubation. For the moderately polluted sediment sample (S22) the TCFU showed an increment up to the fourth week with a sharp increase with the first week of incubation and a sharp decrease occurred throughout the sixth week of incubation. For the heavily polluted sediment sample (S23) the TCFU showed a sharp increase within the first week which continued in increment u to the second week, then remained nearly sustained up to the fourth week with a sharp decline occurred within the sixth week of incubation.

Generally regardless of the initial TPH concentration in the treated samples it is obvious that; the highest viable count was recorded in the biostimulation flasks compared to that of *Staphylococcus gallinarum* NSh37 and bioaugmentation flasks. And also the added bacteria seem to compete poorly with the indigenous population in bioaugmentation flasks, as they show high TCFU, as listed in Table (19). It is difficult, if not impossible, to increase the microbial population over which can be achieved by biostimulation alone. Contaminants are often potential energy sources for microorganisms, and according to Ilyina et al., (2003); microorganisms survive in contaminated habitat because they are metabolically capable of utilizing its resources and can occupy a suitable niche. Reclamation of oil-

contaminated sediment and/or soil by microorganisms is a well-documented process (**Mishra et al., 2004**). Bioremediation of contaminants can be accomplished by two methods, bioaugmentation and/or biostimulation. The process of bioaugmentation, involves the introduction of microorganisms that have been previously isolated from the contaminated site or may be obtained from a stock of microbes that have been previously proven to degrade hydrocarbons. The process of biostimulation introduces additional nutrients mainly nitrogen and phosphorus into the contaminated site to enrich the indigenous microbial populations (**Sarkar et al., 2005**).

Table (19): Total Viable Count (TCFU/ml)

Sample Treatment	Incubation Period (Weeks)				
	Zero time	One	Two	Four	Six
Low polluted Sample (S21)					
Biostimulation	$4X10^6$	$1.4X10^9$	$2.8X10^{10}$	$4X10^6$	$2X10^2$
Staphylococcus gallinarum NSh37	$6X10^7$	$1.2X10^9$	$9X10^9$	$6X10^9$	$3X10^4$
Bioaugmentation	$1X10^9$	$2X10^9$	$1.5X10^{10}$	$2X10^9$	$4X10^3$
Moderately polluted Sample (S22)					
Biostimulation	$2X10^6$	$3X10^{11}$	$5X10^{12}$	$3X10^{11}$	$6X10^9$
Staphylococcus gallinarum NSh37	$6X10^7$	$6X10^8$	$8X10^8$	$1.4X10^{11}$	$1.2X10^{10}$
Bioaugmentation	$9X10^8$	$3.5X10^{10}$	$2.8X10^{11}$	$5X10^{11}$	$3X10^8$
Heavily polluted Sample (S23)					
Biostimulation	$4X10^5$	$2.8X10^{11}$	$5.2X10^{12}$	$2X10^{11}$	$6X10^{10}$
Staphylococcus gallinarum NSh37	$6X10^7$	$2.4X10^9$	$2X10^{10}$	$1.5X10^{11}$	$5.7X10^{10}$
Bioaugmentation	$1X10^8$	$1.2X10^{11}$	$1.4X10^{12}$	$2X10^{12}$	$3.5X10^8$

TPH removal is quantitatively monitored and recorded in Table (20). It is obvious from the results listed in Table (20) that regardless to the initial TPH concentration in the treated sediment samples; there is no or short lag period preceding TPH removal in all the treatment methods, suggesting the

presence of active and well adapted hydrocarbon degrading populations **(Rahman et al., 2003).**

Biostimulation treatment show the best TPH removal indicating that biostimulation treatment accelerate the biodegradation process, as compared to *Staphylococcus gallinarum* NSh37 and bioaugmentation treatments. This demonstrates that the native sediment microbes are capable of degrading hydrocarbons to a large extent. Previous studies have reported indigenous microbial communities could have a considerable potential to remedy the oil-contaminated sediments as they are self adapted to these hard conditions and are well adjusted to their own environment. An immediate increase in the population density of these microbes could ensure rapid degradation of the pollutant which would reduce the cleanup time substantially **(Aldrett et al., 1997&Ramsay et al., 2000& Mrayyan and Battikhi, 2005 & Sabaté et al., 2006).**

Table (20): Total Petroleum Hydrocarbon removal

Sample Treatment	Incubation Period (Weeks)			
	One	**Two**	**Four**	**Six**
Low polluted Sample (S21)				
Biostimulation	12%	72%	74%	77%
Staphylococcus gallinarum **NSh37**	6%	32%	42%	50%
Bioaugmentation	28%	31%	36%	38%
Moderately polluted Sample (S22)				
Biostimulation	17%	20%	43%	53%
Staphylococcus gallinarum **NSh37**	21%	22%	29%	41%
Bioaugmentation	12%	15%	20%	21%
Heavily polluted Sample (S23)				
Biostimulation	50%	51%	66%	74%
Staphylococcus gallinarum **NSh37**	39%	42%	46%	47%
Bioaugmentation	21%	26%	35%	36%

Treatment with Staphylococcus *gallinarum* NSh37 shows better TPH removal than that obtained by bioaugmentation treatment indicating the adaptation of NSh37 to oil-polluted environment as it was previously isolated from hydrocarbon polluted soil **(El-Gendy, 2004)**. Growth and biodegradation potential of NSh37 in the sterilized sediment samples may have been stimulated by the effective death of the indigenous microflora and also due to the changes in physicochemical properties of the sediment after sterilization, a process that can bring about an increase in the concentration of soluble naturally occurring nutrients and organic matter **(Clemente and Durrant, 2005)**. Other investigations, reported that the *Staphylococcus* species have the ability to degrade drilling fluid base oils and oil waste attached to drill cuttings **(Benka-Coker and Olumagin, 1995& Nnubia and Okpokwasili, 1993&Okpokwasili and Nnubia, 1999 & Nweke and Okpokwasili, 2003)**. The results of this work showed that Staphylococcus *gallinarum* NSh37 has potential application in bioremediation of oil polluted sediment samples on the lab scale and it is recommended to be applied in the treatment of oil polluted environments.

