ARCTIC
VOL. 31, NO. 3 (SEPT. 1978), P. 180-191.

Hydrocarbons and Microbial Activities In Sediment Of An Arctic Lake One Year After Contamination With Leaded Gasoline

AMIKAM HOROWITZ, ALAN SEXSTONE and RONALD M. ATLAS

ABSTRACT. Hydrocarbons were found to persist in the sediment of an Arctic lake one year after the lake was accidentally contaminated with leaded gasoline. The contaminating gasoline was continuing to spread from the original site of contamination. High numbers of hydrocarbon utilizing microorganisms were found in the contaminated sediment. Rates of nitrogen fixation did not appear to be affected by hydrocarbon contamination, but potential denitrification activities appeared to be altered by the gasoline. Fertilizer application resulted in a moderate decrease of hydrocarbon concentrations in the sediment.

RÉSUMÉ. On a constaté que des hydrocarbures persistaient dans les sédiments d'un lac arctique, un an après que le lac était pollué accidentellement par de l'essence au plomb (tétraéthyle). De l'essence polluante continuait à se répandre à partir du site originel de pollution. Dans les sédiments contaminés, on trouvait des micro-organismes utilisant toutes sortes d'hydrocarbure. La contamination par les hydrocarbures ne paraissait pas affecter les taux de fixation de l'azote mais l'essence semblait altérer les activités potentielles de dénitrification. A un épendage d'engrais, résultait une décroissance modérée de la concentration d'hydrocarbures dans le sédiment.

Traduit par Alain de Vendigies, Aquitaine Co. of Canada Ltd.

INTRODUCTION

Refined oil spillages, although often of lesser magnitude as the one described here, frequently occur around developing areas in the Arctic and are a serious source of hydrocarbon contamination that has not received sufficient scientific attention. In August, 1976, an accidental gasoline spillage of an estimated 55,000 gallons of leaded MOGAS was detected near a freshwater lake at the Naval Arctic Research Laboratory (NARL) located at Barrow, Alaska. The source of the spillage was a break in a pipeline buried in a gravel pad at the permafrost level. The spilled gasoline moved through the gravel pad over the permafrost and entered the nearby freshwater lake. We previously reported on the immediate effects of the leaded gasoline on the bacterial community and the ability of the microorganisms to degrade the gasoline (Horowitz and Atlas, 1977). The present study extends the previous work by examining the movement and persistence of the gasoline and the effects on microorganisms in the gravel sediments of the lake one year after spillage. A field assessment of fertilizer stimulated biodegradation of gasoline was also made.

Department of Biology, University of Louisville, Louisville, Kentucky 40208

Movement of gasoline was determined by measuring hydrocarbon concentrations in sediment along a transect from the site of contamination to a site beyond where any hydrocarbons had been found during the previous summer. Biodegradation of gasoline was assessed by measuring the hydrocarbon biodegradation potentials of the indigenous microbial populations and by chemically analysing the residual hydrocarbons. The effects of gasoline on the indigenous microbial community was examined by determining levels of selected microbial populations. Since microbiological nitrogen cycling is an essential process in maintaining ecologic balance and supporting hydrocarbon degradation in Arctic ecosystems we measured potential rates of nitrogen fixation and denitrification in sediment.

MATERIALS AND METHODS

Sample Sites

Figure 1 shows the sampling region. Samples were collected from an area of heavy gasoline contamination (shallow pools, sites A-D) and along the northwest shore of the lake. Gasoline had been detected only as far as site F during the previous summer when the spillage occurred. Four areas (sites A, B, K, L) were selected for studying the effects of fertilizer application on the disappearance of gasoline hydrocarbons and on microbial activity. Plastic was placed around these sites to the depth of the permafrost to minimize horizontal movement. Sites A and K were treated with a commercial fertilizer

