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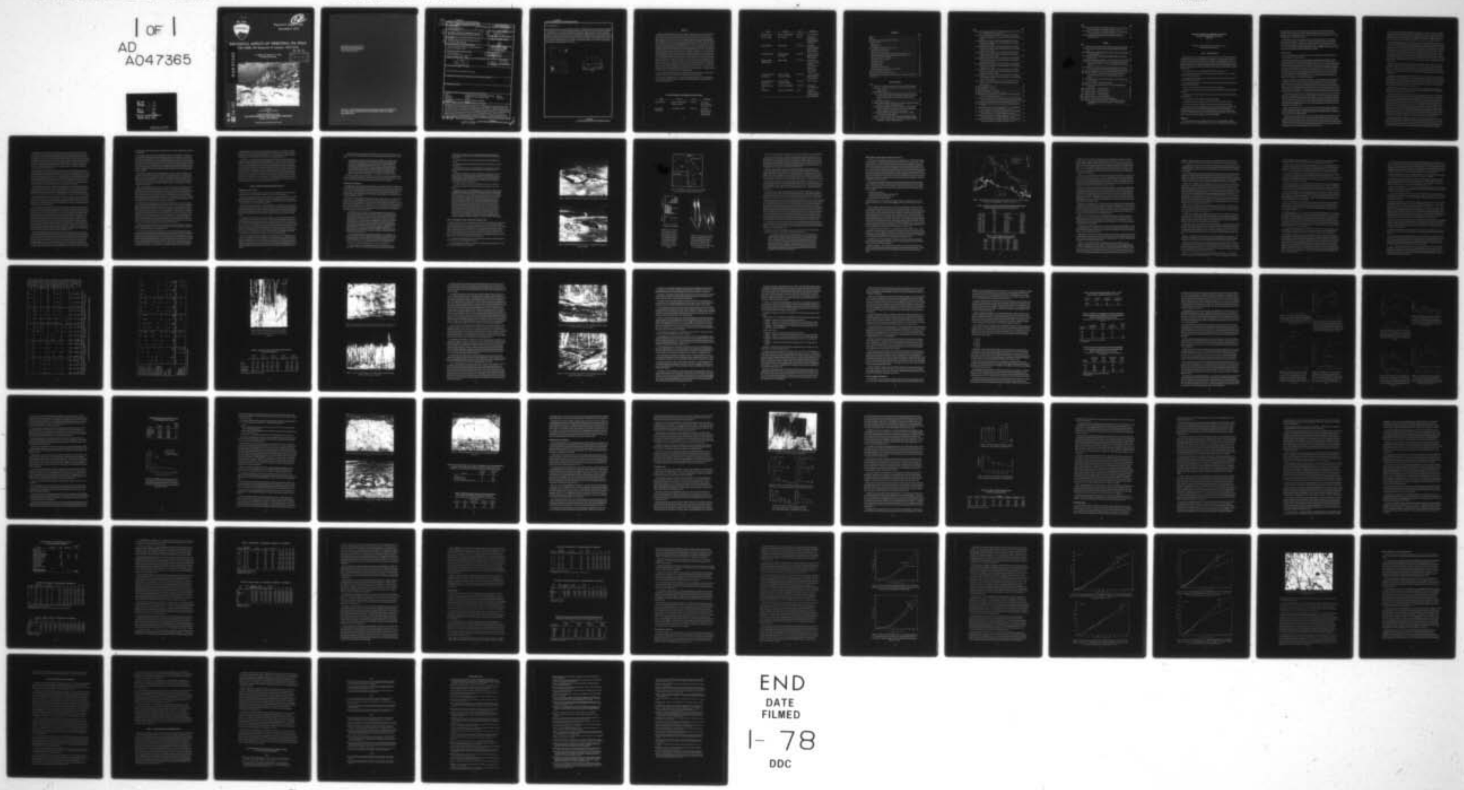

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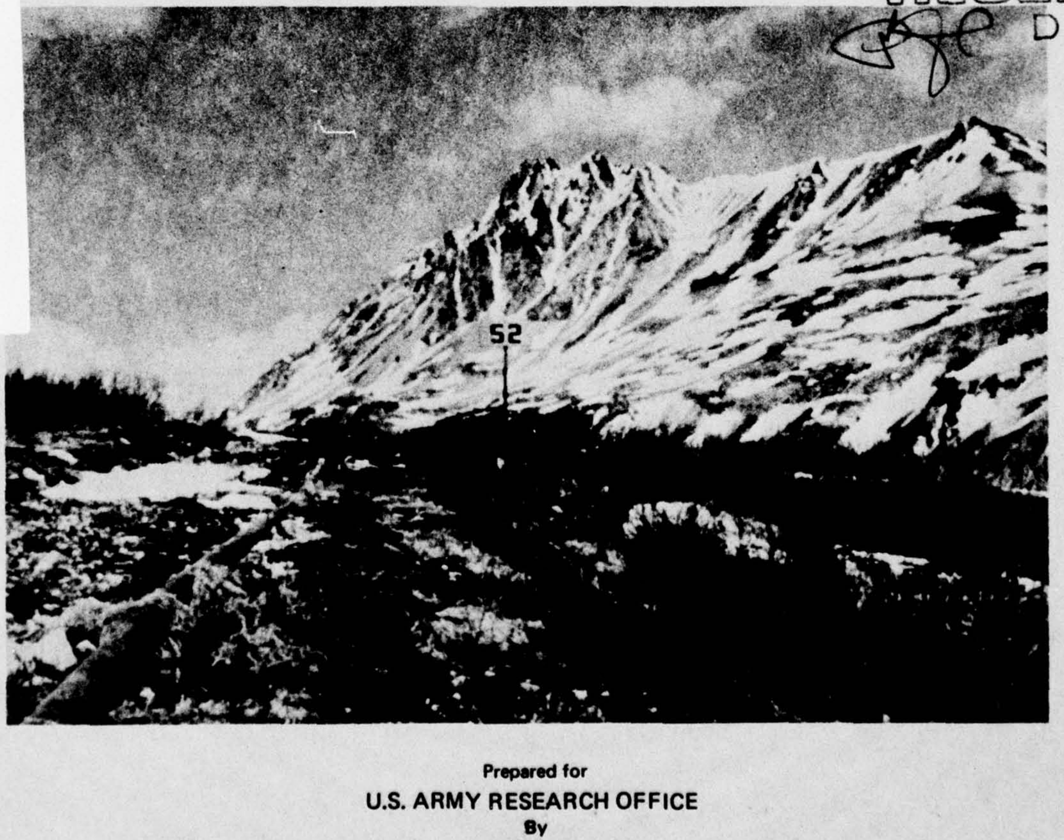
December 1975

BIOLOGICAL ASPECTS OF TERRESTRIAL OIL SPILLS

USA CRREL Oil Research in Alaska, 1970-1974

F.J. Deneke, B.H. McCown, P.I. Coyne,
W. Rickard and J. Brown



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Cover photo: Haines–Fairbanks pipeline near the highest point along the route (Milepost 52), in the vicinity of Three Guardsmen Mountain, British Columbia, Canada, at an elevation of approximately 1000 m.

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Knowledge concerning the biological effects of oil pollution on arctic and subarctic terrestrial ecosystems is limited. USA CRREL research personnel conducted investigations from 1970 through 1974 to expand information in this field. Objectives were to: 1) define the ecosystems most sensitive to the presence of crude oil or its refined products, 2) quantify and understand the injury response, and 3) establish time frames for manifestation of damage and natural restorative processes in arctic and subarctic regions. This was accomplished through: 1) surveys of natural oil seepages and past accidential spills in the Arctic and Subarctic, 2) initiation of controlled oil spills and 3) detailed laboratory investigations. Results demonstrated that terrestrial oil spills will to some degree be detrimental to both

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arctic and subarctic plant communities. Degree and longevity of damage will be influenced primarily by the magnitude of the spill, season of occurrence and existing soil moisture content. Rapid recovery of plant communities subjected to spills will occur only if root systems remain relatively unaffected. Damage will be more extensive and long-term when root systems are saturated with oil. Effects of damage will be manifested gradually over several seasons being influenced by winter stresses. Variation does exist in plant species susceptibility. *Carex aquatilis* a predominant sedge of the arctic is markedly resistant to crude oil damage. In the taiga *Picea mariana* is very susceptible. Plant recovery can be enhanced through the application of fertilizer. Fertilization, in addition to its direct effect on plant nutrition, will stimulate microbial decomposition of crude oil.

PREFACE

This report was prepared by Dr. Frederick J. Deneke, Assistant Professor of Forestry of Kansas State University, Dr. Brent H. McCown, Assistant Professor of Horticulture of the University of Wisconsin, Dr. Patrick I. Coyne, Plant Physiologist of the Lawrence Livermore Laboratory, Warren Rickard, Botanist, formerly of the U.S. Army Cold Regions Research and Engineering Laboratory (USA CRREL) and by Dr. Jerry Brown, Research Soil Scientist of USA CRREL. The work reported here was funded under DA Project 4A061101A91003, *USA CRREL In-House Laboratory Independent Research,* and by DA Project 2N061102B710, *Effect on the Ecology and Biochemistry of Oil Seepages and Spills,* sponsored by the Army Research Office, Life Sciences Division. The assistance and encouragement of Dr. Morthland of the Army Research Office throughout the period of investigation has been greatly appreciated. This project is considered a contribution to the U.S. IBP (International Biological Program) Tundra Biome program. Special appreciation goes to the Naval Arctic Research Laboratory, Institute of Arctic Biology, the University of Alaska, the Alaska Projects Office of USA CRREL located in Fairbanks, and to the U.S. Tundra Biome program for their cooperation. Special appreciation is due to Dr. Jim Anderson of the Institute of Arctic Biology who compiled an early and very comprehensive literature review on terrestrial oil ecology which aided in the literature review portion of this paper. In addition, the cooperation and assistance of British Petroleum, Inc., Alaska, Atlantic Richfield-Exxon and the Alyeska Pipeline Service Company in facilitating research observations and supplying the Prudhoe Bay crude oil is gratefully acknowledged. The contribution by Dr. Richard Schwendinger, Gulf Oil Corp. and Standard Oil Co. of Indiana, through his critical review of this manuscript, is gratefully appreciated.

The U.S. Army Petroleum Distribution, USARAL Support Command Office, provided valuable assistance and information in the Haines-Fairbanks pipeline phase of the study.

The contents of this report are not to be used for advertising, publication, or promotional purposes. Citation of trade names does not constitute an official endorsement or approval of the use of such commercial products.

USA CRREL PROJECT AND PERSONNEL INVOLVEMENT

Phase	*Location*	*Year*	*Individuals*
Literature Review	Hanover, New Hampshire Fairbanks, Alaska	1970-1971	Jerry Brown Brent H. McCown R. Paul Murrmann
Cape Simpson Investigations	Cape Simpson, Alaska	1970-1971	Jerry Brown Brent H. McCown Richard Haugen Richard McGaw

Phase	*Location*	*Year*	*Individuals*
Oil Detection Kit	Hanover, New Hampshire	1970-1971	R. Paul Murrmann
Barrow Oil Spills	Barrow, Alaska	1970-1971	Jerry Brown Brent H. McCown R. Paul Murrmann Don Vietor
Barrow Oil Spills	Barrow, Alaska	1971-1974	Jerry Brown Frederick J. Deneke Brent H. McCown Warren Rickard
Fairbanks Oil Spills	Fairbanks, Alaska Fox, Alaska	1970-1971	Frederick J. Deneke Brent H. McCown Warren Rickard
Haines-Fairbanks Pipeline Studies	Alaska, Canada	1971-1973	Lloyd M. Brown Frederick J. Deneke Patrick Hunt Fleetwood Koutz James Pope Warren Rickard John R. Troth
Fairbanks Laboratory Investigations	Fairbanks, Alaska University of Alaska	1971-1972	Frederick J. Deneke Brent H. McCown Deborah McCown
Fairbanks Laboratory Investigations	Fairbanks, Alaska University of Alaska	1973-1974	Patrick I. Coyne
Microbial Investigations	Hanover, New Hampshire	1971-1972	Patrick Hunt R. Paul Murrmann
Final Report	Hanover, New Hampshire	1974	Jerry Brown Patrick I. Coyne Frederick J. Deneke Brent H. McCown

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BIOLOGICAL ASPECTS OF TERRESTRIAL OIL SPILLS
USA CRREL OIL RESEARCH IN ALASKA
1970–1974

by

Frederick J. Deneke, Brent H. McCown, Patrick I. Coyne,
Warren Rickard and Jerry Brown

PART I. INTRODUCTION

During the period 1970 to 1974 research personnel of the U.S. Army Cold Regions Research and Engineering Laboratory (USA CRREL) were engaged in terrestrial oil pollution research in cold regions. The primary purpose of this work was to gain an understanding of the effects of petroleum spillage on cold-dominated terrestrial ecosystems, particularly plant species and communities. Most studies were conducted in Alaska although some were extended into the Yukon Territory of Canada, and the microbial investigations were conducted at USA CRREL in Hanover, New Hampshire.

The principal objectives of the original USA CRREL research program were:

1. To define the arctic and subarctic terrestrial ecosystems most sensitive to the presence of crude oil and its refined products.

2. To quantify and understand the injury response of these communities to the presence of crude oil and petroleum products.

3. To determine the time interval in which injury would manifest itself and in which natural restoration could be expected.

A variety of approaches were taken to meet these objectives including:

1. Surveys of accidental spills along an existing major petroleum products pipeline in Alaska and Canada.

2. Surveys of accidental petroleum spills on the Arctic Coastal Plain and in southern coastal regions of Alaska.

3. Surveys of natural crude oil seeps at Cape Simpson, Alaska.

4. Contained demonstration spills on the Arctic Coastal Plain and in interior Alaska.

5. Laboratory investigations utilizing controlled environment plant growth chambers.

The results of most of these investigations have been reported and published separately. The purpose of this report is to consolidate the many separate results into one document on terrestrial petroleum pollution in arctic and subarctic regions. The material contained in this report covers USA CRREL oil research completed through September 1974. Some of the work reported in the following pages appears as it was initially published.

Background

In 1969, full development of petroleum resources on the Arctic Coastal Plain of Alaska appeared imminent. Also looming was growing concern over the possible impact that accidental petroleum spills, associated with such development, might have on the cold dominated and potentially sensitive ecosystems of Alaska. Coupled with this concern was the ever increasing dependency upon fossil fuel reserves for energy. Thus a need existed for information on petroleum spill effects in arctic and subarctic regions.

USA CRREL researchers were among the first to recognize the importance of obtaining this information and to address the problem area. They also recognized that military operations in cold regions often result in the accidental introduction of petroleum products (gasoline, diesel fuel, jet fuel) into terrestrial and aquatic environments. Thus it was apparent that the U.S. Army was in need of information to develop regulatory and advisory capabilities in order to deal correctly with terrestrial oil pollution problems encountered in the field.

Literature review

Since USA CRREL research was restricted to terrestrial ecosystems, the following literature review concentrates on land based spills.

Literature about oil spill effects on terrestrial plant communities is limited and is even more limited for oil spill effects in northern latitudes (most research done prior to 1970 centered on spills in temperate regions). Absence of information is due in part to the past lack of environmental concern over accidental petroleum losses on land areas. It also should be recognized that full-scale petroleum resource development did not occur in arctic regions until the mid-1960's. Considering these factors, there follows a review primarily of the existing literature prior to 1970 on studies from both accidental and planned petroleum introduction into terrestrial ecosystems. Research other than CRREL's was conducted during the 1970-1974 period, but rather than include it as a part of the literature it was elected to insert most of it into the results as this work either was parallel to or complementary to CRREL investigations.

Russell and Hutchinson (1909) studied respiration of soil microorganisms as influenced by the introduction of toluene, a petroleum product. Their results indicated that an increase in respiration could be generated by introducing toluene into the soil system. In 1919 Carr studied the effects of oil upon soybean growth and found that growth could be enhanced with the addition of small amounts of oil, although detrimental effects were noted at higher treatment levels. Carr speculated that the latter resulted from oil interference with normal water uptake by the plant. He also speculated that small quantities of crude oil stimulated root nodule development, enhancing growth.

Several German scientists in the early 1900's recognized and reported on the action of soil microorganisms in breaking down petroleum products (Sohngen 1913, Tausz and Peter 1919). Some of their research involved the identification of such organisms. This work was followed by Baldwin in 1922 who found that petroleum products in the soil changed the species compositions of the various microorganisms normally present. He further observed that while the total number of species declined, the overall population increased. This increase probably represented those species using hydrocarbons as an energy source.

Murphy (1929) studied the effects of petroleum on nitrate production, seed germination, and growth of wheat in pot culture. His results indicated that even slight applications of petroleum interfered with nitrate production, delayed seed germination and inhibited growth. At higher rates seed germination was completely lacking.

Ginsberg (1931) observed the effects of oils on plant tissues (apples, peaches, tomatoes). Oils of varying viscosities were applied to leaves. He found that the undersurfaces of leaves were more rapidly penetrated than upper surfaces, and penetration was inversely proportional to oil viscosity. Ginsberg also followed oil uptake in severed stems and found that oils could move through the xylem elements. The amount of movement was again dependent upon viscosity, e.g. greatest movement was in the lighter oils. He found that applications on bark had no effect.

Work on oil-soil microbial relationships was renewed by Stone in 1941. In studies of the degradation of crude oil by soil bacteria, he found no special group of organisms involved and that all common soil microorganisms have the ability to adapt to many different organic compounds. He did find that oils varied in their degree of susceptibility to bacterial attack. Lighter oils were oxidized more readily, as were paraffins when compared to the aromatic or naphthenic fractions.

Plice (1948) investigated the effects of three types of crude oil (paraffin base, asphalt base, basic sediment) on soil fertility. He also studied soil changes due to petroleum and was one of the first researchers to recommend methods for reclaiming soils contaminated with oil residues. He observed yearly production of experimental crops planted in lightly oiled soils. Under the study conditions, very little growth retardation was noted. Plice felt that growth retardation was related to the detrimental effects of oil on soil moisture and aeration relationships, and very little was due to direct toxic effects. He also noted that during the third growing season after contamination, production rates were higher than normally expected. Further work suggested that this increase was related to a decrease in the oxidation-reduction potential of the soil and an increase in the number of nitrogen-fixing microbes. He postulated that these microbes were important in converting petroleum to soil organic matter.

Plice also observed soil-plant relationships associated with actual pipeline spills. He found that the degree of oil penetration was inversely related to soil moisture content. He pointed out that the degree of saturation was also related to the amount of oil spilled, topography and soil texture. He also noted that petroleum degradation was enhanced by warm weather and that areas either unproductive or of low productivity for several years following a spill eventually became more productive than before.

These results were followed with further experimental plot work. Plice recognized certain filtering capabilities of the soil with respect to crude oil and he found that colorless and low viscosity oil fractions penetrated to the lowest depths. He allowed the heavily oiled plots to regenerate naturally, periodically cultivating half of the plots. Revegetation was delayed almost three years on the cultivated halves of the plots and four years on the uncultivated halves, pointing out the value of cultivation in enhancing oil breakdown and soil restoration.

Hunt (1957) observed oil pollution effects on the shores of Lake Michigan and found that, although large areas of marsh vegetation were killed initially, they gradually became re-established to their normal state during a period of four years.

Ellis and Adams (1960, 1961) reported the results of their studies on the effects of petroleum hydrocarbon contamination upon the microbial, chemical and physical properties of soils and plant growth. Most observations involved natural gas contamination. Their studies of hydrocarbon effects on soil properties indicated that, with increases in petroleum contamination, organic matter, total carbon, and nitrogen were increased. This was attributed primarily to microbial activity. A decrease in the oxidation-reduction state of the soil was noted which was also attributed to microbial activity. This was accompanied by an increase in the solubility of iron and manganese compounds present. Their studies on natural gas-contaminated soils indicated that soil pH increased in acidic soils and decreased in basic soils, indicating that natural gas contamination results in a buffering of the soil towards neutrality. Lastly they observed that available phosphorus increased in soils contaminated by natural gas. This increase was deemed a result of more favorable pH levels and increased reducing conditions enhancing the yield of iron phosphates in the soil solution.

Ellis and Adams' studies of soil physical properties, as affected by severe petroleum saturation, indicated that disaggregation and dispersion of soil particles resulted, making the soils very resistant to wetting; however, it was found that once the soil was wetted, water retention was enhanced. The latter condition suggested that more water should be available for plant growth. Soil bulk density was usually lower and soil porosity (usually restricted to the micropores) increased under high petroleum saturation levels. They postulated that these changes resulted from increased organic matter levels. In reviewing their work and the work of others with respect to soil contamination by natural gas or crude oil and resultant plant growth, they concluded that plant growth was usually eliminated or reduced for a period of time, after which vegetative productivity may be enhanced over that of uncontaminated soils. They felt that the early restriction of plant growth was due to the abundance of manganous ions resulting from the reducing conditions created in contaminated soils (since higher levels of available manganese can be toxic to plants). The condition of enhanced growth at later stages was attributed to 1) increased organic matter, 2) enhanced water holding capacity, and 3) increased nitrogen levels.

Kloke and Sahm (1961) and Kloke and Leh (1963) reported on studies involving petroleum products (fuel oil and various mineral oils) and their effects on soil properties and plant growth. They found that small amounts of fuel could reduce the growth of rape to a considerable degree. Attempts made to reverse the adverse effects of the oil by adding binders (straw, peat) and microorganism substrate (sugar) materials proved unsuccessful. Acceptable solutions put forth for cleanup of oil-contaminated areas were the mixing of additional amounts of uncontaminated soils (reducing oil concentration) and the removal and replacement of contaminated soils with new substrate material. It was postulated that natural regeneration could take years, possibly even a decade.

Kloke and Leh (1963), in their experiments with various mineral oils, found detrimental effects on plant growth increased with oil viscosity. It was held that the more viscous oils were retained in the upper portion of the soil horizon for longer periods and the more mobile less viscous oils were readily leached out of the upper soil layers. Seed germination was found to be inhibited more by the low viscosity oils. This was believed due to their ability to penetrate the seed coat more readily.

Dobson and Wilson (1964) reported the results of studies on respiration rates in oil-contaminated and oil-free soils as a function of microbial activity. They showed that the cultivation of contaminated soils could stimulate soil microbe activity with a resultant breakdown of hydrocarbons. This was attributed to increased soil aeration as well as moisture and heat penetration through cultivation which stimulated soil microbial activity.

Grummer (1964) investigated the results of an accidental crude oil pipeline break in the Netherlands. The spill affected approximately 10 hectares of arable land and pasture underlain by a sandy soil. He observed that, although a significant portion of the crude oil seeped into the soil, the more volatile fractions were evaporated rapidly prior to penetration. The spill area was studied for three years. Measures were taken to enhance soil fertility and to monitor damages and changes in the physical and chemical properties of the soil. It was found that in spills of any magnitude, the removal and replacement of soils was not feasible. It was noted that in one area of the spill, where soil was more recently cultivated, less oil accumulated. This was attributed to deeper penetration of heat and oxygen, resulting in a greater evaporation of volatile fractions. Once volatile fractions were lost, remaining crude oil fractions were found to be nontoxic to plant growth. Grummer felt that the major considerations in such spill situations would be the restoration of wettability of the soil and its fertility. This could be done by tillage and addition of organic matter. In this particular situation normal soil-plant relationships were restored after three years.

Schwendinger (1968) published an analysis of the various problems associated with reclamation of oil-contaminated soils. He pointed out that there was a lack of published information of health hazards, land damages and cleanup procedures associated with terrestrial oil pollution. Summarizing the prior literature, he concluded that based upon previous work, small amounts of mineral oil or oil products were not harmful in soil and, in fact, may be beneficial to plants. Large amounts of oil damaged plants, not from toxicity but through the formation of anaerobic and hydrophobic conditions which interfere with plant-soil-water relationships. He felt that the actual level at which oil would damage plants was uncertain, but it probably was above 1 kg oil/m^2 of surface. He noted that, although reclamation practices had received little attention, liming, fertilization and manuring were beneficial.

Schwendinger conducted laboratory experiments on plant growth in relation to oil contamination and resultant damage and symptoms exhibited by plants. He concluded that: 1) even sensitive crops such as vegetables could tolerate considerable quantities of crude oil in the soil; 2) the amount of crude oil tolerated by a crop was species dependent; 3) oil pollution symptoms exhibited by plants were typical of those exhibited in cases of extreme nutrient deficiency; 4) since oil pollution symptoms were inversely proportional to water uptake, plant damage was probably due to disruption of plant-soil-water relationships; and 5) oil pollution damage to plants could be minimized by heavy fertilization. The latter was attributed to simple mass action forcing the necessary nutrients into the plants.

Schwendinger also felt that the microbial breakdown of petroleum could be of benefit in site restoration. He noted that while microbes capable of decomposing petroleum are present in the soil, it is still not their normal substrate and thus a considerable period of time is required for the adaptation of microbial populations to petroleum. He postulated that this time period could be reduced by inoculating the soil with previously adapted organisms. From the results of laboratory experiments it was concluded that this method might be possible for field application, especially if coupled with fertilization.

Baker (1971) published the results of her work on petroleum pollution of salt marsh plant communities in England. This work involved natural vegetation, in contrast to most previous work which dealt with cultivated species and laboratory studies. Her studies of a single oil spill indicated that damage was short-term and was followed by plant regrowth from the roots. Most annual plants, during the recovery period, could be expected to exhibit reduced germination and flowering. With some species, growth stimulation resulted. As a rule, species most susceptible to damage were annuals, having smaller underground root systems and associated smaller food reserves. In successive spillages certain species showed greater resistance than others.

