BIODEGRADABILITY OF NORTHERN CRUDE OILS

by

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The data for this report were obtained as a result of investigations carried out under the Environment-Social Program, Northern Pipelines, of the Task Force on Northern Oil Development, Government of Canada. While the studies and investigations were initiated to provide information necessary for the assessment of pipeline proposals, the knowledge gained is also useful in planning and assessing highways and other development projects.

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1. SUMMARY

Field and laboratory experiments have been carried out to study the factors which influence the microbial utilization of crude oil. Experiments were designed to investigate the effects of fertilizer (urea-phosphate) and oil-utilizing bacteria on the alteration of oil applied to plots in the Norman Wells area, N.W.T. and the Swan Hills area of north central Alberta. The relationship between the chemical composition of three oils originating in the Mackenzie Valley and two from the Saskatchewan area and their microbiological digestibilities, were investigated under laboratory conditions at 4° and 30°C. Soil and muskeg samples from the Mackenzie Valley area were also screened at 4° and 30°C for the presence of micro-organisms capable of using Prudhoe Bay crude oil as their sole carbon source. Preliminary phytoxicity studies of oil-saturated soil from the Swan Hills spill have also been carried out.

The results of the field studies at Norman Wells and Swan Hills showed that the application of urea-phosphate to oil-soaked soil resulted in statistically significant increases in bacterial numbers and in the rate of disappearance of the n-saturate fraction of the crude oil. The results also suggested that the application of oil-utilizing bacteria were benefical under certain soil conditions. Further study of these plots is required to determine if this disappearance of n-saturates is followed by a more rapid return of the native flora. These results also indicated the need to expand the scope of these field experiments to (a) determine the form of nitrogen which is most stimulating and whether phosphate or other nutrients would be beneficial; and (b) to treat an actual spill where the oil concentration is much greater than that applied in our experiments.

The laboratory studies relating chemical composition of oil to biodegradability have shown that bacterial populations developed on high quality, crude oils have very little ability to digest low quality oils. However, populations developed on low quality oils can utilize high quality oils. The temperature at which a population is enriched also has a marked effect on its oil digesting capabilities. Populations enriched at 4°C can utilize the same oil at 30°C but cultures derived at 30°C cannot utilize oil at 4°C. These results point out the need to know the quality of oil being transported by pipelines as well as the prevailing environmental conditions. This information is required if microbial seeding of such spills is contemplated so that populations can be produced which will be effective under the existing conditions.

The results of our preliminary survey of Mackenzie Valley soils for oil-digesting micro-organisms indicate that such activity is not found uniformly amongst the indigenous microbial flora. Unfortunately, not enough samples have been studied to relate this lack of uniformity to any particular area or soil feature. The results do suggest that more of these organisms are present which can utilize Prudhoe Bay oil at 30°C than at 4°C. The soil samples from the burn area near Inuvik are a good illustration of this point: four out of the five samples showed activity at 30°C but only one of the five did so at 4°C. This part of the project should be extended to cover more thoroughly the projected pipeline routes. If large areas of soil are found to be deficient in oil-utilizing bacteria then microbial seeding of oil spills in such areas will be required.

Our preliminary phytoxicity studies indicated a toxic effect of oil-soaked soil on the germination of barley seeds. The experiments also showed an added toxic effect when such oil plots had been treated with fertilizer. However, the design of the experiments (which was set up to determine the effects of amendments on oil disappearance) did not allow for a separation of the interaction of fertilizer and oil on germination.

In summary, the results presented indicate that bacteria are capable of utilizing oil under field and laboratory conditions at 4°C (i.e. temperatures relevant to the Mackenzie Valley area). The data presented also support our working hypothesis that treatment of oil spills to accelerate microbial activity has a major role to play in the ultimate clean-up of such environmental disasters.

2. INTRODUCTION

These investigations compare the biodegradability of northern Canadian and of Alaskan crude oils which might be transported through a Mackenzie Valley pipeline. Studies are being conducted in the field and in the laboratory to establish factors affecting the biodegradability of such oils. Specifically the following problems are being investigated:

a) effect of additives such as fertilizer on the disappearance of oil from oil-soaked soil.
b) distribution in arctic and sub-arctic soils, particularly those of the Mackenzie Valley area, of micro-organisms which can use northern crude oils as the sole carbon source.
c) comparison of the ability of micro-organisms capable of using oil of one quality to use oils of a different quality under psychrophilic and mesophilic conditions. (N.B. quality here refers to the content of paraffins, aromatics, NSO's etc.). This is referred to as the "challenge series".
d) toxicity of oil-treated northern soils to indicator plants, e.g. barley.

These investigations are centered on studies of factors affecting microbial activities, bacteria, fungi and yeasts in these soils, as these are the only biological species known so far, which can metabolise crude oil or its components. It is imperative that knowledge of such factors be obtained - particularly under extreme, northern climatic conditions - if means are to be found to accelerate the rehabilitation of oil-soaked soils.

This work is directed to understanding the factors which affect biodegradation of oil under northern climatic conditions and, correspondingly, to evaluating biodegradation as a means of cleaning up oil spills.

3. RESUME OF CURRENT STATE OF KNOWLEDGE

Existing knowledge on factors affecting the degradation of oil accidentally spilled on soil and water is very limited. The fact that oil spills, whether on water or soil, do disappear is well documented but very little is known about what can be done to accelerate this process. Suggestions have been made for microbial seeding of spills as it is accepted that micro-organisms are the only biological species which have the metabolic capability of utilizing petroleum carbon for cell synthesis. However, to our knowledge this procedure has not been tried under field conditions.

Our hypothesis for treating oil spills is centered around the fact that crude oil is essentially a mixture of carbon and hydrogen. Accidental spills on soil will result in an unbalance in the carbon-nitrogen ratio at the spill site. To grow well, bacteria require about 10 parts carbon to 1 part nitrogen; if the ratio is greater, say 100:1 or 1000:1, the bacteria will not reproduce efficiently and so they will not be able to utilize the new carbon source in and on the soil. (N.B. - fungi require about 20 parts carbon to 1 part nitrogen for growth but our interest has been centered on the activities of bacteria which grow more rapidly). In addition to there being a nitrogen deficiency in oil-soaked soil, there is a distinct possibility that a deficiency of phosphorus or other nutrient could be aggravated by an oil spill. Therefore, in order to test this hypothesis we decided to add the nitrogen, as urea phosphate, thus adding two nutrients in one application. The other problem which exists when oil is applied to or spilled on soil is the question as to whether or not oil-utilizing micro-organisms are present. Thus the much-discussed suggestions to inoculate such spills. Our plot experiments were also designed to test the effect of inoculating such spills with oil-digesting bacteria.

The ability of micro-organisms to digest crude oils of differing quality (i.e. chemical composition) has not been investigated. Most published work has been concerned with the utilization of mixtures of chemically-defined hydrocarbons. Therefore, laboratory studies were designed to compare the efficiency of microbial populations in utilizing oil of different qualities. Such information is essential if micro-organisms are ever going to be used to treat oil spills.

Our knowledge of the microbiological capacity of the northern soils and muskeg is extremely limited. Environmental factors indicate that one would be handling psychrophiles and that

the maximum soil temperature experienced in the summer would be about 20°C. For the rest of the year, the soil would be extremely hazardous for the transport of a material like crude oil. Therefore, in order to try and determine the ability of such soils to degrade oil spills under extreme environmental conditions, laboratory analysis of Mackenzie Valley soils and muskegs was undertaken to assess their oil-utilizing capability at 4°C and 30°C. Such information is required to ascertain if microbial seeding might be considered for oil spilled on this area of the sub-Arctic.

4. STUDY AREAS

The major problem is the relationship between chemical structure and biological activity. The chemical structures we are concerned with are those found in crude oils while the biological activity we are concerned with is the ability of microorganisms to metabolize crude oils of varying chemical composition. This problem is further complicated by the fact that the environmental situation where these factors might be interacting is a psychrophilic one. Therefore, field and laboratory experiments have been established to study the factors controlling this relationship under these extreme conditions.

Geographically, emphasis is being placed on obtaining experimental data which is applicable in the north and particularly in the Mackenzie Valley. Some information is being obtained from plots in north-central Alberta where environmental conditions, while not as harsh, are similar to those experienced in the Mackenzie Valley.

5. METHODS AND SOURCES OF DATA

(a) FIELD STUDIES

Four replicated plots of each treatment (i.e. control; plus oil; plus oil and bacteria; plus oil and fertilizer; plus oil, bacteria and fertilizer), were laid out in the Norman Wells area of the Northwest Territories and in the Swan Hills area of north central Alberta (see Appendix for Swan Hills plan). The plots in the Norman Wells area were laid out on a straight cut-line and thus resolved themselves into bush and cut-line sections. The Swan Hill plots were on an overgrown, unused airstrip. The oil used in each spill was obtained from producing units in the area, that is Imperial Oil at Norman Wells and Shell Oil Company in the Swan Hills. It was applied by sprinkling from watering cans at a rate of 60 l. of crude oil per 9 sq. m. of plot. Urea phosphate fertilizer (27-27-0) was applied to eight plots at a rate calculated to yield 60 gm of nitrogen/m^2 (equivalent to 600 kg nitrogen/hectare). A mixed culture of bacteria which was capable of utilizing Norman Wells oil was applied to eight plots, four of which had received a fertilizer treatment. The cells used were grown in the laboratory, the cells recovered by centrifuging, washed and resuspended in tap water at 4°C. This suspension was diluted to a concentration such that application of six l. per plot yielded an application rate of 10^6 bacteria/cm^2.

Composite soil samples are being taken periodically and analyzed for total bacterial and fungal counts. When samples are taken from plots which received an oil treatment, the oil is extracted and its chemical composition determined by chromatography (see Appendix for details). This technique allows treatments to be statistically analysed as a function of time so that the efficacy of treatments in accelerating the breakdown of oil can be assessed.

The microbial counts were carried out at 4°C or 21°C in quintuplicate using plate count agar (Difco) for bacteria and a malt extract agar (Difco) for molds. Plates at 4°C were incubated for 14 days while those at 21°C were incubated for 4 to 6 days. The data were tested statistically to determine levels of significance.

The chromatographic techniques resolved the crude oil into an asphaltene, a saturate, an aromatic and an NSO fraction. The saturate fraction was further resolved using G.L.C. (see Appendix for details). The results from the liquid chromatographic technique are expressed on a gravimetric basis while the G.L.C. results are initially analyzed by comparing the profiles of

extracted oil from treated plots with those from the untreated ones. The peak area data is also available and may be used for comparative purposes.

The analytical procedures are laborious and time-consuming, therefore sampling has been programmed to provide a continual input of samples which are analyzed as quickly as possible after sampling. All samples are stored at -20°C. The Swan Hills spill has been sampled 12 and 66 days after spilling of the oil and application of additives while the Norman Wells spill was sampled 22 days after application of the oil. Further samples will be taken as soon as environmental conditions permit and they will be subjected to all analytical procedures. Plant succession will be noted on the various plots in the two spill sites.

(b) MICROBIAL ANALYSIS OF SUB-ARCTIC SOILS FOR THE CAPABILITY OF USING PRUDHOE BAY CRUDE OIL AS THE SOLE CARBON SOURCE

The initial bacterial and mold populations of these samples was determined at both 4°C and 21°C using the techniques described in 5a and the Appendix.

The enrichment technique was used to detect the presence of microbes capable of growing on Prudhoe Bay oil. These enrichments consisted of 100 mls of B + N salt medium (see Appendix for composition) plus 1 ml of Prudhoe Bay oil in a 250 ml Erlenmeyer flask. These were set up in duplicate and each inoculated with 1 gm of soil - one flask being incubated at 4°C and the other at 30°C on rotary shakers (radius, one inch eccentricity). At seven day intervals, 25 mls of culture were used to inoculate a fresh flask of medium. The physical appearance of the culture, that is whether emulsification had taken place or not, and the total bacterial counts were determined on the 5th or 7th day of the 4th transfer. The oil was then recovered and its chemical composition determined. A comparison of the gravimetric and G.L.C. profiles was made with untreated oil to determine whether or not utilization had taken place.

(c) CHALLENGE SERIES

Microbial populations which would grow on the following crude oils - Norman Wells, Prudhoe Bay, Atkinson Point, Lost Horse Hill and North Cantal crude oils, were established by the enrichment procedure. The Lost Horse Hill and North Cantal crude oils, which originate in Saskatchewan, were included to provide a more

complete spectrum of oil qualities. Populations were enriched for four transfers at both 4°C and 30°C and then used to test other oils within and between the same temperature range. Total bacterial plate counts as well as chromatographic analysis of the oil were carried out on all samples. In addition, the generic composition of such populations was established (see Appendix for details). The ability of microbial populations to use oils of higher and/or lower quality was therefore comparable on both a microbial and chemical basis.

(d) PHYTOTOXICITY STUDIES

Soil samples were air-dried for two days then spread on aluminum foil trays to provide enough area to accomodate 500 barley seeds. After seeding, the soil was covered with a layer of cheesecloth and brought to field capacity. This level of moisture was maintained throughout the germination period. The flats were incubated at 70°F with a 16 hours per day photo-period. Germinated seeds were counted after 14 days incubation. The effect of oil on germination was obtained by comparing counts from treated and untreated substrates.

6. RESULTS

(a) FIELD STUDIES

(i) Norman Wells Oil Spill

The total microbial counts and pH values recorded in these plots 22 days after treatment are presented in Table A1. The mean microbial counts for the Norman Wells - Cut Line (Table I) and Bush plots (Table II) show that application of fertilizer and oil results in significant increases in bacterial numbers but not in the numbers of molds present in these soils. The pattern of significance (Table III), 95 versus 99% confidence level, varies somewhat with the type of plot, bush or cut-line, and whether the incubation temperature for microbial enumeration was 4°C or 21°C. The results also suggest that bush plots but not line plots responded to the application of bacteria with the oil. No significant change was detectable in the numbers of molds in these experiments.

The complete analysis of the oil recovered from these plots is presented in Table AII. The mean % values (Table IV) suggest that the n-saturate fraction is being utilized faster where fertilizer was applied with the oil. Statistical analysis of the saturate data (Table V) confirms this for the bush plots when bacteria were added with fertilizer. The line plot response was also significant but only at the 95% confidence level. The gas-liquid chromatographic profiles representative of these fractions (Figures 1, 2, 3 and 4) confirm accelerated utilization of saturates when fertilizer was applied with the oil. It is to be noted that the isoprenoids, phytane and pristane, are still present, suggesting a higher degree of resistance to microbial attack than for n-saturates.

The data also indicate a differential response of cut-line and bush plots to additives. The bush plots appear to be responding to fertilizer and bacteria whereas the line plots response could be due to fertilizer alone.

TABLE I - NORMAN WELLS OIL SPILL - Cut-Line Plots

Summary of mean microbial counts and pH values - 22 days after treatment

Treatment	pH	Bacteria x 10^6/gm		Molds x 10^3/gm	
		4°	21°	4°	21°
Control	7.0	36.9	104.7	110.7	268.5
Oil	6.8	46.1	167.5	86.0	162.5
Oil + Bacteria	6.8	89.5	307.5	50.5	169.3
Oil + Fertilizer	6.4	7735.0	14875.0	73.3	103.8
Oil + Bacteria + Fertilizer	6.3	7707.5	12750.0	224.5	824.3

TABLE II - NORMAN WELLS OIL SPILL - Bush Plots

Summary of mean bacterial counts and mean pH values - 22 days after treatment

Treatment	pH	Bacteria x 10^6/gm		Molds x 10^3/gm	
		4°C	21°C	4°C	21°C
Control	6.9	46.0	104.5	104.8	495.0
Oil	6.6	111.5	299.0	46.0	348.0
Oil + Bacteria	6.7	848.0	232.3	36.5	130.8
Oil + Fertilizer	6.4	15,310.0	19,000.0	91.5	230.0
Oil + Bacteria + Fertilizer	6.3	21,125.0	26,375.0	62.0	284.3

TABLE III - NORMAN WELLS OIL SPILL

Statistical analysis[x] of bacterial count date - 22 days after start of treatment

	Confidence Level							
	Bush Plots				Cut Line Plots			
	95%		99%		95%		99%	
Treatment	4°C	21°C	4°C	21°C	4°C	21°C	4°C	21°C
Control	-	-	-	-	-	-	-	-
+ Oil	0	0	0	0	0	0	0	0
+ Oil + Bacteria	1	1	0	1	0	0	0	0
+ Oil + Fertilizer	1	1	0	1	1	1	1	1
+ Oil + Bacteria + Fertilizer	1	1	1	0	1	1	1	1

[x] Control versus treatments; value of 1= significant difference at 95 or 99% confidence level

TABLE IV - MEAN PERCENT COMPOSITION OF NORMAN WELLS OIL AFTER 22 DAYS OF CONTACT WITH SOIL PLUS VARIOUS AMENDMENTS

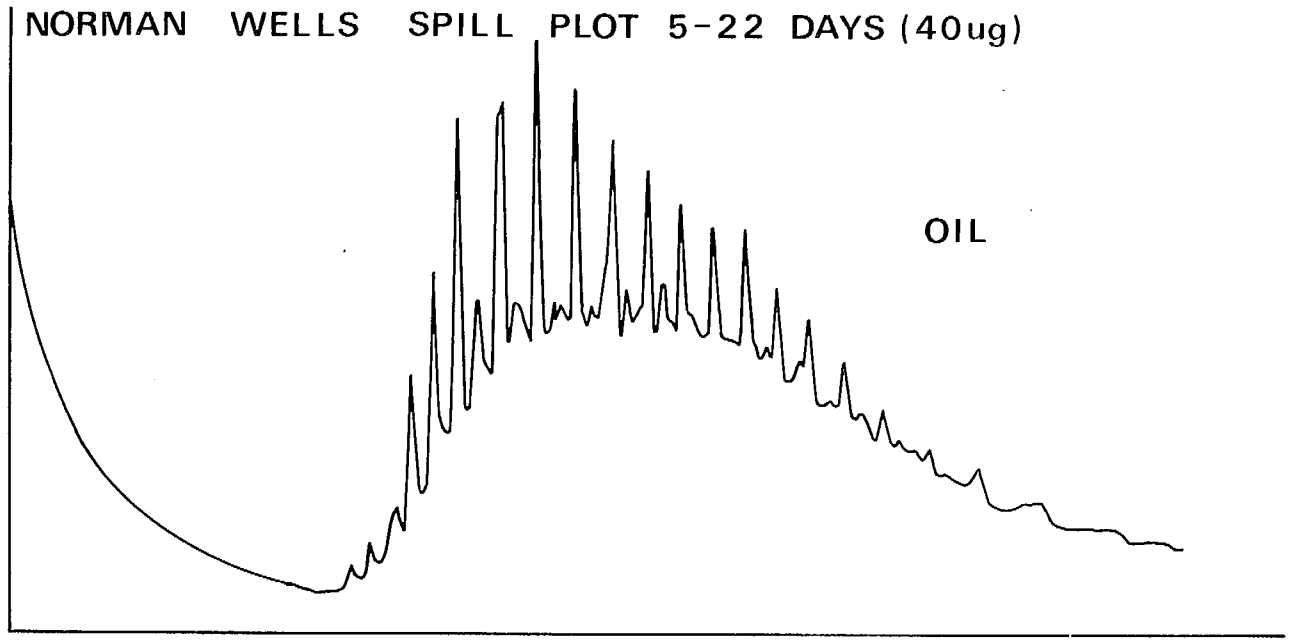
Crude Oil Fraction	% Composition of Oil								
		Soil		+ Bacteria		+ Fertilizer		Bacteria + Fertilizer	
	Barrel	Bush	Line	Bush	Line	Bush	Line	Bush	Line
Asphaltenes, soluble	2.24	4.98	4.30	2.80	4.93	3.72	5.26	4.43	5.20
Asphaltenes, insoluble	8.49	6.86	7.14	6.67	6.17	7.25	7.03	8.45	8.07
Saturates	45.98	47.18	51.51	48.95	48.73	44.92	42.33	43.40	42.55
Aromatics	29.29	26.41	25.77	27.09	26.59	27.69	28.38	27.76	27.47
NSO's, soluble	10.38	8.65	9.11	9.50	9.11	9.88	10.16	10.10	9.73
NSO's, insoluble	3.61	5.97	4.38	4.85	4.53	6.55	6.85	5.86	6.84

TABLE V - STATISTICAL ANALYSIS[x] OF THE SATURATE FRACTION DATA,

NORMAN WELLS SPILL, 22 DAYS AFTER START OF TREATMENT

	CONFIDENCE LEVEL			
	Bush Plots		Line Plots	
Treatment	95%	99%	95%	99%
+ Oil	-	-	-	-
+ Oil + Bacteria	0	0	0	0
+ Oil + Fertilizer	0	0	1	0
+ Oil + Bacteria + Fertilizer	1	1	1	0

[x] Oil versus treatments: value of 1= significant difference at 95 or 99% confidence level.



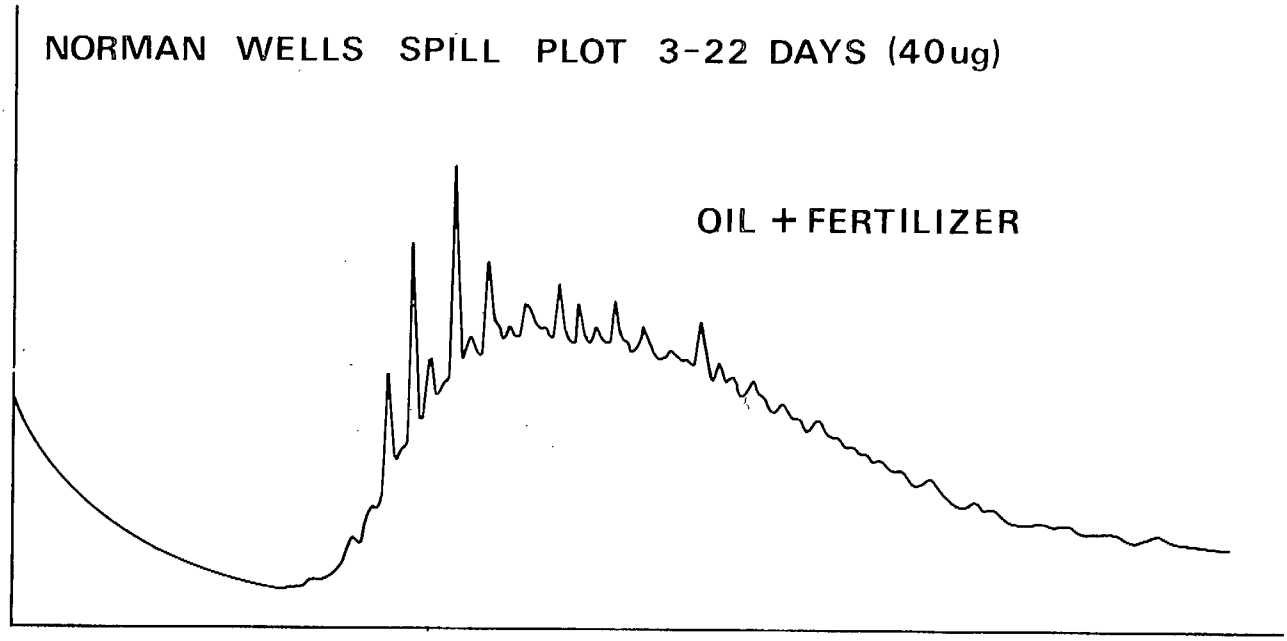
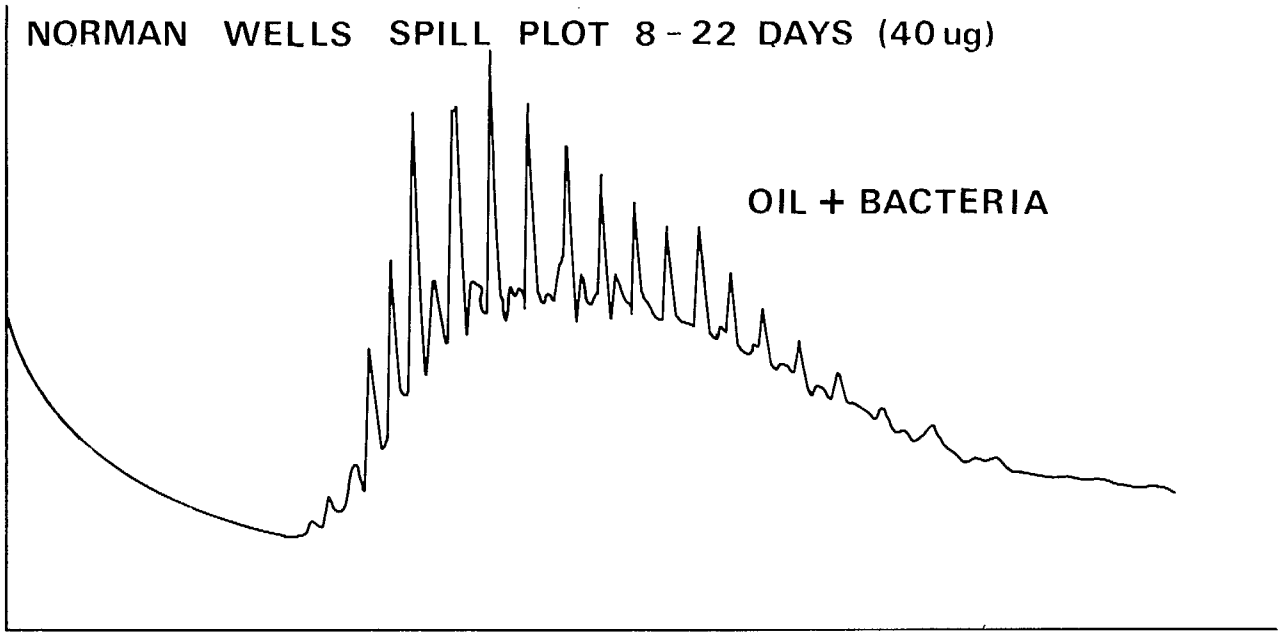


