The bioavailability of selected aromatic hydrocardons : an extension from saturated to variably saturated soils

by Jordan Lee Peccia

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
Montana State University

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Results validate the model for the bioavailability factor, and show that this factor increases with increasing unsaturated soil moisture. They also conclude that the bioavailability model that is written for saturated soils can be extended to unsaturated soils, although the correlation is not as strong for unsaturated soils.

Soil used in this experiment was obtained from a gasoline pipeline release site in western Montana.

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APPROVAL

of a thesis submitted by

Jordan Lee Peccia

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Dr. A.B. Cunningham _____ Dec 7 1995
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ABSTRACT

Significant progress has been made toward understanding how soil moisture affects the bioavailability of selected aromatic hydrocarbons. Bioavailability is a measure of the influence that sorption (as well as the closely related processes of solubility and volatilization) have on biodegradation rates in soil. A dimensionless bioavailability factor can be expressed as either a ratio of soil biodegradation rates to liquid (maximum) biodegradation rates, or as a function of degradation and sorption parameters.

Results validate the model for the bioavailability factor, and show that this factor increases with increasing unsaturated soil moisture. They also conclude that the bioavailability model that is written for saturated soils can be extended to unsaturated soils, although the correlation is not as strong for unsaturated soils.

Soil used in this experiment was obtained from a gasoline pipeline release site in western Montana.

CHAPTER 1: INTRODUCTION

General

Petroleum releases from ruptured underground pipelines and storage tanks (UST) are a serious environmental problem. In the State of Montana alone, more than 2,625 gasoline contaminated sites have been identified by the Underground Storage Tank Program. The release of gasoline, and more specifically BTEX, (benzene, toluene, ethylbenzene, and paraxylene) into the subsurface results in the formation of four contaminant phases. These include a vapor phase in the vadose zone soil air, a dissolved phase in the soil moisture, a phase sorbed onto the soil, and free product or NAPL (non aqueous phase liquid). The imiscible NAPL phase spreads throughout the vadose zone and creates a reservoir of contaminant that is held in the unsaturated soils (Blicker, Wiedemeier, and Guest, 1994). Ultimately, a substantial fraction of the released BTEX is retained by the unsaturated soil.

Research on the bioremediation (removal of contaminant by biological degradation) of BTEX compounds has focused on ground water systems. This is due to the greater human health risk that is associated with ground water contamination. BTEX that remains in the vadose zone, either sorbed, vaporized, or dissolved in soil moisture has traditionally been of less concern. Sorption of contaminant onto soils reduces the toxicity to animals and plants (Alexander, 1994). As a consequence there is a lack of knowledge required to assess

realistic bioremediation end point concentrations in soil and the time required to reach these end points. An improved understanding of this topic is important to keep unrealistic end point goals from costing industry unnecessary time and money.

Remediation Technologies and Limitations

Currently, several physically-based approaches to site remediation are in use including vapor extraction and excavation of contaminated soils. Physical methods only change the contaminant from one physical phase to another and/or may require a high energy input. An attractive alternative is to encourage the mineralization (degradation to carbon dioxide) of contaminant via biological degradation. This alternative not only converts the contaminant to less toxic by-products, carbon dioxide and water, but is generally less expensive than physical remediation methods(Liu et. al.). Two strategies may be taken in order to remediate a site biologically. They are intrinsic and active bioremediation.

Intrinsic bioremediation refers to allowing natural attenuation to remediate a site as long as expansion of the borders of the contaminant plume does not occur. It is clear that at many sites natural bioremediation occurs (Wolfram et. al. 1993), albeit at a slow rate. The second approach is active bioremediation. Active bioremediation enhances the conditions necessary for the biodegradation reaction to occur, and consequently increases the rate of the reaction. Figure 1 denotes the three necessary constituents for the biotransformation reaction. The

interaction among the various physical, biological and chemical processes shown in figure 1 controls the rate at which intrinsic or enhanced bioremediation will occur.

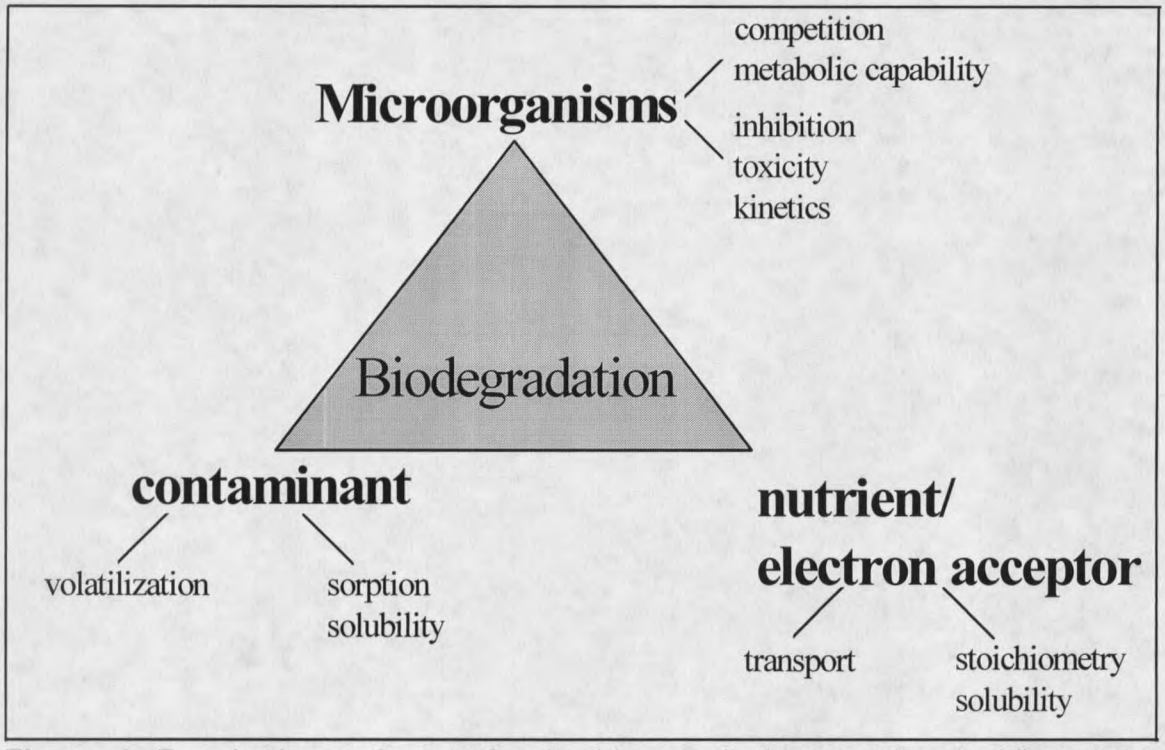

Figure 1. Required constituents for the bioremediation reaction (bold) and the factors which affect the rate and extent of biodegradation.

Field biodegradation rates rarely, if ever, match biological kinetic rates measured in the laboratory. For example, oxygen limitations are often present in the *in_situ* degradation of BTEX compounds. Oxygen's solubility in water is at least an order of magnitude less than that of BTEX (Weast, 1987) Oxygen also has an unfavorable stoichiometry that requires approximately 3.5 times (Sturman et. al., 1992) more oxygen than BTEX for biological mineralization. If one further considers that mass transfer of oxygen into the vadose zone can be much slower

than the degradation reaction (Refsgaard, Christensen, and Ammentorp, 1991), it becomes obvious that an oxygen limitation can exist in the vadose zone.

Bioavailability

In addition to oxygen, substrate availability may also limit biotransformation rates. Depending on the soil characteristics, desorption of contaminant from the soil to the bulk soil water can occur at a much slower rate

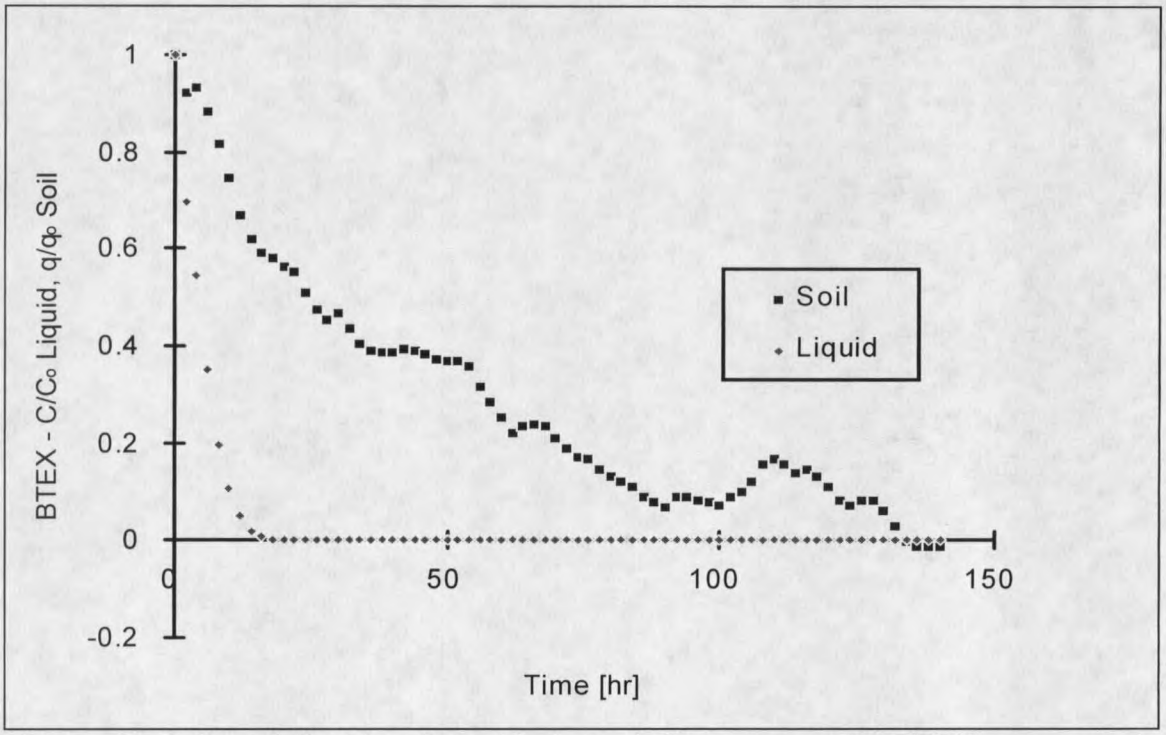

Figure 2. Soil and liquid culture biodegradation curves for BTEX compounds with no nutrient limitations shows slower degradation in the soil culture due to lack of substrate availability.

than the degradation reaction (Zhang and Bouwer, 1995), thereby causing desorption to control the rate at which substrate becomes available for

biotransformation. The effect of bioavailability can be seen in the following comparison of bench scale tests of degradation in soil and liquid cultures. The two samples in figure 2 are not kinetically limited by a lack of nutrients or electron acceptor. The difference in rates is due to a lack of availability of the contaminant. The desorption limitation is reflected in the slower attenuation of BTEX in the soil culture.

Bioavailability is a measure of the influence that sorption (as well as the closely related properties of solubility and volatility) have on degradation rates in soil. Soil moisture is also believed to be an important parameter in desorption phenomena and thus overall bioavailability. This study investigates the influence that vadose zone soil moisture has on the desorption, biodegradation and ultimately the bioavailability of BTEX compounds.

Goals and Objectives

The goal of this project is to extend the concept of bioavailability from saturated soils, as described by Zhang and Bouwer (1995) to unsaturated soils. Objectives used to accomplish this goal are **1)** to determine the effect that soil moisture has on the bioavailability of BTEX compounds in unsaturated soils **2)** to quantify the differences between sorption in unsaturated and saturated soils and **3)** to validate the use of a bioavailability factor (B_f) in unsaturated soils. Bench scale laboratory experiments were performed in order to accomplish these objectives.

CHAPTER 2: BACKGROUND

The Garrison Site

This study on bioavailability is centered around a gasoline contaminated site three miles east of Garrison, Montana. During the fall of 1987, a ruptured underground pipeline released approximately 250,000 gallons of unleaded gasoline down an ephemeral drainage. Vadose zone contamination is confined to the drainage which is approximately 1 km long, 20 meters wide, and varies in depth from 1 to 8 meters.

In September of 1995, a team of student engineers from an environmental engineering course, ENVE 534 (Montana State University) completed an assessment of the Garrison Site. This assessment determined the important physical, chemical and biological parameters required for bioremediation. Parameters determined were nutrient, gasoline range organics, BTEX, and biomass concentrations in soil. Further, temperature, percent soil moisture, organic matter, pH, and soil texture were measured. These analyses were made for varying depth of vadose zone soils, and at various locations throughout the drainage to ensure that contaminated soils as well as uncontaminated soils(for controls) were sampled. Additional field data was obtained from the installation of monitoring piezometers. These piezometers facilitate measurement of oxygen and carbon dioxide concentrations as a function of soil depth.

Data compiled during this field assessment was used to determine appropriate remediation technologies, and to optimize degradation rates through the augmentation of lacking constituents.

The assessment showed the existence of a substantial and metabolically capable microbial population. Nutrients, temperature, and pH were also found to be conducive to natural biodegradation. Soil pore space vapor testing revealed a utilization of oxygen and production of carbon dioxide in contaminated soils. Further, no oxygen utilization or carbon dioxide production was found in uncontaminated soils (figure 3). This lends credibility to a hypothesis that natural bioremediation is occurring.

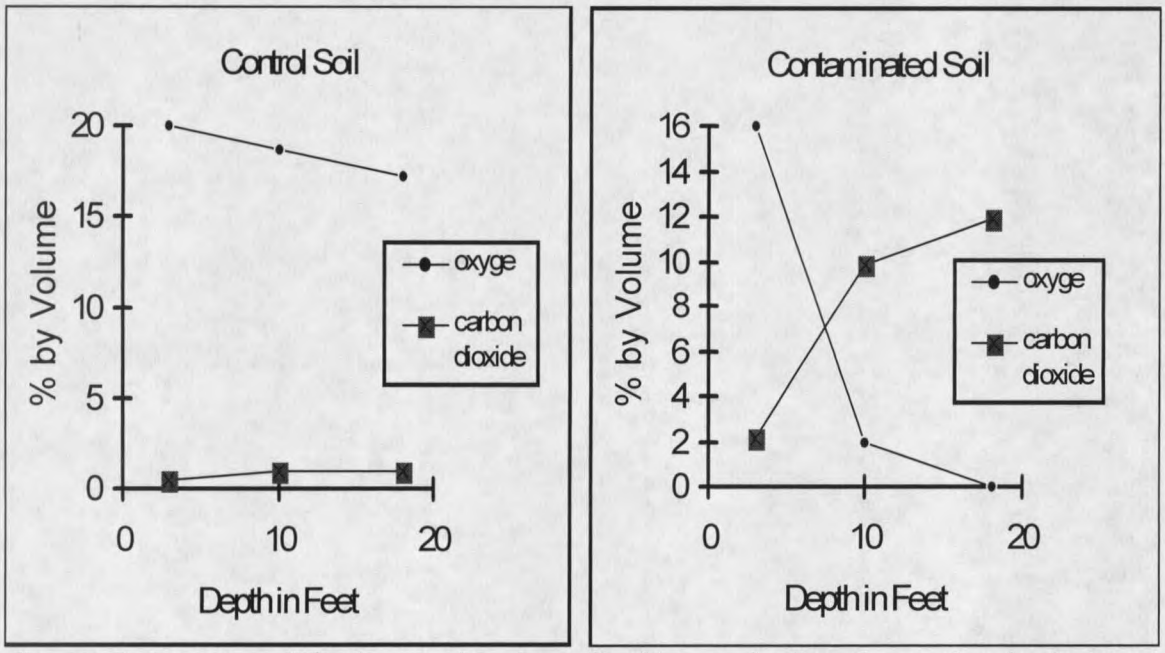

Figure 3. Oxygen utilization and carbon dioxide production for contaminated and control soil show evidence of natural biodegradation at Garrison Site (Hensel,1994).

With evidence of natural degradation, the next step was to consider ways to enhance degradation rates through the augmentation of missing requirements. One parameter of interest was the site's low soil moisture. The site is in the rain shadow of the Flint Creek Mountain Range and therefore very dry. Measured soil moistures varied from 4% to 12.5% (moisture reading taken in late summer). When low soil moisture is coupled with relatively high levels of substrate, biomass, nutrients, and evidence that some biological activity is occurring, a hypothesis was made that the low soil moisture is influencing the rate of natural biodegradation. Issues that were not directly addressed in the original site assessment include the quantification of biodegradation rates, desorption rates and the effect that soil moisture has on these parameters.