Oil pollution was also followed on a seasonal basis and damage was more severe during the hot summer months. Oil-soil relationships were also discussed. Baker pointed out that certain plants allow oxygen diffusion downward from their shoots into the roots. Thus the supply of oxygen is hindered by the application of oil to the plant shoots. Baker also studied comparative toxicities of oils and oil fractions and the effects of oil on physiological processes in plants. Her results indicated that the more volatile fractions of crude oil were more toxic, and thus fresh crude was more damaging than weathered crude oil. In respect to plant physiology, it was theorized that oil penetration into a plant disrupts cell membranes, reduces transpiration rates (stomatal blockage), increases respiration (possibly from mitochondrial damage), and inhibits translocation of water and nutrients. The severity of all of these effects would be dependent on the amount of oil, its chemical properties, environmental conditions and the species of plant affected.

The preceding paragraph describes the effects of petroleum upon plants themselves. However, the more volatile toxic fractions of any petroleum spill are lost very rapidly and have no long-term role in controlling plant growth in contaminated soils.

In summary, the works previously cited show that in oil-contaminated soils microbial populations are changed; total number of microbial species decreases while the total number of organisms increases. The question still unresolved is whether a change in microbial numbers and species affects plant growth, or whether the presence of remaining petroleum fractions brings about resultant changes in the chemical and physical properties of the soil which then affect plant growth. If both occur, to what degree is plant growth affected by each? With respect to changes in physical soil characteristics that initially occur, organic matter is increased, soil particles become disaggregated and dispersed, soil aeration is decreased and water wettability is interfered with. However these conditions generally improve with time. Chemical changes include: 1) the creation of reducing conditions, 2) the forcing of soil pH towards neutrality, 3) a decrease in the oxidation-reduction potential of the soil, 4) an increase in the solubility of iron, 5) an increase in available manganese, 6) an increase in nitrate production, and 7) an increase in available phosphorus.

It is felt that one reason for the decline and death of plants in petroleum-saturated soils is the manganese toxicity resulting from its increased availability. It is also known that seed germination is delayed or inhibited in oil-contaminated soils and that the suspected causes are disrupted soil moisture regimes and possibly ion toxicity. Attempts at reclamation of petroleum-contaminated soils in temperate regions have been generally successful with cultivation (mixing for oxidizing conditions), fertilization, and liming. It is also recognized that periods of enhanced growth can occur at the latter stages of oil degradation. This is generally attributed to an increase in nitrogen and the better water retention by the soil. Some recent investigations (Guseynov 1964, Popoff 1966, Severson 1970, Fattah and Wort 1970, Gudin 1973) indicate that the alkaline extracts of oil have various biological effects and are complex mixtures of molecules which can possibly have auxinic, gibberellinic or inhibitory effects. Gudin (1973) has put forth the hypothesis that the presence of these plant growth regulators in oil is linked to its biological origin.

PART II. CRREL INVESTIGATIONS FROM 1970-1974

Initial literature survey (1970)

A literature survey conducted in late 1969 and early 1970 by B.H. McCown, J. Brown and R.P. Murrmann appeared as a USA CRREL Technical Note in April 1970 (unpublished).

This report provided background information necessary for initiating field and laboratory investigations in Alaska during 1970. It included information on oil pollution in marine environments, cleanup procedures known and established, and information deemed important in determining the effects of oil spills on arctic and subarctic ecosystems.

This survey pointed out the dearth of research information on oil spills and cleanup procedures in temperate and, particularly, in arctic and subarctic regions of the world. Most of the information was gleaned from studies of oil spills in marine environments (tanker and offshore well losses) and the use of refined petroleum products in pesticide formulations. The fate of petroleum in marine environments was discussed and cleanup procedures known at that time were reviewed in some detail.

A theoretical section of the report concerned problems of contamination and subsequent cleanup procedures in arctic and subarctic ecosystems. Critical air-ground heat balances in permafrost areas and possible severe local perturbations that could result from an oil spill were of prime concern. Thus, it was postulated that destruction of the insulating vegetative mat combined with black body absorption of solar radiation could result in increased thaw and permafrost degradation. Possible toxic effects of oil on tundra vegetation and living systems in fresh water streams were also discussed. In considering the effects of oil on plants, it was theorized that some arctic species were more resistant to oil contamination than others. The occurrence of tundra species surrounding natural oil seeps in northern Alaska was cited as evidence for this (Hanna 1963).

Various cleanup procedures were considered for arctic regions. The use of dispersants was discounted due to possible toxic effects and alterations of the natural ecosystem. Burning was mentioned as an alternative; however the ubiquitous wet conditions on the North Slope were seen as a limitation to this procedure, as was the increased thaw that might result in permafrost soils if the insulative vegetative mat were altered or removed. Nontoxic coating and absorbing agents were discussed as plausible alternatives in preventing the spread and reducing the contamination of oil to wildlife.

The literature survey concluded with a discussion of the relevance and need for more work in cold regions. This contained much of the rationale which governed the research work reported here:

> Considering not only the recent oil discoveries in Alaska but also various complex military operations such as the U.S. Army's Haines-Fairbanks petroleum pipeline the occurrence of oil spills on arctic and subarctic terrain is highly probable. The importance of each spill will be dictated by its location. Those in the vicinity of concentrations of wildlife and near fresh water supplies are potentially disastrous. The obvious best course of action is to develop an efficient technology in oil pollution control prior to the occurrence of any major oil pollution incident in the Arctic. In any case, we should not create more destruction in haphazard and unadapted cleanup attempts than would have occurred had we just left the oil alone.

The survey was updated during 1970 (McCown et al. 1971a). Essentially it covered previous research on the effects of oil pollution on terrestrial ecosystems and is not detailed here as it would be a repeat of portions found in the literature review section of this current report.

Oil detection kit development

It is appropriate to note that during the initial literature review phase (1970), Dr. Paul Murrmann (USA CRREL) developed a field-expedient technique to analyze for the presence of petroleum products in soil and water (unpublished). The method utilized the principle that molecular compounds present in crude oil and most petroleum products fluoresce when exposed to ultraviolet light. A portable, battery operated kit based upon this principle was assembled for use in evaluating oil contamination problems in the field.

The approach for analyzing the petroleum products was to extract oil from soil or water using a suitable solvent. For semiquantitative analysis, a series of standard solutions containing known amounts of petroleum was prepared. Test samples were extracted using the same solvent. By matching the fluorescent intensity of the test sample to that of a standard within an ultra-violet light box, it was possible to estimate the amount of oil present. The exact procedure for each use depends on the type of test and test conditions. Example procedures used successfully by USA CRREL for these different applications are indicated below.

> *Determination of petroleum in soil.* The following procedure has been used to detect oil contamination, to evaluate methods for cleanup of oil-contaminated soil, and to determine the extent of movement of oil in soil under different environmental conditions. In evaluating a number of solvents for use in oil extraction, cyclohexane was found to be most suitable. Cyclohexane does not fluoresce, is relatively nonvolatile, and extracts oil from soil with a high degree of efficiency. It has been determined that the soil organic matter extracted does not interfere with the procedure, as was previously a serious problem with less sensitive, tedious, gravimetric methods employed for analysis of oil contamination of soil. It is possible to determine as little as 10 μg of oil per g of soil using the suggested procedure. Care should be taken to avoid contamination with "fingerprint" oil during the procedure. Any contaminating substance such as asphalt from roadways near the test site will seriously interfere with the test.
>
> A. Prepare a series of standard solutions containing from 10 to 10,000 μg of oil per ml of cyclohexane solvent. In preparing the standards, use the same oil contaminating the test area. If this is not practical, use oil as similar as possible to the type of contaminating oil. It is not necessary to add soil to the standard solutions since most of the oil remains in the cyclohexane phase and the test is semiquantitative.
>
> B. Mix 5 ml of cyclohexane solvent with 5 g of oil-contaminated soil.
>
> C. Using a capillary tube, carefully place one drop of each standard solution on a filter paper. Attempt to contain the solution within as small an area as possible.

An alternative procedure is to saturate small filter paper disks (prepared using a paper punch) with the test solution. The solvent evaporates in both cases leaving an oil residue.

D. In the same manner as (C) above, prepare another filter paper using the test solutions.

E. Place the filter papers containing the standards and test samples into the ultraviolet light box.

F. Estimate the amount of oil by matching the fluorescent intensity of the test sample with that of a standard. It may be necessary to interpolate between two standards.

Determination of petroleum in water. The following procedure has been used to detect oil and to determine the amount of oil present as a surface film or as an emulsion in water. In preparing standard solutions, use oil of the type suspected of contaminating the water, being careful to avoid contamination with fingerprints, etc. during the procedure. As little as 0.1 μg of oil per ml of water can be determined using the procedure outlined below.

A. Prepare standards containing 10 to 10,000 μg of oil per ml of cyclohexane.

B. Prepare test samples by extracting 100 ml of test water with 1 ml of cyclohexane.

Identification of type of contaminating petroleum. In addition to detection and semiquantitative analysis of petroleum in soil and water, the test kit can sometimes be used to tentatively identify the type of contaminating oil present. This determination is based upon the chromatographic process which occurs when concentrated or bulk oil is placed on filter paper. The chemical composition of oil varies. When a drop of oil is placed on the filter paper, the various chemical components move away from the point of application at different rates depending on the degree of interaction with the filter paper. Thus, a fluorescent pattern with characteristic size, intensity, and hue is produced for each type of oil. If the oil from the test area can be matched with known samples collected from suspected sources, it is possible to obtain a preliminary identification of the source of oil contamination. If bulk oil cannot be collected from the test soil or water, it may be possible to examine the oil residue remaining after evacuation of a solvent extract. However, care should be used in this approach since aging of oil in the environment may change its fluorescent properties. The above field expedient technique can obviously be made more sophisticated by employing various chromatographic stationary phases and different solvents in development of the characteristic chromatogram.

Survey of Cape Simpson, Alaska, natural crude oil seepages (1970)

Natural crude oil seeps occur at various locations on the Arctic Coastal Plain and, despite knowledge of their existence for better than 70 years, research on these has been primarily geological, with some observations on animal entrapment and bone preservation in the tar (Fig. 1 and 2). Most notable of these reports is that by Hanna (1963). These seeps represent a situation in which oil and its residues have been in contact with the local environment during an extended period of time, offering a natural setting for the observation of crude oil contamination effects in arctic regions. A series of natural seeps near Cape Simpson (Fig. 3) was studied during the summer of 1970 (McCown et al. 1971a). Their objectives were the following:

1. To determine the long-term reaction of tundra plant species and soils to the natural presence of crude oil and its microbial degradation products.

2. To gain information on which arctic plant and microbial species may be adapted to the presence of a hydrocarbon-saturated rhizosphere.

Figure 1. Aerial view of a natural occurring crude oil seep, Cape Simpson, Alaska.

Figure 2. Bull caribou trapped in the tar residue of a natural crude oil seep, Cape Simpson, Alaska.

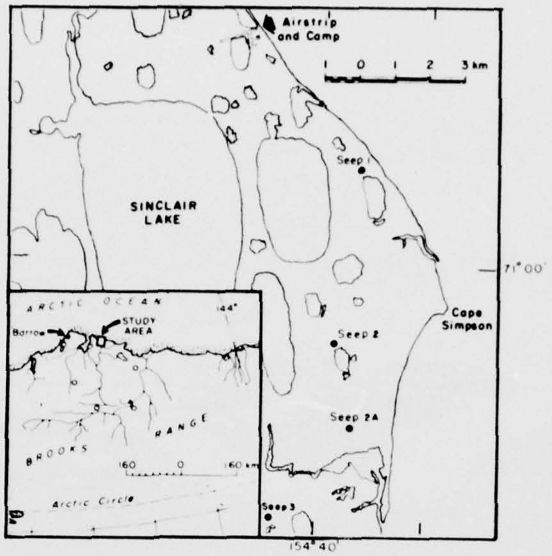

Figure 3. Cape Simpson oil seeps. Locations of a number of oil and gas seeps are indicated (McCown 1971).

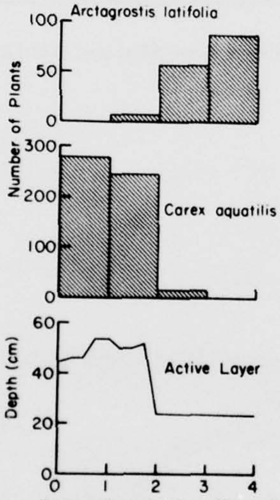

Figure 4a. The correlation between depth of soil active layer (depth of seasonal thaw) and transition from a Carex aquatilis *community to an* Arctagrostis latifolia *community in a transect along the edge of a natural oil seep at Cape Simpson, Alaska.*

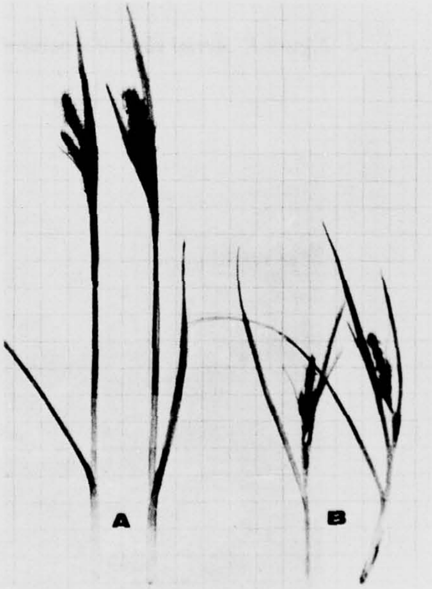

Figure 4b. Growth of Carex aquatilis. *A)* Carex aquatilis *plants showing increased growth and advanced phenology when growing at the edge of the seep; B) as compared to the plants unaffected by the seep (McCown, Brown and Barsdate 1973).*

Due to the relevant findings of this research effort, it will be reported here in considerable detail:

The seeps consisted primarily of an asphaltic material creeping downhill in elongated streams on gently sloping ground, terminating at the shores of small lakes or lagoons. New oil flows were most often in the center of the asphaltic areas but occasionally were found in peripheral areas, frequently flowing along ice wedge contraction cracks into the surrounding tundra. Large areas to the sides of these flows had sparse vegetation over well-oxidized asphaltic materials, suggesting that major active seeps and flows were once in positions different from present flows.

The seeps provided evidence of successive stages in natural oxidation and degradation of oil. Fresh oil was found to be quite fluid but it eventually formed into tar through the evaporation of volatiles. With weathering, an asphalt was formed which appeared to be quite resistant to further changes. When oil, tar or asphalt came into contact with free water, degradation into a gummy light brown material and eventually into a loose brownish humus occurred. The latter appeared to be a water-oil emulsion, with water as the continuous phase. Microorganisms were evident in this emulsion.

When compared to undisturbed surrounding tundra in the area, flows were found to be 3° to 5°C warmer during the day and 1° to 2°C warmer at night at depths of 10 cm. Depth of thaw was measured along separate transects and thaw was greatest near the seep (Fig. 4). Thaw abruptly decreased between 2 and 3 m from the edge of the seeps. Changes in that area were coincident with changes in vegetation.

It was theorized that greater thaw depths observed near the seeps resulted from the creation of a "thaw bulb" by the seep. Here energy absorbed by the black surface of the asphalt, and possibly direct underground heat input by new oil flows, resulted in the thaw of the surrounding permafrost. Drainage of water from the surface of the seep and along its edge aided formation of such a bulb.

Plant species growing in close association with the seeps, both at the periphery and when entrapped by flows, included *Eriophorum scheuchzeri* and *Carex aquatilis*. Plants surrounded by the seeps had their root systems embedded in the tar with no visible deleterious effects. Representative plants of both species growing in areas where flows were in contact with water exhibited more vegetative growth and were more advanced phenologically when compared to plants in the surrounding tundra. Whether this was the result of higher ground temperatures around the seep or other factors, such as nutrient availability or presence of stimulatory chemicals, is unknown. Only when plant foliage was covered with an oil deposit was injury to the plants noticeable. Petroleum hydrocarbon content levels of soils adjacent to seeps were insignificant (< 0.1 mg oil/g wet soil, except where tar was encountered in the profile). Based on the previously reviewed findings (Carr 1910, Ellis and Adams 1961, Schwendinger 1968), this would probably not have a strong influence on plant growth.

Gradual revegetation of inactive seeps was observed. Successional stages appeared to be pioneer lichens and mosses, followed by pockets of *Carex* and *Eriophorum* where water was entrapped and finally to an *Arctagrostis* community when soil conditions became colder and seasonal thaw became shallower due to increased plant cover.

Although not included in the published report, the following is an excerpt from the closing statement of a preliminary draft which seems appropriate and pertinent:

> Injury to plant foliage as well as the physical disturbance of the thermal balance of tundra observed at the seeps may be common results of actual oil spills on arctic terrain. However, the degree to which these effects occur may be more pronounced than was observed at the seeps. The evaporation of volatile fractions of the crude oil, often containing toxic components may have been an important factor at the seeps in reducing short-term damage. Less evaporation before contact with foliage would be expected during actual spills. Physical perturbations, even when first deemed minor, may cause extensive damage to ice-rich tundra and result in permafrost degradation and subsequent erosion. Such disturbances will be an important consideration in subsequent cleanup attempts.

Haines-Fairbanks military pipeline investigations (1971-1973)

The Haines-Fairbanks pipeline was chosen for study beginning in 1971. The line was of interest for several reasons: 1) it was the only major pipeline in the subarctic traversing a variety of vegetation and soil types; 2) it had construction stages both above and below the ground; 3) it was constructed without a protective coating to prevent corrosion (protective coating was available at the time of construction); 4) forty-one recorded spills had occurred at various locations along its route since 1956; 5) spills were of different ages and sizes and on varying sites, which provided opportunity for assessment as to long- and short-term effects on various ecosystems. Partial results of these preliminary investigations have been published previously (Rickard and Deneke 1972, Hunt et al. 1973); however, other results have not been reported. Due to the considerable importance of these investigations and in an attempt to bring all of the information gained into proper perspective, the following detailed summary is presented.

The Haines-Fairbanks pipeline originates in the southeastern Alaska deepwater port of Haines, traverses 1008 surface kilometers through Canada and Alaska and terminates at Fairbanks, Alaska (Fig. 5). It was constructed in 1955-56 with pipe 7 mm thick and 20 cm in diameter. It carried a variety of refined petroleum fuels to military bases in Alaska. The primary fuels pumped through this line included:

1. Diesel Fuel, Grade DFA
2. Aircraft Turbine and Jet Engine Fuel, Grade JP-4
3. Automotive Combat Gasoline, Grade 95C
4. Aviation Gasoline, Grade 114/145

Increasing maintenance costs and decreased strategic importance, combined with recent environmental considerations over recurrent accidental spillages, resulted in the shutdown of the line in 1972.

Upon completion of pipeline construction in 1955, water was pumped into the line for hydrostatic testing. With all systems working, the U.S. Army assumed command of the pipeline operations in October. However, when petroleum was introduced into the line, it would not pass through the pipe. Water used to test the line had not been completely removed and had frozen inside the pipe, blocking petroleum flow in several locations. In January of 1956, efforts were initiated to determine the locations of the ice blockages. When located, the pipe was cut and the line then was purged and the fuel allowed to run onto the frozen surface. In some instances attempts were made to construct catchment basins but these proved impractical because of the frozen ground. The rate of flow from the open end varied, but often exceeded 500 barrels (bbl) per hour. The exact amount of fuel loss could not be assessed with any degree of accuracy. However, unofficial estimates have been put at 50,000 bbl of fuel lost during deicing operations at 28 known cuts.

The methods used in the deicing work were experimental. The main objectives were: 1) clearing of the line as soon as possible; 2) conservation of the product; 3) protection of natural resources; and 4) safety of personnel. Whenever possible, cuts were made away from inhabited areas and with consideration given to drainage systems. In at least one instance, fuel was burned off. This method was later discarded and the fuel was left to dissipate through natural processes.

In addition to the earlier losses resulting from the deicing operations, ruptures of the pipeline have occurred during its operational history. Of these, six were attributed to corrosion and the remaining seven were caused by man (Table I).

Initial reconnaissance – 1971. Between 12 and 16 June 1971, the entire pipeline route was traveled to locate known pipeline breaks and qualitatively assess damage done at each site. Following are the locations that were found and the observations as reported by Rickard and Deneke (1972):

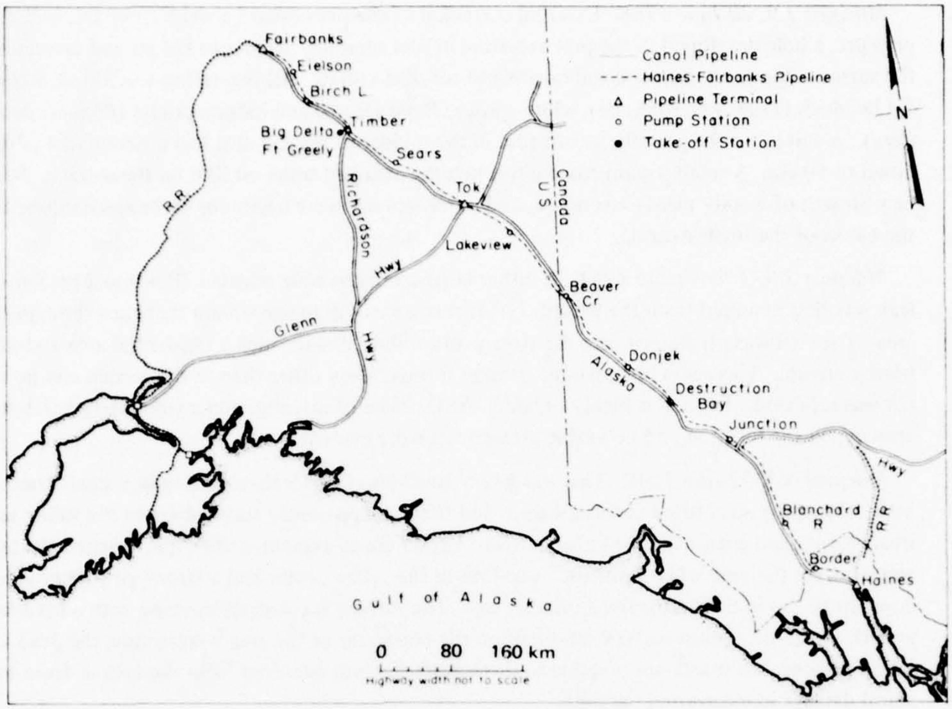

Figure 5. Haines-Fairbanks military pipeline route from Haines terminal to Fairbanks adjacent to the Haines and Alaska Highways (Rickard and Deneke 1972).

Table I. Accidental petroleum losses along the Haines-Fairbanks military pipeline (excluding 1956 deicing losses) (Rickard and Deneke 1972).

	Pipeline milepost locations	*Cause*	*Loss*
Spring 1956	33.5	Bullet hole	Unknown
3 Nov 1964	3.0	Corrosion	Unknown
15 Dec 1967	420.0	Vehicle hit valve	Unknown
Summer 1967	585.0	Power pole auger	Unknown
17 May 1968	119.1	Corrosion	4000 bbl
20 June 1968	1.9	Corrosion	100 bbl
14 July 1968	114.5	Bullet hole	200 bbl
22 July 1968	6.5	Corrosion	50 bbl
3 Dec 1968	17.7	Corrosion	800 bbl
18 May 1969	511.0	Bullet hole	Unknown
12 June 1969	290.0	Bullet hole	100 bbl
28 Sept 1970	19.5	Corrosion	1800 bbl
Oct 1972	580.0	Corrosion	Unknown

Table II. Locations selected for intensive studies (1972), Haines-Fairbanks military pipeline.

Pipeline milepost	*Elevation (m)*	*Slope (%)*	*Aspect (degrees)*	*Parent material*
1.9	122	15	180	Alluvium
119.0	730	20	082	Bedrock
197.1	820	25	060	Alluvium
207.6	790	6	035	Alluvium
217.0	820	3	085	Alluvium

Milepost 1.9, 20 June 1968. External corrosion of the pipe caused a weak spot and, under pressure, a hole developed in the pipe resulting in fuel spraying 30 m into the air and saturating the surrounding vegetation and soil downwind for 30 to 60 m. All vegetation was killed, including hemlock (*Tsuga heterophylla*), white spruce (*Picea glauca*) and balsam poplar (*Populus balsamifera*). A soil pit in the gravelly substratum in the middle of the kill area had a strong fuel odor down to 50 cm. A small stream passing through the area had some oil film on the surface. Some new growth of woody plants was noted, and a few annuals were beginning to be re-established on the banks of the small stream.

Milepost 3.0, 3 November 1964. A rather large corrosion hole released JP-4 into a garden. The leak was first detected from the presence of fuel in a small drainage stream that runs through the area. The fuel was discharged into the stream and followed it through a residential area and into a nearby stream. There was no apparent damage to vegetation other than in the garden and no fish kill was reported. The soil is highly organic. At the time of investigation a strawberry patch was growing on the location and no visible aftereffects were evident.

