ornl

ORNL/TM-12650

OAK RIDGE NATIONAL LABORATORY

MARTIN MARIETTA

Fate of Polycyclic Aromatic Hydrocarbons in Plant-Soil Systems: Plant Responses to a Chemical Stress in the Root Zone

OAK RIDGE NATIONAL LABORATORY
CENTRAL RESEARCH LIBRARY
CIRCULATION SECTION
4500N ROOM 175
LIBRARY LOAN COPY
DO NOT TRANSFER TO ANOTHER PERSON
If you wish someone else to see this report, send in name with report and the library will arrange a loan.
ornl-rm 9-79

Anne M. Hoylman
Barbara T. Walton

Environmental Sciences Division
Publication No. 4206

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

This report has been reproduced directly from the best available copy.

Available to DOE and DOE contractors from the Office of Scientific and Technical Information, P.O. Box 62, Oak Ridge, TN 37831; prices available from (615) 576-8401, FTS 626-8401.

Available to the public from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Rd., Springfield, VA 22161.

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

ORNL/TM-12650

ENVIRONMENTAL SCIENCES DIVISION

FATE OF POLYCYCLIC AROMATIC HYDROCARBONS IN PLANT-SOIL SYSTEMS: PLANT RESPONSES TO A CHEMICAL STRESS IN THE ROOT ZONE[1]

Anne M. Hoylman[2]
Barbara T. Walton

[1] Submitted as a dissertation by Anne M. Hoylman to the Graduate Council of the University of Tennessee, Knoxville in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Graduate Program in Ecology, The University of Tennessee, Knoxville.

Environmental Sciences Division
Publication No. 4206

Date Published - January 1994

Prepared for the
Electric Power Research Institute, Inc.,
Research Project 2879-10

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

MARTIN MARIETTA ENERGY SYSTEMS LIBRARIES
3 4456 0378462 5

ABSTRACT

Hoylman, Anne M. and Barbara T. Walton. 1994. Fate of polycyclic aromatic hydrocarbons in plant-soil systems: Plant responses to a chemical stress in the root zone. ORNL/TM-12650. Oak Ridge National Laboratory, Oak Ridge, TN. 119 pp.

Plant uptake and translocation of polycyclic aromatic hydrocarbons (PAHs) from soil was investigated to explore plant-microbial interactions in response to a chemical stress in the root zone. Plant uptake of individual PAHs was examined under laboratory conditions selected to maximize root exposure. Specially constructed flasks that isolated roots and soil from aboveground tissues, provided a single, unambiguous pathway for PAH uptake. White sweetclover, *Melilotus alba*, was grown in soils dosed with ^{14}C-naphthalene, -phenanthrene, -pyrene, and -fluoranthene. The highest ^{14}C concentrations were associated with roots, with decreasing concentrations observed in stems and leaves; however, the greatest percentage of recoverable ^{14}C remained in the soil ($\geq$ 86%) for all four PAHs. No evidence of bioaccumulation of the individual PAHs was found in *M. alba* over a 5-day exposure period.

Root uptake and translocation of PAHs from soil to aboveground plant tissues, as indicated by ^{14}C-residues in *M. alba* tissues, proved to be a limited mechanism for PAH transport into terrestrial food chains. However, root surface sorption of PAHs may be important for plants in soils containing elevated concentrations of PAHs. Consequently, the root-soil interface may be important for plant-microbial interactions in response to a chemical stress. $^{14}CO_2$ pulse-labeling studies provide evidence of a shift in ^{14}C-allocation from aboveground tissue to the root zone when plants were exposed simultaneously to phenanthrene in soil. In addition, soil respiration and heterotrophic plate counts

of rhizosphere microorganisms increased in plants exposed to phenanthrene as compared to controls.

This study demonstrates the importance of the root-soil interface for plants growing in PAH contaminated soil and provides supportive evidence for a plant-microbial defense response to chemical toxicants in the root zone. Both plants and rhizosphere microorganisms responded quickly to the presence of a PAH in soil: plant exudation and rhizosphere microbial numbers both increased in response to phenanthrene in soil. Thus, lipophilic toxicants in soils may reach high concentrations in the root zone, but rhizosphere microbial communities under the influence of the plant may reduce the amount of the compound that is actually taken up by the root.

TABLE OF CONTENTS

CHAPTER PAGE

List of Tables vii

List of Figures ix

I. INTRODUCTION 1

II. LITERATURE REVIEW 9
- A. Physicochemical Properties of Polycyclic Aromatic Hydrocarbons .. 9
- B. Polycyclic Aromatic Hydrocarbons in the Terrestrial Environment 13
- C. Plant Uptake and Metabolism of Xenobiotic Compounds 20
- D. Plant-Microbial Relationships 28

III. MATERIALS AND METHODS 35
- A. Field Site and Sample Collection 35
- B. Uptake and Translocation of ^{14}C-Labeled PAHs by *Melilotus alba* 37
- C. ^{14}Carbon Allocation in *Melilotus alba* 48
- D. Soil Respiration and Microbial Biomass 52

IV. RESULTS 54
- A. Field Site and Sample Collections 54
- B. Uptake and Translocation of ^{14}C-Labeled PAHs by *Melilotus alba* 54
- C. ^{14}Carbon Allocation in *Melilotus alba* 86
- D. Soil Respiration and Microbial Biomass 92

V. DISCUSSION 99

VI. CONCLUSIONS 106

LIST OF REFERENCES 108

LIST OF TABLES

TABLE PAGE

II - 1. Plant and microbial metabolites released in the rhizosphere and their proposed functions. 30

III - 1. Physicochemical properties of selected polycyclic aromatic hydrocarbons. 40

IV -1. Selected physical and chemical properties of the composite test soil from EBOS-24. 55

IV - 2. Distribution of ^{14}C-PAH derived radioactivity in *Melilotus alba* after 5 days of exposure. 57

IV - 3. Distribution of ^{14}C-PAH derived radioactivity in soil and flask headspace after 5 days of exposure. 74

IV - 4. Plant tissue concentrations of ^{14}C-PAHs after a 5-day exposure period. 76

IV - 5. Percent recoveries achieved in plant uptake experiments with PAHs in soil. 87

IV - 6. Distribution of ^{14}C-photosynthates in *Melilotus alba* after pulse labeling with $^{14}CO_2$ followed by a 5-day chase period. 90

IV - 7. Heterotrophic plate counts of microorganisms cultured on TSA from four separate experiments. CFUs obtained from plants exposed to phenanthrene are shown with data from matched control plants not exposed to phenanthrene. 95

LIST OF FIGURES

FIGURE		PAGE
I - 1.	Proposed interaction of plants and rhizosphere microorganisms in response to a chemical stress in the root zone	5
II - 1.	PAHs may exist in a variety of angular, cluster, and linear arrangements as indicated by the examples shown	10
II - 2.	Pathway for metabolic activation of benzo[*a*]pyrene to ultimate carcinogenic metabolites in mammalian systems	12
II - 3.	Generalized fungal and bacterial oxidative pathways for mineralization of PAHs (after Cerniglia and Heitkamp 1989)	17
III - 1.	Structures of the test compounds, (including Chemical Abstract Numbers), used for PAH uptake studies with *Melilotus alba*	38
III - 2.	Summary diagram of soil, plant tissue, and flask headspace analyses for ^{14}C-residues after 5-day exposure to individual ^{14}C-polycyclic aromatic hydrocarbons	42
III - 3.	Experimental apparatus used to determine plant uptake and fate of ^{14}C-polycyclic aromatic hydrocarbons applied to soil and ^{14}C-allocation in *Melilotus alba*	43
III - 4.	Glass environmental exposure chambers used to house individual plant-soil-flask units during uptake studies with ^{14}C-polycyclic aromatic hydrocarbons, ^{14}C-allocation studies, and to measure ^{14}C-volatiles released into the chamber atmosphere (Jen et al., in review)	44
IV - 1.	^{14}C distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-naphthalene #2 in a composite soil collected from EBOS-24 site	58
IV - 2.	^{14}C distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-phenanthrene #1 in a composite soil collected from EBOS-24 site	59

IV - 3. ^{14}C distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-pyrene #1 in a composite soil collected from EBOS-24 site. 60

IV - 4. ^{14}C distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-fluoranthene #1 in a composite soil collected from EBOS-24 site. 61

IV - 5. ^{14}C distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-naphthalene #1 in a composite soil collected from EBOS-24 site. 62

IV - 6. ^{14}C distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-phenanthrene #2 in a composite soil collected from EBOS-24 site. 63

IV - 7. ^{14}C distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-pyrene #2 in a composite soil collected from EBOS-24 site. 64

IV - 8. ^{14}C distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-fluoranthene #2 in a composite soil collected from EBOS-24 site. 65

IV - 9. ^{14}C distribution of naphthalene #2 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. 66

IV - 10. ^{14}C distribution of phenanthrene #1 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. 67

IV - 11. ^{14}C distribution of pyrene #1 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. 68

IV - 12. ^{14}C distribution of fluoranthene #1 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. 69

IV - 13. ^{14}C distribution of naphthalene #1 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil

vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. 70

IV - 14. ^{14}C-distribution of phenanthrene #2 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. 71

IV - 15. ^{14}C-distribution of pyrene #2 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. 72

IV - 16. ^{14}C-distribution of fluoranthene #2 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. 73

IV - 17. Mineralization of ^{14}C-napthalene #2 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*....78

IV - 18. Mineralization of ^{14}C-phenanthrene #1 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*....79

IV - 19. Mineralization of ^{14}C-pyrene #1 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*....80

IV - 20. Mineralization of ^{14}C-fluoranthene #1 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*....81

IV - 21. Mineralization of ^{14}C-naphthalene #1 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*....82

IV - 22. Mineralization of ^{14}C-phenanthrene #2 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*....83

IV - 23. Mineralization of ^{14}C-pyrene #2 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*....84

IV - 24. Mineralization of ^{14}C-fluoranthene #2 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*....85

IV - 25. Evolution of $^{14}CO_2$ in the headspace of plants exposed to 250 µg phenanthrene and control plants not exposed to phenanthrene....89

IV - 26. *Melilotus alba* tissue concentrations of ^{14}C after a 5-day chase period. 91

IV - 27. Carbon dioxide efflux from vegetated and nonvegetated sterile controls and vegetated treatments exposed to 250 μg phenanthrene/50 g sand. 93

IV - 28. Carbon dioxide efflux from vegetated controls and vegetated treatments exposed to 250, 2500, and 5000 μg phenanthrene/50 g sand. 94

IV - 29. Colony forming units from subsampled sand after a 14-day incubation in sterile controls, vegetated controls, and vegetated sand with 250 μg phenanthrene/50 g sand. 96

IV - 30. Colony forming units from sand after a 14-day incubation with *Melilotus alba* exposed to 0, 250, 2500, and 5000 μg phenanthrene/50 g sand. 98

CHAPTER I
INTRODUCTION

All life forms have, through evolutionary time, encountered injurious chemicals in their environment, and as such, are likely to have developed some level of defense or protection against these chemicals. While defense mechanisms vary among taxa, the cytochrome P-450 detoxicating enzyme system, or similar enzymes, have been isolated from most phyla analyzed to date (Wilkinson 1980). Yet while both animals and plants possess detoxicating enzyme systems, fundamental differences exist between these taxa. Unlike the well developed metabolizing and excretory functions of the vertebrate liver and kidney systems, plants lack a well developed excretory system and must compartmentalize toxicants into vacuoles or storage tissue (Hatzios and Penner 1982) and utilize a physical barrier such as selective root uptake of compounds (Walton and Edwards 1986). An additional profound difference is the inability of a stationary plant to actively avoid a chemical toxicant, which could be distributed in an area larger than the root system.

Yet plants have been exposed to chemical toxicants over evolutionary time, and continue to be exposed to both natural and anthropogenic sources. Natural sources include examples such as allelochemicals (antagonistic plant compounds), mineral deposits, metal outcroppings, and polycyclic aromatic hydrocarbons (PAHs) from forest fires and volcanic activity. Anthropogenic sources represent more recent chemical stresses and include synthetic chemical toxicants generated from human activities.

Studies of plant-microbial interactions in soil have found mutually beneficial exchanges of nutrients, and in some cases, effective defenses

against stresses such as root pathogens (Schippers et al. 1990). Many of these interactions are mediated through plant root excretion of chemical signals in the form of root exudates (Lynch 1990). Beneficial interactions between plants and soil microorganisms may also be present under a chemical stress. The plant-microbial relationship may act, at least in part, as a protective mechanism by which the plant responds to a chemical stress encountered in the root zone by shifting carbon allocation to root exudates to stimulate a microbial response, which in turn acts to alleviate or alter the chemical (Walton et al. in press).

To explore plant-microbe-toxicant interactions, the fate and transport of the toxicant in the soil environment must be determined. Uptake and translocation of soil contaminants by vegetation represents a potential mechanisms for transport of soil contaminants into the terrestrial food chain (Walton and Edwards 1986). Recent studies on the fate of polycyclic aromatic hydrocarbons (PAHs) in sewage-amended agricultural soils indicate that physicochemical interactions of PAHs with soil/sludge organic matter limit plant uptake and translocation of the compounds (Witte et al. 1988; Webber and Goodin in press; O'Connor et al. 1991). However, the fate of PAHs in soil environments with low organic content and/or limited microbial degradation may be quite different (Walton and Hoylman 1992). In addition, substitution of an artificial rooting medium such as sand or nutrient solution may influence the PAH concentration available for root uptake. For example, root uptake or surface sorption may be artificially high in hydroponic systems or artificially low in systems utilizing sewage sludge where sorption to the organic carbon in the sludge could account for low uptake by vegetation. While the significance of plant-toxicant interactions in soil systems has been explored, due to ambiguous routes of exposure and biased influence of experimental designs, the existing

scientific literature is not sufficient to fully evaluate the fate of PAHs in plant-soil systems (Walton and Hoylman 1992). This fundamental information is critical for a comprehensive understanding of the fate of PAHs in plant-soil systems.

The root-influenced area known as the rhizosphere, is a dynamic environment in which the plant influences the composition and physiological activity of microorganisms through the release of diverse compounds (Hedges and Messens 1990). Conversely, microorganisms enhance nutrient availability and uptake by the plant (Curl and Truelove 1986). Plant root exudates quantitatively and qualitatively modify environmental conditions in the rhizosphere such that these conditions are different from the surrounding soil; the result is a distinct microbial community. In addition, root exudates have been found to attract specific microorganisms (reviewed in Lynch 1990). The well studied relationship between leguminous plants and nitrogen-fixing bacteria is one example of plant chemical cues that attract specific microbial members to the rhizosphere (Klein et al. 1990).

While it is clear that the plant affects the rhizosphere community through root exudates, other studies suggest that the rhizosphere community exerts an effect on the plant as well. Examples include alteration of root morphology and increased exudation of organic substances by plants in the presence of rhizosphere bacteria (Barber and Lynch 1977; Kraffczyk et al. 1984). The overall net effect of particular rhizosphere communities may be negative for the plant (Campbell 1985). Yet the fact that many plants have developed mechanisms to attract microorganisms to their roots (Klein et al. 1990) suggests that a net benefit may exist in some cases. Campbell (1985) suggests that an as yet undescribed plant benefit may balance the relationship between plants and the rhizosphere community. One such benefit to the plant may be a

strategy for responding to chemical toxicants through utilization of the rhizosphere community (Walton et al. in press). More specifically, through the release of root exudates, plants may be able to modify or select a specific microbial community when subjected to a variety of environmental stresses. Plants exposed to soil toxicants may respond in this manner by shifting carbon allocation to the rhizosphere to select a microbial community for its degradative capabilities (Figure I - 1).

Recently, laboratory studies have shown an increased rate of degradation of a chlorinated solvent, trichloroethylene (Walton and Anderson 1990), and apparent disappearance of four PAHs (Aprill and Sims 1990) in rhizosphere soils relative to nonvegetated soils. Several possible mechanisms may explain this enhanced degradation, the most prominent being that degradation was the result of an increased microbial biomass found in rhizosphere soils as compared to nonvegetated soils. Additionally, the rhizosphere provides an environment conducive to cometabolism, the oxidation of a compound by a microorganism growing on a different substrate and subsequent utilization of this oxidized compound by other members of the microbial community as an energy source (Atlas and Bartha 1993; Bartha 1990). These studies highlight the potential for vegetation to enhance the microbial degradation of contaminants in surface soils and implicate a plant-microbial interaction in response to a chemical stress. However, there is limited information describing the relationship between a plant and the rhizosphere microflora in the context of chemically induced stresses. Such information could be obtained through studies of changes in plant physiology such as photosynthesis, carbon allocation, and root respiration. In addition, because root exudates are an important factor in the maintenance of rhizosphere

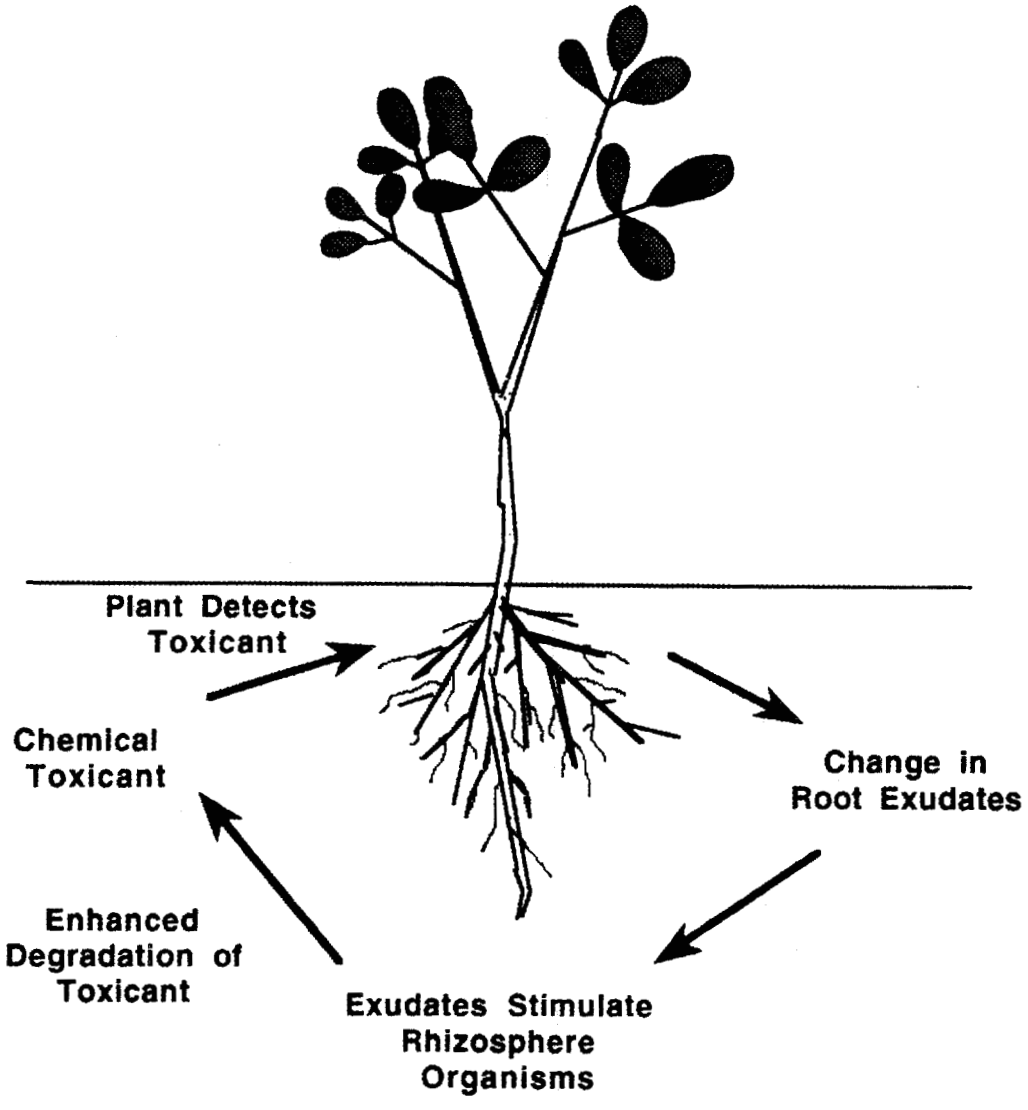


Figure I - 1. Proposed interaction of plants and rhizosphere microorganisms in response to a chemical stress in the root zone.

microbial activity, information on plant-mediated changes in carbon allocation to the root zone could provide possible mechanisms by which rhizosphere degradative capabilities could be manipulated (e.g., selection of plant species that support a particular microbial community). Carbon represents a fundamentally important plant resource as it is the building block of all plant structures (Bazzaz et al. 1987). Therefore, changes in plant carbon allocation represent an investment of the plant to a particular process and should be a sensitive indicator of a plant's response to a chemical stress in the root zone.

While recent studies of enhanced degradation of contaminants in rhizosphere soils implicate the rhizosphere as a possible site of plant-microbial interactions initiated by a chemical stress (Anderson et al. 1993), it is important to fully understand fundamental questions concerning the fate of the chemical in the soil environment. For example, the study of plant response to a chemical stress should focus on the site of the highest concentration of the compound (*i.e.*, roots, stems, and/or leaves). Studies of plant uptake and translocation of a compound provide an overall description of the fate of the compound in environmental compartments (soil, air, plant tissue) and guidance for potential sites of plant-mediated defenses and microbial interactions.

Carbon allocation is considered one of the major mechanisms by which plants respond to changing environmental conditions such as resource imbalances (Chapin et al. 1987). Plants invest carbon and new biomass in the tissue that will provide the greatest acquisition of the limiting resource (e.g., leaves or root tissue) (Fitter and Hay 1987). For example, plants growing under nutrient limited conditions respond by increasing allocation to root growth while plants that experience loss of photosynthetic potential compensate by producing more leaf material (Chapin et al. 1987).

The plant-microbial relationship in the rhizosphere is mediated through both chemical signals in root exudates as well as bacterial signals (Klein et al. 1990). Analysis of root exudation associated with a chemical concentration in the soil should provide evidence of the extent of the plant-microbial relationship. Direct analysis of root exudates, both composition and quantity, is hampered by the myriad of possible compounds which may act as chemical signals. In addition, root exudates are readily metabolized and transformed by microorganisms and thus in essence lost or hidden in the system. However, using a radiolabeled tracer provides an ideal means to determine plant allocation of carbon under chemical stress. Incorporation of $^{14}CO_2$ during photosynthesis results in labeled photosynthates which can then be traced through the plant tissue and soil using radioisotope techniques.

The objectives of this research were to determine (1) the distribution and fate of individual PAHs in plant-soil laboratory systems and (2) the physiological response of vegetation and rhizosphere microorganisms exposed to a chemical stress in the root zone. To address the key issue of the interaction of plants with hazardous chemicals in soil, plant uptake studies were conducted with ^{14}C-PAHs applied to soil and ^{14}C distributions determined in multiple environmental compartments. Studies were conducted to assess plant allocation of carbon when exposed to a PAH stress. Incorporation of $^{14}CO_2$ during photosynthesis produced labeled photosynthates which were traced through plant tissue and soil using radioisotope techniques. In addition, soil respiration studies and characterization of the microbial community were carried out. These experiments were designed to address the potential utilization of the rhizosphere community by plants as an adaptive defense mechanism against a

chemical challenge in the root zone and have important implications for both plant physiology and bioremediation of soil contaminants.

CHAPTER II

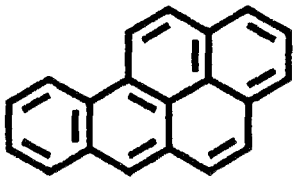
LITERATURE REVIEW

A. Physicochemical Properties of Polycyclic Aromatic Hydrocarbons

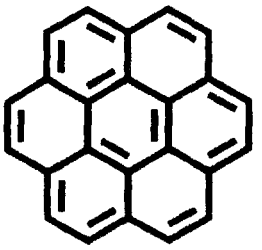
Polycyclic aromatic hydrocarbons (PAHs) are products of incomplete combustion of organic material and are ubiquitous in the environment (Edwards 1983). While PAHs originate from natural pyrolytic processes such as forest fires and volcanoes, the greatest sources today are anthropogenic in origin such as the burning of fossil fuels (Edwards 1983; Sims and Overcash 1983). Endogenous synthesis of PAHs by plants and microorganisms has been proposed (reviewed in Edwards 1983); however, the contribution from biosynthetic origin is probably insignificant relative to combustion processes (Gibson 1984).

Polycyclic aromatic hydrocarbons are composed of carbon and hydrogen arranged in two or more fused aromatic rings. Arrangement of the rings can be linear (rings are in a straight line), angular (rings are on different straight lines with angles always being 120°), or clustered (at least one ring surrounded on three sides) (Figure II - 1) (Sims and Overcash 1983). When substitution of nitrogen, oxygen, or sulfur into the ring occurs, the resultant structure is a heterocyclic hydrocarbon. The latter compounds may have very different properties from PAHs and are excluded from this review. Physical and chemical properties of PAHs depend in part, on size (the number of carbon centers or the number of rings) and topology (type of ring linkage) (Zander 1983).

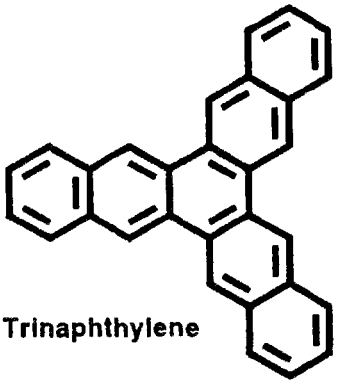
PAHs exhibit a diverse range of ring structures, physicochemical characteristics, biological activities, and environmental behavior. Nonpolar and



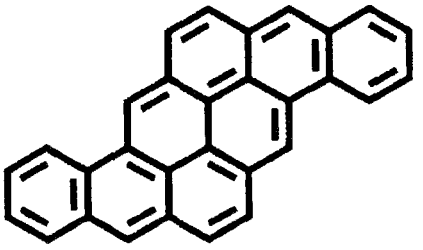
Benzo[*a*]pyrene



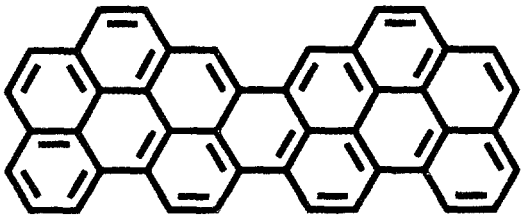
Coronene



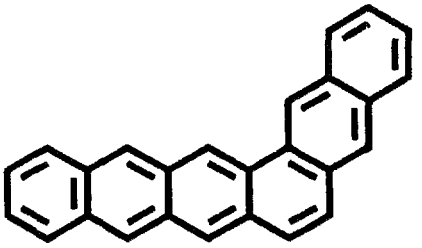
Trinaphthylene



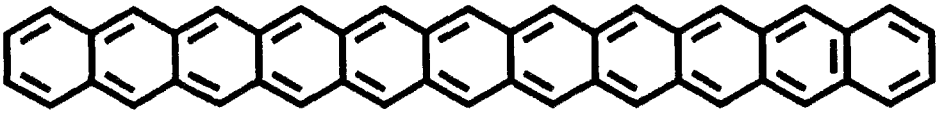
Pyranthrene



Perylo[2,3,4-*bcd*:9,10,11-*e'd'c'*]dipyrene



Hexaphene



Undecacene

Figure II - 1. PAHs may exist in a variety of angular, cluster, and linear arrangements as indicated by the examples shown.

lipophilic in nature, with generally very low water solubilities, PAHs may bioaccumulate in organisms (Bulman et al. 1985). Environmental and health concerns associated with PAHs stem from their carcinogenic/mutagenic effects, bioaccumulation potential, and persistence in the environment (Park et al. 1990). Some members of the class of compounds are carcinogenic and were the first type of chemical carcinogens identified from exposure to chimney soot/tar and snuff use in the late 18th century (Phillips 1985). In addition to health concerns, PAHs are often distributed in complex heterogeneous environmental mixtures which present sampling and analysis challenges when determining the fate of PAHs in the environment.

Metabolic Activation and Carcinogenicity

PAHs are classified as DNA-reactive carcinogens and require metabolic activation of the parent compound to the ultimate carcinogenic metabolite (Williams and Weisburger 1986). Carcinogenicity/mutagenicity has been identified in PAHs having greater than 3-fused benzene rings with increasing carcinogenicity related to the presence of an angular region known as the "Bay region" (Phillips 1985; Williams and Weisburger 1986). The widely studied PAH, B[*a*]P undergoes metabolic activation in the mammalian liver to a reactive bay-region diol-epoxide. Reaction with DNA by the diol-epoxide is believed to be the mechanism resulting in carcinogenicity/mutagenicity (Phillips 1985). The pathway of metabolic activation of B[*a*]P in eukaryotic mammalian systems are shown in Figure II - 2.

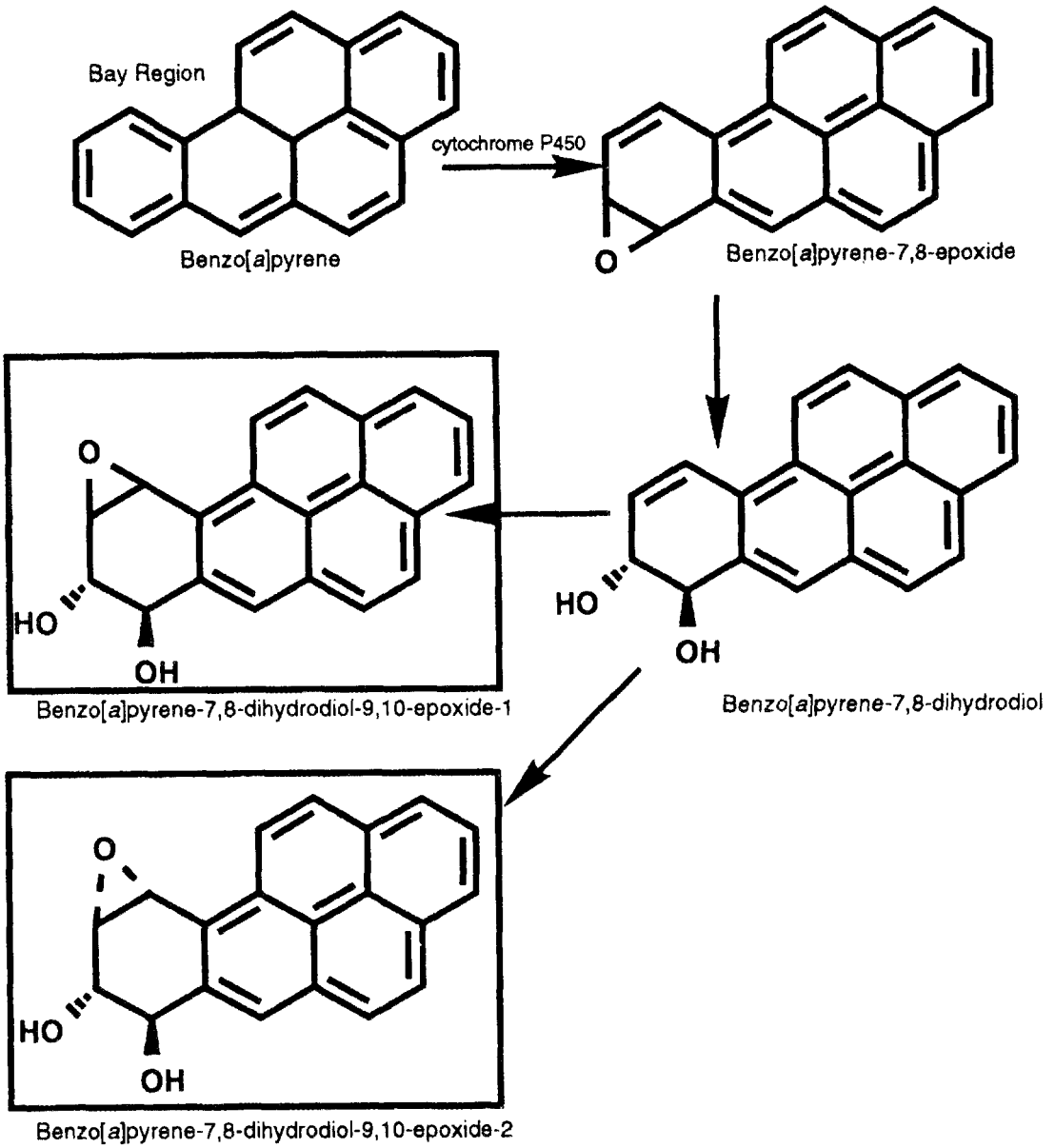


Figure II - 2. Pathway for metabolic activation of benzo[*a*]pyrene to ultimate carcinogenic metabolites in mammalian systems. Reactive carcinogenic metabolites are enclosed in boxes (after Gibson 1982).

B. Polycyclic Aromatic Hydrocarbons in the Terrestrial Environment

Sources and Distributions

PAHs are widely distributed in both aquatic and terrestrial ecosystems and are reported to be detected in all soils at some concentration, primarily because of atmospheric deposition (Wild and Jones 1992). Urban soils in the vicinity of roads and other fossil fuel-related human activities have the highest background levels of PAHs relative to forested and agricultural soils (Linz et al. 1991). In addition, disposal of waste generated by many industrial practices has resulted in locally high concentrations of PAHs. For example, from 1816 to 1960, the manufacture of gas (town gas) from coal, coke, or oil resulted in coal tar by-products that were often disposed of in pits or lagoons both on- and off-site (Murarka 1990).

Domestic and industrial utilization of fossil fuels can release PAHs into the air, water, soil, and sediment. An estimated 900 tons of one B[*a*]P are released into the atmosphere in the U.S. annually (Phillips 1985). Stationary sources such as residential coal burning, fireplaces, forest fires, and coke production plants accounted for 90% of the total particulate PAH emissions into the atmosphere in 1972 (National Academy of Sciences 1972). Concentration and composition of PAHs in emissions or waste products will vary according to the type of fuel used, the fuel origin, and the particular combustion process. For example, smoke generated from coal and wood burning contain concentrations of PAHs orders of magnitude greater than that found in oil or gas emissions (Cooper 1980). The total environmental PAH burden is predicted to increase in the future as use of fossil fuels for transportation, heating, power generation, and production of synthetic fuels grows (Sims and Overcash 1983).

PAH concentrations exhibit a spatial distribution in terrestrial systems. Urban and highly industrialized areas generally have a greater concentration of total PAHs than rural, remote locations. Jones et al. (1989a, 1989b) analyzed surface soils from 49 locations in Wales to assess concentrations of selected PAHs. PAH concentrations increased along a gradient of remote-rural-urban locations. Thus, soils near recent human activity tend to contain higher levels of PAHs than isolated areas. However, even soils in unpopulated areas receive contamination inputs and have elevated PAH concentrations, evidence of regional/global effects of fossil fuel combustion and the long-range transport capabilities of particulate-bound PAHs (Jones et al. 1989a).

