Effects of Hydrocarbons on Microorganisms and Petroleum Biodegradation in Arctic Ecosystems

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Although the occurrence of petroleum deposits in the Arctic has been known for sometime (Hanna, 1963), development of Arctic oil and gas resources did not begin until" the 1970s. The estimated 500 billion barrels of oil and 350 trillion cubic feet of gas to be found in the Arctic may equal the oil reserves of all other areas of the world. Seven nations have land holdings above the Arctic Circle: the United States, the Union of Soviet Socialist Republics, Canada, Greenland, Finland, Sweden and Norway. Approximately 5% of the earth's surface area lies in the Arctic. This region is characterized by severe environmental conditions. In winter temperatures fall below -55°C; there is no sunlight; the seas are ice-covered (the ice being 2+ meters thick); and the tundra is frozen and snow-covered . In summer sunlight is constant; the sea ice recedes from the coastline; and temperatures may reach 30°C. Within these harsh Arctic ecosystems microorganisms play essential roles in energy transfers and nutrient cycling reactions that form the foundations for the existence of higher organisms. Important processes mediated by microorganisms include photosynthetic capture of energy, phytoplankton and detrital food web energy transferee, decomposition of complex polymers, nitrogen fixation and other nitrogen-cyling reactions, and other activities necessary for ecological balance (Atlas and Griffiths, 1984). These critical activities may be impacted by Arctic oil and gas development.

In the Soviet Union, Arctic oilfields have been developed near Nordvik and Dudinka (Kish, 1971). 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 over 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. Test oil wells have been drilled onshore in this area. Around Prudhoe Bay, Alaska the first large commercial Arctic oilfield has been developed. 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 onto ships for transport to the west coast of the U.S. The trans-Alaskan pipeline is designed to transport up to two million barrels of oil per day. The next major phase of Arctic oil and gas development undoubtedly will occur offshore. Oil deposits estimated at 11+ billion barrels are believed to exist offshore in the Beaufort and Chukchi Sea basins; these outer continental shelf deposits are being leased through joint state-federal oil lease sales. Several artificial islands have been constructed to support oil-drilling operations and test wells have been drilled.

The Canadian Arctic probably has even 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. 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.

Greenland is exploring for oil along its western 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. The areas of petroleum development delimit the most likely sites of contamination events. Due to the extensive areas of potential development, coastal marine, freshwater bodies and tundra soils are at risk of hydrocarbon contamination.

Although the development of Arctic oil resources raises the potential for serious environmental hydrocarbon contamination in these fragile-harsh ecosystems, 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. Examination of these natural seeps gives an indicatin of the likely impact of oil spills as well as the possible fate of spilled oil.

Additionally, even though development of petroleum reserves in the Arctic is relatively recent, there have already been several accidental oil spillages; some were crude oil spills and others were of refined oils used in petroleum development activities and support facilities of some of the test wells used in the exploration operation. 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 because the wells were inadequately capped. Spillages have, occurred from ruptured

gasoline and diesel fuel storage tanks that are needed to support petroleum exploration in the area. Sizeable spillages of crude oil have occurred from ruptures of the trans-Alaskan pipeline. Major gasoline spillages in excess of 80,000 gallons each have occurred at Barrow, Alaska, near the former Arctic Research Laboratory. In the development of the Prudhoe Bay oilfield errors in connecting pipes and hose lines have caused spillages. Fuel storage bladders have occasionally ruptured, resulting in sizable localized spillages. Refined oil spillages of unknown magnitudes 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). For example, there was a large spill of Arctic diesel fuel in Melville Sound as a result of a barge mishap.

Future oil and gas development in the Arctic will almost undoubtedly result in additional major contamination incidents. Mackay et al. (1974) have 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 trans-Alaskan pipeline passes through an area of high seismic activity and, even with an extensive safety shutoff system, 50,000 gallons of oil could be spilled in the event of a pipeline breakage before the break is detected and the oil flow stopped. The technology to be used for recovering oil offshore in ice-infested Arctic waters is untried and systems for transport of Arctic oil may be subject to catastrophic events.

When oil spills occur, several physical changes, which immediately begin to occur, greatly affect the eventual fate of the oil and the interaction of the oil contaminants with the local biological systems.

The physico-chemical environment greatly influences the nature of interactions of microorganisms with the contaminating petroleum hydrocarbons. 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. The microbial communities in these different ecosystems vary both in term of species composition and abundance. The effects of petroleum hydrocarbons will vary depending upon which ecosystem is contaminated, the extent of contamination, and the prevailing meteorological conditions (Pfaender and Buckley, 1984; Vestal et al., 1984). As in temperate systems microorganisms will play a key role in mediating the impact of Arctic oil spills. The hydrocarbon biodegradative capacity of microorganisms in large part establishes the duration of the impact. Hydrocarbons are substrates for various microbial populations and microorganisms have the enzymatic capacities to degrade the classes of hydrocarbons found in crude and refined oils (Cerniglia, 1984; Foster, 1962; Gibson, 1971; McKenna and Kalio, 1965; Perry, 1977, 1979, 1984; Pirnik, 1977; Ratledge, 1978; Singer and Finnerty, 1984; Van der Linden and Thijsse, 1965). The role of microorganisms in mitigating the environmental impact of petroleum spillages through biodegradative

removal of the contaminating hydrocarbons has been the subject of several reviews (Atlas, 1978, 1981; 1984a; Colwell and Walker, 1977; Jordan and Payne, 1980; Karrick, 1977; National Academy of Sciences, 1975; Van der Linden, 1978; Zobell, 1964). Recent reviews have considered the fate of petroleum pollutants in soil (Bossert and Bartha, 1984), freshwater (Cooney, 1984), and marine (Floodgate, 1984) ecosystems. The current review will focus specifically on the impact of Arctic oil spills upon microorganisms and the hydrocarbon biodegradative capacities of microorganisms in Arctic terrestrial and aquatic habitats.

EFFECTS OF HYDROCARBONS ON MICROBIAL POPULATION LEVELS

One aspect of concern about the potential introduction of hydrocarbons into Arctic, ecosystems is the relative abundances of hydrocarbon-degrading microorganisms and the ability of these metabolically specialized microbial populations to respond to the presence of hydrocarbon pollutants. The presence of hydrocarbon-degrading microorganisms is essential for biodegradative removal, which is the main natural mechanism for the decontamination of oil polluted environments. Normally, the introduction of hydrocarbons results in a significant increase in the numbers of hydrocarbon-degrading microorganisms. Such an increase serves both as an index of the extent of hydrocarbon impact and as a signal of the onset of hydrocarbon biodegradation (Atlas, 1981). In general, population levels of hydrocarbon utilizers and their proportions within the microbial community appear to be sensitive indices of environmental exposure to hydrocarbons. In unpolluted ecosystems, hydrocarbon utilizers generally constitute less than 0.1% of the microbial

community; in oil-polluted ecosystems, they can constitute up to 100% of the viable microorganisms.

Arctic Marine Ecosystems

Hydrocarbon utilizers have been found to be widely distributed in cold marine ecosystems (Atlas, 1978; Cundell and Traxler, 1973, 1976; Robertson et al., 1973a, 1973b; Tagger et al., 1976; Walker and Colwell, 1976). Bunch and Harland (1976), for example, found that the numbers of hydrocarbon utilizers occurring 'in regions of the Canadian Beaufort Sea were comparable to numbers of hydrocarbon degraders in more temperate marine samples from subarctic Canadian waters; their results substantiate the presence of hydrocarbon-degrading microorganisms in Arctic marine ecosystems and indicate that quantitative differences in the distribution of hydrocarbon utilizers are relatively unimportant over large geographic distances. A similar conclusion was reached by Roubal and Atlas (1978), who used a most probable number procedure based on the mineralization of ^{14}C-labelled hydrocarbons to determine the distribution of hydrocarbon-utilizing microorganisms in Alaskan Continental Shelf regions. They reported that hydrocarbon utilizers were ubiquitously distributed, with no significant overall concentration differences between Arctic and subarctic sampling regions. They also found no significant differences in concentrations of hydrocarbon utilizers in surface water and sediment samples. There were, however, significant seasonal differences in numbers of hydrocarbon utilizers, especially in surface waters. The only samples that failed to yield countable numbers of hydorcarbon-utilizing microorganisms were surface sea-ice samples.