Milepost 6.5, 22 July 1968. This was a very small corrosion leak occurring in a small drainage basin. The pipe was buried at this location and the fuel apparently traveled down the valley into a small stream and eventually into a large river. Repair crews excavated the pipe, repaired the leak and reburied the pipe when finished. A soil pit in the valley center had a strong petroleum odor down to 60 cm in the fine-grained mineral soil. The surface is currently covered with a luxuriant growth of brome. Seven to 10 small birch on the perimeter of the area were among the dead vegetation present, but it was not possible to distinguish if death occurred from the spill or from mechanical damage in excavating the pipe.

Milepost 17.7, 3 December 1968. A small corrosion leak in a buried portion of the pipe resulted in an estimated loss of 800 bbl of fuel. Some large cottonwoods and alders in a nearby depression exhibited severe die-back symptoms apparently from the effects of the fuel. Two of 10 large cottonwoods had a few leaves in some upper branches. In addition, a number of new branches were growing from the bases of the alders. However, the interior of the stand was devoid of lower canopy vegetation, whereas adjacent areas showed luxuriant growth.

Milepost 19.5, 28 September 1970. An Alaska Highway Department employee came upon a pipeline rupture soon after it began and closed a valve that was within 100 m of the break. The spillage flowed directly into a small mountain stream and into other river tributaries. Fisheries biologists found some fish kill and damage to spawning beds and bottom life. Vegetation along the stream appeared to be undamaged.

Milepost 33.5, Spring 1956. This break was caused by a bullet hole in the pipe where it crosses over Little Boulder Creek. Immediate loss of pressure led to the quick detection of the break. The fuel flowed into the rapidly moving stream and apparently was dissipated. No reports of damage to aquatic or terrestrial habitats were made.

Milepost 114.5, 14 July 1968. A bullet hole caused by target practice produced the break at this location (beside a garbage dump on a north-facing 20° slope). The pipe was buried following the incident and the area was greatly disturbed so that no reliable indicators of vegetative damage existed. No vegetation was growing in the area in 1972.

Milepost 119.1, 17 May 1968. The initial spill area was located some 200 m above the west bank of Dezadeash Lake, Yukon, Canada. This spill is considered by the pipeline personnel to be the most significant in the 15-year history of the pipeline, as an estimated 4000 bbl of diesel fuel was lost. Soils in the area are highly acidic and corrosive, resulting in the eventual leak in the pipe. Large quantities of fuel permeated down the slope and out into the lake before the leak was located. Strong prevailing winds scattered fuel along the north shore of the lake up to five or six miles away. Straw was scattered over the water to absorb the fuel and was then collected and burned. A small cleanup party was left at the site for months to clean up any fuel that appeared.

A complaint was received in late June 1970 that the shoreline where fuel had been collected was turning reddish-brown. Analysis of water and soil samples by the Federal Water Quality Laboratory of Portland, Oregon, showed the color to be the result of iron oxides in the water and not from the diesel fuel spill.

The 1972 reconnaissance revealed that all vegetation in the immediate area of the spill was dead. Elevated microrelief in the area, apparently above the greatest oil concentration, had moss cover growing on it. Soil pits at the bottom, middle and top of the slope all had strong odors of petroleum. At least one area was observed where fuel was actively seeping to the surface. Fishing has apparently returned to normal on the lake since the significant fish kill reported at the time of the spill.

Milepost 197.1, 30 January 1956. This spill area was on a north- to northeast-facing slope with the pipe nearly ¼ mile from the bottom of the slope. The fuel was allowed to run down the slope when the line was cut for purging. All species of vegetation were killed at the time of the spill. Some grass was growing in an old tractor trail that traversed the area but very little other new vegetation was evident. A few small white spruce 15 to 30 cm tall were growing within the area. There was one area where the surface organic mat had been washed away leaving a small depression 3 to 4 m in diameter. Other visible evidence of slope movement was present as 5 to 10 cm cracks could be seen where the mat had pulled apart. Strong petroleum odors were found in all soil pits dug on the slope. Some hummocks had moss and Labrador tea (*Ledum groenlandicum*) growing on them within the area. The lower portion of the slope had been disturbed through line construction and abundant white spruce regeneration had resulted.

Milepost 207.6, 2 February 1956. This was a large area 60 m wide near the pipe forming an inverted V-shape 500 m long down a gentle slope. Vegetation was completely lacking in the center of the main spill area. Soil pits were placed in the kill area and on the fringe. Odors of petroleum were present in all with the soil frozen at 20 cm.

Milepost 217.1, 9 February 1956. Spillage here was over an area 30 m wide and 100 m long on a very gentle slope. The soil appeared to be very well drained. A fire had apparently covered the entire area prior to the spill. Areas outside the spill had good spruce and moss growth. Within the spill area a number of young spruce, Labrador tea and other plants were growing. Of all the 1956 spill sites this one had the most abundant amount of vegetation.

Milepost 244.7, 1956. The exact date of this leak was unknown as a valve was left open by workmen when the line was being purged in 1956. An unknown quantity of JP-4 fuel leaked out overnight into a small stream and over an area 30 m wide by 120 m long. Fish taken from the stream reportedly had the taste of petroleum for 4 to 5 years after the spillage occurred. Vegetation appeared to be doing quite well with willow, birch, cottongrass, and moss growing over most of the area.

Milepost 256.8, 2 March 1956. Located on a slope, the spill was within a drainage basin. The appearance of the spill led to the conclusion that the fuel moved uphill on each side of the basin for 60 to 90 cm. Hummocks within the area had some spruce and willows growing on them.

Milepost 268.0, 25 February 1956. The pipeline was cut for deicing in a small flat area drained by a small stream. A soil pit at the edge of the drainage basin had a petroleum odor. A pit in the middle of the drainage area had 10 to 12 cm of organic matter on top with at least 5 cm of volcanic ash. No detectable odor was found there. Some spruce trees on the edge of the spill were killed but a few small birch and spruce were reseeding into the area.

Milepost 273.2, 29 February 1956. This rather large spill area was at least 75 m in diameter. The pipe was purged into a small drainage area on a 1° to 2° slope. All vegetation, of which black spruce predominated, was apparently killed at the time. The main drainage area had vegetation growing in it. However, areas not directly in the drainage basin had no new vegetation.

Milepost 290.8, 12 June 1969. This leak was caused by a bullet. The leakage progressed for an hour before pumping operations were stopped. The pipe in this area was buried following repair operations. As little vegetation was present prior to the spill no assessment of damage could be made. A strong odor was present in the gravelly soil and no vegetation was growing in the area.

Milepost 382.5, 16 March 1956. The fuel spilled at this location was gasoline. The site was on a very steep 40° south-facing slope. Soil was a well-drained silt loam and contained a layer of fine volcanic ash. The spill killed aspen 10 to 12 cm in diameter and all of the surface cover. Brome, small aspen, fireweed and bearberry were growing at scattered locations within the area. Soil pits located at midslope gave a strong odor of gasoline.

Milepost 511.0, 18 May 1959. This location was directly behind a scenic viewpoint at Milepost 1041 of the Alaska Highway. A bullet hole released an unknown amount of fuel. The pipeline had since been buried and no vegetation was growing over the disturbed area. The biggest scar left at this site was a ridge of surface material pushed back into the edge of the wooded area by a bulldozer.

Of the 41 recorded spills, 18 were viewed and sampled; of those visited, 13 exhibited vegetative damage. It was impossible to assess the vegetative response on the remaining sites due to the following: 1) spills were of insufficient quantity to result in noticeable damage; 2) spills fed directly into perennial streams and had no terrestrial impact; 3) spill sites were mechanically disturbed to the point that valid observations were impossible.

The extent of vegetative damage, on sites where detected, was thought to have been influenced by one or more of the following: a) site aspect, b) slope gradient, c) natural drainage patterns, d) soil conditions, e) amount of petroleum loss, and f) amount and type of original ground cover. The role, if any, and degree of importance of each factor was impossible to ascertain during the brief initial reconnaissance trip.

Detailed investigations – 1972. To assess the physical and biological parameters of damage from petroleum spills, and to determine the factors involved in natural rehabilitative processes, an intensive evaluation of five spill sites was made in 1972. The sites were chosen to cover variations in terrain, climatic conditions and degrees of initial damage. Methods by Ohmann and Ream (1971) were utilized to study the vegetative and physical parameters encountered. Spill site and adjacent control areas were examined with respect to slope, aspect and elevation (Table II). Vegetation was characterized as to plant species, frequency of occurrence and percentage cover. Soil was sampled from both spill and control areas and analyzed.

Site 1: The first intensive study site was located at milepost 1.9 near Haines, Alaska. It was approximately 122 m above sea level on a 15% slope with a southern aspect. Conditions at this site would typify spill effects and recovery in moist, cool coastal regions of southern Alaska.

The vegetative community prior to the spill consisted of a young- to intermediate-aged forest stand of Sitka spruce (*Picea sitchensis*), western hemlock (*Tsuga heterophylla*), paper birch (*Betula papyrifera*), black cottonwood (*Populus trichocarpa*) and a luxuriant undergrowth of mosses, ferns, herbs and shrubs common to coastal regions (Table III). The most frequently encountered shrub species in the control area was *Oplopanax horridus* in association with lesser amounts of *Cornus stolonifera* and *Vaccinium alaskensis*. Herb species most frequently found were *Gymnocarpium dryopteris, Cornus canadensis, Athyrium distentifolium, Polygonum bistorta* along with *Dryopteris fragrans*, a fern species. At the time of the spill, fuel was sprayed over the area, saturating the vegetation and soil. All existing vegetation in contact with fuel was killed. Fuel was still found to be present at soil depths of 50 cm.

A luxuriant growth of herbs and shrubs was found on the spill site when investigated (Fig. 6). In fact, the amount of live ground cover was higher within the spill area than in the adjacent control location. The species found to be abundant in the shrub category were *Ribes laxiflorum, Sambucus callicarpa* and *Rubus spectabilis.* Herb species found in abundance were *Gymnocarpium dryopteris, Epilobium angustifolium, Cornus canadensis,* and *Calamagrostis* spp. Two species of the fern genus *Woodsia* were encountered. *Tsuga heterophylla* seedlings were found also (Table III).

Soil analyses indicated a decrease in available phosphorus and increases in calcium and potassium levels in the spill area when compared to the unpolluted control area (Table IV).

It was evident that natural rehabilitative processes were well advanced. Apparently, high precipitation levels had leached the fuel from the upper soil layers, allowing natural revegetation to progress without interference. From the luxuriant appearance of the vegetation, it was apparent that the residual fuel at greater depths was not detrimental to plant growth.

In summary, it appeared that, in coastal regions, initial spill effects could be detrimental to plant communities. However, due to the climate, these effects would be short-lived and natural restorative processes would ensue within a few years.

Site 2: The 1968 spill at milepost 119.0 near Lake Dezadeash, Yukon Territory, Canada, was the second area chosen for intensive investigation (Fig. 7). This site was at 730 m elevation with a 20% slope and an aspect of N 82°E. Diesel fuel gradually permeated the downslope soil as a result of corrosion leaks in the line.

This spill contaminated two different plant communities. The first was a young stand of willow (*Salix* spp.) and alder (*Alnus* spp.). All vegetation was killed except plants on hummocks or high spots within the area. The percentage of ground cover in the plots ranged from 0 to 40%. The remaining live vegetation on the hummocks was composed of various moss and lichen species, Labrador tea (*Ledum groenlandicum*), lowbush cranberry (*Vaccinium vitis-idaea*), rose (*Rosa acicularis*), highbush cranberry (*Viburnum edule*), twin flower (*Linnaea borealis*), and horsetail (*Equisetum scirpoides*), with an occasional wintergreen (*Pyrola* spp.), *Anemone* spp. and grasses (*Graminae* spp.). There was little evidence of revegetation by fireweed (*Epilobium angustifolium*) and reedgrass (*Calamagrostis* spp.) which are pioneer species on disturbed sites in subarctic regions.

The second vegetation type affected was an intermediate-aged stand of white spruce (*Picea glauca*) with an understory of mosses, lichens, Labrador tea, rose and, occasionally, soapberry (*Shepherdia canadensis*). Vegetation kill was dependent upon the channelization of fuel flow. All trees and understory vegetation in the fuel flow path were killed. Existing plant coverage ranged from 0% in kill areas to 85% on hummocks.

Soil nitrogen, phosphorus, calcium and magnesium levels generally were higher in the spill zone than in the control area. Cation exchange capacity of fuel-saturated soils was lower than in the control area.

At this relatively recent fuel spill, there was little evidence to indicate that rehabilitation processes had begun. Except where bare mineral soil had been exposed, no new vegetation was encountered. Erosion problems were nonexistent except where the terrain had been disturbed during cleanup operations (bulldozers had been used to build fuel intercept trenches and a trail to transport collection booms and other equipment to the edge of the lake).

Site 3: The third area chosen for study was located at milepost 197.1. In 1956 a JP-4 (jet fuel) spill occurred at this site. The location was above Lake Kluane in the Yukon Territory of Canada and was characteristic of discontinuous permafrost terrain in interior Alaska. The site was at an elevation of 820 m on a 25% slope with a N 60°E aspect. The spill occurred as a result of a cut during the deicing operations in 1956.

Table III. Haines-Fairbanks pipeline intensive study sites, occurrence and percentage of ground vegetation.*

	Mile Post 1.9				Mile Post 119				Mile Post 197.1				Mile Post 207.6				Mile Post 217			
	Spill zone		Control		Spill zone		Control		Spill zone		Control		Spill zone		Control		Spill zone		Control	
	% Occurrence	% Cover	% Occurrence	% Cover	% Occurrence	% Cover	% Occurrence	% Cover	% Occurrence	% Cover	% Occurrence	% Cover	% Occurrence	% Cover	% Occurrence	% Cover	% Occurrence	% Cover	% Occurrence	% Cover
Ground coverage																				
Live ground cover	100	26.3	71	6.4	87	22.0	90	32.2	100	44.4	100	68.0	100	32.5	100	75.3	100	33.7	100	27.3
Litter	100	64.0	100	86.1	100	68.9	100	62.5	100	49.6	100	29.9	100	36.5	100	22.5	100	63.4	100	70.0
Bare organic soil	14	0.3											80	29.3	20	0.5	10	0.1		
Live wood	100	2.3	57	4.1	60	2.3	90	4.3	73	0.8	100	1.7	40	0.5	100	1.2	100	1.6	100	1.7
Dead wood	86	7.1	29	3.1	73	6.7	40	1.5	60	4.0	20	0.5	93	1.2	47	0.5	100	1.2	70	0.9
Mosses and lichens																				
Total moss cover	100	23.4	71	9.0	80	16.5	45	15.4	100	63.7	100	70.7	100	37.5	100	64.1	100	39.2	100	14.0
Total lichen cover	29	0.3			67	2.8	30	1.4	67	1.1	93	9.3	47	1.0	93	7.2	100	7.9	100	10.3
Herbs																				
Adoxa moschatellina							10	0.2												
Anemone richardsonii							20	0.3												
Arabis hirsuta									40	0.6										
Athyrium distentifolium																				
Calamagrostis spp.																				
Carex spp.									87	4.3	60	1.9	47	0.7	60	0.7	100	1.6	90	2.1
Corallorrhiza trifida											7	0.1			13	0.1				
Cornus canadensis	57	1.4	57	3.6	13	1.0	70	1.8												
Dryopteris fragrans			14	1.1																
Epilobium angustifolium	86	1.7	14	0.2	13	0.1	60	0.8	40	0.8			60	2.0			90	2.3	90	1.6
Equisetum palustre							35	0.4												
Equisetum pratense							5	0.1												
Equisetum scirpoides					13	0.1					14	3.3			100	4.7				
Equisetum sylvaticum																				
Geocaulon lividum							10	1.3			10	3.6								
Gralium triflorum							10	0.1												
Graminae spp.					13	0.7	20	0.4					40	8.0	27	0.3	100	3.3	70	0.8
Gymnocarpium dryopteris	57	10.6	71	9.9																
Hedysarum alpinum											13	4.1	7	0.1	13	2.1				
Lupinus arcticus																	40	0.5	70	11.0
Mertensia paniculata					13	0.3	50	2.8					7	0.1	7	0.1				
Osytropis deflexa							10	0.3			7	0.1					90	2.6	20	0.2
Oxytropis maydelliana									13	0.1										
Pedicularis labradorica							10	0.1												
Petasites hyperboreus							20	1.8												
Platanthera obtusata									7	0.1										
Poa spp.									7	0.1										
Polygonum alaskanum									7	0.1										
Polygonum bistorta			14	1.7	13	0.6	30	2.9												
Pyrola chlorantha					13	0.1														
Pyrola secunda							50	1.1	7	0.1	47	1.5	7	0.1	27	0.3				
Stellaria spp.													27	0.4	13	0.1				
Streptopus amplexifolius	14	0.1																		
Trientalis europaea					7	0.3	50	2.1												
Woodsia scopulina	43	1.7	14	0.2																
Woodsia spp.	43	1.3																		

	% Occurrence	Basal area acre	% Occurrence	Basal area acre	% Occurrence	Basal area acre	% Occurrence	Basal area acre	% Occurrence	Basal area acre	% Occurrence	Basal area acre	% Occurrence	Basal area acre	% Occurrence	Basal area acre	% Occurrence	Basal area acre	% Occurrence	Basal area acre
Half shrubs																				
Arctostaphylos rubra					7 .	0.1	20	2.7	47	2.9	73	9.6	20	0.3	100	16.6	20	0.3	20	0.6
Arctostaphylos uva-ursi							5	0.8					20	0.2	27	1.1	20	0.2	30	6.7
Cornus stolonifera			14	0.3																
Dryas integrifolia									20	0.2	13	0.5					10	0.1		
Empetrum nigrum							15	7.3			13	2.2			100	15.9				
Ledum groenlandicum					47	7.3	40	12.3	13	0.9	33	4.6	7	0.1	80	10.7				
Linnaea borealis					33	2.7	80	14.0	7	1.3	20	0.5			73	2.9				
Oplopanax horridus	14	0.6	14	5.0																
Potentilla fruticosa									13	0.1										
Rhododendron lapponicum									7	0.1										
Ribes hudsonianum							5	0.3												
Ribes lacustre	86	21.9					10	0.1												
Ribes triste							5	0.1												
Rosa acicularis					20	0.2	55	1.6							20	0.3				
Rubus spectabilis	71	1.0																		
Sambucus callicarpa	86	13.4																		
Shepherdia canadensis					7	0.1			20	0.3			40	0.4	7	0.1	90	15.9	100	17.2
Vaccinium alaskensis			14	0.3																
Vaccinium uliginosum															53	0.6				
Vaccinium vitis-idaea					20	0.5	15	0.9			13	0.7			80	4.6				
Viburnum edule	14	0.1			7	0.3	10	0.1												
Tall shrubs																				
Alnus spp.							35	4.0												
Salix spp.							35	4.0	93	2.9	7	0.7	40	4.4	20	0.2	100	12.6	40	0.7
Tree seedlings																				
Picea glauca							5	0.1	87	1.2	20	1.1	40	0.4	7	0.1	100	2.0	100	1.4
Tsuga heterophylla	14	0.1																		
Trees																				
Alnus spp.							20	2.0												
Betula papyrifera			71	79.3																
Picea glauca							80	109.2			100	137.7			100	102.1			100	17.1
Picea sitchensis			43	33.1																
Populus balsamifera																			20	1.5
Salix spp.			71	73.2			20	1.9											30	0.5
Tsuga heterophylla			14	6.6																

* Scientific nomenclature of plant species from Hultén (1968).

Figure 6. Haines-Fairbanks pipeline intensive site 1, milepost 1.9, Haines, Alaska, showing standing dead trees killed at the time of the fuel spill and ensuing revegetation occurring in this coastal region.

Table IV. Soil nutrient analyses, Haines-Fairbanks military pipeline intensive site studies (1972).

	Milepost 1.9		*Milepost 119.0*		*Milepost 197.1*		*Milepost 207.6*		*Milepost 217.0*	
	Control	*Kill*	*Control*	*Kill*	*Control*	*Kill*	*Control*	*Kill*	*Control*	*Kill*
pH	5.30	5.80	6.60	6.50	7.90	8.10	–	6.50	8.20	8.10
% N ppm	0.09	0.10	0.26	0.38	0.16	0.46	–	0.25	0.22	0.21
P ppm	4.91	1.25	1.00	2.13	0.55	0.63	–	1.04	1.76	3.19
CEC meq/100 g	11.73	11.84	92.68	44.11	33.89	53.84	–	33.21	15.18	24.43
Ca meq/100 g	0.56	1.21	29.35	35.46	40.17	62.47	–	24.94	62.71	54.30
Mg meq/100 g	0.20	0.11	0.84	2.59	7.55	7.62	–	3.74	6.03	6.18
K meq/100 g	0.04	0.07	0.08	0.13	0.30	0.24	–	0.10	0.21	0.21

Values expressed on an oven dry weight basis.

Figure 7. Site of diesel fuel spill: Lake Dezadeash, Yukon Territory, milepost 119.0, Haines-Fairbanks Pipeline. Light-colored trees are all dead as a result of the spill.

Figure 8. Site of jet fuel spill, milepost 207.6, Haines-Fairbanks military pipeline. All trees in the foreground were killed.

The plant community that existed was an intermediate-aged white spruce stand with a shrub layer predominated by a bearberry (*Arctostaphylos rubra*), Labrador tea, and crowberry (*Empetrum nigrum*). The herb layer was originally composed of *Hedysarum alpinum*, *Geocaulon lividum*, *Equisetum scirpoides* and *Carex* spp. and the ground had been covered with a deep moss layer and numerous lichens.

The average live groundcover in the control area was 69%, compared to 44% in the spill center. All of the white spruce encountered by the spreading fuel were killed as were the crowberry and species in the other categories. Some of the original moss cover and Labrador tea were found to be alive at high points within the spill area. Although live ground cover 16 years after the spill averaged 44%, individual plot cover averaged from 5 to 60%. Intensive study of the area indicated that the process of vegetative healing had begun. Occasional bearberry and *Dryas integrifolia* were found along with some young willows, fireweed and bromegrass. However, most regrowth was found in areas where the mineral soil had been exposed by falling trees, or where the organic mat was extremely shallow. New spruce seedlings were not encountered in the upper portion of the spill area; however, they were abundant where utility line construction had disturbed the soil at the base of the spill. It was worthwhile to note that, on this site underlain by discontinuous permafrost, erosion had not resulted after 16 years except in one location where falling trees had uplifted the insulating organic mat. A cross-sectional profile of the spill location indicated a definite increase in the permafrost thaw where the fuel killed the vegetation, but no downslope movement or erosional slump had resulted. Analyses of soil samples indicated that nitrogen, calcium and cation exchange capacities were higher in the spill area than in the control. Differences in other parameters were slight.

Site 4: In 1956, a jet fuel spill occurred at milepost 207.6 as a result of a pipeline cut. The site was located at an elevation of 790 m on a 6% slope with an aspect of N 35°E. The site was originally occupied by a mature white spruce community with a shrub layer composed of two bearberry species (*Arctostaphylos rubra* and *Arctostaphylos uva-ursi*), crowberry, Labrador tea, lowbush cranberry and twinflower. The original herb layer was composed of *Equisetum scirpoides, Hedysarum alpinum* and *Carex* spp. A well developed moss layer with numerous lichens was also evident. Live ground cover in the control area ranged from 50 to 90% (average 75%).

It was apparent that all vegetation was killed at the time of the spill (Fig. 8). Live ground cover averaged 36% compared to 75% in the control area. Live ground cover in the oil-polluted areas predominated in swales or drainage channels (average live ground cover 68%) (Fig. 9). Outside of the swales, live ground cover averaged only 9% (Fig. 10). Similar effects were noticed when species compositions were compared. Crowberry, lowbush cranberry and twinflower were nonexistent within the spill area with bearberry species and Labrador tea drastically reduced when compared to the control plots. White spruce seedlings, fireweed and grasses were found in the swale areas; however, no species was abundant within the spill zone.

Temperature and thaw measurements showed that permafrost had receded as a result of the vegetative kill. However, differences were not considered significant enough to indicate a potential permafrost degradation problem. No erosion was encountered. Due to loss of soil samples taken from the control area there was no basis for comparing samples from the area.

Site 5: The fifth study site was located at milepost 217.0 in the Yukon Territory. A jet fuel spill resulted from a pipeline cut during the winter of 1956. However, this spill differed from the others in that the site had been previously burned by a forest fire which removed the normal deep moss and organic layers and exposed bare mineral soil. At the time of the spill, the area was covered by an early successional plant community of white spruce, balsam poplar (*Populus balsamifera*) and willow. Most of these would have been in the seedling or early sapling stage of growth at the time of the spill. Shrub layers probably consisted of arctic lupine (*Lupinus arcticus*), soapberry and bearberry. Fireweed, grasses and sedges presumably were abundant in the herb layers with few moss and lichens present.

Figure 9. Interior of spill area, milepost 207.6, Haines-Fairbanks pipeline, showing revegetation in swales or drainage channels.

Figure 10. Interior of spill area, milepost 207.6, Haines-Fairbanks pipeline, showing lack of vegetative recovery on dry sites.

The spill area was abundantly vegetated, with numerous spruce seedlings along with young willows, soapberry, *Oxytropis deflexa*, grasses, fireweed, sedges and arctic lupine. The soil analyses indicated that phosphorus and cation exchange capacity levels were higher in the spill areas.

Revegetation of this spill was well advanced when compared to the other sites previously studied. It was postulated that prior removal of the deep moss layer and exposure of mineral soil by fire played an important role in advancing vegetative rehabilitation.

