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Comparative Plant Uptake and Microbial Degradation of Trichloroethylene in the Rhizospheres of Five Plant Species–Implications for Bioremediation of Contaminated Surface Soils

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Environmental Sciences Division
Publication No. 3809

MANAGED BY
MARTIN MARIETTA ENERGY SYSTEMS, INC.
FOR THE UNITED STATES
DEPARTMENT OF ENERGY

ORNL/TM--12017

DE92 007780

ENVIRONMENTAL SCIENCES DIVISION

COMPARATIVE PLANT UPTAKE AND MICROBIAL DEGRADATION OF TRICHLOROETHYLENE IN THE RHIZOSPHERES OF FIVE PLANT SPECIES--IMPLICATIONS FOR BIOREMEDIATION OF CONTAMINATED SURFACE SOILS[1]

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Environmental Sciences Division
Publication No. 3809

Date Published - January 1992

Prepared for the
Office of Technology Development
(EW 40 20 10 0)

Prepared by the
OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37831-6285
managed by
MARTIN MARIETTA ENERGY SYSTEMS, INC.
for the
U. S. DEPARTMENT OF ENERGY
under contract DE-AC05-84OR21400

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ACKNOWLEDGMENTS

Several people in the Environmental Sciences Division at Oak Ridge National Laboratory have contributed to the success of this research in specific areas. Bob Luxmoore, Phil Jardine (soils and modeling); Pat Parr, Maureen Cunningham, Linda Mann, Gerry O'Neill, Rich Norby, Nelson Edwards, and Carla Gunderson (plants); Steve Herbes, Tony Palumbo and Mike Morrissey (bacteria and chemical analyses). In addition, we are grateful to colleagues at the Institute for Applied Microbiology (Jim Guckert, David Ringleberg, Tommy Phelps) for help with bacterial community analysis. Dr. Terry Hazen of the Savannah River Laboratory (SRL) served as project officer for this work and provided helpful discussions and advice. Vergil Rogers (SRL) and John Knox (Savannah River Ecology Laboratory) helped with the pedon description and plant survey, respectively.

This research was sponsored by the Office of Technology Development, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. This research was conducted in the Environmental Sciences Division of Oak Ridge National Laboratory, which is managed by Martin Marietta Energy Systems, Inc. for the U.S. Department of Energy. Publication number 3809 of the Environmental Sciences Division.

ABSTRACT

ANDERSON, T. A. and B. T. WALTON. 1992. Comparative plant uptake and microbial degradation of trichloroethylene in the rhizospheres of five plant species--Implications for bioremediation of contaminated surface soils. ORNL/TM-12017. Oak Ridge National Laboratory, Oak Ridge, Tennessee. 206 pp.

The objective of this study was to collect data that would provide a foundation for the concept of using vegetation to enhance *in situ* bioremediation of contaminated surface soils. Soil and vegetation (*Lespedeza cuneata*, *Paspalum notatum*, *Pinus taeda*, and *Solidago* sp.) samples from the Miscellaneous Chemicals Basin (MCB) at the Savannah River Site were used in tests to identify critical plant and microbiological variables affecting the fate of trichloroethylene (TCE) in the root zone.

Microbiological assays including phospholipid fatty acid analyses, and ^{14}C-acetate incorporation were conducted to elucidate differences in rhizosphere and nonvegetated soil microbial communities from the MCB. The microbial activity, biomass, and degradation of TCE in rhizosphere soils were significantly greater than corresponding nonvegetated soils. In addition, vegetation (both indigenous and nonindigenous) had a positive effect on microbial degradation of ^{14}C-TCE in whole-plant experiments. Soils from the MCB containing *Lespedeza cuneata*, *Pinus taeda*, and *Glycine max* mineralized greater than 25% of the ^{14}C-TCE added compared with less than 20% in nonvegetated soils. Collectively, these results provide evidence for the positive role of vegetation in enhancing biodegradation. Furthermore, biodegradation of TCE may be slower than degradation rates for less persistent

waste chemicals such as nonhalogenated aromatics. Therefore, the data are likely to be conservative estimates of the potential for enhanced degradation of waste chemicals in the rhizosphere. In most of the whole plant experiments conducted, total uptake was minimal, ranging from 1%-21%, and was related to water use and plant species. Because the experiments were designed to maximize plant uptake, these data may be overestimates of the potential TCE residues in plants grown in TCE-contaminated soil.

This dissertation represents a systematic approach to answering initial fundamental questions on the soil-plant-microbe-chemical relationship. The results presented provide strong evidence for the potential role of vegetation in remediation of surface soils contaminated with hazardous organic compounds. In addition, the reviewed literature on rhizosphere microbiology, accelerated degradation of agrochemicals in the root zone, and recent research on the fate of nonagricultural chemicals in the rhizosphere, all provide additional impetus for exploring the use of vegetation in bioremediation of contaminated surface soils.

CHAPTER I.

INTRODUCTION

The use of indigenous microbial populations to restore chemically contaminated surface soils is potentially an effective remediation strategy, provided that a sufficient consortia of microorganisms capable of degrading the contaminant(s) are present and their activity is not limited by the existing environmental conditions. The highly versatile metabolic capabilities of fungi and bacteria have been employed to reclaim polluted ecosystems and minimize the potential adverse effects of hazardous chemicals released to the environment (Atlas and Pramer, 1990). Occasionally, environmental conditions onsite significantly hinder microbial degradation of toxicants. In such cases, the environmental conditions may be altered in an attempt to enhance microbial populations and/or their activity, such as through nutrient additions or aeration. The addition of external carbon sources may be especially important in such cases where the contaminant is degraded cometabolically.

The rhizosphere (Figure I - 1) is a zone of increased microbial activity and biomass at the root-soil interface (Curl and Truelove, 1986). The large microbial populations in the rhizosphere are sustained by exudation of carbohydrates and amino acids from the root and sloughing of root epidermis (Rovira et al., 1979). Although modification of the soil environment in the rhizosphere by plant root secretions is an important process that influences microbial populations, the actual structure of the plant root provides microorganisms with a large surface area for colonization (Lynch, 1982). The

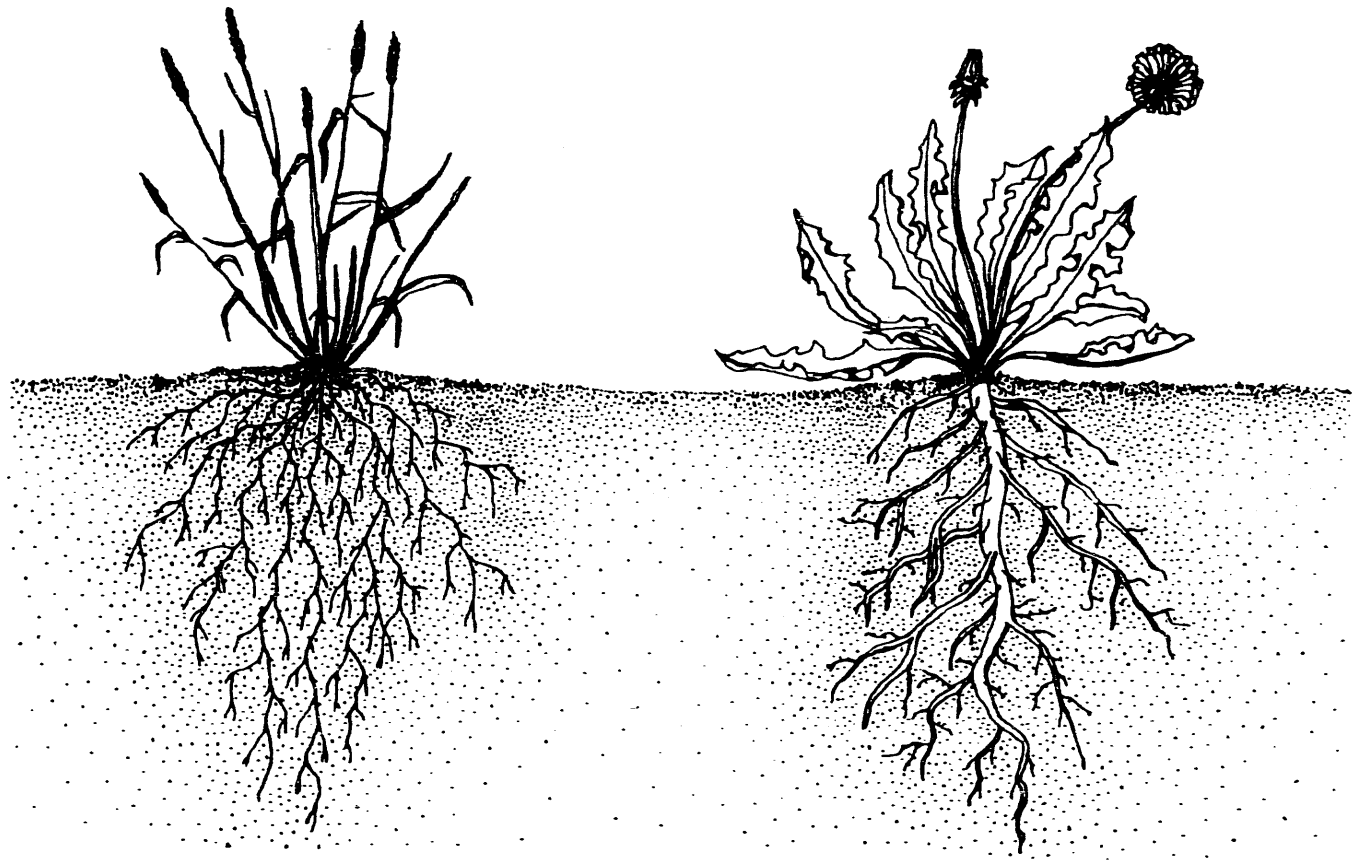

Figure I - 1. Drawing of the plant root-soil interface of two root types: a fibrous root system (left) and a tap root system (right). (Adapted from Stern, 1982)

rhizosphere characteristics briefly described translate into typical microbial populations an order of magnitude or more above microbial populations in nonvegetated soil. In the most simplistic scenario, the increased microbial activity and biomass in the rhizosphere would produce a faster degradation rate compared with nonvegetated soil. More complex scenarios could involve the role of plants in providing an external carbon source (exudates) in situations where the soil is contaminated with wastes that can be degraded cometabolically, such as trichloroethylene (TCE).

The specific use of vegetation to enhance the microbial degradation of surface and near-surface soils contaminated with hazardous wastes such as chlorinated solvents, polycyclic aromatic hydrocarbons (PAHs), or benzene-toluene-xylene (BTX) for example, is not known but could provide a cost-effective remediation strategy for soils containing low to moderate concentrations of these compounds when engineering approaches to cleanup are not practical. In addition, establishing or selectively cultivating vegetation on a contaminated site are relatively simple site management techniques. Overall, vegetation has the potential for providing a cost-effective, low-maintenance, aesthetically pleasing, in situ bioreactor.

The objective of this research was to determine whether vegetation could enhance microbial degradation of hazardous organic compounds in soils as a result of the rhizosphere effect. Trichloroethylene was used as a model compound in studies to identify critical environmental variables affecting the rate of TCE degradation in the root zone. Experiments were carried out using soils and plants from a TCE-contaminated field site. In addition, fundamental

studies on rhizosphere microbiology, microbial degradation of agricultural chemicals in the root zone, and recent research on the fate of non-agricultural chemicals in the rhizosphere are reviewed and presented as affirmation for the potential role of vegetation in enhancing remediation of sites contaminated with hazardous organic compounds.

CHAPTER II.

LITERATURE REVIEW

A. Physicochemical Properties of Trichloroethylene

Trichloroethylene (TCE) is a colorless, nonflammable, volatile liquid with a characteristic etheral odor that was commonly used as an industrial and dry-cleaning solvent, metal degreaser, and fumigant (Merck Index, 1989). These uses of TCE have contributed extensively to its occurance at waste sites and as a contaminant of groundwater (U.S. EPA, 1982). Physicochemical properties of TCE are shown in Table II - 1.

TCE is only slightly soluble in water. Its hydrophobic nature causes it to partition into soils and sediments, both from the liquid and vapor phases. Peterson and coworkers (1988) found soil-liquid partition coefficients for TCE of 0.26 cm^3/g on water-saturated synthetic soil (humic-coated alumina), 53.9 cm^3/g on synthetic soil with 11.6% water content, and 207 cm^3/g on synthetic soil with 8.2% water content. The above observation is consistent with the concept of water competing with organic compounds for sorption sites on soil, where decreasing water content leads to increasing sorption. The influence of moisture content on TCE sorption is further verified by Ong and Lion (1991). Lee et al. (1988) observed TCE sorption coefficients of 0.10 mL/g on Borden aquifer material (0.025% organic carbon) and 0.17 mL/g on Lula aquifer material (0.034% organic carbon).

Table II - 1. Physicochemical properties of trichloroethylene (TCE).

Properties of TCE	
Molecular formula	$Cl_2C=CHCl$
Molecular weight	132
Boiling point	87 °C
Vapor pressure	60 mm Hg at 20 °C
Solubility	1.1 g/L at 25 °C
Density	1.46 g/mL at 20 °C
Log_{10} octanol-water partition coefficient(log K_{ow})	2.36[A]
Henry's law constant (dimensionless)	0.397[B]
Log_{10} adsorption coefficient (log K_{oc})	2.6-2.7[B]

[A] From Mailhot and Peters, 1988
[B] From Lee et al., 1988

B. Mammalian Toxicity and Effects on Microorganisms

Mammalian Toxicity

Most chlorinated hydrocarbons are cytotoxic to the liver and kidney, and TCE is no exception (Williams and Weisburger, 1986). It induces liver neoplasms in mice and rats and can inactivate P-450 (mixed-function oxidase) catalyzed reactions by suicide inactivation (Sipes and Gandolfi, 1986). An enhanced TCE hepatotoxicity is observed after ethanol (Klaassen and Plaa, 1967), isopropanol, and acetone (Plaa et al., 1975) pretreatment. Necrosis and fatty liver are also signs of chronic TCE exposure (Plaa, 1986).

In mammalian systems, TCE undergoes microsomal oxidation via cytochrome P-450 (mixed-function oxidase) enzymes to a bioactivated epoxide metabolite (Miller and Guengerich, 1982). The epoxide intermediate of TCE metabolism may be mutagenic (Heath et al., 1977) and carcinogenic (Miller and Guengerich, 1982), but isolation has been difficult (Miller and Guengerich, 1983) due to its instability. The understanding of TCE metabolism via mixed-function oxidases in mamallian systems has aided in elucidating the pathway for aerobic degradation of TCE by microorganisms employing monooxygenase enzymes.

Effects on Microorganisms

Adsorption of contaminants to soil can result in exposure to soil microorganisms and potential toxicity. Kanazawa and Filip (1986, 1987) have examined the effects of TCE, tetrachloroethylene, and dichloromethane on enzymatic activities, biomass, and microbial counts in soil. They found

significant initial decreases in both ATP content and fungal counts in soil exposed to TCE at 1 µg/g. TCE also initially inhibited aerobic bacteria and actinomycetes at 10 µg/g, the highest concentration tested, although recovery occurred. However, soils exposed to the highest concentration of TCE showed increased microbial counts two months after exposure. The authors did not differentiate between increased microbial counts caused by microorganisms using TCE or its metabolites as a carbon and energy source or to infusion of carbon from microorganisms killed by the TCE. Enzyme assays for ß-glucosidase, ß-acetylglucosaminidase, phosphatase, phosphodiesterase, and proteinase showed no effect from TCE exposure at 0.1 µg/g. An initial inhibition was observed for ß-glucosidase and ß-acetylglucosaminidase at 1 µg/g, however, both enzymes returned to normal or slightly higher than normal levels. Although TCE at 10 µg/g initially inhibited all enzymes tested, their activities had returned to normal levels after two months. The authors did not determine whether residues of TCE were still present after two months.

Wackett and Householder (1989) measured the inhibition of cell growth and covalent modification of cellular macromolecules as indicators of the cytotoxic effects of TCE on *Pseudomonas putida* F1. Although this culture was capable of TCE degradation via the toluene dioxygenase pathway, initial degradation rates declined rapidly, possibly indicating the production of toxic intermediates. Exposure of TCE to mutant microorganisms lacking toluene dioxygenase activity showed no cytotoxic effects. Fractionation of *P. putida* F1 cells exposed to ^{14}C-TCE indicated most of the ^{14}C incorporated was associated with cell protein. Further chemical analyses of the protein fraction

implicated glyoxylic acid and formic acid as components of the protein adducts. The authors speculated that the alkylation of cellular macromolecules by formic and glyoxylic acid produced the observed cytotoxic effects.

The toxicity of TCE to a mixed culture of methanotrophs has also been determined (Broholm et al., 1990). Results from these experiments showed that increasing TCE concentrations from 0 to 65 mg/L translated into decreased methane consumption and decreased bacterial growth as measured by protein concentration. Although the authors did not speculate whether the decreased methane consumption was due to competitive inhibition of methane monooxygenase with TCE or simply to a decline in the population of methanotrophs, these initial results provide an important start to determining the optimal concentrations of methane and TCE to achieve TCE degradation by methanotrophs. In addition, the data have important implications for remediation using methane to enrich for methanotrophs in contaminated soils and groundwater.

C. Microbial Degradation of TCE

Details of several studies involving TCE degradation are shown in Table II - 2. Where possible, complete information on the system involved as well as the experimental conditions employed are provided. In addition, discussions on the significant findings of the experiments are reported below.

The first laboratory experiments on the degradation of TCE failed to show conclusive results (Bouwer et al., 1981). Under both aerobic and anaerobic conditions, no appreciable degradation of TCE was observed, although

Table II - 2. Microbial degradation of trichloroethylene (TCE).

Culture or Organism	Previous TCE exposure[A]	°C[B]	$[TCE]_i$[C]	$t_{1/2}$	O_2 conditions	Additions	Proposed Pathway	Ref.
soil	no	22	2 µg/g	NDO	aerobic	Barley	ND	(1)
Strain G4	yes	26	0.84 µM	12 h[D]	aerobic	NAS H_2O	aromatic	(2)
Strain G4	yes	26	0.70 µM	12 h[D]	aerobic	phenol	aromatic	(2)
Strain G4	yes	26	0.82 µM	70 h[D]	aerobic	none	aromatic	(2)
s ment	no	25	5 mg/L	93 d[D]	anaerobic	none	ND	(3)
sediment	no	25	5 mg/L	200 d[D]	anaerobic	none	ND	(3)
sediment	yes	25	5 mg/L	144 d[D]	anaerobic	none	ND	(3)
sediment	yes	25	5 mg/L	192 d[D]	anaerobic	none	ND	(3)
crushed rock	no	25	5 mg/L	394 d[D]	anaerobic	none	ND	(3)
crushed rock	no	25	5 mg/L	240 d[D]	anaerobic	none	ND	(3)
soil	no	25	150 µg/L	4 d[D]	aerobic	methane	MMO	(4)
soil	no	25	150 µg/L	6 d[D]	aerobic	none	MMO	(4)
mixed culture	no	20	600 ppb	70 h[D]	aerobic	methane	MMO	(5)
l culture	yes	22	50 mg/L	11 d[D]	aerobic	TY	ND	(6)
mixed culture	yes	22	150 mg/L	28 d[D]	aerobic	TY	ND	(6)
mixed culture	yes	22	50 mg/L	11 d[D]	aerobic	glucose	ND	(6)
mixed culture	yes	22	150 mg/L	58 d[D]	aerobic	glucose	ND	(6)
mixed culture	yes	22	50 mg/L	11 d[D]	aerobic	acetate	ND	(6)
mixed culture	yes	22	150 mg/L	44 d[D]	aerobic	acetate	ND	(6)
mixed culture	yes	22	50 mg/L	11 d[D]	aerobic	methanol	ND	(6)
mixed culture	yes	22	150 mg/L	NDO	aerobic	methanol	ND	(6)
mixed culture	yes	22	50 mg/L	NDO	aerobic	TCE	ND	(6)
mixed culture	yes	22	150 mg/L	NDO	aerobic	TCE	ND	(6)
mixed culture	yes	22	50 mg/L	NDO	aerobic	H_2-CO_2	ND	(6)
mixed culture	yes	22	150 mg/L	NDO	aerobic	H_2-CO_2	ND	(6)

Table II - 2 cont'd.

Culture or Organism	Previous TCE exposure[A]	°C[B]	$[TCE]_i$[C]	$t_{1/2}$	O_2 Conditions	Additions	Proposed Pathway	Ref.
mixed culture	yes	22	50 mg/L	NDO	aerobic	propane	ND	(6)
mixed culture	yes	22	150 mg/L	131 d[D]	aerobic	propane	ND	(6)
mixed culture	yes	22	50 mg/L	12 d[D]	aerobic	methane	ND	(6)
mixed culture	yes	22	150 mg/L	46 d[D]	aerobic	methane	ND	(6)
mixed culture	NA	35	180 µg/L	71 d[D]	anaerobic	none	RD	(7)
P. putida F1	NA	30	20 µM	1 h	aerobic	toluene	TDO	(8)
estuary	no	25	200 nM	15 d[D]	aerobic	none	aromatic	(9)
river	no	25	200 nM	25 d[D]	aerobic	none	aromatic	(9)
groundwater	no	25	200 nM	13 d[D]	aerobic	none	aromatic	(9)
estuary	no	25	200 nM	7 d[D]	aerobic	toluene	aromatic	(9)
river	no	25	200 nM	3 d[D]	aerobic	toluene	aromatic	(9)
groundwater	no	25	200 nM	4 d[D]	aerobic	toluene	aromatic	(9)
estuary	no	25	200 nM	29 d[D]	aerobic	phenol	aromatic	(9)
river	no	25	200 nM	7 d[D]	aerobic	phenol	aromatic	(9)
groundwater	no	25	200 nM	3 d[D]	aerobic	phenol	aromatic	(9)
groundwater	no	25	200 nM	2 d[D]	aerobic	PE	aromatic	(9)
sediment	no	25	2.5 mg/L	105 d	aerobic	none	ND	(10)
sediment	no	25	2.5 mg/L	39 d	aerobic	methane	ND	(10)
sediment	no	25	2.5 mg/L	383 d	aerobic	SO_4^{-2}	ND	(10)
sediment	no	25	2.5 mg/L	552 d	aerobic	ethylene	ND	(10)
soil (1-2 ft)	yes	23	2 mg/kg	10 y[D]	anaerobic	soybean	RD	(11)
soil (6-8 ft)	yes	23	2 mg/kg	28 y[D]	anaerobic	soybean	RD	(11)
soil (15-17 ft)	yes	23	2 mg/kg	30 y[D]	anaerobic	soybean	RD	(11)
Strain 46-1	yes	22	400 µg/L	25 d[D]	aerobic	methane	MMO	(12)
P. fluorescens	yes	25	100 µg/mL	25 d[D]	aerobic	MM	ND	(13)
P. mendocina	NA	NA	NA	4 h[D]	aerobic	media	TMO	(14)

Table II - 2 cont'd.

Culture or Organism	Previous TCE exposure[A]	°C[B]	$[TCE]_i$[C]	$t_{1/2}$	O_2 conditions	Additions	Proposed Pathway	Ref.
E. coli *	NA	NA	200 nM	3 h[D]	aerobic	media	TMO	(14)
soil	no	30	30 µg/kg	2.5 d[D]	aerobic	CH_4	MMO	(15)
groundwater	yes	NA	6.7 mg/L	25 d[D]	aerobic	none	ND	(16)
groundwater	yes	NA	0.56 mg/L	19 d[D]	aerobic	none	ND	(16)
sludge	no	35	5.25 mM	10 d[D]	anaerobic	media	RD	(17)
mixed culture	yes	23	0.9 mg/L	2 h[D]	aerobic	CH_4	MMO	(18)
mixed culture	yes	23	1.0 mg/L	2 h[D]	aerobic	CH_4	MMO	(18)
mixed culture	yes	23	1.1 mg/L	2 h[D]	aerobic	CH_4	MMO	(18)
P. oleovorans	no	NA	20 µM	NDO	aerobic	hexane	HMO	(19)
P. aeruginosa	no	NA	20 µM	NDO	aerobic	hexane	HMO	(19)
Mc	no	NA	20 µM	2 d[D]	aerobic	propane	PMO	(19)
Mr	no	NA	20 µM	1 d[D]	aerobic	propane	PMO	(19)
Mv	no	NA	20 µM	2 h[D]	aerobic	propane	PMO	(19)
Mt	no	30	.2 mg/mL	10 h[D]	aerobic	CH_4	MMO	(20)
Mt	no	30	.2 mg/mL	MD	aerobic	methanol	MMO	(20)
Mt	no	30	.2 mg/mL	1 h[D]	aerobic	formate	MMO	(20)
Mt	no	30	.2 mg/mL	MD	aerobic	none	MMO	(20)
sludge	no	35	60 nM	25 d[D]	anaerobic	none	RD	(21)
E. coli **	no	30	200 nmol	7 h[D]	aerobic	toluene	TDO	(22)
Mt	no	30	80 µmol	< 1 h[D]	aerobic	CH_4	MMO	(23)
Ne	NA	NA	1 ppm	1 d[D]	aerobic	NH_3	AMO	(24)
Ae	NA	30	25 µM	1 h[D]	aerobic	phenol	MO	(25)
Ae	NA	30	25 µM	4 h[D]	aerobic	2,4D	MO	(25)
Pc	yes	26	180 µM	6 h[D]	aerobic	phenol	ND	(26)
Pc	yes	26	140 µM	6 h[D]	aerobic	phenol	ND	(26)
Pc	yes	26	80 µM	6 h[D]	aerobic	phenol	ND	(26)

Table II - 2 cont'd.

Culture or Organism	Previous TCE exposure[A]	°C[B]	$[TCE]_i$[C]	$t_{1/2}$	O_2 conditions	Additions	Proposed Pathway	Ref.
Pc	yes	26	20 µM	6 h[D]	aerobic	phenol	ND	(26)
mixed culture	yes	NA	20 mg/L	3 d[D]	aerobic	$CH_4 + C_2H_6$	ND	(27)
mixed culture	yes	NA	20 mg/L	10 d[D]	aerobic	CH_4	ND	(27)
mixed culture	yes	NA	20 mg/L	3 d[D]	aerobic	C_2H_6	ND	(27)
mixed culture	NA	21	17 mg/L	2.5 h[D]	aerobic	CH_4	MMO	(28)
mixed culture	NA	21	6 mg/L	1.5 h[D]	aerobic	CH_4	MMO	(28)
mixed culture	NA	21	3 mg/L	0.6 h[D]	aerobic	CH_4	MMO	(28)
mixed culture	NA	21	0.6 mg/L	0.3 h[D]	aerobic	CH_4	MMO	(28)
Mt	no	30	250 µM	0.3 h[D]	aerobic	CH_4	MMO	(29)
mixed culture	yes	22	8 µg	0.3 h[D]	aerobic	CH_4	MMO	(30)
mixed culture	no	21	500 µg	0.2h[D]	aerobic	CH_4	MMO	(31)

Mc -Mycobacterium convolutum
Mr -Mycobacterium rhodochrous
Mv -Mycobacterium vaccae
Mt -Methylosinus trichosporium OB3b
Ne-Nitrosomonas europaea
Ae-Alcaligenes eutrophus
Pc-Pseudomonas cepacia
*Contains toluene monooxygenase plasmid
**Contains toluene dioxygenase genes
2,4D-2,4-dichloro phenoxyacetic acid
AMO-ammonia monooxygenase
HMO-hexane monooxygenase
MD-minimal degradation
MM-minimal salts media

Table II - 2 cont'd.

MMO-methane monooxygenase
MO-monooxygenase
NA-not available
NAS-Naval Air Station, Pennsacola, FL
NDO-no degradation observed
ND-not determined
PE-phenol enrichment
PMO-propane monooxygenase
RD-reductive dechlorination
TDO-toluene dioxygenase
TMO-toluene monooxygenase
TY-trypiticase plus yeast extract
[A]Contaminated Site
[B]Temperature
[C]Initial concentration
[D]Estimated from literature data
(1) Kloskowski et al., 1981
(2) Nelson et al., 1987
(3) Barrio-Lage et al., 1987a
(4) Henson et al., 1988
(5) Fogel et al., 1986
(6) Fliermans et al., 1988
(7) Bouwer and McCarty, 1983a
(8) Wackett and Gibson, 1988
(9) Nelson et al., 1988
(10) Barrio-Lage et al., 1988
(11) Kleopfer et al., 1985
(12) Little et al., 1988
(13) Vandenbergh and Kunka, 1988
(14) Winter et al., 1989
(15) Uchiyama et al., 1989

Table II - 2 cont'd.

(16) McClellen et al., 1989
(17) Freedman and Gossett, 1989
(18) Strandberg et al., 1989
(19) Wackett et al., 1989
(20) Oldenhuis, et al., 1989
(21) Baek and Jaffe, 1989
(22) Zylstra et al., 1989
(23) Tsien et al., 1989
(24) Vanelli et al., 1990
(25) Harker and Kim, 1990
(26) Folsom et al., 1990
(27) Phelps et al., 1990
(28) Alvarez-Cohen and McCarty, 1991a
(29) Oldenhuis et al., 1991
(30) Henry and Grbic-Galic, 1991
(31) Alvarez-Cohen and McCarty, 1991b

bromodichloromethane, dibromochloromethane, and chloroform were readily removed by mixed cultures from sewage effluent. These results were consistent with field observations at the Palo Alto groundwater recharge project (Roberts et al., 1980), which documented the persistence of TCE in groundwater. However, in subsequent experiments, Bouwer and McCarty (1983a) found that TCE could be degraded under methanogenic conditions in both batch and continuous flow reactors. It was not clear from this paper whether or not the reactors were seeded with cultures from a contaminated site to achieve the nearly 40% reduction of TCE that was observed after 8 weeks. No TCE was lost from sterile (un-innoculated) controls. The accumulation of dichloroethylene (DCE) in the reactor spiked with TCE supported the authors speculation of reductive dehalogenation as the mechanism of degradation. Tracer experiments using ^{14}C-TCE were not performed to confirm the degradation pathway of TCE.

Further proof of reductive dehalogenation as a mechanism for tthe degradation of halogenated aliphatic compounds under anaerobic conditions came from Parsons and co-workers (1984) who observed the disappearance of tetrachloroethylene (PCE) with concommitant accumulations of TCE and cis- and trans-DCE in freshwater organic sediment samples. These studies were initiated in part because of field observations describing the appearance of chloroethene and cis- and trans-DCE in groundwater from areas where these compounds were not used but TCE and PCE were previously used. Although anaerobic or nearly anaerobic conditions were presumed in the microcosms, dissolved oxygen content and redox potential in the samples was not determined. In a study utilizing a system less complex than the organic

sediments, Parsons and Barrio-Lage (1985) used cultures of aquatic bacteria isolated from a TCE spill site to monitor TCE and PCE degradation. Reductive dehalogenation of TCE and PCE occurred, producing both isomers of 1,2-DCE; however, the cis isomer was produced in greater amounts. The authors speculated that the more polar cis isomer was preferentially formed because of the high polarity of the aqueous medium.

Kleopfer and co-workers (1985) used soil from a TCE spill site, ^{13}C-labelled TCE, and gas chromotography/mass spectrometry to conclusively demonstrate reductive dechlorination of TCE in soil. Soybean meal was added to the soil samples to help produce anaerobic conditions although oxygen concentration in the samples was not determined. Only 1,2-DCE was produced from TCE dechlorination, however, the cis and trans isomers could not be differentiated. No 1,1-DCE was found in the samples. Interestingly, microorganisms in soils collected from three different depths in the soil profile were equally effective at dehalogenation of TCE. The authors speculated that in the field, TCE dehalogenation to DCE may be even faster due to the absence of an easily utilized carbon and energy source such as soybean meal. However, the soybean meal may not have been appreciably metabolized under anaerobic conditions.

Vogel and McCarty (1985), using both small (2-day liquid retention time) and large (6-day liquid retention time) continuous-flow, fixed-film, methanogenic columns, observed 99% removal of PCE. In the small-column study, TCE was the major product formed whereas vinyl chloride was the major metabolite in the large-column study. The authors used both liquid scintillation spectroscopy

and gas chromatography/mass spectroscopy to monitor reductive dehalogenation of ^{14}C-PCE and PCE, respectively. These studies further implicated reductive dehalogenation as the degradative mechanism for chlorinated ethylenes in anaerobic systems. In the tracer studies, $^{14}CO_2$ was detected, indicating complete mineralization of ^{14}C-PCE. In addition, the authors proposed a pathway for reductive dehalogenation of PCE to CO_2 by way of TCE, DCE, and vinyl chloride (Figure II - 1). Whether vinyl chloride could be converted to CO_2 under anaerobic conditions is still speculative. However, because of its carcinogenicity (Williams and Weisburger, 1986), the eventual formation of vinyl chloride would not be desirable in situations where in situ remediation of groundwater and/or soils is the goal. Recent work on reductive dechlorination of chlorinated ethylenes has shown that PCE and TCE can be converted to ethylene through DCE and vinyl chloride (Freedman and Gossett, 1989) and vinyl chloride can be metabolized under aerobic conditions by actinomycetes (Phelps et al., 1991b). Unlike the results of Vogel and McCarty (1985, 1987) no significant amounts of CO_2 were produced.

Although the studies described above showed reductive dechlorination of TCE under methanogenic conditions, it is important to note that these studies were undertaken using mixed cultures. Only recently has research on TCE degradation under methanogenic conditions with pure cultures of methanogens been conducted (Baek and Jaffe, 1989). These studies were important for elucidating the potential role of nonmethanogenic anaerobes in TCE degradation. Comparisons of vials innoculated with methanogens and vials innoculated with methanogens and nonmethanogenic anaerobes indicated

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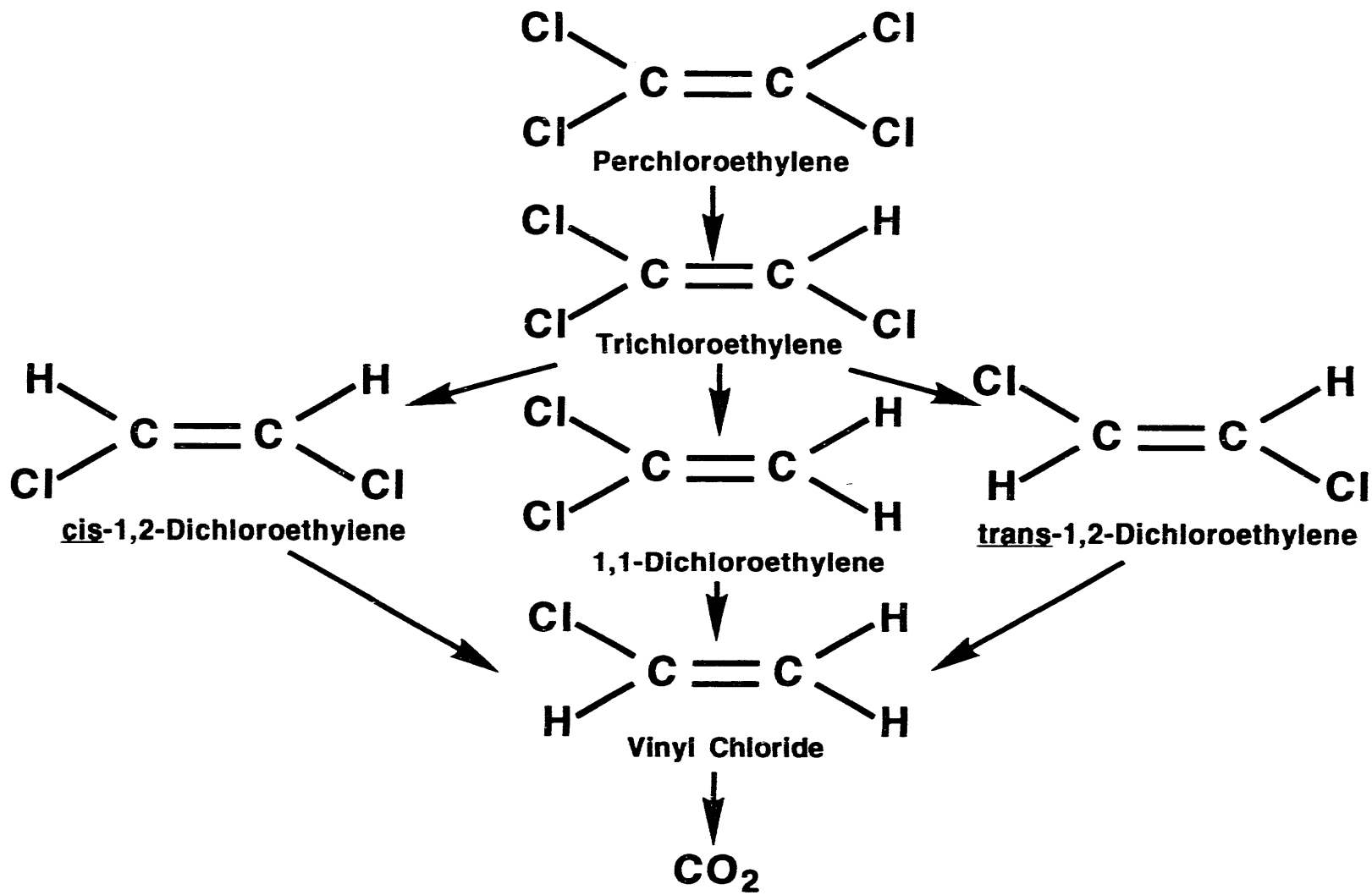

Figure II - 1. Reductive dechlorination of chlorinated ethylenes under anaerobic (methanogenic) conditions.

increased TCE degradation in the vials containing the nonmethanogenic anaerobes indicating their capability to also dechlorinate TCE.

Methane-oxidizing bacteria oxidize and dechlorinate halogenated methanes via methane monooxygenase (Haber et al., 1983). This observation, coupled with the observation of ethylene epoxidation by propane-oxidizing bacteria (Hou et al., 1983) lead Wilson and Wilson (1985) to hypothesize that TCE transformation by methane- and propane-oxidizing bacteria might be possible and may proceed by these same mechanisms. They exposed soil columns to natural gas to enrich for methanotrophs and then compared the fate of TCE in these columns to unexposed soil columns. The 150 cm-long soil columns were moistened daily with H_2O containing TCE (150 µg/L). Less than 5% of the applied TCE passed completely through the natural gas-exposed soil column during the 2-week experimental period. Soil columns not previously exposed to natural gas showed no significant TCE degradation. The authors speculated that the TCE was transformed to CO_2, and that the transformation was an aerobic process. This was the first observation of aerobic TCE metabolism. Because the soil used was sandy, low in organic carbon (<0.45%), and not compacted, aerobic conditions were probably maintained throughout the study. Field applications of this technique may be hampered by the difficulty of maintaining aerobic conditions in the soil profile. However, in the field, water (and the TCE dissolved in it) would not be expected to percolate through the soil as quickly as is observed in packed laboratory soil columns. It is encouraging to note that TCE-degrading populations can be enriched in soil with no previous TCE exposure. Phospholipid ester-linked fatty acid (PLFA)

analyses of the natural gas-exposed soil column indicated characteristic fatty acids typical of type II methanotrophic bacteria (Nichols et al., 1987). In a similar study, Henson and co-workers (1988) also exposed sandy soil to an atmosphere of 4% methane for 6 weeks prior to testing for TCE and PCE degradation. They found significantly greater degradation rates for TCE in the methane-enriched soil, further promoting the role of methanotrophic bacteria in TCE metabolism. In addition, once the soil was enriched with methane, its (methane) presence was not required except for removal of TCE below the detection limits (which were not reported). There was no significant difference between PCE degradation by the methane-enriched and control soils.

Recent research has shown that, in addition to soil, sediment samples may also be enriched with methanotrophic bacteria by exposure to methane (Fliermans et al., 1988; Barrio-Lage et al., 1988; Phelps et al., 1990). Subsurface sediment samples collected from a solvent-contaminated area at the Savannah River Site near Aiken, SC, were used by Fliermans et al. (1988) to determine TCE mineralization by heterotrophic enrichment cultures. They found that methane, methanol, acetate, glucose, and trypticase plus yeast extract stimulated the degradation of TCE (initial concentration 50 mg/L) often leading to 99.8% removal. Degradation of TCE at higher concentrations varied from undetected to 37% among the different enrichments. Additional experiments with the cultures isolated from contaminated sediments at the Savannah River Site were conducted in expanded-bed bioreactors (Phelps et al., 1990; Phelps et al., 1991a). Up to 95% of the TCE added was degraded in the reactors after 5 days. Phelps and coworkers also showed that pulse-feed

reactors had higher TCE degradation rates compared with continuous-feed reactors. These results are very encouraging for groundwater remediation using pump and treat techniques. In similar experiments, Barrio-Lage et al. (1988) used sulfate, methane, and ethylene enrichments to study TCE degradation in sediment microcosms. Methane additions were found to increase the biotransformation rate of TCE, whereas sulfate and ethylene additions had the opposite effect.

