Ecological Distribution and Biodegradational Activities of Oil-Degrading Marine Bacteria in the Arabian Gulf Water at Kuwait

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ABSTRACT. The distribution of oil-degrading bacteria in the Arabian Gulf water at Kuwait during the year January-December 1995 was studied. This group of microorganisms ranged from 0.3 - 15.2 x 10^3 CFU/l in Shuwaikh Station (a commercial harbour) and 0.1 - 5.8 x 10^3 CFU/l in Salmiya (a relatively unpolluted control). Their percentages among total heterotrophic bacteria were in the range of 0.2 - 22.8% in Shuwaikh water and 0.1 - 8.8% in Salmiya water. The ratios of CFU/l of oil-degrading bacteria obtained from Shuwaikh to that obtained from Salmiya are in the range of 1.5 - 57.0.

Autumn and winter were suitable seasons for obtaining high proportions of oil-degrading bacteria, while spring and summer were suitable for the development of large counts of total viable heterotrophic bacteria. The total number of viable fungi was greater during spring and autumn.

Out of 180 bacterial cultures able to grow in the presence of crude oil, 28 cultures showed good growth. They were *Pseudomonas* spp. (32.1%), *Arthrobacter* spp. (23.1%), *Corynebacterium* spp. (17.8%), *Acinetobacter* spp. (10.7%), and *Flavobacterium* spp. (7.1%). Six of the 28 species were selected and inoculated singly and as mixtures into natural sea water. *Arthrobacter* sp. A(1) and *Pseudomonas* sp. P(6) degraded 64% and 60% of the saturates fraction respectively, which were 15.5% and 11.5% above those degraded in presence and in absence of the mixed inoculum of the six species. On the other hand *Pseudomonas* sp. P(8) and *Arthrobacter* sp. A(2) degraded 30% and 29% of the aromatic fractions, which was 10-12% more than the amounts degraded in the presence and absence of the mixed inoculum.

The main topographic features of the Arabian Gulf is a channel on the Iranian side, and shallow areas at the northwest end and off the coasts of Saudi Arabia, Qatar and United Arab Emirates. The Gulf is a region of strong evaporation, leading to salinity values frequently exceeding 40‰ under both summer and winter conditions (Hunter 1986).

Oil pollution in the Arabian Gulf water arises from local exploration, oil-refining and routine handling at ports. Contamination by oil could also originate from heavy maritime transports of crude and refined oil through the Gulf region (El-Samra *et al.* 1986). Through this regoin the heaveist oil tanker traffic in the world, shunting of oil from the Gulf of Arabia to consumers in Europe, North America and Asia.

When oil pollutants enter natural waters they are subjected to numbers of physical, chemical and biological factors, which contribute to the loss or alteration of some of their components. It is well known fact that metabolism by the indigenous microbial community is influenced by numbers of seasonal environmental factors, including light, availability of oxygen, temperature and available nitrogen and phosphorus. Previous pollution history of an ecosystem may also be significant, since systems that receive chronic petroleum pollution are generally enriched in hydrocarbon-using organisms. Davies *et al.* (1981) reported that concentrations as low as 5-15 ppb of hydrocarbons can affect some trophic levels in marine environments.

Adapted communities, *i.e.* those that have been previously exposed to hydrocarbons, exhibit higher biodegradation rates than communities with no history of contamination (Leahy and Colwell 1990). Hydrocarbon degradation by microorganisms depends on the nature and amount of oil, seasonal environmental conditions and on the composition of the community and its adaptive response to the presence of hydrocarbons. Bacteria and fungi are the key agents of degradation, with bacteria assuming the dominant role in marine ecosystems (Leahy and Colwell 1990).

Seeding of petroleum - contaminated aquatic environments has been attempted with mixed results. Tagger *et al.* (1983) observed no increase in petroleum degradation in sea water inoculated with a mixed culture of hydrocarbon-degrading bacteria. In contrast, Atlas and Busdosh (1976) reported increased degradation of oil in saline Arctic ponds after inoculation with an oil-degrading *Pseudomonas* sp., though these treatment gave no improvement in fresh water ponds.

The rate of hydrocarbon biodegradation following an oil spill appears to be

highly dependent on local environmental factors. To predict the rate of hydrocarbon biodegradation in the marine environment, the biodegradation process must be evaluated. This may help in the removal of oil pollutants from the ecosystem.

Most of the available literature, concerning the biodegradation of hydrocarbons in marine environment are describes studies in colder and temperate regions (Leahy and Colwell 1990, Venkateswaran *et al.* 1991). Few reports are available on the biodegradation of crude oil and its fractions in subtropical and tropical waters. Furthermore, there are no studies on the ecological distribution and biodegradation activities of oil-degrading microorganisms in the waters of the Arabian Gulf. Accordingly, this study describes the ecology of the oil-degrading bacteria in the Arabian Gulf at Kuwait; and was conducted to obtain background information on the natural abundance, distribution and seasonal fluctuation of this group of microorganisms in this marine habitat.

Materials and Methods

Collection of Water Samples:

Water samples were collected during January - December, 1995 from two stations: Shuwaikh and Salmiya, both lie 4 and 12 km from Kuwait City on the Arabian Gulf. The first station is a commercial harbour, while the second represents a relatively unpolluted area. From each station, water samples were taken one km off-shore from, at depths of 0.3 m and 5.0 m, using a Nisskin water-sampler. At each station, three 100 ml water samples were collected from three different spots and transferred to the laboratory packed in ice. On arrival to the laboratory , these samples were mixed in 500 ml sterile flasks to form single composite samples. Microbiological analysis was conducted immediately upon arrival and within 3 h of collection.