Conclusions. Based on the field investigations in both 1971 and 1972, it was apparent that refined fuels were toxic to vegetation indigenous to subarctic regions, and that natural rehabilitative processes were slow. From these detailed studies, some general conclusions could be drawn.

First, it appeared that revegetation of spill areas was governed primarily by moisture availability. The coastal site offered evidence that abundant rainfall contributed to leaching of the fuel to greater soil depths. Thus any fuel possibly deleterious to plant establishment would be diluted and gradually removed from the system or reduced to nondeleterious levels. This was also evidenced in interior regions as most of the invading species were in drainage swales.

Second, in areas with a deep organic layer, moisture also appeared to be important in plant germination and growth. Seeds would germinate in the spring following snowmelt. With normal low rainfall and increasing photoperiod (20 to 22 hours of daylight), the dead organic mat would dry rapidly. New seedlings would not have time to produce sufficient root growth to reach necessary moisture in the mineral soil layers. Thus except where mineral soil was exposed, where organic layers were extremely thin, or where moisture was continually abundant during plant establishment and early growth, most invading plants would fail.

Third, channeling of fuel may have controlled damage within spill areas. Vegetation on raised microrelief areas would escape damage from the toxic fuels.

Fourth, spills in permafrost areas having thick organic mats did not result in greatly increased permafrost degradation. Increases in thaw occurred, but even on steep slopes little erosion resulted when the organic mat remained intact. (This is especially important as cleanup procedures could cause more damage than leaving the site undisturbed.) This was verified by erosion associated with disturbance from cleanup procedures at milepost 119.0.

Fifth, fire appeared to play an important role in rapid site restoration. Along with moisture, mineral soil seedbed exposure was a critical requirement for the germination of invading plant species. Fire could be used as a tool in this respect; however, the presence or absence of permafrost along with slope would be important considerations prior to using fire as a rehabilitative tool.

Sixth, soil nutrient relationships were altered by the spills. This was especially true in the case of nitrogen and phosphorus, their levels being higher in areas contaminated by petroleum. These results appeared to be similar to those found by Ellis and Adams (1961) in temperate zone studies.

Revegetation experiments. Since refined fuel spills could result in complete vegetative kills (in some cases revegetation was lacking after 16 years), it was felt that revegetation of spill areas was an aspect that needed attention. Thus it was decided to attempt the establishment of vegetation using artificial methods.

The presence of natural revegetation along drainages in spill areas indicated that moisture availability was an important factor in revegetation. Areas devoid of natural revegetation were characterized by a deep layer of dead moss and organic matter (up to 30 cm). As previously mentioned, this layer became very dry during the summer and was theorized to be a barrier to plant establishment. Lack of revegetation might also have resulted from direct effects of fuel remaining in the soil, inhibiting seed germination and subsequent plant growth.

Two sites were chosen for experimentation in 1972. First was the 1968 spill at pipeline milepost 119.0 adjacent to Lake Dezadeash in the Yukon Territory of Canada. Second was an older (1956) spill in the Yukon Territory near Lake Kluane at pipeline milepost 207.6. Study plots were initiated in two areas at milepost 119.0. The first was once a mixed alder and willow stand, covered with a 10-cm layer of dead moss and organic material over mineral soil. The second was once a mature white spruce stand and covered with a 25- to 30-cm layer of dead moss and organic material. The third site, at pipeline milepost 207.6, once a mature white spruce stand, was covered with a 25- to 30-cm mat of dead moss and organic material.

At each site, 1-m-square plots were established to test the effects of 1) exposing the mineral soil by removal of the organic mat, and 2) fertilization of the soil on the establishment and growth of rye grass (*Lolium multiflorum*), bromegrass (*Bromus inermis*), and red fescue (*Festuca rubra*). Each grass was applied to a third of each plot. All fertilizer applied was commercial 20-10-10 fertilizer. Treatments were applied in two replicates at each location.

Treatments were applied on 18 June 1972 at milepost 119.0 and on 20 June at milepost 207.6, as follows:

Pipeline milepost 119.0. Treatments were replicated in both the alder-willow and spruce stands.

1. Stripped – organic mat removed and soil seeded without fertilizer.
2. Stripped – organic mat removed and soil fertilized at the rate of 112 kg N/ha and seeded.
3. Stripped – organic mat removed and soil fertilized at the rate of 224 kg N/ha and seeded.
4. Unstripped – fertilizer applied at a rate of 224 kg N/ha to the undisturbed surface of the organic mat. No seed was applied.

Pipeline milepost 207.6.

1. Stripped – organic mat removed and soil seeded without fertilizer.
2. Stripped – organic mat removed and soil fertilized at the rate of 112 kg N/ha and seeded.
3. Stripped – organic mat removed and soil fertilized at the rate of 224 kg N/ha and seeded.
4. Unstripped – organic mat left undisturbed and fertilized at the rate of 112 kg N/ha and seeded.
5. Unstripped – organic mat left undisturbed and fertilized at the rate of 224 kg N/ha and seeded.

The last two treatments were aimed at the question of physical barriers to revegetation. The entire series of treatments was replicated twice.

The sites were revisited in August of 1972 and July of 1973. In 1972, at the 119.0 milepost, removal of the organic mat from the plots was associated with good early survival of rye, brome and fescue despite a petroleum fuel odor remaining in these plots. When the organic matter was removed to expose mineral soil, grass establishment and growth were fair to good without fertilization. The addition of fertilizer at the rate of 112 kg/ha improved the establishment and growth of the grass species. The addition of 224 kg/ha of fertilizer did not increase results over the 112 kg/ha rate. The placement of fertilizer on the undisturbed dead organic surface did not enhance establishment of native species. No plots were established which contained fertilizer and seed, or seed alone, on the undisturbed dead organic surface.

The July 1973 observations at the 119.0 milepost revealed that red fescue and ryegrass in the stripped plots had survived the winter and were growing luxuriantly in all plots regardless of fertilizer treatments. Brome was present but only represented 1 or 2% of the cover. Fescue averaged 75% of the cover with ryegrass averaging 5%. Native species such as fireweed and wild rose were found in several of the plots having the organic mat removed. Plots with the organic mat intact were still found to be void of vegetation.

Observations of both years indicated that remaining fuel concentrations at milepost 119.0 were not toxic to germination and growth as plants appeared healthy showing no toxicity symptoms. These results suggest that seed availability combined with moisture availability are the important controllers of revegetation.

Observations of the experimental plots at the 207.6 milepost in August of 1972 found again that removal of the dead organic mat was associated with good establishment and growth of ryegrass, brome and fescue. The response was better at the 112 kg/ha fertilizer rate than with no fertilizer; however, the 224 kg/ha fertilizer rate had the poorest establishment and growth. It was postulated that the heavy rate may have resulted in toxic ion(s) concentrations detrimental to plant growth. Removal of the organic layer stimulated thawing of the underlying permafrost at this location, and due to poor drainage, the plots had some standing water in spots. Coupled with the low rainfall characteristics of this region, the poor drainage may have resulted in high soil concentrations of fertilizer salts.

Fertilizer and seed placed directly on the dead organic surface yielded plant establishment and growth that was less than in plots with the organic mat removed. However, grasses were present in these unstripped plots and 224 kg/ha of fertilizer yielded better results than the 112 kg/ha concentration or the total absence of fertilizer. Interaction of fertilizer salts with moisture and the resulting ion concentration in water were apparently different in terms of plant response under these circumstances. Grass roots had penetrated the dead organic mat to a depth of 15 cm.

Brome yielded best results on the organic surface. Brome and fescue each gave good results when the organic mat was removed. Ryegrass establishment was somewhat thinner. Fuel odor in the mineral soil was distinct in samples removed from the surface of the strip plots. Better growth in the stripped plots was thought indicative of the importance of moisture and related nutrient uptake associated with mineral soil. Fertilizer treatment was of additional benefit at the rate of 224 kg/ha on undisturbed plots, but not on stripped plots.

The July 1973 observations revealed that fescue and ryegrass were doing well on the stripped plots at milepost 207.6. The undisturbed plots also contained ryegrass and fescue but cover was lower and plant growth was reduced. The brome had apparently winterkilled as few plants were found. A most important observation was that the permafrost thaw was associated with removal of the organic mat. In almost all stripped plots slumping had occurred and water was standing. Native vegetation was not encountered in any of the plots.

In summary, removal of the organic mat and subsequent seeding resulted in the establishment of grasses at both sites. With the organic mat removed, fertilizer treatment at 112 kg/ha increased survival and growth, but the 224 kg/ha rate was of no additional benefit. Though seeding and fertilization of undisturbed plots resulted in some grass establishment, it appeared that lack of moisture availability in the dead organic mat was limiting. When fertilizer was added to the dead organic surface, no native species became established; however, native species were observed in the stripped plots in 1973 at the 119.0 milepost location. It was apparent that the presence of fuel residues did not affect grass establishment and growth.

Artificial revegetation could be used in rehabilitating oil spill areas for aesthetic purposes in subarctic regions. However, it would be necessary to remove the organic mat and in areas underlain by permafrost, precautions would have to be taken as considerable damage could result from slumping and erosion, especially if steep slopes were involved.

Barrow investigations (1970-1974)

Previous studies concerning the Haines-Fairbanks pipeline involved refined fuel spills in the subarctic or the interior forests of Alaska and adjacent Canadian areas. Petroleum spills from future pipelines may not be limited to the interior but may also occur on the Arctic Coastal Plain of Alaska. To better understand the sensitivity of arctic ecosystems to crude oil spills and related natural restorative processes, a series of studies was undertaken at Barrow, Alaska (McCown et al. 1973b). The following is similar to the 1973 publication except that it contains greater expansion of detail than in the original publication and includes 1974 observations.

In absence of studies concerned with the effects of oil contamination on arctic ecosystems, only implications and generalizations as to their effects can be made. However, by combining the information learned from research in temperate and marine regions with known characteristics of arctic ecosystems, some aspects become apparent. Heat imbalance and the role of an insulating vegetative mat have already been mentioned. Thus it is possible that oil spilled on tundra landscapes could cause severe local changes in heat flux.

Differences in plant susceptibility to toxic oils are also important as there are indications that some arctic organisms may be able to tolerate the presence of crude oil. However, when considering the potential abrupt changes in microenvironments which may result from oil pollution and the inherent slow recovery rate of arctic ecosystems, the natural development of a vegetative mat of oil-tolerant plants over polluted areas may be unimportant or nonexistent. Therefore it becomes critical to develop efficient techniques for oil removal and subsequent revegetation.

Wet site studies 1970-1974. The first crude oil field studies were initiated at Barrow, Alaska, during the summer of 1970 (Brown and West 1970). The location selected was a site which had standing water at the time of oil application and was underlain by a silt soil. Thaw depth was 12 cm and thickness of the organic mat was more than 4 cm. Prudhoe Bay crude oil was applied manually, keeping oil from direct foliage contact (simulating an oil spill flowing along the ground surface). Five plots were treated as follows:

1. Control
2. 0.7 l/m^2
3. 1.4 l/m^2
4. 5.0 l/m^2
5. 12.0 l/m^2

Application rates were determined in the field as no prior information existed with respect to the quantities of oil that could spread uniformly over the soil surface. The 0.7 l/m^2 rate was the least amount of oil that could be spread and still achieve adequate surface coverage. The 12 l/m^2 rate was the maximum amount of oil that could be contained safely; it literally flooded the surface after application. The 5 l/m^2 rate was selected because it corresponded to the upper limit reported for plant tolerance in temperate regions (Kloke and Sahm 1961, Johansson 1962).

By the end of that growing season several areas of damage were discernible. Plants or leaves physically covered by the oil were dead. This was especially noticeable with the low growing mosses and liverworts in the 5.0 l/m^2 and 12.0 l/m^2 applications. In addition the 12.0 l/m^2 plots exhibited more yellowing than adjacent plots.

Plant production data were collected for three successive seasons, the last being 1972. Preliminary data collected in 1970 indicated that crude oil reduced biomass and chlorophyll production (Table V). In 1971, biomass production and stem counts were recorded at peak season to see what continuing effects, if any, were occurring at the various treatment levels (control through 12.0 l/m^2).

Continuing adverse effects of crude oil on plant survival and growth were evident in 1971. Physical presence of crude oil was still apparent from its odor and visible tar residue. However, early growth of plants in the various treatments was observed, and only in the heavy treatment (12.0 l/m^2)

Table V. Biomass and chlorophyll production (1970) – wet site study area, Barrow, Alaska (from McCown et al. 1971).

Treatment (l/m^2)	*Dry weight (g/m^2)*	*Chlorophyll (mg/m^2)*	*Chlorophyll (mg/g dry wt)*
Control	121.0	452	374
12.0	99.0	338	341

Table VI. The effect of an increasing rate of crude oil contamination on the aboveground biomass production of an arctic grass-sedge tundra at Barrow, Alaska (from McCown et al. 1973).

Plots treated in the 1970 growing season and measured at peak season in 1971 and 1972.

Treatment (l/m^2)	*Dry weight production, 1971 (g/m^2)*	*Decrease over control (%)*	*Dry weight production, 1972 (g/m^2)*	*Decrease from control (%)*
Control	75	–	102	–
0.7	60	19.1	–†	–
1.4	62	17.4	–†	–
5.0	62	18.7	92	9.9
12.0	30*	59.3	27	73.8

* Significantly different from control at 0.05 level.

† No samples taken.

Table VII. The effect of increasing rate of crude oil contamination on the total number of living stems of an arctic grass-sedge tundra at Barrow, Alaska (from McCown et al. 1973).

Plots treated in the 1970 growing season and measured at peak season in 1971 and 1972.

Treatment (l/m^2)	*Total number living stems per m^2 (1971)*	*Decrease over control (%)*	*Total number living stems per m^2 (1972)*	*Decrease from control (%)*
Control	2370	–	2140	–
0.7	1800	24.1	–†	–
1.4	1900	19.8	–†	–
5.0	1430*	40.0	1780	16.8
12.0	820*	65.4	490	77.1

* Significantly different from control at 0.05 level.

† No samples taken.

was there any visual reduction in plant growth. These visual observations were verified by data as the 12.0 l/m^2 treatment resulted in a significant reduction of standing live biomass and number of live stems (Tables VI, VII, Fig. 11). There were no significant differences in standing live biomass among the other levels of contamination (control, 0.7, 1.4 and 5.0 l/m^2); however, there was a significant reduction in the total number of living stems per plot at the 5.0 l/m^2 level. Dry weight percentage decrease in the 0.7, 1.4 and 5.0 l/m^2 plots in 1970 was only about 18.0%, whereas the 12.0 l/m^2 plot resulted in a 59% decrease in dry weight. The total number of living stems was reduced by 20 to 40% in the first three treatments whereas the 12.0 l/m^2 treatment reduced stem count by 65% when compared to the control.

During peak season 1972, three treatment plots were sampled (control, 5.0 and 12.0 l/m^2) for aboveground biomass and stem counts. Aboveground biomass and total number of living stems in the 5.0 l/m^2 treatment plot were again less than those of the control; however, the 1972 biomass production at the 5.0 l/m^2 rate was higher than shown in the 1971 data, indicating that perhaps recovery was occurring. The 12.0 l/m^2 treatment yielded the same trend in 1972 as in 1971; however, the 1972 results indicated a further decline in biomass production. Decrease in 1972 stem count was 77% and dry weight 74%.

Dupontia fischeri, a grass, was studied very intensely for two years in the 1970 wet site plots. The work was conducted through the U.S. Tundra Biome Program and was directed by Dr. Larry Tieszen (McCown et al. 1973b). It was selected because it is one of the predominant grasses of the Arctic Coastal Plain. To follow the more long-term effects, eight individual plants were observed in each plot for average maximum live leaf height (mean of longest vertically measured leaf per plant) and an average number of live leaves during 1970 and 1971 (mean of the number of green leaves per plant).

The 1970 results of studying *Dupontia fischeri* are shown in Figures 12 and 13. The measurements indicated that only the heaviest application (12.0 l/m^2) resulted in a detectable reduction of growth. From this, it was apparent that crude oil in sufficient quantities damages *Dupontia*; however, there appeared to be more tolerance to crude oil than exhibited by many plant communities in the temperate zone.

Results of the 1971 measurements of *Dupontia fischeri* are shown in Figures 14, 15, 16, 17 and 18. Second-year effects of crude oil on the average maximum live leaf height of *Dupontia* differed from the 1970 observations in that both the 5.0 l/m^2 and 12.0 l/m^2 rates altered leaf growth. The 12.0l/m^2 rate very severely restricted leaf growth the second year and, after approximately 10 July, average live leaf height declined; by peak season, the plants were dead. Although leaf growth in the 5.0l/m^2 rate was significantly less than that of the control, the plants did survive.

Data on average number of live leaves per plant of *Dupontia*, when plotted with time, are very similar to those of leaf height (Fig. 15). The graphs of the control and 5.0l/m^2 treatments convey a gradual increase, and both show a decrease in leaf production at about the same time. However, the drop in the 5.0 l/m^2 treatment is more rapid and, in the final stages, the average number of live leaves per plant is about half that of the control. The graph of the 12.0 l/m^2 rate shows severely restricted leaf emergence and indicates that early in the season, emergence ceases and by midseason all plants are dead.

Phenology studies of individual leaves (Figs. 16, 17 and 18) serve to support the previous information and similarities are evident. In the 12.0 l/m^2 treatments, the first leaves senesced and died early, and overall growth was severely restricted. Similar results were encountered with the second leaf, and no third leaf existed at the 12.0 l/m^2 treatment level. Senescence of the first leaf produced in the 5.0 l/m^2 plot was earlier than the control. The overall heights of the second and third leaves were less than the control and senescence also began sooner in these leaves.

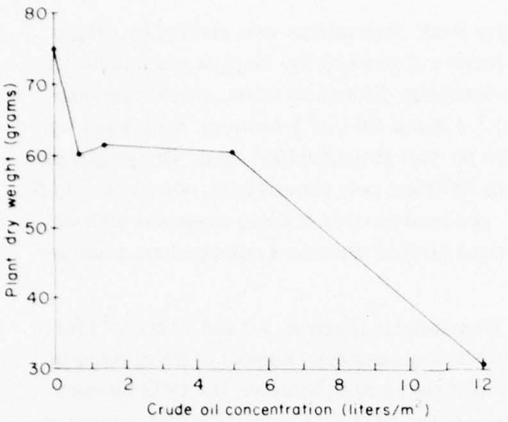

Figure 11. The effect of increasing rates of crude oil contamination on the aboveground biomass production of an arctic grass-sedge tundra at Barrow, Alaska. Plots were treated in the 1970 growing season and measured at season in 1971.

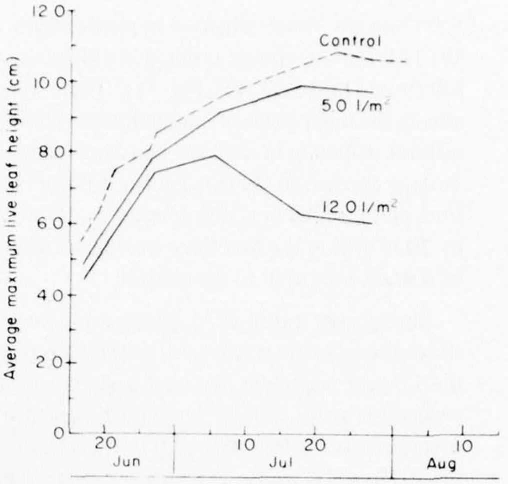

*Figure 12. The influence of two levels of crude oil contamination on the average maximum live leaf height of an arctic grass (*Dupontia fischeri*) growing in a wet grass-sedge tundra at Barrow, Alaska. Measurements taken during the season in which the treatments were made, 1970 (McCown et al. 1973).*

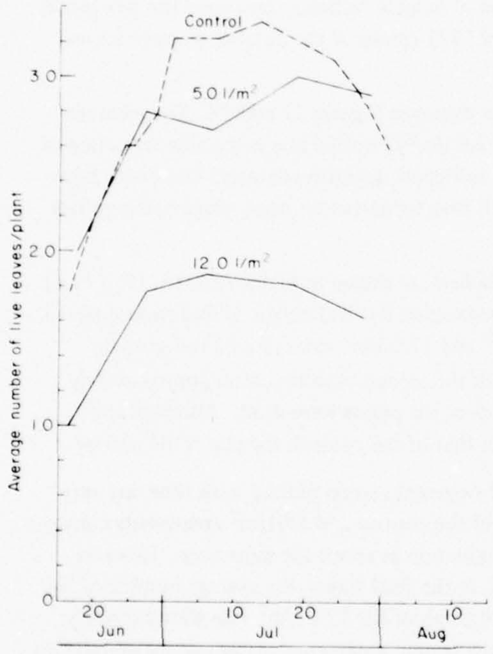

*Figure 13. The influence of two levels of crude oil contamination on the number of live leaves per plant of an arctic grass (*Dupontia fischeri*) growing in a wet grass-sedge tundra at Barrow, Alaska. Measurements taken during the season in which the treatments were made, 1970 (McCown et al. 1973).*

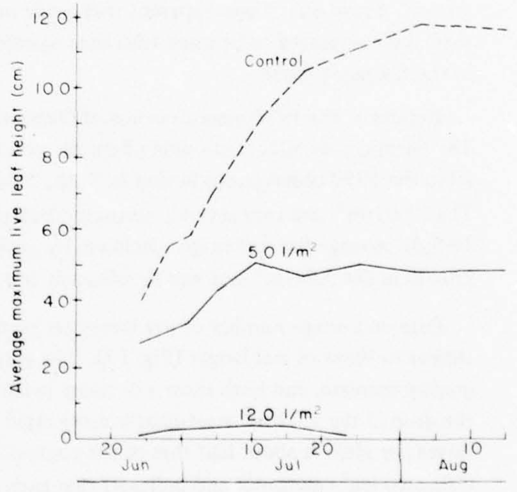

*Figure 14. The influence of two levels of crude oil contamination on the average maximum live leaf height of an arctic grass (*Dupontia fischeri*) growing in a wet grass-sedge tundra at Barrow, Alaska. Treatments were made in 1970 and measurements were taken the season following, 1971 (McCown et al. 1973.*

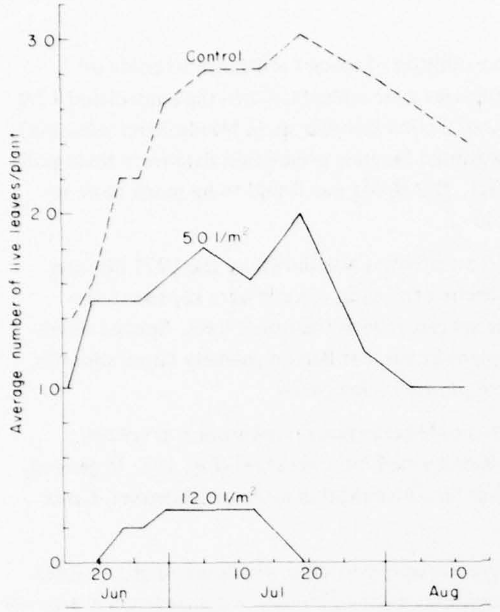

*Figure 15. The influence of two levels of crude oil contamination on the number of live leaves per plant of an arctic grass (*Dupontia fischeri*) growing in a wet grass-sedge tundra at Barrow, Alaska. Measurements taken one season after treatment, 1971 (McCown et al. 1973).*

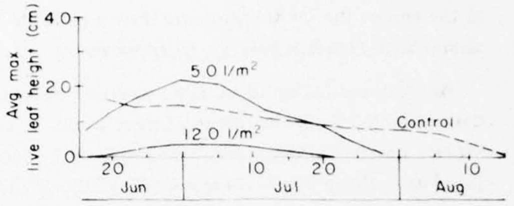

*Figure 16. The influence of two levels of crude oil contamination on the height of the first leaf produced of an arctic grass (*Dupontia fischeri*) growing in a wet grass-sedge tundra at Barrow, Alaska. Treatments were made in 1970 and measurements were taken in 1971.*

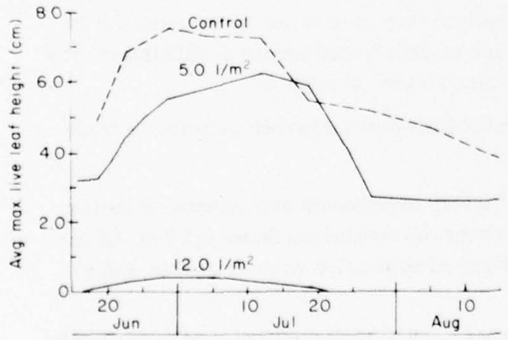

*Figure 17. The influence of two levels of crude oil contamination on the height of the second leaf produced of an arctic grass (*Dupontia fischeri*) growing in a wet grass-sedge tundra at Barrow, Alaska. Treatments were made in 1970 and measurements were taken in 1971.*

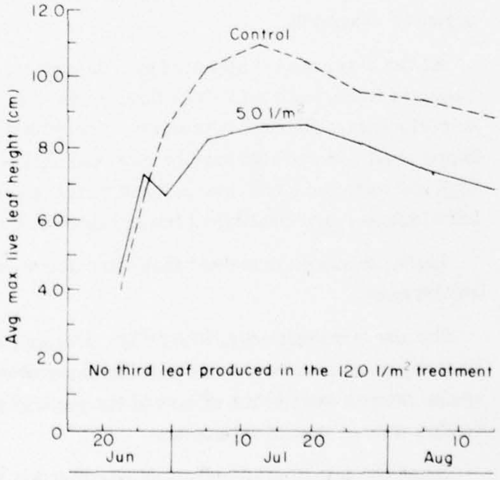

*Figure 18. The influence of two levels of crude oil contamination on the height of the third leaf produced of an arctic grass (*Dupontia fischeri*) growing in a wet grass-sedge tundra at Barrow, Alaska. Measurements were taken in 1971.*

Observations of the 1970 treatments indicated possibilities of species resistance to crude oil. This was first observed when potted plants of three species were embedded into the control and 12.0 l/m^2 plots. The species selections were: 1) *Poa alpina*, 2) *Deschampsia* sp. (a Meade River selection), and 3) *Deschampsia* sp. (an alpine selection). Belowground biomass production data from these pots at the end of the 1970 season are shown in Table VIII. *Poa alpina* was found to be much more resistant than *Deschampsia* sp. to applications of crude oil.