Methane has also been used extensively in enrichments for methanotrophic bacteria in the liquid environments of culture flasks (for example, Fogel et al., 1986; Little et al., 1988) as opposed to soil. Fogel and co-workers (1986) used methane enrichment techniques to determine whether liquid cultures of methane-utilizing bacteria could degrade TCE and PCE. They found that 23% of the added TCE appeared as CO_2 after 2 days. The remaining TCE was transformed to water-soluble products or incorporated as bacterial biomass. Additionally, acetylene, methanol, and 2-propanol inhibited the biodegradation of TCE. PCE transformation during 190 hours of incubation was not observed (0.1 µg/L detection limit). The first observation of TCE biodegradation by pure cultures of methanotrophic bacteria was reported by Little et al. (1988). In addition, they determined the dependence of methane concentration and proposed a pathway for TCE degradation. Strain 46-1, a type I methanotroph, converted 40% of the added TCE after 20 days. The maximum rate of TCE degradation occurred during active cell growth and stopped after methane depletion. In addition, methanol was shown to stimulate biodegradation of TCE by strain 46-1. The pathway proposed by Little and co-

workers (Figure II - 2) involves formation of TCE epoxide from TCE via methane monooxygenase (MMO). TCE epoxide then spontaneously breaks down to form dichloroacetic acid, glyoxylic acid, carbon monoxide, or formate. Previous research has shown that glyoxylic acid and dichloroacetic acid appear during decomposition of TCE epoxide (McKinney et al., 1955; Henschler et al., 1979). Ion chromotographic analysis of the culture medium following the TCE degradation experiments demonstrated the presence of both of these compounds. Strain 46-1 and other organisms from the methanotrophic consortia of Little et al. (1988) has also been used in packed-bed bioreactors (Strandberg et al., 1989) illustrating a possible use of this consortia in pump and treat applications.

The literature on TCE degradation by methanotrophic microorganisms is exciting because of the potential for field applications. Laboratory experiments have shown not only the TCE oxidation ability of these bacteria, but also that enrichment for methanotrophs is easily accomplished. Current research on TCE metabolism by methanotrophic organisms has focused attempts to identify the type(s) of methanotroph(s) (Type I versus. Type II) and enzymatic form(s) of methane monooxygenase (soluble MMO versus. particulate MMO) responsible for TCE degradation (Oldenhuis et al., 1989;Tsien et al., 1989; Uchiyama et al., 1989; Phelps et al., 1990) the influence of TCE toxicity to microorganisms on TCE degradation rates (Broholm et al., 1990; Alvarez-Cohen and McCarty, 1991a, 1991b, 1991d; Oldenhuis et al., 1991; Henry and Grbic-Galic, 1991a and 1991b) the influence of culture growth on treatment of contaminated groundwater in bioreactors (Alvarez-Cohen and McCarty, 1991c) and the fate of

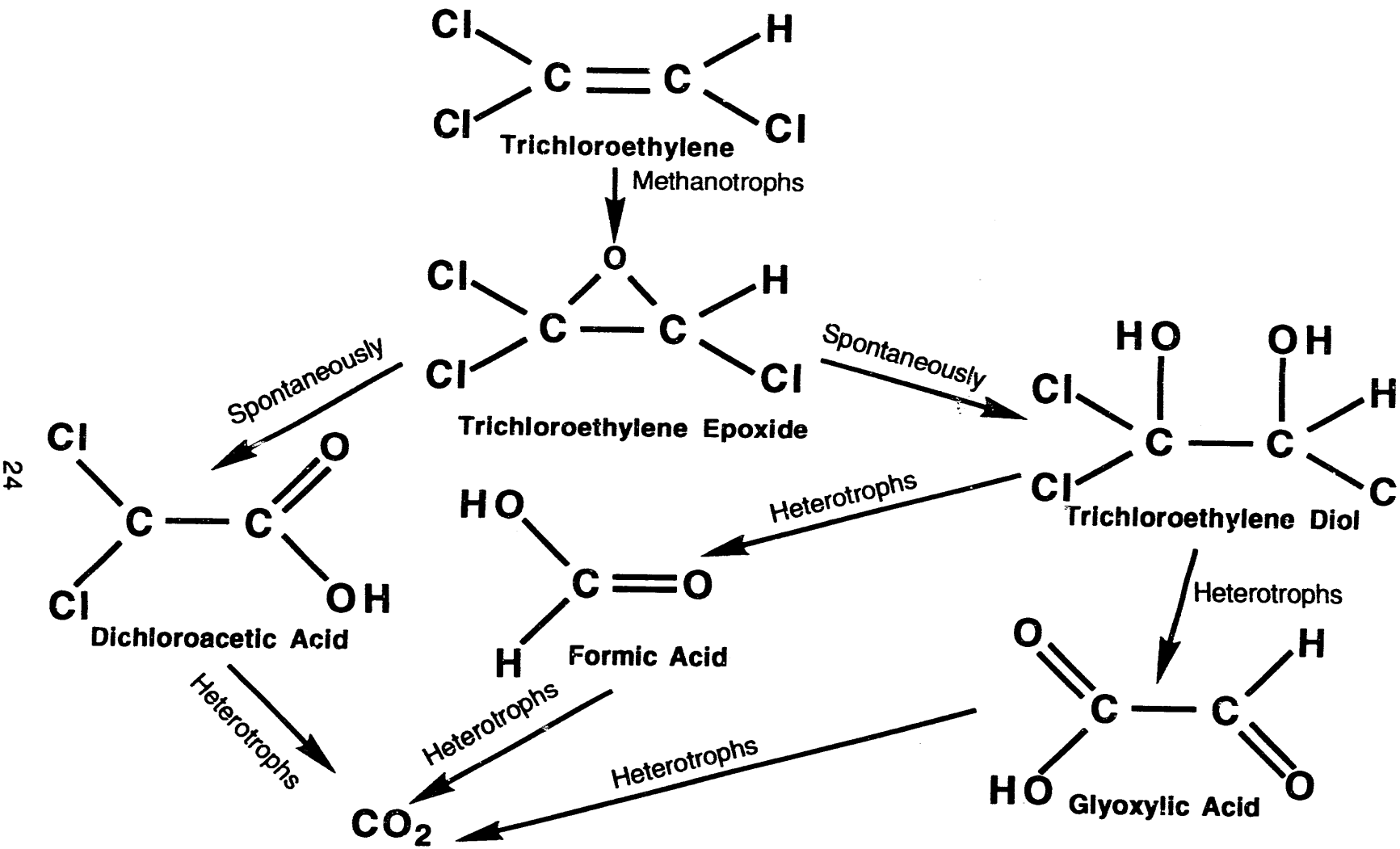

Fig. II - 2. Aerobic microbial degradation of trichloroethylene (TCE).

metabolic products of TCE oxidation in the environment (Newman and Wackett, 1991).

Barrio-Lage et al. (1987b) studied the influence of environmental factors other than TCE toxicity on TCE degradation in sediment samples. They observed a depletion of TCE with simultaneous production of cis-1,2-DCE (via reductive dechlorination) in all 3 types of sediment studied; muck, sand, and rock. In sediments containing significant amounts of organic carbon (>2%), a nonlinear form of Michaelis-Menten kinetics modeled the TCE degradation. However, in the crushed rock microcosms low in organic carbon content (<1%) the linear form of the Michaelis-Menten equation gave the best fit (Barrio-Lage et al., 1987a). The successful use of different kinetic models implies that the rate of TCE degradation varies under different organic carbon conditions. Acetate additions had little or no effect on the rate of TCE degradation, leading the authors to speculate that metnanogens were not involved in the disappearance of TCE. In addition, denitrifying bacteria were not involved in the reductive dehalogenation of TCE in this study as was observed earlier by Bouwer and McCarty (1983b).

In addition to the above research on aerobic TCE degradation by methanotrophic microorganisms, recent research has also implicated the enzymes and pathways involved in degradation of aromatic compounds by microorganisms for the metabolism of TCE (Nelson et al., 1986; 1987; 1988; Wackett and Gibson, 1988). Nelson and co-workers' (1986) observation that TCE was degraded only in the presence of water from an industrial waste treatment facility (U. S. Naval Air Station (NAS), Pensacola, Florida) lead them

to speculate that some component of the NAS water was required for TCE metabolism. This component was later identified as phenol (Nelson et al., 1987). Subsequently, Nelson and co-workers demonstrated that toluene, o-cresol, and m-cresol also stimulated TCE degradation and that a pure culture of toluene degraders (*Pseudomonas putida*) could also metabolize TCE (Nelson et al., 1988). Mutant strains of *P. putida* lacking toluene dioxygenase activity could not degrade TCE; however, revertants regained the TCE-degradative ability. These results provided evidence for the involvement of the aromatic degradative pathway and toluene dioxygenase in TCE metabolism. In addition, these results were independently verified by Wacket and Gibson (1988) during research with *P. putida* F1. Comparison of TCE degradation rates for strain F1 and the methanotroph *Methylosinus trichosporium* OB3b revealed a significantly greater initial rate for strain F1. This observation is based on inspection of graphed data only; the degradation rate for OB3b was never reported. Although the rapid TCE degradation rate by strain F1 is initially exciting, the fact that toluene must be added to induce the toluene dioxygenase activity makes it unattractive for in situ remediation. In addition, the initial rapid TCE degradation rate observed for strain F1 decreased dramatically with time such that a more sustained TCE degradation rate was obtained for OB3b. This observation is encouraging for groundwater or soil remediation applications based on the comparative ease of adding methane rather than toluene to an aquifer or contaminated soil. However, recent work with the Pseudomonads has illustrated their potential in bioreactors (Folsom and Chapman, 1991).

The involvement of the aromatic degradative pathway in TCE metabolism was also illustrated by the results of Winter et al. (1989) in which a recombinant *Escherichia coli* culture containing the toluene monooxygenase (TMO) gene from *Pseudomonas mendocina* was capable of TCE metabolism. Although cultures of *P. mendocina* could metabolize TCE, toluene was required for TMO enzyme induction. Once the TMO gene was transferred to *E. coli*, the gene could be induced by a simple temperature change. This eliminated the need for toluene as an inducer of TCE metabolism in the recombinant *E. coli* cells and probably gave new impetus for exploring the potential for organisms that possess the TMO gene in contaminated site remediation. It certainly would have been unattractive to add toluene to a contaminated aquifer or bioreactor to induce TCE degradation.

Similar results on TCE degradation were obtained by Zylstra and coworkers (1989) using *E. coli* cells containing the cloned toluene dioxygenase (TDO) genes from *Pseudomonas putida*. The recombinant *E. coli* cells required isopropyl-ß-D-thiogalactopyranoside for TDO enzyme induction. The authors did not speculate whether this chemical could be used in contaminated environments to induce TDO enzyme activity during in situ remediation, or whether it could be used to induce TDO activity in bioreactors.

Vandenbergh and Kunka (1988) have reported TCE degradation in vials containing a pure *Pseudomonas fluorescens* culture. Interestingly, no mechanism for TCE removal was proposed although the authors noted that the *P. fluorescens* culture was isolated from soil with a history of 1,2-dichloroethane and 1,2-dichloropropane contamination. Although only 2% of

the TCE was metabolized after 24 hours, these results are encouraging in that the capability to degrade TCE may not be as rare or confined to specialized groups of bacteria as initially expected based on the earlier studies of TCE degradation. The ability to oxidize TCE may be acquired through prolonged exposure to TCE or related compounds. These results are further emphasized by the work of McClellen et al. (1989), who made similar observations with a mixed culture from a TCE-contaminated site. Although no substrate other than TCE was added to the cultures, the microorganisms in the mixed culture were able to degrade 47% and 33% of the TCE at initial concentrations of 0.56 mg/L and 6.7 mg/L, respectively.

With the wealth of information documenting TCE oxidation (in some cases very rapid TCE oxidation) in laboratory experiments, one wonders why TCE would be such a large environmental problem. It appears that conditions in the field (whether it be aeration, temperature, toxicity, etc.) are in some way hindering microbial degradation of TCE. Researchers have realized this, and thus some common themes of laboratory studies during the last two years (1990 and 1991) have been identifying new organisms and pathways for TCE metabolism (Harker and Kim, 1990; Vanelli et al., 1990) and environmental factors such as substrate interactions (Folsom et al., 1990), substrate toxicity and aeration (Alvarez-Cohen and McCarty, 1991a and 1991b), and electron donors (Henry and Grbic-Galic, 1991a) influencing TCE oxidation.

The literature on microbial degradation of TCE reviewed above illustrates some common themes. Under anaerobic (methanogenic) conditions, TCE is converted to vinyl chloride via dichlorethylene (1,1-dichloroethylene, cis-1,2-

dichloroethylene, or trans-1,2-dichloroethylene) by reductive dechlorination (dehalogenation). Vinyl chloride can be converted to CO_2 or ethylene. In contrast, under aerobic conditions, TCE can be converted cometabolically by a variety of oxygenases including methane monooxygenase (MMO), ammonia monooxygenase (AMO), toluene monooxygenase (TMO), toluene dioxygenase (TDO), and propane monooxygenase (PMO) to an epoxide intermediate (TCE epoxide). TCE conversion via MMO is carried out by methanotrophic microorganisms that depend on methane for carbon and energy. Methanotrophic organisms are divided into two groups (Type I and Type II) based on the morphology of there intracellular membranes (Large, 1983). The role that the different types of methanotrophs play in TCE metabolism is currently being debated. There is evidence in the literature for both groups; Type I (Little et al., 1988) and Type II (Wilson and Wilson, 1985; Phelps et al., 1990). In addition, the form of MMO (soluble or particulate) involved in TCE metabolism by methanotrophs is also a topic of discussion. The current consensus is that soluble MMO is the form of the enzyme responsible for the oxidation of TCE (Oldenhuis et al., 1989), although results are influenced by micronutrient concentrations (especially copper) in the culture medium. The propane- and ammonia-oxidizing bacteria that cometabolically degrade TCE accomplish the oxidation via PMO and AMO, respectively. The microorganisms involved in TCE oxidation through the aromatic degradation pathways use both TMO and TDO. These enzymes are induced by a variety of substrates including phenol and toluene.

A shared motif among the oxygenases is their mechanism of action. All have a substrate binding site and an oxygen binding site. Production of the TCE epoxide is accomplished through creation of a reactive oxygen species which epoxidates the double bond between the carbon atoms of TCE. The epoxide intermediate formed from TCE by the variety of oxygenases described above, is very reactive and unstable. Subsequently, it spontaneously breaks down to water-soluble products (formic acid, glyoxylic acid) that can be readily metabolized by heterotrophic microorganisms. Occasionally, when formic acid and glyoxylic acid accumulate, cytotoxic effects are observed.

Continued exploration of the variables affecting TCE degradation including substrate interactions and the myriad of environmental conditions in the micro-environment of bacteria will help identify situations where microbial degradation of TCE is and is not possible. The discovery and identification of novel new microorganisms capable of TCE oxidation could also provide more information on the mechanisms involved as well as potentially supply researchers with more effective microorganisms to employ in site restoration. In addition, continued research on TCE oxidation in bioreactors and eventually field plots will help advance the concept of using bacteria to remediate TCE-contaminated environments.

D. Rhizosphere Microbiology

Plant roots affect the soil in which they grow in a multitude of ways. making the soil more conducive for microbial growth and activity. All roots are protected from abrasion by root cap cells which are sloughed off during root

growth; sometimes as many as 10,000 cells per plant per day in the case of maize (Campbell, 1985). As the root grows downward, cells in the root cap known as dictysomes produce a gel that helps lubricate the root cap (Foster et al., 1983), allowing the root to force its way through the soil. This mucigel (Leiser, 1968), rich in carbohydrates and amino acids, along with other root excretions are classified as root exudate (Rovira et al., 1979). Both of these components, root cap cells and root exudates, are useful sources of nutrients for microorganisms surrounding the root. In addition, microorganisms in the root zone can act as sinks for exudates with the absence of bacteria leading to less production of this material (Campbell, 1985). First described for legumes by Hiltner (1904), the rhizosphere has been the focus of agricultural research for several years, primarily because of its influence on crop productivity. Several excellent comprehensive reviews on the rhizosphere are available (Brock, 1966; Gray and Parkinson, 1968; Campbell, 1985; Curl and Truelove, 1986). The extensive nature of these reviews will limit this review to discussing isolated findings on rhizosphere microbiology within the context of the problem at hand; using the rhizosphere to facilitate bioremediation of contaminated sites.

Continual change at the root-soil interface, both physical and chemical, produces constant alterations in the soil structure and microbial environment. Differences in carbon dioxide and oxygen concentrations, osmotic and redox potentials, pH, and moisture content exist between rhizosphere and bulk soil (Foster et al., 1983). Although the modification of the soil in the rhizosphere by plant root secretions is an important process that influences microbial populations, it is the actual structure of the plant root that provides

microorganisms with a large surface area for colonization (Russell, 1977; Lynch, 1982). Using both simple and electron microscopy, various researchers (Linford, 1942; Campbell and Rovira, 1973; Rovira et al., 1974) have described the increased abundance of microorganisms in the rhizosphere. This rhizosphere effect is often quantified as the ratio of microorganisms in rhizosphere soil to the number of microorganisms in non-rhizosphere soil, the R/S ratio (Katznelson, 1946). R/S ratios from 5 to 20 are common, but occasionally are as high as 100 and above (Katznelson, 1965; Gray and Parkinson, 1968). In the simplest of scenarios, this increase in microbial numbers would translate to increased degradation rates of organic compounds in the rhizosphere.

In addition to the increase in numbers of microorganisms associated with the plant root, the actual composition of rhizosphere microorganisms is dependent on plant species, plant age, and soil type (Campbell, 1985) as well as other selection pressures (Sandmann and Loos, 1984; Abdel-Nasser et al., 1979; Abueva and Bagaev, 1975; Gavrilova et al., 1983). Typically, the rhizosphere is colonized by a predominantly gram negative microbial community (Atlas and Bartha, 1987). The ability of the plants to select for different rhizosphere microbial communities is intriguing from the standpoint of exploring whether this selection translates to differences in the rates of micobial degradation of organic compounds in the rhizosphere. If such relationships exist, it may then be possible to use this knowledge in management of sites contaminated with different types of hazardous organic compounds.

E. Degradation of Organic Compounds in the Rhizosphere

Because of its importance in crop productivity, research on microbial transformations in the rhizosphere has been concerned mainly with agricultural chemicals, such as pesticides and fertilizers. The phenomenon of enhanced microbial degradation of these compounds in the rhizosphere was probable due to the increased density and diversity of microorganisms, their intense activity, and the existence of cometabolic transformations. Table II - 3 lists studies on the degradation of compounds in the rhizosphere. The data presented here, along with the research on rhizosphere microbiology and microbial degradation of TCE provide the foundation for an exploration of the use of vegetation to remediate sites contaminated with hazardous organic compounds.

Several researchers (Hsu and Bartha, 1979; Seibert et al., 1981; Reddy and Sethunathan, 1983; Sandmann and Loos, 1984; Lappin et al., 1985) have described an increased capacity for mineralization of various pesticides in the rhizosphere. Occasionally, this increased mineralization capacity is correlated with increased numbers of pesticide-degrading microorganisms. Sandmann and Loos (1984) found an increase in the number of 2,4-D (2,4-dichlorophenoxyacetate)-degrading bacteria in the rhizospheres of previously untreated African clover and sugarcane. Earlier work by Abdel-Nasser and co-workers (1979) showed higher microbial counts in the rhizospheres of corn, beans, and cotton treated with temik [2-methyl-2(methylthio)propionaldehyde O-(methylcarbamoyl)oxime]. Recently, Sato (1989) found an 8-fold increase in heterotrophic bacteria in rice rhizosphere after benthiocarb (S-p-chlorobenzyl

Table II - 3. Studies on the fate of agricultural chemicals in the rhizosphere.

Rhizosphere	Compound	Concentration	Comments	Reference
Wheat (mixed culture)	Mecoprop[A] 2,4-D[B] MCPA[C]	0.53 and 1.1 g/L 0.57 g/L 0.46 g/L	Mixed culture capable of using compounds as a carbon source.	(1)
Sugarcane (mixed culture)	2,4-D[B]	0.15 g/L	High population of 2,4-D-degrading microorganisms in the rhizosphere.	(2)
Bush bean (mixed culture)	Diazinon[D] Parathion[E]	5 µg/g 5 µg/g	Increased mineralization of both compounds in the rhizosphere.	(3)
Rice (mixed culture)	Carbofuran[F]	10 µg/mL 100 µg/mL	Stimulated ammonium oxidation (nitrification) in rhizosphere soil.	(4)
Rice (mixed culture)	Parathion[E]	20 µg/g	Increased mineralization in the rhizosphere especially under flooded conditions.	(5)
Tobacco (mixed culture)	MH[G]	30%	MH caused an enhanced nitrification and mineralization of organic substances in the rhizosphere.	(6)

Table II - 3 cont'd.

Rhizosphere	Compound	Concentration	Comments	Reference
Corn Beans Cotton	Temik[H]	NA	Higher counts of micro-organisms in treated vs. untreated rhizospheres.	(7)
Wheat Corn Peas	Diazinon[D]	NA	Rhizosphere microbial counts increased by 2 orders of magnitude.	(8)
NA	2,4-D[B]	6 kg	Ammonifying, nitrifying, and cellulose-decomposing bacteria in the rhizosphere increased by 1 to 2 orders of magnitude.	(9)
Corn	Atrazine[I]	NA	Increase in Atrazine degradation by rhizosphere microorganisms in the presence of decomposing roots.	(10)
Rice	Benthiocarb[J]	NA	Eight-fold increased in heterotrophic bacteria.	(11)

Wheat--(*Triticum* sp.)
Sugarcane--(*Saccharum officinarum*)
Bush bean--(*Phaseolus vulgaris*)
Rice--(*Oryza* sp.)
Tobacco--(*Nicotiana* sp.)

Table II - 3 cont'd.

Corn--(*Zea* sp.)
Cotton--(Malvaceae)
Peas--(*Pisum* sp.)
NA--Not Available
[A]2-(2-methyl-4-chlorophenoxy)propionic acid
[B]2,4-dichlorophenoxyacetic acid
[C]2-methyl-4-chlorophenoxyacetic acid
[D]O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate
[E]O,O-diethyl-O-p-nitrophenyl phosphorothioate
[F]2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate
[G]maleic hydrazide (1,2-dihydro-3,6-pyridazinedione)
[H]2-methyl-2(methylthio)propionaldehyde O-(methylcarbamoyl)oxime
[I]2-chloro-4-ethylamino-6-isopropylamino-s-triazine
[J]S-p-chlorobenzyl diethylthiocarbamate
(1) Lappin et al., 1985
(2) Sandmann and Loos, 1984
(3) Hsu and Bartha, 1979
(4) Ramakrishna and Sethunathan, 1982
(5) Reddy and Sethunathan, 1983
(6) Ampova, 1971
(7) Abdel-Nasser et al., 1979
(8) Gavrilova et al., 1983
(9) Abueva and Bagaev, 1975
(10) Seibert et al., 1981
(11) Sato, 1989

diethylthiocarbamate) addition. The numbers of nitrifying bacteria also increased following benthiocarb exposure.

Seibert and co-workers (1981) observed an increase in atrazine (2-chloro-4-ethylamino-6-isopropylamino-S-triazine) degradation by rhizosphere microorganisms in the presence of decomposing roots. They argued that this increase could be attributed to the higher dehydrogenase activity observed in the rhizosphere soil throughout the experimental period. Also, the concentration of unchanged atrazine was lower in the rhizosphere soil, and the concentration of hydroxyatrazine and two other hydroxylated metabolites were 3-fold higher than concentrations outside the rhizosphere. Studies on labelled CO_2 evolution from parathion (O,O-diethyl-O-p-nitrophenyl phosphorothioate) in rice rhizospheres indicated similar results (Reddy and Sethunathan, 1983). Only 5.5% of the ^{14}C in the parathion was evolved as $^{14}CO_2$ in unplanted soils while 9.2% was evolved from rhizospheres under non-flodded conditions. The rice variety in this experiment grew better in flooded soil, thus when flooded conditions prevailed, 22.6% of the radiocarbon was evolved as $^{14}CO_2$. Reddy and Sethunathan argued that the close proximity of the aerobic-anaerobic interface in rice rhizosphere under flooded conditions favored the ring cleavage of parathion.

Parathion and diazinon [O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate] appear to be degraded in soil initially by cometabolic attack (Gunner et al., 1966; Daughton and Hsieh, 1977), a process that requires the presence of a growth substrate other than the compound being degraded. As indicated earlier, root exudates provide microorganisms with a

wide range of organic substrates for use as carbon and energy sources. These factors lead Hsu and Bartha (1979) to hypothesize that the rhizosphere would be especially favorable for cometabolic transformations of pesticides. Using radiolabelled diazinon and parathion, they were able to show accelerated mineralization of these compounds in bean rhizospheres. Beans were chosen because of their published inability to metabolize diazinon (Gunner et al., 1966). Approximately 18% of the parathion and 13% of the diazinon were mineralized in the bean rhizospheres compared with 7.8% and 5.0% in the root-free soil for parathion and diazinon, respectively. Similar results with diazinon were previously found by Gunner and co-workers (1966), although they did not observe an increase in microbial biomass in the rhizosphere after diazinon application. Rather, the diazinon exerted a selective effect, which translated into the enrichment of a particular isolate capable of diazinon metabolism.

Lappin et al. (1985) found that a microbial community isolated from wheat roots was capable of growth on the herbicide mecoprop [2-(2-methyl 4-chlorophenoxy)propionic acid] as the sole carbon and energy source. The authors isolated five species, none of which was capable of growth on mecoprop individually. This microbial community was also shown to degrade 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (2-methyl-4-chlorophenoxy acetic acid).

The increased mineralization capacity of the rhizosphere is not limited to pesticide degradation. Molla and co-workers (1984) showed that phosphate mineralization was maximized in the rhizospheres of wheat and ryegrass while Nakas and Klein (1980) reported an increase in glucose degradation

associated with the rhizospheres of a shortgrass prairie ecosystem. In addition, Ramakrishna and Sethunathan (1982) found enhanced autotrophic ammonium oxidations in rice rhizospheres exposed to carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) and Ampova (1971) observed a stimulation of nitrification and mineralization of organic substances in tobacco rhizospheres exposed to maleic hydrazide (1,2-dihydro-3,6-pyridazinedione).

The increased degradative capability of the rhizosphere is not limited to terrestrial plants. Federle and Schwab (1989) and Federle and Ventullo (1990) have made similar observations of the increased microbial degradation of surfactants in the rhizospheres of aquatic plants. Mineralization of linear alkylbenzene sulfonate (LAS) and linear alcohol ethoxylate (LAE) was more rapid in the rhizosphere of cattails (*Typha latifolia*) than in root free sediments (Federle and Schwab, 1989). Surprisingly, the source of the cattails (plants were obtained from a pristine pond and a pond receiving laundromat wastwater) had no significant influence on the rates of LAS and LAE degradation. Similar results on microbial degradation of LAS and LAE by the microbiota of submerged plant detritus were obtained by Federle and Ventullo (1990).

Although most of the studies described above have dealt with agricultural chemicals, they provided evidence for the accelerated microbial degradation of organic compounds in the rhizosphere and also gave an incentive for exploring the possibility of similar results with hazardous organic compounds. Two recent studies have detailed the accelerated disappearance of nonagricultural chemicals in the root zone; a series of polycyclic aromatic hydrocarbons in

prairie grass rhizospheres (Aprill and Sims, 1990), and the increased degradation of oil residues by microorganisms isolated from oil-polluted rice rhizospheres (Rasolomanana and Balandreau, 1987).

Rasolomanana and Balandreau (1987) appear to be the first researchers to show enhanced microbial degradation of nonagricultural chemicals by rhizosphere microorganisms. These observations were serendipitously discovered while studying the improved growth of rice in soil to which oil residues had been applied. The authors hypothesized that the increased growth was brought about by the initial "removal" of the oil residues from the rhizosphere by capable microorganisms and isolated a *Bacillus* sp. with the ability to grow on the oil residues, but only in the presence of the rice root exudates.

The use of eight prairie grasses for stimulating microbial degradation of four PAHs, benz[a]anthracene, chrysene, benzo[a]pyrene, and dibenz[a,h]anthracene, in soil columns was evaluated by Aprill and Sims (1990). Based on residue analysis of the soil columns, PAH disappearance was consistently greater in the vegetated columns compared with unvegetated controls. Although sterile soil controls were not included in the experiments, the authors speculated that microbial degradation may account for the increased disappearance of the PAHs in the vegetated columns. However, the rhizosphere effect may have been obfuscated by addition of manure to all soil columns during PAH addition. Root uptake of the PAHs may have also obscured the disappearance data. Nonetheless, this research does provide evidence for the accelerated disappearance of hazardous organic compounds

in the rhizosphere and also presents a nice discussion on plant and root biology in relation to stimulating soil microbial activity and enhancing microbial degradation of organic compounds in the root zone.

The literature reviewed above illustrates the increased degradative capacity of the rhizosphere for pesticides and more recently, nonagricultural chemicals. In addition, the literature also provides strong evidence for the potential role of vegetation in remediation of surface and near surface soils contaminated with hazardous organic compounds and certainly suggests that the role of vegetation in remediation of chemically contaminated sites be further explored.

F. Manipulation of Root Exudation

Several factors are known to control the extent of root exudation in the rhizosphere (Rovira and Davey, 1974). Research in this area has primarily been conducted in the laboratory using seedling plants grown in axenic culture. Small-scale field investigations have been tried using $^{14}CO_2$ which is fixed by the plant and released as radiolabelled root exudate, allowing easier detection and more sensitive measurements in the field. In addition, microbial population responses to factors affecting exudation have been used as indirect endpoints (Curl and Truelove, 1986).

One of the important implications of controlling root exudation is testing whether the exudation can control or influence the rate of organic compound degradation in the rhizosphere. The ability to change environmental variables such as light or moisture and thus change root exudation will allow this

hypothesis to be tested. If root exudation is found to have a positive influence on microbial degradation in the rhizosphere, it may be possible to duplicate this phenomenon in the field through site management.

Root exudation is dramatically different among plant species. Rovira (1959) found greater amounts of amino acids in tomato rhizospheres as compared to clover. Vancura (1964) found differences in root exudates between wheat and barley, primarily with regard to certain sugars. Bowen (1969) demonstrated the effect of plant nutrient status on root exudation using pine seedlings. More than 4 times as much root exudate was present in the complete nutrient solution as compared to the N-deficient nutrient solutions. Rovira (1956) found greater amounts of root exudates during the first 10 days of growth.

Environmental factors controlling root exudation include light intensity and temperature (Rovira, 1959), soil moisture (Katznelson et al., 1955), and support medium (Boulter et al., 1966; Barber and Gunn, 1974). Increases in light intensity and temperature have resulted in increases in root exudation for tomatoes and clover, respectively. Katznelson and co-workers found that temporary wilting increased the release of root exudates (especially amino acids) from roots of peas, soybeans, wheat, barley, and tomato. Tremendous increases in root exudation were also observed in plants grown in sand, soil, or glass beads as opposed to liquid media.

G. Lipid Analysis in Microbial Ecology

One of the most important aspects of characterizing a site for bioremediation is the microbial ecology. The density (biomass), diversity, and activity of the microorganisms at the contaminated site are important indices for assessing the potential for bioremediation. The traditional techniques for measuring microbial biomass in pure cultures, such as by filtration and dry weight determination, are usually inadequate for determining viable biomass in environmental samples. Incomplete recoveries from environmental matrices as well as bias viable count procedures illustrate some of the inadequacies (Vestal and White, 1989). In addition, early attempts to study the microbial community structure of natural samples were very cumbersome and suffered from the inadequacies described previously.

Specific biochemical methods have been developed to assay for indicators of microorganisms in soil and sediment samples. These indicators include, ATP (Jenkinson and Oades, 1979), and cell wall components (Millar and Casida, 1970). Membrane phospholipids are present in all cells, have a rapid turnover, and are easily extracted from environmental samples and quantified, making them ideal for determining viable microbial biomass. Essentially identical estimates of microbial biomass were found by the membrane phospholipid and direct count methods (Balkwill et al., 1988). The phospholipid fatty acid assay has been used to describe microbial communities from such environmental samples as plant rhizospheres (Tunlid et al., 1985) and creosote-contaminated soils and sediments (Smith et al., 1986). In addition, the detection of specific phospholipid fatty acids can indicate the

presence and abundance of certain groups of microorganisms. For example, Nichols and coworkers (1987) found relatively high proportions of 18-carbon fatty acids relative to 16-carbon fatty acids in a natural gas-exposed soil column capable of TCE degradation. This indicated the presence of a large population of type II methanotrophic bacteria, rather than type I methanotrophs. Marker fatty acids have also been discovered for, among others, sulfate-reducing bacteria, aerobes, anaerobes, and actinomycetes (Vestal and White, 1989).

Phospholipid fatty acid assays (PLFA) also have the advantage of revealing metabolic changes in a microbial community. During nutrient deprivation and other environmental stresses, fatty acid ratios can shift. For example, Guckert et al. (1986) found increases in the trans:cis ratio of monoenoic 16-carbon fatty acids in *Vibrio cholerae* during nutrient starvation. In addition, the protocol used to extract the lipids from environmental samples simultaneously extracts other biochemical indicators of nutritional status in microbial communities (for example, poly-ß-hydroxyalkonoates).

The "Gaia hypothesis" (Lovelock, 1979) describes the important role microorganisms play in maintaining the environment in its current state through their metabolic activities. However, assessing that role in nature presents a difficult task. Nonetheless, several techniques for measuring microbial metabolic activity in the laboratory have been developed. Respiration (Atlas and Bartha, 1987), ATP energy charge (Davis and White, 1980), and incorporation of radiolabeled substrates into cellular components (Moriarty and Pollard, 1981; White et al., 1977) have all been used successfully to monitor heterotrophic microbial activity in sediments and soils. In addition, these types

of activity measurements permit the evaluation of the effects of environmental factors, such as contaminants, on microbial activity.

Incorporation of ^{14}C-acetate into microbial lipids is a simple yet useful technique for measuring heterotrophic microbial activity. The rate of acetate incorporation into microbial phospholipids has been shown to be an accurate and sensitive measure of growth in sediment samples (Moriarty et al., 1985). The technique has been used to measure activity in sewage sludge (McKinley and Vestal, 1984), marine sediments (White et al., 1977), antarctic rock microbiota (Vestal, 1988), and soils (White et al., 1979). Acetate incorporation into lipids has also been used to assess the effects of toxicants, both inorganic and organic, on microbial metabolic activity (Barnhart and Vestal, 1983).

H. Uptake of Organic Chemicals by Plant Roots

The magnitude of plant uptake is an important variable which must be known (or estimated) for using vegetation in site remediation. Although the possibility for microbial degradation of hazardous organic compounds is high in the rhizosphere, there is also the possibility of plant uptake of the chemical(s) acting as a competing process. In addition, movement of chemicals from soil to vegetation is potentially a critical exposure pathway for humans and wildlife.

Research on the environmental chemistry of pesticides (especially herbicides) has made substantial contributions to our understanding of plant uptake of organic compounds. From this extensive database, some general principles have resulted. Root uptake of organic compounds from soil solution is affected by three factors; (1) physicochemical properties of the compounds,

(2) environmental conditions, and (3) plant characteristics (Ryan et al., 1989; Paterson et al., 1990). Assuming constant plant and environmental characteristics, root uptake from soil solution is directly proportional to the compound's octanol-water partition coefficient (K_{ow}) or inversely proportional to its water solubility. Namely, that more lipophilic compounds readily partition into roots (Briggs et al., 1982). However, highly lipophilic compounds tend to remain in the roots and are not translocated to the shoots. One of the important competing processes for root uptake of lipophilic compounds from soil is sorption (Topp et al., 1986). Compounds with low water solubilities would be expected to readily sorb to soil, making them unavailable for root uptake.

In the context of the present study to explore the potential use of vegetation for remediating TCE-contaminated soil, root uptake and translocation within the plant may be most dependent on environmental conditions. The degradation products of TCE vary with existing environmental conditions in the system. Under aerobic conditions, water-soluble products are formed (Little et al., 1988), whereas under anaerobic (methanogenic) conditions, reductive dechlorination (to dichloroethylene and vinyl chloride) can take place (Vogel and McCarty, 1985). Although the water-soluble products formed from aerobic TCE metabolism can be readily degraded by a variety of heterotrophic organisms, they could be readily translocated to the plant shoots if taken up by the plant. In addition, the lipophilic products formed from reductive dechlorination of TCE under anaerobic conditions (dichloroethylene and vinyl chloride), could be readily taken up by the plant roots.

CHAPTER III.

MATERIALS AND METHODS

A. Site Description and Sample Collection

Site History

The Miscellaneous Chemicals Basin (MCB) at the U.S. Department of Energy's Savannah River Site, Aiken County, South Carolina (Figure III - 1), was used as a chemical disposal site beginning around 1956 (Pickett et al., 1986). Originally the MCB was a small, shallow pit (approximately 6 x 6 x 0.3 m) where waste chemicals (primarily solvents) were poured directly onto the soil. Fill material was added when chemical disposal stopped (around 1974), and the site was graded to an area of approximately 100 by 100 m. Since the site was closed it has become naturally revegetated with weeds, grasses, and small pine trees.

Analyses of soil cores at the site (0.5 m depth) showed residues of TCE, tetrachloroethylene (PCE), and trans-1,2-dichloroethylene (DCE) (Pickett et al., 1986; and T. C. Hazen, Savannah River Site, personal communication). In addition, extensive soil gas analysis of the MCB, completed in 1986, confirmed the soil core analyses. Sediment cores to 100 m and subsequent monitoring wells revealed that the contaminants had not reached the water table and were confined to the upper 10 m of the vadose zone. A risk assessment of the MCB revealed no immediate threat to human health or the environment (Pickett et al., 1986). Thus, several characteristics of the site made it a favorable location for exploring the potential for vegetation to be managed as an integral part of site remediation.

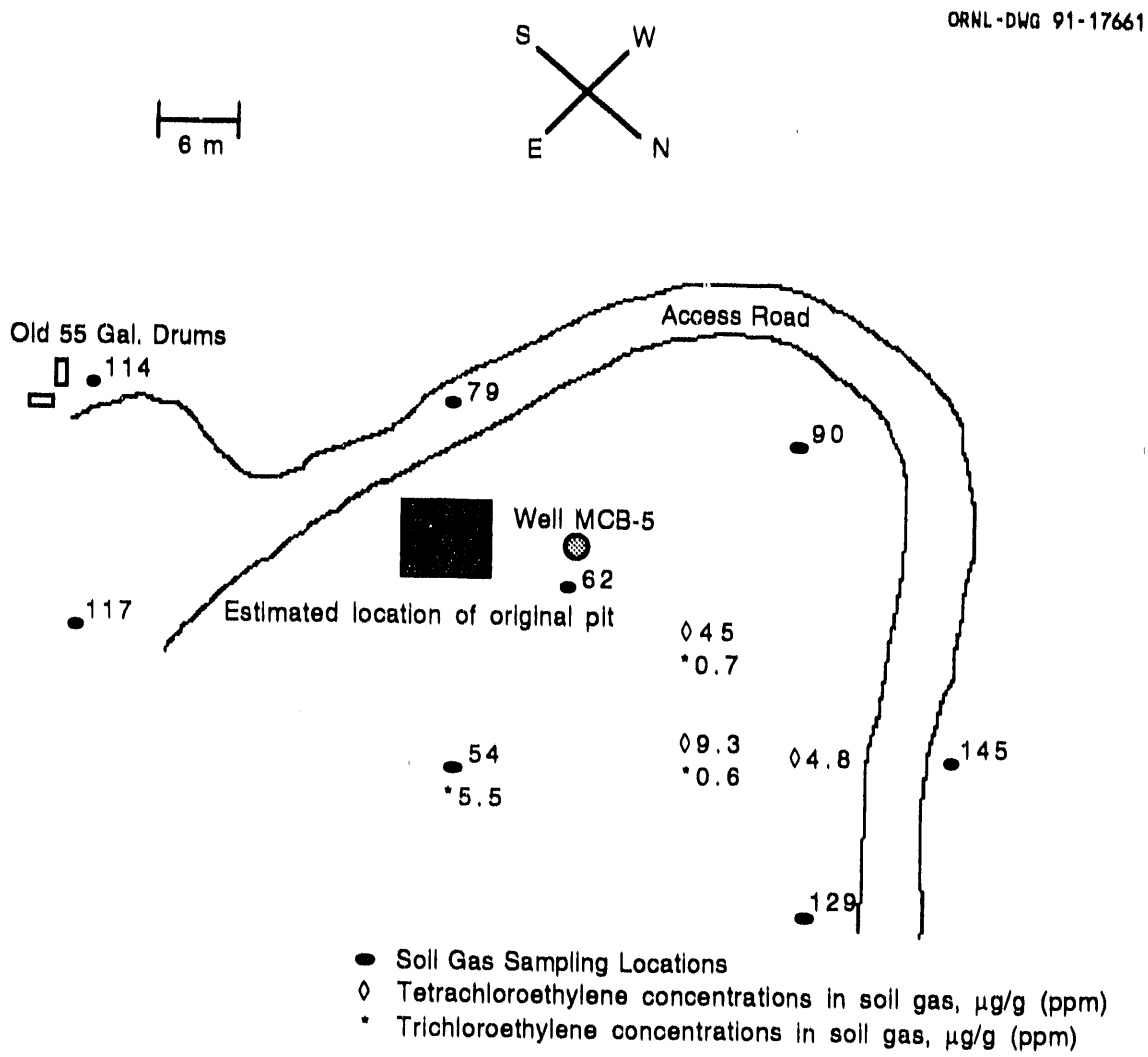

Figure III - 1. Diagram of the Miscellaneous Chemicals Basin showing selected soil gas sampling locations, the original solvent disposal pit, and soil gas concentrations of tetrachloroethylene and trichloroethylene in October, 1986. (Adapted from Microseeps Ltd., 1986)

Specifically, the area is small and inaccessible to the public, TCE and other hazardous chemicals are present in relatively low concentrations, and no imminent human health hazard exists. Moreover, the MCB has become naturally revegetated during the 10^+ years since chemical disposal stopped; thus, sufficient time has passed for populations or consortia of TCE-degrading microorganisms to be enriched in the rhizosphere.

