Proceedings of the Indiana Academy of Science
(1992) Volume 101 p. 75-82

MICROBIAL ACTIVITY IN SOIL-HYDROCARBON MIXTURES AMENDED WITH CHROMIUM

John R. Pichtel and Dale A. Scheidler
Department of Natural Resources
Ball State University
Muncie, Indiana 47306

ABSTRACT: Landfarming of hazardous petroleum wastes has, until recently, been a common disposal practice in the United States. In order to effectively reclaim a decommissioned petroleum waste landfarm, a stable soil is needed. Little is known regarding the influence of metals within oily wastes on soil microbial activity and soil recovery. A Glynwood soil (fine, illitic, mesic Aquic Hapludalf) was treated with 1% (w/w) petroleum or hexadecane in combination with five rates of Cr(III) or Cr(VI) and incubated for 140 days in the laboratory. Soil microbial activity was assessed via respiration and enumeration of microbial populations. Microbial respiration was significantly ($p < 0.05$) increased by addition of the hydrocarbons but partially inhibited by either species of Cr. Numbers of soil fungi increased with the hydrocarbon additions, but bacterial numbers decreased. Fungi were more sensitive to Cr additions than were bacteria. By the completion of the incubation, most of the added Cr(VI) had been reduced to Cr(III). Oxidation of Cr(III) to Cr(VI) was not detected. The results of this study indicate that newly-closed petroleum land treatment facilities may experience impaired organic matter turnover and delayed soil recovery as a result of Cr-induced microbial inhibition.

INTRODUCTION

Soil incorporation (landfarming) of hazardous petroleum industry wastes has been discontinued in the United States as a result of the implementation of the Resource Conservation and Recovery Act and The Toxic Substances Control Act. Until recently, a variety of refinery wastes had been land-applied, including crude and distillate tank cleanings, filter clays from jet oil, and biological sludge from wastewater treatment (Earth Systems, 1985; Hornick, *et al.,* 1983).

A common landfarm closure technique involves conversion of the treated site to permanent vegetative cover. In order to maintain vegetation, however, a stable soil is necessary. An understanding of the interaction of refinery waste components with indigenous microbes capable of degrading the waste constituents and cycling nutrients is needed to assess soil stabilization in such extreme environments. Certain petroleum waste constituents can, if accumulated, alter normal soil chemical and biological reactions (Mott, *et al.,* 1990).

Refinery wastes, in addition to containing a suite of potentially hazardous organics (Speight, 1980; Atlas, 1975), contain variable quantities of chromium (Cr). If landfarming is used for long periods for the disposal of such wastes, Cr will accumulate in the surface soil. Concentrations of Cr may be sufficiently high to inhibit the native soil microbial populations which are critical to the maintenance of a stable soil.

Information is lacking on the specific short-term effects of Cr on microbial activity in soils which have experienced heavy loading of petroleum refinery wastes. It is important to determine whether an accumulation of Cr will be a serious obstacle to soil stabilization, following petroleum landfarm closure. The purpose of this study was to investigate the influence of various combinations of hydrocarbons as well as Cr(III) and Cr(VI) on the activity and numbers of heterotrophic microbes in soil.

