

## **Effects of Petroleum Oil Pollution on the Microbiological Populations of the Desert Soil of Kuwait**

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**ABSTRACT.** During the invasion of Kuwait, Iraqi invaders destroyed oil wells. As a result 60-80 million barrels of crude oil contaminated about 49 Km<sup>2</sup> in the desert of Kuwait. The residual oil in a polluted areas in the desert of Kuwait was measured during the years 1992, 1993 and 1994. Results of the analysis of the residual oil show that the saturates fraction decreased from 23.4% in the first year to 11.9% in the second year, and nearly disappeared in the third year. The aromatic and the resin fractions decreased then increased again, while the asphaltene fractions increased and accumulated during the last two years.

The residual n-alkanes and iso-alkanes of the saturates fraction were followed by gas chromatography. During the first year less quantities were removed during the second year. On the other hand most of the n-alkanes (except traces of some components) were removed during the last year. It was observed that high molecular weight n-alkane (C<sub>31</sub>) was accumulated in the soil samples collected during this year.

Oil-degrading bacteria showed positive response to the exposure to oil pollutants, their ratios of the counts of the polluted samples (SP) to that of control samples (SC) ranged from 3.7-50. In contrast the SP/SC ratios of the total bacteria, actinomycetes, cellulose decomposers and thermophiles were in the range of 0.03-4.2. Fungi were of positive response to oil pollutants than bacteria, their SP/SC ratios ranged from 4.7-11.1.

The microbial mixture activity of each soil sample to biodegrade petroleum oil and its fractions was studied. It was found that biodegradation activity of the microbial population of the polluted soil samples decreased with decreasing the residual oil pollutants. In soil samples of 3.9% residual oil, 37.5% crude oil, 51.2% of the saturates

and 17.4% of the aromatics were biodegraded. When the residual oil was 0.1% in the last year, 17.0% of the added crude oil and 20.3% of its saturates fraction were biodegraded, while none of its aromatic fraction was biodegraded. As a comparison, microbial population of the control soil samples showed less biodegradation activity.

The utilization of n-alkanes of the saturates fraction was followed by gas chromatography. Kuwait crude oil (KOC Blend) contains n-alkanes C<sub>12</sub>-C<sub>34</sub> as well as a number of iso-alkanes. Weathering was found to remove C<sub>12</sub>-C<sub>13</sub>, most of C<sub>14</sub> and slight reduction in the other components.

The pattern of n-alkanes utilization varied according to the structure of the microbial community and the amount of residual oil present in each soil sample. Microbial population present in polluted soil samples collected during 1992 and 1993 resulted in the removal of all n-alkanes except traces of some components. Microbial populations of the soil samples collected during the last year, removed large quantities of such components. On the other hand microbial communities found in the control soil samples also succeeded in removing large quantities of n-alkanes, but slightless than those of the polluted soil samples.

A total of 92 fungal isolates (62 from the polluted samples and 30 from the control samples) were identified. *Aspergillus* spp and *Stemphyllium* spp were sensitive to oil pollution.

*Aspergillus* spp, the dominant fungus in the polluted samples was identified as *Aspergillus terreus*, it was selected and studied for its biodegradation activities. The results show that this fungal species was able to utilize in 21 day 30% of the crude oil, 31.8% of the saturates fraction and 13.8% of the aromatic fraction. It succeeded to utilize most of the components of the n-alkanes.

A total of 90 bacterial isolates were isolated from colonies appeared on oil- silica gel plates. All strains from soil samples collected during 1994 and of control soil samples were *Arthrobacter* spp. *Pseudomonas* was more frequent (80%) in soil samples of 3.7% pollutants. Six isolates, two *Pseudomonas* spp and four *Arthrobacter* spp representing cultures showing very good growth on media containing petroleum as the only carbon source, were selected and studied for their abilities to degrade crude petroleum and its fractions. *Pseudomonas* spp showed biodegradation activity more than the *Arthrobacter* spp. They degraded 11.2-16.8% of the crude oil, 26% of the saturates and 5.6-6.8% of the aromatics.

On the other hand *Arthrobacter* spp isolated from the polluted soil samples showed more activities than *Arthrobacter* spp of the control soil samples. Three strains of *Arthrobacter* were able to degrade 1.3-4.6% of the resin fraction.

Results of the utilization of n-alkanes by the above bacterial strains show that biodegradation accomplished by *Pseudomonas* spp resulted in the removal of most the n-alkanes. On the other hand *Arthrobacter* spp showed less activities than the *Pseudomonas*. *Arthrobacter* spp (1 & 2) isolated from the polluted soil samples were found to remove more n-alkanes than those isolated from the control soil samples. The appearance of an increase of n-alkanes C30 was noted during the growth of *Pseudomonas* spp and *Arthrobacter* spp.

Petroleum hydrocarbons that contaminate an ecosystem, affect the size and composition of the indigenous microbial community. The most widely documented response of microbial community to exposure to petroleum oil is a rapid increase in the size of hydrocarbon utilizing components of the community. Odu (1972) found that in sandy soil, oil contamination resulted in changes in the physical and microbial population of the soil. In heavily polluted sandy soil, a considerable increase in carbon/nitrogen and microbial numbers were obtained.

Jensen (1975) found that a strong increase in bacterial numbers occurred after oil application and this increase occurred within all groups of bacteria except spore formers and streptomycetes. The number of bacteria increased with increasing oil concentration up to a concentration of 4-5%. At higher concentrations, the results showed some variation, but no regular trend. Oudot *et al.* (1987) found that three years after the contamination of an agricultural soil with a fuel spill, biodegradation of the fuel in the soil was achieved, and after five years the residual compounds had no significant effect on the density and specific composition of the microbial populations of the soil. Hydrocarbon oxidizing *Corynebacterium* and *Nocardia* spp. were isolated. Most of the active fungi were *Aspergillus* spp., *Penicillium* spp and strains of *Paecilomyces*, *Acromonium*, *Fusarium* and *Gleocladium*.