Pedon Description and Soil Characterization of the MCB

Characterization of the soil and identification of the plant species at the MCB were conducted with the help of Mr. Vergil Rogers (U.S. Department of Agriculture) and Mr. John Knox (Savannah River Ecology Laboratory), respectively. Pedon descriptions were made down to 200 cm at nine sites within the MCB. This survey revealed that the area is mostly Typic Udorthents with firm substratum. In addition, the basin resulted from removal of much of the developed surface soil leaving a depression known as a borrow pit. The remaining material showed low water permeability and slow infiltration, conditions that produce a perched water table and anaerobic conditions within 1 m of the surface during the rainy season.

The pH and percent organic carbon (%OC) of soil samples were determined at Oak Ridge National Laboratory (ORNL). Carbon analyses were done in triplicate by using a Leco WR12 Carbon Determinator (St. Joseph, Mich.) equipped with an HF20 induction furnace (Nelson and Sommers, 1982). Inorganic carbon sources are insignificant in this soil type, therefore, total carbon represents organic carbon. Soil pH measurements were made in

distilled water at a soil to water ratio of 1 g:2 mL. Further characterization of a composite, nonvegetated soil sample from the MCB included determination of nitrogen (N), phsophorus (P), sulfur (S), and cation-exchange capacity (CEC) by the Analytical Chemistry Division of ORNL. In addition, soil particle size distribution of the composite, nonvegetated soil collected from the MCB was done by the bouyoucos hydrometer method.

Vegetation Survey of the MCB

Plant species and relative abundance were described using a grid system at the site. The predominate herbaceous species in this area were a grass, *Paspalum notatum* var. *saurae* Parodi; a legume, *Lespedeza cuneata* (Dumont); and a composite, *Solidago* sp. Loblolly pine (*Pinus taeda* L.) was also present. All of these species are plentiful in the area surrounding the MCB which is supportive of the idea that the MCB became naturally revegetated since its closure around 1974.

Sample Collection for Laboratory Experiments-Soil and Vegetation

Soil samples were taken from the root zones of the four predominant plant species at the MCB and from nonvegetated areas with TCE contamination and nonvegetated areas where TCE was not present. Herbaceous species were uprooted, and soil was tapped from the roots onto clean plastic sheets. Soils were collected from pine trees by scraping aside the top 1-2 cm and then using a trowel to remove soil adhering to and immediately surrounding the roots. Nonvegetated soils were sampled in a similar manner. Soils of each

type were mixed to produce composite samples, then transferred to sterile Whirl Pak™ bags (NASCO, Fort Wilkinson, Wis.), sealed, and stored on ice for transport to ORNL. The soils, which were fine sieved (2.0 mm) prior to use, were stored in the dark at 4 °C.

Vegetation samples were collected by coring around the base of the specific plant, removing the plant and attached soil and placing it in a plastic container. Water was added as needed during transport from the field site to ORNL. After arrival at ORNL, the vegetation samples were transferred to a greenhouse and maintained there until use in the experiments described below.

B. Microbial Biomass, Community Structure, and Metabolic Activity

CO_2 Biomass

Carbon dioxide efflux was measured from rhizosphere soils for four plant species and the two edaphosphere soils (TCE-contaminated and noncontaminated) to compare respiration among soil sample types and to estimate microbial biomass. Triplicate soil samples (50 g) were moistened to 80% saturation with distilled, deionized water and incubated in the dark at 20 °C in 8- by 5-cm glass jars. An infrared gas analyzer (LIRA Model 3000, Mine Safety Appliances Company, Pittsburgh, Pa.) was used to monitor CO_2 efflux (Edwards, 1982; Walton et al., 1989) at 24-h intervals for 7 days. Carbon dioxide respiration was used to calculate microbial biomass according to the method of Jenkinson and Powlson as modified by Anderson and Domsch (Parkinson and Paul, 1982).

Phospholipid Fatty Acid Analysis

Materials

Glass-distilled solvents (Burdick and Jackson, Muskegon, WI) were used in the extraction, fractionation, and derivitization procedures. Unisil silicic acid (100-200 mesh) was obtained from Clarkson Chemical Co., Williamsport, PA. Gas chromatography columns (fused silica capillary GLC Durabond DB-1) were purchased from J&W Laboratories Inc., Rancho Cordova, CA.

Lipid Extraction

Sample extraction took place in Dr. David White's laboratory at the Institute for Applied Microbiology, The University of Tennessee, Knoxville. The extraction procedure, a modification of Bligh and Dyer (1959), was as follows: Weighed soil samples which had been frozen and lyophilized were extracted for 2 h in glass centrifuge tubes with 1.0:2.0:0.8, v/v/v chloroform:methanol:0.05 M phosphate buffer, pH 7.4. Samples were centrifuged and decanted into 250-mL separatory funnels. Equal amounts of chloroform and water were added to separate the extracts into organic and aqueous phases. The organic (chloroform) phase was filtered through a fluted filter into a round bottom flask and the chloroform was removed using a rotary evaporator.

Lipid Fractionation

Dried lipid extracts were re-dissolved in chloroform and loaded onto silicic acid columns for fractionation (Findlay and White, 1987). The columns

were Pasteur pipettes packed with 0.5 g of activated (heated at 120 °C for 2 h) silicic acid. Neutral lipids, glycolipids, and phospholipids were eluted into test tubes with chloroform, acetone, and methanol, respectively. All lipid samples were dried under nitrogen. Neutral lipid samples were archived, glycolipid samples were stored for use in determining poly-ß-hydroxyalkanoate (PHA), while phospholipid samples were derivatized as described below.

Fatty Acid Derivitization

The phospholipid samples were derivatized to phospholipid fatty acid methyl esters by mild alkaline methanolysis. Dried phospholipid samples were dissolved in 1 mL of toluene:methanol (1:1, v/v). One mL of 0.2M KOH in methanol was added, the samples were vortexed briefly, and heated for 30 minutes at 37 °C. Two mL of hexane:chloroform (4:1, v/v) were added and the samples were neutralized with 0.2 mL of 1N acetic acid and 2 mL distilled water. Samples were vortexed for 5 minutes, centrifuged for 10 minutes and the hexane:chloroform phase (top) was transferred into a clean screw-cap test tube. One mL of hexane:chloroform (4:1, v/v) was added to the aqueous phase (bottom) and the vortex and centrifugation steps were repeated. The hexane:chloroform phase for each sample was pooled and dried under nitrogen.

Quantification

Analysis of phospholipid fatty acid methyl esters (FAME) was done using gas chromatography as described previously (Bobbie and White, 1980). The

procedures and conditions are briefly described henceforth. A Hewlett-Packard 5880 gas chromatograph equipped with a splitless injector and a flame ionization detector was used to quantify FAME based on peak areas and comparisons to a FAME library. Dried FAME samples were re-dissolved in iso-octane containing a 19-carbon fatty acid as an internal standard. Chromatographic conditions were as follows: injection volume, 1 µL; injector temperature, 270 °C; detector temperature, 290 °C; carrier gas, H_2 at 1 mL/minute, make up gas, N_2 at 30 mL/minute; oven temperature, 80 °C for 1 minute then ramped to 150 °C at 10 °C/minute, then to 240 °C at 3 °C/minute, then to 280 °C at 5 °C/minute and held for 5 minutes; column, fused silica capillary GLC Durabond DB-1.

Data Analysis

Total picomoles of phospholipid were converted to active microbial biomass using a conversion factor of 100 µmoles phospholipid/g of bacteria the size of *E. coli* (Smith et al., 1989). Phospholipid fatty acid (PLFA) profiles of the different sample types were also constructed. A cluster analysis of the phospholipid fatty acid profiles of different rhizosphere and nonvegetated soil samples was performed to help in analyzing the different sample types. Interpretation of PLFA profiles was done with the help of Dr. Jim Guckert and Mr. David Ringleberg of the Institute for Applied Microbiology, The University of Tennessee.

Determination of poly-ß-Hydroxyalkanoates

The glycolipid fraction from the total lipid extraction described above was used to determine the endogenous lipid storage products poly-ß-hydroxyalkanoates (PHAs) using the techniques of Findlay and White (1987). Briefly, the glycolipid fraction (acetone) was dried to the side of a test tube under a stream of nitrogen. Lipids other than PHA were removed by washing with ethyl alcohol and diethyl ether. The constituent ß-hydroxy acids from the PHA samples were ethanolyzed and converted to ethyl esters and analyzed by gas chromatography with flame ionization detection (Findlay and White, 1983). Verification of the constituent β-hydroxy acids was done by mass spectroscopy.

^{14}C-Acetate Incorporation into Microbial Lipids

Materials

1-^{14}C-Acetate (19.6 mCi/mmol) was purchased from Sigma Chemical Co., St. Louis, MO. Unisil silicic acid (100-200 mesh, Clarkson Chemical Co., Williamsport, PA) was a gift from Dr. Tom Phelps, Institute for Applied Microbiology, University of Tennessee. Chloroform (EM Science, Cherry Hill, NJ), methanol (Fisher Scientific, Fair Lawn, NJ), and acetone (Malinckrodt Inc., Paris, KY) were used as extraction solvents.

^{14}C-Acetate Incorporation

Soil samples (2 g) from rhizosphere and nonvegetated areas at the MCB were placed in 15-mL polypropylene tubes together with 0.5 mL of sterile, distilled, deionized water. A syringe was used to add 5 µCi of 1-^{14}C-acetate in

such a way as to minimize the disturbance artifact (Findlay et al., 1985). Samples were incubated in the dark at 20 °C. Samples were terminated at predetermined intervals and extracted using the procedure described below. Sterile controls for each sample type were prepared by autoclaving the samples, distilled water, and sample tubes in toto. Laminar-flow hoods were used during addition of the 1-^{14}C-acetate in the sterilized samples.

Extraction

Samples were extracted using a mixture of chloroform, methanol, and water as originally described by Bligh and Dyer (1959) with slight modifications. Briefly, soil samples were initially extracted for 2 h by pipetting 3 mL of methanol:chloroform:phosphate buffer (2:1:0.1, v/v/v) into the sample tubes, vortexing them briefly, and holding them in the dark. After the 2 h extraction, samples were centrifuged at 1500 rpm for 5 minutes and decanted into glass sample tubes. The extracts were then separated into organic and aque. phases by the addition of 2 mL of chloroform:distilled water (1:1, v/v). The lipid-containing chloroform (organic) phase was recovered for further use.

Lipid Fractionation

The chloroform phase was fractionated into neutral, glyco-, and phospho-lipid fractions using column chromotography (Findlay and White, 1987). The columns were Pasteur pipettes packed with 0.5 g of activated (heated at 120 °C for 2 h) silicic acid. The chloroform phase containing the total lipids was loaded

onto the columns and neutral lipids, glycolipids, and phospholipids were eluted with chloroform, acetone, and methanol, respectively.

Quantification

Liquid scintillation spectrometry was used to quantify the radioactivity in the total lipid chloroform phase as well as the radioacticity in the 3 lipid fractions after separation on the silicic acid columns. Aliquots (200 µL) were suspended in 10 mL of Aquasol and counted using a Packard 2000CA liquid scintillation spectrophotometer.

Data Analyses

Data from the experiments on acetate incorporation into total lipids were expressed as picomoles of ^{14}C-acetate incorporated per gram of soil per hour by plotting cumulative radioactivity (disintegrations per minute) over time and determining a rate of incorporation from the plots. Graphs of the percentage of the total radioactivity contained in the 3 lipid fractions at each time point were constructed using Cricket Graph software on an Apple Macintosh Plus personal computer.

C. Toxicity of TCE and PCE to Soil Microorganisms

Successful microbial degradation studies require that the test chemicals be present at concentrations that are not toxic to the microorganisms. Carbon dioxide evolution was monitored as a simple, quick indication of chemical toxicity to soil microorganisms (Zelles et al., 1986; Walton et al., 1989). The

respiration rates of treated and control soils were compared to select a concentration of chemicals that did not alter respiration. An infrared gas analyzer (LIRA Model 3000, Mine Safety Appliances Company, Pittsburgh, PA) was used to measure CO_2 efflux over a 7-d period from incubated (20 °C) 50-g soil samples according to the methods of Edwards (1982) as adapted by Walton et al. (1989). TCE or PCE was introduced into the soils gravimetrically (micrograms of chemical per gram of soil dry weight). However, soils were not mixed so that losses due to volatilization would be minimized. Every 24 h a flow of moist, CO_2-free air was passed through the 8- by 5-cm incubation jars to measure CO_2 efflux and also to maintain aerobic conditions. Toxicity determinations were based on whether microbial respiration in the treatment soils had returned to control levels by the end of the 7-d experiment. High concentrations (500 µg/g and 1000µg/g) of TCE and PCE were chosen so that an effect could be observed.

D. TCE Degradation in Soil Slurries

Initial experiments to monitor the disappearance of TCE from the headspace of soil slurries were undertaken to provide an indication of whether biological degradation of TCE occurred in soil samples. Duplicate soil samples (3 g) from each of the four plant species, as well as from nonvegetated areas of the MCB, were placed in 40-mL glass bottles equipped with screw caps and polytetrafluoroethylene-lined septa. Distilled, deionized water (20 mL), spiked with TCE at either 35 µg/mL or 70 µg/mL was added. Sterile controls for each soil sample type and sterile water controls (no soil) were prepared by

autoclaving samples for 1 h on each of three consecutive days prior to use. Samples were incubated in the dark at 20 °C on a shaker table (125 rpm).

Headspace concentrations of TCE were determined with a Sigma 2000 capillary gas chromatograph (Perkin-Elmer Corp., Norwalk, Conn.) equipped with an electron capture detector. Chromatographic conditions were as follows: Column, SP-1000 (0.32 cm I.D. by 61 cm); carrier gas, N_2 (30 mL/min); injector temperature, 100 °C; column temperature, 100 °C; detector temperature, 350 °C. Concentrations of TCE were quantified by integration of peak areas. The coefficient of variation [(standard deviation x 100)/mean] for measurements of TCE in the headspace of the distilled, deionized water was 7.0%.

E. ^{14}C-TCE Mineralization in Soil Samples

The role of microorganisms in the biodegradation of TCE was further explored by comparing mineralization of ^{14}C-TCE in soil from the root zone of one plant species (*L. cuneata*) with that of the nonvegetated TCE-contaminated soil. Triplicate soil samples (50 g) for both treatment groups plus sterile controls (autoclaved for 1 h on three consecutive days) were moistened with sterile, distilled, deionized water to 80% saturation. The ^{14}C-TCE (specific activity 1.12 x 10^6 Bq/mmol, chemical purity >99%, Sigma, St. Louis, Mo.) was added to the soil at 70 µg/g soil (dry weight) in glass sample jars (8 by 5-cm) and closed with polytetrafluoroethylene-lined neoprene stoppers.

At 24-h intervals, filtered air was used to flush the sample jars and then passed sequentially through 7 mL ethylene glycol monomethyl ether (EGME) (Fisher, Fairlawn, N.J.) and 10 mL $^{14}CO_2$UNT SORB cocktail (Research

Products International, Elk Grove, Ill.) to trap ^{14}C-volatiles and $^{14}CO_2$, respectively (Scheunert et al., 1987). Aliquots (200 µL) of the EGME were counted in Aqueous Counting Scintillant (Amersham, Arlington Heights, Ill.) on a liquid scintillation spectrometer (Packard Tri-Carb 2000 CA). The $^{14}CO_2$ cocktail was counted directly. All samples were monitored for quenching using a quench curve.

F. PCE Degradation in Soil Slurries

Experiments monitoring the disappearance of PCE from the headspace of soil slurries with concomitant production of TCE were undertaken to determine whether biological reductive dechlorination of PCE occurred in soil samples from the MCB. Duplicate samples (3 g) of rhizosphere soils as well as nonvegetated/contaminated soil from the MCB, were placed in 40-mL glass bottles equipped with screw caps and polytetrafluoroethylene-lined septa. Distilled, deionized water (20 mL), spiked with PCE at 50 µg/mL was added. Sterile controls for each soil sample type and sterile water controls (no soil) were prepared by autoclaving samples for 1 hour on three consecutive days prior to use. Resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) was used as an indicator for reduced (anaerobic) conditions. Samples were incubated in the dark at 20 °C.

Initially, PCE and TCE concentrations were determined by headspace analysis with a Sigma 2000 capillary gas chromatograph (Perkin-Elmer Corp., Norwalk, Conn.) equipped with an electron capture detector. Chromatographic conditions were as follows: Column, Carbopak B/1% SP-1000 (0.32 cm I.D. by

61 cm); carrier gas, N_2 (30 mL/min); injector temperature, 100 °C; column temperature, 100 °C; detector temperature, 350 °C. Concentrations of PCE and TCE were quantified by integration of peak areas and checked with external standards containing PCE and TCE. Problems with the Sigma 2000 initiated new analytical methods development in which a Perkin-Elmer 3920 B gas chromatograph with a flame ionization detector was brought on line. New chromatographic conditions were as follows: Column, Carbopak B/1% SP-1000 (0.32 cm I.D. by 60 cm); carrier gas, N_2 (26mL/min); injector temperature, 170 °C; column temperature, 160 °C; detector temperature, 190 °C. External standards of PCE and TCE were used to test the procedure and calibrate the instrument. Because of the decrease in sensitivity in moving from the Sigma 2000 to the 3920 B, headspace analysis could not be employed. Rather, 24 hour methylene chloride extractions were undertaken to remove residual PCE and any TCE (or other metabolites) produced during the experiments and a portion of these extracts was anlayzed.

G. Fate of ^{14}C-TCE in Soil-Plant Systems

Sample Flasks

Specially-designed Erlenmeyer flasks (Figure III - 2) were used for monitoring the fate of ^{14}C-TCE in soil-plant systems. To minimize exposure of TCE to reactive substances, the flasks were equipped with ground-glass joints and stainless steel sampling ports. In addition, all flasks were wrapped with white electrical tape to eliminate the possibility of photooxidation of ^{14}C-TCE. With the exception of *G. max*, and *P. notatum*, plant seedlings were placed in

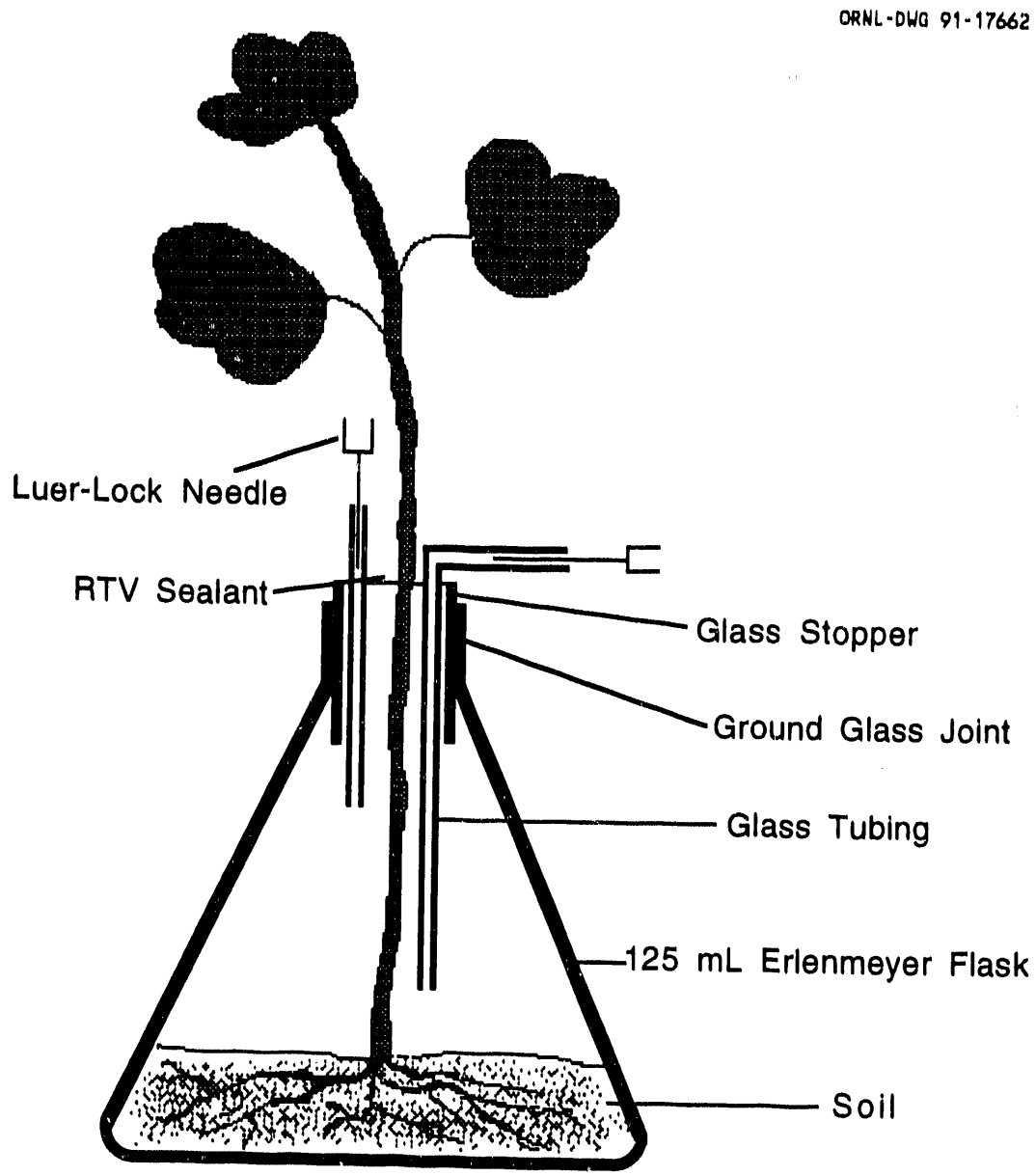

Figure III - 2. Experimental flasks used to measure the fate of ^{14}C-TCE in soil-plant systems.

the flasks and the roots were covered with nonvegetated soil from the field site. These seedlings were acclimated in the greenhouse for two days, followed by two more days in the environmental chamber (described below). Seeds of *G. max* were germinated directly in the flasks and grown in the greenhouse for 12 days, and acclimated in the environmental chambers for two days before initiation of the experiments. Because of its morphology, experiments with *P. notatum* were conducted in 125- by 65-cm glass crystallization dishes in which the soil surface was open to the atmosphere. In addition to the vegetated soil samples, nonvegetated and sterile (autoclaved for 1 hour on three consecutive days) soil samples were also part of each experiment. The ^{14}C-TCE (Sigma Chemical Co., St. Louis, MO, specific activity, 1.12×10^6 Bq/mmol; chemical purity, > 99%) was added directly to the soil and the flasks were sealed by coating a nonreactive silicone rubber sealant (Stotzky et al., 1961; Hsu and Bartha, 1979) between the plant and the flask. Sterile, distilled H_2O was added to help distribute the ^{14}C-TCE within the soil and adjust the soil to field capacity moisture content (water potential, -33 kPa).

Exposure and Environmental Chambers

The Erlenmeyer flasks were placed in two air-tight, glass exposure chambers (Figure III - 3). Air within the exposure chambers was continuously evacuated and passed through charcoal (6-14 mesh, Fisher Scientific, Fair Lawn, NJ) filters to remove any ^{14}C-compounds that had been taken up by the plant roots, translocated through the plant and released into the exposure chamber atmosphere with the evapotranspiration stream. The experimental

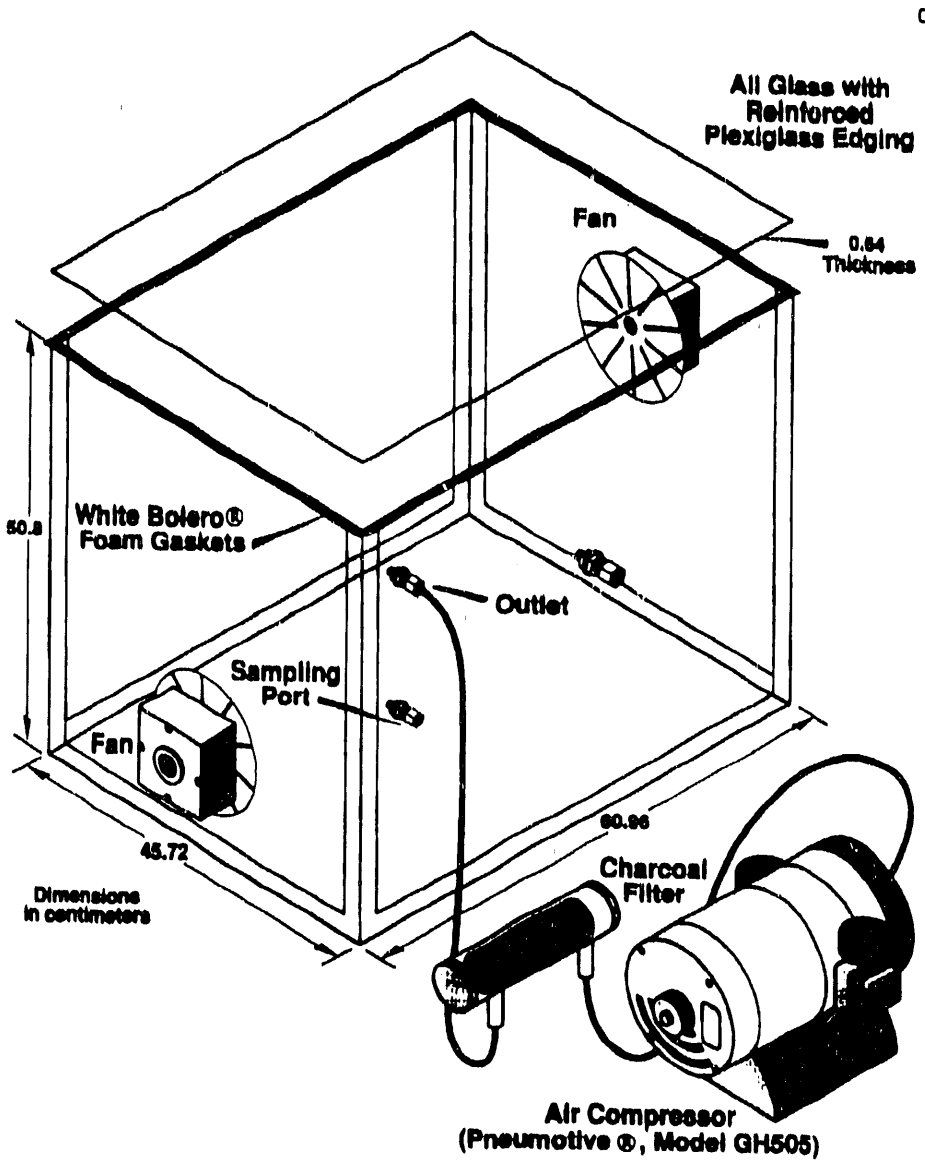

Figure III - 3. Glass exposure chambers used to regulate environmental conditions and quantify ^{14}C released by the plants in whole plant experiments.

design was modified slightly for the experiments with *P. notatum*. In these experiments, the air within the exposure chambers was continuously evacuated and passed through 2 gas-washing bottles containing 0.5 M KOH for trapping $^{14}CO_2$ and a charcoal filter for removing volatile ^{14}C. The exposure chambers were contained within a controlled-environment chamber with a 14-h photoperiod. Diurnal temperatures were 30/22 °C and the CO_2 concentration was ambient (approximately 370 µL/L). Daytime relative humidity and approximate photosynthetic photon flux density in the exposure chambers was 74% and 365 µMoles/m^2s, respectively.

Daily Sampling Procedure

At 24-h intervals, the flasks were flushed with air to help maintain aerobic conditions and determine $^{14}CO_2$ produced from degradation of ^{14}C-TCE. Originally, a 25-mL syringe was used to flush the air inside the flasks through a series of trapping solutions at approximately 10 mL/m. Subsequently, a peristaltic mini-tubing pump was used to provide more accurate air flow control. The exit air from the flasks passed through a trapping system (Hsu and Bartha, 1979) (Figure III - 4) containing an Aquasol (New England Nuclear Corp.) trap to remove ^{14}C-compounds other than $^{14}CO_2$, which was trapped in a vial containing Carbosorb (330 mL/L phenethylamine, 50 mL/L distilled water, 400 mL/L toluene, 220 mL/L methanol, 5.0 g/L 2,5-Diphenyloxazole, 0.3 g/L 1,4-bis-2-[5-phenyloxazolyl]-benzene). Radioactivity in the trapping solutions was determined using a liquid scintillation spectrophotometer (Packard Model 2000CA). The efficiency of the Aquasol trap was determined by using ^{14}C-TCE.

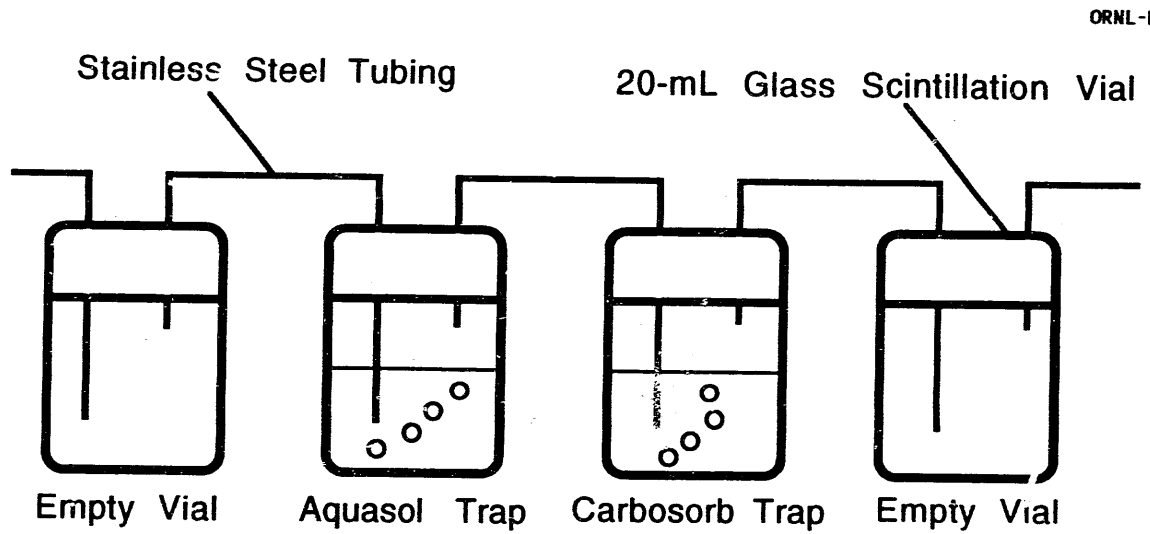

Figure III - 4. System used to trap ^{14}C-volatiles and $^{14}CO_2$ during whole plant experiments.

Originally, a syringe was used to flush the headspace of a flask containing ^{14}C-TCE through the trapping system at 0.5 and 1.5 hours and the radioactive counts (expressed as a percentage of the total counts evolved from the flask) in the Aquasol trap were determined (Figure III - 5). Since subsequent experiements were conducted using a peristaltic pump to flush the flasks, the efficiency of the Aquasol trap was also determined for the flow of air provided by the pump (Figure III - 6). Similarly, the efficiency of the Carbosorb trap was determined using ^{14}C-bicarbonate. A syringe was used to flush the headspace of a flask containing acidified ^{14}C-bicarbonate through the trapping system and the radioactive counts in the carbosorb trap (expressed as a percentage of the total counts evolved) were determined. Acidification of the ^{14}C-bicarbonate was done using two different volumes of HCl to be certain of complete acidification (Figures III - 7 and III - 8). As with the Aquasol trap, the efficiency of the Carbosorb trap was also determined for air flow provided by the peristaltic pump (Figure III - 9). The mean efficiency of the Aquasol trap for all determinations was approximately 92% ± 4%, while that of the Carbosorb trap was approximately 77% ± 7%. All samples were monitored for quenching and auto-fluorescence using a quench curve in the liquid scintillation spectrophotometer.

Soil Extraction Procedures

At the conclusion of each experiment, experimental soil was extracted in toto to determine extractable ^{14}C remaining in the soil. A modified Bligh-Dyer (1959) extraction procedure using 55 mL of methanol:chloroform (2.7:1, v/v)

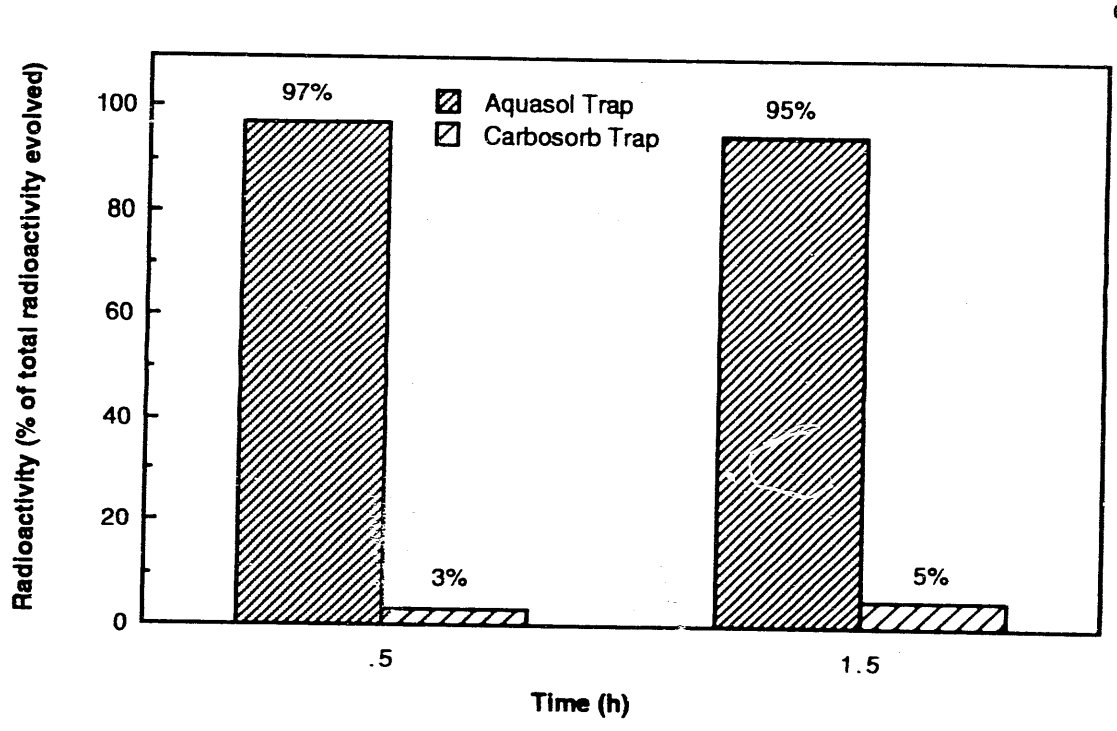

Figure III - 5. Efficiency determination of the aquasol used to trap ^{14}C-volatiles during whole plant experiments. A syringe was used to flush the headspace of a flask containing ^{14}C-TCE through the trapping system at 0.5 and 1.5 hours.

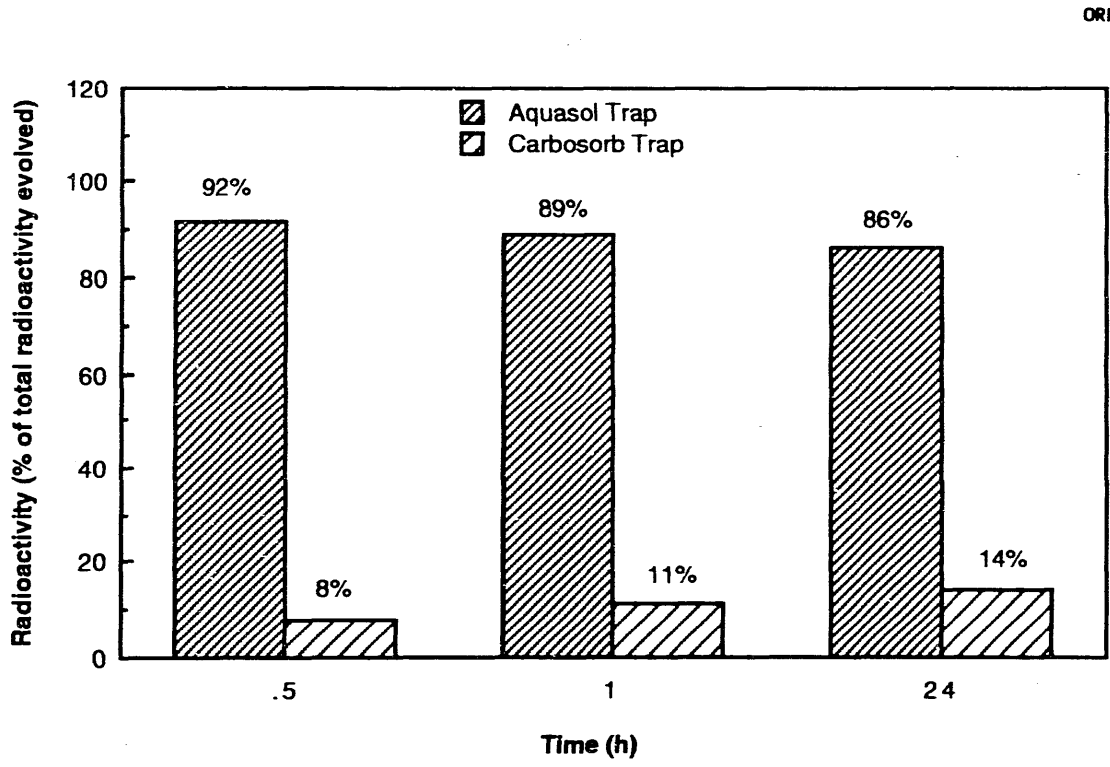

Figure III - 6. Efficiency determination of the aquasol used to trap ^{14}C-volatiles during whole plant experiments. A peristaltic pump was used to flush the headspace of a flask containing ^{14}C-TCE through the trapping system at 0.5, 1, and 24 hours.

