2000

Effects of plant flavonoids on the fate of polynuclear aromatic hydrocarbons in rhizosphere soil

Xiujin Qui
West Virginia University

EFFECTS OF PLANT FLAVONOIDS ON THE FATE OF POLYNUCLEAR AROMATIC HYDROCARBONS IN RHIZOSPHERE SOIL

Xiujin Qiu

Dissertation submitted to the
College of Engineering and Mineral Resource
at West Virginia University
in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy
in
Environmental Engineering

Brian E. Reed, Ph.D., Chair
Roger C. Viadero, Ph.D.
Jeffrey G. Skousen, Ph.D.
Michael J. McFarland, Ph.D.
John W. Zondlo, Ph.D

Department of Civil and Environmental Engineering

Morgantown, West Virginia
2000

Keywords: Fate, PAH, Flavonoids, Rhizosphere Soil, Degradation, Bioavailability

ABSTRACT

EFFECTS OF PLANT FLAVONOIDS ON THE FATE OF POLYNUCLEAR AROMATIC HYDROCARBONS IN RHIZOSPHERE SOIL

Xiujin Qiu

High-molecular-weight polynuclear aromatic hydrocarbons (PAHs) are persistent in the environment although a wide variety of microorganisms can metabolize PAHs. In the past decades, laboratory and filed studies have shown that PAH degradation in soil is often limited by poor bioavailability and oxygen availability. Bound residue formation of PAHs with macromolecules of soil organic matter is an important fate mechanism. More recently, phytoremediation for PAH-contaminated soils is being explored. It is believed that PAH degradation may be enhanced in rhizosphere soil due to the improved aeration condition and the active soil microbial community sustained by root exudates. Whether certain root exudates would influence PAH degradation or bioavailability in soil is not adequately understood. Although various plant flavonoids, important components of root exudates, have been found to activate or inhibit PAH metabolism in mammalian cells, research on the interaction between root flavonoids and the soil microbial activities had been few. The effects of root flavonoids on the fate of PAHs in rhizosphere soil was investigated using ^{14}C-labeled B[a]P and pyrene in slurry phase soil microcosms. A compound nested experiment was designed to evaluate the effects of different types of rhizosphere soil and flavonoids at varied concentration levels on PAH fate via mineralization, water leaching, and bound residue formation. Both synthetic nonhydroxylated and natural hydroxylated flavonoids at low concentration (0.1–1 uM) had no statistically significant effects on PAH fate at 95% confidence level. However, higher flavonoid concentration level (>10-100 uM) or complex root-extracts hindered PAH mineralization but enhanced PAH-soil-bound residue formation in biologically active rhizosphere soils. In contrast, mineralization was negligible and bound residues decreased as flavonoid concentration increased in abiotic control soil. A biologically mediated covalent binding between phenolic moieties may be responsible for the enhanced bound residue formation. Relatively high percentage bound residues were found to be associated with higher clay, soil organic matter, and humus contents in soil. Increased bound residue formation may have reduced the amount of PAH available for biodegradation/mineralization. There were little or no water leachable PAHs and their polar metabolites in all the treatments.

This research was supported by Union Carbide Corporation.

ACKNOWLEDGEMENTS

I greatly appreciate the advice, support, and encouragement throughout the course of the research by my adviser, Dr. Brian E. Reed. I wish to express my sincere gratitude to Dr. Roger C. Viadero, Dr. Jeffrey G. Skousen, Dr. Michael J. McFarland, and Dr. John W. Zondlo for serving on my special committee and for demonstrating special interests in my thesis research.

I am indebted to my supervisor, Mr. Sunil I. Shah, Union Carbide Remediation Technology Manager, for giving me the opportunity to work on this research. Special thanks must be given to Mr. Edward L. Cyrus who provided technical support to the experiments. Thanks are extended to Ms. Sue Holstein and Ms. Darlene Carney who helped in preparing the final draft and submitting the ETD.

This work is dedicated to my mother, whose love and support I felt at each step of my academic career. Finally, thanks to my son Charlie and my siblings, Wendy, Victor, Helen, and Gorden for their love, friendship, and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

DEFINITIONS

The definitions present the essential aspects of a given term used in the context, and therefore are not comprehensive.

PAH Mineralization
: Complete degradation of a PAH compound to CO_2

Soil Bound Residues
: Organic solvent-nonextractable metabolites or parent PAHs retained in soil phase due to humification and intramicropore diffusion

PAH Humification
: PAH metabolites polymerized with soil humus via covalent binding

Water Soluble PAHs
: Hexane-extractable nonpolar parent PAHs dissolved in water phase.

Water Soluble PAH metabolites
: Hexane-nonextractable polar metabolites dissolved in water phase.

Poisened Soil
: Soil mixed with 2% sodium azide (NaN_3) to inhibit microbial metabolism within a soil slurry microcosm used as a pseudo abiotic control treatment

PAH Adsorption
: Organic solvent-extractable parent PAHs partitioning in soil

Sequestration
: A portion of soil adsorbed hydrophobic chemicals which are biounavailable although solvent extractable

Micropore Diffusion and Entrapment
: A portion of solvent-nonextractable soil bound residues, which are entrapped in soil micropore by diffusion

CHAPTER 1. INTRODUCTION

PROBLEM STATEMENT

Polynuclear aromatic hydrocarbon (PAH)-contaminated soils and sludges are frequently present at petrochemical and fuel gas manufacturing sites. High molecular weight PAHs, persistent in the aged contaminated soils are ubiquitous. Microbial degradation was believed to be the primary process affecting the persistence of PAHs in soil, while PAHs may also be removed by chemical oxidation, photolysis, and volatilization (Callahan *et al.* 1979). During the past decade, an increasing number of studies have shown that apparent analytical depletion of extractable PAH in soil is often not only attributed to biodegradation but largely to the bound residue formation with macromolecular soil organic matter and clay particles (Nieman *et al*. 1999, Guthrie and Pfaender 1998, Carmichael and Pfaender 1997, Eschenbach, Wienberg, and Mahro 1998, Sims and Abbott 1992, Qiu and McFarland 1991). Bound residue formation via polymerization and sequestration has been suggested to be a major fate mechanism for PAHs in soil (Richnow *et al*. 1997, Nieman *et al. 1999).* The bound residues are stable and partially slowly degraded to CO_2 (Eschenbach, Wienberg, and Mahro 1998). From environmental risk viewpoint, both complete mineralization of toxic organic chemicals into inorganic products (H_2O, CO_2, Cl^-, etc.) and bound residue formation with soil organic matter are acceptable endpoints.

Although a wide variety of pure cultures of bacteria, fungi and algae have the ability to rapidly metabolize PAHs, degradation of PAHs in soil are slow due to poor bioavailability and PAH insolubility (Cerniglia 1984; Manilal and Alexander 1991; Loehr and Webster 1996; McGroddy, Farrington, and Gschwend 1996). Incorporating molecular oxygen into the PAH ring is known to be the controlling step of PAH metabolism. In the field, poor oxygen availability often inhibits PAH biodegradation. Oxygen transport rate decreases with an increase in soil depth. As a result biodegradation in deep soil diminishes, especially in the saturated zone that is typically anaerobic. Even in the unsaturated zone limited biological activities exist within the intra-micropores of soil particles due to geometrical and mass transfer restrictions (Middleton, Nakles, and Linz 1991; Jones *et al.* 1993). Degradation may also be hindered by the strong adsorption of PAHs onto soils, nutrient deficiencies, and the lack of acclimated microorganisms. Moreover, natural soil organic matter competes with PAHs for electron acceptors (i.e., oxygen). In recent years, researchers have been exploring the potential of phytoremediation for PAH contaminated soils. There are several theoretical premises. First of all, in rhizosphere soil, large population and diversified consortium of bacteria and fungi sustained by plant root exudates (sugars, amino acids, organic acids, phenols, flavonoids, nucleotides, peptides, enzymes, etc.) may possess highly versatile metabolic capabilities that give a great potential of organic contaminant degradation (Atkinson *et al.* 1983). Secondly, plant roots improve aeration in soil by removing water via transpiration and by altering soil structure through agglomeration. Root turnovers (death and grow) create porous soil structure, thus improving soil aeration as well. Thirdly, root exudates may serve as primary substrates to support microbial cometabolism of high-molecular-weight PAHs (i.e., PAHs containing four or more fused benzene rings) (Cerniglia

1984; McFarland and Sims 1991; Keck *et al.* 1989). Meanwhile, increases of soil organic matter content in rhizosphere soil may alter PAH adsorption, bioavailability, and leachability (Walton, Guthrie, and Hoylman 1994). Whether plant root exudates would increase the leachability of PAHs adsorbed in soil remain a concern for groundwater protection.

There are few detailed studies addressing the effects of plant root exudates on PAH metabolism, bioavailability, and fate in soil. Whereas, a number of pharmaceutical studies have shown various plant root flavonoids activate or inhibit the cytochrome P450 enzyme system, which is responsible for the metabolism of PAHs in mammalian cells (Buening 1981, Alexander *et al.*1986). Flavonoids are important components in root exudates. Like PAHs, flavonoids contain multiple benzene rings (but not fused rings). Unlike PAHs, flavonoids also contain hydroxyl, carbonyl, and methoxyl groups. The types and quantities of root flavonoids associated with various plants are highly diversified. If root flavonoids accelerate or inhibit microbial metabolism of PAH, certain plants, rich of root flavonoids, may have greater influences on phytoremediation.

In this study, radioisotopes and mass balance approach were used to evaluate the effects of root flavonoids on the fate and behavior of PAH in soil. The study constructs one of the many building blocks necessary for the development of phytoremediation technology.

GOALS AND OBJECTIVES

The goal of this research is to evaluate the effects of plant root flavonoids on the fate and behavior of high molecular weight PAHs in rhizosphere soil. ^{14}C-PAH fate was determined by measuring ^{14}C radioactivity of $^{14}CO_2$, residual parent ^{14}C-PAHs, and ^{14}C-PAH metabolites associated with gas, soil, and water phases. Specific objectives are to evaluate

1. Effect of flavonoids on PAH degradation/mineralization
2. Effect of flavonoids on soil-bound residue formation and adsorption of PAHs
3. Effect of flavonoids on water leachability of PAHs and metabolites
4. Compound effects of soil types and flavonoids on PAH fate

CHAPTER 2. BACKGROUND

Polynuclear aromatic hydrocarbons (PAHs), mainly formed from the combustion and pyrolysis of fossil fuels are associated with a wide range of hazardous-waste sites including petroleum chemical, coke production, wood preservation, and synthetic oil and gas production. PAHs have shown toxic, mutagenic, and carcinogenic properties (IARC 1983). Also, these compounds are lipophilic and have high bioaccumulation potential. USEPA has listed PAHs among the priority pollutants to be monitored in aquatic and terrestrial ecosystems.

Because of their hydrophobicity, limited water solubility, and low vapor pressure, PAHs are largely partitioned into soils and sediments in the environment. Biological transformation is believed to be the principal process for the removal of PAH constituents in soil/sediment systems (Sims and Over cash 1983). The biodegradability of specific PAHs in the environment depends on their physical and chemical properties and complex environmental conditions.

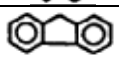
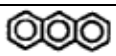
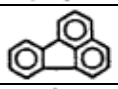
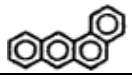
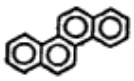
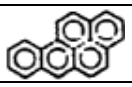
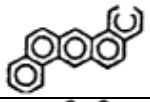
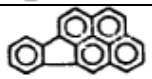
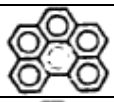
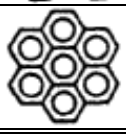
PAH CHARACTERISTICS

Molecular Structure and Physicochemical Properties

Polynuclear aromatic hydrocarbons (PAHs) are composed of fused benzene rings. PAHs range in size from naphthalene ($C_{10}H_8$) to coronene ($C_{24}H_{12}$). Physical and chemical properties of 14 selected PAHs (from 2-ring to 7-ring) are listed in Table 2.1. Aqueous solubility and vapor pressure of PAHs decrease with increasing molecular weight, whereas resistance to oxidation increases with increasing molecular weight (Sutherland 1995). PAH biotransformation rates generally decrease with the increase of the ring number (Park, Sims, and Dupont 1990). Most PAHs are not acutely toxic, but the majority of PAHs with s are potent carcinogens (Cerniglia and Heitkamp 1989).

PAHs with fused benzene rings are persistent in the environment, because of the resonance energy of their structure and their extremely low water solubility (Hall and Grove 1990). Volatilization can be a significant transport process for 2-ring PAHs in environment. PAHs with more than three rings will have insignificant volatile loss under most environmental conditions. PAHs vary in their sensitivity to photooxidation. For example, 60% of Benzo[a]pyrene (B[a]P) in soot particles was degraded under light in 40 minutes, whereas several other PAHs showed little or no photooxidation (Miller and Miller 1985). The photodegradation of surface-sorbed B[a]P is dependent on the oxygen concentration, temperature, and extent of solar radiation, while the potential of photodegradation in subsurface of soil below surface is minimal. Photolysis may transform B[a]P to polar materials (e.g., 7,8-dihydrodiol-9,10-epoxy-B[a]P) that are subject to increased mineralization and binding to humic materials in soil (Millar and Miller 1985).

Table 2.1. Characteristics of selected PAHs[1]

Chemical	Molecular structure	Ring No.	MW	Hc[2] @20°C (atm.•m^3/mol)	Vp[3] @25°C (mm-Hg)	S[4] @25°C (μg/L)	Log K_{ow}	Log BCF	$t_{1/2}$[5] (days)	Carcino genicity
Naphthalene		2	128	$4.8x10^{-4}$	$2.3x10^{-1}$	30000	3.36	1.64-5.0	2	-
Acenaphthene		3	154	$1.46x10^{-4}$	$2.3x10^{-3}$	3470	3.92	2.58		-
Fluorene		3	166	$6.3x10^{-5}$	$6x10^{-4}$	1680	4.12	2.7		-
Anthracene		3	178	$6.51x10^{-5}$	$1.95x10^{-4}$	75	4.45	2.21-3.89	50 - 134	-
Phenanthrene		3	178	$3.9x10^{-5}$	$6.8x10^{-4}$	1002	4.57	0.77-4.52	16 - 35	-
Fluoranthene		4	202	$1.6x10^{-5}$	$5x10^{-5}$	260	5.2	0.76-3.24	268 - 377	+/?
Pyrene		4	202	$1.09x10^{-5}$	$2.5x10^{-6}$	135	5.09	0.72-3.43	199 - 260	-,?
Benz[a]anthracene		4	228	$8x10^{-6}$	$1x10^{-7}$	10	5.61	4.0-4.39	162 - 261	+
Chrysene		4	228	$7.26x10^{-20}$	$6.3x10^{-9}$	2.0	5.91	0.79-3.79	371 - 387	+
Benzo[a]pyrene		5	252	$3.36x10^{-7}$	$5x10^{-9}$	4.0	6.0	2.69-4.0	229 - 309	+
Dibenz[a,h]anthracene		5	278	$1.7x10^{-6}$	$2.78x10^{-12}$	2.5	6.5	3.39-4.63	361 - 420	+
Indeno-[1,2,3-c,d]pyrene		6	276	$2.86x10^{-7}$	$1.01x10^{-10}$	62	5.97		288 - 289	+
Benzo[ghi]perylene		6	276	$5.13x10^{-7}$	$1.01x10^{-10}$	0.26	7.1	4.45	NA	NA
Coronene		7	300	NA	NA	NA	NA	40	NA	+/-,?

[1] Data obtained from (1) Montgomery J. H., Groundwater Chemicals Desk Reference, 2nd Ed., CRC Press, Inc., 1996; (2) Cerniglia, C.E., and M. A. Heitkamp, Microbial Degradation of Polycyclic Aromatic Hydrocarbon in the Aquatic Environment, In Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquartic Environment, Varanasi, U. ed., CRC Press, Inc., 1989, p41-68.

[2] Hc = Henry's Law Constant

[3] Vp = vapor pressure

[4] S = water solubility

[5] $t_{1/2}$ = Biodegradation Half-lives in soil

Aerobic microorganisms present in soil are capable of degrading PAHs. PAHs with 2 and 3 rings often degrade completely to CO_2 and H_2O, whereas 4 or more ring PAHs may form various phenolic and acidic metabolites binding to soil organic matter. High-molecular-weight PAHs are more recalcitrant than the smaller PAHs because of the increased stabilization (resonance) energy associated with the aromatic π electron system. Despite their greater stability, these high-molecular-weight PAHs can be biologically modified through the catalytic actions of cometabolic microorganisms (Cerniglia 1989). Such organisms can insert oxygen atom(s) into the aromatic ring structure through the use of nonspecific oxygenase enzymes. Once an oxygen atom has been incorporated into the PAH compounds, the π electron system is destabilized and the molecule is more susceptible to further biological modification by both cometabolic and noncometabolic microorganisms (Hall and Grover 1990).

Carcinogenicity of PAHs

Many PAHs are known to function as precarcinogens that require metabolic activation before binding to DNA, RNA, and proteins (Hall and Grover 1990). Cancer induction by PAHs is a complex, multi-step process that depends on many factors, such as, size of the PAH molecules, polarity constraints, stereochemistry and chemical reactivity of metabolites, as well as electronic factors that affect the binding of metabolites to macromolecules (Hall and Grover 1990).

Mammalian Metabolism of PAHs by Cytochrome P-450 Enzyme System PAHs are lipophilic. The human body contains enzymes that add epoxide and hydroxyl groups to PAHs (and other xenobiotics) in order to make them more water-soluble so that they may be excreted. The cytochrome P-450 enzyme system is found in the endoplasmic reticulum of many mammalian tissues; it consists of inducible proteins (Gillette, Davis, and Sasame 1972). There are many different cytochrome P-450-dependent isozymes that are induced by different compounds by a poorly understood mechanism (Sutherland *et al.* 1995). The scheme of metabolism of PAHs (Sims et al 1974) by the cytochrome P-450 system involves conversion of PAHs to quinones, alcohols, and conjugates with amino acids and peptides such as glutathione. These are detoxification mechanisms. However, occasionally, by a process referred to as "activation", an epoxide is formed. The epoxide group is hydrolyzed to a dihydrodiol by epoxidehydrase. The resulting dihydrodiol is again a substrate for the cytochrome P-450 so that a diol epoxide is formed (Sims *et al.* 1974). The diol epoxide is an alkylating agent that interacts with cellular macromolecules, such as DNA, and forms a covalent bond between the PAH and DNA (Miller and Miller 1985). The covalent binding of PAH and DNA causes distortion in the DNA of a presently unknown nature, and starts the carcinogenic process (Glusker and Rossi 1986). A schematic representation of the mammalian metabolism of PAHs is shown in Figure 2-1 (Sutherland *et al.* 1995). The distinctive molecular structures of PAHs in regard to carcinogenticity are briefly reviewed in the following.

Stereochemistry of Carcinogen Carcinogen PAHs typically contain 4-5 rings. PAHs that are smaller or much larger are generally not carcinogenic. Each carcinogenic PAH contains a phenanthrene-like group with a "K-region" that is equivalent to the 9-10 double bond of phenanthrene and a "Bay region" at the opposite side. The "Bay-region" and "K-region" in carcinogenic PAHs along with the X-ray diffraction views of two carcinogenic PAHs, i.e., B[a]P and dimethlbenz[a] anthracene (DMBA), are illustrated in Figure 2.1.

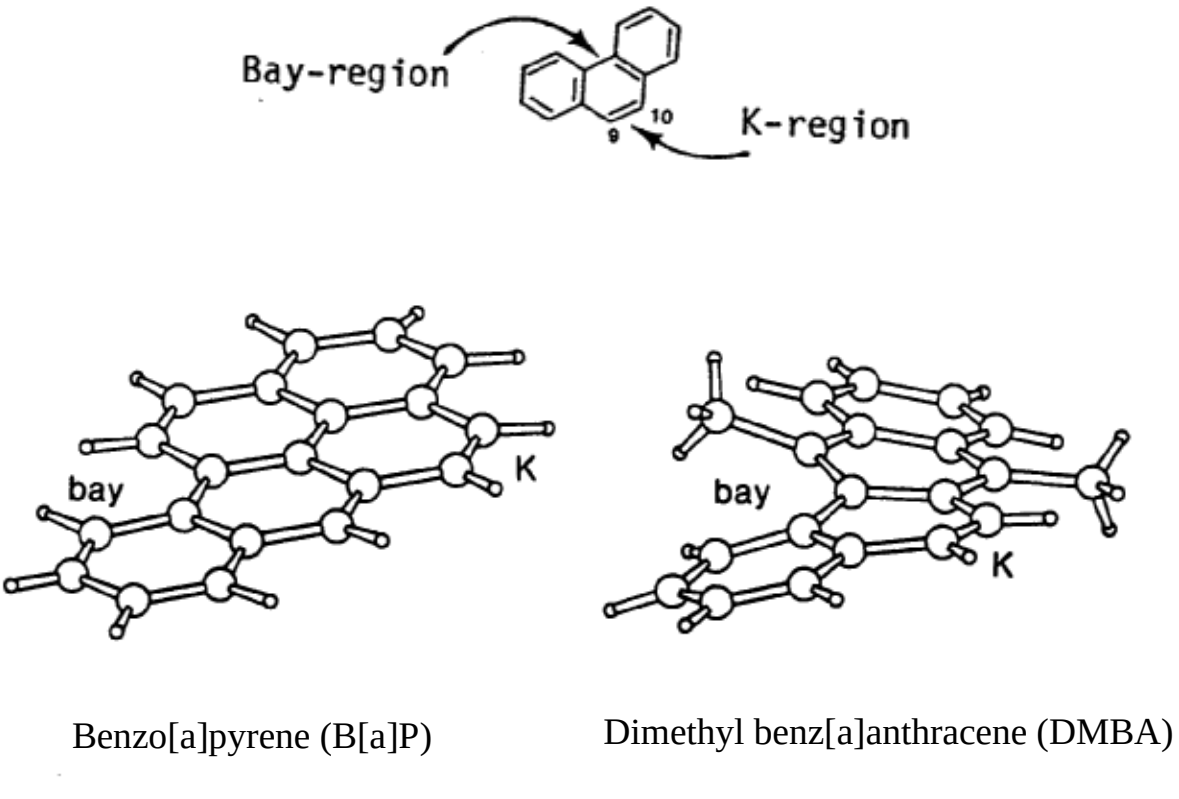


Figure 2.1. Bay-Region and K-Region in Carcinogenic PAHs (X-ray Diffraction Views) (Glusker 1986)

X-ray crystallography showed that PAHs are generally planar (Glusker and Trueblood 1974). PAHs become twisted in the bay-region for steric reasons, when they have a methyl group adjacent to the bay-region (Figure 2.1). The carcinogenicity of such PAHs is enhanced. For example dimethylbenz[a]anthracene is more carcinogen than B[a]P. Activation of PAH, i.e., formation of PAH epoxides, causes distortion from planarity. The crystal structures of B[a]P diol epoxides are nonplanar as shown in Figure 2.2.

The epoxide group lies with the C-O bonds in a plane perpendicular to the plane of the PAH ring system and the hydroxyl groups may lie either in equatorial positions or axial. These hydroxyl groups are trans to each other in the naturally formed diol epoxides. When the diol epoxide interacts with DNA, the epoxide group will open and form a product with DNA substituted on the PAH adjacent to the "Bay region", axial to the plane of the PAH. The most likely targets on DNA are the amino or carbonyl groups of the purine bases. The crystal structure of a portion of DNA alkylated by a diol epoxide has not yet been determined. However, x-ray diffraction studies showed that the PAH portion of the alkylated nucleoside lies intercalated between adenine bases, with the buckled area of the molecule positioned so that it does not stack with the base but lies over a ribose oxygen atom. The structure of B[a]P diol epoxide, which interacts with DNA, is shown in Figure 2.3.

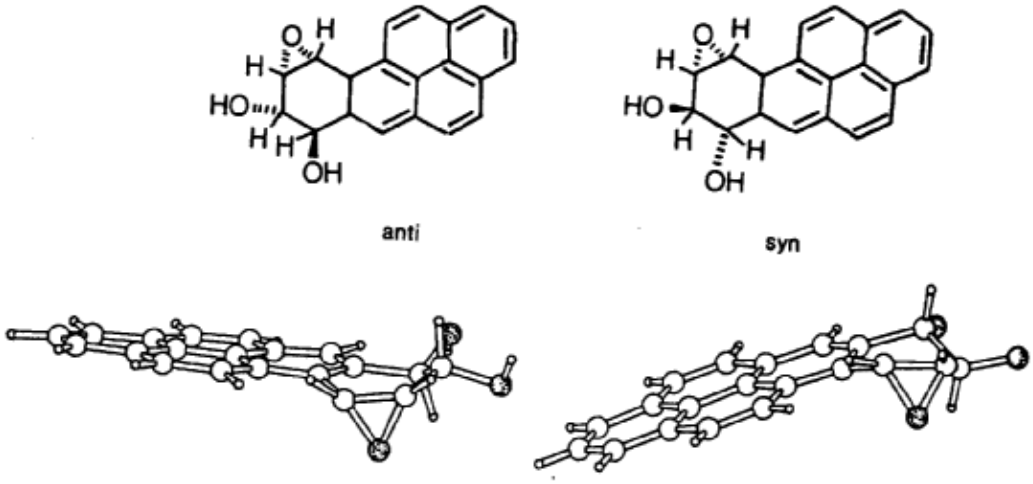


Figure 2.2. Crystal structure of B[a]P diol epoxides, which interacts with DNA (Glusker 1986)

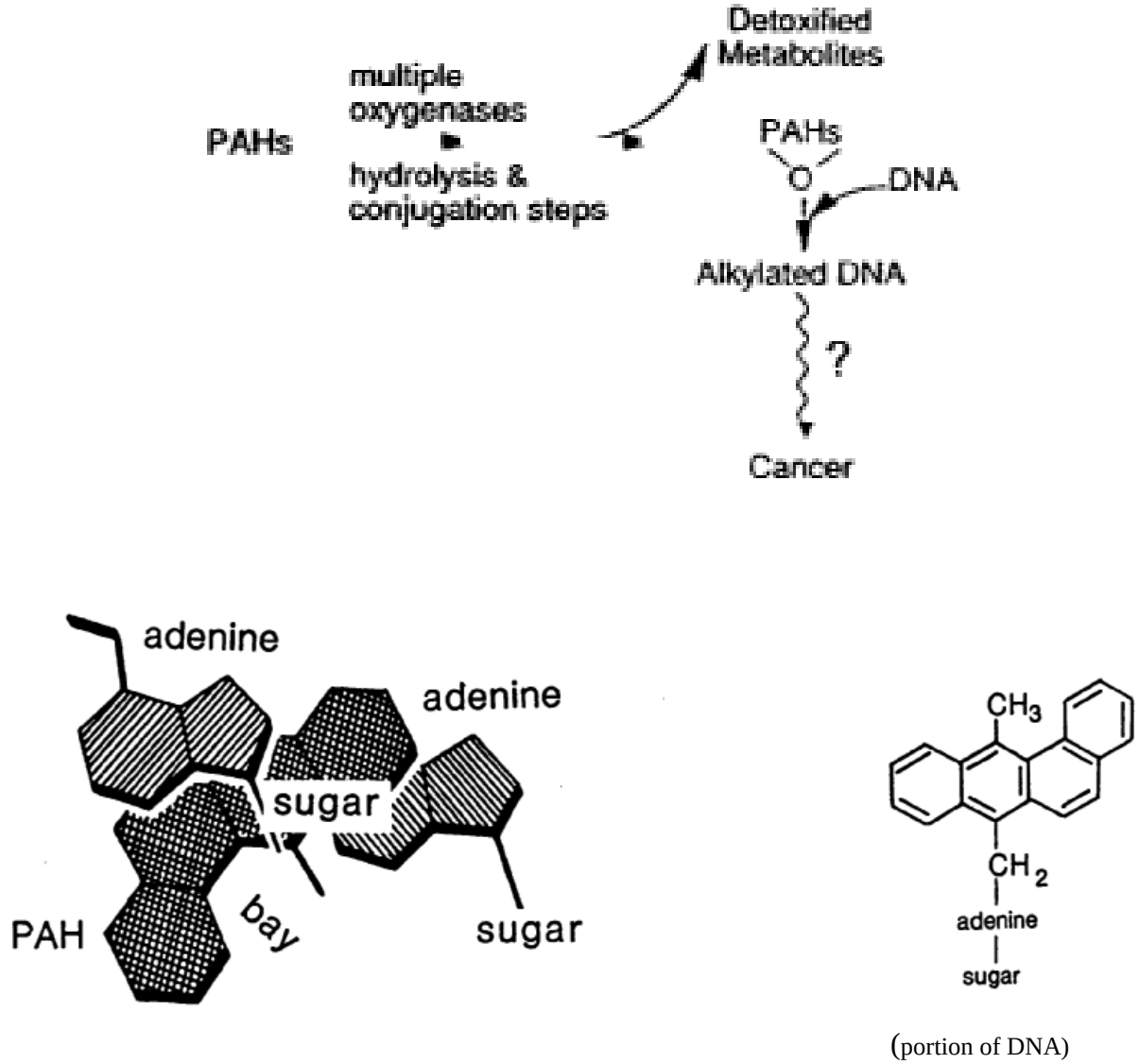


Figure 2.3. Schematic representation of the mammalian metabolism of PAHs (Glusker 1986)

Pathways of metabolic activation in mammalian metabolism of benzo[a]pyrene

Benzo[a]pyrene is among the most potent chemical carcinogens known (Sutherland 1995). Because of its genotoxicity, B[a]P was studied extremely to determine the mechanism of biological activity. Metabolic activation pathways of B[a]P are shown in Figure 2.4. The activation of benzo[a]pyrene to an ultimate carcinogen requires the oxidation of the terminal benzo ring to form B[a]P-7,8diol-9,10-epoxide (Gillette, Davis, and Sasame 1972). Four stereoisomers of B[a]P-7,8diol-9,10-epoxide have been found.

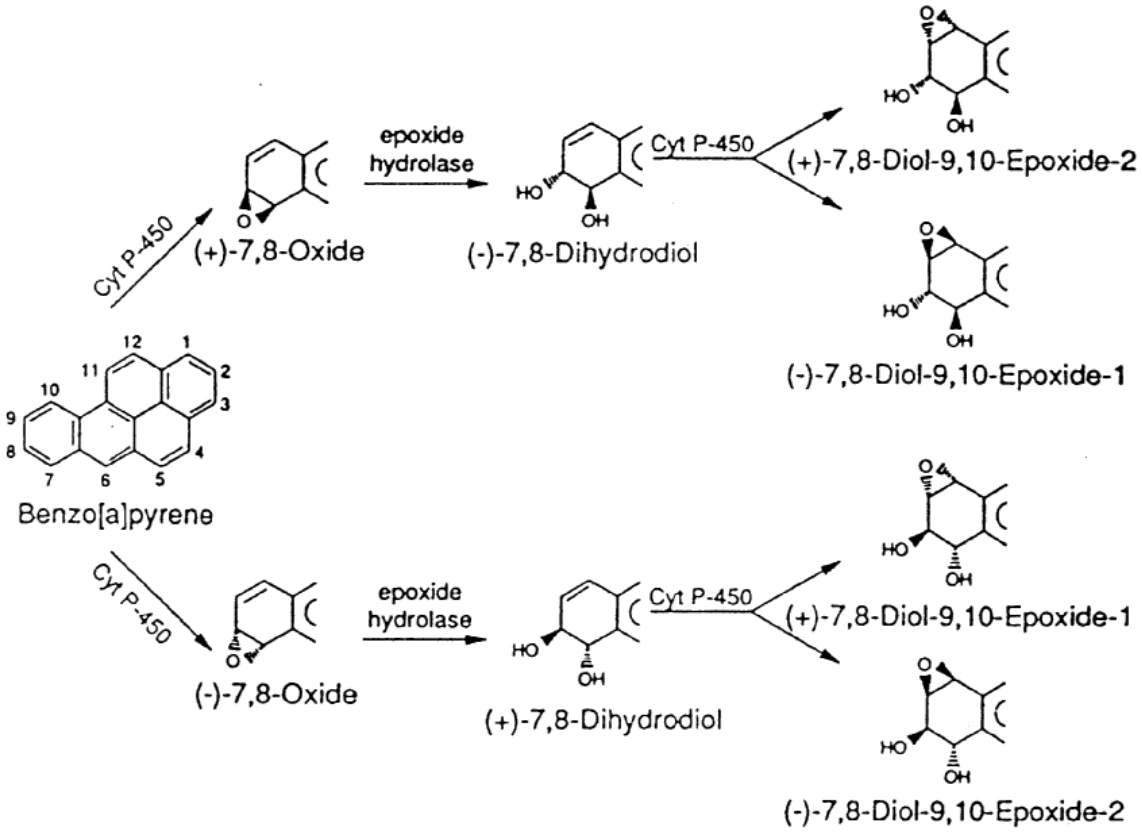


Figure 2.4. Pathways of metabolic activation in the mammalian metabolism of B[a]P (Hall and Grover 1990)

Environmental Fate

PAH fate in the environment involves biodegradation, soil-bound residue formation, soil adsorption, volatilization, photodegradation, and dissolution in soil water. A conceptual model of PAH fate in the environment is illustrated in Figure 2.5. Biodegradation includes complete mineralization to CO_2 or forming intermediate metabolites. Due to hydrophobic interaction, PAHs in the environment are largely adsorbed onto soil organic matter or forming soil-bound-residues (Eschenbach, Wienberg, and Mahro 1998, Carmichael and Pfaender, 1997, Qiu and McFarland, 1991). Laboratory studies have shown that bound residue formation of PAHs is a primary fate mechanism of PAHs (Sims and Abbott 1993, Hurst *et al.* 1996, 1997). Both PAHs and metabolites can diffuse into and be fixed inside soil micropores or soil organic matter voids (Eschenbach, Wienberg, and Mahro 1998). PAH metabolites can also be polymerized to soil humus, a process called humification. Humification and fixation result in soil-bound residues, which are nonextractable by organic solvent. In essence, the soil-bound PAHs and metabolites are not available and no longer toxic to living organisms (Loehr and Webster 1996). Volatilization and dissolution in water are insignificant for four or more ring PAHs but two and three-ring PAHs, such as naphthalene and fluorene. Because four or more ring high-molecular-weight PAHs have very low vapor pressure and water solubility, volatilization and dissolution in water are virtually negligible. In contrast, PAH metabolites, including phenols, acids, alcohols, and ethers, are generally more polar than parent PAHs and therefore are more likely to partition in water phase. Nevertheless, the intermediate metabolites of PAHs, are unstable and are often quickly degraded after formation, and therefore are rarely detected (Sutherland 1992, Sutherland et al. 1993, Cerniglia 1992).

Environmental concerns with regard to PAHs as well as their metabolites are associated with multiple exposure pathways. Residual contaminants associated with soil particles may be exposed to receptors via soil (and vapor) ingestion, inhalation, and dermal contact, while those in water phase may be transported via groundwater to receptors. Other concerns include food chain effects due to plant uptake and animal consumption.

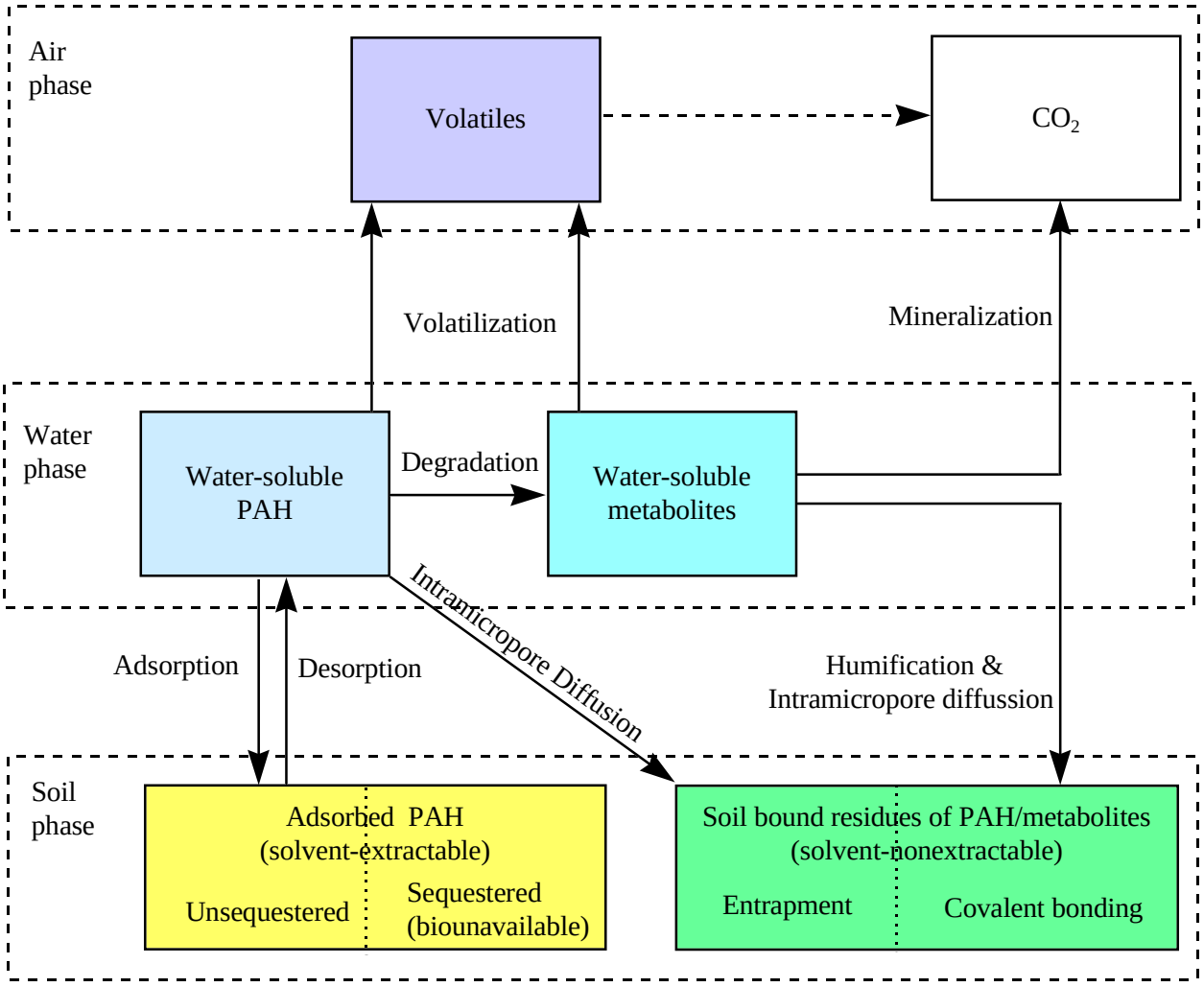


Figure 2.5. A conceptual model of PAH fate in soil

MICROBIAL METABOLISM OF PAHS

Biodegradability of PAHs in the environment depends on their physical and chemical properties and environmental conditions. Many microorganisms are known to readily metabolize 2- and 3-ring PAHs (e.g., naphthalene, phenanthrene, and anthracene) (Sutherland *et al.* 1995). Less is known about the potential of biodegradation of 4 or more ring PAHs (e.g., pyrene, chrysene, and B[a]P).

Biodegradation rates of PAHs are significantly higher under oxic conditions than those under anoxic ones. Two- or three-ring PAHs are amenable to microbial degradation under aerobic and anaerobic denitrifying environment. However, negligible biodegradation occurs under the sulfate-reducing or methanogenic environments (Leduc *et al.* 1992, Bauer and Capoint 1985). According to thermodynamic calculations PAHs are theoretically nondegradable under low redox potential conditions (McFarland and Sims 1991). Moreover, four and more ring PAHs do not serve as a sole substrate for microbial growth, though they may be subject to cometabolic transformation (Gillettee, Davis and Sasame 1972; Davies and Evans1964; Dalton *et al.* 1981; Bulman *et al.* 1985,). Degradation rates decrease as the number of fused rings in PAHs increase (Mihelcic 1988; Bulman *et al.* 1985).

PAH Degradation Pathways

Microorganisms typically degrade PAHs aerobically by incorporating oxygen atoms into the ring structure generating dihydrodiols via oxygenases. The derivative is further mineralized through aromatic ring cleavage and subsequent oxidation (Cerniglia 1984). Microorganisms use several different mechanisms to metabolize PAHs. These mechanisms usually involve enzymatic oxidation to arene oxides, *cis*- and *trans*-dihydrodiols, phenols, quinones, and conjugates. Enzymes for microbial transformation of aromatic hydrocarbons include dioxygenases, methane monooxygenases, cytochrome P-450 monooxygenases, lignin peroxidases, and lactase. The enzymology and genetics of naphthalene metabolism in bacteria are reasonably well understood, and the mechanisms involved in the microbial metabolism of phenanthrene, anthracene, benzo[a]pyrene, and other PAHs are beginning to a yield to investigation (Bauer1985).

Metabolism of PAHs to cis-Dihydrodiols by Bacteria and Green Algae For most bacteria and some green algae, the principal mechanism for the aerobic metabolism of PAHs involves oxidation of the rings by dioxygenases to form cis-dihydrodiols (Cerniglia 1992). These dihydrodiols are transformed further to diphenols, which are cleaved by other dioxygenases. Microorganisms responsible include Gram-negative rod, *Mycobacterium sp., Nocardin sp., Pseudomonas sp., P. acidovorans, P. fluorescens, P.putida, Oscillatoria sp., Beijerinckia sp., flavobacterium sp., Pseudomonas Utida, Streptomyces flavovirens, Agmenellum quadruplicatum, and Selenastrum capricornutum.* For naphthalene, acenaphthalene, fluorene, anthracene, phenanthrene, pyrene, benz[a]anthracene, and benzo[a]pyrene, the initial sites of enzymatic attack have been determined (Cerniglia 1992).

Naphthalene metabolism has been studied more extensively than that of any other PAH. In the metabolism of naphthalene by *Pseudomonas spp*. the initial steps via salicylate are presented in Figure 2.6 (Davies 1964). Naphthalene was oxidized by dioxygenase to naphthalene cis-1,2-dihydrodiol (Eaton 1992). The latter underwent a series of enzymatic reaction and degraded to salicylic acid. Salicylic acid would further degrade to catechol or gentisic acid, which subjects to ring fission and complete mineralization to CO_2 and H_2O. A review paper by Sutherland *et al*. (1995) summarized PAH oxidation to cis-dihydrodiols by bacteria dioxygenase in many other studies. For example, *Beijerinckia* sp. oxidized benzo[a]pyrene to the cis-7,8- and cis-9,10-dihydrodiols (Gibson *et al.*). The green alga *Selenastrum capricornutum* produced benzo[a]pyrene cis-4,5, cis-7,8-, and cis-11,12-dihydrodiols as well as sulfate and glucoside conjugates of the cis-4,5-dihydrodiol (Sutherland 1995). Benzo[a]pyrene was also metabolized by *Pseudomonas* spp. but the products were unknown (Sutherland 1995). Details of the dioxygenase pathways for other PAHs by a variety of microorganisms can be found in many publications (Sutherland 1995).

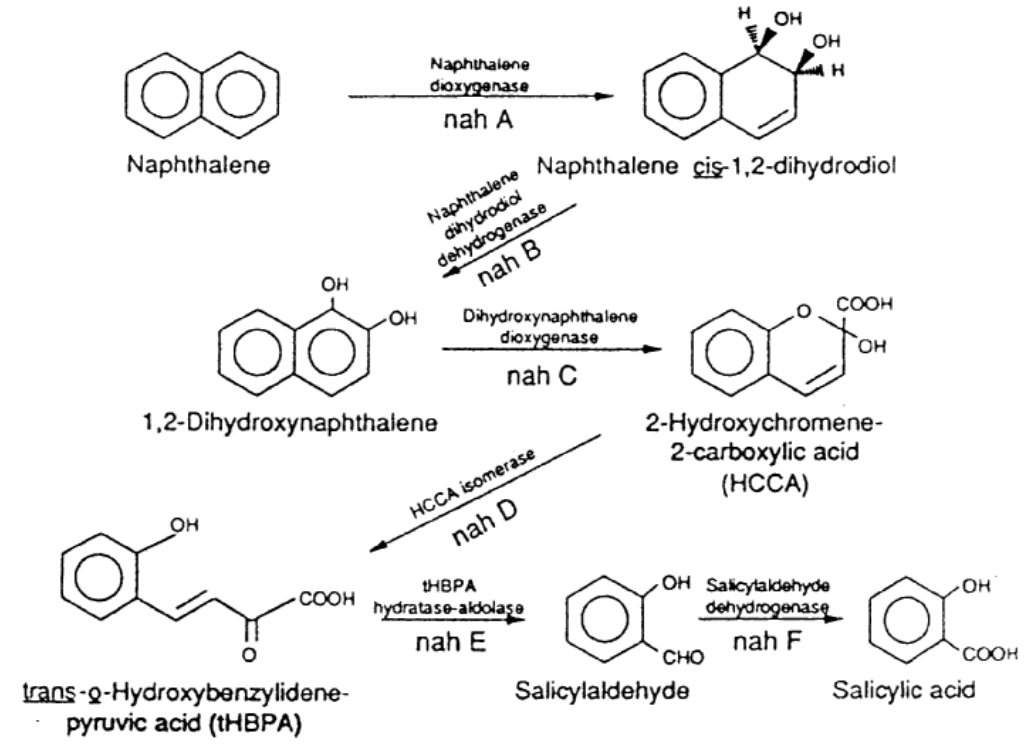


Figure 2.6. Initial steps in the metabolism of naphthalene by *pseudomonas* putida (Davies and Evans 1964; Eaton and Chapman 1992)

Metabolism of PAHs to Phenols by Methylotrophic Bacteria Dalton *et al*. reported that in the presence of NADH, the methane monooxygenase system of *Methylococcus capsulatus*, oxidized naphthalene to 1- and 2-naphthol (Dalton *et al.* 1981).

Metabolism of PAHs to Trans-Dihydrodiols by Fungi, Bacteria, and Cyanobacteria

Many species of fungi, a few bacteria, and some cyanobacteria produce cytochrome P-450 monooxygenases. These enzymes transfer PAHs to arene oxides, which are then either hydrated by epoxide hydrolase to form trans-dihydrodiols or rearranged nonenzymatically to form phenols (Sutherland 1992). In Figure 2.7, metabolism of phenanthrene to phenanthrene trans-1,2-dihydrodiols by different species of fungi is presented (Sutherland 1993). As mentioned earlier incorporation of oxygen into the fused benzene rings is the control step of PAH metabolism, the subsequent reactions are fast. Details of the monooxygenase mediated trans-dihydrodiol pathways for other PAHs mainly by fungi can be found in many publications cited in Sutherland 1995.

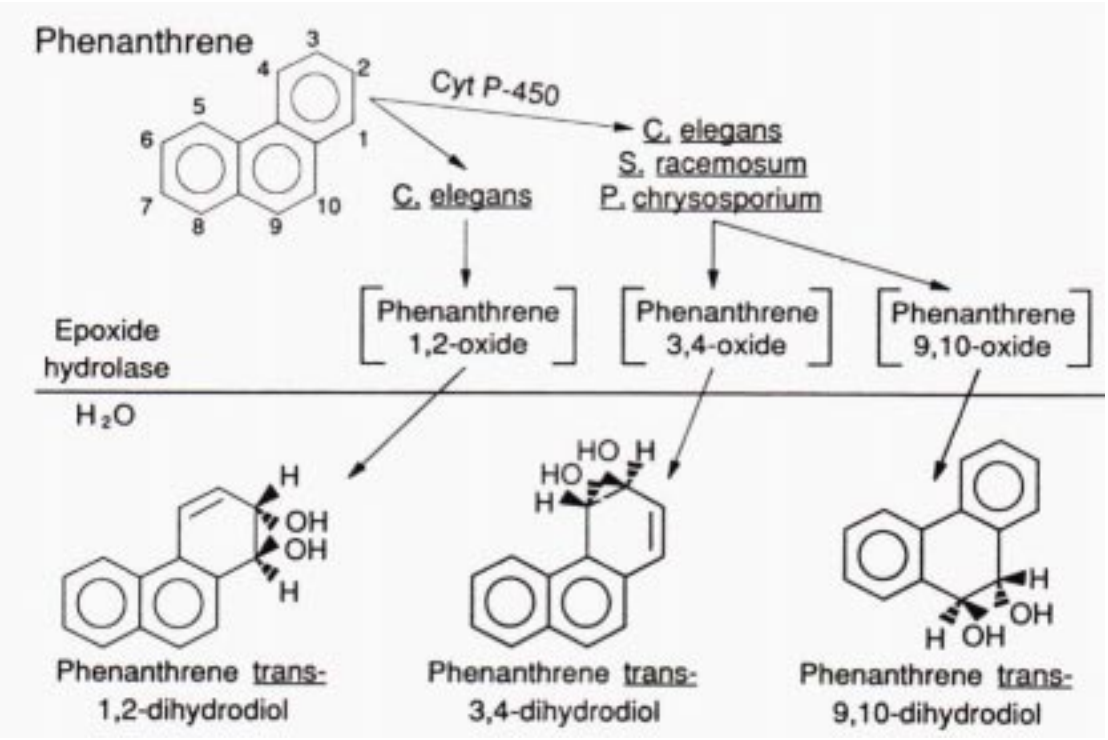


Figure 2.7. Metabolism of Phenanthrene by Different Species of Fungi (Sutherland et al. 1993)

Metabolism of PAHs to Quinones by White-rot Fungi Some white-rot fungi, which decay lignin and cellulose in wood, metabolize PAHs to quinones and other metabolites by mechanisms that do not appear to involve *cis*- or *trans*-dihydrodiols. In some cases, but not all, lignin peroxidases are involved. Oxidation of pyrene, benzo[a]pyrene, anthracene, and phenanthrene by white-rot fungi, *phanerochaete chrysosporium,* to quinones is presented in Figure 2.8 (Sutherland 1995). Quinones are unstable and readily degradable. The observed intermediate metabolites included phthalic acids and diphenic acids, which would completely degrade to CO_2.

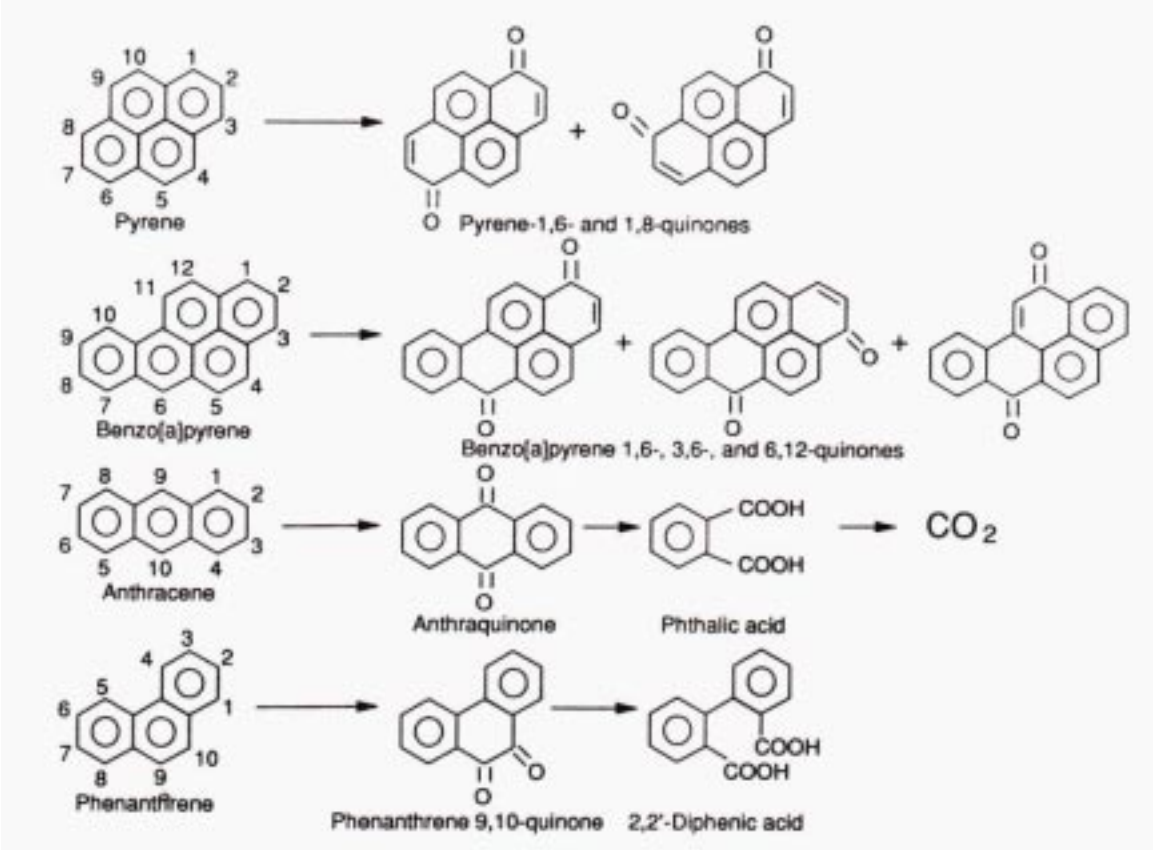


Figure 2.8. Oxidation of PAHs by *Phanerochete chrysosporium* (Sutherland et al. 1993)

Cometabolism

The term cometabolism has been used by many researchers as biodegradation of non-growth substrates (Horvath 1972) in describing the conversion of pesticides. Cometabolism has also been extended to include cooxidation, as well as the utilization of substrates by non-proliferating cellular suspensions. Cooxidation is a technique originally described by Leadbetter and Foster as "Non-growth hycrocarbons are oxidized when present as co-substrates in a medium in which one or more different hydrocarbons are furnished for growth…." (Foster 1962; Leadbetter and Foster 1959). Cooxidation is appropriate in context with etymology, however, cooxidation should not be considered as synonymous with cometabolism when used in describing a biological process, because the definition of metabolism is the sum of processes concerned in the building of protoplasm and its destruction incidental to life. The use of the term cometabolism had been criticized as leading to serious misconceptions about the immediate capacity of micro-organisms to rid the environment of noxious materials (Perry 1979). Despite the controversy, the term cometabolism has been used by researchers to address the disappearance of recalcitrant compounds from the environment in the presence of readily degradable growth substrates (Sims and Overcash 1983; Horvath 1972, Dalton 1981, McKenna 1976). Biological cometabolism has been proposed as a potentially important process for the loss of recalcitrant PAHs from soil (Perry1979; Keck et al. 1989, Alexander et al. 1986).

The cometabolic degradation of PAH differs significantly from mineralizing degradation (Davies and Evans 1964). In cometabolism, organisms do not use PAH for growth and frequently the degradation ceases at a very early stage after initial oxidation. Often the aromatic rings are not split and phenolic, carboxylic, or chinoic derivatives of the PAHs accumulate as dead-end products (Gillette, Davis, and Sasame 1972, Al-Bashir et al. 1990). Except for the white-rot basidiomycetes, all fungi that have been investigated so far transform PAHs into transdiol intermediates under cometabolic conditions. Unlike most bacteria, the reaction used by these fungi to initially oxidize PAHs is catalyzed by monooxygenases instead of dioxygenases (Perry 1979, Davies 1964).

Factors Affecting PAH Degradation in Soil

PAH degradation in soil and sediment is slow, although a wide variety of pure cultures of bacteria, fungi and algae and their purified enzymes have the ability to rather rapidly metabolize PAHs (Cerniglia 1984Kihohar, Nagao, and Nomi 1976; Schocken and Gibson 1984). Microbial degradation of PAHs is constrained in the natural environment by the availability of oxygen and nutrients (Manilal and Alexander 1991). Degradation rates are further limited by soil bound formation, desorption kinetics, the population of acclimated microorganisms, and competing reactions for electron acceptor utilization. Besides, natural soil organic matter competes for electron acceptors with PAHs for degradation. Oxidation of natural soil organic matter is generally thermodynamically more favorable than the oxidation of PAHs (Mihelcic and Luthy 1988a, Raddy, Rao, and Jessup 1982, Al-Bashir et al.). Soil texture, organic content, pH, temperature, and other seasonal effects may have significant impacts on PAH degradation (Carmichael and Pfaender 1997, Cerniglia 1993).

Oxygen Availability Incorporation of molecular oxygen into the PAH ring structure is the rate-limiting step for enzymatic degradation. The follow-up reactions are faster (Sutherland 1995, Cerniglia 1993). The oxidized derivatives (dihydrodiol or quinone) are further mineralized through aromatic ring cleavage and subsequent oxidation as described in a previous section. Biodegradation rates of PAHs are significantly higher under oxic conditions than those under anoxic ones. Two- and three-ring PAHs are amenable to microbial degradation under aerobic and denitrifying (anaerobic) environment. However, negligible biodegradation occurs under the sulfate-reducing or methanogenic environments (Mihelcic and Luthy 1988b; Educ *et al.* 1992; Bauer and Capoint 1985; ,Hurst et al. 1995). According to thermodynamic calculations four or more ring PAHs are theoretically nondegradable at low redox potentials (McFarland and Sims 1991). Degradation of four or more ring PAHs under anoxic conditions has not been reported. The oxygen transfer rate is known to decrease with increasing soil depth and the degree of saturation. PAH biodegradation rate in soil is limited by the low concentrations of oxygen, especially in the wet soils which are frequently under anaerobic conditions.

Bioavailability Soil microorganisms capable of metabolizing PAH compounds are ubiquitous. However, biodegradation of PAHs in soil is often slow due to poor bioavailability and insolubility. Numerous long-term laboratory and field studies showed that PAHs may be bioremediated by microorgnisms to a residual concentration that no longer decreases or decreases very slowly over time with continuing treatment. Earthworm uptake and bacterial minerlaization showed that aging reduces PAH bioavailability to both species. Many studies have been conducted in the past decade. It is now well understood that long-term persistence of residual PAHs in soil is due to poor bioavailability to microorganisms. A number of researchers reported that sorption on soil particles and organic matter caused reduced bioavailability of hydrophobic organic compounds. More details are present in the following section.

SORTION AND ADSORPTION

The term sorption is used when it is not desired, or is experimentally impossible to distinguish between adsorption and absorption. Adsorption is a process of which chemical species passes from bulk phase to the surface of another where it accumulates without penetrating the structure of this second phase. Absorption involves the transfer of a molecule from one phase to another via their interface, and this transfer alters the composition of the second phase. Adsorption is an important fate mechanism of PAHs in soil.

Sorption to natural solids is a primary process affecting the mass transport, degradation, and biological activity of organic compounds in the environment. Although often regarded as instantaneous for modeling purposes, sorption may in fact require weeks to years to reach equilibrium. Fate, transport, and risk assessment models all contain terms for sorption, therefore, an understanding of the dynamics of sorption is crucial. Ignoring slow kinetics can lead to an underestimation of the true extent of sorption, false predictions about the mobility and bioavailability of contaminants.

Mechanism

The predominant mechanism of PAHs adsorption onto soil surfaces is "hydrophobic bonding" also referred to as "partitioning" (Dragun 1988). Soil organic matter coated on clay particles is the major adsorption surface. In most soils, the organic matter intimately binds to clay particle surface forming clay-organic complexes. Clay surfaces also possess hydrophobic regions that can preferentially accumulate organic chemicals (Dragun 1988). Another mechanism for hydrocarbon adsorption is due to van der Waal's force. The larger the molecule, the greater its propensity to exist in the adsorbed state. Generally, van der Waal forces are weak and of very short range. Nevertheless, sorption by organic matter is a key factor in the behavior of many PAHs in soil (Stevenson 1982). Although sorption is considered in general as a reversible process, adsorbed substances tend to become more resistant to extraction and degradation over time (Hatzinger and Alexander 1995).

Soil Organic Matter (SOM) Soil organic matter (SOM) is the most active area of the soil for contaminant partitioning and biodegradation. Partitioning in SOM is the primary mechanism of sorption for hydrophobic organic compounds. Soil organic matter, a mixture of plant and animal residues in various stages of decomposition, consists of humic and nonhumic fractions. The former includes fulvic acid, humic acids, and humins; the latter includes cellulose, starch, proteins, and fats. Plant residues and the associated biomass turnover once every few years. Turnover is the measure of the movement of an element in a biogeochemical cycle. Microbial metabolites and cell wall constituents become stabilized in soil and possess a half-life of 5 to 25 years. Humus is the resistant fractions, which range in age from 250 to 2500 years (Stevenson 1982).

Soils vary greatly in organic matter content depending on soil formation time , climate, vegetation , parent material, topography, etc. A typical prairie grassland soil (e.g., Mollisol) may contain 5 to 6% organic matter in the top 15 cm, but a sandy soil typically contains less than 1% of SOM. Poorly drained soils often have SOM near 10%. The C/N ratio of SOM generally falls in the range of 10 to 12, although higher values are not unusual (Stevenson 1982).

The affinity of SOM for nonpolar organic compounds depends on its origin and geologic history (Luthy *et al.* 1997). Thus different sorptive properties for HOCs can be expected due to the diversity in composition and structure of SOM. Organic matter in unweathered shales and high-grade coals enhanced sorption capacities more than an order of magnitude larger than organic matter in recent soils or geologically immature material or highly weathered SOM (Luthy *et al.* 1997). Variability in the nature of SOM, especially with respect to changes in polarity and aromatic carbon content, appears to be significant in controlling reactivity with HOCs (Kile *et al.* 1995). There are differences in the sorption of organic compounds on different fractions of organic matter (e.g., fulvic and humic acids and humins) (Garbarini and Linon 1986, Gauthier, Seitz, and Grant 1987).

Sorption of Hydrophobic Organic Contaminants onto Soil Domains In soil domains, sorption of hydrophobic organic contaminants is distributed among three principal domains. The first domain is the mineral domain with surface reactivity attributable to (i) exposed external mineral surfaces at the particle scale and surfaces within macropores, (ii) interlayer surfaces of swelling clays at the nanometer scale, and (iii) the surfaces within mesopores and micropores of inorganic mineral matrices. The amorphous and dense soil organic matter (SOM) components constitute a second principal domain at the nanometer scale. Adherent or entrapped nonaqueous phase liquids (NAPLs) comprise a third domain that may not exist in lightly contaminated soils. Hydrophobic organic sorption on external mineral surfaces and on macropore surfaces within mineral matrices is typically a linear and reversible process with equilibrium being attained essentially instantaneously under completely mixed conditions (Huang, Schlautman, and Weber 1996).The organic carbon domain would exhibit some combination of sorption behaviors involving both linear partitioning and nonlinear intramatrix, micropore-filling retention (Xing and Pignatello 1997, Young and Webber 1995). Diffusion of solute molecules into and out of condensed organic matter could be extremely slow, and the associated sorption process would likely be nonlinear, hysteric, and subject to solute competition. At the aggregate and particle scales, SOM and high surface area clay particles may be encapsulated by inorganic precipitates and weathering products. Under such conditions, some SOM may be inaccessible to organic contaminant molecules. Sorption rates are likely to be limited by extremely slow diffusion in micropores within precipitates, mineral particles, and intra-SOM matrix. Overtime, hydrophobic contaminants may be co-encapsulated with the SOM and clay matrices from which they and cannot be readily released.

Although the practicality of dividing contaminated soil metrix into three sorption domains is generally acceptable, the microscopic mechanism is inadequately understood.

Sorption Kinetics

Anomalous Behavior: Fast and Slow Stages Sorption and especially desorption in natural particles can be exceedingly slow. The use of equilibrium expressions for sorption to natural particles in fate and transport models is often invalid due to slow kinetics. Diffusion limitations appear to play a major role. Contending mechanisms include diffusion through natural organic matter matrices and intraparticle nanopores (Pignatello and Xing 1996). These mechanisms probably operate simultaneously, but the relative importance in a given system is indeterminate. Sorption shows anomalous behaviors that can not be explained by simple diffusion models due to concentration dependence of the slow fraction, distributed rate constants, kinetic hysteresis, and possible high-energy adsorption sites within the internal matrix of organic matter and in nanopores (Pignatello and Xing 1996).

In most cases, sorption and desorption of organics on soil occur in fast and slow stages. Weissenfel (1990) found sorption of PAHs onto soil followed a two-phase kinetically distinct process (fast and slow). The initial fast stage was suggested to be rapid adsorption of the hydrophobic PAHs onto hydrophobic areas of soil surfaces, whereas the following slow stage

was suggested due to the slow migration of the PAHs to less accessible sites within the soil matrix (Karickhoff 1980, Robinson 1990). It is well known that hydrophobic interaction is the predominant adsorption mechanism of hydrophobic chemicals such as PAHs. Organic carbon content (major component of SOM) is the most important factor influencing the extent of adsorption of hydrophobic molecules (Karickhoff 1979). Migration of PAHs into SOM increases over time. The migration process should continue until the adsorption capacities of the SOM are exhausted and an equilibrium is reached. The division between them is rather arbitrary, but in many cases it occurs at a few hours to a few days. The magnitude of the slow fraction (in the slow stage) is not trivial, as many long-term studies testify. The reported slow sorption fraction ranged from 30% to 10 fold of the fast stage sorption. Desorption often reveals a major slow fraction (10-96%) following a comparatively rapid release. The slow fraction of some pesticides was found to increase with contact time in the environment.

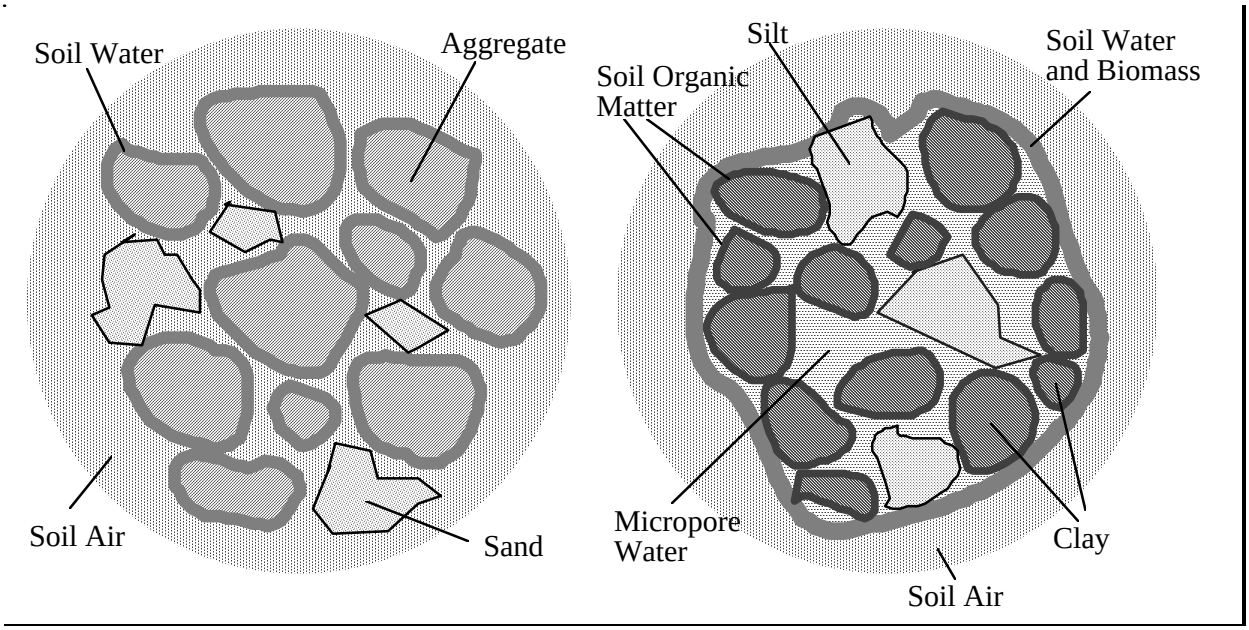
Pignatello and Xing (1996) summarized three features of slow sorption kinetic. Firstly, a single rate constant (1^{st} order) often does not apply over the entire kinetic part of the curve. In desorption studies, the logarithm of fraction remaining vs. time tends to show a progressive decrease in slope, indicating greater resistance to desorption. Secondly, the slow fraction is inversely dependent, often markedly, on the initial contaminant concentration. Thirdly, sorption is often kinetically hysteretic, meaning that the slow stage sorption is faster than desorption. Many examples exist of apparent "irreversible" sorption of some fraction. Diffusion through soil organic matter can be rate-limiting step.

Sorption and Desorption Rates Sorption and desorption for hydrophobic organic contaminants in soils occur on a range of time scales, fast time scales occurring on the order of minutes to days and slow time scales occurring on the order of weeks or even years (Pignatello and Xing 1996). Recent work has attributed these rates to intra-aggregate diffusion and releases from micropores or different forms of soil organic matter relying on macroscopic observations.

In mineral phases, it is quite likely that the slow release kinetics of HOCs is due to diffusion in and out of micropores. Molecular diffusion in hydrophobic microporous materials is governed primarily by steric energy barriers. The diffusion activation energy depends strongly on diffusant and pore sizes, and diffusivities typically fall below 10^{-12} cm^2/s.

Rate Limiting by Slow Diffusion Sorption can occur by physical adsorption on a surface or by partitioning into a phase such as soil organic matter (SOM). The potential causes of slow sorption/desorption are activation energy of sorptive bonds and mass transfer limitations (molecular diffusion). Large molecules (such as PAHs) that can interact simultaneously at multiple points can be more difficult to desorb. There may be steric hindrance to desorption of adsorption. Slow sorption/desorption kinetics is more commonly attributed to diffusion limitation in porous media. Figure 2.9 is a conceptualization of a soil particle aggregate showing possible diffusion processes. Particles are porous by virtue of their aggregated nature and because the lattice of individual grains in the aggregate may be fractured. To reach all sorption site diffusing molecules must traverse bulk liquid film. Film diffusion is potentially rate-limiting for the initial fast stage of sorption; while pore diffusion and matrix diffusion are likely rate limiting steps in slow stage. Diffusion in pores can occur in pore liquids or along pore wall surfaces. Most microorganisms are present at the external surface of soil aggregates and in the

macro pore water. For unsaturated soils, air fills the rest of the macropores and supplies oxygen to the soil water phase. Microorganisms rarely enter the internal micropores due to geometrical and mass transfer restrictions. Limited biological activities exist within the internal micropores; which are typically water saturated and anaerobic (Middleton, Nakles, and Linz 1991, Jones et al. 1993). The interior surface area of the aggregates is orders of magnitude greater than the exterior surface. For aged contaminated soils, the majority of the contaminant mass is sorbed onto interior surfaces. Compounds sorbed at the interior surface area must be transported to biomass-water phase at the exterior surface for degradation to occur. The degradation of sorbed PAHs in soil can be controlled by either desorption, diffusion, or degradation.



(adsorption)

$C_nH_{n\ adsorbed} \rightarrow C_nH_{n\ micropore\ water} \rightarrow C_nH_{n\ biomass\text{-}water} \rightarrow CO_2 + H_2O$

(desorption) *(diffusion)* *(degradation)*

Intraparticle----------→ *External surface of particle*

Figure 2.9. A conceptual model: soil particle structure and PAH adsorption, desorption, diffusion, and degradation processes

In the first step PAH is described from the interior surface of soil particles. In the second step the contaminant diffuses from within the soil micropores to the exterior biomass-water phase in macropores. A relatively small amount of the contaminant desorbs directly to the biomass-water phase from the exterior surface of soil aggregates. The continuous opportunity for surface adsorption and desorption along the micropore (Brusseau and Rao 1989), further complicates the mass transport process. Deeply adsorbed hydrocarbons would transport slowly to the outer surface of the aggregate. In the final step, contaminants are degraded in the exterior biomass-water phase.

It is generally accepted that slow diffusion in a porous particle is at least partially responsible for rate-limited sorption/desorption, the specific nature is not well understood. An emerging view for some researchers is that intraorganic matter diffusion plays a dominant role, however some researchers believe that the arguments for intraorganic matter diffusion are inconclusive (Luthy *et al.* 1997).

The Time Frame of PAH Diffusion in Soil Aggregate The unsteady state continuity equation for compound diffusion through a homogeneous porous spherical particle is given as equation 2.1 and illustrated in Figure 2.10.

$$\frac{dq_r}{dt} = -\frac{D_s}{r^2}\frac{d}{dr}\left(r^2\frac{dq_r}{dr}\right) \qquad \textbf{[Equation 2.1]}$$

where, r = radial distance (L); q_r = sorbed concentration at point r (M/M); t = time (T); D_S = diffusion coefficient (L^2/T); and R = radius of soil aggregate (L). The analytical solution of this equation was presented in detail by Carslaw and Jaeger (1959). An important result obtained from their solution is that the time for 90% of the initial amount of chemical to desorb (t_d) is given approximately by

$$t_d = \frac{d^2}{24D_s} \qquad \textbf{[Equation 2.2]}$$

where d = the diameter of the particle. The difficulty in using this equation is the determination of the correct values for the diameter and diffusion coefficient.

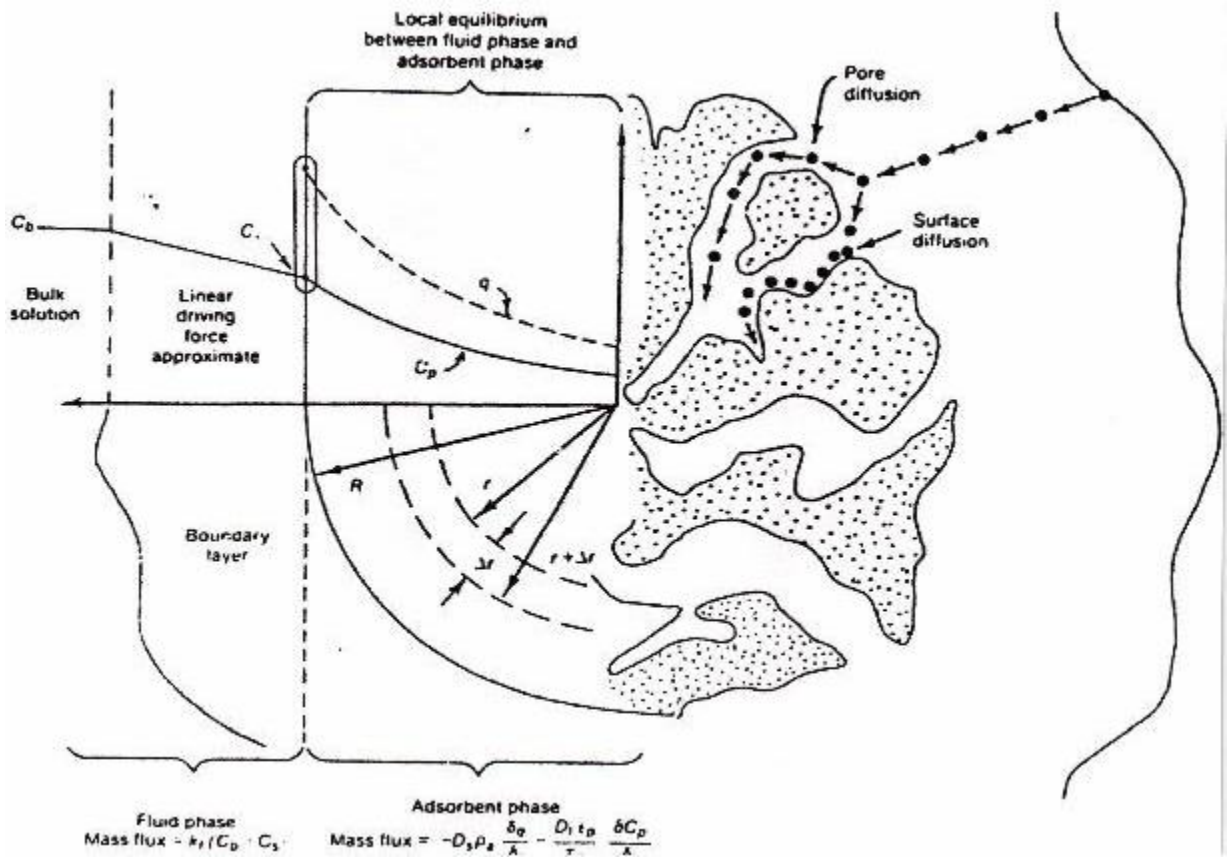


Figure 2.10. Diffusion through a homogeneous porous spherical particle

Lick and Rapaka (1996) applied the diffusion model (Equation 2.1) to the sorption of hydrophobic organic chemicals (HOC) to suspended sediment particles. They advanced the model by including an effective diffusion coefficient based on a hypothesis that diffusion is modified by sorption of the HOC to organic substances within the particle and possibly to mineral surfaces of the particle. If sorption is not rate-limiting and there is no chemical reaction within the particle, then a quasiequilibrium can be assumed. Accordingly, the inter-particle chemical transport can be described by Equation 2.3 with an effective diffusion coefficient, D_s (Berner 1980, Wu and Gschwend 1986).

$$D_s = \frac{D_m f}{1 + \left(\frac{1-\phi}{\phi}\right)\rho_p K_p} \quad \textbf{[Equation 2.3]}$$

where D_m = molecular diffusion coefficient (cm^2/s) of the chemical in the fluid within the particle, f = tortuosity correction factor, ϕ = porosity of the particle, and ρ_p = the mass density of the solid particle (approximately 2.6 kg/L), K_p = solid/liquid partition coefficient. The correction for tortuosity f is not well known. It has been suggested (Lick and Rapaka 1996) that f is proportional to ϕ^n, where n is between 1 and 2. The molecular diffusion coefficient for a dissolved chemical in water is generally about 10^{-6} cm^2/s. For 4-5 ring PAHs, K_p is approximately 10^5 L/kg. Assuming n = 1, ϕ = 0.1 (a reasonable value for a relatively dense floc), Equation 2.3 gives a value for D_s of bout 5 x 10^{-14} cm^2/s. Considering the possible variations of the parameters in Equation 2.3, the best estimates for D_s are between 10^{-13} and 10^{-15} cm^2/s.

The aforementioned expression for D_s is a very general relationship and includes effects of tortuosity and porosity of the particle as well as effects of the chemical/particle property of partitioning (K_p). It also indirectly includes the effects of the organic content of the sediments because the equilibrium partition coefficient is approximately proportional to the organic carbon fraction in the soil.

$$K_p = f_{oc} K_{oc} \quad \textbf{[Equation 2.4]}$$

where f_{oc} is the organic carbon fraction and K_{oc} is the organic carbon partition coefficient. The dependence of K_p on f_{oc} causes D_s to decrease as f_{oc} increases.

Lick and Rapaka (1996) validated the advanced diffusion model by both adsorption and desorption experiments with sediments. For a sediment, the particles have a distribution of sizes and densities. The results of Equations 2.2 and 2.3 was consistent with the experiments using sediments in four sizes with average diameters of approximately 3,7,17, and 40 μm, respectively (Lick and Rapaka 1996).

The diffusion model developed by Lick and Rapaka (1996) can also be used for PAH diffusion in soil. For contaminated soils, aggregates are of highly irregular shape and size. Surface soils often contain small and rounded aggregates typically having diameters less than 10 mm. Compacted backfills and massive clays with cracks can be assumed to be large aggregates. Using Equations 2.2 and 2.3, the time of 90% of the initial amount of PAH to desorb from a soil aggregate with diameter of 10 mm will be approximately 3, 27, 675, 1910, and 314,000 days for benzene, naphthalene, phenanthrene, pyrene, and B[a]P, respectively. Detailed calculations for 17 PAHs to desorb from soil aggregates with diameters of 0.2 cm, 1 cm, and 10 cm are presented in Table 2.2. Notably, t_d is proportional to the square of soil aggregate diameter. As a result, chemical desorbing time increases considerably as soil diameter increases. For clay soil, t_d will be very long because of the massive structure.

Table 2.2. Theoretical calculation: time of 90% of the initial amount of PAH to desorb from a soil particle

Compound	D_{mw}	$f = f^n$ tortuosity	r_p	K_p K_{oc} f_{oc}	K_{oc}	D_s effective	*Time for 90% to desorb* ($t_d = d^2/24D$)		
		f=0.2,n=1		f_{oc} = 0.01			*d = 0.2 cm*	*d = 1 cm*	*d = 10 cm*
	(cm^2/s)		(g/cm3)	(cm3/g)		(cm^2/s)	(days)	(days)	(days)
Naphthalene	6.21E-06	0.2	2.6	6.60E+00	6.60E+02	1.78E-08	1.1	27	2703
Naphthalene,2-methy	5.73E-06	0.2	2.6	6.60E+00	6.60E+02	1.65E-08	1.2	29	2929
Acenaphthene	4.17E-06	0.2	2.6	4.60E+01	4.60E+03	1.74E-09	11	277	27721
Acenaphthylene	4.21E-06	0.2	2.6	4.79E+01	4.79E+03	1.69E-09	11	286	28597
Fluorene	5.48E-06	0.2	2.6	7.30E+01	7.30E+03	1.44E-09	13	334	33443
Phenanthrene	5.20E-06	0.2	2.6	1.40E+02	1.40E+04	7.13E-10	27	676	67617
Anthracene	3.90E-06	0.2	2.6	1.40E+02	1.40E+04	5.36E-10	36	900	90039
Fluoranthene	4.73E-06	0.2	2.6	3.80E+02	3.80E+04	2.39E-10	81	2016	201579
Pyrene	5.00E-06	0.2	2.6	3.80E+02	3.80E+04	2.53E-10	76	1906	190625
Benzo[a]anthracene	4.53E-06	0.2	2.6	2.51E+03	2.51E+05	3.47E-11	556	13892	1389198
Chrysene	4.53E-06	0.2	2.6	2.51E+03	2.51E+05	3.47E-11	556	13892	1389198
Benzo[b]fluoranthene	4.20E-06	0.2	2.6	5.50E+03	5.50E+05	1.47E-11	1313	32813	3281269
Dibenz[ah]Anthracen	4.06E-06	0.2	2.6	5.75E+03	5.75E+05	1.36E-11	1422	35559	3555923
Benzo[k]fluoranthene	4.20E-06	0.2	2.6	4.37E+04	4.37E+06	1.85E-12	10425	260636	26063645
Benzo[a]pyrene	4.39E-06	0.2	2.6	5.50E+04	5.50E+06	1.54E-12	12554	313856	31385608
Benz[ghi]perylene	4.09E-06	0.2	2.6	7.76E+04	7.76E+06	1.01E-12	19047	476165	47616478
Indeno[1,2,3-cd]pyre	4.09E-06	0.2	2.6	8.71E+05	8.71E+07	9.03E-14	213706	5342651	534265063

Influence of Sorption on PAH Bioavailability and Biotoxicity

The aforementioned section has described that slow desorption limits the hydrophobic organic chemicals available to microorganisms in soil. Bioremediation of soil often levels off after an initial rapid decline is believed to be due mostly to the unavailability of an adsorbed fraction. A number of researchers reported that sorption on soil particles and organic matter caused reduced bioavailability of organic compounds (Alexander 1993, Weissenfels 1990, Martin 1978, Ogram 1985). It has been demonstrated that sorption onto activated carbon almost completely prevents dermal uptake and the toxic effects of dioxins in rats (Poiger and schlatter 1980). Thus, bioavailability of soil-sorbed contaminants is related to the effectiveness of microbial degradation as well as on the assessment of toxicological risks. Weissenfel et al. (1990) investigated the relationship of biodegradability and biotoxicity of sorbed PAHs. High degradation rate of PAHs by native microorganism was observed on a sand soil (containing only 1% organic carbon and having a lower specific surface of 1.8 m^2/g-soil). In contrast, PAHs in an organic rich loamy soil (containing 13.6% organic carbon and having a specific surface area of 3.6 m^2/g-soil) were not degraded even after inoculation with bacteria known to effectively degrade PAHs. However, rapid PAH biodegradation in the organic-rich loamy soil was observed after PAHs were extracted from and re-added into the extracted soil. PAH adsorbed into soil appeared to be completely unavailable for biodegradation. Organic carbon content (major component of SOM) is the most important factor influencing the extent of adsorption of hydrophobic molecules (Karickhoff 1979). Migration of PAHs into SOM increases over time. Such deeply sorbed PAHs were suggested to be non-bioavailable and thus non-biodegradable. Weissenfel (1992) further reported that by exhaustive water leaching of the organic rich loamy soil, no biotoxicity, assayed as inhibition of bioluminescence (Microtox test), was detected in the aqueous phase. In contrast, a distinct toxicity was observed with the sandy low organic soil. The toxicity was reduced relative to the amount of activated carbon added to the soil. Weissenfel (1992) suggested that sorption of organic pollutants onto soil organic matter significantly affects biodegradability as well as biotoxicity.

Tang *et al.* (1998) reported that Aging decreased the amount of PAHs available to bacteria in soil as shown by increases in the amount of the compounds remaining after bioremediation and to earth worms (*Eisenia foetida*) as shown by lower tissue concentrations, percentages assimilated, and bioconcentration factors. Aging also diminished the availability of PAH to wheat and barley. PAHs become increasingly more resistant with time to mineralization and extraction (Hatzinger and Alexander). This persistence may result from an initial sorption and subsequent sequestration and unavailability to microorganisms (Alexander 1993).

Sequestration

Sequestration involves slow partitioning of hydrophobic compounds into organic matter (Chiou 1989) or slow diffusion into micropores where their further availability is hindered (Kelsey, Kottler, and Alexander 1997, White 1997, Pignatello 1996). Kelsey, Kottler, and Alexander (1997) defined sequestration as a sorption of hydrophobic organic chemicals that are recoverable by vigorous solvent extraction but not available by living organisms. Because the chemicals can be recovered from soil by vigorous extraction with organic solvents, the chemicals

are not complexed to soil matrix by covalent linkage (Bartha *et al.*1983). The latter will be discussed in detail in the subsequent section.

Soils and sediments are known to have an abundance of pores with diameters appreciably smaller than 1 um (Hassock, 1993, Mayer 1994), and it has been suggested that organic materials that penetrate these nanopores, which have large surface areas, become resistant to degradation (Mayer 1994). Tests with nanopore-containing beads confirmed the possible role of these small pores, provided they have hydrophobic surfaces (Nam and Alexander, 1998). Nam and Alexander (1998) reported that the experimental data indicate that soils in which sequestration was greatest had the largest nanopore volume and surface area. Although this observation may indicate that nanoporosity and surface area are determinants of sequestration, the apparent relationship may simply reflect the greater porosity and larger surface area in soils rich in organic matter. Nam, Chung, and Alexander (1998) reported that phenanthrene mineralization and extractability in soil declined with aging and increased level of soil organic matter. Decline in the rate of biodegradation as a result of aging for 200 days was more marked in soils with >2% organic C. It appeared that a threshold level of organic C is required for sequestration but that the aging effect is independent of additional levels of organic matter, while the extent of sorption was related to the percentage of organic matter in soil. It was suggested that the mechanism of sequestration of hydrophobic compounds entails their partitioning into the organic fraction of soil. To assess the relative importance of these parameters requires a larger number of soils with differences in organic C content, nonoporosity, and surface areas. Organic matter content of soil is a major determinant of sequestration. However, an investigation of 16 soils suggests that other properties of the soil may also contribute to the decline in availability of organic compounds as they age in soil (Chung 1998).

Kelsey, Kottler, and Alexander (1997) challenged the current regulation for assessing exposure risks and toxicity and for setting environmental quality criteria. In USEPA's risk assessment guidance the risk characterization is based on the solvent-extractable contaminant concentrations (USEPA's standard protocol SW846). The fact that vigorous solvent extraction was not correlated with availability of PAHs to bacteria and earthworms indicates the guidance protocol may overestimate the actual exposure risks associated with hydrophobic contaminants in soil. The chemical interactions of hydrophobic organic contaminants with soils and sediments may result in strong binding and slow subsequent release rates that significantly reduce the exposure risks. However, the fundamental physical and chemical phenomena potentially responsible for this apparent sequestration of HOCs by soils are not well understood. Currently there are no definitive data revealing the molecular-scale locations in which hydrophobic organic compounds accumulate when associated with natural soils or sediments, but macroscopic observations are used to make inferences about sorption mechanisms and the chemical factors affecting the sequestration of HOCs by soils.

BOUND RESIDUE FORMATION

A number of recent laboratory studies have revealed that significant fractions of ^{14}C-PAHs added to soil are transformed to bound residues. Bound residues have been defined as "unextractable and chemically unidentifiable residue remaining in soil humus after exhaustive sequential extraction with nonpolar organic and polar solvents" (Kaufman 1976). These bound residues may become associated with components of the soil matrix through several mechanisms including covalent bonding through biologically and abiotically mediated oxidative coupling reactions to soil humus (Bollag 1992, Whelan 1992, Stone 1987, Nieman *et al.* 1999) and intraparticle or intraorganic matter diffusion into organic soil components (Luthy *et al.* 1997). A term of humification is commonly used to describe covalent bonding with soil humus.

Recent studies indicated that bound residue formation represented the most significant mechanism influencing fate and alteration of spiked PAHs (Neiman *et al. 1999*, Guthrie and Pfaender 1998, Carmichael and Pfaender 1997, Sims and Abbot 1992*).* Humification consistently increased with increased time of incubation. Guthrie and Pfaender (1998) reported that approximately 70-80% of ^{14}C-PAH added to soil became bound resides after 285 day incubation. Mineralization and production of polar intermediates of spiked ^{14}C-PAH were less than 5%). Bound residue formation has implications for the bioavailability, toxicity, and transport of xenobiotics in natural environments.

Humification

Plant residues decay rather rapidly in soil and are more or less completely transformed, even the lignin fraction. Freshly incorporated carbon first enters into microbial tissue (soil biomass), the "labile" fraction of SOM, and subsequently into complex humic polymers during advanced stages of humification (Stevenson 1982). Humification is a nonstop polymerization process between humic material and organic molecules. Polymerization of humus material (humification) involves the breakdown, convolution, and transformation of organic matter into long, complex, amorphous organic molecules with numerous reactive functional groups and bridges that are similar to the reactive groups added to aromatic compounds by microbial enzymatic action. Functional groups include hydroxyl, carboxyl, ketonic, phenolic, quinone, ester, ether, carbonyl, and amino groups with dihydrodiol and dione (e.g. quinone). In most soils the major pathway of humification appears to be through condensation reaction involving polyphenols and quinones. Polyphenols derived from lignin, or synthesized by microorgnisms, are enzymatically converted to quinones, which undergo self condensation or combine with amino compounds to form N-containing polymers. Humus structure is highly heterogeneous. A schematic diagram of clay-humate complex is postulated in Figure 2.11 (Stevenson 1994). Electron spin resonance (ESR) spectra of humic substances have revealed the occurrence of stable free radicals. The origin of the free radicals is unknown, but quinone groups of various types are suspect (Stevenson 1982).

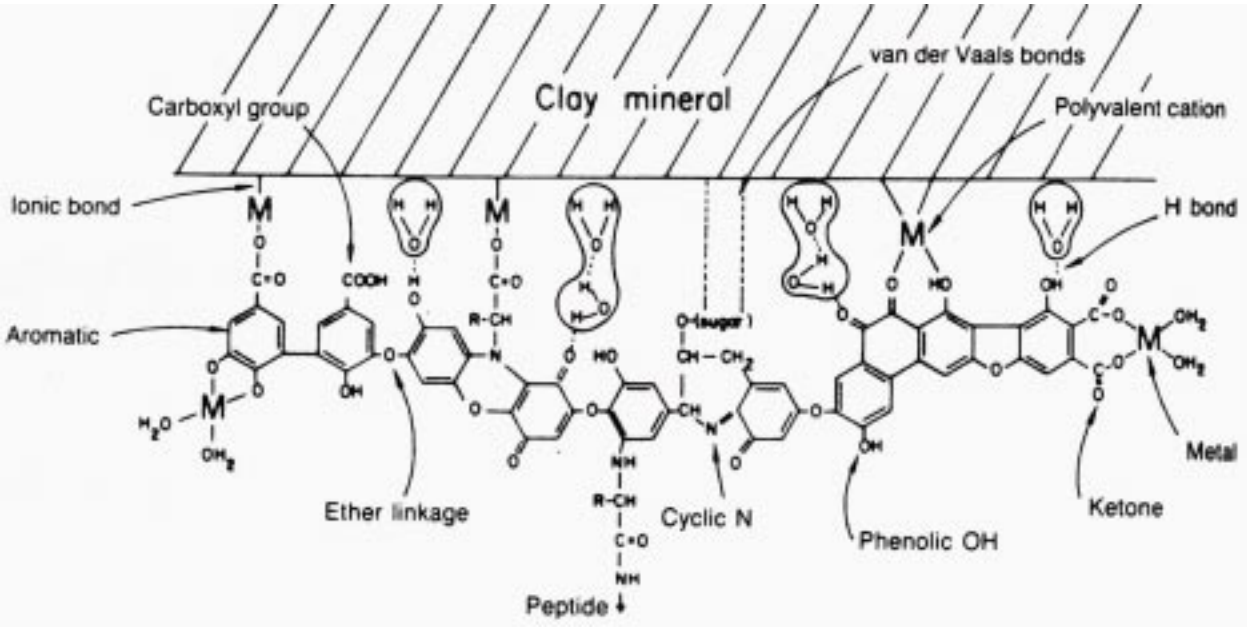


Figure 2.11. Schematic diagram of clay humate complex (Stevenson 1982)

Mechanism of Bound Residue Formation

Studies have shown that apparent depletion of PAHs in contaminated soil is partially due to the formation of stable soil-bound residue that is nonextractable by organic solvent (Eschenbach 1998, Kastner 1995, Qiu 1991). Likewise, substantial evidences indicate that pesticide-derived residues can form stable chemical linkages with components of SOM. These bound residues may become associated with components of the soil matrix through several mechanisms including polymerization through biologically and abiotically mediated covalent bonding to soil humus (Bollag 1992, Whelan 1992, Stone 1987, Neimn *et al.* 1999, Stevenson 1982) and intraparticle or intraorganic matter diffusion into organic soil components (Luthy *et al.* 1997). Adsorption or trapping in the molecular lattice is also possible (Bollag 1992).

Polymerization via Covalent Binding Bollag (1992) suggested that biotic polymerization of xenobiotics in the humification process is possible because many of the degradation products of pesticides and PAHs result in the formation of reactive intermediates with structures and/or functional groups similar to those found in natural humus material. It is well known that humic acid degradation typically yields high concentrations of phenols and a series of alkyl substituted homologues, characteristics of lignin-derivative contribution to humus (Stevenson 1982). Likewise, aromatic alcohols are typical microbiologically derived metabolites of PAHs (Gibson and Subramanian 1984). A number of studies demonstrated that enzymatically-catalyzed bond

formation between various phenols, anilines, and humic materials is primarily of a covalent nature (Sarkar, Malcolm, and Bollag 1988, Martin and Haider 1980, Berry and Boyd 1984, Hatcher *et al.* 1993)). An enzyme-catalyzed oxidative cross-coupling between phenolic moieties may be responsible for the formation of ether- and carbon-carbon bonds within bound residues (Bollag 1983). More recently, scientists have noted that abiotically catalyzed polymerization may also represent an important aspect of humification (Paul and Clark 1996; Sims and Abbot 1992). For example, manganese-bearing silicates have demonstrated catalytic effects in enhancing the polymerization of polyphenols (e.g. hydroquinone) (Whelan 1992).

Since the parent PAH do not possess any coupling groups, PAH may only become susceptible to oxidative coupling if reactive metabolites are produced during degradation. The initial step in microbiological oxidation of PAHs typically results in quinones and dihydrodiols, which may be subsequently transformed to catechols (Cerniglia 1992, Sutherland 1995). Phenanthrols, anthracenols and pyrenols have also been identified as typical metabolites during the biodegradation of phenanthrene (Sutherland *et al.* 1990, Hammel *et al.* 1992), anthracene, and pyrene (Heitkamp *et al.* 1988). Hydroxylated aromatic compounds are chemically more reactive than their precursors. These partially oxidized PAH metabolites, such as quinones and phenols, may then become covalently bound to the SOM (Mahro 1994). In fact, covalent ester bonds, between different PAH metabolites and humic polymers had been identified (Richnow 1994, 1998, Neiman 1999).

Richnow *et al.* (1997) studied ether-link moieties in macromolecular bound residues of PAHs generated in bioremediation experiments using high temperature hydrolysis degradation with subsequent analysis of the products by GC/MS. Ether-bound PAH moieties, which implied a reaction of functionalized PAH-metabolites with humic substances via covalent ether bonds, were identified in the reaction products. A hydrolysis reaction was specifically designed to cleave ether bonds including relatively stable diarylether structures. Among the reaction products Richnow *et al* (1997) found that the concentration of naphthol, phenanthrenol, and pyrenol, and their alkylated homologues in the humus of the PAH-spiked soils were several times higher than that of the non-spiked soil in biodegradation experiments. Significant amount of naphthols and alkylated homologues may originate from aromatic diterpenoids or other plant tissue compounds as well as from PAH motabolites, which subsequently incorporated into humic substances during humification. PAH phenols and their alkylated homologues were not present in the solvent extracts of either PAH-spiked or nonspiked control soils during the biodegradation experiment. Apparently phenanthrols and pyrenols produced during biodegradation were incorporated into the humic substance and nonextractable. It is plausible, that a large portion of the phenols incorporated into humus was derived from the added PAHs. The phenolic metabolites of PAHs may participate in natural condensation processes with humic substances to form relatively stable ether bonds. A scheme of bound residue formation, exemplified by phenanthrene and alkylated homologues, is postulated in Fig 2.12. Microbial metabolism of PAH leads to the formation of reactive phenols that can be incorporated within humic material by the formation of ether-, ester- and C-C bonds. The formation of ether bonds is probably an enzyme-catalyzed process.

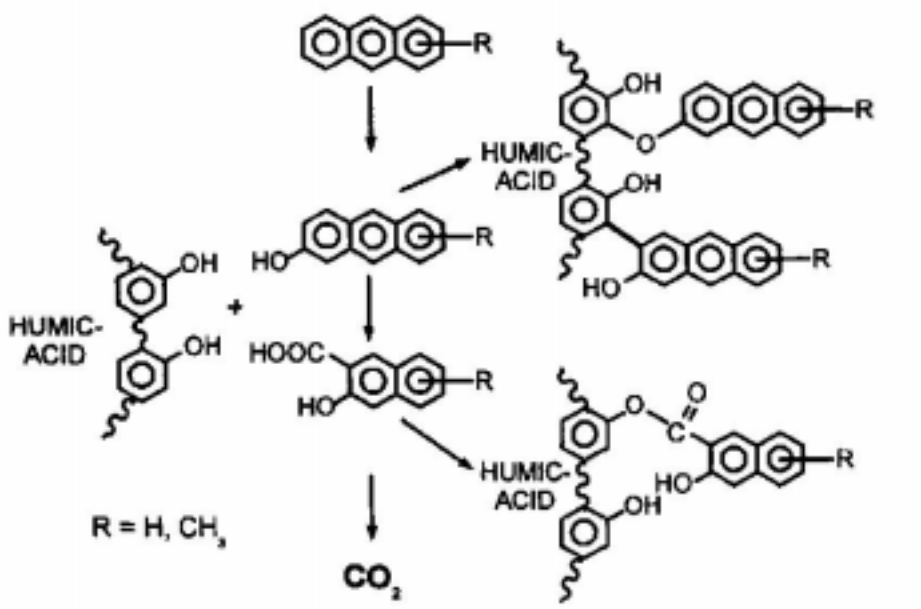


Figure 2.12. A scheme of bound residue formation (Richnow 1997)

Enzyme-Mediated Process The enzyme-catalyzed polymerization of phenol derivatives has been proposed as a major pathway to incorporated xenobiotics into humic material (Bollag, 1992). Oxidoreductase enzymes such as peroxidase, laccase and tyrosinase are known to oxidize phenolic compounds to aryloxy radicals, which then polymerize to form insoluble humic acid-like complexes (Martin and Haider 1980 Sarkar and Bollag 1987, Bollag *et al.* 1988). Phenolic metabolites either derived from SOM or PAH can be cross linked to humic substances via ether- or carbon-carbon bonds.

Sarkar and Bollag (1988) reported that chlorinated phenols can be cross-linked via diarylether and phenyl carbon-carbon-bonds to aquatic humic substances in the presence of various oxidoreductases. Richnow *et al.* (1997) identified peroxidase-mediated cross-coupling of aromatic alcohols to dissolved soil humic substances. With excess humic substances cross-linking of naphthol and humus was more effective than the cross-linking reactions between naphthol and naphthol without humus. Cross-linking between naphthols forming dimmers, oligomers and polymers is not significant when humic substances are present. Humic substance appeared to serve as a preferred substrate in the competition for binding sites during enzymatic cross-coupling reactions. Aliphatic and aromatic hydrocarbons, halocarbons, ketones and various aromatic acids, fatty acids and aliphatic alcohols were found no significant reactivity with peroxidase. However, all species of aromatic alcohols were reactive.

Quinones are important intermediate metabolites derived from the oxidation of PAHs by fungi. Launen (1999) reported that Soil fungus Penicillium janthinellum SFU403 *in vitro*

oxidizes pyrene to pyrenequinones, which subsequently formed nonextractable cell associated products. Almost 100% of the added ^{14}C-pyrene was nonextractable in the presence of SFU403. Approximately 40% of was ^{14}C-pyrene quinones and the rest (~60%) was strong sorption of the parent ^{14}C-pyrene to fungal mycelia. It was hypothesized that the pyrene quinones (PQs) were reduced to pyrene semiquinones (PSQs) by intracellular reductants. PSQs could than polymerize and/or bind covalently to cellular macromolecules. Electron paramagnetic spectroscopy confirmed the hypothesis. 1,6- and 1,8-PQs were reduced by NADPH to the corresponding pyrene semiquinone radical anions *in vitro*.

The kinetics of enzymatic oxidation of phenols and chlorinated derivatives have been studied in various types of soil (Claus and Filip, 1990a). Typical soil constituents can have stimulating or inhibiting effects on the activity of phenoloxidases. Negative effects on the enzyme activity of phenoloxidases have been observed in the case of substances with high cation exchange capacity such as clays and humic acid complexes (Claus and Filip, 1990b). Berry and Boyd (1984) reported structure-activity relationships during oxidative coupling of phenols and anilines by peroxidase. Berry and Boyd found that electron donating substituents enhanced the oxidative coupling, while electron accepting groups hinder the cross-coupling reactions.

Structure of Polymerization To study the structural aspects of polymerization, Richnow (1997) have analyzed the fraction of dimmers resulting from a polymerization experiment that treated phenol and 1-naphthol with horseradish peroxidase. A series of hydroxy diaryl ethers and dihydroxy phenyl derivatives were observed. Dihydroxy biophenyls were the major reaction products and hydroxydiaryl ethers were the minor products. 2,2-dihydroxybiphenyl was found to be the major isomer in the dihydroxybiphenyl fraction indicating the ortho-position to be the most reactive site. The precise structures of the hydroxynaphthylphenyl ethers and dihydroxyphenyl naphthalenes have not been elucidated yet, but, analogous to the phenol dimmers, the two major isomers in this fraction are suggested to be cross-coupled at the ortho-position to the hydroxy group of 1-naphthol and phenol, respectively.

In summary, in soil and sediment, oxidoreductase-like enzymes are suitable microbial-derived catalysts for the formation of C-O-C ether- and C-C linkages and thus contribute to the formation of soil-bound residues.

PHYTOREMEDIATION OF PAH-CONTAMINATED SOIL

Phytoremediation is plant-facilitated *in-situ* bioremediation. *In-situ* bioremediation of PAH-contaminated soils is a challenge, especially for low permeability clay soils. The high adsorption capacity of clay limits the amount of PAHs available to microorganisms. Low flux of nutrients and electron acceptors through low permeability soil also reduce microbial activities. An engineered process may accelerate biodegradation, however a system of distributing electron acceptors, substrates, nutrients, and enzymes to numerous micro-sites can be technically and economically unfeasible. A plant system can facilitate in-situ biodegradation of organic contaminants by taking up chemicals from soil, assimilating chemicals in plant tissue, and/or stimulating rhizosphere degradation, humification, and sequestration.

Plant Assimilation of PAH

Plants use a variety of reactions to degrade complex aromatic structures to more simple derivatives. (Ellis 1974). Benzo[a]pyrene, a five-ring PAH, can be metabolized to oxygenated derivatives in plant tissues (Harms 1977). Although some of these derivatives (e.g., phenols) are known to be more toxic than the original compounds, they appear to be polymerized into the insoluble plant lignin fraction and become nontoxic components. Despite that the intermediate metabolites are not completely mineralized, polymerization is another important detoxification mechanism. With plant seedlings, benzo[a]pyrene was assimilated into organic acids including amino acids (Sims and Overcash 1983). Complete degradation of benzo[a]pyrene to carbon dioxide was also observed for a wide variety of plants (Sims and Overcash 1983).

Plant Uptake

The ability of a plant to take up a chemical from the soil and groundwater and translocate it to its shoots is measured by the chemical's root concentration factor (RCF) and transpiration stream concentration factor (TSCF). RCF is the ratio of chemical concentration in roots to the concentration in external solution. TSCF is the ratio of chemical concentration in xylem sap to the concentration in external solution. Both RCF and TSCCF vary directly with a chemical's octane water partition coefficient (K_{ow}) (Briggs 1982). Contaminants with the highest TSCF are moderately soluble compounds with a log K_{ow} in the range of 0.5 to 3 (Bromilow and Chamberlain 1995; Briggs 1982). These compounds were found to accumulate in plant xylem, but not in phloem. Most chlorinated solvents and BETX have K_{ow} within this range. Plant uptake of organic chemicals decreases as soil organic matter (SOM) contents increase. The influence of soil organic matter content on the plant uptake of xenobiotic organic compounds is illustrated in Figure 2.13 (Briggs 1982).

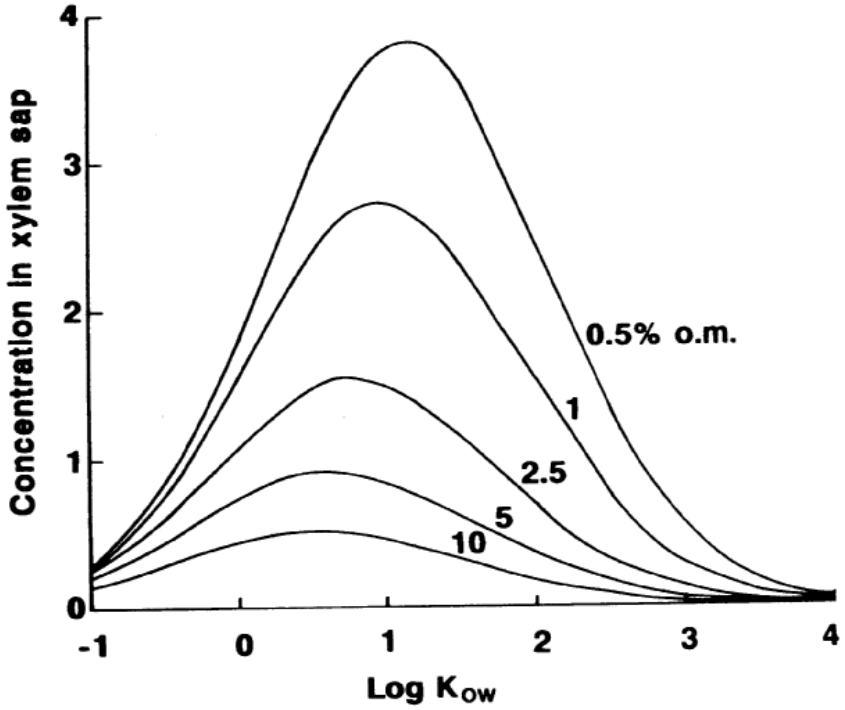


Figure 2.13. Influence of soil organic matter content (o.m.) on the efficiency of uptake by plants of xenobiotics of differing lipophilicities (Briggs 1982)

The "Briggs Curve" in Figure 2.13 indicates that the potential plant uptake of chemicals having K_{ow} greater than 4 are negligible, especially in organic-rich soils. PAHs of which log K_{ow} range from 3.36 to7.66 will be largely adsorbed onto the root surface and will not be translocated to plant shoots except for two-ring PAHs, such as naphthalene. As a result plant uptake and subsequent food web effects are generally not a concern for PAHs, except for small PAHs such as naphthalene and acenaphthene. Fortunately, the small PAHs are less toxic and readily degradable.

Rhizosphere Degradation, Humification and Sequestration

Rhizosphere is the soil region under the immediate influence of plant roots and in which there is proliferation of microorganisms due to the plant roots. The consortium of bacteria and fungi associated with the rhizosphere possess highly versatile metabolic capabilities and great potential of detoxifying organic contaminants (Atkinson et al. 1983). Detoxification mechanism can be through complete mineralization of toxic organic chemicals into innocuous end point products, such as H_2O, CO_2, Cl^-, or formation of soil-bound-residues of parent PAHs or intermediate motabolites. In addition PAHs sequestered in soil micropores were found to be non-bioavailable and non-biotoxic (Weissenfels 1992).

Plant roots have numerous effects on soil biota and the environment. Roots improve aeration in soil by removing water through transpiration and by altering soil structure through agglomeration. Root turnover (growth and death) creates porous soil structure, thus improving soil aeration. Plants release photosynthate by finely distributed plant roots to soil through exudation and sloughing of dead root cells. Roots supply substrates (sugars, organic acids, amino acids, etc.) which sustain a dense microbial community in the rhizosphere, which may enhance degradation, mineralization, and/or polymerization of organic toxicants (Fitter and Hay 1987). The growth substrates may also support active proliferation and action of cometabolism of certain recalcitrant organic compounds which bacteria and fungi cannot use as a sole carbon source Perry 1979; Bossert and Bartha 1984; April and Sims 1990). Additionally, the increased soil organic content in rhizosphere soil may alter the behavior of organic toxicants in soil, by changing the extent of adsorption, soil-bound-residue formation, bioavailability, biodegradability, leachability, and volatility (Walton, Futhrie, and Hoylman 1994).

Effects on Soil Structure Vadose zone soils are often mixtures of sand, silt, clay particles, and natural soil organic matter. The solid phase of soil consists of particles of various shapes and sizes packed together in various ways (Foth and Turk 1972). Soil aggregates are of highly irregular shape and size. Blocky aggregates that are small and rounded (<10 mm diameter) granular or crumb structure are often the characteristics of surface soil of grassland . Large prismatic aggregates rounded tops are described as having columnar structure. Sands of single-grain structure and clays of massive structure are sometimes described as structureless definitions. Many biological organic agents affect the development and stability of aggregates (Foth and Turk 1972). Fungal hyphae growing in soil entangle the bind particles. Earthworms ingest soil and organic matter and void the undigested residues in their casts to form new aggregates. Humus and polysaccharides produced by microbial decomposition of plants, animals, and microorganisms give stability to natural aggregates. Root and microbial secretion (mucilaginous gel, called mucigel) extends into the surrounding clay by long polymerized thread molecules interlaced as a network.

Soils vary greatly in organic matter content depending on soil formation time , climate, vegetation , parent material, topography, etc. A typical prairie grassland soil (e.g., Mollisol) may contain 5 to 6% organic matter in the top 15 cm, but a sandy soil typically contains less than 1% of SOM. Poorly drained soils often have SOM near 10%. The C/N ratio of SOM generally falls in the range of 10 to 12, although higher values are not unusual (Stevenson 1982). Plant roots

affect mineralization of SOM. The effect of moisture removal decreases SOM mineralization under dry conditions. The priming theory of SOM decomposition suggests that the addition of available nutrients to the rhizosphere should increase the decomposition of SOM. It is also argued that plants, by removing nutrients, stimulate decomposition of recalcitrant compounds. Nevertheless, grasses with high root biomass result in mineral soils with the highest SOM.

Effects of Root Exudation Chemicals released from plant roots exert a very strong influence on the soil microorganisms and plant nutrient availability (Rovira 1969).

Root-release chemicals Roots release considerable amounts of organic carbon into the rhizosphere, varying from a few percent to up to 40% of the total dry matter production (Marschner and Römheld 1996). Three major components are involved in the release of organic carbon into the rhizosphere: (1) sloughed-off cells and cell lysates, (2) high molecular weight gelatinous material ("mucilage"), and (3) low molecular weight organic compounds ("free exudates"). The main constituents of the free exudates are sugars and amino sugars, aliphatic, aromatic, and amino acids, amides, and phenolics (Rovira 1969, Paul and Clark 1996). Minor components include nucleotides, peptides, enzymes, vitamins, fungal stimulators, inhibitors and attractants, and many miscellaneous compounds (Rovira 1969; Marschner and Römheld 1996). Phenolic compounds are part of root exudates as well as intermediary products in the metabolism of molecules containing aromatic rings (lignin, tannins, many pesticides). Catechol, 4-hydroxybenzoic acid, 3,4-dihydroxybenoic acid, 3-methoxy-4-hydroxybenzoic acid (Vanillic acid), 2-hydroxyphenylacetic acid, and 3,4-dihydroxycinnamic acid (Caffeic acid) is common in rhizosphere soils. As aforementioned, phenolic compounds are part of root exudates as well as intermediary products in the metabolism of molecules containing aromatic rings (lignin, tannins, many pesticides). Catechol, hydroxybenzoic acids, hydroxyphenylacetic acid, and Caffeic acid are commonly observed in root zone soils.

Both the amounts and the composition of root exudates vary considerably. In five different forest trees, acetic and oxalic acids were found to be the most abundantly excreted organic acids. Forest trees release was 1-3 g compounds per kg dry root per day. In corn plants, 65% of the total exudates were found to be sugars, 33% organic acids (mainly fumaric, oxaloacetic, and malic acids) and 2% amino acids (Bar-Yosef 1996). Organic compounds are released at a rate of 1 ug/cm-root-day, which is equivalent to 5.2×10^{-9} mol citrate/cm-root-day. Citrate was released by alfalfa and rape plants at rates of 13 and 10 nmol/cm-root-day. Forest trees were to release citric acid at a rate of 1- mg/g-dry-root/day. Citrate excretion under such conditions may sustain a total concentration of 0.18 mmol/L/day (Bar-Yosef 1996).

The amount and the composition of the exudation depend on plant species, age, and root environment (Rovira1969; Clayton and Lamberton 1964), Toussoun and Patrick 1963). Temperature, light, nutrition, soil moisture, and soil microorganisms affect exudation. Various forms of stress increase the amount released. For example more organic carbon is released under potassium deficiency, phosphorus deficiency, drought stress, anaerobiosis, or mechanical impedance. The presence of microorganisms also has a distinct enhancement effect on the amount of root exudates. Exudation from intact roots is slight and of the order of 0.1% to 0.4% of the carbon photosynthesized by the plant (McDougal and Rovira 1965). Root damage whether chemical or physical can dramatically increase the quantity of organic substances exuded (Rovira

and McDougall 1967). The physiological process and the mechanisms involved in exudation are not well understood. Several scientists suggested that the roots have a metabolically mediated process by which roots selectively retain and re-absorb the organic compounds. However, other experiments indicated that root exudation is related to cell permeability (Rovira 1969).

Zone of soil influenced by root exudates. The distances that exudates diffuse from roots depend on the amounts exuded, pH, the susceptibility of the compounds to microbial absorption and decomposition, the types and amounts of clay in the soil, and soil moisture content (Katznelson, Rouatt, and Payne 1954, 1955). Considering the increased numbers of bacteria and fungi, researchers have suggested that the zone influenced by root exudates extends from 1 to 2 mm from the root (Rovira and McDougall 1967). The root exuded sugars, organic acids, and amino acids are rapidly metabolized and hence have little opportunity to diffuse farther from the root. Less degradable compounds may diffuse considerable distances from roots. Wallace (Wallace 1961) reported that in a saturated soil root exudates may have diffused away from the root at 5 mm per day indicated by potato eelworm hatching factor. The sensitivity of some nematodes to root exudate has been used in studies to understand the significance of root exudates. Much work has been conducted upon the potato eelworm which is stimulated to hatch from cysts by root exudates

Effects of root exudates on soil microbial community Plants support a prolific population of bacteria and fungi on and around their roots. Due to the large supply of organic carbon by roots, the microbial population in rhizoplane and rhizosphere are 5 - 50 times higher than that in the bulk soil (Lynch and Whipps, 1990). The main organic carbon source for rhizosphere microorganisms is sloughed-off cells. In the rhizosphere there is a selective stimulation of certain fungi and bacteria. The bacteria that colonize roots are predominantly gram negative rods that respond rapidly to glucose and amino acids and are choramphenicol sensitive and resistant to erythromycin and penicillin (Rovira and Brisbane 1967). The specificity shown by plant roots in their selective stimulation of certain bacteria is clearly shown in the symbiotic association between the root nodule bacterium (Rhizobium) and its legume host (Rovira and Brisbane 1967). Adverse allelopathic effects of root exudation have also been evidenced (Rovira 1969). Oppositely, microorganisms may affect root exudation in several ways: (a) the permeability of root cells, (b) the metabolism of roots, and (c) absorption of certain compounds in root exudates by microorganisms and excretion of other compounds (Rovira 1969).

ROOT FLAVONOIDS

Flavonoids, by virtue of their ubiquitous distribution and immense structure diversity, have attracted serious attention of scientists from a wide range of disciplines in life science. Flavonoids comprise a set of biosynthethically related phenolic compounds which are important taxonomic characters of high plants. Flavonoids distribute differently in all parts of the plants. Roots usually contain lower flavonoid concentrations relative to the aerial parts. However, root flavonoids constitute an important class of compounds found in the root exudates (Rao 1990).

Role and Quantity of Root Flavonoids

Synthesized by plant cells, flavonoids are secondary metabolites which are defined as compounds that have no recognized role in the maintenance of fundamental life processes in the organisms that synthesize the compound (Bell 1991). Root flavonoids, interacting with the principal plant hormones, play a significant role in protecting the plants against various pests and diseases, regulating root growth and functions, influencing different aspects of the nitrogen cycle, and exerting allelopathic growth effects. Consumed in the human diet, flavonoids and their synthetic analogs display a variety of biological effects including anticarcinogenic, antiinflammatory, antioxidant and antiallergenic activities (Glusker and Trueblood 1985).

Despite that large number of flavonoids are detected in plant roots, root exudates, and soil extracts, there is very little definitive information regarding the quantitative aspects of flavonoid exudation (Rao 1990). The reported root flavonoid exudation varied from 3.5% to 20% of the total flavonoid content in roots for different plants (Rao 1990). In relation to total photosynthesis, about 2% of the carbon statistically, is assumed to be diverted towards flavonoids and related compounds (Rao 1979). Although these numbers are inadequate to assess the magnitude of flavonoid exudation from plants, it is evident that considerable but highly variable amounts of flavonoids are exuded from the roots. Further, flavonoids are released to soil as a result of root turnover, root injury and root decomposition. It is believed that flavonoids significant biological effects on plants and microorganisms (Rao 1990).

Molecular Structure and Classification

Flavonoids are phenolic compounds. The chemical structures of flavonoids are based on a C_{15} skeleton with a chromane ring bearing a second aromatic ring B in position 2, 3, or 4. The basic structure of most flavonoids is presented in Figure 2.14. In a few cases, the six membered heterocyclic ring C occurs in an isomeric open form or is replaced by a five-membered ring (Hahlbrock 1981). Flavonoids are classified according to the substitution patterns of ring C. Both the oxidation state of the heterocyclic ring and the position of ring B are important in the classification. The six major subgroups of flavonoids are (1) chalcone, (2) flavanone, (3) flavone, (4) flavonol, (5) arithocyanidin, and (6) isoflavone (Hahlbrock 1981). Representative structures of each of the six subgroups are also presented in Figure 2.14.

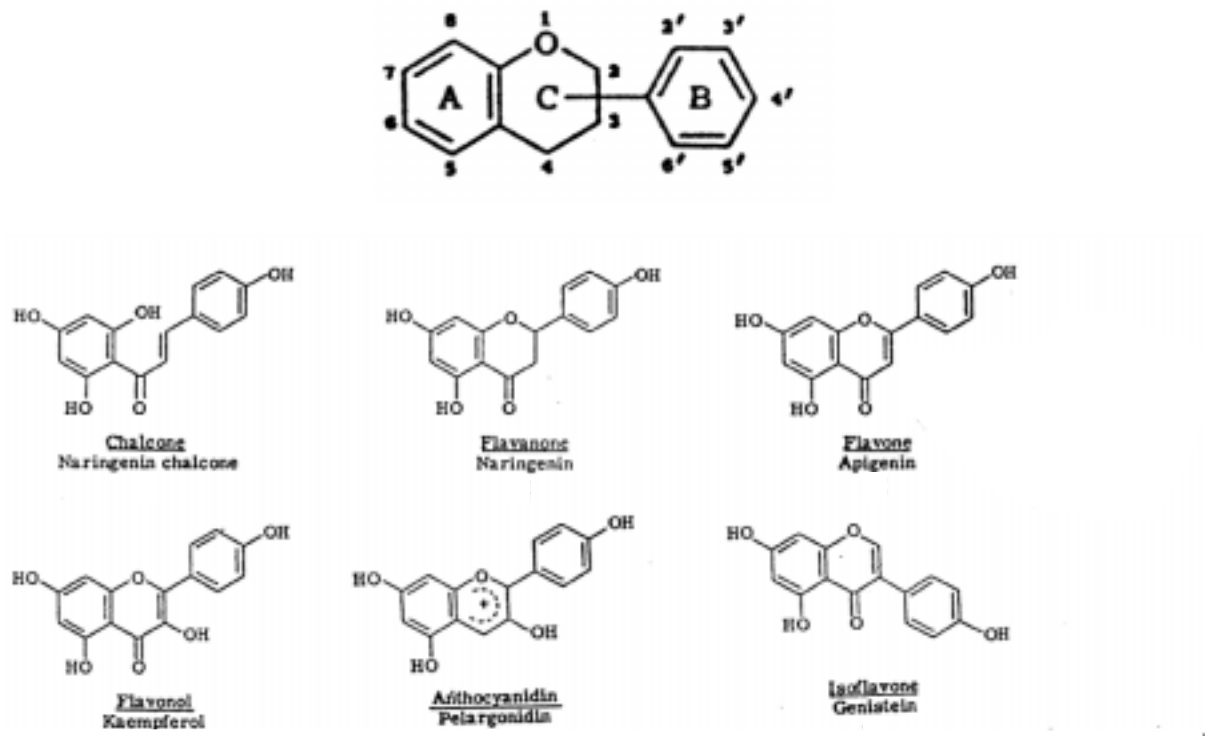


Figure 2.14. Basic structure of most flavonoids and representatives of six major subgroups (adapted from Hahlbrock 1981)

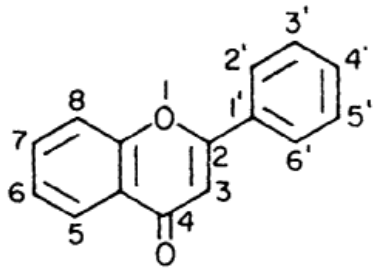
Most of these bear ring B in position 2 of the heterocyclic ring. In isoflavones ring B occupies position 3. Each subgroup contains many different flavonoids differing by the number and attachment positions of hydroxyl and/or alkyl side chains. Flavonoids are widely present in plants as water-soluble glycosides with different combinations of sugars attached to hydroxyl groups. Naturally occurring root flavonoids are Hydroxylated in common and of immense structural diversity. Besides the aforementioned six major subgroups, the major classes of root flavonoids also include chromone, coumarin, aurone, flavane, flavylium, pterocarpan, coumestan, rotenoid, 3-arylcoumarin, coumaronochromone, and some complex root flavonoids (Rao 1990).

Mulberry Root Flavonoids

Mulberry root flavonoids have been studied in great detail because of pharmaceutical and therapeutic significance (Rao 1990). The root of *Morus alba* (white mulberry) is a Chinese herbal drug, "San-Bai-Pi", which has antitussive, antipyretic, diuretic, hypotensive, expectorant and laxative effects. Hydroxylated-prenylated flavones and flavonols with Diols-Alder adducts are characteristic of *Morus* and several other genera belonging to the family Moraceae (Gornall, Bohm, and Dahlgren 1979). These flavonoids, particularly morusin (5,2',4'-trihydroxy-3'(3,3-methylallyl)-2",2"-dimethylpyrano(5",6'-7,8)flavone) possess significant antitumor activity (Rao 1990). *Morus* alba bark contains four flavones: mulberrin, mulberrochromene, cyclomulberrin, and cyclomulberrochromene (Venkataraman 1975). All the four flavones have two prenyl side chains at C-3 and C-5 positions. Characteristic flavones of Mulberry are presented in Figure 2-15. Morus alba flavone have the common feature of hydroxyl groups in the positions 5, 7, 2', 3', and/or 4'. *Morus* nigra (black mulberry) bark does not contain these flavones except mulberrin. Differently, *Morus* rubra (red mulberry) flavones have C_{10} side chains attached at C-3 position (Venkataraman 1975). Morin and many other Hydroxylated flavonoids including myricetin, quercetin, kaempferol, flavonols, apigenin, flavanones, isoflavones, etc. have been isolated from *Morus* bark as well. The structure of simple flavone and morin and a list of naturally occurring common root flavonoids are presented in Figure 2.16 (Gornall, Bohm, and Dahlgren 1979). Some complex root flavonoids, e.g., sanggenons and kuwanons, characteristics of Moraceae plants are presented in Figures 2.17 and 2.18 (Rao 1990).

Morus root flavonoids are Hydroxylated in common and of immense structural diversity. Despite the detailed studies of Morus root flavonoids, no definitive information is available regarding the quantity of Morus flavonoid release.

Naturally occurring flavonoids



FLAVONE

Common name	Chemical name
Apigenin	4′,5,7-Trihydroxyflavone
Chrysin	5,7-Dihydroxyflavone
Fisetin	3,3′.4′,7-Tetrahydroxyflavone
Flavanone	2,3-Dihydroflavone
Galangin	3,5,7-Trihydroxyflavone
Hesperitin	3′,5,7-Trihydroxy-4′-methoxyflavanone
Kaempferol	3,4′,5,7-Tetrahydroxyflavone
Morin	2′,3,4′,5,7-Pentahydroxyflavone
Myricetin	3,3′.4′,5,5′,7-Hexahydroxyflavone
Naringenin	4′,5,7-Trihydroxyflavanone
Nobiletin	5,6,7,8,3′.4′-Hexamethoxyflavone
Quercetin	3,3′.4′,5,7-Pentahydroxyflavone
Tangeretin	5,6,7,8,4′-Pentamethoxyflavone

Morin

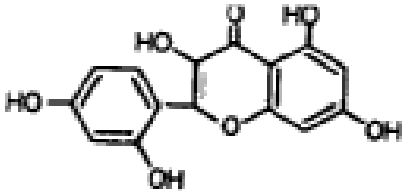
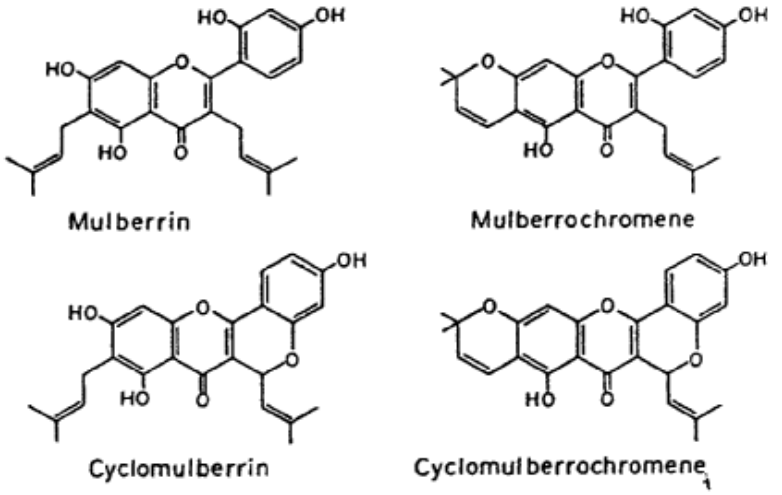
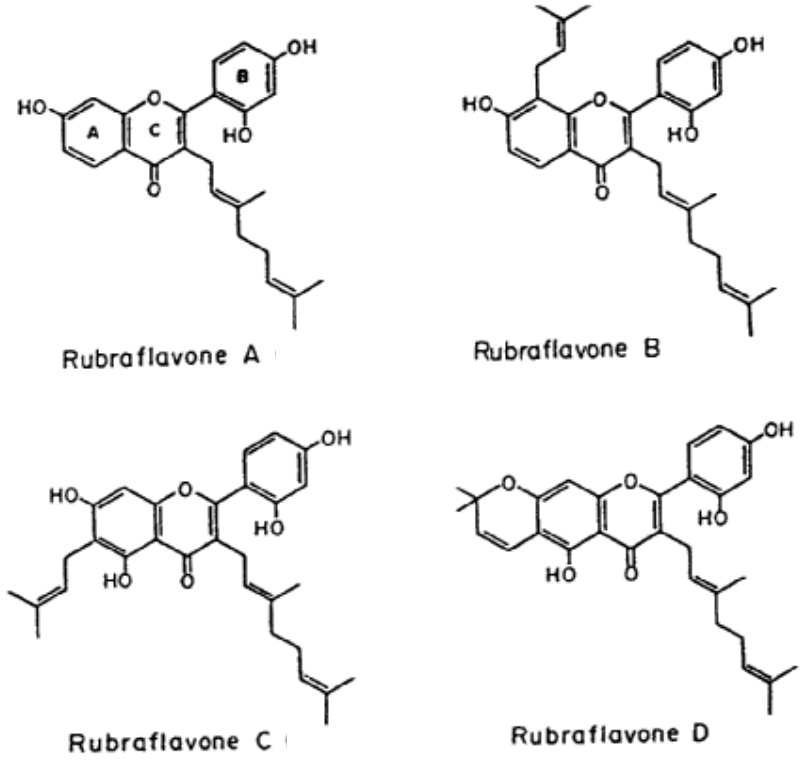


Figure 2.15. Flavone, morin, and other natural occurring flavones (Hahlbrock 1981)



***Morus* Alba Flavones**



***Morus* Rubra Flavones**

Figure 2.16. Characteristic flavones of mulberry (Hahlbrock 1981)

Sanggenons (14 = ^{14}C-α or ^{14}C-β)

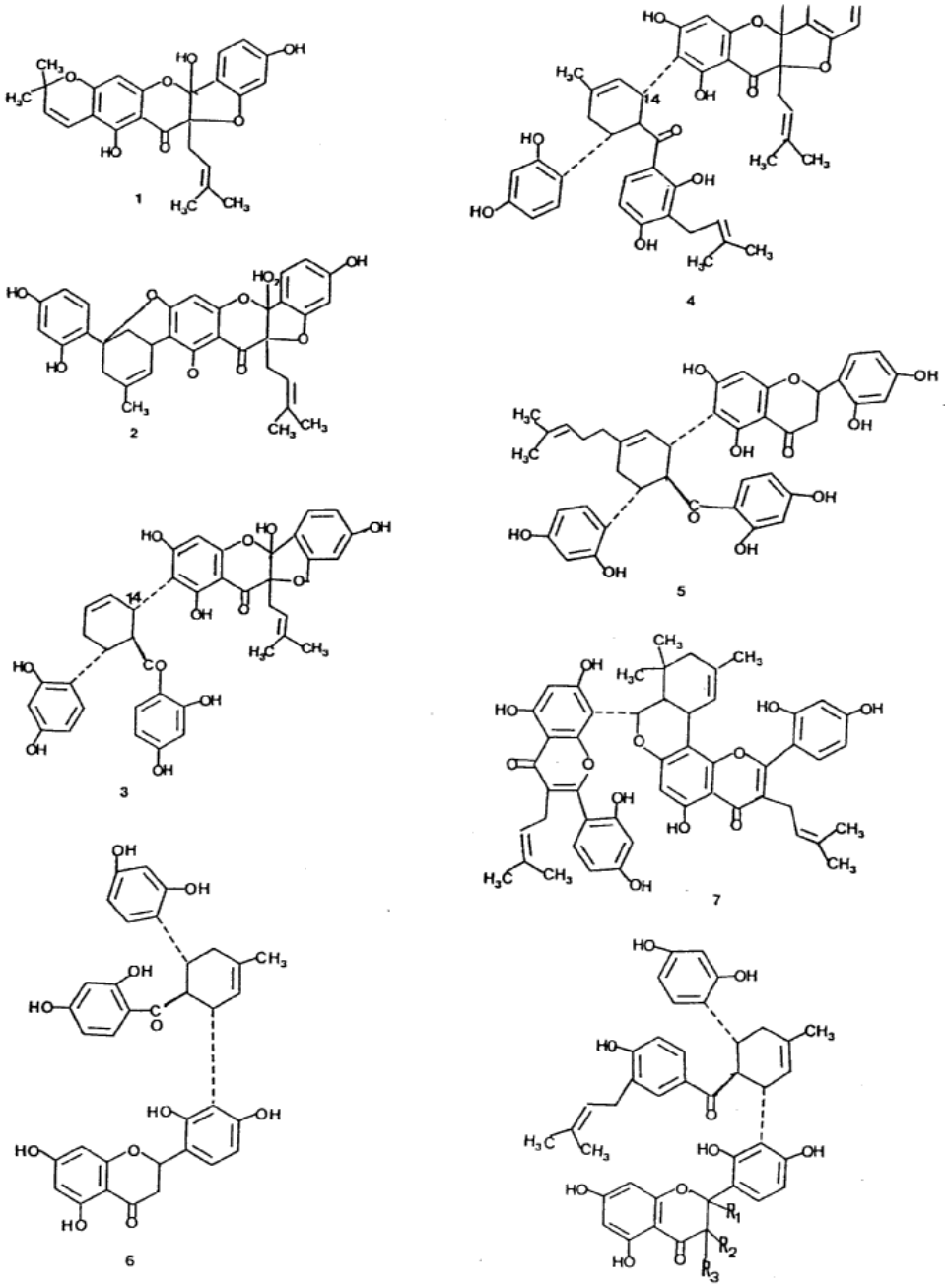


Figure 2.17. Some complex root flavonoids characteristic of *morus* and related species belonging to the family moraceae (Rao 1990)

Kuwanons

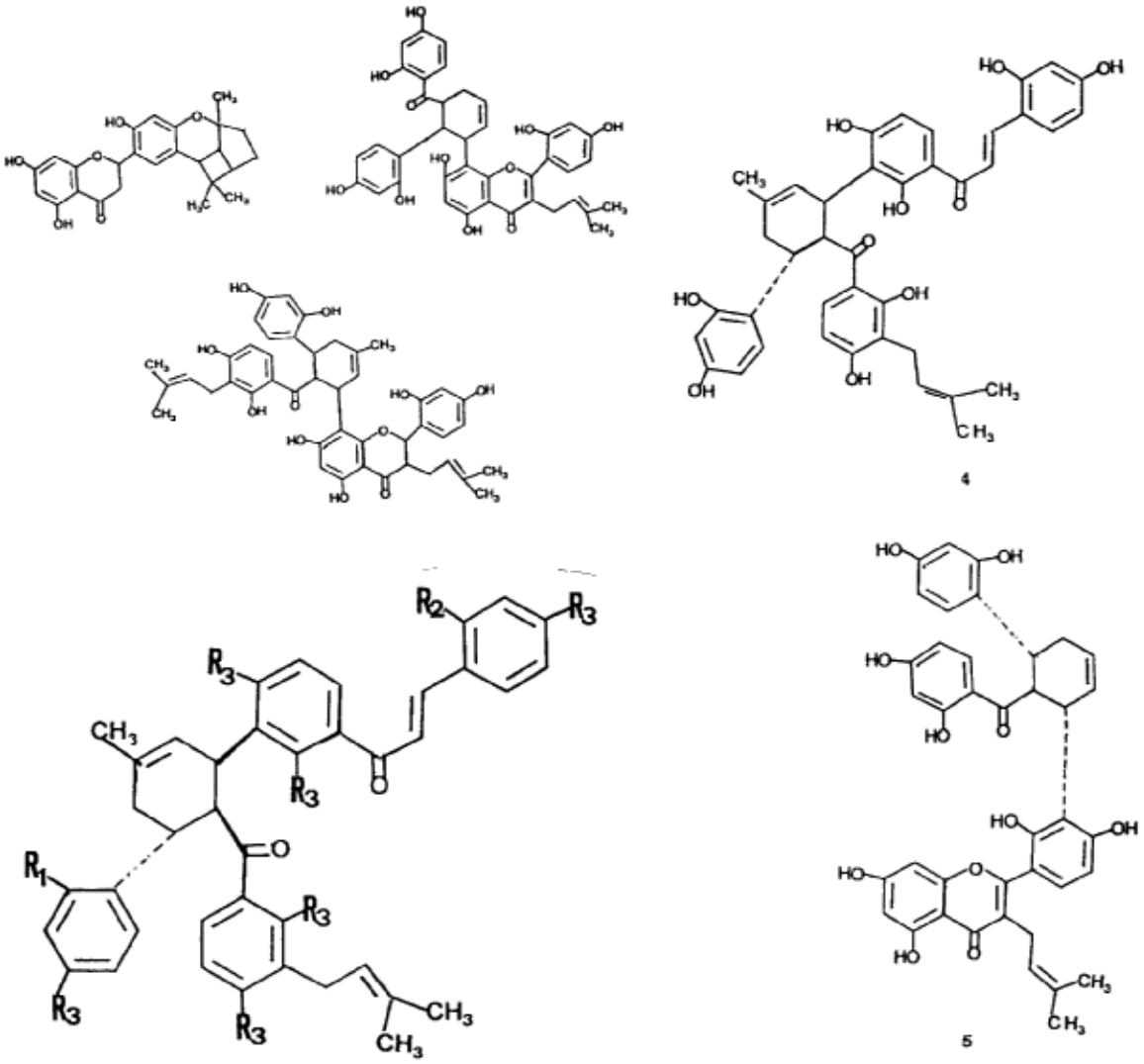


Figure 2.18. Some complex root flavonoids characteristic of *morus* and related species belonging to the family moraceae (cont') (Rao 1990)

FLAVONOID METABOLISM WITH REGARD TO THE POTENTIAL EFFECTS ON PAH DEGRADATION

Flavonoids are often rapidly metabolized after synthesis (Hahlbrock 1981). The metabolic autonomy of the roots in the synthesis, uptake, utilization and storing of various flavonoids remains ambiguous. A conceptual model of anabolic and catabolic pathways of some flavonoids is presented in Figure 2.19. Biosynthesis of flavonoids is derived from acetate and phenylalanine. Flavonoids are converted to benzoic acids prior to completely mineralized to CO_2.

The degradative pathways of various flavonoids and enzymatic reactions involved in higher plants are poorly understood except for some flavones and flavonols. Many flavonoids can be metabolized to epoxides and diols in the same way that PAHs are metabolized. Monooxygenase and/or dioxygenase are responsible for those degradations. Flavonoids are degraded by bacteria or fungi as growth substrates. Microorganisms capable of metabolizing flavonoids are present in rhizosphere soil (Rao 1990).

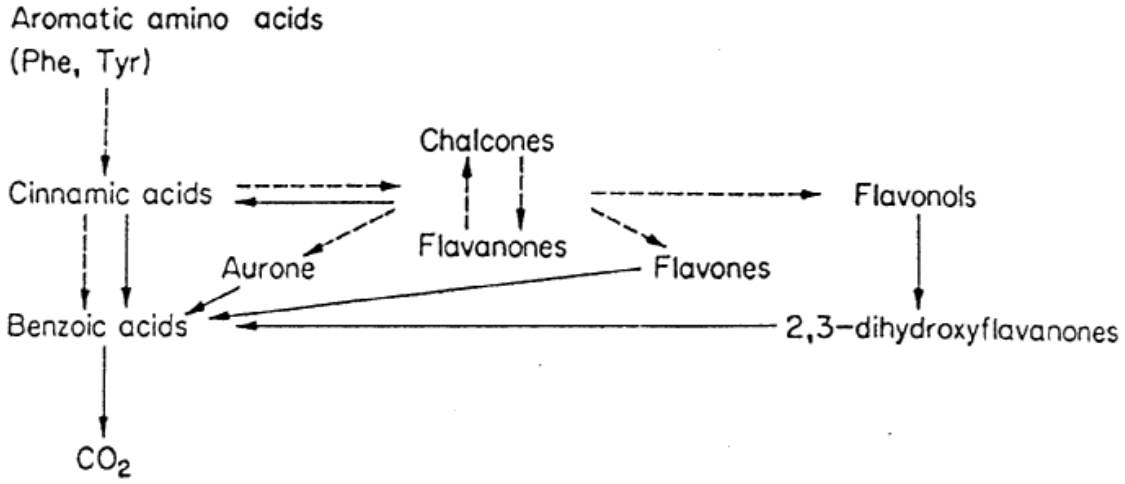


Figure 2.19. Metabolic grid depicting anabolic and catabolic pathways of some flavonoids (Barz 1975)

Degradative Pathways of Flavonoids

Metabolic routes applicable to more than one class of flavonoids are known. The best understood example of flavonoid metabolism by microorganisms is the fungal degradation of flavonols. Rutin (quercetin 3-rutinoside) were shown to be catabolized by *Aspergillus* species. The pathway is illustrated in Figure 2.20. Rutinase hydrolysis rutin (3-rutinoside). Quercetinase, a copper containing dioxygenase, splits flavonol aglycones yielding carbon monoxide and a depside in the presence of oxygen. The ring cleavage occurs between C-2 and C-3, yielding di- and tri-hydroxy benzoic acids (Barz and Hösel 1975).

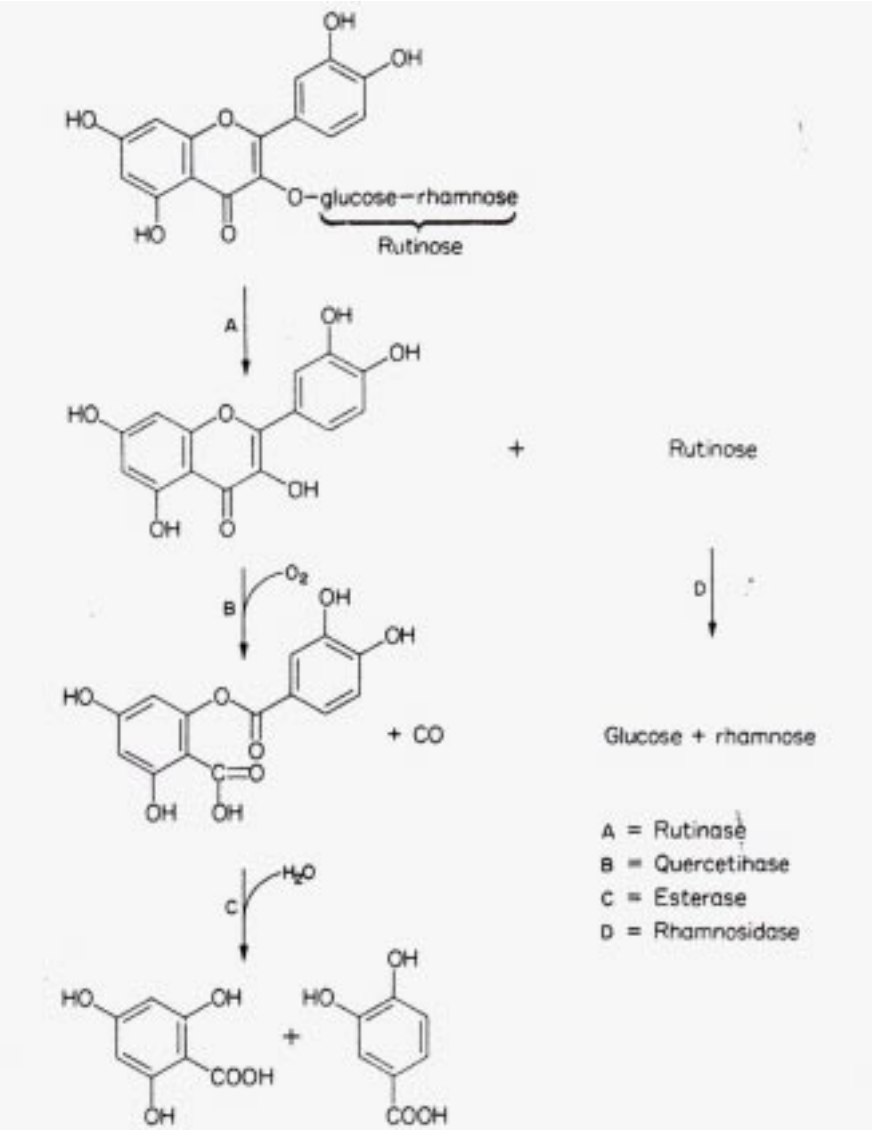


Figure 2.20. Degradative pathway of rutin and flavonols as occurring in fungi (Barz and Hosel 1975)

Barz and Hösel (1975) reported that flavonol, flavone, dihydroflavonol and catechin were metabolized by bacteria, such as *Pseudomonas* species. The first step of the metabolism was hydroxylation at C-8 (ring A). Degradation pathway of flavonols by *Pseudomonas* species is presented in Figure 2.21. The initial hydroxylation requires stoichiometric amounts of oxygen and NADH (reduced nicotinamide adenine dinucleotide). The intermediate metabolites, 7,8-dihydroxyflavonoids, are further degraded by dioxygenases under aerobic conditions. A meta-type ring cleavage occurs between C-8 and C-9, yielding oxaloacetate, hydroxy-benzoic acid, etc.

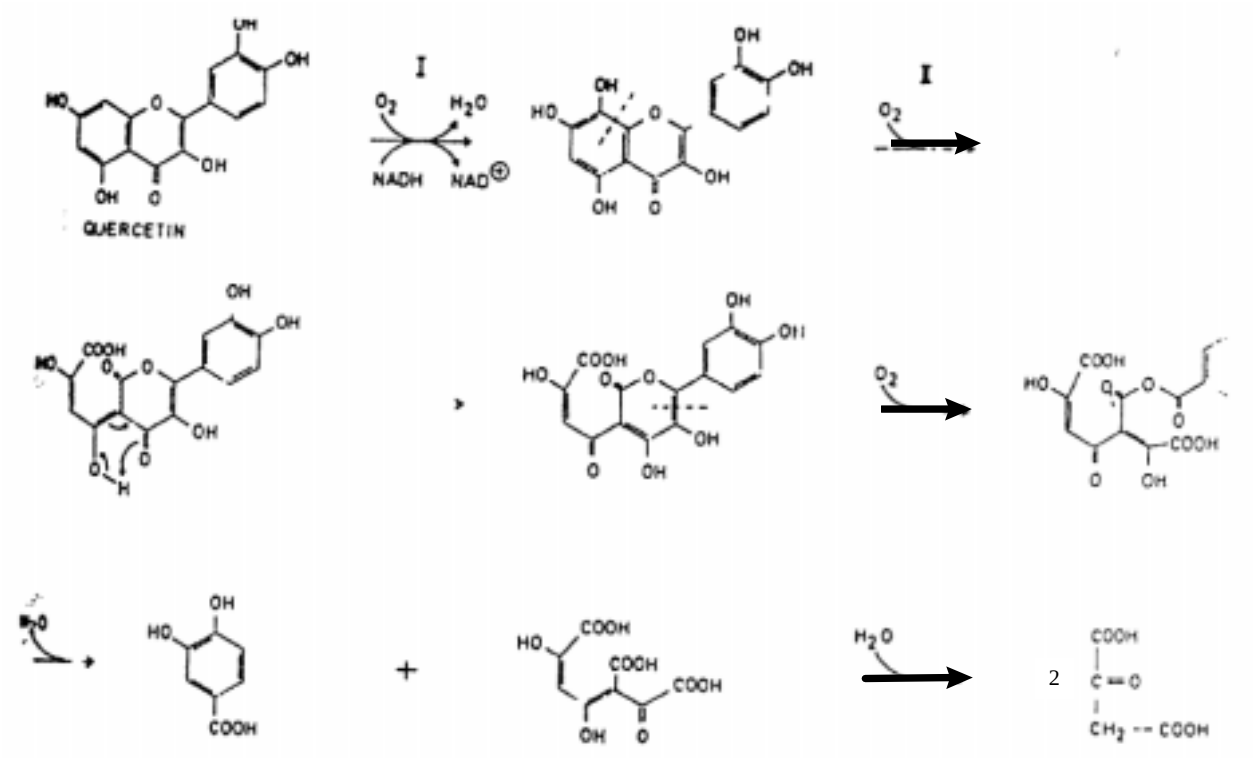


Figure 2.21. Degradation of flavonols by *pseudomonas* species (Barz and Hosel 1975)

Anaerobic dissimilation of flavonoids exists in wet soil, ponds or the mammalian gut. Flavonols are degraded to phenylacetic acids. Degradation pathways of flavones, flavanones, catechins and flavonols by Mammalian gut microflora are presented in Figure 2.22. One of the most active organisms is *Buryrivibrio.* Anaerobic degradation of rutin by is *Buryrivibrio* sp. C_3 is presented in Figure 2.23. Flavones and flavanones give rise to phenylpropionic acids (Barz and Hösel 1975). More details of flavonoid metabolism can be found in Barz and Hösel 1975.

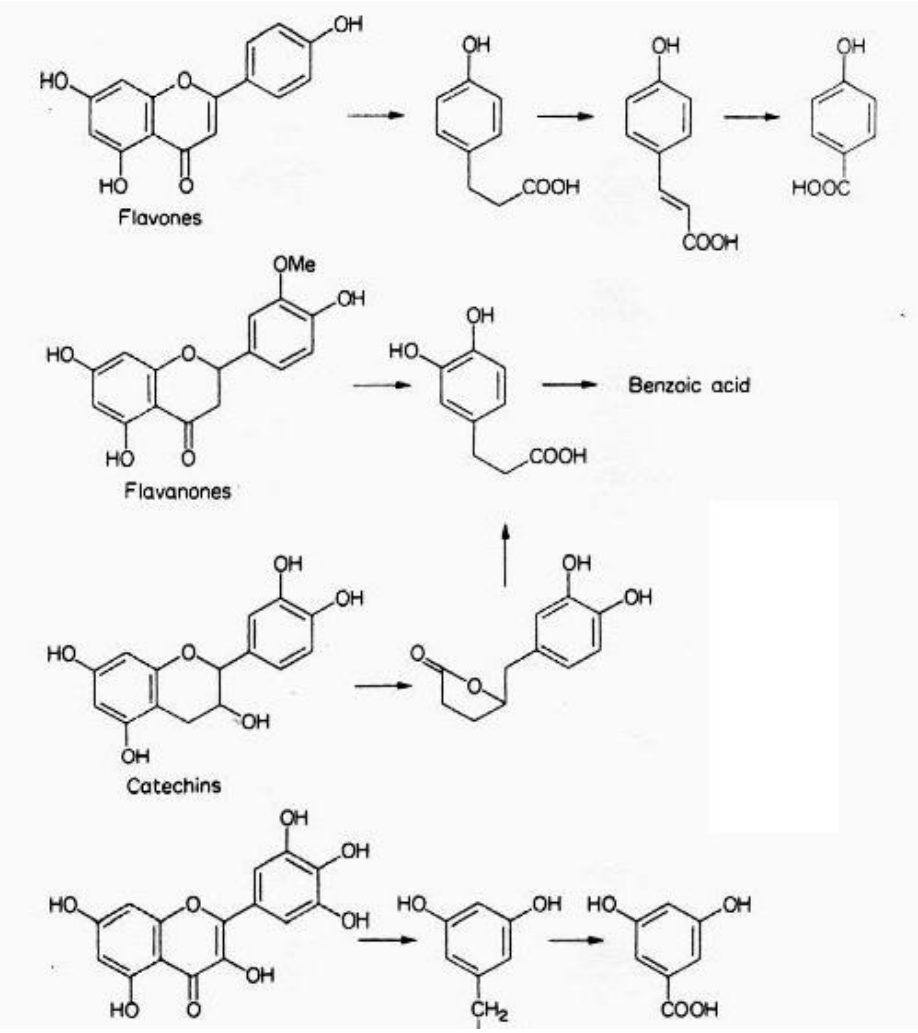


Figure 2.22. Degradation pathways of flavones, flavanones, catechins and flavonols by mammalian gut microflora (Barz and Hosel 1975)

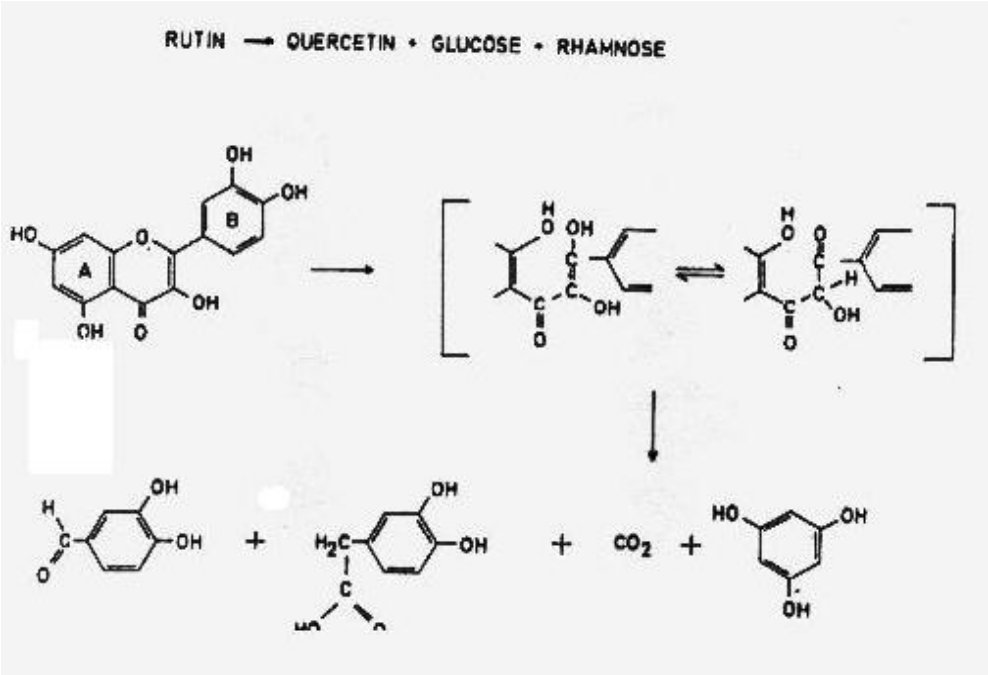
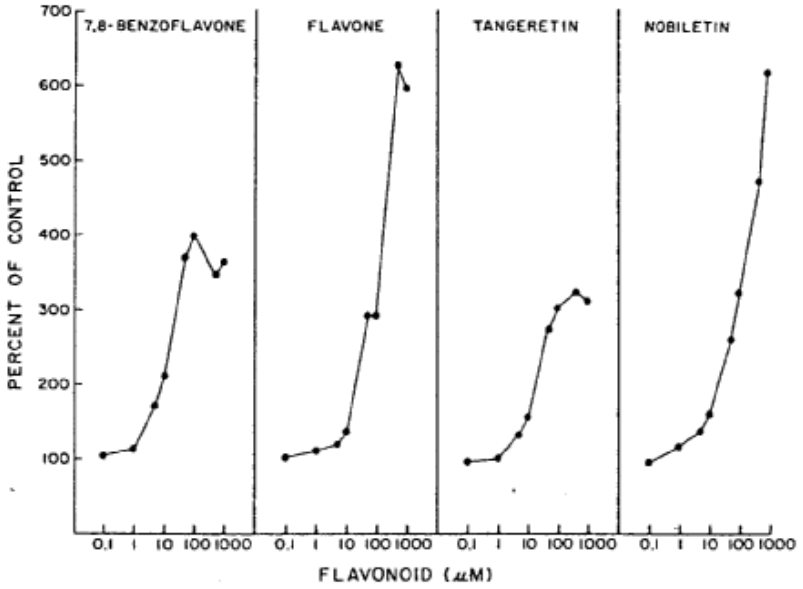


Figure 2.23. Anaerobic degradation of rutin by Butyrivibrio sp.C3 (Barz and Hosel 1975)

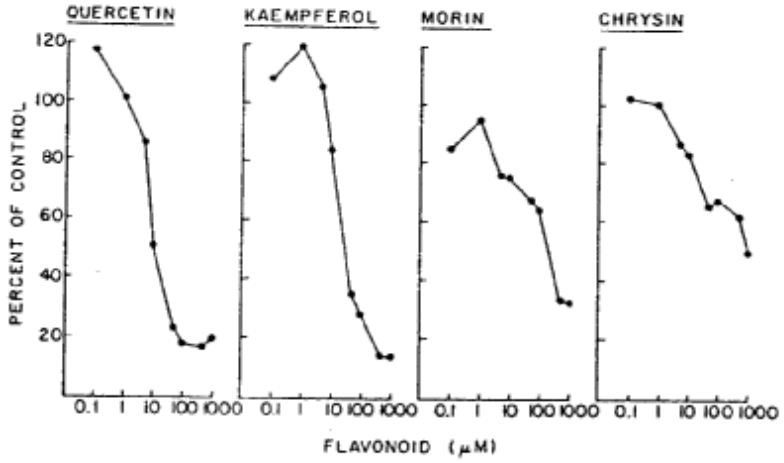
Activation and Inhibition of the Monooxygenase System by Flavonoids in Mammalian Metabolism of PAHs

Research involving the effects of flavonoids on PAH degradation is limited to those studies involving mammalian metabolism. Several naturally occurring and synthetic flavonoids have marked effects on the cytochrome P450-dependent monooxygenase system (Buening *et al.* 1981) including the induced synthesis of specific cytochrome P450 isozymes and the activation or inhibition of these enzymes. The isozymes of the cytochrome P450-dependent monooxygenases often catalyzes the initial step in the oxidative metabolism of PAHs (Sato and Omura 1978). The enzyme system metabolizes PAHs to polar Hydroxylated metabolites that have carcinogenic effects (Miller and Miller 1985). Flavonoids may also directly interact with the PAH metabolite or DNA. Therefore, modulation of the cytochrome P450-dependent monooxygenase system can influence the metabolism of PAHs (Sato and Omura 1978).

Studies *in vitro* indicated that polar polyhydroxylated flavonoids, such as morin, quercetin, chrysin, and kaempferol, inhibit PAH hydroxylation. Whereas, less polar flavonoids, such as 7,8-benzoflavone, flavone, tangeretin, and nobiletin activate PAH hydroxylation (Buening *et al.* 1981). The benzo[a]pyrene hydroxylase activity in human liver microsomes as a function of flavonoid concentrations is plotted in Figure 2.24 (Buening *et al.* 1981).



Effect of flavonoids on benzo[a]pyrene hydroxylase activity in human liver microsomes. Fluorescent phenolic metabolites were measured as described



Inhibitory effect of flavonoids on benzo[a]pyrene hydroxylase activity in human liver microsomes. Fluorescent phenolic metabolites were measured as described

Figure 2.24. Effect of flavonoids on benzo[a]pyrene hydroxylase activity in human liver microsomes (Buening 1981)

Benzo[a]pyrene hydroxylase activity in human liver microsomes decreased from 80-120% to 20-60% (percent of control) as Hydroxylated flavonoids, quercetin, kaempferol, morin, and chrysin concentrations increased from 0.1 μM to 100 μM. In contrast, benzo[a]pyrene hydroxylase activity in human liver microsomes increased from 100% to 300-600% (percent of control) as nonhydroxylated (synthetic) flavonoids, 7,8-benzoflavone, flavone, tangeretin, and nobiletin concentrations increased from 0.1 μM to 100 μM (Buening *et al.* 1981).

Biochemical mechanisms by which flavones activate the cytochrome p450-dependent monooxygenase system have been evaluated in livers from rabbits, humans and rats. Both flavone and 7,8-benzoflavone stimulated hydroxylation of benzo[a]pyrene by enhancing the interaction of cytochrome P450 with NADPH-cytochrome P450 reductase, thereby facilitating the flow of electrons from NADPH to the terminal electron acceptor (Huang *et al.* 1981). However, 7,8-benzoflavone did not enhance the reductase-independent metabolism of benzo[a]pyrene. More detailed studies indicated that flavonoid activation was dependent on the particular isozyme used. Huang *et al.* (1981) studied the mechanisms. With human liver microsomes, the inhibition of benzo[a]pyrene hydroxylation by polyphenolic flavonoids appears partly due to the inhibition of the NADPH cytochrome P450 reductase.

Glusker (1986) reported that the x-ray crystal structures shows that 5,6-benzoflavone and 7,8- benzoflavone are flat or twisted around 23º about the exocyclic carbon-carbon bond, resembling the carcinogenic PAHs B[a]P (flat) and DMBA (buckled with a torsion angle of 23º in the bay region). The crystal structures of 7,8-benzoflavone, 5,6-benzoflavone, quercetin, naringenin, B[a]P, and DMBA are shown in Figure 2.25. In addition the total ring areas are similar for both types of molecules. Many flavonoids can be metabolized by the cytochrome P-450 system to epoxides and diols in the same way that PAHs are metabolized. Benzoflavones may interact with DNA in a manner similar to that of PAHs. Glusker (1986) suggested that benzoflavone activation of PAH metabolism may be related to their molecular structural similarities. However, flavonoids which inhibit PAH metabolism can also be either flat or twisted (e.g., quercetin and naringetin) (Figure 2.25). Whether the analogous flavonoids have directly induced PAH metabolism is not known.

PAH hydroxylation (i.e., the initial incorporation of oxygen into the fused PAH rings, converting PAH to dihydroxy diols) is the rate-limiting step in PAH degradation pathway. Many species of fungi, a few bacteria, and some cyanobacteria produce cytochrome P-450 monooxygenases which degrade PAHs via the same pathways as those involved in mammalian metabolism. Although there are a number of examples of flavonoids that activate monooxygenases in vitro, it is not known whether flavonoids can influence the in vivo metabolism of PAHs.

