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FATE AND EFFECTS OF OIL POLLUTANTS IN EXTREMELY COLD MARINE ENV--ETC(U)
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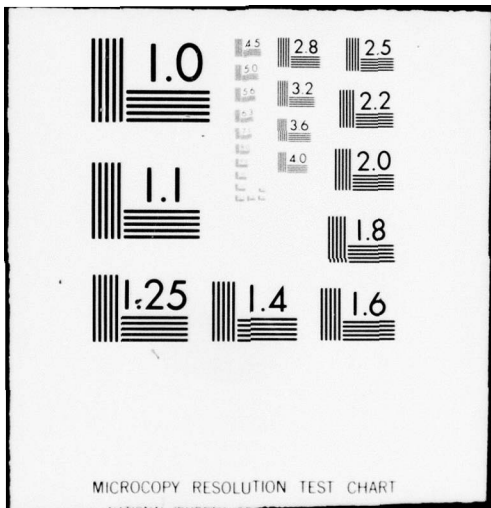
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Fate and Effects of Oil Pollutants in Extremely Cold Marine Environments

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oil biodegradation contamination
pollutants microorganisms degradation
Arctic oil spills hydrocarbons
Alaska petroleum benthos
ice gasoline toxicity

Studies were conducted on the fate and effects of crude and refined oils in Arctic ecosystems. Major conclusions of the study were:

1. Microbial populations respond rapidly to an introduction of hydrocarbons into the environment by an increase of the number of hydrocarbon utilizing bacteria and a decrease in species diversity.

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2. Hydrocarbons will remain in Arctic ecosystems for prolonged periods following contamination. Following initial abiotic weathering, biodegradation occurs slowly. The fate depends on the particular ecosystem that is contaminated. Refined oil spillages may contaminate drinking water supplies for long periods of time;

3. Hydrocarbon biodegradation in the Arctic is limited mainly by nitrogen and phosphorus, and to a lesser extent by low temperatures. Hydrocarbon utilizing microorganisms are widely distributed;

4. When crude oil is exposed on water, biodegradation reduces absolute amounts of petroleum hydrocarbons, but does not appear to alter the relative percentages of oil components. This appears to be a major difference between petroleum biodegradation in the Arctic and in temperate regions: *and*

5. Petroleum contamination of Arctic sediments will result in alterations of the benthic community. Petroleum exhibits differential toxicity to benthic invertebrates. Recovery of impacted areas will begin rapidly following oil spillages but will take a prolonged period for complete recovery. Microbial degradation may reduce levels of hydrocarbons below toxic levels even though complete degradation of the petroleum components is not achieved.

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Final Report

Fate and Effects of Oil Pollutants in
Extremely Cold Marine Environments

by

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July 1, 1979

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Perspective on Fate and Effects of Petroleum Hydrocarbons in the Arctic.

I. Occurrence of Petroleum in the Arctic.

A. Location.

Increasing energy demands have made it necessary to extract petroleum resources from regions previously considered unexploitable. Although the occurrence of petroleum deposits in the Arctic has been known for sometime (Hanna, 1963), development of these resources has only recently begun. The estimated 500 billion barrels of oil and 350 trillion cubic feet of gas to be found in the Arctic (Table I) may perhaps equal the oil reserves of all other areas of the world.

The arctic is that region above 66° N latitude and covers approximately 5% of the earth's surface area. The petroleum industry, however, considers the region above 60° N to be Arctic. This region is characterized by harsh environmental conditions: winter temperatures below -55° C; no sunlight; ice-covered seas (the ice being 2+ meters thick); and frozen, snow-covered tundra. In summer sunlight is constant, the sea ice recedes from the coastline, and temperatures may reach 30° C.

Seven nations have land holdings above the Arctic Circle: the United States, the Union of Soviet Socialist Republics, Canada, Greenland, Finland, Sweden and Norway. Of the countries currently exploring oil and gas deposit potential in the Arctic, the Soviet Union has the greatest oil reserves (Table I). In the Soviet Arctic oilfields have been developed near Nordvik and Dudinka (Kish, 1971), with accumulations of natural gas also developed in locations north

of Dudinka. Oil and gas deposits have also been found (but not yet developed) offshore in the Barents and Kara seas. Additional exploration by Soviet geologists is being conducted in central and western Siberia.

In North America large deposits of oil and natural gas have been discovered north of the Brooks Mountain range with a large land tract in Alaska. This land was originally set aside as Naval Petroleum Reserve #4 but now has been transferred on to the Department of Interior. Gas wells drilled within this area now supply local villages. In this same area test oil wells have been drilled, but no production wells have been developed.

Around Prudhoe Bay the first large commercial Arctic oilfield has been developed by a consortium of American oil companies. The Prudhoe Bay oilfield may contain 10 billion barrels of oil. This oil is transported south through the trans-Alaskan pipeline to Valdez where it is loaded on ships for transport to the west coast of the U.S. The trans-Alaskan pipeline is designed to transport up to 2 million barrels of oil per day.

Oil deposits estimated at 11+ billion barrels are believed to exist offshore in the Beaufort and Chukchi Sea basins (Table I); these outer continental shelf deposits are scheduled for commercial leasing shortly in joint state-federal oil lease sales.

The Canadian Arctic probably has greater oil and gas reserves than the U.S. Arctic; Canadian exploration is centered in the Mackenzie Delta area and the eastern Arctic Islands. The Mackenzie Delta is estimated to have 19 billion barrels of oil and

130 trillion cubic feet of gas, and the Arctic Islands to have 53 billion and 400 trillion respectively (Table I). Most of the explored Mackenzie Delta area has been leased for development. Imperial Oil has found oil at Atkinson Point and adjacent areas. Trans-Canadian gas pipelines are planned from the Mackenzie Delta and Arctic Islands to central Canada. Alternate methods for shipping the oil south are submarine or surface tankers (Dehn and Hughes, 1972). Extensive natural gas deposits have also been discovered in the eastern Canadian Arctic.

Greenland is exploring for oil along its west coast adjacent to the Canadian Arctic Island oil discoveries (Rudkin, 1974). Greenland is also exploring the Greenland Sea for offshore oil deposits. Western European Arctic reserves are estimated at 7 billion barrels of oil and 55 trillion cubic feet of gas. Norway has drilled exploratory wells on its islands in the Barents Sea. It is also believed the Norwegian finds in the North Sea may extend above the Arctic Circle to the Norwegian Sea. Presently, however, Norway is concentrating its oil development activities in the subarctic North Sea.

B. Natural Seepages.

Petroleum contamination of the Arctic is not a new phenomenon. Natural seepages of petroleum occur at several locations in the Arctic (Hanna, 1963). These seepages were the first indications that massive petroleum accumulations were to be found in the Arctic.

Eskimos have been aware of these seepages for sometime but did not utilize the oil and tar seeps as a fuel source until the 1900's when white men explored the Arctic.

Some natural petroleum seepages are quite extensive while others previously reported can no longer be found. At Cape Simpson, Alaska, several major seepages have been extensively explored. Carcasses of animals entrapped in the seeps have been exhumed. Among the skeletons found were several seals. It is not known whether these seal bones were placed in the seep by Eskimos or if the seeps were once sea islands. The Cape Simpson seeps consist of areas of fresh soft oil surrounded by tar-paved soils. Plant cover is absent from the immediate seep areas.

In addition to natural oil seepages there are naturally-occurring gas seepages in the Arctic (Hanna, 1963). Seepages of natural gas have been observed bubbling through tundra ponds, e.g., the Apuk seepage along the Colville River.

These oil and gas seepages provide an opportunity for studying the effects of chronic oil and gas contamination on Arctic terrestrial exosystems. Similar seepages of oil or gas offshore in the Arctic Ocean have not yet been confirmed.

C. Accidental Contamination.

Even though development of petroleum reserves in the Arctic is relatively recent, there have already been many incidents of accidental oil spillages, some being crude oil and others, refined oils used in the petroleum development activities and support of some

of the test wells used in the exploration of U.S. Naval Petroleum Reserve #4 were improperly capped. Thus, at Cape Simpson for example, in addition to the natural seepage areas, small lakes of oil have seeped up around some of the drill sites. Spillages have occurred from ruptured gasoline and diesel fuel storage tanks used in supporting petroleum exploration in the area. Major spillages of crude oil have occurred from ruptures of the trans Alaskan pipeline. Two major gasoline spillages in excess of 80,000 gallons each have occurred at the Naval Arctic Laboratory Research Facility. In the development of the Prudhoe Bay oilfield errors in connecting pipe and hoses have caused spillages. Fuel storage bladders have occasionally ruptured, resulting in sizable localized spillages. Refined oil spillages of unknown magnitude have occurred around construction camps of the trans-Alaskan pipeline. Spillages of oil have also occurred in association with Canadian Arctic oil exploration (Bliss and Peterson, 1973), e.g., there was a large spill of Arctic diesel fuel in Melville Sound as a result of a barge mishap.

Breakage of natural gas lines in the Arctic has also occurred as well as some non-accidental sources of natural gas contamination. For example, the gas supply for Barrow, Alaska, is equipped with a pressure relief system that periodically vents gas over the tundra.

Future oil and gas development in the Arctic will probably result in major contamination incidents. Mackay *et. al.* (1974) estimated that a trans-Canadian pipeline from the Mackenzie Valley would result in two major spillages per year, each in excess of

10,000 barrels. The technology necessary for recovering oil offshore in ice-infested Arctic waters is untried. Novel pipe or ship systems will be necessary for transport of Arctic oil, and these may be subject to catastrophic events. The trans-Alaskan pipeline under construction passes through an area of high seismic activity and, even with an extensive safety shutoff system, 50,000 gallons of oil will be spilled in the event of a pipeline breakage before the break is detected and the oil flow stopped.

It can safely be said that major spillages of crude and refined oil will continue to occur in the Arctic. Some of these spillages threaten human health and safety especially when drinking water supplies are contaminated. All such pollution incidents have detrimental environmental consequences.

II. Physical and Chemical Properties of Oils Found or Used in the Arctic.

A. Crude Oils.

Several crude oils from Arctic and near-Arctic regions have been recovered and subjected to various degrees of analysis. The U.S. Bureau of Mines has performed routine analysis on Prudhoe Bay crude and on other crudes from Naval Petroleum Reserve #4 (Coleman *et al.*, 1973; Gates, 1958; and Robinson, 1964). Although much analytical data from petroleum companies is kept confidential, some information is available (Atlantic-Richfield, personal communication). Partial analyses of northern Canadian crudes have also been published (Cook and Westlake, 1974; and Mackay *et al.*, 1974).