Figure 1 - G L C analysis of the saturate fraction of Norman Wells oil - Line Plots (Plot 5 - oil and Plot 3 - oil plus fertilizer)



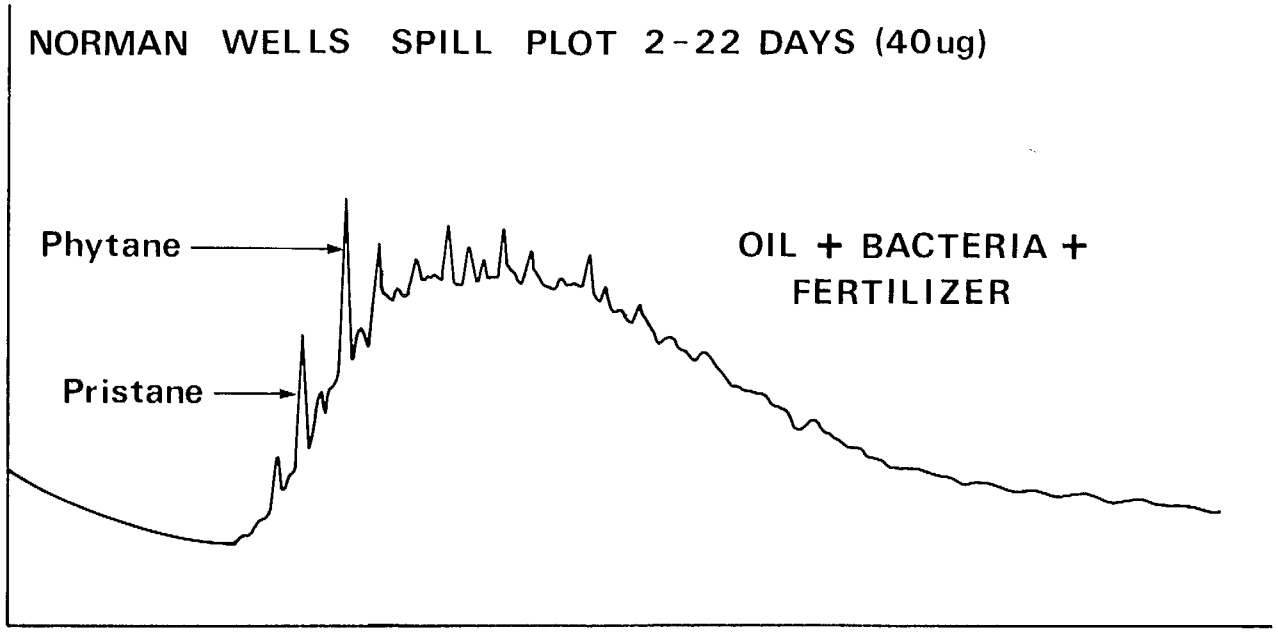


Figure 2 - G L C analysis of the saturate fraction of Norman Wells oil - Line Plots (Plot 8 - oil bacteria and plot 2 - oil plus bacteria plus fertilizer)

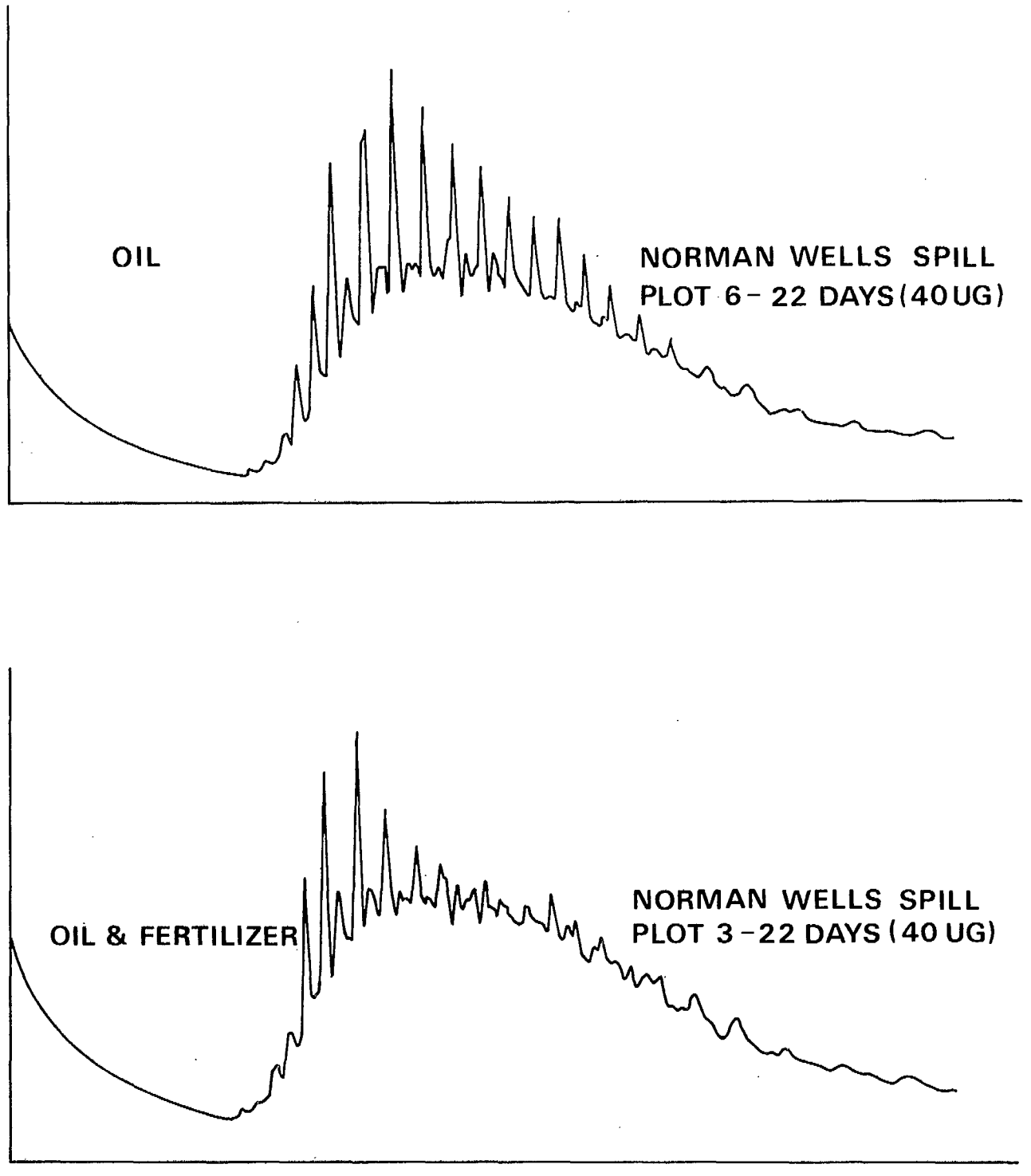
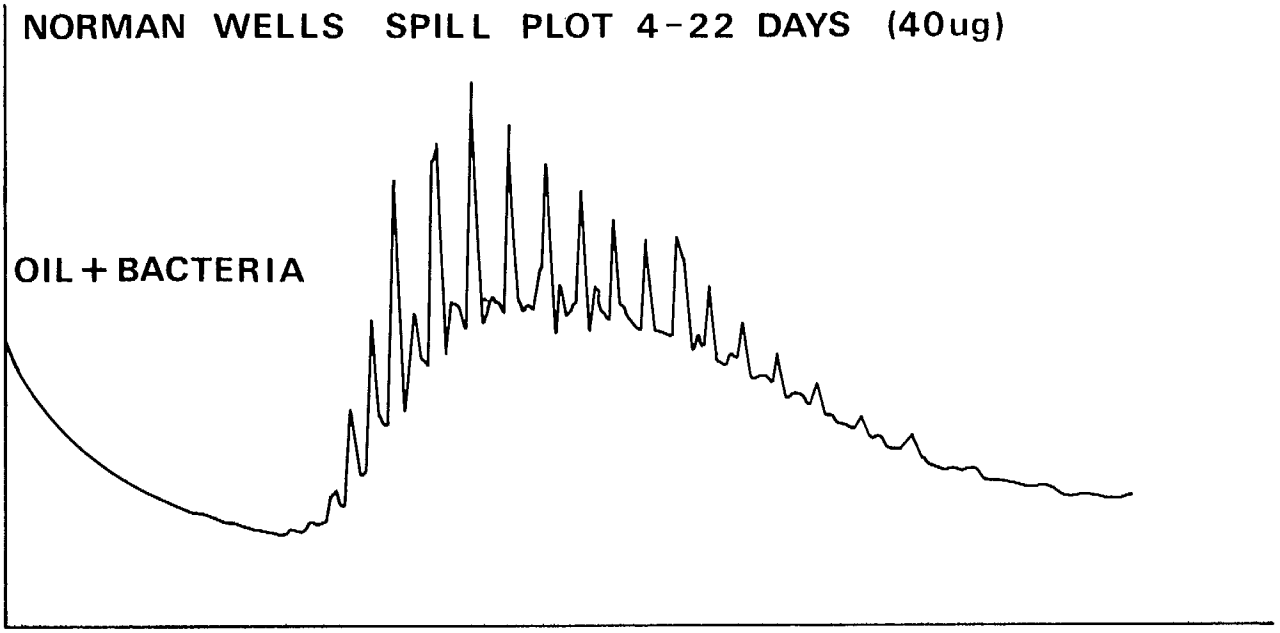


Figure 3 - G L C analysis of the saturate fraction of Norman Wells oil - Bush Plots (Plot 6 - oil and Plot 3 - oil plus fertilizer)



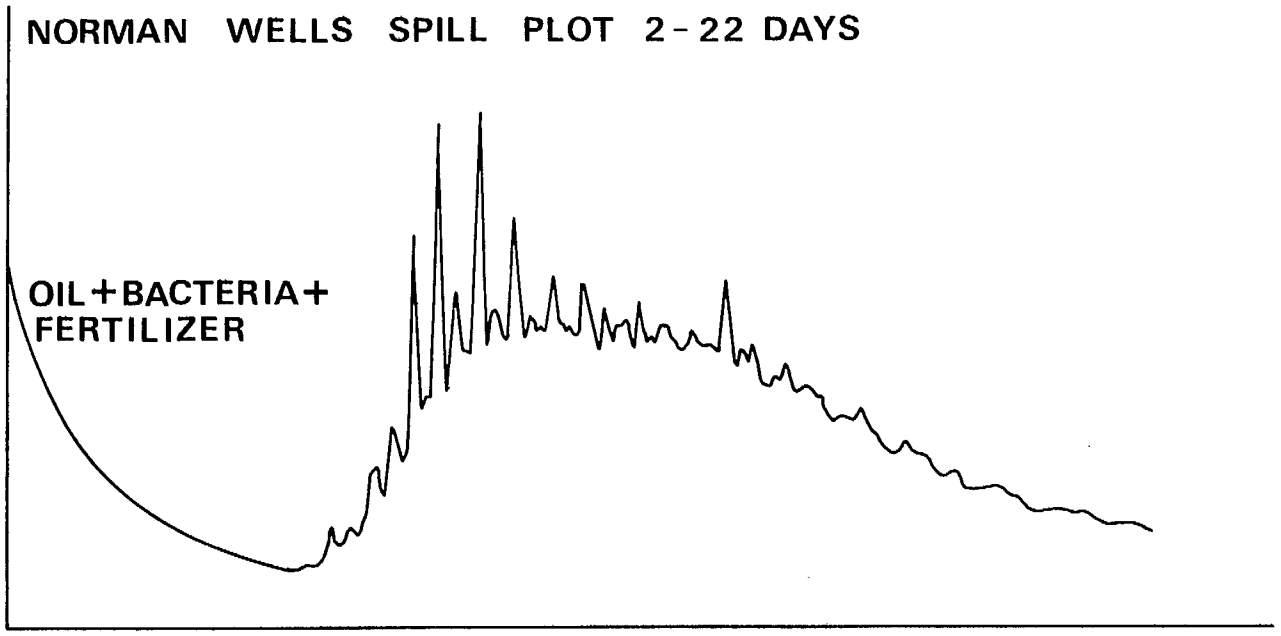


Figure 4 - Analysis of the saturate fraction of Norman Wells oil - Bush Plots (Plot 4 - oil plus bacteria and Plot 2 - oil plus bacteria plus fertilizer)

(ii) Swan Hills Oil Spill

The total microbial counts and pH values for the Swan Hills oil spill at 12 and 66 days after treatment, are presented in Tables AIII and AIV. The results of the 12 day data (Table VI) show a marked stimulation of bacterial numbers but not of molds where fertilizer has been added to oil-soaked plots. Statistical analysis of these results (Table VII), confirms these observations particularly at the 95% confidence level.

The complete chemical analysis of the oil extracted from the plots 12 days after its application is presented in Table AV. The mean values of these data (Table VIII) suggest that very little change has taken place in the oil during this period. Statistical analysis of the saturate fraction showed no significant differences between oil and oil-with-additive treatments. However, gas-liquid chromatographic analysis of these fractions (Figures 5, 6, and 7) indicates that where fertilizer was applied, saturate utilization was taking place at an accelerated rate.

A summary of the microbial counts 66 days after treatment (Table IX) shows increased levels of bacteria but not of molds in those plots which had been treated with oil and fertilizer. Statistical analysis of this data (Table X) confirm the significance (99% level) of this increased count when both fertilizer and bacteria were applied with oil but only at the 95% level when fertilizer was the sole additive.

The complete chemical analysis of the oil extracted from the plots before and after 66 days in the soil is presented in Table XI and indicates accelerated saturate decomposition where fertilizer had been added with the oil. Statistical analysis of data on the saturate fraction (Table XII) confirms the significance at both the 95 or 99% confidence levels. G.L.C. analyses of representative saturate fractions (Figures 8 and 9) confirm the stimulatory effect of fertilizer on the utilization of this fraction of crude oil. The tracings suggest that the isoprenoids phytane and pristane have not yet been completely metabolized. Comparing the tracing of the central (oil only) plot with that of the oil-with-bacteria plot suggests a slightly increased utilization of those saturates of chain length C20 to C25. Some differential response between plots receiving the same treatment is noted (Figures A2 and A3).

TABLE IV - SWAN HILLS OIL SPILL

Summary of mean microbial counts and mean pH values - 12 days after treatment

Treatment	pH	Bacteria x 10^6/gm	Bacteria x 10^3/gm
Control	5.2	13.5	205.5
+ Oil	5.1	59.5	47.8
+ Oil + Bacteria	5.2	77.0	124.2
+ Oil + Fertilizer	5.7	5162.5	20.5
+ Oil + Bacteria + Fertilizer	5.9	3753.3	26.8

TABLE VII - SWAN HILLS OIL SPILL

Statistical analysis[x] of bacterial count data - 12 days after treatment

Confidence Level	95%	99%
+ Oil	-	-
+ Oil + Bacteria	0	0
+ Oil + Bertilizer	1	1
+ Oil + Bacteria + Fertilizer	1	0

[x] Oil versus oil plus treatments;value of 1= significant difference at 95 or 99% confidence levels.

TABLE VIII - CHEMICAL COMPOSITION OF SWAN HILLS OIL AFTER 12 DAYS IN CONTACT WITH SOIL PLUS VARIOUS AMENDMENTS

Crude Oil Fraction	% Composition* of Oil				
	Barrel	Soil	+ Bacteria	+ Fertilizer	+ Bacteria + Fertilizer
Asphaltenes, soluble	2.90	1.00	1.29	1.48	1.62
Asphaltenes, insoluble	9.56	5.04	3.96	4.55	4.70
Saturates	55.08	62.75	62.88	59.12	59.89
Aromatics	21.58	21.18	21.70	23.72	24.13
NSO's, soluble	5.66	6.40	6.23	6.72	7.15
NSO's, insoluble	5.22	3.65	5.37	4.28	4.32

* Average of values from replicate plots.

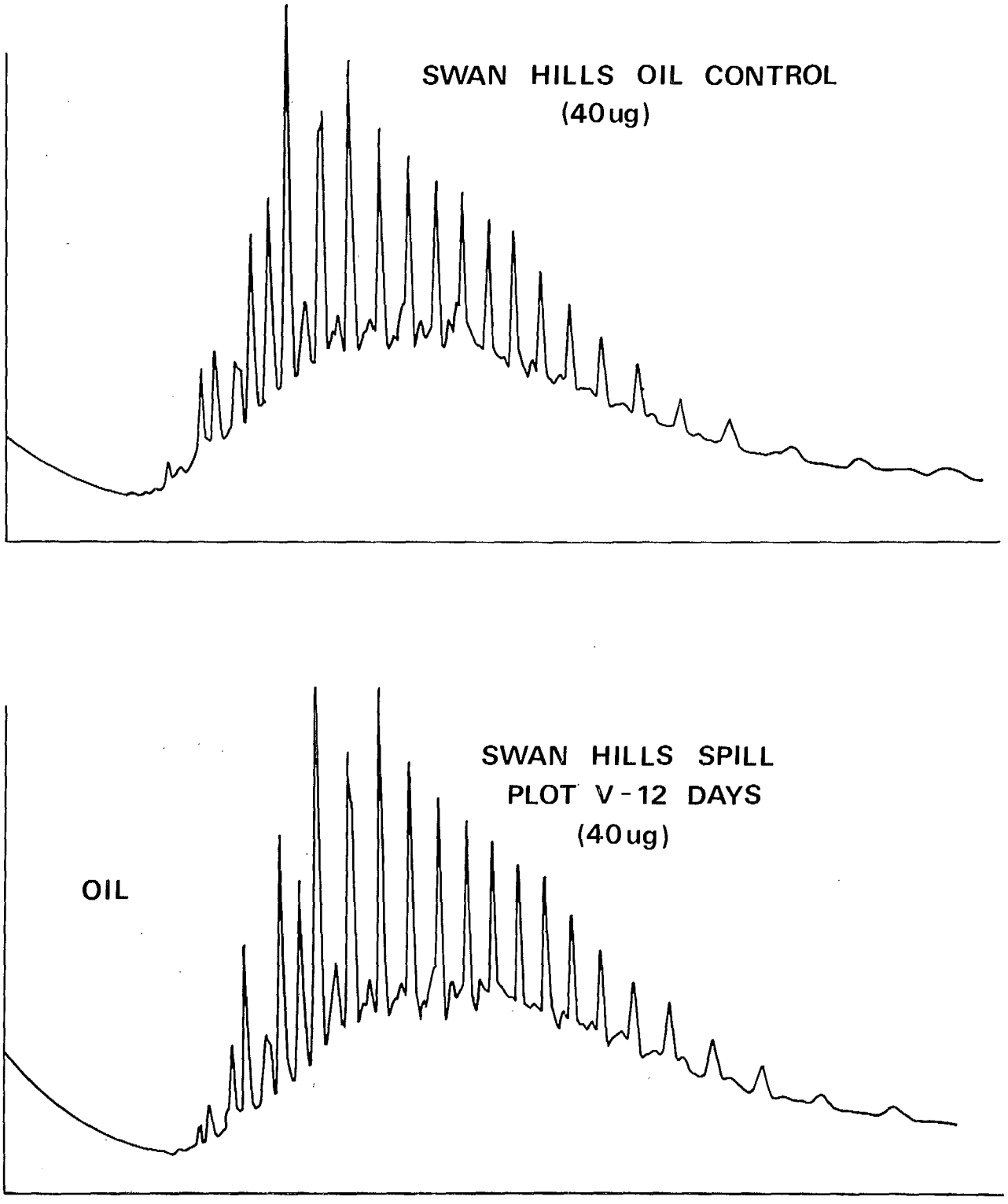


Figure 5 - G L C analysis of the saturate fraction of Swan Hills Oil Control and Plot V (oil alone)

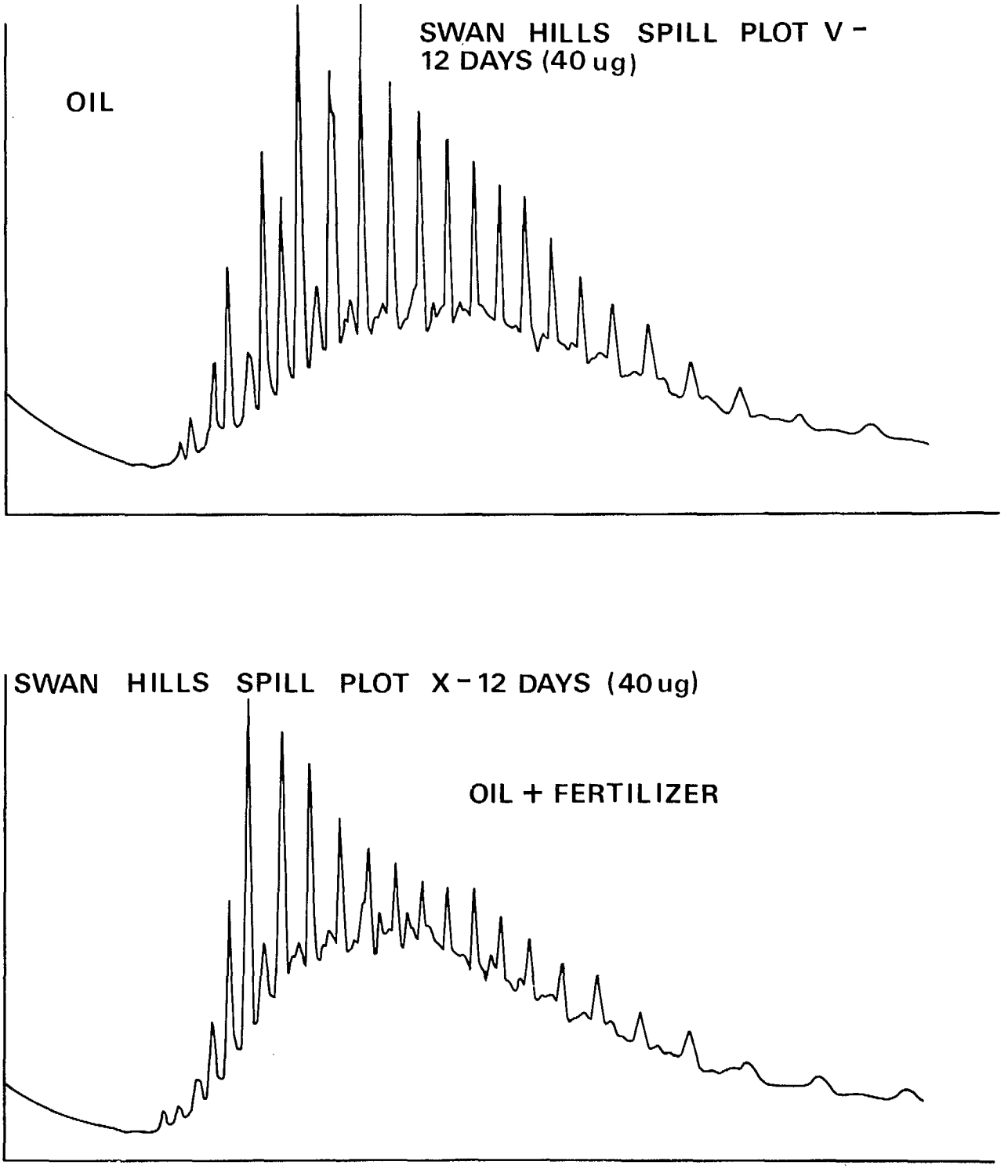


Figure 6 - G L C analysis of saturate fraction of Swan Hills oil
(Plot V, oil alone and plot X, oil and Fertilizer)