Another indication of possible variation in species sensitivities was shown by the 1971 biomass data collected from the five treatment levels. In collecting the data, records were kept as to the average number of live stems per sample plot of four species at each treatment level. Species monitored were those which comprise 60 to 70% of the plant biomass at Barrow, namely *Carex aquatilis, Dupontia fischeri, Eriophorum angustifolium* and *Eriophorum scheuchzeri*.

Dupontia fischeri appeared to be quite sensitive to crude oil as was *Eriophorum scheuchzeri*; however, *Carex aquatilis* and *Eriophorum angustifolium* seemed more resistant (Fig. 19). In general, the average number of live stems tended to decrease as oil concentration increased; however, *Carex* and *Eriophorum* decreased the least.

In summary, it was apparent that initial first season damage to vascular plants was slight. Visible damage was hard to distinguish in the first year and without detailed biomass measurements it may not have been noticeable. Most damage that did occur the first year resulted from direct foliage contact with the crude oil.

Damage became more visible the second year, especially at the higher rates. This was true for all species encountered within the plots. The long-term effects of high concentrations continued to manifest themselves the second year and even more the third year. In general, it can be safely stated that the full effects of oil contamination of cold-dominated regions may not become fully apparent until a period of years have elapsed. This delayed response is thought to result from root injury in which slow deterioration of the affected plant occurs. Such injury may then manifest itself in a high degree of winterkill.

At the lower rates it appeared that damage was stabilized and recovery either was, or could be expected to be, initiated two or three years after contamination (5.0 l/m^2 or less). These data were important since 5.0 l/m^2 rate was considered lethal to plants in temperate zones. Thus, the North Slope plant communities may be more resilient to crude oil than those of temperate zones due to high soil moisture levels, low ambient temperatures and possible species sensitivity differences. The lethal rate was not established but appeared to be around 10 l/m^2 of crude oil.

Lastly, it was quite evident that some degree of individual species sensitivity response to crude oil did exist.

Dry site investigations (1971-1974). During 1971 several experiments were initiated at Barrow to verify or discount some of the preliminary observations and conclusions drawn in 1970. Of particular interest were a) the effects of the method and rate of application on plant survival, and b) further tests of species sensitivities.

Since the rate of crude oil contamination was limited in 1970 by the utilization of an extremely wet site, it was felt that some estimate of higher rates on dry sites was necessary. This was especially true since a drier site is more typical of the vegetation at Prudhoe Bay and the pipeline route southward into the foothills of the Brooks Range.

Another aspect, encountered both at the wet site and in observations of natural oil seeps at Cape Simpson, was that injury to plants was observed to occur only when foliage was in direct contact with crude oil. If this were true then it would be important to assess damage differences from spray

Table VIII. Plant species sensitivities to crude oil, 1970 investigations, Barrow, Alaska.

Species	*Treatment (l/m²)*	*Dry wt. (g)*	*Decrease from control (%)*
Deschampsia sp.	Control	0.1881	–
(Meade River)	12.0 l/m²	0.0993	47.0
Deschampsia sp.	Control	0.4643	–
(Alpine)	12.0 l/m²	0.2488	47.0
Poa alpina	Control	0.3382	–
	12.0 l/m²	0.2530	22.0

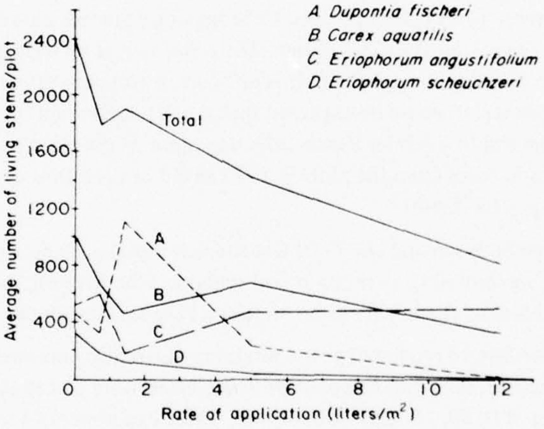

Figure 19. The influence of increasing rates of crude oil contamination on the frequency of occurrence of four arctic grass-sedge tundra species at Barrow, Alaska. Plots were treated in the 1970 growing season and measured at peak season in 1971.

applications as opposed to normal spill flow where root systems (rather than the foliage) are primarily affected. This type of study was also undertaken to assess the regrowth potential of heavily contaminated tundra.

To assess the differences between spray and soak applications, the following experiment was devised. Crude oil was applied to four plots (2 x 2 m) at four treatment levels.

1. Control.
2. Spray – foliage sprayed with warm oil (45°C) at the rate of 5 l/m^2. This rate barely penetrated the soil surface (Fig. 20).
3. Soak – warm oil (45°C) was applied to the soil surface without foliage contamination at the rate of 20 l/m^2 (Fig. 21).
4. Spray and soak – a combination of 2 and 3 above. The total rate was 25 l/m^2 (Fig. 22).

The plots were sampled prior to treatment in order to establish uniformity in plant biomass production. The results of an analysis of variance based on ten 0.01-m^2 clipped plots per treatment area indicated that plot uniformity did exist.

Biomass data from these plots were collected in 1972 (Table IX). The 5.0 l/m^2 spray resulted in a reduction of aboveground biomass production by as much as 70%. This was considerably more than shown with a ground application of 5.0 l/m^2 in the wet site plot. The spray-soak and soak treatments at higher rates resulted in an almost complete vegetative kill; both caused a reduction of almost 96% in aboveground plant production. Visual examinations of the study area in 1973 and 1974 indicated that the spray (only) plot appeared to be making a gradual comeback from the initial kill. *Carex aquatilis* was the principal species found. The other two plots were, from all visual evidence, still in a condition of continuing decline. It is difficult to compare the dry site results with the wet site directly. However, it would be expected that the dry site would suffer more damage as crude oil penetrated the soil to a greater depth, affecting more of the plant's root system. This was evident when taking soil cores from the plots in 1972 as old or dead root channels tended to transport the oil to even greater depths.

Results from both Cape Simpson and the 1970 wet site investigations indicated that tundra species may vary in their susceptibility to injury by oil spillage. Whether such differences exist could be important in predicting the impact of an oil spill on the various tundra ecosystems.

To test species susceptibility to crude oil, pots containing essentially pure stands of *Carex aquatilis, Eriophorum angustifolium* and *Eriophorum scheuchzeri* were placed in the soil and treated with crude oil at the rates of 0, 50, 100 and 200 ml/pot. These experiments were replicated three times.

In 1972 the pots were harvested and the species/pot totals were expressed as dry weight. *Eriophorum angustifolium* suffered the greatest damage with a 92% reduction in dry weight at the 200 ml rate when compared to its control (Table X). *Eriophorum scheuchzeri* was second with a drop of 87% at the 200 ml rate followed by *Dupontia fischeri* with 76% and *Carex aquatilis* with 48%.

Results with *Eriophorum angustifolium* did not substantiate the previous year's observations of resistance; however, *Carex aquatilis* still appeared to be quite resistant when compared to the other species.

In summary, the Barrow studies illustrated that foliage applications of crude oil will result in death of affected plant tissues; however, if root systems remain intact, recovery will begin late in the second season. Heavy oil applications on dry sites, resulting in inundation of the root systems, cause severe damage to the affected plant community. From this and earlier work on wet sites, it is evident that soil moisture content and site drainage are important limiting factors to plant damage from oil contamination. Root channels also appear to be important in the contamination of soil

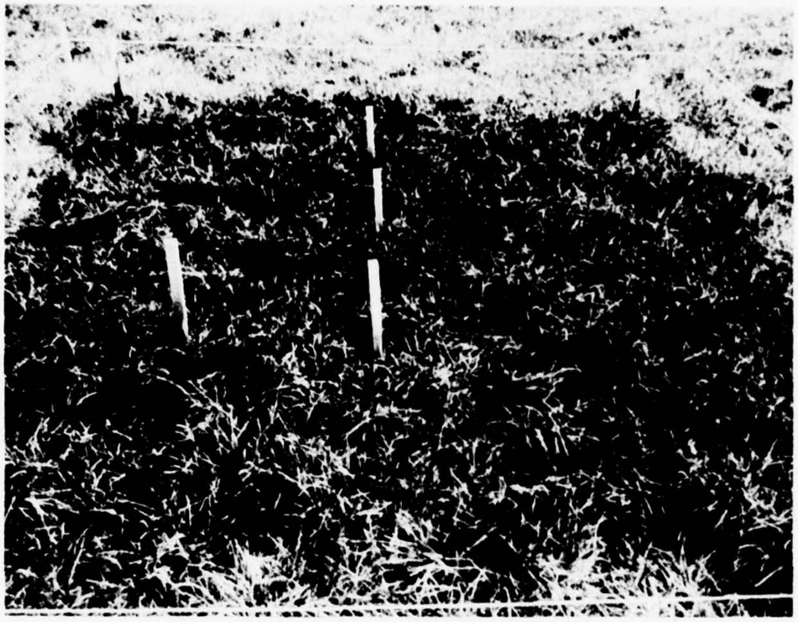

Figure 20. Spray application of crude oil. Dry site investigations, Barrow, Alaska (1971).

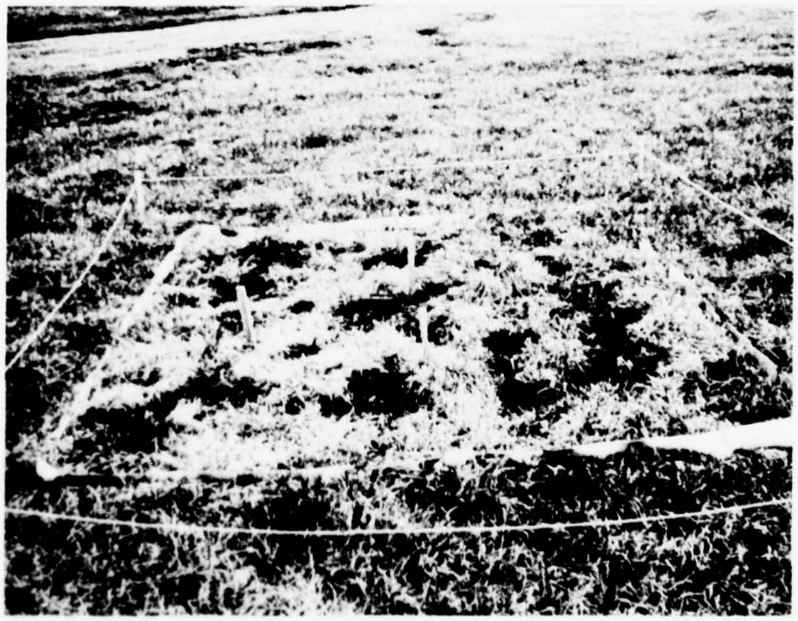

Figure 21. Soak application of crude oil. Dry site investigations, Barrow, Alaska (1971).

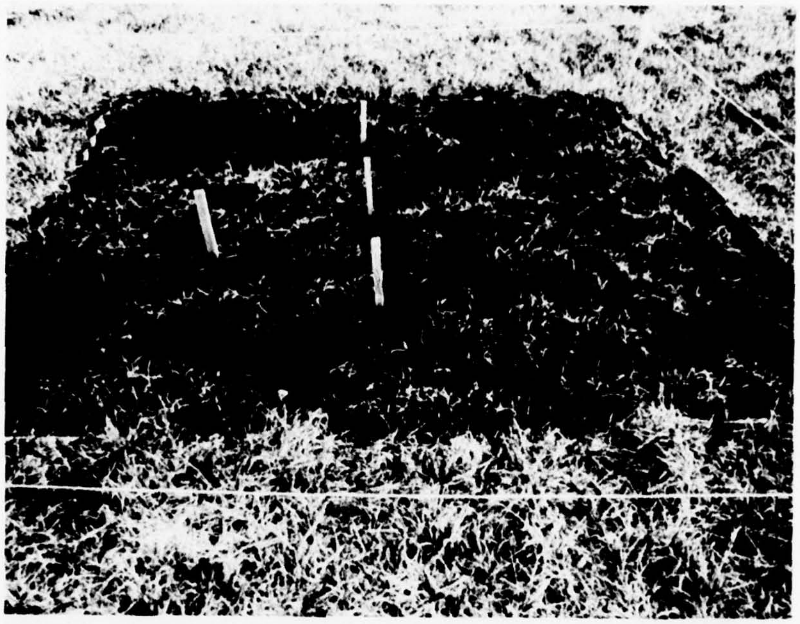

Figure 22. Spray-soak application of crude oil. Dry site investigations, Barrow, Alaska (1971).

Table IX. The effect of different types of crude oil contamination on the aboveground biomass production of a grass-sedge tundra at Barrow, Alaska (from McCown et al. 1973b).

Measurements were taken at peak production during the second growing season following treatment.

Treatment	*Dry weight production (g/m^2)*	*Decrease from control (%)*
Control	79	—
Foliar spray (5 l/m^2)	24	69.2
Ground soak (20 l/m^2)	4	95.3
Foliar spray and ground soak (25 l/m^2)	2	96.9

Table X. Differential response of four arctic tundra species to three levels of crude oil contamination (from McCown et al. 1973b).

Measurements were made at peak production during the second growing season following treatment.

Rate (ml oil/pot)	*Carex aquatilis*	*Eriophorum angustifolium*	*Eriophorum scheuchzeri*	*Dupontia fischeri*
50	28.9	10.1	6.7	40.2
100	30.0	71.9	59.5	40.5
200	48.4	92.1	87.1	75.9

surrounding the plant root system. It was also evident from these investigations that *Carex aquatilis*, a predominant sedge on the North Slope, had some degree of resistance to crude oil contamination.

During the period of studies in Alaska a Canadian research team (R.W. Wein and L.C. Bliss) was conducting similar investigations which involved the treatment of five arctic communities with crude oil during different times of the year. Their results (Wein and Bliss 1973) were similar to those reported in the previous paragraphs. All aboveground plant tissue was killed. Lichens and most moss species failed to recover; however, dwarf woody perennials such as willow and birch appeared to recover rapidly. They found that summer spills were more damaging than winter spills. In addition, they reported very little effect of oil on permafrost thaw and increases in microbial activity. They concluded that in many cases plot recovery was sufficient to warrant the suggestion of leaving contaminated areas undisturbed and allowing natural recovery processes to ensue.

Fairbanks and Fox investigations

After studying plant survival in oil-contaminated areas on the North Slope, it was decided to conduct similar investigations in interior Alaska since the Trans-Alaska pipeline will traverse this region as well. One particular study conducted in Fairbanks involved the treatment of an introduced perennial grass (*Bromus* sp.) with crude oil. Pots were seeded at the initiation of the growing season, placed in soil, and allowed to grow normally until late July 1971. At that time, half were treated with crude oil, and half were left untreated.

Until freeze-up little visible damage was noted other than slight wilting and yellowing of plant tissue in the oiled pots. After freeze-up, all plants were left in the soil for 30 days, then transferred to the greenhouse and allowed to grow for 21 days. The control pots recovered and regrowth was abundant. However, the pots treated with crude oil did not recover (Fig. 23). This substantiated earlier thoughts that the decrease in plant survival, from the first to second season of growth, was primarily the result of death during the winter period.

The investigators also initiated spill studies at Fox, Alaska. The primary research objectives were 1) to understand the effects of crude oil on the vegetation of interior Alaska and 2) to develop procedures and guidelines for restoring areas contaminated by crude oil. The site chosen was a north-facing slope covered with black spruce (*Picea mariana*). Typical understory vegetation consisted of narrow-leafed Labrador tea (*Ledum palustre*), blueberry (*Vaccinium uliginosum*), lowbush cranberry (*Vaccinium vitis-idaea*), sweet coltsfoot (*Petasites hyperboreus*) and cloudberry (*Rubus chamaemorus*) growing along with a deep moss ground cover and numerous lichen species. This location was selected as it was assumed that the greatest and most long-term damage would occur on such a site typically underlain by permafrost.

Six plots, 5 x 10 m, were established on 30 July 1971. Two were treated with crude oil using a point source method of application; that is, crude oil was applied at one spot above each subplot and allowed to run out into the rest of the plot. Two other plots were treated uniformly with crude oil. The remaining plots were retained for controls. In the oiled plots the actual rate of crude oil application was 18 l/m^2. It was interesting to note that on such a site, covered by 15 to 30 cm of moss, the oil disappeared quite rapidly. Thus uniform oil distribution as used on the North Slope was impossible when using comparable rates in the interior.

An unexpected phenomenon of oil flow on permafrost slopes in the interior was that, once the crude oil reached the zone of maximum saturation in the soil, it moved laterally downslope with no significant upward movement into the drier moss layer. Also if the crude oil reached the interface at the top of the permafrost layer it would move laterally downslope along this interface.

At the end of the 1971 growing season, very little damage to the vegetation was noted. However, moss and lichen species saturated with crude oil were killed. Thus direct oil contact was extremely detrimental to these forms of ground cover. Plots were observed in 1971, 1973 and 1974 and, as a general rule, where vegetation of any type was covered or in contact with crude oil, the affected part of the plant (but not necessarily the entire plant) died.

In general, since the oil moved downslope, it was felt that the amount of crude oil necessary to completely saturate the vegetative root zone in the plots was never reached. Thus most of the plants outwardly appeared to be as healthy in succeeding years as they were prior to the application of the oil. However, had the amount of crude oil reached a level of maximum saturation of the root zone, detrimental effects to the vegetation presumably would have increased.

As was previously noted, the Barrow results indicated that arctic tundra communities were extremely susceptible to foliar sprays of crude oil, and even more susceptible when foliar applications were accompanied by quantities sufficient to saturate the plant root zones. To provide similar crude oil sensitivity information on plant species indigenous to interior Alaska, studies were conducted in 1972 adjacent to the 1971 interior spill plots. Two 5- x 5-m plots were selected and all plants within the boundaries were covered with a foliar spray of crude oil and observed for damage.

During the first season (1972), all moss and lichen ground cover was killed and species such as sweet coltsfoot, cloudberry, and willow were defoliated. Little damage could be detected during the initial season on black spruce, Labrador tea, blueberry and lowbush cranberry.

In June 1973, it was observed that all the black spruce had winterkilled and there was no new moss or lichen growth. However, Labrador tea, blueberry, lowbush cranberry, sweet coltsfoot, cloudberry and willow were exhibiting new growth. This area was also observed in July of 1974 and conditions were essentially as observed in 1973. It should be noted that again the root zone was not saturated with crude oil. All species would probably have died had this zone been saturated. From these experiments it was readily apparent that black spruce, a predominant forest species on north slopes in interior Alaska, was extremely sensitive to foliar contact with crude oil.

Germination studies

A problem of concern in terrestrial spillages of crude oil is site rehabilitation. Initial site cleanup is but one step in the restoration process. The most difficult phase of restoration is in returning the contaminated site to a stable condition. In the case of overland spills involving plant communities, revegetation is the process normally used in restoring site stability.

From the literature review, it was apparent that the revegetation of oil-contaminated soils in temperate regions is not difficult. However, in arctic and subarctic regions there is little information in regard to revegetation techniques. In an attempt to gain knowledge in this area, USA CRREL funded research in early 1971 designed to evaluate germination and seedling growth of some northern plant species in petroleum-contaminated soil. A report of this work has been published in detail (McCown and Deneke 1973). Those experiments are described in this report essentially as they appeared in the original publication.

The research on this topic was organized into three areas. The first involved germination of native and introduced species in freshly contaminated soil or media. This was to test species response to fresh oil with its toxic components. The second involved germination of native and introduced species on aged-oil soils. The third area involved monitoring seedling development in crude oil contaminated soils.

As mentioned previously, petroleum can affect plants in several ways. It can disrupt plant-water relationships, indirectly affect plant metabolism or may be directly toxic. It should be recognized that the reaction of the soil to petroleum contamination and resultant changes in physical properties are major factors in the degree of revegetative success. The soil aggregates become coated with a waxy residue and are quite resistant to wetting. However, once saturated they tend to remain wet.

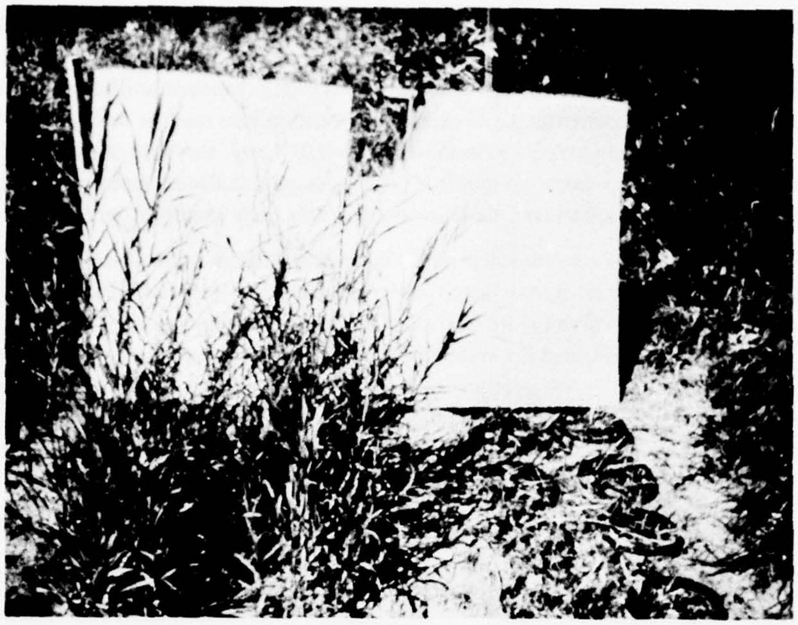

Figure 23. Winterkill of Bromus *sp. treated with crude oil (right), normal condition (no oil) to the left, Fairbanks investigations.*

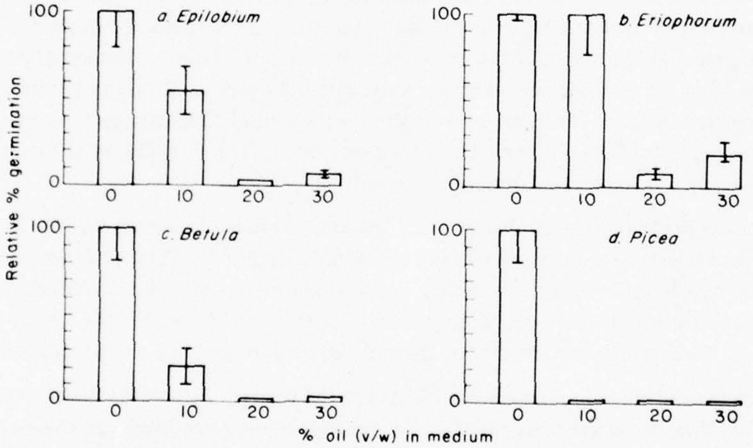

Figure 24. The effect of increasing rates of oil contamination on soil on the germination of four native Alaskan species (McCown and Deneke 1973).

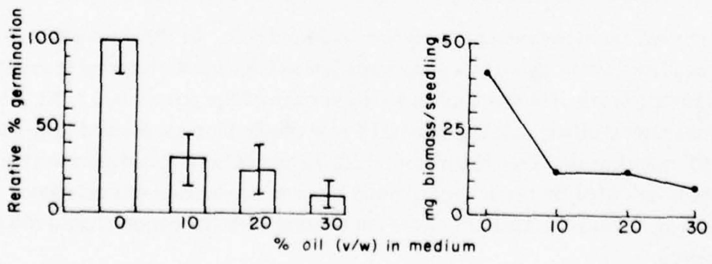

Figure 25. The effect of increasing rates of oil contamination of soil on the germination and subsequent growth of an introduced plant selection to Alaska (McCown and Deneke

It is felt that the disruption of the physical soil-water-plant relationship and anaerobic and hydrophobic conditions are the most important aspects of oil-contaminated soils as they relate to plant germination and growth. There are also other problems such as nutrient uptake interference resulting in either toxicity (excessive uptake of exchangeable manganous ions) or deficiency symptoms (low levels of nitrogen and phosphorus in the early stages). Lastly there are certain components in crude oil, particularly the volatile components (aromatics, naphthalenes, olefins and parafins) which are directly toxic to plants; however, these usually dissipate quite rapidly after a spill.

Germination in fresh oil-contaminated soil. Four native Alaskan species were selected for evaluating germination in crude oil-contaminated soil: *Epilobium* sp. (fireweed), *Eriophorum vaginatum* (cottongrass), *Betula papyrifera* (white birch) and *Picea glauca* (white spruce). In addition, *Trifolium pratense* (red clover) was selected for evaluation, primarily as representatives of this genus were among the first to be used in revegetation of oil-contaminated areas in the temperate zone. The soil used was a Fairbanks silt loam at 60% field moisture capacity when treated. Prudhoe Bay crude oil was evenly mixed with the soil at rates of 0, 10, 20 and 30% (vol/wt). All experiments were conducted in plant growth chambers at 22°C.