Iraqi invaders during their invasion to Kuwait, destroyed petroleum oil wells and as a result, 60-80 million barrels oil gushed from the wells to cover an area of about 49 km<sup>2</sup> in the desert of Kuwait, and more than 70 oil lakes were formed. About one third of the soil in this area was contaminated with 5-20% of the oil.

Remediation of these contaminated soils is needed. The first step in soil remediation is to study the potential impacts of oil pollutants in soils, and this includes measurements of either the effect on the integrated response of the community metabolic activity or the response of specific segments of the community such as cellulose degraders or various specific taxonomic groups (Buckley 1980). Accordingly, the aim of the present work is to study the effect of the spilled petroleum oil on the size and composition of some groups of the microbial community in the untreated polluted soils, and to throw a light on oil biodegradation activity of the microbial content of these soils.

## Materials and Methods

### *Collection of Soil Samples*

Soil samples were collected under aseptic condition from a polluted area in the desert of Kuwait. Control soil samples were collected from a non polluted site. Each sample was a mixture of at least three samples collected from different spots in the same area. Samples were collected from a 5-15 cm depth during April, 1992, 1993 and 1994.

### *Determination of the Residual Oil and its Fractions*

For determination of the residual oil, 25 g. air dried soil was shaken twice with 200 ml chloroform. The extracts were pooled, dried over anhydrous sodium sulphate and evaporated. The residue in each soil sample was calculated as per 100 g soil.

A known weight of the residual oil was suspended in n-hexane and filtered through tared filter paper to remove and to determine the insoluble fraction (asphaltene). The hexane soluble fraction was fractionated into saturates, aromatics and resins by liquid solid chromatography as described by Oudot (1984). The saturates fraction was further resolved by gas chromatography for the separation of the different components of n-alkanes.

### *Microbiological Methods*

Total viable bacteria, actinomycetes, fungi, cellulose decomposers and thermophiles were counted using the usual dilution plate method. Nutrient agar (Difco) supplemented with 0.1% yeast extract and 0.4% soluble starch was used for counting total bacteria and thermophiles. For counting actinomycetes and fungi, inorganic salts starch agar (ISP 4) and malt extract agar were used respectively. Cellulose decomposers were counted as described by Diab and Al-Gounaim (1984). Incubation periods were 5-7 days at 30°C for total bacteria, actinomycetes and fungi, 15 days at 30°C for cellulose decomposers and 2-3 days at 50°C for thermophiles.

For counting oil-degrading bacteria, 50 gm of soil sample was suspended in 100 ml distilled water and shaken for 15 minutes. Ten ml. of the supernatant was filtered on a sterile filter membrane (0.4  $\mu$ m). The membrane was removed and located on a plate of oil-silica gel medium containing crude oil as the only carbon source, and prepared as described by Walker and Colwell (1976). Four plates were used for each sample. The plates were incubated at 30°C for a period of 21 days after which the developed colonies were counted and expressed as counts/g air-dried soil (free from oil). All colonies developed were isolated, purified and subcultured on a suitable medium. Identification of bacteria was carried out using the methods recommended

in *Bergey's Manual of Systematic Bacteriology Vol. 1, 2* (1984, 1986) and *Bergeys Manual of Determinative Bacteriology* (1994).

#### ***Determination of Oil Biodegradation Capacity of the Soil Samples***

To evaluate the oil biodegradation capacity of the microbial mixture found in each soil samples, 50 g soil was suspended in 100 ml sterile distilled water and shaken for 5 minutes. Ten milliliters of the suspension was introduced into a 250 ml conical flask containing 50 ml of the medium used by Fedorak and Westlake (1981), and supplemented with 0.5% w/v of crude petroleum oil (KOC Blend). The oil was sterilized by passing through a 0.45  $\mu$ m membrane. At least six flasks were used for each soil sample, three for the evaluation of the biodegradation capacity and the other three for the evaluation of non-biological factors (each flask was poisoned by the addition of 0.5%  $\text{HgCl}_2$  solution). All flasks were incubated for 21 days at 30°C on a rotary shaker operated at 100 rpm. The same methods were used for the control soil samples.

At the end of the incubation period the residual oil was recovered using the method described by Oudot (1984). The recovered oil was fractionated into saturates, aromatics, resin and asphaltenes by the methods described before. The loss of oil and its fractions during the incubation period can be determined. The saturates fraction in each sample was resolved by gas chromatography to determine the loss of each component of the n-alkanes.

#### ***Selection of Active Oil Degrading Microorganisms***

One milliliter of the bacterial suspension ( $10^6$  cells) of each organism was introduced into 100 ml conical flask containing 20 ml of the same medium used before and overlaid by 0.5% (w/v) sterilized crude oil. The flasks were incubated at 30°C on a rotary shaker operated at 100 rpm for a period of 21 days. Biodegradation activity was indicated by measuring the absorption due to optical density of the turbidity in the liquid phase of the culture at 450 nm. Cultures showing 1-10 in the absorption scale were considered active strains and given (++) , those of 11-20 absorption were moderately active (+) and other strains of (21-30) were considered weakly active ( $\pm$ ). Active strains were selected and studied for the biodegradation of oil and its fractions by the same methods described before.

During the study of the fungal flora of each soil sample, *Aspergillus* sp II was recorded as a dominant species in the polluted samples. It was selected, and studied for the biodegradation of oil and its fractions by the same methods used for bacteria.

### Results and Discussion

The area from which the soil samples were collected represents a sandy soil type with pH 8.1-8.6 for the control samples, and 7.6-7.8 for the polluted samples. The soil samples studied were untreated, deficient in nitrogen and phosphorous content.

