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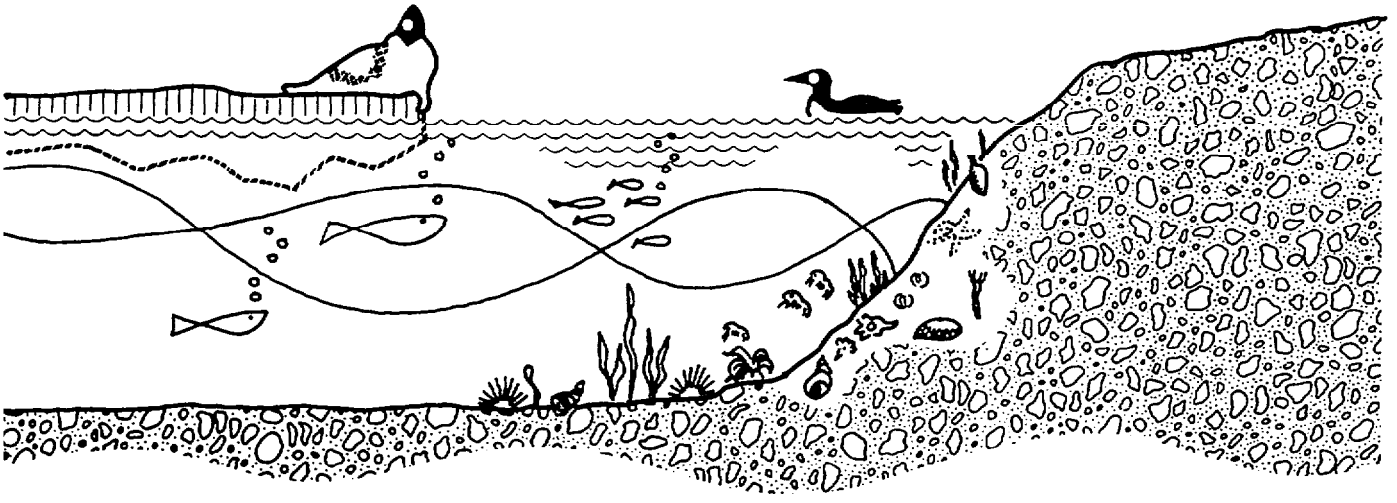
MICROBIOLOGY



Baffin Island Oil Spill Project

WORKING REPORT SERIES
83-6

1983 STUDY RESULTS

BAFFIN ISLAND OIL SPILL PROJECT
WORKING REPORT SERIES

The Baffin Island Oil Spill (BIOS) Project is a multidisciplinary program of research on arctic marine oilspill fate, effects and countermeasures. The Project commenced in the spring of 1980 and has now completed the fourth and final year of planned field work at an experimental site located on the northern end of Baffin Island, Canada. The results of work performed in each of the various study components under the Project, have been made available on a yearly basis through this working report series. This has been done prior to a complete integration of findings and interpretation with respect to the Project objectives. The working report series should therefore be considered as interim or data reports. The contents do no necessarily reflect the views or policies of the BIOS Project management or funders.

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BAFFIN ISLAND OIL SPILL PROJECT

MICROBIAL DEGRADATION OF OIL

ONSHORE ENHANCED BIODEGRADATION EXPERIMENTS IN Z-LAGOON AND ASSESSMENT OF BIODEGRADATION OF OIL IN BAY 11. POSTSPILL SURVEYS.

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ABSTRACT

Three sets of oiled plots for enhanced biodegradation experiments have been established in Arctic backshore sediments.

The effect of artificial fertilizer is positive, but varies depending on the geomorphological conditions of the beach. In fine sediments 10-100 times increased level of oildegrading bacteria relative to unfertilized controls are maintained over a 2 years observation period. Chemical analyses indicate a 3 to 5 times enhancement of the rate of biodegradation, at least for the alkane fraction of oil. In coarse sediments the effect of fertilizer appears to be marginal.

Mechanical mixing of oil and fertilizer into the sediments gives further improvement of the biological selfpurification of the oiled sediment, in spite of a lower level of oildegrading bacteria.

The oil polluted intertidal sediments of Bay 11, the scene of the surface oil release in 1981, have very high levels of oildegrading bacteria. The highest counts of bacteria, $5\text{-}7 \times 10^7$ per ml sediment, are found in the sediments of high oil content. In <u>in situ</u> biodegradation experiments using 1-^{14}C-<u>n</u>-hexadecane as substrate the same sediments also exhibited the highest biological activity for oildegradation, based on the release of $^{14}CO_2$ from the radioactive alkane.

A microbiological survey of the Bays of Cape Hatt indicates quite clearly that the intertidal sediments, particularly inside the Z-lagoon, are affected by oil, presumably due to the activities of the BIOS Project.

RÉSUMÉ

Trois séries de parcelles d'expérience ont été établies clans l'Arctique pour l'étude de la biodégradation des hydrocarbures déversés clans des sédiments d'arrière-plage.

La fertilisation chimique a eu, sur la biodégradation, un effet positif qui a varié selon les conditions géomorphologiques de la plage. Dans les sédiments fins, les bactéries dégradant les hydrocarbures sent demeurées de 10 à 100 fois plus abondantes clans les parcelles fertilisées que clans les parcelles témoins au tours des deux années d'observation. **Les** analyses chimiques indiquent un taux de biodégradation de 3 à 5 fois plus élevé, du moins pour la fraction des alcanes. Dans les sédiments plus grossiers, la fertilisation semble avoir eu un effet marginal.

Le mélange mécanique des hydrocarbures avec l'engrais clans les sédiments a amélioré encore plus l 'autopurification biologique des sédiments pollués malgré une plus faible abondance des bactéries dégradantes.

Dans les sédiments intertidaux de la baie 11, où des hydrocarbures ont été déversés en surface en 1981, les concentrations des bactéries dégradantes étaient très faibles. Les concentrations les plus élevées, soit de 5 **x 107** à **7 x** 10^7 Par ml de sédiments, ont été observées clans des sédiments à forte teneur en hydrocarbures. Dans des expériences de biodégradation in situ avec du n-hexadécane (14C-1) comme substrat, les mêmes sédiments ont également démontré la plus forte activité biologique en ce qui concerne la dégradation d'hydrocarbures d'après la liberation de $^{14}CO_2$ de l'alcane radioactif.

Une étude microbiologique des baies du cap Hatt indique assez clairement que les sédiments intertidaux, plus spécialement à l'intérieur de la lagune en Z, ont été polluées par des hydrocarbures, qui proviennent probablement des activités du projet BIOS.

ACKNOWLEDGEMENTS

On behalf of the Norwegian Authorities we would like to express our gratitude to the Environmental Protection Service for inviting Norwegian scientific groups to participate in this unique experiment. In addition to acquiring scientific results the participation in the BIOS Project has given us experience in arctic biological research and field activities which may be rewarding for work in our own sphere.

At the termination of this Project we want to give sincere thanks to the members of the Project Office, to the staffs of the Cape Hat Camp, to Paul Idlout at Petro-Canada Basecamp at Pond Inlet for very friendly and dependable service during these 4 field seasons, and to all participants of the various components for their cooperation and patience, their gracious chat and encouraging smiles, and their professional advice.

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INTRODUCTION

During the first 3 years of the BIOS Project we participated in both the near shore as well as in the onshore project, in both cases with the main emphasis on the biodegradation of petroleum hydrocarbons in the water column, in the bottom sediments of the near shore and the sediments of oil contaminated tidal or backshore regions.

The near shore studies were performed in direct relation to the two oil spills made in 1981, the surface oil release in Bay 11 and the dispersed oil release in Bay 9, to assess the effect of petroleum hydrocarbons on the natural microbial activity for their degradation in Arctic waters. Baseline measurements in 1980 and during a prespill period in 1981 were compared to situation over an extended postspill period in 1981 immediately succeeding the oil releases and a follow-up period in 1981 to assess the more long-term affects. With the latter period we thought these monitoring studies were brought to a reasonable conclusion and we wanted for the last season on Cape Hatt to concentrate on the experiments on enhanced microbial degradation in the sediments of the backshore and to expand on the biodegradation of the oil in the intertidal zone of Bay 11.

The program for 1983 had the following objectives:

1) To assess the microbial population and the CO_2 producing activity in the backshore plots of Bay 102 established in 1980 and in 1981, i e after 3 respectively 2 years of exposure.

2) To carry out similar analyses of the crude oil backshore control plot at Crude Oil Point, which was established in 1980. Part of this plot was fertilized in 1981.

3) To carry out fairly extensive analyses of the sma11 experimental plots of crude oil and oil-emulsion established in 1982 in the backshore of the low energy beach in Bay 106. These

plots had only been given one year of self-purification and would be the most likely objectives for monitoring during a possible renewed visit to Cape Hatt in 1985-87.

4) Microbial assessment of the oiled intertidal zone of Bay 11. We had particularly intended to carry out experiments to establish unequivocally whether or not biodegradation of petrogenic hydrocarbons takes place in the sediments of the oilpolluted beach.

5) At last we wanted to carry out a microbiological survey of the Bays of Cape Hatt along the Ragged Channel and inside the Z-lagoon for later reference as well as for comparison to the pre-BIOS period.

Our field season lasted 3 weeks from August 8 to 29, with a crew of two.

2. MATERIALS AND METHODS

2.1. SAMPLING PROCEDURES

Samples of sediments or sediment-o ii-mixtures for microbiological analyses were taken by using a sterile 5 ml disposable syringe. The tip of the syringe barrel was cut off to obtain maximal opening and a composite sample of 5 ml ws obtained by collecting 5-8 subsamples from the sampling area. The entire sample was immediately pushed into a plastic tube containing 9 ml sterile seawater/distilled water mixture (1:1). The water reduced the tendency of the oiled sediment to attach to the tube wall and facilitated the later treatment of the sample. The samples were kept as close as possible to zero temperature until analysis 2-4 hours later.

2.2. EXPERIMENTAL AND ANALYTICAL METHODS

2.2.1. Microbiological analyses.

0.5 ml 1% Tween-80 was added to the sediment water sample and shaken by hand for 2 minutes. An aliquot of the supernatant liquid or emulsion was assumed to represent the microflora of the sediment and used as the basis for the determination of the number of total viable heterotrophic batter ia (TVH) and oildegrading bacteria (ODB) as described in (3).

2.2.2. In situ experiments on biodegradation of ^{14}C-n-hexadecane.

In order to obtain direct evidence for the biodegradation of petrogenic alkanes the following experiment was carried out in situ at various spots in the intertidal zone of Bay 11. The tidal sediments of this bay was covered by oil during the surface oil release in 1981.

A chamber (see page 4) made from a 20 cm plexiglass tube was pushed with care into the sediment of the chosen spot to enclose a sediment-oil core. The tidal zone of Bay 11 is very rocky and some of the sediments consist of pebly sand. For that reason we had to search for appropriate spots with sufficiently fine sediments that served the purpose of being suitable for the use of the chamber as well as to give test areas with variable oiling intencity. These requirements were met.

After introduction of the sediment the water level inside the chamber was regulated to about 1.5-2 cm above the sediment surface by the use the the valves build into the upper part of the chamber. With the valves closed the air above the sediment was trapped in the airtight chamber, a situation intended to counteract the drainage of water from the sediment core during the tidal cycles. As the incubation proceeded air could be introduced through the valves to compensate for the biological consumption of oxygen. This was not necessary in the present cases.

The chambers were maintained in fixed position in the tidal zone by lines from 4 poles rammed into the sediment. Over the 15 days of incubation all chambers stayed perfectly in place. During this period only moderate wave action was seen.

The substrate for the experiment, ^{14}C-hexadecane, was absorbed to a glassfibre filter fixed underneath a nylon screen which formed the bottom of a plexiglass cup. In setting up the experiment the tube with the filter was hanging by a string held by the butane rubber gasket in the chamber opening. As the last step in the procedure this string was cut and the filter with the substrate was pressed against the sediment surface. During the incubation the top opening of the chamber was covered by an airtight butane-rubber membrane kept in place by the screw-cap.

Prior to use the inside of the chamber was sterilized by rinsing

with 70% etanol followed by careful drying under aseptic cond it ions.

To each chamber 30 μC (in one case 60 μC) ^{14}C-n-hexadecane, sp. activity 235 μC/mg was applied. By using undiluted ^{14}C-substrate in substantial quantity, the expected dilution by cold n-hexadecane in the oil of the sediment was assumed to be partly compensated to attain a reasonably high specific activity of the n-hexadecane that was biologically degraded to CO_2.

At the termination of the experiment (after 14-15 days) the entire chamber was recovered without losing any of the sediment core and the bottom opening capped. Most of the water inside the chamber was immediately drained by a syringe through the membrane-capped opening and used for extration of $^{14}CO_2$ according to the method described previously (3). The total radioactivity of the water was also measured and the non-CO_2 radioactivity in the water was determined by difference. The chamber with the sediment core was frozen and in this state transported to our laboratory in Trondheim. The core was devided into three approximately equal parts along the length of the core. Each part was dehydrated by methanol, and further treated with toluene to extract the oil components. The oil content of the methanol extract was similarly transferred to toluene by dilution with water. The total toluene extract was used to assess oil-content and radioactivity.