Summaries of some of the available information are shown in tables II through V. The methods used for analysis of the Canadian

crudes differ from those used by the U.S. Bureau of Mines and this variation should be considered in comparing the data.

With that consideration in mind, certain general statements can be made about crude oils found in or near the Arctic. Arctic crude oils are hot (generally greater than 60 C) when pumped to the surface; these crude oils have a low to moderately low sulfur content.

Arctic crude oils, especially those found adjacent to the Beaufort Sea and those found inland, vary greatly. Umiat crude, for example, can be used as a fuel source for diesel engines without refining, while this is certainly not true for other northern crude oils such as Prudhoe Bay crude. Crude oils found in northernmost Alaska and Canada along the Beaufort Sea show greater similarity with each other than with crude oils found nearer the Arctic Circle. Likewise, there is a similarity between oils such as Umiat crude, Alberta crude and Norman Wells crude which are found near the Arctic Circle. Crudes from nearer the Arctic Circle tend to be lighter, i.e., have lower specific gravities, than more northern crudes. They also tend to be less viscous and to have a lower pour point. Oils from near the Arctic Circle have greater percentage of lower boiling components than the more northern crudes. For example, a greater percentage of gasoline and naphtha is found in near-Arctic Circle crudes than in the more northern Arctic crudes while the opposite is true for lubricating distillates.

Fractionation by Cook and Westlake (1974) of several northern crudes into class types did not show major differences between near-Arctic Circle and more northern crudes (Table IV). These class-type

analyses do not however reveal some major compositional differences that do exist between these crude oils. For example, Atkinson Point and Prudhoe Bay crudes show similar percentages of saturate components, but Atkinson Point oil lacks n-alkanes present in Prudhoe crude.

Detailed class compositional data is available for Prudhoe Bay crude (Table X). This detailed analysis showed that the sulfur content of the oil is concentrated in the higher boiling point fractions; that the percentage of paraffins and naphthenes decreases with increased boiling point fractions, and that the percentage of aromatics increases with increased boiling point fractions. This detailed analysis also indicates that Prudhoe Bay crude may have a higher percentage saturates and lower percent aromatics than indicated by the Cook and Westlake analyses.

B. Refined Oils.

Many types of refined oils are used in the Arctic. These include heating oils, such as fuel oil #2, aviation fuels such as JP4 and JP5; and vehicle fuel and oils such as gasoline, Arctic Diesel, various motor oils and lubricants. Large increases in the use of such refined oils in the Arctic has resulted from petroleum development activities and related human expansion into Arctic regions. These oils represent an additional area of concern regarding possible petroleum contamination of the Arctic.

Most of the refined oils used in the Arctic must be shipped from subarctic refineries. The refined oils can be flown in but

only in limited quantities, generally as individual barrels. The bulk of the refined oils are brought in once a year by barge and stored in large tanks. These tanks and the supply lines from them, must withstand the severe Arctic winter and can be easily damaged, resulting in major leakages.

A limited amount of refined oil is produced at Prudhoe Bay, but this refined oil is limited to local use. Refined products from Naval Petroleum Reserve #4 crudes have also been produced and analyzed. The properties of some refined oils used in the Arctic are shown in Table VI. Unlike the crude oils found in the Arctic these refined oils are lighter and less viscous, spreading more readily if spilled and contaminating a larger area than a similar crude oil spill. Many refined oils are clear, and, if they get onto or under ice, they are difficult to detect but will nevertheless contaminate the biota. Many refined oils have toxic additives to prevent microbial contamination. Spills of these oils will present great problems for natural biodegradative removal processes. Refined oils tend to have a higher percentage of volatile components than crude oils. These components, which would volatilize in temperate regions, may be trapped under Arctic ice and would evaporate slowly at Arctic temperatures. For these reasons spillages of refined oils may persist in the Arctic.

III. Physical Fate of Oil Contaminants in the Arctic.

When oil spills, several physical changes immediately begin to occur. These physical changes greatly affect the eventual fate of

the oil and the interaction of the oil contaminants with biological systems. The physical fate of the oil depends on the properties of the oil at the time of the spillage and on the particular environment into which the oil is spilled. Physical changes of importance for oil contaminants include movement of the oil, e.g., flow, spreading, dispersion and emulsification; immobilization of the oil, e.g., absorption, adsorption and sinking; and weathering, e.g., changes in viscosity, density and vapor pressure. Within the Arctic there are several types of environments which may be subject to oil contaminants. These environments include snow, ice, various aquatic environments, such as oceans, lakes and rivers, and various types of tundra soils.

A. Behavior of Oil in Tundra Soils and Snow.

Oil spilled on tundra soils will migrate both laterally and vertically. The surface topography will influence lateral movement of the oil. Flow of oil over the surface will depend largely upon the surface cover, e.g., whether there is plant cover or snow cover; the slope of the land; the presence of channels, e.g., cracks or troughs; the water content of the soil; the temperature; the wind; the viscosity of the oil; the pour point of the oil; the amount of oil spilled; and the ability of the ground to absorb oil (Mackay *et al.*, 1974). Oil will run downhill and into cracks and troughs. Mackay *et al.* (1974) calculated that a spill of 10,000 barrels of Atkinson Point crude occurring over a period of one hour on a slope of 5° with a flow width of 200 feet would form a layer 1.37 cm thick and move at 49

cm/sec. This calculation does not account for vertical movement. Oil will spread less at lower viscosities, which occur at lower temperatures and following chemical weathering. Also, if the ground temperature is below the pour point of the oil, the oil will form pools and spread of the oil will be limited.

Absorption/adsorption effects and vertical movement into the soil will retard the spread of the oil and reduce the area contaminated. Plant cover will absorb spilled oil. The amount of oil that can be absorbed will depend on the dominant plant species. Mackay *et al.* (1974) found that an experimental spill of 160 gallons of Norman Wells crude on moss-covered tundra contaminated a surface area of 50 sq. ft., compared to 1080 sq. ft. on tundra lacking a moss cover. About ten days was required for these spills to reach 80% of total contamination area. From these observations it was calculated that moss-covered tundra absorbed approximately 3 gallons/sq. ft., compared to 0.2 gallon/sq. ft. for the non-moss-covered tundra.

In experiments in the Barrow, Alaska region, K. Everett (unpublished data) found that 10-20 l/sq.m (approximately 0.3-0.6 gallon/sq. ft.) could be absorbed or move vertically into the soil. Except in the case of spills on high-centered polygons, oil in excess of 20 l/sq.m pooled on the surface. These spills were conducted on moss-covered tundra. The mosses at Barrow, however, are different species than those found in the Norman Wells area, which probably accounts for the large differences in absorptive capacity found by Everett and Mackay *et al.*

Everett also found that the vertical flow of oil was highly controlled by the soil horizons and the structural elements of the upper horizons. If there was a vertical crack through the horizons, the oil flowed down the crack to the permafrost. If no cracks were present, the oil moved laterally in the upper structured organic horizon and would not penetrate the silty clay loam mineral soils below, thus restricting oil contamination to the top few inches. If oil moving laterally in this structured layer encountered a crack leading to the surface, it flowed up the crack. Dry soils had more structural elements and oil spilled on these soils showed greater vertical movement. High moisture contents, on the other hand, retarded vertical movement of the oil.

The flow of oil on frozen tundra would be similar to ice and largely involve lateral spreading. The spread of oil on frozen tundra will be highly dependent on surface roughness (Mackay *et al.*, 1974). Oil will spread radially on a smooth surface, but on a rough surface spread of oil will not be uniform.

When the ground is snow-covered, as it is much of the year in the Arctic, the flow of oil will be quite different. Mackay *et al.* (1975) have investigated the behavior of crude oil spilled on snow. They found that when Alberta crude oil was spilled at 0 C, it was readily absorbed by the snow and contaminated an area of about 0.01 sq.m/l of oil. However, hot oil spilled at 60 C melted a channel in the snow and flowed along the ground under the snow, contaminating an area of approximately 0.024 sq.m/liter of oil. Viscosity of the

oil was found to have a marked effect on oil penetration into snow, especially when temperatures were near the pour point: the more viscous the oil, the slower the penetration.

B. Behavior of Oil on Water and Ice.

Great concern has been expressed about the possible interactions of oil and ice in the Arctic (Campbell and Martin, 1973; and Ramseier, 1974). The fear is that the oil will cover a large area and lower the albedo, resulting in large-scale ice melt.

Oil spills may contaminate the ice surface, may become entrapped in the ice matrix or may float under the ice surface (Hoult *et al.*, 1975). The behavior of oil spilled on surface ice is largely dependent on the surface topography of the ice. Glaeser and Vance (1971) found that oil on ice travelled through the upper layer of ice seeking the lowest point, migrating eventually to thaw ponds or leads. They also found that ice could absorb 24% of the oil that they had experimentally spilled. McMinn (1972) reported that oil spreading over ice is dominated by gravity and inertia and is not affected by temperature or by the properties of the oil spilled.

Oil may travel under the ice surface. Hoult *et al.* (1975) found that oil will adhere to the underside of sea ice. They estimated that a spill under sea ice would cover an area only one-fourth that of a spill on the surface of the ice. It was estimated that the spread of oil in the Arctic, whether over or under ice, would be much less than in temperate zones. A spill from a supertanker was predicted to occupy an area of less than one mile.

Whether oil flows over or under ice is determined in part by where the oil is spilled and in part by the density of the oil. Lighter density oils flowing along water that encounter ice will flow over the surface, while heavier oils will tend to flow under the ice (Hoult *et al.*, 1975). Thus spills of northern crude oils are more likely to flow under ice while refined oils such as gasoline and diesel fuel are more likely to flow over the surface of the ice.

As indicated above, oil spilled on ice will migrate to open-water leads. Experiments with Prudhoe Bay crude indicated that a slick in the Arctic would have a thickness between 0.1 and 1.0 cm. Thus, a spill of 2×10^6 barrels, as might occur with a supertanker, could cover an area greater than 24 km^2 .

Glaeser and Vance (1971) reported though that Prudhoe crude oil released on water in the Arctic was significantly affected by wind, showed a negative spreading coefficient, and did not spread out as a film. Little dispersion of oil into the water column was noted. Experimental spills in tundra ponds also have been observed to be piled up by the wind along the shore (R. Vestal, personal communication). From these experimental observations it appears that spills in the Arctic will not cover as extensive an area as they would in temperate regions.

