Appl Microbiol Biotechnol (2001) 57:242–247
DOI 10.1007/s002530100713

ORIGINAL PAPER

W. W. Mohn · C. Z. Radziminski · M.-C. Fortin
K. J. Reimer

On site bioremediation of hydrocarbon-contaminated Arctic tundra soils in inoculated biopiles

Received: 28 August 2000 / Received revision: 23 April 2001 / Accepted: 4 May 2001 / Published online: 11 July 2001

Abstract There is a need to develop technology to allow the remediation of soil in polar regions that have been contaminated by hydrocarbon fuel spills. Bioremediation is potentially useful for this purpose, but has not been well demonstrated in polar regions. We investigated biopiles for on-site bioremediation of soil contaminated with Arctic diesel fuel in two independent small-scale field experiments at different sites on the Arctic tundra. The results were highly consistent with one another. In biopiles at both sites, extensive hydrocarbon removal occurred after one summer. After 1 year in treatments with optimal conditions, total petroleum hydrocarbons were reduced from 196 to below 10 mg per kg of soil at one site, and from 2,109 to 195 mg per kg of soil at the other site. Addition of ammonium chloride and sodium phosphate greatly stimulated hydrocarbon removal and indicates that biodegradation was the primary mechanism by which this was achieved. Inoculation with cold-adapted, mixed microbial cultures further stimulated hydrocarbon removal during the summer immediately following inoculation. At one site, soil temperature was monitored during the summer season, and a clear plastic cover increased biopile soil temperature, measured as degree-day accumulation, by 30–49%. Our results show that on-site bioremediation of fuel-contaminated soil at Arctic tundra sites is feasible.

W.W. Mohn (✉) · C.Z. Radziminski
Department of Microbiology and Immunology,
University of British Columbia, 300–6174 University Blvd.,
Vancouver, BC, Canada V6T 1Z3
e-mail: wmohn@interchange.ubc.ca
Tel.: +1-604-8224285, Fax: +1-604-8226041

M.-C. Fortin
Pacific Agri-Food Research Centre, P.O. Box 1000,
Agassiz, BC, Canada V0M 1A0

K.J. Reimer
Environmental Sciences Group, Royal Military College,
PO Box 17000 Station Forces, Kingston, ON, Canada K7K 7B4

Introduction

Human activity in polar regions relies heavily on fossil fuels for transportation and generation of electricity and heat. Fuel spills have occurred at many sites in polar regions, and there is a need to remediate contaminated soil. Bioremediation is a common means of treating hydrocarbon-contaminated soils (Eweis et al. 1998), but this approach has not been well demonstrated in polar regions. Soils in polar regions have unique properties, including numerous periglacial features (Fitzpatrick 1997), the presence of permafrost and lower microbial populations than other soils (Robinson and Wookey 1997). These features present challenges to the classic bioremediation procedures developed for more temperate climates, and it is important to demonstrate that these soils are amenable to bioremediation before proceeding with this technology.

The potential for biodegradation of hydrocarbons in soil in polar tundra regions has been demonstrated. Studies reviewed by Atlas (1981) showed the presence of hydrocarbon-degrading microorganisms in Arctic tundra soil and their ability to partially degrade crude oil. Crude oil was generally more persistent in Arctic tundra soils than in soils in other regions. Several more recent studies have further characterized hydrocarbon-degrading microorganisms from polar tundra soils (Aislabie et al. 1998; Braddock and McCarthy 1996; Braddock et al. 1997; Kerry 1990; Tumeo and Wolk 1994; Whyte et al. 1996, 1999) or genes encoding hydrocarbon degradation enzymes (Whyte et al. 1999). Some studies have examined biodegradation of refined fuels, which are less recalcitrant than crude oil, in polar tundra soils. The addition of nitrogen, phosphorus and potassium accelerated hydrocarbon removal in an experimental fuel spill in the Vestfold Hills, Antarctica (Kerry 1993). Removal of various hydrocarbons was stimulated by nutrient addition to fuel-contaminated soil in mesocosms incubated on site at Point Barrow, Alaska (Braddock et al. 1997). Studies have also demonstrated biodegradation of refined hydrocarbons in soils from polar regions incubated at low temperatures in laboratories (Aislabie et al. 1998; Brad-

dock and McCarthy 1996; Braddock et al. 1997; Whyte et al. 1999). The above studies indicate the potential for bioremediation of fuel-contaminated soil at polar tundra sites, but there is a need for well-documented reports demonstrating on-site bioremediation of such soil.

