INFLUENCE OF THREE ORGANIC SOLVENTS ON SOIL MICROBIAL ACTIVITY

by

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ABSTRACT

In recent years there has been much concern over the migration of organic pollutants to groundwater. Many organic solvents undergo biodegradation in the soil, but little information is available on the amount of protection soil can provide to groundwater. In this study, the growth response of soil microorganisms was measured when exposed to various concentrations of methyl isobutyl ketone (MIBK), tetrahydrofuran (THF), and xylene. Three Arizona soils were used in the experiment: Canelo loam, Mohave sandy loam, and river sand.

Microbial activity was measured using two methods, carbon dioxide evolution and dilution plate counts. The plate count method was found to have a high degree of experimental error. Because of this error, definite statements could not be made about the influence of the test solvents on microbial numbers using this technique.

The CO_2 evolution experiments indicated that xylene and THF at concentrations of 0.1 and 0.5% were more readily biodegraded than MIBK at similar concentrations. At 1.0%, all three solvents were generally toxic. Overall, the organisms in the Mohave sandy loam were shown to best deal with the solvents followed by Canelo loam and then river sand. Higher soil surface area appeared to enhance the ability of the soil to biodegrade solvents.

INTRODUCTION

Large quantities of organic compounds are produced by the chemical industries. Most of these chemicals are used as solvents, degreasing agents, pesticides, and in the synthesis of other compounds.

The deliberate discharge, accidental spillage or improper disposal of such compounds results largely in their release to the soil. Many of these organic compounds are capable of migrating through soil to contaminate groundwaters at toxic levels. Pollution of high quality water has been demonstrated in numerous recent studies. In California 1,2-dibromo-3-chloropropane (DBCP) was applied regularly to soils as a nematocide from the late 1950's to the late 1970's. In 1979 this compound was detected in underlying groundwater (Peoples et al. 1980). Trichloroethane has been detected in groundwaters of both the United States and Europe, the result of landfill disposal and leaking storage tanks (Beilke, 1985; Giger, 1978).

With growing concern for groundwater quality, it has become necessary to assess the protection that soil can provide to groundwater. Biodegradation is the main process by which these compounds disappear from the environment, although photodegradation also takes place on land surfaces.

The role that microbes play in breaking down organic compounds has been the subject of numerous investigations (Brown et al. 1983; Fuller and Warrick, 1985). Microbes have been described that degrade paraffin, kerosene, gasoline, lubricating oils, and tars. Many other aliphatic and aromatic compounds are decomposed as well. Short chained aliphatics are utilized by Mycobacteria. The longer chained compounds are degraded by several microorganisms such as Mycobacterium, Acinetobacter, Nocardia, Pseudomonas, Streptomyces, Cornybacterium, the yeast Candida and Rhodotortula.

An organism is restricted to a range of molecules which it can metabolize. Some use the short chained compounds while others utilize molecules ranging from eighteen to forty-two carbons (Alexander, 1977; Zobell, 1946). Fredricks (1966) showed that the most rapid utilization of hydrocarbons was in the high molecular weight range. Growth in the low molecular weight range was much less rapid. This could be explained by the known toxicity of lower alkanes to bacteria because of their lipid solvent properties.

Studies have also demonstrated the bacterial degradation of some chlorinated hydrocarbons. For example, Bruner (1980) demonstrated the bacterial degradation of dichloromethane. Janssen et al. (1985) described an organism belonging to the genus Xanthobacter that degraded 1,2-dichloroethane into alcohols and halide ions.

Aromatic compounds including some polycyclic aromatic hydrocarbons (PAH) have been shown to biodegrade. According to McKenna and Heath (1976), and Herbes and Schwall (1978) PAH is utilized by populations of marine and soil bacteria. Species of bacteria that degrade aromatics include Pseudomonas, Mycobacterium, Acinetobacter, Arthrobacter, Bacillus and Nocardia (Alexander, 1977).

Soil microbes possess the ability to attack a wide array of organic pollutants. Because of this ability, soil appears to offer some protection to groundwater. In fact, it has been suggested that the soil is our first and foremost line of defense against pollution of groundwater (Fuller and Warrick, 1985). There is a need to establish certain criteria that will insure groundwater protection yet allow the reasonable utilization of soil as a waste receptor.

The purpose of this investigation was to study the reaction of soil microbes to three common industrial solvents: tetrahydrofuran (THF), methyl isobutyl ketone (MIBK), and xylene.

Environmental Occurrence of THF, MIBK, and Xylene

The presence of THF, MIBK, and xylene in the environment is usually the result of man's activities. Bertch, Chang and Zlatkis (1974) analyzed particulate matter from Houston community air and showed the presence of xylene. Rainwater from the Los Angeles area was found to contain trace quantities of xylene, along with three-hundred other identifiable organic compounds (Kawamura and Kaplan, 1983). Xylene has been found to be a contaminant in several groundwater aquifers of the United States (Tomson et al. 1981; Westrick, Mello and Thomas, 1984). Analyses of leachates from several landfill sites in Connecticut showed the presence of MIBK and THF. Concentrations of these substances were at levels up to three-hundred parts per billion (Sawhney and Kazloski, 1984). Potable water supplies have been contaminated with THF as a result of PVC pipe cement used to join pipe fittings (Wang and Brucher, 1979).

Xylene also occurs naturally in the environment; it has been found in some soils (Simonart and Batistic, 1966). As a component of petroleum, xylene can be introduced to the earth's surface from the natural seepage of hydrocarbons from subterranean surfaces (Perry, 1977). Studies on the combustion products of tobacco smoke have shown the presence of the three isomeric forms of xylene (Sandmeyer, 1982).

Microbiological Utilization of THF, MIBK and Xylene

Information on the microbiological degradation of THF and MIBK is limited. The only work done in this area is in wastewater treatment. MIBK levels in wastewater have been shown to be reduced using standard biological treatment, with a preceding acclimation period (Verschueren, 1977). THF contaminated wastewaters can also be treated using biological treatment (Kasper, 1984).

Xylene, on the other hand, has been studied much more. In 1906, Stormer was the first to demonstrate the microbial assimilation of aromatic hydrocarbons by isolating *Bacillus hexacarbovororum*, an organism capable of utilizing both xylene and toluene (Zobell, 1946). Bacteria known to grow with p- or m-xylene as the sole carbon source have been shown to oxidize these substrates through the corresponding toluic acids (Davis, Hossler, and Stone, 1968; Omari, Horiguchi, and Yamahada, 1967; Omari and Yamahada, 1969). *P. aeruginosa* converts p-xylene into p-methylbenzyl alcohol and possibly further to methylbenzoic acid (Nozaka and Kusumose, 1968). A Pseudomonas strain, P. pxy, can grow on m- and p-xylene and utilize them as a sole source of energy (Figure 1). A mutant P. pxy-82 can transform m-xylene to 3-methylcatechol and 3-methylsalicylic acid (Davey and Gibson, 1974). The xylene fermentation products of Nocardia cultures have been identified as 2,3-dihydroxy-p-toluic acid and 3,6-dimethylpyrocatechol (Raymond, Jamison and Hudson, 1967; Jamison, Raymond, and Hudson, 1969).

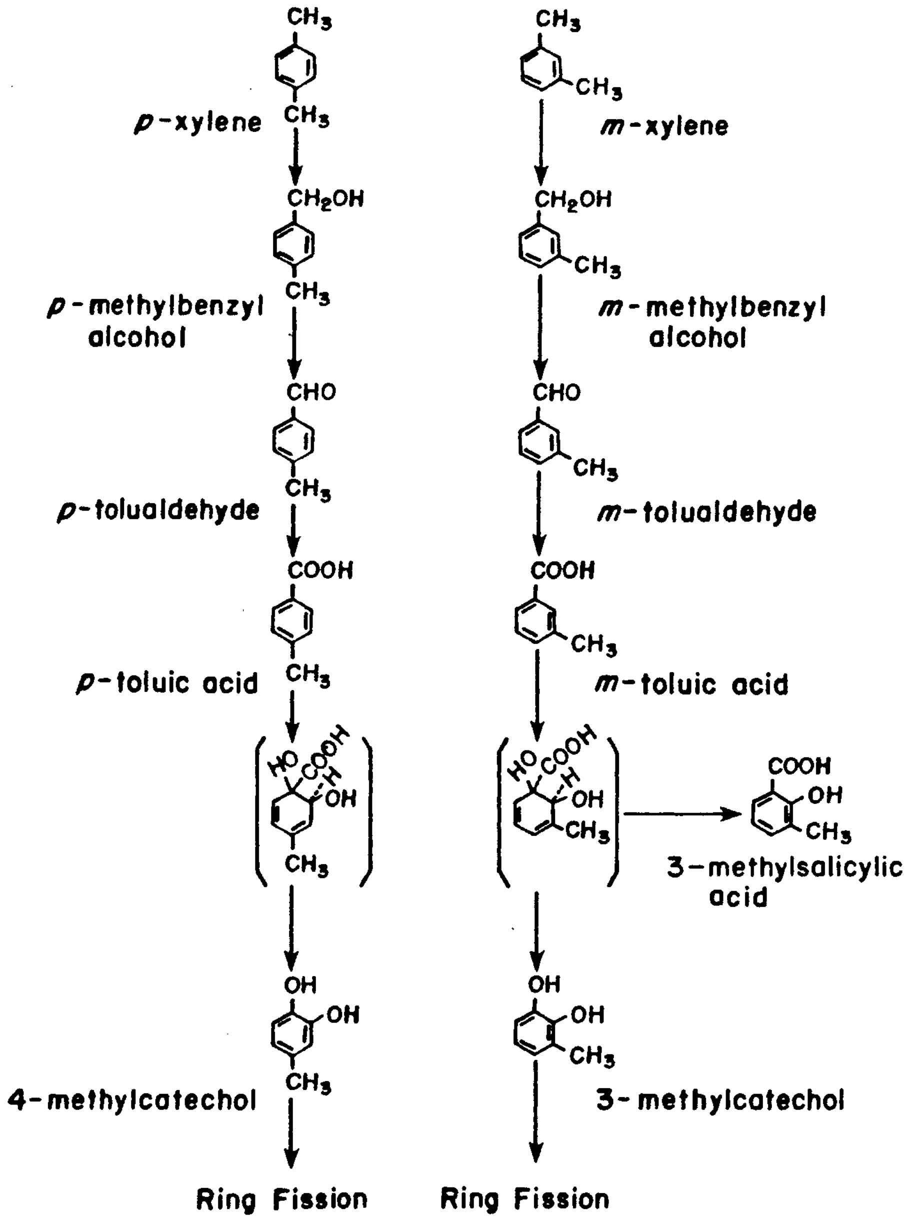

Figure 1. Proposed pathways for the oxidation of p- and m-xylene by Pseudomonas pxy (from Davey and Gibson, 1974).