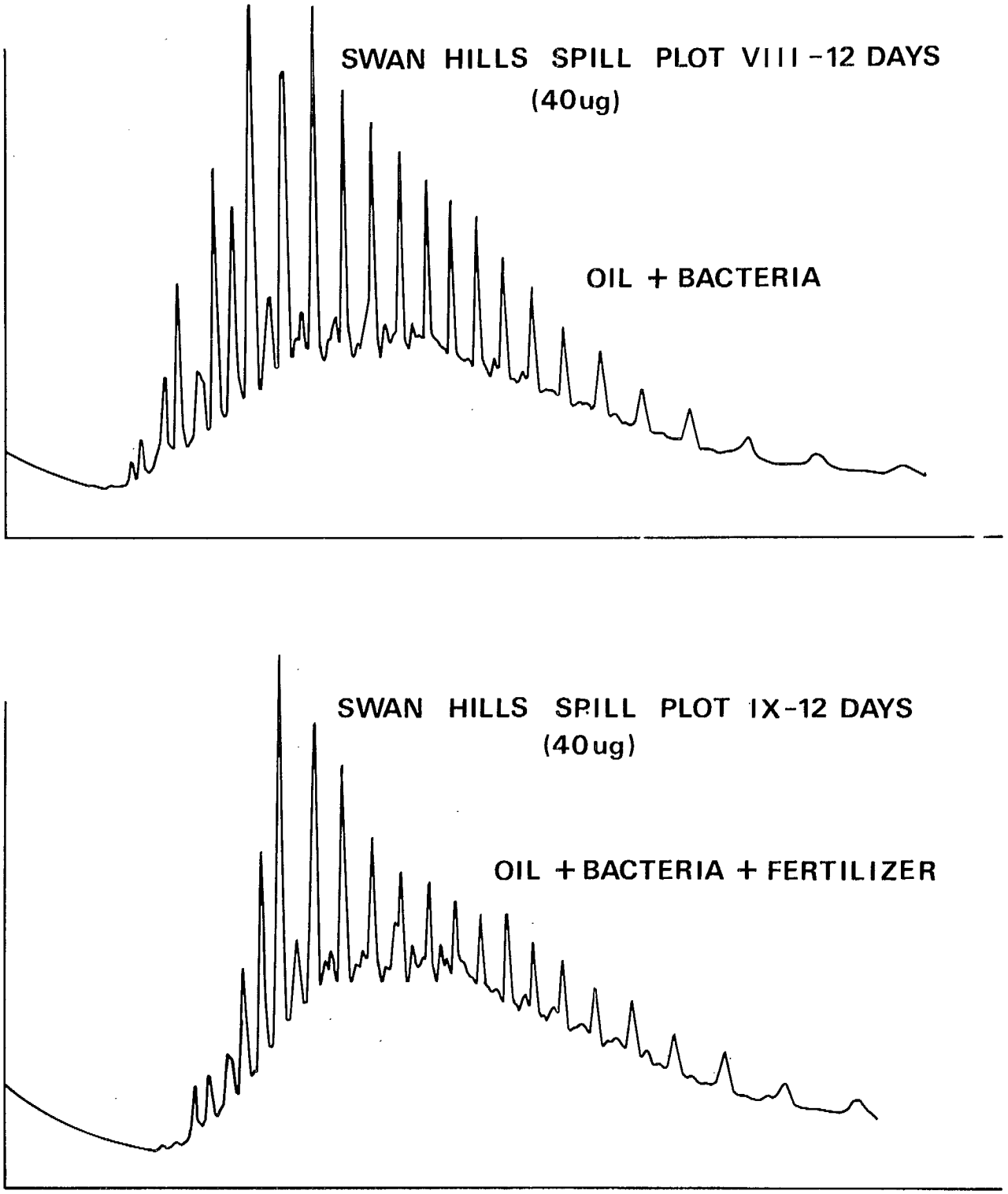


Figure 7 - G L C analysis of saturate fraction of Swan Hills oil (Plot VIII oil plus bacteria and Plot IX oil plus bacteria and fertilizer)

TABLE IX - SWAN HILLS OIL SPILL

Summary of mean microbial counts and mean pH values - 66 days after treatment

Treatment	pH	Bacteria x 10^6/gm	Molds x 10^3/gm
Control	5.4	56.7	53.0
+ Oil	5.5	281.5	182.9
+ Oil + Bacteria	5.5	312.5	131.3
+ Oil + Fertilizer	6.0	574.5	107.0
+ Oil + Fertilizer + Bacteria	5.6	1710.0	59.4

TABLE X - SWAN HILLS OIL SPILL

Statistical analysis[x] of bacterial count date - 66 days after treatment

Confidence Level	95%	99%
+ Oil	-	-
+ Oil + Bacteria	0	0
+ Oil = Fertilizer	1	0
+ Oil + Bacteria + Fertilizer	1	1

[x] Oil versus oil plus treatment;value of 1= significant difference at 95 or 99% confidence levels.

TABLE LX - CHEMICAL COMPOSITION OF SWAN HILLS OIL AFTER 66 DAYS IN CONTACT WITH SOIL PLUS VARIOUS AMENDMENTS

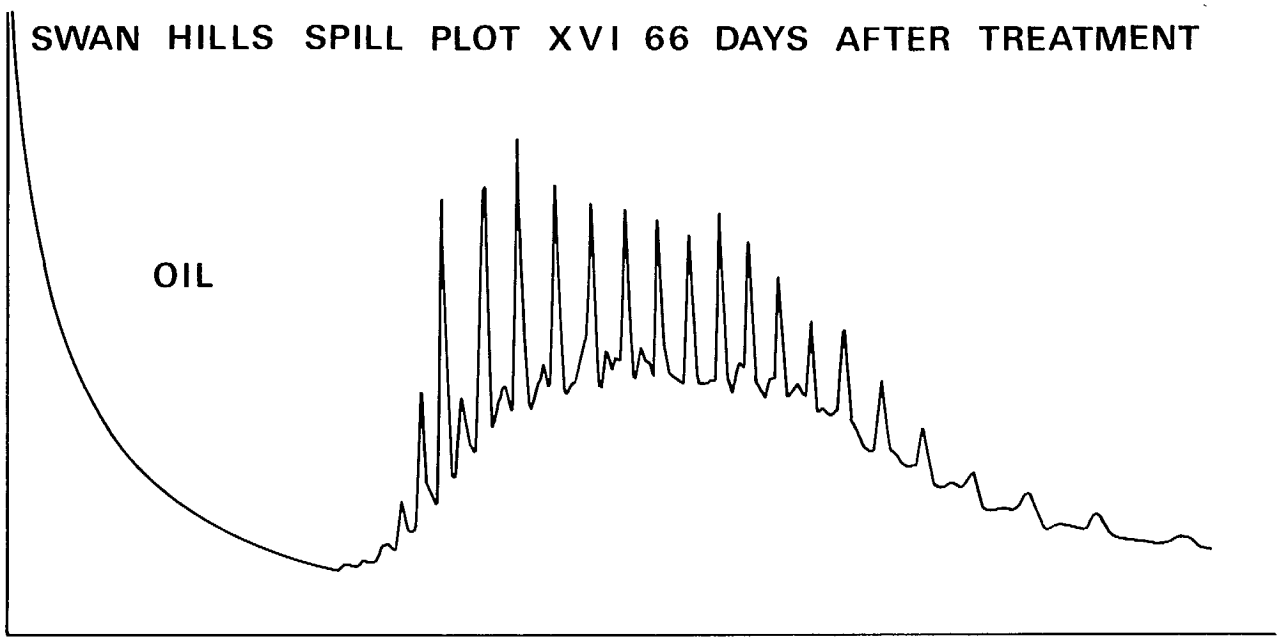
Crude Oil Fraction	% Composition of Oil				
	Barrel	Soil	+ Bacteria	+ Fertilizer	+ Bacteria + Fertilizer
Asphaltenes, soluble	2.90	2.52	2.77	4.34	2.99
Asphaltenes, insoluble	9.56	5.88	6.49	10.81	9.00
Saturates	55.08	61.45	60.78	46.32	46.23
Aromatics	21.58	22.62	20.19	23.23	25.58
NSO's, soluble	5.66	7.09	7.68	10.43	10.27
NSO's, insoluble	5.22	2.12	5.76	4.88	5.93

TABLE XII - STATISTICAL ANALYSIS* OF THE SATURATE FRACTION DATA, SWAN HILLS SPILL

66 DAYS AFTER START OF TREATMENT

Treatment	95%	99%
+ Oil	-	-
+ Oil + Bacteria	0	0
+ Oil + Fertilizer	1	1
+ Oil + Bacteria + Fertilizer	1	1

* Oil versus oil plus treatments; value of 1= significant difference at 95 or 99% confidence level.



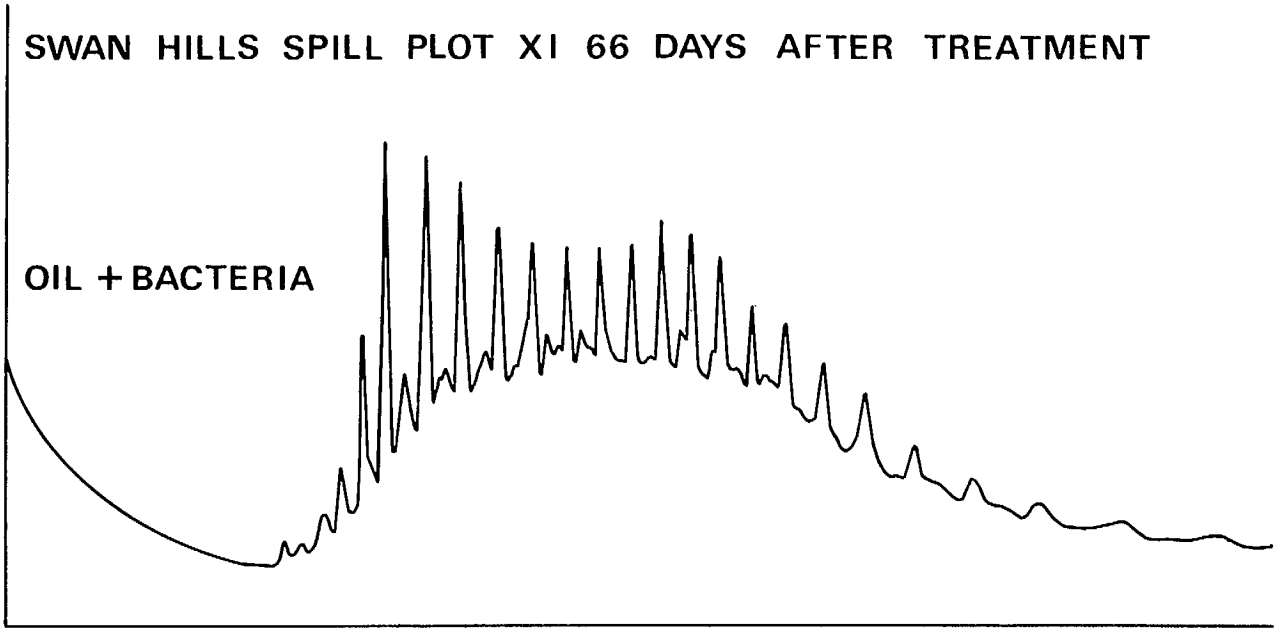
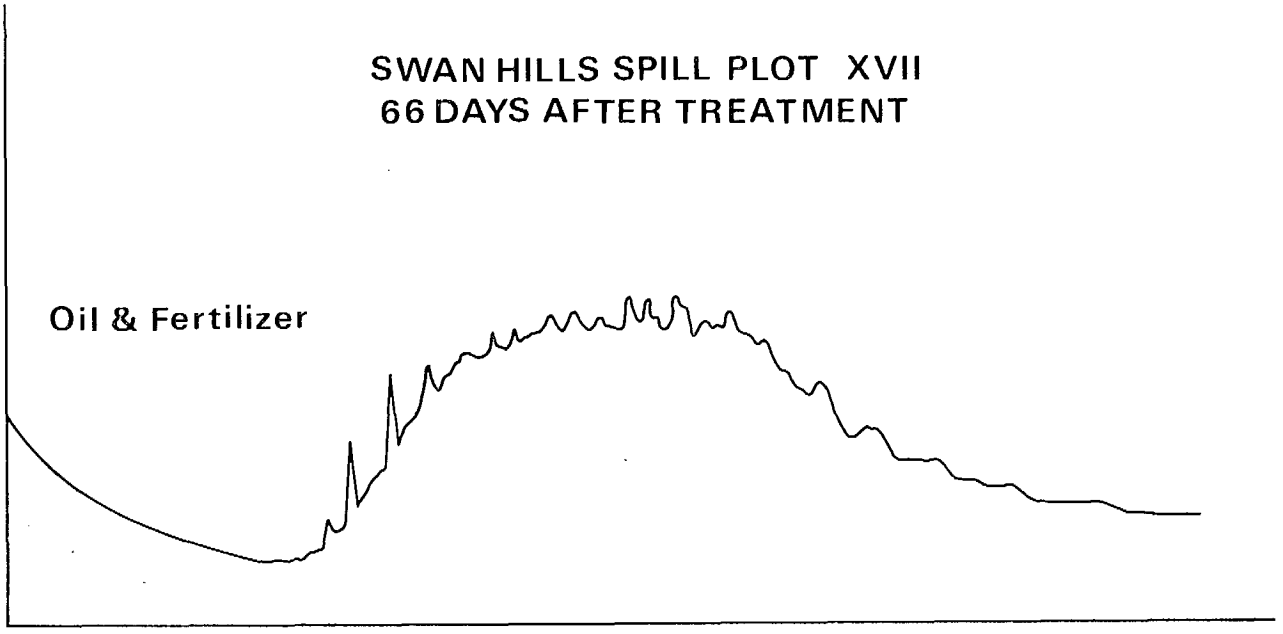


Figure 8 - G L C analysis of the saturate fraction of Swan Hills oil without an amendment (Plot XV1) and with a bacteria amendment (Plot X1) 66 days after treatment



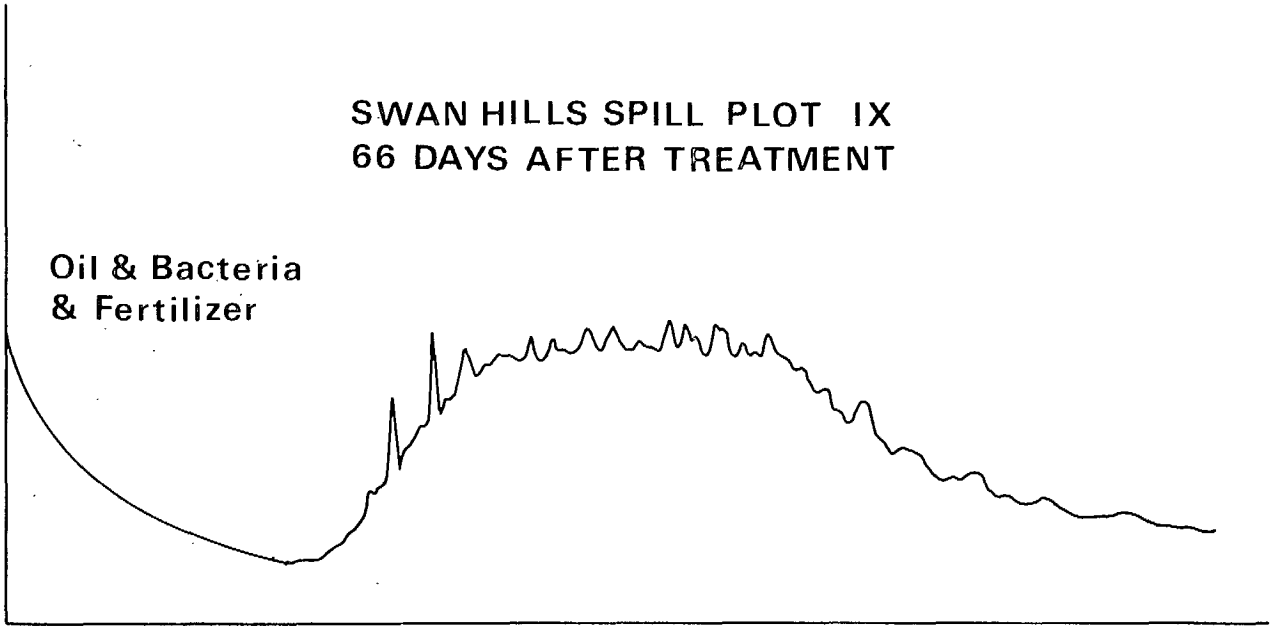


Figure 9 - G L C analysis of the saturate fraction of Swan Hills oil with fertilizer (Plot XVll) and with fertilizer plus bacteria (Plot lX) 66 days after treatment

(b) MICROBIAL ANALYSIS OF ARCTIC SOILS FOR THE CAPABILITY OF USING PRUDHOE BAY CRUDE-OIL AS A SOLE CARBON SOURCE

The numbers of bacteria and molds present in Arctic soil and water samples were presented in Table AVII. All of the samples tested contained reasonable numbers, i.e. 10^6/gm for bacteria and 10^3/gm for molds, while the occasional sample, (duff taken near Old Crow), yielded counts of 10^9 and 10^6 for bacteria and molds respectively.

A comparison of the ability of these samples to yield microbial populations capable of growing on Prudhoe Bay oil at 30°C and 4°C is presented in Table XIII. The observations recorded represent those which existed after the 5th and 7th day of incubation of the fourth transfer at 30°C and 4°C respectively. Previous experience indicated that, if oil-utilizing microbes were present in samples, they would have reached readily-detectable numbers by this transfer. It was previously observed that emulsification is the first visible index of microbial modification of crude oil. By this parameter, there is more activity on crude oil at 30°C than at 4°C (29 soil samples sustained emulsification at 30°C while only 20 did so at 4°C). Further analysis of this phenomenon showed that 14 samples brought about emulsificiation at 30°C but not at 4°C and 6 samples did at 4°C but not at 30°C. Five samples (12% of the total examined) showed no activity on crude oil at either of the temperatures used in these studies.

With only two exceptions, a population of at least 61 X 10^6 cells/ml was observed at 30°C before emulsification of the oil was apparent. The two exceptions, # 2, and the Inuvik soil, prior oil contact was possible in case of sample # 2 while the Inuvik one was soaked with oil. A population of 54 X 10^6 cells was observed at 4°C before emulsification was detected. The significance of pigmentation is not thought to be of importance with regards to oil utilization but was noted for taxonomic purposes.

Permafrost samples (#'s 7, 16, 26, 34 and 39) all contained populations which brought about emulsification of crude oil at 30°C but only two of them (16 and 26) did so at 4°C. The response of samples of the horizon immediately above permafrost samples was too varied to permit valid interpretation.

Moss samples (#40 and Norman Wells) did not yield a population which was capable of emulsifying Prudhoe Bay oil.

Samples from the burn site near Inuvik (#'s 35 to 39 inclusive) showed the presence of oil-emulsifying micro-organisms at 30°C (4 out of 5 samples were positive) but not at 4°C (only 1 out of the five samples positive).

Representative n-alkane profiles of the saturate fraction of the recovered oils are presented in Figures 10, 11 and 12. In figure 10, soils representative of the Norman Wells area, show very little change in the saturate fraction of the oil after incubation with the oil-soaked organic soil from the refinery overflow ditch (#1) and from the permafrost sample (#7). However, the decreased profile for the burned moss layer indicates some saturate utilization has taken place. The greatest amount of n-saturate utilization of all the northern samples tested is shown by the high organic matter sample (#9) obtained at Shell Lake in the Inuvik area. The persistence of phytane and pristane in the profile supports our observation of the inability of bacteria to utilize these isoprenoids readily at low temperatures. Utilization of the saturates, although at a lower level, did occur with the other two samples from this area. Samples from the Old Crow area (Figure 12) showed a varied response - some utilization of n-saturates with the LFH sample (#23) but not with the permafrost sample from the same site. In contrast, the n-saturate profile of Prudhoe Bay oil after incubation with the permafrost sample from the Tuktoyaktuk area shows that considerable utilization has taken place.

In all cases except for sample # 9, there has not been enough utilization to produce changes which would be detectable by our gravimetric procedure.

The utilization of the saturate fraction of crude oil by these soils is much greater at 30°C than at 4°C (Figure 13). The G.L.C. profiles in this figure represent the utilization of n-saturates by micro-organisms in an oil-soaked soil sample obtained near old storage tanks in the Norman Wells area. There has been complete utilization of n-saturates at 30°C but only very little at 4°C.

TABLE XIII - ENRICHMENT STUDIES ON THE UTILIZATION OF PRUDHOE BAY OIL

USING ARCTIC SOILS AS SOURCES OF MICRO-ORGANISMS

TEMPERATURE OF INCUBATION		30°C			4°C		
Sample Number	Location and Description	Emulsification	Pigment	Bacteria* x 10^6/ml	Emulsification	Pigment	Bacteria* x 10^6/ml
	NORMAN WELLS AREA						
1	Oil-soaked organic soil from refinery overflow ditch	+	+	93.0	+	-	57.0
2	Control soil for 1, soil free of oil contamination	+	+	9.4	+	-	63.0
3	Oil-soaked clay	+	+	105.0	+	-	67.0
4	Control clay sample for 3 free of oil	+	-	66.0	+	+	99.0
5	Oil-soaked sample near old Canol storage tanks	+	-	79.0	-	-	38.0
6	Control sample for 5 - free of oil	+	-	80.0	-	-	31.0
7	Clay permafrost sample	+	-	84.0	-	-	31.0
8	Burned moss layer	-	-	38.0	+	-	63.0
8a	Decomposed black organic layer	+	-	61.0	+	+	84.0

TABLE XIII - cont'd

	INUVIK AREA						
9	From bottom edge of Shell Lake (east of Inuvik): degraded algal and fern material present - very black-indicative of FeS_2 presence	+	+	119.0	+	-	85.0
10	Sample site 20' from shore of Shell Lake; LF sample-0-5 inches deep. Ground cover Labrador tea and birch	-	-	60.0	+	+	112.0
11	As for 10 except Ah horizon beneath LF; 5-8" deep; good black coloration	+	+	96.0	+	-	74.0
12	As for 10 except Ah horizon beneath LF; 8-11" deep; yellow material B subsoil	+	+	101.0	-	-	52.0
13	Experimental loop test site west of Inuvik, flat watery muskeg sample	+	+	119.0	+	-	59.0
14	Fisheries Research Board; Inuvik oil spill lakes. Duff LF horizon near L4 lake	+	+	121.0	+	-	86.0
15	As for 14 - mineral soil beneath duff sample; layer only 1 inch thick	+	-	70.0	+	-	69.0
16	As for 14 - permafrost sample beneath mineral soil layer	+	+	94.0	+	-	86.0
17	Mud on shore of L4 lake	-	-	47.0	+	-	74.0
18	'L4 - water sample; 1 inch below surface	not tested			not tested		

TABLE XIII cont'd

19	'as per 18	not tested			not tested		
20	Silt sample, 30 feet from edge of L4 lake; result of spring flooding?	+	+	116.0	-	-	50.0
	OLD CROW AREA						
21	Edge of Old Crow landing strip; top 4" of heavy duff; spruce and willow cover	+	-	80.0	-	-	53.0
22	As for 21, deep Ah and some B very organic, good structure looks fertile	-	-	51.0	-	-	53.0
23	LFH sample (5" deep) 2½ miles north at Old Crow - near tundra	-	-	56.0	-	-	43.0
24	As per 23 - mineral soil 5 to 10" deep beneath LFH horizon	-	-	26.0	+	-	54.0
25	As per 23 - beginning of C-horizon, 12-13" below surface	+	+	92.0	-	-	28.0
26	As per 23 - permafrost 17" below surface	+	-	75.0	+	-	113.0
27	Duff sample 0-12 cm deep; upstream from Old Crow near Porcupine River. Black spruce and older cover	-	-	31.0	-	-	45.0
28	As per 27 - Ah horizon 12-38 cm in depth	+	+	163.0	-	-	42.0
29	Old Crow village, silt layer (recent) 0-5 cm in depth, small amount of organic matter on top	+	-	83.0	-	-	46.0
30	As per 29 - silt material 15-30 cm in depth	+	+	141.0	-	-	35.0

TABLE XIII - cont'd

31	'Water sample - Porcupine River	not tested			not tested		
32	Small island is 10 miles S.W. of Tuktoyaktuk; Duff sample 14 cm deep; cover Reindeer moss	+	-	67.0	+	-	66.0
33	As per 32; organic layer underneath duff and above permafrost	-	-	13.0	+	+	104.0
34	As per 32; permafrost sample	+	-	68.0	-	-	50.0
35	Upland material; ¼ mile from Inuvik; old burn site covered with Fireweed. Duff sample top 5 cm.	+	+	312.0	-	-	15.0
36	As per 35; B horizon 15-29 cm	-	-	31.0	+	-	90.0
37	As per 35; B horizon 15-29 cm	+	-	66.0	-	-	48.0
38	As per 35; C horizon 29 cm	+	+	90.0	-	-	49.0
39	As per 35; permafrost sample	+	-	83.0	-	-	41.0
40	Moss sample near Kelly	-	-	38.0	-	-	29.0
	Norman Wells moss	-	-	55.0	-	-	36.0
	Inuvik (oil soaked soil around power plant)	+	-	6.0	+	-	69.0