The radioactivity remaining in the glass-fibre filter was determined directly by immerging the f i lter into the scintillation cocktail. The chamber and substrate-cup were rinsed with small volumes of toluene and radioactivity measured.

In this way the recovered radioactivity could be compared with the radioactivity applied to each chamber for evaluation of unintentional losses during the incubation.

2.2.3. Measurement of CO_2 production.

The CO_2 evolved by biological degradation of oil and other materials in the sediment was determined by the CO_2-absorption chamber method described in (3]. The measurements were normally carried out over a 20 hours period. The CO_2-content of samples from the NaOH-absorption solution was analyzed using the ASTRO model 1850 TOC-TC-analyzer.

3. RESULTS AND COMMENTS

3.1. ENHANCED BIODEGRADATION EXPERIMENT IN BAY 102.

The experiment in the backshore of the high energy beach of Bay 102 compriced of 7 oiled plots. Two of these (A and B) were established in late August 1980 as unfertilized control plots to give the longest possible observation period within the BIOS Project (TE-l and TE-2 described in (l)) . 102 A (TE-2) contained originally 50% oil-water emulsion of Venezuela Lago Medio crude, 102B (TE-l) the slightly weathered crude Lago Medio. Both plots were set up to reeive the same amount of oil, 10 kg/m^2. In 1980 the plots were situated approximately 1 m above the maximal high water line, intended to be unaffected by tides and waves. Over the years substantial beach erosion has taken place in Bay 102, and in 1983 the lower parts of A and B were sweft by even moderate waves during high tides. Most of the oiled plots appeared to be intact, but the sharp debarkation of the rather solid oil-sediment borderline to the tidal zone sediments indicated continuous breakdown of these sites.

The 5 experimental plots 102 D-H were arranged in early August 1981 to test the effect of 2 levels of common agriculture fertilizer (Norsk Hydro, fullgjødsel C) just spread on top of the oiled sediments (G and H). In one plot (E) with high level of fertilizer, oil and fertilizer was effectively mixed into the sediment by a motor driven rototiller, to break the assumed solid top layer of oil and sediment and thus increase the access to air and oxygen. A fourth plot (D) served the purpose of testing the effect of a commercial absorber product. The fifth plot served as a control. All plots reeived 50% oil-water emulsion with an oiling intensity of 10 kg slightly weathered Lago Medio oil per m^2.

The experimental plots were located just above the assumed high water swash line (see Fig. 1), and were intended to mimic the

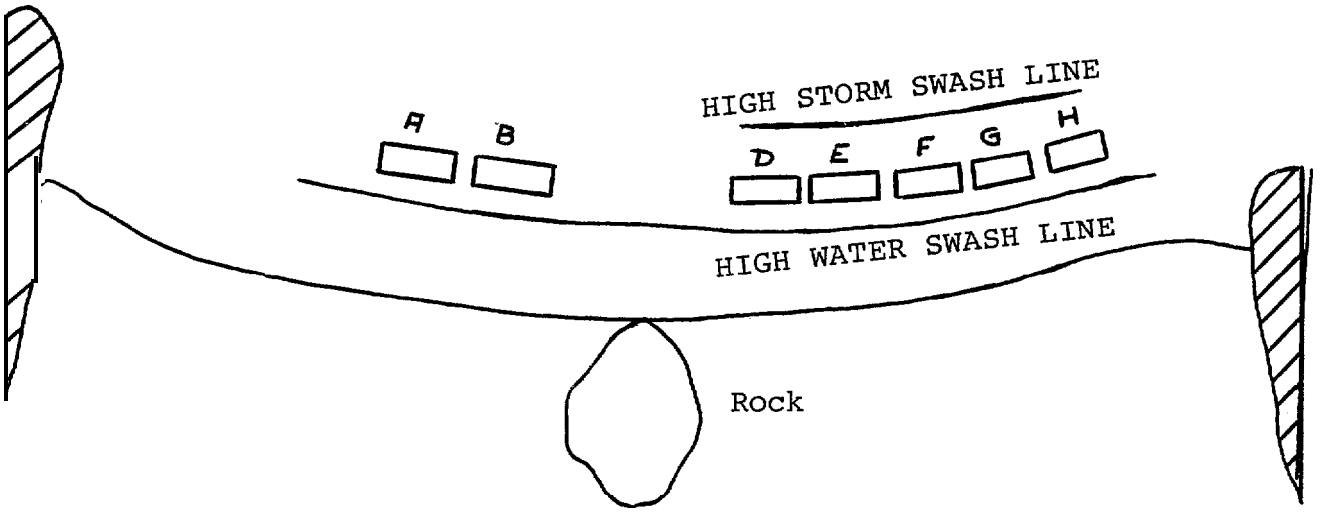

Fig. 1. Location of the backshore test plots in Bay 102. A (oil-emulsion} and B (crude oil) were established in August 1980 and the 5 oil-emulsion test plots (D-H) in August 1981. The oil-sediments in D were completely removed during exceptionally high tides and storm waves in September 1981, and the other plots (E–H) covered by 5–15 cm sand and gravel. A and B remained partly untouched, but their position relative to high water swash line has changed since deposition due to beach erosion.

scenario of oil being washed ashore at high tide under severe wave action. Unfortunately the occasional maximal high tides were underestimated and during at least two periods in September 1981, the first one just 3-4 weeks after the establishment of the plots, a combination of exceptionally high tides and heavy waves completely covered the plots D to H with sand and gravel, 5-20 cm deep. The plots were also partly dismantled. The plots were retraced and remarked the next year by Seakem and sampling for chemical hydrocarbon analyses indicated that plot D had been completely removed. The other plots E-H appeared to be in place, and intact, based on test holes dug at strategic spots. The microbiological analyses in 1982 seemed to support this assumption and in spite of having been continuously hurried by

variable depths of sand it was deemed worth while to monitor these plots in 1983.

The plots were considered too big to permit complete uncovering to expose the oil-sediment layer maximally to air. We decided to use the plots for evaluation of changes in the oil under conditions created by a combination of our efforts as well as those created by Nature. In 1983 we found the holes dug for chemical and microbiological samples in 1982 in a state as if they had been excavated a few days earlier. This clearly indicated that waves and tides had not affected the plots in the intervening period.

At two or three spots in each plot the sand cover was completely removed over areas approx. 50 by 50 cm down to the surface of the oiled sediments (see Fig. 2). These areas were used for microbiological and chemical sampling and for measurement of CO_2-production.

3.1.1. Results of microbiological and chemical analyses.

The data for microbiological analyses (total viable heterotrophic and oildegrading bacteria) for the 1983 season are given in Table 13 and the hydrocarbon analyses (GC) of one sample from each of the plots A, B, and E-H are presented in Table 21.

3.1.1.1. The control plots A and B.

These unfertilized control plots have been exposed to the natural forces of biodeterioration for 3 years. During the first few weeks after deposition the oil-emulsion seemed to offer the best conditions for bacterial growth, with a population of bacteria about 2 orders of magnitude higher than in

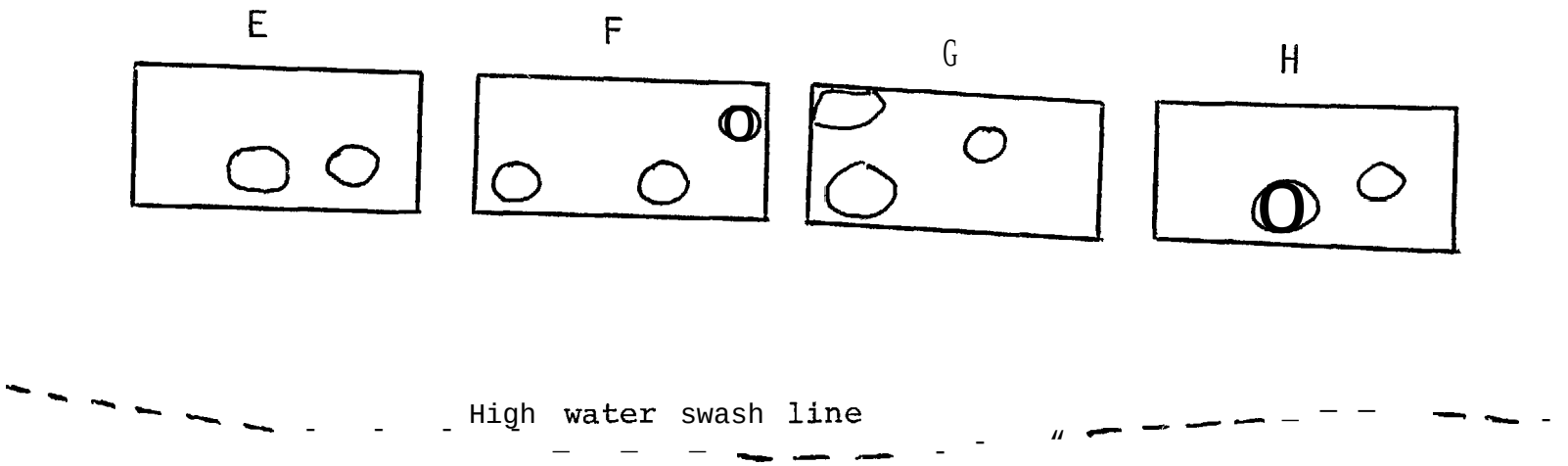

Fig. 2. Retraced outline of the backshore oil-emulsion plots in Bay **102**, with indication of the sampling spots used during the 1983 season. In the indicated areas the sand and gravel was removed down to the oil-sediment surface and the pits left open.

the crude oil plot. We have earlier suggested that this might be due to differences in water activity in the two plots. The same development was found during the initial period in the experimental plots established in Bay 106 in 1982 (4). During the next 2 years the situation in the two plots gradually changed (see Table 1 for ODB) . The highest population of bacteria, total viable heterotrophic (TVH) as well as oil degrading bacteria (ODB), was found in the crude oil plot. The results of 1983 substantiated this fact, the crude oil seem to support the largest population of bacteria.

The absolute number of bacteria found in the oil-sediment was roughly an order of magnitude higher in 1983 than in any previous year. This was not something special for these plots, but seem to be characteristic for all sediment situations analyzed during the 1983 season. We think this is linked to the general climatic conditions of the 1983 summer season.

Over the two years from 1980 to 1982 no change in the biogradation index (alkane/isoprenoid-ratio) was found. In 1983 the alkane/isoprenoid ratio in the oil of both plots dropped from 2.5 to 2.1, indicating a slow, but definite biodegradation of the oil. At the same time the microbiological analyses show that a very substantial part of the viable bacteria in the sediment consists of ODB (see Table 13).

3.1.1.2. Enhanced biodegradation plots E-H.

During the 2-3 weeks following the establishment of the oil-emulsion plots E-H in 1981 the microbial development could be described in this way. Low amounts (40 g/m^2) of deposited nitrogen-phosphorus fertilizer applied to surface increased the bacterial population an order of magnitude or more compared to the control plot. By increasing the amount of fertilizer 10 times, the bacterial population was raised another order of magnitude. A careful mixing of the same amount of fertilizer

into the oiled sand by mechanical means seemed to reduce the overall level of bacteria to approximately the population found in the plot fertilized with the lowest amount of fertilizer. The analytical figures for ODB are given in Table 1, the data for TVH are found in (4).

Table 1. Enhanced biodegradation experiment in Bay 102, supralittoral zone.

Control plots (A and B) were laid in August 1980. The oil-emulsion plots (E, F, G and H) were laid August 1, 1981. 3 weeks later they were covered by sand and gravel due to high tides and waves. The latter plots were treated by N-P-fertilizer (level 1 at 40 g/m^2, level 2 at 400 g/m^2) and one plot with level 2 fertilizer mixed by rototiller after fertilization. The results given are counts of oildegrading bacteria (ODB) and alkane/isoprenoid ratio.

	ODB ml^{-15} sediment x 10^{-5}				ALKANE/ISOPRENOID RATIO**	
	1980*	1981*	1982	1983	1982	1983
Control emulsion (A)	150	1.3	0*7	28	2.5	2*1
control crude (B)	0.95	0.7	7*3	220	2.6	2.1
Control emulsion (F)	-	1*4	130	73	2.6	2.1
Level 1 fertilizer (G)	-	68	43.5	800	2.5	2.2
Level 2 fertilizer (H)	–	730	130	2000	2.4	1.8
Level 2 fertilizer and mixing (E)	–	73	27	250	1.9	1.3

* 17 days after the deposition of the oil

**Data by Boehm (6)

After being covered by sand for one year essentially the same relative situatin was found in 1982. The unexpectedly high value of TVH and ODB in the control plot could be due to an unfortunate cross-contaminated sample. The control plot F is situated (see Fig. 2) between the two fertilized plots E and G, and in 1982 only one sample from each plot was analyzed.

In 1983 at least 2 composite samples from various spots within each plot (see Fig. 2) were analyzed and the same general trend seen in 1981 is still maintained. The microbial population in each plot was approximately an order of magnitude higher than seen the previous year, in line with the general trend found for the summer of 1983 (see Table 1), but the relative proportion between populations in the plots prevails. The plot initially offered the largest amount of fertilizer is, after 2 years, still able to support the largest population of bacteria. The mixed plot with the same amount of fertilizer had about 1/10 the bacterial population, more or less as for the plot initially

Table 1. Enhanced biodegradation experiment in Bay 102, supralittoral zone.