Water samples for chemical analysis were collected in 500 ml brown-glass bottles, and kept refrigerated at 4°C until analysis were performed. Temperature, dissolved oxygen and pH were measured on site.

Extraction of oil from the collected sea water:

The presence of oil in collected sea water samples was measured by the method of Atlas and Bartha (1973).

Microbiological Methods:

Total viable bacteria were counted using the usual dilution plate method and

using nutrient agar granules dissolved in sea water and supplemented with 1.0% (w/v) yeast extract. For counting fungi, 10 - 20 ml of the collected sea water were filtered through a sterile membrane filter (0.45 μm). The filter was placed on a plate of yeast - malt extract agar. Four plates were used on each occasion. All plates were incubated at 28°C for a period of 5 - 7 days, after which colonies of bacteria and of fungi were counted and expressed as CFU/l sea water.

For counting oil-degrading bacteria, 100 ml of the collected sea water was filtered through a sterile membrane filter (0.45 μm). The filter was placed on a plate of oil silica gel medium containing 0.5% filter sterilized crude petroleum oil (Kuwait Oil Company-Blend) as the only carbon source, and prepared as described by Walker and Colwell (1976). Four plates were used for each sample. The plates were transferred to plastic bags and incubated at 28°C for a period of 21 days, after which the developed colonies were counted and expressed as CFU/l sea water. All colonies developed were isolated, purified and subcultured in suitable media. Becteria were identified using methods of Bergey's Manual of Systematic Bacteriology. (1984, 1986) and Bergey's Manual of Determinative Bacteriology (1994).

Selection of the Active Oil-Degrading Bacteria:

For each organism, suspension of 1×10^6 cells was added into a 100 ml conical flask containing 20 ml of sterilized sea water supplemented with 0.15% $NaNO_3$ and 0.1% K_2HPO_4, and overlaid with 0.5% filter-sterilized oil. The flasks were incubated at 28°C for a period of 21 days, on a rotary shaker operated at 100 rpm. Activity was indicated by measuring the absorption due to optical density of the growth turbidity and/or emulsification of the oil in the liquid phase of the culture at 450 nm. Cultures showing values of 1 - 10 in the absorption scale were selected for the study of their biodegradation activities. The selected bacteria were then studied quantitatively for their biodegradation activities using a sterilized sea water medium.

Evaluation of the Biodegradation Capacity of the Selected Bacteria:

One ml sample of bacterial suspension of (10^6 cells) was inoculated into a 250 ml conical flask containing 50 ml of sterilzied sea water medium. At least six flasks were used for each organism, three for the evaluation of biological factors, and the other three, (to which 0.5% (w/v) $HgCl_2$ was added) were used for the evaluation of non-biological factors. All flasks were incubated at 30°C for a period of 21 days on a rotary shaker operated at 100 rpm.

At the end of the incubation period, the residual oil was recovered and fractionated into saturates, aromatics, resins and asphaltenes, using the method

described by Oudot (1984).

From the results of this experiment, six bacterial species showing high biodegradation activities for oil and its fractions were selected to study the effect of seeding natural unsterilized sea water with the six different species, singly or in a mixture.

The loss of the oil and its fractions was determined gravimetrically. The saturates fraction was resolved by gas chromatography to determine the loss of each component of the n-alkanes and iso-ulkanes present.

Results and Discussion

The results of sea water analysis (Table 1) showed seasonal fluctuation of water temperature, varying from 8°C during winter to 31°C during summer. Generally, there were no significant variations between water temperatures recorded either from Shuwaikh or Salmiya stations. At both stations temperature was slightly lower at 5.0 m depth than at 0.3 m depth.

Concentration of dissolved oxygen (DO) in the Arabian Gulf water at Kuwait (Table 1) were in the range of 37.4 μM/l during September to 131.3 μM/l during February. When the concentrations of DO are considered seasonally, there were no significant variations between the results obtained from Shuwaikh and those obtained from Salmiya at both depths. At both stations high DO concentrations were recorded during winter and spring, while lower concentrations were during summer.

The nitrates, nitrites and phosphates concentrations in this marine environment were low. Nitrates ranged from a mean of 18.3 $\pm$ 0.6 μM/l during winter to 21.1 $\pm$ 1.0 μM/l during autumn. Nintrites showed marked decrease during winter (0.05 - 0.07 μM/l), and increased during other seasons, especially during summer. The concentrations of PO_4^- in the Arabian Gulf water at Kuwait did not exceed 1.13 μM/l. In Shuwaikh station no significant difference in PO_4^- concentrations were observed during the different seasons, while in Salmiya station, a marked decrease in PO_4^- concentration was recorded during winter with comparison to other seasons. Water salinity was in the range of 43.6 - 49.0‰ with a pH of 8 - 8.3.

All collected water samples were free from oil films or obvious signs of pollution during the collection period. Analysis of water samples for the detection of hydrocarbons were negative, except for samples collected from Shuwaikh station at 0.3 m depth during June and December in which 8 mg/l were present.

Table. 1. Chemical analysis of water samples collected from Shuwaikh and Salmiya stations during 1995 January - December.

