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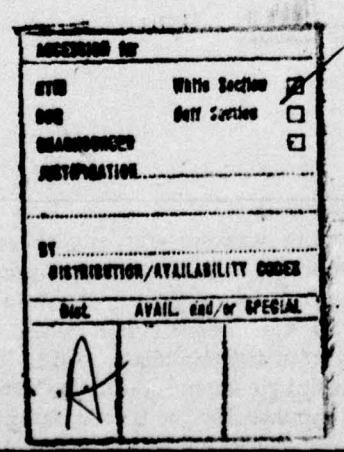

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Fate and Effects of Oil Pollutants in Extremely Cold Marine Environments

by

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ABSTRACT

Oil biodegradation in Arctic coastal marine waters was modelled using a flow through system incubated *in situ*. Rates of natural oil degradation were very slow. Addition of nutrients increased degradative losses. Biodegradation did not result in major changes in the relative concentrations of hydrocarbons in crude oil during one summer's incubation. Oil incubated *in situ* under sea ice showed almost no degradative losses due to chemical or biological weathering during one month's incubation. Low concentrations of soluble/dispersed oil were found to result in sublethal changes in the movement and feeding of Arctic amphipods. Lethality of amphipods exposed to soluble/dispersed oil was concentration dependent. Microbial populations in a lake contaminated by a MOGAS spillage changed in parallel with the movement of the gasoline and appear to be useful as an indication of hydrocarbon contamination. Microorganisms indigenous to the contaminated lake were capable of hydrocarbon degradation and nutrient addition was shown to stimulate degradative losses.

INTRODUCTION

This report will deal with four areas of research conducted to assess the potential interactions of microorganisms with petroleum hydrocarbons in Arctic coastal marine and aquatic ecosystems. The first area involves the use of flow through chambers as a model system to assess changes in microbial populations that encounter petroleum contaminants in coastal marine ecosystems, and to examine oil biodegradation and possible ways of stimulating oil biodegradation in these ecosystems. The second research area involves assessing the fate of oil that becomes entrapped under sea ice. The third area involves developing a bioassay system using amphipods to assess the ecologic consequences of stimulated oil biodegradation. The fourth reseach area involves examining the consequences of actual accidental spillages of petroleum hydrocarbons in Arctic ecosystems with respect to the effects on the microbial community and the ability of the indigenous microbial community to degrade hydrocarbons.

Since it is impossible to replicate in the laboratory all the physical, chemical and biological determinants of any natural ecosystem, and since it is also impossible to quantitate the fate and effects of uncontained oil in the natural ecosystems, an open flow through system was constructed and incubated *in situ* on the coast of the Chukchi Sea at Barrow, Alaska. The system was fed by seawater collected a few meters offshore and affected by the natural abiotic and biotic factors of that nearshore ecosystem, including fluctuation

of temperature, light intensity, wind and biological populations. Since the oil was contained, it was possible to recover and quantitate the compositional changes in the oil brought about by microbial degradation. The system modeled the expected changes in oil spilled into coastal Arctic ecosystems. Unlike other marine ecosystems where the walls of the chamber would greatly alter the natural open situation and lessen the value of the system as a model, in the Arctic ice forms natural "walls" and the flow through chambers are therefore a very good model of the natural ecosystem. Using a flow through system, we examined the ability of nutrient addition to stimulate oil biodegradation processes and the associated changes in microbial populations.

Since Arctic waters are ice covered much of the year, determining the fate of oil that becomes entrapped under ice is very important. Since the ice cover will restrict evaporative losses, dissolution and biodegradation will be the main processes for the removal of oil entrapped under ice. Oil may enter the marine environment from terrestrial sources and become entrapped under ice from mishaps of submarine vessels, ruptures of offshore undersea pipelines or blowouts of offshore oil wells. Oil from an offshore blowout in the Arctic could spread from the U.S. into Canada or vice versa. Offshore test wells have already been drilled in the Canadian Arctic and are planned for U.S. waters in the near future. In our studies on the fate of oil under ice, scuba divers placed small contained experimental oil spills under sea ice near Barrow, Alaska. Periodically the oil was recovered for analysis of the residual oil and

ice samples were taken to examine the microbial population.

Oil spillages in the Arctic will have far reaching ecological effects. It is necessary to have appropriate bioassay systems to assess these effects. Since amphipods are very abundant in Arctic coastal marine ecosystems, we have been assessing the effects of oil contaminants on amphipods. Our aim is to develop measures of oil toxicity to these organisms which we can then use to determine whetner stimulated oil biodegradation results in undesireable side effects to important macro-organisms, or whether enhanced microbial removal of oil will decrease the ecological effects of oil contamination in these ecosystems.

There have already been several accidental spillages of petroleum hydrocarbons in Arctic ecosystems. These generally have been of refined petroleum products. In December, 1975 a storage bladder at Prudhoe Bay ruptured, spilling refined petroleum over the frozen tundra. The spill was removed in large part by physical removal with bulldozers. We obtained soil samples from the contaminated and nearby control areas one week after the spill and nine months after the spill to examine responses of the indigenous microorganisms to oil contaminants.