OBJECTIVE

The objective of this study was to determine the effect of three industrial solvents, THF, MIBK, and xylene, on the native microbial populations of three Arizona soils. The study looked at two effects: solvent toxicity toward the microbes; and solvent utilization by the microbes.

Specific objectives were to:

1) Compare the ability of different soils to break down solvents;

2) Determine if one solvent is more readily biodegraded than another;

3) Determine the effects of solvents on bacteria, fungi, and actinomycete populations; and

4) Compare carbon dioxide evolution and plate counts as techniques for evaluating microbial activity.

MATERIALS

Solvents

The solvents used in these studies, THF, MIBK and xylene, were selected because of their wide use in industry and their diverse chemical structures. Table 1 lists some of the physical and chemical characteristics of these solvents.

MIBK is extensively employed in industry because of its ease of production, low manufacturing costs, excellent solvent properties, and desirable physical properties, such as low viscosity, moderate vapor pressure, high evaporation rates, and a wide range of miscibility with other liquids (Krasavage, O'Donoghue,and Divincenzo, 1982). This compound is widely used in the manufacture of gums, inks, resins and coatings (Hawley, 1981).

Xylene occurs in three isomeric forms, o-, m-, or p-xylenes. Xylene is found in petroleum products, coal naphthas, and as an impurity in petrochemicals. These compounds are widely used as thinners; as solvents for inks, rubber, gums, resins, adhesives, and lacquers; as fuel components; and as intermediates in the chemical industry (Sandmeyer, 1982).

THF is a popular solvent because of its solubility in both water and other organic solvents. It is used as a solvent for natural and synthetic resins, particularly vinyls, cellophane, coatings, adhesives

Table 1. Selected Properties of Solvents Used

Solvent[a]	Methyl isobutyl ketone (MIBK)	Tetrahydrofuran (THF)	Xylene
Other Names	Hexanone Hexone 4-methyl-2-pentanone	Diethyleneoxide Tetramethyleneoxide	Dimethylbenzene
Chemical Formula	$(CH_3)_2CHCH_2COCH_3$	$CH_2CH_2CH_2CH_2O$	$C_6H_4(CH_3)_2$
Molecular Weight[b]	100.2	72.10	106.15
Density g/cc	0.8042	0.888	0.86
Solubility with Water	Slightly soluble	Soluble	Insoluble
Boiling Point °C	115.8	66	137-160

[a] From Hawley 1983, unless otherwise noted.

[b] From Verschueren 1977.

and magnetic tapes. THF is also used as a chemical intermediate in Grignard reactions and polymerizations (Hawley, 1981).

The solvents used in this study were obtained from two sources. The MIBK and THF were distilled-in-glass grade purchased from Burdick and Jackson Laboratories.[a] The xylene was a reagent grade mixture of the o-, m-, and p- isomers, with the last two predominating; it was purchased from Mallinckrodt, Inc.[a]

Soils

Three soils from Arizona representing three soil orders were used in the experiment: Canelo loam (Aeric Ochraqualf), Mohave sandy loam (Typic Haplargid), and river sand (an alluvium Entisol). They were selected because they possess significant differences in physical and chemical properties. Only subsoil horizons (B and C) were taken to avoid the organic matter present in the surface layers. The soils ranged in pH from 5.4 to 7.3. The clay content ranged from 1 to 26%, and soil surface area was 3.6 - 38.3 m^2/g. Some characteristics of these soils are shown in Table 2.

Soils were prepared for testing by air drying, followed by sieving (2 mm wire mesh screen). Soils were stored at room temperature until used. All experiments were run on soil with a moisture content at 70% of field capacity. Soil samples were oven-dried at 110°C for 24 hours for moisture determinations and dry weight corrections. Soil dry weight is used for all calculations.

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[a]The use of trade names does not mean endorsement.

Table 2. Characteristics of Soils Used

Soil[a] Series	Soil Order	Clay	Silt	pH	C.E.C.	Soil Surface Area	Predominant Clay Minerals[b]
		----- % -----			meq $100g^{-1}$	m^2 g^{-1}	
Canelo loam	Alfisol	26	29	5.4	6	35.0	Mica, Kaolinite
Mohave sandy loam	Aridisol	11	37	7.3	10	38.3	Mica, Kaolinite
River Sand sand	Entisol	1	2	7.2	2	3.6	Kaolinite, Mica

[a] From Fuller et al. 1983.

[b] Listed in order of importance.

METHODS

Microbial Activity

Numerous methods can be employed to enumerate microorganisms. Some of these are dilution plate counts, carbon dioxide evolution, biochemical tests and specific enzyme assays. Biochemical tests are used to assay specific biochemicals that are indicative of microorganisms. Examples are protein, ATP, lipopolysaccharide and muramic acid. Enzyme assays are useful for measuring metabolic activities. Some enzymes commonly quantitated are dehydrogenase, phosphatase, cellulase, chitinase and nitrogenase (Atlas and Bartha, 1981; Spare and Merricks, 1981).

In this work microbial activity was measured using two techniques, dilution plate counts and carbon dioxide evolution. Dilution plate counts are a universal technique used in measuring microbial populations to give a direct quantitative measurement of the viable aerobic and facultative anaerobic organisms in the soil (Environmental Protection Agency, 1978). In this study, plate counts were done using selective media, which allowed for the enumeration of actinomycetes, bacteria, and fungi populations. A significant reduction of the selected microorganism would indicate that the test solvents were toxic. On the other hand, significant increases in level would indicate solvent utilization.

Carbon dioxide evolution has long been used to measure microbial activity of soils (Heck, 1929; Fuller and Gauiard, 1954). As microorganisms degrade organic material, oxygen is consumed and CO_2 is liberated. If the amount of CO_2 evolved is lowered in the presence of the test solvent this would indicate toxic effects. Elevated CO_2 levels would indicate biodegradation of the solvent.

Dilution Plate Counts

Plate counts were done on selective media to determine fungi, bacteria, and actinomycete populations. Table 3 lists the media used with appropriate incubation times and microorganisms selected.

Samples were prepared for plate counting by placing 200 g of air-dried soil into 400 ml beakers. Both solvent and water (enough to bring the soil up to 70% of its water holding capacity) were added together and mixed thoroughly into the soil. Table 4 lists the solvent treatments given soils. The beakers were covered with watch glasses during incubation to reduce moisture loss and incubated at room temperature for a total of 14 days.

Plate counts were made at 0, 1, 2, 5, 9, and 14 days. To do the plate counts, 10 g of soil were suspended in 90 ml of 0.1% peptone solution. A serial dilution was continued until a $1:10^6$ dilution was obtained. Aliquots (0.1 ml) of each dilution were cultured in triplicate on each of the selective media using the spread plate technique. The plates were incubated at room temperature and the colony forming units (CFU) were counted.

Table 3. Selective Media Used to Quantitate Bacteria, Fungi and Actinomycetes

Media[a]	Microorganism Selected	Incubation
		days
Thorton's Standardized Medium (Modified)	Bacteria	10
Malt Extract Agar Plus Streptomycin	Fungi	5
Actinomycete Isolation Agar	Actinomycetes	14

[a]Ingredients for all media are listed in Appendix B.

Table 4. Solvent Treatments Applied to Soils

Solvent Concentration[a]	MIBK	THF	Xylene
-- % (W/W) --	--------------- ml ---------------		
0 (control)	0	0	0
0.1	0.12	0.11	0.11
0.5	0.62	0.55	0.60
1.0	1.25	1.10	1.15

[a]Based on 100 g of soil.

Carbon Dioxide Evolution

The amount of CO_2 produced by the soil microbes was measured using a biometer flask which is illustrated in Figure 2. Both the test solvents and water (enough to moisten the soil to 70% of its water-holding capacity) were added to 50 g of soil contained in the flask and thoroughly mixed. Table 4 shows the solvent treatments used. The biometer flask was assembled and 10 ml of 0.2$\underline{N}$ NaOH was injected into the side-arm of the flask. The flask was sealed and incubated at room temperature. CO_2 measurements were made every 24 hours for a period of 21 days. CO_2 readings were made by removing the NaOH from the side-tube and placing it in a 100 ml erlenmeyer flask. To the NaOH contained in the flask 2.0 ml of 2$\underline{N}$ $BaCl_2$ was added to precipitate the carbonate. After precipitation the contents of the flask were titrated with 0.2$\underline{N}$ HCl using phenolphthalein as the indicator. The amount of CO_2 produced is calculated from the difference in alkalinity of the NaOH solution before and after incubation. All CO_2 data is cumulative.

To avoid contamination with CO_2 all stock solutions were prepared from CO_2-free water and stored in bottles fitted with CO_2 absorbing filters and luer lock needles.