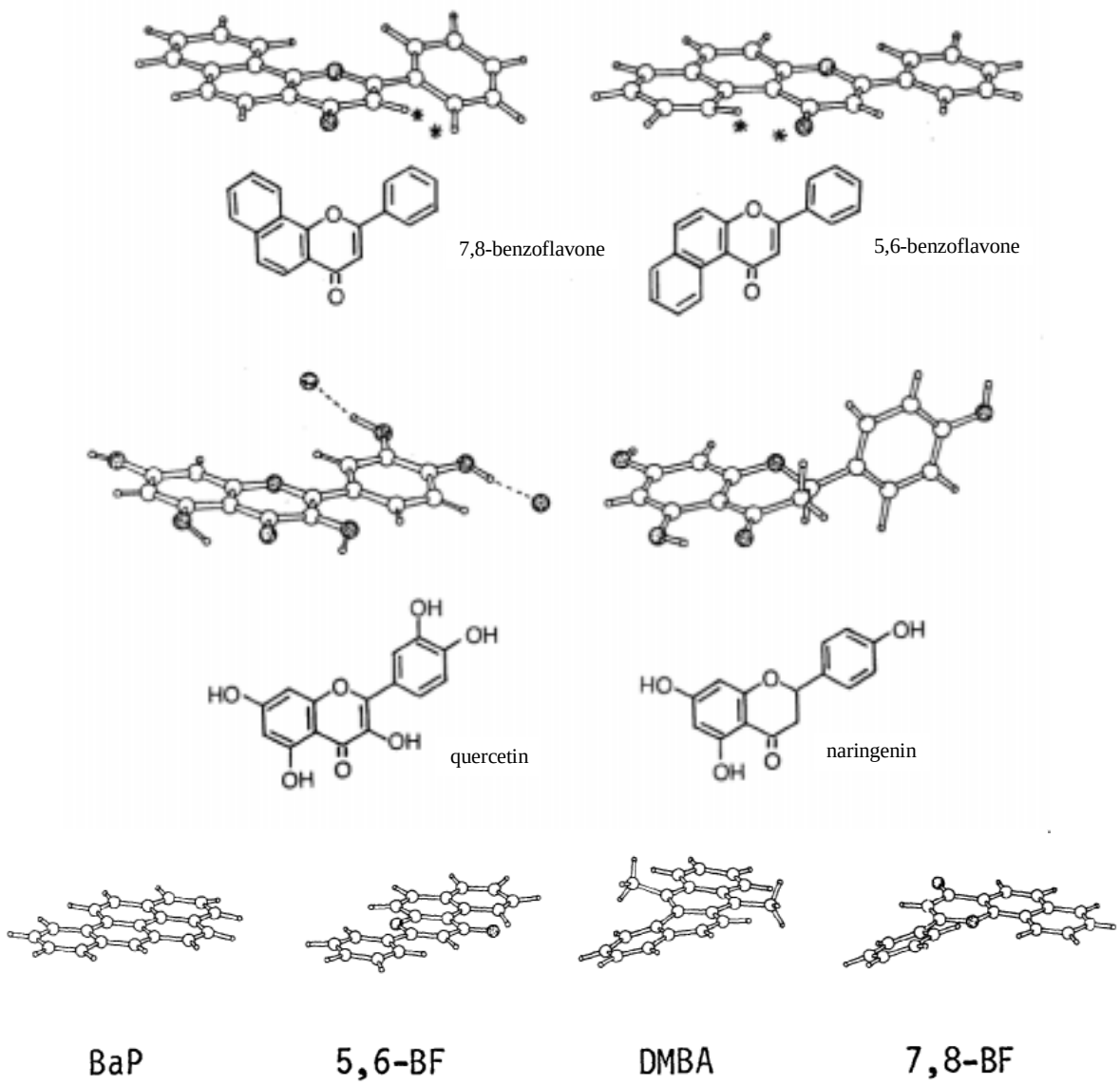


Figure 2.25. Crystal structures of flavonoids and PAHs (Glusker 1986)
(Planer quercetin inhibiting hydroxylation of planar B[a]P; Nonplanar 7,8-benzoflavone and naringenin stimulating planar B[a]P hydroxylation)

Hydroxylated Flavonoids interacting with PAH Diol Epoxide

Hydroxylated flavonoids and other polyphenolic plant constituents have antagonistic effect on the mutagenic and/or tumorigenic activity of bay-region PAH diol epoxides, which are the only known ultimate carcinogenic metabolites of PAHs (Huang et al. 1983, Wood 1986). The polyphenolic compounds interact directly with the diol epoxides in cell free aqueous solution and results in the formation of ether adducts (Sayer et al. 1982). Examination of structure activity relationships indicates that the number and position of the hydroxyl groups of the flavonoids have a marked effect on the ability of these compounds to inhibit the mutagenic activity of the bay-region diol epoxide of benzo[a]pyrene (Wood 1986). Chang *et al.* reported (1985) that several Hydroxylated flavonoids that are effective antimutagens can partially antagonize the tumorigenic activity of bay-region diol epoxides, but activity toward the parent PAHs has not been significant.

Binding and Polymerization

Flavonoids, such as quecetin, contain many more hydroxyl groups which may provide sites for attachment, by hydrogen bonding or metal chelation, to biological macromolecules. Glusker reported that there is a large area in the active site available for binding, with subsequent metabolism, of a PAH or benzoflavone. A hydroxyl group on C5 forms an internal hydrogen bond to the neighboring carbonyl oxygen atom on C4. Alternatively, dimmers may be formed by hydrogen bonding. Adjacent hydroxyl groups on a phenyl ring will form hydrogen bonds to other molecules. Flavonoids with many functional oxygen-containing groups can chelate metals. For example, magnesium forms a complex with flavone-3-monophosphate (Glusker 1986).

Synthesis and further metabolism (turnover, catabolism) of flavonoids in higher plants occur simultaneously. Turnover of flavonoids comprises reactions which transfer the plant products partly into polymers and partly into catabolic pathways. Polymerization is mainly catalyzed by peroxidase and phenolase (Barz and Köster 1985). Polymerization drastically alters the chemical properties of compounds, which may be converted into a metabolically inactive product.

Metabolism of various aromatic and heterocyclic plant constituents and xenobiotics in plants have frequently led to unextractable “bound residues” or “lignin-like material” (Barz and Köster 1981). The association of bound residue with lignin has often been assumed to be oxidative polymerization catalyzed by peroxidase or phenolases. Polymer formation greatly depends on the substitution pattern of the substrates. Though polymerization is a plausible mechanism for inactivating endogenous and exogenous substrates in plants, the chemistry and many essential aspects of cellular localization are inadequately understood. It is known that microbial cometabolism frequently terminates at an early stage after initial oxidation. Often the aromatic rings are not even split and phenolic, carboxylic, or chinoic derivatives of the PAHs accumulate as dead-end products. Meanwhile, metabolism of plant flavonoids often leads to irreversible bounding to protein, polysaccharide, and/or lignin (Barz and Köster 1985). The chemistry is inadequately understood. Whether the presence of natural flavonoids would influence PAH interaction with soil organic matter remains unknown.

CHAPTER 3. INVESTIGATIVE APPROACH

SCOPE OF STUDY

Bench scale soil-slurry microcosm experiment was conducted to evaluate the fate of high-molecular-weight (HMW) PAHs in different types of soil under the influence of root flavonoids. To determine PAH fate via multiple mechanisms including mineralization, volatilization, adsorption, bound residue formation, and water leaching, radioactive ^{14}C-PAHs and flavonoids were added into experimental soil-slurry microcosms. ^{14}C-radioactivity associated with gas, soil, water, and solvent phases in microcosms were measured after 60 days of incubation. Mass balances were calculated to verify the accuracy of the experiment. Analyses of variance of ^{14}C data were performed to determine whether flavonoids had statistical significant effects on PAH fate in soil.

EXPERIMENTAL DESIGN

Compound Nested Experimental Design

A compound nested experimental design model is presented in Figure 3.1. The model was designed to evaluate the effects of three hierarchical factors on the fate of PAHs in soil-slurry microcosms. The first factor is soil type. The second factor is flavonoid type (nested within soil type). The third factor is flavonoid concentration (compound nested within flavonoid and soil types). Two high-molecular-weight PAHs, i.e., pyrene and benzo[a]pyrene were tested in separated sets of microcosms. With three replicates, there were 180 measurements per each of the five fates, a total of 900 fate data points were measured.

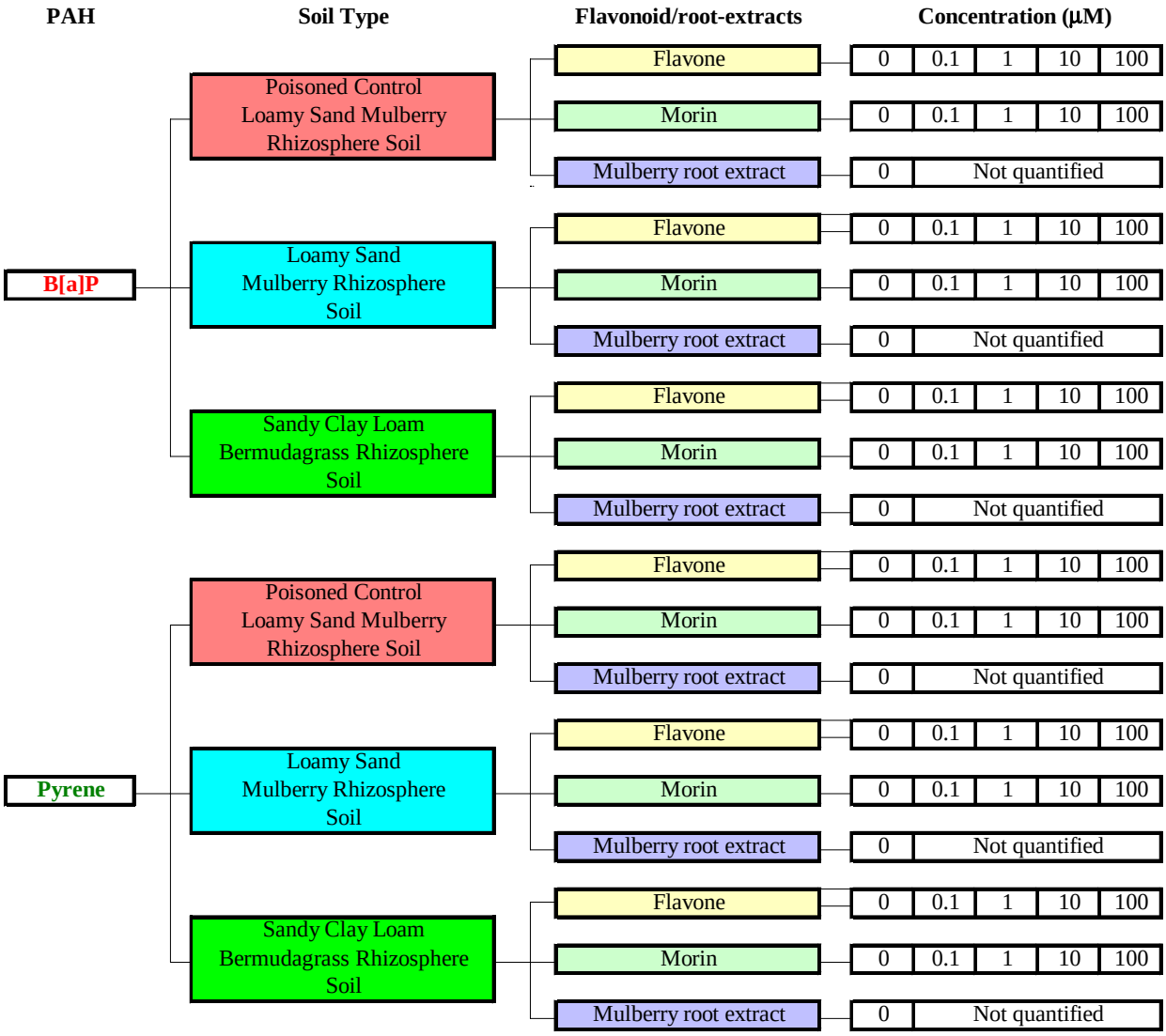


Figure 3.1. A compound nested experimental design

Testing Variables Independent testing variables and experimental conditions are listed in Table 3-1. Three soils used in the experiment were loamy sand Mulberry rhizosphere soil, poisoned loamy sand Mulberry rhizosphere soil, and sandy clay loam Bermudagrass rhizosphere soil. The purpose of including a poisoned Mulberry rhizosphere soil as a metabolically inhibited control was to distinguish the effects of biological-mediated from physico-chemical processes. In the subsequent sections, the poisoned Mulberry rhizosphere soil is abbreviated as "poisoned" soil. The term "poisoned" is more appropriate than "abiotic control", because true abiotic conditions could hardly be managed.

The three flavonoids tested were flavone, morin, and mulberry root extracts. Flavone is a synthetic nonhydroxylated simple flavonoid. Morin is a hydroxylated simple flavonol naturally presented in many plants. Nonhydroxylated and hydroxylated flavonoids were found to stimulate and inhibit PAH metabolism by monooxygenase in mammalian cells, respectively, (see Chapter 2). The purpose of using both nonhydroxylated and hydroxylated simple flavonoids is to explore their effects on PAH metabolism by soil microorganisms which secrete various enzymes, known for PAH degradation, including dioxygenase, monooxygenase, peroxidase, lacase, and perhaps more. To evaluate the effects of complex high-molecular-weight root flavonoids other than simple flavone and morin, mulberry root extracts were used. Mulberry root extract contains simple and complex plant root flavonoids and a variety of other root chemicals including sugars, organic acids, amino acids, phenols, enzymes, etc. Complex root flavonoids were not available from vendors.

Flavone and morin concentrations amended in testing soil slurry ranged from 0.1 μM to 100 μM based on the likely concentration range present in rhizosphere soil (Rao 1990). Microcosms without flavonoids were incorporated as control testing in the evaluation of flavonoid effects. The selected flavonoid concentration range was reported to have transitional effects on the rates of PAH metabolism in human liver microsomes according to relevant pharmaceutical studies (Alexander 1986, Buening 1981). PAH hydroxylase activity decreased significantly as hydroxylated flavonoid concentration increased from 0.1 μM to 100 μM. In contrast, PAH hydroxylase activity increased significantly as nonhydroxylated flavonoid concentration increased from 0.1 μM to 100 μM (see Chapter 2).

Flavonoid concentrations in Mulberry root extracts were not quantified. Differentiation and quantitative analysis of root flavonoids would require specific instruments and techniques, therefore those were beyond the scope of this study.

The two PAHs tested were 4-ring pyrene and 5-ring benzo[a]pyrene. Noncarcinogen pyrene is known to be relatively degradable among the high-molecular-weight PAHs. B[a]P, a potent carcinogen, is one of the most persistent organic contaminants in the environment. Both pyrene and B[a]P have been studied in great deal by researchers. This experiment is the first one to evaluate the effects of root exudates on PAH fate in soil.

Table 3.1. Independent testing variables and experimental conditions

Variables	Levels	
Flavonoids	Flavone Morin, Mulberry root extracts	
Flavonoid concentrations	100 μM 10 μM 1 μM 0.1μM 0 μM	
Soil Types	Mulberry rhizosphere (Loamy sand) Grass rhizosphere (Sandy clay loam) NaN_3-Poisoned (Loamy sand)	
PAHs	Benzo[a]pyrene Pyrene	
^{14}C labeled per microcosm	Benzo[a]pyrene) Pyrene)	17300 dpm (0.1 μg/g-soil) 59300 dpm (0.1μg/g-soil)
Temperature		25ºC
Humidity in environmental. chamber		90%
Microcosm volume		50 ml
Slurry-soil/microcosm	Soil Distilled-deionized water: Nutrients: KH_2PO_4 K_2HPO_4 $Na_2HPO_4 \cdot 7H_2O$ NH_4Cl $MgSO_4 \cdot 7H_2O$ $CaCl_2$ $FeCl_3 \cdot 6H_2O$	1 gram 10 ml 8.5 mg/L 21.75 mg/l 33.4 mg/L 1.7 mg/L 22.5 mg/L 27.5 mg/L 21.75 mg/L
Incubation time		60 days

Dependent variables corresponding to each of the measurement endpoints of PAH fate mechanisms, are listed in Table 3.2. PAH mineralization was determined by measuring $^{14}CO_2$ evolution. PAH adsorption onto soil was determined based on organic solvent-extractable ^{14}C from the soil phase. The ^{14}C residue in soil after solvent extraction was measured as soil bound residue formation of PAHs and metabolites. The nonpolar portion of ^{14}C in water phase extractable by hexane was measured as water-soluble parent PAH. After hexane extraction, the remaining polar portion of ^{14}C in the water phase was measured as the intermediate metabolites of PAH. Highly hydrophobic parent PAHs are nonpolar and tend to partition into organic solvent phase. In contrast, PAH metabolites including quinones, phenols, acids, and alcohols (see Chapter 2), are generally more polar and have a tendency of partitioning into the water phase. PAHs are strongly adsorbed onto soil, largely onto SOM due to the hydrophobic interaction. Portions of PAHs and metabolites were incorporated into soil organic matter (SOM), which are nonextractable by organic solvent. Because four or more ring PAHs have low vapor pressure, volatilization was assumed to be negligible. Therefore, ^{14}C volatiles in the gas phase were generally not measured in the experiment, except for six volatilization-test microcosms.

Table 3.2. Dependent variables to measure

Mechanisms of PAH Fate in Soil[1]	**Phase for measurement**	**Dependent Variables**
Mineralization (complete degradation)	Gas	$^{14}CO_2$ absorbed by potassium hydroxide (KOH)
Volatilization	Gas	^{14}C (gas phase) absorbed by ethylene glycol monomethyl ether (EGME)
Soil bound residue formation of PAH and metabolic intermediates (humification and intraparticle diffusion)	Soil solid	Ethylacetate non-extractable portion of ^{14}C from soil particles
Adsorption of PAH and metabolic intermediates on soil phase	Soil solid	Ethylacetate-extractable portion of ^{14}C from soil particles
Water leaching of parent PAH (dissolution)	Water	Hexane-extractable (nonpolar) portion of ^{14}C from soil water
Water leaching of intermediate metabolites (dissolution)	Water	Hexane non-extractable (polar) portion of ^{14}C from soil water

[1] See Figure 2.5. A conceptual model of PAH fate in soil

Experimental Matrix

Experimental matrix is presented in Table 3.3. A total of 180 microcosms (with triplicates) were used for the measurements of PAH fates in soil, water, and gas phases. In addition, six microcosms were used to estimate volatilization loss of ^{14}C-pyrene and ^{14}C-B[a]P during incubation. Also, six more microcosms spiked with ^{14}C-$NaCO_3$ were used to evaluate $^{14}CO_2$ recovery rates in the experiment. Overall 192 microcosms were used in the experiment.

Table 3.3. Experimental matrix

Factors: (1) Soil types		Sandy clay loam Bermudagrass rhizosphere soil										Loamy sand Mulberry rhizosphere soil										Poisoned Control loamy sand Mulberry rhizosphere soil										Sub total
(2) Flavonoids		None	Mulberry root extract	Flavone				Morin				None	Mulberry root extract	Flavone				Morin				None	Mulberry root extract	Flavone				Morin				
(3) Flav. Conc. (μM)		0	Not quantified	0.1	1	10	100	0.1	1	10	100	0	Not quantified	0.1	1	10	100	0.1	1	10	100	0	Not quantified	0.1	1	10	100	0.1	1	10	100	
Measurements:	**Spike**																															
Mineralization:	^{14}C-Pyrene	3	3	3	3	3	3	3	3	3	3	6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	93
$^{14}CO_2$ sorbed by KOH	^{14}C-B[a]P	3	3	3	3	3	3	3	3	3	3	6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	93
Water leachable parent PAH:	^{14}C-Pyrene	3	3	3	3	3	3	3	3	3	3	6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	93
Hexane extractable ^{14}C in Water phase	^{14}C-B[a]P	3	3	3	3	3	3	3	3	3	3	6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	93
Water leachable Metabolites:	^{14}C-Pyrene	3	3	3	3	3	3	3	3	3	3	6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	93
Hexane nonextractable ^{14}C in Water phase	^{14}C-B[a]P	3	3	3	3	3	3	3	3	3	3	6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	93
PAH remaining in soil:	^{14}C-Pyrene	3	3	3	3	3	3	3	3	3	3	6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	93
Ethylacetate extractable ^{14}C in soil phase	^{14}C-B[a]P	3	3	3	3	3	3	3	3	3	3	6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	93
Bound residues of PAH and metabolites:	^{14}C-Pyrene	3	3	3	3	3	3	3	3	3	3	6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	93
EAc nonextractable ^{14}C in soil	^{14}C-B[a]P	3	3	3	3	3	3	3	3	3	3	6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	93
Volatilization:	^{14}C-Pyrene											3																				3
^{14}C sorbed by EGME	^{14}C-B[a]P											3																				3

Additional six microcosms spiked with ^{14}C-$NaHCO_3$ were used to evaluate $^{14}CO_2$ recovery efficiency.

MATERIAL AND METHODS

Chemicals

Radioactive ^{14}C Isotopes Radio-labeled 4,5,9,10-^{14}C-pyrene (specific activity: 58.7 mCi/mmol), 7,10-^{14}C-B[a]P (specific activity: 18.4 mCi/mmol), and Na_2CO_3-^{14}C (specific activity: 15.1 mCi/mmol) were purchased from Sigma Chemical Company (St. Louis, MO, USA). The position of ^{14}C label on pyrene and B[a]P molecules are presented in Table 3.4.

Flavonoids, Scintillation Cocktail, and Other Chemicals Flavone, morin, sodium azide (NaN_3), were purchased from Sigma Chemical Company (St. Louis, MO). The molecular structure of flavone and morin are also presented in Table 3.4. Scintillation cocktails (Scinti-Safe Gel and Scinti-Safe Plus 50%), Ethanol, hexane, and ethylacetate solvents (HPLC grade), potassium hydroxide, phosphoric acid, and hydrogen peroxide were purchased from Fisher Scientific Inc. (Fairlawn, NJ). Hionic scintillation cocktail was purchased from Packard Instrument Co. (Meriden, CT).

Mulberry Root Extracts Mulberry-root extracts were prepared by soaking Mulberry roots into distilled-deionized water (DDW). The glassware and DDW was sterilized in the autoclave at 1.05 kg/cm^2 and 121ºC for 20 min. Mulberry roots were collected from a big Mulberry tree growing in the PAH contaminated soil. The loamy sand soil particles were removed by air drying and shaking. Approximately 223.6 grams of fine to small Mulberry roots (with diameters of <0.1 - 5 mm) were cut into small pieces and soaked into 854 ml sterilized-distilled-deionized water in a 1000 ml beaker covered with aluminum foil and plastic wrap. The soaking beaker was placed in a refrigerator over night at 5°C. After soaking, the liquid phase was filtered through a Whatman GF/A binds-free glass fiber filter and the filtrate was used as the root extracts in the experiment. Photos of the mulberry roots and root extract preparation are presented in Figures 3.2.

Mulberry root extracts used in the experiment were analyzed for pH, total phenolics, total organic carbon content (TOC), biological oxygen demand (BOD), and chemical oxygen demand (COD). The results are presented in Table 3.5. The relatively high organic concentration in the root extract, indicated by a 1660 mg/l of BOD and 5000 mg/l COD, was due to the high ratio of roots to water used in extract preparation. High root density zones under the tree canopies were visually observed during root zone excavation in the field. The type and quantity of flavonoids and other chemical constituents in Mulberry root-extracts were not analyzed in this study. Isolation and characterization of flavonoids are complex and require a combination of several specific techniques, including high performance liquid chromatography (HPLC) UV spectroscopy, nuclear magnetic resonance spectrometry (NMR) and mass spectrometry (MS) techniques. These analytical tools are not available during the experiment. Furthermore, the special analytical protocols have not been well established for commercialization. As a result, the flavonoid characterization in Mulberry root extracts was beyond the scope of this study.

Table 3.4. Diagram of ^{14}C-pyrene, ^{14}C-B[a]P, flavone, and morin

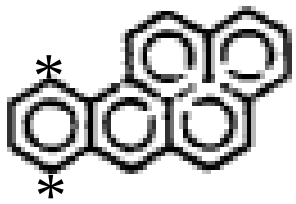
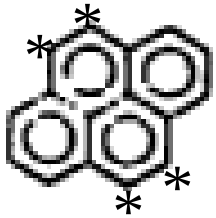
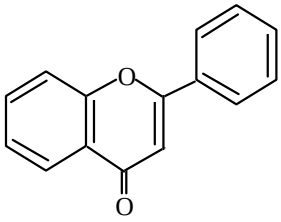
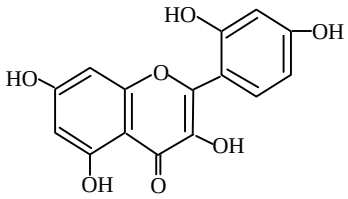
Chemical	Specific radioactivity (mCi/mmol)	Source
[7,10-^{14}C]benzo[a]pyrene 	58.7	Sigma Chemical Company (St. Louis, MO)
[4,5,9,10-^{14}C]pyrene 	18.4	Sigma Chemical Company (St. Louis, MO)
Flavone 	Not applicable	Sigma Chemical Company (St. Louis, MO)
Morin 	Not applicable	Sigma Chemical Company (St. Louis, MO)

Figure 3.2. Mulberry roots and root extract preparation

Table 3.5. Analysis of Mulberry Root Extract

Analysis	Results	Units	Limit of Quantitation	Method
pH	6.73	Std. Units	0.01	EPA 150.1
Total Phenolics	0.131	mg/l	0.005	EPA 420.2
COD	5,000	mg/l	100	HACH 8000
BOD	1,660	mg/l	400	EPA 405.1
TOC (nonpurgible)	855	mg/l	50	EPA 415.1

Rhizosphere Soils

Site Background and Experimental Soil Collection Two soil samples were collected from an inactivated waste disposal basin at a petrochemical manufacturing site. The basin was filled with waste sludge originated from process wastewater primary clarifiers. The sludge consisted of mainly river sediments contaminated with PAHs, aromatics, and traces of other hydrocarbons. Some soils may have been backfilled on the top of the basin sludge after it was inactivated. Over nearly 20 years the sludge was naturally dewatered and the basin was vegetated with forbs, grasses, and trees. The sludge-soil texture in the 1-acre basin area varies. Rhizosphere soils excavated from a big Mulberry tree was characterized as loamy sand. Bermudagrass rhizosphere soil excavated from a close location within the same basin was characterized as sandy clay loam. Rhizosphere soil adhering to the plant roots was collected and placed into clean glass jars (baked ½ hour at 550ºC) and transported to the laboratory in a cooler. Afterwards the soil samples were stored in the dark at 5°C prior to use. The glass jar cover was loosen to allow adequate aeration of the soil. Soil excavation and sampling photos for Mulberry and Bermudagrass soils are presented in Figures 3.3 and 3.4.

Agronomic Assessment The agronomic characteristics of the experimental soil were analyzed by commercial laboratories. Two split soil samples collected from Mulberry and Bermudagrass rhizosphere, used in the experiments, were sent to the Soil and Plant Testing Laboratory, Agricultural Extension Services, at TAMU for agronomic testing. Results are summarized in Table 3.6. The Mulberry rhizosphere soil appeared to be a mild alkaline loamy sand soil, while the grass soil was a mild acidic sandy clay loam soil. Both soils are none saline with low sodium content. Nitrogen concentration was very high in the grass soil but low in the mulberry soil. Common features of the two soils include (1) very high concentrations of phosphorus and calcium but low potassium were common features of the two soils. Other available micronutrients (Mg, Zn, Fe, Mn, Cu, and S) in the soils were plenty. The Bermudagrass soil has higher SOM content (5.2%) than that of the Mulberry soil (3%). Soil cation exchange capacity (CEC) and humic contents were analyzed by SASI laboratory, Collage Station, TX. Both CEC and humic acids of the Bermudagrass soil are higher than those of Mulberry soil.

Figure 3.3. Mulberry tree root zone excavation

Figure 3.4. Bermudagrass root zone excavation

Table 3.6. Summary of soil agronomic properties[1]

Parameter		***Bermudagrass rhizosphere soil***[2] ***(loamy sand)***	***Mulberry tree rhizosphere soil***[2] ***(sandy clay loam)***	*Analytical Methods*[3]
pH		6.6	7.7	Electrode meter
Salinity	(mg/kg)	600	201	Corning chloride analyzer
Available nutrients:				
NO_3-N	(mg/kg)	68	13	ICP-OES[4]
P	(mg/kg)	>8000	2171	ICP-OES
K	(mg/kg)	83	32	ICP-OES
Ca	(mg/kg)	>30000	>30000	ICP-OES
Mg	(mg/kg)	1017	385	ICP-OES
Zn	(mg/kg)	157	66.9	ICP-OES
Fe	(mg/kg)	41.39	54.8	ICP-OES
Mn	(mg/kg)	8.94	2.03	ICP-OES
Cu	(mg/kg)	40.31	32.32	ICP-OES
Na	(mg/kg)	386	282	ICP-OES
S	(mg/kg)	505		ICP-OES
CEC	(meq/100 g)	33.3	19.1	Ammonium acetate method
Organic matter	(%)	5.2	3	Digestion/spectrophotometer
Humic acid	(mg/kg)	5240	3779	MIBK/OC[5]
Fulvic acid	(mg/kg)	3717	3654	MIBK/OC
Texture		sandy clay loam	loamy sand	Hydrometer
Sand	(%)	50	82	Hydrometer
Silt	(%)	23	12	Hydrometer
Clay	(%)	27	6	Hydrometer

[1] Analysis performed by (1) Soil Analytical Services, Inc., Collage Station, TX; (2) Soil and Plant Testing Laboratory, Agricultural Extension Services, TAMU, Collage Station, TX.

[2] Rhizosphere soil samples for agronomic analysis were the split samples of those used in soil microcosms of the study.

[3] Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties, 2nd ed. Agronomy Vol. 9. Am. Soc. Agron. 1982.

[4] ICP-OES = inductively coupled argon plasma-optical emission spectrophotometer

[5] MIBK/OC = methyl isobutyl ketone fractionation/organic carbon analyzer

PAH Analysis PAHs are the primary chemicals of concern in the soil. Concentrations of the 16 PAHs designated as Toxic Priority Pollutants by UAEPA were analyzed by the petroleum laboratory at University of Oklahoma. The reported data[1] are presented in Table 3.7. Both Mulberry and Bermudagrass soil samples used in the experiments contained most of the 16 PAHs. The concentration levels for individual constituents ranged from nondetectable to 300 mg/kg. Four and more-ring PAH concentrations in the Mulberry soil were somewhat higher than those in the Bermudagrass soil. The PAH concentration levels were not likely to be toxic to microorganisms.

Table 3.7. PAH concentrations in study soils[2] (as collected)

PAHs (mg/kg)	*Number of benzene rings*	*Bermudagrass rhizosphere soil (Sandy Clay Loam)*	*Mulberry tree rhizosphere soil (Loamy Sand)*	*Analytical Method[3]*
Naphthalene	2	39.1	104.3	GC/FID
Acenaphthylene	2	248.8	220.5	GC/FID
Acenaphthene	2	52.0	34.9	GC/FID
Fluorene	2	108.5	100.9	GC/FID
Phenanthrene	3	276.5	295.7	GC/FID
Anthracene	3	80.4	79.4	GC/FID
Fluoranthene	3	94.8	127.0	GC/FID
Pyrene	4	196.6	265.6	GC/FID
Benz[a]anthracene	4	34.2	74.9	GC/FID
Chrysene	4	25.1	56.9	GC/FID
Benzo[b]fluranthene	4	7.9	22.3	GC/FID
Benzo[k]fluranthene	4	ND[4]	ND	GC/FID
Benzo[a]pyrene	5	12.7	31.1	GC/FID
Inden[1,2,3-cd]perylene	5	ND	7.3	GC/FID
Dibenz[a,h]anthracene	5	ND	ND	GC/FID
Benzo[ghi]perylene	6	ND	30.6	GC/FID

[1] Data were reported by Dr. Paul Olsen, who was then a Ph.D candidate at University of Oklahoma, collaborating the site research.

[2] Data reported by Dr. Paul Olsen, Univ. of Oklahoma, Norman, OK. (1998). He was then collaborating the study at the site. The soil samples for PAH analysis are split samples of those used in this experiment.

[3] EPA SW84 in "Test Methods for Evaluating Solid Waste Vol. 1B: Laboratory Manual Physical/Chemical Methods"

[4] ND = not detected; Quantitation limit = 2 mg/kg

Metal Analysis Soil metal concentrations reported by Dr. Scott Huling[1] were presented in Table 3.8. The soil samples were taken from the same location and the same depth (i.e., 0.5 - 1 ft below grade surface) as those samples used in the experiments. Generally, metal concentrations in the site soil fall within the background level of the Gulf coast region of TX. Aluminum and chromium concentrations with Bermudagrass soil were significantly higher than those with Mulberry soil. However, the cobalt concentration in the former was significantly lower than that in the latter. There are no significant differences in other metal concentrations between the two soils. The metal concentration levels did not seem to be toxic to microbial organisms and plants. Whereas, significantly higher aluminum and chromium concentrations in Bermudagrass soil than those in Mulberry soil indicate the heterogeneity in sludge within the basin.

Dr. Hulling was then collaborating the study at the site. He investigated the metal contents in the site soils in order to understand the causality of the natural vegetation and concurrent contaminant attenuation. In the absence of baseline data, the lower PAH concentrations in the surface soil than those in the deep sludge are inadequate to prove the occurrence of rhizosphere degradation. To verify whether the less contaminated surface soils and the highly contaminated deep sludge/soils were actually from the same origin or not, soil samples at Mulberry and Bermudagrass locations in the basin were collected from different depth for metal analysis. Metals are generally not subject to biotransformation, therefore can be used as indicators of soil origin. The results showed significant differences in metal compositions between the surface and deep sludge/soils, however, no apparent correlation between the metal concentrations and the sludge/soil depths. As a result, it is not clear whether the surface soil was originally the same material as the deep sludge or not.

[1] Dr. Scott Huling, USEPA R.S. Kerr Lab, was then collaborating the research at the site.

Table 3.8. Metal concentrations in study soil[1]

Metals	***Bermudagrass rhizosphere soil*[2] *(Sandy Clay Loam)***		***Mulberry tree rhizosphere soil*[2] *(Loamy Sand)***		***Analytical Method***
(mg/kg)	#1	#1 (duplicate analysis)	#2	#2 (duplicate analysis)	
Na	831	898	663	664	ICAP[3]
K	2230	2810	1232	1136	ICAP
Ca	40000	36100	29400	26500	ICAP
Mg	5150	5730	3690	3610	ICAP
Fe	33300	35900	41300	41200	ICAP
Mn	300	321	338	332	ICAP
Co	320	317	1040	1030	ICAP
Mo	24.9	22.2	25.9	30.6	ICAP
Al	17700	22600	10500	11400	ICAP
As	41	30	29.7	24.4	ICAP
Se	<52	<56	<64	<64	ICAP
Cd	3.1	4.2	3.31	3.47	ICAP
Be	<0.59	<0.59	<0.34	<0.32	ICAP
Cu	1520	1420	1620	1730	ICAP
Cr	9140	11500	5270	4010	ICAP
Ni	715	660	491	595	ICAP
Zn	4060	4950	2650	2330	ICAP
Ag	<1.2	6.7	3.37	3.03	ICAP
Tl	13.4	16.7	10.8	8.3	ICAP
Pb	236	210	193	230	ICAP
Sr	295	290	242	220	ICAP
V	28.1	34.7	27.6	23.6	ICAP
Ba	590	568	539	506	ICAP
B	40	32.1	31	33	ICAP
Ti	58.7	72.9	96.9	105	ICAP

[1] Analysis were performed by ManTech Environmental Research Services Corporation, contracted by Dr. Scott Huling, National Risk Management Research Laboratory, Subsurface Protection & Remediation Division, USEPA, R.S Kerr Environmental research Lab, Ada, OK.

[2] Rhizosphere soil samples were collected by Paul Olson, University of Oklahoma from the same location and the same depth as those of the study soil.

[3] The samples were hot plate digested and analyzed using ICAP (inductively coupled argon plasma spectroscopy). QA/QC samples included duplicates, blanks, and matrix spikes, in accordance with the standard methods USEPA SW846.

Microbial Enumeration Soil bacteria plate counts reported by Dr. David P. Nagle[1], are presented in Table 3.9. In the soil samples, total bacteria counts were at the order of 10^7 CFU/g-soil, of which the counts with mulberry were higher than those with Bermudagrass. PAH-utilizing bacteria counts associated with Mulberry soil were found to be high, but low or none with the Bermudagrass soils. Fungus enumeration had not been conducted. As mentioned in the analytical method section, the bacteria plate counts gave only a minimum estimate as to the number of bacteria present and were not necessary to be correlated with microbial activity. However, the plate counts did indicate that microbial community in the experimental soil were healthy and were not inhibited by soil chemicals or due to nutrient deficiency. Relatively low bacteria counts with Bermudagrass soil may be related to two reasons. First, the grass soil has a high clay and silt content, most bacteria may not be dislodged from soil surfaces when samples are slurried, especially, this particular assay was conducted by using slurry supernatant instead of slurry. The results could have even more under counted the true microbial population in soil. Second, grass soil are periodically saturated because of seasonal flooding. The counting plates were incubated under aerobic conditions, obligate anaerobes will not be counted. Slow-growing facultative bacteria may be undercounted.

Table 3.9. Microbial enumeration of study soil[2]

Sample ID	Rhizosphere soil description	Total bacteria [3] (CFU/g-wet soil)	PAH-utilizing bacteria[4] (CFU/g-wet soil)
1	Mulberry with high root content	8.9×10^7	8.2×10^7
2	Mulberry near surface	9.7×10^6	2.6×10^6
3	Mulberry	3.1×10^7	2.0×10^7
4	Mulberry	7.6×10^8	4.6×10^6
5	Mulberry	4.4×10^6	1.6×10^6
6	Bermudagrass	5.7×10^6	3.2×10^4
7	Bermudagrass	1.5×10^6	1.2×10^3
8	Bermudagrass	4.3×10^6	0
9	Bermudagrass	4.0×10^5	0
10	Bermudagrass	5.1×10^6	0

[1] Dr. David Nagle, Univ. of Oklahoma, who was then investigating the microbial community associated with different rhizosphere soils at the site.

[2] Analysis performed by Michael D. Kyle, University of Oklahoma. Data published in a post presentation authorized by Dr. David P. Nagle and Michael D. Kyle., University of Oklahoma, at the IBC's 3rd International Conference on Phytoremediation, Houston, 1998. They were then collaborating the site study at the site. Rhizosphere soil samples were collected from the same location and the same depth as those used in this study.

[3] Total bacterial counts on 1/8-strength plate count broth agar (Sack 1997)

[4] PAH-utilizing bacterial counts using Basal Mineral medium solidified with Nobel Agar (Sack 1997)

Soil-Slurry Microcosm Preparation and Incubation

Radioisotope ^{14}C-labelled soil-slurry microcosm experimental procedures developed and verified by Pfaender *et al.* were adopted in this experiment (Carmichael and Pfaender 1997, Dobbins and Pfaender 1988, Pfaender and Bartholomew 1982). Using soil slurry instead of soil ensures more even distribution of ^{14}C-PAHs in the sample soil. Pfaender et al. reported that the soil:water ratio of 1:10 produced higher rates of metabolism than more or less dense slurries. Experiments were carried out in triplicate microcosms constructed from sterile, 40-mL vials closed by caps with Teflon-lined septa. The glass vials were sterilized in autoclave at 121ºC for 20 minutes.. Mulberry and Bermudagrass rhizosphere soil samples collected from field were air-dried and homogenized. Visible fine roots were removed from the soil prior to weighing the soil. Each microcosm contained one gram of either Mulberry or Bermudagrass soil and 10 ml of sterilized-distilled-deionized water. Mineral nutrients were added into the water at the levels specified in the standard BOD test for microbial growth (WEF 1998) (see details in Table 3.1). Sixty poisoned microcosms contained 0.5% NaN_3 (vol/vol). Flavone or morin were added to the designated microcosms (see Table 3.3) to reach a final concentration of 0.1, 1, 10, or 100 uM, respectively. Mulberry root extract solution were added to the nine designated microcosms instead of 10 ml of sterilized-distilled-deionized water. Each microcosm was equipped with a CO_2-trap central well. The CO_2-trap well constructed from a plastic micro centrifuge tube (Kontes Glass Co., Vineland, NJ, USA) contained a fluted 7-cm strip of Whatman 1 chromatography filter paper (Fisher Scientific, Fair Lawn, NJ, USA) saturated with 200 ul of 2N KOH.

Incubation started by spiking 0.0079 uCi (17,300 dpm, disintegration per minute; 1 uCi = 2,200,000 dpm.) of 7,10-^{14}C-benzo[a]pyrene or 0.027 uCi (59300 dpm) 4,5,9,10-^{14}C- pyrene in 200 ul of 50/50% ethanol/H_2O to each slurry microcosm resulting in a final ^{14}C-PAH concentration of approximately 0.1 ug/g-soil (see Table 3-1). Carmichael and Pfaender (1997) reported that the ethanol level did not substantially increase the solubility of the ^{14}C-PAH added to the microcosms. Microcosms were incubated vertically in a vented environmental chamber at 23°C with 90% humidity for 60 days. A photo of soil microcosms set up in the environmental chamber is presented in Figure 3.5. The environmental chamber was ventilated by the laboratory venting system, therefore any leaking of ^{14}C gas from the sealed soil microcosms will not harm the laboratory workers.

Figure 3.5. Soil microcosms setup in the environmental chamber

Sample Preparation and Measurement Procedures

A schematic of the radioactive isotope ^{14}C-PAH sample preparation procedure is presented schematically in Figure 3.6. Details of the phase separation, sample extraction, and liquid scintillation counting sample preparation for each of the measurement parameters are described in the following sections. Photographs of the experimental apparatus, liquid scintillation analyzer, environmental chamber, rotary shaker, and explosive-proof storage refrigerator are presented in Figures 3.7 and 3.8.

Measurement of total ^{14}C-PAH Spike into a Microcosm The amount of radioactivity added to the microcosms were determined by directly counting the same volume of 7,10-14C-benzo[a]pyrene and pyrene-4,5,9,10-^{14}C added in the microcosms in triplicate scintillation vials containing 7 ml of Scintisafe™ gel scintillation cocktail (Fisher Scientific, Fairlawn, NI, USA). ^{14}C activities were analyzed on a Packard Model TriCarb1600 liquid scintillation counter (Packard Instrument Company, Meriden, CT). Counting results are presented in Table 3.10.

Measurement of PAH Mineralization After incubation, microcosms were acidified to pH 2 with 20% (v/v) H_3PO_4 and placed on a rotary shaker at 50 rpm for 24 hours to transfer $^{14}CO_2$ into the gas phase and trap it on the base-soaked filter paper. The filter paper in the $^{14}CO_2$ trap was removed and placed in a 7 ml scintillation vial filled with 7 ml Hionic scintillation cocktail for liquid scintillation counting. Hionic scintillation cocktail (Packard Instruments, Meriden, CT) was compatible with 2N KOH (CO_2 trap solution). $^{14}CO_2$ recovery efficiencies were estimated with triplicate microcosms amended with ^{14}C-$NaHCO_3$ (instead of PAH) that were processed and analyzed in a manner identical to the microcosms containing ^{14}C-PAH. Recovery efficiencies of ^{14}C-$NaHCO_3$ were used to correct mineralization recoveries of ^{14}C-PAH.

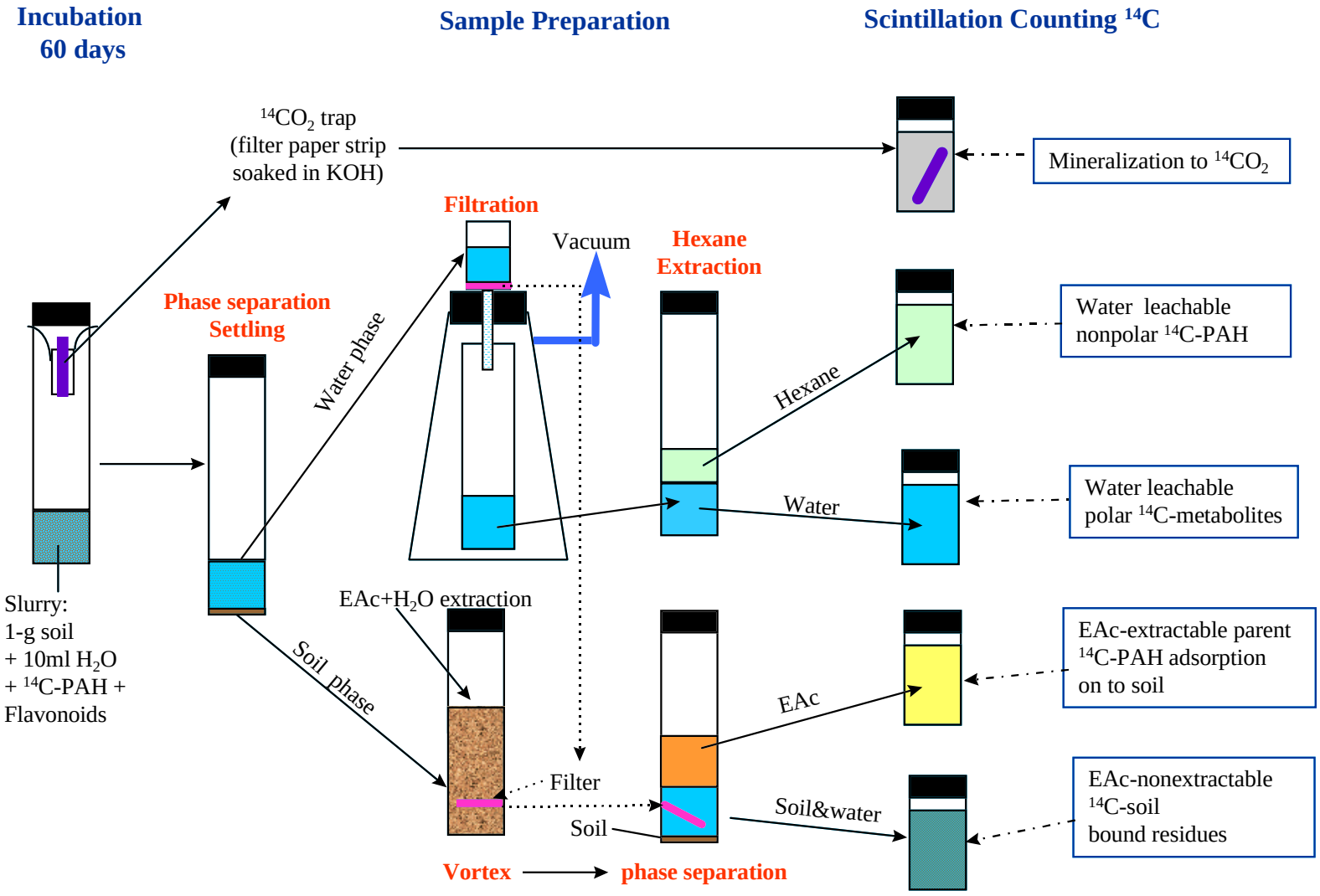
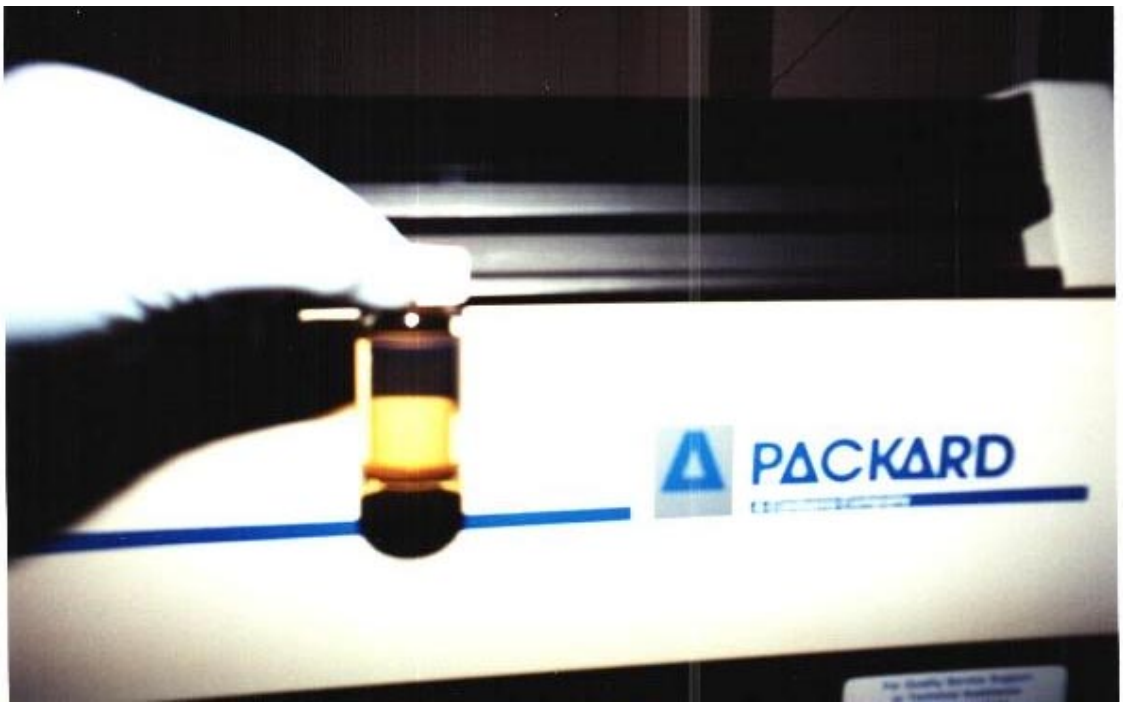


Figure 3.6. Schematic of microcosm experimental procedure

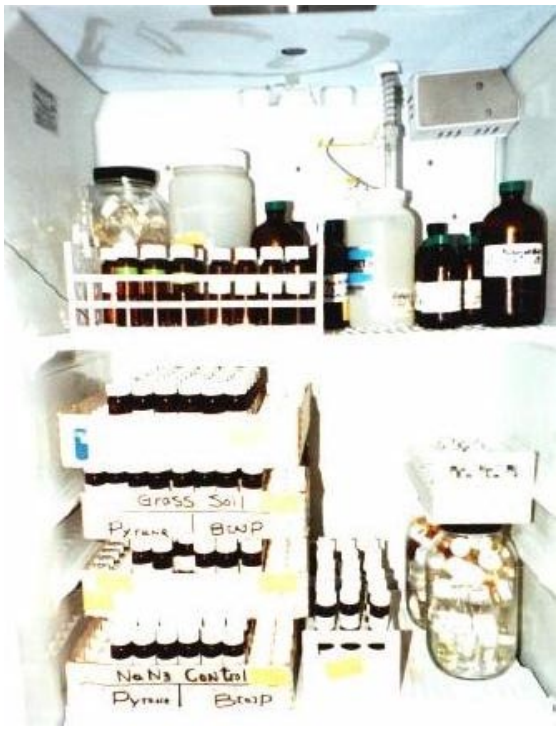
Tri-Carb Liquid Scintillation Analyzer 1600TR



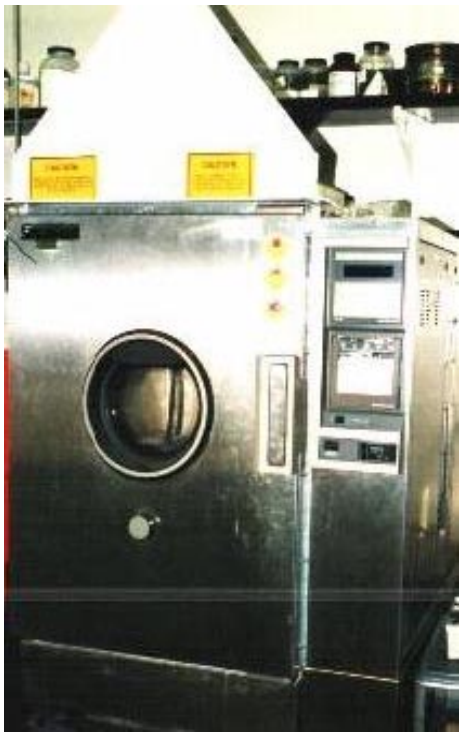
Color-quenched sample

Figure 3.7. Experimental apparatus: liquid scintillation analyzer

Rotary Shaker



Sample storage refrigerator



Environmental Chamber

Figure 3.8. Experimental apparatus: rotary shaker, sample storage refrigerator, and environmental chamber

Table 3.10. The amount of ^{14}C spiked in a soil-slurry microcosm[1]

ID	Replicate	
^{14}C-B[a]P	1	17572
^{14}C-B[a]P	2	16974
^{14}C-B[a]P	3	17469
Average		**17338**
^{14}C-Pyrene	1	57428
^{14}C-Pyrene	2	61140
^{14}C-Pyrene	3	59439
Average		**59336**
^{14}C-Sucrose	1	107272
^{14}C-Sucrose	2	106641
^{14}C-Sucrose	3	102296
Average		**105403**
^{14}C-$NaHCO_3$	1	117471
^{14}C-$NaHCO_3$	2	116199
^{14}C-$NaHCO_3$	3	117567
Average		117079

Measurement of Water Soluble PAHs and Polar Metabolites After the filter paper and the base trap had been removed, the vials were closed with Teflon-lined caps and placed vertically at 4°C overnight for phase separation. Afterwards, the supernatant was filtered[2] through a Whatman GF/A binds-free glass fiber filter and the filtrate was decanted into a clean 40-ml vial. The glass fiber filter was placed back into the soil microcosm. The water phase was extracted, with 2.5 ml of hexane, by being placed horizontally on a rotary shaker at 120 rpm for 30 min. Afterwards the hexane and water were allowed to separate. Using a disposable glass pipette, the hexane portion (2.5 ml) was taken and placed in a 7 ml scintillation vial and filled with 4.5 ml of Scintisafe™ gel scintillation cocktail. The water portion (10 ml) was placed in a 20 ml scintillation vial and filled with 10 ml of rest with Scintisafe™ plus 50% scintillation cocktail. The ^{14}C was counted in each phase by liquid scintillation counter (LSC). Carmichael and Pfaender (1997) reported that the recoveries of PAHs from water by hexane extraction has been

[1] In each soil-slurry microcosm one of those ^{14}C isotopes was spiked (see Table 3-3) at the level listed in the table.
[2] Centrifugation method, which was recommended by Carmichael and Pfaender (1997), failed to separate the water and soil, because the test soil contained lighter plant residues. Filtration method was used instead.

shown to be greater than 98%. As a result the hexane-extractable nonpolar ^{14}C were assumed to be unmetabolized parent PAH, while the polar ^{14}C remaining in the water phase were assumed to be the polar PAH metabolites. A small amount of water remaining in the soil pore was not removed. Based on a soil bulk density of 1.45 g/cm^3 and a porosity of 0.45, the pore water volume in one gram of soil was approximately 0.3 ml, which was about 3% of the 10 ml water added in the slurry microcosm. As a result approximately 3% of the ^{14}C in the water phase was actually erroneously included in the soil phase. Because the water phase ^{14}C was less than 1-2% in all the cases, the related analytical errors (< 0.06%) were negligible.

Measurement of 14C-PAH Associated with Soils. After the supernatant water was removed and filtered, the soil remaining in the microcosm together with the Whatman GF/A binds-free glass fiber filter paper were extracted with simultaneous additions of 5 ml ethylacetate (EAc) and 10 ml deionized water. The vials were vortexed for 30 seconds and then placed at 4°C overnight for phase separation. After separation, 1 ml of the EAc were removed and placed in a vial for LSC to quantified 1/5 of the extractable ^{14}C. The remaining contents in the soil microcosm (including soil, filter, 10 ml deionized water and 4 ml unremoved EAc) were agitated and suspended in 20 ml-scintillation vials filled with Scintisafe™ gel scintillation cocktail. The ^{14}C in the remaining soil-water-EAc mixture were analyzed by LSC to account for nonextractable ^{14}C bound residue in the soil plus the 4/5 of the EAc-extractable ^{14}C. The amount of ^{14}C soil bound residue can be calculated by subtracting 4 times of the ^{14}C measured in the "1 ml of EAc" from the total ^{14}C measured in the "soil-water-EAc mixture". Presumably, the extractable ^{14}C from the soil by ethyl acetate were unmetabolized parent PAHs, while the unextractable ^{14}C-bound residue in the soil can be either the parent PAH or metabolites.

Measurement of ^{14}C-PAH Volatilization Six additional soil microcosms equipped with volatilization trap were established to evaluate the magnitude of pyrene and B[a]P volatilization. The microcosms were prepared the same way as those described in the aforementioned section except for the use of 125 ml serum bottles instead of 40 ml glass vials. The serum bottles were sealed with Teflon lined silicon rubber septum. Once a week the head space was purged by fresh air using two 50 ml glass syringes for five turnover. The purge gas in the syringe was injected into a VOC trap, i.e., a 40 ml vial filled with 20 ml of ethylene glycol monoethyl ether (EGME) solvent. The VOC-trap vial was capped sealed with Teflon-lined septa with an outlet connection to another syringe. The head space gas collected by the outlet syringe was reversely injected into the VOC trap to assure that the ^{14}C-VOC was adequately absorbed by EGME. With ^{14}C-VOC absorbed the 20 ml EGME solvent was divided into two 20 ml scintillation vials. Afterwards, 10 ml of Scinti-Safe Gel was filled into each of the two vials for liquid scintillation counting. At the end of the second week incubation, the measured ^{14}C-VOCs were negligible compared to those at the end of the first week, therefore ^{14}C-VOCs were discontinued.

Radioactive Laboratory Safety Operating Procedures

In compliance with the requirements by NRC and OSHA, laboratory safety operating procedures were established prior to the startup of the experiments. Details can be found in Qiu (1998).

Analytical Methods

Analytical methods for radioisotope ^{14}C counting and experimental soil characterization were summarized in Table 3.11. Soil characterization included soil agronomic properties, the 16 PAHs listed as USEPA's priority pollutants, metals, and bacteria population.

Table 3.11. Summary of analytical methods

Parameter	***Analytical Methods***
Radioactive isotope ^{14}C	Liquid scintillation counting
Soil agronomic properties[1]: (nonstandard protocol published by J. Am. Soc. Agron.)	
pH	Electrode
Anions	Ion chromatography
Cations	ICP-OES (inductively coupled argon plasma-optical emission spectrophotometer)
Cation Exchange Capacity (CEC)	Ammonium acetate method
Soil organic matter (SOM)	$K_2Cr_2O_7/H_2SO_4$ digestion/spectrophotometer
Humic and fulvic acids	MIEK/OC (methyl isobutyl ketone fractionation and organic carbon analyzer)
Soil Texture	Hydrometer
PAHs in soil	Soxhelet extraction and GC/FID (gas chromatography/flame ionization detector)[2] EPA SW 846
Metals in soil	Hot plate digestion and ICAP (inductively coupled argon plasma spectroscopy) EPA SW846
Microbial Enumeration	Plate counting (Sack et al. 1997) (nonstandard protocol)

[1] Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties, 2nd ed. Agronomy Vol. 9. Am. Soc. Agron. 1982.

[2] EPA SW846 in "Test Methods for Evaluating Solid Waste Vol. 1B: Laboratory Manual Physical/Chemical Methods"

Principal of Liquid Scintillation Counting of Radioisotope ^{14}C The experimental samples were analyzed for ^{14}C activity on a Packard Model TriCarb1600 liquid scintillation counter (Packard Instrument Company, Meriden, CT). Liquid scintillation counting (LSC) is an analytical technique by incorporation of radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into emitted photons. The liquid chemical medium, called scintillation fluid or cocktail, consists of solvent and scintillates. To efficiently detect the emitted photon, a photomultiplier tube (PMT) is equipped to amplify the light and transform the detected photons into an electrical pulse. The amplitude of the analog pulse is converted into a digital value by a spectrum analyzer which measure an energy range from 0 to 2000 keV. LSC technique is applicable to all forms of nuclear decay emissions (α,β, and γ). The decay of ^{14}C, a common isotope used in research, results in the emission of β particles of which the maximum energy is 156 KeV (characteristic of ^{14}C). Details can be found in "Tri-Carb Liquid Scintillation analyzers Operation Manual" (Packard 1995).

^{14}C Counting Protocol The LSC counting protocol used in the experiment is presented in Table 3.12. The rationale of this protocol can be found in "Tri-Carb Liquid Scintillation analyzers Operation Manual" (Packard 1995)

^{14}C Counting Efficiency and Detection Limit The LSC instrumental counting efficiency of ^{14}C is approximately 94% - 95%. LSC counting efficiency may be reduced by many different factors. The effect is referred to as quenching, including photon quenching, chemical quenching, and optical quenching. As a result, the energy spectrum detected appears to shift toward lower energies. To compensate for quenching, TriCarb1600 LSC uses the quench indicating parameter (QIP) of the transformed Spectral Index of External Standard (tSIE), monitored in each sample counting. The counting results is independent of sample volume, wall effect, vial size, vial type, and cocktail density. The QIP has been found highly accurate and reproducible over the entire quench range. Detailed discussion can be found in "Tri-Carb Liquid Scintillation analyzers Operation Manual" (Packard 1995).

LSC is extremely sensitive in the detection of radiation. The sensitivity of detecting radioactive events is limited by the presence of background radiation. The instrumental background is approximately 20 CPM (counts per minute, i.e., the observed radioactivity) in ^{14}C counting.

Table 3.12. TriCarb 1600TR scintillation analyzer counting protocol

Protocol Name	^{14}C DPM (disintegration per minute)				
Cycles	1				
Counting time	10 minutes				
Number of Counts/vial	1				
Number of Vials/standard	1				
Number of Vials/sample	1				
Radionuclide	^{14}C				
	LL	UL	Bkg	2 Sigma%	LCR
Region A:	0.0	156	0.00	0.50	0
Region B:	4.0	156	0.00	0.00	0
Region C:	0.0	0.0	0.00	0.00	0
QIP (quench indicate parameter)	tSIE/AEC (transformed spectral index/ automatic efficiency control)				
ES terminator	Count				
% of Reference	no				
Data mode	Single label DPM				

1 micro curie = 2.22×10^6 DPM; 1 curie = 3.7×10^{10} DPS; 1 bacquerel = 1 DPS

Agronomic Assessment The experimental soil samples were analyzed for their agronomic characteristics including texture, pH, electric conductivity (EC), cation exchange capacity (CEC), organic carbon content, humus, salinity, and nutrients. Analytical methods for agronomic parameters can be found in "Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties, 2nd Ed." (Klute 1982).

PAH Analysis Concentrations of the 16 PAHs designated as Toxic Priority Pollutants by UAEPA were analyzed by gas chromatography/flame-ionizing detector (GC/FID) (EPA SW846 standard method 3540 Soxhlet extraction and method 8000 Gas Chromatography). Deuterated phenanthrene d10 was used as internal standards.

Metal Analysis Soil samples were hot plate digested and analyzed for metal concentrations using inductively coupled argon plasma spectroscopy (ICAP).

Microbial Enumeration Soil bacteria population were estimated by plate counting. Extracts were prepared by shaking one gram of soil with sodium phytophosphate buffer and 3 grams of glass beads for 1 hr at 25°C. The supernatants were serially diluted and plated in triplicate. Plate Count Broth Agar containing cycloheximinde were used for total bacteria counting and Basal mineral medium (without yeast extract) solidified with Noble Agar were used for PAH-utilizing bacteria counting. Fungi counting had not been conducted. There are several inherent drawbacks associated with the plate counting method (Chapelle 1993). First, not all bacteria may be dislodged from soil surfaces when samples are slurried. In fact, there is good evidence that many bacteria are not dislodged. Especially, this particular assay was conducted by using slurry supernatant instead of slurry in typical procedures. The results could have even more underestimated the true microbial population in soil. Second, the only bacteria counted with these procedures are those that are capable of growth on the media and under the incubation conditions provided. Fastidious or slow-growing bacteria will be under counted relative to nutritionally diverse and fast-growing microorganism. Also, if the incubations are carried out under aerobic conditions, obligate anaerobes will not be counted with this procedure. Given these problems, which may differ from sample to sample, it is clear that plant counts give a minimum estimate as to the number of bacteria present (Chapelle 1993).

About Cellular Incorporation. Microbial cellular incorporation of ^{14}C were not analyzed in this experiment for several reasons:

1) Cellular incorporation measurement procedures are extremely time-consuming.
2) The amount of cellular incorporation of PAHs is likely to be negligible according to a study conducted by Charmichael and Pfaender (1997).
3) The method developed by Dobbins and Pfaender (1988) is not documented in the publication. The method may need to be improved. Accurate quantification of the cellular incorporation may not be achievable. The analysis are more qualitative than quantitative.

QA/QC Procedures

LSC Performance Verification

Counting efficiency. System Self-Normalization and Calibration (SNC) was performed daily to ensure system accuracy. The acceptable QIP results are 1000±50% for tSIE. The efficiency of ^{14}C counting is calculated as

$$\%\text{Efficiency} = \frac{\text{CPM of Region A}}{\text{DPM of } ^{14}\text{C Standard}} \times 100\%$$

The minimum acceptable efficiency for ^{14}C should be 90%. Results from this study were within the defined limits throughout the experiment. A background check was performed prior to counting for each batch of samples. The instrumental background in the laboratory environment was below 20 CPM throughout the experiment. In this experiment, 20 CPM was equivalent to 0.000116 ug-B[a]P/g-soil or 0.0000337 ug-pyrene/g-soil.

Counting error allowance. Due to the random nature of radioactive disintegration, the number of counts are registered insuccessive increments of time. The true value is more accurately obtained with increased repeated measurements. In the experiment, the protocol was set to continue counting until the 95% confidence limit of the mean counts was within 2% of the mean value.

Heterogeneous sample counting efficiency control. Two types of heterogeneous samples, (1) filter papers and (2) soil particles were measured in this experiment. Heterogeneous samples typically result in loss of physical contact between the radiolabeled analyte and the scintillation cocktail. As a result, counting efficiency reduced. Filter papers immersed in scintillation cocktail for counting is commonly accepted. Typically, suspension method was used for soil samples counting. For better suspension Scinti-safe™ gel cocktail was used. Phase separation is not allowed and only a tiny amount of soil particle can be included in a scintillation vial for reasonably accurate counting. Carmichael and Pfaender (1997) subsampled 1/10 of the soil slurry in a microcosm for soil particle counting. Subsampling of heterogeneous material often results in large errors. To minimize foreseen large errors, the entire soil slurry in a microcosm was divided and placed into eight scintillation vials for counting. As a result, each of the 20 ml vials contained only approximately 0.125 g of soil, which is sufficiently small to not unreasonably hinder the contact between soil particle and scintillation cocktail. The efficiency of the method was validated by spiking a known amount of ^{14}C-B[a]P or ^{14}C-pyrene into a number of soil-containing scintillation vials. The results indicated that a minimum of eight vials for one gram of soil is needed for a reasonably accurate counting. The method verification data are included in Appendix B-4. A more accurate method of counting ^{14}C associated with soil particle is to combust the soil using Harvey Oxidizer (with platinum catalyst) and to count the $^{14}CO_2$ evolved. Unfortunately, the instrument was not available in the lab.

Quench correction Because of sample heterogeneity as well as the presence of chemicals and rich yellow color of flavonoids, heavy quench, indicated by a color flag in the scintillation counting output report, was observed in a number of samples. The default ^{14}C quench curve was not applicable to the heavily quenched samples. Quench correction curve were developed by spiking known amount of ^{14}C into a range of quenched samples to determine the counting efficiencies as a function of QIP (i.e., tSIE) for each type of samples (Packard 1999). Without heavy quench, the default ^{14}C quench curve was applicable to $^{14}CO_2$ and hexane extracted ^{14}C samples. In contrast, heavy color and chemical quench were associated with ethylactate extracts, water, and soil samples. The levels of quench depend on the types of chemicals, solvent, soil particles, and scintillation cocktail present in the counting samples. The counting efficiency quench correction curves are presented in Figure 3.9 through 3.12.

Data Quality Verification Prior to data analysis data accuracy was determined by calculating ^{14}C mass balance and examining the repeatability of measurements. Mass balance measures the possibility of bias or systematic errors and the repeatability measures the precision. Conclusions will be based on the data of reasonable confidence.

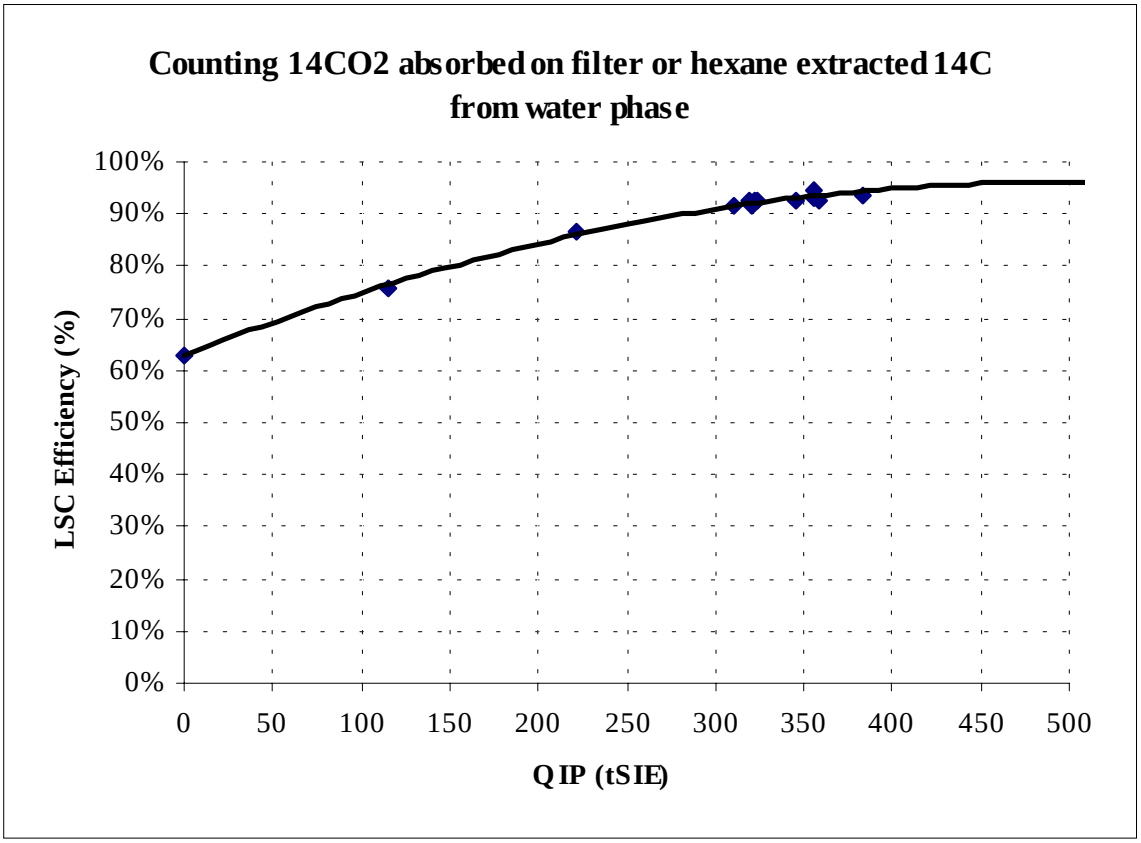


Figure 3.9. Liquid scintillation counting ^{14}C efficiency quench correction curve for $^{14}CO_2$ or hexane-solvent-extracted samples

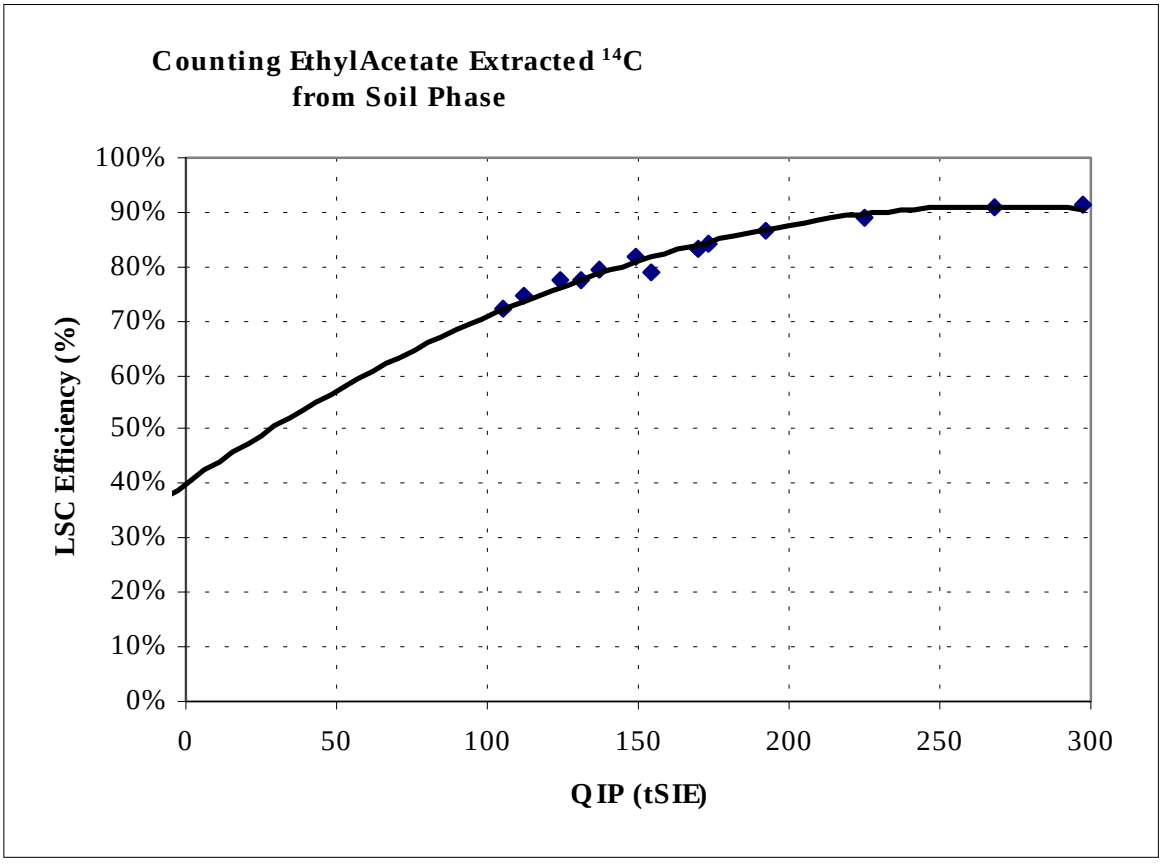
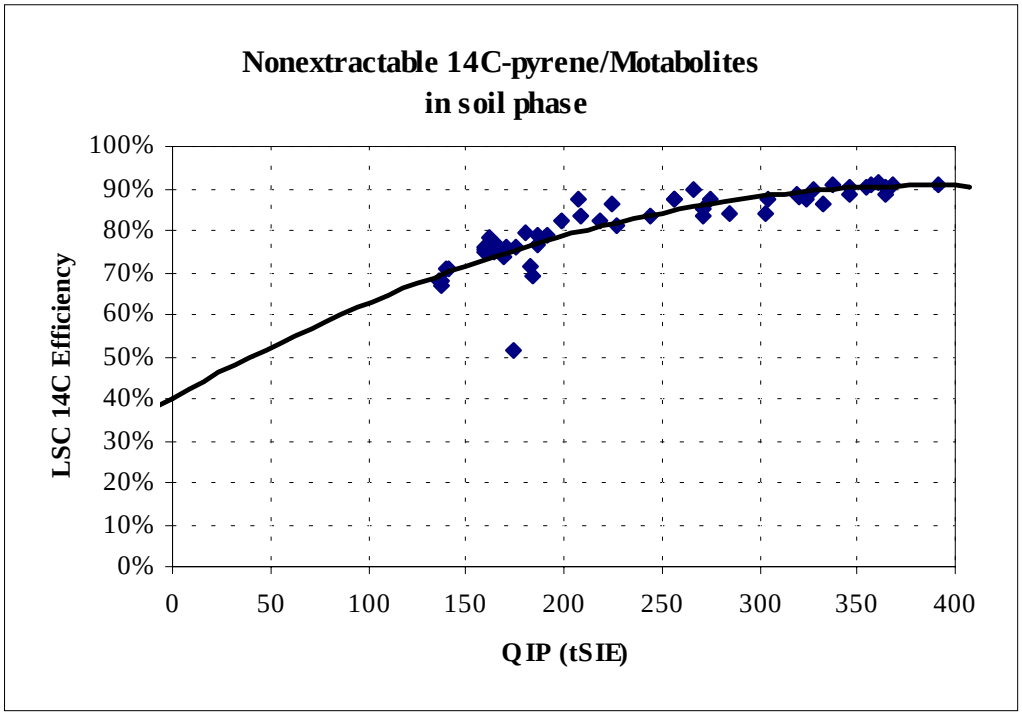


Figure 3.10. Liquid scintillation counting ^{14}C efficiency quench correction curve for ethylacetate-solvent-extracted samples



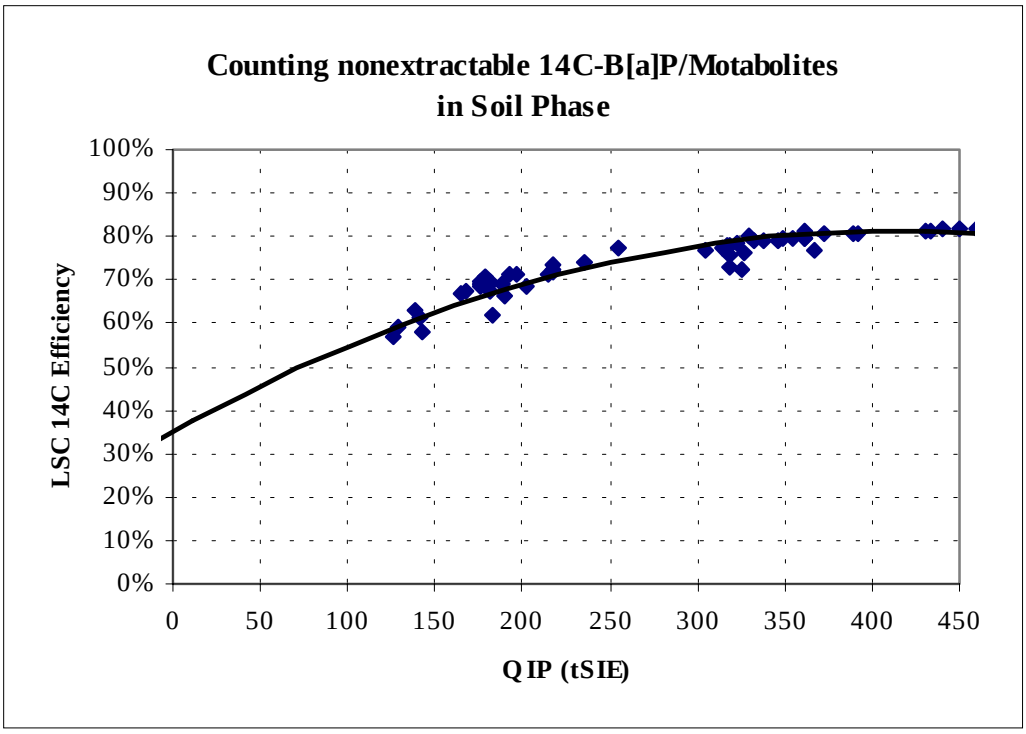


Figure 3.11. Liquid scintillation counting ^{14}C efficiency quench correction curve for ^{14}C soil bound residue formation samples

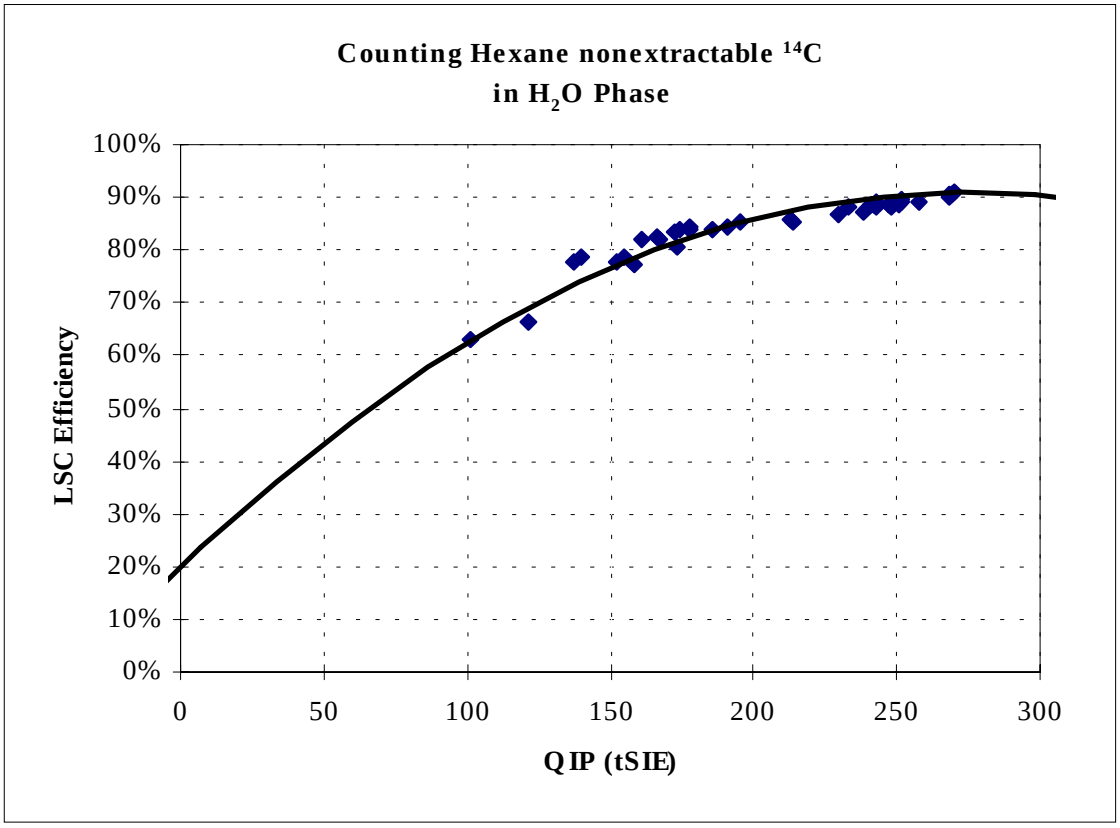


Figure 3.12. Liquid scintillation counting ^{14}C efficiency quench correction curve for ^{14}C-H_2O samples

Mass Balance of ^{14}C Validation of the PAH fate measurements require a ^{14}C mass balance be obtained. ^{14}C mass balance in a soil microcosm was calculated from.

Total ^{14}C added = $^{14}CO_2$ + ^{14}C soil bound residue + ^{14}C adsorption onto soil + ^{14}C-PAH in water + ^{14}C-metabolites in water **[Equation 3.1]**

Where, $^{14}CO_2$ = gas-phase ^{14}C absorbed by CO_2 trap;
^{14}C bound residue = EAc-nonextractable ^{14}C in soil phase;
^{14}C adsorption = EAc-extractable ^{14}C in soil phase;
^{14}C-PAH/H_2O = water soluble parent PAH extracted by hexane;
^{14}C-metabolites/H_2O = water soluble PAH metabolites nonextractable by hexane.

The sum of ^{14}C recovery (dpm) from each soil microcosm at the right side of the equation was calculated as percent of total ^{14}C added (dpm) into the microcosm. Mass balance acceptance criteria was established in reference to the method accuracy reported by the method developer (Carmichael and Pfaender 1997, Dobbins and Pfaender 1988) as well as the relevant, applicable quality control criteria set in USEPA SW 846, "Test Methods for Evaluating Solid Waste".

USEPA SW846 Method 8270, "Polynuclear Aromatic Hydrocarbons", comprises the standard procedures of detecting PAHs by GC/MS and appropriate sample extraction (Method 3540 Soxhlet extraction or Method 3550 Sonication extraction) prior to GC/MS measurement. In the quality control criteria of method 8270, the acceptable range of average recovery for the quality control check sample (test conc. of 100 ug/L) for four recovery measurements were 31.7% - 148% and 69.6% - 100% for B[a]P and pyrene, respectively. The percent recovery ranged from17% to 163% and 52% to 115% for B[a]P and pyrene, respectively. The QC criteria was adapted from 40 CFR Part 136 for Method 625.

The ^{14}C-microcosm experimental method was developed by Dr. Pfaender and his associates. Dobbin and Pfaender (1988) reported that the ^{14}C mass balances for amino acids and m-cresol after 24 hours of incubation exhibited considerable variation with a skewed distribution. The observed mass balances for ^{14}C-amino acids ranged from 65% to 200% with a median of 93%. The observed mass balance for ^{14}C-m-cresol ranged from20% to 180% with a median of 58%. For metabolic-inhibited controls, the median mass balances were 106% and 54% for amino acids and m-cresol, respectively. Low recovery of m-cresol was largely attributed to volatilization during the filtration, when the solution was exposed to the atmosphere. Loss by volatilization was demonstrated by a study using toluene. Toluene loss did not occur during incubation or CO_2 recovery, but during subsequent steps in the procedures of vortex and vacuum filtration. Also, it was found the ^{14}C recovery varied with soil clay content, it is likely that greater adsorption resulted in a less loss from the aqueous phase during the sample handling.

Carmichael and Pfaender (1997) reported that the triplicate mean mass balance of ^{14}C-pyrene with a variety of soils ranged from 30% to 126% after 2 months of incubation. Meanwhile, the triplicate mean mass balance of ^{14}C-B[a]P ranged from 38% to 123% after 2 months of incubation. The standard deviation was less than 15%. Unlike the m-cresol

experiments, the lower ^{14}C recovery was found to be with clay soil and high recovery was observed with sand soils. Dobbins and Pfaender (1988) reported that CO_2 recovery rate in the CO_2-trap was determined using 24-hour incubation of $Ba^{14}CO_2$-spiked controls. The maximum recovery of 78% was observed at 20 hours with a high variability (standard deviation = 33%).

Based on the reported method accuracy and the relevant applicable QC criteria set by USEPA. Acceptance ^{14}C mass balance criteria were established in the following for this experiment.

(1) Triplicate mean mass balance for B[a]P: 100±25% (95%CL of mean)
(2) Triplicate mean mass balance for pyrene: 85±25%
(3) Range of mass balance for B[a]P: 55 – 145%
(4) Rang of mass balance for pyrene: 40 – 130%
(5) At least 90% of the samples meet the above criteria
(6) At least two of the triplicate samples meet criterion (3) or (4)

Repeatability of the measurements Data repeatability was examined based on the degree of scatter of the triplicate measurements. The correlation among the triplicate data sets were examined using JMP® Statistics software. A scatterplot for each pair of replicate data were plotted in a matrix to visualize the data repeatability.

Data Analysis

Statistical analysis is an essential and integral part of the data analysis. Analysis of variance (ANOVA) is a useful tool for breaking down the total variability of designed experiments into interpretable components. For well-designed experiments ANOVA gives clear conclusions drawn from data. JMP® statistics (version 3.26, SAS Institute, Inc.), a powerful, efficient, and user-friendly software, was used to analyze the experimental data. Statistical analysis was conducted in two tiers: (1) screening of multiple factor effects, and (2) detailed one-way ANOVA.

Compound Nested Model A compound nested model is interpreted by

$$y_{ijkl} = \bar{y} + \alpha_i + \beta_j + \gamma_k + \lambda_l + (\text{interaction terms}) + e_i \qquad \textbf{[Equation 3.2]}$$

where, y_{ijkl} = observation (measured ^{14}C-PAH fate data)
$\bar{y}$ = mean observation
α_i = response due to the type of soil
β_j = response due to the type of flavonoid (nested within soil)
γ_k = response due to the level of flavonoid concentration (compound nested within flavonoids and soil)
λ_l = response due to the replicate measurement (triplicate)
e_i = random residual error of the i^{th} observation

Hierarchical structure of the compound nested model is presented in the previous section in Figure 3.1 at the beginning of this chpter.

Screening of Multiple Factor Effects A model fit screening was conducted to assess the compound effects of multiple factors on the ^{14}C-PAH fate measurements. Joint tests were performed on all the parameters. Analysis of variance addresses the problem of identifying which factors contribute significant amounts of variance to measurements. The total variation in the data was assessed and assigned to each of the three factors studied in the experiment and to their interactions. The interaction item indicates whether the variations caused by one factor were independent of or interacted with other factors.

One way analysis of variance (ANOVA): Student's t Test of Paired Mean Comparison
One way analysis of variance was performed to determine whether a particular flavonoid at certain concentration level had significant effects on PAH fate in a particular soil. Paired comparison were conducted between all the flavonoid concentration levels per each of the three soil data groups for the five PAH-fate parameters (i.e., CO_2 production, soil incorporation, soil adsorption, water soluble parent PAH, and water soluble metabolites), respectively. Paired comparison were also conducted among the three soils per each fixed flavonoid concentration level to identify soil effects. Student t-tests were conducted to compare each pair to determine whether the actual differences between the triplicate means was greater than the LSD (least significant difference) at 95% confidence level. The LSD term (for the comparison of triplicate data pairs "a" and "b") is calculated from

$$LSD = t_{n,\alpha/2} S_{pool} \sqrt{\frac{1}{n_a} + \frac{1}{n_b}} \qquad \textbf{[Equation 3.3]}$$

where, t = student t value

$\nu = n_a + n_b - 2$ degrees of freedom

$\alpha = 0.05$

n = number of replicates

$$S_{pool} = \sqrt{\frac{(n_a - 1)S_a^2 + (n_b - 1)S_b^2}{n_a + n_b - 2}} \qquad \textbf{[Equation 3.4]}$$

CHAPTER 4. RESULTS

STUDY OBJECTIVES AND DATA INTERPRETATION

The principal objective of this study was to evaluate the effects of flavonoids on PAH fate in soil via multiple physicochemical and biological pathways. Experimental results include ^{14}C-PAH fate data and experimental soil properties. Influences of soil physicochemical characteristics on PAH fate were evaluated further. PAH fate data, including $^{14}CO_2$ evolution, ^{14}C soil bound, ^{14}C adsorption, and water phase ^{14}C-PAH and metabolites, are interpreted in terms of percentage of the total ^{14}C-PAH spiked onto soil. Data quality was verified against the ^{14}C-mass balance-based quality control criteria prior to statistical analysis.

^{14}C DATA QUALITY VERIFICATION

Data precision and accuracy were determined by calculating ^{14}C mass balances and examining the repeatability of measurements. Mass balance measures the possibility of bias or systematic errors and the repeatability measures the precision.

Mass Balance of ^{14}C

^{14}C mass balance calculation data for each of the 180 soil microcosms are included in Tables A-1 and A-2, Appendix A. In Figures 4.1 and 4.2, mass balances of ^{14}C-B[a]P and ^{14}C-pyrene in each soil microcosm are plotted, respectively. Data per each of the three tested soils are grouped together. The mean, standard deviation, and 95% confidence limits of the ^{14}C mass balance for all microcosms and for each soil group are summarized in Table 4.1. The overall mean of ^{14}C-B[a]P mass balance was 101±4.0% (95% confidence limits). More than 90% of the ^{14}C-B[a]P mass balance data points fell within the acceptable range (55% - 145%, see Chapter 3). Six data points outside the acceptable range were discarded. The triplicate means of ^{14}C-B[a]P mass balances satisfied the acceptable criteria (100±25%) as well. As a result, ^{14}C-B[a]P mass balance met the quality control criteria. In contrast, the overall mean of ^{14}C-pyrene mass balance was 61 ±4% (95% confidence limit). Fourteen out of 90 data points (more than 10%) fell outside the acceptable range (40%-130%, see Chapter 3).

Also, more than 10% of the triplicate means of ^{14}C-pyrene mass balance data were below the acceptable criteria (85±25%, see Chapter 3). As a result, ^{14}C-pyrene mass balance failed to meet the quality control criteria. Majority of the B[a]P and pyrene mass balance data points fell in the lower half of the acceptable range. Apparently, there were some systematic loss of both ^{14}C-pyrene and ^{14}C-B[a]P. To identify the possible root causes of lower ^{14}C recovery, mass balances in different types of soil microcosms were examined.

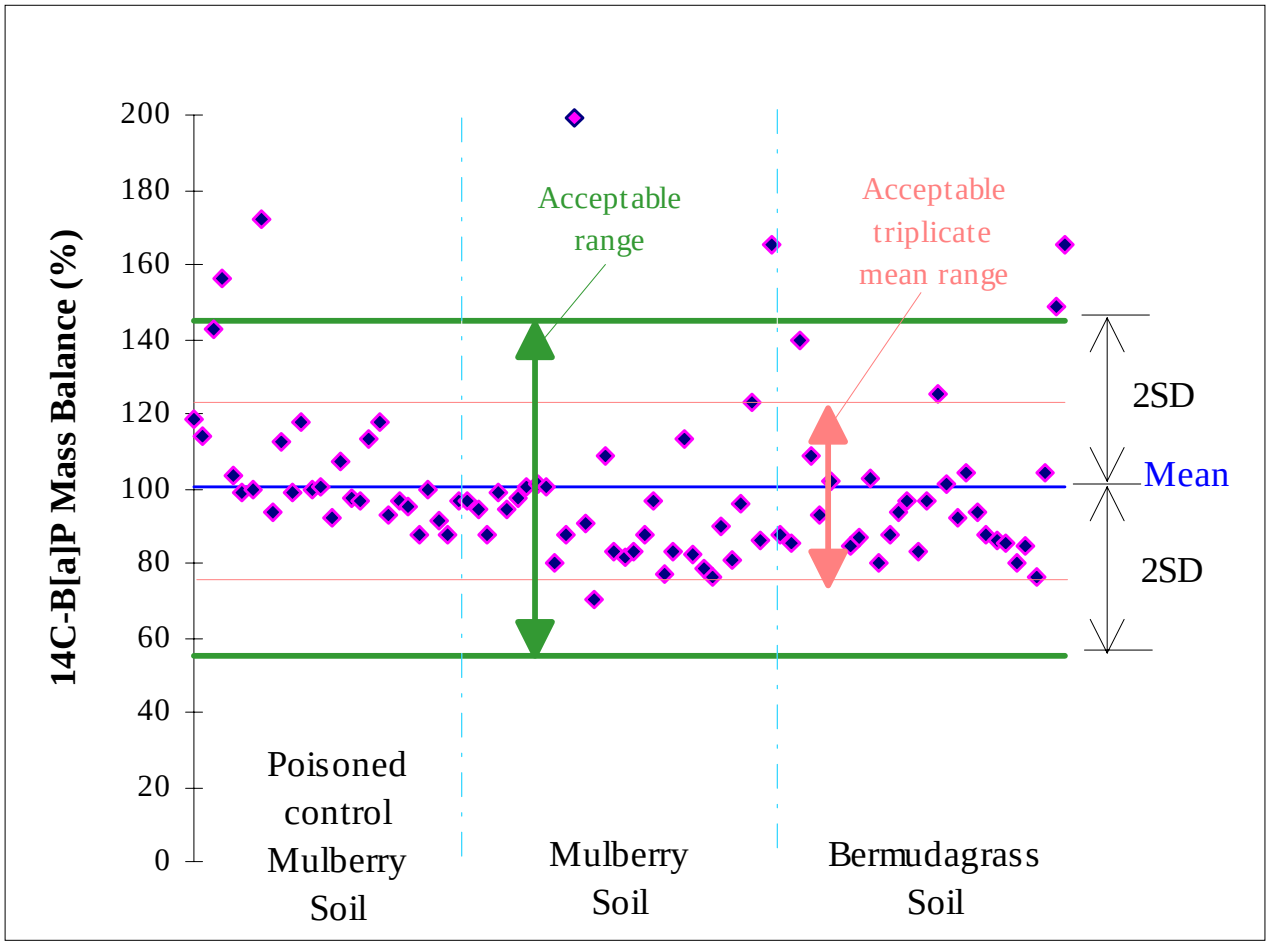


Figure 4. 1. Mass balance of ^{14}C-B[a]P in soil-slurry microcosms

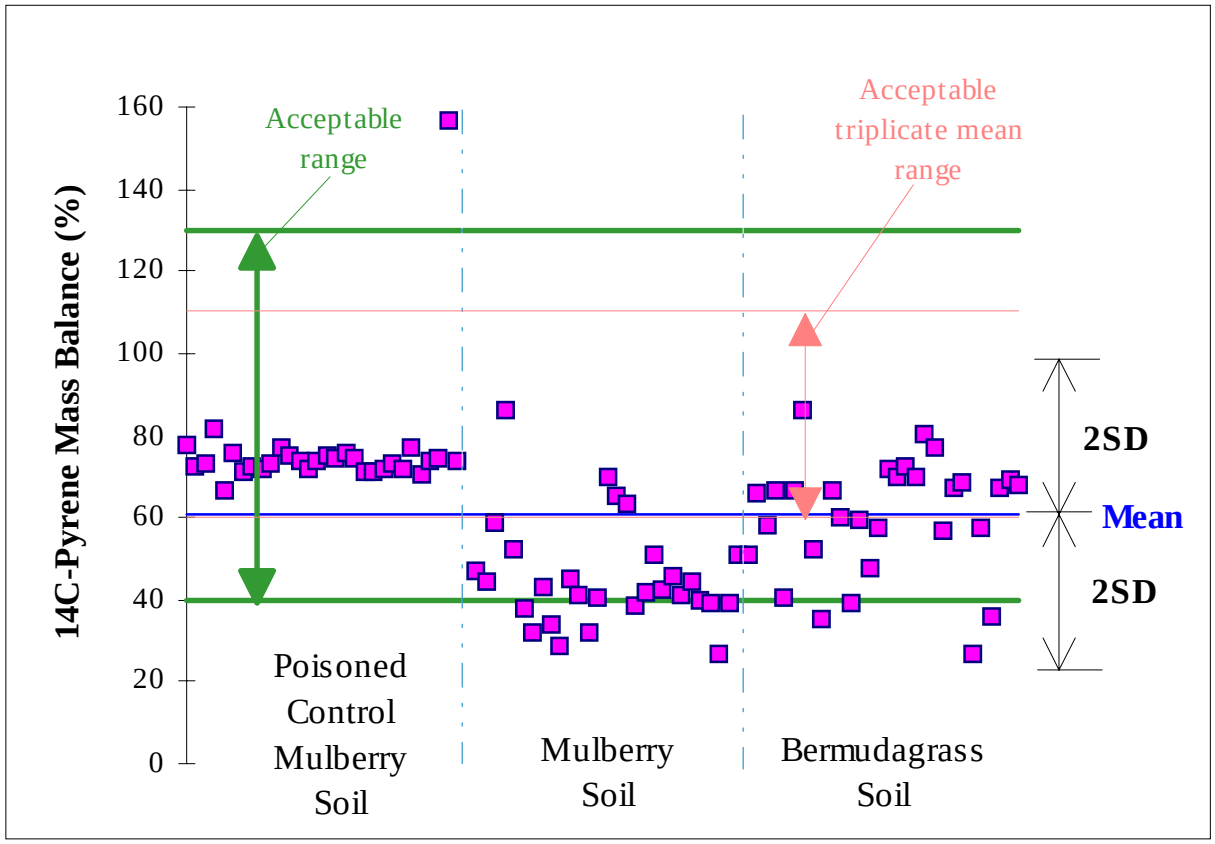


Figure 4.2. Mass balance of ^{14}C-pyrene in soil-slurry microcosms

Table 4.1. Summary of ^{14}C-mass balance

Soil-slurry microcosms	Statistics	Mass balance of pyrene & B[a]P	Mass balance of pyrene	Mass balance of B[a]P	Average mass balance of the triplicates
All	Stdev	28.8	18.9	22.8	25.2
	Count	178.0	89.0	89.0	60.0
	Mean	80.8	61.0	100.6	80.6
	95%UCL Mean	84.3	64.3	104.6	86.0
	95%LCL Mean	77.2	57.7	96.6	75.3
	Min	27.0	27.0	70.4	35.9
	Max	199.1	156.6	199.1	139.3
Poisoned Control	stdev	23.1	15.4	19.7	18.6
	counts	60.0	30.0	30.0	20.0
	Mean	91.4	76.4	106.4	91.4
	95%UCL Mean	96.3	81.0	112.3	98.3
	95%LCL Mean	86.5	71.8	100.5	84.6
Mulberry Rhizosphere	stdev	33.0	12.9	26.4	29.1
	counts	59.0	29.0	30.0	20.0
	Mean	71.5	45.6	96.6	71.1
	95%UCL Mean	78.6	49.5	104.5	81.8
	95%LCL Mean	64.4	41.6	88.6	60.4
Bermudagrass Rhizosphere	stdev	26.3	14.1	21.2	23.7
	counts	59.0	30.0	29.0	20.0
	Mean	79.3	60.4	98.7	79.4
	95%UCL Mean	84.9	64.6	105.2	88.1
	95%LCL Mean	73.6	56.2	92.3	70.7

In poisoned soil microcosms, ^{14}C-B[a]P recoveries were consistently higher than those with Mulberry and Bermudagrass soils. The mean recovery of the former was 106±6% compared to 97±8% and 99±6% for Mulberry and Bermudagrass, respectively. An over 100% mean recovery with poisoned soil was attributed to a few odd high range data points. In fact, majority of the mass balance with poisoned "abiotic"-control soil were about 100%, while those for biotic microcosms were mostly around 90%. Near 100% recovery associated with "abiotic" soil indicates that the loss of ^{14}C-B[a]P in the biotic soils were most likely due to the $^{14}CO_2$ fugitive emission into the atmosphere.

$^{14}CO_2$ Recovery. In the mass balance calculation, the percent of $^{14}CO_2$ production was corrected based on $^{14}CO_2$ trap efficiencies in triplicate microcosms amended with ^{14}C-$NaHCO_3$. Data are presented in Table 4.2. Average $^{14}CO_2$ recovery after 60 days of incubation in the triplicate soil microcosms were 42%. Approximately 2% of the originally added ^{14}C was recovered from soil phase and only a trace (~0.05%) was recovered from water phase. Average ^{14}C recovery in these three microcosms was approximately 44%. To look for the possible reason of $^{14}CO_2$ loss, ^{14}C-$NaHCO_3$ was added into triplicate water phase test tubes equipped with the same CO_2-trap as those in soil-slurry microcosms. Without incubation, 99.8% of the added ^{14}C was recovered after acidification and rotary shaking. Among the 99.8%, approximately 75% was recovered from $^{14}CO_2$ trap and approximately 25% was recovered from water phase. Apparently, the $^{14}CO_2$-trap was effective, however, a significant portion of $^{14}CO_2$ was lost during the 60 days of incubation, most likely via fugitive emission. A minor portion of unaccountable ^{14}C may be due to $^{14}CO_2$ precipitation onto the calcium-rich soil and subsequent sequestration.

Table 4.2. $^{14}CO_2$ recovery efficiency in soil-slurry microcosms after 60 days of incubation

Microcosm ID	Tot. spike (dpm)	$^{14}CO_2$ recovered (dpm)	^{14}C recovered in H_2O (dpm)	^{14}C recovered in soil (dpm)	$^{14}CO_2$ (% recovery)
$NaHCO_3$-1	117079	55886	62	6758	51%
$NaHCO_3$-2	117079	56135	55	6411	51%
$NaHCO_3$-3	117079	34802	65	4394	31%
Average	117079	48941	61	5854	44%
$^{14}CO_2$ recovery (%) in liquid phase test tubes and without incubation					
$NaHCO_3$-1r	38073	27303	8597		93%
$NaHCO_3$-2r	37074	30749	9569		112%
$NaHCO_3$-3	36919	25280	10361		95%
Average	37355	27777	9509		99.8%

It was conceived that the rubber septa with Teflon liner in the microcosm cap may not be completely sealed. Positive gas pressure built up in the headspace during incubation could have caused gas leaking to the environmental chamber, which was continuously ventilated. The more CO_2 production, the higher gas-phase pressure in the headspace and the more $^{14}CO_2$ loss. Meantime, ^{14}C-B[a]P loss from the liquid and solid phases during sample extraction, separation, and adsorption on the sample containers may be insignificant by reason of approximately 100% ^{14}C recovery from the poisoned microcosms.

Similarly, ^{14}C-pyrere mass balance in the poisoned soil was consistently higher than those with Mulberry and Bermudagrass soils. The mean mass balance with poisoned was 81±4% compared to 46±3% and 60±4% for Mulberry and Bermudagrass soils, respectively. Pyrene is much more water soluble and biodegradable than B[a]P. The water solubility of pyrene is 135 μg/L @25°C compared to 4 μg/L @25°C for B[a]P. More $^{14}CO_2$ could have been produced and lost from pyrene mineralization, resulting in average 46% and 60% ^{14}C-pyrene mass balances in mulberry and Bermudagrass soils, respectively. Near 80% mass balance of ^{14}C-pyrere with poisoned "abiotic" soil indicated that loss of ^{14}C other than $^{14}CO_2$ fugitive emission existed.

Volatilization Loss. Volatilization loss during incubation was confirmed to be less than 1% as measured by the VOC tests presented in Table 4.3. Dobbins and Pfaener (1988) found significant volatilization loss of ^{14}C-*m*-cresol in their experiment during sample handling, particularly during vortex and vacuum filtration, however, volatilization loss during incubation was found negligible. Pyrene has a vapor pressure of $2.5x10^{-6}$ mm-Hg @25°C, which is three orders in magnitude higher than that of B[a]P ($5x10^{-9\ 6}$ mm-Hg @25°C). Pyrene could have volatilized somewhat with ethylacetate solvent during vortex. Ethylacetate is highly volatile and water-soluble. Despite that pyrene is more volatile than B[a]P, the volatilization potential of pyrene is generally low. As much as 20% ^{14}C loss via volatilization was very unlikely.

Table 4. 3. ^{14}C data for volatilization test microcosms

Soil	Flavonoid	Concentration	PAH	Tot. spike (DPM)	$^{14}CO_2$ (dpm)	^{14}C/hexane /H2O (dpm)	$^{14}C/H_2O$ (dpm)	^{14}C/Eac- soil (dpm)	^{14}C/soil-bound (dpm)	^{14}C/VOC (dpm)	^{14}C Sum (dpm)	Mass Balance (%)	VOC (%)
Mulberry	None	0	Pyrene	109317	9970	50	495.99	25572	22743	405	59236	54.19	0.37
Mulberry	None	0	Pyrene	109317	9264	64	523.64	22341	38997	346	71535	65.44	0.32
Mulberry	None	0	Pyrene	109317	12141	130	830.69	25744	36726	302	75874	69.41	0.28
Mulberry	None	0	B[a]P	32573	2646	22	80.20	18234	9275	127	30384	93.28	0.39
Mulberry	None	0	B[a]P	32573	1966	25	79.40	20931	11102	80	34184	104.95	0.25
Mulberry	None	0	B[a]P	32573	2997	28	115.46	20237	8866	121	32365	99.36	0.37

Sequestration. Another possible pathway of unaccountable ^{14}C-pyrene was sequestration in soil. A portion of ^{14}C-pyrene and/or metabolites could have been deeply diffused into the soil micropores and resulted in loss of physical contact between the ^{14}C and the scintillation cocktail. As a result, the sequestered ^{14}C within soil particle suspended in scintillation cocktail became uncountable. The fact that ^{14}C-B[a]P was more accountable than ^{14}C-pyrene was not adequately understood. It is suggested that more adsobable and hydrophobic ^{14}C-B[a]P were largely binding onto the soil particle surface without much diffusion into soil micropores. Nevertheless, such ^{14}C counting method (particle suspension) deficiency can be resolved by using an oxidizer, in which heterogeneous samples are completely converted into $^{14}CO_2$ through combustion. The $^{14}CO_2$ evolved is then absorbed by alkaline solution for effective scintillation counting. Unfortunately, the equipment was not available for this study.

In addition, ^{14}C recoveries with Mulberry soil were generally lower than those with Bermudagrass soil. The Bermudagrass soil contained much higher clay, silt, and SOM contents than the mulberry soil. It is suggested that strong adsorption of PAH onto SOM and clay could have attenuated the potential dissolution and subsequent mineralization and volatilization loss. In summary, less than 100% ^{14}C-mass balance was most likely attributed to $^{14}CO_2$ recovery and sequestration in soil micropores. Volatilization loss during sample handling may also cause some unrecoverable ^{14}C. Better mass balance can be achieved by improving CO_2-trap and the seal of soil microcosm vials and using an oxidizer to count ^{14}C in soil phase.

Repeatability of the Measurements

Data repeatability was examined based on the degree of scatter of the triplicate measurements. Highly scattered data are commonly observed in biologically-related and heterogeneous medium tests. The correlation of the triplicate microcosm data was examined using JMP® Statistics software. In Figure 4.3, a matrix of correlation coefficients (0.82 - 0.83) indicates linear relationships between each pair of replications. To visualize the data repeatability, scatterplot for each pair of replicate data were plotted in a matrix. A 95% bivariate normal density ellipse is imposed on each scatterplot. Reasonably good correlation of the replications is seen by the orientation of the ellipse along the diagonal axis. Evidently, the triplicate data sets were consistent throughout the experiment. Analysis of variance for the triplicate data subsets, (i.e., per fate measurement per PAH) indicated that the means of the triplicate data sets were statistically identical at the 95% confidence level. JMP® statistics output report for the analysis of variance is included in Appendix B. Comparisons for each pair using *student's t* indicated the differences between the means of the three replicates were less than the least significant differences (LSD). An example of the comparisons of the triplicate measurements by *student's t* test is presented in Figure 4.4.

In summary, data accuracy and precision were validated by ^{14}C mass balance and the consistency of the triplicate measurements. ^{14}C-B[a]P mass balance met the quality control criteria, while ^{14}C-pyrene mass balance did not. Both ^{14}C-pyrene and ^{14}C-B[a]P fate data are presented and analyzed in Chapter 4. Discussion will rely more on the ^{14}C-B[a]P data, because of the uncertainties associated with poor mass balances of ^{14}C-pyrene.

Correlations

Variable	Replicate 1	Replicate 2	Replicate 3
Replicate 1	1.0000	0.8274	0.8217
Replicate 2	0.8274	1.0000	0.8350
Replicate 3	0.8217	0.8350	1.0000

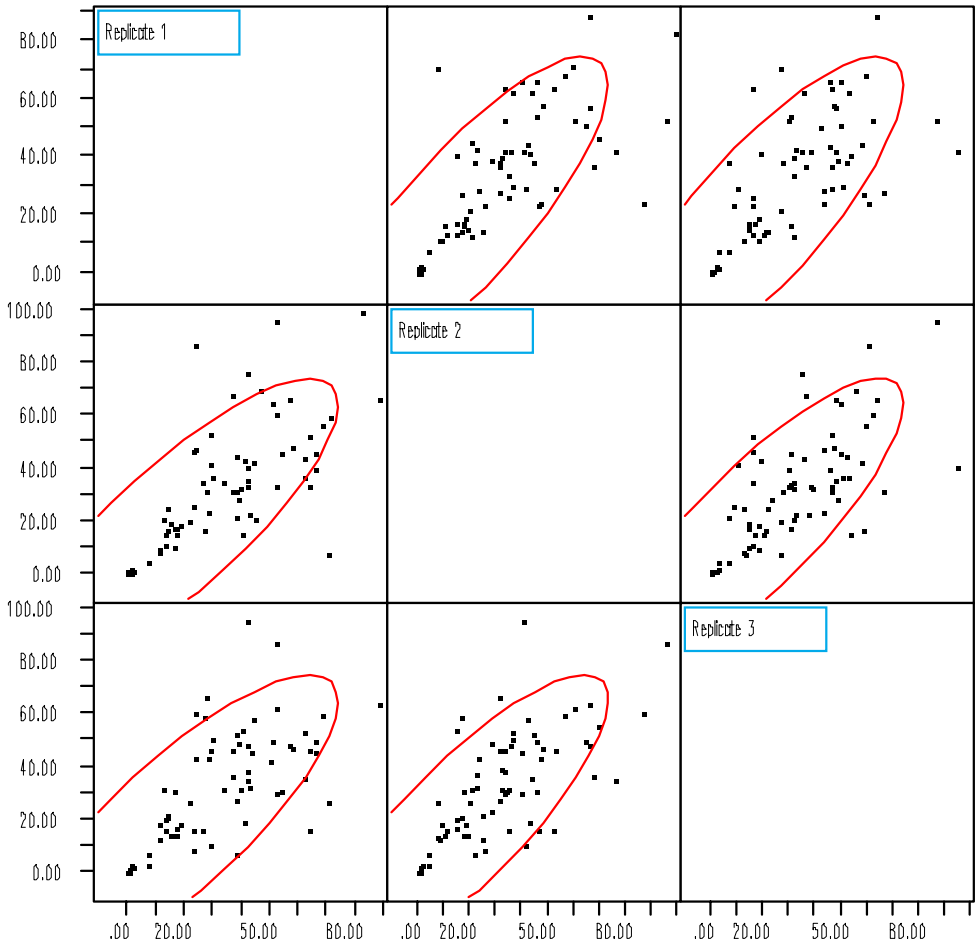
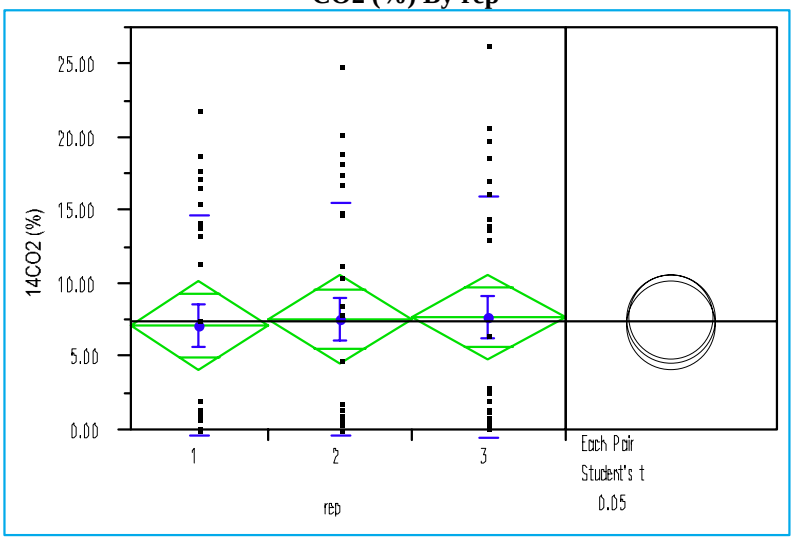


Figure 4.3. Correlation matrix of replicate measurements

$^{14}CO2$ (%) By rep



Oneway Anova
Summary of Fit

RSquare	0.000757
RSquare Adj	-0.02454
Root Mean Square Error	7.994135
Mean of Response	7.525488
Observations (or Sum Wgts)	82

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	3.8244	1.9122	0.0299
Error	79	5048.5890	63.9062	Prob>F
C Total	81	5052.4134	62.3755	0.9705

Means for Oneway Anova

Level	Number	Mean	Std Error
1	26	7.23038	1.5678
2	27	7.56370	1.5385
3	29	7.75448	1.4845

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]	3	2	1
3	0.000000	0.190779	0.524098
2	-0.19078	0.000000	0.333319
1	-0.5241	-0.33332	0.000000
Alpha=	0.05		

Comparisons for each pair using Student's t

t
1.99046

Abs(Dif)-LSD	3	2	1
3	-4.17869	-4.06459	-3.77344
2	-4.06459	-4.33069	-4.03882
1	-3.77344	-4.03882	-4.41319

Positive values show pairs of means that are significantly different.

Figure 4.4. An example of data repeatability: *Student's t* test for paired comparison of $^{14}CO_2$ evolution (%) from the triplicate microcosms (analysis of variance by JMP®)

^{14}C-B[A]P AND ^{14}C-PYRENE FATE DATA

^{14}C-B[a]P and ^{14}C-pyrene fate in soil slurry was determined for five fate mechanisms: mineralization, soil bound residue formation, adsorption, water leaching of parent B[a]P, and water leaching of B[a]P metabolites. In this section fate data are interpreted as percent of the total 7,10-^{14}C-B[a]P or 4,5,9,10-^{14}C-pyrene initially spiked into the soil. All the ^{14}C-B[a]P and ^{14}C-pyrene fate data are summarized in Tables 4.4 and 4.5, respectively. Original liquid scintillation counting of ^{14}C data (dpm) can be found in Tables A-1 and A-2, Appendix A.

Table 4.4. ^{14}C-B[a]P fate data (% of the total ^{14}C added)

ID	rep.	Soil	Flavonoid	Conc. (uM)	PAH	$^{14}CO_2$ (%)	^{14}C-B[a]P in H2O (%)	^{14}C-metabolites in H_2O (%)	^{14}C-adsorption to soil (%)	^{14}C-soil-bound residue (%)	^{14}C mass balance (%)
NNB-0	1	Poison Control	None	0	B[a]P	0.17	0.05	0.33	47.11	71.05	119
NNB-0	2	Poison Control	None	0	B[a]P	0.10	0.04	0.32	70.22	43.59	114
NNB-0	3	Poison Control	None	0	B[a]P	0.74	0.08	0.33	91.06	50.16	142
NRB-0	1	Poison Control	M-Rt-extracts	NQ	B[a]P	0.92	0.47	0.70	122.08	32.30	156
NRB-0	2	Poison Control	M-Rt-extracts	NQ	B[a]P	5.92	0.31	0.63	69.75	26.63	103
NRB-0	3	Poison Control	M-Rt-extracts	NQ	B[a]P	0.46	0.32	0.66	56.04	41.49	99
NMB-0.1	1	Poison Control	Morin	0.1	B[a]P	0.91	0.05	0.31	50.59	47.66	100
NMB-0.1	2	Poison Control	Morin	0.1	B[a]P	0.26	0.13	0.39	70.40	101.27	172
NMB-0.1	3	Poison Control	Morin	0.1	B[a]P	0.21	0.05	0.34	42.89	49.92	93
NMB-1	1	Poison Control	Morin	1	B[a]P	1.12	0.06	0.37	64.18	47.10	113
NMB-1	2	Poison Control	Morin	1	B[a]P	0.96	0.04	0.36	52.86	44.80	99
NMB-1	3	Poison Control	Morin	1	B[a]P	0.46	0.06	0.36	15.94	100.68	117
NMB-10	1	Poison Control	Morin	10	B[a]P	0.24	0.06	0.29	70.68	28.20	99
NMB-10	2	Poison Control	Morin	10	B[a]P	0.12	0.06	0.28	8.04	92.06	101
NMB-10	3	Poison Control	Morin	10	B[a]P	0.18	0.06	0.32	26.88	64.36	92
NMB-100	1	Poison Control	Morin	100	B[a]P	0.20	0.07	0.33	68.65	38.12	107
NMB-100	2	Poison Control	Morin	100	B[a]P	1.09	0.09	0.37	56.82	38.62	97
NMB-100	3	Poison Control	Morin	100	B[a]P	0.27	0.06	0.30	60.23	35.90	97
NFB-0.1	1	Poison Control	Flavone	0.1	B[a]P	3.23	0.09	0.46	42.55	67.23	114
NFB-0.1	2	Poison Control	Flavone	0.1	B[a]P	0.11	0.07	0.29	76.12	41.33	118
NFB-0.1	3	Poison Control	Flavone	0.1	B[a]P	0.24	0.08	0.32	34.96	57.51	93
NFB-1	1	Poison Control	Flavone	1	B[a]P	0.14	0.06	0.30	38.32	58.14	97
NFB-1	2	Poison Control	Flavone	1	B[a]P	0.13	0.07	0.32	44.82	49.48	95
NFB-1	3	Poison Control	Flavone	1	B[a]P	0.42	0.07	0.34	52.29	34.44	88
NFB-10	1	Poison Control	Flavone	10	B[a]P	0.29	0.07	0.33	51.66	47.28	100
NFB-10	2	Poison Control	Flavone	10	B[a]P	0.11	0.07	0.31	65.10	26.09	92
NFB-10	3	Poison Control	Flavone	10	B[a]P	0.54	0.07	0.36	50.18	36.20	87
NFB-100	1	Poison Control	Flavone	100	B[a]P	0.76	0.05	0.29	53.01	42.41	97
NFB-100	2	Poison Control	Flavone	100	B[a]P	0.48	0.06	0.33	60.41	35.69	97
NFB-100	3	Poison Control	Flavone	100	B[a]P	0.36	0.07	0.35	62.54	31.35	95
MNB-0	1	Mulberry	None	0	B[a]P	21.92	0.02	0.25	38.88	26.49	88
MNB-0	2	Mulberry	None	0	B[a]P	20.33	0.01	0.26	28.12	49.90	99
MNB-0	3	Mulberry	None	0	B[a]P	26.44	0.03	0.00	48.84	18.97	94
MRB-0	1	Mulberry	M-Rt-extracts	NQ	B[a]P	1.49	0.32	0.77	24.46	70.15	97
MRB-0	2	Mulberry	M-Rt-extracts	NQ	B[a]P	1.92	0.17	0.57	47.39	50.09	100
MRB-0	3	Mulberry	M-Rt-extracts	NQ	B[a]P	2.75	0.16	0.47	43.18	54.31	101
MMB-0.1	1	Mulberry	Morin	0.1	B[a]P	17.83	0.02	0.32	62.41	19.57	100
MMB-0.1	2	Mulberry	Morin	0.1	B[a]P	14.85	0.02	0.18	44.36	20.24	80
MMB-0.1	3	Mulberry	Morin	0.1	B[a]P	17.20	0.02	0.27	35.64	34.13	87
MMB-1	1	Mulberry	Morin	1	B[a]P	44.53	0.04	0.28	121.09	33.15	199
MMB-1	2	Mulberry	Morin	1	B[a]P	25.01	0.01	0.22	34.60	31.09	91
MMB-1	3	Mulberry	Morin	1	B[a]P	13.14	0.02	0.21	31.03	26.04	70
MMB-10	1	Mulberry	Morin	10	B[a]P	13.39	0.01	0.22	26.81	68.41	109
MMB-10	2	Mulberry	Morin	10	B[a]P	11.31	0.01	0.24	34.85	36.32	83
MMB-10	3	Mulberry	Morin	10	B[a]P	16.28	0.02	0.23	15.75	49.53	82
MMB-100	1	Mulberry	Morin	100	B[a]P	1.07	0.05	0.40	36.87	44.48	83
MMB-100	2	Mulberry	Morin	100	B[a]P	0.87	0.04	0.35	67.75	18.90	88
MMB-100	3	Mulberry	Morin	100	B[a]P	0.93	0.04	0.27	36.79	58.25	96

Table 4.4. ^{14}C-B[a]P fate data (% of the total ^{14}C added) (cont')

ID	rep.	Soil	Flavonoid	Conc. (uM)	PAH	$^{14}CO_2$ (%)	^{14}C-B[a]P in H2O (%)	^{14}C-met-abolites in H_2O (%)	^{14}C-ad-sorption to soil (%)	^{14}C-soil-bound residue (%)	^{14}C mass balance (%)
MFB-0.1	1	Mulberry	Flavone	0.1	B[a]P	15.52	0.02	0.27	30.07	31.05	77
MFB-0.1	2	Mulberry	Flavone	0.1	B[a]P	19.09	0.02	0.27	37.13	26.24	83
MFB-0.1	3	Mulberry	Flavone	0.1	B[a]P	14.60	0.03	0.27	50.74	47.48	113
MFB-1	1	Mulberry	Flavone	1	B[a]P	18.92	0.04	0.26	37.30	25.73	82
MFB-1	2	Mulberry	Flavone	1	B[a]P	18.30	0.03	0.25	31.86	28.34	79
MFB-1	3	Mulberry	Flavone	1	B[a]P	18.69	0.01	0.28	46.76	10.56	76
MFB-10	1	Mulberry	Flavone	10	B[a]P	17.27	0.02	0.15	27.43	44.74	90
MFB-10	2	Mulberry	Flavone	10	B[a]P	17.59	0.02	0.20	16.76	45.92	80
MFB-10	3	Mulberry	Flavone	10	B[a]P	14.11	0.03	0.32	58.89	22.28	96
MFB-100	1	Mulberry	Flavone	100	B[a]P	1.30	0.02	0.24	44.92	76.47	123
MFB-100	2	Mulberry	Flavone	100	B[a]P	0.76	0.03	0.15	21.14	64.01	86
MFB-100	3	Mulberry	Flavone	100	B[a]P	0.71	0.06	0.19	23.86	140.80	166
GNB-0	1	Grasses	None	0	B[a]P	14.03	0.02	0.24	27.82	45.70	88
GNB-0	2	Grasses	None	0	B[a]P	15.00	0.04	0.22	31.69	38.47	85
GNB-0	3	Grasses	None	0	B[a]P	19.96	0.04	0.24	66.67	53.09	140
GRB-0	1	Grasses	M-Rt-extracts	NQ	B[a]P	2.15	0.07	0.16	13.11	93.44	109
GRB-0	2	Grasses	M-Rt-extracts	NQ	B[a]P	0.96	0.17	0.44	20.87	70.52	93
GRB-0	3	Grasses	M-Rt-extracts	NQ	B[a]P	2.06	0.16	0.32	31.57	68.03	102
GMB-0.1	1	Grasses	Morin	0.1	B[a]P						
GMB-0.1	2	Grasses	Morin	0.1	B[a]P	7.98	0.01	0.20	32.78	43.70	85
GMB-0.1	3	Grasses	Morin	0.1	B[a]P	13.89	0.03	0.20	39.44	33.44	87
GMB-1	1	Grasses	Morin	1	B[a]P	16.68	0.02	0.26	42.47	43.48	103
GMB-1	2	Grasses	Morin	1	B[a]P	10.54	0.01	0.22	33.68	35.71	80
GMB-1	3	Grasses	Morin	1	B[a]P	14.57	0.02	0.23	38.03	34.47	87
GMB-10	1	Grasses	Morin	10	B[a]P	7.59	0.02	0.18	53.09	32.61	93
GMB-10	2	Grasses	Morin	10	B[a]P	4.80	0.06	0.17	33.79	57.46	96
GMB-10	3	Grasses	Morin	10	B[a]P	3.04	0.04	0.20	29.98	49.88	83
GMB-100	1	Grasses	Morin	100	B[a]P	0.83	0.05	0.23	38.58	56.94	97
GMB-100	2	Grasses	Morin	100	B[a]P	1.46	0.06	0.34	21.65	102.09	126
GMB-100	3	Grasses	Morin	100	B[a]P	1.57	0.04	0.25	7.23	92.12	101
GFB-0.1	1	Grasses	Flavone	0.1	B[a]P	14.23	0.03	0.22	29.81	47.60	92
GFB-0.1	2	Grasses	Flavone	0.1	B[a]P	16.82	0.04	0.30	41.49	45.86	105
GFB-0.1	3	Grasses	Flavone	0.1	B[a]P	20.76	0.02	0.23	10.49	61.75	93
GFB-1	1	Grasses	Flavone	1	B[a]P	11.46	0.02	0.21	14.73	61.41	88
GFB-1	2	Grasses	Flavone	1	B[a]P	8.66	0.03	0.17	25.25	51.92	86
GFB-1	3	Grasses	Flavone	1	B[a]P	13.09	0.02	0.25	21.94	50.35	86
GFB-10	1	Grasses	Flavone	10	B[a]P	7.65	0.01	0.26	11.44	60.63	80
GFB-10	2	Grasses	Flavone	10	B[a]P	4.87	0.00	0.18	9.39	69.95	84
GFB-10	3	Grasses	Flavone	10	B[a]P	6.61	0.02	0.23	18.39	51.15	76
GFB-100	1	Grasses	Flavone	100	B[a]P	0.83	0.09	0.72	14.41	87.86	104
GFB-100	2	Grasses	Flavone	100	B[a]P	17.38	0.09	0.38	25.08	105.50	148
GFB-100	3	Grasses	Flavone	100	B[a]P	1.31	0.02	0.25	66.64	97.42	166
VOB-0	1	Mulberry	None	0	B[a]P	8.12	0.03	0.14	44.78	53.11	106
VOB-0	2	Mulberry	None	0	B[a]P	6.03	0.08	0.24	64.26	34.08	105
VOB-0	3	Mulberry	None	0	B[a]P	9.20	0.07	0.25	62.13	27.22	99

Table 4.5. ^{14}C-pyrene fate data (% of total ^{14}C-pyrene added)

ID	rep.	Soil	Flavonoid	Conc. (uM)	PAH	$^{14}CO_2$ (%)	^{14}C-Pyene in H_2O (%)	^{14}C-met-abolites in H_2O (%)	^{14}C Ad-sorption to soil (%)	^{14}-/soil-bound residues (%)	^{14}C mass balance (%)
NNP-0	1	Poison Control	None	0	Pyrene	0.24	0.05	0.28	44.99	31.88	77
NNP-0	2	Poison Control	None	0	Pyrene	0.09	0.06	0.22	29.43	42.63	72
NNP-0	3	Poison Control	None	0	Pyrene	0.06	0.03	0.16	53.08	20.13	73
NRP-0	1	Poison Control	M-Rt-extracts	NQ	Pyrene	0.12	0.20	0.38	52.06	29.12	82
NRP-0	2	Poison Control	M-Rt-extracts	NQ	Pyrene	0.11	0.16	0.35	25.83	40.39	67
NRP-0	3	Poison Control	M-Rt-extracts	NQ	Pyrene	0.10	0.11	0.30	45.77	29.15	75
NMP-0.1	1	Poison Control	Morin	0.1	Pyrene	0.31	0.05	0.24	48.27	22.44	71
NMP-0.1	2	Poison Control	Morin	0.1	Pyrene	0.08	0.05	0.22	58.88	13.58	73
NMP-0.1	3	Poison Control	Morin	0.1	Pyrene	0.19	0.06	0.25	63.11	8.33	72
NMP-1	1	Poison Control	Morin	1	Pyrene	0.09	0.06	0.25	32.43	40.55	73
NMP-1	2	Poison Control	Morin	1	Pyrene	0.13	0.05	0.27	58.72	18.06	77
NMP-1	3	Poison Control	Morin	1	Pyrene	0.13	0.06	0.22	56.24	18.27	75
NMP-10	1	Poison Control	Morin	10	Pyrene	0.06	0.06	0.21	33.55	39.69	74
NMP-10	2	Poison Control	Morin	10	Pyrene	0.12	0.04	0.17	18.28	53.13	72
NMP-10	3	Poison Control	Morin	10	Pyrene	0.06	0.06	0.17	20.41	52.83	74
NMP-100	1	Poison Control	Morin	100	Pyrene	0.20	0.08	0.25	57.08	17.20	75
NMP-100	2	Poison Control	Morin	100	Pyrene	0.18	0.06	0.19	18.51	55.58	75
NMP-100	3	Poison Control	Morin	100	Pyrene	0.17	0.07	0.23	37.39	37.79	76
NFP-0.1	1	Poison Control	Flavone	0.1	Pyrene	0.17	0.05	0.44	59.76	14.13	75
NFP-0.1	2	Poison Control	Flavone	0.1	Pyrene	0.20	0.08	0.12	31.63	39.31	71
NFP-0.1	3	Poison Control	Flavone	0.1	Pyrene	0.12	0.05	0.21	52.51	18.12	71
NFP-1	1	Poison Control	Flavone	1	Pyrene	0.10	0.06	0.24	57.22	13.90	72
NFP-1	2	Poison Control	Flavone	1	Pyrene	0.52	0.05	0.27	62.51	10.12	73
NFP-1	3	Poison Control	Flavone	1	Pyrene	0.07	0.06	0.22	61.08	10.63	72
NFP-10	1	Poison Control	Flavone	10	Pyrene	0.12	0.07	0.26	71.92	4.74	77
NFP-10	2	Poison Control	Flavone	10	Pyrene	0.21	0.06	0.31	61.99	7.93	70
NFP-10	3	Poison Control	Flavone	10	Pyrene	0.14	0.08	0.29	53.63	19.47	74
NFP-100	1	Poison Control	Flavone	100	Pyrene	0.12	0.06	0.28	37.54	36.58	75
NFP-100	2	Poison Control	Flavone	100	Pyrene	82.46	0.05	0.23	61.35	12.51	157
NFP-100	3	Poison Control	Flavone	100	Pyrene	0.54	0.06	0.30	45.47	27.19	74
MNP-0	1	Mulberry	None	0	Pyrene						
MNP-0	2	Mulberry	None	0	Pyrene	28.05	0.02	0.81	11.95	6.23	47
MNP-0	3	Mulberry	None	0	Pyrene	25.22	0.01	0.88	5.36	13.04	45
MRP-0	1	Mulberry	M-Rt-extracts	NQ	Pyrene	2.76	0.21	1.28	42.77	11.85	59
MRP-0	2	Mulberry	M-Rt-extracts	NQ	Pyrene	24.25	0.04	0.17	28.61	33.13	86
MRP-0	3	Mulberry	M-Rt-extracts	NQ	Pyrene	2.21	0.07	0.67	37.97	11.43	52
MMP-0.1	1	Mulberry	Morin	0.1	Pyrene	18.21	0.03	0.73	12.02	6.81	38
MMP-0.1	2	Mulberry	Morin	0.1	Pyrene	10.61	0.04	0.76	9.46	11.23	32
MMP-0.1	3	Mulberry	Morin	0.1	Pyrene	23.45	0.01	0.78	11.58	7.04	43
MMP-1	1	Mulberry	Morin	1	Pyrene	16.94	0.03	0.79	9.47	6.70	34
MMP-1	2	Mulberry	Morin	1	Pyrene	8.14	0.03	0.71	10.33	9.30	29
MMP-1	3	Mulberry	Morin	1	Pyrene	28.49	0.01	0.59	10.18	5.89	45
MMP-10	1	Mulberry	Morin	10	Pyrene	16.77	0.04	0.77	10.26	12.99	41
MMP-10	2	Mulberry	Morin	10	Pyrene	13.43	0.01	0.45	10.35	8.00	32
MMP-10	3	Mulberry	Morin	10	Pyrene	13.16	0.01	0.58	14.60	12.02	40
MMP-100	1	Mulberry	Morin	100	Pyrene	0.47	0.03	0.16	36.38	32.57	70
MMP-100	2	Mulberry	Morin	100	Pyrene	0.83	0.07	0.34	37.08	27.24	66
MMP-100	3	Mulberry	Morin	100	Pyrene	0.42	0.07	0.44	16.70	45.57	63

Table 4.5. ^{14}C-pyrene fate data (% of total ^{14}C spike) (cont')

ID	rep.	Soil	Flavonoid	Conc. (uM)	PAH	$^{14}CO_2$ (%)	^{14}C-Pyrene in H_2O (%)	^{14}C-met-abolites in H_2O (%)	^{14}C Ad-sorption to soil (%)	^{14}-/soil-bound residues (%)	^{14}C mass balance (%)
MFP-0.1	1	Mulberry	Flavone	0.1	Pyrene	18.12	0.03	0.94	11.63	8.01	38.73
MFP-0.1	2	Mulberry	Flavone	0.1	Pyrene	23.79	0.01	0.64	8.98	8.22	41.64
MFP-0.1	3	Mulberry	Flavone	0.1	Pyrene	30.78	0.01	0.73	8.93	10.43	50.88
MFP-1	1	Mulberry	Flavone	1	Pyrene	22.77	0.02	0.54	12.38	6.45	42.17
MFP-1	2	Mulberry	Flavone	1	Pyrene	23.43	0.01	0.70	14.06	7.62	45.81
MFP-1	3	Mulberry	Flavone	1	Pyrene	19.43	0.02	0.61	13.19	7.88	41.13
MFP-10	1	Mulberry	Flavone	10	Pyrene	26.79	0.01	0.20	10.78	6.40	44.19
MFP-10	2	Mulberry	Flavone	10	Pyrene	19.76	0.02	0.31	13.29	6.61	39.99
MFP-10	3	Mulberry	Flavone	10	Pyrene	21.93	0.03	0.54	8.67	8.06	39.22
MFP-100	1	Mulberry	Flavone	100	Pyrene	6.48	0.01	0.38	9.75	10.46	27.08
MFP-100	2	Mulberry	Flavone	100	Pyrene	18.79	0.01	0.33	5.19	14.86	39.19
MFP-100	3	Mulberry	Flavone	100	Pyrene	4.46	0.03	0.25	18.33	27.61	50.69
GNP-0	1	Grasses	None	0	Pyrene	31.07	0.02	0.43	9.84	9.74	51.10
GNP-0	2	Grasses	None	0	Pyrene	45.18	0.01	0.55	9.62	10.56	65.92
GNP-0	3	Grasses	None	0	Pyrene	38.84	0.04	0.48	8.57	10.21	58.14
GRP-0	1	Grasses	M-Rt-extracts	NQ	Pyrene	1.32	0.11	0.34	32.11	32.48	66.37
GRP-0	2	Grasses	M-Rt-extracts	NQ	Pyrene	2.88	0.14	0.44	11.75	25.35	40.56
GRP-0	3	Grasses	M-Rt-extracts	NQ	Pyrene	3.46	0.24	1.94	30.25	30.80	66.68
GMP-0.1	1	Grasses	Morin	0.1	Pyrene	66.30	0.04	0.43	8.51	10.82	86.10
GMP-0.1	2	Grasses	Morin	0.1	Pyrene	28.49	0.02	0.52	10.68	12.49	52.20
GMP-0.1	3	Grasses	Morin	0.1	Pyrene	11.68	0.02	0.49	7.96	14.88	35.04
GMP-1	1	Grasses	Morin	1	Pyrene	43.18	0.03	0.40	11.13	11.65	66.39
GMP-1	2	Grasses	Morin	1	Pyrene	40.26	0.03	0.41	10.56	9.13	60.38
GMP-1	3	Grasses	Morin	1	Pyrene	16.67	0.03	0.54	12.81	9.20	39.24
GMP-10	1	Grasses	Morin	10	Pyrene	31.57	0.02	0.29	7.80	19.54	59.22
GMP-10	2	Grasses	Morin	10	Pyrene	20.24	0.04	0.39	13.70	13.38	47.76
GMP-10	3	Grasses	Morin	10	Pyrene	8.84	13.60	17.36	12.57	5.21	57.57
GMP-100	1	Grasses	Morin	100	Pyrene	1.98	0.08	0.59	27.06	42.01	71.72
GMP-100	2	Grasses	Morin	100	Pyrene	1.30	0.07	0.36	45.00	23.19	69.92
GMP-100	3	Grasses	Morin	100	Pyrene	1.83	0.09	0.47	43.79	26.33	72.51
GFP-0.1	1	Grasses	Flavone	0.1	Pyrene	48.89	0.02	0.48	4.81	15.39	69.58
GFP-0.1	2	Grasses	Flavone	0.1	Pyrene	56.62	0.05	0.78	5.02	18.04	80.51
GFP-0.1	3	Grasses	Flavone	0.1	Pyrene	53.00	0.03	0.41	4.27	19.11	76.82
GFP-1	1	Grasses	Flavone	1	Pyrene	35.02	0.03	0.58	4.17	17.17	56.97
GFP-1	2	Grasses	Flavone	1	Pyrene	45.98	0.02	0.56	4.02	16.68	67.27
GFP-1	3	Grasses	Flavone	1	Pyrene	48.13	0.02	0.55	5.07	14.99	68.76
GFP-10	1	Grasses	Flavone	10	Pyrene	0.97	0.02	0.58	6.78	18.66	27.02
GFP-10	2	Grasses	Flavone	10	Pyrene	32.94	0.04	0.57	4.21	20.04	57.79
GFP-10	3	Grasses	Flavone	10	Pyrene	10.14	0.04	0.51	5.16	20.31	36.16
GFP-100	1	Grasses	Flavone	100	Pyrene	0.62	0.11	0.45	46.55	19.54	67.27
GFP-100	2	Grasses	Flavone	100	Pyrene	0.30	0.05	0.36	8.25	60.49	69.45
GFP-100	3	Grasses	Flavone	100	Pyrene	0.49	0.05	0.28	6.97	60.23	68.02

STATISTICAL ANALYSIS

The effects of flavonoids and soil properties on ^{14}C-B[a]P fate were determined based statistical analysis of ^{14}C-B[a]P fate data of the slurry-soil microcosms. Statistical analysis of ^{14}C-pyrene fate data was also conducted and compared with those of ^{14}C-B[a]P fate. It should be noted that ^{14}C-pyrene mass balance data failed quality control criteria (see the previous section "mass balance" in this chapter). As a result, the ^{14}C-pyrene fate data are less reliable than the ^{14}C-B[a]P fate data.

JMP® statistics software was used throughout the analysis. The significant differences were judged at 95% confidence level.

Screening Multiple Factor Effects

A screening analysis of model fit was conducted to test the effects of multiple factors on the measured ^{14}C-B[a]P and ^{14}C-pyrene fate data, respectively. The JMP® output effect test tables are presented in Tables 4.6 and 4.7. Detailed report of fit model summary, analysis of variance, and parameter estimates can be found in Appendices C-1 and C-2. The compound nested model included three hierarchical factors, which are soil, flavonoid (nested within soil), and flavonoid concentration (compound nested within flavonoid and soil). As described in Chapter 3, a general model for the compound nested experimental design is expressed as

$$y_{ijkl} = \bar{y} + \alpha_i + \beta_j + \gamma_k + \lambda_l + (\text{interaction terms}) + e_i$$

where y_{ijkl} = observation (measured ^{14}C data),
$\bar{y}$ = mean observation,
α_i = response due to the type of soil,
β_j = response due to the type of flavonoid,
γ_k= response due to the level of flavonoid concentration,
λ_l = response due to the replicate measurement, and
e_i = random residual error of the ith observation.

Table 4.6. Multiple factor effect test: compound-nested model fit screening of ^{14}C- B[a]P data

Source[1]	Nparm[2]	DF[3]	Sum of Squares[4]	F Ratio[5]	Prob>F[6]
^{14}CO2 (%)					
Soil	2	2	2003.5011	191.3175	<.0001
Flavonoids[Soil]	6	6	37.1226	1.1816	0.3283
Flv Conc.[Soil,Flavonoids]	27	27	3228.0176	22.8332	<.0001
^{14}C-B[a]P soil-bound residues (%)					
Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob>F**
Soil	2	2	4289.181	10.1540	0.0002
Flavonoids[Soil]	6	6	1611.406	1.2716	0.2840
Flv Conc.[Soil,Flavonoids]	27	27	14837.154	2.6018	0.0011
^{14}C-BaP adsorption on soil (%)					
Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob>F**
Soil	2	2	5756.2560	12.8244	<.0001
Flavonoids[Soil]	6	6	1153.1966	0.8564	0.5321
Flv Conc.[Soil,Flavonoids]	27	27	5579.5060	0.9208	0.5821
^{14}C-BaP in H_2O (%)					
Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob>F**
Soil	2	2	0.02976963	32.7139	<.0001
Flavonoids[Soil]	6	6	0.09299620	34.0645	<.0001
Flv Conc.[Soil,Flavonoids]	27	27	0.13470696	10.9652	<.0001
^{14}C-Metabolites in H_2O (%)					
Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob>F**
Soil	2	2	0.13416079	14.2379	<.0001
Flavonoids[Soil]	6	6	0.24820326	8.7803	<.0001
Flv Conc.[Soil,Flavonoids]	27	27	0.67946667	5.3414	<.0001

[1] Source = the name of the effects in the model.

[2] Nparm = the number of parameters associated with the effect.

[3] DF = the degrees of freedom for the effect test..

[4] Sum of squares = the sum of squares for the hypothesis that the listed effect is zero.

[5] F ratio = the F statistic for testing that the effect is zero, equals to the ratio of the mean square for the effect divided by the mean square for error

[6] Prob>F =the significance probability for the F ratio, given that the null hypothesis is true. A value of less than 0.0005 represents a probability that is conceptually zero

Table 4.7. Multiple factor effect test: compound-nested model fit screening of ^{14}C-pyrene data

$^{14}CO_2$ (%)					
Source[1]	**Nparm**[2]	**DF**[3]	**Sum of Squares**[4]	**F Ratio**[5]	**Prob>F**[6]
Soil	2	2	948.342	82.8354	<.0001
Flavonoids[Soil]	6	6	618.683	1.7172	0.1303
Flv Conc.[Soil,Flavonoids]	27	27	12043.672	7.4283	<.0001
^{14}C-pyrene soil-bound residues (%)					
Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob>F**
Soil	2	2	1539.2610	6.0088	0.0040
Flavonoids[Soil]	6	6	1381.2776	1.7974	0.1127
Flv Conc.[Soil,Flavonoids]	27	27	7852.1189	2.2705	0.0034
^{14}C-pyrene adsorption on soil (%)					
Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob>F**
Soil	2	2	17441.284	110.6272	<0.0001
Flavonoids[Soil]	6	6	1644.81	3.4776	0.0047
Flv Conc.[Soil,Flavonoids]	27	27	7626.001	3.5830	<0.0001
^{14}C-pyrene in H_2O (%)					
Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob>F**
Soil	2	2	1.79079	0.4958	0.6113
Flavonoids[Soil]	6	6	6.958321	0.6422	0.6961
Flv Conc.[Soil,Flavonoids]	27	27	48.961803	1.0041	0.4764
^{14}C-pyrene Metabolites in H_2O (%)					
Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob>F**
Soil	2	2	6.810434	1.1823	0.3128
Flavonoids[Soil]	6	6	9.437219	0.5461	0.7713
Flv Conc.[Soil,Flavonoids]	27	27	75.164413	0.9666	0.5233

[1] Source = the name of the effects in the model.

[2] Nparm = the number of parameters associated with the effect.

[3] DF = the degrees of freedom for the effect test..

[4] Sum of squares = the sum of squares for the hypothesis that the listed effect is zero.

[5] F ratio = the F statistic for testing that the effect is zero, equals to the ratio of the mean square for the effect divided by the mean square for error

[6] Prob>F =the significance probability for the F ratio, given that the null hypothesis is true. A value of less than 0.0005 represents a probability that is conceptually zero

The screening includes a multiple factor analysis of variance that interprets the measurement data by breaking down the variances into each item in the model. F statistic tests were performed and the results indicated the following probabilities at 95% confidence level.

1) Soil types had main effects on all the five PAH-fate mechanisms.
2) Flavonoid type had main effects on water phase PAH and metabolites
3) Flavonoid type had no effects on PAH mineralization, soil incorporation, or adsorption.
4) Flavonoid concentration had main effects on all PAH-fate mechanisms except adsorption.

The JMP® Statistics output report of fit model screening include model prediction profiles. The prediction profiles show how the predicted values for each of the five PAH fate mechanisms changes when one of the three factors (soil type, flavonoid type, and flavonoid concentration) changes while the other two are held constant. An example of the model screening prediction file is presented in Figures 4.5. The Y axis is the predicted values of ^{14}C-B[a]P fate measurements and the X axis stands for the testing variable of the three factors. For a predicted value, 95% confidence interval is shown by error bars. The vertical red line can be moved to hold a variable (factor) at a constant level to predict the responses to any combination of the three factors. The horizontal green line shows the predicted responses when the red lines hold the variables constant. The predicted response (fate data) changes as one variable changes while the others are held constant. A matrix of 15 prediction profiles are included in both left and right halves of Figure 4.5, respectively. The 1st column shows the effects of soil types. B[a]P fate changed as soil type changed with 0.1 uM flavone added. The 2nd column shows the effects of flavonoid types. B[a]P fate changed as the types of flavonoid changed when the flavonoid concentration added was held at 0.1 uM. The 3rd column shows the effects of flavonoid concentration. In Bermudagrass soil, ^{14}C-B[a]P fate changed as flavone concentration changed from 0 to 100 uM. Likewise, the effects of multifactors on B[a]P fate are predicted in the right half of Figure 4.5, as the flavonoid type was morin instead of flavone. Complete sets of JMP® output model screening prediction profiles are presented in Appendices C-3 and C-4, for ^{14}C-B[a]P and ^{14}C-pyrene fate data, respectively.

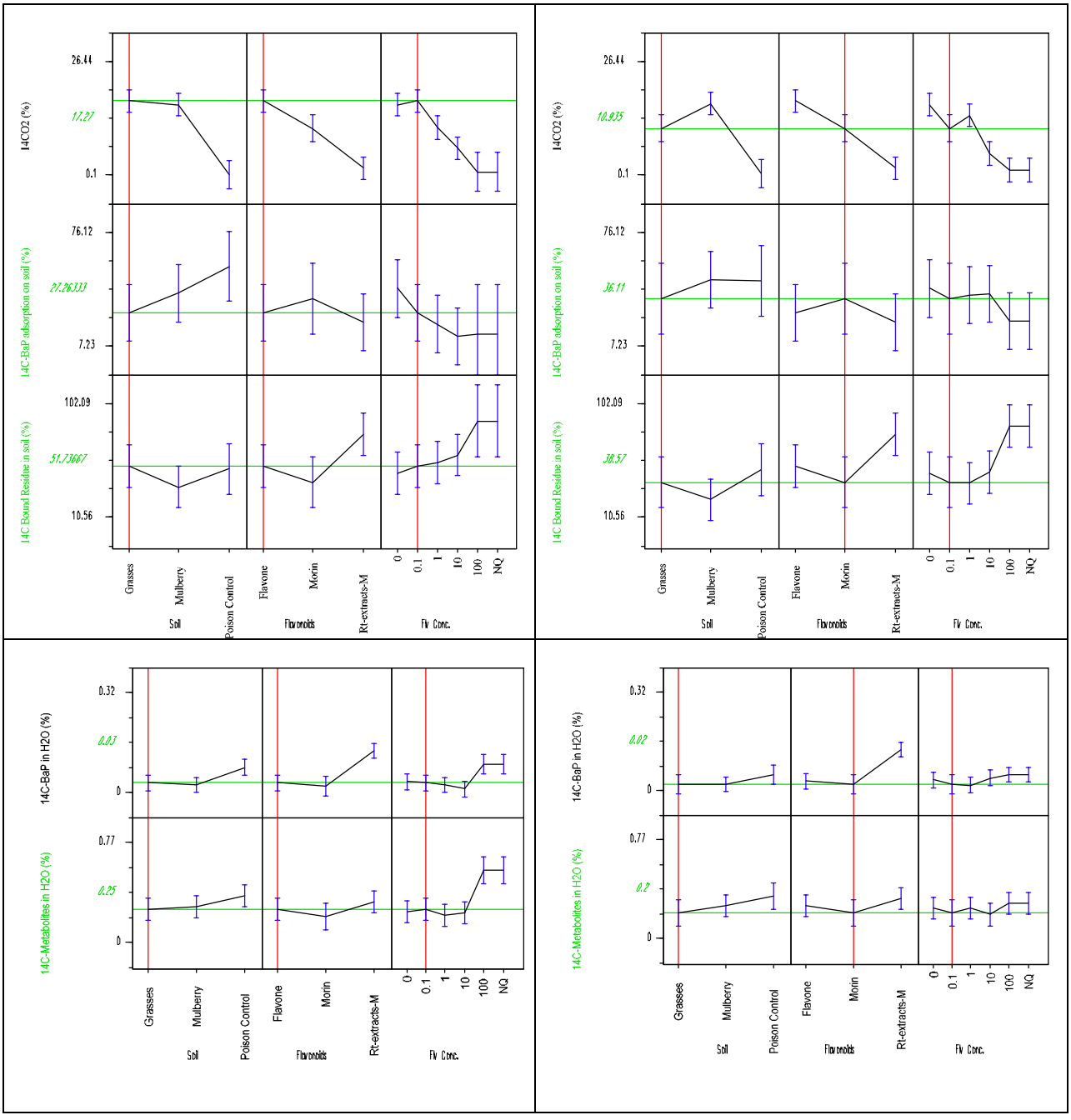


Figure 4.5. An example of multiple factor effect test prediction profile

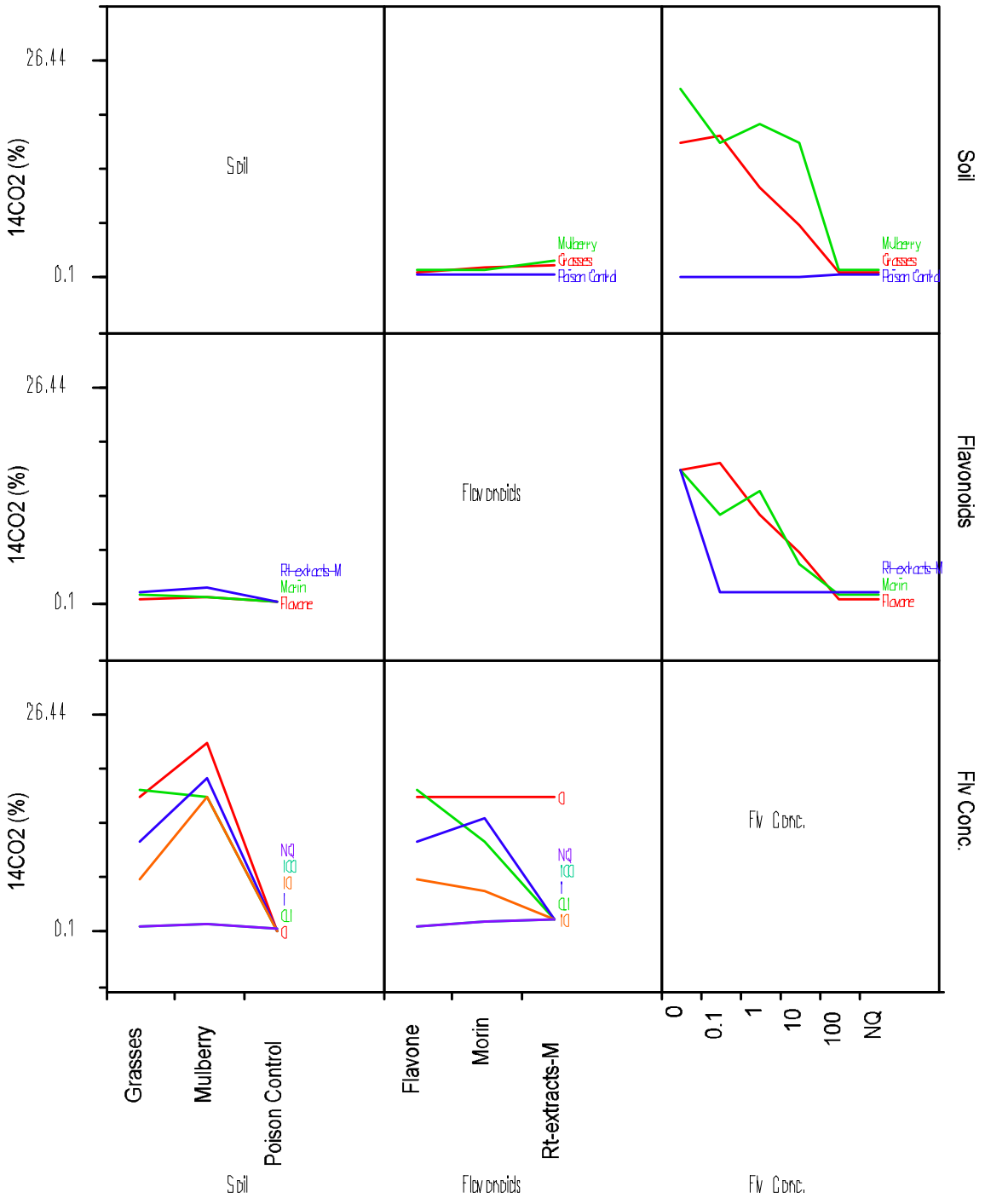


Figure 4.6. An example of multiple factor interaction profiles: $^{14}CO_2$ (%) evolution

The importance of a factor can be assessed to some extent by the steepness of the prediction trace. However, caution must be taken, when assessing multiple factor effects. The effect can be misleading if one factor is interacted with another factor. The traces in the prediction file would shift their slope because of interaction, thus predicting misleading results. In Figures 4.6, a matrix of interaction profiles for $^{14}CO_2$ evolution (i.e., mineralization, one of the B[a]P fate mechanisms) is presented for each two-factor effect. Nonparallel lines indicate the presence of interactions. For example, the upper right profile in Figure 4.6 shows the effect of soil type on CO_2 evolution was very small with high flavonoid concentrations, but it diverged widely with low or zero flavonoid concentration. The interaction of soil with flavonoid concentration complicated the effect of soil as a main effect on CO_2 evolution. Complete sets of interaction profiles including other PAH fate mechanisms (bound residue formation, adsorption, water leaching of PAH and metabolites) are presented in Appendices C-5 and C-6 for ^{14}C-B[a]P and ^{14}C-pyrene fate data, respectively. A visual observation on all the interaction profiles indicates:

1) Major interactions exist between soil type and flavonoid concentration for mineralization and soil bound residue formation mechanisms.
2) Interactions between flavonoid type and soil or flavonoid concentration were minor or none.
3) Notable interactions were not observed for adsorption mechanism.

Although the interaction profiles for water phase B[a]P and metabolites show some nonparallel lines, all the measurement levels were too low for a meaningful assessment. Water phase fraction were mostly less than 0.5% (<90 dpm), which was within five times of the background level (20 dpm). To determine statistical differences more specifically between individual flavonoid concentration level and nonflavonoid treatments for each soil, one way analysis of variance was further conducted.

One Way Analysis of Variance (ANOVA): Paired Comparison of Mean

Multiple factor effect screening indicated that flavonoid effects on PAH fate were dependent on soil type and flavonoid concentrations. One way analysis of variance was further performed to determine the significant effects of individual flavonoid concentration level per flavonoid type per soil. Paired comparison were conducted with each flavonoid concentration level to without flavonoid amendment in respect to each of the three soils for the five PAH-fate parameters, respectively. Those fate parameters are CO_2 production, soil bound residue formation, soil adsorption, water phase parent PAH, and water soluble metabolites. Subsequently, paired comparison among the three soils in respect to fixed flavonoid concentration levels were further conducted to address the effects of soil characteristics on PAH fates. Statistical significant differences were determined by *Student's t* test, with regard to whether the absolute differences between the two triplicate means was greater than the LSD (least significant difference) at 95% confidence level.

Results of the one-way ANOVA of flavonoid effects are summarized in Table 4.8. A summary of one-way ANOVA of soil effects at individual flavonoid concentrations was presented in Table 4.9. For each pair comparison, a "yes" or "no" notation shown in the summary table indicates the presence or absence of statistically significant difference at 95% confidence level. The original JMP® statistics output reports can be found in Appendix D. In the subsequent subsection, ^{14}C-B[a]P fate data and statistical analysis for each fate mechanism are presented as functions of soil types, flavonoid types, and flavonoid concentrations. All the statistical significance described in the following section is meant at 95% confidence level.

Table 4.8. Summary of the statistically significant effects of individual flavonoid concentrations on PAH fate in soil-slurry microcosms

One-Way ANOVA: Significant Differences between with Flavonoid and without Flavonoid

Factors: (1) Soil types			Poisoned Loamy Sand Mulberry Rhizosphere Soil									Loamy Sand Mulberry Rhizosphere Soil									Sandy Clay Loam Bermudagrass Rhizosphere Soil								
(2) Flavonoids			Flavone				Morin				Mulberry root extract	Flavone				Morin				Mulberry root extract	Flavone				Morin				Mulberry root extract
(3) Flav. Conc. (μM)			0.1	1	10	100	0.1	1	10	100	Not quantified	0.1	1	10	100	0.1	1	10	100	Not quantified	0.1	1	10	100	0.1	1	10	100	Not quantified
Measurements:		**Spike**																										
PAHs	*Mineralization:*	^{14}C-Pyrene	No	No	No	No	No	No	No	No	No	No	No	No	Yes (-)	No	No	No	Yes (-)	Yes (-)	No	No	No	Yes (-)	No	No	No	Yes (-)	Yes (-)
Removed,	$^{14}CO_2$ Production	^{14}C-B[a]P	No	No	No	No	No	No	No	No	No	Yes (-)	No	Yes (-)	Yes (-)	Yes (-)	No	Yes (-)	Yes (-)	Yes (-)	No	Yes (-)	Yes (-)	Yes (-)	Yes (-)	No	Yes (-)	Yes (-)	Yes (-)
Detoxified or	*Soil Bound Residues:*	^{14}C-Pyrene	No	No	No	No	No	No	No	No	No	No	No	No	Yes (+)	No	No	No	No	No	No	No	No	Yes (+)	No	No	No	Yes (+)	Yes (+)
Nonextractable	EAc Nonextractable ^{14}C in soil	^{14}C-B[a]P	No	No	No	No	No	No	No	No	No	No	No	No	Yes (+)	No	No	No	No	Yes (+)	No	No	No	Yes (+)	No	No	No	Yes (+)	Yes (+)
PAHs Remaining	*Solvent Extractable PAH in soil:*	^{14}C-Pyrene	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes (+)	Yes (+)	No	No	No	No	No	No	No	Yes (+)	No
in Soil (Extractable)	Ethylacetate Extractable ^{14}C in soil phase	^{14}C-B[a]P	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
PAHs and	*Water Phase Parent PAH:*	^{14}C-Pyrene	No	No	No	No	No	No	No	No	Yes (+)	No	No	No	No	No	No	No	No	Yes (+)	No	No	No	No	No	No	No	Yes (+)	Yes (+)
Metabolites	Hexane Extractable ^{14}C in Water Phase	^{14}C-B[a]P	Yes (+)	No	No	No	No	No	No	No	Yes (+)	No	No	No	No	No	No	No	No	Yes (+)	No	No	No	No	No	No	No	No	Yes (+)
in Water	*Water Soluble Metabolites:*	^{14}C-Pyrene	No	No	No	No	No	No	No	No	Yes (+)	No	No	No	Yes (-)	No	No	Yes (-)	Yes (-)	Yes (+)	No	No	No	No	No	No	No	No	No
Phase	Hexane Nonextractable ^{14}C in Water Phase	^{14}C-B[a]P	No	No	No	No	No	No	No	No	Yes (+)	No	No	No	No	No	No	No	Yes (+)	Yes (+)	No	No	No	Yes (+)	No	No	No	No	No

No = Significant higher between with and without flavonoid additives at 95% confidence level

Yes (+) = With flavonoid additive the measurement was significantly higher than that without flavonoidadditive at 95%

Yes (-) = With flavonoid additive the measurement was significantly lower than that without flavonoid additive at 95% confidence

Table 4.9. Summary of the statistically significant effects of soil types on B[a]P fate in soil-slurry microcosms with or without flavonoids added

One-Way ANOVA: Significant Different Effects on PAH Fate between Soils at a Fixed Flavonoid Concentration															
Fixed Flavonoid Concentration			None of Flavonoids			100 uM of Morin			100 uM of flavone			Mulberry Root Extract		
Paired Comparison:		Soil A	Loamy Sand Mulberry	Sandy Clay Loam Bermudagrass		Loamy Sand Mulberry	Sandy Clay Loam Bermudagrass		Loamy Sand Mulberry	Sandy Clay Loam Bermudagrass		Loamy Sand Mulberry	Sandy Clay Loam Bermudagrass	
Response in Soil A Significantly Greater (+) or Less (-) than that in Soil B?		Soil B	Poisoned Loamy Sand Mulberry		Loamy Sand Mulberry	Poisoned Loamy Sand Mulberry		Loamy Sand Mulberry	Poisoned Loamy Sand Mulberry		Loamy Sand Mulberry	Poisoned Loamy Sand Mulberry		Loamy Sand Mulberry
Measurements (Response):		**Spike**												
PAHs removed detoxified or nonextractable	*Mineralization:*	^{14}C-Pyrene	Yes (+)	Yes (+)	Yes (+)	No	Yes (+)	Yes (+)	No	No	No	No	No	No
	$^{14}CO_2$ Production	^{14}C-B[a]P	Yes (+)	Yes (+)	Yes (-)	No	Yes (+)	No	No	No	No	No	No	No
	Soil Bound Residues	^{14}C-Pyrene	No	No	No	No	No	No	No	No	No	No	No	No
	EAc nonextractable ^{14}C in soil	^{14}C-B[a]P	No	No	No	No	Yes (+)	Yes (+)	Yes (+)	Yes (+)	Yes (+)	No	Yes (+)	No
PAHs and metabolites in water phase	*Water Phase parent PAH:*	^{14}C-Pyrene	No	No	No	No	No	No	No	No	Yes (+)	No	No	No
	Hexane extractable ^{14}C in Water phase	^{14}C-B[a]P	Yes (-)	No	No	Yes (-)	Yes (-)	No	Yes (-)	Yes (-)	Yes (+)	Yes (-)	Yes (-)	No
	Water Phase Metabolites:	^{14}C-Pyrene	Yes (+)	Yes (+)	Yes (-)	No	Yes (+)	No	No	No	No	No	No	No
	Hexane nonextractable ^{14}C in Water phase	^{14}C-B[a]P	No	No	No	No	No	No	No	No	Yes (+)	No	Yes (-)	Yes (-)
PAHs remaining in Soil (extractable)	*Solvent Extractable PAH in soil:*	^{14}C-Pyrene	Yes (-)	Yes (-)	No	No	No	No	No	No	No	No	No	No
	Ethylacetate extractable ^{14}C in soil phase	^{14}C-B[a]P	No	No	No	Yes (-)	Yes (-)	No	Yes (-)	Yes (-)	No	Yes (-)	Yes (-)	No

No = Significant higher between with and without flavonoid additives at 95% confidence level

Yes (+) = With flavonoid additive the measurement was significantly higher than that without flavonoidadditive at 95% confidence level

Yes (-) = With flavonoid additive the measurement was significantly lower than that without flavonoid additive at 95% confidence level

^{14}C-B[a]P Mineralization in Soil Slurry Microcosms

^{14}C-B[a]P Mineralization in Poisoned-Mulberry-Rhizosphere Soil In Figures 4.7, $^{14}CO_2$ evolution from 7,10-^{14}C-B[a]P in poisoned-Mulberry-rhizosphere-soil-slurry microcosms is plotted versus flavonoid concentrations amended in the soil slurry. The X-axis represents flavonoid concentrations ranging from 0, 0.1 uM, 1 uM, 10 uM to 100 uM (micromole) and a separate category of nonquantified Mulberry root extracts. The Y-axis represents the ^{14}C counts as percentage of the total 7,10-^{14}C-B[a]P added into a microcosm. The amount of 7,10-^{14}C-B[a]P added was approximately 17318 dpm that is equivalent to 0.1 ug/g-soil or 0.01 ug/ml-water. In Figure 4.7, the data points represent the mean of triplicate microcosm data. The maximum and minimum of the triplicate data are displayed as Y bars. These plotting rules are applied in all the subsequent charts of this section.