Literature Review

Literature concerning the bioavailability of organic contaminants in soils has concentrated on aquifer or saturated soils. An inherent barrier to unsaturated soil studies is that it is difficult, if not impossible, to measure contaminant concentrations in bulk soil water. Therefore, studies have focused on saturated or slurry systems with a large bulk water phase.

One model for soil desorption in slurries has been developed by Tabak et. al, 1994. The rate of desorption is first order:

$$\frac{dq}{dt} = \frac{k_f A_p}{V_p \rho}(C_{pore} - C) \qquad (1)$$

where:

A_p = external surface area of the soil particle [cm^2]
k_f = mass transfer coefficient for the soil bulk water [cm/hr]
V_p = volume of soil particle [cm^3]
ρ = density of the soil [kg/L]
C = concentration of contaminant in soil water [mg/L]
C_{pore} = soil pore water concentration [mg/L]
q = contaminant concentration sorbed onto the soil [mg/kg]

Using this model approach for different PAH's and soil particle size distributions, Tabak was able to calibrate a dual adsorption/desorption model and show that the two rates, adsorption and desorption, are similar.

Zhang and Bouwer (1995) extended the study of desorption to include a function for bioavailability in terms of soil desorption and biodegradation parameters:

$$B_f = \frac{1}{1 + \frac{m}{V}\frac{dq}{dc}} \tag{2}$$

where:

B_f = bioavailability factor
m = mass of soil [kg]
V = volume of water [L]
q = concentration of BTEX sorbed [mg/kg]
C = concentration of BTEX in soil water [mg/kg]

This bioavailability factor B_f, is thus a non-dimensional number that describes the influence of desorption on contaminant degradation rates in soil slurry (saturated) systems. Overall biotransformation rates may then be calculated as the intrinsic microbial degradation rate expression multiplied by the bioavailability factor. This theory can be extended from saturated to unsaturated

soil systems by determining dq/dC, and thus degradation and desorption parameters for unsaturated systems.

Biodegradation in Soils

Soil biodegradation is a two step process. Contaminant is first desorbed from soil and then degraded once it is dissolved in soil bulk water. The uptake of substrate by microorganisms in vadose zone soils occurs only when substrate is in a dissolved state (Huesemann, 1994; Zhang and Bouwer, 1995). The biodegradation term in the bioavailability factor equation is thus a rate coefficient for a liquid culture. When substrate is dissolved in the soil moisture, assuming there are sufficient amounts of electron acceptor, nutrients, and a microbial population that is metabolically capable of utilizing this substrate, the kinetics of substrate removal will be very similar, if not equal, to the intrinsic microbial kinetics that could be determined from a liquid culture bench study. In this study, the biodegradation step will be described by two different kinetic expressions. They are first order Monod and first order degradation kinetics (equation 3 and 4 respectively). The discussion of first order Monod Kinetics and the bioavailability factor using this kinetic expression is preliminary and therefore left for appendix A. First order degradation kinetics describe biodegradation as a first order reaction, independent of biomass concentration changes.

$$\frac{dC}{dt} = -\frac{\mu_{\max}}{K_c} C \frac{X}{Y_{X/C}} \qquad (3)$$

$$\frac{dC}{dt} = -k_{bliq}C \tag{4}$$

where:

k_{bliq}= liquid phase biodegradation coefficient [1/hr]
X = biomass concentration[mg/L]
μ_{max} = maximum growth rate [1/hr]
K_c = Monod half saturation constant [mg/L]
$Y_{X/C}$= biomass to substrate yield [$mg_{biomass}/mg_{substate}$]

It is hypothesized that high dissolved contaminant levels are seldom seen since biodegradation is faster (in most cases) than the desorption step. In fact, experimental results will be used to determine the magnitude of a ratio of biodegradation rates to desorption rates. Ratios significantly greater than one indicate desorption limited biodegradation or contaminant is degraded as soon as it desorbs. Thus high contaminant concentrations do not exist in vadose zone soil water under this scenario. An empirical validation is difficult to obtain since this requires measuring concentrations in the soil bulk water.

The overall biodegradation in a soil matrix can be described in a manner similar to degradation rates in a liquid matrix. The decrease in sorbed concentration is modeled. Therefore, the effects of desorption are seen in the model for overall degradation in a soil matrix. The model is:

$$\frac{dq}{dt} = -k_{bsoil}q \tag{5}$$

where:

k_{bsoil}= first order degradation coefficient in a soil matrix [1/hr]

The kinetic coefficients k_{bliq} and k_{bsoil} will be required to determine B_f.

Sorption Processes

In most, if not all, vadose zone soils with BTEX contamination, desorption is the rate limiting step and the most profound process that limits bioavailability (Zhang and Bouwer, 1995). It is therefore important to consider sorption processes. When sorptive processes, adsorption and desorption, are rapid compared to the biological degradation reaction in the soil moisture, sorption can be described by an equilibrium isotherm and need not be considered in a degradation rate equation. However, if sorption rates are slow compared to degradation rates, a kinetic sorption model must be considered in order to describe degradation rates.

Sorption processes include adsorption, chemisorption, absorption, and ion exchange. This work does not attempt to delineate between each process but consider their combined effect. Adsorption is thus defined as the process when a solute clings to a solid surface (Wood, Kramer, and Hern, 1990). In a similar way, desorption is the process in which a molecule releases from a solid surface and enters into the liquid phase.

Organic compounds such as BTEX represent a special class of sorption phenomena. While there is a small amount of adsorption of organics on mineral surfaces in soil (Ciccioli et al. 1980), the primary adsorptive surface in soil is the fraction of organic solids. When the fraction of soil organic carbon is 1% or

above, sorption is almost exclusively to the organic carbon in the soil (Karichhoff, Brown, and Scott, 1979) and a linear equilibrium sorption coefficient K_d can be defined as:

$$K_d = K_{oc} \cdot f_{oc} \tag{6}$$

where:

K_d = linear soil/water distribution coefficient [L/kg]
$f_{o/c}$ = fraction of organic carbon
K_{oc} = organic fraction partitioning coefficient [L/kg]

If the fraction of organic carbon is below 1%, K_d must be determined through experimentation.

Sorption Kinetics. Sorption kinetics describe the rates at which a compound adsorbs and desorbs from a surface. Soils may exhibit different sorption kinetics depending on the sorbing compound and surface. Sorption may follow a first order model with respect to concentration, a nonlinear (exponential) model, or other empirical expression.

This study assumes that adsorption and desorption phenomena for BTEX follow the first order relationship in equation 7.

$$\frac{dq}{dt} = -k_m (C_{pore} - C) \tag{7}$$

where:

k_m = first order desorption coefficient for saturated conditions [L/kg·hr], denoted as k_{mu} for unsaturated soils.

It is further assumed that desorption rates and adsorption rates follow the same model, (Tabak et. al., 1994).

CHAPTER 3: MATERIALS, METHODS, AND MODELS

This chapter describes the experimental approach. There are two sections. The first is development of the bioavailability factor model and the experimental design. Second is a detailed discussion of the methods and models used in the determination of degradation, desorption, and partitioning coefficients required for the bioavailability factor.

Experimental Approach

B_f Model Development

The bioavailability factor B_f described by Zhang and Bouwer(1995), (equation 2) is developed for unsaturated soils in this section. B_f can be expressed as either a ratio of soil biodegradation rates to liquid (maximum) biodegradation rates, or as a function of degradation and sorption parameters.

For the first case, a ratio of soil to liquid matrix degradation rates can be determined from setting two different expressions for overall soil matrix degradation equal:

$$\frac{dC}{dt} = -k_{bsoil}C = -B_f k_{bliq}C \tag{8}$$

Solving equation 8 for B_f yields:

$$B_f = \frac{k_{bsoil}}{k_{bliq}} \tag{9}$$

To write B_f as a function of degradation and sorption parameters, a batch reactor mass balance for a soil/water contaminant system is written.

$$\frac{d}{dt}[VC + mq] = -Vk_{bliq}C \tag{10}$$

Equation 10 can be solved for dC/dt such that:

$$\frac{dC}{dt} = -k_{bliq}C \cdot \frac{1}{1 + \frac{m}{V}\frac{dq}{dC}} \tag{11}$$

so that B_f becomes:

$$B_f = \frac{1}{1 + \frac{m}{V}\frac{dq}{dC}} \tag{2}$$

dq/dC in equation 2 is determined by first assuming desorption limited degradation (equation 12).

$$-m\frac{dq}{dt} = Vk_{bliq}C \tag{12}$$

With this primary assumption, the strategy for determining dq/dC is to determine q from equation 12 and differentiate with respect to concentration. Depending on a variety of desorption models used for dq/dt, B_f can take on a number of forms. Using the first order model for dq/dt, where $C_{pore} = q/K_d$, and fist order degradation assumption, dq/dC can be written as:

$$\frac{dq}{dC} = K_d\left(1 + \frac{Vk_{bliq}}{mk_{mu}}\right) \tag{13}$$

Solving the final expression for bioavailability becomes:

$$B_f = \frac{1}{1 + \frac{m}{V} K_d + \frac{k_{bliq}}{k_m} K_d} \tag{14}$$

Equation 14 has several fundamental assumptions associated with it. Foremost is the assumption that the expression is valid only for desorption limited degradation. Further, desorption kinetic coefficients are assumed to follow a first order (with concentration) model, and K_d follows a linear isotherm. The first order degradation coefficient used in equation 14 is valid only if the systems biomass concentration (and hence k_{bliq}) is constant with respect to time. This may be true if degradation is slow and decay is similar to growth. For systems that show increases in biomass concentration over time, an attempt to find a bioavailability factor that accounts for the change in biomass is required to accurately describe the effects of desorption on overall soil matrix biodegradation. The bioavailability factor, as a function of biomass is presented in appendix A. Finally, the bioavailability factor determined from equation 9 is a measured B_f that accounts for every processes that may effect substrate availability. A full discussion of the validity of each assumption is contained in the experimental description for each parameter.

Experimental Design

To achieve the goals set forth in the introductory chapter, the bioavailability factor is determined from equation 9, a ratio of degradation rates, and from equation 14, the model developed by Zhang and Bouwer, 1995. Equation 9 is representative of a measured bioavailability factor, hence equation 14 results are compared to equation 9 results for validation. To determined B_f from equation 9, k_{bliq} and k_{bsoil} must be measured. To determine B_f from equation 14, k_{bliq}, k_m, K_d, m, and V must be measured.

Equation 14 was written for saturated soils. The first set to extending B_f to unsaturated soils is to calculated B_f (from equation 9 and 14) throughout the range of unsaturated soil moistures to create a curve of B_f versus soil moisture for both methods. A close correlation between the two curves indicates that equation 14 is a valid for unsaturated soils.

Experimental Descriptions

Soil Parameters

Soil used in this work was obtained from the Garrison Site. The sampling point is located in the ephemeral drainage at a depth of 1.5 meters. This location was chosen because it showed soil moisture, pH, nutrients and biomass levels that are favorable for the biodegradation reaction. The soil was stored at 4°C.

The following describes the analyses used to determine relevant soil parameters. NO_3^-, NH_4^+, PO_4^{-3}, and organic matter were tested at the Montana State University Plant Soils Analysis Laboratory. Soil pH was determined using a 2:1 paste extraction method. NO_3^- was determined using a KCl extraction, a cadmium reduction to NO_2^-, and a modified Griess-Ilosvay method for measurement (Lowe and Hamilton, 1967) . NH_4^+ was determined using a similar method by Silva and Bremmer, 1966. Olsen phosphate (PO_4^{-3}) was determined using a sodium bicarbonate extraction (Page, Miller, and Keeney, 1982) and ion chromatography (Greenberg et. al., 1985).

Soil concentrations of NO_3^-, NH_4^+, PO_4^{-3} and pH, were measured to determine whether the soils required additional nutrients and whether the soil pH was conducive to biological activity.

Percent organic matter was determined by the Walkley-Black Method (Walkley and Black, 1934). Percent organic matter in soils controls the extent of sorption (Karichhoff, Brown and Scott 1979). Soil organic matter must be measured in order to choose the method in which to determine K_d (see sorption discussion, chapter II).

Numbers of BTEX degrading organisms per gram of soil and milliliter of water were determined. All microbial numbers were obtained from plate counts. A mineral salts solution, msmx, (see appendix D for recipe), and Noble® Agar was used for the growth media. This media was augmented to a concentration

of 100 ppm with BTEX, the sole carbon source. Each constituent of BTEX was added in equal mass portions. Triplicate plates were used, average values reported and standard deviations computed. The pH of the agar was set to match field conditions.

1 gram of soil was diluted with 10 ml of sterile water for dilution tube inoculation. The mixture was vortexed for 1 minute and dilutions 10^{-2} to 10^{-7} were plated. The soil dilution tube procedure was strictly adhered to each time to ensure that the same fraction of biomass was washed off for each sample (vortexing does not remove all of the biomass from soil). Liquid sample dilution tube protocol is similar except 1 ml of sample was added to 9 ml of sterile water.

The plates were incubated at 25°C for 10 days. Controls checked the sterility of dilution tubes and the plating technique.

Initially, biomass measurements were taken to determined if a population of BTEX degraders existed. BTEX degrading organism concentrations play another important role in these studies. Microbial counts were taken before biodegradation experiments to determine initial biomass, and after to look at population increases during respiration. Further, a knowledge of the change in biomass concentration over time is critical in evaluating microbial kinetic assumptions for the bioavailability equation, and to check the sterility of batch sorption reactors

Soil moisture was determined on a dry weight basis by gravimetric analysis. The percent soil moisture is:

$$\%soilmoistrue = \frac{wetsoil - drysoil}{drysoil} \cdot 100 \qquad (15)$$

Wet soil weight was determined on a Mettler PJ400 Balance. Next, the sample was placed in a ceramic crucible and cooked at 105°C for 24 hours. The sample was then re-weighed to determine the dry weight. Finally, the percent soil moisture was determined through the application of equation 15. Soil moisture is an important parameter. Degradation rates and bioavailability were plotted as a function of soil moisture in this study.

Soil texture was determined by a pipette method(Kilmer and Alexander, 1949). Silt, sand, and clay fractions were determined. Soil texture may have profound effects on the bioavailability of a compound. Desorption rate and extent are a function of soil texture. (Tabak et. al., 1994).

Soil field capacity was determined by first saturating a column of soil and allowing it to drain for 48 hours. A gravimetric analysis determined the soil moisture at field capacity. Field capacity is defined as the maximum unsaturated soil moisture or the amount of water held in the soil after excess gravitational water has drained away. (Veihmey and Hendrickson , 1931).

Parameters Required to Determine the Bioavailability Factor

Expressions for the bioavailability factor (equation 9 and 14) are:

(9) (14)

$$B_f = \frac{k_{bsoil}}{k_{bliq}} = \frac{1}{1 + \frac{m}{V} K_d + \frac{k_{bliq}}{k_m} K_d} \quad (16)$$

Methodologies for determining k_{bliq}, k_{bsoil}, k_m, and K_d are discussed in this section.

k_{bliq} and k_{bsoil}. k_{bliq} was required to determine the maximum degradation rate for the microbial consortia with BTEX as the sole carbon source. This coefficient represents the degradation term in the bioavailability equation. k_{bsoil} is the kinetic coefficient for degradation of BTEX in a soil matrix. This parameter , as well as k_{bliq}, was required to determine bioavailability as a ratio of degradation rates. Seven different soil moistures; 6.2, 12.5, 15, 20, 25, 50, and 100% were considered. 6.2% through 25% for unsaturated, 50% and 100% for saturated.

Electrolytic respirometry was used to determine these kinetic coefficients. The respirometer used was a Bioscience® ER100 series (Bethlehem, PA)(figure 4). The operating principle behind the respirometer is a continuously stirred (for liquids) batch culture in which oxygen consumption is monitored in real time. The respirometer measures only the aerobic mineralization of the substrate(BTEX). Figure 5 is a typical respirometry oxygen utilization curve for hydrocarbons in a liquid culture.