' checked for total counts only

* fifth day of incubation during 4th transfer

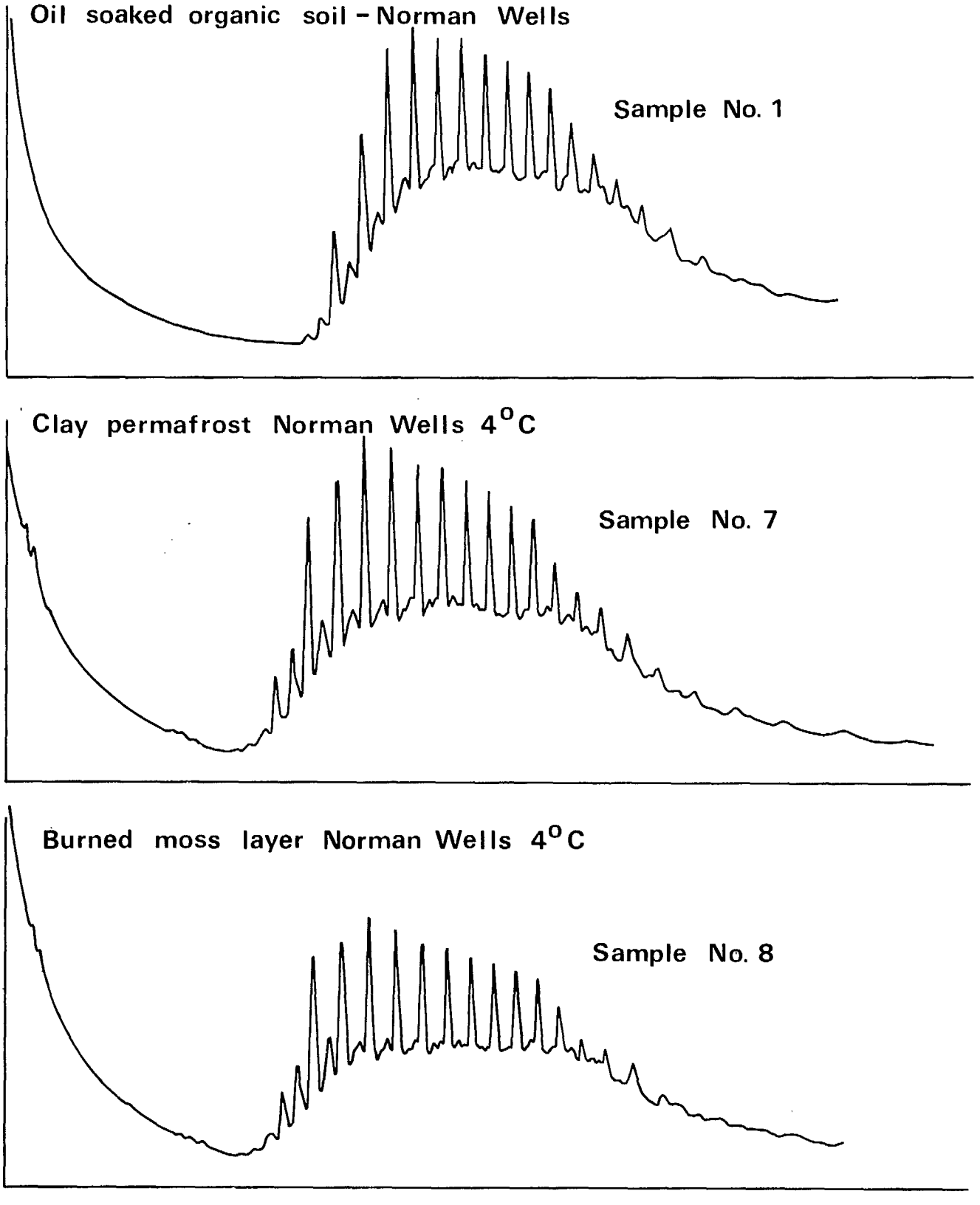


Figure 10 - G L C profile of the saturate fraction of Prudhoe Bay oil after incubation at 4°C with MacKenzie Valley soils

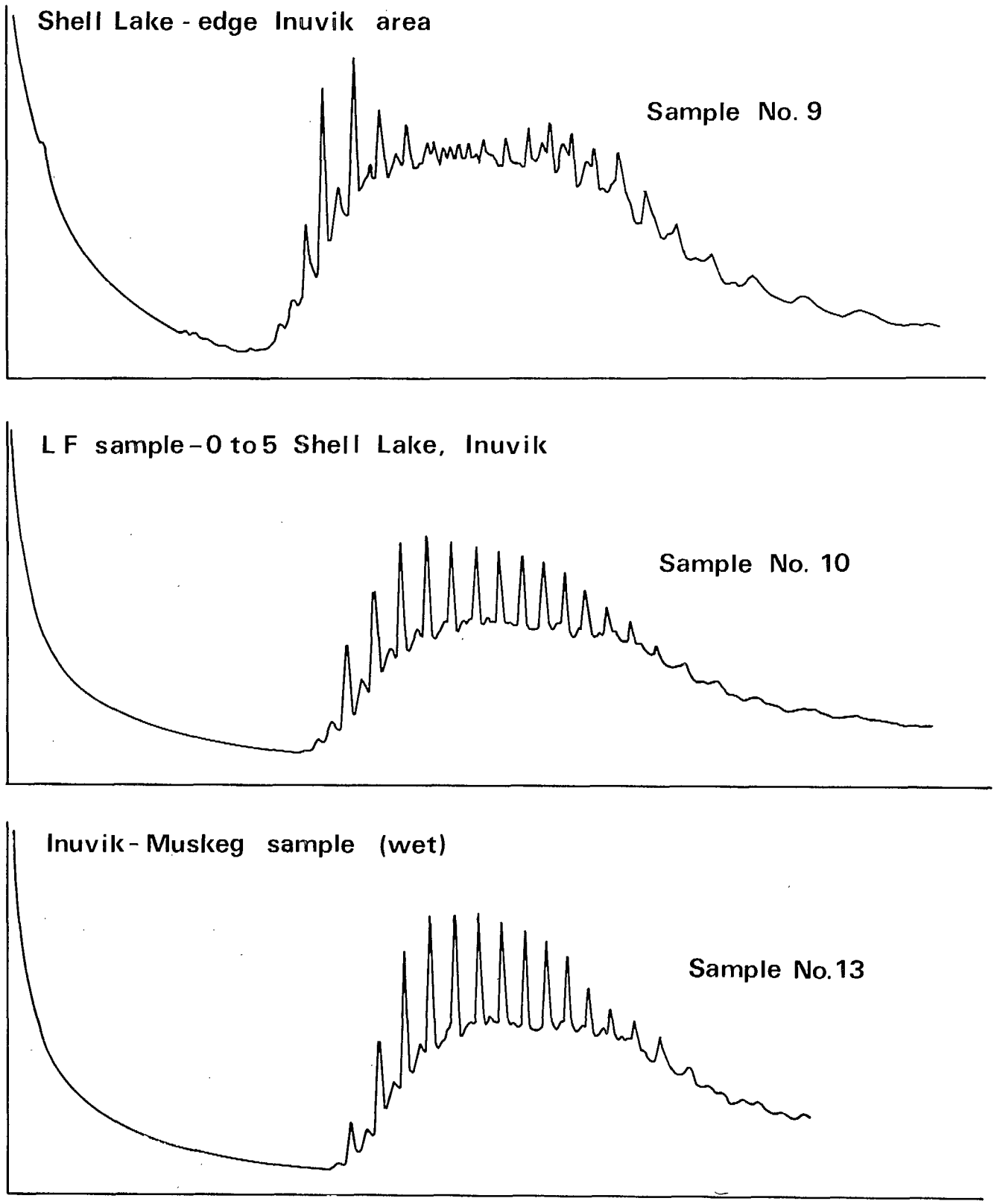


Figure 11 - G L C profile of the saturate fraction of Prudhoe Bay oil after incubation at 4°C with MacKenzie Valley soils

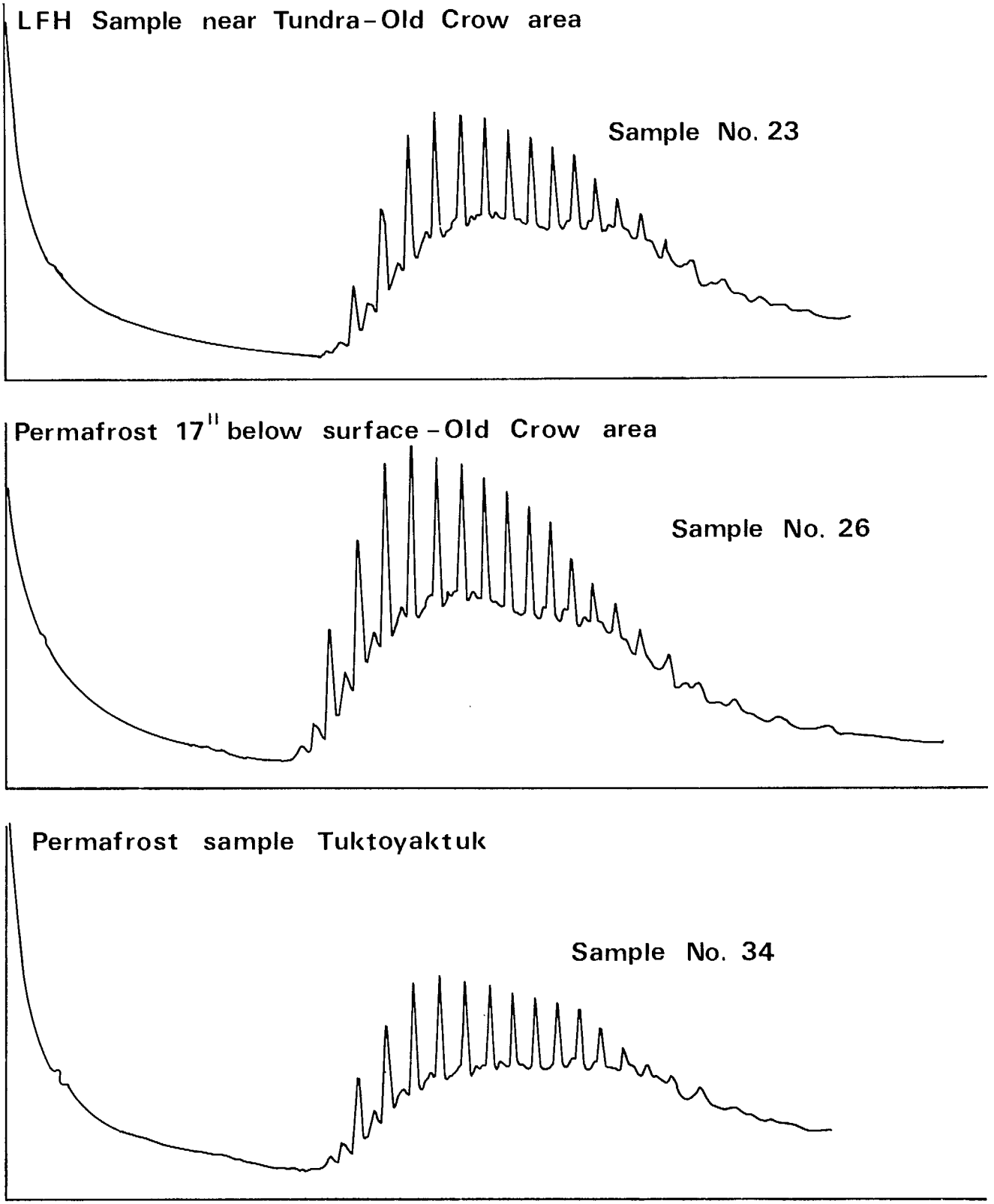
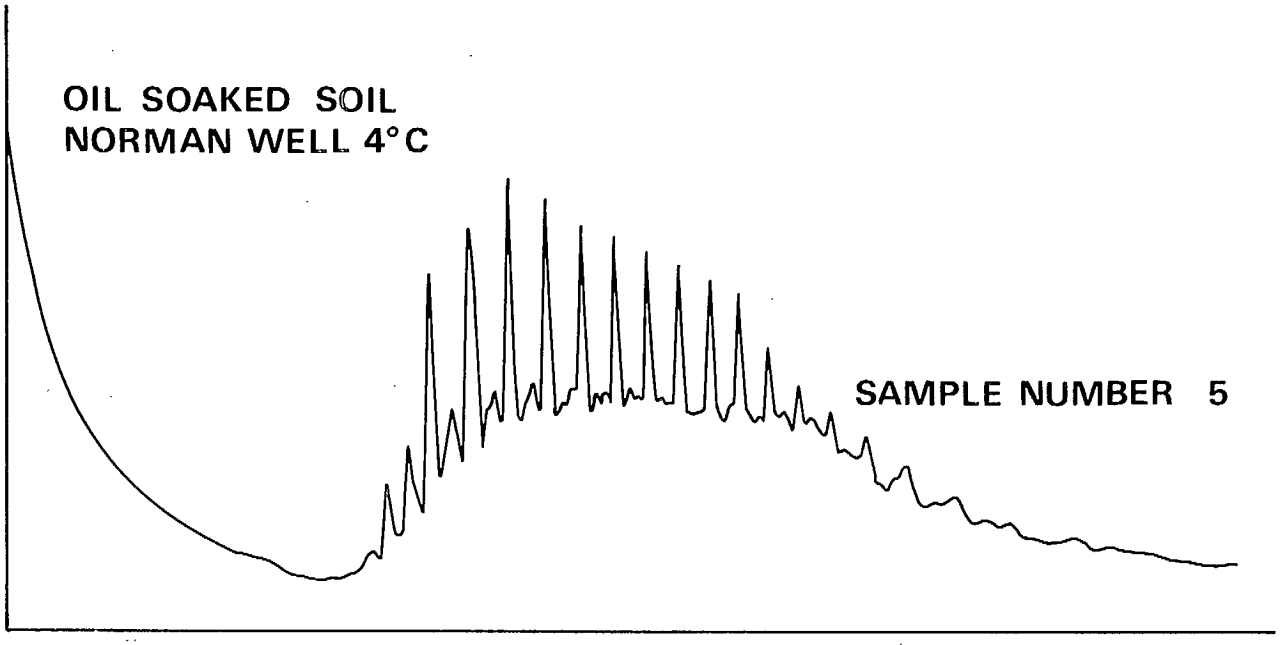


Figure 12 - G L C profiles of the saturate fraction of Prudhoe Bay oil after incubation at 4°C with MacKenzie Valley soils



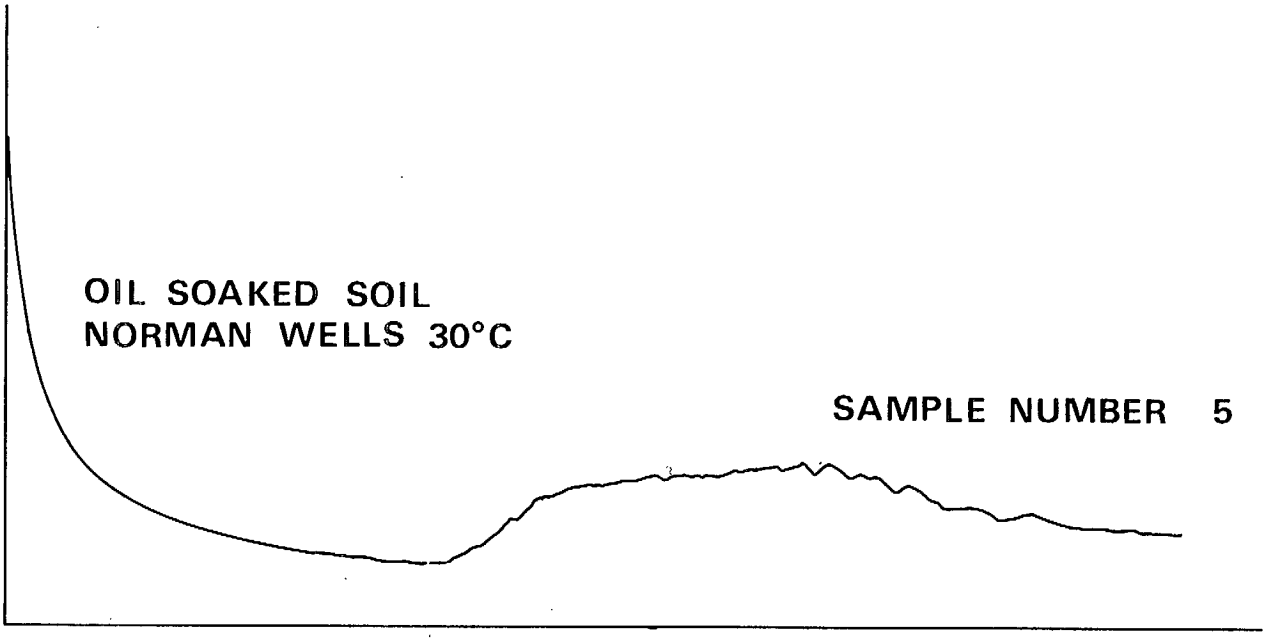


Figure 13 - G L C profile of n-saturate fraction of Prudhoe Bay oil after incubation with an oil soaked from Norman Wells area at $4^{o}C$ and $30^{o}C$

(c) COMPARISON OF THE ABILITY OF MICRO-ORGANISMS CAPABLE OF USING OIL OF ONE QUALITY TO USE OILS OF A LESSER OR HIGHER QUALITY

The oil samples used in this study can be graded for quality, based on their saturate, aromatic and NSO content (Table XIV). A comparison of the G.L.C. profile of the saturate fraction (Figure 4) indicates that this component is missing from Atkinson Point oil.

(i) Studies at 30°C

Data on the growth at 30°C of bacterial populations which utilize crude oil as carbon source are presented in Tables XV, XVI, XVII, XVIII. All of the oils sustained yields of cells in the order of 1 X 10^8cells/ml except Atkinson Point oil which sustained only 2 X 10^7 cells/ml. The inability of this oil to support a high yield of cells was confirmed when the populations enriched on other oils were grown on Atkinson Point oil. The maximum population achieved was only 1/5 to 1/10th of that when other oils were used as substrates. Significant growth on Atkinson Point oil was achieved only when it was used as substrate for enrichment procedures (Table XVIII). When the population enriched on Atkinson Point oil was used as an inoculum for other oils, numbers increased from 30 to 60 fold, whereas on Atkinson Point oil only a 10 fold increase was observed. Thus, this population was able to grow, and grow better, on oils of higher quality than on the poor quality oil which was used as a substrate for the enrichment procedure.

Populations enriched on other crude oils, Prudhoe Bay, Norman Wells and Lost Horse Hill, when tested against each other sustained a 10 to 25 fold increase in number over those which were supplied in the inoculum. Maximum growth was obtained on Prudhoe Bay followed by Norman Wells and Lost Horse oils respectively. However, these populations were relatively ineffective against the low quality Atkinson Point oil and produced only about one-third as much growth as the population enriched on Atkinson Point oil.

The generic composition of the population enriched on these oils at 30°C is presented in Tables XIX, XXI, XXII. The changes in these populations after growth on oil of differing quality are also presented in these tables. The predominant flora enriched on Norman Wells, Lost Horse Hill and Atkinson Point oils consists of gram negative rods whereas on Prudhoe Bay oil gram positive corynebacteria constitute the major components of the

population. The low occurrence of members of this genus in the Norman Wells population is significant. Major changes in populations were noted only when those enriched on the better quality oils were grown on the low quality Atkinson Point oil. For example, the high proportion of corynebacteria in the Prudhoe Bay population was reduced from 46% to 12% after growth on Atkinson Point oil (Table XIX). No major shift in population was observed when the enrichment on Atkinson Point oil was grown on oils of higher quality (Table XXII).

The chemical changes corresponding to the microbial ones are presented (Tables XXIII, XXIV, XXV and XXVI). All populations regardless of their origin readily digested the n-saturate fraction of the higher quality crude oils. Minor changes were also induced in the Atkinson Point oil, however because little growth was observed on this oil such changes could be a result of non-assimilatory oxidations. The GLC profiles in Figure 14 are typical of all high quality oil-microbial enrichment combinations and confirm the utilization of all the n-saturate fraction including the isoprenoids phytane and pristane.

(ii) Studies at 4°C

The results of the growth studies on various oils at 4°C are presented (Tables XXVII, XXVIII, XXIX, XXX and XXXI). As at 30°C, Atkinson Point oil sustained the least amount of growth. The overall results are similar to those obtained at 30°C.

The generic composition of the bacteria growing on the various crude oils are shown in Tables XXXII, XXXIII, XXXIV, XXXV and XXXVI . Major changes in composition occurred primarily when Atkinson Point oil was used as a test oil. Similarly, the population enriched on this oil showed a marked change when grown on the other oils used in the studies. Gram negative rods predominate in all populations induced at this temperature.

The chemical changes in oil composition brought about by microbial action is shown in Tables XXXVII, XXXVIII, XXXIX- XL and XLI . The results show a major utilization of the n-saturate fraction in all oils except the Atkinson Point one where this fraction is absent. As at 30°C, only minor changes were observed in Atkinson Point oil even when the population used was enriched on the oil. Typical G.L.C. profiles are presented in Figure 15 and show utilization of the n-saturate fraction. The isoprenoids phytane and pristane appear in some of these tracings and Table XLII shows their appearance as related to type of oil used for enrichment and test. The population induced on Atkinson Point

oil has very little ability to use these compounds under psychrophilic conditions. The high rate of appearance of phytane and pristane in the residues of the North Cantal and Lost Horse Hill oils as opposed to the Prudhoe Bay and Norman Wells oil suggests oil quality also has an effect on the bacterial utilization of these compounds.

(iii) Comparison of 4°C and 30°C populations

The effect of temperature on the composition of the mixed flora obtained from different quality oils is recorded in Table XLIII. In all of the oils studied, a different population is induced at 4°C and at 30°C. The growth studies (Tables XLIV and XLV) show a limited capability for the 4°C populations to grow at 30°C while the 30°C populations are incapable of growth at 4°C.

Chemical analysis of the residual oils confirms the above growth patterns, major changes were observed only when the 4°C population was used at 30°C (Table XLVI). The 30°C enrichment has little effect on the same oil at 4°C (Table XLVII) except for possibly the Lost Horse Hill enrichment which lost 6% (by weight) of its saturate content under these conditions. Gas-liquid chromatographic analyses confirm this utilization pattern (Figure 16) with the exception that the 6% loss in weight in Lost Horse Hill crude did not result in any change in the G.L.C. profile of the saturate fraction. The reduced ability of populations induced at 4°C to metabolize the isoprenoids phytane and pristane is confirmed.