Entry into Soil Systems

Entry of PAHs into terrestrial systems can arise from atmospheric deposition of rain, snow and particle fallout, runoff from roads (oils, exhaust, tire residues, and weathered asphalt) and direct application of petroleum wastes (tars, oils) and sewage sludge (Edwards 1983; 1991). Archived soil samples collected from the Rothamsted Experimental Station in southeast England indicate a 4-fold increase in total PAH burden of the plough layer (0-23 cm) over the last century (Jones et al. 1988, 1989b, 1989c). In this particular case, the principal source of the PAHs is believed to be atmospheric fallout from local industrial and urban areas.

Long-range transport of particulate aerosols is probably responsible for contamination of remote, isolated areas. Recent analysis of a "brown snow" event in the Canadian Arctic documented the deposition of thousands of tons of particulate matter contaminated with PAHs, chlorinated compounds, and pesticides (Welch et al. 1991). Based on air mass trajectories and particulate

composition, the material probably originated in western China. This single fallout event may have contributed 27% of the total annual loading of PAHs for this region.

Numerous biotic and abiotic processes affect the fate of organic chemicals in soils. Physical, chemical, and biological processes such as leaching, adsorption, desorption, photodegradation, oxidation, hydrolysis, and metabolism by organisms influence the reactivity and eventual fate of PAHs in terrestrial systems (reviewed in Walton and Hoyman 1992). The persistence and ultimate fate of PAHs are strongly related to adsorption, and biotic and abiotic degradation. Varying mixtures of PAHs, exposure histories of soils and microorganisms, soil characteristics, and environmental variables play important roles in determining the proximate and ultimate fate of these compounds.

Biodegradation of PAHs

Prokaryotic and eukaryotic organisms have been isolated that transform the aromatic nucleus of PAHs through incorporation of oxygen into the ring structure. Incorporation of molecular oxygen is considered a prerequisite for biological ring cleavage as this represents an activation step that leaves the molecule reactive and a substrate for fission (Sims and Overcash 1983). Bacteria initialize oxidation of PAHs by incorporation of two atoms of oxygen forming a dihydrodiol with a *cis*-configuration (Cerniglia 1984). Further oxidation leads to catechols and eventually to ring fission. Hydroxylation of PAHs by eukaryotic organisms differs from prokaryotic oxidation in that eukaryotic organisms utilize a monooxygenase that incorporates only one atom of oxygen into the ring structure and produces a reactive arene oxide (Cerniglia

and Heitkamp 1989) (Figure II - 3). Further transformations of this metabolite can result in phenols, *trans*-dihydrodiols, or glutathione conjugates (Cerniglia 1984). Fungi have been found to oxidize B[*a*]P by a monooxygenase system similar to that found in mammalian systems. For example *Cunninghamella elegans* metabolized B[*a*]P to secondary metabolites that almost parallel those produced in mammals (Gibson 1982). The two alternative routes of degradation recognized in prokaryotic and eukaryotic organisms may be a result of fundamentally different enzyme functions (Gibson 1982). Degradation in prokaryotes results in ring cleavege and ultimately into a smaller molecule that could serve as an energy source. In contrast, enzymatic activity in eukaryotes results in metabolites that are more hydrophilic than the parent compounds and as such are more readily excreted (Williams and Weisburger 1986).

Biodegradation of PAHs is a primary route by which PAHs are removed from soil systems. Bacteria, fungi, and algae have the enzymatic capacity to metabolize di- and tricyclic PAHs in soil and aquatic environments (Cerniglia 1984). Through mineralization. microbial mediated degradation of PAHs resulted in removal of 2 and 3 ring PAHs but there was marginal loss of higher molecular weight compounds (Bossert et al. 1984; Park et al. 1990). Until recently, few isolated axenic bacterial isolates have been found that are capable of growing solely on PAHs of more than three condensed rings (Gibson 1984). Weissenfels et al. (1990) isolated a strain of *Alcaligenes denitrificans* that utilizes fluoranthene as a sole carbon source, and Boldren et al. (1993) have isolated a *Mycobacterium* sp. from a coal gasification site that is able to use the four-ringed PAHs, fluoranthene and pyrene, and other PAHs as sole carbon and energy sources. Kelley et al. (1993) have also isolated a

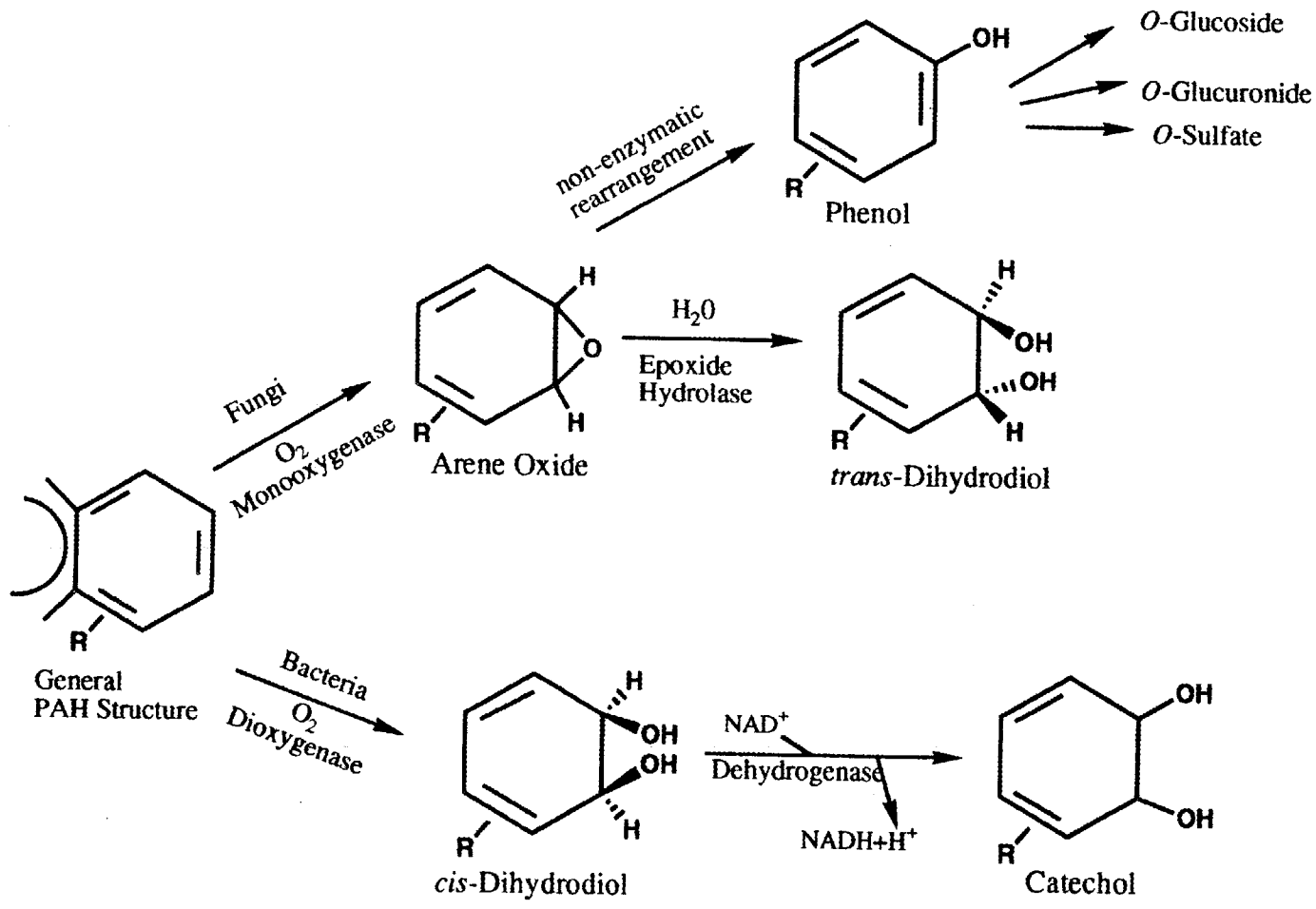


Figure II - 3. Generalized fungal and bacterial oxidative pathways for mineralization of PAHs (after Cerniglia and Heitkamp 1989).

Mycobacterium sp. that mineralizes fluoranthene and have identified several metabolites. They propose that several simultaneously operating pathways exist for the degradation of fluoranthene by this strain of *Mycobacterium*.

Degradation of PAHs of greater than 3-fused benzene rings has primarily been identified in microbial cultures provided with an alternative carbon source (Cerniglia 1984). Termed cometabolism or "fortuitous metabolism", bacteria oxidize the compound concurrently with substrates more suitable as carbon and energy sources (Atlas and Bartha 1993). No energetic benefit to the transforming microorganism is derived from compounds oxidized in this manner. Despite the slower rate of mineralization, cometabolic transformations may be a significant mechanism by which PAHs are removed from the environment and one that may perform efficiently in the heterogeneous environment found in plant-soil systems.

Biodegradation is negatively correlated not only with the molecular weight of the PAH (*i.e.*, number of rings), but also with the topology of the compound. Bossert et al. (1984) found that highly clustered PAHs were more recalcitrant in the environment than PAHs with the same number of rings; for example, pyrene (4 rings, clustered) was more stable than chrysene (4 rings, angular). Scheunert et al. (1987) found that ^{14}C-phenanthrene (3 rings, angular) was marginally degraded in suspended soils in contrast to anthracene (3 rings, linear) which was highly resistant to microbial attack. A clustered arrangement may present a size problem or steric hindrance for microorganisms and/or the absence of the reactive angular region limits sites for metabolic activation.

Previous exposure to a contaminant may provide the needed acclimation or adaptation period to induce the microbial community's degradative abilities.

In aquatic systems, B[*a*]P persisted 2 to 4 times longer in pristine sediments than in sediments chronically exposed to petroleum pollution (Heitkamp and Cerniglia 1987). The length of exposure time neccessary for acclimation varies considerably with the PAH, microbial community, and environmental conditions. After an incubation period of 160 days, Coover and Sims (1989) saw no significant difference in degradation rates of B[*a*]P associated with a second application of the compound to soil microcosms. In contrast, many PAH-utilizing bacteria have been isolated from sites chronically exposed to PAHs (e.g., Boldrin et al. 1993; Kelley et al. 1991; Heitkamp and Cerniglia 1988) and enhanced degradation has been shown in acclimated versus unacclimated samples (e.g., Mihelcic and Luthy 1988; Heitkamp et al. 1987).

Although microbial degradation can reduce the persistence of PAHs, edaphic conditions in soils determine the efficacy of microbial degradation. Sorption is perhaps the single most important factor affecting the fate of organic chemicals in soils (Kaufman 1983; Scheunert 1986) and some critical properties of PAHs and soils that influence sorption are octanol/water partition coefficient, aqueous solubility, vapour pressure, molecular size, chemical reactivity, soil pH, soil organic matter content, clay content and type, and moisture and temperature status. Sorption of PAHs to soil particles reduces further movement of PAHs in soil systems. Jones et al. (1989a) concluded from a long-term agricultural study that PAH losses from sludge-amended soils were due to transport of the solid phase (clay or organic matter) during plowing with little movement attributed to the solution-vapor phase. Verticle transport of PAHs below the plough layer ranged from 0.01-0.14 mg $\bullet$ m^{-2} $\bullet$ $year^{-1}$ and appeared to be a function of concentration in the surface soils. While reduced movement in contaminated soil systems potentially lessens exposure of

terrestrial biota to PAHs, sorption to soil particles also diminishes bioavailability to microorganisms (Bulman et al. 1985) and thus decreases loss through biological and chemical mechanisms.

Soil organic content and physicochemical properties of the PAH also reduce the fraction lost by volatilization from the soil-water phase. Park et al. (1990) monitored the loss of 14 PAHs from soil through volatilization over a 48-hour period and found that only naphthalene exhibited significant loss via volatilization. Parent residues of the other PAHs were associated with the organic solvent fraction of the soil. In general, however, owing to their high lipohilicity, low water solubility and, small Henry's Law constants, the susceptibility of PAHs to volatilization from the soil-water phase is considered low (Bulman et al. 1985) and, with the exception of naphthalene, this is an insignificant removal process.

C. Plant Uptake and Metabolism of Xenobiotic Compounds

Plant uptake of xenobiotic compounds represents a potential mechanism by which soil contaminants may enter terrestrial food chains. This pathway for exposure of humans and wildlife must be considered in comprehensive exposure assessments for PAH-contaminated soil. Research to date has focused primarily on plant uptake and metabolism of agricultural chemicals, such as herbicides and other pesticides. Relatively little is known about the uptake and fate of PAHs in plants. However, fundamental principles regarding uptake of xenobiotic compounds by plant roots and the capabilities of higher plants to metabolically degrade agricultural chemicals, primarily herbicides, provide a basis for extrapolation to the probable uptake and fate of PAHs in plant tissues.

Plant Uptake

The fate of PAHs in plant-soil systems as well as general plant uptake of xenobiotics from soil, have been reviewed in several extensive publications (Edwards 1983; Sims and Overcash 1983; Ryan et al. 1988; Paterson et al. 1990; O'Connor et al. 1991; Bell 1992; and Walton and Hoylman 1992). The number of recent reviews reflects both the continuing interests in plant uptake of xenobiotic compounds and the numerous important questions to be resolved.

Root uptake of xenobiotic compounds is governed by many of the same environmental variables that affect microbial bioavailability and fate of compounds in soil. Namely, sorption of lipophilic compounds to soil organic matter reduces not only availability in soil water and thus root uptake but also transport in the roots to other plant tissue. In addition, the mechanics of root uptake of solutes requires almost contrasting physicochemical requirements of the compounds. Plant-root uptake has been shown to be positively correlated with the n-octanol/water partition coefficent (K_{ow}) (Edwards 1988; Topp et al. 1986; Paterson et al. 1990). Thus, root tissue concentrations increase with increasing lipophilicity of the compounds (*i.e.*, higher K_{ow}'s). However, translocation to aboveground plant tissues from roots has been positively related to aqueous solubility and is most efficient for compounds of intermediate polarity (approximately log K_{ow} = 1.8) (Briggs et al. 1982). Thus, because uptake and translocation are two separate processes, compounds of high lipophilicity such as PAHs may be sorbed by roots, but further movement of the compound may be limited due to low water solubilty.

A recent study with carrots grown in sludges contaminated with a range of PAHs, demonstrates this stratification. Higher molecular weight PAHs such

as pyrene and fluoranthene were identified in the outermost cortex of the carrot root (Wild and Jones 1992), but further translocation of these compounds was not indicated from the plant tissue concentrations. Thus, physicochemical properties such as molecular weight, octanol-water partition coefficient, and aqueous solubility play an important role in the fate of PAHs in plant-soil systems (Ryan et al. 1988). Moreover, the physicochemical characteristics that increase the potential for translocation of compounds in plant tissue, namely water solubility, also increase the potential for losses via biotic and abiotic degradation.

Studies of field-collected plant tissues for PAHs have concluded that plant uptake of PAHs is limited to root uptake from soil, while aboveground contamination of plant tissue primarily arises from atmospheric deposition onto plant surfaces. Jones et al. (1989c) examined archived tissues from crop plants grown over the last century in a semi-rural agricultural environment in south-east England. Despite increases in soil PAH concentrations through time (Jones et al. 1988), total PAH concentrations in plant tissues have declined (Jones et al. 1992). The authors conclude that atmospheric deposition is the primary route of uptake due to higher concentrations yet similar mixtures of PAHs found in broad-leaf herbaceous pasture species versus wheat plants (*i.e.*, the higher concentrations were considered by the authors to be due to the larger aboveground surface area of the pasture species and indicative of atmospheric deposition). Other studies have reached similar conclusions for plants grown in sludge-amended soil. Kampe (1988) found that aboveground shoot material was contaminated via atmospheric deposition whereas belowground crops such as sugarbeets and carrots had slightly higher concentrations of a range of PAHs compared to unamended control soils. In

studies with anthracene sorbed to fly ash, Edwards (1991) found that approximately 90% of the anthracene in bush bean plant tissues remained in the roots. In contrast, plants exposed to anthracene in soil solution assimilated twice as much anthracene with 50% of the anthracene in plant tissues translocated to aboveground foliage. These studies emphasize the significant role of physicochemical processes in determining the fate of PAHs in plant-soil systems. Under specific environmental conditions such as high organic carbon soils or sludge-amended soils, the fate of PAHs in plant-soil systems appears to be dominated by physicochemical interactions with the soil. Therefore, changes in the soil environment and thus bioavailability of the PAH will change the fate of PAHs in plant-soil systems.

Plant Metabolism

Plants have the enzymatic capability to metabolize xenobiotics (Sandermann 1992). The enzyme system of plants generally does not require induction, rather the enzymes that can effectively metabolize xenobiotic compounds are actively present and have broad substrate specificity (Hatzios and Penner 1982).

Plants are similar to vertebrate and invertebrate species in that plants metabolize herbicides and other xenobiotics through a two-stage system of reactions, commonly designated as Phase I and Phase II. However, in plants the lack of a well-developed excretory system precludes the removal of metabolites, as occurs in animals. Alternatively, Phase II conjugation in plants is often followed by immobilization of the metabolite through compartmentalization into storage vacuoles, formation of insoluble salts,

complexation with plant constituents, or binding of the xenobiotic to structural polymers (Hatzios and Penner 1982; Sandermann 1992).

Phase I transformations are oxidation, reduction, and hydrolysis reactions; conjugation, the process by which metabolites react with additional substrates to form larger macromolecules and bound residues, is designated as a Phase II reaction (Shimabukuro 1985; Lamoureux and Frear 1979). Phase I reactions, which usually reduce toxicity of a xenobiotic, may also activate the compound for Phase II conjugation (Shimabukuro 1985). The principal Phase I reactions are mediated primarily through mixed function oxidases, peroxidases, nitroreductases, and hydrolases (Hatzios and Penner 1982). Phase II transformations result in conjugation of Phase I metabolites with water-soluble cell constitutents, such as glutathione, amino acids, glucose, or other thiols and sugars (Hatzios and Penner 1982). Phase II transformations typically increase the polarity, water solubility, and molecular weight of the substrate and reduce phytotoxicity (Hatzios and Penner 1982). In vertebrates and insects this step facilitates excretion of the xenobiotic from the body whereas in plants the final step is compartmentalization.

The three-stage metabolic pathway of plants affords protection to plant cells by first increasing polarity of the xenobiotic, translocating the polar metabolite within the plant, then rendering the metabolite immobile through compartmentalization or polymerization. The overall effect is to reduce the potential toxicity to the plant (Shimabukuro 1985).

Metabolism of aromatic and heterocyclic herbicides by higher plants is generally limited to removal of ring substituents and incorporation of the ring structure into plant polymers as a bound residue (Hatzios and Penner 1982; Ashton and Crafts 1981). Ring cleavage and subsequent oxidation of the

compound to CO_2, although common in microorganisms, does not appear to be a significant route of aromatic metabolism by higher plants (Hatzios 1991).

Evidence of PAH metabolism by higher plants is limited to relatively few plant species and few PAHs. Edwards (1986; 1988) exposed bush bean (*Phaseolus vulgaris* L.) grown in nutrient solution to ^{14}C-anthracene and ^{14}C-benz[*a*]anthracene for 30 days. Parent material and metabolites were characterized through sequential extractions of plant material followed by thin layer chromatography of the extracts. Anthracene, benzo[*a*]anthracene, and unextractable and nonpolar metabolites were primarily associated with the root tissue, whereas polar metabolites were found in proportionally greater concentrations in stems and leaves. The distribution of ^{14}C in the plant tissue suggests that anthracene and benz[*a*]anthracene were taken up by the roots, then were metabolized to polar compounds. Subsequent incorporation of metabolites into bound residues took place in the roots; however, some of the water-soluble metabolites were translocated to the stems and leaves. The possible contribution of microbial degradation of ^{14}C-anthracene and ^{14}C-benz[*a*]anthracene must also be considered because sterile conditions were not maintained during the exposure period. Microbial degradation of ^{14}C-anthracene in the nutrient solution could have produced ^{14}C-metabolites that were subsequently taken up by the plant roots and translocated to the stems and leaves. Extraction of the nutrient solution with potassium hydroxide and ethanol (KOH–ETOH) did not result in increased ^{14}C activity nor did it change the proportion of parent compound and metabolites. The author concluded that had microbial degradation been a significant contributor to degradation of ^{14}C-anthracene, KOH–ETOH digestion would have released these compounds from the microbial tissue and shifted the ^{14}C activity.

Although the fate of PAHs in plant systems is still ambiguous because of the limited number of plant species studied, the small number of PAHs examined, and the lack of clear uptake and metabolic pathways, the existing studies suggest that higher plants have the capability of assimilating PAHs from soil, transforming them into water-soluble, mobile compounds as well as producing bound residues through conjugation with plant polymers. Sakamoto et al. (1984) and Negishi et al. (1987) isolated and identified B[*a*]P-metabolites in extracts of homogenized soybean leaves (*Glycine max*). The predominant metabolites isolated from soybean leaves were B[*a*]P-1,6-, 3,6- and 6,12-quinones (Sakamoto et al. 1984) and two alcohols, B[*a*]P-9,10-diol and B[*a*]P-9-monool (Negishi et al. 1987). The plants were grown in soil treated with B[*a*]P at a rate of 10 µg/g, then covered with a 3-cm layer of untreated soil. Although covered with uncontaminated soil, B[*a*]P may have evaporated from the soil and redeposited on the aboveground foliage. The authors observed that the resultant metabolites were more characteristic of mammalian degradative monooxygenase systems than of metabolites resulting from microbial degradation of B[*a*]P. Although isolation of B[*a*]P-metabolites from leaf homogenates does not confirm production of metabolites by plants, these data provide strong indirect evidence for oxidative degradation by plants.

Toxicity of PAHs to Plants

Very few studies exist that demonstrate a direct toxicity of PAHs to plants. Forrest et al. (1989) showed B[*a*]P or a metabolite of B[*a*]P enhanced growth of fern gametophytes (*Pteridium aquilinum*). At low doses of B[*a*]P (0.1µg/mL), accelerated growth was observed in fern cells. However, growth inhibition was also observed at doses of B[*a*]P higher than 3.2 µg/mL. In addition to changes

in growth rate, morphological changes were observed. Whether these developmental differences are a result of metabolic activation of B[*a*]P or are produced directly by B[*a*]P was not established.

Although the potential toxicity of PAHs to higher plants has not been thoroughly investigated, a study of 150 greenhouse plants exposed to naphthalene showed marginal sensitivity only when atmospheric concentrations exceeded 13% saturation with naphthalene vapor. After 6 h of exposure, only three species [*Capsicum annuum* var. *conoides*, (pepper); *Fagopyrum esculentum*, (buckwheat); and *Glycine max*, (soybean)] showed signs of foliar injury (Hartzell and Wilcoxon 1930). The remaining 147 species showed no visible effects.

Experiments with aquatic algae have shown some indication of the potential toxicity of naphthalene. Growth inhibition was observed in *Chlorella vulgaris* when exposed to naphthalene and an LD_{50} concentration of 33 mg/kg was extrapolated from the data (Kauss and Hutchinson 1975). After an initial lag period, growth resumed at a rate comparable to that of the control. Microbial contamination of the cultures could have resulted in degradation of the naphthalene with subsequent growth of the algae after loss of the naphthalene. Furthermore, algae are physiologically and metabolically more similar to bacteria than to higher plants; thus, the implications of this study for higher plants remain equivocal.

Given the caveat that relatively few plant species and PAHs have been examined, the lack of evidence for toxicity of PAHs to vegetation and the anecdotal nature of the existing reports of phytotoxicity to whole plants are perhaps the strongest evidence that PAHs do not produce profound toxic effects in vegetation.

D. Plant-Microbial Relationships

The rhizosphere defines a zone of root-influenced soil resulting from the interaction between a plant and the microbial community associated with its roots (Curl and Truelove 1986). Actual physical delineation of the rhizosphere is impeded by its dynamic nature and spatial variability (Bazin et al. 1990) but in general extends from the root surface out into the soil for a few millimeters (Campbell and Greaves 1990). The microbial community in the rhizosphere can be composed of saprophytes, mutualistic and antagonistic symbionts, nematodes, and protozoa (Lynch 1986). Key characteristics of the rhizosphere are the maintenance of microbial biomass far greater than outside the root-influenced zone (Bazin et al. 1990) and in some cases unique species composition from that found in root-free soil (Kloepper et al. 1991).

Considerable quantities of carbon fixed by plants are released into the rhizosphere with estimates of up to 30% of the plant's photosynthate (Lynch 1986). Organic matter released from the roots (rhizodeposits or exudates) comprises a diverse array of compounds and varies among plant species and the developmental stage of the plant. Studies with cereal plants found changes in carbon allocation between shoots and roots during a complete growing season. Young barley and wheat plants allocated 33% and 45%, respectively, of the recovered ^{14}C to belowground tissues (Gregory and Atwell 1991). However, as the plants matured, the proportion of ^{14}C recovered from belowground decreased to 5% in barley and 9% in wheat plants. Exudates also vary with the nutritional status of the plant (Kraffczyk et al. 1984), the location along the length of the root (Whipps 1990), and with environmental variables such as light, temperature, and soil moisture (Rovira and Davey

1974). Examples of rhizodeposits include active secretions of polymeric carbohydrates and enzymes; exudation of sugars, amino acids, hormones, vitamins, and organic acids; lyses of sloughed off root cells; and release of volatile substances such as ethylene and carbon dioxide (Whipps 1990; Lynch and Whipps 1990). The organic material released into the root zone may act as chemical signals between the plant and the rhizosphere microorganisms serving to integrate plant-microbial interactions (Hedges and Messens 1990). One of the most highly studied examples of signal exchanges between microorganisms and the host plant are the chemicals exuded by leguminous plants that attract specific rhizobia (Klein et al. 1990).

Plant-Microbial Signals

Studies of plant-microbial interactions have identified a diverse array of compounds produced by plants and microorganisms that produce beneficial and deleterious effects. Table II - 1 presents a selection of some of the plant-microbial metabolites released into the rhizosphere and their proposed functions. Characterizations of the biological activity of plant root exudates and microbial extracellular products are confounded by *in situ* isolation techniques that may not accurately portray plant-microbial interactions under natural conditions as well as rapid loss of exudates through microbial mineralization (Lynch 1990). Nonetheless, specific plant-microbial signals have been identified. For example, studies of the plant-microbial relationship have identifed the role of root exudates in the induction of genes initiating symbiosis and pathogenesis. Plant-derived flavenoid compounds induce the transcription of host-specific bacterial nodulation genes in microorganisms. These chemical signals are critical to the symbiotic legume-rhizobia interaction (Spaink 1992).

Table II - 1 . Plant and microbial metabolites released in the rhizosphere and their proposed functions.

Extracellular Metabolites		Proposed Function
Microbial Metabolites		
	Lipo-polysaccharides	Induce the release of flavenoid compounds by plants, which inturn stimulate morphological changes in roots and increase infection of root by rhizobia (Spaink 1992).
	Siderophores	Iron chelating substances that reduce its availablity for deliterious microorganisms such as root pathogens (Kloepper et al. 1980).
	Polysaccharides	Stabilize soil aggregates beneficial for plant growth (Lynch 1990).
	Lectins and Agglutins	Involved in root attachment of microorganisms (Lynch and Whipps 1990).
	HCN	Induction of plant synthesis of phytoalexins. Suppression of black root rot (Voisard et al. 1989).

Table II - 1. cont'd.

Extracellular Metabolites		Proposed Function
Plant Metabolites		
	Flavenoids	Phenolic compounds which stimulate microbial expression of *nod* genes required for nodulation (Redmond et al. 1986).
		Growth enhancement of *Rhizobia* (Dharmatilake and Bauer 1992).
		Chemoattractants leading to the infectible zone on the root (Dharmatilake and Bauer 1992).
		Induce expression of virulence genes in *Agrobacterium tumefaciens* (Ahmadjian and Paracer 1986).
	Root Cap Cells	Exhibit host specific chemotaxis (Hawes 1990).
		Carbon source for microbial metabolism (Campbell 1985).
	Phytoalexins	Plant defense compounds against phytopathogenic microorganisms (Darvill and Albersheim 1984).
	Salicylic Acid	Chemical signal that induce resistance in tobacco plants to the tobacco mosiac virus (Malamy et al. 1990) and generalized systemic acquired resistance in cucumber plants (Métraux et al. 1990).

A high degree of co-adaptation between plants and their associated microorganism is implied by species specific compounds and variability with the developmental stage of the plant (reviewed in Fisher and Long 1992).

Microbial mediated changes have also been identified in the establishment of legume-rhizobia mutualistic relationships. Release of rhizobial lipo-oligosaccharides induces morphological changes in root structure and the development of nodules (Ahmadjian and Paracer 1986) as well as induction of pre-infection threads (the means by which bacteria enter the root nodule) (Spaink 1992). A further example of plant-induced gene expression in microorganisms, albeit not beneficial, is phenolic root exudate induction of deleterious virulent genes in the plant pathogen *Agrobacterium tumefaciens* (Ahmadjian and Paracer 1986).

Rhizosphere Microorganisms and Plant Stress

Studies of selected plant species subjected to environmental stresses, such as herbivory and microbial root infection, reveal some unique defense mechanisms involving physiologically mediated changes in the root zone. Many of these defense mechanisms are initiated by the exudation of chemical cues from plant roots. While specific studies of the effect of chemical toxicants on plant-microbial relationships are few, studies of the effect of air pollution on plant survival have subsequently considered plant-microbial interactions. A recent review on the impact of two such pollutants, ozone (O_3) and sulfur dioxide (SO_2), showed a wide range of symptoms and responses in plants and root-inhabiting fungi (mycorrhizae) to atmospheric chemical stress (McCool 1988). In general, plants with mycorrhizal associations are more resistant to atmospheric chemical stress than plants of the same species with a

compromised or absent mycorrhizal association. Garrett et al. (1982) determined the effect of O_3 and SO_2 on oxygen uptake by isolated mycorrhizal fungi (mycelial mats), and mycorrhizal and nonmycorrhizal root sections of loblolly pine, *Pinus taeda.* For all treatments examined, oxygen uptake was reduced when compared to control treatments not exposed to the air pollutants. However, nonmycorrhizal root segments and the individual mycelial mats exhibited a far greater reduction in oxygen uptake than the mycorrhizal root segments (*i.e.*, the root with the mycorrhizal fungi). In essence, the root and the mycorrhizal fungi were less susceptible to the damaging effects of the pollutants when in association. This positive benefit of the root-fungal association suggests a protective aspect to this relationship.

Another protective advantage provided to the plant by rhizosphere organisms is the inhibition of deleterious phytopathogenic microorganisms. For example, rhizosphere bacteria of the genus *Pseudomonas* suppress deleterious soil microorganisms such as *Gaeumannomyces graminis* causing "take all" disease in wheat and *Thielaviopsis basicola* affecting tobacco plants (Schippers et al. 1990). Suppression of plant pathogens is believed to involve an array of potential actions by *Pseudomonas* including the synthesis of inhibitory compounds such as antibiotics (Weller 1988) and cyanide (Voisard et al. 1989). While cyanide affects plant root morphology (Défago et al. 1990; reviewed in Schippers et al. 1990) and is toxic to the plant's cytochrome oxidase respiratory system, cyanide also appears to initiate induction of plant production of phytoalexins, which are deleterious to plant pathogens (Défago and Haas 1990). In addition, production of iron-chelating siderophores by rhizosphere microorganisms restricts the availability of iron and suppresses some plant pathogens (Kloepper et al. 1980).

In contrast to the plant defense response stimulated or executed by *Pseudomonas* species, Kloepper et al. (1991) found evidence supporting the plant's role in stimulating a microbial defense response. Analyses of several plant species that exhibit resistance towards a pathogenic nematode found distinct rhizosphere communities compared to less resistant plant species. The authors propose that resistance may involve the plant's selective stimulation of rhizosphere community members, which are in turn antagonistic towards the pathogen.

Perhaps one of the best examples known of a protective role played by microorganisms was found in a study of rice rhizospheres (Joshi and Hollis 1977). Isolated from water-saturated rice fields, the bacterium *Beggiatoa* was found to oxidize hydrogen sulfide, a gas that inhibits oxygen release by rice plants and causes physiological abnormalities in the plants. In the presence of *Beggiatoa*, however, oxygen release was significantly greater than in the absence of this rhizosphere member, suggesting a unique mutually beneficial relationship between the rice plant and the bacterium.

The results of these studies support the concept of plant-microbial interactions in the root zone that extends beyond increased nutrient availability to include complex ecological interactions during times of environmental stress.

CHAPTER III

MATERIALS AND METHODS

A. Field Site and Sample Collection

Site Description

The field site selected for plant uptake studies was a manufactured gas plant (MGP) site in the northeastern United States designated by the Electric Power Research Institute (EPRI), Palo Alto,CA, as EBOS-24 (Environmental Behavior of Organic Substances). This MGP coal tar disposal site was established approximately 25-30 years ago as a disposal pit estimated to have been about 9.2 by 30.5 m.

Results of extensive field investigations carried out by the parent utility company and EPRI identified a plume of naphthalene moving away from the source material, surfacing in a lower, seepy area. Naphthalene, a relatively water-soluble PAH, appeared to be moving with the groundwater in an advective manner and was found in groundwater wells along the entire length of the site. Higher molecular weight compounds did not appear to move as fast, nor as far, as naphthalene nor has movement of PAHs other than naphthalene away from the source area been found.

Recent characterization of microbial patterns at EBOS-24 (Madsen et al. 1991) suggests local microbial adaptations to PAHs. Mineralization of naphthalene and phenanthrene was observed in samples collected from contaminated aquifer boreholes but not from pristine zones adjacent to the source.

Vegetation Samples

An herbaceous plant species, *Melilotus alba* Desr. (Leguminosae), white sweetclover, was selected for uptake studies because of its abundance at the EBOS-24 site and its utilization by wildlife. In addition, *M. alba* has a size and morphology especially suitable to laboratory studies. *M. alba* is an annual that grows singly or in clumps. The plant has a distinct stem, trifoliate leaves, and a tap root. Mature plants were collected at EBOS-24 in May, 1991, transported to Oak Ridge National Laboratory (ORNL), and maintained in a greenhouse. Seeds produced by these plants were collected, stored at 0-5°C, and used to germinate seedlings of uniform ages and sizes for the PAH uptake studies. Young, rapidly growing plants were favored for these studies to maximize uptake potential of the PAHs by the plants. In order to obtain sufficient numbers of *M. alba* plants for experimental use, seeds were also obtained from a commercial supplier (Tennessee Farmer's Cooperative, Lavergne, TN) and raised under the same protocol.