In experimental field studies in the Arctic, Atlas and co-workers have found large increases in hydrocarbon-utilizing microorganisms in marine ecosystems (Atlas, 1978; Atlas et al., 1976; 1978; Atlas and Busdosh, 1976; Atlas and Schofield, 1975; Horowitz and Atlas, 1978). In some cases concentrations of hydrocarbon-utilizing microorganisms have been found to rise rapidly and dramatically in response to acute inputs of petroleum hydrocarbons. Horowitz and Atlas (1977b), using a continuous-flow-through model system, found large increases and shifts to a high percentage of hydrocarbon utilizers in Arctic coastal water when nitrogen and phosphorus were added to oil slicks.

In a longterm experiment on the effects of petroleum hydrocarbons on microorganisms in Arctic marine sediments, Haines and Atlas (1982) found that the increase in hydrocarbon degraders was relatively slow and occurred in stages. Instead of detecting a rise in numbers of hydrocarbon-degrading microorganisms within days, they reported that a one order of magnitude increase of hydrocarbon degraders occurred one month after experimental oiling of the sediment; the numbers of hydrocarbon degraders increased another order of magnitude seven months later, and after two years increased yet another order of magnitude to a maximum concentration of 2.4×10^4. . In an actual spill in a near-Arctic region, Stewart and Marks (1978) found higher numbers of hydrocarbon utilizers in sediment affected by the "Arrow" spill in Chedabucto Bay, Nova Scotia; five years after the spill, only a few of the sites examined had significant concentrations of residual petroleum and elevated counts of hydrocarbon utilizers.

It would appear that at least for sediments the numbers of oil degraders increase relatively slowly over time. This view is supported

by the work of Bunch, Eimjellen, and co-workers (Bunch et al., 1983a, 1983b; Eimjellen et al., 1982) who performed microbiological analyses as part of the Baffin Island oil spill project. In this study oil was released into several embayments in the eastern Canadian Arctic Ocean. Both Bunch et al. (1983a) and Eimjellen et al. (1982) initially found that numbers of oleoclasts (hydrocarbon-degrading bacteria) were unaffected by the release of oil during the the first summer following the experimental release of 8% weathered Lago Medio crude. However, one year after the experimental spillages numbers of oleoclastic bacteria were elevated in the sediments of the oiled embayments (Bunch et al., 1983b).

Arctic Ponds, Lakes, and Rivers

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 Arctic 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 activity in samples from the Colville River. Robertson et al. (1973a, 1973b) reported that they were unable to isolate hydrocarbon-oxidizing bacteria in the Colville River delta region, however, Arthelger and Button (1972) did report hydrocarbon-degrading metabolic activities in these same water samples.

Increases in numbers of hydrocarbon utilizers have not always been found following petroleum contamination of Arctic ponds and lakes. Jordan et al. (1978) added Prudhoe Bay crude oil to a small section of

an oligotrophic Arctic lake which had been enclosed by a sea-curtain. One year later there was no significant difference in the numbers of bacteria in sediment or water; rates of uptake of hexadecane and naphthalene were variable from sample to sample, but values were not significantly different between oiled and nonoiled areas. In this study numbers of oil-degrading bacteria were not specifically determined. Bergstein and Vestal (1978) observed that Prudhoe Bay crude oil alone in an Arctic tundra pond did not increase numbers of oil-degrading or total heterotrophic bacteria over a period of 28 days. They did, however, find a lack of elevated microbial populations in an oil-treated tundra pond unless phosphate also was added.

Atlas and co-workers similarly found that the greatest increases in numbers of hydrocarbon degraders in Arctic coastal ponds occurred when nitrogen and phosphorus fertilizers were added together with petroleum hydrocarbons, but they also found elevated numbers of hydrocarbon degraders occurring in the absence of supplemental nutrients (Atlas et al., 1976; Horowitz and Atlas, 1977a). In a study aimed at examining the effects of a leaded-refined gasoline spillage in the sediment of an Arctic lake, populations of hydrocarbon-utilizing microorganisms were found to be significantly elevated within a few hours of the spill (Horowitz and Atlas, 1977b) through the first year after the spill (Horowitz and Atlas, 1978). The degree of elevation in numbers of microbial hydrocarbon utilizers corresponded with the degree of contamination. Thus, coastal Arctic freshwater bodies have populations of hydrocarbon-degrading microorganisms but the response of these microbial populations to contamination with hydrocarbons varies; in particular, environmental factors related to the nutritional status of

the water body have a major effect on the extent of change in the relative numbers of oleoclastic microbial populations within the communities of freshwater Arctic ecosystems.

Arctic Tundra

Oil-degrading microorganisms have been found to be 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 specifically determined. Using five different soils collected in the Canadian Arctic, Mackenzie Valley area, they 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, and in some soils, modification of Prudhoe crude oil declined with cores below 15 cm depth.

Campbell et al. (1973) found that soils in the Barrow, Alaska, area possessed hydrocarbon-degrading microorganisms. The numbers 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 (1977a, 1977b) found that oil-degrading microorganisms in the Barrow soils varied from

10^{0} to 10^{1}/g soil wet weight. They also found that numbers of hydrocarbon-degrading microorgnaisms were approximately the same in high- and low-centered polygons.

Several studies have shown that the addition of crude oil to Arctic tundra soils results in large increases in total numbers of heterotrophs and of oil-utilizing microorganisms (Sexstone and Atlas, 1977b; Sexstone et al., 1978a, 1978b). In coastal polygonal tundra near Barrow Alaska, and tussock tundra near Prudhoe Bay, Alaska, the response of microbial populations to contaminating oil was found to depend on soil type and depth. Increases in microbial populations in subsurface soils paralleled the downward migration of the oil (Sexstone and Atlas, 1977a), Sparrow et al. (1978) found a rise in oil-utilizing bacterial populations in taiga soils from near Fairbanks, Alaska which were experimentally contaminated with hot Prudhoe Bay crude oil.

Studies in the Swan Hills area of north-central Alberta, Canada, by Cook and Westlake (1974) showed slightly increased bacterial populations 308 and 433 days after treatment with Swan Hills oil at an application rate of 6.5 liters/m^2. 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 liters of Norman Wells crude per m^2. 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 also added.

Elevated numbers of microorganisms have also been found in association with naturally occurring Arctic oil seepages. Oil-degrading microorganisms have been isolated from soils associated with natural oil

seepages. Agosti and Agosti (1973) reported evidence for microbial oil degradation in 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. High numbers of fungi have been found in association with the Cape Simpson, Alaska, oil seeps (Barsdate, 1973; Barsdate et al., 1973). Numbers of filamentous fungi in samples collected 0.2 m from the edge of the seep were reported to be three times higher than those 50 m from the seep; counts for heterotrophic yeasts were 100 times higher close to the seep. Bacterial populations in ponds in contact with the Cape Simpson oil seeps were found to be higher than in unstressed ponds; bacterial populations in soils adjacent to the asphaltic sections of the seeps were higher than those 50 m from the seep. Although not directly examined in these studies, it is reasonable to postulate that the elevated numbers of various groups of microorganisms reflects selective enrichment in numbers of hydrocarbon utilizers.

