BIODEGRADATION OF BENZENE IN SOIL SYSTEMS

by

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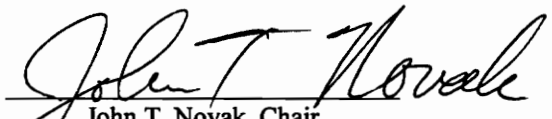

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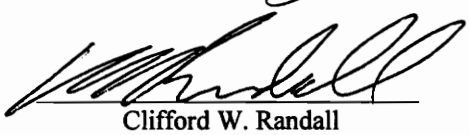

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(ABSTRACT)

The inadvertent release of petroleum hydrocarbons to the subsurface can be an imminent threat to groundwater supplies. Benzene, a water-soluble, carcinogenic petroleum hydrocarbon, is often one of the primary concerns in the cleanup of these underground petroleum leaks. The purpose of this research was to investigate the effect of soil properties, nutrient addition and benzene concentration on the biodegradation of benzene in soil systems. More specifically, a primary objective was to correlate measurable soil properties with benzene biodegradation characteristics in the soil.

Benzene biodegradation was measured in seven uncontaminated Virginia soils and three contaminated soils using laboratory microcosms. Microcosms consisted of 5 grams of soil combined with 5 mL of a sterile benzene solution. Benzene concentrations in the sterile solutions were varied at 1, 10 and 50 ppm initial benzene concentration as well as with and without nutrient supplements in the form of ammonium phosphate and potassium phosphate. Measurable physical, chemical and biological properties of each soil were then correlated with the observed benzene biodegradation characteristics, specifically an acclimation period or lag phase, a zero order biodegradation rate and a final time to degrade the substrate to less then 5 ppb benzene concentration.

Statistical analysis showed an overall increase in zero order degradation rates with the addition of nutrients in uncontaminated soils at 1, 10 and 50 ppm initial benzene concentration. Multiple linear regression analysis also indicated statistically significant relationships between several soil properties (generally pH, % sand, and % organic matter) and benzene biodegradation characteristics. These results indicate that models could be developed to predict the biodegradation of benzene and similar petroleum hydrocarbons in soils based on numerous soil physical, chemical and biological properties, rather than a single microbial degradation rate.

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I. INTRODUCTION

According to the United States Environmental Protection Agency, 10,000 -15,000 newly contaminated sites per year in the United States can be attributed to contamination by petroleum products (Aelion and Long, 1994). Estimates of presently leaking storage tanks containing gasoline and other fuel products are as high as 820,000 in the United States alone (Calabrese *et al.*, 1988). Strict regulations govern the cleanup of these spills including the Resource Conservation and Recovery Act (RCRA) amendments, Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) and the Superfund Amendments and Reauthorization Act (SARA). As more than 40% of the United States relies on groundwater for their drinking water supply, one other major legislative piece governing the cleanup of underground fuel leaks is the Safe Drinking Water Act (Russell, 1990). Health based criteria's, otherwise knowm as maximum contaminant levels (MCL's), are established in this Act which regulate many groundwater contaminants, including benzene, a known human carcinogen.

Through migration from these leaks and spills, gasoline constituents often find their way into surface waters, groundwater supplies and the atmosphere. Due to their high solubility and mobility, the light hydrocarbons, benzene, toluene, ethylbenzene and the xylenes (otherwise known as BTEX) pose the most imminent threat to groundwater supplies when an underground storage tank leak of gasoline occurs. Although benzene comprises only a small fraction of gasoline, it is one of the more water soluble hydrocarbons, with an estimated concentration of 64.80 mg/L in gasoline saturated water (Lyman, 1992). With such a high water soluble fraction, benzene, regulated at 5 ppb in drinking water, often controls site remediation. In a survey of 500 contaminated sites in the United States analyzed for over 1000 organic compounds, benzene was detected at over 120 sites, ranking the 4th most frequently occurring organic contaminant in groundwater in the United States (Plumb, 1992).

These staggering numbers and strict regulations make the need for a cost effective remediation strategy pertinent. Different remediation techniques for contaminated groundwater and soil include pump and treat, isolation and confinement, soil excavation and treatment, soil vapor extraction or enhanced in situ bioremediation (Charbeneau *et al.*, 1992). Certain advantages and disadvantages are associated with each method, as well as site specific characteristics that make one method more feasible than the other. For instance, isolation and confinement is a way of controlling and containing a particular contaminant, but does not effectively reduce its concentration. Similarly, pump and treat technologies are often effective at initial removal of a substance, but removal rates can diminish after a period of time, with no further remediation of the site. Enhanced in situ bioremediation is often the least expensive form of soil and groundwater treatment and has been proven effective in a variety of soils under different conditions.

To better assess and manage in situ bioremediation as a treatment option for the cleanup of underground gasoline leaks, soil factors contributing to the rate and extent of benzene biodegradation need to be identified as well as applicable models for adequately assessing biodegradation rates. Microbial degradation in soils is modeled by numerous techniques, including zero or first order rates, Monod kinetics, nonlinear regression, and others. Laboratory, as well as field studies, involving hydrocarbon degradation in soils may help more accurately depict characteristics of microbial degradation in soils so a standardized system for modeling degradation rates could be adapted. Soil factors that may affect biological degradation include oxygen and nutrient supply, pH, indigenous microorganisms, soil type, and the sorption capacity of the compound. An understanding of the impact of such soil properties on in situ bioremediation may assist engineers and scientists in designing practical remediation systems and modeling techniques for petroleum biodegradation as well as providing regulators with a basis for assessing the adequacy of bioremediation as a feasible treatment option.

Through the collection and analysis of several Virginia soils, this research was designed to correlate soil physical, chemical and biological properties with applicable benzene biodegradation rates as well as identify the effect of nutrient application on individual soils. Specific objectives include:

1. To distinguish critical soil properties, such as particle size distribution, pH, % organic matter, total heterotrophic organisms, indigenous benzene degraders and nutrient levels, that can be correlated to the observed lag phase, benzene biodegradation rate and final degradation time to less than 5 ppb in an aerobic soil system.
2. To determine the effect of initial benzene concentration on the observed lag phase, benzene biodegradation rate and final degradation time to less than 5 ppb in an aerobic soil system.
3. To determine the effect of nutrient addition in the form of $(NH_4)_2HPO_4$ and K_2HPO_4 at initial benzene concentrations of 1, 10 and 50 mg/L.

4. To compare the observed lag phase, benzene biodegradation rate and final degradation time to less than 5 ppb in uncontaminated soils with those of previously contaminated soils.
5. To investigate the adsorptive behavior of benzene in different soil types and its effects on achieving complete biodegradation of the compound.

II. LITERATURE REVIEW

2.1 Properties and environmental significance of benzene

Benzene is an aromatic hydrocarbon commonly used in gasoline, as a chemical solvent for paints and rubber, and in the production of various industrial compounds. Estimated worldwide production of benzene is 15 million tons per year (Maltoni, 1983). Several physical and chemical properties of benzene are listed in Table 1. Being one of the more soluble and volatile aromatic hydrocarbons, benzene often finds its way into drinking water supplies and the atmosphere.

Benzene is a known human carcinogen that has been linked to several forms of leukemia (Sittig, 1991). The maximum contaminant level (MCL) for benzene in drinking water is 5 ppb, with a maximum contaminant level goal (MCLG) of zero (AWWA, 1990). Pathways for exposure to benzene include ingestion of contaminated drinking water, plants, animals or soil, dermal adsorption, and inhalation (Calabrese *et al.*, 1988). Benzene can be accumulated in the body and slowly be released for up to a week after initial exposure (Berlin, 1985). Short term side effects of benzene inhalation include irritation of the nose, throat and lungs, dizziness, headache and even death, depending on the concentration and length of exposure (Sittig, 1991).

Since the majority of benzene production occurs in the manufacture of gasoline, one of the primary pathways for benzene exposure is through gasoline spills, in particular underground storage tank leaks. Although benzene is only about 1.5% of the total volume of gasoline, benzene concentrations are enriched 10 times in the water soluble fraction of gasoline vs. the bulk product. BTEX compounds only make up 23-55% of gasoline but 42-72% of the water soluble fraction of gasoline is BTEX (Guard *et al.* 1983, in Lyman *et al.*, 1992). The water soluble fraction of benzene is doubled in super unleaded gasoline which could be due to methyl t-butyl ether (MTBE), found at especially high concentrations in super

Table 1. Selected physical and chemical characteristics of benzene [a]

Compound	*Chemical Formula*	*Structure*	*Molecular Weight*	*Solubility 25 °C (mg/L)*	*Density 20 °C (g/cm^3)*	*K_H, 25 °C (10^{-3} atm m^3/mol)*	*Saturated Vapor Pressure (atm)*	*log (Kow)*
Benzene	C_6H_6		78.11	1780	0.879	5.4 ± 0.25	0.125	2.13

[a] After Perlinger and Eisenreich, 1991

unleaded gasoline, acting as a cosolvent for benzene (Kramer and Hayes, 1987 in Lyman *et al.*, 1992). Due to its high solubility and known toxicity, benzene is one of the primary concerns in gasoline contaminated groundwater.

2.2 Microbial degradation of benzene in soil systems

2.2.1 Biodegradation of hydrocarbons in soils

Aerobic respiration is the primary mechanism for benzene biodegradation. Aerobic respiration, carried out by the oxidase enzyme, uses oxygen as the terminal electron acceptor in the oxidation-reduction reaction (Dragun, 1988). These enzymatic reactions between the organisms and hydrocarbons can occur with enzymes within the cell (intracellular) or with enzymes located outside the organism in the soil biota (extracellular) (Alexander, 1977), although hydrocarbon degradation primarily involves intracellular enzymatic reactions.

The soil environment for degradation can be viewed as a matrix of surfaces, water, air, microorganisms, and soil organic matter. Bouwer and McCarty (1984) characterized subsurface biodegradation of organic compounds as a biofilm reactor, or an environment "generally characterized by low substrate and nutrient concentrations and high specific surface area" which support attached growth. A contaminant must diffuse through the water or moisture layer, according to Fick's law, to be degraded by organisms within this biofilm. Utilization of the substrate by organisms within the biofilm will follow Monod-type kinetics, but the actual substrate degradation rate may be limited by any of the physical and chemical barriers associated with the biofilm model. Many researchers support this type of growth model for the analysis of soil biodegradation (Brunner and Focht, 1984; Li *et al*, 1993; Novak *et al.*, 1989; Rittman *et al.* 1980; Rogers *et al.*, 1993).

2.2.2 Organisms responsible for biodegradation of benzene in soils

A diverse group of bacteria and fungi in the environment are capable of degrading petroleum hydrocarbons. The most significant of these are the *Pseudomonas*, *Achromobacter* and *Arthrobacter* for bacteria and *Trichoderma*, *Penicillium* and *Aspergillus* for fungi (Atlas, 1977; Dragun, 1988). Spore forming bacteria generally do not play a significant role in hydrocarbon degradation (Dragun, 1988). In a two year study of a moorland soil at a depth of approximately 1 foot and a pH of 3.2 - 5.0, the microbial genera primarily responsible for benzene biodegradation were *Penicillium* and an unidentified sterile mycelium (Jones and Edington, 1968). Some important factors for growth of these organisms are an

available carbon source, pH between 6 and 8, soil temperature (most enzymes are denatured at temperatures above 50 °C), soil moisture, and essential macro- and micronutrients (Dragun, 1988).

Results of using non-indigenous hydrocarbon degraders in the cleanup of oil or gasoline contaminants are inconclusive. These microorganisms are often not capable of competing with indigenous organisms (Rogers *et al.*, 1993). Seeding sometimes works in the marine environment because salt water is naturally low in hydrocarbon degrading organisms; however, seeded organisms must be able to adapt and survive in an environment characterized by very low temperatures and low nutrient levels (Atlas, 1977). Practical problems with seeding include transportation and handling, as well as toxicity of the environment to organisms. Alternatives to seeding are modifying the natural environment to stimulate growth of indigenous organisms (Atlas, 1977).

2.2.3 Distribution of microorganisms in the subsurface environment

The phase in which bacteria exist can impact their ability to degrade organic contaminants. In a study by Arvin *et al.* (1988), it was found that in chemostats containing soil and groundwater from either a non-polluted or a slightly polluted site, a mixture of petroleum hydrocarbons was degraded at the same rate whether the chemostats contained just groundwater or a mixture of soil and groundwater. However, a higher rate was obtained for chemostats with a soil/groundwater mixture from a heavily contaminated site. These results suggested that only soil that has had prolonged or excessive contact with a contaminant will have an active attached biomass that significantly contributes to degradation. Holm *et al.* (1992) found that degradation of aromatic hydrocarbons was more complete in sediment microcosms than groundwater microcosms in the laboratory, but found no significant difference in degradation ability between *in situ* sediment and groundwater microcosms. Attached bacteria may not play a significant role in hydrocarbon degradation due to limited acclimation times, unless they are in constant contact with a contaminated groundwater supply. Since free living bacteria flow with the groundwater, they may become acclimated to pollutants and hence reflect higher degradation rates (Arvin *et al.*, 1988).

The number of bacteria in the attached form in soils is significantly higher than the free living form (Harvey *et al.*, 1984). Holm *et al.* (1992) found that in laboratory studies of sediment and groundwater microcosms, the concentration of microorganisms in the sediment phase was 5 to 7 times higher than the aqueous concentration in the sediment microcosms and 20 to 67 times higher than the aqueous concentration in the groundwater microcosms. Colony counts by Kampfer *et al.* (1993) indicated subsoil sample microbial counts were 100 to 1000 times the levels in groundwater samples.

Microbial counts are often positively correlated with soil grain size. In the smaller particle size soils, such as clay or silt soils, the flow of water, oxygen, and nutrients is restricted, resulting in a less

favorable environment for microbial growth (Paul and Clark, 1989). Konopka and Turco (1991) found microbial counts from sandy sediments greater than those from the overlying strata of clay. Aelion and Long (1994) found high microbial counts by acridine orange enumeration in all soil types, but plate counts and MPN tubes showed significantly less microbial activity for a clay soil.

2.2.4 Pathways for benzene biodegradation

Benzene is degraded primarily by aerobic respiration with oxygen as the terminal electron acceptor. Aerobic respiration of benzene is represented by the chemical equation $C_6H_6 + 7.5\ O_2 = 6\ CO_2 + 3\ H_2O$, with 3.1 grams of oxygen needed per gram of benzene for complete mineralization (Dupont *et al.*, 1990). The degradation of benzene under oxygen limiting conditions has recently been demonstrated in laboratory studies (Edwards and Grbic-Galic, 1992; Grbic-Galic and Vogel, 1987) but there is still substantial data that suggests benzene is resistant to degradation under denitrifying conditions (Hutchins, 1991; Hutchins *et al.*, 1991; Kuhn *et al.* 1988), except when degraded as a cometabolite of toluene (Jensen, *et al.*, 1988; Batterman et al, 1994).

Dihydroxylation, the addition of two hydroxyl groups, is a prerequisite for the enzymatic fission of a benzene ring. Dioxygenase enzymes act to catalyze the hydroxylation of the benzene ring by incorporating both atoms of the oxygen molecule into the substrate (Gibson, 1969). Catechol (Gibson, 1968; Marr and Stone, 1960) and cis-benzene glycol (Gibson and Koch, 1969) have been identified as two early intermediates in benzene degradation while phenol has not (Gibson and Koch, 1969; Marr and Stone, 1960). A proposed pathway for catechol formation in benzene degradation is shown in Figure 1.

2.2.5 Enumeration of benzene degraders

Predicting the number of hydrocarbon degrading microorganisms in soil is difficult due in part to inadequate enumeration techniques. The use of an agar media with hydrocarbons as the only carbon source is questioned due to the presence of some forms of carbon in the agar media or soil sample. Gibson and Koch (1969) were able to culture organisms on xylene vapors using a mineral agar inoculated with K, P, N, bactoagar and mineral medium on xylene vapors. However, Atlas (1981), citing Higashihara (1978), reported plate counts were unsuitable for enumeration, especially in marine environments, due to the ability of colonies to grow on minute amounts of carbon. The use of silica gel as a growth media has been shown to improve counts over traditional spread plate agar media (Walker and Cowell, 1976).

There has been reported success in enumerating hydrocarbon degraders using MPN tubes, although this method has produced lower counts than the use of silica gel (Atlas, 1981). Mills et al.

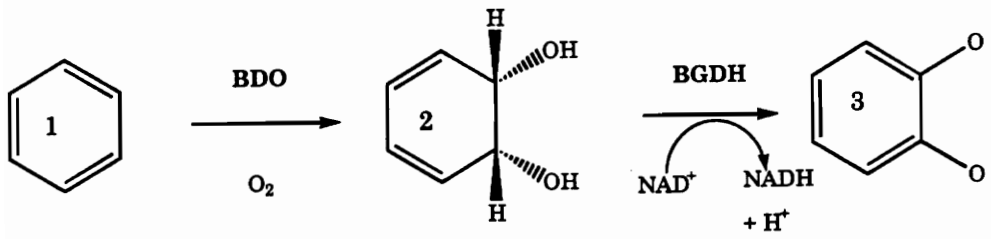

Figure 1. Proposed pathway for catechol formation in benzene degradation

(1978) found ranges of 10^4 - 10^5 and 10^3 for oil degrading bacteria at two oil polluted beaches. Other researchers have reported values of 10^2 for petroleum degrading bacteria in oil contaminated sediments (Walker and Cowell, 1976). Salanitro (1993) found benzene degrader counts as high as 10^7 bacteria/g soil near a hydrocarbon source and as low as 10^1 downgradient from the source. The number of hydrocarbon degraders present is often dependent on the contamination history of the soil (Atlas, 1981).

2.3 Factors affecting benzene biodegradation in soil systems

Factors affecting degradation of BTEX in soils have been extensively reviewed in literature (Atlas 1981; Dragun, 1988; Salanitro, 1993). Several of these properties will be reviewed in the following sections including permeability, moisture content, and soil type; oxygen availability; nutrients; temperature and pH.

2.3.1 Permeability, moisture content and soil type

Permeability, moisture content, and soil grain size all influence the mobility of various compounds in soil. This can have a significant impact on the microbial growth, oxygen transfer and carbon and nutrient availability in the soil matrix. Soil gas permeability is influenced by soil grain size and soil moisture, with moisture content being the most significant. In a study of 103 contaminated sites with greater than 80% by weight silt and clay, sufficient soil gas permeability was obtained for bioremediation applications. However, the combination of high moisture content and fine grained soils severely limited oxygen transfer at 3 out of 103 test sites (Miller *et al.*, 1993). Hydraulic conductivity values greater than 10^{-4} and a homogeneous soil material are generally favorable conditions for in situ bioremediation (Rogers *et al.*, 1993), but the heterogeneous nature of soil can often result in higher permeability values than those predicted from the literature (Miller *et al.*, 1993).

Water content can play a significant role in the physical state of petroleum hydrocarbons in soil. Due to the chemical characteristics of hydrocarbons, no biodegradation of hydrocarbons can occur in the absence of water (Li *et al.*, 1993). Biodegradation of hydrocarbons occurs at the water-hydrocarbon interface (Wodzinski and Larocca, 1977) but not at the hydrocarbon-solid interface. Since spreading of hydrocarbons is limited in soils, there is a limited surface area exposed to water and hence a limited area available for degradation. An increase in the surface area exposed to water should make a hydrocarbon more degradable. Dispertants can increase degradation by increasing surface area (Atlas, 1981).

High soil moisture can also severely limit the oxygen transfer capacity of a soil, while low soil moisture can inhibit microbial activity (Li *et al.*, 1993; Miller *et al.*, 1993). Li *et al.* (1993.) found a

maximum "bioremediation" rate at 40-50% of maximum field holding capacity. At the same time, effective degradation can be obtained at moisture contents as low as 2 - 3% (Miller *et al.*, 1993).

2.3.2 Oxygen

2.3.2.1 Role and significance in benzene biodegradation

Oxygen is the preferred electron acceptor in microbial reactions because reactions with oxygen yield higher energy releases than reactions with other electron acceptors. However, some of the first studies of in situ bioremediation showed that the greatest cause of failure with this treatment option was the lack of adequate oxygen supplies in the subsurface (Brown and Norris, 1994), due to its limited transfer in soils, particularly when water is the carrier fluid (Dupont *et al.*, 1990). The diffusion coefficient of oxygen in water is 1.80×10^{-4} cm^2/sec compared to 0.205 cm^2/sec in air (Paul and Clark, 1989). Table 2 illustrates this point with the relative mass of carrier needed per gram of oxygen for different transport media.

Oxygen is often the limiting nutrient in a contaminated oil spill. Aelion and Bradley (1991) measured oxygen levels in an aquifer near a JP-4 fuel oil contaminated site at 2.9 mg/L in a shallow well near the site but detected zero oxygen in an adjacent well screened from 27 - 32 feet. Other field studies on BTEX degradation suggested that the amount of BTEX remaining at a contaminated site was inversely correlated with the dissolved oxygen levels in the groundwater. At dissolved oxygen concentrations greater than 0.9 ppm, BTEX concentrations were undetectable (Chiang et al., 1989). These oxygen requirements and restrictions make providing an adequate oxygen supply underground an important facet of bioremediation.

2.3.2.2 Methods of supplying oxygen underground

2.3.2.2.1 Hydrogen Peroxide

Hydrogen peroxide has been used extensively as an oxygen source in bioremediation studies (Frago, 1993; Lu, 1994) because it decomposes to generate oxygen. Anid *et al.* (1993) used hydrogen peroxide to stimulate hydrocarbon degradation by indigenous microorganisms. With an initial benzene concentration of 20 mg/L, a medium sized sand soil, and an organic carbon content of 0.03-0.08%, they were able to obtain 90% removal of benzene in hydrogen peroxide amended columns, compared to virtually no removal in unamended columns. Lu (1994) found a removal of 0.265 mg benzene per mg

Table 2. Carrier fluid oxygen supply requirements [a]

Carrier fluid		g carrier/g O_2
Water		
	Air saturated	110,000
	Pure O_2 saturated	22,000
	500 mg/L H_2O_2 (100% utilization)	2,000
Air (20.9%)		13

[a] After Dupont *et al.*, 1990

H_2O_2 added in sand column reactors. However, subsequent increases in this H_2O_2 concentration, only reduced this removal efficiency, suggesting incomplete utilization of the oxygen produced at higher H_2O_2 concentrations.

Hydrogen peroxide is often not effective as an oxygen source due to inefficient transport and limits on oxygen solubility in water (Batterman *et al.*, 1994; Morgan and Watkinson, 1992). Hydrogen peroxide use has limited application in low permeability soil and rapid decomposition and precipitation can occur from reactions with enzyme catalase, iron, and manganese (Brown and Norris, 1994). Hydrogen peroxide can chemically oxidize organic chemicals and lead to overestimates of the biodegradation rate, although this has been shown not to be a significant mechanism for benzene oxidation, even at H_2O_2 concentrations as high as 2,000 mg/L (Lu, 1994). There are also potential toxicity problems at concentrations over 1000 mg/L (Brown and Norris, 1994).

2.3.2.2.2 Bioventing

Another method of providing oxygen underground is through soil bioventing, which is defined as direct air injection or vacuum induction of oxygen rich gas into soil. Bioventing enhances bioremediation by increasing the dissolved oxygen concentration in soil. Expensive off gas treatment is eliminated by putting the vapors of the volatilized fraction through biologically active soil. Reduced flow rates in the use of bioventing can minimize volatilization rates, therefore reducing costs of offgas treatment while maintaining efficient biodegradation on site (Miller *et al.*, 1993). Nevertheless, the application of this technology in the field has not accurately matched laboratory results (Dupont *et al.*, 1990).

Two factors are needed for successful bioventing: 1) air must be able to pass through soil effectively enough to maintain aerobic conditions (influenced heavily by soil type) and 2) natural hydrocarbon degraders must be present in sufficient numbers. A comprehensive look at the use of soil bioventing at numerous contaminated sites throughout the country was performed by Miller *et al.*(1993). In one study at Tyndall Air Force Base, the use of bioventing caused a 40% reduction in TPH levels and a 90% reduction in BTEX. Using oxygen utilization rates to measure degradation, they found a wide variation, between 3-12% /day. Most sites had less than 1 mg/kg benzene, indicating most were JP-4 and diesel spills.

2.3.2.2.3 Alternate electron acceptors

Using alternative electron acceptors, such as NO_3^-, $SO_4^=$ or CO_2 for hydrocarbon degradation is also possible as long as the appropriate organisms and substrate are present for this type of degradation

(Brown and Norris, 1994; Paul and Clark, 1989). Edwards and Gbric-Galic (1992) were able to measure benzene degradation under anaerobic conditions in sediment microcosms from Seal Beach, CA. Using initial benzene concentrations of 40, 90, 140, and 200 uM of benzene they found linear degradation rates of 1, 1.9, 3.7, and 0.4 uM/day, respectively. Sulfate was believed to be the terminal electron acceptor, but the role of CO_2 could not be determined. In laboratory studies under denitrifying conditions, benzene has been degraded as a cometabolite in the degradation of toluene (Batterman *et al.*, 1994) but with significant acclimation periods (Anid *et al.*, 1993; Jensen *et al*, 1988). However encouraging these results may seem, most results are from laboratory studies and have not yet been effectively demonstrated in the field (Salanitro, 1993).

2.3.3 Nutrients

Essential elements or nutrients needed for microbial growth can be subdivided into the macronutrients (hydrogen, carbon, nitrogen, oxygen, magnesium, phosphorus, sulfur, potassium, and calcium) and the micronutrients (boron, chlorine, vanadium, manganese, iron, copper, zinc and molybdenum) (Bohn *et al.* 1985). The nutrients that are generally limiting in soil remediation studies are phosphorus and nitrogen. There are several different reports on the essential ratios of carbon:nitrogen:phosphorus required for optimal microbial growth in hydrocarbon degradation, as shown in Table 3.

There is some conflict over the impact of nutrient addition on the microbial degradation of petroleum hydrocarbons (Atlas 1981, Salanitro 1993). Many studies on nutrient addition in petroleum or fuel oil spills find that the degradation rate is increased with nutrient addition (Atlas and Bartha, 1972b; Dibble and Bartha, 1976). One major successful field application of nutrients to increase oil degradation was the Exxon Valdez oil spill in Prince William Sound, Alaska, where the application of an oleophillic fertilizer increased the degradation rate by approximately two fold (Pritchard *et al.*, 1993). However, there are also conflicting studies that report no enhancement of biodegradation is achieved with nutrient addition (Miller *et al.* 1993; Salanitro, 1993). The reasons for these discrepancies relate mainly to the individual site characteristics, type of contamination and method of nutrient application.

Nutrients may be limiting in the core of a gasoline or oil plume, where hydrocarbons are in the bulk phase rather than the dissolved phase. In the center of an oil or gasoline plume, the amount of carbon available is very high but the rate of diffusion of nitrogen and phosphorus is too slow to establish C/N and C/P ratios for optimal growth. For soluble hydrocarbons, the C/N and C/P ratios are generally not limiting because the solubility of hydrocarbons is low enough that naturally available nutrients are sufficient (Atlas 1981). Salanitro (1993) reports that there is no evidence to support that nutrients in the

Table 3. Optimal nutrient requirements for hydrocarbon biodegradation

Reference	C:N:P
Miller et al., 1993	250:10:1
Rogers et al., 1993	120:10:1
Atlas and Bartha, 1973	100:10:1*
Dibble and Bartha, 1979	800:13:1**

* petroleum hydrocarbon in seawater

** oil sludge degradation

form of NH_4^+, NO_3^- and PO_4^{3-} benefit BTEX degradation at concentrations in the range of < 5000 ppb to 10000 ppb and that nutrient addition may even decrease oxygen availability due to nitrification.

Laboratory studies on nutrient addition periodically conflict with results from field testing. In soil bioventing field studies by Miller *et al.* (1993), a C:N:P ratio less than 250:10:1 was able to sustain microbial activity. Nutrient addition showed an increase in degradation rates in laboratory studies of these soils, but this has not been demonstrated in the field (Miller *et al.*, 1993). However, in other field studies, Allen-King *et al.* (1994) found that hydrocarbons were reduced only 75-90% with natural field conditions, even though adequate oxygen was present. Laboratory results showed nutrients, especially nitrogen in the form of ammonium nitrate, enhanced biodegradation. Upon addition of nutrients in the field, biodegradation proceeded to complete mineralization of the BTEX compounds (Allen-King *et al.*, 1994). There is generally no increase in the degradation rate of petroleum hydrocarbons with nutrient addition if oxygen is limiting (Atlas, 1981).

There are several reasons for the ambiguity between laboratory and field results. Problems occur when excess phosphate and oxygen combine with metals or other inorganic materials in soil such as calcium and iron to form precipitates (Robertson and Alexander, 1992). This can be an even more significant problem when dealing with calcareous soils or when the solution contains an oxidizing compound such as hydrogen peroxide (Aggarwal *et al.*, 1985; Morgan and Watkinson, 1992). These problems lead to reduced availability of the supplemental nutrient as well as plugging problems within the soil. Soil properties such as pH, Eh, and concentration of the compound in question must be considered before adding a nutrient rich solutions to an aquifer due to the affects of these properties on the solubility and speciation of a compound.

Since laboratory studies are generally conducted over a short period of time, slow nutrient precipitation reactions may not be witnessed in the laboratory due to slow reaction kinetics (Aggarwal *et al.*, 1985). Most lab studies are done under aerobic conditions, which are not indicative of field conditions where oxygen is usually only available in sparse quantities. It is not well known what concentrations of phosphate are necessary for hydrocarbon metabolism under oxygen limiting conditions. The excess growth promoted when working under oxygen rich conditions may have much higher demands for phosphorus then under oxygen limiting conditions, where growth is restricted. (Aggarwal *et al.* 1985). Consideration must also be given to the fact that in situ there will be declining phosphorus concentrations at grater distances from the injection point due to reactions in the soil. Polyphosphates have been used to alleviate this problem due to their slower degradation rate (Morgan and Watkinson 1992). Caution should be taken with excess application of nutrients in ground water, because of possible pathogenic organism growth and, in surface waters, because of increased algae production (Atlas, 1977).

2.3.4 Temperature & pH

The relationship between temperature and degradation rate of petroleum hydrocarbons is not straight forward as it depends strongly on the composition of the fuel and the microbial community. Temperature is not often the limiting factor in microbial degradation of hydrocarbons in soil, except when it effects the physical state of the substrate or the availability of water (Atlas, 1981).

Generally, the degradation rate increases with temperature increases from 5°C to 20-25°C (Atlas, 1981). Atlas and Bartha (1972) found differing effects from temperature based on a combined petroleum mixture. Volatilization of low weight hydrocarbons is inhibited at low temperatures, which can lead to a toxic accumulation of these substances. At 20° C, light crude oils were degraded faster than heavy crude oils. At 10°C, however, light volatile components did not evaporate from light crude and caused toxicity and decreased degradation rates. Temperature differences can also play a significant role as seasonal variations may lead to significantly reduced microbial activity in winter (Atlas and Bartha, 1972).

Walker and Colwell (1976) found that "slower but more extensive" degradation occurred at 0°C for a model petroleum hydrocarbon in estuarine water collected during the winter months. Ward and Brock (1976) positively correlated the mean monthly temperature and the rate of disappearance of hydrocarbons from an oil contaminated field. Maximal degradation rates for BTEX has been observed at 35°C (Deeb and Alvarez-Cohen, 1994). However, degradation will still proceed at low temperatures. Microbial degradation of the Exxon Valdez oil spill was possible even at temperatures between 10°C and 15°C (Pritchard *et al.*, 1992).

The pH can affect the number of hydrocarbon degraders naturally present at a site, the dominant organism species (bacteria, fungi, etc.) and the availability of inorganic nutrients. In soil bioventing studies by Miller *et al.* (1993), a pH between the range of 6 - 8 was considered favorable for bioremediation, while McCormick (1991) identified a pH range of 5.4 - 6.6 as the most favorable range for BTEX degradation. Gibson *et al.* (1968) found an optimal pH of 7.5 for the growth of benzene degraders.

2.4 Sorption of benzene in soil systems

2.4.1 Mechanisms for sorption of benzene in soil

Sorption refers to the accumulation of dissolved substances by the solid soil phase (Voice and Weber, 1983). *Ad*sorption describes the process of a compound being located at the interface of a solid, liquid, or gas. *Ab*sorption is the process of an organic compound being "transferred from the bulk state to

one phase or another" (Hassett and Banwart, 1989). Sorption can occur through physical adsorption (van der Waal's forces); hydrogen bonding; ion exchange; coordination bonding, where a nonionic polar compound attaches to metallic ions; chemical bonding between contaminant and soil; and hydrophobic sorption, where a hydrophobic compound partitions into indigenous organic matter or clay minerals (O'Neill *et al.*, 1993).

Organic compounds can become sorbed to numerous sites in a soil including clay minerals, indigenous organic matter and amorphous oxides and hydroxides of metals. Adsorption of organic matter onto clays can subsequently affect properties of the soil, such as hydraulic conductivity, shrinkage and strength (Mitchell, 1993). Sorption to indigenous organic matter, oxides and hydroxides can also be pH dependent, with sorption of humic substances generally increasing with decreasing pH, due to the positive charge on the sorbent (Murphy *et al.* 1990). This pH effect is also dependent on the nature of the organic material being sorbed, i.e. whether it is an acid or base, polar or nonpolar, etc.

Chiou *et al.* (1983) supported that partitioning of nonionic organic compounds to soil organic matter is the most significant form of sorption in soils. Sorption to humic substances in soils may be up to 20 times more than the adsorption to "pure" clay minerals (Rebhun *et al.*, 1992). Minerals generally do not play a significant role due to their affinity for water. Chiou and Shoup (1985) looked at the sorption of organic vapors in soil as a function of relative humidity. In general, in low organic soils (<1.9%), the higher the humidity, the less the sorption of organic vapors, due primarily to competition with water for polar mineral sites. Figure 2 illustrates this relationship for benzene. The strong dipole attraction between the mineral sites and water excludes nonionic organic compounds from sorption in this manner (Chiou and Shoup, 1985).

Despite the dominant behavior of organic matter in the sorption of nonpolar organic compounds, the fraction of organic matter in most unconsolidated aquifers is less than .001, leaving mineral surfaces as the dominant sorption sites. Perlinger and Eisenreich (1991) reported that "sorption to minerals is the major mechanism by which the transport of hydrophobic organic chemicals (HOC) with groundwater is retarded". When clay minerals compose up to 40% of subsurface soils and humic matter only accounts for 0 - 5 %, mineral sorption can play a significant role (Rebhun *et al.* 1992). In addition to surface adsorption on mineral surfaces, sorbed water molecules can orient themselves so they are layered and the HOC can subsequently partition into this water layer (Perlinger and Eisenreich, 1991). McCarty *et al.* (1981) described a critical level of organic carbon below which inorganic matrices are the major contributor to sorption. For benzene, that level is 0.001 or .1% (Lyman *et al.*, 1992). Despite these facts, relatively few studies exist on the sorption of HOC's to mineral surfaces (Perlinger and Eisenreich, 1991).

There is a reported decrease in the sorption coefficient of HOC's with an increase in particle concentration. Perlinger and Eisenreich (1991) found a decrease in the sorption coefficient as particle

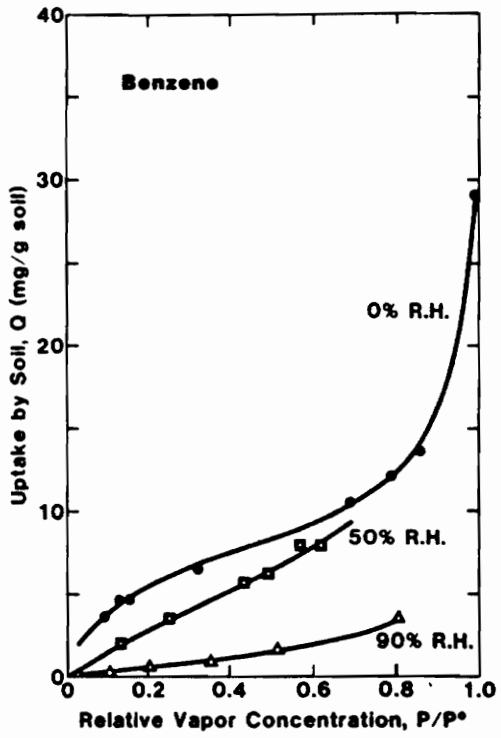

Figure 2. Vapor uptake of benzene as a function of relative humidity *
* After Chiou and Shoup, 1985

concentration increased for benzene. Possible aggregation of particles as the concentration increases may cause decreased surface area and increased diffusional path length, which in turn reduces the sorption coefficient. Soil type is also important in the relative sorption since sorption to fine soil particles is much more significant than sorption to large soil particles (Karickoff, 1979). Sorption on mineral surfaces that are completely void of organic carbon can be very low, but are still measurable in clay minerals with high surface areas (Murphy *et al.* 1990).

Although limited studies have been performed on the kinetics of hydrophobic compound sorption, sorption reactions are generally rapid (Voice and Weber, 1983). Karickoff (1980) proposed a two phase sorption system where initial adsorption occurs in minutes to hours but the second phase of adsorption could take weeks or longer to occur. The second component of adsorption generally involves sorption through diffusive movement to interparticle pores (Karickhoff, 1980) or sorption into organic matter (Perlinger and Eisenreich, 1991).

2.4.2 Sorption and degradation rates

Adsorbed organic material is less available for microbial degradation and this decrease in availability is "a function of the degree of adsorption" (Gordon and Milllero, 1985). Degradation rates for sorbed compounds will be different than those in the aqueous phase (O'Neill *et al.*, 1993). Sorption can be a limiting mechanism in biodegradation, especially in soils high in smectite or any other expansive clay mineral. In a study by Weber and Miller (1989), they found that sorbed compounds are essentially unavailable for biodegradation. If the role of attached bacteria is high, the effect of sorption may not be as strong on the degradation rate (Gordon and Millero, 1985). The influence of sorption on degradation rates will be discussed further in the kinetics section of this chapter.

2.4.3 Desorption and biodegradation

Desorption and diffusion can be a rate limiting step for degradation. Although adsorption kinetics are generally fast, desorption of a compound may be slow, depending on the contact time between the compound and the sorbent. Desorption kinetics can limit the biodegradation of a sorbed compound, especially after fast, initial desorption has occurred. Robinson et al. (1990) reported a two stage desorption process of toluene from soil, with the rate of desorption in the second phase limiting the biodegradation rate of toluene.

These factors have lead to the use of surfactants to enhance the solubility of insoluble organic material that sorb strongly to soil (Rogers *et al.*, 1993). Surfactants, substances with one polar end and

one nonpolar, will attract water to one end and nonpolar organic contaminants to the other end (O'Neill, 1994). Surfactants are used more frequently on highly hydrophobic compounds as they are more strongly sorbed and least likely to be mobile (Hassett and Banwart 1989). Biosurfactants, surfactants produced by microorganisms in the biodegradation process, can also mobilize sorbed contaminants (Batterman *et al.*, 1994). Falatko and Novak (1992) found that biologically produced surfactants through microbial growth on gasoline increased the solubility of gasoline compounds without inhibiting biodegradation of these compounds.

Desorption can occur more readily at high pH since pH induced dispersion breaks up clay sheets and exposes sorbed organic compounds to the aqueous phase. Generally, acids, bases, oxidants, or surfactants can induce desorption of organic contaminants from soil (Hassett and Banwart, 1989).

2.5 Estimating biodegradation rates in soils

2.5.1 Kinetic models for soil biodegradation

Assessing the kinetics of soil systems is different than kinetics generally applied to pure cultures due to the complexity of the system. Many elements of the soil environment such as adsorption, availability of nutrients, oxygen concentration and transport, moisture content, etc. can be limiting in this system where the governing microbial growth kinetic formulas suggest they are not.

Several models depicting substrate degradation are shown in Figure 3. Logarithmic kinetics apply to a single bacterial population and an initial substrate concentration that is much higher than the Ks value (the substrate concentration at half the maximum growth rate). A logistic curve is generally characteristic of an initial substrate concentration much lower than the Ks value and a doubling time of cells dependent on substrate concentration. Hence, as the carbon source decreases, growth rate and degradation rate also decrease. Logistic curves give the characteristic "S" shape curve. When the initial substrate concentration is close to the Ks value, kinetics follow Monod with growth kinetics. If the growth rate falls with an increase in concentration above a certain level, toxicity affects are usually the cause. This is modeled with a Haldane modification of the Monod growth curve which incorporates a toxicity factor (Alexander and Skow, 1989).

The previous models were for growing populations, i.e. substrate concentration is high enough (or cell population is low enough) to warrant the need for population doubling. When the population is high enough relative to the initial substrate concentration, cells do not need to multiply Instead, they produce enzymes that will enable cells to degrade the carbon source. The relationship between enzyme reactions and substrate concentrations is termed Michaelis-Menten equations (Hamaker *et al.*, 1972),

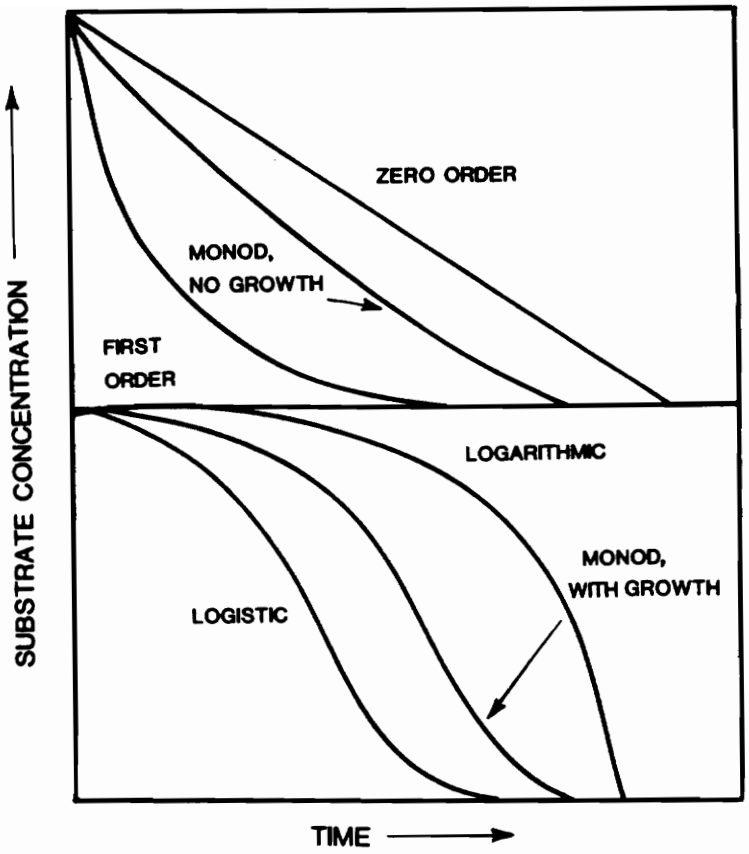

Figure 3. Several kinetic curves for substrate disappearance *
* After Alexander and Skow, 1989

which are a modification of the Monod growth models. In this situation, if the degradation rate stays the same as the concentration decreases, but are still above the Km value (instead of Ks), degradation is termed zero order growth kinetics. If the initial substrate concentration is below Km, then as organisms degrade the substrate, the rate will drop as the concentration does and first order kinetics are applicable (Alexander and Skow, 1989).

2.5.2 Problems in application of kinetic models to soils

Zero order kinetics are often observed in soils, although they should only apply when there is no growth and substrate concentrations are well above the Km value. Reasons why soils may appear to exhibit first order degradation due to factors other than microbial kinetics include (Alexander and Skow, 1989):

1) Generally, when the population density is 10-90% of the maximum (Schmidt *et al.*, 1985), the curve can appear to be a straight line, mimicking zero order degradation, when it is really only the linear portion of a logistic curve.

2) The rate at which nutrients are available may not meet the demand for nutrients at high substrate concentrations. Diffusion of these nutrients limits the degradation rate.

3) The supply of essential nutrients is exhausted and the rate switches from logarithmic to linear (because the curve is following cell growth when degradation begins, but later follows another limiting transfer mechanism such as nutrient availability or adsorption).

4) Acclimated organisms are already present in the soil and a linear rate is observed because organisms do not need to develop.

5) The aqueous phase of the chemical is depleted first as it is easier to degrade. Degradation of the remaining chemical is controlled by diffusion of the chemical from soil surfaces. Growth was unrestricted in the aqueous phase, but degradation of the sorbed compound is more difficult.

First order kinetics are often used when the initial concentration is below the Ks value. There is the question of the validity of this assumption in hydrocarbon degradation. The threshold concentration (the concentration needed for any mineralization to occur) for hydrocarbons is estimated in the ppt range, suggesting the Km value would be very low (Lyman *et al.*, 1992). Salanitro (1993) suggests that a BTEX concentration </= 1 to 500 ppb is much greater than the Ks value. First order kinetics should only apply when the initial concentration is below this value.

Many claims of 1st order transformations are not accurate because they are taken from a limited number of data points that are fit to a straight line. First order models are often incorrectly used because of the ease in which they can be used in other models. Half life values taken from first order kinetics do

not accurately predict the final degradation time because the rate will slow as concentration drops (Alexander and Skow, 1989). Zero and first order models also neglect the presence of an acclimation period before degradation begins.

More confusion in predicting the kinetics of soil biodegradation can be due to multiple organisms with different affinity constants degrading several substrates at one time. Models are developed for a single bacterial population and a single carbon source. In contaminated soils, this is highly unlikely (Lyman *et al.*, 1992). Ks values for each microbial population and substrate could differ and not give the type of relationship depicted by the kinetic models. In addition, organisms that are measured as biomass may not be using the substrate for growth, but only as an energy source. Different kinetics control this type of degradation and there are models to depict it (Alexander and Skow, 1989).

Another problem in applying Monod kinetics to soil biodegradation is the determination of active biomass. Most enumeration techniques detect less than 10 % of the actual microbial population (Rogers *et al.*, 1993). Fungi, enumerated differently than bacteria, may also be significant in reaction kinetics. If enumeration techniques account only for bacteria, there would be an inaccurate prediction of the active biomass. Fungi grow by hyphal lengthening and branching and it is difficult to enumerate and track their growth (Alexander and Skow, 1989). However, due to their larger size, fungi may not be able to access substrates trapped in pore spaces as well as bacteria (Paul and Clark, 1989). The role of fungi in soils is expected to be significant although it has been poorly investigated (Alexander and Skow, 1989).