Flavonoid concentrations in Mulberry root extract, which contains a variety of simple and complex flavonoids as well as other root exudates, were not quantified. However, the total organic carbon concentration in the Mulberry root extract was measured as 885 mg/l (Table 3.4), much higher than that of 100 uM flavone or morin (18 mg/l). Also, Mulberry root extract has a BOD_5 (5 day biological oxygen demand) of 1,660 mg/l and a COD (chemical oxygen demand) of 5,000 mg/l, while the theoretical oxygen demand (ThOD) for 100 uM flavone or morin is less than 50 mg/l. The total phenolics concentration in the Mulberry root extract was measured as 0.131 mg/l. The low phenolic concentration may or may not indicate a low hydroxylated flavonoid concentration, as flavonoids may present as glycosides or binding together via ether and hydrogen bonds.

$^{14}CO_2$ productions under "abiotic" conditions were all below or close to 1% of the total B[a]P-7,10-^{14}C spike (Figure 4.7). There are no statistical significant differences between with flavonoids and without flavonoids (Table 4.8 and Appendix D-1). As a result, abiotic B[a]P mineralization was negligible.

^{14}C-B[a]P Mineralization in Mulberry-Rhizosphere Soil In Figure 4.8, $^{14}CO_2$ production from B[a]P-7,10-^{14}C in Mulberry-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extract. Without flavonoid, the $^{14}CO_2$ production was about 22% of the total 7,10-^{14}C-B[a]P added. $^{14}CO_2$ production decreased to between 15% and 18% as flavone and morin concentration increased to 0.1-10 μM. However, as flavone and morin concentrations increased to 100 μM, $^{14}CO_2$ production reduced significantly to about 1%. Similarly $^{14}CO_2$ production reduced significantly to about 2% as mulberry root extract was added. The amounts of $^{14}CO_2$ production with all concentration levels of flavone and morin as well as Mulberry root extract in Mulberry soil were statistically significantly lower than that without flavonoid except for 1uM flavone and 1 uM morin (see Table 4.8 and Appendix D-2).

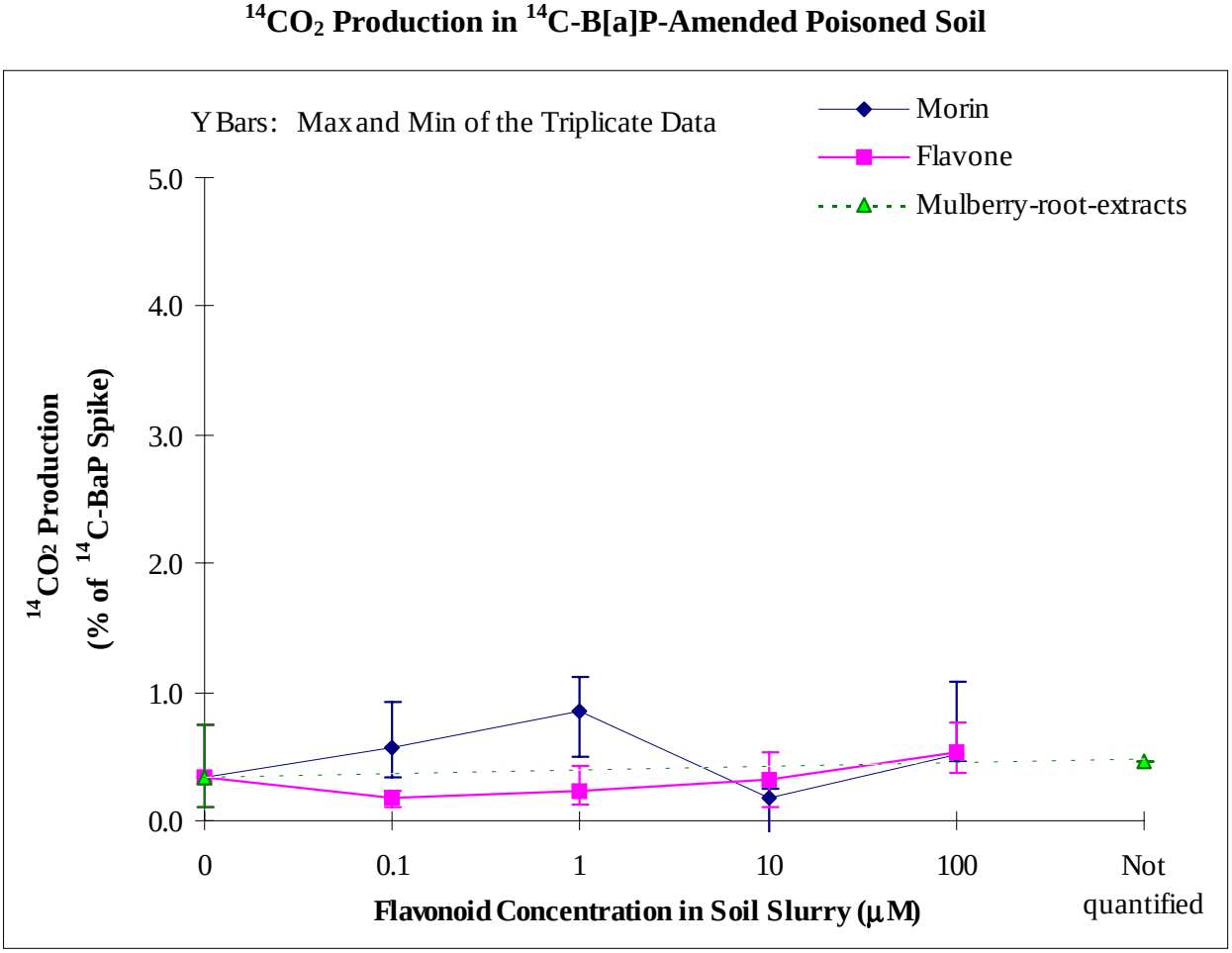


Figure 4.7. ^{14}C-B[a]P (%) mineralization to $^{14}CO_2$[1] versus flavonoid concentrations amended in poisoned[2]-control-Mulberry-rhizosphere-soil-slurry microcosm

1 $^{14}CO_2$ was trapped by a chromatography filter strip soaked in potassium hydroxide

[2] poisoned microcosm simulate metabolic inhibited pseudo-abiotic condition

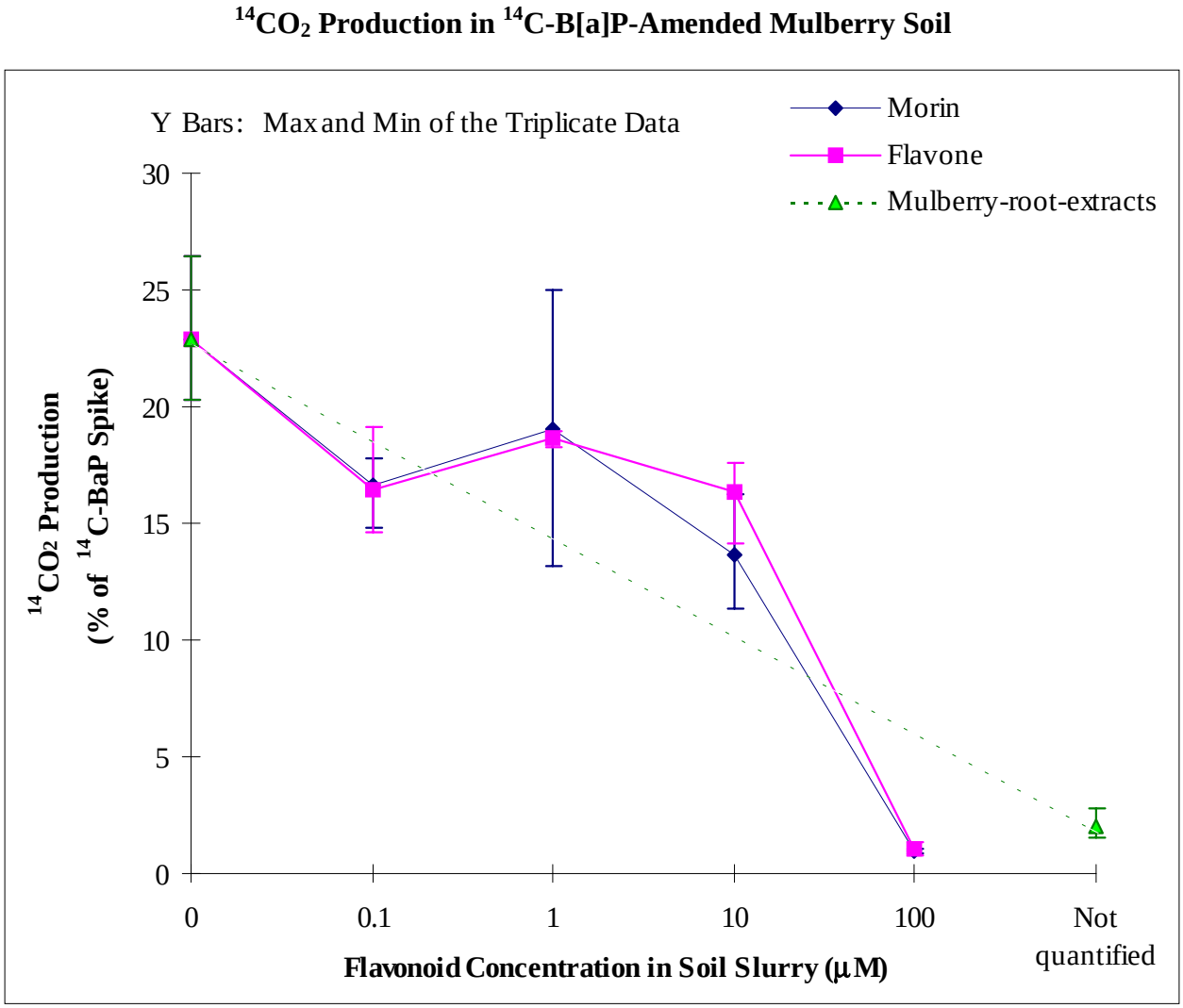


Figure 4.8 ^{14}C-B[a]P (%) mineralization to $^{14}CO_2$[1] versus different flavonoid concentration levels amended in Mulberry-rhizosphere-soil-slurry microcosm

1 $^{14}CO_2$ was trapped by a chromatography filter strip soaked in potassium hydroxide

^{14}C-B[a]P Mineralization in Bermudagrass-Rhizosphere Soil In Figure 4.9, $^{14}CO_2$ production from 7,10-^{14}C- B[a]P in Bermudagrass-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extracts amended in the soil slurry. Without flavonoid the $^{14}CO_2$ production was approximately 17% of the total 7,10-^{14}C- B[a]P added. $^{14}CO_2$ production remained at 11% - 16% as flavone and morin concentration increased to between 0.1 and 1 μM. As flavone and morin concentration increased to 10 μM $^{14}CO_2$ production decreased to about 5% - 6%. As 100 μM flavone, 100 μM morin, or mulberry-root extracts was added, $^{14}CO_2$ production reduced significantly to between 1% and 2%. The amounts of $^{14}CO_2$ production with all concentration levels of flavone and morin as well as Mulberry root extract in Bermudagrass soil were statistically significantly lower than that without flavonoid except for 0.1uM flavone and 1 uM morin (Table 4.6 and Appendix B-5).

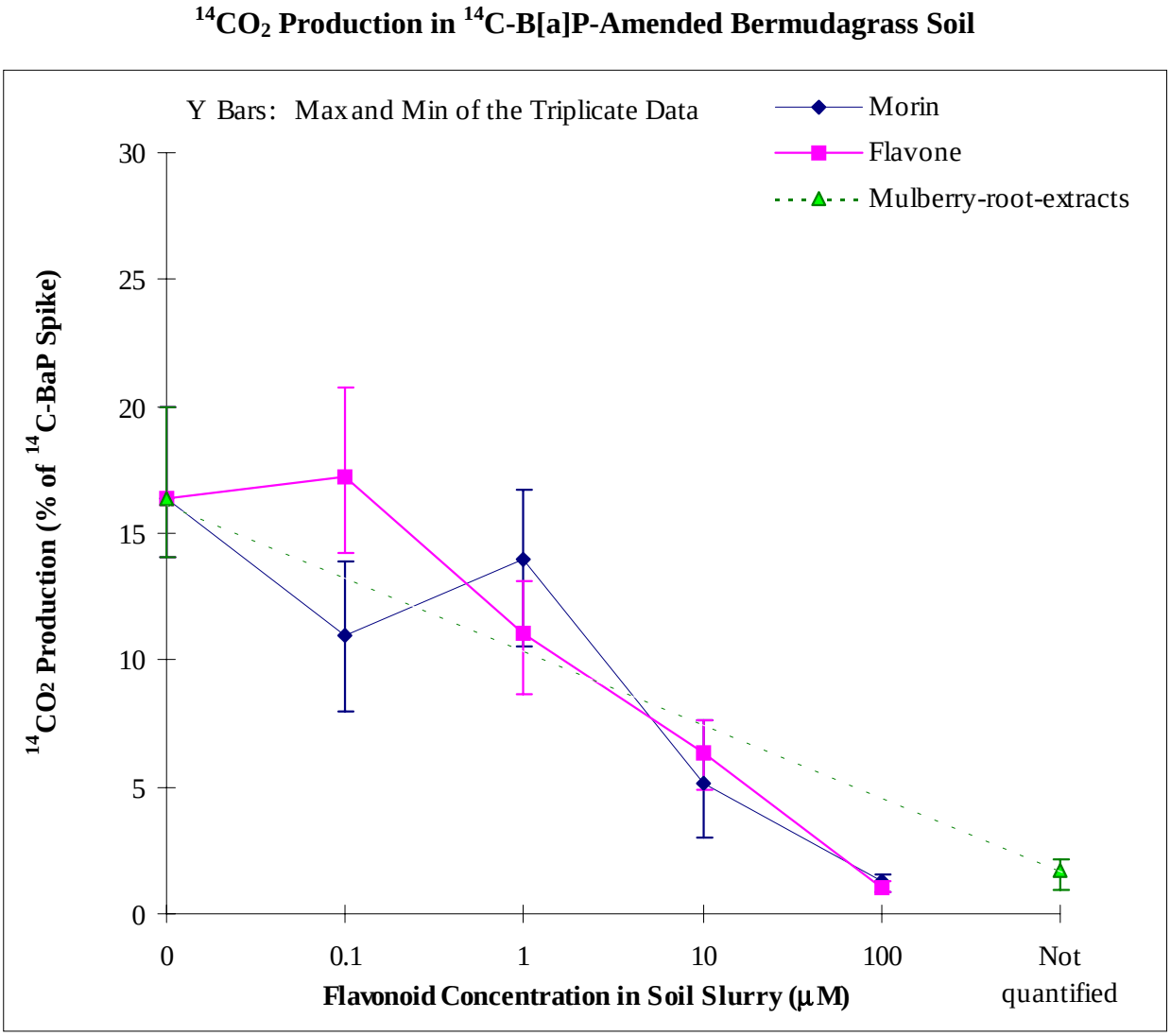


Figure 4.9. ^{14}C-B[a]P (%) mineralization to $^{14}CO_2$[1] versus different flavonoid concentration levels amended in Bermudagrass-rhizosphere-soil-slurry microcosm

[1] $^{14}CO_2$ was trapped by a chromatography filter strip soaked in potassium hydroxide

^{14}C-B[a]P Bound Residue Formation in Soil Slurry Microcosms

^{14}C-B[a]P Bound Residues in Poisoned Mulberry Rhizosphere Soil ^{14}C-bound residues were measured by ethylacetate-nonextractable ^{14}C in soil. ^{14}C bound reside can be either parent ^{14}C-B[a]P diffused into soil micropores or B[a]P metabolites covalently binding to soil humus. In Figure 4.10, 7,10-^{14}C-B[a]P bound residues in poisoned-Mulberry-soil-slurry microcosms are presented versus flavonoid concentration of flavone, morin, and mulberry-root-extracts amended in the soil slurry. Without flavonoid amendment ^{14}C-B[a]P bound residues were approximately 53% of the total 7,10-^{14}C-B[a]P added. ^{14}C-B[a]P bound residues increased somewhat to approximately 65% as morin concentration increased to between 1 uM and 10 uM. In contrast, ^{14}C-B[a]P bound residues decreased somewhat less than 40% as 10 uM - 100 μM flavone or 100 uM morin, or mulberry-root extract was added. However, the differences were not statistically significant at 95% confidence level (Table 4.6 and Appendix B-3).

^{14}C-B[a]P Bound Residues in Mulberry Rhizosphere Soil In Figure 4.11, 7,10-^{14}C-B[a]P bound residues in Mulberry-rhizosphere-soil-slurry microcosms are presented versus flavonoid concentration of flavone, morin, and mulberry-root-extract amended in the soil slurry. Without flavonoid amendment ^{14}C bound residues were approximately 29% of the total 7,10-^{14}C-B[a]P added. As flavonoid concentration increased to between 0.1 uM and 10 μM, ^{14}C bound residues remained at similar levels. As 100 μM flavone or Mulberry-root extract was added ^{14}C bound residues increased to approximately 60%. ^{14}C bound residues was approximately 40% when 100 uM morin was added. There were no statistically significant differences in ^{14}C-B[a]P bound residue formation in Mulberry soil between with and without flavonoid, except that when 100 uM flavone or mulberry root extract was added (Table 4-6 and Appendix B-4).

^{14}C-B[a]P Bound Residues in Bermudagrass Rhizosphere Soil In Figure 4.12, 7,10-^{14}C-B[a]P bound residues in Bermudagrass-rhizosphere-soil-slurry microcosms are presented versus flavonoid concentration of flavone or morin and mulberry-root-extracts added in the soil slurry. Without flavonoid ^{14}C bound residues were approximately 47% of the total ^{14}C added. ^{14}C bound residues remained at between 40% and 50% as flavonoid concentration increased to between 0.1 and10 μM. When Mulberry-root-extract, 100 μM morin or 100 uM flavone was added, ^{14}C bound residues increased to between 75% and 85%. There were no statistically significant differences in ^{14}C-B[a]P bound residue formation in Bermudagrass soil between with and without flavonoid, when flavone and morin concentrations in soil slurry were held between 0.1 uM and 10 uM. When 100 uM flavone, 100 uM morin, or mulberry root extract was added ^{14}C-B[a]P bound residue formation in Bermudagrass soil was statistically significantly higher than that without flavonoid (Table 4.6 and Appendix B-5).

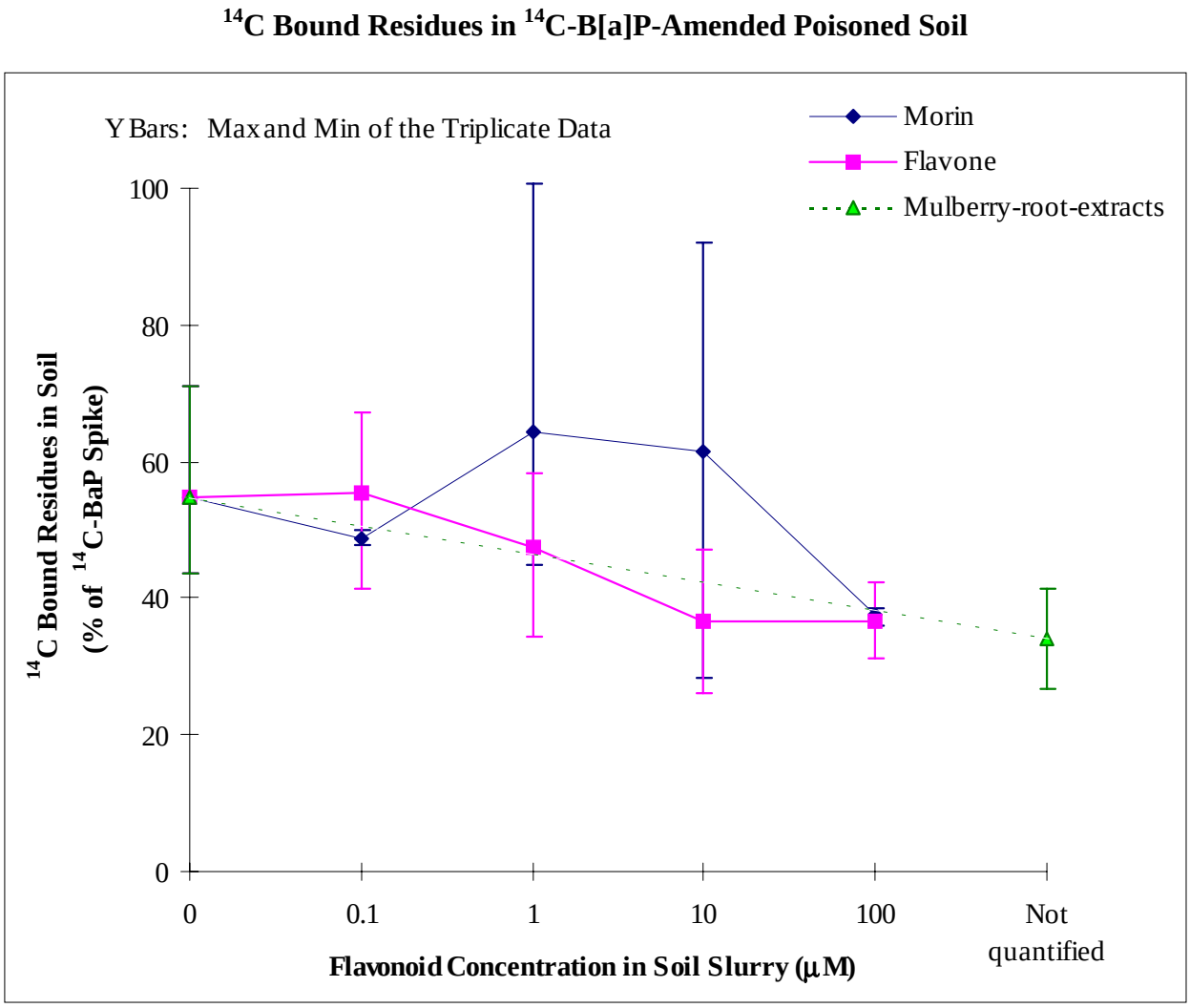


Figure 4.10. Soil bound residue formation[1] of ^{14}C-B[a]P and/or metabolites (%) versus flavonoid concentrations amended in Poisoned-Mulberry-rhizosphere-soil-slurry microcosms[2]

[1] Ethylacetate-non-extractable ^{14}C in the soil phase

[2] poisoned microcosms simulate metabolic inhibited pseudo-abiotic condition

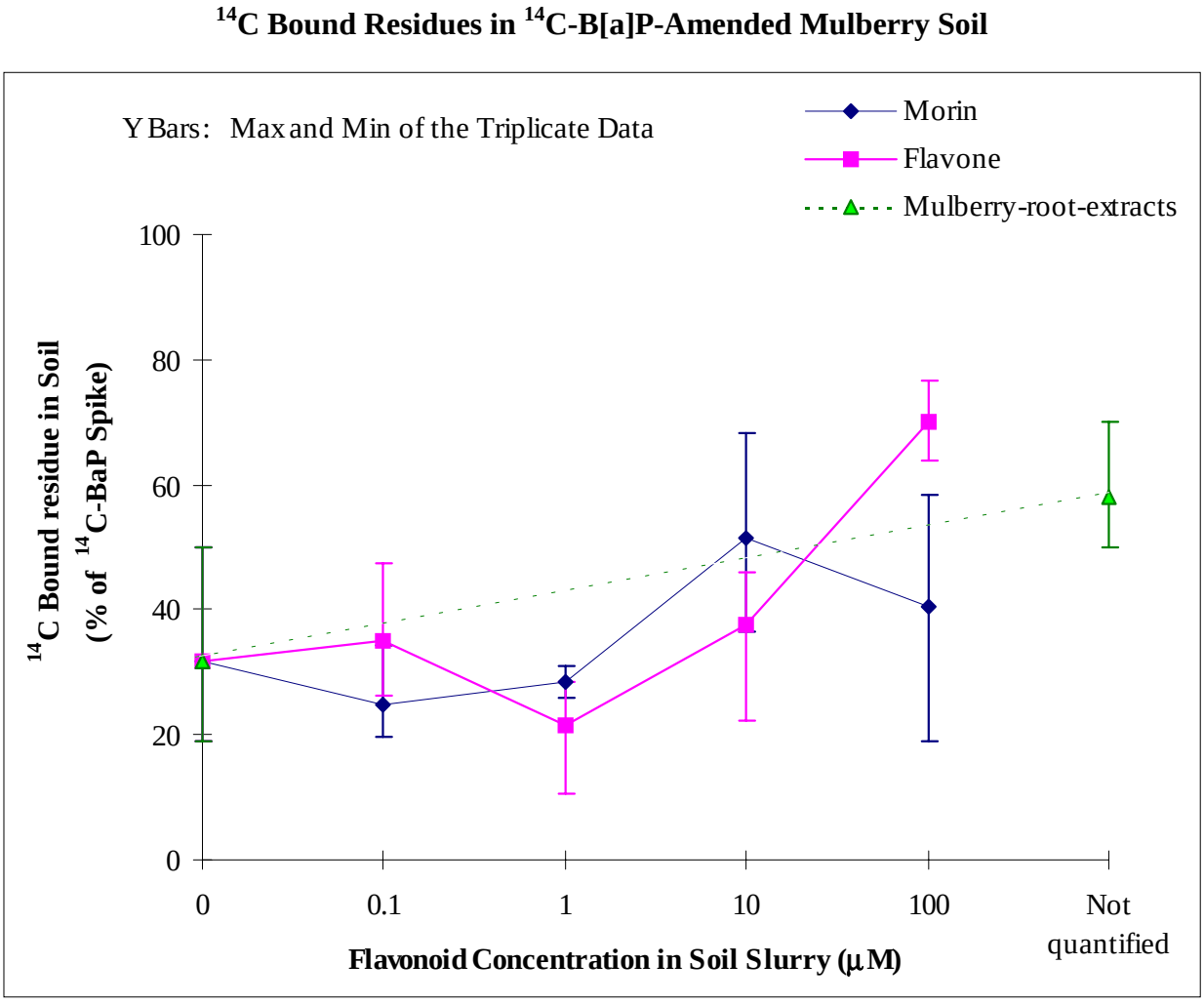


Figure 4.11. Soil bound residue formation[1] of ^{14}C-B[a]P and/or metabolites (%) versus flavonoid concentrations amended in Mulberry-rhizosphere-soil-slurry microcosms

[1] Ethylacetate-non-extractable ^{14}C in the soil phase

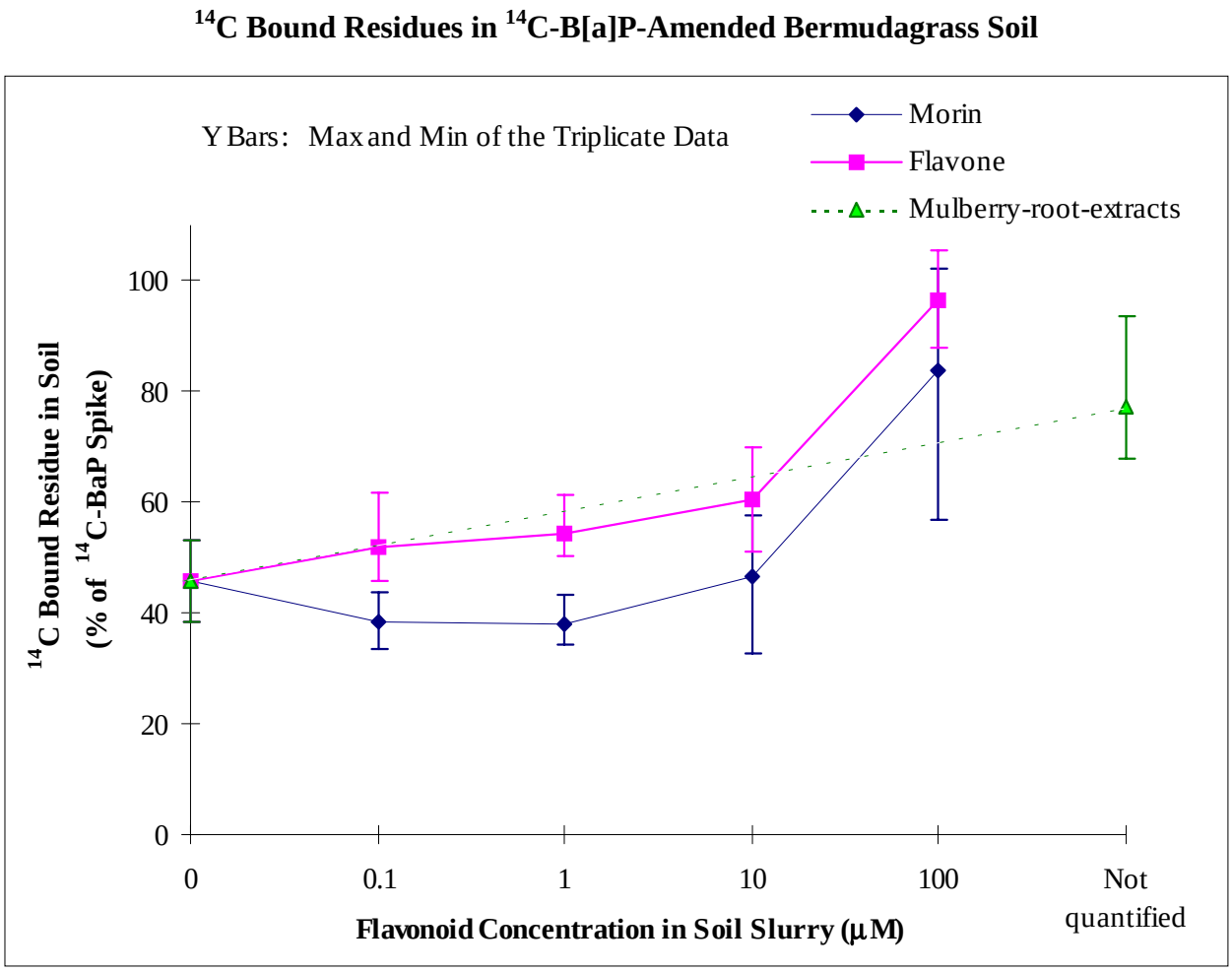


Figure 4.12. Soil bound residue formation[1] of ^{14}C-B[a]P and/or metabolites (%) versus flavonoid concentrations amended in Bermudagrass-rhizosphere-soil-slurry microcosms

[1] Ethylacetate-non-extractable ^{14}C in the soil phase

Adsorption of ^{14}C-B[a]P in Soil Slurry Microcosms

Adsorption of ^{14}C-B[a]P in Poisoned-Mulberry-Rhizosphere Soil Adsorption of ^{14}C-B[a]P was measured by ethylacetate-extractable ^{14}C in soil phase. In Figure 4.13, ethylacetate-extractable B[a]P-7,10-^{14}C in poisoned-Mulberry-rhizosphere soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin and mulberry-root-extract amended in the soil slurry. ^{14}C-B[a]P adsorption to soil ranged from 50% to 70% of the total 7,10-^{14}C-B[a]P added at all the flavonoid amendment levels. There were no statistically significant differences at 95% confidence level in ^{14}C-B[a]P adsorption to poisoned Mulberry soil between with and without flavonoid (Table 4.6 and Appendix B-3).

Adsorption of ^{14}C-B[a]P in Mulberry-Rhizosphere Soil In Figure 4.14, 7,10-^{14}C-B[a]P adsorption to Mulberry-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extracts amended in the soil slurry. Without flavonoid amendment the adsorption was approximately 40% of the total 7,10-^{14}C-B[a]P added. There were no statistically significant differences in B[a]P adsorption as flavone and morin concentration increased from 0.1 to 100 uM or with Mulberry root extract added (Table 4.6 and Appendix B-4).

Adsorption of ^{14}C-B[a]P in Bermudagrass Rhizosphere Soil In Figure 4-15, 7,10-^{14}C-B[a]P adsorption in Bermudagrass-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extracts amended in the soil slurry. Without flavonoid amendment ethylacetate-extractable B[a]P was approximately 42% of the total 7,10-^{14}C-B[a]P added. B[a]P adsorption decreased to approximately 20% when flavone concentrations increased to between 1 and 100 uM. B[a]P adsorption remained at approximately 40% when morin concentrations increased to between 0.1 uM and 10 uM, then decreased to approximately 20% as morin concentration increased to 100 uM. Also, B[a]P adsorption decreased to approximately 20% when Mulberry root extract was added. However, the decreases in B[a]P adsorption were not statistically significant at 95% confidence levels in all the cases (Table 4.6 and Appendix B-5).

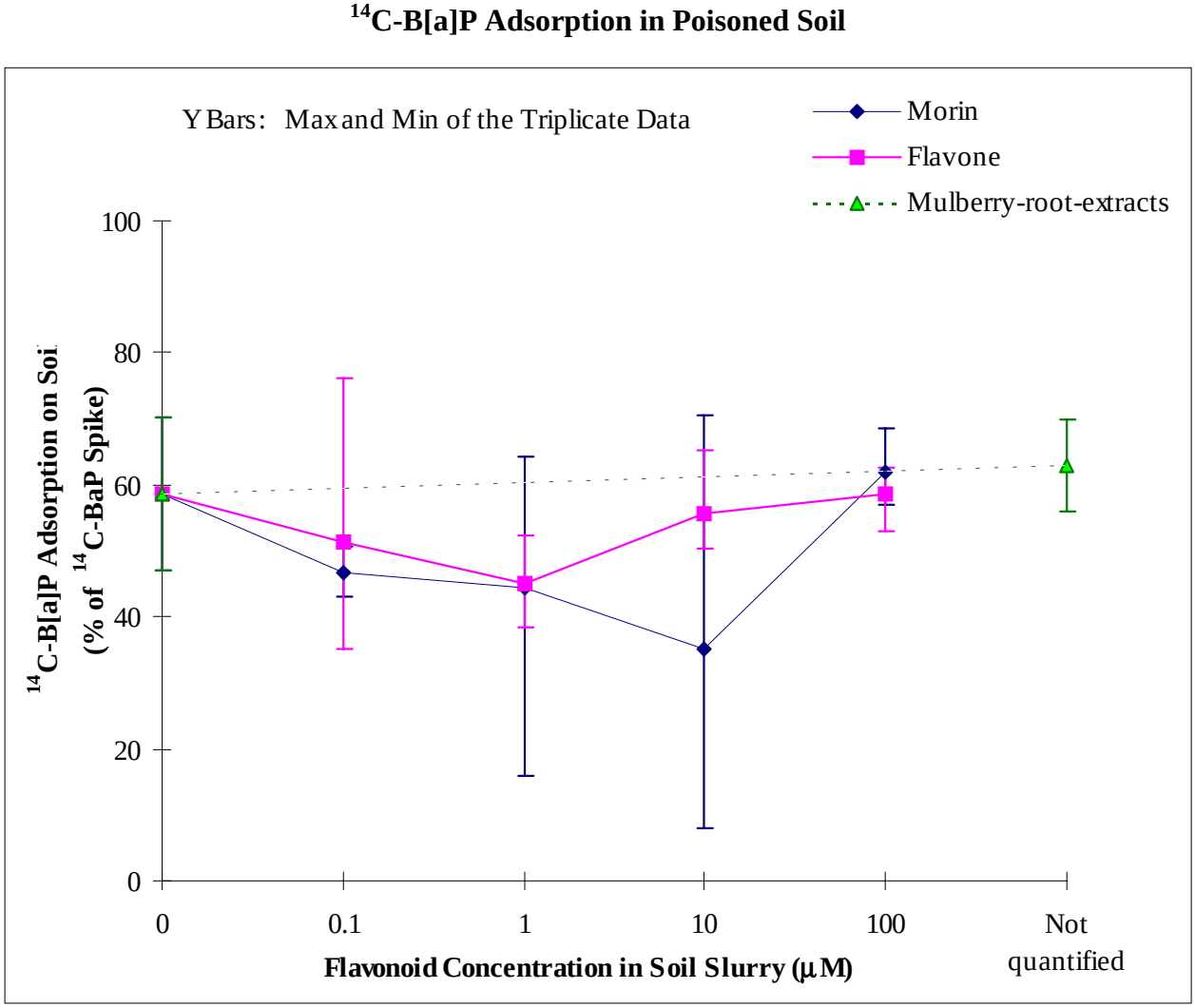


Figure 4.13. Soil adsorption[1] of ^{14}C-B[a]P (%) versus flavonoid concentrations amended in poisoned-Mulberry-rhizosphere-soil-slurry microcosm[2]

[1] ethylacetate-extractable ^{14}C in soil phase

[2] poisoned microcosms simulate metabolic inhibited pseudo-abiotic condition

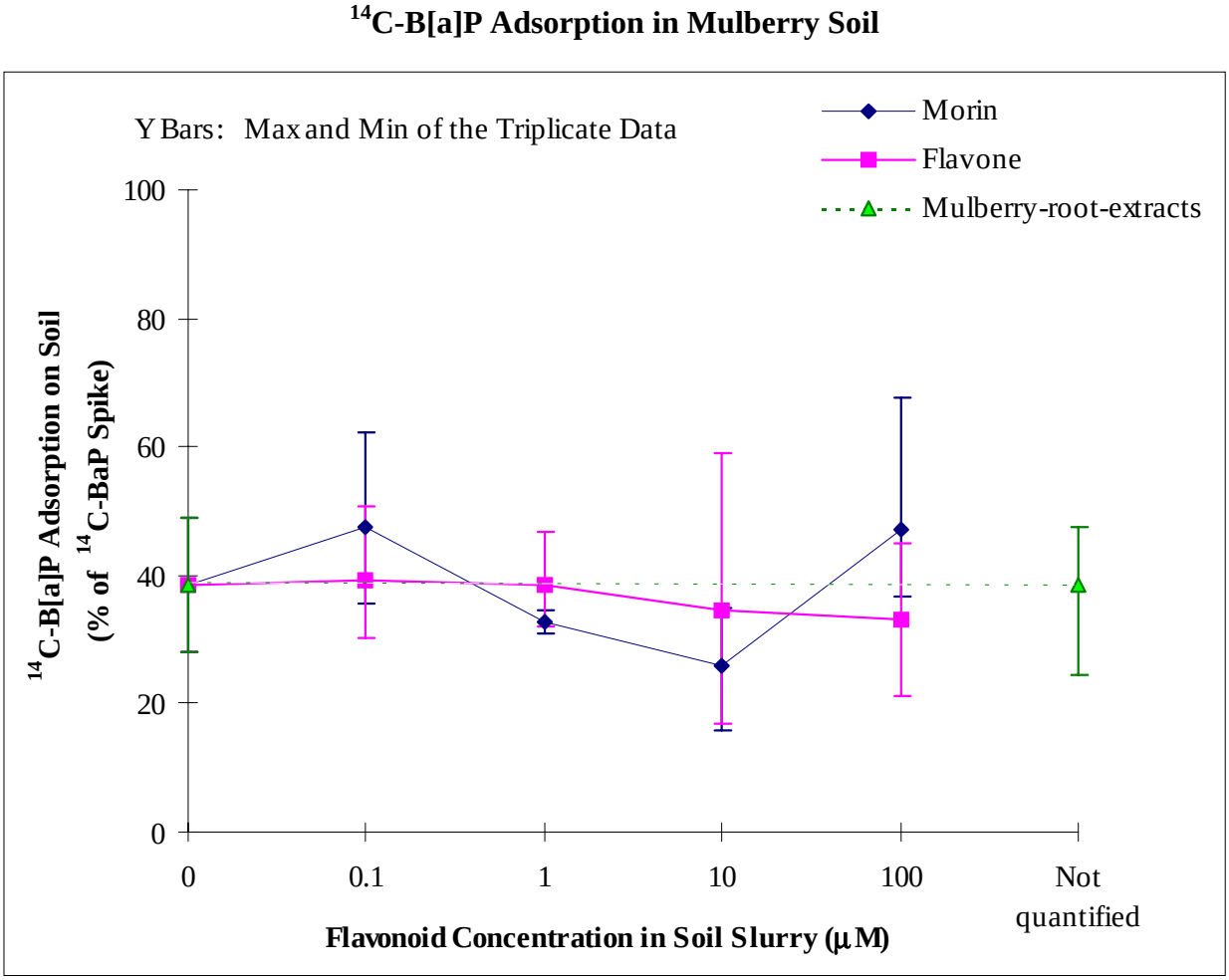


Figure 4. 14. Soil adsorption[1] of ^{14}C-B[a]P (%) versus flavonoid concentrations amended in Mulberry-rhizosphere-soil-slurry microcosm

[1] ethylacetate-extractable ^{14}C in soil phase

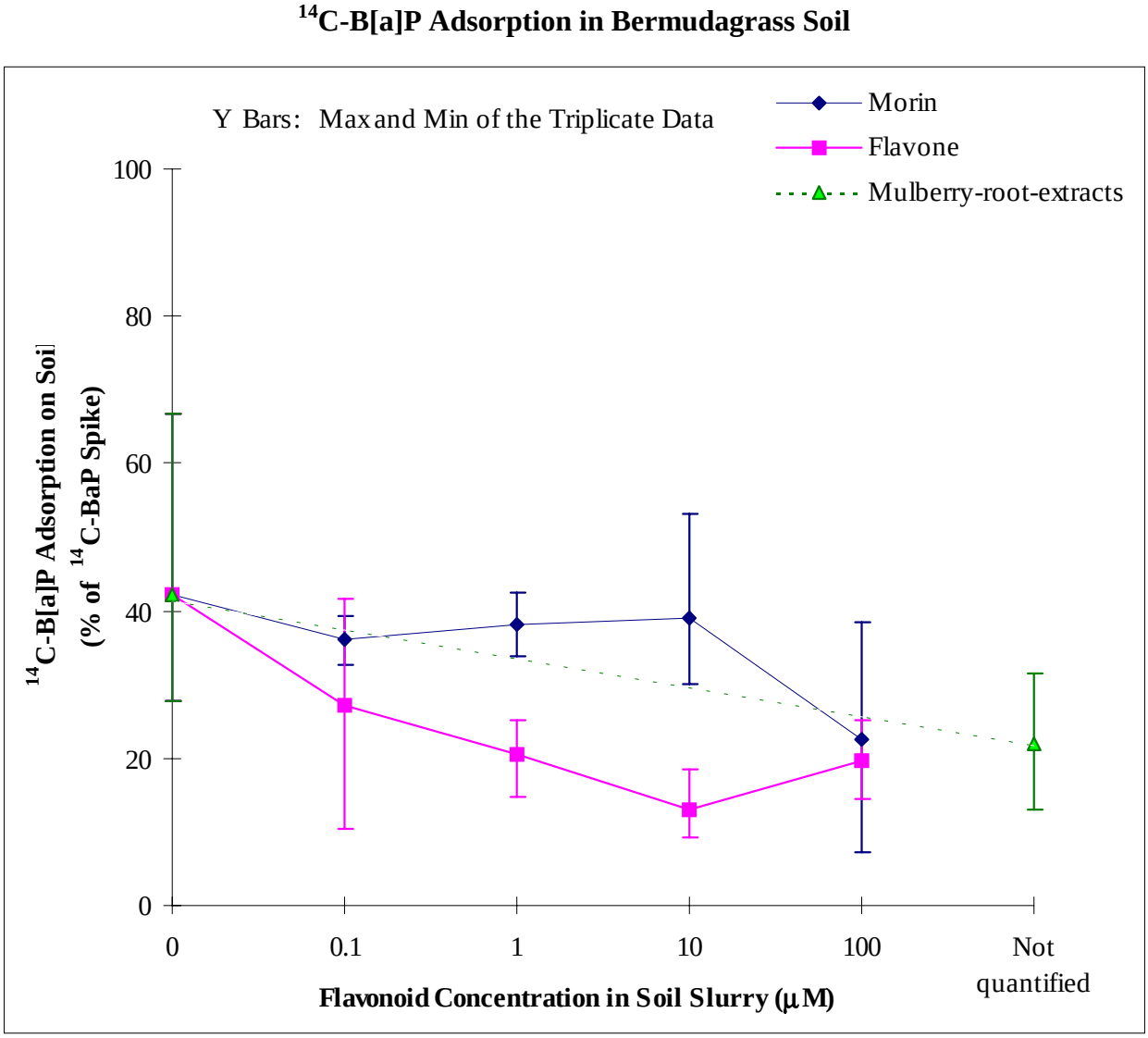


Figure 4.15. Soil adsorption[1] of ^{14}C-B[a]P (%) versus flavonoid concentrations amended in Bermudagrass-rhizosphere-soil-slurry microcosm

[1] ethylacetate-extractable ^{14}C in soil phase

Water Leaching of ^{14}C-B[a]P in Soil Slurry Microcosms

Water-phase ^{14}C-B[a]P in Poisoned Mulberry-Rhizosphere Soil Water phase ^{14}C-B[a]P was measure by hexane-extractable non-polar ^{14}C in water phase. In Figure 4.16, water phase 7,10-^{14}C-B[a]P in poisoned-Mulberry-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extracts amended in the soil slurry. Water phase B[a]P was below 0.1% of the total B[a]P-7,10-^{14}C spike at all the flavonoid amendment levels except that with mulberry-root-extract. When mulberry-root-extract was added water phase 7,10-^{14}C-B[a]P increased to approximately 0.3% (equivalent to 0.03 ug/l). The slight increase with Mulberry root extract was statistically significantly higher than that without flavonoid. Besides, there were no statistically significant differences in water phase ^{14}C-B[a]P between with and without flavone or morin except that 0.1 uM flavone was added. However, the differences were very small. In all the cases, Water-phase ^{14}C-B[a]P in poisoned-Mulberry-rhizosphere soil was negligible.

Water-phase ^{14}C-B[a]P in Mulberry-Rhizosphere Soil In Figure 4.17, water phase 7,10-^{14}C-B[a]P in Mulberry-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extracts amended in the soil slurry. Water phase B[a]P was all below 0.03% of the total 7,10-^{14}C-B[a]P added except that with mulberry-root-extract. When mulberry-root-extract was added water phase ^{14}C-B[a]P increased to approximately 0.2% (equivalent to 0.02 ug/l). There were no statistically significant differences between with and without flavonoid, except that with Mulberry root extract (Table 4.6 and Appendix B-4). In all the cases, Water-phase ^{14}C-B[a]P in Mulberry-rhizosphere soil was negligible and less than that in poisoned-Mulberry-rhizosphere soil.

Water-phase ^{14}C-B[a]P in Bermudagrass-Rhizosphere Soil In Figure 4.18, water phase 7,10-^{14}C-B[a]P in Bermudagrass-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extract amended in the soil slurry. Water phase B[a]P was below 0.05% of the total 7,10-^{14}C-B[a]P added at all the flavonoid amendment levels except that with mulberry-root-extracts. When mulberry-root-extract was added water phase 7,10-^{14}C-B[a]P increased slightly to approximately 0.1% (equivalent to 0.1 ug/l). There were no statistically significant differences between with and without flavonoid, except that with Mulberry root extract (Table 4.6 and Appendix B-4). In all the cases, water-phase ^{14}C-B[a]P in Bermudagrass-rhizosphere soil was negligible and less than that in Mulberry-rhizosphere soil.

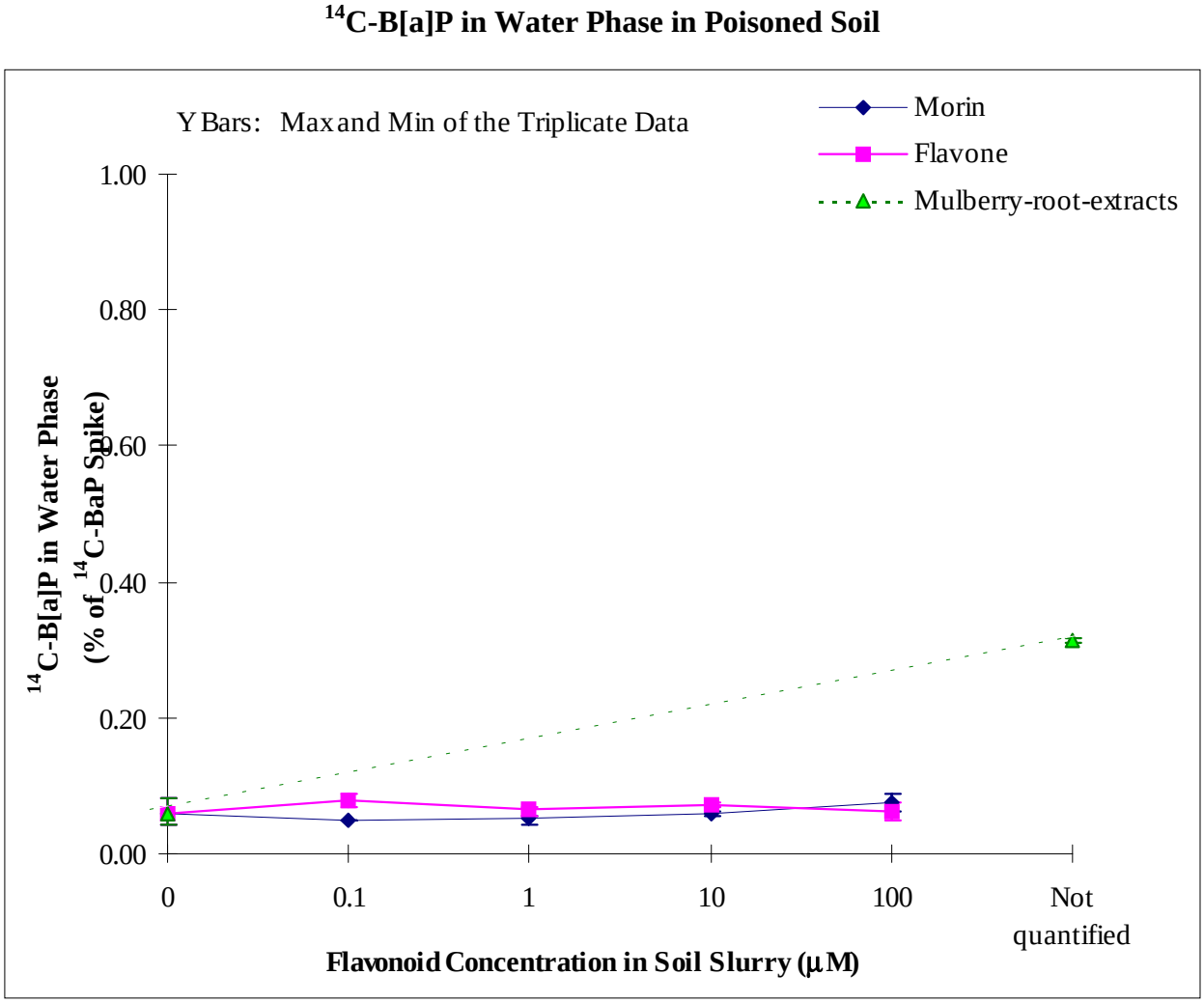


Figure 4.16. Water phase[1] ^{14}C-B[a]P (%) versus flavonoid concentrations amended in poisoned-Mulberry-rhizosphere-soil-slurry microcosm[2]

[1] hexane-extractable, nonpolar ^{14}C in water phase

[2] poisoned microcosms simulate metabolic inhibited pseudo-abiotic condition

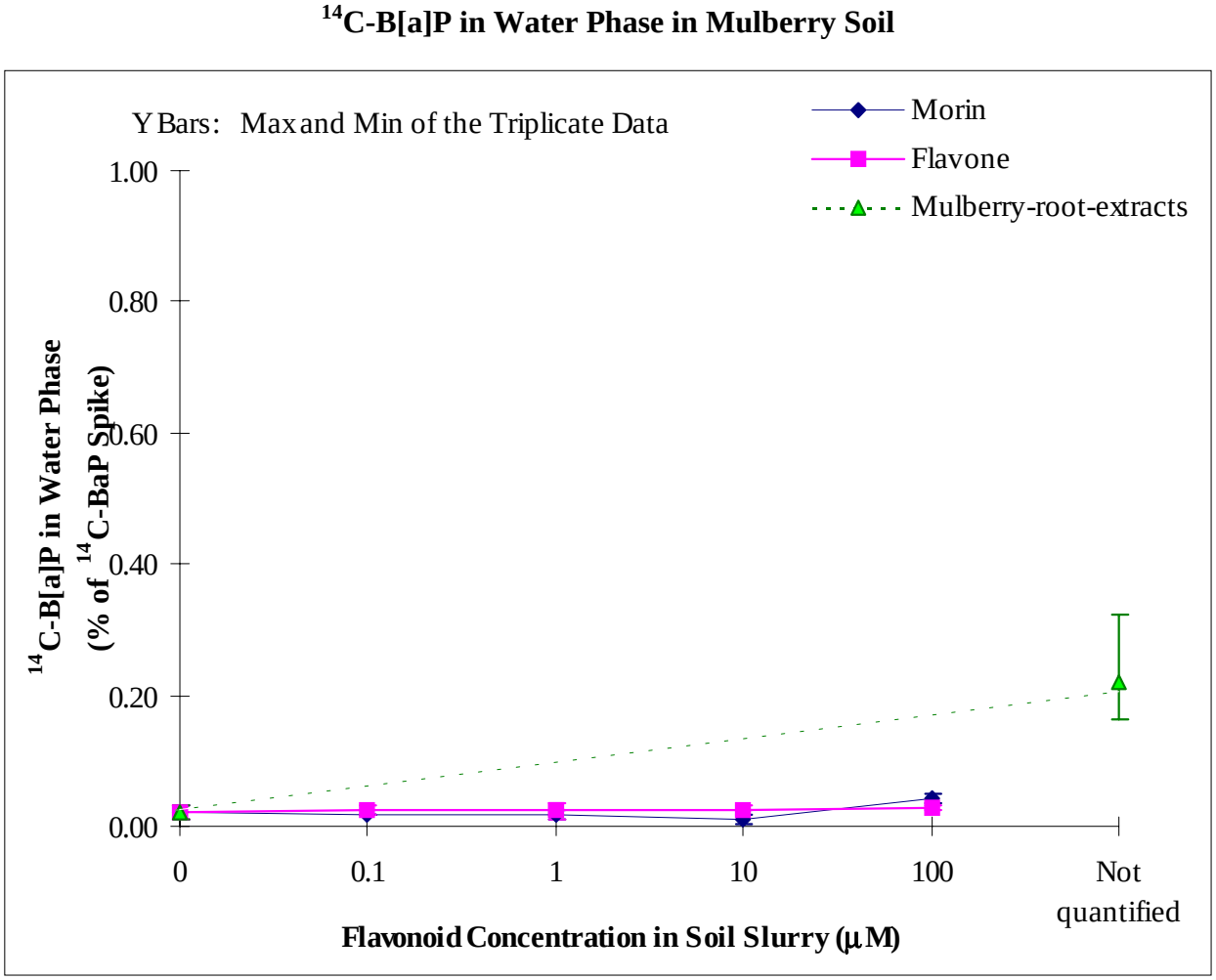


Figure 4.17. Water phase[1] ^{14}C-B[a]P (%) versus flavonoid concentrations amended in Mulberry-rhizosphere-soil-slurry microcosm

[1] hexane-extractable, nonpolar ^{14}C in water phase

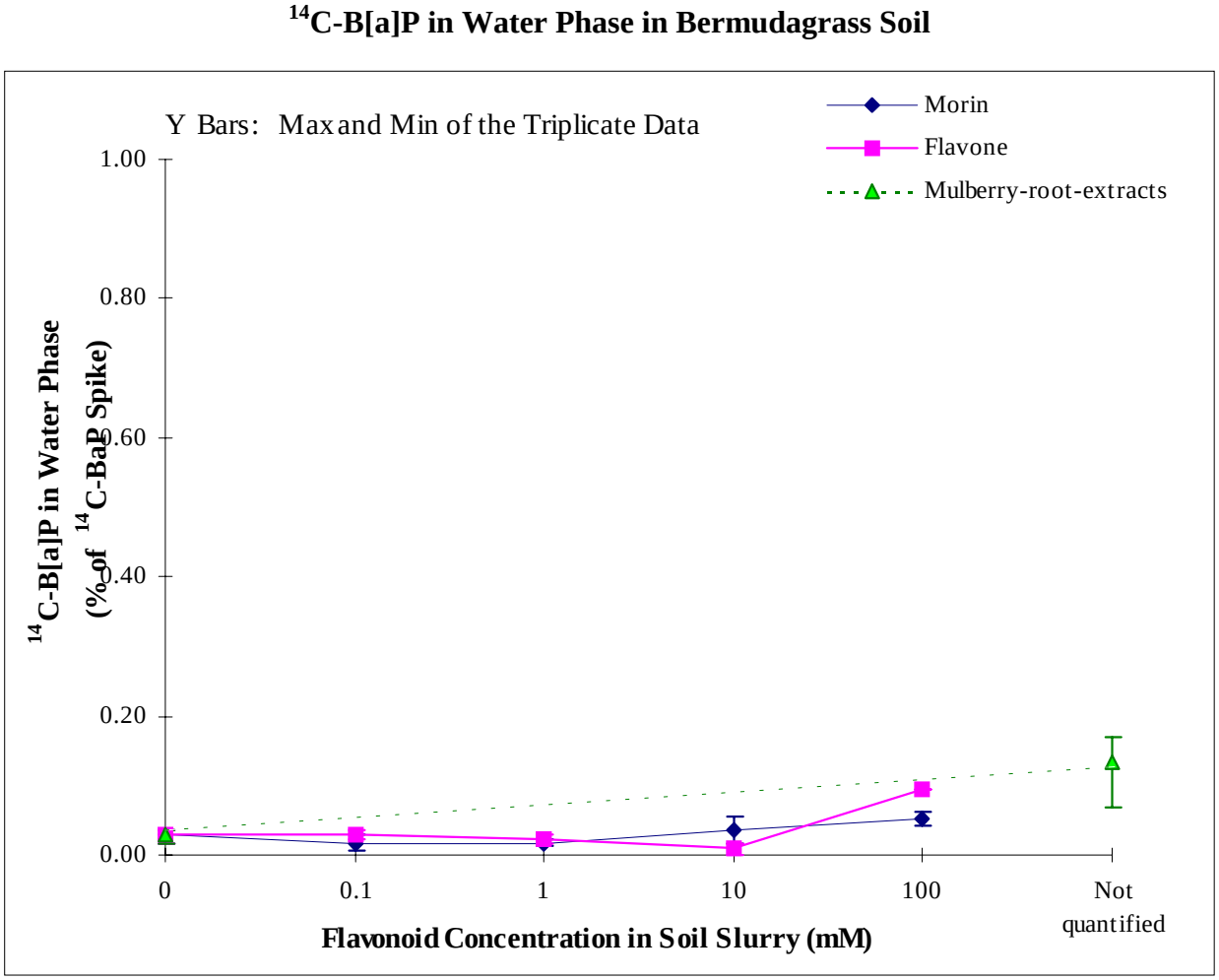


Figure 4.18. Water phase[1] ^{14}C-B[a]P (%) versus flavonoid concentrations amended in Bermudagrass-rhizosphere-soil-slurry microcosm

[1] hexane-extractable, nonpolar ^{14}C in water phase

Water Leaching of ^{14}C-B[a]P Metabolites in Soil Slurry Microcosms

Water-phase ^{14}C-B[a]P Metabolites in Poisoned-Mulberry-Rhizosphere Soil Water-phase ^{14}C-B[a]P metabolites were measured as hexane-nonextractable polar ^{14}C in water phase. In Figure 4.19, water phase metabolites of 7,10-^{14}C-B[a]P in poisoned-Mulberry-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extract amended in the soil slurry. Water-phase B[a]P metabolites were below 0.4% (equivalent to 0.04 ug/l) of the total 7,10-^{14}C-B[a]P added at all the flavonoid amendment levels except that with mulberry-root-extracts. When mulberry-root-extract was added water-phase 7,10-^{14}C-B[a]P increased slightly to approximately 0.65%. When Mulberry root extract was added the increase in water-phase ^{14}C-B[a]P metabolites was statistically significant higher than that without flavonoid. There are no other statistically significant differences between with and without flavonoids. In all the cases, Water-phase ^{14}C-B[a]P in poisoned-Mulberry-rhizosphere soil was negligible.

Water-phase ^{14}C-B[a]P Metabolites in Mulberry-Rhizosphere Soil In Figure 4.20, Water-phase metabolites of 7,10-^{14}C-B[a]P in Mulberry-rhizosphere-soil-slurry microcosms are presented versus flavonoid concentration of flavone or morin and mulberry-root-extract amended in the soil slurry. Water-phase B[a]P metabolites were approximately 0.2% of the total 7,10-^{14}C-B[a]P spike with all the flavonoid amendment levels except that with 100 uM morin or mulberry-root-extract. When 100 uM morin and mulberry-root-extract was added, water phase B[a]P-7,10-^{14}C metabolites increased to approximately 0.3% and 0.6% (equivalent to 0.06 ug/l), respectively, which was statistically significantly higher than that without flavonoid. There are no other statistically significant differences between with and without flavonoids. In all the cases, Water-phase ^{14}C-B[a]P metabolites in Mulberry-rhizosphere soil were negligible and less than that in poisoned-Mulberry-rhizosphere soil.

Water-phase ^{14}C-B[a]P Metabolites in Bermudagrass-Rhizosphere soil In Figure 4.21, water phase metabolites of 7,10-^{14}C-B[a]P in Bermudagrass-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extract amended in the soil slurry. Water-phase B[a]P metabolites were below 0.05% of the total 7,10-^{14}C-B[a]P added except with 100 uM Flavone and Mulberry root extract. When100 uM flavone or Mulberry root extract were added, Water-phase B[a]P metabolites slightly increased to around 0.1% (equivalent to 0.01 ug/l), which was statistically significantly higher than that without flavonoid. There are no other statistically significant differences between with and without flavonoids. In all the cases, water-phase ^{14}C-B[a]P metabolites in Bermudagrass-rhizosphere soil were negligible and less than that in Mulberry-rhizosphere soil.

^{14}C-B[a]P Metabolites in Water Phase in Poisoned Soil

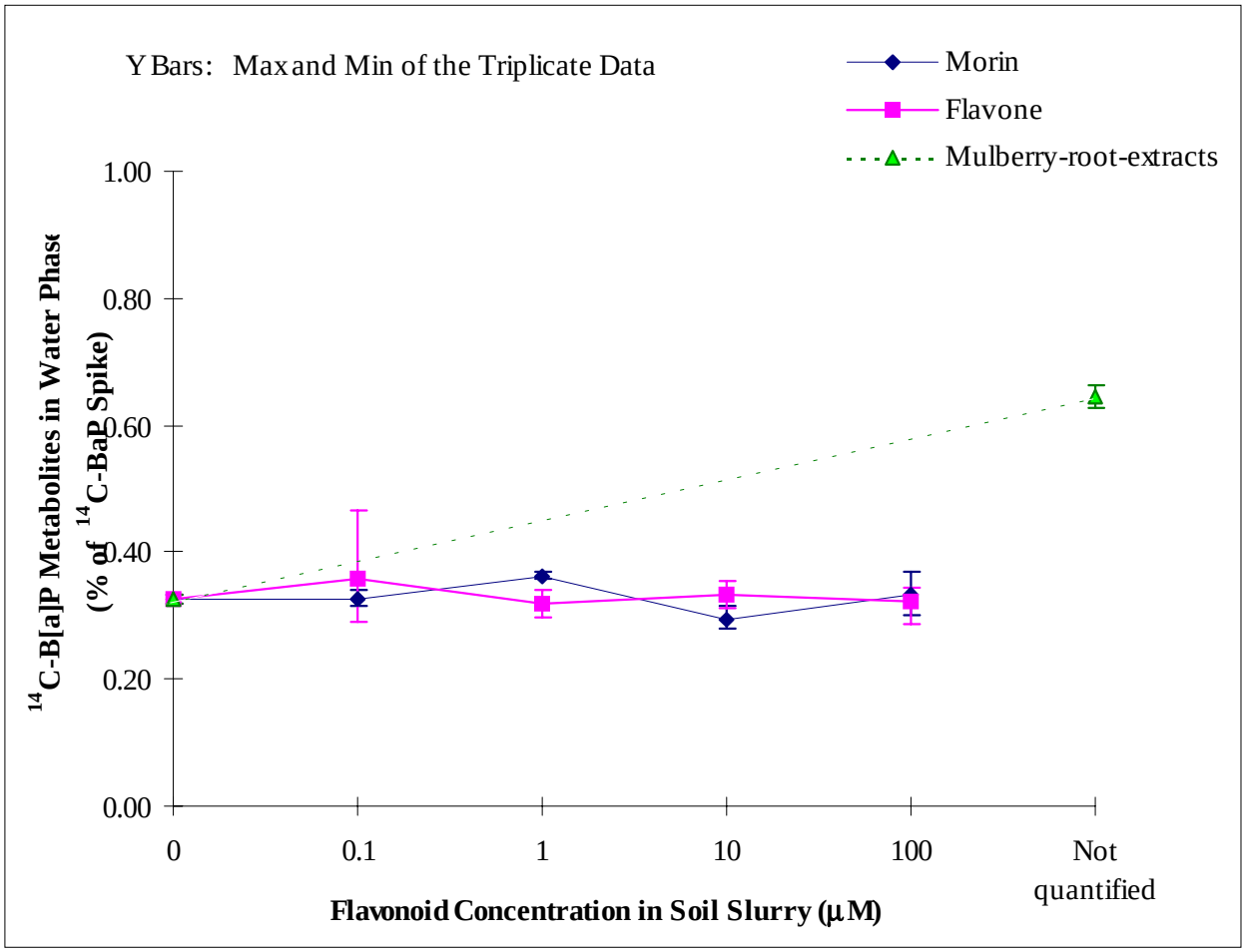


Figure 4.19. Water phase[1] ^{14}C-B[a]P metabolites (%) versus flavonoid concentrations amended in poisoned-Mulberry-rhizosphere-soil-slurry microcosm[2]

[1] hexane-extractable, nonpolar ^{14}C in water phase

[2] poisoned microcosms simulate metabolic inhibited pseudo-abiotic condition

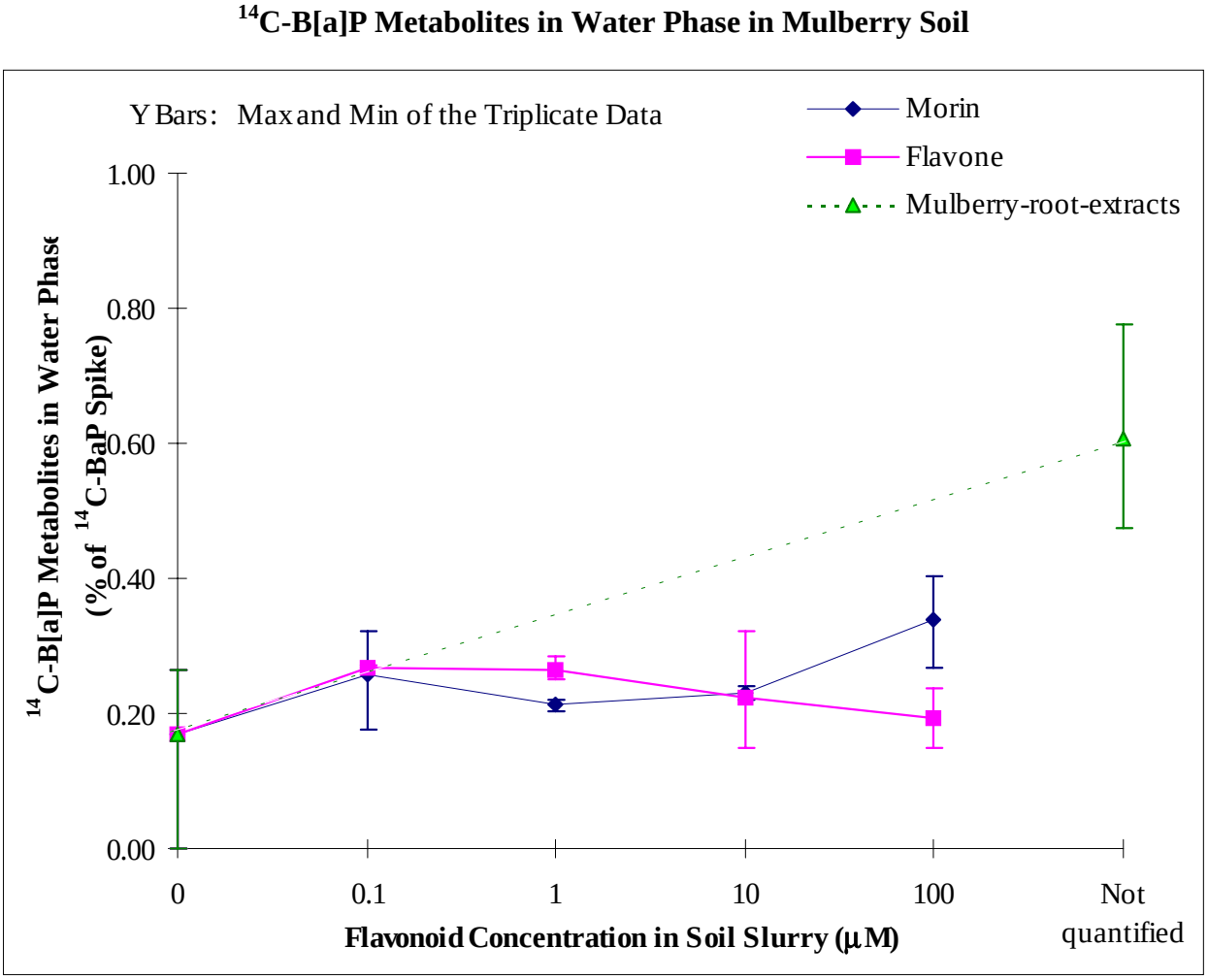


Figure 4.20. Water-phase[1] ^{14}C-B[a]P metabolites (%) versus flavonoid concentrations amended in Mulberry-rhizosphere-soil-slurry microcosm

[1] hexane-extractable, nonpolar ^{14}C in water phase

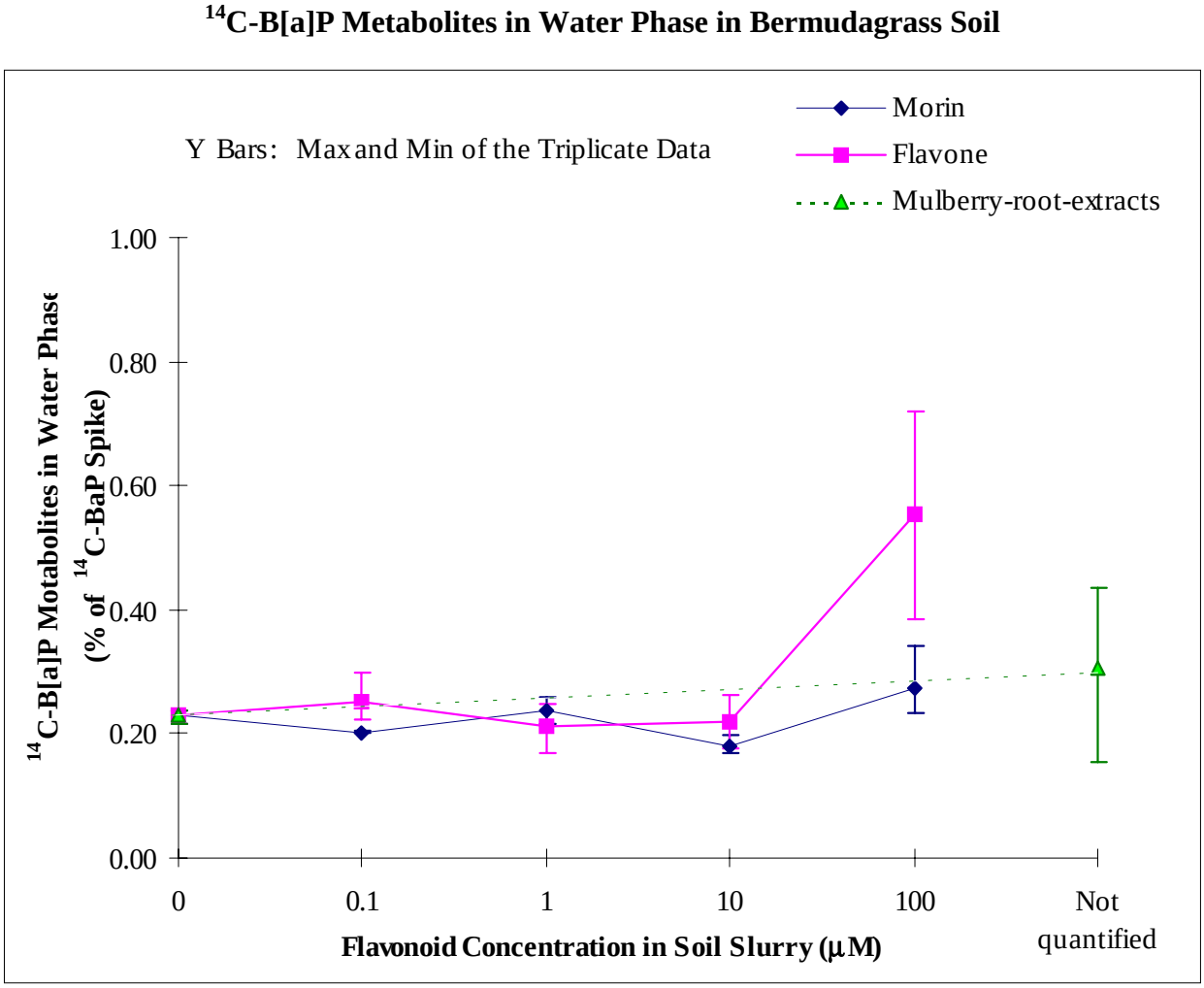


Figure 4.21. Water-phase[1] ^{14}C-B[a]P metabolites (%) versus flavonoid concentrations amended in Bermudagrass-rhizosphere-soil-slurry microcosm

[1] hexane-extractable, nonpolar ^{14}C in water phase

^{14}C-Pyrene Mineralization in Soil Slurry Microcosms

^{14}C-Pyrene Mineralization in Poisoned-Rhizosphere-Soil In Figures 4.22, $^{14}CO_2$ evolution from 7,10-^{14}C-pyrene in poisoned-Mulberry-rhizosphere-soil-slurry microcosms is plotted versus flavonoid concentrations amended in the soil slurry. The amount of 4,5,9,10-^{14}C-pyrene added was approximately 59300 dpm that is equivalent to 0.1 ug/g-soil or 0.01 ug/ml-water. $^{14}CO_2$ productions under "abiotic" conditions were all below 0.5% of the total 4,5,9,10-^{14}C-pyrene added (Figure 4.22). Abiotic pyrene mineralization appeared to be negligible. Similar to those observed in poisoned ^{14}C-B[a]P microcosms (Figure 4.7), there are not statistical significant differences in ^{14}C-pyrene mineralization in poisoned Mulberry soil between with flavonoids and without flavonoids at 95% confidence level (Table 4.8 and Appendix D-4).

^{14}C-Pyrene Mineralization in Mulberry-Rhizosphere Soil In Figure 4.23, $^{14}CO_2$ production from 4,5,9,10-^{14}C-pyrene in Mulberry-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extract. Without flavonoid, the $^{14}CO_2$ production was about 26% of the total 4,5,9,10-^{14}C-Pyrene added. $^{14}CO_2$ production decreased to between 18% and 25% as flavone and morin concentration increased to 0.1-10 μM. However, as flavone and morin concentrations increased to 100 μM, $^{14}CO_2$ production reduced statistically significantly to approximately 10% and 1% (Table 4.8 and Appendix D-5). Also $^{14}CO_2$ production reduced statistically significantly to approximately 10% as mulberry root extract was added (Table 4.8 and Appendix D-5). Similar to those observed in ^{14}C-B[a]P microcosms (Figure 4.8), $^{14}CO_2$ production from ^{14}C-pyrene generally decreased as flavonoid concentrations increased in Mulberry rhizosphere soil (Figure 4.23). The amount of $^{14}CO_2$ production from ^{14}C-pyrene was greater than that from ^{14}C-B[a]P.

^{14}C-Pyrene Mineralization in Bermudagrass-Rhizosphere Soil In Figure 4.24, $^{14}CO_2$ production from 4,5,9,10-^{14}C- Pyrene in Bermudagrass-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extracts amended in the soil slurry. Without flavonoid the $^{14}CO_2$ production was approximately 39% of the total 4,5,9,10-^{14}C-pyrene added. $^{14}CO_2$ production increased to 53% - 43% as flavone concentration increased to between 0.1 and 1 μM. $^{14}CO_2$ production remained at approximately 35% as morin concentration increased to between 0.1 and 1 μM. As flavone and morin concentration increased to 10 μM $^{14}CO_2$ production decreased to approximately 20%. As 100 μM flavone, 100 μM morin, or mulberry-root extracts was added, $^{14}CO_2$ production reduced statistically significantly to between 1% and 2% (Table 4.8 and Appendix D-6). Similar to those observed in ^{14}C-B[a]P microcosms (Figure 4.9), $^{14}CO_2$ production from ^{14}C-pyrene decreased as flavonoid concentration increased (Figure 4.24). The amount of $^{14}CO_2$ production from ^{14}C-pyrene is greater than that from ^{14}C-B[a]P in Bermudagrass rhizosphere soil.

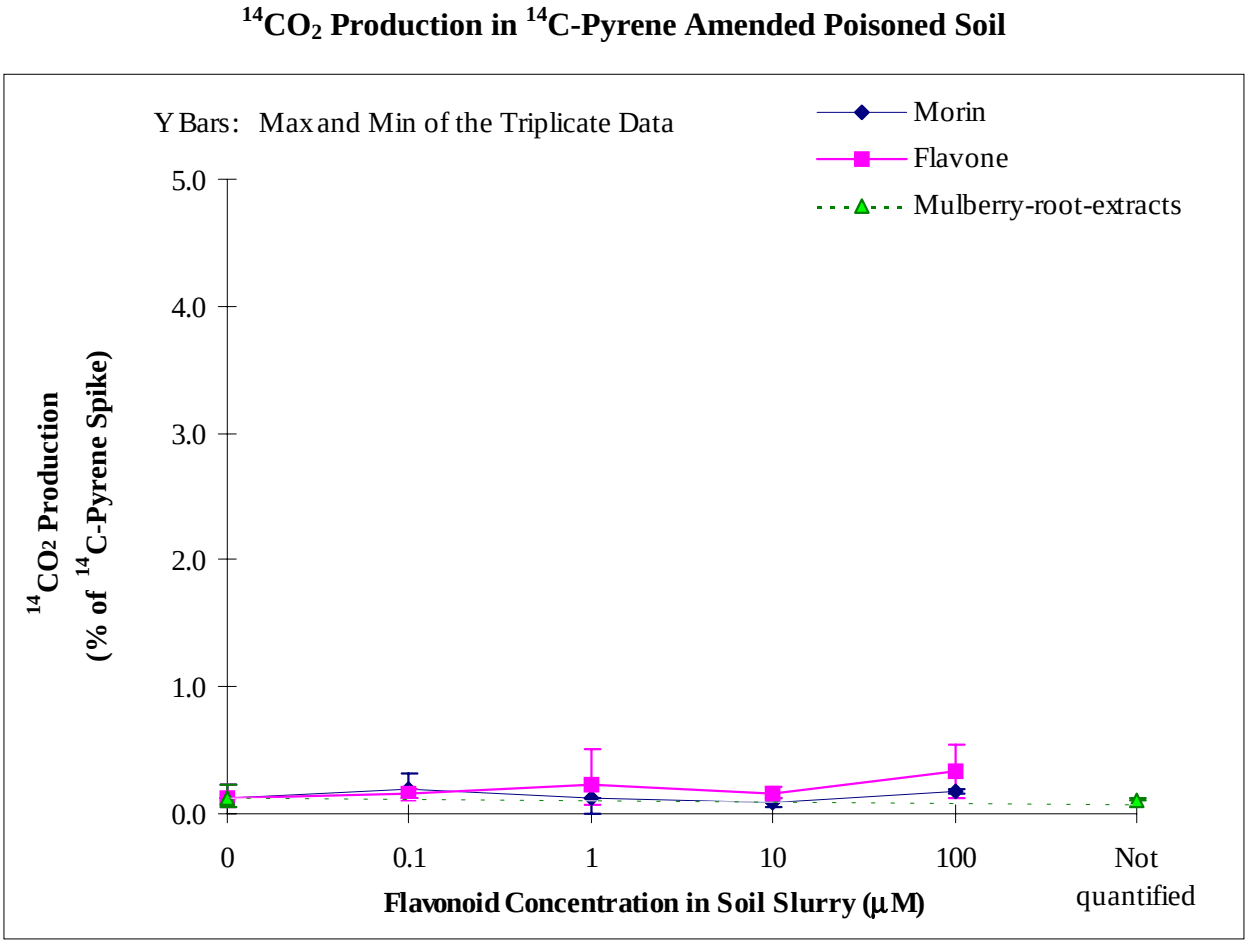


Figure 4.22. ^{14}C-pyrene mineralization[1] to $^{14}CO_2$ (%) versus flavonoid concentrations amended in poisoned-Mulberry-rhizosphere-soil-slurry microcosm[2]

[1] $^{14}CO_2$ was trapped by a chromatography filter strip soaked in potassium hydroxide

[2] poisoned microcosms simulate metabolic inhibited pseudo-abiotic condition

$^{14}CO_2$ Production in ^{14}C-Pyrene Amended Mulberry Soil

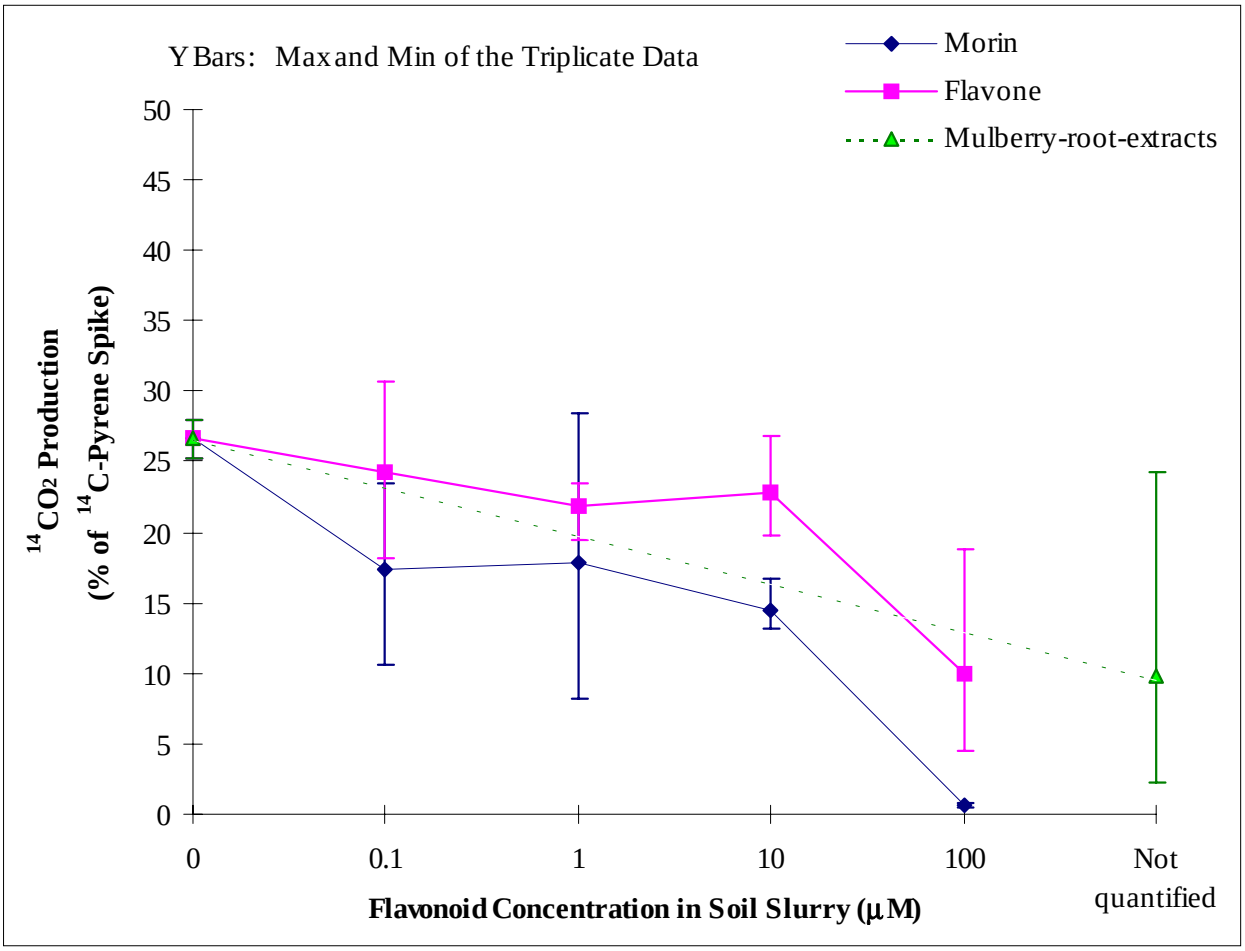


Figure 4.23 ^{14}C-pyrene mineralization[1] to $^{14}CO_2$ (%) versus flavonoid concentrations amended in Mulberry-rhizosphere-soil-slurry microcosm

[1] $^{14}CO_2$ was trapped by a chromatography filter strip soaked in potassium hydroxide

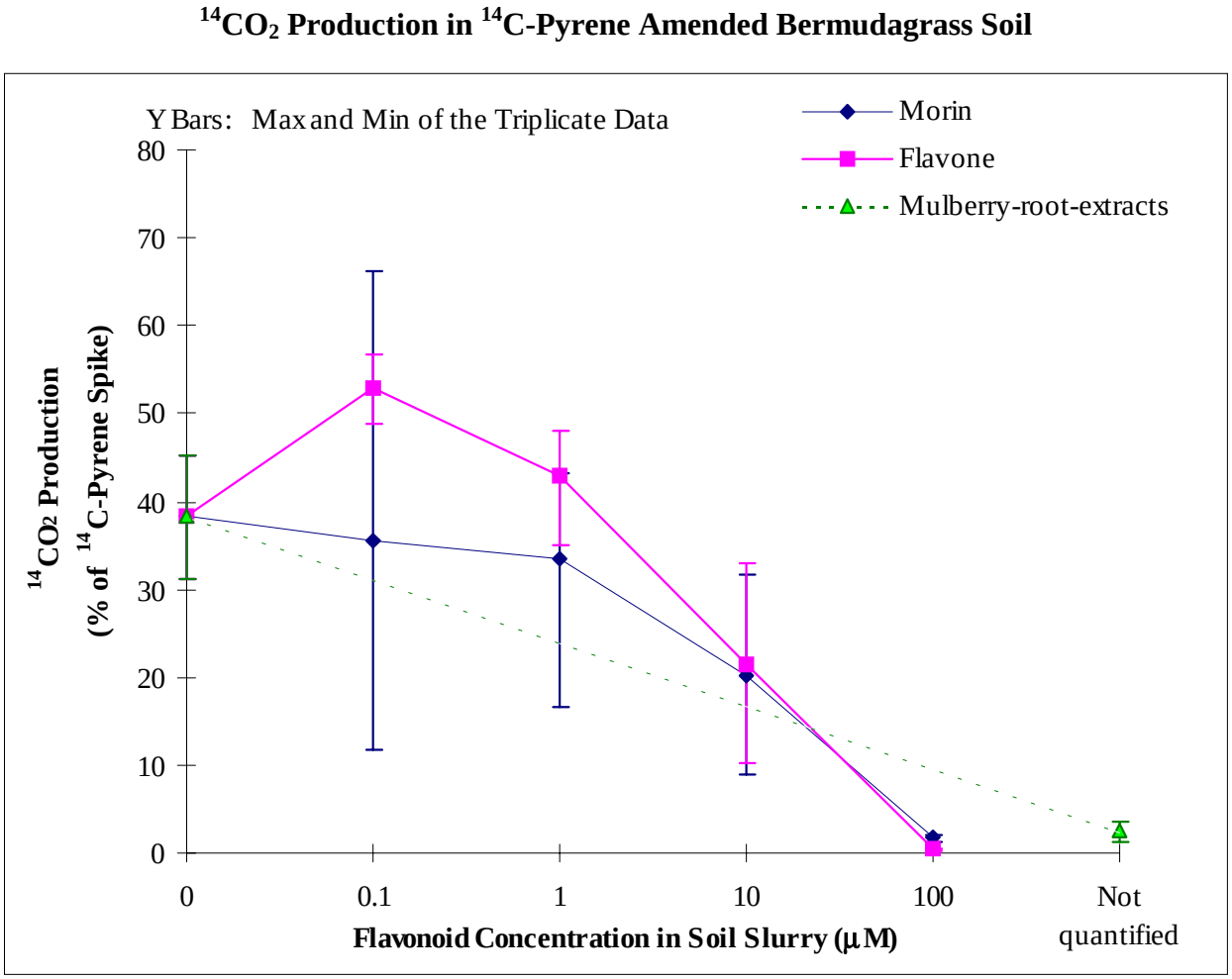


Figure 4.24. ^{14}C-pyrene mineralization[1] to $^{14}CO_2$ (%) versus flavonoid concentrations amended in Bermudagrass-rhizosphere-soil-slurry microcosm

[1] $^{14}CO_2$ was trapped by a chromatography filter strip soaked in potassium hydroxide

^{14}C-Pyrene Bound Residue Formation in Soil Slurry Microcosms

^{14}C-Pyrene Bound Residues in Poisoned-Mulberry-rhizosphere-Soil ^{14}C-bound residues were measured by ethylacetate-nonextractable ^{14}C in soil. ^{14}C bound reside can be either parent ^{14}C-Pyrene diffused into soil micropores or pyrene metabolites covalently binding to soil humus. In Figure 4.25, 4,5,9,10-^{14}C-pyrene bound residues in poisoned-soil-slurry microcosms are presented versus flavonoid concentration of flavone, morin, and mulberry-root-extracts amended in the soil slurry. Without flavonoid added, ^{14}C-pyrene bound residues were approximately 30% of the total ^{14}C-pyrene added. ^{14}C-pyrene bound residues decreased to between 15% and 25%, as morin and flavone concentration increased to between 0.1 uM and 1 uM. As morin concentration increased to 10 uM, ^{14}C-pyrene bound residue increased to approximately 48%. In contrast, as flavone concentration increased to 10 uM, ^{14}C-pyrene bound residues decreased to approximately 10%. ^{14}C-pyrene bound residues were approximately 30%, as 100 μM flavone or morin, or mulberry-root extract was added. Similar to those of ^{14}C-B[a]P bound residues in poisoned-Mulberry-rhizosphere soil (Figure 4.25), there were no statistical significant differences in ^{14}C-pyrene bound residues (Figure 4.10) between with and without flavonoids at 95% Confidence level (Table 4.8 and Appendix D-4).

^{14}C-Pyrene Bound Residues in Mulberry-Rhizosphere Soil In Figure 4.26, 4,5,9,10-^{14}C-Pyrene bound residues in Mulberry-rhizosphere-soil-slurry microcosms are presented versus flavonoid concentration of flavone, morin, and mulberry-root-extract amended in the soil slurry. Without flavonoid amendment, ^{14}C bound residues were approximately 10% of the total 4,5,9,10-^{14}C-pyrene added. As flavonoid concentration increased to between 0.1 uM and 10 μM, ^{14}C bound residues remained at similar levels. As 100 μM flavone or Mulberry-root extract was added ^{14}C bound residues increased to approximately 20%. ^{14}C-pyrene bound residues increased statistically significantly at 95% confidence level to approximately 35% when 100 uM morin was added (Table 4.8 and Appendix D-5). Similar to ^{14}C-B[a]P, ^{14}C-pyrene soil bound residues in Mulberry rhizosphere soil increased as flavone and morin concentration increased (Figure 4.26 and Figure 4.11).

^{14}C-Pyrene Bound Residues in Bermudagrass-Rhizosphere Soil In Figure 4.27, 4,5,9,10-^{14}C-pyrene bound residues in Bermudagrass-rhizosphere-soil-slurry microcosms are presented versus flavonoid concentration of flavone or morin and mulberry-root-extracts added in the soil slurry. Without flavonoid ^{14}C bound residues were approximately 10% of the total ^{14}C added. ^{14}C bound residues remained at between 10% and 20% as flavonoid concentration increased to between 0.1 and10 μM. When Mulberry-root-extract or 100 μM morin was added, ^{14}C bound residues increased statistically significantly (95% confidence level) to between 30%. When 100 uM flavone was added, ^{14}C-pyrene bound residues also increased statistically significantly at 95% confidence level to more than 40% (Table 4.8 and Appendix D-6). Similar to ^{14}C-B[a]P, ^{14}C-pyrene soil bound residues in Bermudagrass rhizosphere soil increased as flavone and morin concentration increased (Figure 4.27 and Figure 4.12).

^{14}C Bound Residues in ^{14}C-Pyrene Amended Poisoned Soil

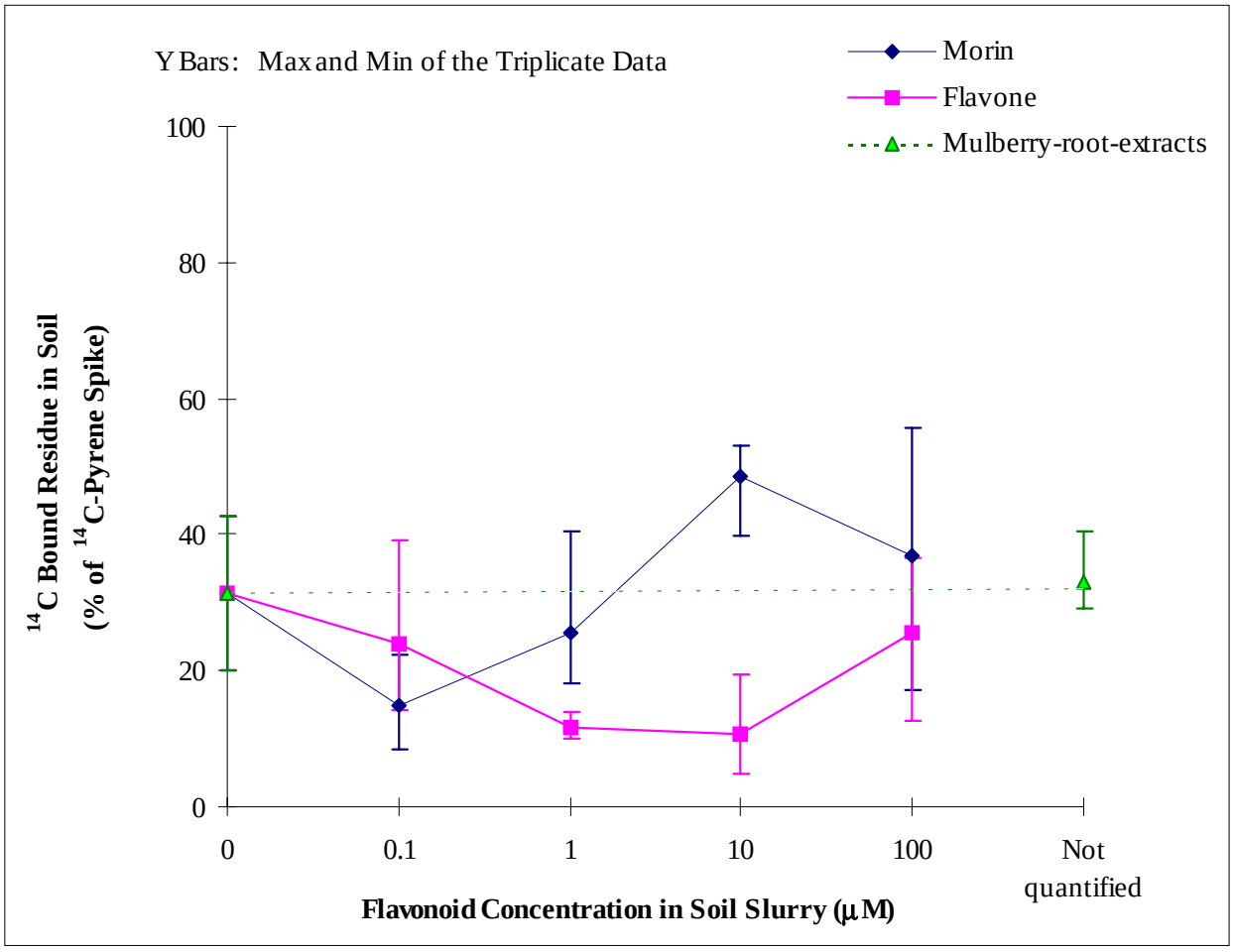


Figure 4.25. ^{14}C-pyrene bound residues[1] in soil (%) versus flavonoid concentrations amended in poisoned-Mulberry-rhizosphere-soil-slurry microcosm[2]

[1] ethylacetate-non-extractable ^{14}C in soil phase

[2] poisoned microcosms simulate metabolic inhibited pseudo-abiotic condition

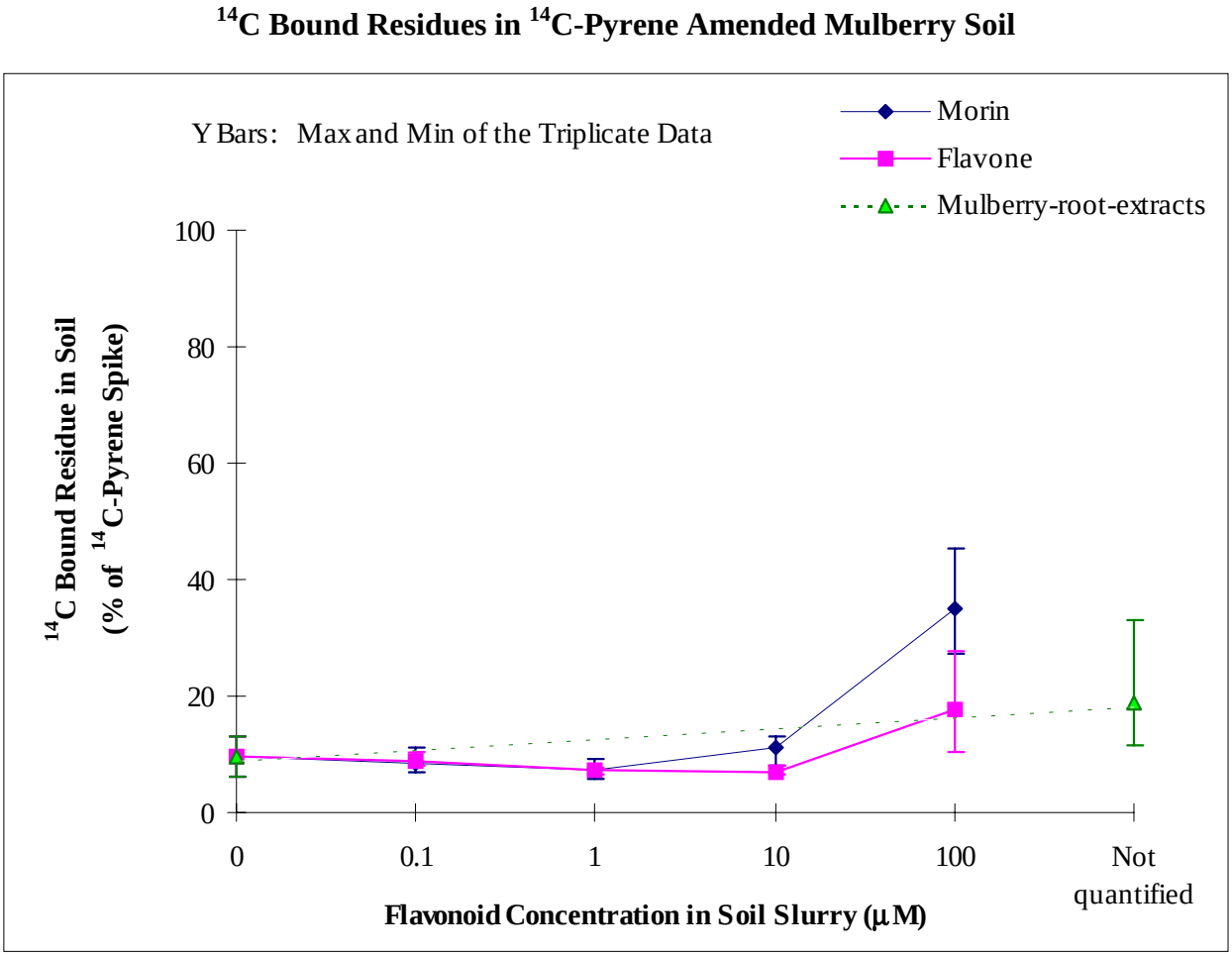


Figure 4.26. ^{14}C-pyrene bound residues[1] in soil (%) versus flavonoid concentrations amended in Mulberry-rhizosphere-soil-slurry microcosm

[1] ethylacetate-non-extractable ^{14}C in soil phase

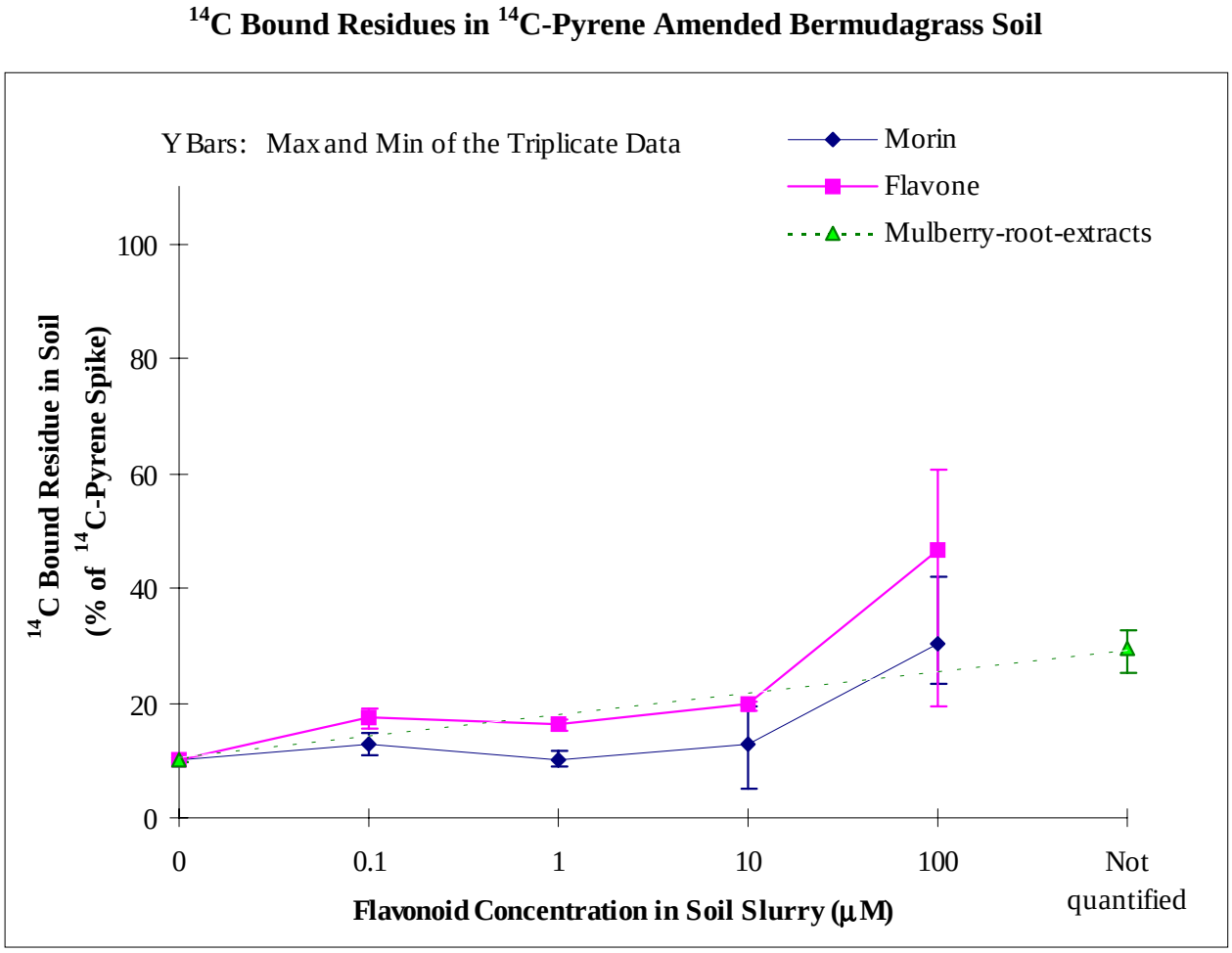


Figure 4.27. ^{14}C-pyrene bound residues[1] in soil (%) versus flavonoid concentrations amended in Bermudagrass-rhizosphere-soil-slurry microcosm

[1] ethylacetate-non-extractable^{14}C in soil phase

Adsorption of ^{14}C-Pyrene in Soil Slurry Microcosms

Adsorption of ^{14}C-Pyrene in Poisoned- Mulberry-Rhizosphere Soil Adsorption of ^{14}C-pyrene was measured by ethylacetate-extractable ^{14}C in soil phase. In Figure 4.28, ethylacetate-extractable 4,5,9,10-^{14}C-pyrene in poisoned-mulberry-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin and mulberry-root-extract amended in the soil slurry. Without flavonoid amendment ^{14}C-pyrene adsorption to soil was approximately 40%. As flavone and morin increased to between 0.1 uM and 1 uM, ^{14}C-pyrene adsorption to soil increased somewhat to approximately 50%. As flavonoid increased further, ^{14}C-pyrene adsorption to soil increased to approximately 60% with 10 uM flavone, but decreased to <30% with 10uM morin. When 100uM flavone, 100 morin, or Mulberry root extract was added the ^{14}C-pyrene adsorption was about 40%, which was similar to that without flavonoid added. Similar to those of ^{14}C-B[a]P, there were no statistical significant differences in ^{14}C-pyrene adsorption onto poisoned-Mulberry-rhizosphere soil between with and without flavonoids (Table 4.8, Appendices D-1 and D-4). The extent of ^{14}C-pyrene adsorption onto poisoned-Mulberry-rhizosphere soil was less than that of ^{14}C-B[a]P ((Figure 4.13).

Adsorption of ^{14}C-Pyrene in Mulberry-Rhizosphere Soil In Figure 4.29, 4,5,9,10-^{14}C-pyrene adsorption in Mulberry-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extracts amended in the soil slurry. Without flavonoid amendment the adsorption was approximately 10% of the total 4,5,9,10-^{14}C-pyrene added. There were little differences in pyrene adsorption as flavone and morin concentration increased from 0.1 to 100 uM, except for 100 uM morin. ^{14}C-Pyrene adsorption increased statistically significantly to approximately 35% when 100 uM morin was added and approximately 30% when Mulberry root extract was added, while there were no statistically significant differences in^{14}C-B[a]P adsorption to Mulberry-rhizosphere soil between with and without flavonoids (Table 4.8, Appendices D-2 and D-5). The amount of ^{14}C-pyrene adsorption onto Mulberry-rhizosphere soil was somewhat less than that of ^{14}C-B[a]P (Figure 4.14).

Adsorption of ^{14}C-Pyrene in Bermudagrass rhizosphere soil In Figure 4.30, 4,5,9,10-^{14}C-pyrene adsorption in Bermudagrass-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extracts amended in the soil slurry. Without flavonoid amendment ethylacetate-extractable pyrene was approximately 10% of the total 4,5,9,10-^{14}C-pyrene added. Pyrene adsorption decreased to approximately 5% when flavone concentrations increased to between 1 and 10 uM, then increased to approximately 20% as flavone concentration increased to 100 uM. Pyrene adsorption remained at approximately 10% when morin concentrations increased to between 0.1 uM and 10 uM, then increased to approximately 40% as morin concentration increased to 100 uM (Table 4.8 and Appendix D-6). Pyrene adsorption increased to approximately 20% when Mulberry root extract was added. The aforementioned increases in adsorption of ^{14}C-pyrene when flavone, morin, or Mulberry root extract was added were statistically insignificant at 95% confidence level except that with 100 uM of morin added (Table 4.8 and Appendices D-6). The amount of ^{14}C-pyrene adsorption onto Bermudagrass-rhizosphere soil was less than ^{14}C-B[a]P adsorption, while both were not much affected by flavonoid amendments (Figure 4.30 and Figure 4.15).

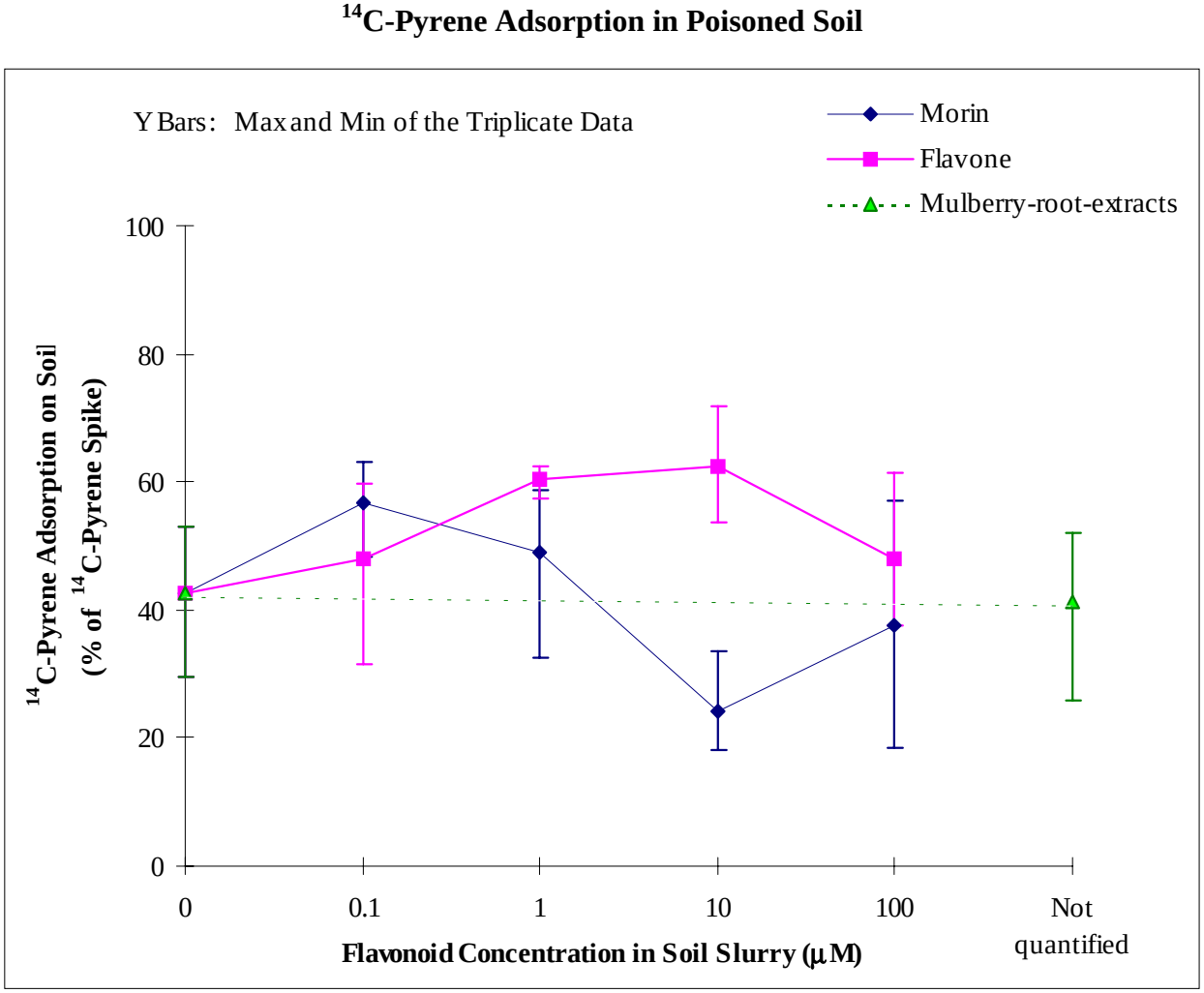


Figure 4.28. ^{14}C-pyrene adsorption[1] (%) versus flavonoid concentrations amended in poisoned-Mulberry-rhizosphere-soil-slurry microcosm[2]

[1] ethylacetate-extractable ^{14}C in soil phase

[2] poisoned microcosms simulate metabolic inhibited pseudo-abiotic condition

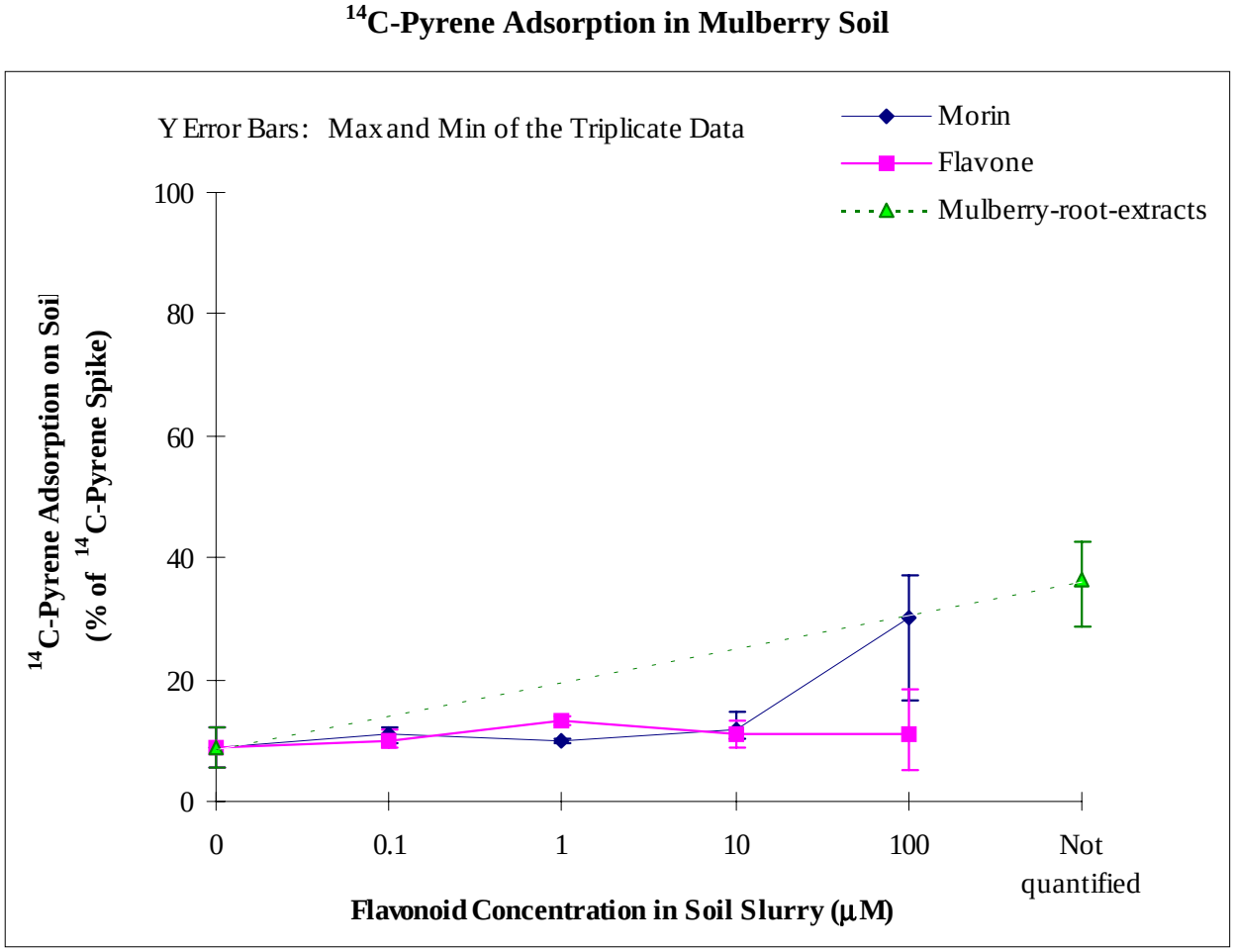


Figure 4.29. ^{14}C-pyrene adsorption [1] (%) versus flavonoid concentrations amended in Mulberry-rhizosphere-soil-slurry microcosm

[1] ethylacetate-extractable^{14}C in soil phase

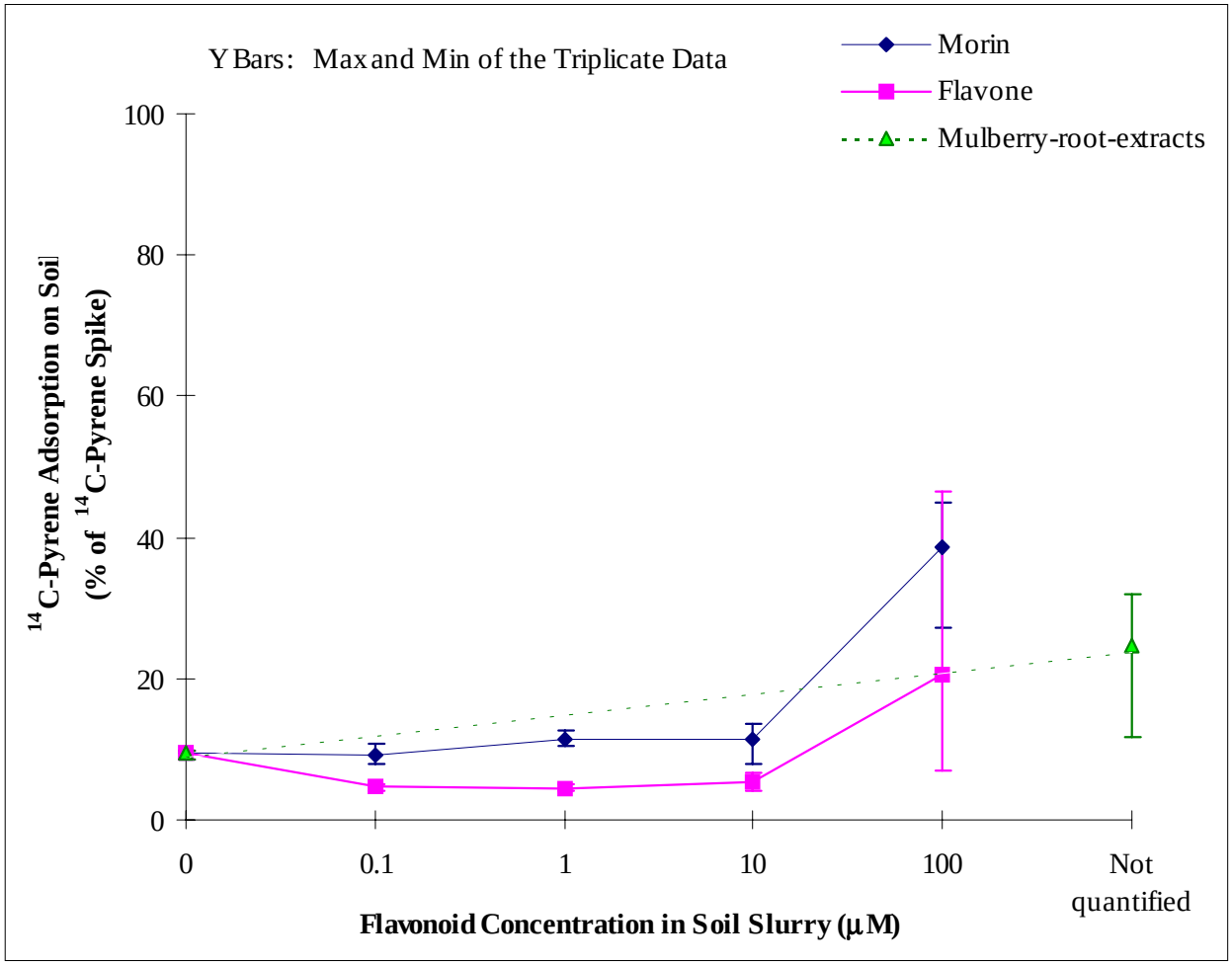


Figure 4.30. ^{14}C-pyrene adsorption[1] (%) versus flavonoid concentrations amended in Bermudagrass-rhizosphere-soil-slurry microcosm

[1] ethylacetate-extractable ^{14}C in soil phase

Water Leaching of ^{14}C-Pyrene in Soil Slurry Microcosms

Water-Phase ^{14}C-Pyrene in Poisoned-Mulberry-Rhizosphere Soil Water-phase ^{14}C-pyrene was measure by hexane-extractable non-polar ^{14}C in water phase. In Figure 4.31, water phase ^{14}C-pyrene in poisoned-Mulberry-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extracts amended in the soil slurry. Water-phase ^{14}C-pyrene was approximately 0.05% of the total 4,5,9,10-^{14}C-pyrene added at all the flavonoid amendment levels except that with mulberry-root-extract. When mulberry-root-extract was added water-phase ^{14}C-pyrene increased somewhat to approximately 0.15% (equivalent to 0.015 ug/l), which was statistically significant at 95% confidence level (Table 4.8 and Appendix D-4). In all the cases, water-phase ^{14}C-pyrene was negligible in the poisoned-Mulberry-rhizosphere soil. In poisoned-Mulberry-rhizosphere soil, water-phase ^{14}C-pyrene was slightly less than water-phase ^{14}C-B[a]P, while both were not influenced by flavone or morin but increased slightly as Mulberry root extract was added (Figure 4.31 and Figure 4.16).

Water-phase ^{14}C-Pyrene in Mulberry Rhizosphere Soil In Figure 4.32, water phase ^{14}C-pyrene in Mulberry-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extracts amended in the soil slurry. Water-phase ^{14}C-pyrene was all below 0.03% of the total 4,5,9,10-^{14}C-pyrene added except that with mulberry-root-extract. When mulberry-root-extract was added, water phase ^{14}C-pyrene increased to approximately 0.1% (equivalent to 0.01 ug/l). Although the increase was very small, it was statistically significant at 95% confidence level (Table 4.8 and Appendix D-5). In all the cases, water-phase ^{14}C-pyrene was negligible in mulberry-rhizosphere soil and less than that in poisoned-Mulberry-rhizosphere soil. In Mulberry-rhizosphere soil the levels of water-phase ^{14}C-pyrene were similar to those of water-phase ^{14}C-B[a]P (Figure 4.32 and Figure 4.17).

Water-phase^{14}C-Pyrene in Bermudagrass Rhizosphere Soil In Figure 4.33, water phase ^{14}C-pyrene in Bermudagrass-rhizosphere-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extract amended in the soil slurry. Water-phase ^{14}C-pyrene was below 0.03% of the total 4,5,9,10-^{14}C-pyrene added, as flavone and morin concentrations increased from 0 to 10 uM. Water-phase ^{14}C-pyrene increased slightly to approximately 0.05% of the total 4,5,9,10-^{14}C-pyrene added as morin concentrations increased to 100 uM. When Mulberry-root-extract was added, water-phase ^{14}C-pyrene increased to approximately 0.1% (equivalent to 0.01 ug/l). Although these increases were very small, those the slight increases were statistically significant at 95% confidence levels (Table 4.8 and Appendix D-6). In all the cases, water-phase ^{14}C-pyrene in Bermudagrass-rhizosphere soil was negligible and less than that in Mulberry-rhizosphere soil. In addition, the levels of water-phase ^{14}C-pyrene were similar to that of water-phase ^{14}C-B[a]P observed in Bermudagrass-rhizosphere soil (Figure 4.33 and Figure 4.18).

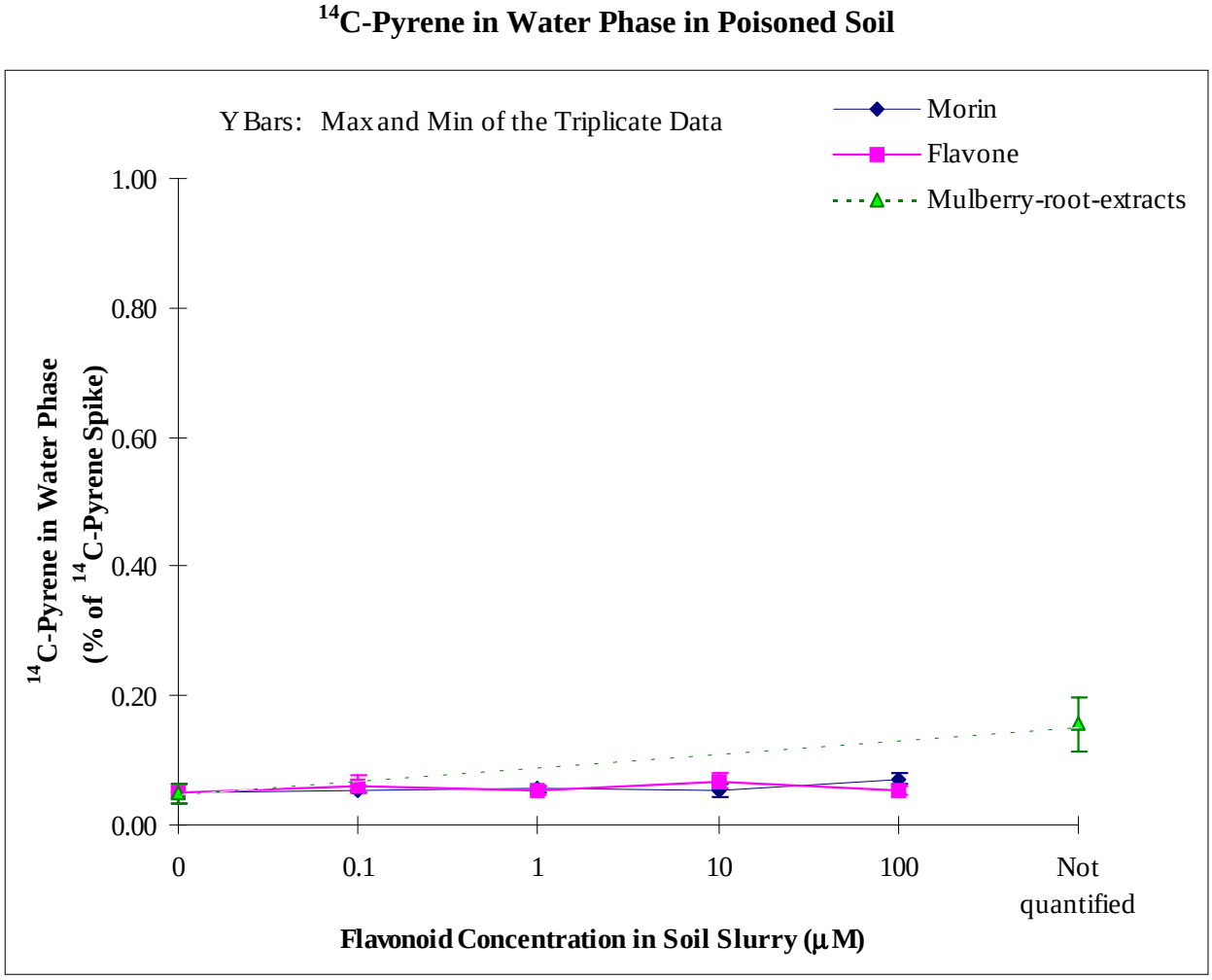


Figure 4.31. Water phase ^{14}C-pyrene[1] (%) versus flavonoid concentrations amended in poisoned-Mulberry-rhizosphere-soil-slurry microcosm[2]

[1] hexane-extractable ^{14}C in water phase

[2] poisoned microcosms simulate metabolic inhibited pseudo-abiotic condition

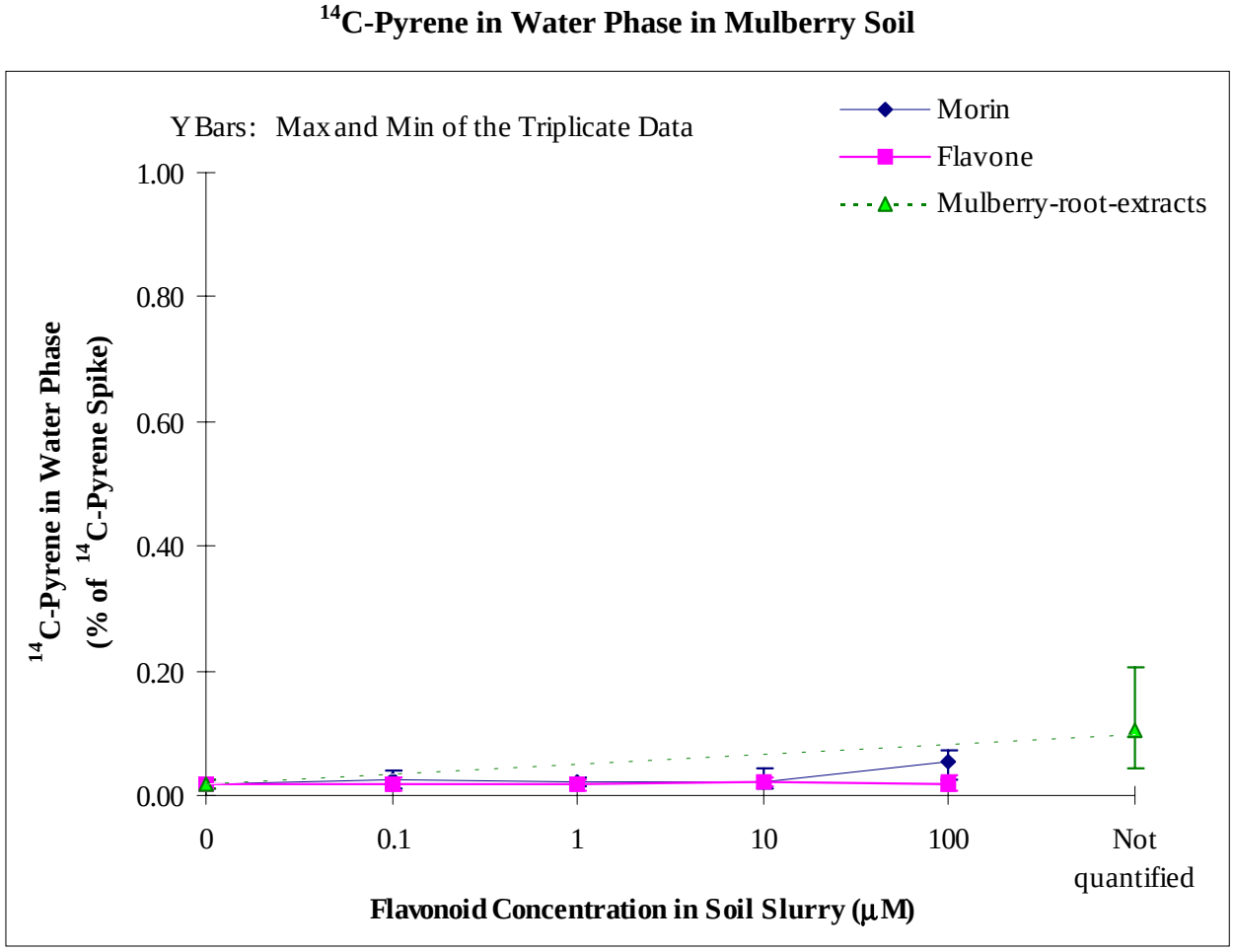


Figure 4.32. Water phase ^{14}C-pyrene[1] (%) versus flavonoid concentrations amended in Mulberry-rhizosphere-soil-slurry microcosm

[1] hexane-extractable, nonpolar ^{14}C in water phase

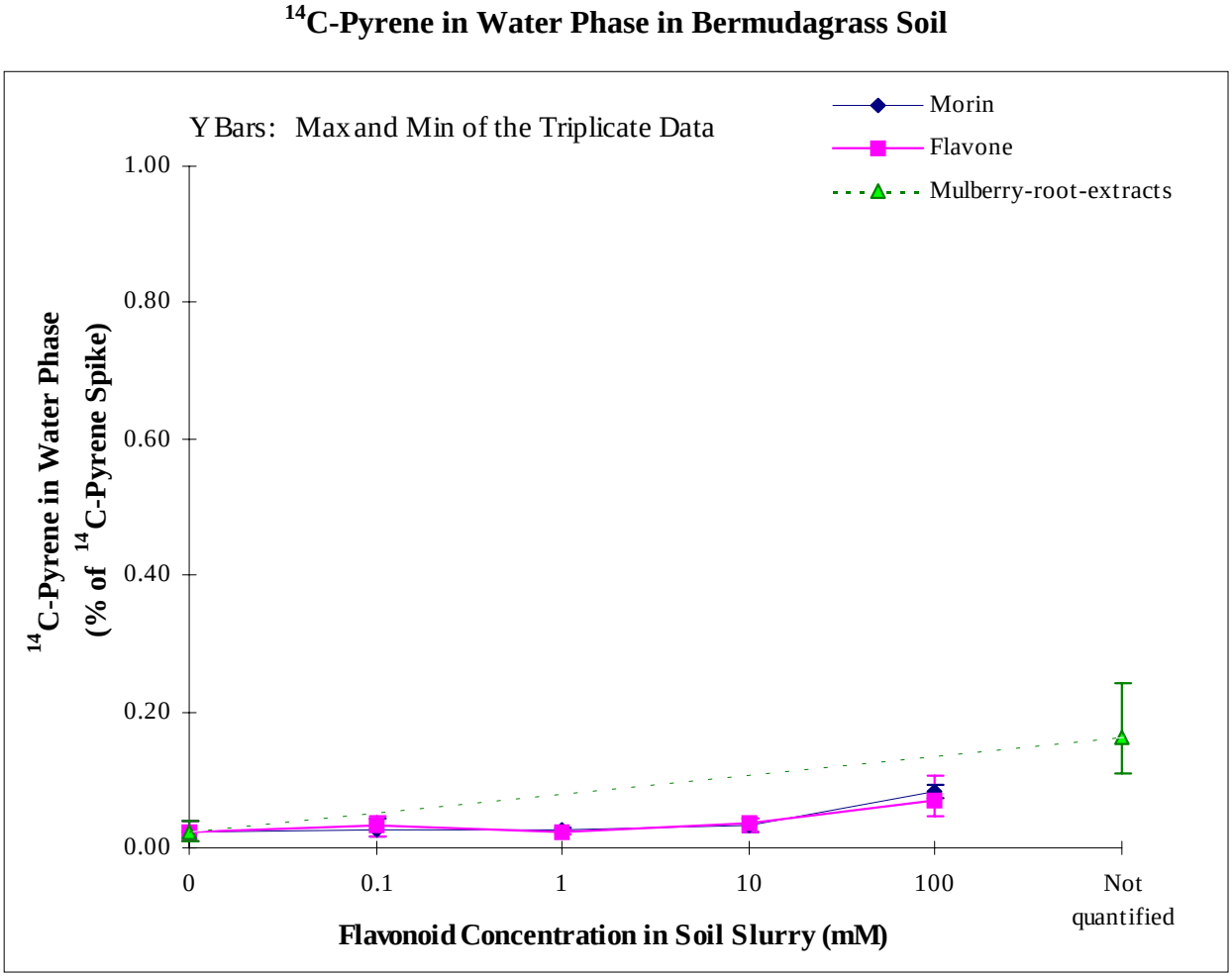


Figure 4.33. Water phase ^{14}C-pyrene[1] (%) versus flavonoid concentrations amended in Bermudagrass-rhizosphere-soil-slurry microcosm[2]

[1] hexane-extractable, nonpolar ^{14}C in water phase

[2] poisoned microcosms simulate metabolic inhibited pseudo-abiotic condition

Water Leaching of ^{14}C-Pyrene Metabolites in Soil Slurry Microcosms

Water-phase ^{14}C-Pyrene Metabolites in Poisoned-Mulberry-Rhizosphere Soil Water phase ^{14}C-pyrene metabolites was measured as hexane-nonextractable polar ^{14}C in water phase. In Figure 4.34, water phase metabolites of ^{14}C-Pyrene in poisoned-Mulberry-soil-slurry microcosms is presented versus flavonoid concentration of flavone, morin, and mulberry-root-extract amended in the soil slurry. Water-phase pyrene metabolites were below 0.3% of the total 4,5,9,10-^{14}C-pyrene added at all the flavonoid amendment levels except that with mulberry-root-extracts. When mulberry-root-extract was added, water phase ^{14}C-pyrene increased slightly to approximately 0.4% (equivalent to 0.04 ug/l), which was statistically significant at the 95% confidence level (Table 4.8 and Appendix D-4). In all the cases, water-phase ^{14}C-pyrene metabolites in poisoned-Mulberry-rhizosphere soil were negligible. In addition, the levels of water-phase ^{14}C-pyrene were slightly less than that of water-phase ^{14}C-B[a]P observed in poisoned-Mulberry-rhizosphere soil (Figure 4.34 and Figure 4.19).

Water-phase ^{14}C-Pyrene Metabolites in Mulberry Rhizosphere Soil In Figure 4.35, water-phase metabolites of 4,5,9,10-^{14}C-pyrene in Mulberry-rhizosphere-soil-slurry microcosms are presented versus flavonoid concentration of flavone or morin and mulberry-root-extract amended in the soil slurry. Without flavonoid added Water-phase ^{14}C-pyrene metabolites were slightly over 0.8% of the total 4,5,9,10-^{14}C-pyrene added. As flavone and morin amendment levels increased from 0 to 100 uM, water-phase ^{14}C-pyrene metabolites decreased from >0.8% to approximately 0.3% (equivalent to 0.03 ug/l). When mulberry-root-extract was added, water phase ^{14}C-pyrene metabolites decreased from >0.8% to approximately 0.7% (equivalent to 0.07 ug/l). Although these decreases were very small, those were statistically significant at the 95% confidence levels (Table 4.8 and Appendix D-5). In all the cases, water-phase ^{14}C-pyrene metabolites in Mulberry-rhizosphere soil were negligible. However, the levels of water-phase ^{14}C-pyrene were slightly more than their counterparts in poisoned-Mulberry rhizosphere soil and slightly higher than the levels of Water-phase ^{14}C-B[a]P observed in Mulberry-rhizosphere soil (Figure 4.35, Figure 4.34 and Figure 4.20).

Water-phase ^{14}C-Pyrene Metabolites in Bermudagrass Rhizosphere Soil In Figure 4.36, water-phase metabolites of ^{14}C-pyrene in Bermudagrass-rhizosphere-soil-slurry microcosms are presented versus flavonoid concentration of flavone, morin, and mulberry-root-extract amended in the soil slurry. Water-phase ^{14}C-pyrene metabolites were below 0.6% of the total 4,5,9,10-^{14}C-pyrene added except that Mulberry root extract. When Mulberry root extract was added, water-phase ^{14}C-pyrene metabolites slightly increased to around 0.9% (equivalent to 0.09 ug/l). This slightl increase was statistically insignificant at the 95% confidence level. In all the cases, water-phase ^{14}C-pyrene metabolites in Bermudagrass-rhizosphere soil were negligible. However, the levels of water-phase ^{14}C-pyrene were slightly more than their counterparts in poisoned-Mulberry rhizosphere soil, but slightly less than their counterparts in Mulberry-rhizosphere soil. In addition, the levels of Water-phase ^{14}C-pyrene were higher than Water-phase ^{14}C-B[a]P observed in Bermudagrass-rhizosphere soil (Figures 4.36, 4.35, 4.34 and Figure 4.21).

^{14}C-Pyrene Metabolites in Water Phase in Poisoned Soil

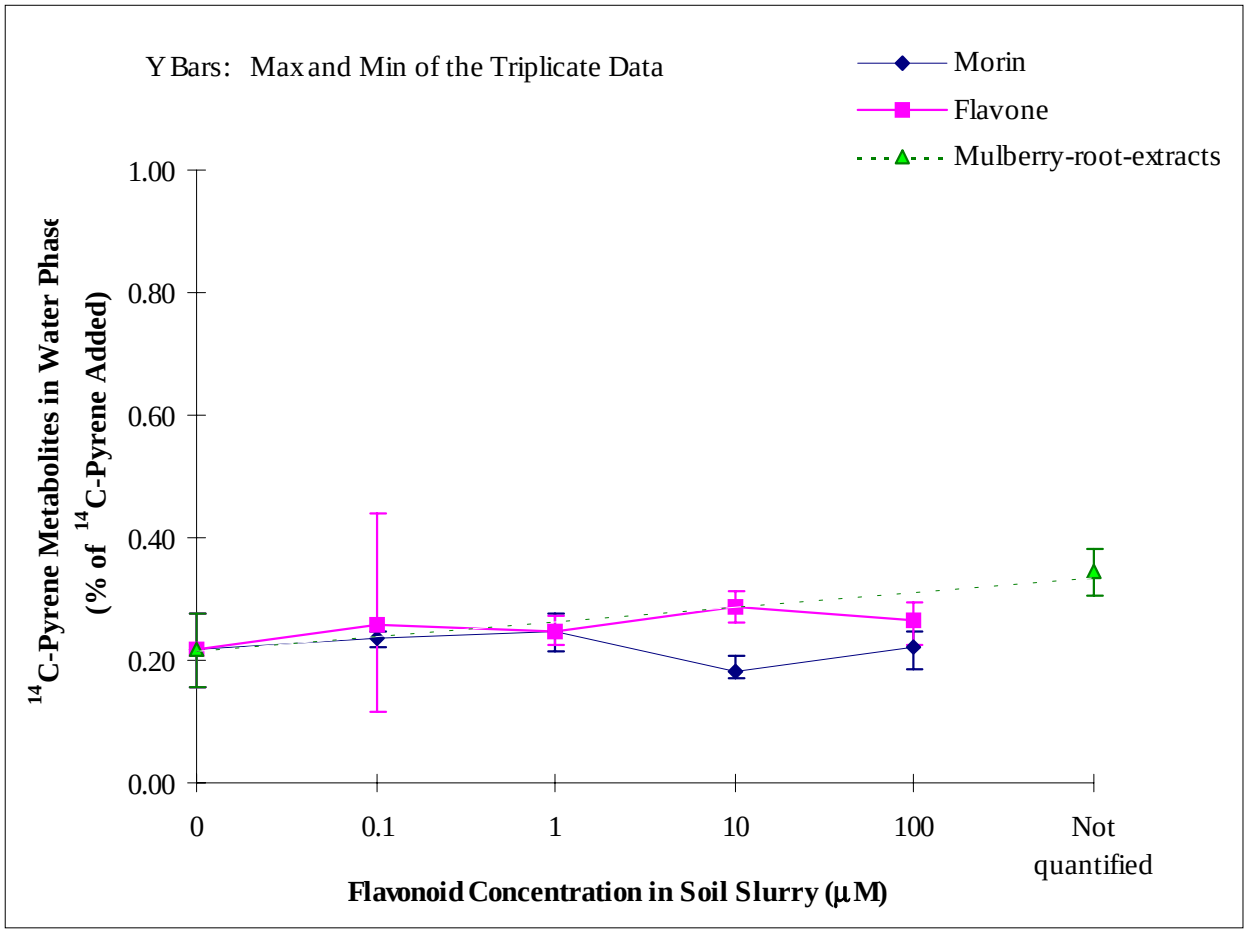


Figure 4.34. Water phase ^{14}C-pyrene metabolites[1] (%) versus flavonoid concentrations amended in poisoned-Mulberry-rhizosphere-soil-slurry microcosm[2]

[1] hexane-nonextractable, nonpolar ^{14}C in water phase

[2] poisoned microcosms simulate metabolic inhibited pseudo-abiotic condition

^{14}C-Pyrene Metabolites in Water Phase in Mulberry Soil

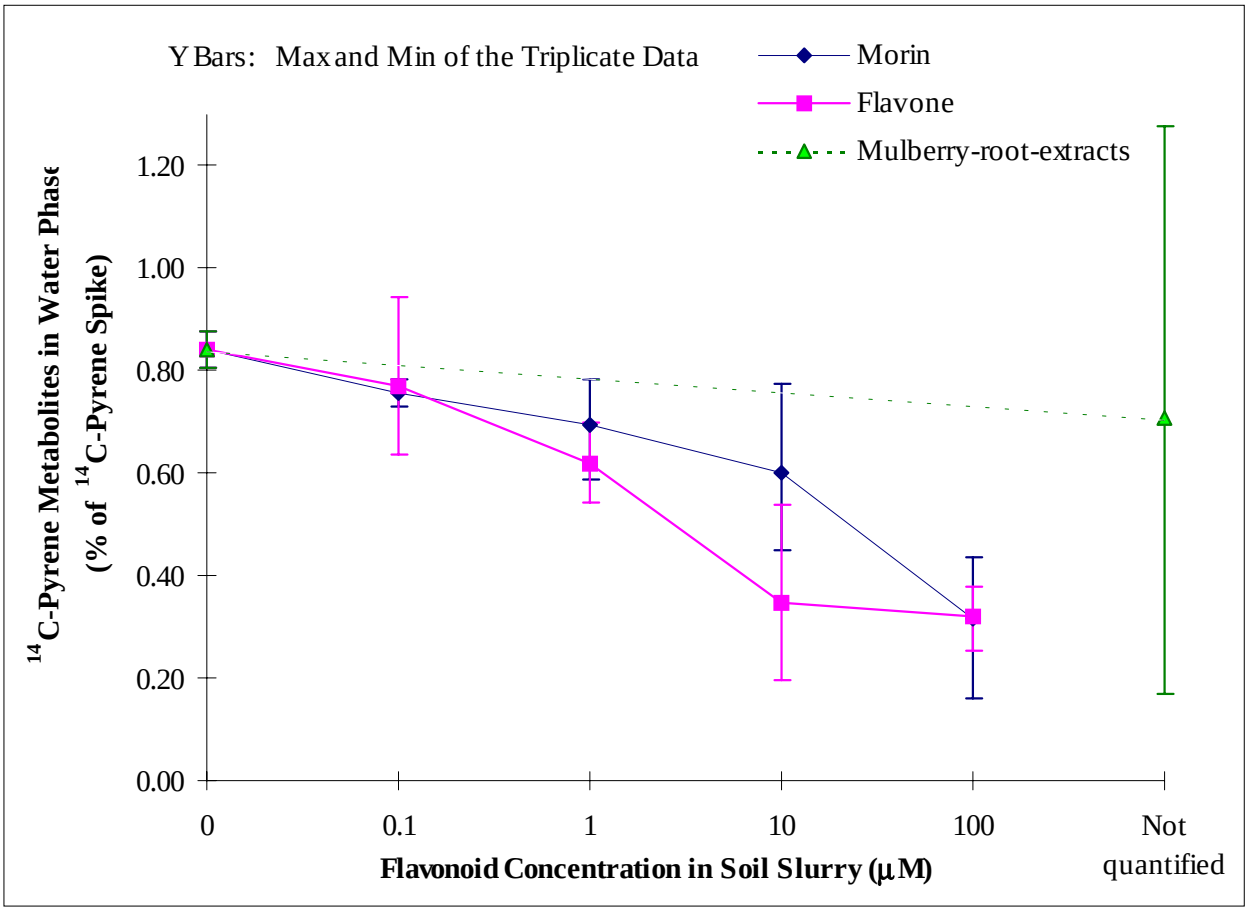


Figure 4.35. Water phase ^{14}C-pyrene metabolites[1] (%) versus flavonoid concentrations amended in Mulberry-rhizosphere-soil-slurry microcosm[2]

[1] hexane-nonextractable, polar ^{14}C in water phase

[2] poisoned microcosms simulate metabolic inhibited pseudo-abiotic condition

^{14}C-Pyrene Metabolites in Water Phase in Bermudagrass Soil

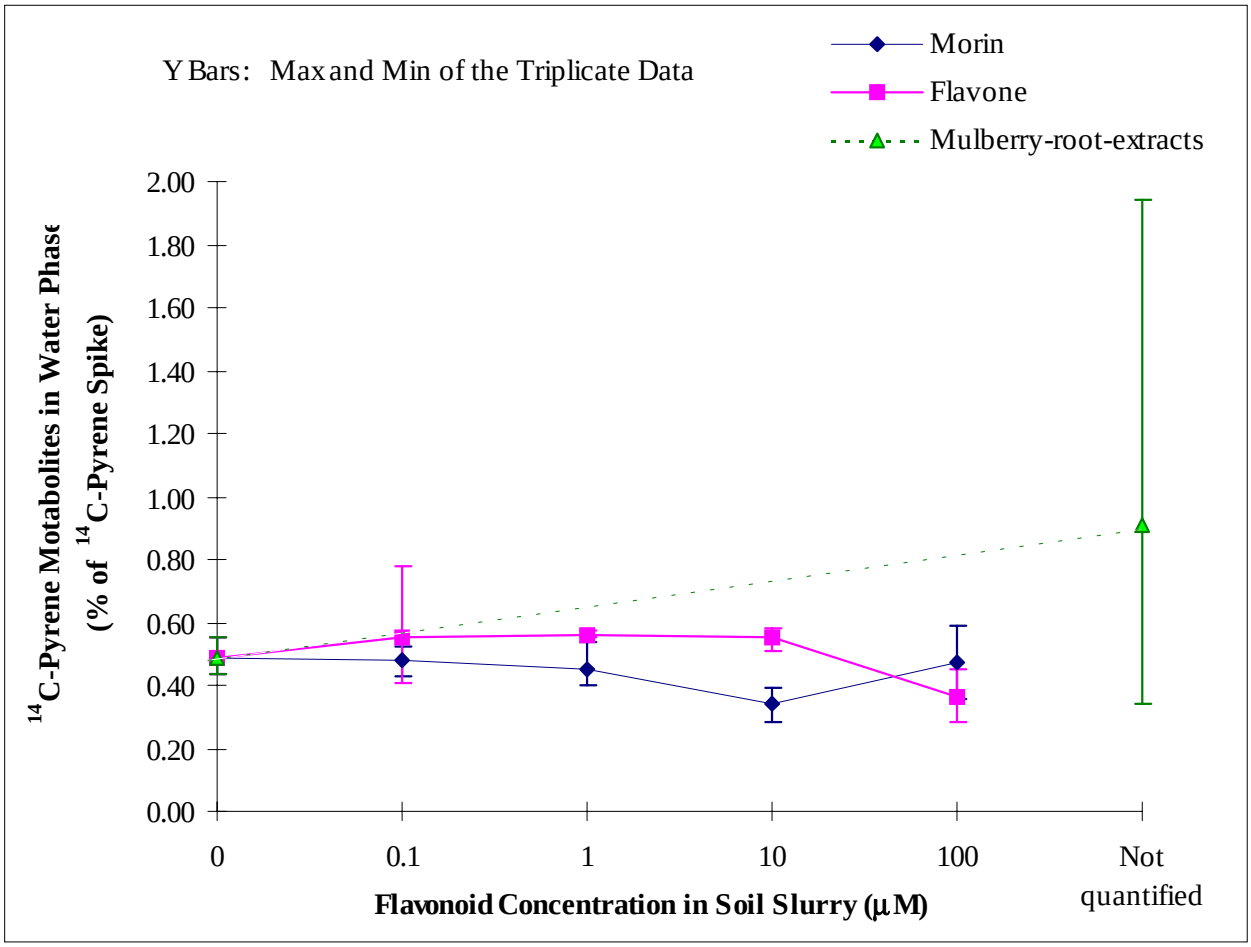


Figure 4.36. Water phase ^{14}C-pyrene metabolites[1] (%) versus flavonoid concentrations amended in Bermudagrass-rhizosphere-soil-slurry microcosm[2]

[1] hexane-nonextractable, polar ^{14}C in water phase

[2] poisoned microcosms simulate metabolic inhibited pseudo-abiotic condition

The aforementioned sections indicated that ^{14}C-pyrene fate in different types of soil was generally consistent with those of ^{14}C-B[a]P, except that water-phase ^{14}C-pyrene metabolites concentrations were slightly higher than their counterparts of ^{14}C-B[a]P. However, water-phase ^{14}C were negligible (<0.1 ug/l) in all the cases and decreased somewhat as flavone and morin concentration increased.

Distribution of ^{14}C-PAH among the Five Fate Mechanisms

Distribution of ^{14}C-B[a]P and 14C-Pyrene in Poisoned Mulberry Rhizosphere Soil Slurry

The percentage of ^{14}C-B[a]P and ^{14}C-pyrene associated with the five aforementioned fate mechanisms in poisoned Mulberry rhizosphere soil slurry microcosms are presented in Figures 4.37 and 4.38, respectively.

With regard to ^{14}C-B[a]P, greater than 99% of the ^{14}C remained associated with soil solid phases as either adsorption onto soil (solvent extractable) or soil bound residues (solvent nonextractable) (Figure 4.37), while mineralization fraction and partitioning to water phase were negligible. Without flavonoid added, approximately 50% was soil bound residues and the other half was adsorption on to soil. The partitioning between bound residue and adsorption remained approximately the same as morin and flavone concentrations increased to between 0.1 uM and 1 uM. Although the average partitioning to bound residues generally decreased with higher flavone or morin concentration or with Mulberry root abstract added, the decrease was statistically insignificant at 95% confidence level (see Appendix B-3). Exceptionally, with 10 uM morin added, partitioning appeared to favor soil bound residues, however, the change in partition was neither statistically significant at 95% confidence level (Appendix B-3).

With regard to ^{14}C-pyrene, greater than 99% of the ^{14}C remained associated with soil solid phases as either adsorption onto soil (solvent extractable) or soil bound residues (solvent nonextractable) (Figure 4.38), while mineralization fraction and partitioning to water phase were negligible. The partitioning of ^{14}C-pyrene between adsorption and bound residue was somewhat different from that of ^{14}C-B[a]P. With morin or Mulberry root extract added, a little over half of the ^{14}C was adsorption onto soil and less than half was soil bound residues. With flavone added, partitioning to adsorption increased and less than one third of the ^{14}C was bound. It is not known whether the differences in partitioning were statistically significant or not, because of the poor mass balances.

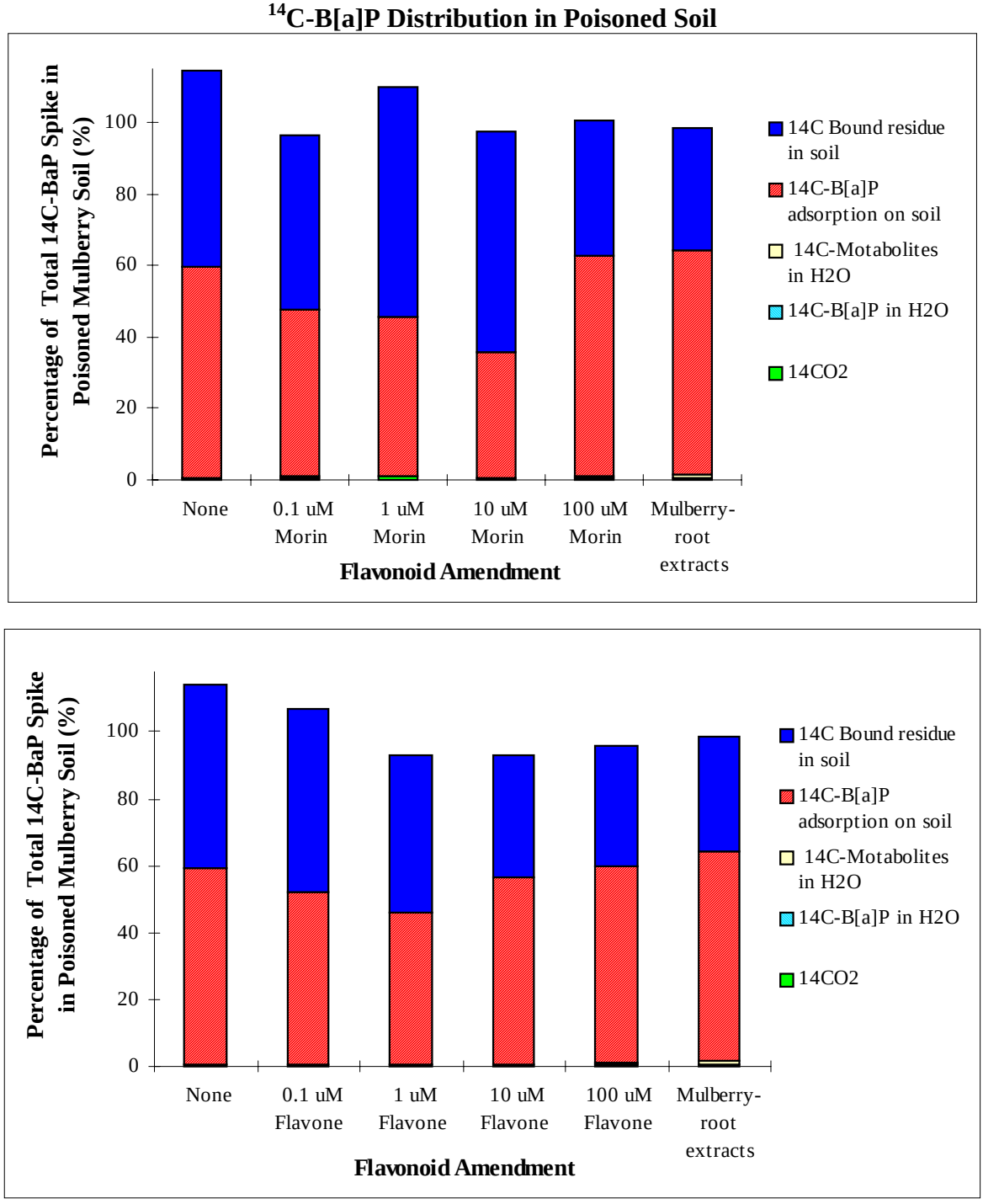


Figure 4.37. ^{14}C-B[a]P distribution among the five fate mechanisms versus flavonoid concentrations in poisoned-Mulberry-rhizosphere-soil-slurry microcosms[1]

[1] poisoned microcosms simulate metabolic inhibited pseudo-abiotic condition

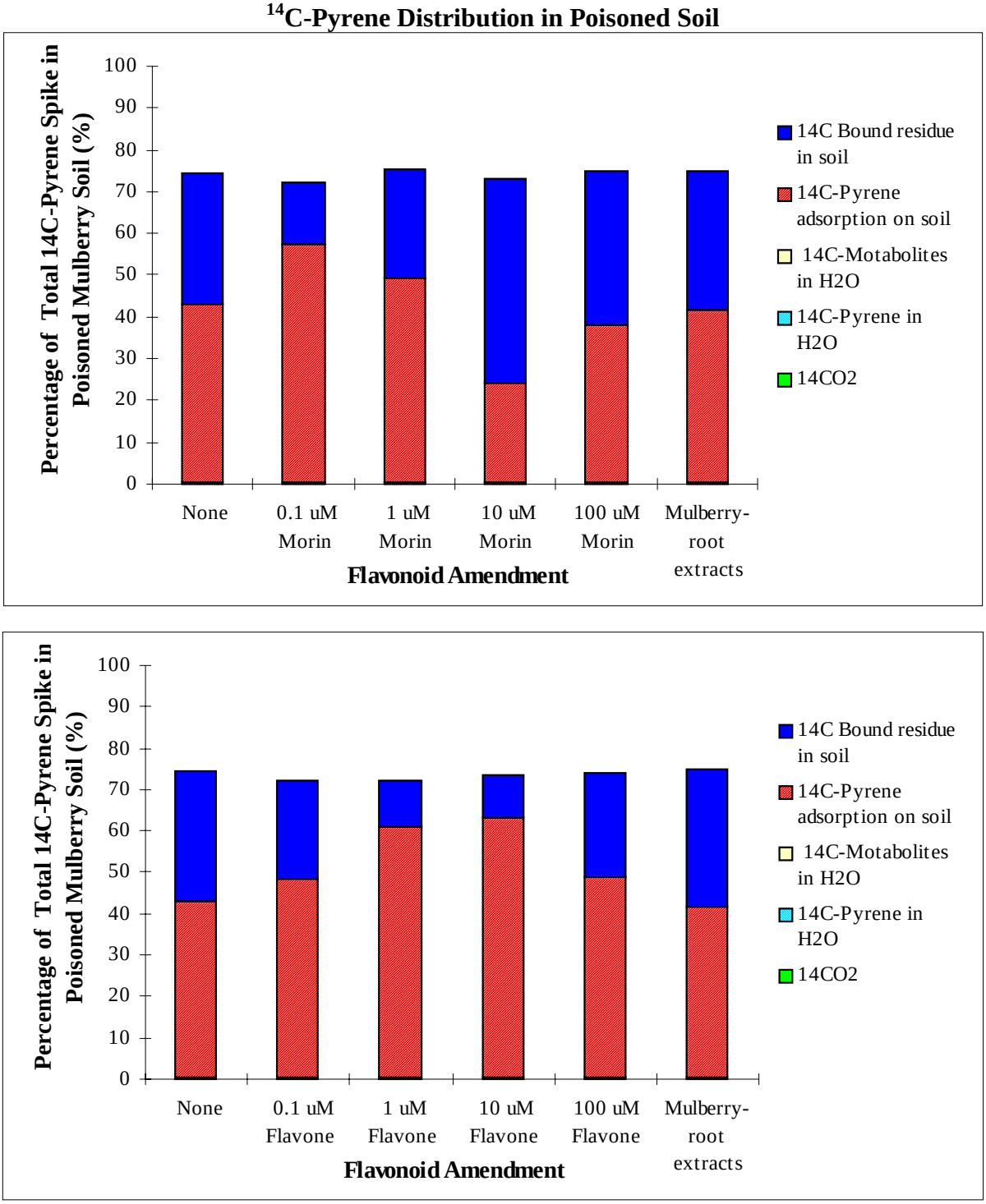


Figure 4.38. ^{14}C-pyrene distribution among the five fate mechanisms versus flavonoid concentrations in poisoned-Mulberry-rhizosphere-soil-slurry microcosms

Distribution of ^{14}C-B[a]P and ^{14}C-Pyrene in Mulberry Rhizosphere Soil Slurry

The percentage of ^{14}C-B[a]P and ^{14}C-pyrene associated with the five fate mechanisms in Mulberry rhizosphere soil slurry microcosms are presented in Figures 4.39 and 4.40, respectively.

