

**A CHEMICAL STUDY OF POLLUTED SOIL AND SALSOLA
PLANT FROM THE OIL - IMPACTED AL-JAIDAN DESERT
AREA OF KUWAIT**

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ABSTRACT

Different polluted soil and plant samples were collected from the oil impacted, (Al-Jaidan) October 1993 and April 1994 and Chemically analysed. The levels of saturated aliphatic, aromatic hydrocarbons, resin and asphaltine in polluted soil and plant (Salsola sp.) were determined by column chromatography.

It was found by using G.C. technique that there was still a detectable amount of aliphatic hydrocarbons in both polluted soil and plants after 36 months of spills.

INTRODUCTION

As a result of violent Iraqi invasion of the state of Kuwait on the second of August 1990 and firing more than seven hundred oil wells; millions of tons of crude oil were spilled in the surrounding areas forming a lot of oil lakes. It was an environmental crisis. Trials could be made to remediate the oil impacted soil as mechanical, chemical or biological methods can be used.

The rate of biodegradation of hydrocarbons in soil can be discussed as a function of three variables; the availability of chemicals to the microorganism that can degrade them, the quantity of these microorganism and the activity of these organisms. Moreover factors such as the soil content of hydrocarbons and clay, moisture level, temperature, pH, aeration and nutrient conditions are of importance as moderators and driving factors (Bartha 1986 and Torstenson 1988).

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Xlaoping Wang (1990) measured and identified polycyclic aromatic hydrocarbon component in a soil contaminated by diesel oil using gas chromatographic analysis. Hydrocarbon residue were also measured quantitatively and correlated in loam soil contaminated by jet fuel spills by Hong-Gyu, Song and Bartha (1990), using gas chromatographic technique. However the possibility of using microorganism for the degradation of oil, and its derivatives for minimizing the pollution due to oil lakes and spills (Walker *et al.* 1992) had promoted us to impliment a comprehensive chemical study of different polluted oil samples from the Kuwaiti impacted area "AL-Jaidan".

This study was undertaken to have an information on the oil pollutants and its derivatives in the soil and plants and to assess the feasibility of analyzing the obtained soil samples.

MATERIALS AND METHODS

Collection of samples and treatment :

The samples of soil collected from the impacted area of Kuwait "Al-Jaidan" at different distances from the plant and at different depths from the surface. The surface crust was removed firstly then samples were taken. The soil was sieved through 2mm - diameter sieves. Control soil and plants "*Solsola sp.*" were taken from the unimpacted area at Al-Ahmady. The roots, stems and leaves of plant were washed with a mixture of water and chloroform then air dried, and crushed in a porcelain mortar.

Analytical Methods :

1. Determination of water content (%) in the collected samples:

Exact weight of the collected soil samples (after sieving) was

put in a drying oven at 104°C overnight. The sample was weighed again after drying in a desiccator (CaCl₂) for two hours, then the % (w/w) of water content was calculated.

2. Chemical analysis of aliphatic and aromatic hydrocarbons in soil and plant :

To determine the quantity of hydrocarbons in the polluted soil and plant samples a method which was described by Oudot (1984) and Diab, (1991) was followed.

a. Soxhlet Extractoin :

An exact weight of the impacted soil (\approx 20g) was ground with about 30 g. of sodium sulphate - that has been heated to 400°C for 12 hours - then put in a thimble. The whole soxhlet extractor was weighed. A solvent of 200 ml. chloroform was used. This thimble was dried and weighed to know the loss in the sample due to extraction. The extract was poured in a pre - weighed beaker then left for evaporation until dryness to constant weight. The oil extract was dissolved in n-hexane. The insoluble residue was weighed. Then n-hexane soluble part of the oil extract was evaporated and dissolved in a least amount of n-hexane.

b. Column Chromatography separation :

Silica Gel (ICN silica 100 - 200) was heated at 130°C overnight. After cooling silica gel was mixed with n-hexane then poured into a glass column (15 cm long, 1.7 cm width) and eluted with additional 60 ml of n-hexane.

The sample which was dissolved in n-hexane (a) was placed at the head column and eluted successively with :

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(a) 60 ml of n-hexane.

(b) 60 ml of benzene.

(c) 60 ml of methanol.

The eluted fraction was collected in 100 ml previously weighed beakers.

The first n-hexane fraction contains aliphatic hydrocarbons (A.H.). The second one contains aromatic hydrocarbons (Ar. H) and the methanol fraction contains resins. The eluted fraction was concentrated by evaporation then analyzed by gas chromatography. Duplicates of experiments was made for each sample.

c. Gas Chromatography :

Gas chromatographic technique was used to analyse the oil residual fraction in order to identify the types of saturated aliphatic hydrocarbons in the collected soil and plant samples.

