



Weathering of a subarctic oil spill over 25 years: the Caribou-Poker Creeks Research Watershed experiment

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Abstract

A small experimental oil spill conducted in an open black spruce forest within the Caribou-Poker Creeks Research Watershed (CPCRW), 48 km north of Fairbanks, AK, in the winter of 1976 was designed to examine the effects of crude oil spills in permafrost terrain. No clean-up was attempted, and the site now provides an opportunity to follow the natural weathering of spilled oil under these conditions. In summer 2001, more than 25 years after the spill, we sampled soils from the spill plot and a nearby reference plot to determine how the oil had weathered, and to assess microbial populations and activity. All samples collected from the oiled plot contained substantial amounts of methylene chloride extractable oil, between 4% and 66% by weight. Using 17 α (H)21 β (H)hopane as a conserved internal marker within the oil, we determined that while some heavily oiled samples were almost unchanged since the spill, others had lost more than 80% of their initial hydrocarbon. Evaporation, biodegradation and photooxidation all seem to have played important roles in this process, but to varying degrees in different samples. Assays of culturable populations of total heterotrophs and crude oil emulsifiers, and mineralization potentials for hexadecane and phenanthrene, indicate that the microbial population in the oiled soils has remained acclimated to degrade hydrocarbons. We conclude that natural weathering processes will eventually lead to the removal of much of the hydrocarbon from these heavily oiled subarctic soils; however, the combination of low rates of nutrient turnover, a short thaw season, and high hydrocarbon concentrations will result in the persistence of oil residue for many more decades. Finding an environmentally appropriate cleanup technology for sites like this remains an important challenge for future research.

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1. Introduction

Arctic and subarctic regions of the world contain significant oil and gas deposits that are expected to account for a considerable portion of the world's future production (IUCN, 1993). Thus, it is of on-going importance to understand the long-term fate of oil spilled in these harsh environments, and to

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offer guidance for remediating any spills that may occur. An experimental oil spill site in the Caribou-Poker Creeks Research Watershed (CPCRW) near Fairbanks, AK, provides an excellent source of samples to follow the natural weathering, including evaporation, biodegradation and photooxidation, of oil spilled in this environment in 1976. In this study, we have examined the changes that have occurred in the composition of the oil, and studied the microbial populations and their activities in soil samples from the site collected over 25 years after the spill.

The CPCRW spill site was developed during construction of the Trans-Alaska Pipeline System in order to examine the potential effects of an oil leak from the pipeline (Johnson et al., 1980). In 1976, two 7570-l spills (one in February and one in July) of hot (57 °C) Prudhoe Bay crude oil were conducted in an open black spruce (*Picea mariana*) forest at the CPCRW. Spill-related effects were evaluated for 2 years following the spill (Sparrow et al., 1978; Jenkins et al., 1978). Three additional follow-up studies were conducted after 10 (Sparrow and Sparrow, 1988; microbiology and chemistry), 15 (Collins et al., 1994; chemistry, and physical and vegetation effects), and 19 years (Lindstrom et al., 1999; microbial community structure). The site has not otherwise been disturbed since the spills occurred in 1976, and no attempt has been made to clean up the soils. Thus, this is an ideal location to observe the long-term effects and evolution of a crude oil spill in the subarctic in the absence of treatment.

We re-visited the site in June 2001 (about 25 years after the spill) to collect samples from the winter oil spill site and from an adjacent reference site. We have monitored the numbers of culturable microorganisms (focussing on hydrocarbon degraders) and their potential to mineralize hydrocarbon substrates in the soils, and conducted an extensive gas chromatography/mass spectrometry analysis on a few samples to assess the degree of oil weathering. No chemical composition data from the site have been published since 1994 (Collins et al., 1994).

The mechanisms for oil loss at this site are likely evaporation, biodegradation and photooxidation. Loss by water-washing does not seem a very likely fate, since there is no evidence for significant

water movements at the site, and the spill area did not expand significantly over the first 13 years (Collins et al., 1994). The three proposed mechanisms of oil loss leave characteristic evidence of their occurrence in the chemical composition of the residual oil. For example, low molecular weight hydrocarbons tend to evaporate preferentially (Finigas, 1995). The biodegradation of oil has been studied extensively, at least under aerobic conditions, and the preferential degradation of linear before branched alkanes, and smaller before larger aromatic compounds, is well established (Prince, 1998). In contrast, photooxidation seems to affect only the aromatic compounds in the oil (Garrett et al., 1998). Thus, if one has a sample of the initially spilled oil, it should be possible to determine the contributions of these different weathering processes in samples collected after the spill, and even perhaps project future losses.

The other well-studied terrestrial spill in a far-northern climate is the Nipisi, Rainbow and Old Peace River pipeline spills in the Lesser Slave Lake area of Northern Alberta (Blenkinsopp et al., 1996; Wang et al., 1998). A variety of treatments were tried at these sites, including burning, tilling and the application of fertilizer, but oil patterns at the site when re-visited about 25 years after the spill were similar despite treatment (Wang et al., 1998). Overall, after 25 years, weathering and degradation were substantial in the samples collected from the surface but little weathering/degradation had occurred in subsurface samples. The specific mechanisms responsible for the weathering/degradation patterns seen were not determined in that study.

Gas chromatography coupled with mass spectrometry, particularly using selected ion monitoring for compounds of specific interest (Douglas et al., 1992; Wang et al., 1998), identifies and quantifies individual hydrocarbons in the residual oil. Rather than relying on absolute quantitation of species of interest, the relative concentration of these species with respect to a conserved internal marker within the oil leads to tighter analytical precision for understanding losses (Prince et al., 1997). We have shown that 17 α (H)21 β (H)hopane is a useful conserved internal marker for following the biodegradation (Prince et al., 1994) and photooxidation (Garrett et al., 1998) of crude oils, and we use it here.