Control plots (A and B) were laid in August 1980. The oil-emulsion plots (E, F, G end H) were laid August 1, 1981. 3 weeks later they were covered by sand and gravel due to high tides and waves. The latter plots were treated by N-P-fertilizer (level 1 at 40 g/m^2, level 2 at 400 g/m^2) and one plot with level 2 fertilizer mixed by rototiller after fertilization. The results given are counts of oildegrading bacteria (ODB) end alkane/isoprenoid ratio.

	ODB ml^{-15} sediment x 10^{-5}				ALKANE/ISOPRENOID RATIO**	
	1980*	1981*	1962	1983	1982	1983
Control emulsion (A)	150	1.3	0.7	28	2.5	2.1
Control crude (B)	0.95	0.7	7.3	220	2.6	2.1
Control emulsion (F)	-	1.4	130	73	2.6	2.1
Level 1 fertilizer (G)	-	68	43.5	800	2.5	2.2
Level 2 fertilizer (H)	-	730	130	2000	2.4	1.8
Level 2 fertilizer and mixing (E)		73	27	250	1.9	1.3

* 17 days after the deposition of the oil

**Data by Boehm (6)

receiving the lowest amount of fertilizer. For both the latter plots the effect of added fertilizer is still very marked, by comparing with the unfertilized plot F.

In all plots the proportion of ODB is 30 to 100 per cent of the total viable count of general heterotrophs (see Table 13), indicating the dominating role of the oil as carbon and energy source for the establishment of these bacterial population.

From these results one has to conclude that the conditions initially set up in these experiments of enhanced biodegradation have not been substantially changed by the incident in September 1981. Furthermore, under the conditions existing in the backshore of the high energy beach at Bay 102 the burial of the plots under 15-20 cm of sand does not seem to have been inhibitory to the development of substantial populations of bacteria in the oiled sediments.

The oil-sediment layers of the experimental plots E-H contain substantial amounts of oil, from 17 to 25 mg total PHC/g sediment (5) based on analysis of samples taken in 1982. These samples represented the entire oil-sediment layer. To observe a significant change in the alkane/isoprenoid ratio indicating biodegradation a substantial absolute amount of the alkane fraction has to be metabolized. In this way the measurement of the alkane/isoprenoid ratio is a dependable, but not a very sensitive indicator of biodegradation. At the same line the size of the microbial population which is biochemically adapted to metabolizing oil-hydrocarbons may serve as an indicator of the potential for biodegradation of oil, but the numerical value of this population gives little information about the absolute rate of the oildegradation carried out by the same bacteria.

After one year of exposure only the mixed fertilized plot (E) had a significant drop in the alkane/isoprenoid ratio of its oil residue (Table 1). This was somewhat surprising, since its population of TVH and ODB was only 1/5 to 1/10 of the

populations in plot H.

The results from 1983 confirm this difference in development. The biodegradation ratio in plot E had further decreased from 1.8 in 1982 to 1.3 in 1983. This has to be considered as an indicator of substantial microbial alteration of the oil in the sediment. But a significant change in the alkane/isoprenoid ratio in the oil of the other plots had also occurred. This change was greatest in plot H with an alkane/isoprenoid ratio of 1.8, the oil sediment containing the largest population of bacteria. In the unfertilized plot (F) and in the plot with low level fertilizer (G) the oil had changed to a lesser degree, but the calculated figures of 2.1 and 2.2 are deemed significant.

In summarizing we may conclude that 1-2 years are needed to observe any significant change in composition and amount of oil in the sediments of the backshore of Arctic high energy beaches.

A combination of fertilization and mechanical mixing of the oil and fertilizer into the sediment seem to offer the best conditions of obtaining the highest rates of biodegradation, possibly due to an improved penetration of oxygen. Although the analyses of populations of oildegrading bacteria cannot be used to assess the absolute rates of biodegradation of oil, they appear to lend themselves to an evaluation of the relative rate of biodegradation.

3.1.2. Results of CO_2-production measurements.

The results of the CO_2-production measurements in the plots A, B, E-H in Bay 102 are given in Table 18 of the Appendix. Due to the uncertainty about the experimental value of the oil plots in Bay 102 prior to the 1983 season only one set of CO_2-measurements was carried out. In view of the results of the other parameters this was regretable.

The differences in the calculated mean rates of CO_2-production in the various plots are not very great. The crude oil in plot B seem to support somewhat higher rates than in the plot A with oil-emulsion, in line with the microbiological data.

In comparing the rate of CO_2-production in the plots E-H, the mixed fertilized plot E had the highest rate, 37 mg C $m^{-2} \cdot h^{-1}$, and the CO_2-production rate in plot H had a value intermediate between the rates observed in the mixed plot and the unfertilized plot. Plot G with low level of added fertilizer did not appear to have CO_2-production over and above the unfertilized control plot. These results are in line with conclusions based on results of microbiological and the hydrocarbon-GC analyses. In spite of a lower concentration of bacteria the mechanical mixing of the oil-emulsion into the sediment seem to make the condition more congenial to high rate in the turnover of the avaialbe nutrient needed for oildegradation, i.e. nitrogen, phosphorus and oxygen.

3.2. ENHANCED BIODEGRADATION EXPERIMENT AT CRUDE OIL POINT

The plots T-1 and T-2 were established in 1980 by Woodward-Clyde (1) at Crude Oil Point (see Fig. 3) as control plots for the intertidal oiling experiments in sediments of similar characteristics. The plots were deliberately placed not to be affected by wave and tidal action and in this way provide a comparison of oil weathering properties not directly affected by active marine processes.

T-1 was oiled with crude Lago Medio oil. During a survey for a contingency enhanced biodegradation experiment in September 1981, this plot was found to be the only one with areas containing sufficiently fine-grained sediments that would be reasonably suitable for microbiological sampling. The T-2 plot

consisted solely of shingle lag deposits and had to be discarded as a test-site for a microbiological experiment, at least with results that might be compared with the other sites. We were at that time not prepared to follow the microbial development in oil-dust films overlaying pebbles and stones, a situation very characteristic for T-2. In T–1 small areas with relatively fine sand could be found in between rocks and pebbles, areas which were sufficiently exposed to have received the full load of oil during the establishment of the plot.

A one meter wide strip of T-1 at the eastern end was fertilized with 80 g of Norsk Hydro fullgjødsel C in early September 1981, one year after the deposition of the oil. Unfortunately samples for microbiological analyses were not taken at that time.

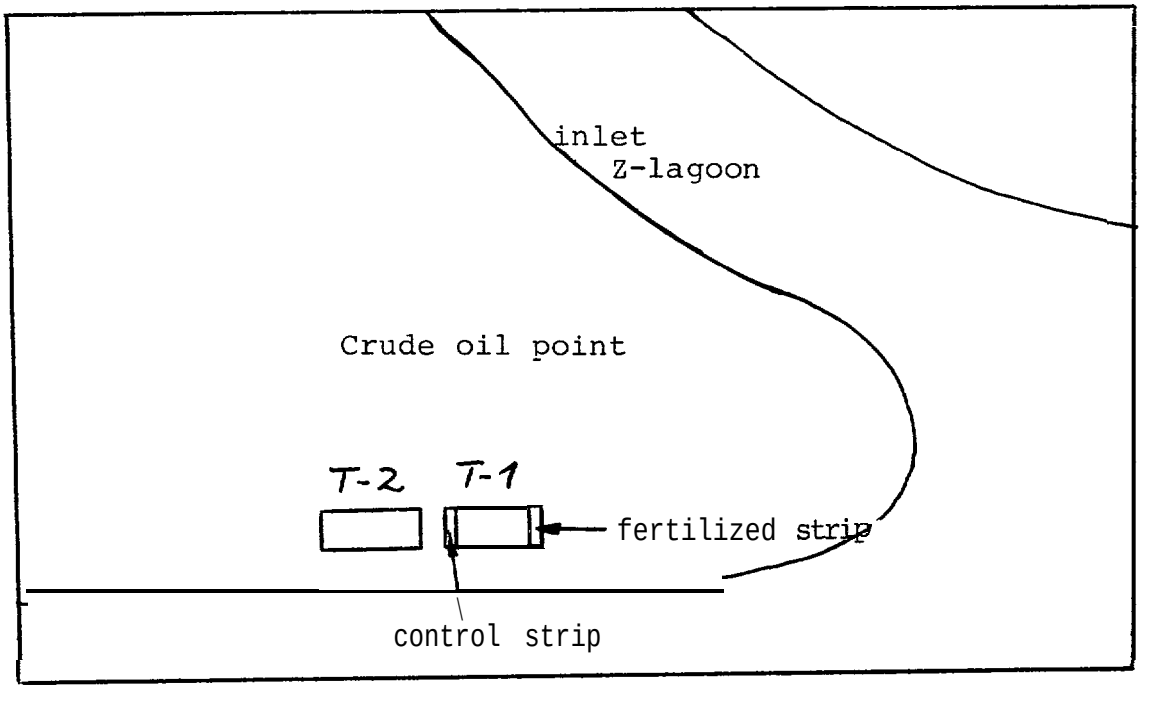

Fig. 3. Location of T-2 (oil-emulsion) and T-1 (crude oil) on the backshore of Crude Oil Point. The strips of T-1 used for the Norwegian enhanced biodegradation experiment are indicated.

During the sampling for microbiological analyses in 1982 the 1 m strip adjoining the fertilized strip was used as unfertilized control area. To minimize the danger of cross contamination and to make identification easier in the future, the control area was moved to the extreme western part of the plot in 1983 (see Fig. 3).

3.2.1. Microbiological and chemical results.

The results of the microbiological analyses in 1982 appeared to indicate a slightly increased level of oildegrading bacteria in the fertilized area compared to the unfertilized control (4). The same applied to the count for total viable heterotrophic bacteria, but due to short incubation period these analyses were not equally dependable.

The results of the microbiological analyses in 1983 are given in Table 14. Table 2 summarizes the data for oildegrading bacteria in 1982 and 1983 and the available data for the alkane/isoprenoid ratio of the oil in this plot from 1980 to 1983. Separate analyses of alkane/isoprenoid ratio for the unfertilized and fertilized part of T-1 are only available for 1983.

The level of oildegrading bacteria in 1983 was reasonably high, in line with what has been found for unfertilized oil-sediment plots during that summer (except for the plots in Bay 106). But no significant difference could be observed between the fertilized and the unfertilized part of T-1.

Over the years from 1980 to 1982 no change in alkane/isoprenoid ratio of the oil of T-1 or T-2 was observed indicating only slight if any biodegradation of oil. From 1982 to 1983 a moderate, but definite decrease in the biodegradation ratio was found, to values of 2.2 to 1.9, based on analyses of 6 separate

samples, 4 taken for this project at T-1 (see Tabll 17) and 2 samples taken in T-1 and T-2 and analyzed for Woodward-Clyde (7). These results do not indicate any significant difference between the fertilized (alkane/isoprenoid ratio 2.1) and the unfertilized part (alkane/isoprenoid ratio 2.0) of T-1. But we take the change in alkane/isoprenoid to be sufficiently significant to show that the oil at T-1 and T-2 is being biologically degraded.

The raised level of oildegrading bacteria in T-1 observed in 1983 seem to coinside with the noticeable change in the composition of the residual oil. But the overall rate of biodegradation in these plots has been low, in comparison to the situation in the fertilized oil-plots in Bay 102 and particularly in Bay 106. The biodegradation seems to be limited by factors other than nutritients. Low water activity may be one possible cause. The site of the experimental plots is very

Table 2. Enhanced biodegradation experiment at Crude Oil Bay.

The plot (T-1) was laid in August 1981 as a backshore crude control plot by Woodward-Clyde (l). In early September 1981 a one meter strip of the eastern side was fertilized with 80 g/m^2 N-P-fertilizer. The corresponding western strip served as control. Results of alkane/isoprenoid-ratio (Boehm (6) and counts of oildegrading bacteria (ODB).

	ALKANE/ISOPRENOID RATIO				ODB ml-l sediment x 10^{-5}	
	1980	1981	1982	1983	1982	1983
Control T-1	2.4	2.1	2.4	2.0	0.28	28-130
c				(1.9)*		(60*60)
Fertilized T-1				2.1	1.3	28-130
						(90*50)

*Additional analysis reported by E. Owens (7).

well drained. At the time of oiling in 1980 the perched groundwater table was found at 0.7 m (1) and the geomorphological characters of these plots differ from the sites at Bay 102 and 106.

3.2.2. CO_2-preduction measurements.

The results of CO_2-production measurements over 2 periods are given in Table 19.

The values for the unfertilized and fertilized oiled plots are not significantly different and they are only slightly higher than the CO_2-production in the chambers incubated in the sand-control area.

3.3. ENHANCED BIODEGRADATION EXPERIMENT IN BAY 106.

At the start of BIOS Project enhanced biodegradation experiments were planned to be established in the backshore of a high energy beach as well as a low energy beach. For economical reasons these plans had to be reduced and only the experimental plots in Bay 102 were set up in 1981.