Soil Collection and Analysis

Soil was collected near the source material at EBOS-24 from a minimally disturbed area (May 1991). After the vegetation was removed from the surface, soil was collected from two depths (0-18 and 25-28 cm), transferred to sterile plastic bags (Whirl-pak™), and transported to ORNL where the soil was sieved to 2 mm and stored in the dark at 5°C until use. The soil from EBOS-24 area is classified by the United States Department of Agriculture as typic Udipsamments, mixed, mesic. This is indicative of young sandy soils that are well drained and have low organic matter. Physically, the soil has a dark gray-brown, loamy sand layer in the top several centimeters and a strong brown to

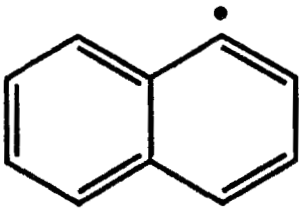
yellowish brown loamy sand substratum. A composite soil made up of soil fractions from the two depths was mixed for use in uptake studies. This composite provided a soil with low organic carbon content, thus reducing PAH adsorption by the soil, and increasing the availability of the compound for root uptake.

Selected physical and chemical properties of the composite soil from EBOS-24 were characterized. Carbon analyses were done in quadruplicate on a LECO Carbon Determinator (St. Joseph, MI). Soil pH measurements were conducted on triplicate soil samples in distilled water with air-dried soil at a soil-to-water ratio of 1 g:5 mL. Soil water holding capacity of the composite soil was determined with 6 replicates using a ceramic plate extractor (Soil Moisture Equipment Co., Santa Barbara, CA) at 1/3 bar. Soil particle size distribution was determined by the bouyoucos hydrometer method (adapted from Day 1965). Further characterization of the composite soil included determination of total nitrogen (N), phosphorus (P), and sulfur (S) by the Analytical Chemistry Division of ORNL.

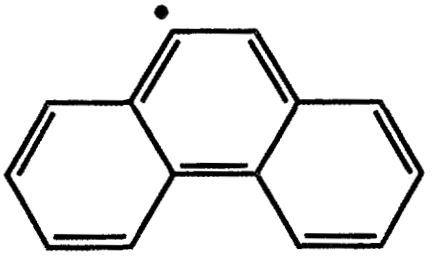
B. Uptake and Translocation of ^{14}C-Labeled PAHs by *Melilotus alba*

Test Chemicals

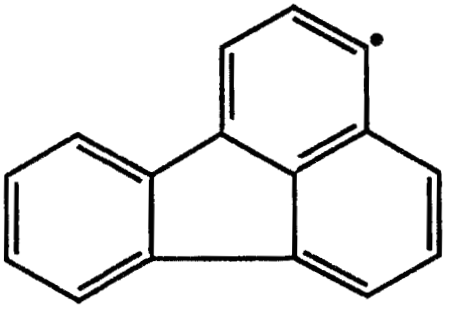
Radiolabeled PAHs were purchased from Sigma Chemical Co. (St. Louis, MO) with radiochemical purity $\geq$ 97%. Four PAHs, [1-^{14}C]naphthalene (obtained from D. Taylor, ORNL) (specific activity 318 x 10^{10} Bq kg^{-1}), [9-^{14}C]phenanthrene (specific activity 270 x 10^{10} Bq kg^{-1}), [4,5,9,10-^{14}C]pyrene (specific activity 101 x 10^{11} Bq kg^{-1}), and [3-^{14}C]fluoranthene (specific activity 101 x 10^{11} Bq kg^{-1}) (Figure III - 1), were selected for plant uptake studies based upon relevance to the MGP study site, and commercial availability and cost of



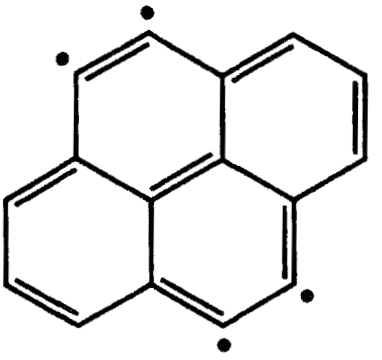
Naphthalene (91-20-3)



Phenanthrene (85-01-8)



Fluoranthene (206-44-0)



Pyrene (129-00-0)

Figure III - 1. Structures of the test compounds, (including Chemical Abstract Numbers), used for PAH uptake studies with *Melilotus alba*. Location of ^{14}C is indicated by •.

^{14}C-compounds. All four PAHs selected are commonly found at MGP sites (phenanthrene, fluoranthene, and pyrene are reported in highest concentrations from EBOS-24 soil, whereas naphthalene is present in the source material) (J. W. Goodrich-Mahoney, EPRI, personal communication). Selected physicochemical properties of the four PAHs are presented in Table III - 1.

Nonradiolabeled PAHs were purchased from Aldrich Chemical Co., Milwaukee, WI (fluoranthene), J.T. Baker Inc., Phillipsburg, NJ (naphthalene), and Sigma Chemical Co., St. Louis, MO (phenanthrene and pyrene). All organic solvents were reagent grade and >99% pure.

Uptake of ^{14}C-PAHs

Prior to initiating uptake experiments, specimens of *M. alba* of approximately the same age (2 months) and size were transferred to uptake flasks to acclimate in a walk-in environmental chamber for a minimum of 7 days. Thus, for the vegetated treatments, ^{14}C-PAHs were applied to soil containing a plant with an established root mass. Otherwise, vegetated, nonvegetated, and sterile controls were treated the same.

Four ^{14}C-PAHs were applied individually to (60-75 g) soil (250 or 500 $\mu g \cdot g^{-1}$) to determine plant uptake and distribution of ^{14}C. ^{14}C-PAHs were dissolved in a minimum toluene volume and applied to soil in vegetated (composite soil and plant), nonvegetated (composite soil, no plant), and sterile treatments (composite soil autoclaved for 1 h over 2 consecutive days, no plant).

The distribution of ^{14}C among root, stem, and leaf tissues was determined as well as ^{14}C-residue concentrations in soils and flask headspace.

Table III - 1. Physicochemical properties of selected polycyclic aromatic hydrocarbons.

PAH	Molecular weight[a]	Aqueous solubility mg/L[c]	Boiling point °C	Melting point °C	Vapor pressure mm Hg @20°C[e]	Octanol/water partition coeff. log K_{ow}	Henry's Law constant
Naphthalene	128.16	30[d]	217.9[a]	80.2[a]	4.92E-2	3.01-3.59[f] 3.37[e]	3.0±0.8E-2[j] 2.0E-02[k]
Phenanthrene	178.22	1.29	340[b]	101[b]	6.8E-4	4.57[g] 4.46[e]	9.6±0.9E-4[j] 1.6E-03[k]
Fluoranthene	202.26[b]	0.2-0.26	375[b]	111[b]	6.0E-6	4.90[h]	4.0E-02[k]
Pyrene	202.24	0.135	393[b]	156[a]	6.85E-7	4.88[i]	2.8E-01[k]

[a]The Merck Index 1989
[b]Weast 1986-87
[c]Mackay and Shiu 1977
[d]Worthing and Walker 1983
[e]Sims and Overcash 1983
[f]Hansch and Leo1979
[g]Karickhoff 1981
[h]USEPA 1980
[i]Hansch and Leo 1984
[j]Fendinger and Glotfelty 1990
[k]Ryan et al. 1988

A flow diagram summarizing plant tissue, soil, and headspace analyses for ^{14}C is presented in Figure III - 2. A detailed description of the experimental procedure and ^{14}C analyses follows.

Experimental Procedures

The ^{14}C-PAH was added to soil in specially fabricated 125-mL Erlenmeyer flasks with glass tops (Figure III - 3). Stainless steel sampling portals in the flask tops allowed the flask headspace to be flushed to maintain aerobic conditions and to trap any volatilized ^{14}C. The vegetated flasks had ground-glass joints and glass tops with openings for the plant stem that were sealed with a nonreactive silicone sealant (General Electric Co., Waterford, NY) (Stotsky et al. 1961; Hsu and Bartha 1979). All flasks were wrapped with Parafilm® and white vinyl tape (Scotch® Brand Tapes, St. Paul, MN) to further ensure an air-tight seal and to minimize the amount of light reaching the soil. This prevented possible algal growth or photooxidation of the PAHs as well as reduced absorption of $^{14}CO_2$ by stem tissue during photosynthesis.

Flasks were kept in a glass exposure chamber (Jen et al., in review) (Figure III - 4) housed in a walk-in environmental chamber. The chamber was programmed for a 14-h photoperiod; diurnal temperatures in the chamber were 32/22 °C. Ambient CO_2 level was 370 $\mu L \bullet L^{-1}$. Glass exposure chambers were kept under negative pressure with exiting air passing through a charcoal filter to trap volatile ^{14}C-compounds released to the atmosphere from the aboveground foliage.

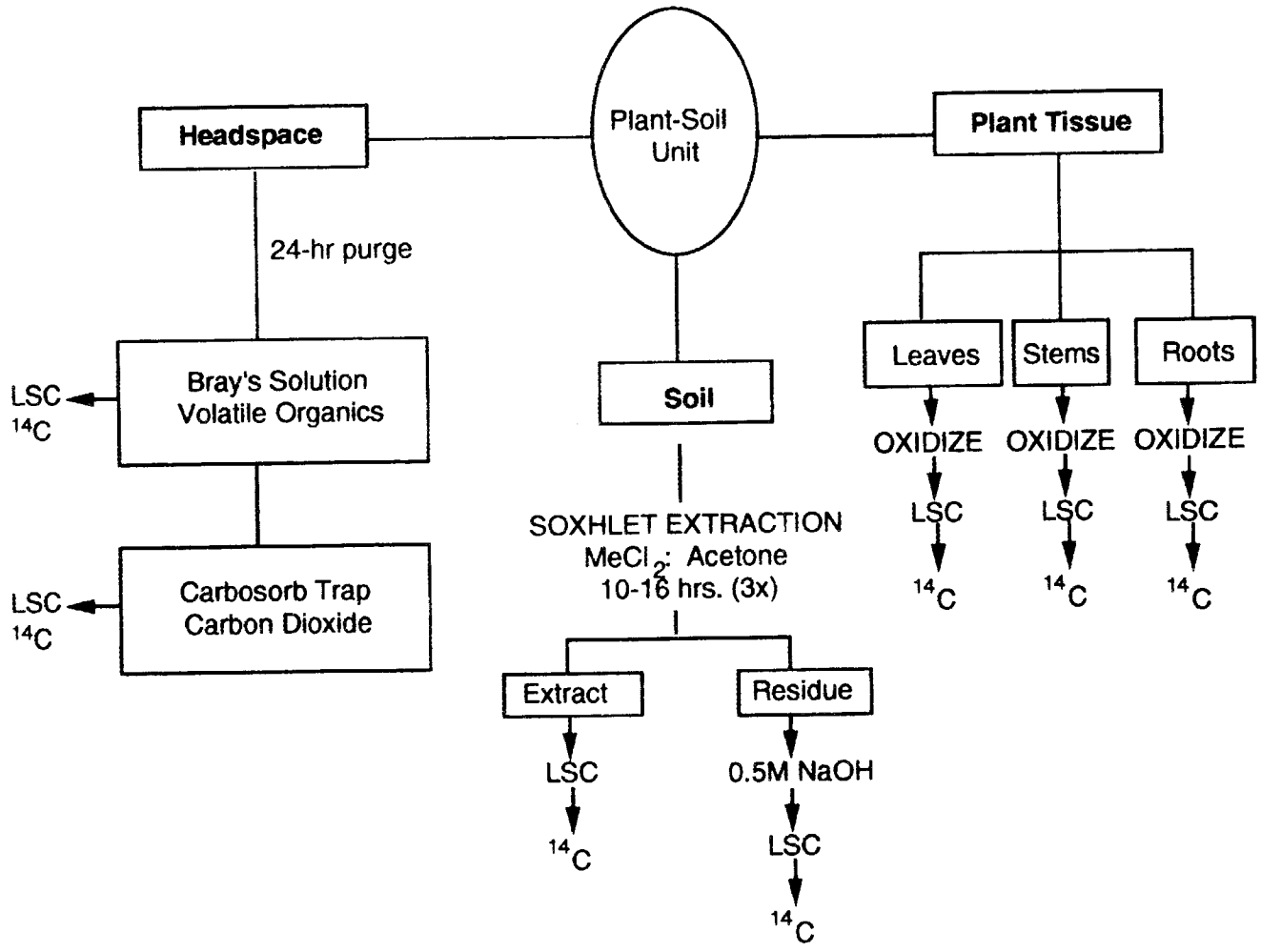


Figure III - 2. Summary diagram of soil, plant tissue, and flask headspace analyses for ^{14}C-residues after 5-day exposure to individual ^{14}C-polycyclic aromatic hydrocarbons.

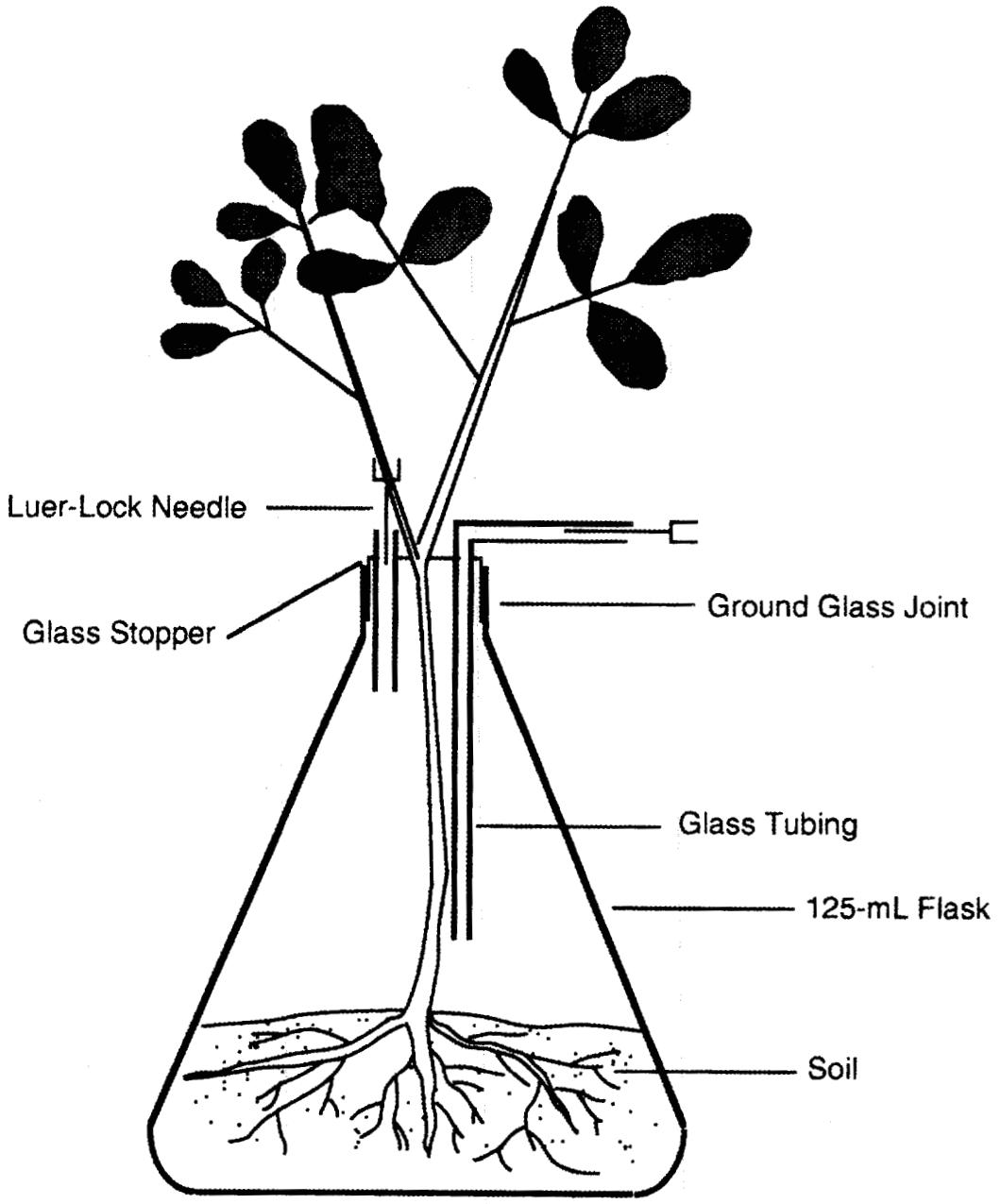


Figure III - 3. Experimental apparatus used to determine plant uptake and fate of ^{14}C-polycyclic aromatic hydrocarbons applied to soil and ^{14}C-allocation in *Melilotus alba*. Aboveground plant tissue was separated from roots and contaminated soil by a silicone sealant. Sampling portals allowed analysis of the headspace for ^{14}C-volatile organics and $^{14}CO_2$ (after Anderson 1991).

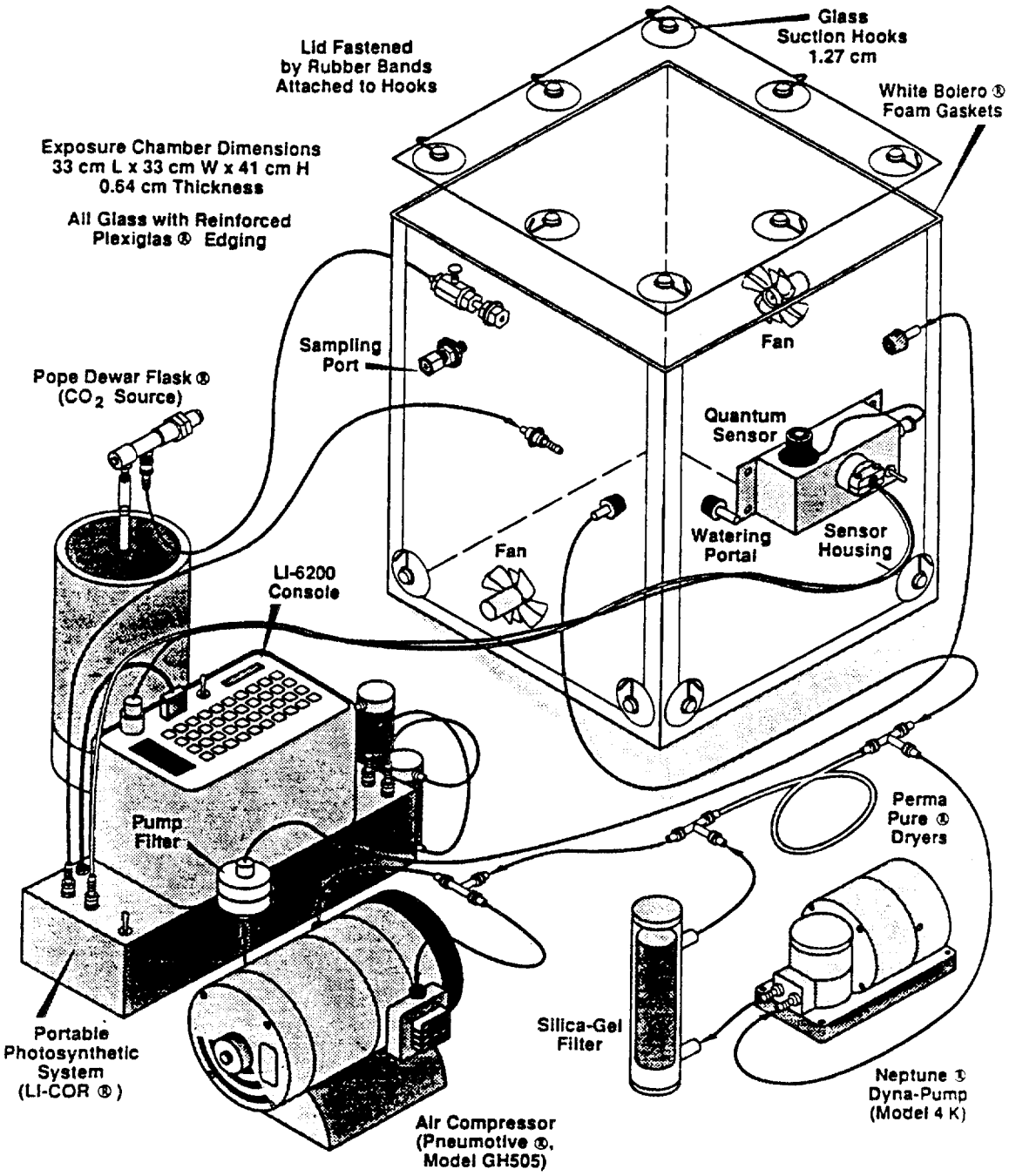


Figure III - 4. Glass environmental exposure chambers used to house individual plant-soil-flask units during uptake studies with ^{14}C-polycyclic aromatic hydrocarbons, ^{14}C-allocation studies, and to measure ^{14}C-volatiles released into the chamber atmosphere (Jen et al., in review).

Headspace Analyses

The air in the flasks (the headspace above the soil) was flushed every 24 h to maintain aerobic conditions and to recover ^{14}C-containing compounds released from the soil. Exit air was first bubbled through two sequential traps containing modified Bray's Solution (Bray 1960) to trap ^{14}C-parent and organic metabolites. A third trap contained a CO_2-trapping scintillation cocktail (Carbosorb). Bray's Solution is a scintillation cocktail based on *p*-dioxane (100 mL ethylene glycol monomethyl ether, 5 g 2,5-diphenyloxazole, 0.3 g 1,4-bis-2-[5-phenyloxazoly]-benzene, 50 g naphthalene, and dioxane to make 1L), whereas Carbosorb is an alkaline scintillation cocktail (330 mL phenethylamine, 50 mL distilled water, 400 mL toluene, 220 mL methanol, 5.0 g 2,5-diphenyloxazole, 0.3 g 1,4-bis-2-[5-phenyloxazolyl]-benzene). Radioactivity in the traps was determined using a liquid scintillation spectrophotometer (Packard Model 2000CA).

Trap Calibration

Efficiency of the Bray's Solution trap was determined using ^{14}C-naphthalene and specially designed glass gas-washing bottles. Regulated air flow was passed through a series of three gas-washing bottles for 30 minutes. The first bottle contained ^{14}C-naphthalene in distilled water and the final two contained Bray's Solution. Two charcoal traps were included in line after the third gas-washing bottle to determine the radioactivity exiting the system and not trapped by the cocktail. Bottles were connected by glass tubing with sleeves of tygon™ tubing holding abutting ends together. The Bray's Solution was effective at trapping 80% of the radioactivity passed through the solution.

In addition, tests were conducted to determine if Bray's Solution would retain $^{14}CO_2$. ^{14}C-methyl methacrylate standards were combusted as described for plant tissue analysis. However, instead of adding the CO_2 trapping solution, Carbosorb, Bray's Solution was added to the flasks. After 2 h the sample was analyzed for radioactivity. Only 3% of the radioactivity was recovered. Thus, Bray's Solution did not retain $^{14}CO_2$.

Efficiency studies with Carbosorb in an identical experimental setup (Anderson 1991) showed that 70% of the $^{14}CO_2$ released from combustion of the standard ^{14}C-methyl methacrylate was trapped by Carbosorb. Therefore, data reported for $^{14}CO_2$ in this study were corrected by this efficiency value.

Soil Extraction

Soils were extracted with methylene chloride and acetone using a soxhlet extraction apparatus and fritted glass extraction thimbles. At the conclusion of the experiments, acetone and methylene chloride (2:1, approximately 112 mL total volume) were added to the soil to aid in separating the roots from the soil. After removal of the plant material, the soils plus 2 consecutive washes with solvents were transferred to polytetrafluoroethylene-lined screw top jars. Soils from each completed experiment were stored at 0°C.

To extract the soil, the solvent stored with the soil sample was poured off into round bottom flasks after centrifugation. Fresh solvents (methylene chloride and acetone) were added to yield a consistent total volume (180 mL). The soil sample was mixed thoroughly before a 30-g portion was added on top of a small plug of glass wool in a fritted glass extraction thimble. Glass wool aids in keeping the fritted surface in the extraction thimble free of soil debris. After approximately 10-16 hours of extraction with an average of 2 cycles/h, a 200-µL

sample of the solvent was counted in Aquasol® (New England Nuclear, Boston, MA) scintillation cocktail using a liquid scintillation spectrophotometer. The solvent volume was determined and fresh solvent added to the flasks to continue soxhlet extraction. A minimum of 2 16-h extractions were conducted. An aliquot of the soil sample remaining after soxhlet extraction was further extracted with a 0.5 M NaOH alkaline solution to determine the remaining radioactivity associated with the soil organic matter.

At the conclusion of the uptake experiment, charcoal traps located on the exit tubing of the exposure chambers were extracted with carbon disulfide for 24 h. A 200-µL sample was counted in Aquasol® scintillation cocktail.

Plant Tissue Analysis

At the conclusion of the uptake studies, plant tissues from each experiment were separated into roots, stems, and leaves, then weighed and stored at -5°C until combustion using a Thomas®-Ogg Infrared Ignitor (Thomas Scientific, Swedesboro, NJ). A known weight of plant tissue was wrapped in black paper sample wrappers, then suspended in platinum baskets in oxygen combustion flasks. After combustion of the tissue, a CO_2 trapping scintillation cocktail was added to the oxygen flasks for a minimum of 2 h. The sample was then transferred to a scintillation vial and radioactivity determined by liquid scintillation spectroscopy.

Mass Balance Calculations

Total recovered radioactivity (disintegrations per minute, DPMs) for each replicate was summed from ^{14}C detected in plant tissues, soil extractions, and trapping solutions for volatile organics and CO_2. Plant tissue analyses and soil

extractions were, in most cases, conducted on a subsample of material with a known weight. Values for total DPMs • g^{-1} of plant tissue and soil were extrapolated from subsample values and total weight of the material determined at the conclusion of the exposure period. The trapping solutions were analyzed directly without subsampling.

Percent recoveries were calculated from total DPMs recovered in each replicate and the amount of radioactivity initially added to the soil. To determine the amount of radioactivity added to the soil, an aliquot of the stock ^{14}C-PAH solution (1-10 µl) was added to Aquasol® scintillation cocktail and radioactivity was determined by liquid scintillation spectroscopy.

C. ^{14}Carbon Allocation in *Melilotus alba*

Experiments were conducted to determine plant carbon allocation in the presence of phenanthrene in the root zone. Plants were exposed to $^{14}CO_2$ and transport of ^{14}C-photosynthates and root exudation of labeled compounds into the rhizosphere were determined. Environmental and experimental conditions were similar to those in previously described PAH-uptake studies (e.g., plant species, PAH concentration) and are detailed below. However, soil for this phase of the research was collected from a local deciduous forest and was used to grow *M. alba* seedlings prior to transplanting to experimental flasks. Plants were transferred with rhizosphere soil (soil adhering to the root surface) to sterile sand dosed with phenanthrene.

Soil Collection

Soil was collected in March 1993 from the Walker Branch Watershed research area at ORNL. Leaf litter and undecomposed organic material (O

horizon) were scraped away and soil collected from 0-5 cm in what was visually distinguished as the A horizon. This soil was selected because of the presence of naphthalene degrading soil microorganisms in samples collected from the same general area (within 3 m) (J. Strong-Gunderson,ORNL, personal communication). Soil was air dried overnight at room temperature then passed through a 2-mm soil seive and stored at 5 °C. Selected soil characterizations were previously determined by Jardine et al. (1989) who found the particle size distribution to be 58.9 % silt, 34.9 % sand, and 6.2 % clay and an organic carbon content of 3.5 %.

Seedling Germination and Growth

Seedlings of *M. alba* were germinated in flats of standard potting soil (Pro-Mix, Premier Brands, Inc., Rochelle, NY) and housed in a walk-in environmental growth chamber. After approximately 5 weeks, seedlings were transplanted to 20-40 g of Walker Branch Watershed (WBW) soil in 30-100 mL beakers. This stimulated the development of a compact root mass which facilitated transfer of the plant to 250-mL flasks (Figure III-3).

Transplants established an extensive root mass within 14 days and were transferred to 250-mL flasks for carbon allocation studies. In order to limit sorption of phenanthrene to soil organic matter, sifted, autoclaved sand (Palmix® Inc., Toledo, OH) was used as the growth media for phenanthrene exposure. Plants were removed from the beakers with most of the root mass and soil in tact. The soil was shaken off the roots leaving only tightly adhering soil on the roots of the plants. Plants were transferred aseptically to flasks containing 70-g of sterile sand previously dosed with 250 µg phenanthrene/g sand (described below). Exposure to phenanthrene began 6-7 days prior to

labeling with $^{14}CO_2$ in order to acclimate the plants and allow new root growth into the dosed sand medium. Control plants were included in all experiments and were processed the same as phenanthrene-exposed plants but no phenanthrene was added to the sand. A minimum of three plants were included for each treatment (phenanthrene exposed plants and control plants).

Phenanthrene Application

A method for dosing the sterile sand was developed to provide a uniform distribution of the PAH among replicates. Depending on the volume of dosed sand required, phenanthrene was mixed with a subsample of the total volume of sand using a sterile mortar and pestle. Grinding the phenanthrene and sand facilitated the distribution of the solid PAH without the introduction of a solvent. This sample of dosed sand was then diluted with the remaining sand in a glass jar by vigorously shaking the mixture for even distribution.

$^{14}CO_2$ Exposure and Chase Period

Plants were transferred to air-tight glass exposure chambers (Figure III-4) and housed in a walk-in environmental chamber. $^{14}CO_2$ was released into the chambers with a specially designed dispensing unit equipped with a gas cylinder of $^{14}CO_2$ (obtained from N. T. Edwards, ORNL, specific activity 19.8 mCi/mL, 358 ppm CO_2, balanced with air), timer, flow meter, CO_2 trapping system, and circulating pump (Cole Parmer, Chicago, IL). Inlet and outlet hoses were hooked up to the glass exposure chambers through polytetrafluoroethylene covered stoppers. $^{14}CO_2$ was released into the chamber for 5 minute (14.0 psi) followed by a 10-minute circulation period. At the end of the circulation period, fresh air was pulled into the glass exposure

chamber as exiting air from the chamber was passed through two consecutive traps of sodium bicarbonate (EM Science, Cherry Hill, NJ). This tagging period was then followed by a chase period of 6 days during which the plants remained in the exposure chambers and the headspace of the flasks were sampled every 24 h. Headspace air was pumped through a trapping system as described earlier for PAH uptake studies. The first trap contained Aquasol® scintillation cocktail to trap volatile compounds other than CO_2, while the second contained the alkaline based CO_2 trapping cocktail, Carbosorb.

Plant Tissue Analysis

At the end of the 6-day chase period, plant shoots (leaves and stems) were cut off, transferred to paper bags, and dried to a constant weight at 70°C in a forced-air convection oven (Blue M, Blue Island, IL) for approximately 9 days. Roots and lower stem tissue (not tagged with $^{14}CO_2$) were washed free of sand *in toto* with 40 mL of methanol before removal from the flasks in order to reduce root loss. The methanol was then used to extract ^{14}C-residues from the sand as described below. Roots and lower stem were then transferred to the drying oven and dried to a constant weight. ^{14}C-residues in plant tissue were determined using a Thomas®-Ogg Infrared Ignitor and liquid scintillation spectroscopy.

Sand Extraction

A modified Bligh-Dyer extraction procedure was used to extract ^{14}C-residues from the sand (Bligh and Dyer 1959) at the end of the chase period. Methanol and chloroform (2.7:1, v/v) were added to the experimental flasks, extracted for at least 2 hours, transferred to 110-mL glass jars with

polytetrafluoroethylene-lined lids, and left overnight. The sample bottles were centrifuged at 2000 rpm for 10 minutes and 10 mL of the supernatant was transferred to glass scintillation vials. Chloroform and water (10 mL, 1:1, v/v) were added, samples agitated, and allowed to separate into a biphasic system overnight. Subsamples of each phase were counted using liquid scintillation spectroscopy.

D. Soil Respiration and Microbial Biomass

Soil Respiration

Total CO_2 evolution from both root and microbial respiration (*i.e.*, soil respiration) in the presence of phenanthrene was determined using an infrared gas analyzer (LIRA® Model 3000, Mine Safety Appliances Co., Pittsburgh, PA). Seedlings of *M. alba* were transplanted from beakers of WBW soil to 110-mL wide-mouth glass jars containing 50 g of sterile, phenanthrene-dosed sand. Analysis of sample headspace for CO_2 was adapted from Edwards (1982). Hard rubber stoppers with twist-cock ports for headspace sampling were modified to include an additional opening for a plant stem. Plant stems were sealed from roots and lower stem with silicon sealant.

Plants (with and without phenanthrene) and sterile control jars (sand with and without phenanthrene) were incubated in a walk-in environmental chamber and sampled at 24-h intervals. Vegetated samples were flushed with a stream of CO_2 free air to remove all CO_2 that had accumulated in the headspace over the 24-h period. The samples were then re-sampled after 15 minutes to obtain mg CO_2/g sand • 15 minutes. Nonvegetated samples were sampled directly without the initial headspace purging.

Heterotrophic Plate Counts

Heterotrophic plate counts of culturable microorganisms were obtained from carbon allocation and respiration experiments at the end of the exposure periods. A 1-g aliquot of sand (wet weight) was removed aseptically from the flasks or sample bottles after removal of the plant or directly from the nonvegetated and sterile treatments and placed in sterile water (9 mL). Serial dilutions were performed on the sample and at least two dilutions plated on Tryptic Soy Agar (TSA) (Difco, Detroit, MI) plates using the spread-plate technique (Johnson and Case, 1992). Plates were inverted and incubated at 23 °C. An automated bacteria colony counter (Model 500A, Spiral System Instruments, Inc., Bethesda, MD) and computer system (CASBA II, Version BEN 4.2, Spiral Biotech, Inc., Bethesda, MD) were used to determine colony forming units (CFUs) at 24 and 36 hs. Dry weights of sand were determined and all data is expressed as CFUs per gram dry weight of sand. Only dilutions with 30-300 colonies were used with preference for the lower dilution. Plates were visually inspected to verify counts obtained from the automated counter and to describe colonies present.

CHAPTER IV

RESULTS

A. Field Site and Sample Collection

Composite Test Soil Description

The composite test soil from the EBOS-24 field site was dominated by sand (94.5%) with 3.3 % and 2.2% concentrations of silt and clay, respectively (Table IV - 1). This is consistent with the USDA soil description of this area and with glaciofluvial geology. The composite soil had a low organic carbon content, <1%, and a pH near neutrality (6.98). No additional nutrients were added to the soil or composite mixture used for uptake studies.