The summer season is very short in polar regions, and the active soil layer above the permafrost typically thaws for a period of 1–2 months. Thus, in these regions, it might be practical and economical to use treatment options that accelerate hydrocarbon biodegradation. Under non-limiting conditions, indigenous hydrocarbon-degrading organisms should multiply and remediate fuel-contaminated soil. Because microbial growth under non-limiting conditions is exponential, most of the substrate degradation occurs at the end of such a period of growth. An increase in the initial population of hydrocarbon degraders, by inoculation of the soil, has the potential to greatly reduce the time required for bioremediation of hydrocarbon contaminants. However, no studies have demonstrated that inoculation can stimulate bioremediation of hydrocarbons in soils from polar regions. In fact, reviewers (Prince 1998; Stotzky 1997; Vogel 1996) conclude that evidence is scarce for the stimulatory effects of inocula on bioremediation in any environment.

Increasing soil temperature is another strategy that could be advantageous for soil bioremediation in cold regions. Summer season temperature and duration are likely to be limiting factors for bioremediation at polar sites. However, very few soil temperature data exist for such sites. A clear plastic cover will reduce convective heat loss from soil, while permitting solar radiation to warm the soil. The resulting temperature increase would probably increase rates of hydrocarbon biodegradation as well as lengthen the period of time in the fall during which the soil remains unfrozen and hydrocarbon biodegradation continues. Additionally, a plastic cover will prevent drying of the soil, a process that would limit hydrocarbon biodegradation.

We conducted field experiments at two military radar sites, Komakuk Beach and Cambridge Bay, on the Canadian Arctic tundra. The main objective was to test the feasibility of bioremediation of hydrocarbon-contaminated soil at these sites. Soil was treated in small-scale biopiles. We tested the effects of different treatments, including fertilization with ammonium chloride and sodium phosphate, addition of peat for soil bulking and inoculation with cold-tolerant hydrocarbon-degrading enrichment cultures. A secondary objective at BAR-1 was to compare the in situ soil temperature with that in biopiles with and without clear plastic covers.

Materials and methods

Field sites

Cambridge Bay (CAM-M), Northwest Territories (69°N, 105°W), is in the Arctic tundra subregion or Cool-Arctic vegetative zone (Longton 1997). Komakuk Beach (BAR-1), Yukon Territory (70°N, 140°W), is in the Subarctic tundra subregion or Mild-Arctic vegetative zone. Historically at BAR-1, June, July, August and September are the only months with mean daily air temperatures above 0°C (3.7°C, 7.6°C, 6.1°C and 0.8°C, respectively) and there is snow cover (>1 cm) for 254 days per year. Weather statistics are not available for CAM-M, but temperatures there are expected to be lower than at BAR-1. Soil at each site was previously contaminated with hydrocarbons by spills of No.1 Arctic diesel fuel, a middle fuel distillate very similar to Jet A-1 fuel. Contamination had probably accumulated at both sites as a result of multiple spills during fuel transfer operations.

Biopiles

Fuel-contaminated soils were excavated and above-ground biopiles constructed in July, when the sites were first clear of snow cover. Soil at CAM-M was excavated from the top 50 cm with a front-end loader; while, that at BAR-1 was excavated from the top 40 cm with shovels. The excavated soil was thoroughly mixed with shovels and by rolling in a steel barrel. The biopiles were constructed on a plastic liner surrounded by a dike to contain any leachate (none was observed). The volume of each biopile was approximately 0.25 m^3. At BAR-1, each biopile had a horizontal layer of gravel (approximately 10 cm thick) in its middle containing three PVC pipes (5 cm diameter) that were perforated (two rows of 1-cm holes spaced every 10 cm) for passive aeration. At CAM-M, each biopile had a similar horizontal layer of gravel at its base with four similar horizontal PVC pipes for passive aeration. All of the biopiles, except for one at BAR-1, were covered with a heavy-duty clear plastic sheet, with the aeration pipes protruding. Construction of the biopiles was completed at CAM-M on 17 July 1996 and at BAR-1 on 9 July 1997.