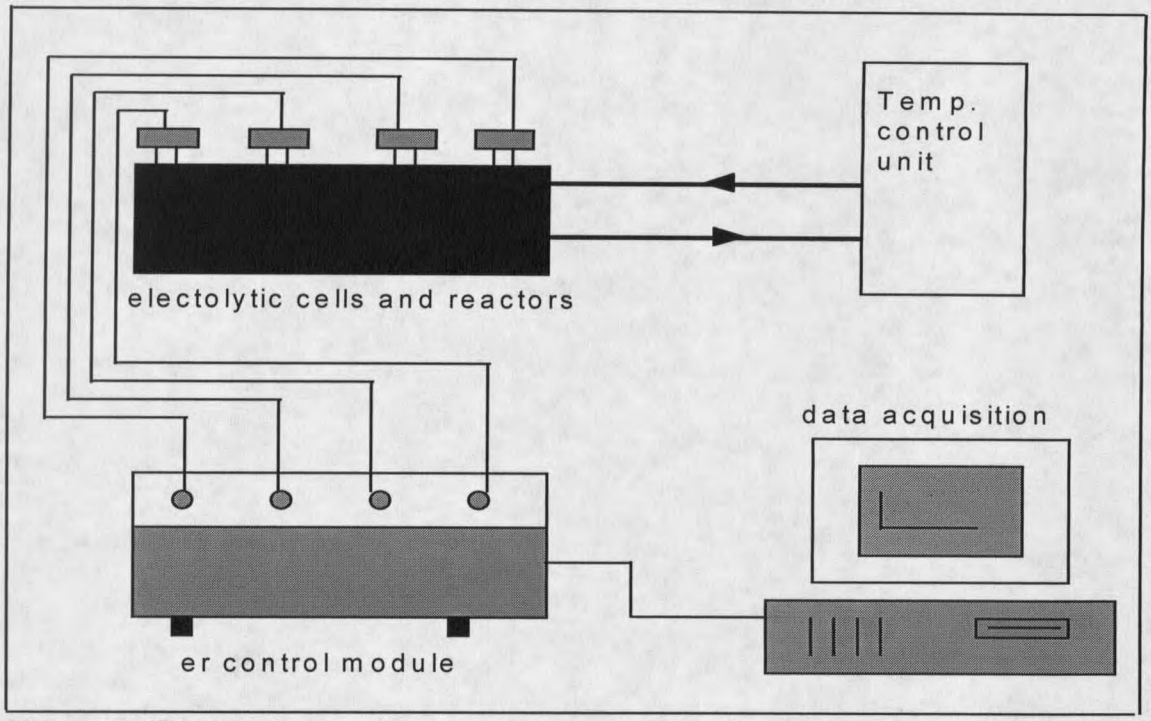

Figure 4. Electrolytic respirometry system schematic.

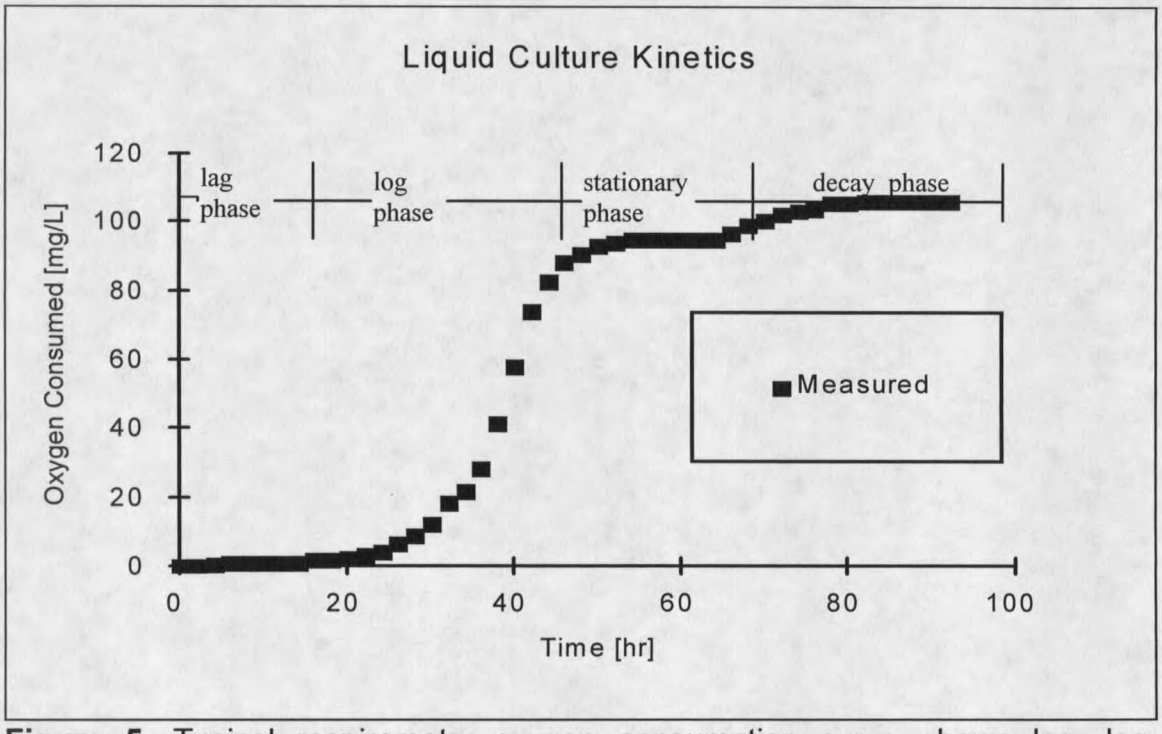

Figure 5. Typical respirometry oxygen consumption curve shows lag, log, stationary, and decay phase indicative of microbial respiration.

The curve follows the form of a growth curve until a stationary phase is reached. At this point, endogenous respiration is reflected in the curve by a slight increase in oxygen consumption.

The advantage of using this equipment is accurate, real time oxygen consumption measurements. The disadvantage is that electrolytic respirometers are sensitive to barometric pressure and temperature changes. Temperature fluctuations were controlled by a Fisher Scientific Isotemp Refrigerated Circulator Model 9100.

The sensitivity to changes in barometric pressure are profound only when degradation rates are very slow, such as in unsaturated soils, or some soil slurries. The respirometer's manufacturer, Bioscience, offers a spreadsheet model in which the apparent oxygen demand due to changes in barometric pressure can be determined and separated from the raw data. Raw barometric pressure data for Bozeman, MT was acquired from the Montana State University Weather Station.

The following is the experimental protocol for liquid cultures. Liquid and soil culture experiments were run at 15°C to simulate vadose zone temperatures.

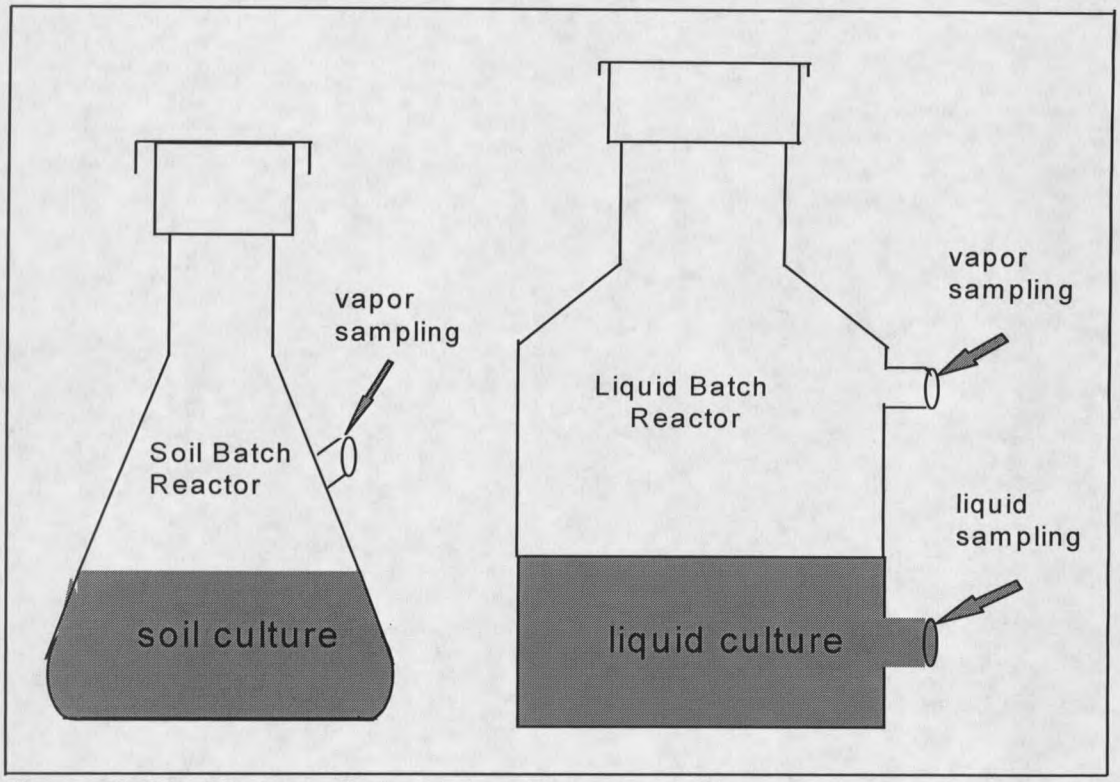

Figure 6. Modified respirometry batch reactors allow non-destructive sampling of liquid and vapor phase BTEX concentrations and decrease the effects of barometric pressure changes on oxygen consumption data.

A one liter volumetric flask modified to hold two Mininert® Valves (figure 6) was sterilized. The top valve was used to sample vapor in the head space, the bottom valve was used for aqueous samples and addition of BTEX.

Next, 25ml of microbial inoculum was added to the reactor. The inoculum for the liquid cultures was obtained from the supernatant of a soil slurry. Soil from the Garrison Site was sifted with a .125 inch sieve. 50 grams of this soil was mixed in a sterile 500ml volumetric flask with 100 ml of sterile distilled water. After .25 hours, mixing was stopped and the soil particles were allowed to settle for 1 hour. 25ml of the supernatant was extracted and used for the microbial

inoculum for the liquid batch reactors. The reactor was then partially filled with 375 ml of sterile mineral salts media (msmx).

The addition of BTEX was the last step in starting respirometry batch cultures. Benzene, toluene, ethyl-benzene, and paraxylene were mixed in equal portions on a mass basis. This BTEX mixture was then added to the reactor through the liquid side Mininert® valve by a 100 µl Hamilton® syringe. The system remained sealed throughout operation.

Soil batch studies were performed in a similar manner. A 125ml flask (figure 6) was partially filled with 70 grams of soil which had been filtered with a .125 inch sieve. The head space volume of this flask is less so that there is a greater sample to head-space ratio, hence less influence from changes in temperature and barometric pressure. Since soil degradation rates are much slower than liquids, soil systems are more vulnerable to the effects of barometric pressure.

Water was added, or the soil dried in order to achieve the correct soil moisture. Water was added before BTEX so that water becomes the wetting fluid for the soil, thus increasing the distribution (by mixing) of BTEX throughout the soil. A final step was to add the BTEX and shake the flask vigorously for 60 seconds. BTEX was added on the glass and then allowed to volatilize throughout the container before mixing. The flasks were placed in the respirometer water bath. Initial and final vapor phase BTEX was sampled.

The contaminant must be sorbed to the soil before accurate biodegradation measurements can be made. Therefore, time to equilibrium must be determined. Sterile soil was added to respirometry reactors and head-space measurements were taken over time. Equilibrium was achieve when the vapor phase concentration became constant with time.

Thorough mixing of liquid cultures in the electrolytic batch reactors ensured that oxygen transport was not a limiting process. However, soil cultures cannot be mixed. If oxygen transport is limiting, degradation rates will reflect this limitation.

This problem can be greatly reduced by maximizing the surface area to volume ratio of the soil in the respirometer. The mass of soil used in this experiment, 70g, ensures that a disk configuration of soil exists in the reactor with a high surface area to volume ratio. Further, when glucose was added to the soil a higher rate of respiration was found than in BTEX contaminated soils, proving that mass transport of oxygen in the slower BTEX degradation was not a limitation.

Oxygen consumption was converted to substrate degradation (to determine k_{bliq} and k_{bsoil}) through an oxygen to substrate yield, $Y_{o/c}$ =.32, for BTEX (Sturman et. al., 1992). The method for determining k_{bliq} was then to solve equation 3 for C. The solution is:

$$C = C_o \exp(-k_{bliq} t) \qquad (17)$$

Equation 4 is solved for q to determine soil degradation coefficients k_{bsoil}. The solution is:

$$q = q_o \exp(-k_{bsoil} t) \tag{18}$$

Equations 17 and 18 were used as a model to fit the measured respirometry data, see figure 7. A sum of least squares is used to find the best fit. All samples were run in triplicate and a standard error analysis was performed.

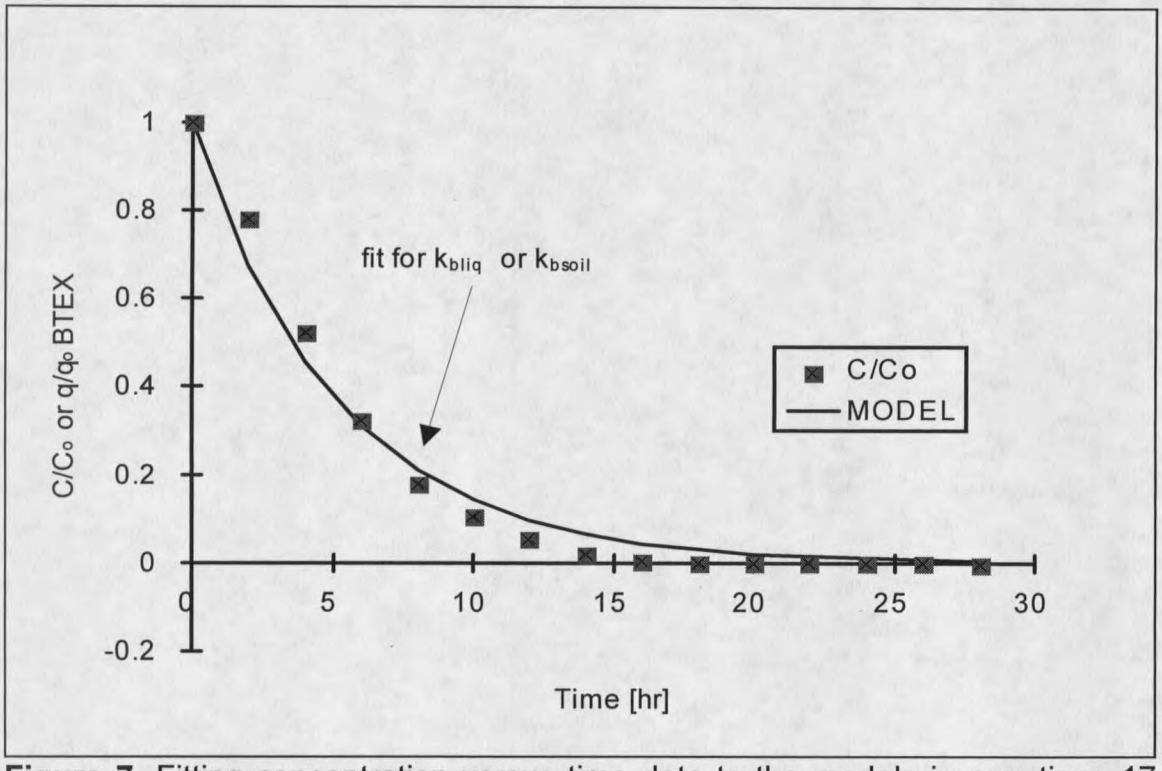

Figure 7. Fitting concentration versus time data to the models in equations 17 and 18 in order to determine k_{bliq} and k_{bsoil}.