In August, 1976 it was discovered that 50,000 gallons of MOGAS were unaccounted for in storage tanks at the Naval Arctic Research Laboratory, Barrow, Alaska. At about that time it was observed that a gasoline slick was covering a portion of a nearby lake, which is used for the base's drinking water supply. It was discovered that a buried small diameter pipe had broken and that gasoline was running downhill thorugh a gravel

pad along the permafrost and entering the lake through the sediment. Immediate action was taken by the base commander to cut off the flow of additional gasoline to the broken pipe and to try to contain and physically remove the gasoline. An earthen and wooden dam was constructed around the area to try to prevent the gasoline from reaching the drinking water inlet. A series of holes were dug and gasoline that seeped into these holes was pumped into barrels. We advised the base commander and laboratory director that an immediate testing program for hydrocarbons in the drinking water should be insituted to insure the health and safety of the base personnel. After consultation with ONR in Washington, D.C., such a monitoring program was begun and hydrocarbons were found to be contaminating the drinking water. Use of the water was discontinued while a charcoal filtering system was activated. After activation of the charcoal filter system, the hydrocarbon levels in the water returned to acceptable levels and use of the water supply was resumed. It is likely that since the gasoline entered the lake through the sediment, that even volatile hydrocarbons may remain as contaminants for many decades. Therefore, routine monitoring for hydrocarbons will have to be continued for an indefinite period. Immediately following discovery of the spill, we began studies to determine what could be done to enhance microbial degradation of the contaminating hydrocarbons. Based on these studies we can make a series of recommendations for action this spring, as soon as ice on the lake disappears, that should decrease the residence time of contaminating hydrocarbons.

MATERIALS AND METHODS

Continuous Flow Through System

Exposure

A continuous flow through system was used during the summers of 1975 and 1976 at Barrow, Alaska to monitor changes in microbial populations exposed to oil, and changes in the oil brought about by microbial degradation. The flow through system was also used to assess the ability of nutrient (fertilizer) addition and microbial seeding to stimulate oil biodegradation in coastal Arctic waters. A diagram of the apparatus was shown in the last annual report.

A summary of the different treatments added in replicate to the various growth chambers is shown in Table 1. Crude oil additions were 150 ml (1% v/v) of Prudhoe crude oil, specific density 0.889 g/ml. Oleophilic fertilizers were added as 12.0 g paraffinized urea (Sun Oil Co.) and 7.5 ml octylphosphate per growth chamber (Atlas and Bartha, 1973). Water-soluble (W.S.) fertilizers were added as 12.75 g $NaNO_3$, 8.025 g NH_4Cl, and 0.945 g $NaHPO_4$. Water soluble fertilizers were either added weekly or only once at the beginning of the experiment. Addition of the oleophilic fertilizers was only at the beginning of the experiment. Bacterial seeding was with a mixture of psychrotrophic hydrocarbonoclastic microorganisms previously isolated from coastal Arctic waters for their ability to utilize hydrocarbons at low temperature. Temperature, salinity, nitrogen concentrations, phosphorus concentrations, numbers of heterotrophic microorganisms and numbers of oil degrading micro-

organisms were monitored regularly.

Analysis of Oil

Residual oil was extracted from the growth chambers with diethyl-ether. All chambers were extracted after 51 days for the 1975 summer samples. In summer, 1976 replicate chambers were extracted every 2 weeks. The sediment from the second experimental chamber was extracted separately. The oil was dried over sodium sulfate, and weighed.

Column chromatographic analysis was based on the work of Coleman *et al.* (1973). One ml of oil was placed on a 19x300 mm column, packed with 1:1 (w/w) silica gel on top of alumina gel. The following fractions were obtained: saturate fraction - 4 volumes of pentane; monoaromatic fraction - 4 volumes of 5% benzene, 95% pentane (v/v); diaromatic fraction - 4 volumes of 15% benzene, 85% pentane (v/v); first polyaromatic-polar fraction - 2/3 volume of 20% diethyl-ether, 20% benzene, 50% methanol (v/v), followed by 1.5 volumes of methanol; and second polyaromatic fraction - 4 volumes of chloroform. The fractions were dried over sodium sulfate, and weighed.

Gas liquid chromatographic analysis was performed with a model 5830A Hewlett-Packard reporting gas chromatograph. Residual oils were adjusted with diethyl-ether to the initial volume (150 ml). One µl of a 1:10 dilution was injected into a 2 m column packed with 10% apiezon L on chromosorb W 60-80 mesh. Temperature was programmed at 100 C isothermal for 8 min., 8 C per min. to 250 C and isothermal at 250 C for 20 min.

Nuclear magnetic spectroscopic analysis was performed by Continental Oil Co. using a Varion HA/100 NMR spectrometer. Computerized mass spectral

analyses were also performed by Continental Oil using a CEC 2-103C mass spectrometer to give the weight percents of fifteen classes of hydrocarbons.

Oil Under Ice

Exposure

In May, 1976 a 4'x8' hole was cut through 6' of ice in Elson Lagoon. Scuba divers implanted stainless steel cylinders, 3" in diameter, X 3" long, into the underside of the ice. The stanless steel cylinders were fitted with a styrofoam collar to hold them in place. The underside of the ice was smooth and covered by extensive pockets of gas. One ml Prudhoe crude oil was injected into each cylinder and floated up against the ice surface. Periodically the cylinders were screwed 1" into the ice, stoppered on the bottom and an ice core broken off. The ice cores were recovered and returned on ice to the laboratory for analysis.

Analysis

Oil was recovered from the ice cores by extraction with diethylether, weighed and analysed by gas chromatography. Microorganisms at the ice-water interface were enumerated by viable plate counting on marine agar 2216 for total heterotrophs and on oil agar for oil utilizers, and incubated at 5 C. Ice exposed to oil for various time periods was melted, placed in a Gilson respirometer and the rate of oxygen consumption by the microorganisms in the ice was measured.