With regard to ^{14}C-B[a]P, the ^{14}C associated with soil solid phases varied from greater than 75% to 98% (Figure 4.39), while mineralization varied from less than 25% to 2% and water phase fraction remained negligible. The amount of ^{14}C associated with soil generally increased as flavonoid concentration increased, meanwhile mineralization decreased. Partitioning between soil bound residues and adsorption remained approximately even, except with 100 uM flavone or Mulberry root extract added. Soil bound residue increased to over 60% with 100 uM flavone or Mulberry root extract. The increase was statistically significant at 95% confidence level (see Appendix B-4).

With regard to ^{14}C-pyrene, approximately half or a little over than half of the ^{14}C mineralized to CO_2 and less than half remained associated with soil solid phases as either adsorption onto soil (solvent extractable) or soil bound residues (solvent nonextractable) (Figure 4.38) when flavone and morin concentrations were between 0 and 10 uM. With 100 uM flavone or Mulberry root extract added, over two thirds of the ^{14}C became associated with soil and the mineralization reduced significantly. With 100 uM of morin, greater than 99% of the ^{14}C became associated with soil and the mineralization was negligible. Partitioning to water phase was negligible in all the cases. The partitioning of ^{14}C-pyrene between adsorption and bound residues was generally even except with Mulberry root extract added. With Mulberry root extract, approximately two thirds of the ^{14}C was adsorption onto soil, while only one third was soil bound residues. It is not known whether the differences in ^{14}C-pyrene partitioning were statistically significant or not, because of the poor mass balances.

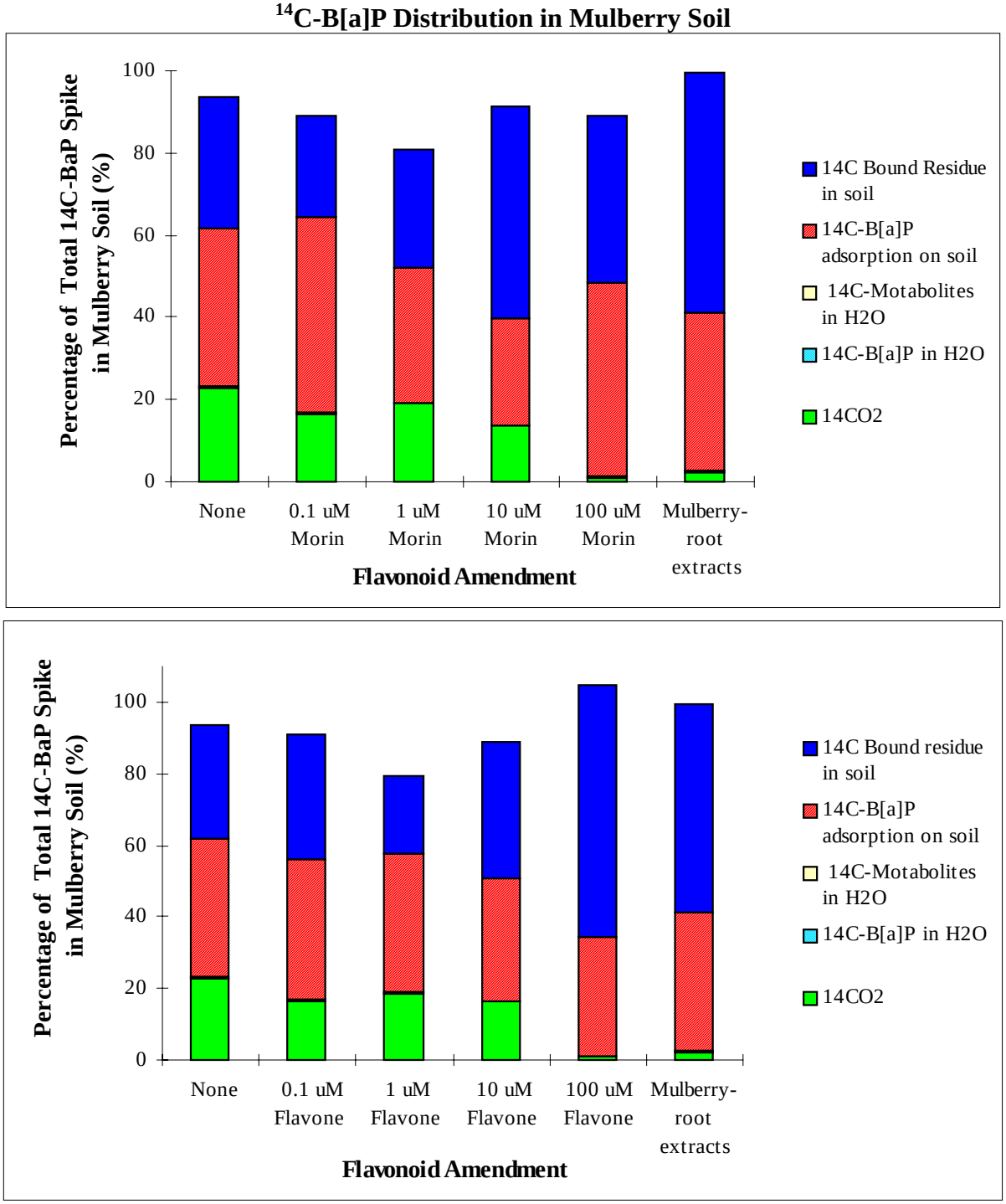


Figure 4.39. ^{14}C-B[a]P distribution among the five fate mechanisms versus flavonoid concentrations in Mulberry-rhizosphere-soil-slurry microcosms

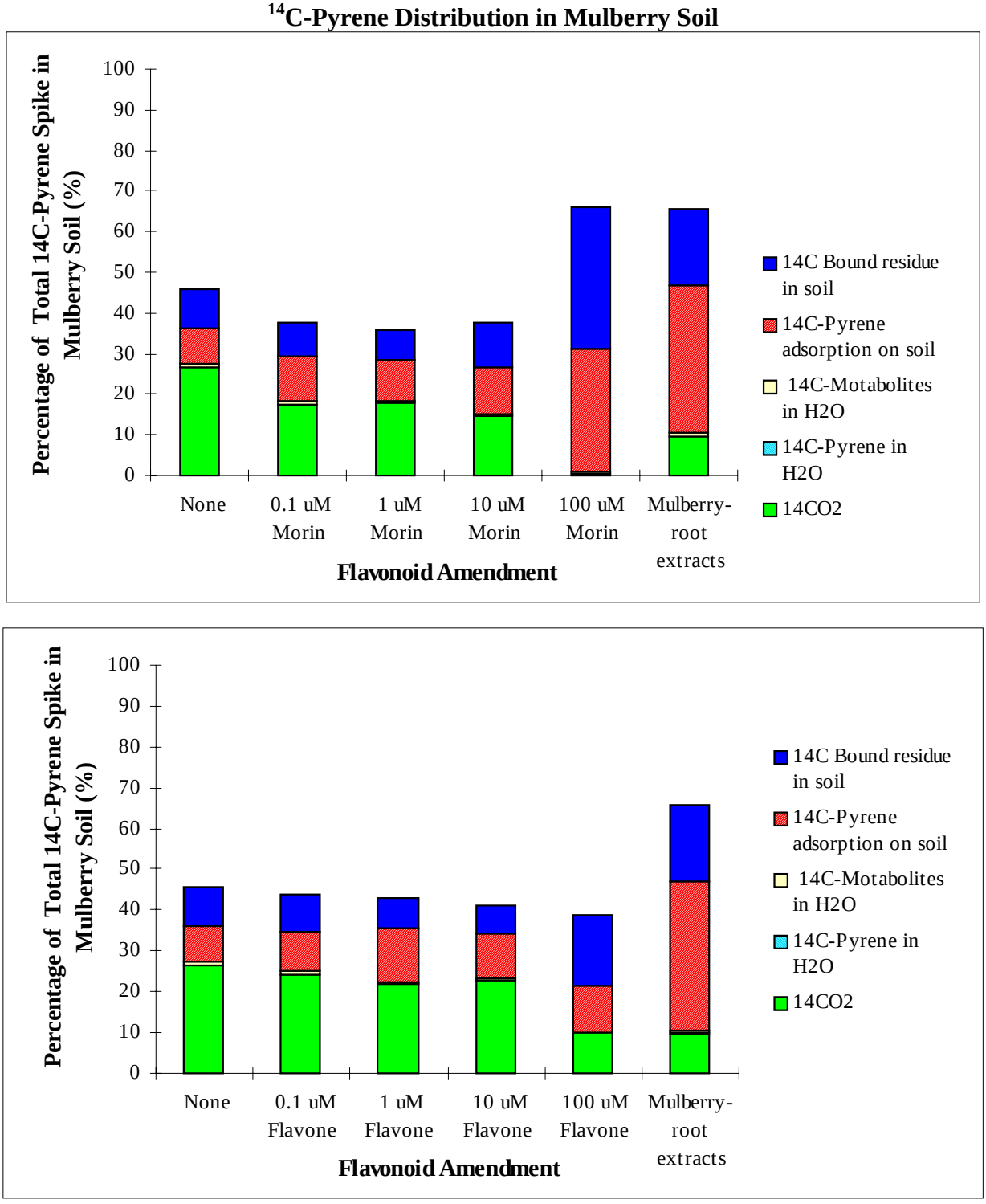


Figure 4. 40. ^{14}C-pyrene distribution among the five fate mechanisms versus flavonoid concentrations in Mulberry-rhizosphere-soil-slurry microcosms

Distribution of ^{14}C-B[a]P and ^{14}C-Pyrene in Bermudagrass Rhizosphere Soil Slurry

The percentage of ^{14}C-B[a]P and ^{14}C-pyrene associated with the five fate mechanisms in Bermudagrass rhizosphere soil slurry microcosms are presented in Figures 4.41 and 4.42, respectively.

With regard to ^{14}CB[a]P, the ^{14}C associated with soil solid phases varied from greater than 80% to 98% (Figure 4.41), while mineralization varied from less than 20% to 2% and water phase fraction remained negligible. The amount of ^{14}C associated with soil generally increased as flavonoid concentration increased, meanwhile mineralization decreased. Partitioning between soil bound residues and adsorption remained approximately even without flavonoid added. Soil bound residue increased as flavone concentration increased. With 100 uM flavone, 100 uM morin, or Mulberry root extract added soil bound residue increased to over 75%. The increase was statistically significant at 95% confidence level (see Appendix B-5).

With regard to ^{14}C-pyrene, approximately two thirds of the ^{14}C mineralized to CO_2 and one third remained associated with soil solid phases as either adsorption onto soil (solvent extractable) or soil bound residues (solvent nonextractable) when flavone and morin concentrations were between 0 and 1 uM (Figure 4.42). With 10 uM flavone or morin, approximately one half of the ^{14}C mineralized to CO_2 and one half remained associated with soil solid phases. With 100 uM flavone, 100 uM morin, or Mulberry root extract added, over 95% of the ^{14}C became associated with soil and the mineralization reduced significantly. Partitioning to water phase was negligible in all the cases. The partitioning of ^{14}C-pyrene between adsorption and bound residues was generally even with morin or Mulberry root extract added. With Flavone added, over two thirds of the ^{14}C associated with soil were bound residues, while less than one third was adsorption onto soil. It is not known whether the differences in ^{14}C-pyrene partitioning were statistically significant or not, because of the poor mass balances.

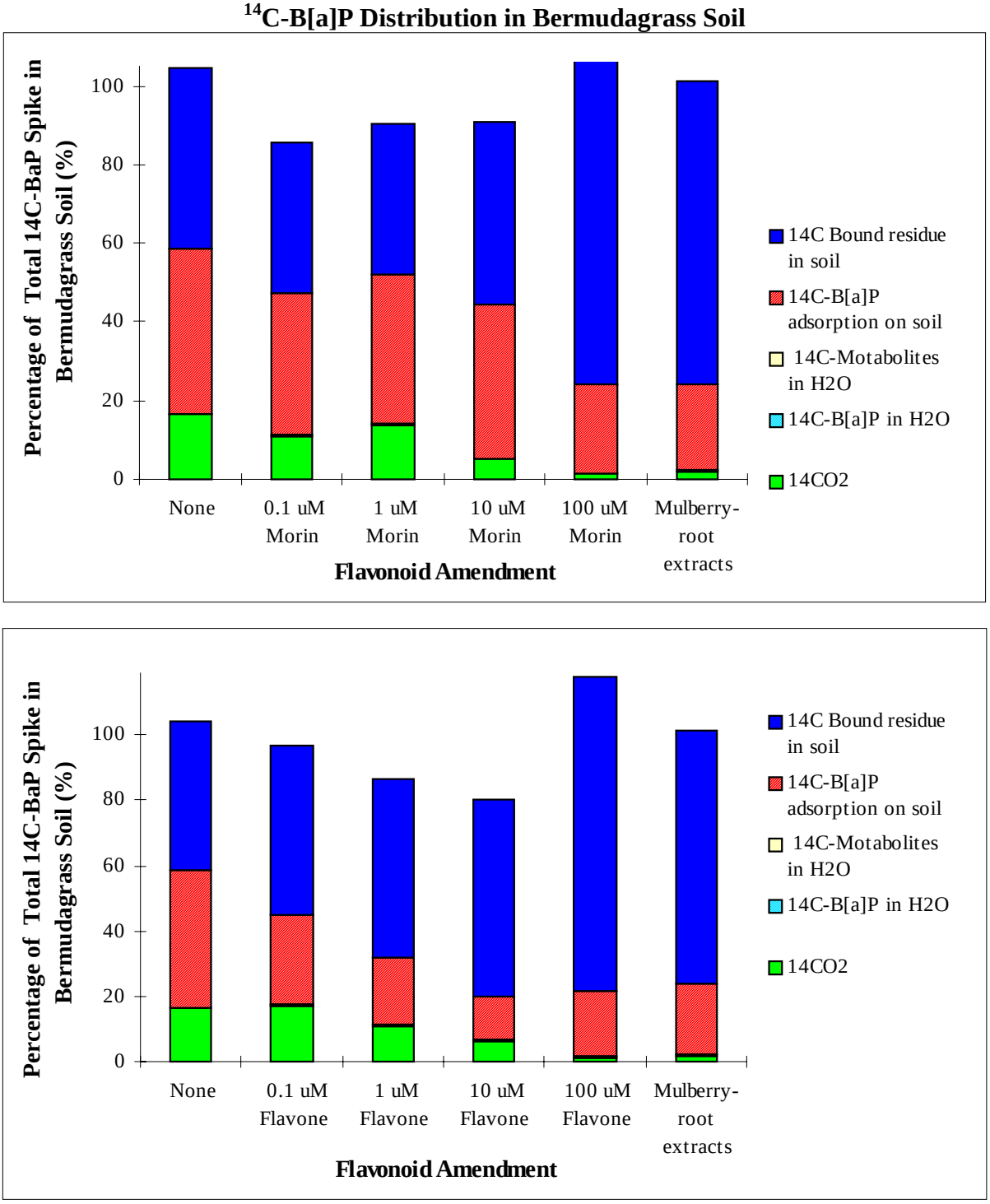


Figure 4.41. ^{14}C-B[a]P distribution among the five fate mechanisms versus flavonoid concentrations in Bermudagrass-rhizosphere-soil-slurry microcosms

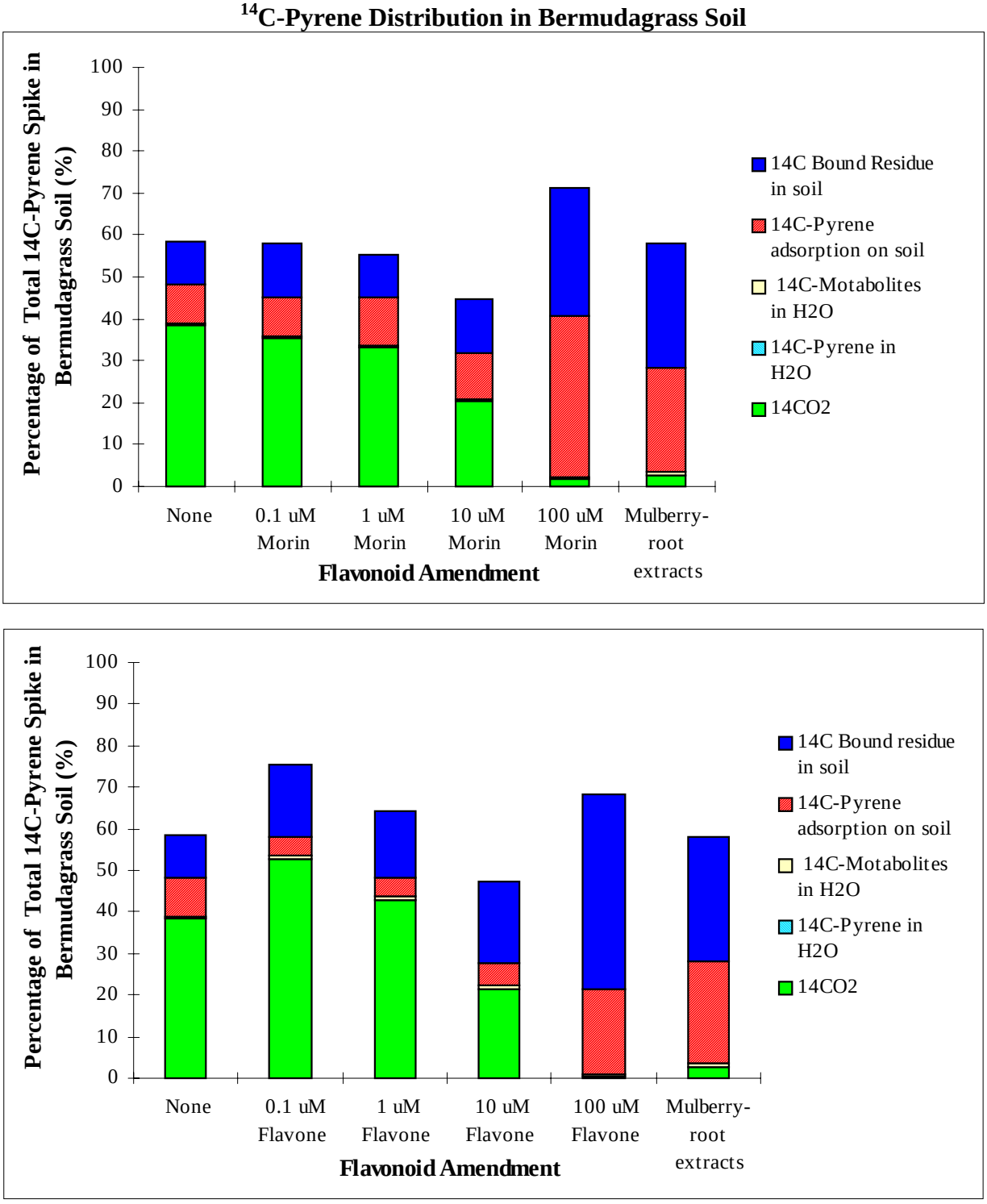


Figure 4.42. ^{14}C-pyrene distribution among the five fate mechanisms versus flavonoid concentrations in Bermudagrass-rhizosphere-soil-slurry microcosms

CHAPTER 5. DISCUSSION

Data presented in Chapter 4 indicate that a majority of the ^{14}C-PAH added into the soil were associated with soil. Partitioning of B[a]P and pyrene and/or metabolites in water phase was negligible. A minor fraction of ^{14}C was transformed into gas phase $^{14}CO_2$ via mineralization and negligible vapor via volatilization. One-way analysis of variance of ^{14}C-B[a]P and ^{14}C-pyrene fate date confirmed that flavonoid had major effects on PAH fate in soil, however, only at adequate concentration levels. In Chapter 5, the compound effects of flavonoid types, concentration, and soil types are evaluated and plausible mechanisms are discussed. Discussion relies more on ^{14}C-B[a]P data, because of poor mass balance in ^{14}C-pyrene-amended soil slurry microcosms. Possible reason causing poor ^{14}C-pyrene mass balance is further explored.

EFFECTS OF FLAVONOIDS ON SOIL BOUND RESIDUE FORMATION AND ADSORPTION OF B[A]P

Results from ^{14}C-B[a]P-amended soil-slurry microcosms indicated that over 70% to 99% of the radiolabeled carbon (^{14}C) remained associated with soil solids as either solvent extractable (adsorption) or solvent nonextractable (bound residue) fractions after 60 days of incubation for non-poisoned and poisoned treatments, respectively (Figures 4.37, 4.39, and 4.41). Soil bound residues consist of considerable amounts of the soil-associated ^{14}C-B[a]P. Although the measurable ^{14}C associated with ^{14}C-pyrene-amended soil were significantly less than those with ^{14}C-B[a]P, approximately 20%-40% unaccountable^{14}C was very likely to be soil bound resides.

Effects of Flavonoid Types on Bound Residue Formation from 7,10-^{14}C-B[a]P in Biologically Active Rhizosphere Soils versus "Poisoned" Soil

The effects of flavone, morin, and Mulberry root extract on soil-association of PAHs are determined based on statistical analysis at 95% confidence levels. Three types of flavonoids were used in the experiments. Flavone, a synthetic nonhydroxylated flavonoid, is not naturally present in plant roots. Morin (2',3,4',5,7-pentahydroxyflavone) is a common natural hydroxylated root flavonoid. Mulberry root extracts contains multiple hydroxylated flavones, complex root flavonoids, and many other root exudates. There were no statistically significant difference in bound residue formation of ^{14}C-B[a]P between flavone and morin when amended at the same concentration levels for either biologically active or poisoned soil treatment, except that soil bound residue with 100 uM of flavone was significantly higher than that with 100 uM morin in Mulberry rhizosphere soil slurry (Figure 4.11 and 4.39). Meanwhile, there were no statistically significant differences in ^{14}C-B[a]P adsorption between flavone and morin when amended at the same concentration levels for either biologically active or poisoned treatments, except that ^{14}C-B[a]P adsorption to Bermudagrass soil with 10 uM of flavone was significantly lower than that with 10 uM morin (Figure 4.15 and 4.41). There were no statistically significant differences in ^{14}C-pyrene adsorption between flavone and morin when amended at the same concentration levels for either biologically active or poisoned treatments, except that ^{14}C-pyrene adsorption to poisoned Mulberry soil with 10 uM of flavone was significantly higher than that

with 10 uM morin (Figures 4.15 and 4.41). The amount of soil bound residues in Mulberry root extract-amended soils were generally as high as those with 100 uM morin or Flavone added.

In Figures 4.12 and 4.27, the average ^{14}C-B[a]P- and ^{14}C-pyrene-soil-bound residues in Bermudagrass soil amended with flavone appear to be somewhat higher than those amended with morin, however, the differences were not statistically significant at 95% confidence levels. In Figures 4.15 and 4.30 the average ^{14}C-B[a]P adsorption onto Bermudagrass soil amended with flavone appears to be slightly lower than those amended with morin, however, the differences are not statistically significant except at 10 uM concentration levels.

Although consistently statistically significant different effects as a whole between flavone and morin were not observed with regard to soil bound residue formation and adsorption of B[a]P or pyrene in this experiment, nonhydroxylated flavone amendment appeared to result relatively more bound residues and less adsorption of ^{14}C-B[a]P than hydroxylated morin and Mulberry root extracts.

Effects of Flavonoid Concentrations on Bound Residue Formation from 7,10-^{14}C-B[a]P in Biologically Active Rhizosphere Soils versus "Poisoned" Soil

Statistical analyses indicate that ^{14}C bound residue formation from 7,10-^{14}C-B[a]P and 4,5,9,10-^{14}C-pyrene in the two biologically active rhizosphere soil slurry microcosms (Table 4.6 and Figures 4.11 and 4.12) increased significantly when 100 uM flavone (100 uM) was added. When 100 uM morin was added ^{14}C bound residue was significantly increased in Bermudagrass rhizosphere soils, but not in Mulberry rhizosphere soil. When Mulberry root extracts (855 mg-TOC/L) was added, ^{14}C-B[a]P-bound residues were also significantly increased in the two biologically active soils. With Mulberry root extract added ^{14}C-pyrene-soil-bound residues increased in Bermudagrass rhizosphere soils, but not in Mulberry rhizosphere soil. In contrast, 100 uM flavone, 100 uM morin, or Mulberry root extract did not increase bound residue formation in ^{14}C-B[a]P- and ^{14}C-pyrene-amended-poisoned Mulberry rhizosphere soil. At low to medium concentrations (0.1 uM, 1 uM, and 10 uM) neither morin nor flavone, had statistically significant effects on ^{14}C bound residue formation in biologically active or poisoned (Table 4.6). In ^{14}C-B[a]P amended Bermudagrass-rhizosphere soil, average ^{14}C bound residues of the triplicate microcosms increased slightly as flavone concentration increased from zero to 10 uM (Figure 4.12), however, the increase was statistically insignificant at 95% confidence level. Further, average bound residue formation in ^{14}C-B[a]P amended poisoned Mulberry rhizosphere soil decreased slightly as flavone amendment increased, however, the decrease was statistically insignificant at 95% confidence level (Figures 4.10 and Table 4.6). The aforementioned results indicate that flavone, morin and Mulberry root extract amendments had increased ^{14}C-PAH soil bound residue formation, however, only at the higher concentration level (100 uM). The enhanced bound residue formation was observed in biologically active soils but not in poisoned soil. The amounts of bound residues in biologically active soils were significantly higher than that in poisoned soil with high concentration flavonoids, but not without flavonoids. As a result, microbial activity is likely the agent of enhanced soil bound residue formation from ^{14}C-B[a]P.

The Amount of Soil Bound Residue Formation in Loamy Sand Soil versus Sandy Clay Loam Soil

Aforementioned discussion has shown that in biologically active rhizosphere soils both nonhydroxylated flavone and hydroxylated morin promoted ^{14}C bound residue formation of ^{14}C-PAH, however, only at higher (100 uM) concentration level. So did Mulberry root extract (855 mg-TOC/L). Whereas, the degree of effects depended on the types of soil. The influence of soil types on B[a]P bound residue formation in soil was further evaluated based on One way ANOVA *Student's t* tests presented in Appendix D-7 through D-14. The Mulberry rhizosphere soil used in this experiment is a loamy sand soil containing 6% clay, 12% silt, and 82% sand, while the Bermudagrass soil is a sandy clay loam soil containing 27% clay, 23% silt, and 50% sand. In addition, Mulberry soil contains 3% SOM, 3779 mg/kg of humic acids, and 3653 mg/kg of fulvic acids. These numbers are consistently lower than 5.2% SOM, 5240 mg/kg of humic acids and 3717 mg/kg of fulvic acids for the Bermudagrass soil.

In Figure 5.1, ^{14}C bound residue formation from parent ^{14}C-B[a]P in the biologically active Bermudagrass and Mulberry rhizosphere soils, and poisoned Mulberry rhizosphere soil, amended with none, Mulberry root extract, 100 μM morin, and 100 μM flavone, are compared. Without flavonoid, there were no statistically significant differences among the ^{14}C-soil bound resides in ^{14}C-B[a]P amended poisoned Mulberry (55%), non poisoned Mulberry (32%), and Bermudagrass (45%) rhizosphere soils (see table 4-9, and Appendix D-7). With 100 uM morin, average ^{14}C bound residues in the poisoned Mulberry rhizosphere soil slightly increased to 43%, which was not significantly different from the 37% in the poisoned counterpart. In contrast, with 100 uM morin, average ^{14}C bound residues in the sandy clay loam Bermudagrass rhizosphere soil increased significantly to approximately 83%, which statistically significantly higher than those in the loamy sand nonpoisoned Mulberry and the poisoned Mulberry rhizosphere soils (see Fig 5.1, Table 4.9, and Appendix D-8). With 100 μM flavone, average ^{14}C bound residue formation in poisoned Mulberry soil decreased approximately 36%, while the bound residues increased significantly to approximately 70% and 95% in nonpoisoned Mulberry and Bermudagrass rhizosphere soils, respectively. The differences between Bermudagrass soil and Mulberry soil as well as between poisoned and nonpoisoned Mulberry soils were statistically significant at 95% confidence levels (Table 4.9 and Appendix D-10). Likewise, amended with Mulberry root extract the average ^{14}C bound residues decreased in poisoned Mulberry soil, but increased in nonpoisoned Mulberry and Bermudagrass soils. With Mulberry root extract, average ^{14}C bound residues in the loamy sand nonpoisoned Mulberry rhizosphere soil was approximately 58%, which was not significantly different from the 34% in the poisoned counterpart (Table 4.9 and Appendix D-9). In contrast, amended with Mulberry root extract, average ^{14}C bound residues in the sandy clay loam Bermudagrass rhizosphere soil was approximately 75%, which was statistically significantly higher than that in the poisoned loamy sand Mulberry rhizosphere soil (Table 4.9 and Appendix D-9).

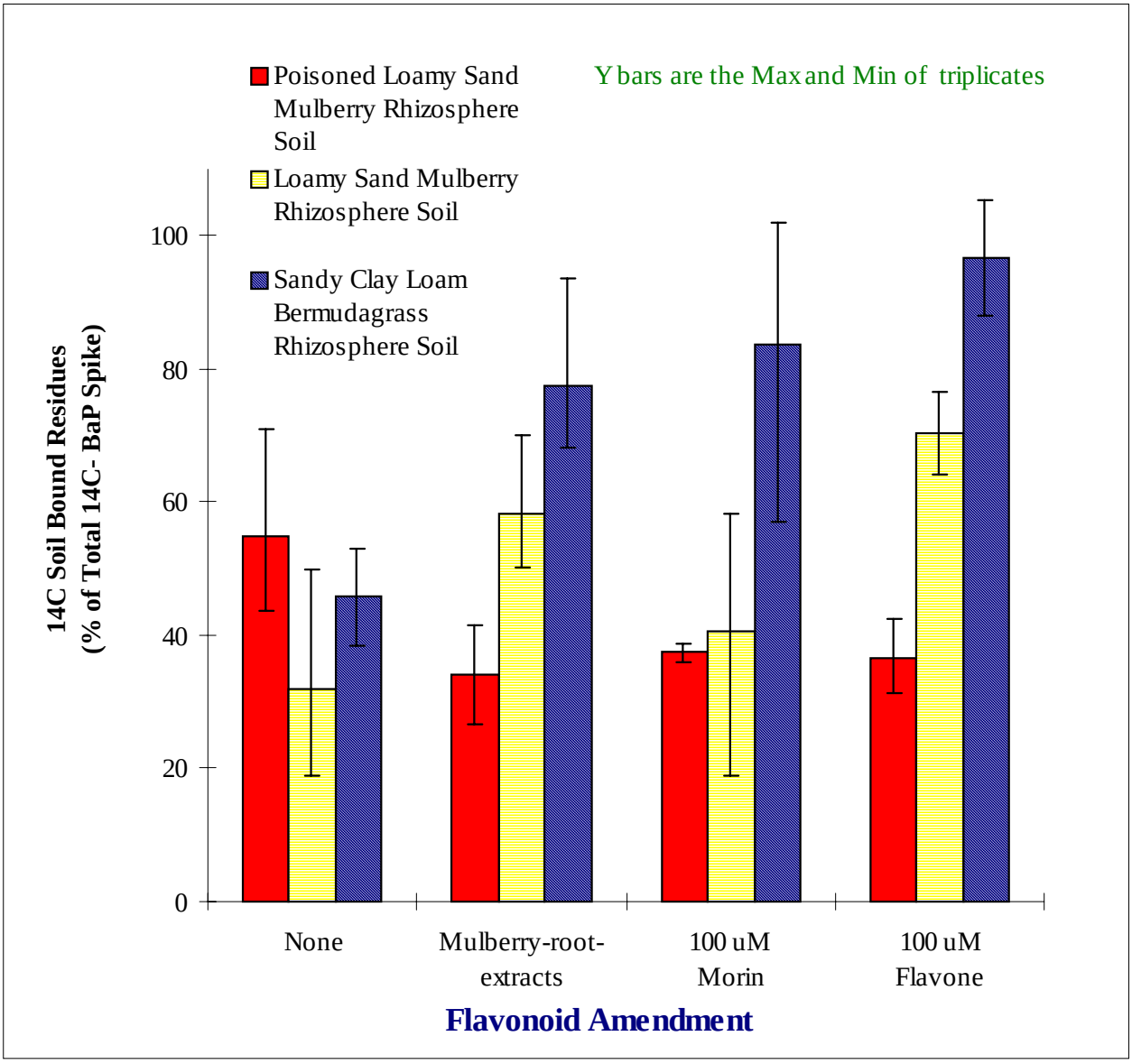


Figure 5.1. Comparison of soil bound residue formation from ^{14}C-B[a]P in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone

These data indicate ^{14}C-B[a]P-soil-bound residue formation was enhance significantly in the sandy clay loam Bermudagrass rhizosphere soil when amended with 100 uM morin or Mulberry root extract, while it was not enhanced or significantly less enhanced in the loamy sand Mulberry rhizosphere soil. Soil organic matter (SOM), humus, and clay contents may have attributed to the different degree of bound residue formation between the loamy sand Mulberry rhizosphere soil and sandy clay loam Bermudagrass rhizosphere soil. The Mulberry soil contains 6% clay, 12% silt, and 82% sand, while the Bermudagrass soil contains 27% clay, 23% silt, and 50% sand. In addition, Mulberry soil contains 3% SOM, 3779 mg/kg of humic acids, and 3653 mg/kg of fulvic acids. These numbers are consistently lower than 5.2% SOM, 5240 mg/kg of humic acids and 3717 mg/kg of fulvic acids for the Bermudagrass soil.

As a result the overall bound residue formation in the relatively clayey and organic-rich Bermudagrass soil was higher than that in the loamy sand Mulberry soil. Further, Bermudagrass also has higher cation exchange capacity, which may provide more binding sites.

These findings are consistent with that reported in literature (Nieman *et al.* 1999, Carmichael and Pfaender 1997). Nieman *et al.* (1999) reported the humic acid fraction of soil organic carbon was the primary accumulator of ^{14}C in biologically active microcosms, although an increase was observed in all organic carbon fractions over time. The Bermudagrass soil used in the experiment contained 5240 mg/kg of humic acids compared to 3779 mg/kg in the Mulberry soil.

In Figure 5.2, ^{14}C bound residue formation from parent ^{14}C-pyrene in the biologically active Bermudagrass and Mulberry rhizosphere soils, and poisoned Mulberry rhizosphere soil, amended with none, Mulberry root extract, 100 μM morin, and 100 μM flavone, are compared. There were no statistically significant differences at 95% confidence levels among the ^{14}C-pyrene-soil bound resides in poisoned Mulberry, non poisoned Mulberry, and Bermudagrass rhizosphere soils (see table 4-9, and Appendices D-11 through D-14). The amount of ^{14}C-pyrene-soil-bound residues was apparently less than their counterparts of ^{14}C-B[a]P-soil bound residues. It is suggested that a portion of ^{14}C-pyrene-soil-bound residues diffused deep into soil micropores or SOM may not be accountable by Liquid Scintillation Analyzer. More details are discussed is in the subsequent section.

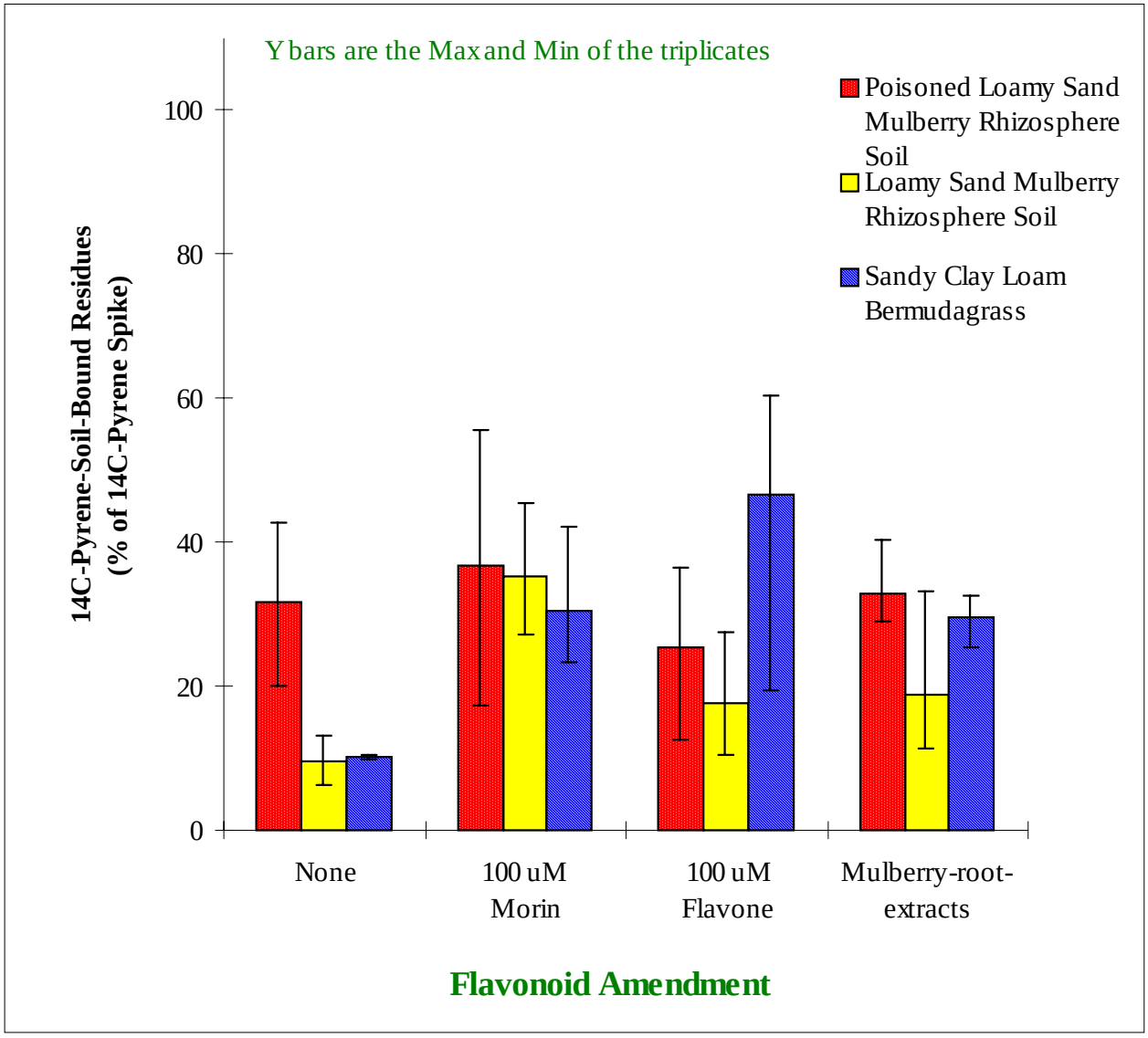


Figure 5.2. Comparison of soil bound residue formation from ^{14}C-pyrene in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone

Adsorption of ^{14}C-B[a]P and ^{14}C-Pyrene onto Poisoned and Nonpoisoned Loamy Sand Mulberry Soils versus Sandy Clay Loam Bermudagrass Soil

Solvent-nonextractable ^{14}C-bound residues changed significantly as flavonoid concentration increased to high levels in biologically active soils. Meanwhile, solvent-extractable ^{14}C-B[a]P, that is adsorption onto soil, generally did not change in either biologically active or poisoned soil slurry soils (Figures 4.13, 4.14, and 4.15). Although the average extractable ^{14}C-B[a]P decreased as flavone and morin concentration increased or as Mulberry root extract was added in sandy clay loam Bermudagrass soil, the decrease was statistically insignificant at 95% confidence levels.

Comparison of ^{14}C-B[a]P adsorption in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone are illustrated in Figure 5.3. Without flavonoid, ^{14}C-B[a]P adsorption in the three soils are not significantly different at 95% confidence levels (Table 4.9 and Appendix D-7). When adequate amount of flavone, morin, or Mulberry root extract was added into biologically active soils, less ^{14}C became extractable by solvent. With Mulberry root extract and 100 uM Flavone, average ^{14}C-B[a]P adsorption in the biologically active Bermudagrass (approximately 20%) and Mulberry (35-40%) soils was significantly lower than that in the poisoned Mulberry soil (Figure 5.3, Appendices D-9 and D-10). With 100 uM morin, average ^{14}C-B[a]P adsorption (20%) in the sandy clay loam Bermudagrass soil was statistically significantly lower than those (50%) in the nonpoisoned and (65%) nonpoisoned loamy sand Mulberry soils (Figure 5.3, Appendix D-8). With small amounts (0.1 - 1 uM) or without flavonoid, approximately 50% or more ^{14}C-B[a]P was solvent extractable in all soils (Figures 4.37, 4.38, and 4.39). Evidently, flavonoid amendment significantly reduced solvent extractable B[a]P in biologically active soils, especially in clayey Bermudagrass soil. In other words, flavonoid amendment enhanced B[a]P stabilization in soil. Carmichael and Pfaender (1997) reported that a majority amount of the ^{14}C-B[a]P added was extractable by ethylacetate solvent in the abiotic control microcosms. The finding was generally consistent with those in this experiment, however, less (< 60%) ^{14}C-B[a]P was found extractable by ethylacetate in the poisoned Mulberry rhizosphere soil in this experiment. B[a]P stabilization may have been enhanced by abiotic interaction with SOM in the rhizosphere soil used in this experiment compared to nonrhizosphere soil used by Carmichael and Pfaender.

Comparison of ^{14}C-pyrene adsorption in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone are illustrated in Figure 5.4. Without flavonoid, ^{14}C-B[a]P adsorption in the two biologically active soils were significantly less that that in the poisoned Mulberry soil at the 95% confidence levels (Table 4.9 and Appendix D-11). When adequate amount of flavone, morin, or Mulberry root extract was added into biologically active soils, more ^{14}C became extractable by solvent. As a result, there were no statistically significant differences at 95 confidence levels in ^{14}C-pyrene adsorption among the poisoned, nonpoisoned Mulberry, and Bermudagrass soils (Table 4.9 and Appendices D-12, 13, and 14). However, this observation may be uncertain because of the poor mass balance.

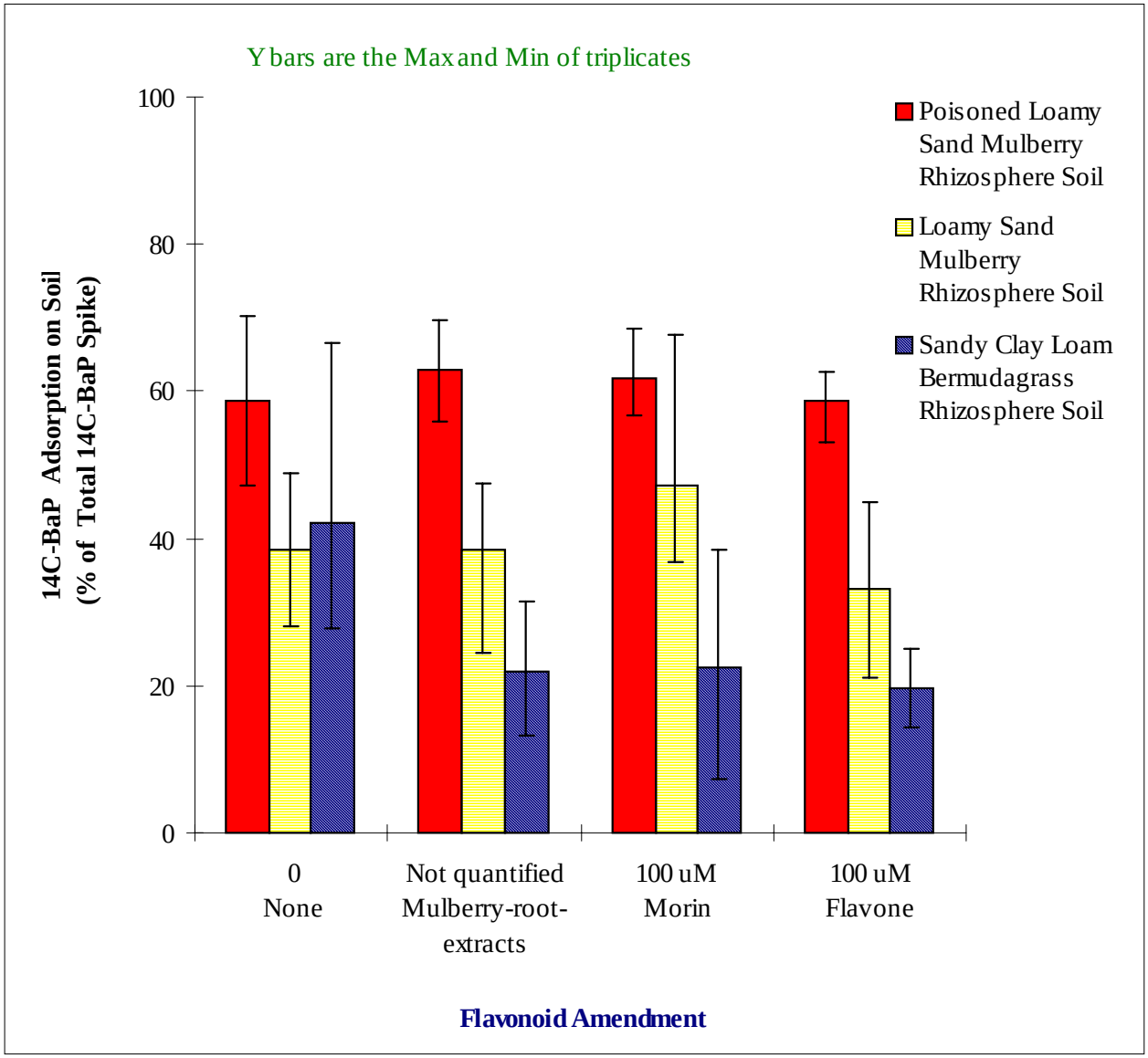


Figure 5.3. Comparison of ^{14}C-B[a]P adsorption in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone

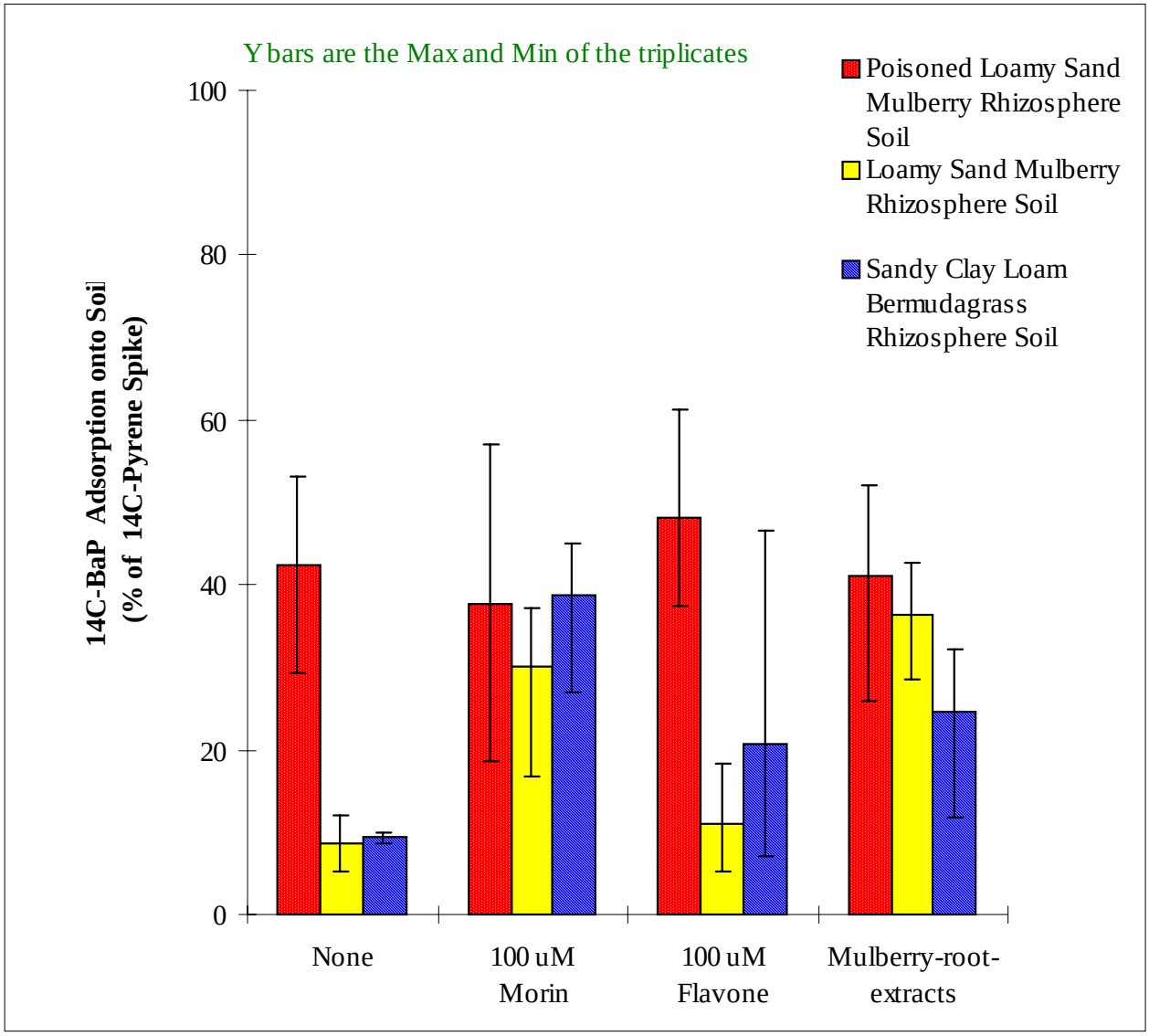


Figure 5.4. Comparison of ^{14}C-pyrene adsorption in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone

Possible Mechanism of Enhanced Bound Residue Formation with Flavonoids

Soil bound residues may become associated with components of the soil matrix through several mechanisms including covalent bonding via biologically and abiotically mediated oxidative coupling reactions to humic substances (Bollag and Myers 1992, Whelan and Sims 1992, Stone 1987) and intramicropore or intra-organic-matter diffusion into organic soil components (Luthy *et al.* 1997).

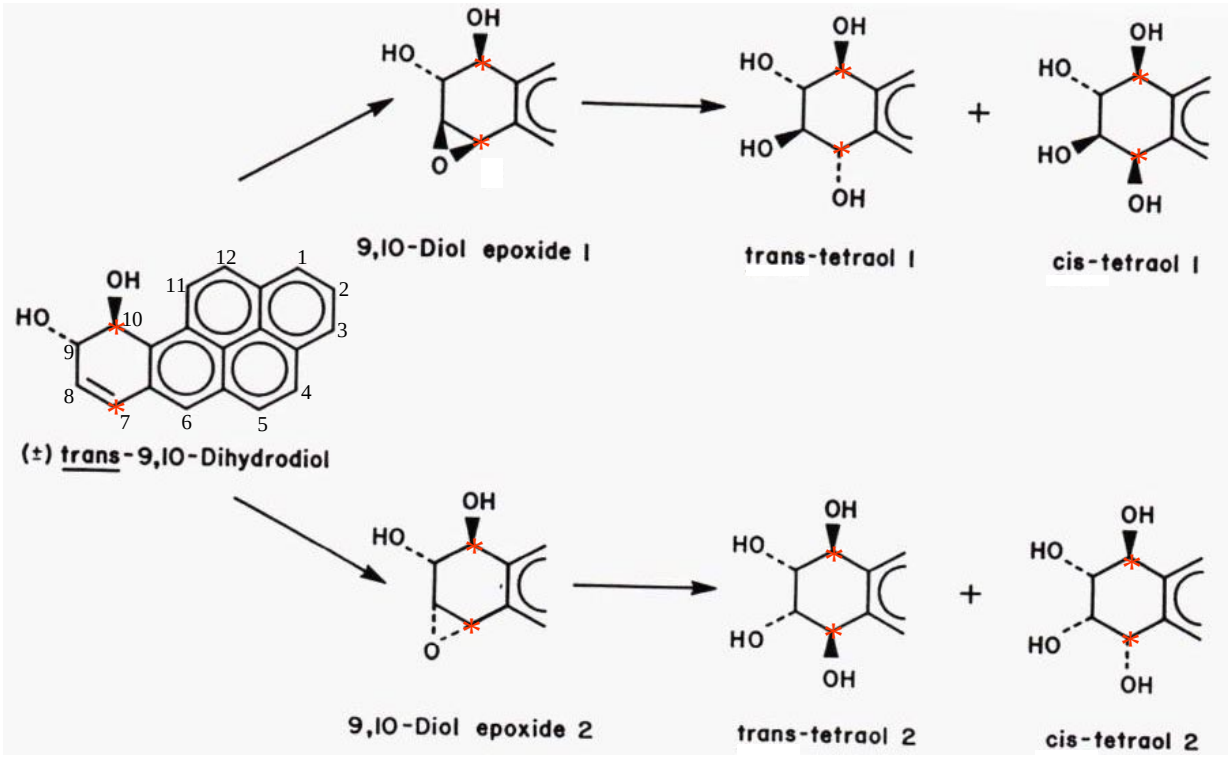
Humification The term humification has been used by researchers to address the polymerization of xenobiotic chemical metabolites with soil humus via covalent binding (Sims and Abbott 1992, Whelan and Sims 1992, Nieman *et al.* 1999). Soil bound residues may be associated but not limited to humic acids and perhaps other organic carbon fractions in biological active soil due to covalent and noncovalent bonding (Burgos, Novak, and Berry 1996). Covalent bonding through oxidative coupling would result in stable metabolite-organic matter complexes that would be sufficiently stable and biounavailable (Bollag 1992, Whelan and Sims 1992, Loehr and Webster 1997). Noncovalent metabolite-organic matter interactions may allowing soil bound organics to release slowly and be mineralized by the microbial community. Resistance to organic solvent extraction indicates that B[a]P metabolite-bound residues that increased under biologically active conditions may be covalent in nature. Nieman *et al*. (1999) reported that the bound lipid component of the soil humin was the primary sink of bound ^{14}C under biologically inhibited conditions in a loam soil (50% sand, 38% silt, and 12% clay) with 1.43% organic carbon, previously contaminated with PAHs and PCP (pentachlorophenol). In this experiment, the enhanced bound residue formation was most likely biologically mediated, because bound residue increase was not observed in metabolically inhibited poisoned Mulberry soil.

Microbial organisms convert polynuclear aromatic hydrocarbons to intermediate arene oxides, which then either isomerize to a phenol or undergo enzymatic hydration to a dihydrol. These products may completely degrade to CO_2 and H_2O (Miller and Miller 1985, Cerniglia 1993, Sutherland 1995). Alternatively, intermediate metabolites may bind to biofilm or humic substances forming biological inactive products (Yang 1988). For example, lignin-degrading microoorganisms secrete phenol-polymerizing enzymes to bind degraded lignin-derived phenols and detoxify their environment (Richnow *et al.* 1997). The presence of ether-linked xenobiotic moieties in humic substances may indicate that oxidoreductases are involved in the polymerization processes (Richnow *et al.* 1997). The ability of soil-borne microorganisms to detoxify their habitats by binding natural toxic substances to humic substances may lead to the formation of soil-bound residues with xenobiotics. Ether- and C-C linkages are relatively stable chemical bonds. Therefore, these types of humic substance-bound residues appear to be a sink for xenobiotic detoxification.

Since PAH do not possess any coupling groups, PAH may only become susceptible to oxidative coupling if reactive metabolites are produced during degradation. Partially oxidized PAH metabolites, such as phenols, may then become covalently bound to the soil organic matter (Eschenbach, Wienberg, and Mahro 1998). Covalent ester bonds between different PAH metabolites and humic polymers had been identified (Richnow *et al.* 1997). These bound

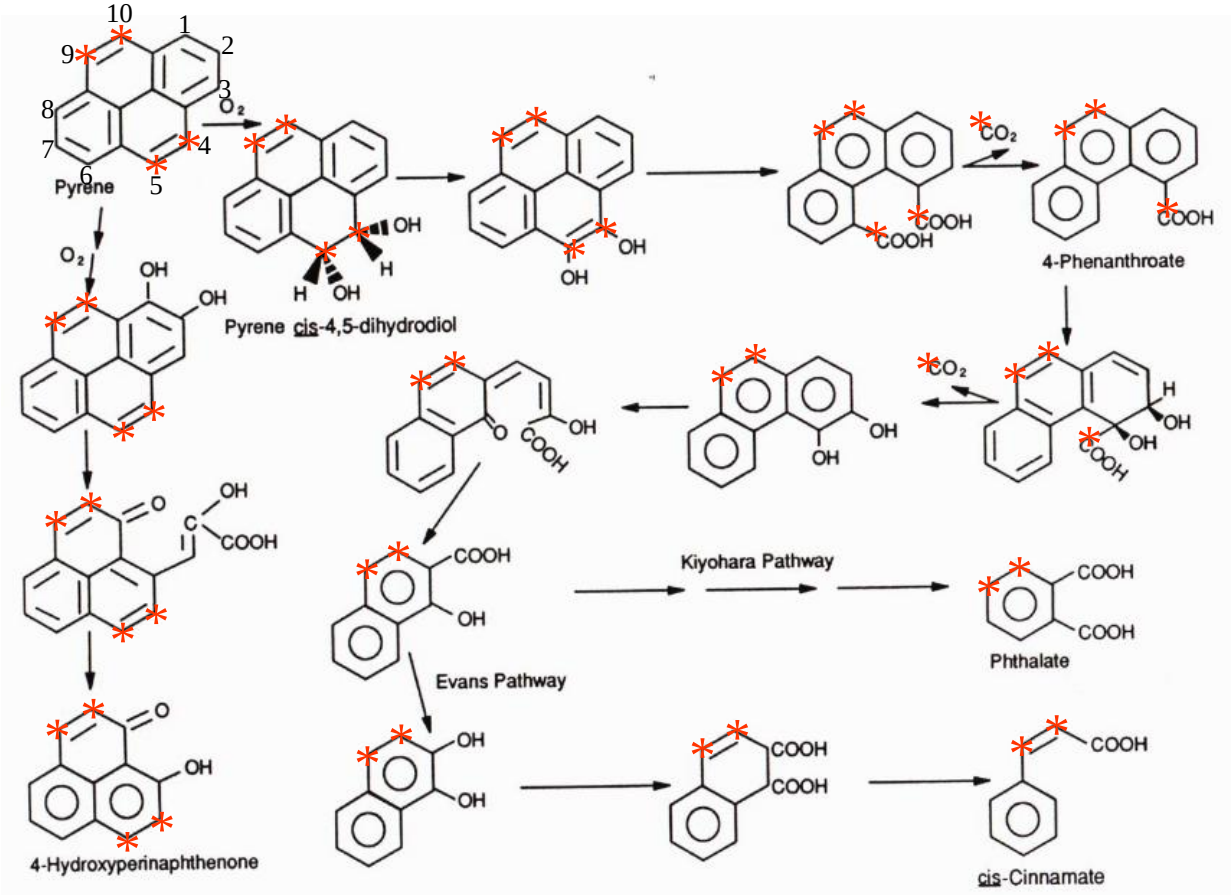
residues may range from simple transformation products, which could be released in forms similar to parent PAHs, to more extensively degraded metabolites, as shown in Figures 5.5 and 5.6.

The enzyme-catalyzed polymerization of phenol derivatives has been proposed as a major pathway to incorporate xenobiotics into humic material (Bollag 1992). Oxidoreductase enzymes such as peroxidase, laccase and tyrosinase are known to oxidize phenolic compounds to aryloxy radicals, which then polymerize to form insoluble humic acid like complexes (Martin and Haider 1980, Sarkar and Bollag 1988). Phenolic metabolites either derived from SOM or PAH can be cross linked to humic substances via ether or carbon-carbon bonds (Figure 2.12).



* indicates radiolabeled carbon position on 7,10-^{14}C-B[a]P used in this experiment

Figure 5.5. B[a]P metabolites of typical microbial degradation pathways



* indicates radiolabeled carbon position on 4,5,9,10-^{14}C-pyrene used in this experiment

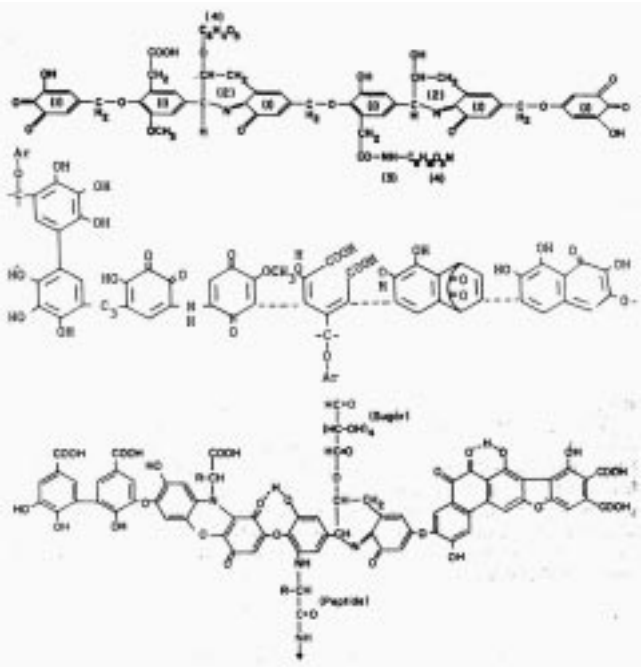
Figure 5.6. Pyrene metabolites of typical microbial degradation pathways

In this experiment, when adequate amount of morin, flavone, or Mulberry root extract was added into the biologically active Mulberry or Bermudagrass rhizosphere soils, soil bound residue increased significantly. The nonextractable ^{14}C bound residue can either the entrapment of parent ^{14}C-B[a]P or covalently binding of oxidative metabolites to SOM or both. The role of flavonoids in enhanced bound residue formation of B[a]P was not defined. However, it is suggested that flavonoids and their metabolic products could have provided numerous binding sites as bridges for B[a]P metabolites binding and polymerization to SOM. Many plant flavonoids occur in the form of conjugates where they may be attached to a rather wide variety of different monomeric or oligomeric compounds (Barz and Hösel 1975). Conjugation drastically alters the chemical properties of compounds, which may be converted into a metabolically

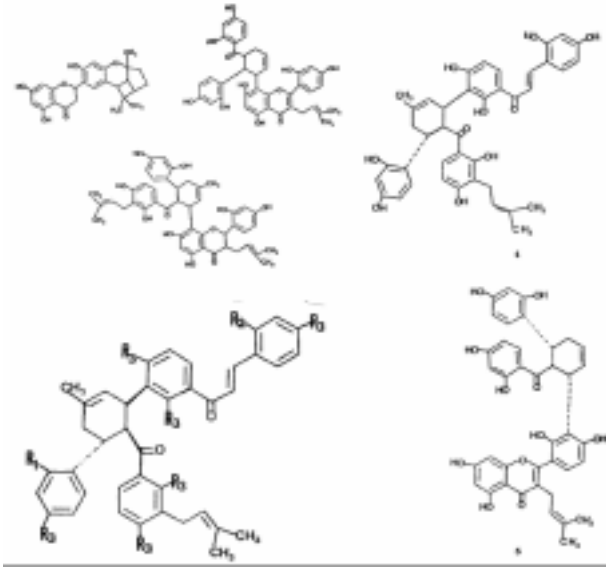
inactive detoxification product. Metabolism of various aromatic and heterocyclic plant constituents and xenobiotics in plants have frequently led to “insoluble”, or “bound” or “unextractable” or “lignin-like material” (Barz and Köster 1981). A portion of such alcohol-insoluble material often consists of metabolites bound to protein or polysaccharide structures (Barz and Köster 1985) (See Figure 2.19 through 2.23). Natural flavonoids, simple or complex, and their metabolites typically contain many hydroxyl groups that provide sites for attachment by hydrogen bonding or metal chelation, to biological macromolecules (Barz and Köster 1985) (See Figure 2.14 through 2.18). It is known that metabolism of plant flavonoids often leads to irreversible bounding to protein polysaccharide, and/or lignin, however, the chemistry is not adequately understood (Barz and Köster 1985). Schematic diagrams of humus, complex root flavonoids and bound residue formation are shown in Figure 5.7.

The type of PAH-SOM interaction will significantly affect long-term contaminant fate and bioavailability (Pignatello and Xing 1996). Irreversible binding of pesticide residues in soil, as result of either biological or abiotic oxidative coupling reactions, has been proposed to limit residue desorption and transport (Verstraete and Devliegher 1996, Bollag 1992). Several studies have noted higher than expected sorption values, as defined by distribution coefficients (Kd) and partition coefficients (Koc), for soils and sediments contaminated with PAH for extensive periods of time (Carmichael, Christman, and Pfaender 1997, McGroddy and Farrington 1995).

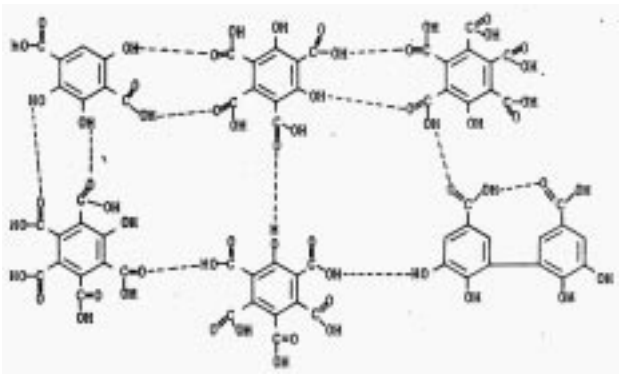
HUMIC ACID



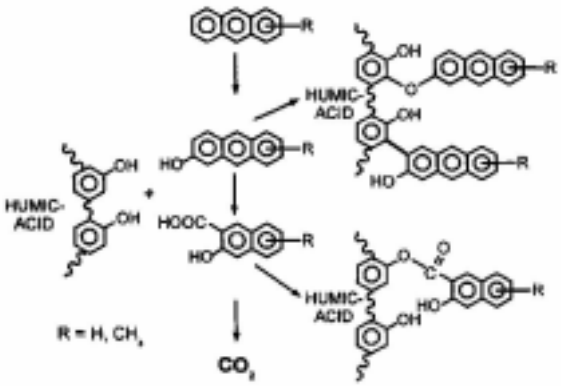
COMPLEX ROOT FLAVONOIDS



Fulvic Acids



Hypothetical scheme of bound reside formation



Clay Humate Complex

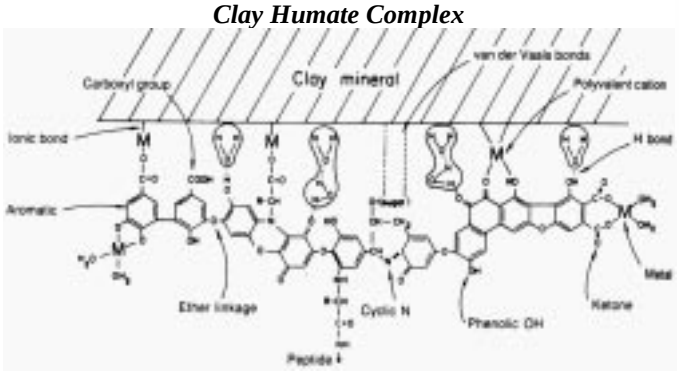


Figure 5.7. Schematic diagrams of humus, complex root flavonoids, and bound residue formation

Intramicropore diffusion, sequestration, and entrapment In addition to covalent binding, intraparticle diffusion, sequestration, and entrapment of the hydrophobic organic contaminants (HOCs) in macromolecular humus substances has received more attention recently (Eschenbach, Weinberg, and Mahro 1998). Here sequestration is defined as sorption of HOCs, which are biounavailable but organic solvent-extractable. Entrapment is defined as sorption of HOCs, which diffused into soil micropores and become biounavailable and solvent-nonextractable. It is believed that the entrapment involves slow partitioning of the hydrophobic compounds into organic matter or slow diffusion into micropores where their further availability is hindered (Echenbach Weinberg, and Mahro 1998). Recent observations suggest that hydrophobic organic carbon interactions with soils and sediments comprise different inorganic and organic surfaces and matrices, particularly with regard to the roles of inorganic micropores (Luthy *et al. 1997).* Mixed sorption phenomena complicate the interpretation of macroscopic data regarding diffusion of hydrophobic organic carbons into and out of different matrices and the hysteretic sorption and aging effects for soils (Luthy *et al. 1997).*

In this experiment, ^{14}C-pyrene mass balances in microcosms were consistently low at less than 70%, which may largely attribute to intraparticle diffusion, sequestration, and entrapment of ^{14}C-pyrene and/or metabolites. When the soil samples were suspended in scintillation fluid for ^{14}C bound reside measurement, the deeply entrapped ^{14}C could have lost contact with Scintillation fluid and therefore not accountable by liquid scintillation analyzer. The 4,5,9,10-^{14}C-pyrene used in the experiments had ^{14}C labels at the numbers 4, 5, 9, and 10 carbons (Figure 5.6). Initial enzymatic attack of pyrene ring is typically at the ^{14}C-4 and ^{14}C-5 position (Figure 5.6). Pyrene will have to be metabolized extensively before the radiolabeled carbon, ^{14}C-9 and ^{14}C-10, can be removed as $^{14}CO_2$. Partially degraded ^{14}C-pyrene metabolites containing ^{14}C-9 and ^{14}C-10 could have diffused into soil micropore and became solvent nonextractable and unaccountable by liquid scintillation analyzer.

Luthy *et al.* (1997) reported the using complementary spectroscopic and spectroelectric techniques revealed at the micro-scale on the sequestration of PAH contaminants in sediments (Gohosh*et al.* 1999). A strong correlation of soil organic matter location with PAH location is observed for Milwaukee harbor sediments. PAH level on the black carbonaceous particles are two orders of magnitude higher than on the white siliceous particles. Additionally, most PAHs were found to be associated with the external surface regions of sediment carbonaceous particles indicating near surface sorption mechanisms. Unlike ^{14}C-pyrene, ^{14}C-B[a]P had near 100% mass balance, which was likely due to the different positions of radioactive ^{14}C labels on ^{14}C-B[a]P. In the 7,10-^{14}C-B[a]P used in the experiments, ^{14}C is labeled at the numbers 7 and 10 carbons (Figure 5.5). Enzymatic attack of the B[a]P ring containing the labeled carbon at either the 7-8 or 9-10 position are some of the most energetically favorable in the B[a]P molecule (Cerniglia 1992). Many of the ^{14}C-B[a]P metabolites that have been identified resulted from initial oxidation at the 7-8 or 9-10 positions by both bacteria and fungi and cleavage of the oxidized aromatic ring. Once ring oxidation occurs, PAHs will become more degradable. After the initial oxidation and 7,10-^{14}C-labeled ring cleavage, the remaining four non-labeled rings will further degrade to smaller molecules that may diffuse into and to be entrapped in soil mircropores. By

contrast, the parent ^{14}C-B[a]P was likely to interact with SOM at particle surface without diffusing into micropores. The diffusion/entrapment of non-radio-labeled fraction of molecules would not affect the ^{14}C measurement by LSC. It is important to note that PAH fate experiments using ^{14}C-PAHs with ^{14}C labels at different positions of the molecular structure could generate different results. For example, 4,5-^{14}C-B[a]P is used instead of 7,10-^{14}C-B[a]P, the resulting B[a]P mineralization may be very little, because the ^{14}C on position 4 and 5 of B[a]P would be much less susceptible to microbial degradation. The intermediate metabolites of 4,5-^{14}C-B[a]P would be more likely associated with soil. To fully evaluate the fate and behavior of PAH in soil, B[a]P with ^{14}C labeled at various position should be evaluated. Meanwhile, the 7,10-^{14}C-B[a]P appeared to be a good candidate of the fate study to identify the most important initial step of B[a]P degradation.

Effects of Flavonoid Concentration on B[a]P and Pyrene Mineralization in Biologically Active Rhizosphere Soils versus "Abiotic" Soil

^{14}C-B[a]P and ^{14}C-pyrene were shown to be biologically transformed and mineralized through active $^{14}CO_2$ production in biologically active microcosms. Abiotic $^{14}CO_2$ production was less than 1% (near background level) of the total ^{14}C-B[a]P or ^{14}C--pyrene added in all poisoned Mulberry soil-slurry microcosms regardless the type and amount of flavonoid amendment (Figure 4.7).

$^{14}CO_2$ evolutions from ^{14}C-B[a]P in the biologically active Mulberry and Bermudagrass rhizosphere soil microcosms were statistically significantly greater than those in the "abiotic" poisoned Mulberry rhizosphere soil without flavonoid or Mulberry root extract amendment (Table 4.9, Appendices D-7 and D-11). Evidently, native microbial consortia were actively degrading B[a]P in the rhizosphere soil slurry. Mineralization of B[a]P and pyrene was an important fate mechanism in rhizosphere soil. In contrast, abiotic degradation of B[a]P was negligible. Without flavonoid, the range of B[a]P mineralization (15% -25%) in soil slurry observed in this experiment was consistent with those reported by Carmichael and Pfaender (1997). Carmichael and Pfaender found the extent of B[a]P mineralization ranging from <1% to 25% with a variety of soils and environmental conditions. Pyrene mineralization ranged from 25% to 40% without flavonoid in this experiment. Considerable B[a]P and pyrene mineralization found in this experiments were likely due to the presence of plenty oxygen, nutrients, and acclimated microorganisms in aged PAH-contaminated rhizosphere soil.

Flavone, morin, as well as Mulberry root extract inhibited B[a]P and pyrene mineralization in the biologically active Mulberry and Bermudagrass rhizosphere soils (Figures 4.8, 4.9, 4.23, and 4.24; Table 4.8; Appendices D-2, D-3, D-5, and D6). Average B[a]P mineralization reduced gradually from approximately 20% to approximately 2% as flavone and morin concentrations increased from 0 to 100 uM (Figures 4.8 and 4.9) or with Mulberry root extract added. As morin and flavone concentrations increased to between 0.1 uM and 1 uM, the decreases in $^{14}CO_2$ production were either statistically insignificant or marginally different (Table 4.8, Appendices D-2 and D-3). As flavone and morin concentrations increased from 10 uM to 100 uM, average $^{14}CO_2$ evolution from ^{14}C-B[a]P decreased dramatically to between 1% and 2% (Figures 4.8 and 4.9). The decreases were statistically significant at 95% confidence levels (Table 4.8, Appendices D-2 and D-3). When Mulberry root extract (855 mg-TOC/L) was

amended, $^{14}CO_2$ evolution from ^{14}C-B[a]P in both Mulberry and Bermudagrass Rhizosphere soils decreased significantly to between 1% and 2% (Figures 4.8 and 4.9, Table 4.8, Appendices D-2, and D-3). Similar trends were observed for pyrene mineralization (Figures 4.23, 4.24, Table 4.8, Appendices D-5 and D-6). Statistically significant different differences in the amount of $^{14}CO_2$ productions from different groups of soil microcosms confirmed that flavone, morin, or Mulberry root extract inhibited microbial mineralization of B[a]P in biologically active Mulberry and Bermudagrass soil slurry microcosms.

B[a]P Minerlization in Loamy Sand Mulberry Soil versus in Sandy Clay Loam Soil

In Figures 5.8 and 5.9, $^{14}CO_2$ production from parent 7,10-^{14}C-B[a]P and 4,5,9,10-^{14}C-pyrene in the biologically active Bermudagrass and Mulberry rhizosphere soils, and poisoned Mulberry rhizosphere soil, amended with none, Mulberry root extract, 100 μM morin, and 100 μM flavone, are compared, respectively.$^{14}CO_2$ production from poison Mulberry soil was consistently below 1%. Without flavonoid amendment, the average $^{14}CO_2$ production (23%) from ^{14}C-B[a]P in Mulberry soil was statistically significantly higher than that (17%) in Bermudagrass rhizosphere soils (Table 4.9, Appendix D-7). Whereas, without flavonoid amendment, the average $^{14}CO_2$ production (26%) from ^{14}C-pyrene in Mulberry soil was statistically significantly lower than that (39%) in Bermudagrass rhizosphere soils (Table 4.9, Appendix D-7). $^{14}CO_2$ evolution decreased as flavone and morin concentrations increased (Figures 4.8 and 4.9). When 100 uM flavone, 100 uM morin, or Mulberry root extract was amended $^{14}CO_2$ evolution from ^{14}C-B[a]P in both Mulberry and Bermudagrass Rhizosphere soils decreased to between 1% and 2% (Figure 5.8). In the same way, $^{14}CO_2$ evolution decreased to below 10% and 3% in Mulberry and Bermudagrass soils, respectively. There were no statistically significant differences between $^{14}CO_2$ productions among the two biologically active and the poisoned soils with high concentration flavonoids or Mulberry root extracts (Table 4.9, Appendices D-8 through D-14).

Without flavonoid amendment, greater B[a]P mineralization in loamy sand Mulberry soil than that in sandy clay loam Bermudagrass soil was likely due to soil clay, silt, and organic matter contents. The Mulberry soil contained 6% of clay 12% of silt and 3% of SOM compared with 27% of clay, 23% of silt, and 5.2% of SOM in the Bermudagrass soil. Carmichael and Pfaender (1997) reported that the extent of mineralization and soil bound residue formation of chrysene and B[a]P in soil was found to be significantly correlated to soil organic carbon content (f_{oc}), the fraction of silt and clay in the soils. The reduced mineralization was believed to be attributed to the increased interaction of PAHs with organic matter coated on the clay surface and with the hydrophobic region on mineral surfaces. Silt and clay have larger surface areas than sand in several orders of magnitude. As a result, more PAH molecules partition onto the soil and became unavailable for biodegradation (Kan, Fu, Tomson 1994, Karickhoff, Brown, Scott 1979).

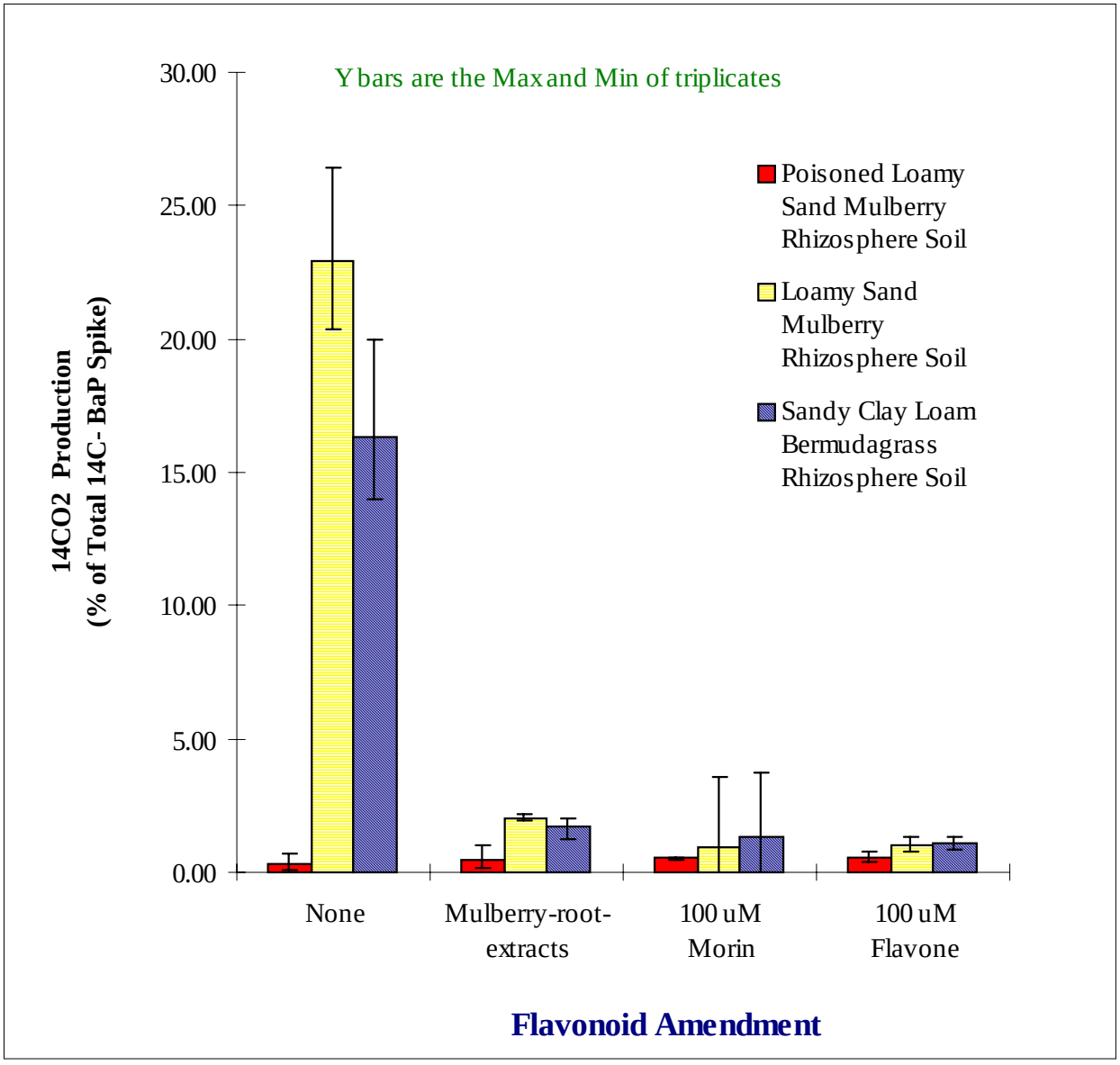


Figure 5.8. Comparison of $^{14}CO_2$ production from ^{14}C-B[a]P in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone

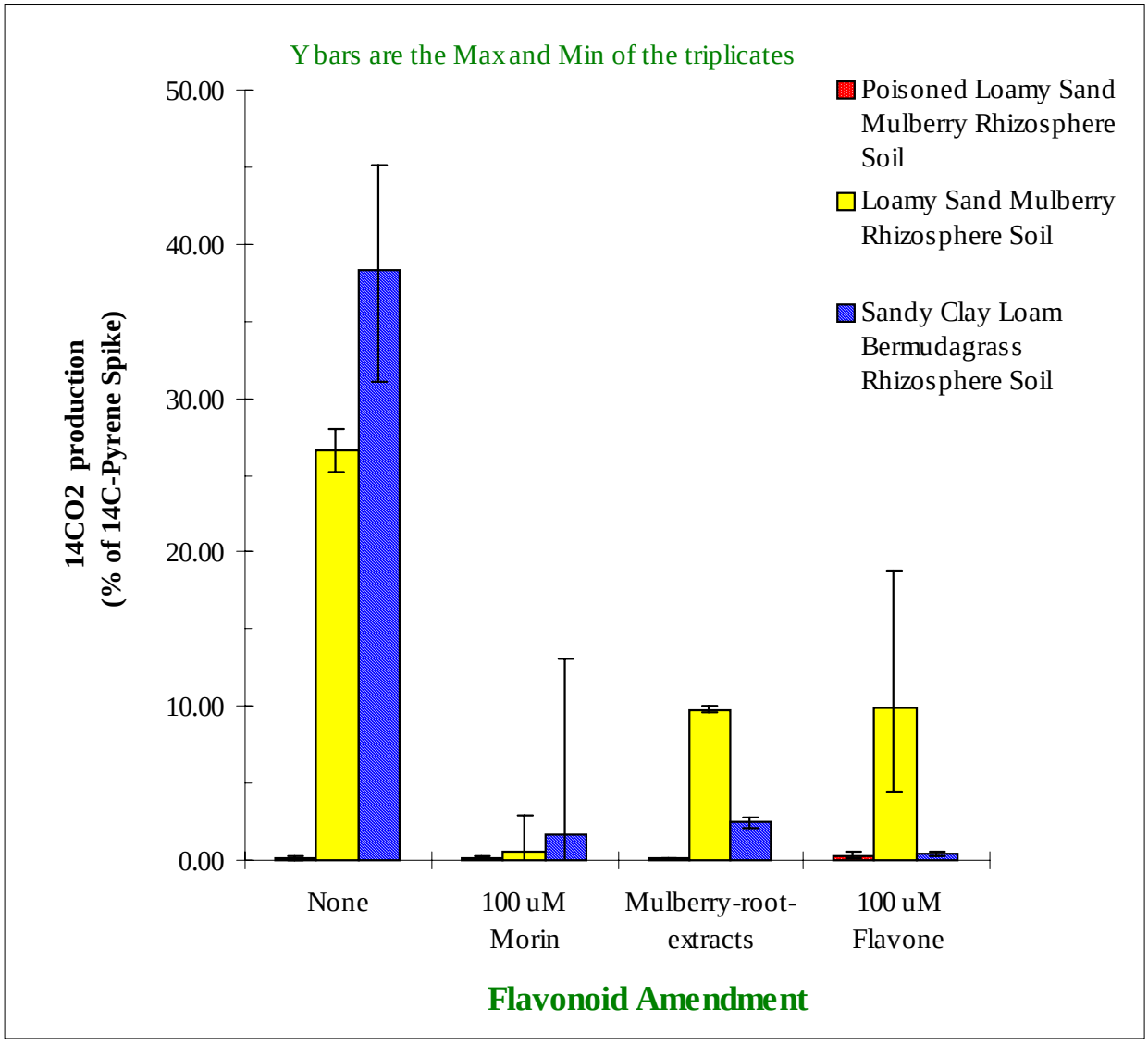


Figure 5.9. Comparison of $^{14}CO_2$ production from ^{14}C-pyrene in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone

Without flavonoids, $^{14}CO_2$ production from ^{14}C-pyrene in Mulberry soil was statistically significantly lower than that in Bermudagrass soils. In contrast, the former becomes higher as 100 uM morin was added. Enhanced binding with soil may have reduced pyrene mineralization more in higher clay and SOM content Bermudagrass soil. Note that, there were uncertainties associated with pyrene data due to the poor mass balance.

Microbial population in Bermudagrass rhizosphere soil is not likely a limiting factor for PAH mineralization. Both Bermudagrass and Mulberry rhizosphere soils contained active heterotrophic microbial communities as measured by CFU counts on 1/8-strength Plate Count Broth Agar (Table 3.8). The total bacteria counts for both soils were on the order of 10^7 CFU/g-wet soil (Table 3.8).. Very little or no PAH-utilizing bacteria was counted in Bermudagrass soil sample, whereas a majority of the bacteria counted in the Mulberry soil were PAH-utilizing (Table 3.8). Actual metabolic activity in the Bermudagrass soil, as indicated by considerable $^{14}CO_2$ production from both B[a]P and pyrene, did not agree with the low/no PAH-utilizing bacteria plate counts (Table 3.8). As mentioned in Chapter 3, a number of studies have shown the inconsistent relationship between the number of PAH-degrading microorganisms and the extent of PAH, because most of the community assays depend on the growth of microorganisms on a specific media or substrate and the degree of dislodging microbes attached on soil (Carmichael and Pfaender 1997, Chapelle 1992).

Effects of Flavonoids on Water Soluble ^{14}C-B[A]P, ^{14}C-Pyrene AND Metabolites

PAHs are highly hydrophobic and nonpolar. Presence of polar water soluble ^{14}C indicated that PAH are degrading to polar metabolites. In all the three soils tested, water soluble ^{14}C-B[a]P and ^{14}C-pyrene were less than 0.1% (near back ground level), except that when Mulberry root extract was added (Figure 4.16, 4.17, and 4.18). Generally, water soluble ^{14}C-B[a]P in poisoned Mulberry soil was slightly higher than those in biologically active Mulberry and Bermudagrass soils. With Mulberry root extract average water soluble ^{14}C-B[a]P counts increased to between 0.2% and 0.4%. Likewise, water soluble ^{14}C-pyrene increased marginally to less than 0.2%. These slight increases in water soluble ^{14}C-B[a]P and ^{14}C-pyrene were statistically significantly higher than those without flavonoid amendment in both biologically active and poisoned soils, although very low.

In Figures 5.10 and 5.11, water soluble ^{14}C-B[a]P and ^{14}C-pyrene in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone are compared. Statistical analyses indicate that, water soluble ^{14}C-B[a]P fractions in biologically active soils were statistically significantly higher than those in poisoned soil, but there were no significant difference in water soluble ^{14}C-pyrene among the three soil treatment (Table 4.9, Appendices B-7 through B-10).

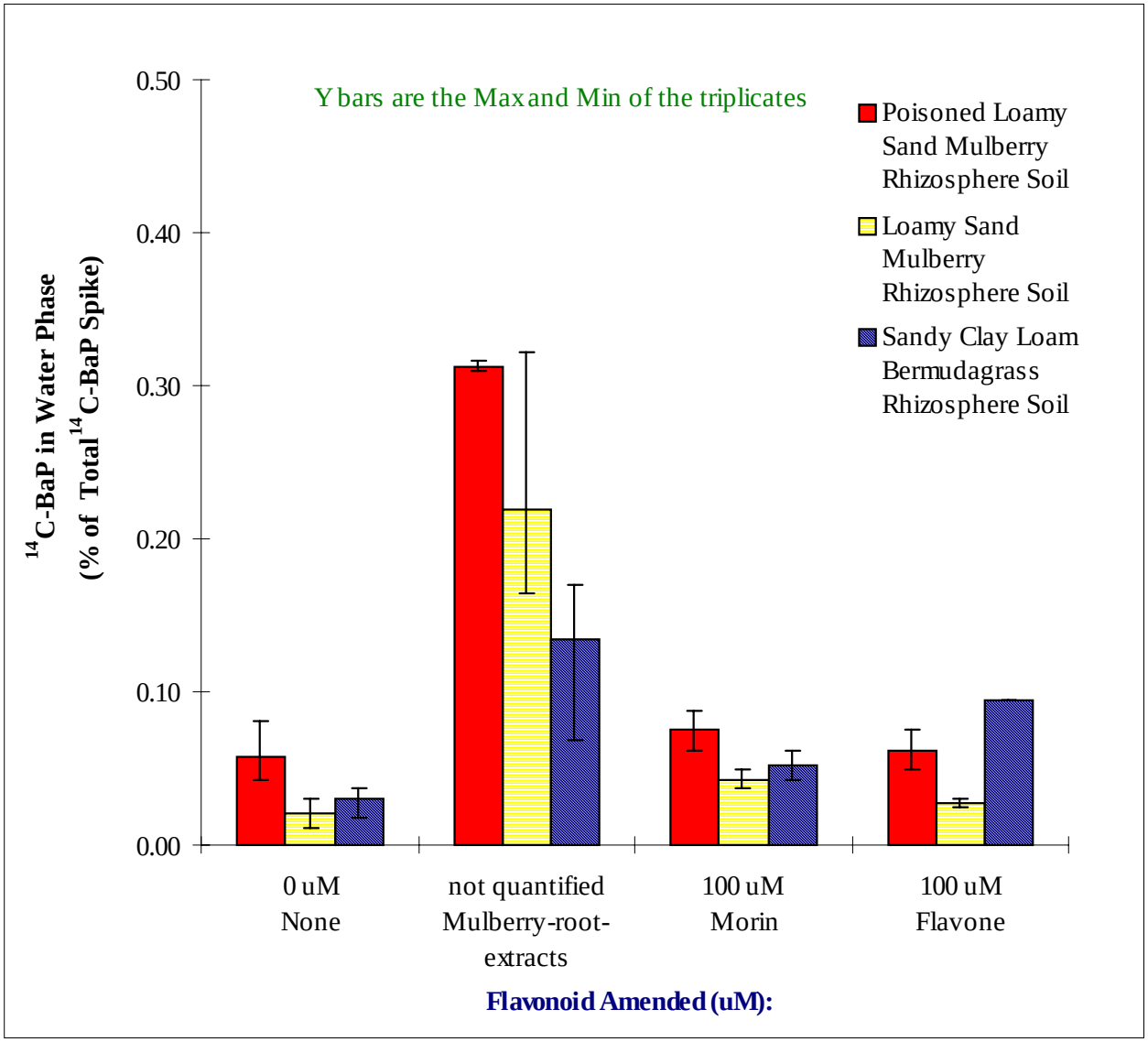


Figure 5.10. Comparison of water-phase ^{14}C-B[a]P in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone

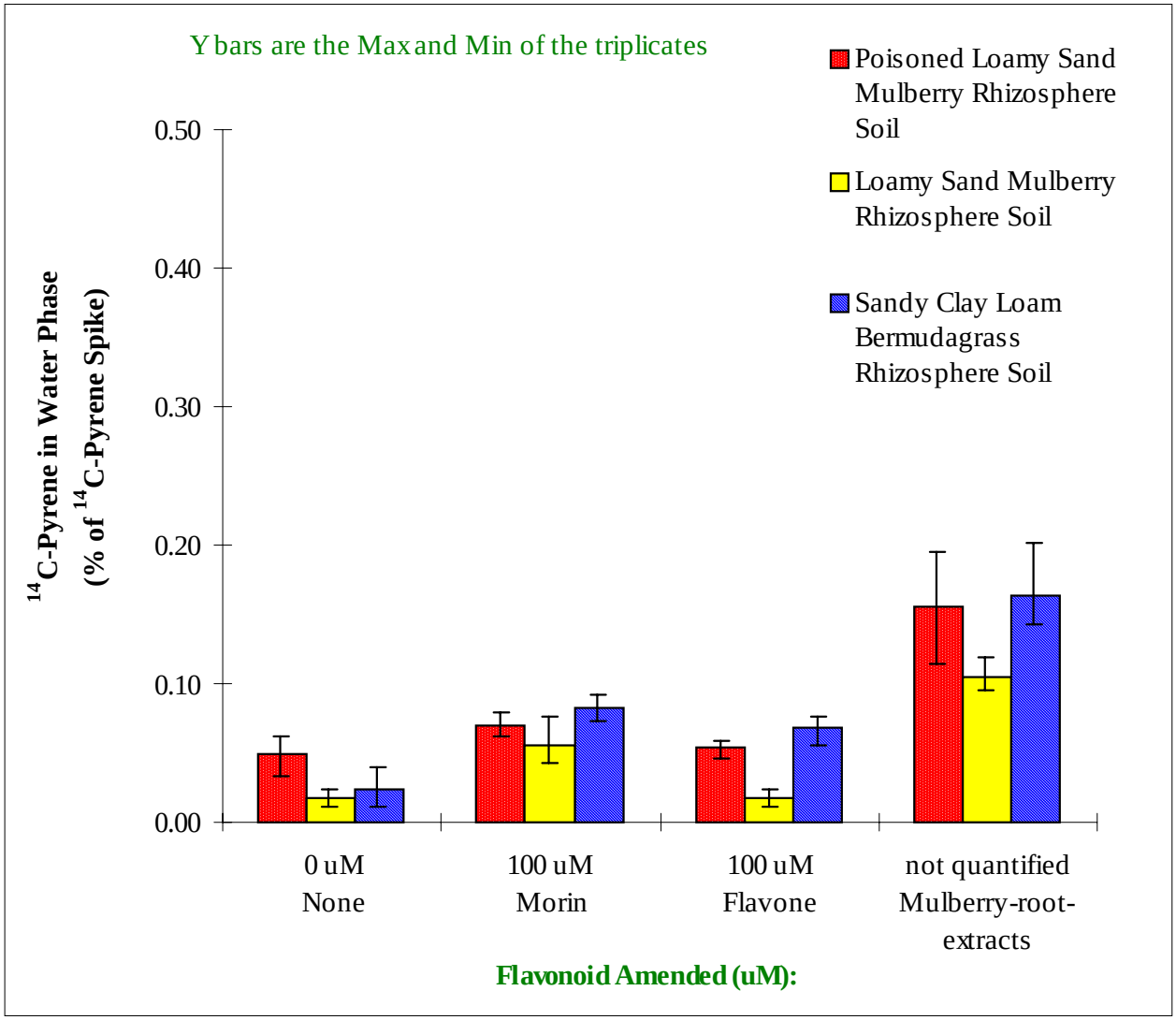


Figure 5.11. Comparison of water-phase ^{14}C-pyrene in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone

Water soluble ^{14}C-B[a]P and ^{14}C-pyrene metabolites were little in all the treatments. Average water soluble ^{14}C-B[a]P metabolites ranged between 0.2% and 0.3% except that when Mulberry root extract was added (Figures 4.19, 4.20, and 4.21). Average water soluble ^{14}C-pyrene metabolites ranged between 0.2% and 0.8% (Figure 4.34, 4.35, and 4.36). Generally, water soluble ^{14}C-B[a]P and ^{14}C-pyrene metabolites were slightly higher than their parents. With Mulberry root extract average water soluble ^{14}C-B[a]P metabolites increased to approximately 0.6%, that was statistically significantly higher than those without flavonoid amendment in both biologically and poisoned soil treatments. Likewise, water soluble ^{14}C-pyrene increased slightly with Mulberry root extract amendments, however, the increases were statistically insignificant.

In Figures 5.12 and 5.13, water soluble ^{14}C-B[a]P metabolites in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone are compared. Without flavonoid or with 100 uM morin there were no statistically significant differences in water soluble ^{14}C-B[a]P metabolites among poisoned Mulberry, biologically active Mulberry, and Bermudagrass soils. With Mulberry root extract, water soluble ^{14}C-B[a]P metabolites in Bermudagrass soil were statistically significantly lower than those in the poisoned and nonpoisoned Mulberry soils (Table 4.9, Appendices D-7 through D-10). By contrast, water soluble ^{14}C-B[a]P metabolites in Bermudagrass soil were statistically significantly higher than those in the poisoned and nonpoisoned Mulberry soils when 100 uM Flavone was added. It is not clear whether this exceptional increase of water soluble ^{14}C-B[a]P in the biologically active Bermudagrass soil was a random incidence or not.

Without flavonoid or Mulberry root extract, water soluble ^{14}C-pyrene metabolites in poisoned Mulberry were statistically significantly less than those in nonpoisoned Mulberry, and Bermudagrass rhizosphere soils. With flavonoids or Mulberry root extract added, there were generally no statistically significant differences in water soluble ^{14}C-pyrene metabolites among the three soil treatments (Table 4.9, Appendices D-11 through D-14).

Water-phase ^{14}C fractions were negligible under all the experimental conditions. Slightly higher water-phase ^{14}C fractions in Mulberry root extract treatment indicated that certain root constituents may have increased the solubility of ^{14}C-PAH and metabolites. However, slightly lower water soluble ^{14}C-B[a]P fractions in biologically active soils than those in poisoned soil indicated the occurrence of active biodegradation of ^{14}C-B[a]P in water phase. In other word, water phase ^{14}C-B[a]P will be degraded without accumulation when dissolved or released from solid phase.

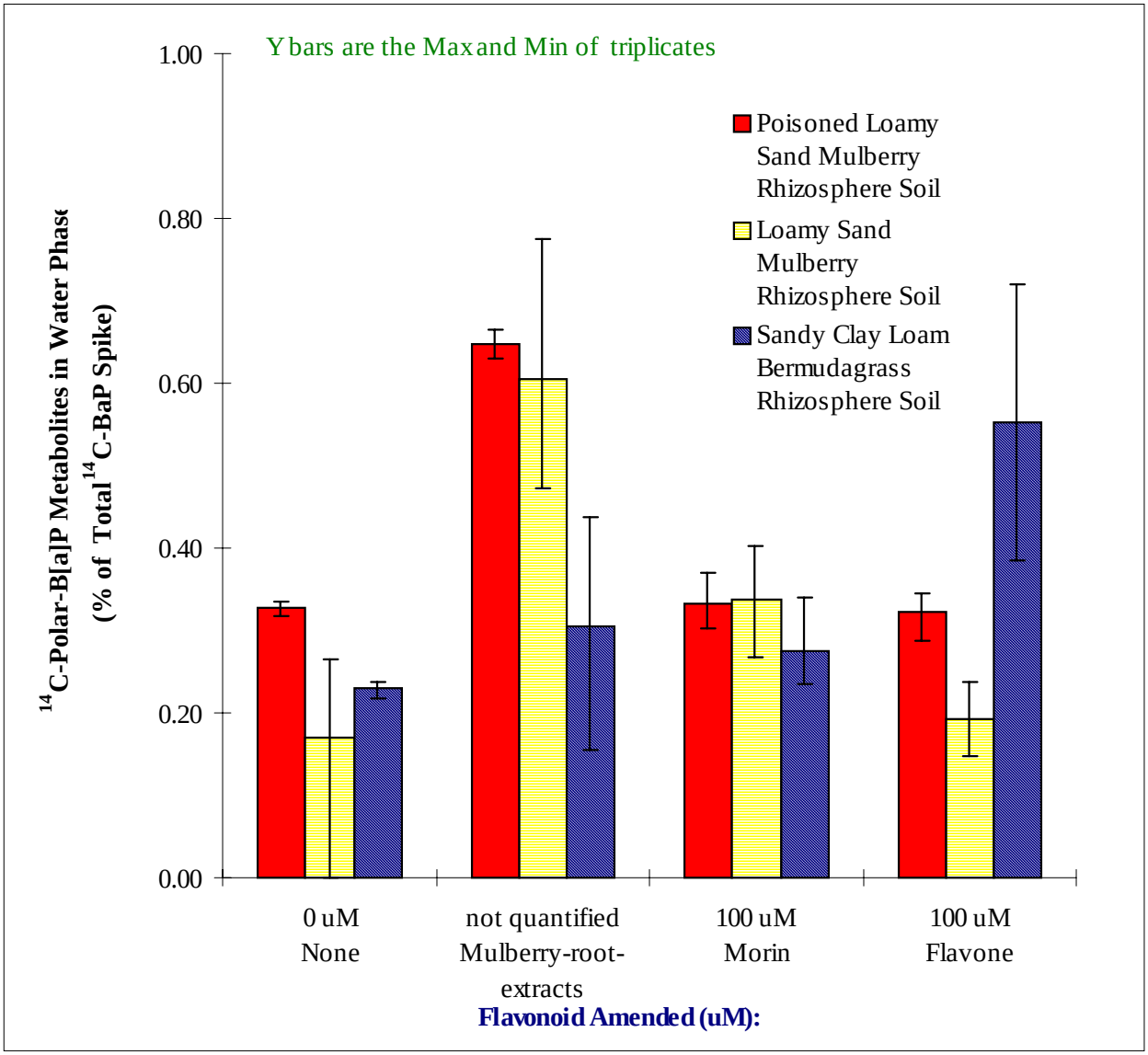


Figure 5.12. Comparison of water-phase ^{14}C-B[a]P metabolites in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone

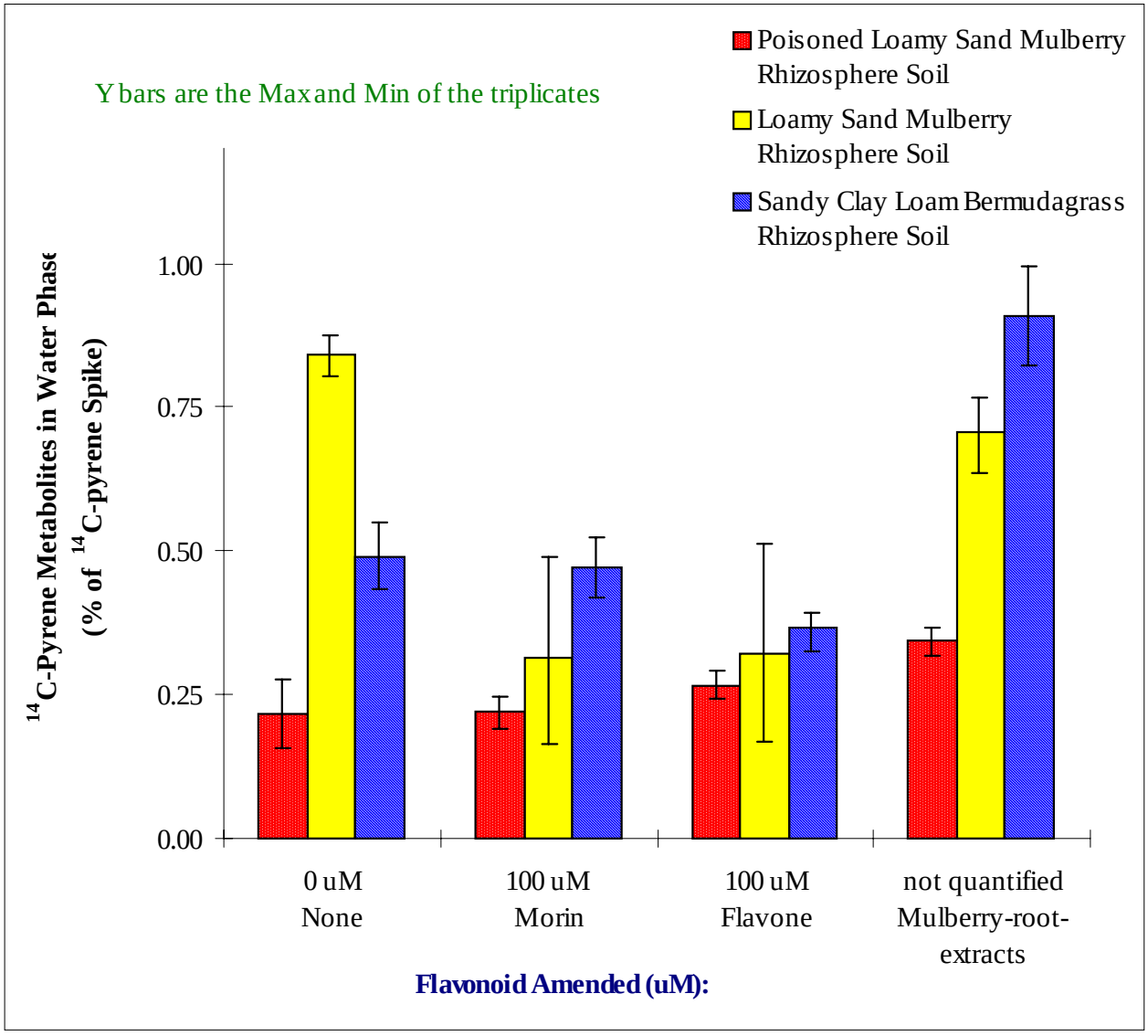


Figure 5.13. Comparison of water-phase ^{14}C-pyrene metabolites in poisoned Mulberry, nonpoisoned Mulberry, and Bermudagrass rhizosphere soils amended with none, Mulberry root extract, 100 uM morin, or 100 uM flavone

Possible Mechanism of Inhibited PAH degradation/mineralization with Flavonoids

In the biological active soil slurry microcosms, B[a]P mineralization decreased, as increased amounts of flavone, morin, or Mulberry root extract was added into the soil slurry microcosms, meanwhile soil bound residues increased (Figures 4.37, 4.39, and 4.41). Whereas, B[a]P adsorption onto soil generally did not change as flavone, morin, or Mulberry root extract was added. Water soluble B[a]P and metabolite were negligible under all the experimental conditions. The reduced microbial degradation/mineralization of B[a]P was mostly likely attributed to the reduced bioavailablity as more B[a]P was binding to soil organic matter.

Lesage *et al.* (1999) Reported the addition of humic acids enhanced the dissolution of hydrocarbons from diesel fuel and retarded the degradation of the PAH (phenanthrene, pyrene, and B[a]P) spiked onto soil, but this effect was reversed when the petroleum product was also added. This indicated that biodegradation was dependent on the relative sorption of PAHs onto soil, or humic acids in soil. Studies of PAH fate have shown that microbial mineralization of PAHs, especially PAHs with four or more benzene rings, decreases with increasing contaminant residence time in soils. Decreased microbial mineralization is often attributed to PAH association with the soil organic matrix (SOM) (Hatzinger and Alexander 1995, Mihelcic, and Luthy 1991) due to sorption (McCarthy and Jimenez 1985, Weber, and Huang 1996, Maruya *et al.* 1996), partitioning (Pignatello and Xing 1996), and covalent binding (Verstraete and Devliegher 1996, Bollag 1992).

Sorption and partitioning processes reduce PAH mineralization by slowing PAH desorption from SOM into soil aqueous phases where biodegradation is believed to occur. Covalent bonding through oxidative coupling would result in stable metabolite-organic matter complexes that would likely be stable and biounavailable (Bollag 1992, Whelan and Sims 1992, Loehr and Webster 1997).

Neither hydroxylated, nonhydroxylated flavonoids, nor Mulberry root extract was found to stimulated B[a]P degradation/mineralization in this experiments. As a result, B[a]P degradation/mineralization was not likely limited by the primary substrates or specific root-exudates which foster the growth of specific PAH-degrading microbial organisms.

Although the metabolic pathway of B[a]P degradation/mineralization has not been fully understood, it is known that complete mineralization is associated with a number of enzymes and microbial consortia. In this experiment, it is not clear which enzymatic reactions are limiting; however, negligible ^{14}C-B[a]P and metabolic products in the water phase shows no evidence that either initial oxidation or the subsequent enzymatic reaction could have controlled B[a]P degradation/mineralization. Oxygen content in the experimental microcosms was adequate for PAH degradation. Stoichiometry calculation indicates that oxygen was not depleted in the experimental soil slurry microcosms. Hurst *et al.*(1996) reported that the microbial degradation/mineralization of PAHs was enhanced under soil gas oxygen concentration between 2% and 21% in the contaminated soil. No statistically significant mineralization was found to occur at oxygen concentration of 0%. Mineralization of B[a]P at 21% oxygen was actually less than those at 2% and 5% of oxygen. Although adequate oxygen content was maintained in the

experiment, organic constituents in soil may compete oxygen at the microsites where microbial degradation of organic compounds occurred.

Buening *et al.* (1981) reported that nonhydroxylated (e.g., flavone) and hydroxylated (e.g., morin) flavonoids were found to promote and inhibit the initial oxidation of B[a]P metabolism in mammalian cells (see Chapter 2), which was not observed in this experiment. However, Buening's study was conducted in liquid phase, in this experiments reactions and PAH behavior may be complicated. PAH associated with soil were generally not readily available for degradation/mineralization. Flavone, morin, and Mulberry root extract may or may not have inhibited the initial oxidation of B[a]P. B[a]P degradation/mineralization may have been hindered by increased association between B[a]P and SOM, presumably, enhanced bound residue formation.

ENGINEERING IMPLICATION

Phytoremediation

One of the theoretical premises of applying phytoremediation to PAH-contaminated soils was that plant-root-exudates may enhance the rhizosphere degradation. However, this study indicates that plant flavonoid and mulberry root extracts hindered B[a]P degradation/mineralization. Although PAH degradation /mineralization is an important fate mechanism, only small amounts of PAHs added into soil are available for biodegradation/mineralization. PAHs added into soil are largely associated with soil organic matter either adsorbed onto soil or forming soil bound residues. Soil bound residue formation is the primary fate mechanism of PAHs in soil. Flavone, morin, and Mulberry root extract significantly enhance soil bound residue formation, particularly in clay and organic-rich soils. Solvent nonextractable bound residues may be a metabolic inactive detoxification product. As a result, PAHs may essentially be stabilized in rhizosphere through bound residue formation, enhanced by plant root exudates. Indeed, the term phytostabilization may be more informative for plant-facilitated remediation of PAH-contaminated soils. Note that phytostabilization of PAH-contaminated soil may be more appropriate for clay and organic-rich soil rather than low organic matter sandy soil.

The experimental results indicate that the enriched Mulberry root extract in soil did not enhance PAH degradation/mineralization. Although Mulberry was the predominant plant species naturally growing at the site, the tree may not be the most suitable for PAH-contaminated soil remediation everywhere. Plant natural succession in the disturbed land is a random process, it depends on the ever changing climate, air pollution, soil water, insects, seed bank, and many other influential factors. Understanding the causality of ecological recovery at a contaminated site may be important in selecting suitable plants for phytoremediation. Considerable mineralization from both Mulberry and Bermudagrass soils indicate rhizosphere degradation of PAHs is not necessary attribute to specific plants. Acclimated PAH-degrading microbial consortia seemed to be ubiquitous.

Recent years more and more investigators have found differences between the extent of metabolism for freshly added and aged PAH contamination. This difference is usually attributed

to minimal availability and mass transfer limitations of aged PAHs in soil (Erickson, Loehr, Neuhauser 1993). High concentrations of PAH remaining in the aged contaminated soils were observed everywhere, even low molecular weight PAH such as naphthalene and phenanthrene, which have been shown to be readily degradable. Therefore in some contaminated soils, the bioavailability of PAHs is a controlling factor for in-situ remediation. Phytoremediation should be carefully designed to accommodate site-specific properties.

Another important implication in this study was negligible water soluble fraction for both parent B[a]P and metabolites. In all the experimental microcosms, water phase fractions of ^{14}C-B[a]P and metabolites were well below 1% of the total original spike of 7,10-^{14}C-B[a]P, equivalent to approximately 0.1 μg/L. It was significantly lower than the reported B[a]P water solubility 4 μg/L@25°C. The fact indicates that B[a]P was degradable and not persistent in water phase. The 0.1 μg/L B[a]P was also below the human-health-risk-based drinking water aquifer standard 0.2 μg/L. PAH migration via rainwater infiltration and groundwater is not likely a concern with regard to phytoremediation.

Environmentally Acceptable Endpoints

Both nonhydroxylated and hydroxylated flavonoids as well as Mulberry root extract were found to enhance soil bound residue formation of PAHs. A number of recent laboratory studies have shown soil bound residue formation of PAHs and metabolites is a primary fate mechanism of PAHs in soil (Sims and Abbott 1992, Hurst et al. 1997, Guthrie and Pfaender 1998, Carmichael and Pfaender 1997, Qiu and McFarland 1991). The nonextractable soil bound residue, primarily associated with soil organic matter, was found to be stable, non-bioavailable, and possibly nontoxic (Eschenbach, Weinberg, and Mahro 1998, Richnow *et al.* 1998, Weissenfels, Klewer, and Langhoff 1992, Pignatello 1996, Loehr and Webster 1997, Chung and Alexander 1998, Santini, Bureau, and Deschênes 1999). Bound residue formation is believed to be an environmentally acceptable endpoint in the remediation of contaminated soil (Erickson, Loehr, and Neuhauser 1993, Alexander 1995). Studies have shown evidences that adsorbed substances tend to become more resistant to extraction and degradation the longer they are in the soil.

Bound residue formation includes covalent bonding through oxidative coupling and intramicropore diffusion and entrapment. In this study, flavonoid-enhanced soil bound residues were more likely formed by covalent bonding rather than intramicropore diffusion, because bound residue formation was not increased in metabolically inhibited poisoned soil slurry microcosms. Covalent bonding would result in stable metabolite-organic matter complexes that would likely be stable low in bioavailabilty and toxicity (Bollag 1992, Whelan and Sims 1992, Loehr and Webster 1997). Covalently bonding will significantly affect long-term PAH fate in soil (Pignatello and Xing 1996).

Sims and Abbott (1992) reported that occurrence of detoxification was observed through incubation time for non-poisoned PAH-contaminated soil, while no detoxification trend was apparent for poisoned soil. Micortox™ assay was used to evaluate changes in toxicity of soil water extracts through incubation time for PAH-contaminated and non-contaminated soils. With regard to contaminated soil, all poisoned soil samples were consistently toxic through incubation

time. However, a decrease in toxicity of water extracts was observed for non-poisoned soil through time of incubation. Santini reported a biostimulation of a PAH-contaminated soil. Residual toxicity was measure through earthworm mortality (*Eisenia foetida)* and growth of watercress *(Lepidiu7j sativum)* (Santini, Bureau, and Deschênes 1999). Despite the fact that a few PAH had not reach the selected chemical criterion after 245 days of incubation, an important reduction of the toxicity was observed, It is then advisable to use the relationship between detoxification and decontamination to better assess a bioremediation process.

With regard to Environmentally Acceptable Endpoints, additional concern may be the potential release of the nonextractable PAH residues from the soil in the long term. Eschenbach *et al*. (1998) had conducted a long-term stability study of ^{14}C labeled naphthalene, anthracene, pyrene, and B[a]P under different ecological stress conditions. They found that a considerable fraction of the nonextractable and extractable ^{14}C-PAH biodegraded to $^{14}CO_2$. The degradation rate was as slow as natural turnover rates of humic substances. Neither the addition of humus degrading microorganisms nor freezing and thawing led to a mobilization of the nonextractable ^{14}C-PAH residues. However, a significant mobilization of the nonextractable ^{14}C occurred when EDTA was added to the soil. The metal-organic soil complexes were destabilized by this complexing agent and released ^{14}C that was attached to colloidal or dissolved organic matter.

CHAPTER 6. SUMMARY AND CONCLUSIONS

A compound-nested experiment was conducted to investigate the effects of flavonoid types, concentration, and soil types on PAH fate and behavior in ^{14}C-B[a]P-amended soil slurry microcosms. Nonhydroxylated flavone, hydroxylated morin, and complex Mulberry root extract were amended into biologically active Mulberry and Bermudagrass as well as "pseudo abiotic" poisoned Mulberry rhizosphere soil microcosms. ^{14}C-B[a]P mineralization, bound residue formation, adsorption, and water soluble ^{14}C-B[a]P and metabolites were measured. Statistical analyses of the experimental data lead to the following conclusion.

Bound Residue Formation - the Most Important PAH Fate Mechanisms in Rhizosphere Soil

Soil bound residue formation and adsorption were predominant fate mechanism for ^{14}C-B[a]P added into soil slurry microcosms. Mineralization of PAHs is also an important mechanism, however, only a small portion (2% - 23%) of ^{14}C-B[a]P in soil was available for biodegradation in the experimental soils. Abiotic mineralization was minimal (<1%) in the metabolically inhibited poisoned soil slurry microcosms. Water soluble ^{14}C-B[a]P and metabolites were negligible (<0.35% and <0.65%) under all the experimental conditions.

Flavonoids Enhanced PAH-Soil-Bound Residue Formation and Hindered PAH Mineralization

At adequate concentration level, either hydroxylated or nonhydroxylated flavonoids (100 uM morin or flavone), or Mulberry root extract (TOC = 855 mg/L) enhanced solvent-nonextractable soil bound residue formation of ^{14}C-B[a]P in biologically active soil microcosms, meanwhile hindered ^{14}C-B[a]P mineralization. Further, average solvent-extractable ^{14}C-B[a]P, (adsorption to soil) did not change in the loamy sand Mulberry soil , but decreased in sandy clay loam Bermudagrass soil. However, the decrease was statistically insignificant at 95% confidence levels. The degree of soil bound residue formation and mineralization depends on the types of soil. Soil bound residue formation was significantly higher in the organic rich sandy clay loam Bermudagrass rhizosphere soil than that in the loamy sand Mulberry rhizosphere soil. Flavonoid and Mulberry root extract had no effects on PAH fate in the "pseudo abiotic" poisoned soil microcosms, except that Mulberry root extract increased water soluble ^{14}C-B[a]P and metabolites slightly. The increase was statistically significant at 95% confidence level.

Hypothetical Mechanisms of Flavonoid Effects on PAH Fate

Flavonoid-enhanced soil bound residue formation of ^{14}C-B[a]P is believed to be mainly attributed to covalent bounding of ^{14}C-B[a]P metabolites to SOM, a process called humification. Intramicropore diffusion and entrapment of parent ^{14}C-B[a]P and metabolites may be also be responsible. Flavonoid and metabolites may have provided more binding sites as bridges promoting ^{14}C-B[a]P bound residue formation. Increased bound residue formation reduced the bioavailability of ^{14}C-B[a]P and metabolite for microbial degradation. As a result mineralization

of ^{14}C-B[a]P was hindered. Flavonoids and other constituents in Mulberry root extract may competed oxygen with ^{14}C-B[a]P at the microsite for microbial degradation. PAH-degrading microbial consortia was not limited in the rhizosphere soils. Metabolic activity was most likely related to the amount of PAH that is bioavailable.

Implication of Phytostabilization for PAH-Contaminated Soils

Both soil bound residue formation and degradation/mineralization are environmentally acceptable endpoints for PAH-contaminated soil remediation. Soil bound residues limit contaminant release from soil. Trace of water-soluble PAHs, if any, slowly released from soil phase, will be quickly degraded. In essence, the solvent-nonextractable soil-bound PAHs and metabolite residues are not available and no longer toxic to living organisms. Flavonoid-enhanced soil bound residue formation and reduced bioavailability implicate potential phytostabilization of PAH-contaminated soils to attain environmentally acceptable endpoints. Manipulating PAH bioavailablity through appropriate agricultural management may be a significant challenge to achieve the most cost-effective and environmentally sound solution.

CHAPTER 7. FUTURE RESEARCH

Results from this study suggest that soil bound residue formation, a predominant fate mechanism of PAHs, may be enhanced in rhizosphere soil. With regard to environmentally acceptable endpoints the following studies are recommended for the potential of utilizing risk-based phytostabilization technology to remediate PAH-contaminated soils.

Root Exudation and the Pertinent Microbiological and Biochemical Process

A systematic research on the root exudation and the pertinent microbiological and biochemical process for a variety of plants are needed to fully understand rhizodegradation and rhizostabilization. Fundamental studies should include

(1) Identification and characterization of plant root exudate
(2) The rate of chemical release from root exudation and plant root turnover at various growth periods
(3) Metabolic pathways of plant root-releasing chemicals
(4) Influence of root-releasing chemicals and their metabolites on PAH bioavailability and biodegradability
(5) Competitive behavior for oxygen and nutrients between plant root-releasing chemicals and PAHs
(6) Parallel field monitoring to verify laboratory studies

Long term fate and behavior of PAHs in Rhizosphere Soil

To fully evaluate the validity and consequences of phytostabilization, the long term fate and behavior of PAHs in rhizosphere soil should be elaborated. Studies must address

(1) Identification and characterization of PAH metabolites and their behavior over time
(2) SOM-PAH interactions with respect to both parent compound, contaminant intermediate product, and the soil organic matrix
(3) PAH-soil bound residue formation mechanisms and the long term stability
(4) PAH bioavailability and biotoxicity in aged soils with regard to long-term sorption in soil with or without active microbial communities

Development and Application of Mathematical Fate and Transport Model to justify Soil and Sediment Cleanup Criteria

A mathematical fate and transport model will be very beneficial to assess long-term PAH fate and behavior, exposure and toxicity, to justify soil and sediment cleanup criteria. The model should include processes in relation to contaminant interactions with soil organic matter and mineral particles, biological reactions, biotoxicity, bioavailability, under realistic exposure scenarios.

REFERENCES

Al-Bashir, B.; Cseh, T.; Lecuc, R.; Samson, R., "Effect of Soil/Contaminant Interactions o the Biodegradation of Naphthalene in Flooded Soil under Denitrifying Conditions," *Appl. Miocrobiol. Biotechnol*. 1990, 34:414-419

Alexander, W.W., Smith, D. S., Chang, R.L., Huang, M.T., and Conney, A.H., "Effects of Flavonoids on the Metabolism of Xenobiotics," *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmocological, and Structure Activity Relationships*, pp. 195-210, Alan R. Liss, Inc., 1986.

Alexander, M., "How Toxic are Toxic Chemicals in Soil?", *Environ. Sci. Technol.,* 1995, 29:2713-2715.

April, W; Sims, R.C., "Evaluation of the Use of Prairie Grasses for Stimulating Polycyclic Aromatic Hydrocarbon Ttreatment in Soil," *Chemosphere*, 1990, *20(1-2)*:253-265.

Atkinson, D., Bhat., L.K.S., Coutts, M.P., Mason, P.A., Read, D.J. Eds., *The Root System and their Mycorrhizae*. Martinus Nijhoff/Dr. W. Junk Publ., The Hague, 1983.

Bartha, R., You, I.S., Saxena, A., In *Pesticide Chemistry: Human Welfar and the Environment*, Matsunaka, S., Hutson,D.H., Murphy, S.D., Eds., Pergamon Press, Oxford, 1983, pp 345-350.

Barz, W. and Köster, J.,"Turnover and Degradation of Secondary (Natural) Products, *The Biochemistry of plants, Secondary Plant Products*, Conn, E.E. ed., Vol. 7, p 35-48, Academic Press, New York, NY, 1981.

Barz, W., Koster, J., Weltring, K-M., and Strack, D., "Recent Advances in the Metabolism and Degradation of Phenolic Compounds in Plants and Animals," *The Biochemistry of Plant Phenolics,* Van Sumere C.F., Lea, P.J. eds., Clarendon Press, Oxford, pp. 307-348, 1985.

Barz, W., Hösel, W., "Metabolism of Flavonoids," *"The Flavonoids Vol. 2,"*Harborne, J.B., Mabry, H., eds., Academic Press, New York, NY, p916-969, 1975.

Bauer, J.E.; Capoint, D.G., "Degradation and Mineralization of the Polycyclic Aromatic Hydrocarbons Anthracene and Naphthalene in Intertidal Marine Sediments," *Appl. Environ. Microbiol.* 1985, 50:81-90

Bell, E.A., "The Physiological Roles of Secondary Natural Products," *The Biochemistry of Plants*, Conn, E.E. Ed., Academic Press, 1981.

Berner, R.A., "Early Diagenesis, A Theoretical Approach," Princeton University Press, Princeton, NJ, 1980.

Berry, D.F., S.A. Boyd, "Oxydative coupling of phenols and anilines by perxidase: structure-activity relationships", *J. Soil Sci. Soc. Am.*,1984,*48:*565-569.

Bollag, J.M., "Decontaminating soils with enzymes", *Environmental Science and Technology,* 1992, *26*:1877-1881.

Bollag, J.M., "Cross-coupling of humus constituents and xenobiotic substances", In *Aquatic and Terrestrial Humic Materials*, R.F. Christman and E.T. Gjessing, Eds., Ann Arbor, Michigan, 1983, p127-141.

Bollag, J. M., Myers, C., "Detoxification of Aquatic and Terrestrial Sites through Binding of Pollutants to Humic Substances", *Sci. Total Environ.*, 1992, 117:357-366.

Bossert, I., Bartha, R., "Microbial Ecology: Fundamentals and Applications," Addison-Wesley, Reading, Mass, 1984.

Briggs, G.G., Bromilow, R.H., and Evans, A.A., "Relationships between Lipophilicity and Root Uptake and Translocation of Non-ionized Chemicals by Barkey, Pestic, Sci., 1982, 13: 495. 1982

Bromilow and Chamberlain, "Principles Governing Uptake and Transport of Chemicals", *Plant contamination, modeling and simulation of organic chemical processes*, Trapp, S. and McFarlane, J.C., eds., Lewis Publishers, 1995.

Brusseau, M. L., Rao, P. S. C., "Sorption Nonideality during Organic Contaminant Transport in Porous Media," *CRC Cri t. Rev. Environmental Control* 19:33-99, 1989.

Buening, M.K., Chang, R.L., Huang, M_T, Fortner, J.G., Wood, A.W., and Conney, A.H., "Activation and Inhibition of Benzo[a]pyrene and Aflatoxin B1 Metabolism in Human Liver Microsomes by Naturally Occurring Flavonoids," *Cancer Research* 41:67-72, 1981.

Bulman, T.; Lesage, S.; Fowlie P.J.A.; Weber, M.D., "The Persistence of Polynuclear Aromatic Hydrocarbons in Soil," *PACE Report;* Petroleum Association for Conservation of the Canadian Environment, Ottawa, Canada, 1985, *85-92.*

Burgos, W.D., Novak, J.T., Berry, D.B., "Reversible Sorption and Irreversible Binding of Naphthalene and α-Naphthol to Soil: Elucidation of Processes", *Environ. Sci. Technol.*, 1996, 30:1205-1211.

Callahan M.A., Slimak, M.W., Gabel, N.W., May, I.P., Fowler, C.F., Freed, J.R., Jennings, P., Durfee, R.C., Whitmore, F.C., Maestri, B., Mabey, W.R., Holt, B.R., Gould C., "Water Related Environmental Fate of 129 Priory Pollutants, Vol. II. Halogenated Aliphatic Hydrocarbons, Halogenated Ethers, Monocyclic Aromatics, Phthalate Esters, Polycyclic Aromatic Hydrocarbons, Nitrosoamines, and Misecllaneous Compounds", EPA-440/4-79-029 b, 1997.

Carmichael, L.M., and Pfaender, F.K., "Polynuclear Aromatic Hydrocarbon Metabolism in Soils: Relationship to Soil Characteristics and Preexposure," *Envir. Toxicol. And Chem.*, Vol. 16, No. 4. 1997, p. 666-675.

Carmichael, L.C., Christman, R.F., Pfaender, F.K., *Environ. Sci. Technol.*, 1997, 31:126-132.

Carslaw, H.S., Jaeger, J.C., "Conduction of Hear in Solids," Oxford University Press, Oxford, UK. 1959.

Cerniglia, C.E., "Biodegradation of Polycyclic Aromatic Hydrocarbons," *Curr Opin Biotechnol*., Vol. 4, 1993, pp.331-338.

Cerniglia, C.E., "Microbial metabolism of polycyclic aromatic hydrocarbons," *Adv. Appl. Microbiol.*, 1984, 30:31-71.

Cerniglia, C.E., and M. A. Heitkamp, "Microbial Degradation of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment," *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment*, Varanasi, U. Ed., CRC Press, Inc., 1989, pp. 41-68.

Cerniglia, C.E., Biodegradation of Polycyclic Aromatic Hydrocarbons, *Biodegradation,* Vol. 3., 1992, pp. 351-368.

Chang RL, Huang MT, Wood AW, Wong CQ, Newmark HL, Hagi H, Sayer JM, Jerina DM, Conney AH, 1985, Effect of Ellagic Acid and Hydroxylated Flavonoids on the Tumorigenicity of Benxo[a]pyrene and dihydroxy-epoxy-tetrahydrobenzo[a]pyrene on mouse skin and in the newborn mouse. *Carcinogenesis* 6:1127.

Chiou, C.T., In *Reaction and Movement of Organic Chemicals in Soils,* Sawhney, B.L., Brown, K., Eds., *Soil Sci. Soc. Am.*, Madison, WI, 1989, pp1-29.

Chung, N, alexander, M., " Differences in Sequestration and Bioavailability of Organic Compounds Aged in Dissimilar Soils", *ES&T,* 32:855-860.

Claus, H. and Z. Filip, "Effects of clays and other solids on the activity of phenoleoxidases produced by some fungi and actinomycetes. *Soil Biology and Biochemistry,* 1990a, *22:*483-488.

Claus, H. and Z. Filip, "Enzymatic oxidation of some substituted phenols and aromatic amines, and the behavior of some phenoleoxidase in the presence of soil related adsorbents. *Water Science and Technology,* 1990b, *22*:69-77.

Clayton, M. F., Lamberton, J.A., "A Study of Root Exudates by the Fog-Box Technique," *Aust. Jour. Biol. Sci.,* Vol. 17, 1964, pp. 855-866.

Chapelle, F. H., "Groundwater Microbiology and Geochemistry", John Wiley & Sons, Inc., 1993, 424 pp.

Dalton, H., Golding, B.T., Waters, B.W., Higgins, R., Taylor, J.A., "Oxidations of Cyclopropane, Methylcyclopropane, and Arenes with the Mono-oxygenase System from *Methylococcus capsulatus," J. Chem. Soc. Chem Commun.,* 1981, pp.482-483.

Davies, J.I., Evans, W.C., Oxidative Metabolism of Naphthalene by Soil *Pseudomonads*: The Ring-Fission Mechanism, *Biochem. J.,* Vol. 91, 1964, pp. 251-261.

Dobbins, D.C., Pfaender, F.K., "Methodology for Assessing Respiration and Cellular Incorporation of Radio-labeled Substrates by Soil Microbial Communities," *Microbiol. Ecol.,* Vol. 15, 1988, pp.257-273.

Dragun, J, "The Soil Chemistry of Hazardous Materials," The Hazardous Materials Control Research Institute, Silver Spring, MD, 458pp, 1988.

Eaton, R.W., Chapman, P.J., Bacterial Metabolism of Naphthalene: Construction and Use of Recombinant Bacteria to Study Ring Cleavage of 1,2-dihydroxynaphthalene and subsequent reactions, *J. Bacteriol.,* Vol. 114, 1992, pp. 974-979.

Ellis, B.E., "Degradation of Aromatic Compounds in Plants," *Lloydia*. 1974, *37(2)*:168-184

Erickson, D.C., R.C. Loehr, and E.F. Neuhauser. "PAH Loss during Bioremediation of Manufactured Gas Plant Soils," *Water Res.* 5:911-919, 1993.

Eschenbach, A., R. Wienberg, and B. Mahro, "Fate and Stability of Nonextractable Residues of [^{14}C]PAH in Contaminated Soils under Environmental Stress Conditions," *Environ. Sci. Technol*., 1998, 32,2585-2590.

Fitter, A.H.; Hay, R.K.M., "Envir. Physiology, 2nd Ed.," Academic Press, London, UK, 1987, pp 423.

Foth, H. D., Turk L.M., "Fundamentals of Soil Science" 5th ed.," John Wiley &Sons, Inc. New York, NY, 1972.Foster, J.W., "Bacterial Oxidation of Hydrocarbons," *Oxygenases,* Hayaishi O. ed., Academic Press, New York, 1962.

Garbarini, D.R., Lion, L.W., "Influence of the Nature of Soil Organics on the Sorption of Toluene and Trichloroethylene", *ES&T,* 1986, 20:, 1263-1269.

Gauthier, T.J., Seitz, W.R., Grant, C.L.,"Effects of Structural and Compositional Variations of Dissolved Humic Materials on Pyrene K_{oc} Values", *ES&T,* 21:243-248.

Gibson, D.T., Mahadevan, V., Jerina, D.M., Yagi, H., Yeh HJC., "Oxidation of the Carcinogens Benzo[a]anthracene to Dihydrodiols by a Bacterium," *Science,* Vol. 189, pp.295-297.

Gillette, J.R., Davis, D.C., Sasame, H.A., "Cytochrome P-450 and its Role in Drug Metabolism," *Annu Rev Pharmacol,* Vol. 12, 1972, pp. 57-84.

Glusker, J.P., Rossi M., "Molecular Aspects of Chemical Carcinogens and Bioflavonoids, Plant," *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships*, pp.395, Alan R. Liss, Inc., 1986.

Glusker, J.P.,Trueblood K.N., "Crystal Structure Analysis: A Primer," 2nd Ed., New York: Oxford University Press, 1985.

Gohosh, U., Luthy, R.G., Gillette, J.S., Zare, R.N., Talley, J.W., "Microscale Characterization of PAH Sequestration on Sediments," *Bioremediation technologies for polycyclic aromatic hydrocarbon compounds,* Leeson A. and B.C. Alleman eds., Battelle, The Fifth International In-Situ and On-Site Bioremediation Symposium, SanDiego, CA. pp.289-294, 1999.

Gornall, R.J., Bohm, B.A., and Dahlgren, R., "The Distribution of Flavonoids in Angiosperms," *Bot. Notiser,* Vol. 132, p1-30, 1979.

Guthrie, E.A., Pfaender, F.K., "Reduced Pyrene Bioavailability in Microbially Active Soils", *Environ. Sci. Technol.,* 1998, 32:501-508.

Hall, M. Grover P.L., "Polycyclic Aromatic Hydrocarbons: Metabolism, Activation and Tumor Initiation," *Chemical Carcinogenesis and Mutagenesis,* Cooper, C.S. and Grover, P.L., eds., Berlin: Springer-Verlag, Vol 1, 1990, pp. 327-372.

Hahlbrock, K., "Flavonoids," *The Biochemistry of Plants*, Conn, E.E. Ed., Academic Press, 1981.

Harms, H.; Dehren, W.; Monch, W., "Benzo[a]pyrene Metabolites Formed in Plant Cells," *Z. Naturforsch.* 1977**,** *32*:321-326.

Hatcher, P.G., J.M. Bortiatynski, R.D. Minard, J. Dec, and J.M. Bollag, "Use of high resolution ^{13}C NMR to examine enzymatic covalent binding of ^{13}C-labelled 2,4 dichlorophenol to humic substances. *ES&T,* 1993, *27*:2089-2103.

Hatzinger P.B., Alexander, M., "Effect of Aging of Chemicals in Soil on Their Biodegradability and Extractability" *ES&T,* 1995, 29:537-545.

Haufman, D.D. In *Bound and conjugated Pesticide Residues; ACS Sumposium Series 29:* Kaufmann, D.D., G.G. Still, G.D. Paulson, S.K. Bandal, Eds; American Chemical Society: Washington, D.C., 1976, p1-10.

Horvath, R.S., "Microbial Co-Metabolism and the Degradation of Organic Compounds in Nature," *Bacteriol. Rev.,* Vol. 36,1972, pp.146-155.

Huang, W., Schlautman, M.A., and Weber, W.J. Jr., *ES&T,* 1996, 30:2993-3000.Manilal, V.B.;

Alexander, M., "Factors Affecting the Microbial Degradation of Phenanthrene in Soil," *Appl. Microbial Biotechnol*. 1991, 35:401-405.

Huang MT, Johnson EF, Muller-Eberhard U, Koop DR, Coon MJ, Conney AH, 1981, Specificity in the activtion and inhibition by flavonoids of benxo[a]pyrene hydroyxlation by cytochrome P-450 isozymes from rabbit liver microsomes. *J. Biol. Chem.* 256:10897.

Huang MT, Wood AW, Newmark HL, Sayer JM, Yagi H, Jerina DM, Conney, AH, 1983, Inhibition of the mutagenicity of bay-region diol-epoxides of polycyclic aromatic hydrocarbons by phenolic plant flavonoids, *Carcinogenesis* 4:1637.

Hurst, C.J., R.C. Sims, J.L. Sims, D.L. Sorensen, J.L. McLean, S.J. Huling, *J. Environ. Eng.* 1997, 123:364-370

Hurst, C.J., R.C. Sims, J.L. Sims, D.L. Sorensen, J.L. McLean, S.J. Huling, "Polycyclic Aromatic Hydrocarbon Biodegradation as a Function of Oxygen Tension in Contaminated Soil *J. Hazard. Mater.* 1996, 51:193-208.

IARC (International Agency for Research on Cancer): IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. "Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental and Experimental Data," vol 32. Geneva: World Health Organization, 1983.

Jones, W.L., Dockery, J.D., Bogel, C.R., and Sturmen, P.J., "Diffusion and Reaction within Porous Packing Media: a Phenomenological Model," *Biotechnology and Bioengineering*, Vol. 41, 1993, p. 947-956.

Kan, A.T., G. Fu, Tomson, M.B., "Adsorption/Desorption Hysteresis in Organic Pollutant and Soil/Sediment," *Environ. Sci. Technol.* 28:859-867, 1994.

Karickhoff, S.W., Brown, D.S., Scott, T.A., "Sorption of hydrophobic pollutants on natural sediments," *Water Res.* 13:241-248, 1979.

Karickhoff, S.W., "Sorption Kinetics of Hydrophobic Pollutants in Natural Sediments", In: Baker R.S.(Ed.) "Contaminants and Sediments Vol. 2.", Ann Arbor Science, Ann Arbor, 1980, p193-125.

Kästner, M., Lotter, S., Heerenklage, J., Brueer-Jammali, M., Stegmann, R., Mahro, B. *Appl. Microbiol. Biotechnol.,* 1995, 43: 1128-1135.

Katznelson, H., Rouatt, J.W., Payne, T.M.B., "Liberation of Amino Acids by Plant Roots in Relation to Desiccation," *Nature,* Vol. 174, 1954, pp.1110-1111.

Katznelson, H., Rouatt, J.W., Payne, T.M.B., "Liberation of Amino Acids and Reducing Compounds by Plant Roots," *Plant and Soil,* Vol. 7, 1955, pp.35-48.

Keck, J.; Sims, R.C.; Coover, M.; Park, K. Symons, B., "Evidence for Cooxidation of Polynuclear Aromatic Hydrocarbons in Soil," *Water Res.*, 1989, *23(12)*:1467-1476.

Kelsey, JW., Kottler, B.D., Alexander, M., "Selective Chemical extractants to predict Bioavailability of Soil-Aged Organic Chemicals, *ES&T*, 1997, 31:214-217.

Kihohar, H., Nagao, K, Nomi, R., "Degradation of Phenanthrene through o-Phthalate by an Aeromonas sp.," *Agric Biol Chem.*, Vol. 40, 1976, pp.1075-1082.

Kile, D.E., Chiou, C.T., Zhou, H., Li, H., Xu, O., "Partition of Nonpolr Organic Pollutants from Water to Soil and Sediment Organic Matters", *ES&T*, 1995, 29:1401-1406.

Klute, A. Ed. "Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties, 2nd

ed.", Agronomy vol. 9. Am. Soc. Agron., Madison, Wisconsin, 1982, 1216 pp.

Launen, L.A, Percival, P., Lam, S., Pinto, L., Moore, M., "Pure Cultures of a Penicillium Species Metabolize Pyrenequinones to Inextractable Products," *Bioremediation technologies for polycyclic aromatic hydrocarbon compounds*, Leeson A. and B.C. Alleman eds., Battelle, The Fifth International In-Situ and On-Site Bioremediation Symposium, SanDiego, CA. Pp. 75-80, 1999.

Leadbetter, E.R., Foster, J.W., "Oxidation Products Formed from Gaseous Alkanes," *Aarchs Biochem, Biophys*, Vol. 82, 1959, pp 491-492.

Lesage, S., Li, W.C., Millar, K., Liu, D., "Effect of Humic Acids on the Bioavailability of PAHs from Weathered Soils," *Bioremediation technologies for polycyclic aromatic hydrocarbon compounds*, Leeson A. and B.C. Alleman eds., Battelle, The Fifth International In-Situ and On-Site Bioremediation Symposium, SanDiego, CA. pp.161-166, 1999.

Leduc,R.; Samson,R.; Al-Bashir,B.; Al-Hawari, J.; Cseh, T. "Biotic and Abiotic Disappearance of Four PAH Compound from Flooded Soil under Various Redox Conditions," *Wat. Sci. Tech*., 1992, 26(1-2):51-60

Lick, W., Rapaka, V., "A quantitative Analysis of the Dynamics of the Sorption of Hydrophobic Organic Chemicals of Suspended Sediments," *Environ., Toxicol. Chemi.,* Vol. 15., 1996, pp. 1038-1048.

Livingston, D., 1993, Biotechnology and Pollution Monitoring: Use of Molecular Biomarkers in the Aquatic Environment. *J. Chem. Tech. and Biotech. 57*:195-211.

Loehr, R.C., Webster M.T., "Behavior of Fresh vs. Aged Chemicals in Soil," *Journal of Soil Contamination*, 1996, 5(4):361-383.

Loehr, R.C., Webster, M.T., "Changes in Toxicity and Mobility Resulting from Bioremediation Processes", *Biorem. J.*, 1997, 1:149-163.

Luthy R.G., Aiken, G.R., Brusseau, M.L., Cunningham, S.D., Gschwend, O.M., Pignatello, J.J., Reinhard, M., Traina, S.J., Weber, W.J. Jr., and Westall, J.C., "Critical Review: Sequestration of Hydrophobic Organic Contamiants by Geosorbents", *ES&T*, 1997, 31:3341-3347.

Mahro, B.; Schaeger, G., Kasterner, M., In "Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds", Hinchee, R.E., Leeson, A., Semprini, L., Ong, S.K. Eds., Lewis Publishers, 1994, p203-217.

Marschner, H and V. Römheld., "Root-Induced Changes in the Availability of Micronutrients in the Rhizosphere," *Plant Roots: The Hidden Half, 2nd Ed.*, Waisel, Y., Eshel, A., and Kafkafi, U. eds., Marcel Dekker, Inc. 1996, New York. 1002 pp.

Martin, J.P. and K. Haider, "A comparison of the use of Phenolase and peroxidase for the synthesis of model humic acid-type polyners", *J. Soil. Sci. Soc. Am.*, 1980, *44*:983-988.

Martin, J.P., Parsa, A.A., Haider, K., "Influence of Intimate Association with Humic Polymner on Biodegradation of ^{14}C-labelled Organic Substances in Soil", *Soil Biochem.*, 1978, 10:483-486.

Maruya, K.A., Risebrough, R.W., Horne, A.J., "Partitioning of Polynuclear Aromatic Hydrocarbons between Sediments from San Francisco Bay and Their Porewaters", *Environ. Sci. Technol.*, 1996, 30: 2942-2947.

McCarthy, J.F., Jimenez, B.D., "Interactions between Polycyclic Aromatic Hydrocarbons and Dissolved Humic Material: Binding and Dissociation", *Environ. Sci. Technol.*, 1985, 19:1072-1076.

McDougall, B.M., Rovira, A.D., "Carbon^{14} Labeled Photosynthate in Wheat Root Exudates," *Nature*, Vol. 207, 1965, 1104-1105.

McFarland, M.J.; Sims, R.C, "Thermodynamic Framework for Evaluating PAH Degradation in the Subsurface," *Journal of Groundwater*, 1991, *29(6)*:885-698.

McGroddy, S.E., Farrington, J.W., Gschwend, P.M., "Comparison of the in Situ and Desorption Sediment-Water Partitioning of Polycyclic Aromatic Hydrocarbons and Polychlorinated Biphenyls," *Env. Sci. Technol.*, Vol. 30, 1996, pp.172-177.

McKenna, E.J., "Biodegradation of Polynuclear Aromatic Hydrocarbon Pollutants by Soil and Water Microorganisms," *WRC Research Report No. 113*, University of Illinois, Water Research Center, Urbana, Ill. 1976.

Mihelcic, J.R., Luthy, R.G., "Microbial Degradation of Acenaphthene and Naphthalene under Denitrification Conditions in Soil-Water Systems," *Appl. Envir. Microbiol.*, 1988, 54(5):1182-1187.

Mihelcic, J.R., Luthy, R.G., "Adsorption and Microbial Degradation of Naphthalene in Soil-Water suspensions under Denitrification Conditions", *Envir. Sci. Technol.*, 1991, 25:169-177.

Middleton, C.A., Nakles, D.V., and Linz, D.G., "The Influence of Soil Composition on Bioremediation of PAH-Contaminated Soils," *Remediation /Autum*, 1991.

Miller, E.C., and Miller, J.A., "Some Historical Perspectives on the Metabolism of Xenobiotic Chemicals to Reactive Electrophiles," *Bioactivation of Foreign Compounds*, Anders, M.W. Ed., Orlando, FL: Academic Press, 1985, pp. 3-28.

Nam, K., Chung, N., and Alexander, M., "Relationship between Organic Matter Content of Soil and the Sequestration of Phenanthrene", *ES&T*, 1998, 32:3785-3788.

Nieman , J.K.C., Sims, R.C., Sims, J.L., Sorensen, D.L., McLean, J.E., and Rice, J.A., "[^{14}C]Pyrene Bound Residue Evaluation Using MIBK Fractionation Method for Cresote-Contaminated Soil", *Environ. Sci. Technol., 1999*, 33:776-781.

Orgam, A.V., Jessup, R.E., Ou, L.T., Rao, P.S.C., "Effects of Sorption on Biological Degradation Rates of (2,4-dichlorophenoxy)acetic Acid in Soils", *Appl. Environ. Microbiol.*, 1985, 49:582-587.

Packard, Tri-Carb® Liquid Scintillaiton analyzers Operation Manual, No. 7001334, Packard Instrument Co., Inc. 1995.

Packard Instrument Company Technical Service. Personnel Communication.

Park, K.S., R.C. Sims, and R.R. Dupont, "Transformation of PAHs in Soil Systems," *J. Env. Eng.*, 116:3, 1990, p 632-640.

Paul, E.A., Clark F.E., "Soil Microbiology and Biochemistry, 2nd Ed.", Academic Press, 1996, 340 pp.

Pfaender, F. K., Bartholomew, G.W., "Measurement of Aquatic Degradation Rates by Determining Heterotrophic Uptake of Radiolabeled Pollutants," *Appl. Environ. Microbiol.*, Vol. 44:1, 1982, pp. 59-164.

Pignatello, J.J., and Xing, B.S., "Mechanisms of Slow Sorption of Organic Chemicals to Natural Particles", *ES&T*, 1996, 30:1-11.

Poiger, H. Schlatter, C., "Influence of Sovents and Adsorbents on Dermal and Intestinal Absorption of TCDD", *Food Cosmet Toxicol.*, 1980, 18:477-481.

Qiu, X. "PAH Phytoremediation Lab Study Operating Procedures (OPs)", Union Carbide Corporation, Tech Center, South Charleston, WV, 1996.

Qiu, X., McFarland, M.J., "Bound Residue Formation in PAH-Contaminated Soil Composting using *phanerochaete* chrysosporium", Hazardous. *Waste Hazard. Mater.*, 1991, 8:115-126.

Raddy, K.R.; Rao, P.S.C.; Jessup, R.E., "The Effect of Carbon Mineralization of Denitrification Kinetics in Mineral and Organic Soils," *Soil Sci. Soc. Am. J.* 1982, 46:62-68

Richnow, H.H., R. Seifert, J. Hefter, M. Link, W, Francke, G. Schaefer, and W. Michaelis, "Organic Pollutants Associated with Macromolecular Soil Organic Matter: Mode of Binding", *Geochem.* 1997, 26:745-758.

Richnow, H.H., Seifert, R., Hefter, J., Kastner, M.,Mahro, B., Michaelis, W., *Chemosphere* 1998, 36:2211-2224.

Richnow, H.H., Eschenbach, A., Seifert, R., Mahro, B., Wehrung, P., Albrecht, P.,Michaelis, W., "Metabolites of xenobiotic and mineral oil consitituents linked to macromolecular organic matter in polluted environments", *Adv. Org. Geochem.* , 1994, 22:671-681.

Robinson, K.G., Farmer, W.S., Novak, J.T., "Availability of Sorbed Toluene in Soils for Biodegradation by Acclimated Bacteria"" *Water Res.*, 1990, 24:345-350.

Rovira, A.D., "Residues on Root Rot of Bean," *Phytopathology*, Vol. 53, 1963, pp. 265-270.

Rovira, A.D., "Plant Root Exudates," *Bot. Rev.*, Vol. 35, 1969, pp.35-69.

Rovira, A.D., McDougall, B.M., "Microbiological and Biochemical Aspects of the Rhizosphere," *Soil Biochemistry*, McLaren, A.D. & Peterson, G.F. eds., Marcel Dekker, New York, 1967.

Rovira, A.D., Brisbane, P.G., "Numerical Taxonomy and Soil Bacteria," *The Ecology of Soil Bacteria*, Gray, T.R.G., Parkinson, D., eds., Liverpool Univ. Press, 1967, pp. 337-350.

Santini, K., Bureau, J., Deschênes, L., "Relationship Between Detoxification and Decontamination During the Treatment of a PAH-contaminated Soil," *Bioremediation technologies for polycyclic aromatic hydrocarbon compounds,* Leeson A. and B.C. Alleman eds., Battelle, The Fifth International In-Situ and On-Site Bioremediation Symposium, SanDiego, CA. pp.185-190, 1999.

Sack, U., T.M. Heinze, J. Deck, C.E. Cerniglia, R. Martens, F. Zadrazil, and W. Fritsche. Comparison of Phenanthrene and pytene degradation by different wood-decaying fungi. Appl. Environ. Microbiol. 1997, 63: 3919-3925

Sarkar, J.M., R.L. Malcolm, and J. M. Bollag, "Enzymatic coupling of 2,4 dichlorophenol to stream fulvic acid in the presence of oxidoreductase", *J. Soil. Sci. Soc. Of Am.*, 1988, *52*:688-694.

Sato, R., and Omura, T., "Cytochrome P-450." Academic Press, New York, 1978.

Sayer JM, Hagi H, Wood AW, Conney AH, Jerina DM, 1982, Extremely facile reaction between the ultimate carcinogene benxo[a]pyrene 7,8-diol-9,10-epoxide and ellagic acid, *J. Am. Chem. Soc.* 64: 5562.

Schocken, M.J., Gibson, D.T., "Bacterial Oxidation of the Polycyclic Aromtic Hydrocarbons Acenaphthene and Acenaphthylene," *Appl. Environ. Microbiol.,* Vol. 48, 1984, pp.10-16.

Sims, R.C. and C.K. Abbott, "Evaluation of Mechanisms of Alteration and Humification of PAHs for Water Quality Management", *USGS* 14-08-0001-G1723, NTIS PB93-118313, 1993.

Sims, R.C., and M.R. Overcash, "Rate of Polynuclear Aromatic Compounds in Soil-Plant Systems," *Residue Rev., 88, p1-68,* 1983

Sims P., Frover P.L., Swaisland A., Pal,K., Hewer, A, "Metabolic Activation of Benzo{a]pyrene Proceeds by a Diol-Epoxide," *Nature*, London, Vol. 252, 1974, pp. 326.

Stevenson, F.J., "Humus Chemistry – Genesis, Composition, Reactions", John Wiley and Sons, Inc., NY, 1982, 443 pp.

Stevenson, F.J., "Humus Chemistry – Genesis, Composition, Reactions", 2nd Ed., Wiley, NY, 1994.

Stone, A.T., "Reductive Dissolution of Manganese (III/IV) Oxides by Substituted Penols", *Environ. Sci., Technol.,* 1987, 21:979-988.

Sutherland, J.B.; Rafii, F.; Khan, A.A.; Cerniglia, C.E., "Microbial Transformation and Degradation of Toxic Organic Chemicals," Young, L.Y., Cerniglia, C.E. Eds., Wiley-Liss: New York, 1995; pp269-306.

Sutherland, J.B., "Detoxification of Polycyclic Aromatic Hydrocarbons by Fungi," *J. Ind. Microbiol.* Vol. 9, 1992, pp.53-62.

Sutherland, J.B., Fu, P.P., Yang, S.K., Selby A.L., Von Tungeln, L.S., Casillas, R.P., Crow, S.A., Cerniglia, C.E., "Enantiomeric composition of the *Trans*-dihydrodiols Produced from Phenanthrene by Fungi," *Appl. Environ. Microbiol.,* Vol. 59, 1993, pp.2145-2149.

Tang J.X., M.J. Carroquino, B.K. Rovertson, and M. Alexander, "Combined Effect of Sequestration and Bioremediation in Reducing the Bioavailability of Polycyclic Aromatic Hydrocarbons in Soil", *ES&T,* 1998, 32:3586-3590.

Toussoun, T.A., Patrick, Z.A., "Effect of Phytotoxic Substances from Decomposing Plant Venkataraman, K., "Flavones", *The Flavonoids,* Vol. 1, Harborne, J.B. and Mabry, H.. eds., pp. 267-295, Academic Press, New York, NY, 1975.

Verstraete, W., Devliegher, W., *Biodegradation,* 1996, 7:471-485.

Walton B.T.; Guthrie, E.A.; Hoylman, A.M., "Toxicant Degradation in the Rhizosphere," *Bioremediation through Rhizosphere Technology;* Anderson T. A.; Coats,J.R. Eds.; ACS Symposium Series 563; American Chemical Society: Washington, DC, 1994, pp. 11-27..

Weber, W.J., Huang, W., "A Distributed Reactivity Model for Sorption by Soils and Sediments. $. Intrapartical Heterogeneity and Phase-Distribution Relationships under Noneequilibrium Conditions", *Environ. Sci. Technol.,* 1996, 30:881-888.

WEF, "Standard Method for the Examination of Water and Wastewater", 19 Ed., 1998.

Wu, S., Gschwend, P.M., "Sorption Kinetics of Hydrophobic Organic Compounds to Natural Sediments and Soils," *Environ. Sci. Technol.,* Vol. 20, 1986, pp.717-725.

Wallace, H.R., "Factors influencing the Ability of *Heteodera* Larvae to Reach Host Plant Roots," *Recent Advances in Botany*, University Toronto Press, 1961, pp. 407.

Walton B.T.; Guthrie, E.A.; Hoylman, A.M., "Toxicant Degradation in the Rhizosphere," *Bioremediation through Rhizosphere Technology;* Anderson T. A.; Coats,J.R. Eds.; ACS Symposium Series 563; American Chemical Society: Washington, DC, 1994, pp. 11-27..

Weissenfels W.D, Beyer, M., Klein, J., "Degradation of Phenanthrene, Fluorene, and Fluoranthere by Pure Bacterial Cultures", *App". Microbiol. Biortechnol.*, 1990, 32:479-484.

Weissenfels, W.D, Klewer, H-J, and Langhoff J., "Adsorption of Polycyclic Aromatic Hydrocarbons (PAHs) by Soil Particles: Influence on Biodegradability and Biotoxicity", *Appl. Microbiol. Biotechnol., 1992, 36:689-696.*

Whelan, G., Sims, R.C., "Oxidation of Recalcitrant Organics in Subsurface Systems", *Haz, Waste & Haz. Mat.*, 1992, 9(3): 245-265.

White, J.C., Kelsey, J.W., Hatzinger, P.B., Alexander, M., "Factors Afecting Sequestration and Bioavailability of Phenanthrene in Soil:, *Environ. Toxicol. Chem.*, 1997, 16:2040-2045.

Xing, B., Pignatello, J.J., "Dual-Mode Sorption of Low-Polarity Compounds in Glassy Poly(Vinyl Chloride) and Soil Organic Matter", *ES&T*, 1997, 31-792-799

Young, T.M., Weber, W.J., Jr., "A Distributed Reactivity Model for Sorption by Soils and Sediments. 3. Effects of Diagenetic Processes on Sorption Energetics", *ES&T*, 1995, 29-92-97.

Yang, S.K., 1988, "Metabolism and activation of benz[a]anthracene and methylbenz[a]anthracene. In *Polycyclic aromatic Hydrocarbon Carcinogenesis: Structure-Activity Relationships*, eds. S.K. Yang and B.D. Silverman, CRC Press, Boca Raton, FL.