IV. Effects of Oil on Arctic Microorganisms.

A. Protozoa.

Few investigations have examined the interaction of protozoa and petroleum in any ecosystem (Andrews and Floodgate, 1974). Pro-

tozoa have been characterized in tundra soils and the soils were found to contain a mean protozoan biomass of approximately 0.5 g/m² (Cameron *et al.*, 1975). Protozoan ciliates were less abundant than flagellates or amoeboids. Similar species of protozoa have been found in Barrow tundra soils and in tundra ponds at Prudhoe Bay. Ponds characterized as being under medium oil stress from one of the Cape Simpson oil seepages have been found to have high protozoan populations, especially a flagellate, *Monosiga* sp., as compared to unoiled and heavily-oiled ponds (Barsdate *et al.*, 1973).

B. Algae.

Algae play especially important roles in terrestrial and aquatic tundra ecosystems because of their activities in primary production and nitrogen fixation (Alexander and Schell, 1972). Green, blue-green, yellow-green, and diatom algae have been reported to be widely distributed in tundra soils (Cameron *et al.*, 1975) and ponds (Alexander *et al.*, 1972).

Application of Prudhoe crude oil to a small tundra pond resulted in no immediate change in the phytoplankton (Barsdate, 1973). However, one year after oil application productivity of phytoplankton and benthic algae had decreased. Low algal productivity was still observable two years after oil application. A decrease of ATP in tundra soil treated with 12 l/m² Prudhoe crude oil was attributed in part to a postulated decrease in diatoms and green algae by Campbell *et al.*, 1973.

The effect of oil spilled on Alaskan freshwater phytoplankton populations was studied by Miller *et al.*, 1978 in waters affected by natural oil seeps, by controlled crude oil spills in tundra thaw ponds and in a morainal lake, by subpond manipulations and bioassay experiments. The studies were carried out over a period of seven years. Regardless of dose the effects of oil were predictable in the small ponds. The zooplankton populations were virtually eliminated, and after an initial depression of primary productivity the photosynthetic rates returned to approximately prespill levels with a small increase in algal biomass. A markedly altered algal composition was an invariable effect of the response, with the elimination of a dominant flagellated form, *Rhodomonas* spp., in the case of the ponds. From the results of subpond manipulation experiments, evidence supports the hypothesis that elimination of grazers is the principal cause of altered species composition and increased biomass in these ponds. In our lake system there was a severe reduction in primary production during the season of the experimental spill. During the second year only the spring boom was suppressed by the added oil. Bioassay experiments supported the hypothesis that in such lakes, direct inhibition of algal photosynthesis may be important, although zooplankton were greatly reduced.

C. Zooplankton.

Bioassay experiments were conducted by O'Brien (1978) to determine the relative susceptibilities of three arctic zooplankton species to

oil pollution, and the results were compared with the effects of an actual oil spill on a pond near Barrow. In both the bioassays and the pond, the addition of Prudhoe Bay crude oil was toxic to fairy shrimp (*Branchionecta paladosa* O. F. Müller), which seemed most sensitive, *Daphnia middendorffiana* Fischer, which was next most susceptible and *Heterocope septentrionalis* Juday and Muttkowski, which appeared somewhat resistant to the effects of oil. Cyclopoid copepods were the only common zooplankters able to survive the pond oil spill, and these were still present two and one half weeks after the spill. The rapid deaths of the other species, especially the branchiopods, suggest that zooplankton may be the most susceptible of all arctic freshwater organisms to oil pollution.

D. Fungi.

Fungi are important decomposers in tundra soils (Scarsborough and Flanagan, 1973). One year after application of Prudhoe crude oil, 12 l/m², to wet meadow tundra soil at Barrow, numbers of yeasts were higher, but filamentous fungal populations were lower than control plots (Campbell *et al.*, 1973). In experiments at Prudhoe Bay, an increase in fungal biomass was reported following oiling of mechanically-disturbed tundra soil (Scarsborough and Flanagan, 1973). Increased numbers of both yeasts and filamentous fungi accounted for this increased biomass. Species diversity of fungi however declined slightly in the perturbed plots. Some disturbed plots contained fungal species not found in unperturbed areas. In tests with

Canadian crude oils, populations of soil molds showed slight increases that were not significant at the 95% confidence level (Cook and Westlake, 1974) in contaminated soils.

High numbers of fungi have been found in association with the Cape Simpson oil seeps. Numbers of filamentous fungi 0.2 m from the edge of the seep were reported to be three times higher than those 50 m from the seep (Barsdate *et al.*, 1973). Yeast counts were two times higher in the site adjacent to the oil seep than in a site 50 m away. Miller *et al.*, 1978 examined the effects of two Prudhoe Bay crude oil treatments of 5 and 12 l/m² on fungal hyphae/gm dry wt of soil and on the grams of mycelium/m² were followed in polygonal tundra for three seasons. They found a significant depressing effect of oil on fungal hyphae was evident over three seasons. However, no significant difference between oil treatments was recorded. The moisture content of the soil appeared to influence the mobility of the oil. Shifts occur in fungal populations in the presence of oil and the presence of oil biodegradation by filamentous fungi was detected. Sparrow *et al.*, 1978 conducted a study on the short-term effects of seasonal spills of hot Prudhoe Bay crude oil on microorganisms in a taiga soil in interior Alaska. Following a winter spill, the filamentous fungal populations were inhibited whereas the heterotrophic bacterial populations were stimulated. After a summer spill there was an initial depression of both the filamentous fungal and bacterial populations followed by a general enhancement. In both oil spill plots, yeasts; along with the denitrifying, proteolytic, oil-utilizing, and cellulose-utilizing microorganisms; were favorably

affected by the oil. Soil respiration was also enhanced in the oiled plots. They concluded that an extended period of study is required to fully evaluate the impact of oil on the soil microflora and the role of these microorganisms in recovery of oil-inundated areas in sub-arctic ecosystems.

E. Bacteria.

Several studies on the interactions of microorganisms and oil have dealt with bacteria. Many studies have been concerned with oil biodegradation; these will be discussed in a later section. This section will consider the effects of oil on bacteria and on the general metabolic activities of the microbiota of an ecosystem.

As generally reported, experimental application of oil results in increased numbers of bacteria. One year after application of 12 l/m² crude oil to Barrow wet meadow tundra, bacterial numbers in the surface soil increased slightly (0-2 cm) with greater increase in the subsurface soil (2-7 cm) (Campbell *et al.*, 1973). The higher numbers of bacteria were attributed to an increase in numbers of *Pseudomonas* sp. Examination of these plots for two years showed lower ATP content in heavily-oiled soils than in control plot soils. The respiration rates of oil-treated soils were several times higher than control soils. The greatest difference in respiration rates between oiled and control soils occurred one year after oil application. Two years after application respiration rates in the oiled soils were still higher than in control soils but the difference was not as marked. Increased soil respiration of microorganisms in Fairbanks silt loam

soil was also found in response to addition of 15% (by weight) Prudhoe crude (Hunt *et al.*, 1973). However, when ammonium nitrate in excess of 100 ppm was added with the oil, soil respiration declined.

Respiration increased in near Arctic soils that had been exposed to refined oil spills from a pipeline (Hunt *et al.*, 1972). Automotive gasoline (MO gas), jet fuel (JP4) and diesel fuel were all examined. Lower respiration rates were observed with a JP4 spill area, as compared to a diesel fuel spill, probably because of the presence of antimicrobial agents in the JP4.

Studies in the Swan Hills area of north central Alberta, Canada, showed slightly increased bacterial populations 308 and 433 days after treatment with Shell Swan Hills oil at an application rate of 6.5 l/m^2 (Cook and Westlake, 1974). Increases in numbers of bacteria were significantly higher when the plots were also treated with urea-phosphate fertilizer. Similar results were obtained at Norman Wells 321 and 416 days after treatment with 6.5 l/m^2 Norman Wells crude. As with the Swan Hills spill slight increases in bacterial numbers occurred when oil alone was added and significantly higher increases occurred when fertilizer was added in addition to oil.

The chemical composition of different northern crude oils was found to affect the generic composition of microbial populations in soils similar to those in the Mackenzie Delta (Cook and Westlake, 1974). At 4 C major genera of bacteria associated with Prudhoe crude were *Acrhomobacter*, *Alcaligenes*, *Flavobacterium* and *Cytophaga*; with Atkinson Point crude were *Acinetobacter*, *Pseudomonas* and unidentified gram negative cocci; with Norman Wells crude, *Flavobacterium*, *Cytophaga*, *Pseudomonas* and *Xanthomonas*; and with Lost Horse Hill crude

Alcaligenes and *Pseudomonas*. At 30 C the major genera were *Achromobacter*, *Arthrobacter* and *Pseudomonas* with Prudhoe crude; with Atkinson Point crude, *Achromobacter*, *Alcaligenes* and *Xanthomonas*; with Norman Wells crude, *Acinetobacter*, *Arthrobacter*, *Xanthomonas* and other gram negative rods; and with Lost Horse Hill crude, *Achromobacter*, *Acinetobacter* and *Pseudomonas*.

Application of Prudhoe crude oil to soils has been found to alter nitrogen-cycling activities. An increase in *Axotobacter* was reported in Barrow tundra plots that had been oiled and fertilized (Campbell *et al.*, 1973). From this it was hypothesized that oil enhanced rates of nitrogen fixation. Increased denitrification rates were reported in Fairbanks soils that had also been physically perturbed and treated with Prudhoe crude (Lindholm and Norell, 1973). There was an apparent decrease in nitrification rates, conversion of NH_3 to NO_2^- , in these perturbed oiled soils, but this could have been due to the increased removal of NO_2^- by denitrification. Oil application to soils that were not physically perturbed showed no alteration in nitrification rates or denitrification.

Bacterial populations in ponds in contact with the Cape Simpson oil seeps were found to be higher than in unstressed ponds (Barsdate *et al.*, 1973). Bacterial populations in soils adjacent to the asphaltic sections of the seeps were higher than those 50 m from the seep. Elevated bacterial counts have been observed near northern regions that have been exposed to accidental oil spillages (Cook and Westlake, 1974; Hunt *et al.*, 1972).