Bioaugmentation treatment show the lowest efficiency in TPH removal; about approximately half that can be obtained in biostimulation treatment, approximately 30% lower than that can be obtained with Staphylococcus *gallinarum* NSh37 treatment for the low (S21) and heavy (S23) polluted sediment samples, but it shows approximately half that can be obtained with NSh37 treatment for moderately polluted sediment sample (S22).

Bioaugmentation is often used to enhance the rate of biodegradation of organic substances **(Vogel, 1996)**. However, other studies showed that bioaugmentation did not improve and even slow down the degradation processes **(Launen et al., 2002& Saponaro et al., 2002)**. The low efficiency of bioaugmentation might indicate that the activity of the indigenous microorganisms in the treated samples limit the activity of the introduced bacteria. Similar results were obtained by **Devenny and Chang, (2000) and Bento et al., (2004)**.They explained this as the success of bioremediation is dependent of the level of metabolic and genetic adaptation of the microbial

populations to their environment. According to **Yu et al., 2005**; such negative interaction between the augmented NSh37 and indigenous microbial community may be due to competition for resources, in particular nutrients. The interactions between inoculum and indigenous microbes are complicated and further work on monitoring the survival and activity of inoculum will be essential and recommended.

Generally from Tables (19 and 20), independent of the initial TPH concentration it is obvious that with sustainability or decline in TCFU there is a consequent sustainability and/or decrease in the TPH removal rate. Changes in microbial population and TPH degradation efficiency throughout the experiment incubation period could be due to interactions between various microbial populations and resulting changes to the physicochemical properties of the treated sediment sample slurries for example the accumulation of toxic intermediates and/or end metabolites during the biodegradation process (**Frankenberger, 1992 & Delille et al., 2002**). A lack of correlation between the degradation success and the toxicity reduction has been reported in previous studies (**Pelletier et al., 2003 &Coulon et al., 2004**) but it is not yet clear how toxicity could increase with time. Formation of toxic metabolites has been suggested in many instances, but the search for toxic metabolites has not yet been successful. However, the high TCFU did not necessarily translate to significant differences in TPH degradation which suggests existence of a certain threshold level to nutrient supplementation that might be able to accelerate hydrocarbon degradation during the essential stages of bioremediation (**Sarkar et al., 2005**). According to **Trindad et al., 2005** this probably caused by the beginning of the recalcitrant compounds degradation or by the inability of the microorganisms in degrading those compounds.

Results listed in Table (20) show that during the first two weeks of incubation; the rate of TPH removal was high in the three treatment methods and regardless to the initial TPH concentration. This is followed with a small and continual increase in degradation rate until the end of the experiment (6 weeks). Similar observation was reported by **Bento et al., 2004 and Sarkar et al., 2005.** They explained this as at the beginning; oil-degrading

microorganisms were stimulated by labile hydrocarbon sources (probably linear and open-chain hydrocarbons) that induced a high percentage of degradation. As those forms decreased, microbial populations had to use the more recalcitrant hydrocarbons (probably aromatic hydrocarbons with higher molecular weight) less efficiently. With the decrease in labile carbon sources, nutrients were most likely limited in supporting microbial growth. It is also possible that degradation of high molecular weight hydrocarbons may produce toxic intermediates that can inhibit oil-degrading microorganisms.

It is well known that as the concentration of the contaminant increases the degree of biodegradation decreases, but in this study as listed in Table (20) the efficiency of biodegradation at the end of incubation period (6 weeks) independent on the type of treatment is nearly the same for low and heavily polluted sediment samples; for biostimulation treatment, the degree of TPH removal reaches 77% and 74% for S21 and S23, respectively. For treatment with NSh37, the degree of TPH removal reaches 50% and 47% for S21 and S23, respectively. For bioaugmentation treatment, the degree of TPH removal reaches 38% and 36% for S21 and S23, respectively. But for the moderately polluted sediment sample S22, it shows the lowest degree of TPH removal at the end of incubation period; for biostimulation treatment the degree of TPH removal reaches 53%, for NSh37 treatment it reaches 41% and for bioaugmentation treatment it reaches 21%. Independent of the fraction of TPH (light or heavy) and the bioremediation treatment, the soils themselves have varying effects on degradation of oil and on microbial populations and activity (Bento et al., 2004& Aichberger et al., 2006). According to Rahman et al., 2003, the rate and quantity of hydrocarbon degraded depend on physical characteristics of the treated sample, chemical structure of the pollutant compounds, type and amount of oil present at the treated samples. It is obvious as discussed in the GC analysis for chemical characterization of the treated samples that each sediment sample has its own properties; S23 mainly contains petrogenic hydrocarbons and weathered crude oil. But S21 and S22 have mixture of petrogenic and biogenic hydrocarbons; S21 is characterized by highly weathered crude oil as it has the highest UCM and lowest TRP compared to S22 and S23, as listed in Table (21), it shows bimodal distribution indicating the diverse sources of oil

pollution. S22 shows a well defined and broad UCM with mainly predominant peaks C_{17}-C_{25} this might indicate contamination with mid range distillates. Mid range distillates; kerosene, jet fuel and diesel products are typically products in a relative broad carbon range (C6-C26) and the UCM is well defined **(Wang and Fingas, 2003). Dorn and Salanitro (2000)** observed that diesel has higher toxic signals to microorganisms and consequently to the TPH removal than crude oil in soil bioremediation studies; toxicity is greater with lighter oils containing higher concentrations of saturated hydrocarbons compared to heavier oils. The HPLC analysis monitoring the 16 PAHs currently regulated by the US EPA, found that S22 is characterized by the highest concentration of PAHs (22,623ppb) specially the six membered ring compounds; Benzo[g,h,i]perylene (BP) and Indeno[1,2,3-cd]Pyrene (IP). Then comes S21 (6,741ppb) and followed by S23 (2,341ppb) as listed in Tables (22, 23 and 24).