Statistical Analyses

Data were analyzed using the Statistical Analysis System (SAS) computer program (SAS Institute, 1982). The CO_2 and plate count data were analyzed using a 3x3x3 analysis of variance (ANOVA) factorial

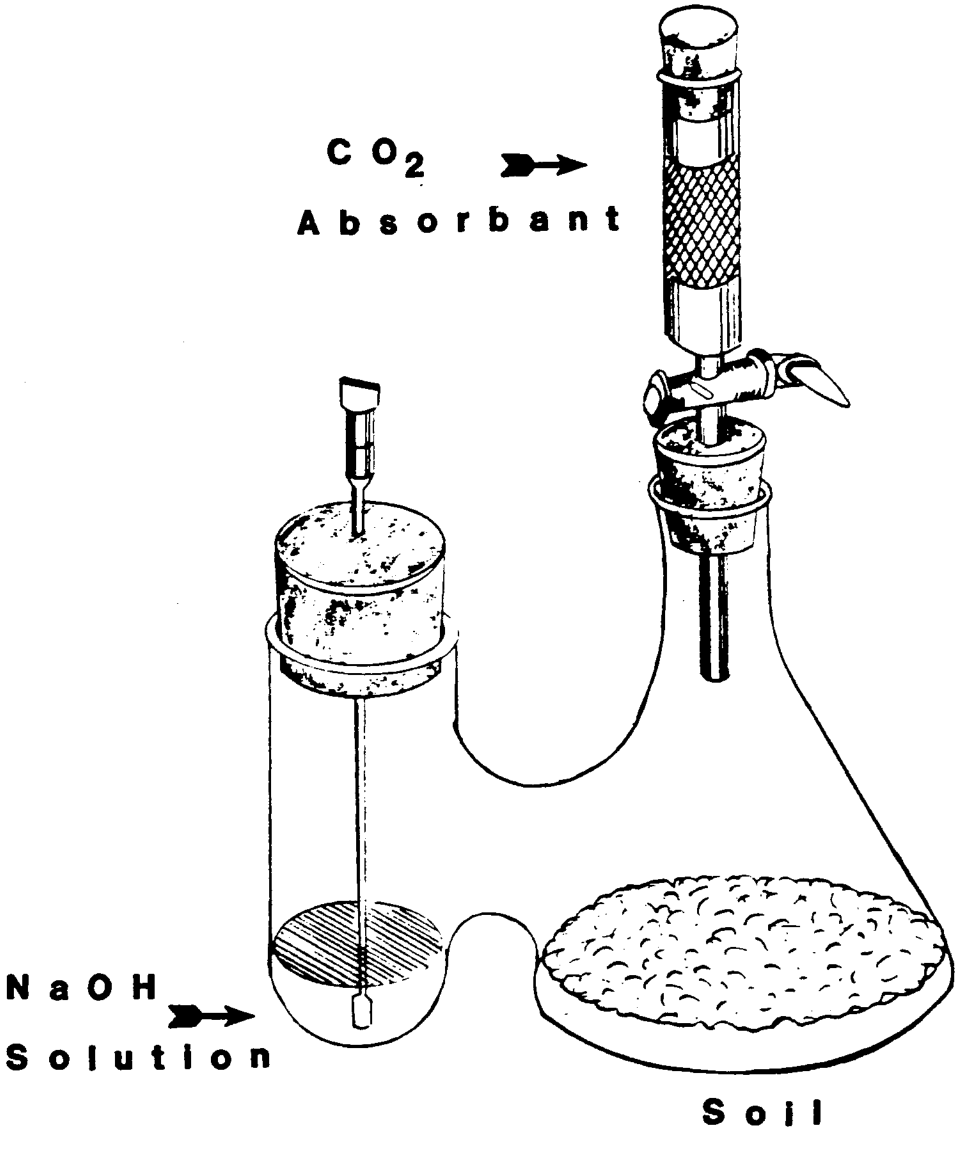

Figure 2. Biometer flask for monitoring the production of CO_2 by soil (from Bartha and Pramer, 1965).

design with comparisons of means using the Duncan's Multiple Range Test (DMRT). The DMRT test was used to determine three things: if significant reductions or increases occurred in CO_2 production or microbial growth; if one solvent was more biodegradable than another; and if one soil type was better at treating the solvents.

DMRT results for the CO_2 experiments are in table form where a "+" would indicate a significant increase over the control, "-" would indicate a significant decrease, while a "0" would mean no significant difference from the control value.

Because of the large volume of data generated in these experiments not all of it were analyzed. For the plate count experiments microbial levels were tested at days 5 and 14. The CO_2 data was analyzed at days 5, 14, and 21.

All ANOVA and DMRT results are at the 0.05 level of significance. Significance at the 0.05 level indicates 95% certainty that the treatment was responsible for the observed difference between two values.

RESULTS AND DISCUSSION

Plate Count Experiments

The data collected from the plate count experiments are presented in Tables 12-20 (Appendix B). Results are graphically presented in Figures 3-5.

Analysis of variance results which are summarized in Table 5 reveal that soil was the only significant (.05) factor affecting actinomycete, bacteria, and fungi populations at five days. However, at 14 days soil type, solvent and concentration were found to be significant factors influencing microbial populations (Table 6).

A subsequent Duncan range test for multiple comparisons was applied to the between-treatment means. This test was used to give an overall view of which soil and solvent concentrations were producing significant effects. The DMRT test could not be applied to the individual treatment means because of missing data. Reasons for these missing data will be discussed later in this section.

The day 5 data indicate that none of the test solvents at any concentration had a significant effect on microbial populations. However, the results indicate that microbial levels differ significantly among the three soil types. Actinomycete populations were higher in the river sand than in the Mohave sl. (The numbers in Canelo l could not be determined because of missing data.) Fungi were found to grow better in the Canelo l than the Mohave sl and river sand. Bacterial numbers did

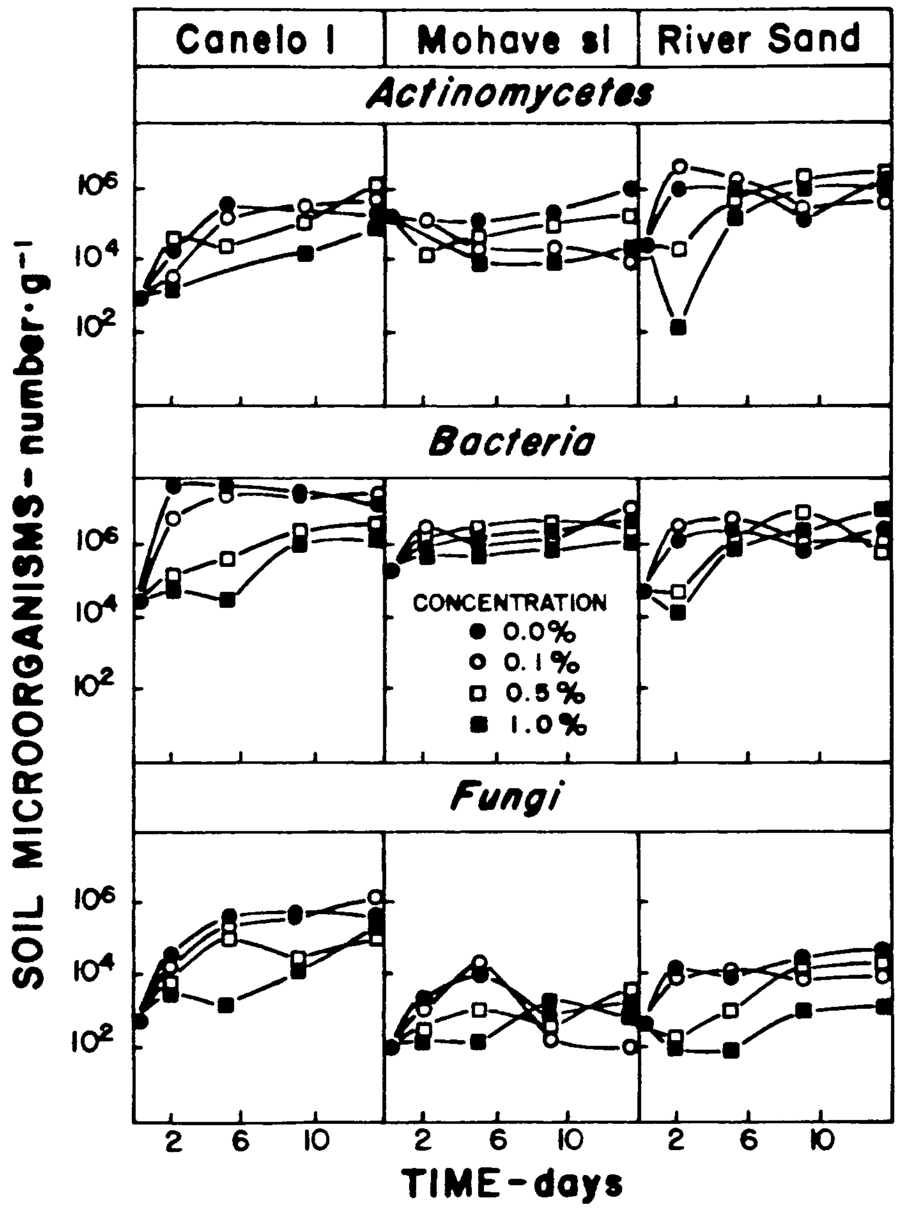

Figure 3. The effect of various concentrations of MIBK on actinomycete, bacteria and fungi populations of Canelo l, Mohave sl, and river sand soils.

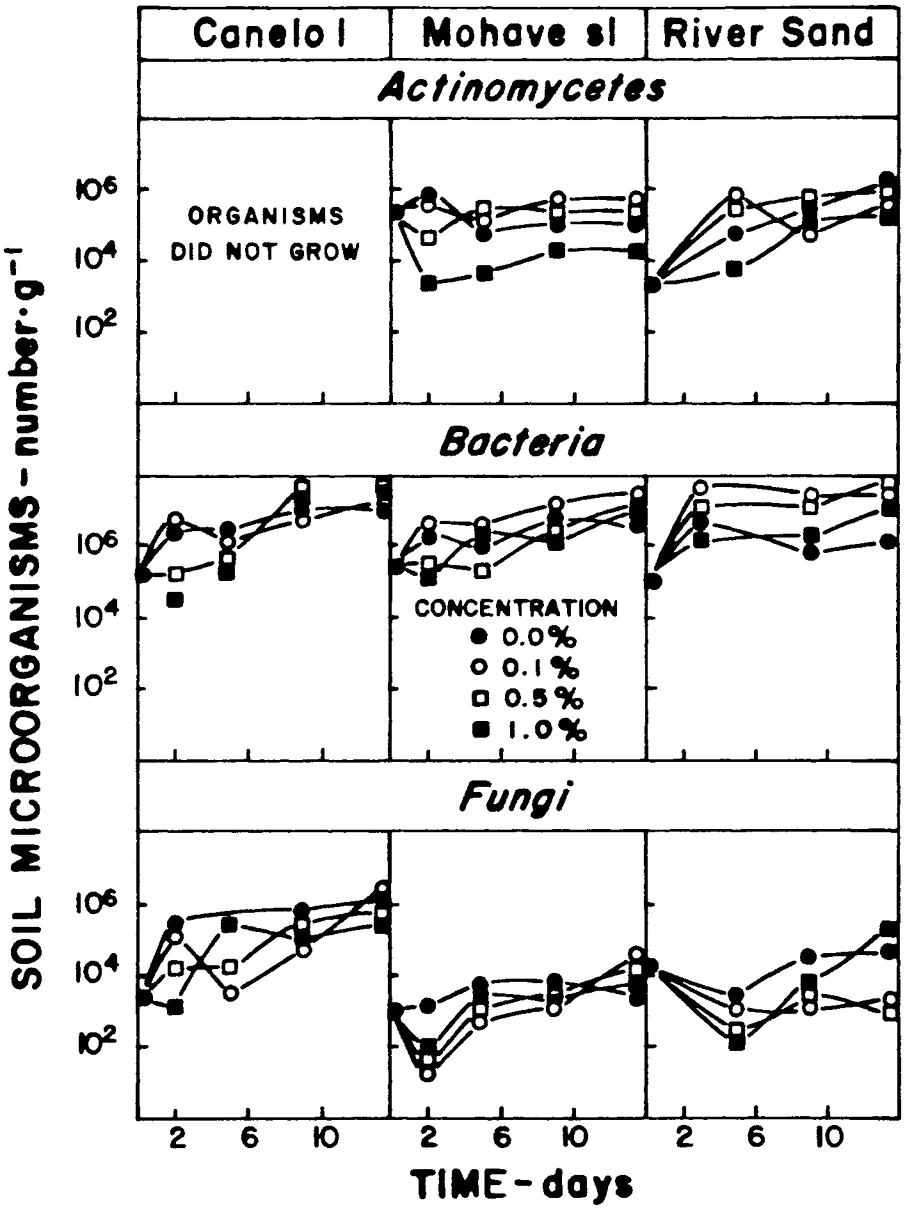

Figure 4. The effect of various concentrations of Xylene on actinomycete, bacteria and fungi populations of Canelo 1, Mohave sl, and river sand soils.