Bioassay

Test Organisms

Amphipods were chosen for use as bioassay organisms in this toxicity study because of their abundance in Arctic marine ecosystems. *Boeckosimus (=Onisimus auct.) affinis Hansen* were selected since they are the dominant amphipod species in the lagoon system near Barrow, Alaska (Busdosh and Atlas, 1975). *B. affinis* was captured in wire mesh traps from Elson Lagoon, just south of Plover Point, six miles west of Barrow, Alaska.

Exposure to Water Soluble/Dispersed Oil

Water containing soluble/dispersed oil components was prepared by adding crude oil to 15 l 27°/oo Instant Ocean to give six mixing ratios: one part crude oil:one million parts seawater; 1:100,000; 1:10,000; 1:1,000 and 1:100. The water plus oil was mixed with a Teflon coated magnetic stir bar for 6 hours; the mixture was allowed to stand for 12 hours and water was siphoned from beneath the slick; no water was collected from within 5 cm of the slick. Mixing and siphoning were done at 5 C. The actual concentrations of the oil in solution or dispersed into the water column were much lower than the mixing ratios, and probably not separted by factors of ten as in the mixing ratios. Measurements were not made to determine the actual concentrations of hydrocarbons in the water because of the complexity of interpreting analytical data at such low concentrations. Thirty individuals of *B. affinis* were added to replicate trays containing 5 l oil treated water. Water was replaced every two weeks with freshly prepared oil-water mixtures.

Lethal Effects

Mortality was checked daily. Organisms that appeared dead, *i.e.*, noted by lack of pleopod movement and failure to respond to touch, were

removed to separated chambers and checked for an additional day. No recovery was ever detected. Analyses of variance were performed. A level of 0.05 was used for determining significance.

Sublethal Effects

Movement was measured in an open field apparatus, a square tray, 50 cm on a side and 10 cm deep, with a grid marked on the bottom. The grid was composed of 100 squares, each 5 cm on a side. Each square was identified by a letter and a number, *e.g.* A3, L6. An oil-water mixture corresoponding to each exposure concentration was placed in the open field and five animals were introduced. After ten minutes acclimation, each animal was given a gentle touch with a glass rod. As the animal moved, the grid square it occupied was noted every five seconds for three minutes. For quantitative purposes an animal was always presumed to be in the center of the square at the time of recording. An animal was assumed to travel in a straight line between squares. The distance traveled for each five second interval was then determined and expressed as units of movement (units of movement x 5 = cm moved).

The ability to find and recognize food was measured in a glass tray, similar to the open field tray, filled with the appropriate oil-water mixture. Animals were placed in the tray and after ten minutes acclimation, a cube of fresh meat, approximately 2 cm on a side, was placed in the middle of the tray. The number of animals feeding was noted at ten minute intervals for one hour. Animals had not been offered food for ten days prior to these tests.

Respiration rates were determined using a Gilson respirometer.

One amphipod and a 5 ml oil-water mixture were placed in each flask. Following a one hour acclimation period, data were collected for six hours. Animals were removed, rinsed in distilled water and dried at 70 C for twelve hours. Dry weight was determined using a Cahn electrobalance. Results were recorded as µl O_2 consumed/hr/mg amphipod dry weight.

Accidental Oil Spillages

Prudhoe Bay Spill

Surface soil and snow samples were collected from oil contaminated and non-contaminated control areas shortly after and 9 months after the spillage. Viable microorganisms in the samples were enumerated as total heterotrophs, plated on trypticase soy agar at 5 C and hydrocarbon utilizers, plated on oil or gasoline agar at 5 C.

NARL MOGAS Spill

Samples were collected periodically for one month following the gasoline spillage at NARL. Sediment and water samples were collected from a very heavily contaminated area. Sediment and water samples were also collected from the moderately contaminated shallow portion of the lake near where the gasoline initially entered the lake. Sediment and water samples were finally collected from an area away from the heavily contaminated area that did not show visible signs of hydrocarbon contamination. Heterotrophic microorganisms in the samples were enumerated on trypticase soy agar. Gasoline utilizing microorganisms were enumerated on gasoline agar. Respiration rates were measured in a Gilson respirometer.

Gasoline soaked sediment samples were also collected in the heavily contaminated area. Replicate weighed samples were left *in situ* adjacent to the lake. Replicate samples were also treated with ammonium, nitrate and phosphate ions alone or in combination with inoculation with a mixture of hydrocarbon degrading microorganisms. Periodically the residual gasoline hydrocarbons were recovered by solvent extraction, weighed and stored for further analysis.

RESULTS

Flow Through System

Addition of oil to sea water changes not only the total number of microorganisms, but also the composition of the community. The ratios of oil-degrading bacteria, as enumerted on oil agar, versus heterotrophic bacteria, enumerated on marine agar, were calculated for the 1975 chambers and are shown in Table 2. The presence of oil, as was expected, enriched for oil-degrading bacteria versus heterotrophic proteolytic bacteria. The presence of water-soluble fertilizers, added once, somewhat decreased the ratio in comparison to the additon of oil alone. When added weekly, water-soluble fertilizers maintained the same ratio in comparison to the addition of oil alone in the case of 5 C, and at 25 C increased it. On the other hand, oleophilic fertilizers enriched significantly for oil-degrading bacteria. In fact, addition of oleophilic fertilizers resulted in higher counts of bacteria on oil agar than on marine agar.

Oil Residues

The weight of the oil residues and percentage of loss in comparison to fresh Prudhoe crude oil following 51 days of exposure during summer 1975 are shown in Table 3. Without addition of fertilizers, the oil lost about 15% of its weight. This loss appears to be largely abiotic. Treatments with water-soluble fertilzers, either added once or weekly, increased the loss to about 25%. The greatest loss was observed when oleophilic fertilizers were added, 32%. No oil was recovered by diethyl-

ether or chloroform extractions from the sediments of any of the second experimental chambers. Similarly, low weight losses were found in the 1976 exposures.