EFFECTS OF HYDROCARBONS ON TAXONOMIC COMPOSITION OF MICROBIAL COMMUNITIES

Arctic Tundra

Oil-degrading microorganisms associated with the Cape Simpson, Alaska oil seeps have been isolated and their taxonomic status studied. Cundell and Traxler (1974) studied 15 isolates from an asphaltic flow near this natural seepage. The isolates were psychrotrophic and utilized paraffinic, aromatic, and asphaltic petroleum components. The isolates belonged to the bacterial genera Pseudomonas, Brevibacterium, Spirillum, Xanthomonas, Alcaligenes, and Arthrobacter.

Cook and Westlake (1974) isolated Achromobacter, Alcaligenes, Flavobacterium, and Cytophaga at 4°C on a substrate of Prudhoe Bay crude oil; Acinetobacter, Pseudomonas, and unidentified Gram-negative cocci at 4°C on a substrate of Atkinson Point crude oil; Flavobacterium, Cytophaga, Pseudomonas, and Xanthomonas were isolated at 4°C with Norman Wells crude oil as substrate; and Alcaligenes and Pseudomonas were isolated on Lost Horse crude oil at 4°C. At 30°C, the major genera isolated on Prudhoe Bay crude oil were Achromobacter, Arthrobacter, and Pseudomonas; on Atkinson Point crude oil, the major genera were Achromobacter, Alcaligenes, and Xanthomonas; on Norman Wells crude oil, the major genera were Acinetobacter, Arthrobacter, Xanthomonas, and other Gram-negative rods; and on Lost Horse crude oil, they were Achromobacter, Acinetobacter, and Pseudomonas.

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 condida, e.g. Penicillium and Verticillium spp. Oil-degrading strains of Beauveria bassiana, Mortierella sp., Phoma sp., Scolecobasidium obovatum, and Tolypocladium 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.

Arctic Marine Ecosystems

Mulkins-Phillips and Stewart. (1974) reported finding hydrocarbon-utilizing bacteria of the genera Nocardia, Pseudomonas, Flavobacterium, Vibrio, and Achromobacter in northwest Atlantic coastal waters and sediment. Atlas et al. (1982) collected the marine amphipod Boeckosimus affinis from Arctic waters and examined the microorganisms associated with the animals during exposure to aquatic extracts of Prudhoe Bay crude oil. The genera of hydrocarbon-degrading bacteria associated with the amphipods tentatively were identified as Vibrio and Acinetobacter. These same genera of hydrocarbon-utilizing bacteria were found in low numbers in water and sediment samples collected throughout the western Beaufort Sea (Kaneko et al., 1979).

In studies on the diversity of Arctic microbial communities, Atlas and co-workers (Atlas, 1983; Atlas 1984b) have found that an influx of hydrocarbons generally results in lower diversity but in some cases the diversity of the microbial community increases. For example, exposure to petroleum hydrocarbons accelerated the successional process within the guts of amphipods, resulting in greater diversity in the associated microbial community than occurred in controls that were not exposed to hydrocarbons (Atlas et al., 1982); the dominance of Vibrio-like organisms clearly diminished. It was not clear whether the changes were due to the toxicity of hydrocarbons to Vibrio-like organisms or to an alteration in the successional process. In contrast to the effects of oil on the microbial communities associated with amphipods, in water and sediment exposed to Prudhoe crude oil, the diversities of the microbial communities decreased (Atlas, 1983, 1984b). For example, the diversity of the microbial community as measured with the Shannon index declined

from 3.5 to 2.4 in surface water exposed to Prudhoe Bay crude oil during a six week period {Atlas, 1983).

Arctic Ponds and Lakes

An even more dramatic decline in diversity, from a Shannon index of greater than 3 to a Shannon index of 0 during a one week period, occurred in a coastal lake contaminated with leaded gasoline (Horowitz and Atlas, unpublished data). The total bacterial biomass remained constant during this time period in the heavily contaminated region of the lake but the normal diverse bacterial community was replaced by a single hydrocarbon-utilizing Pseudomonas species. This drastic reduction in bacterial diversity undoubtedly reflects the presence of toxic components in the refined oil and the ability of the surviving Pseudomonas species to tolerate these toxic compounds and to metabolize the hydrocarbons in the refined fuel.

Hellebust et al. (1975) set up experimental chambers in a lake in the Mackenzie Valley near Norman Wells, Northwest Territories, Canada to study the effects of Norman Wells crude oil on the attached algal community. Crude oil was spilled on these subponds and the effects of the oil on the algae that would attach to an artificial substrate were studied. Samples were collected during the ice-free season and the composition of the algal community was determined using preserved samples. Numbers and species composition of the periphyton were greatly reduced after exposure to 15 l/m^2 of oil. However, the treatment caused a "bloom" of the cyanobacterium Oscillatoria angustissima.

In experimentally oiled ponds near Barrow, Alaska, Miller et al., (1978b) found that the movement of the spilled oil resulted in the formation of a slick around the edges of the pond that led to coating

with oil of the macrophytes growing at the edges of the pond. Algal biomass and species composition changed dramatically from a predominance of cryptophytes before the spill to a dominance of chrysophytes after the spill. There were no significant changes in species composition by the other algal species. Federle et al. (1979) found that the addition of oil to Arctic ponds killed the indigenous zooplankton within the first five days. Planktonic algal composition changed rapidly and again, the chrysophytes almost completely replaced the cryptophytes within fifteen days. Rhodomonas minuta, a cryptophyte, decreased in number by 95-98% within the first five clays post-spill and was replaced by the chrysophyte Uroglena within 41 days (Miller et al., 1978a). 'It was concluded that the crude oil causes an initial drastic inhibition in planktonic primary production; eventually the primary production recovers but the recovered composition of the community of primary producers changes to relatively oil resistant algae.

EFFECTS OF HYDROCARBONS ON MICROBIAL ACTIVITIES

Effects of Hydrocarbons on Primary Productivity

A number of controlled field studies have been performed to examine the effects of hydrocarbon perturbants on natural primary production. Many of these studies concerning the effects of oil on phytoplankton have been reviewed by O'Brien and Dixon (1976). In studies with Arctic marine phytoplankton and various crude oils, Hsiao (1978) found that exposure to various crude oils inhibited all the algae that were examined. They found, though, that the degree of inhibition was dependent on temperature. At 0°C the oils were generally less toxic to growth than at 5°C as compared to 10"C, indicating that an accidental

oil spillage in the Arctic could lead to severe damage to the marine phytoplankton community.

In studies on the effect of the detergent Corexit on natural marine phytoplankton in the presence of crude oil, Hsiao et al. (1978) surveyed the natural phytoplankton of the Beaufort Sea in northern Canada. They exposed seawater samples to Atkinson Point, Norman Wells, Pembina, and Venezuela crude oils in the presence and absence of the detergent Corexit, and measured photosynthesis by $H^{14}CO_3$ uptake. When all of the data from the various sample sites were combined, it was shown that the dispersant accentuated the inhibitory effects of the four crude oils. Hsiao and co-workers, therefore, concluded that the detergent allowed the toxic fractions of the oils to penetrate and impair the cells more easily than the oil alone.