At CAM-M, there were four biopiles constituting the following treatments: (1) control with no amendment, (2) fertilizer plus peat, (3) fertilizer plus inoculum and (4) fertilizer plus peat plus inoculum. At CAM-M, fertilizer consisted of 50 g of NH_4Cl plus 14 g of Na_2HPO_4 per m^3 of soil, and peat was added to 25% v/v. At BAR-1, there were five biopiles constituting the following treatments: (1) uncovered control with no amendment, (2) control with no amendment, (3) fertilizer, (4) fertilizer plus peat, and (5) fertilizer plus peat plus inoculum. At BAR-1, fertilizer consisted of 125 g of NH_4Cl, 13 g of NaH_2PO_4 and 21 g of Na_2HPO_4 per m^3 of soil, and peat was added to 14% v/v. After excavation, the soils began to drain, and when the biopiles were constructed, soils at CAM-M and BAR-1 were at 65% and 64% of water holding capacity, respectively.

Inocula

Some biopiles were inoculated with cold-adapted, mixed microbial enrichment cultures from soil. Enrichment

Table 1 Soil temperature (°C) at BAR-1 for the period 9 July to 30 August 1997

Location	Average	Average daily range	Cumulative degree-days
In situ, 2 cm depth	11.5	8.7	605
In situ, 5 cm depth	11.2	7.0	585
In situ, 10 cm depth	10.9	5.2	563
In situ, 20 cm depth	10.5	3.2	540
Uncovered biopile, upper section	10.8	6.7	562
Uncovered biopile, lower section	9.4	1.7	483
Covered biopile, upper section	16.0	10.2	835
Covered biopile, lower section	12.2	2.7	629

cultures were grown on mineral medium (Bedard et al. 1986) with 1 g/l Jet A-1 fuel and incubated at 7°C on a shaker. Lyophilized cultures were transported to the field sites and re-hydrated with 4 l of pond water from the sites. Approximate initial inoculum densities, per gram of soil, were 7.2×10^6 cells at CAM-M and 2.5×10^6 cells at BAR-1. After 39 days, the innoculum density at CAM-M was 4.8×10^6 cells.

Analyses

A composite sample of approximately 1.0 kg, consisting of 10 sub-samples, was taken from the stockpile of soil at each site after it was excavated and before it was used to construct biopiles. This sample was well mixed, stored and transported on ice or refrigerated (4°C to 10°C) and analyzed within 3 weeks. The soil at BAR-1 was a loamy sand and that at CAM-M a sandy loam (Canadian Soil Classification System). Both soils contained substantial amounts of rock and gravel. Large rocks (greater than 10 cm long) were removed from soil placed in the biopiles. Both soils had neutral pH, as determined in a slurry with distilled water. The total soil C (Leco analyzer) was 3.8% at CAM-M and 2.7% at BAR-1.Total N (semi-micro Kjeldahl digest method) was 0.5 and 0.4 g/kg, respectively, and available P (Bray extract method) was 2.0 and 1.3 mg/kg, respectively.

Samples for analysis of total petroleum hydrocarbons (TPH) were taken from biopiles at CAM-M as follows. Vertical cores (2.5 cm diameter) were taken from random locations in the biopiles. From each biopile, the spatially random soil cores were used to sequentially fill three 250-ml sample jars. At BAR-1, horizontal cores were taken from three locations: (1) from north to south through the center of the upper section of the biopile (above the gravel bed), (2) from northeast to southwest through the center of the lower section and (3) from northwest to southeast through the center of the lower section. Three separate sample jars were filled with spatially-distinct sample cores for each biopile. TPH was measured by the Analytical Services Unit, Queen's University (Kingston, ON) as previously described (Mohn and Stewart 2000). The TPH data were analyzed using the ANOVA procedure of SAS v.6.4 (SAS Institute Inc., Cary, NC), using two statistical models (Steel and Torrie 1980). The model for the CAM-M experiment accounted for a completely random sample procedure without subsamples. That for the BAR-1 experiment accounted for subsampling. An alpha value of 0.10 was chosen to define significant differences, due to the large variability typical of soil TPH contamination.