The oil residues were studied by gas-liquid and column chromatography, and the results are shown in Tables 4 and 5 respectively. Gas-liquid chromatographic analysis showed the same percentage of decrease or increase for each component for all treatments. Normal paraffins were still present and extensive biodegradation was not apparent in any case. Column chromatography showed a decrease in the percent saturated and monoaromatic factions, the same percentage of diaromatic fraction and an increase in the two polyaromatic-polar compounds for each of the treatments.

NMR and mass spectrometric analyses also showed that the oil residues had similar composition to fresh oil, Tables 6 and 7. The NMR analysis showed a slight increase in the percent of branched compounds, as shown by the slight increase in the percent of alpha-H. The mass spectral analysis showed no significant compositional differences regardless of treatment.

Oil Under Ice

Effect on Microbial Population

The heterotrophic bacterial population on the underside of ice was approximately 10 colony forming units/g enumerated either at 5 or 20 C. The hydrocarbon utilizing microorganisms on the bottomside of the ice was less than 0.1 colony forming unit/g. Water at the ice-water inter-

face had the same numbers of enumerable microorganisms as the bottom ice layer. Exposure to crude oil did not result in any significant change in the numbers of total heterotrophs or hydrocarbon utilizing microorganisms.

Rates of oxygen consumption were 1.7 µl O_2 consumed/h/g for the bottom ice layer and 1.3 µl/h/g for water from the ice-water interface. Exposure to crude oil did not result in an immediate change in rates of oxygen consumption, but after 2 weeks of exposure to Prudhoe crude oil the rate of oxygen consumption for bottom ice rose to 4.2 µl O_2 consumed/h/g. Rates of oxygen consumption in water from the ice-water interface did not change even after 2 weeks exposure to oil. During the 2 week exposure, oil penetrated into the ice layer to a height of 1-2 cm. In comparison, rates of oxygen consumption were less than 0.1 µl O_2 consumed/h/g in surface ice even after exposure to crude oil for 2 weeks.

Oil Losses

During 21 days of exposure oil placed on the botton of sea ice lost 9% (90 mg) of its weight. This weight loss is slightly lower than the 12% previously found for surface ice at the same time of the year, or the 15% found for surface water in summer and reflects the restricted evaporative losses for oil entrapped under ice. Compositional analyses of the recovered oil have not yet been completed, but it does not appear that biodegradative losses were significant.

Bioassay

Survival rates of *B. affinis* exposed to six concentrations of soluble/dispersed oil are shown in Fig. 1. Survival of organisms exposed to 1:10 to 1:1,000,000 mixtures was signficantly lower than controls. No significant difference was detected between concentrations of 1:100 to 1:1,000,000, but more rapid death did occur in organisms exposed to the 1:10 mixture.

Sublethal Effects

Movement. Results of open field measurement of movement are summarized in Table 8. After 2 weeks the 1:1,000,000 exposed organisms showed a slight increase in movement; 1:1,000 showed a slight decrease; and 1:10 showed a large decrease. At 6 weeks of exposure, movement of 1:1,000,000 organisms was very similar to that of the control, and 1:10 concentration showed greatly reduced movement. After 12 weeks, animals exposed to 1:1,000,000 and 1:1,000 moved less than one-fourth the distance the controls did.

Food Finding/Recognition. The percentage of animals feeding after 1 hour's exposure to food is summarized in Table 9. Throughout the 12 week experiment, 58 to 70% of the control animals were feeding after 1 hour. After 4 weeks exposure to 1:1,000,000 oil, animals were slow in responding, with 49% feeding, but at 8 and 10 weeks there were 65 and 75% feeding, respectively. The twelfth week showed a drop to 45%. Amphipods exposed to 1:1,000 oil responded normally at 4 weeks, 85% feeding successfully, but the percentage of feeding animals dropped to approximately 30% after 8 weeks. Only about 20% of the 1:10 exposed

amphipods successfully fed after 4 weeks, and this percentage further decreased to 0% after 10 weeks.

Respiration. Respiration data for the water soluble/dispersed oil experiments are summarized in Table 10. Unlike oil slick experiments, no statistically significant decrease in respiration rate could be detected during the 45 days of exposure to the soluble/dispersed oil.

Accidental Spills

Prudhoe Bay Spill

Within one week following the accidental diesel fuel spill at Prudhoe Bay, numbers of microorganisms were higher in snow and soil from beneath snow that had been contaminated with the fuel than in uncontaminated areas. In contaminated snow, initial counts were $2x10^3$ heterotrophs/g compared to less than 1 heterotroph/g in uncontaminated snow. Seventy percent of the heterotrophic population of contaminated snow were hydrocarbon utilizers. In contaminated soil from beneath the snow, initial counts were $1x10^6$ heterotrophs/g compared to $3x10^5$ in uncontaminated soil. Twelve percent of the heterotrophs in the contaminated soil could utilize hydrocarbons compared to 1% in the uncontaminated soil. Ten months after the spill, numbers of microorganisms were still higher in the contaminated area. Heterotrophs in the contaminated soil collected in late summer had $1.6x10^8$ CFU/g compared to $3.3x10^6$ CFU/g for uncontaminated soil. Nine percent of the heteroptrophs from the contaminated area were able to utilize hydrocarbons compared to 1% from the uncontaminated area. It is not known whether the initially high counts could

have resulted from the growth of the indigenous microorganisms, but it would seem much more likely, considering the low temperature, that the fuel that was spilled was contaminated with high numbers of bacteria.