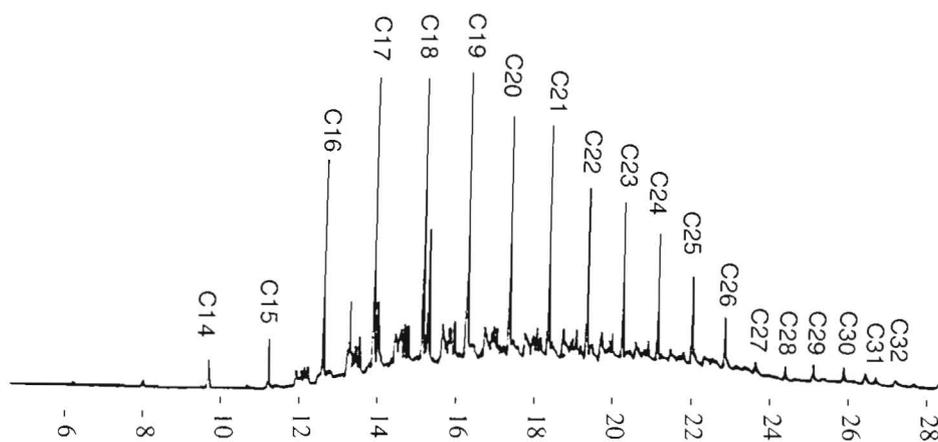
Results of chloroform extractable materials (Table 1) show that soil samples collected during 1992 contained 3.9% residue, while sample collected during the years 1993, 1994 contained 0.7% and 0.1% respectively.

Results of the analysis of the residual oil into its fractions (Table 1) show that the saturates fraction decreased from 32.4% (g/100 g residue) in the first year to 11.9% in the second year, and 0.01% in the third year. On the other hand the aromatic fraction was 25.4% (g/100 g residue) in the first year, then decreased to 7.5% in the second year and increased again in the third year (this increase was due to the disappearance of the saturates fraction). The same trend was observed for the resin fraction. Asphaltene fraction, on the other hand, increased and accumulated during the last two years (66.8% - 55.0%).

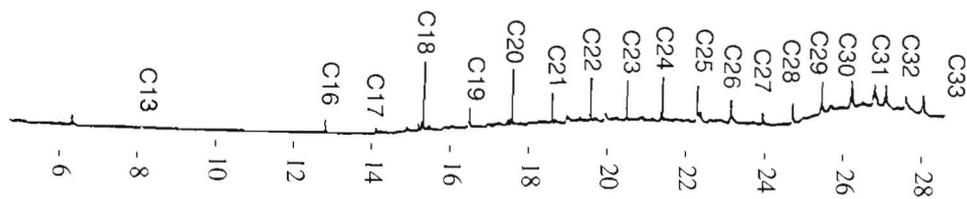
**Table 1.** Residual contents (g/100g soil) of petroleum oil and its fractions (g/100g) residue) in different soil samples collected during 1992, 1993 and 1994. From each polluted soil sample 200 mg of the residual oil was fractionated into saturates, aromatics, resins and asphaltene fractions

Residual oil and its fractions	Residual Content / 100g		
	1992	1993	1994
Oil	3.90	0.70	0.10
Saturates	32.40	11.90	0.01
Aromatics	25.40	7.50	15.00
Resins	26.70	13.80	30.00
Asphaltene	15.50	66.80	55.50

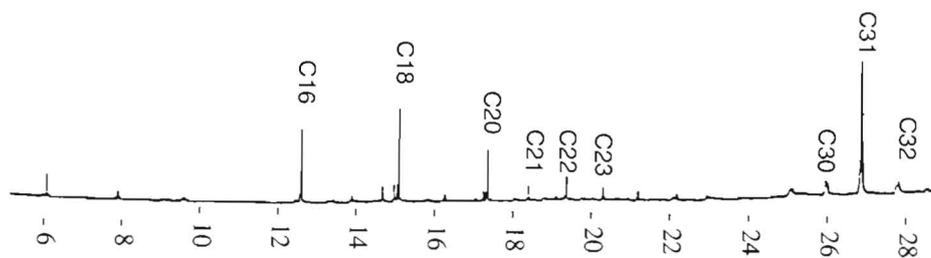
The residual n-alkanes and iso-alkanes of the saturates fraction were followed by gas chromatography and the results are found in Figs. 1-3. During the first year less quantities were removed from the soil (Fig. 1), while large quantities of such compounds were removed during the second year. On the other hand most of the n-alkanes (except traces of some components) were removed during the last year (Fig. 3). It was observed that high molecular weight n-alkane (C31) was accumulated in the soil samples collected during this year.



**Fig. 1.** Gas chromatogram of residual n-alkanes showing the removal of less quantities during the year, 1992.



**Fig. 2.** Gas chromatogram of residual n-alkanes showing the removal of large quantities during the year, 1993.



**Fig. 3.** Gas chromatogram of residual n-alkanes showing removal of most of the components during the year, 1994.

Results of total viable bacterial counts (Table 2) show that during the first year (1992) when the residual oil was 3.9%, higher counts were recovered ( $84.4 \times 10^4/\text{g}$  soil), and the SP/SC was 4.2. This was followed by a sharp decrease with decreasing oil residue, so that the number of total viable bacteria during the last year was nearly at the same level as in the control-nonpolluted samples. Jensen (1975) found that the number of bacteria increased with increasing oil concentration up to a concentration of 4-5%. Other investigators (*e.g.* Jones *et al.* 1970, Gudin and Syrratt 1975, Sextone and Atlas 1977, 1978, Pinholt *et al.* 1979, Diab 1991, Al-Gounain *et al.* 1992), also found that petroleum application to soil increased total microbial counts as well as caused shifts in the composition of the microbial community.

**Table 2.** Counts/g soil of the different groups of microorganisms in the polluted soil samples as compared to the control, nonpolluted samples, during April, 1992, 1993 and 1994