Month	Station	Depth	Salinity ‰	pH	DO µM/l	NO_3^- µM/l	NO_2^- µM/l	PO_4^- µM/l	Temper-ature
January	Shuwaikh	0.3	44	8.1	131.0	17.7	0.04	0.70	15
		5.0	44	8.1	124.8	19.4	0.04	0.74	12
	Salmiya	0.3	42	8.0	134.2	18.4	0.06	0.24	14
		5.0	43	7.9	131.0	19.4	0.05	0.26	11
February	Shuwaikh	0.3	46	8.0	137.3	17.7	0.04	0.47	12
		5.0	45	7.9	127.9	19.4	0.04	0.63	9
	Salmiya	0.3	44	8.0	134.2	19.4	0.07	0.20	12
		5.0	44	8.1	121.7	17.7	0.07	0.19	8
March	Shuwaikh	0.3	45	8.1	106.1	19.4	0.12	0.96	16
		5.0	44	7.9	106.1	19.4	0.10	0.82	13
	Salmiya	0.3	46	8.2	99.8	19.4	0.04	0.25	16
		5.0	44	8.1	99.8	17.7	0.04	0.47	13
Mean	Shuwaikh	0.3	45.0	8.1	124.7 ± 9.5	18.3 ± 0.6	0.07 ± 0.03	0.71 ± 0.14	14.3 ± 1.2
		5.0	44.3	8.0	119.6 ± 6.6	19.4 ± 0.0	0.06 ± 0.02	0.73 ± 0.05	11.3 ± 1.2
	Salmiya	0.3	44.0	8.1	122.7 ± 11.5	19.1 ± 0.3	0.06 ± 0.01	0.23 ± 0.01	14.0 ± 1.1
		5.0	43.6	8.0	117.5 ± 9.2	18.3 ± 0.6	0.05 ± 0.01	0.31 ± 0.08	10.7 ± 1.4
April	Shuwaikh	0.3	44	8.2	109.2	19.4	0.15	0.63	24
		5.0	44	8.2	103.0	19.4	0.19	0.68	22
	Salmiya	0.3	42	8.2	99.8	21.0	0.08	0.69	23
		5.0	43	8.1	96.8	21.0	0.06	0.49	22
May	Shuwaikh	0.3	45	8.2	118.6	19.4	0.23	0.74	26
		5.0	46	8.2	115.4	17.7	0.15	0.63	24
	Salmiya	0.3	44	8.3	112.3	21.0	0.23	0.89	27
		5.0	45	8.2	105.2	19.4	0.18	1.01	24
June	Shuwaikh	0.3	46	8.4	103.0	21.0	0.26	0.74	29
		5.0	47	8.5	99.8	21.0	0.33	0.89	27
	Salmiya	0.3	47	8.2	90.5	21.0	0.29	0.79	27
		5.0	45	8.2	87.4	21.0	0.35	0.84	25
Mean	Shuwaikh	0.3	45.0	8.3	110.3 ± 4.5	19.9 ± 0.5	0.21 ± 0.03	0.70 ± 0.04	26.3 ± 1.4
		5.0	45.7	8.3	106.1 ± 4.8	19.4 ± 0.7	0.22 ± 0.05	0.73 ± 0.08	24.3 ± 1.4
	Salmiya	0.3	44.3	8.2	110.9 ± 6.3	21.0 ± 0.0	0.20 ± 0.06	0.79 ± 0.06	25.7 ± 1.3
		5.0	44.3	8.2	96.5 ± 5.1	20.5 ± 0.5	0.20 ± 0.08	0.78 ± 0.15	23.7 ± 0.8

Table. 1. – *(Continued).*

Month	Station	Depth	Salinity ‰	pH	DO $\mu M/l$	NO_3^- $\mu M/l$	NO_2^- $\mu M/l$	PO_4^- $\mu M/l$	Temper-ature
July	Shuwaikh	0.3	46	8.2	90.5	17.4	0.25	0.94	27
		5.0	47	8.2	84.2	21.4	0.38	0.84	25
	Salmiya	0.3	46	8.2	87.4	21.0	0.33	1.01	29
		5.0	46	8.3	81.1	21.0	0.33	0.84	25
August	Shuwaikh	0.3	46	8.3	43.7	21.0	0.33	0.79	30
		5.0	46	8.3	40.6	19.4	0.33	0.89	28
	Salmiya	0.3	45	8.2	43.7	21.0	0.36	0.84	30
		5.0	45	8.2	40.6	21.0	0.30	1.01	28
September	Shuwaikh	0.3	55	8.2	40.7	19.4	0.33	0.84	31
		5.0	46	82	37.4	19.4	0.38	0.84	30
	Salmiya	0.3	47	8.2	43.7	21.0	0.39	0.94	30
		5.0	46	8.2	40.6	19.7	0.32	0.79	29
Mean	Shuwaikh	0.3	49.0	8.2	58.3 ± 16.1	19.3 ± 1.0	0.30 ± 0.03	0.86 ± 0.04	29.3 ± 1.2
		5.0	46.3	8.3	54.1 ± 15.1	20.0 ± 0.7	0.33 ± 0.03	0.86 ± 0.07	27.7 ± 1.4
	Salmiya	0.3	46.3	8.2	58.3 ± 14.6	21.0 ± 0.0	0.36 ± 0.03	0.93 ± 0.05	29.7 ± 0.3
		5.0	45.6	8.2	54.1 ± 13.5	20.6 ± 0.4	0.32 ± 0.01	0.88 ± 0.07	27.3 ± 1.2
October	Shuwaikh	0.3	46	8.1	43.6	22.6	0.34	0.89	27
		5.0	46	8.2	40.6	22.6	0.25	0.89	25
	Salmiya	0.3	45	8.2	43.6	22.6	0.42	1.00	27
		5.0	44	8.2	40.6	21.0	0.30	1.02	26
November	Shuwaikh	0.3	45	8.1	90.5	22.6	0.25	1.02	23
		5.0	44	8.2	84.2	19.4	0.15	1.00	21
	Salmiya	0.3	44	8.2	87.7	22.6	0.30	1.13	22
		5.0	44	8.2	81.1	21.0	0.21	1.00	21
December	Shuwaikh	0.3	44	8.1	99.8	19.4	0.26	0.79	19
		5.0	44	8.1	93.6	21.0	0.25	0.74	17
	Salmiya	0.3	45	8.2	87.4	21.0	0.38	0.79	19
		5.0	46	8.3	81.1	21.0	0.33	1.00	18
Mean	Shuwaikh	0.3	45	81	78.0 ± 17.4	21.5 ± 1.1	0.28 ± 0.03	0.90 ± 0.06	23 ± 2.3
		5.0	45.7	82	72.8 ± 16.3	20.9 ± 0.9	0.22 ± 0.03	0.88 ± 0.07	21 ± 2.3
	Salmiya	0.3	45.6	8.2	72.9 ± 14.7	22.1 ± 1.0	0.37 ± 0.03	0.97 ± 0.07	22.7 ± 2.3
		5.0	44.6	8.2	67.6 ± 13.5	21.0 ± 0.0	0.28 ± 0.04	1.00 ± 0.01	21.7 ± 2.3