APPENDICES

APPENDIX A. EXPERIMENTAL DATA

Table A-1. Liquid scintillation counting data for 14C-B[a]P microcosms

Soil	Flavonoid	Concentration	PAH	Tot. spike (DPM)	$^{14}CO_2$ (dpm)	^{14}C/hexane /H2O (dpm)	$^{14}C/H_2O$ (dpm)	^{14}C/Eac- soil (dpm)	^{14}C/soil-bound (dpm)	^{14}C Sum (dpm)	Mass Balance (%)
Poison Control	None	0	B[a]P	17318	29	9	57	8158	12305	20558	1.19
Poison Control	None	0	B[a]P	17318	18	7	55	12161	7549	19791	1.14
Poison Control	None	0	B[a]P	17318	127	14	58	15770	8686	22457	1.30
Poison Control	M-Rt-extrac	Not quantified	B[a]P	17318	159	82	122	21142	5594	27100	1.56
Poison Control	M-Rt-extrac	Not quantified	B[a]P	17318	1025	54	109	12080	4612	17880	1.03
Poison Control	M-Rt-extrac	Not quantified	B[a]P	17318	80	55	115	9705	7186	17141	0.99
Poison Control	Morin	0.1	B[a]P	17318	158	9	54	8762	8254	17237	1.00
Poison Control	Morin	0.1	B[a]P	17318	46	22	67	12192	17539	29865	1.72
Poison Control	Morin	0.1	B[a]P	17318	36	9	59	7429	8645	16178	0.93
Poison Control	Morin	1	B[a]P	17318	193	10	64	11114	8157	19538	1.13
Poison Control	Morin	1	B[a]P	17318	166	7	62	9155	7758	17149	0.99
Poison Control	Morin	1	B[a]P	17318	80	10	62	2760	17437	20349	1.17
Poison Control	Morin	10	B[a]P	17318	42	11	50	12241	4883	17226	0.99
Poison Control	Morin	10	B[a]P	17318	21	11	48	1392	15944	17416	1.01
Poison Control	Morin	10	B[a]P	17318	31	10	55	4655	11146	15896	0.92
Poison Control	Morin	100	B[a]P	17318	35	13	57	11888	6602	18594	1.07
Poison Control	Morin	100	B[a]P	17318	188	15	64	9840	6689	16796	0.97
Poison Control	Morin	100	B[a]P	17318	46	11	52	10432	6217	16757	0.97
Poison Control	Flavone	0.1	B[a]P	17318	560	15	80	7369	11642	19667	1.14
Poison Control	Flavone	0.1	B[a]P	17318	19	12	51	13183	7157	20422	1.18
Poison Control	Flavone	0.1	B[a]P	17318	41	14	55	6054	9959	16123	0.93
Poison Control	Flavone	1	B[a]P	17318	24	10	52	6636	10068	16789	0.97
Poison Control	Flavone	1	B[a]P	17318	23	12	56	7762	8568	16421	0.95
Poison Control	Flavone	1	B[a]P	17318	73	12	59	9055	5965	15163	0.88
Poison Control	Flavone	10	B[a]P	17318	50	13	58	8947	8189	17256	1.00
Poison Control	Flavone	10	B[a]P	17318	19	12	54	11274	4519	15879	0.92
Poison Control	Flavone	10	B[a]P	17318	93	12	62	8690	6269	15125	0.87
Poison Control	Flavone	100	B[a]P	17318	131	9	50	9181	7344	16714	0.97
Poison Control	Flavone	100	B[a]P	17318	82	11	58	10462	6181	16794	0.97
Poison Control	Flavone	100	B[a]P	17318	63	13	60	10830	5429	16396	0.95
Mulberry	None	0	B[a]P	17318	3796	3	43	6733	4588	15162	0.88
Mulberry	None	0	B[a]P	17318	3520	2	46	4871	8641	17080	0.99
Mulberry	None	0	B[a]P	17318	4580	5		8459	3285	16328	0.94
Mulberry	M-Rt-extrac	Not quantified	B[a]P	17318	259	56	134	4236	12149	16834	0.97
Mulberry	M-Rt-extrac	Not quantified	B[a]P	17318	333	29	98	8207	8675	17343	1.00
Mulberry	M-Rt-extrac	Not quantified	B[a]P	17318	477	28	82	7479	9405	17471	1.01
Mulberry	Morin	0.1	B[a]P	17318	3088	3	56	10808	3390	17344	1.00
Mulberry	Morin	0.1	B[a]P	17318	2572	3	30	7683	3505	13794	0.80
Mulberry	Morin	0.1	B[a]P	17318	2979	3	47	6172	5911	15112	0.87
Mulberry	Morin	1	B[a]P	17318	7712	7	49	20971	5741	34479	1.99
Mulberry	Morin	1	B[a]P	17318	4332	2	38	5992	5384	15748	0.91
Mulberry	Morin	1	B[a]P	17318	2276	4	36	5374	4510	12200	0.70
Mulberry	Morin	10	B[a]P	17318	2319	1	38	4642	11848	18848	1.09
Mulberry	Morin	10	B[a]P	17318	1959	1	42	6035	6290	14327	0.83
Mulberry	Morin	10	B[a]P	17318	2820	3	40	2727	8578	14168	0.82
Mulberry	Morin	100	B[a]P	17318	186	9	70	6386	7704	14353	0.83
Mulberry	Morin	100	B[a]P	17318	151	6	60	11733	3274	15224	0.88
Mulberry	Morin	100	B[a]P	17318	161	7	46	6372	10087	16674	0.96

Table A-1. Liquid scintillation counting data for ^{14}C-B[a]P microcosms (cont')

Soil	Flavonoid	Concentration	PAH	Tot. spike (DPM)	$^{14}CO_2$ (dpm)	^{14}C/hexane /H2O (dpm)	$^{14}C/H_2O$ (dpm)	^{14}C/Eac- soil (dpm)	^{14}C/soil-bound (dpm)	^{14}C Sum (dpm)	Mass Balance (%)
Mulberry	Flavone	0.1	B[a]P	17318	2688	3	46	5207	5377	13322	0.77
Mulberry	Flavone	0.1	B[a]P	17318	3306	4	47	6431	4545	14333	0.83
Mulberry	Flavone	0.1	B[a]P	17318	2528	5	47	8787	8223	19590	1.13
Mulberry	Flavone	1	B[a]P	17318	3276	6	45	6460	4456	14244	0.82
Mulberry	Flavone	1	B[a]P	17318	3170	5	44	5518	4907	13644	0.79
Mulberry	Flavone	1	B[a]P	17318	3238	2	49	8097	1829	13215	0.76
Mulberry	Flavone	10	B[a]P	17318	2991	4	26	4751	7749	15521	0.90
Mulberry	Flavone	10	B[a]P	17318	3046	4	35	2903	7952	13940	0.80
Mulberry	Flavone	10	B[a]P	17318	2444	5	56	10198	3858	16561	0.96
Mulberry	Flavone	100	B[a]P	17318	226	4	41	7780	13243	21295	1.23
Mulberry	Flavone	100	B[a]P	17318	131	5	26	3661	11085	14908	0.86
Mulberry	Flavone	100	B[a]P	17318	123	11	32	4132	24385	28683	1.66
Grasses	None	0	B[a]P	17318	2429	3	41	4818	7914	15205	0.88
Grasses	None	0	B[a]P	17318	2598	6	38	5488	6662	14792	0.85
Grasses	None	0	B[a]P	17318	3456	6	41	11546	9195	24245	1.40
Grasses	M-Rt-extrac	Not quantified	B[a]P	17318	372	12	27	2271	16183	18865	1.09
Grasses	M-Rt-extrac	Not quantified	B[a]P	17318	166	29	76	3614	12213	16098	0.93
Grasses	M-Rt-extrac	Not quantified	B[a]P	17318	357	28	56	5468	11781	17690	1.02
Grasses	Morin	0.1	B[a]P	17318							
Grasses	Morin	0.1	B[a]P	17318	1382	1	35	5677	7568	14663	0.85
Grasses	Morin	0.1	B[a]P	17318	2405	5	35	6831	5792	15068	0.87
Grasses	Morin	1	B[a]P	17318	2889	3	45	7356	7529	17822	1.03
Grasses	Morin	1	B[a]P	17318	1825	2	38	5832	6185	13882	0.80
Grasses	Morin	1	B[a]P	17318	2524	4	40	6586	5970	15124	0.87
Grasses	Morin	10	B[a]P	17318	1314	3	31	9194	5648	16189	0.93
Grasses	Morin	10	B[a]P	17318	832	10	29	5852	9952	16674	0.96
Grasses	Morin	10	B[a]P	17318	526	6	34	5193	8639	14398	0.83
Grasses	Morin	100	B[a]P	17318	143	9	41	6681	9861	16735	0.97
Grasses	Morin	100	B[a]P	17318	253	11	59	3749	17680	21752	1.26
Grasses	Morin	100	B[a]P	17318	271	7	43	1252	15954	17527	1.01
Grasses	Flavone	0.1	B[a]P	17318	2464	5	39	5163	8244	15914	0.92
Grasses	Flavone	0.1	B[a]P	17318	2913	6	52	7186	7941	18099	1.05
Grasses	Flavone	0.1	B[a]P	17318	3595	4	40	1816	10694	16149	0.93
Grasses	Flavone	1	B[a]P	17318	1985	3	37	2550	10636	15211	0.88
Grasses	Flavone	1	B[a]P	17318	1500	5	29	4374	8991	14900	0.86
Grasses	Flavone	1	B[a]P	17318	2267	3	43	3799	8719	14832	0.86
Grasses	Flavone	10	B[a]P	17318	1324	2	45	1981	10501	13853	0.80
Grasses	Flavone	10	B[a]P	17318	843	0	31	1626	12114	14614	0.84
Grasses	Flavone	10	B[a]P	17318	1145	3	39	3185	8858	13230	0.76
Grasses	Flavone	100	B[a]P	17318	143	16	125	2496	15215	17995	1.04
Grasses	Flavone	100	B[a]P	17318	3010	16	66	4344	18272	25708	1.48
Grasses	Flavone	100	B[a]P	17318	227	4	44	11541	16872	28688	1.66

Table A-2. Liquid scintillation counting data for ^{14}C-pyrene microcosms

Soil	Flavonoid	Concentration	PAH	Tot. spike (DPM)	$^{14}CO_2$ (dpm)	^{14}C/hexane /H2O (dpm)	$^{14}C/H_2O$ (dpm)	^{14}C/Eac- soil (dpm)	^{14}C/soil-bound (dpm)	^{14}C Sum (dpm)	Mass Balance (%)
Poison Control	None	0	Pyrene	59316	140	31	164	26688	18909	45931	0.77
Poison Control	None	0	Pyrene	59316	53	37	131	17454	25286	42962	0.72
Poison Control	None	0	Pyrene	59316	34	20	93	31486	11939	43571	0.73
Poison Control	M-Rt-ex	Not quantified	Pyrene	59316	72	116	227	30879	17273	48567	0.82
Poison Control	M-Rt-ex	Not quantified	Pyrene	59316	62	93	206	15323	23958	39643	0.67
Poison Control	M-Rt-ex	Not quantified	Pyrene	59316	58	68	180	27150	17292	44748	0.75
Poison Control	Morin	0.1	Pyrene	59316	186	31	140	28629	13309	42295	0.71
Poison Control	Morin	0.1	Pyrene	59316	48	29	131	34923	8053	43185	0.73
Poison Control	Morin	0.1	Pyrene	59316	115	36	147	37432	4940	42670	0.72
Poison Control	Morin	1	Pyrene	59316	51	35	150	19237	24050	43522	0.73
Poison Control	Morin	1	Pyrene	59316	78	29	163	34831	10714	45815	0.77
Poison Control	Morin	1	Pyrene	59316	75	35	128	33357	10837	44433	0.75
Poison Control	Morin	10	Pyrene	59316	36	38	123	19900	23540	43637	0.74
Poison Control	Morin	10	Pyrene	59316	71	26	101	10841	31513	42552	0.72
Poison Control	Morin	10	Pyrene	59316	36	33	101	12105	31336	43610	0.74
Poison Control	Morin	100	Pyrene	59316	118	47	146	33859	10202	44372	0.75
Poison Control	Morin	100	Pyrene	59316	108	37	110	10979	32969	44204	0.75
Poison Control	Morin	100	Pyrene	59316	98	40	137	22179	22415	44870	0.76
Poison Control	Flavone	0.1	Pyrene	59316	100	31	262	35447	8379	44219	0.75
Poison Control	Flavone	0.1	Pyrene	59316	117	45	69	18761	23317	42308	0.71
Poison Control	Flavone	0.1	Pyrene	59316	73	32	126	31146	10749	42125	0.71
Poison Control	Flavone	1	Pyrene	59316	59	35	145	33938	8247	42424	0.72
Poison Control	Flavone	1	Pyrene	59316	306	27	161	37079	6004	43577	0.73
Poison Control	Flavone	1	Pyrene	59316	42	33	133	36231	6307	42746	0.72
Poison Control	Flavone	10	Pyrene	59316	69	40	155	42662	2811	45738	0.77
Poison Control	Flavone	10	Pyrene	59316	123	33	184	36769	4705	41815	0.70
Poison Control	Flavone	10	Pyrene	59316	85	48	173	31813	11548	43668	0.74
Poison Control	Flavone	100	Pyrene	59316	73	35	164	22264	21696	44232	0.75
Poison Control	Flavone	100	Pyrene	59316	48912	27	134	36392	7419	92885	1.57
Poison Control	Flavone	100	Pyrene	59316	322	34	175	26970	16131	43632	0.74
Mulberry	None	0	Pyrene								
Mulberry	None	0	Pyrene	59316	16636	14	478	7088	3693	27908	0.47
Mulberry	None	0	Pyrene	59316	14961	6	520	3178	7732	26397	0.45
Mulberry	M-Rt-ex	Not quantified	Pyrene	59316	1635	122	757	25367	7028	34909	0.59
Mulberry	M-Rt-ex	Not quantified	Pyrene	59316	14384	26	101	16972	19650	51133	0.86
Mulberry	M-Rt-ex	Not quantified	Pyrene	59316	1314	39	398	22521	6783	31055	0.52
Mulberry	Morin	0.1	Pyrene	59316	10804	17	432	7131	4039	22423	0.38
Mulberry	Morin	0.1	Pyrene	59316	6296	23	452	5609	6662	19042	0.32
Mulberry	Morin	0.1	Pyrene	59316	13911	7	465	6866	4174	25423	0.43
Mulberry	Morin	1	Pyrene	59316	10049	16	466	5619	3972	20122	0.34
Mulberry	Morin	1	Pyrene	59316	4828	16	421	6127	5515	16908	0.29
Mulberry	Morin	1	Pyrene	59316	16900	9	348	6041	3494	26791	0.45
Mulberry	Morin	10	Pyrene	59316	9944	26	459	6085	7703	24218	0.41
Mulberry	Morin	10	Pyrene	59316	7967	6	267	6136	4746	19122	0.32
Mulberry	Morin	10	Pyrene	59316	7804	9	343	8661	7127	23944	0.40
Mulberry	Morin	100	Pyrene	59316	282	15	95	21579	19321	41291	0.70
Mulberry	Morin	100	Pyrene	59316	495	40	204	21993	16160	38891	0.66
Mulberry	Morin	100	Pyrene	59316	251	43	260	9903	27031	37487	0.63

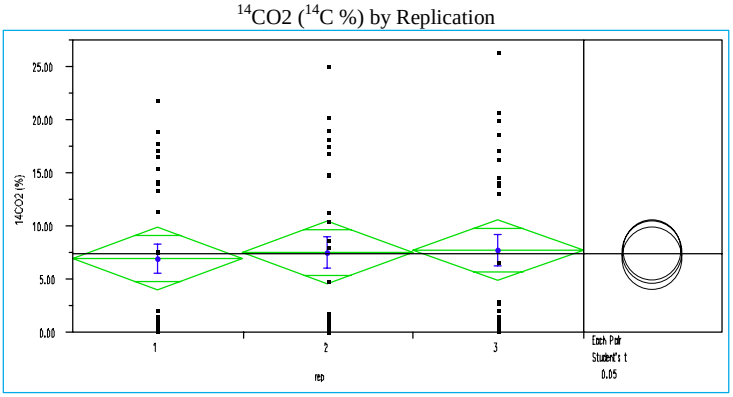
Table A-2. Liquid scintillation counting data for ^{14}C-pyrene microcosms (cont')

Soil	Flavonoid	Concentration	PAH	Tot. spike (DPM)	$^{14}CO_2$ (dpm)	^{14}C/hexane /H2O (dpm)	$^{14}C/H_2O$ (dpm)	^{14}C/Eac- soil (dpm)	^{14}C/soil-bound (dpm)	^{14}C Sum (dpm)	Mass Balance (%)
Mulberry	Flavone	0.1	Pyrene	59316	10747	17	560	6897	4752	22974	0.39
Mulberry	Flavone	0.1	Pyrene	59316	14112	5	378	5328	4877	24700	0.42
Mulberry	Flavone	0.1	Pyrene	59316	18260	7	431	5295	6188	30182	0.51
Mulberry	Flavone	1	Pyrene	59316	13508	14	323	7341	3828	25013	0.42
Mulberry	Flavone	1	Pyrene	59316	13898	4	415	8338	4518	27173	0.46
Mulberry	Flavone	1	Pyrene	59316	11526	11	360	7823	4677	24396	0.41
Mulberry	Flavone	10	Pyrene	59316	15892	9	117	6396	3798	26212	0.44
Mulberry	Flavone	10	Pyrene	59316	11719	12	185	7882	3920	23718	0.40
Mulberry	Flavone	10	Pyrene	59316	13006	16	320	5141	4782	23264	0.39
Mulberry	Flavone	100	Pyrene	59316	3842	7	225	5786	6201	16062	0.27
Mulberry	Flavone	100	Pyrene	59316	11147	4	197	3081	8813	23243	0.39
Mulberry	Flavone	100	Pyrene	59316	2648	18	150	10874	16374	30065	0.51
Grasses	None	0	Pyrene	59316	18431	11	257	5837	5775	30311	0.51
Grasses	None	0	Pyrene	59316	26797	6	326	5707	6263	39100	0.66
Grasses	None	0	Pyrene	59316	23036	24	286	5083	6059	34487	0.58
Grasses	M-Rt-e	Not quantified	Pyrene	59316	782	65	202	19049	19267	39365	0.66
Grasses	M-Rt-e	Not quantified	Pyrene	59316	1710	83	259	6971	15035	24058	0.41
Grasses	M-Rt-e	Not quantified	Pyrene	59316	2051	144	1152	17940	18266	39553	0.67
Grasses	Morin	0.1	Pyrene	59316	39325	26	254	5048	6418	51071	0.86
Grasses	Morin	0.1	Pyrene	59316	16899	11	310	6337	7409	30966	0.52
Grasses	Morin	0.1	Pyrene	59316	6929	13	293	4723	8827	20786	0.35
Grasses	Morin	1	Pyrene	59316	25614	18	239	6601	6908	39380	0.66
Grasses	Morin	1	Pyrene	59316	23880	15	241	6262	5418	35816	0.60
Grasses	Morin	1	Pyrene	59316	9886	15	320	7595	5457	23273	0.39
Grasses	Morin	10	Pyrene	59316	18725	14	170	4628	11589	35127	0.59
Grasses	Morin	10	Pyrene	59316	12006	25	233	8125	7938	28327	0.48
Grasses	Morin	10	Pyrene	59316	5242	8064	10295	7456	3090	34147	0.58
Grasses	Morin	100	Pyrene	59316	1172	47	348	16052	24919	42538	0.72
Grasses	Morin	100	Pyrene	59316	772	44	211	26691	13756	41474	0.70
Grasses	Morin	100	Pyrene	59316	1083	56	279	25972	15621	43011	0.73
Grasses	Flavone	0.1	Pyrene	59316	28998	11	283	2850	9127	41269	0.70
Grasses	Flavone	0.1	Pyrene	59316	33587	27	464	2975	10700	47753	0.81
Grasses	Flavone	0.1	Pyrene	59316	31437	20	241	2530	11337	45565	0.77
Grasses	Flavone	1	Pyrene	59316	20775	15	343	2476	10185	33793	0.57
Grasses	Flavone	1	Pyrene	59316	27274	12	332	2386	9896	39899	0.67
Grasses	Flavone	1	Pyrene	59316	28549	12	328	3005	8892	40786	0.69
Grasses	Flavone	10	Pyrene	59316	577	14	345	4024	11069	16030	0.27
Grasses	Flavone	10	Pyrene	59316	19536	26	336	2496	11885	34278	0.58
Grasses	Flavone	10	Pyrene	59316	6016	25	303	3060	12047	21451	0.36
Grasses	Flavone	100	Pyrene	59316	371	62	268	27610	11588	39899	0.67
Grasses	Flavone	100	Pyrene	59316	178	31	216	4893	35879	41196	0.69
Grasses	Flavone	100	Pyrene	59316	289	28	167	4134	35728	40345	0.68

APPENDIX B. STATISTICAL ANALYSIS: DATA REPEATABILITY (JMP OUTPUT REPORTS)

Appendix B-1. Student's t Test: Paired Comparison of Mean Data for Triplicate B[a]P-microcosms[1]

The left side chart show data points, group data mean dots, standard error bars, and 95% confidence interval diamond. The horizontal line cross the chart is the mean of all sample data



The right side chart shows comparison circles. LSD is what the distance would be if the two mean circles intersected at right angles. Circles for means that are significantly different either do not intersect or intersect slightly so that the outside angle of intersection is <90°. If the circles intersect by an outside angle of >90° or if they are nested, the means are not significantly different.

Oneway Anova
Summary of Fit

RSquare	0.001685
RSquare Adj	-0.02327
Root Mean Square Error	7.974126
Mean of Response	7.445904
Observations (or Sum Wgts)	83

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	8.5851	4.2925	0.0675
Error	80	5086.9351	63.5867	Prob>F
C Total	82	5095.5202	62.1405	0.9348

Means for Oneway Anova

Level	Number	Mean	Std Error
1	27	6.99667	1.5346
2	27	7.56370	1.5346
3	29	7.75448	1.4808

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
1	27	6.99667	7.57741	1.4583
2	27	7.56370	8.00536	1.5406
3	29	7.75448	8.29771	1.5408

Means Comparisons[2]

Dif=Mean[i]-Mean[j]		3	2	1
3	0.000000	0.190779	0.757816	
2	-0.19078	0.000000	0.567037	
1	-0.75782	-0.56704	0.000000	

Alpha= 0.05

Comparisons for each pair using Student's t[3] t 1.99007

Abs(Dif)-LSD		3	2	1
3	-4.16742	-4.05312	-3.48608	
2	-4.05312	-4.31902	-3.75198	
1	-3.48608	-3.75198	-4.31902	

Negative values show pairs of means that are not significantly different.[4]

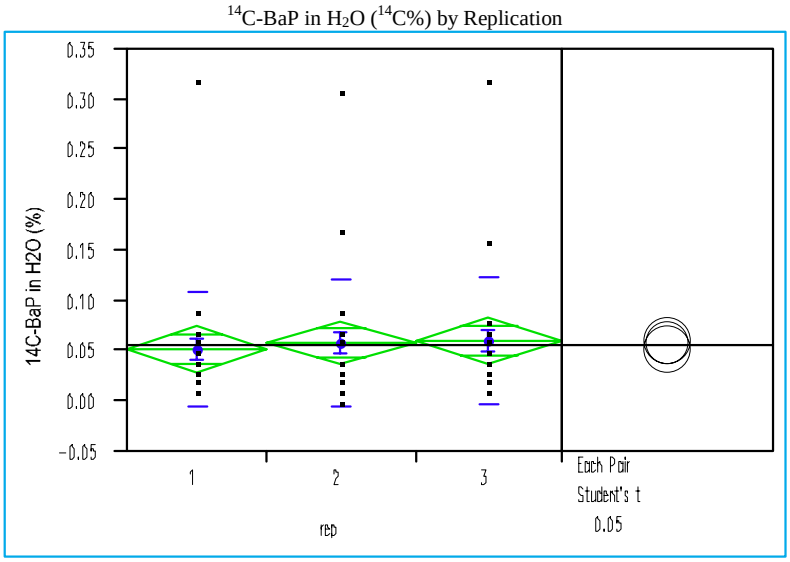
---

[1] The means comparison indicates whether the actual difference in the means is greater than the least significant difference (LSD).

[2] All means comparisons with the differences between each pair. The groups are listed with the differences sorted in descending order.

[3] The LSDs for different sample sizes are shown on the diagonal.

[4] There are no significant differences among the mean of the triplicate data sets.



Oneway Anova Summary of Fit

RSquare	0.002652
RSquare Adj	-0.02197
Root Mean Square Error	0.061988
Mean of Response	0.056667
Observations (or Sum Wgts)	84

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.00082771	0.000414	0.1077
Error	81	0.31123896	0.003842	Prob>F
C Total	83	0.31206667	0.003760	0.8980

Means for Oneway Anova

Level	Number	Mean	Std Error
1	27	0.052222	0.01193
2	29	0.057931	0.01151
3	28	0.059643	0.01171

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]		3	2	1
3	0.000000	0.001712	0.007421	
2	-0.00171	0.000000	0.005709	
1	-0.00742	-0.00571	0.000000	

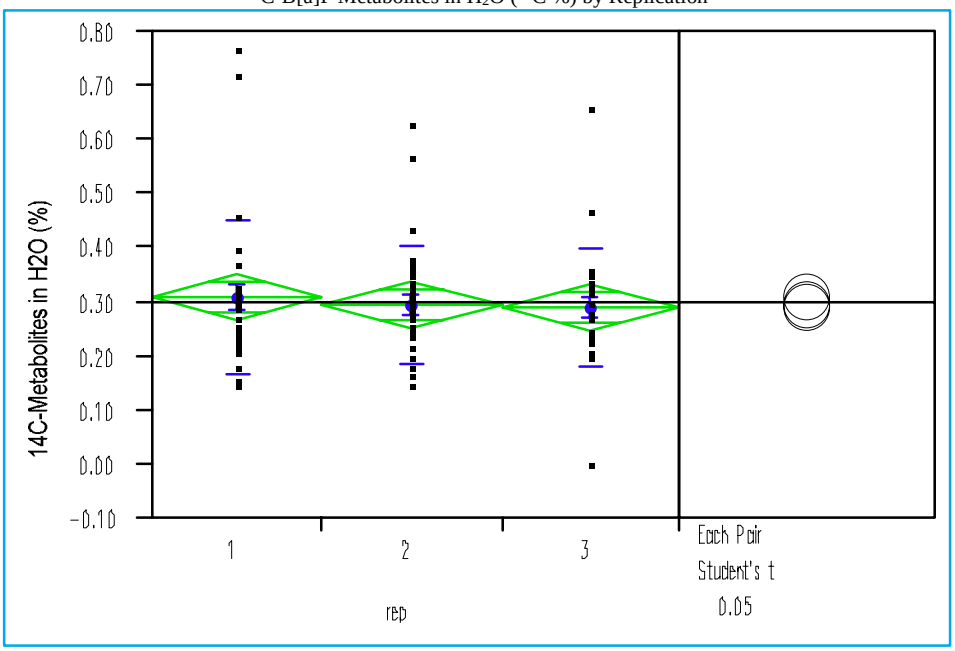
Alpha= 0.05

Comparisons for each pair using Student's t t 1.98969

Abs(Dif)-LSD		3	2	1
3	-0.03296	-0.03097	-0.02585	
2	-0.03097	-0.03239	-0.02728	
1	-0.02585	-0.02728	-0.03357	

Negative values show pairs of means that are not significantly different.

^{14}C-B[a]P Metabolites in H_2O (^{14}C %) by Replication



Oneway Anova
Summary of Fit

RSquare	0.004718
RSquare Adj	-0.01986
Root Mean Square Error	0.122311
Mean of Response	0.298095
Observations (or Sum Wgts)	84

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.0057446	0.002872	0.1920
Error	81	1.2117506	0.014960	Prob>F
C Total	83	1.2174952	0.014669	0.8257

Means for Oneway Anova

Level	Number	Mean	Std Error
1	27	0.310000	0.02354
2	29	0.293793	0.02271
3	28	0.291071	0.02311

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]		1	2	3
1	0.000000	0.016207	0.018929	
2	-0.01621	0.000000	0.002722	
3	-0.01893	-0.00272	0.000000	

Alpha= 0.05

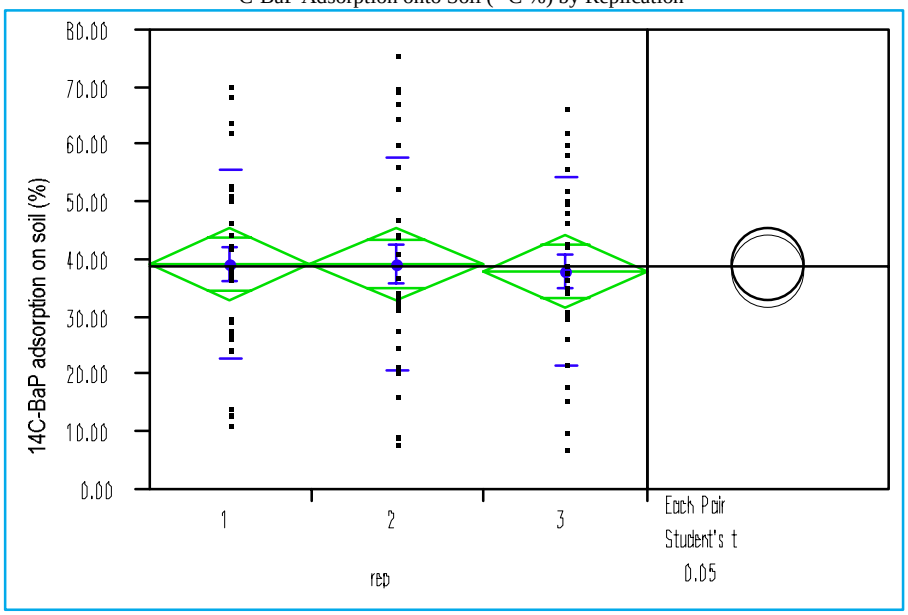
Comparisons for each pair using Student's t

t 1.98969

Abs(Dif)-LSD		1	2	3
1	-0.06623	-0.04888	-0.04671	
2	-0.04888	-0.06391	-0.06176	
3	-0.04671	-0.06176	-0.06504	

Positive values show pairs of means that are significantly different.

^{14}C-BaP Adsorption onto Soil (^{14}C %) by Replication



Oneway Anova
Summary of Fit

RSquare	0.000928
RSquare Adj	-0.02405
Root Mean Square Error	17.42119
Mean of Response	39.01735
Observations (or Sum Wgts)	83

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	22.546	11.273	0.0371
Error	80	24279.821	303.498	Prob>F
C Total	82	24302.367	296.370	0.9636

Means for Oneway Anova

Level	Number	Mean	Std Error
1	27	39.3096	3.3527
2	29	39.4403	3.2350
3	27	38.2707	3.3527

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]		2	1	3
2	0.00000	0.13072	1.16960	
1	-0.13072	0.00000	1.03889	
3	-1.16960	-1.03889	0.00000	

Alpha= 0.05

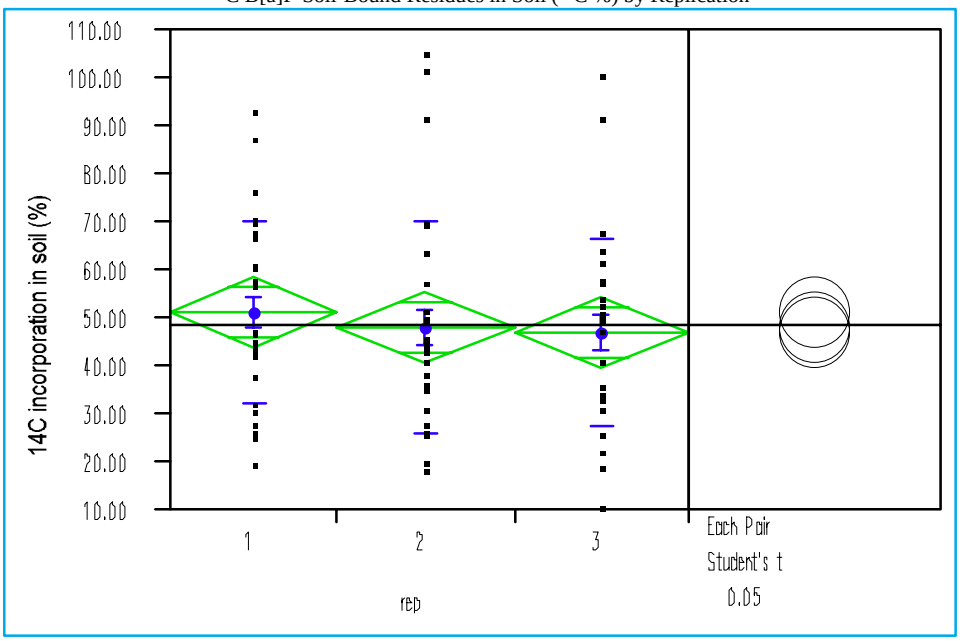
Comparisons for each pair using Student's t

t 1.99007

Abs(Dif)-LSD		2	1	3
2	-9.10463	-9.14098	-8.10210	
1	-9.14098	-9.43581	-8.39692	
3	-8.10210	-8.39692	-9.43581	

Negative values show pairs of means that are not significantly different.

^{14}C B[a]P-Soil-Bound Residues in Soil (^{14}C %) by Replication



Oneway Anova
Summary of Fit

RSquare	0.007827
RSquare Adj	-0.01667
Root Mean Square Error	20.46814
Mean of Response	48.71798
Observations (or Sum Wgts)	84

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	267.704	133.852	0.3195
Error	81	33934.528	418.945	Prob>F
C Total	83	34202.232	412.075	0.7274

Means for Oneway Anova

Level	Number	Mean	Std Error
1	27	51.2574	3.9391
2	29	47.9490	3.8008
3	28	47.0657	3.8681

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
1	27	51.2574	18.9841	3.6535
2	29	47.9490	22.3231	4.1453
3	28	47.0657	19.8244	3.7465

Means Comparisons

Dif=Mean[i]-Mean[j]		1	2	3
1	0.00000	3.30844	4.19169	
2	-3.30844	0.00000	0.88325	
3	-4.19169	-0.88325	0.00000	

Alpha= 0.05

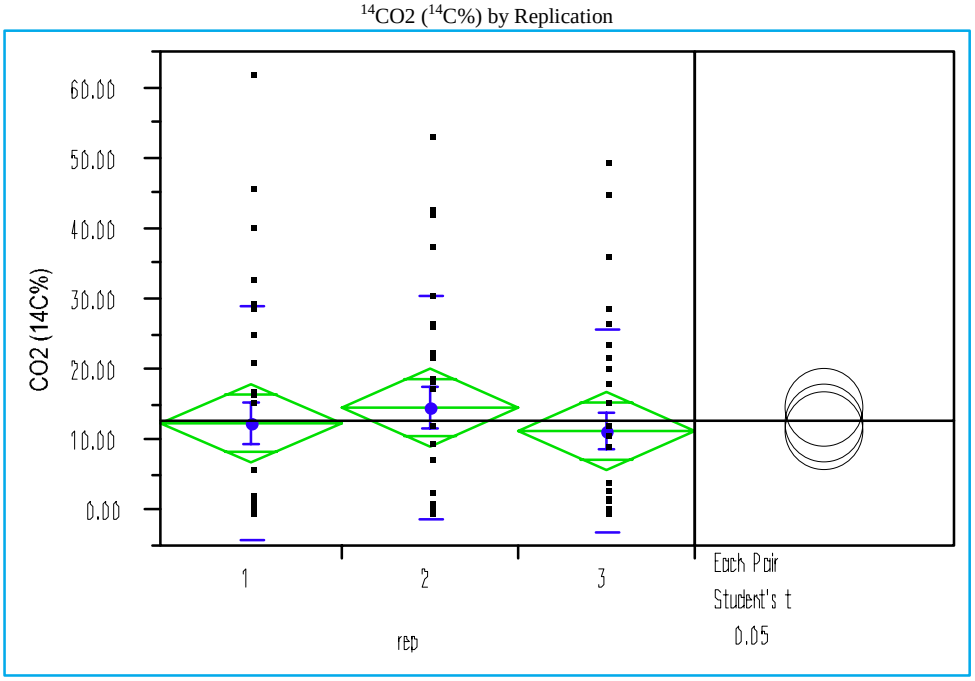
Comparisons for each pair using Student's t

t 1.98969

Abs(Dif)-LSD		1	2	3
1	-11.0840	-7.5828	-6.7929	
2	-7.5828	-10.6950	-9.9068	
3	-6.7929	-9.9068	-10.8843	

Negative values show pairs of means that are not significantly different.

Appendix B-2. Student's t Test: Paired Comparison of Mean Data for Triplicate Pyrene-Microcosms



Oneway Anova

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	137.863	68.932	0.2761
Error	85	21221.723	249.667	Prob>F
C Total	87	21359.586	245.512	0.7594

Means for Oneway Anova

Level	Number	Mean	Std Error
1	30	12.3323	2.8848
2	29	14.5428	2.9341
3	29	11.5790	2.9341

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]		2	1	3
2	0.00000	2.21043	2.96379	
1	-2.21043	0.00000	0.75337	
3	-2.96379	-0.75337	0.00000	

Alpha= 0.05

Comparisons for each pair using Student's t

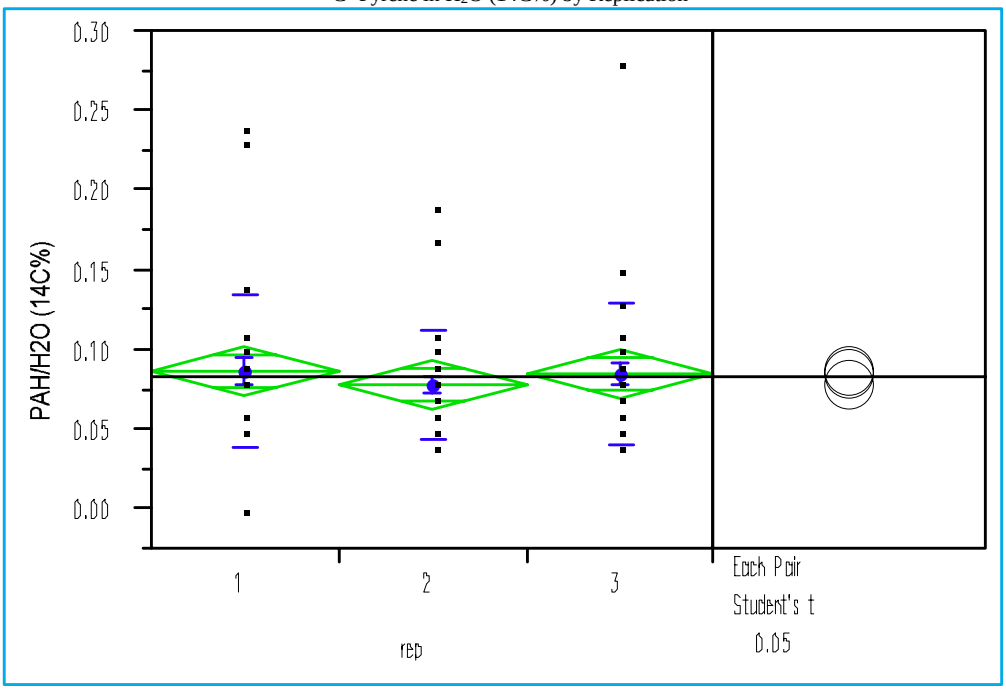
t

1.98828

Abs(Dif)-LSD		2	1	3
2	-8.25037	-5.97091	-5.28658	
1	-5.97091	-8.11170	-7.42796	
3	-5.28658	-7.42796	-8.25037	

Positive values show pairs of means that are significantly different.

^{14}C -Pyrene in H_2O (14C%) by Replication



Oneway Anova
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.00096032	0.000480	0.2507
Error	86	0.16474080	0.001916	Prob>F
C Total	88	0.16570112	0.001883	0.7789

Means for Oneway Anova

Level	Number	Mean	Std Error
1	30	0.087000	0.00799
2	30	0.079333	0.00799
3	29	0.085172	0.00813

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]		1	3	2
1	0.000000	0.001828	0.007667	
3	-0.00183	0.000000	0.005839	
2	-0.00767	-0.00584	0.000000	

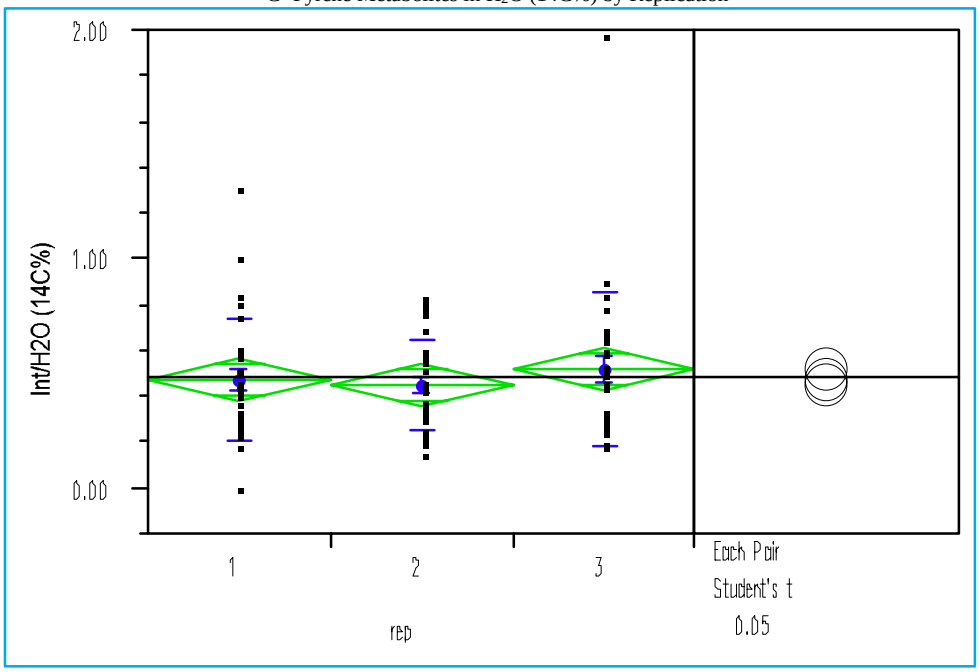
Alpha= 0.05

Comparisons for each pair using Student's t

		t		
		1.98794		
Abs(Dif)-LSD		1	3	2
1	-0.02247	-0.02083	-0.0148	
3	-0.02083	-0.02285	-0.01682	
2	-0.0148	-0.01682	-0.02247	

Positive values show pairs of means that are significantly different.

^{14}C -Pyrene Metabolites in H_2O (14C%) by Replication



Oneway Anova
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.0742327	0.037116	0.4812
Error	86	6.6332168	0.077130	Prob>F
C Total	88	6.7074494	0.076221	0.6197

Means for Oneway Anova

Level	Number	Mean	Std Error
1	30	0.478333	0.05071
2	30	0.456333	0.05071
3	29	0.525862	0.05157

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]		3	1	2
3	0.000000	0.047529	0.069529	
1	-0.04753	0.000000	0.022000	
2	-0.06953	-0.022	0.000000	

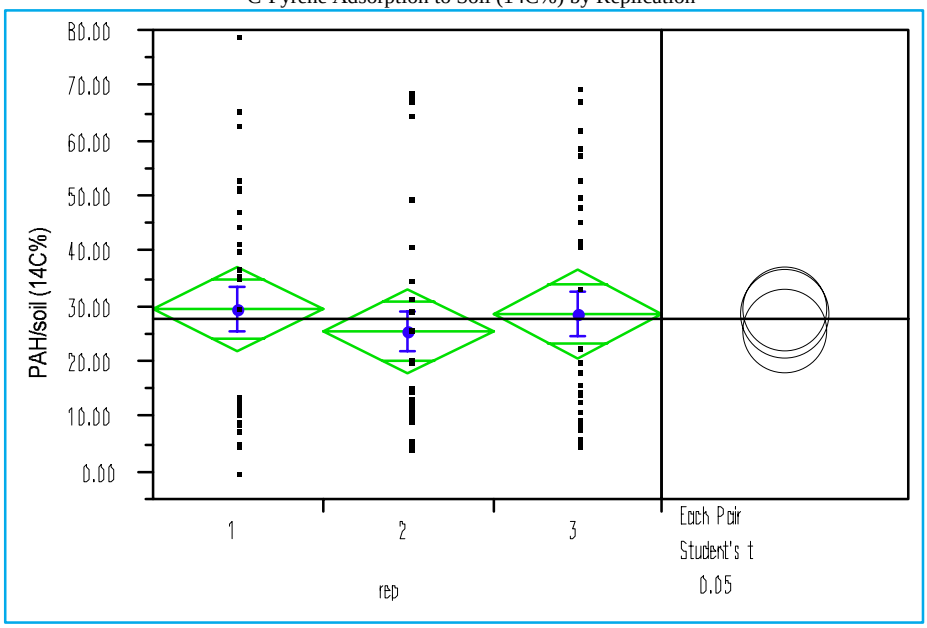
Alpha= 0.05

Comparisons for each pair using Student's t

		t		
		1.98794		
Abs(Dif)-LSD		3	1	2
3	-0.14499	-0.09625	-0.07425	
1	-0.09625	-0.14255	-0.12055	
2	-0.07425	-0.12055	-0.14255	

Positive values show pairs of means that are significantly different.

^{14}C-Pyrene Adsorption to Soil (14C%) by Replication



Oneway Anova
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	247.012	123.506	0.2545
Error	86	41726.713	485.194	Prob>F
C Total	88	41973.725	476.974	0.7758

Means for Oneway Anova

Level	Number	Mean	Std Error
1	30	29.6437	4.0216
2	30	25.7693	4.0216
3	29	28.7638	4.0903

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]		1	3	2
1	0.00000	0.87987	3.87433	
3	-0.87987	0.00000	2.99446	
2	-3.87433	-2.99446	0.00000	

Alpha= 0.05

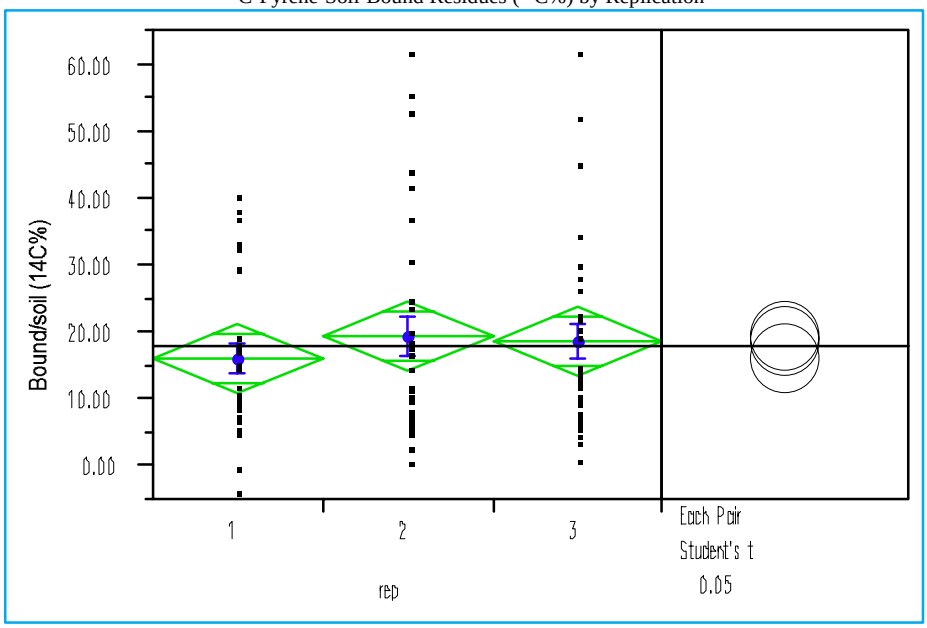
Comparisons for each pair using Student's t

t
1.98794

Abs(Dif)-LSD		1	3	2
1	-11.3062	-10.5234	-7.4319	
3	-10.5234	-11.4995	-8.4088	
2	-7.4319	-8.4088	-11.3062	

Positive values show pairs of means that are significantly different.

^{14}C-Pyrene-Soil-Bound Residues (^{14}C%) by Replication



Oneway Anova
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	172.251	86.126	0.3966
Error	86	18673.961	217.139	Prob>F
C Total	88	18846.212	214.162	0.6738

Means for Oneway Anova

Level	Number	Mean	Std Error
1	30	16.1613	2.6903
2	30	19.4087	2.6903
3	29	18.6334	2.7363

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]		2	3	1
2	0.00000	0.77522	3.24733	
3	-0.77522	0.00000	2.47211	
1	-3.24733	-2.47211	0.00000	

Alpha= 0.05

Comparisons for each pair using Student's t

t
1.98794

Abs(Dif)-LSD		2	3	1
2	-7.56358	-6.85328	-4.31624	
3	-6.85328	-7.69288	-5.15639	
1	-4.31624	-5.15639	-7.56358	

Positive values show pairs of means that are significantly different.

APPENDIX C. STATISTICAL ANALYSIS: COMPOUND NESTED MODEL SCREENING FIT (JMP STATISTICS OUTPUT REPORT

(

Appendix C-1. Compound-Nested Model Screening Fit Results (^{14}C-B[a]P Data)

$^{14}CO_2$ (%)

Summary of Fit

RSquare	0.954976
RSquare Adj	0.928712
Root Mean Square Error	2.288244
Mean of Response	8.863958
Observations (or Sum Wgts)	96

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	35	6663.4931	190.386	36.3604
Error	60	314.1638	5.236	Prob>F
C Total	95	6977.6569		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	
Intercept		8.0140185	0.276381	29.00	<.0001
Soil[Grasses-Poison]		1.6283148	0.375538	4.34	<.0001
Soil[Mulberr-Poison]		6.0446481	0.370338	16.32	<.0001
Soil[Grasses]:Flavonoi[Flavone-Rt-extr]		0.733	0.597351	1.23	0.2246
Soil[Grasses]:Flavonoi[Morin-Rt-extr]		-0.117333	0.567381	-0.21	0.8369
Soil[Mulberr]:Flavonoi[Flavone-Rt-extr]		0.9993333	0.557032	1.79	0.0778
Soil[Mulberr]:Flavonoi[Morin-Rt-extr]		0.5843333	0.557032	1.05	0.2984
Soil[Poison]:Flavonoi[Flavone-Rt-extr]		-0.063722	0.671478	-0.09	0.9247
Soil[Poison]:Flavonoi[Morin-Rt-extr]		0.1072778	0.671478	0.16	0.8736
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0-100]		5.9546667	1.239319	4.80	<.0001
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0.1-100]		6.8946667	1.239319	5.56	<.0001
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[1-100]		0.6946667	1.239319	0.56	0.5772
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[10-100]		-3.998667	1.239319	-3.23	0.0020
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0-100]		7.8386667	1.196324	6.55	<.0001
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0.1-100]		1.3453333	1.196324	1.12	0.2653
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[1-100]		3.5786667	1.196324	2.99	0.0040
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[10-100]		1.2653333	1.196324	1.06	0.2944
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0-100]		-0.142333	1.410569	-0.10	0.9200
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0.1-100]		-0.102333	1.410569	-0.07	0.9424
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[1-100]		-0.047333	1.210825	-0.04	0.9689
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[10-100]		0.036	1.210825	0.03	0.9764
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0-100]		6.805	1.196324	5.69	<.0001
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0.1-100]		1.41	1.39814	1.01	0.3173
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[1-100]		4.405	1.196324	3.68	0.0005
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[10-100]		-4.381667	1.196324	-3.66	0.0005
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0-100]		8.2536667	1.196324	6.90	<.0001
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0.1-100]		1.9836667	1.196324	1.66	0.1025
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[1-100]		4.432	1.39814	3.17	0.0024
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[10-100]		-0.983	1.196324	-0.82	0.4145
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0-100]		-0.313333	1.410569	-0.22	0.8250
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0.1-100]		0.1116667	1.410569	0.08	0.9372
Flavonoi[Morin]:Soil[Poison]:Flv Conc[1-100]		0.3983333	1.210825	0.33	0.7433
Flavonoi[Morin]:Soil[Poison]:Flv Conc[10-100]		-0.268333	1.210825	-0.22	0.8254
Flavonoi[Rt-extr]:Soil[Grasses]:Flv Conc[0-NQ]		7.3033333	0.934172	7.82	<.0001
Flavonoi[Rt-extr]:Soil[Mulberr]:Flv Conc[0-NQ]		10.421667	0.934172	11.16	<.0001
Flavonoi[Rt-extr]:Soil[Poison]:Flv Conc[0-NQ]		-0.1625	1.401258	-0.12	0.9081

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Soil	2	2	2003.5011	191.3175	<.0001
Flavonoids[Soil]	6	6	37.1226	1.1816	0.3283
Flv Conc.[Flavonoids,Soil]	27	27	3228.0176	22.8332	<.0001

^{14}C-BaP in H_2O (%)

Summary of Fit

RSquare	0.891741
RSquare Adj	0.828591
Root Mean Square Error	0.021331
Mean of Response	0.048854
Observations (or Sum Wgts)	96

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	35	0.22487396	0.006425	14.1208
Error	60	0.02730000	0.000455	Prob>F
C Total	95	0.25217396		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	
Intercept	0.0687963	0.002576	26.70	<.0001	
Soil[Grasses-Poison]	-0.017907	0.003501	-5.12	<.0001	
Soil[Mulberr-Poison]	-0.014019	0.003452	-4.06	0.0001	
Soil[Grasses]:Flavonoi[Flavone-Rt-extr]	-0.013556	0.005568	-2.43	0.0179	
Soil[Grasses]:Flavonoi[Morin-Rt-extr]	-0.018889	0.005289	-3.57	0.0007	
Soil[Mulberr]:Flavonoi[Flavone-Rt-extr]	-0.031111	0.005193	-5.99	<.0001	
Soil[Mulberr]:Flavonoi[Morin-Rt-extr]	-0.032444	0.005193	-6.25	<.0001	
Soil[Poison]:Flavonoi[Flavone-Rt-extr]	-0.037389	0.006259	-5.97	<.0001	
Soil[Poison]:Flavonoi[Morin-Rt-extr]	-0.044389	0.006259	-7.09	<.0001	
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0-100]	-0.004	0.011553	-0.35	0.7304	
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0.1-100]	-0.007333	0.011553	-0.63	0.5280	
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[1-100]	-0.014	0.011553	-1.21	0.2303	
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[10-100]	-0.027333	0.011553	-2.37	0.0212	
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0-100]	-0.003667	0.011152	-0.33	0.7435	
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0.1-100]	-0.000333	0.011152	-0.03	0.9763	
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[1-100]	0.003	0.011152	0.27	0.7888	
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[10-100]	-0.000333	0.011152	-0.03	0.9763	
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0-100]	-0.018333	0.013149	-1.39	0.1684	
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0.1-100]	0.0116667	0.013149	0.89	0.3785	
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[1-100]	0.0033333	0.011287	0.30	0.7688	
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[10-100]	0.0066667	0.011287	0.59	0.5570	
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0-100]	0.0013333	0.011152	0.12	0.9052	
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0.1-100]	-0.012	0.013033	-0.92	0.3609	
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[1-100]	-0.015333	0.011152	-1.37	0.1743	
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[10-100]	0.008	0.011152	0.72	0.4759	
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0-100]	-0.002333	0.011152	-0.21	0.8350	
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0.1-100]	-0.002333	0.011152	-0.21	0.8350	
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[1-100]	-0.007333	0.013033	-0.56	0.5758	
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[10-100]	-0.009	0.011152	-0.81	0.4228	
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0-100]	-0.011333	0.013149	-0.86	0.3922	
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0.1-100]	-0.006333	0.013149	-0.48	0.6318	
Flavonoi[Morin]:Soil[Poison]:Flv Conc[1-100]	-0.003	0.011287	-0.27	0.7913	
Flavonoi[Morin]:Soil[Poison]:Flv Conc[10-100]	0.0036667	0.011287	0.32	0.7464	
Flavonoi[Rt-extr]:Soil[Grasses]:Flv Conc[0-NQ]	-0.05	0.008708	-5.74	<.0001	
Flavonoi[Rt-extr]:Soil[Mulberr]:Flv Conc[0-NQ]	-0.098333	0.008708	-11.29	<.0001	
Flavonoi[Rt-extr]:Soil[Poison]:Flv Conc[0-NQ]	-0.1375	0.013062	-10.53	<.0001	

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Soil	2	2	0.02976963	32.7139	<.0001
Flavonoids[Soil]	6	6	0.09299620	34.0645	<.0001
Flv Conc.[Flavonoids,Soil]	27	27	0.13470696	10.9652	<.0001

^{14}C-B[a]P Metabolites in H_2O (%)
Summary of Fit

Rsquare	0.775422
RSquare Adj	0.644419
Root Mean Square Error	0.06864
Mean of Response	0.280833
Observations (or Sum Wgts)	96

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	35	0.9760500	0.027887	5.9191
Error	60	0.2826833	0.004711	Prob>F
C Total	95	1.2587333		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	
Intercept		0.3131667	0.00829	37.77	<.0001
Soil[Grasses-Poison]		-0.038944	0.011265	-3.46	0.0010
Soil[Mulberr-Poison]		-0.028722	0.011109	-2.59	0.0122
Soil[Grasses]:Flavonoi[Flavone-Rt-extr]		0.0531111	0.017918	2.96	0.0043
Soil[Grasses]:Flavonoi[Morin-Rt-extr]		-0.048889	0.017019	-2.87	0.0056
Soil[Mulberr]:Flavonoi[Flavone-Rt-extr]		-0.060111	0.016709	-3.60	0.0007
Soil[Mulberr]:Flavonoi[Morin-Rt-extr]		-0.042111	0.016709	-2.52	0.0144
Soil[Poison]:Flavonoi[Flavone-Rt-extr]		-0.0595	0.020142	-2.95	0.0045
Soil[Poison]:Flavonoi[Morin-Rt-extr]		-0.052167	0.020142	-2.59	0.0120
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0-100]		-0.094	0.037175	-2.53	0.0141
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0.1-100]		-0.077333	0.037175	-2.08	0.0418
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[1-100]		-0.117333	0.037175	-3.16	0.0025
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[10-100]		-0.104	0.037175	-2.80	0.0069
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0-100]		-0.054333	0.035886	-1.51	0.1353
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0.1-100]		0.0456667	0.035886	1.27	0.2081
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[1-100]		0.039	0.035886	1.09	0.2815
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[10-100]		-0.001	0.035886	-0.03	0.9779
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0-100]		0.0036667	0.042312	0.09	0.9312
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0.1-100]		-0.016333	0.042312	-0.39	0.7008
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[1-100]		-0.001333	0.036321	-0.04	0.9708
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[10-100]		0.012	0.036321	0.33	0.7423
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0-100]		0.008	0.035886	0.22	0.8243
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0.1-100]		-0.025333	0.041939	-0.60	0.5481
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[1-100]		0.0113333	0.035886	0.32	0.7532
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[10-100]		-0.042	0.035886	-1.17	0.2465
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0-100]		-0.072333	0.035886	-2.02	0.0483
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0.1-100]		0.0143333	0.035886	0.40	0.6910
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[1-100]		-0.027333	0.041939	-0.65	0.5171
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[10-100]		-0.012333	0.035886	-0.34	0.7323
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0-100]		-0.003667	0.042312	-0.09	0.9312
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0.1-100]		-0.003667	0.042312	-0.09	0.9312
Flavonoi[Morin]:Soil[Poison]:Flv Conc[1-100]		0.0346667	0.036321	0.95	0.3437
Flavonoi[Morin]:Soil[Poison]:Flv Conc[10-100]		-0.032	0.036321	-0.88	0.3818
Flavonoi[Rt-extr]:Soil[Grasses]:Flv Conc[0-NQ]		-0.036667	0.028022	-1.31	0.1957
Flavonoi[Rt-extr]:Soil[Mulberr]:Flv Conc[0-NQ]		-0.216667	0.028022	-7.73	<.0001
Flavonoi[Rt-extr]:Soil[Poison]:Flv Conc[0-NQ]		-0.1675	0.042033	-3.98	0.0002

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Soil	2	2	0.13416079	14.2379	<.0001
Flavonoids[Soil]	6	6	0.24820326	8.7803	<.0001
Flv Conc.[Flavonoids,Soil]	27	27	0.67946667	5.3414	<.0001

14C-BaP Adsorption onto Soil (%)

Summary of Fit

RSquare	0.510169
RSquare Adj	0.224435
Root Mean Square Error	14.98083
Mean of Response	39.78917
Observations (or Sum Wgts)	96

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	35	14024.622	400.703	1.7855
Error	60	13465.515	224.425	Prob>F
C Total	95	27490.138		0.0239

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	
Intercept		40.673796	1.809426	22.48	<.0001
Soil[Grasses-Poison]		-10.34769	2.458598	-4.21	<.0001
Soil[Mulberr-Poison]		-2.794685	2.424554	-1.15	0.2536
Soil[Grasses]:Flavonoi[Flavone-Rt-extr]		-6.836778	3.910776	-1.75	0.0855
Soil[Grasses]:Flavonoi[Morin-Rt-extr]		5.2078889	3.714566	1.40	0.1661
Soil[Mulberr]:Flavonoi[Flavone-Rt-extr]		-1.087778	3.646817	-0.30	0.7665
Soil[Mulberr]:Flavonoi[Morin-Rt-extr]		0.4885556	3.646817	0.13	0.8939
Soil[Poison]:Flavonoi[Flavone-Rt-extr]		0.9135	4.396079	0.21	0.8361
Soil[Poison]:Flavonoi[Morin-Rt-extr]		-4.449833	4.396079	-1.01	0.3155
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0-100]		18.570667	8.113656	2.29	0.0256
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0.1-100]		3.774	8.113656	0.47	0.6435
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[1-100]		-2.849333	8.113656	-0.35	0.7267
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[10-100]		-10.416	8.113656	-1.28	0.2042
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0-100]		1.822	7.832171	0.23	0.8168
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0.1-100]		2.522	7.832171	0.32	0.7486
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[1-100]		1.8486667	7.832171	0.24	0.8142
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[10-100]		-2.431333	7.832171	-0.31	0.7573
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0-100]		3.9353333	9.234804	0.43	0.6715
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0.1-100]		0.8103333	9.234804	0.09	0.9304
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[1-100]		-9.586333	7.92711	-1.21	0.2313
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[10-100]		0.917	7.92711	0.12	0.9083
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0-100]		6.526	7.832171	0.83	0.4080
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0.1-100]		0.576	9.153438	0.06	0.9500
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[1-100]		2.526	7.832171	0.32	0.7482
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[10-100]		3.4193333	7.832171	0.44	0.6640
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0-100]		0.2456667	7.832171	0.03	0.9751
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0.1-100]		9.1023333	7.832171	1.16	0.2498
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[1-100]		-5.552667	9.153438	-0.61	0.5464
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[10-100]		-12.56433	7.832171	-1.60	0.1139
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0-100]		9.2986667	9.234804	1.01	0.3180
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0.1-100]		-2.626333	9.234804	-0.28	0.7771
Flavonoi[Morin]:Soil[Poison]:Flv Conc[1-100]		-5.039667	7.92711	-0.64	0.5274
Flavonoi[Morin]:Soil[Poison]:Flv Conc[10-100]		-14.16633	7.92711	-1.79	0.0790
Flavonoi[Rt-extr]:Soil[Grasses]:Flv Conc[0-NQ]		10.105	6.115898	1.65	0.1037
Flavonoi[Rt-extr]:Soil[Mulberr]:Flv Conc[0-NQ]		0.135	6.115898	0.02	0.9825
Flavonoi[Rt-extr]:Soil[Poison]:Flv Conc[0-NQ]		1.3125	9.173847	0.14	0.8867

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Soil	2	2	5756.2560	12.8244	<.0001
Flavonoids[Soil]	6	6	1153.1966	0.8564	0.5321
Flv Conc.[Flavonoids,Soil]	27	27	5579.5060	0.9208	0.5821

^{14}C-B[a]P-Soil-Bound Residues (%)

Summary of Fit

RSquare	0.6275
RSquare Adj	0.410208
Root Mean Square Error	14.53292
Mean of Response	47.26365
Observations (or Sum Wgts)	96

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	35	21347.351	609.924	2.8878
Error	60	12672.345	211.206	Prob>F
C Total	95	34019.696		0.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	
Intercept		48.940296	1.755326	27.88	<.0001
Soil[Grasses-Poison]		8.4444815	2.385089	3.54	0.0008
Soil[Mulberr-Poison]		-9.071852	2.352063	-3.86	0.0003
Soil[Grasses]:Flavonoi[Flavone-Rt-extr]		2.7125556	3.793848	0.71	0.4774
Soil[Grasses]:Flavonoi[Morin-Rt-extr]		-6.869444	3.603504	-1.91	0.0614
Soil[Mulberr]:Flavonoi[Flavone-Rt-extr]		-0.640444	3.537781	-0.18	0.8570
Soil[Mulberr]:Flavonoi[Morin-Rt-extr]		-4.476111	3.537781	-1.27	0.2107
Soil[Poison]:Flavonoi[Flavone-Rt-extr]		-4.147667	4.264641	-0.97	0.3347
Soil[Poison]:Flavonoi[Morin-Rt-extr]		4.3103333	4.264641	1.01	0.3162
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0-100]		-14.344	7.871066	-1.82	0.0734
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0.1-100]		-8.360667	7.871066	-1.06	0.2924
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[1-100]		-5.537333	7.871066	-0.70	0.4845
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[10-100]		0.4793333	7.871066	0.06	0.9516
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0-100]		-7.441333	7.597998	-0.98	0.3313
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0.1-100]		-4.304667	7.597998	-0.57	0.5731
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[1-100]		-17.68467	7.597998	-2.33	0.0233
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[10-100]		-1.581333	7.597998	-0.21	0.8358
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0-100]		11.9	8.958693	1.33	0.1891
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0.1-100]		4	8.958693	0.45	0.6568
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[1-100]		1.9333333	7.690098	0.25	0.8024
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[10-100]		-8.896667	7.690098	-1.16	0.2519
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0-100]		-4.762	7.597998	-0.63	0.5332
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0.1-100]		-11.94533	8.87976	-1.35	0.1836
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[1-100]		-12.62867	7.597998	-1.66	0.1017
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[10-100]		-3.865333	7.597998	-0.51	0.6128
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0-100]		-3.605667	7.597998	-0.47	0.6368
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0.1-100]		-10.74567	7.597998	-1.41	0.1624
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[1-100]		-6.827333	8.87976	-0.77	0.4450
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[10-100]		16.027667	7.597998	2.11	0.0391
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0-100]		3.442	8.958693	0.38	0.7022
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0.1-100]		-5.088	8.958693	-0.57	0.5722
Flavonoi[Morin]:Soil[Poison]:Flv Conc[1-100]		10.315333	7.690098	1.34	0.1849
Flavonoi[Morin]:Soil[Poison]:Flv Conc[10-100]		7.662	7.690098	1.00	0.3231
Flavonoi[Rt-extr]:Soil[Grasses]:Flv Conc[0-NQ]		-15.78833	5.933039	-2.66	0.0100
Flavonoi[Rt-extr]:Soil[Mulberr]:Flv Conc[0-NQ]		-13.19833	5.933039	-2.22	0.0299
Flavonoi[Rt-extr]:Soil[Poison]:Flv Conc[0-NQ]		7.915	8.899559	0.89	0.3774

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Soil	2	2	4289.181	10.1540	0.0002
Flavonoids[Soil]	6	6	1611.406	1.2716	0.2840
Flv Conc.[Flavonoids,Soil]	27	27	14837.154	2.6018	0.0011

Appendix C-2. Compound-Nested Model Screening Fit Results (^{14}C-Pyrene Data)

$^{14}CO_2$ (%)

Summary of Fit

RSquare	0.861865
RSquare Adj	0.790766
Root Mean Square Error	7.749121
Mean of Response	14.81135
Observations (or Sum Wgts)	104

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	35	25477.045	727.916	12.1221
Error	68	4083.324	60.049	Prob>F
C Total	103	29560.369		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	
Intercept	14.588944	0.844349	17.28	<.0001	
Soil[Grasses-Poison]		10.799833	1.174792	9.19	<.0001
Soil[Mulberr-Poison]		3.6348889	1.225748	2.97	0.0042
Soil[Grasses]:Flavonoi[Flavone-Rt-extr]		4.4905556	1.826485	2.46	0.0165
Soil[Grasses]:Flavonoi[Morin-Rt-extr]		0.4398889	1.826485	0.24	0.8104
Soil[Mulberr]:Flavonoi[Flavone-Rt-extr]		2.8718333	1.958687	1.47	0.1472
Soil[Mulberr]:Flavonoi[Morin-Rt-extr]		-2.8355	1.958687	-1.45	0.1523
Soil[Poison]:Flavonoi[Flavone-Rt-extr]		0.0477778	1.874559	0.03	0.9797
Soil[Poison]:Flavonoi[Morin-Rt-extr]		-0.013556	1.838622	-0.01	0.9941
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0-100]		8.484	4.001629	2.12	0.0376
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0.1-100]		22.957333	4.001629	5.74	<.0001
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[1-100]		13.164	4.001629	3.29	0.0016
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[10-100]		-15.196	4.001629	-3.80	0.0003
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0-100]		5.5393333	4.734791	1.17	0.2461
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0.1-100]		3.1343333	4.051341	0.77	0.4418
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[1-100]		0.781	4.051341	0.19	0.8477
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[10-100]		1.731	4.051341	0.43	0.6705
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0-100]		-0.072	4.051341	-0.02	0.9859
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0.1-100]		-0.038667	4.051341	-0.01	0.9924
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[1-100]		0.028	4.051341	0.01	0.9945
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[10-100]		-0.045333	4.051341	-0.01	0.9911
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0-100]		12.534667	4.001629	3.13	0.0026
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0.1-100]		9.6613333	4.001629	2.41	0.0185
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[1-100]		7.5413333	4.001629	1.88	0.0638
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[10-100]		-5.612	4.001629	-1.40	0.1653
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0-100]		11.246667	4.734791	2.38	0.0204
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0.1-100]		2.035	4.051341	0.50	0.6171
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[1-100]		2.4683333	4.051341	0.61	0.5444
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[10-100]		-0.935	4.051341	-0.23	0.8182
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0-100]		-0.010667	4.001629	-0.00	0.9979
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0.1-100]		0.0526667	4.001629	0.01	0.9895
Flavonoi[Morin]:Soil[Poison]:Flv Conc[1-100]		-0.024	4.001629	-0.01	0.9952
Flavonoi[Morin]:Soil[Poison]:Flv Conc[10-100]		-0.060667	4.001629	-0.02	0.9879
Flavonoi[Rt-extr]:Soil[Grasses]:Flv Conc[0-NQ]		17.905	3.163566	5.66	<.0001
Flavonoi[Rt-extr]:Soil[Mulberr]:Flv Conc[0-NQ]		8.4475	3.536974	2.39	0.0197
Flavonoi[Rt-extr]:Soil[Poison]:Flv Conc[0-NQ]		0.01	3.163566	0.00	0.9975

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Soil	2	2	9948.342	82.8354	<.0001
Flavonoids[Soil]	6	6	618.683	1.7172	0.1303
Flv Conc.[Flavonoids,Soil]	27	27	12043.672	7.4283	<.0001

^{14}C -Pyrene in H_2O(^{14}C%)

Summary of Fit

RSquare	0.325501
RSquare Adj	-0.02167
Root Mean Square Error	1.343856
Mean of Response	0.178077
Observations (or Sum Wgts)	104

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	35	59.26327	1.69324	0.9376
Error	68	122.80455	1.80595	Prob>F
C Total	103	182.06782		0.5736

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	
Intercept		0.1550185	0.146427	1.06	0.2935
Soil[Grasses-Poison]		0.202537	0.203733	0.99	0.3237
Soil[Mulberr-Poison]		-0.11963	0.21257	-0.56	0.5754
Soil[Grasses]:Flavonoi[Flavone-Rt-extr]		-0.320889	0.31675	-1.01	0.3146
Soil[Grasses]:Flavonoi[Morin-Rt-extr]		0.5851111	0.31675	1.85	0.0691
Soil[Mulberr]:Flavonoi[Flavone-Rt-extr]		-0.018389	0.339676	-0.05	0.9570
Soil[Mulberr]:Flavonoi[Morin-Rt-extr]		-0.007056	0.339676	-0.02	0.9835
Soil[Poison]:Flavonoi[Flavone-Rt-extr]		-0.013444	0.325087	-0.04	0.9671
Soil[Poison]:Flavonoi[Morin-Rt-extr]		-0.016111	0.318855	-0.05	0.9598
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0-100]		-0.013333	0.693964	-0.02	0.9847
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0.1-100]		-0.003333	0.693964	-0.00	0.9962
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[1-100]		-0.013333	0.693964	-0.02	0.9847
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[10-100]		-0.003333	0.693964	-0.00	0.9962
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0-100]		-0.002	0.82111	-0.00	0.9981
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0.1-100]		-0.000333	0.702585	-0.00	0.9996
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[1-100]		-0.000333	0.702585	-0.00	0.9996
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[10-100]		0.003	0.702585	0.00	0.9966
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0-100]		-0.012	0.702585	-0.02	0.9864
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0.1-100]		0.0013333	0.702585	0.00	0.9985
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[1-100]		-0.002	0.702585	-0.00	0.9977
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[10-100]		0.0113333	0.702585	0.02	0.9872
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0-100]		-0.919333	0.693964	-1.32	0.1897
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0.1-100]		-0.916	0.693964	-1.32	0.1913
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[1-100]		-0.912667	0.693964	-1.32	0.1929
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[10-100]		3.6106667	0.693964	5.20	<.0001
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0-100]		-0.013333	0.82111	-0.02	0.9871
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0.1-100]		-0.001667	0.702585	-0.00	0.9981
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[1-100]		-0.005	0.702585	-0.01	0.9943
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[10-100]		-0.008333	0.702585	-0.01	0.9906
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0-100]		-0.009333	0.693964	-0.01	0.9893
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0.1-100]		-0.002667	0.693964	-0.00	0.9969
Flavonoi[Morin]:Soil[Poison]:Flv Conc[1-100]		0.0006667	0.693964	0.00	0.9992
Flavonoi[Morin]:Soil[Poison]:Flv Conc[10-100]		-0.002667	0.693964	-0.00	0.9969
Flavonoi[Rt-extr]:Soil[Grasses]:Flv Conc[0-NQ]		-0.07	0.548627	-0.13	0.8988
Flavonoi[Rt-extr]:Soil[Mulberr]:Flv Conc[0-NQ]		-0.045833	0.613384	-0.07	0.9407
Flavonoi[Rt-extr]:Soil[Poison]:Flv Conc[0-NQ]		-0.055	0.548627	-0.10	0.9204

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Soil	2	2	1.790790	0.4958	0.6113
Flavonoids[Soil]	6	6	6.958321	0.6422	0.6961
Flv Conc.[Flavonoids,Soil]	27	27	48.961803	1.0041	0.4764

^{14}C-Pyrene Motabolites in H_2O(14C%)

Summary of Fit

RSquare	0.325004
RSquare Adj	-0.02242
Root Mean Square Error	1.697084
Mean of Response	0.620577
Observations (or Sum Wgts)	104

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	35	94.29828	2.69424	0.9355
Error	68	195.84648	2.88010	Prob>F
C Total	103	290.14477		0.5766

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	
Intercept		0.6159444	0.184915	3.33	0.0014
Soil[Grasses-Poison]		0.3113889	0.257283	1.21	0.2304
Soil[Mulberr-Poison]		0.0502222	0.268443	0.19	0.8521
Soil[Grasses]:Flavonoi[Flavone-Rt-extr]		-0.422667	0.400007	-1.06	0.2944
Soil[Grasses]:Flavonoi[Morin-Rt-extr]		0.6533333	0.400007	1.63	0.1070
Soil[Mulberr]:Flavonoi[Flavone-Rt-extr]		-0.085833	0.428959	-0.20	0.8420
Soil[Mulberr]:Flavonoi[Morin-Rt-extr]		-0.023833	0.428959	-0.06	0.9559
Soil[Poison]:Flavonoi[Flavone-Rt-extr]		0.005	0.410535	0.01	0.9903
Soil[Poison]:Flavonoi[Morin-Rt-extr]		-0.032333	0.402665	-0.08	0.9362
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0-100]		-0.018	0.876371	-0.02	0.9837
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0.1-100]		0.052	0.876371	0.06	0.9529
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[1-100]		0.0586667	0.876371	0.07	0.9468
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[10-100]		0.0486667	0.876371	0.06	0.9559
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0-100]		0.2646667	1.036936	0.26	0.7993
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0.1-100]		0.1896667	0.887258	0.21	0.8314
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[1-100]		0.0363333	0.887258	0.04	0.9675
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[10-100]		-0.230333	0.887258	-0.26	0.7960
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0-100]		-0.039333	0.887258	-0.04	0.9648
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0.1-100]		-0.002667	0.887258	-0.00	0.9976
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[1-100]		-0.016	0.887258	-0.02	0.9857
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[10-100]		0.0273333	0.887258	0.03	0.9755
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0-100]		-1.094	0.876371	-1.25	0.2162
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0.1-100]		-1.100667	0.876371	-1.26	0.2134
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[1-100]		-1.130667	0.876371	-1.29	0.2014
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[10-100]		4.4326667	0.876371	5.06	<.0001
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0-100]		0.2026667	1.036936	0.20	0.8456
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0.1-100]		0.1143333	0.887258	0.13	0.8978
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[1-100]		0.0543333	0.887258	0.06	0.9513
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[10-100]		-0.042333	0.887258	-0.05	0.9621
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0-100]		-0.002	0.876371	-0.00	0.9982
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0.1-100]		0.0146667	0.876371	0.02	0.9867
Flavonoi[Morin]:Soil[Poison]:Flv Conc[1-100]		0.0246667	0.876371	0.03	0.9776
Flavonoi[Morin]:Soil[Poison]:Flv Conc[10-100]		-0.038667	0.876371	-0.04	0.9649
Flavonoi[Rt-extr]:Soil[Grasses]:Flv Conc[0-NQ]		-0.21	0.692832	-0.30	0.7627
Flavonoi[Rt-extr]:Soil[Mulberr]:Flv Conc[0-NQ]		0.0691667	0.774609	0.09	0.9291
Flavonoi[Rt-extr]:Soil[Poison]:Flv Conc[0-NQ]		-0.061667	0.692832	-0.09	0.9293

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Soil	2	2	6.810434	1.1823	0.3128
Flavonoids[Soil]	6	6	9.437219	0.5461	0.7713
Flv Conc.[Flavonoids,Soil]	27	27	75.164413	0.9666	0.5233

^{14}C-Pyrene Adsorption onto Soil (^{14}C%)

Summary of Fit

RSquare	0.861576
RSquare Adj	0.790328
Root Mean Square Error	8.878576
Mean of Response	24.65587
Observations (or Sum Wgts)	104

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	35	33363.979	953.257	12.0927
Error	68	5360.379	78.829	Prob>F
C Total	103	38724.358		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	
Intercept		24.922981	0.967416	25.76	<.0001
Soil[Grasses-Poison]		-10.96165	1.346021	-8.14	<.0001
Soil[Mulberr-Poison]		-9.060593	1.404404	-6.45	<.0001
Soil[Grasses]:Flavonoi[Flavone-Rt-extr]		-5.074	2.0927	-2.42	0.0180
Soil[Grasses]:Flavonoi[Morin-Rt-extr]		2.012	2.0927	0.96	0.3397
Soil[Mulberr]:Flavonoi[Flavone-Rt-extr]		-5.119389	2.244171	-2.28	0.0257
Soil[Mulberr]:Flavonoi[Morin-Rt-extr]		-1.570722	2.244171	-0.70	0.4864
Soil[Poison]:Flavonoi[Flavone-Rt-extr]		6.0057778	2.147781	2.80	0.0067
Soil[Poison]:Flavonoi[Morin-Rt-extr]		-2.920556	2.106605	-1.39	0.1702
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0-100]		0.456	4.584877	0.10	0.9211
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0.1-100]		-4.187333	4.584877	-0.91	0.3643
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[1-100]		-4.467333	4.584877	-0.97	0.3333
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[10-100]		-3.504	4.584877	-0.76	0.4474
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0-100]		-2.088	5.424899	-0.38	0.7015
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0.1-100]		-0.896333	4.641834	-0.19	0.8475
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[1-100]		2.467	4.641834	0.53	0.5968
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[10-100]		0.1703333	4.641834	0.04	0.9708
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0-100]		-8.451	4.641834	-1.82	0.0731
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0.1-100]		-2.984333	4.641834	-0.64	0.5224
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[1-100]		9.319	4.641834	2.01	0.0487
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[10-100]		11.562333	4.641834	2.49	0.0152
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0-100]		-6.63	4.584877	-1.45	0.1528
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0.1-100]		-6.923333	4.584877	-1.51	0.1357
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[1-100]		-4.473333	4.584877	-0.98	0.3327
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[10-100]		-4.616667	4.584877	-1.01	0.3175
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0-100]		-5.636667	5.424899	-1.04	0.3025
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0.1-100]		-3.271667	4.641834	-0.70	0.4833
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[1-100]		-4.298333	4.641834	-0.93	0.3577
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[10-100]		-2.555	4.641834	-0.55	0.5838
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0-100]		0.4753333	4.584877	0.10	0.9177
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0.1-100]		14.728667	4.584877	3.21	0.0020
Flavonoi[Morin]:Soil[Poison]:Flv Conc[1-100]		7.1053333	4.584877	1.55	0.1258
Flavonoi[Morin]:Soil[Poison]:Flv Conc[10-100]		-17.94467	4.584877	-3.91	0.0002
Flavonoi[Rt-extr]:Soil[Grasses]:Flv Conc[0-NQ]		-7.68	3.624663	-2.12	0.0378
Flavonoi[Rt-extr]:Soil[Mulberr]:Flv Conc[0-NQ]		-13.8975	4.052497	-3.43	0.0010
Flavonoi[Rt-extr]:Soil[Poison]:Flv Conc[0-NQ]		0.64	3.624663	0.18	0.8604

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Soil	2	2	17441.284	110.6272	<.0001
Flavonoids[Soil]	6	6	1644.810	3.4776	0.0047
Flv Conc.[Flavonoids,Soil]	27	27	7626.001	3.5830	<.0001

Total ^{14}C -Pyrene-Soil-Bound Residue (^{14}C%)
(*Including LSC unaccountable ^{14}C)*

Summary of Fit

RSquare	0.592142
RSquare Adj	0.382215
Root Mean Square Error	11.31743
Mean of Response	59.73452
Observations (or Sum Wgts)	104

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	35	12645.083	361.288	2.8207
Error	68	8709.729	128.084	Prob>F
C Total	103	21354.812		0.0001

Parameter Estimates

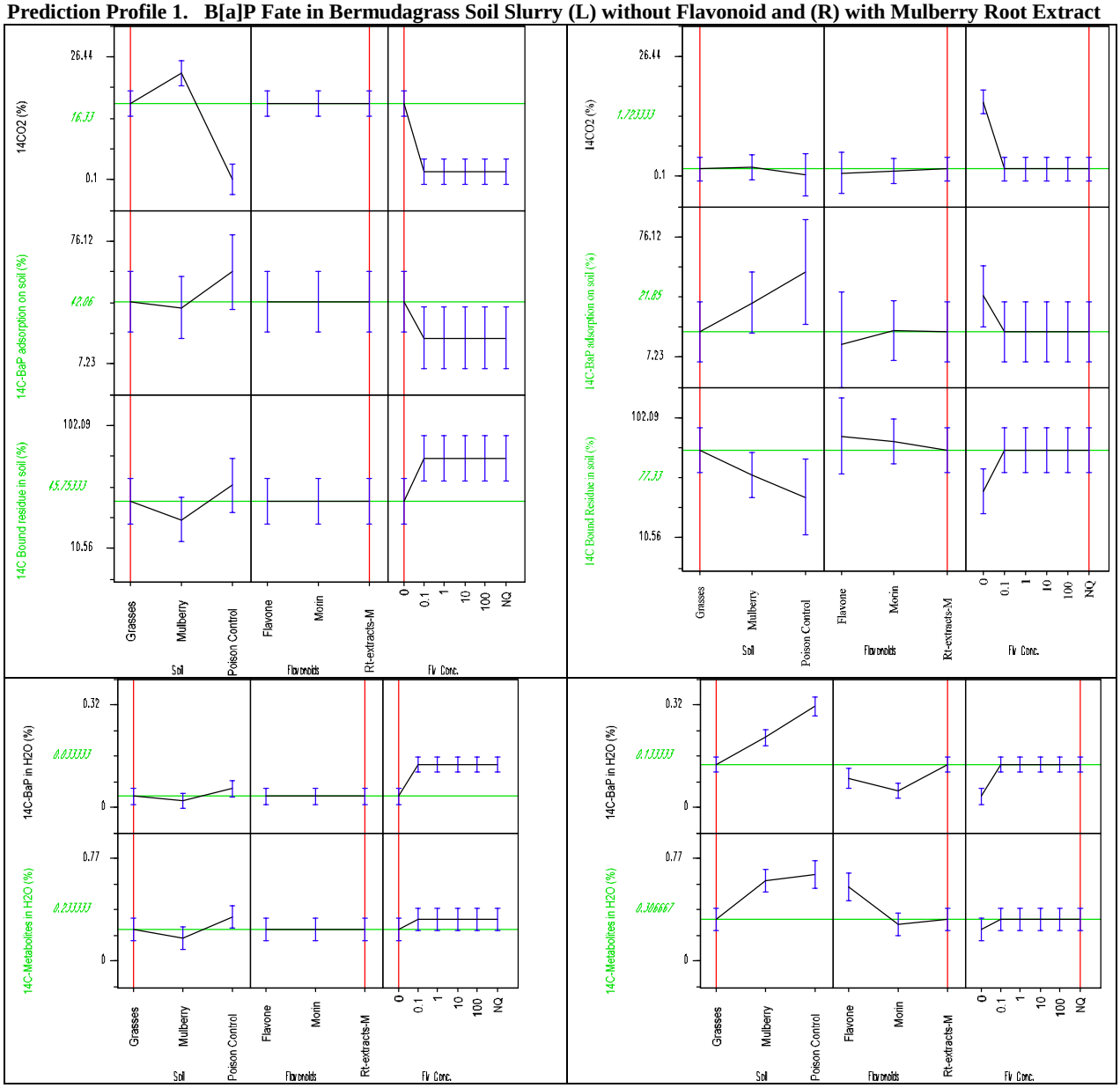
Term	Estimate	Std Error	t Ratio	Prob>|t|	
Intercept		59.71737	1.233155	48.43	<.0001
Soil[Grasses-Poison]		-0.352259	1.715759	-0.21	0.8379
Soil[Mulberr-Poison]		5.4944074	1.790179	3.07	0.0031
Soil[Grasses]:Flavonoi[Flavone-Rt-extr]		1.3275556	2.667544	0.50	0.6203
Soil[Grasses]:Flavonoi[Morin-Rt-extr]		-3.689111	2.667544	-1.38	0.1712
Soil[Mulberr]:Flavonoi[Flavone-Rt-extr]		2.3515556	2.860622	0.82	0.4139
Soil[Mulberr]:Flavonoi[Morin-Rt-extr]		4.4368889	2.860622	1.55	0.1255
Soil[Poison]:Flavonoi[Flavone-Rt-extr]		-6.043556	2.737755	-2.21	0.0307
Soil[Poison]:Flavonoi[Morin-Rt-extr]		2.9821111	2.685269	1.11	0.2707
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0-100]		-8.912667	5.844296	-1.53	0.1319
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[0.1-100]		-18.816	5.844296	-3.22	0.0020
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[1-100]		-8.742667	5.844296	-1.50	0.1393
Flavonoi[Flavone]:Soil[Grasses]:Flv Conc[10-100]		18.654	5.844296	3.19	0.0021
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0-100]		-3.713333	6.915065	-0.54	0.5930
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[0.1-100]		-2.426667	5.916899	-0.41	0.6830
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[1-100]		-3.28	5.916899	-0.55	0.5812
Flavonoi[Flavone]:Soil[Mulberr]:Flv Conc[10-100]		-1.673333	5.916899	-0.28	0.7782
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0-100]		8.5716667	5.916899	1.45	0.1520
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[0.1-100]		3.0216667	5.916899	0.51	0.6112
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[1-100]		-9.328333	5.916899	-1.58	0.1195
Flavonoi[Flavone]:Soil[Poison]:Flv Conc[10-100]		-11.55833	5.916899	-1.95	0.0549
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0-100]		-3.896	5.844296	-0.67	0.5073
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[0.1-100]		-0.726	5.844296	-0.12	0.9015
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[1-100]		-1.019333	5.844296	-0.17	0.8621
Flavonoi[Morin]:Soil[Grasses]:Flv Conc[10-100]		2.1873333	5.844296	0.37	0.7094
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0-100]		-5.798667	6.915065	-0.84	0.4047
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[0.1-100]		1.1246667	5.916899	0.19	0.8498
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[1-100]		1.778	5.916899	0.30	0.7647
Flavonoi[Morin]:Soil[Mulberr]:Flv Conc[10-100]		3.5413333	5.916899	0.60	0.5515
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0-100]		-0.454	5.844296	-0.08	0.9383
Flavonoi[Morin]:Soil[Poison]:Flv Conc[0.1-100]		-14.794	5.844296	-2.53	0.0137
Flavonoi[Morin]:Soil[Poison]:Flv Conc[1-100]		-7.107333	5.844296	-1.22	0.2281
Flavonoi[Morin]:Soil[Poison]:Flv Conc[10-100]		18.049333	5.844296	3.09	0.0029
Flavonoi[Rt-extr]:Soil[Grasses]:Flv Conc[0-NQ]		-9.946667	4.620322	-2.15	0.0349
Flavonoi[Rt-extr]:Soil[Mulberr]:Flv Conc[0-NQ]		5.4266667	5.165677	1.05	0.2972
Flavonoi[Rt-extr]:Soil[Poison]:Flv Conc[0-NQ]		-0.533333	4.620322	-0.12	0.9084

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Soil	2	2	1539.2610	6.0088	0.0040
Flavonoids[Soil]	6	6	1381.2776	1.7974	0.1127
Flv Conc.[Flavonoids,Soil]	27	27	7852.1189	2.2705	0.0034

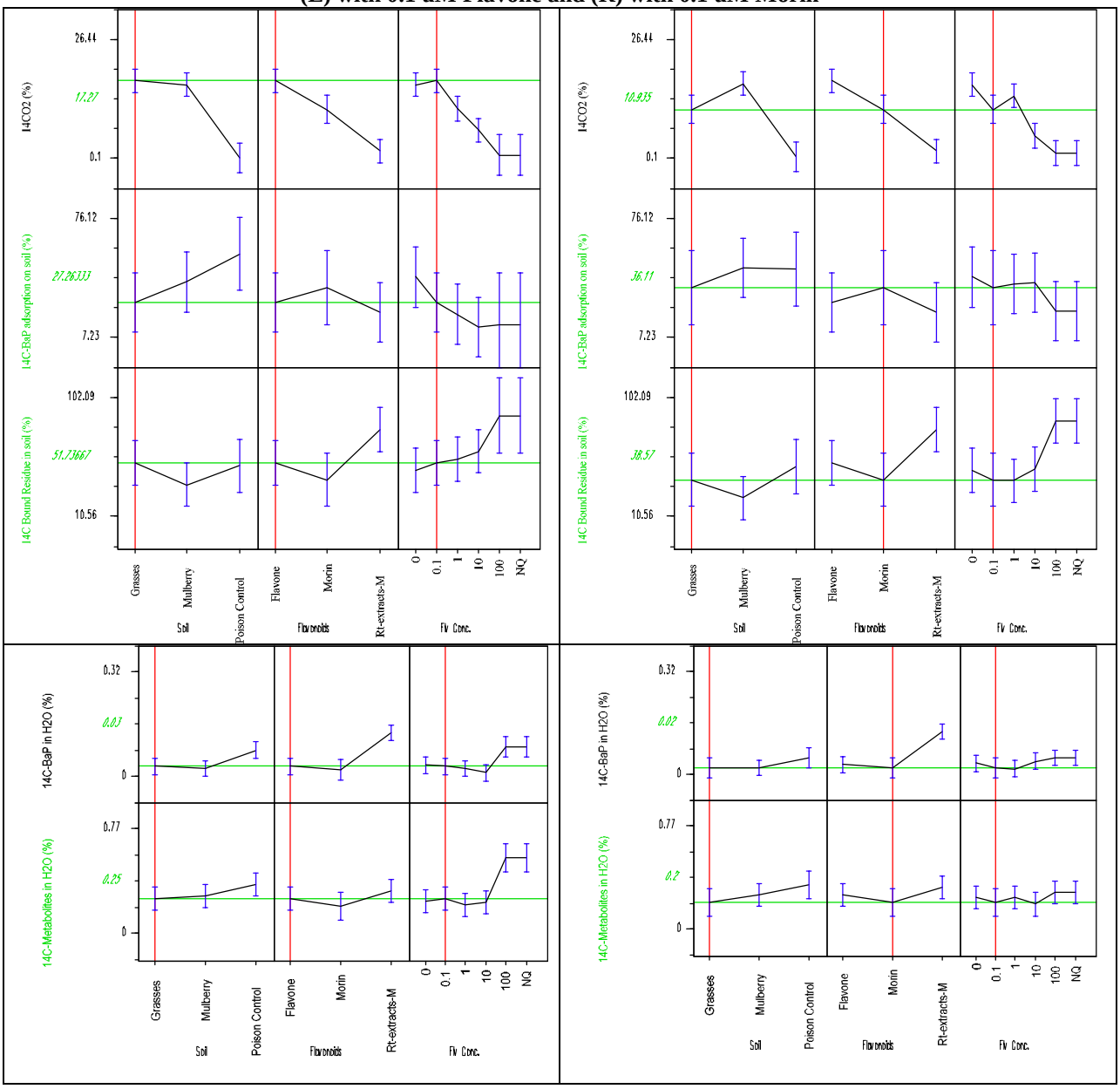
Appendix C-3. Compound-Nested Model Screening Fit Prediction Profiles (B[a]P Data)

Prediction Profile 1. B[a]P Fate in Bermudagrass Soil Slurry (L) without Flavonoid and (R) with Mulberry Root Extract

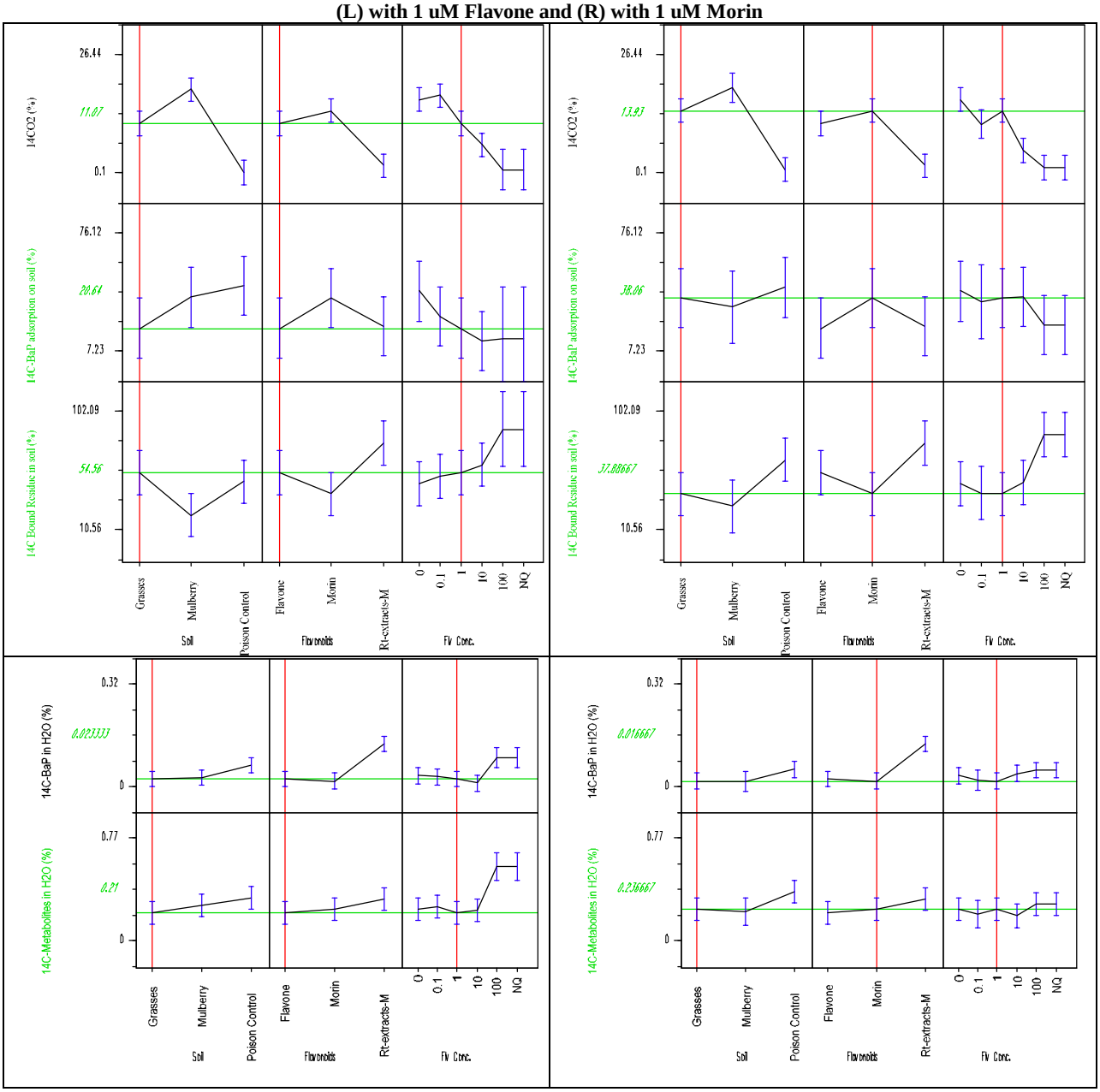


The prediction profiles show how the predicted values for each of the five PAH fate mechanisms changes when each of the three factors (soil type, flavonoid type, and flavonoid concentration) changes. The Y axis is the predicted values of ^{14}C-B[a]P fate measurements and the X axis stands for the testing variable of the three factors. For a predicted value, 95% confidence interval is shown by error bars. The vertical red line holds a variable (factor) at a constant level to predict the responses to any combination of the three factors. The horizontal green line shows the predicted responses when the red lines hold the variables constant. The predicted response (i.e., fate data) changes as one variable (i.e., factor) changes while the others are held constant. A matrix of 15 prediction profiles are included in both left and right halves, respectively. The 1st column shows the effects of soil types. B[a]P fate changed as soil type changed without flavonoid added. The 2nd column shows the effects of flavonoid types. B[a]P fate did not change with the types of flavonoid when the flavonoid concentration was zero. The 3rd column shows the effects of flavonoid concentration. In Bermudagrass soil, ^{14}C-B[a]P fate changed as mulberry root extract concentration changed. Likewise, the effects of multifactors on B[a]P fate as any one of the variables changed were presented in the following prediction profiles.

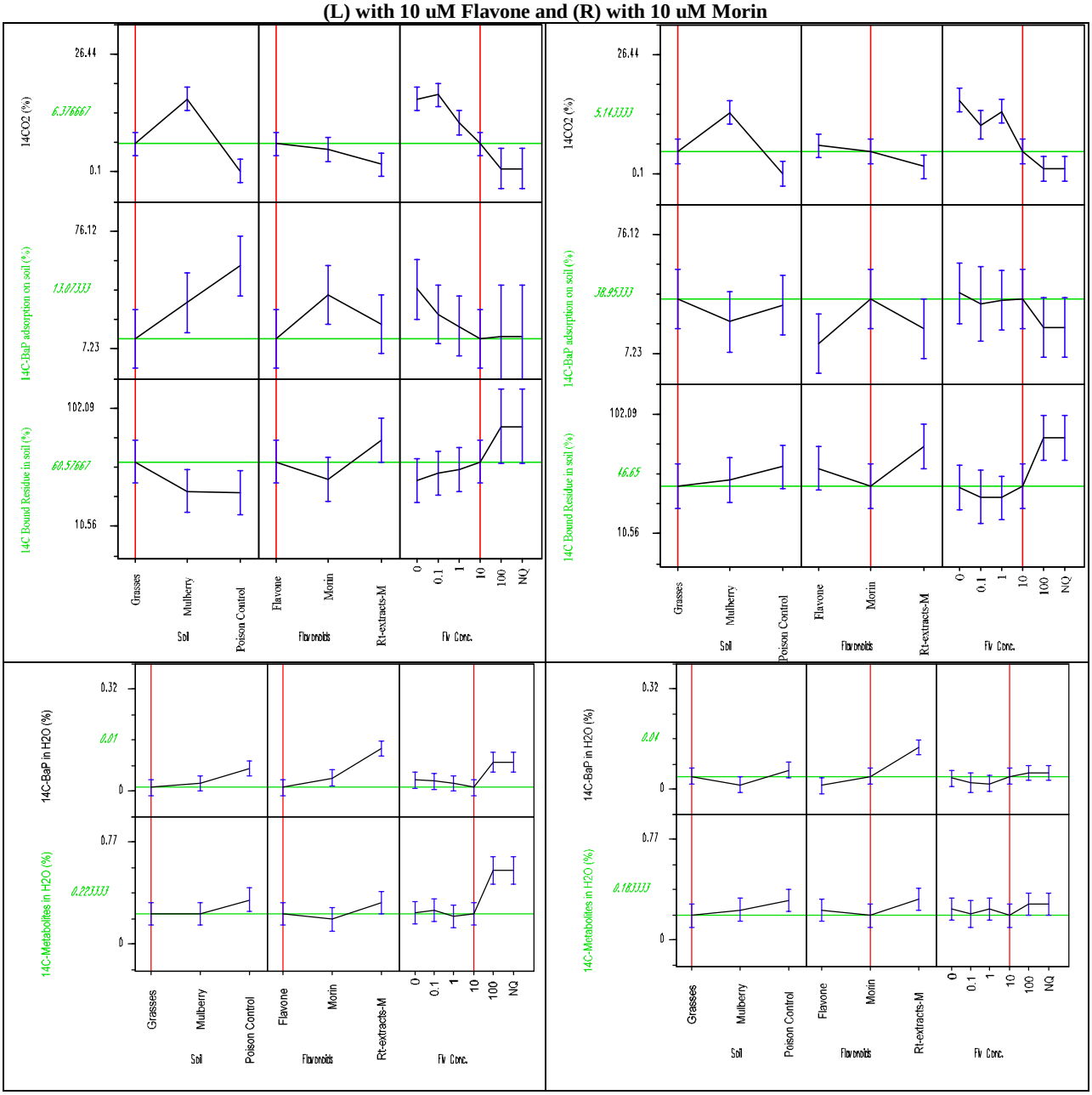
Prediction Profile 2. B[a]P Fate in Bermudagrass Soil Slurry (L) with 0.1 uM Flavone and (R) with 0.1 uM Morin



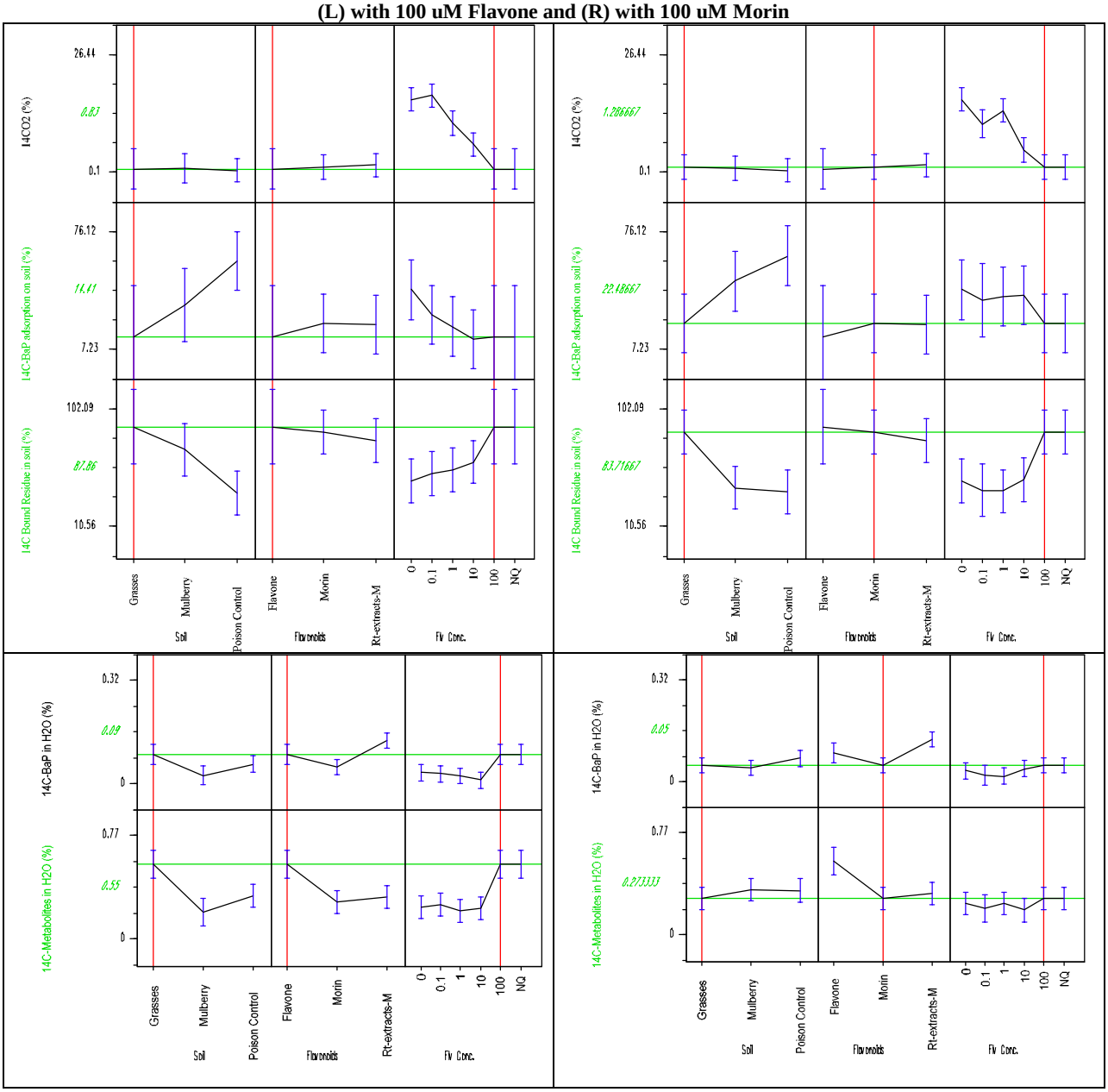
Prediction Profile 3. B[a]P fate in Bermudagrass Soil Slurry (L) with 1 uM Flavone and (R) with 1 uM Morin



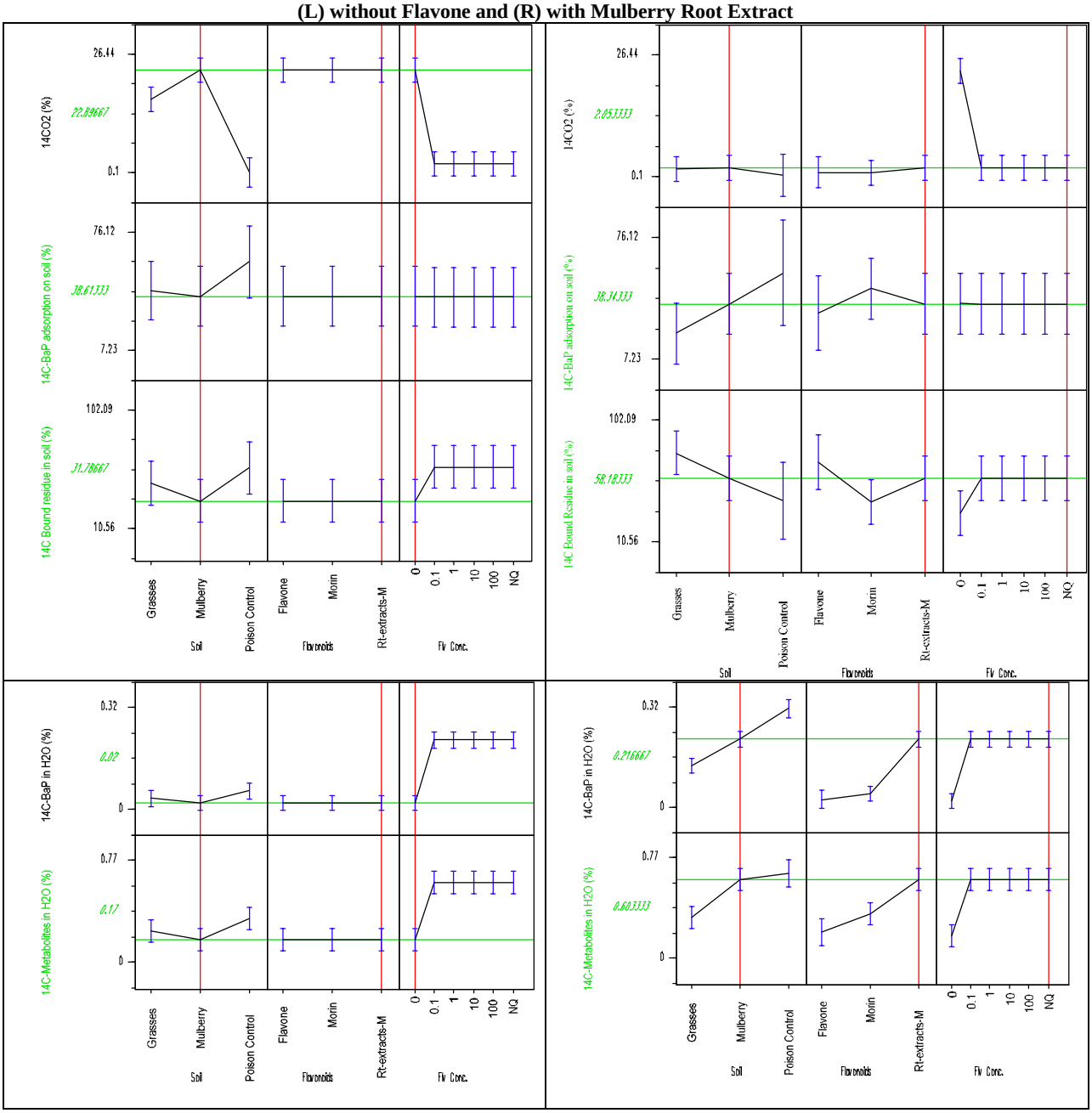
Prediction Profile 4. B[a]P Fate in Bermudagrass Soil Slurry (L) with 10 uM Flavone and (R) with 10 uM Morin



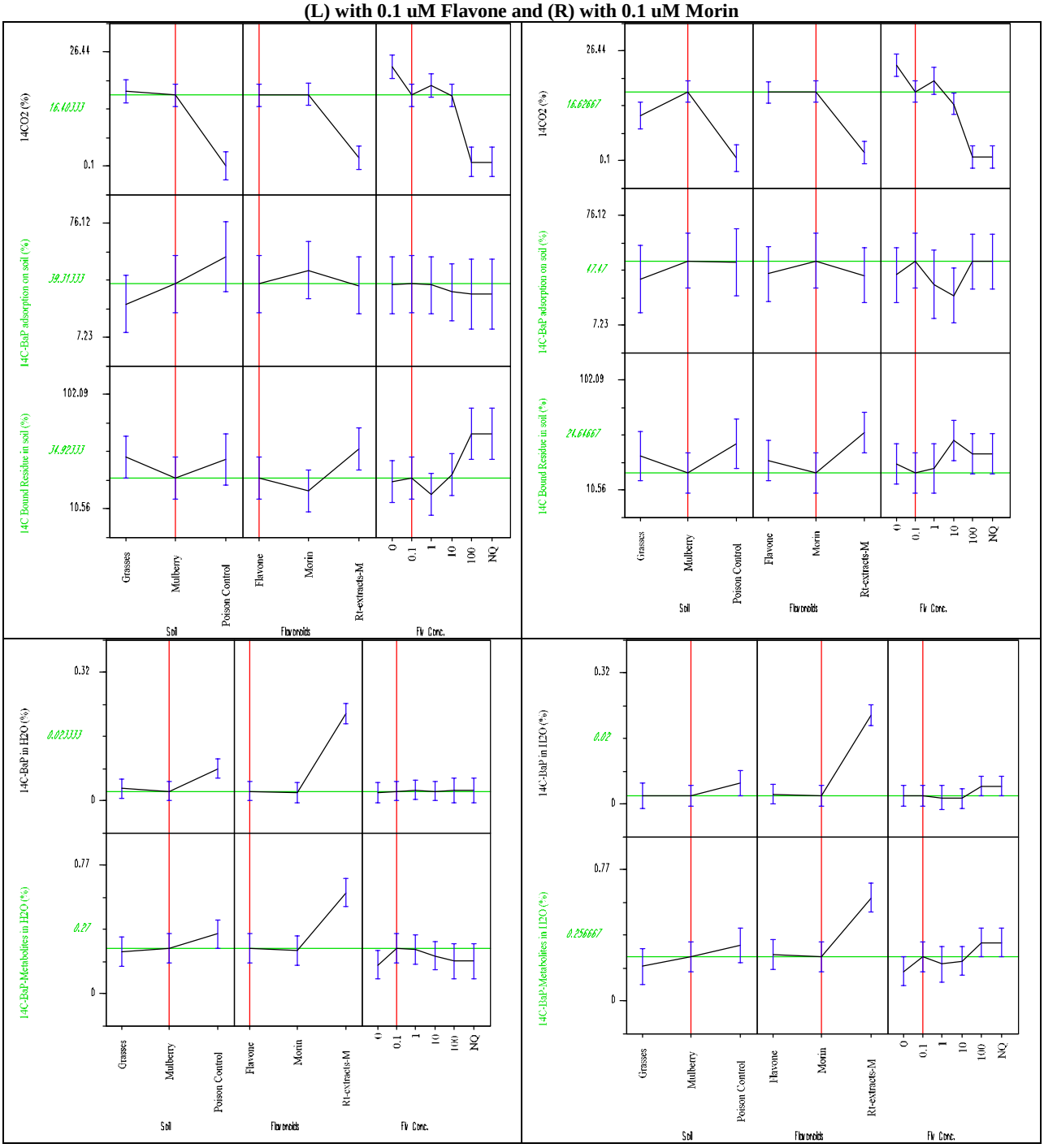
Prediction Profile 5. B[a]P Fate in Bermudagrass Soil Slurry (L) with 100 uM Flavone and (R) with 100 uM Morin



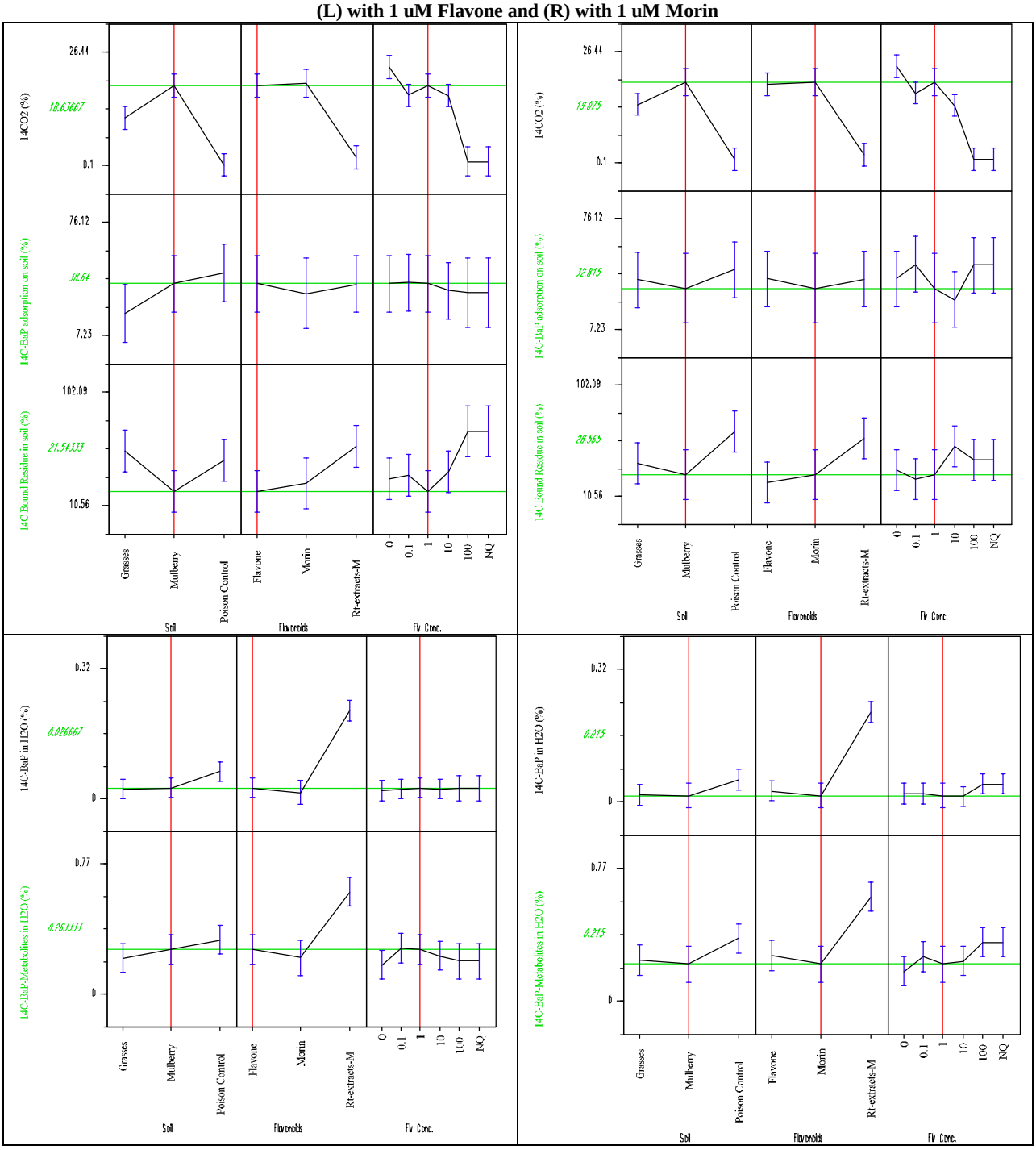
Prediction Profile 6. B[a]P Fate in Mulberry Soil Slurry
(L) without Flavone and (R) with Mulberry Root Extract



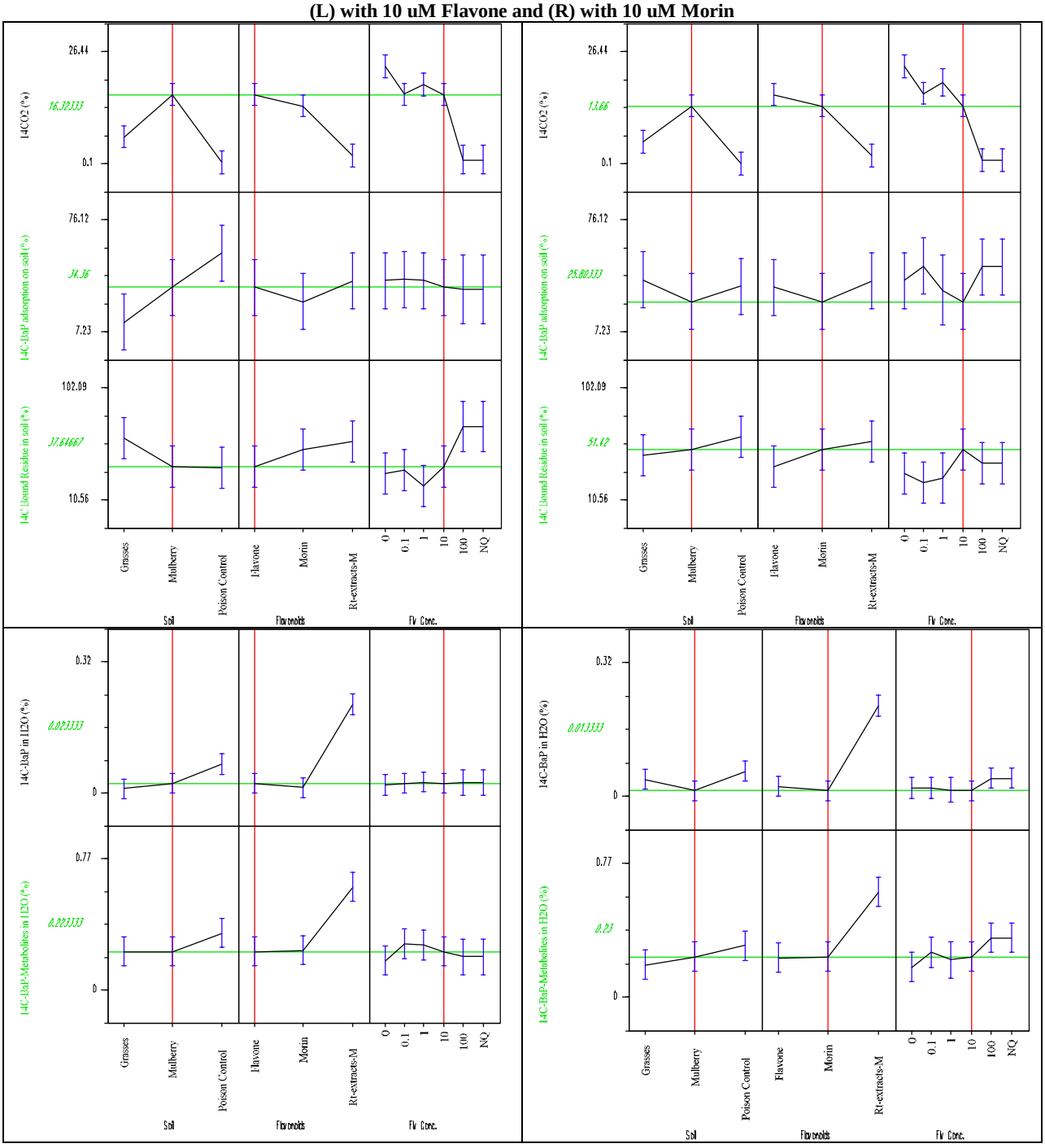
Prediction Profile 7. B[a]P Fate in Mulberry Soil Slurry (L) with 0.1 uM Flavone and (R) with 0.1 uM Morin



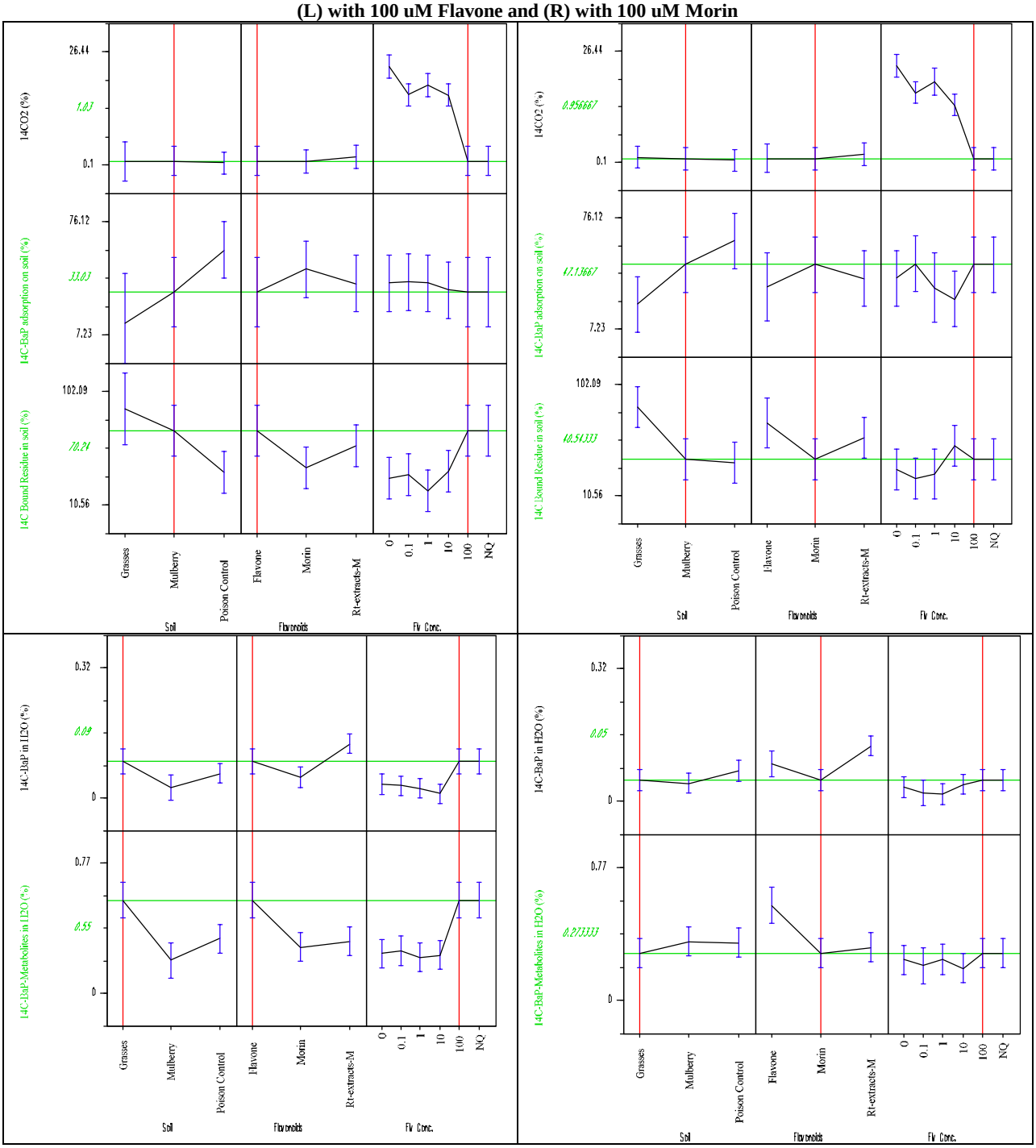
Prediction Profile 8. B[a]P Fate in Mulberry Soil Slurry (L) with 1 uM Flavone and (R) with 1 uM Morin



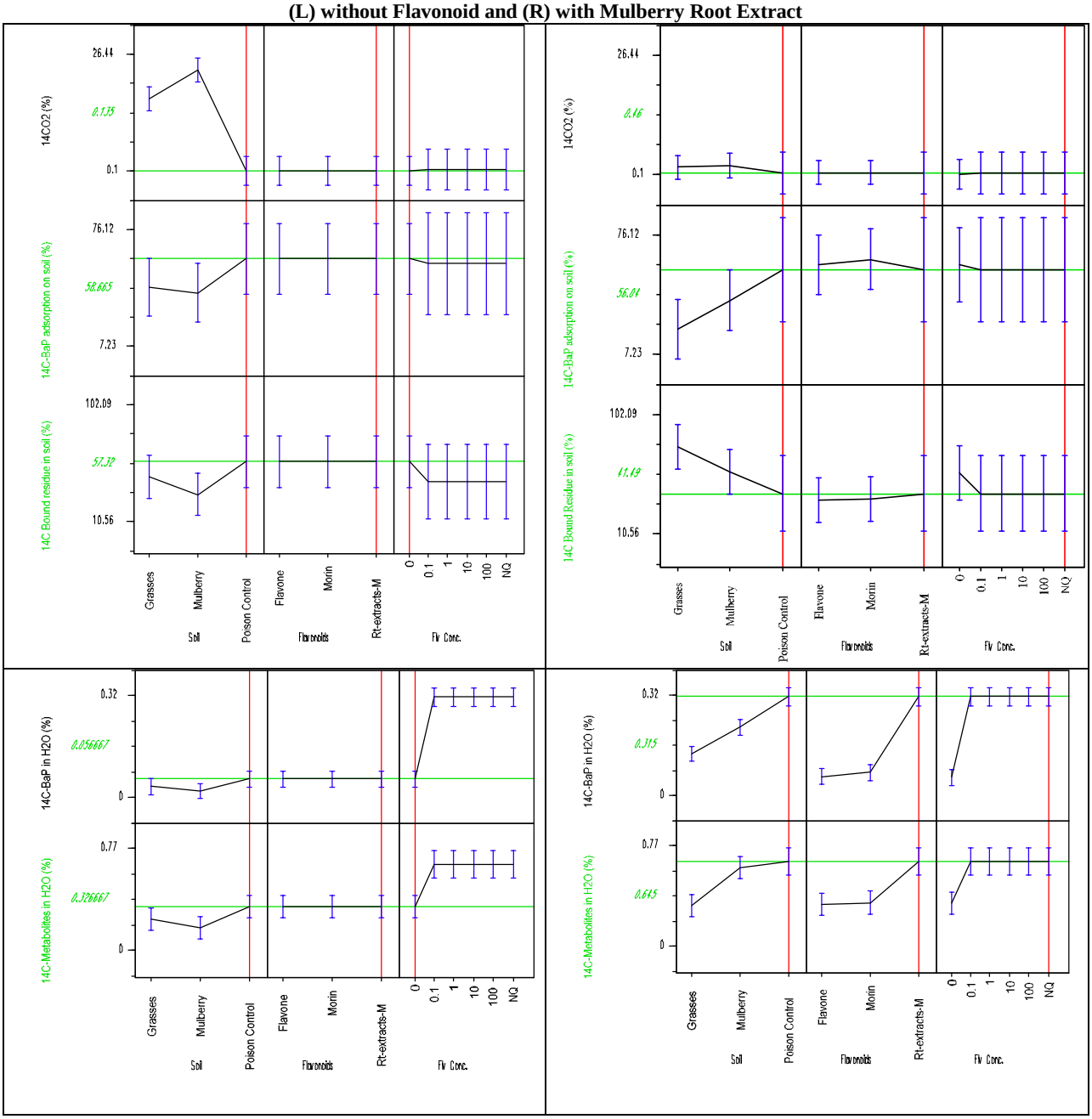
Prediction Profile 9. B[a]P fate in Mulberry Soil Slurry (L) with 10 uM Flavone and (R) with 10 uM Morin



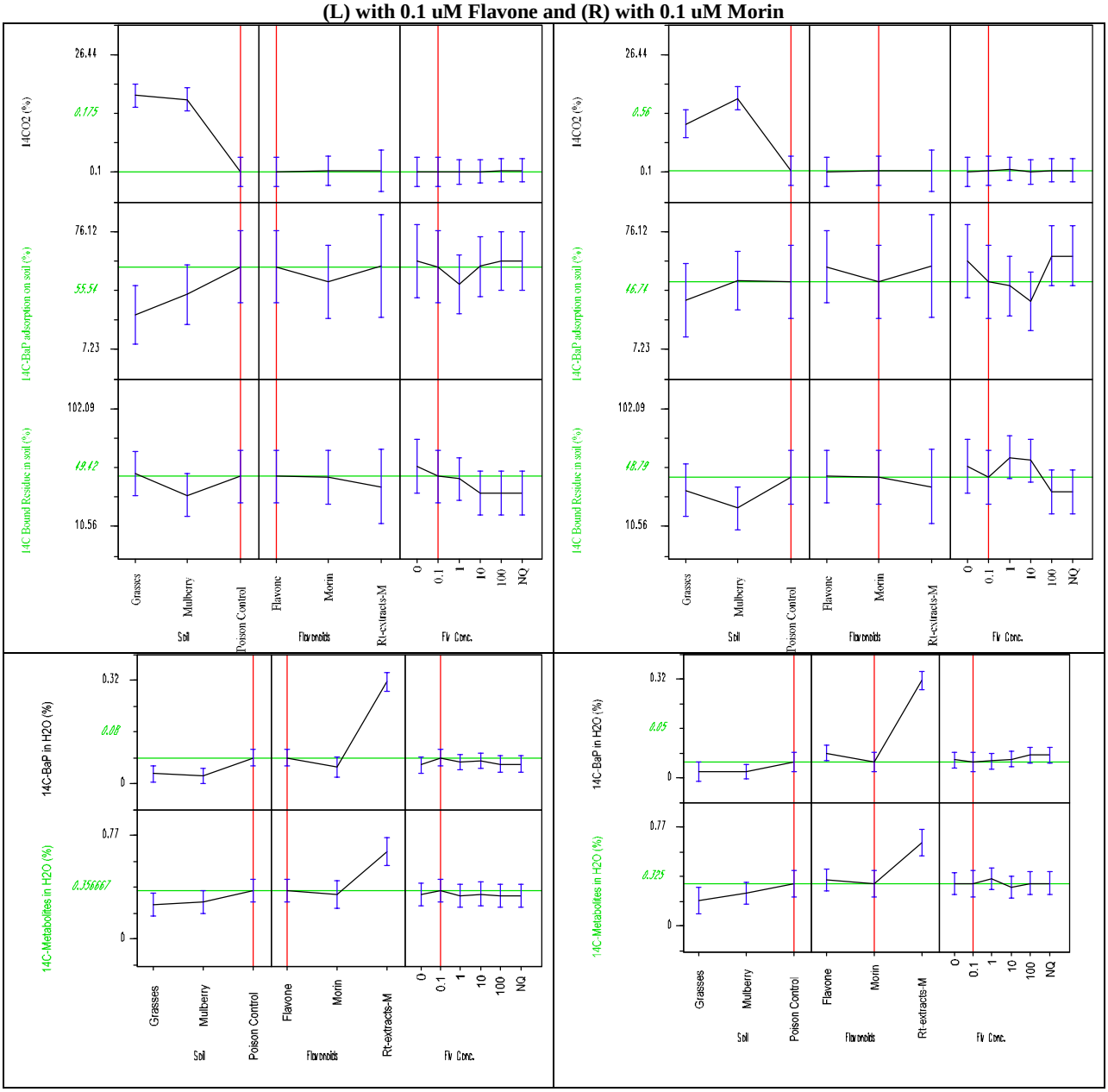
Prediction Profile 10. B[a]P fate in Mulberry Soil Slurry (L) with 100 uM Flavone and (R) with 100 uM Morin



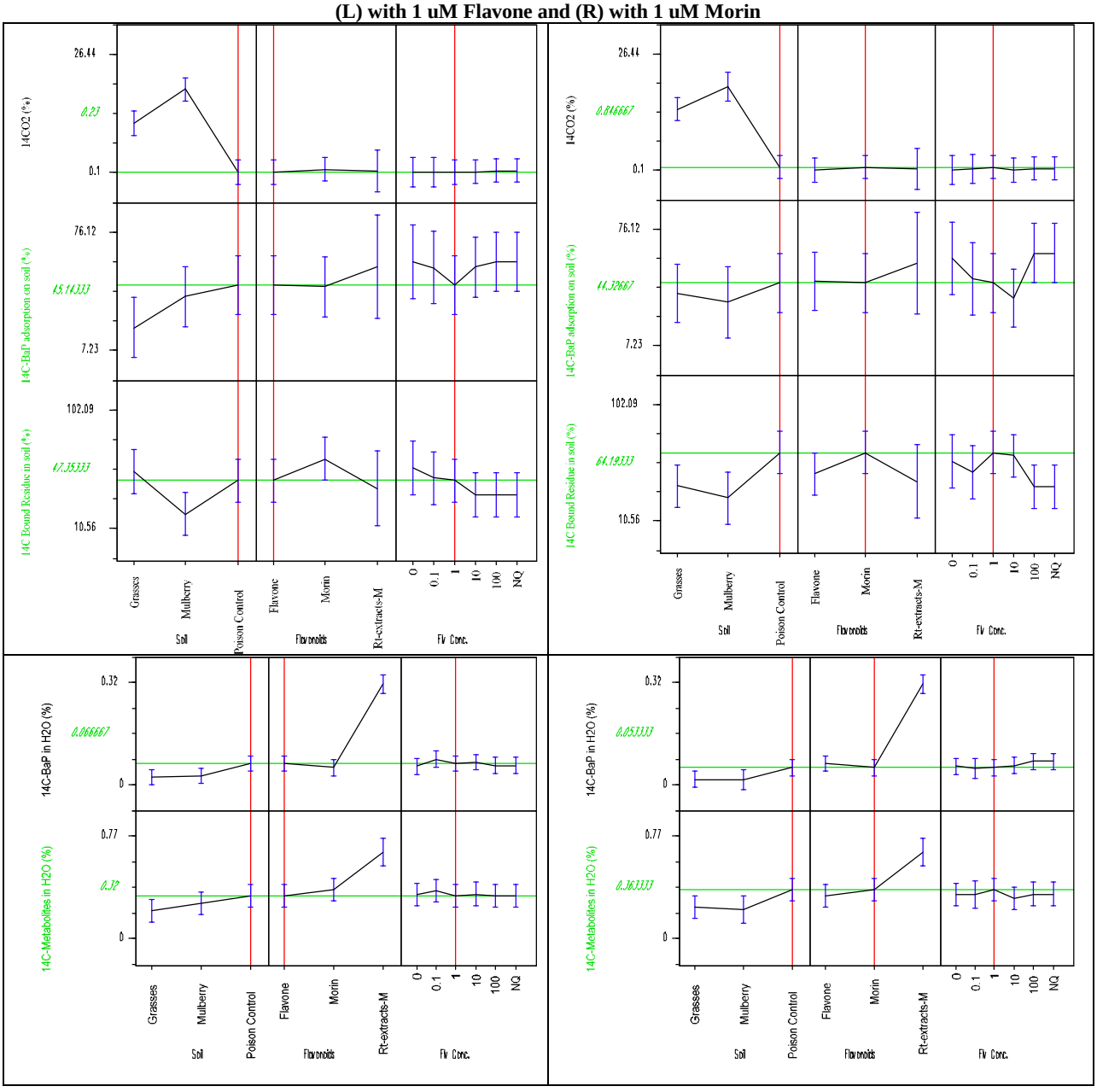
Prediction Profile 11. B[a]P fate in Poisoned Control Mulberry Soil Slurry (L) without Flavonoid and (R) with Mulberry Root Extract



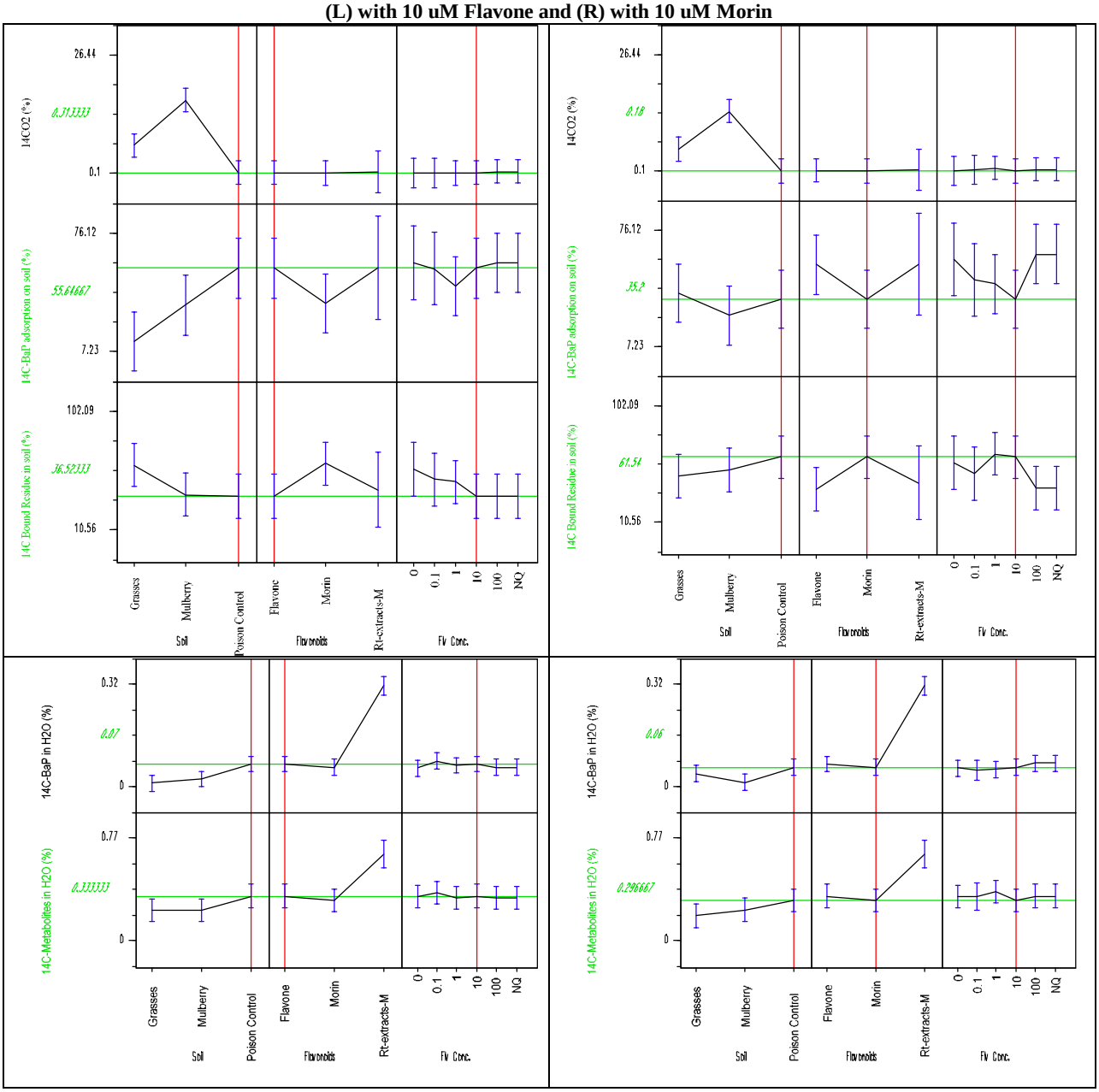
Prediction Profile 12. B[a]P Fate in Poisoned Control Mulberry Soil Slurry (L) with 0.1 uM Flavone and (R) with 0.1 uM Morin



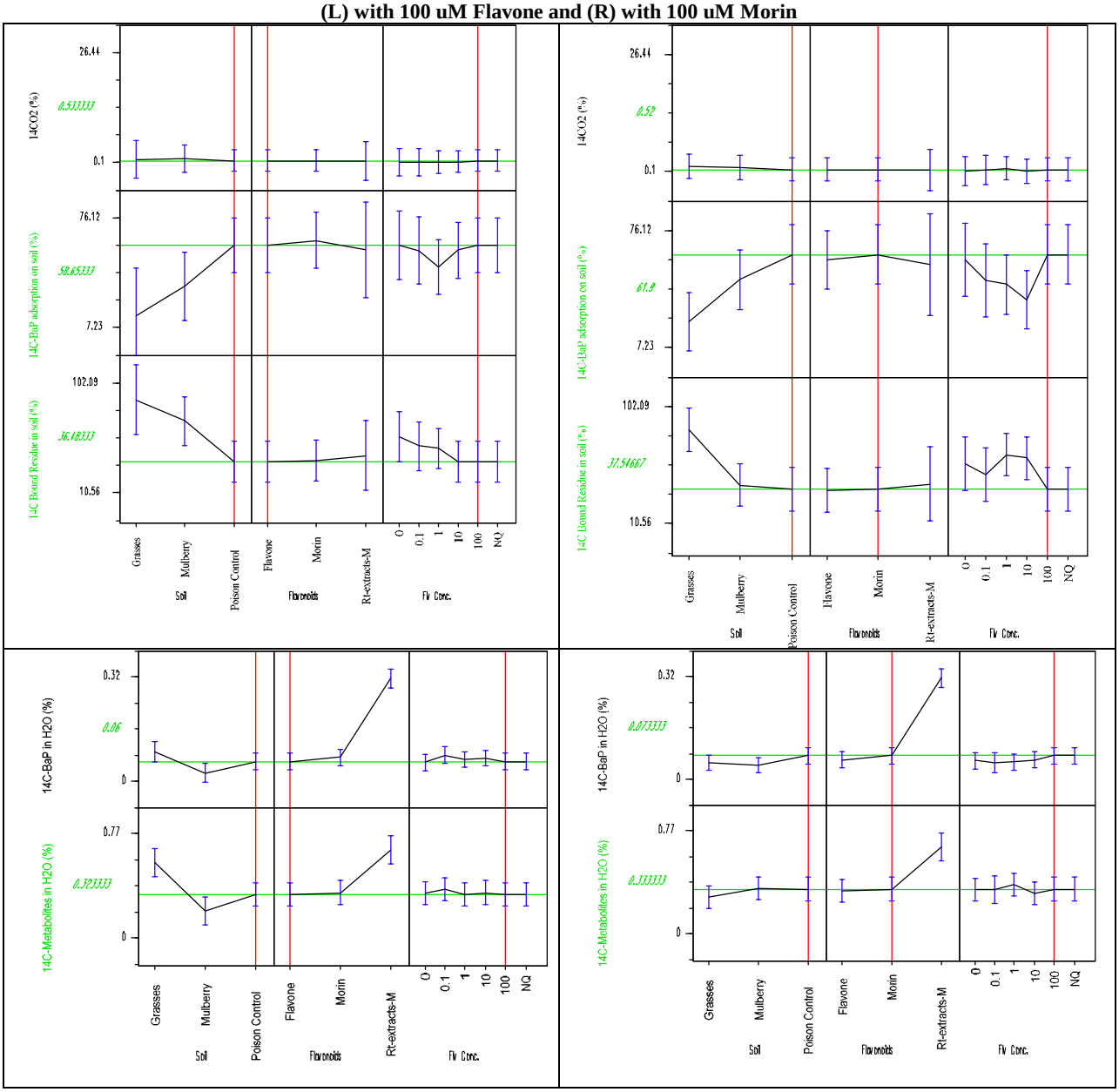
Prediction Profile 13. B[a]P fate in Poisoned Control Mulberry Soil Slurry (L) with 1 uM Flavone and (R) with 1 uM Morin



Prediction Profile 14. B[a]P fate in Poisoned Control Mulberry Soil Slurry (L) with 10 uM Flavone and (R) with 10 uM Morin

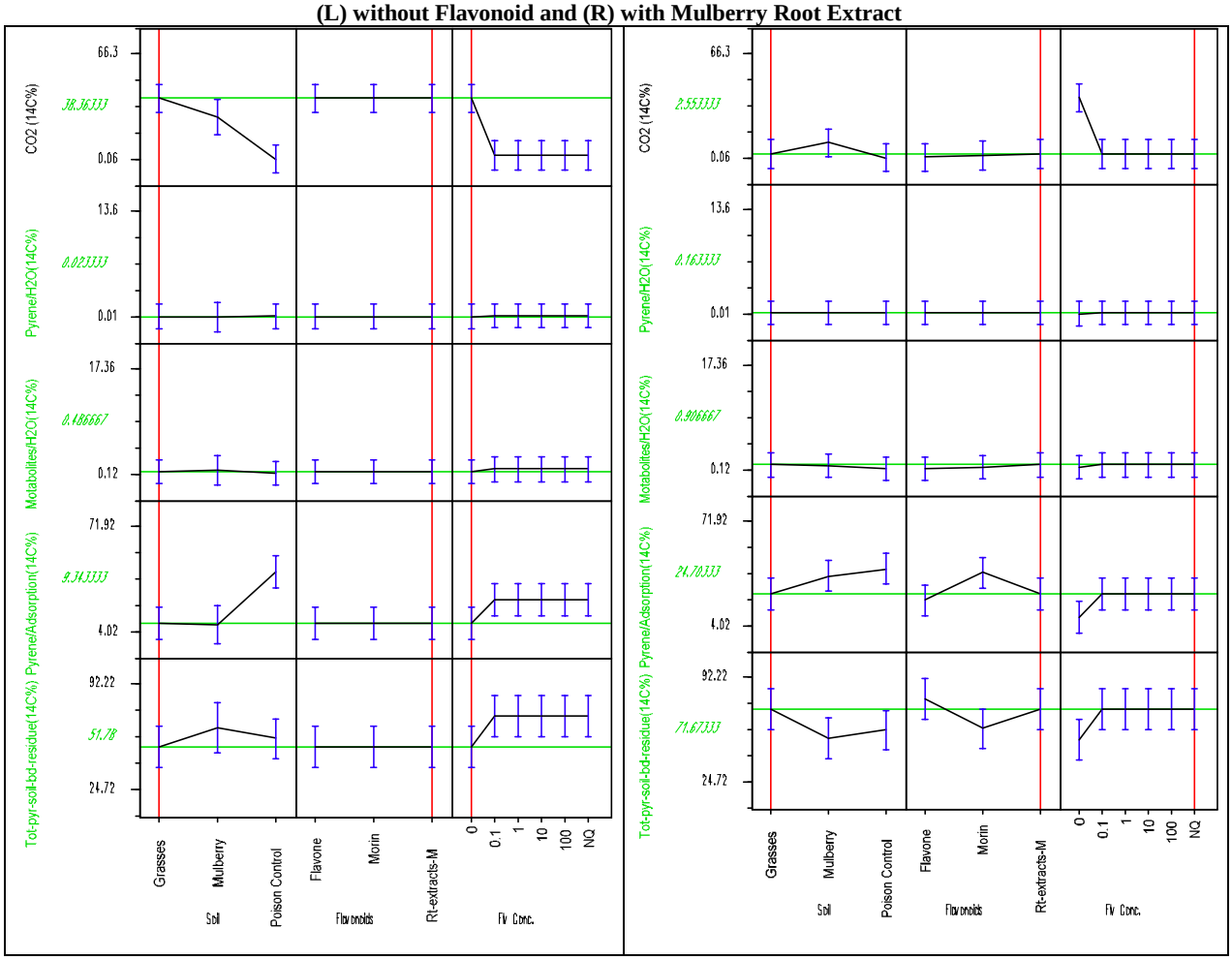


Prediction Profile 15. B[a]P fate in Poisoned Control Mulberry Soil Slurry (L) with 100 uM Flavone and (R) with 100 uM Morin



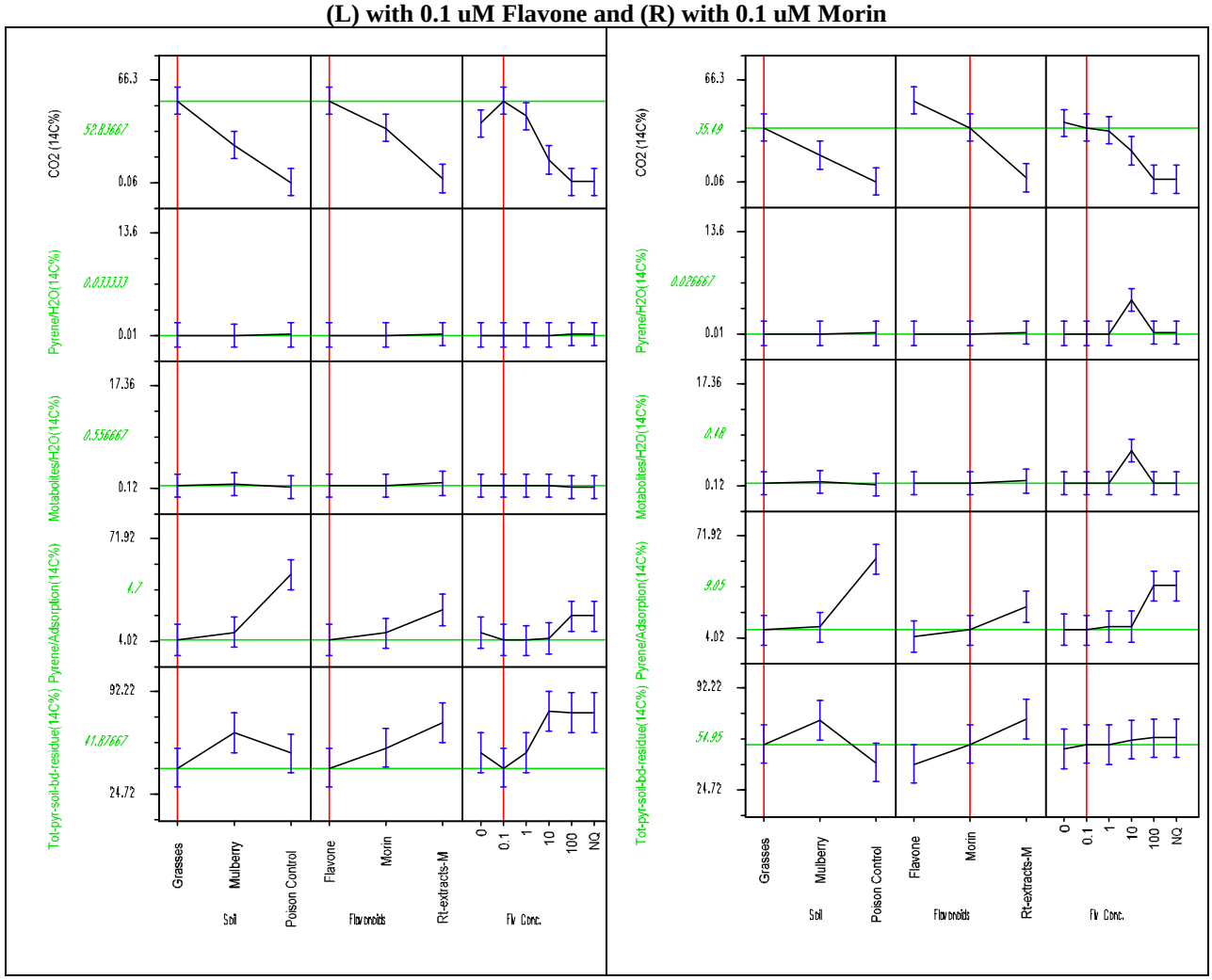
Appendix C-4. Compound-Nested Model Screening Fit Prediction Profiles (Pyrene Data)

Prediction Profile 16. ^{14}C-Pyrene Fate in Bermudagrass Soil Slurry (L) without Flavonoid and (R) with Mulberry Root Extract

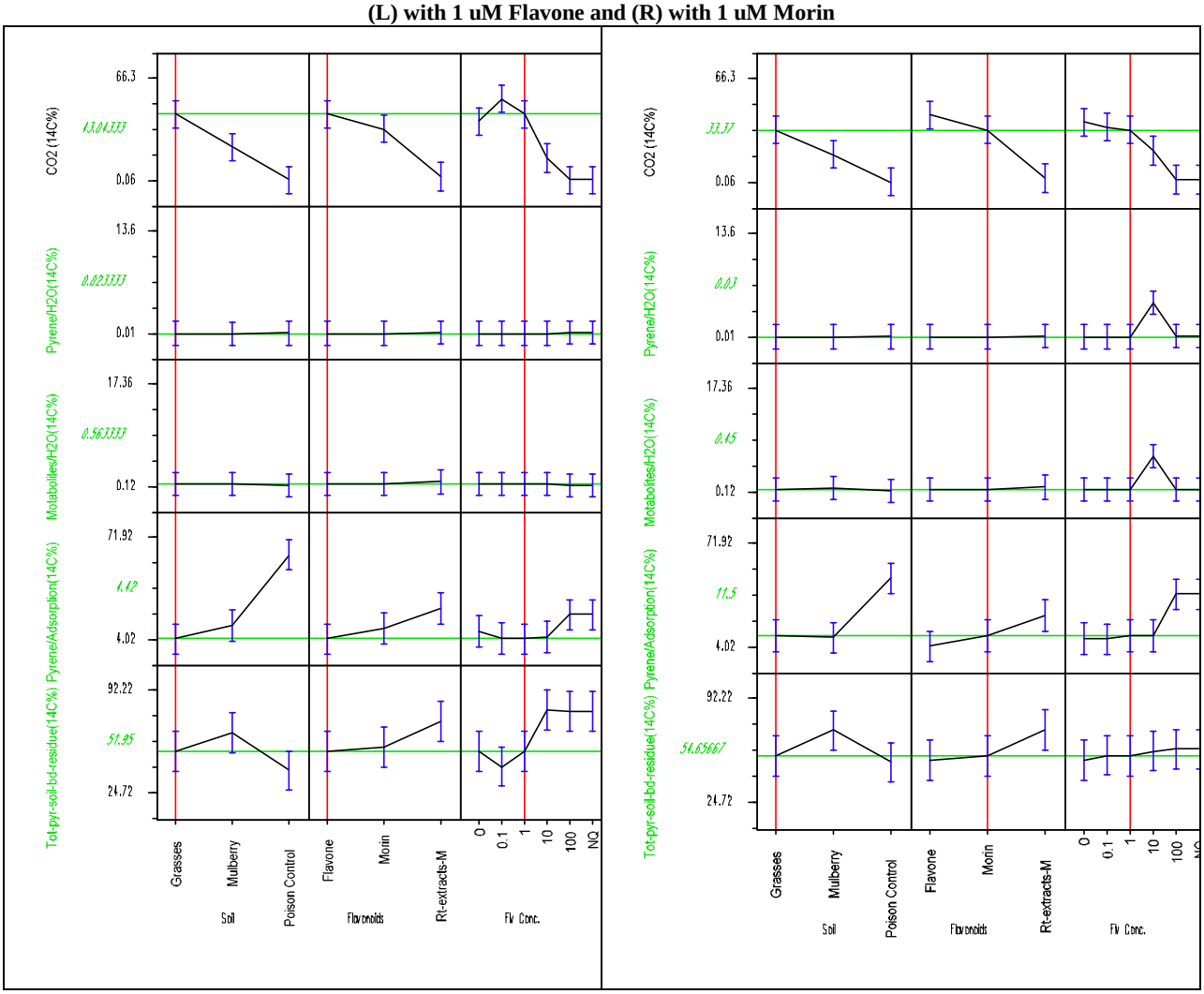


The prediction profiles show how the predicted values for each of the five PAH fate mechanisms changes when each of the three factors (soil type, flavonoid type, and flavonoid concentration) changes. The Y axis is the predicted values of ^{14}C-B[a]P fate measurements and the X axis stands for the testing variable of the three factors. For a predicted value, 95% confidence interval is shown by error bars. The vertical red line holds a variable (factor) at a constant level to predict the responses to any combination of the three factors. The horizontal green line shows the predicted responses when the red lines hold the variables constant. The predicted response (i.e., fate data) changes as one variable (i.e., factor) changes while the others are held constant. A matrix of 15 prediction profiles are included in both left and right halves, respectively. The 1st column shows the effects of soil types. B[a]P fate changed as soil type changed without flavonoid added. The 2nd column shows the effects of flavonoid types. B[a]P fate did not change with the types of flavonoid when the flavonoid concentration was zero. The 3rd column shows the effects of flavonoid concentration. In Bermudagrass soil, ^{14}C-B[a]P fate changed as mulberry root extract concentration changed. Likewise, the effects of multifactors on B[a]P fate as any one of the variables changed were presented in the following prediction profiles.