Microorganisms		1992	1993	1994
Total bacteria $\times 10^{-4}$	SP	$84.4 \pm 2.1$	$22.3 \pm 0.81$	$14.9 \pm 0.37$
	SC	$20.8 \pm 1.5$	$32.7 \pm 0.6$	$16.8 \pm 0.15$
	SP/SC	4.2	0.7	0.9
Actinomycetes $\times 10^{-3}$	SP	$0.8 \pm 0.04$	$5.0 \pm 0.5$	$40 \pm 0.4$
	SC	$31.1 \pm 2.0$	$128.0 \pm 4.0$	$59.0 \pm 3.0$
	SP/SC	0.03	0.04	0.07
Fungi $\times 10^{-3}$	SP	$15.5 \pm 0.18$	$6.0 \pm 0.15$	$3.8 \pm 0.48$
	SC	$1.4 \pm 0.01$	$0.9 \pm 0.09$	$0.8 \pm 0.01$
	SP/SC	11.1	6.7	4.7
Thermophiles $\times 10^{-3}$	SP	$3.8 \pm 0.15$	$7.8 \pm 0.3$	$1.4 \pm 0.16$
	SC	$62.0 \pm 2.0$	$60.4 \pm 2.4$	$7.2 \pm 0.30$
	SP/SC	0.06	0.13	0.19
Cellulose decomposers $\times 10^{-3}$	SP	$0.3 \pm 0.01$	$0.5 \pm 0.04$	$1.0 \pm 0.01$
	SC	$2.6 \pm 0.10$	$3.3 \pm 0.28$	$3.1 \pm 0.25$
	SP/SC	0.1	0.15	0.32
Oil-degrading bacteria $\times 10^{-2}$	SP	$25.0 \pm 1.2$	$4.8 \pm 0.4$	$2.2 \pm 0.2$
	SC	$0.5 \pm 0.07$	$0.4 \pm 0.07$	$0.6 \pm 0.07$
	SP/SC	50.0	12.0	3.7

SP/SC = ratios of the counts of the polluted samples (SP) to that of the control samples (SC).

Actinomycetes were found to be sensitive to oil pollution their number increased with decreasing oil content. Their SP/SC ratios were in the range of 0.03-0.07.

Fungi exhibited a more positive response to oil pollution than bacteria as indicated from their SP/SC ratios (11.1 – 4.7). Jensen (1975) reported that the activity of fungi tends to persist long after bacterial activity has tapered off. Perry and Cerniglia (1973) reported that though fungi are lower in number, they adapt more rapidly to adverse environmental conditions, such as limiting nitrogen and phosphorus. Davies and Westlake (1978) suggested that filamentous fungi may enhance oil biodegradation in soil indirectly by their mycelial invasion of the soil, thus providing increased surface contact area for bacteria capable of initiating hydrocarbon biodegradation. The same suggestion may be applicable to actinomycetes. Jensen (1975) found that a number of actinomycetes have been shown to have hydrocarbon degrading ability, though these organisms do not seem to compete as successfully in contaminated soil. Their slower growth, however, may infer a more dominant role in the later stages of hydrocarbon biodegradation.

Cellulose decomposers and thermophiles were of negative response to oil contamination and their SP/SC ratios were less than one. Walker *et al.* (1974, 1975) found that oil would decrease utilization of chitin, cellulose, lipids and proteins.

The results of the counts of oil-degrading microorganisms (Table 2) show that this group of microorganisms had a positive response to the exposure to oil pollutants. Their SP/SC ratios ranged from 50 during the first year to 3.7 during the last year. The counts of oil degraders in this polluted arid desert soil, as compared to other habitats, were low, ranging from 25.0 to  $2.2 \times 10^2$ /g soil, probably due to limiting nitrogen and phosphorus. Vecchioli *et al.* (1990) found that in untreated clay loam soil, hydrocarbon degrading bacteria were in the  $10^5$  range and the addition of a 10% loading rate of an aromatic hydrocarbon mixture increased the number of total viable soil bacteria and hydrocarbon degraders above the counts in untreated soil.

Results of the capacity of the microbial population in each soil sample to biodegrade oil and its fraction are found in Table 3. It was found that the biodegradation activity of the microbial population of the polluted soil samples decreased with decreasing the residual oil pollutants (Table 3). In soil samples of 3.9% residual oil, 37.5% crude oil, 51.2% of the saturates and 17.4% of the aromatics were biodegraded. Decreasing the residual oil to 0.1% in the last year, 17.0% of the crude oil and 20.3% of the saturates were biodegraded, while none of

the aromatic fraction was biodegraded. As a comparison, the microbial population of the control soil samples showed less biodegradation activity (Table 3).

**Table 3.** Biodegradation (%) of crude petroleum oil and its fractions due to microbial mixture activities of each soil sample

Crude oil and its fractions	Biodegradation (%)					
	1992		1993		1994	
	SP	SC	SP	SC	SP	SC
Crude oil	37.5 ±	8.2 ±	26.0 ±	6.8 ±	17.0 ±	5.9 ±
Saturates	51.2 ± 2.1	18.4 ± 0.8	48.8 ± 1.0	15.9 ± 1.0	20.3 ± 0.9	17.1 ± 0.9
Aromatics	17.4 ± 0.8	—	11.9 ± 0.7	6.1 ± 0.6	—	—
Resins	—	—	—	—	—	—

SP = Polluted soil, SC = Control, not polluted soil.

The utilization of n-alkanes of the saturates fraction was followed by gas chromatography and the results are presented in Figs. 4-11. Kuwait crude oil (KOC Blend) contains n-alkanes C<sub>12</sub> - C<sub>34</sub> as well as a number of iso-alkanes. Weathering was found to remove C<sub>12</sub> - C<sub>13</sub>, most of C<sub>14</sub> and slight reduction in the other components (Fig. 4).

The pattern of n-alkane utilization varied according to the structure of the microbial community and the amount of residual oil present in each soil sample. The microbial population present in polluted soil samples collected during 1992 and 1993 resulted in the removal of all n-alkanes except traces of some components (Fig. 5-6). Microbial populations of the soil samples collected during the last year, removed large quantities of such components (Fig. 7). On the other hand microbial communities found in the control soil samples also succeeded in removing large quantities of n-alkanes, but slightly less than those of the polluted soil samples (Figs. 8-10).