Temperature measurements

At BAR-1, two biopiles were equipped with thermocouples to monitor soil temperature. Three thermocouples were placed horizontally, 10 cm apart, across the center of the upper section of each biopile, and three more were placed, 10 cm apart, across the center of the lower section of each biopile. A thermocouple was placed horizontally 10 cm from each of the north, east, south and west edges of the lower section of each biopile. Additionally, a vertical temperature profile of undisturbed reference soil near the biopiles was monitored with duplicate thermocouples placed horizontally at 20 and 10 cm depths and quadruplicate thermocouples placed horizontally at 5 and 2 cm depths. The thermocouples were connected to a multiplexer and datalogger (Campbell Scientific, Edmonton, AB). Temperatures were recorded every 2 h from 9 July to 30 August 1997. Degree-days were calculated according to the following equation:

$$(T_{D,max}\text{-}T_{D,min})/2$$

where $T_{D,max}$ is the daily maximum temperature (°C) and $T_{D,min}$ is the daily minimum temperature (°C). Average temperatures were calculated for the upper and lower central sections of biopiles from the triplicate thermocouples in each section. Average temperatures were calculated for soil in situ at 2, 5, 10 and 20 cm depth and, using all thermocouples at these depths, for the 2 to 20 cm depth range.

Results

Soil temperature at BAR-1

At BAR-1 from 9 July to 30 August 1997, the soil in situ (from 2 to 20 cm depth) had an average daily temperature of 11°C and a degree-day accumulation of 573°. Predictably, the soil temperature decreased with depth (Table 1). The degree day accumulation at 20 cm depth was 11% lower than at 2 cm depth. The daily temperature range

(daily variation in temperature) was greatest near the surface.

Soil temperatures in the covered biopile were consistently higher than those in situ; whereas temperatures in the uncovered biopile were slightly lower than those in situ (Table 1). The daily temperature range was greater in the covered biopile than in the uncovered biopile, mainly because of the higher daily maxima in the latter. Degree-day accumulation in the upper part of the covered biopile was 45% higher than in situ (from 2 to 20 cm depth); whereas degree-day accumulation in the upper part of the uncovered biopile was similar to that in situ (Table 1). Degree-day accumulation in the lower part of the covered biopile was also 10% higher than in situ, whereas degree-day accumulation in the lower part of the uncovered biopile was 16% lower than in situ. Edge effects on soil temperature in the biopiles were relatively small (<2°C).

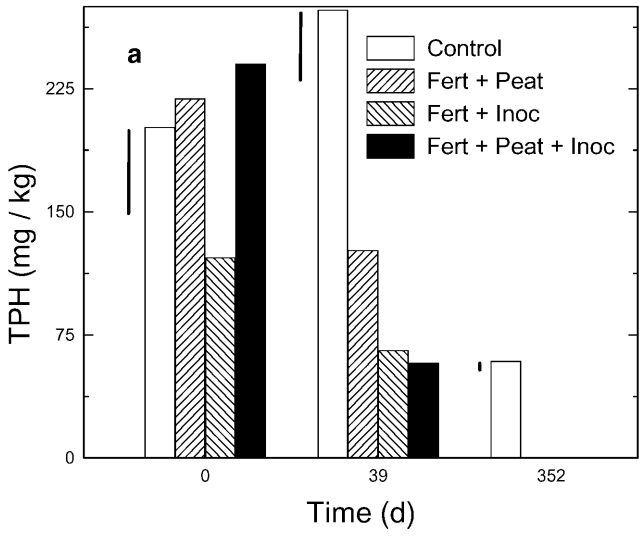
Hydrocarbon removal

The mean initial TPH concentration in the soil at CAM-M was 196 mg/kg (SE 19). Initially, there was a significant difference (P<0.05) among these treatments: the fertilizer plus inoculum treatment having lower TPH than the others (Fig. 1). This difference presumably resulted from heterogeneity of the TPH contamination, despite the thorough mixing. The mean initial TPH concentration in the soil at BAR-1 was 2,109 mg/kg (SE 211). There was no significant difference (P≥0.10) between these treatments at the start of the experiment. As the experiment at BAR-1 progressed, mean TPH values for the uncovered control treatment displayed extremely large standard errors. Thus, this treatment was excluded from the statistical analysis to enable meaningful comparison of the other treatments. After 39 and 53 days at CAM-M and BAR-1, respectively, there was extensive hydrocarbon removal in all amended (non-control) biopiles, with maximum TPH removal of 72% and 85%, respectively. At these times, all amended treatments had significantly (P<0.05) less TPH than the respective controls. After 352 and 365 days at CAM-M and BAR-1, respectively, further removal had occurred, with maximum TPH removal of 100% and 92%, respectively. At these times, all amended treatments still had significantly (P<0.05) less TPH than the respective controls.