Temperature of water also included. DO = Dissolved oxygen. HC = Hydrocarbons.

The results of total viable bacterial counts (Table 2) showed that within each station the CFU/l sea water varied according to season and depth. Lower counts were recorded during winter in both stations. Seasons of high counts differed according to station and depth. In Shuwaikh a high count of $16.2 \pm 0.7 \times 10^4$ CFU/l was recorded during summer from depth 5.0 m; at 0.3 m depth on the other hand, high counts of $15.9 \pm 0.9 \times 10^4$ CFU/l were obtained during spring. As for Salmiya station, reverse results took place, the seasons of high counts were found during summer at a depth of 0.3 m ($14.4 \pm 0.3 \times 10^4$ CFU/l) and at spring at depth of 5.0 m ($11.4 \pm 0.2 \times 10^4$ CFU/l). It can be clearly observed that high counts of total viable bacteria are represented by two peaks, one peak represents summer and the other represents spring. Om-Kalthoum (1984) found that CFU of bacteria in the Mediterranean Sea at Alexandria were in the range of 2.2×10^4/100 ml during March to 2.4×10^6/100 ml during June at 5 - 10 m depth. She also reported that seasonal variation did not induce significant variation in total viable bacterial counts, except for winter during which counts were significantly lower as compared to other seasons. The same author reported that the results were difficult to be correlated with the physical and chemical analysis of sea water. Walker and Colwell (1973) found 1500 CFU/l in waters of the eastern Bay and Baltimore harbour, compared to 15000 CFU/l in Chesapeake Bay.

In the north eastern Japanese coastal water, Venkateswaran *et al.* (1991) found that total viable bacterial counts were in the range of 4.4×10^3 - 7.9×10^4 CFU/ml. In the north Pacific and in the south Pacific, Venkateswaran *et al.* (1993) found that total viable bacteria were in the range of 1.3×10^2 - 6.2×10^3 CFU/ml and 9.5×10^1 - 4.0×10^3 CFU/ml respectively.

Results of the distribution of fungi (mostly yeasts) are represented in Table (2). The results taken from the two stations at all seasons of the year showed no significant variations between the counts at 0.3 m depth and those at 5.0 m depth. (*i.e.* no effect of depth). CFU/l of fungi ranged from $0.7 \pm 0.0 \times 10^2$ - $4.8 \pm 0.5 \times 10^2$ in water samples collected from Shuwaikh, on the other hand in water samples collected from Salmiya, the counts were in the range of $0.5 \pm 0.0 \times 10^2 - 11.0 \pm 0.4 \times 10^2$/l.

Counts of CFU/l of fungi were higher in water samples of Salmiya than those of Shuwaikh during spring and autumn, while during other seasons no significant variations between counts were observed. The high counts recorded from Shuwaikh were during winter, while during other seasons no significant difference could be obtained.

Oil-degrading bacteria in the Arabian Gulf water at Kuwait (Table 3) ranged from 0.3 - 15.2×10^3/l at Shuwaikh and 0.1 - 5.8×10^3/l at Salmiya. While the

Table. 2. Mean counts of CFU of total viable bacteria and fungi / litre sea water collected during 1995 January - December.