The samples were analysed with a SHIMADZU Gas chromatograph GC - 14B.

The experimental condition was as follows :

Column 15 m X 0.25 mm i.d. film thickness 0.25 U db-5 (J & W Scientific).

Temperature 5°C for 1 min.

Program 5 - 300°C at 8 min.

 300°C for 10 min.

Source temperature 200°C

GC interface 280°C

The same experiments (a,b and c) were done on :

- Control soil sample
- Roots of plant (*Salsola sp.*), from the impacted area and leaves.
- Stems of plants (*Salsola sp.*), from the impacted area.
- Control plant "*Salsola sp.*", from an unpollutant area.

The obtained data is depicted in Tables 3 (a,b).

3. Determination of the pH, CO_3^{2-} , HCO_3^- , Cl^- , Ca^{2+} Mg^{2+} in different collected samples of the soil.

The levels of bicarbonate, carbonate, chloride, calcium, magnesium as well as the pH in different collected soil samples were determined in laboratory of the Public Authority for Agriculture Affairs and Fish Resources.

Chemical characterization of the soil :

(a) Moisture content of the soil (% Wt) :

Table (1) illustrates the % (Wt) of the moisture of the collected soil samples. Moisture content of the soil which was collected in April 1994 was more than that in October 1993. The polluted soil samples (E1S, E3S)* can hold relatively more moisture than that of the unpolluted soil samples.

* E1S : Soil sample (10/93) E1 Stem : Stem sample (10/93)
E3S : Soil sample (4/94) E1L : Leaf Sample (10/93)
E1R : Root sample (10/93) E1S1 : Soil sample (10/93) No. 1.
E3R : Root sample (4/94) E1S2 : Soil sample (10/93) No. 2.

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Table (1) : Percentage of the moisture content (wt) for the collected soil samples :

Soil samples	% (Wt) of moisture	Date of collection
Polluted samples ;		
* E1S1 : 20 cm depth 20 cm off root	0.493	10 / 93
* E1S2 : 20 cm depth colse to the root	0.313	10 / 93
* E3S : 20 cm depth 20 cm off root	1.950	4 / 94
Unpolluted samples :		
* E1S : (control) 20 cm depth 20 cm off root (Al-Ahmady)	0.231	10 / 93
* E3S : (control) 20 cm depth 20 cm off root	1.372	4 / 94

(b) Determination of pH, CO_3^{2-} , HCO_3^- , Ca^{2+} , Mg^{2+} ,

Table 2 shows the concentration of CO_3^{2-} , HCO_3^- , Cl^- , Ca^{2+} , Mg^{2+} (mg/l) as well as % (Wt) of the total nitrogen and pH of the collected soil samples.

The pH of the polluted soil samples ranged between 7.6 - 8.62, indicating that was an alkaline soil. The higher concentration of Ca^{2+} and Mg^{2+} cation can be observed.

Determination of Residual Oil and its fraction in the collected soil and plant samples :

Table 3 (a & b) represents % (Wt) of saturated, aromatic hydrocarbons, resin and asphaltine referred to the soil and plant samples as well as the % (Wt) of the residual oil to the weight of soil or plant.

Results of analysis of the residual oil fraction extracted from the impacted soil samples collected in October 1994 show that the samples E1S2 which was collected close to the root of the plant (rhizosphere) and 20 cm depth from the surface, by removing the crust surface, had less saturated aliphatic hydrocarbons (6.03) than that of E1S1 (7.15). However, E1S2 has more residual oil (0.81%) than E1S1 (0.64). This may be attributed to the secretion of plant root which has higher relative moisture around it. The crust surface sample has more asphaltine than the samples E1S1 and E1S2 because the aliphatic and aromatic hydrocarbons are much more volatile. Moreover, the crust surface samples, has much more residual oil. The residual oil analysis for the sample, E3S, collected in April 1994, indicates that it has nearly no aliphatic and aromatic hydrocarbons but it has more resin (methanol fraction) (17.28%) and more asphaltine. However, the polluted soil samples, as a whole, contain a very small

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Table (3 a) : Percentage of saturates, aromatics, resin and asphaltine (Wt) being in the soil relative to the amount of residual oil and soil.