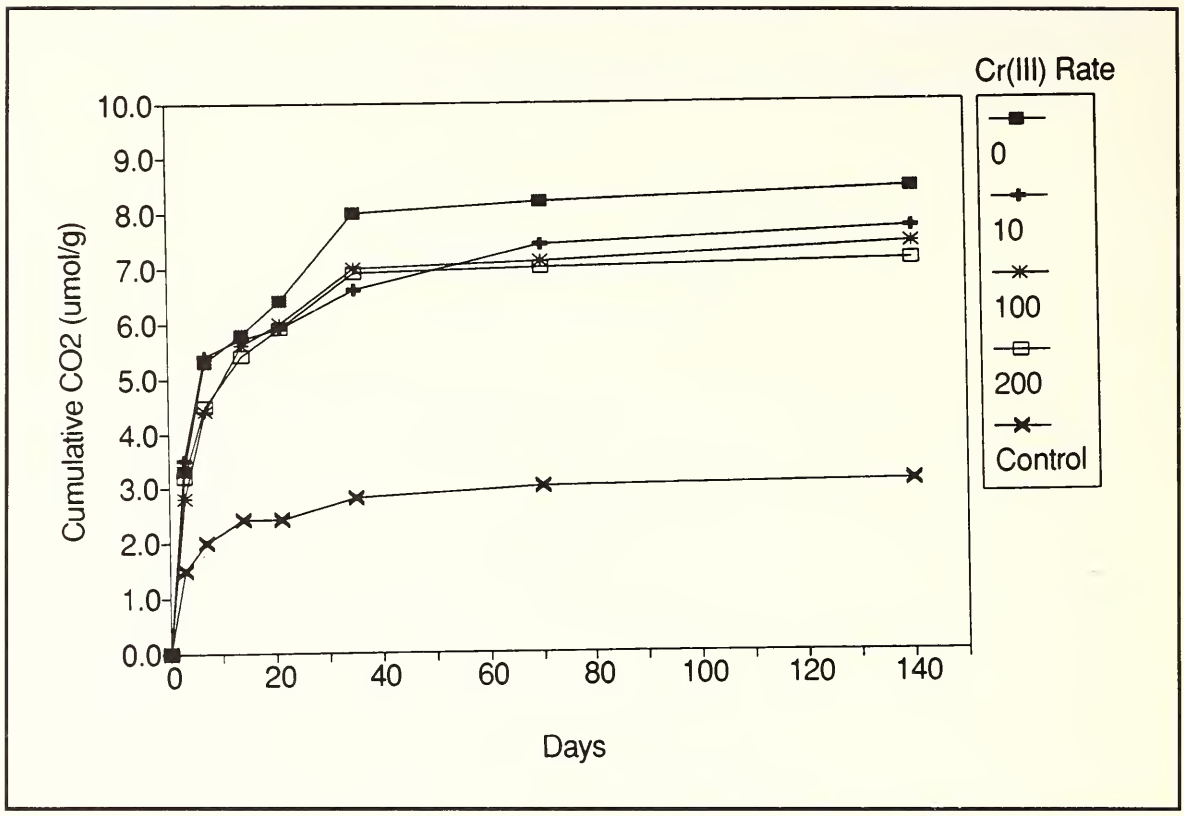

Figure 1. Carbon dioxide evolution from the Glynwood soil amended with petroleum and five rates of Cr(III).