In field studies, Miller and co-workers (Barsdate et al., 1980; Federle et al., 1979; Miller et al., 1978a, 1978b) extensively studied the effects of Prudhoe Bay crude oil on the primary production of Arctic tundra thaw ponds and some deep lakes on the North Slope of the Brooks Range. In the ponds near Barrow, Alaska, planktonic primary production initially was drastically inhibited after addition of oil, but slowly recovered to normal levels before the ponds froze (Miller et al., 1978b). During a study of an oil spill contained by a curtain on a deep tundra lake, Miller et al., (1978a) found that algal biomass, measured by chlorophyll A concentration, was severely inhibited inside the oil curtain as compared to outside the curtain. Planktonic primary production was also inhibited in the portion of the lake containing spilled oil at depths of 0.5 m and 3.0 m. Planktonic primary production

in the oil spilled portion of the lake did not return to control rates until after the spring bloom.

Federle et al. (1979) used oil contained within subponds to examine the effects of oil on primary productivity. In some subponds the normal grazing zooplankton was removed and in other subponds, a normal level of zooplankton, Daphnia middendorphania and Brachinecta paludosa, were included. After addition of 1 ml/l Prudhoe Bay crude oil {240 ml oil/m^2), primary production decreased to zero or near zero in the oil spilled subponds within five days. Production slowly improved, but did not reach control (no oil) levels within fifteen days.

Effects of Hydrocarbons on Heterotrophic Activity

Heterotrophic activity, measured by the rate of substrate uptake, is an index of the productivity of heterotrophic microorganisms; such secondary productivity is very important in detrital food webs. Perhaps the most extensive series of experiments on the effects of oil on heterotrophic activity have been carried out around the coast of Alaska. In an extensive survey by Griffiths et al. (1981a), 215 water samples and 162 sediment samples were incubated with Alaskan crude oil (0.1% v/v) for periods of between 1 and 12 hours. In all cases there was a significant reduction in glucose uptake in the oiled samples compared with control samples, but the reduction was less in the sediments than in the water columns. In addition to the reduction of rates of glucose uptake, the uptake of glutamic acid was inhibited by crude oil. The results off the northern, western and southern coasts of Alaska were similar. Some of the data suggests that inhibition was least where the bacterial population had been previously exposed to oil. The adverse effect of crude oil on glucose uptake was reduced by increasing exposure

time until, after a few days, there was no measurable effect. This observation suggests that the microbial population adjusted very rapidly to the presence of crude oil.

Although studies on pelagic microheterotrophs have shown that these populations may rapidly adjust to the presence of crude oil, this was not the case in long-term studies of marine sediments (Griffiths et al., 1981b; 1982b). The effect of crude oil was less marked in sediments than in water samples; glucose uptake was inhibited 37 to 58% in water samples and 14 to 36% in sediments. In studies with subarctic benthic samples exposed to both fresh and weathered crude oil significant reductions in glucose uptake rates and significant increases in the proportion respired were Found. It was concluded that bacterial production was depressed by the presence of these oils; however, it was not possible to determine whether the mechanism responsible for the change was a direct toxic effect or an indirect effect resulting from the observed reduction in the redox potential (Griffiths et al., 1982b).

The studies on microbial activity in Alaskan waters have clearly shown that the impact of crude oil in the water column is different from that observed in the sediments (Griffiths et al., 1981a, 1981b; 1982b). In the water column fresh crude oil has only a transitory effect on glucose and glutamate uptake rates with no significant change in the proportion respired. These results suggest that the initial toxic effects are most probably associated with substrate transport mechanisms; analysis of the uptake kinetics did not indicate competitive inhibition. Experiments conducted in situ on the long-term effect of both fresh and weathered crude oil at various concentrations indicated that both glucose and glutamate uptake rates were depressed even after

18 months exposure. (Griffiths et al., 1981b; 1982a, 1982b). Competitive inhibition did not seem to be the cause of reduced uptake rates, but the effects were of a much longer duration than in the water column. Additionally, there was a significant increase in the proportion of substrate respired. Hence, it appears that in sediments microbial uptake rates are reduced and therefore oil contamination will reduce rates of microbial biomass production.

Hodson et al. (1977), as part of a controlled oil release experiment conducted in the northern Pacific Ocean, examined the effects of Kuwait and Louisiana crude oil, #2 fuel oil, and Bunker C oil on the assimilation and mineralization of radiolabelled glucose by 1 µm filterable populations from Saanich Inlet, British Columbia, Canada. Water samples were enclosed within large p"lastic containers and suspended in the water column. They demonstrated that glucose utilization was inhibited by the presence of all types of oil, although the refined petroleum products, #2 fuel oil and Bunkder C oil, produced significantly greater degrees of inhibition than either of the crude oils. Both uptake and mineralization of glucose appeared to be inhibited to a similar extent by the concentrations of #2 fuel oil added. No significant inhibition was observed at. concentrations of aqueous oil below 300 µg/l. At concentrations above this level, a good dose-response relationship was obtained with up to 80% inhibition of glucose assimilation by concentrations in the range of 1200 to 1800 µg/l . In this study there appeared to be a correlation between the degree of inhibition of glucose utilization and the concentration of naphthalene present in the various oils utilized. These results are comparable to those of Griffiths et al. (1981a, 1981b, 1982a, 1982b).

Bunch et al. (1983a, 1983b), working in the Baffin Island region of the Canadian Arctic Ocean, found results contradictory to those of Griffiths et a?. (1981a, 1981b; 1982a, 1982b) with respect to the impact of oil on water and sediment microbial activities. Bunch's in vitro experiments showed that the respiration of glutamic acid was not significantly affected by 0.1% by volume petroleum crude. In the dispersed oil release experiments of the Baffin Island oil spill field study, the V_{max} for glutamic acid uptake initially decreased with respect to the control bay (Bunch et al., 1983a). Perturbation of bacterial uptake of glutamic acid in the water column, however, was short term. This effect could be attributed to the light fraction of the dispersed Lago Medio, but the effect of Corexit cannot be discounted.

Both 0.01% Corexit alone and a mixture of petroleum crude and Corexit caused significant decreases in V_{max} and significant increases in turnover and (K+S). At the concentrations of oil entered into the water by the surface and dispersed oil releases, the effect on measured bacterial activity was transient and minimal. No changes in bacterial activity in the sediments could be ascribed to either oil release. Addition of Corexit 9527, however, significantly altered kinetic parameters of glutamic acid uptake in laboratory experiments. This effect could not be determined during the dispersed release where the concentration of Corexit was three orders of magnitude lower than in the laboratory experiments.

The lack of effects of the oil releases in the water column was not unexpected since the waters of the bays were rapidly flushed of oil after both releases (Bunch et al., 1983a, 1983b). Although significant

interactions were found in the kinetic parameters of turnover and (K+S) of glutamic acid uptake in a supplementary sampling two days after the surface release of 8% weathered Lago Medio crude, these changes could not be ascribed with certainty to the surface oil release. In laboratory experiments, glutamate mineralization was not significantly different in the presence or absence of 0.1% weathered crude. Similar results were obtained by Alexander and Schwarz (1980) with 0.1% fresh crude for much warmer Gulf of Mexico samples. As already discussed, Griffiths et al. (1981a, 1981b; 1982a, 1982b) found that glucose and glutamic acid uptake were reduced significantly in water samples amended with 0.1% by volume fresh crude. Hodson et al. (1977) found 15% inhibition of glucose assimilation in the presence of 0.8 mg/l of aqueous extracts of petroleum crudes, i.e., that fraction of fresh petroleum which is usually considered toxic.