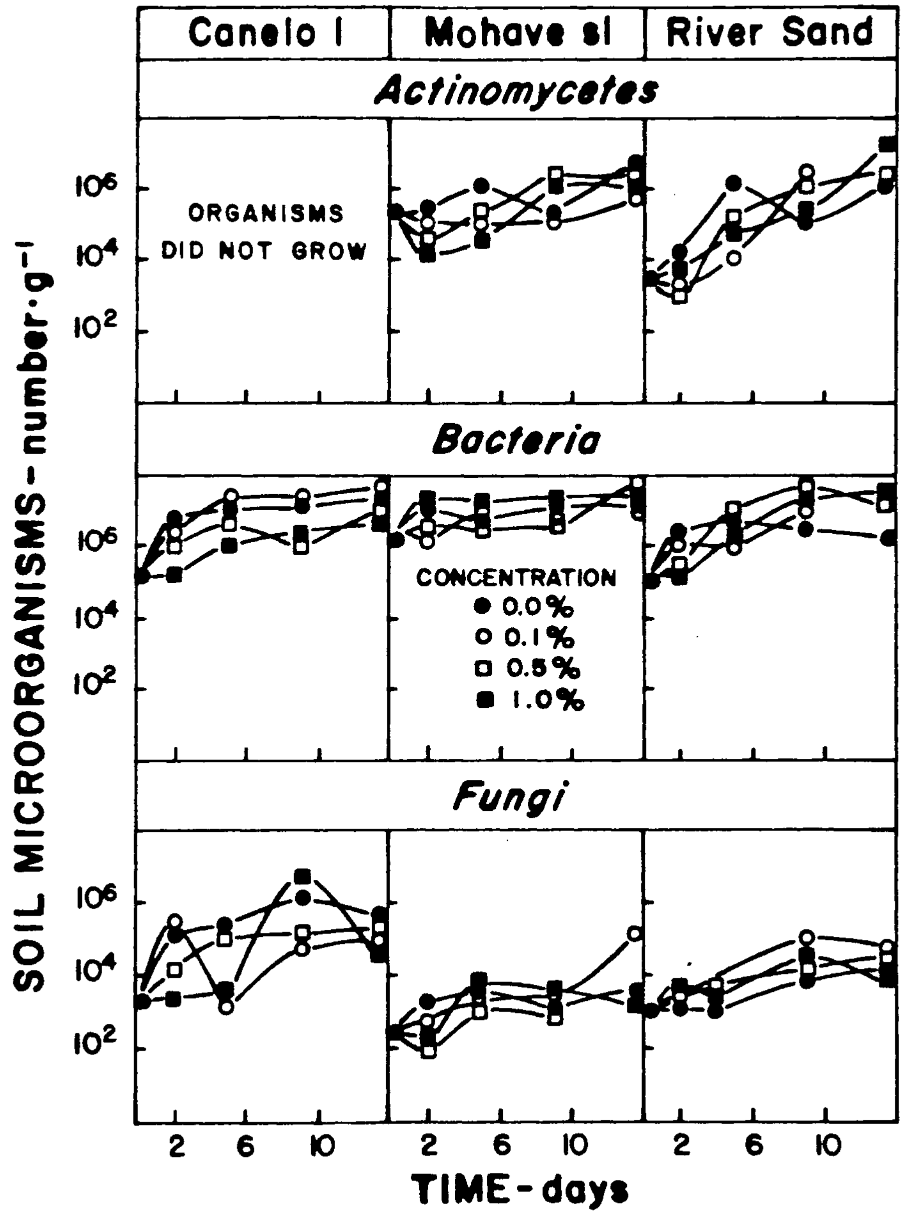

Figure 5. The effect of various concentrations of THF on actinomycete, bacteria and fungi populations of Canelo 1, Mohave sl, and river sand soils.

Table 5. Analysis of Variance Summary for Dilution Plate Count Experiments at Day 5

Source of Variation	df	Bacteria F	df	Fungi F	df	Actinomycetes F
Soil	2	5.66[a]	2	4.86[a]	1	15.70[a]
Solvent	2	0.82	2	1.90	2	1.56
Level	3	1.19	2	0.61	3	1.78
Soil x Solvent	2	3.83	4	0.53	2	4.94
Soil x Level	5	0.59	4	1.69	3	1.24
Solvent x Level	6	1.19	4	1.21	6	2.06

[a] $p < 0.05$.

Table 6. Analysis of Variance Summary for Dilution Plate Count Experiments at Day 14

Source of Variation	df	Bacteria F	df	Fungi F	df	Actinomycetes F
Soil	2	3.06	2	35.06[a]	1	2.12
Solvent	2	20.95[a]	2	5.27[a]	2	1.88
Level	2	9.08[a]	2	2.66	3	0.74
Soil x Solvent	4	17.88[a]	4	2.39	2	0.99
Soil x Level	4	1.98	4	5.73[a]	3	0.95
Solvent x Level	4	4.41[a]	4	0.31	6	1.06

[a] $p < 0.05$.

not vary with soil type. Variations in microbial numbers are common between different soils.

After 14 days of solvent exposure, actinomycete populations in the river sand and Mohave sl were found not to be significantly affected by the presence of MIBK, THF or xylene. Missing data did not allow Canelo l to be evaluated. Fungi populations were found to be greater in the Canelo l than the river sand and Mohave sl. The effect of THF on fungi was significantly different than that of xylene; MIBK could not be statistically distinguished from the other two. More fungi were produced in the presence of xylene. Soil type was not a factor influencing bacterial growth. MIBK was more toxic than THF and xylene. For bacteria, concentration effects were significant. Growth at 0.5% were significantly higher than the control indicating that solvents may be breaking down. From the limited data, which solvent was breaking down could not be determined.

More specific statements are difficult to make since data are missing, and a limited DMRT was done. There are two reasons for missing data, the loss of plates and the failure of the organisms to form colonies on the media. This was especially true when isolating actinomycetes from the Canelo l. This could be attributed to the fact that actinomycetes do not proliferate well in acid environments (Alexander, 1978).

The plate count method is a time-consuming technique and seems to have a high rate of experimental error. One source of error is in obtaining a representative soil sample. A slight difference in moisture or organic matter can significantly change microbial levels. Another source of error is assuming all organisms will grow on the selected media. Different microbes have different nutrient requirements which are difficult to account for in one media. Counting the colonies on the plates can be another problem. For example, fungi were difficult to enumerate because certain colonies would grow faster than others, overgrowing the plate. Shaking can introduce error when enumerating fungi, agitation can rupture the mycelium into a number of fragments each of which form a single colony. On the other hand, bacteria may be underestimated, because bacteria adhere to soil particles and may not be released to the solution before plating.

Carbon Dioxide Evolution Experiments

The data collected from the CO_2 evolution experiments are presented in Tables 9-11 (Appendix A). Results are graphically shown in Figure 6-8.

Analyses of variance results showed soil type, solvent type, and concentration to be significant factors affecting CO_2 evolution (Table 7). In order to determine where these significant differences lie, the Duncan multiple range test was applied to individual treatment means. Table 8 summarizes the effects of the test solvents at varying concentrations on CO_2 production at 5, 14, and 21 days for the Mohave sl, Canelo l and river sand soils.

River sand was the most adversely affected by the presence of the solvents. Microbial activity in the river sand was either inhibited