Of the soil treatments, fertilization with N and P had the greatest stimulatory effect on hydrocarbon removal (Fig. 1). The addition of peat as a bulking agent had no clear stimulatory effect on hydrocarbon removal. When TPH samples were taken at BAR-1 after 53 days and after 365 days, soil in the uncovered biopile was visibly drier than that in the covered control.

Effect of inoculation

Inoculation had a consistent, significant stimulatory effect on hydrocarbon removal in both experiments. This effect was in addition to, but smaller than, the effect of fertilization with N plus P. After 39 days at CAM-M, the treatment with fertilizer plus peat plus inoculum had significantly (P<0.05) less TPH than that with fertilizer plus peat (Fig. 1a). After 53 days at BAR-1, the treatment with fertilizer plus peat plus inoculum had significantly (P<0.10) less TPH than that with fertilizer plus peat (Fig. 1b). The inocula only affected hydrocarbon removal during the first summer of treatment. After 1 year at both sites, TPH concentrations in the inoculated treatments were no longer significantly different from those in the other amended treatments. It is unclear whether re-inoculation could stimulate hydrocarbon removal after the first year. This was tried after 1 year at CAM-M, but by that time TPH was completely removed from all amended biopiles.



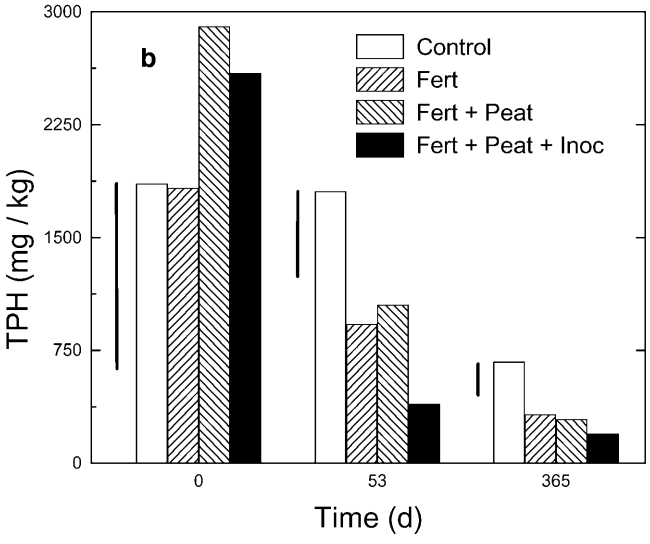


Fig. 1a, b Total petroleum hydrocarbons in biopiles at CAM-M (**a**) and BAR-1 (**b**); n=3; bars indicate least significant differences, LSD (0.10), for each sampling date; *Control* covered control; *Fert* fertilizer (N plus P); *Inoc* microbial inoculum; TPH detection limit 10 mg/kg

Discussion

The clear plastic biopile covers had the desired effects of increasing soil temperature (Table 1) and conserving moisture. Logistical limitations precluded additional treatments testing the effect of the plastic cover on hydrocarbon removal in amended biopiles. However, it is likely that fuel biodegradation was stimulated by the measured increase in degree-days in covered biopiles. In microcosms with similar Arctic soils, an increase of temperature from 7°C to 15°C greatly increased hydrocarbon degradation rates (Mohn and Stewart 2000). The dimensions of a biopile would affect its temperature regime. The warming effect found in this study would probably not occur if the height of biopiles were greatly increased, considering the differences in temperature observed between the upper and lower sections of biopiles at BAR-1. One interesting question that remains to be addressed is how the increased diurnal temperature variation due to the cover on such a system affects soil microbial populations and their metabolic activities. It is unclear whether the visible conservation of moisture attributable to the cover also stimulated fuel biodegradation.