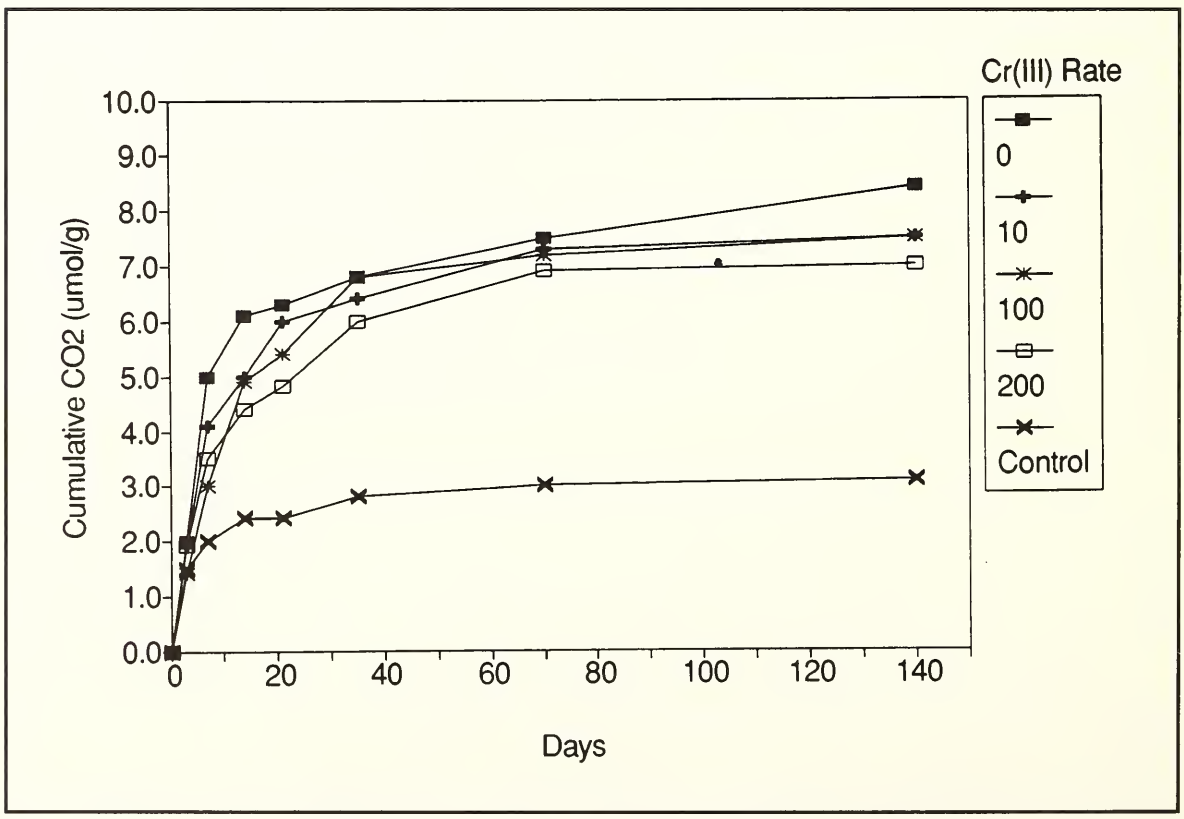

Figure 2. Carbon dioxide evolution from the Glynwood soil amended with hexadecane and five rates of Cr(III).

MATERIALS AND METHODS

Sample collection and preparation. Glynwood silt loam (fine, illitic, mesic Aquic Hapludalf; pH 6.2, 0.85% organic carbon) was collected from the upper 25 cm of a field cropped to a corn (*Zea mays* L.)-soybean (*Glycine max* (L.) Merr.) rotation near New Albany, Indiana. Soil samples were composited, air-dried, and sieved through a 2-mm mesh sieve.

Incubations and measurements. Crude petroleum (0.2% N, pH 5.5) (Country Mart Cooperative, Inc., Mt. Vernon, IN) and a pure hydrocarbon, n-hexadecane ($CH_3(CH_2)_{14}CH_3$), were applied to the soil in the laboratory on a 1% weight basis as hydrocarbon test substances. Petroleum characteristically contains a broad array of aliphatics, aromatics, and alicyclics (Speight, 1980), and the hexadecane has been employed in other studies (Jensen, 1977) to simulate the aliphatic petroleum fraction. The hydrocarbons were added dropwise evenly over the soil surface. Chromium was added either as a $CrCl_3$ or $K_2Cr_2O_7$ solution leading to final concentrations of 0, 10, 100, and 200 µg Cr/g soil (dry weight basis). All treatments were run in triplicate.

Microbial activity within the hydrocarbon- and Cr-treated soil samples was assessed via soil microbial respiration and enumeration of microbial populations. Respiration was measured within airtight soil microcosms (Gillet and Witt, 1978). To 0.95-liter (1 qt.) mason jars, distilled water (or Cr solution) was added to triplicate 200 g samples to bring the mixtures to 0.03 MPa field capacity as measured by a soil tensiometer. All microcosms were incubated at room temperature (22 ± 2° C) and opened at weekly intervals to allow for exchange of gases.

The CO_2 produced was trapped in a vial containing 10 mL of 1M NaOH that was removed from each microcosm at Day 3 and Weeks 1, 2, 3, 5, 10, and 20. A solution of 3M $BaCl_2$ was added to the vials, and the CO_2 trapped in the alkali was determined by titration with 1M HCl (Stotzky, 1965).

At 20 weeks, microbial numbers were estimated using a spread plate procedure (Koch, 1981). Soil samples (1 g) were removed from each microcosm, diluted in distilled H_2O, and plated in triplicate. Standard plate count agar (Difco, Detroit, MI) was used for total bacterial counts and malt extract agar (Difco) for total fungi. Plates were incubated in the dark at room temperature for 6 days.