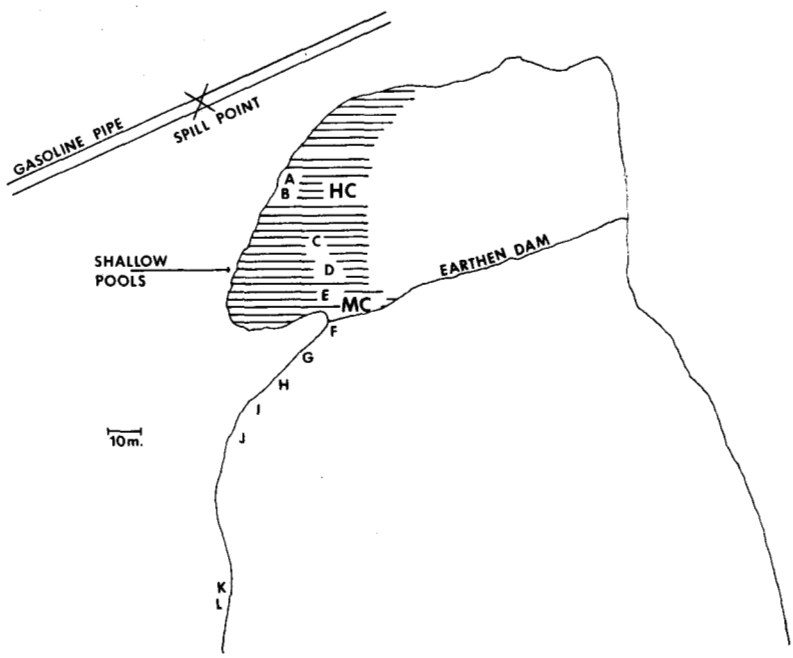

FIG. 1. Sampling sites

(American Green 10-6-4, Free Flow Fertilizers Co., Maumee, Ohio) at an application rate of 38 g/m^2. Sediment gravel samples were collected weekly for eight weeks starting on June 10, 1977. Samples were collected in sterile glass containers and immediately returned to the laboratory for processing.

Hydrocarbon Analysis

Portions of the sediment samples were transferred to sterile whirlpak bags, poisoned with 3% w/v KCN, and frozen for later analysis. One hundred gram samples (dry weight basis) were thawed and extracted with 250 ml diethyl ether using a Soxhlet extractor with an extraction time of 2 hours. Samples were concentrated (10 or 100 fold) at 25 °C under a dry hydrocarbon-free air stream to a fixed volume but were not brought to dryness. Samples were analysed by gas liquid chromatography using a Hewlett Packard model 5830 reporting gas chromatograph operated with dual 2 m x 0.3 cm stainless steel columns packed with 3% OV 1 on 80/100 Supelcoport, nitrogen carrier flow = 22 ml/min and temperature = 80 °C isothermal (2 min), program to 250 °C (8 °C/min), 250 °C isothermal (20 min). Duplicate samples were analysed in each case. Area response was corrected using the solvent peak as internal standard to adjust for size variations of automatic injections. Total integrated area units were converted to weight units using a standard curve derived from gas chromatographic area response to known amounts of gasoline.

Enumeration of Microorganisms

Viable heterotrophs were enumerated by plating samples on a medium designated TGA (0.75% trypticase peptone, 0.25% phytone peptone, 0.25% NaCl, 0.1% unleaded gasoline, 1.5% agar). Gasoline hydrocarbon utilizing microorganisms were enumerated on medium BA-G (Bushnell Haas agar exposed to volatile gasoline hydrocarbons; Horowitz and Atlas 1977). Gasoline utilizing and tolerant microorganisms were enumerated on medium GA (Bushnell Haas agar with 0.5% emulsified leaded MOGAS; Horowitz and Atlas 1977). Incubation was at 15 °C for one week. Presumptive heterotrophic denitrifiers were enumerated on Difco nitrate agar incubated at 15 °C for one week under an atmosphere of helium. All plate counts were performed in duplicate.