2. Site description

The study area is located in the CPRW 48 km northeast of Fairbanks, AK. The sampling areas for our study included the site of the 7570-l experimental crude oil spill conducted in February 1976 to investigate the long-term effects of crude oil contamination on subarctic taiga (see Collins et al., 1994). The study was designed to mimic the effects of a leak in the trans-Alaska oil pipeline. Oil warmed to 57 °C was applied at the top of a plot established on a west-facing, low angle slope and allowed to flow down the slope. The distribution of the oil following the spill was mapped and can be found in Collins et al. (1994). Visually, the upper 0–4-m portion of the winter oil plot in 2001 still looked much like this map. Visible signs of oiling (dark soil, dead vegetation) remain over about 50% of the upper 4 m of the plot. We collected soil samples from both the oiled plot (winter oil spill; organic and mineral horizons) and an adjacent oil-free reference site (organic soil only) located just up the slope from the spill area. Collection of mineral soil in the reference plot was not possible due to the shallow frozen soils in the active layer in June. Local vegetation at the site is open black spruce (*P. mariana*) forest (total tree canopy cover less than 60%) with a shrub understory of blueberry (*Vaccinium uliginosum*), Labrador tea (*Ledum decumbens*), resin birch (*Betula glandulosa*) and willow (*Salix* spp.). The ground is covered by mosses and lichens, with scattered (10% of cover) cotton grass (*Eriophorum vaginatum*) tussocks (Collins et al., 1994). The area is underlain by permafrost, with an active layer thickness of 40–60 cm, and the soil is classified as a histic pergelic cryaquept (Jenkins et al., 1978). A typical soil profile (unoiled) consists of a 5-cm moss-and-lichen surface layer above a 15-cm undecomposed peat horizon (O1), a 5-cm decomposed dark brown peat horizon (O2), a 5-cm dark grayish-brown silt loam horizon (A1), and a grayish-brown silt loam mineral soil (C; Collins et al., 1994).

During the initial spill, in the winter, most of the oil flowed between *Eriophorum* tussocks. As a result, almost all mosses, lichens and shrubs in the zone 10 to 15 m below the spill were killed by the oil. In 1995, moss and lichen surface vegetation was generally dead and incorporated into the O2 and A1 horizons. With few exceptions, the only surviving vegetation

was scattered *E. vaginatum* tussocks, some of which had apparently “increased dramatically in size” since the spill (Collins et al., 1994). The site looked much the same in summer 2001, although some mosses and lichens are beginning to creep across the surface of the spill zone from unoiled areas adjacent to the site.

A physical consequence of the oil spill is a substantial increase in average thaw depths. For example, in 1991 the average thaw depth for the winter spill site 1-m downslope from the spill was 193 cm versus only 70 cm in the reference plot. This is likely attributable to decreased soil surface albedo and loss of the insulating moss and surface vegetation layer in the oiled plot (Collins et al., 1994). Oiled soil temperatures are on average significantly warmer than reference soils during the summer months. Average soil temperatures as high as 13 °C were observed 10 cm below the surface in the oil plot, while mean soil temperatures above 8 °C were not seen in the reference soils even in late summer (August; Lindstrom, unpublished Ph.D. thesis, University of Alaska Fairbanks, 1997). By October, low sun angles and cool ambient air temperatures provide little energy for soil heating, and temperature differences between oiled and reference soils disappear. Mean oiled soil temperatures greater than 5 °C were measured in early July, dropping to about 1 °C by mid-October; we therefore estimate that soil temperatures in the oil plot may be sufficiently warm to allow microbial populations to be active for perhaps 100 days each year. However, microbial metabolic activity (detected by incorporation of ¹⁴C labeled acetate into lipids) has recently been measured at temperatures as low as –20 °C in Siberian permafrost samples (Rivkin et al., 2000) indicating that a limited amount of activity may occur throughout the rest of the year.

Mean soil pH at this site is fairly low (ca. 4.5), though typical of this forest type with its low concentrations of basic ions (calcium, magnesium, potassium; Van Cleve et al., 1983). There are no differences in pH between the oiled and reference soils (Johnson et al., 1980; Sparrow and Sparrow, 1988), an observation consistent with other oil spill studies in Alaska (e.g., Sexstone et al., 1978). A previous study at this site demonstrated that total N on a soil dry mass basis was very similar between the oiled and reference soils, with mean values of ca. 0.65 % for each (Lindstrom et al., 1999). In that study, soil total inorganic N (essen-

tially all NH_4^+ -N) was higher in reference than in oiled soil samples, but averaged about 1000-fold lower than total soil N; in other words, almost all N present was organically bound. Mean NO_3^- -N concentrations in both soil types were low (averaging $< 1 \mu\text{g g dry soil}^{-1}$) and showed no oil treatment effects. Extractable Phosphorus values also exhibited no differences according to treatment, averaging $3.7 (\pm 1.0)$ and $3.2 (\pm 0.3) \mu\text{g g dry soil}^{-1}$ for reference and oiled soils, respectively (Lindstrom et al., 1999). The ratio of soil total C to total N did not vary significantly with respect to season, and oiled soil ratios were more than twice those seen in reference soil samples. The mean ratio of C to N was $52.7 (\pm 3.8)$ for oiled soils and $23.0 (\pm 0.8)$ for reference site soils.

3. Methods and materials

3.1. Sample collection

Samples were collected on 11 June 2001 from the top 3–4 m of the winter oil spill plot and in an adjacent reference plot. Similarly to the map published by Collins et al. (1994), about 40–50% of the upper 4 m of the oil plot has visible signs of oiling (dark color and dead vegetation) although tussocks containing live vegetation are scattered through the plot. Samples from the oil plot were collected from areas containing signs of persistent oil. In the reference plot, samples were collected through the moss/lichen layer, between *E. vaginatum* tussocks. Samples from both the oiled and reference plots were collected

with a hand corer (2.5 cm diameter). Cores were collected to the depth of thaw. For microbial analyses, five clustered cores were collected at three locations within each plot. The cores were stored at about 4°C in a cooler until returned to the laboratory for processing. Cores from each location were separated into organic and mineral horizons (oil plot); mineral soil was generally not present in reference soil cores. The five cores from each location were then mixed and sieved (2 mm mesh) to yield three replicate samples from each plot (oil and reference) and two horizons from the oil plot (organic and mineral). The sieved composite samples were placed in sterile plastic bags and stored at 4°C until processed. Subsamples of the sieved soil were also placed in pre-cleaned glass jars (Scientific Specialties Service, Randallstown, MD) for chemical analysis. Other samples for chemical analysis were collected in the field with a metal spoon (pre-rinsed with methylene chloride) and placed directly in pre-cleaned glass jars. These samples were placed in a cooler upon collection and stored at about 4°C until shipped to the ExxonMobil Research and Engineering laboratory in Annandale, NJ, for chemical analysis at which time they were stored at -20°C until extraction. Samples were frozen within a week of collection. Table 1 provides a description of the samples and their degree of oiling.