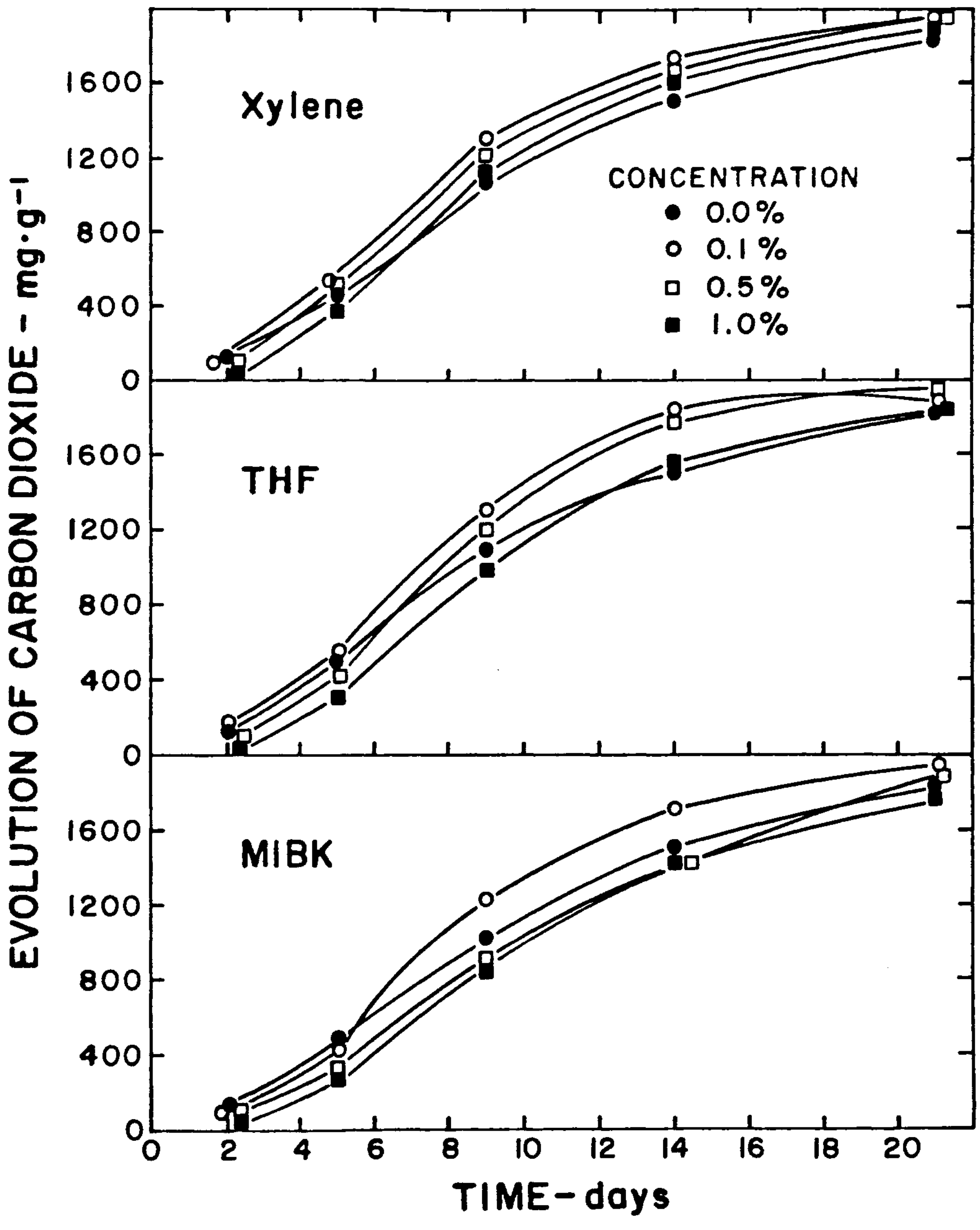

Figure 6. Cumulative CO_2 evolved from Mohave sl treated with various concentrations of MIBK, THF, and Xylene.

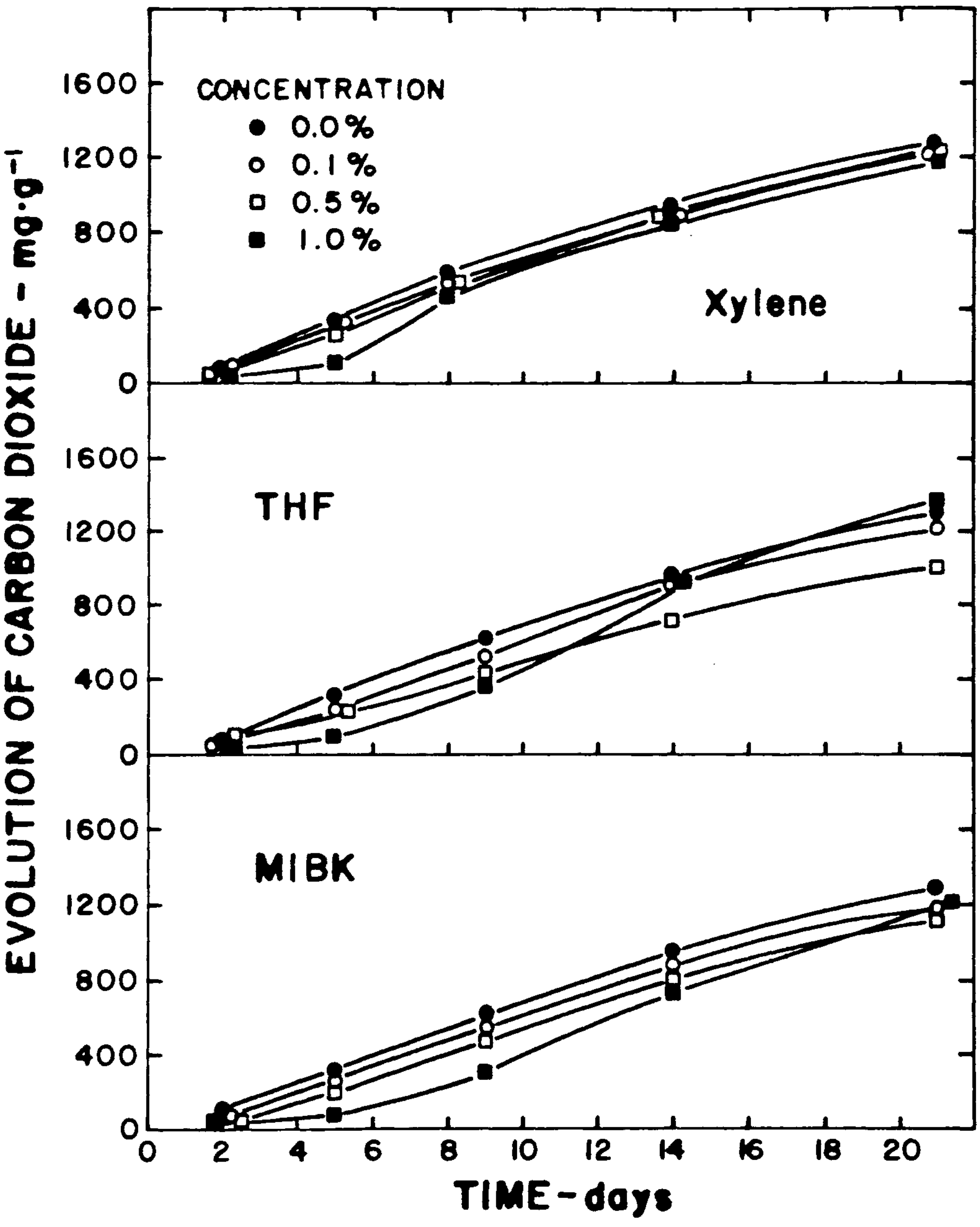

Figure 7. Cumulative CO_2 evolved from river sand treated with various concentrations of MIBK, THF, and Xylene.

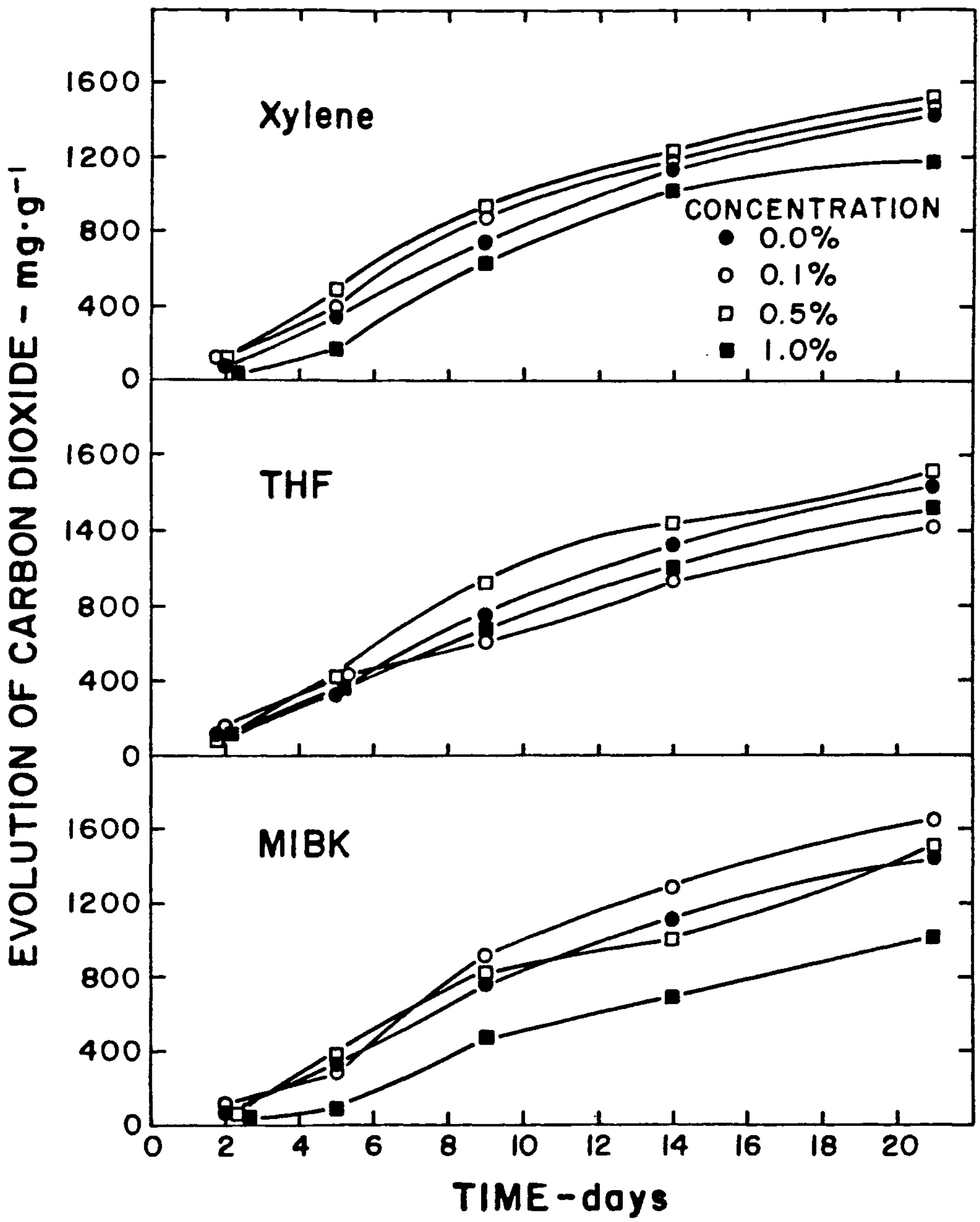

Figure 8. Cumulative CO_2 evolved from Canelo 1 treated with various concentrations of MIBK, THF, and Xylene.

Table 7. Analysis of Variance for CO_2 Evolution Experiments at Day 5, 14, and 21

Source of Variation	df	Day 5 F	Day 14 F	Day 21 F
Soil	2	762.37[a]	529.10[a]	391.01[a]
Solvent	2	110.81[a]	9.47[a]	1.65
Soil x Solvent	4	28.01[a]	1.99	1.22
Concentration	2	558.00[a]	30.33[a]	10.31[a]
Soil x Concentration	4	25.38[a]	7.45[a]	9.52[a]
Solvent x Concentration	4	12.79[a]	5.89[a]	2.78[a]
Soil x Solvent x Concentration	8	22.19[a]	3.42[a]	3.73[a]

[a] $p < 0.05$.