Vegetation

Melilotus alba seedlings germinated from seeds from plants collected at EBOS-24 and from a commercial source worked well under laboratory conditions. Seedlings reached an ideal size (approximately 30 cm) for use in the laboratory in approximately 2 months. Although a great deal of handling of the plants was necessary for each experiment, the seedlings were resilient and hardy. Root surfaces exhibited pinkish nodules indicative of the presence of nitrogen fixing bacteria associated with the sweetclover genus *Melilotus*.

B. Uptake and Translocation of ^{14}C-Labeled PAHs by *Melilotus alba*

Plant tissue concentration of ^{14}C-PAHs and ^{14}C-metabolic products varied with the specific PAH and tissue type. However, for all tested PAHs, the highest concentration of ^{14}C was found associated with root tissue with

Table IV - 1. Selected physical and chemical properties of the composite test soil from EBOS-24.

Parameter	Value
Sand (%)	94.5
Silt (%)	3.3
Clay (%)	2.2
Organic Carbon (%)	0.93 ± 0.04
Field Capacity[a] (mg/g)	2.37 ± 0.16
Nitrogen[b](%)	0.066
Sulfur (μg/g)	60
Phosphorus (μg/g)	598
pH	6.98 ± 0.16

[a] Gravimetric water content at 1/3 bar, ml/g

[b] Total Kjeldahl nitrogen

decreasing concentrations translocated to stem and leaf tissues (Table IV - 2 and Figures IV - 1 through IV - 8). This difference was significantly different in both fluoranthene experiments and in pyrene experiment #2 (Student's t-test, paired comparison, $p \leq 0.05$).

Differences in root tissue concentrations and translocation of the labeled material from the root tissue to the aboveground tissue were observed between test compounds. Plants exposed to ^{14}C-fluoranthene had the lowest root ^{14}C concentrations relative to the other PAHs tested (Figure IV - 8) and exhibited the lowest translocation of ^{14}C to aboveground plant tissues. Plants exposed to ^{14}C-naphthalene had the highest root tissue ^{14}C concentrations and the greatest translocation of ^{14}C to aboveground plant tissues.

For all PAHs tested the greatest percentage of ^{14}C ($\geq$ 86 % of total recovered radioactivity) remained in the soil after 5 days and was recovered during soxhlet extraction (Figure IV - 9 through IV - 16). No significant differences were observed between treatments (vegetated, nonvegetated, and sterile) for this compartment (Student's t-test, $p > 0.05$). Alkaline extraction for soil ^{14}C-residues after soxhlet extraction recovered only an additional $\leq$1.6% with vegetated naphthalene treatments having the highest concentration in this compartment (Table IV - 3).

Concentrations of ^{14}C-PAH in plant tissues and soil were estimated from the specific activity and molecular weight of each ^{14}C-PAH (Table IV - 4). For these calculations, all ^{14}C was assumed to be present as unaltered parent compound, an assumption that was probably not valid. Bioaccumulation factors (µg PAH/g plant tissue/µg/g soil) were <1 for all tested PAHs indicating that bioaccumulation of PAHs from soil to plant tissue did not occur. BCFs ranged from 0.011 ± 0.0083 for fluoranthene to 0.039 ± 0.02 for naphthalene. However,

Table IV - 2. Distribution of ^{14}C-PAH derived radioactivity in *Melilotus alba* after 5 days of exposure.

PAH (250 µg/g)	n	Total $^{14}C^{a}$		
		Leaf	Stem	Root
Naphthalene #1	3	0.014 ± 0.007	0.14 ± 0.071	2.35 ± 1.34
Naphthalene #2	3	0.23 ± 0.12	0.26 ± 0.15	3.2 ± 1.2
Phenanthrene #1[b]	2	0.065	0.035	2.8
Phenanthrene #2	3	0.002 ± 0.001	$0.005 \pm 2.3 \times 10^{-4}$	0.82 ± 0.58
Pyrene #1	2	0.001	0.004	3.2
Pyrene #2	3	0.005 ± 0.003	0.007 ± 0.005	0.024 ± 0.006
Fluoranthene #1	3	$2.0 \times 10^{-4} \pm 1.2 \times 10^{-4}$	$4.4 \times 10^{-4} \pm 3.8 \times 10^{-4}$	1.7 ± 0.44
Fluoranthene #2	3	$1.5 \times 10^{-4} \pm 4.6 \times 10^{-5}$	$8.8 \times 10^{-5} \pm 3.4 \times 10^{-5}$	0.006 ± 0.002

[a] Expressed as a mean percent of the total recovered ^{14}C ± one standard error.

[b] Total concentration of Phenanthrene #1 added to soil was 500 µg/g.

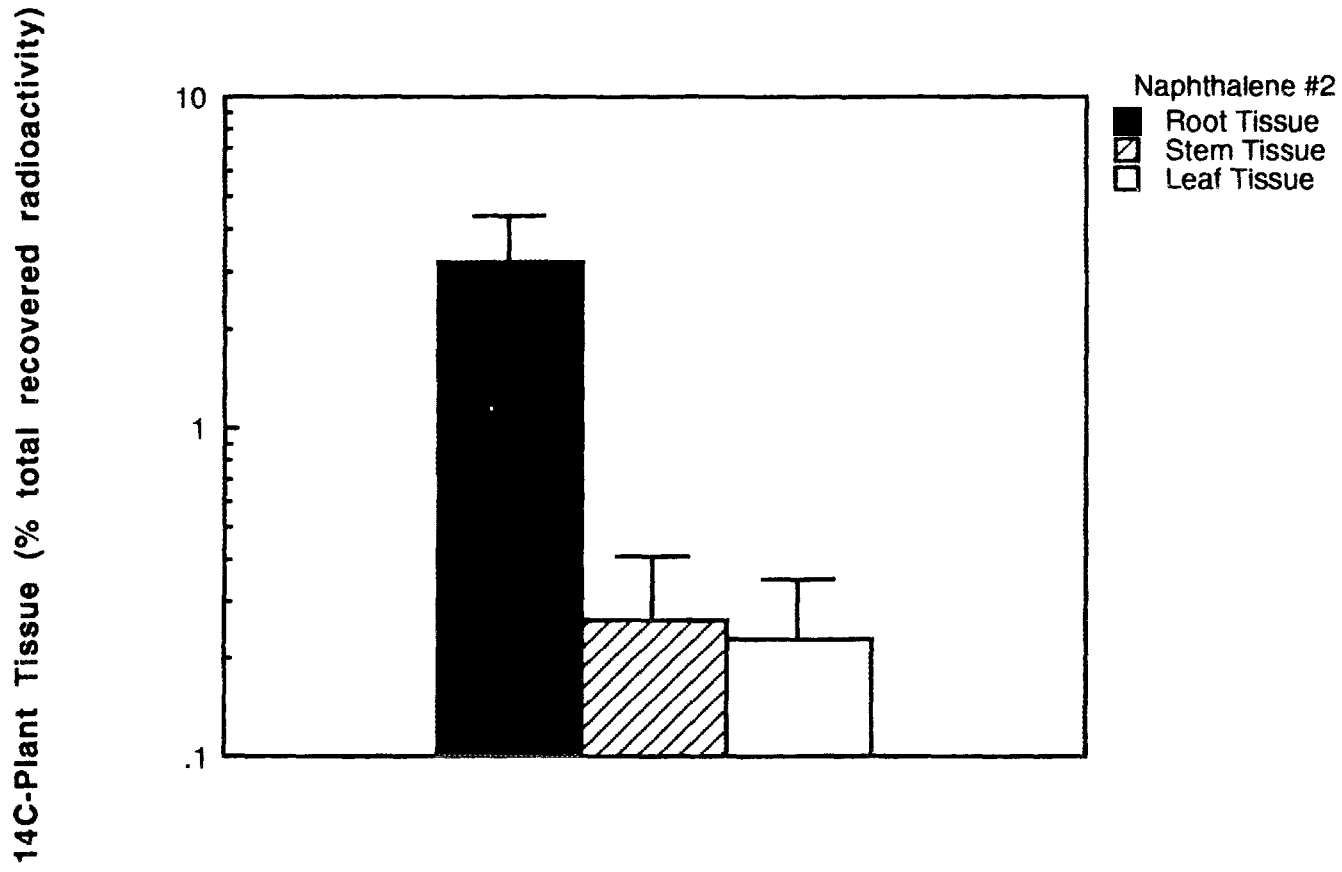


Figure IV - 1. ^{14}C-distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-naphthalene #2 in a composite soil collected from EBOS-24 site. Data are expressed as the mean percent ± one standard error of the total radioactivity recovered (n = 3).

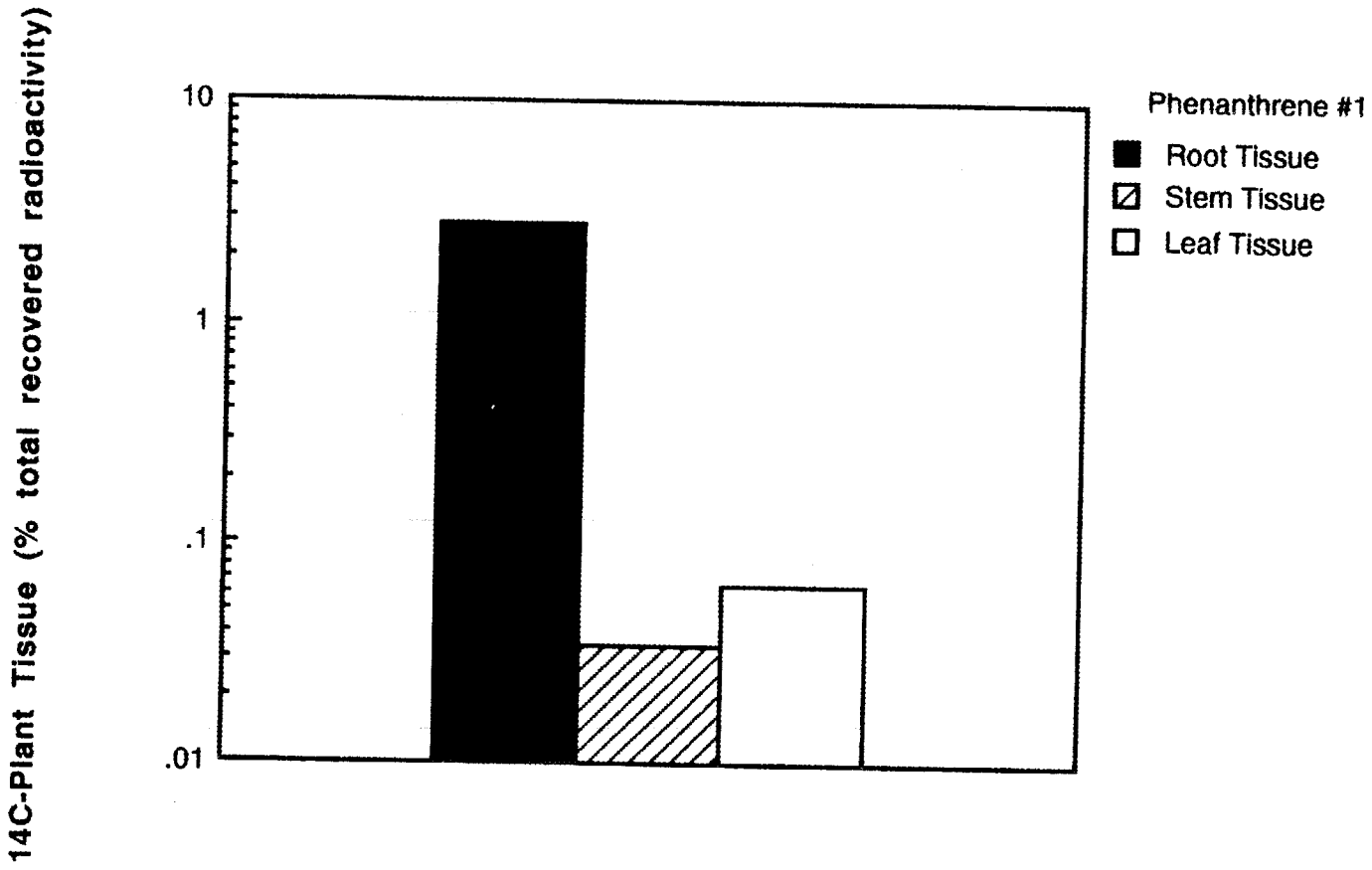


Figure IV - 2. ^{14}C-distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-phenanthrene #1 in a composite soil collected from EBOS-24 site. Data are expressed as the mean percent of the total radioactivity recovered (n = 2).

Figure IV - 3. ^{14}C-distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-pyrene #1 in a composite soil collected from EBOS-24 site. Data are expressed as the mean percent of the total radioactivity recovered (n = 2).

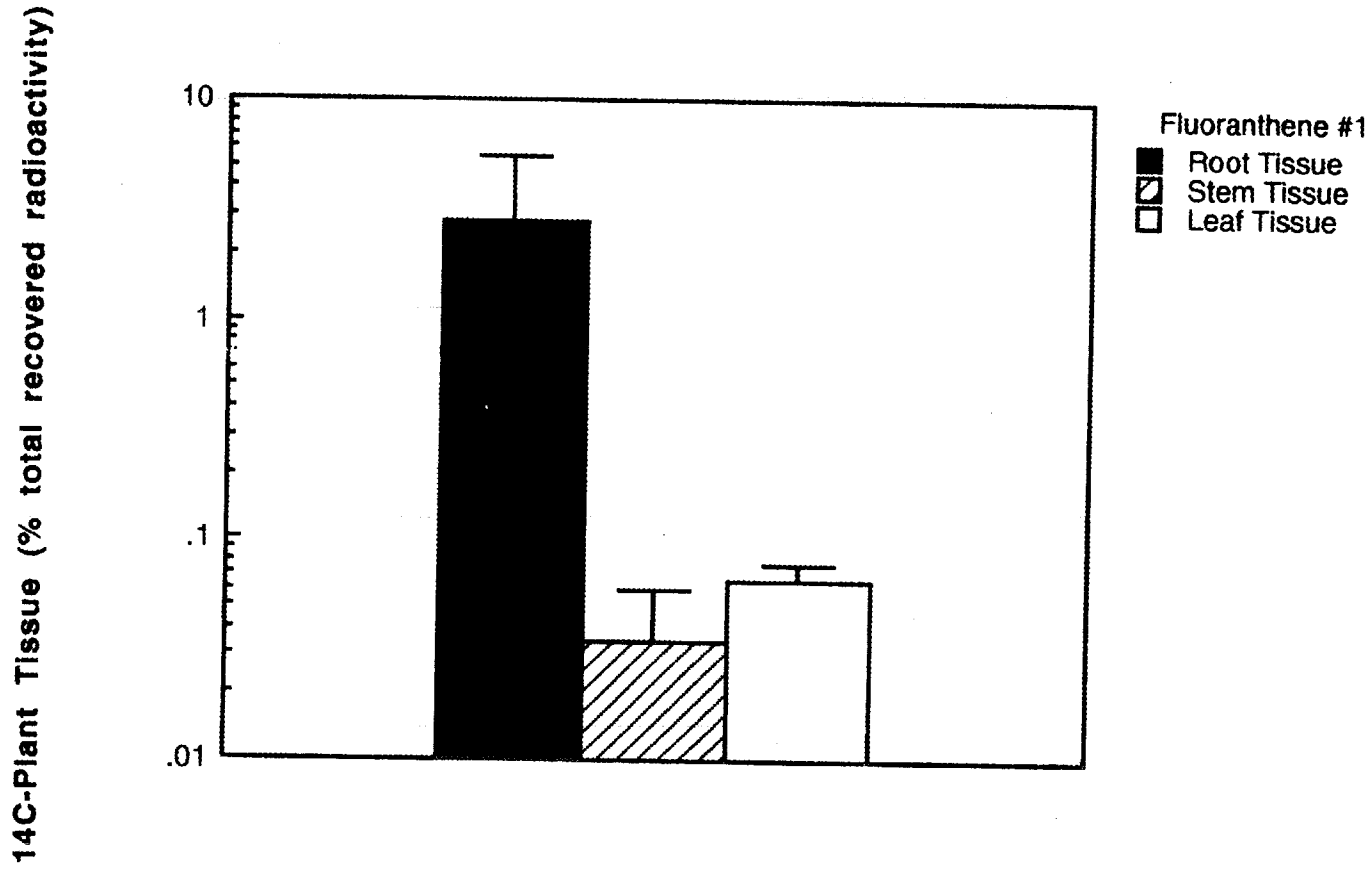


Figure IV - 4. ^{14}C-distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-fluoranthene #1 in a composite soil collected from EBOS-24 site. Data are expressed as the mean percent ± one standard error of the total radioactivity recovered (n = 3) .

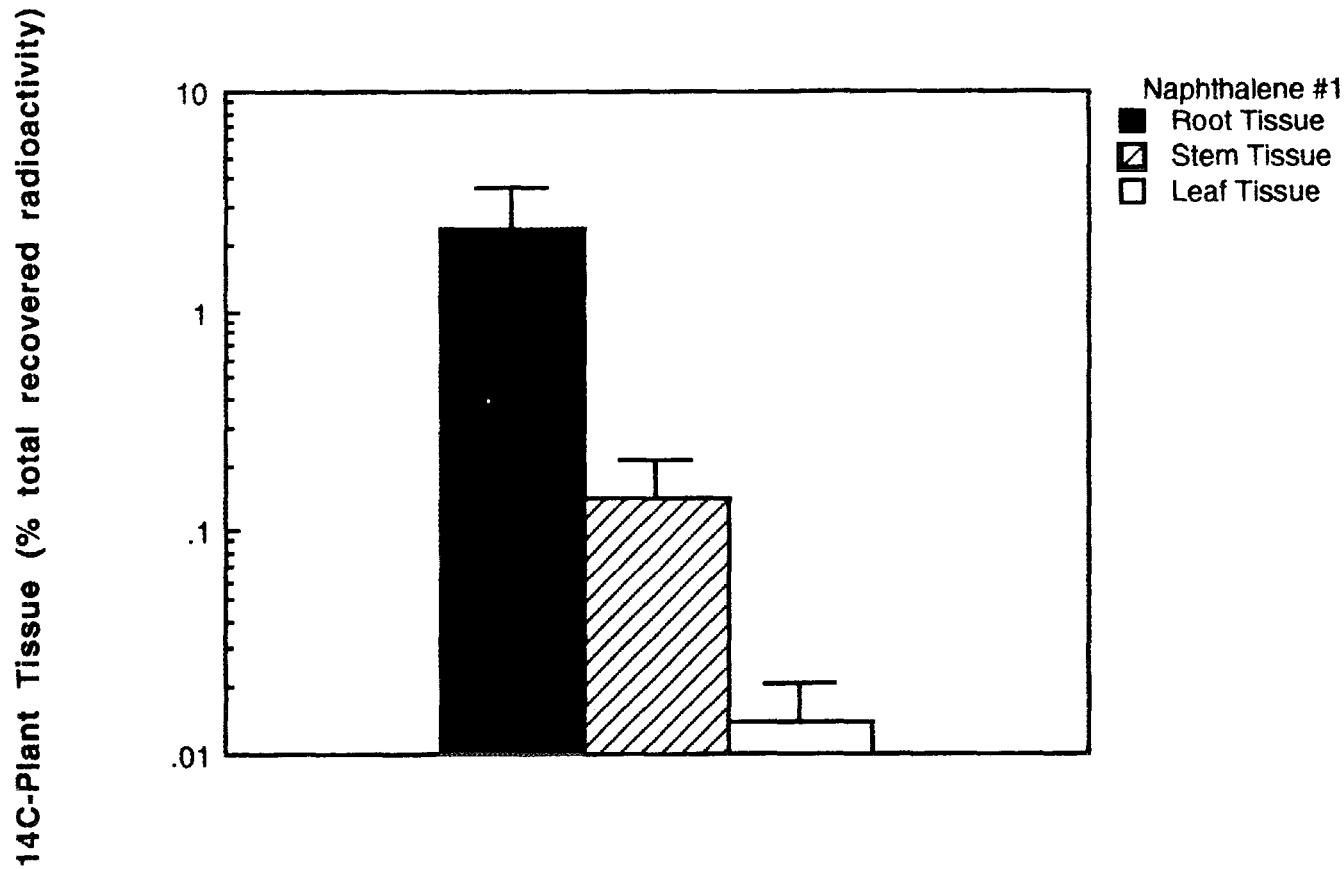


Figure IV - 5. ^{14}C-distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-naphthalene #1 in a composite soil collected from EBOS-24 site. Data are expressed as the mean percent ± one standard error of the total radioactivity recovered (n = 3).

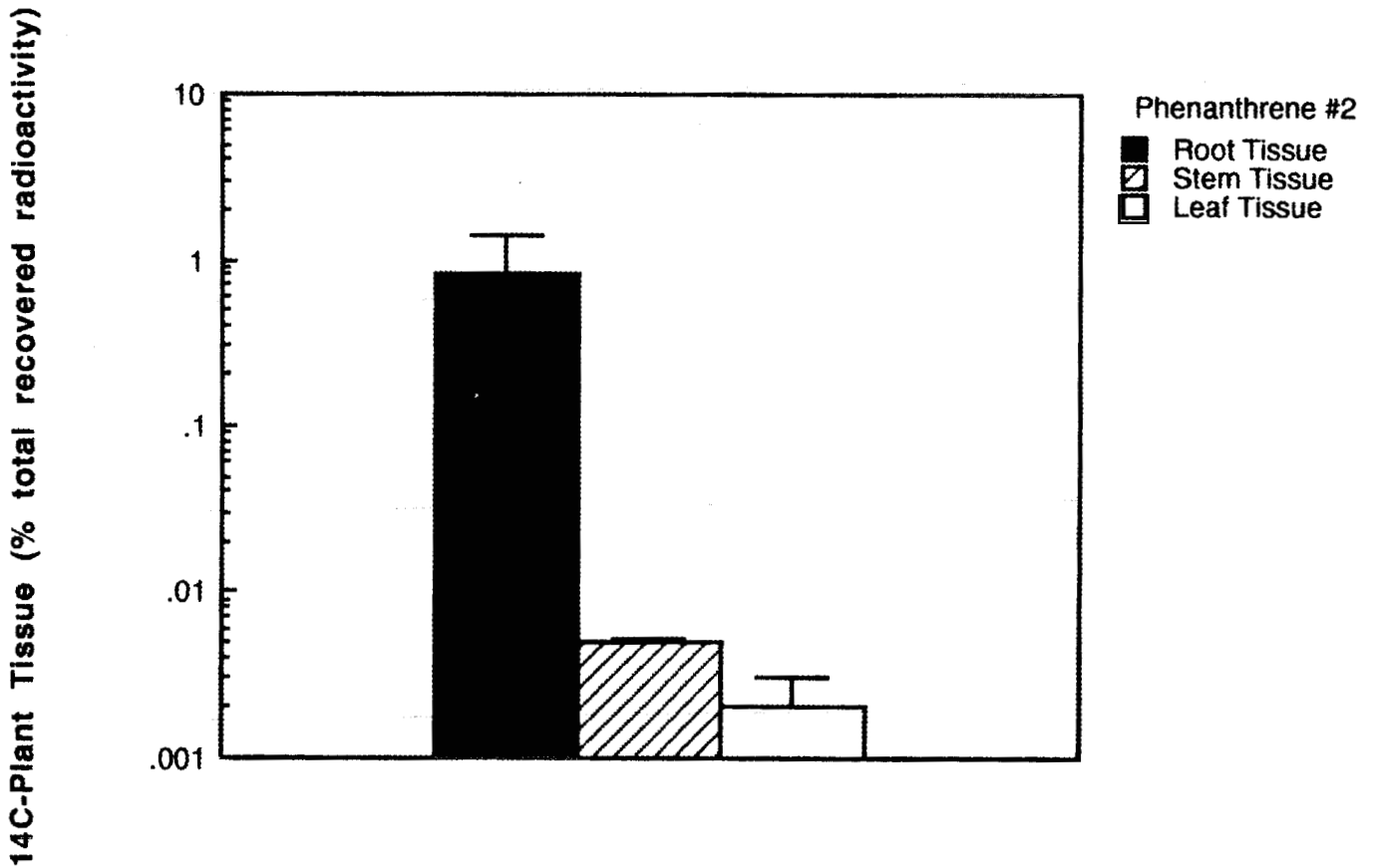


Figure IV - 6. ^{14}C-distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-phenanthrene #2 in a composite soil collected from EBOS-24 site. Data are expressed as the mean percent ± one standard error of the total radioactivity recovered (n = 3).

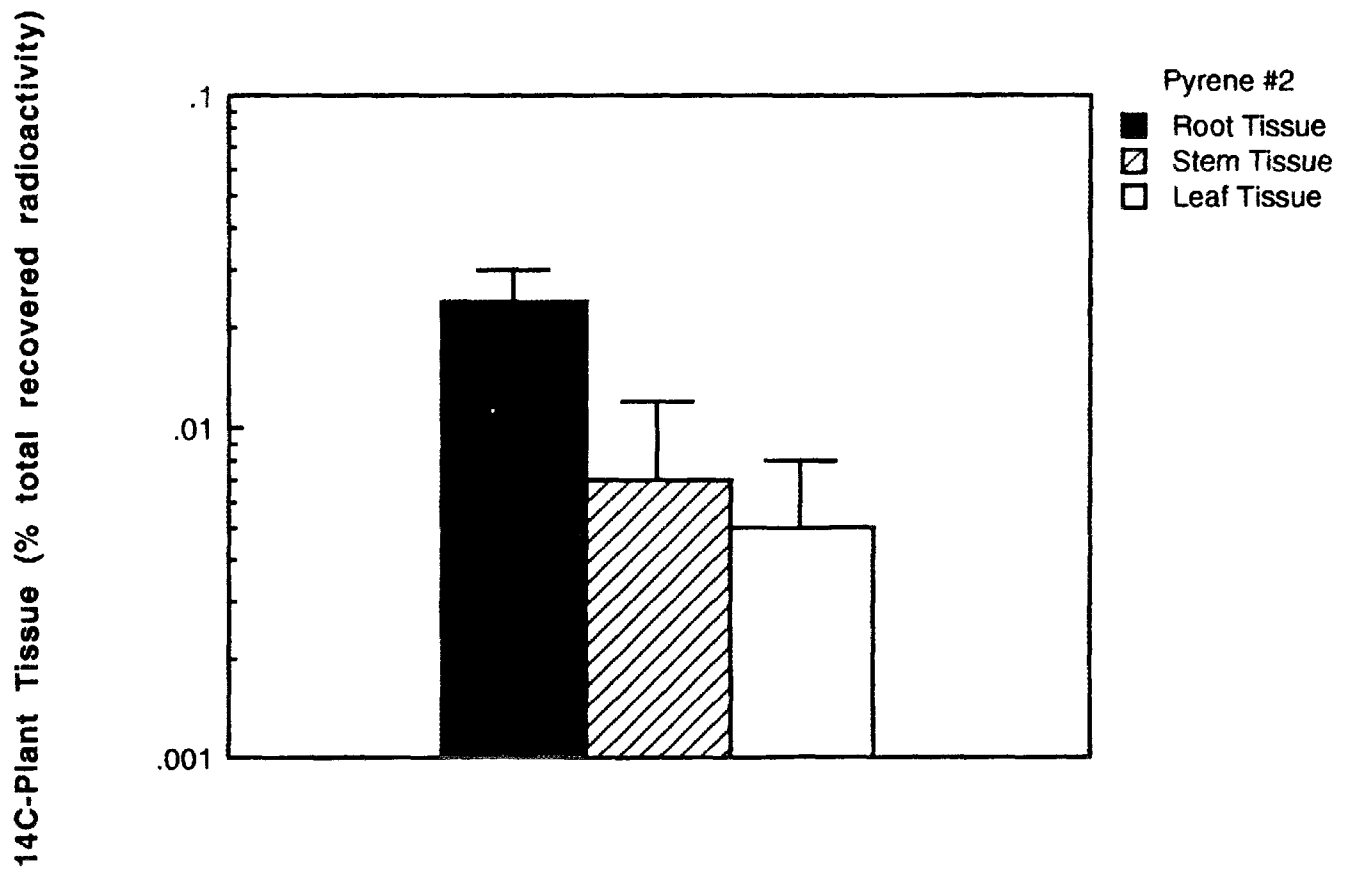


Figure IV - 7. ^{14}C-distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-pyrene #2 in a composite soil collected from EBOS-24 site. Data are expressed as the mean percent ± one standard error of the total radioactivity recovered (n = 3). * Denotes a statistically significant difference (p = 0.0013) between root and aboveground tissue (Student's t-test, paired comparison).

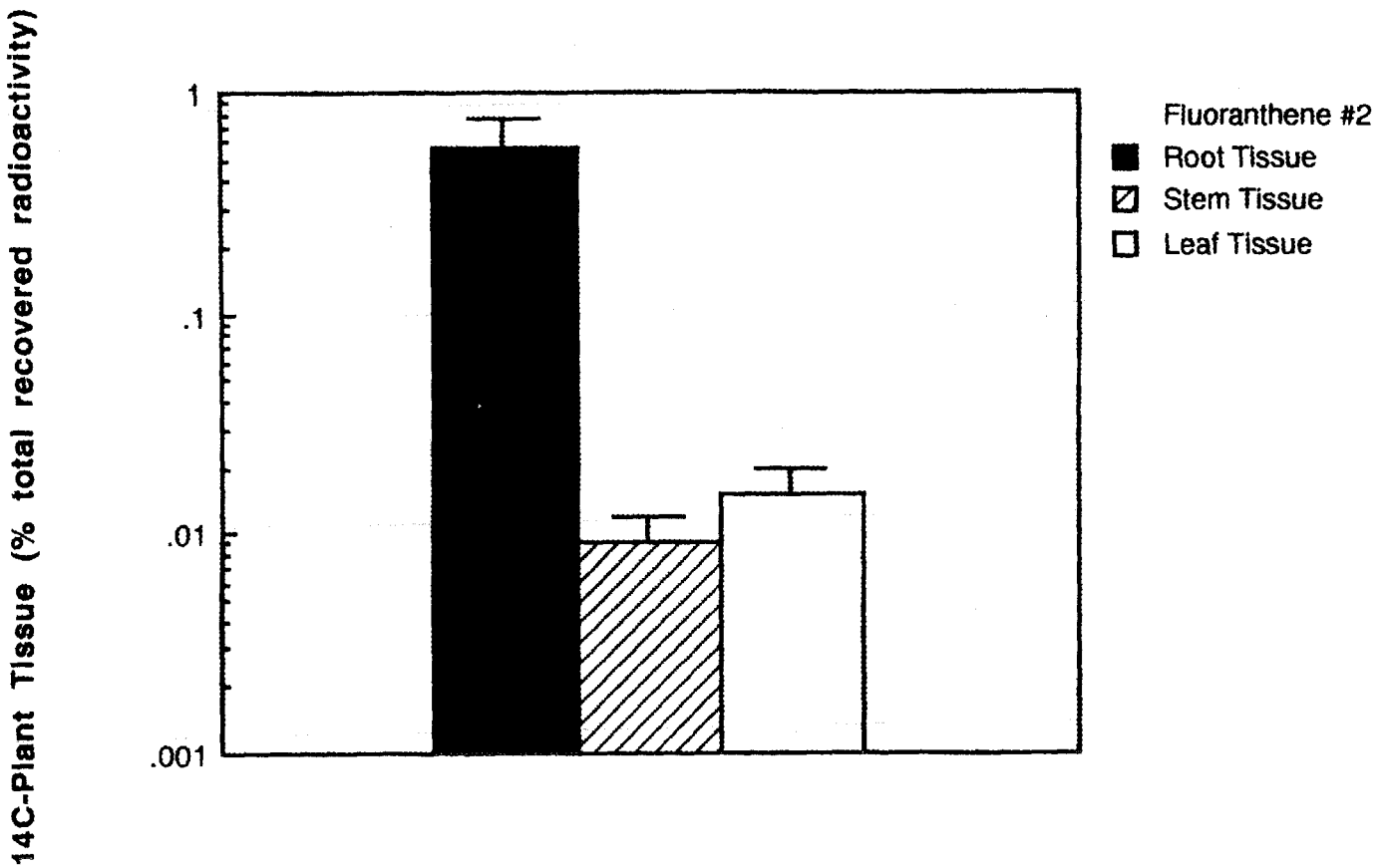


Figure IV - 8. ^{14}C-distribution in *Melilotus alba* tissue after 5 days of exposure to ^{14}C-fluoranthene #2 in a composite soil collected from EBOS-24 site. Data are expressed as the mean percent ± one standard error of the total radioactivity recovered (n = 3). * Denotes a statistically significant difference (p = 0.0191) between root and aboveground tissue (Student's t-test, paired comparison).

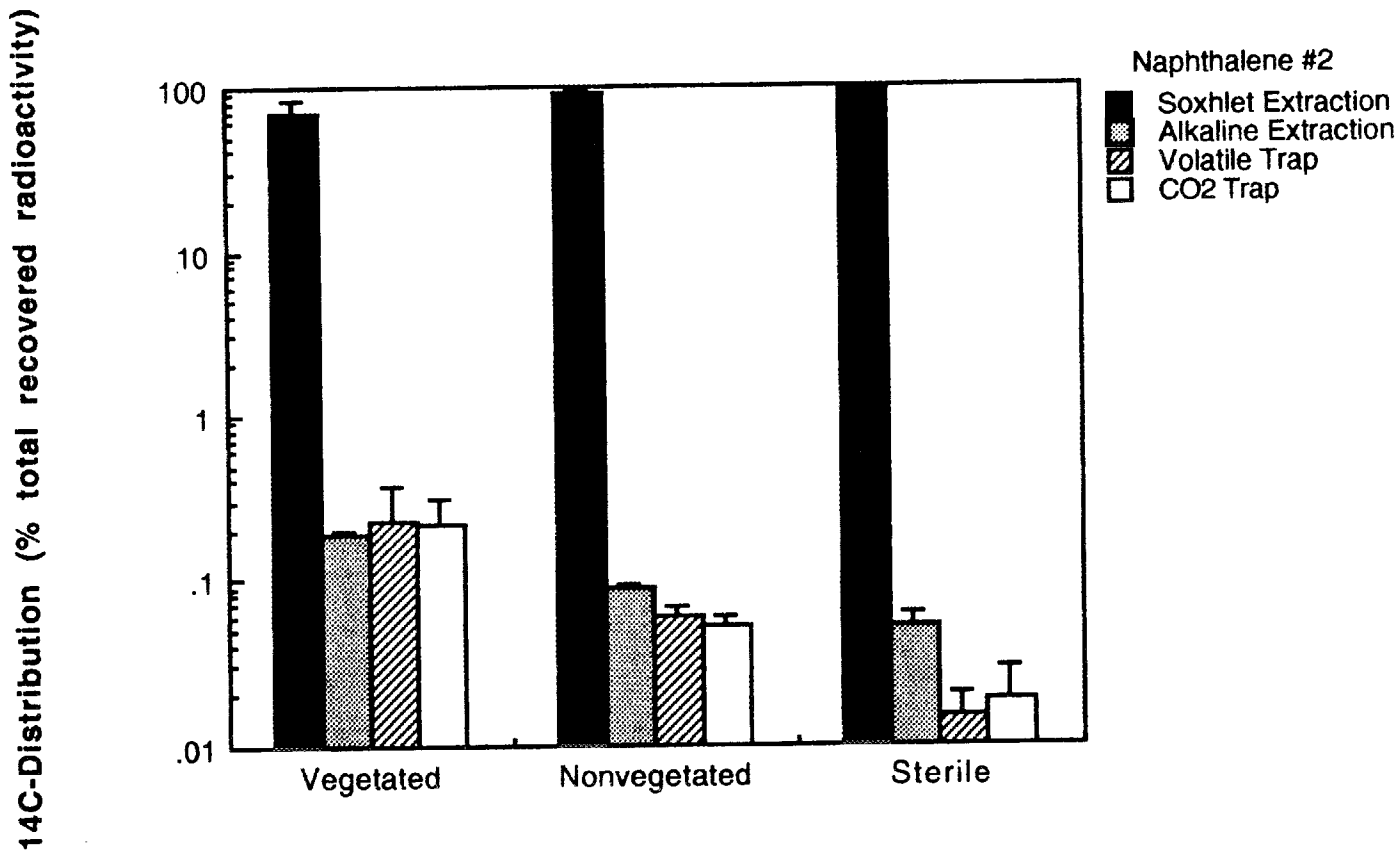


Figure IV - 9. ^{14}C-distribution of naphthalene #2 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. Data are expressed as a mean ± one standard error (n = 3).