Soil samples	Fraction	%(Wt / g oil Residue)	%(oil Residue /g soil
Polluted soil :			
EIS1 :	(A) Saturates	7.15	0.46
20 cm depth	(B) Aromatics	11.09	
20 cm off root	(C) Resin	27.09	
	(D) Asphaltine	53.81	
EIS2 :	(A) Saturates	6.03	0.81
20 cm depth	(B) Aromatics	10.92	
colse to the root	(C) Resin	30.21	
(Rhizosphere)	(D) Asphaltine	45.80	
EIS :	(A) Saturates	0.02	88.60
Crust surface	(B) Aromatics	0.23	
	(C) Resin	40.62	
	(D) Asphaltine	59.13	
E3S :	(A) Saturates	0.005	0.10
20 cm depth	(B) Aromatics	2.77	
20 cm off the root	(C) Resin	17.28	
	(D) Asphaltine	78.25	
Non polluted soil :			
E1S : (control)	(A) Saturates	18.80	0.071
20 cm depth,	(B) Aromatics	23.80	
20 cm off root)	(C) Resin	23.56	
(Al-Ahmady)	(D) Asphaltine	74.48	
* E3S : (control)	(A) Saturates	15.21	0.032
20 cm depth	(B) Aromatics	19.32	
20 cm off root	(C) Resin	34.12	
(Al-Ahmady)	(D) Asphaltine	31.45	

Table (3 b) : Percentage of saturates, aromatics, resin and asphaltine (Wt) found in plant relative to the amount of residual oil and weight of plant..

Parts of the plant	Fraction	% (Wt / g oil Residue)	% (oil Residue /g. soil
Polluted samples :			
E1R :	(A) Saturates	0.85	0.72
(Root)	(B) Aromatics	0.33	
(Washed with wter and dried by air)	(C) Resin	3.32	
	Residue	94.62	
E3R :			
(Root)	(A) Saturates	0.087	0.69
(Water wash. and air dried)	(B) Aromatics	13.90	
	(C) Resin	24.00	
	Residue	61.02	
E3R :			
(Root)	(A) Saturates	0.066	0.13
(Washed with water and CHCl ₃ and dried by air)	(B) Aromatics	12.82	
	(C) Resin	26.42	
	Residue	60.00	
E3 stem :			
(Washed with water and dried by air)	(A) Saturates	7.65	0.36
	(B) Aromatics	19.50	
	(C) Resin	46.17	
	Residue	72.12	
E3 stem :			
(Washed with water and CHCl ₃ and then dried by air)	(A) Saturates	19.44	0.31
	(B) Aromatics	22.50	
	(C) Resin	38.62	
	Residue	39.41	

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Table (3 b) : Continued

Parts of the plant	Fraction	% (Wt / g oil Residue)	%. (oil Residue /g. soil
EIL :			
(Leaves)	(A) Saturates	0.085	3.94
Washed with wter	(B) Aromatics	0.74	
	(C) Resin	4.40	
	Residue	94.86	
Non polluted part of the plant			
E3R : (Control)	(A) Saturates	11.60	0.37
(Al-Ahmady)	(B) Aromatics	12.95	
(Washed with water only. air dried))	(C) Resin	45.85	
	Residue	29.20	
EZR : (control	(A) Saturates	10.62	0.31
Al-Ahmady	(B) Aromatics	12.21	
(Washed with water and CHCl ₃ , ir and dried	(C) Resin	45.63	
	Residue	31.44	
EIS	Soil sample. (10/93)		
E3S	Soil sample. (4/94)		
EIR	Root sample. (10/93)		
E3R	Root sample. (4/94)		
EI Stem	Stem sample. (10/93)		
EIL	Leaf sample. (10/93)		

amount of oil (0.1%), but it has ten times more than the control soil.