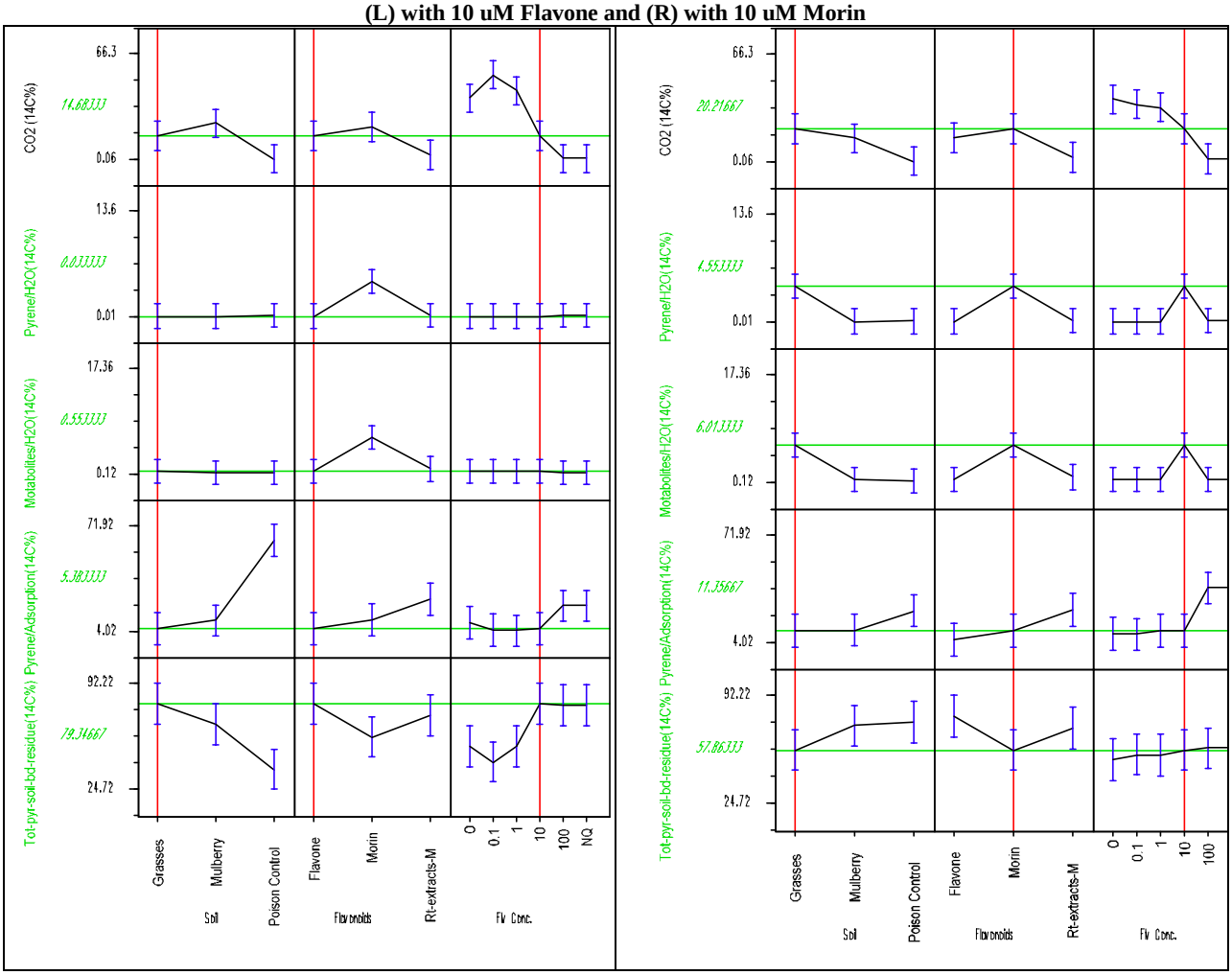
Prediction Profile 17. ^{14}C-Pyrene Fate in Bermudagrass Soil Slurry (L) with 0.1 uM Flavone and (R) with 0.1 uM Morin



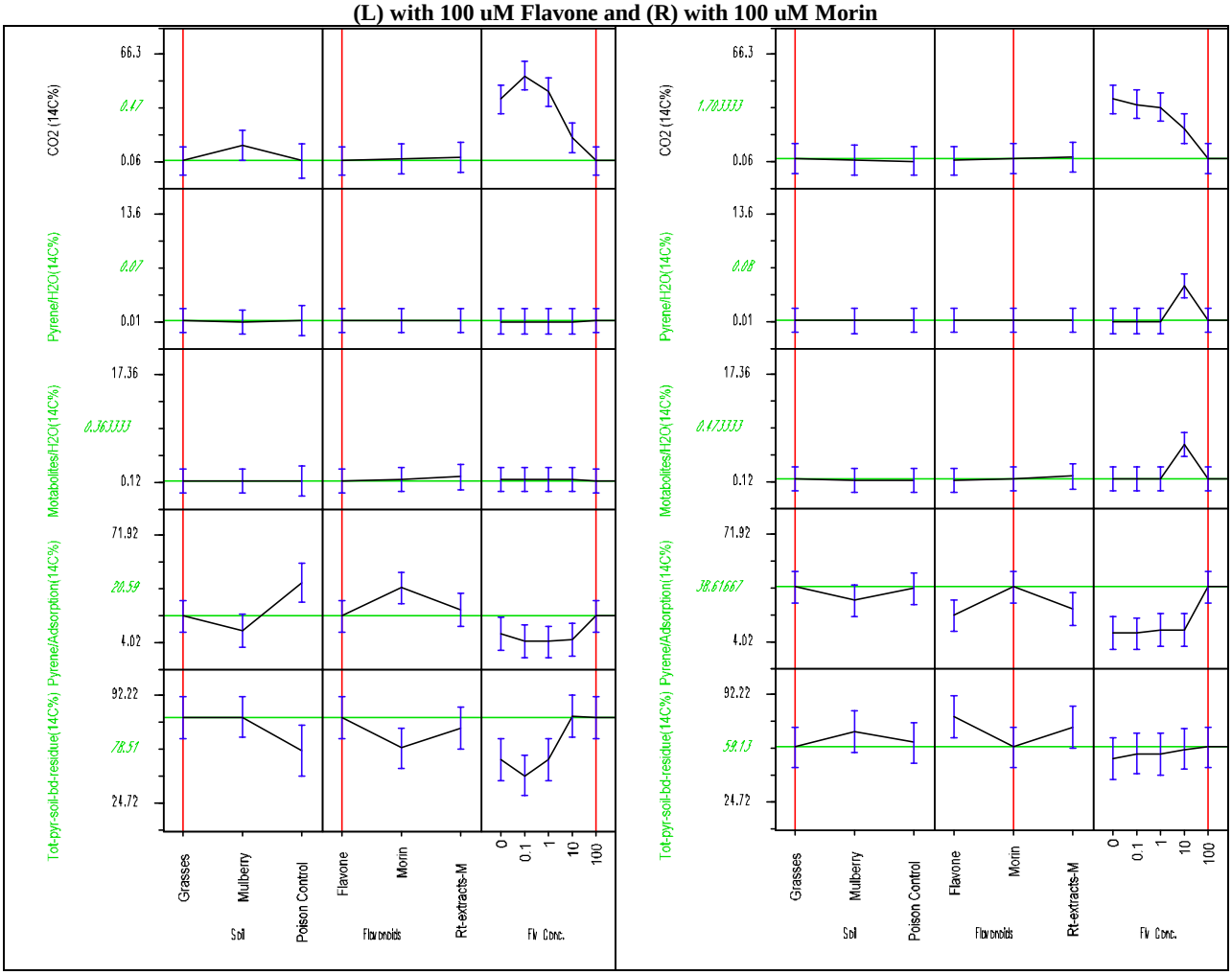
Prediction Profile 18. Pyrene fate in Bermudagrass Soil Slurry (L) with 1 uM Flavone and (R) with 1 uM Morin



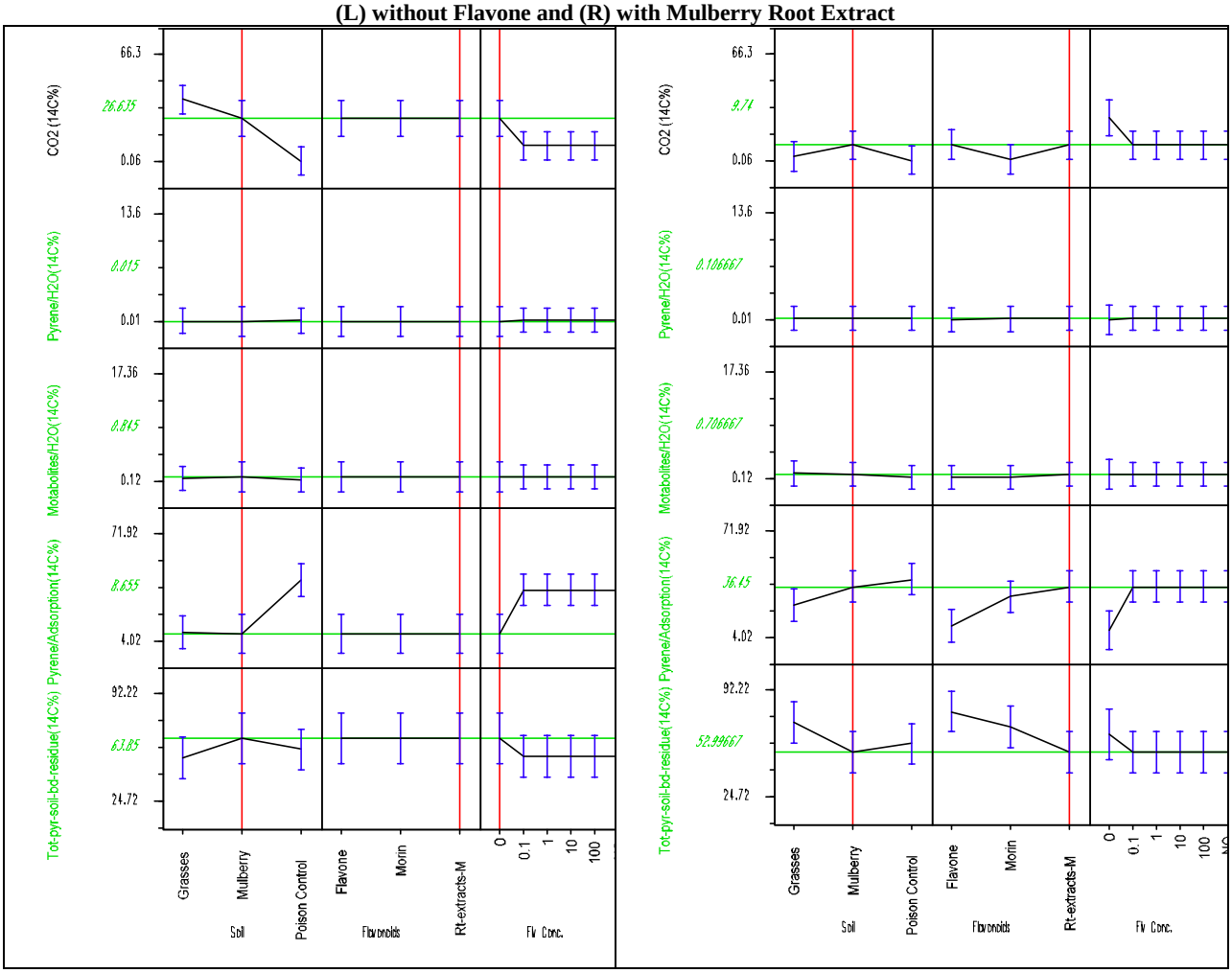
Prediction Profile 19. ^{14}C-Pyrene Fate in Bermudagrass Soil Slurry (L) with 10 uM Flavone and (R) with 10 uM Morin



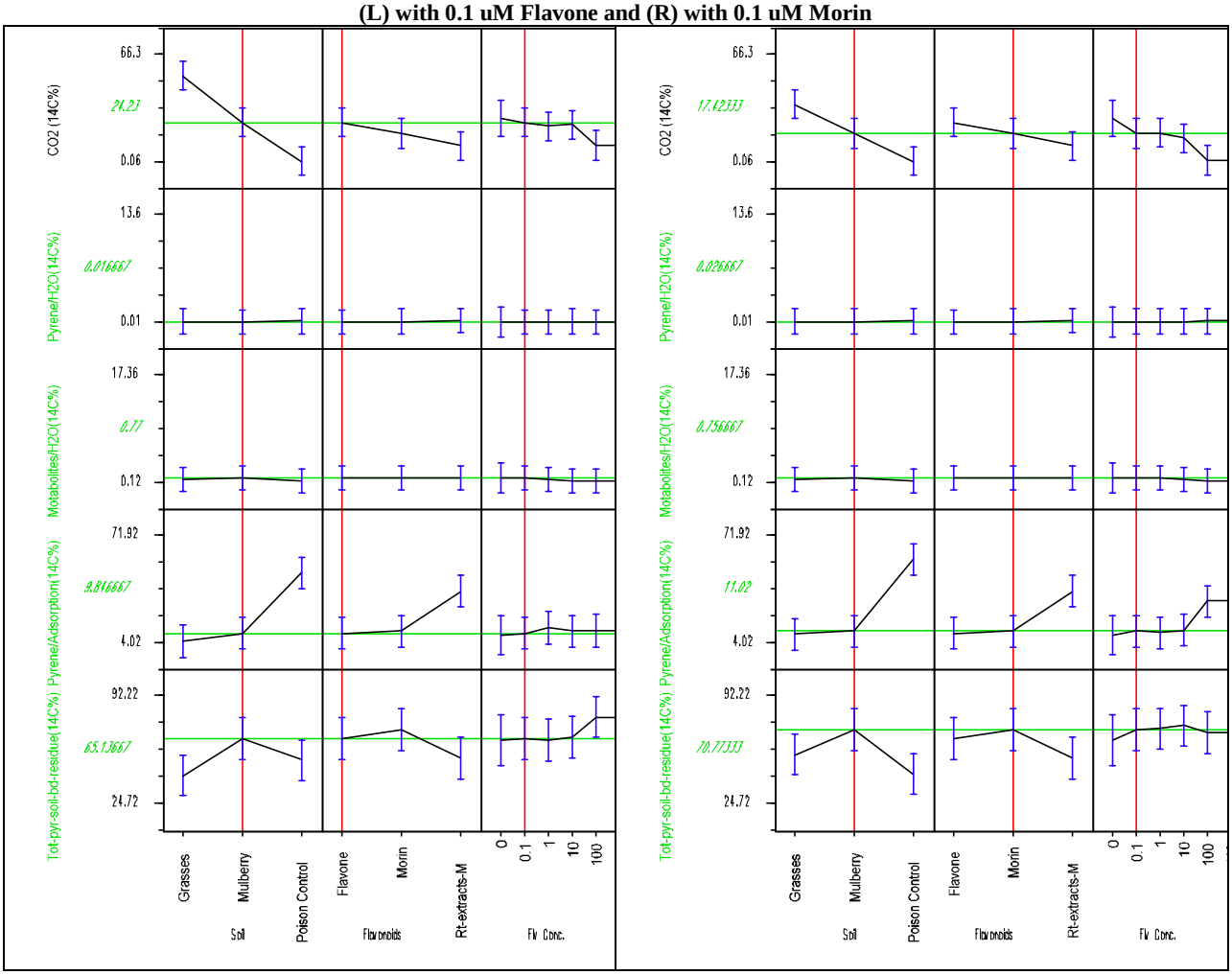
Prediction Profile 20. ^{14}C-Pyrene Fate in Bermudagrass Soil Slurry (L) with 100 uM Flavone and (R) with 100 uM Morin



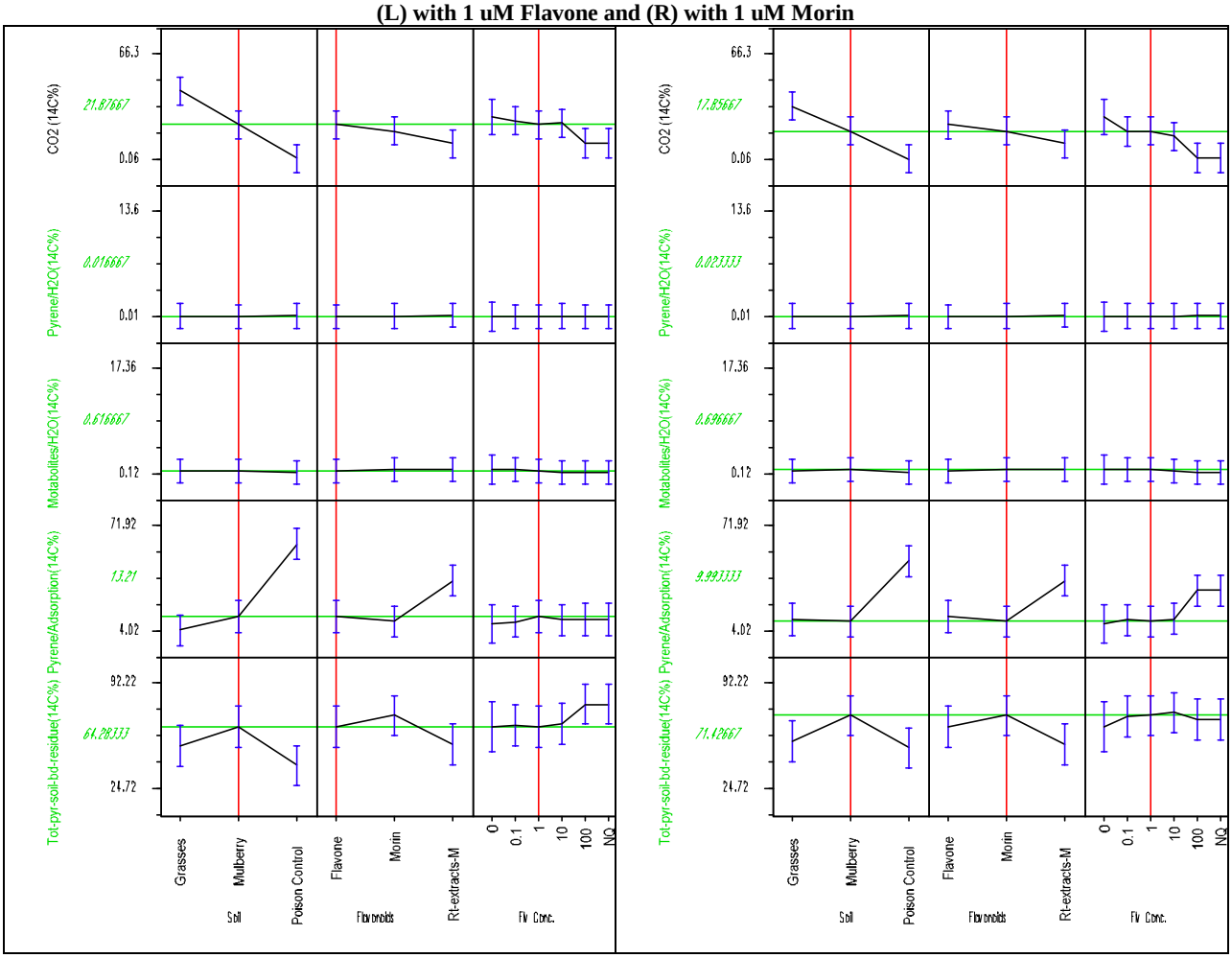
Prediction Profile 21. ^{14}C-Pyrene Fate in Mulberry Soil Slurry
(L) without Flavone and (R) with Mulberry Root Extract



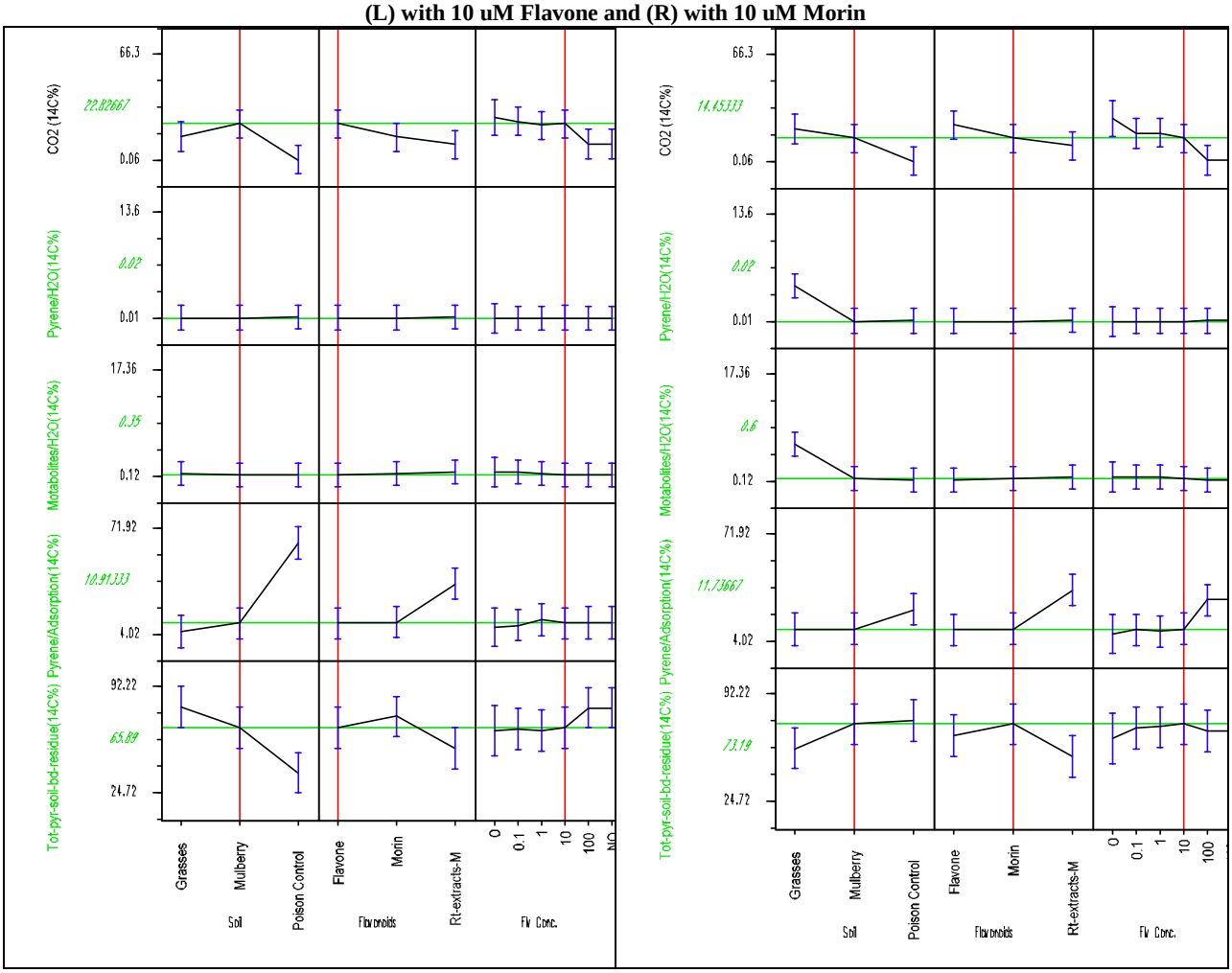
Prediction Profile 22. ^{14}C-Pyrene Fate in Mulberry Soil Slurry (L) with 0.1 uM Flavone and (R) with 0.1 uM Morin



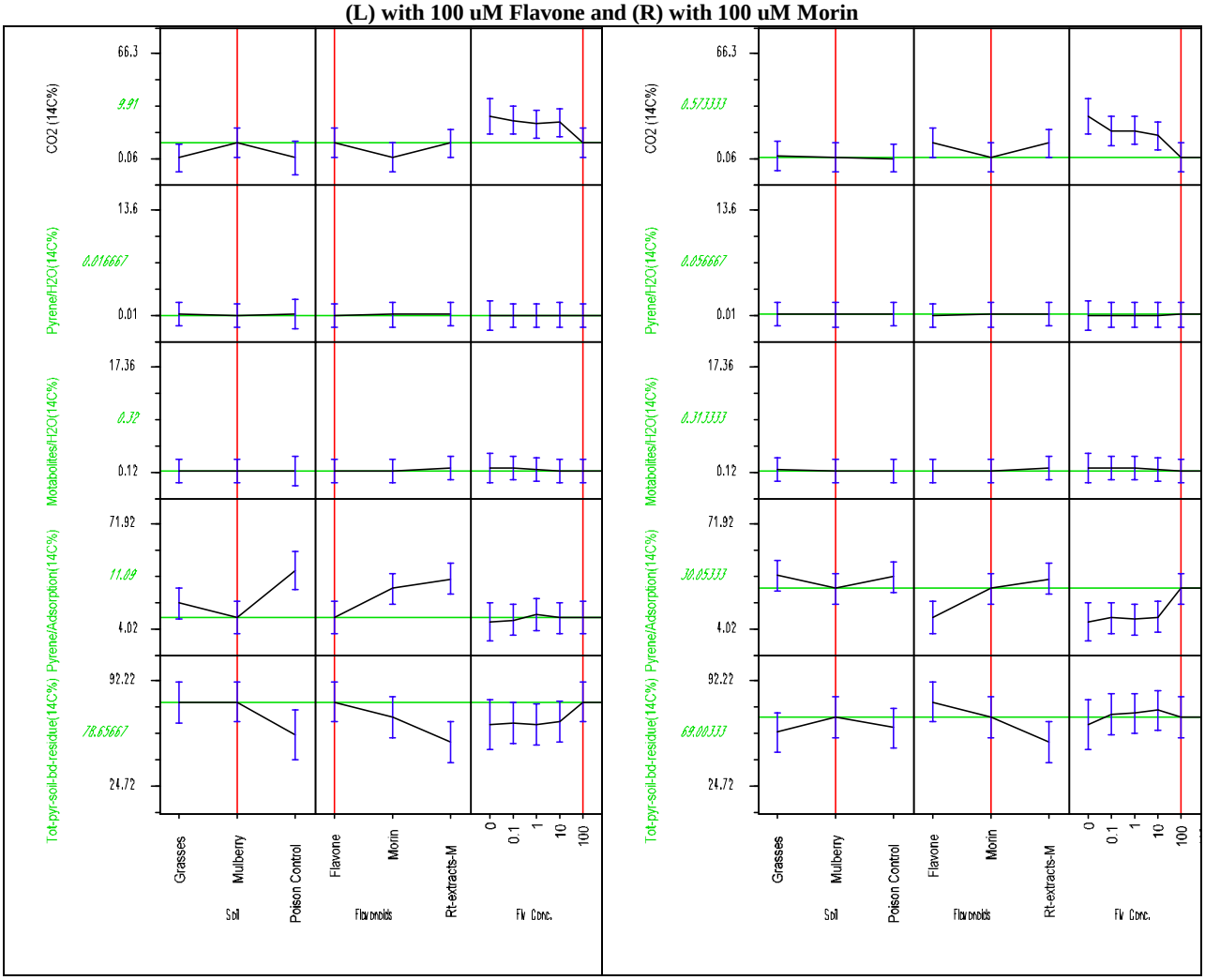
Prediction Profile 23. ^{14}C-Pyrene Fate in Mulberry Soil Slurry (L) with 1 uM Flavone and (R) with 1 uM Morin



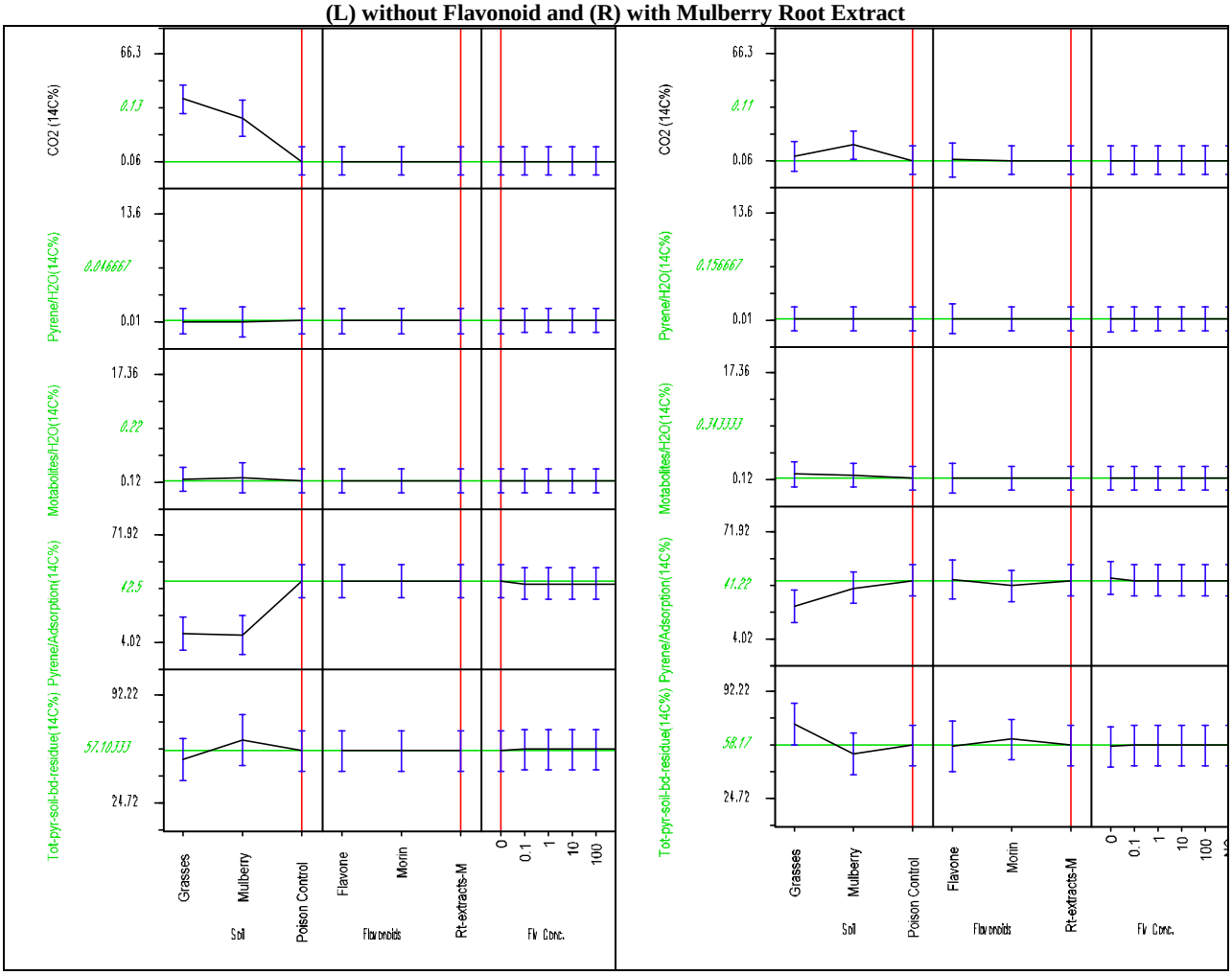
Prediction Profile 24. ^{14}C-Pyrene fate in Mulberry Soil Slurry (L) with 10 uM Flavone and (R) with 10 uM Morin



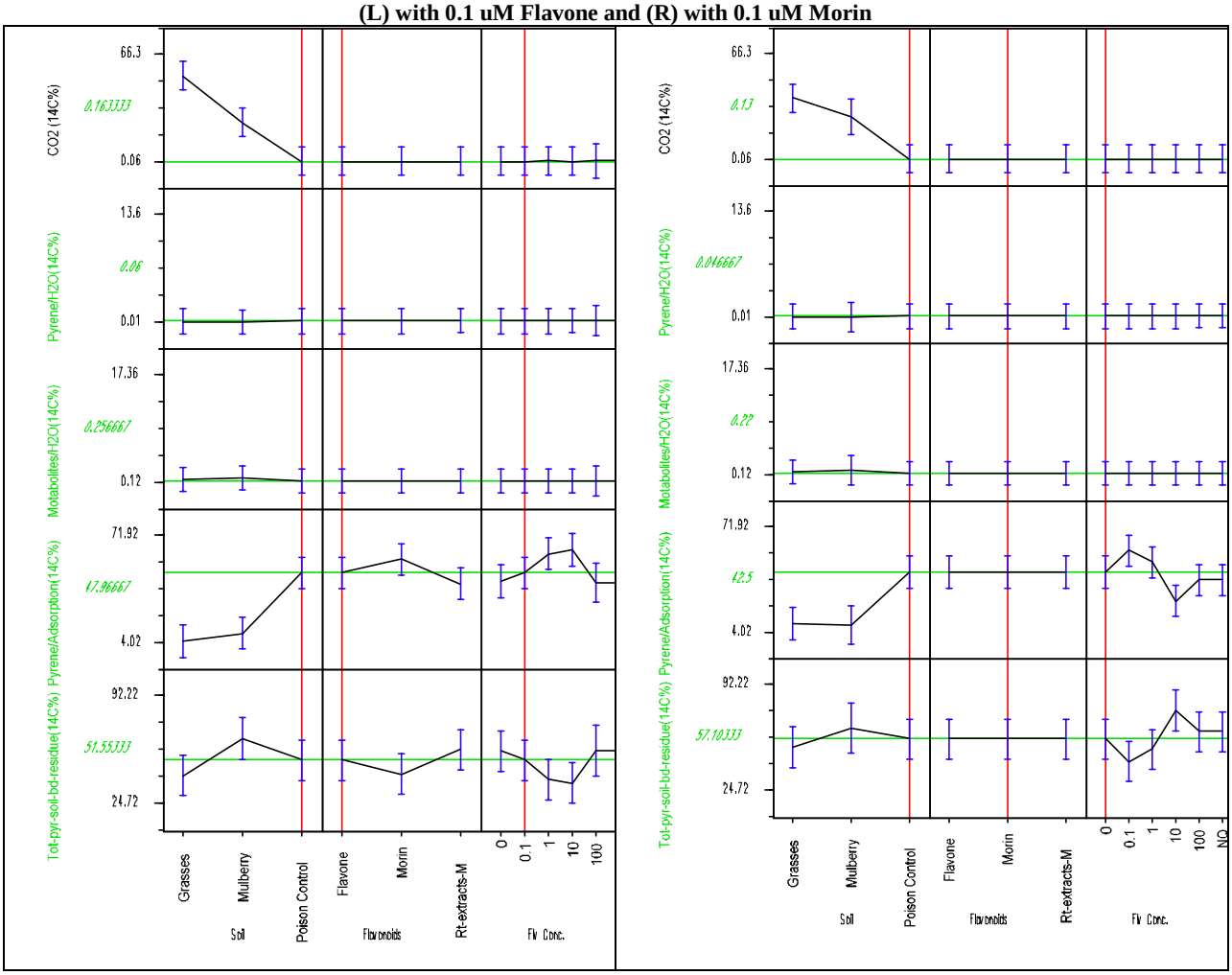
Prediction Profile 25. ^{14}C-Pyrene fate in Mulberry Soil Slurry (L) with 100 uM Flavone and (R) with 100 uM Morin



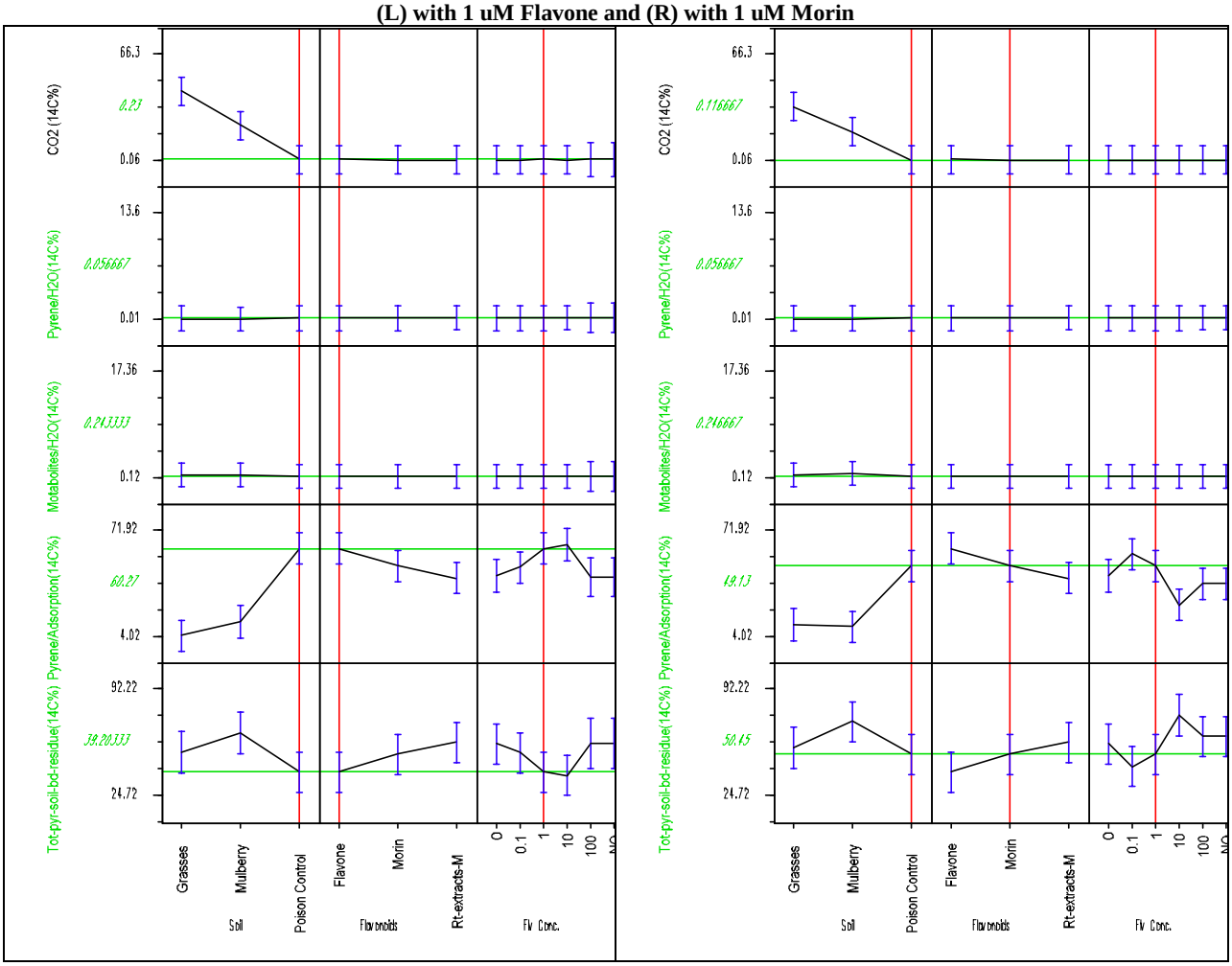
Prediction Profile 26. ^{14}C-Pyrene fate in Poisoned Control Mulberry Soil Slurry (L) without Flavonoid and (R) with Mulberry Root Extract



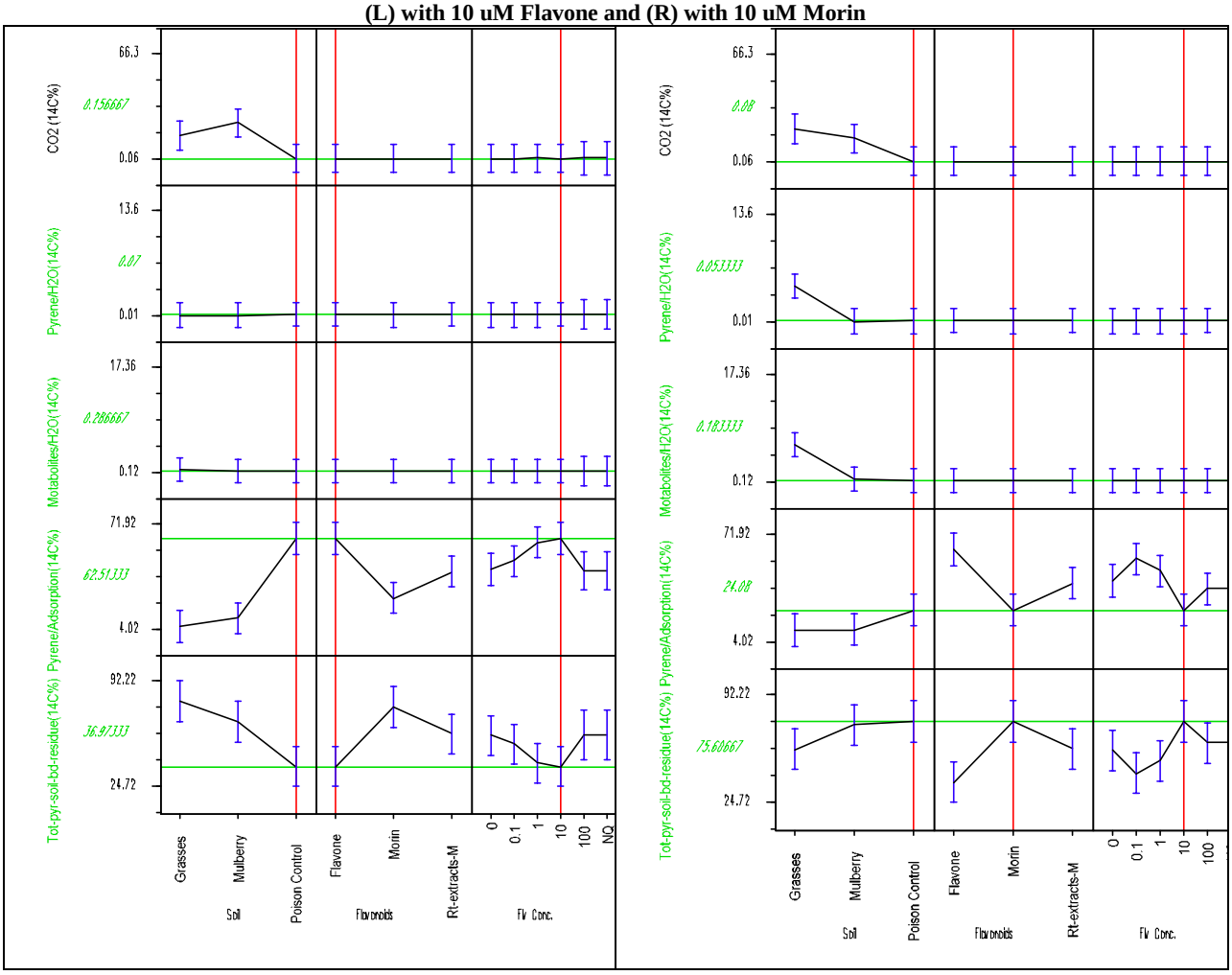
Prediction Profile 27. ^{14}C-Pyrene Fate in Poisoned Control Mulberry Soil Slurry (L) with 0.1 uM Flavone and (R) with 0.1 uM Morin



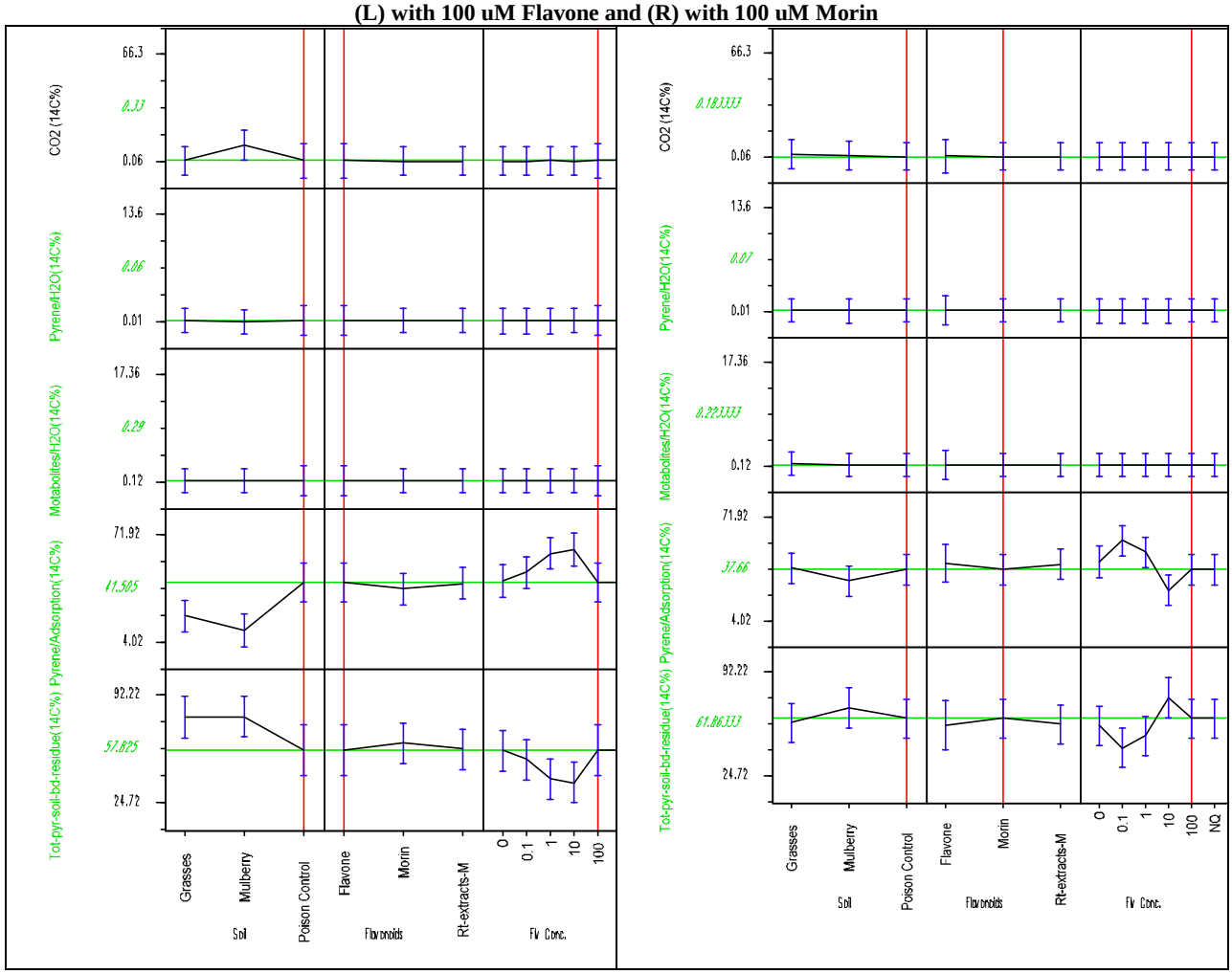
Prediction Profile 28. ^{14}C-Pyrene fate in Poisoned Control Mulberry Soil Slurry (L) with 1 uM Flavone and (R) with 1 uM Morin



Prediction Profile 29. ^{14}C-Pyrene fate in Poisoned Control Mulberry Soil Slurry (L) with 10 uM Flavone and (R) with 10 uM Morin

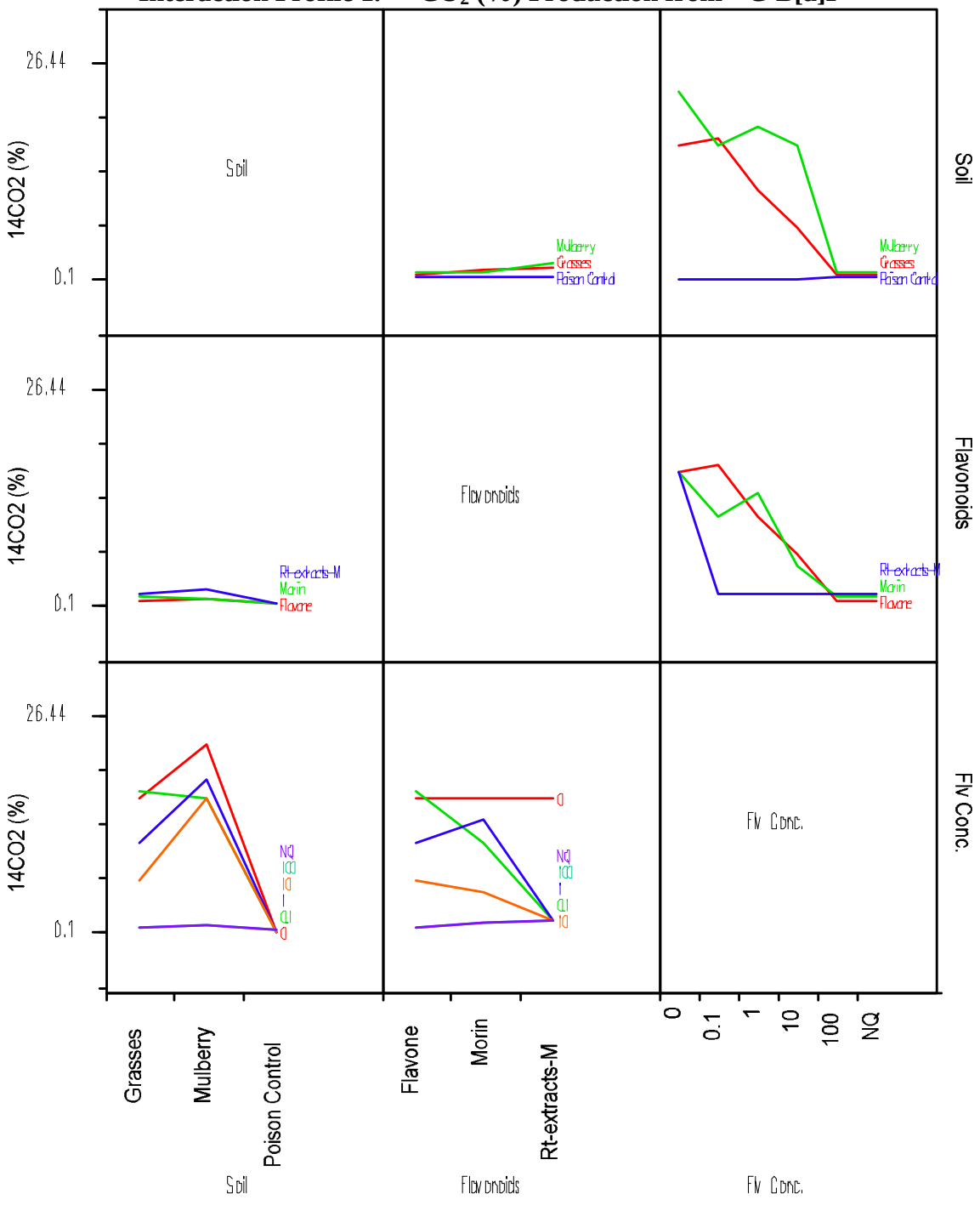


Prediction Profile 30. ^{14}C-Pyrene fate in Poisoned Control Mulberry Soil Slurry (L) with 100 uM Flavone and (R) with 100 uM Morin

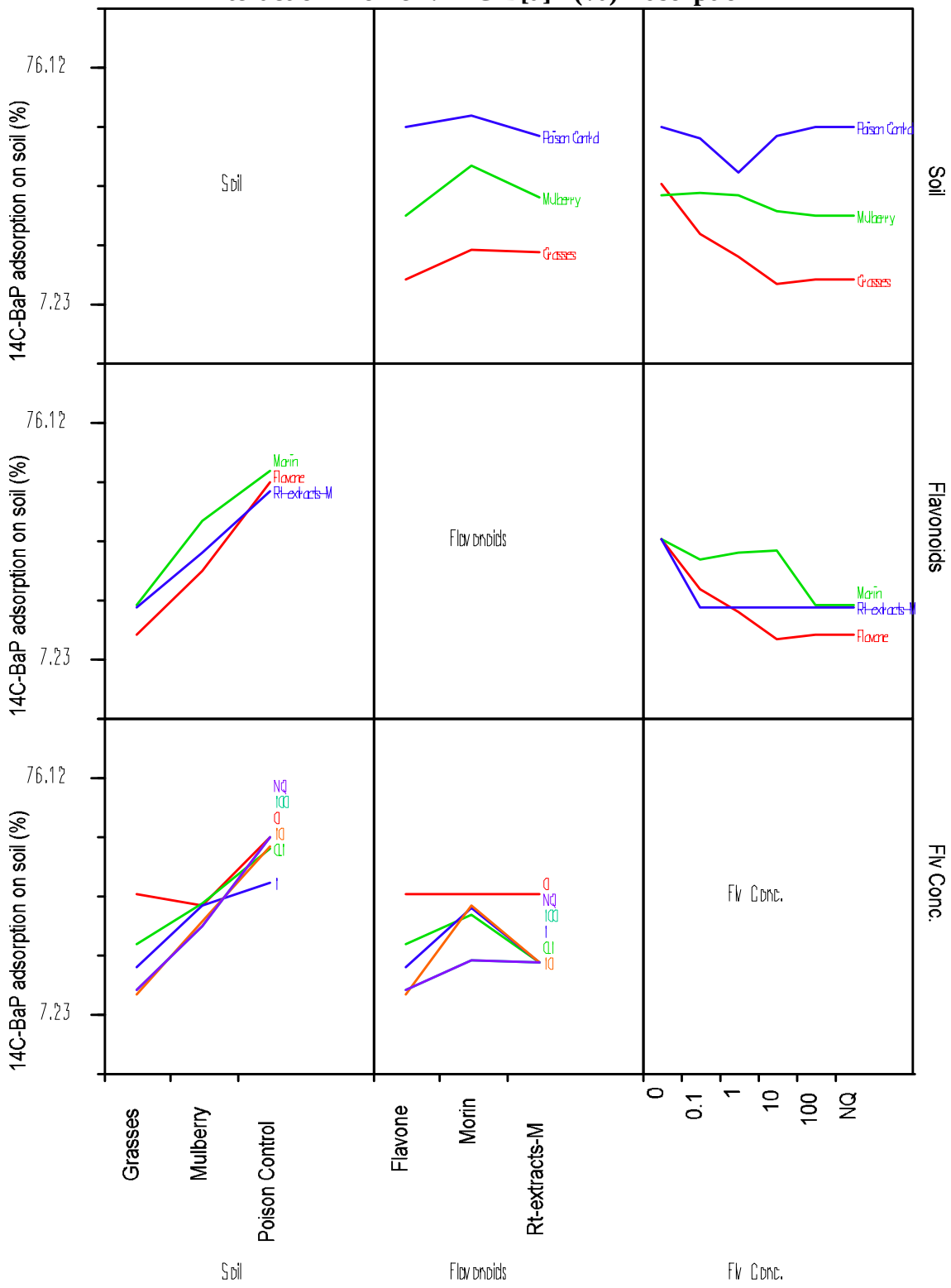


Appendix C-5. Compound Nested Model Screening Fit Interaction Profiles (^{14}C-B[a]P Data)

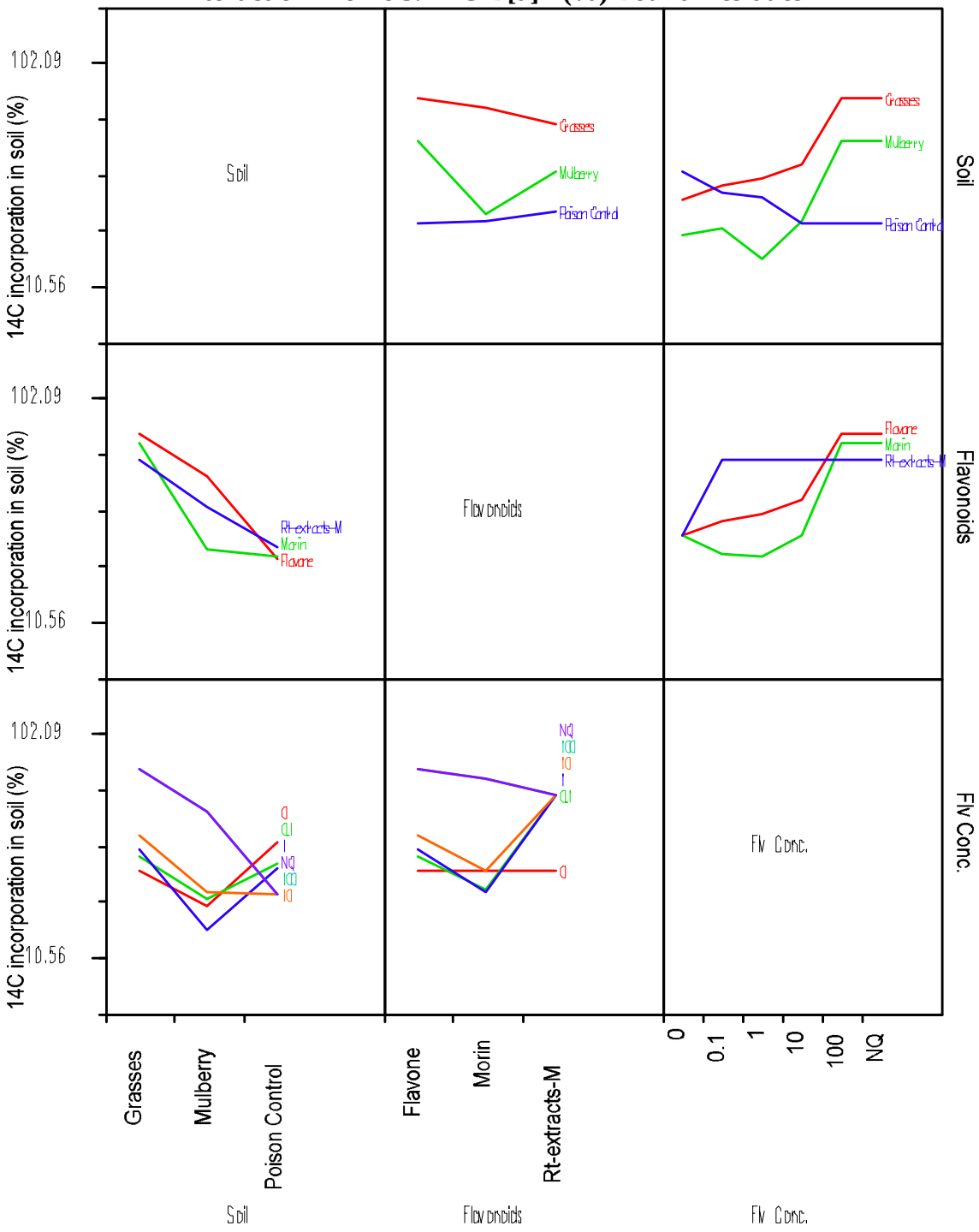
Interaction Profile 1. $^{14}CO_2$ (%) Production from ^{14}C-B[a]P



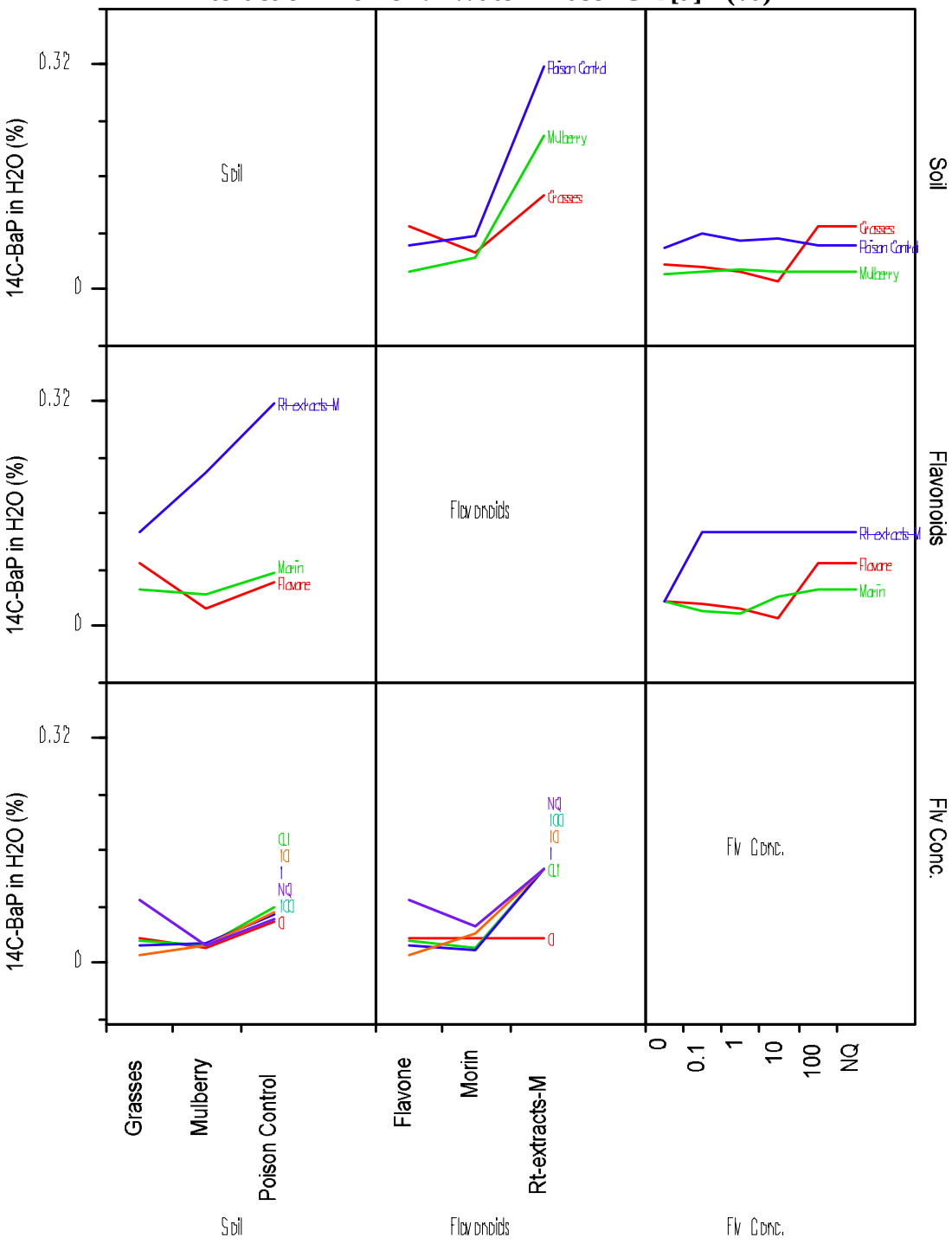
Interaction Profile 2. ^{14}C-B[a]P (%) Adsorption



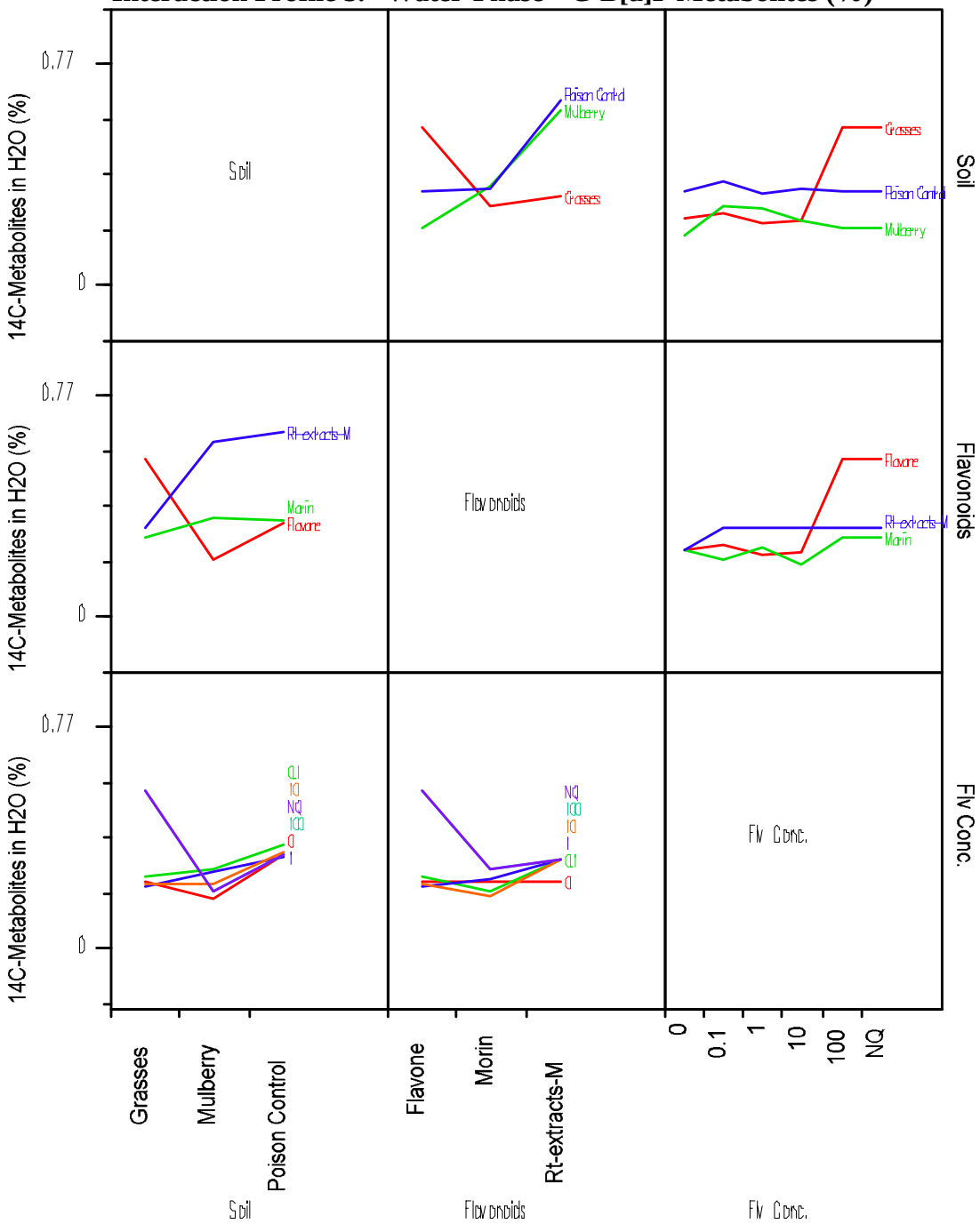
Interaction Profile 3. ^{14}C-B[a]P (%) Bound Residues



Interaction Profile 4. Water-Phase^{14}C-B[a]P (%)

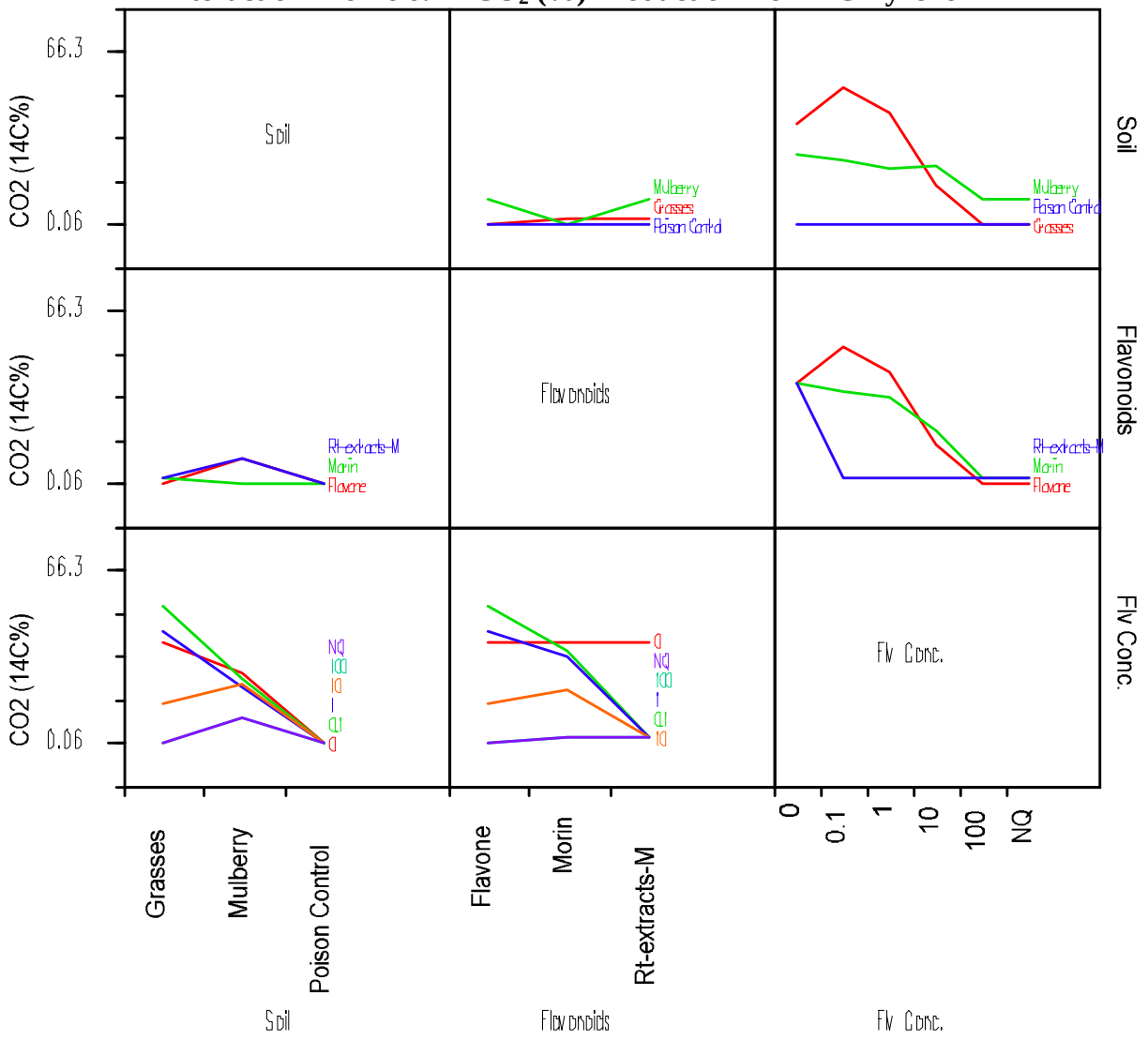


Interaction Profile 5. Water-Phase ^{14}C-B[a]P Metabolites (%)

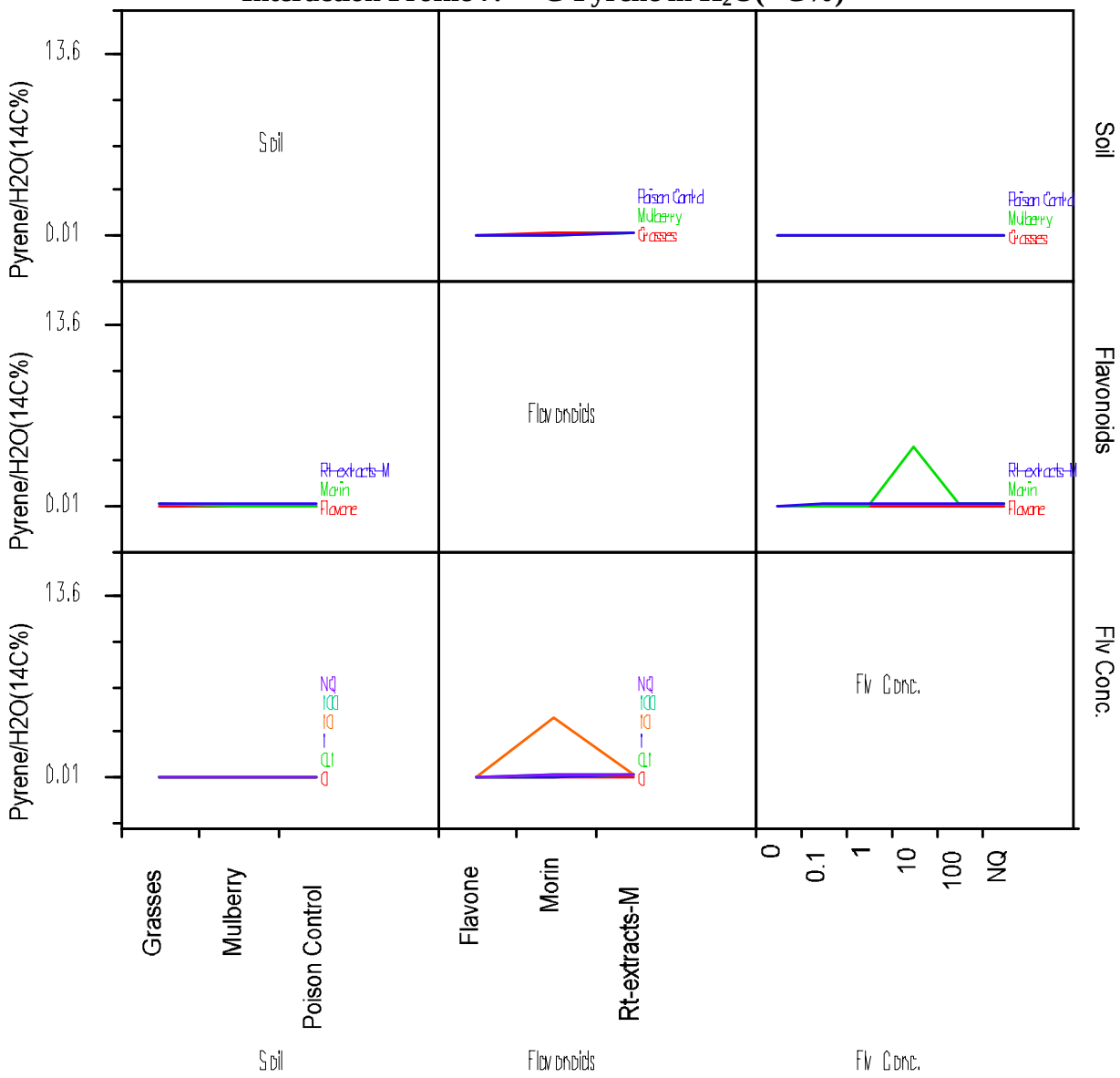


Appendix C-6. Compound Nested Model Screening Fit Interaction Profiles (^{14}C-Pyrene Data)

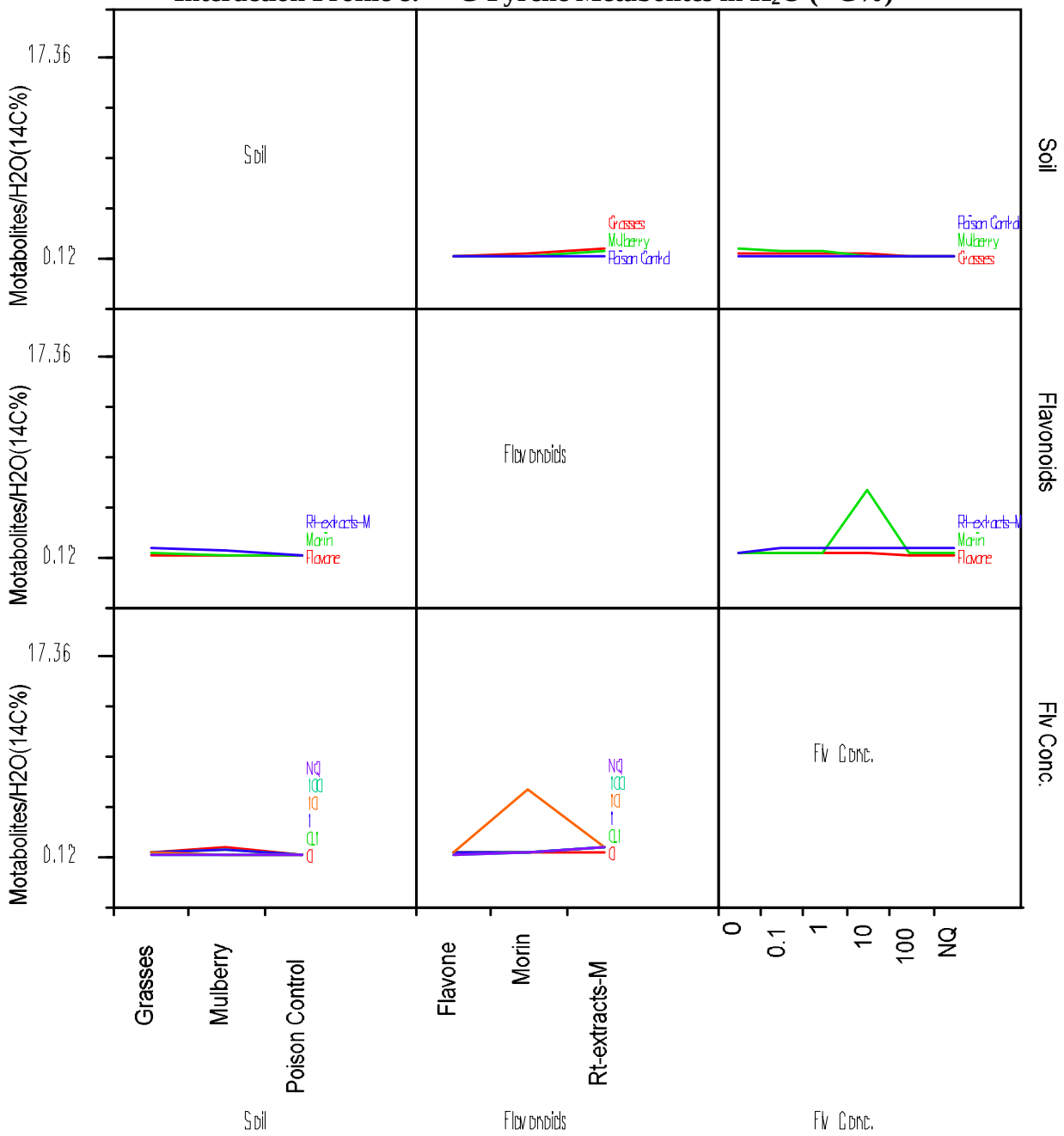
Interaction Profile 6: $^{14}CO_2$ (%) Production from ^{14}C-Pyrene



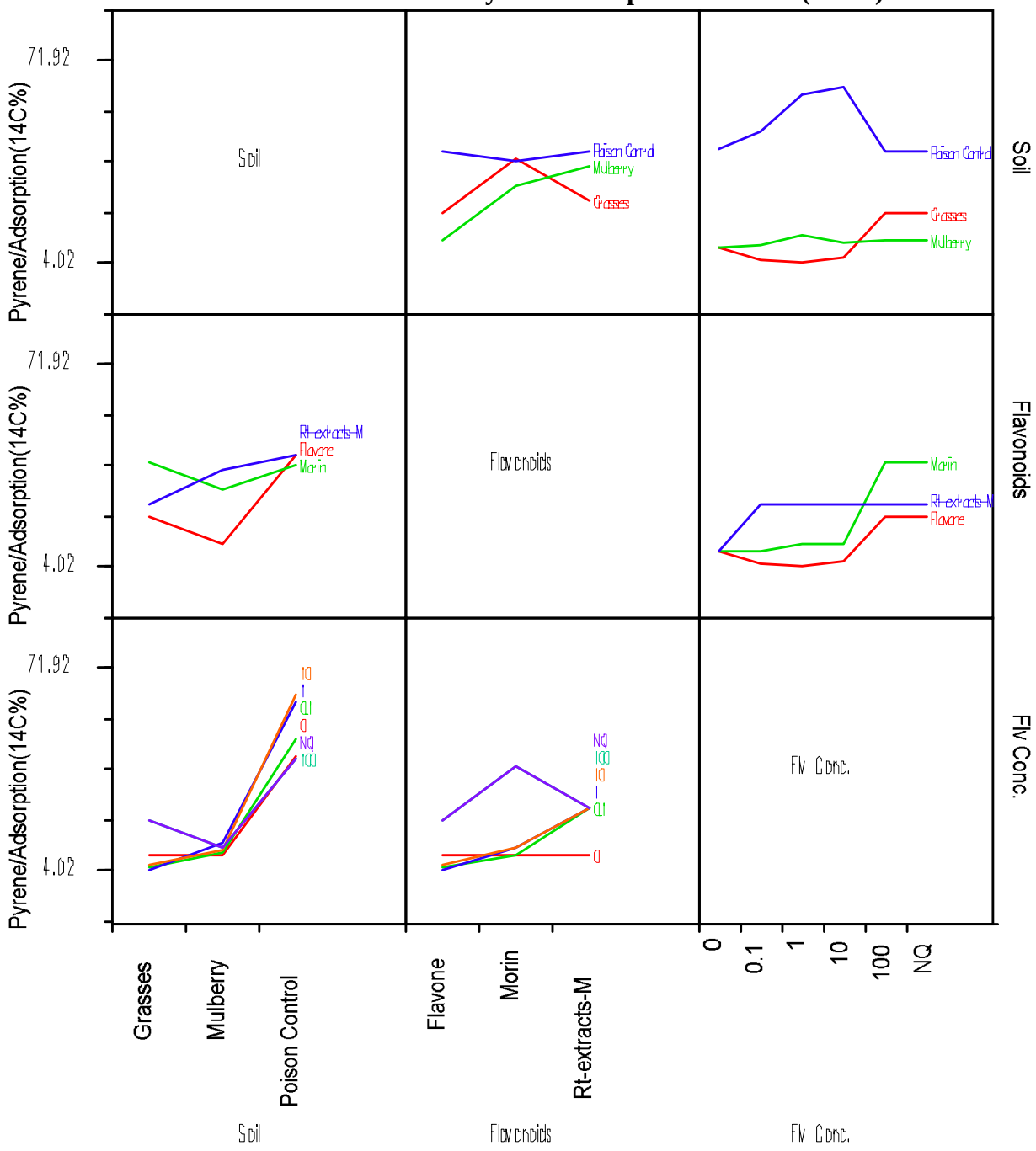
Interaction Profile 7. ^{14}C-Pyrene in H_2O(^{14}C%)



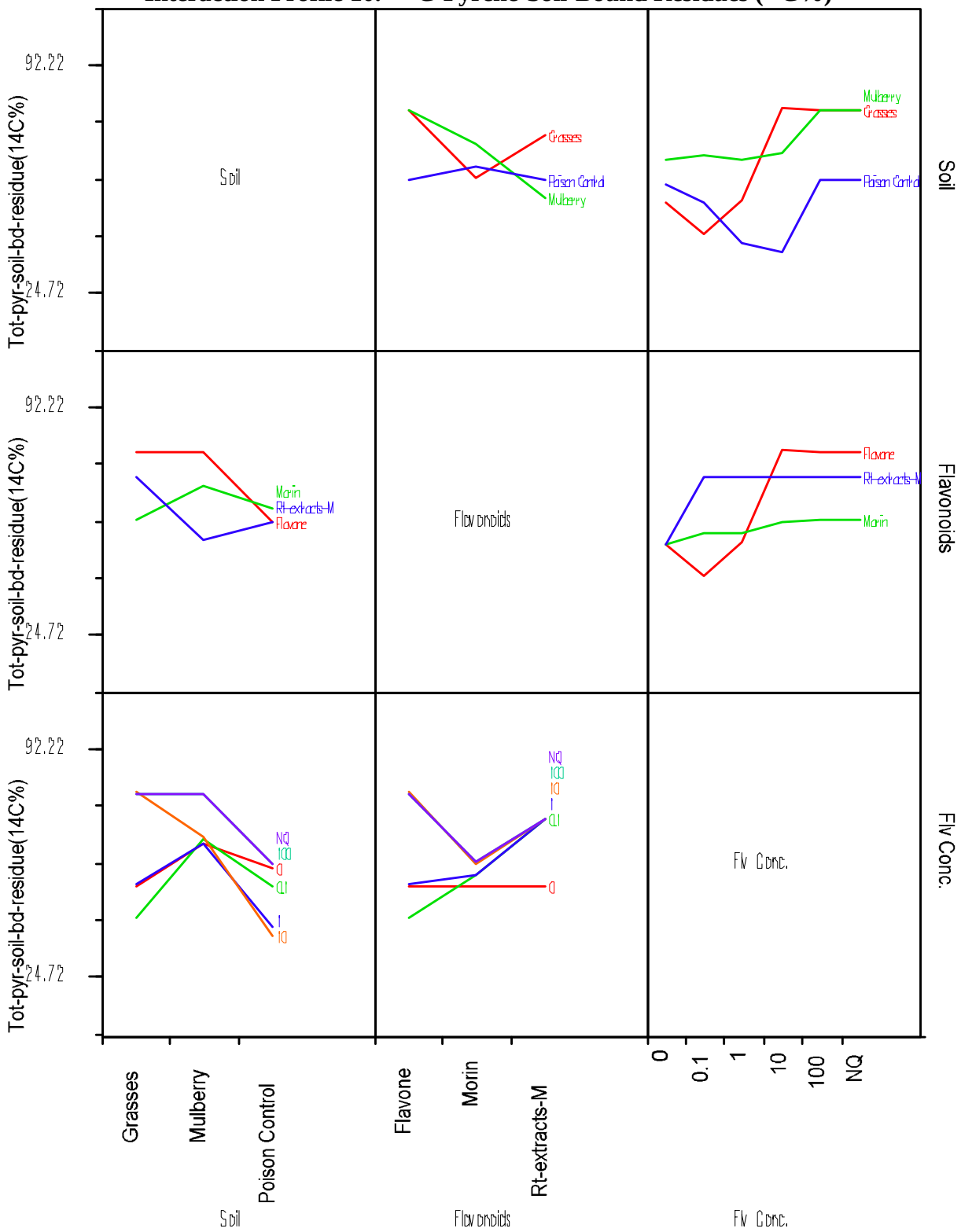
Interaction Profile 8. ^{14}C-Pyrene Metabolites in H_2O (^{14}C%)



Interaction Profile 9. ^{14}C-Pyrene Adsorption onto Soil (^{14}C%)



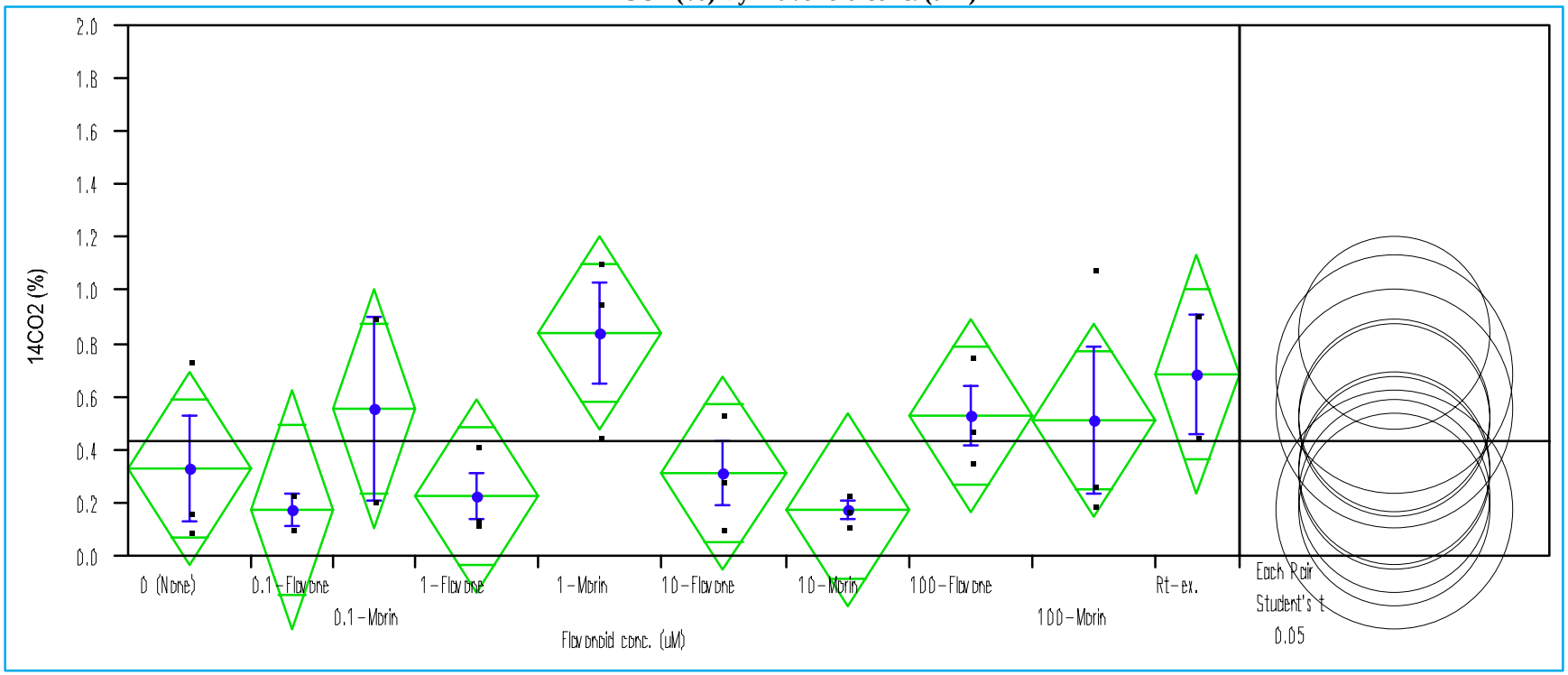
Interaction Profile 10. ^{14}C-Pyrene-Soil-Bound Residues (^{14}C%)



APPENDIX D. STATISTICAL ANALYSIS: ONE-WAY ANALYSIS OF VARIANCE (JMP OUTPUT REPORTS)

Appendix D-1. Student's t Test: Paired Comparison of Mean ^{14}C-B[a]P Fate Data in Poisoned Mulberry Rhizosphere Soil with or without Flavonoid Amendment [1]

14CO2 (%) By Flavonoid conc. (uM)[2]



[1] The means comparison indicates whether the actual difference in the means is greater than the least significant difference (LSD).

[2] The left side chart show data points, group data mean dots, standard error bars, and 95% confidence interval diamond.
The horizontal line cross the chart is the mean of all sample data. The righ side chart shows comparison circles. LSD is what the distance would be if the two mean circles intersected at right angles. Circles for means that are significantly different either do not intersect or intersect slightly so that the outside angle of intersection is <90°. If the circles intersect by an outside angle of >90° or if they are nested, the means are not significantly different.

Oneway Anova
Summary of Fit

RSquare	0.443054
RSquare Adj	0.1482
Root Mean Square Error	0.304043
Mean of Response	0.434444
Observations (or Sum Wgts)	27

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	1.2501500	0.138906	1.5026
Error	17	1.5715167	0.092442	Prob>F
C Total	26	2.8216667	0.108526	0.2244

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	0.336667	0.17554
0.1-Flavone	2	0.175000	0.21499
0.1-Morin	2	0.560000	0.21499
1-Flavone	3	0.230000	0.17554
1-Morin	3	0.846667	0.17554
10-Flavone	3	0.313333	0.17554
10-Morin	3	0.180000	0.17554
100-Flavone	3	0.533333	0.17554
100-Morin	3	0.520000	0.17554
Rt-extracts-M	2	0.690000	0.21499

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	0.336667	0.351046	0.20268
0.1-Flavone	2	0.175000	0.091924	0.06500
0.1-Morin	2	0.560000	0.494975	0.35000
1-Flavone	3	0.230000	0.164621	0.09504
1-Morin	3	0.846667	0.344287	0.19877
10-Flavone	3	0.313333	0.215948	0.12468
10-Morin	3	0.180000	0.060000	0.03464
100-Flavone	3	0.533333	0.205264	0.11851
100-Morin	3	0.520000	0.494874	0.28572
Rt-extracts-M	2	0.690000	0.325269	0.23000

Means Comparisons

Dif=Mean[i]-Mean[j]	1-Morin	Rt-extracts-M	0.1-Morin	100-Flavone	100-Morin	0 (None)	10-Flavone	1-Flavone	10-Morin	0.1-Flavone
1-Morin	0.000000	0.156667	0.286667	0.313333	0.326667	0.510000	0.533333	0.616667	0.666667	0.671667
Rt-extracts-M	-0.15667	0.000000	0.130000	0.156667	0.170000	0.353333	0.376667	0.460000	0.510000	0.515000
0.1-Morin	-0.28667	-0.13	0.000000	0.026667	0.040000	0.223333	0.246667	0.330000	0.380000	0.385000
100-Flavone	-0.31333	-0.15667	-0.02667	0.000000	0.013333	0.196667	0.220000	0.303333	0.353333	0.358333
100-Morin	-0.32667	-0.17	-0.04	-0.01333	0.000000	0.183333	0.206667	0.290000	0.340000	0.345000
0 (None)	-0.51	-0.35333	-0.22333	-0.19667	-0.18333	0.000000	0.023333	0.106667	0.156667	0.161667
10-Flavone	-0.53333	-0.37667	-0.24667	-0.22	-0.20667	-0.02333	0.000000	0.083333	0.133333	0.138333
1-Flavone	-0.61667	-0.46	-0.33	-0.30333	-0.29	-0.10667	-0.08333	0.000000	0.050000	0.055000
10-Morin	-0.66667	-0.51	-0.38	-0.35333	-0.34	-0.15667	-0.13333	-0.05	0.000000	0.005000
0.1-Flavone	-0.67167	-0.515	-0.385	-0.35833	-0.345	-0.16167	-0.13833	-0.055	-0.005	0.000000

Alpha= 0.05

Comparisons for each pair using Student's t[1]

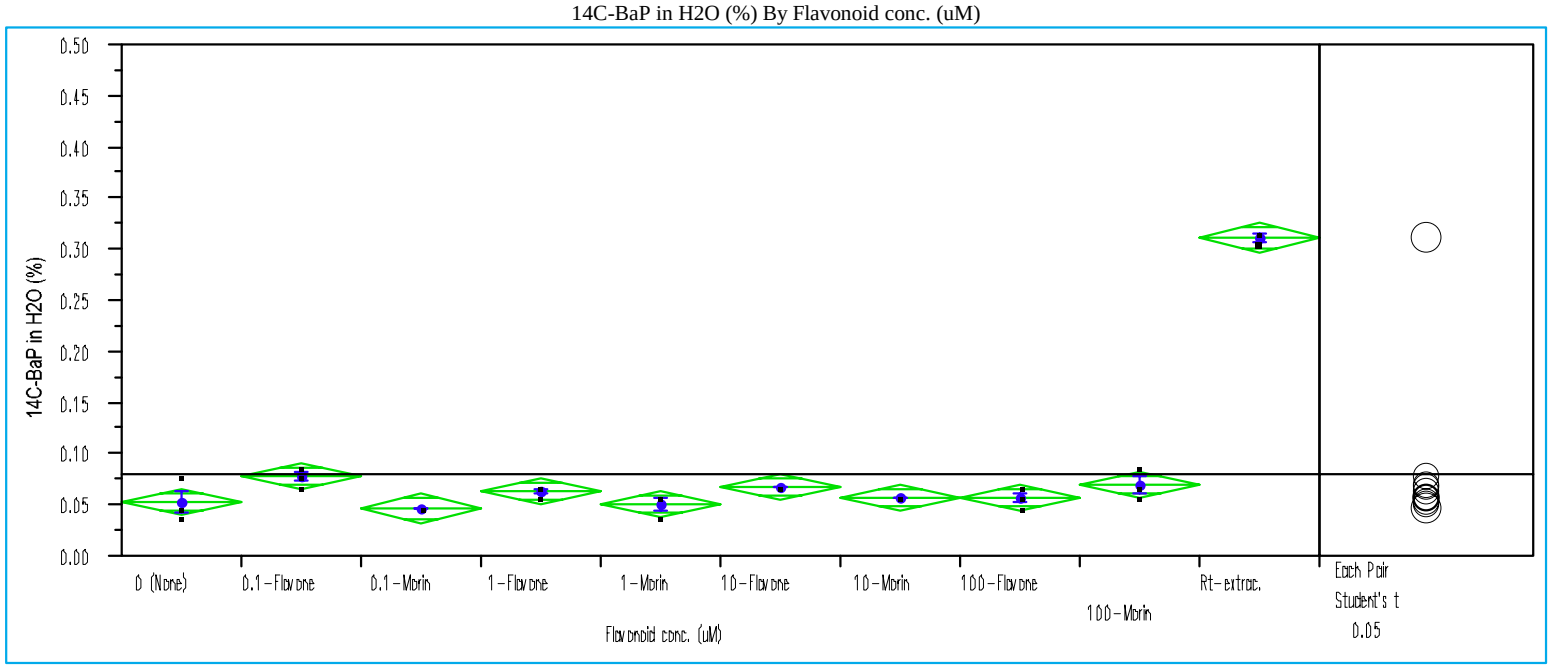
t = 2.10980)

Abs(Dif)-LSD	1-Morin	Rt-extracts-M	0.1-Morin	100-Flavone	100-Morin	0 (None)	10-Flavone	1-Flavone	10-Morin	0.1-Flavone
1-Morin	-0.52376	-0.42891	-0.29891	-0.21043	-0.19709	-0.01376	0.009575	0.092908	0.142908	0.086087
Rt-extracts-M	-0.42891	-0.64147	-0.51147	-0.42891	-0.41558	-0.23225	-0.20891	-0.12558	-0.07558	-0.12647
0.1-Morin	-0.29891	-0.51147	-0.64147	-0.55891	-0.54558	-0.36225	-0.33891	-0.25558	-0.20558	-0.25647
100-Flavone	-0.21043	-0.42891	-0.55891	-0.52376	-0.51043	-0.32709	-0.30376	-0.22043	-0.17043	-0.22725
100-Morin	-0.19709	-0.41558	-0.54558	-0.51043	-0.52376	-0.34043	-0.31709	-0.23376	-0.18376	-0.24058
0 (None)	-0.01376	-0.23225	-0.36225	-0.32709	-0.34043	-0.52376	-0.50043	-0.41709	-0.36709	-0.42391
10-Flavone	0.009575	-0.20891	-0.33891	-0.30376	-0.31709	-0.50043	-0.52376	-0.44043	-0.39043	-0.44725
1-Flavone	0.092908	-0.12558	-0.25558	-0.22043	-0.23376	-0.41709	-0.44043	-0.52376	-0.47376	-0.53058
10-Morin	0.142908	-0.07558	-0.20558	-0.17043	-0.18376	-0.36709	-0.39043	-0.47376	-0.52376	-0.58058
0.1-Flavone	0.086087	-0.12647	-0.25647	-0.22725	-0.24058	-0.42391	-0.44725	-0.53058	-0.58058	-0.64147

Positive values show pairs of means that are significantly different. [2]

[1] The LSDs for different sample sizes are shown on the diagonal.

[2] There were no differences in $^{14}CO_2$ (%) with and without flavonoids at 95% confidence level (see all the negative values in the column of None). However, $^{14}CO_2$ (%) is low with 1 uM of Moring compared with 0.1 - 10 uM of Flavone and 10 uM of Morin.



Oneway Anova

Summary of Fit

RSquare	0.982566
RSquare Adj	0.973849
Root Mean Square Error	0.010844
Mean of Response	0.081786
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	0.11929405	0.013255	112.7188
Error	18	0.00211667	0.000118	Prob>F
C Total	27	0.12141071	0.004497	<.0001

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	0.056667	0.00626
0.1-Flavone	3	0.080000	0.00626
0.1-Morin	2	0.050000	0.00767
1-Flavone	3	0.066667	0.00626
1-Morin	3	0.053333	0.00626
10-Flavone	3	0.070000	0.00626

10-Morin	3	0.060000	0.00626
100-Flavone	3	0.060000	0.00626
100-Morin	3	0.073333	0.00626
Rt-extracts-M	2	0.315000	0.00767

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	0.056667	0.020817	0.01202
0.1-Flavone	3	0.080000	0.010000	0.00577
0.1-Morin	2	0.050000	0.000000	0.00000
1-Flavone	3	0.066667	0.005774	0.00333
1-Morin	3	0.053333	0.011547	0.00667
10-Flavone	3	0.070000	0.000000	0.00000
10-Morin	3	0.060000	0.000000	0.00000
100-Flavone	3	0.060000	0.010000	0.00577
100-Morin	3	0.073333	0.015275	0.00882
Rt-extracts-M	2	0.315000	0.007071	0.00500

Means Comparisons

Dif=Mean[i]-Mean[j]	Rt-extracts-M	0.1-Flavone	100-Morin	10-Flavone	1-Flavone	100-Flavone	10-Morin	0 (None)	1-Morin	0.1-Morin
Rt-extracts-M	0.000000	0.235000	0.241667	0.245000	0.248333	0.255000	0.255000	0.258333	0.261667	0.265000
0.1-Flavone	-0.235	0.000000	0.006667	0.010000	0.013333	0.020000	0.020000	0.023333	0.026667	0.030000
100-Morin	-0.24167	-0.00667	0.000000	0.003333	0.006667	0.013333	0.013333	0.016667	0.020000	0.023333
10-Flavone	-0.245	-0.01	-0.00333	0.000000	0.003333	0.010000	0.010000	0.013333	0.016667	0.020000
1-Flavone	-0.24833	-0.01333	-0.00667	-0.00333	0.000000	0.006667	0.006667	0.010000	0.013333	0.016667
100-Flavone	-0.255	-0.02	-0.01333	-0.01	-0.00667	0.000000	0.000000	0.003333	0.006667	0.010000
10-Morin	-0.255	-0.02	-0.01333	-0.01	-0.00667	0.000000	0.000000	0.003333	0.006667	0.010000
0 (None)	-0.25833	-0.02333	-0.01667	-0.01333	-0.01	-0.00333	-0.00333	0.000000	0.003333	0.006667
1-Morin	-0.26167	-0.02667	-0.02	-0.01667	-0.01333	-0.00667	-0.00667	-0.00333	0.000000	0.003333
0.1-Morin	-0.265	-0.03	-0.02333	-0.02	-0.01667	-0.01	-0.01	-0.00667	-0.00333	0.000000

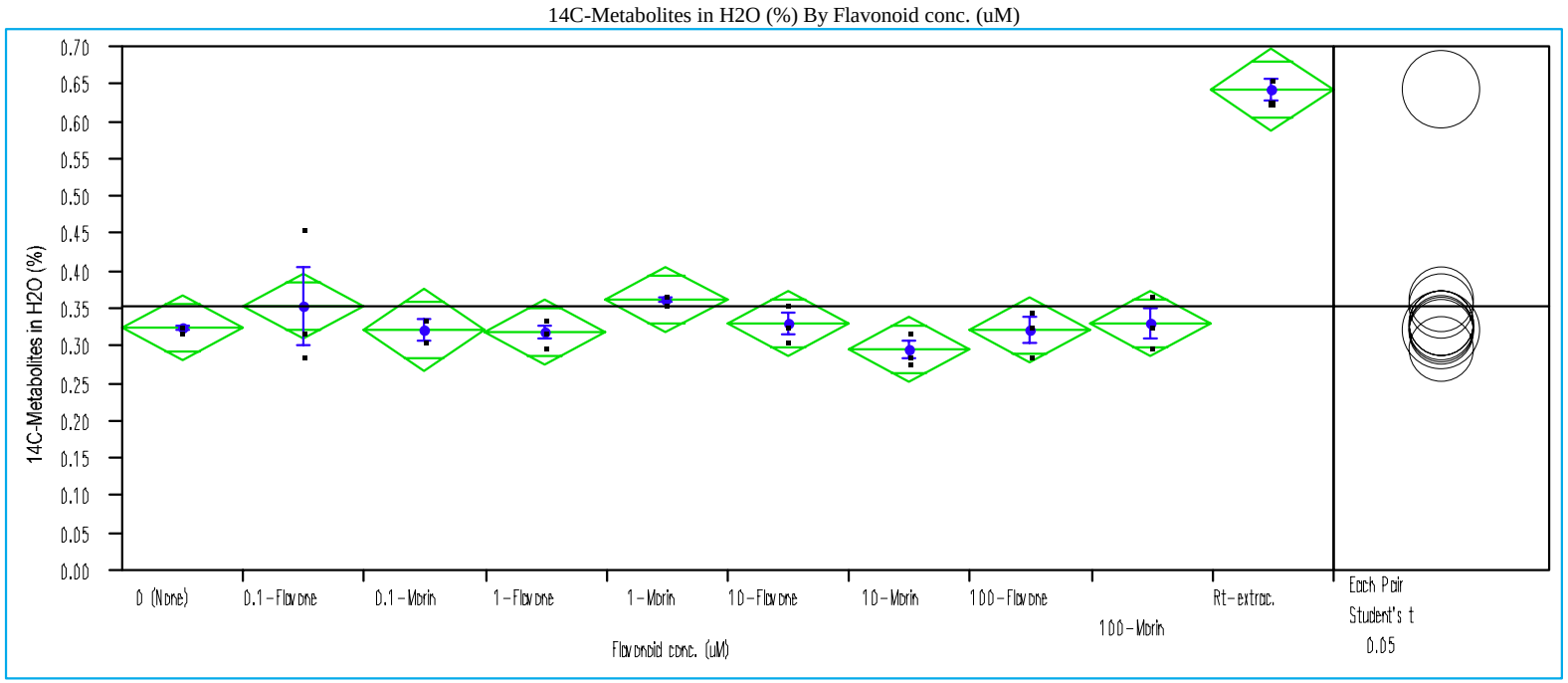
Alpha= 0.05

Comparisons for each pair using Student's t

t 2.10091

Abs(Dif)-LSD	Rt-extracts-M	0.1-Flavone	100-Morin	10-Flavone	1-Flavone	100-Flavone	10-Morin	0 (None)	1-Morin	0.1-Morin
Rt-extracts-M	-0.02278	0.214203	0.220869	0.224203	0.227536	0.234203	0.234203	**0.237536**	0.240869	0.242218
0.1-Flavone	0.214203	-0.0186	-0.01193	-0.0086	-0.00527	0.001398	0.001398	**0.004732**	0.008065	0.009203
100-Morin	0.220869	-0.01193	-0.0186	-0.01527	-0.01193	-0.00527	-0.00527	-0.00193	0.001398	0.002536
10-Flavone	0.224203	-0.0086	-0.01527	-0.0186	-0.01527	-0.0086	-0.0086	-0.00527	-0.00193	-0.0008
1-Flavone	0.227536	-0.00527	-0.01193	-0.01527	-0.0186	-0.01193	-0.01193	-0.0086	-0.00527	-0.00413
100-Flavone	0.234203	0.001398	-0.00527	-0.0086	-0.01193	-0.0186	-0.0186	-0.01527	-0.01193	-0.0108
10-Morin	0.234203	0.001398	-0.00527	-0.0086	-0.01193	-0.0186	-0.0186	-0.01527	-0.01193	-0.0108
0 (None)	0.237536	0.004732	-0.00193	-0.00527	-0.0086	-0.01527	-0.01527	-0.0186	-0.01527	-0.01413
1-Morin	0.240869	0.008065	0.001398	-0.00193	-0.00527	-0.01193	-0.01193	-0.01527	-0.0186	-0.01746
0.1-Morin	0.242218	0.009203	0.002536	-0.0008	-0.00413	-0.0108	-0.0108	-0.01413	-0.01746	-0.02278

Positive values show pairs of means that are significantly different.



Oneway Anova

Summary of Fit

RSquare	0.88589
RSquare Adj	0.828836
Root Mean Square Error	0.037093
Mean of Response	0.353571
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	0.19227619	0.021364	15.5270
Error	18	0.02476667	0.001376	Prob>F
C Total	27	0.21704286	0.008039	<.0001

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	0.326667	0.02142
0.1-Flavone	3	0.356667	0.02142
0.1-Morin	2	0.325000	0.02623
1-Flavone	3	0.320000	0.02142

1-Morin	3	0.363333	0.02142
10-Flavone	3	0.333333	0.02142
10-Morin	3	0.296667	0.02142
100-Flavone	3	0.323333	0.02142
100-Morin	3	0.333333	0.02142
Rt-extracts-M	2	0.645000	0.02623

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	0.326667	0.005774	0.00333
0.1-Flavone	3	0.356667	0.090738	0.05239
0.1-Morin	2	0.325000	0.021213	0.01500
1-Flavone	3	0.320000	0.020000	0.01155
1-Morin	3	0.363333	0.005774	0.00333
10-Flavone	3	0.333333	0.025166	0.01453
10-Morin	3	0.296667	0.020817	0.01202
100-Flavone	3	0.323333	0.030551	0.01764
100-Morin	3	0.333333	0.035119	0.02028
Rt-extracts-M	2	0.645000	0.021213	0.01500

Means Comparisons

Dif=Mean[i]-Mean[j]	Rt-extracts-M	1-Morin	0.1-Flavone	10-Flavone	100-Morin	0 (None)	0.1-Morin	100-Flavone	1-Flavone	10-Morin
Rt-extracts-M	0.000000	0.281667	0.288333	0.311667	0.311667	0.318333	0.320000	0.321667	0.325000	0.348333
1-Morin	-0.28167	0.000000	0.006667	0.030000	0.030000	0.036667	0.038333	0.040000	0.043333	0.066667
0.1-Flavone	-0.28833	-0.00667	0.000000	0.023333	0.023333	0.030000	0.031667	0.033333	0.036667	0.060000
10-Flavone	-0.31167	-0.03	-0.02333	0.000000	0.000000	0.006667	0.008333	0.010000	0.013333	0.036667
100-Morin	-0.31167	-0.03	-0.02333	-1.1e-16	0.000000	0.006667	0.008333	0.010000	0.013333	0.036667
0 (None)	-0.31833	-0.03667	-0.03	-0.00667	-0.00667	0.000000	0.001667	0.003333	0.006667	0.030000
0.1-Morin	-0.32	-0.03833	-0.03167	-0.00833	-0.00833	-0.00167	0.000000	0.001667	0.005000	0.028333
100-Flavone	-0.32167	-0.04	-0.03333	-0.01	-0.01	-0.00333	-0.00167	0.000000	0.003333	0.026667
1-Flavone	-0.325	-0.04333	-0.03667	-0.01333	-0.01333	-0.00667	-0.005	-0.00333	0.000000	0.023333
10-Morin	-0.34833	-0.06667	-0.06	-0.03667	-0.03667	-0.03	-0.02833	-0.02667	-0.02333	0.000000

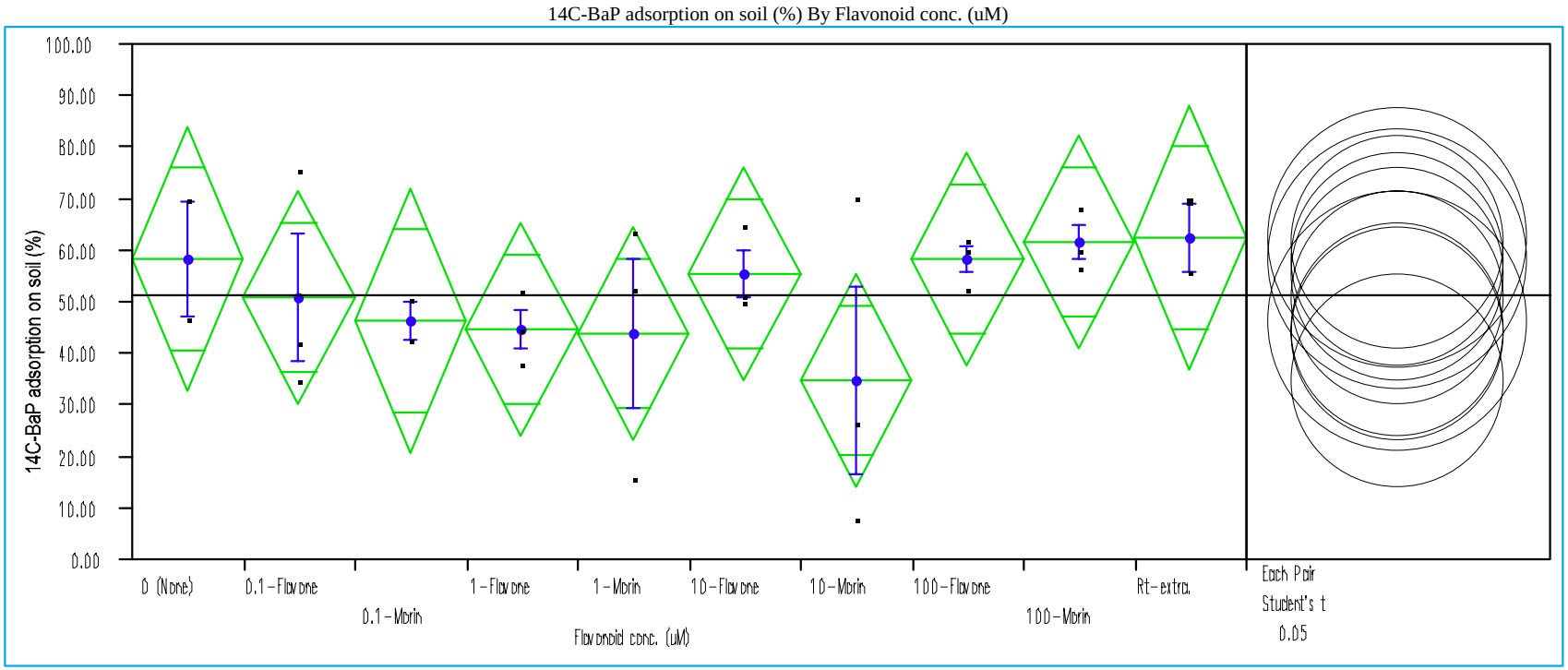
Alpha= 0.05

Comparisons for each pair using Student's t

t 2.10091

Abs(Dif)-LSD	Rt-extracts-M	1-Morin	0.1-Flavone	10-Flavone	100-Morin	0 (None)	0.1-Morin	100-Flavone	1-Flavone	10-Morin
Rt-extracts-M	-0.07793	0.210527	0.217193	0.240527	0.240527	**0.247193**	0.242070	0.250527	0.253860	0.277193
1-Morin	0.210527	-0.06363	-0.05696	-0.03363	-0.03363	-0.02696	-0.03281	-0.02363	-0.0203	0.003037
0.1-Flavone	0.217193	-0.05696	-0.06363	-0.0403	-0.0403	-0.03363	-0.03947	-0.0303	-0.02696	-0.00363
10-Flavone	0.240527	-0.03363	-0.0403	-0.06363	-0.06363	-0.05696	-0.06281	-0.05363	-0.0503	-0.02696
100-Morin	0.240527	-0.03363	-0.0403	-0.06363	-0.06363	-0.05696	-0.06281	-0.05363	-0.0503	-0.02696
0 (None)	0.247193	-0.02696	-0.03363	-0.05696	-0.05696	-0.06363	-0.06947	-0.0603	-0.05696	-0.03363
0.1-Morin	0.242070	-0.03281	-0.03947	-0.06281	-0.06281	-0.06947	-0.07793	-0.06947	-0.06614	-0.04281
100-Flavone	0.250527	-0.02363	-0.0303	-0.05363	-0.05363	-0.0603	-0.06947	-0.06363	-0.0603	-0.03696
1-Flavone	0.253860	-0.0203	-0.02696	-0.0503	-0.0503	-0.05696	-0.06614	-0.0603	-0.06363	-0.0403
10-Morin	0.277193	0.003037	-0.00363	-0.02696	-0.02696	-0.03363	-0.04281	-0.03696	-0.0403	-0.06363

Positive values show pairs of means that are significantly different.



Oneway Anova

Summary of Fit

RSquare	0.284869
RSquare Adj	-0.09373
Root Mean Square Error	17.22823
Mean of Response	51.58667
Observations (or Sum Wgts)	27

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	2009.9680	223.330	0.7524
Error	17	5045.8010	296.812	Prob>F
C Total	26	7055.7690	271.376	0.6592

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	2	58.6650	12.182
0.1-Flavone	3	51.2100	9.947
0.1-Morin	2	46.7400	12.182

1-Flavone	3	45.1433	9.947
1-Morin	3	44.3267	9.947
10-Flavone	3	55.6467	9.947
10-Morin	3	35.2000	9.947
100-Flavone	3	58.6533	9.947
100-Morin	3	61.9000	9.947
Rt-extracts-M	2	62.8950	12.182

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	2	58.6650	16.3412	11.555
0.1-Flavone	3	51.2100	21.9040	12.646
0.1-Morin	2	46.7400	5.4447	3.850
1-Flavone	3	45.1433	6.9906	4.036
1-Morin	3	44.3267	25.2267	14.565
10-Flavone	3	55.6467	8.2202	4.746
10-Morin	3	35.2000	32.1381	18.555
100-Flavone	3	58.6533	5.0020	2.888
100-Morin	3	61.9000	6.0892	3.516
Rt-extracts-M	2	62.8950	9.6944	6.855

Means Comparisons

Dif=Mean[i]-Mean[j]	Rt-extracts-M	100-Morin	0 (None)	100-Flavone	10-Flavone	0.1-Flavone	0.1-Morin	1-Flavone	1-Morin	10-Morin
Rt-extracts-M	0.0000	0.9950	4.2300	4.2417	7.2483	11.6850	16.1550	17.7517	18.5683	27.6950
100-Morin	-0.9950	0.0000	3.2350	3.2467	6.2533	10.6900	15.1600	16.7567	17.5733	26.7000
0 (None)	-4.2300	-3.2350	0.0000	0.0117	3.0183	7.4550	11.9250	13.5217	14.3383	23.4650
100-Flavone	-4.2417	-3.2467	-0.0117	0.0000	3.0067	7.4433	11.9133	13.5100	14.3267	23.4533
10-Flavone	-7.2483	-6.2533	-3.0183	-3.0067	0.0000	4.4367	8.9067	10.5033	11.3200	20.4467
0.1-Flavone	-11.6850	-10.6900	-7.4550	-7.4433	-4.4367	0.0000	4.4700	6.0667	6.8833	16.0100
0.1-Morin	-16.1550	-15.1600	-11.9250	-11.9133	-8.9067	-4.4700	0.0000	1.5967	2.4133	11.5400
1-Flavone	-17.7517	-16.7567	-13.5217	-13.5100	-10.5033	-6.0667	-1.5967	0.0000	0.8167	9.9433
1-Morin	-18.5683	-17.5733	-14.3383	-14.3267	-11.3200	-6.8833	-2.4133	-0.8167	0.0000	9.1267
10-Morin	-27.6950	-26.7000	-23.4650	-23.4533	-20.4467	-16.0100	-11.5400	-9.9433	-9.1267	0.0000

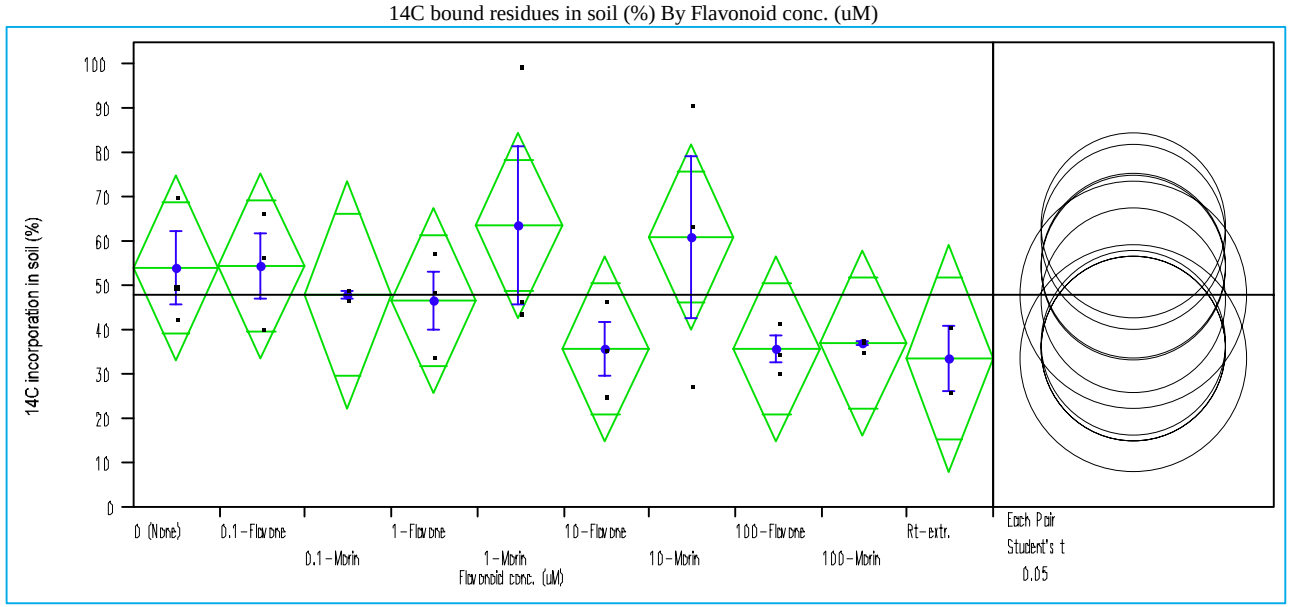
Alpha= 0.05

Comparisons for each pair using Student's t

t 2.10980

Abs(Dif)-LSD	Rt-extracts-M	100-Morin	0 (None)	100-Flavone	10-Flavone	0.1-Flavone	0.1-Morin	1-Flavone	1-Morin	10-Morin
Rt-extracts-M	-36.3482	-32.1862	-32.1182	-28.9395	-25.9329	-21.4962	-20.1932	-15.4295	-14.6129	-5.4862
100-Morin	-32.1862	-29.6782	-29.9462	-26.4315	-23.4248	-18.9882	-18.0212	-12.9215	-12.1048	-2.9782
0 (None)	-32.1182	-29.9462	-36.3482	-33.1695	-30.1629	-25.7262	-24.4232	-19.6595	-18.8429	-9.7162
100-Flavone	-28.9395	-26.4315	-33.1695	-29.6782	-26.6715	-22.2348	-21.2679	-16.1682	-15.3515	-6.2248
10-Flavone	-25.9329	-23.4248	-30.1629	-26.6715	-29.6782	-25.2415	-24.2745	-19.1748	-18.3582	-9.2315
0.1-Flavone	-21.4962	-18.9882	-25.7262	-22.2348	-25.2415	-29.6782	-28.7112	-23.6115	-22.7948	-13.6682
0.1-Morin	-20.1932	-18.0212	-24.4232	-21.2679	-24.2745	-28.7112	-36.3482	-31.5845	-30.7679	-21.6412
1-Flavone	-15.4295	-12.9215	-19.6595	-16.1682	-19.1748	-23.6115	-31.5845	-29.6782	-28.8615	-19.7348
1-Morin	-14.6129	-12.1048	-18.8429	-15.3515	-18.3582	-22.7948	-30.7679	-28.8615	-29.6782	-20.5515
10-Morin	-5.4862	-2.9782	-9.7162	-6.2248	-9.2315	-13.6682	-21.6412	-19.7348	-20.5515	-29.6782

Positive values show pairs of means that are significantly different.



Oneway Anova

Summary of Fit

RSquare	0.364699
RSquare Adj	0.047048
Root Mean Square Error	17.47325
Mean of Response	48.12464
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	3154.8158	350.535	1.1481
Error	18	5495.6575	305.314	Prob>F
C Total	27	8650.4733	320.388	0.3816

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	54.9333	10.088
0.1-Flavone	3	55.3567	10.088
0.1-Morin	2	48.7900	12.355
1-Flavone	3	47.3533	10.088
1-Morin	3	64.1933	10.088
10-Flavone	3	36.5233	10.088
10-Morin	3	61.5400	10.088

100-Flavone	3	36.4833	10.088
100-Morin	3	37.5467	10.088
Rt-extracts-M	2	34.0600	12.355

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	54.9333	14.3388	8.279
0.1-Flavone	3	55.3567	13.0836	7.554
0.1-Morin	2	48.7900	1.5981	1.130
1-Flavone	3	47.3533	11.9923	6.924
1-Morin	3	64.1933	31.6193	18.255
10-Flavone	3	36.5233	10.5987	6.119
10-Morin	3	61.5400	32.0233	18.489
100-Flavone	3	36.4833	5.5725	3.217
100-Morin	3	37.5467	1.4478	0.836
Rt-extracts-M	2	34.0600	10.5076	7.430

Means Comparisons

Dif=Mean[i]-Mean[j]	1-Morin	10-Morin	0.1-Flavone	0 (None)	0.1-Morin	1-Flavone	100-Morin	10-Flavone	100-Flavone	Rt-extracts-M
1-Morin	0.0000	2.6533	8.8367	9.2600	15.4033	16.8400	26.6467	27.6700	27.7100	30.1333
10-Morin	-2.6533	0.0000	6.1833	6.6067	12.7500	14.1867	23.9933	25.0167	25.0567	27.4800
0.1-Flavone	-8.8367	-6.1833	0.0000	0.4233	6.5667	8.0033	17.8100	18.8333	18.8733	21.2967
0 (None)	-9.2600	-6.6067	-0.4233	0.0000	6.1433	7.5800	17.3867	18.4100	18.4500	20.8733
0.1-Morin	-15.4033	-12.7500	-6.5667	-6.1433	0.0000	1.4367	11.2433	12.2667	12.3067	14.7300
1-Flavone	-16.8400	-14.1867	-8.0033	-7.5800	-1.4367	0.0000	9.8067	10.8300	10.8700	13.2933
100-Morin	-26.6467	-23.9933	-17.8100	-17.3867	-11.2433	-9.8067	0.0000	1.0233	1.0633	3.4867
10-Flavone	-27.6700	-25.0167	-18.8333	-18.4100	-12.2667	-10.8300	-1.0233	0.0000	0.0400	2.4633
100-Flavone	-27.7100	-25.0567	-18.8733	-18.4500	-12.3067	-10.8700	-1.0633	-0.0400	0.0000	2.4233
Rt-extracts-M	-30.1333	-27.4800	-21.2967	-20.8733	-14.7300	-13.2933	-3.4867	-2.4633	-2.4233	0.0000

Alpha= 0.05

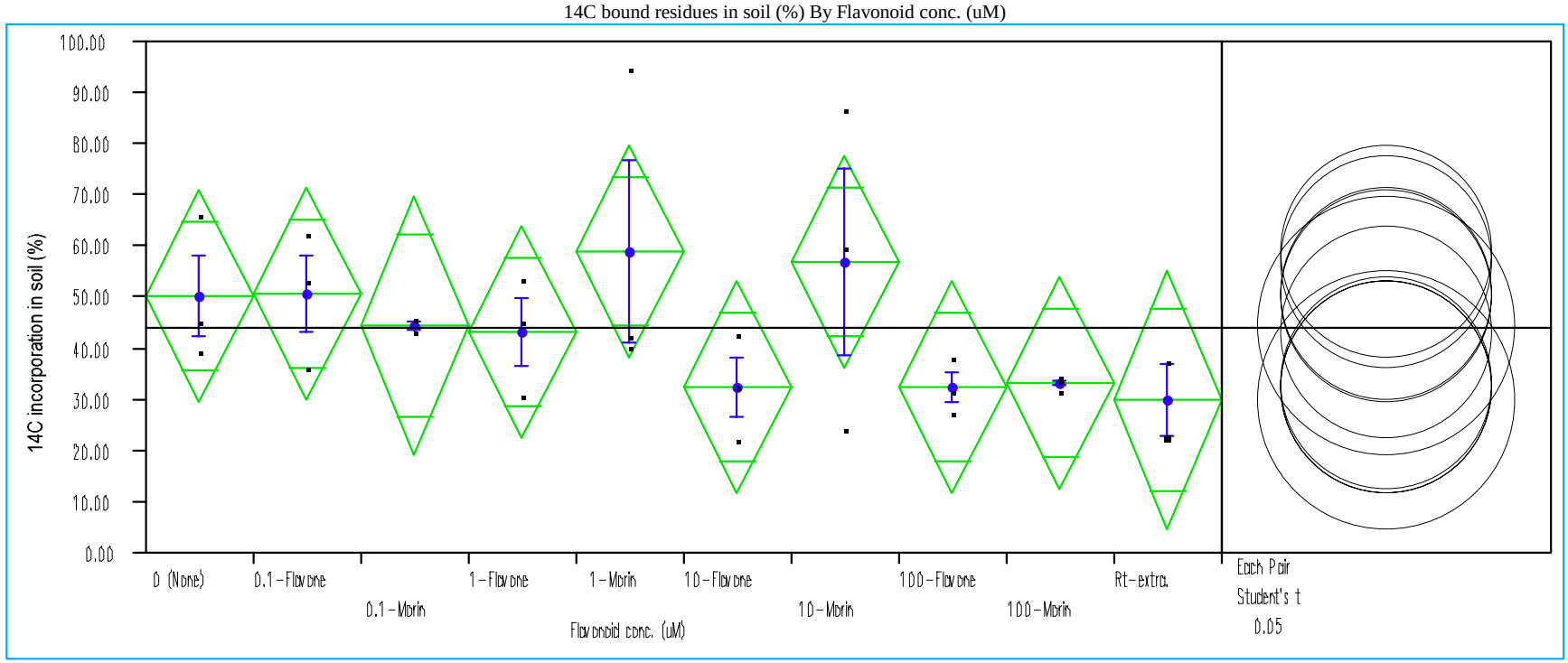
Comparisons for each pair using Student's t

t

2.10091

Abs(Dif)-LSD	1-Morin	10-Morin	0.1-Flavone	0 (None)	0.1-Morin	1-Flavone	100-Morin	10-Flavone	100-Flavone	Rt-extracts-M
1-Morin	-29.9733	-27.3200	-21.1367	-20.7133	-18.1079	-13.1333	-3.3267	-2.3033	-2.2633	-3.3779
10-Morin	-27.3200	-29.9733	-23.7900	-23.3667	-20.7612	-15.7867	-5.9800	-4.9567	-4.9167	-6.0312
0.1-Flavone	-21.1367	-23.7900	-29.9733	-29.5500	-26.9445	-21.9700	-12.1633	-11.1400	-11.1000	-12.2145
0 (None)	-20.7133	-23.3667	-29.5500	-29.9733	-27.3679	-22.3933	-12.5867	-11.5633	-11.5233	-12.6379
0.1-Morin	-18.1079	-20.7612	-26.9445	-27.3679	-36.7097	-32.0745	-22.2679	-21.2445	-21.2045	-21.9797
1-Flavone	-13.1333	-15.7867	-21.9700	-22.3933	-32.0745	-29.9733	-20.1667	-19.1433	-19.1033	-20.2179
100-Morin	-3.3267	-5.9800	-12.1633	-12.5867	-22.2679	-20.1667	-29.9733	-28.9500	-28.9100	-30.0245
10-Flavone	-2.3033	-4.9567	-11.1400	-11.5633	-21.2445	-19.1433	-28.9500	-29.9733	-29.9333	-31.0479
100-Flavone	-2.2633	-4.9167	-11.1000	-11.5233	-21.2045	-19.1033	-28.9100	-29.9333	-29.9733	-31.0879
Rt-extracts-M	-3.3779	-6.0312	-12.2145	-12.6379	-21.9797	-20.2179	-30.0245	-31.0479	-31.0879	-36.7097

Positive values show pairs of means that are significantly different.



Oneway Anova

Summary of Fit

RSquare	0.361443
RSquare Adj	0.042165
Root Mean Square Error	17.2191
Mean of Response	44.02821
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	3020.8842	335.654	1.1321
Error	18	5336.9546	296.497	Prob>F
C Total	27	8357.8388	309.550	0.3909

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	50.7300	9.941
0.1-Flavone	3	50.8867	9.941
0.1-Morin	2	44.7950	12.176

1-Flavone	3	43.4833	9.941
1-Morin	3	59.5633	9.941
10-Flavone	3	32.7800	9.941
10-Morin	3	57.3667	9.941
100-Flavone	3	32.6267	9.941
100-Morin	3	33.5200	9.941
Rt-extracts-M	2	30.1650	12.176

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	50.7300	14.0603	8.118
0.1-Flavone	3	50.8867	13.1557	7.595
0.1-Morin	2	44.7950	1.7183	1.215
1-Flavone	3	43.4833	11.6790	6.743
1-Morin	3	59.5633	31.0311	17.916
10-Flavone	3	32.7800	10.3454	5.973
10-Morin	3	57.3667	31.6862	18.294
100-Flavone	3	32.6267	5.4907	3.170
100-Morin	3	33.5200	1.3846	0.799
Rt-extracts-M	2	30.1650	10.3733	7.335

Means Comparisons

Dif=Mean[i]-Mean[j]	1-Morin	10-Morin	0.1-Flavone	0 (None)	0.1-Morin	1-Flavone	100-Morin	10-Flavone	100-Flavone	Rt-extracts-M
1-Morin	0.0000	2.1967	8.6767	8.8333	14.7683	16.0800	26.0433	26.7833	26.9367	29.3983
10-Morin	-2.1967	0.0000	6.4800	6.6367	12.5717	13.8833	23.8467	24.5867	24.7400	27.2017
0.1-Flavone	-8.6767	-6.4800	0.0000	0.1567	6.0917	7.4033	17.3667	18.1067	18.2600	20.7217
0 (None)	-8.8333	-6.6367	-0.1567	0.0000	5.9350	7.2467	17.2100	17.9500	18.1033	20.5650
0.1-Morin	-14.7683	-12.5717	-6.0917	-5.9350	0.0000	1.3117	11.2750	12.0150	12.1683	14.6300
1-Flavone	-16.0800	-13.8833	-7.4033	-7.2467	-1.3117	0.0000	9.9633	10.7033	10.8567	13.3183
100-Morin	-26.0433	-23.8467	-17.3667	-17.2100	-11.2750	-9.9633	0.0000	0.7400	0.8933	3.3550
10-Flavone	-26.7833	-24.5867	-18.1067	-17.9500	-12.0150	-10.7033	-0.7400	0.0000	0.1533	2.6150
100-Flavone	-26.9367	-24.7400	-18.2600	-18.1033	-12.1683	-10.8567	-0.8933	-0.1533	0.0000	2.4617
Rt-extracts-M	-29.3983	-27.2017	-20.7217	-20.5650	-14.6300	-13.3183	-3.3550	-2.6150	-2.4617	0.0000

Alpha= 0.05

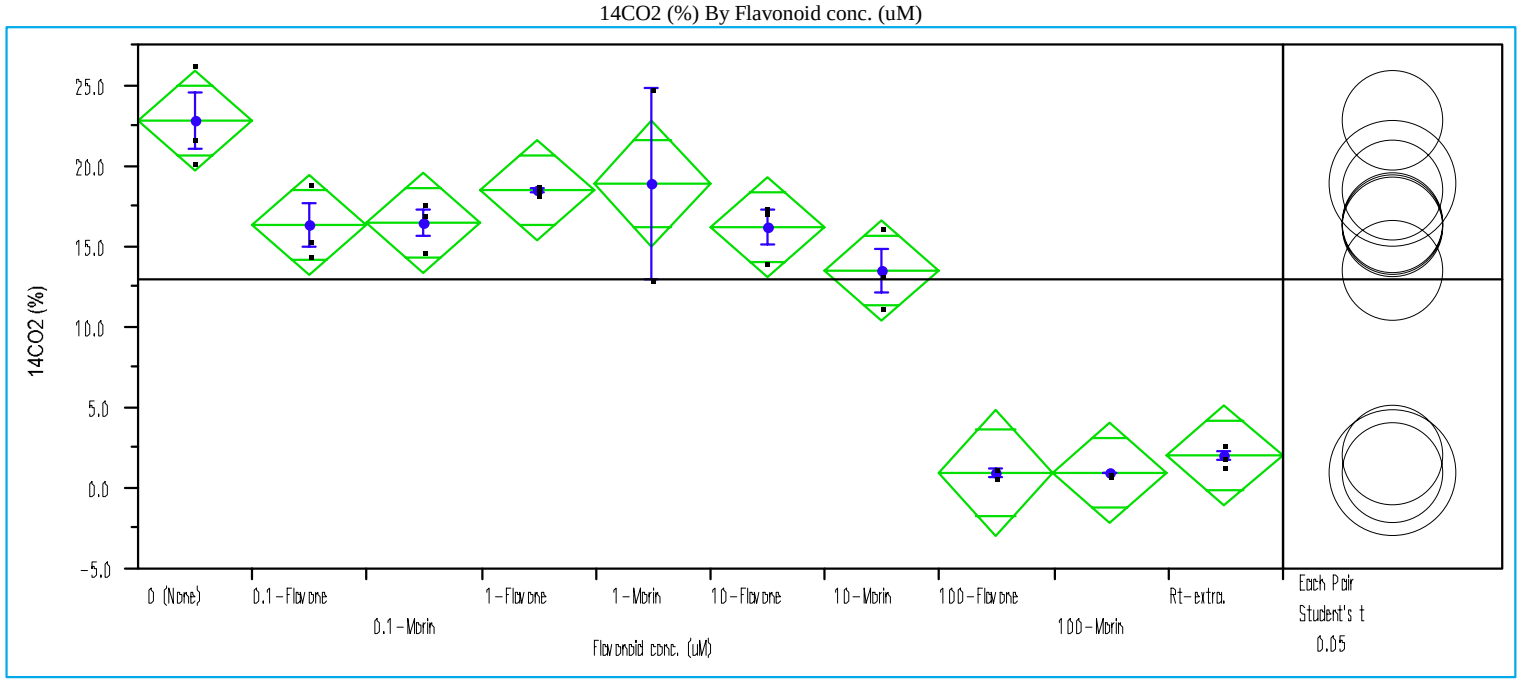
Comparisons for each pair using Student's t

t 2.10091

Abs(Dif)-LSD	1-Morin	10-Morin	0.1-Flavone	0 (None)	0.1-Morin	1-Flavone	100-Morin	10-Flavone	100-Flavone	Rt-extracts-M
1-Morin	-29.5374	-27.3407	-20.8607	-20.7040	-18.2555	-13.4574	-3.4940	-2.7540	-2.6007	-3.6255
10-Morin	-27.3407	-29.5374	-23.0574	-22.9007	-20.4521	-15.6540	-5.6907	-4.9507	-4.7974	-5.8221
0.1-Flavone	-20.8607	-23.0574	-29.5374	-29.3807	-26.9321	-22.1340	-12.1707	-11.4307	-11.2774	-12.3021
0 (None)	-20.7040	-22.9007	-29.3807	-29.5374	-27.0888	-22.2907	-12.3274	-11.5874	-11.4340	-12.4588
0.1-Morin	-18.2555	-20.4521	-26.9321	-27.0888	-36.1758	-31.7121	-21.7488	-21.0088	-20.8555	-21.5458
1-Flavone	-13.4574	-15.6540	-22.1340	-22.2907	-31.7121	-29.5374	-19.5740	-18.8340	-18.6807	-19.7055
100-Morin	-3.4940	-5.6907	-12.1707	-12.3274	-21.7488	-19.5740	-29.5374	-28.7974	-28.6440	-29.6688
10-Flavone	-2.7540	-4.9507	-11.4307	-11.5874	-21.0088	-18.8340	-28.7974	-29.5374	-29.3840	-30.4088
100-Flavone	-2.6007	-4.7974	-11.2774	-11.4340	-20.8555	-18.6807	-28.6440	-29.3840	-29.5374	-30.5621
Rt-extracts-M	-3.6255	-5.8221	-12.3021	-12.4588	-21.5458	-19.7055	-29.6688	-30.4088	-30.5621	-36.1758

Positive values show pairs of means that are significantly different.

Appendix D-2. Student's t Test: Paired Comparison of ^{14}C-B[a]P Fate Data in Mulberry Rhizosphere Soil with or without Flavonoid Amendment



Oneway Anova Summary of Fit

RSquare	0.928238
RSquare Adj	0.892358
Root Mean Square Error	2.664292
Mean of Response	12.96
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	1652.7371	183.637	25.8701
Error	18	127.7721	7.098	Prob>F
C Total	27	1780.5092	65.945	<.0001

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	22.8967	1.5382
0.1-Flavone	3	16.4033	1.5382
0.1-Morin	3	16.6267	1.5382

1-Flavone	3	18.6367	1.5382
1-Morin	2	19.0750	1.8839
10-Flavone	3	16.3233	1.5382
10-Morin	3	13.6600	1.5382
100-Flavone	2	1.0300	1.8839
100-Morin	3	0.9567	1.5382
Rt-extracts-M	3	2.0533	1.5382

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	22.8967	3.16993	1.8302
0.1-Flavone	3	16.4033	2.37176	1.3693
0.1-Morin	3	16.6267	1.57055	0.9068
1-Flavone	3	18.6367	0.31342	0.1810
1-Morin	2	19.0750	8.39336	5.9350
10-Flavone	3	16.3233	1.92347	1.1105
10-Morin	3	13.6600	2.49598	1.4411
100-Flavone	2	1.0300	0.38184	0.2700
100-Morin	3	0.9567	0.10263	0.0593
Rt-extracts-M	3	2.0533	0.64049	0.3698

Means Comparisons

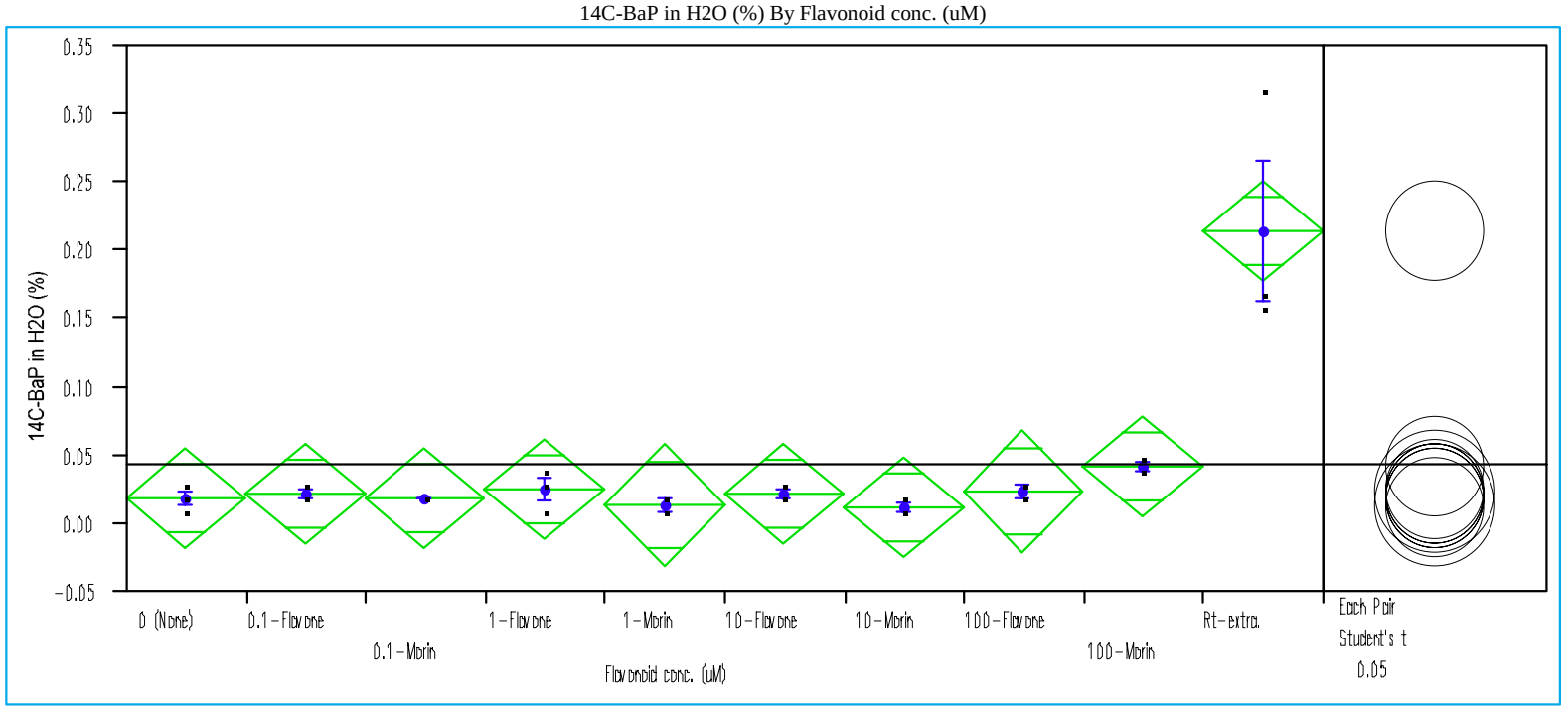
Dif=Mean[i]-Mean[j]	0 (None)	1-Morin	1-Flavone	0.1-Morin	0.1-Flavone	10-Flavone	10-Morin	Rt-extracts-M	100-Flavone	100-Morin
0 (None)	0.0000	3.8217	4.2600	6.2700	6.4933	6.5733	9.2367	20.8433	21.8667	21.9400
1-Morin	-3.8217	0.0000	0.4383	2.4483	2.6717	2.7517	5.4150	17.0217	18.0450	18.1183
1-Flavone	-4.2600	-0.4383	0.0000	2.0100	2.2333	2.3133	4.9767	16.5833	17.6067	17.6800
0.1-Morin	-6.2700	-2.4483	-2.0100	0.0000	0.2233	0.3033	2.9667	14.5733	15.5967	15.6700
0.1-Flavone	-6.4933	-2.6717	-2.2333	-0.2233	0.0000	0.0800	2.7433	14.3500	15.3733	15.4467
10-Flavone	-6.5733	-2.7517	-2.3133	-0.3033	-0.0800	0.0000	2.6633	14.2700	15.2933	15.3667
10-Morin	-9.2367	-5.4150	-4.9767	-2.9667	-2.7433	-2.6633	0.0000	11.6067	12.6300	12.7033
Rt-extracts-M	-20.8433	-17.0217	-16.5833	-14.5733	-14.3500	-14.2700	-11.6067	0.0000	1.0233	1.0967
100-Flavone	-21.8667	-18.0450	-17.6067	-15.5967	-15.3733	-15.2933	-12.6300	-1.0233	0.0000	0.0733
100-Morin	-21.9400	-18.1183	-17.6800	-15.6700	-15.4467	-15.3667	-12.7033	-1.0967	-0.0733	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t t 2.10091

Abs(Dif)-LSD	0 (None)	1-Morin	1-Flavone	0.1-Morin	0.1-Flavone	10-Flavone	10-Morin	Rt-extracts-M	100-Flavone	100-Morin
0 (None)	-4.5703	-1.2881	-0.3103	1.6997	1.9230	2.0030	4.6664	16.2730	16.7569	17.3697
1-Morin	-1.2881	-5.5974	-4.6714	-2.6614	-2.4381	-2.3581	0.3053	11.9119	12.4476	13.0086
1-Flavone	-0.3103	-4.6714	-4.5703	-2.5603	-2.3370	-2.2570	0.4064	12.0130	12.4969	13.1097
0.1-Morin	**1.6997**	-2.6614	-2.5603	-4.5703	-4.3470	-4.2670	-1.6036	10.0030	10.4869	11.0997
0.1-Flavone	**1.9230**	-2.4381	-2.3370	-4.3470	-4.5703	-4.4903	-1.8270	9.7797	10.2636	10.8764
10-Flavone	**2.0030**	-2.3581	-2.2570	-4.2670	-4.4903	-4.5703	-1.9070	9.6997	10.1836	10.7964
10-Morin	**4.6664**	0.3053	0.4064	-1.6036	-1.8270	-1.9070	-4.5703	7.0364	7.5203	8.1330
Rt-extracts-M	**16.2730**	11.9119	12.0130	10.0030	9.7797	9.6997	7.0364	-4.5703	-4.0864	-3.4736
100-Flavone	**16.7569**	12.4476	12.4969	10.4869	10.2636	10.1836	7.5203	-4.0864	-5.5974	-5.0364
100-Morin	**17.3697**	13.0086	13.1097	11.0997	10.8764	10.7964	8.1330	-3.4736	-5.0364	-4.5703

Positive values show pairs of means that are significantly different.



Oneway Anova

Summary of Fit

RSquare	0.855922
RSquare Adj	0.783883
Root Mean Square Error	0.030822
Mean of Response	0.044286
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	0.10158571	0.011287	11.8814
Error	18	0.01710000	0.000950	Prob>F
C Total	27	0.11868571	0.004396	<.0001

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	0.020000	0.01780
0.1-Flavone	3	0.023333	0.01780
0.1-Morin	3	0.020000	0.01780

1-Flavone	3	0.026667	0.01780
1-Morin	2	0.015000	0.02179
10-Flavone	3	0.023333	0.01780
10-Morin	3	0.013333	0.01780
100-Flavone	2	0.025000	0.02179
100-Morin	3	0.043333	0.01780
Rt-extracts-M	3	0.216667	0.01780

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	0.020000	0.010000	0.00577
0.1-Flavone	3	0.023333	0.005774	0.00333
0.1-Morin	3	0.020000	0.000000	0.00000
1-Flavone	3	0.026667	0.015275	0.00882
1-Morin	2	0.015000	0.007071	0.00500
10-Flavone	3	0.023333	0.005774	0.00333
10-Morin	3	0.013333	0.005774	0.00333
100-Flavone	2	0.025000	0.007071	0.00500
100-Morin	3	0.043333	0.005774	0.00333
Rt-extracts-M	3	0.216667	0.089629	0.05175

Means Comparisons

Dif=Mean[i]-Mean[j]	Rt-extracts-M	100-Morin	1-Flavone	100-Flavone	0.1-Flavone	10-Flavone	0 (None)	0.1-Morin	1-Morin	10-Morin
Rt-extracts-M	0.000000	0.173333	0.190000	0.191667	0.193333	0.193333	0.196667	0.196667	0.201667	0.203333
100-Morin	-0.17333	0.000000	0.016667	0.018333	0.020000	0.020000	0.023333	0.023333	0.028333	0.030000
1-Flavone	-0.19	-0.01667	0.000000	0.001667	0.003333	0.003333	0.006667	0.006667	0.011667	0.013333
100-Flavone	-0.19167	-0.01833	-0.00167	0.000000	0.001667	0.001667	0.005000	0.005000	0.010000	0.011667
0.1-Flavone	-0.19333	-0.02	-0.00333	-0.00167	0.000000	0.000000	0.003333	0.003333	0.008333	0.010000
10-Flavone	-0.19333	-0.02	-0.00333	-0.00167	0.000000	0.000000	0.003333	0.003333	0.008333	0.010000
0 (None)	-0.19667	-0.02333	-0.00667	-0.005	-0.00333	-0.00333	0.000000	0.000000	0.005000	0.006667
0.1-Morin	-0.19667	-0.02333	-0.00667	-0.005	-0.00333	-0.00333	0.000000	0.000000	0.005000	0.006667
1-Morin	-0.20167	-0.02833	-0.01167	-0.01	-0.00833	-0.00833	-0.005	-0.005	0.000000	0.001667
10-Morin	-0.20333	-0.03	-0.01333	-0.01167	-0.01	-0.01	-0.00667	-0.00667	-0.00167	0.000000

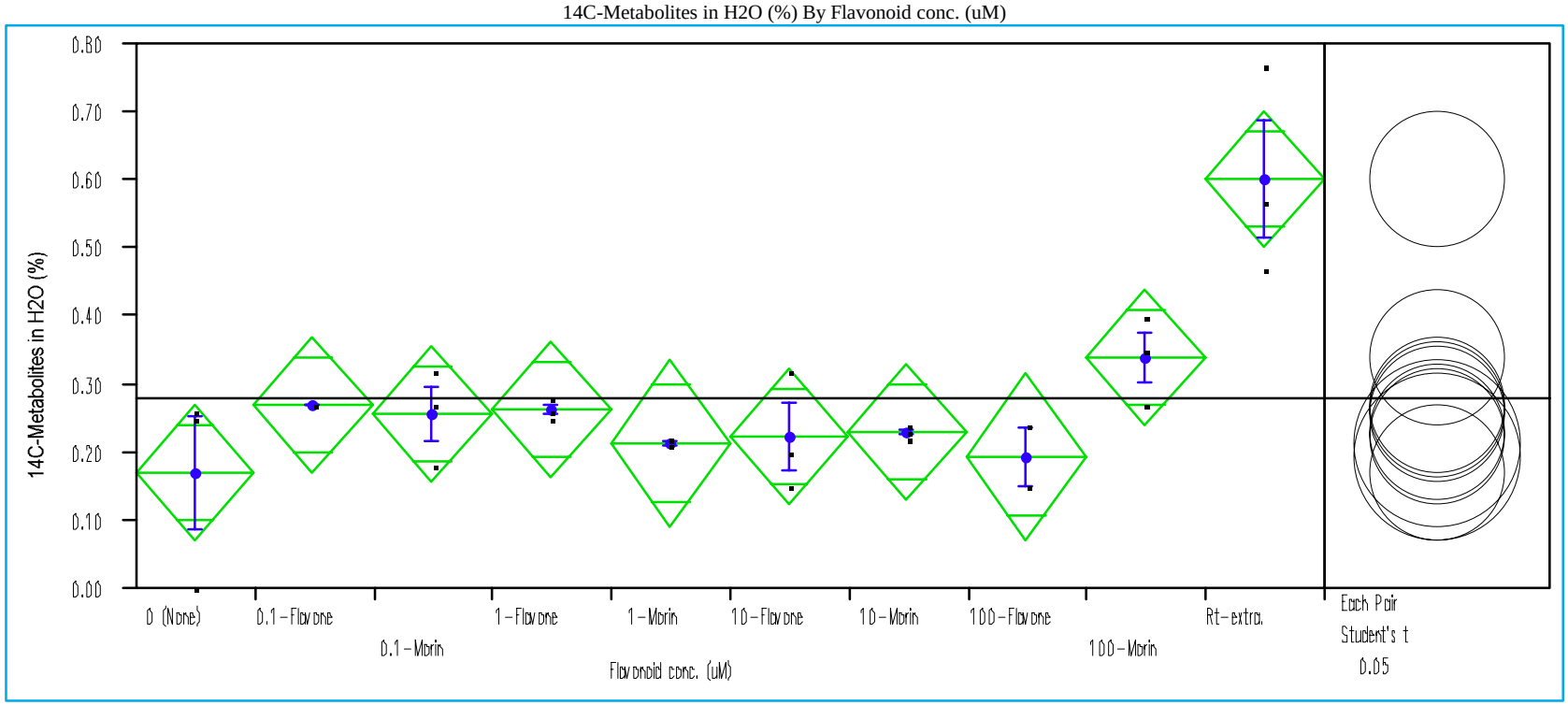
Alpha= 0.05

Comparisons for each pair using Student's t

t 2.10091

Abs(Dif)-LSD	Rt-extracts-M	100-Morin	1-Flavone	100-Flavone	0.1-Flavone	10-Flavone	0 (None)	0.1-Morin	1-Morin	10-Morin
Rt-extracts-M	-0.05287	0.120462	0.137128	0.132554	0.140462	0.140462	**0.143795**	0.143795	0.142554	0.150462
100-Morin	0.120462	-0.05287	-0.03621	-0.04078	-0.03287	-0.03287	-0.02954	-0.02954	-0.03078	-0.02287
1-Flavone	0.137128	-0.03621	-0.05287	-0.05745	-0.04954	-0.04954	-0.04621	-0.04621	-0.04745	-0.03954
100-Flavone	0.132554	-0.04078	-0.05745	-0.06475	-0.05745	-0.05745	-0.05411	-0.05411	-0.05475	-0.04745
0.1-Flavone	0.140462	-0.03287	-0.04954	-0.05745	-0.05287	-0.05287	-0.04954	-0.04954	-0.05078	-0.04287
10-Flavone	0.140462	-0.03287	-0.04954	-0.05745	-0.05287	-0.05287	-0.04954	-0.04954	-0.05078	-0.04287
0 (None)	0.143795	-0.02954	-0.04621	-0.05411	-0.04954	-0.04954	-0.05287	-0.05287	-0.05411	-0.04621
0.1-Morin	0.143795	-0.02954	-0.04621	-0.05411	-0.04954	-0.04954	-0.05287	-0.05287	-0.05411	-0.04621
1-Morin	0.142554	-0.03078	-0.04745	-0.05475	-0.05078	-0.05078	-0.05411	-0.05411	-0.06475	-0.05745
10-Morin	0.150462	-0.02287	-0.03954	-0.04745	-0.04287	-0.04287	-0.04621	-0.04621	-0.05745	-0.05287

Positive values show pairs of means that are significantly different.



Oneway Anova Summary of Fit

RSquare	0.758053
RSquare Adj	0.63708
Root Mean Square Error	0.08458
Mean of Response	0.281786
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	0.40344405	0.044827	6.2663
Error	18	0.12876667	0.007154	Prob>F
C Total	27	0.53221071	0.019712	0.0005

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	0.170000	0.04883
0.1-Flavone	3	0.270000	0.04883
0.1-Morin	3	0.256667	0.04883

1-Flavone	3	0.263333	0.04883
1-Morin	2	0.215000	0.05981
10-Flavone	3	0.223333	0.04883
10-Morin	3	0.230000	0.04883
100-Flavone	2	0.195000	0.05981
100-Morin	3	0.340000	0.04883
Rt-extracts-M	3	0.603333	0.04883

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	0.170000	0.147309	0.08505
0.1-Flavone	3	0.270000	0.000000	0.00000
0.1-Morin	3	0.256667	0.070946	0.04096
1-Flavone	3	0.263333	0.015275	0.00882
1-Morin	2	0.215000	0.007071	0.00500
10-Flavone	3	0.223333	0.087369	0.05044
10-Morin	3	0.230000	0.010000	0.00577
100-Flavone	2	0.195000	0.063640	0.04500
100-Morin	3	0.340000	0.065574	0.03786
Rt-extracts-M	3	0.603333	0.152753	0.08819

Means Comparisons

Dif=Mean[i]-Mean[j]	Rt-extracts-M	100-Morin	0.1-Flavone	1-Flavone	0.1-Morin	10-Morin	10-Flavone	1-Morin	100-Flavone	0 (None)
Rt-extracts-M	0.000000	0.263333	0.333333	0.340000	0.346667	0.373333	0.380000	0.388333	0.408333	0.433333
100-Morin	-0.26333	0.000000	0.070000	0.076667	0.083333	0.110000	0.116667	0.125000	0.145000	0.170000
0.1-Flavone	-0.33333	-0.07	0.000000	0.006667	0.013333	0.040000	0.046667	0.055000	0.075000	0.100000
1-Flavone	-0.34	-0.07667	-0.00667	0.000000	0.006667	0.033333	0.040000	0.048333	0.068333	0.093333
0.1-Morin	-0.34667	-0.08333	-0.01333	-0.00667	0.000000	0.026667	0.033333	0.041667	0.061667	0.086667
10-Morin	-0.37333	-0.11	-0.04	-0.03333	-0.02667	0.000000	0.006667	0.015000	0.035000	0.060000
10-Flavone	-0.38	-0.11667	-0.04667	-0.04	-0.03333	-0.00667	0.000000	0.008333	0.028333	0.053333
1-Morin	-0.38833	-0.125	-0.055	-0.04833	-0.04167	-0.015	-0.00833	0.000000	0.020000	0.045000
100-Flavone	-0.40833	-0.145	-0.075	-0.06833	-0.06167	-0.035	-0.02833	-0.02	0.000000	0.025000
0 (None)	-0.43333	-0.17	-0.1	-0.09333	-0.08667	-0.06	-0.05333	-0.045	-0.025	0.000000

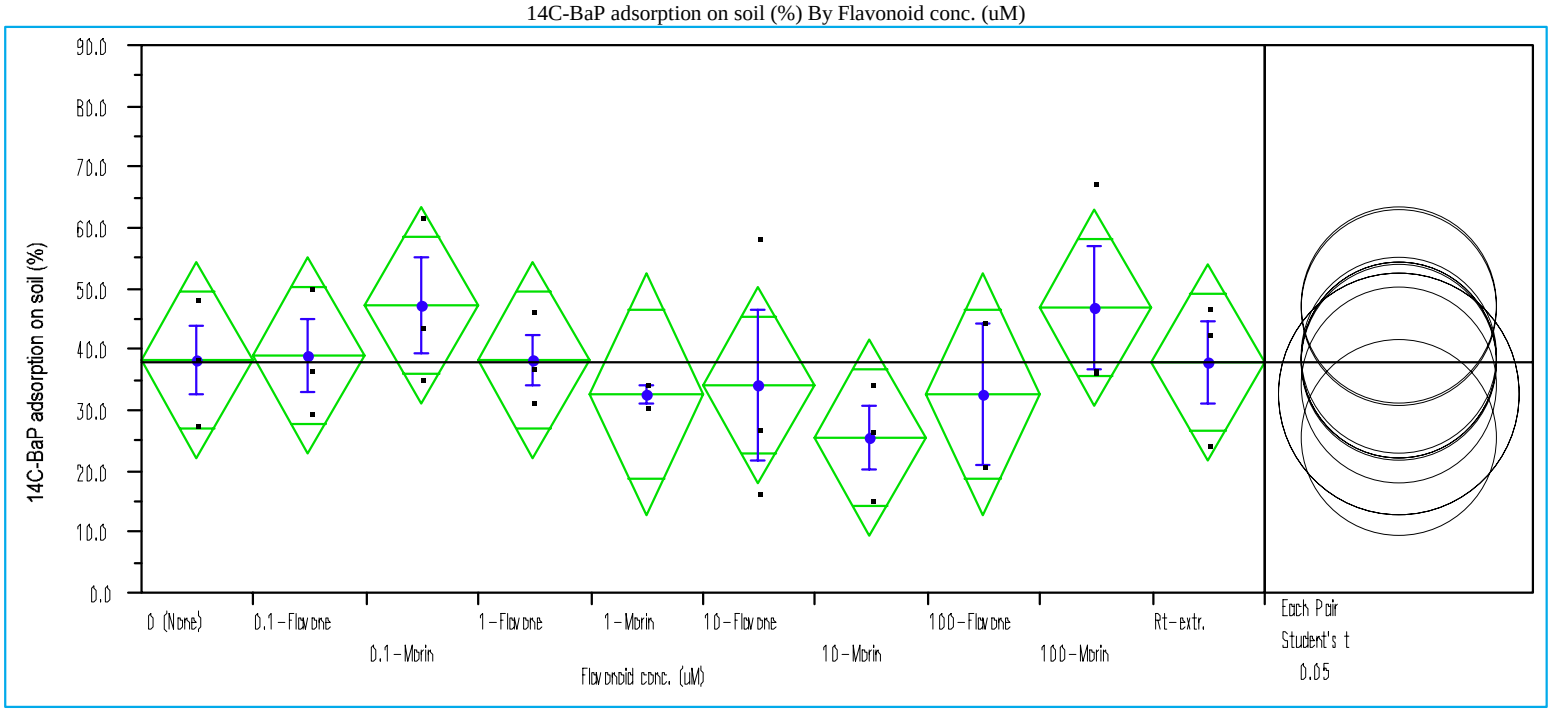
Alpha= 0.05

Comparisons for each pair using Student's t

t 2.10091

Abs(Dif)-LSD	Rt-extracts-M	100-Morin	0.1-Flavone	1-Flavone	0.1-Morin	10-Morin	10-Flavone	1-Morin	100-Flavone	0 (None)
Rt-extracts-M	-0.14509	0.118247	0.188247	0.194914	0.201580	0.228247	0.234914	0.226122	0.246122	**0.288247**
100-Morin	0.118247	-0.14509	-0.07509	-0.06842	-0.06175	-0.03509	-0.02842	-0.03721	-0.01721	**0.024914**
0.1-Flavone	0.188247	-0.07509	-0.14509	-0.13842	-0.13175	-0.10509	-0.09842	-0.10721	-0.08721	-0.04509
1-Flavone	0.194914	-0.06842	-0.13842	-0.14509	-0.13842	-0.11175	-0.10509	-0.11388	-0.09388	-0.05175
0.1-Morin	0.201580	-0.06175	-0.13175	-0.13842	-0.14509	-0.11842	-0.11175	-0.12054	-0.10054	-0.05842
10-Morin	0.228247	-0.03509	-0.10509	-0.11175	-0.11842	-0.14509	-0.13842	-0.14721	-0.12721	-0.08509
10-Flavone	0.234914	-0.02842	-0.09842	-0.10509	-0.11175	-0.13842	-0.14509	-0.15388	-0.13388	-0.09175
1-Morin	0.226122	-0.03721	-0.10721	-0.11388	-0.12054	-0.14721	-0.15388	-0.17769	-0.15769	-0.11721
100-Flavone	0.246122	-0.01721	-0.08721	-0.09388	-0.10054	-0.12721	-0.13388	-0.15769	-0.17769	-0.13721
0 (None)	0.288247	0.024914	-0.04509	-0.05175	-0.05842	-0.08509	-0.09175	-0.11721	-0.13721	-0.14509

Positive values show pairs of means that are significantly different.



Oneway Anova

Summary of Fit

RSquare	0.253323
RSquare Adj	-0.12002
Root Mean Square Error	13.51945
Mean of Response	37.88321
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	1116.1774	124.020	0.6785
Error	18	3289.9612	182.776	Prob>F
C Total	27	4406.1386	163.190	0.7185

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	38.6133	7.8055
0.1-Flavone	3	39.3133	7.8055
0.1-Morin	3	47.4700	7.8055
1-Flavone	3	38.6400	7.8055

1-Morin	2	32.8150	9.5597
10-Flavone	3	34.3600	7.8055
10-Morin	3	25.8033	7.8055
100-Flavone	2	33.0300	9.5597
100-Morin	3	47.1367	7.8055
Rt-extracts-M	3	38.3433	7.8055

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	38.6133	10.3626	5.983
0.1-Flavone	3	39.3133	10.5065	6.066
0.1-Morin	3	47.4700	13.6533	7.883
1-Flavone	3	38.6400	7.5398	4.353
1-Morin	2	32.8150	2.5244	1.785
10-Flavone	3	34.3600	21.9033	12.646
10-Morin	3	25.8033	9.5897	5.537
100-Flavone	2	33.0300	16.8150	11.890
100-Morin	3	47.1367	17.8517	10.307
Rt-extracts-M	3	38.3433	12.2062	7.047

Means Comparisons

Dif=Mean[i]-Mean[j]	0.1-Morin	100-Morin	0.1-Flavone	1-Flavone	0 (None)	Rt-extracts-M	10-Flavone	100-Flavone	1-Morin	10-Morin
0.1-Morin	0.0000	0.3333	8.1567	8.8300	8.8567	9.1267	13.1100	14.4400	14.6550	21.6667
100-Morin	-0.3333	0.0000	7.8233	8.4967	8.5233	8.7933	12.7767	14.1067	14.3217	21.3333
0.1-Flavone	-8.1567	-7.8233	0.0000	0.6733	0.7000	0.9700	4.9533	6.2833	6.4983	13.5100
1-Flavone	-8.8300	-8.4967	-0.6733	0.0000	0.0267	0.2967	4.2800	5.6100	5.8250	12.8367
0 (None)	-8.8567	-8.5233	-0.7000	-0.0267	0.0000	0.2700	4.2533	5.5833	5.7983	12.8100
Rt-extracts-M	-9.1267	-8.7933	-0.9700	-0.2967	-0.2700	0.0000	3.9833	5.3133	5.5283	12.5400
10-Flavone	-13.1100	-12.7767	-4.9533	-4.2800	-4.2533	-3.9833	0.0000	1.3300	1.5450	8.5567
100-Flavone	-14.4400	-14.1067	-6.2833	-5.6100	-5.5833	-5.3133	-1.3300	0.0000	0.2150	7.2267
1-Morin	-14.6550	-14.3217	-6.4983	-5.8250	-5.7983	-5.5283	-1.5450	-0.2150	0.0000	7.0117
10-Morin	-21.6667	-21.3333	-13.5100	-12.8367	-12.8100	-12.5400	-8.5567	-7.2267	-7.0117	0.0000

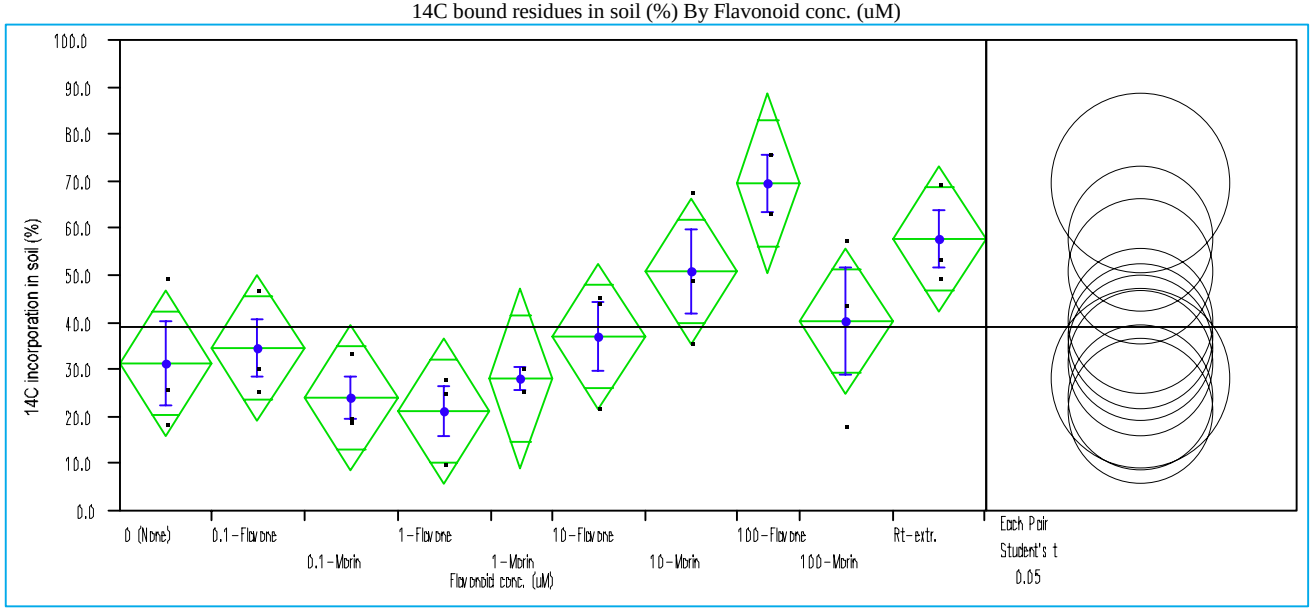
Alpha= 0.05

Comparisons for each pair using Student's t

t 2.10091

Abs(Dif)-LSD	0.1-Morin	100-Morin	0.1-Flavone	1-Flavone	0 (None)	Rt-extracts-M	10-Flavone	100-Flavone	1-Morin	10-Morin
0.1-Morin	-23.1911	-22.8577	-15.0344	-14.3611	-14.3344	-14.0644	-10.0811	-11.4884	-11.2734	-1.5244
100-Morin	-22.8577	-23.1911	-15.3677	-14.6944	-14.6677	-14.3977	-10.4144	-11.8217	-11.6067	-1.8577
0.1-Flavone	-15.0344	-15.3677	-23.1911	-22.5177	-22.4911	-22.2211	-18.2377	-19.6451	-19.4301	-9.6811
1-Flavone	-14.3611	-14.6944	-22.5177	-23.1911	-23.1644	-22.8944	-18.9111	-20.3184	-20.1034	-10.3544
0 (None)	-14.3344	-14.6677	-22.4911	-23.1644	-23.1911	-22.9211	-18.9377	-20.3451	-20.1301	-10.3811
Rt-extracts-M	-14.0644	-14.3977	-22.2211	-22.8944	-22.9211	-23.1911	-19.2077	-20.6151	-20.4001	-10.6511
10-Flavone	-10.0811	-10.4144	-18.2377	-18.9111	-18.9377	-19.2077	-23.1911	-24.5984	-24.3834	-14.6344
100-Flavone	-11.4884	-11.8217	-19.6451	-20.3184	-20.3451	-20.6151	-24.5984	-28.4031	-28.1881	-18.7017
1-Morin	-11.2734	-11.6067	-19.4301	-20.1034	-20.1301	-20.4001	-24.3834	-28.1881	-28.4031	-18.9167
10-Morin	-1.5244	-1.8577	-9.6811	-10.3544	-10.3811	-10.6511	-14.6344	-18.7017	-18.9167	-23.1911

Positive values show pairs of means that are significantly different.