Hydrocarbon Biodegradation Potential Activity

Hydrocarbon biodegradation potential activities were measured using unleaded gasoline spiked with either 1-^{14}C-toluene (sp. act. 0.013 μCi/ml), 1-^{14}C-n-decane (sp. act. 0.01 μCi/ml) or 1-^{14}C-napthalene (sp. act. 0.013 μCi/ml). Sediment samples (10 ml of a 1:10 dilution) were placed in 60 ml serum bottles containing 10 ml sterile nutrient solution (10 mM NH_4NO_3, 0.5 mM Na_2HPO_4). The bottles were sealed with rubber serum stoppers and 50 μl of gasoline injected into each vial. Fifty μl 1-^{14}C sodium acetate (sp. act. 10 μCi/ml) was injected into separate vials. Acetate was used because it is an intermediate metabolite in hydrocarbon and lipid metabolism. Replicate samples were incubated in the dark at 15 °C for 48 hours. Incubation was

terminated by addition of buffered formaldehyde, pH 7, final concentration 10%. $^{14}CO_2$ production was quantitated by liquid scintillation counting. Samples were acidified with 0.5 ml concentrated H_2SO_4 to release $^{14}CO_2$. Chloroform (1 ml) was added to each sample to remove labelled hydrocarbons from the atmosphere which could give false positive counts especially with volatile hydrocarbons. $^{14}CO_2$ was recovered by purging the serum bottles with air and trapping the CO_2 in 1 ml hyamine hydroxide (Atlas and Hubbard 1974). Both the trapped $^{14}CO_2$ and the chloroform extracted material were counted using Omnifluor (New England Nuclear) and a Beckman model LS 100 counter. Counts of $^{14}CO_2$ were corrected for background by subtracting counts obtained from the poisoned controls. Counts of $^{14}CO_2$ hydrocarbons that were utilized were determined by subtracting the counts in the chloroform extracts of active samples from the counts in the chloroform extracts of the poisoned controls. $^{14}CO_2$ production (mineralization) and hydrocarbon disappearance (utilization) are reported as percentages of available substrates.

Denitrification and Nitrogen Fixation Potential Activities

Denitrification in the gravel sediments was examined using the acetylene blockage of N_2O reduction technique (Balderson *et al.* 1976; Yoshinari *et al.* 1976). Nitrogen fixation was estimated by the acetylene reduction method (Hardy *et al.* 1973). A 5 ml portion of a 1:10 diluted sediment sample was added to a 20 ml glass vial containing 5 ml of either H_2O, 0.05% KNO_3, 0.25% glucose, 0.05% KNO_3 plus 0.25% glucose, or 0.05% KNO_3 plus 0.25% Bacto peptone plus 0.15% Bacto beef extract. Vials were sealed with rubber serum stoppers and flushed with helium. Vials were injected with C_2H_2 generated from CaC_2 (Alpha Lux Company) to give a final concentration of 0.02 atm. Vials were incubated in the dark at 15 °C for one week. Gas samples were removed from the sample vials with 5 ml Vacutainer evacuated glass tubes (Becton Dickinson) for later analysis. For gas analysis, 250 μl of gas from the vacutainer sub-samples were injected with a gas tight syringe (Glenco Scientific) into a Hewlett Packard 5830 reporting gas chromatograph equipped with both a thermal conductivity detector for N_2O and N_2 analysis, and a dual flame ionization detector for C_2H_2 and C_2H_4 analysis. Separation of gases was achieved on a stainless steel column (6 m x 0.3 cm) packed with 50/80 mesh Poropak Q (Waters Associates, Inc.). Operation was at 30 °C with a carrier gas flow of 35 ml He/min. Integrated area response units were converted to μl units by comparison with the area response of known concentrations of standard gases.

Statistical Analysis

Analyses of variance were performed using the SPSS ANOVA procedure (Nie *et al.* 1975). The Duncan's multiple range test option with an alpha value of 0.05 was used in these analyses. Wherever the term "significant differences" is used in this paper it refers to differences shown in these tests to be significant at the 0.05 probability level.