After the misfortune in September 1981 and before we know anything about the consequences of the burial of these plots, it became a need to compensate for the loss of the experimental sites in Bay 102. On the one hand we wanted to have a look at the situation of oil stranded in the low energy area of a coastal confinement, at the same time we felt that this type of experimental site would be the safest place for an undisturbed long-term study of biodegradation of oil on an Arctic shoreline.

For these reasons two small experimental plots were set up in Bay 106 of the Z-lagoon in 1982, very close to the so called mixed oil-plots of Woodward-Clyde (2), see Fig. 4.

Each plot had the dimension of 1x2 m, one sprayed with slightly weathered crude Lago Medio and the other with a 50 per cent water emulsion of the same oil. A total of 10 kg oil/m^2 was applied. Each plot was divided into two equal parts and "Norsk Hydro fullgjødsel C" grains, a commercial nitrogen-phosphorus fertilizer with trace element supplement, was evenly spread on the oiled surface to give 100 g/m^2.

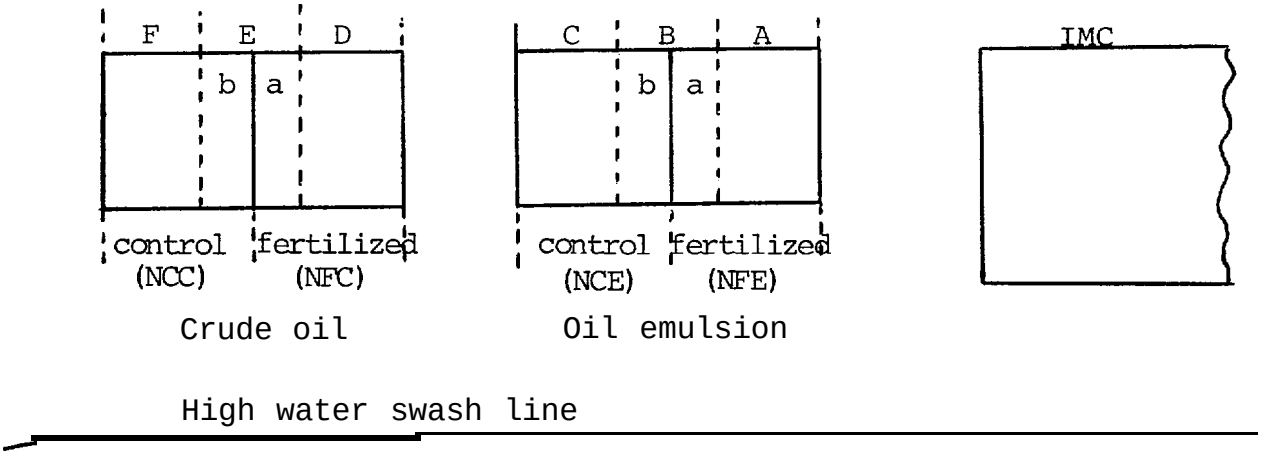

Fig. 4. Location of the backshore Norwegian test plots in Bay 106. A-F indicate the sampling sectors after dividing each plot into three equal parts, B and E representing the trasition zone between the fertilized and control part of the emulsion and the crude oil plots. The small letters 'a" and "b" indicate areas of sub-sampling within these zones. IMC shows the position of the backshore crude oil mixed test plots of

Admittedly the plots were very small, but shortage of oil prevented a more appropriate design of the experiment. For reasons unexplained no area of separation between the fertilized and the unfertilized control plot were made to reduce the danger

Of cross contamination. The sampling pattern in 1983 was designed to elucidate this question in some detail.

3.3.1. Results of microbiological and hydrocarbon analyses.

During the post-oiling period in 1982 the microbial population in the oiled sediments developed rapidly over the 16 days of observation. A maximum of $2.7 \cdot 10^{a}$ ODB per ml of sediment was found for the fertilized oil emulsion plot, an order of

Table 3. Enhanced biodegradation experiment in Bay 106.

The plots (see Pig. 3) of oil-emulsion end crude oil were established in August 1982 and half of each plot fertilized with 100 g/m² N-P-fertilizer. For sampling in 1983 each plot was divided into three equal parts, the center part in each plot (B and E) representing the transition zone between the fertilized area and the unfertilized control area. The results from these zones are given in Table 4.

These results summarizes the results from samples taken in the outer zones, with the least possibility of cross contaminant ion. The 1983 values for oildegrading bacteria (ODB) are numerical mean of 4 analyses, the range given in parenthesis.

	ODB ml-l sediment x 10^{-5}		ALKANE/ISOPRENOID RATIO*
	1982	1983	1983
106A, fertilized oil-emulsion	2750	12000 (32000/1300)	0.9
106C, control oil-emulsion	130	1420 (2800/280)	2.0
106D, fertilized crude oil	275	9600 (13000/2800)	1.6
106F, control crude oil	7*3	900 (2800/280)	2.2

*Composite sample

magnitude higher than in the unfertilized control. As observed in 1980 for the experimental plots A and B in Bay 102, the bacterial development appears to proceed faster and to higher population in the oil-emulsion than in the sediment impregnated with crude oil. Although the fertilization raises the population of oildegrading bacteria as well as the count of total viable heterotrophs approximately an order magnitude in both types of oil, the relative differences in bacterial activity between the oil-emulsion and the crude oil plot prevails. The oil-emulsion seems to offer the best conditions for growth. Some data for ODB to illustrate this are given in Table 3 and more data may be found in (4). The results of the microbiological analyses made in 1983 are given in Table 15 and in Table 17 some of the hydrocarbon analytical data supplied by Boehm (6) on samples from our plots in Bay 106 are summarized.

Table 4. Enhanced biodegradation experiment in Bay 106; degree of cross contamination by fertilizer.

The transition zone (See Fig. 3) in each plot was sub-sampled in "a"-area (closest to the unfertilized control) and two samples from each area were analyzed for oildegrading bacteria (ODB). The values are compared to ODB-counts of the outer regions of each plot.

	ODB ml-l sediment x 10^{-5}
106A, fertilized oil-emulsion	12000
106B-a, 106B-b, transition zone	7300, 800
106c, unfertilized oil-emulsion	1420
106D, fertilized crude oil	9600
106E-a, 106E-b, transition zone	8100, 2800
106F, unfertilized crude oil	900

In an attempt to evaluate the degree of cross contamination between the fertilized and unfertilized part of each plot due to the lack of physical separation, each plot was divided into 3 equal zones for sampling and analysis (CO_2) (see Fig. 4). The middle part represents the transition zone where any cross contamination ought to be strongest. The results of the microbiological analyses (summarized for ODB in Table 4) appear to indicate very clearly that the zone of contamination must be very short and can for practical reasons be neglected. Samples from the trasition zone had counts of ODB which coinsided very much with the sampling area on each side of the line of division. Samples marked a in Table 4 or Table 15 were taken from area closest to the fertilized part of the plot, and samples marked b were collected from the corresponding unfertilized part. In all cases the b samples had counts of TVH and ODB close to or identical to the unfertilized part furthest away from the line of division. These results indicate that the water soluble inorganic salts of nitrate and phosphate initially applied to the oiled sediments must have been rapidly assimilated and physically immobilized to resist redistribution by diffusion or convection during the wet periods of the year.

During the summer season of 1983 very high levels of bacteria were observed in the fertilized as well as the unfertilized plots, some of the values of ODB and TVH are the highest that were recorded during the BIOS Project for any oiled sediment. For TVH maximal values of in excess of $3 \cdot 10^{10}$ per ml of sediment sample were found, with an average of $1.2.10^{9}$ per ml of sediment, and maximal and average counts of ODB at $3.2 \cdot 10^{9}$ and $1.2 \cdot 10^{9}$ respectively, all based on analyses of the fertilized oil-emulsion plot. The counts in the fertilized crude oil plot were only slightly lower. As mentioned earlier we think these exceptionally high figures have to do with the exceptional conditions during the spring and summer season of 1983. In some cases we did underestimate the bacterial content and only minimal values for the sample could be obtained (Table 15).

The data for ODB and the alkane/isoprenoid ratios are summarized *in* Table 3. The favorable season of 1983 compared to 1982 is amply demonstrated. The the oil emulsion as well as the crude oil the 1983 level of ODB is substantially higher than during the 1982 season.

The effect of fertilization is very clearly demonstrated. For both types of oil the artificial fertilization supports a population of ODB approximate one order higher than what is possible under unfertilized conditions. And this difference in bacterial biomass seems to cause a significantly increased rate of biodegradation of oil, based on the evaluation of the alkane/isoprenoid ratio of residual oil. In the unfertilized oil plots marginal alterations in the alkane/isoprenoid ratio had taken place. In particular the change in the oil of the control crude oil plot was very slight, and may not be significant. In the fertilized plots the change was very mar ked, indicating degradation of 30-60% of the biologically vulnerable alkane fraction of the oil in one year. For an Arctic situation this has to be considered remarkable.

The extent of biodegradation seems to be strongest in the fertilized plot with oil emulsion. Based on the high level of ODB observed in this plot in 1982 relative to the situation in the crude oil plot, this may not be surprising. But the difference between the two types of oil, fertilized or not, is no longer as marked as during the initial stages of development. Further monitoring is needed to show whether the same reversion of development as was seen in the crude oil/oil emulsion plots in Bay 102 (A-B) will repeat itself in these cases.

In the previous working report (4) we questioned the sampling procedure for the samples used for the determination of alkane/isoprenoid ratio. A composite sample of the entire oil-sediment might not be representative for evaluating the extent of biodegradation in case the latter process preferentially occurred in the surface layer maximally exposed

to the atmosphere. TWO types of samples were therefore taken from the experimental plots in Bay 106 and at Crude Oil Point, one very top surface sample and one composite sample at the same spot . The results of the analyses are given in Table 17. Within the error of analysis each pair of samples gave the same value, with a maximal deviation of 10%.

The biodegradation thus seems to be fairly uniform throughout the oil sediment column.

3.3.2. Results of CO_2-production measurements.

The results of the CO_2-productive measurements are presented in Table 20.

The fertilized oil plots had 50-100% higher CO_2-production than the unfertilized oil plots. The CO_2-production of the latter plots could not be distinguished from the sand control measurements. In general terms this support the data which indicate a more rapid biodegradation of oil in the plots of Bay 106 compared to the experimental plots assessed in Bay 102 and at Crude Oil POint and at the same time they stress the difference between the fertilized and unfertilized plot in this experimental set-up. The presition of the analyses probably only lends itself to this kind of conclusion.

3.4. BIODEGRADATION OF OIL IN BAY 11.

The entire tidal zone of this bay was covered with oil during the surface oil release August 19, 1981. After 2 years patches of the sediment have been cleansed, particularly in areas of strong penetration of fresh water, either from the small streams that enter the bay at 2-3 places or by subterranean upwellings

that drain directly into the tidal zone. Approximately 2/3 of the tidal zone is still massively affected by oil, but extensive areas of apparently clean sediments are obvious by their bright, grey color in the otherwise brown colored mixture of oiled sediments and oiled cobbles and stones. By superficial inspection the heaviest oil deposits are located in the upper intertidal zone and on ridges which may occur anywhere in the tidal zone. But even in sediments where no visible oil is apparent, a sheen of oil is immediately seen on the surface of the water that is squeezed out by your footstep.

3.4.1. Microbiological analyses of tidal sediments.

In 1982 a few samples taken at random in the tidal zone of Bay 11 were analyzed for TVH and ODB (4). They showed very high counts of bacteria and the counts were particularly high in samples containing visible quantities of oil. Compared to oil sediments in the backshore the bacterial counts were equivalent to lightly fertilized sediments, which indicated the tidal zone to have a fair access to nutrients.

In 1983 a more extensive survey of the microbial situation in the oiled tidal zone of Bay 11 was carried out. Numerous samples were taken along the sampling profiles laid by Woodward-Clyde (2), along Profile 4 and 8 (see Fig. 5) and along Profile 6 (see Fig. 6). In the latter case the sampling started in the supralittoral zone and samples were taken at approximately even distances down to the low water. For comparison a similar set of samples were taken along a profile in Bay 12, the latter to represent a bay of approximately similar topography and geomorphological composition with no obvious oil pollution.

The results of the microbiological analyses of these 4 sampling profiles are given in Table 5 (Profile 4 and 8), Table 6 (Profile 6) and Table 7 (Profile in Bay 12).