A sea-curtain enclosed section of a lake 240 km south of Prudhoe Bay, Alaska was exposed to Prudhoe crude oil in July 1976 by Jordan *et al.*, (1978). One year following exposure to the oil, no significant differences were detected between the waters or sediments of the oiled versus control area in rates of turnover of glucose. Total numbers of bacteria were slightly higher in oiled than in control waters. There were no differences in numbers of sediment bacteria.

Rates of uptake of hexadecane and naphthalene by sediment microbes were not linear with time. Hexadecane was taken up sooner and faster than was naphthalene. In some incubations, significantly (88 - 95% probability level) greater rates of hydrocarbon uptake were measured for oiled than for control sediments.

V. Biodegradation of Petroleum in the Arctic.

A. Occurrence of Oil-degrading Microorganisms.

1. In Tundra Soils.

Oil-degrading microorganisms have been found widely distributed in Arctic soils. Cook and Westlake (1974) examined soil samples collected in northern Alberta and the Northwest Territories above the Arctic Circle. All samples contained microorganisms capable of altering the chemical composition of Prudhoe crude oil. The chemical composition of Prudhoe crude oil, however, varied markedly following exposure to microbially active soils collected at different locations. Total heterotrophic microorganisms in these soils capable of growth at 4 and 21 C varied from 10^5 to 10^8 . Numbers of oil-degrading microorganisms were not determined.

Using five different soils collected in the Canadian Arctic, Mackenzie Valley area, Cook and Westlake (1974) found microorganisms capable of chemically modifying Prudhoe crude oil throughout the soil profiles. Numbers of microorganisms decreased with increasing depth, but even at 32 cm there were oil-degrading microorganisms present. The degree of oil modification varied with soils of different depths. With some soils modification of Prudhoe crude oil declined with cores below 15 cm depth.

Sixty fungal isolates, 34 obtained by a static enrichment technique from soils of northern Canadian oil-producing areas and 26 from culture collections, were screened by Davies and Westlake (1979) for their ability to grow on *n*-tetradecane, toluene, naphthalene, and seven crude oils of varying composition. Forty cultures, including 28 soil isolates, were capable of growth on one or more crude oils. The genera most frequently isolated from soils were those producing abundant small conidia. e.g. *Penicillium* and *Verticillium* spp. Oil-degrading strains of *Beauveria bassiana*, *Mortieriella* sp., *Phoma* sp., *Scolecobasidium obovatum*, and *Tolyposcladium inflatum* were also isolated. Qualitative and quantitative differences were noted among the capacities of different crude oils to sustain the growth of individual fungal isolates. The ability to grow on a pure *n*-alkane was not a good indicator of ability to grow on crude oil. Degradation of Rainbow Lake crude oil by individual isolates was demonstrated by gravimetric and gas-chromatographic techniques.

Campbell *et al.* (1973) found that soils in the Barrow, Alaska, area possessed hydrocarbon-degrading microorganisms. The growth of

these microorganisms increased in soils that had been treated with Prudhoe crude oil. A number of psychrotrophic oil-degrading bacteria were isolated from oil-treated soils. Sexstone and Atlas (unpublished data) found that oil-degrading microorganisms in the Barrow soils varied from 10^0 to 10^1 /g soil wet weight. Numbers of hydrocarbon-degrading microorganisms were approximately the same in high- and low-centered polygons.

Oil-degrading microorganisms have been isolated from soils associated with natural oil seepages. Agosti and Agosti (1973) reported evidence for microbial oil degradation with soil near a seep at Umiat, Alaska. ZoBell (1973) reported evidence for an indigenous oil-degrading microbial population in soils that had been collected near the Umiat seepage by Agosti and Agosti. Oil-degrading microorganisms associated with the Cape Simpson oil seeps have also been studied. Agosti (personal communication) found that bacterial isolants from a Cape Simpson seep could oxidize waste oils. Cundell and Traxler (1973) studied 15 isolants from an asphaltic flow near one of the Simpson seeps. The isolants were psychrotrophic and utilized paraffinic, aromatic and asphaltic compounds. The isolants belonged to the genera *Pseudomonas*, *Brevibacterium*, *Sprillum*, *Xanthomonas*, *Alcaligenes* and *Arthrobacter*. Polynuclear aromatic compounds were degraded by many of the isolants. Decalin was reported to be readily degraded by fourteen of the fifteen isolants, this was surprising, considering previous reports on the resistance of decalin to microbial degradation (Pelz and Rehm, 1971).

Sexstone *et al.* 1978a applied Prudhoe Bay crude oil and refined diesel fuel to five topographically distinct tundra soils at Prudhoe Bay, Alaska. The penetration of hydrocarbons into the soil column depended on soil moisture and drainage characteristics. Biodegradation, shown by changes in the pristane to heptadecane and resolvable to total gas chromatographic area ratios, appeared to be greatly restricted in drier tundra soils during one year exposure. Some light hydrocarbons, C₉-C₁₀, were recovered from soils one year after spillages. Hydrocarbons were still present in soils at Fish Creek, Alaska, contaminated by refined oil spillages 28 years earlier, attesting to the persistence of hydrocarbons in North Slope soils.

Oil also was recovered by Sexstone *et al.* (1978b) from tundra soils two and seven years after spillage. Oil persisted in the upper soil layer. The depth of penetration appears to depend on soil moisture and drainage characteristics. Maximal penetration seems to occur within one year of spillage. Biodegradation of the oil was indicated by changes in the ratio of gas chromatographically resolved to unresolved components. Individual components appear to be preferentially degraded, but no evidence was found for significant preferential degradation of structural classes of hydrocarbons. Numbers of microorganisms were different in oil contaminated and reference soils generally showing continued enrichment, but in some soils showing inhibition of microbial populations.

2. In Water.

Although much coastal tundra is covered by ponds, relatively few

studies on the occurrence of hydrocarbon-degrading microorganisms in these ecosystems have been conducted.

ZoBell (1973) reported that a freshwater stream had limited microbial oil-degrading activity, indicating low numbers of oil-degrading microorganisms. Oil-degrading microorganisms have been isolated from the Saganavirtok and Putaligayuk rivers near Prudhoe Bay (Atlas, unpublished data). ZoBell (1973) found microbial oil-degradation activities in samples from the Colville River.

Robertson *et al.* (1973) reported that they were unable to isolate hydrocarbon-oxidizing bacteria in the Colville River delta area of the Arctic Ocean. However, Arthelger and Button (1972) did report hydrocarbon-degrading metabolic activities in these same water samples. The degradation of Prudhoe crude oil was studied in arctic tundra ponds by Bergstein and Vestal 1978. Contained subponds were treated with oil and/or oleophilic phosphate or inorganic phosphate fertilizers in an attempt to enhance the degradation of the oil by the indigenous microflora. Enumeration studies of water and sediment samples indicated that oil treatment alone did not increase numbers of total heterotrophic or oil-degrading bacteria over a short period (28 days). It was also shown that oil spilled years previously on 2 whole ponds at a high (10 l/m^2) and a low dose (0.24 l/m^2) did not alter the microflora quantitatively, except in a small core spilled with oil. Although oil alone seemed to exhibit neither stimulatory nor toxic effects, oleophilic phosphate, added weekly at a concentration of 0.1 mM , significantly stimulated the microflora in the presence or absence of oil. Since equal concentrations of

inorganic phosphate failed to induce this effect, the stimulation was attributed to the hydrocarbon portion of the organic phosphate molecule. ^{14}C -hydrocarbon mineralization studies demonstrated that the microflora would mineralize the saturate fraction of the oil before the polyaromatic fraction. It was concluded that oleophilic fertilizers may provide a useful tool to enhance the biodegradation of crude oil spilled on oligotrophic waters.

3. In Sediment.

Although much of any oil spilled on water eventually may enter the sediment, only a very few Arctic sediment samples have been examined for the presence of oil-degrading microorganisms. ZoBell (1973) did report that sediment from the Colville River and from an unidentified lake possessed oil-degrading microorganisms, but the numbers of oil degraders were not determined.

4. In Ice.

Studies on bacterial populations of ice on the Arctic have not been published. When ice forms, particles, including microorganisms, freeze out. Microorganisms can be found in high numbers in localized regions of ice. In other regions of the ice no viable microorganisms can be enumerated. The highest concentrations of microorganisms associated with Arctic sea ice are found on the underside of the ice (Horner, 1972).

B. Factors Influencing Microbial Oil-degrading Activities.

1. Temperature.

Low temperatures depress rates of enzyme activity. In the Arctic

microbial hydrocarbon-degrading activities are therefore expected to be severely limited. Some have predicted that low temperature will prevent any microbial degradation of petroleum in the Arctic (Campbell and Martin, 1973).

However, some microorganisms are capable of metabolic activities at subzero temperatures (Morita, 1975). Also, as mentioned earlier, summer temperatures in the Arctic can be high enough for even mesophilic microbial metabolic activities.

Cook and Westlake (1974) compared the effects of temperature on the degradation of several crude oils. They found that n-alkanes were completely removed from crude oil at 30 C but were only partially degraded at 4 C. They also found that oil-degrading microorganisms enriched for at 4 C were also capable of oil degradation at 30 C but that microorganisms enriched for at 30 C were not active at 4 C.

ZoBell (1973) found that when various crude oils were incubated with psychrophilic bacteria, an average of 38, 44, and 55% of the oil was degraded at -1.1, 4 and 8 C respectively. At -1.1 C the rate of hydrocarbon degradation was 18 mg/l/day. Hydrocarbon degradation was more rapid at -1.1 C when ice was present than at the same temperature when ice was absent. The solid surface provided by the ice was hypothesized as the stimulating factor for microbial activity.

From the above findings it would appear that oil will be subject to slow biodegradation in aqueous environments even during the winter. It is not known whether oil-degrading microorganisms will be active in frozen soil or ice. It was noted above that oil-degrading microorganisms may be absent from regions of ice. Oil-degrading microorganisms have also been reported to be more sparsely distributed in frozen soils than in unfrozen soils (Cook and Westlake, 1974).

2. Nutrients.

It has been predicted that at low Arctic temperatures oil biodegradation would not be nutrient-limited (Kinney *et al.*, 1969). Work by Kinney *et al.* (1969) showed that oil degradation in Cook Inlet in southern Alaska, where there is extensive water turnover, was not nutrient-limited.