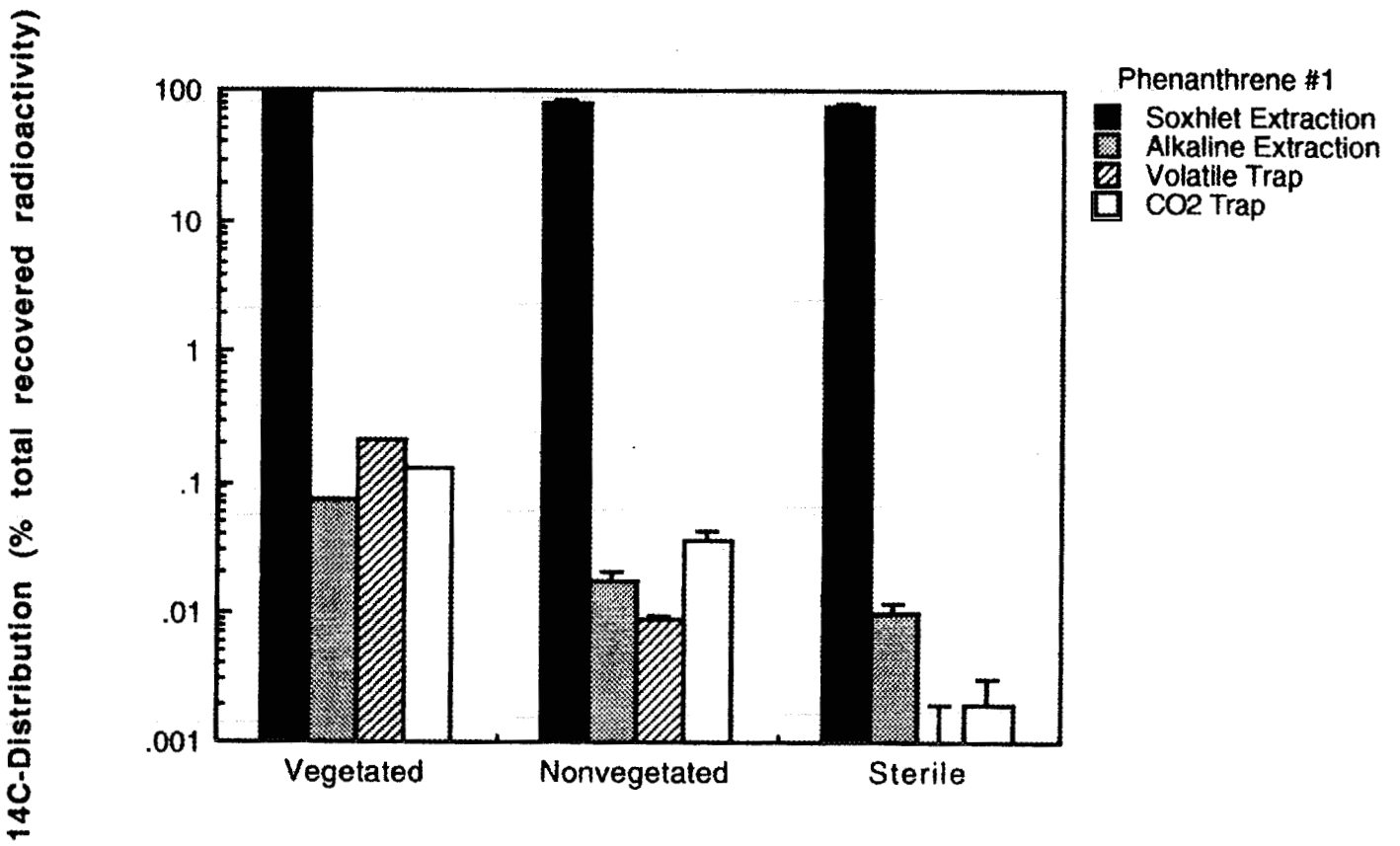


Figure IV - 10. ^{14}C-distribution of phenanthrene #1 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. Vegetated samples are the mean of two replicates whereas the remainder are the mean of three replicates ± one standard error.

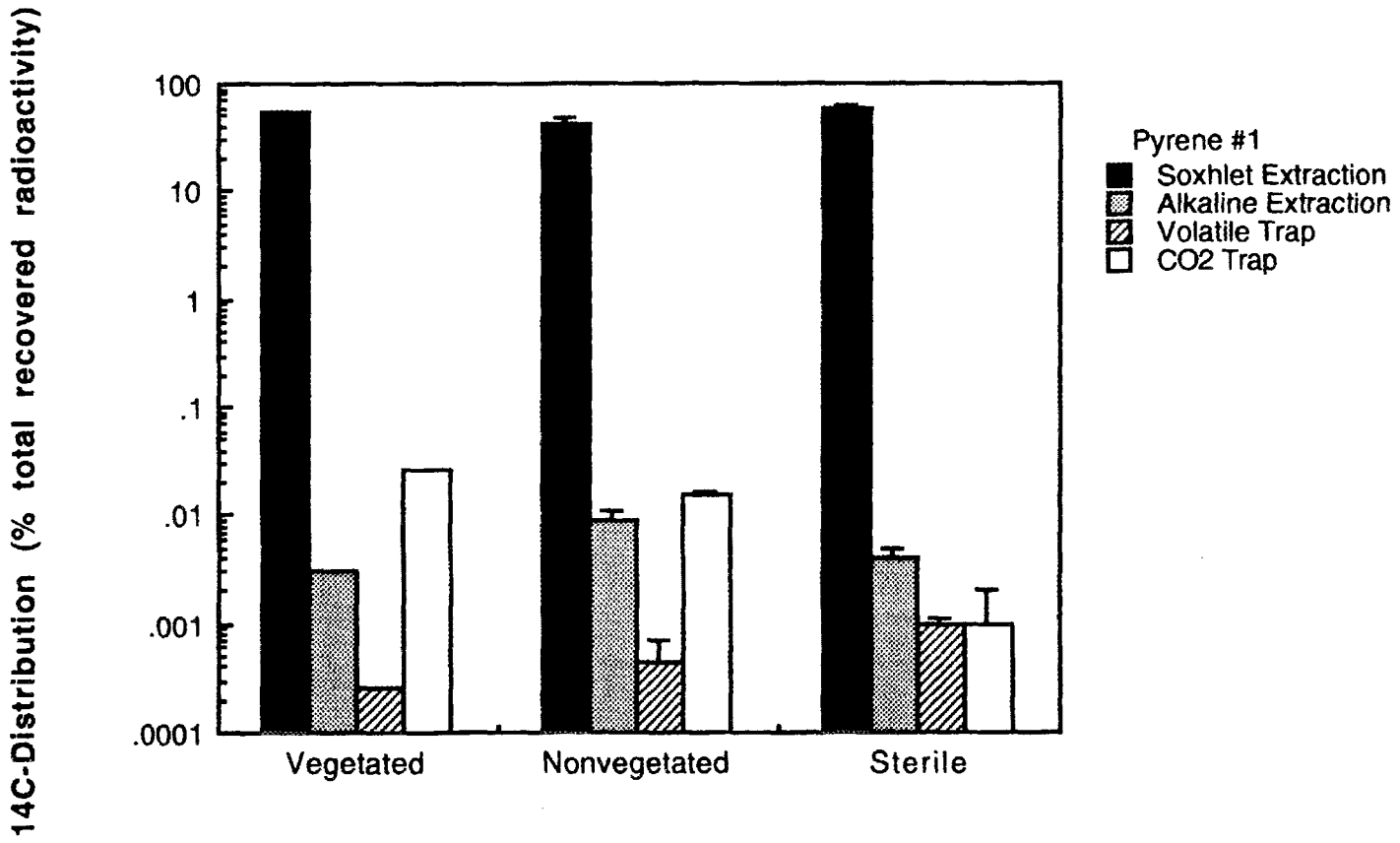


Figure IV - 11. ^{14}C-distribution of pyrene #1 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. Vegetated samples are the mean of two replicates whereas the remainder are the mean of three replicates ± one standard error.

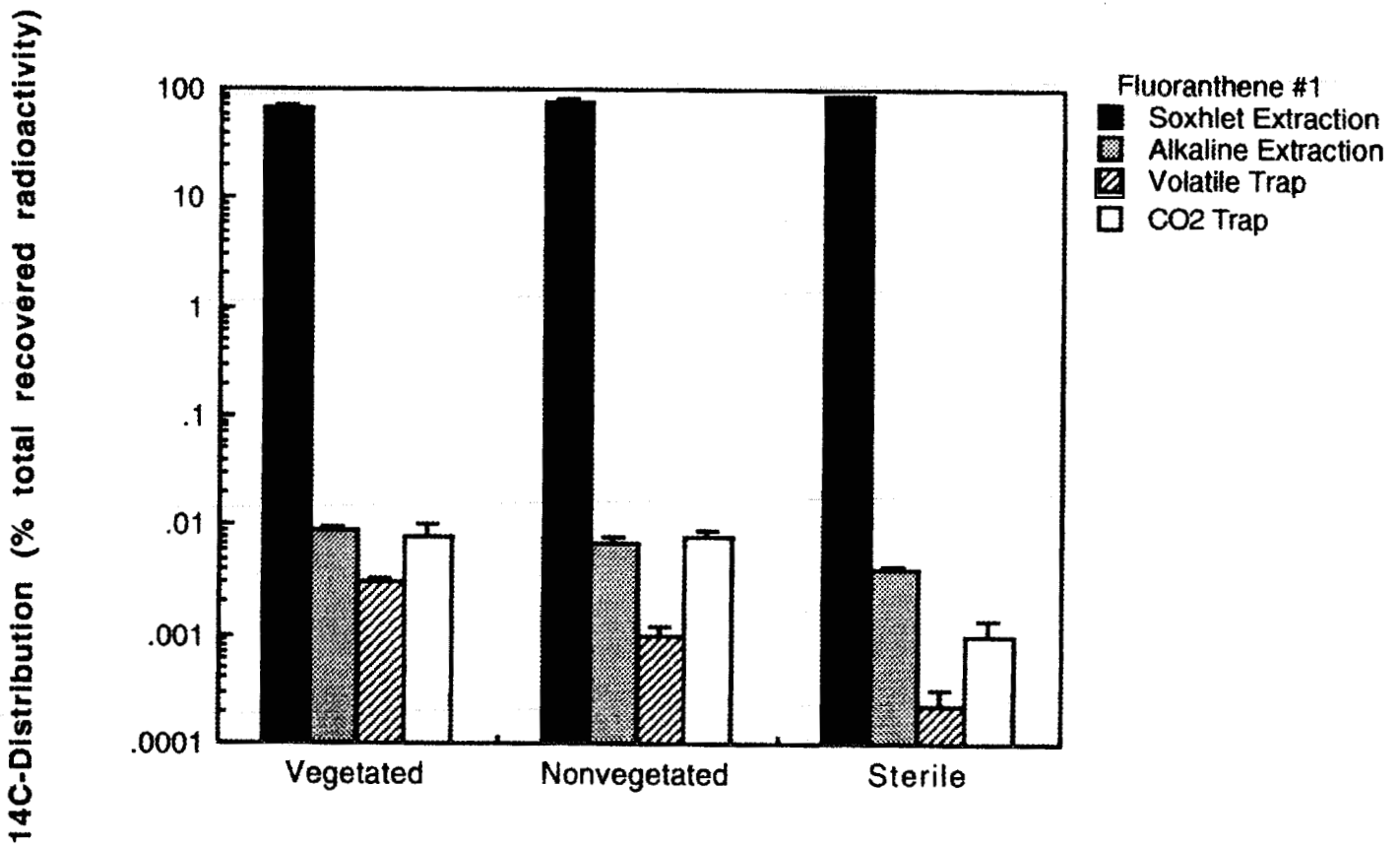


Figure IV - 12. ^{14}C-distribution of fluoranthene #1 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. Data are expressed as the mean ± one standard error (n = 3).

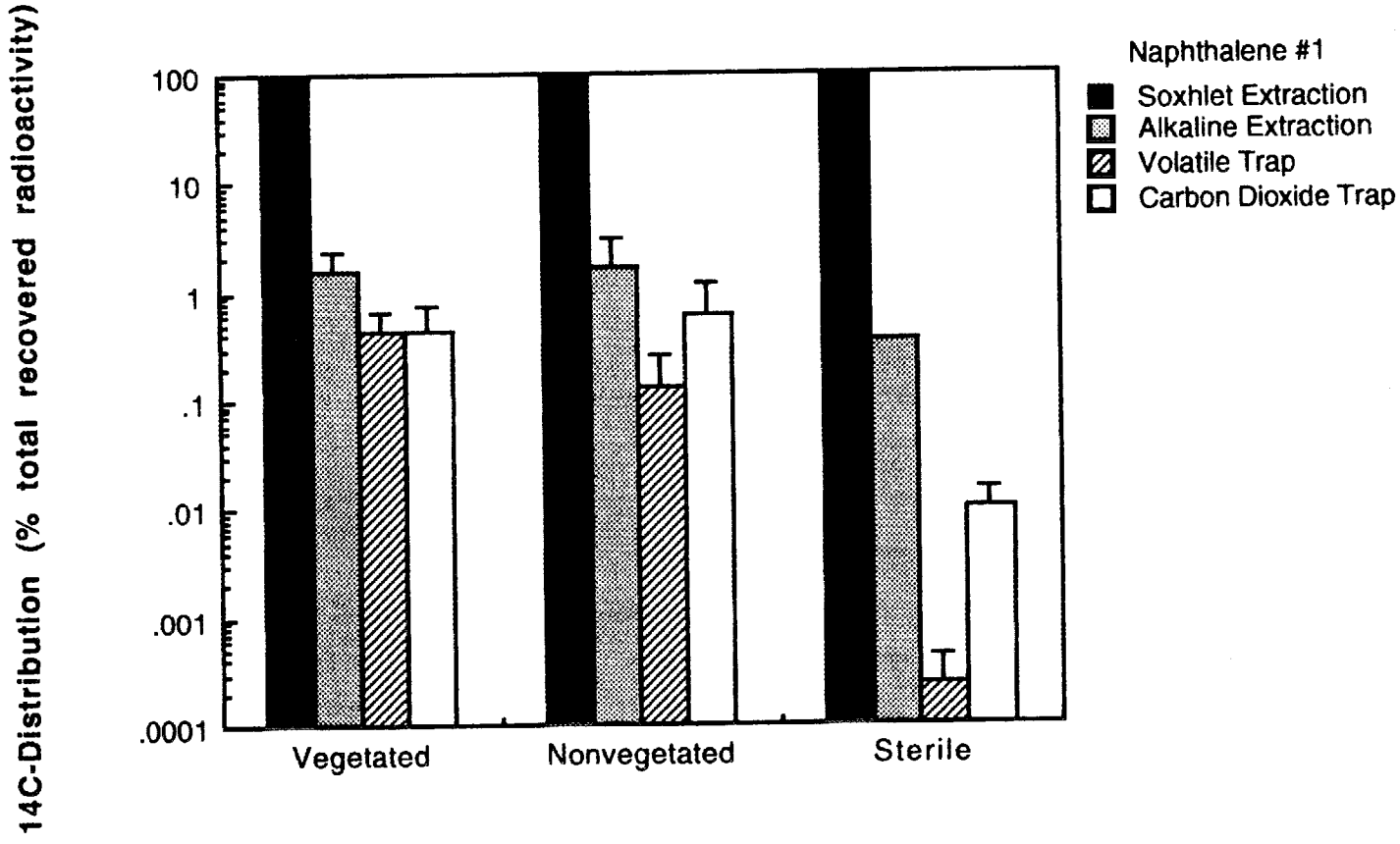


Figure IV - 13. ^{14}C-distribution of naphthalene #1 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. Data are expressed as a mean ± one standard error (n = 3).

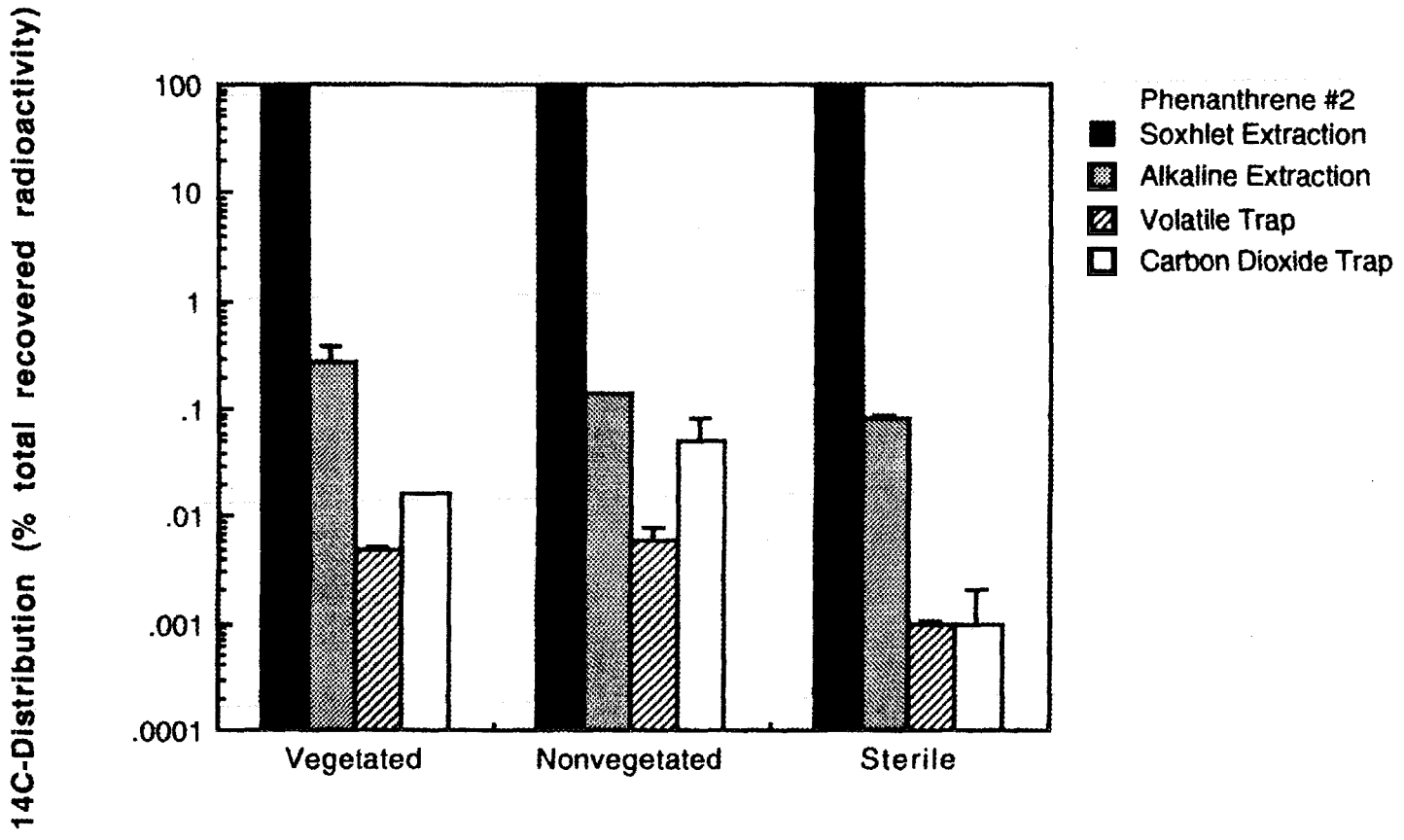


Figure IV - 14. ^{14}C-distribution of phenanthrene #2 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. Data are expressed as a mean ± one standard error (n = 3).

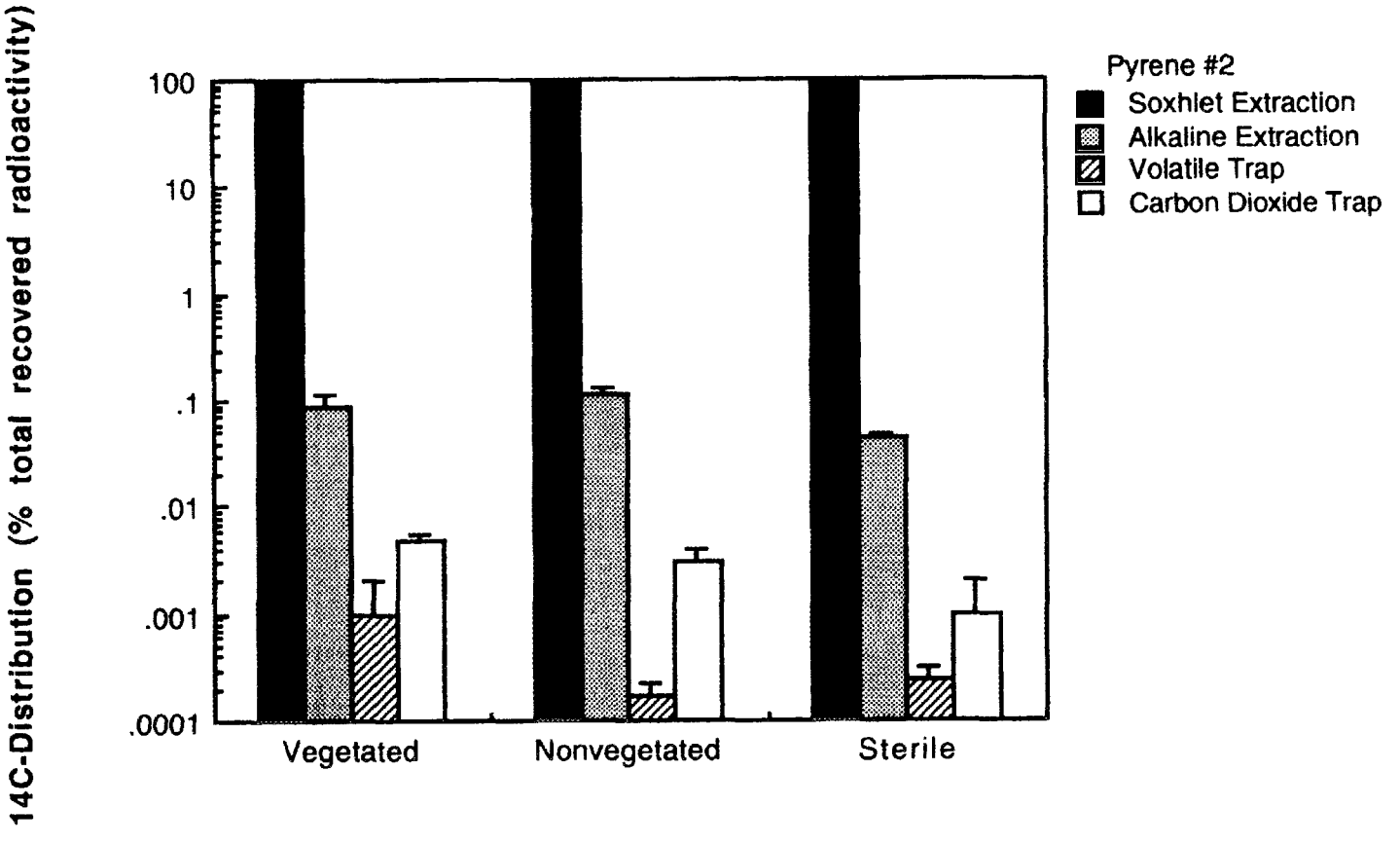


Figure IV - 15. ^{14}C-distribution of pyrene #2 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. Data are expressed as a mean ± one standard error (n = 3).

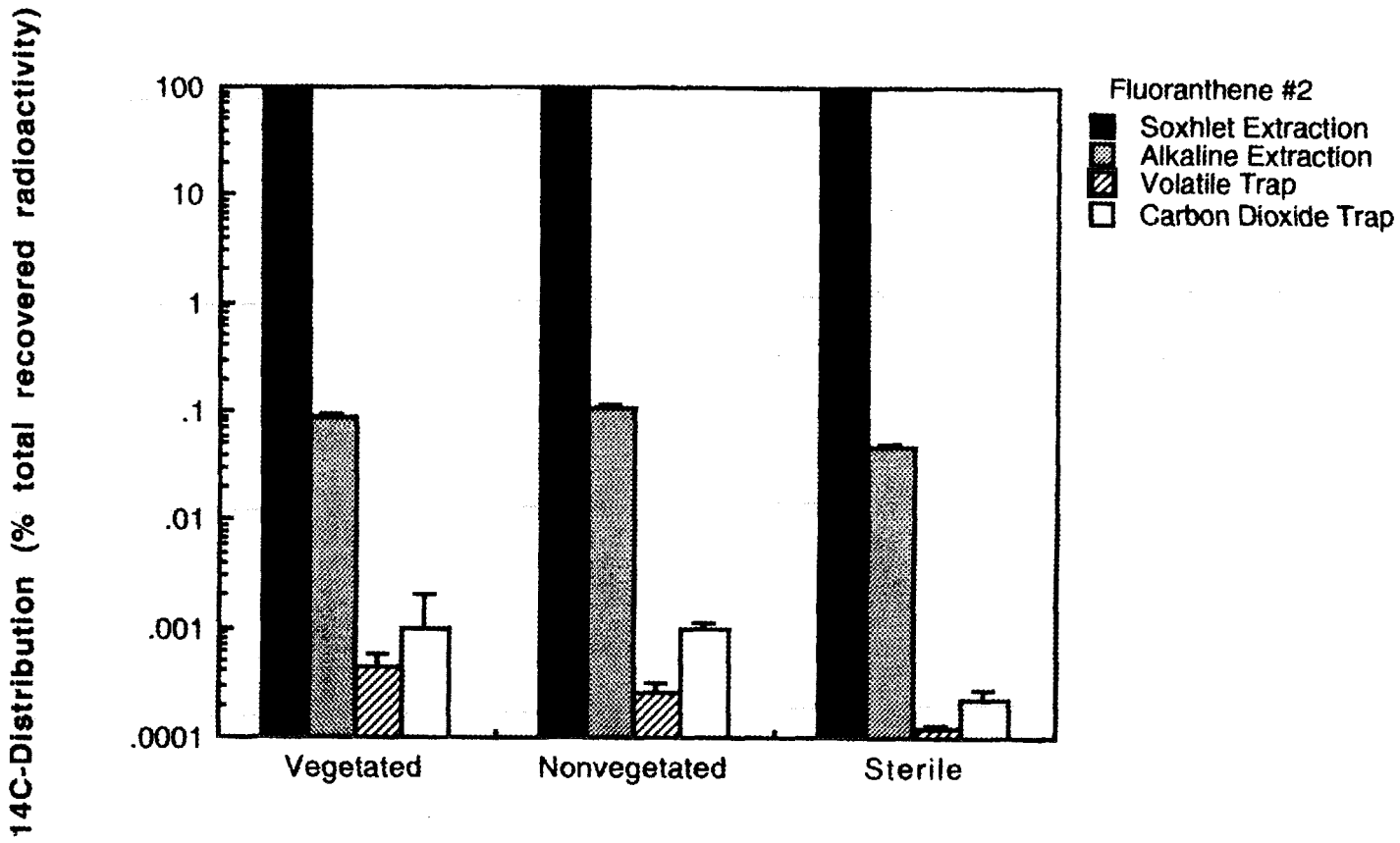


Figure IV - 16. ^{14}C-distribution of fluoranthene #2 derived radioactivity in soil extracts and traps of volatile organics and CO_2 from soil vegetated with *Melilotus alba*, nonvegetated soil, and autoclaved sterile soil. Data are expressed as a mean ± one standard error (n = 3).

Table IV - 3. Distribution of ^{14}C-PAH derived radioactivity in soil and flask headspace after 5 days of exposure.

PAH (250 µg/g)	n	% Total Recovered Radioactivity[a]			
		Soxhlet Extraction	Alkaline Extraction	Volatile Organic Trap	CO_2 Trap
Naphthalene #1					
vegetated	3	95.0 ± 2.5	1.6 ± 0.8	0.44 ± 0.2	0.45 ± 0.3
nonvegetated	3	97.6 ± 2.0	1.7 ± 1.33	0.13 ± 0.1	0.61 ± 0.6
sterile	3	99.6 ± 0.01	0.4 ± 0.01	2.4×10^{-4} ± 0.0004	0.01 ± 0.005
Naphthalene #2					
vegetated	3	71.1 ± 13.3	0.19 ± 0.01	0.23 ± 0.15	0.20 ± 0.09
nonvegetated	3	91.7 ± 2.2	0.09 ± 0.003	0.06 ± 0.01	0.053 ± 0.007
sterile	3	95.1 ± 0.3	0.05 ± 0.01	0.015 ± 0.005	0.019 ± 0.01
Phenanthrene #1[b]					
vegetated	2	93.2	0.075	0.21	0.13
nonvegetated	3	80.0 ± 2.3	0.017 ± 0.003	0.009 ± 0.0003	0.035 ± 0.008
sterile	3	76.7 ± 4.4	0.01 ± 0.002	0.001 ± 0.001	0.002 ± 0.001
Phenanthrene #2					
vegetated	3	99.7 ± 0.11	0.27 ± 0.11	$0.005 \pm 1.4 \times 10^{-4}$	$0.016 \pm 4.2 \times 10^{-4}$
nonvegetated	3	99.9 ± 0.006	0.14 ± 0.006	0.006 ± 0.002	0.051 ± 0.029
sterile	3	99.9 ± 0.003	0.08 ± 0.003	$0.001 \pm 2.9 \times 10^{-5}$	0.001 ± 0.001
Pyrene #1					
vegetated	2	54	0.003	2.5×10^{-4}	0.026
nonvegetated	3	43 ± 5.9	0.009 ± 0.002	$4.32 \times 10^{-4} \pm 2.6 \times 10^{-4}$	0.015 ± 0.001
sterile	3	60 ± 3.9	0.004 ± 0.001	$0.001 \pm 8.9 \times 10^{-5}$	0.001 ± 0.001

Table IV - 3 cont'd.

PAH	n	% Total Recovered Radioactivity[a]			
		Soxhlet Extraction	Alkaline Extraction	Volatile Organic Trap	CO_2 Trap
Pyrene #2					
vegetated	3	99.9 ± 0.025	0.09 ± 0.025	0.001 ± 0.001	0.005 ± 4.9 x 10^{-4}
nonvegetated	3	99.9 ± 0.012	0.12 ± 0.012	1.7 x 10^{-4} ± 6.1 x 10^{-5}	0.003 ± 0.001
sterile	3	100 ± 0.003	0.05 ± 0.003	2.4 x 10^{-4} ± 6.1 x 10^{-5}	0.001 ± 0.001
Fluoranthene #1					
vegetated	3	67.2 ± 5.3	0.009 ± 3.4 x 10^{-4}	0.003 ± 2.5 x 10^{-4}	0.008 ± 0.002
nonvegetated	3	76.3 ± 2.9	0.007 ± 0.001	0.001 ± 0.001	0.008 ± 0.001
sterile	3	88.1 ± 0.73	0.004 ± 3.3 x 10^{-4}	2.3 x 10^{-4} ± 9.3 x 10^{-5}	0.001 ± 4.0 x 10^{-4}
Fluoranthene #2					
vegetated	3	99 ± 0.19	0.088 ± 0.007	4.3 x 10^{-4} ± 1.3 x 10^{-4}	0.001 ± 3.0 x 10^{-4}
nonvegetated	3	99.9 ± 0.003	0.107 ± 0.004	2.6 x 10^{-4} ± 4.6 x 10^{-5}	0.001 ± 1.4 x 10^{-4}
sterile	3	99.95 ± 0.003	0.048 ± 0.003	1.2 x 10^{-4} ± 1.5 x 10^{-5}	2.2 x 10^{-4} ± 4.7 x 10^{-5}

[a] Expressed as a mean percent of the total recovered ^{14}C ± one standard error.

[b] Total concentration of Phenanthrene #1 added to soil was 500 µg/g.

Table IV - 4. Plant tissue concentrations of ^{14}C-PAHs after a 5-day exposure period.

	Plant Tissue Concentrations (µg/g plant tissue)[a]				
PAH	Root Tissue	Stem Tissue	Leaf Tissue	Soil	BCF[b]
Naphthalene	1.35 ± 1.02	0.084 ± 0.05	0.07 ± 0.04	37 ± 12	0.039 ± 0.02
Phenanthrene	4.56	0.066	0.051	174	0.0302
Pyrene	2.93	7.05×10^{-3}	1.25×10^{-3}	89	0.033
Fluoranthene	0.86 ± 0.37	$2.1 \times 10^{-4} \pm 2.4 \times 10^{-4}$	$4.8 \times 10^{-4} \pm 3.3 \times 10^{-5}$	50 ± 6.2	0.011 ± 0.008

[a] Plant tissue concentrations represent the mean of three replicates ± one standard error. Plant tissue concentrations for phenanthrene and pyrene represent the mean of two replicates.

[b]Bioaccumulation Factor = µg PAH in plant tissue/µg PAH in soil. ^{14}C residues in plant tissue and soil were assumed to be present as parent compound.

bioaccumulation values obtained from these studies were probably not at equilibrium, and as a result, these BCF measurements may be an underestimation of accumulation at equilibrium (Byshe 1990).