During the period of study, 92 fungal isolates were isolated, 62 from the polluted soil samples and 30 from the control samples. Results of the identification

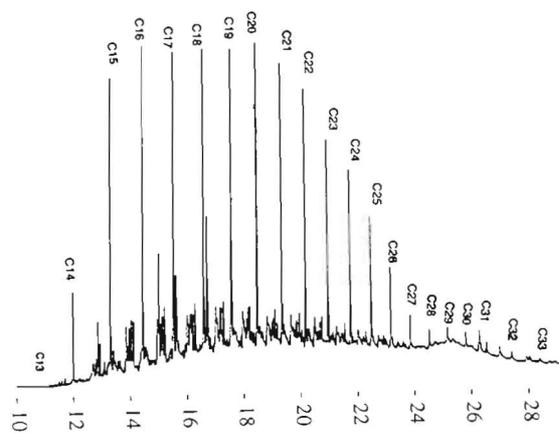


Fig. 4

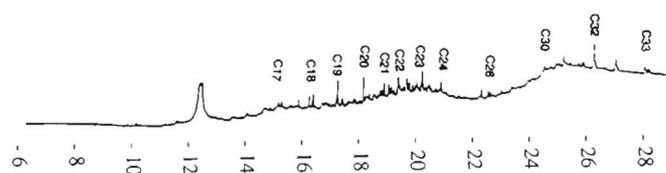


Fig. 5

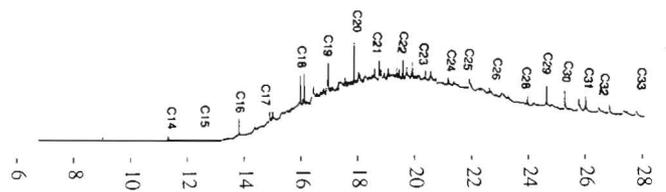


Fig. 6

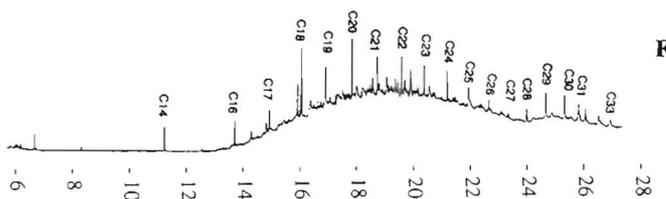


Fig. 7

- Fig. 4. Gas chromatogram showing pattern of n-alkanes components found in weathered crude oil (KOC-Blend).
- Fig. 5-6. Gas chromatogram showing the utilization of most of the components of n-alkanes (except traces) by microbial population found in soil samples collected during 1992, 1993.
- Fig. 7. Gas chromatogram showing the utilization of large quantities of the components of n-alkanes as a result of activities of microbial community found in soil sample collected during 1994.

(Table 4) show that *Aspergillus* spp I and *Stemphyllium* spp were sensitive to oil pollutants. They were absent from the polluted soil samples during the period of

**Table 4.** Numbers and percentages of fungal species isolated from the different soil samples during the period of studies

Fungal Species		1992		1993		1994		Total	
		No	%	No	%	No	%	No	%
1- <i>Aspergillus</i> spp II	SP	20	100	19	86.4	15	75.0	54	87.1
	SC	4	26.7	2	22.2	1	16.7	7	23.3
2- <i>Aspergillus</i> spp I	SP	-	-	-	-	-	-	-	-
	SC	5	33.3	4	44.4	3	50.0	12	40.0
3- <i>Aspergillus niger</i>	SP	-	-	1	4.5	-	-	1	1.6
	SC	-	-	-	-	-	-	-	-
4- <i>Stemphyllium</i> spp	SP	-	-	-	-	-	-	-	-
	SC	3	20.0	1	11.1	-	-	4	13.3
5- <i>Alternaria</i> spp	SP	-	-	1	4.5	3	-	4	6.5
	SC	1	6.7	1	11.1	-	-	2	6.7
6- <i>Fusarium</i> spp	SP	-	-	1	4.5	-	-	1	1.6
	SC	-	-	1	11.1	1	16.7	2	6.7
7- <i>Penicillium</i> spp	SP	-	-	-	-	2	-	2	3.2
	SC	-	-	-	-	-	-	-	-
8- <i>Halocladium</i> spp	SP	-	-	-	-	-	-	-	-
	SC	1	6.7	-	-	1	16.7	2	6.7
9- <i>Stachybotrys</i> spp	SP	-	-	-	-	-	-	-	-
	SC	1	6.7	-	-	-	-	1	3.3
Total	SP	20	32.2	22	35.5	20	32.2	62	-
	SC	15	50.0	9	30.0	6	20.0	30	-

SP = Polluted samples, SC = Control samples.

studies. On the other, hand *Aspergillus* sppII was the dominant fungus (75-100%) in the polluted samples. Other species of low frequency such as *Aspergillus niger*, *Alternaria* spp, *Fusarium* spp and *Penicillium* spp were also recorded in the polluted samples. Oudot *et al.* (1987) reported that most of the oil-degrading fungi were *Aspergillus* spp and *Penicillium* spp, but strains of *Paecilomyces*, *Acremonium*, *Fusarium*, and *Gleocladium* were also identified.

*Aspergillus* sppII, the dominant fungus in the polluted samples was tentatively identified as *Aspergillus terreus*, it was selected and studied for its biodegradation activities. The results (Table 5) show that this fungal species was able to utilize in 21 days 30% of the crude oil, 31.4% of the saturates fraction and 13.8% of the aromatic fraction. It succeeded in utilizing most of the components of the n-alkanes (Fig. 11).

A total of 90 bacterial isolates were isolated from the colonies which appeared on the silica gel plates, 66 from the polluted samples and 24 from the control samples. All strains (20) isolated from polluted soil samples collected during 1994, and all strains obtained from the control soil samples were Gram positive rods, and could be identified as *Arthrobacter* spp. From soil samples collected during 1992, sixteen isolates (80%) were *Pseudomonas* spp and four strains (20%) were *Arthrobacter* spp. On the other hand, from samples of 1993, two strains (7.7%) were *Pseudomonas* and 24 strains (92.3%) were *Arthrobacter* spp. Jensen (1975) reported

**Table 5.** Biodegradation of petroleum oil and its fractions by certain microorganisms isolated from polluted and non polluted soil samples