3.2. Microbiology

Processing of the samples for microbiological analysis occurred within 48 h of sieving. Culturable microbial populations (total heterotrophs, crude oil

Table 1
Sample descriptions and results of analysis by gas chromatography coupled with mass spectrometry

Sample number	Description (soil type)	Approximate depth below surface (cm)	g methylene chloride extractable material/10 g sample	% depleted, based on hopane in GC-detectable oil
1	Mineral horizon of oiled plot (composite)	8–18	0.45	10
2	Organic horizon of oiled plot (composite)	0–8	3.38	40
3	Organic horizon of reference plot (composite)	0–8	0.01	–
4	Surface sample from oiled plot	0–4	6.59	66
5	Surface sample from oiled plot	0–4	3.97	83
6	Surface oiled twigs from oiled plot	Not applicable	5.10	75
7	Oiled tussock from oiled plot	Not applicable	5.36	62
8	Subsurface sample from oiled plot	4–8	6.43	54

Approximate depths at which samples were collected are indicated. Note that in the reference soil (sample 3), a moss layer of about 5 cm was removed and soil was collected below that layer.

emulsifiers, hexadecane degraders and phenanthrene degraders) as well as their mineralization potentials (for three substrates; glutamate, hexadecane and phenanthrene) were measured. Populations were assayed using miniaturized most probable number techniques following dilution of samples in carbon-free Bushnell–Haas broth (BH; modified from Atlas, 1993 by only adding 1/10th the FeCl_3). Duplicate plates were set up for each sample for heterotrophs and single plates for all other population assays. Ninety-six well plates were used for the heterotroph, hexadecane and phenanthrene assays as previously described (Braddock and Catterall, 1999). R2A medium (Atlas, 1993) was used as growth medium for the heterotroph plates. Phenanthrene plates were prepared by placing 10 μl of a phenanthrene solution (13 g/l in methanol) in each well of a 96-well plate, allowing the methanol to evaporate and adding 100- μl BH to each well, then inoculating the plates. Hexadecane plates were prepared by adding 5 μl hexadecane to each well of inoculated plates containing BH. Crude oil emulsifiers (Sheen Screen method) were determined using 24-well plates and Prudhoe Bay crude oil as the carbon source (Brown and Braddock, 1990). Mineralization potentials were determined in soil slurries (1:10; soil/BH) to which radiolabeled substrates were added (Brown et al., 1991). Briefly, 10-ml aliquots of soil slurry were added to 40-ml VOA vials (I-Chem Research, Hayward, CA), and then spiked with 50 μl of a 2-g/l solution of radiolabeled substrate (about 50,000 dpm/vial). The radiolabeled substrates included [9- ^{14}C]-phenanthrene and [1- ^{14}C]-*n*-hexadecane (Sigma, St. Louis, MO) diluted in acetone, and [UL- ^{14}C]-L-glutamic acid diluted in sterile deionized water. Triplicate vials were prepared for each sample. Following incubation at 10 °C, the $^{14}\text{CO}_2$ evolved was recovered and counted by liquid scintillation (Brown et al., 1991). Time courses were run for each substrate to determine optimum incubation times of 24 h for glutamate assays and 72 h for hexadecane and phenanthrene assays (details on determining optimum incubation times are found in Brown et al., 1991). Data for both enumerations and mineralization potentials were corrected to soil dry weight. However, due to the high oil content, particularly of the organic layer soils from the oil plot where oil may actually now be part of the soil matrix, this

correction underestimates the values for the oiled soils because it overestimates the amount of soil material present in those samples.

Differences among soils were tested using analysis of variance followed by Bonferroni probability-adjusted pairwise comparisons (Systat version 5.05, SPSS, Chicago, IL).

3.3. Chemistry

Samples were extracted with methylene chloride in an automated solvent extractor (ASE200, Dionex, Sunnyvale, CA 94086), dried with anhydrous sodium sulfate, and diluted to approximately 10 mg extractable material/ml methylene chloride. The samples were not evaporated to dryness, so volatile hydrocarbons present in the samples should have been conserved. For comparison, we also analyzed a sample of the initially spilled oil, which has been stored at -20 °C since the spill at the U.S. Army Corps of Engineers Cold Regions Research and Engineering Laboratory (CRREL) in Hanover, NH.

Gas chromatography and mass spectrometry in total scan and selected ion monitoring modes was carried out essentially as described by Douglas et al. (1992). We used a Hewlett Packard HP 5890 gas chromatograph fitted with a 30 m \times 0.25 mm fused silica capillary column with 5% crosslinked phenyl methyl silicone as the stationary phase. Helium served as the carrier gas at a flow rate of 1 ml/min. Mass spectral data were obtained with a Hewlett Packard 5972 mass selective detector at an electron energy of 70 eV. Spectral tuning with perfluorotributylamine followed USEPA method 8270C.

4. Results

4.1. Microbiology

Culturable populations of total heterotrophs and crude oil emulsifiers (Sheen Screen test) were significantly greater ($p < 0.05$) in the oiled soil (both organic and mineral) than in the reference soil (Table 2). Neither phenanthrene- nor hexadecane-degrader populations were significantly different among the soils at the 95% confidence level. However, two of the three samples from the organic layer in the oil

Table 2

Culturable microbial populations in soils from the winter oil spill plot (organic and mineral horizons) and from an adjacent reference plot (organic horizon only)

Population	Most Probable Number (log ₁₀ cells/g dry weight soil)		
	Soil type		
	Reference (organic)	Oil (organic)	Oil (mineral)
Heterotrophs	6.15 ± 0.17 ^a	6.92 ± 0.13 ^b	6.83 ± 0.08 ^b
Hexadecane degraders	4.13 ± 0.42 ^a	5.27 ± 0.26 ^a	3.90 ± 0.17 ^a
Phenanthrene degraders	3.57 ± 0.13 ^a	3.81 ± 0.18 ^a	3.50 ± 0.03 ^a
Crude oil emulsifiers	2.43 ± 0.07 ^a	5.41 ± 0.21 ^b	4.87 ± 0.27 ^b