The distribution of ^{14}C in the flask headspace as indicated by the volatile organic trap and the CO_2 trap was less than 1% of the total radioactivity recovered for all PAHs tested. Table IV - 3 summarizes the mean values for ^{14}C-labeled volatiles and CO_2 released during the individual uptake experiments. In general, ^{14}C-volatile organics and $^{14}CO_2$ production in vegetated and nonvegetated soil treatments were elevated over sterile soil treatments for all tested PAHs (Figure IV - 17 through IV - 24). Naphthalene uptake experiments exhibited the greatest evolution of ^{14}C-volatile organics and $^{14}CO_2$ relative to the other tested PAHs, while fluoranthene uptake experiments had the least.

The evolution of $^{14}CO_2$, indicative of mineralization, in vegetated and nonvegetated treatments over the 5-day exposure period exhibited high variability, with nonsignificant differences in the four experiments involving naphthalene and phenanthrene. In general, evolution of $^{14}CO_2$ was greatest in the presence of plants exposed to naphthalene and approximately equal or greater in nonvegetated treatments exposed to phenanthrene (Figure IV - 17, IV - 18, IV - 21, and IV - 22). Uptake experiments with pyrene consistently showed an enhanced evolution of $^{14}CO_2$ in the presence of vegetation over nonvegetated treatments (Figure IV - 19 and Figure IV - 23), while uptake experiments with fluoranthene consistently showed no difference in mineralization of fluoranthene between vegetated and nonvegetated treatments (Figure IV - 20 and Figure IV - 24).

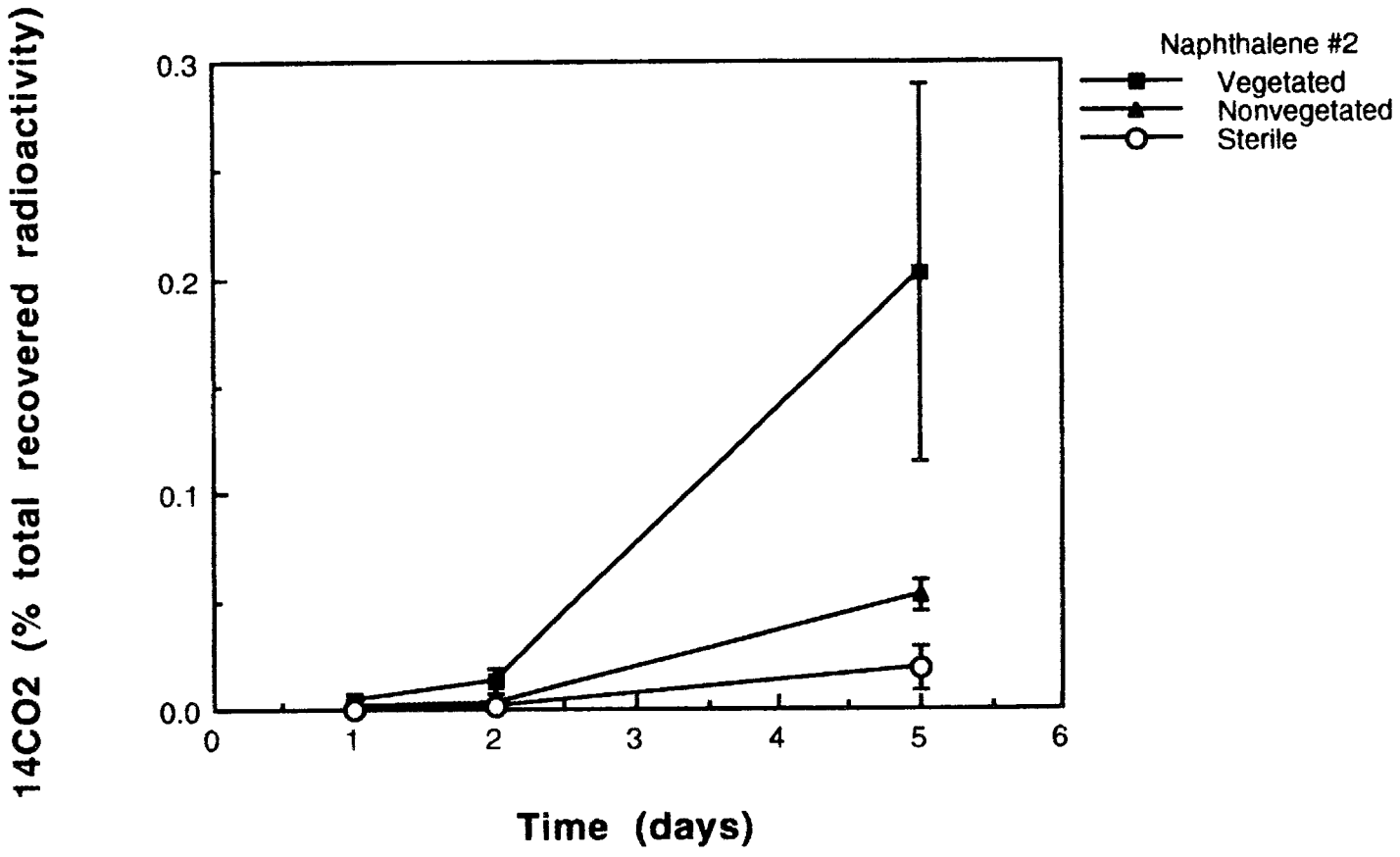


Figure IV - 17. Mineralization of ^{14}C-naphthalene #2 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*. Data points are the mean ± one standard error (n = 3).

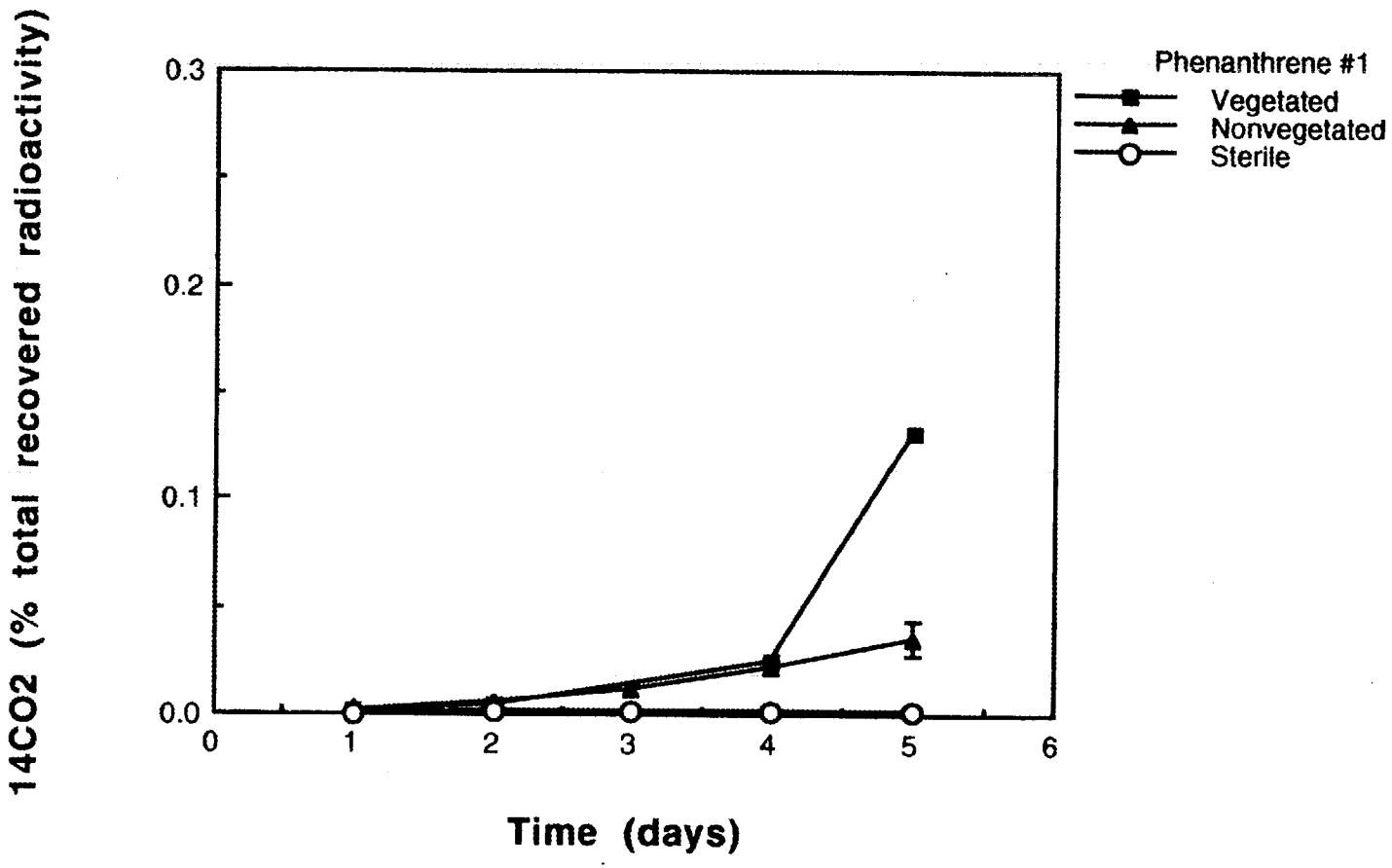


Figure IV - 18. Mineralization of ^{14}C-phenanthrene #1 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*. Vegetated values are the mean of two replicates while the remainder data points are the mean ± one standard error (n = 3).

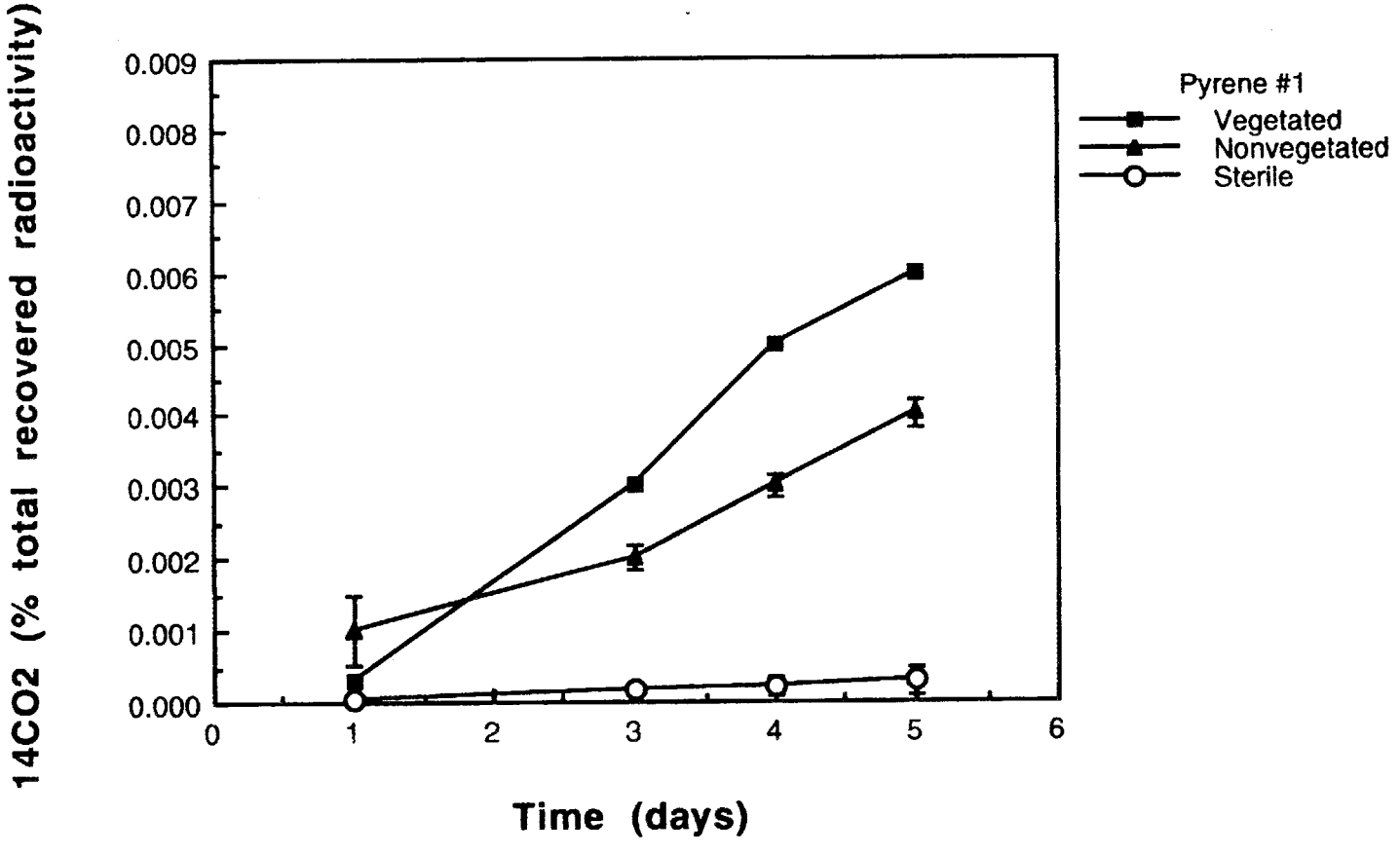


Figure IV - 19 Mineralization of ^{14}C-pyrene #1 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*. Vegetated values are the mean of two replicates while the remainder data points are the mean ± one standard error (n = 3).

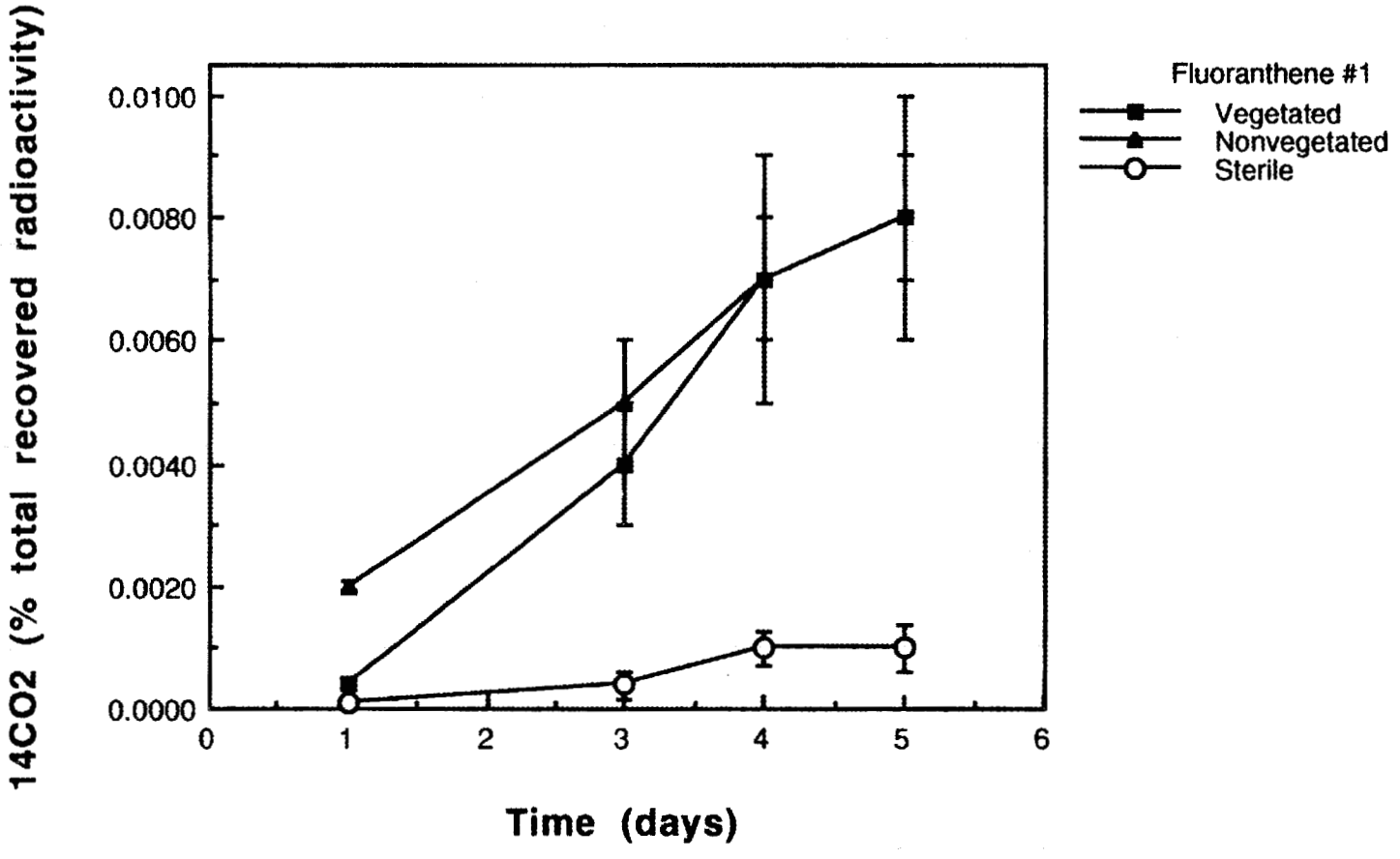


Figure IV - 20. Mineralization of ^{14}C-fluoranthene #1 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*. Data points are the mean ± one standard error (n = 3).

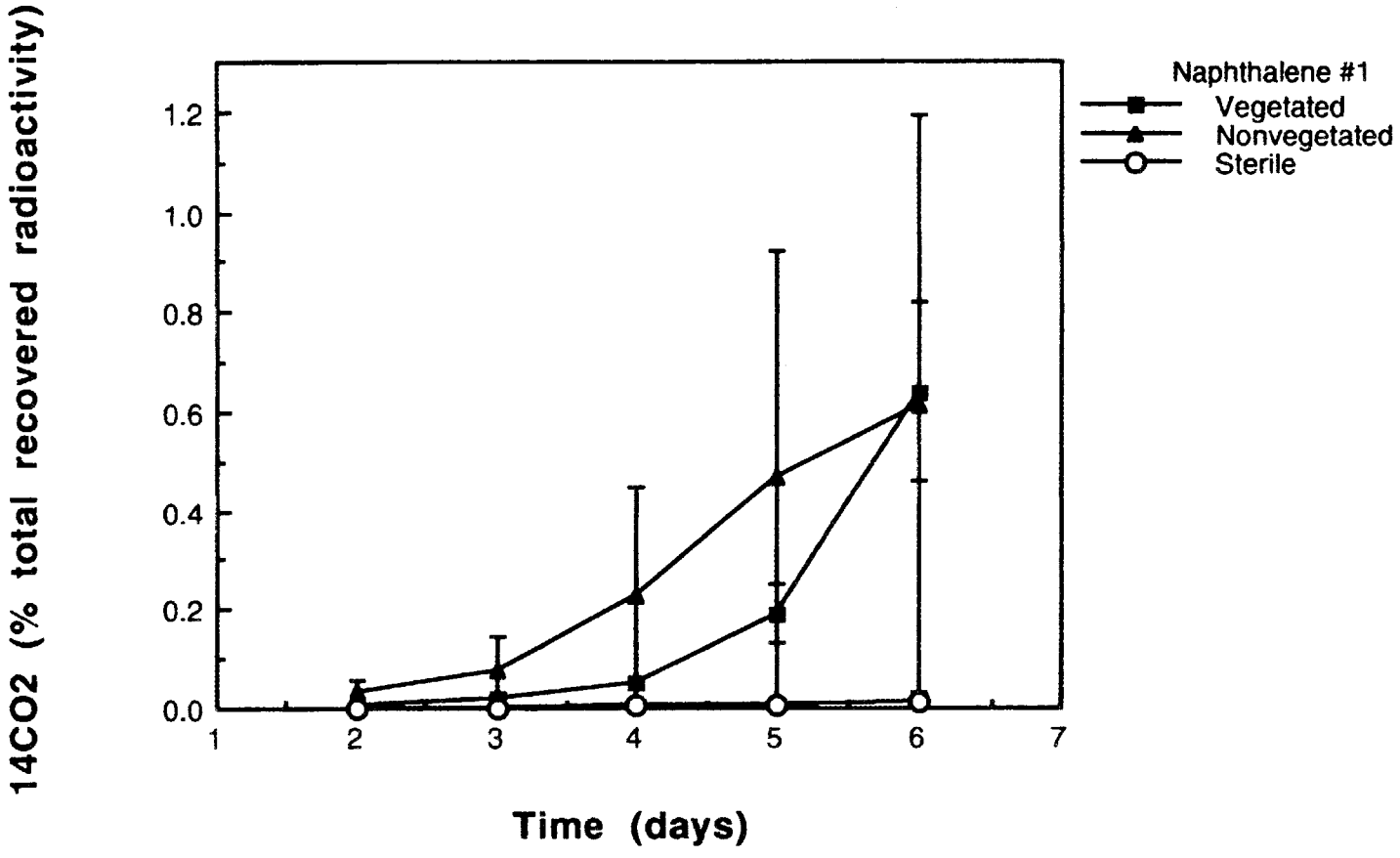


Figure IV - 21. Mineralization of ^{14}C-naphthalene #1 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*. Data points are the mean ± one standard error (n = 3).

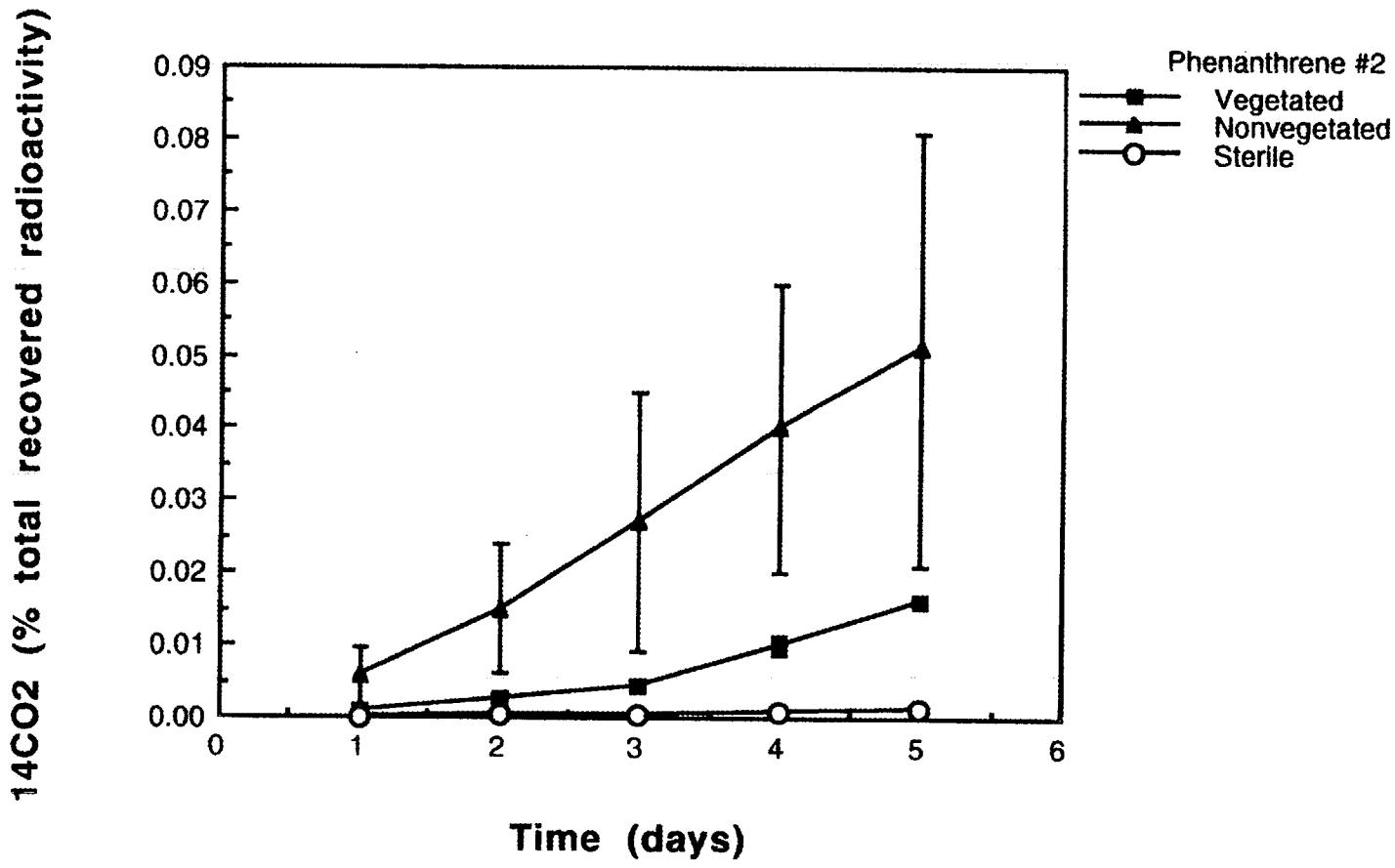


Figure IV - 22. Mineralization of ^{14}C-phenanthrene #2 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*. Data points are the mean ± one standard error (n = 3).

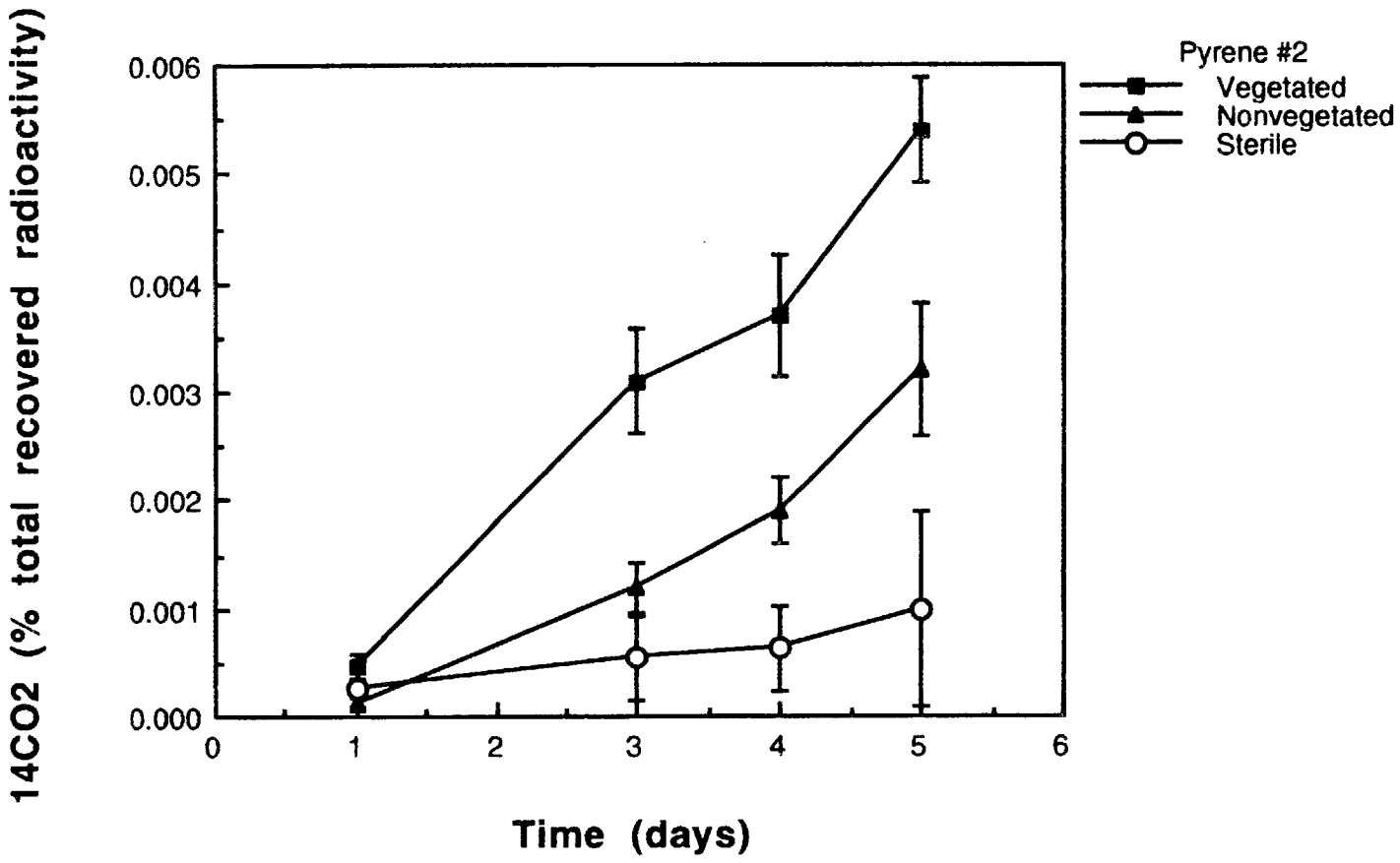


Figure IV - 23. Mineralization of ^{14}C-pyrene #2 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*. Data points are the mean ± one standard error (n = 3).

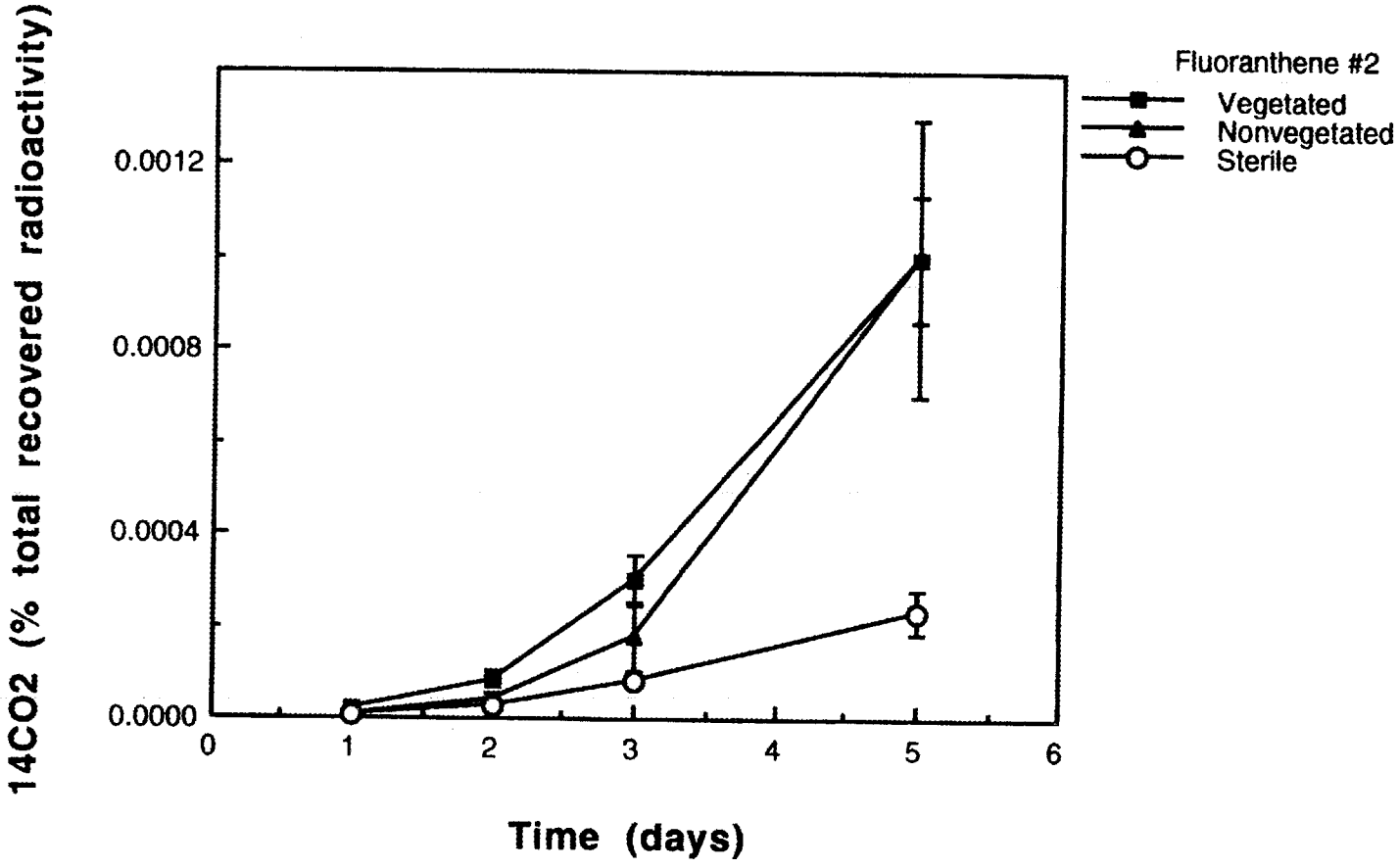


Figure IV - 24. Mineralization of ^{14}C-fluoranthene #2 in sterile control soil, nonvegetated soil, and soil containing *Melilotus alba*. Data points are the mean ± one standard error (n = 3).

Mass Balance

Initial percent recoveries of PAHs ranged from a mean of 72% for sterile phenanthrene treatments (n = 3) to a mean of 101% for nonvegetated phenanthrene treatments (n = 3). A mean mass balance of 85 ± 10% (one standard deviation of the mean) was obtained for the first 18 experiments completed, which included all four PAHs. Based on this percent recovery, all experimental measurements were completed for the four PAHs. Calculations from subsequent experiments consistently yielded percent recoveries greater than 100%. Exhaustive extraction of the residual soil (subsampled previously for soxhlet extractions) for four of the eight experiments using organic solvent washes followed by alkaline and acidic extractions (Walton et al., in press) provided a more accurate assessment of mass balance. Table IV - 5 summarizes recovery data obtained with exhaustive extraction of the soil. Recoveries ranged from 46 ± 19 % for naphthalene vegetated treatments (n = 3) to 128 ± 8 % for pyrene nonvegetated treatments (n = 3). Because of the descrepancy between the two sets of recovery data, all data were normalized by reporting ^{14}C on the basis of percent total recovered ^{14}C for each individual experiment, allowing for comparison of relative amounts of ^{14}C in each compartment (soil, plant, and air).

C. ^{14}Carbon Allocation in *Melilotus alba*

Evidence of a change in carbon allocation in plants exposed to a chemical stress in the root zone was found in experiments using pulse-labeling techniques with $^{14}CO_2$ and phenanthrene. Analysis of flask headspace for release of $^{14}CO_2$ over the 5-day chase period showed elevated levels of ^{14}C in the alkaline traps of plants exposed to phenanthrene in the root zone compared

Table IV - 5. Percent recoveries achieved in plant uptake experiments with PAHs in soil.