For modeling purposes it is easier and more accurate to determine k_{bsoil} from q/q_o versus time data than C/C_o versus time data. Note that the k_{bsoil} used in equation 4 and equation 7 are equal. Since the degradation in the soil water

cannot occur until removal(desorption) from the soil occurs, the rates of removal and thus the dimentionless concentrations C/C_o and q/q_o versus time can be modeled as equal(since dq/dC is constant). Hence, the curve fitting coefficient for C/C_o and q/q_o versus time, k_{bsoil}, must be the same for both cases.

k_m and K_d. A mathematical model for describing first order sorption phenomena was proposed by Zhang and Bouwer (1995). Adsorption is described by:

$$\frac{dq}{dt} = -k_m(C_{pore} - C) \tag{7}$$

where:

C_{pore} = q/Kd = intraparticle concentration [mg/L]

A mass balance for sorption in a soil/water batch system, with organic contaminant is :

$$-m\frac{dq}{dt} = V\frac{dC}{dt} \tag{12}$$

Solving equation 6 for C and substituting into equation 12 yields an expression for d^2q/dt^2 in terms of constants and q(equation 19).

$$\frac{K_d}{k_m}\frac{d^2q}{dt^2} + (1 + K_d\frac{m}{V})\frac{dq}{dt} = 0 \tag{19}$$

Solving the differential equation for the boundary conditions :

1) at t=0; q_i=0 (where q_i = q @ time i)and,

2) at $t\rightarrow\infty$; $q=q_{equilibrium}=q_e$

yields an expression for the sorbed concentration of contaminant on soil as a function of K_d, k_m, q_e, and time:

$$q_i = \frac{1 - \exp(\frac{-k_m t}{K_d})}{q_e} \tag{20}$$

where:

q_e = equilibrium concentration of sorbed contaminant

The procedure for determining k_m was to plot q_i/q_e versus time from experimental data. Next the model for q/q_e as a function of time (equation 20) was plotted on the same axis. k_m determined by fitting the model to measured data and using a sum of least squares analysis to find the best fit (figure 8).

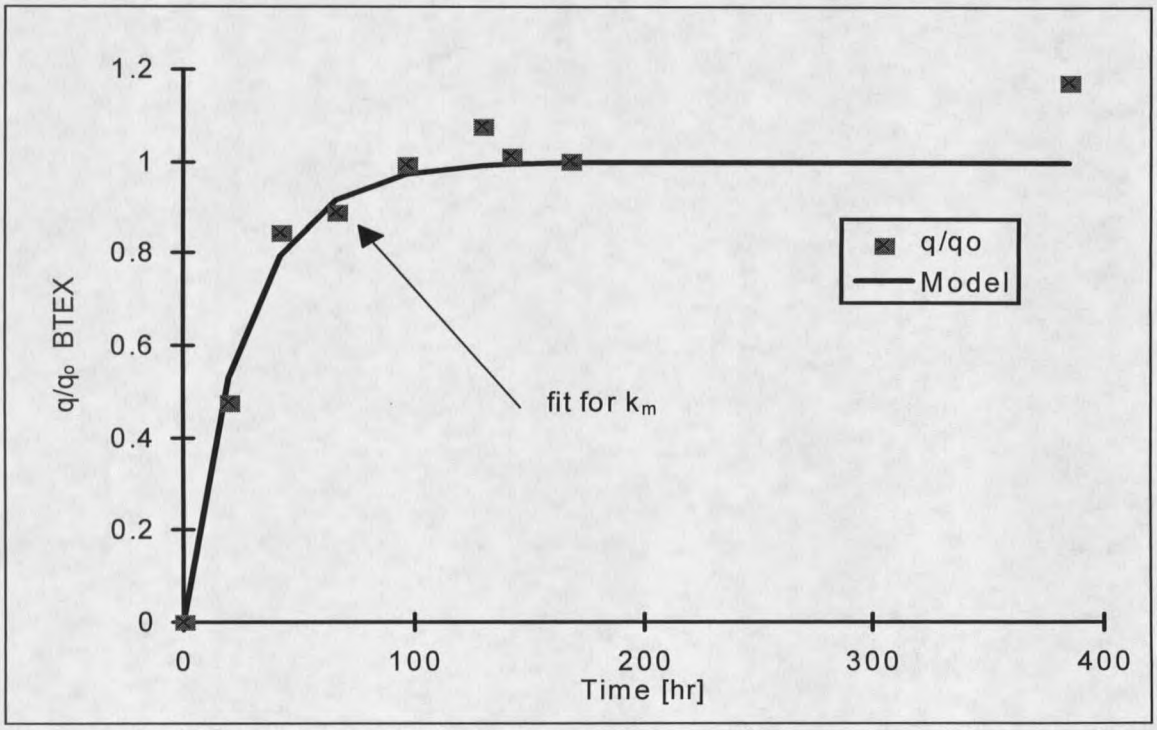

Figure 8. Fitting the saturated desorption coefficient, k_m shows a close agreement between the first order model prediction and measured soil sorbed concentration versus time.

Batch reactors were used to determine the experimental values q_i, q_e, and K_d. Teflon®centrifuge tubes were used in all sorption studies. BTEX compounds will not sorb to Teflon®.

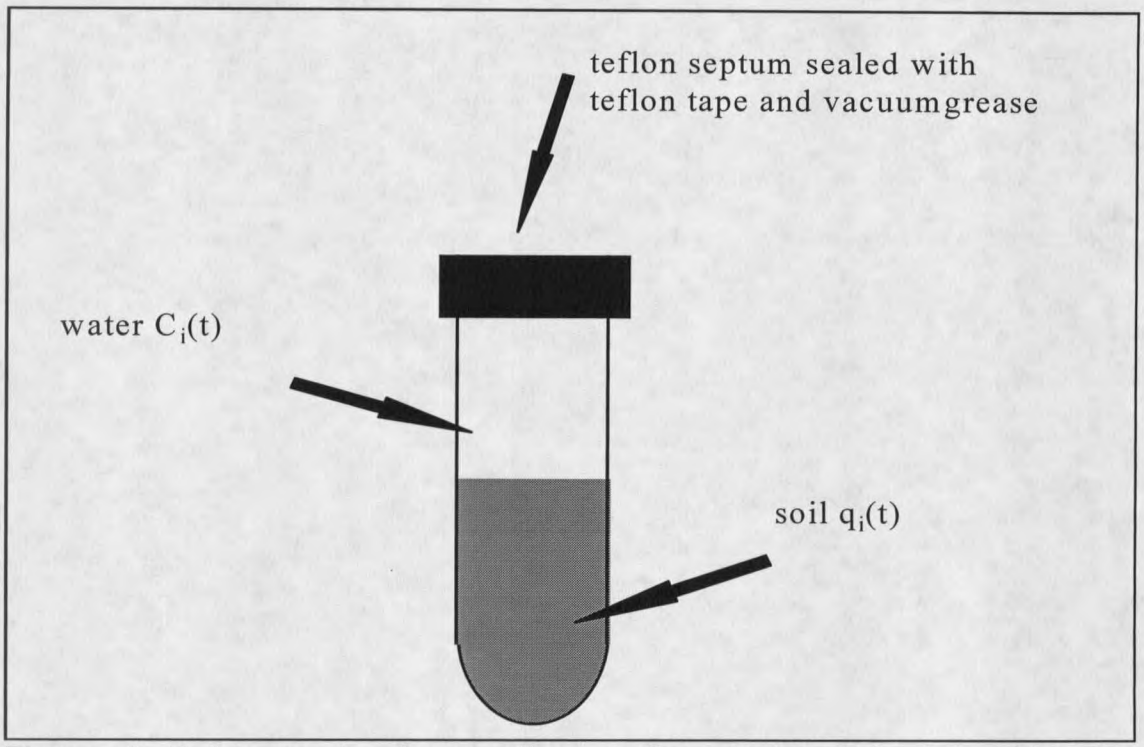

Figure 9 Batch reactor for sorption studies designed to be resistant to volatilization.

40ml tubes were filled with an approximate 50/50 soil-water mixture by weight. To separate the effects of biodegradation, the soil liquid slurry was pasteurized by first setting the mixture in a water bath at 72°C for 1 hour. Next, the reactor was allowed to cool for 24 hours, then re-immersed for 1 hour to kill off any newly formed vegetative cell from spores created during the first heating.

Pasteurization greatly reduces the population of BTEX degrading organisms in the slurry.

Sterile water was then added to the tubes to fill them completely and a Supelco® 20mm Teflon® septum and screw cap were added to seal the tube. There was no head-space in the reactor. This lack of head space also ensured that less than two percent of the BTEX added could be degraded aerobically. Further, initial microbial plate counts were performed to check the sterility of the reactors.

Once the reactor was filled and sealed BTEX was added through the septum such that the liquid concentration was 150ppm. The system is then placed in a 15°C water bath and not stirred to best simulate a vadose zone soil system.

The measurement of sorbed soil concentration depends on a closed mass balance. No BTEX can be lost through volatilization or the sorbed concentrations and ultimately k_m and K_d will be incorrectly calculated. Sterile liquid controls with no soil were amended with BTEX and placed with the sorption reactors. Initial and final concentrations were measured to ensure that there was no volatilization loss or sorption to Teflon® of BTEX.

Sampling of BTEX was done on the Hewlett Packard 5890 series II gas chromatograph. To sample, the centrifuge tubes were first placed in a size SS34 rotor and spun at 6,000 rpm for 10 minutes to separate the soil and water. The

centrifuge used was a DuPont Sorvall® RC5C. Samples were taken at time 4, 8, 12, finally 24 hour increments until the liquid phase had reached an equilibrium for at least 50 hours. Liquid phase concentrations were converted to sorbed phase concentrations through a mass balance:

$$mass_{sorbed} = mass_{total} - mass_{liquid} \quad (21)$$

A similar procedure was followed for K_d (figure 10). In this method, concentrations of 50, 75, 100, and 150 ppm BTEX were set and measurements were taken once the soil/water system had reached equilibrium. This point was determined by the k_m kinetic studies and was approximately 60 hours. A linear regression was performed on sorbed concentration versus liquid concentration to

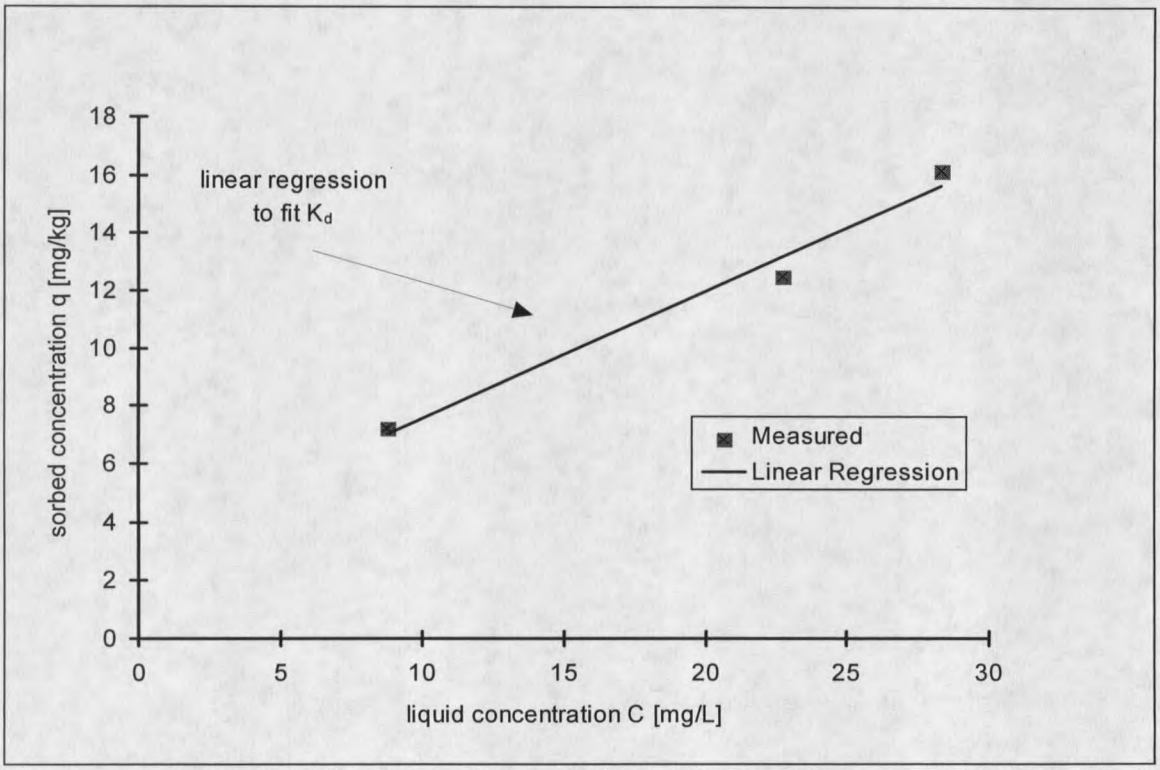

Figure 10. Fitting distribution coefficient K_d shows close agreement with a linear isotherm model.

determine a final K_d. The isotherm followed a linear model throughout the range considered. All K_d and k_m samples were run in triplicate and standard errors computed.

Gas Chromatography

All BTEX sampling was performed on a Hewlett Packard 5890 Series II gas chromatograph utilizing a .1% Alltech AT-1000 (Graph Pack) column. The temperature was held constant at 70°C for 3 minutes, then ramped at 10°C per minute to 186°C. The ramp was then slowed to 5C per minute until 210°C was reached. The final ramp was to ensure better separation between ethylbenzene and paraxylene. Concentration was determined through a standard curve for BTEX (appendix D). A four point calibration curve 150ppm, 100 ppm, 50 ppm, and 25 ppm BTEX was constructed and updated monthly. The standards were made with a methanol solvent in accordance with section IV of the USEPA Statement of Work for Organic Analysis, 1987. Each component of BTEX was measured separately rather than as a group.

CHAPTER 4: RESULTS

The first section of this chapter presents soil parameter results relevant to the biodegradation reaction. The second section presents results for the coefficients required to compute the bioavailability factor. In addition, results validating the bioavailability factor model are presented.

Soil Parameter Results

Soil parameters were determined in order to asses the soil's conduciveness to biodegradation and to determine if augmentation of nutrients was necessary. NO_3^-, NH_4^+, and PO_4^{-3} concentrations were found to be .9, 5.8, and 1 ppm, respectively. The soil pH was 8.5.

Organic matter content and soil texture are indicative of the extent and rate of desorption. Percent soil organic matter was .58%. The soil texture was determined to be a sandy clay loam, 49% sand, 24% silt and 27% clay. Field capacity was determined to be 26% soil moisture by dry weight of soil.

BTEX degrading microorganisms were found. Microbial enumerations revealed populations on Garrison Soil to be to be $6.7 \pm 0.8 \times 10^5$ CFU/g_{soil}.

Bioavailability Factor Coefficient Results

This section contains results for the required coefficients in the bioavailability factor equation:

$$B_f = \frac{1}{1 + \frac{m}{V} K_d + \frac{k_{bliq}}{k_m} K_d} \tag{14}$$

In addition, results of the experiments used to test and validate the bioavailability factor model are given, the sensitivity of the bioavailability factor to desorption and degradation coefficients is determined, and the bioavailability factor is applied to Garrison Soils to predict clean-up times.

Soil/Water Distribution Coefficient: K_d

The soil/water distribution coefficient describes the ratio of the concentration of BTEX sorbed to soil to the dissolved BTEX concentration in the soil moisture. K_d is a required coefficient in the bioavailability equation and in the model for determining the first order desorption coefficient k_m (equation 7). Results for individual BTEX compounds appear in figure 11. When considering BTEX as one compound K_d = .713±.068 L/kg.

The error bars represent the standard error for three replicates. Raw data for each replicate is presented in appendix C.

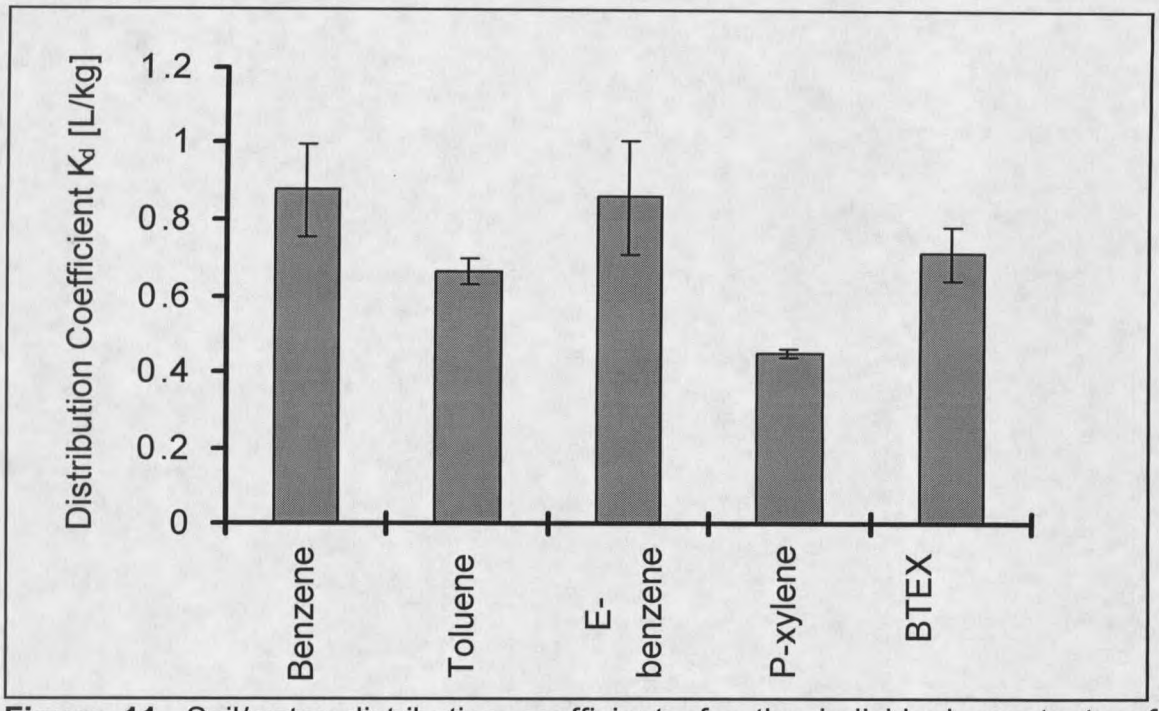

Figure 11. Soil/water distribution coefficients for the individual constants of BTEX and BTEX as on compound on Garrison Soil. Error bars represent standard error in three replicates.