TABLE XIV - CHEMICAL COMPOSITION OF OILS USED IN "CHALLENGE SERIES" OF EXPERIMENTS

Crude Oil Fraction	OIL				
	Norman Wells	Prudhoe Bay	Atkinson Point	Lost Horse Hill	North Cantal
Asphaltenes, soluble	2.24	4.43	3.31	7.80	2.5
Asphaltenes, insoluble	8.49	5.54	6.48	9.90	6.8
Saturates	45.98	35.68	32.09	36.80	51.0
Aromatics	29.29	35.30	39.63	37.90	31.2
NSO's, soluble	10.38	17.29	13.73	13.10	8.6
NSO's, insoluble	3.61	3.04	4.78	0.20	0.3

TABLE XV - CAPABILITY OF A BACTERIAL POPULATION ENRICHED ON NORMAN WELLS OIL TO GROW ON OILS OF HIGHER OR LOWER QUALITY (30°C)

TIME (days)	TEST OILS							
	Bacteria x 10^5/ml							
	Atkinson Point		Lost Horse Hill		Norman Wells		Prudhoe Bay	
	Count	Increase	Count	Increase	Count	Increase	Count	Increase
0	45	-	44	-	42	-	44	-
1	90	2.0	380	8.6	420	10.0	690	15.8
2	120	2.7	530	12.1	570	13.6	1100	25.0
3	170	3.8	630	14.3	720	17.1	1110	25.2
4	130	2.9	590	13.4	770	18.3	950	21.6
7	155	3.4	370	8.4	640	15.2	620	14.1
10	146	3.2	161	3.7	264	6.3	370	8.4

TABLE XVI - CAPABILITY OF A BACTERIAL POPULATION ENRICHED ON LOST HORSE HILL OIL TO GROW ON OILS OF HIGHER OR LOWER QUALITY (30°C)

TIME (days)	TEST OILS							
	Bacteria x 10^5/ml							
	Atkinson Point		Lost Horse Hill		Norman Wells		Prudhoe Bay	
	Count	Increase	Count	Increase	Count	Increase	Count	Increase
0	52	-	52	-	50	-	47	-
1	76	1.5	330	6.4	430	8.6	420	8.9
2	95	1.8	670	12.9	610	12.2	640	13.6
3	96	1.9	720	13.9	980	18.9	600	12.8
4	110	2.1	770	14.8	550	11.0	670	14.3
7	105	2.0	810	15.6	520	10.4	510	10.9
10	117	2.3	800	15.4	390	7.8	540	11.5

TABLE XVII - CAPABILITY OF A BACTERIAL POPULATION ENRICHED ON PRUDHOE BAY OIL TO USE OILS OF HIGHER OR LOWER QUALITY (30°C)

TIME (days)	TEST OILS							
	Bacteria x 10^5/ml							
	Atkinson Point		Lost Horse Hill		Norman Wells		Prudhoe Bay	
	Count	Increase	Count	Increase	Count	Increase	Count	Increase
0	97	-	85	-	94	-	91	-
1	200	2.1	770	9.1	1080	11.5	116	1.3
2	210	2.2	970	11.4	1270	13.5	1580	17.4
3	190	2.0	830	9.8	1010	10.7	1580	17.4
4	199	2.1	940	11.1	1040	11.1	1250	13.7
7	190	2.0	680	8.0	820	8.7	960	10.6
10	200	2.1	890	10.5	770	8.2	860	9.5

TABLE XVIII - CAPABILITY OF A BACTERIAL POPULATION ENRICHED ON ATKINSON POINT CRUDE OIL TO GROW ON OILS OF HIGHER QUALITY (30°C)

TIME (days)	TEST OILS							
	Bacteria x 10^5/ml							
	Atkinson Point		Lost Horse Hill		Norman Wells		Prudhoe Bay	
	Count	Increase	Count	Increase	Count	Increase	Count	Increase
0	4.5	-	4.4	-	3.7	-	5.1	-
1	36.2	8.0	130.0	29.5	117.8	31.8	235.0	46.1
2	34.5	7.7	118.0	26.8	121.8	32.9	280.0	54.9
3	38.0	8.4	125.0	28.4	117.8	31.6	336.0	65.9
4	37.0	8.2	150.0	34.1	140.0	37.8	250.0	49.0
7	43.0	9.6	160.0	36.4	210.0	56.8	240.0	47.1
10	46.0	10.2	144.0	32.7	159.0	43.0	168.0	32.9

TABLE XIX - CHANGE IN COMPOSITION OF MICROBIAL POPULATION AS AFFECTED BY CRUDE OIL QUALITY.

(Original population induced on PRUDHOE BAY crude oil plus Salmon Arm soaked soil).

TEMPERATURE= 30°C

GENUS	% of Population			
	Prudhoe Bay	Atkinson Point	Norman Wells	Lost Horse Hill
Achromobacter sp.	18	17	20	15
Alcaligenes sp.	8	10	5	7
Corynebacterium sp.	46	12	45	44
Pseudomonas sp.	21	27	23	20
Unidentified Gram *Negative rods	2	3	1	3
*Unidentified Gram Negative rods	5	31	6	11

* Differing in at least one characteristic.

TABLE XX - CHANGE IN COMPOSITION OF MICROBIAL POPULATION AS AFFECTED BY CRUDE OIL QUALITY.

(original population induced on LOST HORSE HILL crude oil plus Salmon Arm oil soaked soil). TEMPERATURE= 30°C.

GENUS	% of Population			
	Lost Horse Hill	Norman Wells	Prudhoe Bay	Atkinson Point
Achromobacter sp.	67	56	56	35
Acinetobacter sp.	16	20	16	38
Flavobacterium sp.	2	5	7	10
Pseudomonas sp.	15	19	21	17

TABLE XXI - CHANGE IN COMPOSITION OF MICROBIAL POPULATION AS AFFECTED BY CRUDE OIL QUALITY.

(Original population induced on NORMAN WELLS crude oil plus Salmon Arm oil soaked soil). TEMPERATURE= 30°C

GENUS	% of Population			
	Norman Wells	Prudhoe Bay	Atkinson Point	Lost Horse Hill
Acinetobacter sp.	28	20	22	16
Corynebacterium sp.	8	10	9	4
Xanthomonas sp.	16	26	3	26
*Unidentified Gram Negative Rods	16	11	19	11
*Unidentified Gram Negative Rods	9	17	31	8
*Unidentified Gram Negative Rods	24	15	15	34

* Differing in at least one characteristic.

TABLE XXII - CHANGE IN COMPOSITION OF MICROBIAL POPULATION AS AFFECTED BY CRUDE OIL QUALITY.

(Original population induced on ATKINSON POINT crude oil plus Salmon Arm oil soaked soil). TEMPERATURE= 30°C

GENUS	% of Population			
	Atkinson Point	Norman Wells	Prudhoe Bay	Lost Horse Hill
Achromobacter sp.	34	27	44	28
Alcaligenes sp.	27	24	20	29
Xanthomonas sp.	39	49	36	43

TABLE XXIII - CHEMICAL COMPOSITION OF CRUDE OILS AFTER 10 DAYS INCUBATION WITH A POPULATION ENRICHED ON PRUDHOE BAY CRUDE OIL (30°C)

	OIL			
Crude Oil Fraction	Prudhoe Bay Difference*	Norman Wells Difference	Atkinson Point Difference	Lost Horse Hill Difference
Asphaltenes, soluble	+ 4.37	+ 2.51	+ 1.56	+ 6.36
Asphaltenes, insoluble	+ 0.16	- 2.82	- 1.81	- 1.49
Saturates	-10.11	- 8.88	- 0.29	-20.83
Aromatics	- 0.03	+ 3.16	- 3.41	- 4.61
NSO's, soluble	+ 2.56	+ 5.00	+ 4.55	+ 9.43
NSO's, insoluble	+ 1.90	+ 1.05	- 0.62	+ 5.32

* Percentage in microbially treated crude oil less that in untreated oil.

TABLE XXIV - CHEMICAL COMPOSITION OF CRUDE OILS AFTER 10 DAYS INCUBATION WITH A POPULATION ENRICHED ON LOST HORSE HILL CRUDE OIL (30°C)

Crude Oil Fraction	OIL			
	Lost Horse Hill Difference*	Norman Wells Difference	Atkinson Point Difference	Prudhoe Bay Difference
Asphaltenes, soluble	+ 6.06	+ 5.07	+ 3.60	+ 5.72
Asphaltenes, insoluble	+ 4.24	- 3.03	- 2.98	- 0.78
Saturates	-23.01	-10.13	- 2.32	-10.45
Aromatics	- 6.63	+ 1.78	- 3.30	- 0.34
NSO's soluble	+ 7.87	+ 5.62	+ 4.18	+ 2.59
NSO's insoluble	+ 5.86	+ 0.70	+ 0.81	+ 1.88

* Percentage in microbially treated crude oil less that in untreated oil.

TABLE XXV - CHEMICAL COMPOSITION OF CRUDE OILS AFTER 10 DAYS INCUBATION WITH A POPULATION ENRICHED ON NORMAN WELLS CRUDE OIL (30°C)

	OIL			
Crude Oil Fraction	Norman Wells Difference*	Atkinson Point Difference	Prudhoe Bay Difference	Lost Horse Hill Difference
Asphaltenes, soluble	- 0.01	+ 1.52	+ 2.31	+ 2.65
Asphaltenes, insoluble	+ 0.47	- 0.52	+ 0.38	+ 2.71
Saturates	- 9.04	- 0.93	- 6.65	-19.41
Aromatics	+ 3.57	- 1.99	+ 1.30	- 0.97
NSO's soluble	+ 4.99	+ 2.80	+ 0.73	+ 5.86
NOS's insoluble	+ 0.02	- 0.90	+ 0.64	+ 3.88

* Percentage in microbially treated crude oil less that in untreated oil.

TABLE XXVI - CHEMICAL COMPOSITION OF CRUDE OILS AFTER 10 DAYS INCUBATION WITH A POPULATION ENRICHED ON ATKINSON POINT CRUDE OIL (30°C)

Crude Oil Fraction	OIL			
	Atkinson Point Difference*	Norman Wells Difference	Prudhoe Bay Difference	Lost Horse Hill Difference
Asphaltenes, soluble	+ 2.43	+ 1.15	+ 2.68	+ 2.83
Asphaltenes, insoluble	- 1.68	+ 0.90	+ 5.15	+ 5.89
Saturates	- 2.54	- 9.22	-10.13	-20.61
Aromatics	+ 3.37	+ 2.87	- 0.41	- 3.85
NSO's soluble	+ 4.10	+ 3.44	- 0.27	+ 5.51
NSO's insoluble	- 1.13	+ 0.97	+ 1.70	+ 4.42

* Percentage in microbially treated crude oil less that in untreated oil.

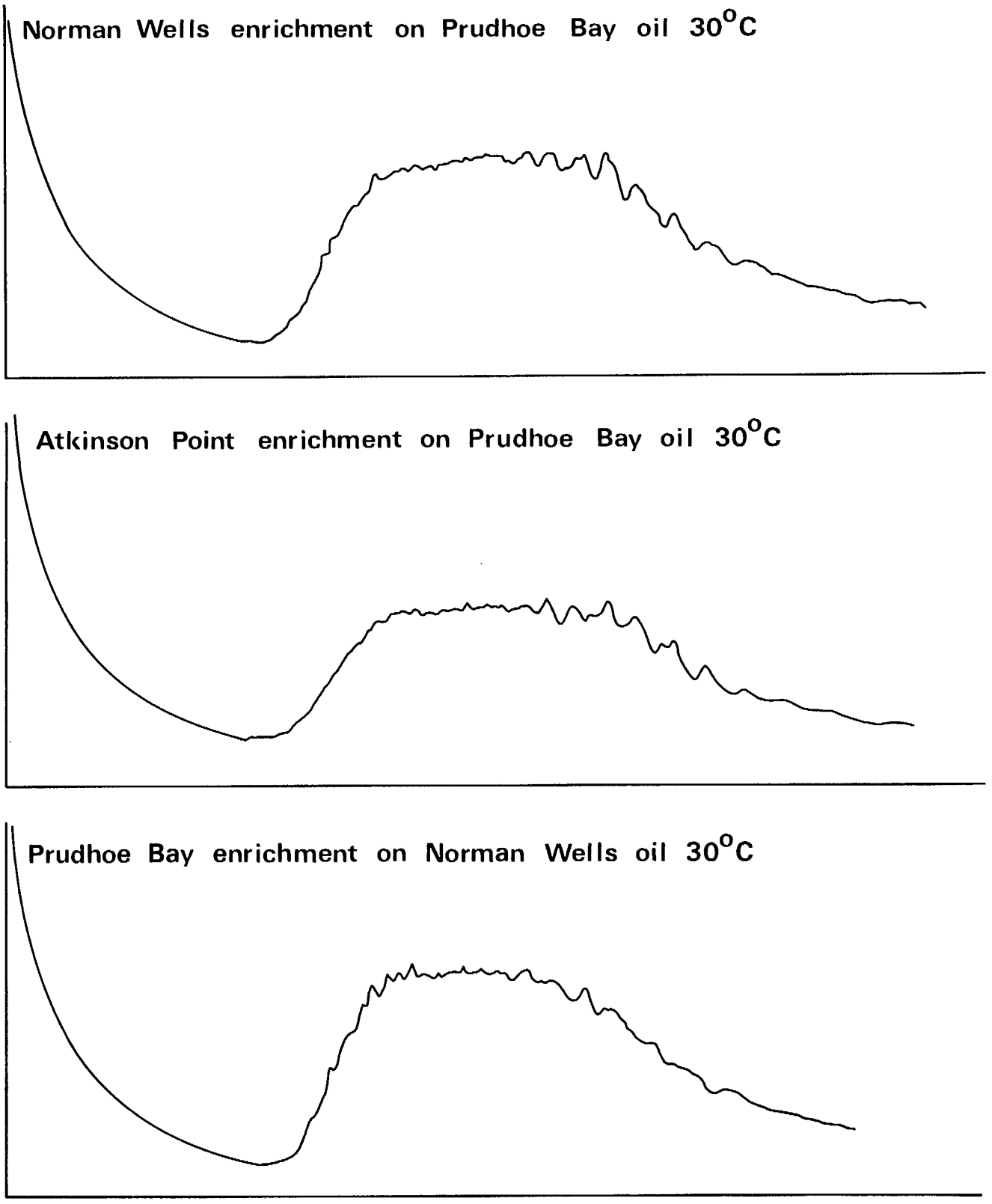


Figure 14 - G L C analysis of saturate fraction of Prudhoe Bay and Norman Wells oil after growth at 30°C of populations enriched on various oils

TABLE XVII - CAPABILITY OF A POPULATION ENRICHED ON PRUDHOE BAY OIL TO GROW ON OILS OF HIGHER OR LOWER QUALITY (4°C)

TIME (days)	TEST OILS									
	Bacteria x 10^5/ml									
	Prudhoe Bay		Atkinson Point		Norman Wells		North Cantal		Lost Horse Hill	
	Count	Increase	Count	Increase	Count	Increase	Count	Increase	Count	Increase
0	51	-	55	-	42	-	40	-	51	-
1	67	1.3	57	1.0	60	1.4	65	1.6	56	1.1
2	280	5.5	121	2.2	240	5.7	400	10.0	220	4.3
3	340	6.7	94	1.7	690	16.4	800	20.0	350	10.8
4	880	17.3	104	1.9	860	20.5	1090	27.3	690	13.5
7	990	19.4	220	4.0	870	20.7	900	22.5	820	16.1
10	800	15.7	280	5.1	990	23.6	1230	30.8	870	17.1

TABLE XXVIII - CAPABILITY OF A POPULATION ENRICHED ON NORMAN WELLS OIL TO GROW ON OILS AT HIGHER OR LOWER QUALITY (4°C)

TIME (days)	TEST OILS									
	Bacteria x 10^5/ml									
	Norman Wells		Prudhoe Bay		Atkinson Point		North Cantal		Lost Horse Hill	
	Count	Increase	Count	Increase	Count	Increase	Count	Increase	Count	Increase
0	62	-	76	-	74	-	68	-	79	-
1	98	1.6	95	1.3	93	1.3	82	1.2	116	1.5
2	230	3.7	340	4.5	130	1.8	260	3.8	189	2.4
3	400	6.5	440	5.8	143	1.9	540	7.9	380	4.8
4	500	8.1	450	5.9	190	2.6	630	9.3	460	5.8
7	670	10.8	670	8.8	230	3.1	730	10.7	540	6.8
10	650	10.5	580	7.6	170	2.3	790	11.6	470	6.0

TABLE XXIX - CAPABILITY OF A POPULATION ENRICHED ON ATKINSON POINT OIL TO GROW ON OILS OF HIGHER QUALITY (4°C)

TIME (days)	TEST OILS									
	Bacteria x 10^5/ml									
	Atkinson Point		Prudhoe Bay		Norman Wells		North Cantal		Lost Horse Hill	
	Count	Increase	Count	Increase	Count	Increase	Count	Increase	Count	Increase
0	37	-	37	-	56	-	22	-	28	-
1	60	1.6	46	1.2	34	0.6	28	1.3	42	1.5
2	48	1.3	65	1.8	47	0.8	52	2.4	38	1.4
3	98	2.6	93	2.5	94	1.7	75	3.4	116	4.1
4	47	1.3	200	5.4	141	2.5	141	6.4	182	6.5
7	212	5.7	340	9.2	490	8.8	209	9.5	300	10.7
10	320	8.7	480	13.0	700	12.5	600	27.3	590	21.1

TABLE XXX - CAPABILITY OF A POPULATION ENRICHED ON LOST HORSE HILL OIL TO GROW ON OILS OF HIGHER OR LOWER QUALITY (4°C)

TIME (days)	TEST OILS									
	Bacteria x 10^5/ml									
	Lost Horse Hill		North Cantal		Atkinson Point		Prudhoe Bay		Norman Wells	
	Count	Increase	Count	Increase	Count	Increase	Count	Increase	Count	Increase
0	51	-	68	-	70	-	78	-	68	-
1	74	1.5	88	1.3	99	1.4	98	1.3	90	1.3
2	280	5.5	212	3.5	106	1.5	184	2.4	183	2.7
3	380	7.5	470	6.9	118	1.7	440	5.6	640	9.4
4	490	9.6	510	7.5	113	1.6	460	5.9	350	5.2
7	730	14.3	680	10.0	138	2.0	570	7.3	670	9.6
10	720	14.1	860	12.7	134	1.9	750	9.6	670	9.6

TABLE XXXI - CAPABILITY OF A POPULATION ENRICHED ON NORTH CANTAL OIL TO GROW ON OILS OF HIGHER OR LOWER QUALITY (4°C)

TIME (days)	TEST OILS									
	Bacteria x 10^5/ml									
	North Cantal		Lost Horse Hill		Prudhoe Bay		Norman Wells		Atkinson Point	
	Count	Increase	Count	Increase	Count	Increase	Count	Increase	Count	Increase
0	92	-	111	-	132	-	91	-	92	-
1	127	1.4	104	0.9	146	1.1	143	1.6	135	1.4
2	570	6.2	350	3.2	530	4.0	500	5.5	147	1.6
3	900	9.8	580	5.2	500	3.8	570	6.3	128	1.4
4	1040	11.3	680	6.1	820	6.2	780	8.6	270	2.9
7	1030	11.2	690	6.2	870	6.6	880	9.7	280	3.0
10	840	9.1	460	4.1	640	4.9	740	8.1	210	2.3

TABLE XXXII - CHANGES IN COMPOSITION OF MICROBIAL POPULATION AS AFFECTED BY CRUDE OIL QUALITY. (Original population induced on PRUDHOE BAY crude oil plus Salmon Arm Oil - soaked soil TEMPERATURE= 4°C

GENUS	% of Population				
	Prudhoe Bay	Norman Wells	Atkinson Point	Lost Horse Hill	North Cantal
Achromobacter sp.	22	21	23	15	17
Alcaligenes sp.	22	23	43	29	31
Flavobacterium sp.	53	53	31	51	48
Unidentified Gram Negative Rod	3	3	3	5	4

TABLE XXX111 - CHANGE IN COMPOSITION OF MICROBIAL POPULATION AS AFFECTED BY CRUDE OIL QUALITY.

(Original population induced on NORMAN WELLS crude oil plus Salmon Arm oil soaked soil. TEMPERATURE = 4° C

GENUS	% of Population				
	Norman Wells	Prudhoe Bay	Atkinson Point	North Cantal	Lost Horse Hill
Pseudomonas sp.	34	33	23	33	23
Xanthomonas sp.	10	12	18	6	11
Unidentified Gram Negative - maybe an Alcaligenes sp.	9	9	23	10	9
Unidentified Gram Negative Rods - maybe a Flavobacterium sp.	41	41	29	46	52
Unidentified Gram Positive irregular staining branched rods	6	5	7	5	5

TABLE XXX1V - CHANGE IN COMPOSITION OF MICROBIAL POPULATION AS AFFECTED BY CRUDE OIL QUALITY.

(Original population induced on Atkinson Point crude oil plus Salmon Arm oil soaked soil). TEMPERATURE =4°C.

GENUS	% of Population				
	Atkinson Point	Norman Wells	Prudhoe Bay	Lost Horse Hill	North Cantal
Pseudomonas sp.	14	11	5	7	11
Unidentified Gram Negative Rods - maybe Acinetobacter sp.	46	77	81	78	75
Unidentified Gram Negative Coccus- maybe Neiserria sp.	40	12	14	15	14

TABLE XXXV - CHANGE IN COMPOSITION OF MICROBIAL POPULATION AS AFFECTED BY CRUDE OIL QUALITY.

(Original population induced on LOST HORSE HILL crude oil plus Salmon Arm oil soaked soil). TEMPERATURE = 4° C.

GENUS	% of Population				
	Lost Horse Hill	North Cantal	Norman Wells	Prudhoe Bay	Atkinson Point
Acinetobacter sp.	5	2	4	7	3
Alcaligenes sp.	50	46	33	37	42
Corynebacterium sp.	2	0	2	0	2
Pseudomonas sp.	41	49	58	53	51
Unidentified Gram Negative Polar Flagellated Rod	2	3	3	3	2

TABLE XXXV1 - CHANGE IN COMPOSITION OF MICROBIAL POPULATION AS AFFECTED BY CRUDE OIL QUALITY.

(Original population induced on NORTH CANTAL crude oil plus Salmon Arm oil soaked soil). TEMPERATURE = 4°C.

GENUS	% of Population				
	North Cantal	Lost Horse Hill	Prudhoe Bay	Norman Wells	Atkinson Point
Achromobacter sp.	48	42	40	42	24
Alcaligenes sp.	39	42	32	33	52
Pseudomonas sp.	23	16	28	25	24

TABLE XXXV11 - CHEMICAL COMPOSITION OF CRUDE OILS AFTER 10 DAYS INCUBATION WITH A POPULATION ENRICHED ON PRUDHOE BAY CRUDE OIL (4°C)

	OIL				
Crude Oil Fraction	Norman Wells Difference*	Atkinson Point Difference	Prudhoe Bay Difference	Lost Horse Hill Difference	North Cantal Difference
Asphaltenes, soluble	+ 1.55	+ 1.33	+ 1.88	- 0.16	+ 0.15
Asphaltenes, insoluble	- 3.17	- 2.11	+ 1.21	- 0.45	+ 1.06
Saturates	- 6.37	- 1.63	- 7.96	-18.26	-15.41
Aromatics	+ 2.99	- 4.43	+ 0.40	- 0.65	+ 0.68
NSO,s soluble	+ 6.19	+ 2.94	+ 1.60	+ 7.43	+ 3.09
NSO,s insoluble	- 1.18	+ 3.88	+ 1.46	+ 6.39	+10.04

*Percentage in microbially treated crude oil less that in untreated oil

TABLE XXXVIII - CHEMICAL COMPOSITION OF CRUDE OILS AFTER 10 DAYS INCUBATION WITH A POPULATION ENRICHED ON NORMAN WELLS CRUDE OIL (4°C)

Crude Oil Fraction	OIL				
	Norman Wells Difference*	Atkinson Point Difference	Prudhoe Bay Difference	Lost Horse Hill Difference	North Cantal Difference
Asphaltenes, soluble	+ 1.66	+ 1.34	+ 2.66	+ 1.57	+ 1.13
Asphaltenes, insoluble	- 2.52	- 2.00	+ 0.86	- 0.79	+ 0.65
Saturates	- 5.70	+ 1.95	- 6.60	-17.14	-12.40
Aromatics	+ 2.48	- 3.48	- 0.62	- 1.25	+ 2.50
NSO's soluble	+ 3.19	+ 3.58	+ 2.30	+ 6.70	+ 3.09
NSO's insoluble	+ 1.41	- 1.40	+ 0.13	+ 5.20	+ 4.63

*Percentage in microbially treated crude oil less that in untreated oil

TABLE XXXIX - CHEMICAL COMPOSITION OF CRUDE OILS AFTER 10 DAYS INCUBATION WITH A POPULATION ENRICHED ATKINSON POINT CRUDE OIL (4°C)

Crude Oil Fraction	OIL				
	Norman Wells Difference*	Atkinson Point Difference	Prudhoe Bay Difference	Lost Horse Hill Difference	North Cantal Difference
Asphaltenes, soluble	+ 1.26	+ 1.31	+ 1.81	+ 2.30	+ 1.66
Asphaltenes, insoluble	- 5.20	- 3.60	- 2.28	- 5.09	- 1.44
Saturates	- 5.65	+ 1.26	- 5.98	-16.62	-12.29
Aromatics	+ 3.64	- 2.98	+ 0.55	+ 0.25	+ 3.33
NSO's soluble	+ 5.76	+ 3.52	+ 2.55	+ 7.99	+ 3.80
NSO's insoluble	+ 0.21	+ 0.47	+ 2.06	+ 5.47	+ 4.86

*Percentage in microbially treated crude oil less that in untreated oil

TABLE XL - CHEMICAL COMPOSITION OF CRUDE OILS AFTER 10 DAYS INCUBATION WITH A POPULATION ENRICHED ON LOST HORSE HILL CRUDE OIL (4°C).