Addition of N plus P was very effective in stimulating hydrocarbon removal (Fig. 1). This indicates that one or both of these nutrients is a limiting factor. The effect of N plus P also supports the hypothesis that the soils contain indigenous microflora capable of degrading hydrocarbons and supports the conclusion that biodegradation was the primary mechanism of hydrocarbon removal. Laboratory microcosm experiments with soil from BAR-1 further support the above conclusions, as we observed that individually added N or P both stimulated dodecane mineralization, and that autoclaving completely inhibited dodecane mineralization (Mohn and Stewart 2000). The stimulatory effect of N and P on hydrocarbon removal is consistent with other studies of soils from polar regions (Braddock et al. 1997; Kerry 1993) and elsewhere. The fact that peat did not stimulate hydrocarbon removal is consistent with the fact that the soil types used are coarse, presumably allowing adequate drainage and air diffusion.

Drainage of excavated soil, and the resulting aeration, might have been sufficient to permit substantial hydrocarbon removal. Soils that were bioremediated at both CAM-M and BAR-1 were in low areas that were saturated with water before excavation, suggesting that each soil was anoxic and therefore unfavorable for aerobic fuel biodegradation. The permafrost probably interferes with the drainage of soil at each of the sites, therefore contributing to the persistence of fuel in the soil. The effect of drainage on hydrocarbon removal was not specifically tested by comparing TPH concentrations in biopiles to TPH concentrations of untreated soil in situ, but there was evidence that drainage stimulated hydrocarbon biodegradation. The history of contamination of soils at CAM-M and BAR-1 is not recorded, but the fuel is known to have persisted in the soils for many years before these experiments, suggesting that it was not being extensively degraded in situ. However, after approximately 1 year, substantial hydrocarbon removal occurred in the unamended control biopiles at both sites (Fig. 1). This observation does not prove that biodegradation was the major mechanism of removal in the control treatments, but other common mechanisms of removal appear unlikely. Volatile losses did not appear to be the major mechanism, as the biopiles were covered and hydrocarbon concentrations did not significantly decrease in either control treatment during the first sampling period (summer). Leaching of hydrocarbons was probably not substantial, because the biopiles were covered and no leachate was observed after construction of the biopiles.

This study is a rare case in which inocula have been shown to have significant stimulatory effects on bioremediation in field experiments. Consistent with this result, we have observed that inoculation stimulated dodecane mineralization in laboratory microcosms containing three Arctic tundra soils from military radar sites with similar characteristics to the soils used in this study (Mohn and Stewart 2000). Several factors could have contributed to the efficacy of inocula in the field experiments. First, the sandy soils, low in organic content, might have had relatively small populations of hydrocarbon-degrading and total microorganisms. Low microbial populations are thought to be typical of Arctic soils (Robinson and Wookey 1997). Second, the short summer season might have substantially limited the growth of indigenous hydrocarbon-degrading populations. Third, the inocula used might have been particularly well adapted for their purpose. These inocula were psychrotolerant, mixed microbial cultures, enriched from soil. Further, the inoculum used at BAR-1 was enriched from hydrocarbon-contaminated soil of an Arctic site similar to BAR-1.

Logistical considerations prevented additional treatments testing killed inocula, so we cannot rule out a nutritional effect of the inocula on the native soil population, as Prince (1998) suggested can occur. Unlike some commercial inocula, the inocula in these experiments contained only lyophilized microorganims and no nutrients or surfactants. The inocula in these experiments were extremely small and contributed less than 2.5 µg biomass, 2.4 µg sucrose (used as a cryoprotectant) and 0.10 pg N plus 0.23 pg P (from the mineral medium) per g of soil. Thus, we conclude that the most probable effect of the inocula was the intended one, to provide microorganisms that multiplied and degraded hydrocarbons.

The results of the two independent field experiments were highly consistent with one another. These results suggest that fuel-contaminated Arctic tundra soil can be effectively bioremediated on site in biopiles. Addition of nutrients (N plus P) is essential for maximizing the hydrocarbon removal rate, and inoculation can further increase the initial hydrocarbon removal rate. In determining the value of inoculation in treatment systems similar to these, the benefit of time saved will have to be

weighed against the expense of inoculation. Because the treatment season is so short in polar regions, eliminating a few months of treatment time can potentially reduce the site clean up time by 1 or 2 years. Heating biopiles would probably be an alternative way to reduce the clean up time.