First Order Saturated Desorption Coefficient k_m

The coefficient k_m describes the saturated desorption rate of BTEX compounds from soil to the bulk soil water. BTEX as one compound was determined to have a k_m of .039±.01[L/kg·hr] (figure 12). k_m for saturated soil is used in equation 14.

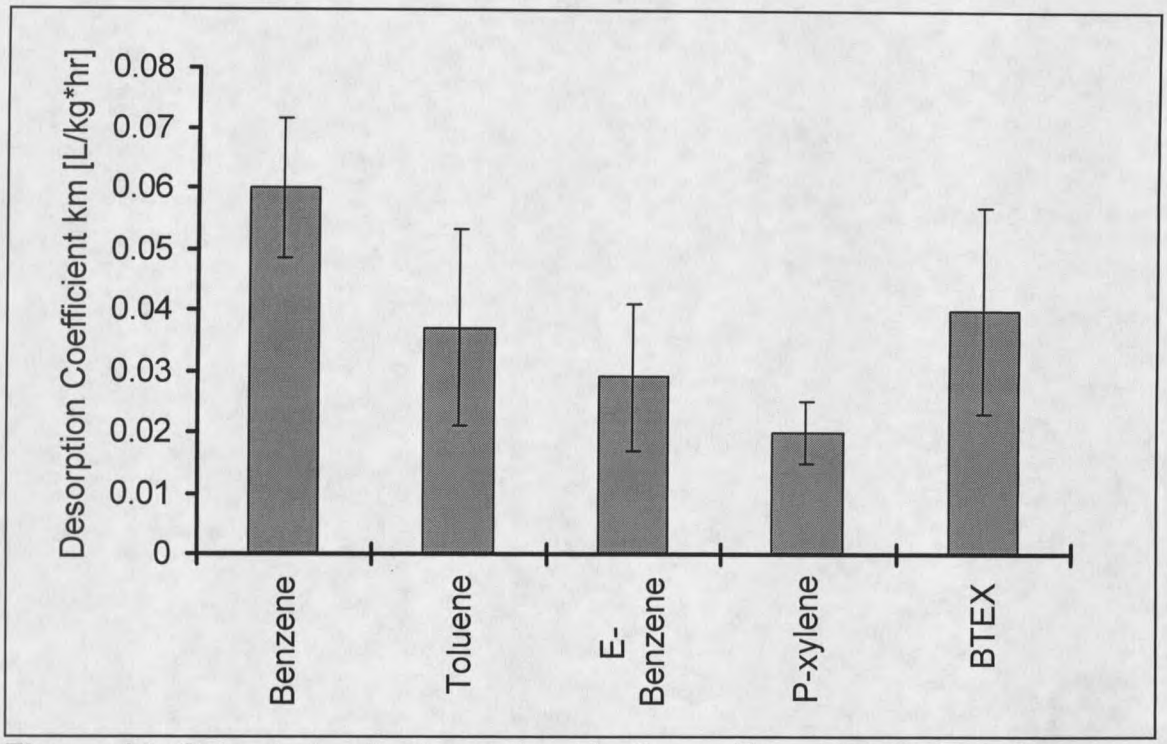

Figure 12. Saturated soils desorption kinetic coefficient k_m for constituents of BTEX as well as BTEX as one compound on Garrison Soils. Error bars represent standard error in three replicates.

Controls showed no loss of BTEX due to volatilization or sorption in the batch reactors used sorption tests. Figure 13 shows before and after BTEX concentrations in the volatilization control reactors (no soil) for a sorption test of 426 hours. The higher final levels are due to measurement error.

Microbial enumerations were performed at the beginning of the sorption test ensuring that the pasteurization process was sterilizing the soil. Biomass counts decreased four orders of magnitude.

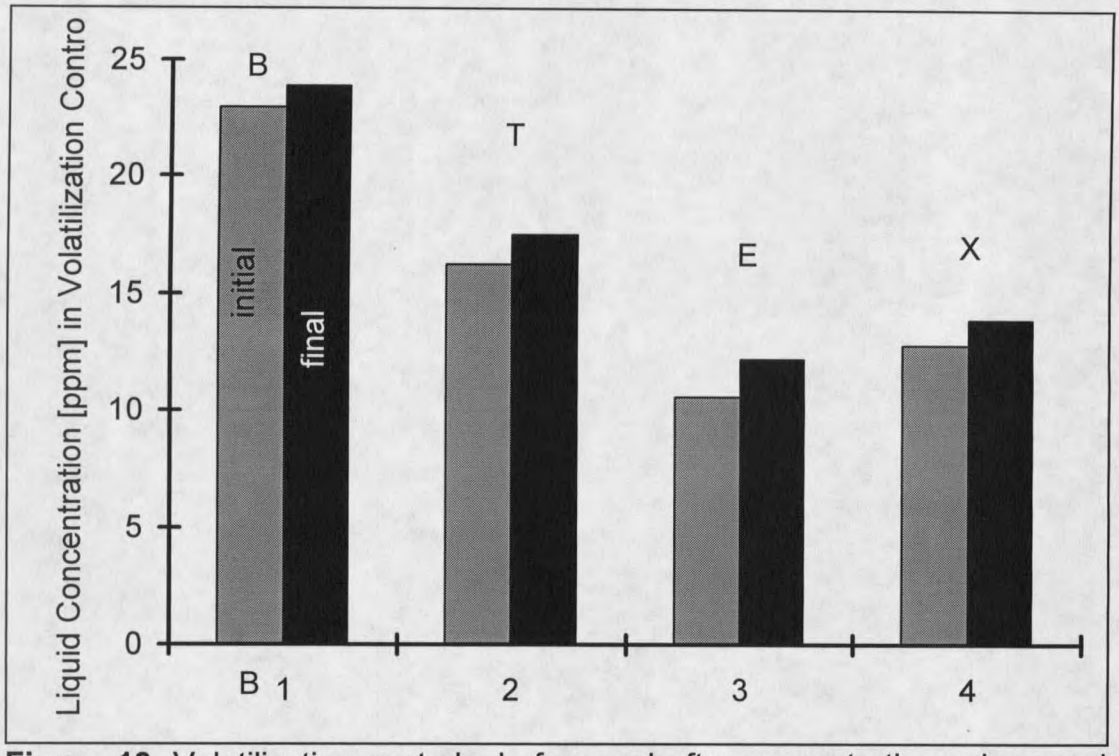

Figure 13. Volatilization controls, before and after concentrations show no loss of BTEX due to volatilization or sorption to Teflon® in soil batch sorption reactors.

Liquid Kinetic Rates k_{bliq}

The intrinsic microbial kinetic coefficient for liquid cultures, k_{bliq}, represents the maximum aerobic degradation rate of the microbial consortia with BTEX as the sole carbon source. This coefficient was determined via electrolytic respirometry, as described in chapter III to be, .188 1/hr ± .007. Figure 14 shows BTEX utilization versus time for the first replicate. k_{bliq} is fit to measured data using equation 3. The k_{bliq} data on the graph is the average for all three replicates. Remaining replicates can be found in appendix B.

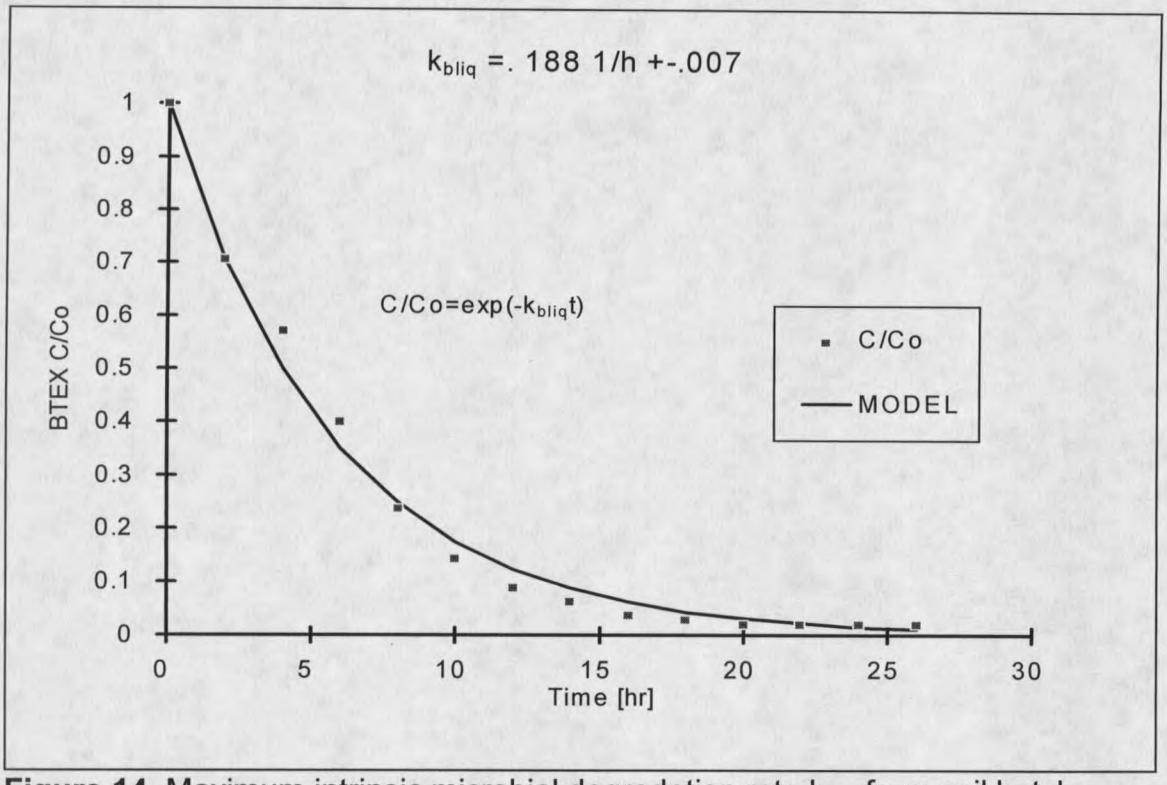

Figure 14. Maximum intrinsic microbial degradation rate k_{bliq} from soil batch studies and the fit of measured data and the first order model.

Soil Kinetic Rates k_{bsoil}

BTEX degradation kinetic coefficients for soil cultures were measured for a variety of unsaturated soil moistures. A range of moistures was chosen in order to incorporate the entire unsaturated regime, 4% to 25%. Figure 14 shows the kinetic coefficients that were determined via electrolytic respirometry. Soil slurry data is included in figure 15 to show a comparison with the saturated region. Saturated soil moistures of 50 and 100% were run. Kinetic values increase with increasing soil moistures. The k_{bsoil} data on figure 15 is the average

of three replicates. Error bars represent the standard error. All replicates can be found in appendix B.

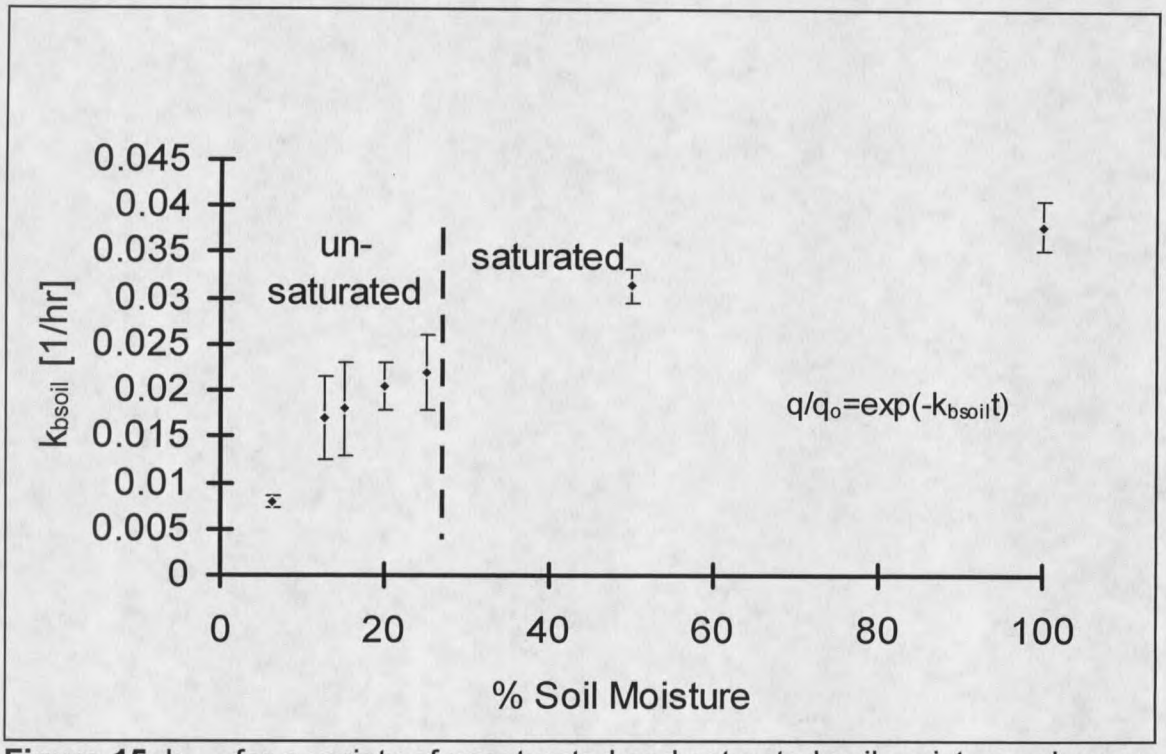

Figure 15. k_{bsoil} for a variety of unsaturated and saturated soil moistures show an increase with increasing soil moisture.

To ensure that a desorption limited scenario occurs, the time to sorption, or equilibrium was determined (figure 16). Equilibrium occurs at approximately 48 hours. No measurements for biodegradation data were taken until 24 hours after equilibrium occurred, time for sorption and for the BTEX originally dissolved in the soil moisture to be degraded.