Table 8. The Effect of MIBK, THF, and Xylene at Various Concentrations on CO_2 Evolution from Three Soils

Solvent	Concentration	River sand			Mohave sl			Canelo l		
	%	day			day			day		
		5	14	21	5	14	21	5	14	21
THF	0.1	0	0	-[a]	+[b]	+	+	+	-	-
	0.5	0	0	0[c]	0	+	0	0	+	+
	1.0	-	0	0	-	0	0	0	-	0
MIBK	0.1	0	0	-	0	+	+	-	+	+
	0.5	-	-	0	-	0	+	0	0	0
	1.0	-	-	0	-	0	0	-	-	-
Xylene	0.1	0	0	0	+	+	0	+	0	0
	0.5	0	0	-	0	+	+	0	+	+
	1.0	-	0	0	-	0	0	-	-	-

[a] Amount of CO_2 evolved was significantly less than control.

[b] Amount of CO_2 evolved was significantly greater than control.

[c] Amount of CO_2 evolved was not significantly different than control.

or the same as the control for the entire 21 day exposure period for all three test solvents.

Organisms in Canelo l performed better in the presence of all three solvents than river sand. On day 5 both xylene and THF at a concentration of 0.1% were found to produce elevated levels of CO_2. By day 14 xylene and THF at 0.1% no longer stimulated microbial activity. The THF appeared to cause toxic effects while the xylene produced CO_2 at the same rate as the control. The reason for this is not clear. At the 1% concentration all three solvents exhibited toxic effects toward the soil microbes. All three solvents at either the 0.1 or 0.5% concentration appeared to biodegrade. By day 21, 1% MIBK and THF were still inhibitory to soil microorganisms. The lower concentrations of THF, MIBK and xylene were generally shown to biodegrade.

The organisms in Mohave sl were found to utilize the solvents better than those of the other two soils. On day 5, solvent biodegradation was occurring for all three solvents at some of the lower concentrations. Higher solvent concentrations (1.0%) were inhibitory. By days 14 and 21, all solvents were found to be biodegrading or exhibit no increase in the CO_2 evolution from the Mohave sl.

Soil surface area appeared to play an important role in the soil organisms ability to break down solvents. Soils with larger surface areas have two advantages. One is that microbes have a larger area to establish themselves. The other is that a large surface area allows the solvent to form a thinner layer over the soil which presumably would make the solvent more susceptible to biological attack. Mohave sl was the least affected by the presence of the solvent followed by Canelo l, then river sand. The Mohave and Canelo soils have a similar surface area of 38.3 and 35.0 m^2/g respectively whereas river sand has a surface area of 3.6 m^2/g. The Mohave sl was shown to be significantly better at treating the solvents than the Canelo l. This may be attributed to the low soil pH of Canelo l. Microbial activity is often severely inhibited in acid subsoils.

In general, solvent concentration of 1% were more toxic than the lower concentration of 0.1 and 0.5%. Overall, THF and xylene were more susceptible to biological attack than the MIBK. This is interesting because MIBK is the only one of the three solvents that is not a ring structure. Usually ring structures are more difficult to biodegrade because the ring must first be cleaved.

Use of the biometer flask is a good technique to measure CO_2 evolution; the flasks are easy to use and contribute little experimental error. However, their use may be limited if large amounts of organic matter are present, because oxygen is not continually supplied to these flasks unless special innovations in the equipment are developed for this purpose. The nonbiological production of CO_2 may also interfere with this test. Carbon dioxide may be produced by chemical decarboxylation, by cell-free enzymes, or by the action of organic acids on soil carbonates.

SUMMARY

The dilution plate count method was found to have a high degree of experimental error. Because of this error, definite statements could not be made about the influence of the test solvents on microbial numbers. It was determined that soil was the only significant factor affecting actinomycete, bacteria, and fungi populations at day 5. At 14 days, soil type, solvent and concentration were found to influence microbial numbers. Actinomycetes were not affected by any of the test solvents in the river sand and Mohave sl. Bacteria were found to be more affected by MIBK than THF and xylene. These effects did not vary with soil type. Fungi growth was found to be more affected by the presence of THF than xylene. Fungi grew better in the acid Canelo l than river sand and slightly alkaline Mohave sl.

Carbon dioxide evolution proved to be a simple, reliable technique for measuring microbial activity. The CO_2 experiments indicated that xylene and THF were less toxic than MIBK. Concentrations at 1.0% were generally more toxic than those at 0.1 and 0.5%. Compared with the other soils, river sand microbial populations were the most adversely affected by the presence of the solvents. Microbial activity in river sand was either inhibited or the same as the control at the end of the 21 day incubation period for all three solvents. The Canelo l performed better than the river sand in the presence of the solvents. At the end of the exposure period all three solvents at the

0.1 or 0.5% concentrations were biodegrading. At concentrations of 1%, MIBK and THF were still inhibitory. The organisms associated with Mohave sl were shown to best treat the solvents. After the complete exposure period all solvents were shown to be breaking down or exhibiting no effect in Mohave sl. Higher soil surface area appeared to enhance the ability of the soils to biodegrade the solvents.

Appendix A

COLLECTED DATA - CARBON DIOXIDE EVOLUTION

Table 9. Cumulative Amount of CO_2 Evolved from Mohave sl as Influenced by Treatment with Various Concentrations of MIBK, THF, and Xylene

Exposure Period	MIBK				THF			Xylene		
	%									
	0	0.1	0.5	1.0	0.1	0.5	1.0	0.1	0.5	1.0
d	$mg\ CO_2 \cdot g^{-1}$									
1	73[a]	70	34	18	77	52	15	70	62	15
2	155	147	96	72	155	120	67	144	150	61
3	249	252	174	136	268	196	145	256	246	140
4	363	362	232	216	386	300	226	381	361	244
5	492	485	306	278	545	455	312	530	521	394
6	645	688	495	325	763	659	447	770	739	641
7	819	903	693	445	953	863	615	970	930	859
8	980	1093	789	668	1141	1044	818	1159	1112	1032
9	1104	1257	958	843	1316	1205	995	1317	1282	1182
10	1214	1401	1091	970	1465	1359	1145	1459	1435	1311
11	1307	1497	1206	1096	1575	1504	1282	1514	1514	1408
12	1382	1591	1311	1228	1676	1610	1371	1604	1581	1489
13	1451	1675	1412	1337	1761	1708	1489	1683	1637	1565
14	1519	1752	1511	1432	1830	1799	1582	1761	1721	1635
15	1577	1820	1607	1514	1895	1877	1664	1807	1797	1699
16	1637	1852	1690	1585	1958	1946	1732	1855	1868	1756
17	1690	1905	1760	1645	2017	2005	1791	1905	1929	1805
18	1725	1955	1831	1712	2060	2061	1848	1949	1986	1848
19	1771	2001	1892	1777	2104	2113	1898	1994	2013	1887
20	1815	2051	1953	1837	2149	2165	1952	2035	2066	1931
21	1862	2097	2006	1899	2190	2211	2002	2079	2125	1975

[a]Mean of Duplicate Measurements.

Table 10. Cumulative Amount of CO_2 Evolved from River Sand as Influenced by Treatment with Various Concentrations of MIBK, THF, and Xylene

Exposure Period	MIBK				THF			Xylene		
	%									
	0	0.1	0.5	1.0	0.1	0.5	1.0	0.1	0.5	1.0
d	$mg\ CO_2 \cdot g^{-1}$									
1	35[a]	15	10	5	22	16	6	29	10	3
2	90	63	55	14	84	58	13	79	63	13
3	160	107	99	29	135	120	22	150	123	29
4	235	182	167	63	212	189	64	230	188	58
5	312	273	235	113	265	257	108	313	254	128
6	379	339	294	158	332	286	161	391	341	200
7	453	403	353	232	407	266	221	462	423	273
8	529	477	421	297	480	352	308	530	507	367
9	611	551	501	360	557	422	384	602	587	452
10	696	634	578	425	637	485	511	675	664	548
11	770	708	648	495	723	543	628	755	744	638
12	845	782	717	584	799	607	731	822	820	724
13	912	848	778	683	869	678	837	887	888	801
14	981	911	835	779	929	742	928	947	956	875
15	1046	972	884	871	986	803	1007	1004	1022	943
16	1106	1032	938	954	1032	857	1079	1054	1087	1008
17	1146	1085	989	1026	1073	901	1148	1105	1143	1069
18	1195	1130	1037	1095	1107	945	1211	1153	1186	1132
19	1239	1173	1081	1155	1143	984	1269	1200	1224	1189
20	1283	1193	1118	1210	1177	1021	1298	1237	1264	1242
21	1330	1254	1154	1259	1208	1056	1349	1275	1298	1293

[a]Mean of Duplicate Measurements.

Table 11. Cumulative Amount of CO_2 Evolved from Canelo 1 as Influenced by Treatment with Various Concentrations of MIBK, THF, and Xylene

Exposure Period	MIBK				THF			Xylene		
	%									
	0	0.1	0.5	1.0	0.1	0.5	1.0	0.1	0.5	1.0
d	$mg\ CO_2 \cdot g^{-1}$									
1	35[a]	44	29	11	76	48	43	71	53	15
2	82	84	73	20	147	99	96	135	117	33
3	133	136	123	33	234	153	174	203	190	59
4	225	227	188	65	323	245	271	303	305	104
5	368	337	373	155	415	407	372	407	491	183
6	510	480	547	277	472	605	484	524	676	249
7	586	686	649	392	544	750	581	637	822	373
8	678	858	726	444	615	854	658	752	909	547
9	782	950	797	517	688	933	729	876	981	633
10	864	1035	867	583	756	1007	789	981	1048	741
11	938	1118	932	642	818	1075	758	1058	1115	780
12	1005	1192	995	688	883	1141	897	1119	1179	851
13	1069	1261	1046	724	939	1200	948	1146	1232	914
14	1130	1328	1088	756	992	1254	997	1200	1284	968
15	1189	1391	1126	792	1042	1302	1052	1250	1338	995
16	1248	1453	1162	836	1088	1341	1111	1299	1383	1022
17	1302	1506	1190	857	1133	1382	1158	1345	1425	1056
18	1355	1546	1217	920	1151	1421	1202	1367	1464	1091
19	1380	1581	1247	956	1176	1462	1234	1408	1502	1127
20	1422	1618	1263	988	1217	1503	1265	1446	1527	1146
21	1459	1651	1293	1015	1258	1534	1291	1465	1563	1180

[a]Mean of Duplicate Measurements.