Compared to these marine studies, relatively little work on glucose uptake has been carried out in other Arctic habitats, but Jordan et al. (1978) did look at the suspended and sediment bacterial activities in an Arctic lake a year after crude oil was added to it. Turnover rates of glucose were not significantly different to the control in either the water column or the lake sediment. -

Effects of Hydrocarbons on Cellulose Decomposition

In addition to studies on the effects of oil on general microbial activities, such as heterotrophic uptake, some studies have examined the effects of hydrocarbons on specific microbial activities such as cellulose decomposition. McKinley et al. (1982) showed that exposure of the microflora of an Arctic freshwater lake to Prudhoe Bay crude oil, diesel fuel, or toluene had little effect on acetate incorporation into

lipid or on ATP levels in the 12 hour period following addition of hydrocarbon. But over a 21-day period, mineralization of labelled plant litter material was affected in different ways by several petroleum materials. ^{14}C-(cellulose)-lignocellulose mineralization was inhibited by motor oil or toluene, but diesel fuel stimulated mineralization. Mineralization of the lignin component of lignocellulose was more sensitive than mineralization of the cellulose component. Inhibition of plant litter decomposition could seriously affect the cycling of carbon and energy in freshwater ecosystems.

In a study on the effects of oil on soil cellulase activity Linkins et al. (1978) added Prudhoe Bay crude oil to polygonal tundra soils at Barrow, Alaska. They found significantly depressed cellulase activities in oiled soils over a three year period as compared to reference soils. There was a greater loss of exoglucanase activity as compared to endoglucanase. The greater relative loss in exoglucanase activity supports the hypothesis that soil microbial populations are not utilizing the hydrolysis products of recalcitrant plant materials, whereas the continued presence of some endoglucanase activity suggests that low numbers of organisms capable of carbohydrate polysaccharide hydrolysis and utilization are still- present in the soils. These hypotheses are supported by the declines in fungal populations (Miller et al., 1978c) which are thought to be the major sources for cellulytic enzymes in soils.

Effects of Hydrocarbons on Nitrogen Cycling

The first studies on the effects of oil on nitrogen fixation in Arctic ecosystems were reported by Knowles and Wishart (1977). These workers added weathered Canadian crude to marine sediment samples from

stations off the northwestern coast of Canada. With the addition of oil at 1, 3, and 5% v/w, no significant alteration in the rates of nitrogen fixation were found during incubation periods of up to 35 days.

In tundra soils, however, addition of oil significantly lowered rates of nitrogen fixation (Atlas and Schofield, 1975). This finding may reflect a primary inhibition of photosynthetic activity because much of the nitrogen fixing activity in tundra soils is associated with cyanobacteria which are inhibited by hydrocarbons. Sexstone and Atlas (unpublished data) also found that oil addition to tundra soils enhanced rates of denitrification. The increased rates of denitrification may be due to lowered oxygen concentrations resulting from extensive decomposition after oiling. Assuming both decreased inputs of nitrogen, because of inhibition of nitrogen fixation, and increased losses of nitrogen, because of enhanced denitrification, oil contamination of tundra soils can severely reduce the availability of soil nitrogen and thereby decrease productivity in tundra soils.

Griffiths et al. (1982b), using oil concentrations of 0.1 and 5.0% v/v and exposure periods of up to 18 months, found that fresh oil significantly decreased nitrogen fixation rates and potential denitrification rates in coastal sediments of Alaska. However, short term exposure to oil did not reduce nitrogen fixation rates (Haines et al., 1981). However, weathered crude oil did not produce a significant decrease in nitrogen fixation. Natural rates of denitrification (i.e., the rate measured in the absence of added nitrate) was generally below the detectable limit using gas chromatographic methods. Little work has been carried out on the effects of oil on nitrogen cycling in freshwater, but rates of nitrogen fixation were unaffected in Arctic

pond sediment (Horowitz et al., 1978) a year after it was severely polluted with gasoline. Similarly, preliminary observations on a stream sediment mixed with crude oil (Baker and Morita, 1983) showed little difference in nitrogen fixation rates from the control samples.

Interestingly there is agreement between two different groups of workers (Griffiths et al., 1982b; Haines et al., 1981) on the effect of weathered crude on nitrogen fixation rates. Both studies report no significant change in Arctic marine sediments treated with similar concentrations of oil, Griffiths et al. (1982b) also applied fresh crude oil and found reduced fixation rates. These experiments emphasize the importance of using weathered crude oil as a perturbant where this is the most likely state of the material under "natural" conditions. Not unexpectedly, rates of denitrification are often undetectable in unperturbed marine sediments which are generally of low nitrogen content. Studies on denitrification potentials, measured after addition of nitrate, also showed a lack of impact by petroleum hydrocarbons; rates of denitrification in control and sediments with added Prudhoe Bay crude oil were not significantly different even during prolonged exposure (Haines et al., 1981).

MICROBIAL DEGRADATION OF PETROLEUM HYDROCARBONS

Biodegradation in Tundra Soils

The fate of petroleum hydrocarbons in soil ecosystems has been reviewed by Bossert and Bartha (1984). As discussed in this review, a number of studies have been conducted on the fate of oil in cold Arctic soils. Sexstone and colleagues (Sexstone and Atlas, 1978; Sexstone et al., 1978a, 1978b) have reported very long persistence times for oil in tundra soils. It appears that degradation of hydrocarbons ceases during

winter when tundra soils are frozen. Westlake and colleagues (Cook and Westlake, 1974; Jobson et al., 1972; Westlake et a?., 1978) found that the microbial populations in northern soils were able to degrade hydrocarbons at the ambient temperatures found during the warmer seasons.

Predictably, in frozen soil no oil biodegradation was observed (Atlas et al., 1978; Sexstone et al., 1978a, 1978b) and the overall persistence of oil in Arctic soils was very long. In one instance substantial amounts of hydrocarbons were found 28 years after a spill. However, the same investigation showed that hydrocarbon-degrading microorganisms isolated from Alaskan oil-contaminated soils were able to grow at 5°C. Fungal isolates generally required higher temperatures (10"C) for growth. Incubation temperatures up to 25°C produced no enhanced hydrocarbon biodegration. In agreement with Baker and Smith (1972), the study demonstrated that psychrotrophic microorganisms abound in tundra soils, but obligate psychrophiles are relatively few.

In another study involving Arctic soils, Hunt et al. (1973) investigated the biodegradation of a Prudhoe Bay crude oil in a Fairbanks silt loam. Samples were inoculated with hydrocarbon-degrading microorganisms isolated from a Cape Simpson natural oil seep and incubated at a representative summer temperature (15°C). Biodegradation of the oil, as measured by $C0_2$ evolution, was stimulated by the addition of fertilizer and an inoculum of hydrocarbon-degrading microorganisms. The artificial seeding of hydrocarbonoclastic microorganisms into a polluted environment is generally considered ineffective for increasing biodegradation rates due to limitations by inorganic nutrients and the presence of indigenous hydrocarbon degraders (Atlas, 1977a), but the

inoculum in this case produced a measurable stimulation of oil biodegradation. This was explained by the slow response of the indigenous microorganisms to the oil in a cold environment, and by increasing the number of hydrocarbon-degrading microorganisms in the soil, the need for a long acclimatization period was avoided. This may be important in Arctic regions where summer temperatures prevail for short time periods only. Jobson et al. (1972) and Cundell and Traxler (1974) showed that microorganisms enriched at low temperatures exhibited longer lag periods in cell growth and in hydrocarbon utilization, respectively, than did mesophilic microorganisms. Generally, the greatest range of hydrocarbon utilization occurred with psychrotrophic microorganisms at 16-24°C.