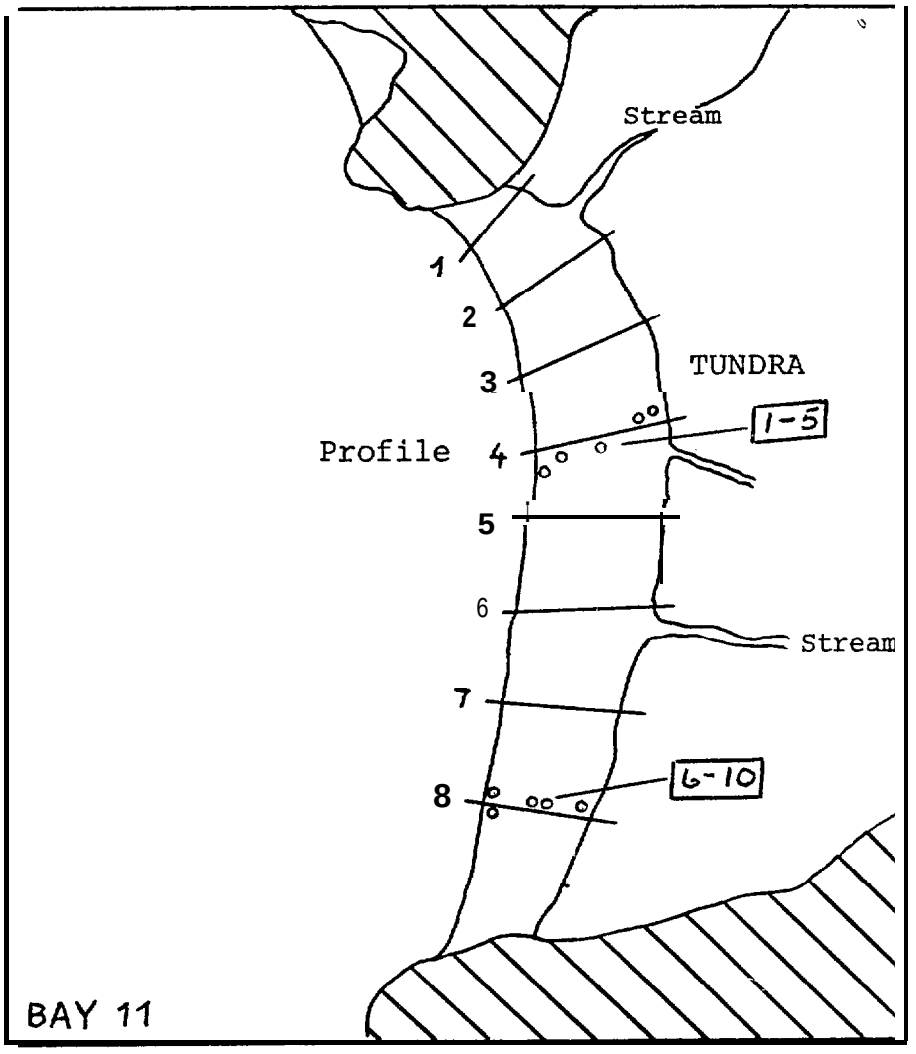

Fig. 5. Sampling sites for microbiological analyses of the sediment in the oil polluted intertidal zone of Bay 11, August 19, 1983. Results, see Table 9. The numbers in the box refer to sample number in Table 5.

Any sediment with heavy concentration of oil had counts of oildegrading bacteria in the 10^7-10^8 per ml sediment region, normally constituting 10 to 30 per cent of the count for total viable heterotrophs. Particularly coarse sand samples occasionally had lower levels of ODB, probably because of less solid surface area. In extensive sediment areas with no visible oil (samle 9 in Table 5 and sample 7 in Table 6) the ODB-count was markedly lower, probably because of constant drainage of fresh water. But other areas lacking visible oil still contained high levels of ODB (sample 4 and 10 in Table 5 and sample 3 in Table 6), because they represented small areas closely surrounded by oil-containing sediments and stones. The general trend was consistent, any oil-infested sediment harboured high levels of oildegrading bacteria.

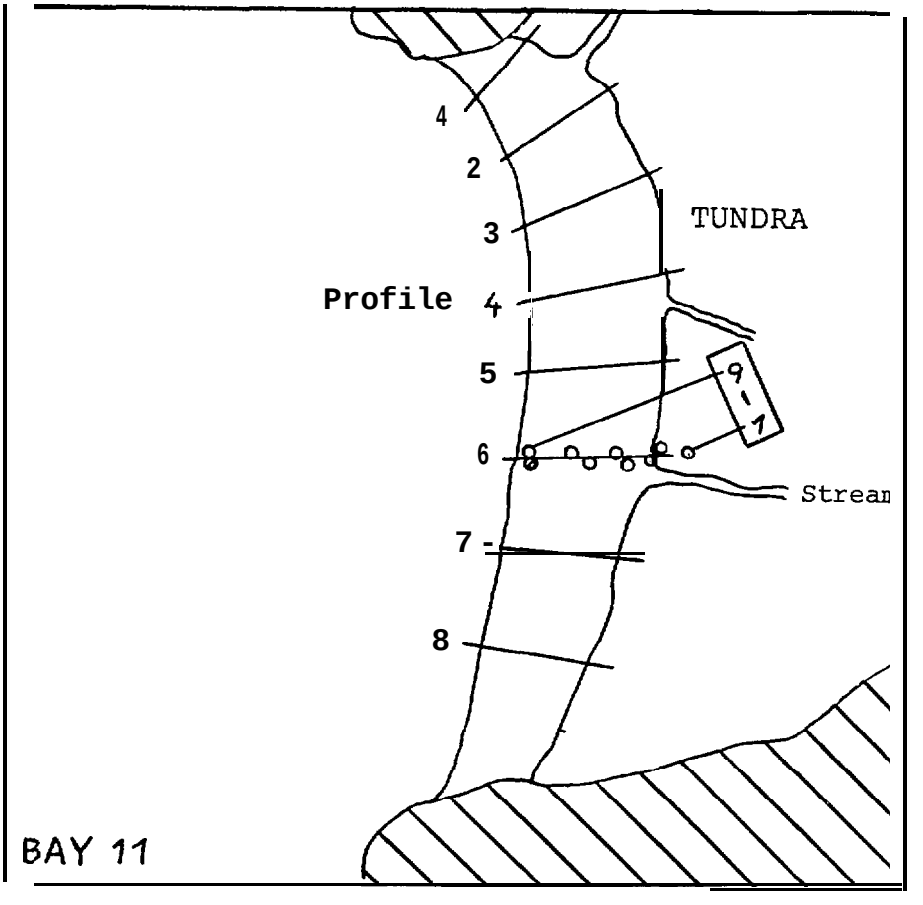

Fig. 6. Sampling sites for microbiological analyses of the sediment in the oil polluted zone of Bay 11, August 20, 1983. Results, see Table 10. The numbers in the box refer to the sample number in Table 6.

Table 5. Enumeration of total viable heterotrohps (TVH) and oil degrading bacteria (ODB) of the surface layer (O-2 cm) in the intertidal zone within the test area in Bay 11, Cape Hatt, August 19, 1983.

The enumeration was done along two transects, one northern, along Woodward-Clyde-profile 4 (samples 1-5), and one southern along Woodward-Clyde-profile 8 (samples 6-10) . The surface oil spill was carried out August 19, 1981.

Site	Sample	TVH No.ml^{-1}x10^{-5}	ODB No.ml^{-1}x10^{-5}	Comments
Upper intertidal	1	64.000	730	Heavily oiled sand
	2	26.000	730	Coarse, heavily oiled sand
Mid intertidal	3	5.500	730	Oiled sand
Lower intertidal	4	550	73	Coarse sand, no visible oil
	5	2.600	320	Loamy soil, no visible oil
Upper intertidal	6	4.400	1.300	Coarse, heavily oiled sand
Mid intertidal	7	700	130	Coarse, oiled sand
	8	230	730	Coarse, oiled sand
Lower intertidal	9	70	13	Sand in a dry brook bed, no visible oil
	10	2.600	320	Sand, no visible oil

Approximately 95% confidence limit MPN/4.68 to MPN x 4.68.

Table 6. Enumeration of total viable heterotrophs (TVH) and oil degrading bacteria (ODB) of the surface layer (O-2 cm) along a profile from supralittoral to lower intertidal zone in Bay 11, Cape Hatt, August 20, 1983.

The transect was along Woodward-Clyde sampling profile 6. The distance from high water mark (i.e. upper limit of visible oil) to low water mark was approx. 25 m.

Sample	Distance below high water mark (m)	TVH No.ml^{-1}x10^{-5}	ODB No.ml^{-1}x10^{-5}	Comments
1		4.4	0.73	Supralittoral, dry sand
2	-0.15	12	0.0073	Supralittoral, dry sand
3	0	870	580	Heavily oiled sand
4	3	8.700	280	Wet sand, in an area with fresh water drainage
5	6	2.300	280	Very coarse sand with some oil
6	9	870	130	Sand, no visible oil
7	12	2.600	28	Wet sand in an area with fresh water drainage, no visible oil
8	18	64.000	730	Coarse, oiled sand on a low ridge
9	18	4.400	280	Sand below ridge, no visible oil

Approximately 95% confidence limit: MPN/4 .68 to MPN x 4.68.

Table 7. Enumeration of total viable heterotrophs (TVH) and oil degrading bacteria (ODB) of the surface layer (0-2 cm) along a transect from supralittoral to lower intertidal zone in Bay 12, Cape Hatt, August 20, 1983.

Total distance from vegetation to low water mark was 65-70 m. The distance from high to low water mark was 40-45 m.

Sample	Distance from high water mark (m)	TVH $No.ml^{-1}x10^{-5}$	ODB $No.ml^{-1}x10^{-5}$	% ODB (ODB x 100/TVH)	Comments
1	-17	2.3	0.013	0.6	Dry sand, supralittoral
2	- 7.5	1200	>41	>3.4	Dry sand in wave-zone, some seaweed in the area
3	0	2600	>41	>1.6	Coarse sand, approximately at normal high water mark
4	6	550	2.8	0.5	Wet sand in an area with fresh water drainage
5	12	44	1.3	3.0	Sand
6	21	55	0.12	0.2	Sand
7	28	55	2.8	5.1	Sand
8	34	440	0.73	0.2	Sand

Approximately 95% confidence limit MPN/4.68 to MPN x 4.68

The samples of Bay 12 contrasted these findings. With the exception-of two samples in the upper intertidal and in the high water swash zone, which had ODB-counts in excess of $4 \cdot 10^5$ per ml sediment (cell-count underestimated), the sediment samples of this bay contained 10^4-10^5 ODB per ml sediment. This level of ODB probably still indicates some influence of petrogenic hydrocarbons, but the level is only 1/100-1/1000 of the oiled sediment in Bay 11.

From these analyses one may assume that a fairly extensive biodegradation of oil taking place in the oiled sediment of Bay 11.

A number of intertidal samples from profile 2, 4, 6 and 8 have been analyzed by Boehm (6) for total hydrocarbons and determination of the weathering ratio and the biodegradation ratio (alkane/isoprenoid ratio). These results seem to indicate that sediment samples with high content of oil exhibited high alkane/isoprenoid ratios in the range 1.9 to 2.5, whereas sediment samples with low amounts of oil had exceptionally low alkane/isoprenoid ratio indicating extensive degradation of their high molecular alkane fraction. All our samples with clearly copious amounts of oil and high counts of ODB most likely belong in the first category of the samples analyzed by Boehm. The question is where does the oil in the low-oil-containing samples come from? Decomposed *in situ*? or washed out from the biodegraded surface layer of oil in heavily infested sediment areas?

3.4.2. *In situ* biodegradation of ^{14}C-*n*-hexadecane in Bay 11.

The indications of microbial degradation of the oil in the tidal sediments of Bay 11 and the bottom sediments of Bay 9, 10 and 11 were not strong based on the results in 1982. For that reason we wanted as part of the work in Bay 11 during the 1983 season to make an attempt in establishing unequivocally whether

biodegradation of oil takes place in the tidal sediments or not.

The experimental set-up is described in Materials and Methods, including a drawing of the *in situ* incubation chamber (page 4) used for these experiments.

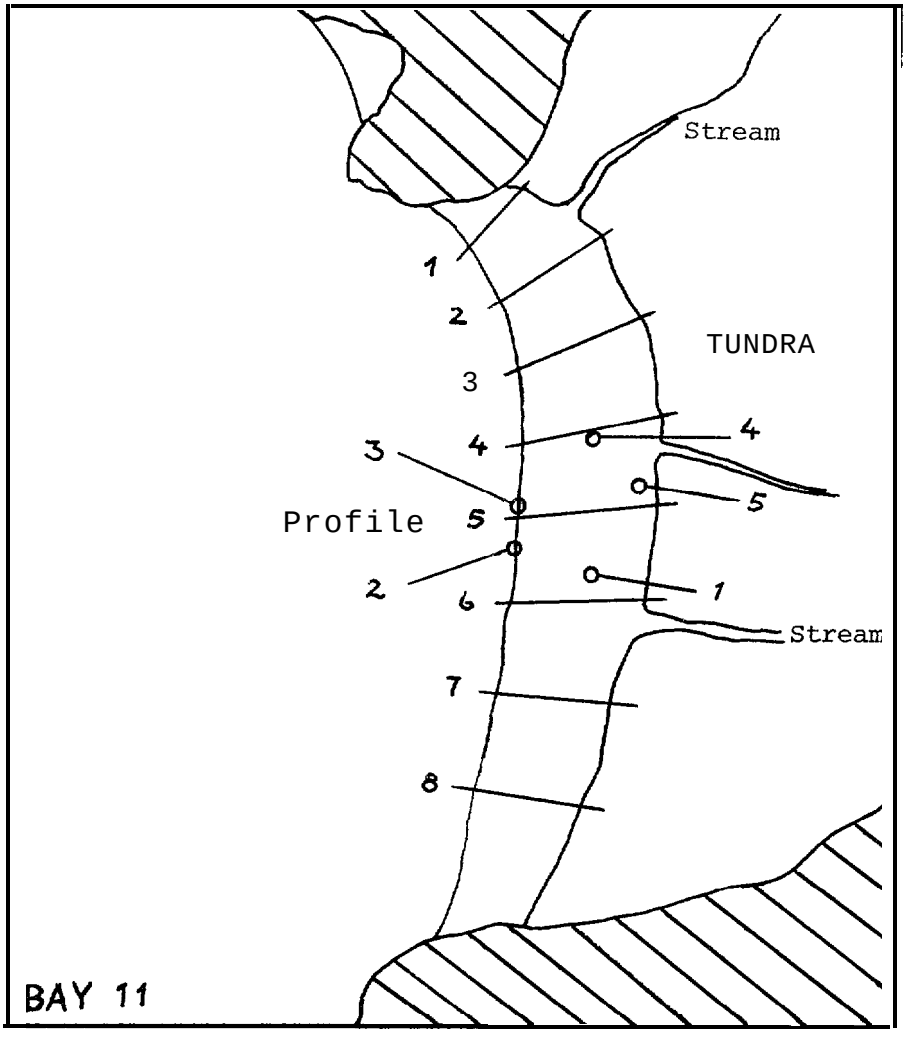

Fig. 7. Location of the ^{14}C-n-hexadecane incubation chambers in oil polluted intertidal zone of Bay 11, August 10-25, 1983. Results, see Table 6, 7 and 8. Number at each site refers to chamber number.