Organisms	Biodegradation (%)			
	Oil	Saturates	Aromatics	Resins
<i>Pseudomonas</i> spp1	11.2 ± 0.8	25.8 ± 0.45	5.8 ± 0.51	–
<i>Pseudomonas</i> spp2	16.8 ± 0.8	25.7 ± 0.8	6.8 ± 0.48	–
<i>Arthrobacter</i> spp1	16.5 ± 0.5	16.8 ± 0.8	–	–
<i>Arthrobacter</i> spp2	15.8 ± 0.4	17.5 ± 1.0	–	4.6 ± 1.2
<i>Arthrobacter</i> spp3	5.6 ± 0.40	16.4 ± 0.1	–	5.7 ± 1.7
<i>Arthrobacter</i> spp4	6.6 ± 0.35	15.0 ± 1.0	–	2.8 ± 0.6
<i>Aspergillus terreus</i>	30.0 ± 1.0	31.4 ± 1.5	13.8 ± 0.6	–

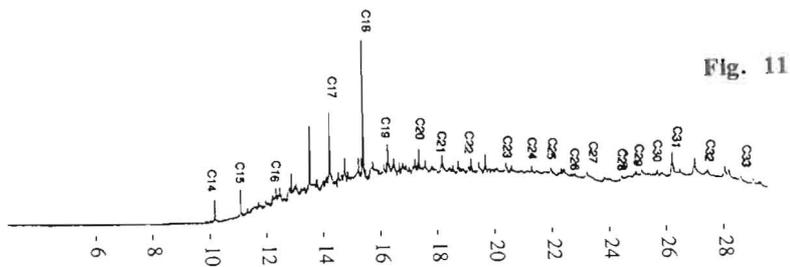
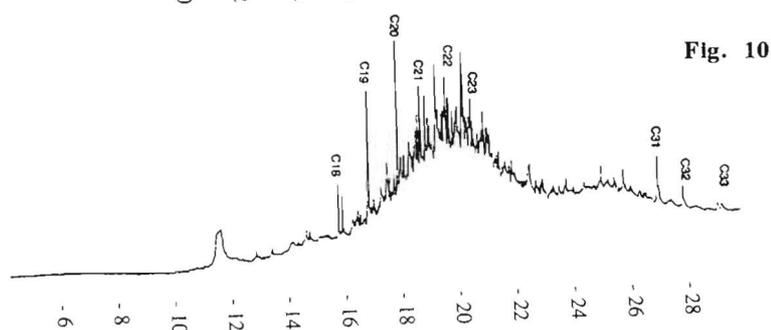
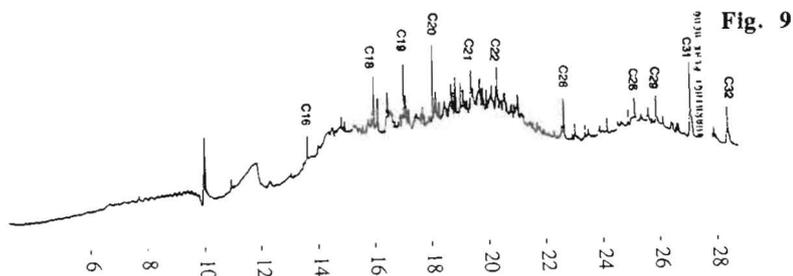
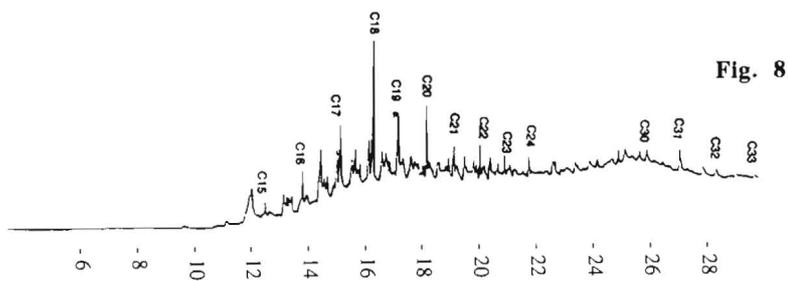


Fig. 8-10. Gas chromatogram of n-alkanes showing the removal of large quantities of n-alkanes components as a result of activities of the microbial population found in non polluted soil samples collected during 1992, 1993 and 1994 respectively.

Fig. 11. Gas chromatogram of n-alkanes showing the utilization of most of the components by *Aspergillus terreus*.

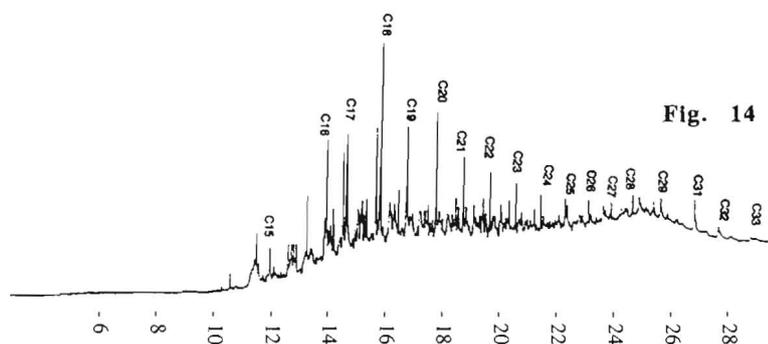
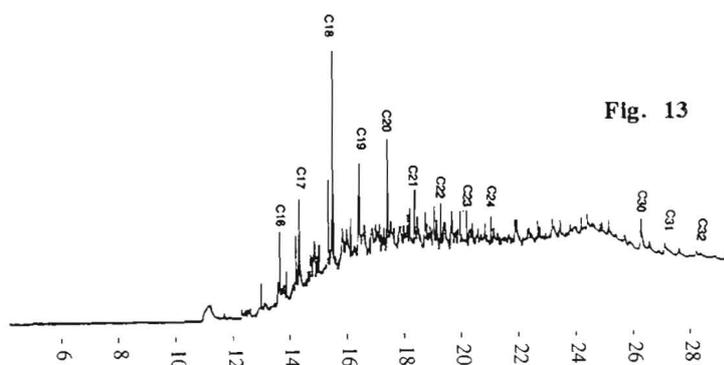
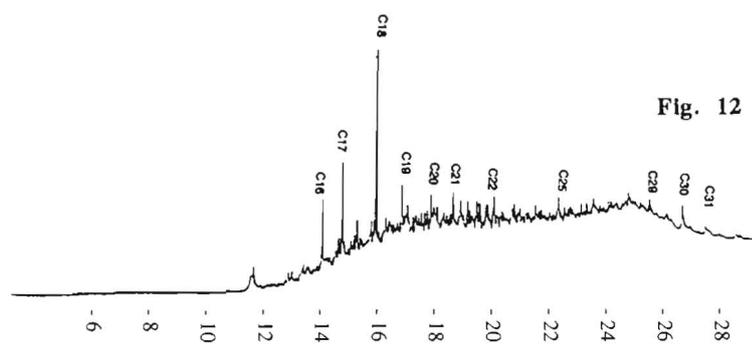