RESULTS

Hydrocarbons in Sediment Samples

Gasoline hydrocarbons were found at all sampling sites. Hydrocarbon concentrations were not significantly different during the sampling period at sites C-L (Table 1). Hydrocarbon concentrations were significantly higher at site B than other sites. Gas chromatographic analysis showed the presence of compounds that co-chromatographed with the following standard compounds: *n*-nonane, *n*-decane, *m*-xylene, naphthalene and 1-methyl-naphthalene. Concentrations of hydrocarbons increased during the sampling period at sites K and L (Table 2). There was apparent movement of gasoline into this previously uncontaminated area during this study. The application of fertilizer did not affect the concentration of hydrocarbons in this newly contaminated area. Fertilizer application did result in significantly lower concentrations of hydrocarbons at site A compared to the unfertilized reference site B. Hydrocarbon concentrations were significantly lower after the fourth sampling time at sites A and B. During the sixth to ninth sampling time period hydrocarbon concentrations were significantly lower at site A with fertilizers than at reference site B. Sites A and B had been contaminated with higher concentrations of gasoline for a much longer period of time than sites K and L, allowing for adaptation of microbial populations.

Microbial Populations

The mean colony forming units showed that microbial populations were higher at the highly contaminated sites A and B than at sites D, H and J (Table 3). Populations were not significantly different in the fertilizer treated site A and the reference site B. The ratios of gasoline utilizing and MOGAS tolerant organisms (enumerated on media GA and BA-G) to viable heterotrophs (enumerated on medium TGA) indicated that hydrocarbon degrading bacterial populations had developed at sites A-J in response to the presence of gasoline hydrocarbons (Table 4). All ratios were greater than 0.3. Ratios for uncontaminated regions of this lake have been found previously to be less than 0.002 (Horowitz and Atlas 1977). The presumptive counts of denitrifiers showed no differences between any sites. The mean probable number of denitrifiers was 3×10^6 CFU/g dry wt sediment.

Biodegradation Potential Activity Measurements

The hydrocarbon biodegradation potential activity measurements showed that only small amounts of the labelled hydrocarbons were completely mineralized to $^{14}CO_2$ (Table 5). No significant utilization of toluene could be measured. Decane and naphthalene utilization was greater at sites A and B than at sites D, H and J. Fertilizer application to site A did not have any significant effect on the hydrocarbon biodegradation potentials. Acetate was more readily mineralized than any of the hydrocarbon substrates.

TABLE 1. Mean hydrocarbon concentrations in sediment samples (μg hydrocarbon/g dry wt. sediment — ppm) during the summer, 1 year after detection of the gasoline spillage

Site	Mean	Standard Error
B	76.2	15.6
C	8.6	2.7
D	8.6	2.7
E	2.7	0.2
F	3.5	0.6
G	4.3	0.5
H	3.1	0.2
I	4.3	0.6
J	2.7	0.6
L	2.2	1.0

TABLE 2. Hydrocarbon concentrations in fertilizer treated and reference sediment samples (μg hydrocarbon/g dry wt sediment — ppm)

	SITE			
Sampling Time*	A	B	K	L
1	160	167	—	—
2	246	84	—	—
3	49	138	—	—
4	287	176	—	—
5	15	11	—	—
6	1	55	1	0
7	2	14	4	3
8	2	12	3	2
9	3	29	18	14

*Fertilizer application was at sampling time 1. Sampling times are at 1 week intervals.

(— = not determined.)

TABLE 3. Mean colony forming units in sediment samples during sampling period (CFU/g dry wt)

	Medium		
Site	TGA	GA	BA-G
A	$3.2x10^6$	$4.6x10^5$	$1.4x10^6$
B	$2.5x10^6$	$8.4x10^5$	$2.6x10^6$
D	$9.0x10^4$	$3.0x10^4$	$4.9x10^4$
H	$9.1x10^4$	$8.2x10^3$	$2.8x10^4$
J	$2.9x10^4$	$5.1x10^3$	$1.5x10^4$

TGA = Trypticase soy agar + 0.1% unleaded gasoline.
GA = Bushnell Haas agar + 0.5% leaded gasoline.
BA-G = Bushnell Haas agar incubated with gasoline hydrocarbon vapors.