Month	Depth (m)	Counts of total viable bacteria x 10^{-3}/l		Counts of fungi x 10^{-2}/l	
		Shuwaikh	Salmiya	Shuwaikh	Salmiya
January	0.3	65.5 ± 4.0	45.2 ± 1.5	3.2 ± 0.4	1.8 ± 0.4
	5.0	36.8 ± 1.6	32.2 ± 1.5	4.8 ± 0.7	4.3 ± 0.3
February	0.3	91.4 ± 4.3	44.3 ± 0.8	2.5 ± 0.3	2.5 ± 0.5
	5.0	85.8 ± 5.3	35.5 ± 2.0	2.3 ± 0.3	2.0 ± 0.4
March	0.3	82.8 ± 3.8	24.3 ± 0.6	3.0 ± 0.4	1.2 ± 0.2
	5.0	35.7 ± 4.1	13.0 ± 1.5	3.3 ± 0.4	2.2 ± 0.2
Mean	0.3	79.9 ± 4.2	37.9 ± 1.6	2.9 ± 0.2	1.8 ± 0.4
	5.0	52.8 ± 2.5	26.9 ± 1.7	3.8 ± 0.5	2.8 ± 0.3
April	0.3	156.0 ± 9.2	91.2 ± 2.4	1.7 ± 0.3	11.0 ± 0.4
	5.0	136.6 ± 3.3	192.0 ± 4.0	3.3 ± 0.6	9.2 ± 0.6
May	0.3	54.0 ± 2.9	177.0 ± 3.8	2.0 ± 0.4	1.2 ± 0.2
	5.0	43.0 ± 1.9	129.3 ± 1.2	1.3 ± 0.2	3.2 ± 0.6
June	0.3	266.6 ± 14.2	41.6 ± 1.9	1.4 ± 0.2	1.1 ± 0.1
	5.0	25.6 ± 1.1	19.4 ± 1.3	0.7 ± 0.0	0.8 ± 0.1
Mean	0.3	158.9 ± 8.8	103.3 ± 2.7	1.1 ± 0.3	4.5 ± 0.3
	5.0	68.4 ± 2.1	113.6 ± 2.2	1.8 ± 0.3	4.4 ± 0.3
July	0.3	137.7 ± 2.6	75.7 ± 1.9	1.4 ± 0.1	0.6 ± 0.1
	5.0	191.2 ± 4.2	23.2 ± 1.9	1.5 ± 0.2	0.5 ± 0.0
August	0.3	76.3 ± 2.5	300.0 ± 8.2	1.3 ± 0.1	0.8 ± 0.1
	5.0	146.5 ± 4.7	89.2 ± 4.2	0.9 ± 0.1	0.5 ± 0.0
September	0.3	82.3 ± 3.1	57.5 ± 1.9	1.1 ± 0.1	1.1 ± 0.1
	5.0	147.7 ± 4.3	103.0 ± 7.7	2.0 ± 0.2	1.3 ± 0.1
Mean	0.3	98.8 ± 8.4	144.4 ± 3.3	1.3 ± 0.7	0.8 ± 0.1
	5.0	161.8 ± 6.5	71.8 ± 1.1	1.5 ± 0.2	0.8 ± 0.1
October	0.3	54.0 ± 5.3	35.5 ± 0.6	0.9 ± 0.1	1.8 ± 0.3
	5.0	129.0 ± 5.2	41.3 ± 1.4	2.0 ± 0.3	2.0 ± 0.4
November	0.3	160.0 ± 7.1	37.8 ± 1.8	1.9 ± 0.1	6.6 ± 0.5
	5.0	78.8 ± 4.3	25. 0 ± 2.0	1.9 ± 0.1	9.4 ± 0.7
December	0.3	70.3 ± 3.1	84.5 ± 1.8	1.9 ± 0.4	2.3 ± 0.1
	5.0	39.0 ± 1.5	47.5 ± 1.4	1.6 ± 0.1	0.9 ± 0.1
Mean	0.3	94.7 ± 14.3	52.6 ± 8.6	1.6 ± 0.2	3.5 ± 0.6
	5.0	82.3 ± 11.3	37.9 ± 1.1	1.8 ± 0.1	4.1 ± 0.6

'n' for each month = 6; 'n' for the mean = 12

percentages of this group of bacteria (Table 3) among total heterotrophic bacteria ranged from 0.2 - 22.8% in Shuwaikh water and 0.1 - 8.8% in Salmiya water samples. Venkateswaran *et al.* (1991) found that oil-degrading microorganisms counts ranged from as low as 3 cells to 2.3 x 10^2/100 ml in north eastern Japanese coastal waters. While the percentage of oil-degraders among total heterotrophs was less than 0.1%. At the north and south Pacific stations, Venkateswaran *et al.* (1993) found 0.1 - 1.3 x 10^3 and 0.09 - 1.7 x 10^3 CFU/ml water respectively. Walker and Colwell (1973) found 50 and 600 CFU/l of water collected from two stations in Chesapeake Bay. Eastern Bay and Baltimore harbour respectively with 3.3% and 4% respectively. Atlas and Bartha (1973) reported that the numbers of oil-degraders during a one year period, varied from as low as 20 to as high as 3400 CFU/l of Raritan Bay sea water. Atlas (1981) reported that population levels of hydrocarbon utilizers and their properties within the microbial community appear to be a sensitive index of environmental exposure to hydrocarbons. In unpolluted ecosystem, hydrocarbon utilizers generally constitute less than 0.1% of the microbial counts; in oil polluted ecosystems, they can constitute up to 100% of the viable microorganisms.

The ratios of CFU/l of oil-degrading bacteria obtained from Shuwaikh to that obtained from Salmiya (Table 3) are in the range of 1.5 - 57.0 times. The degree of elevation above unpolluted compared reference sites appears to quantitatively reflect the degree of extent of exposure of that ecosystem to hydrocarbon contaminants (Atlas 1981).

The high counts and percentages of oil-degrading bacteria were recorded during autumn from the two depths of the two stations studied. The low counts obtained from Salmiya were during spring followed by winter, while from Shuwaikh low counts were during summer. In both stations, the depth of 0.3 m as compared to the depth of 5.0 m supported high proportions of oil-degrading bacteria.

It appears from the above results that autumn and winter were suitable seasons for obtaining high proportions of oil-degrading bacteria, while spring and summer were suitable for the development of high counts of total viable heterotrophic bacteria. Total viable fungi on the other hand were stimulated more during spring and autumn. It is difficult to correlate the present results with the results of physical and chemical analysis of sea water.

From Shuwaikh water samples, 240 bacterial isolates were isolated from the colonies appeared on silica gell plates, during the period of study. Out of the 240 isolates, 180 showed good growth (as indicated from their 1 - 10 absorption scale) in liquid medium containing oil as the only carbon source. These isolates were selected

Table. 3. Mean counts of CFU of oil-degrading bacteria / litre sea water obtained from Shuwaikh and Salmiya stations during 1995 January - December.