Fig. 12. Gas chromatogram of n-alkanes showing the utilization of most of n-alkanes components as a result of *Pseudomonas* sp.

Fig. 13. Gas chromatogram showing the removal of n-alkanes by *Arthrobacter* sp. isolated from polluted soil samples.

Fig. 14. Gas chromatogram showing the removal of n-alkanes by *Arthrobacter* sp. isolated from the non-polluted soil samples.

that populations of *Arthrobacter*, *Corynebacterium*, *Mycobacterium*, and *Nocardia* showed strong positive responses to oil contamination. Soil pseudomonads represent another bacterial group with a major role in soil biodegradation. *Pseudomonas* strains were often isolated from oil contaminated environments (Cooper and Hedrick 1976, Diab 1991a) and have been shown to degrade a wide variety of hydrocarbons including aromatic hydrocarbons (Traxler 1962, Diab 1991b).

Six isolates, two *Pseudomonas* spp and four *Arthrobacter* spp, representing cultures showing very good growth on media containing petroleum oil as the only carbon source, were selected and studied for their abilities to degrade crude petroleum and its fractions. *Pseudomonas* spp showed biodegradation activity more than the *Arthrobacter* spp (Table 5). They degraded 11.2-16.8% of the crude oil, 26% of the saturates and 5.6-6.8 of the aromatics.

On the other hand *Arthrobacter* spp isolated from the polluted soil samples showed more activity than *Arthrobacter* spp of the control soil samples. Three strains of *Arthrobacter* were able to degrade 1.3-4.6% of the resin fraction.

Result of the utilization of n-alkanes by the above bacterial strains (Figs. 12-14) show that biodegradation accomplished by *Pseudomonas* spp resulted in the removal of most of the n-alkanes (Fig. 12). On the other hand *Arthrobacter* spp showed less activity than the *Pseudomonas*. *Arthrobacter* spp (1 & 2) isolated from the polluted soil samples were found to remove more n-alkanes than those isolated from the control soil samples (Figs. 13-14). The appearance of an increase of n-alkanes C<sub>30</sub> was noted during the growth of *Pseudomonas* spp and *Arthrobacter* spp.

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## References

- Al-Gounaim, M.Y., Diab, A. and El-Shinawi, K. (1992) Proceedings of Third World Academy of Science. Fourth General Conference, Kuwait, 23-26 Nov. 1992. Effect of oil spilled in desert of Kuwait on the microbial flora of the soil.
- Bergey's Manual of Systematic Bacteriology (1984) Vol. (1). (Krieg, N.R. and Holt, J.G. (eds.) Editorial board: Murray, Brenner, Bryant, Holt, Krieg, Moulder, Pfenning, Sneath and Staly). Williams & Wilkins, Baltimore.
- Bergey's Manual of Systematic Bacteriology (1984) Vol. (2). (Sneath, P.H.A., Mair, M.S., Sharple, M.E. and Holt, J.G. (eds.) Editorial board: Murray, Brenner, Bryant, Holt, Krieg, Moulder, Sneath and Staly). Williams & Wilkins, Baltimore.
- Bergey's Manual of Determinative Bacteriology (1994) 9<sup>th</sup> ed. (Holt, J.G., Krieg, N.R. Sneath, P.H.A., Staly J.T. and Williams, S.T.) Williams & Wilkins, Baltimore.
- Buckley, E.N. (1980) *Effects of petroleum hydrocarbons on metabolic activity and community composition of suspended heterotrophic salt marsh bacteria*. Ph.D. Thesis, University of North Carolina, Chapel Hill.
- Cooper, R.E. and Hedrick, H.G. (1976) Activity of soil bacteria on petroleum waste adjacent to an active oil well. *Soil Science*, **122**: 331-339.
- Davies, J.S. and Westlake, D.W.S. (1978) Crude oil utilization by fungi. *Can. J. Microbiol.* **25**: 146-156.
- Diab, A. (1991) Biodegradation of petroleum oil by microbial population in the desert soil of Kuwait. *A.Z. J. Microbiol.* **11**: 118-138.
- Diab, A. (1991) Distribution and activities of oil - degrading bacteria in the Arabian Gulf water at Kuwait. *A.Z. J. Microbiol.* **11**: 139-160.
- Diab, A. and Al-Gounaim, M.Y. (1984) Distribution of Azotobacter, actinomycetes, cellulose-degrading, acid producing, and phosphate dissolving bacteria in desert and salt marsh soils of Kuwait, *Zbl Microbiol.* **139**: 425-433.
- Fedorak, P.M. and Westlake, D.W.S. (1981) Degradation of aromatics and saturates in crude oil by soil enrichment. *Water, Air, and soil Pollution*, **16**: 367-375.
- Gudin, C. and Syrratt, W.J. (1975) Biological aspects of land rehabilitation following hydrocarbon contamination. *Environ. Pollut.* **8**: 107-112.
- Jensen, V. (1975) Bacterial flora of soil after application of oily waste *OIKOS*, **26**: 152-158.
- Jones, J.G., Knight, M. and Byrom, J.A. (1970) Effect of gross pollution by kerosene hydrocarbons on the microflora of a moorland soil, *Nature*, **227**: 11-66.
- Odu, C.T. (1972) Microbiology of soils contaminated with petroleum hydrocarbons. Extent of contamination and some soil and microbial properties after contamination. *J. of the Institute of Petroleum*, **58**: 201-208.
- Oudot, J. (1984) Rates of microbial degradation of petroleum components as determined by computerized mass - spectrometry. *Mar Environ. Res.*, **13**: 277-302.
- Oudot, J., Fusey, P., Abdelouahid, D.E., Haloui S. and Roquebert, M.F. (1987) Capacites degradative de bacteries et de champignons isoles dum sol contamine par un fuel. *Can J. Microbiol.* **33**: 232-243.
- Perry, J.J. and Cerniglia, C.E. (1973) Studies on the degradation of petroleum by filamentous