After 20 weeks, free and adsorbed Cr(VI) was measured in the Cr(III)- and Cr(VI)-treated microcosms using the s-diphenyl carbazide method (Bartlett and James, 1979). In the method, a soil sample is extracted with 0.06*M* KH_2PO_4, and the Cr(VI) in the extract is reacted with s-diphenyl carbazide reagent to form a magenta color.

Respiration and microbial population data were subjected to one-way analysis of variance and a t-test for independent means, respectively. All statistical tests were conducted using SPSSX through a VAX 11/785 operation system (SPSS, 1986).

RESULTS AND DISCUSSION

Carbon dioxide evolution. Microbial respiration patterns in all hydrocarbon-Cr mixtures were similar, regardless of whether the soil was treated with petroleum or hexadecane (Figs. 1-4). Respiration was maximal up through Day 7, due to the initial wetting of the soil and subsequent reactivation of microbial activities, and increased only gradually thereafter. Respiration also leveled off due to depletion of easily degradable substrate (e.g., short-chain linear alkanes). The microflora then utilized organic matter

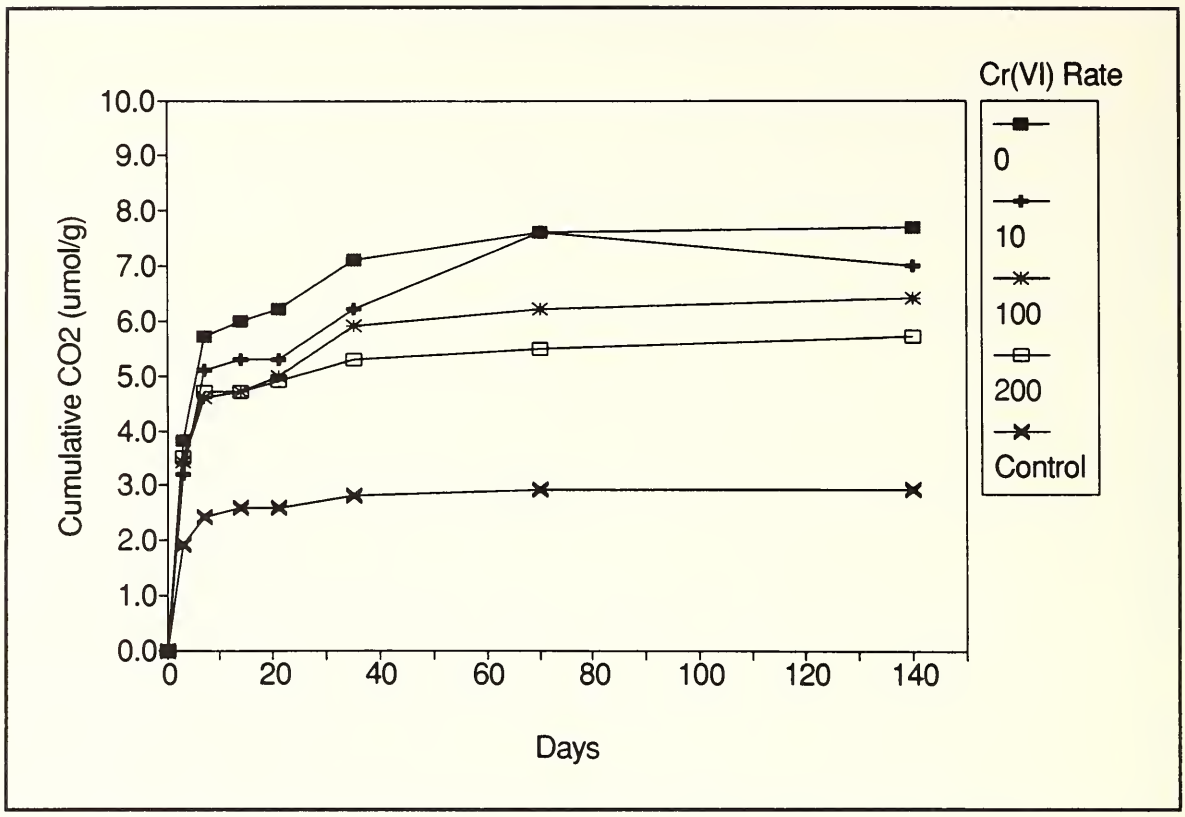

Figure 3. Carbon dioxide evolution from the Glynwood soil amended with petroleum and five rates of Cr(VI).