The effects of sorption are another significant barrier in applying Monod kinetics to soil. Sorption is modeled as a two phase process where the second phase is reportedly associated with diffusion to internal pores in the soil matrix (Karickoff, 1980). Sorption may control degradation rates, especially when the substrate becomes trapped in small pores and organisms do not have direct access to these pores. Most organisms are located on the outside of soil aggregates or in the pore spaces between them; some studies suggest that less than 1% of the pore space is occupied by soil microorganisms (Paul and Clark, 1989). The Monod models assume all substrate is freely available to organisms, which is not the case in soils; attached bacteria may not have free access to the dissolved substrate and the sorbed substrate may not be available to free bacteria. Alexander and Skow (1989) call this the "two compartment model" where the substrate is categorized in two different niches within the soil. One is the sorbed substrate, one is free in solution. After the substrate is depleted in solution, what is limiting is diffusion of the sorbed substrate to the active organisms. If a compound is highly hydrophobic and nonpolar it has a lower solubility and will be more strongly sorbed. Less degradation occurs because most of the compound is in the sorbed phase. For these reasons, the soil water partition coefficient and the solubility for any particular compound can be important (Rogers *et al.*, 1993). Compared to other gasoline components,

benzene has a high solubility (1780 mg/L) and a low K_{ow} (2.13) so it would probably be one of the less adsorbent gasoline constituents (Lyman *et al.*, 1992).

In summary, the choice of a model may be arbitrary. Models are often used because they fit a certain set of data adequately, not because they explain the mechanisms that control biodegradation. The assumptions and restrictions that go into each individual model need to be considered. It is very rare that one model will apply under all conditions, but it may be helpful to identify which models are appropriate for certain physical, biological and chemical characteristics of the soil and chemical.

2.6 Laboratory techniques for estimating field degradation rates

Numerous techniques are used in laboratory studies to approximate biodegradation rates in the field. These include flow through aquifer columns, slurry reactors and soil water microcosms. Microcosms are often used to minimize volatilization losses and to mimic slow moving groundwater environments.

Alvarez and Vogel (1991) used a similar analytical technique to the one applied here. They employed a Hewlett Packard 5890 gas chromatograph and 19395A headspace sampler to measure BTEX concentrations in slurry microcosms. They first removed aqueous samples from the microcosms and transferred them to 5 mL vials before taking samples for GC analysis. The minimum detection limit by this method was 0.01 ppm.

Aelion and Bradley (1991) used soil microcosms (20 mL glass vials) containing 3 g of soil on a dry weight basis and 10 mL of sterilized distilled water to study the aerobic biodegradation potential of indigenous microorganisms at an 83,000 gallon leak of JP-4 fuel oil in north Charleston, SC. GC analysis was used to measure CO_2 production and O_2 consumption to measure biological activity under aerobic conditions. Numerous other researchers have used soil microcosms and BTEX losses over time from active microcosms as compared to sterile controls to quantify biodegradation of benzene (Frago, 1993; Novak, 1989; Salanitro, 1993).

2.7 Observed benzene biodegradation rates

Reported benzene biodegradation rates vary from study to study and from the laboratory to the field. There is the complicating feature of nomenclature and procedures that make it difficult to compare rates as well as the models used to obtain the kinetic data from one study to the next. There is a need for a standardized way of measuring biodegradation rates in soils as one has not yet been established (Dragun, 1988). Many studies use combinations of the kinetic models previously mentioned, diffusion or sorption

rates, acclimation periods and final degradation times to adequately assess the biodegradation of an organic compound.

In theory, microbial kinetics switch from zero to first order as concentration decreases (Alexander and Skow, 1989). First order decay models may be appropriate for very low concentrations (< 1 ppm). It is also a more conservative estimate of degradation rates than zero order models. Buscheck *et al.*(1993) used a first order model for temporal and spatial analysis of biodegradation of several BTEX compounds. A good fit of the data was obtained at several different sites ($R^2 = 0.85 - 0.98$) but this was for a very limited number of data points. Buscheck (1993), citing Wilson *et al.* (1993b), reported the use of a first order model to estimate biodegradation rates below 1 ppm for aromatic hydrocarbons and, citing Tucker and Zavala (1992), found that switching from zero to first order at low BTEX concentrations was an accurate method for predicting degradation rates. Borden and Bedient (1986) determined that under oxygen limited aerobic degradation, rates may approximate first order, whereas Miller *et al.* (1993) found that oxygen consumption rates followed zero order kinetics down to 2 to 4% oxygen at a site previously contaminated with JP-4 fuel oil. Greater-than first order kinetics are also commonly observed in soils (Hamaker, 1972).

In a study on the interactions of benzene, toluene, and p-xylene, Alvarez and Vogel (1991) reported pseudo zero-order benzene degradation rates of 25 mg/L/day for benzene alone and in the presence of toluene and p-xylene. Lag times for benzene degradation were 6 days in the presence of toluene and xylene, 4 days in the presence of xylene and 2 days alone and with toluene. Initial concentrations of all compounds were 50 mg/L and the setup consisted of 16 g of aquifer material with 50 mL of a mineral medium in 120 mL serum vials. Although no detectable levels of BTEX were present in the aquifer samples used in the experiment, the aquifer itself had an overall BTEX concentration of 200 ug BTEX/L.

Arvin *et al.* (1988) investigated the role of attached bacteria vs. free living bacteria in the biodegradation of aromatic hydrocarbons. For slightly or uncontaminated soils, they found an "S" shaped curve but for highly contaminated soils they found a linear relationship between concentration and time. This suggests that linear degradation may be observed when there is an acclimated supply of organisms. To analyze the data, they used an "adaptation time" and "degradation time to less than 1 ppb" for comparative analysis.

Brunner and Focht (1984) used a nonlinear regression model for biodegradation in soils. When they tried to fit the data for CO_2 evolution over time, linear regression gave a better fit of the data than exponential models. Non exponential growth is usually treated as out of the ordinary since it is generally not used in modeling growth in liquid cultures, but degradation in the soil biota is much different than that of a liquid culture. Diffusion limitations are often caused by the soil matrix. If it is modeled as a

biofilm reactor where organisms exist as a thin film on a soil particle, diffusion of nutrients to outer cells on a soil particle may be limited and diffusion of the carbon substrate to any inner cells may also be limited (Brunner and Focht, 1984). The combination of these two diffusion-limiting situations may give some rate that appears to be zero order or linear growth.

Rogers *et al.* (1993) used slurry reactors to find kinetic factors in a worst case situation, assuming that if biodegradation did not occur under these conditions, it would probably not occur in situ. To approximate Monod kinetics, zero order growth was used at high substrate concentrations (compared to Km) and first order growth was assumed at low concentrations. They also modeled the soil as a biofilm reactor, where kinetics would be controlled by the mass transfer of the substrate, nutrients and oxygen through the biofilm. They found that typically the rate was associated with some other parameter such as diffusion, adsorption or availability of substrate rather than growth of organisms.

Li *et al.* (1993) modeled bioremediation through the use of Monod kinetics and mass transfer rates of oxygen and hydrocarbons within the soil matrix. They modeled it as a biofilm process where the biodegradation rate is controlled by the transfer rate of the organic contaminant or oxygen, or the biochemical reaction rate. Whichever was the slowest dictated the "bioremediation" rate. In this model, it was also assumed inorganic nutrients, although required for biodegradation, are not limiting because their solubility's are high compared to the solubility's of organic contaminants. A simple model was introduced for the prediction of the "bioremediation" rate when soil parameters such as total surface area, mass transfer rates for hydrocarbons and oxygen, and Km values are known. If the controlling factor can be identified, whether it is the mass transfer of hydrocarbons or oxygen, or the microbial degradation rate, necessary steps can be taken to increase the bioremediation rate.

Allen-King *et al.* (1994) were able to model batch experiments with substrate limited growth kinetics, when sufficient oxygen was present, and did see an increase in degradation rates with an increase in initial substrate concentrations. However, when nutrients or oxygen were limiting, degradation did not follow growth kinetics. They concluded that inorganic nutrients should be considered in kinetic formulas for soil degradation and variables that are going to limit growth and degradation should be identified in order to determine kinetic models and parameters.

The preceding examples demonstrated the variety of laboratory techniques used for obtaining kinetic data as well as the methods used to interpret this data. It is also evident that researchers do not always agree on what are parameters are critical in modeling biodegradation in soils or what kinetic models should be used. No comparisons can be made between reported rates from individual studies without first considering all the laboratory and field techniques and kinetic models that were used in each study.

2.8 Literature Summary

Although research has been extensive and comprehensive on microbial degradation of hydrocarbons in the soil environment, there are still many perplexing issues. The value of bioremediation as a viable treatment option has been accepted, but not without the problems and limitations associated with it. Oxygen has been identified as an often limiting nutrient in achieving desired degradation in the field. Phosphorus and nitrogen have also been identified as possible limiting agents in bioremediation studies. The technology to successfully supply these nutrients to subsurface microbial populations in all soil conditions has not yet been developed and additional work is needed in this area.

As modeling the fate and transport of underground petroleum contaminants becomes a larger aspect of remediation schemes, models for successfully predicting the bioremediation of these contaminants becomes even more important. Models that only describe the microbial kinetics may not be adequate for assessing bioremediation rates in the soil environment. The complexity and many other mechanisms that control substrate availability and microbial populations may have a significant impact on biodegradation of underground contaminants. Models that include these soil factors in the prediction of degradation rates may more accurately describe the mechanisms that control biodegradation of organic contaminants in soil systems.

III. METHODS AND MATERIALS

3.1 Experimental Design

The purpose of this study was 1) to determine the effect of initial benzene concentration and nutrient addition on benzene biodegradation in soil systems and 2) attempt to correlate measurable soil properties with observed benzene biodegradation characteristics. The first of these two tasks was accomplished through the use of static soil microcosms in which the initial benzene concentration was varied with and without the addition of nutrients for each soil investigated. The second task required the determination of kinetic parameters for benzene biodegradation in seven Virginia soils and the measurement of physical, chemical, and biological properties within each of these soils. These properties were then correlated through multiple linear regression analysis with kinetic parameters obtained from the biodegradation data for each soil. Biodegradation data was obtained through the measurement of benzene losses in active static soil microcosms over time as compared with benzene losses from sterile soil microcosms identically prepared.

3.2 Soils sampled

Seven non-contaminated Virginia soils and three contaminated soils (2 from Virginia and 1 from Texas) were sampled. The general locations of these soils and their series names are shown in Figure 4 (information on series names of contaminated soils was not available). Non-contaminated samples were collected using a hand auger and sterile sampling bags. Approximately 5 pounds of each soil was collected and transported quickly back to the laboratory in a cooler. Samples were stored at approximately 10°C until testing was complete. Contaminated soils were obtained from three different sites with three different types of contamination. Supplemental information on contaminated and uncontaminated soils is summarized in Table 4.

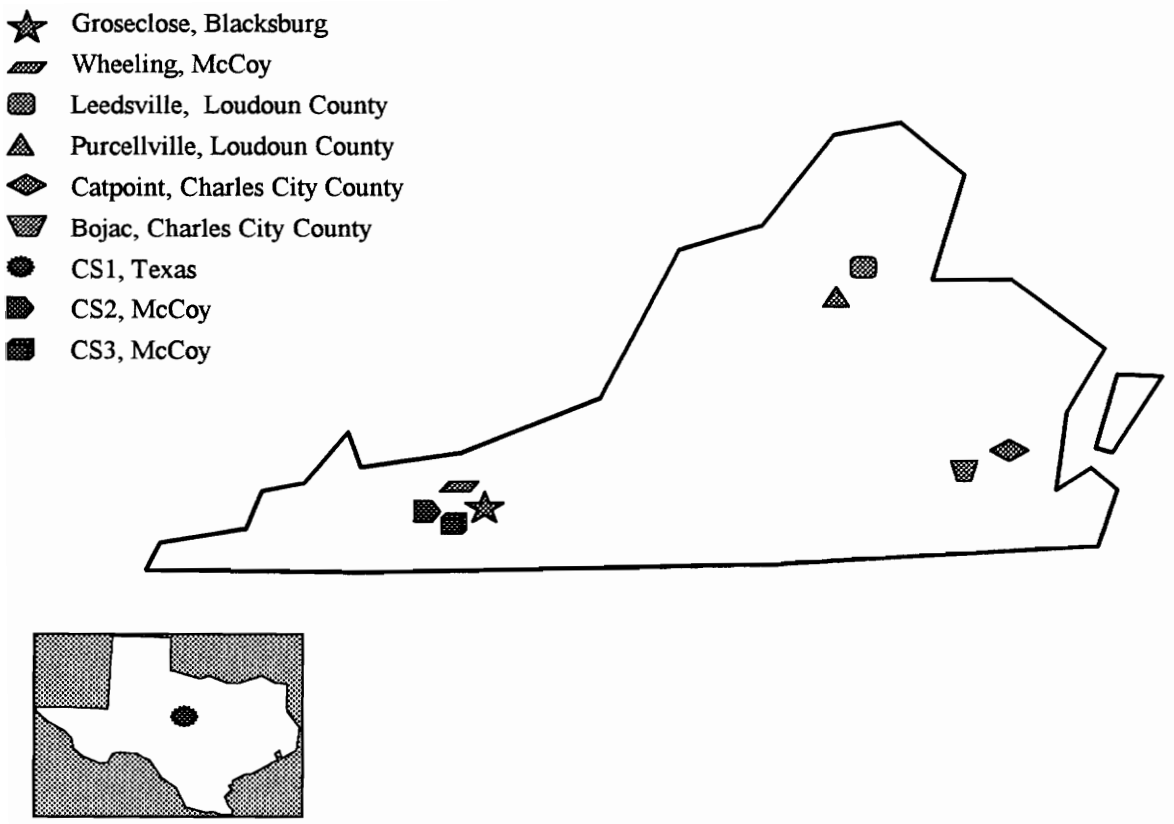

Figure 4. Series name and location of each soil sample

Table 4. Supplementary soil data

Series name[a]	Soil Type	Depth of sample (ft)	Color[b]	Drainage	Primary use[c]	Comments
Bojac	sand	4 - 5	SB (7.5YR5/6)	Mod. Rapid	WD	0 - 2% slope
Catpoint	sand	3.5 - 5.5	YB (10YR6/4)	Rapid	AG	0 - 4 % slope
Leedsville	silt loam	3 - 5	RB (2.5YR4/4)	Mod	LF	Adjacent to landfill (tree stump disposal pile)
Purcellville	sandy loam	3 - 4	YR (5YR5/8)	Mod	AG	Wheat; 2-7% slope
Groseclose loam	loam	9 - 10.5	YB (10YR5/6)	Mod	AG	Dairy farm
Groseclose clay	clay	5 - 6	YB (10YR5/8)	Mod	AG	Dairy farm
Wheeling	sandy loam	9	BR (7.5YR4/4)	Well	AG	Hay; 0 - 2 % slope
CS1	sand	No data	YB	No data	No data	Gasoline contaminated soil; previously inoculated with nutrients and gasoline degrading bacteria
CS2	sandy loam	12	BR	Well	IND	Diesel spill adjacent to New River
CS3	sandy loam	4 - 5	BR	Well	IND	Fuel oil leak at military arsenal

[a] **Bold** denotes contaminated soil (Series names not available)

[b] BR = brown; BY = brownish yellow; RB = reddish brown; SB = strong brown; YB = yellowish brown; YR = yellowish-red

[c] AG = agricultural; LF = landfill; WD = wooded; IND = industrial

The Groseclose loam and clay were sampled once in September of 1993 and again in May of 1994. These two sampling dates are denoted as G. loam and G. clay 1 and 2, respectively. Although each second soil sample was located in the same area as the first sample, most characterization tests were repeated due to the heterogeneous nature of soil and differences that can occur even in adjacent samples.

3.3 Analytical Technique

Benzene biodegradation was measured through the use of static soil/water microcosms. Soil microcosms consisted of 22 mL glass vials containing five grams of soil (± .05 g) on a wet weight basis and 5 mL of a benzene spiked solution. Benzene was obtained through Aldrich chemical company (99.9+%; Milwaukee, Wisconsin) chemical company. Microcosm solutions were autoclaved at 120°C and 15 psi for 15 minutes before being inoculated with the appropriate volume of benzene to bring the concentration of the solution to either 1, 10 or 50 ppm. Microcosms were sealed with a Teflon lined crimp cap and incubated at 20°C in the dark. For each test, 24 soil microcosms were identically prepared and 3 microcosms were sacrificed for each sampling point. Abiotic controls were prepared in the same way except soil was autoclaved 5 times over a five day period before addition to the microcosm.

The selection of an analytical method was based on the ability to obtain minimum detection levels of 5 ppb of benzene, have minimal losses of benzene and the least disruption of the microcosm upon sampling. Aqueous benzene concentrations were measured using a Hewlett-Packard 19395A headspace analyzer in conjunction with a Hewlett Packard 5880A gas chromatograph equipped with a flame ionization detector.

The temperature program for the GC was selected based on past experience with BTEX programs on the HPGC 5880A. However, temperature programs for the headspace sampler needed to be determined experimentally. Testing was done on the headspace sampler to determine the optimum program by varying the temperature, equilibration time and auxiliary pressure. The temperature was varied at 50, 60 and 70 °C with varying equilibration times from 30 to 180 minutes. The highest GC response was obtained with a temperature of 60°C, an equilibration time of 60 minutes and an auxiliary pressure of 1 bar. Specific program parameters for both the headspace sampler and gas chromatograph are given in Table 5.

Standard curves were prepared in order to correlate aqueous benzene concentrations with measured headspace concentrations. The analytical technique used to prepare and analyze the standards was identical to that used for the microcosms, except 5 g of sterile sand was used in place of the soil. This sand was used to make up for the volume difference caused by the soil in the microcosms without significant adsorption of the benzene on the sand surface. Standard curves were also prepared for

Table 5. Headspace sampler and gas chromatograph parameters

Headspace Sampler - HP 19395A

Headspace Method	1
Equilibration Time	0
Bath Temperature, °C	60
Valve Temperature, °C	65
Injections/Vial	1
Carrier Gas	Nitrogen
Carrier Gas Flow Rate, mL/min	30
Aux. Pressure, bars	1
Servo Air, bars	3.3 - 3.7

Gas Chromatograph (GC) - HP 5880A

Detector	FID
Column	Stainless steel, packed
Length, ft	6
Outside diameter, in	1/8
Stationary phase, wt %	5% SP 1200, 1.75% Bentone-34, 100/120 Supelco Port
Temperature:	
Injector 1, °C	100
Injector 2, °C	130
Detector 1, °C	100
Detector 2, °C	200
Column temperature program:	
Initial, °C	50
Initial time, min	2
Program rate, °C/min	6
Final, °C	70
Final time, min	1
Carrier gas:	Nitrogen
Flow rate, mL/min	40 (Total flow)

GC Integrator and Chart Recorder

Attenuation	0
Threshold	0
Peak Width	0.08
Signal	C

solutions with and without nutrients to account for effects of high salt concentrations in the nutrient solution. These curves, response factors and R^2 values are shown in Appendix A. Standard curves were prepared at several points over the sampling period to adjust for changes in the performance of analytical equipment.

3.4 Soil property tests

In order to allow comparative analysis of soil characteristics, standard soil property tests were needed for all soils investigated. These tests were divided into three different categories: physical, chemical and biological.

3.4.1 Physical soil properties

Physical soil properties measured included pH, particle size distribution, % organic matter, % adsorbed water and % moisture.

The pH was measured using a soil/water slurry for each soil sample and a pH meter equipped with a glass electrode. Twenty grams of air dried, sieved soil (No. 10 sieve) was equilibrated with 20 mL of distilled water for 15 minutes to 2 hours before the measurement was taken. Two independent pH measurements were taken for each soil.

Soils were classified for % particle size by the Soil Science laboratory at Virginia Tech using the pipette method. This method followed standardized procedures as outlined in Methods of Soil Analysis, Part 1 (Klute, 1982). Soil classifications followed the guidelines of the United States Department of Agriculture (USDA) and were based on the textural triangle, as shown in Figure 5.

The percent adsorbed water and the percent moisture represented two different water contents of the soil. Percent adsorbed water was calculated by oven drying an air-dried, sieved (No. 10) soil for 24 hours at 110°C. Percent moisture was a measurement of the % moisture in the soil as it was brought in directly from the field. This was measured using the same technique used for adsorbed water, only the soil was not air dried or sieved before oven drying. This measurement was necessary to calculate the dry weight of the wet soil used in the soil microcosms and in biological tests.

The loss on ignition test was used to measure % organic matter in soil samples. Sieved, oven dried soils (110°C for 24 hours) were baked in a muffle furnace for 24 hours at 430°C. The % organic matter was calculated as the mass of soil lost between baking at 430°C and 110°C divided by the initial weight of soil. In the use of the loss-on-ignition test for measuring soil organic matter, Davies (1974) found that measurements were not effected by the presence of calcium carbonate ($CaCO_3$) in soils,

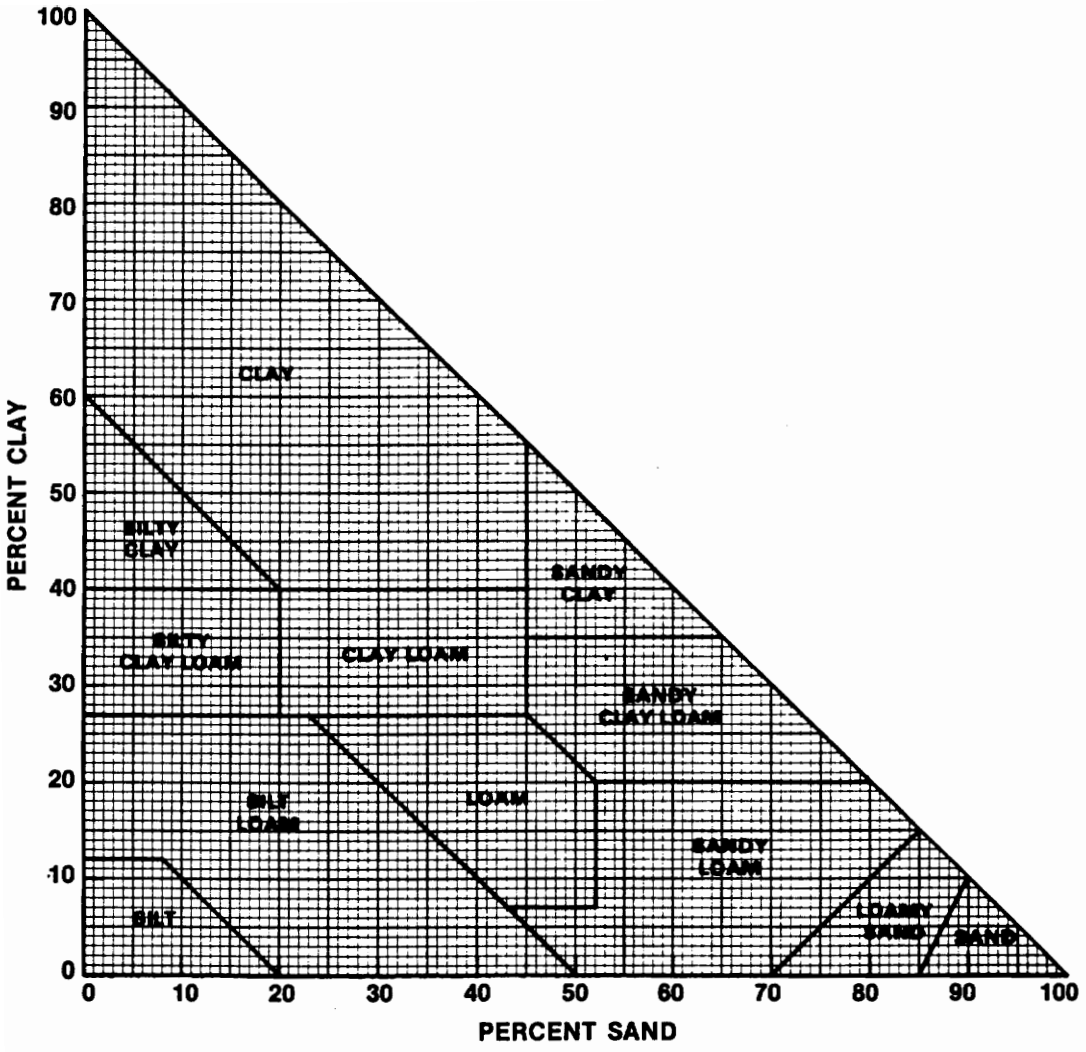

Figure 5. Textural triangle for soil classifications based on USDA guidelines*
* After Klute, 1982

although high levels of gibbsite can distort measurements due to significant water losses at 300°C. Ball (1963) reported that at 375°C the loss-on-ignition method was reliable for predicting soil organic matter in noncalcareous soils.

The loss-on-ignition method may result in values higher than expected for organic matter in subsurface soils. This test can often include some of the water of constitution (Page *et al.*, 1982), even though it is oven baked prior to the test to remove most of this water. Because these soils will be compared relative to each other, this error should not introduce substantial problems. If comparisons are to be made with soils outside of this study, corrections may be needed for the effect of adsorbed water on this calculation.

3.4.2 Chemical soil properties

Soils were characterized for several elements, soluble salts, nitrate-nitrogen and cation exchange capacity (CEC). The elemental, soluble salt and nitrate-nitrogen analyses were conducted by the Soil Science laboratory at Virginia Tech. All soils were air dried and passed through a No. 10 sieve before all chemical analyses.

Chemical analysis for the contaminated soils were not performed due to limitations in the quantity of soil available for analysis as well as analytical problems in testing contaminated soil. The Soil Science Laboratory at Virginia Tech is not equipped to handle contaminated soil samples. If different techniques were used to analyze the contaminated soils, the validity of making comparative analysis between these results and results from uncontaminated soils would be questioned. For these reasons, chemical tests were not conducted for the contaminated soils.

Phosphorus, potassium, calcium, magnesium, zinc, and manganese were measured by acid extraction of the soils with Mehlich No. 1 extracting solution (0.05 N HCl in 0.025 N H_2SO_4). Samples were shaken on a reciprocating shaker for 5 minutes at 180 oscillations per minute and filtered through Whatman No. 1 filter paper. Analysis was performed with a Jarrell Ash ICAP 9000 (Inductively Coupled Argon Plasma Emission Simultaneous Spectrometer) equipped with a modified Technicon Autosampler IV. Results are reported as ppm of element in soil assuming a bulk density of 1.25 g/mL.

Extraction with a 0.02 N solution of $CuSO_4$ was used to measure NO_3-N in soil. Samples were equilibrated with the extractant on a wrist action shaker for 10 minutes then filtered with Whatman No. 1 filter paper. The NO_3-N in the sample was measured with an Orion SA 720 ionalyzer equipped with an Orion Nitrate Specific Ion Electrode. Results are reported as ppm NO_3-N in soil.

Soluble salts were extracted using distilled water and a volume to volume ratio of soil:water of 1:2. The soil solution was quiescently equilibrated for one hour. The electrical conductivity of the

solution was determined and compared to a potassium chloride standard solution. Results were reported in ppm soluble salts in soil.

CEC was measured using techniques from Methods of Soil Analysis, Part 2 for an acid soil (Page *et al.*, 1982). The soil is equilibrated with a barium chloride solution to displace retained cations from the soil matrix. Subsequent displacement of barium from the soil with a concentrated $MgSO_4$ solution and measurement of the retained magnesium results in a measurement of the CEC of the soil.

3.4.3 Biological soil properties

Bacterial counts were made in order to assess the intrinsic biological activity of the soil. Total heterotrophic bacteria and benzene degraders were enumerated using heterotrophic plate counts (HPC) and the most probable number method (MPN) tubes, respectively. All laboratory equipment was autoclaved at 120°C and 15 psi for 15-20 minutes and laboratory benchtops sprayed with a 70% methanol/water mixture prior to any microbial work.

Soil dilutions were prepared using a modified method from Methods of Soil Analysis, Part 2 (Page *et al.*, 1982) and a method outlined by Kanazwa (1986). Ten grams of soil were combined with 95 mL of sterile distilled water and blended in an automatic blender on low speed for 3 minutes at 1 minute intervals with 30 seconds of cool down between each blending. This represented the 10^{-1} soil dilution. Subsequent dilutions were prepared by combining 0.5 mL of the dilution with 4.5 mL of sterile distilled water and mixing on a vortex mixer.

Methods for enumeration of total heterotrophs using aerobic spread plates followed those outlined in Methods of Soil Analysis, Part 2 (Page *et al.*, 1982). Plate media was prepared using the recipe shown in Table 6. Soil extract was prepared by combining 50 g of soil with 500 mL of water and autoclaving for 20 minutes at 120°C and 15 psi. The extract was then filtered through 2 μm filter paper. All other ingredients for the media were then combined, mixed and autoclaved. The final pH of the solution was adjusted to 7.4 ± 0.2. The agar was poured into sterile glass petri dishes when the temperature of the agar solution reached 48°C. Approximately 15 mL of agar solution was placed in each dish. The plates were then cured for two days at room temperature. After curing, 0.1 mL of each dilution was added to each petri dish to represent a 10 fold dilution. (i.e. 0.1 mL of 10^{-3} on a spread plate would equal a 10^{-4} soil dilution). Dilutions were distributed on the spread plate using a glass spreader stick, which was flame-alcohol sterilized between each plate. Plates were prepared in increasing concentration of soil dilution and in duplicate for each dilution series. Plates were inverted and incubated at 35°C for 5 days before colony forming unit counts were made.

Table 6. Agar media for spread plates and MPN tubes

Amount*	Compound
1.0 g	peptone
1.0 g	yeast extract
0.4 g	K_2HPO_4
0.5 g	$(NH_4)_2HPO_4$
0.05 g	$MgSO_4 \bullet 7H_2O$
0.01 g	$FeCl_3$
0.1 g	$CaCl_2$
250 mL	soil extract (sterile)
15.0 g	agar
750 mL	distilled water
as needed to maintain pH	1 N NaOH

* Per liter of solution

MPN tubes, used for the enumeration of benzene degraders, were prepared using the same solution used for the spread plates, with the omission of the bactopeptone, agar and yeast extract. Benzene, at a concentration of 10 ppm, was the sole carbon source in the MPN tubes. Soil dilutions ranged from 10^{-2} to 10^{-5}. MPN tubes were prepared by combining 0.5 mL of the soil dilution with 4.5 mL of the benzene mineral media in 22 mL headspace vials sealed with a Teflon lined crimp cap. After incubation at 35°C for 3 weeks, benzene concentrations were measured by GC headspace analysis, with a measurement of less than 1 mg/L benzene indicating a positive reading.

3.5 Benzene biodegradation studies

Preliminary benzene biodegradation studies were conducted on the first two soils collected, a Groseclose loam and clay. These studies consisted of:

1. varying initial benzene concentrations at 10, 50, 100 and 300 mg/L
2. adding nutrients in the form of ammonium phosphate, ammonium sulfate and ammonium nitrate
3. adding the salts Ca^{++}, Mg^{++} and K^{+} to an initial benzene concentration of 50 mg/L
4. varying oxygen concentrations in microcosms by varying the headspace concentrations of oxygen in the microcosms at 1%, 21% (air) and 100% or amending with H_2O_2

The purpose of these experiments was to determine the final soil matrix to use on all soils tested.

Oxygen headspace variation was accomplished by purging the microcosms with the appropriate oxygen concentration prior to setup. Using compressed gas at either 1 or 100% oxygen, an air bag was then filled with the appropriate gas mixture and equilibrated for one hour before microcosm preparation. Another oxygen test conducted was with the addition of H_2O_2. Hydrogen peroxide addition consisted of adding approximately 2 uL of hydrogen peroxide through the septum top with a 5 uL syringe after benzene degradation had begun. In order to account for volatilization through the punctured septum top, some additional microcosms were punctured but no hydrogen peroxide was added. After 1-2 days, the microcosms were sampled to test for the effects of the hydrogen peroxide amendment.

Based on the results of these tests (discussed later), the final soil test matrix for all uncontaminated soils was established and consisted of:

- varying initial benzene concentrations at 1, 10 and 50 mg/L
- adding nutrients in the form of 200 mg/L $(NH_4)_2HPO_4$-NH_4 and 25 mg/L K_2HPO_4-K at 1, 10 and 50 mg/L initial benzene concentration

For some selected uncontaminated soils, benzene degraders and heterotrophic plate counts were done throughout the degradation study. For the contaminated soils, biodegradation studies were conducted at 10 and 50 mg/L with and without nutrient addition.

3.6 Microbial growth tracking over degradation

Microbial growth was tracked for certain soils at selected points throughout the degradation study. As benzene degradation began, microcosms were sacrificed for microbial analysis. 0.5 mL of the soil/water/benzene solution was removed from the microcosm and was combined with 4.5 mL of sterile distilled water to form the 10^{-1} soil dilution. Subsequent dilutions, spread plates and MPN tubes were prepared in the same manner as previously described.

3.7 Correlation of properties and statistical analysis

The primary objective of this research was to attempt to draw statistically significant correlations between measurable soil properties and biodegradation rates of benzene within a soil system. Correlations were performed on soil physical, chemical and biological properties as they related to measurable kinetic parameters. In this case, these parameters were a lag phase, linear biodegradation rate and time to degrade benzene to less than 5 ppb. Multiple linear regression was done using Microsoft Excel© for three soil properties simultaneously as they related to each of the dependent kinetic variables. Statistical verification of the empirical regression models was done using an Analysis of Variance F test.

Due to the large number of tests and many soil properties measured, there were numerous possible combinations of correlations that could be made in this study. In order to identify any statistical correlations between kinetic parameters and soil properties, a screening process was first needed to determine which factors on their own had any significant correlation with lag phase, degradation rate, or final time to degrade to less than 5 ppb. A "correlation coefficient" was determined for almost all the physical, chemical and biological properties for their correlation with the lag phase, degradation rate and final time to degrade to less than 5 ppb. The value for the correlation coefficient ranges from -1 to +1, with a value close to -1 or +1 indicating a high negative correlation or a high positive correlation, respectively. A correlation of zero indicates no relationship between the two parameters.

Correlation coefficients only indicated relationships between a single soil characteristic and degradation data. In order to correlate more than one soil parameter simultaneously, multiple linear regression analysis was used. Multiple linear regression analysis is, as the name implies, a way of

predicting a dependent variable (in this case, the lag phase, degradation rate or time to degrade to less than 5 ppb) through the linear relationship of several independent variables. The equation is of the form

$$y=B_o+B_1x_1+B_2x_2+B_3x_3+...+B_nx_n$$

where B_o represents a y-intercept, if applicable, x_n represents the independent variable and B_n represents the corresponding coefficient. The magnitude of the coefficients, m_n, is not significant due the different units used for each independent variable (Mendenhall and Sincich, 1988).

Regression analysis was done on uncontaminated soils for both 1 and 10 ppm initial benzene concentrations with and without nutrient addition. Contaminated soil regressions were not performed due to the limited number of soils analyzed and the fact that chemical analyses were not done for these soils. However, regression analysis was done for the 10 ppm concentration for contaminated and uncontaminated soils combined, with and without nutrient addition for physical and biological soil properties.

Statistical significance of the multiple linear regressions was determined by the F test. The F test is an analysis of variance test used to see if at least one of the variable coefficients, B_n, differs from zero (Mendenhall and Sincich, 1988). It allows the entire model, encompassing all B parameters, to be analyzed for their statistical significance simultaneously. Although t tests were also conducted on individual parameters, the significance of individual parameters alone is not as relevant as the overall significance of the parameters combined. However, the t and p values give an indication of the level of significance of each individual parameter within the model. The lower the p value for the variable, the more significant role the parameter plays in the model.

IV. RESULTS & DISCUSSION

4.1 Soil characterization tests

The results for the soil physical, chemical and biological characterization tests are presented in Tables 7-9. Results for most of these soil parameters covered a broad range of values. The pH of the soils varied from 4.3 to 7.7, indicating strongly acidic to fairly alkaline soil conditions. Soil types also ranged from clay soils (78.6 % clay) to coarse sand soils (98.5% sand). However, some gaps exist in the range of % clay in the soils, with the highest value being 78.6% and the next highest value of 23.9%. Other physical and chemical properties such as % organic matter, % moisture, CEC, and other elements varied over a wide range.

HPC's and benzene degrader counts were generally higher for the contaminated soils than uncontaminated soils. While values for HPC's were fairly similar for all uncontaminated soils (within one order of magnitude), values for benzene degrader counts varied over 3 orders of magnitude (2.0×10^1 to 6.9×10^3 cells/g dry soil). Due to the large range of values for all soil parameters measured, any correlations between these properties and degradation rates should be discernible from the research conducted here.

4.2 Preliminary biodegradation studies

In order to design a final test matrix for all the soil samples that would maximize degradation rates through nutrient, salt and oxygen supplements, preliminary tests were conducted on soils from the Virginia Tech dairy farm and the Whithorne Research Center. Experiments were conducted using salt

Table 7. Soil physical properties

Soil name	Soil type	pH	PSA			% organic matter	% adsorbed water	% moisture (for biology)
			% sand	% silt	% clay			
Bojac	sand	5.3	90.2	7.3	2.5	1.11	0.25	8.7
Catpoint	sand	4.5	98.5	1.1	0.4	0.22	0.1	1.8
Leedsville	silt loam	4.3	26.5	55.3	18.2	3.44	3.29	22
Purcellville	sandy loam	4.5	58.2	30.9	10.9	2.73	2.72	14
G. loam 1	loam	6.9	42.7	33.4	23.9	---	10	23
G. loam 2	loam	7.7	33.9	43.4	22.7	1.87	2.3	29
G. clay 1	clay	5.8	23.4	32.7	44	4.78	12	25
G. clay 2	clay	6.7	3.1	18.3	78.6	6	5.3	34
Wheeling	sandy loam	6.3	62.7	22.8	14.5	3.12	1.5	18
CS1	sand	5.8	89.3	10.6	0.1	1.58	--	8.1
CS2	sandy loam	6.6	70.1	23.4	6.4	3.77	--	19.29
CS3	sandy loam	7.6	54.1	38.1	7.9	2.98	--	37.32

Table 8. Soil chemical properties

Soil name	CEC	NO3-N	P	K	Ca	Mg	Zn	Mn	Fe	Al
	(meq/100 g)	(ppm)								
Bojac	0.8	5	3	11	216	25	0.6	2.5	10	36.8
Catpoint	0.2	3	8	3	48	8	0.5	0.5	9.4	144
Leedsville	4	3	0	25	84	32	0.7	0.2	5.8	155
Purcellville	6	23	1	34	372	108	0.6	0.2	5.7	163
G. loam 1	10	15	-	31	1040	317	1.1	13	19.6	116
G. loam 2	--	10	1	40	3560	967	0.1	11.3	0.6	0
G. clay 1	13	--	1	63	564	362	0.6	3	8.4	70.8
G. clay 2	--	10	3	42	1200	548	0.7	16.1	9.6	99
Wheeling	7	3	5	23	624	175	1	2	23.3	120

Table 9. Soil biological properties

Soil name	HPC (CFU/g dry soil)	benzene degraders (cells/g dry soil)
Bojac	9.0 x 10^4	3.6 x 10^2
Catpoint	6.3 x 10^4	<20
Leedsville	1.5 x 10^4	24
Purcellville	2.4 x 10^4	<20
G. loam 1	--	--
G. loam 2	6.7 x 10^4	<20
G. clay 1	--	6.9 x 10^3
G. clay 2	2.0 x 10^4	1.2 x 10^2
Wheeling	1.9 x 10^4	8.2 x 10^2
CS1	1.0 x 10^7	>2.6 x 10^5
CS2	6.3 x 10^4	9.4 x 10^3
CS3	4.1 x 10^5	4.5 x 10^3

addition, nutrient addition and oxygen concentration variation as outlined in Methods and Materials (Section 3.5).

Results for the first experiment involving nutrient addition using different ammonium compounds are shown in Figure 6. It is evident that the addition of ammonium phosphate had the most significant impact on acclimation period and degradation rate over the use of ammonium sulfate and ammonium nitrate. This is not surprising as this compound contains both phosphorus and nitrogen, not just nitrogen. These two nutrients are often cited as limiting agents in the biodegradation of organic contaminants.

As shown in Figures 7-8, addition of Ca^{2+} or Mg^{2+} seemed to have no effect or be slightly inhibitory on the benzene degradation rate as compared to the unamended (no salt addition) microcosms for both the loam and the clay soil. It was difficult to determine if potassium had any favorable impact on the degradation rate because of the variation in the initial benzene concentration for the microcosms containing 50 mg/L K^+. To further investigate the impact of potassium, a second experiment was conducted using 50 and 25 mg/L K_2SO_4-K in a Wheeling sandy loam (Figure 9). These results suggest that potassium addition at 25 mg/L K_2SO_4-K may increase the degradation rate over unamended microcosms.

The mass of oxygen present in the headspace of the soil microcosms was calculated to be adequate for complete mineralization of over 50 mg/L of benzene. However, as the availability of this depends on the transfer of oxygen between the air, water and soil matrix, experiments were conducted with variations in the headspace oxygen concentration to observe any effects on the benzene degradation rate. The results for varying the initial headspace concentration of oxygen are shown in Figure 10. It appears from these results that the degradation rate was approximately the same for each concentration of oxygen in the microcosm headspace, although tailing occurred at different levels in each experiment.

Another experiment on oxygen availability was conducted using hydrogen peroxide as an oxygen source (Figures 11-12). Hydrogen peroxide addition was done after degradation had begun or at a point where tailing had started, suggesting oxygen may be a limiting factor. In both studies for two different initial concentrations, the effect of hydrogen peroxide addition was not discernible from unamended or punctured microcosms (punctured microcosms were those microcosms punctured with a needle through the Teflon liner but not injected with hydrogen peroxide). These results suggested that oxygen was not limiting in the soil microcosms.

Based on the results of these initial experiments, the final test matrix to be used on all uncontaminated soils was designed incorporating initial benzene concentrations of 1, 10 and 50 mg/L, with and without nutrient addition in the form of ammonium and potassium phosphate. Initial concentrations were selected based more on a review of literature (Salanitro, 1993) and concentrations

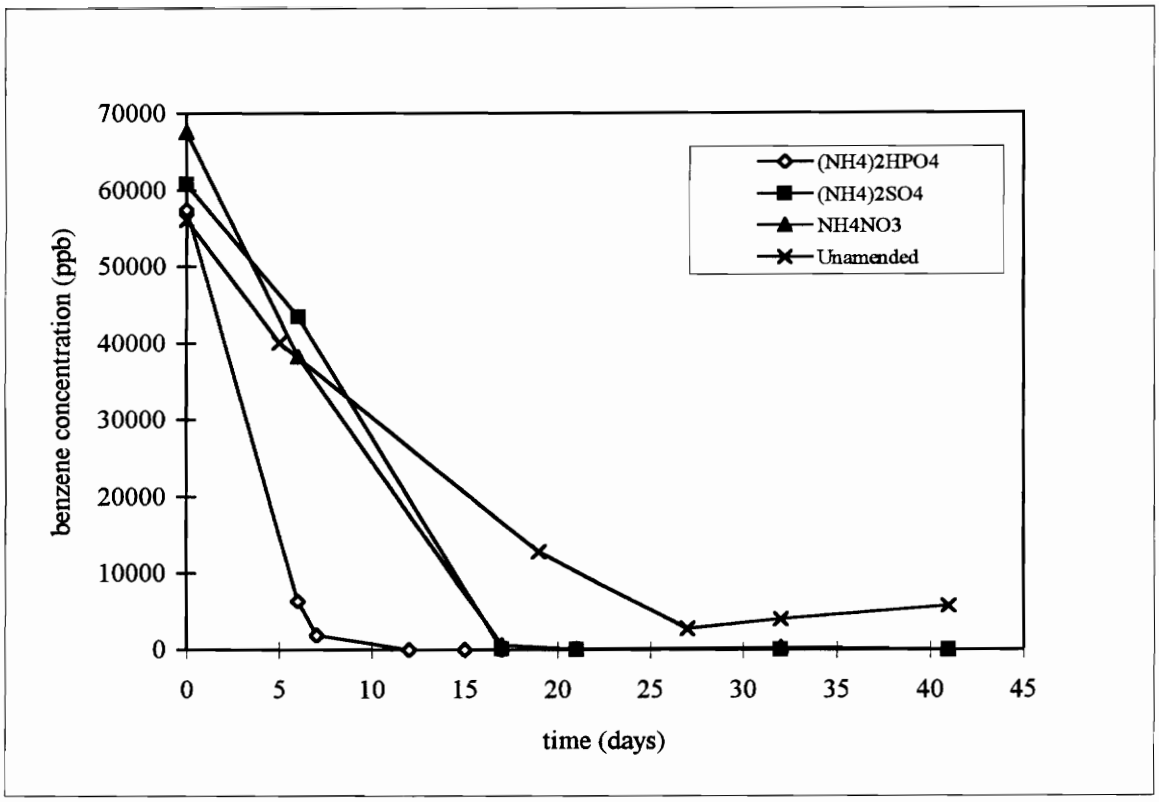

Figure 6. Effect of various ammonium compounds on benzene biodegradation at initial benzene concentration of 50 mg/L in Groseclose loam.