TABLE 4. Mean ratios of colony forming units

Station	GA/TGA	BA-G/TGA
A	0.49	0.64
B	0.52	0.74
D	0.65	1.47
H	0.32	0.64
J	0.37	0.48

TGA = Trypticase soy agar + 0.1% unleaded gasoline.
GA = Bushnell Haas agar + 0.5% leaded gasoline.
BA-G = Bushnell Haas agar incubated with gasoline hydrocarbon vapors.

TABLE 5. Mean biodegradation potential activities

Site	% Utilized	% Mineralized
	n-decane	
A	45	1.3
B	40	2.4
D	14	0.5
H	4	0.4
J	3	0.3

TABLE 5. (Cont'd)

Site	% Utilized	% Mineralized
	toluene	
A	0.5	1.5
B	0.5	3.5
D	0.0	0.5
H	0.0	1.5
J	0.0	6.0
	naphthalene	
A	30	2.2
B	37	2.1
D	11	0.4
H	9	0.3
J	5	0.3
	acetate	
A	—	10
B	—	10
D	—	9
H	—	9
J	—	8

Denitrification Activity

The production of N_2O was not detectable unless NO_3- was added to the sediment, i.e., endogenous rates of denitrification were below detection levels. Nitrate concentrations could have been low in sediment even following fertilizer application. The evolution of N_2O was detectable at sites A and B at the sixth and eighth sampling times when NO_3- was added to the sediment (Fig. 2A). When glucose was added as a carbon source together with NO_3-, N_2O evolution was greater than when only NO_3- was added (Fig. 2B). With added glucose N_2O accumulation was higher at sites A and K which had been fertilized than the reference sites B and L. When peptones were added, instead of glucose as a carbon source, rates of N_2O accumulation were also higher than with NO_3- alone (Fig. 2C). With added peptones rates of denitrification were similar at sites A and B and at sites K and L, but different between the A/B location and the K/L location, i.e., with added peptones differences between heavily contaminated and recently contaminated

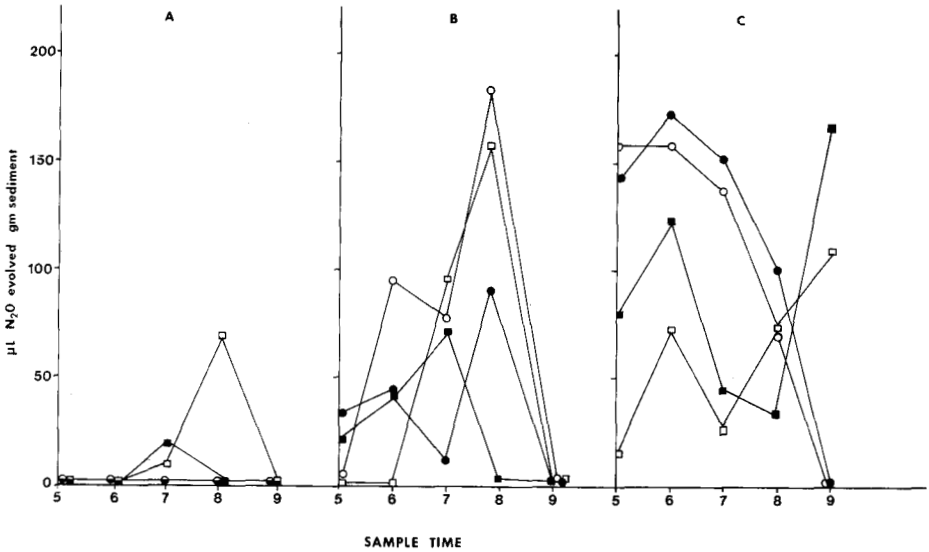

FIG. 2. Denitrification potential activities.
A. NO_3- added
B. NO_3- + glucose added
C. NO_3- + peptone added
□ Site A
■ Site B
○ Site K
● Site L

sediment were apparent but not differences between fertilized and unfertilized sites. Similar maximum rates of N_2O evolution were obtained for glucose and peptone amended sediment. Significant N_2 production was not detected in any case.