However, it has now been shown that oil biodegradation in many Arctic ecosystems is nutrient-limited. Nitrogen and phosphorus have been determined to be major factors limiting the rate of oil biodegradation in Arctic estuarine, freshwater and soil ecosystems.

In Arctic terrestrial environments, nitrogen and phosphorus also have been found to be limiting factors for oil biodegradation. Cook and Westlake (1974) found that numbers of oil-degrading microorganisms in Arctic soils increased if nitrogen and phosphorus were added to oil-contaminated soils. Addition of urea-phosphate fertilizer was found to increase oil biodegradation in these soils.

C. Changes in Oil Contaminants in the Arctic.

1. Abiotic.

Even at Arctic temperature oil spilled in the Arctic is subject to abiotic weathering. Mackay *et al.* (1974), using Norman Wells crude oil, found that 25% of the oil had been lost from a lake surface within one hour and 45% was lost within six days due to weathering. Similar results were found with oil-impregnated moss. The maximum losses due to abiotic weathering during summer months occurred within six days. At lower temperatures weathering losses were slower. Laboratory experiments showed it took five to 10 times as long to reach

the same degree of weathering at 3 C than it did at 19 C. Mackay *et al.* (1974) predicted that it would take one day to reach 35% weathering at 25 C, four days at 0 C, 10 days at -15 C, and 30 days at -35 C.

2. Biological.

Residual northern crude oils that had been subjected to biodegradation by mixed soil communities have been extensively characterized (Cook and Westlake, 1974; Jobson *et al.*, 1972; and Westlake *et al.*, 1974). Following degradation by a variety of microbial populations enriched from soil samples, Prudhoe crude oil generally had a higher weight percent of soluble asphaltenes, insoluble asphaltenes and soluble nitrogen, sulfur and oxygen components and a lower percent insoluble nitrogen, sulfur and oxygen components, saturates and aromatics. Studies with Norman Wells crude, Lost Horse Hill crude and Atkinson Point crude also showed that biodegraded oil had a lower percentage of saturates and a higher percentage of N, S, O components. There were variations in the percent aromatics and asphaltenes in these oils depending upon the source of enrichment for the degradation; with some enrichments percent aromatics and/or asphaltenes increased while in others, they decreased. Similar compositional changes were observed in oils recovered from field spills as were found in laboratory studies.

Within the saturate fractions of these oils *n*-paraffins were preferentially degraded. Some preference for different chain length paraffins was found, depending upon incubation temperature. Pristane and phytane were utilized at mesophilic temperatures. However, at 4 C these isoprenoid components were only partially utilized or spared

completely with some soil enrichments. Microbial populations, originally enriched for using Atkinson Point crude which lacks pristane and phytane, lacked the ability to degrade isoprenoid compounds at 4 C in Prudhoe crude, Norman Wells crude and Lost Horse Hill crude.

D. Stimulated Petroleum Biodegradation.

Several approaches have been considered for stimulating microbial degradation of oil in the Arctic: the oil may be modified physically, e.g., by addition of emulsifiers; or chemically, e.g., by burning; the environment may be modified, e.g., by fertilization; or the microbial community may be modified, e.g., by seeding.

Emulsification is not a practical approach in the Arctic (McMinn, 1972). Burning has been considered as a means of removing oil pollutants in the Arctic (Glaeser and Vance, 1971). Burning has also been suggested as a method for removing volatile toxic components that are present in some oils and which are inhibitory to microbial oil degradation (Atlas and Bartha, 1973). Crude oils found in the Arctic lack such toxic components. The residue left after burning may be more resistant to microbial degradation than the originally spilled oil because many of the readily biodegradable substrates will have been removed.

The most promising method for stimulated petroleum biodegradation is by fertilization. As already noted, nitrogen and phosphorus are limiting factors for microbial oil degradation. Microbial oil-degrading activities have been found to increase in many Arctic ecosystems if nitrogen and phosphorus are added (Atlas various publica-

tions discussed later, Campbell *et al.*, 1973; Cook and Westlake, 1974). Urea-phosphate fertilizer application to oil-contaminated soils resulted in large increases in numbers of microorganisms and in enhanced modification of oil components (Cook and Westlake, 1974). Fertilization of oil-contaminated Arctic soils was concluded to be a promising method for removal of such pollutants. In Arctic aquatic systems fertilization with an oleophilic nitrogen and phosphorus fertilizer, paraffinized urea and octyl phosphate, was found to greatly increase biodegradative losses from experimental oil slicks.

Seeding with oil-degrading microorganisms to enhance biodegradation has received attention by the popular news media. In most ecosystems examined, however, the naturally-occurring microbial populations capable of oil degradation have been found to be adequate enough to preclude the need for seeding. In the Arctic though, some ecosystems have low numbers of microorganisms and generation times are generally slow because of the low prevalent temperatures. Seeding with effective psychrotrophic or psychrophilic oil-degrading microorganisms may therefore reduce the time course of biodegradation of Arctic oil pollutants. If seeding is ever to be considered for stimulated removal of Arctic oil pollutants, the proper microorganisms for the particular ecosystem will have to be used. Microbial seeding would have to be coupled with addition of appropriate nutrient fertilizer as seeding alone is ineffective.

It does appear that in situations found in the Arctic seeding can stimulate oil biodegradation. Extensive studies on the potential

toxicity or pathogenicity of seed microorganisms will have to be conducted before such seeding would be possible.

VI. Prospects for Cleanup of Oil Pollutants in the Arctic.

The physical removal of oil pollutants in the Arctic will likely be precluded by environmental and logistical limitations. Ship operations in the Arctic Ocean are prevented at most times of the year by the presence of sea ice. Cleanup operations in the winter will have to be performed in darkness. Vehicles though can operate in cleanup operations on snow- and ice-covered tundra during winter. During summer, however, operation of vehicles on tundra would result in the death of plant cover and leave barren areas void of plant cover for decades. Physical cleanup of terrestrial oil spills in summer therefore would likely result in more widespread ecologic damage than the spilled oil.

For these reasons leaving oil spilled in the Arctic to natural degradative processes may result in minimal ecologic damage. Oil so left will persist for long periods of time, but only a limited area will be contaminated. Hunt (1972) predicted that natural recovery from a terrestrial oil spill might take 15 to 20 years, but would result in the least total environmental damage.

It may be possible to shorten the recovery rate by stimulating natural degradative losses. Fertilization with nitrogen and phosphorus in an appropriate form is one method that has been demonstrated to enhance biodegradative losses. Seeding with oil-degrading microorganisms has also been shown to be beneficial when used in certain

situations. All natural degradative processes studied to date still leave residual oil which would have to be incorporated into the soil or sediment organic matter. The levels of hydrocarbons, however, may be reduced below toxic levels by natural biodegradation.

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Discussion of Results of Studies Conducted During This Project

I. Survey of the Coastal Marine Environment for Hydrocarbon Utilizers.

The greatest ecologic impact of Arctic oil spills will probably be in coastal waters. Oil spilled inland may be washed down rivers into coastal waters. Oil spilled offshore may be trapped by sea ice in coastal areas, reaching the coastal sediment or being washed onto the shore. This study showed the presence of oil degrading microorganisms at each of the coastal sites which were examined along the Alaskan coast. Determinations of number of microorganisms in waters along the coast near Point Barrow, Alaska, including Elson Lagoon, Beaufort Sea and Chukchi Sea sites showed that numbers of oil degrading microorganisms and the proportion of the heterotrophic bacterial populations composed of oil degraders were significantly different between summer 1975 and summer 1976. Oil degrading populations were in very high numbers during the ice dominated summer of 1975. Oil degradation processes can thus be expected to be highly variable from one year to the next. Ice conditions appear to be very important, since ice can prevent spreading of hydrocarbons and decreases the area of free water surface, thus, increasing hydrocarbon concentrations in localized areas. There were seasonal differences in the microbial populations within each summer, indicating specific time of oil contamination could be an important factor in degradation rates of oil in Arctic coastal waters. There were differences in numbers of oil degraders and the proportion of the heterotrophic bacterial populations composed of oil degraders between the Chukchi Sea, Beaufort Sea and Elson Lagoon

sites. The most notable differences were found in 1975. This would indicate that oil degradation processes may vary even at coastal sites that are in close proximity to each other, i.e., oil degradation processes may be site specific. Differences in the ratios and numbers of hydrocarbon utilizers may have arisen from differential natural and/or unnatural inputs of hydrocarbons.

Presence of oil degrading bacteria in an area has an important role in case of hydrocarbon spill. Even if oil degrading bacteria occupy only a small part of the total microbial population, they can increase their numbers in a matter of hours or days and participate actively in removal of the hydrocarbons.

II. Continuous Open Flow-through System Studies.

It is impossible to replicate in the laboratory, all the physical, chemical, and biological determinants of any natural ecosystem. Batch cultures have been used to study hydrocarbon biodegradation by many investigators, but these studies are inadequate to predict the fate of oil in a natural ecosystem. Better modeling of oil biodegradation can be achieved using chemostats which permit a constant influx of nutrients and efflux of products.

The flow-through system used in these studies which was incubated *in situ*, is a good model for studying oil biodegradation. It reflects natural changes in environmental factors such as temperature, salinity, light intensity, and variability of natural microbial populations. Such a model is particularly suited for the Arctic where the chambers produce similar wall effect naturally produced by sea ice. The flow

rate of one flushing per day appears to be suitable for this system in this environment. The fluctuations in the bacterial numbers indicated that this rate of flushing did not wash the bacteria out of the system and did not cause a stationary situation in the system.

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. The summer of 1976 was different. Presence of offshore winds pushed the ice away from shore. Presence of ice in 1975 lowered the water temperature and caused a gradual increase of salinity while an abrupt rise in salinity was observed when the ice was pushed away from shore, giving the opportunity of rapid mixing of saline bottom waters with ice melted top waters.

Concentration of nitrogen and phosphorus in the nearshore waters of the Arctic Ocean also varied between the years, showing higher amounts of nutrients during the summer of 1976 in comparison to 1975.

In each case of oil addition (without fertilizers) to sea water, an increase of total number of viable counts of bacteria was observed. The increase is generally rapid, reaches its peak within 2-4 weeks and then declines, usually to the level of the control chambers that did not receive oil. This phenomenon indicates that this environment is primarily carbon limited.