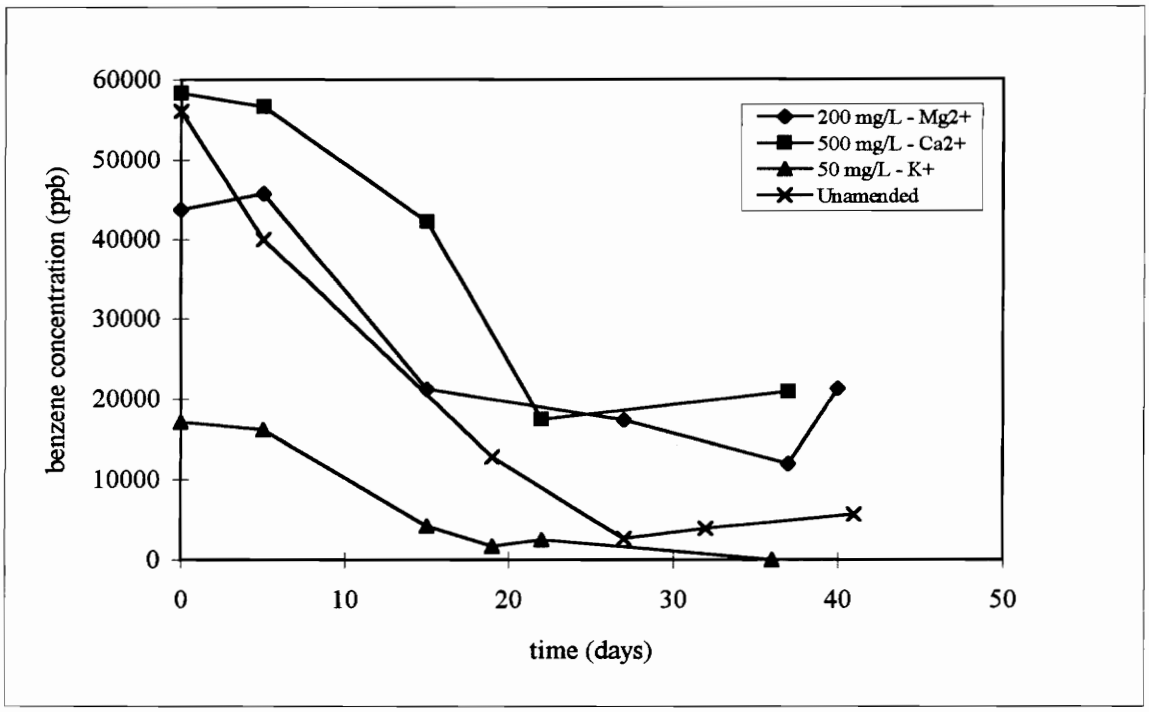

Figure 7. Effect of salt addition on benzene degradation in Groseclose loam at 50 mg/L initial benzene concentration

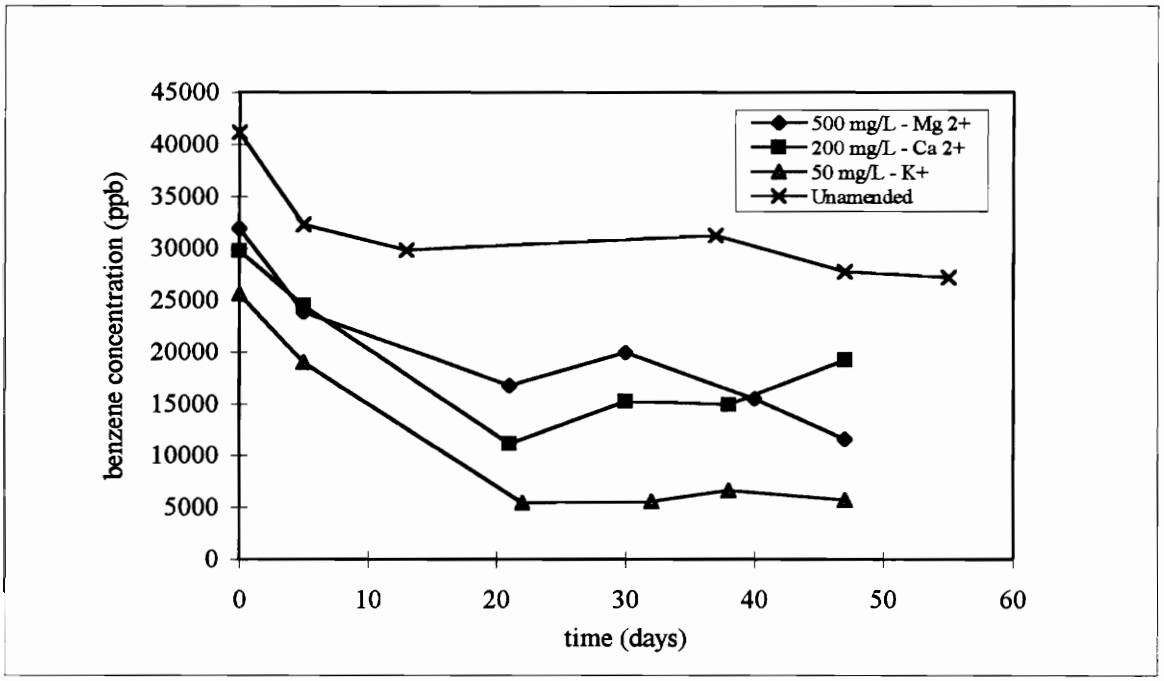

Figure 8. Effect of salt addition on benzene degradation rate in Groseclose clay at 50 mg/L initial benzene concentration

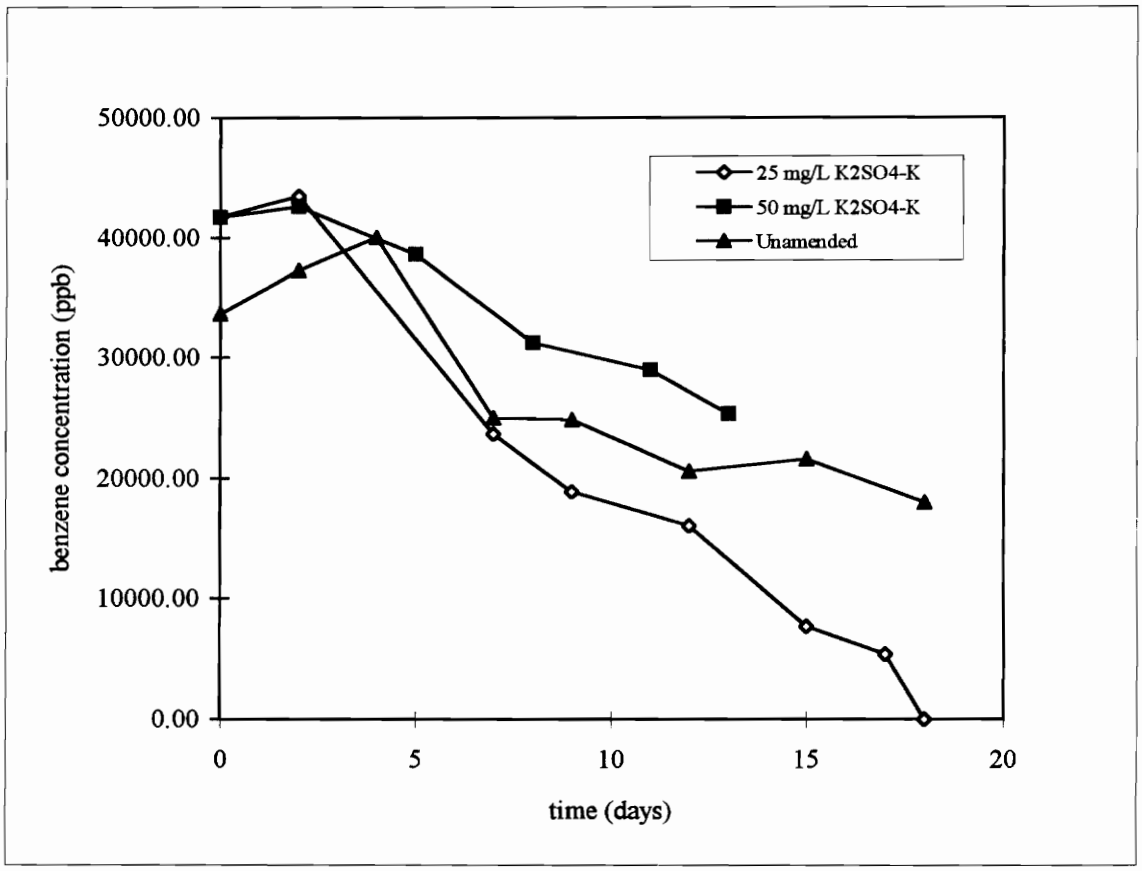

Figure 9. Effect of K_2SO_4 addition at 25 and 50 mg/L at initial benzene concentration of 50 mg/L in Wheeling sandy loam

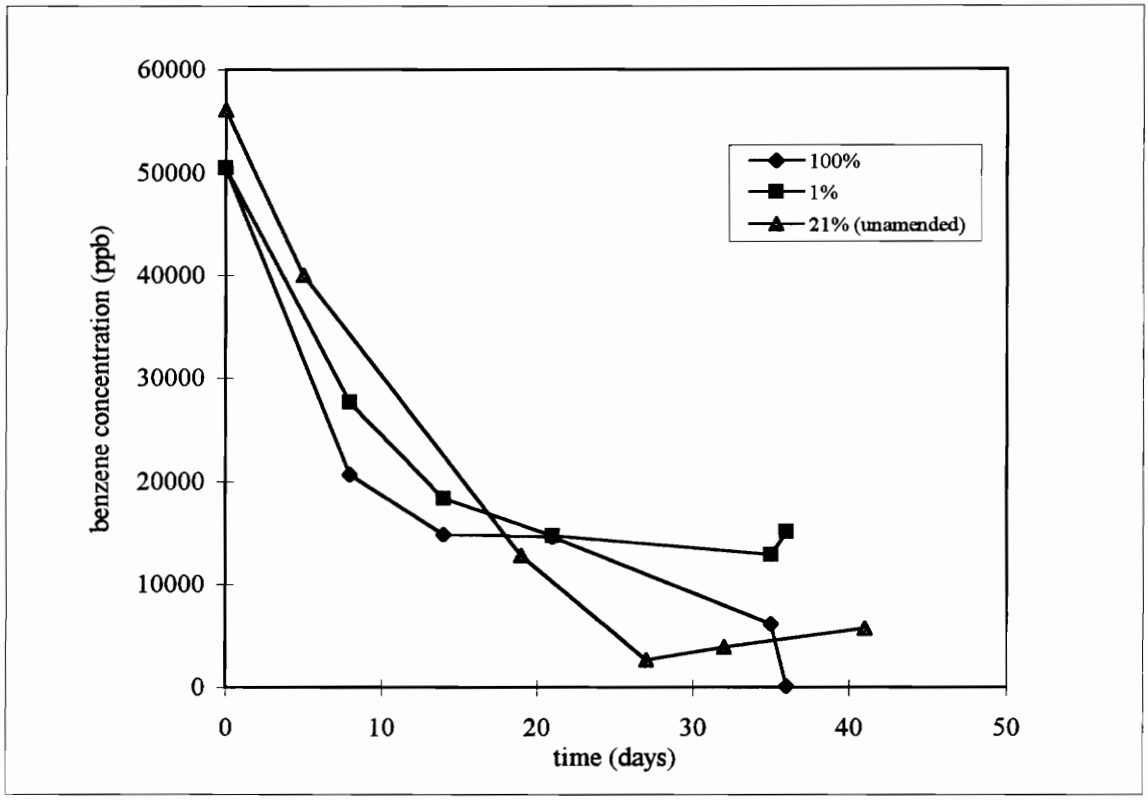

Figure 10. Effect of % oxygen in headspace on benzene degradation in Groseclose loam at 50 mg/L initial benzene concentration

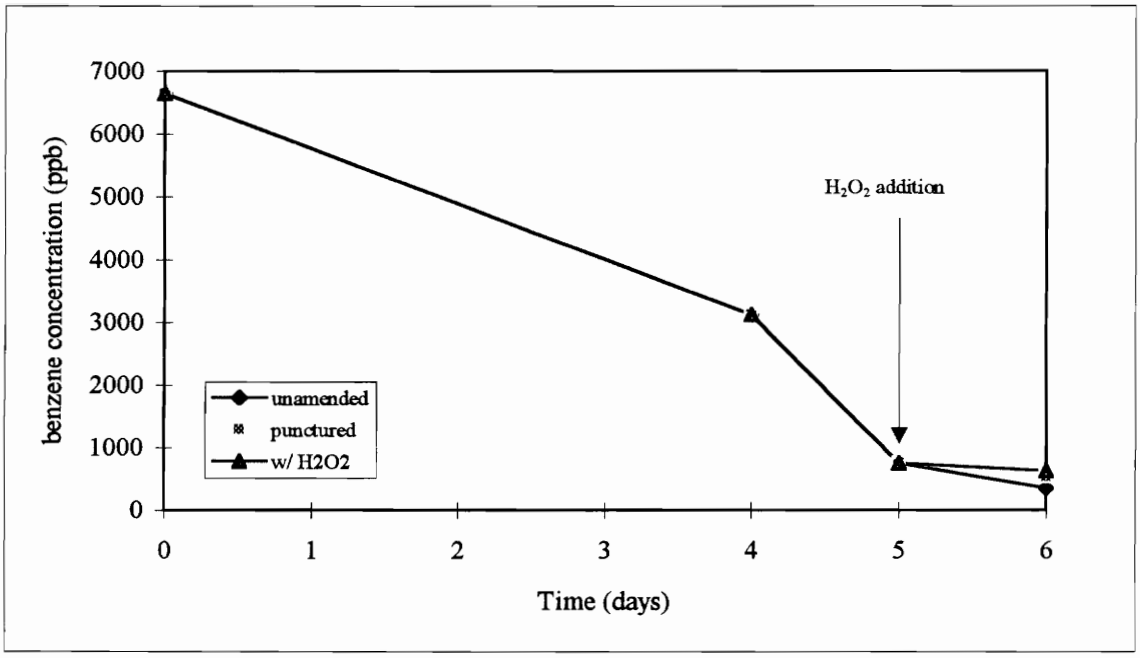

Figure 11. Effect of H_2O_2 addition in Wheeling sandy loam at initial benzene concentration of 10 mg/L.

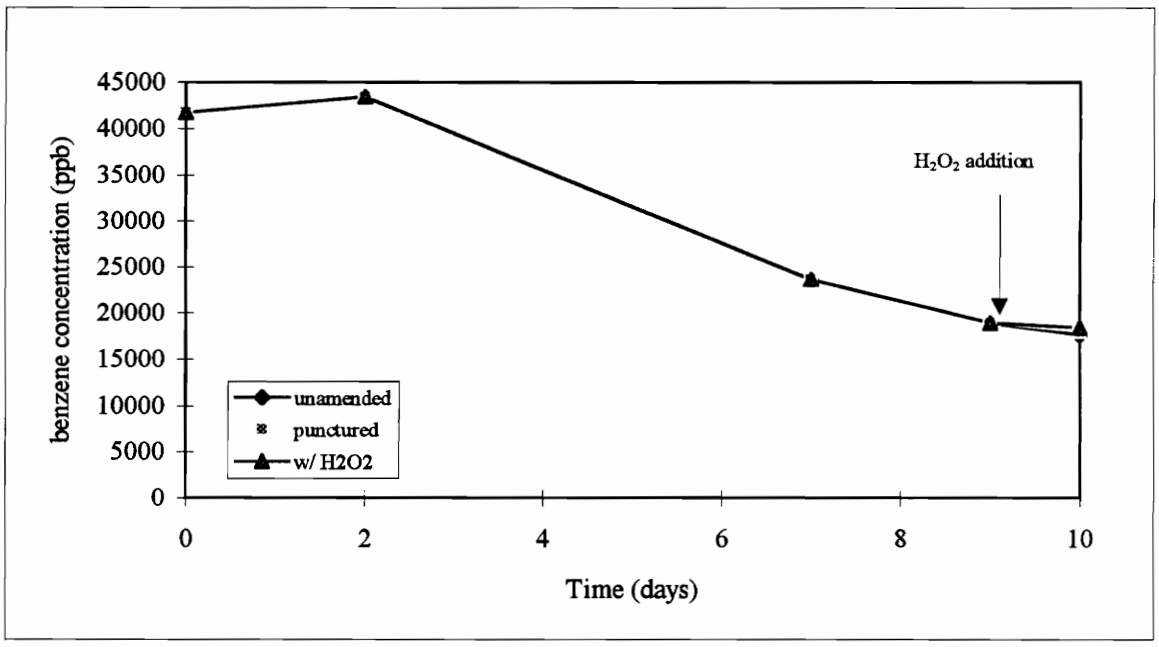

Figure 12. Effect of H_2O_2 addition in Wheeling sandy loam at initial benzene concentration of 50 mg/L.

indicative of actual field conditions. Because preliminary studies indicated oxygen was not limiting in the soil microcosms, no oxygen addition was used in the final test matrix.

All results were compared to autoclaved controls to verify that decreases in benzene concentration were due to biological degradation. Figures 13-14 display volatilization and abiotic losses in sterile soil microcosms for each soil sampled at two different initial benzene concentrations. It should be noted that the time and concentration scale are different on each graph, with the 1 ppm sampled for only 20 days, while the 50 ppm controls were sampled for 40 days. Each of the control studies were conducted for the same period of time as the active microcosms studies and prepared in the same manner as the active microcosms with the substitution of sterilized soil for fresh soil.

Although losses in individual microcosms were up to a maximum of 25% over 35 days, average losses from each different soil type were approximately the same. For the purposes of this study, where only relative comparisons are made within the study for correlations, volatilization and abiotic losses could be neglected. If comparisons of degradation rates with outside studies are to be made, corrections may be needed, particularly at higher concentrations, for losses shown in the control microcosms.

4.3 Determination of kinetic parameters

In order to compare benzene biodegradation under each test condition, a method for analyzing and interpreting the results was needed. In soil biodegradation studies, several methods are used to interpret degradation rates and other soil biodegradation data. Although several models could be used for this data, there was a limited number of data points available from this study to assess which type of model would fit the data best. It appears the degradation, although it varies from one soil to another and between concentrations, may be following a logistic type curve (See Section 2.5). The area around the inflection point of a logistic curve can be adequately approximated by a straight line (Schmidt et al., 1985). Using this reasoning, biodegradation curves were separated into three different sections as shown in Figure 15. These sections included a "lag phase" or acclimation period before degradation began, a linear degradation rate, and a final time at which the concentration of benzene dropped below 5 ppb, the drinking water standard. Using these three parameters, the shape of the curve could be adequately described without the use of a higher order or nonlinear regression model. It is not to say that these higher models are not applicable and useful for soil biodegradation analysis, but for simplicity and practicality and because of the limited number of data points, this "three parameter" method was chosen instead.

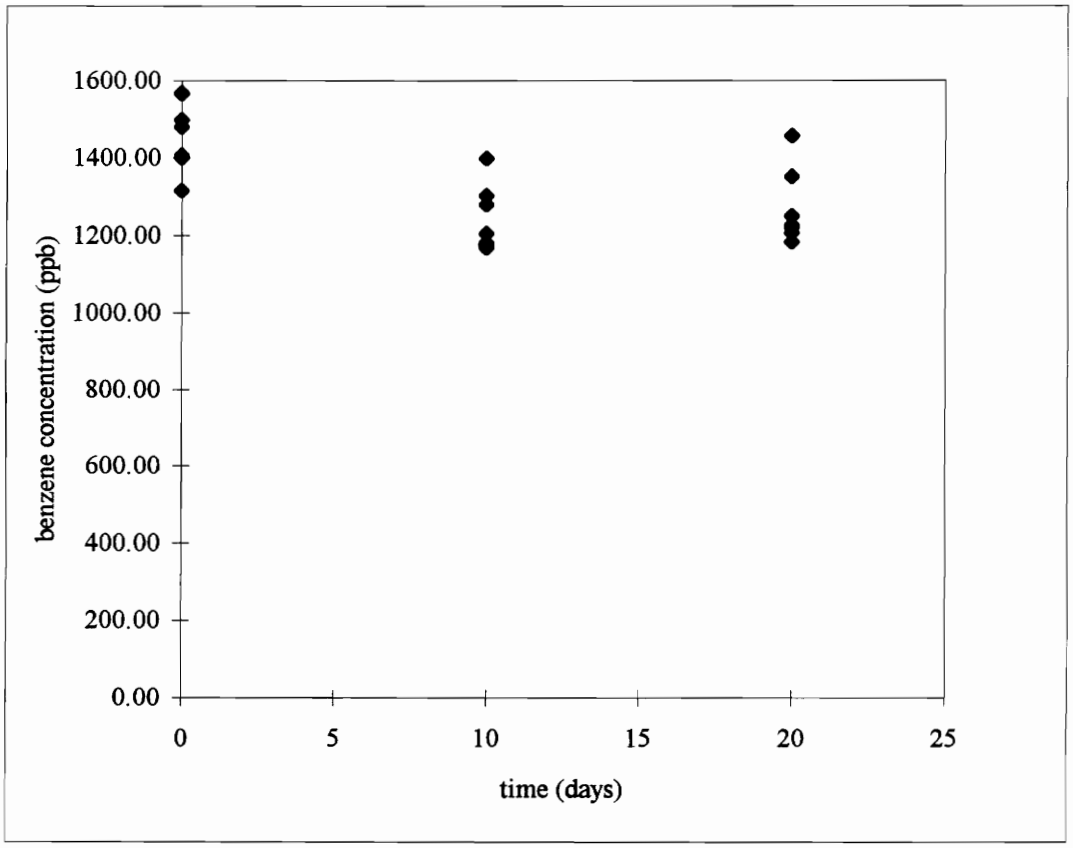

Figure 13. Losses from sterile soil microcosms for all soils at initial benzene concentration of 1 ppm.

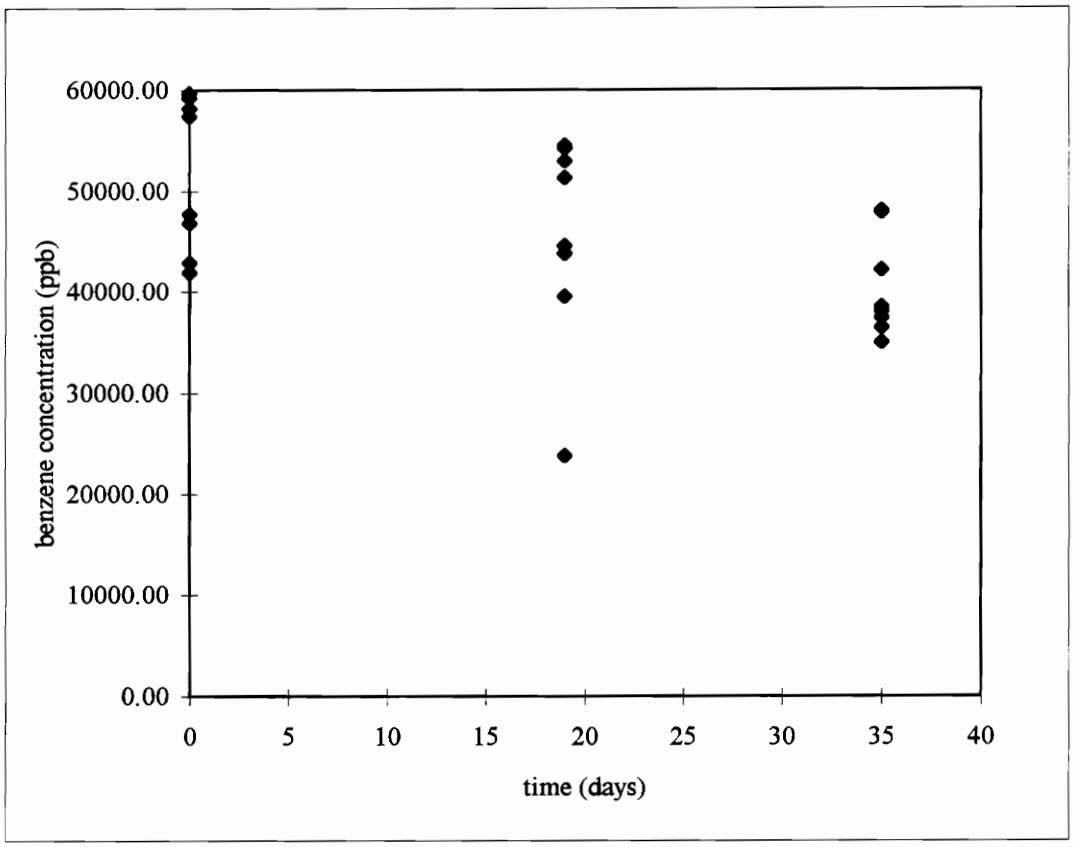

Figure 14. Losses from sterile soil microcosms for all soils at initial benzene concentration of 50 ppm.

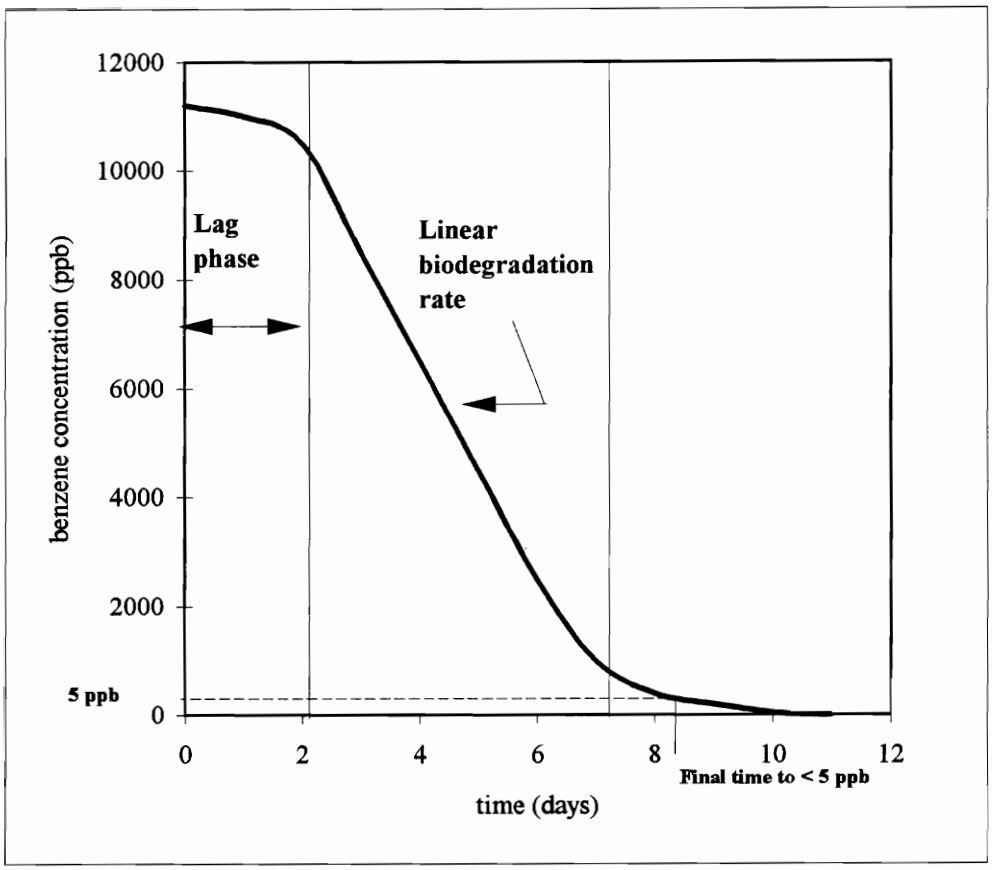

Figure 15. Determination of kinetic parameters from soil microcosm biodegradation data.

The "lag phase" or acclimation period, seen in almost all the soil microcosms results, was calculated as the time to degrade approximately 20% of the initial benzene concentration, unless the start of degradation could be easily identified. This lag phase or acclimation period is often associated with the growth of substrate specific organisms that were not initially present in the soil or the enzymatic transformation of already existent organisms.

A pseudo zero-order or linear degradation rate was chosen for several reasons. All the data was plotted on a linear and logarithmic scale to determine the best fit between a zero or first order decay rate. In general, neither zero or first order adequately described the shape of all the degradation curves but each set of data could fit a pseudo zero order rate over some period of time. Although microbial growth kinetics would not support or suggest zero order degradation, within the soil biota processes other than microbial growth, such as diffusional barriers and nutrient limitations, may limit biodegradation and these processes may follow linear, not exponential, kinetics. Zero order rate constants are often used to describe hydrocarbon degradation in soils in laboratory and field studies under both aerobic and anaerobic conditions (Edwards and Grbic-Galic, 1992; Holm et al., 1992; Hutchins, 1991). Zero order rates are also commonly associated with concentrations above the Ks value while first order rates pertain to degradation at values below the Km or Ks value (Alexander and Skow, 1989). Buscheck (1993) citing Tucker and Zavala (1992) reported the successful use of zero order rates at hydrocarbon concentrations as low as 1 ppm and first order rates at values below this level. Although first order decay rates would give a more conservative estimate of degradation, the absolute value of the rate is not necessarily as significant as the use of the same methods for all soils tested, in order to allow relative comparisons within soils.

These zero order rates were obtained by taking the slope of the linear portion of the curve, as indicated conceptually in Figure 15. These rates were calculated per microcosm, which contained 5 grams of soil on a wet weight basis. The rates were normalized for the dry weight of soil in each microcosm, but on comparison, no significant difference in the relative degradation rate observed in each microcosm was observed with normalization. For analysis purpose in the correlation studies, degradation rates per microcosm were used.

The final time to degrade to 5 ppb was included to account for the tailing effect often exhibited in soil biodegradation studies, particularly at high contaminant concentrations. This tailing, attributed to numerous mechanisms within the soil such as adsorption, nutrient limitations, reduced microbiological activity and soil heterogeneity, can often cause substantial problems in practice when an asymptotic level is reached just slightly in excess of the remediation standard. For these important reasons, a final degradation time was also included in the kinetic parameters used to describe the degradation data.

4.4 Initial benzene concentration variation and nutrient addition

4.4.1 Uncontaminated soils

The degradation pattern at several different initial benzene concentrations (without nutrient addition) in soil microcosms are shown in Figures 16-17. The y-axis is a logarithmic scale on both of these figure in order to facilitate the comparison of all three initial concentrations. In Figure 16, results for the two sandy soils, Bojac and Catpoint, are shown. At 1 and 10 ppm, the degradation rate increases with increasing concentrations for both soils, while at 50 ppm, the soils do not appreciably degrade although it is difficult to judge by this graph because of the logarithmic scale. In Figure 17, the same trend is noted for the Groseclose clay and loam. The Groseclose loam, which had the highest benzene attenuation rate overall for all the soils investigated, displayed a significant increase in the degradation rate with an increase in initial benzene concentration. The Groseclose clay also exhibited these characteristics but benzene was degraded at a slower rate than in the Groseclose loam at all three initial benzene concentrations. The effect of initial concentration on degradation rates, with and without nutrients, will be discussed in more detail in the next section.

In Figures 18 - 23, the degradation of benzene in all uncontaminated soil microcosms is shown for 1, 10 and 50 ppm with and without nutrients. Raw data used to construct these graphs are shown in Appendix B. These figures are more useful for analysis as they are on a linear scale instead of a log scale and small changes can be seen. Note that the time scale is only 20 days for the 1 ppm study while it is 40 and 45 days for the 10 and 50 ppm, respectively.

For all the soils investigated, nutrient addition caused a statistically significant increase in the biodegradation rate at all concentrations, a significant decrease in the time to degrade to 5 ppb benzene at all concentrations and a significant decrease in the lag phase at 50 ppm. Statistical significance was verified through the two sampled paired t-test. These results indicate a nutrient deficiency in these soils at NO_3-N levels as high as 23 ppm, P at 8 ppm and K at 42 ppm. From preliminary studies, phosphorus had the greatest impact on degradation rates. Suggested levels of available phosphorus for field biodegradation of organic chemicals is 20 mg/L (Aggarwal et al., 1985). All the soils from this study fall below this phosphorus level for total phosphorus in soils.

Nutrient addition has been shown to increase petroleum hydrocarbon degradation rates (Dibble and Bartha, 1979; Ward and Brock, 1976), particularly for oil contamination in marine environments (Atlas and Bartha, 1972; Dibble and Bartha, 1976; Pritchard, 1992). However, the effect of nutrient addition on petroleum hydrocarbon degradation in soils is constantly debated. Salanitro (1993) states that there is no data, field or laboratory, that suggests nutrient addition stimulates the degradation of BTEX at

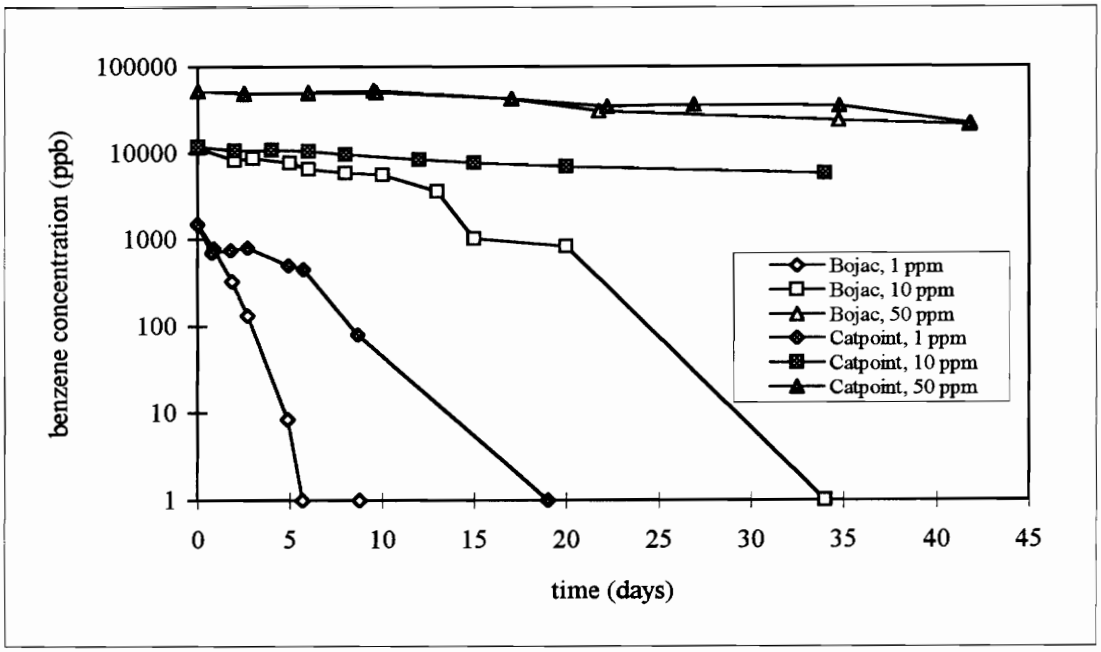

Figure 16. Effect of initial benzene concentration on benzene biodegradation in Bojac and Catpoint soils.

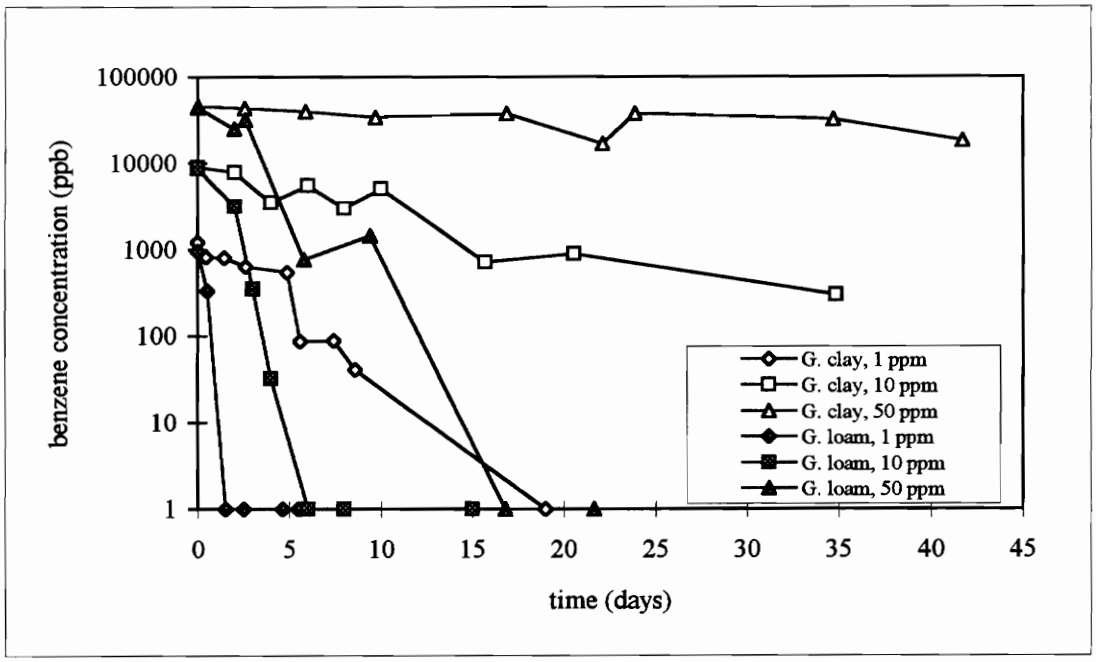

Figure 17. Effect of initial benzene concentration on degradation in Groseclose clay and Groseclose loam soils.

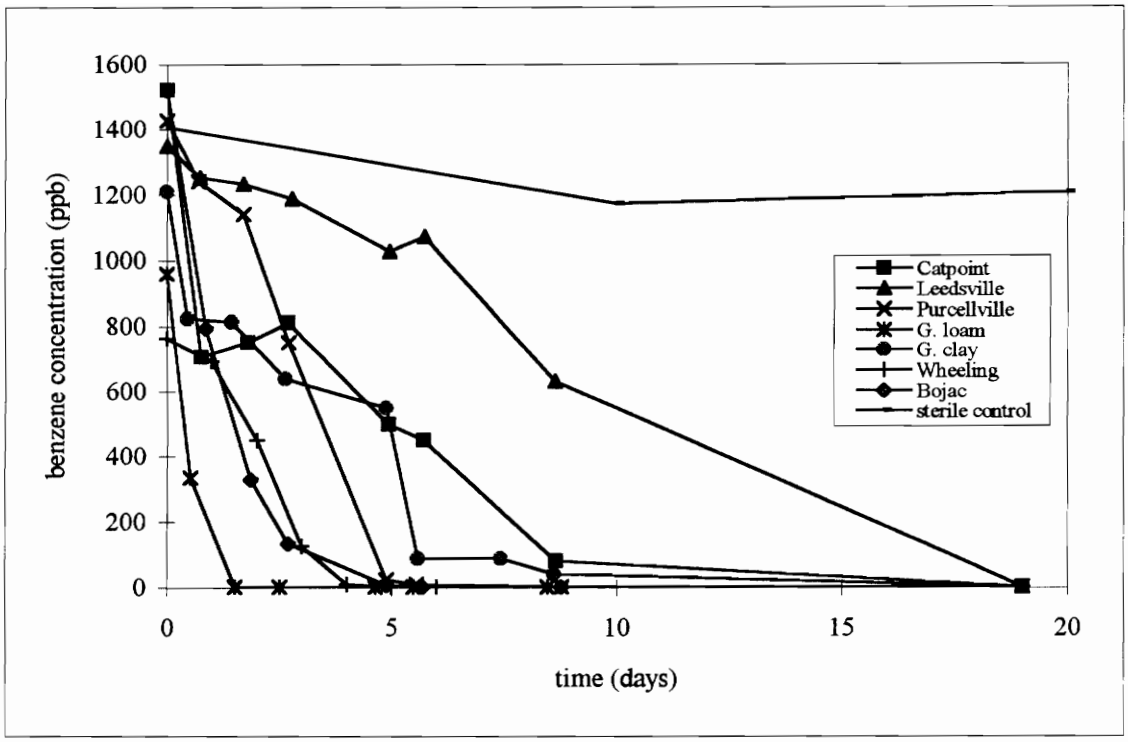

Figure 18. Benzene degradation in all uncontaminated soils at initial benzene concentration of 1 ppm.

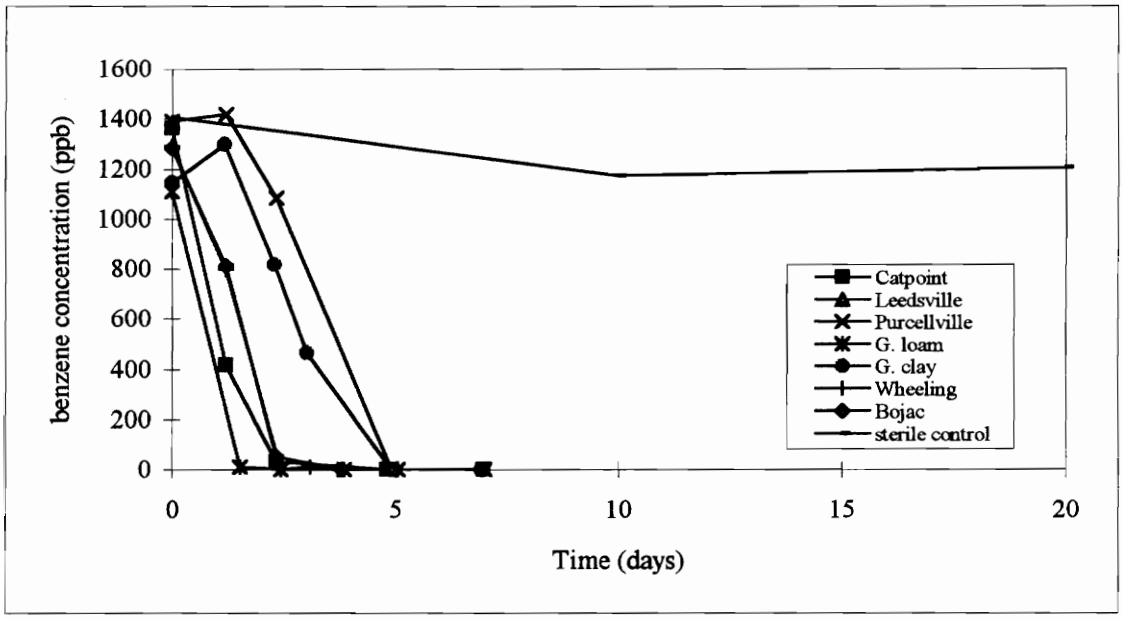

Figure 19. Benzene degradation in all uncontaminated soils amended with 200 mg/L $(NH_4)_2HPO_4$-NH_4 and 25 mg/L K_2HPO_4-K at initial benzene concentration of 1 ppm.

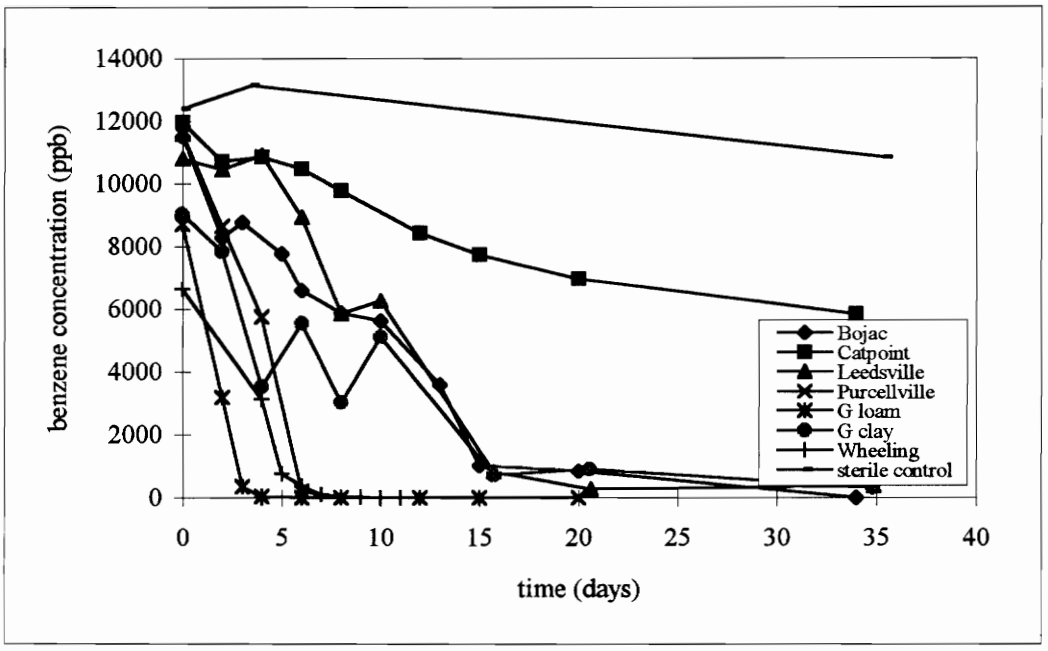

Figure 20. Benzene degradation in all uncontaminated soils at initial benzene concentration of 10 ppm.

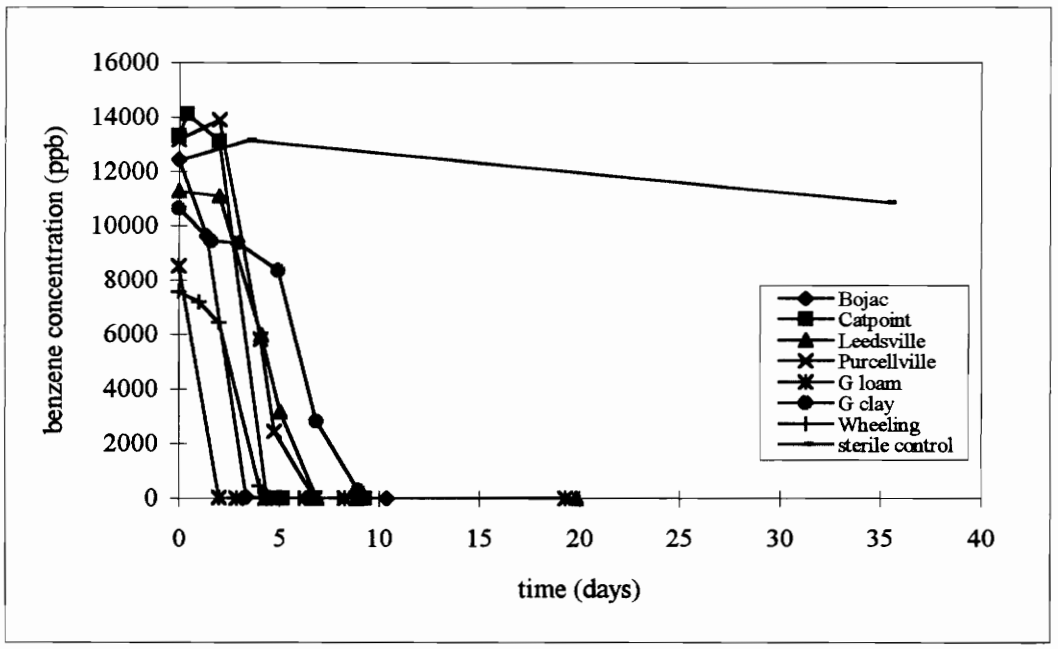

Figure 21. Benzene degradation in all uncontaminated soils amended with 200 mg/L $(NH_4)_2HPO_4$-NH_4 and 25 mg/L K_2HPO_4-K at initial benzene concentration of 10 ppm.