Unlike the conventional treatment technologies, bioremediation technique must be tailored specifically to each polluted site. Each waste site has unique characteristics, and thus requires individual attention, so an official criterium for evaluating the success or failure of a particular strategy is needed. In addition, a successful biodegradation program requires a multidisciplinary approach, integrating fields such as microbiology, engineering, geology, and soil science and project management.

The effect of microbial treatment on the hydrocarbon skeleton of the resolvable components (TRP) of the oil contaminating the sediment samples was also studied using GC-FID analysis. Figs.(32, 33 and 34) represent the GC chromatogram of the samples before and after treatment at the end of the experiment (six weeks).

Table (21): Biotreatment effect on TRP and UCM using different GC-parameters

Low polluted sample (S21)

parameter	Initial C.No.	Final C.No.	C_{17}/Pr	C_{18}/Ph	Pr/Ph	CPI	UCM%	TRP%	UCM/TRP
-ve control of low polluted sample	C_{14}	C_{41}	2.6	23.9	33.23	1.25	89.58	10.4	8.6
Biostimulation of low polluted sample	C_{16}	C_{41}	2.24	2	0.67	4.2	56.73	43.27	1.3
Staphylococcus gallinarum NSh37	C_{16}	C_{41}	5.12	0.48	1	7.96	67.58	32.42	2
Bioaugmentation of low polluted sample	C_{16}	C_{41}	0.9	2.34	1.7	3.45	70.9	29.1	2.4

Moderately polluted sample (S22)

parameter	Initial C.No.	Final C.No.	C_{17}/Pr	C_{18}/Ph	Pr/Ph	CPI	UCM%	TRP%	UCM/TRP
-ve control of moderately polluted sample	C_{17}	C_{41}	1.23	1.34	1.07	1.34	84.76	15.24	5.54
Biostimulation of moderately polluted sample	C_{16}	C_{41}	0.19	0.3	0.77	1.1	91.75	8.25	11.12
Staphylococcus gallinarum NSh37	0	0	0	0	0	0	99.04	0.96	103.16
Bioaugmentation of moderately polluted sample	C_{16}	C_{41}	1.25	0.2	0.9	1.49	90.77	9.23	9.83

Highly polluted sample (S23)

parameter	Initial C.No.	Final C.No.	C_{17}/Pr	C_{18}/Ph	Pr/Ph	CPI	UCM%	TRP%	UCM/TRP
-ve control of highly polluted sample	C_{17}	C_{44}	1.1	1	0.8	0.96	88.25	11.75	7.5
Biostimulation of highly polluted sample	0	0	0	0	0	0	99.4	0.55	180.7
Staphylococcus gallinarum NSh37	C_{17}	C_{44}	2.6	6.1	1.3	0.97	92.2	7.8	11.8
Bioaugmentation of highly polluted sample	C_{17}	C_{44}	0.15	0.2	0.2	1	94.46	5.54	17
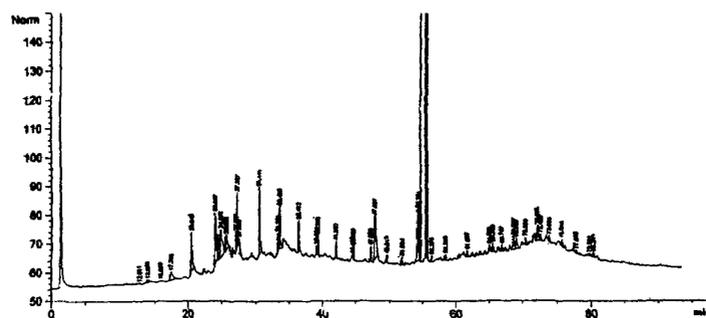
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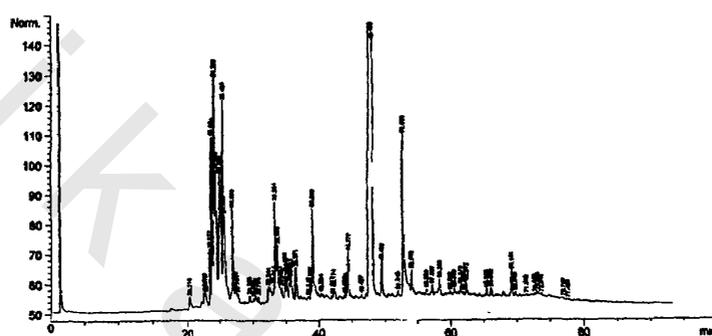
Biostimulation

***Staphylococcus gallinarum* NSh37**

Bioaugmentation

Fig.(32): Gas chromatographic profile of extracted oil before and after biotreatment of Low polluted sample (S21)