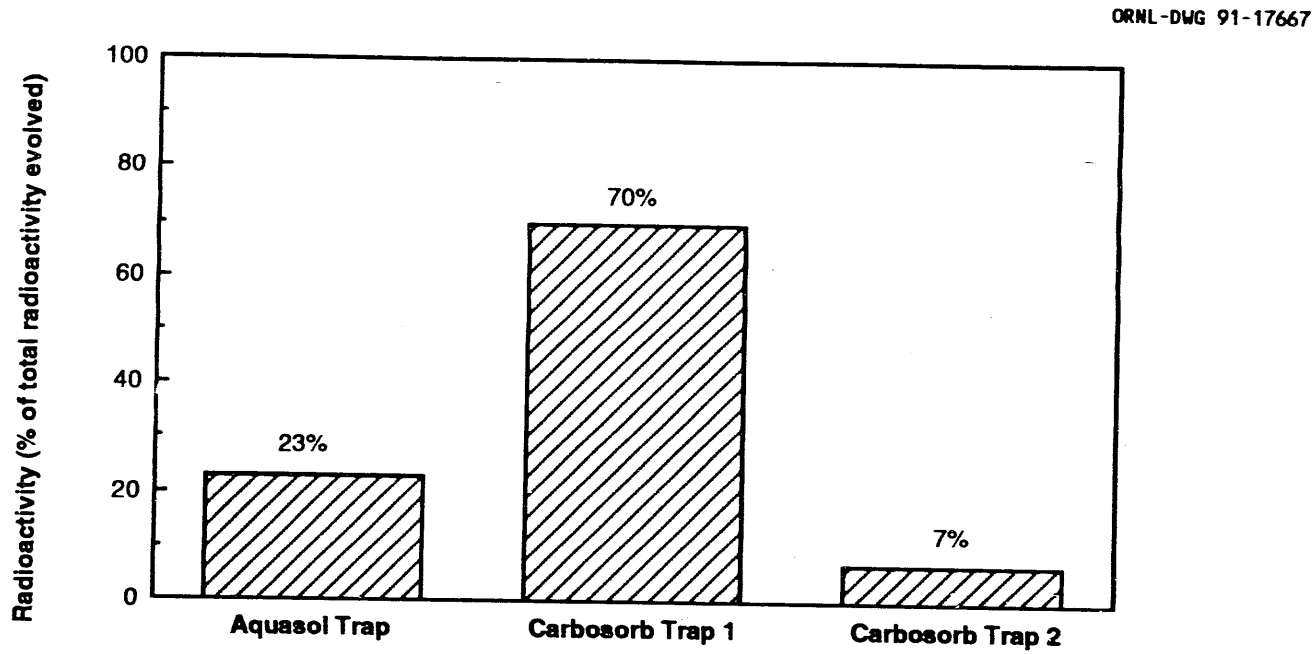

Figure III - 7. Efficiency determination of the carbosorb used to trap $^{14}CO_2$ during whole plant experiments. A syringe was used to flush the headspace of a flask containing acidified ^{14}C-bicarbonate through the trapping system.

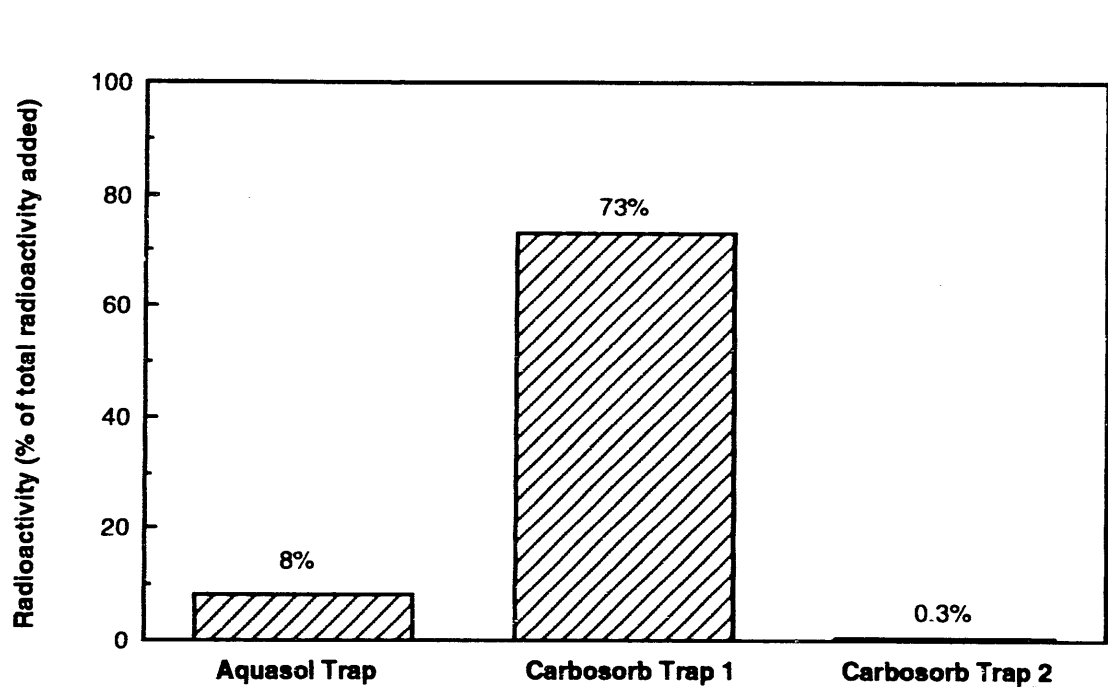

Figure III - 8. Efficiency determination of the carbosorb used to trap $^{14}CO_2$ during whole plant experiments. A syringe was used to flush the headspace of a flask containing acidified ^{14}C-bicarbonate through the trapping system.

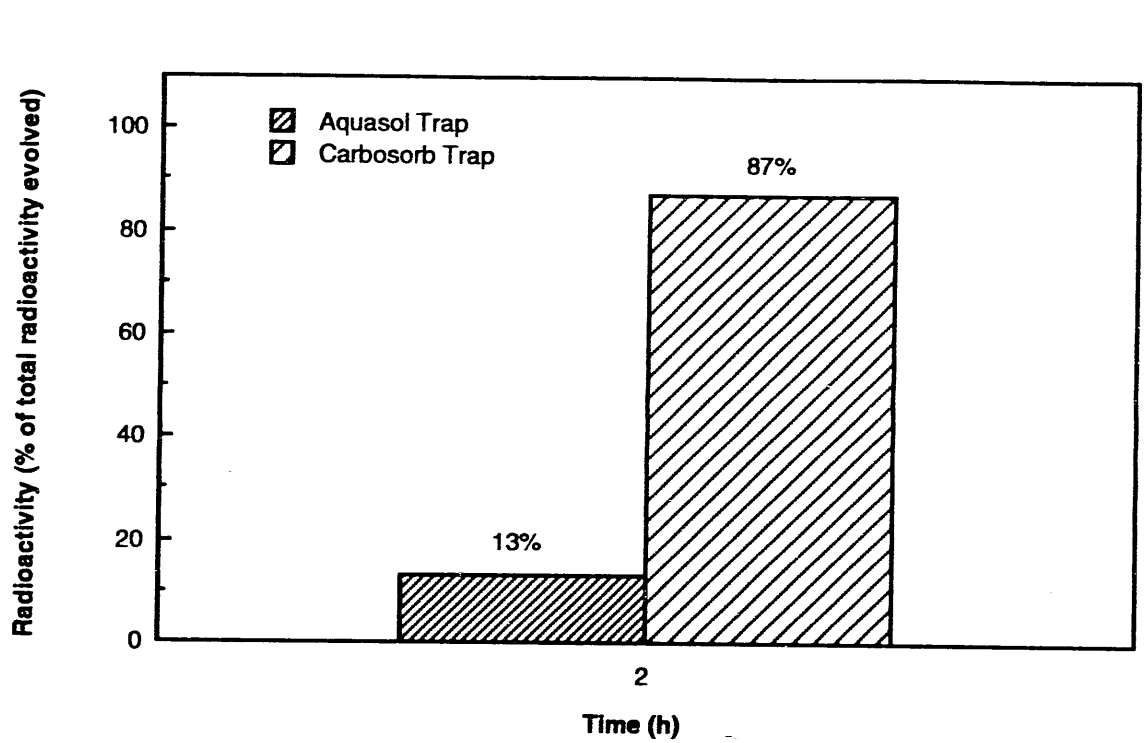

Figure III - 9. Efficiency determination of the carbosorb used to trap $^{14}CO_2$ during whole plant experiments. A peristaltic pump was used to flush the headspace of a flask containing acidified ^{14}C-bicarbonate through the trapping system.

was employed. Soils were extracted in the sample flasks for at least two hours before being transferred to 110-mL glass bottles equipped with polytetrafluoroethylene-lined caps. The bottles were centrifuged and 10 mL of the supernatant was decanted into a polypropylene vial. The supernatant was split into two phases (aqueous and organic), by adding 5 mL of chloroform and 5 mL of distilled water, and allowed to stand for 24 h. Aliquots (200 µL) of each phase were added to Aquasol and analyzed for ^{14}C. A portion of the soil pellet from the centrifugation step was removed for use in the alkaline extraction procedure described below. Different ratios of methanol:chloroform and sequential extractions did not increase recovery of ^{14}C from the soil (data not shown). The solvent-extracted soil samples from the centrifugation step described above were weighed, and then extracted with 0.5 M NaOH overnight, and radioactivity in aliquots of each NaOH extract was determined. All samples were monitored closely for quenching (physical and chemical) and auto-fluorescence.

Plant Analysis for ^{14}C

In order to determine ^{14}C in plant tissues for describing uptake and calculating a mass balance, plant tissue samples were combusted using a Thomas Ogg Infrared Ignitor (Thomas Scientific). Weighed samples of leaf, stem, and root tissue were enclosed in black paper sample wrappers (Thomas Scientific), placed in platinum baskets, and combusted in specially designed sealed flasks. Leaf and stem samples were combusted fresh, however, root samples were air dried before combustion. After cooling, an alkaline

scintillation cocktail (described as Carbosorb above) was introduced into the flasks. The cocktail remained in the flasks for approximately 1 hour before being transferred to scintillation vials and counted using a Packard 2000CA Liquid Scintillation Spectrometer. Methyl methacrylate ^{14}C standards were used to determine the efficiency of the combustion procedure.

Data Analysis

In most cases, data from the whole plant experiments was expressed as a percentage of the total recovered radioactivity at the conclusion of each experiment. For example, mineralization of ^{14}C-TCE over time is expressed as the percentage of the total radioactivity contained in the carbosorb traps. Data from all the experiments were recorded using an Apple Macintosh Plus personal computer and Cricket Graph software.

H. Influence of Root Exudation on ^{14}C-TCE Mineralization

The potential role that exudation rate plays in ^{14}C-TCE mineralization in rhizosphere soils was tested using materials and methods identical to the whole plant experiments described above with one major deviation. The light intensity (photosynthetic photon flux density) in chamber one was reduced to 249.4 $\mu Moles/m^2s \pm 6.0$. This represented a statistically signficant reduction ($p < 0.05$) in light intensity compared with chamber two (387.8 $\mu Moles/m^2s \pm 9.2$). Importantly, temperature within the two chambers varied only slightly (29.2 °C ± 0.2 and 29.9 °C ± 0.3 for chambers 1 and 2, respectively) and was not

significantly different ($p > 0.05$). Experiments were conducted using *G. max*, *P. taeda*, and *L. cuneata*.

I. Pesticide Root Zone Model (PRZM)

The Pesticide Root Zone Model (PRZM) is an interactive software program developed for estimating the movement and fate of pesticides and other hazardous organic compounds within and below the plant root zone (Carsel et al., 1984). The model allows the user to input soil, chemical, and environmental data based on calculated, literature, and/or experimental measurements. Output from the model simulations is assessed based on both abiotic and biotic factors. Data from the whole plant experiments described above were incorporated into the PRZM to estimate the fate of TCE at the MCB under vegetated and nonvegetated conditions.

CHAPTER IV.

RESULTS

A. Site Description and Sample Collection

Soil at the MCB

The soil samples from the Miscellaneous Chemicals Basin (MCB) proved to be low in organic carbon and slightly acidic (Table IV - 1), which is typical of areas where rainfall is abundant and warm, humid conditions prevail. Percent organic carbon was lower ($p \leq 0.05$) for the nonvegetated soils (non-TCE contaminated, 0.20% OC; TCE contaminated, 1.06% OC) than for the rhizosphere soils, which ranged from 1.20% to 1.43% OC. With the exception of *Solidago* sp., the rhizosphere soils had higher pHs than the nonvegetated soils. Differences in these parameters (especially %OC) were expected and were consistent with the fact that some soils received waste solvent input and were vegetated whereas others were not. Because the primary purpose of the experiments was to determine whether degradation of TCE was enhanced in the rhizosphere, without regard at this time for the specific factors controlling such an effect, no attempt was made to normalize differences in %OC or pH between soil samples.

Further soil characterization of the composite, nonvegetated soil sample (Table IV - 2) used in the experiments on the fate of ^{14}C-TCE in soil-plant systems provided additional confirmation of the initial pedon description. Namely, the MCB soil is dominated (> 90%) by sand (> 67%) and clay (> 23%). Cation-exchange capacity of the composite soil was 13.5 ± 0.52 cmol (+)/kg while nitrogen, sulfur, and phosphorus content were 10.02% ± 0.001%, 26.7

Table IV - 1. Percent organic carbon (%OC) and pH of soil samples from the Miscellaneous Chemicals Basin at the Savannah River Site.

Soil source	% OC ± SD (n = 3)	pH ± SD (n = 3)
Nonvegetated, noncontaminated[A]	0.20 ± 0.04[B]	5.20 ± 0.10[B]
Nonvegetated	1.06 ± 0.08[B,C]	6.39 ± 0.09[B]
L. cuneata	1.35 ± 0.01[B,D]	6.81 ± 0.01[E]
Solidago sp.	1.20 ± 0.05[D]	5.53 ± 0.15[B]
P. notatum	1.43 ± 0.05[B,D]	6.75 ± 0.05[E]
P. taeda	1.26 ± 0.03[D]	6.87 ± 0.09[E]

[A]No detectable TCE; however, trace dichloroethylene was detected.
[B]Significantly different from all other soils ($p \leq 0.05$)
[C]Significantly greater than nonvegetated, noncontaminated soil ($p \leq 0.05$).
[D]Significantly greater than nonvegetated soil and nonvegetated, noncontaminated soil ($p \leq 0.05$).
[E]Significantly different from the *Solidago* sp., nonvegetated soil, and nonvegetated, noncontaminated soil ($p \leq 0.05$).

Table IV - 2. Selected physical and chemical properties of the composite, nonvegetated soil from the Miscellaneous Chemicals Basin at the Savannah River Site.

Parameter	Value
Sand (%)	67.5
Silt (%)	9.0
Clay (%)	23.5
Cation-exchange capacity [cmol (+)/kg]	13.5 ± 0.52
Nitrogen (%)	10.02 ± 0.001
Sulfur (μg/g)	26.7 ± 9.3
Phosphorus (μg/g)	99.0 ± 5.1

µg/g ± 9.3 µg/g, and 99.0 µg/g ± 5.1 µg/g, respectively. Determination of soil physical and chemical properties in this study was used strictly for soil characterization. No additional nutrients were added to the soil used in these experiments.

Vegetation at the MCB

Results of the vegetation survey of the Miscellaneous Chemicals Basin conducted in August, 1988, are summarized in Table IV - 3. As described earlier, the predominant plant species are a legume, *Lespedeza cuneata* (Dumont); a grass, *Paspalum notatum* var. *saurae* (Parodi); and a composite, *Solidago* sp. The estimated cover for these three species was greater than 84% of the total area of the MCB. In addition, approximately 500 trees (mostly loblolly pine, *Pinus taeda* with diameters at breast height ranging from <1 to 20 cm) were found throughout the MCB. Miscellaneous vegetation, which covered about 7% of the total area of the MCB included broom sedge (*Andropogon virginicus*), dwarf sumac (*Rhus copallina*), and consumption weed (*Baccharis halimifolia*). Nonvegetated areas at the MCB were estimated at 8% of the total area.

B. Microbial Biomass, Community Structure, and Metabolic Activity

Carbon dioxide efflux from incubated soils collected in August, 1988, (late summer) illustrated a pattern consistent with the enhanced microbial activity in the plant rhizosphere, in that higher respiration rates ($p \leq 0.05$) were observed in all soils from the rhizosphere compared to soils from nonvegetated

Table IV - 3. Vegetation survey of the Miscellaneous Chemicals Basin at the Savannah River Site.

Species (common name)	Estimated % Cover[A]	Comments
Lespedeza cuneata (sericea)	46	Tap root system Leguminous
Paspalum notatum var. *saurae* (bahia grass)	32	Fibrous root system
Solidago sp. (goldenrod)	6.5	Tap root system
Miscellaneous[B]	7	
Pinus taeda (loblolly pine)	~500 trees[C]	Tap root system Ectomycorrhizae

[A]The vegetation survey of the MCB was conducted in August, 1988. Nonvegetated areas within the MCB were estimated at 8% of the total area.
[B]Miscellaneous vegetation included *Eupatorium capillifolium* (dog fennel), *Andropogon virginicus* (broom sedge), *Pteridium aquilinum* (bracken fern), *Baccharis halimifolia* (consumption weed), *Rhus copallina* (dwarf sumac), and *Hypericum* sp.
[C]Trees were almost exclusively loblolly pine with <10 trees being long-leaf pine (*Pinus australis*)

areas within the MCB (Figures IV - 1 through IV - 6). For example on day 3 of soil incubation, CO_2 efflux was from 4 to 9 times greater in rhizosphere soils than in nonvegetated soils (Table IV - 4). Consequently, the calculated biomass based on CO_2 efflux was consistently higher in rhizosphere soils than in nonvegetated soils (Table IV - 4).

Microbial biomass measurements based on CO_2 efflux from nonvegetated soils collected in March, 1989, (spring) were elevated over nonvegetated soils from the previous summer (Table IV - 5). In addition, biomass estimates for rhizosphere soils collected in March, 1989, also varied slightly from previous estimates (Table IV - 5). Microbial biomass estimates calculated from phospholipid fatty acid (PLFA) analysis were fairly consistent with the estimates based on CO_2 efflux and also illustrated the increased microbial biomass associated with the rhizosphere soils (Table IV - 5). Consistency between biomass based on PLFA and other techniques has been previously reported (Balkwill et al., 1988). Vestall and White (1989) believe that PLFA gives a better indication of viable biomass because phospholipids are a cellular component common to all cells of the microbiota and are quickly degraded on cell death.

In addition to microbial biomass, PLFA analysis was also used to determine the microbial community structure of the soil samples from the MCB. Phospholipid fatty acid profiles of the different rhizosphere and nonvegetated soil samples are shown in Table IV - 6. The most abundant PLFA among both rhizosphere and nonvegetated soils was palmitate (16:0), a common saturated fatty acid. It was also concluded that the communities of both sample types

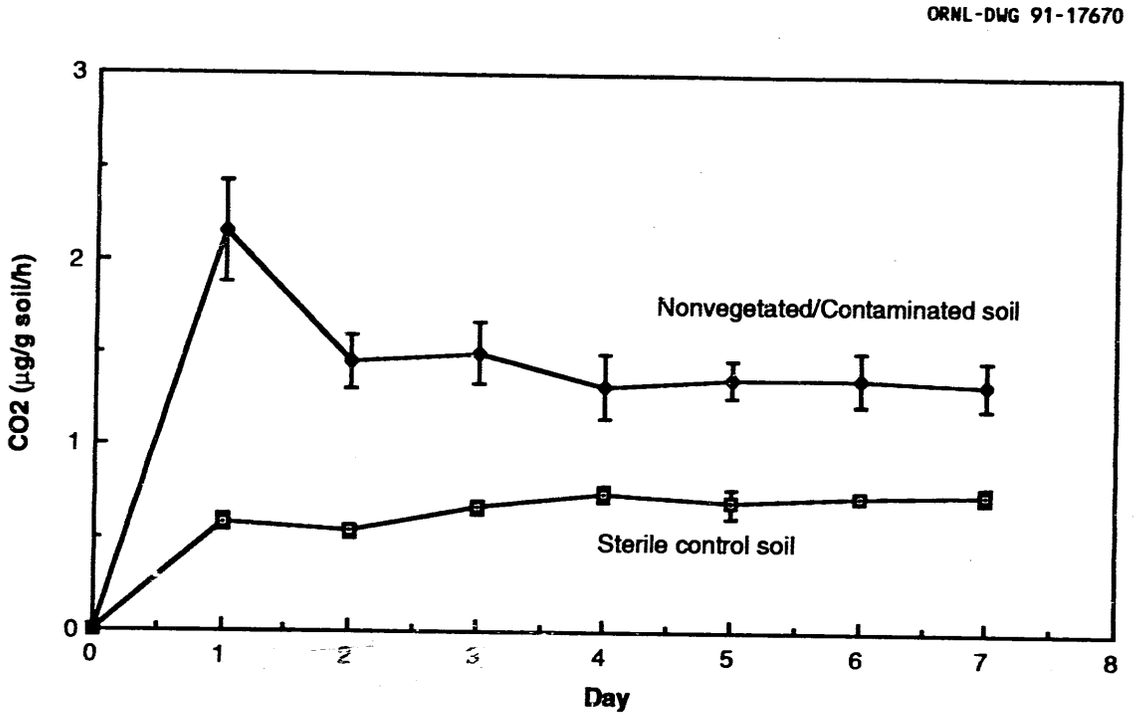

Figure IV - 1. Carbon dioxide efflux from experimental soils collected from a nonvegetated/noncontaminated area of the Miscellaneous Chemicals Basin. Sterile controls were made by autoclaving experimental soil for one hour on three consecutive days. Each datum point is the mean of three replicates. Bars indicate one standard deviation of the mean.

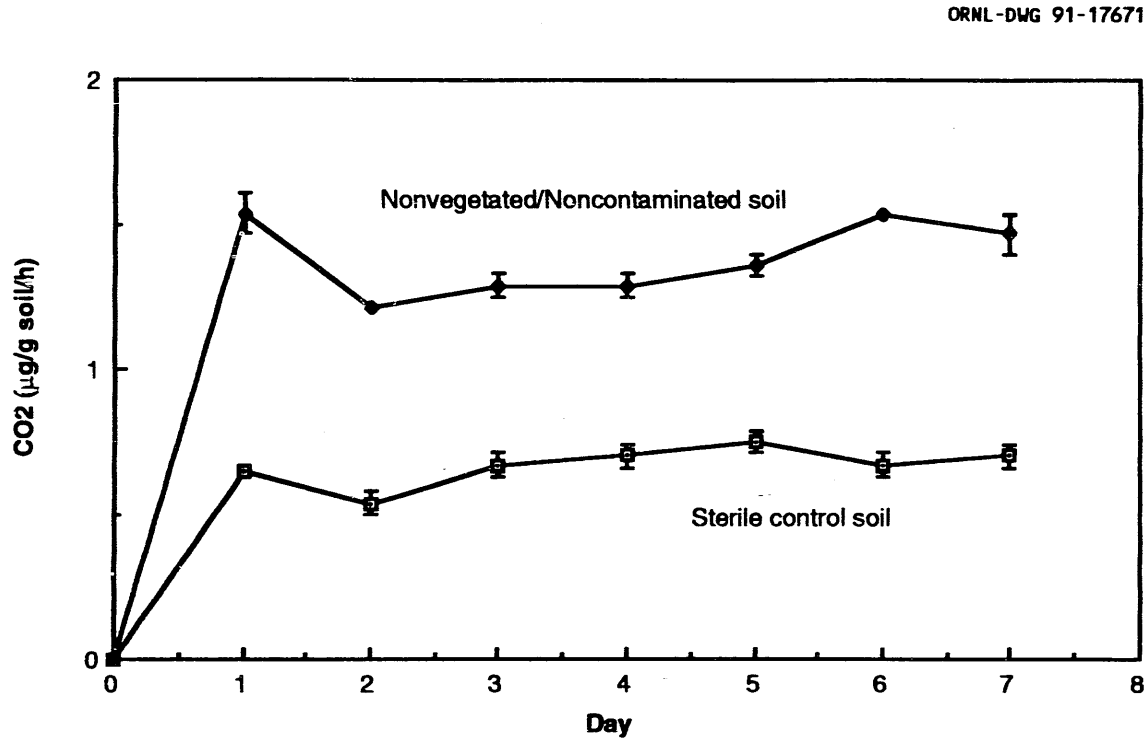

Figure IV - 2. Carbon dioxide efflux from experimental soils collected from a nonvegetated/contaminated area of the Miscellaneous Chemicals Basin. Sterile controls were made by autoclaving experimental soil for one hour on three consecutive days. Each datum point is the mean of three replicates. Bars indicate one standard deviation of the mean.

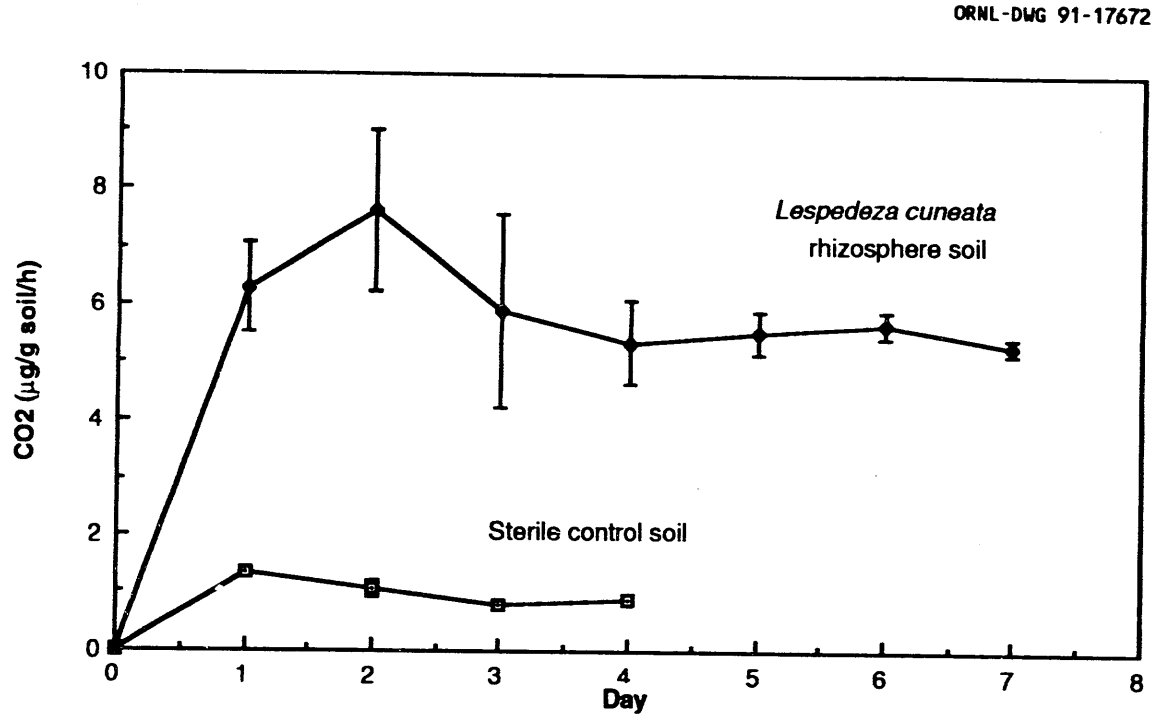

Figure IV - 3. Carbon dioxide efflux from experimental soils collected at the Miscellaneous Chemicals Basin from the rhizosphere of *Lespedeza cuneata*. Sterile controls were made by autoclaving experimental soil for one hour on three consecutive days. Each datum point is the mean of three replicates. Bars indicate one standard deviation of the mean.

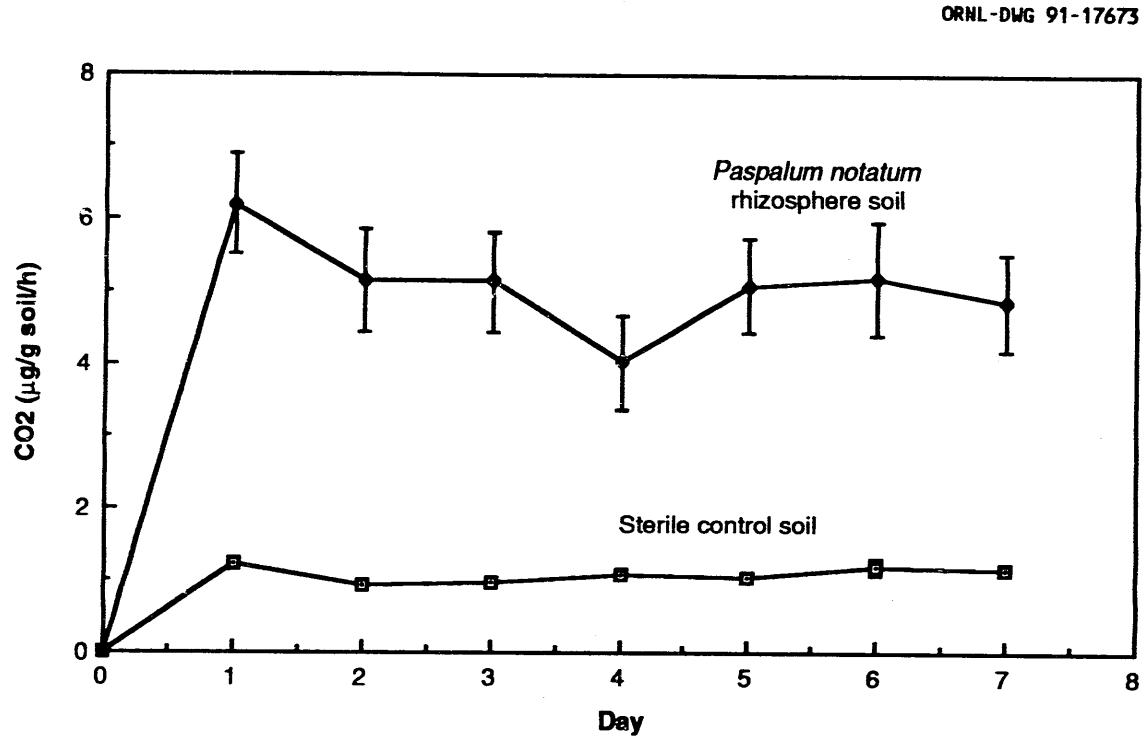

Figure IV - 4. Carbon dioxide efflux from experimental soils collected at the Miscellaneous Chemicals Basin from the rhizosphere of *Paspalum notatum*. Sterile controls were made by autoclaving experimental soil for one hour on three consecutive days. Each datum point is the mean of three replicates. Bars indicate one standard deviation of the mean.

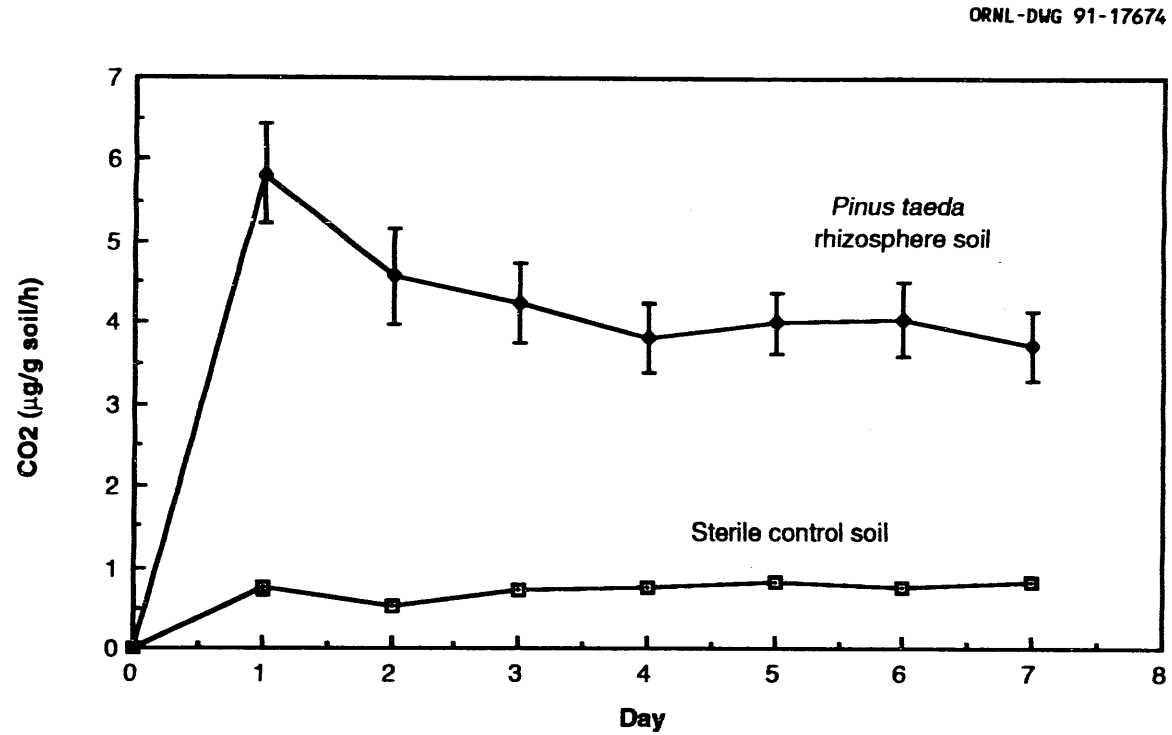

Figure IV - 5. Carbon dioxide efflux from experimental soils collected at the Miscellaneous Chemicals Basin from the rhizosphere of *Pinus taeda*. Sterile controls were made by autoclaving experimental soil for one hour on three consecutive days. Each datum point is the mean of three replicates. Bars indicate one standard deviation of the mean.

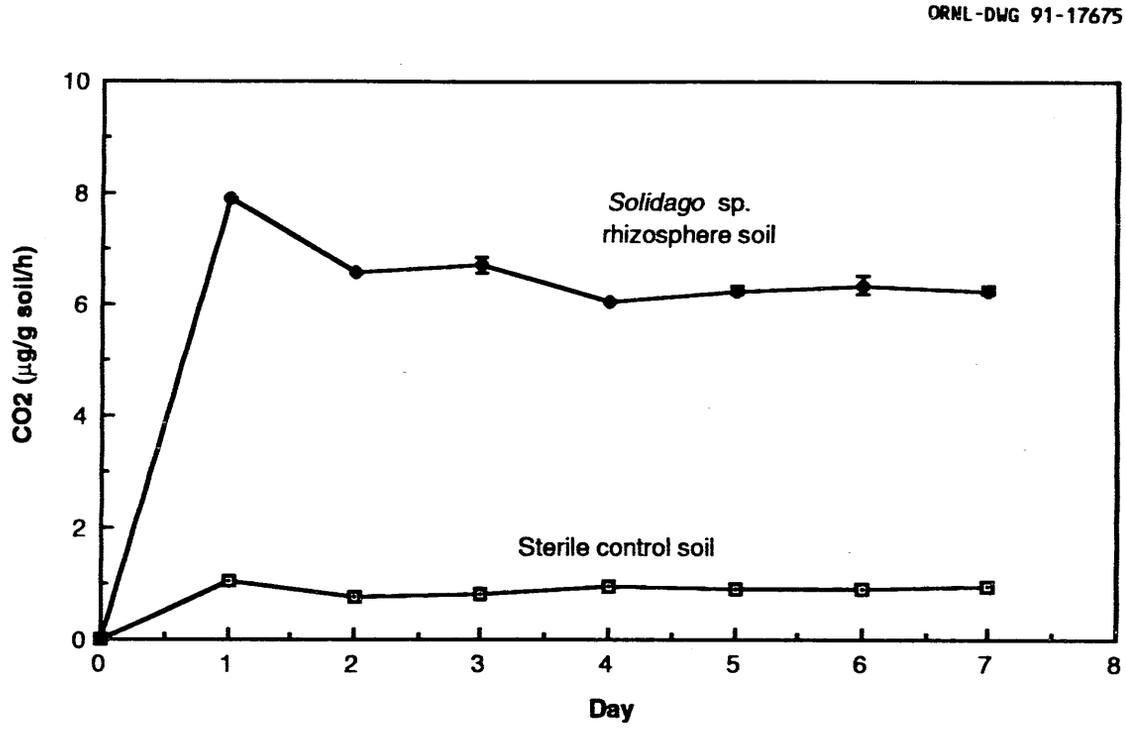

Figure IV - 6. Carbon dioxide efflux from experimental soils collected at the Miscellaneous Chemicals Basin from the rhizosphere of *Solidago* sp. Sterile controls were made by autoclaving experimental soil for one hour on three consecutive days. Each datum point is the mean of three replicates. Bars indicate one standard deviation of the mean.

Table IV - 4. Carbon dioxide efflux on day 3 of incubation from rhizosphere and nonvegetated soils collected at the Miscellaneous Chemicals Basin at the Savannah River Site.

Soil source[A]	Net CO_2 efflux[B] (µg/g of soil per h) ± SD (n = 3)	Microbial Biomass (µg/g of soil)[C]
Nonvegetated, noncontaminated[D]	0.62 ± 0.04	372
Nonvegetated	0.83 ± 0.17	498
L. cuneata	4.93 ± 1.66[E]	3072
Solidago sp.	5.90 ± 0.14[E]	3540
P. notatum	4.17 ± 0.68[E]	2502
P. taeda	3.52 ± 0.49[E]	2112

[A]All samples were collected from the MCB in August, 1988.
[B]Net CO_2 efflux = CO_2 efflux from sieved soil sample (triplicate determinations) - CO_2 efflux from matched, sterile (autoclaved) soil (triplicate determinations).
[C]Microbial biomass was calculated according to methods in Parkinson and Paul, 1982.
[D]No detectable TCE; however, trace dichloroethylene was detected.
[E]Significantly greater than CO_2 efflux from nonvegetated soil and nonvegetated, noncontaminated soil ($p \leq 0.05$).

Table IV - 5. Comparisons of microbial biomass determinations of soil collected from the Miscellaneous Chemicals Basin at the Savannah River Site.

Soil source	CO_2 Biomass[A] (µg/g soil)	CO_2 Biomass[B] (µg/g soil)	PLFA Biomass[C] (µg/g soil)
Nonvegetated, noncontaminated	372 ± 24	2016 ± 0	---
Nonvegetated	498 ± 102	1524 ± 0	680 ± 423
L. cuneata	3072 ± 996	3150 ± 264	1449 ± 316
Solidago sp.	3540 ± 0	2676 ± 294	5825 ± 1176
P. notatum	2502 ± 408	2262 ± 240	3624 ± 522
P. taeda	2112 ± 294	2670 ± 90	1252 ± 175

[A]Biomass based on CO_2 efflux from samples collected August, 1988.
[B]Biomass based on CO_2 efflux from samples collected March, 1989.
[C]Biomass of samples collected March, 1989 with a conversion factor of 100 µmoles phospholipid/g of bacteria the size of *E. coli* (Smith et al., 1989).

Table IV - 6. Phospholipid fatty acid (PLFA) profiles of soil samples collected from the Miscellaneous Chemicals Basin at the Savannah River Site.

PLFA[A]	Mole % ± SD				
	Nonvegetated	*L. cuneata*	*Solidago* sp.	*P. notatum*	*P. taeda*
a13:0	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
i14:0	0.7 ± 0.6	0.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.2
14:0	1.4 ± 0.9	1.4 ± 0.2	1.4 ± 0.1	1.3 ± 0.1	1.8 ± 0.3
i15:1	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
a15:1	0.4 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
i15:0	7.1 ± 2.1	8.8 ± 0.4	10.9 ± 0.4	8.6 ± 0.4	10.3 ± 0.2
a15:0	2.7 ± 0.9	3.8 ± 0.3	4.5 ± 0.1	3.8 ± 0.1	5.3 ± 0.4
15:1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
15:0	1.0 ± 0.7	0.9 ± 0.2	0.9 ± 0.1	1.1 ± 0.0	1.2 ± 0.1
16:3	0.5 ± 0.8	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
16:1	0.7 ± 0.8	0.0 ± 0.0	0.5 ± 0.0	0.6 ± 0.1	0.6 ± 0.0

Table IV - 6. cont'd.

PLFA[A]	Mole % ± SD				
	Nonvegetated	*L. cuneata*	*Solidago* sp.	*P. notatum*	*P. taeda*
i16:0	4.1 ± 1.0	2.2 ± 0.1	2.4 ± 0.1	2.7 ± 0.2	2.7 ± 0.1
16:1ω9c	1.1 ± 0.7	1.3 ± 0.1	1.7 ± 0.1	1.9 ± 0.2	2.2 ± 0.2
16:1ω7c	5.3 ± 0.5	6.2 ± 0.7	7.6 ± 0.6	5.6 ± 0.1	6.2 ± 0.7
16:1ω7t	0.2 ± 0.3	0.3 ± .01	0.3 ± 0.0	0.3 ± 0.0	0.1 ± 0.1
16:1ω5c	2.8 ± 0.8	3.8 ± 0.3	4.9 ± 0.2	4.1 ± 0.1	4.1 ± 0.2
16:1ω13t	0.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
16:0	13.0 ± 2.3	14.9 ± 1.2	14.9 ± 0.4	14.1 ± 0.4	14.8 ± 0.4
i17:1	2.1 ± 0.9	2.3 ± 0.1	2.7 ± 0.1	2.6 ± 0.2	2.7 ± 0.6
10Me16:0	3.8 ± 1.0	4.6 ± 0.8	3.5 ± 0.2	5.6 ± 0.4	5.4 ± 0.4
i17:0	2.4 ± 0.6	1.6 ± 0.1	1.3 ± 0.0	1.6 ± 0.1	1.7 ± 0.1
a17:0	2.4 ± 0.9	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.3

Table IV - 6. cont'd.