Month	Depth (m)	Counts of CFU of oil-degrading bacteria x 10^{-3}/l				Sh / Sa
		Shuwaikh	(%)	Salmiya	(%)	
January	0.3	8.4 ± 0.5	12.8	4.0 ± 0.20	8.8	2.1
	5.0	4.0 ± 0.2	10.9	0.7 ± 0.07	2.2	5.7
February	0.3	3.6 ± 0.3	3.9	0.2 ± 0.02	0.5	18.0
	5.0	1.6 ± 0.1	1.9	0.5 ± 0.10	1.4	3.2
March	0.3	5.7 ± 0.5	6.9	0.1 ± 0.02	0.4	57.0
	5.0	3.8 ± 0.6	10.6	0.1 ± 0.02	0.8	38.0
Mean	0.3	5.9 ± 0.4	7.4	1.4 ± 0.07	3.7	4.2
	5.0	3.1 ± 0.3	5.9	0.4 ± 0.07	1.5	7.7
April	0.3	1.9 ± 0.1	1.2	1.1 ± 0.10	1.2	1.7
	5.0	0.3 ± 0.1	0.2	0.2 ± 0.01	0.1	1.5
May	0.3	2.0 ± 0.20	3.7	0.9 ± 0.07	0.5	2.2
	5.0	1.5 ± 0.20	3.5	0.2 ± 0.01	0.2	7.5
June	0.3	15.2 ± 0.2	5.7	0.6 ± 0.07	1.4	25.3
	5.0	1.5 ± 0.1	5.9	0.4 ± 0.09	2.1	3.8
Mean	0.3	6.4 ± 0.30	4.0	0.8 ± 0.07	0.8	8.0
	5.0	1.8 ± 0.07	2.6	0.6 ± 0.07	0.5	3.0
July	0.3	1.9 ± 0.3	1.4	0.5 ± 0.05	0.7	3.8
	5.0	1.3 ± 0.2	0.7	0.3 ± 0.05	1.3	4.3
August	0.3	8.9 ± 0.4	11.7	4.1 ± 0.20	1.4	2.3
	5.0	4.6 ± 0.5	3.1	2.6 ± 0.20	2.9	1.8
September	0.3	3.4 ± 0.2	4.1	1.6 ± 0.20	2.8	2.1
	5.0	1.6 ± 0.1	1.1	0.9 ± 0.10	0.9	1.8
Mean	0.3	4.7 ± 0.4	4.7	2.1 ± 0.50	1.5	2.2
	5.0	2.5 ± 0.3	1.5	1.3 ± 0.30	1.8	1.9
October	0.3	5.6 ± 0.6	10.4	2.0 ± 0.20	5.6	2.8
	5.0	3.8 ± 0.2	2.9	1.4 ± 0.20	3.4	2.7
November	0.3	4.5 ± 0.3	2.8	1.3 ± 0.10	3.4	3.5
	5.0	3.6 ± 0.2	4.6	1.8 ± 0.10	7.2	2.0
December	0.3	12.0 ± 0.4	17.1	5.8 ± 0.10	6.9	2.1
	5.0	8.9 ± 0.1	22.8	4.1 ± 0.10	8.6	2.2
Mean	0.3	7.4 ± 0.6	7.8	3.0 ± 0.30	5.7	2.5
	5.0	5.4 ± 0.4	6.6	2.4 ± 0.30	6.3	2.3

Sh / Sa = Ratios of the counts of Shuwaikh to that of Salmiya.

Table. 4. Biodegradation of crude oil and its fractions by different bacterial species, when sterilized sea water medium was used.

Bacterial Inocula	Biodegradation (%)			
	Crude oil	Saturates	Aromatics	Resins
Pseudomonas sp. (P$_1$ 810)	10.5	25.7	8.0	–
Pseudomonas sp. (P$_2$ 840)	11.9	19.4	8.5	–
Pseudomonas sp. (P$_3$ 786)	11.2	17.6	9.7	1.8
Pseudomonas sp. (P$_4$ 792)	14.7	33.7	10.5	1.0
Pseudomonas sp. (P$_5$ 722)	10.7	19.6	4.2	–
Pseudomonas sp. (P$_6$ 781)	13.5	29.4	11.2	3.1
Pseudomonas sp. (P$_7$ 1021)	12.5	25.0	–	3.4
Pseudomonas sp. (P$_8$ 1036)	19.0	24.9	17.3	–
Pseudomonas sp. (P$_9$ 1038)	9.6	22.1	11.8	–
Arthrobacter sp. (A (1) 613)	17.6	38.5	11.7	–
Arthrobacter sp. (A (2) 771)	17.8	23.4	14.8	–
Arthrobacter sp. (A (3) 701)	9.2	18.3	10.0	2.0
Arthrobacter sp. (A (4) 716)	9.9	11.1	6.0	2.7
Arthrobacter sp. (A (5) 717)	10.5	22.1	6.6	–
Arthrobacter sp. (A (6) 971)	9.7	19.3	12.9	–
Arthrobacter sp. (A (7) 990)	9.8	27.6	2.9	–
Arthrobacter sp. (A (8) 1104)	9.2	19.0	4.0	–
Arthrobacter sp. (A (9) 784)	9.7	16.6	11.5	–
Acinetobacter sp. (Act (1) 812)	9.8	25.3	7.2	–
Acinetobacter sp. (Act (2) 831)	9.8	27.5	3.7	–
Acinetobacter sp. (Act (3) 808)	12.8	23.3	13.3	–
Corynebacterium sp. (C (1) 833)	10.0	12.9	14.0	–
Corynebacterium sp. (C (2) 707)	8.8	24.8	–	–
Corynebacterium sp. (C (3) 968)	11.2	32.8	–	–
Corynebacterium sp. (C (4) 999)	10.7	21.7	7.8	–
Corynebacterium sp. (C (5) 1078)	11.0	24.3	2.5	–
Flavobacterium sp. (F (1) 788)	10.0	19.6	4.0	–
Flavobacterium sp. (F (2) 1023)	10.8	20.0	11.0	–