Oneway Anova
Summary of Fit

RSquare	0.640804
RSquare Adj	0.461206
Root Mean Square Error	13.06958
Mean of Response	39.27464
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	5485.1662	609.463	3.5680
Error	18	3074.6489	170.814	Prob>F
C Total	27	8559.8151	317.030	0.0104

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	31.7867	7.5457
0.1-Flavone	3	34.9233	7.5457
0.1-Morin	3	24.6467	7.5457
1-Flavone	3	21.5433	7.5457
1-Morin	2	28.5650	9.2416
10-Flavone	3	37.6467	7.5457

10-Morin	3	51.4200	7.5457
100-Flavone	2	70.2400	9.2416
100-Morin	3	40.5433	7.5457
Rt-extracts-M	3	58.1833	7.5457

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	31.7867	16.1309	9.313
0.1-Flavone	3	34.9233	11.1372	6.430
0.1-Morin	3	24.6467	8.2196	4.746
1-Flavone	3	21.5433	9.6009	5.543
1-Morin	2	28.5650	3.5709	2.525
10-Flavone	3	37.6467	13.3210	7.691
10-Morin	3	51.4200	16.1283	9.312
100-Flavone	2	70.2400	8.8106	6.230
100-Morin	3	40.5433	19.9682	11.529
Rt-extracts-M	3	58.1833	10.5761	6.106

Means Comparisons

Dif=Mean[i]-Mean[j]	100-Flavone	Rt-extracts-M	10-Morin	100-Morin	10-Flavone	0.1-Flavone	0 (None)	1-Morin	0.1-Morin	1-Flavone
100-Flavone	0.0000	12.0567	18.8200	29.6967	32.5933	35.3167	38.4533	41.6750	45.5933	48.6967
Rt-extracts-M	-12.0567	0.0000	6.7633	17.6400	20.5367	23.2600	26.3967	29.6183	33.5367	36.6400
10-Morin	-18.8200	-6.7633	0.0000	10.8767	13.7733	16.4967	19.6333	22.8550	26.7733	29.8767
100-Morin	-29.6967	-17.6400	-10.8767	0.0000	2.8967	5.6200	8.7567	11.9783	15.8967	19.0000
10-Flavone	-32.5933	-20.5367	-13.7733	-2.8967	0.0000	2.7233	5.8600	9.0817	13.0000	16.1033
0.1-Flavone	-35.3167	-23.2600	-16.4967	-5.6200	-2.7233	0.0000	3.1367	6.3583	10.2767	13.3800
0 (None)	-38.4533	-26.3967	-19.6333	-8.7567	-5.8600	-3.1367	0.0000	3.2217	7.1400	10.2433
1-Morin	-41.6750	-29.6183	-22.8550	-11.9783	-9.0817	-6.3583	-3.2217	0.0000	3.9183	7.0217
0.1-Morin	-45.5933	-33.5367	-26.7733	-15.8967	-13.0000	-10.2767	-7.1400	-3.9183	0.0000	3.1033
1-Flavone	-48.6967	-36.6400	-29.8767	-19.0000	-16.1033	-13.3800	-10.2433	-7.0217	-3.1033	0.0000

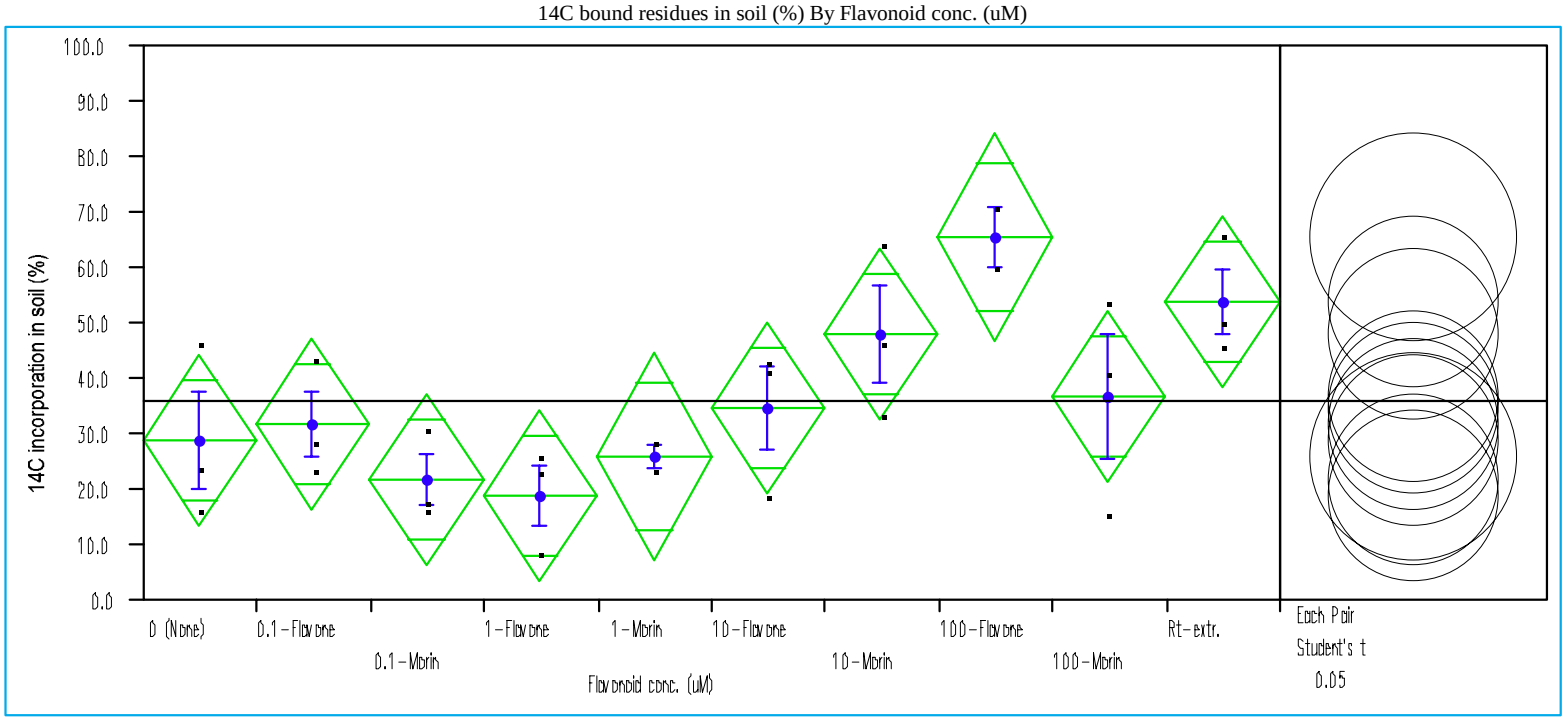
Alpha= 0.05

Comparisons for each pair using Student's t

t 2.10091

Abs(Dif)-LSD	100-Flavone	Rt-extracts-M	10-Morin	100-Morin	10-Flavone	0.1-Flavone	0 (None)	1-Morin	0.1-Morin	1-Flavone
100-Flavone	-27.4580	-13.0089	-6.2456	4.6311	7.5277	10.2511	13.3877	14.2170	20.5277	23.6311
Rt-extracts-M	-13.0089	-22.4193	-15.6560	-4.7793	-1.8827	0.8407	3.9773	4.5527	11.1173	14.2207
10-Morin	-6.2456	-15.6560	-22.4193	-11.5427	-8.6460	-5.9227	-2.7860	-2.2106	4.3540	7.4573
100-Morin	4.6311	-4.7793	-11.5427	-22.4193	-19.5227	-16.7993	-13.6627	-13.0873	-6.5227	-3.4193
10-Flavone	7.5277	-1.8827	-8.6460	-19.5227	-22.4193	-19.6960	-16.5593	-15.9839	-9.4193	-6.3160
0.1-Flavone	10.2511	0.8407	-5.9227	-16.7993	-19.6960	-22.4193	-19.2827	-18.7073	-12.1427	-9.0393
0 (None)	13.3877	3.9773	-2.7860	-13.6627	-16.5593	-19.2827	-22.4193	-21.8439	-15.2793	-12.1760
1-Morin	14.2170	4.5527	-2.2106	-13.0873	-15.9839	-18.7073	-21.8439	-27.4580	-21.1473	-18.0439
0.1-Morin	20.5277	11.1173	4.3540	-6.5227	-9.4193	-12.1427	-15.2793	-21.1473	-22.4193	-19.3160
1-Flavone	23.6311	14.2207	7.4573	-3.4193	-6.3160	-9.0393	-12.1760	-18.0439	-19.3160	-22.4193

Positive values show pairs of means that are significantly different.



Oneway Anova

Summary of Fit

RSquare	0.633278
RSquare Adj	0.449917
Root Mean Square Error	12.75488
Mean of Response	36.0475
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	5056.8731	561.875	3.4537
Error	18	2928.3648	162.687	Prob>F
C Total	27	7985.2379	295.750	0.0121

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	28.8833	7.3640
0.1-Flavone	3	31.8500	7.3640
0.1-Morin	3	21.7567	7.3640
1-Flavone	3	19.1200	7.3640

1-Morin	2	26.0250	9.0191
10-Flavone	3	34.6233	7.3640
10-Morin	3	48.0633	7.3640
100-Flavone	2	65.7350	9.0191
100-Morin	3	36.9200	7.3640
Rt-extracts-M	3	54.0533	7.3640

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	28.8833	15.7406	9.088
0.1-Flavone	3	31.8500	10.2900	5.941
0.1-Morin	3	21.7567	8.2299	4.752
1-Flavone	3	19.1200	9.4143	5.435
1-Morin	2	26.0250	3.3022	2.335
10-Flavone	3	34.6233	13.4821	7.784
10-Morin	3	48.0633	15.4898	8.943
100-Flavone	2	65.7350	7.7852	5.505
100-Morin	3	36.9200	19.6434	11.341
Rt-extracts-M	3	54.0533	10.5286	6.079

Means Comparisons

Dif=Mean[i]-Mean[j]	100-Flavone	Rt-extracts-M	10-Morin	100-Morin	10-Flavone	0.1-Flavone	0 (None)	1-Morin	0.1-Morin	1-Flavone
100-Flavone	0.0000	11.6817	17.6717	28.8150	31.1117	33.8850	36.8517	39.7100	43.9783	46.6150
Rt-extracts-M	-11.6817	0.0000	5.9900	17.1333	19.4300	22.2033	25.1700	28.0283	32.2967	34.9333
10-Morin	-17.6717	-5.9900	0.0000	11.1433	13.4400	16.2133	19.1800	22.0383	26.3067	28.9433
100-Morin	-28.8150	-17.1333	-11.1433	0.0000	2.2967	5.0700	8.0367	10.8950	15.1633	17.8000
10-Flavone	-31.1117	-19.4300	-13.4400	-2.2967	0.0000	2.7733	5.7400	8.5983	12.8667	15.5033
0.1-Flavone	-33.8850	-22.2033	-16.2133	-5.0700	-2.7733	0.0000	2.9667	5.8250	10.0933	12.7300
0 (None)	-36.8517	-25.1700	-19.1800	-8.0367	-5.7400	-2.9667	0.0000	2.8583	7.1267	9.7633
1-Morin	-39.7100	-28.0283	-22.0383	-10.8950	-8.5983	-5.8250	-2.8583	0.0000	4.2683	6.9050
0.1-Morin	-43.9783	-32.2967	-26.3067	-15.1633	-12.8667	-10.0933	-7.1267	-4.2683	0.0000	2.6367
1-Flavone	-46.6150	-34.9333	-28.9433	-17.8000	-15.5033	-12.7300	-9.7633	-6.9050	-2.6367	0.0000

Alpha= 0.05

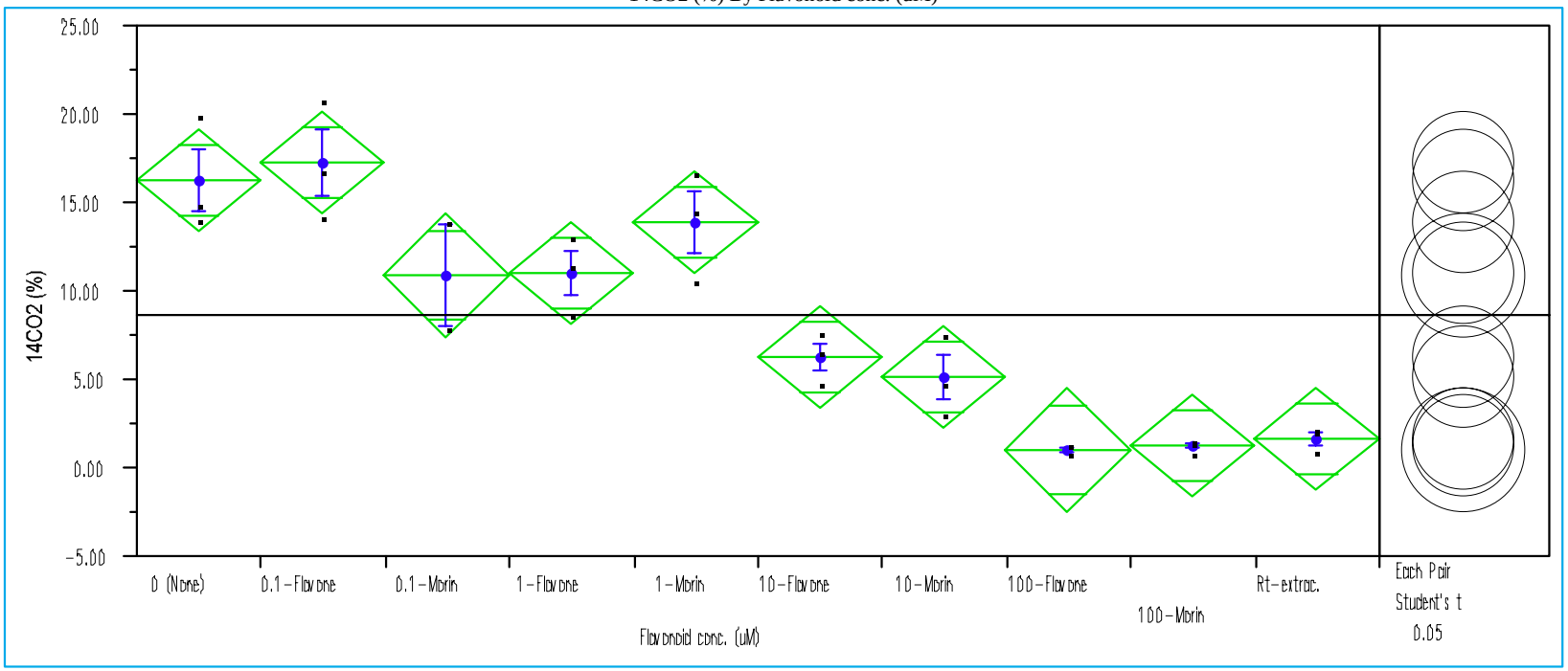
Comparisons for each pair using Student's t

t 2.10091

Abs(Dif)-LSD	100-Flavone	Rt-extracts-M	10-Morin	100-Morin	10-Flavone	0.1-Flavone	0 (None)	1-Morin	0.1-Morin	1-Flavone
100-Flavone	-26.7968	-12.7804	-6.7904	4.3530	6.6496	9.4230	**12.3896**	12.9132	19.5163	22.1530
Rt-extracts-M	-12.7804	-21.8795	-15.8895	-4.7462	-2.4495	0.3238	**3.2905**	3.5663	10.4171	13.0538
10-Morin	-6.7904	-15.8895	-21.8795	-10.7362	-8.4395	-5.6662	-2.6995	-2.4237	4.4271	7.0638
100-Morin	4.3530	-4.7462	-10.7362	-21.8795	-19.5829	-16.8095	-13.8429	-13.5670	-6.7162	-4.0795
10-Flavone	6.6496	-2.4495	-8.4395	-19.5829	-21.8795	-19.1062	-16.1395	-15.8637	-9.0129	-6.3762
0.1-Flavone	9.4230	0.3238	-5.6662	-16.8095	-19.1062	-21.8795	-18.9129	-18.6370	-11.7862	-9.1495
0 (None)	12.3896	3.2905	-2.6995	-13.8429	-16.1395	-18.9129	-21.8795	-21.6037	-14.7529	-12.1162
1-Morin	12.9132	3.5663	-2.4237	-13.5670	-15.8637	-18.6370	-21.6037	-26.7968	-20.1937	-17.5570
0.1-Morin	19.5163	10.4171	4.4271	-6.7162	-9.0129	-11.7862	-14.7529	-20.1937	-21.8795	-19.2429
1-Flavone	22.1530	13.0538	7.0638	-4.0795	-6.3762	-9.1495	-12.1162	-17.5570	-19.2429	-21.8795

Positive values show pairs of means that are significantly different.

Appendix D-3. Student's t Test: Paired Comparison of ^{14}C-B[a]P Fate Data in Bermudagrass Rhizosphere Soil with or without Flavonoid Amendment



Oneway Anova Summary of Fit

RSquare	0.90402
RSquare Adj	0.856031
Root Mean Square Error	2.410797
Mean of Response	8.692857
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	985.3551	109.484	18.8378
Error	18	104.6149	5.812	Prob>F
C Total	27	1089.9700	40.369	<.0001

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	16.3300	1.3919
0.1-Flavone	3	17.2700	1.3919
0.1-Morin	2	10.9350	1.7047
1-Flavone	3	11.0700	1.3919

1-Morin	3	13.9300	1.3919
10-Flavone	3	6.3767	1.3919
10-Morin	3	5.1433	1.3919
100-Flavone	2	1.0700	1.7047
100-Morin	3	1.2867	1.3919
Rt-extracts-M	3	1.7233	1.3919

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	16.3300	3.18086	1.8365
0.1-Flavone	3	17.2700	3.28818	1.8984
0.1-Morin	2	10.9350	4.17900	2.9550
1-Flavone	3	11.0700	2.24060	1.2936
1-Morin	3	13.9300	3.11963	1.8011
10-Flavone	3	6.3767	1.40461	0.8110
10-Morin	3	5.1433	2.29435	1.3246
100-Flavone	2	1.0700	0.33941	0.2400
100-Morin	3	1.2867	0.39929	0.2305
Rt-extracts-M	3	1.7233	0.66260	0.3825

Means Comparisons

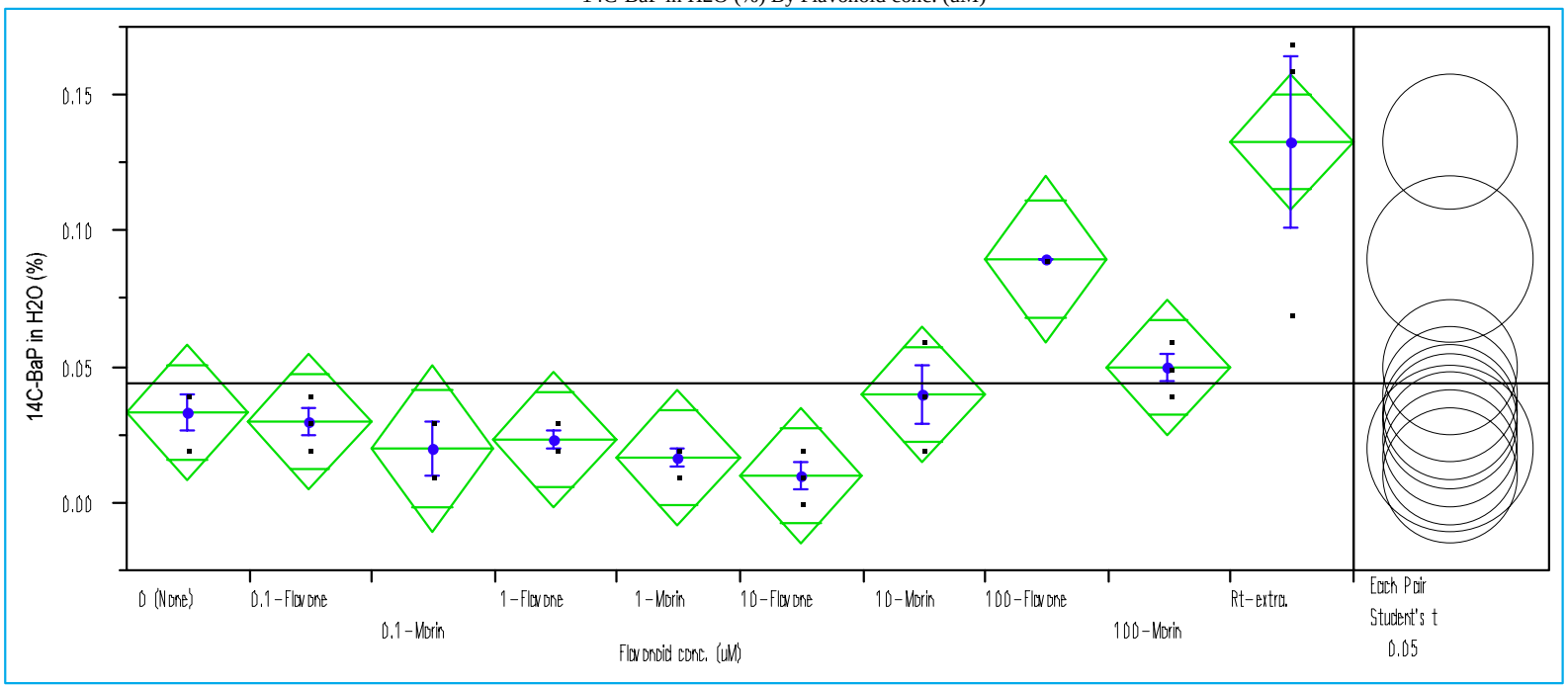
Dif=Mean[i]-Mean[j]	0.1-Flavone	0 (None)	1-Morin	1-Flavone	0.1-Morin	10-Flavone	10-Morin	Rt-extracts-M	100-Morin	100-Flavone
0.1-Flavone	0.0000	0.9400	3.3400	6.2000	6.3350	10.8933	12.1267	15.5467	15.9833	16.2000
0 (None)	-0.9400	0.0000	2.4000	5.2600	5.3950	9.9533	11.1867	14.6067	15.0433	15.2600
1-Morin	-3.3400	-2.4000	0.0000	2.8600	2.9950	7.5533	8.7867	12.2067	12.6433	12.8600
1-Flavone	-6.2000	-5.2600	-2.8600	0.0000	0.1350	4.6933	5.9267	9.3467	9.7833	10.0000
0.1-Morin	-6.3350	-5.3950	-2.9950	-0.1350	0.0000	4.5583	5.7917	9.2117	9.6483	9.8650
10-Flavone	-10.8933	-9.9533	-7.5533	-4.6933	-4.5583	0.0000	1.2333	4.6533	5.0900	5.3067
10-Morin	-12.1267	-11.1867	-8.7867	-5.9267	-5.7917	-1.2333	0.0000	3.4200	3.8567	4.0733
Rt-extracts-M	-15.5467	-14.6067	-12.2067	-9.3467	-9.2117	-4.6533	-3.4200	0.0000	0.4367	0.6533
100-Morin	-15.9833	-15.0433	-12.6433	-9.7833	-9.6483	-5.0900	-3.8567	-0.4367	0.0000	0.2167
100-Flavone	-16.2000	-15.2600	-12.8600	-10.0000	-9.8650	-5.3067	-4.0733	-0.6533	-0.2167	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t t 2.10091

Abs(Dif)-LSD	0.1-Flavone	0 (None)	1-Morin	1-Flavone	0.1-Morin	10-Flavone	10-Morin	Rt-extracts-M	100-Morin	100-Flavone
0.1-Flavone	-4.1354	-3.1954	-0.7954	2.0646	1.7114	6.7579	7.9912	11.4112	11.8479	11.5764
0 (None)	-3.1954	-4.1354	-1.7354	1.1246	0.7714	5.8179	7.0512	10.4712	10.9079	10.6364
1-Morin	-0.7954	-1.7354	-4.1354	-1.2754	-1.6286	3.4179	4.6512	8.0712	8.5079	8.2364
1-Flavone	2.0646	**1.1246**	-1.2754	-4.1354	-4.4886	0.5579	1.7912	5.2112	5.6479	5.3764
0.1-Morin	1.7114	**0.7714**	-1.6286	-4.4886	-5.0649	-0.0652	1.1681	4.5881	5.0248	4.8001
10-Flavone	6.7579	**5.8179**	3.4179	0.5579	-0.0652	-4.1354	-2.9021	0.5179	0.9546	0.6831
10-Morin	7.9912	**7.0512**	4.6512	1.7912	1.1681	-2.9021	-4.1354	-0.7154	-0.2788	-0.5502
Rt-extracts-M	11.4112	**10.4712**	8.0712	5.2112	4.5881	0.5179	-0.7154	-4.1354	-3.6988	-3.9702
100-Morin	11.8479	**10.9079**	8.5079	5.6479	5.0248	0.9546	-0.2788	-3.6988	-4.1354	-4.4069
100-Flavone	11.5764	**10.6364**	8.2364	5.3764	4.8001	0.6831	-0.5502	-3.9702	-4.4069	-5.0649

Positive values show pairs of means that are significantly different.



Oneway Anova

Summary of Fit

RSquare	0.822585
RSquare Adj	0.733878
Root Mean Square Error	0.02117
Mean of Response	0.043929
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	0.03740119	0.004156	9.2730
Error	18	0.00806667	0.000448	Prob>F
C Total	27	0.04546786	0.001684	<.0001

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	0.033333	0.01222
0.1-Flavone	3	0.030000	0.01222
0.1-Morin	2	0.020000	0.01497
1-Flavone	3	0.023333	0.01222

1-Morin	3	0.016667	0.01222
10-Flavone	3	0.010000	0.01222
10-Morin	3	0.040000	0.01222
100-Flavone	2	0.090000	0.01497
100-Morin	3	0.050000	0.01222
Rt-extracts-M	3	0.133333	0.01222

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	0.033333	0.011547	0.00667
0.1-Flavone	3	0.030000	0.010000	0.00577
0.1-Morin	2	0.020000	0.014142	0.01000
1-Flavone	3	0.023333	0.005774	0.00333
1-Morin	3	0.016667	0.005774	0.00333
10-Flavone	3	0.010000	0.010000	0.00577
10-Morin	3	0.040000	0.020000	0.01155
100-Flavone	2	0.090000	0.000000	0.00000
100-Morin	3	0.050000	0.010000	0.00577
Rt-extracts-M	3	0.133333	0.055076	0.03180

Means Comparisons

Dif=Mean[i]-Mean[j]	Rt-extracts-M	100-Flavone	100-Morin	10-Morin	0 (None)	0.1-Flavone	1-Flavone	0.1-Morin	1-Morin	10-Flavone
Rt-extracts-M	0.000000	0.043333	0.083333	0.093333	0.100000	0.103333	0.110000	0.113333	0.116667	0.123333
100-Flavone	-0.04333	0.000000	0.040000	0.050000	0.056667	0.060000	0.066667	0.070000	0.073333	0.080000
100-Morin	-0.08333	-0.04	0.000000	0.010000	0.016667	0.020000	0.026667	0.030000	0.033333	0.040000
10-Morin	-0.09333	-0.05	-0.01	0.000000	0.006667	0.010000	0.016667	0.020000	0.023333	0.030000
0 (None)	-0.1	-0.05667	-0.01667	-0.00667	0.000000	0.003333	0.010000	0.013333	0.016667	0.023333
0.1-Flavone	-0.10333	-0.06	-0.02	-0.01	-0.00333	0.000000	0.006667	0.010000	0.013333	0.020000
1-Flavone	-0.11	-0.06667	-0.02667	-0.01667	-0.01	-0.00667	0.000000	0.003333	0.006667	0.013333
0.1-Morin	-0.11333	-0.07	-0.03	-0.02	-0.01333	-0.01	-0.00333	0.000000	0.003333	0.010000
1-Morin	-0.11667	-0.07333	-0.03333	-0.02333	-0.01667	-0.01333	-0.00667	-0.00333	0.000000	0.006667
10-Flavone	-0.12333	-0.08	-0.04	-0.03	-0.02333	-0.02	-0.01333	-0.01	-0.00667	0.000000

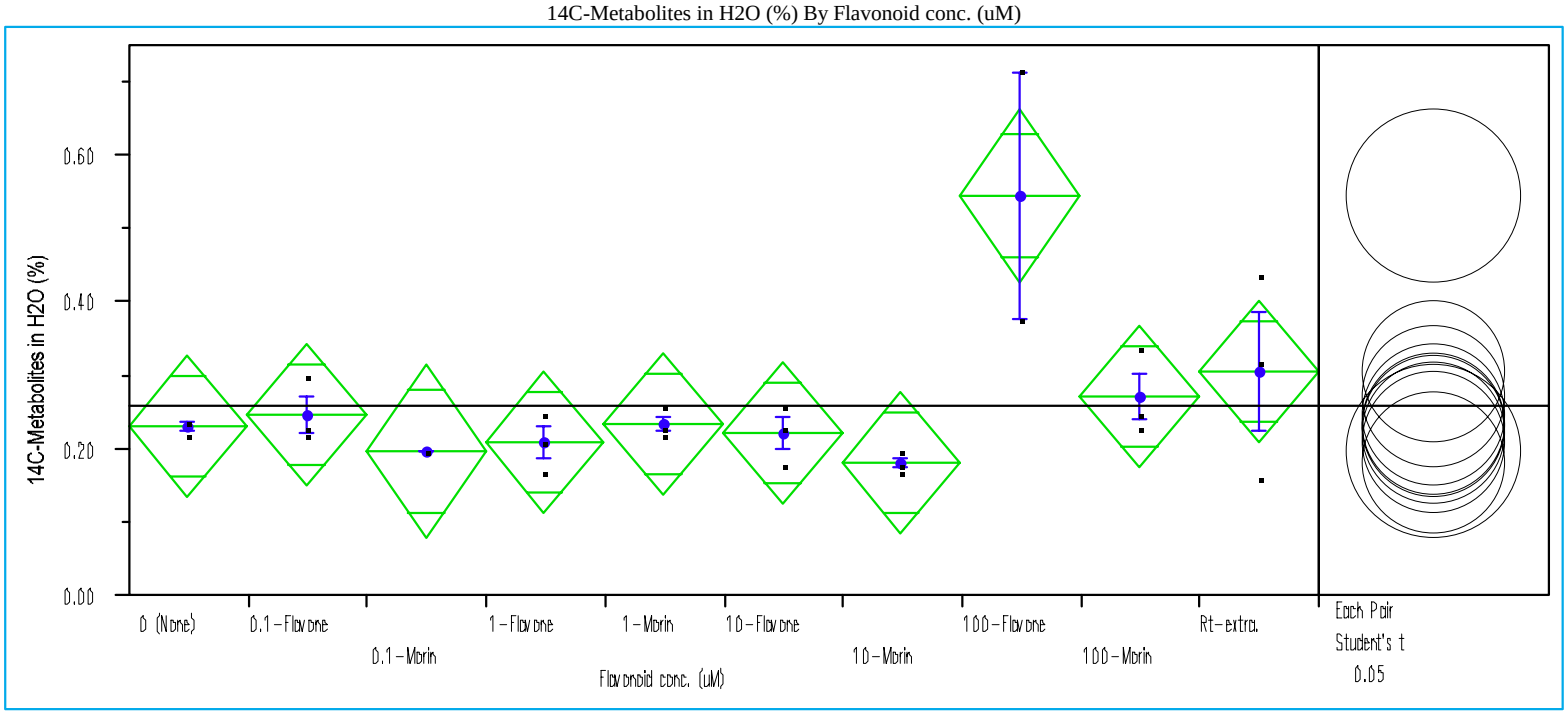
Alpha= 0.05

Comparisons for each pair using Student's t

t 2.10091

Abs(Dif)-LSD	Rt-extracts-M	100-Flavone	100-Morin	10-Morin	0 (None)	0.1-Flavone	1-Flavone	0.1-Morin	1-Morin	10-Flavone
Rt-extracts-M	-0.03631	0.002733	0.047019	0.057019	**0.063686**	0.067019	0.073686	0.072733	0.080353	0.087019
100-Flavone	0.002733	-0.04448	-0.0006	0.009400	**0.016067**	0.019400	0.026067	0.025525	0.032733	0.039400
100-Morin	0.047019	-0.0006	-0.03631	-0.02631	-0.01965	-0.01631	-0.00965	-0.0106	-0.00298	0.003686
10-Morin	0.057019	0.009400	-0.02631	-0.03631	-0.02965	-0.02631	-0.01965	-0.0206	-0.01298	-0.00631
0 (None)	0.063686	0.016067	-0.01965	-0.02965	-0.03631	-0.03298	-0.02631	-0.02727	-0.01965	-0.01298
0.1-Flavone	0.067019	0.019400	-0.01631	-0.02631	-0.03298	-0.03631	-0.02965	-0.0306	-0.02298	-0.01631
1-Flavone	0.073686	0.026067	-0.00965	-0.01965	-0.02631	-0.02965	-0.03631	-0.03727	-0.02965	-0.02298
0.1-Morin	0.072733	0.025525	-0.0106	-0.0206	-0.02727	-0.0306	-0.03727	-0.04448	-0.03727	-0.0306
1-Morin	0.080353	0.032733	-0.00298	-0.01298	-0.01965	-0.02298	-0.02965	-0.03727	-0.03631	-0.02965
10-Flavone	0.087019	0.039400	0.003686	-0.00631	-0.01298	-0.01631	-0.02298	-0.0306	-0.02965	-0.03631

Positive values show pairs of means that are significantly different.



Oneway Anova

Summary of Fit

RSquare	0.650253
RSquare Adj	0.475379
Root Mean Square Error	0.080277
Mean of Response	0.258929
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	0.21566786	0.023963	3.7184
Error	18	0.11600000	0.006444	Prob>F
C Total	27	0.33166786	0.012284	0.0085

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	0.233333	0.04635
0.1-Flavone	3	0.250000	0.04635
0.1-Morin	2	0.200000	0.05676
1-Flavone	3	0.210000	0.04635

1-Morin	3	0.236667	0.04635
10-Flavone	3	0.223333	0.04635
10-Morin	3	0.183333	0.04635
100-Flavone	2	0.550000	0.05676
100-Morin	3	0.273333	0.04635
Rt-extracts-M	3	0.306667	0.04635

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	0.233333	0.011547	0.00667
0.1-Flavone	3	0.250000	0.043589	0.02517
0.1-Morin	2	0.200000	0.000000	0.00000
1-Flavone	3	0.210000	0.040000	0.02309
1-Morin	3	0.236667	0.020817	0.01202
10-Flavone	3	0.223333	0.040415	0.02333
10-Morin	3	0.183333	0.015275	0.00882
100-Flavone	2	0.550000	0.240416	0.17000
100-Morin	3	0.273333	0.058595	0.03383
Rt-extracts-M	3	0.306667	0.140475	0.08110

Means Comparisons

Dif=Mean[i]-Mean[j]	100-Flavone	Rt-extracts-M	100-Morin	0.1-Flavone	1-Morin	0 (None)	10-Flavone	1-Flavone	0.1-Morin	10-Morin
100-Flavone	0.000000	0.243333	0.276667	0.300000	0.313333	0.316667	0.326667	0.340000	0.350000	0.366667
Rt-extracts-M	-0.24333	0.000000	0.033333	0.056667	0.070000	0.073333	0.083333	0.096667	0.106667	0.123333
100-Morin	-0.27667	-0.03333	0.000000	0.023333	0.036667	0.040000	0.050000	0.063333	0.073333	0.090000
0.1-Flavone	-0.3	-0.05667	-0.02333	0.000000	0.013333	0.016667	0.026667	0.040000	0.050000	0.066667
1-Morin	-0.31333	-0.07	-0.03667	-0.01333	0.000000	0.003333	0.013333	0.026667	0.036667	0.053333
0 (None)	-0.31667	-0.07333	-0.04	-0.01667	-0.00333	0.000000	0.010000	0.023333	0.033333	0.050000
10-Flavone	-0.32667	-0.08333	-0.05	-0.02667	-0.01333	-0.01	0.000000	0.013333	0.023333	0.040000
1-Flavone	-0.34	-0.09667	-0.06333	-0.04	-0.02667	-0.02333	-0.01333	0.000000	0.010000	0.026667
0.1-Morin	-0.35	-0.10667	-0.07333	-0.05	-0.03667	-0.03333	-0.02333	-0.01	0.000000	0.016667
10-Morin	-0.36667	-0.12333	-0.09	-0.06667	-0.05333	-0.05	-0.04	-0.02667	-0.01667	0.000000

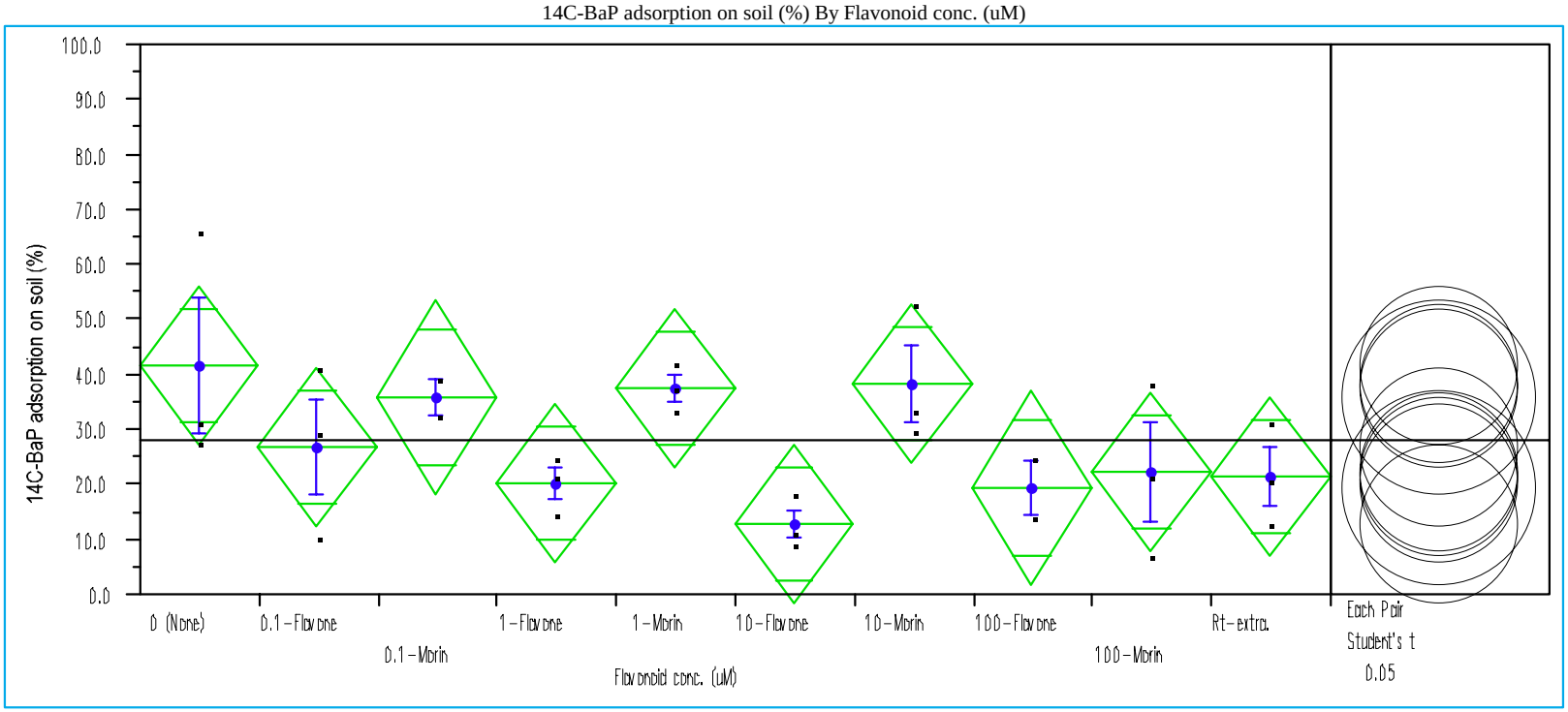
Alpha= 0.05

Comparisons for each pair using Student's t

t 2.10091

Abs(Dif)-LSD	100-Flavone	Rt-extracts-M	100-Morin	0.1-Flavone	1-Morin	0 (None)	10-Flavone	1-Flavone	0.1-Morin	10-Morin
100-Flavone	-0.16866	0.089373	0.122706	0.146040	0.159373	**0.162706**	0.172706	0.186040	0.181345	0.212706
Rt-extracts-M	0.089373	-0.13771	-0.10437	-0.08104	-0.06771	-0.06437	-0.05437	-0.04104	-0.04729	-0.01437
100-Morin	0.122706	-0.10437	-0.13771	-0.11437	-0.10104	-0.09771	-0.08771	-0.07437	-0.08063	-0.04771
0.1-Flavone	0.146040	-0.08104	-0.11437	-0.13771	-0.12437	-0.12104	-0.11104	-0.09771	-0.10396	-0.07104
1-Morin	0.159373	-0.06771	-0.10104	-0.12437	-0.13771	-0.13437	-0.12437	-0.11104	-0.11729	-0.08437
0 (None)	0.162706	-0.06437	-0.09771	-0.12104	-0.13437	-0.13771	-0.12771	-0.11437	-0.12063	-0.08771
10-Flavone	0.172706	-0.05437	-0.08771	-0.11104	-0.12437	-0.12771	-0.13771	-0.12437	-0.13063	-0.09771
1-Flavone	0.186040	-0.04104	-0.07437	-0.09771	-0.11104	-0.11437	-0.12437	-0.13771	-0.14396	-0.11104
0.1-Morin	0.181345	-0.04729	-0.08063	-0.10396	-0.11729	-0.12063	-0.13063	-0.14396	-0.16866	-0.13729
10-Morin	0.212706	-0.01437	-0.04771	-0.07104	-0.08437	-0.08771	-0.09771	-0.11104	-0.13729	-0.13771

Positive values show pairs of means that are significantly different.



Oneway Anova

Summary of Fit

RSquare	0.49651
RSquare Adj	0.244765
Root Mean Square Error	12.01299
Mean of Response	28.03107
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	2561.6064	284.623	1.9723
Error	18	2597.6161	144.312	Prob>F
C Total	27	5159.2225	191.082	0.1052

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	42.0600	6.9357
0.1-Flavone	3	27.2633	6.9357
0.1-Morin	2	36.1100	8.4945

1-Flavone	3	20.6400	6.9357
1-Morin	3	38.0600	6.9357
10-Flavone	3	13.0733	6.9357
10-Morin	3	38.9533	6.9357
100-Flavone	2	19.7450	8.4945
100-Morin	3	22.4867	6.9357
Rt-extracts-M	3	21.8500	6.9357

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	42.0600	21.4005	12.356
0.1-Flavone	3	27.2633	15.6561	9.039
0.1-Morin	2	36.1100	4.7093	3.330
1-Flavone	3	20.6400	5.3791	3.106
1-Morin	3	38.0600	4.3951	2.537
10-Flavone	3	13.0733	4.7171	2.723
10-Morin	3	38.9533	12.3900	7.153
100-Flavone	2	19.7450	7.5448	5.335
100-Morin	3	22.4867	15.6917	9.060
Rt-extracts-M	3	21.8500	9.2689	5.351

Means Comparisons

Dif=Mean[i]-Mean[j]	0 (None)	10-Morin	1-Morin	0.1-Morin	0.1-Flavone	100-Morin	Rt-extracts-M	1-Flavone	100-Flavone	10-Flavone
0 (None)	0.0000	3.1067	4.0000	5.9500	14.7967	19.5733	20.2100	21.4200	22.3150	28.9867
10-Morin	-3.1067	0.0000	0.8933	2.8433	11.6900	16.4667	17.1033	18.3133	19.2083	25.8800
1-Morin	-4.0000	-0.8933	0.0000	1.9500	10.7967	15.5733	16.2100	17.4200	18.3150	24.9867
0.1-Morin	-5.9500	-2.8433	-1.9500	0.0000	8.8467	13.6233	14.2600	15.4700	16.3650	23.0367
0.1-Flavone	-14.7967	-11.6900	-10.7967	-8.8467	0.0000	4.7767	5.4133	6.6233	7.5183	14.1900
100-Morin	-19.5733	-16.4667	-15.5733	-13.6233	-4.7767	0.0000	0.6367	1.8467	2.7417	9.4133
Rt-extracts-M	-20.2100	-17.1033	-16.2100	-14.2600	-5.4133	-0.6367	0.0000	1.2100	2.1050	8.7767
1-Flavone	-21.4200	-18.3133	-17.4200	-15.4700	-6.6233	-1.8467	-1.2100	0.0000	0.8950	7.5667
100-Flavone	-22.3150	-19.2083	-18.3150	-16.3650	-7.5183	-2.7417	-2.1050	-0.8950	0.0000	6.6717
10-Flavone	-28.9867	-25.8800	-24.9867	-23.0367	-14.1900	-9.4133	-8.7767	-7.5667	-6.6717	0.0000

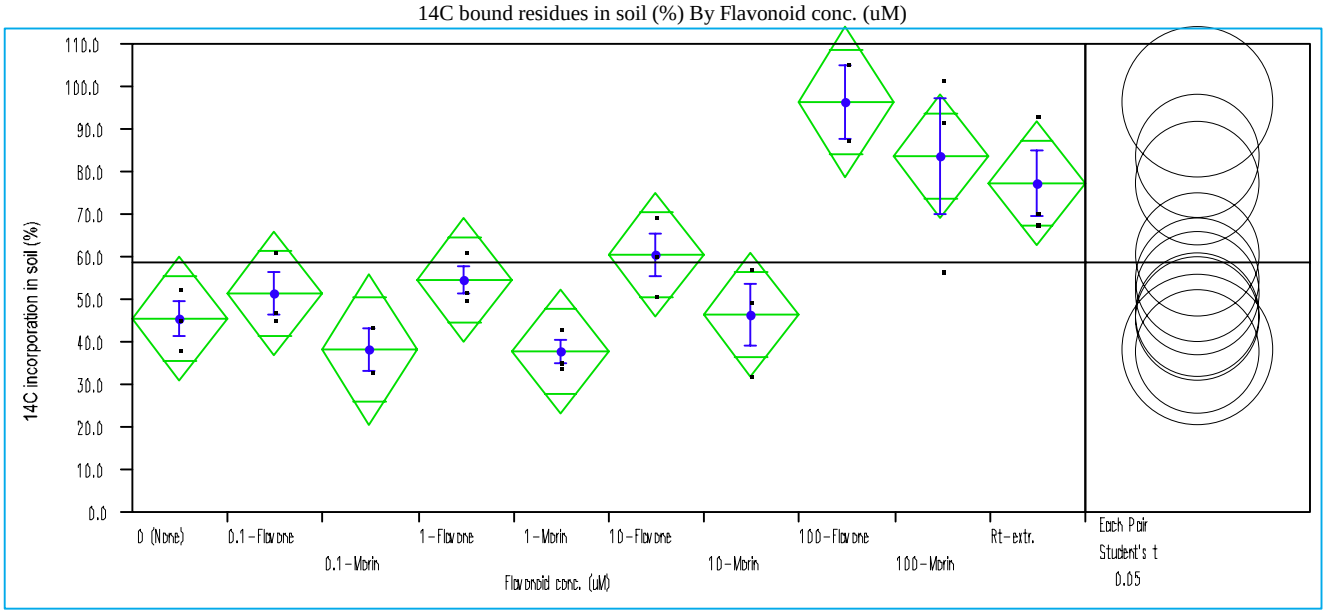
Alpha= 0.05

Comparisons for each pair using Student's t

t 2.10091

Abs(Dif)-LSD	0 (None)	10-Morin	1-Morin	0.1-Morin	0.1-Flavone	100-Morin	Rt-extracts-M	1-Flavone	100-Flavone	10-Flavone
0 (None)	-20.6069	-17.5002	-16.6069	-17.0892	-5.8102	-1.0336	-0.3969	0.8131	-0.7242	8.3798
10-Morin	-17.5002	-20.6069	-19.7136	-20.1959	-8.9169	-4.1402	-3.5036	-2.2936	-3.8309	5.2731
1-Morin	-16.6069	-19.7136	-20.6069	-21.0892	-9.8102	-5.0336	-4.3969	-3.1869	-4.7242	4.3798
0.1-Morin	-17.0892	-20.1959	-21.0892	-25.2382	-14.1925	-9.4159	-8.7792	-7.5692	-8.8732	-0.0025
0.1-Flavone	-5.8102	-8.9169	-9.8102	-14.1925	-20.6069	-15.8302	-15.1936	-13.9836	-15.5209	-6.4169
100-Morin	-1.0336	-4.1402	-5.0336	-9.4159	-15.8302	-20.6069	-19.9702	-18.7602	-20.2975	-11.1936
Rt-extracts-M	-0.3969	-3.5036	-4.3969	-8.7792	-15.1936	-19.9702	-20.6069	-19.3969	-20.9342	-11.8302
1-Flavone	**0.8131**	-2.2936	-3.1869	-7.5692	-13.9836	-18.7602	-19.3969	-20.6069	-22.1442	-13.0402
100-Flavone	-0.7242	-3.8309	-4.7242	-8.8732	-15.5209	-20.2975	-20.9342	-22.1442	-25.2382	-16.3675
10-Flavone	**8.3798**	5.2731	4.3798	-0.0025	-6.4169	-11.1936	-11.8302	-13.0402	-16.3675	-20.6069

Positive values show pairs of means that are significantly different.



Oneway Anova
Summary of Fit

RSquare	0.776671
RSquare Adj	0.665006
Root Mean Square Error	12.03015
Mean of Response	58.75464
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	9059.532	1006.61	6.9554
Error	18	2605.041	144.72	Prob>F
C Total	27	11664.572	432.02	0.0003

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	45.7533	6.9456
0.1-Flavone	3	51.7367	6.9456
0.1-Morin	2	38.5700	8.5066
1-Flavone	3	54.5600	6.9456
1-Morin	3	37.8867	6.9456
10-Flavone	3	60.5767	6.9456

10-Morin	3	46.6500	6.9456
100-Flavone	2	96.6800	8.5066
100-Morin	3	83.7167	6.9456
Rt-extracts-M	3	77.3300	6.9456

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	45.7533	7.3101	4.221
0.1-Flavone	3	51.7367	8.7153	5.032
0.1-Morin	2	38.5700	7.2549	5.130
1-Flavone	3	54.5600	5.9840	3.455
1-Morin	3	37.8867	4.8835	2.819
10-Flavone	3	60.5767	9.4001	5.427
10-Morin	3	46.6500	12.7360	7.353
100-Flavone	2	96.6800	12.4734	8.820
100-Morin	3	83.7167	23.7190	13.694
Rt-extracts-M	3	77.3300	14.0071	8.087

Means Comparisons

Dif=Mean[i]-Mean[j]	100-Flavone	100-Morin	Rt-extracts-M	10-Flavone	1-Flavone	0.1-Flavone	10-Morin	0 (None)	0.1-Morin	1-Morin
100-Flavone	0.0000	12.9633	19.3500	36.1033	42.1200	44.9433	50.0300	50.9267	58.1100	58.7933
100-Morin	-12.9633	0.0000	6.3867	23.1400	29.1567	31.9800	37.0667	37.9633	45.1467	45.8300
Rt-extracts-M	-19.3500	-6.3867	0.0000	16.7533	22.7700	25.5933	30.6800	31.5767	38.7600	39.4433
10-Flavone	-36.1033	-23.1400	-16.7533	0.0000	6.0167	8.8400	13.9267	14.8233	22.0067	22.6900
1-Flavone	-42.1200	-29.1567	-22.7700	-6.0167	0.0000	2.8233	7.9100	8.8067	15.9900	16.6733
0.1-Flavone	-44.9433	-31.9800	-25.5933	-8.8400	-2.8233	0.0000	5.0867	5.9833	13.1667	13.8500
10-Morin	-50.0300	-37.0667	-30.6800	-13.9267	-7.9100	-5.0867	0.0000	0.8967	8.0800	8.7633
0 (None)	-50.9267	-37.9633	-31.5767	-14.8233	-8.8067	-5.9833	-0.8967	0.0000	7.1833	7.8667
0.1-Morin	-58.1100	-45.1467	-38.7600	-22.0067	-15.9900	-13.1667	-8.0800	-7.1833	0.0000	0.6833
1-Morin	-58.7933	-45.8300	-39.4433	-22.6900	-16.6733	-13.8500	-8.7633	-7.8667	-0.6833	0.0000

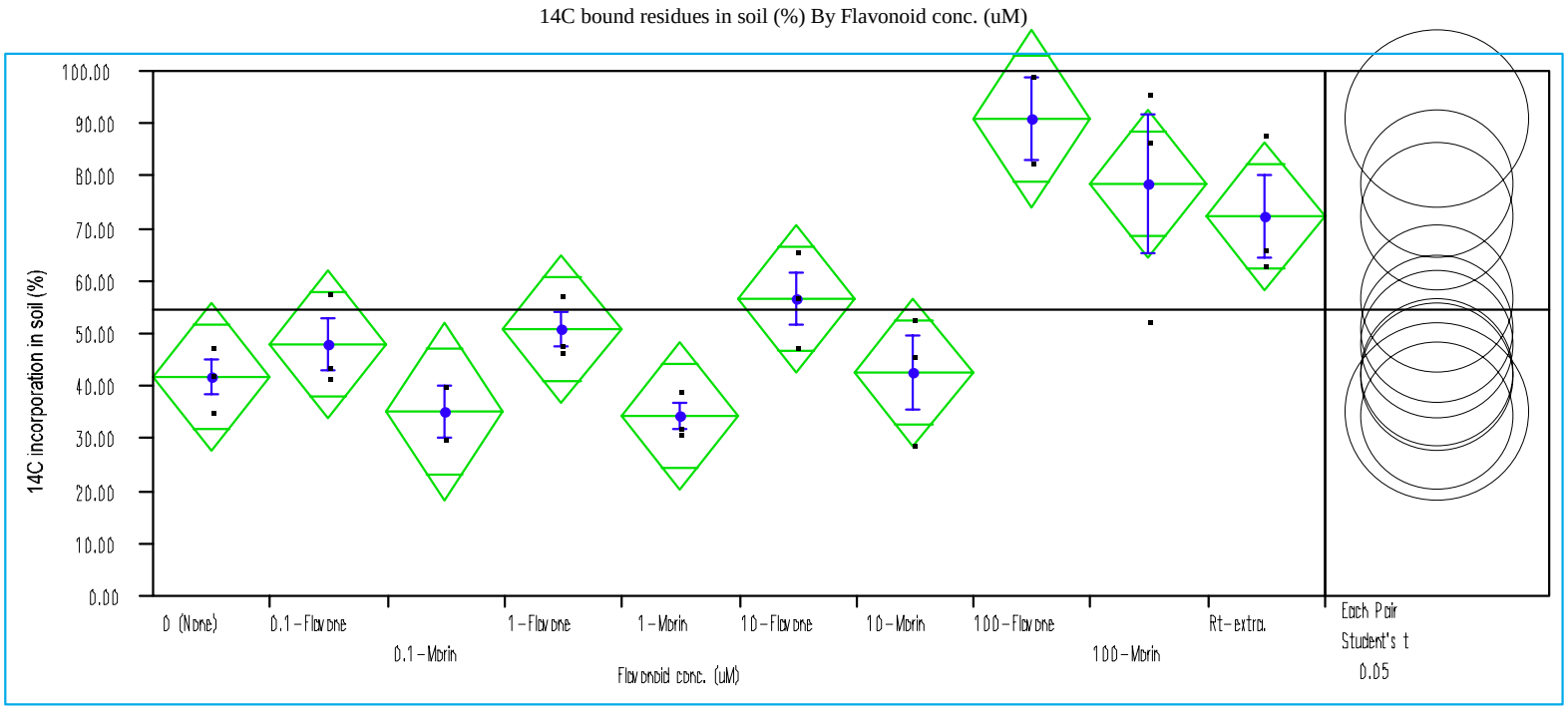
Alpha= 0.05

Comparisons for each pair using Student's t

T 2.10091

Abs(Dif)-LSD	100-Flavone	100-Morin	Rt-extracts-M	10-Flavone	1-Flavone	0.1-Flavone	10-Morin	0 (None)	0.1-Morin	1-Morin
100-Flavone	-25.2742	-10.1088	-3.7221	13.0312	19.0479	21.8712	26.9579	27.8546	32.8358	35.7212
100-Morin	-10.1088	-20.6363	-14.2497	2.5037	8.5203	11.3437	16.4303	17.3270	22.0746	25.1937
Rt-extracts-M	-3.7221	-14.2497	-20.6363	-3.8830	2.1337	4.9570	10.0437	10.9403	15.6879	18.8070
10-Flavone	13.0312	2.5037	-3.8830	-20.6363	-14.6197	-11.7963	-6.7097	-5.8130	-1.0654	2.0537
1-Flavone	19.0479	8.5203	2.1337	-14.6197	-20.6363	-17.8130	-12.7263	-11.8297	-7.0821	-3.9630
0.1-Flavone	21.8712	11.3437	4.9570	-11.7963	-17.8130	-20.6363	-15.5497	-14.6530	-9.9054	-6.7863
10-Morin	26.9579	16.4303	10.0437	-6.7097	-12.7263	-15.5497	-20.6363	-19.7397	-14.9921	-11.8730
0 (None)	27.8546	17.3270	10.9403	-5.8130	-11.8297	-14.6530	-19.7397	-20.6363	-15.8888	-12.7697
0.1-Morin	32.8358	22.0746	15.6879	-1.0654	-7.0821	-9.9054	-14.9921	-15.8888	-25.2742	-22.3888
1-Morin	35.7212	25.1937	18.8070	2.0537	-3.9630	-6.7863	-11.8730	-12.7697	-22.3888	-20.6363

Positive values show pairs of means that are significantly different.



Oneway Anova

Summary of Fit

RSquare	0.775496
RSquare Adj	0.663245
Root Mean Square Error	11.6648
Mean of Response	54.97429
Observations (or Sum Wgts)	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	8460.265	940.029	6.9085
Error	18	2449.217	136.068	Prob>F
C Total	27	10909.482	404.055	0.0003

Means for Oneway Anova

Level	Number	Mean	Std Error
0 (None)	3	42.0700	6.7347
0.1-Flavone	3	48.3133	6.7347
0.1-Morin	2	35.4400	8.2483

1-Flavone	3	51.2433	6.7347
1-Morin	3	34.7200	6.7347
10-Flavone	3	57.2567	6.7347
10-Morin	3	43.0400	6.7347
100-Flavone	2	91.4250	8.2483
100-Morin	3	78.9700	6.7347
Rt-extracts-M	3	72.9033	6.7347

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	42.0700	6.3381	3.659
0.1-Flavone	3	48.3133	8.8578	5.114
0.1-Morin	2	35.4400	7.0852	5.010
1-Flavone	3	51.2433	5.8834	3.397
1-Morin	3	34.7200	4.5279	2.614
10-Flavone	3	57.2567	9.1205	5.266
10-Morin	3	43.0400	12.5475	7.244
100-Flavone	2	91.4250	11.6178	8.215
100-Morin	3	78.9700	23.0524	13.309
Rt-extracts-M	3	72.9033	13.6469	7.879

Means Comparisons

Dif=Mean[i]-Mean[j]	100-Flavone	100-Morin	Rt-extracts-M	10-Flavone	1-Flavone	0.1-Flavone	10-Morin	0 (None)	0.1-Morin	1-Morin
100-Flavone	0.0000	12.4550	18.5217	34.1683	40.1817	43.1117	48.3850	49.3550	55.9850	56.7050
100-Morin	-12.4550	0.0000	6.0667	21.7133	27.7267	30.6567	35.9300	36.9000	43.5300	44.2500
Rt-extracts-M	-18.5217	-6.0667	0.0000	15.6467	21.6600	24.5900	29.8633	30.8333	37.4633	38.1833
10-Flavone	-34.1683	-21.7133	-15.6467	0.0000	6.0133	8.9433	14.2167	15.1867	21.8167	22.5367
1-Flavone	-40.1817	-27.7267	-21.6600	-6.0133	0.0000	2.9300	8.2033	9.1733	15.8033	16.5233
0.1-Flavone	-43.1117	-30.6567	-24.5900	-8.9433	-2.9300	0.0000	5.2733	6.2433	12.8733	13.5933
10-Morin	-48.3850	-35.9300	-29.8633	-14.2167	-8.2033	-5.2733	0.0000	0.9700	7.6000	8.3200
0 (None)	-49.3550	-36.9000	-30.8333	-15.1867	-9.1733	-6.2433	-0.9700	0.0000	6.6300	7.3500
0.1-Morin	-55.9850	-43.5300	-37.4633	-21.8167	-15.8033	-12.8733	-7.6000	-6.6300	0.0000	0.7200
1-Morin	-56.7050	-44.2500	-38.1833	-22.5367	-16.5233	-13.5933	-8.3200	-7.3500	-0.7200	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

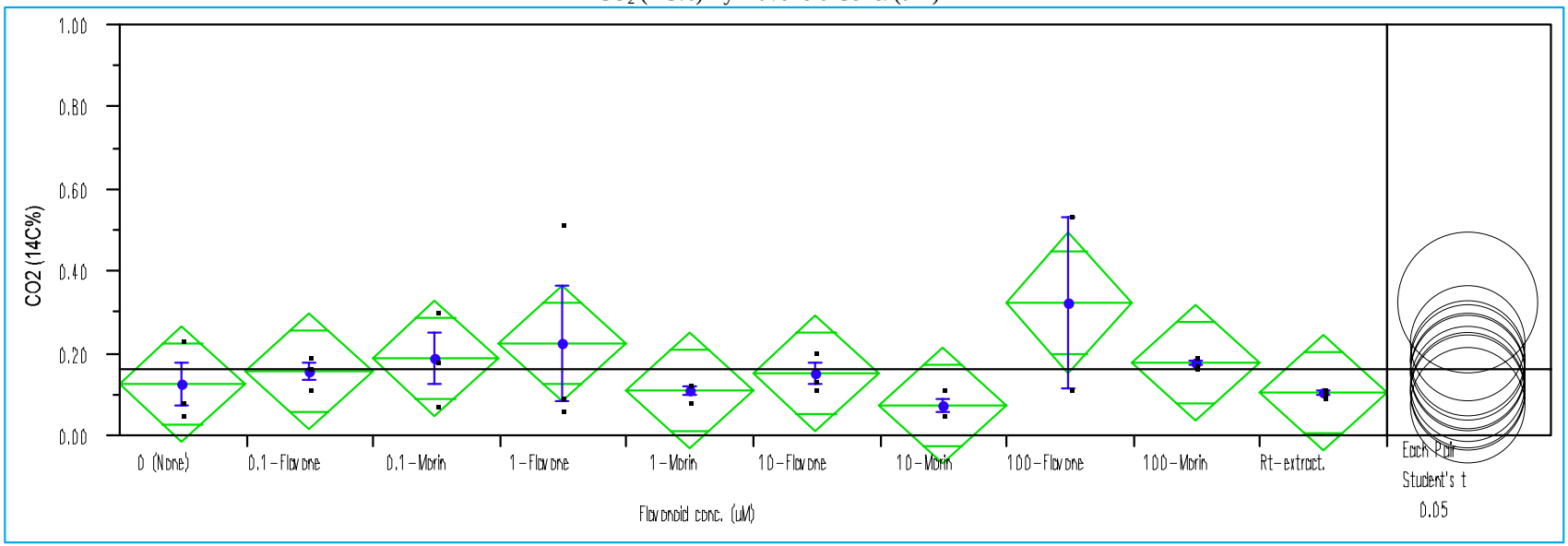
t 2.10091

Abs(Dif)-LSD	100-Flavone	100-Morin	Rt-extracts-M	10-Flavone	1-Flavone	0.1-Flavone	10-Morin	0 (None)	0.1-Morin	1-Morin
100-Flavone	-24.5067	-9.9164	-3.8498	11.7969	17.8102	20.7402	26.0136	**26.9836**	31.4783	34.3336
100-Morin	-9.9164	-20.0096	-13.9430	1.7037	7.7170	10.6470	15.9204	**16.8904**	21.1586	24.2404
Rt-extracts-M	-3.8498	-13.9430	-20.0096	-4.3630	1.6504	4.5804	9.8537	**10.8237**	15.0919	18.1737
10-Flavone	11.7969	1.7037	-4.3630	-20.0096	-13.9963	-11.0663	-5.7930	-4.8230	-0.5548	2.5270
1-Flavone	17.8102	7.7170	1.6504	-13.9963	-20.0096	-17.0796	-11.8063	-10.8363	-6.5681	-3.4863
0.1-Flavone	20.7402	10.6470	4.5804	-11.0663	-17.0796	-20.0096	-14.7363	-13.7663	-9.4981	-6.4163
10-Morin	26.0136	15.9204	9.8537	-5.7930	-11.8063	-14.7363	-20.0096	-19.0396	-14.7714	-11.6896
0 (None)	26.9836	16.8904	10.8237	-4.8230	-10.8363	-13.7663	-19.0396	-20.0096	-15.7414	-12.6596
0.1-Morin	31.4783	21.1586	15.0919	-0.5548	-6.5681	-9.4981	-14.7714	-15.7414	-24.5067	-21.6514
1-Morin	34.3336	24.2404	18.1737	2.5270	-3.4863	-6.4163	-11.6896	-12.6596	-21.6514	-20.0096

Positive values show pairs of means that are significantly different.

Appendix D-4. Student's t Test: Paired Comparison of Mean ^{14}C-Pyrene Fate Data in Poisoned Mulberry Rhizosphere Soil with or without Flavonoid Amendment [1]

CO_2 (^{14}C%) By Flavonoid Conc. (uM) [2]



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean

[1] The means comparison indicates whether the actual difference in the means is greater than the least significant difference (LSD).

[2] The left side chart show data points, group data mean dots, standard error bars, and 95% confidence interval diamond.

The horizontal line cross the chart is the mean of all sample data. The righ side chart shows comparison circles. LSD is what the distance would be if the two mean circles intersected at right angles. Circles for means that are significantly different either do not intersect or intersect slightly so that the outside angle of intersection is <90°. If the circles intersect by an outside angle of >90° or if they are nested, the means are not significantly different.

0 (None)	3	0.130000	0.096437	0.05568
0.1-Flavone	3	0.163333	0.040415	0.02333
0.1-Morin	3	0.193333	0.115036	0.06642
1-Flavone	3	0.230000	0.251595	0.14526
1-Morin	3	0.116667	0.023094	0.01333
10-Flavone	3	0.156667	0.047258	0.02728
10-Morin	3	0.080000	0.034641	0.02000
100-Flavone	2	0.330000	0.296985	0.21000
100-Morin	3	0.183333	0.015275	0.00882
Rt-extracts-M	3	0.110000	0.010000	0.00577

Means Comparisons

Dif=Mean[i]-Mean[j]	100-Flavone	1-Flavone	0.1-Morin	100-Morin	0.1-Flavone	10-Flavone	0 (None)	1-Morin	Rt-extracts-M	10-Morin
100-Flavone	0.000000	0.100000	0.136667	0.146667	0.166667	0.173333	0.200000	0.213333	0.220000	0.250000
1-Flavone	-0.1	0.000000	0.036667	0.046667	0.066667	0.073333	0.100000	0.113333	0.120000	0.150000
0.1-Morin	-0.13667	-0.03667	0.000000	0.010000	0.030000	0.036667	0.063333	0.076667	0.083333	0.113333
100-Morin	-0.14667	-0.04667	-0.01	0.000000	0.020000	0.026667	0.053333	0.066667	0.073333	0.103333
0.1-Flavone	-0.16667	-0.06667	-0.03	-0.02	0.000000	0.006667	0.033333	0.046667	0.053333	0.083333
10-Flavone	-0.17333	-0.07333	-0.03667	-0.02667	-0.00667	0.000000	0.026667	0.040000	0.046667	0.076667
0 (None)	-0.2	-0.1	-0.06333	-0.05333	-0.03333	-0.02667	0.000000	0.013333	0.020000	0.050000
1-Morin	-0.21333	-0.11333	-0.07667	-0.06667	-0.04667	-0.04	-0.01333	0.000000	0.006667	0.036667
Rt-extracts-M	-0.22	-0.12	-0.08333	-0.07333	-0.05333	-0.04667	-0.02	-0.00667	0.000000	0.030000
10-Morin	-0.25	-0.15	-0.11333	-0.10333	-0.08333	-0.07667	-0.05	-0.03667	-0.03	0.000000
Alpha=	0.05									

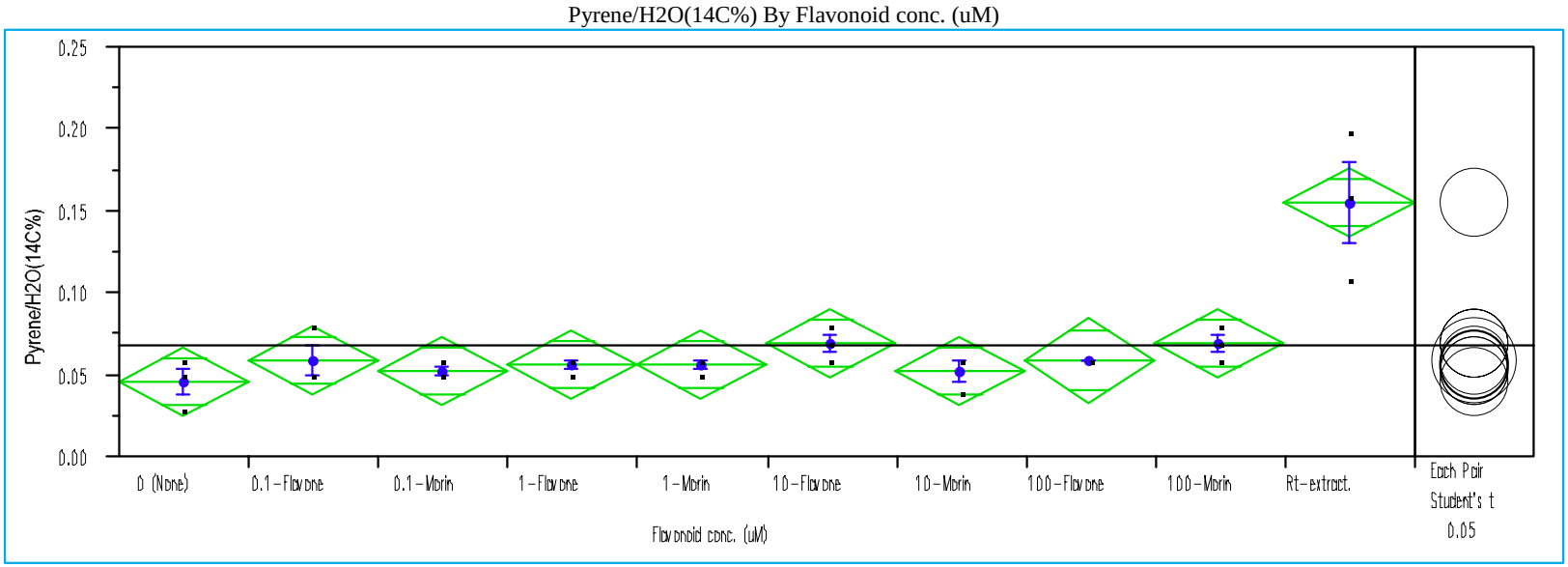
Comparisons for each pair using Student's t

t

2.09301

Abs(Dif)-LSD	100-Flavone	1-Flavone	0.1-Morin	100-Morin	0.1-Flavone	10-Flavone	0 (None)	1-Morin	Rt-extracts-M	10-Morin
100-Flavone	-0.2503	-0.12849	-0.09183	-0.08183	-0.06183	-0.05516	-0.02849	-0.01516	-0.00849	0.021506
1-Flavone	-0.12849	-0.20437	-0.1677	-0.1577	-0.1377	-0.13104	-0.10437	-0.09104	-0.08437	-0.05437
0.1-Morin	-0.09183	-0.1677	-0.20437	-0.19437	-0.17437	-0.1677	-0.14104	-0.1277	-0.12104	-0.09104
100-Morin	-0.08183	-0.1577	-0.19437	-0.20437	-0.18437	-0.1777	-0.15104	-0.1377	-0.13104	-0.10104
0.1-Flavone	-0.06183	-0.1377	-0.17437	-0.18437	-0.20437	-0.1977	-0.17104	-0.1577	-0.15104	-0.12104
10-Flavone	-0.05516	-0.13104	-0.1677	-0.1777	-0.1977	-0.20437	-0.1777	-0.16437	-0.1577	-0.1277
0 (None)	-0.02849	-0.10437	-0.14104	-0.15104	-0.17104	-0.1777	-0.20437	-0.19104	-0.18437	-0.15437
1-Morin	-0.01516	-0.09104	-0.1277	-0.1377	-0.1577	-0.16437	-0.19104	-0.20437	-0.1977	-0.1677
Rt-extracts-M	-0.00849	-0.08437	-0.12104	-0.13104	-0.15104	-0.1577	-0.18437	-0.1977	-0.20437	-0.17437
10-Morin	0.021506	-0.05437	-0.09104	-0.10104	-0.12104	-0.1277	-0.15437	-0.1677	-0.17437	-0.20437

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	0.046667	0.015275	0.00882
0.1-Flavone	3	0.060000	0.017321	0.01000
0.1-Morin	3	0.053333	0.005774	0.00333
1-Flavone	3	0.056667	0.005774	0.00333
1-Morin	3	0.056667	0.005774	0.00333
10-Flavone	3	0.070000	0.010000	0.00577
10-Morin	3	0.053333	0.011547	0.00667
100-Flavone	2	0.060000	0.000000	0.00000
100-Morin	3	0.070000	0.010000	0.00577
Rt-extracts-M	3	0.156667	0.045092	0.02603

Means Comparisons

Dif=Mean[i]-Mean[j]	Rt-extracts-M	100-Morin	10-Flavone	0.1-Flavone	100-Flavone	1-Morin	1-Flavone	0.1-Morin	10-Morin	0 (None)
Rt-extracts-M	0.000000	0.086667	0.086667	0.096667	0.096667	0.100000	0.100000	0.103333	0.103333	0.110000
100-Morin	-0.08667	0.000000	0.000000	0.010000	0.010000	0.013333	0.013333	0.016667	0.016667	0.023333
10-Flavone	-0.08667	0.000000	0.000000	0.010000	0.010000	0.013333	0.013333	0.016667	0.016667	0.023333
0.1-Flavone	-0.09667	-0.01	-0.01	0.000000	0.000000	0.003333	0.003333	0.006667	0.006667	0.013333
100-Flavone	-0.09667	-0.01	-0.01	0.000000	0.000000	0.003333	0.003333	0.006667	0.006667	0.013333
1-Morin	-0.1	-0.01333	-0.01333	-0.00333	-0.00333	0.000000	0.000000	0.003333	0.003333	0.010000
1-Flavone	-0.1	-0.01333	-0.01333	-0.00333	-0.00333	0.000000	0.000000	0.003333	0.003333	0.010000
0.1-Morin	-0.10333	-0.01667	-0.01667	-0.00667	-0.00667	-0.00333	-0.00333	0.000000	0.000000	0.006667
10-Morin	-0.10333	-0.01667	-0.01667	-0.00667	-0.00667	-0.00333	-0.00333	0.000000	0.000000	0.006667
0 (None)	-0.11	-0.02333	-0.02333	-0.01333	-0.01333	-0.01	-0.01	-0.00667	-0.00667	0.000000

Alpha= 0.05

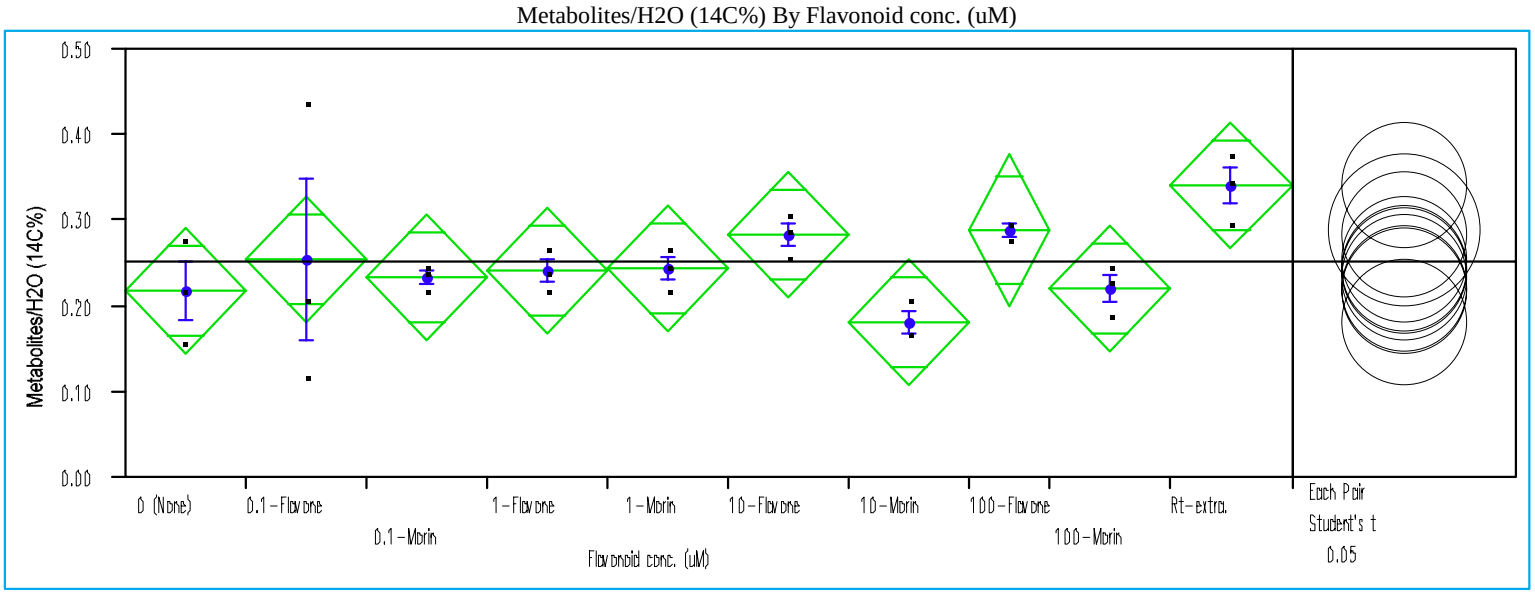
Comparisons for each pair using Student's t

t

2.09301

Abs(Dif)-LSD	Rt-extracts-M	100-Morin	10-Flavone	0.1-Flavone	100-Flavone	1-Morin	1-Flavone	0.1-Morin	10-Morin	0 (None)
Rt-extracts-M	-0.03037	0.056298	0.056298	0.066298	0.062714	0.069631	0.069631	0.072965	0.072965	0.079631
100-Morin	0.056298	-0.03037	-0.03037	-0.02037	-0.02395	-0.01704	-0.01704	-0.0137	-0.0137	-0.00704
10-Flavone	0.056298	-0.03037	-0.03037	-0.02037	-0.02395	-0.01704	-0.01704	-0.0137	-0.0137	-0.00704
0.1-Flavone	0.066298	-0.02037	-0.02037	-0.03037	-0.03395	-0.02704	-0.02704	-0.0237	-0.0237	-0.01704
100-Flavone	0.062714	-0.02395	-0.02395	-0.03395	-0.03719	-0.03062	-0.03062	-0.02729	-0.02729	-0.02062
1-Morin	0.069631	-0.01704	-0.01704	-0.02704	-0.03062	-0.03037	-0.03037	-0.02704	-0.02704	-0.02037
1-Flavone	0.069631	-0.01704	-0.01704	-0.02704	-0.03062	-0.03037	-0.03037	-0.02704	-0.02704	-0.02037
0.1-Morin	0.072965	-0.0137	-0.0137	-0.0237	-0.02729	-0.02704	-0.02704	-0.03037	-0.03037	-0.0237
10-Morin	0.072965	-0.0137	-0.0137	-0.0237	-0.02729	-0.02704	-0.02704	-0.03037	-0.03037	-0.0237
0 (None)	0.079631	-0.00704	-0.00704	-0.01704	-0.02062	-0.02037	-0.02037	-0.0237	-0.0237	-0.03037

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	0.220000	0.060000	0.03464
0.1-Flavone	3	0.256667	0.165025	0.09528
0.1-Morin	3	0.236667	0.015275	0.00882
1-Flavone	3	0.243333	0.025166	0.01453
1-Morin	3	0.246667	0.025166	0.01453
10-Flavone	3	0.286667	0.025166	0.01453
10-Morin	3	0.183333	0.023094	0.01333
100-Flavone	2	0.290000	0.014142	0.01000
100-Morin	3	0.223333	0.030551	0.01764
Rt-extracts-M	3	0.343333	0.040415	0.02333

Means Comparisons

Dif=Mean[i]-Mean[j]	Rt-extracts-M	100-Flavone	10-Flavone	0.1-Flavone	1-Morin	1-Flavone	0.1-Morin	100-Morin	0 (None)	10-Morin
Rt-extracts-M	0.000000	0.053333	0.056667	0.086667	0.096667	0.100000	0.106667	0.120000	0.123333	0.160000
100-Flavone	-0.05333	0.000000	0.003333	0.033333	0.043333	0.046667	0.053333	0.066667	0.070000	0.106667
10-Flavone	-0.05667	-0.00333	0.000000	0.030000	0.040000	0.043333	0.050000	0.063333	0.066667	0.103333
0.1-Flavone	-0.08667	-0.03333	-0.03	0.000000	0.010000	0.013333	0.020000	0.033333	0.036667	0.073333
1-Morin	-0.09667	-0.04333	-0.04	-0.01	0.000000	0.003333	0.010000	0.023333	0.026667	0.063333
1-Flavone	-0.1	-0.04667	-0.04333	-0.01333	-0.00333	0.000000	0.006667	0.020000	0.023333	0.060000
0.1-Morin	-0.10667	-0.05333	-0.05	-0.02	-0.01	-0.00667	0.000000	0.013333	0.016667	0.053333
100-Morin	-0.12	-0.06667	-0.06333	-0.03333	-0.02333	-0.02	-0.01333	0.000000	0.003333	0.040000
0 (None)	-0.12333	-0.07	-0.06667	-0.03667	-0.02667	-0.02333	-0.01667	-0.00333	0.000000	0.036667
10-Morin	-0.16	-0.10667	-0.10333	-0.07333	-0.06333	-0.06	-0.05333	-0.04	-0.03667	0.000000

Alpha= 0.05

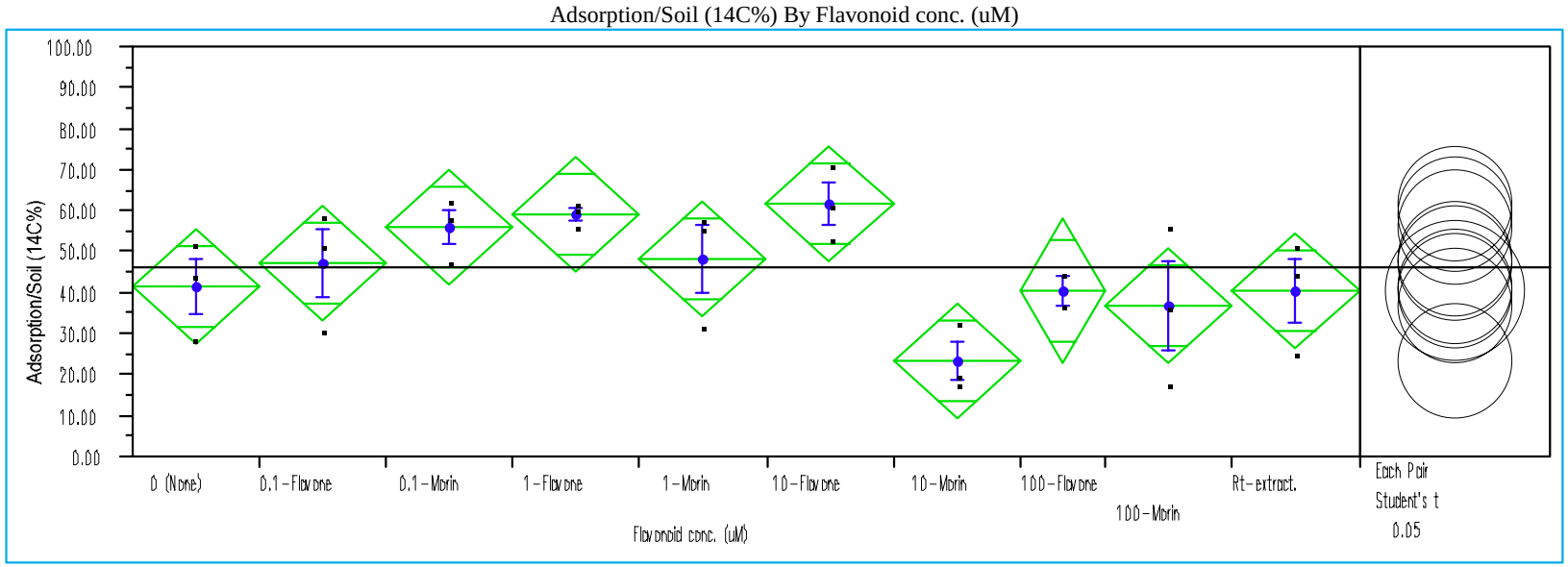
Comparisons for each pair using Student's t

t

2.09301

Abs(Dif)-LSD	Rt-extracts-M	100-Flavone	10-Flavone	0.1-Flavone	1-Morin	1-Flavone	0.1-Morin	100-Morin	0 (None)	10-Morin
Rt-extracts-M	-0.10544	-0.06456	-0.04878	-0.01878	-0.00878	-0.00544	0.001224	0.014557	0.017890	0.054557
100-Flavone	-0.06456	-0.12914	-0.11456	-0.08456	-0.07456	-0.07122	-0.06456	-0.05122	-0.04789	-0.01122
10-Flavone	-0.04878	-0.11456	-0.10544	-0.07544	-0.06544	-0.06211	-0.05544	-0.04211	-0.03878	-0.00211
0.1-Flavone	-0.01878	-0.08456	-0.07544	-0.10544	-0.09544	-0.09211	-0.08544	-0.07211	-0.06878	-0.03211
1-Morin	-0.00878	-0.07456	-0.06544	-0.09544	-0.10544	-0.10211	-0.09544	-0.08211	-0.07878	-0.04211
1-Flavone	-0.00544	-0.07122	-0.06211	-0.09211	-0.10211	-0.10544	-0.09878	-0.08544	-0.08211	-0.04544
0.1-Morin	0.001224	-0.06456	-0.05544	-0.08544	-0.09544	-0.09878	-0.10544	-0.09211	-0.08878	-0.05211
100-Morin	0.014557	-0.05122	-0.04211	-0.07211	-0.08211	-0.08544	-0.09211	-0.10544	-0.10211	-0.06544
0 (None)	0.017890	-0.04789	-0.03878	-0.06878	-0.07878	-0.08211	-0.08878	-0.10211	-0.10544	-0.06878
10-Morin	0.054557	-0.01122	-0.00211	-0.03211	-0.04211	-0.04544	-0.05211	-0.06544	-0.06878	-0.10544

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	42.5000	12.0200	6.940
0.1-Flavone	3	47.9667	14.6050	8.432
0.1-Morin	3	56.7533	7.6452	4.414
1-Flavone	3	60.2700	2.7364	1.580
1-Morin	3	49.1300	14.5157	8.381
10-Flavone	3	62.5133	9.1562	5.286
10-Morin	3	24.0800	8.2701	4.775
100-Flavone	2	41.5050	5.6074	3.965
100-Morin	3	37.6600	19.2864	11.135
Rt-extracts-M	3	41.2200	13.6942	7.906

Means Comparisons

Dif=Mean[i]-Mean[j]	10-Flavone	1-Flavone	0.1-Morin	1-Morin	0.1-Flavone	0 (None)	100-Flavone	Rt-extracts-M	100-Morin	10-Morin
10-Flavone	0.0000	2.2433	5.7600	13.3833	14.5467	20.0133	21.0083	21.2933	24.8533	38.4333
1-Flavone	-2.2433	0.0000	3.5167	11.1400	12.3033	17.7700	18.7650	19.0500	22.6100	36.1900
0.1-Morin	-5.7600	-3.5167	0.0000	7.6233	8.7867	14.2533	15.2483	15.5333	19.0933	32.6733
1-Morin	-13.3833	-11.1400	-7.6233	0.0000	1.1633	6.6300	7.6250	7.9100	11.4700	25.0500
0.1-Flavone	-14.5467	-12.3033	-8.7867	-1.1633	0.0000	5.4667	6.4617	6.7467	10.3067	23.8867
0 (None)	20.0133	-17.7700	-14.2533	-6.6300	-5.4667	0.0000	0.9950	1.2800	4.8400	18.4200
100-Flavone	-21.0083	-18.7650	-15.2483	-7.6250	-6.4617	-0.9950	0.0000	0.2850	3.8450	17.4250
Rt-extracts-M	-21.2933	-19.0500	-15.5333	-7.9100	-6.7467	-1.2800	-0.2850	0.0000	3.5600	17.1400
100-Morin	-24.8533	-22.6100	-19.0933	-11.4700	-10.3067	-4.8400	-3.8450	-3.5600	0.0000	13.5800
10-Morin	-38.4333	-36.1900	-32.6733	-25.0500	-23.8867	-18.4200	-17.4250	-17.1400	-13.5800	0.0000

Alpha= 0.05

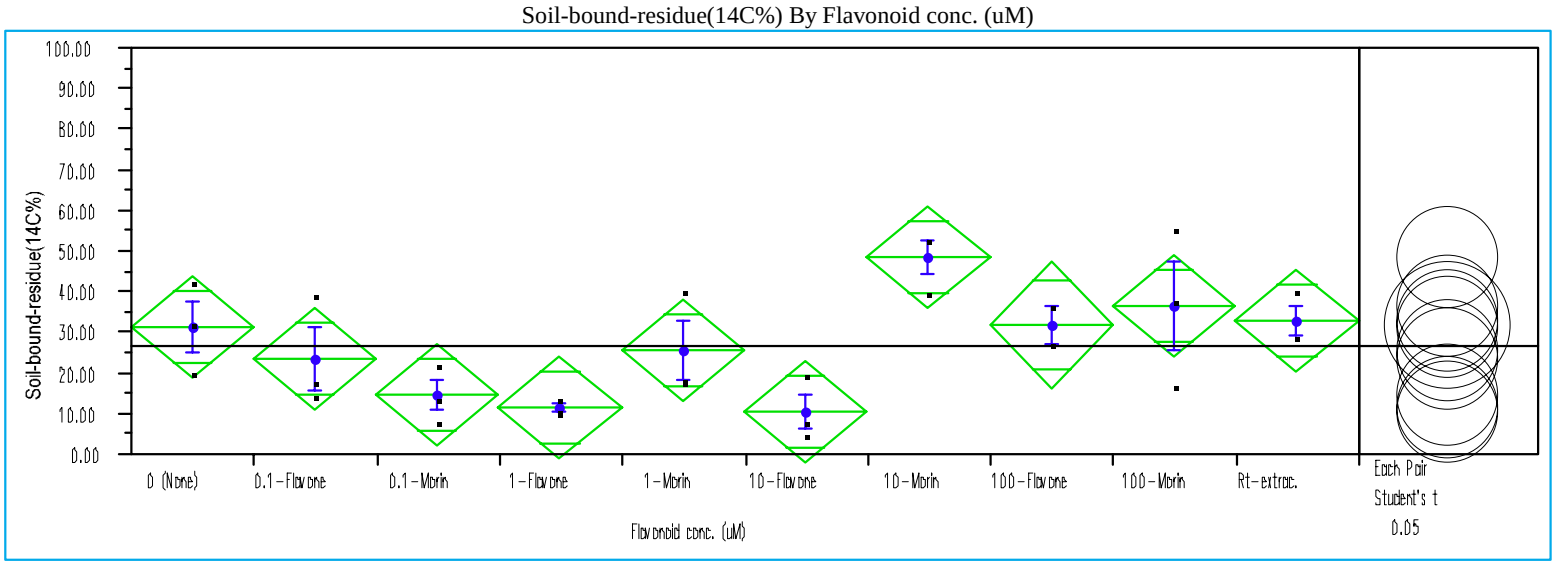
Comparisons for each pair using Student's t

t

2.09301

Abs(Dif)-LSD	10-Flavone	1-Flavone	0.1-Morin	1-Morin	0.1-Flavone	0 (None)	100-Flavone	Rt-extracts-M	100-Morin	10-Morin
10-Flavone	-20.4613	-18.2179	-14.7013	-7.0779	-5.9146	-0.4479	-1.8681	0.8321	4.3921	17.9721
1-Flavone	-18.2179	-20.4613	-16.9446	-9.3213	-8.1579	-2.6913	-4.1114	-1.4113	2.1487	15.7287
0.1-Morin	-14.7013	-16.9446	-20.4613	-12.8379	-11.6746	-6.2079	-7.6281	-4.9279	-1.3679	12.2121
1-Morin	-7.0779	-9.3213	-12.8379	-20.4613	-19.2979	-13.8313	-15.2514	-12.5513	-8.9913	4.5887
0.1-Flavone	-5.9146	-8.1579	-11.6746	-19.2979	-20.4613	-14.9946	-16.4147	-13.7146	-10.1546	3.4254
0 (None)	-0.4479	-2.6913	-6.2079	-13.8313	-14.9946	-20.4613	-21.8814	-19.1813	-15.6213	-2.0413
100-Flavone	-1.8681	-4.1114	-7.6281	-15.2514	-16.4147	-21.8814	-25.0598	-22.5914	-19.0314	-5.4514
Rt-extracts-M	0.8321	-1.4113	-4.9279	-12.5513	-13.7146	-19.1813	-22.5914	-20.4613	-16.9013	-3.3213
100-Morin	4.3921	2.1487	-1.3679	-8.9913	-10.1546	-15.6213	-19.0314	-16.9013	-20.4613	-6.8813
10-Morin	17.9721	15.7287	12.2121	4.5887	3.4254	-2.0413	-5.4514	-3.3213	-6.8813	-20.4613

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	31.5467	11.2537	6.497
0.1-Flavone	3	23.8533	13.5337	7.814
0.1-Morin	3	14.7833	7.1316	4.117
1-Flavone	3	11.5500	2.0511	1.184
1-Morin	3	25.6267	12.9244	7.462
10-Flavone	3	10.7133	7.7494	4.474
10-Morin	3	48.5500	7.6745	4.431
100-Flavone	2	31.8850	6.6397	4.695
100-Morin	3	36.8567	19.2070	11.089
Rt-extracts-M	3	32.8867	6.4981	3.752

Means Comparisons

Dif=Mean[i]-Mean[j]	10-Morin	100-Morin	Rt-extracts-M	100-Flavone	0 (None)	1-Morin	0.1-Flavone	0.1-Morin	1-Flavone	10-Flavone
10-Morin	0.0000	11.6933	15.6633	16.6650	17.0033	22.9233	24.6967	33.7667	37.0000	37.8367
100-Morin	-11.6933	0.0000	3.9700	4.9717	5.3100	11.2300	13.0033	22.0733	25.3067	26.1433
Rt-extracts-M	-15.6633	-3.9700	0.0000	1.0017	1.3400	7.2600	9.0333	18.1033	21.3367	22.1733
100-Flavone	-16.6650	-4.9717	-1.0017	0.0000	0.3383	6.2583	8.0317	17.1017	20.3350	21.1717
0 (None)	-17.0033	-5.3100	-1.3400	-0.3383	0.0000	5.9200	7.6933	16.7633	19.9967	20.8333
1-Morin	-22.9233	-11.2300	-7.2600	-6.2583	-5.9200	0.0000	1.7733	10.8433	14.0767	14.9133
0.1-Flavone	-24.6967	-13.0033	-9.0333	-8.0317	-7.6933	-1.7733	0.0000	9.0700	12.3033	13.1400
0.1-Morin	-33.7667	-22.0733	-18.1033	-17.1017	-16.7633	-10.8433	-9.0700	0.0000	3.2333	4.0700
1-Flavone	-37.0000	-25.3067	-21.3367	-20.3350	-19.9967	-14.0767	-12.3033	-3.2333	0.0000	0.8367
10-Flavone	-37.8367	-26.1433	-22.1733	-21.1717	-20.8333	-14.9133	-13.1400	-4.0700	-0.8367	0.0000

Alpha= 0.05

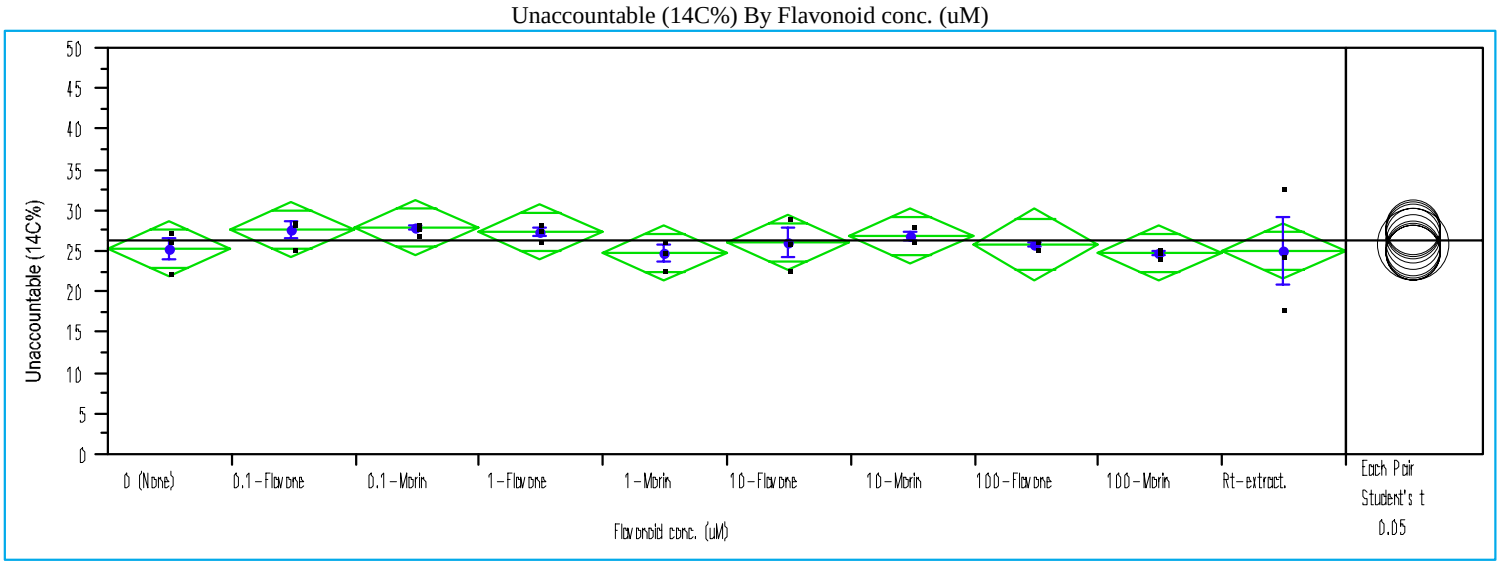
Comparisons for each pair using Student's t

t

2.09301

Abs(Dif)-LSD	10-Morin	100-Morin	Rt-extracts-M	100-Flavone	0 (None)	1-Morin	0.1-Flavone	0.1-Morin	1-Flavone	10-Flavone
10-Morin	-18.2552	-6.5619	-2.5919	-3.7449	-1.2519	4.6681	6.4415	15.5115	18.7448	19.5815
100-Morin	-6.5619	-18.2552	-14.2852	-15.4383	-12.9452	-7.0252	-5.2519	3.8181	7.0515	7.8881
Rt-extracts-M	-2.5919	-14.2852	-18.2552	-19.4083	-16.9152	-10.9952	-9.2219	-0.1519	3.0815	3.9181
100-Flavone	-3.7449	-15.4383	-19.4083	-22.3580	-20.0716	-14.1516	-12.3783	-3.3083	-0.0749	0.7617
0 (None)	-1.2519	-12.9452	-16.9152	-20.0716	-18.2552	-12.3352	-10.5619	-1.4919	1.7415	2.5781
1-Morin	4.6681	-7.0252	-10.9952	-14.1516	-12.3352	-18.2552	-16.4819	-7.4119	-4.1785	-3.3419
0.1-Flavone	6.4415	-5.2519	-9.2219	-12.3783	-10.5619	-16.4819	-18.2552	-9.1852	-5.9519	-5.1152
0.1-Morin	15.5115	3.8181	-0.1519	-3.3083	-1.4919	-7.4119	-9.1852	-18.2552	-15.0219	-14.1852
1-Flavone	18.7448	7.0515	3.0815	-0.0749	1.7415	-4.1785	-5.9519	-15.0219	-18.2552	-17.4185
10-Flavone	19.5815	7.8881	3.9181	0.7617	2.5781	-3.3419	-5.1152	-14.1852	-17.4185	-18.2552

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	25.5600	2.64013	1.5243
0.1-Flavone	3	27.7000	1.95471	1.1286
0.1-Morin	3	27.9833	0.75791	0.4376
1-Flavone	3	27.6500	1.00683	0.5813
1-Morin	3	24.8267	1.94839	1.1249
10-Flavone	3	26.2567	3.30673	1.9091
10-Morin	3	27.0567	1.04242	0.6018
100-Flavone	2	25.9350	0.71418	0.5050
100-Morin	3	25.0067	0.58688	0.3388
Rt-extracts-M	3	25.2833	7.55103	4.3596

Means Comparisons

Dif=Mean[i]-Mean[j]	0.1-Morin	0.1-Flavone	1-Flavone	10-Morin	10-Flavone	100-Flavone	0 (None)	Rt-extracts-M	100-Morin	1-Morin
0.1-Morin	0.00000	0.28333	0.33333	0.92667	1.72667	2.04833	2.42333	2.70000	2.97667	3.15667
0.1-Flavone	-0.28333	0.00000	0.05000	0.64333	1.44333	1.76500	2.14000	2.41667	2.69333	2.87333
1-Flavone	-0.33333	-0.05000	0.00000	0.59333	1.39333	1.71500	2.09000	2.36667	2.64333	2.82333
10-Morin	-0.92667	-0.64333	-0.59333	0.00000	0.80000	1.12167	1.49667	1.77333	2.05000	2.23000
10-Flavone	-1.72667	-1.44333	-1.39333	-0.80000	0.00000	0.32167	0.69667	0.97333	1.25000	1.43000
100-Flavone	-2.04833	-1.76500	-1.71500	-1.12167	-0.32167	0.00000	0.37500	0.65167	0.92833	1.10833
0 (None)	-2.42333	-2.14000	-2.09000	-1.49667	-0.69667	-0.37500	0.00000	0.27667	0.55333	0.73333
Rt-extracts-M	-2.70000	-2.41667	-2.36667	-1.77333	-0.97333	-0.65167	-0.27667	0.00000	0.27667	0.45667
100-Morin	-2.97667	-2.69333	-2.64333	-2.05000	-1.25000	-0.92833	-0.55333	-0.27667	0.00000	0.18000
1-Morin	-3.15667	-2.87333	-2.82333	-2.23000	-1.43000	-1.10833	-0.73333	-0.45667	-0.18000	0.00000

Alpha= 0.05

Comparisons for each pair using Student's t

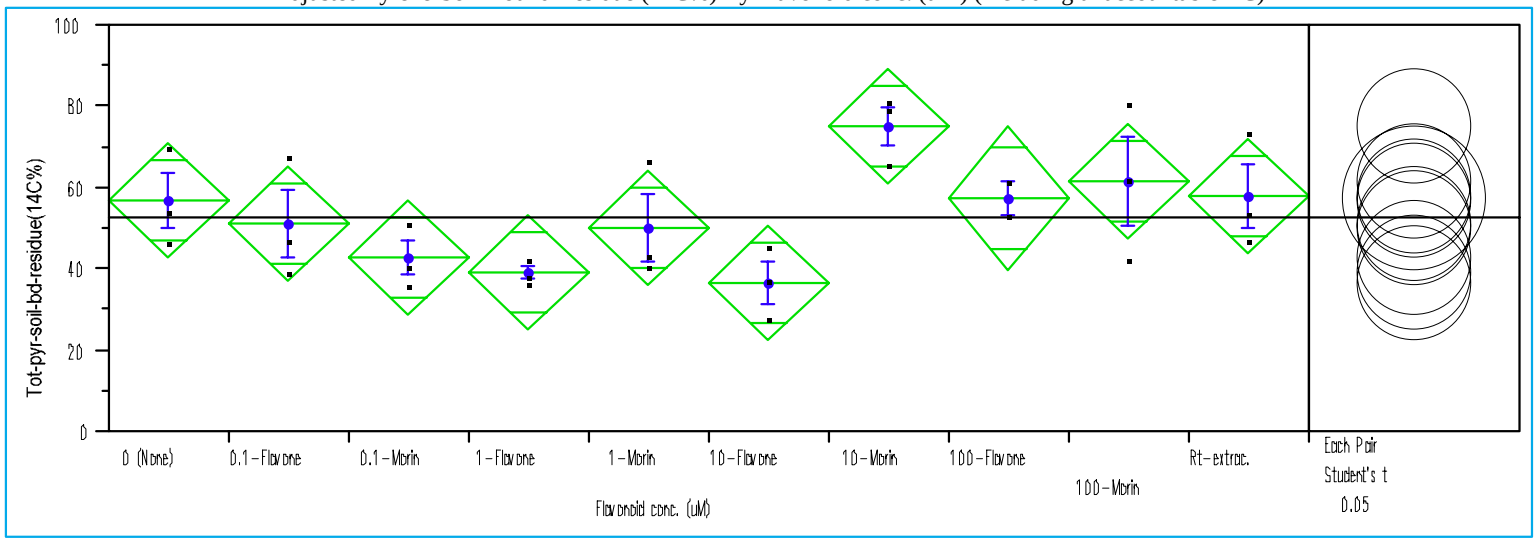
t

2.09301

Abs(Dif)-LSD	0.1-Morin	0.1-Flavone	1-Flavone	10-Morin	10-Flavone	100-Flavone	0 (None)	Rt-extracts-M	100-Morin	1-Morin
0.1-Morin	-5.13621	-4.85288	-4.80288	-4.20955	-3.40955	-3.69413	-2.71288	-2.43621	-2.15955	-1.97955
0.1-Flavone	-4.85288	-5.13621	-5.08621	-4.49288	-3.69288	-3.97746	-2.99621	-2.71955	-2.44288	-2.26288
1-Flavone	-4.80288	-5.08621	-5.13621	-4.54288	-3.74288	-4.02746	-3.04621	-2.76955	-2.49288	-2.31288
10-Morin	-4.20955	-4.49288	-4.54288	-5.13621	-4.33621	-4.62079	-3.63955	-3.36288	-3.08621	-2.90621
10-Flavone	-3.40955	-3.69288	-3.74288	-4.33621	-5.13621	-5.42079	-4.43955	-4.16288	-3.88621	-3.70621
100-Flavone	-3.69413	-3.97746	-4.02746	-4.62079	-5.42079	-6.29055	-5.36746	-5.09079	-4.81413	-4.63413
0 (None)	-2.71288	-2.99621	-3.04621	-3.63955	-4.43955	-5.36746	-5.13621	-4.85955	-4.58288	-4.40288
Rt-extracts-M	-2.43621	-2.71955	-2.76955	-3.36288	-4.16288	-5.09079	-4.85955	-5.13621	-4.85955	-4.67955
100-Morin	-2.15955	-2.44288	-2.49288	-3.08621	-3.88621	-4.81413	-4.58288	-4.85955	-5.13621	-4.95621
1-Morin	-1.97955	-2.26288	-2.31288	-2.90621	-3.70621	-4.63413	-4.40288	-4.67955	-4.95621	-5.13621

Positive values show pairs of means that are significantly different.

Adjusted Pyrene-Soil-Bound Residue (14C%) By Flavonoid conc. (uM) (including unaccountable ^{14}C)



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	57.1033	11.9890	6.922
0.1-Flavone	3	51.5533	14.7144	8.495
0.1-Morin	3	42.7633	7.5695	4.370
1-Flavone	3	39.2033	2.9121	1.681
1-Morin	3	50.4500	14.5355	8.392
10-Flavone	3	36.9733	9.1190	5.265
10-Morin	3	75.6067	8.2813	4.781
100-Flavone	2	57.8250	5.9185	4.185
100-Morin	3	61.8633	19.3365	11.164
Rt-extracts-M	3	58.1700	13.7151	7.918

Means Comparisons

Dif=Mean[i]-Mean[j]	10-Morin	100-Morin	Rt-extracts-M	100-Flavone	0 (None)	0.1-Flavone	1-Morin	0.1-Morin	1-Flavone	10-Flavone
10-Morin	0.0000	13.7433	17.4367	17.7817	18.5033	24.0533	25.1567	32.8433	36.4033	38.6333
100-Morin	-13.7433	0.0000	3.6933	4.0383	4.7600	10.3100	11.4133	19.1000	22.6600	24.8900
Rt-extracts-M	-17.4367	-3.6933	0.0000	0.3450	1.0667	6.6167	7.7200	15.4067	18.9667	21.1967
100-Flavone	-17.7817	-4.0383	-0.3450	0.0000	0.7217	6.2717	7.3750	15.0617	18.6217	20.8517
0 (None)	-18.5033	-4.7600	-1.0667	-0.7217	0.0000	5.5500	6.6533	14.3400	17.9000	20.1300
0.1-Flavone	-24.0533	-10.3100	-6.6167	-6.2717	-5.5500	0.0000	1.1033	8.7900	12.3500	14.5800
1-Morin	-25.1567	-11.4133	-7.7200	-7.3750	-6.6533	-1.1033	0.0000	7.6867	11.2467	13.4767
0.1-Morin	-32.8433	-19.1000	-15.4067	-15.0617	-14.3400	-8.7900	-7.6867	0.0000	3.5600	5.7900
1-Flavone	-36.4033	-22.6600	-18.9667	-18.6217	-17.9000	-12.3500	-11.2467	-3.5600	0.0000	2.2300
10-Flavone	-38.6333	-24.8900	-21.1967	-20.8517	-20.1300	-14.5800	-13.4767	-5.7900	-2.2300	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

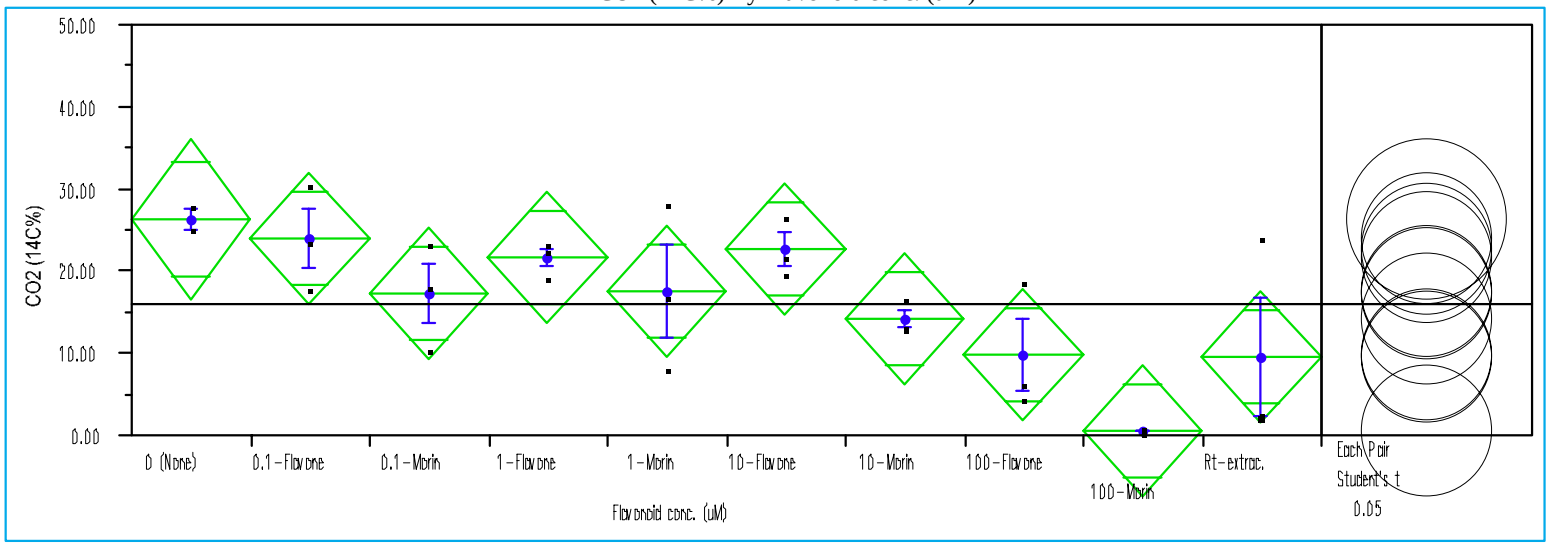
t

2.09301

Abs(Dif)-LSD	10-Morin	100-Morin	Rt-extracts-M	100-Flavone	0 (None)	0.1-Flavone	1-Morin	0.1-Morin	1-Flavone	10-Flavone
10-Morin	-20.5114	-6.7681	-3.0748	-5.1508	-2.0081	3.5419	4.6452	12.3319	15.8919	18.1219
100-Morin	-6.7681	-20.5114	-16.8181	-18.8941	-15.7514	-10.2014	-9.0981	-1.4114	2.1486	4.3786
Rt-extracts-M	-3.0748	-16.8181	-20.5114	-22.5875	-19.4448	-13.8948	-12.7914	-5.1048	-1.5448	0.6852
100-Flavone	-5.1508	-18.8941	-22.5875	-25.1213	-22.2108	-16.6608	-15.5575	-7.8708	-4.3108	-2.0808
0 (None)	-2.0081	-15.7514	-19.4448	-22.2108	-20.5114	-14.9614	-13.8581	-6.1714	-2.6114	-0.3814
0.1-Flavone	3.5419	-10.2014	-13.8948	-16.6608	-14.9614	-20.5114	-19.4081	-11.7214	-8.1614	-5.9314
1-Morin	4.6452	-9.0981	-12.7914	-15.5575	-13.8581	-19.4081	-20.5114	-12.8248	-9.2648	-7.0348
0.1-Morin	12.3319	-1.4114	-5.1048	-7.8708	-6.1714	-11.7214	-12.8248	-20.5114	-16.9514	-14.7214
1-Flavone	15.8919	2.1486	-1.5448	-4.3108	-2.6114	-8.1614	-9.2648	-16.9514	-20.5114	-18.2814
10-Flavone	18.1219	4.3786	0.6852	-2.0808	-0.3814	-5.9314	-7.0348	-14.7214	-18.2814	-20.5114

Positive values show pairs of means that are significantly different.

Appendix D-5. Student's t Test: Paired Comparison of Mean ^{14}C-Pyrene Fate Data in Mulberry Rhizosphere Soil with or without Flavonoid Amendment



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	2	26.6350	2.0011	1.4150
0.1-Flavone	3	24.2300	6.3415	3.6612
0.1-Morin	3	17.4233	6.4560	3.7274
1-Flavone	3	21.8767	2.1444	1.2381
1-Morin	3	17.8567	10.2059	5.8924
10-Flavone	3	22.8267	3.5998	2.0783
10-Morin	3	14.4533	2.0108	1.1610
100-Flavone	3	9.9100	7.7563	4.4781
100-Morin	3	0.5733	0.2237	0.1291
Rt-extracts-M	3	9.7400	12.5690	7.2567

Means Comparisons

Dif=Mean[i]-Mean[j]	0 (None)	0.1-Flavone	10-Flavone	1-Flavone	1-Morin	0.1-Morin	10-Morin	100-Flavone	Rt-extracts-M	100-Morin
0 (None)	0.0000	2.4050	3.8083	4.7583	8.7783	9.2117	12.1817	16.7250	16.8950	26.0617
0.1-Flavone	-2.4050	0.0000	1.4033	2.3533	6.3733	6.8067	9.7767	14.3200	14.4900	23.6567
10-Flavone	-3.8083	-1.4033	0.0000	0.9500	4.9700	5.4033	8.3733	12.9167	13.0867	22.2533
1-Flavone	-4.7583	-2.3533	-0.9500	0.0000	4.0200	4.4533	7.4233	11.9667	12.1367	21.3033
1-Morin	-8.7783	-6.3733	-4.9700	-4.0200	0.0000	0.4333	3.4033	7.9467	8.1167	17.2833
0.1-Morin	-9.2117	-6.8067	-5.4033	-4.4533	-0.4333	0.0000	2.9700	7.5133	7.6833	16.8500
10-Morin	-12.1817	-9.7767	-8.3733	-7.4233	-3.4033	-2.9700	0.0000	4.5433	4.7133	13.8800
100-Flavone	-16.7250	-14.3200	-12.9167	-11.9667	-7.9467	-7.5133	-4.5433	0.0000	0.1700	9.3367
Rt-extracts-M	-16.8950	-14.4900	-13.0867	-12.1367	-8.1167	-7.6833	-4.7133	-0.1700	0.0000	9.1667
100-Morin	-26.0617	-23.6567	-22.2533	-21.3033	-17.2833	-16.8500	-13.8800	-9.3367	-9.1667	0.0000

Alpha= 0.05

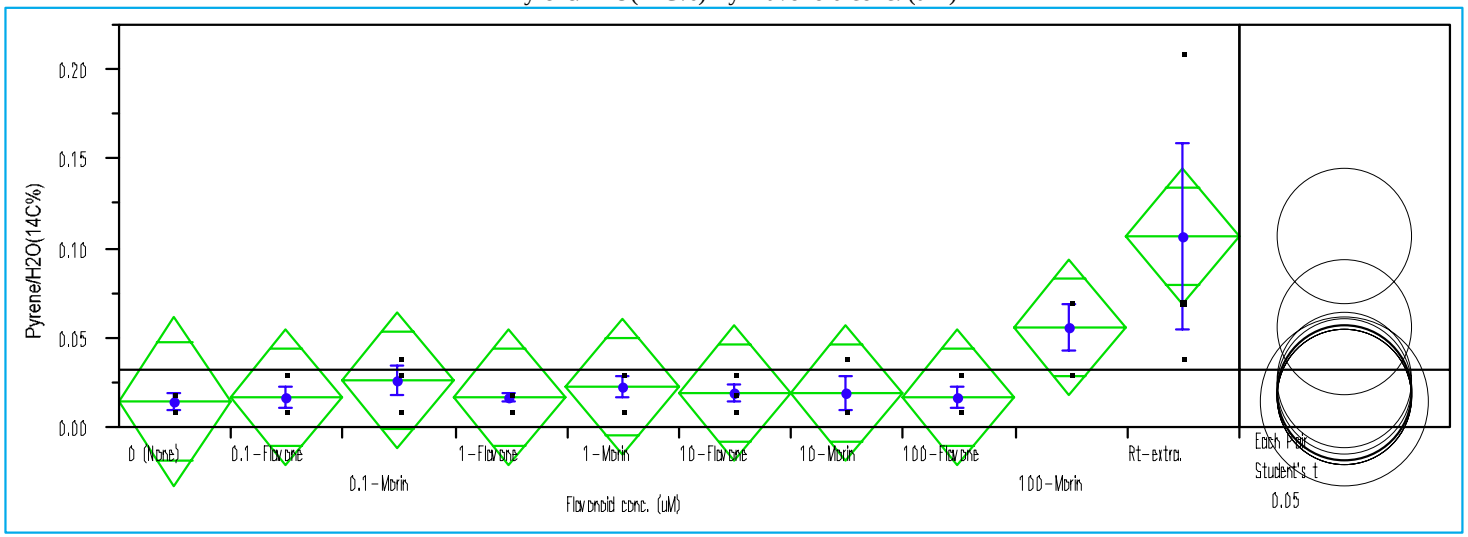
Comparisons for each pair using Student's t

t

2.09301

Abs(Dif)-LSD	0 (None)	0.1-Flavone	10-Flavone	1-Flavone	1-Morin	0.1-Morin	10-Morin	100-Flavone	Rt-extracts-M	100-Morin
0 (None)	-14.0461	-10.4172	-9.0139	-8.0639	-4.0439	-3.6106	-0.6406	3.9028	4.0728	13.2394
0.1-Flavone	-10.4172	-11.4686	-10.0652	-9.1152	-5.0952	-4.6619	-1.6919	2.8514	3.0214	12.1881
10-Flavone	-9.0139	-10.0652	-11.4686	-10.5186	-6.4986	-6.0652	-3.0952	1.4481	1.6181	10.7848
1-Flavone	-8.0639	-9.1152	-10.5186	-11.4686	-7.4486	-7.0152	-4.0452	0.4981	0.6681	9.8348
1-Morin	-4.0439	-5.0952	-6.4986	-7.4486	-11.4686	-11.0352	-8.0652	-3.5219	-3.3519	5.8148
0.1-Morin	-3.6106	-4.6619	-6.0652	-7.0152	-11.0352	-11.4686	-8.4986	-3.9552	-3.7852	5.3814
10-Morin	-0.6406	-1.6919	-3.0952	-4.0452	-8.0652	-8.4986	-11.4686	-6.9252	-6.7552	2.4114
100-Flavone	3.9028	2.8514	1.4481	0.4981	-3.5219	-3.9552	-6.9252	-11.4686	-11.2986	-2.1319
Rt-extracts-M	4.0728	3.0214	1.6181	0.6681	-3.3519	-3.7852	-6.7552	-11.2986	-11.4686	-2.3019
100-Morin	13.2394	12.1881	10.7848	9.8348	5.8148	5.3814	2.4114	-2.1319	-2.3019	-11.4686

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	2	0.015000	0.007071	0.00500
0.1-Flavone	3	0.016667	0.011547	0.00667
0.1-Morin	3	0.026667	0.015275	0.00882
1-Flavone	3	0.016667	0.005774	0.00333
1-Morin	3	0.023333	0.011547	0.00667
10-Flavone	3	0.020000	0.010000	0.00577
10-Morin	3	0.020000	0.017321	0.01000
100-Flavone	3	0.016667	0.011547	0.00667
100-Morin	3	0.056667	0.023094	0.01333
Rt-extracts-M	3	0.106667	0.090738	0.05239

Means Comparisons

Dif=Mean[i]-Mean[j]	Rt-extracts-M	100-Morin	0.1-Morin	1-Morin	10-Flavone	10-Morin	0.1-Flavone	100-Flavone	1-Flavone	0 (None)
Rt-extracts-M	0.000000	0.050000	0.080000	0.083333	0.086667	0.086667	0.090000	0.090000	0.090000	0.091667
100-Morin	-0.05	0.000000	0.030000	0.033333	0.036667	0.036667	0.040000	0.040000	0.040000	0.041667
0.1-Morin	-0.08	-0.03	0.000000	0.003333	0.006667	0.006667	0.010000	0.010000	0.010000	0.011667
1-Morin	-0.08333	-0.03333	-0.00333	0.000000	0.003333	0.003333	0.006667	0.006667	0.006667	0.008333
10-Flavone	-0.08667	-0.03667	-0.00667	-0.00333	0.000000	0.000000	0.003333	0.003333	0.003333	0.005000
10-Morin	-0.08667	-0.03667	-0.00667	-0.00333	0.000000	0.000000	0.003333	0.003333	0.003333	0.005000
0.1-Flavone	-0.09	-0.04	-0.01	-0.00667	-0.00333	-0.00333	0.000000	0.000000	0.000000	0.001667
100-Flavone	-0.09	-0.04	-0.01	-0.00667	-0.00333	-0.00333	0.000000	0.000000	0.000000	0.001667
1-Flavone	-0.09	-0.04	-0.01	-0.00667	-0.00333	-0.00333	0.000000	0.000000	0.000000	0.001667
0 (None)	-0.09167	-0.04167	-0.01167	-0.00833	-0.005	-0.005	-0.00167	-0.00167	-0.00167	0.000000

Alpha= 0.05

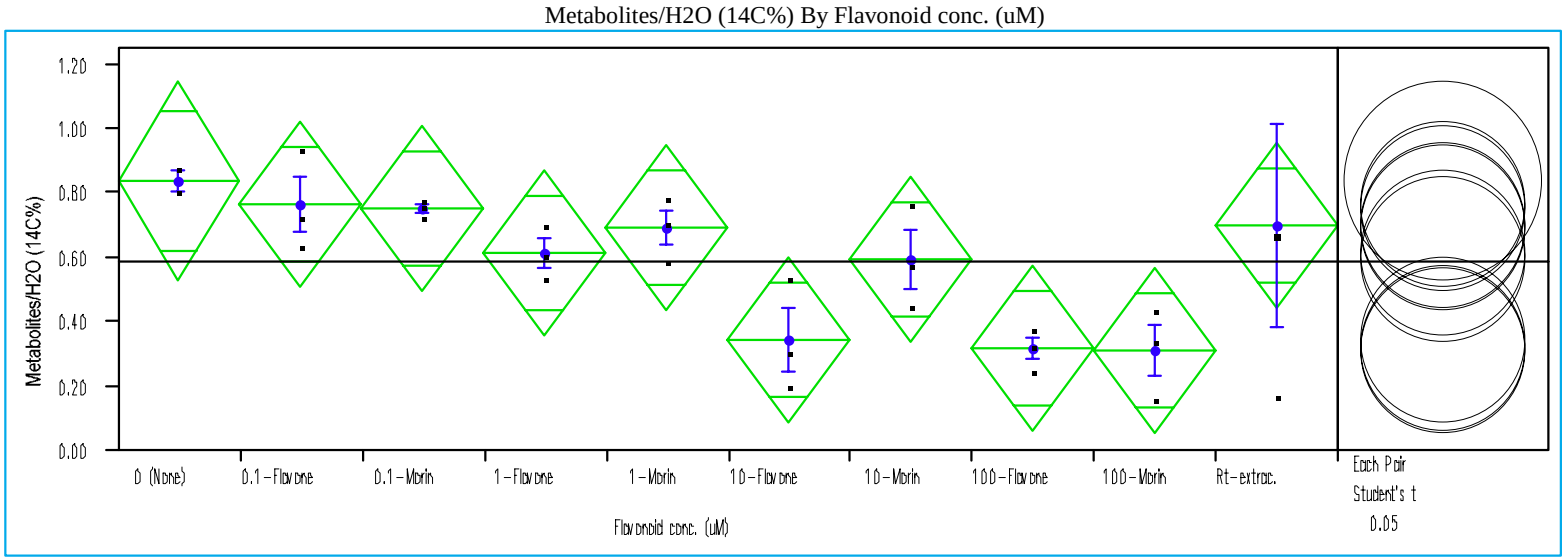
Comparisons for each pair using Student's t

t

2.09301

Abs(Dif)-LSD	Rt-extracts-M	100-Morin	0.1-Morin	1-Morin	10-Flavone	10-Morin	0.1-Flavone	100-Flavone	1-Flavone	0 (None)
Rt-extracts-M	-0.05505	-0.00505	0.024949	0.028282	0.031616	0.031616	0.034949	0.034949	0.034949	0.030118
100-Morin	-0.00505	-0.05505	-0.02505	-0.02172	-0.01838	-0.01838	-0.01505	-0.01505	-0.01505	-0.01988
0.1-Morin	0.024949	-0.02505	-0.05505	-0.05172	-0.04838	-0.04838	-0.04505	-0.04505	-0.04505	-0.04988
1-Morin	0.028282	-0.02172	-0.05172	-0.05505	-0.05172	-0.05172	-0.04838	-0.04838	-0.04838	-0.05322
10-Flavone	0.031616	-0.01838	-0.04838	-0.05172	-0.05505	-0.05505	-0.05172	-0.05172	-0.05172	-0.05655
10-Morin	0.031616	-0.01838	-0.04838	-0.05172	-0.05505	-0.05505	-0.05172	-0.05172	-0.05172	-0.05655
0.1-Flavone	0.034949	-0.01505	-0.04505	-0.04838	-0.05172	-0.05172	-0.05505	-0.05505	-0.05505	-0.05988
100-Flavone	0.034949	-0.01505	-0.04505	-0.04838	-0.05172	-0.05172	-0.05505	-0.05505	-0.05505	-0.05988
1-Flavone	0.034949	-0.01505	-0.04505	-0.04838	-0.05172	-0.05172	-0.05505	-0.05505	-0.05505	-0.05988
0 (None	0.030118	-0.01988	-0.04988	-0.05322	-0.05655	-0.05655	-0.05988	-0.05988	-0.05988	-0.06742

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	2	0.845000	0.049497	0.03500
0.1-Flavone	3	0.770000	0.153948	0.08888
0.1-Morin	3	0.756667	0.025166	0.01453
1-Flavone	3	0.616667	0.080208	0.04631
1-Morin	3	0.696667	0.100664	0.05812
10-Flavone	3	0.350000	0.173494	0.10017
10-Morin	3	0.600000	0.160935	0.09292
100-Flavone	3	0.320000	0.065574	0.03786
100-Morin	3	0.313333	0.141892	0.08192
Rt-extracts-M	3	0.706667	0.555908	0.32095

Means Comparisons

Dif=Mean[i]-Mean[j]	0 (None)	0.1-Flavone	0.1-Morin	Rt-extracts-M	1-Morin	1-Flavone	10-Morin	10-Flavone	100-Flavone	100-Morin
0 (None)	0.000000	0.075000	0.088333	0.138333	0.148333	0.228333	0.245000	0.495000	0.525000	0.531667
0.1-Flavone	-0.075	0.000000	0.013333	0.063333	0.073333	0.153333	0.170000	0.420000	0.450000	0.456667
0.1-Morin	-0.08833	-0.01333	0.000000	0.050000	0.060000	0.140000	0.156667	0.406667	0.436667	0.443333
Rt-extracts-M	-0.13833	-0.06333	-0.05	0.000000	0.010000	0.090000	0.106667	0.356667	0.386667	0.393333
1-Morin	-0.14833	-0.07333	-0.06	-0.01	0.000000	0.080000	0.096667	0.346667	0.376667	0.383333
1-Flavone	-0.22833	-0.15333	-0.14	-0.09	-0.08	0.000000	0.016667	0.266667	0.296667	0.303333
10-Morin	-0.245	-0.17	-0.15667	-0.10667	-0.09667	-0.01667	0.000000	0.250000	0.280000	0.286667
10-Flavone	-0.495	-0.42	-0.40667	-0.35667	-0.34667	-0.26667	-0.25	0.000000	0.030000	0.036667
100-Flavone	-0.525	-0.45	-0.43667	-0.38667	-0.37667	-0.29667	-0.28	-0.03	0.000000	0.006667
100-Morin	-0.53167	-0.45667	-0.44333	-0.39333	-0.38333	-0.30333	-0.28667	-0.03667	-0.00667	0.000000

Alpha= 0.05

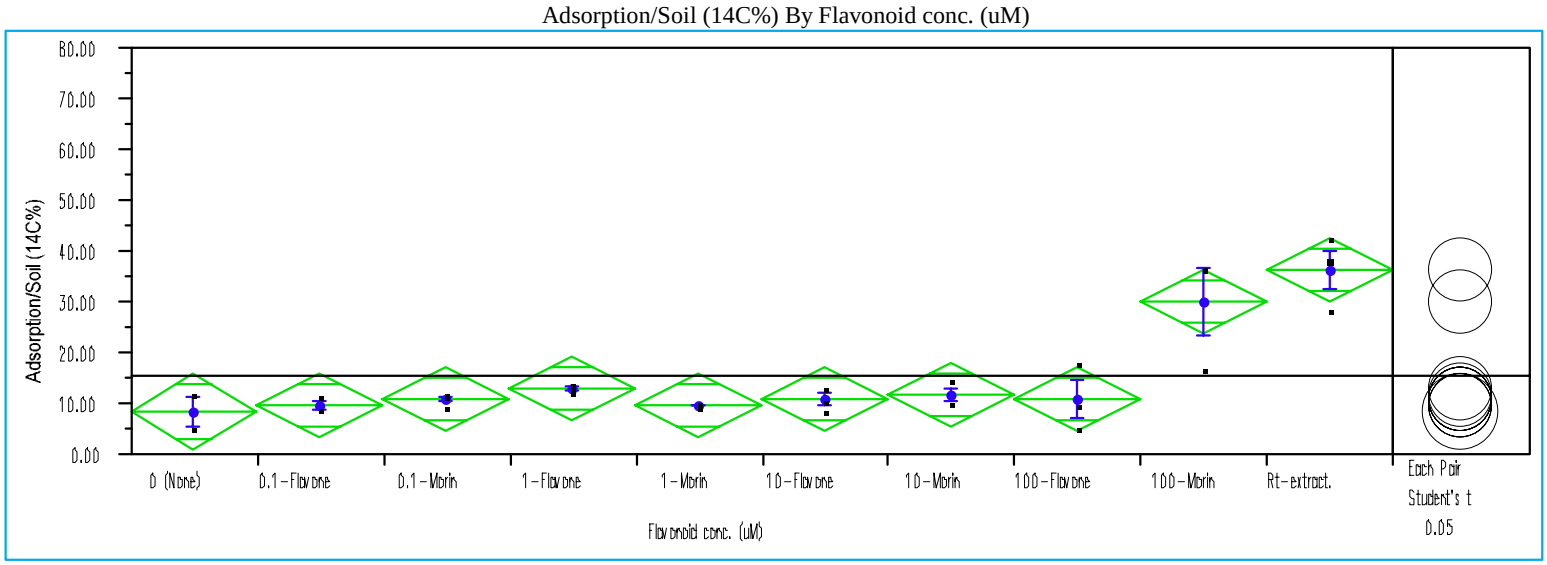
Comparisons for each pair using Student's t

t

2.09301

Abs(Dif)-LSD	0 (None)	0.1-Flavone	0.1-Morin	Rt-extracts-M	1-Morin	1-Flavone	10-Morin	10-Flavone	100-Flavone	100-Morin
0 (None)	-0.44611	-0.33224	-0.31891	-0.26891	-0.25891	-0.17891	-0.16224	0.087756	0.117756	0.124422
0.1-Flavone	-0.33224	-0.36425	-0.35092	-0.30092	-0.29092	-0.21092	-0.19425	0.055749	0.085749	0.092416
0.1-Morin	-0.31891	-0.35092	-0.36425	-0.31425	-0.30425	-0.22425	-0.20758	0.042416	0.072416	0.079083
Rt-extracts-M	-0.26891	-0.30092	-0.31425	-0.36425	-0.35425	-0.27425	-0.25758	-0.00758	0.022416	0.029083
1-Morin	-0.25891	-0.29092	-0.30425	-0.35425	-0.36425	-0.28425	-0.26758	-0.01758	0.012416	0.019083
1-Flavone	-0.17891	-0.21092	-0.22425	-0.27425	-0.28425	-0.36425	-0.34758	-0.09758	-0.06758	-0.06092
10-Morin	-0.16224	-0.19425	-0.20758	-0.25758	-0.26758	-0.34758	-0.36425	-0.11425	-0.08425	-0.07758
10-Flavone	0.087756	0.055749	0.042416	-0.00758	-0.01758	-0.09758	-0.11425	-0.36425	-0.33425	-0.32758
100-Flavone	0.117756	0.085749	0.072416	0.022416	0.012416	-0.06758	-0.08425	-0.33425	-0.36425	-0.35758
100-Morin	0.124422	0.092416	0.079083	0.029083	0.019083	-0.06092	-0.07758	-0.32758	-0.35758	-0.36425

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	2	8.6550	4.6598	3.2950
0.1-Flavone	3	9.8467	1.5446	0.8918
0.1-Morin	3	11.0200	1.3688	0.7903
1-Flavone	3	13.2100	0.8402	0.4851
1-Morin	3	9.9933	0.4594	0.2652
10-Flavone	3	10.9133	2.3129	1.3353
10-Morin	3	11.7367	2.4801	1.4319
100-Flavone	3	11.0900	6.6717	3.8519
100-Morin	3	30.0533	11.5696	6.6797
Rt-extracts-M	3	36.4500	7.2013	4.1577

Means Comparisons

Dif=Mean[i]-Mean[j]	Rt-extracts-M	100-Morin	1-Flavone	10-Morin	100-Flavone	0.1-Morin	10-Flavone	1-Morin	0.1-Flavone	0 (None)
Rt-extracts-M	0.0000	6.3967	23.2400	24.7133	25.3600	25.4300	25.5367	26.4567	26.6033	27.7950
100-Morin	-6.3967	0.0000	16.8433	18.3167	18.9633	19.0333	19.1400	20.0600	20.2067	21.3983
1-Flavone	-23.2400	-16.8433	0.0000	1.4733	2.1200	2.1900	2.2967	3.2167	3.3633	4.5550
10-Morin	-24.7133	-18.3167	-1.4733	0.0000	0.6467	0.7167	0.8233	1.7433	1.8900	3.0817
100-Flavone	-25.3600	-18.9633	-2.1200	-0.6467	0.0000	0.0700	0.1767	1.0967	1.2433	2.4350
0.1-Morin	-25.4300	-19.0333	-2.1900	-0.7167	-0.0700	0.0000	0.1067	1.0267	1.1733	2.3650
10-Flavone	-25.5367	-19.1400	-2.2967	-0.8233	-0.1767	-0.1067	0.0000	0.9200	1.0667	2.2583
1-Morin	-26.4567	-20.0600	-3.2167	-1.7433	-1.0967	-1.0267	-0.9200	0.0000	0.1467	1.3383
0.1-Flavone	-26.6033	-20.2067	-3.3633	-1.8900	-1.2433	-1.1733	-1.0667	-0.1467	0.0000	1.1917
0 (None)	-27.7950	-21.3983	-4.5550	-3.0817	-2.4350	-2.3650	-2.2583	-1.3383	-1.1917	0.0000

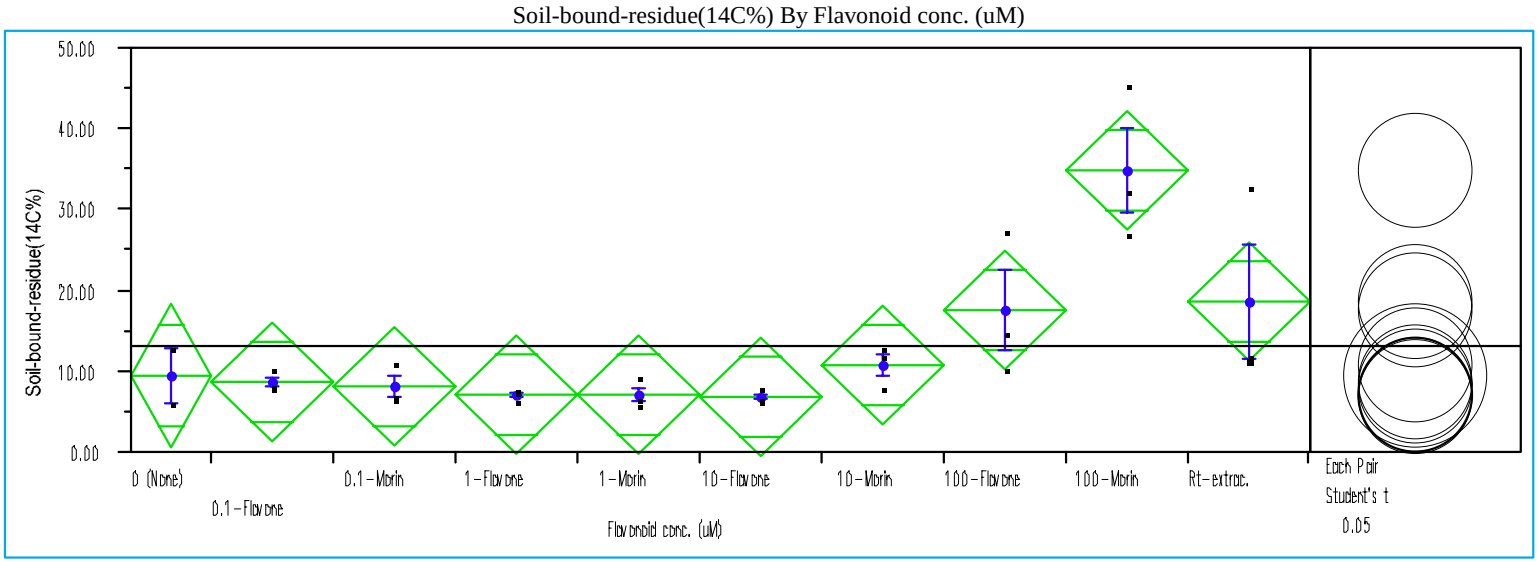
Alpha= 0.05

Comparisons for each pair using Student's t

t
2.09301

Abs(Dif)-LSD	Rt-extracts-M	100-Morin	1-Flavone	10-Morin	100-Flavone	0.1-Morin	10-Flavone	1-Morin	0.1-Flavone	0 (None)
Rt-extracts-M	-8.9017	-2.5050	14.3383	15.8117	16.4583	16.5283	16.6350	17.5550	17.7017	17.8426
100-Morin	-2.5050	-8.9017	7.9417	9.4150	10.0617	10.1317	10.2383	11.1583	11.3050	11.4459
1-Flavone	14.3383	7.9417	-8.9017	-7.4283	-6.7817	-6.7117	-6.6050	-5.6850	-5.5383	-5.3974
10-Morin	15.8117	9.4150	-7.4283	-8.9017	-8.2550	-8.1850	-8.0783	-7.1583	-7.0117	-6.8707
100-Flavone	16.4583	10.0617	-6.7817	-8.2550	-8.9017	-8.8317	-8.7250	-7.8050	-7.6583	-7.5174
0.1-Morin	16.5283	10.1317	-6.7117	-8.1850	-8.8317	-8.9017	-8.7950	-7.8750	-7.7283	-7.5874
10-Flavone	16.6350	10.2383	-6.6050	-8.0783	-8.7250	-8.7950	-8.9017	-7.9817	-7.8350	-7.6941
1-Morin	17.5550	11.1583	-5.6850	-7.1583	-7.8050	-7.8750	-7.9817	-8.9017	-8.7550	-8.6141
0.1-Flavone	17.7017	11.3050	-5.5383	-7.0117	-7.6583	-7.7283	-7.8350	-8.7550	-8.9017	-8.7607
0 (None)	17.8426	11.4459	-5.3974	-6.8707	-7.5174	-7.5874	-7.6941	-8.6141	-8.7607	-10.9023

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	2	9.6350	4.8154	3.4050
0.1-Flavone	3	8.8867	1.3407	0.7740
0.1-Morin	3	8.3600	2.4882	1.4365
1-Flavone	3	7.3167	0.7617	0.4398
1-Morin	3	7.2967	1.7816	1.0286
10-Flavone	3	7.0233	0.9039	0.5219
10-Morin	3	11.0033	2.6458	1.5276
100-Flavone	3	17.6433	8.9073	5.1427
100-Morin	3	35.1267	9.4287	5.4436
Rt-extracts-M	3	18.8033	12.4090	7.1644

Means Comparisons

Dif=Mean[i]-Mean[j]	100-Morin	Rt-extracts-M	100-Flavone	10-Morin	0 (None)	0.1-Flavone	0.1-Morin	1-Flavone	1-Morin	10-Flavone
100-Morin	0.0000	16.3233	17.4833	24.1233	25.4917	26.2400	26.7667	27.8100	27.8300	28.1033
Rt-extracts-M	-16.3233	0.0000	1.1600	7.8000	9.1683	9.9167	10.4433	11.4867	11.5067	11.7800
100-Flavone	-17.4833	-1.1600	0.0000	6.6400	8.0083	8.7567	9.2833	10.3267	10.3467	10.6200
10-Morin	-24.1233	-7.8000	-6.6400	0.0000	1.3683	2.1167	2.6433	3.6867	3.7067	3.9800
0 (None)	-25.4917	-9.1683	-8.0083	-1.3683	0.0000	0.7483	1.2750	2.3183	2.3383	2.6117
0.1-Flavone	-26.2400	-9.9167	-8.7567	-2.1167	-0.7483	0.0000	0.5267	1.5700	1.5900	1.8633
0.1-Morin	-26.7667	-10.4433	-9.2833	-2.6433	-1.2750	-0.5267	0.0000	1.0433	1.0633	1.3367
1-Flavone	-27.8100	-11.4867	-10.3267	-3.6867	-2.3183	-1.5700	-1.0433	0.0000	0.0200	0.2933
1-Morin	-27.8300	-11.5067	-10.3467	-3.7067	-2.3383	-1.5900	-1.0633	-0.0200	0.0000	0.2733
10-Flavone	-28.1033	-11.7800	-10.6200	-3.9800	-2.6117	-1.8633	-1.3367	-0.2933	-0.2733	0.0000

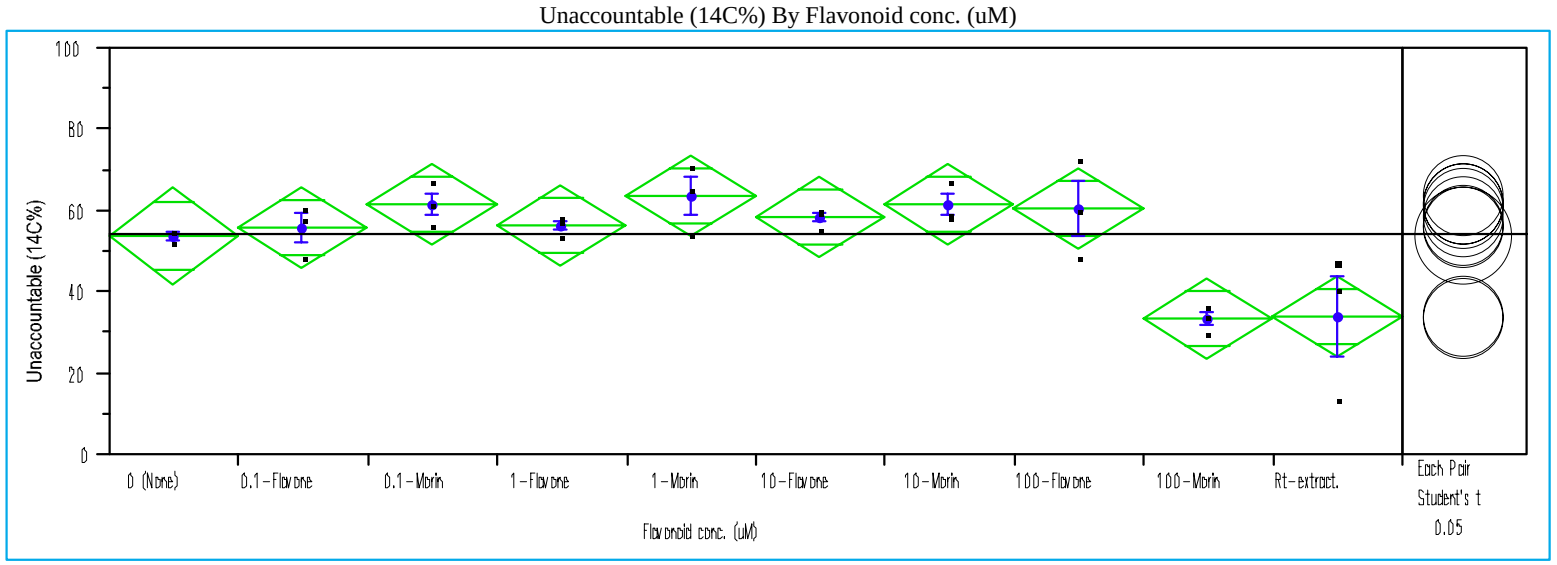
Alpha= 0.05

Comparisons for each pair using Student's t

t
2.09301

Abs(Dif)-LSD	100-Morin	Rt-extracts-M	100-Flavone	10-Morin	0 (None)	0.1-Flavone	0.1-Morin	1-Flavone	1-Morin	10-Flavone
100-Morin	-10.4228	5.9005	7.0605	13.7005	13.8386	15.8172	16.3439	17.3872	17.4072	17.6805
Rt-extracts-M	5.9005	-10.4228	-9.2628	-2.6228	-2.4847	-0.5061	0.0205	1.0639	1.0839	1.3572
100-Flavone	7.0605	-9.2628	-10.4228	-3.7828	-3.6447	-1.6661	-1.1395	-0.0961	-0.0761	0.1972
10-Morin	13.7005	-2.6228	-3.7828	-10.4228	-10.2847	-8.3061	-7.7795	-6.7361	-6.7161	-6.4428
0 (None)	13.8386	-2.4847	-3.6447	-10.2847	-12.7653	-10.9047	-10.3780	-9.3347	-9.3147	-9.0414
0.1-Flavone	15.8172	-0.5061	-1.6661	-8.3061	-10.9047	-10.4228	-9.8961	-8.8528	-8.8328	-8.5595
0.1-Morin	16.3439	0.0205	-1.1395	-7.7795	-10.3780	-9.8961	-10.4228	-9.3795	-9.3595	-9.0861
1-Flavone	17.3872	1.0639	-0.0961	-6.7361	-9.3347	-8.8528	-9.3795	-10.4228	-10.4028	-10.1295
1-Morin	17.4072	1.0839	-0.0761	-6.7161	-9.3147	-8.8328	-9.3595	-10.4028	-10.4228	-10.1495
10-Flavone	17.6805	1.3572	0.1972	-6.4428	-9.0414	-8.5595	-9.0861	-10.1295	-10.1495	-10.4228

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	2	54.2250	1.8031	1.275
0.1-Flavone	3	56.2500	6.3439	3.663
0.1-Morin	3	62.4133	5.3832	3.108
1-Flavone	3	56.9633	2.4574	1.419
1-Morin	3	64.1333	8.4989	4.907
10-Flavone	3	58.8667	2.6750	1.544
10-Morin	3	62.1867	4.8321	2.790
100-Flavone	3	61.0133	11.8063	6.816
100-Morin	3	33.8733	3.2411	1.871
Rt-extracts-M	3	34.1933	17.9653	10.372

Means Comparisons

Dif=Mean[i]-Mean[j]	1-Morin	0.1-Morin	10-Morin	100-Flavone	10-Flavone	1-Flavone	0.1-Flavone	0 (None)	Rt-extracts-M	100-Morin
1-Morin	0.0000	1.7200	1.9467	3.1200	5.2667	7.1700	7.8833	9.9083	29.9400	30.2600
0.1-Morin	-1.7200	0.0000	0.2267	1.4000	3.5467	5.4500	6.1633	8.1883	28.2200	28.5400
10-Morin	-1.9467	-0.2267	0.0000	1.1733	3.3200	5.2233	5.9367	7.9617	27.9933	28.3133
100-Flavone	-3.1200	-1.4000	-1.1733	0.0000	2.1467	4.0500	4.7633	6.7883	26.8200	27.1400
10-Flavone	-5.2667	-3.5467	-3.3200	-2.1467	0.0000	1.9033	2.6167	4.6417	24.6733	24.9933
1-Flavone	-7.1700	-5.4500	-5.2233	-4.0500	-1.9033	0.0000	0.7133	2.7383	22.7700	23.0900
0.1-Flavone	-7.8833	-6.1633	-5.9367	-4.7633	-2.6167	-0.7133	0.0000	2.0250	22.0567	22.3767
0 (None)	-9.9083	-8.1883	-7.9617	-6.7883	-4.6417	-2.7383	-2.0250	0.0000	20.0317	20.3517
Rt-extracts-M	-29.9400	-28.2200	-27.9933	-26.8200	-24.6733	-22.7700	-22.0567	-20.0317	0.0000	0.3200
100-Morin	-30.2600	-28.5400	-28.3133	-27.1400	-24.9933	-23.0900	-22.3767	-20.3517	-0.3200	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

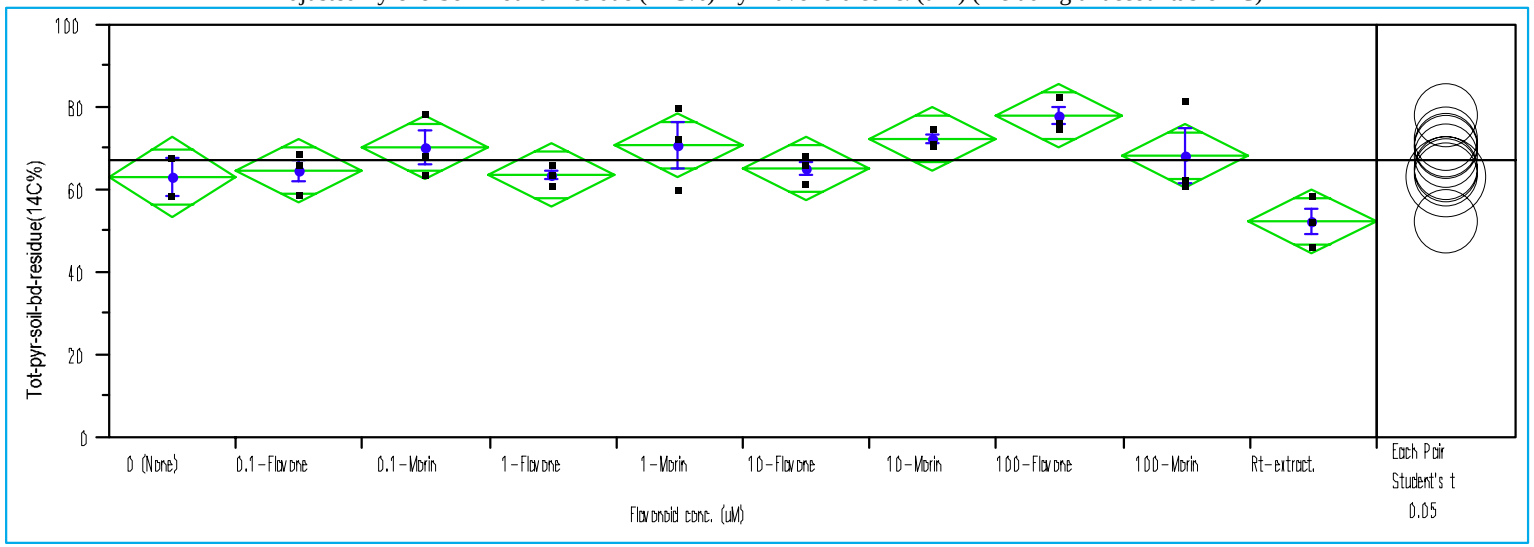
t

2.09301

Abs(Dif)-LSD	1-Morin	0.1-Morin	10-Morin	100-Flavone	10-Flavone	1-Flavone	0.1-Flavone	0 (None)	Rt-extracts-M	100-Morin
1-Morin	-14.1605	-12.4405	-12.2138	-11.0405	-8.8938	-6.9905	-6.2771	-5.9235	15.7795	16.0995
0.1-Morin	-12.4405	-14.1605	-13.9338	-12.7605	-10.6138	-8.7105	-7.9971	-7.6435	14.0595	14.3795
10-Morin	-12.2138	-13.9338	-14.1605	-12.9871	-10.8405	-8.9371	-8.2238	-7.8702	13.8329	14.1529
100-Flavone	-11.0405	-12.7605	-12.9871	-14.1605	-12.0138	-10.1105	-9.3971	-9.0435	12.6595	12.9795
10-Flavone	-8.8938	-10.6138	-10.8405	-12.0138	-14.1605	-12.2571	-11.5438	-11.1902	10.5129	10.8329
1-Flavone	-6.9905	-8.7105	-8.9371	-10.1105	-12.2571	-14.1605	-13.4471	-13.0935	8.6095	8.9295
0.1-Flavone	-6.2771	-7.9971	-8.2238	-9.3971	-11.5438	-13.4471	-14.1605	-13.8069	7.8962	8.2162
0 (None)	-5.9235	-7.6435	-7.8702	-9.0435	-11.1902	-13.0935	-13.8069	-17.3429	4.1998	4.5198
Rt-extracts-M	15.7795	14.0595	13.8329	12.6595	10.5129	8.6095	7.8962	4.1998	-14.1605	-13.8405
100-Morin	16.0995	14.3795	14.1529	12.9795	10.8329	8.9295	8.2162	4.5198	-13.8405	-14.1605

Positive values show pairs of means that are significantly different.

Adjusted Pyrene-Soil-Bound Residue (14C%) By Flavonoid conc. (uM) (including unaccountable ^{14}C)



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	2	63.8500	6.6185	4.6800
0.1-Flavone	3	65.1367	5.0230	2.9000
0.1-Morin	3	70.7733	7.6294	4.4048
1-Flavone	3	64.2833	2.4750	1.4289
1-Morin	3	71.4267	10.1022	5.8325
10-Flavone	3	65.8900	3.3747	1.9484
10-Morin	3	73.1900	2.2402	1.2934
100-Flavone	3	78.6567	4.1380	2.3891
100-Morin	3	69.0033	11.5936	6.6935
Rt-extracts-M	3	52.9967	6.0800	3.5103

Means Comparisons

Dif=Mean[i]-Mean[j]	100-Flavone	10-Morin	1-Morin	0.1-Morin	100-Morin	10-Flavone	0.1-Flavone	1-Flavone	0 (None)	Rt-extracts-M
100-Flavone	0.0000	5.4667	7.2300	7.8833	9.6533	12.7667	13.5200	14.3733	14.8067	25.6600
10-Morin	-5.4667	0.0000	1.7633	2.4167	4.1867	7.3000	8.0533	8.9067	9.3400	20.1933
1-Morin	-7.2300	-1.7633	0.0000	0.6533	2.4233	5.5367	6.2900	7.1433	7.5767	18.4300
0.1-Morin	-7.8833	-2.4167	-0.6533	0.0000	1.7700	4.8833	5.6367	6.4900	6.9233	17.7767
100-Morin	-9.6533	-4.1867	-2.4233	-1.7700	0.0000	3.1133	3.8667	4.7200	5.1533	16.0067
10-Flavone	-12.7667	-7.3000	-5.5367	-4.8833	-3.1133	0.0000	0.7533	1.6067	2.0400	12.8933
0.1-Flavone	-13.5200	-8.0533	-6.2900	-5.6367	-3.8667	-0.7533	0.0000	0.8533	1.2867	12.1400
1-Flavone	-14.3733	-8.9067	-7.1433	-6.4900	-4.7200	-1.6067	-0.8533	0.0000	0.4333	11.2867
0 (None)	-14.8067	-9.3400	-7.5767	-6.9233	-5.1533	-2.0400	-1.2867	-0.4333	0.0000	10.8533
Rt-extracts-M	-25.6600	-20.1933	-18.4300	-17.7767	-16.0067	-12.8933	-12.1400	-11.2867	-10.8533	0.0000

Alpha= 0.05

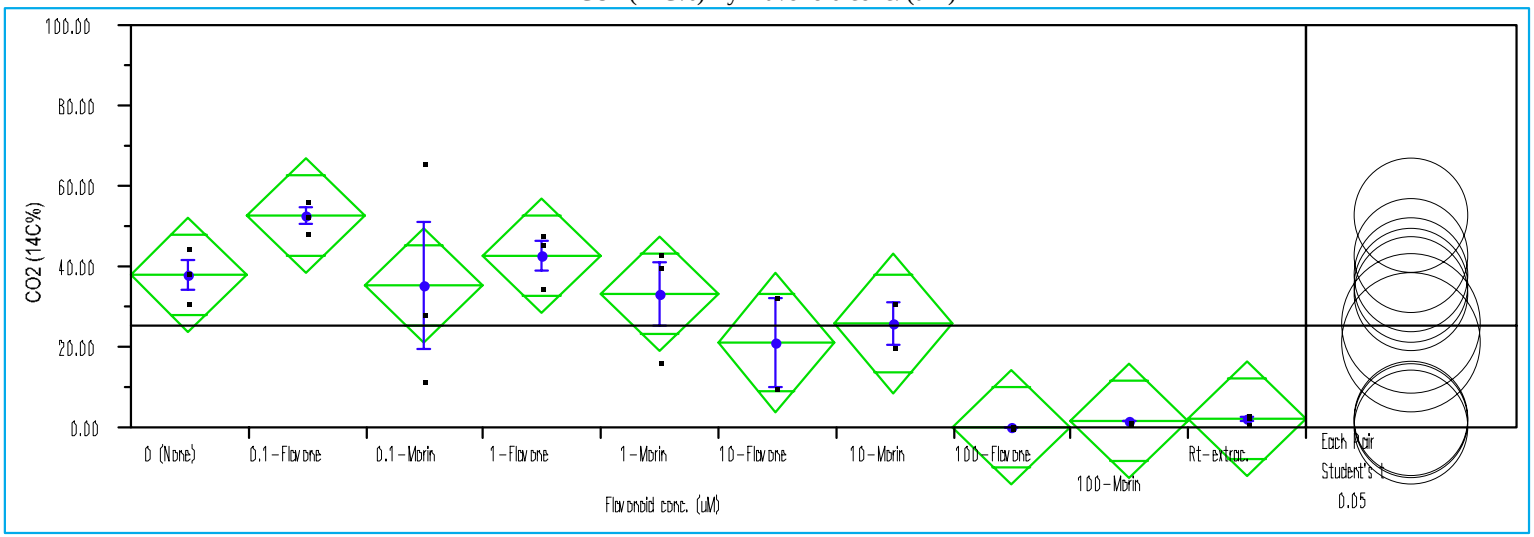
Comparisons for each pair using Student's t

t
2.09301

Abs(Dif)-LSD	100-Flavone	10-Morin	1-Morin	0.1-Morin	100-Morin	10-Flavone	0.1-Flavone	1-Flavone	0 (None)	Rt-extracts-M
100-Flavone	-11.3416	-5.8749	-4.1116	-3.4583	-1.6883	1.4251	2.1784	3.0317	2.1264	14.3184
10-Morin	-5.8749	-11.3416	-9.5783	-8.9249	-7.1549	-4.0416	-3.2883	-2.4349	-3.3403	8.8517
1-Morin	-4.1116	-9.5783	-11.3416	-10.6883	-8.9183	-5.8049	-5.0516	-4.1983	-5.1036	7.0884
0.1-Morin	-3.4583	-8.9249	-10.6883	-11.3416	-9.5716	-6.4583	-5.7049	-4.8516	-5.7570	6.4351
100-Morin	-1.6883	-7.1549	-8.9183	-9.5716	-11.3416	-8.2283	-7.4749	-6.6216	-7.5270	4.6651
10-Flavone	1.4251	-4.0416	-5.8049	-6.4583	-8.2283	-11.3416	-10.5883	-9.7349	-10.6403	1.5517
0.1-Flavone	2.1784	-3.2883	-5.0516	-5.7049	-7.4749	-10.5883	-11.3416	-10.4883	-11.3936	0.7984
1-Flavone	3.0317	-2.4349	-4.1983	-4.8516	-6.6216	-9.7349	-10.4883	-11.3416	-12.2470	-0.0549
0 (None)	2.1264	-3.3403	-5.1036	-5.7570	-7.5270	-10.6403	-11.3936	-12.2470	-13.8906	-1.8270
Rt-extracts-M	14.3184	8.8517	7.0884	6.4351	4.6651	1.5517	0.7984	-0.0549	-1.8270	-11.3416

Positive values show pairs of means that are significantly different.