PLFA[A]	Mole % ± SD				
	Nonvegetated	*L. cuneata*	*Solidago* sp.	*P. notatum*	*P. taeda*
17:1ω6	0.6 ± 0.9	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
cy17:0	2.8 ± 1.1	3.8 ± 0.3	3.2 ± 0.2	3.5 ± 0.2	3.5 ± 0.2
17:1	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
17:0	0.8 ± 0.5	0.9 ± 0.3	0.6 ± 0.0	0.6 ± 0.0	0.8 ± 0.3
18:3ω6	0.4 ± 0.3	0.2 ± 0.1	0.7 ± 0.0	0.8 ± 0.0	0.4 ± 0.3
18:2ω6	4.5 ± 2.4	3.9 ± 1.1	5.4 ± 1.1	6.6 ± 1.3	5.3 ± 1.0
18:3ω3	2.4 ± 1.5	0.0 ± 0.0	0.9 ± 0.1	1.0 ± 0.2	0.6 ± 0.4
18:1ω9c	5.9 ± 1.8	4.0 ± 0.5	3.9 ± 0.3	3.1 ± 0.1	3.2 ± 0.0
18:1ω7c	6.7 ± 1.2	8.5 ± 0.8	8.4 ± 0.6	7.9 ± 0.1	6.4 ± 0.4
18:1ω7t	0.0 ± 0.0	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	0.0 ± 0.0
18:1ω5c	0.7 ± 0.6	1.0 ± 0.2	0.7 ± 0.1	0.9 ± 0.0	0.7 ± 0.1

Table IV - 6. cont'd.

PLFA[A]	Mole % ± SD				
	Nonvegetated	*L. cuneata*	*Solidago* sp.	*P. notatum*	*P. taeda*
18:1	0.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
18:0	2.7 ± 0.7	2.9 ± 0.2	2.1 ± 0.6	1.7 ± 0.0	1.6 ± 0.1
br19:1	0.4 ± 0.4	1.3 ± 0.5	0.8 ± 0.0	0.7 ± 0.0	0.7 ± 0.1
10Me18:0	1.8 ± 0.6	0.8 ± 0.1	0.7 ± 0.0	1.1 ± 0.0	1.2 ± 0.4
cy19:0	8.0 ± 2.2	9.7 ± 1.2	4.2 ± 0.2	4.6 ± 0.2	5.2 ± 0.5
20:4ω6	0.5 ± 0.6	1.9 ± 0.5	0.8 ± 0.0	0.8 ± 0.0	0.4 ± 0.1
20:5ω3	0.2 ± 0.4	0.7 ± 0.2	0.4 ± 0.0	0.4 ± 0.1	0.0 ± 0.0
20:3ω6	0.4 ± 0.5	0.7 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:2ω3	0.1 ± 0.1	0.5 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
20:1ω9c	0.6 ± 0.5	0.3 ± 0.3	0.1 ± 0.0	0.3 ± 0.0	0.0 ± 0.0
20:0	2.5 ± 1.6	2.0 ± 0.2	0.7 ± 0.1	0.5 ± 0.4	0.7 ± 0.5

Table IV - 6. cont'd.

PLFA[A]	Mole % ± SD				
	Nonvegetated	*L. cuneata*	*Solidago* sp.	*P. notatum*	*P. taeda*
22:0	0.5 ± 0.5	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
23:0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
24:0	0.2 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.2

[A]The shorthand nomenclature used to describe fatty acids is a follows. The number before the colon indicates the number of carbon atoms in the fatty acid. The number after the colon indicates the number of double bonds in the fatty acid chain. The position of the initial double bond is indicated by the number of carbon atoms from the methyl, or ω, end of the molecule. The configuration of the double bond is shown by the **c** for cis and **t** for trans. Because almost all unsaturations are cis, **c** is often omitted. Methyl branching can be indicated as iso (**i**; the second carbon from the methyl end), anteiso (**a**; the third carbon) or **br** (branch) if the position is unknown. When a branch is known but not in the **i** or **a** position, it is indicated by the position from the carboxyl end followed by **Me** before the carbon chain length. Cyclopropane fatty acids are indicated as **cy**. (Adapted from Vestal and White, 1989).

(rhizosphere and nonvegetated) are composed of gram negative and gram positive microorganisms in approximately equal abundance as indicated by the strong presence of monounsaturated (16:1ω7c and 18:1ω7c) and branched phospholipid fatty acids (i15:0 and i16:0 for example), respectively, in the samples. Further review of the PLFA profiles indicated that ratios of cis fatty acids to trans fatty acids (specifically 16:1ω7c to 16:1ω7t and 18:1ω7c to 18:1ω7t), which are indicative of stress or unbalanced growth (Vestall and White, 1989), were not significantly different among the rhizosphere and nonvegetated soils. In addition, cyclopropyl fatty acids such as cy17:0 and cy19:0, which are indicative of unbalanced growth and/or anaerobic microorganisms (Guckert et al., 1985), were present in all sample types. These results are probably best explained by the soil conditions at the MCB. Namely that the soil is low in organic carbon and fluctuates between aerobic and anaerobic conditions.

Additional evaluation of the PLFA profiles of rhizosphere and nonvegetated soils from the MCB was done using standard cluster analysis techniques. A dendogram of the cluster analysis results is shown in Figure IV - 7. The figure illustrates the primary clustering of nonvegetated soil samples with *Lespedeza cuneata* soil samples and *Solidago* sp. soil samples with *Pinus taeda* soil samples and *Paspalum notatum* soil samples. Secondary clustering occurred between *Pinus taeda* soil samples and *Paspalum notatum* soil samples. A principal components analysis indicated that the fatty acid which most determines the primary clustering (separation of the nonvegetated soil and *L. cuneata* rhizosphere soil from the others) was cy19:0. The secondary

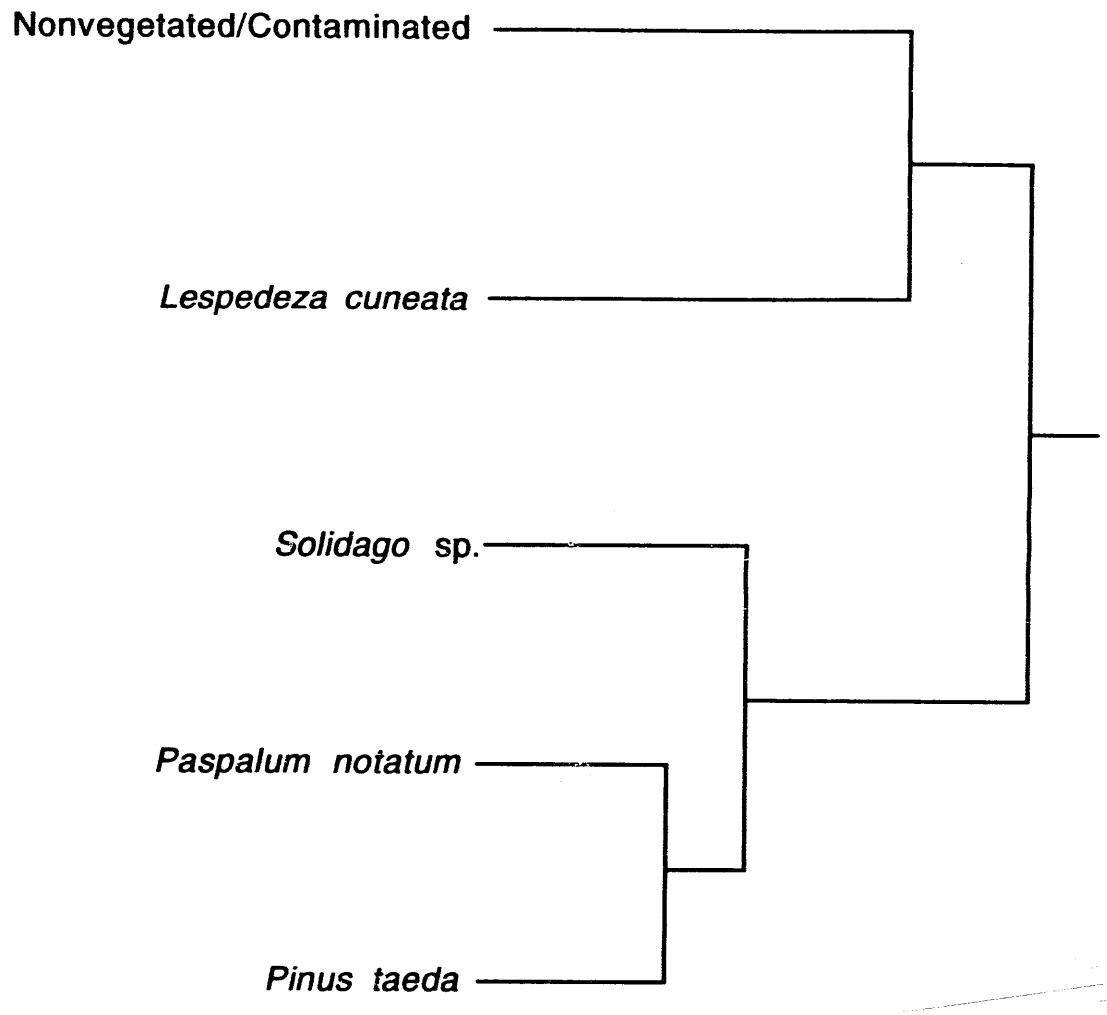

Figure IV - 7. Dendogram of cluster analysis results from phospholipid fatty acid profiles of rhizosphere and nonvegetated soils from the Miscellaneous Chemicals Basin.

clustering which separates *P. notatum* and *P. taeda* from *Solidago* sp. was explained by the greater presence of 10Me16:0 and 10Me18:0 in samples of *P. taeda* and *P. notatum* rhizosphere soil. These fatty acids (10Me16:0 and 10Me18:0) are characteristic of Actinomycetes (Vestall and White, 1989).

The microbial metbolic activity of soil samples from the MCB was determined by measuring the incorporation of ^{14}C-acetate into cellular lipids. In initial studies, the rate of ^{14}C-acetate incorporation into cellular lipids (pMoles/g soil/h) was determined for the four rhizosphere soils and the nonvegetated soil (Figure IV - 8). The metabolic activity of the four rhizosphere samples, as determined by ^{14}C-acetate incorporation rate, was significantly greater ($p \leq 0.05$) than metabolic activity in the nonvegetated soil (1407 ± 10, 905 ± 35, 599 ± 33, 1727 ± 180 pMoles/g soil/h for *L. cuneata*, *P. taeda*, *P. notatum*, and *Solidago* sp., respectively vs. 445 ± 86 pMoles/g soil/h for the nonvegetated soil). In addition, ^{14}C-acetate incorporation rates among the four rhizosphere samples were significantly different ($p \leq 0.05$) from each other, with *Solidago* sp. rhizosphere soil having the highest rate. The results indicating greater metabolic activity among the rhizosphere samples is consistent with the biomass estimates reported earlier and also consistent with what traditionally has been observed in rhizosphere samples.

In addition to monitoring acetate incorporation into total microbial lipids, the incorporation of ^{14}C-acetate into the three lipid classes (neutral lipids, glycolipids, and phospholipids) was also determined for rhizosphere and nonvegetated soils from the MCB (Figures IV - 9 through IV - 13). Soil from nonvegetated areas within the MCB incorporated ^{14}C-acetate predominantly

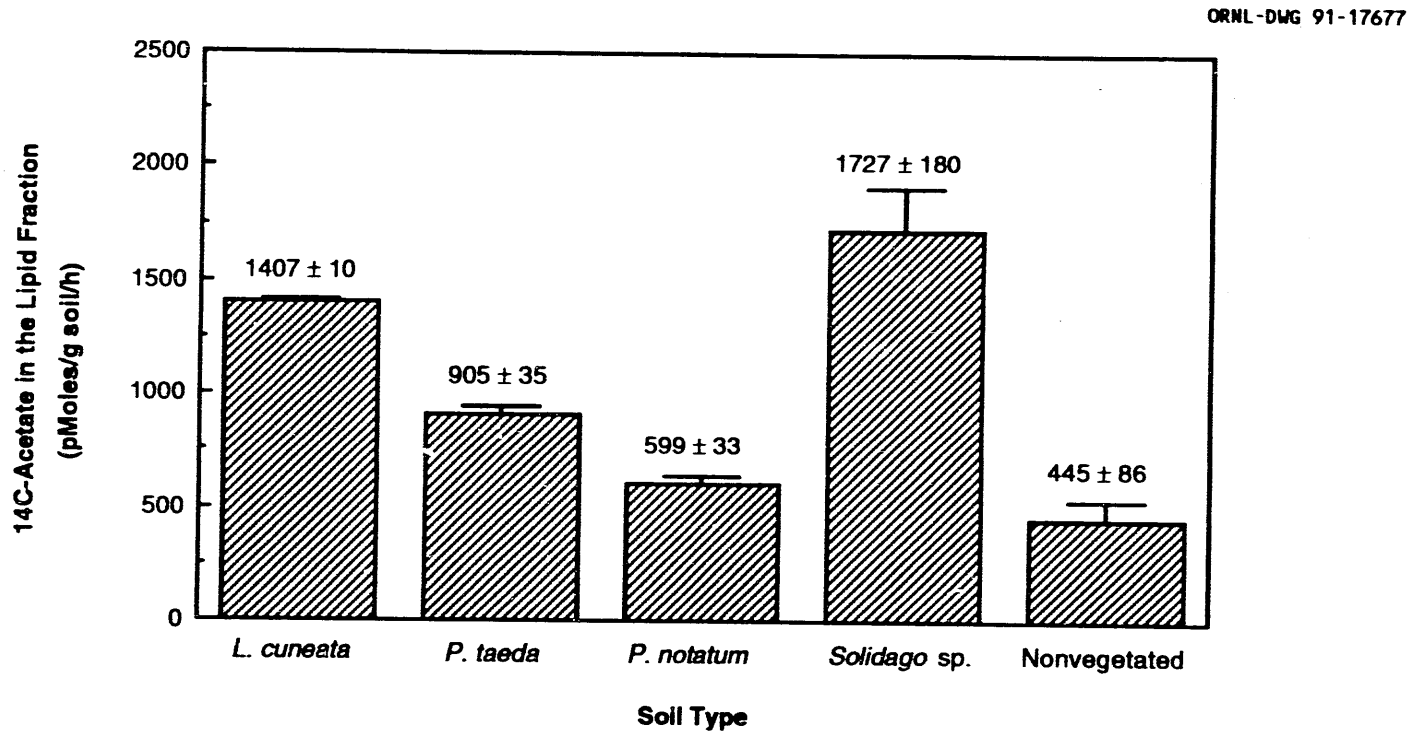

Figure IV - 8. Rates of ^{14}C-acetate incorporation into total microbial lipids of soil samples collected from rhizosphere and nonvegetated areas at the Miscellaneous Chemicals Basin.

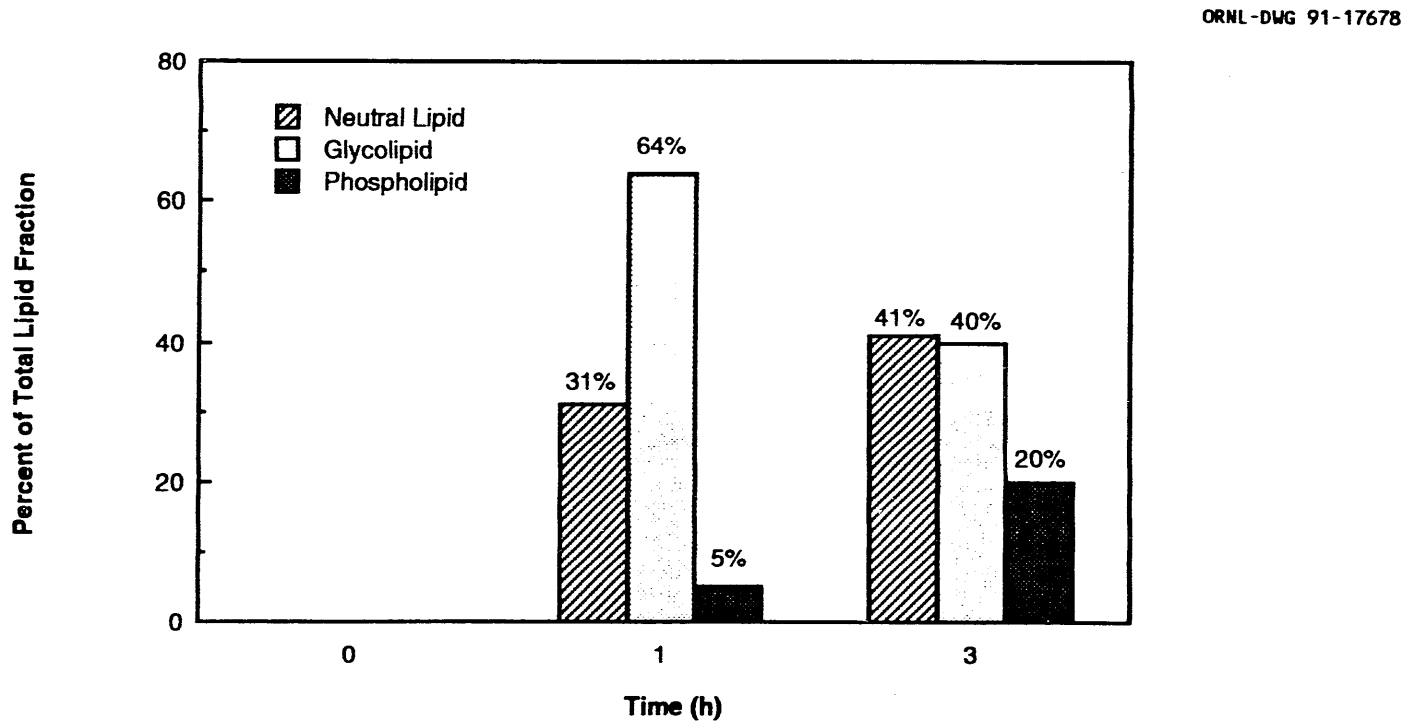

Figure IV - 9. Incorporation of ^{14}C-acetate into the lipids of microorganisms from nonvegetated soil collected at the Miscellaneous Chemicals Basin.

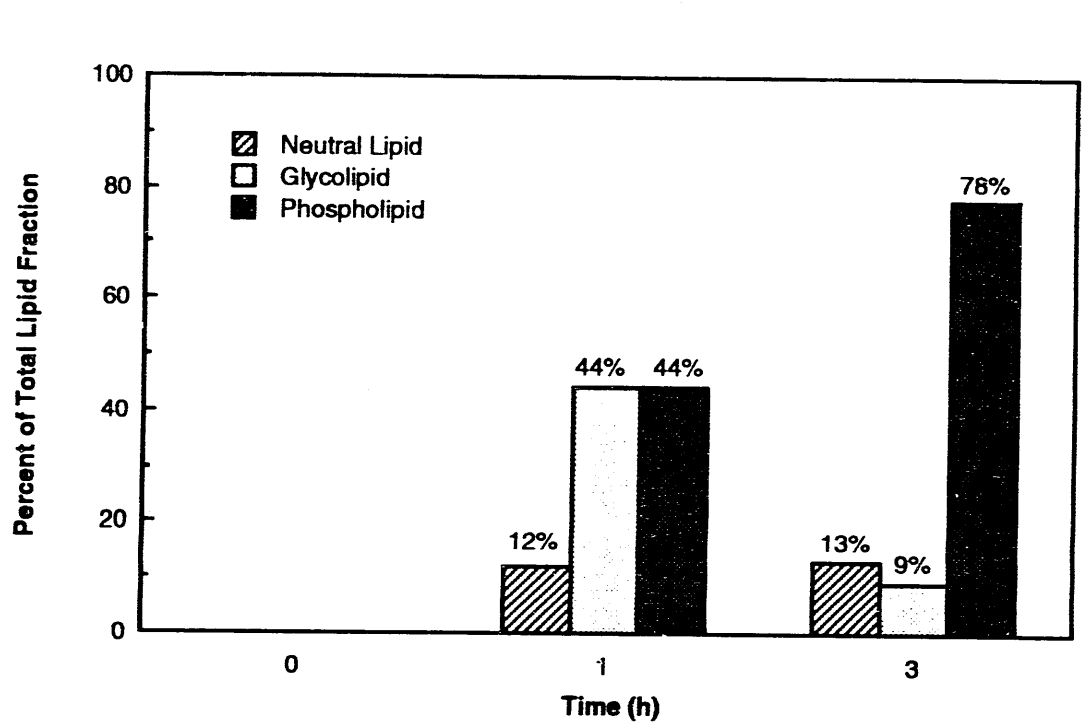

Figure IV - 10. Incorporation of ^{14}C-acetate into the lipids of microorganisms from the rhizosphere of *Lespedeza cuneata* soil collected at the Miscellaneous Chemicals Basin.

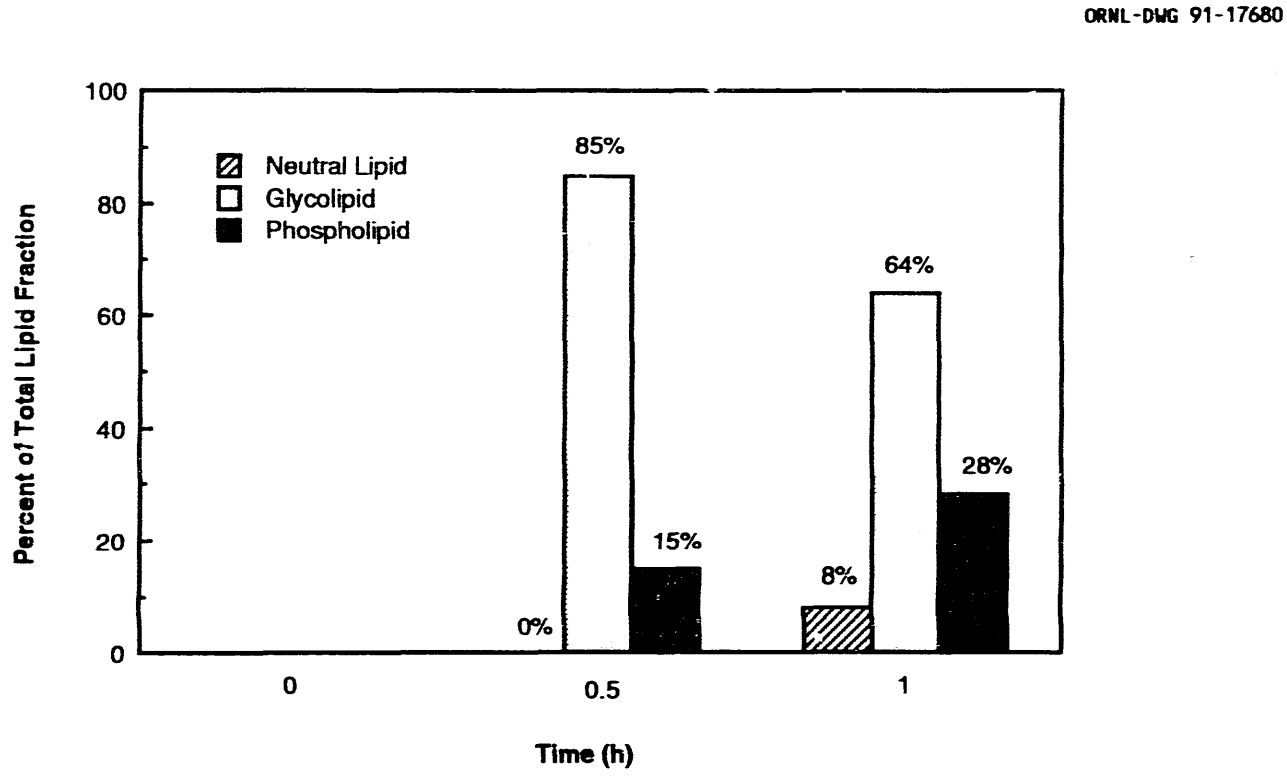

Figure IV - 11. Incorporation of ^{14}C-acetate into the lipids of microorganisms from the rhizosphere of *Paspalum notatum* soil collected at the Miscellaneous Chemicals Basin.

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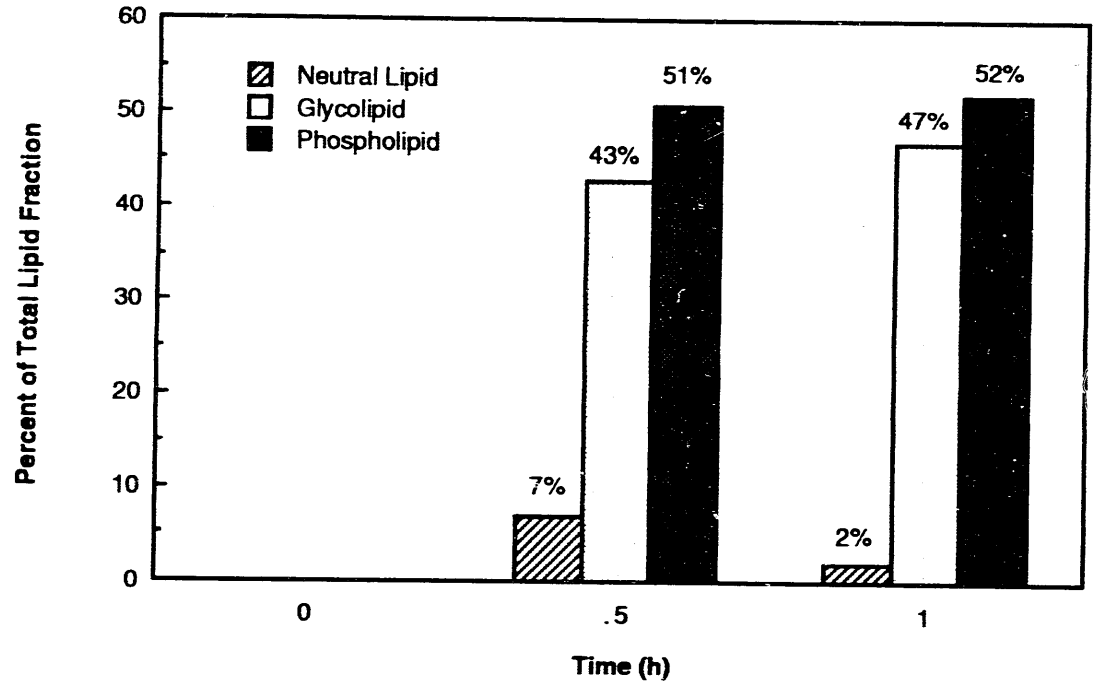

Figure IV - 12. Incorporation of ^{14}C-acetate into the lipids of microorganisms from the rhizosphere of *Pinus taeda* soil collected at the Miscellaneous Chemicals Basin.

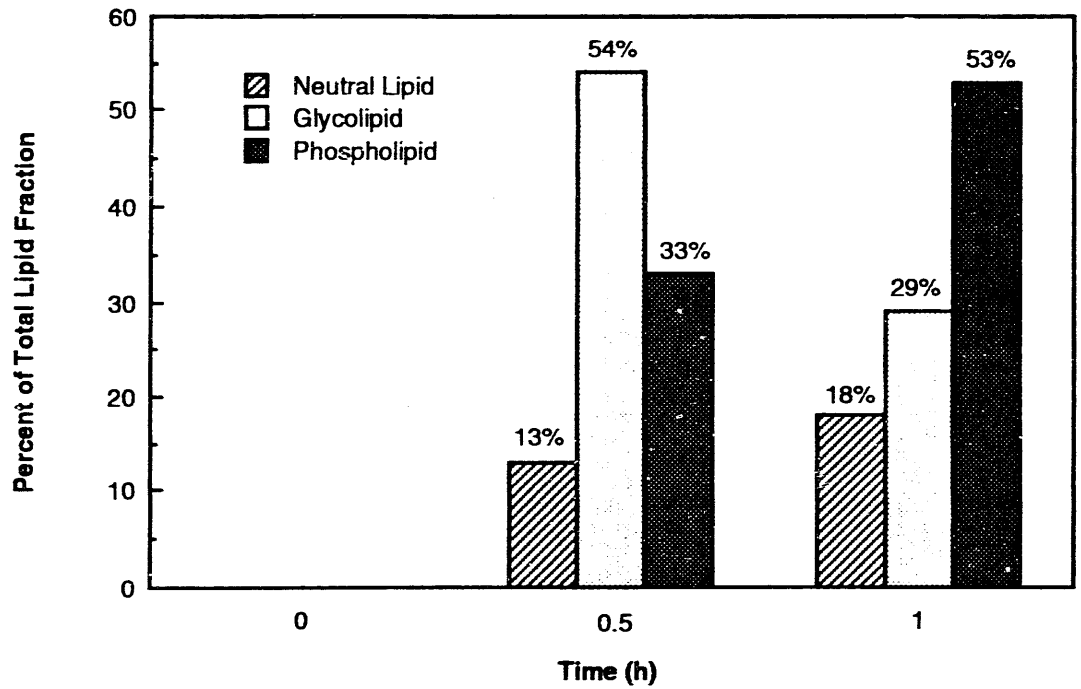

Figure IV - 13. Incorporation of ^{14}C-acetate into the lipids of microorganisms from the rhizosphere of *Solidago* sp. soil collected at the Miscellaneous Chemicals Basin.

into neutral and glycolipids (storage lipids) during the first three hours of incubation possibly indicating unbalanced growth of the microbial community in this soil. Only about 20% of the radioactivity was found in the phospholipid (membranes) fraction after 3 hours (Figure IV - 9). In contrast, soils from *L. cuneata* (Figure IV - 10), *P. taeda* (Figure IV - 12), and *Solidago* sp. (Figure IV - 13) incorporated the acetate into membrane lipids (phospholipids). Radioactivity in the phospholipid fraction for *P. taeda* and *Solidago* sp. rhizosphere soil was greater than 50% of the total radioactivity in all lipid fractions after 1 hour and greater than 80% for *L. cuneata* rhizosphere soil after 3 hours. Soils collected from *P. notatum* rhizosphere soil incorporated most of the ^{14}C-acetate into the glycolipid fraction after 1 hour (Figure IV - 11), similar to the results of the nonvegetated soil.

Production of the endogenous storage lipids, polybetahydroxyalkanoates (PHAs) was also used as an indicator of the nutritional status of rhizosphere and nonvegetated soils from the MCB. Mass spectroscopy revealed that almost all of the constituent beta-hydroxy acids from the PHAs was betahydroxybutyrate (PHB) although very small amounts of betahydroxy hexanoate were also detected. All samples tested contained significant amounts of PHA, however, PHA levels varied among sample types (Table IV - 7). Consistently greater amounts of PHA were detected in rhizosphere soils compared with the nonvegetated soils, although the amounts were significantly greater ($p \leq 0.05$) only for soils from *Paspalum notatum* rhizosphere. The ratios of phospholipid fatty acid (PLFA) to PHA were also determined for the

Table IV - 7. Results of polybetahydroxyalkanoate analyses of rhizosphere and nonvegetated soils from the Miscellaneous Chemicals Basin.

Soil sample	PLFA[A]	PHA[B]	PLFA/PHA[C]
Nonvegetated	3400 ± 2115	276 ± 132	12 ± 7.7
Lespedeza cuneata rhizosphere	7245 ± 1580	621 ± 321	12 ± 2.5
Paspalum notatum rhizosphere	18120 ± 2610	514 ± 78	35 ± 5.1
Pinus taeda rhizosphere	6260 ± 875	455 ± 161	14 ± 1.9
Solidago sp. rhizosphere	29125 ± 5880	297 ± 57	98 ± 19.8

[A]Mean nanomoles of phospholipid fatty acid (PLFA) per 50 g soil sample ± one standard deviation of the mean
[B]Mean nanomoles of polybetahydroxyalkanoates (PHA) per 50 g soil sample ± one standard deviation of the mean
[C]Mean ratio of PLFA to PHA ± one standard deviation of the mean

rhizosphere and nonvegetated soils as an indicator of the relative amounts of these membrane (PLFA) and storage (PHA) lipid components (Table IV - 7).

C. Toxicity of TCE and PCE to Soil Microorganisms

The influence of TCE on CO_2 efflux (used as an indicator of toxicity) in soil samples collected from the MCB varied with TCE concentration and sample type (Figures IV - 14 through IV - 18). Only the *Solidago* sp. rhizosphere soil showed a statistically significant decrease ($p \leq 0.05$ at 1000 µg/g TCE) in CO_2 efflux (Figure IV - 18) although *P. notatum* rhizosphere soil (Figure IV - 16), and the nonvegetated soil (Figure IV - 14) also showed decreased respiration at 1000 µg/g TCE, and 500 µg/g and 1000 µg/g TCE, respectively. Respiration in *L. cuneata* (Figure IV - 15) or *P. taeda* (Figure IV - 17) rhizosphere soils was not affected by TCE at the two concentrations tested.

Toxicity to soil samples from the MCB was also determined for tetrachloroethylene (PCE) (Figures IV - 19 through IV - 24). Soil gas concentrations of PCE at the MCB are higher than all of the other chlorinated solvents and it was hypothesized that PCE may be inhibiting TCE degradation because of its toxicity. Nonvegetated soil samples collected from an area within the MCB shown in earlier soil gas analyses to be uncontaminated showed a significant decrease ($p \leq 0.05$) in CO_2 efflux at PCE concentrations of 500 and 1000 µg/g (Figure IV - 19) while nonvegetated soils collected from contaminated areas showed no statistically significant effect (Figure IV - 20). Tetrachloroethylene concentrations of 500 µg/g and 1000 µg/g had a stimulatory effect on CO_2 efflux from *L. cuneata* (Figure IV - 21) and *Solidago*

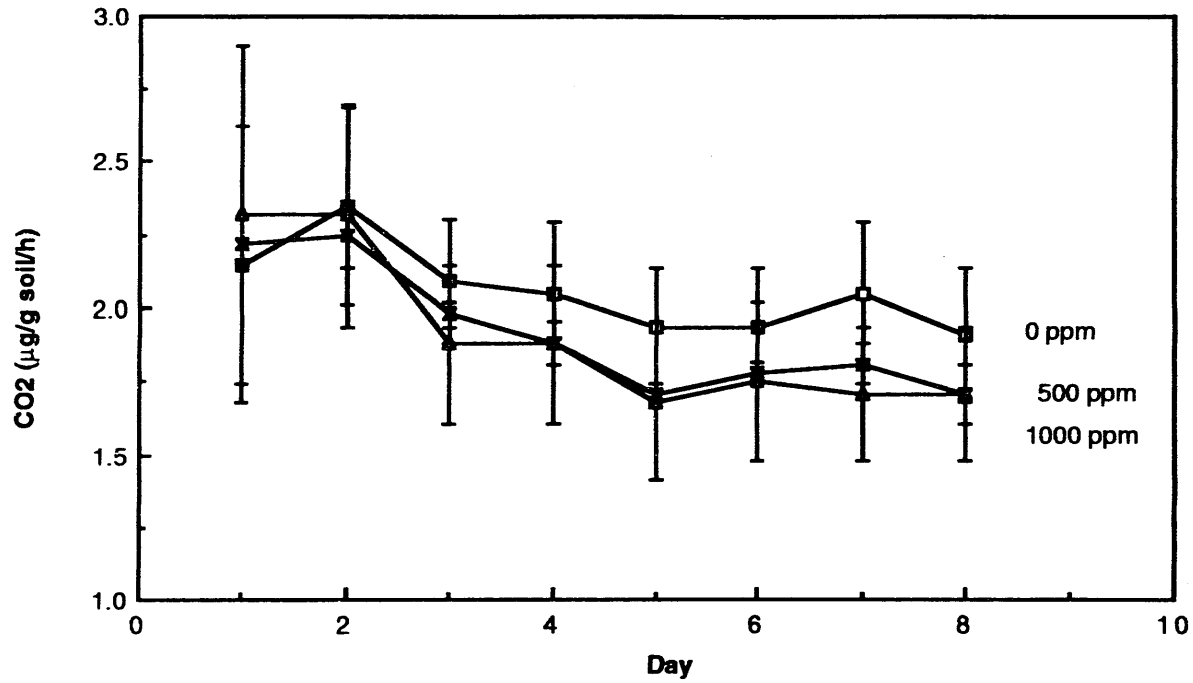

Figure IV - 14. Carbon dioxide efflux from nonvegetated/contaminated soil collected from the Miscellaneous Chemicals Basin and treated with 0, 500, and 1000 µg/g (ppm) trichloroethylene. Each datum point is the mean of three replicates. Error bars represent one standard deviation of the mean.

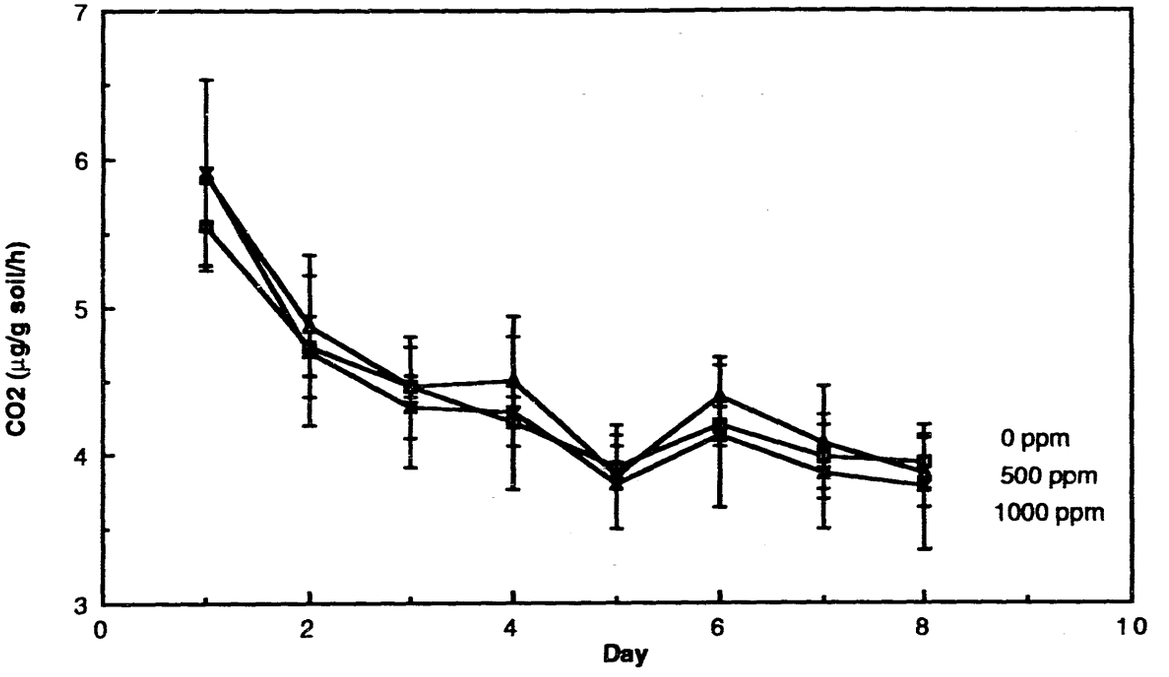

Figure IV - 15. Carbon dioxide efflux from *Lespedeza cuneata* rhizosphere soil collected from the Miscellaneous Chemicals Basin and treated with 0, 500, and 1000 µg/g (ppm) trichloroethylene. Each datum point is the mean of three replicates. Error bars represent one standard deviation of the mean.