-ve control

Biostimulation

Staphylococcus gallinarum NSh37

Bioaugmentation

Fig.(33): Gas chromatographic profile of extracted oil before and after biotreatment of moderately polluted sample (S22)
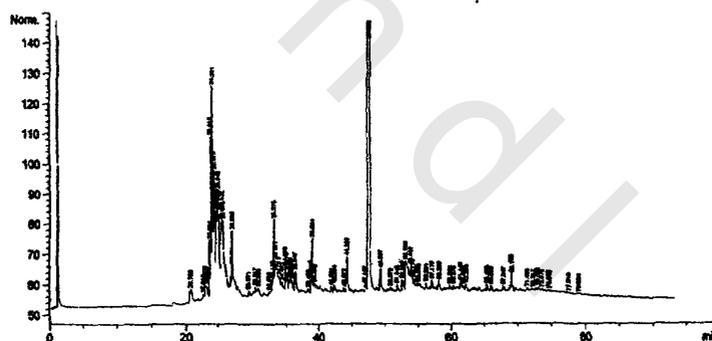
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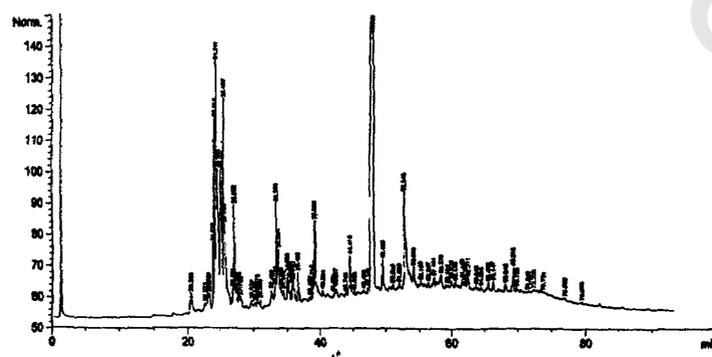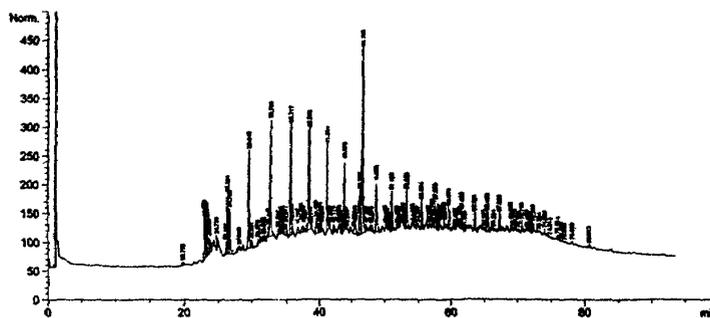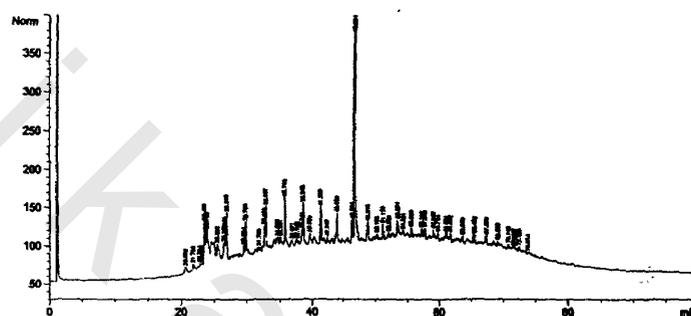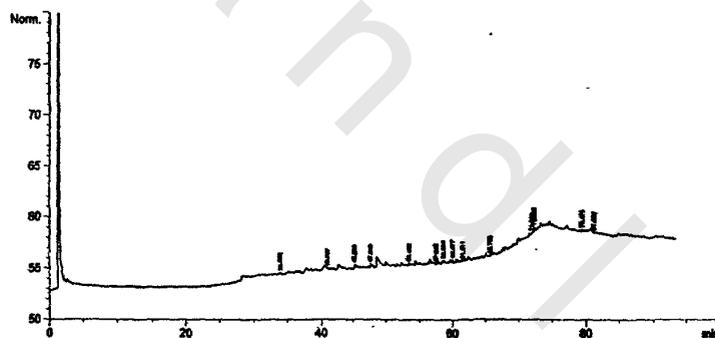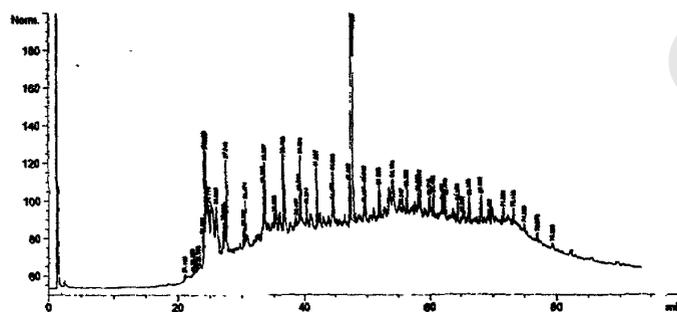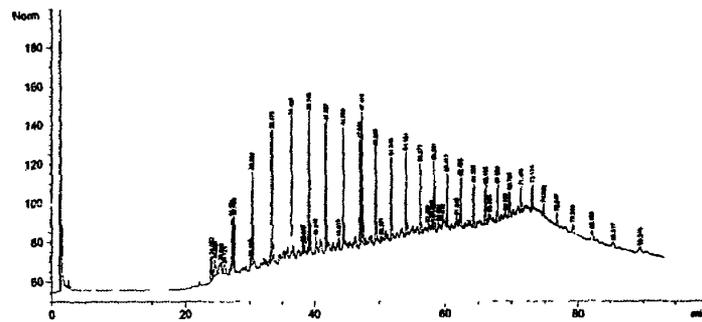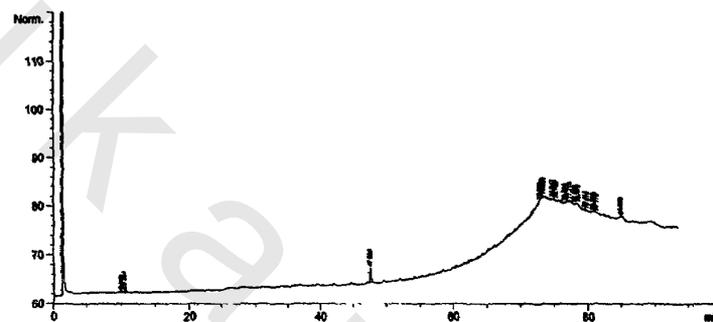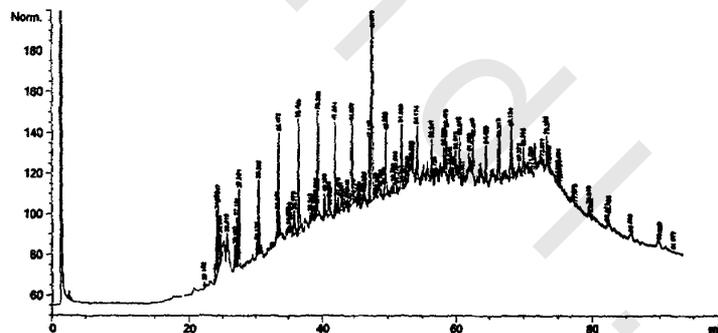
Biostimulation