Acknowledgments We thank the many people who assisted in these experiments, including Barb Zeeb, Sarah Silver, Allyson Campbell, Zou Zou Kuzyk and Bianca Kuipers for helping to construct the biopiles at CAM-M; the Frontec employees at CAM-M for logistical assistance; Bianca Kuipers, Barbara Zeeb, Jennifer Rogers and Michelle Adams for sampling; Gary Myers of Environment Canada for providing summary weather data for BAR-1; and John Hall of Agriculture and Agri-Food Canada for statistical advice. This research was supported by a Strategic Grant from the Canadian Natural Sciences and Engineering Research Council and by the Canadian Department of National Defence.

References

Aislabie J, McLeod M, Fraser R (1998) Potential for biodegradation of hydrocarbons in soil from the Ross Dependency, Antarctica. Appl Microbiol Biotechnol 49:210–214

Atlas RM (1981) Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiol Rev 45:180–209

Bedard DL, Unterman R, Bopp LH, Brennan MJ, Haberl ML, Johnson C (1986) Rapid assay for screening and characterizing microorganisms for the ability to degrade polychlorinated biphenyls. Appl Environ Microbiol 51:761–768

Braddock JF, McCarthy KA (1996) Hydrologic and microbiological factors affecting persistence and migration of petroleum hydrocarbons spilled in a continuous-permafrost region. Environ Sci Technol 30:2626–2633

Braddock JF, Ruth ML, Catterall PH, Walworth JL, McCarthy KA (1997) Enhancement and inhibition of microbial activity in hydrocarbon-contaminated Arctic soils: implications for nutrient-amended bioremediation. Environ Sci Technol 31:2078–2084

Eweis JB, Ergas SJ, Chang DPY, Schroeder ED (1998) Bioremediation principles. McGraw-Hill, Boston

Fitzpatrick EA (1997) Arctic soils and permafrost. In: Woodin SJ, Marquiss M (eds) Ecology of Arctic environments. Blackwell, Oxford, pp 1–39

Kerry E (1990) Microorganisms colonizing plants and soil subjected to different degrees of human activity, including petroleum contamination in the Vestfold Hills and MacRobertson Land Antarctica. Polar Biol 10:423–430

Kerry E (1993) Bioremediation of experimental petroleum spills on mineral soils in the Vestfold Hills, Antarctica. Polar Biol 13:163–170

Longton RE (1997) The role of bryophytes and lichens in polar ecosystems. In: Woodin SJ, Marquiss M (eds) Ecology of Arctic environments. Blackwell, Oxford, pp 69-96

Mohn WW, Stewart GR (2000) Limiting factors for hydrocarbon biodegradation at low temperature in Arctic tundra soils. Soil Biology Biochem 32:1161–1172

Prince RC (1998) Bioremediation. In: Kroschwitz JI (ed) Kirk-Othmer encyclopedia of chemical technology, 4th edn, Supplement. Wiley, New York, pp 48–89

Robinson CH, Wookey PA (1997) Microbial ecology, decomposition and nutrient cycling. In: Woodin SJ, Marquiss M (eds) Ecology of Arctic environments. Blackwell, Oxford, pp 41–68

Steel RGD, Torrie JH (1980) Principles and procedures of statistics. McGraw-Hill, San Francisco

Stotzky G (1997) Soil as an environment for microbial life. In: van Elsas JD, Trevors J, Wellington EMH (eds) Modern soil microbiology. Dekker, New York, pp 1–20

Tumeo MA, Wolk AE (1994) Assessment of the presence of oil-degrading microbes at McMurdo Station. Antarct J US 29: 375–377

Vogel TM (1996) Bioaugmentation as a soil bioremediation approach. Curr Opin Biotechnol 7:311–316

Whyte LG, Greer CW, Inniss WE (1996) Assessment of the biodegradation potential of psychrotrophic microorganisms. Can J Microbiol 42:99–106

Whyte LG, Bourbonniere L, Bellerose C, Greer CW (1999) Bioremediation assessment of hydrocarbon-contaminated soils from the High Arctic. Bioremediation J 3:69–79