	OIL				
Crude Oil Fraction	Norman Wells Difference	Atkinson Point Difference	Prudhoe Bay Difference	Lost Horse Hill Difference	North Cantal Difference
Asphaltenes, soluble	+ 1.14	+ 2.52	+ 2.26	+ 3.12	+ 0.69
Asphaltenes, insoluble	- 0.10	- 0.27	+ 4.49	+ 1.31	+ 0.37
Saturates	- 8.91	- 2.89	- 9.36	-18.76	- 9.84
Aromatics	+ 1.97	- 3.50	- 1.17	- 0.58	+ 2.49
NSO's soluble	+ 4.55	+ 3.04	+ 0.60	+ 4.97	+ 1.08
NSO's insoluble	+ 1.36	+ 1.07	+ 0.89	+ 4.32	+ 4.80

*Percentage in microbially treated crude oil less that in untreated oil

TABLE XL1 - CHEMICAL COMPOSITION OF CRUDE OILS AFTER 10 DAYS INCUBATION WITH A POPULATION ENRICHED ON NORTH CANTAL CRUDE OIL (4°C)

Crude Oil Fraction	OIL				
	Norman Wells Difference*	Atkinson Point Difference	Prudhoe Bay Difference	Lost Horse Hill Difference	North Cantal Difference
Asphaltenes, soluble	+ 1.16	+ 1.49	+ 2.93	+ 3.15	+ 1.79
Asphaltenes, insoluble	- 3.38	- 2.29	+ 2.39	+ 0.32	- 0.77
Saturates	- 5.22	+ 1.34	- 9.39	-17.83	-11.87
Aromatics	+ 2.05	- 3.85	- 0.83	- 3.25	- 1.00
NSO's soluble	+ 5.11	+ 3.00	+ 1.09	+ 6.81	+ 2.90
NSO's insoluble	+ 0.28	+ 0.29	+ 2.48	+ 5.10	+ 8.56

*Percentage in microbially treated crude oil less that in untreated oil

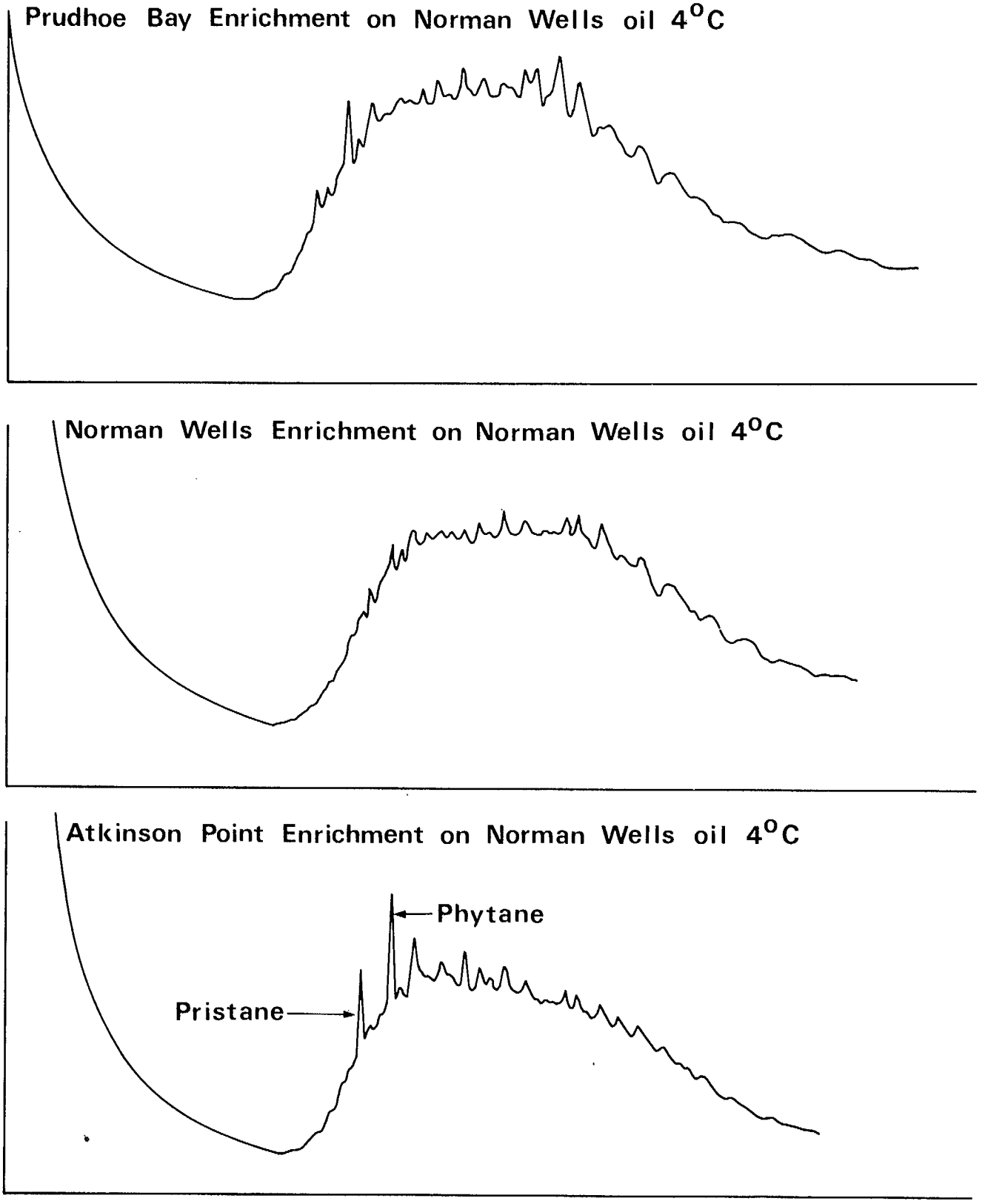


Figure 15 - G L C analysis of saturate fraction of Norman Wells oil after growth of populations enriched on Prudhoe Bay, Norman Wells and Atkinson Point oils

TABLE XL11 - APPEARANCE OF ISOPRENOID (PHYTANE AND PRISTANE) IN GLC TRACING OF n-SATURATE FRACTION OF VARIOUS CRUDE OILS AFTER 10 DAYS GROWTH AT 4°C

	TEST OIL*			
Enrichment Oil	Prudhoe Bay	Norman Wells	North Cantal	Lost Horse Hill
Atkinson Point	+'	+	+	+
Prudhoe Bay	±	±	±	+
Norman Wells	-	-	+	±
North Cantal	-	-	±	-
Lost Horse Hill	±	-	+	+

*Atkinson Point oil not used as it does not have an n-saturate fraction

'Apperance of phytane and pristane peaks in GLC tracing (see Figure 11). + = strong signal, ± = weak signal, - = utilization.

TABLE XL111 - EFFECT OF TEMPERATURE ON GENERIC COMPOSITION OF MIXED MICROBIAL POPULATION OBTAINED BY ENRICHMENT CULTURE ON OILS OF DIFFERENT QUALITY

GENUS	OIL							
	Prudhoe Bay		Atkinson Point		Norman Wells		Lost Horse Hill	
	4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C
Achromobacter sp.	22	18	0	27	0	0	0	67
Acinetobacter sp.	0	0	46	0	0	37	5	16
Alcaligenes sp.	22	8	0	34	0	0	50	0
Corynebacterium sp.	0	46	0	3	0	10	2	0
Flavobacterium sp.	53	0	0	0	40	0	0	2
Pseudomonas sp.	0	21	14	0	34	0	41	15
Xanthomonas sp.	0	0	0	39	10	21	0	0
Unidentified Gram Negative Rods	3	8	0	0	15	64	2	0
Unidentified Gram Negative Cocci	0	0	40	0	0	0	0	0

TABLE XL1V - GROWTH OF 4°C ENRICHMENT POPULATION ON THE SAME OIL AT 30°C

	TEST OILS									
	Bacteria x 10^5/ml									
	Prudhoe Bay		Norman Wells		Atkinson Point		North Cantal		Lost Horse Hill	
TIME (days)	Count	Increase	Count	Increase	Count	Increase	Count	Increase	Count	Increase
0	180	-	190	-	77	-	260	-	200	-
1	230	1.3	210	1.1	89	1.2	300	1.2	210	1.1
2	450	2.5	240	1.3	82	1.1	280	1.1	410	3.6
3	480	2.7	390	2.1	82	1.1	410	1.6	250	1.3
4	560	3.1	360	1.9	75	1	500	1.9	330	1.7
7	580	3.2	330	1.7	89	1.2	390	1.5	430	2.2
10	450	2.5	370	2.0	92	1.2	390	1.5	420	2.1

TABLE XLV - GROWTH OF 30°C ENRICHMENT POPULATION ON THE SAME OIL AT 4°C.

	TEST OILS							
	Bacteria x 10^5/ml							
TIME (days)	LOST HORSE HILL		NORMAN WELLS		PRUDHOE BAY		ATKINSON POINT	
	Count	Increase	Count	Increase	Count	Increase	Count	Increase
0	67	-	93	-	89	-	6	-
1	77	1.2	92	1.0	86	1.0	6	1
2	73	1.1	95	1.0	75	0.8	6	1
3	82	1.2	73	0.8	135	1.5	8	1.3
4	96	1.4	107	1.2	80	0.9	17	2.8
7	84	1.3	80	0.9	71	0.8	12	2.0
10	57	0.9	56	0.6	45	0.5	16	2.7

TABLE XLV1 - CHEMICAL COMPOSITION OF CRUDE OILS AFTER 10 DAYS INCUBATION AT 30°C WITH A POPULATION ENRICHED ON THE SAME OIL AT 4°C.

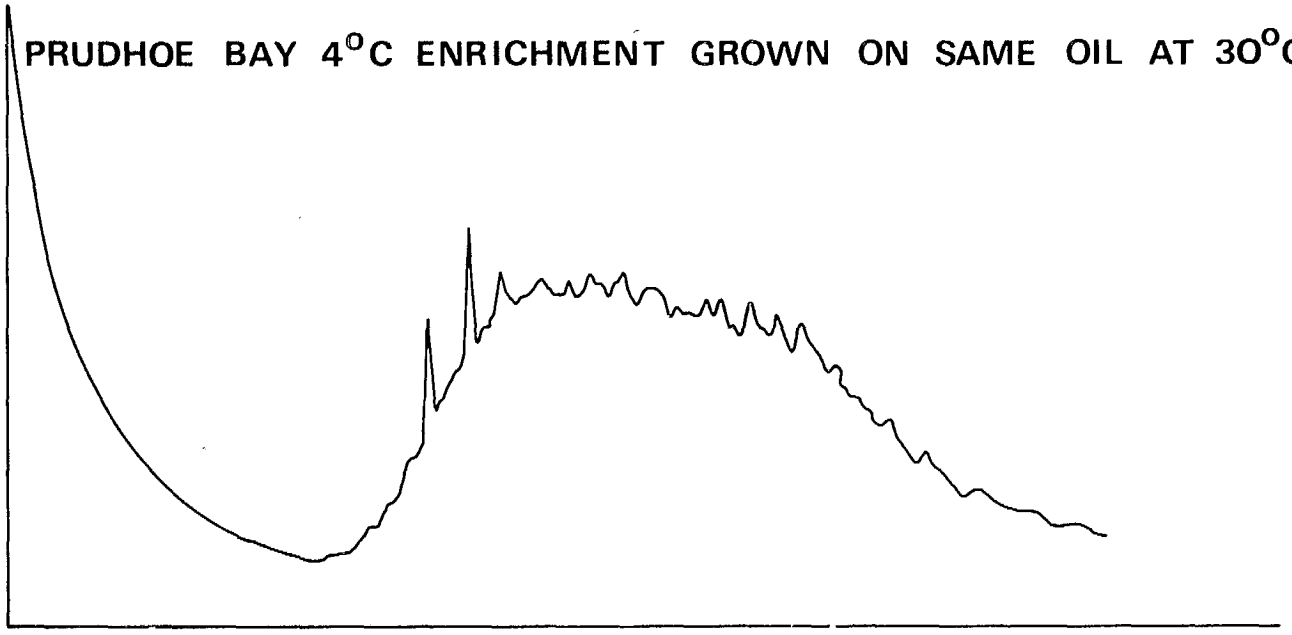
Crude Oil Fraction	OIL				
	Norman Wells Difference*	Atkinson Point Difference	Prudhoe Bay Difference	Lost Horse Hill Difference	North Cantal Difference
Asphaltenes, soluble	+ 0.31	+ 1.11	+ 2.52	+ 3.45	+ 1.30
Asphaltenes, insoluble	+ 0.86	- 3.60	- 0.15	- 2.59	- 1.02
Saturates	- 4.31	+ 1.72	- 5.93	-17.89	-10.28
Aromatics	+ 0.95	- 3.52	0	+ 0.09	+ 3.36
NSO's soluble	+ 1.65	+ 3.96	- 0.68	+ 5.95	- 2.58
NSO's insoluble	+ 0.55	+ 0.32	+ 1.61	+ 5.29	+ 3.65

*Percentage in microbially treated crude oil less that in untreated oil.

TABLE XLV11 - CHEMICAL COMPOSITION OF CRUDE OILS AFTER 10 DAYS INCUBATION AT 4°C WITH A POPULATION ENRICHED ON THE SAME OIL AT 30°C.

Crude Oil Fraction	OIL			
	Norman Wells Difference*	Atkinson Point Difference	Prudhoe Bay Difference	Lost Horse Hill Difference
Asphaltenes, soluble	- 0.05	+ 1.10	+ 1.35	+ 1.48
Asphaltenes, insoluble	- 4.84	- 3.46	- 1.81	+ 5.03
Saturates	+ 2.30	+ 2.89	+ 0.40	- 6.38
Aromatics	+ 0.66	+ 2.29	- 0.65	- 1.49
NSO's soluble	+ 1.35	- 2.99	+ 1.14	+ 3.71
NSO's insoluble	+ 0.60	- 1.25	+ 0.58	+ 2.00

*Percentage in microbially treated crude oil less that in untreated oil



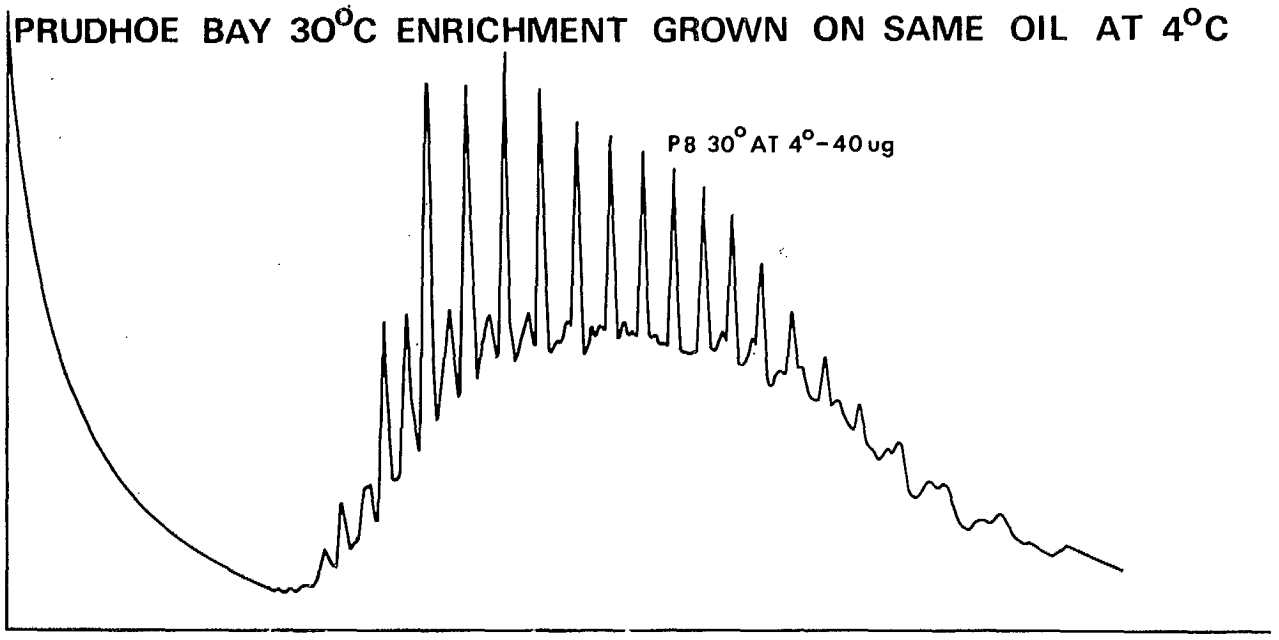


Figure 16 - G L C analysis of saturate fraction of Prudhoe Bay crude oil using populations enriched at 4° cna 30° respectively

(d) PHYTOTOXICITY STUDIES

The results of the phytotoxicity studies are presented in Tables XLVIII and XLIX. The low level of germination with the Swan Hills soils is a result of the use of poor quality barley seed. The level of germination in the Norman Wells study (93%) was obtained with a better quality seed. Statistical analysis (Table XLIX) indicates that, with one exception, the application of oil to soil inhibited germination. Analysis of the affects of treatments on the toxicity of oil indicated that there was no effect in the Swan Hills experiment or in the Cut-line samples from the Norman Wells experiment. However analysis of the results from the Bush-soils (Table XLVIII) suggests that the application of fertilizer enhanced the toxic effect of the oil.

TABLE XLVIII - PHYTOTOXICITY STUDIES - MEAN COUNT OF GERMINATED BARLEY

PLUS STATISTICAL ANALYSIS OF DATA

	Swan Hills Soils			Norman Wells Soils					
				Cut-Line			Bush		
Treatment	Seeds Germinated (mean)	Significance* 95%	99%	Seeds Germinated (mean)	Significance* 95%	99%	Seeds Germinated (mean)	Significance* 95%	99%
Control	249	-	-	468.3	-	-	423.3	-	-
+ Oil	168.5	1	1	403.3	1	1	327.5	1	0
+ Oil + Bacteria	175.8	1	0	406.3	0	0	259.0	1	1
+ Oil + Fertilizer	132.3	1	1	299.8	1	0	141.5	1	1
+ Oil + Fertilizer + Bacteria	143	1	0	337.0	1	0	181.5	1	1

* Control versus treatments; value of 1 = significant difference at 95 or 99% confidence level

' Significance difference also exists between these values and that of the oil application alone at the 95% confidence level

TABLE XLV1X - PHYTOTOXICITY STUDIES - STATISTICAL ANALYSIS OF NORMAN WELLS (BUSH) OIL TREATED PLOTS WITH THOSE WHICH HAD FERTILIZER AND OR BACTERIA APPLIED AS WELL.

Treatment	Confidence Level	
	95%	99%
Oil	-	-
Oil + Bacteria	0	0
Oil + Fertilizer	1	0
Oil + Bacteria + Fertilizer	1	0

7. DISCUSSION

The results of the field studies show that the utilization of crude oil in northern soils can be accelerated by the application of urea-phosphate fertilizer. The results obtained also indicate that under conditions such as Norman Wells bush plots the application of bacteria also has a stimulatory effect. These observations suggest that further field studies are warranted. They should help define which treatments can be used to accelerate the decomposition of oil in soil and in muskeg. These studies should ascertain which kinds and amounts of fertilizer and what concentrations and types of bacteria are needed to produce stimulatory effects.

The lack of reproducibility and sensitivity of the gravimetric procedure when applied to oil recovered from soil should be investigated. An increase in the efficiency of this section of our analytical procedures would aid in the determination of changes in the composition of the other fractions of crude oil occurring in soil.

Analysis of the ability of Arctic soils to digest Prudhoe Bay oil under laboratory conditions indicates a considerable variation in this ability. The results also suggest that emulsification of oil by a micro-organism does not necessarily indicate utilization of the n-saturate fraction. The presence of a relatively high number of bacteria in soil (e.g. 10^7 to 10^8/gm) does not necessarily mean the presence of oil-digesting bacteria. For example, the duff sample (#27) had approximately 10^8 bacteria/gm but was not capable of utilizing Prudhoe Bay oil. This point is further illustrated in comparing permafrost samples 7, 26 and 34. All contained between 10^5 to 10^6 bacteria per gram yet only one sample, i.e. #34 showed any ability to use the saturate fraction in Prudhoe Bay oil. These results suggest that a more detailed study of the presence of oil-utilizing micro-organisms in Arctic soils is warranted. It is also suggested that this survey be extended to water bodies and to the effects of burning on the presence of oil-digesting micro-organisms. This latter suggestion arises from the apparent loss of micro-organisms capable of utilizing oil under psychrophilic conditions in the Inuvik burn. If this is a general phenomena of burns in the north, then means of re-establishing this flora should be investigated if burning is going to be used to clean up oil spills. Emphasis should be placed on the temperature range 5°C to 15°C as this range is more relevant to northern conditions than the 30°C one used in parts of this study. Samples which did not contain micro-organisms capable of using the Prudhoe Bay oil at either temperature should be checked to see if this phenomenon is

an aspect of soil structure.

The results of the challenge series of experiments show that the chemical composition of an oil, its quality, has a marked effect on the types and total numbers of bacteria which can be produced on an oil. The presence of saturates, while not the sole factor controlling the microbiological response, is a major one. For example, the Prudhoe Bay oil contains less saturates (still has a normal saturate profile) and more aromatics and soluble NSO's than Norman Wells oil, but, of the oils studied, it is the most susceptible to microbial attack. The Lost Horse Hill crude has a saturate content similar to that of Prudhoe Bay yet weight losses of the order of 20% were common with this crude, as opposed to only 10% for Prudhoe Bay crude. This difference could lie in the nature of the saturate fraction itself or in some of the components in other fractions.

The temperature used for enrichment is also a major factor in determining the types of bacteria found growing on an oil. The major types enriched at both 4^{O} and $30^{o}C$ consisted of gram negative rods. Only on Prudhoe Bay crude at the higher temperature used did gram positive bacteria constitute a major portion of the population. On the information so far available, it is not possible to state whether or not an isolate by an Achromobacter genus isolated at $4^{o}C$ and $30^{o}C$ represents different species. It is also interesting to note that the population induced at $4^{o}C$ can utilize the same oil at $30^{o}C$ but those induced at $30^{o}C$ cannot grow at $4^{o}C$. Therefore, it would be useless to use mesophilic temperatures to induce populations to apply to oil spills where psychrophilic conditions exist.

It is interesting to note that microbial populations induced on high quality oils can readily utilize oils of similar quality but not a low quality crude like Atkinson Point oil. Therefore, if bacteria are being prepared for field application the quality of the crude spilled has to be taken into consideration when establishing the growth conditions.

The isoprenoids, phytane and pristane, are more resistant to utilization by bacterial populations induced at $4^{o}C$ than by those induced at $30^{o}C$. The appearance of these isoprenoids in G.L.C. profiles of oils extracted from soils suggests that the temperatures reached in these areas are those of a psychrophilic rather than a mesophilic nature. This observation confirms our previous statement about the need for work to be carried out under psychrophilic rather than mesophilic conditions.

The results of the phytotoxicity studies are too variable to be interpreted realistically. At the best, we have shown that the application of oil inhibits the germination of barley seeds. This is not new. However, there is a very clear indication that the application of fertilizer to the bush plots, but not the cut-line ones, resulted in an enhanced retardation of the germination of barley seed. Unfortunately, the appropriate control is lacking, that is fertilizer application alone. However, the failure to observe a similar effect on the cut-line plots suggests that it may be the result of an interaction between fertilizer and oil. This section of our work is not being continued as it consumes too much of our soil samples and the plots are not large enough to support active sampling at this level. The results also are too variable and so far the treatments have not had any effect on germination levels. It is our opinion that it is doubtful if soils from spills in the northern area would ever be used for agricultural purposes. Therefore, we are not sure as to the value of such data and plan to spend the time following the rate of the re-establishment of the natural flora of the areas where the spills were carried out and try to relate this to treatment.

8. CONCLUSIONS

The following conclusion can be drawn from the data presented in this initial report.

(1) The application of fertilizer can accelerate the decomposition of oil applied to soil. Such applications result in marked increases in bacterial numbers and increases in the rate of disappearance of the saturate fraction of crude oil. The application of oil-utilizing bacteria can also be beneficial under certain soil conditions. The application of fertilizer and oil was without effect on the numbers of molds present in the soils.

(2) Considerable variability exists on the presence, in Arctic soils, of bacteria capable of growing on the high quality Prudhoe Bay oil as the sole carbon source. The results obtained depend to some degree on the temperature of incubation used for the enumeration of the bacteria. Only 50% of the samples tested contained bacteria which were able to bring out emulsification of Prudhoe Bay oil under psychrophilic 4°C conditions. G.L.C. analysis of the saturate fraction recovered from these experiments indicates that, under these psychrophilic conditions, with one exception there had been only a very low degree of utilization of this component of Prudhoe Bay oil. In contrast, the analysis of recovered oils from similar experiments at 30°C showed a complete utilization of the n-saturates present.

(3) Considerable variation exists in the capabilities of mixed bacterial populations to use crude oils of different qualities. Populations enriched on low quality oils, (e.g. Atkinson Point) have some ability to use higher quality crudes, (e.g. Prudhoe Bay). However, the converse is not true; a population enriched on a high quality oil has very little ability to metabolize a low quality oil. The type of population enriched is dependent in part on the temperature of incubation, oil quality and, undoubtedly, by the microbial composition of the soil used in the enrichment procedure.

(4) A comparison of the changes brought about in oil under laboratory and field conditions emphasizes the need for future work to be centered on psychrophilic conditions; that is, field data complemented the laboratory results at 4°C and not those noted at 30°C.

(5) Gram negative rods predominate in populations capable of using crude oils at both 4°C and 30°C.