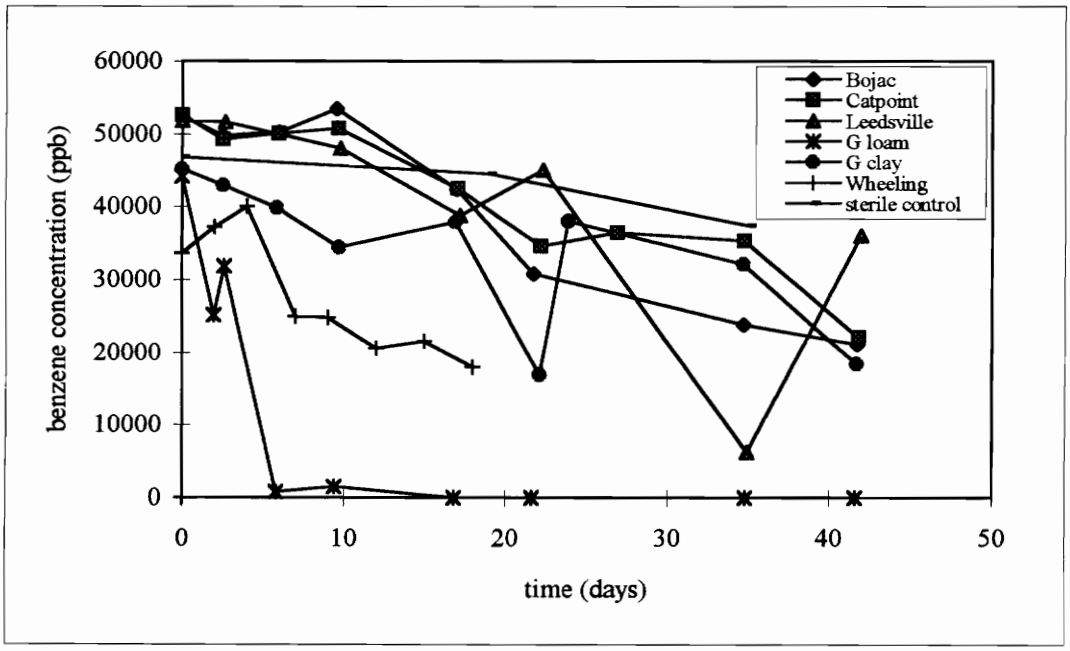

Figure 22. Benzene degradation in all uncontaminated soils at initial benzene concentration of 50 ppm.

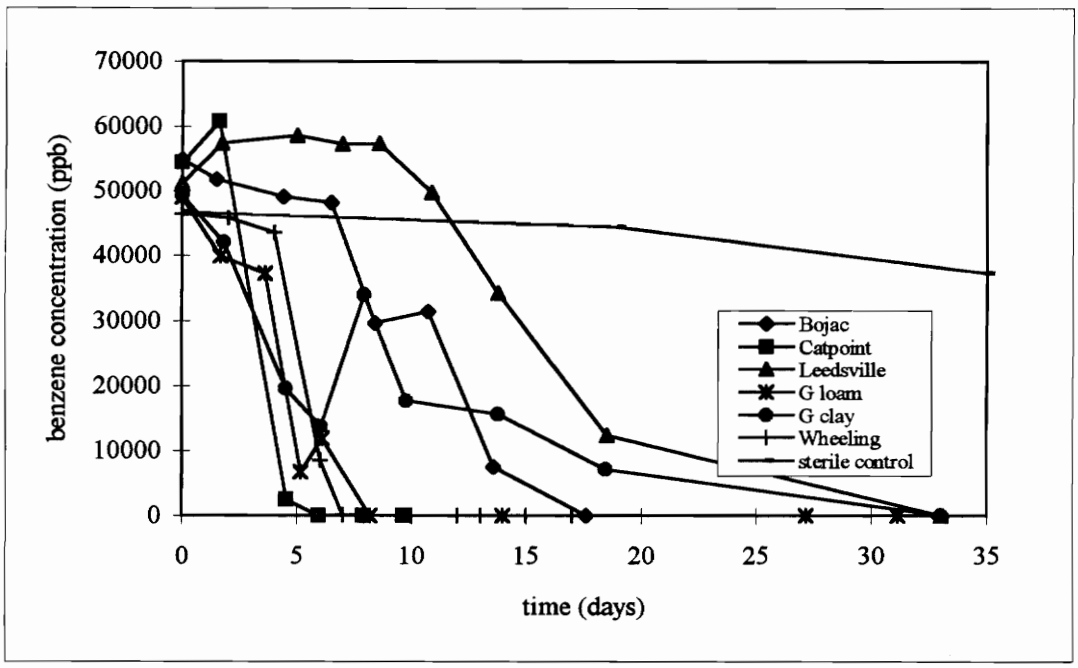

Figure 23. Benzene degradation in all uncontaminated soils amended with 200 mg/L $(NH_4)_2HPO_4$-NH_4 and 25 mg/L K_2HPO_4-K at initial benzene concentration of 50 ppm.

concentrations in the range of < 5000 to 10000 ppb. This contradicts results from this study where nutrients have had a stimulatory effect at concentrations as low as 1 ppm. Other researchers claim that the solubility of inorganic nutrients, such as nitrogen or phosphorus, is so high, compared to those of organic contaminants, that their concentrations seldom limit biodegradation rates (Li et al., 1993).

The validity of laboratory results on nutrient effects on petroleum hydrocarbon biodegradation is often questioned due to the lack of supporting field data. Miller *et al.* (1993) found that nutrient addition increased the degradation rate of petroleum hydrocarbons in laboratory studies, but found no increase in degradation rates with nutrient addition in field studies. Other researchers, (Allen-King et al., 1994), have found that nutrients helped achieve complete mineralization of hydrocarbons, even in field studies. Dupont et al. (1990) found that in laboratory studies, both moisture and nutrient addition stimulated the degradation of BTEX compounds but when applied in the field, only moisture addition had a statistically significant effect on degradation rates over unamended soils.

Reasons for these differences lie mainly in the availability of nutrients once they are applied in the field. Precipitation or ion exchange can immobilize nutrients, making them unavailable to microorganisms as well as causing potential plugging problems (Robertson and Alexander, 1992). Ammonium ions may be bound through cations exchange reactions while phosphate ions may complex with nutrients, often in proportion to the clay content of the soil (Morgan and Watkinson, 1992). Because many of these are slow precipitation reactions, short term laboratory studies may not adequately address these problems. Oxygen rich conditions often employed in laboratory studies, which promote excess microbial growth, can also overestimate nutrient requirements (Aggarwal et al., 1985).

To properly assess the applicability of the results seen from this study, either in situ or column studies in parallel could give a better indication if the results are applicable to actual field conditions. However, since this was a relative study between different soils in the laboratory, artifacts introduced in the movement and handling of the soils from the field to the laboratory may be approximately the same in all soils. Comparisons and correlations between soils may adequately represent relationships that would be found in field studies. In any case, laboratory studies still offer the advantage of controlled experimentation with numerous samples.

Lag phases, biodegradation rates and final time to degrade to less than 5 ppb benzene concentration are presented in Tables 10-11 for all the uncontaminated soils at 1, 10 and 50 ppm with and without nutrient addition. These parameters were determined using Figures 18-23 and the method outlined in Section 4.4. These values will be used later in this chapter (Section 4.7) to perform correlations between these parameters and soil properties.

Table 10. Lag phase, biodegradation rates and time to < 5 ppb for **nutrient amended** soil microcosms.

		G. loam	G. clay	Wheel	Bojac	Catpoint	Leedsville	Purcellville
1 ppm								
	Lag phase (days)	0.25	1.5	0.25	0.5	0.5	3.1	2.1
	Bio. rate (mg/L/day)	0.72	0.34	0.41	0.53	0.57	0.30	0.39
	< 5 ppb (days)	1.8	4.5	2.2	3.2	3.8	6.4	4.4
10 ppm								
	Lag phase (days)	0.6	3.8	2.1	0.7	2.4	2.8	2.8
	Bio. rate (mg/L/day)	4.24	1.64	2.36	3.76	5.45	2.30	4.11
	< 5 ppb (days)	2.4	11.3	5.7	5	4.4	6.5	6.4
50 ppm								
	Lag phase (days)	1.9	2.2	4.2	7.1	2.9	10	no data
	Bio. rate (mg/L/day)	6.78	1.06	14.94	4.24	19.94	4.63	no data
	< 5 ppb (days)	8	30.5	never reached	18.8	8.5	32.5	no data

Table 11. Lag phase, biodegradation rates and time to < 5 ppb for **unamended** soil microcosms.

		G. loam	G. clay	Wheel	Bojac	Catpoint	Leedsville	Purcellville
1 ppm								
	Lag phase (days)	0.25	1.5	1.1	0.4	2.7	3.9	1.7
	Bio. rate (mg/L/day)	0.59	0.13	0.22	0.29	0.12	0.09	0.28
	< 5 ppb (days)	1.1	12	4.2	5	13	15.5	6.5
10 ppm								
	Lag phase (days)	0.9	2.5	2	3	7.5	5.5	1.5
	Bio. rate (mg/L/day)	2.78	0.39	1.08	0.58	0.17	0.65	1.85
	< 5 ppb (days)	5	>35	9.5	30.5	>35	>35	7.5
50 ppm								
	Lag phase (days)	1.1	9.6	5.8	18	18	16.1	no data
	Bio. rate (mg/L/day)	2.50	0.40	1.28	0.98	0.62	0.77	no data
	< 5 ppb (days)	15	>42	>18	>42	>42	>42	no data

Comparison of these values with others reported in the literature is difficult due to the use of various laboratory methods as well as different types of rate calculations for different studies. One similar study by Alvarez and Vogel (1991), using 16 g of drained aquifer material that had been previously contaminated with BTEX and 50 mL of a mineral medium in soil microcosms, found benzene was degraded at 25 mg/L/day with a 6 day lag period for an initial benzene concentration of 50 mg/L. For comparison with this study, this would correspond to a biodegradation rate of 7.8 mg/L/day/5 g aquifer material, which is compatible with degradation rates observed in this study at 50 mg/L initial benzene concentration.

In order to facilitate the comparison of the changes in degradation rates within soils at different initial concentrations with and without nutrients, Figures 24-25 were prepared. These graphs mimic the hyperbolic curve representative of Monod's mathematical relationship for growth of a bacterium (Monod, 1949). As indicated in these graphs, there is an increase in degradation rate with an increase in initial benzene concentration for all the soils. However, the rate increases much more significantly from 1 to 10 ppm than from 10 to 50 ppm. This increase in degradation rate from 1 to 10 ppm suggests that substrate concentration limits the growth of cells below 10 ppm. As the concentration increases, the growth rate increases in proportion to the increase in concentration. However, between 10 and 50 ppm, this increase in rate slows down appreciably. The degradation rate has reached its maximum value and is no longer influenced by changes in substrate concentration.

When comparing the two figures, with and without nutrient addition, it is evident that the maximum degradation rate is at a higher level for the nutrient amended soils over the unamended soils. This suggests that nutrients are a limiting factor in the degradation of benzene in these soils. The Ks or Km value (the concentration at which the rate is equal to one-half of the maximum degradation rate) would lie somewhere between 0 and 10 ppm based on these graphs, except for the Catpoint sand and the Wheeling sandy loam, which still showed increasing degradation rates at 50 ppm with the addition of nutrients. To better estimate the Km or Ks value for most of the soils, more data at other initial benzene concentrations would be necessary to better define the shape of the curve.

4.4.2 Contaminated soils

Results for different experiments involving contaminated soils are shown in Figures 26-27. Microcosm data used to construct these graphs are tabulated in Appendix C. CS1 was only tested at 10 ppm with and without nutrients because there was only enough sample available to perform two tests. CS2 and CS3 microcosms were prepared for both 10 and 50 ppm with and without nutrients.

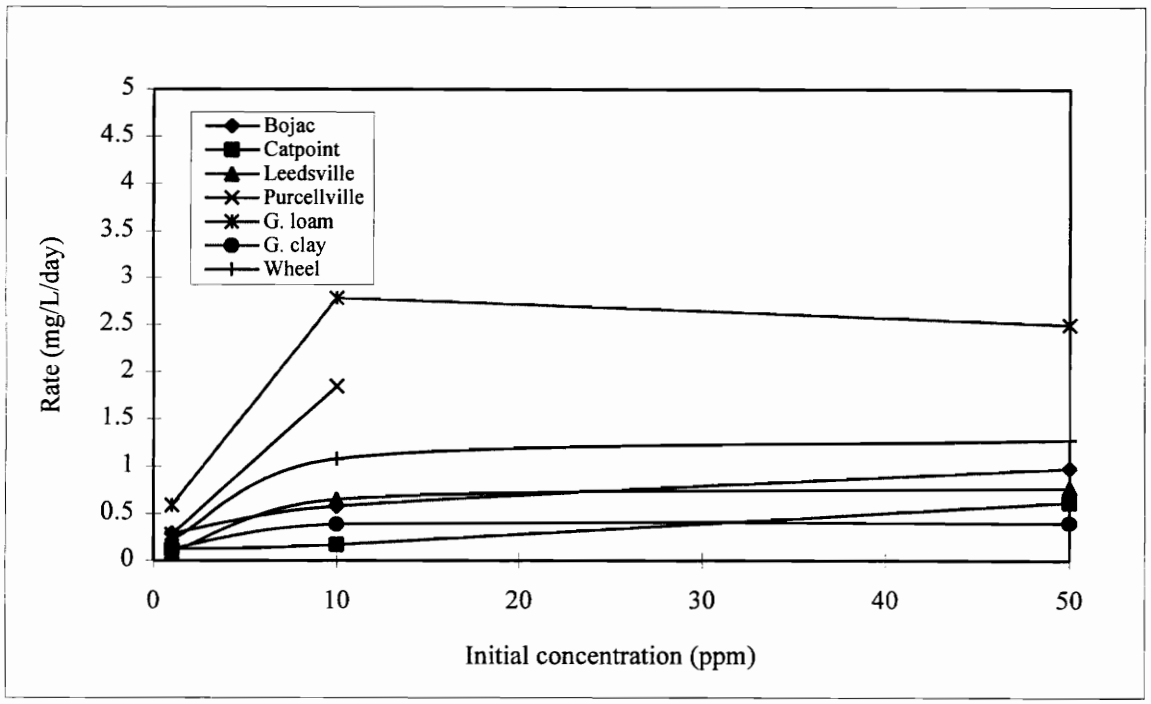

Figure 24. Effect of initial concentration on biodegradation rate in **unamended** soil microcosms.

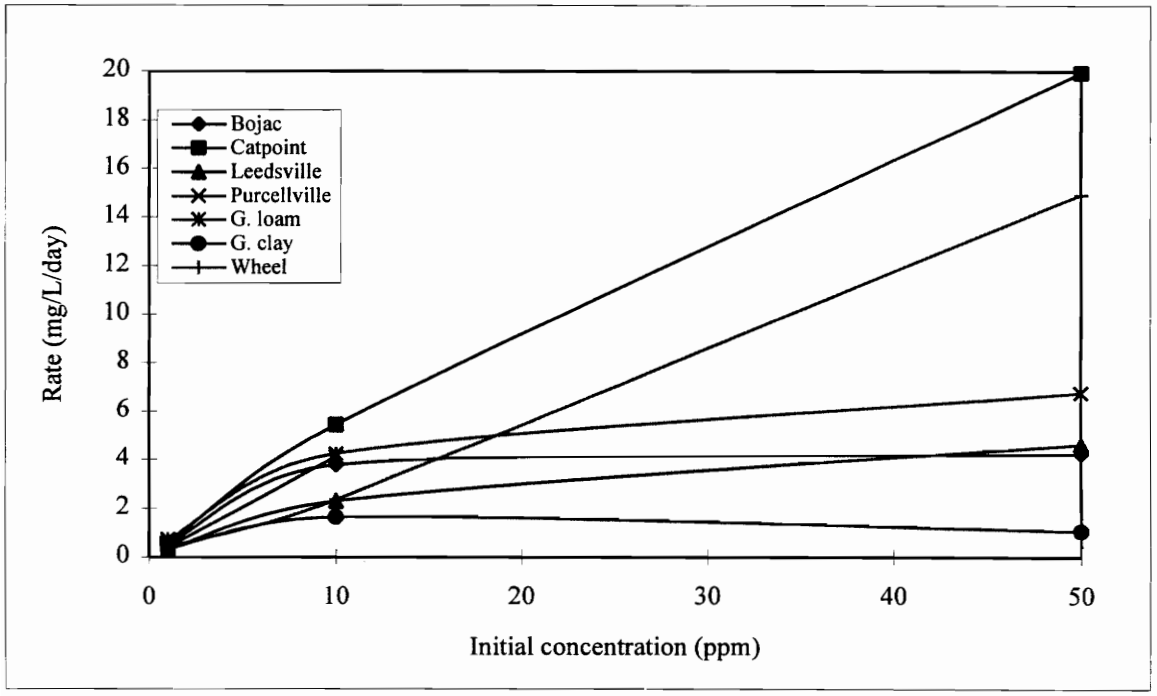

Figure 25. Effect of initial concentration on biodegradation rate in **nutrient amended** soil microcosms.

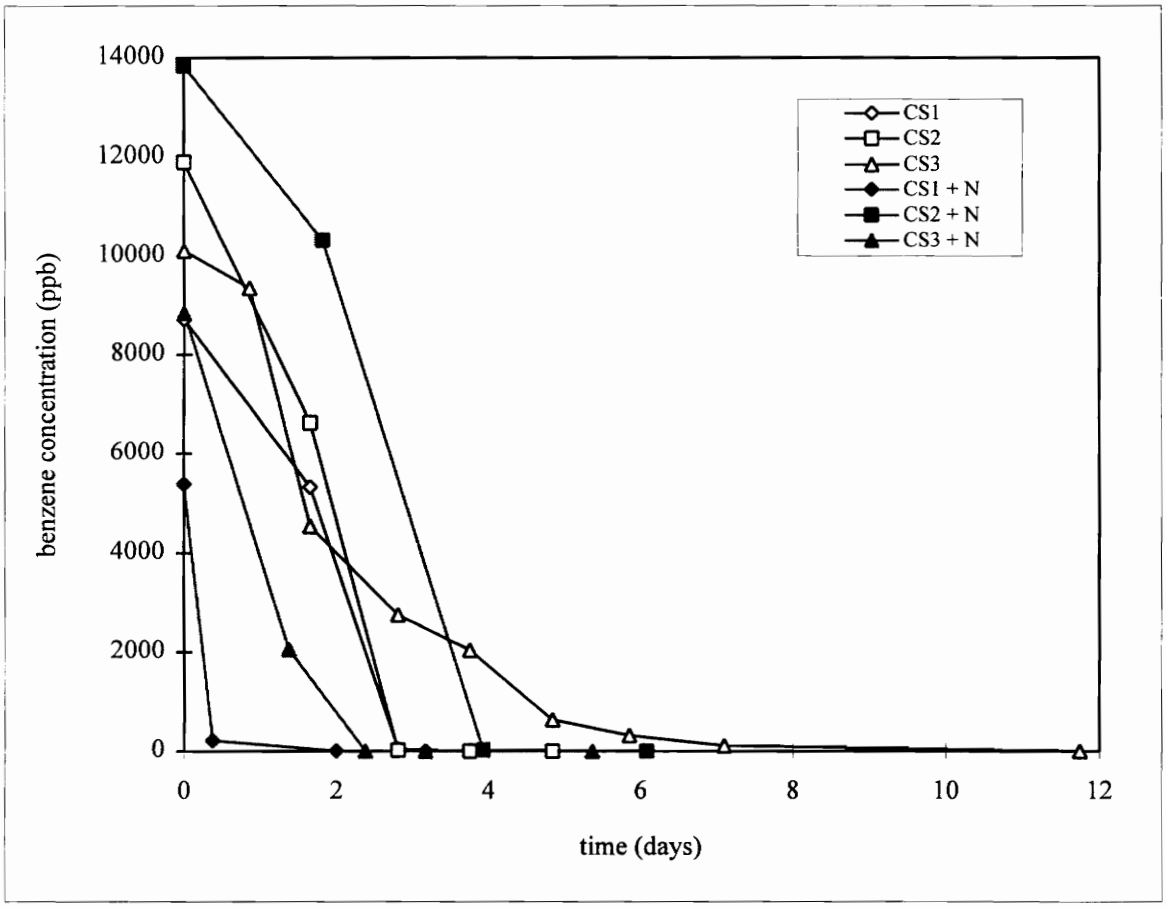

Figure 26. Benzene degradation in 3 contaminated soils with and without nutrient addition at initial benzene concentration of 10 ppm. "+N" denotes nutrient addition in the form of 200 mg/L $(NH_4)_2HPO_4$-NH_4 and 25 mg/L K_2HPO_4-K.

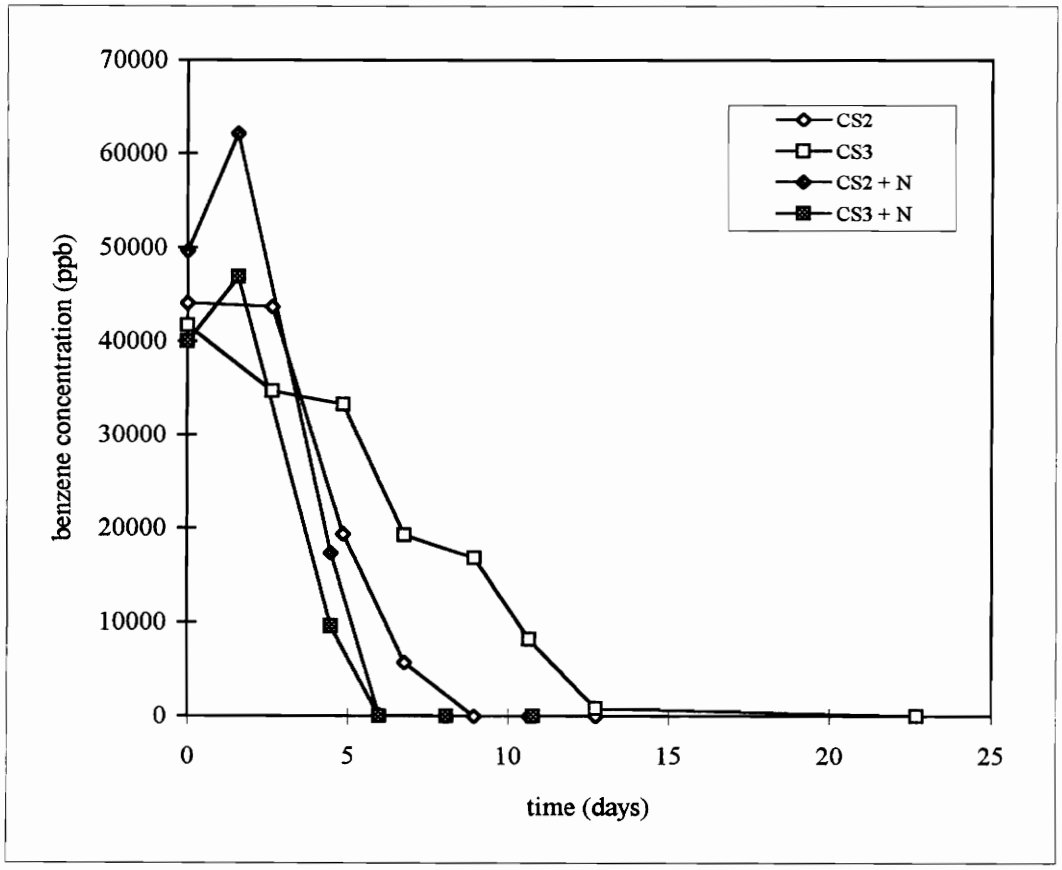

Figure 27. Benzene degradation in 2 contaminated soils with and without nutrient addition at initial benzene concentration of 50 ppm. "+N" denotes nutrient addition in the form of 200 mg/L $(NH_4)_2HPO_4$-NH_4 and 25 mg/L K_2HPO_4-K.

For 10 ppm (Figure 26), nutrient addition had a positive effect on the degradation rate in all but the CS2 soil. However, at an initial benzene concentration of 50 mg/L (Figure 27), nutrient addition increased benzene degradation in both the CS2 and CS3 soils. In situ nutrient concentrations in the CS2 soil may have been sufficient for benzene biodegradation at 10 ppm but not at 50 ppm. In the 10 ppm study, the difference in the initial concentrations in the nutrient amended and unamended CS2 soil as well as the lack of sampling points between 2 and 4 days may make it difficult to compare these two tests and adequately determine the degradation rate.

At an initial benzene concentration of 50 ppm, nutrient addition decreased the lag phase over unamended microcosms. The tailing observed in the CS3 soil was also significantly reduced with the addition of nutrients. This tailing is more pronounced in both the CS2 and CS3 soils at an initial benzene concentration of 50 ppm over 10 ppm, which may be attributable to an increased lag phase at higher concentrations.

Calculated lag phases, rates and final time to degrade to less than 5 ppb for the contaminated soils are shown in Tables 12-13. In order to facilitate comparisons of degradation rates with and without nutrients in uncontaminated and contaminated soils at an initial benzene concentration of 10 ppm, Figure 28 was constructed. It was expected that the contaminated soils would have higher degradation rates associated with them since initial benzene degrader counts were higher in the contaminated soils. For unamended soils, degradation rates were higher in the contaminated soils for all samples. However, with the addition of nutrients, with the exception of soil CS1, several of the uncontaminated soils had degradation rates that surpassed those of contaminated soils. This suggests that nutrients were limiting the growth of benzene degraders in the unamended, uncontaminated soils. With the addition of nutrients, benzene degrader growth was no longer inhibited, and degradation rates were maximized at values similar to those in the contaminated soils.

The variation in the results for the contaminated soils for nutrient addition may pertain to the type of contamination present in each soil sample. Soil CS1 was from a contaminated gasoline site being treated with nutrients and engineered organisms. During the storage of the soil, these organisms may have continued to grow and metabolize carbon and available nutrients. When nutrients were added, degradation was immediately stimulated as nutrients had become limiting in the soil. CS2 was from a fuel oil spill and CS2 was from a diesel spill, both of which contain little benzene. It would be expected that nutrient addition would increase the degradation rate associated with these soils as benzene degrader growth may be necessary if indigenous microorganisms were not capable of degrading benzene. This is the case in the both soils at 50 ppm, but no significant increase in the degradation rate is evident in the CS2 soil at 10 ppm. Petroleum degraders in the CS2 soil may have easily assimilated the degradation of benzene at 10 ppm, but not so easily at 50 ppm. Nutrients may have played a more significant role in

Table 12. Lag phase, biodegradation rates and time to < 5 ppb for **nutrient amended** contaminated soil microcosms

		CS1	CS2	CS3
10 ppm				
	Lag phase (days)	0.1	1.4	0.5
	Bio. rate (mg/L/day)	13.77	3.55	3.79
	< 5 ppb (days)	2.2	5.1	2.6
50 ppm				
	Lag phase (days)	--	2.8	2.8
	Bio. rate (mg/L/day)	--	14.21	10.90
	< 5 ppb (days)	--	7.7	7.3

Table 13. Lag phase, biodegradation rates and time to < 5 ppb for **unamended** contaminated soil microcosms.

		CS1	CS2	CS3
10 ppm				
	Lag phase (days)	0.9	0.8	1.1
	Bio. rate (mg/L/day)	3.01	4.16	1.95
	< 5 ppb (days)	3.4	3.3	10.2
50 ppm				
	Lag phase (days)	--	3.4	5.1
	Bio. rate (mg/L/day)	--	6.95	3.42
	< 5 ppb (days)	--	11.5	20.2

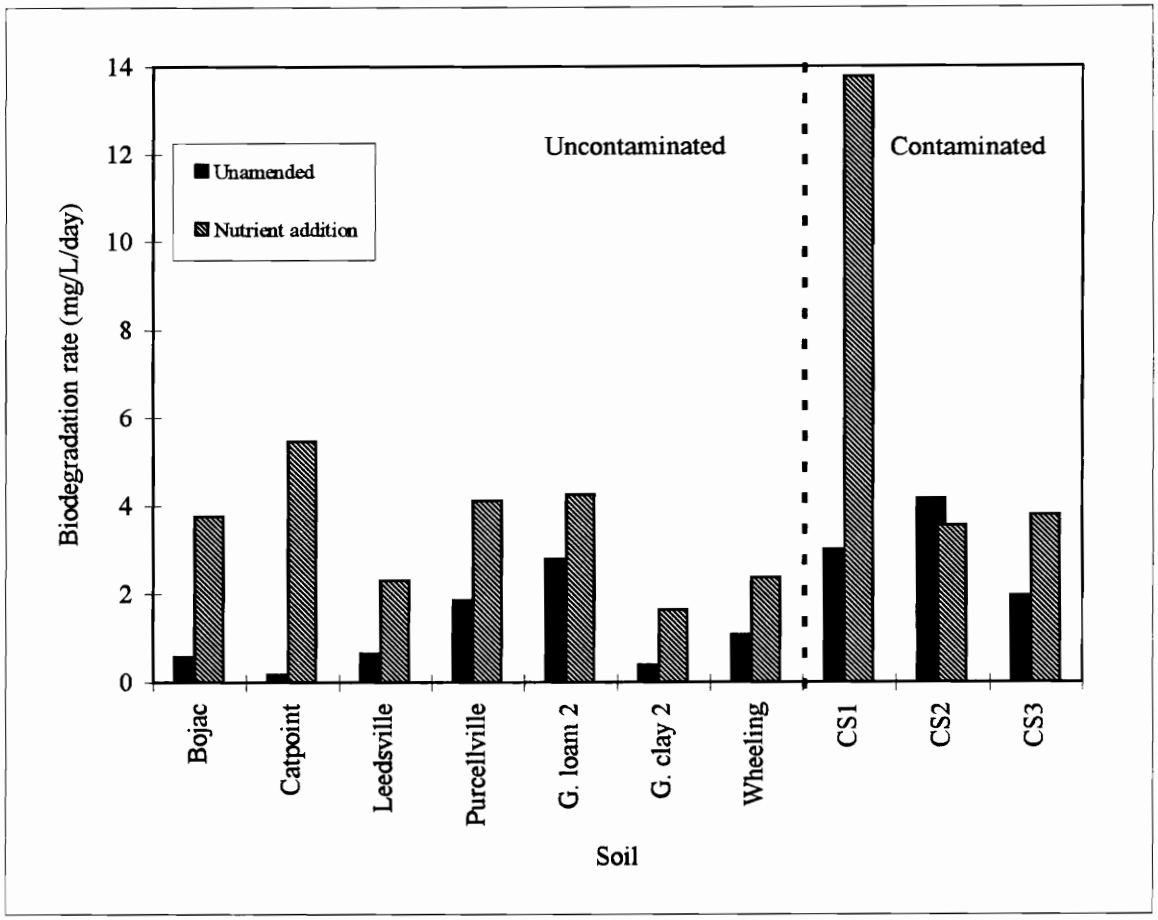

Figure 28. Effect of nutrient addition on noncontaminated and contaminated soils at initial benzene concentration of 10 ppm.

supporting growth of new organisms at 50 ppm. No inferences could be made about natural nutrient levels in the soil as chemical analyses were not done on the contaminated soils.

4.5 Tracking of microbial growth

The purpose of this experiment was to investigate the growth of benzene degraders and heterotrophic microorganisms over time as benzene degradation proceeded. As described in Methods and Materials, the benzene degraders were enumerated by the MPN method and the heterotrophs by HPC's (heterotrophic plate counts). These studies were conducted using the Wheeling sandy loam and Bojac sand. For the Wheeling sandy loam, only benzene degraders were enumerated as shown in Figure 29, but both benzene degraders and HPC's were tracked for the Bojac sand, as shown in Figures 30-31. Note that the Wheeling sandy loam was for an initial benzene concentration of 10 ppm with the addition of nutrients while the Bojac sand was at 10 ppm initial benzene concentration without the addition of nutrients. The growth of benzene degraders occurred in less then 5 days in the Wheeling sandy loam while it required approximately 13 days in the Bojac sand. This decreased lag in benzene degrader growth in the Wheeling sandy loam may be attributable to the addition of nutrients.

In interpreting the changes in the number of benzene degraders over time, in view of the corresponding HPC's for the Bojac sand, there are probably two valid arguments that could be made to explain what is occurring in the microcosms. First, the HPC's could be viewed as having a general upward trend. If an average of the three values found around day five is used as an initial value (about 45,000) and an average for the two readings located around day 15 are used as a final value (about 56,000), the difference between these is about 11,000 CFU/g dry soil. At the same time, the difference from the first benzene degrader reading to the third is 13,000 benzene degrader cells/g dry soil. Therefore, it could be surmised that the growth in heterotrophs is due to the growth of new benzene degrader cells.

Alternatively, the variation in the data for the HPC's from day 0 to day 16 is enough that one could conclude there was no significant change in the level of heterotrophs during the course of the degradation study. In preparing spread plates, there are numerous occasions for errors to occur. Some growth media may be more enriched than others, some dilution preparations may more adequately remove attached bacteria than others, the amount of dilution water applied to the plate may vary, cross contamination could occur, etc. Due to the possibility of concentrated bacterial colonies, a small error may lead to substantial differences in the results. In light of the method used and an erroneous data point at day 11, this may be a valid argument. If the results are interpreted this way, the benzene degrader growth may be attributable to enzymatic transformation of the existing bacterial population rather than

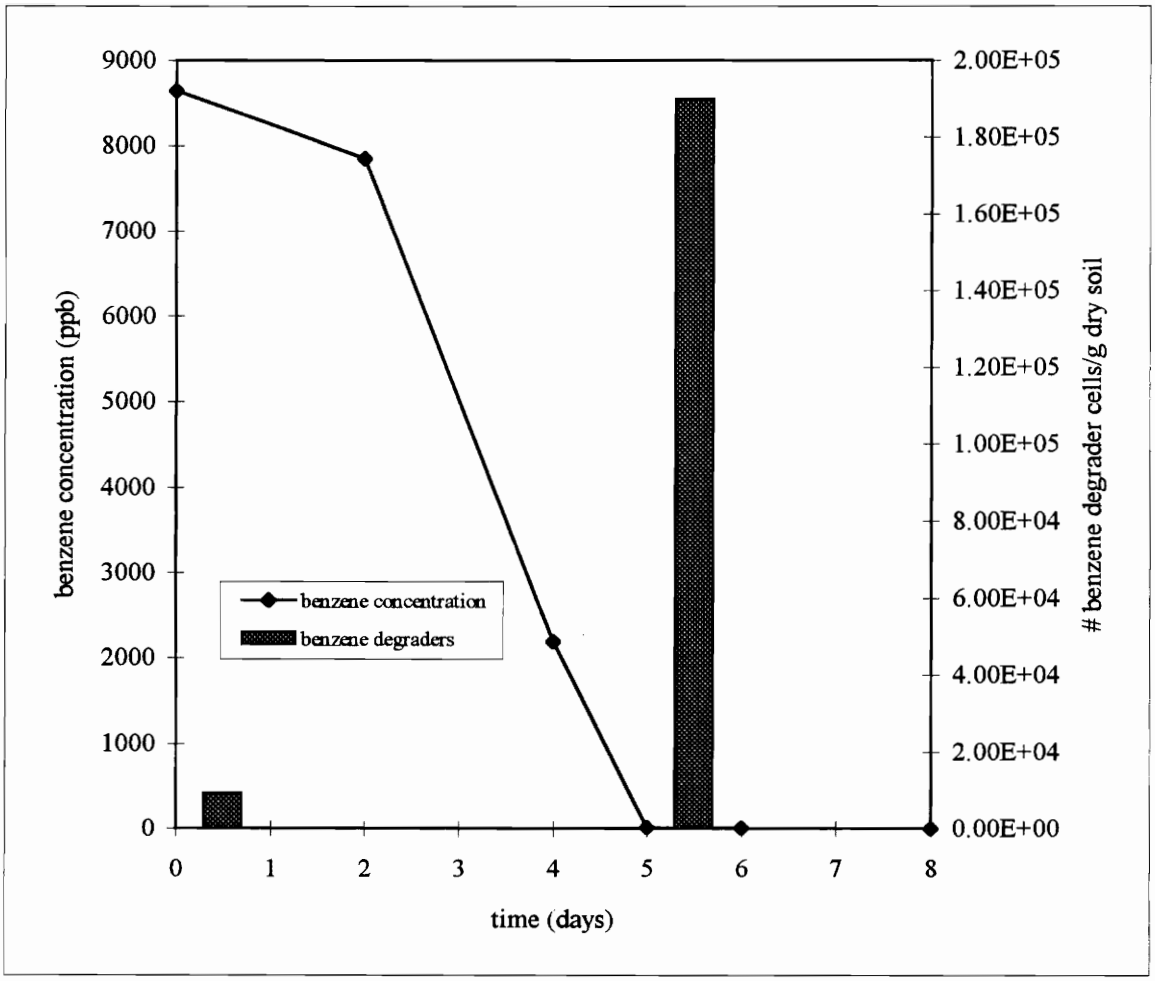

Figure 29. Benzene degradation and benzene degrader growth in Wheeling sandy loam amended with 200 mg/L $(NH_4)_2HPO_4$-NH_4 and 25 mg/L K_2HPO_4-K at initial benzene concentration of 10 ppm.

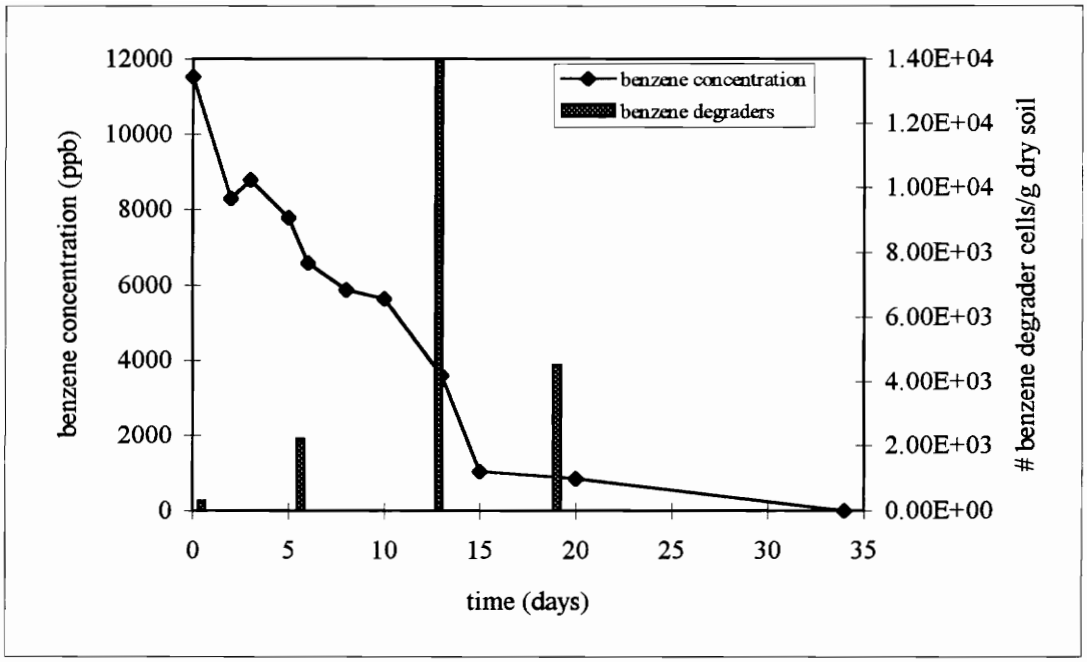

Figure 30. Benzene degradation and benzene degrader growth in Bojac sand at an initial benzene concentration of 10 ppm.

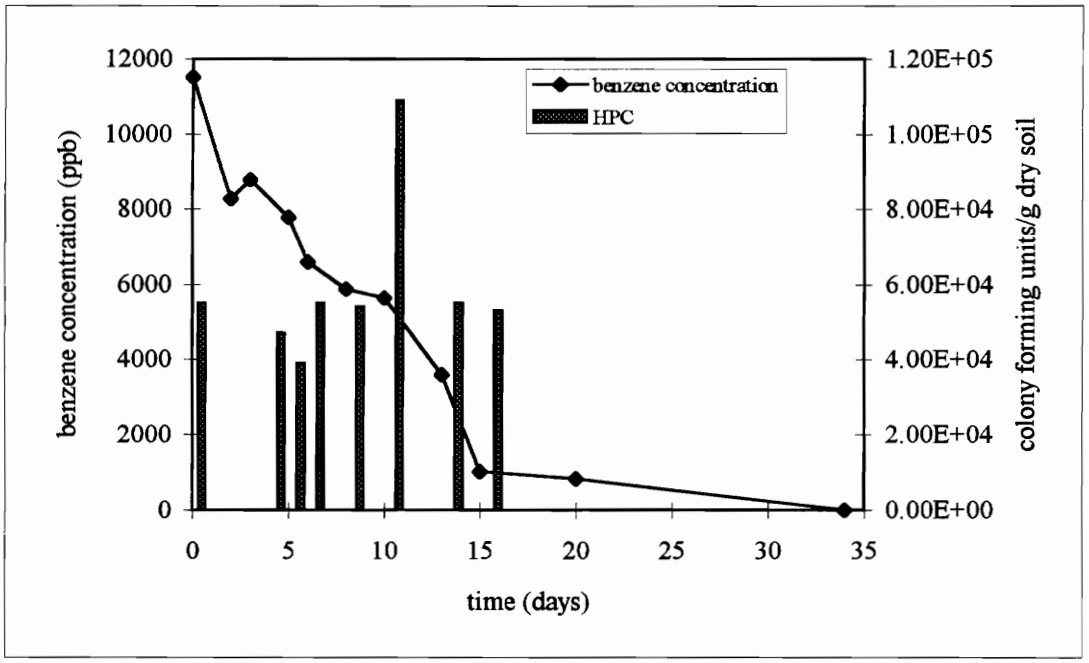

Figure 31. Benzene degradation and heterotrophic microorganism growth in Bojac sand at an initial benzene concentration of 10 ppm.

growth of new cells. This would mean a transformation in the percent distribution of benzene degraders/total heterotrophs rather than an increase in the total number of bacteria.

The interpretation of these results could subsequently effect the selection of a kinetic model for degradation. Many models are based upon the assumption of a growing or non-growing organism population. The logistic or logarithmic models assume there is a growing organism population whereas the zero and first order decay models are associated with a sufficient population going through enzymatic transformations (Alexander and Skow, 1989). It is probably true that both mechanisms are at work in the soil microcosms studies used in this research. The data from one soil for one test is not enough to decide either way. More studies, with perhaps a contaminated soil as well, could provide more information on the role of growth vs. enzymatic changes in the degradation of benzene.

4.6 Adsorption, tailing and soil heterogeneity

4.6.1 Adsorption

In order to better characterize the kinetics of adsorption within the soil microcosms, a study was conducted using microcosms containing sterile soil, active soil, organic free soil and no soil. The results for this study using the Groseclose clay are shown in Figure 32. As the results from the control experiment suggested, the most significant losses occurred in the beginning of the study (within one day) with a subsequent leveling off from day 1 to 21.

Since the test soil was a clay, the high percentage of fine material and organic matter (6% by loss-on-ignition) was anticipated to increase adsorption. Initial adsorption was higher for this soil than control results from other soils. However, the effect of organic matter removal from the soil is not clear based on these results. After 21 days, the autoclaved clay soil which still contained organic matter actually had a higher aqueous concentration than the organic free soil. With a high organic matter content, the removal of organic matter should significantly effect sorption. It is possible that organic matter was lower than measured and sorption into minerals was the dominant mechanism by which adsorption occurred. Benzene that had initially sorbed to the organic matter may have started to equilibrate with the soil/water mixture while benzene in the organic free soil microcosm had continued to adsorb to mineral sites within the soil.