PAH		% Recovered[a]
Naphthalene		
	Vegetated	46 ± 19
	Nonvegetated	120 ± 15
	Sterile	112 ± 20
Phenanthrene		
	Vegetated[b]	48
	Nonvegetated	62 ± 4
	Sterile	88 ± 29
Pyrene		
	Vegetated[b]	108
	Nonvegetated	128 ± 8
	Sterile	124 ± 4
Fluoranthene		
	Vegetated	65 ± 10
	Nonvegetated	59 ± 2
	Sterile	58 ± 5

[a] Percent recoveries were calculated from triplicate samples and are reported as the mean ± one standard error from the mean.

[b] Percent recoveries were calculated from duplicate samples and are reported as the mean.

to plants not exposed to phenanthrene (Figure IV - 25). Aboveground plant tissues (leaves and stem) were separated from the lower stem (stem tissue below the top of the flask), roots, and soil. Therefore, evolution of $^{14}CO_2$ in the headspace of the flasks represents recently fixed and translocated ^{14}C-photosynthates. The analyses used in this study could not distinguish between root respiration and microbial degradation of ^{14}C-photosynthates released into the rhizosphere. Thus, the increased evolution of $^{14}CO_2$ in the headspace of plants indicates a shift in carbon allocation by the plant to the roots, and increased root respiration and/or increased exudation of recently fixed photosynthates.

The distribution of ^{14}C in plant tissue for both treatments (control plants and plants exposed to phenanthrene) exhibited leaf to root translocation of ^{14}C-photosynthates with decreasing concentrations of ^{14}C in leaf, stem, lower stem, root tissue, and sand extracts. Both phenanthrene treatments and matched controls had similar quantities of ^{14}C-labeled material in leaf tissues, that is 84.33 ± 5.1 and 81.26 ± 3.1 % of the total recovered $^{14}C \pm$ one standard error for control and phenanthrene treated plants, respectively (Student's t-test, unpaired comparison, $p = 0.6098$) (Table IV - 6). This finding suggests a minimum effect of phenanthrene on the rate of carbon fixation during the exposure period. However, plants exposed to phenanthrene exhibited a higher ^{14}C concentration in the lower stem and root tissues and in sand extracts at the end of the exposure period (Table IV - 6, Figure IV - 26). The higher ^{14}C-residues in belowground tissues and sand are evidence of a shift in carbon allocation to the root tissue of plants exposed to phenanthrene.

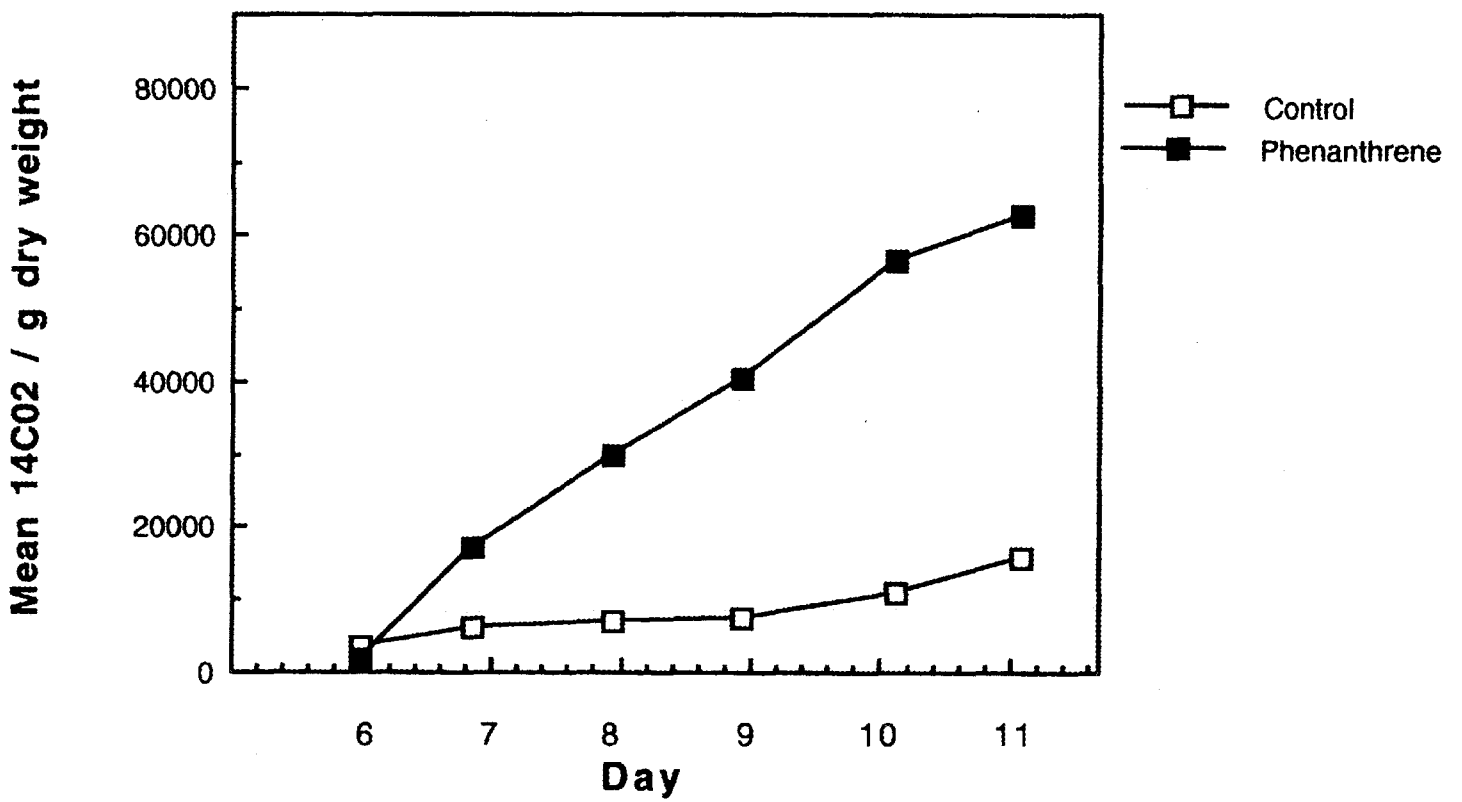


Figure IV - 25. Evolution of $^{14}CO_2$ in the headspace of plants exposed to 250 µg phenanthrene and control plants not exposed to phenanthrene. Plants were pulse-labeled with $^{14}CO_2$ after a 5-day exposure followed by a 5-day chase period.

Table IV - 6. Distribution of ^{14}C-photosynthates in *Melilotus alba* after pulse labeling with $^{14}CO_2$ followed by a 5-day chase period.

		Total ^{14}C[a]				
Treatment	n	Leaf	Upper Stem	Lower Stem[b]	Root	Sand
Vegetated Control	4	84.33 ± 5.1	15.45 ± 5.2	0.161 ± 0.07	0.012 ± 0.002	0.0034
Vegetated 250 µg/g Phenanthrene	4	81.26 ± 3.1	17.88 ± 2.9	0.272 ± 0.18	0.174 ± 0.17	0.005 ± 0.004

[a] Expressed as a mean percent of the total recovered ^{14}C ± one standard error.

[b] Stem tissue below the top of the flask, thus not exposed to $^{14}CO_2$ during the pulse period.

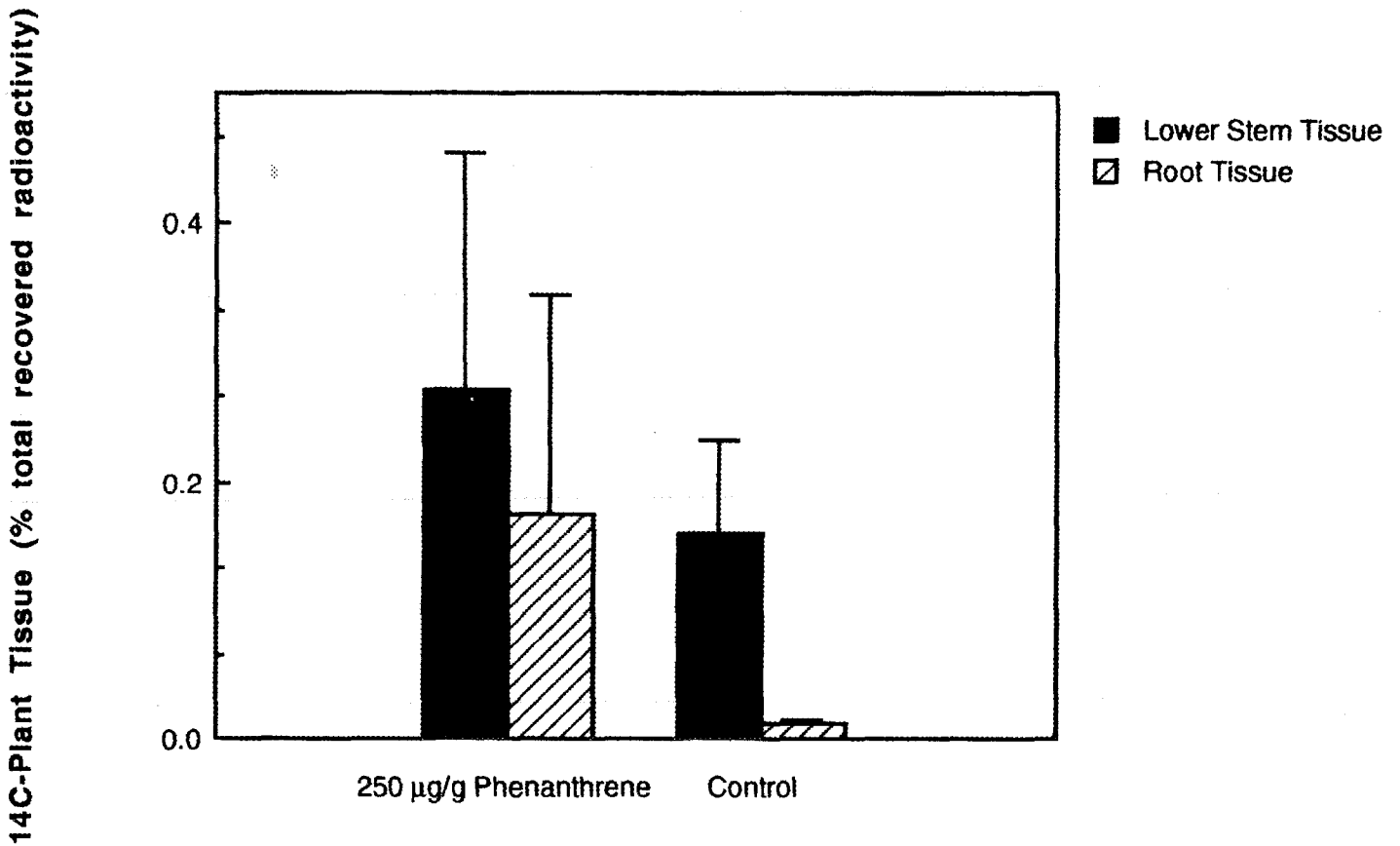


Figure IV - 26. *Melilotus alba* plant tissue concentrations of ^{14}C after a 5-day chase period. Data are expressed as the mean percent of the total radioactivity recovered ± one standard error (n = 4).

D. Soil Respiration and Microbial Biomass

Soil Respiration

Total CO_2 efflux from plant root and microbial respiration was elevated in plants exposed to 250 μg phenanthrene/50 g sand compared to control plants not exposed to phenanthrene (Figure IV - 27). There was no apparent difference between the two treatments after 6-7 days of incubation. Subsequent experiments with 250 μg phenanthrene and two additional concentrations of phenanthrene, 2500 and 5000 μg phenanthrene/50 g sand, did not exhibit this trend, although respiration was greater in plants exposed to the highest concentration of phenanthrene (Figure IV - 28).

Microbial Biomass

Heterotrophic plate counts of subsampled sand from plants exposed to 250 μg phenanthrene/g sand and from control plants not exposed to phenanthrene showed a higher number of microorganisms in the presence of phenanthrene. Table IV - 7 summarizes the CFUs obtained in matched vegetated controls and vegetated treatments with phenanthrene after a 10- or 14-day incubation period in four separate experiments. In some experiments the difference in CFUs obtained from the control plants and exposed plants were an order of magnitude greater when phenanthrene was present.

After 14 days of incubation, CFUs from subsampled sand were significantly greater in vegetated treatments with phenanthrene (Student's t-test, unpaired comparison, $p = 0.0088$) (Figure IV- 29). After removal of the plants and subsequent respike with phenanthrene (250 μg phenanthrene/10 g sand) to the sample jars, control treatments without previous exposure to phenanthrene showed a significant increase in CFUs after 10 days of

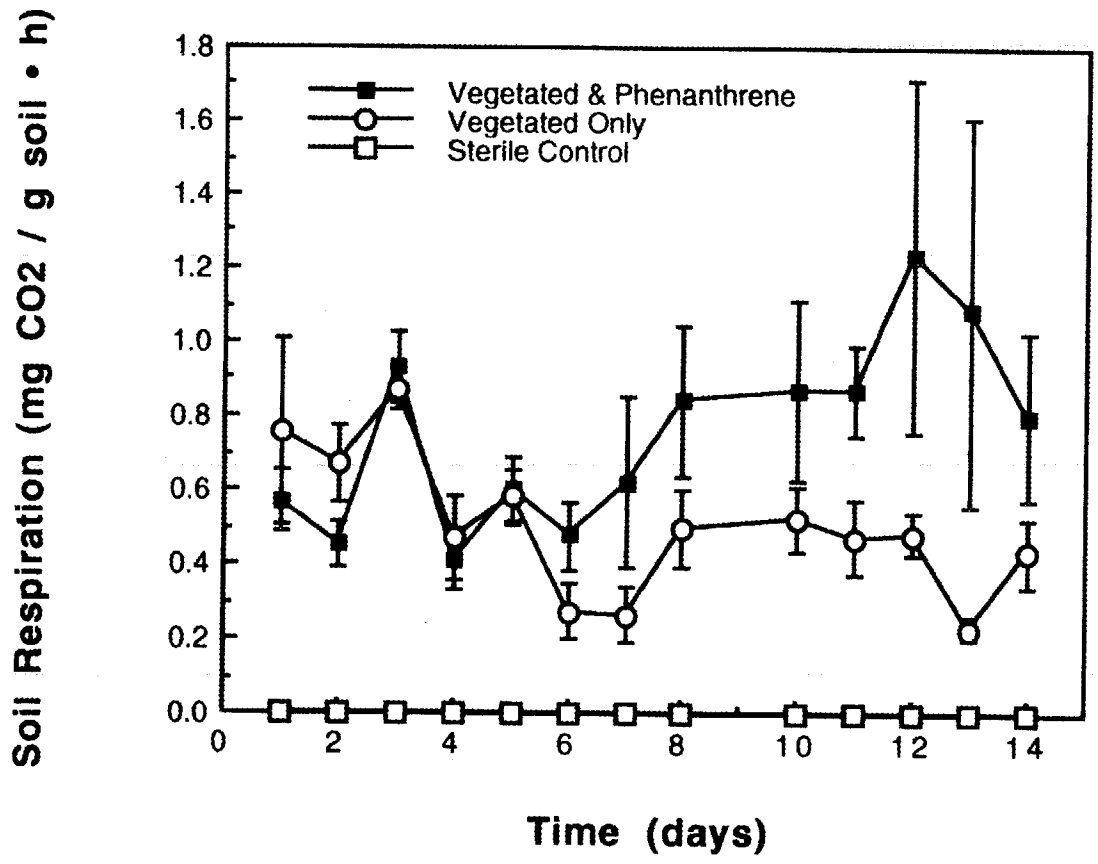


Figure IV - 27. Carbon dioxide efflux from vegetated and nonvegetated sterile controls and vegetated treatments exposed to 250 µg phenanthrene/50 g sand. Data are expressed as the mean ± one standard error (n = 3).

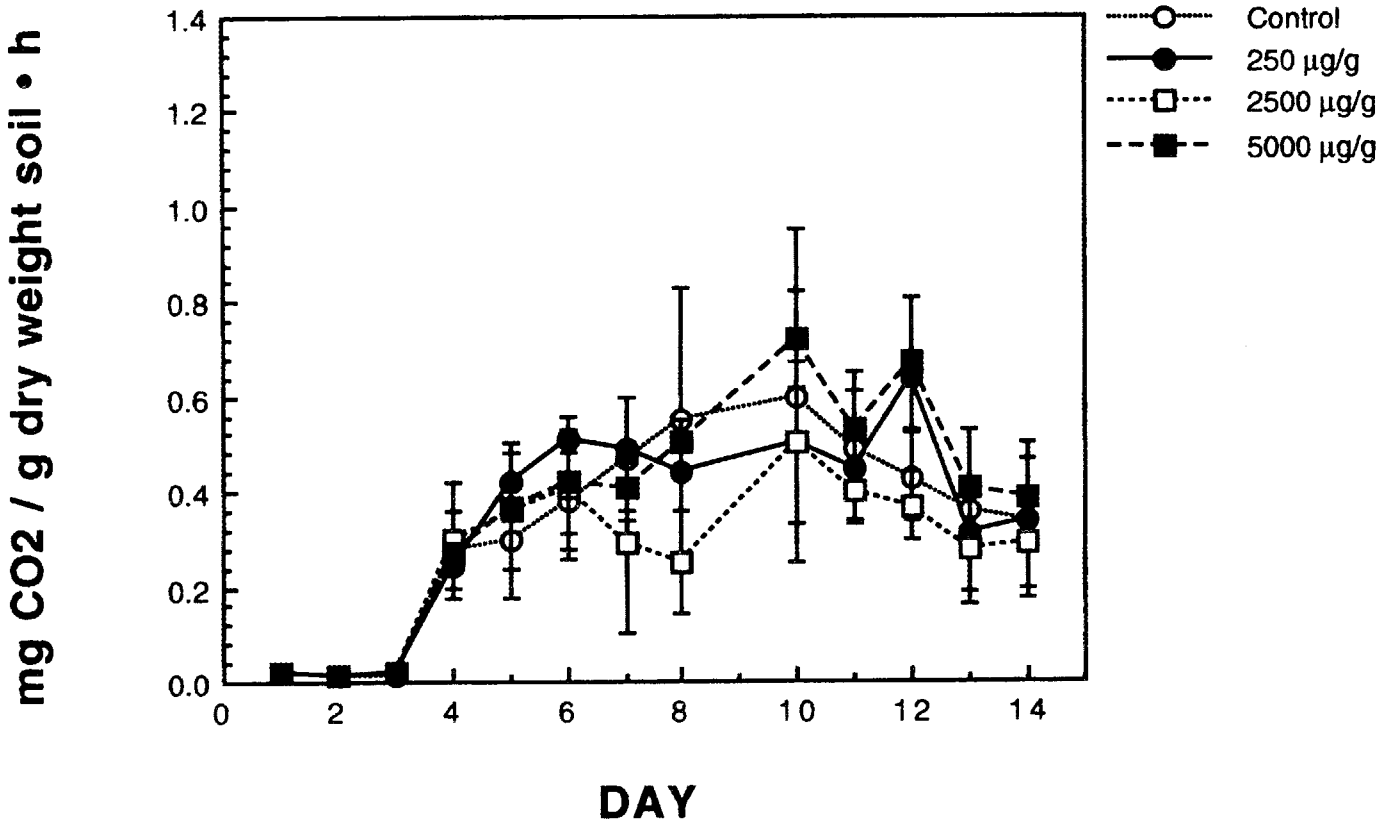


Figure IV - 28. Carbon dioxide efflux from vegetated controls and vegetated treatments exposed to 250, 2500, and 5000 µg phenanthrene/50 g sand. Data are expressed as the mean ± one standard error (n = 3).

Table IV - 7. Heterotrophic plate counts of microorganisms cultured on TSA from four separate experiments. CFUs obtained from plants exposed to phenanthrene are shown with data from matched control plants not exposed to phenanthrene.

Treatment	n	Plant Exposure Time	CFU/g sand
Vegetated Control	2	14	2.01×10^6
Vegetated 250 ppm Phenanthrene	2	14	2.8×10^6
Vegetated Control	3	14	$3.45 \times 10^6 \pm 0.86 \times 10^6$
Vegetated 250 ppm Phenanthrene	3	14	$8.85 \times 10^6 \pm 1.7 \times 10^6$
Vegetated Control	3	10	$3.42 \times 10^6 \pm 2.62 \times 10^6$
Vegetated 250 ppm Phenanthrene	2	10	4.04×10^7
Vegetated Control	3	14	$4.17 \times 10^6 \pm 4.4 \times 10^6$
Vegetated 250 ppm Phenanthrene	3	14	$9.2 \times 10^6 \pm 3.1 \times 10^6$
Vegetated 2500 ppm Phenanthrene	3	14	$5.8 \times 10^6 \pm 2.43 \times 10^6$
Vegetated 5000 ppm Phenanthrene	3	14	$1.68 \times 10^7 \pm 1.89 \times 10^7$

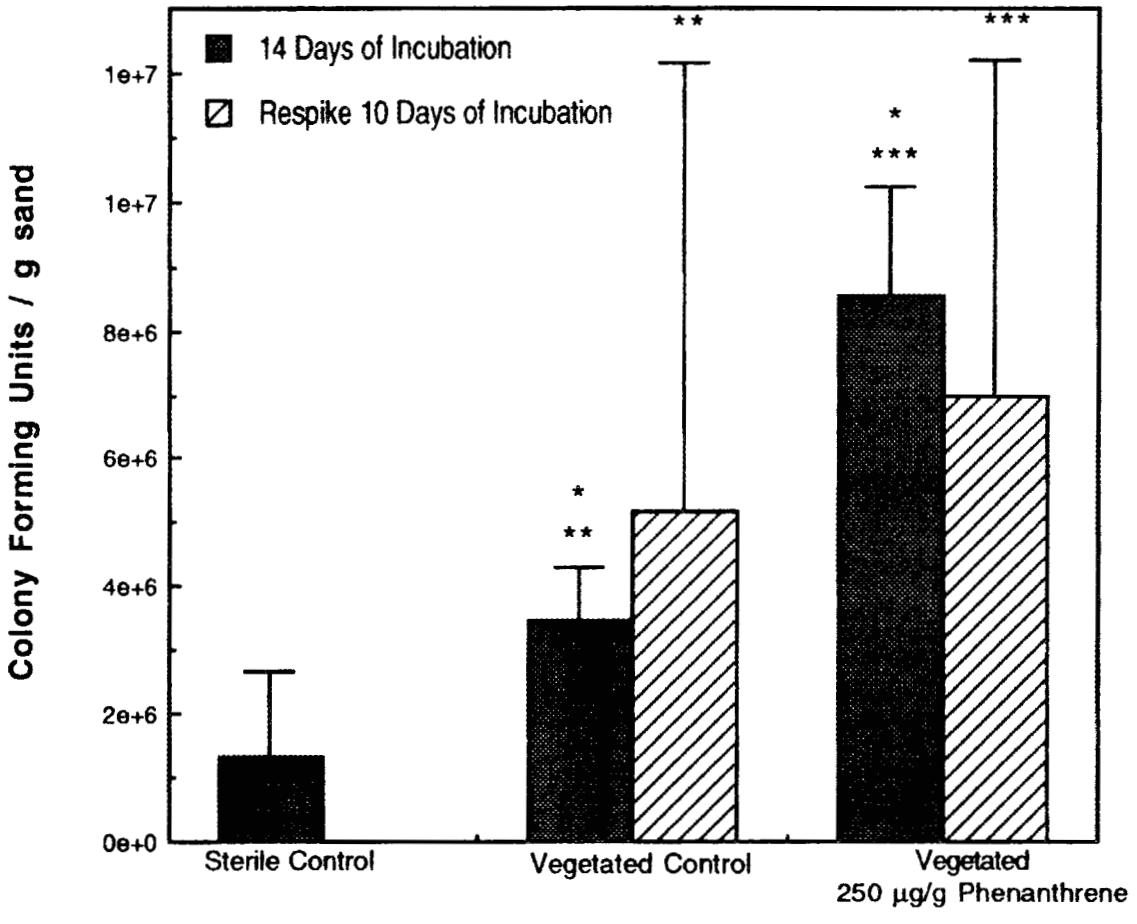


Figure IV - 29. Colony forming units from subsampled sand after a 14-day incubation in sterile controls, vegetated controls, and vegetated sand with 250 µg phenanthrene/50 g sand. Respike CFUs were obtained 10 days after removal of the plant and addition of phenanthrene. *, **, and *** Denote a statistically significant difference (p=0.0088, p=0.0488, and p=0.0031, respectively) (Student's t-test). Data are expressed as the mean ± one standard error (n = 3).

incubation (Student's t-test, paired comparison, $p = 0.0488$). Treatments previously exposed to phenanthrene had a significantly lower number of microorganisms in the absence of the plant compared to earlier counts obtained from the same jars but incubated in the presence of a plant (Student's t-test, paired comparison, $p = 0.0031$).

Using heterotrophic plate counts as an indication of toxicity, phenanthrene toxicity to rhizosphere microorganisms was not exhibited at three concentrations of phenanthrene (Figure IV - 30), although CFUs were lower at 2500 μg/50 g sand. At the highest dose tested, 5000 μg phenanthrene, microbial numbers were elevated over all other treatments, thus, no dose response relationship was established.

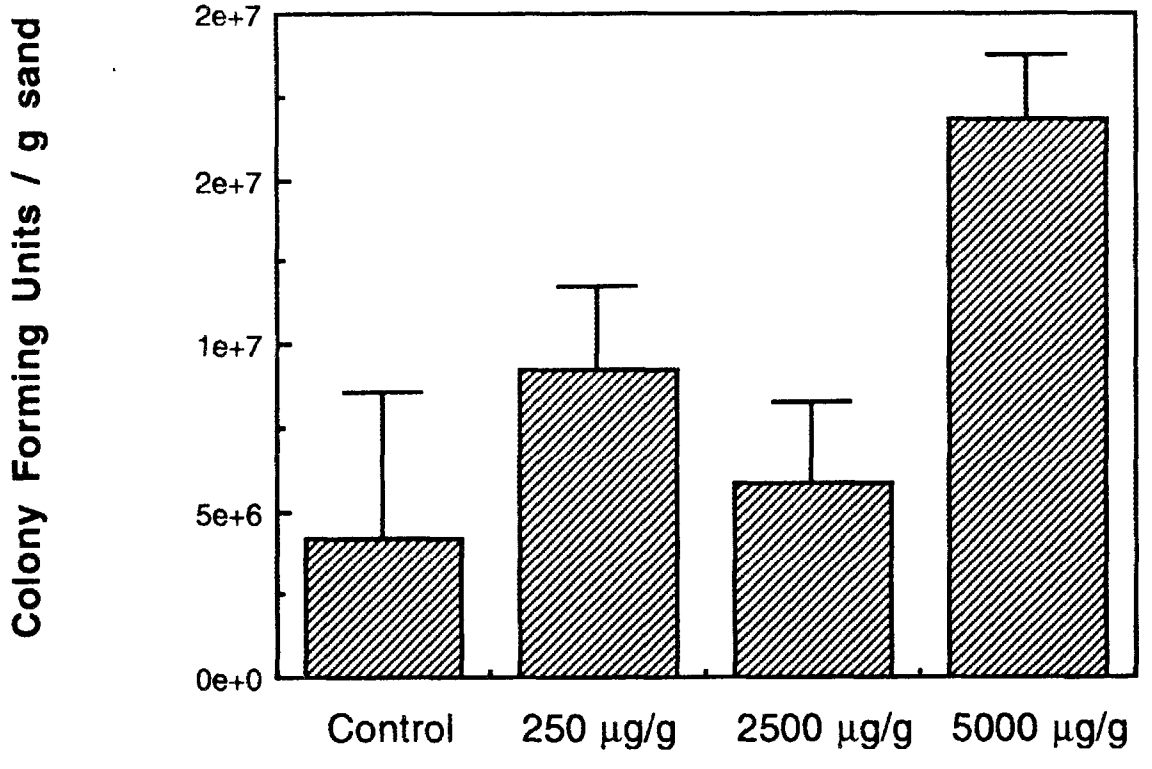


Figure IV - 30. Colony forming units from sand after a 14-day incubation with *Melilotus alba* exposed to 0, 250, 2500, and 5000 µg phenanthrene/50 g sand. Data are expressed as the mean ± one standard error (n = 3).

CHAPTER V
DISCUSSION

The objective of this study was to examine the potential role vegetation may play in the transport of PAHs from soil to the terrestrial food chain. Specifically, laboratory studies were conducted to (1) measure the individual uptake of four ^{14}C-PAHs from soil by a single plant species, and (2) determine the distribution of ^{14}C in plant tissues, soil, and air in PAH uptake experiments. In addition, studies were conducted to were to determine physiological changes in plants and microorganisms exposed to a PAH in the root zone. Experiments were designed to address fundamental questions concerning PAHs in plant-soil systems and the response of plants and microorganisms to a chemical stress in the root zone.

Results from plant uptake and translocation studies of PAHs from soil are confounded by ambiguous routes of plant-tissue exposure and transport, and utilization of high organic carbon soils or sludges that reduce PAH availability (Walton and Hoylman 1992). This study was designed to optimize the potential for root uptake of PAHs from soil and provide a clear route of transport from soil to the aboveground plant tissues. In addition, low organic carbon soil was used and loss from microbial degradation was likely limited due to the short (5-day) exposure period. Results from this study with four PAHs showed the highest concentration of ^{14}C-PAH derived radioactivity in *Melilotus alba* root tissue, with decreasing concentrations translocated to stems and leaves. Plant tissue concentrations and distribution patterns of ^{14}C were consistent with physicochemical properties of the individual PAHs and root uptake processes. For example, the highest root and shoot tissue

concentrations were found in uptake studies with ^{14}C-naphthalene, the most water-soluble PAH tested (30 mg naphthalene • L^{-1} water). Conversely, uptake studies with the least water-soluble PAHs, ^{14}C-pyrene and ^{14}C-fluoranthene, had the lowest plant tissue concentrations of ^{14}C, while ^{14}C-phenanthrene uptake experiments showed intermediate tissue concentrations. Edwards (1988), Topp et al. (1986), and Paterson et al. (1990) found plant root tissue concentrations of lipophilic compounds were proportional to the octanol-water partition coefficient of the compound. However, translocation to aboveground tissues was positively related to aqueous solubility (inversely proportional to octanol-water partition coefficient). The present findings of this study are in keeping with this generalization; that is, aqueous solubility favors translocation of organic compounds from roots to aboveground foliage, whereas lipophilicity favors sorption to organic material such as root tissue.

Results from headspace analyses in vegetated and nonvegetated samples from pyrene experiments strongly suggest an enhanced mineralization of ^{14}C-pyrene in vegetated treatments. In contrast, no apparent difference was indicated between vegetated and nonvegetated treatments exposed to fluoranthene, while results were inconclusive in experiments with naphthalene and phenanthrene. Although the absence of conclusive mineralization data for phenanthrene is possibly due to the placement of the labeled carbon, ring cleavage and subsequent oxidation of the carbon chain for the remaining compounds should have been detected. However, these data must be evaluated in light of the radiochemical purity of the test compounds, which was $\geq$ 97%. In addition, the contributions of microorganisms to the fate of PAHs in plant-soil systems were evaluated under conditions specifically selected to promote plant uptake and were not

conducive to microbial degradation. For example, the soil samples were not collected from a chronically contaminated area, such as the source plume where Madsen et al. (1991) found the highest concentration of PAH-degrading organisms. Other studies have also shown that the highest rates of microbial degradation are found in populations chronically exposed to PAHs (Heitkamp et al. 1988). In addition, many microorganisms capable of degrading PAHs require an initial period for induction of enzyme systems, some as long as 2-3 weeks (Heitkamp et al. 1988). Therefore, the influences of microbial degradation and the role of rhizosphere populations in vegetated soil were not present in this study and warrant a more specific study of these interactions.

Root tissue concentrations presented in this study do not distinguish between surface sorption of the ^{14}C to the outer root tissue and actual uptake of the labeled compound into the root. However, given the minimal translocation to aboveground tissue for phenanthrene, pyrene, and fluoranthene derived radioactivity, surface sorption to the root is the most likely explanation for these larger molecular weight compounds. For example, Briggs et al. (1982) found that the partitioning of chemicals to lipophilic root solids (*i.e.*, surface sorption) was the dominant process for lipophilic compounds such as PAHs. A recent study with carrots grown in sludges contaminated with a range of PAHs, also indicated that higher molecular weight PAHs such as pyrene and fluoranthene were limited to the outermost surface of the carrot root (Wild and Jones 1992).

The largest percentage of recoverable ^{14}C was associated with the soil and was removed with solvent extractions. As exposure time increases, the PAH concentration available to the plant would be expected to decrease due to other competing processes such as increasing sorption to soil material.

However, because the uptake study provided conditions that optimized the potential for root uptake (e.g., low soil organic carbon content, high root to soil ratio, rapidly growing young plants, and exposure to freshly applied compounds), the uptake data presented in this study suggest that root uptake and translocation of the four PAHs tested is a limited process. Site to site variations, plant species differences, and length of exposure must be considered when extrapolating these data to field conditions.

Although aboveground plant tissue ^{14}C concentrations were small relative to soil concentrations, translocation of the labeled material did occur for all PAHs examined. However, bioaccumulation factors for all tested PAHs were <1, and are consistent with findings in the literature (Edwards 1989; Wang and Meresz 1982). A bioaccumulation factor >1 would indicate preferential movement of the compound from the soil into the plant tissues. The data from this study are based on the assumption that the ^{14}C-labeled compound in the plant tissue is parent compound, thus the tissue concentrations may be overestimated. Nonetheless, under laboratory conditions, bioaccumulation of the tested PAHs is not indicated.

While root sorption of PAHs appears to effectively limit translocation of PAHs to aboveground tissues, sorbed PAHs could have a negative effect on the plant root. No indication of toxicity was evident in plants exposed to three concentrations of phenanthrene (250, 2500, and 5000 μg phenanthrene/50 g sand) using soil respiration as an endpoint for toxicity.

Microbial degradation of PAHs may play a significant role in determining the fate of PAHs in plant-soil systems. For example, the potential for plant uptake of lower molecular weight PAHs, such as naphthalene and phenanthrene, is high relative to larger PAHs. Similarly, the physicochemical

characteristics of these compounds also favor microbial degradation. Analyses of sand after incubation with *M. alba* show an increased number of culturable microorganisms in treatments exposed to phenanthrene over control treatments, which may be indicative of microbial utilization of phenanthrene. In addition, the addition of fresh phenanthrene after removal of the plants resulted in an increase in microbial numbers in the control treatments not previously exposed to phenanthrene. In contrast, plate counts from the previously exposed treatments showed a decrease in microbial numbers in the absence of the plant. This contrasting response in "pristine" versus previously exposed microbial communities may signify plant support of a particular microbial community when exposed to phenanthrene and the removal of the plant alters the microbial community previously established.