Values are the mean ($n=6$ for heterotrophs; $n=3$ for other populations) log₁₀ cells/g dry weight soil estimated by most probable number methods ± one standard error. Different letters following the values indicate significant differences ($p < 0.05$) among the soil types for a given population type.

plot had populations of hexadecane degraders greater than $3 \times 10^5 \text{ g}^{-1}$ dry soil, which were the highest two values measured for any of the samples (individual values not shown). Acclimation of the microbial community in the oiled plot (organic layer) for hexadecane utilization is supported by the mineralization potentials measured (Table 3). Mineralization of both hexadecane and phenanthrene was significantly greater in the oil (organic layer only) plot than in the reference plot. However, mineralization potentials for phenanthrene in all soils were low compared to values measured for aromatic hydrocarbons by us at other sites. For example, we found a mean mineralization potential of $4500 \pm 300 \text{ ng/g dry weight soil day}$ for naphthalene for soils collected from an Arctic fuel oil contaminated site near Barrow, AK, while hexadecane mineralization potentials were similar to those measured in this study (Braddock et al., 1997). Glutamate mineralization activities were highly variable among replicates; there were no significant differences among the soils.

4.2. Oil chemistry

The CPRW soil samples contained significant amounts of methylene chloride extractable oil, as shown in Table 1. Indeed, several were more than 50% oil by weight. Even cursory inspection of the total ion gas chromatograms (Fig. 1) reveals that

some samples, such as 4, 5 and 6, are extensively degraded, while sample 1 still seems remarkably “fresh”, retaining detectable levels of compounds expected to be volatile, such as undecane ($n\text{C}_{11}\text{H}_{24}$). Crude oils contain biomarkers (Peters and Moldowan, 1993) that are the molecular fossils of the original biomass that gave rise to the oil. These biomarkers can be detected with selected ion mass spectrometry. Fig. 2 shows chromatograms of the terpanes in the initial oil, and in the sample of the reference (un-oiled) soil (sample 3). As expected, the oil contains the hopane series (Peters and Moldowan, 1993), including $17\alpha(\text{H})21\beta(\text{H})$ hopane, a compound that has proven to be a useful conserved internal marker for monitoring biodegradation (Prince et al., 1994) and photooxidation (Garrett et al., 1998). The suite of hopanes seen in the initial oil (Fig. 2) was found in all the oil samples analyzed here. There is no evidence that any of these hopanes have been biodegraded or otherwise lost since the spill.

Since $17\alpha(\text{H})21\beta(\text{H})$ hopane showed no evidence of biodegradation, is not generated during oil biodegradation in laboratory experiments (Prince et al., 1994), and is not present in the un-oiled soil (Fig. 2), we have used it as a conserved internal marker within the oil. We can calculate the percent depletion of other analytes within the oil using the equation:

$$\% \text{Loss} = [(A_0/H_0) - (A_s/H_s)] / (A_0/H_0) \times 100$$

where: A_s and H_s are the concentrations of the target analyte and hopane in the oil sample, respectively, and A_0 and H_0 are the concentrations in the initially spilled

Table 3

Mean (± one standard error) mineralization potentials for phenanthrene, hexadecane, and glutamate for soil slurries ($n=9$) incubated at 10 °C of samples collected from the oil and reference plots near Fairbanks, AK

Substrate	Mineralization potential (ng/g dry weight soil day)		
	Soil type		
	Reference (organic)	Oiled (organic)	Oiled (mineral)
Glutamate	8900 ± 5600 ^a	12,000 ± 3200 ^a	3800 ± 200 ^a
Hexadecane	230 ± 33 ^a	900 ± 130 ^b	500 ± 100 ^a
Phenanthrene	33 ± 0 ^a	130 ± 33 ^b	100 ± 33 ^{a,b}

Incubation times were 24 h for glutamate and 72 h for hexadecane and phenanthrene. Letters following the values indicate significant differences ($p < 0.05$) among the soil types for substrate use.

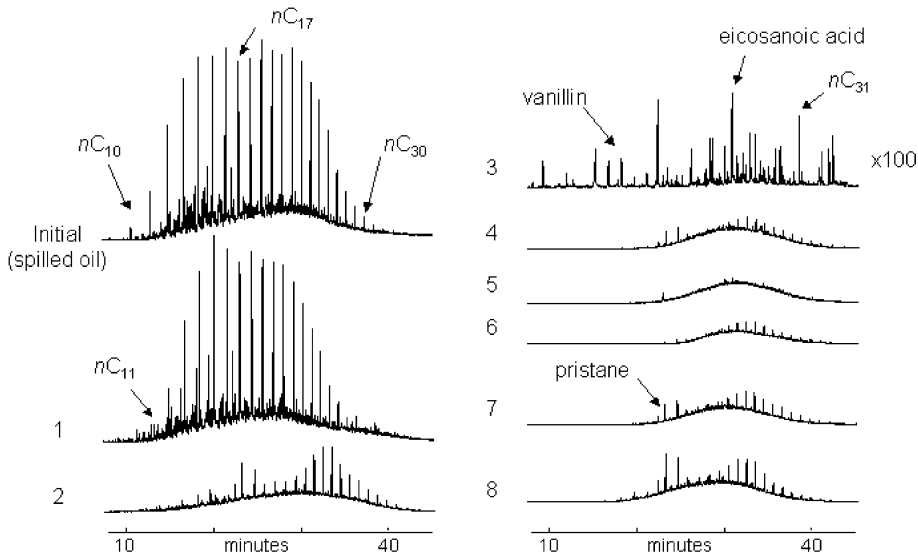


Fig. 1. Total ion gas chromatograms. Samples include the initial spilled oil, a composite of mineral horizon soil samples from the oiled plot (1), a composite of organic horizon soil samples from the oiled plot (2), a composite of organic horizon soil samples from the reference plot (3), surface samples from the oiled plot (4 and 5), oiled twigs from the surface of the oiled plot (6), oiled tussock material from the oiled plot (7) and a subsurface sample from the oiled plot (8). Tentative identifications of reference compounds are indicated.

oil. We have used the sample of initially spilled oil (stored frozen since the spill) to determine values for A_0 and H_0 . Comparing this oil to a sample of fresh Alaska North Slope oil collected in 1990 suggests the stored sample is some 8% depleted of total hydro-

carbon compared to fresh oil. This is consistent with the fact that it was heated to 57 °C before application onto snow (Collins et al., 1994). As can be seen in Fig. 1, the spilled oil contained some decane ($nC_{10}H_{22}$), but had lost lighter components.