(6) The phytotoxicity studies using an agriculturally-important plant have confirmed the already-known fact that crude oil inhibits germination. The enhanced effect of fertilizer on the inhibitory effects of oil-treated Norman Wells bush plots is noted, but it is not felt to be worthwhile to pursue this observation unless such soils might be used for agricultural purposes.

9. IMPLICATION AND RECOMMENDATIONS

(a) GENERAL SCIENTIFIC IMPORTANCE

These investigations are unique in that they have attempted to establish relationships between oil composition and susceptibility to microbial degradation in experimental procedures with results which can be tested statistically. In addition, the investigations of some of the chemical factors (carbon to nitrogen ratio with or without the application of oil, introducing microbes to oil-soaked field plots in attempts to accelerate the disappearance of oil under natural conditions) are of prime importance in studying the oil-microbe relationship. The results suggest that the fertilization (urea-phosphate) of oil-soaked soils could accelerate the decomposition of the oil, emphasizing the need to know the fertility of the oil-soaked soil when projecting treatment procedures. The quality of the oil is also important when considering such recommendations, particularly if bacterial seeding is contemplated. The population used should be induced on a similar quality oil and under psychrophilic conditions.

The results of the field studies confirm the previous laboratory observation that the n-saturate fraction is the first one to be utilized during the microbial attack of oil. The observations that oils with similar saturate content can lose different amounts of this fraction during microbial attack suggest that other components of the crude could control the degree of metabolism of n-saturates. It is also equally probable that the composition of the n-saturate fraction itself is of critical importance as the typical saturate profile cannot be correlated with a given weight loss.

Future studies on phytotoxicity, particularly if agricultural plants are being used as test materials, have to examine the possible increased toxicity of oil brought about by interaction with fertilizer applications.

(b) All applicants, more specifically the users of pipelines, should be required to provide samples of oil being transported so that its biodegradability and other characteristics can be studied. If future work shows that certain types of Arctic soils are devoid of oil-utilizing micro-organisms, then consideration should be given to (i) inoculation of such areas with bacteria which can utilize the oil; (ii) use of alternate routes where the soils have the capability of biodegrading such an oil; (iii) extra care in the construction of pipelines through such terrain to minimize the possibility of such spills.

(c) The major point here is the possible advantages of the application of oil-utilizing bacteria to spill sites. We now realize that our level of application was very low when one considers the numbers present in the soil in its natural state. Therefore we feel, and some data support this, that stimulation of the rate of utilization of the added oil was achieved by the low level of bacteria applied. This procedure should be investigated to try and resolve its ultimate effect on the environment. None of us are enthusiastic about the introduction of large numbers of "foreign" microbes especially as this would probably involve the use of gram negative rods (e.g. Psuedomonas sp.) which are known to be pathogenic to man and other mammals under certain conditions. This is particularly true if one considers them as secondary invaders, not the primary causes of diseases but opportunists which flourish in animals under weakened conditions. Their antibiotic resistance patterns also complicate their projected use. However, one cannot rule out the possibility that they would only survive as long as the oil was present and, once it had been consumed, would die off leaving a 'normal' flora present in the soil. Such possibilities also have to be considered in the application of bacterial treatment of oil-water spills.

10. NEEDS FOR FURTHER STUDY

(a) Field Studies

We recommend continued monitoring of existing plots to determine if the additives stimulate the recovery of a normal plant cover and the extension of those plots to ascertain whether nitrogen or phosphate or both were required to bring about the observed results. The form of nitrogen, urea or ammonia, the rate of application and so on should also be considered as possible subjects for study. Similarly, the use of oil-utilizing bacteria should be further investigated.

It would be highly desirable to try our additive experiment to an existing oil spill if the environmental conditions are similar to those in the Mackenzie Valley. The spill would have to be found south of the 60th parallel because of the lack of oil lines north of it. A carefully chosen site would yield results which would be just as applicable north of 60 as south of it.

The results obtained so far are extremely encouraging.

(b) Survey of Arctic soils for oil-utilizing capability

The project should be developed more fully to try and delineate those areas where oil-utilizing micro-organisms cannot be found. The apparent lack of these micro-organisms when the experiments were carried out at 4°C, should be extended to other soil types. The marked decrease in oil-utilizing psychrophiles in the old burn site near Inuvik if confirmed would be of major interest when one considers the widespread use of the burning technique to "clean-up" spilled oil. It would also be very interesting to obtain samples from oil-worked sites in the central and high Arctic to see if the findings of the Mackenzie Valley studies are applicable elsewhere in the north.

(c) The mixed populations from the challenge series of experiments should be resolved to see if it is only the major components of the population (e.g. those comprising 20% or more of the population) which are required to bring about the observed changes in the oil. The maintenance of these populations should be examined in order to have a collection of microbes which could be quickly brought forth to be applied to spills of different quality oils. If this were possible, the time required to treat a spill with oil-utilizing microbes would be greatly reduced. It would also be of interest to see if such populations could readily metabolize oil from other areas of the Arctic and other parts of the world. That is, would oils of similar saturate content from difference oil fields be equally susceptible to biodegradation.

(d) Phytotoxicity Studies

There is need for further work in this area to resolve the apparent enhancement of oil toxicity when fertilizer has been applied to plots. It cannot be ruled out that the oil induced a toxicity in the fertilizer which was applied at the time the soils were treated with oil. This problem is particularly important if the land is to be used for agricultural purposes.

(e) Further studies relating the chemical composition of oil to biodegradability.

The studies have been concerned with the utilization of only one of the main components of high quality oils, the n-saturate fraction. Undoubtedly we are getting utilization of compounds in the aromatic fraction as well. It is also reasonable that the most bio-toxic components of oil reside in this and the NSO fraction. The data presented have suggested that the composition of the other fractions of crude oil have an effect on the utilization of n-saturates. It is in these studies that new and possibly valuable new chemical compounds will be found.

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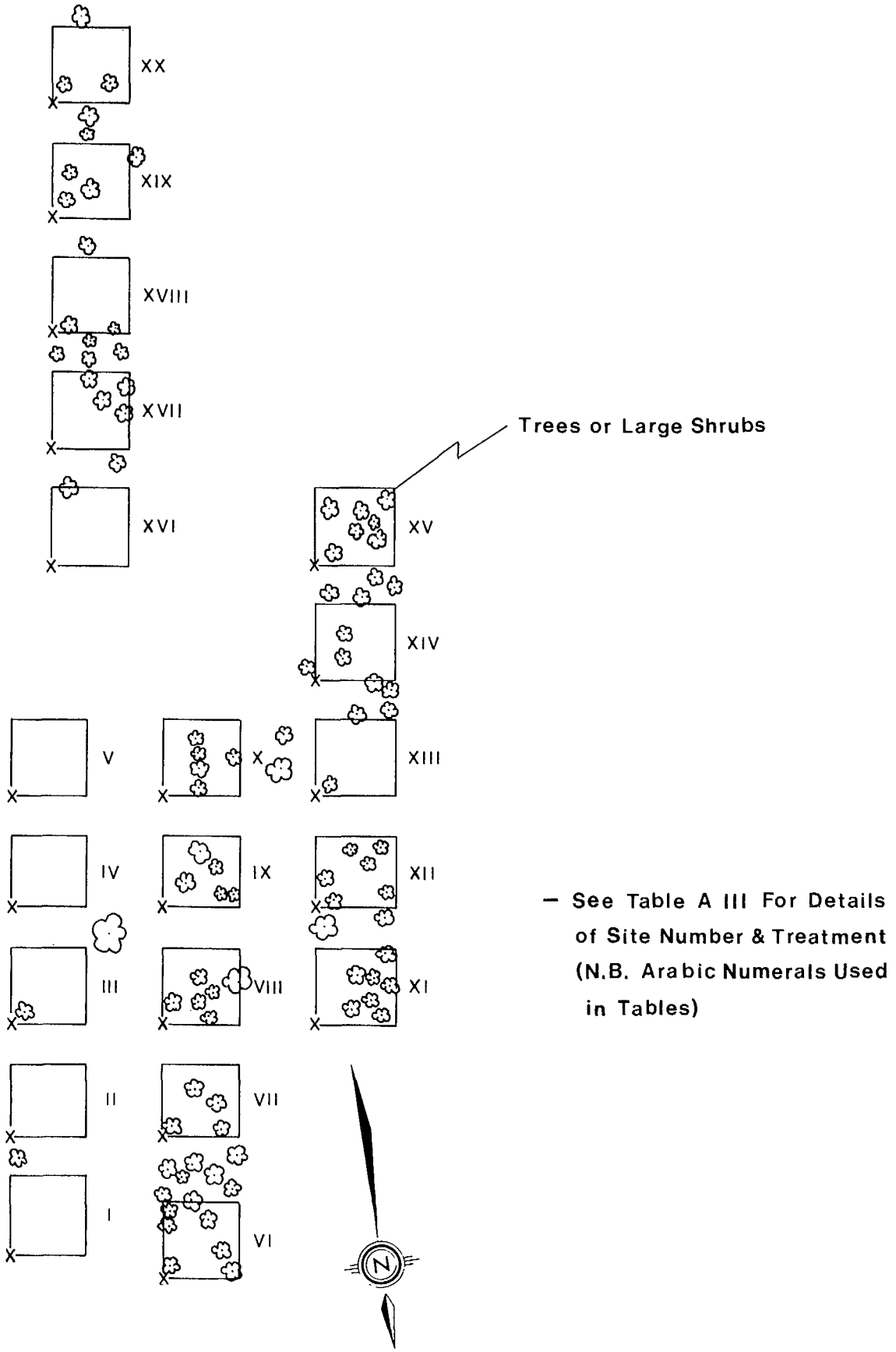
12. APPENDIX

-PLOT PLAN - SWAN HILLS SPILL



– See Table A III For Details of Site Number & Treatment (N.B. Arabic Numerals Used in Tables)

ANALYTICAL

Residual crude oils were extracted from cultures using n-pentane as a solvent. Cultures were divided into two equal fractions and extracted three times with a volume of solvent equal to 30% of the original divided culture volume. The aqueous phase was removed each time using separatory funnels and draining to the inerfacial area which contained the salts, asphaltenes and solvent. This interfacial material was removed after the last solvent extraction, pooled with the n-pentane washes, and evaporated to dryness in a fume hood. The residual petroleum was recovered from the beakers using 10-20 mls of benzene. The walls of the beaker were rigorously washed with this benzene prior to its removal and storage for analysis.

Liquid chromatographic fractionation: Samples containing 0.05-0.4 gms of residual or crude petroleum were placed in a tared beaker and "topped" by exposing them to forced draft conditions for 19 hours at either 32^{o} or 36.5^{o}. This treatment removed volatile materials e.g. light aromatics, napthenes, and n-alkanes up to and including c_{15} chain length, leaving a weight referred to as the "topped weight of oil". The asphaltenic component of the "topped oil" was precipitated by addition of n-pentane. The pentane solubles and precipitated asphaltenes were then applied to a 1 X 15 cm bed of Hyflo Super-Cell (Fisher Scientific Co.) suspended in n-pentane. The column was sequentially developed using 50 mls of n-pentane and 40 mls of benzene to elute the deasphaltened oil and benzene-soluble asphaltenes. The weight of benzene-insoluble asphaltenes remaining on the column was calculated by difference. The deasphaltened oil (n-pentance-soluble) was fractionated by adsorption chromatography using a dual phase column (1 X 40 cm) containing in the bottom half 10 $\pm$ 0.2 gms of activated 28-200 mesh silica gel (Matheson) and in the upper half 10 $\pm$ 0.2 gms of activated F-20 alumina gel (Matheson). Both phases were suspended in n-pentane. The deasphaltened oil was layered on the top of the column and then eluted sequentially with 65 mls of n-pentane, 100 mls of benzene, and 100 mls of a 1:1 mixture of benzene-methanol. This procedure (Fig. A1) eluted the saturate, aromatic, and soluble *NSO components in that order. The sum of these three weights subtracted from the deasphaltened weight originally applied to the dual phase column yielded the insoluble NSO component weight. This procedure is similar to that used by Imperial Oil Research Laboratories, Calgary, Alberta.

*NSO component - fraction recovered by elution with a 1:1 benzene:methanol mixture. This fraction should contain more polar compounds than those eluted with benzene which yields the aromatic fraction of crude oil.

Gas-Liquid chromatographic analysis of saturate hydrocarbons: The separation and determination of n-saturate alkanes was achieved using a Varion Aerograph Chromatograph Model 1740-1 equipped with a flame ionization detector and a ten foot 1/16" I.D. stainless steel column containing (100-200 mesh) chromosorb P pre-coated with 3% OV-1. Columns were conditioned at 325° for 72 hours prior to use, and all carrier gases were purified by passage through Hydro-Purge Molecular Sieve 5A filters prior to passage through column or detector. The instrument was programmed as follows:

Linear temperature program: 50° - 325°
Rate of programming: 10°/minute
Injection block temperature: 300°
Nitrogen flow rate: 12.0 mls/minute
Hydrogen flow rate: 15.0 mls/minute
Air Flow rate: 300 mls/minute

Forty micrograms of saturates could be readily resolved using these conditions. Benzene was used as solvent as it passed through the OV-1 column well in advance of all other components and is less volatile than n-pentane.

TABLE AI NORMAN WELLS OIL SPILL

Microbial counts and pH values - 22 days after treatment

LOCATION		CUT LINE				BUSH				
			Bacteria		Molds			Bacteria		Molds	
			4°C	21°C	4°C	21°C		4°C	21°C	4°C	21°C
Number	Treatment	pH	x 10^6/gm		x 10^3/gm		pH	x 10^6/gm		x 10^6/gm	
1	Control	6.5	21.3	36.6	28	97	6.7	36.0	42.0	57	380
7		7.1	26.6	57.1	91	171	6.8	38.7	127	109	220
12		6.7	35.5	192	300	700	6.6	27.3	131	128	700
19		7.8	64.0	133	24	106	7.6	82	118	125	680
4	Oil	6.6	42.0	142	110	184	6.3	48	115	55	120
6		6.5	47.2	188	36	85	6.6	176	630	56	400
14		7.1	51.5	248	103	131	6.8	127	171	48	780
16		6.9	43.7	92	95	250	6.8	95	280	25	92
4	Oil	6.5	39.6	179	34	108	6.3	72	236	61	230
8	Bacteria	6.5	127	572	42	250	6.6	67	221	33	60
11		6.7	27.2	168	84	280	6.6	97	296	34	83
20		7.5	164	311	42	39	103	103	176	18	150
3	Oil	6.0	12,000	17,600	37	117	6.3	13,300	21,600	210	370
10	Urea-PO_4	6.6	10,100	20,700	114	153	7.1	25,800	25,000	51	140
13		6.1	3,000	10,300	90	76	5.5	3,140	5,300	79	350
17		6.9	5,840	10,900	52	69	6.5	18,700	24,100	26	60
2	Oil	6.1	12,700	20,200	690	2,820	6.1	45,600	54,300	60	27
9	Bacteria	6.4	7,400	13,200	138	315	6.4	8,400	13,100	94	300
15	Urea-PO_4	6.1	7,710	13,800	49	66	6.0	9,700	14,200	84	460
18		6.6	3,020	3,800	20.9	96	6.5	20,800	23,900	100	350

TABLE AII % COMPOSITION OF NORMAN WELLS OIL AFTER 22 DAYS OF CONTACT WITH SOIL PLUS VARIOUS AMENDMENTS

Crude Oil Fraction	Barrel	Soil		+ Bacteria		+ Fertilizer		+ Bacteria + Fertilizer	
		Bush	Line	Bush	Line	Bush	Line	Bush	Line
Asphaltenes, soluble		4.59	2.85	3.72	4.66	3.58	4.60	3.18	2.98
	2.24	3.61	2.87	2.60	3.08	4.09	5.41	5.46	7.15
		6.89	4.81	4.63	5.23	2.54	3.01	3.35	4.91
		4.85	6.66	0.26	6.75	4.66	8.00	5.74	3.78
Asphaltenes, insoluble	8.49	7.61	6.62	7.02	6.87	7.51	7.49	5.95	9.65
		9.33	6.64	6.64	6.27	9.84	13.40	11.01	8.30
		4.82	5.66	5.95	5.76	3.97	5.22	9.55	8.68
		5.68	9.78	7.06	5.67	7.68	2.02	7.27	6.12
Saturates		46.89	48.76	47.99	49.83	47.16	42.73	45.65	41.59
	45.98	46.74	48.06	47.07	47.77	42.57	38.75	42.48	42.43
		47.69	50.73	50.92	47.58	46.17	44.14	41.88	41.84
		47.40	58.47	49.83	49.74	43.77	43.68	43.59	44.35
Aromatics		26.81	27.49	26.78	26.58	28.08	30.88	30.62	28.67
	29.29	26.81	27.77	27.07	27.05	27.08	25.93	26.89	27.27
		25.86	26.27	26.90	26.12	29.28	28.67	28.38	26.59
		26.16	21.56	27.60	26.60	26.30	28.04	27.16	27.35
NSO's, soluble		8.88	9.63	8.50	8.61	9.31	10.32	10.75	9.95
	10.38	8.21	8.86	9.24	8.93	8.81	8.60	9.15	9.31
		7.98	10.01	8.79	8.91	11.28	11.44	9.99	9.09
		9.53	7.95	11.47	9.97	10.12	10.27	10.50	10.55
NSO's, insoluble		5.22	4.65	5.99	3.45	4.36	3.98	3.84	2.98
	3.61	5.50	5.97	6.78	6.97	7.62	7.90	5.00	7.65
		6.77	2.52	2,80	6.41	6.77	7.53	8.85	8.89
		6.37	0	3.82	1.27	7.46	7.99	5.74	7.85

TABLE A111 SWAN HILLS OIL SPILL

Microbial counts and pH - 12 days after treatment

Site Number	Treatment	pH	Bacteria x 10^6/gm	Molds x 10^3/gm
1	Control	5.2	17.0	210
7		5.2	9.0	280
12		4.9	17.8	290
19		5.4	9.5	42
5	Oil	5.2	16.9	20
6		5.2	34.0	110
14		4.9	169	30
16		5.2	19.8	31
4	Oil + Bacteria	5.5	21.1	33
8		5.3	25.2	320
11		5.0	170	140
20		9.2	92	4
3	Oil + Urea-PO_4	6.3	6300	14
10		5.8	6670	17
13		4.7	2080	6
17		6.1	5600	45
2	Oil	5.8	7150	11
9	+ Bacteria	5.7	193	80
15	+ Urea-PO_4	6.5	6320	13
18		5.6	1350	3

TABLE AlV SWAN HILLS OIL SPILL

Microbial counts and pH values - 66 days after treatment

Site Number	Treatment	21°C Bacteria x 10^6/gm	21°C Molds x 10^3/gm	pH
1	Control	20.8	51	5.3
7		133	50	5.5
12		58.0	59	5.3
19		15.1	22	5.4
5	Oil	153	9.6	5.6
6		159	50.0	5.6
14		670	650	5.5
16		114	22	5.4
4	Oil + Bacteria	71	45	5.5
8		89	210	5.5
11		600	140	5.3
20		490	130	5.5
3	Oil	1370	85	6.1
10	+ Fertilizer	1380	15	5.6
13		2070	310	5.7
17		148	18	6.7
2	Oil	1610	NA*	6.0
9	+ Bacteria	1210	7	5.7
15	+ Fertilizer	2800	170	5.2
18		1220	1.2	5.5

TABLE AV CHEMICAL COMPOSITION OF SWAN HILLS OIL AFTER 12 DAYS

IN CONTACT WITH SOIL PLUS VARIOUS AMENDMENTS

Crude Oil Fraction	% Composition of Oil				
	Barrel	Soil	Bacteria	Fertilizer	Bacteria Fertilizer
Asphaltenes, soluble		1.05	1.23	2.49	1.91
		0.95	0.99	1.58	2.15
	2.90	1.36	1.80	1.20	1.42
		0.62	1.15	0.66	0.99
Asphaltenes, insoluble		2.98	7.50	4.74	5.78
	9.56	4.21	1.64	6.11	4.89
		4.49	3.52	3.66	4.43
		8.46	3.16	3.68	3.70
Saturates		61.74	59.74	55.47	58.35
		65.93	60.84	56.57	55.39
	55.08	61.85	62.48	60.62	57.94
		61.47	68.47	63.85	67.89
Aromatics		21.10	21.22	23.68	23.14
	21.58	21.46	20.79	23.65	25.52
		22.19	22.37	23.54	24.66
		19.95	22.42	24.02	23.18
NSO's, soluble		6.87	6.22	8.22	7.12
		6.35	7.62	6.68	8.02
	5.66	5.42	5.14	5.99	6.30
		6.95	5.92	5.98	0
NSO's, insoluble		6.26	4.09	5.40	3.68
		1.11	8.11	5.40	4.03
	5.22	4.68	3.91	4.50	5.25
		2.55	0	1.81	0

TABLE AV1 CHEMICAL COMPOSITION OF SWAN HILLS OIL AFTER 66 DAYS IN CONTACT WITH OIL PLUS VARIOUS AMENDMENTS

Crude Oil Fraction	% Composition of Oil				
	Barrel	Soil	Bacteria	Fertilizer	Bacteria Fertilizer
Asphaltenes, soluble	2.90	3.27	3.91	5.44	3.52
		2.20	2.33	6.27	3.48
		1.87	2.50	3.42	2.62
		2.72	2.33	2.22	2.35
Asphaltenes, insoluble	9.56	5.00	6.32	9.35	9.36
		3.47	6.76	15.66	9.97
		4.65	7.09	11.70	6.17
		10.19	5.79	6.53	10.50
Saturates	55.08	58.56	58.70	45.04	44.51
		61.49	64.36	42.20	41.96
		65.47	54.43	43.26	48.54
		60.26	65.61	54.78	49.91
Aromatics	21.58	21.70	19.89	21.92	25.17
		23.96	21.05	20.15	24.98
		24.06	19.51	25.17	26.86
		20.76	20.32	25.67	25.30
NSO's soluble	5.66	9.04	9.66	12.20	10.70
		6.81	0	11.41	12.12
		6.24	6.46	10.93	10.82
		6.38	6.91	7.17	7.44
NSO's, insoluble	5.22	2.17	1.5.	6.07	6.74
		2.07	0 (?)	4.30	7.49
		0	10.0	5.52	4.99
		0	0	3.63	4.50

TABLE AV11 - MICROBIAL ANALYSIS OF ARCTIC SOILS

Sample Number	Location and Description	Bacteria x 10^5/gm		Molds x 10^3/gm	
		4°C	21°C	4°C	21°C
	NORMAN WELLS AREA				
1	Oil soaked organic soil from refinery overflow ditch	23.2	105.0	NA	489.0
2	Control soil for 1 taken near this first sampling site-free of oil contamination	209.0	77.0	9.4	21.0
3	Oil soaked clay	155.0	49.2	0.4	5.9
4	Control clay sample for 3 free of oil	0.6	19.5	19.6	24.2
5	Oil soaked sample near old Canol storage tanks	0.4	0.2	0.2	11.4
6	Control sample for 5 - free of oil	9.7	31.2	2.6	18.9
7	Clay permafrost sample	3.4	4.7	$>10^2$	0.3
8	Burned moss layer	389.0	10,700.0	123	665
8a	Decomposed black organic layer	310	765.0	8.3	4.0

TABLE CONT'D

Sample Number	Location and Description	Bacteria x 10^5/gm 4°C	Bacteria x 10^5/gm 21°C	Molds x 10^3/gm 4°C	Molds x 10^3/gm 21°C
9	From bottom edge of Shell Lake (east of Inuvik): degraded algal and fern material present - very black-indicative of FeS_2 presence	235.0	379.0	1.3	8.2
10	Sample site 20' from shore of Shell Lake; LF sample-0-5 inches deep. Ground cover Labrador tea and birch	55.0	81.0	2.0	2.8
11	As for 10 except Ah horizon beneath LF; 5-8" deep; good black coloration	29.0	76.0	1.1	1.7
12	As for 10 except Ah horizon beneath LF; 8-11" deep; yellow material B subsoil	4.0	289.0	$>10^2$	0.1
13	Experimental loop test site west of Inuvik, flat watery muskeg sample	452.0	1342.0	32.4	16.9
14	Fisheries Research Board; Inuvik oil spill lakes. Duff LF horizon near L4 lake.	220.0	265.0	9.0	14.0
15	As for 14 - mineral soil beneath duff sample; layer only 1 inch thick	59.0	84.0	2.3	24.0
16	As for 14 - permafrost sample beneath mineral soil layer	14.0	32.0	$>10^2$	16.6
17	Mud on shore of L4 lake	55.0	86.0	1	81.0
18	'L4 - water sample; 1 inch below surface	1.3	2.4	$>10^2$	$>10^2$