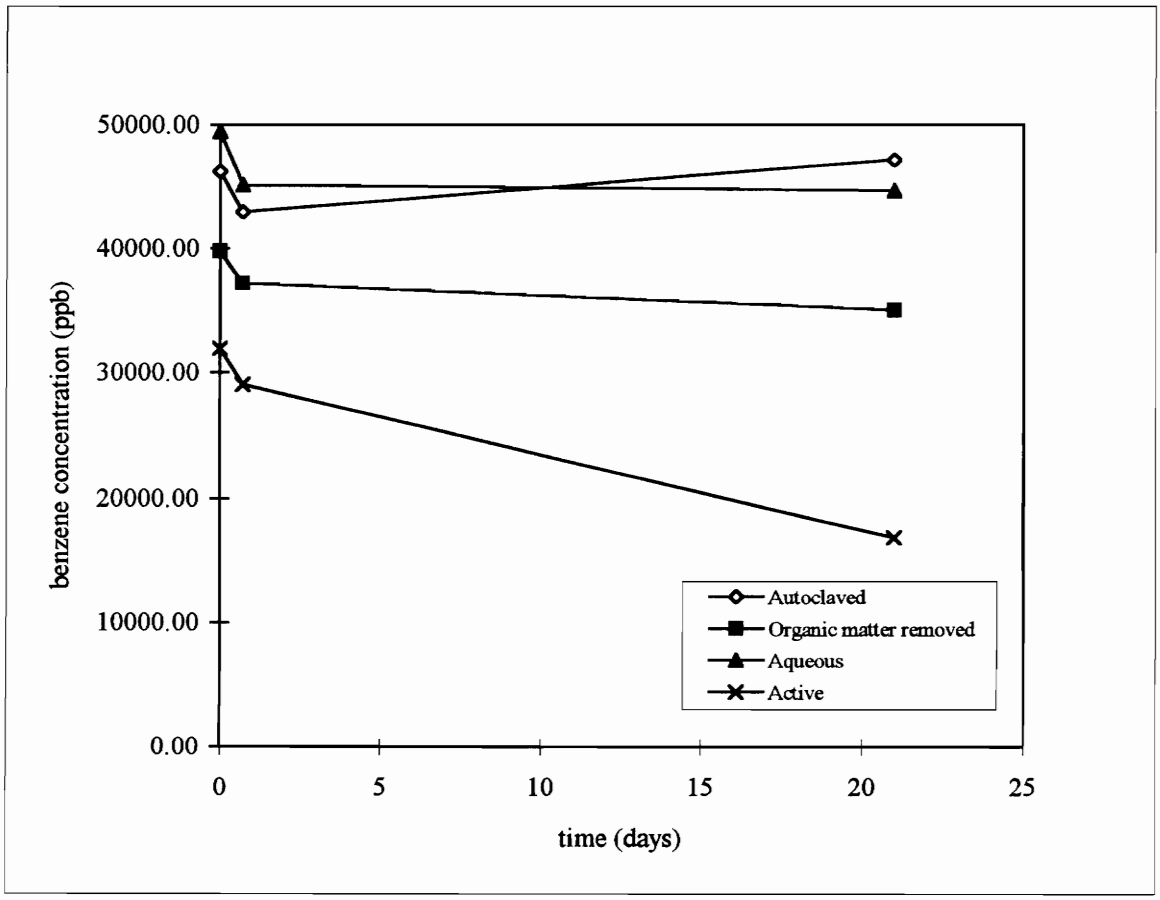

Figure 32. Adsorption and volatilization of benzene in Groseclose clay soil microcosms with an initial benzene concentration of 50 ppm.

4.6.2 Tailing and soil heterogeneity

Tailing, characterized by drastic declines in degradation rates of organic compounds as the substrate concentration decreases, has had significant implications in achieving final substrate concentrations below health based standards. This tailing effect was a significant factor in a few of the soils investigated, particularly at high initial benzene concentrations.

Results for particular studies are shown in Figures 33-34. It is evident from these curves that tailing is significant at 50, 100 and 300 mg/L in two different soils, even with the addition of nutrients. As discussed previously, there was some speculation that this tailing may be due to insufficient nutrients for complete mineralization of the substrate. This does not seem likely based on these results because tailing occurs at all three concentrations and at about the same level (10 ppb). In order to investigate this point further, three different nutrient concentrations were added to soil microcosms at an initial benzene concentration of 100 ppm. The results for two of these tests, shown in Figure 35-38 on a logarithmic scale, indicated that the degree of tailing was independent of nutrient concentration at 100, 200 and 400 mg/L $(NH_4)_2HPO_4$-NH_4 and 25 mg/L K_2HPO_4-K. In fact, soil microcosms amended with the highest nutrient concentration exhibited the lowest degradation rate and the least overall benzene degradation. Figures 35-38 also represent the effect of soil heterogeneity on the interpretation of results from soil biodegradation studies. As indicated in the Methods discussion, each data point is representative of the average of three identically prepared microcosms sampled at the same time. Figures 35 and 37 represent the average values of the data points shown in Figures 36 and 38, respectively. It is evident that biodegradation may not occur in all the soil microcosms for the same test. Some soil microcosms may have completely degraded the substrate while degradation has not even begun in other identically prepared microcosms. This is characteristic of the heterogeneity of soils, where many microcommunities may exist within the soil biota. Harvey et al. (1984) found that greater than 95% of the bacterial population in a freshwater aquifer was associated with particle surfaces. More specifically, 90% of this fraction was associated with those particles less than 60 μm in size, despite the fact that greater then 97% of the total mass of the soil was due to particles greater than 105 μm. Microscopic evaluations of these populations indicated a dominance of microbial clusters in sediment samples. This indicates the heterogeneity of microbial populations in soils, particularly in fine grained soils, where small microcolonies may exist that are responsible for a significant portion of the degradation of organic compounds.

There is no obvious relationship between observed tailing in the soil microcosms and nutrient limitations. As the initial benzene concentration increases from 1 to 50 mg/L, tailing is more significant.

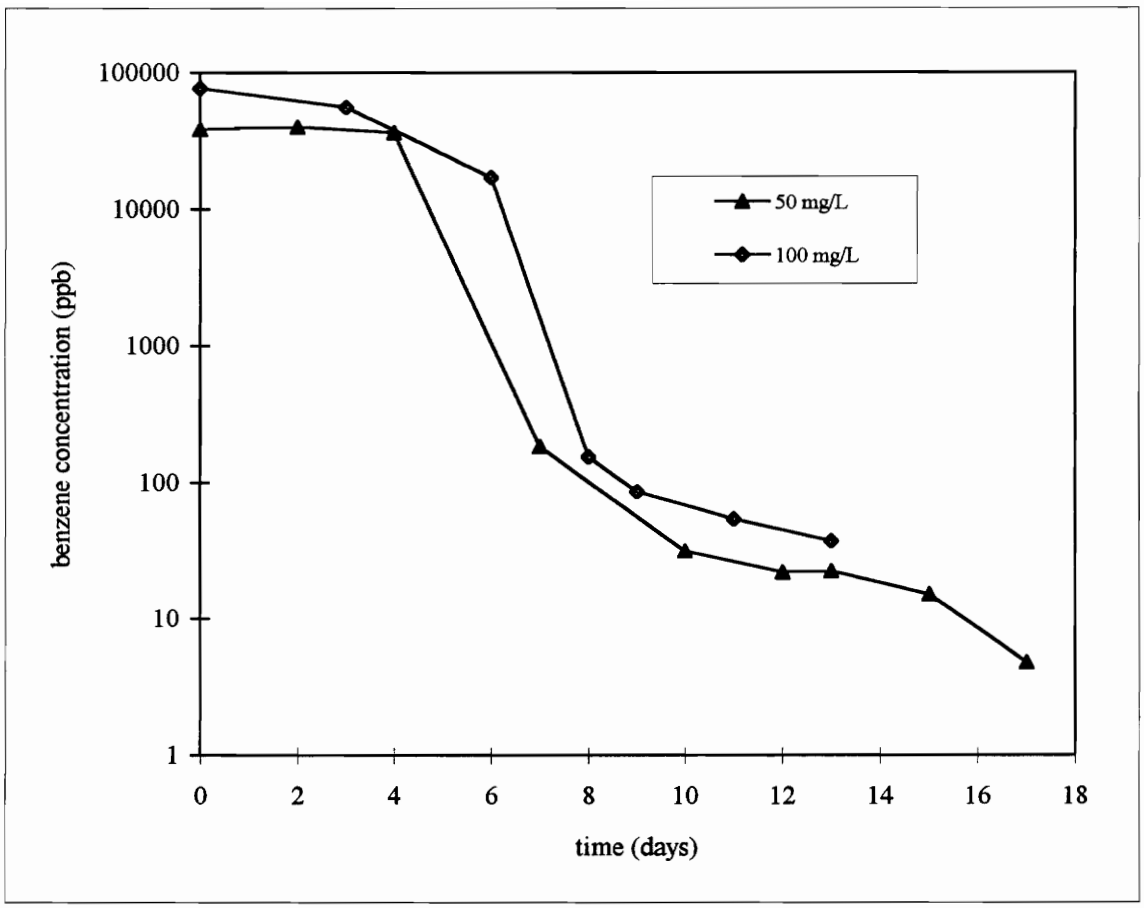

Figure 33. Benzene degradation in Wheeling sandy loam soil microcosms at 50 and 100 ppm initial benzene concentration with nutrient addition. Nutrient addition was 200mg/L $(NH_4)_2HPO_4$-NH_4.

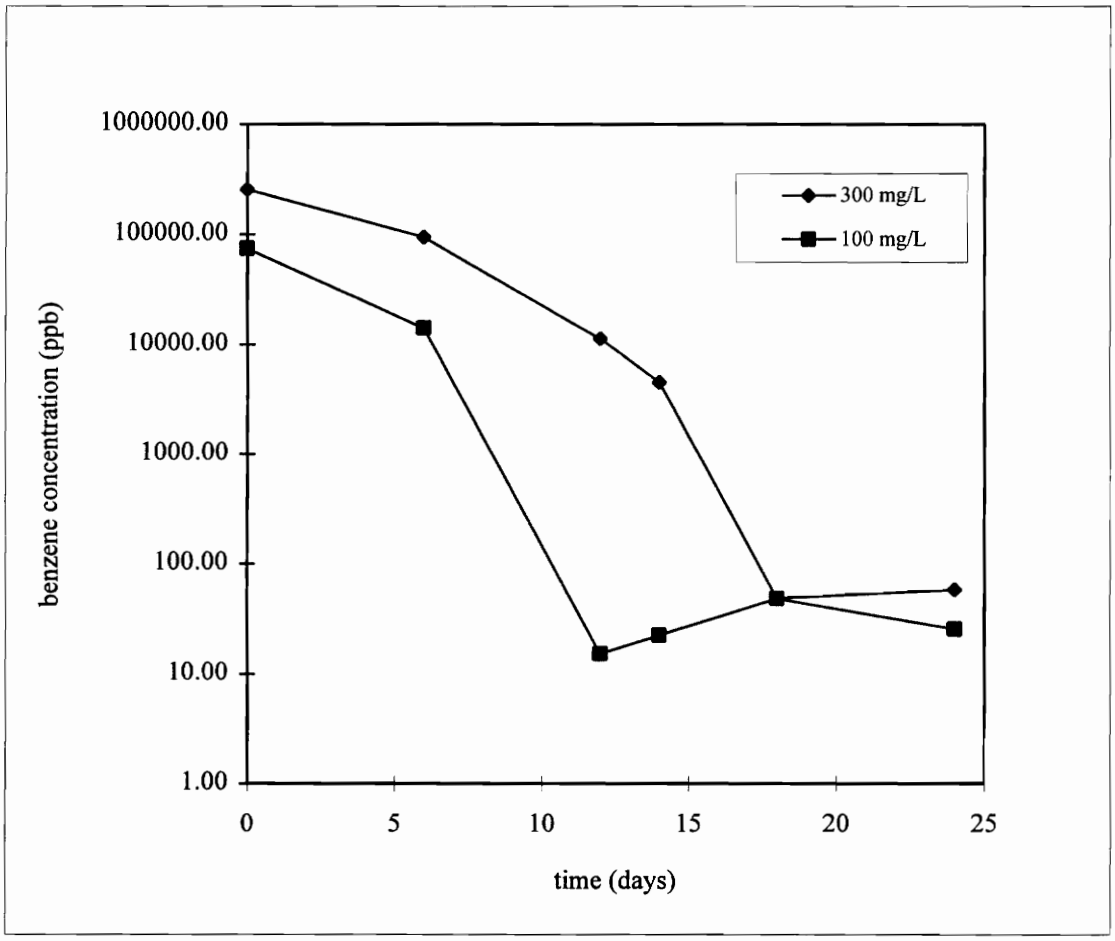

Figure 34. Benzene degradation in Groseclose loam at initial benzene concentrations of 100 and 300 mg/L with nutrient addition. Nutrient addition was 200mg/L $(NH_4)_2HPO_4$-NH_4.

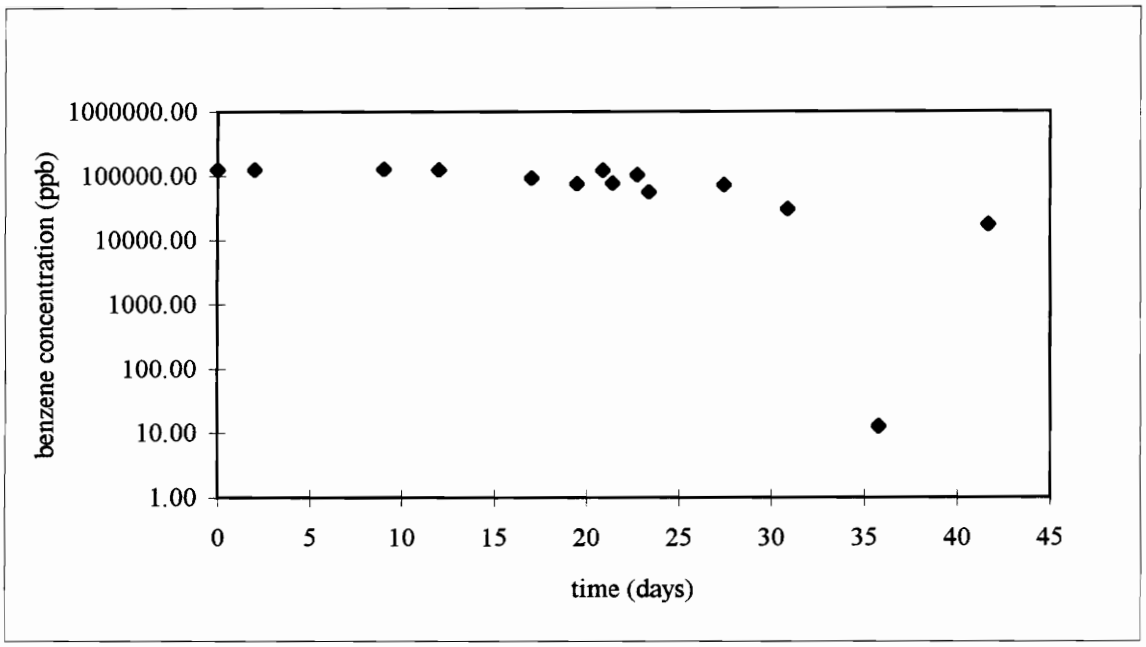

Figure 35. Benzene degradation in Purcellville soil at 100 ppm initial benzene concentration with nutrient addition (average points). Nutrient addition was 100mg/L $(NH_4)_2HPO_4$-NH_4 and 25 mg/L K_2HPO_4-K

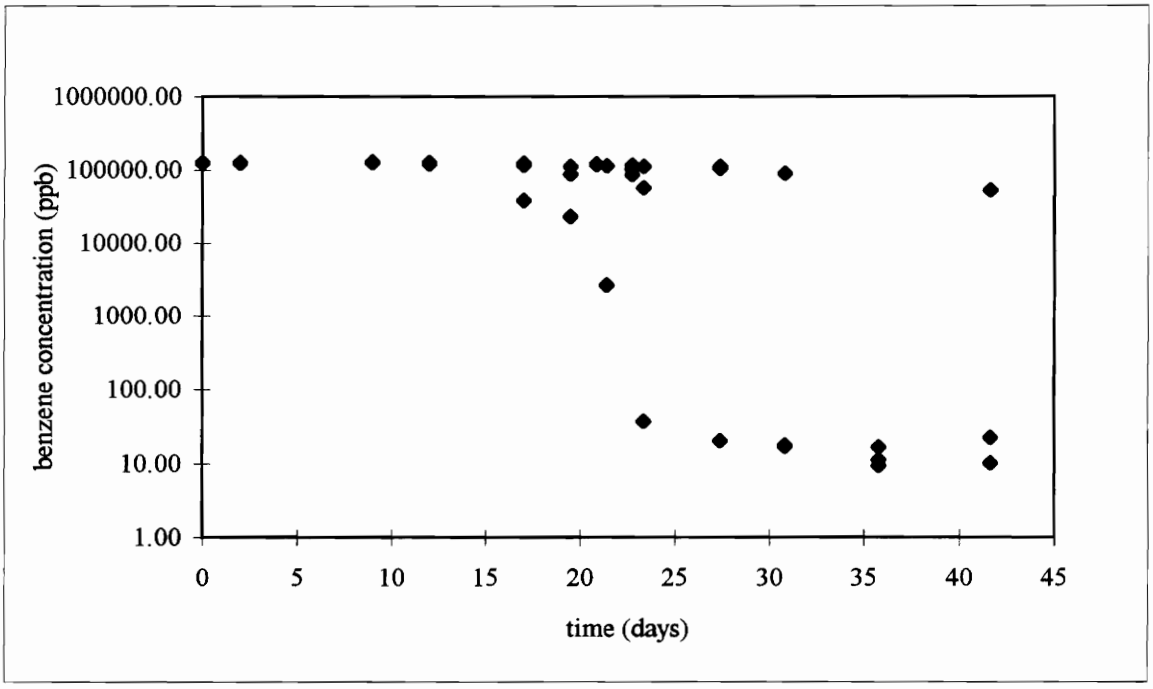

Figure 36. Benzene degradation in Purcellville soil at 100 ppm initial benzene concentration with nutrient addition (all data points). Nutrient addition was 100mg/L $(NH_4)_2HPO_4$-NH_4 and 25 mg/L K_2HPO_4-K.

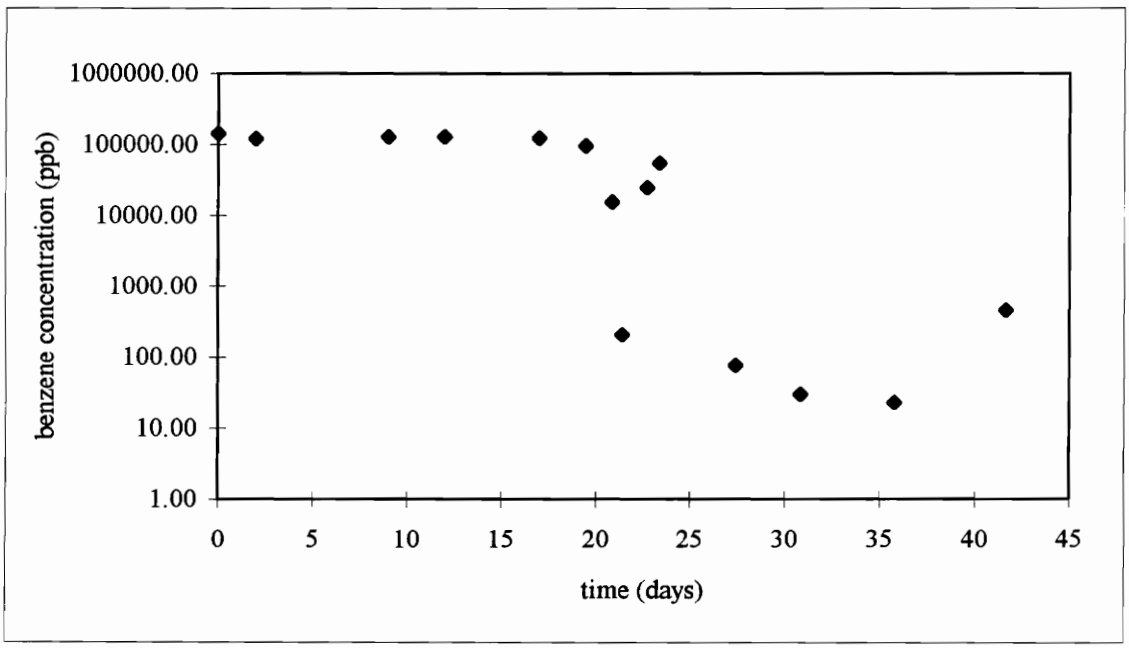

Figure 37. Benzene degradation in Purcellville soil at 100 ppm initial benzene concentration with nutrient addition (all data points). Nutrient addition was 200mg/L $(NH_4)_2HPO_4$-NH_4 and 25 mg/L K_2HPO_4-K.

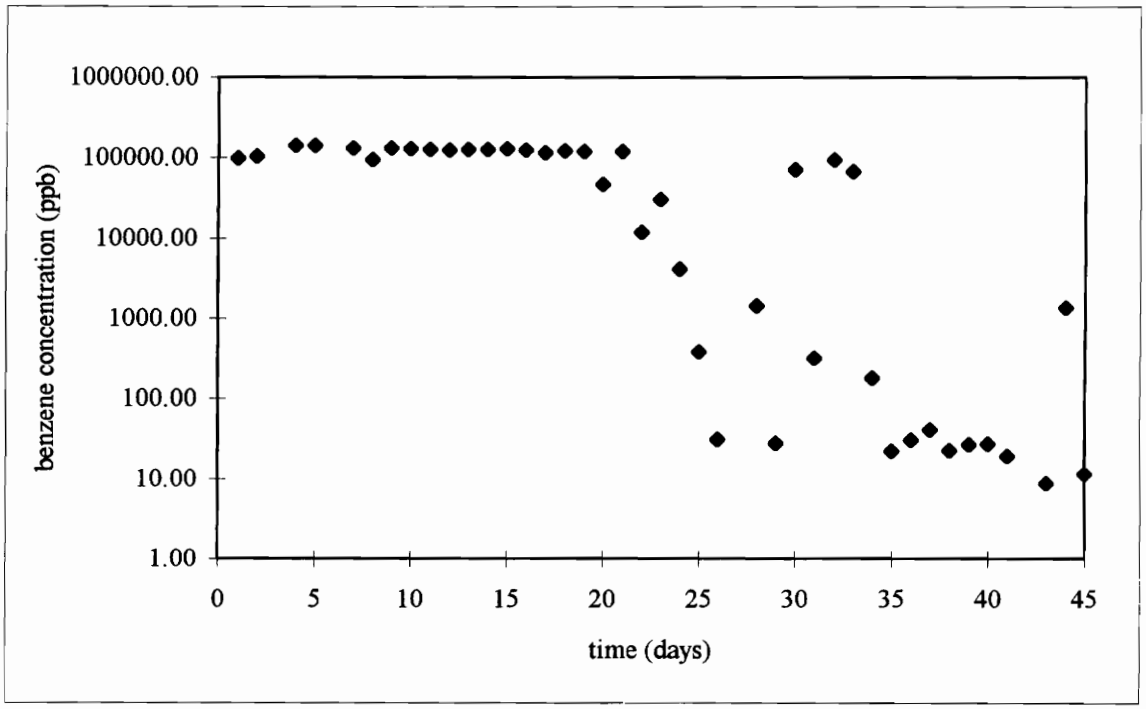

Figure 38. Benzene degradation in Purcellville soil at 100 ppm initial benzene concentration with nutrient addition (all data points). Nutrient addition was 200mg/L $(NH_4)_2HPO_4$-NH_4 and 25 mg/L K_2HPO_4-K.

Although this may suggest tailing is related to nutrient limitations, this is probably not the case because at initial benzene concentrations above 50 ppm, where nutrient limitations should be even more pronounced, tailing occurs at the same level. From three different soils at three different benzene concentrations and two different levels of nutrients, this tailing effect is still observed and it strangely levels off at about 10 ppb for each soil, independent of the initial benzene concentration.

This tailing is probably the result of the long lag phase associated with each of these experiments. In Karickhoff's two phase adsorption kinetics model (1980), chemicals that were initially adsorbed, within the first few minutes to hours, were easily extractable and filled approximately half of the available sorptive sites. The second period of sorption, which involved penetration into the interparticle areas, produced organic compounds that were more difficult to extract as a result of the long initial equilibration time with the sediment. This type of sorption may be dominant when the lag phase of the soil microcosms is long and benzene has an extended initial contact time. The tailing phenomenon seen in this research may be more dependent on the length of the lag phase then the initial concentration of benzene, which would explain why all the microcosms seem to tail at the same level. Karickhoff (1980), also sates that the fast, initial stage of adsorption is independent of the contaminant concentration and the type of sediment. Nutrient addition, therefore, at higher initial benzene concentrations, will induce degradation, but does not sufficiently reduce the tailing effect because of the persistence of a long lag phase. Hence, tailing is primarily dependent on the length of the lag phase or initial exposure time.

Another concept that may explain the tailing around 10 ppb could be that of a minimum concentration or "S_{min} value" as presented by Bouwer and McCarty (1984). This theory, applied when modeling the system as an active biofilm, supports the existence of a minimum concentration below which energy and synthesis requirements for microbial degradation cannot be supported. If benzene had an S_{min} value around 10 ppb, the tailing seen here could be attributed to insufficient concentrations to support further microbial degradation. In conjunction with this concept is the theory of secondary utilization, where a substrate at higher concentrations ($>>S_{min}$), would act as the primary substrate for microbial synthesis and the organic substance at concentrations around S_{min} could be cometabolized. If degradation of benzene does not yield enough energy for microbial growth at this low concentration, perhaps in the presence of a higher yielding reaction, benzene can be degraded as a secondary substrate. To investigate this point further, microcosms could either be established with initial benzene concentrations near 10 ppb or supply a cosubstrate at higher concentrations as the tailing occurs.

4.7 Correlations

4.7.1 Correlation coefficients

The values for correlation coefficients for different combinations of soils with and without nutrient addition are tabulated in Tables 14-17. The correlations that seemed to follow a reasonable trend (positive correlation with lag phase and final time and a negative correlation with degradation rate, or vice versa) with numbers fairly close to 1 (0.50 or higher) were highlighted in gray and coefficients with weaker relationships (0.30 - 0.50) were highlighted in light gray. Correlations were, in general, more numerous and more significant for nutrient amended soils and for lower benzene concentrations. This may be due to error in the interpretation of the data at higher initial benzene concentrations because of difficulty in obtaining a linear degradation rate from the data.

General trends between soil characteristics and degradation parameters were as expected for some of the more significant relationships. These trends will be discussed in more detail in the following section on multiple linear regression analysis although some discussion will be afforded here to the correlation of benzene degraders with degradation parameters.

Benzene degrader counts and lag phase were two parameters that were expected to correlate well. In all combinations, this correlation was not significant except in that of the contaminated soils. This could be due in some part to the techniques used for measuring benzene degraders. Measurements of soil heterotrophs and benzene degraders could be influenced by the adsorption of microbial cells to soil particles. Microbial cell adsorption can be effected by numerous soil properties including pH, soil type, microbial isoelectric point, cations and specific adsorption of microorganisms. When the net negative charge on clay particles or microorganisms within the soil becomes neutralized, adsorption of microorganisms to soil particles can become more significant (Gammack et al., 1992). Bacteria can even show attachment to sand grains through fibrallae and extracellular growths (Paul and Clark, 1989). These factors may play important roles in the enumeration of HPC's and benzene degraders by the techniques employed here. Viable microbial counts can be low due to insufficient removal of these attached bacteria and also through selectivity of the agar media itself.

In the research reported here, initial benzene degrader counts may not correlate well because enumeration techniques did not account for benzene degrading yeast or fungi. Walker and Cowell (1976) reported that MPN tubes poorly accounted for petroleum degrading yeast and fungi. They had more favorable results enumerating these microorganisms using a yeast agar media. The high pH values of the soils used here are not generally favorable for fungal growth (Paul and Clark, 1989). However, pH measurements reflect the pH of the bulk solution and pH gradients can often exist, with low pH being

Table 14. Correlation coefficients between selected soil properties and degradation lag phases, linear degradation rates and final time to degrade to less than 5 ppb for **unamended, uncontaminated** soils at initial benzene concentrations of 1, 10 and 50 ppm.
* data for final time at 50 ppb ommitted because only the G. loam degraded below 5 ppb

		Physical						**Chemical**						
Initial benzene concentration	Degradation parameter	*pH*	*% sand*	*% silt*	*% clay*	*% organic matter*	*% adsorbed water*	*CEC (meq/100 g)*	*NO3-N*	*P*	*K*	*Ca*	*Mg*	*Al*
									(ppm)					
1 ppm	Lag phase	-0.71	-0.11	0.28	-0.06	0.10	0.18	-0.31	-0.22	0.00	-0.24	-0.57	-0.53	0.79
	Rate	0.64	-0.02	0.25	-0.15	-0.29	-0.15	0.28	0.32	-0.34	0.38	0.84	0.71	-0.77
	Final time	-0.61	-0.18	0.01	0.22	0.26	0.29	-0.21	-0.27	0.13	-0.24	-0.59	-0.47	0.67
10 ppm	Lag phase	-0.65	0.38	-0.27	-0.29	-0.36	-0.33	-0.66	-0.56	0.51	-0.73	-0.59	-0.59	0.48
	Rate	0.50	-0.22	0.54	-0.10	-0.09	0.08	0.41	0.54	-0.53	0.58	0.77	0.66	-0.44
	Final time	-0.78	0.91	-0.91	-0.86	-0.71	-0.92	-0.94	-0.43	0.30	-0.91	-0.53	-0.56	-0.27
50 ppm	Lag phase	-0.91	0.49	-0.40	-0.35	-0.32	-0.35	-0.78	-0.59	0.28	-0.75	-0.85	-0.85	0.48
	Rate	0.70	-0.21	0.44	-0.05	-0.21	0.00	0.38	0.57	-0.39	0.48	0.94	0.83	-0.74

		Biological	
Initial benzene concentration	Degradation parameter	*HPC (CFU/g dry soil)*	*Benzene degraders (cells/g dry soil)*
1 ppm	Lag phase	-0.51	-0.52
	Rate	0.48	-0.01
	Final time	-0.40	-0.32
10 ppm	Lag phase	0.08	-0.20
	Rate	0.07	-0.33
	Final time	0.68	0.93
50 ppm	Lag phase	0.21	0.25
	Rate	0.33	-0.33

Table 15. Correlation coefficients between selected soil properties and degradation lag phases, linear degradation rates and final time to degrade to less than 5 ppb for **nutrient amended, uncontaminated** soils at initial benzene concentrations of 1, 10 and 50 ppm.

		Physical						Chemical						
Initial benzene concentration	Degradation parameter	*pH*	*% sand*	*% silt*	*% clay*	*% organic matter*	*% adsorbed water*	*CEC (meq/100 g)*	*NO3-N*	*P*	*K*	*Ca*	*Mg*	*Al*
									(ppm)					
1 ppm	Lag phase	-0.55	-0.48	0.59	0.20	0.46	0.57	0.04	0.27	-0.60	0.27	-0.38	-0.31	0.61
	Rate	0.45	0.39	-0.20	-0.36	-0.68	-0.55	-0.13	-0.08	0.22	-0.16	0.63	0.49	-0.72
	Final time	-0.69	-0.31	0.31	0.18	0.35	0.44	-0.16	0.05	-0.32	-0.01	-0.57	-0.48	0.67
10 ppm	Lag phase	-0.31	-0.46	0.03	0.57	0.68	0.66	0.31	0.20	0.05	0.25	-0.42	-0.23	0.76
	Rate	-0.25	0.72	-0.35	-0.68	-0.88	-0.71	-0.57	0.14	0.37	-0.50	0.05	-0.10	-0.10
	Final time	-0.04	-0.56	-0.09	0.79	0.85	0.75	0.47	0.16	-0.08	0.37	-0.32	-0.09	0.39
50 ppm	Lag phase	-0.69	0.07	0.38	-0.36	-0.05	-0.09	-0.51	-0.59	-0.44	-0.31	-0.58	-0.64	0.34
	Rate	-0.28	0.68	-0.41	-0.60	-0.60	-0.66	-0.48	-0.59	0.81	-0.64	-0.25	-0.35	0.39
	Final time	-0.25	-0.62	0.35	0.55	0.79	0.70	0.27	-0.04	-0.49	0.33	-0.40	-0.25	0.45

		Biological	
Initial benzene concentration	Degradation parameter	*HPC (CFU/g dry soil)*	*Benzene degraders (cells/g dry soil)*
1 ppm	Lag phase	-0.62	-0.39
	Rate	0.80	0.00
	Final time	-0.49	-0.29
10 ppm	Lag phase	-0.80	-0.39
	Rate	0.66	-0.22
	Final time	-0.61	0.08
50 ppm	Lag phase	-0.14	0.23
	Rate	0.10	-0.25
	Final time	-0.76	0.12

Table 16. Correlation coefficients between selected soil properties and degradation lag phases, linear degradation rates and final time to degrade to less than 5 ppb for **nutrient amended** and **unamended contaminated** soils at initial benzene concentration of 10 ppm.

		Physical					**Chemical**	**Biological**	
Initial benzene concentration	Degradation parameter	*pH*	*% sand*	*% silt*	*% clay*	*% organic matter*	*CEC (meq/100 g)*	*HPC (CFU/g dry soil)*	*Benzene degraders (cells/g dry soil)*
10 ppm	Lag	0.239	-0.350	0.262	0.602	0.929	0.170	-0.757	-0.726
with nutrients	rate	-0.821	0.882	-0.834	-0.980	-0.942	-0.779	1.000	0.999
	final	0.063	-0.179	0.088	0.452	0.850	-0.007	-0.630	-0.593
10 ppm	Lag	0.702	-0.614	0.654	0.364	-0.173	0.750	-0.159	-0.205
unamended	Rate	-0.535	0.433	-0.514	-0.158	0.378	-0.593	-0.054	-0.007
	final	0.891	-0.832	0.879	0.639	0.146	0.920	-0.462	-0.504

Table 17. Correlation coefficients between selected soil properties and degradation lag phases, linear degradation rates and final time to degrade to less than 5 ppb for **nutrient amended** and **unamended contaminated and uncontaminated** soils at initial benzene concentration of 10 ppm.

		Physical					**Chemical**	**Biological**	
Initial benzene concentration	Degradation parameter	*pH*	*% sand*	*% silt*	*% clay*	*% organic matter*	*CEC (meq/100 g)*	*HPC (CFU/g dry soil)*	*Benzene degraders (cells/g dry soil)*
10 ppm	Lag	-0.422	-0.512	0.071	0.624	0.548	-0.296	-0.480	-0.472
with nutrients	rate	-0.083	0.547	-0.381	-0.443	-0.491	-0.105	0.949	0.947
	final	-0.219	-0.531	-0.037	0.793	0.730	-0.197	-0.405	-0.395
10 ppm	Lag	-0.676	0.153	-0.187	-0.064	-0.315	-0.530	-0.271	-0.277
unamended	Rate	0.464	0.132	0.177	-0.305	0.029	0.431	0.363	0.384
	final	-0.322	0.435	-0.473	-0.306	-0.528	-0.316	-0.301	-0.315

associated with particle surfaces (Gammack et al., 1992). If fungi at the particle surface were the predominant mechanism in benzene biodegradation, it is logical that MPN tubes may not adequately enumerate these degraders and a low correlation is subsequently obtained between these degraders and benzene biodegradation.

As these correlation coefficients are only for relationships between a single soil parameter and observed kinetic estimators, they were only used as a tool to select parameters to be used in the multiple linear regression analysis. The coefficients can be compared to tabulated values for significant r values for a known number of observations, but it will be shown that although these values may not be statistically valid based on individual correlation coefficients, they can be significant when combined together with other soil properties. For these reasons, the statistical verification for these correlations was not done until the next stage of analysis, multiple linear regression.

4.7.2 Multiple linear regression analysis

Multiple linear regression was performed on over 25 different combinations of physical, chemical and biological properties as the independent variables with corresponding dependent variables of lag phase, degradation rate, and final time to degrade to less than 5 ppb. The results of all these attempted combinations are shown in Appendix D. A summary of the statistically significant results from this analysis for all uncontaminated soils at 1 and 10 ppm with and without nutrients is given in Table 18. As it is evident from this Table, statistically significant relationships were only found for those soil microcosms amended with nutrients. The table gives values for coefficients for each variable investigated, the t statistic and p-value associated with each variable as well as the observed F-value, the probability greater than F and the R^2 value. Considering seven observations and three independent variables, the critical F value (at p=0.05) above which a relationship is deemed significant was determined to be 9.28 (Mendenhall and Sincich, 1988). The tests for which this occurred are denoted with bold lettering in the table.

Using these variables and corresponding coefficients, empirical models were developed and are shown in Table 19. Although all variables for each model are shown in this table, not all parameters within each model were statistically significant. Statistical significance of a single parameter was indicated by a p-value less than 0.05 in Table 18 for the individual parameter. This p-value indicates that, with a 95% degree of confidence, the coefficient for this particular variable is statistically different from zero. When certain parameters occur in a model such that it intuitively does not make sense, such as benzene degraders having a negative effect on rate, the p-value is at such a level that this may be described as "noise" within the model. Since three parameters were regressed simultaneously in order to get the maximum possible

Table 18. Multiple linear regression analysis results shown to be statistically significant via the F- test for all uncontaminated soils with and without nutrient addition at 1 and 10 ppm initial benzene concentration. Equation is of the form: $y=x_o+m_1x_1+m_2x_2+...+m_nx_n$ (where $x_o = m_n$ value for intercept). **Bold** denotes $F_{obs} > F_{crit}$ (= 9.28).

Test	y parameter	Variables				F_{obs}	Prob > F	R^2
		x_n	m_n	t-value	p value			
10 ppm with nutrients	Lag phase	Intercept	5.3039	2.8874	0.0631	4.2986	0.1310	0.8113
		pH	-0.7166	-2.7742	0.0693			
		% sand	-0.0002	-0.0112	0.9918			
		% clay	0.0426	2.0742	0.1297			
	Rate	Intercept	1.8262	0.5590	0.6152	1.2512	0.4291	0.5558
		pH	0.1509	0.3284	0.7642			
		% sand	0.0203	0.7458	0.5099			
		% clay	-0.0164	-0.4475	0.6848			
	Final time	Intercept	8.8774	3.0966	0.0534	**11.0606**	0.0395	0.9171
		pH	-1.2276	-3.0451	0.0556			
		% sand	0.0210	0.8810	0.4432			
		% clay	0.1351	4.2113	0.0245			
10 ppm with nutrients	Lag phase	Intercept	5.2665	5.8741	0.0098	**11.1991**	0.0388	0.9180
		pH	-0.5463	-2.8671	0.0642			
		% clay	0.0306	2.9375	0.0606			
		HPC[1]	-0.1583	-1.9767	0.1425			
	Rate	Intercept	3.4964	1.5762	0.2131	1.6781	0.3406	0.6266
		pH	-0.1040	-0.2207	0.8395			
		% clay	-0.0208	-0.8076	0.4784			
		HPC[1]	0.2198	1.1093	0.3482			
	Final time	Intercept	10.5647	4.5126	0.0203	8.9192	0.0527	0.8992
		pH	-1.1739	-2.3593	0.0995			
		% clay	0.1076	3.9579	0.0288			
		HPC[1]	-0.0680	-0.3252	0.7664			
10 ppm with nutrients	Lag phase	Intercept	5.1958	2.1690	0.1186	4.0420	0.1407	0.8017
		pH	-0.8411	-1.7296	0.1821			
		CEC	0.1686	0.7927	0.4859			
		% organic	0.2691	0.7263	0.5202			
	Rate	Intercept	8.5177	5.3620	0.0127	**14.4067**	0.0275	0.9351
		pH	-0.7243	-2.2460	0.1103			
		CEC	0.3742	2.6537	0.0767			
		% organic	-1.2244	-4.9830	0.0155			
	Final time	Intercept	3.7146	0.7498	0.5078	5.5350	0.0968	0.8470
		pH	-0.1782	-0.1772	0.8707			
		CEC	-0.2571	-0.5847	0.5998			
		% organic	1.7979	2.3461	0.1007			

Table 18. (continued) Multiple linear regression analysis results shown to be statistically significant via the F- test for all uncontaminated soils with and without nutrient addition at 1 and 10 ppm initial benzene concentration. Equation is of the form: $y=x_o+m_1x_1+m_2x_2+...+m_nx_n$ (where $x_o = m_n$ value for intercept). **Bold** denotes $F_{obs} > F_{crit}$ (= 9.28).

Test	y parameter	Variables				F_{obs}	Prob > F	R^2
		x_n	m_n	t-value	p value			
1 ppm with nutrients	Lag phase	Intercept	3.8374	4.4342	0.0213	**9.4507**	0.0488	0.9043
		pH	-0.6423	-4.0806	0.0266			
		% organic	-0.2247	-0.7662	0.4993			
		% water	0.6943	2.3122	0.1038			
	Rate	Intercept	0.2104	4.7568	0.0176	**71.3456**	0.0027	0.9862
		pH	0.0806	10.0253	0.0021			
		% organic	-0.1183	-7.8955	0.0042			
		% water	0.0521	3.3985	0.0425			
	Final time	Intercept	8.3626	7.0284	0.0059	**10.0107**	0.0452	0.9092
		pH	-1.0376	-4.7948	0.0173			
		% organic	-0.1434	-0.3556	0.7456			
		% water	0.7238	1.7534	0.1778			
1 ppm with nutrients	Lag phase	Intercept	7.9360	8.9492	0.0029	**40.4651**	0.0063	0.9759
		pH	-0.8235	-9.5859	0.0024			
		% sand	-0.0346	-5.4878	0.0119			
		% organic	-0.1124	-1.0302	0.3787			
	Rate	Intercept	0.3763	2.3432	0.1009	**21.8525**	0.0153	0.9562
		pH	0.0732	4.7049	0.0182			
		% sand	-0.0014	-1.2634	0.2957			
		% organic	-0.0926	-4.6875	0.0184			
	Final time	Intercept	12.6313	6.8725	0.0063	**18.2243**	0.0198	0.9480
		pH	-1.2264	-6.8876	0.0063			
		% sand	-0.0360	-2.7578	0.0703			
		% organic	-0.0258	-0.1142	0.9163			
1 ppm with nutrients	Lag phase	Intercept	7.4544	10.6333	0.0018	**34.2370**	0.0080	0.9716
		pH	-0.8465	-7.7307	0.0045			
		% sand	-0.0316	-6.0952	0.0089			
		HPC[1]	0.0360	0.6717	0.5499			
	Rate	Intercept	-0.0231	-0.0905	0.9336	3.7917	0.1513	0.7913
		pH	0.0548	1.3714	0.2638			
		% sand	0.0011	0.5669	0.6104			
		HPC[1]	0.0292	1.4954	0.2317			
	Final time	Intercept	13.1719	13.1843	0.0009	**33.5548**	0.0083	0.9711
		pH	-1.3561	-8.6903	0.0032			
		% sand	-0.0433	-5.8591	0.0099			
		HPC[1]	0.1187	1.5543	0.2180			

Table 18. (continued) Multiple linear regression analysis results shown to be statistically significant via the F- test for all uncontaminated soils with and without nutrient addition at 1 and 10 ppm initial benzene concentration. Equation is of the form: $y=x_o+m_1x_1+m_2x_2+...+m_nx_n$ (where $x_o = m_n$ value for intercept). **Bold** denotes $F_{obs} > F_{crit}$ (= 9.28).

Test	y parameter	Variables				F_{obs}	Prob > F	R^2
		x_n	m_n	t-value	p value			
1 ppm with nutrients	Lag phase	Intercept	7.2610	10.5375	0.0018	**29.7673**	0.0098	0.9675
		pH	-0.8095	-7.8960	0.0042			
		% sand	-0.0292	-7.0975	0.0058			
		benz deg[2]	0.0001	0.1159	0.9150			
	Rate	Intercept	-0.2728	-1.1114	0.3474	3.3843	0.1717	0.7719
		pH	0.1024	2.8050	0.0676			
		% sand	0.0040	2.7364	0.0716			
		benz deg[2]	-0.0005	-1.3383	0.2732			
	Final time	Intercept	12.6478	10.9507	0.0016	**20.8099**	0.0164	0.9541
		pH	-1.2559	-7.3080	0.0053			
		% sand	-0.0367	-5.3156	0.0130			
		benz deg[2]	0.0011	0.6467	0.5639			
1 ppm with nutrients	Lag phase	Intercept	4.3367	2.5150	0.0866	1.7227	0.3330	0.6327
		pH	-0.4017	-1.3524	0.2691			
		HPC[1]	-0.1871	-1.3128	0.2807			
		benz deg[2]	-0.0011	-0.3201	0.7699			
	Rate	Intercept	0.0829	0.8468	0.4593	**14.2772**	0.0279	0.9345
		pH	0.0425	2.5217	0.0860			
		HPC[1]	0.0469	5.7917	0.0102			
		benz deg[2]	-0.0005	-2.7549	0.0705			
	Final time	Intercept	8.9006	3.7102	0.0340	1.8015	0.3204	0.6430
		pH	-0.7504	-1.8159	0.1670			
		HPC[1]	-0.1998	-1.0074	0.3880			
		benz deg[2]	-0.0008	-0.1624	0.8813			
1 ppm with nutrients	Lag phase	Intercept	3.7373	3.7960	0.0321	7.8141	0.0626	0.8865
		pH	-0.6687	-3.4823	0.0400			
		% water	0.5042	2.6539	0.0767			
		HPC[1]	0.0176	0.1590	0.8838			
	Rate	Intercept	0.1143	0.6679	0.5520	4.2714	0.1320	0.8103
		pH	0.0537	1.6087	0.2060			
		% water	-0.0267	-0.8087	0.4779			
		HPC[1]	0.0256	1.3270	0.2765			
	Final time	Intercept	8.0253	7.1243	0.0057	**12.4092**	0.0338	0.9254
		pH	-1.1364	-5.1721	0.0140			
		% water	0.7366	3.3890	0.0428			
		HPC[1]	0.1139	0.8986	0.4351			

[1]HPC = Heterotrophic plate counts = Colony forming units / g dry soil

[2]benz deg = initial benzene degrader counts = number of cells / g dry soil

Table 19. Multiple linear regression models.

Y parameter: Lag phase

Test: 1 ppm with nutrients

Model 1: $y = 3.84 - 0.6423\,pH - 0.2247\,\%\,organic\ matter + 0.6943\,\%\,adsorbed\ water$

Model 2: $y = 7.94 - 0.8235\,pH - .0346\,\%sand - 0.1124\,\%organic\ matter$

Model 3: $y = 7.45 - 0.8465\,pH - 0.0316\,\%\,sand + 0.0360\,HPC$

Model 4: $y = 7.26 - 0.8095\,pH - 0.0292\,\%\,sand + 0.0001\,benzene\ degraders$

Test: 10 ppm with nutrients

Model 1: $y = 5.27 - 0.5463\,pH + 0.0306\,\%\,clay - 0.1583\,HPC$

Y parameter: Rate

Test: 1 ppm with nutrients

Model 1: $y = 0.2104 + 0.0806\,pH - 0.1183\,\%\,organic\ matter + 0.0521\,\%\,adsorbed\ water$

Model 2: $y = 0.3763 + 0.0732\,pH - 0.0014\,\%sand - 0.0926\,\%organic\ matter$

Model 3: $y = 0.0829 + 0.0425\,pH + 0.0469\,HPC - 0.0005\,benzene\ degraders$

Test: 10 ppm with nutrients

Model 1: $y = 8.52 - 0.7243\,pH + 0.3742\,CEC - 1.2244\,\%\,organic\ matter$

Y parameter: Final time to degrade to < 5 ppb

Test: 1 ppm with nutrients

Model 1: $y = 8.36 - 1.0376\,pH - 0.1434\,\%\,organic\ matter + 0.7238\,\%\,adsorbed\ water$

Model 2: $y = 12.63 - 1.2264\,pH - 0.0360\,\%sand - 0.0258\,\%organic\ matter$

Model 3: $y = 13.17 - 1.3561\,pH - 0.0433\,\%\,sand + 0.1187\,HPC$

Model 4: $y = 12.65 - 1.2559\,pH - 0.0367\,\%\,sand + 0.0011\,benzene\ degraders$

Model 5: $y = 8.02 - 1.1364\,pH + 0.07366\,\%\,water + 0.1139\,HPC$

Test: 10 ppm with nutrients

Model 1: $y = 8.87 - 1.2276\,pH + 0.0210\,\%\,sand + 0.1351\,\%\,clay$

parameters in each model, some of this type of noise is expected, but the model is still overall statistically significant.