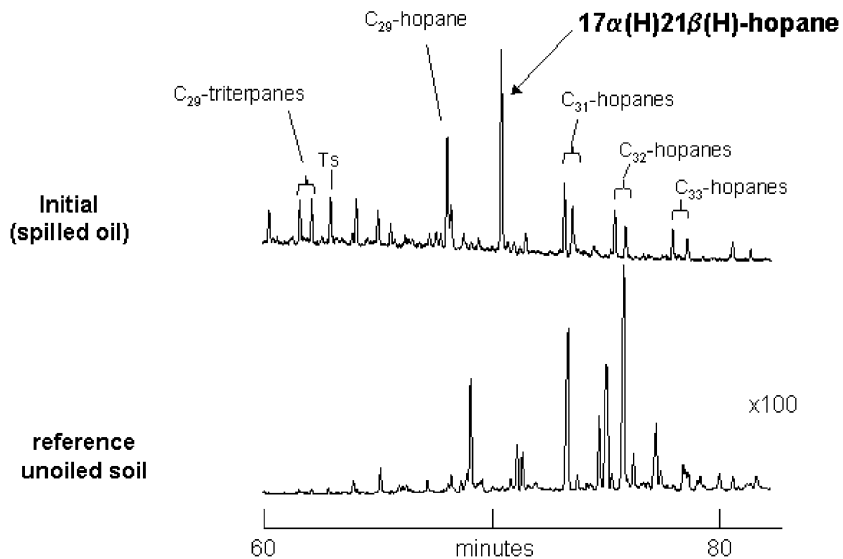


Fig. 2. Chromatograms of terpanes in the initial spilled oil and in extractable material from an unoiled reference plot soil sample (sample 3).

The unoiled soil (sample 3) contained some methylene chloride extractable material (Table 1, Fig. 1), but this material was not petroleum. Tentative identifications of three compounds in this sample, based on comparison to reference libraries of spectra, are indicated in Fig. 1. Some long waxes of biological origin are present. The concentrations (note that chromatogram of this sample has been amplified $\times 100$) are so low, however, that we do not believe they contribute significantly to the hydrocarbons measured in the oiled samples, and we have ignored them in the following analyses.

Gas chromatography coupled with mass spectrometry allows a molecular analysis of the oil samples. We have focussed on the linear and branched alkanes because these are the most abundant species in the oil (making up some 5.2% of the sample of the initial oil sample), and the two-, three- and four-ring polycyclic aromatic hydrocarbons and their alkylated congeners, because these are on the USEPA list of priority pollutants (Keith and Telliard, 1979).

Fig. 3 shows the percentage loss of heptadecane (nC_{17}), pristane (2,6,10,14-tetramethylpentadecane), tricosane (nC_{23}) and triacontane (nC_{30}) as representative alkanes. All are biodegradable, but the n -alkanes are typically degraded preferentially to the

branched alkanes, and shorter alkanes are typically degraded preferentially to the longer ones (see Prince, 1998). This pattern is seen here in all the samples except sample 1, where there has been apparently greater loss of the largest compounds. We note that while there has been substantial depletion of heptadecane and tricosane in all the samples, significant depletion of triacontane was only seen in samples 1, 5 and 6. Nevertheless, there was substantial degradation of the branched compound, pristane, in all but samples 1 and 8.

Fig. 4 shows the percentage loss of phenanthrene, a three-ring aromatic compound, and its alkylated congeners. The identification of 4-methyl groups, for example, includes tetramethyl-, ethyl-dimethyl-, propyl-methyl-, and butyl-forms. The unsubstituted parent is typically biodegraded most readily, and increasing alkylation slows biodegradation. In all of our samples, the percent losses decreased with the degree of alkylation (Fig. 4) indicating biodegradation or evaporation as a major removal mechanism for these compounds. Very similar depletion patterns were seen for dibenzothiophene and its alkylated forms (data not shown). This result is consistent with findings reported in samples from marine environments (Douglas et al., 1996), which showed that

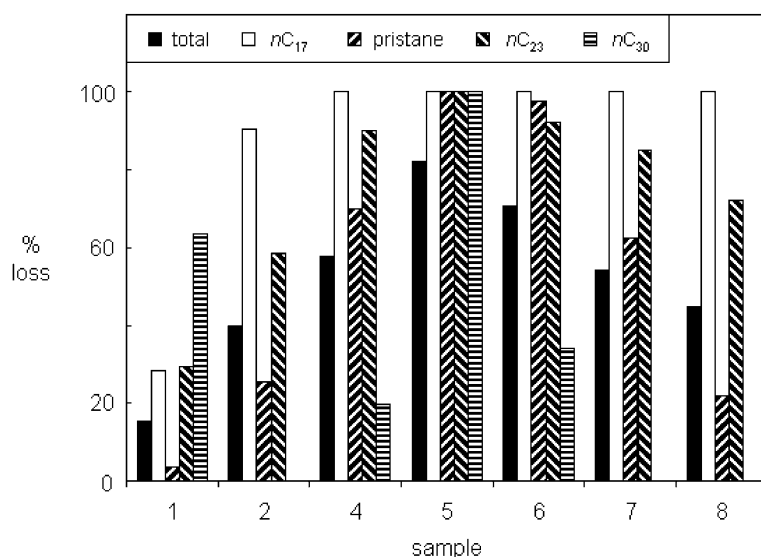


Fig. 3. Percentage loss of total saturates (measured as the ion with $m/z=57$) and representative alkanes; heptadecane (nC_{17}), pristane (2,6,10,14-tetramethylpentadecane), tricosane (nC_{23}) and triacontane (nC_{30}). Sample numbers are described in Table 1 and Fig. 1.