NARL Spill

Effects on Microorganisms. Within 2 weeks of discovery of the gasoline spillage, elevated numbers of microorganisms were found in the contaminated water and sediment (Table 11). The magnitude of the elevation paralleled the degree of contamination. In the heavily contaminated area, the ratio of organisms enumerated on gasoline agar to total heterotrophs was high throughout the sampling period in both sediment and water. In the moderately contaminated area, this ratio was high in sediment throughout the sampling period and gradually rose in the water samples about 5 weeks after the spill. In the area of lightest contamination, this ratio remained low in water, but rose in the sediment, starting 3 weeks after the leakage.

Rates of oxygen consumption were also elevated in sediment samples collected from heavily contaminated areas (Table 12). Rates of oxygen consumption in the heavily contaminated area continuously rose until mid-September and then abruptly declined in the last sample. This abrupt decrease occurred at the time that freezing of the lake occurred. Respiration rates in the moderately and lightly contaminated areas did not rise during the sampling period and were typtical of rates of oxygen consumption in sediment of this lake in previous summers.

Weight losses of hydrocarbons from heavily contaminated sediment

samples are shown in Table 13. If untreated, 86 and 90% of the hydrocarbons were lost after 3 and 5 weeks respectively due to combined abiotic and biotic factors. Addition of nutrients with or without seeding with hydrocarbon utilizing bacteria increased these losses to 94 and 96% respectively after the 3 and 5 week time periods. A slight further enhancement was found when nutrient addition was coupled with seeding. In the best case only 3% of the extractable hydrcarbons remained after 5 weeks if treated and 10% if untreated. Nutrient addition would seem advantageous to enhance removal of sediment bound gasoline from this ecosystem and thus remove the potential health hazard from continued use of water from this lake as a drinking water supply.

DISCUSSION

The described flow through system, incubated *in situ*, is a good model for studying oil biodegradation. It reflects natural changes in environmental factors such as temperature, salinity and variability of natural microbial populations. Such a model is particularly suited for the Arctic where the chambers produce the same wall effect naturally produced by sea ice. The Arctic summer of 1975 was particularly cold and lacked offshore winds which, unlike normal years, resulted in the presence of shorefast ice all summer. It is possible that such sustained cold and lack of air movement resulted in lower biodegradation and abiotic losses of the crude oil in this system as compared to other reports (Atlas, 1975; Atlas and Schofield, 1975). The Arctic summer of 1976 was warmer than 1975, but we still found very low rates of oil biodegradation. Although summer 1976 was warmer than 1975, much of the flow thorugh system experiment in 1976 was conducted while the sea at Barrow was still ice covered.

An increase in bacterial population densities was obtained when crude oil with no fertilizers was added. This indicates that in the studied area of the Arctic Ocean organic matter is a primary limiting growth factor. When oil was added, nitrogen and phosphorus became the limiting growth factors. Addition of these two nutrients, either in the water-soluble or in the oleophilic form, further increased the bacterial numbers. The presence of oil resulted in a shift within the microbial population to a greater percentage capable of utilizing petroleum hydro-

carbons.

Unlike other reports (Colwell *et al.*, 1976; Davis, 1967; Jobson *et al.*, 1972; and Mechalas *et al.*, 1973), no preference, in terms of degradation, of any fraction of the oil was observed in this experiment. Examination of the data obtained from gas-liquid and dual column chromatography, NMR and mass spectrometry shows no difference in the percent composition of the residual oils. It appears that regardless of the total amount of degradation, which varied with treatment, individual components within the oil were similarly degraded, *i.e.*, following weathering, both general class fractions, shown by column chromatography, and specific components, shown by gas-liquid chromatography, retained the same relative percent concentrations. The exceedingly low rates of biodegradation, in spite of the very high numbers of bacteria, may be due to temperature restrictions or other unidentified nutritional restrictions. Addition of nitrogen and phosphorus fertilizers stimulated biodegradation as has previously been shown for other environments. A lack of extractable hydrocarbons in the sediment of the second experimental chambers indicates that oil probably was not lost from the system by emulsification.

Based on the experiments conducted with the flow through system, it appears that oil spilled in coastal waters near Barrow, Alaska will persist for much longer periods of time than originally predicted based on our earlier work in Prudhoe Bay and that the composition of the residual oil will closely resemble fresh oil for long periods of time. We have allowed the flow through system to freeze *in situ* and will follow

the fate of the oil through another summer. We expect to determine whether any changes occur in oil that has been frozen into the ice matrix and whether microorganisms require an extensive lag period following thawing of the ice to again establish active biodegradation, or whether oil degrading microorganisms survive the winter in the oil-ice matrix and are metabolically active immediately following spring melt.

Based on our oil on and under ice experiment, we would predict that little change in oil will occur during the ice covered winter. We have found only negligible degradative losses during one month exposure of oil over or under sea ice. Numbers of hydrocarbon degrading microorganisms appear to be extremely low in sea ice and initially limit rates of biodegradation of oil on or under ice. Experiments conducted for longer exposure times will be necessary to predict how long oil will persist under ice and whether it will be possible to enhance removal of oil under ice by stimulated biodegradation.

In the amphipod bioassay experiments, the water contained soluble oil components and may have also had some dispersed (emulsified) oil components, as would occur in a natural situation with wind and current mixing. As expected, mortality was greatest in the tests using the highest oil concentration, 1:10. Even the lowest concentration tested, 1:1,000,000, caused increased mortality, but no difference in lethality was observed for concentrations between 1:100 and 1:1,000,000. One would expect low concentrations of soluble and dispersed oil, approximating our 1:1,000,000 test, in a wide area around any major spill and reaching well down into the water column. One would also expect to

find such low concentrations of soluble/dispersed hydrocarbons around chromic sources of oil pollutants, *e.g.*, around loading docks and drilling rigs.