The soil characteristics that were statistically significantly in almost all the models where they appeared were pH, % sand, % organic matter and HPC's. This is not surprising as these parameters are often controlling factors in the bioremediation of soils. The pH can limit the presence or growth of benzene degraders as well as precipitation of nutrients, % sand can limit the influx and availability of nutrients and substrate, % organic matter can significantly influence adsorption and HPC dictates the total number of heterotrophic organisms in the soil. Since these factors only correlate when nutrients are added is not unexpected if, in fact, nutrients are limiting in the unamended soils. If nutrients are limiting, it would be difficult to correlate observed degradation rates when the limiting factor is not even measurable. When nutrients are present at such low levels, as they were in many of the soils investigated, changes in nutrient levels will not necessarily correlate with changes in degradation rates. Only when this limitation is removed can other important soil characteristics become evident as controlling or limiting the degradation rate.

On further investigation of the models, it would appear that pH was the most significant parameter, sometimes overwhelmingly so, in predicting the three degradation parameters at both 1 and 10 ppm. This may suggest that pH is really the only soil parameter worth measuring in order to predict degradation characteristics of a soil. In order to investigate this point, linear regression analysis was performed using only pH as the independent parameter to predict lag phase and degradation rate and is shown in Figures 39-40. As seen in these figures, despite its high statistical significance within the multiple linear regression models, pH does not individually correlate well with either degradation rate or lag phase in the soil microcosms. This illustrates that biodegradation rates of organic compounds in a soil system cannot be described by a single soil parameter. Due to the complexity of the soil environment, several parameters must be considered simultaneously to accurately predict a biodegradation rate.

Using both the multiple linear regression models and the correlation factors for individual soil characteristics, some general trends can be identified between the observed degradation of benzene and soil parameters. Percent sand generally had a positive effect on benzene biodegradation while organic matter and % clay tended to have a negative influence on the overall degradation of benzene. In the pH range of these soils, the degradation rate increased and both the lag phase and time to degrade benzene to less than 5 ppb decreased as pH increased. As previously indicated, however, no single factor should be used to predict kinetic parameters. Although pH may be high and indicate a high degradation rate, other

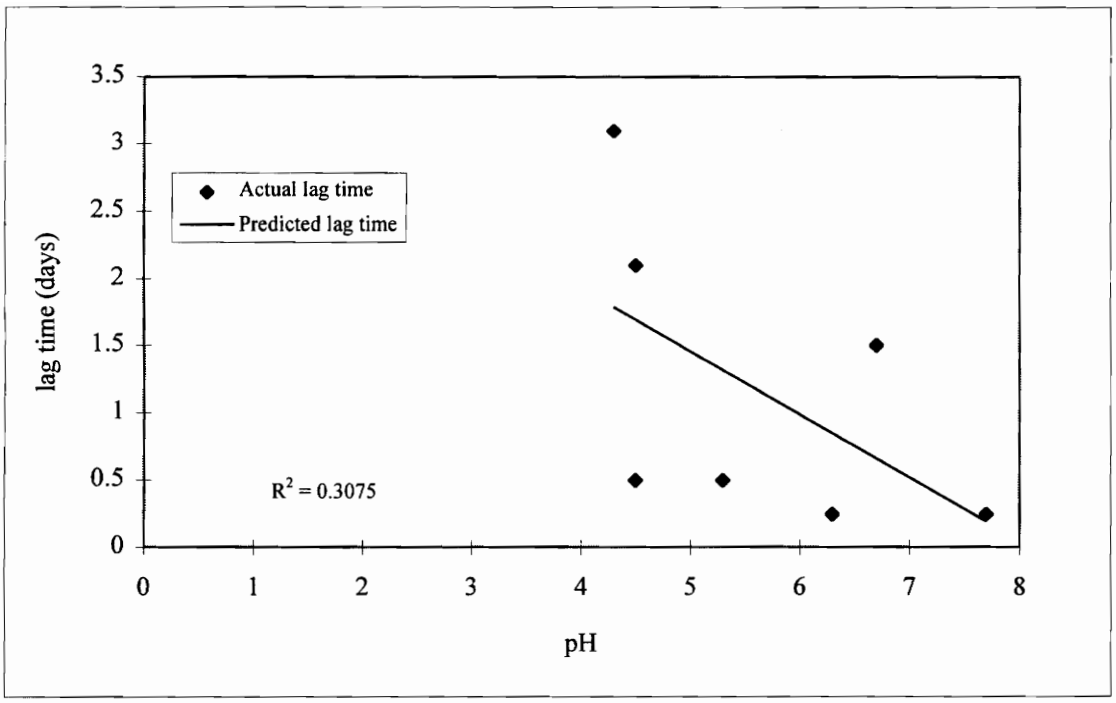

Figure 39. Comparison of predicted **lag times as a function of pH** to actual lag times in uncontaminated soil microcosms amended with nutrients at an initial benzene concentration of 1 ppm.

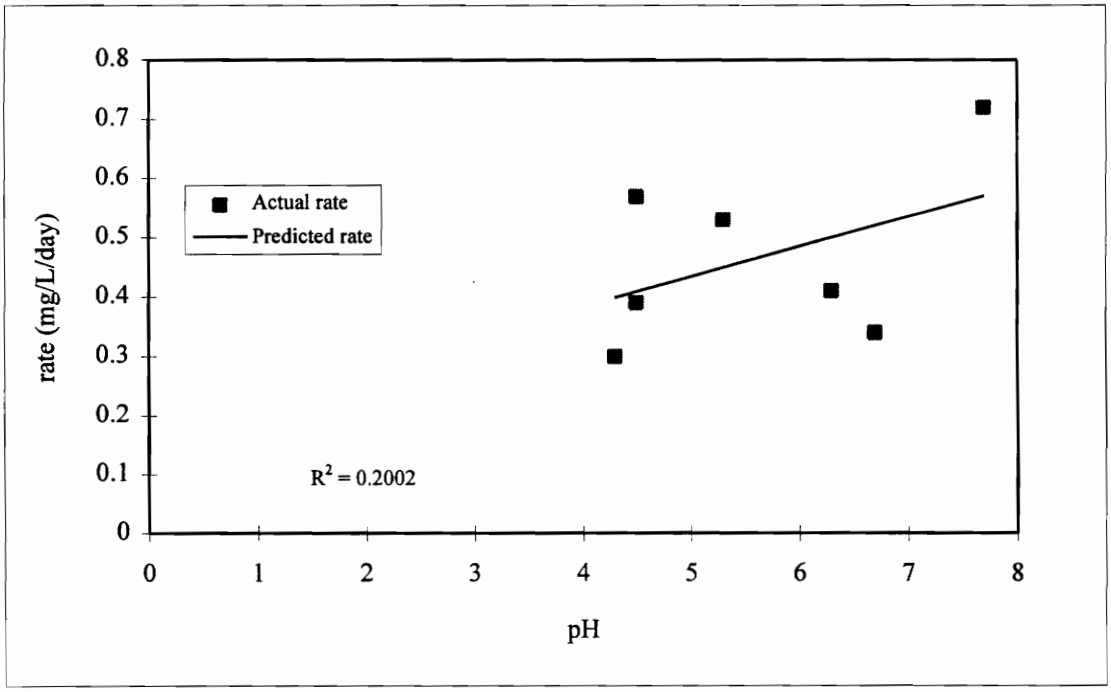

Figure 40. Comparison of predicted **rates as a function of pH** to actual rates in uncontaminated

soil factors such as % sand (or % clay) or high organic matter, may be limiting the degradation rate in a system where pH is not limiting.

These models are only estimates based on the data presented here. In order to prove their validity, they would have to be confirmed with other soil degradation data from a different set of similarly analyzed soil. The model would also need validation through the use of field studies and it would be interesting to see how the model compared when parameters are assessed differently, such as using first order rates instead of zero order. However analyzed, the results do indicate the feasibility of predicting biodegradation rates based on measurable soil properties at an individual site. This would offer tremendous benefits over the currently employed tactic of applying degradation rates generated from laboratory or field data from studies where experimental or soil conditions may differ greatly from those to which they are applied. The analysis of numerous soils in the same fashion as described here and with a wide range of soil characteristics could yield more general and widely applicable models that could be used at contaminated sites where accurate degradation rates are desired.

V. CONCLUSIONS

The purpose of this study was to correlate measurable soil properties with observed benzene biodegradation by indigenous microorganisms in a soil system. The effect of initial benzene concentration as well as nutrient addition on benzene biodegradation was also investigated. The following conclusions were suggested by the research conducted here:

1. For seven Virginia uncontaminated subsurface soils and three contaminated soils, benzene biodegradation by indigenous microorganisms occurred at 1 and 10 ppm with and without the addition of nutrients and at 50 ppm with the addition of nutrients.

2. Nutrient addition in the form of ammonium phosphate and potassium phosphate accelerated benzene biodegradation in all but one soil at 1 ppm initial benzene concentration and in all soils at 10 and 50 ppm initial benzene concentration.

3. With nutrient addition, zero order benzene biodegradation rates increased with increases in initial benzene concentration from 1 to 50 pmm. However, the final time to degrade the substrate also increased, due mainly to longer acclimation periods and more significant tailing at higher initial benzene concentrations.

4. Without nutrient addition, zero order degradation rates increased from 1 to 10 ppm but did not significantly increase in most soils at 50 ppm. Again, however, the final time to degrade the substrate also increased.

5. Contaminated soils tended to have higher degradation rates than uncontaminated soils without the addition of nutrients at an initial benzene concentration of 10 ppm. However, with nutrient addition, degradation rates were similar in all soils, with some uncontaminated soil biodegradation rates being higher than those of contaminated soils.

6. Using an Analysis of Variance F test, statistically significant ($R^2 > 0.90$) multiple linear regression relationships were found between several soil properties and degradation characteristics (lag phase, zero order biodegradation rate and final time to degrade to less than 5 ppb) for initial benzene concentrations of 1 and 10 ppm with the addition of nutrients.

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APPENDIX A: STANDARD CURVES

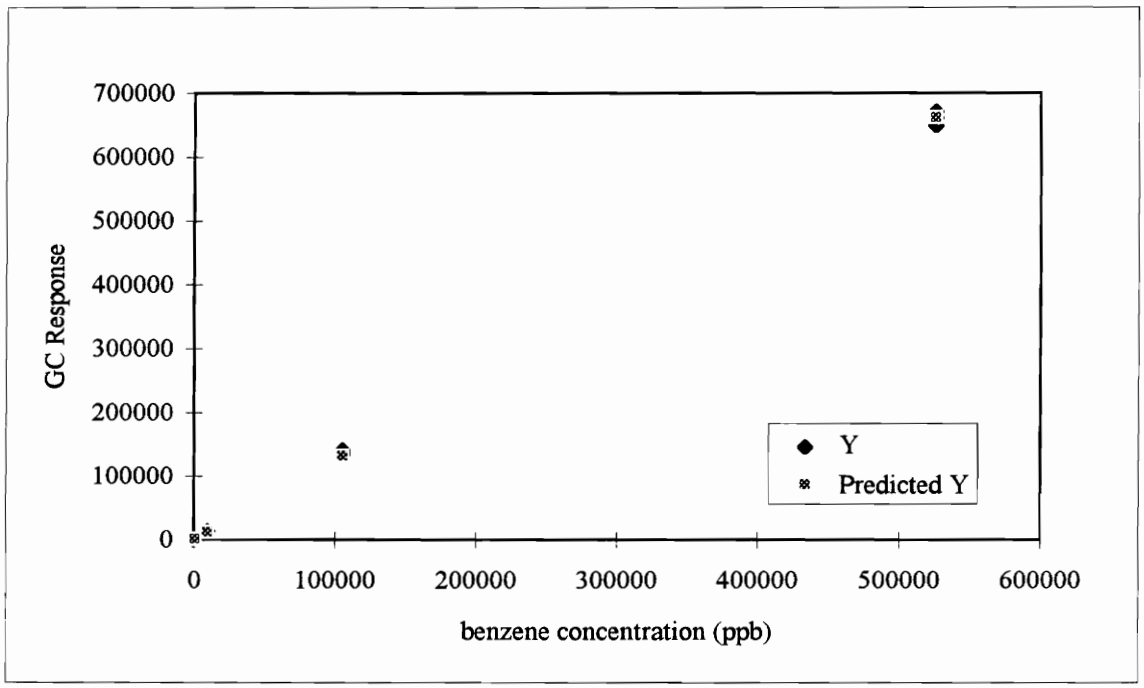


Figure A1. Standard curve for aqueous benzene concentration (no nutrients) vs. GC response; concentration range = 8 ppb to 53000 ppb; conversion factor = 1.26; R^2=0.999

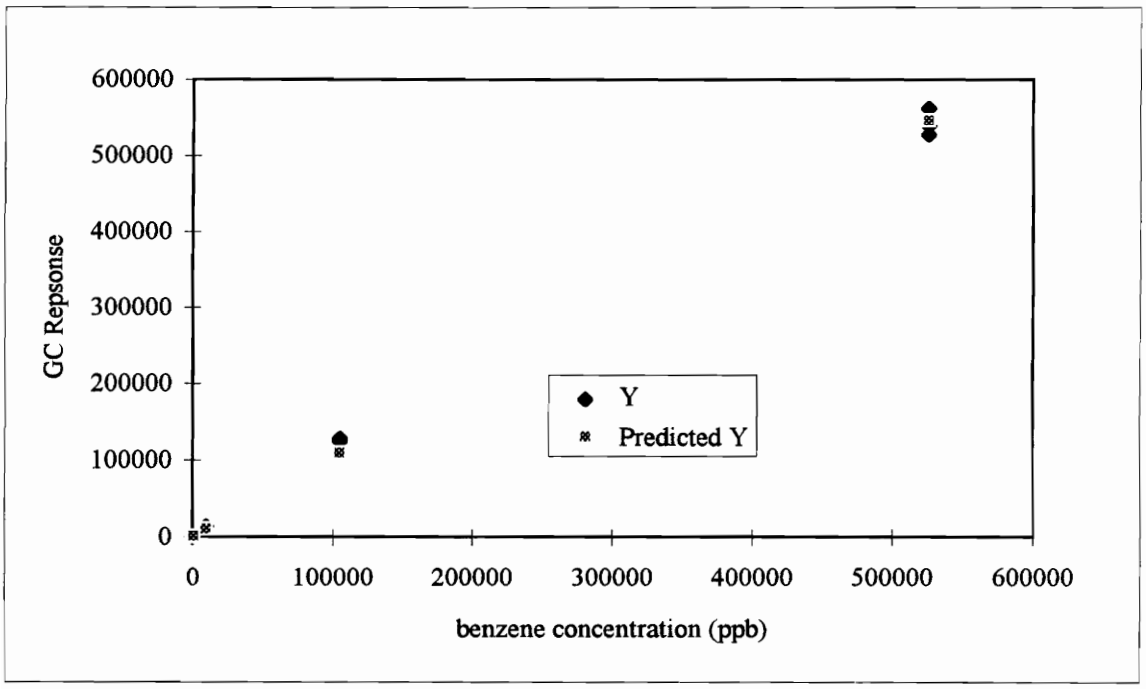


Figure A2. Standard curve for aqueous benzene concentration (with nutrients) vs. GC response; concentration range = 8 ppb to 53000 ppb; conversion factor = 1.04; R^2=0.998

APPENDIX B: UNCONTAMINATED SOIL DATA

Table B1. Bojac soil data at 1 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Conc (ppb)
0	Soln only	7/12/94 18:02	1898.47	0.00	1506.72	0.00	1521
	Soln only	7/12/94 18:02	1794.43	0.00	1424.15	0.86	794
	Soln only	7/12/94 18:02	1785.02	0.00	1416.68	1.86	329
	Soln + soi	7/12/94 18:52	1920.51	0.00	1524.21	2.69	133
	Soln +soil	7/12/94 19:09	1921.32	0.00	1524.86	4.88	8
	Soln +soil	7/12/94 19:25	1907.03	0.00	1513.52	5.68	0
1	1	7/13/94 15:49	1000.59	0.86	794.12	7.45	0
	2	7/13/94 15:49	1046.31	0.86	830.40		
	3	7/13/94 15:49	953.32	0.86	756.60		
2	4	7/14/94 15:53	435.49	1.86	345.63		
	5	7/14/94 15:53	474.03	1.86	376.21		
	6	7/14/94 15:53	332.28	1.86	263.71		
3	7	7/15/94 11:44	184.14	2.69	146.14		
	8	7/15/94 11:44	150.31	2.69	119.29		
	9	7/15/94 11:44	168.28	2.69	133.56		
4	10	7/17/94 16:23	0.00	4.88	0.00		
	11	7/17/94 16:23	20.54	4.88	16.30		
	12	7/17/94 16:23	11.22	4.88	8.90		
5	13	7/18/94 11:31	0.00	5.68	0.00		
	14	7/18/94 11:31	0.00	5.68	0.00		
	15	7/18/94 11:31	0.00	5.68	0.00		
6	16	7/20/94 6:00	0.00	7.45	0.00		
	17	7/20/94 6:00	0.00	7.45	0.00		
	18	7/20/94 6:00	0.00	7.45	0.00		

Table B2. Bojac soil data at 10 ppm initial benzene concentrstion unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Conc (ppb)
0	Soln only	7/27/94 0:00	14560.10	0.00	11555.63	0.00	11518
	Soln only	7/27/94 0:00	14952.80	0.00	11867.30	2.00	8285
	Soln only	7/27/94 0:00	11943.20	0.00	9478.73	3.00	8783
	Soln + soi	7/27/94 0:00	14484.80	0.00	11495.87	5.00	7776
	Soln +soil	7/27/94 0:00	14533.40	0.00	11534.44	6.00	6583
	Soln +soil	7/27/94 0:00	14519.00	0.00	11523.02	8.00	5878
1	1	7/29/94 0:00	11319.20	2.00	8983.49	10.00	5629
	2	7/29/94 0:00	8600.37	2.00	6825.69	13.00	3586
	3	7/29/94 0:00	11399.00	2.00	9046.83	15.00	1027
2	4	7/30/94 0:00	11533.90	3.00	9153.89	20.00	842
	5	7/30/94 0:00	10904.00	3.00	8653.97	34.00	0
	6	7/30/94 0:00	10761.90	3.00	8541.19		
3	7	8/1/94 0:00	9738.12	5.00	7728.67		
	8	8/1/94 0:00	9883.65	5.00	7844.17		
	9	8/1/94 0:00	9771.75	5.00	7755.36		
4	10	8/2/94 0:00	8093.71	6.00	6423.58		
	11	8/2/94 0:00	7860.96	6.00	6238.86		
	12	8/2/94 0:00	8930.88	6.00	7088.00		
5	13	8/4/94 0:00	7242.10	8.00	5747.70		
	14	8/4/94 0:00	7835.61	8.00	6218.74		
	15	8/4/94 0:00	7139.74	8.00	5666.46		
6	16	8/6/94 0:00	6805.76	10.00	5401.40		
	17	8/6/94 0:00	7435.84	10.00	5901.46		
	18	8/6/94 0:00	7036.96	10.00	5584.89		
7	19	8/9/94 0:00	4350.40	13.00	3452.70		
	20	8/9/94 0:00	4686.82	13.00	3719.70		
	21	8/9/94 0:00		13.00			
8	22	8/11/94 0:00	0.00	15.00	0.00		
	23	8/11/94 0:00	2719.51	15.00	2158.34		
	24	8/11/94 0:00	1162.12	15.00	922.32		
9	25	8/16/94 0:00	405.51	20.00	321.83		
	26	8/16/94 0:00	2245.41	20.00	1782.07		
	27	8/16/94 0:00	531.25	20.00	421.63		
10	28	8/30/94 0:00	0.00	34.00	0.00		
	29	8/30/94 0:00	0.00	34.00	0.00		
	30	8/30/94 0:00	0.00	34.00	0.00		

Table B3. Bojac soil data at 50 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Conc (ppb)
0	Soln only	8/19/94 20:58		0.00	0.00	0.00	52463
	Soln only	8/19/94 20:58		0.00	0.00	2.49	49801
	Soln only	8/19/94 20:58		0.00	0.00	5.97	50245
	Soln + soi	8/19/94 20:58	68559.40	0.00	54412.22	9.53	53520
	Soln +soil	8/19/94 20:58	65056.60	0.00	51632.22	16.99	42431
	Soln +soil	8/19/94 20:58	64695.10	0.00	51345.32	21.76	30777
1	1	8/22/94 8:37	62041.70	2.49	49239.44	34.73	23877
	2	8/22/94 8:37	63398.10	2.49	50315.95	41.75	21098
	3	8/22/94 8:37	62808.60	2.49	49848.10		
2	4	8/25/94 20:18	62614.80	5.97	49694.29		
	5	8/25/94 20:18	64910.70	5.97	51516.43		
	6	8/25/94 20:18	62399.80	5.97	49523.65		
3	7	8/29/94 9:42	59655.80	9.53	47345.87		
	8	8/29/94 9:42	70416.50	9.53	55886.11		
	9	8/29/94 9:42	72231.50	9.53	57326.59		
4	10	9/5/94 20:47	57492.90	16.99	45629.29		
	11	9/5/94 20:47	46909.00	16.99	37229.37		
	12	9/5/94 20:47	55989.00	16.99	44435.71		
5	13	9/10/94 15:07	58679.60	21.76	31718.70		
	14	9/10/94 15:07	56490.60	21.76	30535.46		
	15	9/10/94 15:07	55644.60	21.76	30078.16		
6	16	9/23/94 14:30	44904.90	34.73	24272.92		
	17	9/23/94 14:30		34.73			
	18	9/23/94 14:30	43440.40	34.73	23481.30		
7	19	9/30/94 15:01	42948.10	41.75	23215.19		
	20	9/30/94 15:01	29497.90	41.75	15944.81		
	21	9/30/94 15:01	44646.60	41.75	24133.30		

Table B4. Bojac soil data at 1 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Conc (ppb)
0	Soln only	7/18/94 15:48	1390.99	0.00	1337.49	0.00	1282
	Soln only	7/18/94 15:48	1356.61	0.00	1304.43	1.21	813
	Soln only	7/18/94 15:48	1371.71	0.00	1318.95	2.33	52
	Soln + soi	7/18/94 17:28	1303.62	0.00	1253.48	3.80	0
	Soln +soil	7/18/94 17:28	1346.41	0.00	1294.63	4.99	0
	Soln +soil	7/18/94 17:28	1351.33	0.00	1299.36	6.94	0
1	1	7/19/94 22:28	764.70	1.21	735.29		
	2	7/19/94 22:28	890.89	1.21	856.63		
	3	7/19/94 22:28	879.99	1.21	846.14		
2	4	7/21/94 1:29	69.40	2.33	66.73		
	5	7/21/94 1:29	54.31	2.33	52.22		
	6	7/21/94 1:29	38.78	2.33	37.29		
3	7	7/22/94 12:34	0.00	3.80	0.00		
	8	7/22/94 12:34	0.00	3.80	0.00		
	9	7/22/94 12:34	0.00	3.80	0.00		
4	10	7/23/94 17:18	0.00	4.99	0.00		
	11	7/23/94 17:18	0.00	4.99	0.00		
	12	7/23/94 17:18	0.00	4.99	0.00		
5	13	7/25/94 16:01	0.00	6.94	0.00		
	14	7/25/94 16:01	0.00	6.94	0.00		
	15	7/25/94 16:01	0.00	6.94	0.00		

Table B5. Bojac soil data at 10 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Conc (ppb)
0	Soln only	8/9/94 11:34	14441.70	0.00	13886.25	0.00	12450
	Soln only	8/9/94 11:34	14162.20	0.00	13617.50	1.38	9607
	Soln only	8/9/94 11:34		0.00		3.38	39
	Soln + soi	8/9/94 8:00	12806.40	0.00	12313.85	4.36	11
	Soln +soil	8/9/94 8:00	13089.40	0.00	12585.96	6.36	0
	Soln +soil	8/9/94 8:00		0.00		10.37	0
1	1	8/10/94 17:00	9432.36	1.38	9069.58	20.67	0
	2	8/10/94 17:00	10357.50	1.38	9959.13		
	3	8/10/94 17:00	10184.10	1.38	9792.40		
2	4	8/12/94 17:00	30.79	3.38	29.61		
	5	8/12/94 17:00	29.63	3.38	28.49		
	6	8/12/94 17:00	62.23	3.38	59.84		
3	7	8/13/94 16:33	10.41	4.36	10.01		
	8	8/13/94 16:33	14.00	4.36	13.46		
	9	8/13/94 16:33	9.02	4.36	8.67		
4	10	8/15/94 16:44	0.00	6.36	0.00		
	11	8/15/94 16:44	0.00	6.36	0.00		
	12	8/15/94 16:44	0.00	6.36	0.00		
5	13	8/19/94 16:47	0.00	10.37	0.00		
	14	8/19/94 16:47	0.00	10.37	0.00		
	15	8/19/94 16:47	0.00	10.37	0.00		
6	16	8/30/94 0:00	0.00	20.67	0.00		
	17	8/30/94 0:00	0.00	20.67	0.00		
	18	8/30/94 0:00	0.00	20.67	0.00		

Table B6. Bojac soil data at 50 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Conc (ppb)
0	Soln only	9/3/94 1:44	57274.30	0.00	55071.44	0.00	54934
	Soln only	9/3/94 1:44	50158.70	0.00	48229.52	1.50	51843
	Soln only	9/3/94 1:44		0.00		4.40	49142
	Soln + soi	9/3/94 1:44	56894.30	0.00	54706.06	6.50	48205
	Soln +soil	9/3/94 1:44	57489.70	0.00	55278.56	8.40	29672
	Soln +soil	9/3/94 1:44	57008.60	0.00	54815.96	10.71	31430
1	1	9/4/94 13:50	50626.00	1.50	48678.85	13.57	7512
	2	9/4/94 13:50	54225.80	1.50	52140.19	17.62	6
	3	9/4/94 13:50	56899.10	1.50	54710.67	32.93	0
2	4	9/7/94 11:25	50828.00	4.40	48873.08		
	5	9/7/94 11:25	51117.00	4.40	49150.96		
	6	9/7/94 11:25	51377.70	4.40	49401.63		
3	7	9/9/94 13:43	50570.60	6.50	48625.58		
	8	9/9/94 13:43	50955.50	6.50	48995.67		
	9	9/9/94 13:43	48874.80	6.50	46995.00		
4	10	9/11/94 11:17	40758.10	8.40	39190.48		
	11	9/11/94 11:17	4663.50	8.40	4484.13		
	12	9/11/94 11:17	47156.30	8.40	45342.60		
5	13	9/13/94 18:52	32788.50	10.71	31527.40		
	14	9/13/94 18:52	2652.00	10.71	2550.00		
	15	9/13/94 18:52	62620.60	10.71	60212.12		
6	16	9/16/94 15:32	23413.50	13.57	22512.98		
	17	9/16/94 15:32	10.59	13.57	10.18		
	18	9/16/94 15:32	13.52	13.57	13.00		
7	19	9/20/94 16:40	10.68	17.62	10.27		
	20	9/20/94 16:40	7.54	17.62	7.25		
	21	9/20/94 16:40	0.00	17.62	0.00		
8	22	10/6/94 0:00	0.00	32.93	0.00		
	23	10/6/94 0:00	0.00	32.93	0.00		
	24	10/6/94 0:00	0.00	32.93	0.00		

Table B7. Catpoint soil data at 1 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	7/12/94 18:02	1898.47	0.00	1506.72	0.00	1521
	Soln only	7/12/94 18:02	1794.43	0.00	1424.15	0.76	705
	Soln only	7/12/94 18:02	1785.02	0.00	1416.68	1.79	751
	Soln + soi	7/12/94 19:59	1920.51	0.00	1524.21	2.69	811
	Soln +soil	7/12/94 19:59	1921.32	0.00	1524.86	4.93	498
	Soln +soil	7/12/94 19:59	1907.03	0.00	1513.52	5.72	449
1	1	7/13/94 14:08	954.90	0.76	757.86	7.56	215
	2	7/13/94 14:08	752.33	0.76	597.09	8.66	80
	3	7/13/94 14:08	957.40	0.76	759.84	19.17	0
2	4	7/14/94 15:03	787.30	1.79	624.84		
	5	7/14/94 15:03	966.12	1.79	766.76		
	6	7/14/94 15:03	1085.83	1.79	861.77		
3	7	7/15/94 12:34	986.89	2.69	783.25		
	8	7/15/94 12:34	1042.89	2.69	827.69		
	9	7/15/94 12:34	1037.29	2.69	823.25		
4	10	7/17/94 18:25	617.38	4.93	489.98		
	11	7/17/94 18:25	736.33	4.93	584.39		
	12	7/17/94 18:25	527.90	4.93	418.97		
5	13	7/18/94 13:11	817.59	5.72	648.88		
	14	7/18/94 13:11	590.99	5.72	469.04		
	15	7/18/94 13:11	287.47	5.72	228.15		
6	16	7/20/94 9:19	167.54	7.56	132.97		
	17	7/20/94 9:19	131.02	7.56	103.98		
	18	7/20/94 9:19	512.41	7.56	406.67		
7	19	7/21/94 11:50	98.64	8.66	78.29		
	20	7/21/94 11:50	92.20	8.66	73.17		
	21	7/21/94 11:50	111.05	8.66	88.13		
8	22	8/1/94 0:00	0.00	19.17	0.00		
	23	8/1/94 0:00	0.00	19.17	0.00		
	24	8/1/94 0:00	0.00	19.17	0.00		

Table B8. Catpoint soil data at 10 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	7/27/94 0:00	14560.10	0.00	11555.63	0.00	11957
	Soln only	7/27/94 0:00	14952.80	0.00	11867.30	2.00	10708
	Soln only	7/27/94 0:00	11943.20	0.00	9478.73	4.00	10853
	Soln +soil	7/27/94 0:00	15125.60	0.00	12004.44	6.00	10476
	Soln +soil	7/27/94 0:00	15151.60	0.00	12025.08	8.00	9775
	Soln +soil	7/27/94 0:00	14919.50	0.00	11840.87	12.00	8433
1	1	7/29/94 0:00	13879.80	2.00	11015.71	15.00	7737
	2	7/29/94 0:00	13551.00	2.00	10754.76	20.00	6967
	3	7/29/94 0:00	13044.80	2.00	10353.02	34.00	5834
2	4	7/31/94 0:00	14111.50	4.00	11199.60		
	5	7/31/94 0:00	14027.20	4.00	11132.70		
	6	7/31/94 0:00	12885.30	4.00	10226.43		
3	7	8/2/94 0:00	13335.40	6.00	10583.65		
	8	8/2/94 0:00	13503.50	6.00	10717.06		
	9	8/2/94 0:00	12761.00	6.00	10127.78		
4	10	8/4/94 0:00	12066.40	8.00	9576.51		
	11	8/4/94 0:00	12011.60	8.00	9533.02		
	12	8/4/94 0:00	12869.90	8.00	10214.21		
5	13	8/8/94 0:00	9945.11	12.00	7892.94		
	14	8/8/94 0:00	11867.00	12.00	9418.25		
	15	8/8/94 0:00	10066.20	12.00	7989.05		
6	16	8/11/94 0:00	11970.40	15.00	9500.32		
	17	8/11/94 0:00	7834.66	15.00	6217.98		
	18	8/11/94 0:00	9441.43	15.00	7493.20		
7	19	8/16/94 0:00	6581.35	20.00	5223.29		
	20	8/16/94 0:00	8528.06	20.00	6768.30		
	21	8/16/94 0:00	11225.60	20.00	8909.21		
8	22	8/30/94 0:00	9189.70	34.00	7293.41		
	23	8/30/94 0:00	4786.66	34.00	3798.94		
	24	8/30/94 0:00	8076.17	34.00	6409.66		

Table B9. Catpoint soil data at 50 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	8/19/94 21:48		0.00		0.00	52649
	Soln only	8/19/94 21:48		0.00		2.51	49297
	Soln only	8/19/94 21:48		0.00		5.97	50084
	Soln +soil	8/19/94 21:48	65927.30	0.00	52323.25	9.66	50773
	Soln +soil	8/19/94 21:48	66519.30	0.00	52793.10	17.05	42536
	Soln +soil	8/19/94 21:48	66565.20	0.00	52829.52	22.21	34568
1	1	8/22/94 10:01	63423.10	2.51	50335.79	26.92	36413
	2	8/22/94 10:01	61375.90	2.51	48711.03	34.77	35295
	3	8/22/94 10:01	61544.90	2.51	48845.16	41.82	22080
2	4	8/25/94 21:00	64829.20	5.97	51451.75		
	5	8/25/94 21:00	59612.90	5.97	47311.83		
	6	8/25/94 21:00	64873.70	5.97	51487.06		
3	7	8/29/94 13:34	64324.30	9.66	51051.09		
	8	8/29/94 13:34	65045.10	9.66	51623.10		
	9	8/29/94 13:34	62550.90	9.66	49643.57		
4	10	9/5/94 23:06	57572.80	17.05	45692.70		
	11	9/5/94 23:06	51467.00	17.05	40846.83		
	12	9/5/94 23:06	51746.70	17.05	41068.81		
5	13	9/11/94 2:51	29413.10	22.21	23343.73		
	14	9/11/94 2:51	49679.30	22.21	39428.02		
	15	9/11/94 2:51	51575.10	22.21	40932.62		
6	16	9/15/94 19:58	41996.10	26.92	33330.24		
	17	9/15/94 19:58	47195.80	26.92	37456.98		
	18	9/15/94 19:58	48447.80	26.92	38450.63		
7	19	9/23/94 16:12	46278.30	34.77	36728.97		
	20	9/23/94 16:12	44296.80	34.77	35156.19		
	21	9/23/94 16:12	42840.30	34.77	34000.24		
8	22	9/30/94 17:32	38034.70	41.82	30186.27		
	23	9/30/94 17:32	8883.96	41.82	7050.76		
	24	9/30/94 17:32	36545.10	41.82	29004.05		

Table B10 Catpoint soil data at 1 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentratio (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	7/18/94 15:48	1390.99	0.00	1337.49	0.00	1362
	Soln only	7/18/94 15:48	1356.61	0.00	1304.43	1.21	417
	Soln only	7/18/94 15:48	1371.71	0.00	1318.95	2.33	31
	Soln + soi	7/18/94 19:59	1417.40	0.00	1362.88	4.82	0
	Soln +soil	7/18/94 19:59	1406.25	0.00	1352.16	6.97	0
	Soln +soil	7/18/94 19:59	1426.42	0.00	1371.56	8.17	0
1	1	7/20/94 0:58	500.61	1.21	481.36		
	2	7/20/94 0:58	457.52	1.21	439.92		
	3	7/20/94 0:58	342.44	1.21	329.27		
2	4	7/21/94 4:01	0.00	2.33	0.00		
	5	7/21/94 4:01	0.00	2.33	0.00		
	6	7/21/94 4:01	97.51	2.33	93.76		
3	7	7/23/94 15:38	0.00	4.82	0.00		
	8	7/23/94 15:38	0.00	4.82	0.00		
	9	7/23/94 15:38	0.00	4.82	0.00		
4	10	7/25/94 19:22	0.00	6.97	0.00		
	11	7/25/94 19:22	0.00	6.97	0.00		
	12	7/25/94 19:22	0.00	6.97	0.00		
5	13	7/27/94 0:00	0.00	8.17	0.00		
	14	7/27/94 0:00	0.00	8.17	0.00		
	15	7/27/94 0:00	0.00	8.17	0.00		

Table B11 Catpoint soil data at 10 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentratio (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	8/9/94 8:00	14441.70	0.00	13886.25	0.00	13311
	Soln only	8/9/94 8:00	14162.20	0.00	13617.50	0.38	14120
	Soln only	8/9/94 8:00		0.00		2.00	13118
	Soln + soi	8/10/94 8:00	13944.20	0.00	13407.88	4.40	4
	Soln +soil	8/10/94 8:00	13742.30	0.00	13213.75	5.19	0
	Soln +soil	8/10/94 8:00		0.00		9.30	0
1	1	8/10/94 17:00	15004.00	0.38	14426.92		
	2	8/10/94 17:00	15024.50	0.38	14446.63		
	3	8/10/94 17:00	14024.80	0.38	13485.38		
2	4	8/12/94 8:00	14169.40	2.00	13624.42		
	5	8/12/94 8:00	12904.00	2.00	12407.69		
	6	8/12/94 8:00	13855.60	2.00	13322.69		
3	7	8/14/94 17:43	12.08	4.40	11.62		
	8	8/14/94 17:43	0.00	4.40	0.00		
	9	8/14/94 17:43	0.00	4.40	0.00		
4	10	8/15/94 12:33	0.00	5.19	0.00		
	11	8/15/94 12:33	0.00	5.19	0.00		
	12	8/15/94 12:33	0.00	5.19	0.00		
5	13	8/19/94 15:06	0.00	9.30	0.00		
	14	8/19/94 15:06	0.00	9.30	0.00		
	15	8/19/94 15:06	0.00	9.30	0.00		

Table B12 Catpoint soil data at 50 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentratio (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	9/3/94 0:54	57274.30	0.00	55071.44	0.00	54499
	Soln only	9/3/94 0:54	50158.70	0.00	48229.52	1.61	60765
	Soln only	9/3/94 0:54		0.00		4.53	2482
	Soln + soi	9/3/94 0:54	56873.40	0.00	54685.96	5.96	20
	Soln +soil	9/3/94 0:54	56484.80	0.00	54312.31	7.87	8
	Soln +soil	9/3/94 0:54	57947.90	0.00		9.64	0
1	1	9/4/94 15:31	63339.00	1.61	60902.88	12.96	0
	2	9/4/94 15:31	63349.30	1.61	60912.79	26.96	0
	3	9/4/94 15:31	62897.60	1.61	60478.46		
2	4	9/7/94 13:39	6645.35	4.53	6389.76		
	5	9/7/94 13:39	999.80	4.53	961.35		
	6	9/7/94 13:39	99.55	4.53	95.72		
3	7	9/8/94 23:58	24.34	5.96	23.40		
	8	9/8/94 23:58	12.87	5.96	12.38		
	9	9/8/94 23:58	23.99	5.96	23.07		
4	10	9/10/94 21:51	16.46	7.87	15.83		
	11	9/10/94 21:51	6.96	7.87	6.69		
	12	9/10/94 21:51	0.00	7.87	0.00		
5	13	9/12/94 16:09	0.00	9.64	0.00		
	14	9/12/94 16:09	0.00	9.64	0.00		
	15	9/12/94 16:09	0.00	9.64	0.00		
6	16	9/16/94 0:00	0.00	12.96	0.00		
	17	9/16/94 0:00	0.00	12.96	0.00		
	18	9/16/94 0:00	0.00	12.96	0.00		
7	19	9/30/94 0:00	0.00	26.96	0.00		
	20	9/30/94 0:00	0.00	26.96	0.00		
	21	9/30/94 0:00	0.00	26.96	0.00		

Table B1 Leedsville soil data at 1 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	7/12/94 18:02	1898.47	0.00	1506.72	0.00	1347
	Soln only	7/12/94 18:02	1794.43	0.00	1424.15	0.73	1252
	Soln only	7/12/94 18:02	1785.02	0.00	1416.68	1.70	1233
	Soln + soi	7/12/94 21:22	1657.48	0.00	1315.46	2.77	1187
	Soln +soil	7/12/94 21:22	1724.74	0.00	1368.84	4.95	1027
	Soln +soil	7/12/94 21:22	1710.32	0.00	1357.40	5.73	1072
1	1	7/13/94 14:58	1563.68	0.73	1241.02	7.57	808
	2	7/13/94 14:58	1604.05	0.73	1273.06	8.64	632
	3	7/13/94 14:58	1564.50	0.73	1241.67	19.11	0
2	4	7/14/94 14:13	1568.75	1.70	1245.04		
	5	7/14/94 14:13	1531.66	1.70	1215.60		
	6	7/14/94 14:13	1561.03	1.70	1238.91		
3	7	7/15/94 15:55	1484.99	2.77	1178.56		
	8	7/15/94 15:55	1483.03	2.77	1177.01		
	9	7/15/94 15:55	1520.53	2.77	1206.77		
4	10	7/17/94 20:06	1294.56	4.95	1027.43		
	11	7/17/94 20:06	--	4.95			
	12	7/17/94 20:06	--	4.95			
5	13	7/18/94 14:52	1374.68	5.73	1091.02		
	14	7/18/94 14:52	1426.84	5.73	1132.41		
	15	7/18/94 14:52	1250.59	5.73	992.53		
6	16	7/20/94 10:59	953.16	7.57	756.48		
	17	7/20/94 10:59	835.49	7.57	663.09		
	18	7/20/94 10:59	1265.62	7.57	1004.46		
7	19	7/21/94 12:40	799.40	8.64	634.44		
	20	7/21/94 12:40	922.34	8.64	732.02		
	21	7/21/94 12:40	667.05	8.64	529.40		
8	22	8/1/94 0:00	0.00	19.11	0.00		
	23	8/1/94 0:00	0.00	19.11	0.00		
	24	8/1/94 0:00	0.00	19.11	0.00		

Table B1 Leedsville soil data at 10 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)	
0	Soln only	7/27/94 0:00	14560.10	0.00	11555.63	0.00	10786	
	Soln only	7/27/94 0:00	14952.80	0.00	11867.30	2.00	10446	
	Soln only	7/27/94 0:00	11943.20	0.00	9478.73	4.00	10914	
	Soln + soi	7/27/94 0:00	13656.90	0.00	10838.81	6.00	8940	
	Soln +soil	7/27/94 0:00	13526.90	0.00	10735.63	8.00	5861	
	Soln +soil	7/27/94 0:00	13588.20	0.00	10784.29	10.00	6274	
1	1	7/29/94 0:00	13138.20	2.00	10427.14	15.71	810	
	2	7/29/94 0:00	13166.60	2.00	10449.68	20.62	269	
	3	7/29/94 0:00	13182.60	2.00	10462.38	34.88	359	
2	4	7/31/94 0:00	13850.70	4.00	10992.62			
	5	7/31/94 0:00	13651.10	4.00	10834.21			
	6	7/31/94 0:00	13752.80	4.00	10914.92			
3	7	8/2/94 0:00	9368.58	6.00	7435.38			
	8	8/2/94 0:00	12789.70	6.00	10150.56			
	9	8/2/94 0:00	11634.20	6.00	9233.49			
4	10	8/4/94 0:00	9388.35	8.00	7451.07			
	11	8/4/94 0:00	8776.12	8.00	6965.17			
	12	8/4/94 0:00	3988.51	8.00	3165.48			
5	13	8/6/94 0:00	1374.68	10.00	5425.75		2nd run	
	14	8/6/94 0:00	1426.84	10.00	8858.91		2nd run	
	15	8/6/94 0:00	1250.59	10.00	4538.08		2nd run	
6	16	8/11/94 17:00	2041.95	15.71	1620.60			
	17	8/11/94 17:00	0.00	15.71	0.00			
	18	8/11/94 17:00		15.71				
7	19	8/16/94 14:52	0.00	20.62	0.00			
	20	8/16/94 14:52	39.98	20.62	31.73			
	21	8/16/94 14:52	978.09	20.62	776.26			
8	22	8/30/94 21:04	0.00	34.88	0.00			
	23	8/30/94 21:04	0.00	34.88	0.00			
	24	8/30/94 21:04	1355.20	34.88	1075.56			

Table B1 Leedsville soil data at 50 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)	
0	Soln only	8/19/94 20:07		0.00		0.00	51788	
	Soln only	8/19/94 20:07		0.00		2.66	51640	
	Soln only	8/19/94 20:07		0.00		9.76	48016	
	Soln + soi	8/19/94 20:07	65895.50	0.00	52298.02	17.17	38825	
	Soln +soil	8/19/94 20:07	65194.30	0.00	51741.51	22.32	45046	
	Soln +soil	8/19/94 20:07	64667.50	0.00	51323.41	34.94	6274	
1	1	8/22/94 11:58	64832.40	2.66	51454.29	41.93	35941	
	2	8/22/94 11:58	65843.20	2.66	52256.51			
	3	8/22/94 11:58	64525.40	2.66	51210.63			
2	4	8/29/94 14:24	60185.40	9.76	47766.19			
	5	8/29/94 14:24	59873.90	9.76	47518.97			
	6	8/29/94 14:24	61440.30	9.76	48762.14			
3	7	9/6/94 0:13	54618.20	17.17	43347.78			
	8	9/6/94 0:13	43221.30	17.17	34302.62			
	9	9/6/94 0:13		17.17				
4	10	9/11/94 3:42	56994.00	22.32	45233.33			
	11	9/11/94 3:42	57415.50	22.32	45567.86			
	12	9/11/94 3:42	55865.80	22.32	44337.94			
5	13	9/23/94 18:43	49437.20	34.94	5425.75		2nd run	
	14	9/23/94 18:43	48798.40	34.94	8858.91		2nd run	
	15	9/23/94 18:43	50120.00	34.94	4538.08		2nd run	
6	16	9/30/94 18:23	44114.60	41.93	35011.59			
	17	9/30/94 18:23	43545.90	41.93	34560.24			
	18	9/30/94 18:23	48195.60	41.93	38250.48			
7	19	9/30/94 18:23		41.93				
	20	9/30/94 18:23		41.93				
	21	9/30/94 18:23		41.93				
8	22	9/30/94 18:23		41.93				
	23	9/30/94 18:23		41.93				
	24	9/30/94 18:23		41.93				