Addition of hydrocarbons changed the composition of the bacterial populations. Oil is selectively toxic to some portions of the microbial population and at the same time enriches other portions that can utilize the hydrocarbons as a carbon source. Changes in the composition of the bacterial populations were found when samples were plated

on agar plates with crude oil as a sole source of carbon, in addition to nutritionally rich plates which yielded what is considered to be "total" viable counts of marine bacteria (Marine agar, Difco 2216). Comparison of the results which were obtained on these two media (obtained by dividing the numbers of oil utilizing bacteria by the "total" numbers) showed shifts in the microbial populations. Hydrocarbons in the environment resulted in a larger ratio. Similar results were obtained by Walker and Colwell (1976c) in temperate areas.

A random selection of 225 colonies from different oil agar plates, showed that 81% could metabolize a simple mixture of hydrocarbons as a sole source of carbon in a liquid medium of Bushnell-Haas broth. It is assumed that a more complex mixture as a crude oil could support an even higher percentage of the population. Since some of the growth did not reach very high levels, and crude oil interferes with the protein measurements, the simpler colorless hydrocarbon mixture had to be used.

Addition of nitrogen and phosphorus containing fertilizers to oil resulted in further increases in the number of colony forming units (CFU). This shows that when oil was added to the natural sea waters, the limiting factor of bacterial growth was shifted from carbon to other nutrients -- in this case nitrogen and phosphorus.

The experiments which were done using the open flow-through system during the first year (1975) served several purposes. Aside from a valuable comparison between two climatically different summers, it helped to simplify the system, and thus provided the possibility and the opportunity to have more growth chambers, enough so each two weeks

a group could be recovered and the rate of degradation was determined as a function of time. The lack of detectable oil in the sediment of the second chamber may be due to a lack of oil reaching the sediment, may be due to the lack of sorption of any oil that did reach the sediment or may be due to biodegradation of oil that reached the sediment.

Based on total amounts of crude oil which were lost by weight when water soluble or oleophilic fertilizers were added, it is concluded that oleophilic fertilizers support more extensive degradation than water soluble fertilizers. Oleophilic fertilized growth chambers did not have higher numbers of viable counts of bacteria than water soluble fertilizer treated ones. Being coated by hydrocarbons or attached to hydrocarbon-like compounds, oleophilic fertilizers remain at the oil water interface and are available mostly to organisms that can degrade this type of molecule. While there was no difference in total numbers of microorganism between chambers treated with water soluble and oleophilic fertilizers there was a difference in the ratios of oil utilizing to "total" heterotrophs. The highest ratios were obtained when oleophilic fertilizers were added. Addition of water soluble fertilizers, however, decreased this ratio in comparison to oil added alone, showing that these fertilizers fail to selectively enrich for hydrocarbon degrading bacteria.

Sinusoidal fluctuations were observed in the population densities. This phenomenon was observed earlier in laboratory studies, when mixed cultures were grown on crude oils in batch culture. Changes in temperature and salinity showed no correlation with these fluctuations. The crude oil or crude oil plus fertilizers were added, the sinusoidal fluctuations of bacterial populations were of greater magnitude than

TABLE I Potential Arctic Petroleum Reserves

	Oil (billion barrels)	Gas (trillion cu.ft.)
U.S.S.R.....	200 ¹ -350 ²	1,600 ¹ -2,000 ²
Western Europe.....	7 ¹ -20 ²	55 ¹ -120 ²
U.S.A.-Alaska.....	40 ¹ -75 ²	300 ¹ -450 ²
Bering Sea.....	27.4 ¹	
Chukchi Sea.....	8.4 ¹	
Beaufort Sea.....	2.7 ¹	
Prudhoe Bay.....	10.0 ⁴	
Canada.....	72 ¹ -105 ²	530 ¹ -630 ²
Baffin Shelf.....	6.0 ¹	
Hudson Bay.....	1.0 ¹	
Mainland Northwest Territory and Yukon (incl. Mackenzie Delta).....	7.0 ¹ -3.8 ³	20 ³
Beaufort Basin.....	12.0 ¹ -8.0 ³	64 ³
Arctic Islands.....	46.0 ¹ -22.5 ³	203 ³
Lowlands.....	7.0 ¹ -6.0 ³	16 ³
Fold Belt.....	8.0 ¹	
Sverdrop Basin....	25.0 ¹	
Arctic Coastal Plain.....	6.0 ¹	
Totals.....Oil:	319 ¹ -530 ²	Gas: 2,485 ¹ -3,200 ²

¹ Rudin, 1974.
² McCaslin, 1972.
³ West, 1974.
⁴ Aleyska Pipeline
Service Co., 1973

those of the control chambers. Microbial predators are present in all aquatic ecosystems and this may account for sinusoidal fluctuations in levels of bacterial populations. Another possible cause of these fluctuations would be competition between microbial populations.

Hydrocarbon biodegradation potential activities were measured with bacterial populations some of which had previously been exposed to the presence of crude oils. Radio-labelled ^{14}C hexadecane or pristane were added to crude oil. Populations exposed to crude oil for 30 days prior to this experiment, showed higher hydrocarbon biodegradation potential activities than populations that had no previous history of exposure to hydrocarbons. Both hexadecane and pristane appeared to be degraded at the same rate.

A comparison of the amounts of oil which were lost in both years, shows a marked difference in the unfertilized growth chambers -- 15% in 1975 versus 24% in 1976 during roughly the same length of time of exposure. The difference was much smaller when oleophilic fertilizers were added -- 33% in 1975 and 36% in 1976.

During much of the year the Arctic Ocean is covered by a thick layer of ice, and oil contaminating from oil well blowouts may contaminate over or under-ice ecosystems, depending on the time of year and the location of such an occurrence. Under-ice experiments showed only changes in composition of exposed crude oil in comparison to fresh oil as was analyzed by gas liquid chromatography. The residual oil from the under-ice experiments showed a gradual disappearance of light hydrocarbons. However, even after three weeks

TABLE II Selected Properties of Some Northern Crude Oils

Crude Oil	Sp.Gr.	API Gravity ^o	Saybolt Universal Viscosity 100 ^o F (sec)	Viscosity Centipoise	Pour Point (oF)	S %	Color
Cape Simpson ¹	0.932	20.3	670	135.0	5	0.44	green
Prudhoe Bay ²	0.893	27.0	84	15.3	15	0.82	brown-black
Prudhoe Bay ³	0.897	28.0	67	--	5	0.92	brown-black
Atkinson Pt. ⁴	---	--	---	20.0	--	--	--
Umiat ⁵	0.841	36.8	37	2.7	5	0.01	Nat.Pet.Assn#4
Norman Wells ⁴	---	--	--	3.8	67	--	--
Alberta ⁴	0.830	--	---	3.3	65	--	--
Fish Creek ⁶	0.973	13.9	2080	--	25	1.92	brown-black

¹Robinson, 1964.

²Coleman et al., 1973.

³Atlantic-Richfield, personal communication.

⁴Mackay et al., 1974.

⁵Gates, 1958.

⁶Robinson and Collins, 1959.

exposure, significant quantities of light hydrocarbons remained in the oil. The ice cover probably limits evaporative losses, and dissolution probably accounts for the loss of light hydrocarbons. If the toxic fraction of crude oil resides in this light fraction, oil spilled under ice will retain toxic properties for prolonged periods.

Failures of buried pipelines would initially contaminate benthic ecosystems. Also, in the nearshore areas, when oil interacts with broken ice, it may migrate from the ice to the sediment. Loss of about 45% of the oil weight was observed after 60 days of exposure of the oil in trays incubated on the bottom of Elson Lagoon. Gas liquid chromatographic analysis showed a decrease in lightweight compounds which may be due to biodegradation and physical disappearance by solubility.

Allowing the oil to freeze into the ice matrix effectively models what would naturally occur to oil floating on the surface during the period of ice formation. In fact, in nearshore shallow regions where oil may concentrate along the beach, ice freezes to the bottom. Degradative losses under these conditions during winter were found to be nil. Obviously, annual rates of oil disappearance are much lower than would be predicted from only summer studies.

Unlike other reports (e.g. Colwell, *et al.*, 1976; Jobson, *et.al.*, 1972), no preference for degradation of any fraction of the oil was observed in these experiments. Examination of data obtained from gas liquid chromatography, dual column chromatography, and mass spectral analyses, showed no difference in the percent composition of the residual oils. It appears that regardless of the total amount of

TABLE III Constituents of Some Northern Crude Oils

Constituent	Prudhoe Bay ¹		Cape Simpson ²		Umiat ³		Fish Creek ⁴		Atkinson Pt. ⁵		Norman Wells ⁵	
	%	Sp.gr.	%	Sp.gr.	%	Sp.gr.	%	Sp.gr.	%	Sp.gr.	%	Sp.gr.
Light gasoline	4.7	0.710	0	---	6.2	0.730	-	---	2.0	---	9.0	---
Total gasoline and naphtha	19.0	0.762	0	---	34.4	0.777	1.9	0.820	19.0	---	34.0	---
Kerosene distillate	4.3	0.818	0	---	6.2	0.822	-	---	4.0	---	4.0	---
Gas oil	18.4	0.860	21.8	0.893	29.3	0.857	16.9	0.875	15.0	---	27.0	---
Nonviscous lubricating distillate												
	11.0	0.911	7.5	0.924	11.5	0.890	7.8	0.921	-	---	-	---
Medium lubricating distillate	8.1	0.922	8.2	0.930	5.6	0.899	10.0	0.935	-	---	-	---
Viscous lubricating distillate	1.8	0.924	14.5	0.939	0.9	0.901	9.8	0.944	-	---	-	---
Residuum	36.3	0.990	47.4	0.951	12.0	0.915	53.8	1.019	-	---	-	---
Distillation loss	1.1	---	0.6	---	0.1	---	0.1	---	-	---	-	---

¹Coleman et al., 1973.

²Robinson, 1964.

³Gates, 1958.

⁴Robinson and Collins, 1959.

⁵Mackay et al., 1974.

degradation, which varied with the treatments, 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 relatively low rates of degradative loss during an Arctic summer may not be entirely due to low temperatures, insufficient concentrations of nitrogen and phosphorus, lack of enzymatic capability of the microbial population on oil composition. Part of this may be explained by the possibility that other nutrients become limiting following the first few weeks of oil exposure. It is also possible that bacterial populations that colonize oil droplets prevent diffusion of nutrients and/or oxygen to the oil, effectively preventing further rapid degradation. Lack of oil spreading could also have contributed to limited surface area available for degradation.