Appendix D-6. Student's t Test: Paired Comparison of Mean ^{14}C-Pyrene Fate Data in Bermudagrass Rhizosphere Soil with or without Flavonoid Amendment



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	38.3633	7.0671	4.080
0.1-Flavone	3	52.8367	3.8676	2.233
0.1-Morin	3	35.4900	27.9747	16.151
1-Flavone	3	43.0433	7.0311	4.059
1-Morin	3	33.3700	14.5361	8.392
10-Flavone	2	21.5400	16.1220	11.400
10-Morin	2	25.9050	8.0115	5.665
100-Flavone	3	0.4700	0.1609	0.093
100-Morin	3	1.7033	0.3573	0.206
Rt-extracts-M	3	2.5533	1.1068	0.639

Means Comparisons

Dif=Mean[i]-Mean[j]	0.1-Flavone	1-Flavone	0 (None)	0.1-Morin	1-Morin	10-Morin	10-Flavone	Rt-extracts-M	100-Morin	100-Flavone
0.1-Flavone	0.0000	9.7933	14.4733	17.3467	19.4667	26.9317	31.2967	50.2833	51.1333	52.3667
1-Flavone	-9.7933	0.0000	4.6800	7.5533	9.6733	17.1383	21.5033	40.4900	41.3400	42.5733
0 (None)	-14.4733	-4.6800	0.0000	2.8733	4.9933	12.4583	16.8233	35.8100	36.6600	37.8933
0.1-Morin	-17.3467	-7.5533	-2.8733	0.0000	2.1200	9.5850	13.9500	32.9367	33.7867	35.0200
1-Morin	-19.4667	-9.6733	-4.9933	-2.1200	0.0000	7.4650	11.8300	30.8167	31.6667	32.9000
10-Morin	-26.9317	-17.1383	-12.4583	-9.5850	-7.4650	0.0000	4.3650	23.3517	24.2017	25.4350
10-Flavone	-31.2967	-21.5033	-16.8233	-13.9500	-11.8300	-4.3650	0.0000	18.9867	19.8367	21.0700
Rt-extracts-M	-50.2833	-40.4900	-35.8100	-32.9367	-30.8167	-23.3517	-18.9867	0.0000	0.8500	2.0833
100-Morin	-51.1333	-41.3400	-36.6600	-33.7867	-31.6667	-24.2017	-19.8367	-0.8500	0.0000	1.2333
100-Flavone	-52.3667	-42.5733	-37.8933	-35.0200	-32.9000	-25.4350	-21.0700	-2.0833	-1.2333	0.0000

Alpha= 0.05

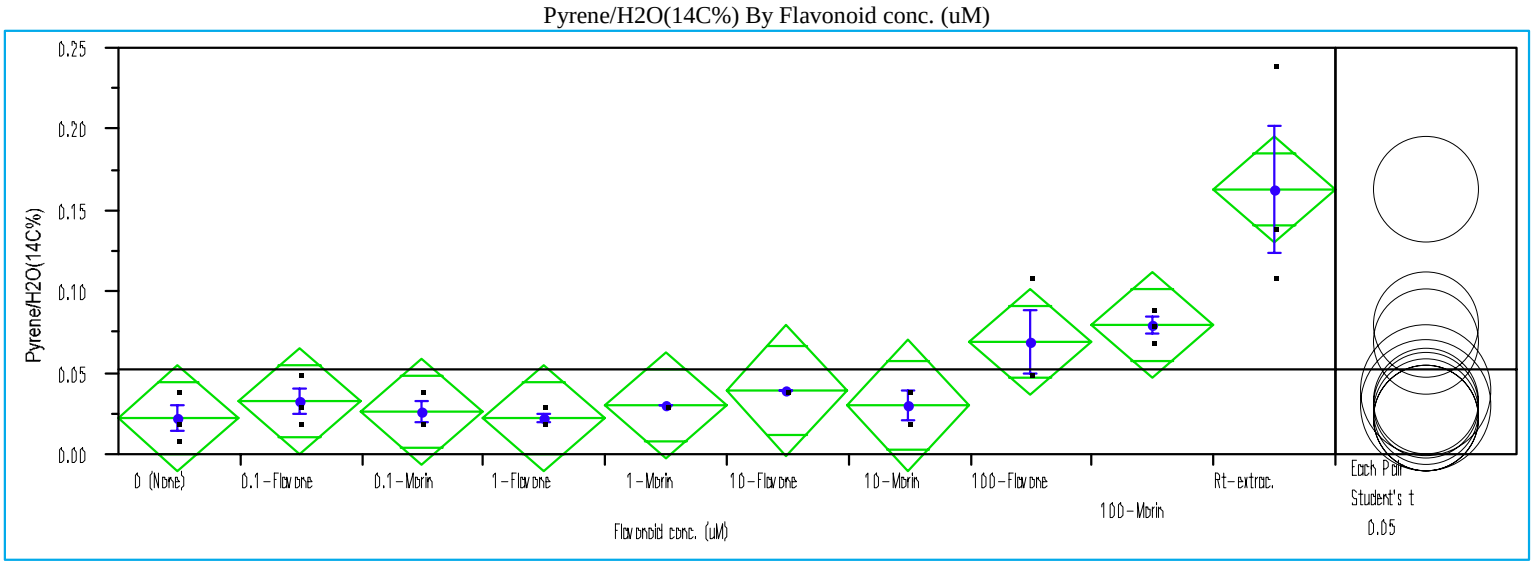
Comparisons for each pair using Student's t

t

2.10091

Abs(Dif)-LSD	0.1-Flavone	1-Flavone	0 (None)	0.1-Morin	1-Morin	10-Morin	10-Flavone	Rt-extracts-M	100-Morin	100-Flavone
0.1-Flavone	-20.3903	-10.5970	-5.9170	-3.0437	-0.9237	4.1346	8.4996	29.8930	30.7430	31.9763
1-Flavone	-10.5970	-20.3903	-15.7103	-12.8370	-10.7170	-5.6588	-1.2938	20.0997	20.9497	22.1830
0 (None)	-5.9170	-15.7103	-20.3903	-17.5170	-15.3970	-10.3388	-5.9738	15.4197	16.2697	17.5030
0.1-Morin	-3.0437	-12.8370	-17.5170	-20.3903	-18.2703	-13.2121	-8.8471	12.5463	13.3963	14.6297
1-Morin	-0.9237	-10.7170	-15.3970	-18.2703	-20.3903	-15.3321	-10.9671	10.4263	11.2763	12.5097
10-Morin	4.1346	-5.6588	-10.3388	-13.2121	-15.3321	-24.9730	-20.6080	0.5546	1.4046	2.6379
10-Flavone	8.4996	-1.2938	-5.9738	-8.8471	-10.9671	-20.6080	-24.9730	-3.8104	-2.9604	-1.7271
Rt-extracts-M	29.8930	20.0997	15.4197	12.5463	10.4263	0.5546	-3.8104	-20.3903	-19.5403	-18.3070
100-Morin	30.7430	20.9497	16.2697	13.3963	11.2763	1.4046	-2.9604	-19.5403	-20.3903	-19.1570
100-Flavone	31.9763	22.1830	17.5030	14.6297	12.5097	2.6379	-1.7271	-18.3070	-19.1570	-20.3903

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	0.023333	0.015275	0.00882
0.1-Flavone	3	0.033333	0.015275	0.00882
0.1-Morin	3	0.026667	0.011547	0.00667
1-Flavone	3	0.023333	0.005774	0.00333
1-Morin	3	0.030000	0.000000	0.00000
10-Flavone	2	0.040000	0.000000	0.00000
10-Morin	2	0.030000	0.014142	0.01000
100-Flavone	3	0.070000	0.034641	0.02000
100-Morin	3	0.080000	0.010000	0.00577
Rt-extracts-M	3	0.163333	0.068069	0.03930

Means Comparisons

Dif=Mean[i]-Mean[j]	Rt-extracts-M	100-Morin	100-Flavone	10-Flavone	0.1-Flavone	1-Morin	10-Morin	0.1-Morin	1-Flavone	0 (None)
Rt-extracts-M	0.000000	0.083333	0.093333	0.123333	0.130000	0.133333	0.133333	0.136667	0.140000	0.140000
100-Morin	-0.08333	0.000000	0.010000	0.040000	0.046667	0.050000	0.050000	0.053333	0.056667	0.056667
100-Flavone	-0.09333	-0.01	0.000000	0.030000	0.036667	0.040000	0.040000	0.043333	0.046667	0.046667
10-Flavone	-0.12333	-0.04	-0.03	0.000000	0.006667	0.010000	0.010000	0.013333	0.016667	0.016667
0.1-Flavone	-0.13	-0.04667	-0.03667	-0.00667	0.000000	0.003333	0.003333	0.006667	0.010000	0.010000
1-Morin	-0.13333	-0.05	-0.04	-0.01	-0.00333	0.000000	0.000000	0.003333	0.006667	0.006667
10-Morin	-0.13333	-0.05	-0.04	-0.01	-0.00333	0.000000	0.000000	0.003333	0.006667	0.006667
0.1-Morin	-0.13667	-0.05333	-0.04333	-0.01333	-0.00667	-0.00333	-0.00333	0.000000	0.003333	0.003333
1-Flavone	-0.14	-0.05667	-0.04667	-0.01667	-0.01	-0.00667	-0.00667	-0.00333	0.000000	0.000000
0 (None)	-0.14	-0.05667	-0.04667	-0.01667	-0.01	-0.00667	-0.00667	-0.00333	0.000000	0.000000

Alpha= 0.05

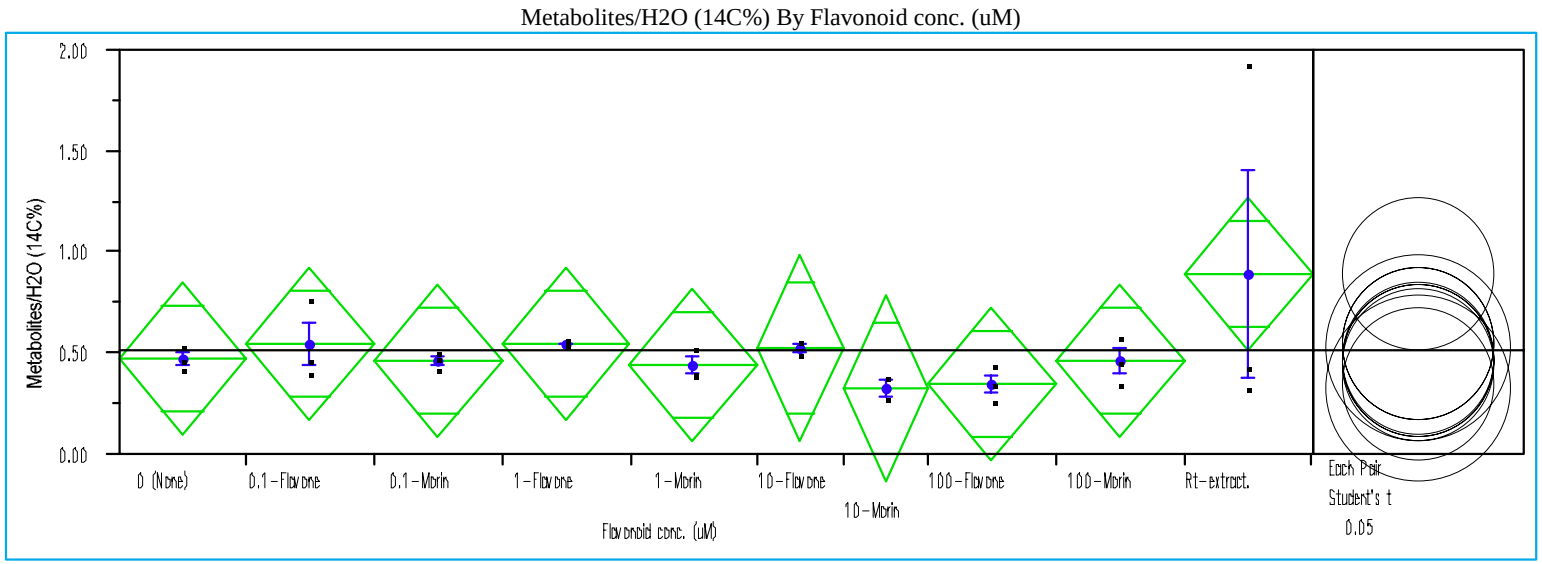
Comparisons for each pair using Student's t

t

2.10091

Abs(Dif)-LSD	Rt-extracts-M	100-Morin	100-Flavone	10-Flavone	0.1-Flavone	1-Morin	10-Morin	0.1-Morin	1-Flavone	0 (None)
Rt-extracts-M	-0.04669	0.036646	0.046646	0.071136	0.083313	0.086646	0.081136	0.089980	0.093313	0.093313
100-Morin	0.036646	-0.04669	-0.03669	-0.0122	-0.00002	0.003313	-0.0022	0.006646	0.009980	0.009980
100-Flavone	0.046646	-0.03669	-0.04669	-0.0222	-0.01002	-0.00669	-0.0122	-0.00335	-0.00002	-0.00002
10-Flavone	0.071136	-0.0122	-0.0222	-0.05718	-0.04553	-0.0422	-0.04718	-0.03886	-0.03553	-0.03553
0.1-Flavone	0.083313	-0.00002	-0.01002	-0.04553	-0.04669	-0.04335	-0.04886	-0.04002	-0.03669	-0.03669
1-Morin	0.086646	0.003313	-0.00669	-0.0422	-0.04335	-0.04669	-0.0522	-0.04335	-0.04002	-0.04002
10-Morin	0.081136	-0.0022	-0.0122	-0.04718	-0.04886	-0.0522	-0.05718	-0.04886	-0.04553	-0.04553
0.1-Morin	0.089980	0.006646	-0.00335	-0.03886	-0.04002	-0.04335	-0.04886	-0.04669	-0.04335	-0.04335
1-Flavone	0.093313	0.009980	-0.00002	-0.03553	-0.03669	-0.04002	-0.04553	-0.04335	-0.04669	-0.04669
0 (None)	0.093313	0.009980	-0.00002	-0.03553	-0.03669	-0.04002	-0.04553	-0.04335	-0.04669	-0.04669

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	0.486667	0.060277	0.03480
0.1-Flavone	3	0.556667	0.196554	0.11348
0.1-Morin	3	0.480000	0.045826	0.02646
1-Flavone	3	0.563333	0.015275	0.00882
1-Morin	3	0.450000	0.078102	0.04509
10-Flavone	2	0.540000	0.042426	0.03000
10-Morin	2	0.340000	0.070711	0.05000
100-Flavone	3	0.363333	0.085049	0.04910
100-Morin	3	0.473333	0.115036	0.06642
Rt-extracts-M	3	0.906667	0.896289	0.51747

Means Comparisons

Dif=Mean[i]-Mean[j]	Rt-extracts-M	1-Flavone	0.1-Flavone	10-Flavone	0 (None)	0.1-Morin	100-Morin	1-Morin	100-Flavone	10-Morin
Rt-extracts-M	0.000000	0.343333	0.350000	0.366667	0.420000	0.426667	0.433333	0.456667	0.543333	0.566667
1-Flavone	-0.34333	0.000000	0.006667	0.023333	0.076667	0.083333	0.090000	0.113333	0.200000	0.223333
0.1-Flavone	-0.35	-0.00667	0.000000	0.016667	0.070000	0.076667	0.083333	0.106667	0.193333	0.216667
10-Flavone	-0.36667	-0.02333	-0.01667	0.000000	0.053333	0.060000	0.066667	0.090000	0.176667	0.200000
0 (None)	-0.42	-0.07667	-0.07	-0.05333	0.000000	0.006667	0.013333	0.036667	0.123333	0.146667
0.1-Morin	-0.42667	-0.08333	-0.07667	-0.06	-0.00667	0.000000	0.006667	0.030000	0.116667	0.140000
100-Morin	-0.43333	-0.09	-0.08333	-0.06667	-0.01333	-0.00667	0.000000	0.023333	0.110000	0.133333
1-Morin	-0.45667	-0.11333	-0.10667	-0.09	-0.03667	-0.03	-0.02333	0.000000	0.086667	0.110000
100-Flavone	-0.54333	-0.2	-0.19333	-0.17667	-0.12333	-0.11667	-0.11	-0.08667	0.000000	0.023333
10-Morin	-0.56667	-0.22333	-0.21667	-0.2	-0.14667	-0.14	-0.13333	-0.11	-0.02333	0.000000

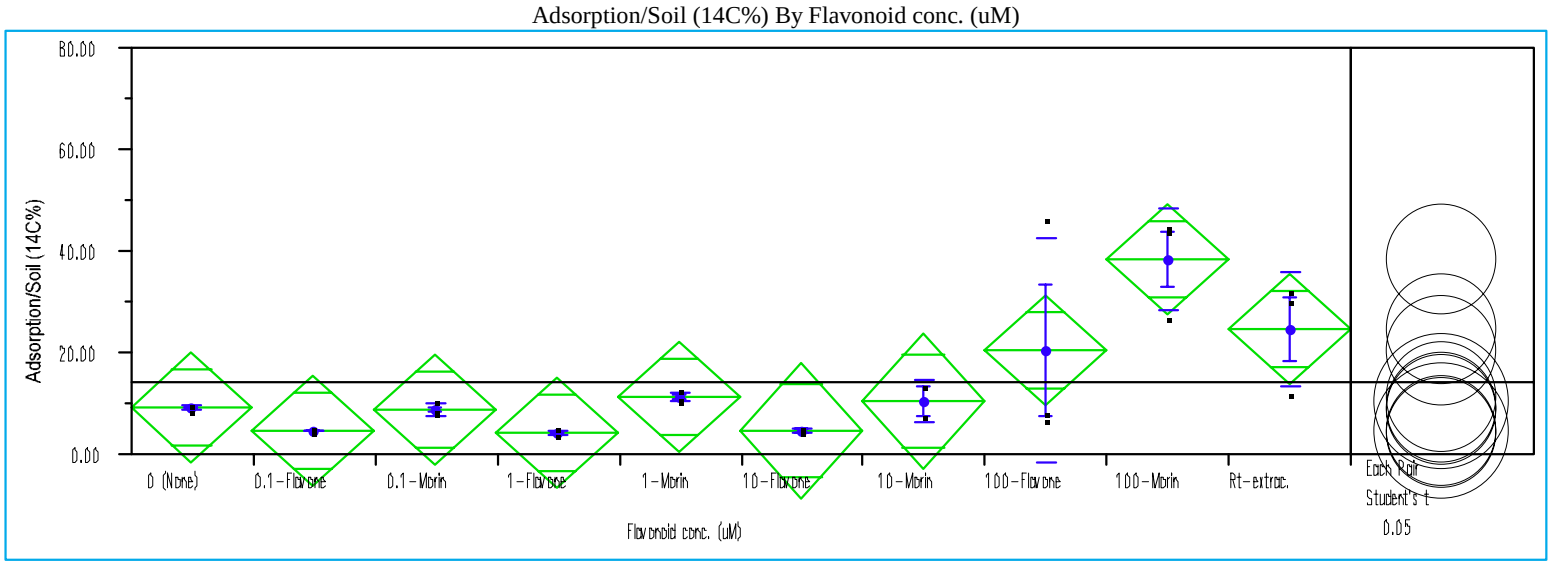
Alpha= 0.05

Comparisons for each pair using Student's t

t
2.10091

Abs(Dif)-LSD	Rt-extracts-M	1-Flavone	0.1-Flavone	10-Flavone	0 (None)	0.1-Morin	100-Morin	1-Morin	100-Flavone	10-Morin
Rt-extracts-M	-0.53575	-0.19242	-0.18575	-0.23232	-0.11575	-0.10908	-0.10242	-0.07908	0.007583	-0.03232
1-Flavone	-0.19242	-0.53575	-0.52908	-0.57565	-0.45908	-0.45242	-0.44575	-0.42242	-0.33575	-0.37565
0.1-Flavone	-0.18575	-0.52908	-0.53575	-0.58232	-0.46575	-0.45908	-0.45242	-0.42908	-0.34242	-0.38232
10-Flavone	-0.23232	-0.57565	-0.58232	-0.65616	-0.54565	-0.53899	-0.53232	-0.50899	-0.42232	-0.45616
0 (None)	-0.11575	-0.45908	-0.46575	-0.54565	-0.53575	-0.52908	-0.52242	-0.49908	-0.41242	-0.45232
0.1-Morin	-0.10908	-0.45242	-0.45908	-0.53899	-0.52908	-0.53575	-0.52908	-0.50575	-0.41908	-0.45899
100-Morin	-0.10242	-0.44575	-0.45242	-0.53232	-0.52242	-0.52908	-0.53575	-0.51242	-0.42575	-0.46565
1-Morin	-0.07908	-0.42242	-0.42908	-0.50899	-0.49908	-0.50575	-0.51242	-0.53575	-0.44908	-0.48899
100-Flavone	0.007583	-0.33575	-0.34242	-0.42232	-0.41242	-0.41908	-0.42575	-0.44908	-0.53575	-0.57565
10-Morin	-0.03232	-0.37565	-0.38232	-0.45616	-0.45232	-0.45899	-0.46565	-0.48899	-0.57565	-0.65616

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	9.3433	0.6787	0.392
0.1-Flavone	3	4.7000	0.3869	0.223
0.1-Morin	3	9.0500	1.4382	0.830
1-Flavone	3	4.4200	0.5679	0.328
1-Morin	3	11.5000	1.1697	0.675
10-Flavone	2	4.6850	0.6718	0.475
10-Morin	2	10.7500	4.1719	2.950
100-Flavone	3	20.5900	22.4911	12.985
100-Morin	3	38.6167	10.0266	5.789
Rt-extracts-M	3	24.7033	11.2564	6.499

Means Comparisons

Dif=Mean[i]-Mean[j]	100-Morin	Rt-extracts-M	100-Flavone	1-Morin	10-Morin	0 (None)	0.1-Morin	0.1-Flavone	10-Flavone	1-Flavone
100-Morin	0.0000	13.9133	18.0267	27.1167	27.8667	29.2733	29.5667	33.9167	33.9317	34.1967
Rt-extracts-M	-13.9133	0.0000	4.1133	13.2033	13.9533	15.3600	15.6533	20.0033	20.0183	20.2833
100-Flavone	-18.0267	-4.1133	0.0000	9.0900	9.8400	11.2467	11.5400	15.8900	15.9050	16.1700
1-Morin	-27.1167	-13.2033	-9.0900	0.0000	0.7500	2.1567	2.4500	6.8000	6.8150	7.0800
10-Morin	-27.8667	-13.9533	-9.8400	-0.7500	0.0000	1.4067	1.7000	6.0500	6.0650	6.3300
0 (None)	-29.2733	-15.3600	-11.2467	-2.1567	-1.4067	0.0000	0.2933	4.6433	4.6583	4.9233
0.1-Morin	-29.5667	-15.6533	-11.5400	-2.4500	-1.7000	-0.2933	0.0000	4.3500	4.3650	4.6300
0.1-Flavone	-33.9167	-20.0033	-15.8900	-6.8000	-6.0500	-4.6433	-4.3500	0.0000	0.0150	0.2800
10-Flavone	-33.9317	-20.0183	-15.9050	-6.8150	-6.0650	-4.6583	-4.3650	-0.0150	0.0000	0.2650
1-Flavone	-34.1967	-20.2833	-16.1700	-7.0800	-6.3300	-4.9233	-4.6300	-0.2800	-0.2650	0.0000

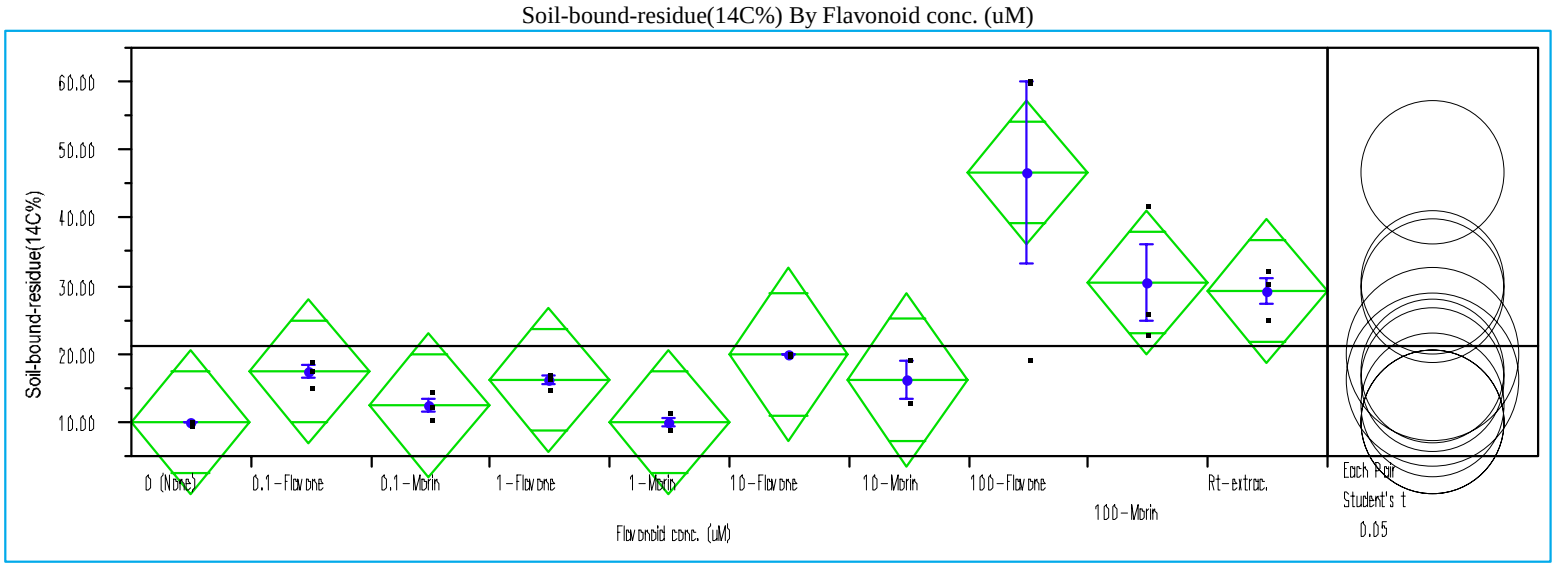
Alpha= 0.05

Comparisons for each pair using Student's t

t
2.10091

Abs(Dif)-LSD	100-Morin	Rt-extracts-M	100-Flavone	1-Morin	10-Morin	0 (None)	0.1-Morin	0.1-Flavone	10-Flavone	1-Flavone
100-Morin	-15.6215	-1.7082	2.4052	11.4952	10.4013	13.6518	13.9452	18.2952	16.4663	18.5752
Rt-extracts-M	-1.7082	-15.6215	-11.5082	-2.4182	-3.5120	-0.2615	0.0318	4.3818	2.5530	4.6618
100-Flavone	2.4052	-11.5082	-15.6215	-6.5315	-7.6254	-4.3748	-4.0815	0.2685	-1.5604	0.5485
1-Morin	11.4952	-2.4182	-6.5315	-15.6215	-16.7154	-13.4648	-13.1715	-8.8215	-10.6504	-8.5415
10-Morin	10.4013	-3.5120	-7.6254	-16.7154	-19.1323	-16.0587	-15.7654	-11.4154	-13.0673	-11.1354
0 (None)	13.6518	-0.2615	-4.3748	-13.4648	-16.0587	-15.6215	-15.3282	-10.9782	-12.8070	-10.6982
0.1-Morin	13.9452	0.0318	-4.0815	-13.1715	-15.7654	-15.3282	-15.6215	-11.2715	-13.1004	-10.9915
0.1-Flavone	18.2952	4.3818	0.2685	-8.8215	-11.4154	-10.9782	-11.2715	-15.6215	-17.4504	-15.3415
10-Flavone	16.4663	2.5530	-1.5604	-10.6504	-13.0673	-12.8070	-13.1004	-17.4504	-19.1323	-17.2004
1-Flavone	18.5752	4.6618	0.5485	-8.5415	-11.1354	-10.6982	-10.9915	-15.3415	-17.2004	-15.6215

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	10.1700	0.4115	0.238
0.1-Flavone	3	17.5133	1.9151	1.106
0.1-Morin	3	12.7300	2.0406	1.178
1-Flavone	3	16.2800	1.1437	0.660
1-Morin	3	9.9933	1.4351	0.829
10-Flavone	2	20.1750	0.1909	0.135
10-Morin	2	16.4600	4.3558	3.080
100-Flavone	3	46.7533	23.5678	13.607
100-Morin	3	30.5100	10.0823	5.821
Rt-extracts-M	3	29.5433	3.7274	2.152

Means Comparisons

Dif=Mean[i]-Mean[j]	100-Flavone	100-Morin	Rt-extracts-M	10-Flavone	0.1-Flavone	10-Morin	1-Flavone	0.1-Morin	0 (None)	1-Morin
100-Flavone	0.0000	16.2433	17.2100	26.5783	29.2400	30.2933	30.4733	34.0233	36.5833	36.7600
100-Morin	-16.2433	0.0000	0.9667	10.3350	12.9967	14.0500	14.2300	17.7800	20.3400	20.5167
Rt-extracts-M	-17.2100	-0.9667	0.0000	9.3683	12.0300	13.0833	13.2633	16.8133	19.3733	19.5500
10-Flavone	-26.5783	-10.3350	-9.3683	0.0000	2.6617	3.7150	3.8950	7.4450	10.0050	10.1817
0.1-Flavone	-29.2400	-12.9967	-12.0300	-2.6617	0.0000	1.0533	1.2333	4.7833	7.3433	7.5200
10-Morin	-30.2933	-14.0500	-13.0833	-3.7150	-1.0533	0.0000	0.1800	3.7300	6.2900	6.4667
1-Flavone	-30.4733	-14.2300	-13.2633	-3.8950	-1.2333	-0.1800	0.0000	3.5500	6.1100	6.2867
0.1-Morin	-34.0233	-17.7800	-16.8133	-7.4450	-4.7833	-3.7300	-3.5500	0.0000	2.5600	2.7367
0 (None)	-36.5833	-20.3400	-19.3733	-10.0050	-7.3433	-6.2900	-6.1100	-2.5600	0.0000	0.1767
1-Morin	-36.7600	-20.5167	-19.5500	-10.1817	-7.5200	-6.4667	-6.2867	-2.7367	-0.1767	0.0000

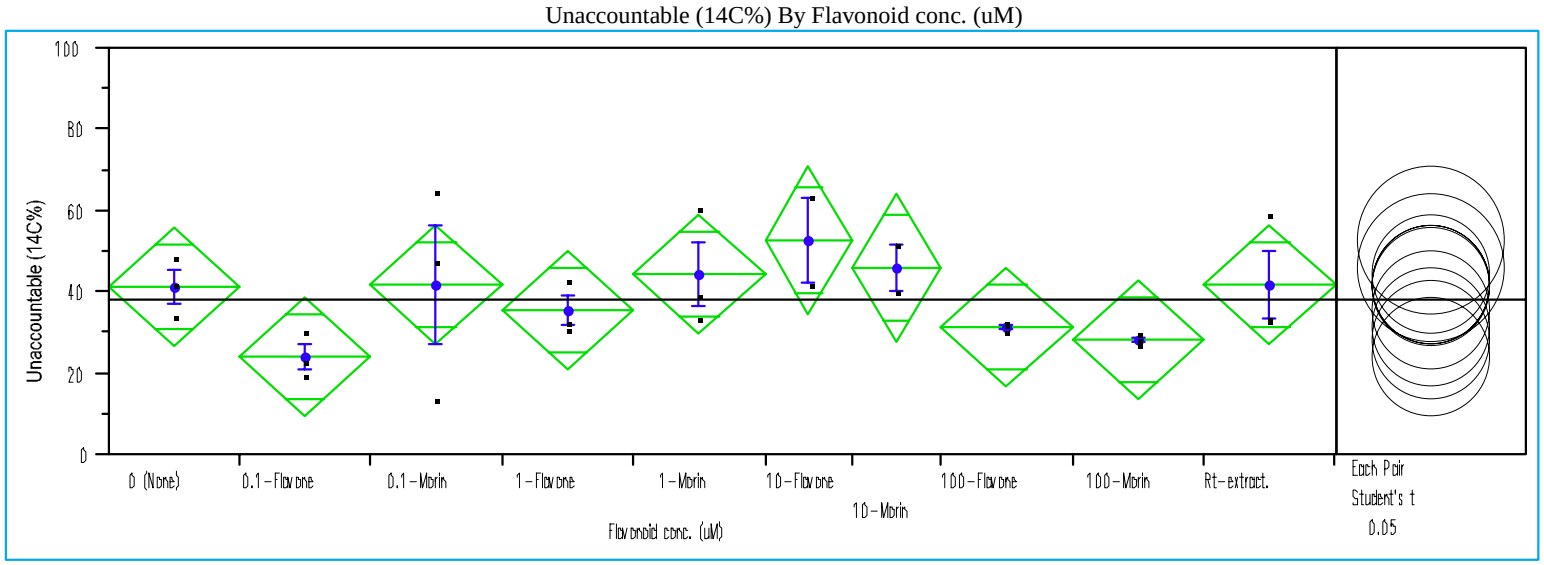
Alpha= 0.05

Comparisons for each pair using Student's t

t
2.10091

Abs(Dif)-LSD	100-Flavone	100-Morin	Rt-extracts-M	10-Flavone	0.1-Flavone	10-Morin	1-Flavone	0.1-Morin	0 (None)	1-Morin
100-Flavone	-15.0401	1.2033	2.1699	9.7630	14.1999	13.4780	15.4333	18.9833	21.5433	21.7199
100-Morin	1.2033	-15.0401	-14.0734	-6.4803	-2.0434	-2.7653	-0.8101	2.7399	5.2999	5.4766
Rt-extracts-M	2.1699	-14.0734	-15.0401	-7.4470	-3.0101	-3.7320	-1.7767	1.7733	4.3333	4.5099
10-Flavone	9.7630	-6.4803	-7.4470	-18.4202	-14.1536	-14.7052	-12.9203	-9.3703	-6.8103	-6.6336
0.1-Flavone	14.1999	-2.0434	-3.0101	-14.1536	-15.0401	-15.7620	-13.8067	-10.2567	-7.6967	-7.5201
10-Morin	13.4780	-2.7653	-3.7320	-14.7052	-15.7620	-18.4202	-16.6353	-13.0853	-10.5253	-10.3486
1-Flavone	15.4333	-0.8101	-1.7767	-12.9203	-13.8067	-16.6353	-15.0401	-11.4901	-8.9301	-8.7534
0.1-Morin	18.9833	2.7399	1.7733	-9.3703	-10.2567	-13.0853	-11.4901	-15.0401	-12.4801	-12.3034
0 (None)	21.5433	5.2999	4.3333	-6.8103	-7.6967	-10.5253	-8.9301	-12.4801	-15.0401	-14.8634
1-Morin	21.7199	5.4766	4.5099	-6.6336	-7.5201	-10.3486	-8.7534	-12.3034	-14.8634	-15.0401

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	41.6133	7.4131	4.280
0.1-Flavone	3	24.3633	5.5603	3.210
0.1-Morin	3	42.2200	25.9833	15.001
1-Flavone	3	35.6667	6.4202	3.707
1-Morin	3	44.6633	14.2603	8.233
10-Flavone	2	53.0250	15.2947	10.815
10-Morin	2	46.5100	8.1034	5.730
100-Flavone	3	31.7533	1.1075	0.639
100-Morin	3	28.6167	1.3274	0.766
Rt-extracts-M	3	42.1300	14.9917	8.655

Means Comparisons

Dif=Mean[i]-Mean[j]	10-Flavone	10-Morin	1-Morin	0.1-Morin	Rt-extracts-M	0 (None)	1-Flavone	100-Flavone	100-Morin	0.1-Flavone
10-Flavone	0.0000	6.5150	8.3617	10.8050	10.8950	11.4117	17.3583	21.2717	24.4083	28.6617
10-Morin	-6.5150	0.0000	1.8467	4.2900	4.3800	4.8967	10.8433	14.7567	17.8933	22.1467
1-Morin	-8.3617	-1.8467	0.0000	2.4433	2.5333	3.0500	8.9967	12.9100	16.0467	20.3000
0.1-Morin	-10.8050	-4.2900	-2.4433	0.0000	0.0900	0.6067	6.5533	10.4667	13.6033	17.8567
Rt-extracts-M	-10.8950	-4.3800	-2.5333	-0.0900	0.0000	0.5167	6.4633	10.3767	13.5133	17.7667
0 (None)	-11.4117	-4.8967	-3.0500	-0.6067	-0.5167	0.0000	5.9467	9.8600	12.9967	17.2500
1-Flavone	-17.3583	-10.8433	-8.9967	-6.5533	-6.4633	-5.9467	0.0000	3.9133	7.0500	11.3033
100-Flavone	-21.2717	-14.7567	-12.9100	-10.4667	-10.3767	-9.8600	-3.9133	0.0000	3.1367	7.3900
100-Morin	-24.4083	-17.8933	-16.0467	-13.6033	-13.5133	-12.9967	-7.0500	-3.1367	0.0000	4.2533
0.1-Flavone	-28.6617	-22.1467	-20.3000	-17.8567	-17.7667	-17.2500	-11.3033	-7.3900	-4.2533	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

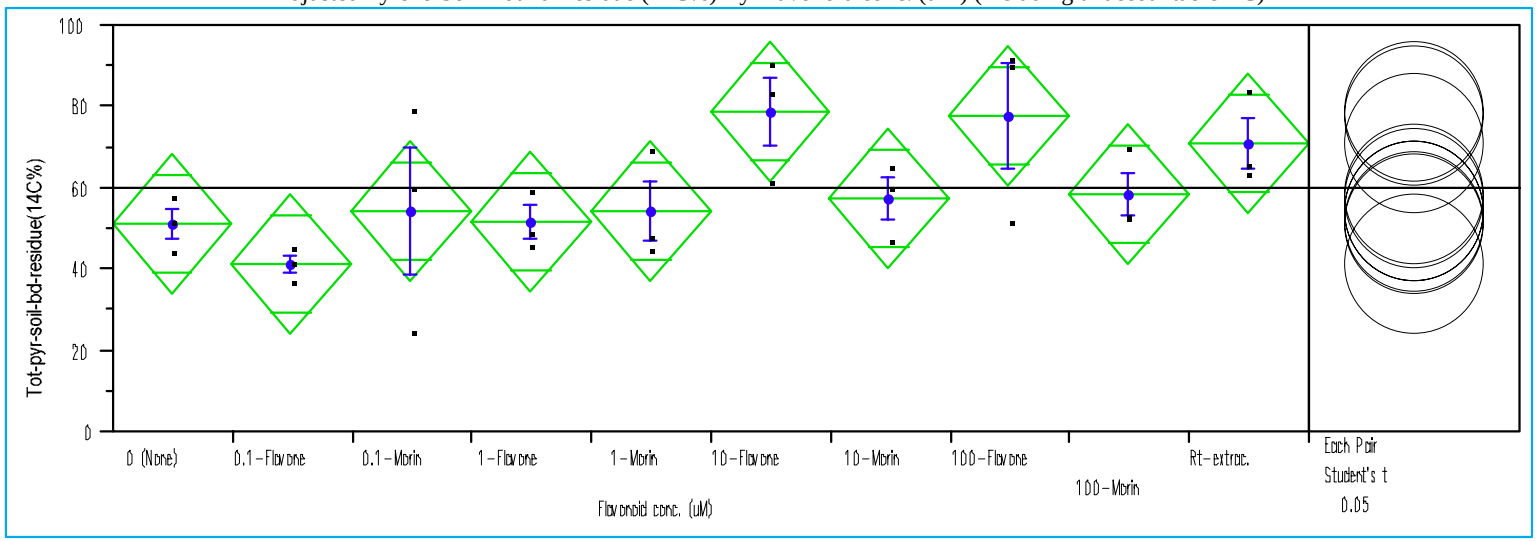
t

2.10091

Abs(Dif)-LSD	10-Flavone	10-Morin	1-Morin	0.1-Morin	Rt-extracts-M	0 (None)	1-Flavone	100-Flavone	100-Morin	0.1-Flavone
10-Flavone	-26.0444	-19.5294	-15.4136	-12.9702	-12.8802	-12.3636	-6.4169	-2.5036	0.6331	4.8864
10-Morin	-19.5294	-26.0444	-21.9286	-19.4852	-19.3952	-18.8786	-12.9319	-9.0186	-5.8819	-1.6286
1-Morin	-15.4136	-21.9286	-21.2652	-18.8219	-18.7319	-18.2152	-12.2685	-8.3552	-5.2185	-0.9652
0.1-Morin	-12.9702	-19.4852	-18.8219	-21.2652	-21.1752	-20.6585	-14.7119	-10.7985	-7.6619	-3.4085
Rt-extracts-M	-12.8802	-19.3952	-18.7319	-21.1752	-21.2652	-20.7485	-14.8019	-10.8885	-7.7519	-3.4985
0 (None)	-12.3636	-18.8786	-18.2152	-20.6585	-20.7485	-21.2652	-15.3185	-11.4052	-8.2685	-4.0152
1-Flavone	-6.4169	-12.9319	-12.2685	-14.7119	-14.8019	-15.3185	-21.2652	-17.3519	-14.2152	-9.9619
100-Flavone	-2.5036	-9.0186	-8.3552	-10.7985	-10.8885	-11.4052	-17.3519	-21.2652	-18.1285	-13.8752
100-Morin	0.6331	-5.8819	-5.2185	-7.6619	-7.7519	-8.2685	-14.2152	-18.1285	-21.2652	-17.0119
0.1-Flavone	4.8864	-1.6286	-0.9652	-3.4085	-3.4985	-4.0152	-9.9619	-13.8752	-17.0119	-21.2652

Positive values show pairs of means that are significantly different.

Adjusted Pyrene-Soil-Bound Residue (14C%) By Flavonoid conc. (uM) (including unaccountable ^{14}C)



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
0 (None)	3	51.7800	6.9995	4.041
0.1-Flavone	3	41.8767	4.1554	2.399
0.1-Morin	3	54.9500	27.9453	16.134
1-Flavone	3	51.9500	7.3206	4.227
1-Morin	3	54.6567	13.3675	7.718
10-Flavone	3	79.3467	15.2724	8.818
10-Morin	3	57.8633	9.2432	5.337
100-Flavone	3	78.5100	22.7322	13.124
100-Morin	3	59.1300	9.6774	5.587
Rt-extracts-M	3	71.6733	11.4037	6.584

Means Comparisons

Dif=Mean[i]-Mean[j]	10-Flavone	100-Flavone	Rt-extracts-M	100-Morin	10-Morin	0.1-Morin	1-Morin	1-Flavone	0 (None)	0.1-Flavone
10-Flavone	0.0000	0.8367	7.6733	20.2167	21.4833	24.3967	24.6900	27.3967	27.5667	37.4700
100-Flavone	-0.8367	0.0000	6.8367	19.3800	20.6467	23.5600	23.8533	26.5600	26.7300	36.6333
Rt-extracts-M	-7.6733	-6.8367	0.0000	12.5433	13.8100	16.7233	17.0167	19.7233	19.8933	29.7967
100-Morin	-20.2167	-19.3800	-12.5433	0.0000	1.2667	4.1800	4.4733	7.1800	7.3500	17.2533
10-Morin	-21.4833	-20.6467	-13.8100	-1.2667	0.0000	2.9133	3.2067	5.9133	6.0833	15.9867
0.1-Morin	-24.3967	-23.5600	-16.7233	-4.1800	-2.9133	0.0000	0.2933	3.0000	3.1700	13.0733
1-Morin	-24.6900	-23.8533	-17.0167	-4.4733	-3.2067	-0.2933	0.0000	2.7067	2.8767	12.7800
1-Flavone	-27.3967	-26.5600	-19.7233	-7.1800	-5.9133	-3.0000	-2.7067	0.0000	0.1700	10.0733
0 (None)	-27.5667	-26.7300	-19.8933	-7.3500	-6.0833	-3.1700	-2.8767	-0.1700	0.0000	9.9033
0.1-Flavone	-37.4700	-36.6333	-29.7967	-17.2533	-15.9867	-13.0733	-12.7800	-10.0733	-9.9033	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

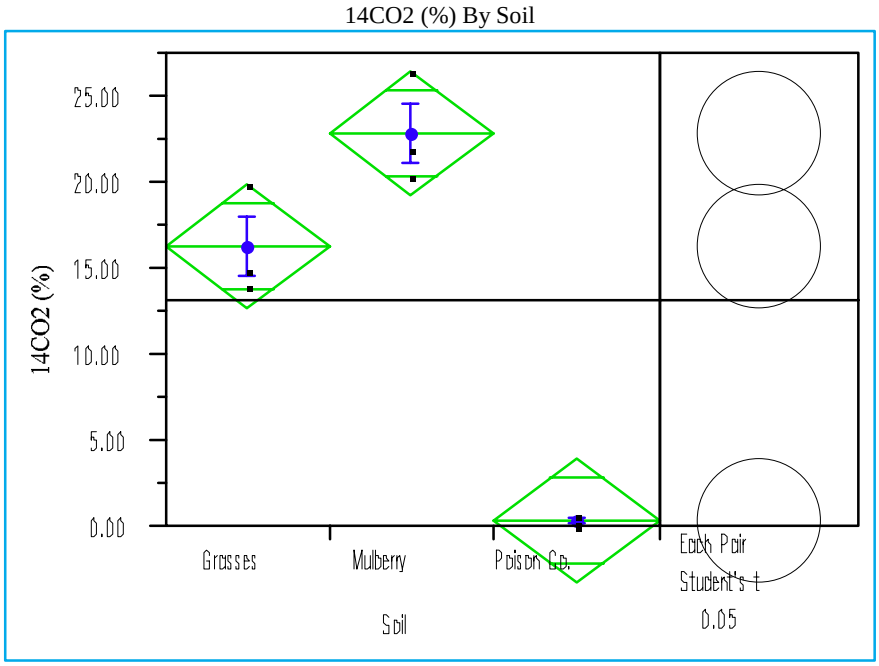
t

2.08595

Abs(Dif)-LSD	10-Flavone	100-Flavone	Rt-extracts-M	100-Morin	10-Morin	0.1-Morin	1-Morin	1-Flavone	0 (None)	0.1-Flavone
10-Flavone	-24.9071	-24.0705	-17.2338	-4.6905	-3.4238	-0.5105	-0.2171	2.4895	2.6595	12.5629
100-Flavone	-24.0705	-24.9071	-18.0705	-5.5271	-4.2605	-1.3471	-1.0538	1.6529	1.8229	11.7262
Rt-extracts-M	-17.2338	-18.0705	-24.9071	-12.3638	-11.0971	-8.1838	-7.8905	-5.1838	-5.0138	4.8895
100-Morin	-4.6905	-5.5271	-12.3638	-24.9071	-23.6405	-20.7271	-20.4338	-17.7271	-17.5571	-7.6538
10-Morin	-3.4238	-4.2605	-11.0971	-23.6405	-24.9071	-21.9938	-21.7005	-18.9938	-18.8238	-8.9205
0.1-Morin	-0.5105	-1.3471	-8.1838	-20.7271	-21.9938	-24.9071	-24.6138	-21.9071	-21.7371	-11.8338
1-Morin	-0.2171	-1.0538	-7.8905	-20.4338	-21.7005	-24.6138	-24.9071	-22.2005	-22.0305	-12.1271
1-Flavone	2.4895	1.6529	-5.1838	-17.7271	-18.9938	-21.9071	-22.2005	-24.9071	-24.7371	-14.8338
0 (None)	2.6595	1.8229	-5.0138	-17.5571	-18.8238	-21.7371	-22.0305	-24.7371	-24.9071	-15.0038
0.1-Flavone	12.5629	11.7262	4.8895	-7.6538	-8.9205	-11.8338	-12.1271	-14.8338	-15.0038	-24.9071

Positive values show pairs of means that are significantly different.

Appendix D-7. Student's t Test: Paired Comparison of B[a]P Fate Data in different Soils without Flavonoid Amendment



Means Comparisons

Dif=Mean[i]-Mean[j]	Mulberry	Grasses	Poison Control
Mulberry	0.0000	6.5667	22.5600
Grasses	-6.5667	0.0000	15.9933
Poison Control	-22.5600	-15.9933	0.0000

Alpha= 0.05

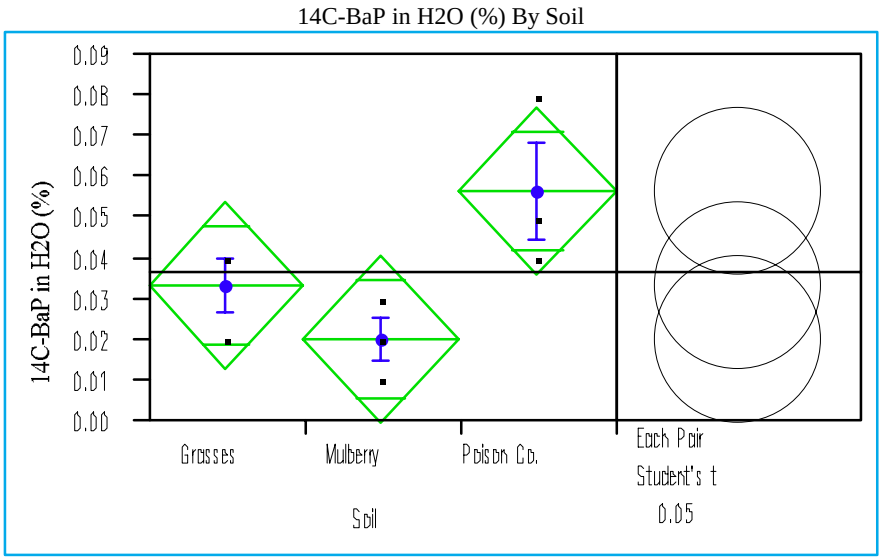
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Mulberry	Grasses	Poison Control
Mulberry	-5.1958	1.3709	17.3642
Grasses	1.3709	-5.1958	10.7976
Poison Control	17.3642	10.7976	-5.1958

Positive values show pairs of means that are significantly different.



Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Grasses	Mulberry
Poison Control	0.000000	0.023333	0.036667
Grasses	-0.02333	0.000000	0.013333
Mulberry	-0.03667	-0.01333	0.000000

Alpha= 0.05

Comparisons for each pair using Student's t

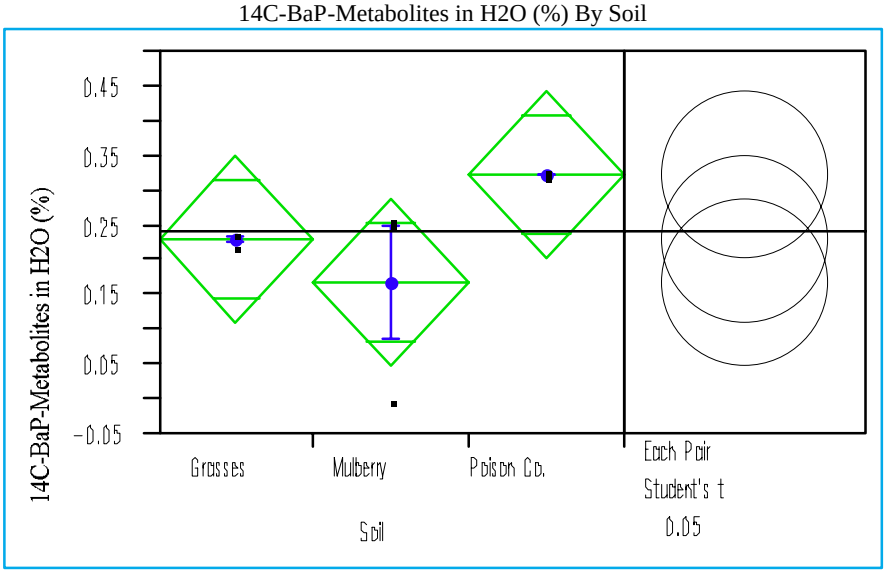
t

2.44691

Abs(Dif)-LSD	Poison Control	Grasses	Mulberry
Poison Control	-0.02978	-0.00645	0.006884
Grasses	-0.00645	-0.02978	-0.01645
Mulberry	0.006884	-0.01645	-0.02978

Positive values show pairs of means that are significantly different.

14C-BaP-Metabolites in H2O (%) By Soil



Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Grasses	Mulberry
Poison Control	0.000000	0.093333	0.156667
Grasses	-0.09333	0.000000	0.063333
Mulberry	-0.15667	-0.06333	0.000000

Alpha= 0.05

Comparisons for each pair using Student's t

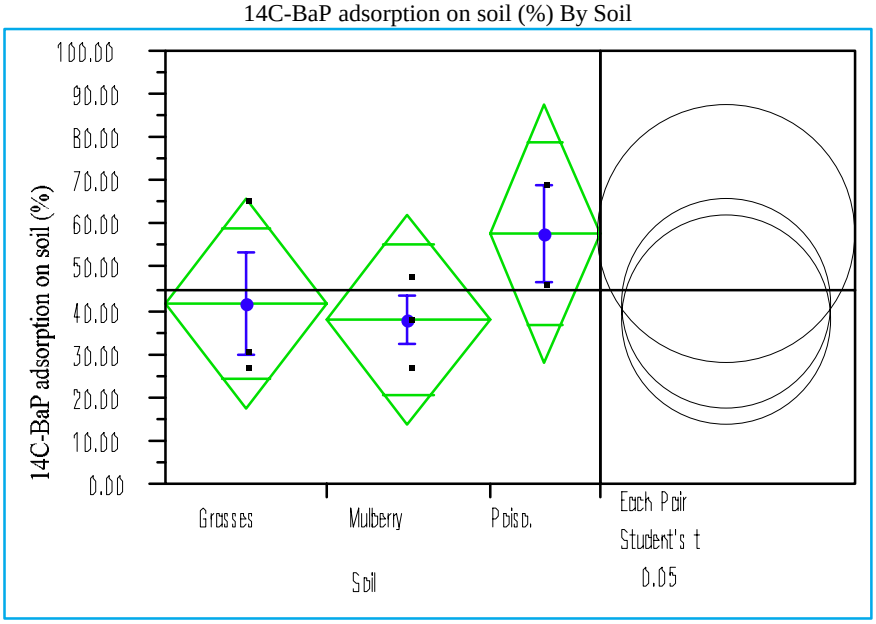
t

2.44691

Abs(Dif)-LSD	Poison Control	Grasses	Mulberry
Poison Control	-0.17057	-0.07724	-0.0139
Grasses	-0.07724	-0.17057	-0.10724
Mulberry	-0.0139	-0.10724	-0.17057

Positive values show pairs of means that are significantly different.

14C-BaP adsorption on soil (%) By Soil



Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Grasses	Mulberry
Poison Control	0.0000	16.6050	20.0517
Grasses	-16.6050	0.0000	3.4467
Mulberry	-20.0517	-3.4467	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

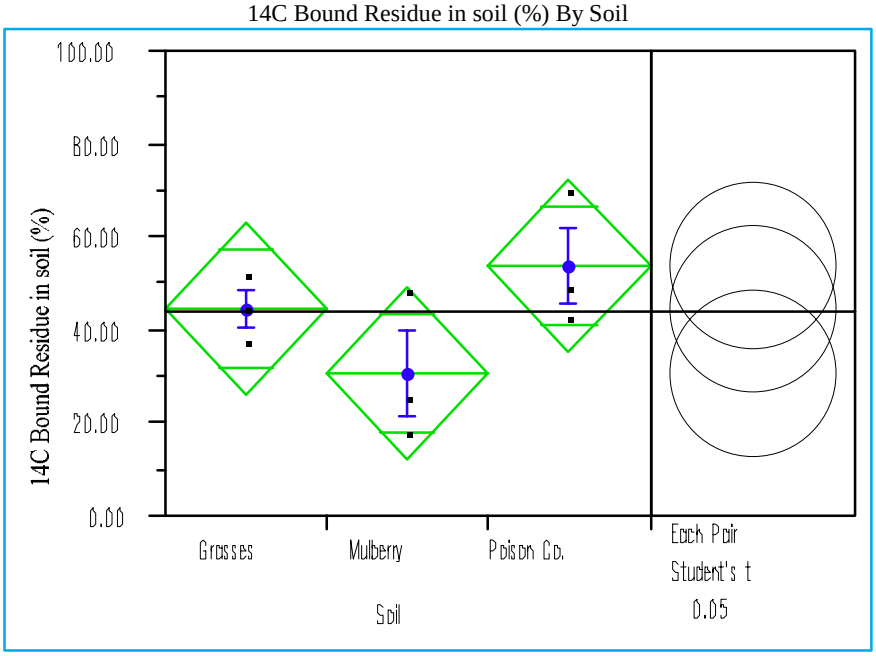
t

2.57054

Abs(Dif)-LSD	Poison Control	Grasses	Mulberry
Poison Control	-42.9791	-22.6294	-19.1827
Grasses	-22.6294	-35.0923	-31.6457
Mulberry	-19.1827	-31.6457	-35.0923

Positive values show pairs of means that are significantly different.

14C Bound Residue in soil (%) By Soil



Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Grasses	Mulberry
Poison Control	0.0000	9.1800	23.1467
Grasses	-9.1800	0.0000	13.9667
Mulberry	-23.1467	-13.9667	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

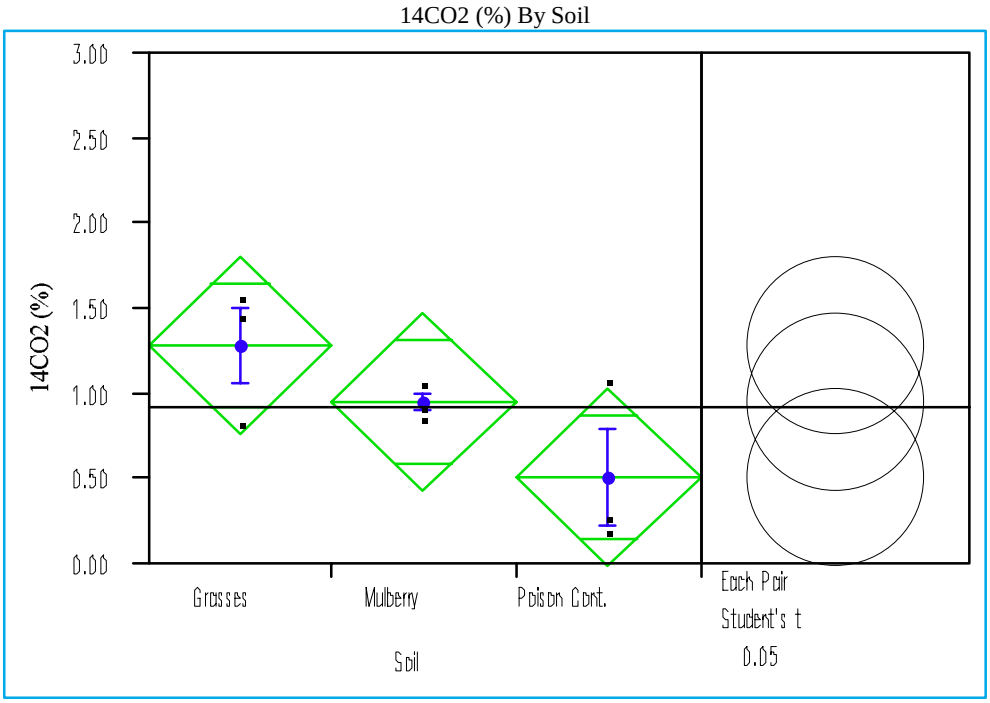
t

2.44691

Abs(Dif)-LSD	Poison Control	Grasses	Mulberry
Poison Control	-26.2845	-17.1045	-3.1378
Grasses	-17.1045	-26.2845	-12.3178
Mulberry	-3.1378	-12.3178	-26.2845

Positive values show pairs of means that are significantly different.

Appendix D-8. Student's t Test: Paired Comparison of B[a]P Fate in different Soils Amended with 100 uM morin



Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Mulberry	Poison Control
Grasses	0.000000	0.330000	0.766667
Mulberry	-0.33	0.000000	0.436667
Poison Control	-0.76667	-0.43667	0.000000

Alpha= 0.05

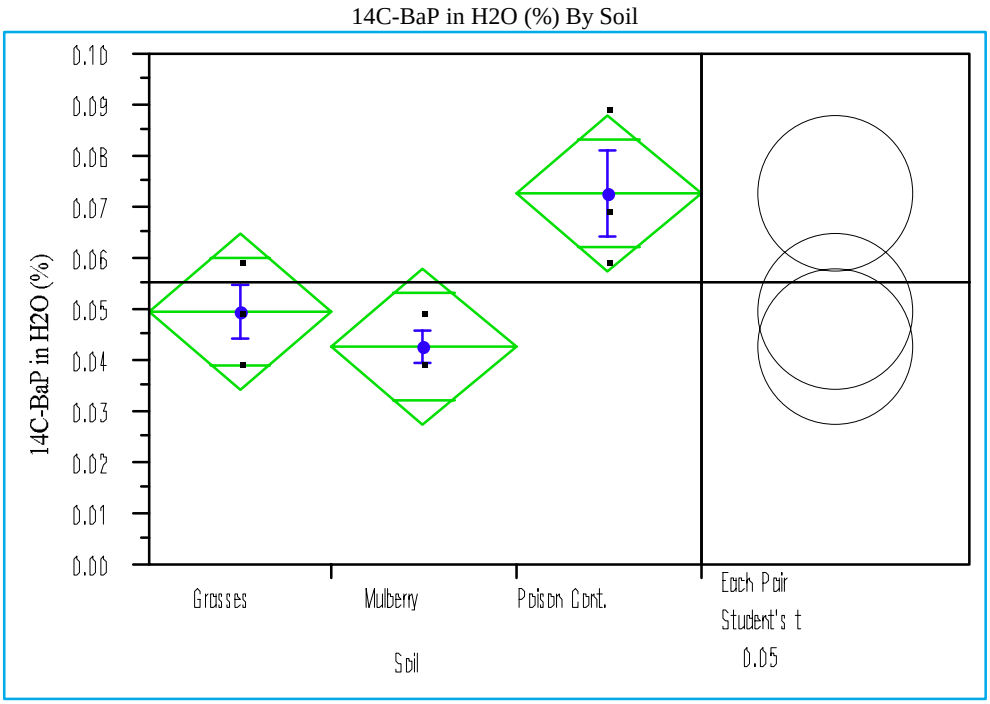
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Grasses	Mulberry	Poison Control
Grasses	-0.74296	-0.41296	0.023705
Mulberry	-0.41296	-0.74296	-0.3063
Poison Control	0.023705	-0.3063	-0.74296

Positive values show pairs of means that are significantly different.



Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Grasses	Mulberry
Poison Control	0.000000	0.023333	0.030000
Grasses	-0.02333	0.000000	0.006667
Mulberry	-0.03	-0.00667	0.000000

Alpha= 0.05

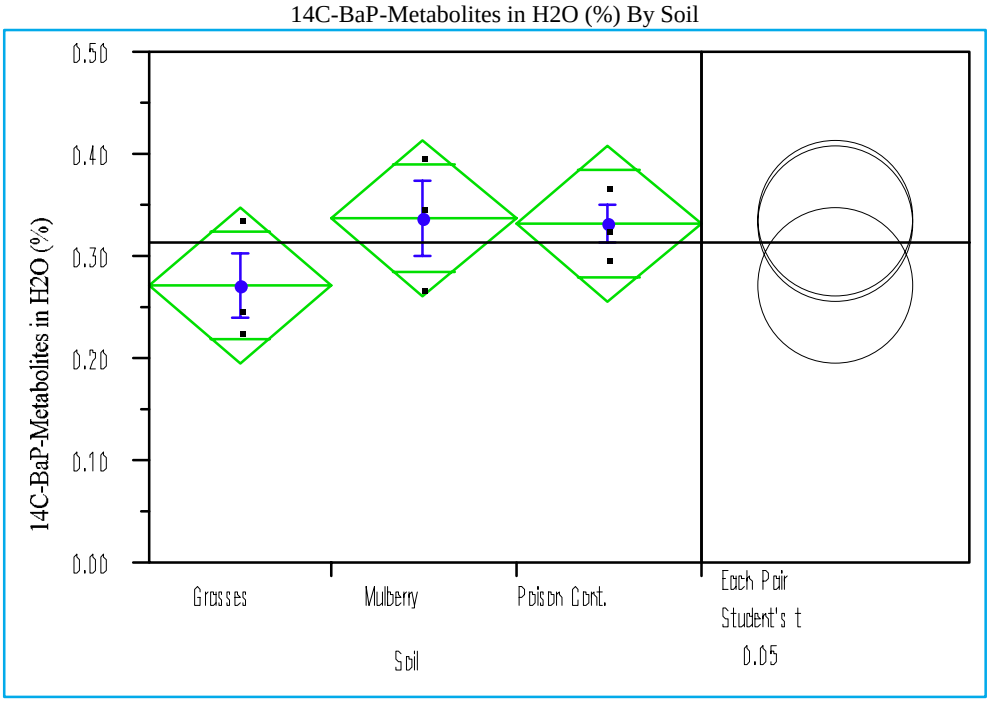
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Poison Control	Grasses	Mulberry
Poison Control	-0.02209	0.001246	0.007912
Grasses	0.001246	-0.02209	-0.01542
Mulberry	0.007912	-0.01542	-0.02209

Positive values show pairs of means that are significantly different.



Means Comparisons

Dif=Mean[i]-Mean[j]	Mulberry	Poison Control	Grasses
Mulberry	0.000000	0.006667	0.066667
Poison Control	-0.00667	0.000000	0.060000
Grasses	-0.06667	-0.06	0.000000

Alpha= 0.05

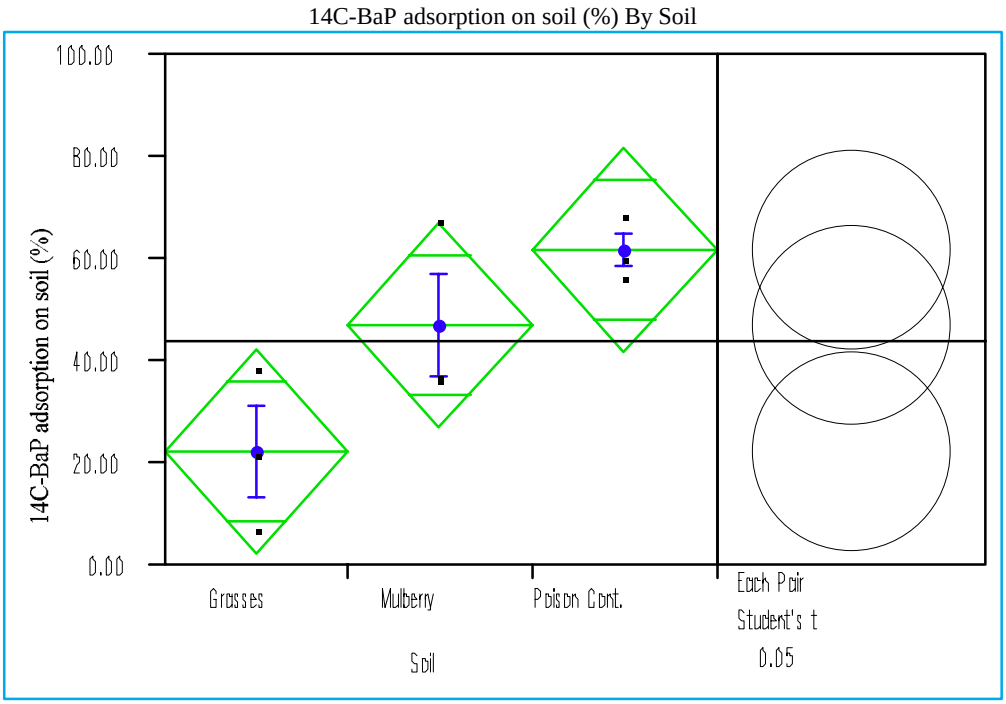
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Mulberry	Poison Control	Grasses
Mulberry	-0.10923	-0.10256	-0.04256
Poison Control	-0.10256	-0.10923	-0.04923
Grasses	-0.04256	-0.04923	-0.10923

Positive values show pairs of means that are significantly different.



Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Mulberry	Grasses
Poison Control	0.0000	14.7633	39.4133
Mulberry	-14.7633	0.0000	24.6500
Grasses	-39.4133	-24.6500	0.0000

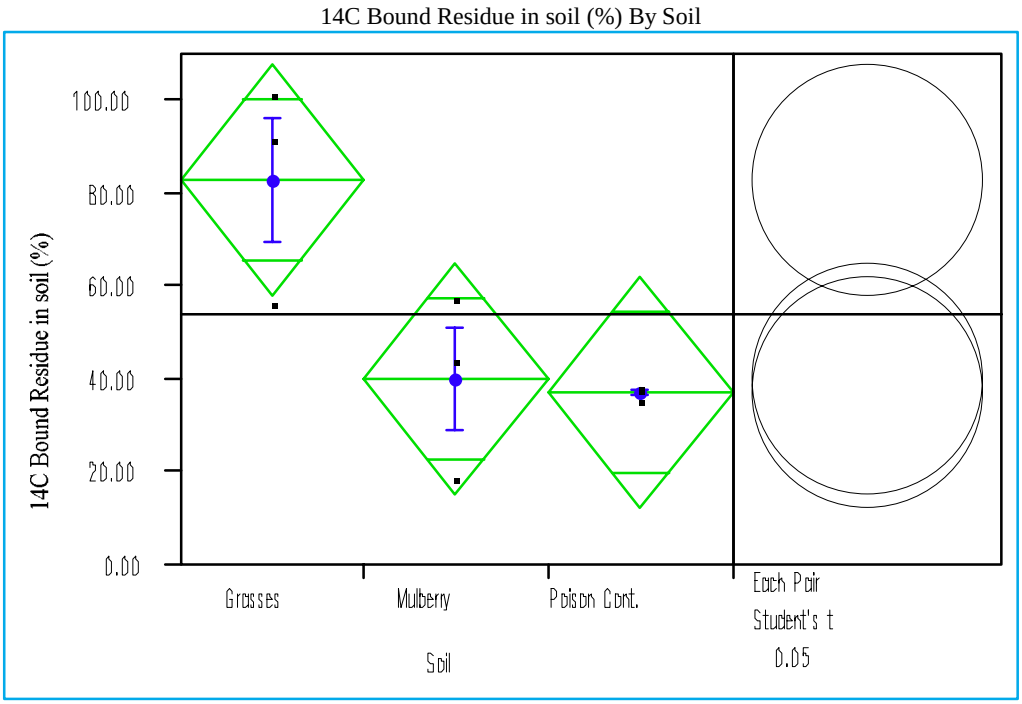
Alpha= 0.05

Comparisons for each pair using Student's t

t
2.44691

Abs(Dif)-LSD	Poison Control	Mulberry	Grasses
Poison Control	-28.3014	-13.5381	11.1119
Mulberry	-13.5381	-28.3014	-3.6514
Grasses	11.1119	-3.6514	-28.3014

Positive values show pairs of means that are significantly different.



Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Mulberry	Poison Control
Grasses	0.0000	43.1733	46.1700
Mulberry	-43.1733	0.0000	2.9967
Poison Control	-46.1700	-2.9967	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

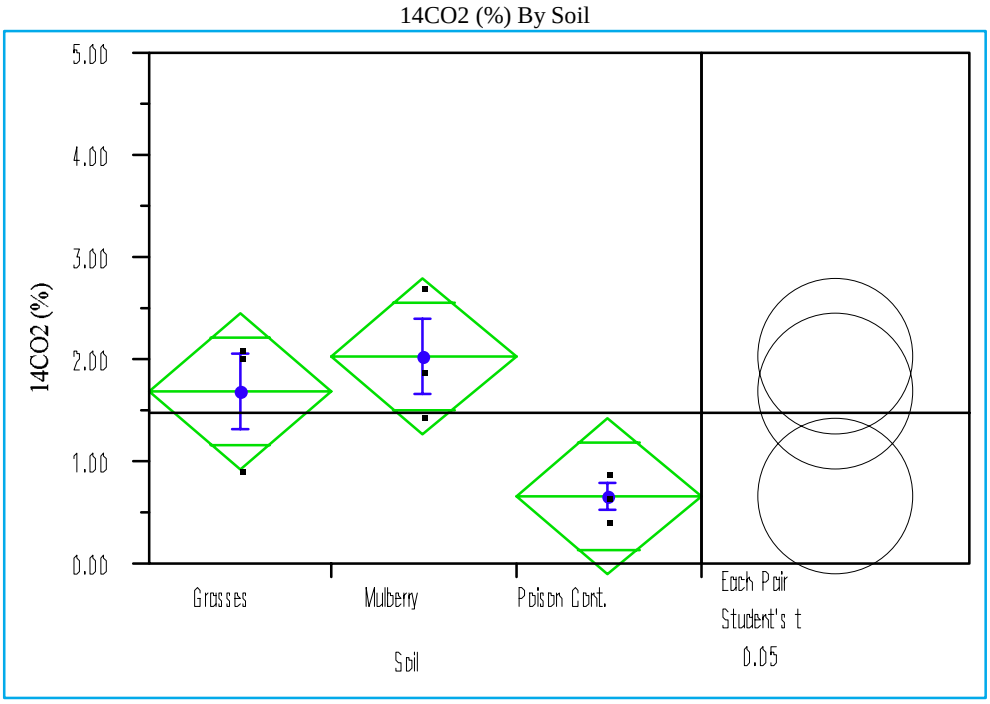
t

2.44691

Abs(Dif)-LSD	Grasses	Mulberry	Poison Control
Grasses	-35.8030	7.3703	10.3670
Mulberry	7.3703	-35.8030	-32.8064
Poison Control	10.3670	-32.8064	-35.8030

Positive values show pairs of means that are significantly different.

Appendix D-9. Student's t Test: Paired Comparison of B[a]P Fate Data in different Soils Amended with Mulberry Root Extract



Means Comparisons

Dif=Mean[i]-Mean[j]	Mulberry	Grasses	Poison Control
Mulberry	0.00000	0.33000	1.36333
Grasses	-0.33000	0.00000	1.03333
Poison Control	-1.36333	-1.03333	0.00000

Alpha= 0.05

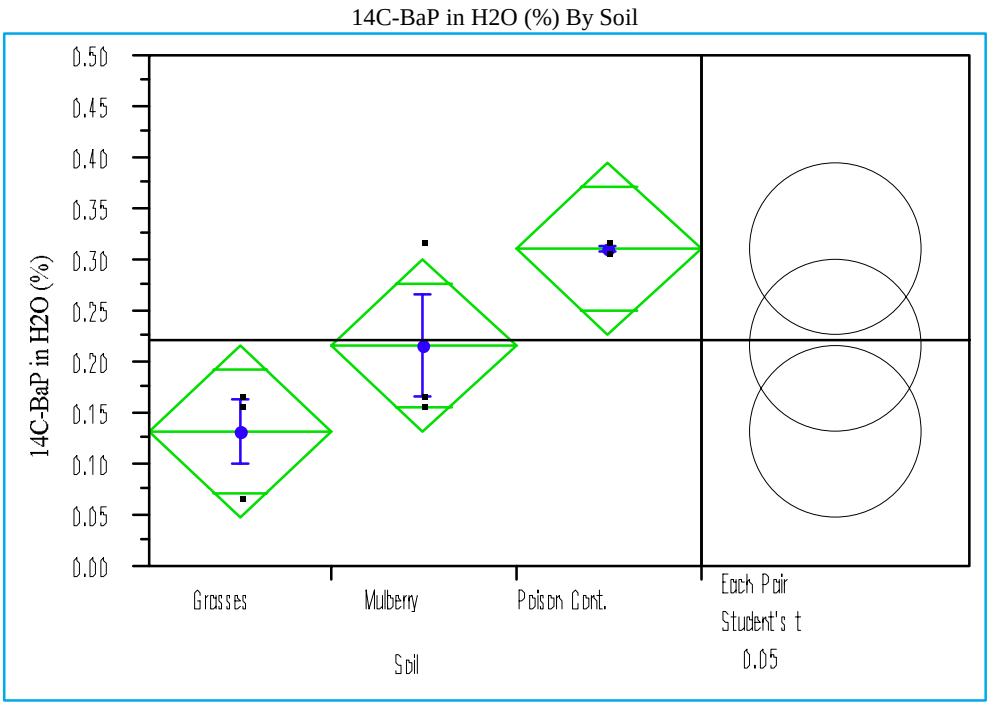
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Mulberry	Grasses	Poison Control
Mulberry	-1.09561	-0.76561	0.26772
Grasses	-0.76561	-1.09561	-0.06228
Poison Control	0.26772	-0.06228	-1.09561

Positive values show pairs of means that are significantly different.



Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Mulberry	Grasses
Poison Control	0.000000	0.096667	0.180000
Mulberry	-0.09667	0.000000	0.083333
Grasses	-0.18	-0.08333	0.000000

Alpha= 0.05

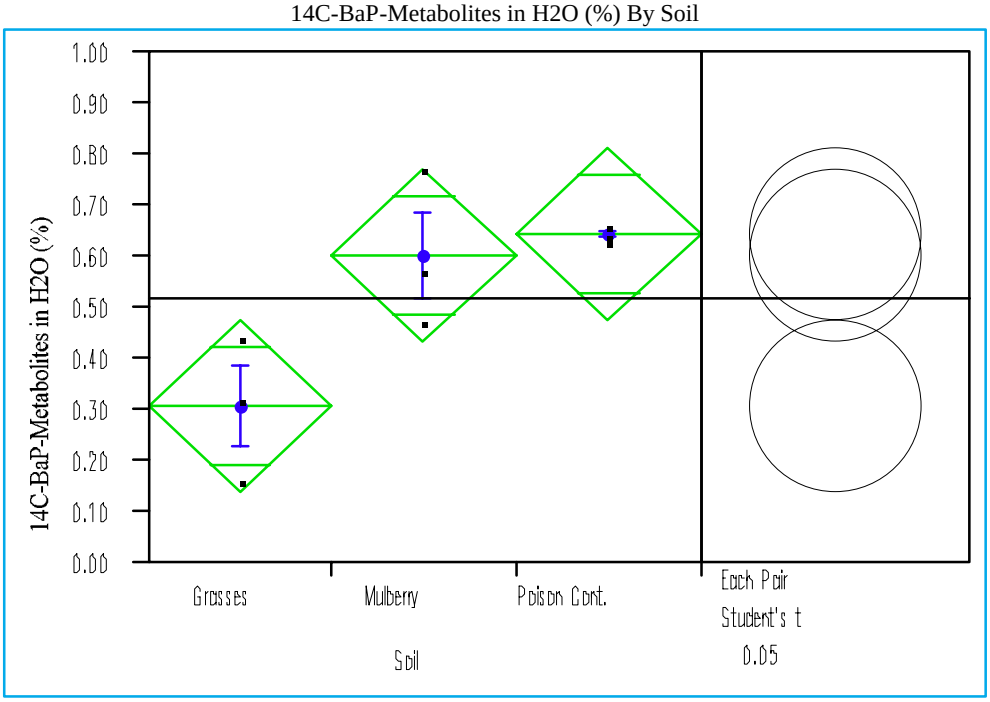
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Poison Control	Mulberry	Grasses
Poison Control	-0.12153	-0.02486	0.058473
Mulberry	-0.02486	-0.12153	-0.03819
Grasses	0.058473	-0.03819	-0.12153

Positive values show pairs of means that are significantly different.



Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Mulberry	Grasses
Poison Control	0.000000	0.040000	0.336667
Mulberry	-0.04	0.000000	0.296667
Grasses	-0.33667	-0.29667	0.000000

Alpha= 0.05

Comparisons for each pair using Student's t

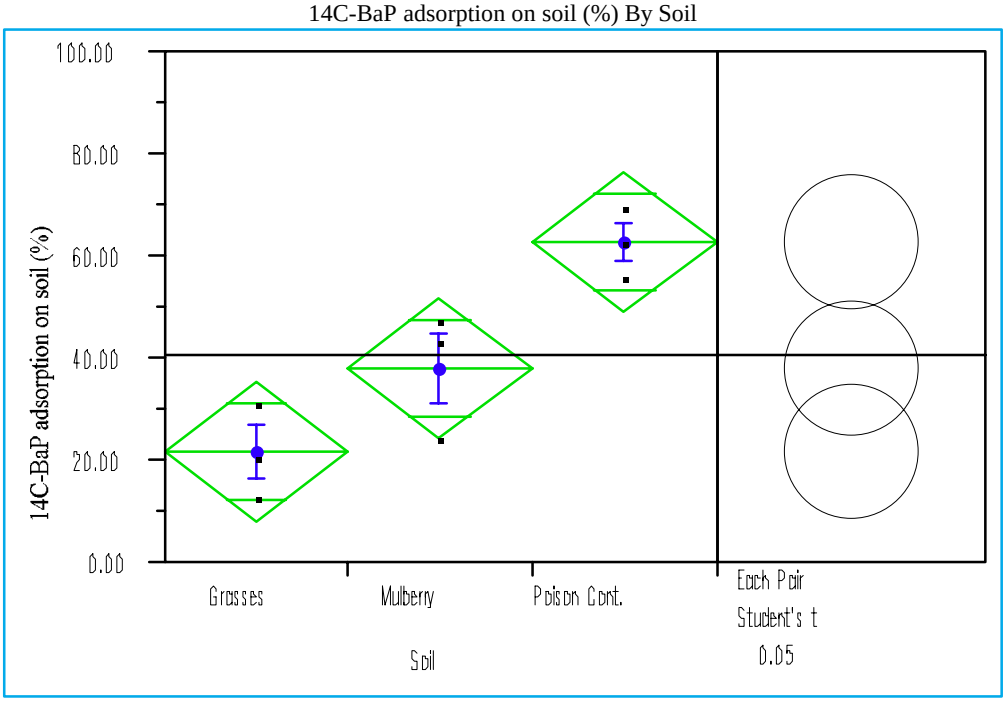
t

2.44691

Abs(Dif)-LSD	Poison Control	Mulberry	Grasses
Poison Control	-0.24002	-0.20002	0.096642
Mulberry	-0.20002	-0.24002	0.056642
Grasses	0.096642	0.056642	-0.24002

Positive values show pairs of means that are significantly different.

14C-BaP adsorption on soil (%) By Soil



Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Mulberry	Grasses
Poison Control	0.0000	24.5867	41.0800
Mulberry	-24.5867	0.0000	16.4933
Grasses	-41.0800	-16.4933	0.0000

Alpha= 0.05

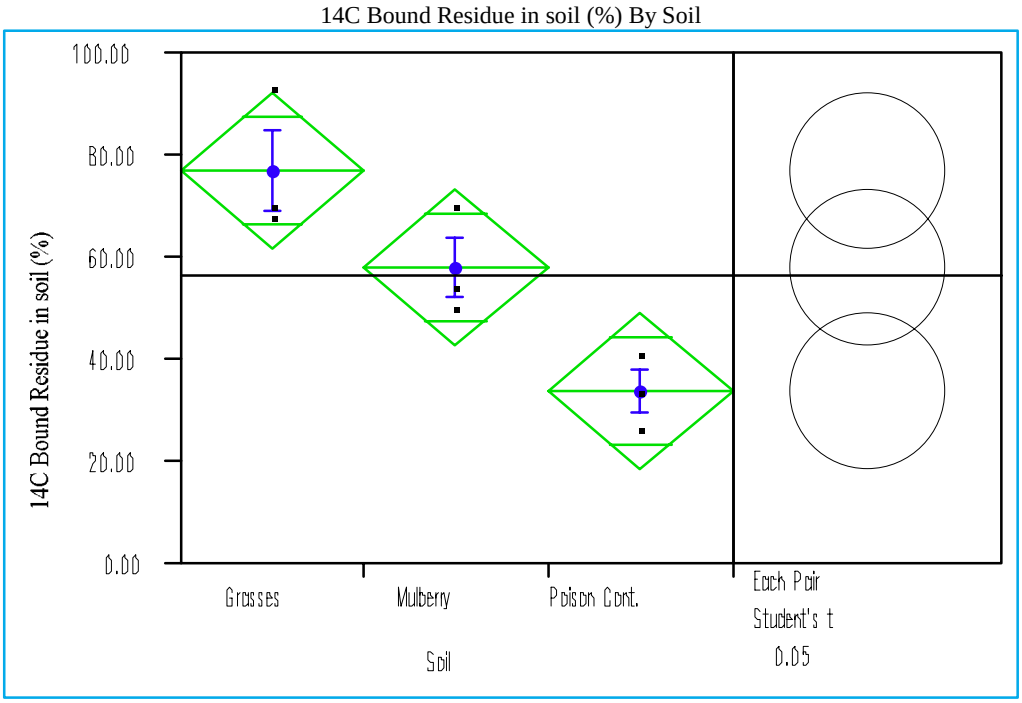
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Poison Control	Mulberry	Grasses
Poison Control	-19.3669	5.2198	21.7131
Mulberry	5.2198	-19.3669	-2.8735
Grasses	21.7131	-2.8735	-19.3669

Positive values show pairs of means that are significantly different.



Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Mulberry	Poison Control
Grasses	0.0000	19.1467	43.2700
Mulberry	-19.1467	0.0000	24.1233
Poison Control	-43.2700	-24.1233	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

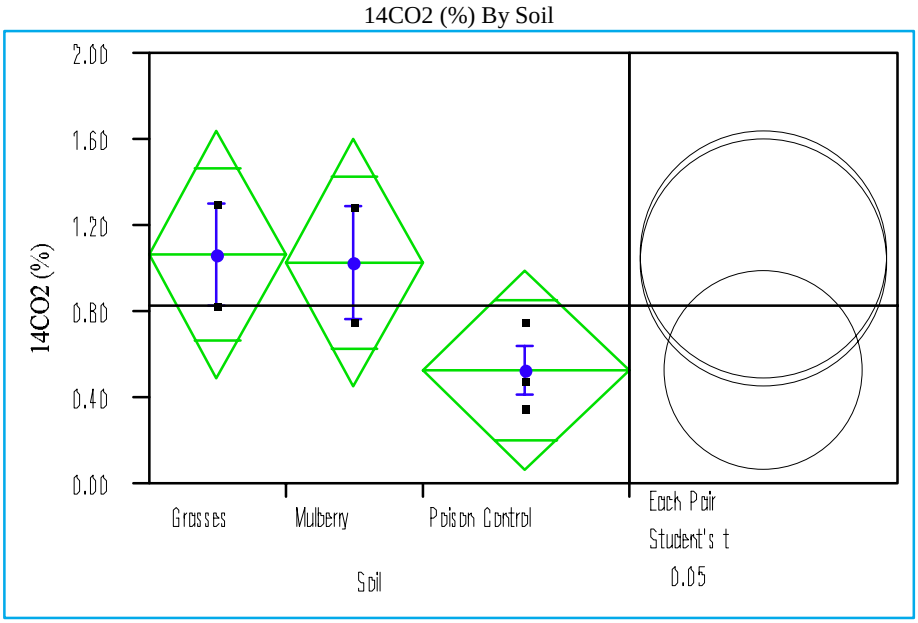
t

2.44691

Abs(Dif)-LSD	Grasses	Mulberry	Poison Control
Grasses	-21.9846	-2.8380	21.2854
Mulberry	-2.8380	-21.9846	2.1387
Poison Control	21.2854	2.1387	-21.9846

Positive values show pairs of means that are significantly different.

Appendix D-10. Student's t Test: Paired Comparison of B[a]P Fate Data in Different Soils Amended with 100 uM Flavone



Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Mulberry	Poison Control
Grasses	0.000000	0.040000	0.536667
Mulberry	-0.04	0.000000	0.496667
Poison Control	-0.53667	-0.49667	0.000000

Alpha= 0.05

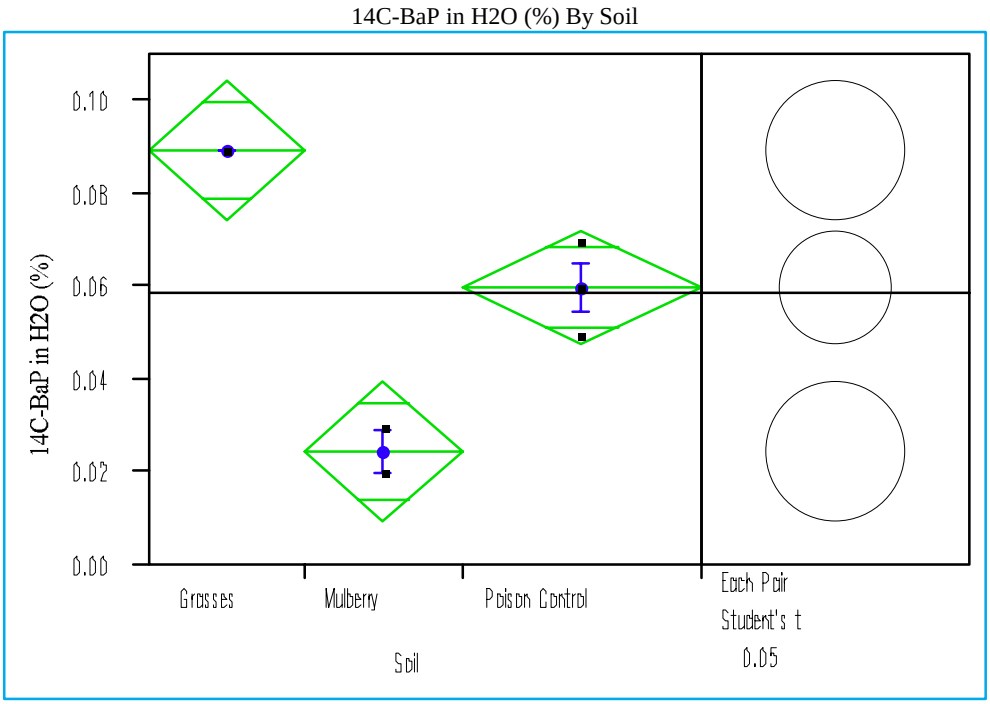
Comparisons for each pair using Student's t

t

2.77641

Abs(Dif)-LSD	Grasses	Mulberry	Poison Control
Grasses	-0.8157	-0.7757	-0.20796
Mulberry	-0.7757	-0.8157	-0.24796
Poison Control	-0.20796	-0.24796	-0.66602

Positive values show pairs of means that are significantly different.



Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Poison Control	Mulberry
Grasses	0.000000	0.030000	0.065000
Poison Control	-0.03	0.000000	0.035000
Mulberry	-0.065	-0.035	0.000000

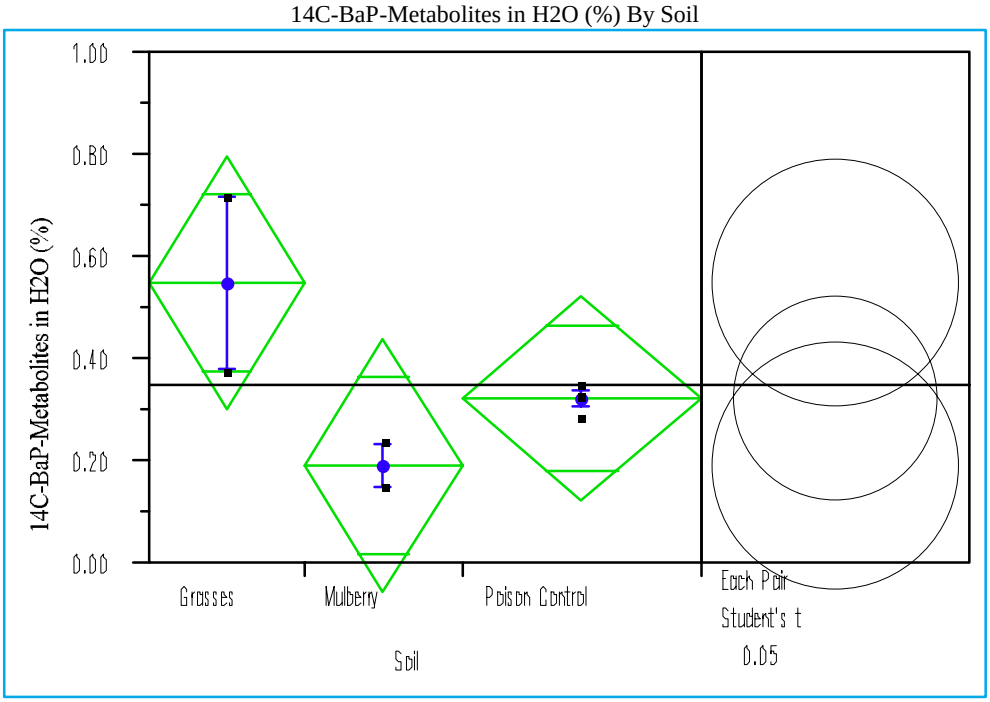
Alpha= 0.05

Comparisons for each pair using Student's t

t
2.77641

Abs(Dif)-LSD	Grasses	Poison Control	Mulberry
Grasses	-0.02195	0.009963	0.043051
Poison Control	0.009963	-0.01792	0.014963
Mulberry	0.043051	0.014963	-0.02195

Positive values show pairs of means that are significantly different.



Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Poison Control	Mulberry
Grasses	0.000000	0.226667	0.355000
Poison Control	-0.22667	0.000000	0.128333
Mulberry	-0.355	-0.12833	0.000000

Alpha= 0.05

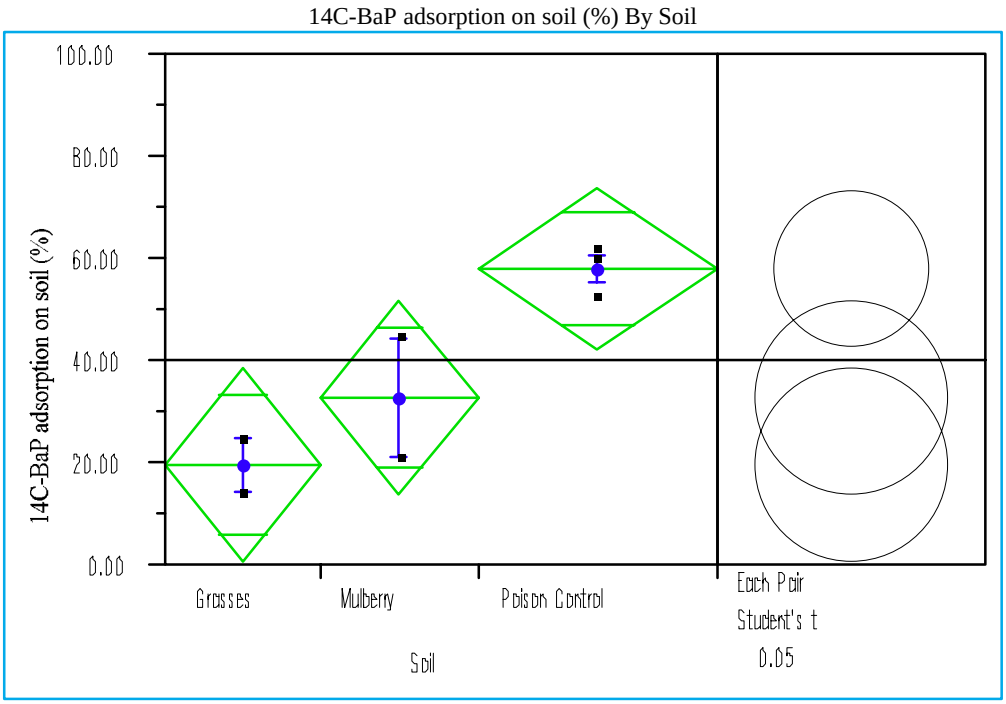
Comparisons for each pair using Student's t

t

2.77641

Abs(Dif)-LSD	Grasses	Poison Control	Mulberry
Grasses	-0.35041	-0.09321	0.004587
Poison Control	-0.09321	-0.28611	-0.19155
Mulberry	0.004587	-0.19155	-0.35041

Positive values show pairs of means that are significantly different.



Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Mulberry	Grasses
Poison Control	0.0000	25.6233	38.9083
Mulberry	-25.6233	0.0000	13.2850
Grasses	-38.9083	-13.2850	0.0000

Alpha= 0.05

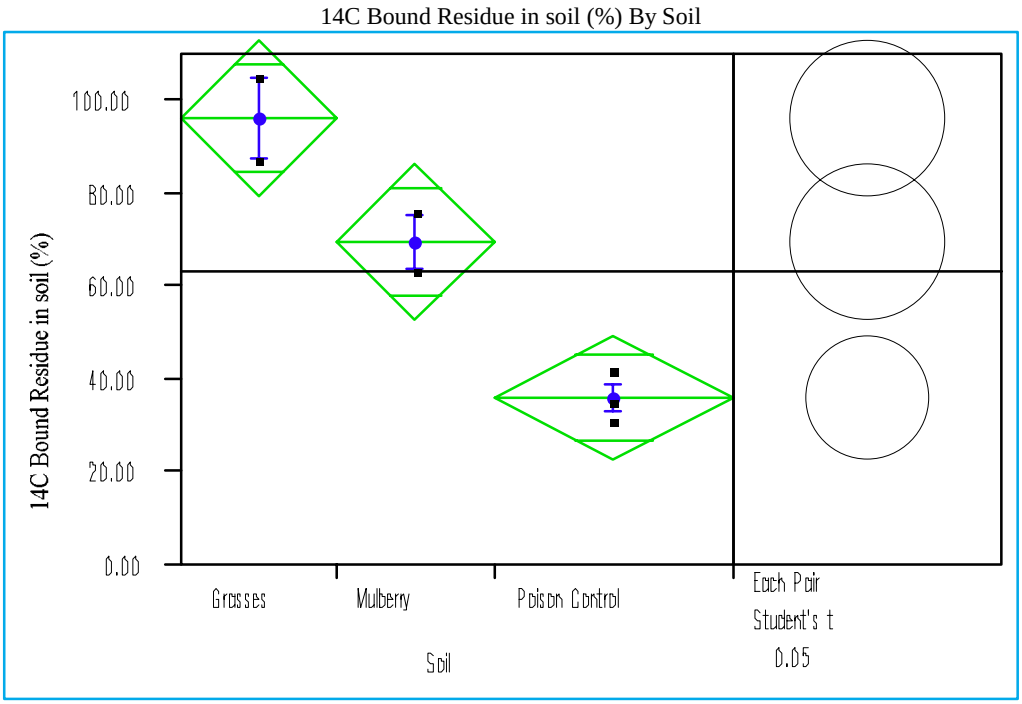
Comparisons for each pair using Student's t

t

2.77641

Abs(Dif)-LSD	Poison Control	Mulberry	Grasses
Poison Control	-22.3757	0.6065	13.8915
Mulberry	0.6065	-27.4046	-14.1196
Grasses	13.8915	-14.1196	-27.4046

Positive values show pairs of means that are significantly different.



Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Mulberry	Poison Control
Grasses	0.0000	26.4400	60.1967
Mulberry	-26.4400	0.0000	33.7567
Poison Control	-60.1967	-33.7567	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

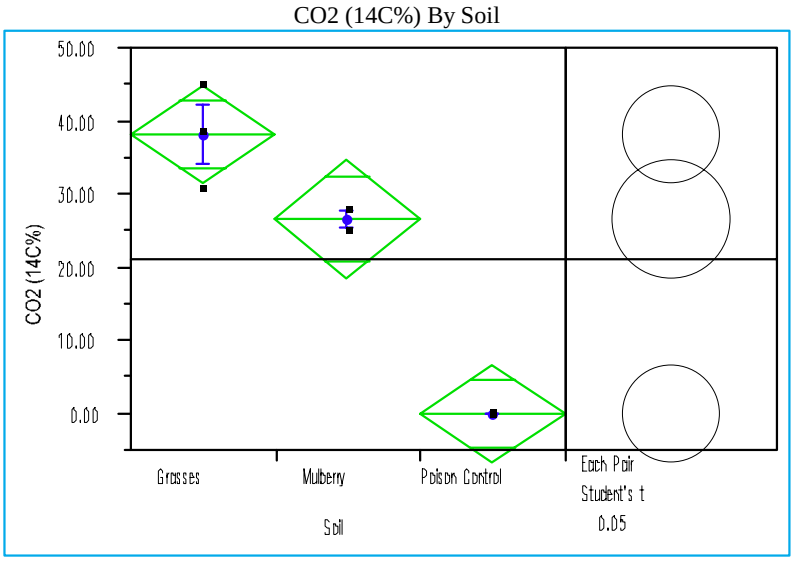
t

2.77641

Abs(Dif)-LSD	Grasses	Mulberry	Poison Control
Grasses	-23.8560	2.5840	38.4192
Mulberry	2.5840	-23.8560	11.9792
Poison Control	38.4192	11.9792	-19.4783

Positive values show pairs of means that are significantly different.

Appendix D-11. Student's t Test: Paired Comparison of Pyrene Fate Data in Different Soils without Flavonoid Amendment



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	38.3633	7.06707	4.0802
Mulberry	2	26.6350	2.00111	1.4150
Poison Control	3	0.1300	0.09644	0.0557

Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Mulberry	Poison Control
Grasses	0.0000	11.7283	38.2333
Mulberry	-11.7283	0.0000	26.5050
Poison Control	-38.2333	-26.5050	0.0000

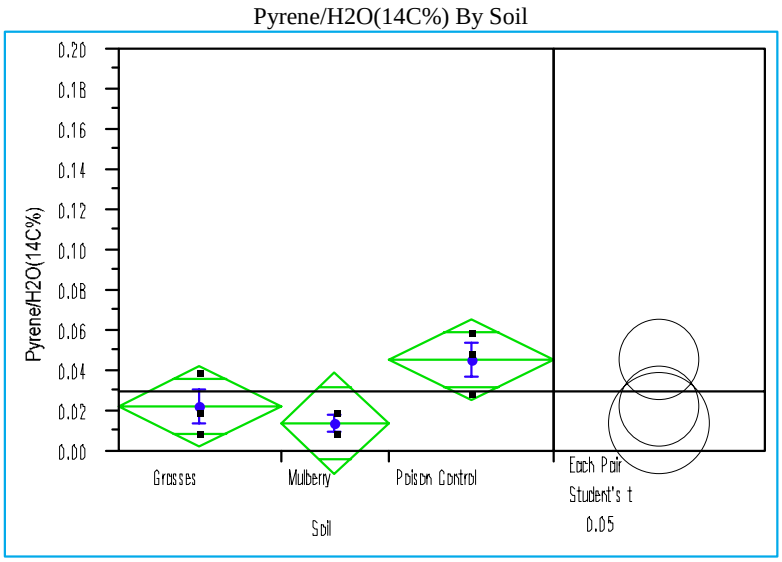
Alpha= 0.05

Comparisons for each pair using Student's t

t
2.57054

Abs(Dif)-LSD	Grasses	Mulberry	Poison Control
Grasses	-9.5680	1.0309	28.6653
Mulberry	1.0309	-11.7184	15.8076
Poison Control	28.6653	15.8076	-9.5680

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	0.023333	0.015275	0.00882
Mulberry	2	0.015000	0.007071	0.00500
Poison Control	3	0.046667	0.015275	0.00882

Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Grasses	Mulberry
Poison Control	0.000000	0.023333	0.031667
Grasses	-0.02333	0.000000	0.008333
Mulberry	-0.03167	-0.00833	0.000000

Alpha= 0.05

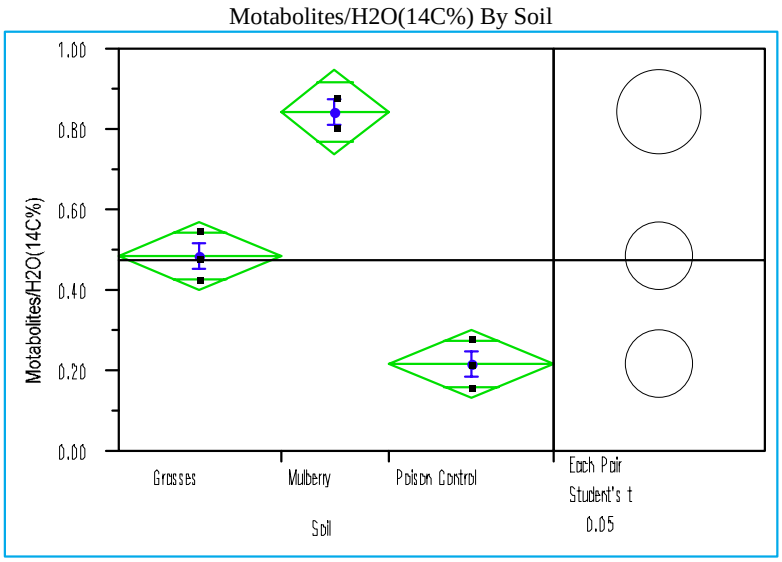
Comparisons for each pair using Student's t

t

2.57054

Abs(Dif)-LSD	Poison Control	Grasses	Mulberry
Poison Control	-0.02943	-0.0061	-0.00124
Grasses	-0.0061	-0.02943	-0.02457
Mulberry	-0.00124	-0.02457	-0.03605

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	0.486667	0.060277	0.03480
Mulberry	2	0.845000	0.049497	0.03500
Poison Control	3	0.220000	0.060000	0.03464

Means Comparisons

Dif=Mean[i]-Mean[j]	Mulberry	Grasses	Poison Control
Mulberry	0.000000	0.358333	0.625000
Grasses	-0.35833	0.000000	0.266667
Poison Control	-0.625	-0.26667	0.000000

Alpha= 0.05

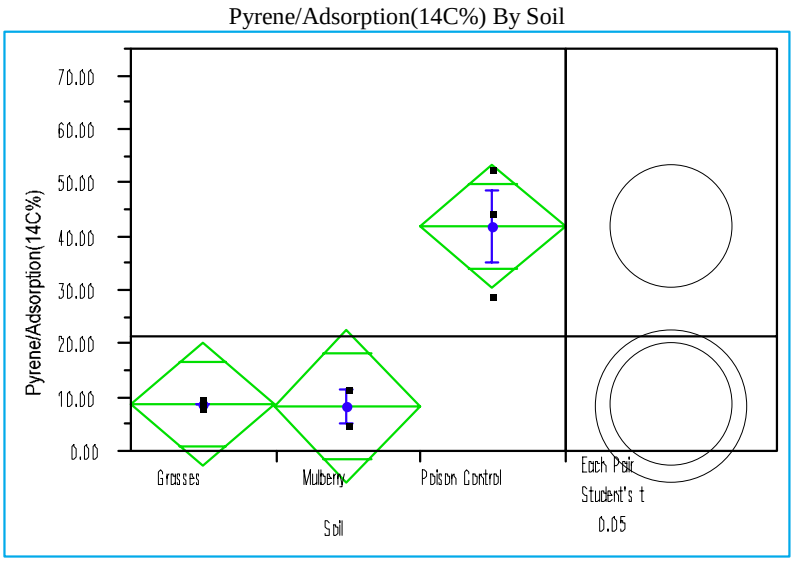
Comparisons for each pair using Student's t

t

2.57054

Abs(Dif)-LSD	Mulberry	Grasses	Poison Control
Mulberry	-0.14952	0.221841	0.488508
Grasses	0.221841	-0.12208	0.144585
Poison Control	0.488508	0.144585	-0.12208

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	9.3433	0.6787	0.3918
Mulberry	2	8.6550	4.6598	3.2950
Poison Control	3	42.5000	12.0200	6.9398

Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Grasses	Mulberry
Poison Control	0.0000	33.1567	33.8450
Grasses	-33.1567	0.0000	0.6883
Mulberry	-33.8450	-0.6883	0.0000

Alpha= 0.05

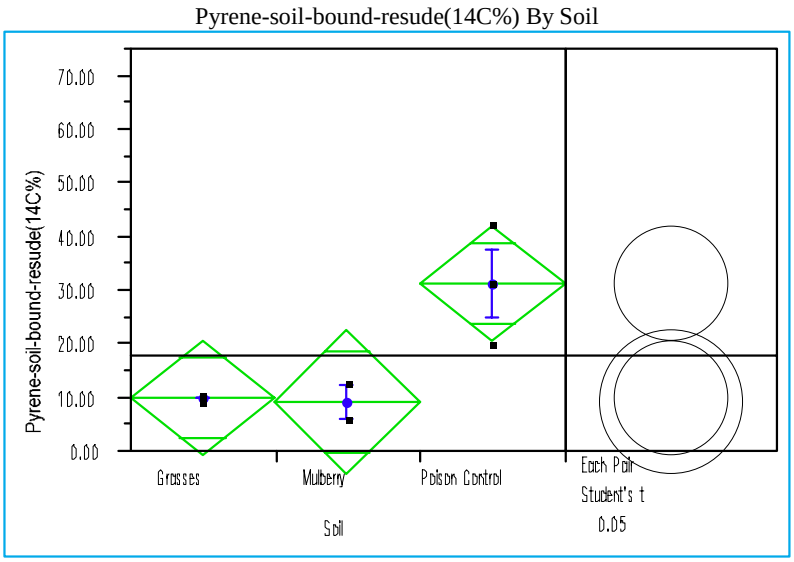
Comparisons for each pair using Student's t

t

2.57054

Abs(Dif)-LSD	Poison Control	Grasses	Mulberry
Poison Control	-16.5688	16.5879	15.3205
Grasses	16.5879	-16.5688	-17.8361
Mulberry	15.3205	-17.8361	-20.2925

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	10.1700	0.4115	0.2376
Mulberry	2	9.6350	4.8154	3.4050
Poison Control	3	31.5467	11.2537	6.4973

Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Grasses	Mulberry
Poison Control	0.0000	21.3767	21.9117
Grasses	-21.3767	0.0000	0.5350
Mulberry	-21.9117	-0.5350	0.0000

Alpha= 0.05

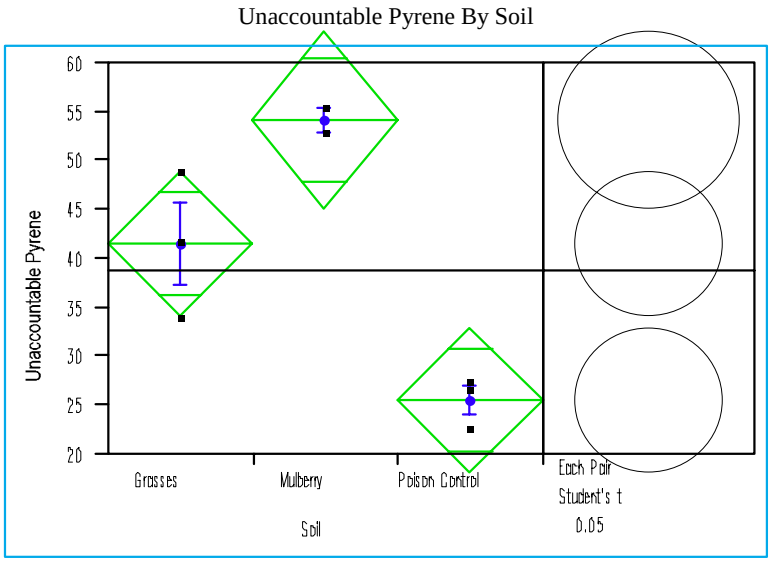
Comparisons for each pair using Student's t

t

2.57054

Abs(Dif)-LSD	Poison Control	Grasses	Mulberry
Poison Control	-15.6168	5.7599	4.4516
Grasses	5.7599	-15.6168	-16.9251
Mulberry	4.4516	-16.9251	-19.1266

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	41.6133	7.41308	4.2799
Mulberry	2	54.2250	1.80312	1.2750
Poison Control	3	25.5600	2.64013	1.5243

Means Comparisons

Dif=Mean[i]-Mean[j]	Mulberry	Grasses	Poison Control
Mulberry	0.0000	12.6117	28.6650
Grasses	-12.6117	0.0000	16.0533
Poison Control	-28.6650	-16.0533	0.0000

Alpha= 0.05

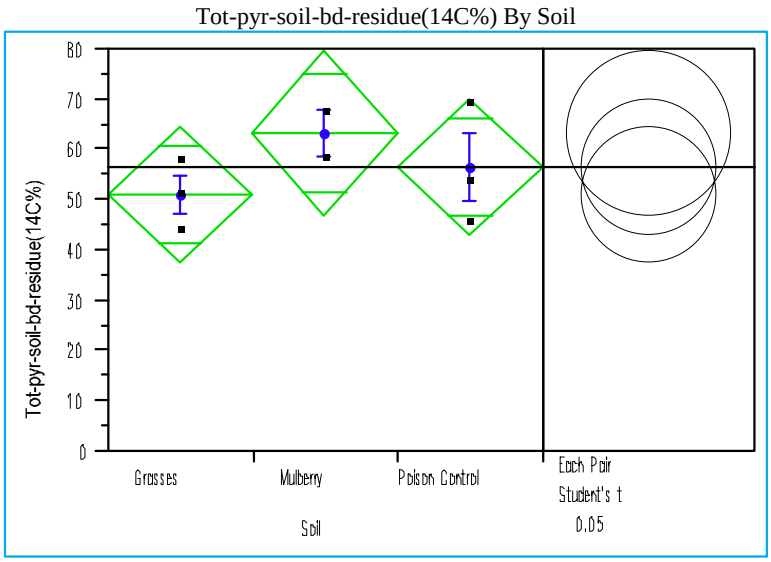
Comparisons for each pair using Student's t

t

2.57054

Abs(Dif)-LSD	Mulberry	Grasses	Poison Control
Mulberry	-12.9602	0.7807	16.8340
Grasses	0.7807	-10.5820	5.4714
Poison Control	16.8340	5.4714	-10.5820

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	51.7800	6.9995	4.0412
Mulberry	2	63.8500	6.6185	4.6800
Poison Control	3	57.1033	11.9890	6.9218

Means Comparisons

Dif=Mean[i]-Mean[j]	Mulberry	Poison Control	Grasses
Mulberry	0.0000	6.7467	12.0700
Poison Control	-6.7467	0.0000	5.3233
Grasses	-12.0700	-5.3233	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

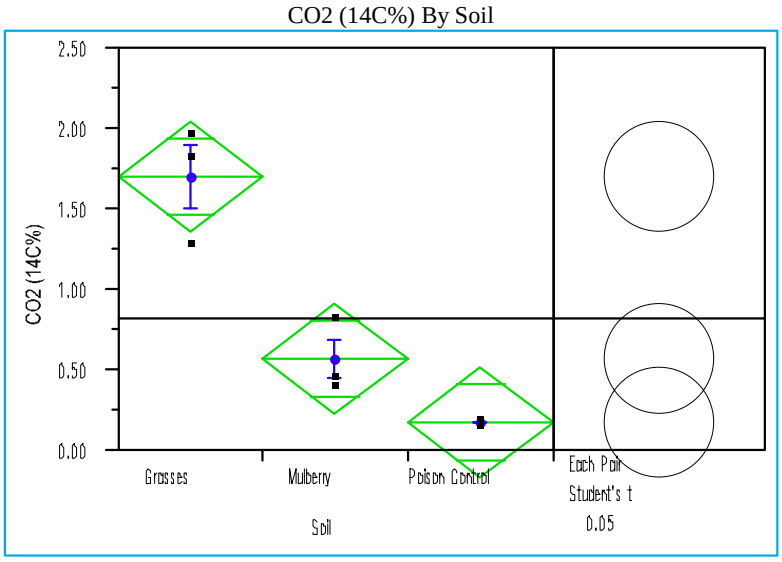
t

2.57054

Abs(Dif)-LSD	Mulberry	Poison Control	Grasses
Mulberry	-23.8178	-14.9959	-9.6725
Poison Control	-14.9959	-19.4471	-14.1238
Grasses	-9.6725	-14.1238	-19.4471

Positive values show pairs of means that are significantly different.

Appendix D-12. Student's t Test: Paired Comparison of Pyrene Fate Data in Different Soils Amended with 100 uM of Morin



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	1.70333	0.357258	0.20626
Mulberry	3	0.57333	0.223681	0.12914
Poison Control	3	0.18333	0.015275	0.00882

Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Mulberry	Poison Control
Grasses	0.00000	1.13000	1.52000
Mulberry	-1.13000	0.00000	0.39000
Poison Control	-1.52000	-0.39000	0.00000

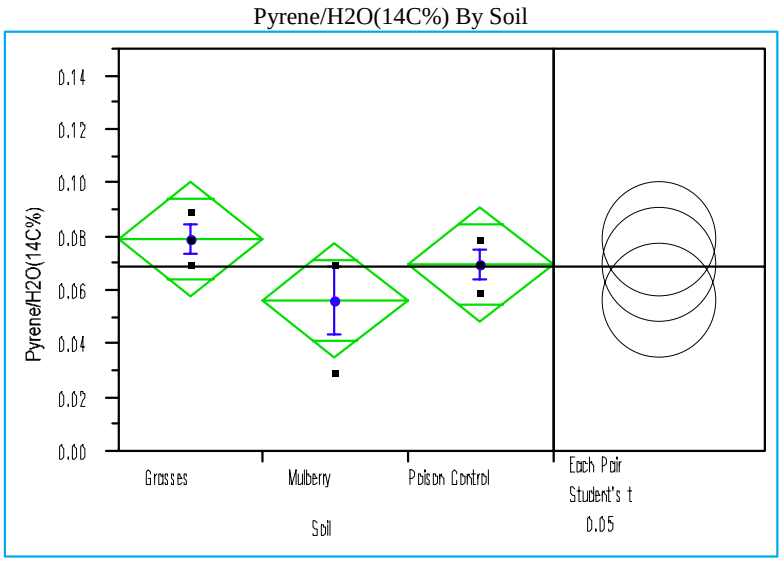
Alpha= 0.05

Comparisons for each pair using Student's t

t
2.44691

Abs(Dif)-LSD	Grasses	Mulberry	Poison Control
Grasses	-0.48652	0.64348	1.03348
Mulberry	0.64348	-0.48652	-0.09652
Poison Control	1.03348	-0.09652	-0.48652

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	0.080000	0.010000	0.00577
Mulberry	3	0.056667	0.023094	0.01333
Poison Control	3	0.070000	0.010000	0.00577

Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Poison Control	Mulberry
Grasses	0.000000	0.010000	0.023333
Poison Control	-0.01	0.000000	0.013333
Mulberry	-0.02333	-0.01333	0.000000

Alpha= 0.05

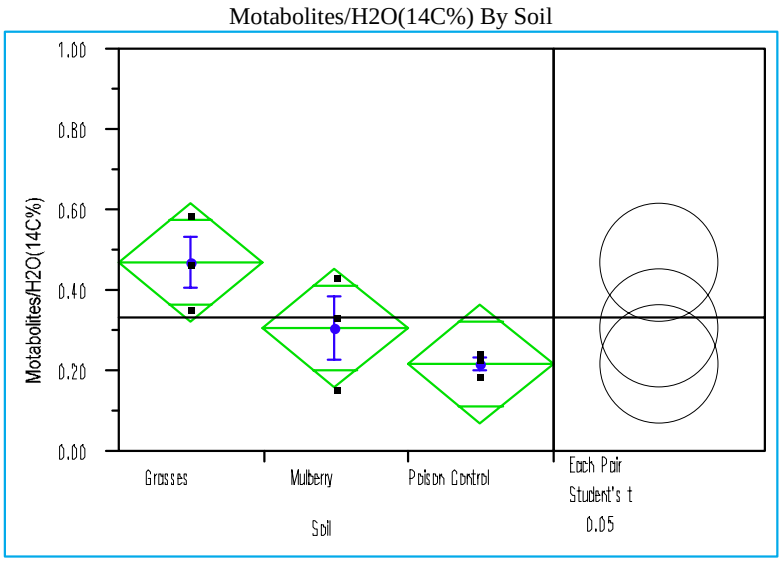
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Grasses	Poison Control	Mulberry
Grasses	-0.03124	-0.02124	-0.0079
Poison Control	-0.02124	-0.03124	-0.0179
Mulberry	-0.0079	-0.0179	-0.03124

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	0.473333	0.115036	0.06642
Mulberry	3	0.313333	0.141892	0.08192
Poison Control	3	0.223333	0.030551	0.01764

Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Mulberry	Poison Control
Grasses	0.000000	0.160000	0.250000
Mulberry	-0.16	0.000000	0.090000
Poison Control	-0.25	-0.09	0.000000

Alpha= 0.05

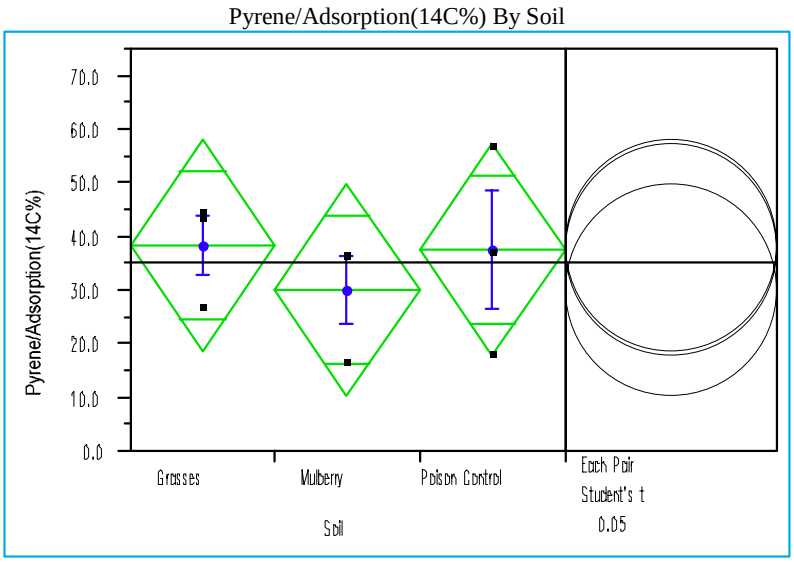
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Grasses	Mulberry	Poison Control
Grasses	-0.21363	-0.05363	0.036371
Mulberry	-0.05363	-0.21363	-0.12363
Poison Control	0.036371	-0.12363	-0.21363

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	38.6167	10.0266	5.789
Mulberry	3	30.0533	11.5696	6.680
Poison Control	3	37.6600	19.2864	11.135

Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Poison Control	Mulberry
Grasses	0.00000	0.95667	8.56333
Poison Control	-0.95667	0.00000	7.60667
Mulberry	-8.56333	-7.60667	0.00000

Alpha= 0.05

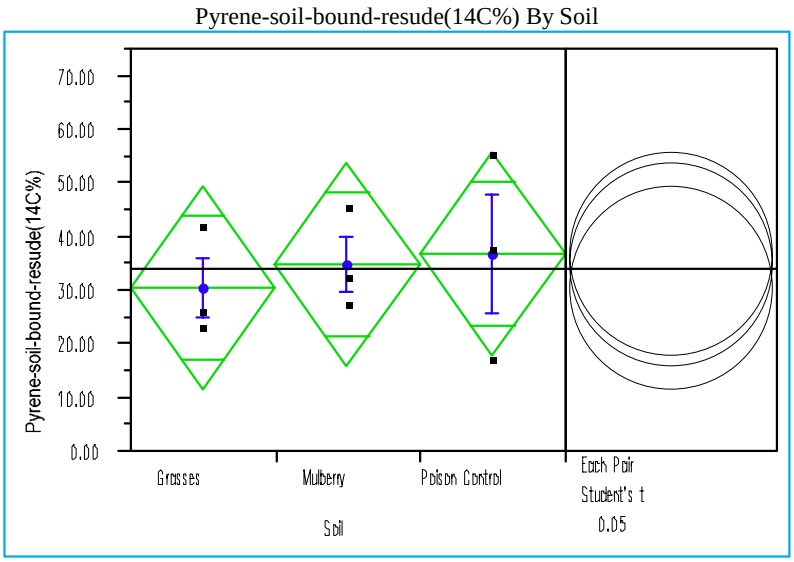
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Grasses	Poison Control	Mulberry
Grasses	-28.4038	-27.4471	-19.8404
Poison Control	-27.4471	-28.4038	-20.7971
Mulberry	-19.8404	-20.7971	-28.4038

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	30.5100	10.0823	5.821
Mulberry	3	35.1267	9.4287	5.444
Poison Control	3	36.8567	19.2070	11.089

Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Mulberry	Grasses
Poison Control	0.00000	1.73000	6.34667
Mulberry	-1.73000	0.00000	4.61667
Grasses	-6.34667	-4.61667	0.00000

Alpha= 0.05

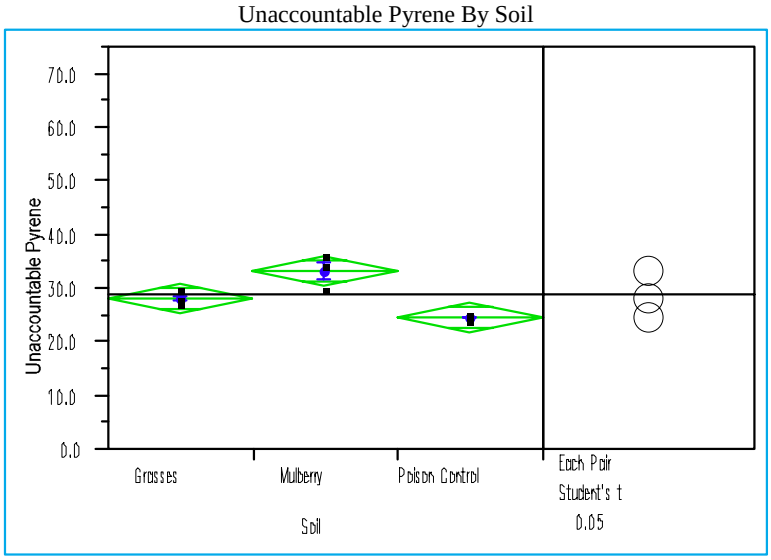
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Poison Control	Mulberry	Grasses
Poison Control	-27.2833	-25.5533	-20.9367
Mulberry	-25.5533	-27.2833	-22.6667
Grasses	-20.9367	-22.6667	-27.2833

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	28.6167	1.32742	0.7664
Mulberry	3	33.8733	3.24105	1.8712
Poison Control	3	25.0067	0.58688	0.3388

Means Comparisons

Dif=Mean[i]-Mean[j]	Mulberry	Grasses	Poison Control
Mulberry	0.00000	5.25667	8.86667
Grasses	-5.25667	0.00000	3.61000
Poison Control	-8.86667	-3.61000	0.00000

Alpha= 0.05

Comparisons for each pair using Student's t

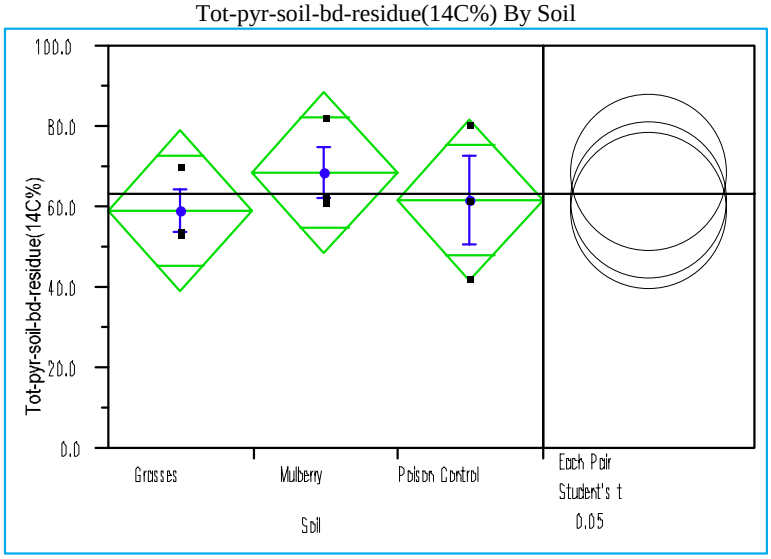
t

2.44691

Abs(Dif)-LSD	Mulberry	Grasses	Poison Control
Mulberry	-4.09624	1.16043	4.77043
Grasses	1.16043	-4.09624	-0.48624
Poison Control	4.77043	-0.48624	-4.09624

Positive values show pairs of means that are significantly different.

Tot-pyr-soil-bd-residue(14C%) By Soil



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	59.1300	9.6774	5.587
Mulberry	3	69.0033	11.5936	6.694
Poison Control	3	61.8633	19.3365	11.164

Means Comparisons

Dif=Mean[i]-Mean[j]	Mulberry	Poison Control	Grasses
Mulberry	0.00000	7.14000	9.87333
Poison Control	-7.14000	0.00000	2.73333
Grasses	-9.87333	-2.73333	0.00000

Alpha= 0.05

Comparisons for each pair using Student's t

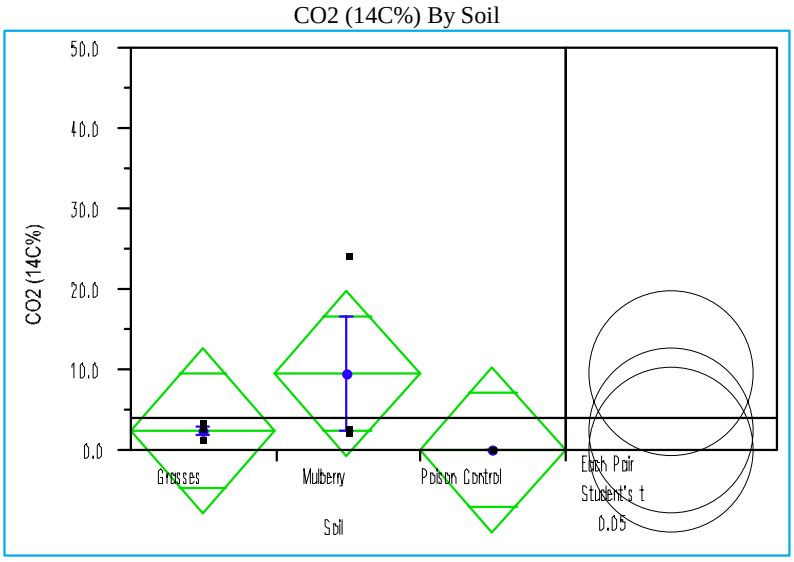
t

2.44691

Abs(Dif)-LSD	Mulberry	Poison Control	Grasses
Mulberry	-28.3007	-21.1607	-18.4274
Poison Control	-21.1607	-28.3007	-25.5674
Grasses	-18.4274	-25.5674	-28.3007

Positive values show pairs of means that are significantly different.

Appendix D-13. Student's t Test: Paired Comparison of Pyrene Fate Data in Different Soils Amended with Mulberry Root Extract



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	2.55333	1.1068	0.6390
Mulberry	3	9.74000	12.5690	7.2567
Poison Control	3	0.11000	0.0100	0.0058

Means Comparisons

Dif=Mean[i]-Mean[j]	Mulberry	Grasses	Poison Control
Mulberry	0.00000	7.18667	9.63000
Grasses	-7.18667	0.00000	2.44333
Poison Control	-9.63000	-2.44333	0.00000

Alpha= 0.05

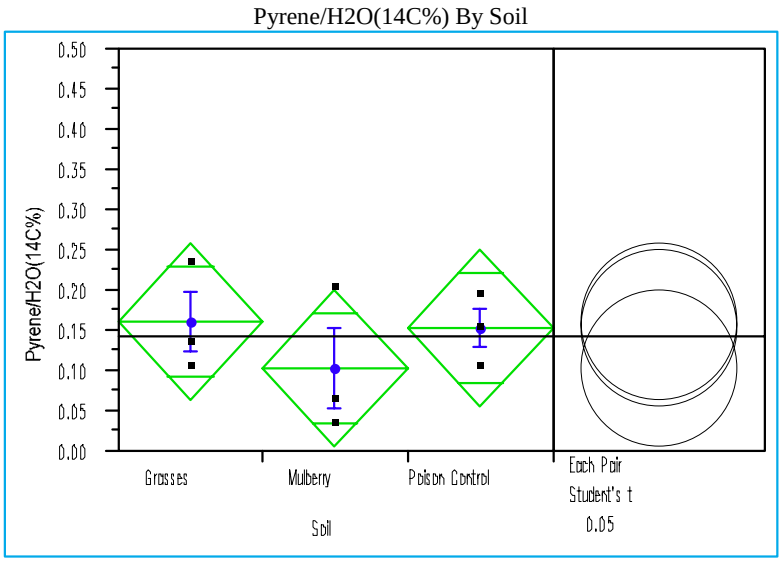
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Mulberry	Grasses	Poison Control
Mulberry	-14.5543	-7.3676	-4.9243
Grasses	-7.3676	-14.5543	-12.1110
Poison Control	-4.9243	-12.1110	-14.5543

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	0.163333	0.068069	0.03930
Mulberry	3	0.106667	0.090738	0.05239
Poison Control	3	0.156667	0.045092	0.02603

Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Poison Control	Mulberry
Grasses	0.000000	0.006667	0.056667
Poison Control	-0.00667	0.000000	0.050000
Mulberry	-0.05667	-0.05	0.000000

Alpha= 0.05

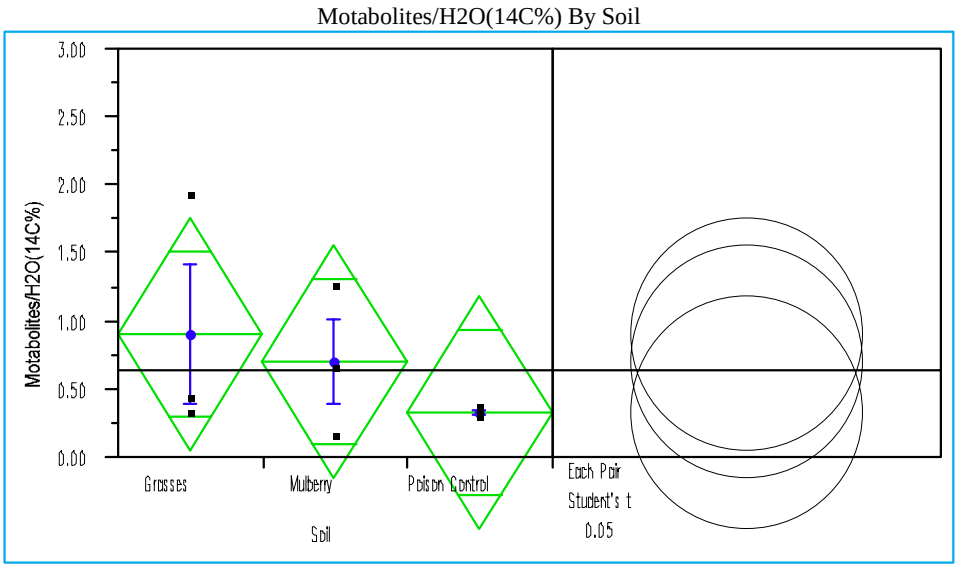
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Grasses	Poison Control	Mulberry
Grasses	-0.1408	-0.13413	-0.08413
Poison Control	-0.13413	-0.1408	-0.0908
Mulberry	-0.08413	-0.0908	-0.1408

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	0.906667	0.896289	0.51747
Mulberry	3	0.706667	0.555908	0.32095
Poison Control	3	0.343333	0.040415	0.02333

Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Mulberry	Poison Control
Grasses	0.000000	0.200000	0.563333
Mulberry	-0.2	0.000000	0.363333
Poison Control	-0.56333	-0.36333	0.000000

Alpha= 0.05

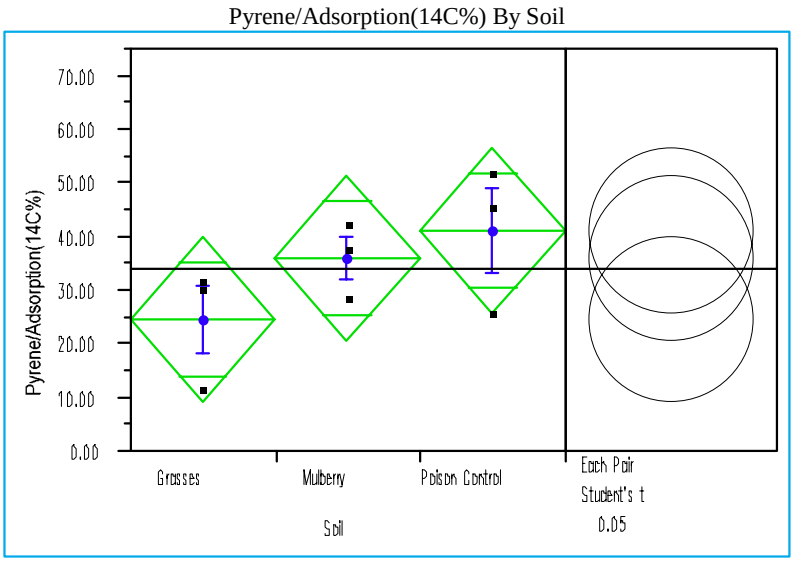
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Grasses	Mulberry	Poison Control
Grasses	-1.21746	-1.01746	-0.65413
Mulberry	-1.01746	-1.21746	-0.85413
Poison Control	-0.65413	-0.85413	-1.21746

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	24.7033	11.2564	6.4989
Mulberry	3	36.4500	7.2013	4.1577
Poison Control	3	41.2200	13.6942	7.9063

Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Mulberry	Grasses
Poison Control	0.0000	4.7700	16.5167
Mulberry	-4.7700	0.0000	11.7467
Grasses	-16.5167	-11.7467	0.0000

Alpha= 0.05

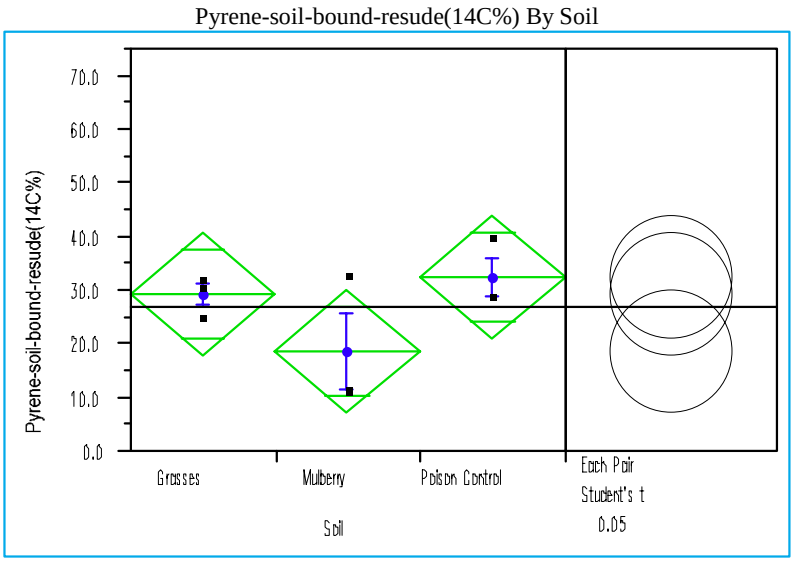
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Poison Control	Mulberry	Grasses
Poison Control	-22.0704	-17.3004	-5.5537
Mulberry	-17.3004	-22.0704	-10.3237
Grasses	-5.5537	-10.3237	-22.0704

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	29.5433	3.7274	2.1520
Mulberry	3	18.8033	12.4090	7.1644
Poison Control	3	32.8867	6.4981	3.7517

Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Grasses	Mulberry
Poison Control	0.0000	3.3433	14.0833
Grasses	-3.3433	0.0000	10.7400
Mulberry	-14.0833	-10.7400	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

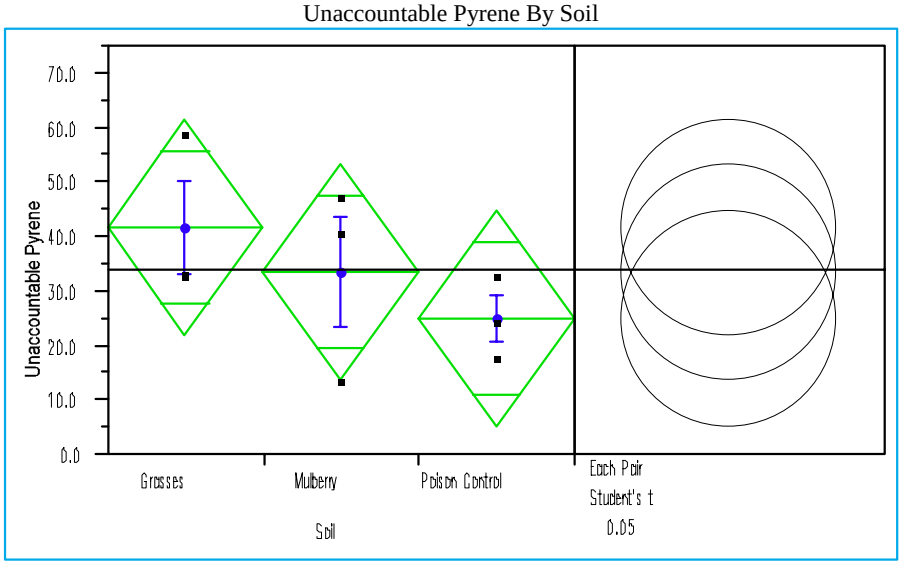
t

2.44691

Abs(Dif)-LSD	Poison Control	Grasses	Mulberry
Poison Control	-16.7197	-13.3764	-2.6364
Grasses	-13.3764	-16.7197	-5.9797
Mulberry	-2.6364	-5.9797	-16.7197

Positive values show pairs of means that are significantly different.

Unaccountable Pyrene By Soil



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	42.1300	14.9917	8.655
Mulberry	3	34.1933	17.9653	10.372
Poison Control	3	25.2833	7.5510	4.360

Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Mulberry	Poison Control
Grasses	0.0000	7.9367	16.8467
Mulberry	-7.9367	0.0000	8.9100
Poison Control	-16.8467	-8.9100	0.0000

Alpha= 0.05

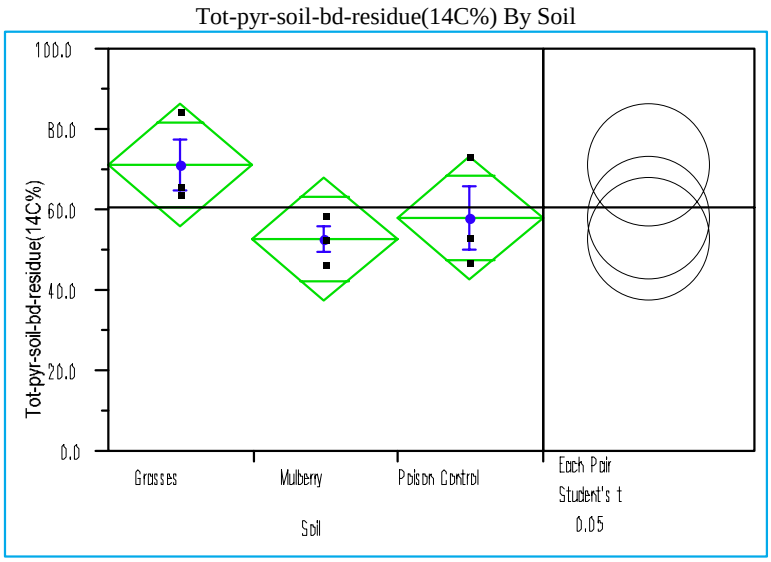
Comparisons for each pair using Student's t

t

2.44691

Abs(Dif)-LSD	Grasses	Mulberry	Poison Control
Grasses	-28.3608	-20.4241	-11.5141
Mulberry	-20.4241	-28.3608	-19.4508
Poison Control	-11.5141	-19.4508	-28.3608

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	71.6733	11.4037	6.5840
Mulberry	3	52.9967	6.0800	3.5103
Poison Control	3	58.1700	13.7151	7.9184

Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Poison Control	Mulberry
Grasses	0.0000	13.5033	18.6767
Poison Control	-13.5033	0.0000	5.1733
Mulberry	-18.6767	-5.1733	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

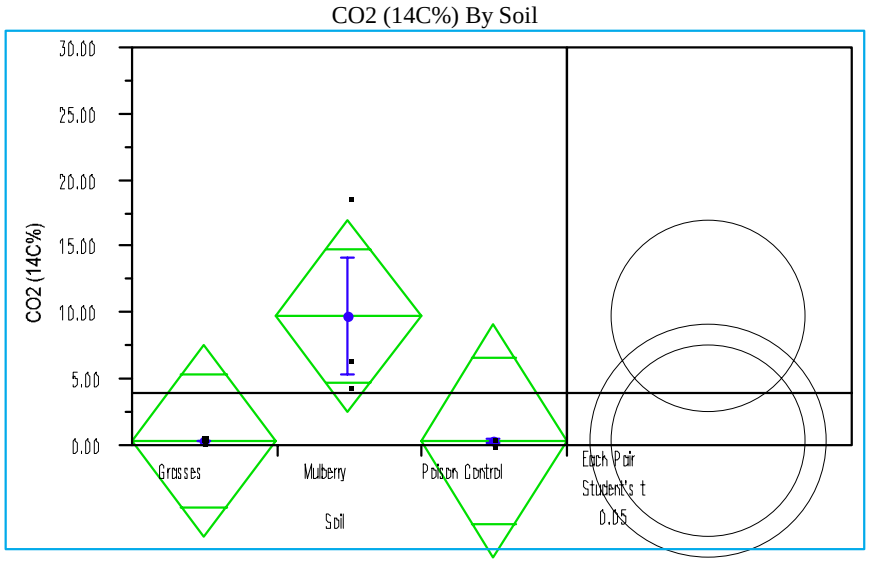
t

2.44691

Abs(Dif)-LSD	Grasses	Poison Control	Mulberry
Grasses	-21.7369	-8.2336	-3.0602
Poison Control	-8.2336	-21.7369	-16.5636
Mulberry	-3.0602	-16.5636	-21.7369

Positive values show pairs of means that are significantly different.

Appendix D-14. Student's t Test: Paired Comparison of Pyrene Fate Data in Different Soils Amended with 100 uM of Flavone



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	0.47000	0.16093	0.0929
Mulberry	3	9.91000	7.75635	4.4781
Poison Control	2	0.33000	0.29698	0.2100

Means Comparisons

Dif=Mean[i]-Mean[j]	Mulberry	Grasses	Poison Control
Mulberry	0.00000	9.44000	9.58000
Grasses	-9.44000	0.00000	0.14000
Poison Control	-9.58000	-0.14000	0.00000

Alpha= 0.05

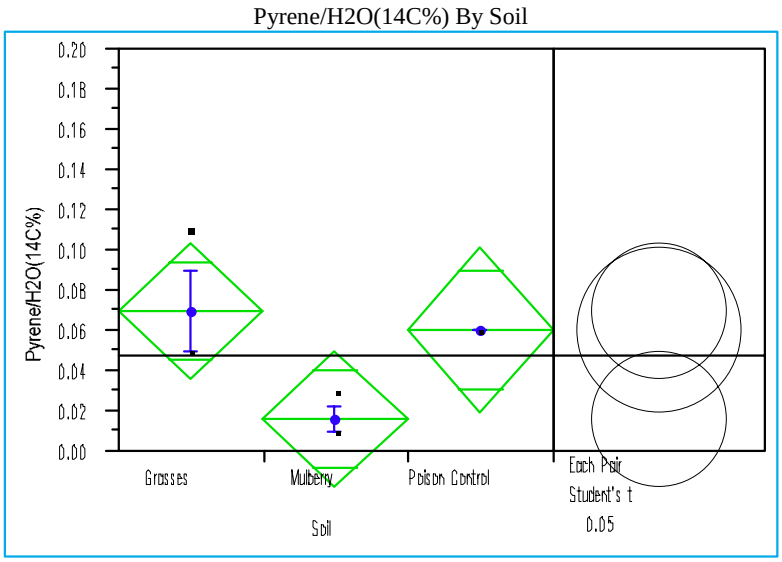
Comparisons for each pair using Student's t

t

2.57054

Abs(Dif)-LSD	Mulberry	Grasses	Poison Control
Mulberry	-10.3019	-0.8619	-1.9379
Grasses	-0.8619	-10.3019	-11.3779
Poison Control	-1.9379	-11.3779	-12.6173

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	0.070000	0.034641	0.02000
Mulberry	3	0.016667	0.011547	0.00667
Poison Control	2	0.060000	0.000000	0.00000

Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Poison Control	Mulberry
Grasses	0.000000	0.010000	0.053333
Poison Control	-0.01	0.000000	0.043333
Mulberry	-0.05333	-0.04333	0.000000

Alpha= 0.05

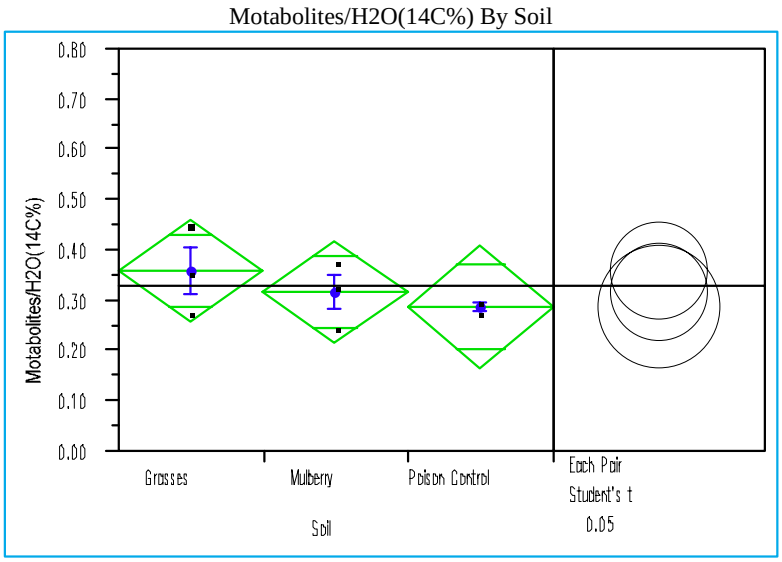
Comparisons for each pair using Student's t

t

2.57054

Abs(Dif)-LSD	Grasses	Poison Control	Mulberry
Grasses	-0.04847	-0.04419	0.004863
Poison Control	-0.04419	-0.05936	-0.01086
Mulberry	0.004863	-0.01086	-0.04847

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	0.363333	0.085049	0.04910
Mulberry	3	0.320000	0.065574	0.03786
Poison Control	2	0.290000	0.014142	0.01000

Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Mulberry	Poison Control
Grasses	0.000000	0.043333	0.073333
Mulberry	-0.04333	0.000000	0.030000
Poison Control	-0.07333	-0.03	0.000000

Alpha= 0.05

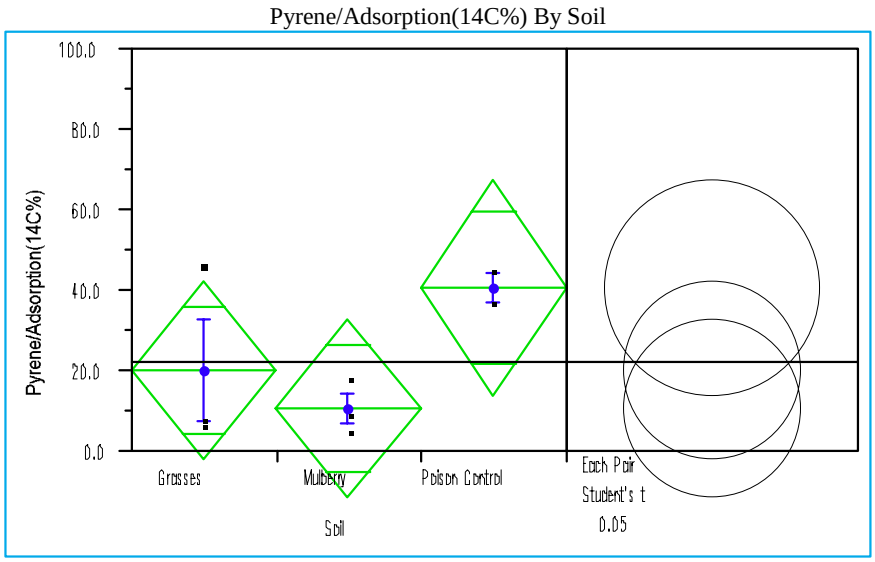
Comparisons for each pair using Student's t

t

2.57054

Abs(Dif)-LSD	Grasses	Mulberry	Poison Control
Grasses	-0.14317	-0.09984	-0.08674
Mulberry	-0.09984	-0.14317	-0.13007
Poison Control	-0.08674	-0.13007	-0.17535

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	20.5900	22.4911	12.985
Mulberry	3	11.0900	6.6717	3.852
Poison Control	2	41.5050	5.6074	3.965

Means Comparisons

Dif=Mean[i]-Mean[j]	Poison Control	Grasses	Mulberry
Poison Control	0.0000	20.9150	30.4150
Grasses	-20.9150	0.0000	9.5000
Mulberry	-30.4150	-9.5000	0.0000

Alpha= 0.05

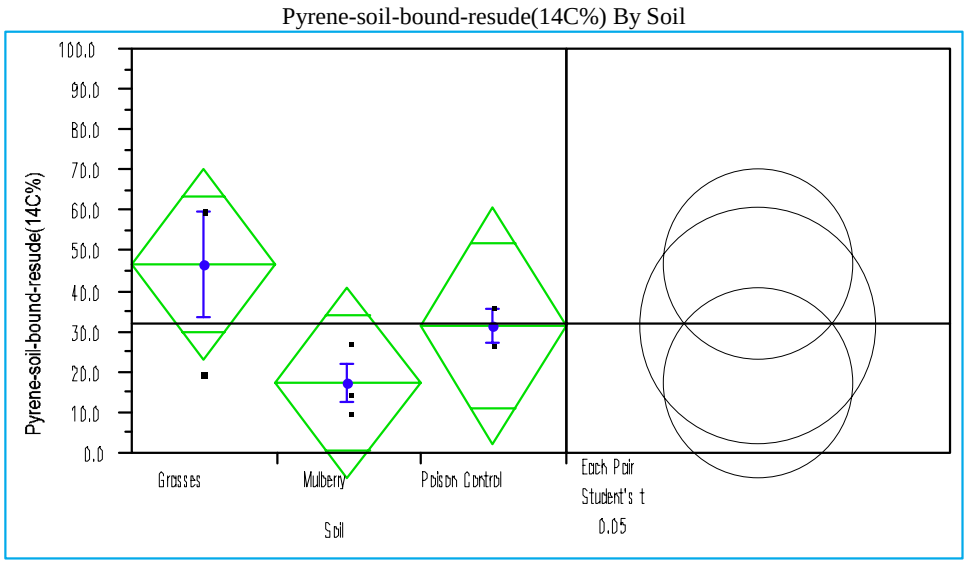
Comparisons for each pair using Student's t

t

2.57054

Abs(Dif)-LSD	Poison Control	Grasses	Mulberry
Poison Control	-38.6808	-14.3956	-4.8956
Grasses	-14.3956	-31.5827	-22.0827
Mulberry	-4.8956	-22.0827	-31.5827

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	46.7533	23.5678	13.607
Mulberry	3	17.6433	8.9073	5.143
Poison Control	2	31.8850	6.6397	4.695

Means Comparisons

Dif=Mean[i]-Mean[j]	Grasses	Poison Control	Mulberry
Grasses	0.0000	14.8683	29.1100
Poison Control	-14.8683	0.0000	14.2417
Mulberry	-29.1100	-14.2417	0.0000

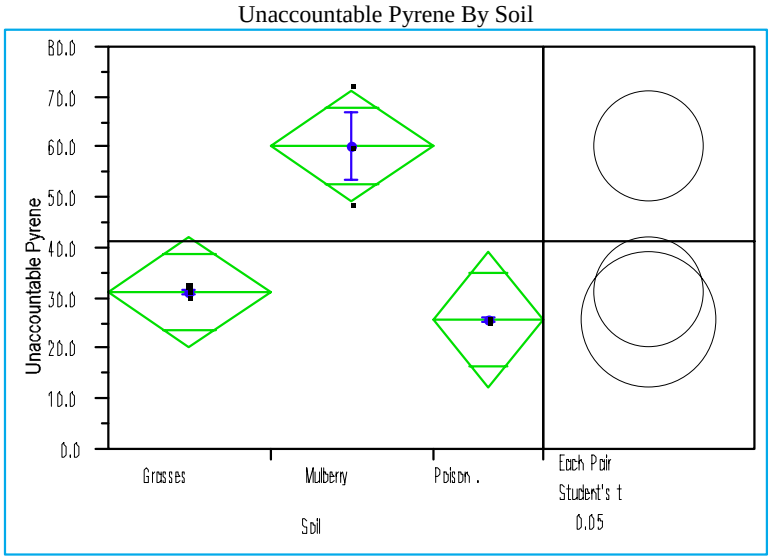
Alpha= 0.05

Comparisons for each pair using Student's t

t
2.57054

Abs(Dif)-LSD	Grasses	Poison Control	Mulberry
Grasses	-34.0200	-23.1672	-4.9100
Poison Control	-23.1672	-41.6658	-23.7938
Mulberry	-4.9100	-23.7938	-34.0200

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	31.7533	1.1075	0.6394
Mulberry	3	61.0133	11.8063	6.8164
Poison Control	2	25.9350	0.7142	0.5050

Means Comparisons

Dif=Mean[i]-Mean[j]	Mulberry	Grasses	Poison Control
Mulberry	0.0000	29.2600	35.0783
Grasses	-29.2600	0.0000	5.8183
Poison Control	-35.0783	-5.8183	0.0000

Alpha= 0.05

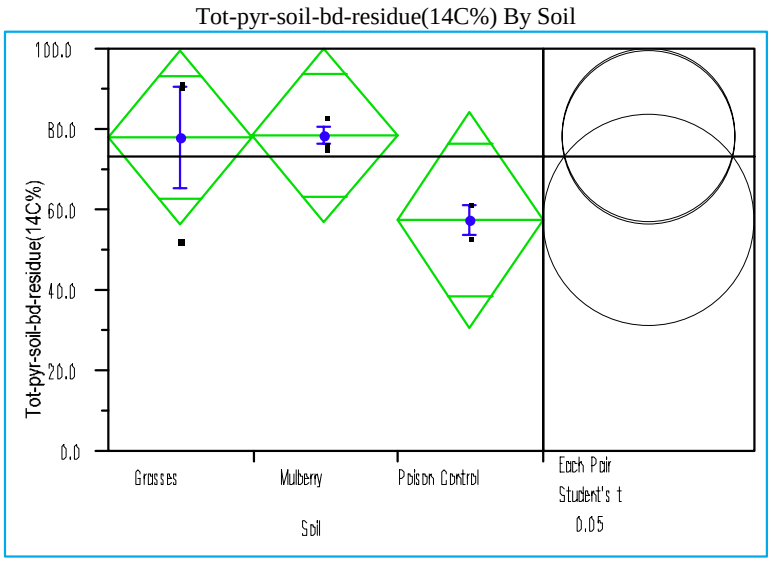
Comparisons for each pair using Student's t

t

2.57054

Abs(Dif)-LSD	Mulberry	Grasses	Poison Control
Mulberry	-15.7551	13.5049	17.4637
Grasses	13.5049	-15.7551	-11.7963
Poison Control	17.4637	-11.7963	-19.2959

Positive values show pairs of means that are significantly different.



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
Grasses	3	78.5100	22.7322	13.124
Mulberry	3	78.6567	4.1380	2.389
Poison Control	2	57.8250	5.9185	4.185

Means Comparisons

Dif=Mean[i]-Mean[j]	Mulberry	Grasses	Poison Control
Mulberry	0.0000	0.1467	20.8317
Grasses	-0.1467	0.0000	20.6850
Poison Control	-20.8317	-20.6850	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

t

2.57054

Abs(Dif)-LSD	Mulberry	Grasses	Poison Control
Mulberry	-31.1701	-31.0234	-14.0176
Grasses	-31.0234	-31.1701	-14.1642
Poison Control	-14.0176	-14.1642	-38.1754

Positive values show pairs of means that are significantly different.

VITA

Xiujin Qiu

Education

BS	Civil Engineering	Tongji University, Shanghai, PRC	1979
MS	Sanitary Engineering	Tongji University, Shanghai, PRC	1982
MS	Environmental Engineering	Utah State University, Logan, UT	1991
Ph.D	Environmental Engineering	West Virginia University, Morgantown, WV	2000

Professional Experience

1982 - 1984	Engineer	Yizheng Chemical Fibre Company Incorporation, Yizheng, Jiangsu, PRC
1984 - 1986	Engineer	Suzhou Institute of Civil and Environmental Engineering, Suzhou, Jiangsu, PRC
1986 - 1988	Visiting Researcher	Waste Lab, Cornell University, Ithaca, NY
1988 - 1991	Research Assistant	Utah Water Research Lab, Logan, UT
1991 - present	Engineer	Union Carbide Corporation, South Charleston Tech Center, South Charleston, WV

Professional Engineer

West Virginia (since 1992)