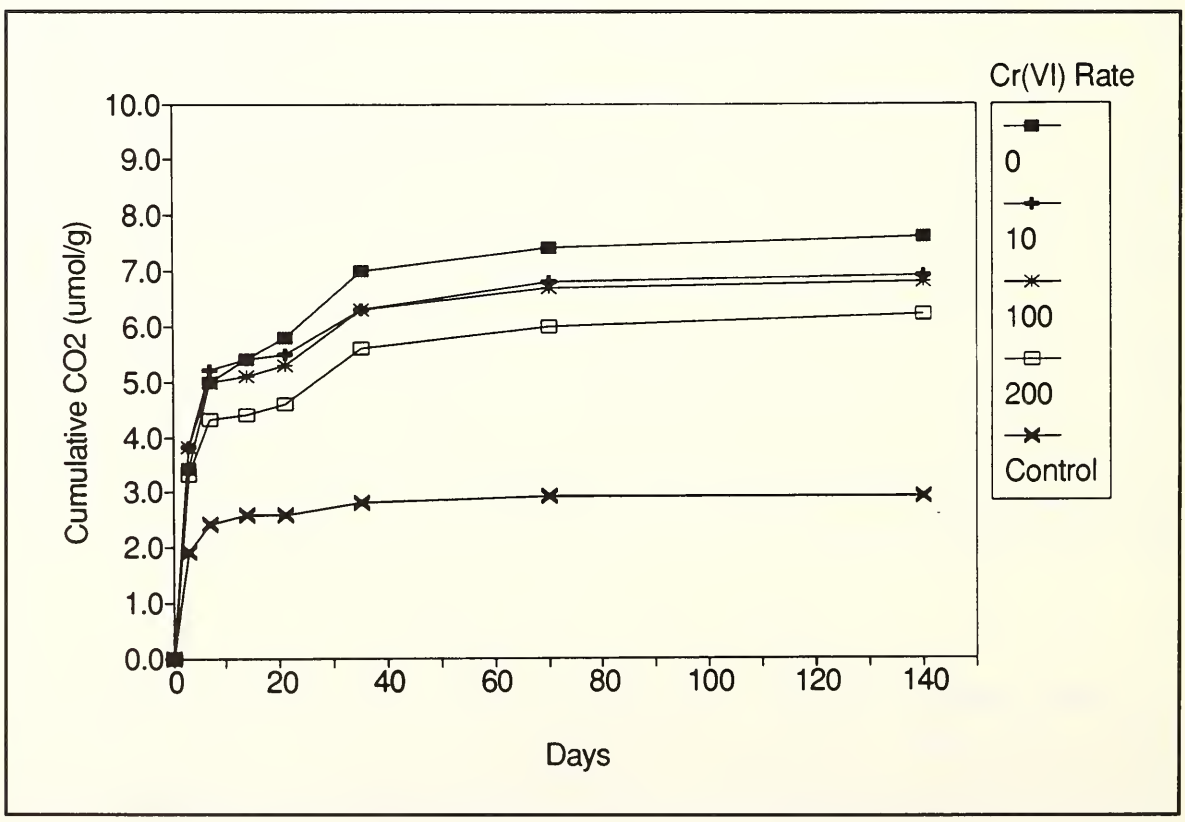

Figure 4. Carbon dioxide evolution from the Glynwood soil amended with hexadecane and five rates of Cr(VI).

Table 1. Microbial numbers in the Glynwood soil as affected by hydrocarbon and Cr(III) treatment.