The possibility of artificial seeding of oil spills in aquatic environments to stimulate hydrocarbon biodegradation has been frequently suggested. Questions have been raised concerning the need for seeding, and the possible toxicity or pathogenicity that seeding could cause. Seeding would be useful only in ecosystems with inadequate hydrocarbon degrading bacteria and where physical recovery of oil was not possible. Arctic aquatic ecosystems may meet these criteria for establishing the need for bacterial seeding of oil spills.

Due to the complexity of crude oils, no single organism naturally contains the genetic information for utilizing all the hydrocarbons found in oil. It is therefore necessary to utilize the genetic information from several bacteria to extensively degrade a crude oil. Nat-

TABLE IV Class Composition of Some Northern Crude Oils¹

Class Type	Atkinson Point	Prudhoe Bay	Norman Wells	Pan- Arctic	Kuparuk	Lost Horse Hill
Asphaltene -soluble	6.32	5.68	3.26	2.16	15.50	8.56
Asphaltene -insoluble	7.23	1.57	4.89	2.98	8.95	5.31
Saturate	31.80	38.70	49.50	65.40	27.80	30.50
Aromatic	37.40	36.40	30.00	21.60	33.10	39.20
NSO - soluble	15.90	12.80	8.40	5.17	11.40	12.00
NSO - insoluble	2.33	5.94	5.91	3.65	4.30	5.49

¹Cook and Westlake, 1974

urally occurring microbial communities that respond to the presence of contaminating hydrocarbons normally have more than one type of hydrocarbon utilizing microorganisms.

For seeding oil slicks a mixture of hydrocarbon utilizing microorganisms or a genetically engineered microorganism should be considered. In the present study the mixed culture approach was used. We did not exhaustively search for hydrocarbon degrading microorganisms and make no claims that the bacterial strains used in this study were superior hydrocarbon degrading bacteria. The two strains used in this study are naturally found in Arctic aquatic ecosystems, are capable of degrading a wide range of hydrocarbons. The latter two properties can be considered essential features for effective seed microorganisms for Arctic ecosystems.

In open water tests, an uncoated bacterial inoculum was washed rapidly from the oil and did not enhance degradation. The use of octylphosphate as a fertilizer lowered the pH in early summer tests apparently killing the seed bacteria. Rendering the bacteria oleophilic by coating with octadecane and delayed seeding solved these problems. The octadecane coated seed mixture visibly floated with the oil. Coating with octadecane at 32⁰ C following lyophilization did not kill even these psychrotrophic bacterial strains. The experiments with oleophilic fertilizers and oleophilic seed bacteria demonstrated that biodegradation of oil could be stimulated in Arctic ecosystems. Much work remains to determine optimal bacterial seed cultures, optimal fertilizers, optimal modes of application, ways

TABLE V Detailed Analysis of Prudhoe Crude Oil¹

Fraction No.	°C	°F	Vol. % of Crude	Aromatics %	Naphtrenes %	Paraffins %	PN % (saturate)	Sulfur %	Nitrogen %
1	50	122	--	--	--	--	--	--	--
2	75	167	2.1	3.4	23.1	73.5	96.6	0.10	--
3	100	212	2.6	11.3	22.3	66.3	88.7	0.01	--
4	125	257	3.5	14.0	38.5	47.5	86.0	0.01	--
5	150	302	3.6	19.6	39.1	41.3	80.4	0.01	--
6	175	347	3.7	23.0	36.3	40.8	77.0	0.02	--
7	200	392	3.5	25.0	29.4	45.6	75.0	0.03	--
8	225	437	4.3	27.1	--	--	72.9	0.07	--
9	250	482	4.8	26.9	--	--	73.1	0.13	--
10	275	527	5.0	26.6	--	--	73.4	0.23	--
11	308	587	2.8	27.6	--	--	72.4	0.27	--
12	336	637	6.5	32.9	--	--	67.1	0.54	--
13	364	687	6.8	--	--	--	--	0.78	--
14	392	737	6.0	--	--	--	--	0.97	--
15	420	787	7.4	--	--	--	--	1.06	--
Residue	--	--	36.3	--	--	--	--	1.36	0.468
Crude Oil	--	--	--	--	--	--	--	0.82	0.230

¹Coleman *et al.*, 1973.

of avoiding undesirable toxic side effects, and under what conditions seeding should be considered for treating oil spillages.

III. Toxicity to Invertebrates.

The toxicology of Prudhoe crude oil to the arctic amphipod crustaceans *Boeckosimus* (= *Onisimus*) *affinis* Hansen and *Gammarus zaddachi* Sexton was examined. Laboratory experiments and in situ experiments were set up to model the effects of oil slicks, water containing dissolved oil, and sediment polluted with crude oil.

Amphipods were exposed directly to the surface slicks of whole crude oil, of the paraffinic, aromatic, or asphaltic fractions of Prudhoe crude oil, and of Arctic diesel. Other amphipods were allowed close proximity to each of the slicks, but direct contact was prevented by a nylon mesh screen. *B. affinis* and *G. zaddachi* suffered total mortality by the tenth day regardless of whether or not they were prevented from contacting the slick of the whole crude oil. Respiration rates dropped to unmeasurable levels after seven days of exposure.

G. zaddachi was also tested for toxicity of whole crude oil slicks in situ in Nuwuk Lake. At the end of the 23-day experiment 93% of the animals were alive. Only animals that had become mired in oil suffered mortality.

Access to the paraffinic fraction slick caused total mortality to *B. affinis* after 4 days. Shielding from the slick prolonged the LT_{100} to 12 days. Animals exposed to the paraffinic fraction became sluggish and lost coordination. Respiration rates in animals directly exposed to the slick dropped below measurable levels after seven days.

TABLE VI Analysis of Refined Oils Used in the Arctic

Oil	Gravity °API	Sp. Gr.	% Wt. S	Viscosity			Pour Pt.	Freeze Pt.	% Volume	
				Sayboul 100F	Kinematic 100F	Kinematic -30F			Olefin	Aromatic
Diesel Fuel from Fish Creek Crude ¹	25.4	0.902	1.17	67.1	--	--	-50	--	14.6	26.6
Lubricating Distillate from Fish Creek Crude ¹	19.8	0.935	1.57	787.0	--	--	--	--	--	--
JP4 ²	54.3	--	0.035	--	--	2.68	--	-81	0.9	10.6
JP5 ²	41.6	--	0.065	--	--	10.5	--	-58	1.0	16.0
Diesel Fuel for Trucks and Tractors Grade 1D ³	39.5	--	0.16	32.9	2.0	--	-15	--	--	--
Diesel Fuel for Trucks and Tractors Grade 2D ³	44.8	--	0.001	--	1.3	--	-60	--	--	--
No.2 Fuel Oil ⁴	40.1	--	0.005	--	2.0	--	-15	--	--	--
Gasoline, Regular ⁵	61.8	--	0.010	--	--	--	--	--	--	--
Arctic-grade Diesel Fuel ⁶	--	--	-0.15	--	1.4- 1.5	--	-60	-55	--	--
Two-cycle Oil ⁷	--	0.880	0.3- 0.6	--	--	--	-25	--	--	8.0- 12.0

¹ Robinson and Collins, 1959.

⁵ Shelton, 1975b.

² Shelton, 1974a.

⁶ Federal Specifications

³ Shelton, 1974b.

Document VVP-800, 1968.

⁴ Shelton, 1975a.

⁷ Exxon Aquaglide DG-2D

Data Sheet, 1973.

A 61% decrease was experienced by animals shielded from the slick.

Both direct and shielded exposures to the aromatic fraction slicks reduced survival rates to 20% after at the end of the 20-day experiment, but did not cause total mortality. The exposed amphipods exhibited uncoordinated, erratic movements. Respiration rates were decreased by 65% after one week of exposure.

Neither direct nor shielded exposures to the asphaltic fraction of crude oil caused mortality in *B. affinis*. Respiration rates decreased 20%-50% after one week of exposure.

Direct exposure to Arctic diesel slicks caused total mortality of *B. affinis* within two days; shielding the animals extended this time to 15 days. Animals developed coordination problems similar to those of animals exposed to the aromatic fraction. Respiration rates dropped to below measurable levels after 7 days.

Six mixtures of WSF portion of aromatic fraction were prepared for test exposures by mixing crude oil and sea water. Proportions were 1 part oil:10 parts water, 1:100, 1:1000, 1:10,000, 1:100,000, 1:1,000,000; water containing the WSF components was siphoned from beneath the slick. *B. affinis* was exposed to these mixtures for 16 weeks. Three-day and ten-day exposures were conducted using 1:10, 1:1000, and 1:1,000,000 mixtures; after exposure, amphipods were moved to clean seawater. In the first 6-weeks, amphipods continuously exposed to WSF suffered mortality rates positively correlated with oil amount in mixture. In 1:10 mixture, total mortality was reached by 12th week. In remaining 5 mixtures, 75%-85% mortality was seen by 16th week; survival rates were about 1/3 of those in controls. In 3-day and 10-day exposures, 15% and

20% mortality was observed in 1:10 and 1:1000 mixtures; only the strongest concentration caused significant mortality after 2 weeks recovery.

After 2-weeks constant exposure, food search success was reduced 50% in the 3 most dilute WSF mixtures. Search success increased to 90% of control rate after this time. Only amphipods in the strongest oil mixture fed at a greatly reduced rate. In 3-day and 10-day exposures, feeding success rate reduction was inversely proportional to oil strength of WSF mixture. Full recovery occurred after 2 weeks with light and medium mixtures; with heavy oil mixture, 70% recovery was observed. Respiration rates of animals exposed to WSF mixtures exhibited no discernible trends.

B. affinis was exposed to sediment in which all of the surface sediment was clean, 50% was contaminated, or 100% was contaminated. Fresh crude oil and weathered crude oil were the two contaminants used. Survival rate of animals was reduced by 11% in the animals exposed to fresh crude oil, and by 3% in the amphipods exposed to weathered crude oil, compared to the control rate, at the end of 14 weeks.

Animals exposed to fresh oil in sediment for 2 weeks showed a feeding rate reduction of two-thirds. Exposure to weathered oil reduced the rate by one-third after 2 weeks. After 6 weeks all exposed animals fed at at least 85% of the control rate. Rates were lower in the fully contaminated sediment than in the partially contaminated sediment for both pollutants.

Initially one-half of the animals exposed to sediments containing fresh oil did not burrow. With time, increasing numbers of animals did burrow. If given a choice between clean or fresh-oiled sediments, animals preferred clean sediment.