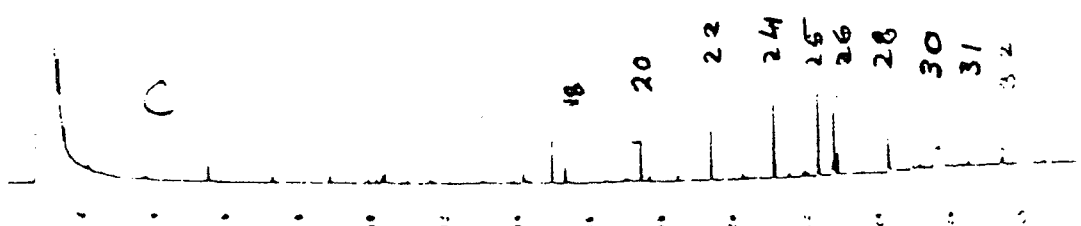
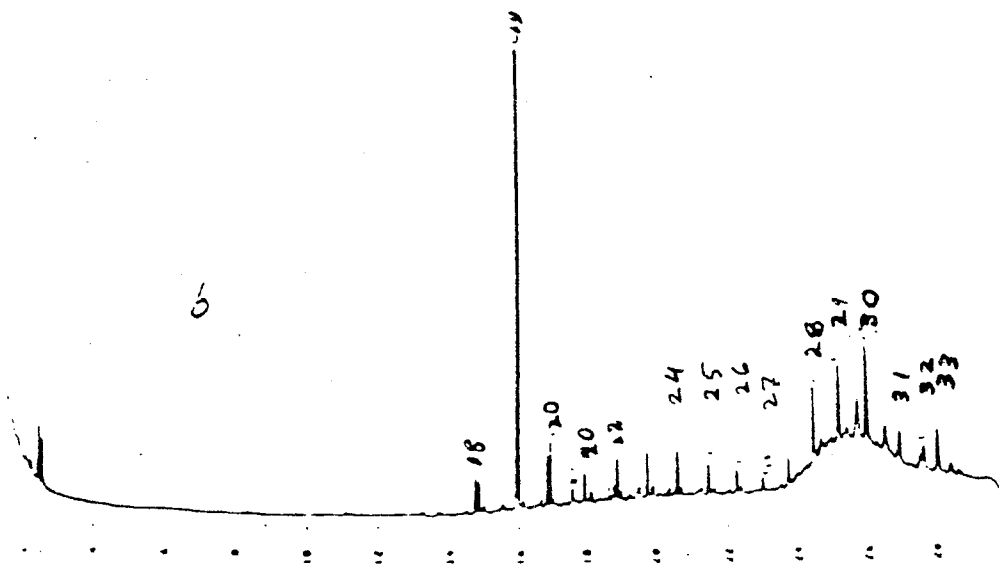
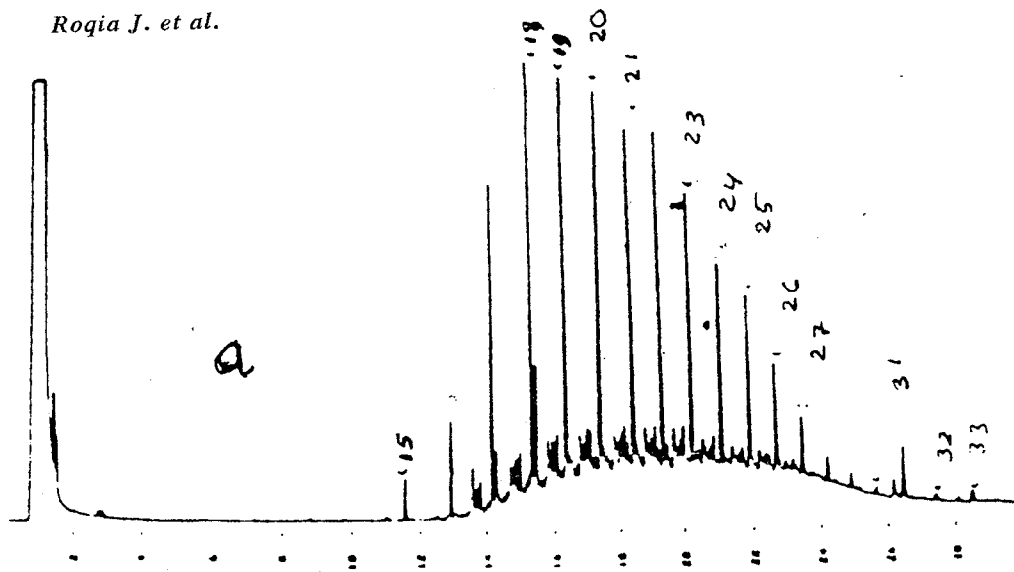
Al-Gonaim *et al.* (1992) measured the residual oil in three soil samples from the impacted Kuwait desert area after ten months of spills and found that there was a significant loss in saturated hydrocarbons, while the aromatics were more degraded. Now, after 36 months of spills the samples, however, contains very little amount of aliphatic hydrocarbons, but having more resin and asphaltine.