and studied for their ability to degrade crude oil. The results show that they fall into three groups. One group containing 90 isolates degraded less than 6% of the oil; the second group comprising 62 isolates degraded 6 - 9.5% of the oil. The third group containing 28 isolates degraded 10 - 17% crude oil; this group was selected, identified to genus level and further studied for the biodegradation of the saturates, aromatics and resins fractions of oil. The results of identification (Table 4) showed that the 28 bacterial isolates belonged to five genera; *Pseudomonas* (32.1%), *Arthrobacter* (23.1%), *Corynebacterium* (17.8%), *Acinetobacter* (10.7%) and *Flavobacterium* (7.1%). Results of the biodegradation activitie (Table 5) show that up to 38.5% of the saturates degraded by *Arthrobacter* sp.A(1), this was followed by 33.7% by *Pseudomonas* sp. P (4) and 32.8% by *Corynebacterium* sp. C(3). The high percentages degraded from the aromatic fraction were 17.3%, 14.8% and 14% by *Pseudomonas* sp. P (8), *Arthrobacter* sp. A (2) and *Corynebacterium* sp C(1) respectively. It is of interest to observe that *Pseudomonas* spp. (P (3), P (4), P (5), P (6)) were able to degrade 0.8 - 3.4% of the resin fraction. This was followed by 1.5 - 2.7% of the resin degraded by *Arthrobacter* spp. (A (2), A (3), A (4)).

Table. 5. Effect of seeding natural sea water with certain bacterial species on the biodegradation of petroleum oil and its fractions.

Bacterial Inocula	Biodegradation (%)			
	Crude oil	Saturates	Aromatics	Resins
Arthrobacter sp. A (1)	29.8 ± 1.3	64.0 ± 1.4	27.5 ± 1.1	–
Arthrobacter sp. A (2)	22.9 ± 1.3	41.3 ± 2.0	29.0 ± 1.4	–
Arthrobacter sp. A (4)	27.9 ± 1.9	51.2 ± 3.1	26.5 ± 2.1	–
Corynebacterium sp. (C 3)	23.5 ± 1.3	42.9 ± 2.5	15.8 ± 1.1	–
Pseudomonas sp. P (6)	31.5 ± 1.8	60.0 ± 1.7	27.3 ± 1.2	4.1
Pseudomonas sp. P (8)	27.9 ± 0.3	59.4 ± 1.4	30.0 ± 1.4	–
Mixture of inocula	24.7 ± 1.7	48.6 ± 2.0	18.2 ± 1.5	–
Sea water without inoculation	24.7 ± 1.7	48.5 ± 2.2	19.0 ± 1.4	–
Sea water without inoculation and without nutrients	6.6 ± 1.5	14.0 ± 1.7	11.6 ± 1.5	2.3

It is well known that the resin fraction of crude oil is hardly biodegradable. The first report describing microorganisms that were able to degrade resins was by Venkateswaran *et al.* (1995). They described a *Pseudomonas* sp. that grew on the resin fraction as a sole source of carbon and energy.

Six different species out of the 28 were selected on the basis of their biodegradation activities (Table 5). *Arthrobacter* sp. A(1), *Pseudomonas* sp. P(4) and *Corynebacterium* sp. C(3) were characterized by the degradation of high percentages of the saturates fraction. *Arthrobacter* sp. A(2) and *Pseudomonas* sp. P (8) degraded high amounts of the aromatic fraction; while *Pseudomonas* sp. P(7) was able to degrade 3.7% of the resins. The selected six species were singly seeded and in a mixture into natural sea water of the Arabian Gulf supplemented by nitrogen and phosphorus nutrients, and with 0.5% filter sterilized crude oil (Kuwait Oil Company-Blend). The results (Table 5) showed that *Arthrobacter* sp. A(1) and *Pseudomonas* sp. P(6) degraded 64% and 60% of the saturates fraction, respectively, which are 15.5% and 11.5% more than the amount degraded in sea water with and without inoculation with the mixture of the six organisms. On the other hand, *Pseudomonas* sp. P(8) and *Arthrobacter* sp. A(2) degraded 30% and 29% of the aromatic fraction, which are 10 - 12% more than the amounts degraded in the presence and in absence of the mixed inoculum. Only one out of the six species, *Pseudomonas* sp. P(6), was able to degrade 4.1% of the resins in natural sea water. Harayama *et al.* (1996) reported that seeding of microorganisms in environments contaminated by hydrocarbons may be effective in bioremediation. From the genetic stability and ease of handling points of views, the use of pure strains may be preferable than of mixed population of unidentified microorganisms.