Table B16 Leedsville soil data at 1 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	7/18/94 15:48	1390.99	0.00	1337.49	0.00	1308
	Soln only	7/18/94 15:48	1356.61	0.00	1304.43	1.24	1343
	Soln only	7/18/94 15:48	1371.71	0.00	1318.95	2.40	1160
	Soln + soi	7/18/94 20:49	1376.67	0.00	1323.72	3.07	1184
	Soln +soil	7/18/94 20:49	1330.69	0.00	1279.51	4.99	263
	Soln +soil	7/18/94 20:49	1373.64	0.00	1320.81	7.01	0
1	1	7/20/94 2:39	1420.73	1.24	1366.09	8.13	0
	2	7/20/94 2:39	1415.99	1.24	1361.53	9.13	0
	3	7/20/94 2:39	1352.63	1.24	1300.61		
2	4	7/21/94 6:31	1332.91	2.40	1281.64		
	5	7/21/94 6:31	1024.05	2.40	984.66		
	6	7/21/94 6:31	1262.36	2.40	1213.81		
3	7	7/21/94 22:30	1216.27	3.07	1169.49		
	8	7/21/94 22:30	1219.38	3.07	1172.48		
	9	7/21/94 22:30	1258.05	3.07	1209.66		
4	10	7/23/94 20:39	218.65	4.99	210.24		
	11	7/23/94 20:39	405.16	4.99	389.58		
	12	7/23/94 20:39	197.93	4.99	190.32		
5	13	7/25/94 21:02	0.00	7.01	0.00		
	14	7/25/94 21:02	0.00	7.01	0.00		
	15	7/25/94 21:02	0.00	7.01	0.00		
6	16	7/27/94 0:00	0.00	8.13	0.00		
	17	7/27/94 0:00	0.00	8.13	0.00		
	18	7/27/94 0:00	0.00	8.13	0.00		
7	19	7/28/94 0:00	0.00	9.13	0.00		
	20	7/28/94 0:00	0.00	9.13	0.00		
	21	7/28/94 0:00	0.00	9.13	0.00		

Table B17 Leedsville soil data at 10 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	8/10/94 17:00	14441.70	0.00	13886.25	0.00	11268
	Soln only	8/10/94 17:00	14162.20	0.00	13617.50	2.00	11067
	Soln only	8/10/94 17:00		0.00		4.10	5975
	Soln +soil	8/10/94 17:00	11652.30	0.00	11204.13	5.04	3188
	Soln +soil	8/10/94 17:00	11727.40	0.00	11276.35	6.88	0
	Soln +soil	8/10/94 17:00	11776.70	0.00	11323.75	8.89	0
1	1	8/12/94 17:00	11555.50	2.00	11111.06	19.87	0
	2	8/12/94 17:00	11471.00	2.00	11029.81		
	3	8/12/94 17:00	11502.50	2.00	11060.10		
2	4	8/14/94 19:23	3327.43	4.10	3199.45		
	5	8/14/94 19:23	6199.48	4.10	5961.04		
	6	8/14/94 19:23	9115.88	4.10	8765.27		
3	7	8/15/94 17:54	1016.80	5.04	977.69		
	8	8/15/94 17:54	4829.86	5.04	4644.10		
	9	8/15/94 17:54	4099.79	5.04	3942.11		
4	10	8/17/94 14:01	0.00	6.88	0.00		
	11	8/17/94 14:01	0.00	6.88	0.00		
	12	8/17/94 14:01	0.00	6.88	0.00		
5	13	8/19/94 14:16	0.00	8.89	0.00		
	14	8/19/94 14:16	0.00	8.89	0.00		
	15	8/19/94 14:16	0.00	8.89	0.00		
6	16	8/30/94 13:52	0.00	19.87	0.00		
	17	8/30/94 13:52	0.00	19.87	0.00		
	18	8/30/94 13:52	0.00	19.87	0.00		

Table B18 Leedsville soil data at 50 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	9/2/94 23:13	57274.30	0.00	55071.44	0.00	51175
	Soln only	9/2/94 23:13	50158.70	0.00	48229.52	1.71	57428
	Soln only	9/2/94 23:13		0.00		4.99	58650
	Soln +soil	9/2/94 23:13	55646.80	0.00	53506.54	6.97	57307
	Soln +soil	9/2/94 23:13	50749.50	0.00	48797.60	8.57	57406
	Soln +soil	9/2/94 23:13	53270.80	0.00	51221.92	10.85	49878
1	1	9/4/94 16:21	59312.90	1.71	57031.63	13.78	34283
	2	9/4/94 16:21	57985.00	1.71	55754.81	18.51	12343
	3	9/4/94 16:21	61877.40	1.71	59497.50	33.03	3
2	4	9/7/94 22:52	61781.00	4.99	59404.81		
	5	9/7/94 22:52	59951.70	4.99	57645.87		
	6	9/7/94 22:52	61254.10	4.99	58898.17		
3	7	9/9/94 22:24	57393.00	6.97	55185.58		
	8	9/9/94 22:24	60998.80	6.97	58652.69		
	9	9/9/94 22:24	60406.30	6.97	58082.98		
4	10	9/11/94 12:57	57461.00	8.57	55250.96		
	11	9/11/94 12:57	60522.30	8.57	58194.52		
	12	9/11/94 12:57	61124.50	8.57	58773.56		
5	13	9/13/94 19:42	57731.60	10.85	55511.15		
	14	9/13/94 19:42	40332.20	10.85	38780.96		
	15	9/13/94 19:42	57556.50	10.85	55342.79		
6	16	9/16/94 18:03	52643.60	13.78	50618.85		
	17	9/16/94 18:03	4533.27	13.78	4358.91		
	18	9/16/94 18:03	49786.50	13.78	47871.63		
7	19	9/21/94 11:25	41.65	18.51	40.05		
	20	9/21/94 11:25	36723.10	18.51	35310.67		
	21	9/21/94 11:25	1745.77	18.51	1678.63		
8	22	10/6/94 0:00		33.03			
	23	10/6/94 0:00	0.00	33.03	0.00		
	24	10/6/94 0:00	7.19	33.03	6.91		

Table B19. Purcellville soil data at 1 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	7/12/94 18:02	1898.47	0.00	1506.72	0.00	1429
	Soln only	7/12/94 18:02	1794.43	0.00	1424.15	0.70	1241
	Soln only	7/12/94 18:02	1785.02	0.00	1416.68	1.70	1142
	Soln + soi	7/12/94 20:32	1793.18	0.00	1423.16	2.70	750
	Soln +soil	7/12/94 20:32	1780.01	0.00	1412.71	4.88	24
	Soln +soil	7/12/94 20:32	1828.45	0.00	1451.15	5.55	10
1	1	7/13/94 13:18	1552.17	0.70	1231.88	7.43	0
	2	7/13/94 13:18	1497.48	0.70	1188.48		
	3	7/13/94 13:18	1640.17	0.70	1301.72		
2	4	7/14/94 13:22	1426.74	1.70	1132.33		
	5	7/14/94 13:22	1390.12	1.70	1103.27		
	6	7/14/94 13:22	1498.17	1.70	1189.02		
3	7	7/15/94 13:24	1052.97	2.70	835.69		
	8	7/15/94 13:24	1052.23	2.70	835.10		
	9	7/15/94 13:24	730.04	2.70	579.40		
4	10	7/17/94 17:35	40.58	4.88	32.21		
	11	7/17/94 17:35	34.20	4.88	27.14		
	12	7/17/94 17:35	14.83	4.88	11.77		
5	13	7/18/94 9:50	15.94	5.55	12.65		
	14	7/18/94 9:50	20.09	5.55	15.94		
	15	7/18/94 9:50	0.00	5.55	0.00		
6	16	7/20/94 6:50	0.00	7.43	0.00		
	17	7/20/94 6:50	0.00	7.43	0.00		
	18	7/20/94 6:50	0.00	7.43	0.00		

Table B20. Purcellville soil data at 10 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	7/27/94 0:00	14560.10	0.00	11555.63	0.00	11587
	Soln only	7/27/94 0:00	14952.80	0.00	11867.30	2.00	8648
	Soln only	7/27/94 0:00	11943.20	0.00	9478.73	4.00	5759
	Soln +soil	7/27/94 0:00	14528.60	0.00	11530.63	6.00	197
	Soln +soil	7/27/94 0:00	14860.00	0.00	11793.65	8.00	0
	Soln +soil	7/27/94 0:00	14408.60	0.00	11435.40	12.00	0
1	1	7/29/94 0:00	10875.30	2.00	8631.19	15.00	0
	2	7/29/94 0:00	11128.70	2.00	8832.30		
	3	7/29/94 0:00	10686.70	2.00	8481.51		
2	4	7/31/94 0:00	8238.56	4.00	6538.54		
	5	7/31/94 0:00	7619.31	4.00	6047.07		
	6	7/31/94 0:00	5910.02	4.00	4690.49		
3	7	8/2/94 0:00	118.09	6.00	93.72		
	8	8/2/94 0:00	432.09	6.00	342.93		
	9	8/2/94 0:00	195.65	6.00	155.28		
4	10	8/4/94 0:00	0.00	8.00	0.00		
	11	8/4/94 0:00	0.00	8.00	0.00		
	12	8/4/94 0:00	0.00	8.00	0.00		
5	13	8/8/94 0:00	0.00	12.00	0.00		
	14	8/8/94 0:00	0.00	12.00	0.00		
	15	8/8/94 0:00	0.00	12.00	0.00		
6	16	8/11/94 0:00	0.00	15.00	0.00		
	17	8/11/94 0:00	0.00	15.00	0.00		
	18	8/11/94 0:00	0.00	15.00	0.00		

Table B21. Purcellville soil data at 1 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	7/18/94 15:48	1390.99	0.00	1337.49	0.00	1390
	Soln only	7/18/94 15:48	1356.61	0.00	1304.43	1.21	1418
	Soln only	7/18/94 15:48	1371.71	0.00	1318.95	2.33	1084
	Soln + soi	7/18/94 18:18	1443.99	0.00	1388.45	4.92	2
	Soln +soil	7/18/94 18:18	1451.44	0.00	1395.62	7.01	0
	Soln +soil	7/18/94 18:18	1442.77	0.00	1387.28	8.24	0
1	1	7/19/94 23:18	1451.51	1.21	1395.68	9.24	0
	2	7/19/94 23:18	1482.22	1.21	1425.21		
	3	7/19/94 23:18	1491.28	1.21	1433.92		
2	4	7/21/94 2:20	1189.86	2.33	1144.10		
	5	7/21/94 2:20	972.75	2.33	935.34		
	6	7/21/94 2:20	1219.20	2.33	1172.31		
3	7	7/23/94 16:28	5.39	4.92	5.18		
	8	7/23/94 16:28	0.00	4.92	0.00		
	9	7/23/94 16:28	0.00	4.92	0.00		
4	10	7/25/94 18:31	0.00	7.01	0.00		
	11	7/25/94 18:31	0.00	7.01	0.00		
	12	7/25/94 18:31	0.00	7.01	0.00		
5	13	7/27/94 0:00	0.00	8.24	0.00		
	14	7/27/94 0:00	0.00	8.24	0.00		
	15	7/27/94 0:00	0.00	8.24	0.00		
6	16	7/28/94 0:00	0.00	9.24	0.00		
	17	7/28/94 0:00	0.00	9.24	0.00		
	18	7/28/94 0:00	0.00	9.24	0.00		

Table B22. Purcellville soil data at 10 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	8/10/94 17:00	14441.70	0.00	13886.25	0.00	13163
	Soln only	8/10/94 17:00	14162.20	0.00	13617.50	2.00	13909
	Soln only	8/10/94 17:00		0.00		4.06	5830
	Soln +soil	8/10/94 17:00	13554.70	0.00	13033.37	4.75	2453
	Soln +soil	8/10/94 17:00	13437.10	0.00	12920.29	6.81	0
	Soln +soil	8/10/94 17:00	14077.90	0.00	13536.44	8.85	0
1	1	8/12/94 17:00	14529.60	2.00	13970.77	19.29	0
	2	8/12/94 17:00	14142.10	2.00	13598.17		
	3	8/12/94 17:00	14725.20	2.00	14158.85		
2	4	8/14/94 18:33	5875.98	4.06	5649.98		
	5	8/14/94 18:33	6666.09	4.06	6409.70		
	6	8/14/94 18:33	5647.44	4.06	5430.23		
3	7	8/15/94 10:53	1315.35	4.75	1264.76		
	8	8/15/94 10:53	1353.51	4.75	1301.45		
	9	8/15/94 10:53	4984.21	4.75	4792.51		
4	10	8/17/94 12:20	0.00	6.81	0.00		
	11	8/17/94 12:20	0.00	6.81	0.00		
	12	8/17/94 12:20	0.00	6.81	0.00		
5	13	8/19/94 13:26	0.00	8.85	0.00		
	14	8/19/94 13:26	0.00	8.85	0.00		
	15	8/19/94 13:26	0.00	8.85	0.00		
6	16	8/30/94 0:00	0.00	19.29	0.00		
	17	8/30/94 0:00	0.00	19.29	0.00		
	18	8/30/94 0:00	0.00	19.29	0.00		

Table B23. Groseclose loam soil data at 1 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Sol'n only	7/12/94 18:02	1898.47	0.00	1506.72	0.00	959
	Sol'n only	7/12/94 18:02	1794.43	0.00	1424.15	0.52	333
	Sol'n only	7/12/94 18:02	1785.02	0.00	1416.68	1.52	0
	Sol'n + so	7/12/94 23:03	1281.62	0.00	1017.16	2.50	0
	Sol'n +soi	7/12/94 23:03	1217.55	0.00	966.31	4.64	0
	Sol'n +soi	7/12/94 23:03	1127.73	0.00	895.02	5.48	0
1	1	7/13/94 11:37	512.31	0.52	406.60	7.25	0
	2	7/13/94 11:37	468.89	0.52	372.13		
	3	7/13/94 11:37	278.13	0.52	220.74		
2	4	7/14/94 11:25	0.00	1.52	0.00		
	5	7/14/94 11:25	0.00	1.52	0.00		
	6	7/14/94 11:25	0.00	1.52	0.00		
3	7	7/15/94 11:10	0.00	2.50	0.00		
	8	7/15/94 11:10	0.00	2.50	0.00		
	9	7/15/94 11:10	0.00	2.50	0.00		
4	10	7/17/94 14:25	0.00	4.64	0.00		
	11	7/17/94 14:25	0.00	4.64	0.00		
	12	7/17/94 14:25	0.00	4.64	0.00		
5	13	7/18/94 10:41	0.00	5.48	0.00		
	14	7/18/94 10:41	0.00	5.48	0.00		
	15	7/18/94 10:41	0.00	5.48	0.00		
6	16	7/20/94 5:10	--	7.25	--		
	17	7/20/94 5:10	0.00	7.25	0.00		
	18	7/20/94 5:10	0.00	7.25	0.00		

Table B24. Groseclose loam soil data at 10 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Sol'n only	7/27/94 0:00	14560.10	0.00	11555.63	0.00	8709
	Sol'n only	7/27/94 0:00	14952.80	0.00	11867.30	2.00	3185
	Sol'n only	7/27/94 0:00	11943.20	0.00	9478.73	3.00	352
	Sol'n + so	7/27/94 0:00	10982.20	0.00	8716.03	4.00	32
	Sol'n +soi	7/27/94 0:00	10699.70	0.00	8491.83	6.00	0
	Sol'n +soi	7/27/94 0:00	11238.90	0.00	8919.76	8.00	0
1	1	7/29/94 0:00	5375.53	2.00	4266.29	15.00	0
	2	7/29/94 0:00	3418.26	2.00	2712.90	20.00	0
	3	7/29/94 0:00	3243.72	2.00	2574.38	34.00	0
2	4	7/30/94 0:00	1065.32	3.00	845.49		
	5	7/30/94 0:00	265.49	3.00	210.71		
	6	7/30/94 0:00	0.00	3.00	0.00		
3	7	7/31/94 0:00	0.21	4.00	0.17		
	8	7/31/94 0:00	0.00	4.00	0.00		
	9	7/31/94 0:00	122.56	4.00	97.27		
4	10	8/2/94 0:00	0.00	6.00	0.00		
	11	8/2/94 0:00	0.00	6.00	0.00		
	12	8/2/94 0:00	0.00	6.00	0.00		
5	13	8/4/94 0:00	0.00	8.00	0.00		
	14	8/4/94 0:00	0.00	8.00	0.00		
	15	8/4/94 0:00	0.00	8.00	0.00		
6	16	8/11/94 0:00	--	15.00	--		
	17	8/11/94 0:00	0.00	15.00	0.00		
	18	8/11/94 0:00	0.00	15.00	0.00		
7	19	8/16/94 0:00	0.00	20.00	0.00		
	20	8/16/94 0:00	0.00	20.00	0.00		
	21	8/16/94 0:00	0.00	20.00	0.00		
8	22	8/30/94 0:00	0.00	34.00	0.00		
	23	8/30/94 0:00	0.00	34.00	0.00		
	24	8/30/94 0:00	0.00	34.00	0.00		

Table B25. Groseclose loam soil data at 50 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Sol'n only	8/19/94 22:38		0.00		0.00	44205
	Sol'n only	8/19/94 22:38		0.00		1.98	25123
	Sol'n only	8/19/94 22:38		0.00		2.59	31870
	Sol'n + so	8/19/94 22:38	56649.19	0.00	44959.67	5.80	763
	Sol'n +soi	8/19/94 22:38	54388.00	0.00	43165.08	9.43	1455
	Sol'n +soi	8/19/94 22:38	56057.10	0.00	44489.76	16.82	0
1	1	8/21/94 22:07	27095.80	1.98	21504.60	21.65	0
	2	8/21/94 22:07	19342.50	1.98	15351.19	34.80	4
	3	8/21/94 22:07	48527.30	1.98	38513.73	41.58	6
2	4	8/22/94 12:53	45323.60	2.59	35971.11		
	5	8/22/94 12:53	48064.70	2.59	38146.59		
	6	8/22/94 12:53	27078.50	2.59	21490.87		
3	7	8/25/94 17:47	33.38	5.80	26.49		
	8	8/25/94 17:47	2852.15	5.80	2263.61		
	9	8/25/94 17:47	0.00	5.80	0.00		
4	10	8/29/94 8:52	5491.71	9.43	4358.50		
	11	8/29/94 8:52	0.00	9.43	0.00		
	12	8/29/94 8:52	7.51	9.43	5.96		
5	13	9/5/94 18:17	0.00	16.82	0.00		
	14	9/5/94 18:17	0.00	16.82	0.00		
	15	9/5/94 18:17	0.00	16.82	0.00		
6	16	9/10/94 14:17	14.25	21.65	--		
	17	9/10/94 14:17	0.00	21.65	0.00		
	18	9/10/94 14:17	0.00	21.65	0.00		
7	19	9/23/94 17:53	15.99	34.80	12.69		
	20	9/23/94 17:53	0.00	34.80	0.00		
	21	9/23/94 17:53	0.00	34.80	0.00		
8	22	9/30/94 12:31	24.25	41.58	19.25		
	23	9/30/94 12:31	0.00	41.58	0.00		
	24	9/30/94 12:31	0.00	41.58	0.00		

Table B2 Groseclose loam soil data at 1 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Sol'n only	7/18/94 15:48	1390.99	0.00	1337.49	0.00	1108
	Sol'n only	7/18/94 15:48	1356.61	0.00	1304.43	1.52	10
	Sol'n only	7/18/94 15:48	1371.71	0.00	1318.95	2.44	0
	Sol'n + so	7/18/94 16:38	1125.57	0.00	1082.28	3.87	0
	Sol'n +soi	7/18/94 16:38	1185.78	0.00	1140.17	5.06	0
	Sol'n +soi	7/18/94 16:38	1145.94	0.00	1101.87	7.01	0
1	1	7/20/94 5:10	31.31	1.52	30.11	8.31	0
	2	7/20/94 5:10	0.00	1.52	0.00		
	3	7/20/94 5:10	0.00	1.52	0.00		
2	4	7/21/94 3:10	0.00	2.44	0.00		
	5	7/21/94 3:10	0.00	2.44	0.00		
	6	7/21/94 3:10	0.00	2.44	0.00		
3	7	7/22/94 13:25	0.00	3.87	0.00		
	8	7/22/94 13:25	0.00	3.87	0.00		
	9	7/22/94 13:25	0.00	3.87	0.00		
4	10	7/23/94 18:09	0.00	5.06	0.00		
	11	7/23/94 18:09	0.00	5.06	0.00		
	12	7/23/94 18:09	0.00	5.06	0.00		
5	13	7/25/94 16:51	0.00	7.01	0.00		
	14	7/25/94 16:51	0.00	7.01	0.00		
	15	7/25/94 16:51	0.00	7.01	0.00		
6	16	7/27/94 0:00	0.00	8.31	0.00		
	17	7/27/94 0:00	0.00	8.31	0.00		
	18	7/27/94 0:00	0.00	8.31	0.00		

Table B2 Groseclose loam soil data at 10 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Sol'n only	8/10/94 17:00	14441.70	0.00	13886.25	0.00	8499
	Sol'n only	8/10/94 17:00	14162.20	0.00	13617.50	2.00	17
	Sol'n only	8/10/94 17:00		0.00		2.87	0
	Sol'n + so	8/10/94 17:00	12213.20	0.00	11743.46	4.29	0
	Sol'n +soi	8/10/94 17:00	12589.00	0.00	12104.81	8.29	0
	Sol'n +soi	8/10/94 17:00	1714.30	0.00	1648.37	19.29	0
1	1	8/12/94 17:00	18.81	2.00	18.09		
	2	8/12/94 17:00	19.19	2.00	18.45		
	3	8/12/94 17:00	16.50	2.00	15.87		
2	4	8/13/94 13:48	0.00	2.87	0.00		
	5	8/13/94 13:48	0.00	2.87	0.00		
	6	8/13/94 13:48	0.00	2.87	0.00		
3	7	8/15/94 0:00	0.00	4.29	0.00		
	8	8/15/94 0:00	0.00	4.29	0.00		
	9	8/15/94 0:00	0.00	4.29	0.00		
4	10	8/19/94 0:00	0.00	8.29	0.00		
	11	8/19/94 0:00	0.00	8.29	0.00		
	12	8/19/94 0:00	0.00	8.29	0.00		
5	13	8/30/94 0:00	0.00	19.29	0.00		
	14	8/30/94 0:00	0.00	19.29	0.00		
	15	8/30/94 0:00	0.00	19.29	0.00		

Table B2 Groseclose loam soil data at 50 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Sol'n only	9/2/94 19:52	57274.30	0.00	55071.44	0.00	49023
	Sol'n only	9/2/94 19:52	50158.70	0.00	48229.52	1.68	39896
	Sol'n only	9/2/94 19:52		0.00		3.60	37248
	Sol'n + so	9/2/94 19:52	48143.60	0.00	46291.92	5.16	6741
	Sol'n +soi	9/2/94 19:52	52784.30	0.00	50754.13	6.10	11914
	Sol'n +soi	9/2/94 19:52	52023.20	0.00	50022.31	8.19	0
1	1	9/4/94 12:10	57295.00	1.68	55091.35	13.96	5
	2	9/4/94 12:10	28356.90	1.68	27266.25	27.17	0
	3	9/4/94 12:10	38822.80	1.68	37329.62	31.17	0
2	4	9/6/94 10:15	51346.50	3.60	49371.63		
	5	9/6/94 10:15	22057.00	3.60	21208.65		
	6	9/6/94 10:15	42809.40	3.60	41162.88		
3	7	9/7/94 23:42	139.28	5.16	133.92		
	8	9/7/94 23:42	20877.60	5.16	20074.62		
	9	9/7/94 23:42	16.39	5.16	15.76		
4	10	9/8/94 22:17	0.00	6.10	0.00		
	11	9/8/94 22:17	37090.60	6.10	35664.04		
	12	9/8/94 22:17	80.05	6.10	76.97		
5	13	9/11/94 0:21	0.00	8.19	0.00		
	14	9/11/94 0:21	0.00	8.19	0.00		
	15	9/11/94 0:21	0.00	8.19	0.00		
6	16	9/16/94 18:53	15.54	13.96	14.94		
	17	9/16/94 18:53	0.00	13.96	0.00		
	18	9/16/94 18:53	0.00	13.96	0.00		
7	19	9/30/94 0:00	0.00	27.17	0.00		
	20	9/30/94 0:00	0.00	27.17	0.00		
	21	9/30/94 0:00	0.00	27.17	0.00		
8	22	10/4/94 0:00	0.00	31.17	0.00		
	23	10/4/94 0:00	0.00	31.17	0.00		
	24	10/4/94 0:00	0.00	31.17	0.00		

Table B29 Groscdose clay soil data at 1 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Sol'n only	7/12/94 18:02	1898.47	0.00	1506.72	0.00	1210
	Sol'n only	7/12/94 18:02	1794.43	0.00	1424.15	0.45	822
	Sol'n only	7/12/94 18:02	1785.02	0.00	1416.68	1.45	812
	Sol'n + so	7/12/94 23:53	1509.99	0.00	1198.40	2.63	640
	Sol'n +soi	7/12/94 23:53	1525.18	0.00	1210.46	4.88	549
	Sol'n +soi	7/12/94 23:53	1536.83	0.00	1219.71	5.59	89
1	1	7/13/94 10:47	1009.97	0.45	801.56	7.43	89
	2	7/13/94 10:47	1056.73	0.45	838.67	8.60	42
	3	7/13/94 10:47	1040.15	0.45	825.50	19.00	0
2	4	7/14/94 10:35	1044.72	1.45	829.14		
	5	7/14/94 10:35	1008.67	1.45	800.53		
	6	7/14/94 10:35	1016.77	1.45	806.96		
3	7	7/15/94 15:05	935.13	2.63	742.17		
	8	7/15/94 15:05	505.00	2.63	400.79		
	9	7/15/94 15:05	978.11	2.63	776.28		
4	10	7/17/94 20:56	911.03	4.88	723.04		
	11	7/17/94 20:56	658.08	4.88	522.29		
	12	7/17/94 20:56	506.51	4.88	401.99		
5	13	7/18/94 14:02	106.31	5.59	84.37		
	14	7/18/94 14:02	36.75	5.59	29.17		
	15	7/18/94 14:02	191.66	5.59	152.11		
6	16	7/20/94 10:09	285.04	7.43	226.22		
	17	7/20/94 10:09	0.00	7.43	0.00		
	18	7/20/94 10:09	49.84	7.43	39.56		
7	19	7/21/94 14:22	55.92	8.60	44.38		
	20	7/21/94 14:22	80.07	8.60	63.55		
	21	7/21/94 14:22	21.63	8.60	17.17		
8	22	8/1/94 0:00	0.00	19.00	0.00		
	23	8/1/94 0:00	0.00	19.00	0.00		
	24	8/1/94 0:00	0.00	19.00	0.00		

Table B30 Groscdose clay soil data at 10 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Sol'n only	7/27/94 0:00	14560.10	0.00	11555.63	0.00	9036
	Sol'n only	7/27/94 0:00	14952.80	0.00	11867.30	2.00	7841
	Sol'n only	7/27/94 0:00	11943.20	0.00	9478.73	4.00	3527
	Sol'n + so	7/27/94 0:00	10719.50	0.00	8507.54	6.00	5559
	Sol'n +soi	7/27/94 0:00	11526.60	0.00	9148.10	8.00	3031
	Sol'n +soi	7/27/94 0:00	11908.70	0.00	9451.35	10.00	5115
1	1	7/29/94 0:00	9759.86	2.00	7745.92	15.71	715
	2	7/29/94 0:00	9830.75	2.00	7802.18	20.55	898
	3	7/29/94 0:00	10048.90	2.00	7975.32	34.84	302
2	4	7/31/94 0:00	7595.94	4.00	6028.52		
	5	7/31/94 0:00	2204.54	4.00	1749.63		
	6	7/31/94 0:00	3531.07	4.00	2802.44		
3	7	8/2/94 0:00	5383.04	6.00	4272.25		
	8	8/2/94 0:00	8235.20	6.00	6535.87		
	9	8/2/94 0:00	7396.12	6.00	5869.94		
4	10	8/4/94 0:00	7351.60	8.00	5834.60		
	11	8/4/94 0:00	3813.80	8.00	3026.83		
	12	8/4/94 0:00	290.01	8.00	230.17		
5	13	8/6/94 0:00	6385.42	10.00	5067.79		
	14	8/6/94 0:00	7626.27	10.00	6052.60		
	15	8/6/94 0:00	5323.92	10.00	4225.33		
6	16	8/11/94 17:00	0.00	15.71	0.00		
	17	8/11/94 17:00	907.16	15.71	719.97		
	18	8/11/94 17:00	1795.70	15.71	1425.16		
7	19	8/16/94 13:11	0.00	20.55	0.00		
	20	8/16/94 13:11	37.90	20.55	30.08		
	21	8/16/94 13:11	3356.72	20.55	2664.06		
8	22	8/30/94 20:14	1140.37	34.84	905.06		
	23	8/30/94 20:14	0.00	34.84	0.00		
	24	8/30/94 20:14	0.00	34.84	0.00		

Table B31 Groscdose clay soil data at 50 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Sol'n only	8/19/94 22:38		0.00		0.00	45259
	Sol'n only	8/19/94 22:38		0.00		2.54	42940
	Sol'n only	8/19/94 22:38		0.00		5.87	39854
	Sol'n +soi	8/19/94 22:38	56349.80	0.00	44722.06	9.69	34370
	Sol'n +soi	8/19/94 22:38	57791.50	0.00	45866.27	16.89	37898
	Sol'n +soi	8/19/94 22:38	56938.70	0.00	45189.44	22.11	16848
1	1	8/22/94 11:41	53593.10	2.54	42534.21	23.87	37969
	2	8/22/94 11:41	54391.50	2.54	43167.86	34.70	32135
	3	8/22/94 11:41	54327.90	2.54	43117.38	41.72	18403
2	4	8/25/94 19:27	55372.60	5.87	43946.51		
	5	8/25/94 19:27	42164.20	5.87	33463.65		
	6	8/25/94 19:27	53113.20	5.87	42153.33		
3	7	8/29/94 15:14	55124.50	9.69	43749.60		
	8	8/29/94 15:14	40922.90	9.69	32478.49		
	9	8/29/94 15:14	33871.90	9.69	26882.46		
4	10	9/5/94 19:57	48154.50	16.89	38217.86		
	11	9/5/94 19:57	44265.10	16.89	35131.03		
	12	9/5/94 19:57	50835.10	16.89	40345.32		
5	13	9/11/94 1:11	51215.40	22.11	40647.14		
	14	9/11/94 1:11	4469.40	22.11	3547.14		
	15	9/11/94 1:11	8001.20	22.11	6350.16		
6	16	9/12/94 19:30	51226.20	23.87	40655.71		
	17	9/12/94 19:30	46156.80	23.87	36632.38		
	18	9/12/94 19:30	46138.60	23.87	36617.94		
7	19	9/23/94 15:21	44769.80	34.70	35531.59		
	20	9/23/94 15:21	43078.40	34.70	34189.21		
	21	9/23/94 15:21	33622.20	34.70	26684.29		
8	22	9/30/94 15:52	34489.40	41.72	27372.54		
	23	9/30/94 15:52	508.81	41.72	403.82		
	24	9/30/94 15:52	34564.30	41.72	27431.98		

Table B32. Groscdose clay soil data at 1 ppm initial benzene concentration with nutrient addition.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Sol'n only	7/18/94 15:48	1390.99	0.00	1337.49	0.00	1145
	Sol'n only	7/18/94 15:48	1356.61	0.00	1304.43	1.17	1298
	Sol'n only	7/18/94 15:48	1371.71	0.00	1318.95	2.30	817
	Sol'n + so	7/18/94 21:39	1158.67	0.00	1114.11	3.01	465
	Sol'n +soi	7/18/94 21:39	1206.02	0.00	1159.63	4.92	0
	Sol'n +soi	7/18/94 21:39	1207.20	0.00	1160.77	6.94	0
1	1	7/20/94 1:49	1384.39	1.17	1331.14	8.10	0
	2	7/20/94 1:49	1318.95	1.17	1268.20	0.00	#DIV/0!
	3	7/20/94 1:49	1345.04	1.17	1293.31		
2	4	7/21/94 4:51	1013.66	2.30	974.67		
	5	7/21/94 4:51	685.03	2.30	658.68		
	6	7/21/94 4:51	850.94	2.30	818.21		
3	7	7/21/94 21:56	378.41	3.01	363.86		
	8	7/21/94 21:56	589.60	3.01	566.92		
	9	7/21/94 21:56	--	3.01			
4	10	7/23/94 19:49	0.00	4.92	0.00		
	11	7/23/94 19:49	0.00	4.92	0.00		
	12	7/23/94 19:49	0.00	4.92	0.00		
5	13	7/25/94 20:12	0.00	6.94	0.00		
	14	7/25/94 20:12	0.00	6.94	0.00		
	15	7/25/94 20:12	0.00	6.94	0.00		
6	16	7/27/94 0:00	0.00	8.10	0.00		
	17	7/27/94 0:00	0.00	8.10	0.00		
	18	7/27/94 0:00	0.00	8.10	0.00		

Table B33. Groscdose clay soil data at 10 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Sol'n only	8/10/94 17:00	14441.70	0.00	13886.25	0.00	10622
	Sol'n only	8/10/94 17:00	14162.20	0.00	13617.50	1.63	9421
	Sol'n only	8/10/94 17:00		0.00		2.96	9366
	Sol'n +soi	8/10/94 17:00	11443.20	0.00	11003.08	4.95	8337
	Sol'n +soi	8/10/94 17:00	11132.50	0.00	10704.33	6.84	2824
	Sol'n +soi	8/10/94 17:00	10564.90	0.00	10158.56	8.96	317
1	1	8/12/94 8:00	11049.60	1.63	10624.62		
	2	8/12/94 8:00	8545.96	1.63	8217.27		
	3	8/12/94 8:00		1.63			
2	4	8/13/94 15:59	8577.34	2.96	8247.44		
	5	8/13/94 15:59	10419.40	2.96	10018.65		
	6	8/13/94 15:59	10226.60	2.96	9833.27		
3	7	8/15/94 15:54	9680.38	4.95	9308.06		
	8	8/15/94 15:54	8098.89	4.95	7787.39		
	9	8/15/94 15:54	8232.33	4.95	7915.70		
4	10	8/17/94 13:11	7060.40	6.84	6788.85		
	11	8/17/94 13:11	24.19	6.84	23.26		
	12	8/17/94 13:11	1726.28	6.84	1659.88		
5	13	8/19/94 15:56	17.54	8.96	16.87		
	14	8/19/94 15:56	0.00	8.96	0.00		
	15	8/19/94 15:56	971.11	8.96	933.76		

Table B34. Groscdose clay soil data at 50 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Sol'n only	9/2/94 22:23	57274.30	0.00	55071.44	0.00	49291
	Sol'n only	9/2/94 22:23	50158.70	0.00	48229.52	1.79	42158
	Sol'n only	9/2/94 22:23		0.00		4.51	19563
	Sol'n +soi	9/2/94 22:23	52624.00	0.00	50600.00	6.03	13757
	Sol'n +soi	9/2/94 22:23	50855.10	0.00	48899.13	7.94	34059
	Sol'n +soi	9/2/94 22:23	50309.30	0.00	48374.33	9.78	17731
1	1	9/4/94 17:24	42322.50	1.79	40694.71	13.78	15612
	2	9/4/94 17:24	45615.90	1.79	43861.44	18.47	7105
	3	9/4/94 17:24	43594.30	1.79	41917.60	33.07	0
2	4	9/7/94 10:35	13344.90	4.51	12831.63		
	5	9/7/94 10:35	30588.20	4.51	29411.73		
	6	9/7/94 10:35	17104.20	4.51	16446.35		
3	7	9/8/94 23:07	32.94	6.03	31.67		
	8	9/8/94 23:07	35932.20	6.03	34550.19		
	9	9/8/94 23:07	6955.90	6.03	6688.37		
4	10	9/10/94 21:01	37990.10	7.94	36528.94		
	11	9/10/94 21:01	30737.70	7.94	29555.48		
	12	9/10/94 21:01	37534.80	7.94	36091.15		
5	13	9/12/94 16:59	5127.40	9.78	4930.19		
	14	9/12/94 16:59	41805.60	9.78	40197.69		
	15	9/12/94 16:59	8387.04	9.78	8064.46		
6	16	9/16/94 17:13	379.36	13.78	364.77		
	17	9/16/94 17:13	27576.10	13.78	26515.48		
	18	9/16/94 17:13	20755.60	13.78	19955.38		
7	19	9/21/94 9:45	22002.70	18.47	21156.44		
	20	9/21/94 9:45	165.24	18.47	158.88		
	21	9/21/94 9:45	0.00	18.47	0.00		
8	22	10/6/94 0:00	0.00	33.07	0.00		
	23	10/6/94 0:00	0.00	33.07	0.00		
	24	10/6/94 0:00	0.00	33.07	0.00		

Table B35. Wheeling soil data at 1 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	3/30/94	1872.12	0.00	1011.96	0.00	761
	Soln + sand	3/30/94	1841.66	0.00	995.49	1.00	692
	Soln +soil	3/30/94	1408.05	0.00	761.11	2.00	450
1	1	3/31/94	1339.33	1.00	723.96	3.00	125
	2	3/31/94	1293.19	1.00	699.02	4.00	10
	3	3/31/94	1209.49	1.00	653.78	5.00	0
2	4	4/1/94	818.32	2.00	442.34	6.00	0
	5	4/1/94	931.88	2.00	503.72		
	6	4/1/94	747.56	2.00	404.09		
3	7	4/2/94	249.10	3.00	134.65		
	8	4/2/94	278.95	3.00	150.78		
	9	4/2/94	168.44	3.00	91.05		
4	10	4/3/94	0.00	4.00	0.00		
	11	4/3/94	22.49	4.00	12.16		
	12	4/3/94	35.33	4.00	19.10		
5	13	4/4/94	0.00	5.00	0.00		
	14	4/4/94	0.00	5.00	0.00		
	15	4/4/94	0.00	5.00	0.00		
6	16	4/5/94	0.00	6.00	0.00		
	17	4/5/94	0.00	6.00	0.00		
	18	4/5/94	0.00	6.00	0.00		

Table B3 Wheeling soil data at 1 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	7/18/94 15:48	1390.99	0.00	1337.49	0.00	1290
	Soln only	7/18/94 15:48	1356.61	0.00	1304.43	1.21	796
	Soln only	7/18/94 15:48	1371.71	0.00	1318.95	2.44	0
	Soln + soil	7/18/94 19:09	1348.16	0.00	1296.31	3.09	10
	Soln +soil	7/18/94 19:09	1332.87	0.00	1281.61	4.99	0
	Soln +soil	7/18/94 19:09	1344.93	0.00	1293.20	6.94	0
1	1	7/20/94 0:08	797.74	1.21	767.06	8.20	0
	2	7/20/94 0:08	715.49	1.21	687.97		
	3	7/20/94 0:08	971.33	1.21	933.97		
2	4	7/21/94 5:41	0.00	2.44	0.00		
	5	7/21/94 5:41	0.00	2.44	0.00		
	6	7/21/94 5:41	0.00	2.44	0.00		
3	7	7/21/94 21:23	0.00	3.09	0.00		
	8	7/21/94 21:23	21.60	3.09	20.77		
	9	7/21/94 21:23	--	3.09	--		
4	10	7/23/94 18:59	0.00	4.99	0.00		
	11	7/23/94 18:59	0.00	4.99	0.00		
	12	7/23/94 18:59	0.00	4.99	0.00		
5	13	7/25/94 17:41	0.00	6.94	0.00		
	14	7/25/94 17:41	0.00	6.94	0.00		
	15	7/25/94 17:41	0.00	6.94	0.00		
6	16	7/27/94 0:00	0.00	8.20	0.00		
	17	7/27/94 0:00	0.00	8.20	0.00		
	18	7/27/94 0:00	0.00	8.20	0.00		

Table B36. Wheeling soil data at 10 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	3/24/94	12280.00	0.00	6637.84	0.00	6638
	Soln + sand	3/24/94		0.00	0.00	4.00	3131
	Soln +soil	3/24/94		0.00	0.00	5.00	755
1	1	3/28/94	5895.72	4.00	3186.88	6.00	362
	2	3/28/94	5814.40	4.00	3142.92	7.00	94
	3	3/28/94	5667.47	4.00	3063.50	8.00	25
2	4	3/29/94	1635.00	5.00	883.78	9.00	12
	5	3/29/94	1630.12	5.00	881.15	10.00	3
	6	3/29/94	925.00	5.00	500.00	11.00	4
3	7	3/30/94	113.05	6.00	61.11		
	8	3/30/94	1167.65	6.00	631.16		
	9	3/30/94	728.98	6.00	394.04		
4	10	3/31/94	220.86	7.00	119.38		
	11	3/31/94	234.14	7.00	126.56		
	12	3/31/94	66.25	7.00	35.81		
5	13	4/1/94	66.44	8.00	35.91		
	14	4/1/94	25.72	8.00	13.90		
	15	4/1/94	--	8.00	--		
6	16	4/2/94	51.27	9.00	27.71		
	17	4/2/94	16.90	9.00	9.14		
	18	4/2/94	0.00	9.00	0.00		
7	19	4/3/94	17.23	10.00	9.31		
	20	4/3/94	0.00	10.00	0.00		
	21	4/3/94	0.00	10.00	0.00		
8	22	4/4/94	20.01	11.00	10.82		
	23	4/4/94	0.00	11.00	0.00		
	24	4/4/94	0.00	11.00	0.00		

Table B3 Wheeling soil data at 10 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	3/24/94	14000.00	0.00	7567.57	0.00	7568
	Soln + sand	3/24/94		0.00	0.00	1.00	7195
	Soln + soil	3/24/94		0.00	0.00	2.00	6432
1	1	3/25/94	13285.60	1.00	7181.41	4.00	446
	2	3/25/94	13335.96	1.00	7208.63	5.00	13
	3	3/25/94	--	1.00	--	6.00	3
2	4	3/26/94	7590.44	2.00	4102.94	7.00	1
	5	3/26/94	13904.20	2.00	7515.78	8.00	0
	6	3/26/94	14204.10	2.00	7677.89	9.00	0
3	7	3/28/94	724.49	4.00	391.62		
	8	3/28/94	1262.16	4.00	682.25		
	9	3/28/94	488.00	4.00	263.78		
4	10	3/29/94	37.94	5.00	20.51		
	11	3/29/94	16.61	5.00	8.98		
	12	3/29/94	17.80	5.00	9.62		
5	13	3/30/94	9.27	6.00	5.01		
	14	3/30/94	7.74	6.00	4.18		
	15	3/30/94	0.00	6.00	0.00		
6	16	3/31/94	7.92	7.00	4.28		
	17	3/31/94	0.00	7.00	0.00		
	18	3/31/94	0.00	7.00	0.00		
7	19	4/1/94	0.00	8.00	0.00		
	20	4/1/94	0.00	8.00	0.00		
	21	4/1/94	0.00	8.00	0.00		
8	22	4/2/94	0.00	9.00	0.00		
	23	4/2/94	0.00	9.00	0.00		
	24	4/2/94	0.00	9.00	0.00		

Table B37. Wheeling soil data at 50 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	3/26/94	91487.20	0.00	49452.54	0.00	33644
	Soln + sand	3/26/94	92295.50	0.00	49889.46	2.00	37250
	Soln +soil	3/26/94	62240.85	0.00	33643.70	4.00	40018
1	1	3/28/94	63065.40	2.00	34089.41	7.00	24994
	2	3/28/94	74759.50	2.00	40410.54	9.00	24853
	3	3/28/94	--	2.00	--	12.00	20569
2	4	3/30/94	72802.80	4.00	39352.86	15.00	21583
	5	3/30/94	75262.80	4.00	40682.59	18.00	17976
	6	3/30/94	--	4.00	--		
3	7	4/2/94	48468.50	7.00	26199.19		
	8	4/2/94	42133.30	7.00	22774.76		
	9	4/2/94	48115.40	7.00	26008.32		
4	10	4/4/94	44469.60	9.00	24037.62		
	11	4/4/94	44370.10	9.00	23983.84		
	12	4/4/94	49096.20	9.00	26538.49		
5	13	4/7/94	38891.00	12.00	21022.16		
	14	4/7/94	39702.30	12.00	21460.70		
	15	4/7/94	35566.30	12.00	19225.03		
6	16	4/10/94	37223.50	15.00	20120.81		
	17	4/10/94	34735.20	15.00	18775.78		
	18	4/10/94	47825.20	15.00	25851.46		
7	19	4/13/94	41360.20	18.00	22356.86		
	20	4/13/94	--	18.00	--		
	21	4/13/94	25152.20	18.00	13595.78		

Table B4 Wheeling soil data at 50 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	3/26/94	96000.90	0.00	51892.38	0.00	46501
	Soln + sand	3/26/94	97999.30	0.00	52972.59	2.00	45893
	Soln +soil	3/26/94	86027.20	0.00	46501.19	4.00	43645
1	1	3/28/94	84253.20	2.00	45542.27	6.00	8476
	2	3/28/94	85549.70	2.00	46243.08	7.00	147
	3	3/28/94	--	2.00	--	8.00	58
2	4	3/30/94	77904.60	4.00	42110.59	10.00	40
	5	3/30/94	83581.90	4.00	45179.41	12.00	29
	6	3/30/94	--	4.00	--	13.00	24
3	7	4/1/94	26947.00	6.00	14565.95	15.00	16
	8	4/1/94	336.49	6.00	181.89	17.00	8
	9	4/1/94	19759.30	6.00	10680.70		
4	10	4/2/94	257.01	7.00	138.92		
	11	4/2/94	128.24	7.00	69.32		
	12	4/2/94	428.61	7.00	231.68		
5	13	4/3/94	108.19	8.00	58.48		
	14	4/3/94	105.01	8.00	56.76		
	15	4/3/94	110.35	8.00	59.65		
6	16	4/5/94	64.71	10.00	34.98		
	17	4/5/94	82.19	10.00	44.43		
	18	4/5/94	74.22	10.00	40.12		
7	19	4/7/94	66.81	12.00	36.11		
	20	4/7/94	48.86	12.00	26.41		
	21	4/7/94	43.54	12.00	23.54		
8	22	4/8/94	53.42	13.00	28.88		
	23	4/8/94	41.44	13.00	22.40		
	24	4/8/94	39.28	13.00	21.23		
9	25	4/10/94	31.41	15.00	16.98		
	26	4/10/94	18.62	15.00	10.06		
	27	4/10/94	40.58	15.00	21.94		
10	28	4/12/94	10.00	17.00	5.41		
	29	4/12/94	13.82	17.00	7.47		
	30	4/12/94	19.83	17.00	10.72		