The presence of oil generally depressed germination rates. Of the native species tested in freshly oiled soils, white spruce was the most sensitive as there was no germination in the contaminated treatments (Figs. 24 and 25). Red clover appeared to be less sensitive than the native species to crude oil-contaminated soils; however, germination rates were significantly reduced when compared with the controls. Perhaps even more important was the decrease in biomass per seedling with increasing oil concentrations for all plants tested.

A grass mixture consisting of 10 parts annual rye, five parts brome, two parts meadow foxtail, and two parts red fescue was also evaluated for germination in contaminated medium. The treatments were prepared as outlined above except that Jiffy-mix, a uniform commercial potting medium, was used and the seed was mixed with a water saturated vermiculite slurry before seeding on the contaminated surface. However, even employing these more favorable germination conditions, results were similar to those obtained with the other species. The germination of the perennial species especially was reduced in all treatments with oil.

Germination in aged oil-contaminated soil. The second phase of research was the evaluation of germination of selected species in aged crude oil-contaminated soils. Pots with freshly oiled soil were aged in a greenhouse for approximately one year before testing. The rate of oil used in these experiments was approximately equivalent to the highest rate, 30%, used in the freshly oiled soil experiments. Seeds were sown directly on the soil surface and kept moist with plastic covers.

White spruce and annual ryegrass were selected as the test species because the earlier experiments had indicated these to be the most and the least sensitive to oil, respectively, of the species analyzed. Although some germination of *Picea* did occur in the contaminated aged soil, the rate was only 35% that of the control. The germination of annual ryegrass on the contaminated soil was not reduced but the aboveground biomass was only 10% of the control values (Fig. 26).

Seedling growth and development in oil-contaminated soils. In the third phase of this work, one-year-old *Picea* plants were treated at a stage intended to coincide with spring growth, i.e. the breaking of new candle growth. Oil was applied to the surface of the pots at 0, 10, 20, 30 and 60% (total pot saturation) rates (vol/wt). Measurements of new candle biomass showed little difference between the 0 and 10% rates but the three higher rates (20, 30 and 60%) reduced growth (Fig. 27). Examination of the pots indicated that new aboveground biomass production was correlated with the depth of penetration of oil into the medium. Roots in contact with the contaminated soil were brown and appeared injured.

Established annual ryegrass plants were also treated with a surface application of crude oil at 0, 10, 20 and 30% rates. Plants showed initial wilting when the crude oil was applied but revived with

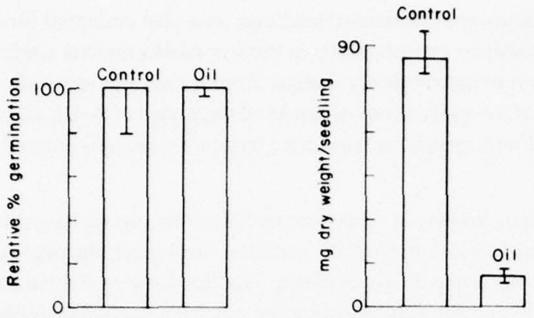

Figure 26. The response of annual ryegrass to aged oil-contamination of the soil (McCown and Deneke 1973).

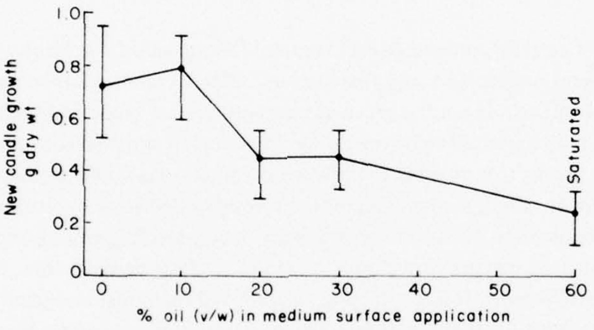

Figure 27. The response of white spruce to increasing rates of oil contamination of the soil (McCown and Deneke 1973).

Table XI. The effect of crude oil on plant nutrient levels (McCown and Deneke 1973).

	*Element**						
Treatment	*Nitrogen*	*Phosphorus*	*Potassium*	*Calcium*	*Magnesium*	*Manganese*	*Zinc*
Control	2.34	0.37	2.38	1.12	0.50	0.032	0.001
10%	1.86	0.29	2.14	0.74	0.50	0.017	0.006
20%	1.87	0.27	1.94	0.65	0.38	0.014	0.008
30%	0.43	0.39	2.04	0.56	0.42	0.014	0.010

* Data expressed as % dry weight of shoot.

subsequent watering. Little reduction in general growth was observed over a four-week period, even at the 30% concentration.

Established *Taraxacum* sp. (dandelion) seedlings were also evaluated for their sensitivity to crude oil. *Taraxacum* are common pioneer plants in interior Alaska and are characterized by a thick, fleshy tap root. This is morphologically distinct from spruce and especially annual ryegrass which has fibrous roots. Surface applications of crude oil were used at 0, 10, 20 and 30% (vol/wt) concentrations. In all oil treatments, root and stem bases were severely injured and the eventual death of the plant resulted.

Several species, when growing in oil-contaminated media, showed a red to reddish-purple coloration, frequently associated with mineral deficiencies. In general, the degree of red color in the leaves increased with increasing crude oil in the media. Species showing the red coloration included *Betula, Epilobium, Taraxacum* and the grasses. Inorganic nutrition, including availability and plant uptake, has often been implicated as an important secondary effect of oil contamination. Annual rye leaf tissues, taken from plants treated with a surface application of crude oil and allowed to grow for four weeks, were analyzed for nitrogen, potassium, phosphorus, calcium, magnesium, manganese and zinc. Data represented in Table XI show no major change in the amount of P, K, Mg or Zn with increasing crude contamination. There was, however, a reduction of N, Ca and Mn with increasing crude oil contamination.

Summary of plant germination and growth results. Disruption of the plant-water balance, indirect influences on plant metabolism, and toxicity have all been reported as consequences of oil-contaminated soil on plants. From the previous observations, no single factor could be identified as the primary effect; all appeared to be operating. Although it is difficult to predict precisely how crude oil will affect plants, it is possible to draw some conclusions on the potential success of seed germination and growth. First, seed germination on freshly oiled soil can be expected to be low. Second, although germination on aged oiled soils may occur, seedling growth and survival will be limited. Mineral nutrition may be very closely linked to seedling survival; thus, the long-term effects of low levels of essential elements like nitrogen, calcium and manganese on plant fitness in a natural environment are difficult to predict. It is interesting to note that in previous literature (Ellis and Adams 1960, 1961; Schwendinger 1968) levels of nitrogen, phosphorus and manganese increase in oil-contaminated soils. In this experiment soil nutrient levels were not measured. One might expect to find increased levels of these nutrients in the plant tissue if indeed they are more abundant in the soil solution. This was not found. However, the disruption of normal plant-water relationships by the oil could certainly impede normal nutrient uptake by the plants.

There is certainly a variation in species sensitivity to crude oil contamination, and the long-term result of a terrestrial oil spill may be a shift in species composition. Root morphology may be an important selective characteristic and taprooted species would probably be eliminated. The observation that fibrous rooted species (in particular, grasses) may be more tolerant of crude oil-contaminated soils is important since grasses are most commonly selected for revegetation.

It should be reiterated that these experiments were conducted in the laboratory under more or less ideal environmental conditions. The additional or moderating effects that cold soil temperatures, drought or other environmental variables may have cannot be projected from the results.

Physiological studies

Some physiological responses of plants to low volumes of crude oil applied to the soil surface. Plant performance under a variety of oil and environmental conditions has generally been evaluated in terms of whole plant response. Parameters measured include dry matter production, shoot/root ratios, seed germination, seedling survival, and mineral nutrient composition. Oil-contaminated systems have been further stressed by soil nutrient additions, soil temperature alterations, and surface perturbations. Information is generally lacking, however, on the physiological bases for observed plant responses to oil.

The general pattern which emerges is that oil can both stimulate and retard plant growth, depending on the rates of oil application and the time elapsed since application. The response of a plant to a given level of oil application is species- and environment-dependent. In relative terms, low levels of oil may stimulate plant growth while higher levels may inhibit growth or rapidly kill the plant. For any given level of oil which does not immediately kill the plant, the effect may be to inhibit plant growth for a time after application but later to stimulate growth after partial oil decomposition has occurred.

In the absence of the directly toxic volatile components of crude oil, sufficient information does not yet exist to adequately resolve the question of whether oil affects plant growth primarily through direct effects on the soil microbial populations, or whether the primary effect is on soil physical and chemical properties. While a number of soil properties do change in response to oil, the bulk of the information would suggest that microbial influence is most important. Growth inhibition or death of plants soon after soil contamination is generally associated with Mn availability at toxic levels, concurrent with a decrease in soil oxidation-reduction potentials. Enhanced growth at later stages of oil decomposition has been attributed to an increase in soil N and water holding capacity. However, a number of more recent studies cited in this report suggest that the presence of complex molecules at more advanced stages of oil decomposition may have growth regulatory properties.

Schwendinger's (1968) review of the literature supported the conclusion that small amounts of oil may be beneficial to plants but larger quantities of oil may induce hydrophobic conditions which interrupt normal plant-water relations rather than being directly toxic to the plants. In Schwendinger's (1968) report, he found that even sensitive crops could withstand appreciable oil in the soil if they were already well established prior to contamination. Oil pollution symptoms of the foliage were similar to those caused by severe nutrient deficiencies, and the development of the symptoms was inversely related to water uptake. He concluded that oil-induced damage was due to disruption of plant water uptake. Finally, he found that the effects of oil contamination could, at least partially, be offset by heavy fertilization, and he attributed this to mass action effects on nutrient uptake.

A series of experiments was initiated at USA CRREL to investigate the effects of low concentrations of oil on plant growth at the process level. Specifically, the physiological basis by which dry matter production is reduced by oil was of primary interest. Since photosynthesis is the basis of all dry matter yield, several components of the photosynthetic apparatus were chosen for study. These included: the activity of the principal carboxylating enzyme of the carbon reduction cycle (ribulose-1, 5-diphosphate carboxylase, or RuDPC), the concentrations of chlorophylls, and leaf resistances to CO_2 diffusion as a function of oil. Conclusions concerning oil effects on leaf resistance did not materialize because of the very high variability encountered. The choice of this parameter as an indicator of plant response was derived from the observations of previous investigators that oil affected normal plant-water relations. The hypothesis was that the stomatal resistance increased as a result of oil-induced water stress. The stomatal resistance in turn decreased photosynthesis and possibly elevated leaf temperatures. Therefore leaf respiration was also elevated with the end result being a reduction in net dry matter accumulation.

The positive correlation between tissue N content and chlorophyll has long been known (Brown and Ashley 1974). In addition, reduction in nitrogen supply to plant roots has been shown to drastically reduce the activity of RuDPC (Medina 1971). In view of Schwendinger's (1968) observations that heavy fertilization at least partially offsets the effects of oil, it was hypothesized in this study that oil exerts its effect on dry matter production by impeding nitrogen availability in the soil and/or nitrogen uptake by the roots, which in turn decreases the chlorophyll concentration and RuDPC activity. If the photosynthetic potential is affected in this way by oil, then nitrogen fertilizer might overcome all or part of the antagonistic effects of oil. In general, the experiments supported this hypothesis.

Methods applicable to all experiments will be described here. Methods peculiar to a particular experiment will be included with the discussion for that experiment.

Plants were started from seed and grown in soil and were well established prior to treatment with oil. The soil was a Tanana silt loam topsoil from the University of Alaska Experimental Farm in Fairbanks. A soil analysis is given in Table XII. The soil has a long history of fertilization. It is relatively high in Mn, Cu, Fe and Mg, but is low in P and K. The exchange complex is saturated with bases and reaction is slightly acid. The soil was mixed to obtain a homogeneous mixture, heat sterilized (100°C) to kill soil borne pathogens, and then contained in 16.5-cm-diam pots having a depth of either 12 or 17 cm. Two or 2.5 kg of dry soil was used in the 12- and 17-cm pots respectively. Equal quantities of distilled water were used to keep the soil moist. Pots were nested in tin cans so that the leachate and/or oil which came through the drain holes could be collected and added back to the surface of the soil. In this way oil and fertilizer contamination by adjacent pot drainage was prevented.

Crude oil used in these experiments was produced at Prudhoe Bay in 1972 by the Atlantic Richfield Co. An analysis of a sample of Prudhoe Bay crude was presented in McCown and Deneke (1972). The oil was applied to the surface of the soil at room temperature and care was taken to prevent oil from coming in contact with the foliage except at the soil surface. Microscopic examination at the time of the application showed the oil to be free of microorganisms.

Plants (grasses) were grown on a growth table 1.2 × 2.4 m. Lighting was provided by four banks of fluorescent fixtures each containing four white tubes 2.4 m long. Irradiance at plant level varied from 190 microeinsteins/m^2 sec (400–700 nm) at the edge of the table to 240 microeinsteins/m^2 sec at the center. The gradient in irradiance occurred in spite of the light banks extending well beyond the sides of the table. To compensate experimentally for this gradient, a randomized complete block design was used with the treatments blocked in the directions of changing irradiance. Treatments were randomized within the blocks. The photoperiod was 16 hours long. There was no control of temperature *per se*, but a thermoperiod did result from cycling the lights. Temperature fluctuated between about 20° and 28°C. Relative humidities were generally between 35 and 60%. A fan was used above the lights to increase circulation but did not blow directly on the plants.

Fertilizers used in this study were either ammonium nitrate (33⅓% N) or treble superphosphate (20% P). The fertilizer was applied to the appropriate treatments at the same time oil was applied.

Live leaf blade tissue was used for the chlorophyll, RuDPC, and mineral nutrient analysis. Chlorophylls were extracted according to the procedure of Tieszen (1972). Concentrations of chlorophylls a and b were calculated from the absorbance values at 645, 652 and 663 nm (Arnon 1949) as measured in a Beckman DB-G spectrophotometer. The extraction and assay of RuDPC followed the method of Tieszen and Sigurdson (1973). Nutrient analyses were done according to Jackson (1958).

It was intended to use barley (*Hordeum vulgare*) initially to explore techniques since this species is easy to establish and grows very rapidly. Also, the leaves are wider than 3.5 mm, the minimum width required to measure leaf resistance with the diffusion porometer described by Kanemasu et al. (1969). However, barley proved to be extremely sensitive to even very low levels of oil and did not live long enough after treatment to allow physiological effects to be determined. Therefore, two species of introduced grasses which have shown varying degrees of success in Alaskan revegetation studies, foxtail and bromegrass, were used.

1. *Foxtail (Nitrogen – oil)*: Seeds of Garrison creeping foxtail (*Alopecurus arundinacea*), a tall rhizomatous perennial, were stratified between moist filter papers at 5°C for nine days in the dark

and planted 1 cm deep in moist soil. Eight seedlings were established per pot. The plants were clipped back to pot level after 45 days to prevent flowering and to stimulate pod production. Twenty days after clipping the treatments were applied to the pots while the plants were still in a vegetative stage. Regrowth at this time was about 12 cm high with up to three leaves exserted.

Treatments consisted of all factorial combinations of 2 levels of N and 4 levels of oil. The N levels were control and 22.5 g N/m^2 equivalent. The oil rates were control and 0.5, 1.0 and 2.0 l/m^2 equivalent. The eight treatments were replicated twice in a randomized block design. RuDPC activity and chlorophyll concentrations were determined five days after treatment and plants were again clipped to pot level. The regrowth was again sampled 40 days later for RuDPC and chlorophyll, and nutrient concentrations and aboveground dry matter production were determined.

Some effects of oil and nitrogen at the levels applied are shown by the treatment means of nine parameters (Table XIII). Significant sources of variation are shown in Table XIV. Chlorophylls a and b generally followed the same trends as total chlorophyll (a + b) and these data were not included in Table XIII. Nitrogen did cause a slight but significant increase in the chlorophyll a to b ratio (3.0/3.2) at the second sampling period, but oil had no effect on the relative proportions of these two pigments.

Nitrogen produced highly significant increases in the levels of all parameters in Table XIII except Mg, Ca and Mn. These effects of nitrogen on chlorophyll and RuDPC agree with the literature as cited earlier. Nitrogen effects on the other parameters, particularly dry matter production and leaf nitrogen content, also followed the expected. The slight increase in Mg due to nitrogen was not significant although nitrogen applications did result in increases in chlorophyll concentrations.

In general, the low levels of oil used in this study had little effect on most parameters measured. Oil levels were high enough to cause significant reductions in dry matter production from 6.8 g/pot for the controls to 4.1 g/pot at the highest level of oil (2 l/m^2). Oil did decrease RuDPC activity at the second sampling period and a nitrogen - oil interaction was apparent for dry matter and for RuDPC at both sampling periods. Dry matter production declined almost linearly as oil increased either with or without nitrogen. However, the rate of decline was slightly greater in the presence of nitrogen. The oil - nitrogen interaction for RuDPC showed that a synergistic effect on enzyme activity may result by certain combinations of oil and nitrogen. In this experiment, oil had almost no effect on RuDPC in the absence of nitrogen. In the presence of nitrogen, RuDPC activity declined from its control through the level 3 of oil but then increased again at the highest level of oil. This was observed for both sampling periods.

Although increasing levels of oils resulted in a corresponding decrease in dry matter production either with or without nitrogen, even at the highest level of oil N application resulted in over twice as much production as the control. This supports Schwendinger's (1968) observation that fertilization can overcome some effects of oil on plant growth.

Concentrations of inorganic nutrients in leaf blade tissue did not respond to low levels of oil. The highly significant interaction for calcium resulted because Ca concentrations increased up to the third level of oil and then declined slightly in the absence of N. In the presence of N, Ca concentrations generally decreased as oil rates increased.

A comparison of RuDPC activities and chlorophyll concentrations between the first and second sampling periods showed no significant differences for RuDPC. For chlorophyll, a highly significant decrease in the mean among pots not receiving nitrogen did occur. While it is tempting to invoke N as the factor which prevented a corresponding decline in N-treated plants, the data are insufficient to make this conclusion. There may be other factors interacting with nitrogen not tested in this experiment.

Table XII. Results of soil nutrient analysis for the soil used in the physiological experiments.

Property	*Percentage*	*ppm*	*Meq/100 q*	*Saturation (%)*
Organic matter	2.4			
Phosphorus (spray no. 1)		21		
Sulfur (available)		35		
Potassium (exchangeable)		35	0.1	0.4
Magnesium (exchangeable)		742	6.2	30.7
Calcium (exchangeable)		2680	13.4	66.5
Sodium (exchangeable)		113	0.5	2.4
Zinc (available)		3.9		
Manganese (available)		2.0		
Copper (available)		3.8		
Iron (available)		123.0		
Boron (available)		0.6		
Molybdenum (available)		0.3		
Soil pH	6.7			
Cation exchange capacity			20.2	—
Percent base saturation				100.0

Table XIII. Analytical results – foxtail (nitrogen - oil) experiments.

Treatment		*Chlorophyll a+b (mg/g dry wt)*		*RuDPC (mg CO_2/g dry wt hr)*		*Dry wt (g/pot)*	*N (%)*	*P (%)*	*K (%)*	*Ca (%)*	*Mg (%)*	*Mn (%)*
*N**	*Oil†*	*1*	*2*	*1*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*
0	0	11.0	7.4	2.7	3.5	2.97	1.26	0.28	1.68	0.33	0.24	0.04
0	1	10.9	7.5	2.8	2.2	2.83	1.35	0.23	1.41	0.43	0.30	0.04
0	2	11.5	9.7	3.0	2.6	2.45	1.65	0.25	1.46	0.53	0.38	0.46
0	3	12.0	7.5	2.9	2.6	1.70	1.10	0.25	1.34	0.48	0.29	0.04
Mean		11.3	8.0	2.9	2.7	2.49	1.34	0.25	1.47	0.44	0.30	0.04
1	0	15.8	17.4	13.0	12.3	10.56	2.96	0.31	1.79	0.38	0.42	0.02
1	1	15.2	13.7	11.0	11.4	8.54	2.81	0.34	2.36	0.30	0.30	0.02
1	2	13.8	13.6	3.9	4.1	7.81	2.33	0.31	1.95	0.30	0.28	0.02
1	3	13.9	14.4	8.1	6.1	6.41	2.69	0.31	1.72	0.28	0.26	0.02
Mean		14.7	14.8	9.0	8.5	8.33	2.69	0.31	1.96	0.32	0.31	0.02

Note: The 1 or 2 under each column heading refers to first or second sampling period.

*Nitrogen treatment was always 22.5 g/m^2.

†Oil treatment was 0.5 l/m^2 for level 1, 1.0 l/m^2 for level 2, and 2.0 l/m^2 for level 3.

Table XIV. Analysis of variance – foxtail (nitrogen - oil) experiments.

Source of variation	*Degrees of freedom*	*Chlorophyll a+b*		*RuDPC*		*Dry wt*	*N*	*P*	*K*	*Ca*	*Mg*	*Ma*
		1	*2*	*1*	*2*	**2**	*2*	*2*	*2*	*2*	*2*	*2*
Blocks	1	4.22	54.02†	8.37	0.77	2.34*	0.66	0.001	0.17	0.001	0.015*	0.000
Nitrogen (N)	1	44.29†	183.26†	151.23†	134.21†	136.53†	7.34†	0.018†	0.94†	0.063†	0.001	0.003†
Oil (O)	3	0.48	2.46	14.84	17.69†	5.10†	0.04	0.000	0.09	0.003	0.003	0.000
N X O	3	2.07	6.25	16.64	14.59†	1.54*	0.21	0.001	0.12	0.015†	0.013*	0.000
Error	7	1.60	4.37	3.82	0.75	0.34	0.20	0.001	0.07	0.001	0.002	0.0001
Total	15											

*Significant at 0.05 level .

†Significant at 0.01 level.

2. *Foxtail (Nitrogen - phosphorus - oil):* The plants used in this experiment were from the same set used in experiment 1 except treatment occurred 66 days after clipping rather than 20. Plants were about 20 cm high and in the fourth leaf stage.

The treatments consisted of a 2^3 factorial arranged in two blocks. The factors were nitrogen (0 and 22.5 g N/m^2 equivalent), phosphorus (0 and 11.2 g P/m^2 equivalent), and oil (0 and 2 l/m^2 equivalent). The pots were sampled for RuDPC and chlorophyll analyses 15 days after the treatments were applied. Also at this time, the aboveground production was determined by clipping, drying and weighing. The leaf blade material was ground and subsampled for nutrient analyses.

Summarized results and the analysis of variance appear in Tables XV and XVI respectively. Oil had little effect on the chlorophyll a to b ratio or on leaf blade Mg. Oil significantly increased leaf blade Ca but only in the presence of N giving a significant N - oil interaction. In all other parameters, the effect of oil was to reduce the level of the parameter regardless of the presence or absence of N and P (Table XV). Reductions by oil were either significant or highly significant in all but RuDPC. Nitrogen significantly affected all parameters but leaf Ca, while P only had a significant effect on leaf P. Although oil generally had antagonistic effects on the parameters measured with or without nitrogen, nitrogen more than overcame the effects of oil in comparison to the control, and this result agrees with Schwendinger (1968).

Phosphorus had little influence on oil effects and there were no significant interactions of N and P. The one significant interaction of oil and P occurred in the leaf P data, as might be expected. Oil decreased leaf P levels with or without the addition of P fertilizer. Although P fertilizer increased leaf P by 40% in the absence of oil, leaf P was the same in the presence of oil. There were several significant N - oil interactions. For the cases of dry weight production, fresh weight/dry weight ratio of the leaf blades, N, and K, interaction resulted because reductions in the respective parameters due to oil were greater in the presence of nitrogen. However, in all cases, parameter levels under the oil and nitrogen treatment exceeded levels of the control treatment. Calcium in the leaf behaved differently. Oil produced no effect on leaf Ca in the absence of nitrogen fertilizer. In the presence of N, oil interacted to cause a synergistic effect on leaf Ca causing it to increase by about 25%. Oil and nitrogen had opposite effects on tissue succulence (fresh weight/dry weight ratio). Nitrogen increased succulence but oil decreased it. This would suggest that oil did interfere with normal plant-water relationships as suspected. It may reflect a relatively greater synthesis of cellulose and lignin and less protein synthesis under conditions of a slower rate of growth.

In comparison with experiment 1, parameter levels were generally lower. This probably reflects the age of the plants and the development processes of senescence. This may also explain oil response differences between the two experiments with relation to significant effects, although differences in numbers of factors, factor levels and degrees of freedom would also affect significant differences. Oil significantly lowered RuDPC activity at the second sampling period of experiment 1 but had not produced an effect at the first sampling (Table XIV). Whether this response resulted from aging or from increased time of exposure to oil is not known.

Sixty days after the plants were clipped to soil level for sampling, treatments were ranked based on a qualitative assessment of vegetative regrowth and recovery. The array from best recovery to worst was (N, N-P), (control, P), N-P-oil, N-oil, P-oil, and oil. Differences could not be delineated between treatments in parentheses. Recovery ranged essentially from death in the oil only treatment to a very lush and thick regrowth in N- and N-P-treated plants. The ranking definitely depicted what the quantitative data had shown earlier, that P had little beneficial effect on oil-induced plant responses and that nitrogen greatly facilitated recovery of oil-treated plants subsequent to clipping.