Appendix B

COLLECTED DATA - DILUTION PLATE COUNTS

Table 12. Numbers of Soil Actinomycetes, Bacteria, and Fungi Colonies Found on Agar Plates as Influenced by Treatment of Mohave sl with MIBK at 0.1, 0.5, and 1.0 Percentages

Exposure Period	MIBK - % (w/w)			
	0 (control)	0.1	0.5	1.0
d	Actinomycetes			
	----CFU.g^{-1}----			
0	4.3×10^5 [a]	4.3×10^5	4.3×10^5	4.3×10^5
1	1.2×10^6	1.6×10^6	1.2×10^4	1.4×10^5
2	NA	3.7×10^5	3.2×10^4	NA
5	1.2×10^5	2.2×10^4	8.6×10^4	1.1×10^4
9	4.8×10^6	3.9×10^4	1.7×10^5	1.7×10^4
14	7.7×10^6	1.4×10^5	6.1×10^5	1.9×10^4
	Bacteria			
	----CFU.g^{-1}----			
0	1.1×10^5	1.1×10^5	1.1×10^5	1.1×10^5
1	9.0×10^6	7.9×10^6	3.4×10^5	2.0×10^7
2	1.0×10^7	1.9×10^7	1.3×10^7	8.4×10^5
5	1.2×10^6	1.0×10^6	2.0×10^6	9.4×10^5
9	4.8×10^6	3.1×10^6	5.0×10^6	2.8×10^6
14	7.7×10^6	1.0×10^7	6.2×10^6	3.4×10^6
	Fungi			
	----CFU.g^{-1}----			
0	1.0×10^2	1.0×10^2	1.0×10^2	1.0×10^2
1	2.2×10^3	1.3×10^3	5.6×10^2	1.1×10^2
2	1.2×10^3	1.2×10^3	5.6×10^2	2.3×10^2
5	1.2×10^4	1.6×10^4	1.1×10^3	1.1×10^2
9	1.0×10^3	5.7×10^2	8.0×10^2	1.3×10^3
14	1.4×10^3	1.1×10^2	2.4×10^3	1.2×10^3

NA = Not Available.

[a]Mean of Duplicate Measurements.

Table 13. Numbers of Soil Actinomycetes, Bacteria, and Fungi Colonies Found on Agar Plates as Influenced by Treatment of Mohave sl with Xylene at 0.1, 0.5, and 1.0 Percentages

Exposure Period	Xylene - % (w/w)			
	0 (control)	0.1	0.5	1.0
d	Actinomycetes			
	CFU.g^{-1}			
0	3.2×10^5 [a]	3.2×10^5	3.2×10^5	3.2×10^5
1	6.2×10^4	7.9×10^4	6.0×10^3	8.3×10^3
2	1.6×10^5	1.5×10^5	8.6×10^4	6.1×10^3
5	9.1×10^4	1.3×10^5	2.3×10^5	8.4×10^3
9	1.1×10^5	6.4×10^5	2.8×10^5	2.6×10^4
14	1.1×10^5	6.4×10^5	2.8×10^5	2.6×10^4
	Bacteria			
	CFU.g^{-1}			
0	4.7×10^5	4.7×10^5	4.7×10^5	4.7×10^5
1	3.0×10^6	3.7×10^6	3.5×10^5	3.0×10^5
2	3.7×10^6	6.2×10^6	5.6×10^5	2.9×10^5
5	1.3×10^6	4.5×10^6	3.5×10^5	1.4×10^6
9	3.3×10^6	3.7×10^7	3.0×10^6	2.6×10^6
14	7.2×10^6	6.9×10^7	2.8×10^7	1.4×10^7
	Fungi			
	CFU.g^{-1}			
0	2.6×10^3	2.6×10^3	2.6×10^3	2.6×10^3
1	1.7×10^3	6.8×10^2	<100	<100
2	1.1×10^3	<100	<100	<100
5	3.3×10^3	1.1×10^3	1.9×10^3	2.1×10^3
9	6.9×10^3	1.8×10^3	4.7×10^3	4.4×10^3
14	5.7×10^3	3.1×10^4	2.5×10^4	5.9×10^3

NA = Not Available.

[a]Mean of Duplicate Measurements.

Table 14. Numbers of Soil Actinomycetes, Bacteria, and Fungi Colonies Found on Agar Plates as Influenced by Treatment of Mohave sl with THF at 0.1, 0.5, and 1.0 Percentages

Exposure Period	THF - % (w/w)			
	0 (control)	0.1	0.5	1.0
d	Actinomycetes			
	CFU.g^{-1}			
0	2.4×10^5 [a]	2.4×10^5	2.4×10^5	2.4×10^5
1	3.5×10^5	5.2×10^5	1.3×10^5	9.2×10^3
2	3.2×10^5	1.5×10^5	5.5×10^4	1.8×10^4
5	1.3×10^6	1.2×10^5	1.5×10^5	5.2×10^4
9	1.5×10^6	1.4×10^5	1.3×10^6	1.0×10^6
14	1.9×10^6	9.2×10^5	1.7×10^6	1.3×10^6
	Bacteria			
	CFU.g^{-1}			
0	1.1×10^6	1.1×10^6	1.1×10^6	1.1×10^6
1	7.0×10^6	1.0×10^7	5.8×10^6	5.7×10^6
2	1.6×10^7	2.8×10^6	8.7×10^6	1.8×10^7
5	9.6×10^6	1.1×10^7	9.6×10^6	2.0×10^7
9	1.9×10^7	9.2×10^6	7.2×10^6	2.6×10^7
14	3.4×10^7	1.4×10^7	4.2×10^7	3.6×10^7
	Fungi			
	CFU.g^{-1}			
0	5.6×10^2	5.6×10^2	5.6×10^2	5.6×10^2
1	1.8×10^3	1.2×10^3	2.2×10^2	5.9×10^3
2	2.5×10^3	9.1×10^2	2.2×10^2	2.3×10^2
5	2.1×10^3	1.7×10^3	1.7×10^3	4.8×10^3
9	2.6×10^3	4.1×10^4	2.1×10^2	4.4×10^3
14	3.7×10^3	1.4×10^5	NA	3.6×10^3

NA = Not Available.

[a]Mean of Duplicate Measurements.

Table 15. Numbers of Soil Actinomycetes, Bacteria, and Fungi Colonies Found on Agar Plates as Influenced by Treatment of River Sand with MIBK at 0.1, 0.5, and 1.0 Percentages

Exposure Period	MIBK - % (w/w)			
	0 (control)	0.1	0.5	1.0
d	Actinomycetes			
	CFU.g^{-1}			
0	4.4×10^{3} [a]	4.4×10^{3}	4.4×10^{3}	4.4×10^{3}
1	1.2×10^{5}	2.0×10^{4}	2.8×10^{3}	1.2×10^{3}
2	1.2×10^{6}	7.3×10^{6}	4.3×10^{4}	2.2×10^{3}
5	1.1×10^{6}	3.3×10^{6}	9.0×10^{5}	3.7×10^{5}
9	3.6×10^{5}	7.1×10^{5}	2.4×10^{6}	1.2×10^{6}
14	1.5×10^{6}	8.0×10^{5}	1.9×10^{6}	1.3×10^{6}
	Bacteria			
	CFU.g^{-1}			
0	9.3×10^{4}	9.3×10^{4}	9.3×10^{4}	9.3×10^{4}
1	1.4×10^{5}	8.7×10^{4}	2.7×10^{4}	4.5×10^{4}
2	2.4×10^{6}	5.3×10^{4}	9.8×10^{4}	2.2×10^{4}
5	1.9×10^{6}	5.1×10^{6}	1.5×10^{6}	1.1×10^{6}
9	2.8×10^{6}	2.8×10^{6}	1.5×10^{7}	5.4×10^{6}
14	4.5×10^{6}	3.7×10^{6}	1.7×10^{6}	7.2×10^{6}
	Fungi			
	CFU.g^{-1}			
0	9.1×10^{2}	9.1×10^{2}	9.1×10^{2}	9.1×10^{2}
1	1.7×10^{3}	5.1×10^{3}	1.0×10^{2}	NA
2	2.3×10^{4}	1.0×10^{3}	1.0×10^{2}	1.0×10^{2}
5	1.0×10^{4}	1.2×10^{4}	2.0×10^{2}	1.0×10^{2}
9	2.5×10^{4}	1.1×10^{4}	2.1×10^{4}	1.8×10^{3}
14	2.5×10^{4}	1.3×10^{4}	2.0×10^{4}	2.4×10^{3}

NA = Not Available.

[a] Mean of Duplicate Measurements.

Table 16. Numbers of Soil Actinomycetes, Bacteria, and Fungi Colonies Found on Agar Plates as Influenced by Treatment of River Sand with THF at 0.1, 0.5, and 1.0 Percentages

Exposure Period	THF - % (w/w)			
	0 (control)	0.1	0.5	1.0
d	Actinomycetes			
	---CFU.g^{-1}---			
0	4.8×10^{3} [a]	4.8×10^{3}	4.8×10^{3}	4.8×10^{3}
1	6.3×10^{4}	1.3×10^{3}	9.8×10^{2}	5.4×10^{2}
2	1.1×10^{5}	2.3×10^{3}	1.3×10^{3}	8.4×10^{3}
5	1.3×10^{6}	1.0×10^{4}	2.3×10^{5}	8.0×10^{4}
9	2.2×10^{5}	1.8×10^{6}	1.4×10^{6}	2.3×10^{5}
14	1.3×10^{6}	3.1×10^{6}	2.4×10^{6}	2.6×10^{7}
	Bacteria			
	---CFU.g^{-1}---			
0	1.0×10^{5}	1.0×10^{5}	1.0×10^{5}	1.0×10^{5}
1	2.5×10^{6}	5.4×10^{5}	4.6×10^{5}	6.8×10^{4}
2	1.8×10^{6}	1.3×10^{6}	6.5×10^{5}	2.2×10^{5}
5	2.4×10^{6}	1.2×10^{7}	1.3×10^{5}	3.4×10^{6}
9	4.4×10^{6}	2.1×10^{7}	2.4×10^{7}	2.4×10^{7}
14	2.3×10^{6}	NA	3.2×10^{7}	3.2×10^{7}
	Fungi			
	---CFU.g^{-1}---			
0	6.5×10^{3}	6.5×10^{3}	6.5×10^{3}	6.5×10^{3}
1	2.6×10^{3}	5.4×10^{2}	5.4×10^{2}	2.2×10^{2}
2	1.7×10^{4}	1.9×10^{3}	NA	2.0×10^{3}
5	1.2×10^{3}	1.9×10^{4}	2.1×10^{4}	2.1×10^{4}
9	1.0×10^{4}	1.7×10^{5}	2.6×10^{4}	3.4×10^{4}
14	3.3×10^{4}	1.8×10^{5}	3.4×10^{4}	3.1×10^{4}

NA = Not Available.