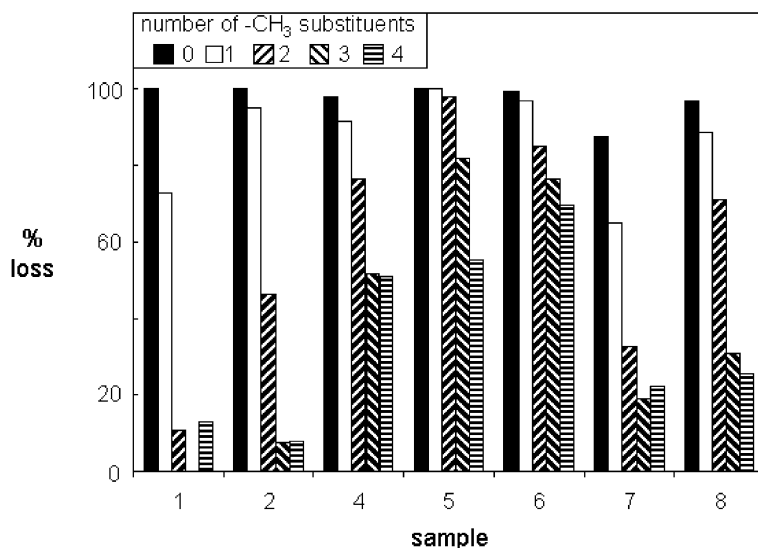


Fig. 4. Percentage loss of phenanthrene and its alkylated congeners. Sample numbers are described in Table 1 and Fig. 1.

biodegradation of these two families of compounds is so similar that ratios of, for example, trimethylphenanthrenes to trimethyldibenzothiophenes remain diagnostic fingerprints even in heavily degraded samples. We also examined naphthalene and its alkylated homologs, but these had been essentially completely lost from all the field samples (least loss was 96% of

the trimethyl naphthalenes from sample 8) in the 25 years since the spill took place.

We also examined the percentage loss of chrysene, a four-ring aromatic compound, and its alkylated congeners (Fig. 5). Losses were less than seen for the phenanthrenes, particularly for samples 1, 2, 7 and 8 that had lost only a little of these com-

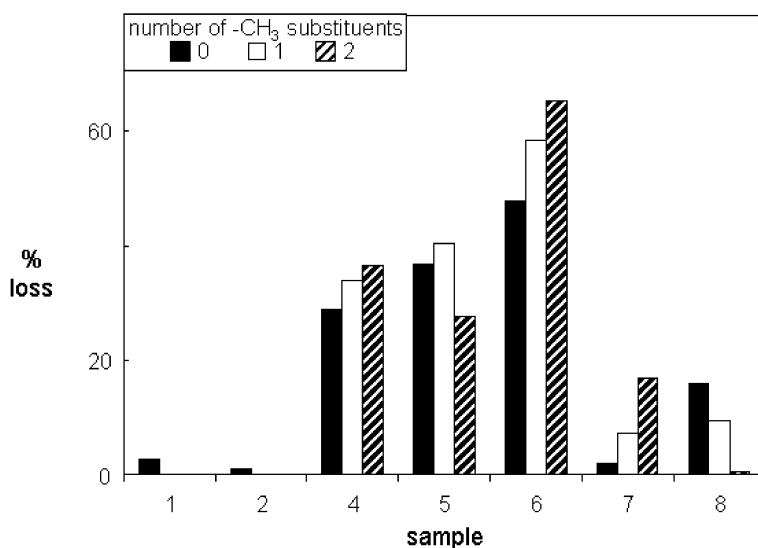


Fig. 5. Percentage loss of chrysene and its alkylated congeners. Sample numbers described in Table 1 and Fig. 1.

pounds. Nevertheless, there seem to be two patterns of loss in the samples. The pattern exhibited by sample 8 (subsurface soil) is akin to that seen for the phenanthrenes, with the parent being the most depleted. This is the pattern expected for biodegradation (Prince, 1998). In contrast, samples 4, 6 and 7 (all collected from the surface) show a reverse pattern, with the greatest loss of the more alkylated forms. This is the pattern seen for photooxidation (Garrett et al., 1998). Sample 5 (surface sample) shows substantial losses, but with no clear pattern. We interpret this lack of pattern to indicate contributions of both biodegradation and photooxidation to the observed losses.

5. Discussion and conclusions

After 25 years of “exposure to the elements”, some of the samples (4–8) collected from the CPCRW site have lost more than 50% of their initial hydrocarbon. Some of this loss must have been due to evaporation (Fingas, 1995), although this has not been a very rapid process at our site where the oil has become incorporated into the subsurface (Collins et al., 1994). Sample 1, a composite of subsurface mineral horizon soils (Table 1) still contains about 17% of its initial undecane, a relatively volatile compound.

Sample 5, a surface sample, is the most degraded sample we collected; it has lost 82% of the total hydrocarbons present in the initially spilled oil, and 86% of the saturates (measured as the m/z ion = 57). It has lost (Fig. 3) essentially all of its initial heptadecane, phytane, tricosane and triacontane. It has also lost (Fig. 4) essentially all of its initial phenanthrene and dibenzothiophene, and even the trimethyl forms are more than 80% depleted. We attribute the majority of these losses to biodegradation, although we note that photooxidation may have played a role in the loss of the alkylated phenanthrenes. As shown in Fig. 5, there has also been substantial loss of chrysene and its alkylated forms up to at least dimethyl that we attribute to both biodegradation and photooxidation. Even in this most degraded sample, however, we saw no evidence for the loss of the terpane and sterane biomarkers, even though loss of these compounds has

been seen (although not for C_{29} and C_{30} hopanes) in extensively degraded samples collected from some marine environments (Wang et al., 1995). No degradation of terpanes and steranes was seen after 25 years at the Nipisi spill site, near the Lesser Slave Lakes in northern Alberta, either (Wang et al., 1998).

The other samples were less degraded than sample 5 (Table 1), and sample 1, a composite of oiled mineral horizon soils is almost unchanged since the spill. It has lost all of its phenanthrene and most of the methyl phenanthrenes, but very little of its alkanes with the exception of triacontane. We have no good explanation for this unexpected loss pattern, although it may perhaps be due to a chromatographic separation of the waxiest components as the oil slowly percolated to the subsurface. In light of the minimal loss of the most biodegradable alkanes, the loss of phenanthrenes in this sample would be consistent with evaporation. However, this mineral soil sample came from a generally water saturated environment not exposed to a gas phase. Thus, the exact mechanisms leading to loss of phenanthrenes and/or inhibition of alkane degradation in this sample are not clear. Sample 2 is a composite collected from the organic horizon of the oiled plot, and its gas chromatogram is reminiscent of the most degraded sample collected from this plot in 1991 (Collins et al., 1994). We note that this sample has lost more than 25% of its pristane (Fig. 3), so estimates of biodegradation using pristane as a conserved internal marker (Collins et al., 1994, Table 1) are underestimates of the true extent of biodegradation.