Staphylococcus gallinarum NSh37

Bioaugmentation

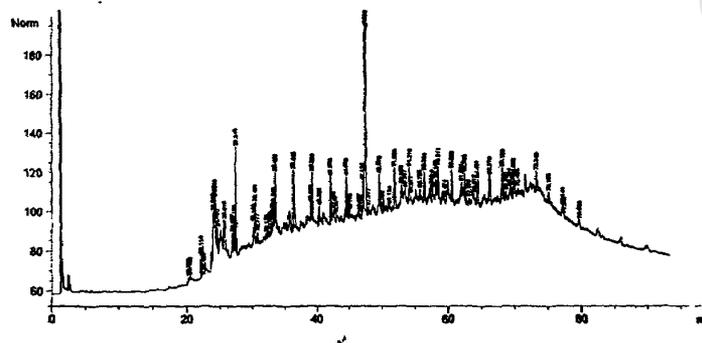**Fig.(34):** Gas chromatographic profile of extracted oil before and after biotreatment of highly polluted sample (S23)

All the treatments independent on the initial TPH concentration express a great change in the TRP (linear and branched alkanes) and persistence of UCM (aromatics, naphthenes and cycloalkanes) of the contaminating oil, but with different efficiency and trend Table (21) summarizes these changes.

Several investigators used a number of parameters depending on gas chromatographic analysis which can be used as an indication for biodegradation. Among these are the following; nC_{17}/Pr, nC_{18}/Ph, Pr/Ph and UCM/TRP ratios **(Tolosa et al., 2005)**.

Biostimulation treatment generally shows a great decrease in TRP as listed in Table (21) and shown in Fig. (32, 33 and 34), indicating the efficiency of the indigenous microbial population for degrading the TRP. This is very obvious in heavily polluted sediment sample S23 which showed a sharp increase of UCM/TRP ratio and CPI, nC_{17}/Pr, nC_{18}/Ph and Pr/Ph values of zero and TRP% of 0.55% indicating a complete degradation of TRP and complete removal of Pr and Ph. For treatment of low polluted sample (S21) and moderately polluted one (S22); the decrease in nC_{17}/Pr, nC_{18}/Ph than the control samples indicates the degradation of nC17 and nC18. The decrease of Pr/Ph than the control samples indicates the degradation of Pr and/or the degradation of Pr is much higher than that of Ph. The decrease of TRP%, CPI and increase of UCM/TRP than the control sample of S22 indicates the degradation of TRP. For treatment of S21 the decrease of UCM% and UCM/TRP than the control sample indicates the degradation efficiency of indigenous microbial population of S21 towards the UCM components. The increase of CPI value than the control sample of S21 can be explained by the obvious biogenic addition occurred on nC_{23} and nC_{25} after six weeks of incubation, as shown in Fig.(32) **(Wang et al., 1995 and Wang et al., 1999)**

Treatment with Staphylococcus *gallinarum* NSh37 generally shows a great decrease in TRP as listed in Table (21) and shown in Figs (32, 33 and 34), indicating the efficiency of NSh37 for degrading the TRP. This is very obvious in the treatment of moderately polluted sediment sample S22 which showed a sharp increase of UCM/TRP ratio and CPI, nC_{17}/Pr, nC_{18}/Ph and

Pr/Ph values of zero and TRP% of 0.96% indicating a complete degradation of TRP and complete removal of Pr and Ph. For treatment of heavily polluted sediment sample S23; the decrease of TRP% and increase of UCM/TRP than the control sample indicates the degradation of TRP. The increase of nC_{17}/Pr, nC_{18}/Ph and Pr/Ph values indicating the efficiency of NSh37 for degrading the isoprenoids and preference of degrading Ph than Pr. For treatment of S21 the decrease of UCM% and UCM/TRP than the control sample indicates the degradation efficiency of NSh37 in S21 towards the UCM components. The increase of CPI value than the control samples in S21 can be explained by the obvious biogenic addition occurred on nC_{23} although the iso paraffin appears after nC_{25} was obviously degraded which predominating the GC chromatogram of its negative control. The increase of nC_{17}/Pr value than the control sample in treatment of S21 indicating the high efficiency of NSh37 for degrading Pr which is confirmed by the decrease of Pr/Ph. In conclusion Staphylococcus *gallinarum* NSh37 has good efficiency in biodegradation of branched alkanes. Bioaugmentation treatment, generally shows a great decrease in TRP but lower than that can be obtained by biostimulation as listed in Table (21) and shown in Figs.(32, 33 and 34) indicating negative interaction between augmented bacteria (Staphylococcus *gallinarum* NSh37) and indigenous microbial populations in the treated samples this confirms the results obtained in TPH removal, as discussed previously. There is a general decrease in nC_{17}/Pr, nC_{18}/Ph and Pr/Ph values except for nC_{17}/Pr in treatment of S22, which remained nearly the same as the control sample; the decrease in these values confirms the efficiency of bioaugmentation treatment in degrading the TRP. The increase in CPI value in all samples indicates biogenic addition, which is very obvious in treatment of S21 through the biogenic addition on nC_{23}. The decrease of TRP% and increase of UCM/TRP value in treatment of S22 and S23 indicates the degradation of TRP. While for treatment of S21 the decrease of UCM% and UCM/TRP than the control sample indicates the degradation efficiency towards the UCM components.