Westlake et al. (1974) compared the biodegradability of four northern crude oils under mesophilic (30°C) and psychrophilic (4°C) conditions and showed that the types of hydrocarbons biodegraded may vary at different temperatures. Cell growth and yields were comparable at both temperatures. However, unlike the enriched mesophilic populations, the ones enriched at 4°C were unable to degrade the isoprenoid and branched paraffin components of all crude oils studied. Enumeration of the microbial populations enriched at both temperatures demonstrated that different microorganisms were predominant in similar media with incubation temperatures being the only variable. Similarly, Jobson et al. (1972) reported the isolation of mesophilic and psychrotrophic populations of microorganisms able to utilize two types of crude oil as growth substrates. The psychrotrophic populations possessed minimal ability to degrade the aromatic fraction of the crude oils, but had a capability similar to that of mesophilic populations for

utilization of the saturate fraction. Bailey et al. (1973) reported a similar phenomenon occurring in the biodegradation of a North Cantal crude by soil microorganisms isolated from a northern producing well. Incubation with a mixed mesophilic population produced preferential attack on branched and linear alkanes of less than C_{25}. Two psychrotrophic enrichments from different soils were able to biodegrade n-paraffins up to C_{31}, but they failed to attack the isoprenoids. The above-described differences in metabolic capabilities were attributed to different types of bacteria enriched at the respective temperatures.

Westlake et al. (1978) examined the in situ degradation of oil in a soil of the boreal region of the Northwest Territories of Canada. When "fertilizer containing nitrogen and phosphorus was applied to the oil, there was a rapid increase in bacterial numbers, but no increase in fungal propagules. This was followed by a rapid disappearance of n-alkanes and isoprenoids and a continuous loss of weight of saturated compounds in the recovered oil. The seeding of oil slick plots with oil-degrading bacteria had no effect on the composition of the recovered oil. Jobson et al. (1974) similarly found that nitrogen and phosphorus addition stimulated hydrocarbon degradation in oil applied to soil but that seeding did not, stimulate degradation. Hunt et al. (1973) found that fertilizer application to subarctic soils enhanced microbial hydrocarbon degradation. They found in laboratory tests, however, that nitrogen addition caused an initial negative response in microbial activity, followed by the biodegradation of the oil in the sediment as shown by an alteration in the the ratio of n-paraffins to isoprenoid hydrocarbons. Within 1 year, most of the oil was gone and rapid

biodegradation appeared to contribute to the removal of contaminating hydrocarbons.

Several Arctic terrestrial oil spills have been examined. Cook and Westlake (1974) found evidence for extensive utilization of n-alkanes in oils applied in the Norman Wells area of the Northwest Territories and in the Swan Hill area of northern Alberta, They also found evidence for biodegradation of oil of the Nipisi spill in northern Alberta. The spill was on a sphagnum bog. Sexstone et al. (1978a), and Atlas et al. (1979), however, found evidence for greatly restricted rates of biodegradation in northern soils. They found that hydrocarbons were still present in soils at Fish Creek, Alaska, 28 years after contamination by a spillage of refined oil. This finding suggests that hydrocarbons will persist in Arctic tundra soils for decades following spillage. It appears that compositional changes in spilled oil begin shortly after spillage (Sexstone and Atlas, 1977a). These changes include both abiotic weathering and biodegradation. After initial biodegradative modification , rates of oil biodegradation significantly decreased. Experimental crude oil spillages less than 12 liters/m^2 showed disappearance of n-paraffins but not branched paraffins after 7 years exposure. Similar spillages of 20 liters/m^2 or more showed persistence of n-paraffins. Spillages in excess of 20 liters/m^2 occurred at Franklin Bluffs and Fairbanks, Alaska from breaks in the Trans-Alaskan Pipeline.

They concluded that biodegradation of oil pollutants appears to be limited in large part by environmental constraints. Concentrations of hydrocarbon-utilizing microorgnaisms do not appear to be a limiting factor in these soils. Hydrocarbon-utilizing microorganisms were

present in all soils examined and increased in numbers in response to oil spills, and the indigenous bacteria and fungi utilized a wide range of hydrocarbon substrates. Cellulose input. was found to inhibit hydrocarbon mineralization. Since oil causes plant mortality in Arctic ecosystems, extensive oil biodegradation may be prevented until after decomposition of the plant residue. Also tundra soils are severely limited in available nitrogen and phosphorus (Bunnell et al., 1975) which can limit rates of degradation of crude oil (Atlas, 1977).

A modeling effort was conducted to elucidate the crucial factors which determine the fate of petroleum hydrocarbon pollutants in Arctic tundra soils (Dauffenbach et al., 1981). The preliminary tundra model considers as important factors: tundra plant biomass, litter, standing dead and belowground dead plant material, decomposes and hydrocarbon-utilizing microorganisms, nitrogen, phosphorus and carbon storages, petroleum hydrocarbons, solar radiation, temperature, precipitation, runoff and drainage from other areas, and nutrient inputs of various sorts. The critical processes which appear to affect the removal of hydrocarbons include, biodegradation, abiotic weathering, migration of oil into the soil, decomposition of dead plant material (which is a competitive process with oil degradation), and nitrogen and phosphorus cycling, including uptake by plants and microbes.

Biodegradation in Ponds and Lakes

Terrestrial Arctic oil spills may contaminate ponds and lakes which are quite abundant. The fate of petroleum pollutants in freshwater ecosystems has been reviewed by Cooney (1984). Compared to soil and marine studies, less attention has been given to the fate of oil in freshwater bodies, and this is particularly true for Arctic ponds and

lakes. Bergstein and Vestal (1978) studied the biodegradation of crude oil in Arctic tundra ponds. They concluded that oleophilic fertilizer may provide a useful tool to enhance the biodegradation of crude oil spilled on such oligotrophic waters. Without addition of nitrogen and phosphorus, hydrocarbon biodegradation was limited. Atlas and Bartha (1973a) described an oleophilic nitrogen and phosphorus fertilizer which could overcome the limitation of nitrogen and phosphorus and stimulate petroleum biodegradation in seawater. The fertilizer consisted of paraffinized urea and octylphosphate; optimal C/N and-C/P ratios were 10:1 and 100:1, respectively.

Horowitz and Atlas (1977a, 1978) examined the biodegradation of hydrocarbons in an Arctic lake following an accidental spillage of 55,000 gallons of leaded gasoline. The hydrocarbon biodegradation potential experiments showed that the microorganisms exposed to leaded gasoline were capable of extensively converting contaminating hydrocarbons to $C0_2$. The lower rate of ^{14}C incorporation into the cells in the area of heaviest gasoline contamination and the decreasing activities with time of exposure to leaded gasolinein all three areas could be due to several reasons, including the diluting effect of the nonlabelled leaded gasoline hydrocarbons or increased concentrations of toxic leaded gasoline components after weathering and biodegrative losses. The lower activities could also be due to the production of toxic compounds from biodegradation or to the depletion of an essential element such as iron.

The in situ biodegration experiments showed that abiotic weathering and biodegradation resulted in extensive losses from the spilled leaded gasoline, but when the lake began to freeze for the winter, 10% of the

extractable material still remained in the untreated sediment. Nutrient addition effectively increased losses, and inoculation, 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 leaded gasoline 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 addition of fertilizers would appear to be effective in the abatement of the effects of the gasoline spillage.

Biodegradation in Marine Waters and Sediments

The fate of oil in marine ecosystems has been the subject of several reviews (Atlas, 1981; Atlas and Bartha, 1973b; Bartha and Atlas, 1977; Colwell and Walker, 1977; Crow et al., 1974; Floodgate, 1972, 1984; Jordan and Payne, 1980; Lee and Ryan, 1976; National Academy of Sciences, 1975; Van der Linden, 1978; ZoBell, 1964, 1969). Unlike the limited numbers of studies on the fate of oil in Arctic freshwater ecosystems, extensive studies have been conducted on the weathering of petroleum in coastal regions of the Arctic ocean. Two major interdisciplinary studies, the U.S. Outer Continental Shelf Environmental Assessment Program and the Canadian Baffin Island Oil Spill Project, have yielded a great deal of information on the fate of petroleum hydrocarbons in Arctic marine ecosystems.