Biodegradation seems to have been a significant weathering process in all the samples except number 1, demonstrated by the loss of alkanes; evaporation, water washing and photooxidation do not lead to this pattern. This conclusion is supported by the microbial data collected. Culturable populations of total heterotrophs and crude oil emulsifiers remain elevated in the oiled soils relative to soils from an adjacent unoiled plot. Furthermore, hexadecane mineralization activity has been clearly demonstrated in the composite samples, which still contain this compound. Taken together, these data provide evidence of a microbial population that has remained acclimated to the degradation of petroleum hydrocarbons. We found a relatively low phenanthrene-mineralizing

activity in the composite samples, but we note that these samples have lost essentially all of their initial complement of this compound, and its methyl-forms, and so this may reflect the fact that phenanthrene-degraders are absent due to the depletion of available substrate.

Since oil biodegradation has been an important contributor to the observed weathering, it is appropriate to consider what might be limiting the process, particularly in sample 1. While cold soil conditions probably inhibit biodegradation for 8 to 9 months per year, this has not prevented substantial biodegradation in the other samples. This is in line with recent work that suggests that low temperatures (0–7 °C) can still accommodate oil biodegradation by indigenous organisms (Gibb et al., 2001; Eriksson et al., 2001). When the area is thawed it is likely that oxygen diffusion is impeded by the high moisture content and subsequent lack of air exchange in these soils.

It is also likely that inorganic nutrients, perhaps biologically available nitrogen and phosphate, are at sub-optimal levels to support microbial growth. In the black spruce forests of interior Alaska, low soil temperatures, low substrate quality, and relatively high C to N ratios generally explain the reduced nutrient flux of this vegetation type (Van Cleve et al., 1983). Another study at this site found that measured *in vitro* soil C mineralization potentials were not different between oiled and reference soils (Lindstrom et al., 1999). This indicates that similar amounts of labile carbon were available to decomposer populations in both soils, further evidence that microbial populations in the oiled soils are well acclimated to the added petroleum substrates. In that study, net N mineralization potentials were higher in reference soil samples, and nitrification (net) was never observed in oiled soils. Given the similar amounts of carbon substrate available to microbial communities in the oiled and reference soils, the much higher C to N ratio of the oiled soils suggests N immobilization associated with heterotrophic activity would be higher in the oiled than in reference soils. The low nutrient turnover and decomposition rates that result in the generally low productivity of interior Alaska black spruce forests may also be limiting crude oil biodegradation at the site.

These observations suggest that a bioremediation strategy of carefully providing oxygen and/or nutrients might stimulate biodegradation (Eriksson et al., 2001; Mohn et al., 2001). We also note that it is unlikely that any significant spill from a pipeline would be left so entirely untreated as the CPRW spill. Nevertheless, exactly what clean-up technologies should be used remains an open question. The other well-studied terrestrial spill in a far-northern climate is the Nipisi, Rainbow and Old Peace River pipeline spills in the Lesser Slave Lake area of Northern Alberta (Blenkinsopp et al., 1996; Wang et al., 1998). A variety of treatments were tried on these sites, including burning, tilling and the application of fertilizer, but none seem to have been particularly effective. Blenkinsopp et al. (1996) concluded that “well-intentioned but misinformed efforts appeared to aggravate the situation and are still causing great difficulties in rehabilitation after more than 20 years”.

Photooxidation seems to have played an important role in removing larger polycyclic aromatic hydrocarbons from exposed surface samples (4–7), and not in subsurface ones (1, 8) as exemplified by the pattern of loss of the alkylated chrysenes (Garrett et al., 1998, Fig. 5).

None of the samples has yet lost all of its biodegradable and/or photooxidizable hydrocarbon, so we may anticipate that the processes that have occurred over the past 25 years will continue until these processes approach completion. Predicting a time scale, however, is problematic, since it is likely that the remaining hydrocarbons are both less biodegradable (e.g., chrysenes compared to phenanthrenes) and more deeply buried in the soil, and so less amenable to photooxidation. Despite the substantial losses of hydrocarbons from some samples, soils at the site still appear heavily oiled with dark oil. This is not unanticipated, since the color centers in oil are associated with heteroatom species that are not volatile, and so cannot be analyzed by gas chromatography (Tissot and Welte, 1984). There is no evidence for substantial biodegradation of these molecules, as exemplified by the longevity of asphalt road materials and tar mats at natural seeps, so they are unlikely to leave the site. Whether plants can recolonize the site when the majority of hydrocarbons have gone through natural weathering remains

to be seen. Interestingly, it appears that some mosses and lichens are beginning to creep across the surface to the spill zone from unoiled areas adjacent to the site, and it is possible that the oiled layers may eventually be buried by these plants, and then provide a substratum for succession of other species. Should plant growth become widespread it might also affect the relative importance of photooxidation as a removal mechanism at this site.