Although the branched alkanes; pristane and phytane have been used as conservative biomarkers in oil bioremediation studies, their recalcitrance to biodegradation has been questioned in a lot of reports due to their susceptibility to biodegradation (**Huang et al., 2004 and Chaîneau et al.,**

2005). The change in ratio of nC_{17}/Pr, $nC18/Ph$, Pr/Ph and UCM/TRP have been long recognized and used as indicators of biodegradation **(Wang and Fingas, 2003and Tolosa et al., 2005)**.

Measuring the success of bioremediation of oil spills is based on several parameters, among them the degradation of PAHs in the contaminated site. So in this piece of work we have focused on the effect of the used biotreatment methods on the PAHs found in the contaminated sediment samples.

The US-EPA had classified 16 PAHs as priority pollutants whose remediation is considered indispensable for environmental clean up and human health **(Ke et al., 2002 and Anayakora et al., 2005)**.The concentration of the 16-PAHs in the extracted oil from the three treated samples before and after treatment were monitored and recorded in Tables (22, 23 and 24). Analysis the three- to six-ring PAHs exhibit appreciable differences in pollutant distribution pattern between the three investigated sediment samples; S21, S22 and S23. The moderately polluted sample; S22 is characterized by the highest concentration of PAHs especially the five and six membered ring compounds as listed in Tables (22, 23 and 24). It is obvious from the results listed in Tables (22, 23 and 24) that Staphylococcus *gallinarum* NSh37 has broad versatility in its action on PAHs. Biostimulation and bioaugmentation show very good results on removal of PAHs, indicating that the indigenous microbial populations in the studied sediment samples displayed a good capability to degrade 2-6 ring PAHs. These findings indicate that addition of nutrients or inoculation of enriched bacteria enhanced the sizes of total aerobic heterotrophic bacteria and PAH-degrading bacteria in the slurry. A higher increase in microbial number (TCFU), corresponding to the higher PAHs removal as illustrated in Tables (19, 22, 23 and 24) was recorded in the first week of treatment in all the methods of treatment in the three sediment samples independent of the initial concentration of TPH or PAHs. Microbial population in contaminated sediment samples, because of the acclimation and adaptation, may have developed some capabilities to degrade PAHs. The PAHs are known to be utilized as sole carbon and energy sources, or degraded co-metabolically by a diverse group of bacteria.

Table (22): Biodegradation potential of 16-PAHs in low polluted sediment sample (S21)

Biotreatment	[PAHs]ppb through out the incubation period (weeks)														
	Biostimulation					Staphylococcus gallinarum NSh37					Bioaugmentation				
PAHs time	Zero	1	2	4	6	Zero	1	2	4	6	Zero	1	2	4	6
Naphthalene	17	Nil	Nil	Nil	Nil	17	14	0.12	Nil	Nil	17	1.5	Nil	Nil	Nil
Acenaphthylene	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Acenaphthene	1.2	0.56	Nil	Nil	Nil	1.2	0.11	Nil	Nil	Nil	1.2	1.2	0.63	Nil	Nil
Fluorene	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Phenanthrene	5.8	0.4	0.15	Nil	Nil	5.8	Nil	Nil	Nil	Nil	5.8	1.4	Nil	Nil	Nil
Anthracene	140	28	1.8	0.06	Nil	140	0.16	Nil	Nil	Nil	140	20	4.5	0.8	Nil
Fluoranthene	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Pyrene	36	0.2	Nil	Nil	Nil	36	10	5.5	4.9	Nil	36	20	11	Nil	Nil
Benzo[a]anthracene	6.7	1.7	1.1	1	Nil	6.7	Nil	Nil	Nil	Nil	6.7	3.8	0.11	0.05	Nil
Chrysene	26	0.9	0.11	Nil	Nil	26	Nil	Nil	Nil	Nil	26	14	Nil	Nil	Nil
Benzo[b]fluoranthene	15.6	0.11	Nil	Nil	Nil	15.6	Nil	Nil	Nil	Nil	15.6	10	4.9	Nil	Nil
Benzo[k]fluoranthene	5.7	0.1	Nil	Nil	Nil	5.7	0.006	Nil	Nil	Nil	5.7	0.44	0.3	0.2	Nil
Benzo[a]pyrene	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Dibenzo[a,h]anthracene	27	0.11	Nil	Nil	Nil	27	0.16	Nil	Nil	Nil	27	1.7	0.9	Nil	Nil
Benzo[g,h,i]perylene	5580	4	Nil	Nil	Nil	5580	130	Nil	Nil	Nil	5580	870	140	139.6	130
Indeno[1,2,3-cd]pyrene	880	65	17	4.3	Nil	880	7.3	Nil	Nil	Nil	880	560	300	172	108.4
Total PAHs	6741	101.08	20.16	5.36	0	6741	161.74	5.62	4.9	0	6741	1504.04	462.34	312.65	238.4

Table (23): Biodegradation potential of 16-PAHs in moderately polluted sediment sample (S22)