3. *Bromegrass (Nitrogen – oil):* This study was similar to experiment 1. Seeds of Manchar brome (*Bromus inermis*), a tall rhizomatous perennial, were suspended above distilled water on a hardware cloth platform covered with large Kimwipes (paper towels). These acted as wicks to keep the seed

Table XV. Analytical results – foxtail (nitrogen - phosphorus - oil) experiment.

Treatment			Chlorophyll (mg/g dry wt)		RuDPC	Dry wt	Fresh wt/Dry wt	N	P	K	Ca	Mg
N	P	Oil	a+b	a/b	(mg CO_2/g dry wt hr)	(g/pot)	(g/g)	(%)	(%)	(%)	(%)	(%)
0	0	0	6.6	3.2	1.2	2.44	2.77	1.04	0.25	1.36	0.35	0.28
0	0	1	4.9	3.0	1.0	2.30	2.68	1.05	0.23	1.17	0.34	0.29
Mean			5.8	3.1	1.1	2.37	2.72	1.04	0.24	1.26	0.35	0.28
0	1	0	8.3	3.1	1.5	2.51	3.02	1.22	0.32	1.50	0.31	0.29
0	1	1	5.7	3.0	1.1	2.22	2.62	1.06	0.20	1.18	0.32	0.26
Mean			7.0	3.0	1.3	2.36	2.82	1.14	0.26	1.34	0.31	0.28
1	0	0	17.5	3.3	9.9	5.36	4.94	4.33	0.32	2.19	0.33	0.32
1	0	1	15.5	3.4	8.6	2.73	3.53	3.99	0.31	1.36	0.38	0.31
Mean			16.5	3.3	9.2	4.04	4.23	4.16	0.31	1.77	0.36	0.31
1	1	0	18.6	3.1	11.7	5.39	5.00	4.49	0.53	2.29	0.28	0.39
1	1	1	14.4	3.4	8.1	2.44	2.78	3.58	0.33	1.21	0.42	0.37
Mean			16.5	3.3	9.9	3.91	3.89	4.03	0.43	1.75	0.35	0.38

Nitrogen treatment was 22.5 g/m^2.
Phosphorus treatment was 11.2 g/m^2.
Oil treatment was 2.0 l/m^2.

Table XVI. Analysis of variance – foxtail (nitrogen - phosphorus - oil) experiment.

Source of variation	Degrees of freedom	Chlorophyll (mg/g dry wt)		RuDPC (mg CO_2/ g wt hr)	Dry wt. (g/pot)	Fresh wt./ dry wt. (g/g)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
		a+b	a/b								
Blocks	1	3.49	0.073	0.47	0.01	0.018	0.00	0.000	0.00	0.008*	0.004
Nitrogen	1	411.99†	0.226*	279.39†	10.40†	6.656†	36.21†	0.058†	0.85†	0.002	0.015†
Phosphorus (P)	1	1.51	0.023	0.67	0.02	0.060	0.00	0.018*	0.00	0.002	0.004
Oil (O)	1	27.07†	0.001	8.04	9.06	4.244†	0.49*	0.032†	1.48†	0.009*	0.001
NP	1	1.47	0.001	0.19	0.02	0.194	0.05	0.008	0.01	0.001	0.005
NO	1	0.89	0.133	4.50	6.60†	2.449	0.31*	0.001	0.50†	0.010*	0.000
PO	1	2.35	0.017	1.55	0.06	0.314	0.14	0.020*	0.04	0.003	0.001
NPO	1	0.41	0.000	1.00	0.01	0.065	0.04	0.002	0.00	0.001	0.001
Error	7	1.05	0.030	2.42	0.07	0.113	0.04	0.002	0.03	0.001	0.001
Total	15										

* Significant at 0.05 level.
† Significant at 0.01 level.

moist but not anaerobic. The seeds were incubated in the dark at 10°C for three days and at 25°C for two days. During the latter incubation, the seeds germinated rapidly. On the fifth day after the start of incubation, 10 germinating caryopses were transplanted to pots containing the same soil mix as in experiment 1. Small holes were made in the soil about 1 cm deep and caryopses were inserted by forceps and lightly covered with moist soil. The day after transplantation, coleoptiles were showing above the soil surface. Seventeen days after transplantation, the treatments were applied when the plants were in the sixth leaf stage and about 25 cm in height. The oil treatments were applied again 10 days after the first but only the initial fertilizer application was used. The experimental design and rates of N and oil followed that outlined in experiment 1. The plants were sampled for chlorophyll, RuDPC, nutrients and aboveground production 10 days after the second oil application.

Results of the laboratory analyses and analysis of variance are in Tables XVII and XVIII. Nitrogen caused significant increases in all variables measured except leaf P. Oil significantly reduced RuDPC activities in leaves of this species alone or in combination with N. However, at the highest level of oil, N-treated plants had more than twice the activity of the controls, once again showing the beneficial effects of fertilizer in overcoming negative responses of plants to oil. The reduction of RuDPC activity due to oil was accelerated in the presence of N and caused the highly significant N - oil interaction.

Unlike experiment 2 oil in this experiment apparently had no effect on succulence. Magnesium declined in the leaves in response to oil in contrast to the other experiment. Variability in the chlorophyll data prevented a significant main effect of oil on chlorophyll, so covariance of these two variables in response to oil cannot be shown (in spite of their close association in the plant). As in the other experiments, oil reduced dry matter yields, but in this case, the effect of oil had begun to reverse at the highest level of oil in the nitrogen-treated pots. More work would be required to ascertain if this trend is real or artifactual.

The treatments were qualitatively ranked (as in experiment 2) 60 days after clipping the plants for analyses. In order of decreasing recovery the array was N, control, (N-oil 1, N-oil 2), (oil 2, oil 3), N-oil 3, oil 3. Treatments in parentheses showed similar recovery. The least recovered treatments (N-oil 3 and oil 3) had about the same number of live shoots, but in the former, the shoots were twice as long as in the latter. The effects of N in hastening recovery under the experimental conditions are shown in this ranking.

Conclusions. These experiments are only a starting point for discerning effects of oil at the process level. They indicate that oil can adversely affect enzyme and pigment systems in the plants' photosynthesis apparatus and that nutrient uptake, as evidenced by leaf tissue concentrations, can be impeded by oil. The possibility of differential physiological responses of plants as a function of species, age and exposure time was suggested. It is realized that greater replication and environmental control must be used to form definite conclusions in this regard and that to have applicability in Alaska, temperature must be superimposed as a variable (since temperature interactions can be expected). In addition, more levels of the independent variables must be used to delineate response surface; more interactions might have been observed in the present study if logistics had permitted more levels of nitrogen and oil.

In these studies, rates of oil application were insignificant in comparison to those which would be experienced by plants from an actual spill. Their purpose, however, was to ascertain some of the chronic effects of oil which might have application in rehabilitation of spill areas. It is realized that plants exposed to actual spills will very likely be killed and little can be done to save them. Thus it would have been more realistic, had time permitted, to use soil-oil mixtures of various ages as did McCown and Deneke (1972) to study physiological response of plant species which successfully germinate and establish. Possible adaptations or tolerances at the process level could be determined and used in selecting plants for rehabilitation.

Surface applications of oil as done in these experiments did not penetrate appreciably into the soil. The roots themselves were generally not directly in contact with oil-contaminated soil. Oil appeared to enter the plant through the leaf sheath bases as evidenced by a blackened zone of 4 to 6 cm above the soil surface on the stems. The length of this zone was several times greater than the depth of oil at the time of application. Perhaps this lack of soil penetration explains the lack of response of leaf manganese to oil. Although the soil surface of oil treatments was hard and hydrophobic, it is unlikely that this condition resulted in an anaerobic rooting zone, due to the small volume of the pots. While oil prevented water infiltration at the surface, water easily moved into the rooting zone from the sides and bottom of the pots. Thus, severe oil toxicity and water stress symptoms were not visibly noticeable. Limited porometer data indicated a greater leaf resistance for barley plants growing in contact with oil but variability was high and prevented statistical conclusions. It is probable that the effect of oil on chlorophyll concentrations, RuDPC activities and nitrogen metabolism would have been more pronounced and less variable had the oil been mixed with the soil.

Further work should concentrate on duplicating natural post-spill conditions and ultimately should include a variety of substrates and plant species to enable more direct applicability to rehabilitation problems. In addition, a leaf or plant gas exchange monitoring capability (photosynthesis, transpiration) would make possible the concurrent and immediate assessment of plant physiological responses to oil. Oxygen tensions and soil-water potentials should be measured and manipulated to determine the mechanism of oil-induced effects on plant-water relations and nutrient uptake.

Dispersant studies

Numerous chemical compounds have been developed to assist in the washing of oil residues from various surfaces contaminated by accidental petroleum spills (Canevari 1970). These compounds are commonly known as dispersants. Their use in terrestrial spills has been pointed toward the removal of crude oil from plant surfaces. Recent investigations indicate that the removal of crude oil from plant surfaces solves only one part of the terrestrial oil pollution problem. The previous sections of this report have discussed the problems associated with plant germination and growth, i.e. direct toxicity, hydrophobic soil surfaces, etc. As was mentioned earlier, an oil-soaked soil is simply not conducive to plant seed germination. Thus some method for removing crude oil from the soil surface is needed to provide conditions more conducive to germination.

One method to aid revegetation of soils saturated with crude oil would be the use of chemical dispersants to aid in the removal of the oil from the upper soil layers and thus to promote hydrophyllic conditions, particularly in the surface layer. Naturally one criterion in selecting such a dispersant would be whether or not it also had properties which inhibit seed germination and plant development.

Corexit 7664 (trade name for the chemical dispersant developed by Esso Research and Engineering Company, Houston, Texas) is a commercially developed chemical dispersant. This compound is relatively nontoxic to marine life (Canevari 1970a) and established terrestrial plants (Westfall 1971), but its effects on seed germination and early seedling development have not been evaluated prior to this study. This experiment was designed to provide information in this regard.

Esso Chemical Company, Houston, Texas, furnished the dispersant, Corexit 7664, used in this study. Rye and bromegrass seed were selected for this experiment as these species have been used quite extensively in revegetation studies.

Stock solutions of dispersant were made up in 1/50 (Corexit to water), 1/100, 1/250, 1/500, 1/1000, 1/3000 ratios, and distilled water was used as the control medium. The Corexit/water

Table XVII. Analytical results – bromegrass (nitrogen - oil) experiment.

Treatment		*Chlorophyll (mg/g dry wt)*		*RuDPC*	*Dry wt*	*Fresh wt/ dry wt †*	*N*	*P*	*K*	*Ca*	*Mg*
*N**	*Oil*	*a+b*	*a/b*	*(mg CO_2/g dry wt hr)*	*(g/pot)*	*(g/g)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
0	0	9.8	3.1	3.0	7.9	3.17	1.49	0.21	2.09	0.56	0.44
0	1	7.1	3.0	1.8	6.8	3.14	1.41	0.21	2.00	0.55	0.36
0	2	6.3	3.1	1.6	6.3	3.16	1.53	0.21	1.79	0.47	0.34
0	3	7.5	3.1	1.2	6.1	2.99	1.29	0.23	1.85	0.55	0.32
Mean		7.7	3.1	1.9	6.8	3.11	1.43	0.21	1.93	0.53	0.36
1	0	15.1	3.3	10.6	11.6	3.52	3.46	0.20	1.16	0.60	0.55
1	1	14.6	3.3	9.6	9.1	3.61	3.85	0.26	1.63	0.71	0.62
1	2	16.6	3.2	7.8	9.5	3.66	3.40	0.24	1.36	0.75	0.54
1	3	15.1	3.2	6.2	10.3	3.54	2.84	0.23	1.43	0.70	0.44
Mean		15.3	3.2	8.6	10.1	3.58	3.39	0.23	1.39	0.69	0.53

*Nitrogen treatment was 22.5 g/m^2.

†Oil treatment 0.5 l/m^2 for level 1, 1.0 l/m^2 for level 2, 2.0 l/m^2 for level 3.

Table XVIII. Analysis of variance results – bromegrass (nitrogen - oil) experiment.

Source of variation	*Degrees of freedom*	*Chlorophyll (mg/g dry wt)*		*RuDPC (mg CO_2/ g dry wt hr)*	*Dry wt. (g/pot)*	*Fresh wt./ dry wt (g/g)*	*N (%)*	*P (%)*	*K (%)*	*Ca (%)*	*Mg (%)*
		a+b	*a/b*								
Blocks	1	0.10	0.083	0.14	0.56	0.005	0.01	0.000	0.00	0.001	0.003
Nitrogen (N)	1	234.93†	0.101*	177.89†	44.56†	0.870†	15.31†	0.001	0.16†	0.095†	0.119†
Oil (O)	3	1.85	0.001	6.87†	3.05†	0.014	0.24	0.000	0.04	0.002	0.012†
N × O	3	4.40	0.003	1.68†	0.67	0.006	0.13	0.001	0.07	0.010	0.005
Error	7	1.44	0.013	0.20	0.33	0.078	0.14	0.001	0.05	0.004	0.001
Total	15										

* Significant at 0.05 level.

† Significant at 0.01 level.

Table XIX. Mean percentage germination and mean coleoptile length of rye and bromegrasses as influenced by the presence of the oil dispersant, Corexit 7664.

	Brome				*Rye*			
Treatment (Corexit/water)	*Seed germination (%)*		*Coleoptile length (mm)*		*Seed germination (%)*		*Coleoptile length (mm)*	
Distilled H_2O	84.3	c*	1.361	c	97.5	abc	2.361	e
1:3000	81.9	bc	1.131	c	98.1	bc	2.113	de
1:1000	73.8	ab	0.701	b	95.6	abc	1.688	cd
1:500	76.9	bc	0.600	b	95.0	abc	1.313	bc
1:250	67.5	a	0.126	a	96.9	abc	1.125	ab
1:100	79.4	bc	0.070	a	96.9	abc	1.001	ab
1:50	65.5	a	0.000	a	92.5	a	0.674	a

* Means that are followed by the same letter are not significant at 0.05 level of confidence by Duncan's New Multiple Range Test (NMRT).

mixtures used were based upon Westfall's (1971) research. Twenty seeds of each species were selected and placed on filter paper in sterile petri dishes for each of the seven treatments. Each treatment was replicated eight times. The appropriate solution mixture was applied to each petri dish until the filter paper was saturated and then placed in a controlled environment growth chamber. Petri dishes were positioned in the chamber on a completely randomized basis.

The growth chamber was set on a 16-hour day with day temperature set at 22°C and night temperature at 13°C. Rye germination was evaluated after four days in the chamber and brome germination after nine days.

Early plant development in rye and brome was assessed at the time of germination by evaluating the length of coleoptile emergence from the seed. Coleoptiles were severed at the point of emergence from the seed and measured in millimeters.

An analysis of variance was applied to the data resulting from both germination and coleoptile measurements to evaluate the effect of the treatments upon the variance exhibited. Significance was determined by application of an F-test (Fryer 1966). Significant differences among mean treatment values for germination and coleoptile development in each species were determined by Duncan's New Multiple Range Test (NMRT) as discussed in Fryer (1966).

The dispersant Corexit 7664 had no significant effect on rye germination. However, a significant reduction in the germination of brome was shown by the analysis of variance (Table XIX). This reduction was most notable at the 1/50 (Corexit/water) treatment level. Brome germination was less than rye at all treatment levels.

Corexit did have a significant effect on coleoptile development in both species. As the Corexit concentration increased in the germinating medium, coleoptile development became increasingly inhibited. In general, treatment levels of 1/50, 1/100 and 1/250 were the most inhibitory, with development increasing at the 1/500 and 1/1000 levels. The 1/3000 treatment level showed very little inhibition of coleoptile development and was not significantly different from the control in both species tests.

This investigation shows that the oil dispersant Corexit 7664 has no detrimental effect on seed germination except at relatively high concentrations. However, it does have a definite detrimental effect on early seedling development. It is not yet known what levels of Corexit are necessary for optimum removal of crude oil from plant surfaces. Westfall's 1971 studies did not indicate any recommended rates for use. It appears that if Corexit concentrations are too high, the soil could be saturated with a mixture that would be inhibitory to future plant germination and early growth. Studies should be conducted using various Corexit-water mixtures to remove crude oil from various plant surfaces. These would establish the appropriate rates necessary for removal on various species. If the established rates would happen to fall in the concentrations of 1/250 (Corexit/water) which were shown inhibitory in this study, it seems appropriate to then determine what levels of Corexit in soil are inhibitory to plant growth. In the case of revegetation it would also be important to establish if the presence of Corexit in the soil is of short-term importance, whether effects decrease with time due to biological degradation or leaching.

Microbiological investigations

The following studies on microbiological aspects of oil pollution and the use of microbes in the enhancement of natural environmental recovery processes were performed by Dr. Patrick Hunt when he was assigned to USA CRREL (currently with Corps of Engineers Waterways Experiment Station, Vicksburg, Mississippi). The report as contained here is essentially as it appeared in several other CRREL reports and in the *Proceedings of the Joint Conference on Prevention and Control of Oil Spills*, 13-15 March 1973, Washington, D.C. (Hunt et al. 1973).

Soils contain many types of microorganisms capable of decomposing petroleum compounds, especially straight chain hydrocarbons up to C18 (Hunt 1972, McKenna and Kallio 1964, McKenna and Kallio 1965, Zobell 1969). Aromatic and branched compounds are most resistant and some compounds in crude oil may even be considered recalcitrant to microbial degradation, but these compounds may be neutralized by their incorporation with soil organic matter. In arctic and subarctic regions, the time required for microbial degradation of spilled petroleum may be extremely long due to low soil temperatures and the short summer season during which microbiological processes are active.

To determine the feasibility of enhancing the rate of microbial degradation of petroleum in cold region soils, laboratory studies were conducted on the effects of nutrient addition, pH control, and inoculation by oil-degrading microorganisms. The soil was Fairbanks silt loam collected from the Glenn Creek watershed near Fairbanks, Alaska (Allen et al. 1969, Dingman 1971). The terrain at the site was characterized by shallow permafrost with a vegetative cover of black spruce and sphagnum moss. The soil had a pH of 5 and an organic matter content of 10%.

In conducting initial experiments, 200 g of soil was placed in quart mason jars used as incubation chambers at 15°C. Prudhoe Bay crude oil was added at a level of 15% by weight to the soil. One hundred ppm phosphorus as calcium monobasic phosphate was added to each sample. The amounts of crude oil and phosphorus added were determined by preliminary experiments. Nitrogen treatments were 100, 200 and 300 ppm nitrogen as ammonium nitrate. Half of the samples received an inoculation of oil-degrading microorganisms of 10^4 microorganisms per g of dry soil. The microbial inoculum was made by isolating microorganisms that utilized Prudhoe Bay crude oil as their sole source of carbon. The isolation technique involved the incubation at 15°C for about eight weeks of 0.1 g of soil from samples taken around oil seeps at Cape Simpson, Alaska, in 50 ml of a solution that contained 0.1% diammonium phosphate, 0.05% magnesium sulfate, and 10 ml of Prudhoe Bay crude oil. The evolution of carbon dioxide was used as a measure of biological activity and as an indicator of microbial degradation of crude oil. The CO_2 was determined by titration of a standard base in a collection flask. The use of respiration as an indicator of oil degradation was substantiated by gas chromatographic analysis of extracts from the soil samples.

Figure 28 shows the increase in respiration as measured by CO_2 evolution in response to inoculation. The two curves represent the means of the three nitrogen levels for the inoculated and uninoculated treatments, respectively. There was no significant nitrogen level-inoculation interaction; response to each nitrogen level was in the same relative order for the inoculated and uninoculated samples. The lag in respiration in the inoculated soils during the first two weeks probably reflected an adjustment period for the inoculation microorganisms. Positive response to inoculation was unexpected since organisms capable of degrading oil are normally present in Fairbanks silt loam as well as in most other types of soil. The lag is thought to be related to generation time of the microorganisms which is lengthened at lower temperatures (Lamanna and Mallette 1965). In the case of an actual oil spill in the Alaskan interior, there would be no more than 12 weeks in which the soil temperature would approach 15°C. This would limit the number of microbial generations.

There was a definite decrease in microbial respiration with increased amounts of nitrogen (Fig. 29). Although not shown here, this unusual response was more pronounced for samples inoculated with the oil-degrading microorganisms than for the uninoculated samples. This effect of nitrogen addition was verified in an independent experiment in which respiration was measured as O_2 consumption using a differential respirometer. The suppression of microbial activity by nitrogen could be explained by either ammonia or nitrite toxicity. A soil pH of 5 should be too low for significant evolution of ammonia. Thus, nitrite seemed to be the more likely candidate. Accumulation of nitrite at a toxic level could occur during nitrification if *Nitrobacter* were more sensitive to some toxic substance than *Nitrosomonas*.

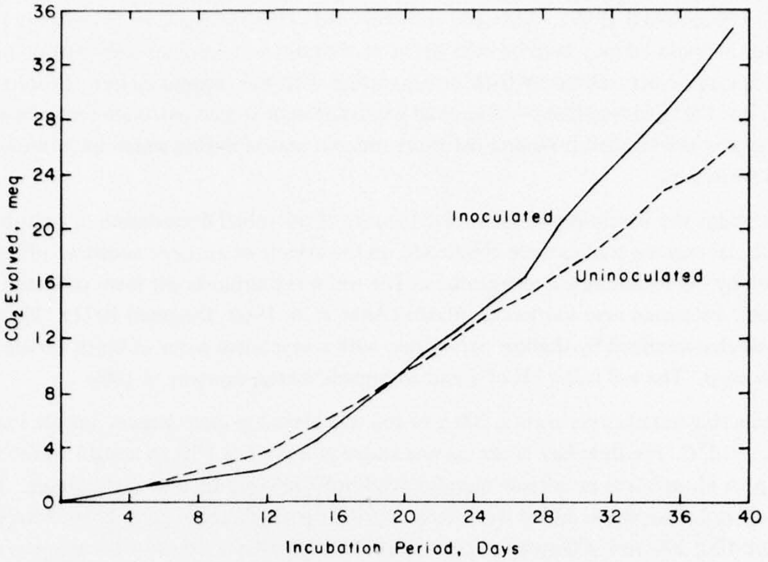

Figure 28. Effects of inoculum on cumulative respiration at 15°C in Fairbanks silt loam containing 15% by weight Prudhoe Bay crude oil. Soil pH was 5. Samples were also treated with NH_4NO_3 and $Ca(H_2PO_4)_2$.

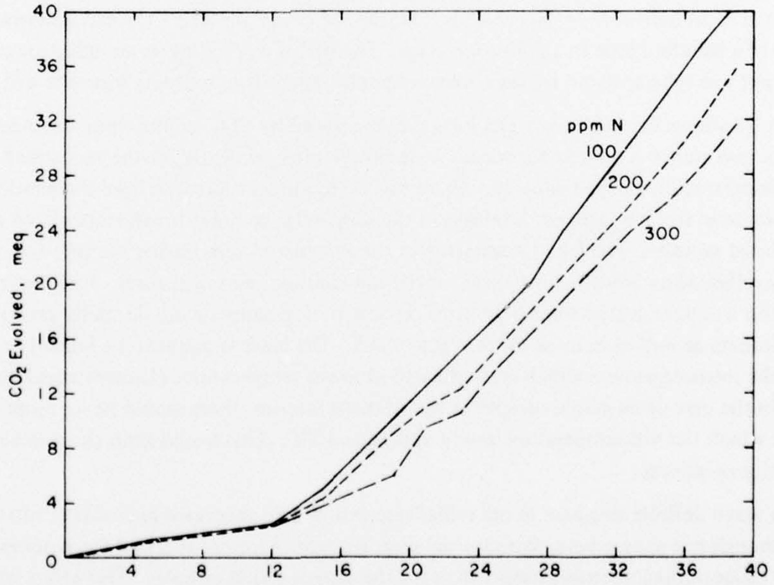

Figure 29. Effects of nitrogen (NH_4NO_3) levels on cumulative respiration at 15°C in Fairbanks silt loam containing 15% by weight Prudhoe Bay crude oil. Soil pH was 5. Samples were also treated with $Ca(H_2PO_4)_2$ and inoculum (Hunt et al. 1973).

A second set of experiments was conducted to further investigate the anomalous results with nitrogen. The experimental procedure was similar to that previously described. All samples were inoculated and treated with phosphorus at a level of 100 ppm. Nitrogen was added at 100, 200 and 300 ppm but as either ammonium chloride or sodium nitrate instead of ammonium nitrate. The pH of the samples was adjusted to 5, 6, and 7 using calcium carbonate. If the nitrate toxicity hypotheses were correct, the suppression in respiration should have been eliminated in samples containing nitrate as the nitrogen source. Alternatively, ammonia toxicity would be accentuated at the higher pH values for samples to which ammonium was added. The experimental results for pH values of 5 and 7 are given in Figures 30-33.