- fungi. In: The microbiol degradation of oil pollutants, **Ahearn, D.G.** and **Meyers, S.P.** (eds.), pp. 89-94. Publ. No. Lsu - SG - 73-01 Center for wetland Resorces, Louisiana State University, Baton Range, Louisiana, U.S.A.
- Pinholt, Y., Struwe, S.** and **Kjoller, A.** (1979) Microbial changes during oil decomposition in soil. *Holarctic Ecol.* **2**: 195-200.
- Sextone, A.J.** and **Atlas, R.M.** (1977) Mobility and biodegradability of crude oil in arctic tundra soils. *Dev. Ind. Microbiol.* **18**: 673-684.
- Sextone, A.J.** and **Atlas, R.M.** (1978) Persistence of oil in tundra soils. *Dev. Ind. Microbiol.* **19**: 507-515.
- Traxler, R.W.** (1962) Microbiol degradation of asphalt. *Biotechnol. Bioeng.* **4**: 369-379.
- Vecchioli, G.I., Del Panno, M.T.** and **Painceira, M.T.** (1990) Use of selected autochthonous soil bacteria to enhance degradation of hydrocarbons in soil. *Environ. Pol.* **67**: 249-258.
- Walker, J.D.** and **Colwell, R.R.** (1976) Enumeration of petroleum - degrading microorganisms. *Appl. Environ. Microbiol.* **3**: 198-207.
- Walker, J.D., Seasman, P.A.** and **Colwell, R.R.** (1974) Effects of petroleum on estuarine bacteria. *Mar. Poll. Bull.* **5**: 186-189.
- Walker, J.D., Seasman, P.A.** and **Colwell, R.R.** (1975) Effects of South Louisiana crude oil and No. 2 fuel oil on growth of heterotrophic microorganisms, including proteolytic, lipolytic, chitinolytic and cellulytic bacteria. *Environ. Pollut.* **9**: 13-32.

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## تأثير التلوث النفطي على المجتمعات الميكروبية في تربة صحراء الكويت

مرزوق يوسف الغنيم و علي دياب و رقية العبد الله و نبيله الزامل

قسم العلوم - كلية التربية الأساسية  
ص. ب. ٣٤٠٥٣ - العدلية - الكويت

خلال الغزو العراقي دمر الغزاة العراقيون آبار النفط وكان من نتيجتها تلوث حوالي ٤٩ كم<sup>٢</sup> من تربة صحراء الكويت بحوالي ٦٠-٨٠ مليون برميل من النفط الخام . وقد تم تعيين هذا التلوث خلال السنوات ٩٢/٩٣/٩٤ م . وقد أظهرت التحاليل أن المركبات المشبعة كانت نسبتها ٤, ٢٣٪ في السنة الأولى وأصبحت ٩, ١١٪ في السنة الثانية ، واختفت تقريباً في السنة الثالثة - أما المركبات العطرية والراتنجية فهي قلت ثم ازدادت مرة أخرى ، على حين أن المركبات الاسفلتينية زادت وتراكمت في السنتين الاخيرتين . وقد اظهرت البكتريا المحللة للنفط منحاً ايجابياً عندما تعرضت للملوثات النفطية ، وكانت نسبة الكائنات المحللة للنفط في التربة الملوثة إلى محتوى الكائنات المحللة للنفط في التربة غير الملوثة قد تراوحت بين ٧, ٣-٥٠ بمقارنتها بالبكتريا الكلية والاكثينومايسيتات ومحللات السليلوز والبكتريا المحبة للحرارة العالية التي تراوحت بين ٠, ٣-٢, ٤ .

أما الفطريات فقد اظهرت تجاوزاً أكثر من البكتريا حيث كانت نسبة محتوى الفطريات في التربة الملوثة إلى محتواها في التربة غير الملوثة يتراوح بين ٧, ٤ - ١١, ١ .

وقد درست قدرة المجتمع الميكروبي لكل عينة من التربة على تحليل النفط ومشتقاته حيث وجد أن سرعة تحليل هذه الملوثات تقل كلما قلت كميتها في التربة . وقد تم عزل وتعريف ٩٢ مزرعة من الفطريات (٦٢ من عينات التربة الملوثة و ٣٠ من عينات التربة غير الملوثة) . وقد اظهرت كل من الفطره *Aspergillus spp* و *Stemphyllium spp* حساسية للموثرات النفطية - حيث تواجدت فقط في التربة غير الملوثة- اما الفطره *Aspergillus tereus* فقط كانت هي السائدة في التربة الملوثة ولذلك فقد عزلت ودرست قدرتها على تحليل النفط ومشتقاته . ومن الاطباق المحتوية على السليكا جل وزيت البترول تم عزل وتعريف ٩٠ مزرعة بكتيرية ووجد ان كل المزارع المعزولة من التربة الملوثة التي جمعت خلال ١٩٩٤م وأيضاً جميع عينات التربة غير الملوثة تتبع الجنس *Arthrobacter spp* . أما البكتيريا *Pseudomonas spp* فقد كانت نسبتها ٨٠٪ في التربة المحتوية على ٧, ٣٪ ملوثات نفطيه .

وقد اظهرت مزرعتين من الجنس *Pseudomonas spp* ، ٤ مزارع من الجنس *Arthrobacter spp* نموا كثيفاً على الأوساط الغذائية المحتوية على زيت البترول كمصدر وحيد للكربون ، ولذلك فقد درست قدرة هذه المزارع على تحليل النفط ومشتقاته .