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References

- Atlas, R.M., 1993. Handbook of Microbiological Media CRC Press, Boca Raton, FL.
- Blenkinsopp, S., Sergy, G., Lambert, P., Wang, Z., Zoltai, S.C., Sitanen, M., 1996. Long-term recovery of peat bogs oiled by pipeline spills in Northern Alberta. Proc. 19th Arctic and Marine Oilspill Program Technical Seminar. Environment Canada, Calgary, Alberta, pp. 1335–1354.
- Braddock, J.F., Catterall, P.H., 1999. A simple method for enumerating gasoline- and diesel-degrading microorganisms. *Bio-rem. J.* 3, 81–84.
- Braddock, J.F., Ruth, M.L., Catterall, P.H., Walworth, J.L., McCarthy, K.A., 1997. Enhancement and inhibition of microbial activity in hydrocarbon-contaminated arctic soils: implications for nutrient-amended bioremediation. *Environ. Sci. Technol.* 31, 2078–2084.
- Brown, E.J., Braddock, J.F., 1990. Sheen Screen: a miniaturized Most Probable Number technique for oil-degrading microorganisms. *Appl. Environ. Microbiol.* 56, 3895–3896.
- Brown, E.J., Resnick, S.M., Rebstock, C., Luong, H.V., Lindstrom, J., 1991. UAF radiorespirometric protocol for assessing hydrocarbon mineralization potential in environmental samples. *Bio-degradation* 2, 121–127.
- Collins, C.M., Racine, C.H., Walsh, M.E., 1994. The physical, chemical and biological effects of crude oil spills after 15 years on a black spruce forest, interior Alaska. *Arctic* 47, 164–175.
- Douglas, G.S., McCarthy, K.J., Dahlen, D.T., Seavey, J.A., Steinhauer, W.G., Prince, R.C., Elmendorf, D.L., 1992. The use of hydrocarbon analyses for environmental assessment and remediation. *J. Soil Contam.* 1, 197–216.
- Douglas, G.S., Bence, A.E., Prince, R.C., McMillen, S.J., Butler, E.L., 1996. Environmental stability of selected petroleum hydrocarbon source and weathering ratios. *Environ. Sci. Technol.* 30, 2332–2339.
- Eriksson, M., Ka, J.A., Mohn, W.W., 2001. Effects of low temperature and freeze–thaw cycles on hydrocarbon biodegradation in Arctic tundra soil. *Appl. Environ. Microbiol.* 67, 5107–5112.
- Fingas, M.F., 1995. A literature review of the physics and predictive modeling of oil spill evaporation. *J. Hazard. Mater.* 42, 157–175.
- Garrett, R.M., Pickering, I.J., Haith, C.E., Prince, R.C., 1998. Photooxidation of crude oils. *Environ. Sci. Technol.* 32, 3719–3723.
- Gibb, A., Chu, A., Wong, R.C.K., Goodman, R.H., 2001. Bioremediation kinetics of crude oil at 5 degrees C. *J. Environ. Eng. ASCE* 127, 818–824.
- International Union for the Conservation of Nature (IUCN), 1993. Oil and gas exploration and production in Arctic and Subarctic Onshore Regions. IUCN Gland, Switzerland and Cambridge, UK, 1993 with E&P Forum, London, UK, vol. viii. 56 pp.
- Jenkins, T.F., Johnson, L.A., Collins, C.M., McFadden, T.T., 1978. The physical, chemical and biological effects of crude oil spills on black spruce forest, interior Alaska. *Arctic* 31, 305–323.
- Johnson, L.A., Sparrow, E.B., Jenkins, T.F., Collins, C.M., Davenport, C.V., McFadden, T.T., 1980. The Fate and Effects of Crude Oil Spilled on Subarctic Permafrost Terrain in Interior Alaska. CRREL Report 80-29. U.S. Cold Regions Research and Engineering Laboratory, Hanover, New Hampshire.
- Keith, L.H., Telliard, W.A., 1979. Priority pollutants: I. A perspective view. *Environ. Sci. Technol.* 13, 416–423.
- Lindstrom, J.E., Barry, R.P., Braddock, J.F., 1999. Long-term effects on microbial communities after a subarctic oil spill. *Soil Biol. Biochem.* 31, 1677–1689.
- Mohn, W.W., Radziminiski, C.Z., Fortin, M.C., Reimer, K.J., 2001. On site bioremediation of hydrocarbon-contaminated Arctic tundra soils in inoculated biopiles. *Appl. Microbiol. Biotechnol.* 57, 242–247.
- Peters, K.E., Moldowan, J.M., 1993. The Biomarker Guide: Interpreting Molecular Fossils in Petroleum and Ancient Sediments. Prentice-Hall, Englewood Cliffs, NJ.
- Prince, R.C., 1998. Crude oil biodegradation. *The Encyclopedia of Environmental Analysis and Remediation*, vol. 2. Wiley, New York, pp. 1327–1342.
- Prince, R.C., Elmendorf, D.L., Lute, J.R., Hsu, C.S., Haith, C.E., Senius, J.D., Dechert, G.J., Douglas, G.S., Butler, E.L., 1994. 17 α (H),21 β (H)-hopane as a conserved internal marker for estimating the biodegradation of crude oil. *Environ. Sci. Technol.* 28, 142–145.
- Prince, R.C., Drake, E.N., Madden, P.C., Douglas, G.S., 1997. Biodegradation of polycyclic aromatic hydrocarbons in a histori-

- cally contaminated site. In: Alleman, B.C., Leeson, A. (Eds.), *In Situ and On-Site Bioremediation*, vol. 2. Battelle Press, Columbus, OH, pp. 205–210.
- Rivkin, E.M., Friedmann, E.I., McKay, C.P., Gilichinsky, D.A., 2000. Metabolic activity of permafrost bacteria below the freezing point. *Appl. Environ. Microbiol.* 66, 3230–3233.
- Sexstone, A., Everett, K., Jenkins, T., Atlas, R.M., 1978. Fate of crude and refined oils in North Slope soils. *Arctic* 31, 339–347.
- Sparrow, S.D., Sparrow, E.B., 1988. Microbial biomass and activity in a subarctic soil ten years after crude oil spills. *J. Environ. Qual.* 17, 304–309.
- Sparrow, E.B., Davenport, C.V., Gordon, R.C., 1978. Response of microorganisms to hot crude oil spills on a subarctic taiga soil. *Arctic* 31, 324–338.
- Tissot, B.P., Welte, D.H., 1984. *Petroleum Formation and Occurrence*. Springer, Berlin.
- Van Cleve, K., Dyrness, C.T., Vierick, L.A., Fox, J., Chapin III, F.S., Oechel, W., 1983. Taiga ecosystems in interior Alaska. *BioScience* 33, 39–44.
- Wang, Z., Fingas, M., Sergy, G., 1995. Chemical characterization of crude oil residues from an Arctic beach by GC/MS and GC/FID. *Environ. Sci. Technol.* 29, 2622–2631.
- Wang, Z., Fingas, M., Blenkinsopp, S., Sergy, G., Landriault, M., Sigouin, L., Lambert, P., 1998. Study of the 25-year-old Nipisi Oil spill: persistence of oil residues and comparisons between surface and subsurface sediments. *Environ. Sci. Technol.* 32, 2222–2232.