The suppression in respiration at nitrogen levels above 100 ppm nitrogen was again evident for the first several weeks, regardless of soil pH and nitrogen source. In these experiments an inversion occurred after several weeks in which respiration first was highest for the 200 ppm N treatments rather than for the 300 ppm N treatments. This inversion was pH-dependent since it occurred earlier at pH 7 in comparison to pH 5. Intermediate times were observed for the inversions at pH 6. There is no apparent explanation for the failure to observe this inversion during the initial experiments at pH 5 in which ammonium nitrate was applied to the soil. The longer lag period may have been related in some way to nitrogen source. Soil extracts had nitrite levels less than 2 ppm. The absence of nitrite accumulation, the insensitivity of respiration to the nitrogen source, and the increase in respiration at the higher nitrogen levels with increasing pH tend to eliminate both ammonia and nitrite from consideration as the toxic substances responsible for the initial suppression of respiration at the higher nitrogen levels. Alternative possibilities include nitrogen oxides or organonitrogen compounds. Nitrogen oxides are evolved under oxygen stress and acidic conditions and can be toxic to biological systems (Alexander 1961). Considering the large number of organic compounds in the oil and nitrogen-treated soil, formation of toxic organonitrogen compounds seems possible.

Although reasons for the unusual response of microbial activity to nitrogen treatment are uncertain, there is no doubt that microbial activity can be enhanced through proper soil treatment. This is illustrated in Figure 21, in which respiration is shown for a sample which received no treatment except for the addition of crude oil. The combination of inoculation, adjustment of pH to 7, and application of phosphorus and nitrogen at levels of 100 ppm and 300 ppm respectively, increased microbiological activity by at least a factor of 4 after 40 days. This should be of practical significance in enhancing the natural recovery of oil-contaminated terrain in cold regions when easily biodegradable compounds are present in the soil. Although compounds resistant to degradation or colatilization should predominate in time, the rate of degradation of these compounds should be proportional to the total microbiological activity (Davis 1967).

Laboratory results were extended to a field study along the Haines to Fairbanks pipeline. A site was selected near milepost 382.5 where a gasoline spill occurred in 1956. Stimulating revegetation was equal in interest to enhancing biodegradation of residual petroleum compounds. Thirty-six 2 X 1.5-m plots were prepared, providing three replicates of 12 combinations of treble superphosphate and ammonium nitrate fertilizer applications. However, no attempt was made to inoculate the soils with oil-degrading microorganisms. Nitrogen was applied at rates of 56, 112, 224, 336, 448 and 672 kg/ha. Phosphorus was applied at rates of 224 and 448 kg/ha. A seed mixture which included rye, brome, fescue and foxtail was also used.

Sparse vegetation cover existed at the time of application, and one year later considerable revegetation occurred. Actually, most of the treatment response took place during the 1972 growing season. Native volunteer species were much more successful than the introduced seed mixture, in part because much of the 1971 seed application was eaten by birds. In the individual plots, the density of vegetation and microbial activity was increased by fertilizer treatment; however,

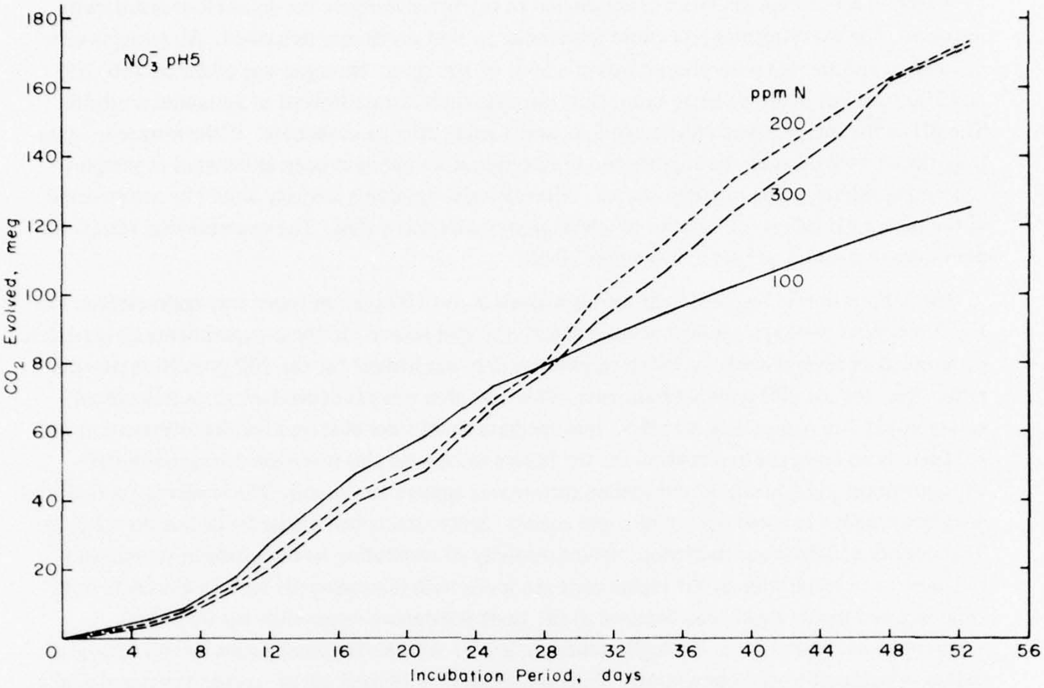

Figure 30. Effect of nitrogen (NaNO$_3$) levels on cumulative respiration at 15°C in Fairbanks silt loam containing 15% by weight Prudhoe Bay crude oil. Soil pH was 5. Samples were also treated with Ca(H$_2$PO$_4$)$_2$ and inoculum (Hunt et al. 1973).

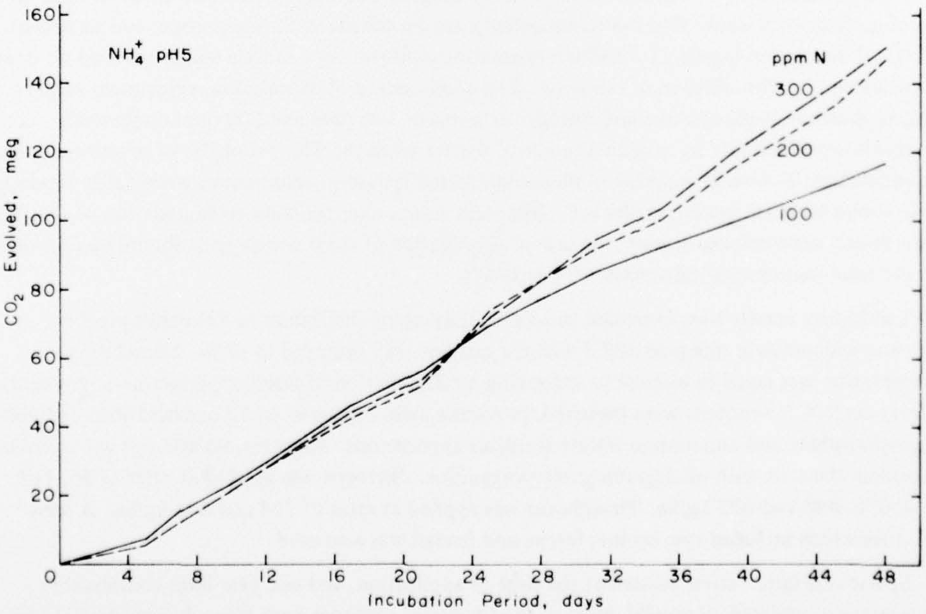

Figure 31. Effects of nitrogen (NH$_4$Cl) levels on cumulative respiration at 15°C in Fairbanks silt loam containing 15% by weight Prudhoe Bay crude oil. Soil pH was 5. Samples were also treated with Ca(H$_2$PO$_4$)$_2$ and inoculum (Hunt et al. 1973).

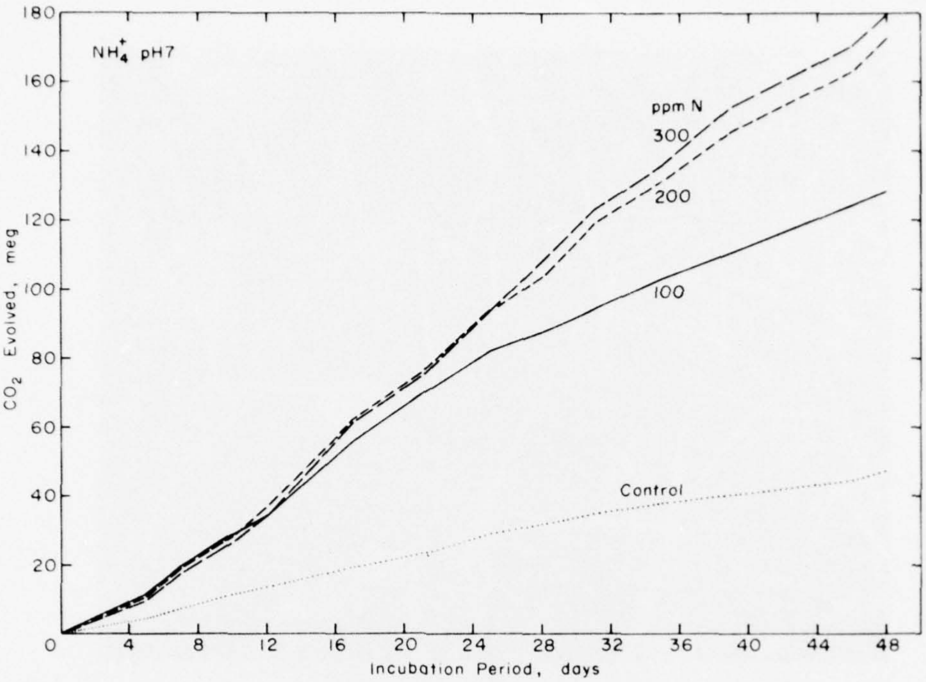

Figure 32. Effects of nitrogen ($NaNO_3$) levels on cumulative respiration at 15°C in Fairbanks silt loam containing 15% by weight Prudhoe Bay crude oil. Soil pH was 7. Samples were also treated with $Ca(H_2PO_4)_2$ and inoculum (Hunt et al. 1973).

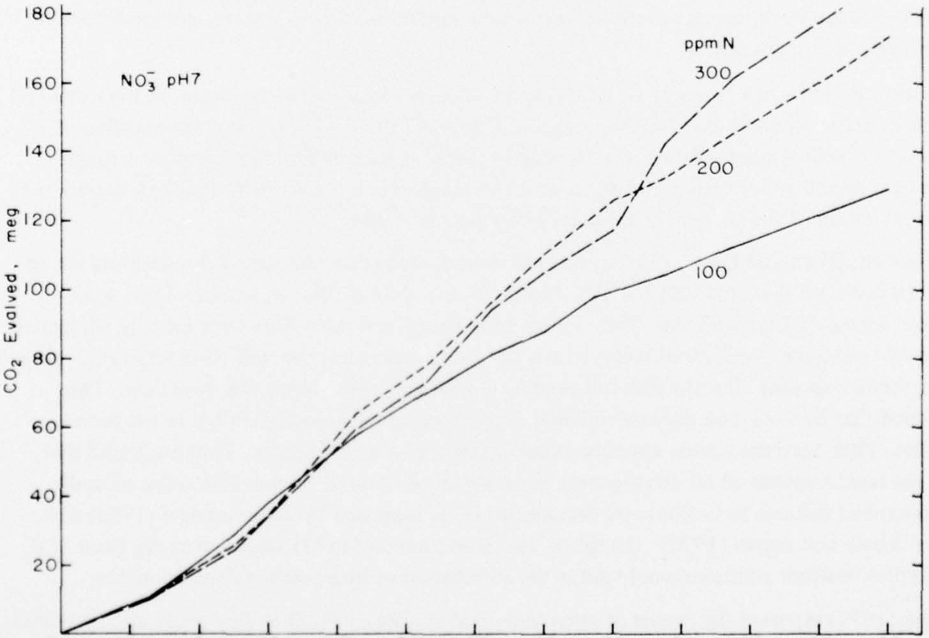

Figure 33. Effects of nitrogen (NH_4Cl) levels on cumulative respiration at 15°C in Fairbanks silt loam containing 15% by weight Prudhoe Bay crude oil. Soil pH was 7. Samples were also treated with $Ca(H_2PO_4)_2$ and inoculum (Hunt et al. 1973).

Figure 34. Carex aquatilis *growing vigorously in soil saturated with crude oil, Prudhoe Bay, 1971.*

preliminary analysis of the data available at this time implies that there is little additional benefit at the higher treatment rates.

In addition to Hunt's (Hunt et al. 1973) report other work has been conducted on the microbial aspects of arctic oil pollution. Scarborough and Flanagan (1973) reported that the addition of oil to the arctic environment resulted in a decrease in fungal species diversity although new fungal species did appear and overall populations were increased. Their results indicated that, despite the cold-domination of the ecosystem, oil breakdown does take place.

Campbell, Harris and Benoit (1973) reported on soil respiration rates and soil microflora found in association with the joint USA CRREL-Tundra Biome spills at Barrow, Alaska. Their work centered on the 12-l/m^2 spill site. They found that, though soil microflora were initially eliminated by the oil treatment, populations began to increase one month after the spill. Soil respiration peaked during the second year after the spill followed by a gradual decline during the third year. They concluded that hydrocarbon respiration could occur from late May until October in the Barrow vicinities. High bacterial counts were also observed on the oil-treated plots. They suggested that, given the rapid response of oil decomposers there would be no need to inoculate arctic oil spills with microbial cultures to facilitate oil decomposition as suggested by Schwendinger (1968) and also by Agosti and Agosti (1973). Campbell, Harris and Benoit (1973) suggested as did Hunt et al. (1973) that fertilizer additions would aid in the stimulation of hydrocarbon decomposition.

Atlas (1974) reported the results of microbiological studies on Prudhoe Bay crude oil. He found that biodegradation of crude oil was related to composition and external temperature conditions, heavy oils being less biodegradable than light oils, and decomposition of light oils being reduced at lower temperatures. Contrary to Campbell et al. (1973), he established that degradation of Prudhoe Bay crude oil could be increased by the introduction of microbial cultures. However, success was dependent upon the use of fertilization for stimulation.

Field investigations of accidental petroleum losses

In addition to the previous experimental investigations over a period of four years working in Alaska, other observations of spills (controlled and accidental) were made and conclusions were drawn.

In conjunction and in cooperation with mutual USA CRREL and Tundra Biome terrestrial spill work in 1970, an experimental spill was conducted on a small inland lake at Barrow, Alaska. Barsdate (1973) abstracted the results of this study. He reported that physical effects were minor and of short duration. With the oil slick, just after the spill was conducted, water temperatures were lowered but after three days returned to normal. Measurements of oxygen levels under the oil layer showed that they were reduced but had little noticeable effect in 1970 on phytoplankton or emergent vegetation production. However, there was high zooplankton mortality. In 1971 (2nd year) the productivity of all plant life was low along with that of zooplankton. The 1972 observations gave preliminary indications that there was little change from 1971.

In biomass measurements of emergent plants at this site in 1971, it was very apparent that productivity was lowered in the affected areas of the pond. It did not appear that there were any toxic effects from the crude oil on the emergent plants, but instead they were usually killed by the physical action of the oil coating the leaf surfaces and causing the previously emergent plant parts to become submerged.

Prudhoe Bay was visited in 1971, and a spill adjacent to a well platform was observed. The site was on dry soil with a deep active layer. Oil had been gradually lost by seepage from a drilling pad and covered an area about 20 X 20 m. Straw had been used to soak up the excess oil and then the site was burned over. At the time of observation, one year from the occurrence, no increase in the depth of excessive permafrost thaw was apparent; however, field measurements were never taken to validate this. A slight depression had been left where the straw and organic mat were removed by burning. The entire surface was black; however, it was moist. Plant growth extended to the edge of the burn area and *Carex aquatilis*, the sedge which indicated resistance to crude oil at Cape Simpson and in the Barrow experiments, was growing quite lushly while surrounded with crude oil at the periphery of actual spill (Fig. 34).

Another experiment involved the application of crude oil in sub-zero weather conditions (−30°C) on snow-covered terrain. The experiment was designed to determine the effects of oil pollution on tundra plants when occurring in the winter prior to spring breakup. It was also designed to study cleanup techniques under such conditions. Although the former purpose was never observed, the latter was. Under the conditions described, crude oil was applied to 15 cm of snow having a very hard surface crust. At the time of application the oil congealed in the upper snow layers, roughly 2 to 4 cm, and failed to penetrate to the ground surface. However, if the snow surface structure were physically disrupted, the individual snow particles, being quite granular, compacted readily and the oil moved rapidly on through. It appeared that spills occurring under arctic winter conditions could be cleaned up very rapidly by moving the congealed snow-oil mixture from the surface, provided that the mixture was allowed to freeze thoroughly prior to cleanup.

During the course of four years, a log of oil spill incidents in Alaska and Canada has been compiled. Other than those mentioned in this report, most remained unresearched. It is particularly noteworthy that there were cases of crude oil spills in arctic areas dating back to World War II. The Canol Oil line, which at one time originated in Norman Wells (Yukon Territory, Canada), apparently had a substantial spill at one of the tank farm locations along it. This might possibly yield some worthwhile information if the site were to be revisited today. Also, several small spills at the Project Chariot site, Cape Thompson, Alaska, are reported to have occurred.

PART III. RECENT RELATED LITERATURE

In the span of time encompassing the development and conclusion of the foregoing USA CRREL experiments, there have been considerable parallel research efforts in the field of terrestrial oil pollution in arctic and subarctic regions. Generally these have been conducted by Canadian research teams in relation to the proposed MacKenzie River Valley pipeline route.

Hutchinson and Hellebust published an initial report in early 1974 on their investigations of oil spills on terrestrial and aquatic ecosystems in the Norman Wells region of the Northwest Territories, Canada. Vegetation upon which controlled spills were conducted was apparently very similar to that of the USA CRREL study area at Fox, Alaska. The spills were conducted during the period of July 1972 to February 1974 and results appear to be very similar to those of interior Alaska. Hutchinson and Hellebust carefully detailed the study area prior to conducting actual controlled spills of crude oil. Crude oil was then applied at the rate of approximately 8 $\mathrm{l/m^2}$. They reported that foliage contact with crude oil entirely eliminated some species (herbicide effect). Mosses and lichens were severely affected. Species having waxy evergreen leaves (thick protective leaf cuticles) were least affected. The only two species to show new regeneration during the growing season of initial oil application were *Ledum groenlandicum* and *Potentilla fruticosa.* In their initial report Hutchinson and Hellebust indicated that *Picea mariana* appeared relatively resistant to foliage applications of crude oil. However, a follow-up report (Hutchinson et al. 1974a) reported that *Picea mariana* died during the 2nd year after application (as in the USA CRREL Fox spill).

The first report (Hutchinson and Hellebust 1974) also indicated that permafrost thaw was increased on the sites where crude oil was applied. This was most evident on an area treated which had previously been burned by natural wildfire. This area presumably had less insulating vegetation at the time of the spill than did a corresponding unburned treatment site. The initial studies also involved some aquatic investigations (crude oil spills on open lake cylinders). They found that such spills did not affect lake phytoplankton. In contrast, oil did affect plant parts above the waterline for species such as *Carex* and *Equisetum*; however, belowground parts were relatively unaffected. They also found that aquatic mosses were less affected by oil spills than those on terrestrial areas.

The follow-up report was published in August of 1974 (Hutchinson et al. 1974). Second-year effects on *Picea mariana* have already been mentioned. They also found that vegetative recovery in the terrestrial spill plots was very low, with the exception of some species having the ability to produce new shoots or foliage from underground rhizomes (examples were *Arctostaphylos rubra, Ledum groenlandicum*, and *Vaccinium vitis-idaea*). Fertilizer studies indicated little benefit was derived during the first year following the oil spills.

They also experimented with a winter spill, finding that despite several months of weathering prior to plant growth, the oil was quite phytotoxic. However, winter spill effects on vegetation were still less severe than those of the summer spill.

In their report Hutchinson et al. (1974) also compared results of tundra oil spills with oil spills in a black spruce forest community. They concluded that, even though mosses, lichens and specific plant species were severely affected, damage was less severe than in taiga communities. They based their conclusion on the fact that *Eriophorum vaginatum* and *Salix glauca*, two important species in the tundra community, appeared somewhat oil resistant and were able to initiate new short growth during the season following the spill.

A phase of their study involved monitoring soil arthropod populations. They did find that oil spills reduced these populations. However, they also found a similar reduction with construction disturbances. They concluded that arctic pipeline construction may be potentially more destructive to soil arthropod populations than isolated oil spills.

MacKay et al. (1974) issued a third Canadian report and found vegetative results that were essentially the same as those reported by Hutchinson and Hellebust (1974), and Hutchinson et al. (1974). However, their experiments indicated little or no change in the thermal regime of the soil after spills. They found that the thermal characteristics of dead vegetation differed little from those of live vegetation.

Their studies included laboratory experiments related to oil-snow interactions during winter spills. In addition they determined absorptive capacities for various surface areas and found, as also observed in the USA CRREL Fox experimental spill, that oil would flow along the water table beneath the surface. A very interesting part of their report included predictive models of the behavior of a 50,000-bbl oil spill under summer and winter conditions. These models included areas affected and amounts volatilized. They also discussed clean-up technology which included burying, concluding that it had too many associated problems to warrant its use.

Another report (MacKay et al. 1975) followed, dealing with crude oil spills on snow. They found that snow served as an excellent absorbent, allowing coverage of 0.01 m^2/l of cold oil or about $^1/_8$ of the area contaminated by a summer spill. However, they also found that after thawing, the original winter spill may exceed the summer spill by a factor of 2, unless cleanup is undertaken shortly afterward and during the winter months. MacKay et al. also studied an experimental spill of hot oil, which indicated that contamination rates were higher (0.024 m^2/l of oil) or about twice that of oil spilled at ambient temperatures. Their report also included additional work on oil-ice-air surface effects and flow rates in snow. In these they found that hot oil spills differ from ambient spills as the heat of the oil generates an oil-water mix that penetrates the snow in advance of the hot oil, and if snow temperatures are cool enough, this mix may freeze and prevent further oil penetration.

PART IV. CONCLUSIONS AND RECOMMENDATIONS

Based upon our investigations and those of others, it has been demonstrated that the introduction of petroleum, whether refined products or crude oil, will to some degree be detrimental to both subarctic and arctic plant communities. The degree and longevity of damage will be influenced by a number of environmental factors. Initial damage will be related primarily to the time of year and the soil moisture conditions prevailing at the time of the spill (high soil moisture, less damage). Recovery from foliar contact with petroleum, which usually results in death of the affected foliage, usually occurs, but only if the root systems are relatively unaffected. Generally high root/shoot ratios encountered in the arctic, combined with high soil moisture levels should result in high potentials for recovery. The previous conditions may, in fact, make arctic terrestrial regions less vulnerable to severe and long-term damage than terrestrial regions in subarctic or taiga areas. However, when penetration of petroleum into the soil does occur in arctic regions, damage may be more extensive and effects more long-term. It should be noted that in experiments on the North Slope at sublethal levels of contamination, death of vegetation continued to increase over several seasons. This was apparently influenced by winter stresses. The observations at Cape Simpson prove that at some point in time recovery will commence, but the time factor involved is still an unknown. Because of this, there is a need to follow annually all of the controlled spills in order that changes can be monitored as they occur.

In subarctic regions, many of the same conditions apply except that, due to the hot, dry conditions through much of the growing season, spills can result in a deeper and more thorough saturation of organic and soil layers; here inundation of the plant root zone would occur. The resultant dead, highly hydrophobic organic mat inhibits plant germination and growth. Thus damage may be extremely long-term. Where moisture is seasonally high, as in depressions and lower ends of drainage basins, recovery rates will be accelerated. In any case, the prolonged existence of the dead, but relatively stable, organic mat preserves thermal equilibrium of permafrost zones in underlying strata and inhibits soil erosion.

There does appear to be a sensitivity spectrum of arctic and subarctic species to crude oil contamination. Thus spills probably will cause temporary changes in species composition in the affected areas. Further work needs to be done on species sensitivity. Which native species are tolerant to the presence of petroleum products in the soil? Can selections of genetic material be made in areas like Cape Simpson which are even more highly resistant? If *Carex aquatilis* is resistant, as it appears to be, what is known about its seeding habits, its germination requirements, its growth requirements? This information will be necessary if a given species is to be used in revegetation of spill areas. The area of microbial degradation of petroleum needs to be addressed fully. Results of recent experiments in microbe-oil relationships indicate that microbial seeding combined with fertilization has definite possibilities in arctic regions. Further study of fertilization in stimulating the plant-soil nutrient cycle is warranted from the results of the physiological studies reported earlier.

In addition, it is imperative that further studies be initiated in the area of cleanup procedures in northern ecosystems. In areas devoid of permafrost and having gentle slopes, mechanical scarification followed by reseeding with prepared plant seed mixtures can be used, as they are in temperate zones. However, this will be restricted to non-permafrost areas in the taiga. In more severe situations, burning might be used to remove surface contamination and aid in stimulating natural revegetative processes. The work along the Haines-Fairbanks pipeline proves the validity of this. However, precautions must be taken in the use of this method where zones of discontinuous and continuous permafrost exist until further investigations determine the effects of burning on possible permafrost degradation and subsequent soil erosion.

Research in the above areas needs to be undertaken as such technology is essential if effective contingency plans are to be formulated for oil spills in terrestrial regions of the north. The inadequacies described should provide impetus for such research. If the Haines line and others are used as examples, it is only a matter of time until a spill of major magnitude occurs along the Trans-Alaska pipeline. The success in dealing with such a spill will depend upon a working knowledge of all of the variables involved. Research will be necessary, not only in developing the proper techniques to minimize site impact during the phase of cleanup activities, but also to develop revegetation methodology in order to minimize the recovery time span.

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