Nitrogen Fixation Activity

Rates of nitrogen fixation measured by the acetylene reduction method showed maximal rates during the seventh and eighth sampling times (Table 6). Before and after this period acetylene reduction was not detected in unamended sediment. Addition of NO_3- blocked acetylene reduction. Acetylene reduction was detectable in additional early season samples when glucose was added to the sediment samples. There were no apparent site specific or fertilizer induced differences between the sampling sites.

DISCUSSION

The gasoline contaminated lake serves as the drinking water supply for the Naval Arctic Research Laboratory. Our results indicate that continued use of this lake as a water supply may present a human health hazard. Gasoline

TABLE 6. Nitrogen fixation — C_2H_2 accumulation (μl C_2H_4/g sediment)

Sampling Time	Station: Unsupplemented A	B	K	L	With Glucose A	B	K	L
5	0.0	0.0	0.0	0.0	1.3	8.3	0.0	0.0
6	0.0	0.0	0.0	1.9	1.9	1.0	10.2	0.9
7	4.9	5.9	0.0	4.2	16.8	7.7	3.2	4.0
8	6.4	3.0	8.5	4.9	1.3	0.0	2.4	7.2
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

hydrocarbons have spread from the initial point of contamination and persist in the sediment of the lake. Hydrocarbon analyses of the water intake have occassionally shown the presence of excessive concentrations of hydrocarbons (G. Laursen, personal communication). An activated charcoal filter has been installed to remove hydrocarbons from the drinking water.

The spread of hydrocarbons was directly measureable by gas chromatographic analyses and indirectly by monitoring microbial populations in the sediment. Sites K and L were contaminated starting only one year after the original spillage. These sites were originally selected as uncontaminated controls. However, gasoline spread into this area during the study period. We do not know whether sediment in any part of the lake remains uncontaminated. We have not established yet whether transport of gasoline across the lake is directly through the sediment or whether gasoline in low concentrations moves across the water surface and is concentrated in the shoreline sediment by adsorption.

The presence of gasoline resulted in changes in microbial populations and metabolic activities. Numbers of hydrocarbon utilizing microorganisms were high in sediment one year after spillage. Ratios of hydrocarbon utilizers to viable heterotrophs showed the dominance of hydrocarbon utilizers in the gasoline contaminated sediment. The relative occurrence of hydrocarbon utilizers in the microbial community can be used to monitor the presence of hydrocarbons in the environment.

Productivity of many Arctic ecosystems are nitrogen limited. Alterations in microbial cycling of nitrogen can greatly change the ecologic balance. Nitrogen fixation did not appear to be affected by the presence of gasoline hydrocarbons or fertilizers. The assay system used, however, probably did not measure nitrogen fixation by cyanobacteria. It is difficult to determine whether gasoline or fertilizers altered the rates of denitrification. Denitrification could only be estimated as potential activities when NO_3- and carbon sources were added. Denitrification activity, measured with NO_3- alone added, could be interpreted as showing that prolonged exposure to hydrocarbons results in increased rates of denitrification activity. Results with

NO_3- plus peptone may indicate that as leaded gasoline migrated into sediment at sites K and L, it was toxic to a segment of the microbial community resulting in the observed sharp decreases in denitrification potential activities measured in samples collected after gasoline was detected. Organisms at sites A and B may have adapted to the presence of gasoline. Results in the presence of glucose could be interpreted as showing that fertilizer application enhances denitrification potential activity. It is likely that the presence of nitrogen in peptones but not in glucose is the reason that different results were obtained with the two carbon sources. Measurement of denitrification potential activities in the presence of NO_3- and added carbon is a valid model of what occurs in a localized natural environmental niche when allochthonous or autochthonous material enters the sediment. Factors that influence environmental conditions, such as 0_2 tension, can greatly alter rates of nitrogen cycling. Hydrocarbon contamination can result in greatly reduced oxygen tension. Our interpretation of the measured denitrification activities is that both gasoline contamination and fertilizer application altered potential rates of denitrification under different conditions.