Hydrocarbon	Cr(III) Rate (μg/g)	Total Bacteria (10^5/g soil)	Total Fungi (10^5/g soil)
Petroleum	0	7.4 ± 4.7	38.0 ± 3.7
	10	7.0 ± 0.45	5.3 ± 1.7
	100	5.3 ± 0.050	6.9 ± 2.1
	200	5.9 ± 0.11	0.4 ± 0.10
Hexadecane	0	1.2 ± 0.04	2.2 ± 0.50
	10	0.080 ± 0.01	1.8 ± 0.90
	100	0.050 ± 0.01	0.20 ± 0.05
	200	0.040 ± 0.01	0.40 ± 0.08
Control	-	21.7 ± 1.1	3.3 ± 0.60

more resistant to decomposition.

Respiration in all hydrocarbon treatments, regardless of the Cr species or application rate, was significantly ($p < 0.05$) above the control at all sampling dates after Day 3. By Day 140, respiration in the hexadecane/Cr and petroleum/Cr treatments (zero Cr rate) both averaged 2.7-times that of the control.

Both Cr(VI) and Cr(III) were inhibitory to soil microflora (Figs. 1-4). Cumulative respiration decreased with increased rates of Cr, whether as trivalent or hexavalent. The lowest respiration observed in the hydrocarbon treatments was at 200 μg Cr(VI)/g soil: 6.2 and 5.7 μmol CO_2/g in the hexadecane and petroleum treatments, respectively.

Microbial respiration was greater in the Cr(III)- compared to the Cr(VI)-treated soil (Figs. 1-4), although the differences were not significant. Hexavalent Cr was found to be more toxic to microbes incubated in a soil extract than was trivalent Cr (Ross, *et al.*, 1981).

Microbial populations. Microbial numbers were affected by both hydrocarbon type and Cr application rate (Table 1). Bacterial numbers were 66% lower in the petroleum treatment, zero Cr rate, compared to the soil-only control. In contrast, the fungal numbers were 91% higher compared to the control. Soil fungi are known to be more efficient than bacteria in oily waste decomposition, and a wide array of fungi are known to metabolize hydrocarbons, including crude oil (Perry and Cerniglia, 1973; Llanos and Kjoller, 1976; Hornick, *et al.*, 1983).

With increased Cr application rate, the numbers of bacteria and fungi decreased (Table 1). Populations of bacteria and fungi were significantly different in both hydrocarbon treatments. Fungi were more sensitive to Cr than were the bacteria. An 86% decrease in the number of fungal colonies occurred in the petroleum-treated soil with application of 10 μg Cr/g, while bacterial numbers were essentially unchanged. At the 200 μg Cr/g rate, fungi and bacteria decreased by 99% and 20%, respectively.

Chromium transformations. At the completion of the incubation, most of the

Table 2. Cr(VI) remaining in microcosms at the completion of incubation.

Hydrocarbon	Cr(VI) added	Cr(VI) remaining	Cr(VI) reduced
	(——— µg/g dry soil ———)		(% of added)
Petroleum	0	0.60	-
	10	0.39	97
	100	35.0	65
	200	48.0	76
Hexadecane	0	0.40	-
	10	1.0	90
	100	43.0	57
	200	60.0	70
Control	-	0.90	-

added Cr(VI) was not recovered (Table 2). The Cr(VI) not recovered had either been reduced to an insoluble form of Cr(III) (e.g., $Cr(OH)_3$) or had been precipitated or tightly adsorbed by the soil as anionic Cr(VI) (e.g., $HCrO_4^-$, CrO_4^{-2}) (James and Bartlett, 1983; Rai, *et al.,* 1989). Chromium(VI) is strongly oxidizing, as shown by its stability only under high redox potentials (Rai, *et al.,* 1989). In aerobic soils, easily oxidized organic compounds act as reducing agents for Cr(VI). The hexadecane and various components of the petroleum may have served as reducing agents.

Chromium(III) in the microcosms may also have been oxidized. Chromium(III) oxidation to Cr(VI) is plausible in aerobic soils, the main oxidants being the higher valent Mn oxides (Rai, *et al.,* 1989). Total Mn in the petroleum-treated soil, however, measured only 0.2 µg/g, which is insignificant in terms of oxidizing Cr(III) (Bartlett and James, 1979). Nascent Cr(VI) was not detected in the Cr(III)-treated soils.