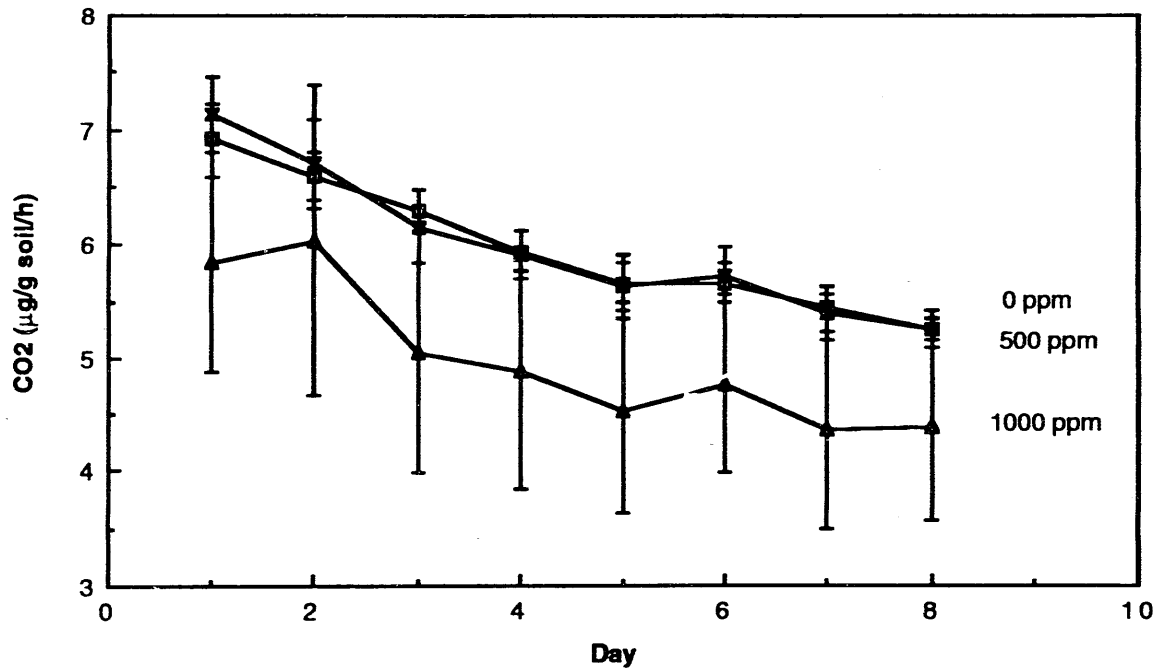

Figure IV - 16. Carbon dioxide efflux from *Paspalum notatum* rhizosphere soil collected from the Miscellaneous Chemicals Basin and treated with 0, 500, and 1000 µg/g (ppm) trichloroethylene. Each datum point is the mean of three replicates. Error bars represent one standard deviation of the mean.

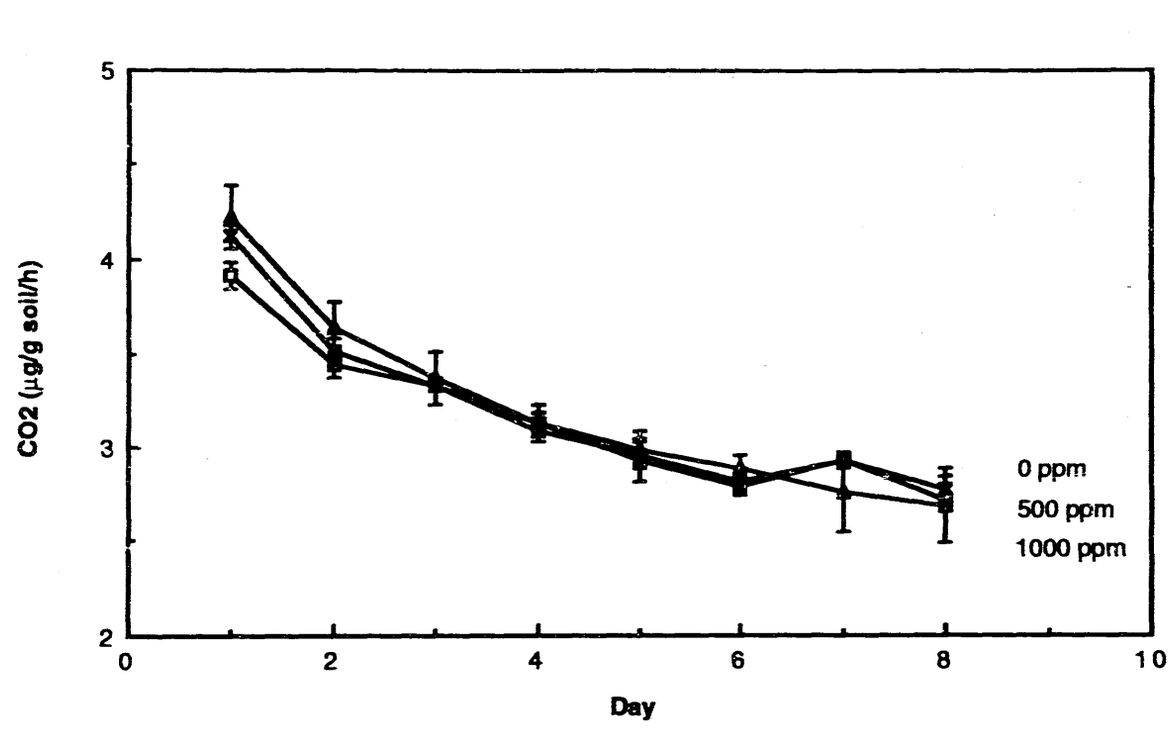

Figure IV - 17. Carbon dioxide efflux from *Pinus taeda* rhizosphere soil collected from the Miscellaneous Chemicals Basin and treated with 0, 500, and 1000 µg/g (ppm) trichloroethylene. Each datum point is the mean of three replicates. Error bars represent one standard deviation of the mean.

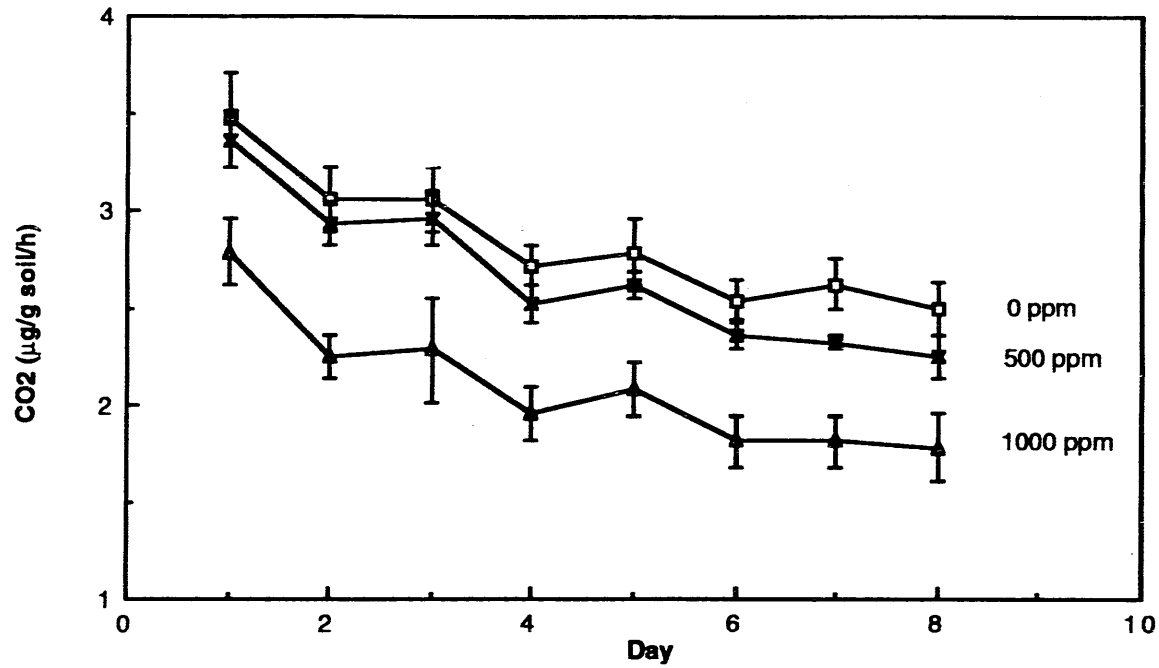

Figure IV - 18. Carbon dioxide efflux from *Solidago* sp. rhizosphere soil collected from the Miscellaneous Chemicals Basin and treated with 0, 500, and 1000 µg/g (ppm) trichloroethylene. Each datum point is the mean of three replicates. Error bars represent one standard deviation of the mean.

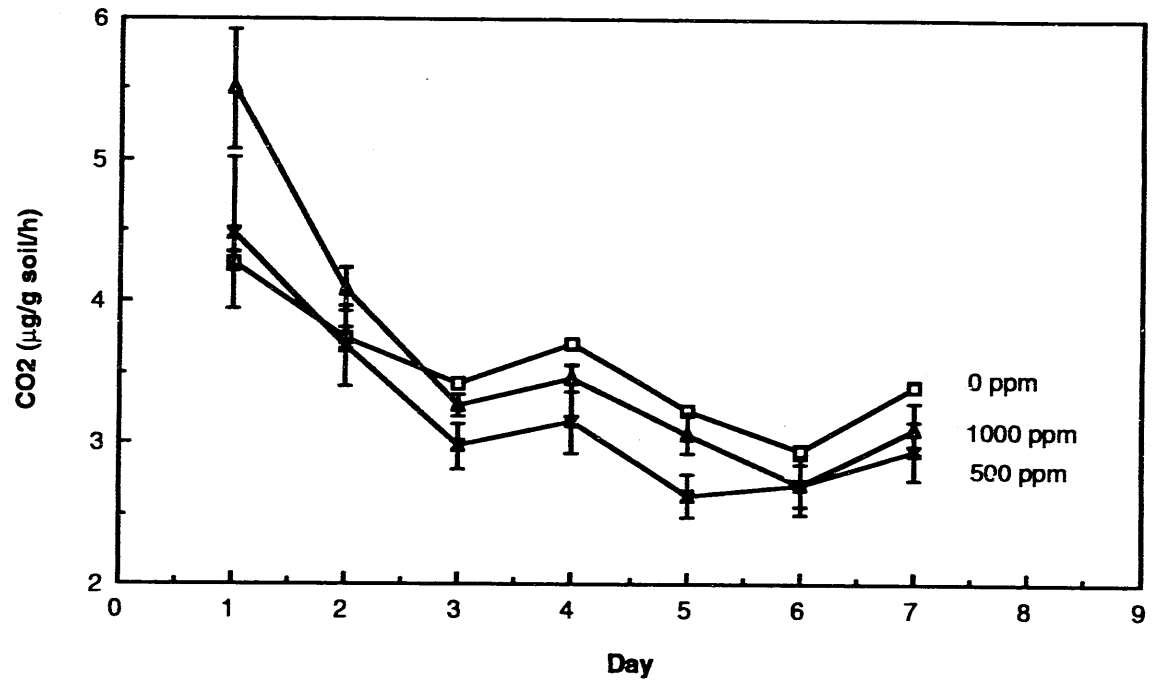

Figure IV - 19. Carbon dioxide efflux from nonvegetated/noncontaminated soil collected from the Miscellaneous Chemicals Basin and treated with 0, 500, and 1000 µg/g (ppm) tetrachloroethylene. Each datum point is the mean of three replicates. Error bars represent one standard deviation of the mean.

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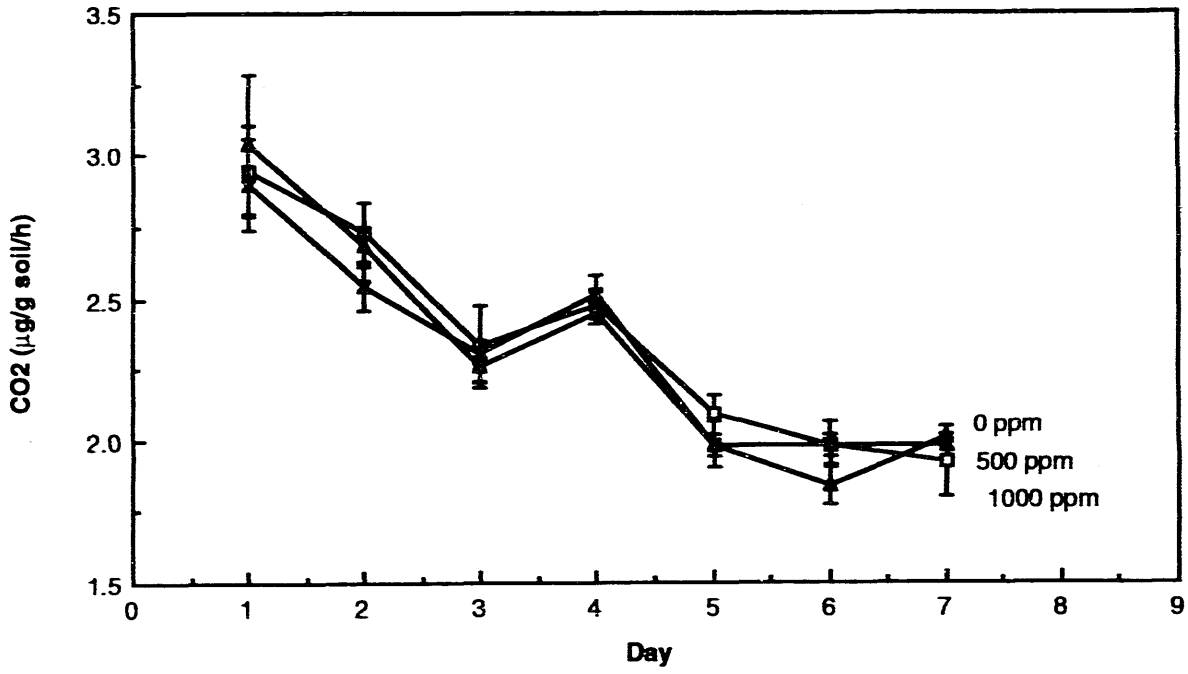

Figure IV - 20. Carbon dioxide efflux from nonvegetated/contaminated soil collected from the Miscellaneous Chemicals Basin and treated with 0, 500, and 1000 µg/g (ppm) tetrachloroethylene. Each datum point is the mean of three replicates. Error bars represent one standard deviation of the mean.

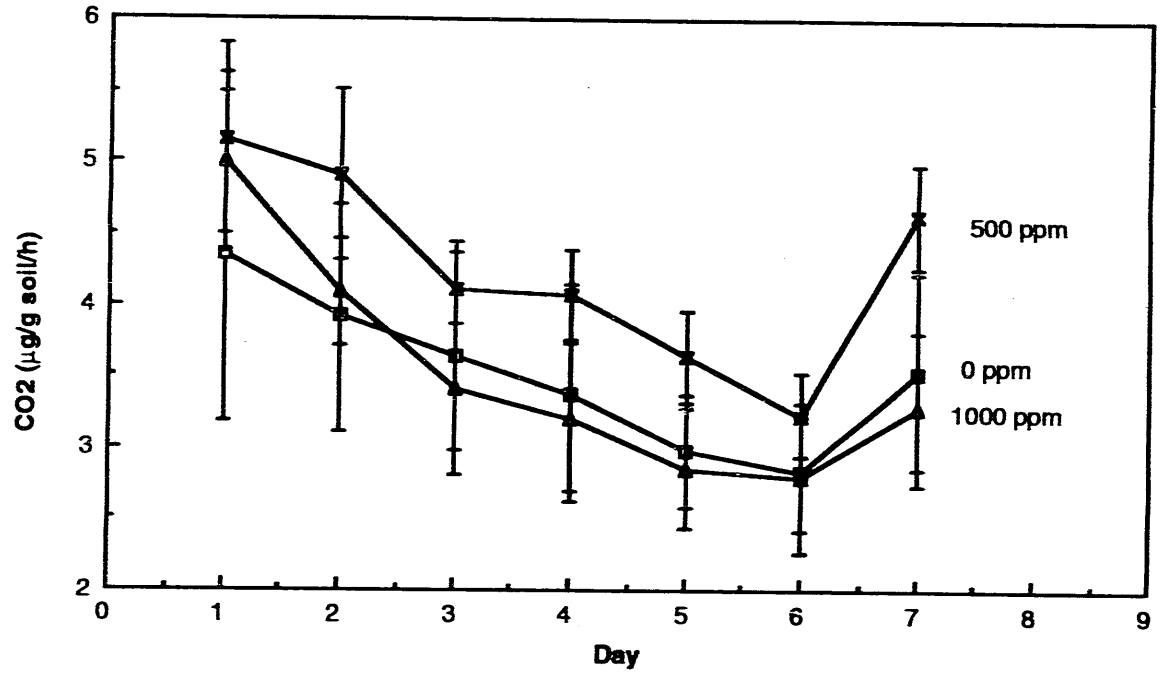

Figure IV - 21. Carbon dioxide efflux from *Lespedeza cuneata* rhizosphere soil collected from the Miscellaneous Chemicals Basin and treated with 0, 500, and 1000 µg/g (ppm) tetrachloroethylene. Each datum point is the mean of three replicates. Error bars represent one standard deviation of the mean.

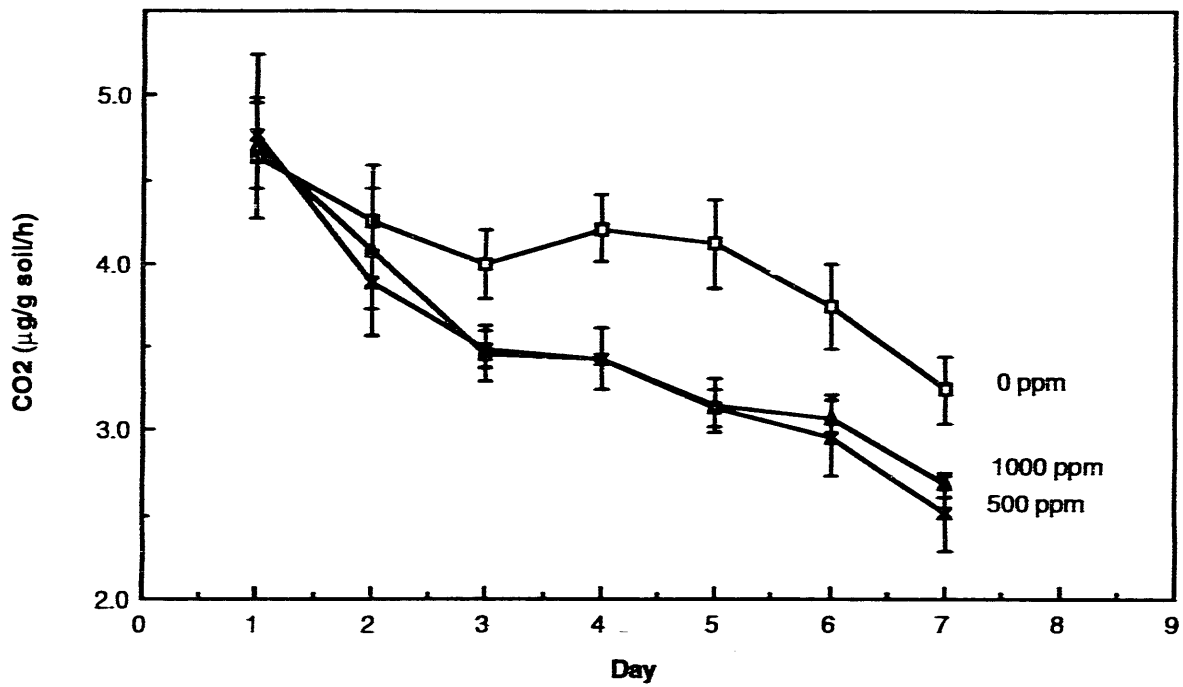

Figure IV - 22. Carbon dioxide efflux from *Paspalum notatum* rhizosphere soil collected from the Miscellaneous Chemicals Basin and treated with 0, 500, and 1000 µg/g (ppm) tetrachloroethylene. Each datum point is the mean of three replicates. Error bars represent one standard deviation of the mean.

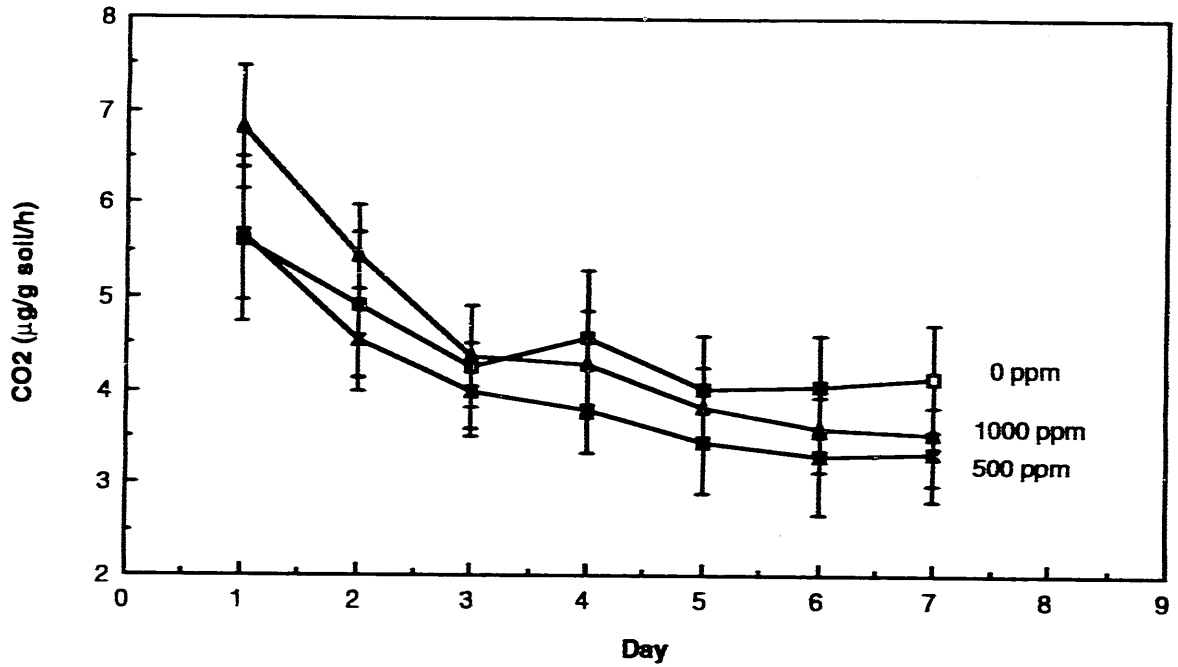

Figure IV - 23. Carbon dioxide efflux from *Pinus taeda* rhizosphere soil collected from the Miscellaneous Chemicals Basin and treated with 0, 500, and 1000 µg/g (ppm) tetrachloroethylene. Each datum point is the mean of three replicates. Error bars represent one standard deviation of the mean.

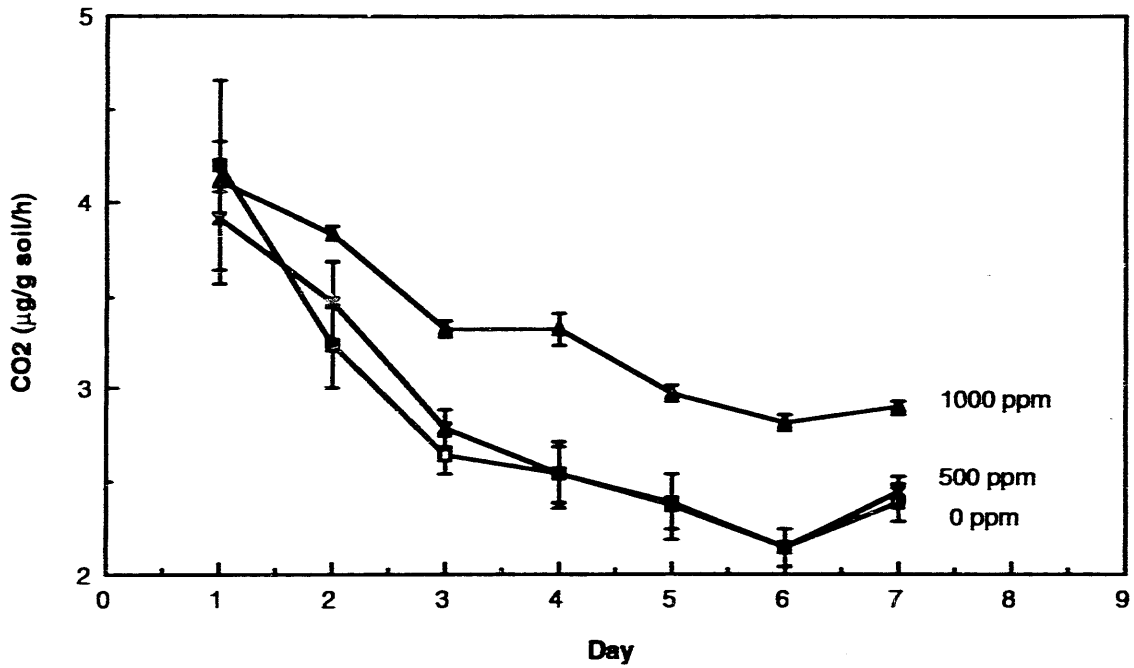

Figure IV - 24. Carbon dioxide efflux from *Solidago* sp. rhizosphere soil collected from the Miscellaneous Chemicals Basin and treated with 0, 500, and 1000 µg/g (ppm) tetrachloroethylene. Each datum point is the mean of three replicates. Error bars represent one standard deviation of the mean.

sp. (Figure IV - 24) rhizosphere soils, respectively. Although PCE concentrations of 500 and 1000 µg/g caused a decrease in CO_2 efflux from *P. notatum* (Figure IV - 22) and *P. taeda* (Figure IV - 23) rhizosphere soils, only the decrease observed in *P. notatum* rhizosphere soil was statistically significant ($p \leq 0.05$). The toxic effect of TCE and PCE to soil samples from the MCB were not similar as illustrated by results from *Solidago* sp. Although respiration in *Solidago* sp. rhizosphere was significantly depressed by TCE at 1000 µg/g, PCE at the same concentration had a stimulatory effect on CO_2 efflux. The fact that high concentrations of TCE are toxic to microorganisms in soil samples from nonvegetated areas and *Solidago* sp. and *Paspalum notatum* rhizosphere could provide an explanation for the decreased degradative ability of these soils should such an observation be made. In addition, the lack of a toxic effect (or stimulatory effect) from TCE and/or PCE exposure could be used to help interpret results on TCE degradation in the laboratory as well as describing field situations where toxicity may affect degradation rates. Results from these toxicity experiments with TCE and PCE will be discussed further following the presentation of results on microbial degradation of ^{14}C-TCE in soil plant systems.

D. Degradation of TCE in Soil Slurries

Analysis of the headspace above aqueous soil slurries spiked with TCE showed that the solvent was lost from the headspace of rhizosphere soils more quickly (Figures IV - 27 through IV - 30) than from nonvegetated soils (Figures IV - 25 and IV - 26) in all cases as evidenced by TCE concentrations on the last

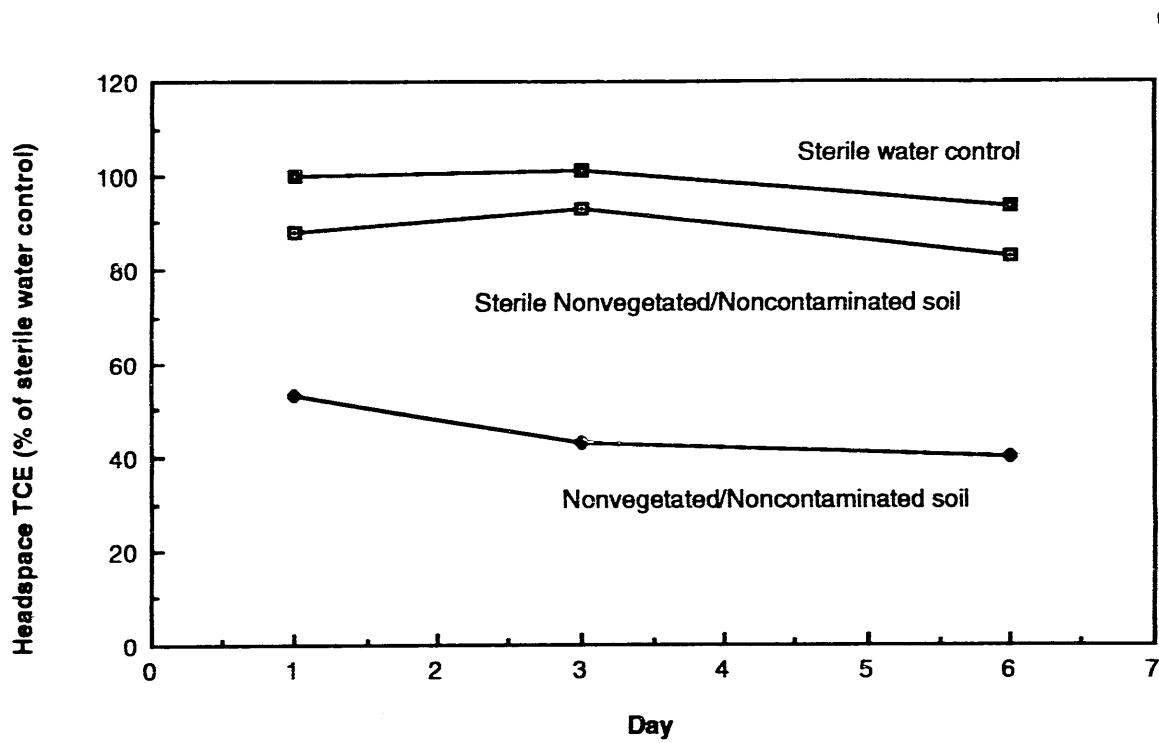

Figure IV - 25. Headspace concentrations of TCE in sterile, distilled water and soil slurries prepared from nonvegetated/noncontaminated soil. The coefficient of variation ([standard deviation x 100]/mean) for measurements of TCE in the headspace of the water controls was 7.0%.

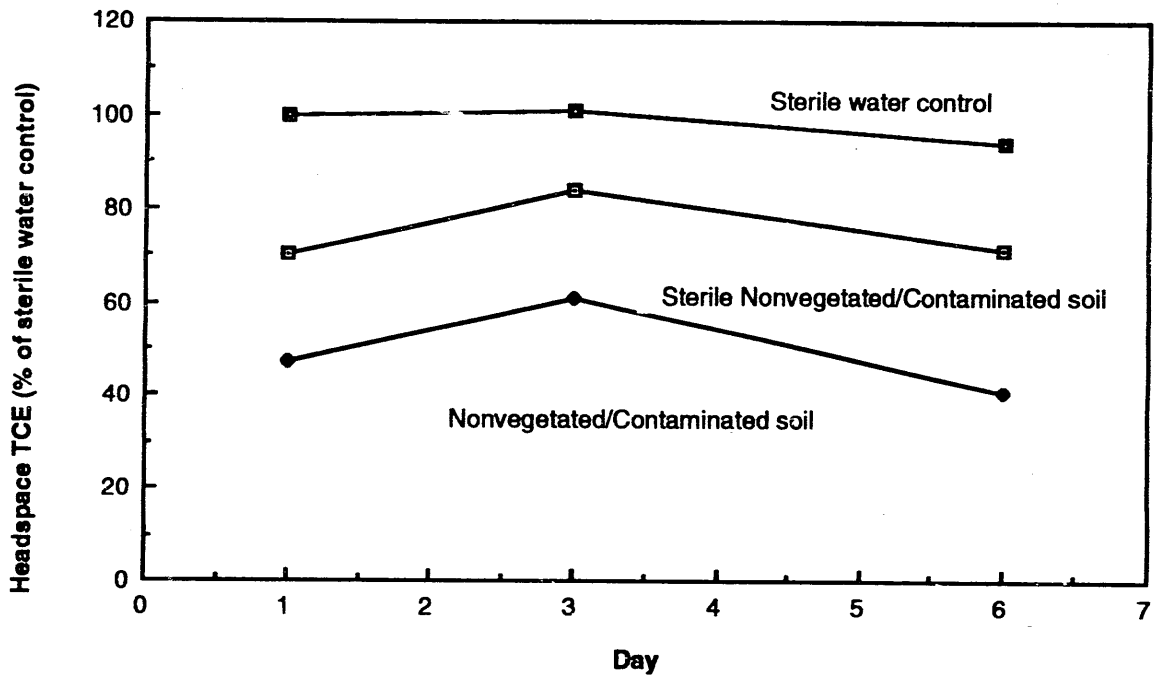

Figure IV - 26. Headspace concentrations of TCE in sterile, distilled water and soil slurries prepared from nonvegetated/contaminated soil. The coefficient of variation ([standard deviation x 100]/mean) for measurements of TCE in the headspace of the water controls was 7.0%.

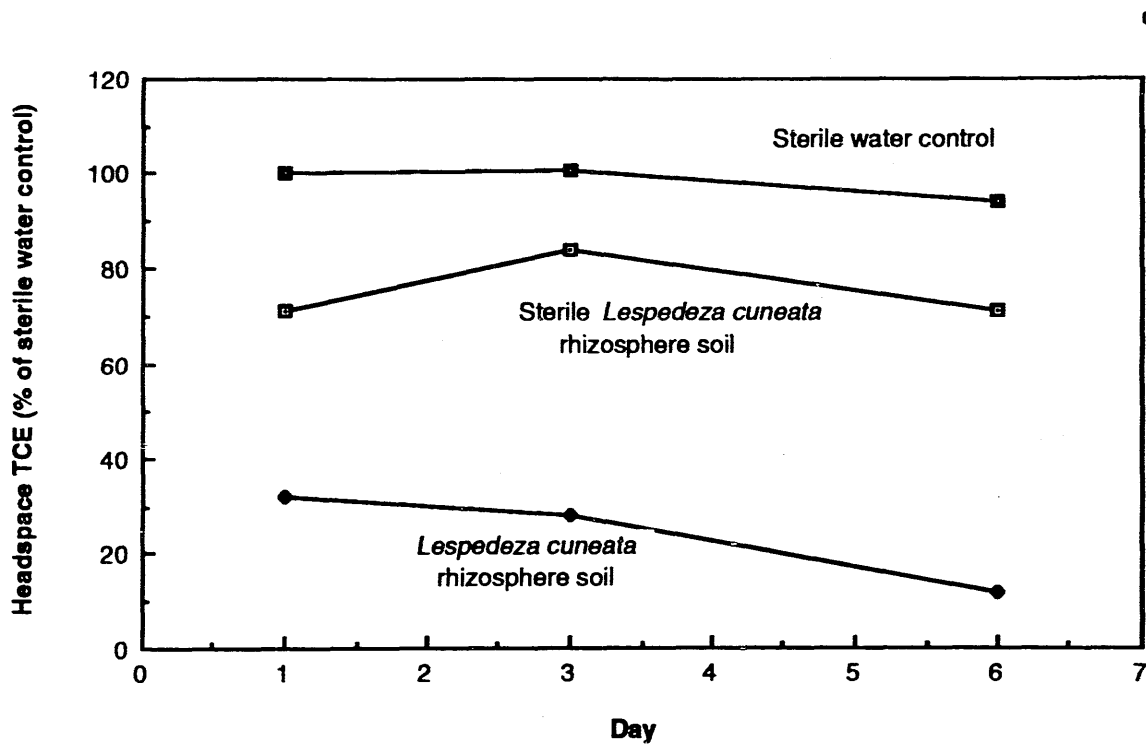

Figure IV - 27. Headspace concentrations of TCE in sterile, distilled water and soil slurries prepared from *Lespedeza cuneata* rhizosphere soil. The coefficient of variation ([standard deviation x 100]/mean) for measurements of TCE in the headspace of the water controls was 7.0%.

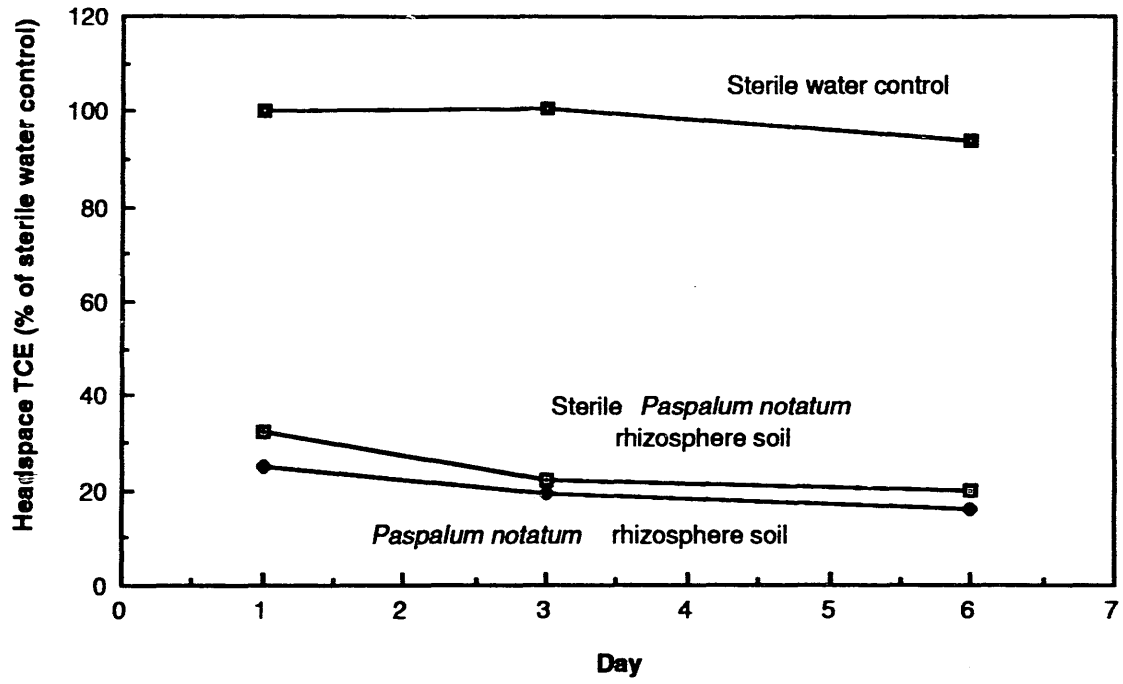

Figure IV - 28. Headspace concentrations of TCE in sterile, distilled water and soil slurries prepared from *Paspalum notatum* rhizosphere soil. The coefficient of variation ([standard deviation x 100]/mean) for measurements of TCE in the headspace of the water controls was 7.0%.

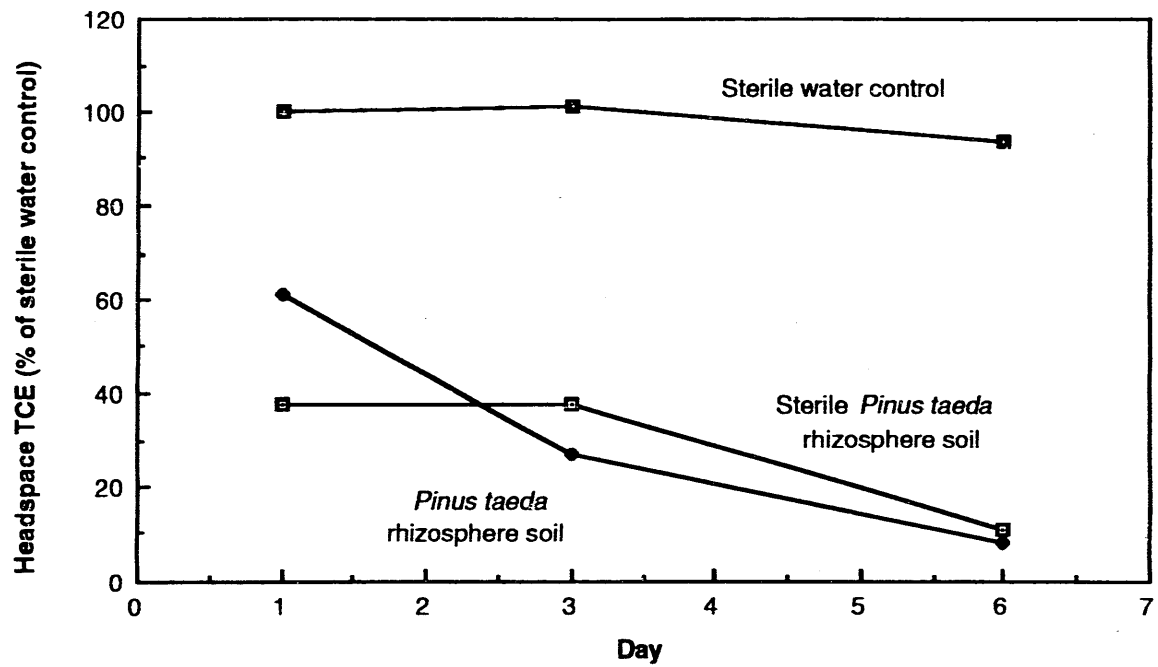

Figure IV - 29. Headspace concentrations of TCE in sterile, distilled water and soil slurries prepared from *Pinus taeda* rhizosphere soil. The coefficient of variation ([standard deviation x 100]/mean) for measurements of TCE in the headspace of the water controls was 7.0%.