Table 8. Description of position and sites for the sediment ^{14}C-n-hexadecane degradation chambers in Bay 11 and Bay 12. Profile number as used by Owens et al. (2).

Chamber	Location; sediment conditions
1	Bay 11; in mid-tidal zone close to profile 6; in heavily oiled sediment.
2	Bay 11; at minimal lower tidal mark close to profile 5, actually submerged during entire incubation period; no visible oil.
3	Bay 11; as for 2.
4	Bay 11; in mid-tidal zone close to profile 4, on a heavily oiled ridge. Sediment very coarse.
5	Bay 11; in upper tidal zone in heavily oiled coarse sediments; position close to profile 4.
6	Bay 12; in lower tidal zone, in fine sediments with no visible oil.

The use of radioactive hydrocarbons as substrate in an environment with extremely high concentration of oil may of course have very limited application. We have used 1-^{14}C-n-hexadecane in our experiments and intended at least to some degree to compensate for the expected dilution of the radioactive substrate with cold n.-hexadecane from the oil deposits, by the following means:

1) the use of reasonably high total dosage of radioactive material
2) to maintain incubation over an extended period of 2-3 weeks to accumulate maximal amount of CO_2 as the recoverable metabolic product
3) to minimize losses during the incubation

The results will answer to what extent our intensions have been met. In case the results would give positive results as to biodegradation, we assumed they would be primarily of qualitative value and only in a very measured way could be used for quantitative evaluations.

5 of the *in situ* incubation chambers were placed in the tidal zone of Bay 11 (see Fig. 7) and one in the middle intertidal zone of Bay 12, the latter to serve as a reference control. A description of the sites are given in Table 8.

The very simple anchoring procedure kept the chamber in perfect position throughout the 14-15 days of incubation and the intended immobilization of the water phase of the chamber appeared to be successful. Chamber 2 and 3 were actually completely submerged during the entire incubation period, since they, by pure chance, were placed at the low water line at minimal low tide. The other 4 chambers were submerged to a variable extent during each tidal cycle depending on their intertidal position. The chamber in the upper intertidal position (chamber 5) was only submerged 2-4 hours per day.

The main body of water in the chambers was collected at the termination of the experiment and the radioactivity in the carbon dioxide recovered from these watersamples are given in Table 9.

In chamber 1, 4 and 5 substantial amounts of $^{14}CO_2$ was found. They were all situated in areas with the most heavily oiled sediment. The highest yield of $^{14}CO_2$ was recorded in chamber 4 which contained the largest dose of ^{14}C-*n*-hexacedane, 60 μCi. The other chambers contained 30 μCi. The chambers positioned in sediments with no visible oil produced much less, but still easily verifiable amounts of $^{14}CO_2$. These results clearly demonstrate that biodegradation of hydrocarbons do take place in the intertidal sediments and the biological activity appears to be strongest in the sediments most heavily infested by oil. The

Table 9. Biodegradation of ^{14}C-n-hexadecane in sediments of Bay 11 and 12, in the period of August 10 to 25, 1983.

The tidal zone of Bay 11 was oiled by surface oil spill in 1981. The radioactivity in CO_2 recovered from the waterphase of sediments is recorded. The chambers had been in place 15 days and their sediment content exposed to ^{14}C-n-hexadecane.

Chamber	Radioactivity in C02 cpm	Radioactivity in C02 µC	^{14}C-n-hexadecane dosage µC	Oiling index
1	221.780	0.1	30	heavy
2	52.050	0.02	30	not visible
3	3.010	0.001	30	not visible
4	363.300	0.16	60	heavy
5	98.530	0.04	30	heavy
6	39.850	0.02	30	(Bay 12) not visible

microbiological analyses have already shown these sediments to possess the highest counts of oildegrading bacteria. In Table 11 some of the relevant data are assembled. It is somewhat unfortunate that in 5 of the 6 cases only minimal counts of ODB can be given, due to underestimation of the bacterial content at the time of the analyses.

The residual radioactivity of ^{14}C-n-hexadecane and non-CO_2-products from this substrate accumulated inside the chamber has been measured. The distribution of this activity between filter, chamber wall and sediment fractions is given in Table 10.

The total recovery of radioactivity varies from 93 to 104%. Except for chamber 1 which has the lowest recovery, the other

yield recoveries are satisfactory to show that no major losses of substrate or products have taken place during the incubation. We tend to think that the low recovery of radioactivity of chamber 1 is due to faults in setting up the experiment and/or in the analyses, and not due to a greater loss during the incubation.

Table 10. Biodegradation *in situ* of ^{14}C-hexadecane in the sediments of the oiled tidal zone of Bay 11, Cape Hatt, Aug. 10 to 25, 1983. Distribution of ^{14}C-radioactivity at the termination of the experiments.

^{14}C-hexadecane was initially introduced to the enclosed sediment system adsorbed to a sterile glassfibre filter. The length of the sediment column was devided into 3 equal parts prior to the analyses.

Chamber No. (and dosage)	/.4C.i·^{14}C recovered from filter	chamber walls	water	sediment ** upper	middle	lower	Per cent recovery
1 (30)	18.98	5.50	0.11	3.01 (9.5)	0.16 (0.4)	0.09 (80.2)	93.2
2 (30)	28.22	1.86	0.04	0.21 (0.5)	0.01 (<0.2)	0.01 (<0.2)	101.2
3 (30)	27.52	2.88	0.009	0.57 (<0.2)	0.06 (<0.2)	0.13 (<0.2)	103.9
5 (30)	16.33	6.15	0,54	6.94 (43.5)	0.52 (19.4)	0.07 (12.1)	102.0
6* (30]	25.53	2.94	0.02	0.74 (<0.2)	0.03 (<0.2)	<0.01 (<0.2)	97.6

*Chamber placed in tidal zone of Bay 12.

**Numbers in parenthesis indicate concentration of oil in mg/g.

During the incubation the radioactive n-hexadecane is confined to the glass fibre filter held in place on the surface of the sediment. From this filter the substrate leaks into the environment of the chamber.

After 15 days most of the ^{14}C-n-hexadecane is still associated with the glass-fibre filter (see Table 10), and particularly where the chamber is situated in sediments with very low amounts of oil. In the chambers with high oil content in the sediment roughly 50% have been transported to other places, and in these cases a fair amount of radioactivity is recovered from the oil sediment layer. This was expected. This will, however, affect the quantitative evaluation of the results.

Table 11. Biodegradation of ^{14}C-n-hexadecane and the microflora of the sediments of the tidal zone of Bay 11 and 12.

2-3 samples of the sediment close to each in situ sediment incubation chamber were analyzed for oildegrading (ODB) and total viable heterotrophic (TVH) bacteria.

Chamber	TVH $ml^{-1} \times 10^{-5}$	ODB	μC $^{14}CO_2$
1	950	500	0.1
2	380	> 41	0.02
3	115	13	0.001
4	590	>410	0.16
5	960	>410	0.04
6	185	> 41	0.02

In the chamber with oil sediments the n-hexadecane being metabolized is expected to have a lower, probably a very much lower, specific activity than in chambers (2, 3, 6) with no visible oil in the sediment. For that reason the values for radioactive $C0_2$ produced in the sediments may not give a true relative impression of the differences in the biochemical activities in the same sediment. In a direct comparison the bacterial activity in the heavily oiled sediments may be grossly underestimated.

The water phase of the chambers (see column "water" in Table 10) contained radioactive material of non-carbonate nature. This may represent ^{14}C-n-hexadecane or decomposition products of this hydrocarbon. The chambers exhibiting the highest biological activity based on the production of $^{14}CO_2$, also had the largest quantities of radioactive non-CO_2 carbon in their water phase; actually in quantity, this fraction exceeded the radioactivity in C!02 in each case. It is suggested that this phenomenon is also directly or indirectly associated with the biological processes involved the conversion of n-hexadecane or other types of hydrocarbons.

In conclusion the experiments with ^{14}C-n-hexadecane unequivocally demonstrate that hydrocarbons of the alkane type is being biologically transformed in the intertidal sediments of Bay 11 and the most active degradation appears to take place in the sediment with the highest concentration of oil.

3.5. Microbiological survey of the Bays of Cape Hatt.

The beaches of Cape Hatt, along the Ragged Channel and inside the Z-lagoon, have been to variable degree exposed to petrogenic

hydrocarbons by intensional oil release experiments and other activities. We have very few analyses of the virgin situation prior to any BIOS activity, but we thought it would be of interest to monitor the microbial situation in the tidal sediments as one parameter in evaluating the degree of oil-pollution at the termination of the project.

We chose to collect sediment samples from the upper intertidal zone, assuming this to represent the most sensitive area for detecting the influence of petrogenic hydrocarbons on the microflora in the sediment. Polluting material of this kind would most likely be transported as a surface slick and any material deposited in the intertidal zone would have the longest residence time in the upper portion of the zone.

The data from the survey carried out August 26, 1983 are given in Table 21. The same data as well as the calculated per cent oildegrading bacteria are presented according to smpling sites in the Fig. 8, 9 and 10.

The count of oildegrading bacteria and their relative proportion in the microflora are of particular interest in the evaluation of the results. Based on both parameters all beaches appear to be influenced by oil, but to a very variable degree.

Except for Bay 11 and Bay 12 the number of ODB in the Bays along the Ragged Channel *are* moderate to low, with a marked trend of less affected bays from north to south. But the proportion of ODB appear to be less influenced.

The beaches of the Z-lagoon are by far most strongly affected. The per centage of ODB in excess of 10% as well as high counts of ODB clearly indicates this.

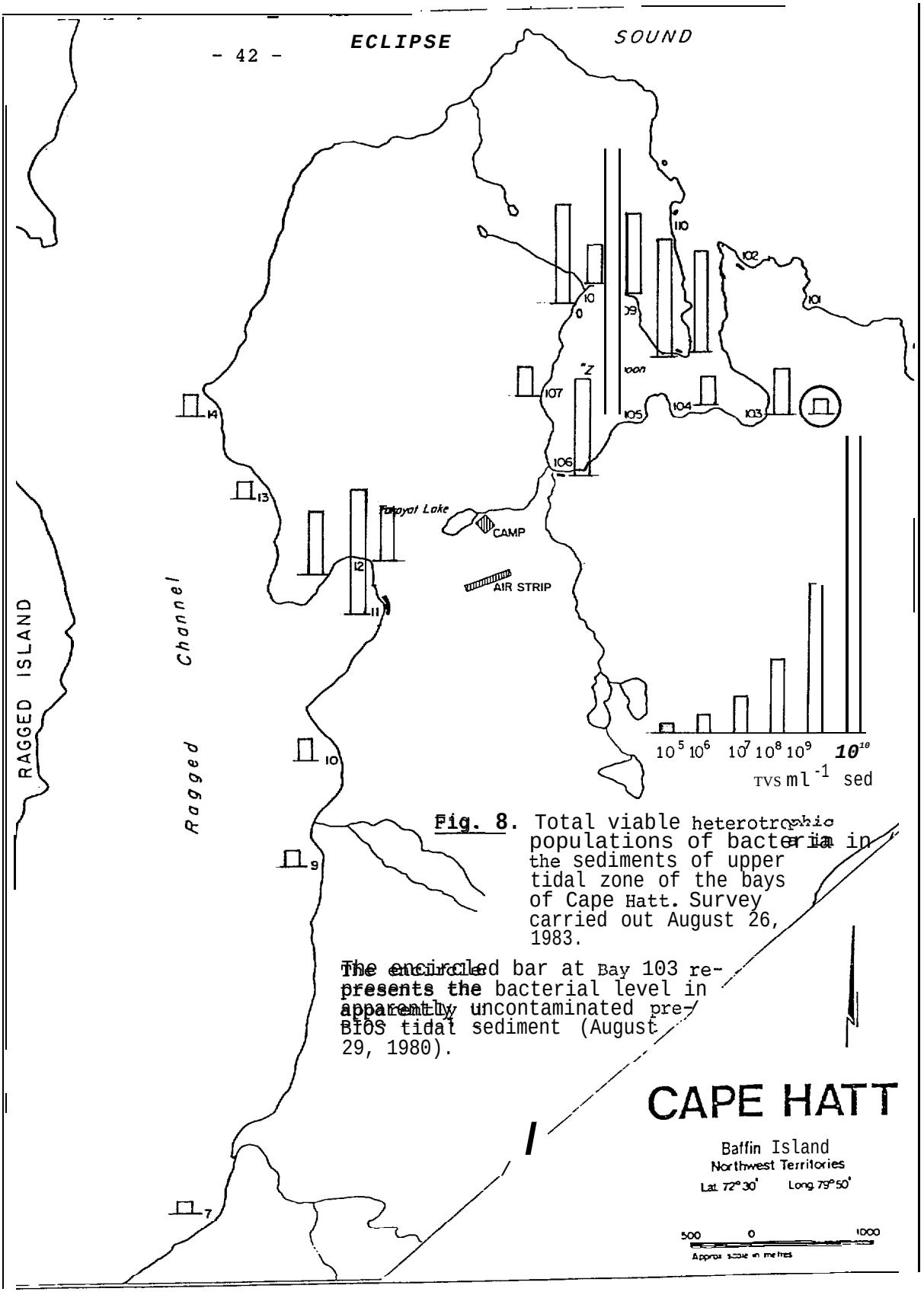

Fig. 8. Total viable heterotrophic populations of bacteria in the sediments of upper tidal zone of the bays of Cape Hatt. Survey carried out August 26, 1983.