Enhanced $^{14}CO_2$ evolution in the headspace of plants exposed to phenanthrene, along with greater ^{14}C-root tissue concentrations, indicated a plant shift in carbon allocation in the presence of phenanthrene in the root zone. In addition, the increase in heterotrophic plate counts suggest a microbial response to the increased carbon allocation. Analogous shifts in carbon allocation are observed in plants growing under nutrient-limited conditions and are the means by which plants compensate for the nutrient imbalance (Chapin et al. 1987). Phenotypic plasticity, the alteration of physiological and/or morphological processes in response to environmental conditions is an important evolutionary adaptation in plants (Schlichting 1986). The ability to alter specific physiological characteristics or processes enables plants to persist under dynamic environmental conditions, a particularly adaptive attribute for a sessile organism (Schlichting 1986).

Allocating resources to defense mechanisms, for example defense against herbivory, is an energetic cost and as such, investment by the plant results in decreased resource allocation to growth or reproduction. Independent biotic and abiotic ecological parameters are good predictors of defense allocation (Bazzaz et al. 1987). For example, if the defense mechanism is nitrogen based, plants growing under low nutrient conditions allocate less towards defense compounds while plants with carbon-based defense growing under high light will allocate more carbon to defense compounds (Bazzaz et al. 1987). Plant defense against chemical stress in the root zone may also be determined by ecological parameters such as nutrient availability.

The shift in carbon allocation to belowground processes may result from a carbon sink (movement of carbon from an area of high concentration to an area of low concentration). In studies with the herbicide mecoprop (2-[(4-chloro-*o*-toyl)oxy]propyionic acid), Whipps and Greaves (1986) found an increased allocation of carbon to the roots after spraying aboveground foliage. Concurrently, the authors observed an increase in microbial colonization of the roots by *Pseudomona* sp. and degradation of root cells. The interaction of the herbicide, translocated from the leaves to the roots, with the root tissue resulted in an increase in root exudation and translocation of carbon. These exudates were shown to act as chemical cues for soil microorganisms. The resulting increased colonization and degradation of the root tissue stimulated continued root exudation and translocation. Anthropogenic compounds such as PAHs may act on the root tissue in similar manner and may have resulted in an increased allocation of carbon to the roots in the present study. Thus, plant defense mechanisms against a chemical stress in the root zone may be

initiated by damaged root tissue and subsequent shift in carbon distribution to the site of injury. The microbial response may be secondary and cued by the increase in exudates. Degradation of the chemical inducing stress may occur concurrently with increased colonization of the root and possibly with increased microbial damage to the root tissues.

The variability among replicates and between duplicate experiments using naphthalene and phenanthrene may indicate the need for an increased number of replicates to unequivocally determine the effect of vegetation on microbial degradation of these compounds. Variability among replicates may be a reflection of differences in plant size, resource availability, and the heterogeneous plant-soil environment. However, if this variability is due to inherent inter-individual differences, rather than experimental-based artifacts, increasing the number of replicates under these laboratory conditions will not result in decreased variability and thus may not improve overall statistical significance.

The results of this study strongly suggest that plant uptake of PAHs is a limited mechanism for transport of these contaminants from soil to the terrestrial ecosystem. Consequently, plants may be subjected to high concentrations of PAHs sorbed to the root tissue. Evidence of a shift in carbon allocation to the root zone and elevated soil respiration in *Melilotus alba* when exposed to phenanthrene indicate a change in physiological processes in response to a chemical stress. This shift in carbon allocation lends support to a hypothesized plant-microbial interaction during exposure to a chemical stress in the root zone and impetus for future considerations.

CHAPTER VI
CONCLUSIONS

Under laboratory conditions selected to maximize root uptake, plant tissue distribution of PAH-derived ^{14}C was largely limited to root tissue of *Melilotus alba*. These results suggest that plant uptake of PAHs from contaminated soil via roots, and translocation to aboveground plant tissues (stems and leaves), is a limited mechanism for transport into terrestrial food chains. However, these data also indicate that root surface sorption of PAHs may be important for plants grown in soils containing elevated concentrations of PAHs.

In the present experiments, naphthalene was taken up by white sweetclover to a greater extent than phenanthrene, pyrene, or fluoranthene. Root tissue concentrations of ^{14}C-labeled material were, on average, three times greater in naphthalene uptake experiments compared to the other PAHs tested. In addition to ^{14}C-concentrations being highest in root tissue from naphthalene experiments, ^{14}C-concentrations were also highest in leaves and stems of ^{14}C-naphthalene exposed plants. Nonetheless, even in naphthalene experiments < 0.5 % of the total recovered ^{14}C reached aboveground foliage. For all tested PAHs, no evidence of bioaccumulation in *Melilotus alba* tissues after a 5-day exposure was found. However, because only radiocarbon was measured in these experiments, ^{14}C-PAHs and ^{14}C-PAH metabolites are indistinguishable, no further inferences are possible regarding the toxicological consequences of the trace quantities of compounds found in vegetation. Furthermore, under actual field conditions, microbial degradation of PAHs and

soil sorption could both limit the availability of PAHs for plant uptake and help to mitigate any potential toxicity.

Root surface sorption of PAHs may be an important route of exposure for plants in soils containing elevated concentrations of PAHs. Consequently, the root-soil interface may be the site of plant-microbial interactions in response to a chemical stress. In this study, evidence of a shift in carbon allocation to the root zone of plants exposed to phenanthrene and corresponding increases in soil respiration and heterotrophic plate counts provide evidence of a plant-microbial response to a chemical stress.

Future areas of research into plant-defense mechanisms should determine (1) plant species differences, (2) isolation of specific chemical signals, (3) time-series assessment of plant carbon allocation and microbial community characterization, (4) separation of the chemical's effect on rhizosphere microorganisms from the plant's effect, and (5) the effect of nutrient status and carbon:nitrogen balance on allocation of plant resources while exposed to a chemical stress in the root zone.

The results of this study establish the importance of the root-soil interface for plants growing in PAH contaminated soil and indicate the existence of plant-microbial interactions in response to a chemical stress. These results may provide new avenues of inquiry for studies of plant toxicology, plant-microbial interactions in the rhizosphere, and environmental fates of soil contaminants. In addition, the utilization of plants to enhance the biodegradation of soil contaminants may require evaluation of plant physiological changes and plant shifts in resource allocation.

LIST OF REFERENCES

CHAPTER VII

LIST OF REFERENCES

Ahmadjian, V. and S. Paracer. 1986. Symbiosis: An Introduction to Biological Associations. University Press of New England, Hanover and London, 212 p.

Anderson, T. A. 1991. Comparitive Plant Uptake and Microbial Degradation of Trichloroethylene in the Rhizosphere of Five Plant Species–Implications for Bioremediation of Contaminated Surface Soils. Ph. D. Thesis, University of Tennessee, Knoxville, TN.

Anderson, T. A., E. A. Guthrie, and B. T. Walton. 1993. Bioremediation in the rhizosphere. Environ. Sci. Technol. (in press)

Aprill, W. and R. C. Sims. 1990. Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere. 20:253-265.

Ashton, F. M. and A. S. Crafts. 1981. Mode of Action of Herbicides. John Wiley & Sons, New York. 525 p.

Atlas, R. and R. Bartha. 1993. Microbial Ecology: Fundamentals and Applications. Benjamin/Cummings Publishing Co., Menlo Park, CA. 563 p.

Barber, D. A. and J. M. Lynch. 1977. Microbial growth in the rhizospere. Soil Biol. Biochem. 9:305-308.

Bartha, R. 1990. Isolation of Microorganisms that Metabolize Xenobiotic Compounds. *In* (D. P. Labeda, ed.) Isolation of Biotechnological Organisms from Nature. McGraw-Hill Publishing Co., New York. pp. 283-397.

Bazin, M. J., P. Markham, E. M. Scott and J. M. Lynch. 1990. Population Dynamics and Rhizosphere Interactions. *In* (J. M. Lynch, ed.) The Rhizosphere. John Wiley & Sons, New York. pp. 99-127.

Bazzaz, F. A., N. R. Chiariello, P. D. Coley, and L. F. Pitelka. 1987. Allocating resources to reproduction and defense. BioScience. 37:58-67.

Bell, R. M. 1992. Higher Plant Accumulation of Organic Pollutants from Soils. U.S. Environmental Protection Agency. Office of Research and Development. U.S. Department of Commerce, NTIS, Springfield, VA. EPA-600/R-92/138.

Bligh, E. G. and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochemic. Physiol.. 37:911-917.

Boldrin, B., A. Tiehm, and C. Fritzsche. 1993. Degradation of phenanthrene, fluorene, fluoranthene, and pyrene by a *Mycobacterium* sp. Appl. Environ. Microbiol. 59:1927-1930.

Bossert, I. D., W. M. Kachel and R. Bartha. 1984. Fate of hydrocarbons during oily sludge disposal in soil. Appl. Environ. Microbiol. 47:763-767.

Bray, G. A. 1960. Anal. Biochem. 1:279

Briggs, G. G., R. H. Bromilow, and A. A. Evans. 1982. Relationships between lipophilicity and root uptake and tanslocation of non-ionised chemicals by barley. Pestic. Sci. 13:495-504.

Bulman, T. L., S. Lesage, P. Fowler and M. Webber. 1985. The Persistence of Polynuclear Aromatic Hydrocarbons in Soil. Environment Canada, Pace Report No. 85-2.

Byshe, S. E. 1990. Bioconcentration Factor in Aquatic Organisms. *In* (W. J. Lyman, W. F. Reehl, and D. H. Rosanblatt, eds.) Handbook of Chemical Property Estimation Methods. American Chemical Society, Washington, D.C. pp.

Campbell, R. 1985. Plant Microbiology. Edward Arnold, Baltimore, MD. 191 p.

Campbell, R. and M. P. Greaves. 1990. Anatomy and Community Structure of the Rhizosphere. *In* (J. M. Lynch, ed.) The Rhizosphere. John Wiley & Sons, New York. pp. 11-34.

Cerniglia,C. E. and M. A. Heitkamp. 1989. Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAH) in the Aquatic Environment. *In* (U. Varanasi, ed.) Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. CRC Press, Inc., Boca Raton, FL. pp. 41-68.

Cerniglia, C. E. 1984. Microbial Transformation of Aromatic Hydrocarbons. *In* (R. M. Atlas, ed.) Petroleum Microbiology. Macmillan, New York. pp. 99-128

Chapin, F. S., A. J. Bloom, C. B. Field, and R. H. Waring. 1987. Plant response to multiple environmental factors. BioScience. 37:49-57.

Coover, M. P. and R. C. Sims. 1989. The rate of benzo[a]pyrene apparant loss in a natural and manure amended clay loam soil. Haz. Waste Haz. Mat. 4:151-158.

Curl, E. A. and B. Truelove. 1986. The Rhizosphere. Springer-Verlag, Berlin Heidelberg. 288 p.

Darvill, A. G. and P. Albersheim. 1984. Phytoalexins and their elicitors–a defense against microbial infection in plants. Ann. Rev. Plant Physiol. 35:243-275.

Day, P. R. 1965. Particle Fractionation and Particle-Size Analyses. *In* (C. A. Black, ed.) Methods of Soil Analysis-Part I. Agronomy 9. American Society of Agronomy, Madison, WS. pp. 545-567.

Défago, G. and D. Haas. 1990. Pseudomonads as antagonists of soilborne plant pathogens: mode of action and genetic analysis. Soil Biochem. 6:249-291.

Défago, G., C. H. Berling, U. Burger, D. Haas, G. Kahr, C. Keel, C. Voisard, Ph. Wirthner, and B. Wütrich. 1990. Suppression of Black root rot of Tobacco by a Pseudomonas Strain: Potential Applications and Mechanisms. *In* (D. Hornby, ed.) Biological Control of Soil-borne Plant Pathogens. CAB Internation. pp. 93-108.

Dharmatilake, A. J., and W. D. Bauer. 1992. Chemotaxis of *Rhizobium meliloti* towards nodulation gene-inducing compounds from alfalfa roots. Appl. Environ. Microbiol. 58:1153-1158.

Edwards, N. T. 1992. A timesaving technique for measuring respiration rates in incubated soil samples. Soil Sci. Am. J. 46:1114-1116.

Edwards, N. T. 1983. Polycyclic aromatic hydrocarbons (PAH's) in the terrestrial environment: A review. J. Environ. Qual. 12:427-441.

Edwards, N. T. 1986. Uptake translocation and metabolism of anthracene in bush bean (*Phaseolus vulgaris* L.). Environ. Toxicol. Chem. 5:659-665.

Edwards, N. T. 1988. Assimilation and Metabolism of Polycyclic Aromatic Hydrocarbons by Vegetation — An Approach to This Controversial Issue and Suggestions for Future Research. *In* (M. Cooke and A. J. Dennis, eds.) Polynuclear Aromatic Hydrocarbons: A Decade of Progress, Battelle Press, Columbus, OH. pp. 211-229.

Edwards, N. T. 1991. Fate and Effects of PAHs in the Terrestrial Environment: An Overview. *In* (T. J. Moser, J. R. Barker, and D. T. Tingey, eds.) Ecological Exposure and Effects of Airborne Toxic Chemicals: An Overview. EPA Report No. 600/3-91/001. pp. 48-59.

Fendinger, J. J. and D. E. Glotfelty. 1990. Henry's Law constants for selected pesticides, PAHs and PCBs. Environ. Tox. Chem. 9:731-735.

Fisher, R. F. and S. R. Long. 1992. *Rhizobium*–plant signal exchange. Nature. 357:655-660.

Fitter, A. H. and R. K. M. Hay. 1987. Environmental Physiology of Plants. Academic Press, London. 423 p.

Forrest, V., T. Cody, J. Caruso, and D. Warshawsky. 1989. Influence of the carcinogenic pollutant benzo(a)pyrene on plant development: Fern gametophytes. Chem.-Biol. Interactions. 72:295-307.

Garrett, H. E., J. L. Carney and H. G. Hedrick. 1982. The effects of ozone and sulfur dioxide on respiration of ectomycorrhizal fungi. Can. J. For. Res. 12:141-145.

Gibson, D. T. 1982. Microbial degradation of hydrocarbons. Environ. Toxicol. Chem. 5:237-250.

Gibson, D. T. 1984. Microbial Degradation of Organic Compounds. Marcel Dekker, New York. 535 p.

Gregory, P. J. and B. J. Atwell. 1991. The fate of carbon in pulse-labelled crops of barley and wheat. Plant Soil. 136:205-213.

Hance, R. J. Adsorption and Bioavailability. *In* (R. Grover, ed.) Environmental Chemistry of Herbicides, Vol I. Boca Raton, Fla.: CRC Press, 1988, pp. 1-19.

Hansch, C. H. and A. Leo. 1979. Substituent Constants for Correlations Analysis in Chemistry and Biology. John Wiley & Sons, New York. 249 p.

Hansch, C. H. and A. Leo. 1984. Log P Database. Pomona College, Claremont CA.

Hartzell, A. and F. Wilcoxon. 1930. Naphthalene fumigation at controlled concentrations. J. Econom. Entomol. 23:608-618.

Hatzios, K. K. 1991. Biotransformations of Herbicides in Higher Plants. *In* (R. Grover and A. J. Cessna, eds.) Environmental Chemistry of Herbicides. Vol. II. CRC Press, Boca Raton, Fl. pp. 141-187.

Hatzios, K. K. and D. Penner. 1982. Metabolism of Herbicides in Higher Plants. Burgess Publishing Company, Minneapolis, MN. pp. 15-74.

Hawes, M. C. 1990. Living plant cells released from the root cap: A regulator of microbial populations in the rhizosphere? Plant Soil. 129:19-27.

Hedges, R. W. and E. Messens. 1990. Genetic Aspects of Rhizosphere Interactions. *In* (J. M. Lynch, ed.) The Rhizosphere. John Wiley & Sons, New York. pp. 129-176.

Heitkamp, M. A., J. P. Freeman, and C. E. Cerniglia. 1987. Naphthalene biodegradation in environmental microcosms: Estimates of degradation rates and characterization of metabolites. Appl. Environ. Microbiol. 53:129-136.

Heitkamp, M. A. and C. E. Cerniglia. 1987. Effects of chemical structure and exposure on the microbial degradation of polycyclic aromatic hydrocarbons in freshwater and estuarine ecosystems. Environ. Toxicol. Chem. 6:535-546.

Heitkamp, M. A. and C. E. Cerniglia. 1988. Mineralization of polycyclic aromatic hydrocarbons by a bacterium isolated from sediment below an oil field. Appl. Environ. Microbiol. 54:1612-1614.

Heitkamp, M. A., W. Franklin, and C. E. Cerniglia. 1988. Microbial metabolism of polycyclic aromatic hydrocarbons: Isolation and characterization of a pyrene-degrading bacterium. Appl. Environ. Microbiol. 54:2549-2555.

Hsu, T. S. and R. Bartha. 1979. Accelerated mineralization of two organophosphate insecticides in the rhizosphere. Appl. Environ. Microbiol. 37:36-41.

Jardine, P. M., N. L. Weber, and J. F. McCarty. 1989. Mechansisms of dissolved organic carbon adsorption on soil. Soil Sci. Am. J. 53:1378-1385.

Jen, M. S., A. M. Hoylman, N. T. Edwards, and B. T. Walton. Gaseous deposition of ^{14}C-toluene to soybean (*Glycine max*) foliage. (submitted to Environ. Exper. Bot.)

Johnson, T. R. and C. L. Case. 1992. Laboratory Experiments in Microbiology, 3rd ed., Benjamin/Cummings Publishing Co., Redwood City, CA. 372 p.

Jones, K. C., J. A. Stratford, K. S. Waterhouse, E. T. Furlong, W. Giger, R. A. Hites, C. Schaffner and A. E. Johnston. 1988. Increases in the polynuclear aromatic hydrocarbon content of an agricultural soil over the last century. Environ. Sci. Technol. 23:95-101.

Jones, K. C., J. S. Stratford, K. S. Waterhouse, and N. B. Vogt. 1989a. Organic contaminants in Welsh soils: Polynuclear aromatic hydrocarbons. Environ. Sci. Technol. 23:540-550.

Jones, K. C., J. A. Stratford, P. Tidridge, K. S. Waterhouse and A. E. Johnston. 1989b. Polynuclear aromatic hydrocarbons in an agricultural soil: Long-term changes in profile distribution. Environ. Poll. 56:337-351.

Jones, K. C., G. Grimmer, J. Jacob, and A. E. Johnston. 1989c. Changes in the polynuclear aromatic hydrocarbon content of wheat grain and pasture grassland over the last century from one site in the U.K. Sci. Total Environ. 78 :117-130.

Jones, K. C., G. Sanders, S. R. Wild, V. Burnett, and A. E. Johnston. 1992. Evidence for a decline of PCB's and PAHs in rural vegetation and air in the United Kingdom. Nature. 356:137-140.

Joshi, M. M. and J. P. Hollis. 1977. Interaction of *Beggiatoa* and rice plant: Detoxification of hydrogen sulfide in the rice rhizosphere. Science. 191:179-180.

Kampe, W. 1988. Organic Substances in Soils and Plants After Intensive Applications of Sewage Sludge. *In* (A. H. Dirkzwager and P. L'Hermite, eds.) Sewage Sludge Treatment and Use: New Developments, Technological Aspects and Environmental Effects. Elsevier Applied Science, London. pp. 180-185.

Karickhoff, S. W. 1981. Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. Chemosphere. 10:833-46.

Kaufman, D. D. 1983. Fate of Toxic Organic Compounds in Land-Applied Wastes. *In* (J. F. Parr, P. B. Marsh, and J. M. Kla, eds.) Land Treatment of Hazardous Wastes. Noyes Data Corp., Park Ridge, NJ. pp. 77-151.

Kauss, P. B. and T. C. Hutchinson. 1975. The effects of water-soluble petroleum components on the growth of *Chlorella vulgaris* beijerinck. Environ. Pollut. 9:157-174.

Kelley, I., J. P. Freeman, F. E. Evans, and C. E. Cerniglia. 1991. Identification of a carboxylic acid metabolite from catabolism of fluoranthene by a *Mycobacterium* sp. Appl. Environ. Microbiol. 57:636-641.

Kelley, I., J. P. Freeman, F. E. Evans, and C. E. Cerniglia. 1993. Identification of metabolites from the degradation of fluoranthene by *Mycobacterium* sp. strain PYR-1. Appl. Environ. Microbiol. 59:800-806.

Klein, D. A., J. L. Salzwedel, and F. B. Dazzo. 1990. Microbial Colonization of Plant Roots. *In* (J. P. Nakas and C. Hagedorn, eds.) Biotechnology of Plant-Microbe Interactions. McGraw-Hill Publishing Company, New York. p. 190.

Kloepper, J. W., J. Leong, M. Teintze, and M. N. Schroth. 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature. 286:885-886.

Kloepper, J. W., R. Rodriguez-Kábana, J. A. McInroy, and D. J. Collins. 1991. Analysis of populations and physiological characterization of microorganisms in rhizospheres of plants with antagonistic properties to phytopathogenic nematodes. Plant Soil. 136:95-102.

Kraffczyk, I., G. Trolldenier and H. Beringer. 1984. Soluble root exudates of maize: Influence of potassium supply and rhizosphere microorganisms. Soil Biol Biochem. 16:315-322.

Lamoureux, G. L. and D. S. Frear. 1979. Pesticide Metabolism in Higher Plants: In Vitro Enzyme Studies. *In* (G. D. Paulson, D. S. Frear and E. P. Marks, eds.) Xenobiotic Metabolism: In Vitro Methods. American Chemical Society, Washington, D. C. pp. 77-128.

Linz, D. G., E. F. Neuhauser, and A. C. Middleton. 1991. Perspectives on Bioremediation in the Gas Industry. *In* (G. S. Sayler, R. Fox, and J. W. Blackburn, eds.) Environmental Biotechnology for Waste Treatment. Plenum Press, New York. pp. 25-36.

Lynch, J. M. 1986. Rhizosphere microbiology and its manipulation. Biol. Agric. Horticult. 3:143-152.

Lynch, J. M. 1990. Microbial Metabolites. *In* (J. M. Lynch, ed.) The Rhizosphere. John Wiley & Sons, New York. pp. 177-206.

Lynch, J. M. and J. M. Whipps. 1990. Substrate flow in the rhizosphere. Plant Soil. 129:1-10.

Mackay, D. and W. Y. Shiu. 1977. A Critical Review of Henry's Law Constants for Chemicals of Environmental Interest. J. Chem. Eng. Data. 22:399.

Madsen, E. L., J. L. Sinclair, and W. C. Ghiorse. 1991. In Situ biodegradation: Microbiological patterns in a contaminated aquifer. Science. 252:830-833.

Malamy, J., J. P. Carr, D. F. Klessig, and I. Raskin. 1990. Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. Science. 250:1002-1004.

McCool, P. M. 1988. Effect of Air Pollutants on Mycorrhizae. *In* (S. Schulte-Hosted, N. M. Darrall, L. W. Blank, and A. R. Wellburn, eds.) Air Pollution and Plant Metabolism. Elsevier Applied Science Publishers, LTD, New York, 356 p.

Métraux, J. P., H. Signer, J. Ryals, E. Ward, M. Wyss-Benz, J. Gaudin, K. Raschdorf, E. Schmid, W. Blum, and B. Inverardi. 1990. Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. Science. 250:1004-1006.

Mihelcic, J. R. and R. G. Luthy. 1988. Degradation of polycyclic aromatic hydrocarbon compounds under various redox conditions in soil-water systems. Appl. Environ. Microbiol. 54:1182-1187.

Murarka, I. P. 1990. Manufactured gas plant site investigations. EPRI Journal. September:40-45.

National Academy of Sciences. 1972. Committee on Biological Effects of Atmospheric Pollutants: Particulate Polycyclic Organic Matter. NAS, Washington, D.C.

Negishi T., M. Nakano, S. Kobayashi, and C. H. Kim. 1987. Isolation and determination of benzo-a-pyrene glucuronide and sulfate cunjugates in soybean leaves. Bull. Environ. Contam. Toxicol. 39 :294-298.

O'Connor, G. A., R. L. Chaney, and J. A. Ryan. 1991. Bioavailability to plants of sludge-borne toxic organics. Rev. Environ. Contamn. Toxicol. 121:129-155.

Park, K. S., R. C. Sims, R. R. Dupont, W. J. Doucette and J. E. Matthews. 1990. Fate of polycyclic aromatic hydrocarbon compounds in two soil types: Influence of volatilization, abiotic loss and biological activity. Environ. Toxicol. Chem. 9:187-195.

Paterson, S., D. Mackay, D. Tam, and W. Y. Shiu. 1990. Uptake of organic chemicals by plants: A review of processes, correlations and models. Chemosphere. 21:297-331.

Phillips, D. H. 1985. Chemical Carcinogens. *In* (P. B. Farmer and J. M. Walker, eds.) The Molecular Basis of Cancer. John-Wiley & Sons, New York. pp. 133-179.

Redmond, J. W., M. Batley, M. A. Djordjevic, R. W. Innes, P. L. Kuempel, and B. G. Rolfe. 1986. Flavones induce expression of nodulation genes in Rhizobium. Nature. 323:632-635.

Rovira, A. D. and C. B. Davey. 1974. Biology of the Rhizosphere. *In* (E. W. Carson, ed.) The Plant Root and its Environment. University Press of Virginia, Charlottesville. pp. 154-204.

Ryan, J. A., R. M. Bell, J. M. Davidson, and G. A. O'Connor. 1988. Plant uptake of non-ionic organic chemicals from soils. Chemosphere. 17:2299-2323.

Sakamoto, M., S. Kobayashi, M. Nakano, and T. Negishi. 1984. Identification of benzo(a)pyrene quinone in soybean leaves. Sci. Total Environ. 37:247-251.

Sandermann, H., Jr. 1992. Plant metabolism of xenobiotics. Tr. Biochemic. Sci. 17:82-84.

Scheunert, I. 1986. Prediction of Chemicals' Behavior in Soil-Plant Systems from Physico-Chemical Properties. *In* Environmental Modelling for priorty setting among existing chemicals: Workshop, 11-13 Nov. 1985. Munchen-Neuherberg: Proceedings, c1986, pp.279-298

Scheunert, I., D. Vockel, J. Schmitzer and F. Korte. 1987. Biomineralization rates of ^{14}C-labelled organic chemicals in aerobic and anaerobic suspended soil. Chemosphere 16:1031-1041.

Schippers, B., A. W. Bakker, P. A. H. M. Bakker, and R. Van Peer. 1990. Beneficial and deleterious effects of HCN-producing pseudomonads on rhizosphere interactions. Plant Soil. 129:75-83.

Schlichting, C. D. 1986. The evolution of phenotypic plasticity in plants. Ann. Rev. Ecol. Syst. 17:667-693.

Shimabukuro, R. H. 1985. Detoxication of Herbicides. *In* (S. O. Duke, ed.) Weed Physiology, Vol. II: Herbicide Physiology. CRC Press, Boca Raton, Fl. pp. 215-240.

Sims, R. C. and M. R. Overcash. 1983. Fate of polynuclear aromatic compounds (PNAs) in soil-plant systems. Res. Rev. 88:1-68.

Smith, I. M. PAH from Coal Utilisation--Emissions and Effects, London, 1984. IEA Coal Research Report No. ICTIS/TR29.

Spaink, H. P. 1992. Rhizobial lipo-oligosaccharides: Answers and questions. Plant Mol. Biol. 20:977-986.

Stotsky, G., W. Culbreth, and L. B. Mish. 1961. A sealing compound for use in biological work. Nature. 191:410.

The Merck Index. 1989. 11th ed., Merck & Co., Inc, Rahway, N. J.

Topp, E., I. Scheunert, A. Attar and F. Korte. 1986. Factors affecting the uptake of ^{14}C-labeled organic chemicals by plants from soil. Ecotox. Environ. Saf. 11:219-228.

USEPA. 1980. Ambient Water Quality Criteria Doc: Polynuclear Aromatic Hydrocarbons.

Voisard, C., C. Keel, D. Haas, and G. Défago. 1989. Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. EMBO J. 8:351-358.

Walton, B. T. and A. M. Hoylman. 1992. Uptake, Translocation, and Accumulation of Polycyclic Aromatic Hydrocarbons in Vegetation. Electric Power Research Company, Palo Alto, CA. EPRI TR-101651.

Walton, B. T. and N. T. Edwards. 1986 Accumulation of Organic Waste Constituents in Terrestrial Biota. *In* (R. C. Loehr and J. F. Malina, Jr., eds.) Land treatment: A Hazardous Waste Management Alternative. University of Texas Press, Austin, Tx. pp. 73-86.

Walton, B. T. and T. A. Anderson. 1990. Microbial degradation of trichloroethylene in the rhizosphere: Potential application to biological remediation of waste sites. Appl. Environ. Microbiol. 56:1012-1016.

Walton, B. T., A. M. Hoylman, M. M. Perez, T. A. Anderson, T. R. Johnson, E. A. Guthrie, and R. F. Christman. Rhizosphere Microbial Communities as a Plant Defense Against Toxic Substances in Soils. *In* (T. A. Anderson and J. R. Coates, eds.) Microbial Degradation of Organic Chemicals in the Rhizosphere: Implications for Bioremediation. American Chemical Society, Washington, D.C. (in press)

Wang, D. T. and O. Meresz. 1982. Occurrence and Potential Uptake of Polynuclear Aromatic Hydrocarbons of Highway Traffic Origin by Proximally Grown Food Crops. *In* (M. Cooke, A. J. Dennis, and G. L. Fisher, eds.) Polynuclear Aromatic Hydrocarbons: Physical And Biological Chemistry. Battelle Press, Columbus, OH. pp. 885-896.

Weast, R. C. 1986-87. Handbook of Chemistry and Physics. 67th ed., CRC Press, Boca Raton, FL.

Webber, M. D. and J. D. Goodin. Studies on the fate of organic contaminants in sludge treated soils. Proceedings of the European Communities Land Application of Sludge Working Group. Braunschweig Germany (1990). (in press)

Weissenfels, W. D., M. Beyers, and J. Klein. 1990. Degradation of phenanthrene, fluorene and fluoranthene by pure bacterial cultures. Appl. Microbiol. Biotechnol. 32:479-484.

Welch, H. E., D. C. G. Muir, B. N. Billeck, W. L. Lockhart, G. J. Brunskill, H. J. Kling, M. P. Olson and R. M. Lemoine. 1991. Brown snow: A long-range transport event in the Canadian Arctic. Environ. Sci. Technol. 25:280-286.

Weller, D. M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopath. 26:379-407.

Whipps, J. M. 1990. Carbon Economy. *In* (J. M. Lynch, ed.) The Rhizosphere. John Wiley & Sons, New York, 129 p.

Whipps, J. M. and M. P. Greaves. 1986. Effect of mecoprop on plant growth and distribution of photosynthate in wheat (*Triticum aestivum* L.) seedlings. Weed Res. 26:227-232.

Wild, S. R. and K. C. Jones. 1992. Polynuclear aromatic hydrocarbon uptake by carrots grown in sludge-amended soil. J. Environ. Qual. 21:217-225.

Wilkinson, C. F. 1980. The Metabolism of Xenobiotics: A Study in Biochemical Evolution. *In* (H. Witschi, ed.) The Scientific Basis of Toxicity Assessment. Elsevier/North-Holland Biomedical Press, Amsterdam. pp. 251-268.

Williams, G. M. and J. H. Weisburger. 1986. Chemical Carcinogens. *In* (C. D. Klaassen, M. O. Amdur and J. Doull, eds.) Casarett and Doull's Toxicology: The Basic Science of Poisons. Third Edition. Macmillan Publishing Company, New York. pp. 99-173.

Witte, H., T. Langenohl, and G. Offenbacher. 1988. Investigation of the entry of organic pollutants into soils and plants through the use of sewage sludge in agriculture. Korrespondenz Abwasser. 13(35 Jahrgang):118-136.

Worthing, C. R. and S. B. Walker. 1983. Pesticide Manual—A World Compendium. 7th ed. The Lavanham Press Limited, Great Britain, 390 p.

Zander, M. 1983. Physical and Chemical Properties of Polycyclic Aromatic Hydrocarbons. *In* (A. Bjorseth, ed.) Handbook of Polycyclic Aromatic Hydrocarbons. Marcel Dekker, Inc., New York. pp. 1-25.

INTERNAL DISTRIBUTION

1. L. D. Bates
2. J. H. Cushman
3. N. T. Edwards
4. D. E. Fowler
5. C. W. Gehrs
6. S. E. Herbes
7. S. G. Hildebrand
8-12. A. M. Hoylman
13. P. Kanciruk
14. A. V. Palumbo
15. D. E. Reichle
16. F. E. Sharples
17. D. S. Shriner
18. S. H. Stow
19-22. B. T. Walton
23. Central Research Library
24-38. ESD Library
39-40. Laboratory Records Dept.
41. Laboratory Records, ORNL-RC
42. ORNL Patent Section
43. ORNL Y-12 Technical Library

EXTERNAL DISTRIBUTION

44. T. A. Anderson, Department of Entomology, Iowa State University, Insectary Building, Ames, IA 50011-3140

45. R. N. Farvolden, Department of Earth Sciences, University of Waterloo, Waterloo, Ontario N2L 3G1 Canada

46. J. Goodrich-Mahoney, Electric Power Research Institute, 1019 19th Street, N.W., Suite 1001, Washington, DC 20036

47. E. A. Guthrie, Institute for Environmental Studies, Department of Environmental Sciences and Engineering, University of North Carolina, CB#7400, Chapel Hill, NC 27599-7400

48. R. C. Harriss, Institute for the Study of Earth, Oceans, and Space, Science and Engineering Research Building, University of New Hampshire, Durham, NH 03824

49. T. C. Hazen, Environmental Sciences Division, Building 773-442A, Savannah River Laboratory, Aiken, SC 29808

50. T. R. Johnson, Department of Biology, St. Olaf College, Northfield, MN 55057

51. G. Y. Jordy, Office of Program, Analysis, Office of Energy Research, ER-30, G-226, U. S. Department of Energy, Washington, DC 20545

52. E. F. Neuhauser, Niagara Mohawk Power Corporation, 300 Erie Boulevard West, Syracuse, NY 13202

53. A. Patrinos, Environmental Sciences Division, Office of Health and Environmental Research, ER-74, U. S. Department of Energy, Washington, DC 20585

54. F. K. Pfaender, Institute for Environmental Studies, Department of Environmental Sciences and Engineering, University of North Carolina, CB#7400, Chapel Hill, NC 27599-7400

55. Office of Assistant Manager for Energy Research and Development, Oak Ridge Operations, P. O. Box 2001, U. S. Department of Energy, Oak Ridge, TN 37831-8600

56–57. Office of Scientific and Technical Info r mation, P. O. Box 62, Oak Ridge, TN 37831