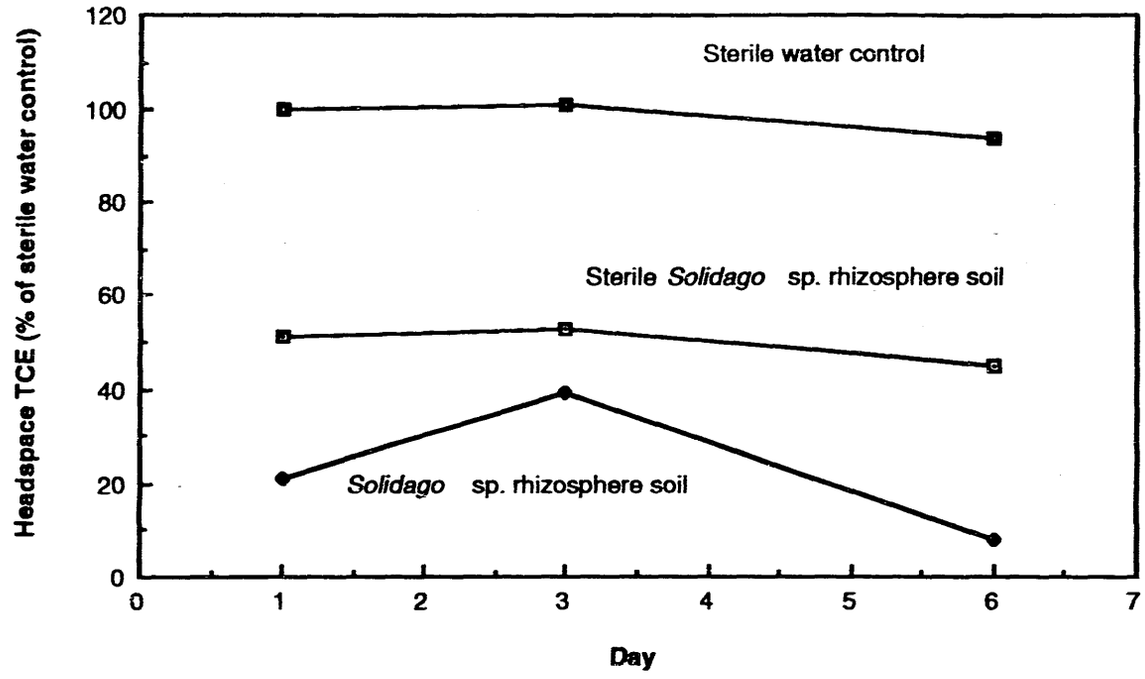

Figure IV - 30. Headspace concentrations of TCE in sterile, distilled water and soil slurries prepared from *Solidago* sp. rhizosphere soil. The coefficient of variation ([standard deviation x 100]/mean) for measurements of TCE in the headspace of the water controls was 7.0%.

sampling day. Throughout the 6-day sampling period, TCE concentrations were higher in the sterile water controls (no soil) than in sterile soil slurries for all soils indicating either nonbiological transformation of TCE or simply sorption of TCE to the soil. The fact that TCE concentrations decreased rapidly from day 0 to day 1 but changed only slightly from day 1 to day 6 in all sterile soil samples indicates that abiotic losses occurred rapidly in the first day of the experiment but contributed very little to losses thereafter. This observation is consistent with sorption as the predominant abiotic loss process.

Biological transformation of the TCE was most evident as an important loss process in *L. cuneata* (Figure IV - 27) and nonvegetated, noncontaminated soils (Figure IV - 25), because the TCE loss from the soil was greatest compared with the matched sterile control for each of these samples. On day 6, there was 55% less TCE in the headspace of the *L. cuneata* soil compared to its matched sterile control (Figure IV - 27), whereas, the nonvegetated, noncontaminated soil showed 40% less TCE in the headspace compared to its matched sterile control (Figure IV - 25) on day 6. These differences can be attributed directly to biological transformation of TCE in each soil.

Differences in TCE concentrations were marked but less pronounced between sterile and nonsterile soil slurries of *Solidago* sp. (Figure IV - 30) and nonvegetated, contaminated soil (Figure IV - 26). Once again the faster disappearance of TCE from the nonautoclaved soils indicates that biological processes contribute to the disappearance of TCE. Rapid loss of TCE was also observed for *P. taeda* (Figure IV - 29) and *P. notatum* (Figure IV - 28); however, the contribution of microbial transformation could not be inferred because TCE

was also lost from the headspace of matched, sterile soils. Nonetheless, the rate of TCE disappearance from *P. taeda* and *P. notatum* exceeded that of nonvegetated soils (Figures IV - 25 and IV - 26). Thus, enhanced microbial degradation may still occur in these rhizosphere soils, but microbial degradation may not be distinquishable from other losses unless a higher TCE concentration is present.

E. Mineralization of ^{14}C-TCE in Soil Samples

The hypothesis that microbial degradation of TCE is faster in the rhizosphere than in nonvegetated soil was also supported by the comparison of ^{14}C-TCE mineralization in *L. cuneata* and non-vegetated, TCE-contaminated soils. Mineralization to $^{14}CO_2$ occurred in both soils; however, a significantly greater amount of $^{14}CO_2$ ($p \leq 0.05$) was produced in the rhizosphere soil from *L. cuneata* (mean $\pm$ one standard deviation = 3.1 $\pm$ 0.42 ng/50 g soil over 30 days) than in the nonvegetated, TCE-contaminated soil (1.3 $\pm$ 0.68 ng $^{14}CO_2$/50 g soil). Moreover, $^{14}CO_2$ efflux from the latter soil was not significantly greater than its matched, sterile control or than the sterile *L. cuneata* control. Although these results were encouraging for providing further evidence for the role of rhizosphere microorganisms in degrading TCE, experiments with other rhizosphere types were not attempted. Rather, experiments exploring ^{14}C-TCE mineralization in soil samples containing a living plant were conducted based on the hypothesis that the estimates of ^{14}C-TCE mineralization in rhizosphere soil samples were conservative due to the absence of a living plant.

F. Degradation of PCE in Soil Slurries

Initial results on microbial degradation of tetrachloroethylene (PCE) in rhizosphere and nonvegetated soils from the MCB were somewhat encouraging although equivocal (Table IV - 8). Reductive dechlorination of PCE to trichloroethylene (TCE) was observed in both nonvegetated and *Solidago* sp. rhizosphere soil as determined by gas chromatography after 2 days. However, more TCE was produced through reductive dechlorination in the rhizosphere soil. Control (sterile) samples for both the nonvegetated and *Solidago* sp. rhizosphere soil produced 0.0 and 0.1 mg of TCE, respectively. In addition, no TCE was produced in the sterile water controls. Somewhat troubling was the failure to detect PCE in all samples (sterile and nonsterile) on day 2. It was hypothesized that leaks in the sample vials, some type of reaction with the redox indicator (resazurin), or sorption may have lead to headspace concentrations of PCE below detection.

More rigorous experiments on PCE degradation failed to confirm the results of the initial tests (Table IV - 9). No TCE was detected in any of the samples tested over 18 days. Tetrachloroethylene concentrations remained constant in sterile water controls, nonvegetated soils (sterile and nonsterile) and *Solidago* sp. rhizosphere soil (sterile and nonsterile) throughout the 18-day experiment.

The PCE degradation experiments were conducted with nonvegetated soil and *Solidago* sp. rhizosphere soil simply because of their availability at the time. No additional experiments on microbial degradation of PCE in other rhizosphere soil samples from the MCB were attempted. Thus, it is possible that

Table IV - 8. Tetrachloroethylene (PCE) and trichloroethylene (TCE) concentrations in soil samples used in preliminary studies on reductive dechlorination of PCE in soils from the Miscellaneous Chemicals Basin at the Savannah River Site.

Sample type	Day 0		Day 2	
	PCE (mg/g)	TCE (mg/g)	PCE (mg/g)	TCE (mg/g)
Sterile water	0.27[A]	0.00[B]	ND	ND
Nonvegetated (sterile)	0.27[A]	0.00[B]	ND	ND
Nonvegetated	0.27[A]	0.00[B]	ND	0.07[C]
Solidago sp. (sterile)	0.27[A]	0.00[B]	ND	0.03[C]
Solidago sp.	0.27[A]	0.00[B]	ND	0.27[C]

[A]Amount of PCE initially added.
[B]Concentration of TCE at time 0.
[C]TCE concentration calculated from calibration curve.
ND--not detected

Table IV - 9. Tetrachloroethylene (PCE) and trichloroethylene (TCE) concentrations in soil samples used in studies on reductive dechlorination of PCE in soils from the Miscellaneous Chemicals Basin at the Savannah River Site.

Sample type	Day 3		Day 6		Day 11		Day 18	
	PCE (mg/g)	TCE (mg/g)	PCE (mg/g)	TCE (mg/g)	PCE (mg/g)	TCE (mg/g)	PCE (mg/g)	TCE (mg/g)
Sterile water	0.60 ± 0.10	ND	–	–	0.57 ± 0.07	ND	–	–
Nonvegetated (sterile)	0.33 ± 0.03	ND	0.27 ± 0.10	ND	0.27 ± 0.03	ND	0.23 ± 0.03	ND
Nonvegetated	0.60 ± 0.03	ND	0.27 ± 0.03	ND	0.23 ± 0.07	ND	0.27 ± 0.00	ND
Solidago sp. (sterile)	0.13 ± 0.03	ND	0.13 ± 0.00	ND	0.13 ± 0.00	ND	0.13 ± 0.03	ND
Solidago sp.	0.17 ± 0.03	ND	0.17 ± 0.03	ND	0.17 ± 0.03	ND	0.13 ± 0.00	ND

ND--not detected

microbial degradation of PCE occurs in the untested soils. Certainly PCE degradation via reductive dechlorination is favorable in soil at the MCB because of the anaerobic conditions that exist as a result of the soils poor drainage. I addition, recent work by Kastner (1991) showed that reductive dechlorination of TCE and PCE occurred in anaerobic soils only after the soils had gone from aerobic to anaerobic conditions. Soils under strictly anaerobic conditions failed to show any dechlorination of TCE or PCE. This phenomenon (fluctuation between aerobic and anerobic conditions) certainly occurs at the MCB.

G. Fate of ^{14}C-TCE in Soil-Plant Systems

The first goal of the ^{14}C-TCE soil-plant experiments was to demonstrate the ability to achieve a good mass balance of ^{14}C in the experimental systems. Using the sampling, extraction, and plant analysis techniques described in the Materials and Methods section, consistent recovery of >70% (range 40% - 96%) of the total ^{14}C initially added to the samples was achieved. Analysis of the Carbosorb traps from the whole plant experiments showed that $^{14}CO_2$ production in the vegetated soils was elevated compared with either the nonvegetated soil or the sterile control soil (Figures IV - 31 through IV - 36). In the experiments with soils containing *Lespedeza cuneata* (Figures IV - 31 and IV - 32) and *P. taeda* (Figures IV - 33 and IV - 34), $^{14}CO_2$ production at the conclusion of the experiment was significantly greater ($p \leq 0.05$) than $^{14}CO_2$ production in nonvegetated and sterile (autoclaved) control soils. In experiments with soils containing *Solidago* sp. (Figures IV - 35 and IV - 36),

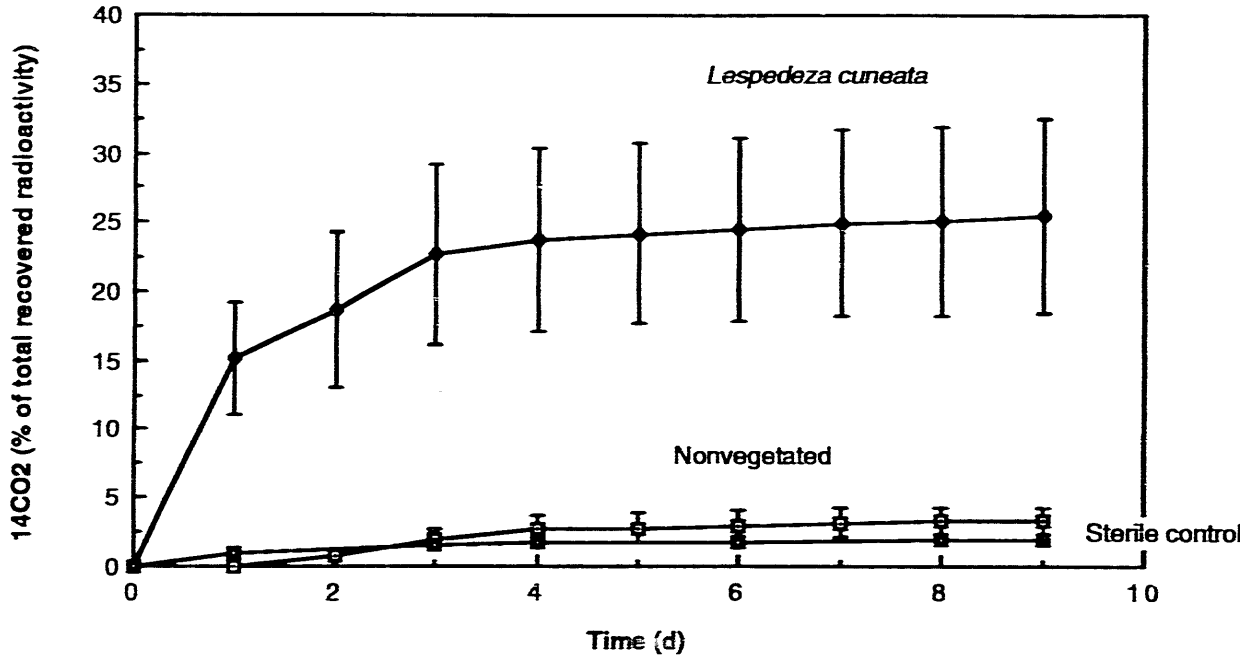

Figure IV - 31. Mineralization of ^{14}C-TCE in sterile control soil, nonvegetated soil, and soil containing *Lespedeza cuneata*. Data points for sterile control samples are the mean of two replicates whereas the nonvegetated and vegetated samples are the mean of three replicates. Error bars indicate one standard deviation of the mean.

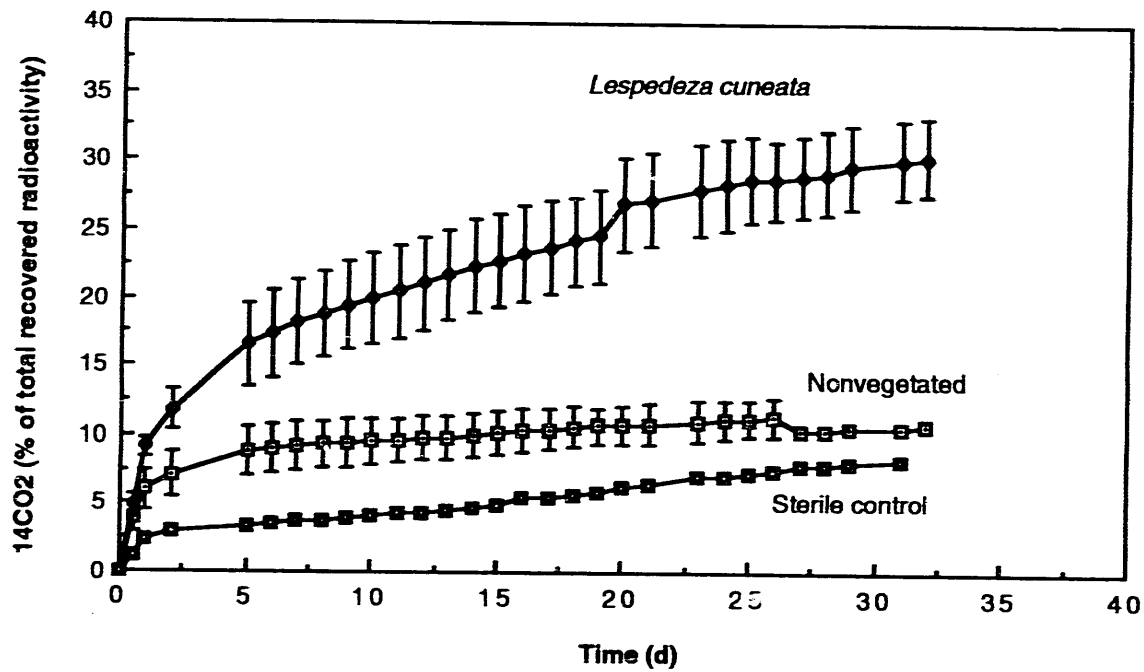

Figure IV - 32. Mineralization of ^{14}C-TCE in sterile control soil, nonvegetated soil, and soil containing *Lespedeza cuneata*. Data points for sterile control samples are the mean of two replicates whereas the nonvegetated and vegetated samples are the mean of three replicates. Error bars indicate one standard deviation of the mean.

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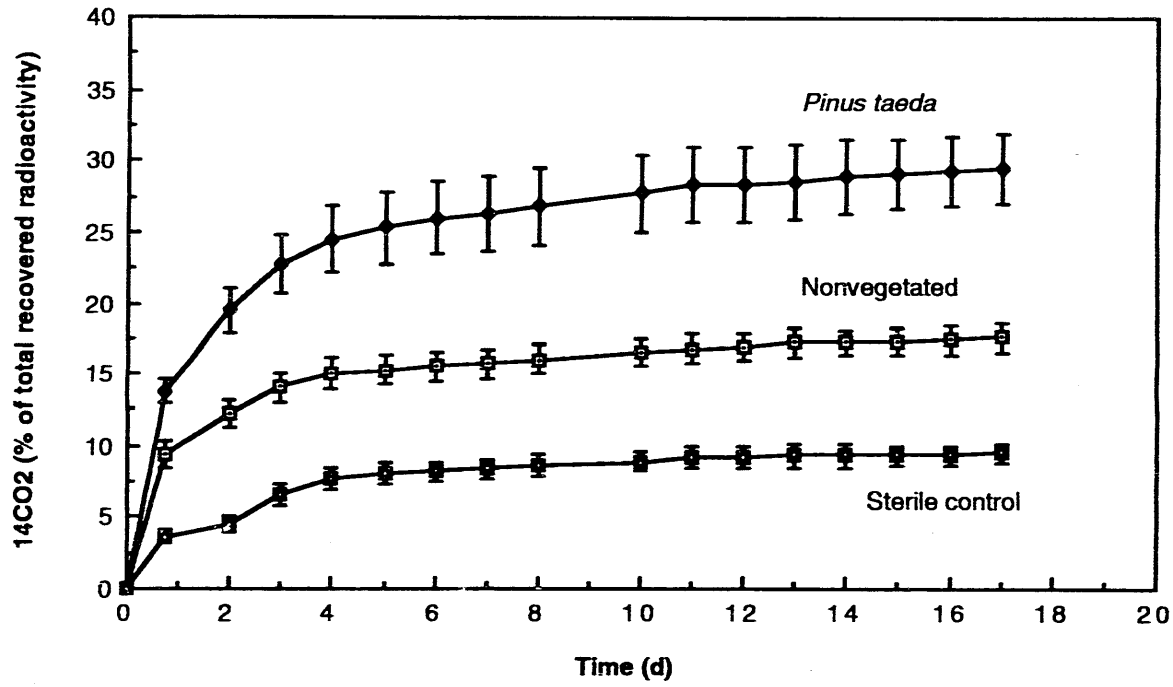

Figure IV - 33. Mineralization of ^{14}C-TCE in sterile control soil, nonvegetated soil, and soil containing *Pinus taeda*. Each datum point is the mean of three replicates. Error bars indicate one standard deviation of the mean.

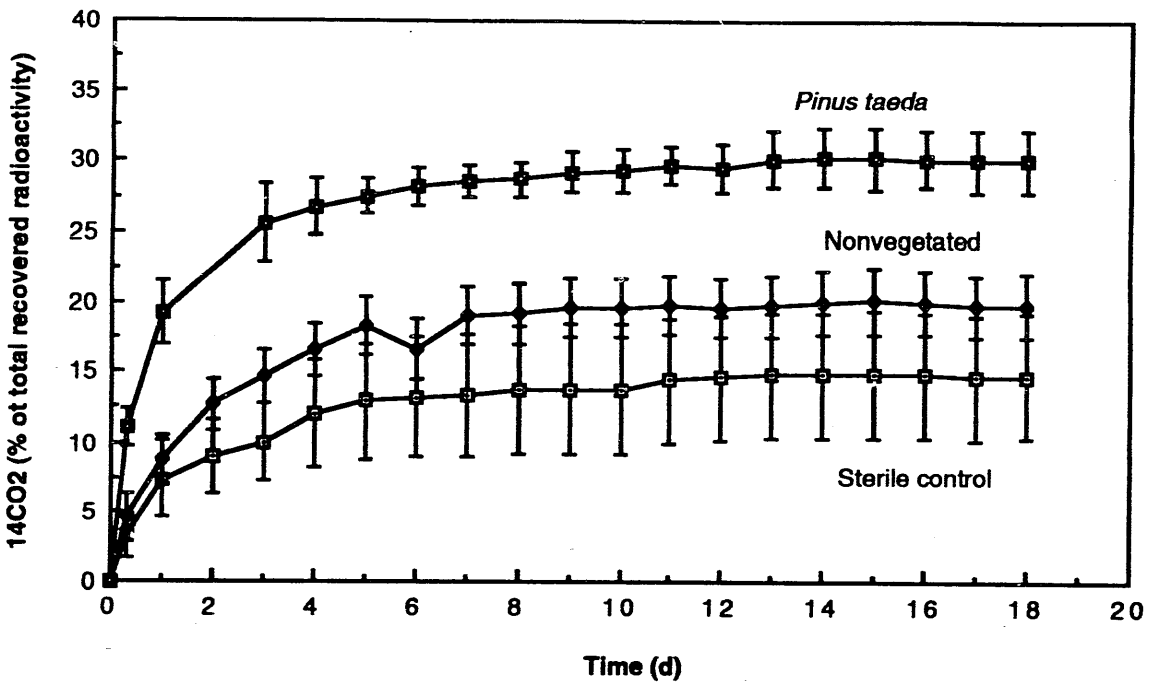

Figure IV - 34. Mineralization of ^{14}C-TCE in sterile control soil, nonvegetated soil, and soil containing *Pinus taeda*. Each datum point is the mean of three replicates. Error bars indicate one standard deviation of the mean.

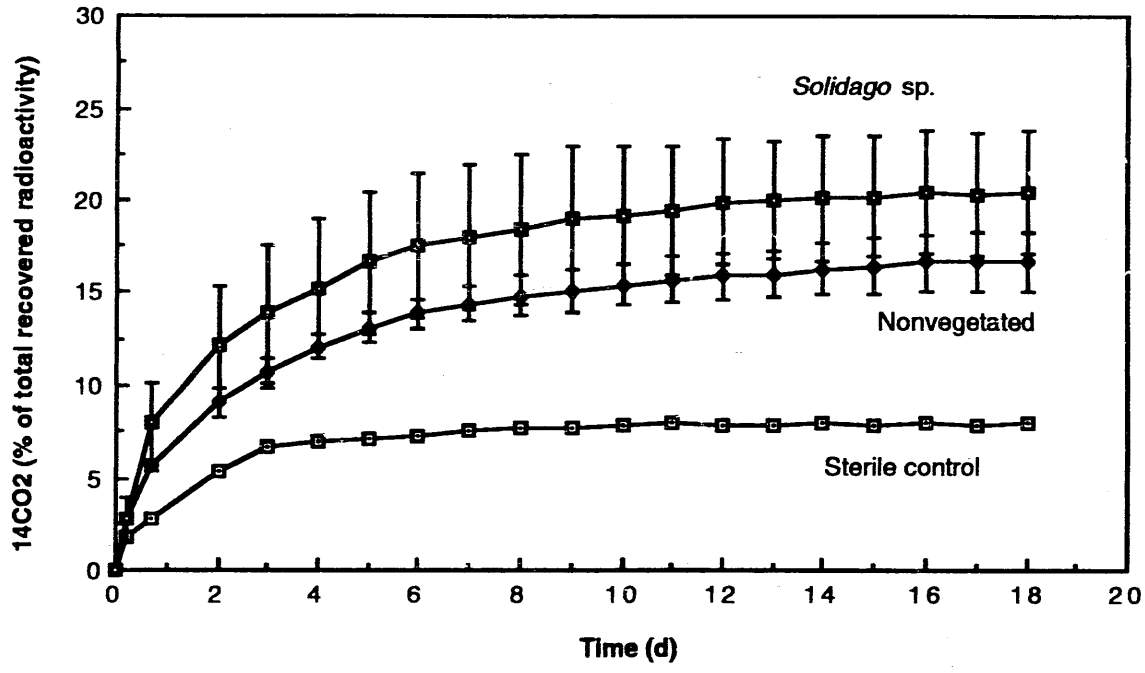

Figure IV - 35. Mineralization of ^{14}C-TCE in sterile control soil, nonvegetated soil, and soil containing *Solidago* sp. Data points for sterile control samples are the mean of two replicates whereas the nonvegetated and vegetated samples are the mean of three replicates. Error bars indicate one standard deviation of the mean.

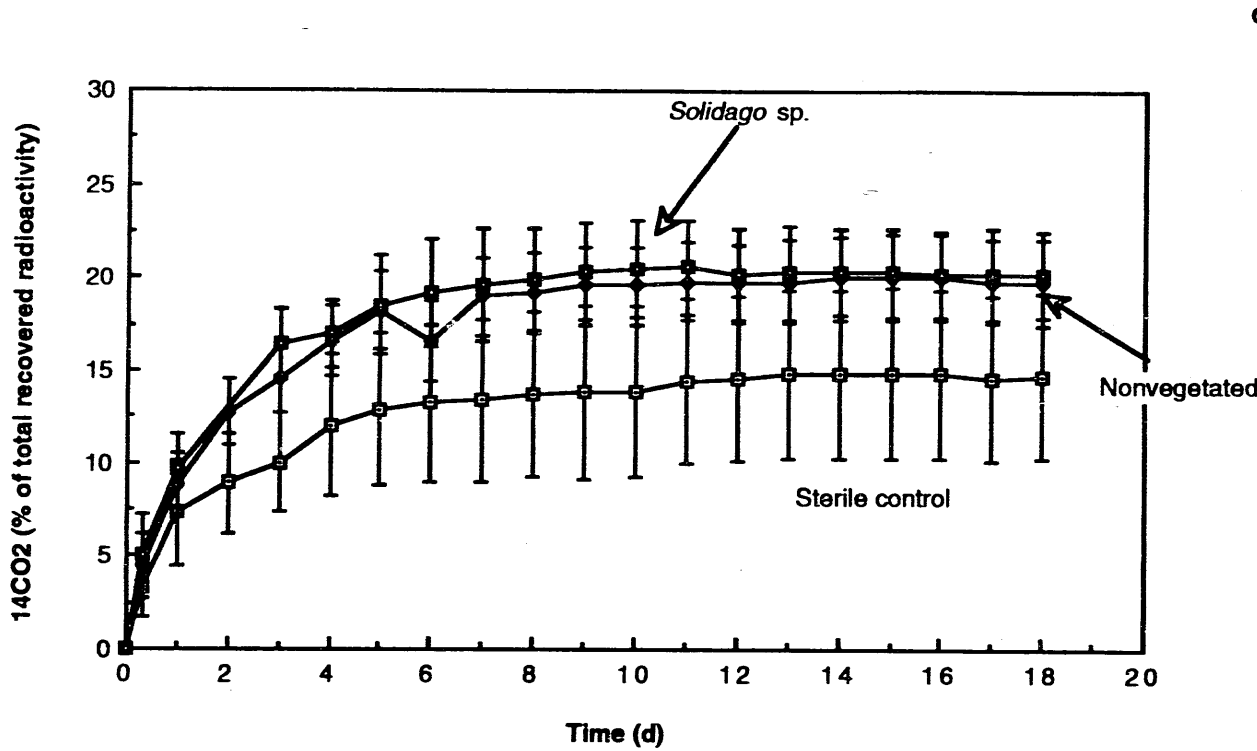

Figure IV - 36. Mineralization of ^{14}C-TCE in sterile control soil, nonvegetated soil, and soil containing *Solidago* sp. Each datum point is the mean of three replicates. Error bars indicate one standard deviation of the mean.

$^{14}CO_2$ production was elevated in the vegetated samples but not statistically different ($p \leq 0.05$) from $^{14}CO_2$ production in the nonvegetated soil. Most of the $^{14}CO_2$ produced from ^{14}C-TCE in the vegetated soils was evolved during the first three days of the experiment. This is in agreement with earlier observations on the initial rapid disappearance of TCE from the headspace above aqueous slurries of rhizosphere soil (Walton and Anderson, 1990). In addition, comparisons of the percentage of ^{14}C-TCE mineralized in the whole plant systems with previous data on ^{14}C-TCE mineralization in *L. cuneata* rhizosphere soil (Walton and Anderson, 1990), appeared to confirm the hypothesis that the mineralization rates based on soil slurries gave conservative estimates of mineralization rates that would occur in soil containing a live plant.

As described in the Materials and Methods section, slight modification of the experimental apparatus was necessary for the grass, *Paspalum notatum*, because of its different morphology, that is, the lack of a stem. Glass crystallization dishes were used to contain the plants. Although mineralization of ^{14}C-TCE was detected in soil containing *P. notatum* during two different experiments, $^{14}CO_2$ production in the vegetated dishes was slightly less than $^{14}CO_2$ production in an identical dish containing soil but no vegetation (Figure IV - 37). However, these results should be taken with caution due to the low mass balance of radioactivity and the difficulty of measuring $^{14}CO_2$ production in these "open" systems.

Mineralization experiments were also conducted with soybean, *Glycine max*. These tests were done to obtain data on the fate of ^{14}C-TCE in the

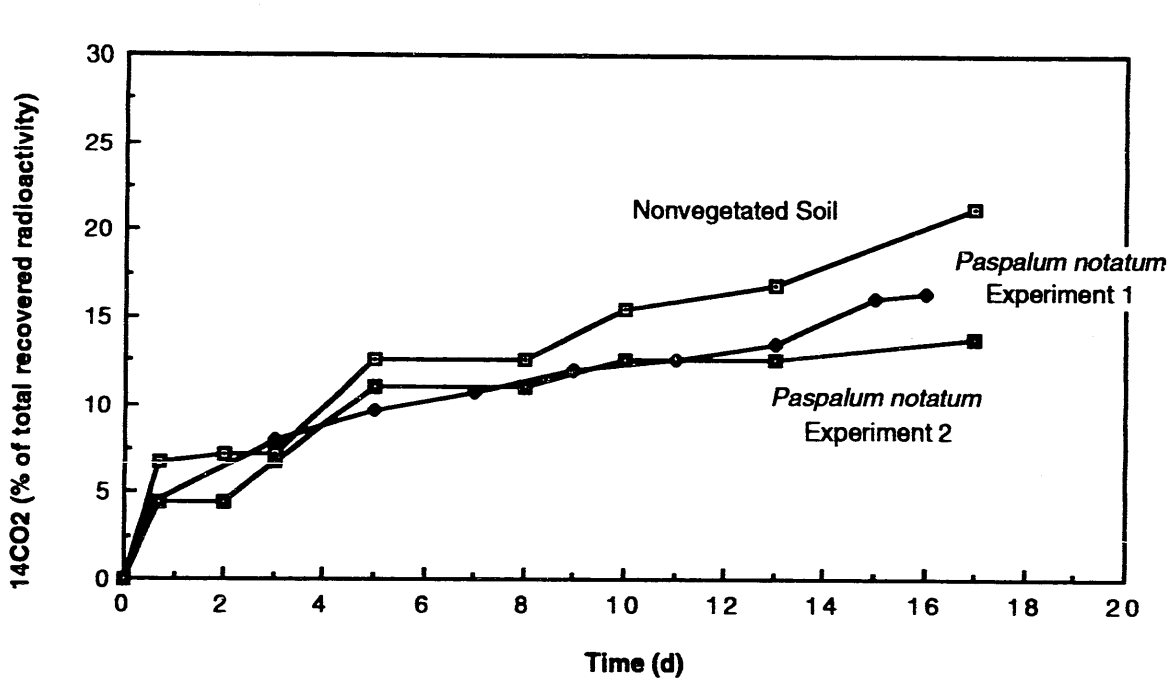

Figure IV - 37. Mineralization of ^{14}C-TCE in nonvegetated soil and soil containing *Paspalum notatum* from two different experiments.

rhizosphere of a common experimental plant not indigenous to the MCB and also determine whether previous exposure of the plant to TCE was necessary for enhanced microbial degradation of ^{14}C-TCE in the rhizosphere. Results of these tests were somewhat inconclusive. Although elevated levels of $^{14}CO_2$ were detected from flasks containing soybean plants compared with nonvegetated flasks and sterile control flasks (Figures IV - 38 and IV - 39), the $^{14}CO_2$ levels were significantly greater ($p \leq 0.05$) in only the second experiment (Figure IV - 39). Although environmental conditions monitored were identical during both experiments, the soybean plants in the second experiment grew much better, as indicated by water use, visual estimates of foliage, and weight of the plant tissue at the conclusion of the experiment. Because one of the effects of increased growth can be increased root exudation, these results imply a possible role of root exudation in TCE degradation in the rhizosphere. In addition, if the results of the second soybean experiment are correct, it appears that previous exposure of TCE to the plant may not be necessary for enhanced degradation of TCE to occur in the rhizosphere.

The initial rapid $^{14}CO_2$ production in soils containing vegetation as observed in previous tests, was further confirmed by respiking sterile control, nonvegetated, and vegetated (*Pinus taeda*) flasks with ^{14}C-TCE after $^{14}CO_2$ production had leveled off. Once again, a burst of $^{14}CO_2$ production was observed, but only in the vegetated sample (Figure IV - 40). These results are consistent with the initial rapid mineralization of 14C-TCE observed previously. In addition, the results also provide evidence for the importance of bioavailability of the TCE, as would be the case in these respiked samples.

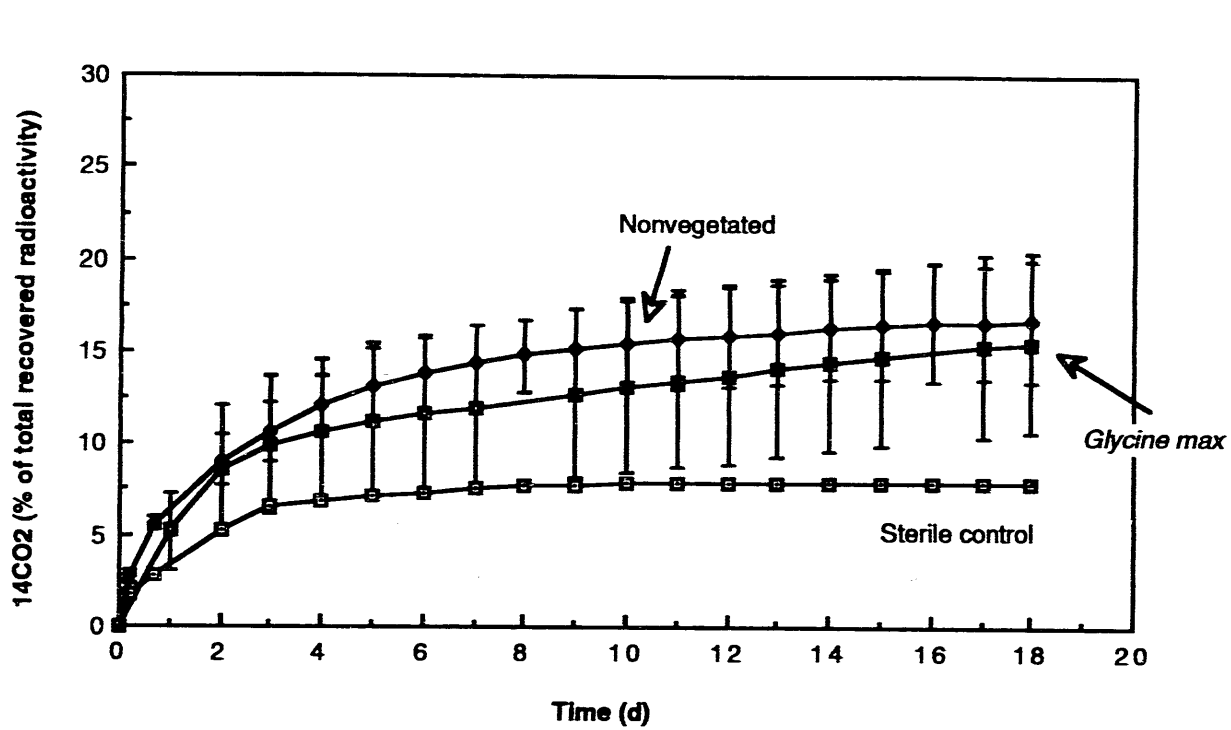

Figure IV - 38. Mineralization of ^{14}C-TCE in sterile control soil, nonvegetated soil, and soil containing *Glycine max*. Data points for sterile control samples are the mean of two replicates whereas the nonvegetated and vegetated samples are the mean of three replicates. Error bars indicate one standard deviation of the mean.

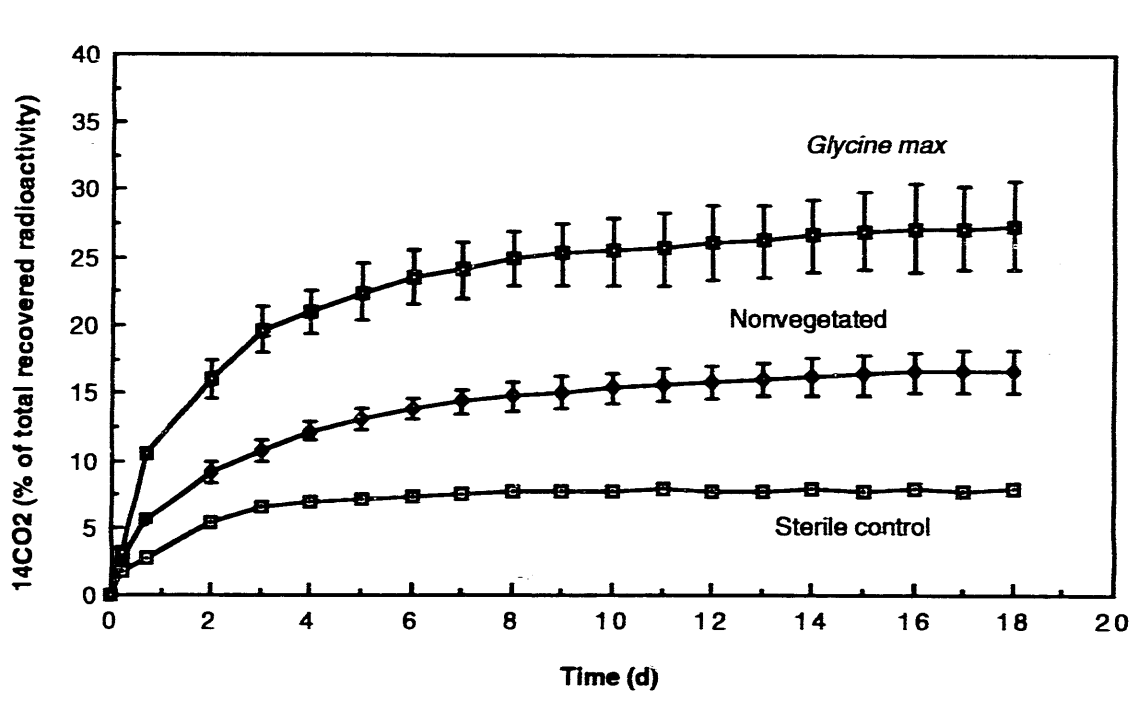

Figure IV - 39. Mineralization of ^{14}C-TCE in sterile control soil, nonvegetated soil, and soil containing *Glycine max*. Data points for sterile control samples are the mean of two replicates whereas the nonvegetated and vegetated samples are the mean of three replicates. Error bars indicate one standard deviation of the mean.