It was also possible to detect sublethal changes in behavior of amphipods exposed to soluble/dispersed oil components. Reduced movement and feeding activity occurred following exposure to soluble/dispersed oil. The percent reduction was dependent on concentration and time of exposure. Surviving animals showed decreased movement and ability to find and recognize food after two weeks exposure to 1:10 and 1:1,000 concentrations of soluble/dispersed oil. Decreased movement and food recognition was also observed in 1:1,000,000 exposed organisms but not until after twelve weeks of exposure. As amphipods are important omnivorous scavengers in Arctic ecosystems, this decrease in movement and feeding could seriously affect various ecological processes, in addition to leading to the eventual death of the amphipods. No significant change in rates of respiration could be detected, however, when amphipods were exposed to soluble/dispersed oil. It would appear that rates of movement and food finding ability can be used as sublethal measures of toxicity in future bioassay systems. We are presently examining whether the same sublethal effects are observed in cases where the oil is bound to sediment.

Based on our examination of samples from the lake at NARL that was contaminated by the accidental MOGAS leakage, we were able to inform the base commander and the laboratory director of the movement toward the drinking water inlet and insist that safety testing of the water supply

be initiated. We can also say that residual, potentially toxic, hydrocarbons may remain bound to sediment for a prolonged period, slowly being released into the water supply. We have found that high numbers of hydrocarbon degrading microorganisms are present in the contaminated areas and that nutrient addition will increase degradative losses and reduce the sediment bound hydrocarbons. We recommend that the Navy institute a program to add nutrients to sediment in heavily contaminated areas with the necessary approval of other federal and state agencies and that a monitoring program be established to follow movement of oil in the lake and its removal by weathering and microbial degradation. We further recommend that testing of the drinking water for hydrocarbons be continued.

ACKNOWLEDGEMENTS

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Fig. 1. Mortality of *B. affinis* exposed to soluble/dispersed crude oil components.

- control
- 1:1,000,000
- 1:100,000
- 1:10,000
- 1:1,000
- 1:100
- 1:10

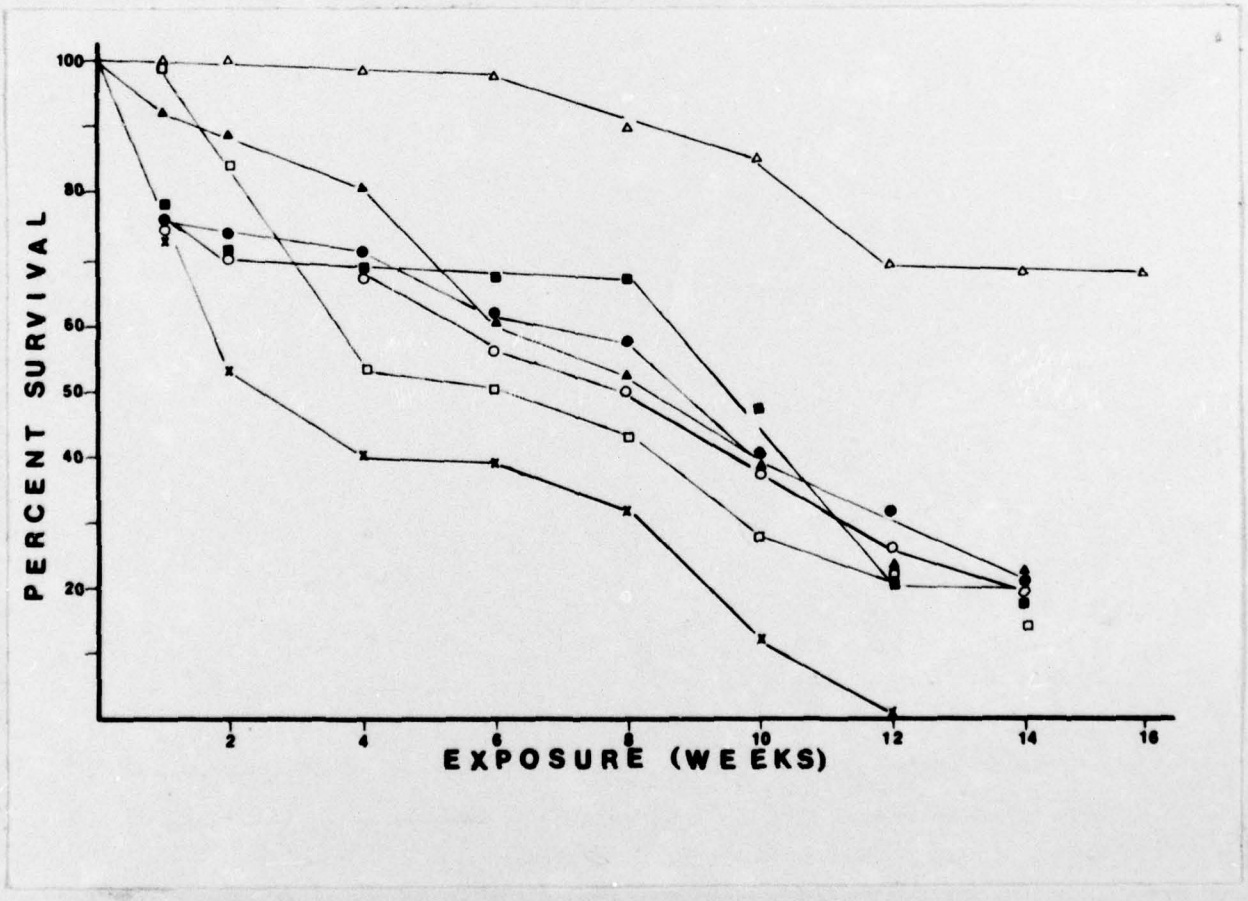

Table 1. Summary of the treatments added to the growth chambers.