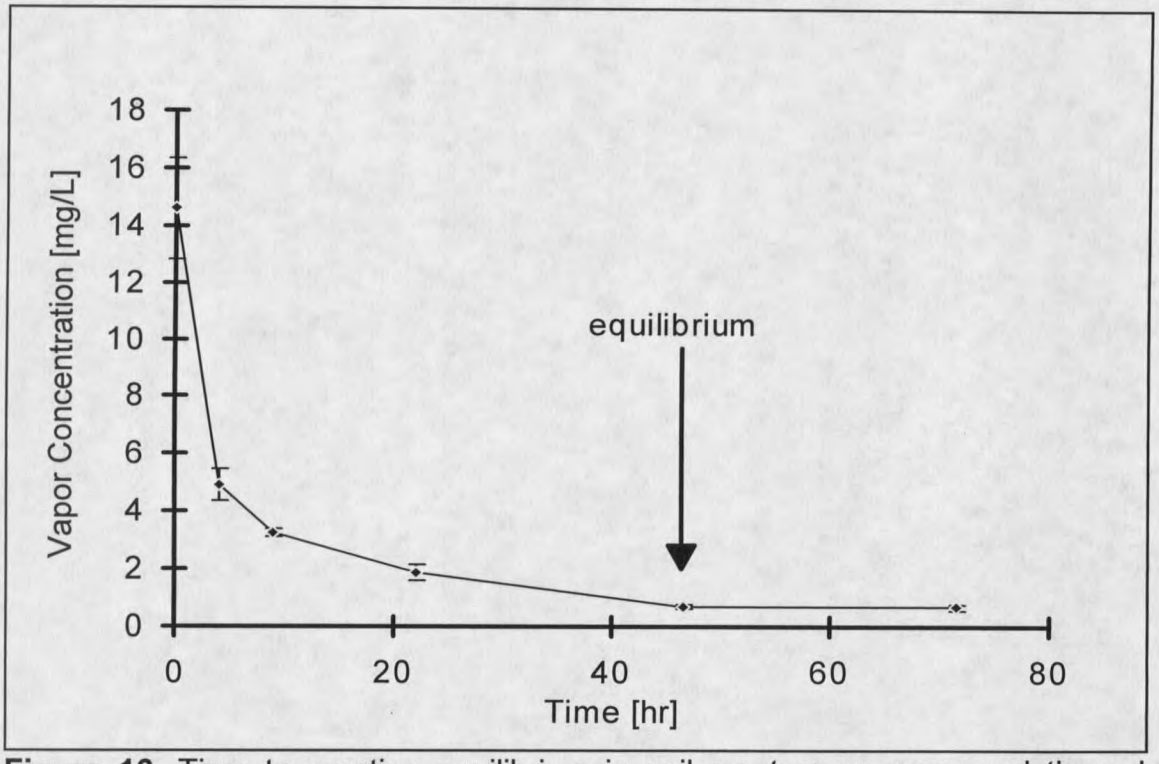

Figure 16. Time to sorption equilibrium in soil reactors as measured through vapor phase concentrations. Desorption limited degradation states at t=48 hours.

Biomass was monitored as a function of time to determine if a substantial increase occurred. Figure 17 shows more than a log increase in each reactor. BTEX vapor phase concentration was measured initially and finally as an indicator of microbial degradation (figure 18). BTEX concentrations were reduced to below measurable levels in three of the reactors and more than an order of magnitude in the remaining reactors.

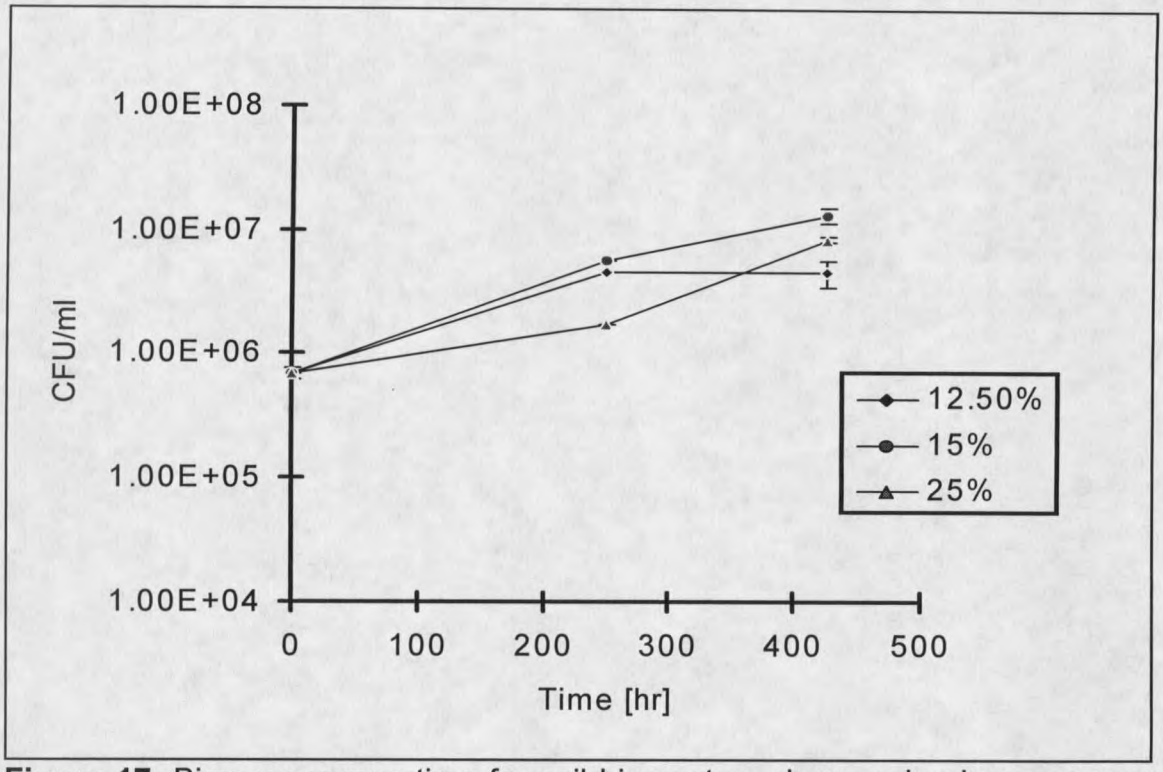

Figure 17. Biomass versus time for soil bioreactors shows a log increase over the life of the experiment.

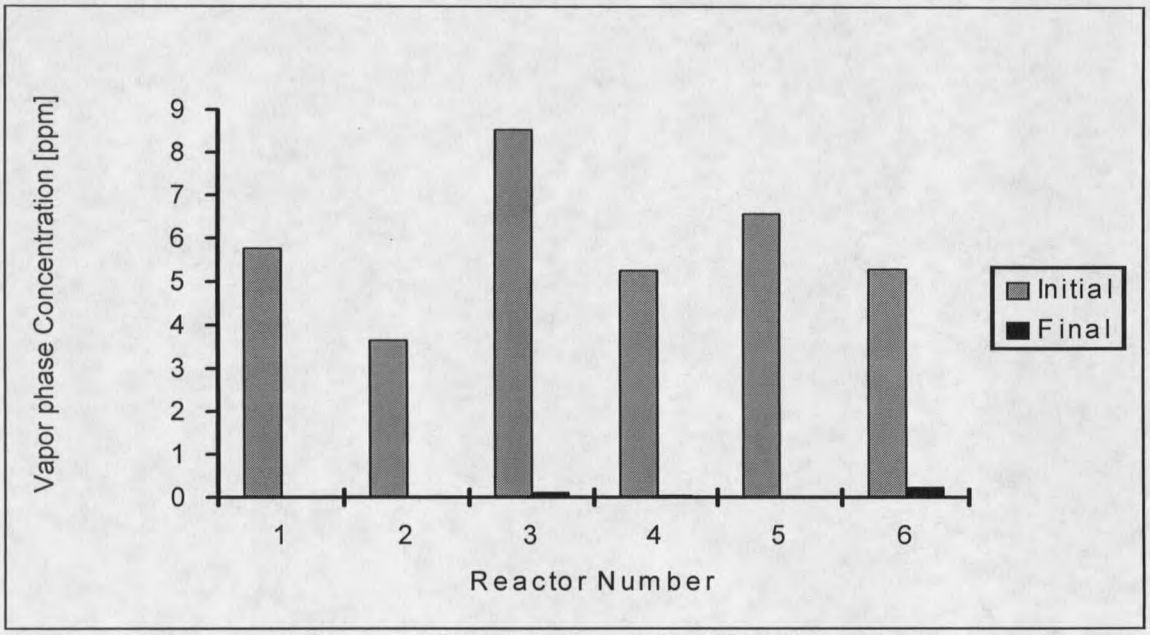

Figure 18. The depletion of BTEX; initial and final BTEX head space readings in soil reactors shows at least a 90% removal of BTEX.

Bioavailability

Once degradation and desorption coefficients have been determined, the bioavailability factor may be computed from the B_f model. Bioavailability is a strong function of soil moisture. Figure 19 shows that the bioavailability factor, determined from the coefficients calculated from Garrison Soils, increases throughout the unsaturated region and then shows asymptotic behavior as 100%, (half soil, half water) is approached.

The bioavailability factor was also calculated by a ratio of degradation coefficients, k_{bsoil}/k_{bliq}(figure 20). Of worthy note is the similar increase with increasing soil moisture and the similar asymptotic behavior at 100% soil moisture. To show this satistically, a null set that assumed the average values for soil biodegradation rates are equal was discredited through an analysis of variance (Hamilton 1995). Standard errors were computed for the variance of the ratio k_{bsoil}/k_{bliq} (method from Rice, 1988).

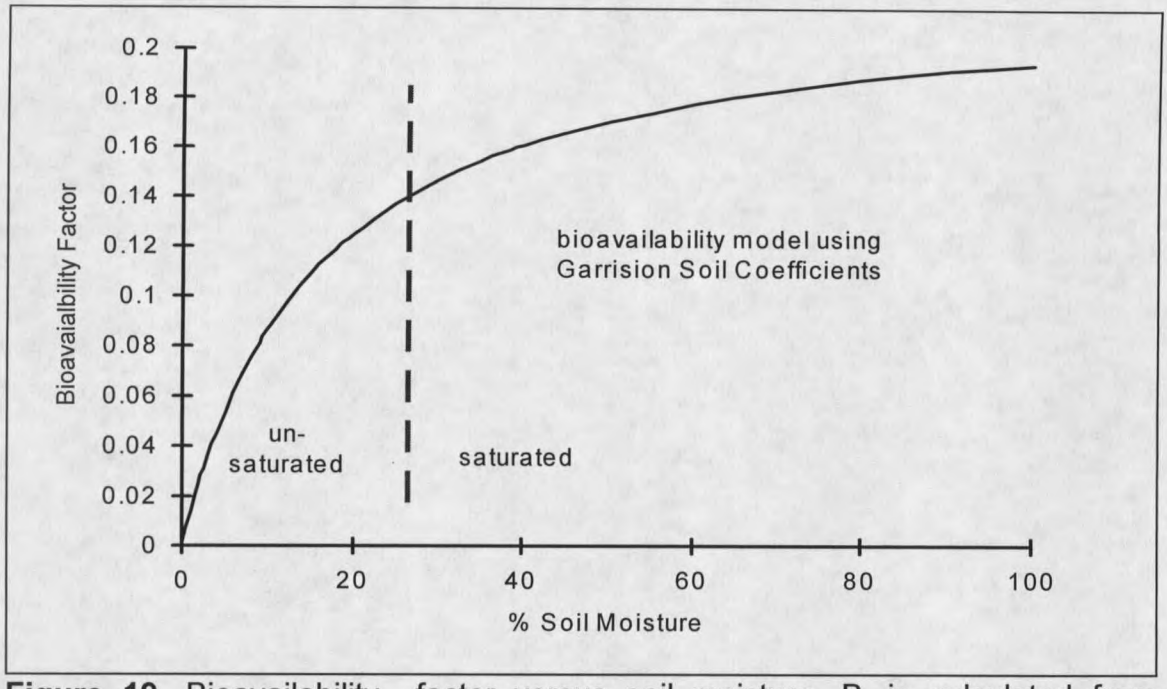

Figure 19. Bioavailability factor versus soil moisture. B_f is calculated from equation 14, the model proposed by Zhang and Bouwer (1995).

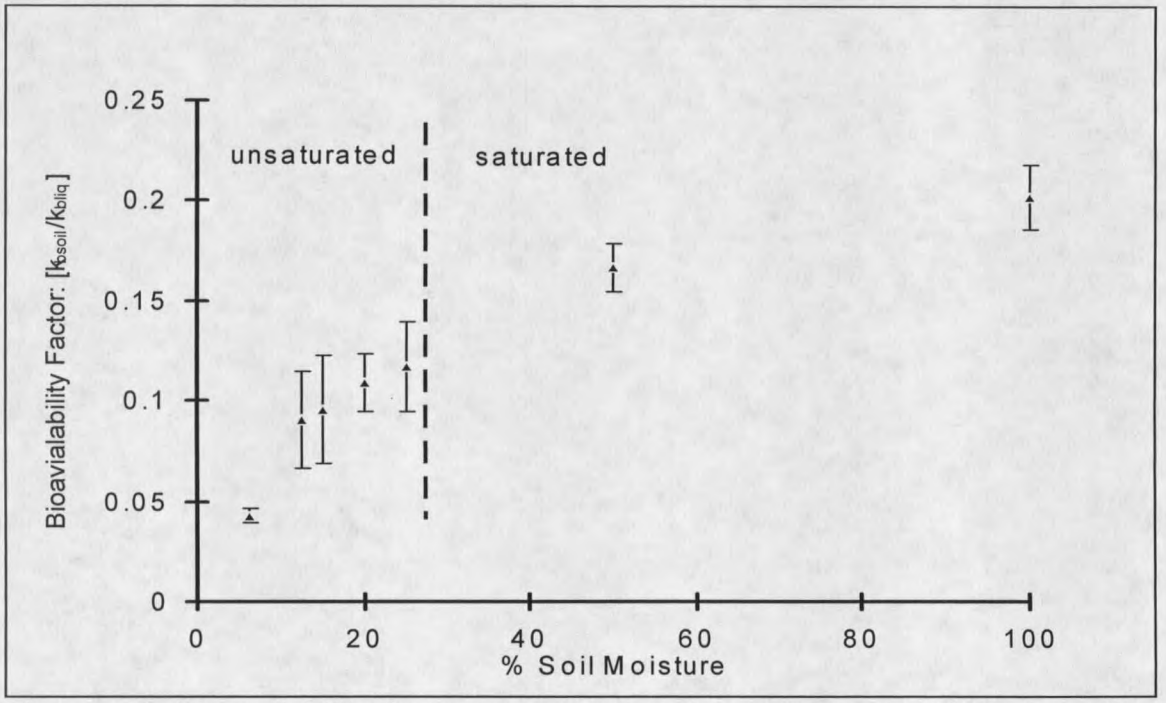

Figure 20. Bioavailability factor versus soil moisture: computed from (equation 9.) a ratio of degradation rates. Error bars represent standard error in three replicates.

Figure 21 shows a comparison between B_f calculated by a ratio of degradation rates and B_f calculated from the model.

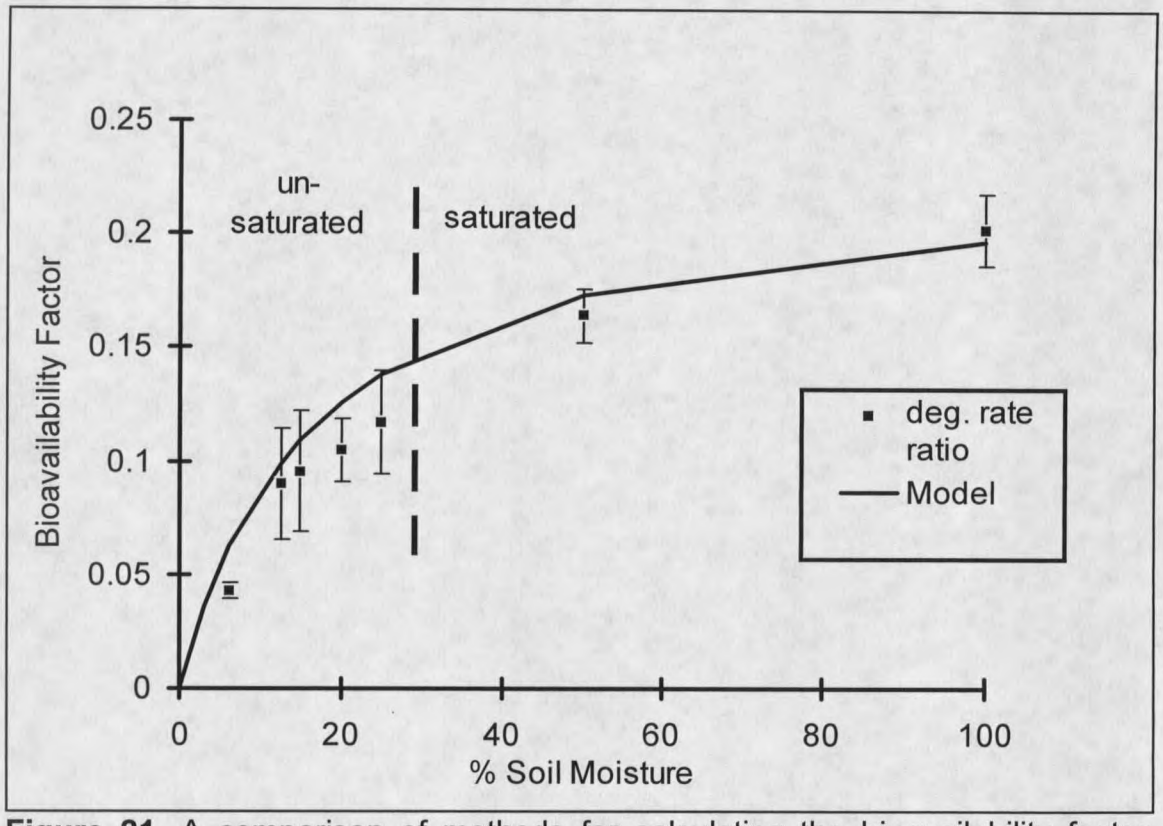

Figure 21. A comparison of methods for calculating the bioavailability factor. The line represents equation 14, the dots represent equation 9. Agreement of the two models validates the use of equation 14 for unsaturated soils.