The utilization of n-alkanes and iso-alkanes of the saturates fraction was followed by gas chromatography (GC), and the results are presented in Figs. 1-2. Kuwait crude oil (Kuwait Oil Company-Blend) contains n-alkanes C_{11}-C_{34} as well as a number of iso-alkanes (Fig. 1). Wheathering was found to remove C_{11} - C_{13} and most of C_{14}, large quantity of C_{15} and slight reduction in the other components. The microbial population found in natural sea water (without inoculation) but supplemented with nutrients (P and N salts) succeeded in the removing of all n-alkanes and iso-alkanes, except traces (Fig. 2). Inoculation of tnatural sea waters with pure or mixed cultures attained the same results. It has been recognized that in microbial cultures, easily biodegradable compounds such as n-alkanes are decomposed initially (Leahy and Colwell 1990).
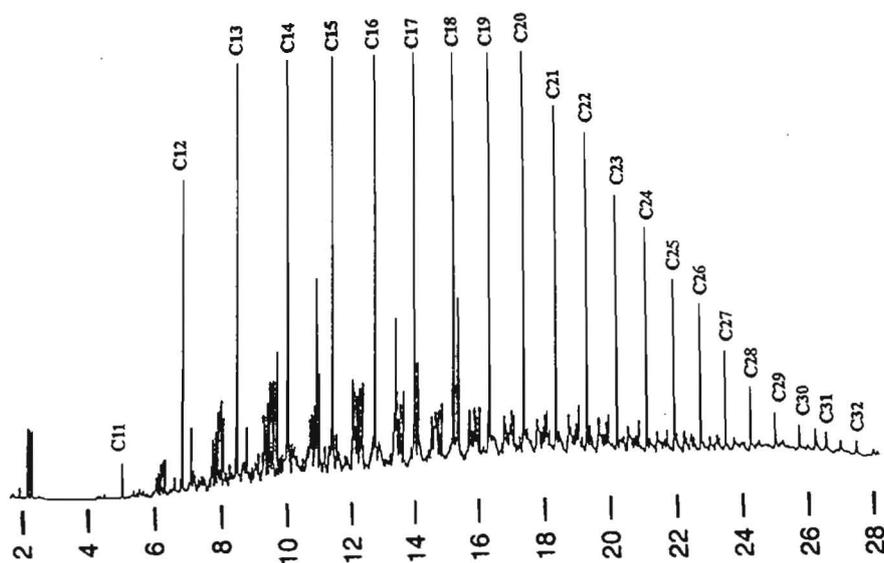
Fig. 1. Gas chromatogram showing pattern of n-alkanes and iso-alkanes components found in crude petroleum oil (Kuwait Oil Company-Blend).

Fig. 2. Gas chromatogram showing the removal of all n-alkanes and iso-alkanes (except traces) by microbial population found in sea water.

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التوزيع البيئي والنشاط التحللي للبكتيريا البحرية المحللة لزيت البترول في مياه الخليج العربي بالكويت

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قسم العلوم - كلية التربية الأساسية - ص .ب(٣٤٠٥٣) - العديلية - الكويت

تمت دراسة توزيع البكتيريا المحللة لزيت البترول في مياه الخليج العربي بالكويت ، وذلك خلال الفترة من يناير حتى ديسمبر ١٩٩٥ . ووجد أن هذه المجموعة من الكائنات الدقيقة تتراوح بين ٣ , ٠ - ٢ , ١٥ × ١٠^3 من الوحدات المكونة للمستعمرات في اللتر الواحد في محطة الشويخ (ميناء تجاري) ، و ١ , ٠ - ٨ , ٥ × ١٠^3 من الوحدات المكونة للمستعمرات في اللتر الواحد في محطة السالمية (منطقة غير ملوثة) ، وكانت النسبة المئوية للبكتيريا المحللة لزيت البترول تتراوح بين ٢ , ٠ - ٢٢ , ٨٪ في الشويخ ، و ١ , ٠ - ٨ , ٨٪ في السالمية ، كما وجد أن نسبة أعداد البكتيريا المحللة لزيت البترول في اللتر الواحد من مياه الشويخ إلى تلك الموجودة في اللتر الواحد من مياه السالمية تتراوح بين ١ , ٥ - ٥٧ مرة .

بينما وجد أن فصلي الخريف والشتاء مناسبان لتواجد أعداداً كبيرة من البكتيريا المحللة لزيت البترول ، نجد أن فصلي الربيع والصيف مناسبان للتواجد أكبر عدد من البكتيريا الكلية ، وأن فصلي الربيع والخريف مناسبان لتواجد أكثر اعداد الفطريات . وقد استطاعت ١٨٠ مزرعة بكتيرية النمو في وجود زيت البترول الخام ، منها ٢٨ مزرعة من تلك المزارع أظهرت نمواً جيداً ، وهذه المزارع تنتمي للأجناس التالية :

Corynebacterium ، (٪۲۳,۱) Arthrobacter ، (٪۳۲,۱) Pseudomonas
(٪۱۷,۸) ، Acinetobacter (۷, ۱۰٪) و Flavobacterium (٪۷,۱) . وحين
تم اختيار ستة أنواع من هذه المزارع وحقنها منفردة أو مختلطة في ماء البحر ،
وجد أن (1)Arthrobacter sp.A و (6)Pseudomonas sp.P استطاعا أن يحللا ٦٠ –
٦٤٪ من المركبات المشبعة بزيادة قدرها ٥ , ١١ – ٥ , ١٥ ٪ عن الكمية التي
تحللت في وجـود أو عـدم وجـود خليط مـن المزارع الست . بينمـا حلل كل من
(8)Pseudomonas sp.P و (2)Arthrobacter sp.A ۳۰٪ و ۲۹٪ على التـوالي ،
بزيادة قدرها ۱۰ – ۱۲٪ أعلى من الكمية التي حُللت في وجود أو عدم وجود
خليط من المزارع الست .