APPENDIX C: CONTAMINATED SOIL DATA

TableC1. CS1 data at 10 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Conc (ppb)
0	Sol'n only	09/08/1994 18:23	16839.90	0.00	13365.00	0.00	8708
	Sol'n only	09/08/1994 18:23	17408.20	0.00	13816.03	1.65	5321
	Sol'n only	09/08/1994 18:23		0.00		2.81	47
	Sol'n+ soi	09/08/1994 18:23	15921.00	0.00	12635.71	3.75	0
	Sol'n +soi	09/08/1994 18:23	15474.30	0.00	12281.19	11.75	0
	Sol'n +soi	09/08/1994 18:23	1519.70	0.00	1206.11	17.23	0
1	1	09/10/1994 10:05	6096.60	1.65	4838.57		
	2	09/10/1994 10:05	6969.70	1.65	5531.51		
	3	09/10/1994 10:05	7046.30	1.65	5592.30		
2	4	09/11/1994 13:50	38.41	2.81	30.48		
	5	09/11/1994 13:50	22.36	2.81	17.75		
	6	09/11/1994 13:50	118.45	2.81	94.01		
3	7	09/12/1994 12:28	0.00	3.75	0.00		
	8	09/12/1994 12:28	0.00	3.75	0.00		
	9	09/12/1994 12:28	0.00	3.75	0.00		
4	10	09/20/1994 12:19		11.75			
	11	09/20/1994 12:19	0.00	11.75	0.00		
	12	09/20/1994 12:19	0.00	11.75	0.00		
5	13	09/26/1994 00:00	0.00	17.23	0.00		
	14	09/26/1994 00:00	0.00	17.23	0.00		
	15	09/26/1994 00:00	0.00	17.23	0.00		

Table C2. CS1 data at 10 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene oncentrati (ppb)	Average values Time (days)	Conc (ppb)
0	Sol'n only	08/10/1994 08:00	13947.30	0.00	13410.87	0.00	5405
	Sol'n only	08/10/1994 08:00		0.00		0.38	216
	Sol'n only	08/10/1994 08:00		0.00		2.00	6
	Sol'n+ soil	08/10/1994 08:00	5887.10	0.00	5660.67	3.17	0
	Sol'n +soil	08/10/1994 08:00	5301.27	0.00	5097.38	5.36	0
	Sol'n +soil	08/10/1994 08:00	5675.52	0.00	5457.23	9.37	0
1	1	08/10/1994 17:00	78.55	0.38	75.53		
	2	08/10/1994 17:00	83.10	0.38	79.90		
	3	08/10/1994 17:00	511.98	0.38	492.29		
2	4	08/12/1994 08:00	10.31	2.00	9.91		
	5	08/12/1994 08:00	9.86	2.00	9.48		
	6	08/12/1994 08:00	0.00	2.00	0.00		
3	7	08/13/1994 12:07	0.00	3.17	0.00		
	8	08/13/1994 12:07	0.00	3.17	0.00		
	9	08/13/1994 12:07	0.00	3.17	0.00		
4	10	08/15/1994 16:44	0.00	5.36	0.00		
	11	08/15/1994 16:44	0.00	5.36	0.00		
	12	08/15/1994 16:44	0.00	5.36	0.00		
5	13	08/19/1994 16:47	0.00	9.37	0.00		
	14	08/19/1994 16:47	0.00	9.37	0.00		
	15	08/19/1994 16:47	0.00	9.37	0.00		

Table C3. CS2 data at 10 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	9/8/94 18:23	16839.9	0.00	13365.00	0.00	11899
	Soln only	9/8/94 18:23	17408.2	0.00	13816.03	1.65	6620
	Soln only	9/8/94 18:23		0.00		2.81	21
	Soln+ soil	9/8/94 18:23	15152.2	0.00	12025.56	3.75	0
	Soln +soil	9/8/94 18:23	15006.8	0.00	11910.16	4.84	0
	Soln +soil	9/8/94 18:23	14820.8	0.00	11762.54	5.85	0
1	1	9/10/94 10:05	7769.5	1.65	6166.29		
	2	9/10/94 10:05	9630.8	1.65	7643.48		
	3	9/10/94 10:05	7624.8	1.65	6051.41		
2	4	9/11/94 13:50	38.4	2.81	30.48		
	5	9/11/94 13:50	22.4	2.81	17.75		
	6	9/11/94 13:50	18.5	2.81	14.64		
3	7	9/12/94 12:28	0.0	3.75	0.00		
	8	9/12/94 12:28	0.0	3.75	0.00		
	9	9/12/94 12:28	0.0	3.75	0.00		
4	10	9/13/94 14:32	0.0	4.84	0.00		
	11	9/13/94 14:32	0.0	4.84	0.00		
	12	9/13/94 14:32	0.0	4.84	0.00		
5	13	9/14/94 14:49	0.0	5.85	0.00		
	14	9/14/94 14:49	0.0	5.85	0.00		
	15	9/14/94 14:49	0.0	5.85	0.00		

Table C4. CS2 data at 50 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	9/7/94 21:11	68194.4	0.00	54122.54	0.00	44083
	Soln only	9/7/94 21:11	67315.2	0.00	53424.76	2.64	43676
	Soln only	9/7/94 21:11		0.00	0.00	4.86	19409
	Soln+ soil	9/7/94 21:11	58258.4	0.00	46236.83	6.77	5709
	Soln +soil	9/7/94 21:11	57001.0	0.00	45238.89	8.95	18
	Soln +soil	9/7/94 21:11	51374.0	0.00	40773.02	10.66	11
1	1	9/10/94 12:36	55850.7	2.64	44325.95	12.74	0
	2	9/10/94 12:36	53267.0	2.64	42275.40	18.74	0
	3	9/10/94 12:36	55978.8	2.64	44427.62	22.70	0
2	4	9/12/94 17:49	24029.8	4.86	19071.27		
	5	9/12/94 17:49	24210.0	4.86	19214.29		
	6	9/12/94 17:49	25125.8	4.86	19941.11		
3	7	9/14/94 15:40	9083.3	6.77	7208.97		
	8	9/14/94 15:40	10086.5	6.77	8005.16		
	9	9/14/94 15:40	2411.7	6.77	1914.05		
4	10	9/16/94 20:00	30.9	8.95	24.48		
	11	9/16/94 20:00	25.8	8.95	20.46		
	12	9/16/94 20:00	13.2	8.95	10.45		
5	13	9/18/94 12:57	43.0	10.66	23.22		
	14	9/18/94 12:57	10.2	10.66	5.49		
	15	9/18/94 12:57	7.1	10.66	3.81		
6	16	9/20/94 15:00	0.0	12.74	0.00		
	17	9/20/94 15:00	0.0	12.74	0.00		
	18	9/20/94 15:00	0.0	12.74	0.00		
7	19	9/26/94 15:00	0.0	18.74	0.00		
	20	9/26/94 15:00	0.0	18.74	0.00		
	21	9/26/94 15:00	0.0	18.74	0.00		
8	22	9/30/94 13:54	0.0	22.70	0.00		
	23	9/30/94 13:54	0.0	22.70	0.00		
	24	9/30/94 13:54	0.0	22.70	0.00		

Table C5. CS2 data at 10 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	8/15/94 18:45	14728.70	0.00	14162.21	0.00	13840
	Soln only	8/15/94 18:45	14967.10	0.00	14391.44	1.81	10314
	Soln only	8/15/94 18:45		0.00		3.93	28
	Soln+ soil	8/15/94 19:18	14787.00	0.00	14218.27	6.08	0
	Soln +soil	8/15/94 19:18	13999.70	0.00	13461.25	14.20	0
	Soln +soil	8/15/94 19:18	14148.70	0.00			
1	1	8/17/94 14:51	10196.20	1.81	9804.04		
	2	8/17/94 14:51	11283.00	1.81	10849.04		
	3	8/17/94 14:51	10701.40	1.81	10289.81		
2	4	8/19/94 17:37	28.49	3.93	27.39		
	5	8/19/94 17:37	27.76	3.93	26.69		
	6	8/19/94 17:37	30.53	3.93	29.36		
3	7	8/21/94 21:17	0.00	6.08	0.00		
	8	8/21/94 21:17	0.00	6.08	0.00		
	9	8/21/94 21:17	0.00	6.08	0.00		
4	10	8/30/94 0:00	0.00	14.20	0.00		
	11	8/30/94 0:00	0.00	14.20	0.00		
	12	8/30/94 0:00	0.00	14.20	0.00		

Table C6. CS2 data at 50 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	9/2/94 21:33	80852.90	0.00	77743.17	0.00	49603
	Soln only	9/2/94 21:33	80710.00	0.00	77605.77	1.57	62132
	Soln only	9/2/94 21:33		0.00		4.47	17366
	Soln + soi	9/2/94 21:33	52630.60	0.00	50606.35	6.00	131
	Soln +soil	9/2/94 21:33	51752.10	0.00	49761.63	8.08	3
	Soln +soil	9/2/94 21:33	50378.60	0.00	48440.96	10.78	0
1	1	9/4/94 11:19	64135.00	1.57	61668.27	13.10	0
	2	9/4/94 11:19	64798.00	1.57	62305.77	27.10	0
	3	9/4/94 11:19	64918.50	1.57	62421.63	31.10	0
2	4	9/7/94 8:54	8219.19	4.47	7903.07		
	5	9/7/94 8:54	12549.90	4.47	12067.21		
	6	9/7/94 8:54	33411.60	4.47	32126.54		
3	7	9/8/94 21:27	191.18	6.00	183.83		
	8	9/8/94 21:27	114.27	6.00	109.88		
	9	9/8/94 21:27	103.99	6.00	99.99		
4	10	9/10/94 23:34	0.00	8.08	0.00		
	11	9/10/94 23:34	0.00	8.08	0.00		
	12	9/10/94 23:34	8.65	8.08	8.32		
5	13	9/13/94 16:13	0.00	10.78	0.00		
	14	9/13/94 16:13	0.00	10.78	0.00		
	15	9/13/94 16:13	0.00	10.78	0.00		
6	16	9/16/94 0:00	0.00	13.10	0.00		
	17	9/16/94 0:00	0.00	13.10	0.00		
	18	9/16/94 0:00	0.00	13.10	0.00		
7	19	9/30/94 0:00	0.00	27.10	0.00		
	20	9/30/94 0:00	0.00	27.10	0.00		
	21	9/30/94 0:00	0.00	27.10	0.00		
8	22	10/4/94 0:00	0.00	31.10	0.00		
	23	10/4/94 0:00	0.00	31.10	0.00		
	24	10/4/94 0:00	0.00	31.10	0.00		

Table C7. CS3 data at 10 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	9/8/94 18:23	16839.90	0.00	13365.00	0.00	10103
	Soln only	9/8/94 18:23	17408.20	0.00	13816.03	0.86	9348
	Soln only	9/8/94 18:23		0.00		1.65	4534
	Soln+ soil	9/8/94 18:23	12729.60	0.00	10102.86	2.81	2748
	Soln +soil	9/8/94 18:23		0.00		3.75	2035
	Soln +soil	9/8/94 18:23		0.00		4.84	635
1	1	9/9/94 15:00	12403.70	0.86	9844.21	5.85	318
	2	9/9/94 15:00	10107.90	0.86	8022.14	7.10	108
	3	9/9/94 15:00	12824.40	0.86	10178.10	11.75	0
2	4	9/10/94 10:05	7783.40	1.65	6177.30		
	5	9/10/94 10:05	856.18	1.65	679.51		
	6	9/10/94 10:05	8498.89	1.65	6745.15		
3	7	9/11/94 13:50	2207.27	2.81	1751.80		
	8	9/11/94 13:50	3548.80	2.81	2816.51		
	9	9/11/94 13:50	4629.99	2.81	3674.60		
4	10	9/12/94 12:28	380.85	3.75	302.26		
	11	9/12/94 12:28	4692.81	3.75	3724.45		
	12	9/12/94 12:28	2619.73	3.75	2079.15		
5	13	9/13/94 14:32	1171.78	4.84	929.98		
	14	9/13/94 14:32	0.00	4.84	0.00		
	15	9/13/94 14:32	1228.87	4.84	975.29		
6	16	9/14/94 14:49	165.66	5.85	131.48		
	17	9/14/94 14:49	501.56	5.85	398.06		
	18	9/14/94 14:49	533.15	5.85	423.13		
7	19	9/15/94 20:48	97.90	7.10	77.70		
	20	9/15/94 20:48	41.10	7.10	32.62		
	21	9/15/94 20:48	270.40	7.10	214.60		
8	22	9/20/94 12:19	0.00	11.75	0.00		
	23	9/20/94 12:19	0.00	11.75	0.00		
	24	9/20/94 12:19	0.00	11.75	0.00		

Table C8. CS3 data at 50 ppm initial benzene concentration unamended.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	9/7/94 21:11	68194.40	0.00	54122.54	0.00	41690
	Soln only	9/7/94 21:11	67315.20	0.00	53424.76	2.64	34636
	Soln only	9/7/94 21:11		0.00		4.86	33241
	Soln+ soil	9/7/94 21:11	52019.70	0.00	41285.48	6.77	19254
	Soln +soil	9/7/94 21:11	51666.70	0.00	41005.32	8.95	16819
	Soln +soil	9/7/94 21:11	53901.20	0.00	42778.73	10.66	8219
1	1	9/10/94 12:36	41673.70	2.64	33074.37	12.74	839
	2	9/10/94 12:36	46317.60	2.64	36760.00	22.70	0
	3	9/10/94 12:36	42932.50	2.64	34073.41		
2	4	9/12/94 17:49	43583.20	4.86	34589.84		
	5	9/12/94 17:49	42367.40	4.86	33624.92		
	6	9/12/94 17:49	39700.80	4.86	31508.57		
3	7	9/14/94 15:40	35992.10	6.77	28565.16		
	8	9/14/94 15:40	1759.50	6.77	1396.43		
	9	9/14/94 15:40	35027.70	6.77	27799.76		
4	10	9/16/94 20:00	25518.50	8.95	20252.78		
	11	9/16/94 20:00	18797.20	8.95	14918.41		
	12	9/16/94 20:00	19261.10	8.95	15286.59		
5	13	9/18/94 12:57	9474.68	10.66	5121.45		
	14	9/18/94 12:57	16303.70	10.66	8812.81		
	15	9/18/94 12:57	19837.40	10.66	10722.92		
6	16	9/20/94 15:00	4270.24	12.74	2308.24		
	17	9/20/94 15:00	0.00	12.74	0.00		
	18	9/20/94 15:00	384.42	12.74	207.79		
7	19	9/30/94 13:54	0.00	22.70	0.00		
	20	9/30/94 13:54	0.00	22.70	0.00		
	21	9/30/94 13:54	0.00	22.70	0.00		

Table C9. CS3 data at 10 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	8/10/94 8:00	13947.3	0.00	13410.87	0.00	8843
	Soln only	8/10/94 8:00		0.00		1.38	2050
	Soln only	8/10/94 8:00		0.00		2.38	9
	Soln+ soil	8/10/94 8:00	9170.5	0.00	8817.83	3.17	0
	Soln +soil	8/10/94 8:00	9222.1	0.00	8867.43	5.36	0
	Soln +soil	8/10/94 8:00	9456.4	0.00		9.37	0
1	1	8/11/94 17:00	4631.4	1.38	4453.24	19.67	0
	2	8/11/94 17:00	1610.9	1.38	1548.91		
	3	8/11/94 17:00	153.7	1.38	147.81		
2	4	8/12/94 17:00	14.2	2.38	13.61		
	5	8/12/94 17:00	2.2	2.38	2.15		
	6	8/12/94 17:00	12.9	2.38	12.42		
3	7	8/13/94 12:07	0.0	3.17	0.00		
	8	8/13/94 12:07	0.0	3.17	0.00		
	9	8/13/94 12:07	0.0	3.17	0.00		
4	10	8/15/94 16:44	0.0	5.36	0.00		
	11	8/15/94 16:44	0.0	5.36	0.00		
	12	8/15/94 16:44	0.0	5.36	0.00		
5	13	8/19/94 16:47	0.0	9.37	0.00		
	14	8/19/94 16:47	0.0	9.37	0.00		
	15	8/19/94 16:47	0.0	9.37	0.00		
6	16	8/30/94 0:00	0.0	19.67	0.00		
	17	8/30/94 0:00	0.0	19.67	0.00		
	18	8/30/94 0:00	0.0	19.67	0.00		

Table C10. CS3 data at 50 ppm initial benzene concentration with nutrients.

Sample point	Sample	Date	GC Area	Time (days)	Benzene Concentration (ppb)	Average values Time (days)	Average values Conc (ppb)
0	Soln only	9/2/94 21:33	80852.9	0.00	77743.17	0.00	39961
	Soln only	9/2/94 21:33	80710.0	0.00	77605.77	1.57	46922
	Soln only	9/2/94 21:33		0.00		4.47	9600
	Soln + soi	9/2/94 21:33	47145.6	0.00	45332.31	6.00	24
	Soln +soil	9/2/94 21:33	36831.1	0.00	35414.52	8.08	2
	Soln +soil	9/2/94 21:33	40700.6	0.00	39135.19	10.78	0
1	1	9/4/94 11:19	48534.7	1.57	46667.98	13.10	0
	2	9/4/94 11:19	51496.2	1.57	49515.58		
	3	9/4/94 11:19	46365.9	1.57	44582.60		
2	4	9/7/94 8:54	17194.0	4.47	16532.69		
	5	9/7/94 8:54	12614.6	4.47	12129.42		
	6	9/7/94 8:54	143.9	4.47	138.37		
3	7	9/8/94 21:27	34.9	6.00	33.54		
	8	9/8/94 21:27	15.0	6.00	14.44		
	9	9/8/94 21:27	25.5	6.00	24.48		
4	10	9/10/94 23:34	0.0	8.08	0.00		
	11	9/10/94 23:34	0.0	8.08	0.00		
	12	9/10/94 23:34	6.2	8.08	5.95		
5	13	9/13/94 16:13	0.0	10.78	0.00		
	14	9/13/94 16:13	0.0	10.78	0.00		
	15	9/13/94 16:13	0.0	10.78	0.00		
6	16	9/16/94 0:00	0.0	13.10	0.00		
	17	9/16/94 0:00	0.0	13.10	0.00		
	18	9/16/94 0:00	0.0	13.10	0.00		

APPENDIX D: ALL MULTIPLE LINEAR REGRESSION TRIALS

Table D1. Multiple linear regression analysis of all uncontaminated soils at 1 ppm initial benzene concentration. (No nutrient addition)

y parameter	Variables				F_{obs}	F_{crit}	R^2
	x_n	m_n	t-value	p value			
Lag phase	Intercept	8.2436	2.1346	0.1225	1.7315	0.3316	0.6339
	pH	-1.1149	-1.5042	0.2296			
	N + P	-0.0673	-0.9387	0.4171			
	Ca + Mg	0.0004	0.6032	0.5890			
Rate	Intercept	0.2573	0.5505	0.6203	2.2742	0.2587	0.6946
	pH	-0.0303	-0.3377	0.7578			
	N + P	0.0032	0.3738	0.7334			
	Ca + Mg	0.0001	1.4646	0.2393			
Final time	Intercept	25.0589	1.2821	0.2899	0.8133	0.5654	0.4485
	pH	-2.5504	-0.6799	0.5454			
	N + P	-0.2306	-0.6352	0.5704			
	Ca + Mg	0.0000	0.0044	0.9967			
Lag phase	Intercept	0.7812	0.4917	0.1858	2.3096	0.2548	0.6979
	pH	-0.8583	0.4538	0.3535			
	Ca + Mg	0.6371	0.5693	0.9615			
	Al	1.3325	0.2749	0.6012			
Rate	Intercept	0.6639	1.6494	0.1976	4.5252	0.1234	0.8190
	pH	-0.0706	-1.0344	0.3770			
	Ca + Mg	0.0001	1.8641	0.1592			
	Al	-0.0013	-1.2696	0.2938			
Final time	Intercept	8.3278	0.3908	0.7220	0.9098	0.5301	0.4764
	pH	-0.6984	-0.1933	0.8590			
	Ca + Mg	-0.0004	-0.1096	0.9196			
	Al	0.0404	0.7227	0.5221			
Lag phase	Intercept	6.9710	4.2260	0.0242	3.9614	0.1440	0.7984
	pH	-0.6650	-2.5803	0.0818			
	N + P	-0.0664	-1.2825	0.2898			
	(CFU/g dry soil)	-0.1990	-1.7635	0.1760			
Rate	Intercept	-0.3915	-1.3855	0.2599	1.9672	0.2962	0.6630
	pH	0.0792	1.7937	0.1708			
	N + P	0.0078	0.8749	0.4460			
	(CFU/g dry soil)	0.0249	1.2894	0.2877			
Final time	Intercept	27.1401	2.7668	0.0698	1.3860	0.3975	0.5809
	pH	-2.3579	-1.5385	0.2215			
	N + P	-0.2635	-0.8555	0.4551			
	(CFU/g dry soil)	-0.6532	-0.9734	0.4022			
Lag phase	Intercept	7.5646	2.4934	0.0882	2.5885	0.2277	0.7213
	pH	-0.9642	-1.5964	0.2087			
	Ca + Mg	0.0004	0.5996	0.5910			
	(CFU/g dry soil)	-0.1982	-1.4758	0.2365			
Rate	Intercept	0.2346	0.7134	0.5271	4.3617	0.1288	0.8135
	pH	-0.0329	-0.5032	0.6494			
	Ca + Mg	0.0001	1.9504	0.1462			
	(CFU/g dry soil)	0.0177	1.2147	0.3114			
Final time	Intercept	21.4753	1.2599	0.2968	0.9333	0.5220	0.4827
	pH	-1.8485	-0.5447	0.6238			
	Ca + Mg	-0.0006	-0.1543	0.8871			
	(CFU/g dry soil)	-0.5675	-0.7521	0.5066			
Lag phase	Intercept	6.0848	3.8593	0.0307	3.4742	0.1668	0.7765
	pH	-0.6339	-2.3339	0.1018			
	(CFU/g dry soil)	-0.1229	-0.9428	0.4153			
	(benz degraders/g dry soil)	-0.0033	-1.0903	0.3553			
Rate	Intercept	-0.2875	-1.1028	0.3507	1.8710	0.3099	0.6517
	pH	0.0796	1.7722	0.1745			
	(CFU/g dry soil)	0.0304	1.4087	0.2537			
	(benz degraders/g dry soil)	-0.0004	-0.8021	0.4811			
Final time	Intercept	23.6208	2.4175	0.0944	0.9811	0.5061	0.4952
	pH	-2.2714	-1.3495	0.2700			
	(CFU/g dry soil)	-0.4823	-0.5971	0.5925			
	(benz degraders/g dry soil)	-0.0059	-0.3141	0.7740			
Lag phase	Intercept	3.0719	0.9963	0.3925	3.6574	0.1576	0.7853
	pH	0.0018	0.0026	0.9981			
	Ca + Mg	-0.0008	-1.0238	0.3813			
	(benz degraders/g dry soil)	-0.0071	-1.9288	0.1493			
Rate	Intercept	0.6791	2.2338	0.1116	7.4089	0.0671	0.8811
	pH	-0.1307	-1.9061	0.1527			
	Ca + Mg	0.0002	3.4058	0.0423			
	(benz degraders/g dry soil)	0.0007	2.0048	0.1387			
Final time	Intercept	5.9029	0.3392	0.7568	1.4864	0.3763	0.5978
	pH	1.6363	0.4168	0.7049			
	Ca + Mg	-0.0046	-1.1012	0.3512			
	(benz degraders/g dry soil)	-0.0260	-1.2593	0.2970			

Table D2. Multiple linear regression analysis of all uncontaminated soils at 1 ppm initial benzene concentration. (WITH nutrient addition)

y parameter	Variables				F_{obs}	F_{crit}	R^2
	x_n	m_n	t-value	p value			
Lag phase	Intercept	3.8374	4.4342	0.0213	9.4507	0.0488	0.9043
	pH	-0.6423	-4.0806	0.0266			
	% organic matter	-0.2247	-0.7662	0.4993			
	% water	0.6943	2.3122	0.1038			
Rate	Intercept	0.2104	4.7568	0.0176	71.3456	0.0027	0.9862
	pH	0.0806	10.0253	0.0021			
	% organic matter	-0.1183	-7.8955	0.0042			
	% water	0.0521	3.3985	0.0425			
Final time	Intercept	8.3626	7.0284	0.0059	10.0107	0.0452	0.9092
	pH	-1.0376	-4.7948	0.0173			
	% organic matter	-0.1434	-0.3556	0.7456			
	% water	0.7238	1.7534	0.1778			
Lag phase	Intercept	7.9360	8.9492	0.0029	40.4651	0.0063	0.9759
	pH	-0.8235	-9.5859	0.0024			
	% sand	-0.0346	-5.4878	0.0119			
	% organic matter	-0.1124	-1.0302	0.3787			
Rate	Intercept	0.3763	2.3432	0.1009	21.8525	0.0153	0.9562
	pH	0.0732	4.7049	0.0182			
	% sand	-0.0014	-1.2634	0.2957			
	% organic matter	-0.0926	-4.6875	0.0184			
Final time	Intercept	12.6313	6.8725	0.0063	18.2243	0.0198	0.9480
	pH	-1.2264	-6.8876	0.0063			
	% sand	-0.0360	-2.7578	0.0703			
	% organic matter	-0.0258	-0.1142	0.9163			
Lag phase	Intercept	7.4544	10.6333	0.0018	34.2370	0.0080	0.9716
	pH	-0.8465	-7.7307	0.0045			
	% sand	-0.0316	-6.0952	0.0089			
	(CFU/g dry soil)	0.0360	0.6717	0.5499			
Rate	Intercept	-0.0231	-0.0905	0.9336	3.7917	0.1513	0.7913
	pH	0.0548	1.3714	0.2638			
	% sand	0.0011	0.5669	0.6104			
	(CFU/g dry soil)	0.0292	1.4954	0.2317			
Final time	Intercept	13.1719	13.1843	0.0009	33.5548	0.0083	0.9711
	pH	-1.3561	-8.6903	0.0032			
	% sand	-0.0433	-5.8591	0.0099			
	(CFU/g dry soil)	0.1187	1.5543	0.2180			
Lag phase	Intercept	3.7373	3.7960	0.0321	7.8141	0.0626	0.8865
	pH	-0.6687	-3.4823	0.0400			
	% water	0.5042	2.6539	0.0767			
	(CFU/g dry soil)	0.0176	0.1590	0.8838			
Rate	Intercept	0.1143	0.6679	0.5520	4.2714	0.1320	0.8103
	pH	0.0537	1.6087	0.2060			
	% water	-0.0267	-0.8087	0.4779			
	(CFU/g dry soil)	0.0256	1.3270	0.2765			
Final time	Intercept	8.0253	7.1243	0.0057	12.4092	0.0338	0.9254
	pH	-1.1364	-5.1721	0.0140			
	% water	0.7366	3.3890	0.0428			
	(CFU/g dry soil)	0.1139	0.8986	0.4351			
Lag phase	Intercept	7.2610	10.5375	0.0018	29.7673	0.0098	0.9675
	pH	-0.8095	-7.8960	0.0042			
	% sand	-0.0292	-7.0975	0.0058			
	(cells/g dry soil)	0.0001	0.1159	0.9150			
Rate	Intercept	-0.2728	-1.1114	0.3474	3.3843	0.1717	0.7719
	pH	0.1024	2.8050	0.0676			
	% sand	0.0040	2.7364	0.0716			
	(cells/g dry soil)	-0.0005	-1.3383	0.2732			
Final time	Intercept	12.6478	10.9507	0.0016	20.8099	0.0164	0.9541
	pH	-1.2559	-7.3080	0.0053			
	% sand	-0.0367	-5.3156	0.0130			
	(cells/g dry soil)	0.0011	0.6467	0.5639			
Lag phase	Intercept	4.3367	2.5150	0.0866	1.7227	0.3330	0.6327
	pH	-0.4017	-1.3524	0.2691			
	(CFU/g dry soil)	-0.1871	-1.3128	0.2807			
	(cells/g dry soil)	-0.0011	-0.3201	0.7699			
Rate	Intercept	0.0829	0.8468	0.4593	14.2772	0.0279	0.9345
	pH	0.0425	2.5217	0.0860			
	(CFU/g dry soil)	0.0469	5.7917	0.0102			
	(cells/g dry soil)	-0.0005	-2.7549	0.0705			
Final time	Intercept	8.9006	3.7102	0.0340	1.8015	0.3204	0.6430
	pH	-0.7504	-1.8159	0.1670			
	(CFU/g dry soil)	-0.1998	-1.0074	0.3880			
	(cells/g dry soil)	-0.0008	-0.1624	0.8813			

Table D3. Multiple linear regression analysis of all uncontaminated soils at 10 ppm initial benzene concentration. (No nutrient addition)

y parameter	Variables				F_{obs}	F_{crit}	R^2
	x_n	m_n	t-value	p value			
Lag phase	Intercept	11.2624	2.1442	0.1214	1.7159	0.3341	0.6318
	pH	-1.0597	-1.0380	0.3756			
	CEC	-0.0639	-0.2170	0.8421			
	N +P	-0.1496	-1.0631	0.3657			
Rate	Intercept	-2.1348	-0.8303	0.4673	0.7460	0.5923	0.4273
	pH	0.4834	0.9673	0.4048			
	CEC	-0.0408	-0.2831	0.7955			
	N +P	0.0657	0.9531	0.4109			
Lag phase	Intercept	12.3821	2.1610	0.1195	2.7132	0.2171	0.7307
	pH	-1.3559	-1.1867	0.3208			
	N +P	-0.1490	-1.8307	0.1646			
	Ca+Mg	0.0005	0.5232	0.6370			
Rate	Intercept	2.3731	1.0280	0.3796	2.5242	0.2335	0.7162
	pH	-0.4301	-0.9344	0.4190			
	N +P	0.0248	0.7558	0.5047			
	Ca+Mg	0.0007	1.6945	0.1887			
Lag phase	Intercept	10.0409	3.0384	0.0559	2.4706	0.2385	0.7119
	pH	-0.7965	-1.2766	0.2916			
	N +P	-0.1437	-1.6347	0.2006			
	(CFU/g dry soil)	-0.0672	-0.2447	0.8225			
Rate	Intercept	-1.1112	-0.6480	0.5632	0.9853	0.5047	0.4963
	pH	0.1914	0.5910	0.5961			
	N +P	0.0551	1.2087	0.3134			
	(CFU/g dry soil)	0.0790	0.5546	0.6178			
Lag phase	Intercept	8.6404	1.0781	0.3600	0.8482	0.5522	0.4589
	pH	-1.0218	-0.6352	0.5704			
	Ca+Mg	-0.0002	-0.1437	0.8949			
	(CFU/g dry soil)	0.1374	0.4017	0.7148			
Rate	Intercept	3.0231	1.2305	0.3062	2.0300	0.2878	0.6700
	pH	-0.4874	-0.9885	0.3958			
	Ca+Mg	0.0008	1.9515	0.1461			
	(CFU/g dry soil)	-0.0278	-0.2656	0.8078			
Lag phase	Intercept	12.6069	3.4047	0.0423	2.2899	0.2569	0.6960
	pH	-1.1881	-2.0463	0.1332			
	N +P	-0.1903	-1.5721	0.2140			
	(cells/g dry soil)	-0.0051	-0.8313	0.4668			
Rate	Intercept	-1.4099	-0.7499	0.5078	0.9658	0.5111	0.4913
	pH	0.3915	1.3279	0.2762			
	N +P	0.0458	0.7459	0.5099			
	(cells/g dry soil)	-0.0021	-0.6839	0.5431			
Lag phase	Intercept	9.5944	2.2198	0.1131	1.0149	0.4953	0.5037
	pH	-1.2012	-1.6133	0.2051			
	(CFU/g dry soil)	0.2117	0.5925	0.5952			
	(cells/g dry soil)	-0.0045	-0.5414	0.6259			
Rate	Intercept	-0.9372	-0.5170	0.6409	0.7643	0.5848	0.4332
	pH	0.3731	1.1946	0.3181			
	(CFU/g dry soil)	0.0656	0.4380	0.6910			
	(cells/g dry soil)	-0.0034	-0.9820	0.3985			

Table D4. Multiple linear regression analysis of all uncontaminated soils at 10 ppm initial benzene concentration. (WITH nutrient addition)

y parameter	Variables				F_{obs}	F_{crit}	R^2
	x_n	m_n	t-value	p value			
Lag phase	Intercept	5.3039	2.8874	0.0631	4.2986	0.1310	0.8113
	pH	-0.7166	-2.7742	0.0693			
	% sand	-0.0002	-0.0112	0.9918			
	% clay	0.0426	2.0742	0.1297			
Rate	Intercept	1.8262	0.5590	0.6152	1.2512	0.4291	0.5558
	pH	0.1509	0.3284	0.7642			
	% sand	0.0203	0.7458	0.5099			
	% clay	-0.0164	-0.4475	0.6848			
Final time	Intercept	8.8774	3.0966	0.0534	11.0606	0.0395	0.9171
	pH	-1.2276	-3.0451	0.0556			
	% sand	0.0210	0.8810	0.4432			
	% clay	0.1351	4.2113	0.0245			
Lag phase	Intercept	-0.7705	-0.2879	0.7922	1.1862	0.4459	0.5426
	% sand	0.0191	0.6880	0.5409			
	% clay	0.0003	0.0067	0.9950			
	% organic matter	0.7272	1.1888	0.3200			
Rate	Intercept	5.6846	3.0495	0.0554	5.0544	0.1081	0.8348
	% sand	-0.0032	-0.1636	0.8804			
	% clay	0.0236	0.8830	0.4423			
	% organic matter	-0.9863	-2.3148	0.1036			
Final time	Intercept	-3.2025	-1.0628	0.3659	8.5588	0.0556	0.8954
	% sand	0.0665	2.1333	0.1226			
	% clay	0.0424	0.9822	0.3984			
	% organic matter	1.7865	2.5937	0.0808			
Lag phase	Intercept	5.2665	5.8741	0.0098	11.1991	0.0388	0.9180
	pH	-0.5463	-2.8671	0.0642			
	% clay	0.0306	2.9375	0.0606			
	(CFU/g dry soil)	-0.1583	-1.9767	0.1425			
Rate	Intercept	3.4964	1.5762	0.2131	1.6781	0.3406	0.6266
	pH	-0.1040	-0.2207	0.8395			
	% clay	-0.0208	-0.8076	0.4784			
	(CFU/g dry soil)	0.2198	1.1093	0.3482			
Final time	Intercept	10.5647	4.5126	0.0203	8.9192	0.0527	0.8992
	pH	-1.1739	-2.3593	0.0995			
	% clay	0.1076	3.9579	0.0288			
	(CFU/g dry soil)	-0.0680	-0.3252	0.7664			
Lag phase	Intercept	3.5700	2.3848	0.0972	2.4619	0.2394	0.7111
	% clay	0.0239	0.7456	0.5100			
	water	-0.2488	-0.4290	0.6968			
	(CFU/g dry soil)	-0.3179	-1.7003	0.1876			
Rate	Intercept	2.8478	1.4362	0.2465	1.6516	0.3451	0.6229
	% clay	-0.0295	-0.6948	0.5372			
	water	0.1049	0.1365	0.9001			
	(CFU/g dry soil)	0.2236	0.9028	0.4332			
Final time	Intercept	6.9934	2.0479	0.1330	2.6882	0.2191	0.7289
	% clay	0.0949	1.2984	0.2849			
	water	-0.5694	-0.4303	0.6960			
	(CFU/g dry soil)	-0.4188	-0.9818	0.3986			
Lag phase	Intercept	4.5274	2.6774	0.0752	2.1592	0.2718	0.6835
	pH	-0.1919	-0.6589	0.5570			
	(CFU/g dry soil)	-0.2915	-2.0855	0.1283			
	(cells/g dry soil)	-0.0004	-0.1076	0.9211			
Rate	Intercept	4.0025	2.8549	0.0648	5.1756	0.1051	0.8381
	pH	-0.3116	-1.2903	0.2874			
	(CFU/g dry soil)	0.4288	3.7003	0.0343			
	(cells/g dry soil)	-0.0063	-2.3285	0.1023			
Final time	Intercept	7.9589	1.6167	0.2044	1.0664	0.4795	0.5161
	pH	0.0207	0.0245	0.9820			
	(CFU/g dry soil)	-0.7213	-1.7724	0.1744			
	(cells/g dry soil)	0.0090	0.9425	0.4155			
Lag phase	Intercept	1.3248	0.4882	0.6589	2.3539	0.2502	0.7018
	% sand	0.0153	0.7034	0.5325			
	matter	0.3891	0.8109	0.4768			
	(CFU/g dry soil)	-0.2340	-1.2659	0.2949			
Rate	Intercept	5.9139	2.2513	0.1098	3.8094	0.1505	0.7921
	% sand	-0.0079	-0.3743	0.7331			
	matter	-0.7755	-1.6697	0.1936			
	(CFU/g dry soil)	-0.0087	-0.0487	0.9642			
Final time	Intercept	-4.0590	-0.9624	0.4068	6.6976	0.0763	0.8701
	% sand	0.0603	1.7849	0.1723			
	matter	2.3720	3.1808	0.0501			
	(CFU/g dry soil)	0.1261	0.4391	0.6903			

Table D5. Multiple linear regression analysis of all soils (contaminated and uncontaminated) at 10 ppm initial benzene concentration with and without nutrient addition.

y parameter	Variables				F_{obs}	F_{crit}	R^2
	x_n	m_n	t-value	p value			
Lag phase	Intercept	1.4793	1.5332	0.1858	1.4007	0.3545	0.5284
	% clay	0.0298	1.0225	0.3535			
	% organic matter	-0.0221	-0.0507	0.9615			
	(CFU/g dry soil)	-0.0181	-0.5575	0.6012			
	(benz degraders/g dry soil)	0.0001	0.5168	0.6274			
Rate	Intercept	5.2355	8.4353	0.0004	48.3218	0.0003	0.9748
	% clay	0.0050	0.2682	0.7993			
	% organic matter	-0.6809	-2.4294	0.0594			
	(CFU/g dry soil)	-0.0054	-0.2572	0.8073			
	(benz degraders/g dry soil)	0.0001	0.7201	0.5037			
Final time	Intercept	3.7700	2.3720	0.0638	3.3495	0.1086	0.7282
	% clay	0.0766	1.5936	0.1719			
	% organic matter	0.1627	0.2266	0.8297			
	(CFU/g dry soil)	-0.0428	-0.7990	0.4605			
	(benz degraders/g dry soil)	0.0002	0.7665	0.4780			
Lag phase	Intercept	9.2657	2.4519	0.0578	1.8291	0.2609	0.5940
	pH	-1.0471	-1.4431	0.2086			
	CEC	-0.0331	-0.3937	0.7100			
	(CFU/g dry soil)	0.0366	0.7420	0.4915			
	(benz degraders/g dry soil)	-0.0001	-0.7843	0.4684			
Rate	Intercept	-0.5547	-0.2968	0.7785	3.1309	0.1212	0.7147
	pH	0.2922	0.8144	0.4524			
	CEC	0.0417	1.0029	0.3619			
	(CFU/g dry soil)	-0.0576	-2.3568	0.0650			
	(benz degraders/g dry soil)	0.0002	2.4246	0.0598			

Table D6. Multiple linear regression analysis of all noncontaminated soils at 1 ppm initial benzene concentration with nutrient addition using **two** parameters.

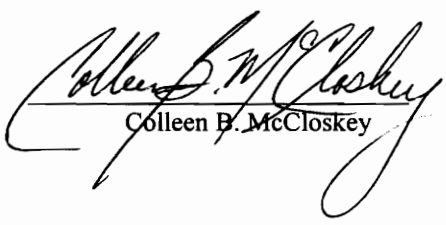
y parameter	Variables				F_{obs}	Prob > F	R^2
	x_n	m_n	t-value	p value			
Lag phase	Intercept	3.7842	4.6326	0.0098	15.4809	0.0131	0.8856
	pH	-0.6547	-4.4157	0.0115			
	% ads. water	0.4811	4.4955	0.0109			
Rate	Intercept	0.1824	1.0237	0.3638	4.6434	0.0906	0.6989
	pH	0.0741	2.2918	0.0837			
	% ads. water	-0.0601	-2.5744	0.0617			
Final time	Intercept	8.3287	7.9431	0.0014	19.1304	0.0090	0.9053
	pH	-1.0455	-5.4939	0.0053			
	% ads. water	0.5878	4.2788	0.0129			
Lag phase	Intercept	7.2422	12.4592	0.0002	59.2602	0.0011	0.9674
	pH	-0.8060	-9.4995	0.0007			
	% sand	-0.0290	-8.9912	0.0008			
Rate	Intercept	-0.1954	-0.7487	0.4957	3.4906	0.1327	0.6357
	pH	0.0877	2.3013	0.0828			
	% sand	0.0032	2.1871	0.0940			
Final time	Intercept	12.4719	12.0190	0.0003	36.2822	0.0027	0.9478
	pH	-1.2223	-8.0704	0.0013			
	% sand	-0.0347	-6.0345	0.0038			
Lag phase	Intercept	3.6899	2.9598	0.0416	5.5128	0.0709	0.7338
	pH	-0.6384	-2.8082	0.0484			
	% org. matter	0.4035	2.5307	0.0646			
Rate	Intercept	0.1993	2.3692	0.0769	27.8329	0.0045	0.9330
	pH	0.0809	5.2755	0.0062			
	% org. matter	-0.0711	-6.6124	0.0027			
Final time	Intercept	8.2088	5.6139	0.0049	8.8761	0.0338	0.8161
	pH	-1.0336	-3.8762	0.0179			
	% org. matter	0.5115	2.7353	0.0522			

Table D7. Multiple linear regression analysis of all noncontaminated soils at 10 ppm initial benzene concentration with nutrient addition using **two** parameters.

y parameter	Variables				F_{obs}	Prob > F	R^2
	x_n	m_n	t-value	p value			
Lag phase	Intercept	5.2901	4.4905	0.0109	8.5968	0.0356	0.8113
	pH	-0.7165	-3.2074	0.0327			
	% clay	0.0428	3.8860	0.0178			
Rate	Intercept	3.4636	1.5185	0.2035	1.7982	0.2773	0.4734
	pH	0.1322	0.3057	0.7751			
	% clay	-0.0378	-1.7707	0.1513			
Final time	Intercept	10.5748	5.1266	0.0069	17.1630	0.0109	0.8956
	pH	-1.2470	-3.1882	0.0333			
	% clay	0.1129	5.8523	0.0043			
Lag phase	Intercept	1.1476	1.8373	0.1400	3.0490	0.1569	0.6039
	CEC	-0.1516	-1.1835	0.3021			
	% org. matter	0.7237	2.2640	0.0863			
Rate	Intercept	5.0318	10.4835	0.0005	9.4910	0.0303	0.8260
	CEC	0.0985	1.0008	0.3735			
	% org. matter	-0.8329	-3.3911	0.0275			
Final time	Intercept	2.8570	3.1091	0.0359	10.9346	0.0239	0.8454
	CEC	-0.3250	-1.7246	0.1597			
	% org. matter	1.8942	4.0281	0.0158			
Lag phase	Intercept	3.6073	2.8856	0.0448	6.3376	0.0575	0.7601
	pH	-0.5056	-2.2177	0.0908			
	% org. matter	0.5310	3.3210	0.0294			
Rate	Intercept	4.9914	3.6191	0.0224	7.2052	0.0472	0.7827
	pH	0.0206	0.0821	0.9385			
	% org. matter	-0.6431	-3.6460	0.0218			
Final time	Intercept	6.1380	2.4736	0.0687	9.7327	0.0291	0.8295
	pH	-0.6901	-1.5252	0.2019			
	% org. matter	1.3984	4.4066	0.0116			
Lag phase	Intercept	0.9659	1.1649	0.3088	1.7764	0.2805	0.4704
	% clay	-0.0068	-0.1986	0.8523			
	% org. matter	0.5110	1.0449	0.3551			
Rate	Intercept	5.3969	10.0095	0.0006	10.0017	0.0278	0.8334
	% clay	0.0247	1.1063	0.3306			
	% org. matter	-0.9505	-2.9891	0.0404			
Final time	Intercept	2.8603	2.0776	0.1063	5.5953	0.0693	0.7367
	% clay	0.0176	0.3088	0.7729			
	% org. matter	1.0315	1.2705	0.2728			

VITA

Colleen McCloskey was born on October 14, 1970 to Mr. and Mrs. William and Barbara McCloskey, taking her place as the youngest of eight children. After finishing high school, she attended Temple University, pursuing a degree in Environmental Engineering Technology. Having had enough of the city life and deciding to pursue an engineering instead of a technology degree, in the Spring of 1991, Colleen decided to transfer to Virginia Tech in beautiful Blacksburg, Virginia. Before graduating from Tech, she was fortunate enough to spend two summers at North Carolina State University studying the biodegradation of gasoline in the subsurface. She obtained her Bachelor of Science in Civil Engineering in 1993 and her Master of Science in Environmental Engineering in 1995, both from Virginia Tech. She is currently employed as an Environmental Engineer, working on computer modeling of underground soil and groundwater contaminants. She specializes in the biodegradation of these contaminants in the subsurface.



Colleen B. McCloskey