Chromium(VI) is considered the species most toxic to biota. However, microbial respiration (Figs. 3-4) did not increase as the Cr(VI) disappeared. Possible reasons include: 1) the Cr(VI), although present for only a short time, may have permanently injured some component of the soil microbiota; 2) Cr(III) may have formed cross-linkages between organic compounds in the soil, rendering them unavailable to microbial utilization; or 3) the presence of Cr(III) resulted in inhibited respiration (Figs. 1-2), because an unstudied member of the soil microbial population (e.g., the actinomycetes) may have been sensitive to Cr(III) (Ross, *et al.,* 1981).

The toxicity of Cr(VI) to biota is established, whereas Cr(III) has only recently been considered a hazard. The current study demonstrates that both Cr(III) and Cr(VI) additions are inhibitory to soil microbiota. Additional study is needed to assess the mechanism of the toxicity of both Cr species in soils.

ACKNOWLEDGMENTS

Financial support from Ball State University and the Indiana Academy of Science is gratefully acknowledged.

LITERATURE CITED

Atlas, R.M. 1975. Effects of temperature and crude oil composition on petroleum biodegradation. Appl. Microbiol. 30: 396-403.

Bartlett, R. and B. James. 1979. Behavior of chromium in soils: III. Oxidation. J. Environ. Qual. 8: 31-35.

Earth Systems. 1985. Field and laboratory evaluation of petroleum land treatment system closure. Earth Systems Associates, Marietta, Georgia, 88 pp.

Gillet, J.W. and J.M. Witt. 1978. Terrestrial microcosms. NAS/RA 790034, National Science Foundation, Washington, D.C., 35 pp.

Hornick, S.B., R.H. Fisher, and P.A. Paolini. 1983. Petroleum wastes. *In:* J.F. Parr, P.B. Marsh, and J.M. Kla (Eds.), *Land Treatment of Hazardous Wastes,* pp. 321-337, Noyes Data Corp., Park Ridge, New Jersey, 425 pp.

James, B.R. and R.J. Bartlett. 1983. Behavior of chromium in soils: VII. Adsorption and reduction of hexavalent forms. J. Environ. Qual. 12: 177-181.

Jensen, V. 1977. Effects of lead on biodegradation of hydrocarbons in soil. Oikos. 28: 220-224.

Koch, A.L. 1981. Growth measurement. *In:* P. Gerhardt (Ed.), *Manual of Methods for General Bacteriology,* pp. 179-207, Amer. Soc. Microbiol., Washington, D.C., 524 pp.

Llanos, C. and A. Kjoller. 1976. Changes in the flora of soil fungi following oil waste application. Oikos. 27: 377-382.

Mott, S.C., P.H. Groenevelt, and R.P. Voroney. 1990. Biodegradation of a gas oil applied to aggregates of different sizes. J. Environ. Qual. 19: 257-260.

Perry, J.J. and C.E. Cerniglia. 1973. Studies on the degradation of petroleum by filamentous fungi. *In:* D.G. Ahearn and S.P. Meyers (Eds.), *The Microbial Degradation of Oil Pollutants,* pp. 89-94, Baton Rouge, Louisiana, 322 pp.

Rai, D., L.E. Eary, and J.M. Zachara. 1989. Environmental chemistry of chromium. Sci. Tot. Environ. 86: 15-23.

Ross, D.S., R.E. Sjogren, and R.J. Bartlett. 1981. Behavior of chromium in soils: IV. Toxicity to microorganisms. J. Environ. Qual. 10: 145-148.

Speight, J.G. 1980. The chemistry and technology of petroleum. Marcel Dekker, New York, 498 pp.

SPSS. 1986. SPSSx user's guide, second edition. McGraw Hill, New York, 988 pp.

Stotzky, G. 1965. Soil respiration. *In:* C.A. Black (Ed.), *Methods of Soil Analysis, Part 2,* pp. 1550-1572, Amer. Soc. Agronomy, Madison, Wisconsin, 1572 pp.