By solving equation 14 explicitly for k_m a desorption coefficient can be back calculated from bioavailability equation data. Desorption coefficients calculated by saturated soil batch reactors are compared with saturated desorption coefficients calculated from solving equation 14 explicitly for k_m. A close agreement between the two k_ms indicates a close agreement for the two B_f models.

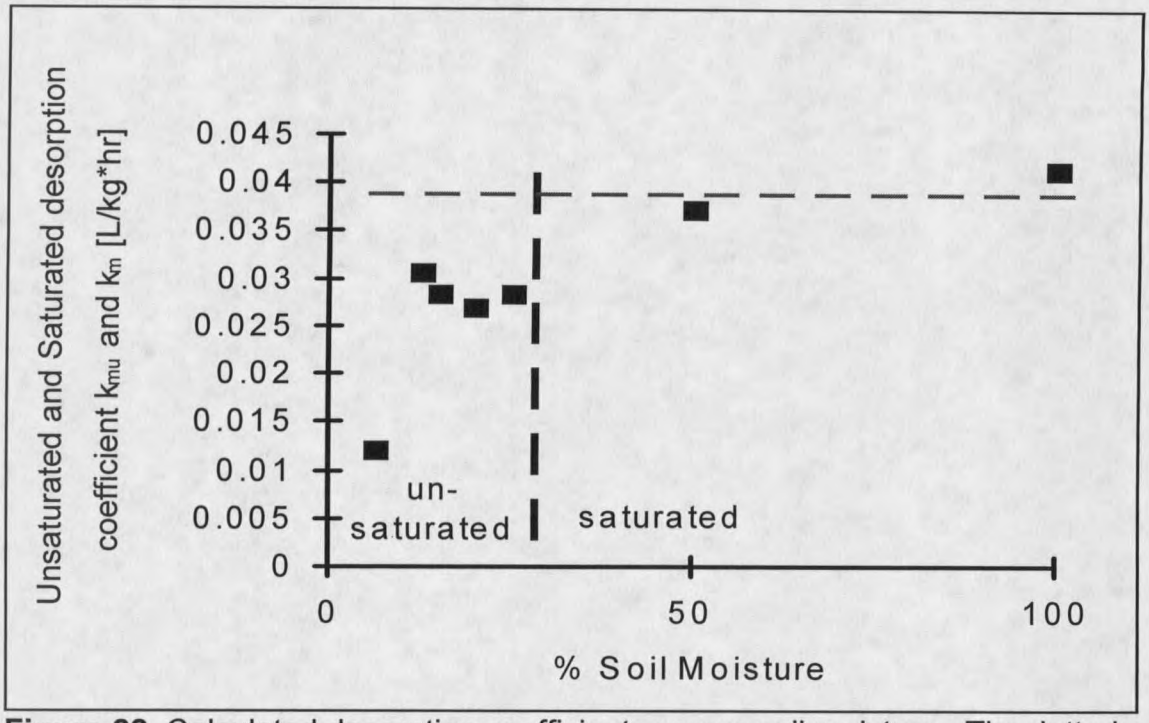

Figure 22. Calculated desorption coefficients versus soil moisture. The dotted line denotes the desorption coefficient measured in the sorption batch reactors.

The value for the saturated soil desorption coefficient from soil batch desorption studies is .039±.01 L/kg·hr. Values (seen in figure 21) range from .037 to .041 L/kg·hr for saturated soils. A decrease (k_m for unsaturated is approximately 75% of k_m for saturated), was seen between unsaturated (range of 12.5% to 25%) and saturated desorption coefficients. k_m for 6.2% soil moisture is approximately 35% of the rest of saturated k_m values, denoting a poorer correlation for the unsaturated regime.

The model B_f can be used to determine the effects that different sorption and degradation coefficients have on the bioavailability factor. Figure 23 models the effects variable soil moistures and distribution coefficients have

on the bioavailability factor. For the Garrison Soils sampled, the effects of K_d on B_f are profound provided K_d is below 5 L/kg. Figure 24 denotes the effects of the ratio of desorption coefficients to degradation coefficients and soil moisture have on the bioavailability factor for the soils that were tested. The effect is more profound for higher soil moistures. Lines of soil moisture represent 1,5,10,15,20,30,50 and 100% for figures 23,24 and 25

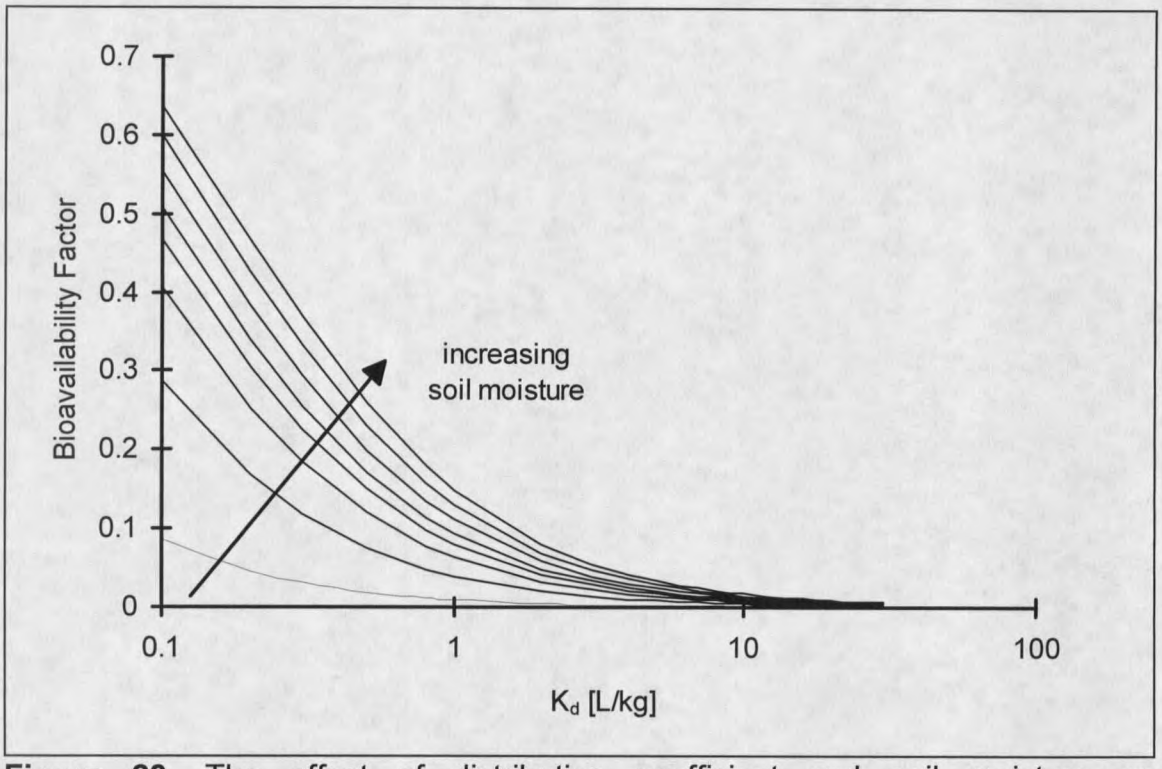

Figure 23. The effect of distribution coefficient and soil moisture on bioavailability. The lowest soil moisture is 1%, the highest is 10)%. Beyond a K_d of 100, soil moisture is of little consequence to the bioavailability factor.

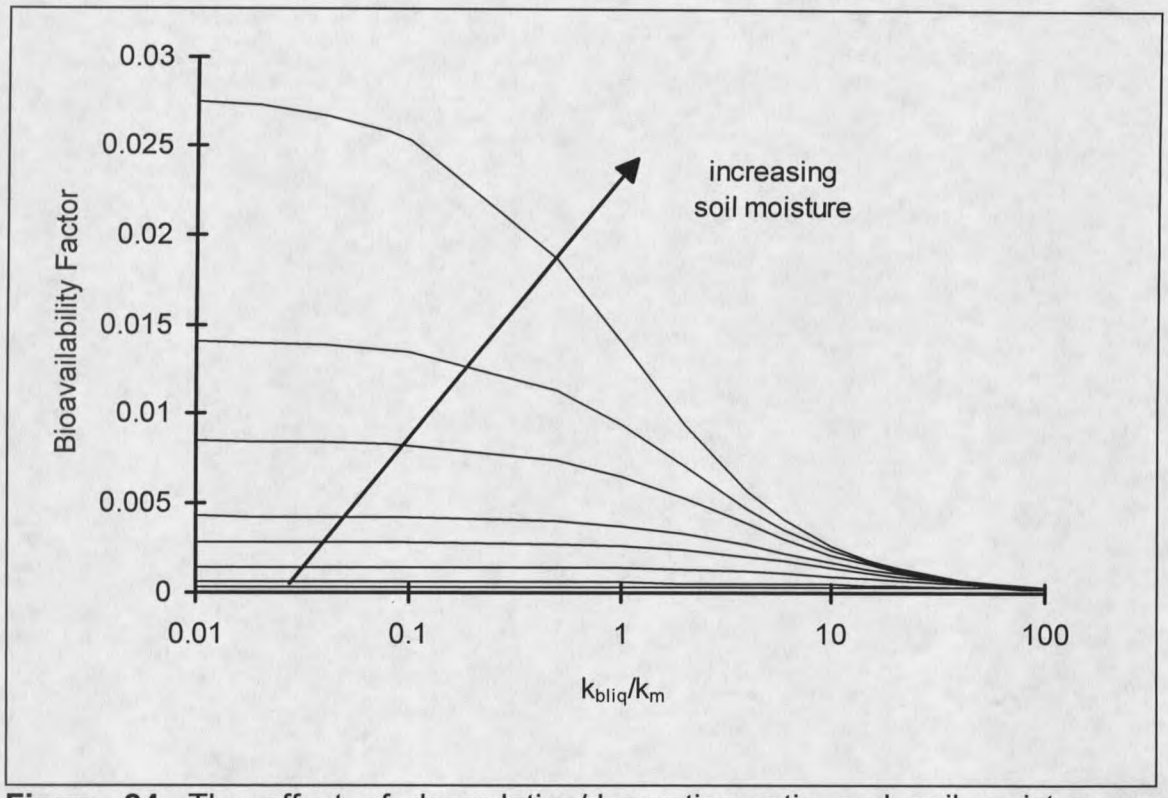

Figure 24. The effect of degradation/desorption ratio and soil moisture on bioavailability. The lowest soil moisture is 1%, the highest is 100%. Note that when degradation is 10 times faster than desorption, the addition of water has no effect of the bioavailability factor.

A validated model for the bioavailability factor can be used to predict clean up times for differing soil parameters. Figure 25 denotes the effect of soil moisture (due to the effect of soil moisture on bioavailability) on predicted cleanup times. Figure 26 denotes the effect of K_d and soil moisture on the time required to biologically degrade 95% of the contaminant.

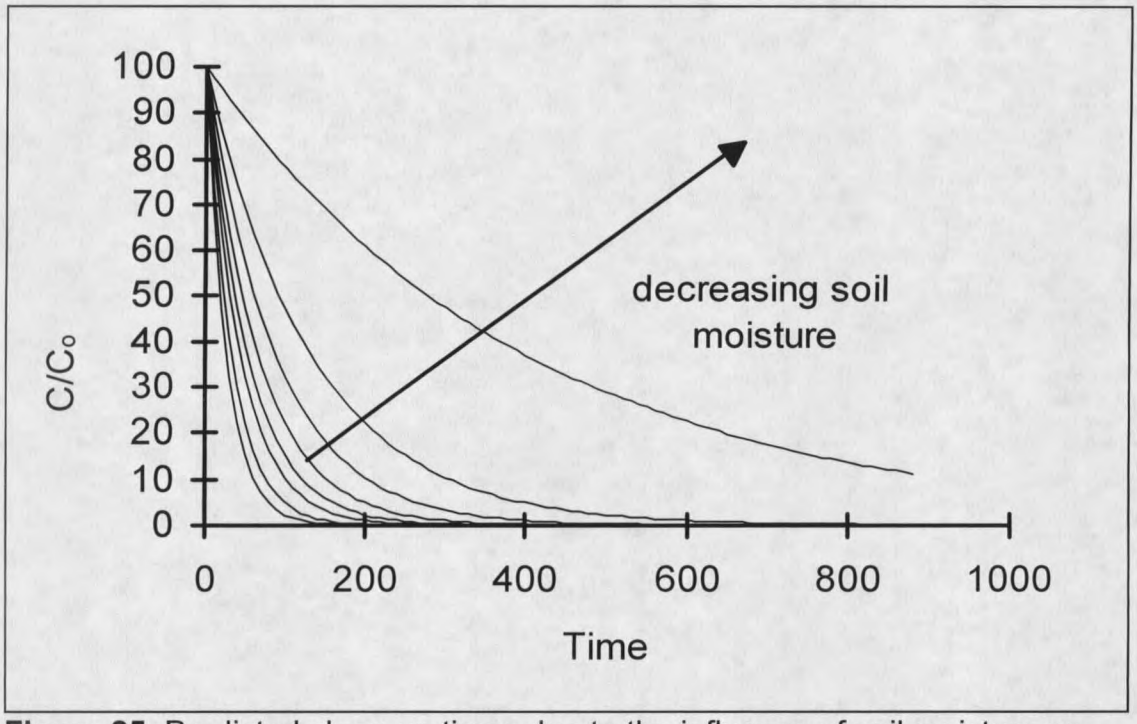

Figure 25. Predicted clean-up times due to the influence of soil moisture on bioavailability. The lowest soil moisture is 1% the highest is 100%.

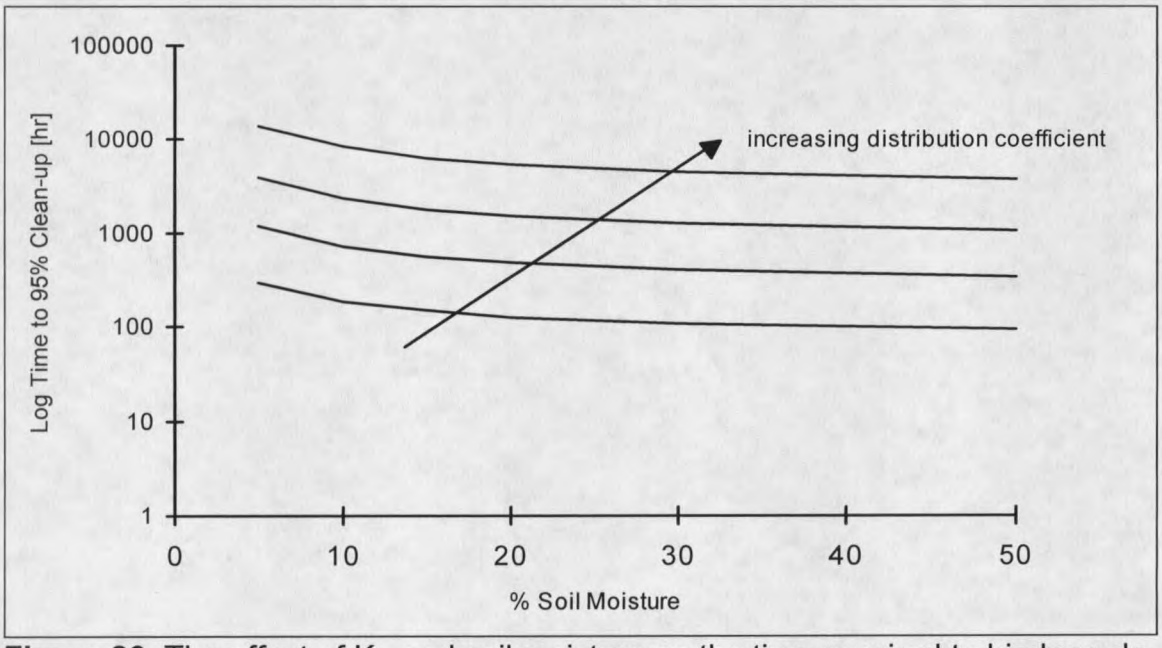

Figure 26. The effect of K_d and soil moisture on the time required to biodegrade 95% of the contaminant. For K_d's of .713, 3, 10, and 35 [L/kg].