The encircled bar at Bay 103 represents the bacterial level in apparently uncontaminated pre-BIOS tidal sediment (August 29, 1980).

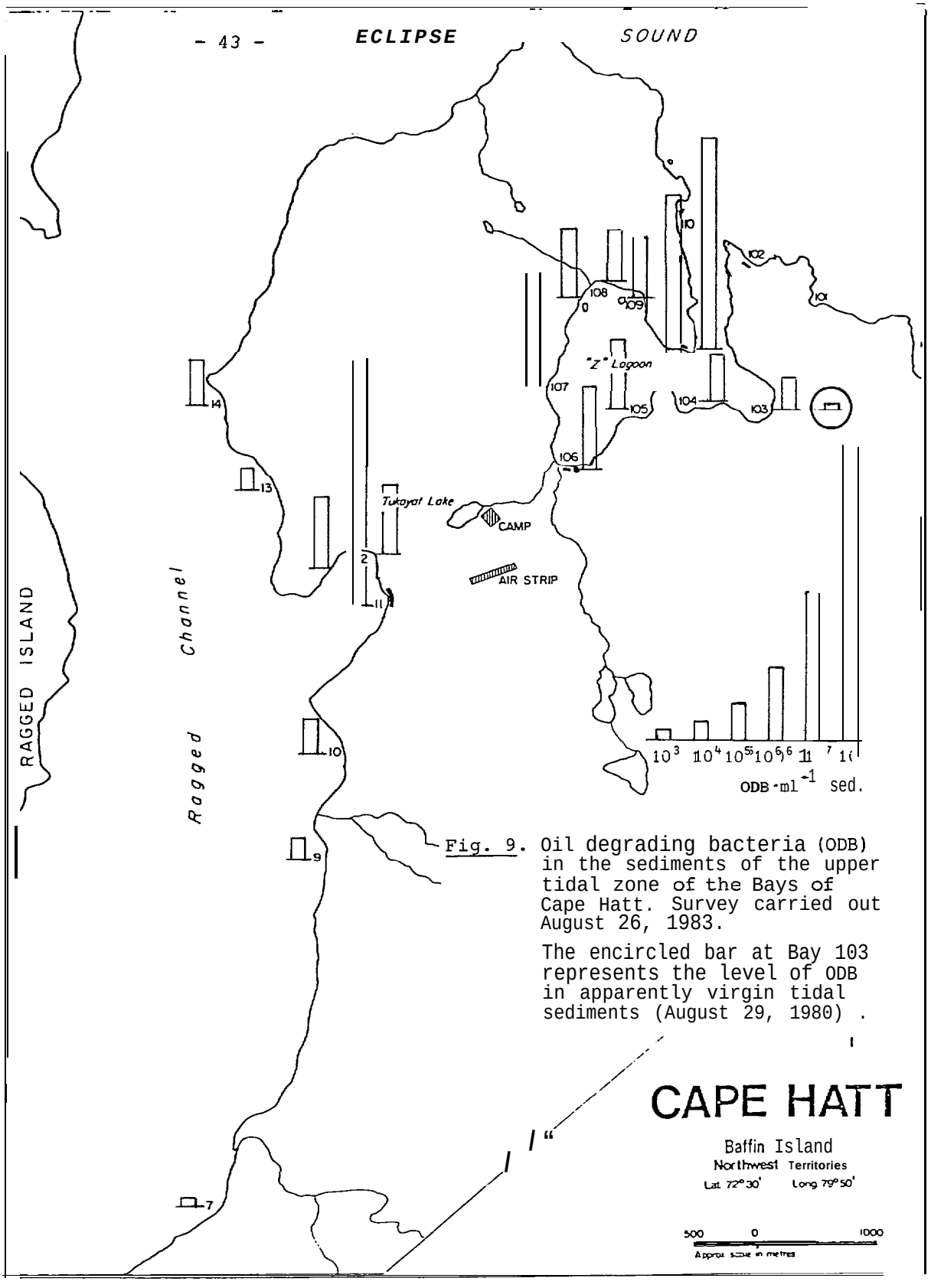

Fig. 9. Oil degrading bacteria (ODB) in the sediments of the upper tidal zone of the Bays of Cape Hatt. Survey carried out August 26, 1983.

The encircled bar at Bay 103 represents the level of ODB in apparently virgin tidal sediments (August 29, 1980) .

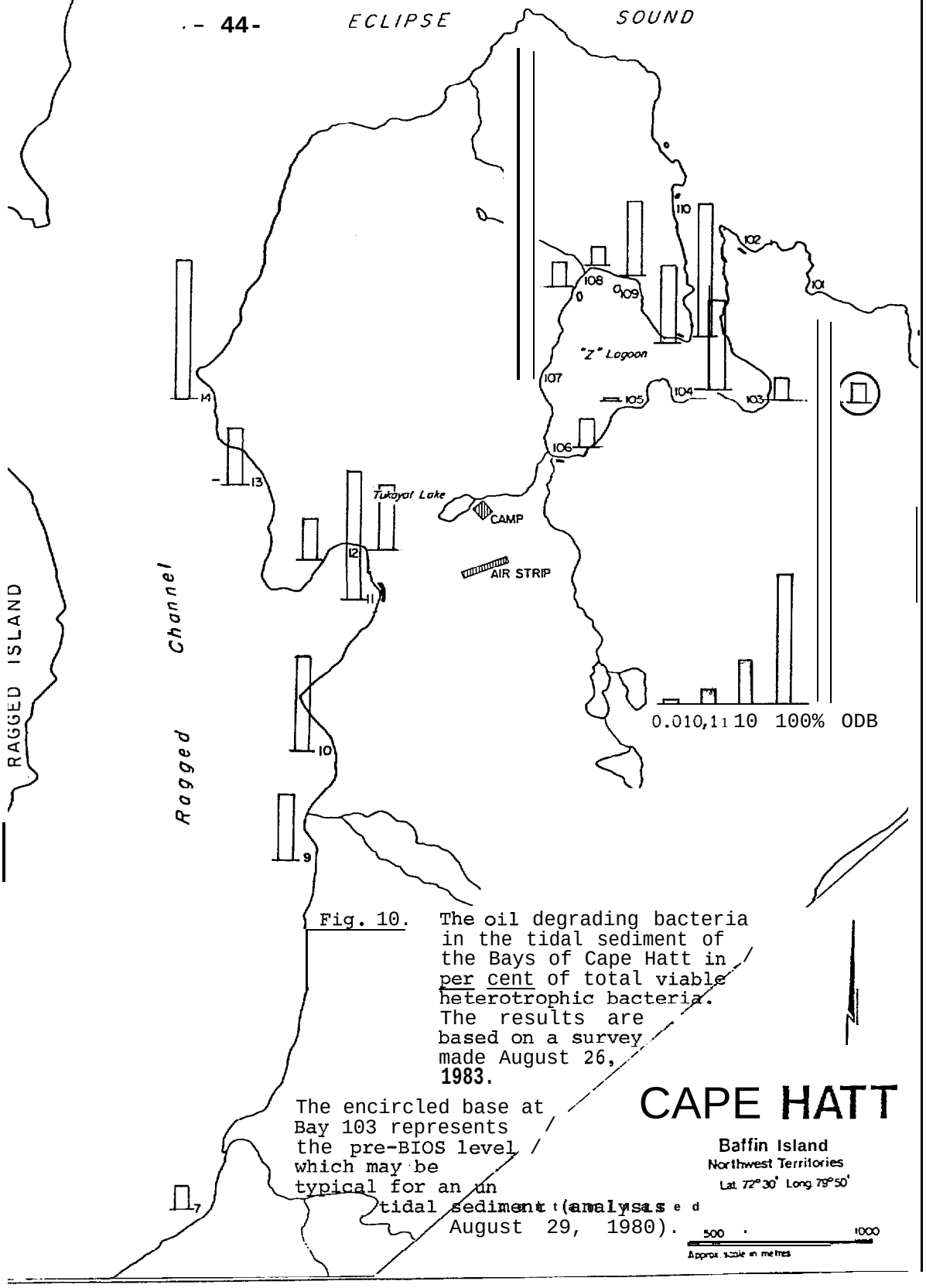

Fig. 10. The oil degrading bacteria in the tidal sediment of the Bays of Cape Hatt in per cent of total viable heterotrophic bacteria. The results are based on a survey made August 26, 1983.

The encircled base at Bay 103 represents the pre-BIOS level which may be typical for an un tidal sediment (analysed August 29, 1980).

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APPENDIX

DATA FOR MICROBIOLOGICAL AND CHEMICAL ANALYSES

Table 12. Relationship between oil in bottom sediment and the microbial activity for oil degradation.

Data for microbiological analyses and hydrocarbon chemistry along a transect in Bay 11 (from H2) and at various depths at random in Bay 7, August 1983. The results for hydrocarbon chemistry are from Boehm (6). The transect was established by Bunch (8) and the sediment samples were taken by divers from L.G.L.

Our analyses of total viable heterotrophic (TVH) and oildegrading bacteria (ODB) covers the stations taken August 10, 1983, and each sediment sample represents a composite of 8 individual samples.

		$ml^{-1} x 10^{-5}$		Estimated	
Station	depth,m	TVH*	ODB	petroleum, µg/g	Phytane/C-18 ratio
Bay 11:1	11.3	120 (4)	4.5	1.7	1.6
4	9.1	170 (14)	25	1.2	0.9
5	7.6	46 (21)	9.6	1.7	1.2
7	6.4	130 (19)	25	4.4	1.7
9	5.5	490 (3)	15	29	2.7
10	4.5	110 (41)	45	40.7	2.7
13	3.9	800 (14)	110	120	2.6
15	1.5	330 (14)	45	44	3.6
Bay 7: 1	2	1100 (4)	45	1.8	2.2
2	4	490 (1)	4.5	< .5	
3	6	800 (1)	7.5	< .5	
4	8	110 (9)	9.5	< .5	0.25
5	10	170 (2)	2.5	< .5	0.6

*Number in parenthesis indicates percent ODB.

Undegraded oil has a phytane/C-18 ratio of 0.61.

Table 13. Enhanced biodegradation experiment in the supralittoral zone of Bay 102, Cape Hatt.

Results of the enumeration of total viable heterotrophs (TVH) and oildegrading bacteria (ODB) of the oil containing sediment, August 14, 1983.

Sample K (control) was taken south and east of the test plots 102 E-H. The test plots 102A and B were laid August 23, 1980, the others August 1, 1981; all plots initially received a load of 10 kg/m^2 crude Lago Medio. In plot 102A and plots 102D-H the oil was emulsified (oil/water 1:1).

Plot	Sample	TVH No.ml^{-1}·10^{-5}	ODB No.ml^{-1}·10^{-5}
102A (emulsion)	a	14	28
102B (crude oil)	a	230	220
102E	a	200	280
(fertilized (0.4 kg m^{-2}) and mixed)	b	320	220
102F	a	19	73
(oiled control)	b	200	73
102G	a	670	1300
(low level (0.04 kg m^{-2}) fertilized)	b	130	280
102H	a	1400	2100
(high level (0.4 kg m^{-2}) fertilized)	b	2300	730
	c	960	730
	d	670	1300
102K	a	6.7	<0.41
(control)	b	6.?	<0.41
	c	1.4	<0.41

Approximate 95% confidence limits:

TVH: MPN/3.30 to MPN X 3.30

ODB : MPN/4.68 to MPN X 4.68

Table 14. Enhanced biodegradation exper iment in the supra-littoral zone on Crude oil point (Z-lagoon), Cape Hatt.