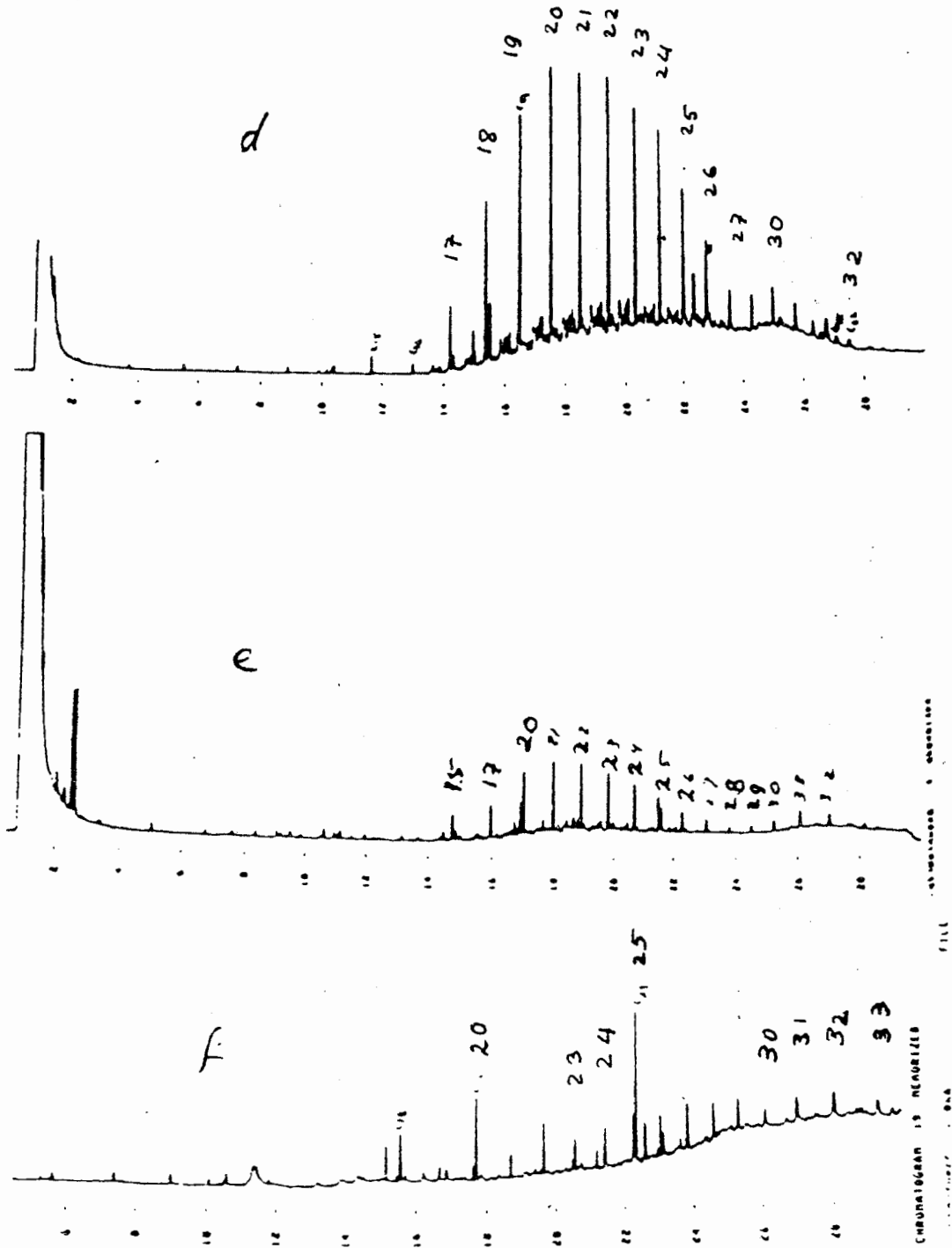
Regarding the results of analysis of the contaminated parts of plants which were collected from the oil impacted area (*Salsola sp*) in October 1993 and April 1994, indicated that traces of aliphatic hydrocarbons, which may be dependent on the way of preparing parts of plants before extraction, the amount of extracted residual oil from roots, leaves and stems in October 1993 was slightly greater (or nearly the same) than that from parts of plants which were collected in April 1994. This can be attributed to the fact that the plant can get rid of these hydrocarbons very slowly which can be translocated through the plant tissue.