If given a choice between clean sediment or sediment containing weathered oil, *B. affinis* preferred clean sediments in the first weeks of study, with a gradual decline in preference to approximately equal levels after 14 weeks. Failure to burrow would expose animals to predation in a natural situation.

Contaminated sediment significantly reduced the distance the amphipods moved with both oil contaminants at both concentrations. Animals also moved significantly less of the time. Effects of weathered oil on movement were constant throughout the experiment, but fresh oil caused a decrease in movement with the interaction of pollutant and time exposed.

Plexiglas trays were filled with clean or oiled sediment and placed on the bottom of Elson Lagoon. Trays were recovered after 1, 2, 3, 4, 8, and 30 weeks. Animals were removed, identified, and counted.

The amphipods overwhelmingly preferred clean sediment to oiled sediment throughout the study. The two species of Isopoda collected did not discriminate between the oiled and clean trays. Both of these groups' members are highly mobile, and moved in and out of the trays in minutes.

An unidentified anemone species was recovered from both oiled and control trays throughout the experiment. The animal's distribution showed no substrate preference at any time during the 30-week experiment.

Of the Polychaeta recovered oil only a *Nephytes* sp. preferred oiled sediment to clean sediment. Although *Capitella capitata* has been reported as being abundant in oil contaminated sediment, it was found only in the clean sediment in the present study. The other polychaete species taken preferred clean sediment.

No distinct substrate preference was exhibited by either gastropod or bivalve Mollusca in the in situ recolonization study.

IV. Effects of Air Pollutants and Hydrocarbons on Arctic Lichens.

Although Arctic ecosystems were generally found to be free of atmospheric and fossil fuel-related pollutants, localized areas are subject to contamination. Examples include petroleum pollution around spillages at Prudhoe Bay, atmospheric pollution by natural gas around the Barrow gas well, and SO₂ pollution around areas of fossil fuel combustions. The present study showed that exposure to such pollutants could inhibit essential metabolic activities of lichens and of an alga in Arctic Alaska. Natural gas in concentrations up to 10 ppm was generally not inhibitory to the test lichens or algae, but exposure of these organisms to SO₂ or Prudhoe crude oil at concentrations as low as 0.01 ppm SO₂ or 0.1 ml oil did result in inhibition of metabolic functions.

Although dark fixation of CO₂ occurs in all organisms, CO₂ fixation in the light was at least two orders of magnitude higher than dark CO₂ fixation for the lichens and algae used in this study. CO₂ fixation by the test organisms measured in the light may be considered as a measure of algal photosynthetic activity. Both exposure to SO₂ and crude

oil appear to inhibit photosynthetic activity. The inhibition of light fixation of CO_2 by crude oil may be due to the opacity of the oil which prevents penetration of light to the alga.

Greater inhibition of CO_2 assimilation occurred with the free alga than with the alga in symbiotic lichen association. Interestingly, periodic exposure to SO_2 may be cumulative, as shown by the identical inhibition of CO_2 fixation by lichens chronically exposed to SO_2 and lichens exposed for only one day per week for seven weeks.

Oxygen consumption in the dark may be used as a measure of respiration. In lichens respiration is largely due to fungal metabolism. The initial increase in the rate of oxygen consumption following exposure to SO_2 or oil may reflect increased fungal metabolic activity associated with oxidation of the pollutant or utilization of degraded algal components. The change in rates of oxygen consumption may also represent a shift in metabolic pathways or in changes or residual light algal O_2 production. The return to control levels of O_2 consumption by *C. nivalis* suggests that SO_2 did not inhibit fungal respiration. Similarly, no evidence for inhibition of fungal respiration was observed with *P. aphthosa* exposed to either SO_2 , natural gas, or Prudhoe crude oil.

Inhibition of nitrogen fixation in *Nostoc* and *Peltigera* that was observed following prolonged exposure to 10 ppm SO_2 may have been a secondary effect resulting from the inhibition of photosynthetic activity, e.g. from reduced levels of NADPH. The effects of crude oils on nitrogen fixation were more extensive and appeared sooner. The only inhibitory effects shown by natural gas on lichen or algal metabolism

was shown as a moderate decline in the rate of N_2 fixation after prolonged exposure to 10 ppm gas. It is possible that some hydrocarbons found in crude oil and natural gas directly inhibit nitrogen fixation. Further work would be needed to distinguish direct effects of pollutants on nitrogen fixation from their secondary effects.

V. Gasoline Contamination of a Freshwater Lake.

Microbial populations in an Arctic fresh-water ecosystem were found to respond rapidly to the presence of contaminating MOGAS. The number of viable bacteria increased significantly from lightly to moderately to highly contaminated areas. The results show that not only the presence but also the concentration of contaminating MOGAS affected the number of bacteria. There was a rapid population shift to high numbers of gasoline hydrocarbon-utilizing and leaded MOGAS-tolerant in contaminated areas. The ratio of the bacterial colonies (CFU) on hydrocarbon plates to the CFU on a rich carbon source plates appears to be a useful, sensitive indicator of environmental hydrocarbon concentrations for accidental spillages.

Use of these ratios of hydrocarbon degrading bacteria to total heterotrophic bacterial populations should be restricted to sediment, since the water phase may be affected by wind causing the concentrations of hydrocarbons (which would mainly be floating on the surface) to change rapidly. The sediment, on the other hand, is more stable and will retain hydrocarbons for longer periods of time. This was the reason why water samples were not examined one year after the spill occurred. Upon detection of the spill, the lightly contaminated

area (LC), which seemed not to contain any hydrocarbons in the first two weeks, started to show an increase in the ratios of GA/TSA and BA-G/TSA. In other words, the proportions of lead tolerant and gasoline utilizing bacteria increased within the population. A sharp increase in this ratio indicates an introduction of hydrocarbons into an area which initially did not have hydrocarbons in it.

The observation of higher numbers of microorganisms enumerated on MOGAS vapors than on media containing emulsified MOGAS possibly reflects the toxicity of lead, and possibly other compounds in MOGAS, to hydrocarbon-utilizing microorganisms. In the highly contaminated (HC) area, the difference was less between counts on emulsified MOGAS and MOGAS vapors than in other areas, suggesting that the microorganisms proliferating in this area had undergone selection for tolerance to MOGAS components as well as for ability to utilize hydrocarbons.

Microorganisms in the contaminated lake were found to be capable of degrading hydrocarbons in MOGAS. There have been relatively few reports on the ability of microorganisms to degrade gasoline (Jamison, et al., 1975; 1976). There have been, however, a number of accidental gasoline and other refined oil spillages into Arctic ecosystems associated with construction of the trans-Alaskan pipeline as well as similar spillages in more temperate ecosystems.

The *in situ* biodegradation experiments showed that abiotic weathering and biodegradation rapidly resulted in extensive losses from the spilled MOGAS. When the lake began to freeze for the winter though, 10% of the extractable material still remained in the untreated sediment. Nutrient addition effectively increased losses, and inocula-

tion, together with nutrient supplementation further enhanced degradative losses. Enhanced biodegradation resulted in only 3% extractable material left when the lake began to freeze; the residue had a very different compound distribution than MOGAS prior to extensive biodegradation. The residue after extensive biodegradation had a predominance of higher-retention-time compounds that may have been synthesized during biodegradation. The appearance of such higher-retention-time compounds has previously been reported in laboratory experiments; (Prichard, et al., 1976; Walker and Colwell, 1976d). The persistence and potential toxicity of these compounds is unknown.

One year after the spill occurred, gasoline hydrocarbons had spread from the initial point of contamination. Gasoline hydrocarbons still persisted in the sediment of the lake one year after spillage. Gasoline components persisted in the sediment near the spill site even 2 years after the spill. Hydrocarbon analyses of the drinking water intake have shown the presence of excessive concentrations of hydrocarbons. An activated charcoal filter has been installed to remove hydrocarbons from the drinking water. The persistence and spread of hydrocarbons in the sediment were directly measured by gas chromatography. It appears that transport of gasoline across the lake is not directly through the sediment but rather gasoline moves across the water surface and is concentrated in the shoreline sediment. Fertilizer application did appear to increase *in situ* degradation of gasoline hydrocarbons. Five weeks after fertilizer application hydrocarbon concentrations in the fertilizer treated area were 10% of the concentrations in the reference site. Further degradation may have been

limited by oxygen concentrations. The presence of gasoline resulted in major taxonomic shifts within the microbial community. There was a great decrease in microbial diversity. The dominant microbial populations present in the contaminated area following the spillage were gasoline hydrocarbon utilizing bacteria.

The problem of persistence from refined oil spillages in the Arctic is a serious health concern. Toxic light aromatic hydrocarbons such as toluene and naphthalene do not evaporate when sorped onto sediment particles. Even light fuels which would rapidly evaporate in temperate regions can persist for long periods of time in Arctic ecosystems. Microbial degradation of hydrocarbons is an active process in Arctic ecosystems and can be stimulated by nutrient addition but rates of biodegradation and number of months during the year when biodegradation occurs results in only slow removal of contaminated hydrocarbons from ecosystems such as the one studied.

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GENERAL CONCLUSIONS

1. Microbial populations respond rapidly to an introduction of hydrocarbons into the environment by an increase of the number of hydrocarbon utilizing bacteria and a decrease in species diversity.
2. Hydrocarbons will remain in Arctic ecosystems for prolonged periods following contamination. Following initial abiotic weathering, biodegradation occurs slowly. The fate depends on the particular ecosystem that is contaminated. Refined oil spillages may contaminate drinking water supplies for long periods of time.
3. Hydrocarbon biodegradation in the Arctic is limited mainly by nitrogen and phosphorus, and to a lesser extent by low temperatures. Hydrocarbon utilizing microorganisms are widely distributed.
4. When crude oil is exposed on water, biodegradation reduces absolute amounts of petroleum hydrocarbons, but does not appear to alter the relative percentages of oil components. This appears to be a major difference between petroleum biodegradation in the Arctic and in temperate regions.
5. Petroleum contamination of Arctic sediments will result in alterations of the benthic community. Petroleum exhibits differential toxicity to benthic invertebrates. Recovery of impacted areas will begin rapidly following oil spillages but will take a prolonged period for complete recovery. Microbial degradation may reduce levels of hydrocarbons below toxic levels even though complete degradation of the petroleum components is not achieved.

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