The oiled test plots were laid August, 1980. One part of the plot (T1FC) was fertilized September 7, 1981 (80 g/m^2). Results of the enumeration of total viable heterotrophs (TVH) and oil degrading bacteria (ODB), August 1983.

Date	Plot	Sample	TVH $No.ml^{-1}x10^{-5}$	ODB $No.ml^{-1}x10^{-5}$
08.17	T 1 SC	a	4.1	1.3
	(sand control)	b	0.49	>4.1
08.23	do.	a	>81	7.2
08.17	T 1 CC	a	96	28
	(crude control)	b	96	73
08.23	do.	a	2600	130
08.17	T 1 FC	a	140	130
	(fertilized crude)	b	67	130
08.23	do.	a	260	44
		b	2600	130
		c	150	28

Approximate 95% confidence limits:

TVH (08.17): MPN/3 .30 to MPN X 3.30

TVH (08.23) and ODB: MPN/4 .68 to MPN X 4.68

Table 15. Enhanced biodegradation experiment in the supralittoral zone of Bay 106, Cape Hatt.

Results of the enumeration of total *viable* heterotrophs (TVH) and oil degrading bacteria (ODB) of oil containing and of the upper o-2 cm of the surface layer, August 1983. The oiled test plots were laid August 19, 1982.

Date	Plot	Sector	sample	TVH No.ml^{-1}x10^{-5}	ODB No.ml^{-1}x10^{-5}
08.17	106 NSC			9.6	32
	(sand control)			96	> 4.1
08.23	do.			870	28
				44	7.3
08.17	106 NFE	A		9.600	>4.100
	(fertilized emulsion)			49.000	32.000
		B	a	9.600	>4.100
08.23	do.	A		4.400	7.300
				>810.000	1.300
				260.000	7.300
		B	a	87.000	7.300
08.17	106 NCE	B	b	2.300	1.300
	(crude emulsion)	c		6.700	1.300
				3.800	1.300
08.23	do.	B	b	2.600	280
		C		2.600	280
		c		2.600	280
				1.500	2.800
08.17	106 NFC	D		32.000	>41 .000
	(fertilized crude)				2.800
		E	a	2.300	3.200
08.23	do.	D		4.400	13.000
				15.000	13.000
		E	a	5.500	13.000
08.17	106 NCC	E	b	490	2.800
				1.400	730
				1.300	440
08.23	do.	E	b	1.500	2.800
		F		2.600	220
				2.600	2.200
			c*	870	73

Approximate 95% confidence limit
TVH (08.17): MPN/3.30 to MPN X 3.30
TVH (08.23) and ODB: MPN/4.68 to MPN X 4.68

*This sample was taken in a spot within sector F where all visible oil had been removed lest year (for chemical analysis). The diameter of the area was approximately 10 cm.

Table 16. Enumeration of the total viable heterotrophs (TVH) and oil degrading bacteria (ODB) of the surface layer (0–2 cm) of the proximate area around the "^{14}C-hexadecane-chambers".

Chambers 1-5 were situated in Bay 11. Chambers 2 and 3 were in the low intertidal zone, chamber 1 and 4 in the mid intertidal zone and chamber 5 in the high intertidal zone. Chamber 6 was situated in the mid/lower intertidal zone in Bay 12. Sample a was taken as close as possible to the chamber, sample b was taken in a 0.5 m radius around the chamber.

Chamber	Date	Sample	TVH No.ml^{-1}x10^{-5}	ODB No.ml^{-1}x10^{-5}	Comments
1	08.10	a	960	>410	Heavily oiled, coarse sand/gravel
		b	1400	730	
	08.12	a	510	280	
2	08.12	a	380	> 41	Sand, no visible oil
		b	380	> 41	
3	08.12	a	140	13	Sand no visible oil
		b	88	> 41	
4	08.12	a	690	>410	Heavily oiled gravel
		b	490	>410	
5	08.12	a	960	>410	Heavily oiled gravel
6	08.12	a	140	> 41	Sand
		b	230	> 41	

Approximate 95% confidence limit
TVH MPN/3.30 to MPN X 3.30
ODB MPN/4.68 to MPN X 4.68

Table 17. Hydrocarbon chemistry data on sediment samples from enhanced biodegradation experiments in Bay 102, Crude Oil Point and Bay 106.

Analyses based on GC of samples indicated; data by Boehm (6).

		HYDROCARBON (PHC) mg/g	SHWR	ALKANE/ ISOPRENOID RATIO
Bay 102 A	emulsion, composite	19.5	1.57	2.1
B	crude oil, composite	17.9	1.47	2.1
E	emulsion, composite	8.7	1.36	1.3
F	" "	9.2	1.80	2.1
G	" "	17.9	1.76	2.2
H	" "	30.5	1.74	1.8
Crude oil, Bay				
T1	crude oil, top surface	48.4	1.40	2.0
T1	crude oil, composite	25.4	1.75	2.2
T1	fertilized crude, top surface	28.0	1.31	2.1
T1	fertilized crude, composite	28.3	1.11	2.1
Bay 106 A	fertilized emulsion, top surface	47.3	1.33	1.1
"	fertilized emulsion, composite	8.9	1.%	0.9
106 C	emulsion, top surface	36.8	1.42	2,0
"	emulsion, composite	6.7	1.53	2.0
106 D	fertilized crude, top surface	38.8	1.44	1,5
"	fertilized crude, composite	21.1	1.67	1.6
106 F	crude oil, top surface	15.5	1.40	2.0
"	crude oil, composite	19.8	1.84	2.2

Table 18. Enhanced biodegradation exper iment in the supra-littoral zone of Bay 102, Cape Hatt. $C0_2$ production in oiled sediments August 13-14, 1983.

The test plots A and B were laid August 24, 1980, the others August 1, 1961; all plots initially receiving a load of 10 kg/m^2 crude Lago medio. In plot A and plots E-H the oil was emulsified (oil/water 1:1). An area east of plot E served as a sand control.

All the test plots were originally situated in the supralittoral zone. But since then the conditions in Bay 102 have changed, and plot A and parts of plot B are now swept by waves at high tide. The incubation period for all the CO_2 production measurement chambers on plot A, and four of the chambers on plot B were therefore only 10 hours. The rest of the chambers were incubated for 23 hours (from 1 p.m. August 13 to 12 a.m. August 14). The weather during the incubation period was clear and calm, and the average mean temperature* inside the $C0_2$ chambers was 8.4°C (max. 18°C, min. 0°C).

Plot	Number of measurements	CO_2 production (mg m^{-2} h-l) mean	span
102 A (emulsion)	5	25	(15-41)
102 B (crude oil)	7	41	(25-70)
102 E (fertilized (0.4 kg m^{-2}) and mixed)	7	37	(28-%)
102 F (oiled control)	7	25	(13-30)
102 G (low level (0.04 kg m^{-2}) fertilized)	7	24	(12-30)
102 H (high level (0.4 kg m^{-2}) fertilized)	9	30	(20-39)
102 K (sand control)	6	32	(26-26)

*) Mean temperature in a CO_2-chamber is calculated as half the sum of the highest end the lowest recorded temperature. Ten CO_2-chambers were fitted with thermometers, and the temperatures were recorded every fifth hour.

Table 19. Enhanced biodegradation experiment in the supralittoral zone at Crude Oil Point, Cape Hatt. $C0_2$ production in oiled sediments August, 1983.

The test plots were laid August, 1980. One part of the plot (T1FC) was fertilized (0.08 kg m^{-2}) September 7, 1981.

The period of measurement was from approximately 10 a.m. the first day to 9. a.m. the next day. During the first measurement period the weather was clear and sunny, while the sky was clouding during the second period. The CO_2 production measurements in the oiled sediments were performed in exactly the same spots at both times. The control plot, however, was, due to an inuit camp, different on the two occasions.

	August 16-17			August 22-23		
	Average mean temperature 7.3°C* (max. 17°C, min. −2°C)			Average mean temperature 5.7°C* (max. 10°C, min. 2°C)		
Plot	No. of measurements	$C0_2$ production ($mg\ m^{-2}h^{-1}$) mean	span	No. of measurements	$C0_2$ production ($mg\ m^{-2}h^{-1}$) mean	span
T1 SC (sand control)	6	37±1.8	(34-38)	6	34*5.4	(27-34)
T1 CC (crude oil)	6	42±1.6	(40-44)	6	33±3.9	(30-36)
T1 FC (fertilized crude)	7	40±6.7	(32-51)	7	38±5.2	(32-%)

*) The temperatures were measured inside the $C0_2$ chambers. Mean temperature in a chamber is half the sum of the highest and the lowest recorded temperature. Five chambers were fitted with thermometers, and the temperatures were recorded every fifth hour.

Table 20. Enhanced biodegradation experiments in the supralittoral zone in Bay 106, Cape Hatt. $C0_2$ production in oiled sediments August 1983.

The test plots were laid August 19, 1982, all spots initially receiving a level of 10 kg Oil/m^2. In plot NCE and NFE the oil was emulsified (oil/water 1:1). The plots NFC and NFE were fertilized (0.1 kg/m^2) with Fullgjødsel C (Norsk Hydro).

The period of measurement was approximately from 10 a.m. the first day to 9 a.m. the next day (23 hours). The weather during the first measurement period was clear and sunny, while it was cloudy during the second period. The mean average temperature inside the CO_2 measurement chambers is given in the table. During the first measurement period the temperature in the upper centimeter of "clean" and oil covered sediment was also recorded. The mean average temperature in the upper centimeter of oil covered sediment was 6.7°C (*max.* 12.3°C, min. 1.0°C). The mean average temperature in "clean" sediment was 5.0°C (max. 9.5°C, min. 0.5°C).

	August 16-17			August 22-23		
	Average mean temperature 7.2°C* (max. 15.5°C, min. 0.2°C)			Average mean temperature 5.2°C* (max. 9.8°C, min. 1.9°C)		
Plot	No. of measurements	$C0_2$ production ($mg\ m^{-2}h^{-1}$) mean	span	No. of measurements	$C0_2$ production ($mg\ m^{-2}h^{-1}$) mean	span
NSC (sand control)	5	16±7.4	(9-28)	6	25±8	(15-38)
NCC (crude oil)	5	20*3.8	(16-26)	5	32±9	(22-45)
NFC (fertilized crude)	5	41±9.9	(29-56)	5	44*7	(34-53)
NCE (crude emulsion)	6	26±10.4	(13-42)	6	32±8	(17-%)
NFE (fertilized emulsion)	6	42*11.1	(29-59)	6	48±14	(29-56)
NCC**	1	11		1	*10*	
NFC**	*1*	27		1	26	

*) Mean temperature inside a CO_2 chamber is equal to the half value of highest and lowest recorded temperature.

**)These measurements were performed on sites within the plots where all visible oil (NCC) or almost all visible oil (NFC, small spots left) had been removed the previous year.

Table 21. Enumeration of total viable heterotrophs (TVH) and oil degrading bacteria (ODB) of the surface layer (0-2 cm) in the intertidal zone of beaches in Z-lagoon and Ragged Channel, Cape Hatt, August 1983.

All samples were taken in the upper intertidal zone. In Ragged channel (Bay 7-14) the samples were either taken close to high water mark (a) or at approximately 2/3 of the high tide (b). The sample (5 ml) usually consisted of 3-4 subsamples, sampled within a few meters of each other. For positions of the sampling sites, see map page

Date	Site	TVH $No.ml^{-1}x10^{-5}$	ODB $No.ml^{-1}x10^{-5}$	% ODB (ODBx100/TVH)
Z-lagoon				
08.24	Bay 103	260	0.73	0.3
**	" 104	55	2.2	4.0
"	" 105	64.000	7.3	0.01
"	" 106	2.600	13	0.5
08.23	" 107	55	44	80.0
"	" 108	2.600	7.3	0.3
"	" 109 w	1.200	2.8	0.2
"	" 109 E	150	4.4	2.9
"	crude oil point, W	4.400	130	3.0
"	crude oil point, E	2.600	320	10.8
Ragged Channel				
08.26	Bay 14 a	15	1.3	8.7
"	" 14 b	15	2.8	18.7
"	" 13 a	1.5	0.28	18.7
"	" 13 b	87	0.13	0.2
"	" 12 W a	1.200	7.3	0.6
"	" 12 W b	440	7.3	1.7
08.20	" 12 E a*	2.600	>41	>1.6
"	" 12 E b*	44	1.3	3.0
08.26	" 10 a	15	0.73	4.9
"	" 10 b	26	1.3	5.0
"	" 9 a	12	0.28	2.3
**	" 9 b	5.5	0.13	2.4
**	" 7 a	2.3	0.073	3.2
"	" 7 b	5.5	0.13	2.4
Z-lagoon				
08.24	Bay 103, supralittoral	550	13	2.4

Approximate 95% confidence limit MPN/4.68 to MPN x 4.68.

*)Bay 12 E a and b are identical with samples 3 and 5 in Table 7.

	TVH		ODB		% ODB
	-1	-5	-1	-5	