1. Sea water
2. Sea water + crude oil
3. Sea water + crude oil + oleophilic fertilizers
4. Sea water + crude oil + water-soluble fertilizers (only the first day)
5. Sea water + crude oil + water-soluble fertilizers (weekly)
6. Sea water + crude oil + oleophilic fertilizers plus bacterial seeding

Table 2. Average ratios of oil degrading bacteria to heterotrophic bacteria.

Treatment	25°C	5°C
Control, sea water only	0.124	0.358
Crude oil	0.758	0.740
Crude oil + oleophilic fertilizers	2.920	1.174
Crude oil + water-soluble fertilizers (once)	0.628	0.516
Crude oil + water-soluble fertilizers (weekly)	1.345	0.712

Table 3. Solvent extractable material.

Treatment	Weight (g)	% of loss (a)
Oil	113.28	15.05
Oil + *W.S. fertilizers (once)	98.70	25.98
Oil + *W.S. fertilizers (weekly)	100.70	24.48
Oil + oleophilic fertilizers	89.98	32.52

(a) In comparison to fresh Prudhoe crude oil.
* water-soluble

Table 4. Gas chromatographic analysis of residual oil, showing relative percentages of the resolved components.

*R.T.	Fresh crude oil	Crude oil	Oil + oleoph. fertil.	Oil + **W.S. fert. (once)	Oil + **W.S. fert. (weekly)	Ident. Paraffins
13.00	54.74(a)					
13.31	6.26		0.24			
14.98	1.28					
15.95	4.20	1.74	2.24		2.26	nC_{12}
16.53	0.07					
17.13	2.29	0.72	1.06		0.29	
17.94	3.43	5.55	6.71	2.92	6.64	nC_{13}
18.83	6.08		0.06			
19.31	1.90	3.39	3.22	2.52	3.89	
19.84	3.09	9.31	9.56	7.57	9.95	nC_{14}
20.77	0.86	2.37	2.35	2.64	1.84	
21.15	0.62					
21.70	3.82	15.19	14.80	14.89	14.81	nC_{15}
22.61	0.41	0.06	0.06	2.29	0.17	
23.43	1.61	9.23	8.52	8.56	7.95	nC_{16}
24.00	0.58	3.23	3.42	3.99	3.65	
25.03	2.78	15.99	15.19	18.33	15.63	nC_{17} (b)
26.55	2.38	14.17	12.66	15.63	13.37	nC_{18}
27.17	0.04		0.17		1.95	
28.83	1.24	7.01	6.98	8.06	7.24	nC_{19}
30.44	1.12	6.52	6.31	6.49	6.22	nC_{20}
33.45	1.18	6.23	5.90	6.12	5.12	nC_{21}
37.49	0.18		0.36			

* Retention Time (min.)
** Water-soluble
(a) represents 16 major components
(b) + Pristane

Table 5. Column chromatographic analysis of residual oils showing relative percentages of the eluted classes.

Treatment	Saturate fraction	Monoaromatic fraction	Diaromatic fraction	Polyaromatic-polar fraction I	Polyaromatic-polar fraction II
Fresh crude oil	49.56%	16.27%	8.83%	19.55%	5.80%
Crude oil	41.65%	13.73%	8.37%	27.60%	8.66%
Crude oil +*W.S. fertilizers (once)	39.94%	13.69%	7.65%	29.23%	9.50%
Crude oil +*W.S. fertilizers (weekly)	42.04%	15.85%	7.93%	25.59%	8.60%
Crude oil + oleophilic fertilizers	42.56%	14.50%	8.77%	26.11%	8.05%

* water soluble

Table 6. NMR Spectral Analysis

	Hydrogen Distribution Normalized to 100 Total				
Treatment	CH_3	CH_2	Alpha-H	Olefinic	Aromatic
Fresh crude oil	30.1	53.1	9.7	0.0	7.1
Crude oil	24.7	56.3	11.3	0.0	7.7
Crude oil + *W.S. fertilizers (once)	25.9	56.7	10.3	0.0	7.1
Crude oil + *W.S. fertilizers (weekly)	27.0	56.3	9.9	0.0	6.8
Crude oil + oileophilic fertilizers	26.5	54.6	11.0	0.0	7.9

* water soluble

Table 7. Mass Spectral Analysis.