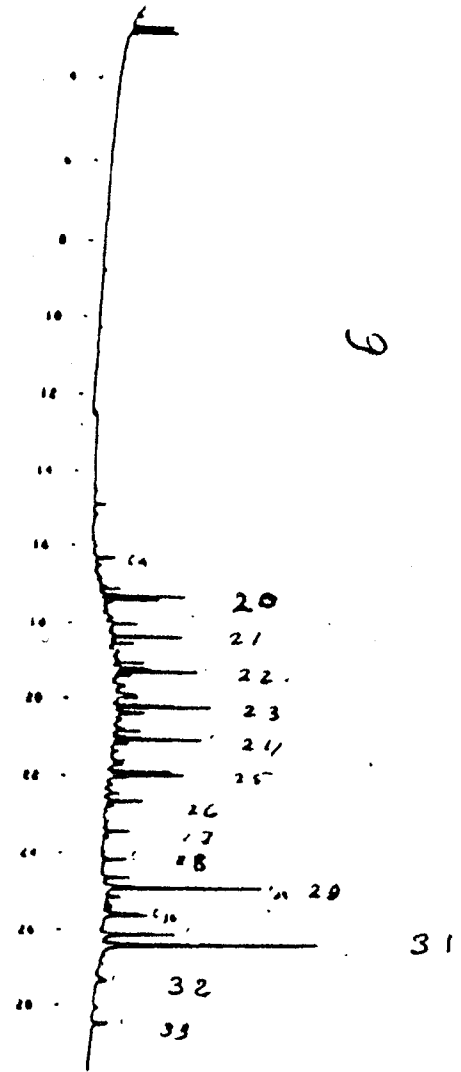
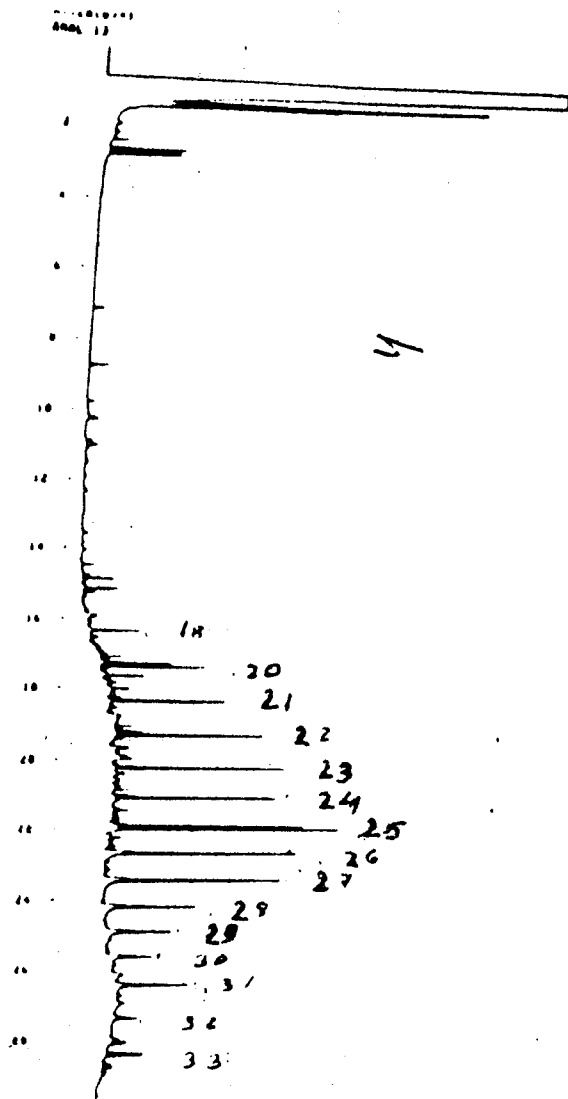
Gas Chromatographic Analysis of Oil Residual Fractions :

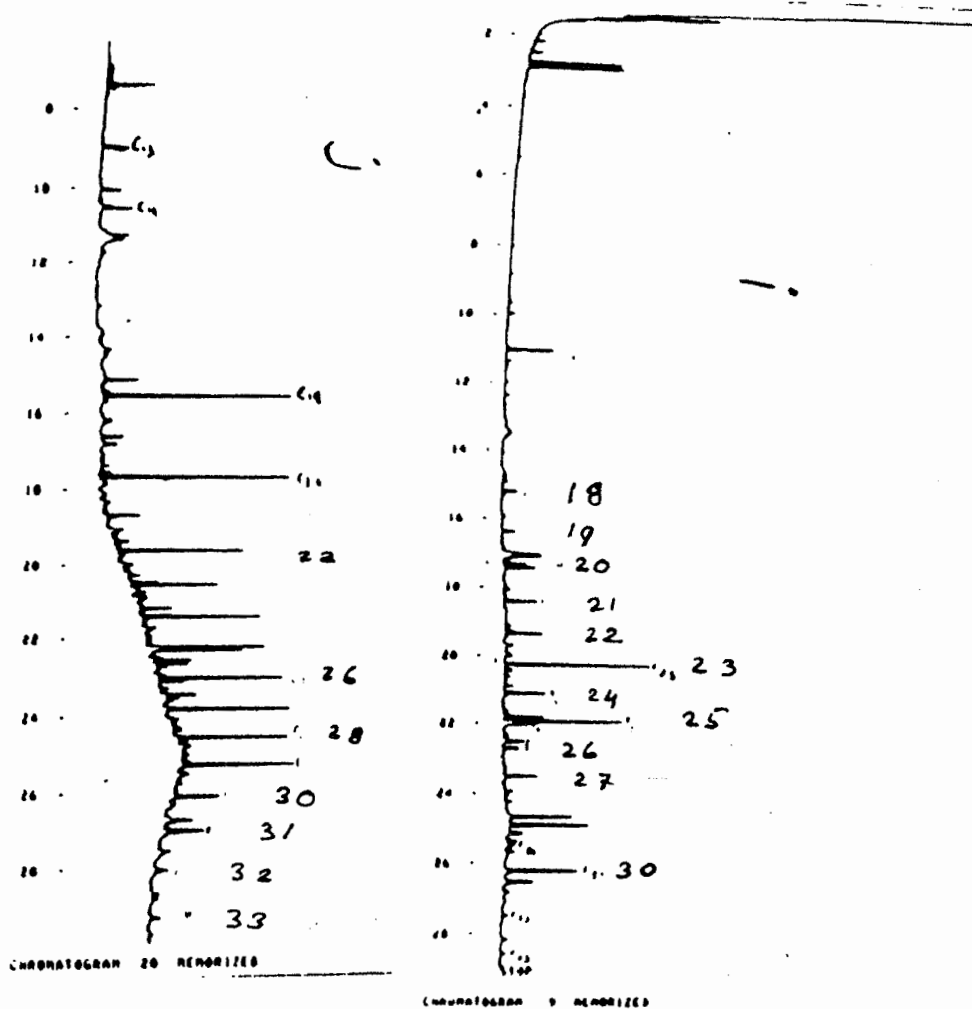
The results are given Figs. 1 (a - 1) comparing Fig. 1 (b), for the soil sample which was collected from the polluted area in October 1993, 20 cm and 20 cm depth under the *Salsola sp.* plant, with that of Fig. 1 (a) (KOC - blend : contains aliphatic n-alkanes (C 12 - C34) number of branched alkane was used as a control sample, and that of the control sample (EIS control), one can deduce that, different saturated aliphatic hydrocarbons (C18, C33), still present in the soil with a relatively low concentrations after nearly 36 months from the date of spill. When a soil sample (E3S) was collected from the same impacted area in April 1994 and analysed [Fig. 1(c)], low concentrations of saturates were still present.