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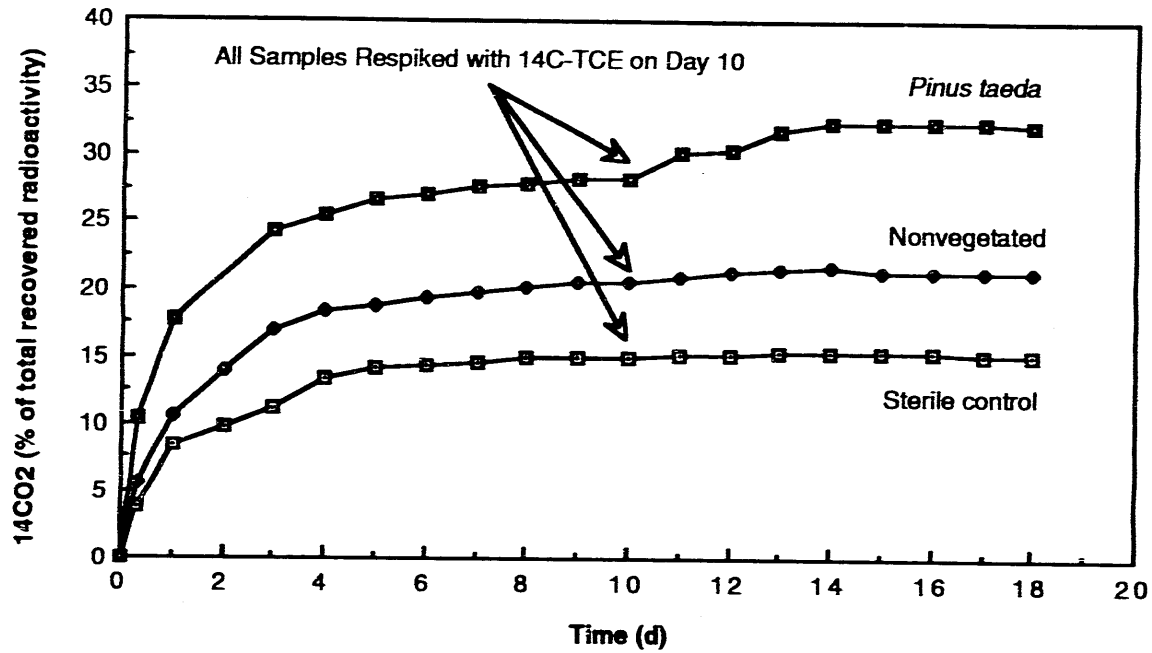

Figure IV - 40. Mineralization of ^{14}C-TCE in sterile control soil, nonvegetated soil, and soil containing *Pinus taeda*. All samples were respiked with ^{14}C-TCE on day 10.

To further explore whether the enhanced degradation of ^{14}C-TCE observed in vegetated samples was catalyzed by microorganisms or by the plant itself, fate experiments were conducted under sterile conditions. The fate of ^{14}C-TCE in flasks containing sterile soil and flasks containing sterile soil and loblolly pine seedlings treated with NaOCl to reduce bacterial activity on the plant roots (Norby et al., 1987) was monitored. In two separate experiments (Figures IV - 41 and IV - 42), $^{14}CO_2$ production in both sample types was not significantly different ($p \leq 0.05$). These results provide strong evidence for the role of soil microorganisms in the rhizosphere in microbial degradation of ^{14}C-TCE.

H. Influence of Root Exudation on ^{14}C-TCE Mineralization

In addition to monitoring microbial degradation of ^{14}C-TCE in the rhizosphere, preliminary studies were also conducted on the influence of root exudation on microbial degradation of TCE. Specifically, root exudation was reduced (by reducing light intensity) to determine the effect on mineralization of ^{14}C-TCE. Experiments were conducted with soils containing *Glycine max*, *Pinus taeda*, and *Lespedeza cuneata*. Although mineralization of ^{14}C-TCE in soils containing *G. max* grown under the two different lighting conditions was significantly greater ($p \leq 0.05$) than mineralization in the nonvegetated and sterile control soils, the two light intensities had no significant effect on mineralization in the vegetated samples (Figure IV - 43). Similar results were obtained in soils containing *P. taeda* (Figure IV - 44) and *L. cuneata* (Figure IV - 45). Although plants grown under reduced light showed elevated levels of

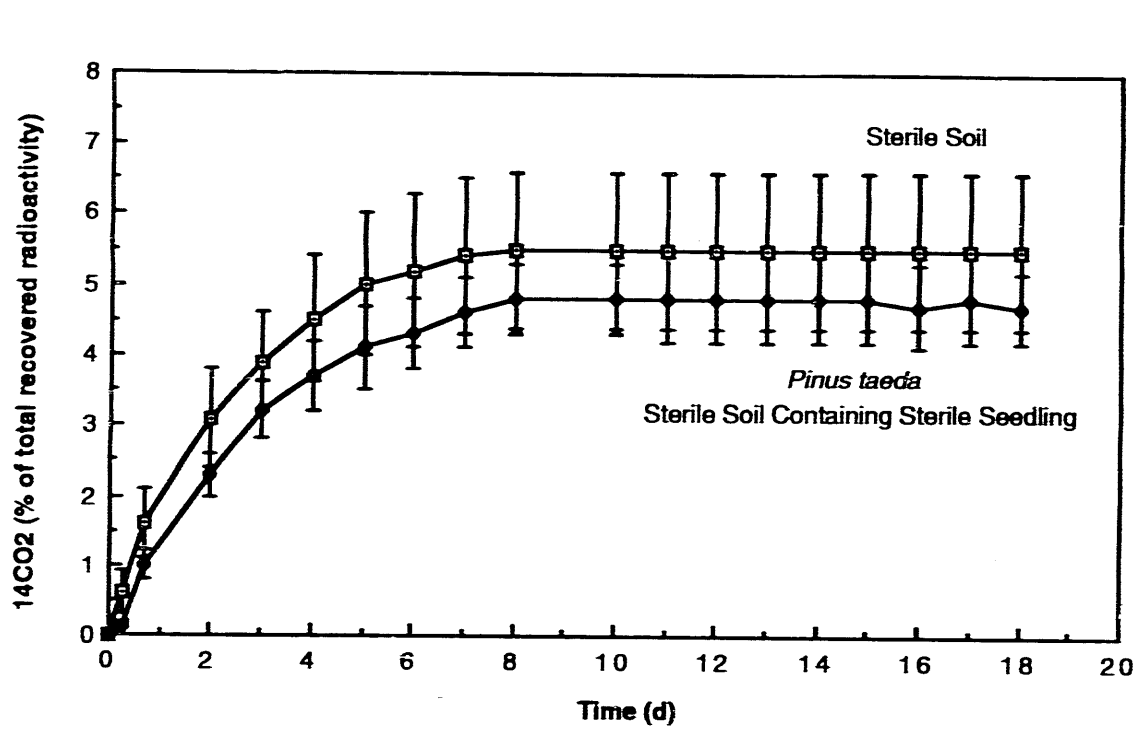

Figure IV - 41. Mineralization of ^{14}C-TCE in sterile control soil and sterile soil containing a *Pinus taeda* seedling treated with NaOCl. Each datum point is the mean of three replicates. Error bars indicate one standard deviation of the mean.

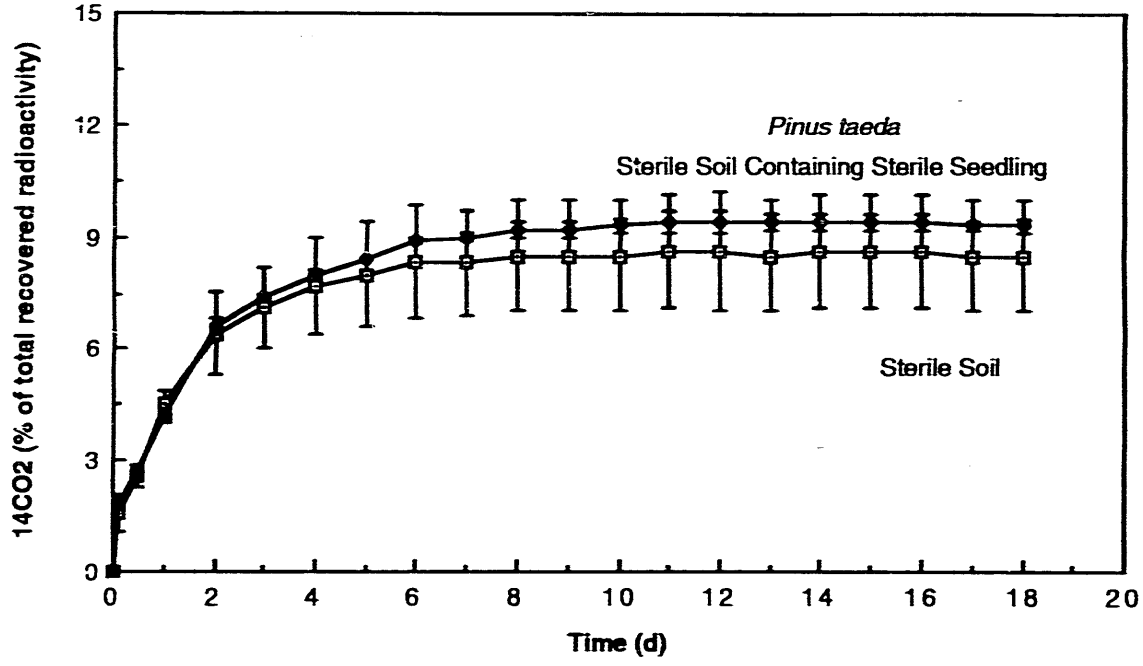

Figure IV - 42. Mineralization of ^{14}C-TCE in sterile control soil and sterile soil containing a *Pinus taeda* seedling treated with NaOCl. Each datum point is the mean of three replicates. Error bars indicate one standard deviation of the mean.

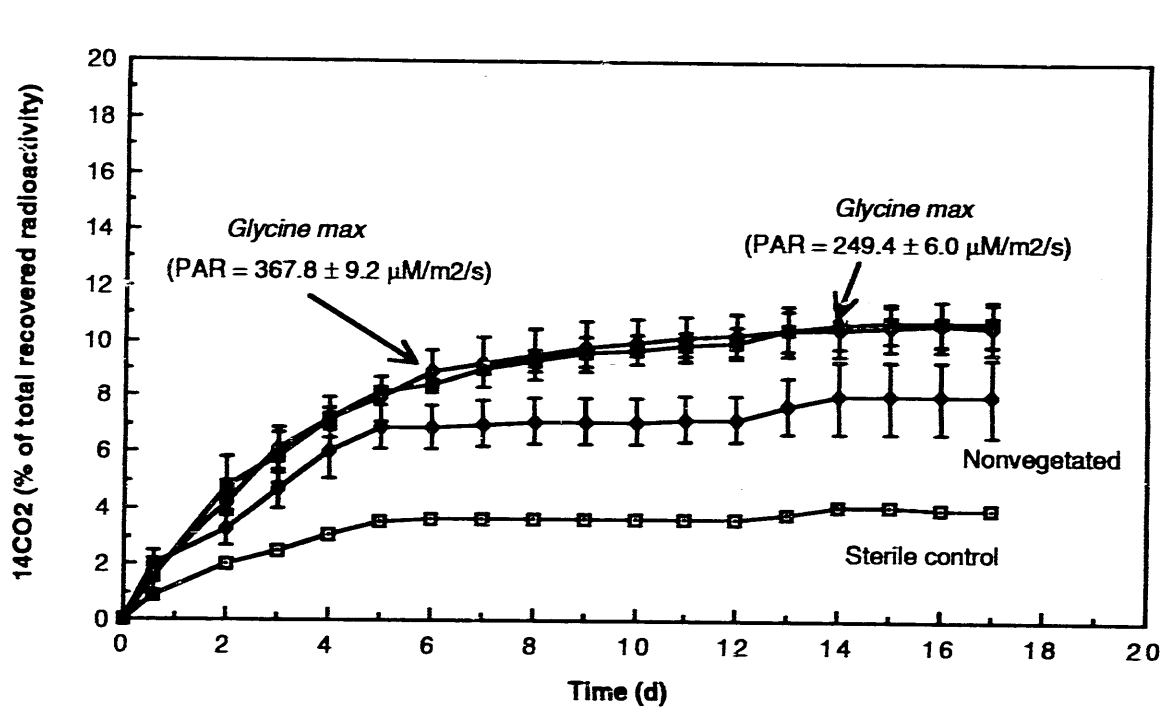

Figure IV - 43. Mineralization of ^{14}C-TCE in sterile control soil, nonvegetated soil, and soil containing *Glycine max* grown under two different lighting conditions. Data points for sterile control samples are the mean of two replicates whereas the nonvegetated and vegetated samples are the mean of three replicates. Error bars indicate one standard deviation of the mean.

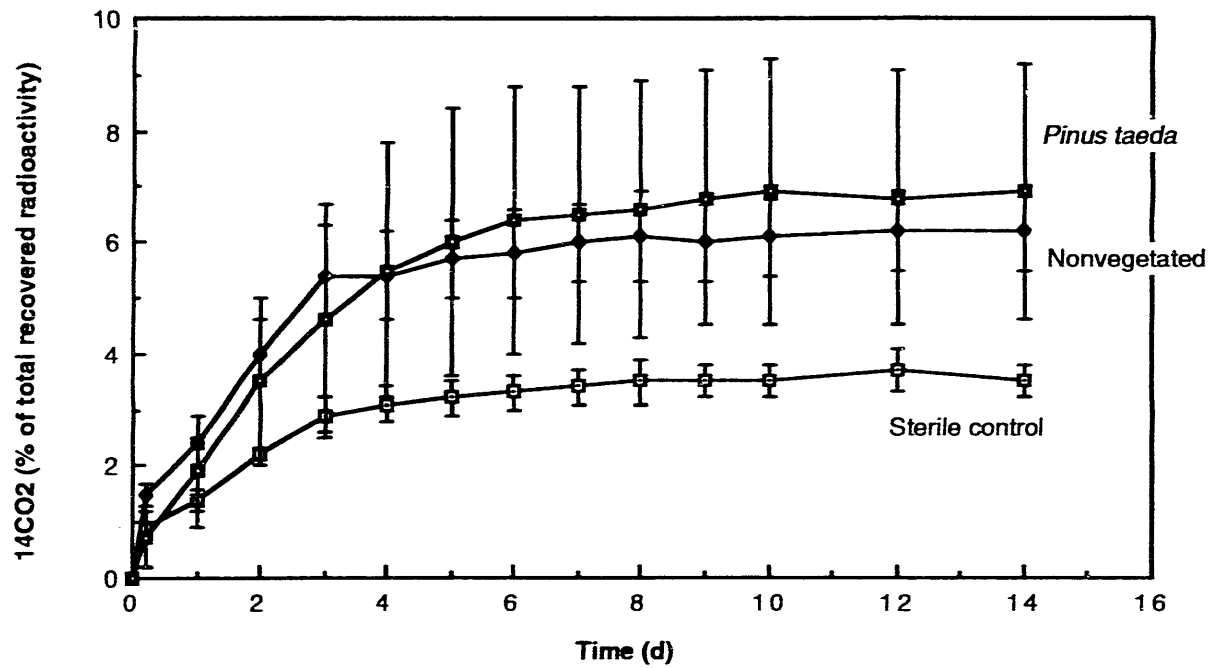

Figure IV - 44. Mineralization of ^{14}C-TCE in sterile control soil, nonvegetated soil, and soil containing *Pinus taeda* grown under reduced light conditions. Each datum point is the mean of three replicates. Error bars indicate one standard deviation of the mean.

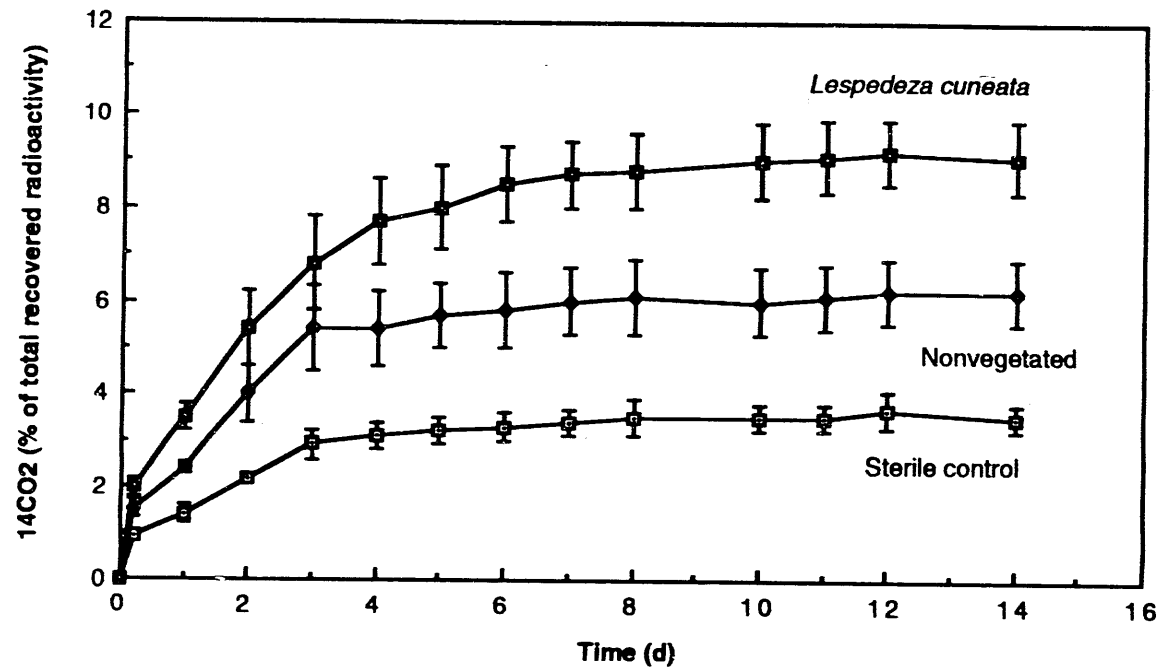

Figure IV - 45. Mineralization of 14C-TCE in sterile control soil, nonvegetated soil, and soil containing *Lespedeza cuneata* grown under reduced light conditions. Each datum point is the mean of three replicates. Errors bars indicate one standard deviation of the mean.

$^{14}CO_2$ production (significantly elevated in the case of *L. cuneata*), the percentage of ^{14}C-TCE mineralized was greater than 15% less than in previous tests with *P. taeda* and *L. cuneata*. Although preliminary, the results indicate that root exudation influences microbial degradation of ^{14}C-TCE in the root zone. The implications of such an influence could be important for managing sites where vegetation is being used in bioremediation.

I. Root Uptake of ^{14}C-TCE-Derived Compounds

The experimental apparatus used isolated the top portion of the plants from the bottom portion, thus enabling the determination of the route of entry of any radioactivity contained in the plant tissues. Root uptake of ^{14}C and translocation in plant tissues ranged from 1% to 22% for the total percentage contained in leaves (or needles), stems, and roots (Table IV - 10). It appeared that there were differences within a species from separate experiments, but when the uptake data was normalized for water use those differences disappeared, providing strong evidence for the direct relationship between water use and uptake. In only one case (soybean) did normalization for water use fail to explain the differences within a species (Table IV - 11). Consistently greater amounts of radioactivity were contained in the root tissue compared with the leaves (or needles) and stems. The last column of Table IV - 11 (charcoal) represents the percentage of recovered radioactivity in the exit air from the chamber. In cases where elevated water use within the same plant species from different experiments was observed, increased carbon 14 being released through evapotranspiration and captured in the charcoal trap was concomitantly

Table IV - 10. Total root uptake of ^{14}C-trichloroethylene-derived radioactivity in leaves (or needles), stems, and roots of plants used in studies on microbial degradation of ^{14}C-TCE in the rhizosphere.

Plant	Time[A]	^{14}C[B]
L. cuneata	9	2.5 ± 2.1
L. cuneata	32	1.3 ± 1.3
P. taeda	17	14.4 ± 4.0
P. taeda	18	4.2 ± 1.6
G. max	18	2.2 ± 1.1
G. max	18	21.2 ± 11.1
G. max	17	13.7 ± 3.8
P. notatum	16	5.8 ± 3.4
P. notatum	16	6.6 ± 1.6
P. notatum	17	1.4 ± 0.7
Solidago sp.	18	14.8 ± 5.7
Solidago sp.	18	8.3 ± 3.6

[A]Length of experiment in days
[B]Radioactivity (% ot total recovered radioactivity ± one standard deviation) contained in leaves, stem^, and roots

Table IV - 11. Distribution of ^{14}C-trichloroethylene-derived radioactivity in leaves (or needles), stems, and roots of plants used in studies on microbial degradation of TCE in the rhizosphere.

Plant	Time[B]	H_2O[C]	Total ^{14}C[A]			
			Leaf	Stem	Root	Charcoal[D]
L. cuneata	9	5.0	0.6 ± 0.7	1.1 ± 0.9	0.8 ± 0.5	0.0
L. cuneata	32	5.0	0.2 ± 0.1	0.2 ± 0.1	0.9 ± 1.1	0.2
P. taeda[E]	17	8.5	4.5 ± 0.4	1.3 ± 0.6	8.8 ± 3.0	10.1
P. taeda[E]	18	2.0	0.5 ± 0.3	0.4 ± 0.1	3.3 ± 1.2	0.2
G. max	18	3.0	0.3 ± 0.01	0.9 ± 1.0	1.0 ± 0.1	0.1
G. max[E]	18	9.0	3.2 ± 0.5	4.0 ± 0.6	14.0 ± 10.0	9.8
G. max[E,F]	17	5.4	5.6 ± 0.7	2.4 ± 1.2	5.7 ± 1.9	9.3
P. notatum	16	6.8	2.7 ± 1.4	---	3.1 ± 2.0	---
P. notatum	16	6.8	2.9 ± 0.9	---	3.7 ± 0.7	---
P. notatum	17	8.0	0.5 ± 0.2	---	0.9 ± 0.5	---
Solidago sp.[E]	18	7.0	4.8 ± 1.8	2.7 ± 0.4	7.3 ± 3.5	4.3

Table IV - 11. cont'd.

Plant	Time[B]	H_2O[C]	Total ^{14}C[A] Leaf	Stem	Root	Charcoal[D]
Solidago sp.	18	7.0	1.4 ± 1.1	3.3 ± 1.6	3.6 ± 0.9	2.0

[A]Expressed as a percent of the total recovered ^{14}C ± one standard deviation
[B]Length of experiment in days
[C]Water (in mL/plant/day)
[D]Carbon 14 in the air leaving the exposure chamber per plant
[E]Significantly greater ($p \leq 0.05$) total uptake than *L. cuneata*
[F]Significantly greater ($p \leq 0.05$) total uptake within species after normalization for water use

observed. The amount of ^{14}C in the plant tissues also appears to be dependent on plant species as illustrated by the significant differences in total uptake ($p \leq 0.05$) in *P. taeda*, *G. max*, and *Solidago* sp. compared with *L. cuneata* and *P. notatum*. No attempt was made to qualitatively identify the form of the ^{14}C in the plant tissues.

Although this system for monitoring uptake is more complex than most techniques employing plants or isolated roots in sterile nutrient solutions, simple relationships describing chemical uptake into plants can be developed from the soil-plant system. However, if environmental conditions are changed, the relationships became more complex as can be seen in Table IV - 12 illustrating uptake data for plants grown under reduced light and in sterile soil. One of the effects of reduced light is decreased growth, which could have a negative effect on TCE uptake. However for *L. cuneata* and *P. taeda*, reduction of the light caused an increase in uptake of ^{14}C derived from ^{14}C-TCE compared to identical plants grown under normal light. A possible explanation for this is that another effect of reduced light is decreased root exudation which could translate to decreased microbial activity and an increase in the amount of ^{14}C available for uptake due to the absence of microorganisms competing (through biodegradation) for the TCE. This explanation appears to be further illustrated by the increased uptake of ^{14}C in loblolly pine seedlings under sterile conditions.

Table IV - 12. Comparison of root uptake of ^{14}C-trichloroethylene-derived radioactivity in leaves (or needles), stems, and roots of plants grown under variable light and soil conditions.

Plant	Time[B]	H_2O[C]	Total ^{14}C[A] Leaf	Stem	Root	Charcoal[D]
L. cuneata[E] (reduced light)	14	0.8	3.2 ± 1.6	0.3 ± 0.2	8.7 ± 1.7	18.0
L. cuneata	32	5	0.2 ± 0.1	0.2 ± 0.1	0.9 ± 1.1	0.2
P. taeda[E] (sterile soil)	18	0.6	4.8 ± 1.2	3.9 ± 1.3	9.0 ± 0.1	1
P. taeda[E] (sterile soil)	18	0.9	2.0 ± 0.8	1.6 ± 0.1	3.6 ± 0.2	6.0
P. taeda[E] (reduced light)	14	1.0	1.1 ± 0.1	0.5 ± 0.1	13.9 ± 5.3	9.1
P. taeda	18	2.0	0.5 ± 0.3	0.4 ± 0.1	3.3 ± 1.2	0.2
G. max (reduced light)	17	4.5	2.6 ± 0.6	2.0 ± 0.7	4.2 ± 0.2	11.3
G. max	17	5.4	5.6 ± 0.7	2.4 ± 1.2	5.7 ± 1.9	9.3

[A]Expressed as a percent of the total recovered ^{14}C ± one standard deviation
[B]Length of experiment in days

Table IV - 12. cont'd.

[C]Water (in mL/plant/day)
[D]Carbon 14 in the air leaving the exposure chamber per plant
[E]Significantly greater ($p \leq 0.05$) total uptake than corresponding control after normalization for water use

J. Pesticide Root Zone Model (PRZM)

The Pesticide Root Zone Model (PRZM) was chosen for its ability to simulate the decay and transport of pesticides in and below the plant root zone. Because the model was developed for monitoring the fate of nonvolatile compounds such as pesticides, it lacks the ability to simulate volatilization, a very important component of TCE fate. Although this presented no problem for actually conducting the simulation, the modeling results should be viewed with this shortcoming in mind. The complex matrix of input data included properties of TCE (such as sorption coefficient and degradation half-life), hydrology parameters for Aiken, SC (such as daily temperature and evapotranspiration), soil properties for the MCB (such as organic carbon content and bulk density), and vegetation characteristics (such as root depth and aerial coverage) for all four plant species and nonvegetated conditions.

In all of the simulations for the four plant species and nonvegetated conditions, the percentage of TCE remaining at the conclusion of the simulation (109 days) was consistently less under vegetated conditions (Table IV - 13). In plots planted with *L. cuneata* and *P. taeda*, the decay (The "decay" compartment in PRZM includes microbial degradation and losses due to runoff and erosion) of TCE was simulated as >90%, uptake into both plants was <1%, and <0.1% of the TCE initially applied had migrated past the root zone (40 cm) after 109 days. For plots planted with *Solidago* sp. and *P. notatum*, the decay was >75%, while uptake was minimal but slightly larger (1.6% and 1.4% for *P. notatum* and *Solidago* sp., respectively). In plots left unvegetated, 72% of the TCE initially added had decayed after 109 days. Although the presence or absence of

Table IV - 13. Results of process control analyses after 109 days of simulation using the Pesticide Root Zone Model (PRZM) (Carsel et al., 1984) for TCE applied to soil at the Miscellaneous Chemicals Basin at the Savannah River Site under vegetated and nonvegetated conditions.

Soil condition	Decay[A] (% of total)	Uptake[B] (% of total)	Root Zone[C] (% of total)	Breakthrough[D]
Vegetated *Lespedeza cuneata*	94	0.7	0.04	yes
Vegetated *Pinus taeda*	92	0.9	0.06	yes
Vegetated *Paspalum notatum*	75	1.6	0.15	yes
Vegetated *Solidago* sp.	80	1.4	0.12	yes
Nonvegetated	72	--	0.31	yes

[A]Percentage of the total applied TCE described as "Decay" by PRZM after 109 days of simulation. The decay compartment in PRZM includes microbial degradation and losses due to runoff and erosion.

[B]Percentage of the total applied TCE described as "Root Uptake" by PRZM after 109 days of simulation. The root uptake compartment in PRZM is determined from evapotranspiration and a plant uptake efficiency factor (Briggs et al., 1982).

Table IV - 13. cont'd.

[C]Percentage of the total applied TCE described as moving out of the root zone by PRZM after 109 days of simulation. The root zone was set at 40 cm depth.

[D]Occurence of TCE breakthrough at 150 cm depth after 109 days of simulation using PRZM. Although TCE breakthrough did occur under all conditions tested (indicating vegetation had no effect on the speed with which the TCE front moved through the soil profile), the concentration of TCE breakthrough was at least an order of magnitude greater under nonvegetated conditions than under vegetated conditions.

vegetation appeared to have no influence on the speed in which the TCE front migrated through the simulated soil profile (150 cm), concentrations of TCE breakthrough were at least one order of magnitude greater under nonvegetated conditions.

CHAPTER V.

DISCUSSION

The indices of microbial biomass and activity measured in the present study all provide evidence that microbial activity is greater in rhizosphere soils than in nonvegetated soils at the MCB. Results from two different techniques for measuring microbial biomass indicate that there is a substantial increase in microbial numbers associated with the plant rhizosphere. Although biomass estimates were useful for describing the microbiological properties of the MCB soils, they were not good predictors of TCE degradation. Rhizosphere soils from P. notatum and Solidago sp. had comparatively high levels of microbial biomass, yet both rhizosphere types did not degrade TCE as well as other samples with less microbial biomass.

Measurements of ^{14}C-acetate incorporation rates, used as a measure of heterotrophic microbial activity, provide evidence for the increased microbial activity associated with the plant root zone. In addition, acetate incorporation also appeared to be a good predictor for the ability to degrade TCE in whole-plant experiments. Rhizosphere samples from *L. cuneata* and and *P. taeda* had relatively high rates of acetate incorportation and also had the highest TCE degradation rates, while samples of *P. notatum* rhizosphere and nonvegetated areas had lower acetate incorporation rates and lower TCE degradation rates.

In addition to providing biomass estimates, phospholipid fatty acid (PLFA) analysis also gave an indication of differences in the microbial community structure of nonvegetated and rhizosphere soil samples. Although taxonomic characterization of the microorganisms from the soil samples was not

undertaken, it is possible to compare qualitative differences between the groups of microorganisms present using PLFA analysis. Both gram positive and gram negative microorganisms were present in all samples tested. In addition, cluster analysis of PLFA profiles indicated a stong presence of actinomycetes in samples from *P. taeda* and *P. notatum* rhizospheres. Previous PLFA analysis of a TCE-degradading soil column enriched with natural gas (Wilson and Wilson, 1985) revealed the strong presence of type II methanotrophs as indicated by the fatty acid 18:1ω8c (Nichols et al., 1987). Because methanotrophs are likely to be found in zones that fluctuate between aerobic and anerobic conditions, such as surface soils that periodically flood and drain and subsurface soils at the capillary fringe, these bacteria are likely to be present at the MCB and also to contribute to TCE degradation in soils from the MCB. However, the characteristic fatty acid for type II methanotrophs (18:1ω8c) was not detected in soil samples from the MCB. Nonetheless, these microorganisms are undoubtedly present based on their ubiquity in nature and the favorable conditions for their proliferation at the MCB.

Analyses for the endogenous storage lipid polybetahydroxyalkanoate (PHA) indicated its presence in all samples primarily as polybetahydroxybutyrate. Samples of *L. cuneata* and *P. taeda* rhizosphere soils contained elevated levels of PHA and were also very capable of degrading TCE in whole-plant experiments. Correlations between TCE degradation rate and PHA production have been observed previously in bioreactors (David Ringleberg and Dr. Tommy Phelps, Institute for Applied Microbiology, personal communication). Soil samples from *Solidago* sp.

rhizosphere and nonvegetated areas within the MCB had the lowest levels of PHA and also degraded TCE more slowly in whole-plant experiments than rhizosphere soils of *L. cuneata* and *P. taeda*. Only soils from the rhizosphere of *P. notatum* failed to follow the correlation between degradation of TCE and production of PHA. Soils from *P. notatum* rhizosphere had comparatively high levels of PHA but did not degrade TCE as well as soils from the rhizosphere of *L. cunata* and *P. taeda*.

Toxicity experiments with TCE were very helpful in interpreting the data on biodegradation of TCE. Soil samples from *P. notatum* and *Solidago* sp. rhizosphere and nonvegetated areas had decreased respiration after exposure to TCE at 1000 µg/g. These samples also had lower ^{14}C-TCE degradation rates campared with the rhizosphere soil of *L. cuneata* and *P. taeda*. Both of these samples showed no effect from TCE at 100 µg/g. Somewhat conflicting results were obtained from toxicity experiments with PCE indicating that only in certain cases may the PCE be influencing ^{14}C-TCE degradation rates.

The results obtained from the degradation experiments indicate that the presence of vegetation enhances ^{14}C-TCE mineralization in soil from a contaminated field site. Furthermore, these findings corroborate interpretations of earlier data indicating an initial rapid degradation of TCE in the rhizosphere soil slurries and mineralization of ^{14}C-TCE in rhizosphere soil from *L. cuneata* (Walton and Anderson, 1990). In soils containing *L. cuneata*, *P. taeda*, and *G. max*, the levels of $^{14}CO_2$ produced were significantly greater than $^{14}CO_2$ production in both nonvegetated and sterile control soils. Radiolabelled CO_2 production in soil containing *Solidago* sp. and *P. notatum* was elevated,

however, there was no statistically significant difference from $^{14}CO_2$ produced in the respective nonvegetated soils. The plants tested represent a variety of root types--fibrous, tap, leguminous, and mycorrhizal. Both of the legumes, *L. cuneata* and *G. max*, enhanced soil microbial mineralization of ^{14}C-TCE, although only *L. cuneata* is indigenous to the contaminated site. In addition, the enhanced mineralization observed in soil containing loblolly pine seedlings, which traditionally have abundant ectomycorrhizal associations (Curl and Truelove, 1986), raises the question of whether mycorrhizae contribute to the degradation of TCE or other hazardous organic compounds. Because elevated $^{14}CO_2$ production was observed in soils containing soybean germinated from commercially available seeds, prolonged TCE exposure to the plant does not appear to be a requirement for enhanced ^{14}C-TCE mineralization. This may be a possible explanation for the lack of a statistically significant difference between $^{14}CO_2$ production in soil containing the indigenous composite, *Solidago* sp. compared with nonvegetated soil. Although prolonged exposure of the plant to TCE may not be essential for enhanced degradation in the rhizosphere, prolonged exposure of the soil to TCE may be required for enhanced degradation of TCE. It is not clear whether the enhanced mineralization is most influenced by the structure or surface area of the root system (tap vs. fibrous), the selective influence of the root exudates, or the kind(s) of root associations present (N_2-fixing, mycorrhizal). Attempts to control the ^{14}C-TCE degradation in the rhizosphere by influencing root exudation were successful but very preliminary.

The results of degradation experiments described above were obtatined for soil samples and freshly added TCE. In these instances, the TCE is readily available to the microorganisms. In the latter stages of the degradation experiments, the TCE degradation rate decreases, probably because through sorption and volatilization, less of the TCE is available to the microorganisms. Thus, whether similar degradation rates could be obtained with soil contaminated with TCE for some time or at low TCE concentrations is a question that has yet to be answered and could possibly limit the application of vegetation to remediate contaminated surface soils. However, evidence from the literature on the phenomenon of secondary substrate utilization (Schmidt and Alexander, 1985; Schmidt et al., 1987) is encouraging. Schmidt and coworkers found that dissolved organic substrates such as glucose could be used to enhance biodegradation of organic contaminants such as nitrophenol at low concentrations or after initial biodegradation of these compounds had stopped. Additionally, it may be possible to manipulate other environmental variables (Thorton-Manning et al., 1987) to positively influence TCE degradation in the field.

Root uptake of ^{14}C into plant tissues at the conclusion of the experiments (in most cases 18 days) was minimal for most of the plants tested. Uptake of organic compounds from soil solution is primarily affected by three factors; (1) physicochemical properties of the compounds, (2) environmental conditions, and (3) plant characteristics (Paterson et al., 1990). In these experiments where environmental conditions such as temperature, relative humidity, soil organic carbon content, and soil moisture content remained relatively constant, root

uptake appeared to be dependent on plant characteristics and physicochemical properties of TCE and/or its metabolites. The plants used in this study represent a variety of root and leaf characteristics. Additionally, the metabolic products of TCE degradation are dependent on the existing environmental conditions in the system. Under aerobic conditions, TCE is oxidized by a variety of oxygenases to an epoxide which spontaneously breaks down to water soluble metabolites (formic acid, glyoxylic acid) (Little et al., 1988). These water-soluble metabolites could be readily taken up by plants or could be metabolized quite easily by a wide variety of microoganisms. Under anaerobic conditions, TCE is metabolized through reductive dechlorination (Vogel and McCarty, 1985) in which more lipophilic metabolites (dichloroethylene, vinyl chloride) are formed. These metabolites would have different fates in the soil-plant systems than the water soluble metabolites of aerobic TCE metabolism. Paterson and co-workers (1990), in a recent review on the uptake of organic compounds by plants, hypothesized that root uptake from soil solution was a function of the compound's octanol-water partition coefficient (K_{ow}). Namely, that more lipophilic compounds readily partition into roots, but tend to remain there and are not translocated. However, compounds with high K_{ow} would also be expected to partition from soil solution into soil organic matter, thus making them less available for uptake into the root. The $\log_{10} K_{ow}$ for TCE is 2.3-2.4 (Lyman et al., 1990). The increased ^{14}C observed in stems, leaves, and the charcoal trap during experiments when elevated water use was observed, is consistent with the concept of bulk water flow, induced by evapotranspiration, as being the driving force behind translocation (Mengel, 1974).

Simulations using the Pesticide Root Zone Model (PRZM) (Carsel et al., 1984) and microbial degradation data from laboratory experiments were successful in illustrating the influence of vegetation on the biodegradation of TCE in soil at the MCB. However, the model lacked the ability to incorporate volatilization into the overall fate simulations. Thus the analyses for a highly volatile compound such as TCE using PRZM were somewhat compromised. Uptake into plant tissues was underestimated by PRZM compared with uptake experiments with ^{14}C-TCE in the laboratory. Root uptake in the model is based on a root uptake concentration factor (Briggs et al., 1982) related to Kow, the evapotranspiration rate, and the amount of chemical in soil solution. Under conditions where the degradation rate was decreased, such as with *P. notatum* and *Solidago* sp., uptake was elevated. It appeared that degradation in the model was also based in part on the amount of chemical in the soil solution. This may account for the underestimation of uptake by the model. Surprisingly, the presence or absence of vegetation had little influence on the speed in which the front of TCE migrated through the soil profile. It was hypothesized that the presence of vegetation, because of its influence on the microorganisms and soil in the root zone, would significantly retard breakthrough of TCE. The simulations indicated that the front of TCE would reach 150 cm after 109 days under all conditions. However, there was at least one order of magnitude more breakthrough of TCE under nonvegetated conditions.

The data from the literature on microbial activity of the rhizosphere, the observation of enhanced pesticide degradation in the root zone, and the results of TCE degradation in the rhizosphere presented here provides a strong

incentive for continued exploration of the soil-root-microbial interaction and the variables that may influence biodegradation of waste chemicals in surface and near-surface soils.

CHAPTER VI.

CONCLUSIONS

The objective of this study was to collect data that would provide a foundation for the concept of using vegetation to enhance *in situ* bioremediation of contaminated surface soils. The Miscellaneous Chemicals Basin at the Savannah River Site provided an ideal area for testing this hypothesis because of its previous exposure history and its present condition (naturally revegetated). This dissertation represents a systematic approach to answering initial fundamental questions which were critical to meeting the overall objective and providing a foundation for further studies. The results presented provide strong evidence for the potential role of vegetation in remediation of surface soils contaminated with hazardous organic compounds. In addition, the reviewed literature on rhizosphere microbiology, accelerated microbial degradation of agricultural chemicals in the root zone, and recent research on similar observations with hazardous organic compounds provide additional impetus for further exploring bioremediation of contaminated soils using vegetation.

The microbial activity, biomass, and degradation of TCE in rhizosphere soils from the MCB was found to be significantly greater than corresponding nonvegetated soils. In addition, the presence of vegetation had a positive effect on microbial degradation of ^{14}C-TCE providing additional evidence for the positive role of vegetation in remediation of the MCB. Furthermore, biodegradation of TCE may be much slower than biodegradation rates for less persistent waste chemicals such as nonhalogenated monoaromatics and

diaromatics. Therefore, the data presented herein are likely to be a conservative estimate of the potential for enhanced degradation of waste chemicals in the plant rhizosphere. Certainly, a critical part of using vegetation in remediating contaminated surface soils is determining the magnitude of uptake of parent compound or hazardous metabolite into the plant tissue. In most of the whole plant experiments conducted, uptake was minimal and was related to water use and plant species.

Issues which must be addressed further include (1) the role of mycorrhizae in degradation in the root zone, (2) the influence of different root morphologies, (3) identifying the microbial communities associated with different plant species and the role of exudates in selection of those communities, (4) determining if different rhizosphere communities exist under different conditions (contaminated vs. uncontaminated) and (5) the influence of multiple contaminants, as would occur at waste sites, on microbial degradation in the rhizosphere.

Certainly, there may be limitations to using vegetation in site remediation, as there are limitations to bioremediation as a whole. However, continued exploration of critical environmental variables affecting the soil-plant-microbe-chemical relationship will help to identify situations in which bioremediation using vegetation may be inappropriate.

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CHAPTER VII.

LIST OF REFERENCES

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DATE FILMED

4/08/92