TABLE CONT'D

Sample Number	Location and Description	Bacteria x 10^5/gm 4°C	Bacteria x 10^5/gm 21°C	Molds x 10^3/gm 4°C	Molds x 10^3/gm 21°C
19	'as per 18	0.2	0.2	$>10^2$	$>10^2$
20	Silt sample, 30 feet from edge of L4 Lake; result of spring flooding?	8.0	33	0.3	1.1
21	Edge of Old Crow landing strip; top 4" of heavy duff; spruce and willow cover	36.2	1000	0.2	0.9
22	As for 21, deep Ah and some B - very organic, good structure looks fertile	25	47	0.3	2.4
23	LFH sample (5" deep) 2 1/2 miles north at Old Crow - near tundra	17.7	700	3.1	19.0
24	As per 23 - mineral soil 5 to 10" deep beneath LFH horizon	3.8	12.3	$>10^2$	2.0
25	As per 23 - beginning of C-horizon, 12-13" below surface	0.9	14.6	$>10^2$	$>10^2$
26	As per 23 - permafrost 17 inches below surface	8.8	20.1	$>10^2$	$>10^2$
27	Duff sample 0-12 cm deep; upstream from Old Crow near Porcupine River Black spruce and older cover	2610	3020	480.0	420.0
28	As per 27 - Ah horizon 12-28 cm in depth	4.5	17.9	$>10^2$	1.0
29	Old Crowvillage, silt layer (recent) 0-5 cm in depth, small amount of organic matter on top	10.5	175	$>10^2$	$>10^2$
30	As per 29 - silt material 15-30 cm in depth	16.2	52	$>10^2$	$>10^2$

TABLE CONT'D

Sample Number	Location and Description	Bacteria x 10^5/gm 4°C	Bacteria x 10^5/gm 21°C	Molds x 10^3/gm 4°C	Molds x 10^3/gm 21°C
31	'Water sample - Porcupine River	$>10^3$	$>10^3$	$>10^2$	$>10^2$
32	Small island is 10 miles S.W. of Tuktoyaktuk; Duff sample 14 cm deep; cover Reindeer moss	1.4	47.3	0.9	44.0
33	As per 32; organic layer underneath duff and above permafrost	1.5	21.9	1.6	1.0
34	As per 32; permafrost sample	5.8	1783	41.0	$>10^2$
35	Upland material; 1/4 mile from Inuvik; old burn site covered with Fireweed. Duff sample top 5 cm	1.3	2.7	1.3	3.6
36	As per 35; B horizon 15-29 cm	0.6	2.8	0.9	1.8
37	As per 35; B horizon 15-29 cm	2.2	3.1	$>10^2$	$>10^2$
38	As per 35; C horizon 29 cm	2.9	5.1	$>10^2$	$>10^2$
39	As per 35; permafrost sample	36.6	70	$>10^2$	0.6
40	Moss sample near Kelly Norman Wells moss Inuvik (oil soaked soil around power plant)	0.7	9.2	$>10^2$	12.6

' checked for total counts only

* fifth day of incubation during 4th transfer

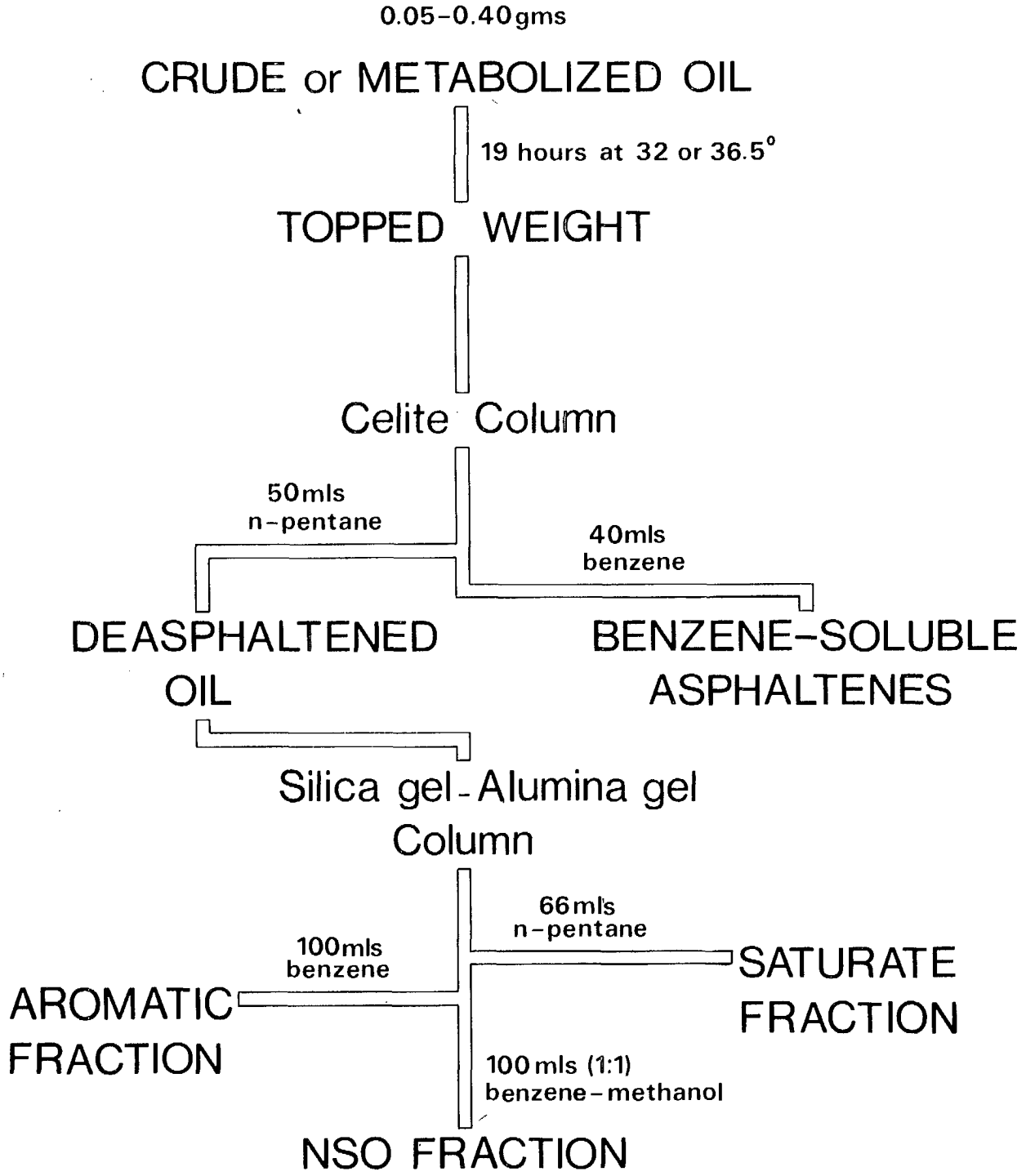
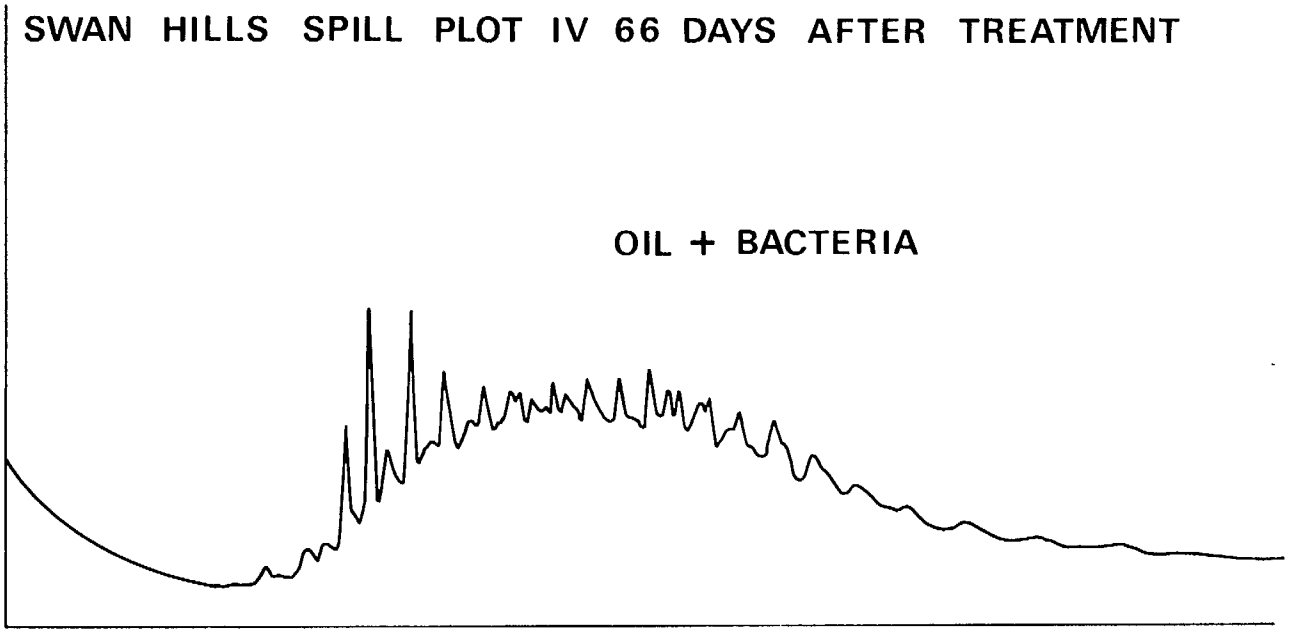


Figure A1 - Flow sheet liquid chromatographic analysis of petroleum



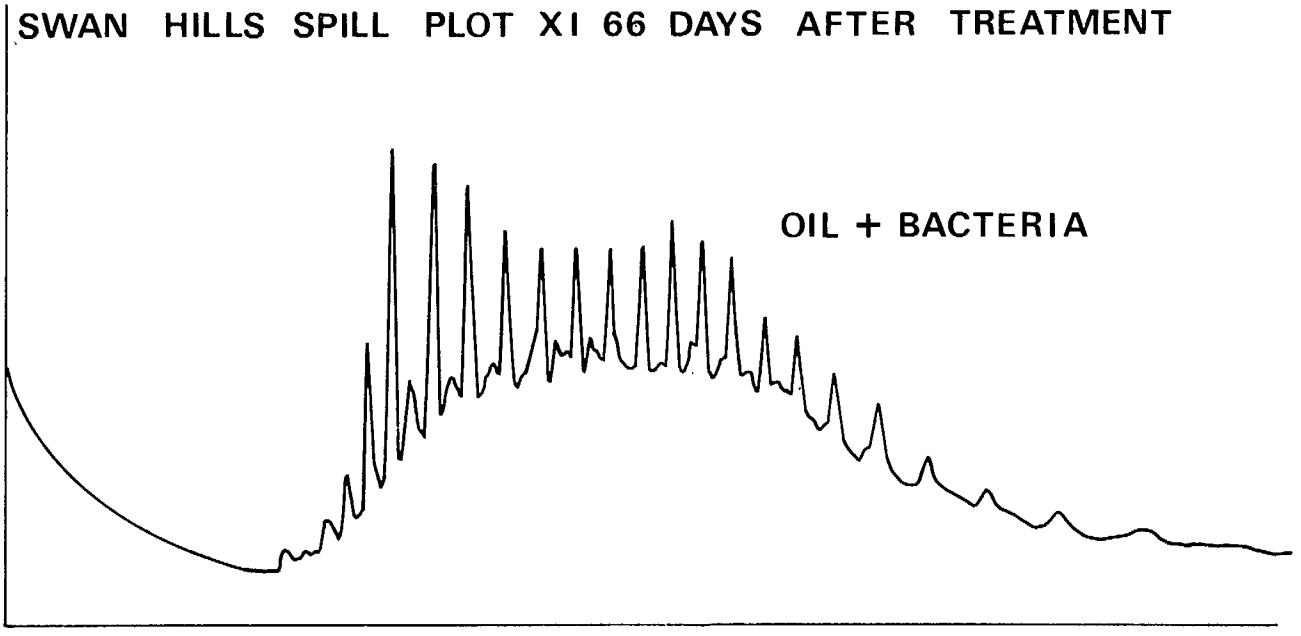
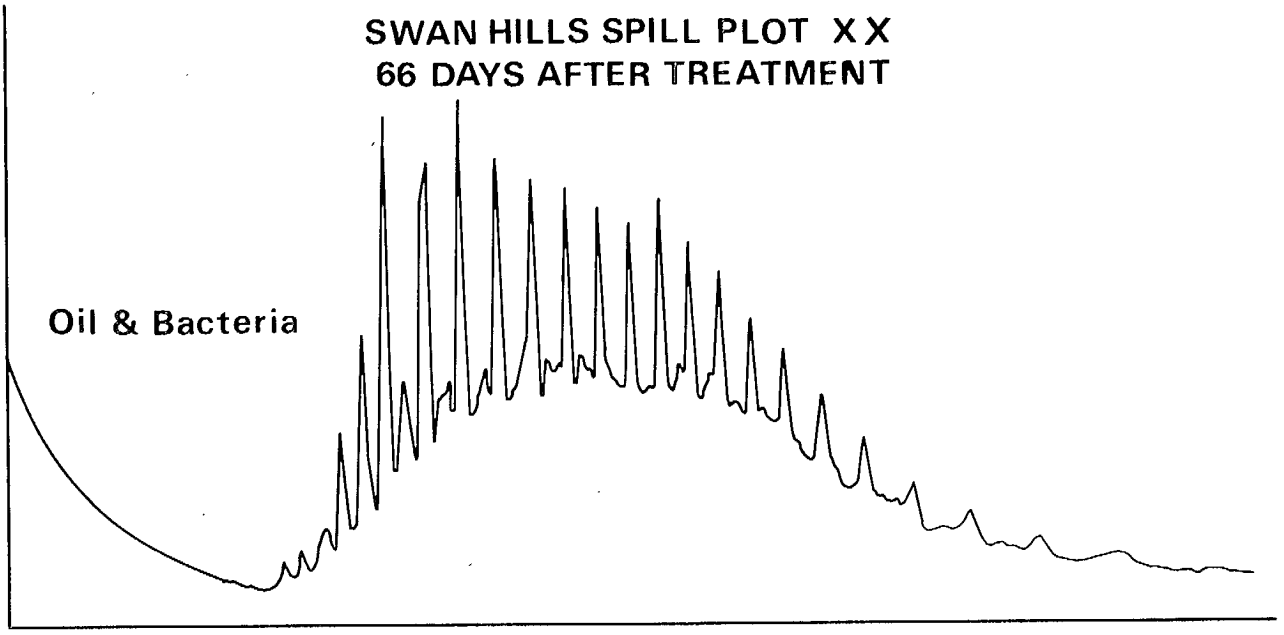


Figure A2 - G L C analysis of saturate fraction of Swan Hills oil 66 days after application to soil with a bacterial amendment



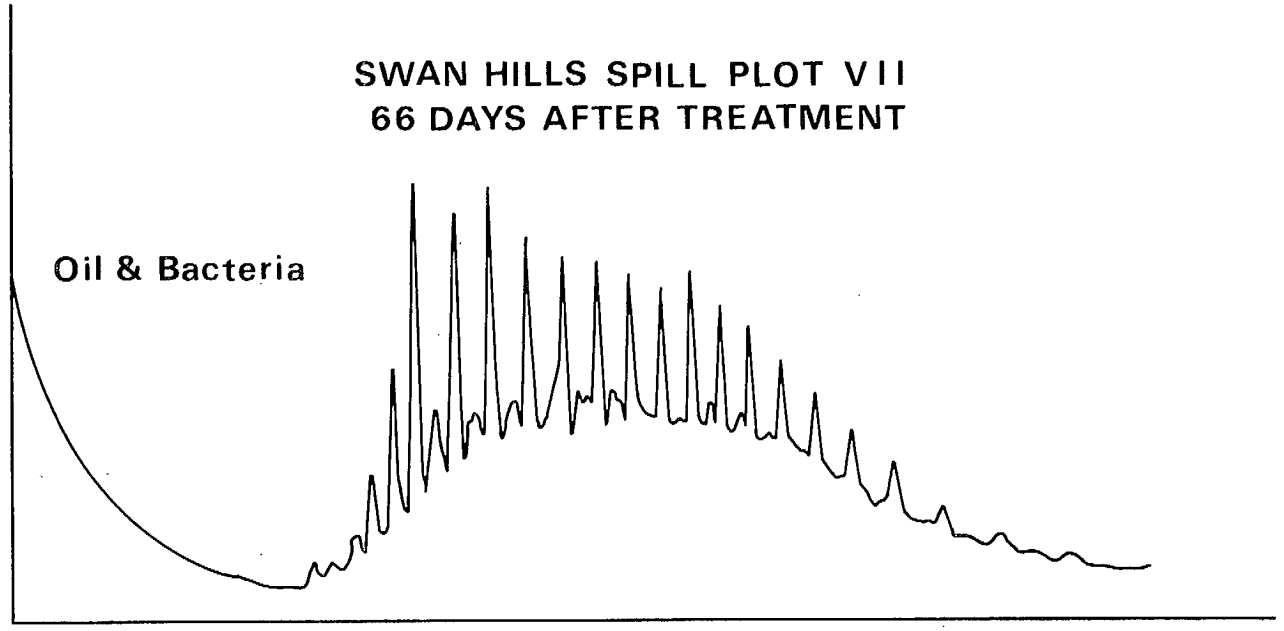


Figure A3 - G L C analysis of saturate fraction of Swan Hills oils 66 days after application to soil with bacterial amendment

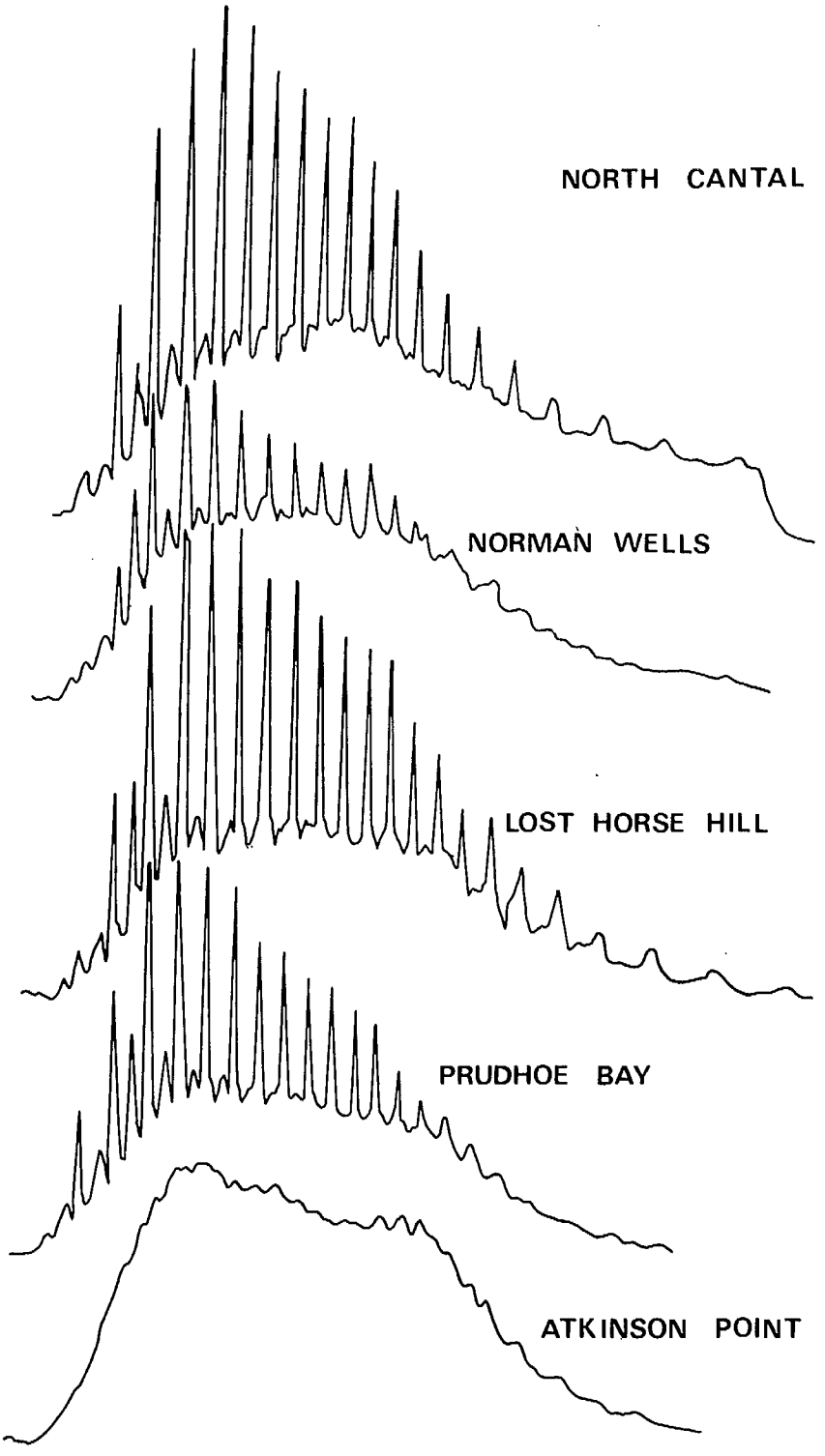


Figure 4 - G L C profile of n-saturate fraction of crude oils used in these studies

TAXOMETRIC PROCEDURES

All of the colonies appearing on a plate in the dilution series counted for the enumeration of micro-organisms in the challenge experiments were grouped on the basis of their colonial morphology. Samples of each colony were then inoculated into tubes of plate count liquid medium and incubated at 30°C. As soon as sufficient growth occurred, the culture was used to inoculate test media necessary to characterize the cultures. The procedure used was as follows:

1. Colonies were inoculated into tubes of plate count liquid medium and incubated at 30°C.

2. After growth was observed, the culture was used to inoculate 6 tubes of O.F. (oxidation-reduction) medium and 6 tubes of holding medium.

3. Three of the O.F. tubes were incubated under aerobic conditions and three under anaerobic conditions at 30°C, and scored for acid alkaline and/or gas production after a suitable incubation period.

4. Flagellar arrangement determined by electron-microscopy.

5. Plates were spread for catalase, oxidase and 3-ketolactose formation.

6. Determination of Gram reaction.

7. All isolates which were classifiable as Pseudomonads by the above tests were streaked on Bacto Pseudomonas, F agar to test for pigment production (fluorescein and/or pyocyanin formation).

8. All isolates which were Gram positive, non-motile rods and which did not ferment sugars were tested for acid-fastness and/or the presence of metachromatic granules.

COMPOSITION OF MEDIA AND REACTIONS OBSERVED

(a) Sugar utilization - Bacto OF (oxidation-fermentation) media with sugars tested being sterilized separately and added aseptically prior to inoculation. Reaction noted - acid or alkaline with or without gas formation.

(b) Oxidase and catalase activity - isolates streaked on plate count agar (Difco) and a Difco oxidase disc placed on a streak - oxidase positive cultures develop a pink color. When isolates had grown a few drops of 5% H_2O_2 were added to as streak to test for catalase - positive reaction - bubble formation.

(c) Test for 3-ketolactose formation. Isolates were streaked on yeast extract, lactose agar plates and when growth occurred the plates were flooded with Benedict's solution. A positive reaction is indicated by the formation of a ring of reduced Cu_2O around a colony.

(d) Acid-fast stain for non-motile Gram + rods - Zehl - Nielsen method.

(e) Metachromatic granule stain as per Laybourn, R.L. 1924. J. Amer. Med. Ass. 83, 121.

Data presented in Table A show the relationship between morphology and the reactions and generic classification.

TABLE A - CULTURE CHARACTERISTICS* AND GENERIC CLASSIFICATION

Gram Reaction	Shape	Motility	Flagella	SUGAR REACTION aerobic pH	aerobic gas	anaerobic pH	anaerobic gas	3 keto-lactose production	Catalase	Oxidase	GENUS
negative	Rod	±	polar	A	-				+	+	Pseudomonas
negative	rod	±	polar	A	-	A	+		+	+	Aeromonas
negative	rod			A±	-				+		Acinetobacter
negative	rod	±	peritrichous	A±	-				+	+	Achromobacter
negative	rod	+	polar or peritrichous	Alk-					+	+	Alcaligenes
negative	rod	±	peritrichous	A±					+	+	Flavobacterium
negative	rod	+	peritrichous	-					+		Chromobacterium
negative	rod	+	polar	A±					+	+	Xanthomonas
negative	rod	±		A±				+	+	+	Agrobacterium
negative	rod	+	peritrichous	A+		A	±				Enterobacteria
negative	comma	+	polar	A+					+	+	Vibrio
positive	coccus			A		A			+		Staphylococcus
positive	coccus			A±					+		Micrococcus
positive	coccus			A±					-		Streptococcus
positive	rod	±	peritrichous	A		A	±		+		Bacillus (± spores)
positive	rod			A		A					Lactobacillus
positive	rod	±	polar	A±					+		Corynebacterium (metachromatic granules)
positive	rod (irregular)			A±					+		Mycobacterium (acid fast)

*+ positive response; ± variable response; A= acid; Alk= alkaline; blanks not valid test or negative response

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