Arhelger et al. (1977) compared Arctic and subarctic hydrocarbon biodegradation. In situ [^{14}C]dodecane oxidation rates based on $^{14}CO_2$ production were: Port Valdez, 0.7 g/liter per day; Chuckchi Sea, 0.5 g/liter per day; and Arctic Ocean, 0.001 g/liter per day. This study

indicates that rates of hydrocarbon degradation show a definite climatic shift and are lower in the Arctic Ocean than in more southerly Alaskan regions. Roubal and Atlas (1978) also compared biodegradation potentials in Arctic and subarctic Alaskan seas. They concluded that there was no siginficant correlation between numbers of hydrocarbon utilizers and hydrocarbon biodegradation potentials. The biodegradation potentials showed large seasonal variations in the Beaufort Sea, probably due to seasonal depletion of available nutrients. Non-nutrient-limited biodegradation potentials followed the order hexadecane > naphthalene >> pristane > benzanthracene.

Atlas and co-workers (Atlas, 1977b; Atlas and Busdosh, 1976; Atlas et al., 1978) examined the degradation of Prudhoe Bay crude oil in Arctic marine ice, water, and sediment ecosystems. Petroleum hydrocarbons were degraded slowly. They found that ice greatly restricted losses of light hydrocarbons and that biodegradation of oil on the surface of ice or under sea ice was negligible. Hydrocarbon biodegradation potentials were lower in ice than in water or sediment. Natural rates of degradation were slow, and maximal losses from experimental oil spills were less that 50% during the Arctic summer due to combined abiotic and biodegradative losses. Rates of biodegradation were found to be limited by temperature and concentration of available nitrogen and phosphorus. Residual oil had similar percentages of hydrocarbon classes as fresh oil; i.e., biodegradation of all oil component classes, including paraffinic and aromatic fractions, apparently proceeded at similar rates. They concluded that petroleum hydrocarbons will remain in cold Arctic ecosystems for long periods of time after oil contamination. In these studies, however, temperature

was not specifically elucidated as a major factor limiting hydrocarbon degradation], except as it related to the occurrence of ice.

Haines and Atlas (1982) examined the fate of Prudhoe Bay crude oil in nearshore sediments of the Beaufort Sea, in situ, with emphasis on the role of microorganisms in the weathering process. In these studies divers placed oil contaminated sediment in trays at the bottom of a coastal lagoon and periodically recovered replicate trays for extraction of oil and detailed chemical analyses. These studies clearly indicate that weathering of oil in Beaufort Sea sediments will be a slow process. Microbial degradation of petroleum hydrocarbons occurred in contaminated Arctic sediments but only after significant exposure periods; evidence for biological modification of petroleum in experimentally oil contaminated Beaufort Sea sediment was not observed until after a year.

Several factors probably contributed to the slow rate of microbial weathering. The hydrocarbon-degrading microbial population did not increase rapidly following addition of oil to the sediments. Only after 8 months of exposure were there sufficient numbers of hydrocarbon utilizers to establish a significant response within the microbial community to the presence of the oil. This initial lack of response could not have been entirely due to -the low temperature. The hydrocarbon-utilizing microorganisms were psychrotrophic or psychrophilic; such microorganisms are capable of active growth and metabolism at temperatures below 0°C (Morita, 1975; Robertson et al., 1973a; Traxler, 1973; ZoBell, 1973). Also Prudhoe Bay crude oil has not been shown to contain a toxic fraction inhibitory to microbial hydrocarbon biodegradation at low temperatures (Atlas, 1975). The nitrogen and phosphorus concnetrations were adequate to support. only

limited hydrocarbon biodegradation; optimal rates of hydrocarbon biodegradation typically occur at C:N and C:P ratios of 10:1 and 30:1 (Atlas and Bartha, 1972) which would have required several orders of magnitude higher concentrations of N and P; concentrations of available N and P which were actually measured, were below those needed to support maximal rates of biodegradation of the hydrocarbons added to the sediments. Oxygen availability also may have been a rate-limiting factor. Gibbs and Davis (1976) have shown that 0_2 and N are limiting factors in fine grained sediment columns. The sediments in Elson Lagoon, the westernmost lagoon of the Beaufort Sea, located near Barrow, Alaska, are very fine grained (silty-clay). Rates of exchange of nutrients and oxygen between the interstitial water of the sediment and the overlying water column are likely to be quite low in such sediments. It is likely that all of the above factors contributed to the limited rates of oil biodegradation. The fact that the inorganic N and P concentrations remained relatively constant in the interstitial water during the experimental period indicates that these inorganic nutrients were not being rapidly utilized during oil degradation; otherwise the concentration of N and P in the interstitial water would have declined with time. The concentrations of inorganic N and P in the interstitial water may have been below the threshold concentrations needed to support rapid microbial utilization of hydrocarbons or that hydrocarbon biodegradation was blocked by some other factor. Alternatively, hydrocarbon degradation could have been proceeding slowly throughout the experimental period, but the population of hydrocarbon degraders was low enough that chemical changes in the oil were not observed; the delay of observable changes in C_{17}/pristane ratios would be due to the length of

time needed to develop a relatively large degrading population and for that population to utilize enough oil to be detectable by current analytical techniques.

Not only was biodegradation limited but abiotic weathering also was restricted. Low molecular weight aliphatic and aromatic compounds, which normally are rapidly lost from surface oil slicks by evaporation and dissolution, remained as a significant feature of the residual hydrocarbon mixture for over one year. Oil which becomes entrained in Arctic sediments without significant surface weathering, thus, would retain toxic low molecular weight aromatic hydrocarbons for prolonged periods of time. This would retard ecological recovery of benthic Arctic sediments which become oiled by ruptures of buried oil transfer pipelines. Weathering of the oil was atypical in several ways. Loss of low molecular weight compounds (aromatics such as naphthalene and aliphatics through $\underline{n}$-C_{14}) was not the first event which would have resulted in major modification of the hydrocarbon mixture. Aliphatic hydrocarbons were not preferentially degraded over aromatic hydrocarbons; aromatic compounds were lost at rates comparable to those for aliphatic compounds. There was preferential biodegradation of $\underline{n}$-alkanes $\leq nC_{17}$ over alkanes $\geq nC_{18}$. These were significant deviations from the normal oil weathering process.

An important feature of the oil weathering process was the patchy oil distribution. The appearance of isolated pockets of crude oil within the sediment would reduce the surface area/volume ratio of the oil exposed to microbiological weathering. The original sediment-oil mixture had a uniform distribution of oil throughout the sediment. Accumulation of the oil in pockets reduced the interracial surface area

of the oil available for microbial degradation of the petroleum hydrocarbons. The mechanisms by which oil in sediments would gather into small pools may be due to differential volubility. The implication of this observation is that accidentally spilled oil would tend to gather into small pools and prolong the time for recovery of the ecosystem from oil contamination. A patchy distribution of hydrocarbons was a characteristic of Chedabucto Bay sediments following weathering of oil spilled by the tanker "Arrow"; degradation of oil in Chedabucto Bay, Nova Scotia, Canada, was particularly slow in low wave energy environments (Rashid, 1974). This may have been due to a lack of resupply of oxygen and nutrients needed to support microbial hydrocarbon biodegradation. In polar seas, such as the Beaufort Sea, ice dampens wave action contributing to the limitation of nutrient and oxygen resupply to the sediments.