Biotreatment	[PAHs]ppb through out the incubation period (weeks)														
	Biostimulation					Staphylococcus gallinarum NSh37					Bioaugmentation				
PAHs time	Zero	1	2	4	6	Zero	1	2	4	6	Zero	1	2	4	6
Naphthalene	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Acenaphthylene	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Acenaphthene	14	0.8	Nil	Nil	Nil	14	8.9	0.067	Nil	Nil	14	Nil	Nil	Nil	Nil
Fluorene	9.3	1.9	1.8	Nil	Nil	9.3	7.3	4.9	Nil	Nil	9.3	8.1	7.2	1.7	Nil
Phenanthrene	1.4	0.02	Nil	Nil	Nil	1.4	1.4	0.7	Nil	Nil	1.4	1.4	1.38	1.2	Nil
Anthracene	200	6	2	Nil	Nil	200	5	0.1	Nil	Nil	200	6	0.5	Nil	Nil
Fluoranthene	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Pyrene	100	100	1	Nil	Nil	100	35	0.08	Nil	Nil	100	15	15	2.3	Nil
Benzo[a]anthracene	4.1	4.1	3.9	Nil	Nil	4.1	11	0.74	Nil	Nil	4.1	0.66	0.41	0.09	Nil
Chrysene	166	68	Nil	Nil	Nil	166	42	12	Nil	Nil	166	Nil	Nil	Nil	Nil
Benzo[b]fluoranthene	95	54	3.6	Nil	Nil	95	4	Nil	Nil	Nil	95	Nil	Nil	Nil	Nil
Benzo[k]fluoranthene	5.7	Nil	Nil	Nil	Nil	5.7	1.1	0.55	0.004	Nil	5.7	0.21	0.02	0.02	Nil
Benzo[a]pyrene	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Dibenzo[a,h]anthracene	137	37	Nil	Nil	Nil	137	24	3.9	0.037	Nil	137	32	31	0.036	Nil
Benzo[g,h,i]perylene	11700	Nil	Nil	Nil	Nil	11700	2240	Nil	Nil	Nil	11700	6500	990	2	Nil
Indeno[1,2,3-cd]pyrene	10190	2200	200	Nil	Nil	10190	1880	Nil	Nil	Nil	10190	1000	570	Nil	Nil
Total PAHs	22623	2471.8	212.3	Nil	Nil	22623	4259.7	23.04	0.041	Nil	22623	7563.4	1615.51	7.35	Nil

Table (24): Biodegradation potential of 16-PAHs in heavily polluted sediment sample (S23)

| Biotreatment | [PAHs]ppb through out the incubation period (weeks) | | | | | | | | | | | | | | |
| | Biostimulation | | | | | Staphylococcus gallinarum NSh37 | | | | | Bioaugmentation | | | | |
PAHs \ time	Zero	1	2	4	6	Zero	1	2	4	6	Zero	1	2	4	6
Naphthalene	0.3	Nil	Nil	Nil	Nil	0.3	Nil	Nil	Nil	Nil	0.3	Nil	Nil	Nil	Nil
Acenaphthylene	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Acenaphthene	29	7.9	4.3	Nil	Nil	29	9.73	2.67	0.69	Nil	29	Nil	Nil	Nil	Nil
Fluorene	61	19.4	3.7	Nil	Nil	61	11	5.5	Nil	Nil	61	1.7	1.6	0.9	0.02
Phenanthrene	7	1.6	0.65	0.5	Nil	7	2	0.7	0.07	Nil	7	0.8	0.2	Nil	Nil
Anthracene	100	29.1	8.84	3	Nil	100	6	Nil	2	Nil	100	2	9	0.3	Nil
Fluoranthene	5.5	3.9	0.09	Nil	Nil	5.5	1.5	Nil	Nil	Nil	5.5	4.2	0.23	0.11	Nil
Pyrene	54	11	4	1	Nil	54	44	4	1.3	Nil	54	15	4.2	0.23	0.11
Benzo[a]anthracene	149	3.8	0.16	Nil	Nil	149	2.2	0.14	Nil	Nil	149	6.8	1.3	0.8	Nil
Chrysene	7.8	5	Nil	Nil	Nil	7.8	Nil	Nil	Nil	Nil	7.8	2.6	Nil	Nil	Nil
Benzo[b]fluoranthene	250	5.1	2.3	Nil	Nil	250	18	0.11	Nil	Nil	250	1	0.8	0.2	Nil
Benzo[k]fluoranthene	2	1.8	1.3	0.02	Nil	2	0.8	0.02	0.01	Nil	2	0.4	0.06	Nil	Nil
Benzo[a]pyrene	10.46	Nil	Nil	Nil	Nil	10.46	5.63	Nil	Nil	Nil	10.46	7.60	6.67	5.47	Nil
Dibenzo[a,h]anthracene	5.4	1	0.9	Nil	Nil	5.4	1.6	0.047	0.044	Nil	5.4	1.1	0.5	0.03	Nil
Benzo[g,h,i]perylene	1160	430	65	Nil	Nil	1160	380	9	Nil	Nil	1160	1025	Nil	Nil	Nil
Indeno[1,2,3-cd]pyrene	500	Nil	Nil	Nil	Nil	500	360	66.19	5	1	500	439	Nil	Nil	Nil
Total PAHs	2341.3	519.8	91.24	4.52	Nil	2341.3	842.46	87.68	9.11	1	2341.3	1506	1986	6.41	0.02

Each sediment sample has its own trend in bioremediation capabilities of the 16-PAHs in the three treating methods. The present study did not show any significant correlation between PAHs concentrations in the sediment samples and degradation ability of the augmented bacteria NSh37 and the enriched indigenous microbial populations in the biostimulation or bioaugmentation flasks. Biodegradation kinetics in PAH-contaminated environments is complicated by the possibility of substrate interactions. Impacts of PAHs on the rate of disappearance of other PAHs in sediments revealed that interactions may result from enzyme inductions that occur due to pre-exposure and adaptation of a culture to PAHs. The presence of different microbial communities could explain the different behavior of degrading the 16-PAHs. Interactions between microorganisms, including co-metabolism, synergism or antagonism, are significant in PAHs degradation and deserve more in-depth studies. Also the type of PAHs in the contaminated samples; with the type of soils, physical and chemical properties of the sediment all of these factors could explain the difference in behavior of PAHs biodegradation **(Guo et al., 2005& Sabaté et al., 2006 &Abbondanzi et al., 2006)**.