[a]Mean of Duplicate Measurements.

Table 17. Numbers of Soil Actinomycetes, Bacteria, and Fungi Colonies Found on Agar Plates as Influenced by Treatment of River Sand with Xylene at 0.1, 0.5, and 1.0 Percentages

Exposure Period	Xylene - % (w/w)			
	0 (control)	0.1	0.5	1.0
d	Actinomycetes			
	---CFU.g^{-1}---			
0	3.7×10^3 [a]	3.7×10^3	3.7×10^3	3.7×10^3
1	4.3×10^4	9.6×10^3	1.1×10^3	3.7×10^2
2	NA	NA	NA	NA
5	7.7×10^4	6.1×10^5	6.1×10^5	7.0×10^3
9	2.0×10^5	7.0×10^4	6.5×10^5	1.1×10^5
14	1.3×10^6	8.6×10^5	8.8×10^5	7.6×10^5
	Bacteria			
	---CFU.g^{-1}---			
0	1.0×10^5	1.0×10^5	1.0×10^5	1.0×10^5
1	6.6×10^6	4.2×10^6	7.1×10^4	1.5×10^6
2	NA	NA	NA	NA
5	8.2×10^6	2.9×10^7	2.5×10^7	1.3×10^6
9	7.8×10^5	3.1×10^7	2.9×10^7	3.2×10^6
14	1.0×10^6	7.7×10^7	1.9×10^8	2.1×10^7
	Fungi			
	---CFU.g^{-1}---			
0	1.2×10^4	1.2×10^4	1.2×10^4	1.2×10^4
1	1.5×10^3	4.4×10^2	<100	<100
2	NA	NA	NA	NA
5	2.4×10^3	1.5×10^3	3.3×10^2	<100
9	6.5×10^4	2.1×10^3	2.9×10^3	7.6×10^3
14	7.7×10^4	3.0×10^3	1.2×10^3	2.3×10^5

NA = Not Available.

[a]Mean of Duplicate Measurements.

Table 18. Numbers of Soil Actinomycetes, Bacteria, and Fungi Colonies Found on Agar Plates as Influenced by Treatment of Canelo 1 with MIBK at 0.1, 0.5, and 1.0 Percentages

Exposure Period	MIBK - % (w/w)			
	0 (control)	0.1	0.5	1.0
d	Actinomycetes			
	CFU.g^{-1}			
0	1.1×10^{3}[a]	1.1×10^{3}	1.1×10^{3}	1.1×10^{3}
1	7.3×10^{3}	5.2×10^{3}	8.4×10^{3}	3.1×10^{3}
2	1.9×10^{4}	4.7×10^{3}	5.2×10^{4}	2.5×10^{3}
5	2.5×10^{5}	2.3×10^{5}	5.5×10^{4}	NA
9	NA	5.1×10^{5}	1.8×10^{5}	2.3×10^{4}
14	3.1×10^{5}	9.6×10^{5}	1.1×10^{6}	1.0×10^{5}
	Bacteria			
	CFU.g^{-1}			
0	4.2×10^{4}	4.2×10^{4}	4.2×10^{4}	4.2×10^{4}
1	7.3×10^{5}	2.2×10^{5}	8.3×10^{4}	5.5×10^{4}
2	1.8×10^{7}	8.1×10^{6}	1.2×10^{5}	7.8×10^{4}
5	1.6×10^{7}	1.5×10^{7}	7.7×10^{5}	5.7×10^{4}
9	NA	1.5×10^{7}	1.3×10^{6}	1.0×10^{6}
14	1.5×10^{7}	1.6×10^{7}	8.2×10^{6}	1.1×10^{6}
	Fungi			
	CFU.g^{-1}			
0	8.4×10^{2}	8.4×10^{2}	8.4×10^{2}	8.4×10^{2}
1	1.7×10^{3}	1.4×10^{4}	1.2×10^{3}	3.0×10^{3}
2	5.0×10^{4}	2.9×10^{4}	7.9×10^{3}	6.3×10^{3}
5	4.0×10^{5}	3.7×10^{5}	2.2×10^{5}	1.4×10^{3}
9	NA	7.5×10^{5}	2.1×10^{5}	2.1×10^{4}
14	5.6×10^{5}	1.2×10^{6}	2.5×10^{5}	2.6×10^{5}

NA = Not Available.

[a]Mean of Duplicate Measurements.

Table 19. Numbers of Soil Actinomycetes, Bacteria, and Fungi Colonies Found on Agar Plates as Influenced by Treatment of Canelo 1 with THF at 0.1, 0.5, and 1.0 Percentages

Exposure Period	THF - % (w/w)			
	0 (control)	0.1	0.5	1.0
d	Actinomycetes			
	CFU.g^{-1}			
0				
1				
2	Organisms Did Not Grow			
5				
9				
14				
	Bacteria			
	CFU.g^{-1}			
0	1.7×10^5 [a]	1.7×10^5	1.7×10^5	1.7×10^5
1	3.0×10^6	2.3×10^6	3.1×10^5	1.9×10^5
2	2.2×10^7	1.9×10^6	1.3×10^6	3.1×10^5
5	1.9×10^7	3.0×10^7	8.4×10^6	1.0×10^6
9	2.2×10^7	3.0×10^7	1.5×10^6	2.3×10^6
14	3.2×10^7	5.1×10^7	1.5×10^7	7.2×10^6
	Fungi			
	CFU.g^{-1}			
0	2.1×10^3	2.1×10^3	2.1×10^3	2.1×10^3
1	2.7×10^4	3.7×10^4	3.4×10^4	1.2×10^4
2	1.7×10^5	2.5×10^5	3.6×10^4	4.8×10^3
5	3.9×10^5	2.2×10^3	1.0×10^5	6.5×10^3
9	3.8×10^6	9.2×10^4	1.1×10^5	6.3×10^6
14	4.3×10^5	1.6×10^5	2.4×10^5	8.3×10^4

NA = Not Available.

[a]Mean of Duplicate Measurements.

Table 20. Numbers of Soil Actinomycetes, Bacteria, and Fungi Colonies Found on Agar Plates as Influenced by Treatment of Canelo 1 with Xylene at 0.1, 0.5, and 1.0 Percentages

Exposure Period	Xylene - % (w/w)			
	0 (control)	0.1	0.5	1.0
d	Actinomycetes			
	CFU.g^{-1}			
0	Organisms Did Not Grow			
1				
2				
5				
9				
14				
	Bacteria			
	CFU.g^{-1}			
0	1.9×10^{5} [a]	1.9×10^{5}	1.9×10^{5}	1.9×10^{5}
1	1.7×10^{6}	4.0×10^{5}	7.7×10^{4}	7.4×10^{4}
2	4.4×10^{6}	7.3×10^{6}	2.5×10^{5}	6.6×10^{4}
5	1.9×10^{7}	1.2×10^{7}	6.6×10^{5}	2.5×10^{5}
9	1.8×10^{7}	8.2×10^{6}	2.6×10^{7}	2.2×10^{7}
14	2.6×10^{7}	3.8×10^{7}	7.3×10^{7}	4.7×10^{7}
	Fungi			
	CFU.g^{-1}			
0	2.3×10^{3}	2.3×10^{3}	2.3×10^{3}	2.3×10^{3}
1	7.0×10^{4}	2.7×10^{4}	3.5×10^{3}	3.1×10^{3}
2	1.3×10^{5}	1.3×10^{5}	2.4×10^{4}	1.3×10^{3}
5	NA	5.8×10^{3}	3.7×10^{4}	9.0×10^{5}
9	7.2×10^{5}	9.8×10^{4}	3.1×10^{5}	1.3×10^{5}
14	1.1×10^{6}	1.4×10^{6}	8.3×10^{5}	7.3×10^{5}

NA = Not Available.

[a]Mean of Duplicate Measurements.

Appendix C

INGREDIENTS FOR MEDIA

Table 21. Ingredients for Actinomycete Isolation Agar Used for the Isolation and Quantification of Actinomycetes

Ingredient[a]	Amount added to 2-Liter Flask
	g
Sodium caseinate	2.0
Asparagine	0.1
Sodium propionate	4.0
Dipotassium phosphate	0.5
Magnesium sulfate	0.1
Ferrous sulfate	0.001
Streptomycin[b]	0.003
Glycerol	5.0
Agar	15.0
Deionized water	1000

[a] All ingredients except streptomycin were autoclaved for 15 minutes at 15 psi (121°C).

[b] Streptomycin solution was prepared by dissolving 1.0 g of streptomycin sulfate in 100 ml of deionized water. The solution was sterilized by passing through a 0.45 micron filter. This solution was added to the media after it was cooled to approximately 50°C.

Table 22. Ingredients for Malt Extract Agar Used for the Isolation and Quantification of Fungi

Ingredient[a]	Amount added to 2-Liter Flask
	g
Maltose	12.75
Dextrin	2.75
Glycerol	2.35
Peptone	0.78
Agar	20.0
Streptomycin[b]	0.003
Deionized Water	1000

[a] All ingredients were autoclaved for 15 minutes at 15 psi (121°C).

[b] Prepared by dissolving 1 g of streptomycin sulfate in 100 ml of deionized water. Solution was sterilized by passing through a 0.45 micron filter. This solution was added to the media after it was cooled to approximately 50°C.

Table 23. Ingredients for Thorton's Standardized Medium (Modified) for the Isolation and Quantification of Bacteria

Ingredient[a]	Amount added to 2-Liter Flask
	g
K_2HPO_4	1.0
$MgSO_4$ $7H_2O$	0.2
$CaCl_2$	0.05
NaCl	0.1
$FeCl_3 \cdot 6H_2O$[b]	0.002
KNO_3	0.5
Asparagine	0.5
Mannitol	1.0
Agar	20.0
Deionized water	1000

[a]All ingredients were autoclaved for 15 minutes at 15 psi (121°C).

[b]Made up as 0.002 g/ml stock solution.

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