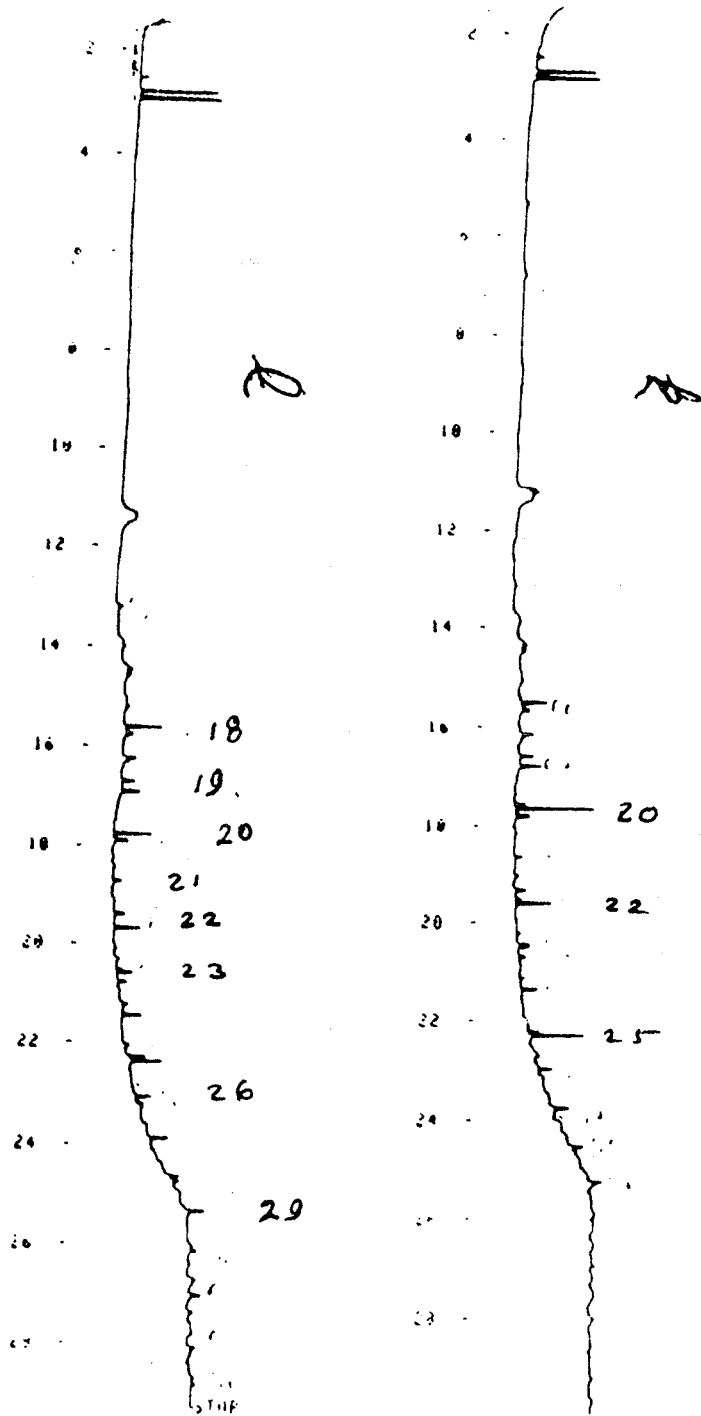
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The disappearance or the low of concentration of saturates can be attributed to the effect of oil degrading bacteria present in the soil (Bartha 1986; Bergay's 1989, Atlas 1981 and the references cited therein). Figures 1 (F - 1) illustrate the Gas chromatographic analysis obtained for roots, stems and leaves of "*Salsola sp.*" plant collected from polluted and unpolluted area (AL-Jaidan). It can be deduced, from figure 1 (g, j, k, l) that, there are a detectable amount of saturates being in the roots of the plant which were collected from the polluted area. The quantity of saturates of the samples collected in April 1994, differ slightly from that collected at October 1993. Also a very small amount of saturates are found in stems and leaves of "*Salsola sp.*" plant.