It is obvious from Fig.(35) that generally biostimulation expresses the highest rate in 16-PAHs removal followed by treatment with Staphylococcus *gallinarum* NSh37 while bioaugmentation shows the lowest efficiency in 16-PAHs removal which agrees with results obtained in TPH removal previously discussed. Similar observation was demonstrated by **Launen et al., 2002 and Sabaté et al., 2006**. Generally there is a continual removal of the PAHs up to the six week of incubation although there is a decrease in TCFU as listed in Table (19) starting from the fourth week of incubation in all treatments except for S22 and S23 treatments with NSh37 and bioaugmentation, where the TCFU decreased within the sixth week of incubation. Frequently, interactive effects such as inhibition and cometabolism are observed in addition to simultaneous utilization. Cometabolism consists of the transformation of nongrowth substrate by growing cells in the presence of a growth substrate, as well as transformation by resting cells in the absence of growth substrate and resting cells in the presence of an energy substrate.

Enhancement in the rate of utilization of one PAH in the presence of a growth substrate has been frequently observed and attributed to cometabolism **(Dean-Ross et al., 2002 & Xia et al., 2006)**. The degradation of HMwt. PAHs, especially those PAHs with more than 4-rings are always degraded by means of co-metabolism; LMwt. PAHs (di- and tri- rings) or other matrix components could serve as a co-metabolic substrate to enhance the biodegradation process of HMwt. PAHs **(Aitken et al., 2005 & Abbondanzi et al., 2006)**. This might explain the complete degradation of the HMwt. PAHs in all the treatment slurries throughout the six weeks of incubation, as shown in Tables (22, 23and 24), although the bacterial growth was inhibited as shown in Table (19).

The inhibition of growth and decrease of TPH degradation rate, could be due to the accumulation of toxic intermediates formed from the biodegradation of the HMwt. PAHs compounds since many PAHs intermediates affects the survival and activity of microorganisms **(Kazunga and Aitken, 2000)**.

In conclusion, bioremediation is a proven alternative treatment tool that can be used in certain-oil contaminated environments. Typically, it can be used as a polishing step after conventional mechanical clean up options have been applied.
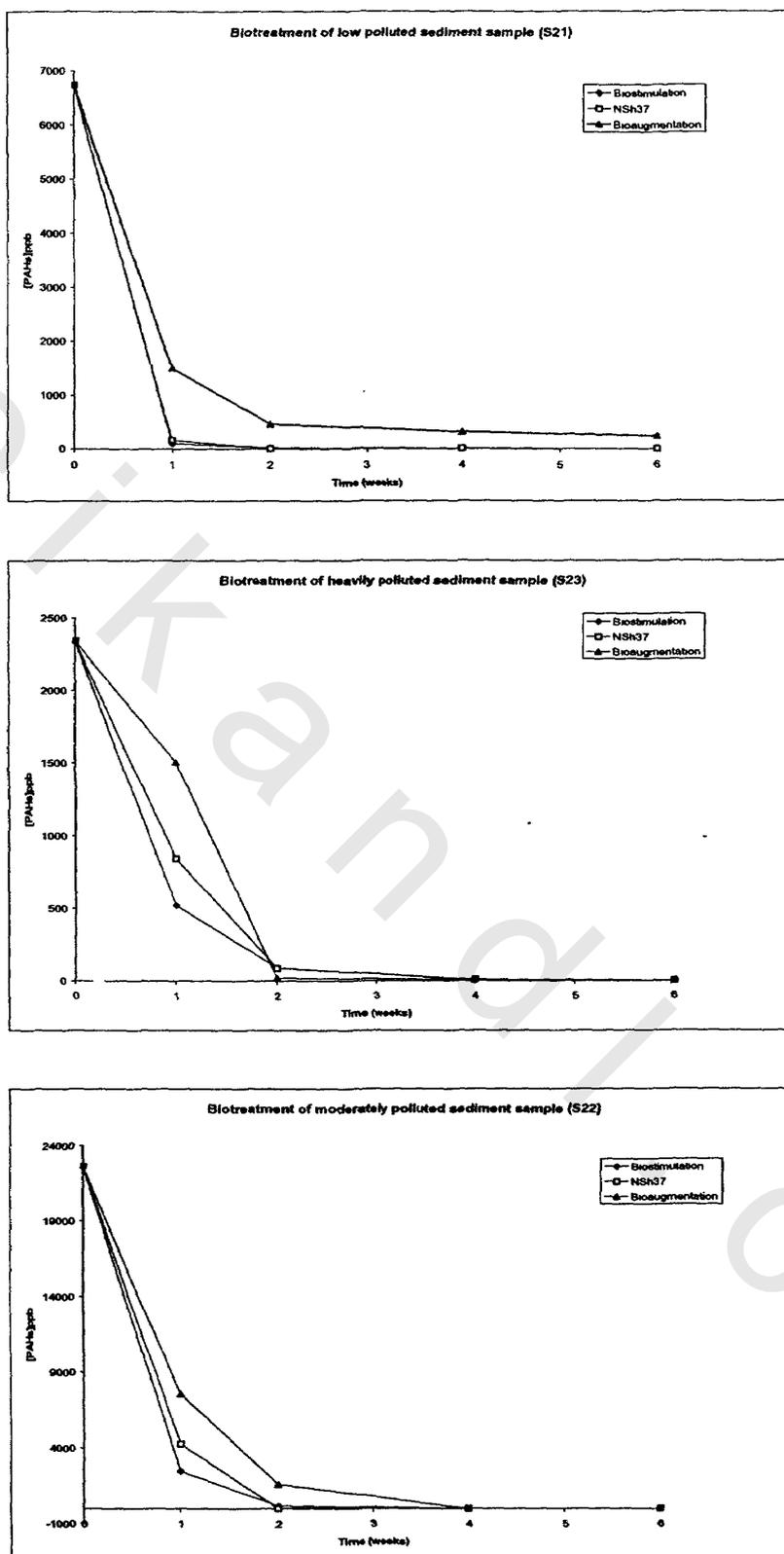
Fig.(35): Bioremoval of PAHs in contaminated sediments