Treatment	Approximate Concentration (%)														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Fresh oil	14.4	11.7	8.3	6.4	4.9	0.3	0.2	8.4	6.2	5.1	8.2	10.2	7.0	6.5	2.0
Crude oil	14.5	11.9	8.2	6.2	4.8	0.3	0.2	8.8	6.0	5.2	8.1	10.5	7.1	6.2	2.0
Crude oil + *W.S. fertilizers (once)	12.8	11.4	8.2	6.3	5.1	0.4	0.2	8.4	6.9	5.4	8.7	10.5	7.0	6.8	2.0
Crude oil + *W.S. fertilizers (weekly)	11.7	10.2	7.7	6.4	6.4	0.5	0.3	7.5	7.6	6.4	8.7	10.4	7.2	6.9	2.1
Crude oil + oleophilic fertilizers	14.8	12.2	8.5	6.5	4.4	0.3	0.2	8.7	6.0	4.9	8.3	10.5	6.9	5.9	2.0

* water soluble

A - Paraffins
B - Monocycloparaffins
C - Dicycloparaffins
D - Tricycloparaffins
E - Tetracylcoparaffins
F - Pentacycloparaffins
G - Hexacycloparaffins
H - Alkylbenzenes/$C_N H_{2N-22}$ cpds.
I - $C_N H_{2N-8}/C_N H_{2N-22}$ aromatics eg. tetralin, pyrene
J - $C_N H_{2N-10}/C_N H_{2N-24}$ aromatics eg. chrysene
K - $C_N H_{2N-12}/C_N H_{2N-26}$ aromatics eg. naphthalene
L - $C_N H_{2N-14}$ aromatics eg. acenaphthene
M - $C_N H_{2N-16}$ aromatics eg. fluorene, dihydroanthracene
N - $C_N H_{2N-18}$ aromatics eg. anthracene, phenanthrene
O - $C_N H_{2N-20}$ aromatics eg. phenylnaphthalene, ovalene

Table 11. Enumeration of microoganisms from sediment and water of gasoline contaminated lake at NARL.

Time	TSA	Gasoline agar	Gasoline agar / TSA	TSA	Gasoline agar	Gasoline agar / TSA
			Lightly Contaminated Area			
8/16	1E3	<1E1	<0.001	1E6	5E3	0.005
8/23	1E2	3E1	0.07	2E4	4E3	0.05
8/31	2E2	<1E1	<0.04	2E4	1E4	0.44
9/7	1E3	2E1	0.02	2E5	3E5	1.88
9/14	1E2	1E2	0.08	2E7	1E6	0.10
9/21	2E2	5E0	0.02	1E5	1E5	1.30
			Moderately Contaminated Area			
8/16	2E4	2E4	0.15	9E5	5E4	0.56
8/23	4E3	4E3	0.04	1E7	4E5	0.35
8/31	6E2	6E2	0.07	7E5	1E5	0.13
9/7	3E1	3E1	0.01	1E6	4E5	0.32
9/14	2E3	2E3	0.21	9E5	3E4	0.03
9/21	3E3	3E3	0.59	4E5	9E4	0.20
			Heavily Contaminated Area			
8/16	7E5	8E5	1.14	1E7	1E7	1.00
8/23	2E7	6E6	0.38	7E6	3E6	0.37
8/31	1E7	1E7	1.29	2E8	8E7	0.45
9/7	2E6	8E5	0.35	1E8	1E7	0.12
9/14	2E6	1E6	0.74	4E7	2E7	0.56
9/21	4E6	6E5	0.13	4E8	5E7	0.14

Table 12. Rates of Oxygen consumption in sediment collected from the gasoline contaminated lake (μl/h/g)

Time	Heavily contaminated	Moderately contaminated	No visible contamination
8/16	11.0	7.5	5.0
8/23	17.0	5.5	4.0
9/7	24.5	6.0	7.5
9/14	46.5	6.5	8.2
9/21	7.4	6.5	6.2

Table 8. Percent Deviation from Movement of Control Organisms

Exposure (wks)	Ratio of oil:water mixture 1:1,000,000	1:1,000	1:10
2	+11.9%	-12.0%	-60.0%
6	+ 4.9%	-	-95.3%
12	-78.9%	-75.0%	-

Table 9. Percent Animals Feeding after One Hour Exposure to Food

Exposure (wks)	Ratio of Oil:Water Mixture Control	1:1,000,000	1:1,000	1:10
4	69%	49%	85%	19%
8	63%	65%	30%	20%
10	58%	75%	29%	0%
12	70%	45%	36%	0%

Table 10

Mean Respiration Rates of Amphipods Exposed to Soluble/Dispersed Oil (μl O_2 consumed/hr/mg dry wt)

Oil Concentration	Exposure (days) 7	15	30	45
0	1.42	1.81	1.98	1.39
1:1,000,000	1.24	1.83	2.31	0.88
1:1,000	1.55	1.53	1.51	1.75
1:10	1.64	1.46	1.69	1.13

Table 13. Weight of gasoline hydrocarbons remaining in heavily contaminated sediment after 3 and 5 weeks.

Exposure time	Treatment	Weight of extractable hydrocarbon	Wt. % loss
3 weeks	Control	0.06	86
3 weeks	+ nutrients	0.03	94
3 weeks	+ nutrients + seeding	0.02	96
5 weeks	Control	0.04	90
5 weeks	+ nutrients	0.02	96
5 weeks	+ nutrients + seeding	0.01	97

Personnel

In addition to the Principal Investigator, Dr. Ronald M. Atlas, major contributions to this project were made by Mr. Amikam Horowitz and Mr. Michael Busdosh. Both Mr. Horowitz and Mr. Busdosh are doctoral candidates at the University of Louisville.

Plans for Future

The flow through system is presently frozen *in situ* with oil and ice in the chambers. The fate of the oil and changes in microbial populations will be followed through spring melt and part of the summer. We will continue the amphipod bioassay system development and are at present examining sublethal effects of sediment bound oil. Some *in situ* testing is planned for May. In May we intend to establish a number of small under ice experimental spills and to follow the fate of the oil for a period of up to two months. It is hoped that prolonged incubation will allow us to detect changes in the oil. We will follow up our work on the NARL gasoline spill. If ONR wishes, we will assist in the addition of fertilizer this spring to contaminated sediment in an effort to remove as much of the remaining hydrocarbons as possible by stimulated microbial degradation.

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