The hydrocarbon biodegradation potential activity measurements showed differences in degradability of several hydrocarbons found in gasoline. Toluene resisted biodegradation whereas decane and naphthalene were degraded, although not extensively converted to CO_2. Sites with the greatest exposure to gasoline showed the greatest hydrocarbon biodegradation activities. The biodegradation potential activities did not show any effect of fertilizer aplication. The biodegradation potential measurements were performed in the presence of added nutrients, so that nitrogen and phosphorous were not limiting during the assay period.

Fertilizer application did appear to increase *in situ* degradation of gasoline hydrocarbons. Five weeks after fertilizer application hydrocarbon concentrations in the fertilizer treated area were 10% of the concentrations in the reference site. Further degradation may have been limited by oxygen concentrations. Hydrocarbon concentrations decreased during the summer at both sites A and B. Horizontal movement was restricted at these sites, but vertical movement was not restricted. Fertilizer application and physical collection from the heavily contaminated region should decrease further movement of gasoline across the lake toward the drinking water inlet. The hydrocarbon biodegradation potentials, which measure relative potential rates of hydrocarbon degradation, failed to predict the differences in extent of hydrocarbon degradation between fertilized and reference sites. Extent of degradation may have been controlled by abiotic factors which were not modelled in the hydrocarbon biodegradation potential measurements.

The problem of persistence from refined oil spillages in the Arctic remains. Toxic light aromatic hydrocarbons such as toluene and naphthalene do not evaporate when sorbed onto sediment particles. Even light fuels which would rapidly evaporate in temperate regions can persist for long periods of time in Arctic ecosystems. Microbial degradation of hydrocarbons is an active process in Arctic ecosystems and can be stimulated by nutrient addition.

However, rates of biodegradation and number of months during the year when biodegradation occurs results in only slow removal of contaminated hydrocarbons from ecosystems such as the one studied.

ACKNOWLEDGEMENTS

This work was supported by the Office of Naval Research. We wish to thank Mrs. Anne Bronner for her technical assistance.

REFERENCES

ATLAS, R. M., and J. S. HUBBARD. 1974. Applicability of radioactive tracer methods of measuring $^{14}CO_2$ assimilation for determining microbial activity in soil. *Microbial Ecology. 1:* 145-163.

BALDERSTON, W. L., B. SHERR, and W. J. PAYNE. 1976. Blockage by acetylene of nitrous oxide reduction in *Pseudomonas perfectomarinus*. *Applied Environmental Microbiology. 31:* 504-508.

HARDY, R. W. F., R. C. BURNS, and R. D. HOLSTEN. 1973. Application of the acetylene-ethylene assay for measurement of nitrogen fixation. *Soil Biology and Biochemistry. 5:* 47-81.

HOROWITZ, A., and R. M. ATLAS. 1977. Response of microorganisms to an accidental gasoline spillage in an Arctic freshwater ecosystem. *Applied Environmental Microbiology. 33:* 1252-1258.

NIE, N. H., C. H. HULL, J. G. JENKINS, K. STEINBRENNER, and D. H. BENT. 1975. *Statistical Package for the Social Sciences*. McGraw Hill, New York 675 p.

YOSHINARI, T., R. HYNES, and R. KNOWLES. 1977. Acetylene inhibition of nitrous oxide reduction and measurements of denitrification and nitrogen fixation in soil. *Soil Biology and Biochemistry. 9:* 177-183.