The losses of hydrocarbons from Beaufort Sea sediments, from both abiotic and biotic weathering, based on in situ exposure experiments, are estimated at a rate of about 0.2 mg oil degrading/g dry wt sediment/year. This value is calculated as the difference in the mean values of the resolvable hydrocarbons at the start and end of the experiment divided by the total time. of experimental exposure. At the observed rate of degradation it will take many years to remove hydrocarbons from heavily oiled Arctic sediments by natural weathering processes.

The prediction of slow Arctic oil biodegradation is supported by actual observations of oil, from several accidental spills, weathered in cold sediments. Colwell et al. (1978) found that biodegradation of oil spilled near the Straits of Magellan by the tanker "Metula" was slow,

with marked persistence 2 years after the spill. Mayo et al. (1978) found that petroleum residues, from a pipeline spill at Searsport, Maine, weathered particularly slowly in the cold anoxic fine grained sediments of a contaminated cove. In October 1977, the Soviet tanker "Tsesis" struck a rock in the northern Baltic off Sodertalje, Sweden and released about 1100 tons of No. 5 fuel oil. Clean-up operations recovered most of the oil leaving only about 400 tons within an enclosed archipelago, visibly oiling an area of 34 km^2. There was significant transport of oil to the benthos. Studies by Boehm et al. (1980) on the weathering of the oil showed that oil remaining at the water's surface apparently underwent only slow degradation due to chemical and microbial weathering until landfall occurred. However, petroleum material dispersed in the water 'column underwent rapid bacterial degradation of the aliphatic hydrocarbons with n-alkanes being rapidly depleted relative to the isoprenoid compounds and rapid removal of the lighter aromatic fraction due to dissolution. Measurements of the bacterial populations following the spill indicate an increase in the bacterial population in the water column possibly due in part to the availability of oil as a carbon source.

In March 1977, there was a spill from the tanker "Potomac" into the ice-laden waters of Melville Bay in the northeastern part of Baffin Bay, off western Greenland. About 107,000 gallons (ea. 406,600 liters) of Bunker C fuel oil was lost. The fate of the oil was investigated by a team Of scientists (Grouse et al, 1979). Biodegradation of the oil at the low water temperatures was found to proceed very slowly if at all. . There was no significant increase in numbers of hydrocarbon utilizers within a few weeks after the spill. During this period there was also

almost no change in the C_{17}/pristane ratio in the oil, indicating that biodegradation was not occurring at a significant rate.

An extensive field study in the Baffin Island region has been conducted in which oil has been released in several contained Bays. In some cases dispersant was added together with crude oil. As part of this multidisciplinary study, Eimjellen et al. (1982), using tritiated Lago Medio weathered crude oil, assessed the rate of oil mineralization in samples of sediments and in water of Ragged Channel, Baffin Island, Canada. In early August the average mineralizing activity in the water of the bays rapidly reached a plateau level of about V_{10} = 35 $\mu g/m^3/day$ (V_{10} = rate determined in water at equilibrium with 1 ppmof oil), which unexpectedly dropped to half this value just prior to the first oil spill (surface spill). During the first two weeks of September the activity again rose to 18-25 $\mu g/m^3/day$ in all but one of the bays. The mineralizing activity in the sediment ranged from 15-39 $\mu g/l/day$. All water and sediment samples analyzed could mineralize ^{3}H-Lago Medio oil as well as n-(1-^{14}C)-hexadecane. The average V_{10}-rates for n-hexadecane mineralization for the various bays ranged from 9.5-43.8 $\mu g/m^3/day$ for water (highest rates in early August), from 4 to 114 $\mu g/l/day$ for the sediment samples. Only a minor percentage of the samples exhibited ability to mineralize ^{14}C-naphthalene (28%) and benz(a)pyrene (10%).

These Baffin Island oil spill studies also showed that the heterotrophic respiration of oil slicks in the supralittoral zone of Arctic beaches can be enhanced up to about five times the normal rates of beach sediments. It was also demonstrated that the microbiological community responds to heavy loads of fertilizers. Only the fertilizer that is actually mixed into the oil film affected oil biodegradation.

Fertilizing combined with land tilling does create a dry soil surface, but rates of heterotrophic respiration are approximately the same as without this treatment. The use of sorbents without the addition of fertilizers did not enhance rates of respiration in this study. The total decomposition rate of oil during the summer following the spill was reported to be approximately 25 g oil/kg soil and 10 g oil/kg soil for aged and emulsified oil, respectively. These rates are comparable to subarctic soil studies where rates between 9 to 165 g oil/kg soil were reported when oil content varied between 5% and 10% in the soil (Sparrow et al., 1978). These studies show that, by simple means, it is possible to enhance the oil biodegradation and restrict the negative environmental effects of oil, even in the cold climate of Arctic shorelines.

CONCLUSIONS

Comapared to more temperate ecosystems, the impact of petroleum hydrocarbons on microorganisms and microbial activities in Arctic ecosystems appears to be more variable. Often factors other than the presence of contaminating hydrocarbons appear to determine the response of microorganisms to oil contamination. In most cases the composition of the microbial community changes following contamination with hydrocarbons; generally numbers of hydrocarbon degraders increase and species diversity declines. However, there are instances where the elevation of hydrocarbon-degrading populations is transitory or delayed for some time following the input of hydrocarbons. In contrast to temperate zones where the numbers of hydrocarbon degraders may rise within hours or days (Atlas and Bronner, 1981; Gundlach et al., 1983), the increase in numbers of hydrocarbon degraders in Arctic ecosystems

may take months or years (Bunch et al., 1983a, 1983b; Haines and Atlas, 1982).

As with the effects on the levels of microbial populations the impact of oil on critical microbial activities varies. It appears in many cases that the localized environmental conditions of the ecosystem are important mediating factors. In some cases Arctic hydrocarbon contamination clearly reduces rates of microbial activities such as primary production, heterotrophic uptake (secondary productivity), nitrogen fixation, and polymer decomposition. In other cases, however, such interference with normal microbial functions have not been detected. It appears that oil contamination of Arctic ecosystems will not cause major widespread alterations in critical microbial functions, in part because oil spills are not likely to spread over wide areas of Arctic ecosystems. Permafrost and sea ice tend to restrict the spread of hydrocarbons and the areas of impact. Within heavily oiled localized regions, however, the normal contributions of microorganisms to ecological functions, which include energy transfers and nutrient cycling reactions, are likely to be disrupted.

With respect to the biodegradative removal of oil, this microbially mediated process will undoubtedly be slow in Arctic ecosystems. Both experimental oil release field studies, in Arctic tundra, freshwater, and marine ecosystems, and follow-up studies after Arctic and subarctic oil spillages indicate long persistence times for hydrocarbon contaminants and slow rates of microbial biodegradation. The slow rates of petroleum biodegradation in Arctic ecosystems are not due to a lack of indigenous hydrocarbon-degrading microorganisms. Virtually all Arctic ecosystems contain adequate numbers of naturally occurring hydrocarbon-degrading

microorganisms. Low temperatures alone also can not explain the limited rates of hydrocarbon biodegradation. Rather the limitation to microbial degradation of petroleum hydrocarbons in Arctic ecosystems appears to be due to nutritional considerations, that is, to the availability of nitrogen and phosphorus, and to a lack of oxygen. In many cases the lack of an adequate oxygen supply appears to be the major limiting factor. Compared to temperate ecosystems, Arctic tundra and coastal marine ecosystems are relatively stagnant; ice dampens reaeration due to wave action in marine ecosystems and standing water in tundra soils limits inputs of oxygen. The result is that, although the potential for hydrocarbon degradation exists, the actual rates of hydrocarbon biodegradation in Arctic ecosystems are slow. Eventually microbial hydrocarbon degradation can decontaminate Arctic ecosystems but the time frame after a major spillage will be decades rather than years.

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