However the same types of saturates are found in roots, stems and leaves of the plant, but roots and stems can hold more saturates than leave [Fig. 1 (h, l)]. The oil loss during the period from 10/93 to 4/94 could be attributed to the evaporation. Photodecomposition, adsorption, percolation and biodegradation, although these processes are known to occur, there exists a generally accepted belief that microbial decomposition is the primary cause of reduction in oil concentration.

However GAS chromatograms of the contaminated (collected) samples contain n-alkanes and branched alkanes, C18 to C339 (Fig. 1), which means that n-alkanes of low carbon atom (less than 18) was removed by biodegradation (Walker and Colwell 1976). Moreover, it can be noticed from, Fig. 1, for the oil contaminated samples, that the n-alkanes C24 to C31, have not disappeared or having low concentration than that of KOC - Blend control oil sample. This may be explained as the microorganism which are there in the samples (soil and rhizosphere) are not able to degrade such hydrocarbons or there is a type of polymerisation taking place during that period of time (36 months).

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REFERENCES

- Al-Gonaim, M.Y., Diab, A. and El-Shinawi, K. (1992) : Effect of Oil spilled in desert of Kuwait and Microbial Flora of Soil : Proceeding of the third world Academy of Sciences. Fourth General Conference, Kuwait, 23 - 26 - Nov.
- Atlas, R.M., (1981) : Microbial degradation of Petroleum Hydrocarbons. An Environmental Prospective. *Microbial Rev.* 45 I 180 - 209.
- Diab, A., (1991) : Biodegradation of Petroleum Oil by Microbial Population in the Desert of Soil of Kuwait; *Az. J. Microbiol.* 11, 118 - 138.
- Bartha, R. (1986) Biotechnology of Petroleum Pollutant Biodegradation. *Microb. Ecol.* 12 : 155 - 127.
- Bergays, Manual of Systematic Bacteriology (1984), Vol. 4 (S.T. Williams ed.), Boord : Murry, Brenner, Holt. Krieg, Moulder, Piening, Sneath, Stantly, and S.T. Williams.
- Hong-Gyu Song and Bartha, R. (1990) : Applied and Environmental Microbiology, 464 - 651.
- Oudot, J. (1984) : Rates of Microbial degradation of Petroleum Components as determined by Computerized Capillary Gas Chromatography and Computerized mas Spectrometry; *Mar. Environ. Rev.* 277 - 302.
- Torstenson L. (1988) : Microbial decomposition of Herbicides in the Soil Outlook in Agriculture 17, 120 - 4 and the references cited in.
- Walker J.D. and R.R. Colwell, (1976) : Long - Chain - alkanes occuring during Microbial degradation of Petroleum, *can. J. Microbiol.* 22; 886 - 891.
- Xlaoping Wang, Xlaoping Yu and Richard Bortha (1990) : Effect of Biodegradation on Polycyclic Aromatic Hydrocarbon Residues *Environ. Sci. Techn.* Vol. 24, No. 7, 1087.

Figure 1. Gas Chromatogram of saturate

Fractions (n-hexane fraction) for :

- a. KOC - Blend (control oil sample).
- b. Polluted oil sample (E1S), depth = 20 cm, 20 cm off plant, (10 - 93)
- c. Polluted soil (E3S), depth = 20 cm, 20 cm, off plant, (4 - 94).
- d. Polluted soil with surface crust (E2S), 20 cm of plant (2.94).
- e. Unpolluted soil, Al-Ahmady, 20 cm depth, 20 cm away off plant (E1S control).
- f. Unpolluted soil, Al-Ahmady, 20 cm, depth, plant, (E3S control), (4.94).
- g. Root of *Salsola sp.* from polluted area (10 - 93).
- h. Stem of *Salsola sp.* from polluted area (10 - 93).
- i. Leaves of *Salsola sp.* from polluted area (10 - 93).
- j. Root of *Salsola sp.* from polluted area (4 - 94).
- k. Root of *Salsola sp.* from unpolluted area (Al-Ahmady), washed with water and then dried by air, (4 - 49).
- l. Root of *Salsola sp.* from unpolluted area (Al- Ahmady), Washed by water and chloroform, 4 - 94.