CHAPTER 5: DISCUSSION

The discussion of results is contained in this chapter. The discussion and conclusion apply only to the soils and contaminants used in this study.

Bioavailability Factor

The bioavailability factor that was determined from equation nine accounts for limitations not only due to desorption and degradation rates, but every process that occurs in soils. These processes may include solubility limitations, volatility, or any other process that is not easily measured. The B_f for soil therefore acts as a true or measured B_f.

The bioavailability factor that was determined through equation 14 accounts for the processes and characteristics or soil/water ratio, sorption rates, and biodegradation rates. This relationship for B_f is based on the assumption that the B_f can be closely estimated by accounting only for the processes mentioned previously.

While this estimate is most certainly true under saturated conditions, it may not be true under unsaturated conditions. Microorganisms may be hindered by salt stress or the soil may be variably wet with discontinuous water films on the soil peds under unsaturated conditions.

Figure 21 is a comparison between the two values for bioavailability factor as a function of soil moisture. There is a good correlation between the two

models. Five of seven data point fit the model within the standard error while the remaining two points are at least 73% of the value predicted by the B_f model. These points are in the unsaturated regime, where the correlation is good, but not as strong as the saturated regime. Equation 14 is therefore a valid relationship for the bioavailability factor in unsaturated soils and B_f can be written as a function of sorption and degradation and soil moisture parameters.

Although small and negligible in this case, there does appear to be a slight departure of the two models at low unsaturated soil moistures. The author hypothesis that there may be decreased desorption rates in the unsaturated zone. The unsaturated desorption coefficient that was calculated from the B_f equation does not correspond as closely to the k_m found in batch reactors. There are two different levels of disagreement. Soil moistures 12.5% to 25% have a k_{mu} that is approximately 75% of k_m(from abiotic batch studies). The lowest soil moisture, 6.2% shows a k_{mu} that is approximately 35% of k_m(from abiotic batch studies).

There may be several factors contributing to the discrepancies in desorption coefficients. The levels of disagreement coincide with distinctly different levels of unsaturation. The first is a change from saturated, all soil pore space filled with water, to field capacity(the maximum amount of water soil can hold against gravity). This change occurs at approximately 26% soil moisture. The second level of change occurs between 6.2% and 12.5%. At 12.5% all of the soil is visibly wet. The 6.2% soil is variably wet.

The effects of these two unsaturated soil moisture conditions is visible in the desorption coefficient since a hypothesis made in the introduction maintains that k_m is the only coefficient in the B_f equation that may be effected by soil moisture. This is most certainly incorrect. There may be a number of factors contributing to the above disparity. For the first case, a change from unsaturated to saturated, the thermodynamic activity of ions in soil solution may effect the desorption and/or the degradation coefficients. The second case's discrepancies are due to a variable wetting of the soil as well as thermodynamic activity. Microbial and mass transfer activity occur only in the wet portions of soil.

Practical Implication of The Bioavailability Factor in Unsaturated Soils

The advantage of the bioavailability equation is that given the degradation, sorption, and soil moisture parameters, the effect of desorption on biotransformation can be easily and accurately quantified. With a tool to quantify overall soil degradation rates versus soil moisture, optimization of degradation rates can be achieved through the manipulation of the soil moisture.

There are two important terms in equation 14. The first, $m \cdot K_d/V$ is sensitive to the soil moisture and the soil water distribution coefficient and insensitive to desorption and degradation rates. The second term is $k_{bliq} \cdot K_d/k_m$. This term is insensitive to soil moisture. When soil moisture is low, the first term is large and controls the bioavailability factor. At high soil moistures, the first

term is low and the bioavailability factor is controlled by the second term(sorption and degradation parameters).

Figure 23, 24, 25, and 26 model the dependency of the bioavailability factor on k_{bsoil}/k_m and K_d and m/V.

The only soil characteristic that can be changed in the field is the soil moisture. Bioavailability is a strong function of soil moisture in only specific scenarios. Figure 23 through 26 address each scenarios. For example, figure 24 shows that if K_d is approximately 35 [L/kg], the bioavailability factor is mostly insensitive to soil moisture. However, from figure 26, with a K_d of 35, the estimated time for biological remediation of 95% of the contaminant is approximately 416 days for a soil moisture of 10%, but 250 days for a soil moisture of 30%. There may be a variety of desorption, degradation and moisture conditions at any site and for any contaminant, figure 23-27 are tools that can be used to determine the effects of water addition on the bioavailability factor and ultimately cleanup times.

Figure 27 is a protocol for enhancing bioavailability and ultimately speeding clean-up rates by the manipulation of soil moisture.

The bioavailability factor may or may not be able to be increased through the addition of water. For the specific conditions, the use of figures 23 through 27 is the only way to accurately determine the need for more water.

This study does not account for losses in mass transfer rates due to the addition of water. The addition of water often corresponds to an oxygen

limitation since oxygen from air must be transported through a greater amount of soil water, rather than air. Diffusive coefficients in water for oxygen are at least two orders of magnitude lower than diffusion coefficient in air. The addition of water to enhance *in_situ* biodegradation should be considered only if oxygen is not, nor becomes, a limitation.

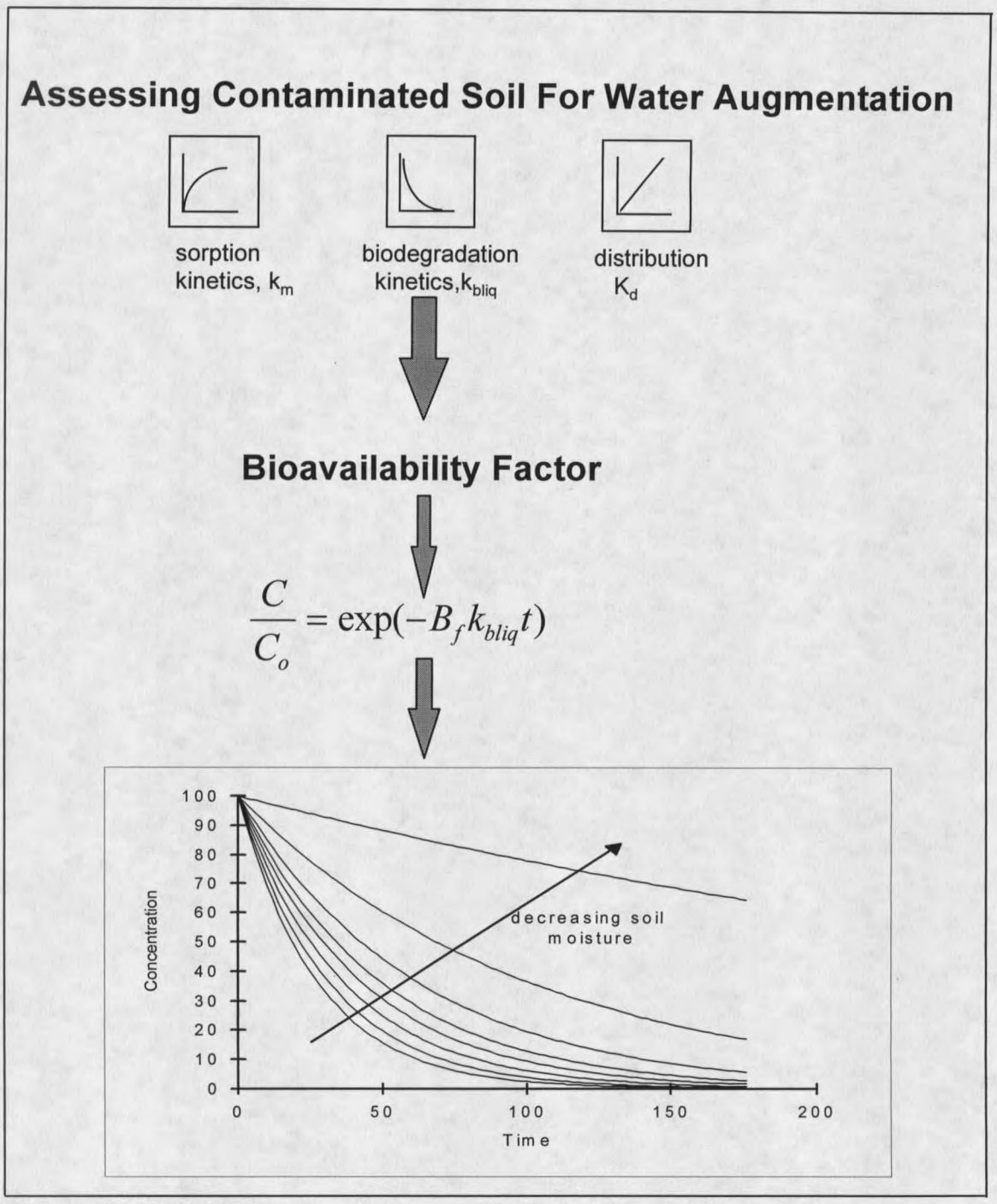

Figure 27. decision protocol for the addition of water

CHAPTER 6: CONCLUSIONS

Conclusions in this study apply only to the Garrison soils and range of soil moistures that were considered. The bioavailalility factor is soil specific.

1) The bioavailability factor, as determined by a ratio of kinetic rate coefficients, corresponds well with the bioavailability factor that is calculated from the analytical model. Saturated B_f 's correlate closer than unsaturated B_f's. An extension of the bioavailability factor from saturated to unsaturated soils is possible, however, a degree of accuracy is lost.

2) The bioavailability factor increases with increasing soil moisture in the unsaturated zone, then behaves asymptotically in the saturated zone.

3) The desorption coefficient appears to be a function of soil moisture in the unsaturated regime.

4) *In-situ* biodegradation rates in soils can be enhanced through the control of soil moisture in only specific cases, depending on K_d, k_m, and k_{bliq}.

5) The influence of desorption accounts for at least 80% of the bioavailability factor.

CHAPTER 7: FUTURE WORK

Future work on the bioavailability of organic contaminants in unsaturated soils should focus on three issues.

There are two issues immense importance in the biodegradation of contaminants in soil. The first is bioavailability. The second is oxygen transport. Incorporation of oxygen vadose zone transport and bioavailability theory in a large scale model is desirable.

The second issue is the prediction of soil degradation end-points. This is a natural extension of bioavailability factor theory. The endpoint can be predicted by a traditional minimum substrate concentration such that growth is equal to or less than decay. However, the minimum concentration in the soil water is a function of desorption rates and liquid degradation rates(bioavailability factor). The minimum substrate that is predicted would be the substrate in which the rate of degradation becomes markedly slow (since biomass is no longer being generated in the degradation of the compound).

Finally, the bioavailability equation assumption of a first order degradation coefficient must be thoroughly examined. The literature is not consistent with the effects of biomass on degradation rates in soils. Further, our studies indicate a log increase in biomass during soil degradation. An expression for the bioavailability that utilizes Monod Kinetics, or another biomass dependent kinetic

expression should be proposed and tested in the B_f model. Appendix A deals with this issue in greater detail.

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APPENDICES

APPENDIX A

Incorporation of Biomass in the B_f

A Preliminary Consideration of a Biomass Dependency in the Bioavailability Factor Equation

Equation 14 uses degradation coefficients that are independent of biomass. The topic of this appendix is to present a preliminary model for the bioavailability factor that is a function of biomass concentration. The assumption of a first order degradation coefficient is valid when growth rates are very close in magnitude to decay rates since there is low of no biomass increase. However, situation may occur (low organic matter or anaerobic degradation rates)when the growth rate is high compared to the decay rate. A model for B_f is needed to correctly model this scenario.

For the biodegradation reaction, the first order Monod Kinetic expression is:

$$\frac{dC}{dt} = -k_{Mliq} C \frac{X}{Y_{x/c}} \tag{22}$$

where:

X = concentration of biomass [mg/L]

k_{Mliq} = first order Monod approximation[L/mg·hr]:

$$\mu = \frac{\mu_{\max} C}{K_c + C} \tag{23}$$

where:

μ=specific growth rate [1/hr]

K_c=Monod half saturation constant [mg/L]

and k_{Mliq} is for the assumption that when $K_c >> C$, $\mu=\mu_{max}/K_c = k_{Mliq}$.

The method for determining B_f as a function of soil parameters is then the same as for determination of equation 14. However, an expression for biomass as a function of concentration is required. The expression used is:

$$X = X_o + Y_{x/c}(C_o - C) \tag{24}$$

where:

X_o and C_o = initial biomass and contaminant concentrations[mg/L]

Using these parameters, the final expression for bioavailability becomes:

$$B_f = \frac{1}{1 + \frac{m}{V}K_d + \frac{k_{Mliq}K_d[X_o + C_o Y_{x/c} - 2CY_{x/c}]}{k_{mu}Y_{x/c}}} \tag{25}$$

The advantage of using kinetic coefficients that are dependent on biomass (Monod Kinetics) is that B_f then becomes a function of biomass, and if biomass is transient, then B_f is not constant with time and this will be reflected in its value. The disadvantages include that B_f is now no longer a true constant but a function of biomass which is a function of concentration and that there no longer exist a simple analytical solution for concentration versus time with bioavailability

incorporated into it. Further, decay of biomass is not incorporated into this model.

The effects of biomass in equation 24 are seen in the change in substrate concentrations. The sensitivity of this equation is studied in figure 28. For low substrate concentrations, there is little difference in the behavior and the produced bioavailability than the in equation 13.

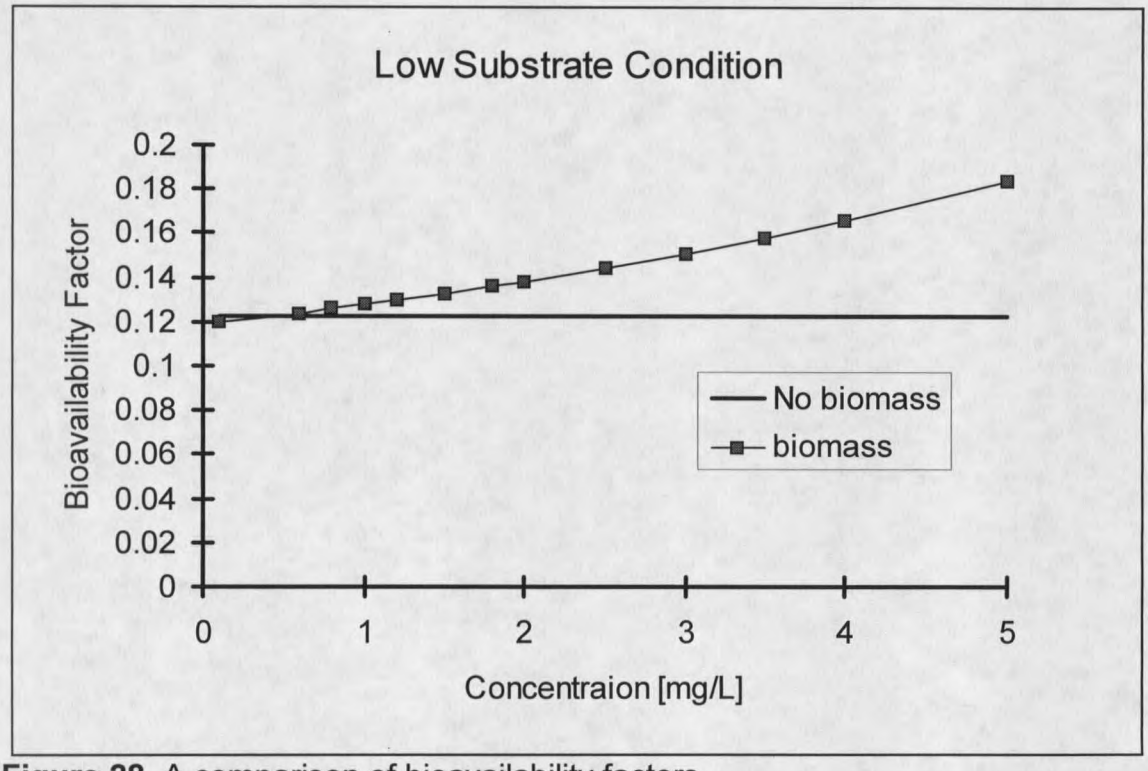
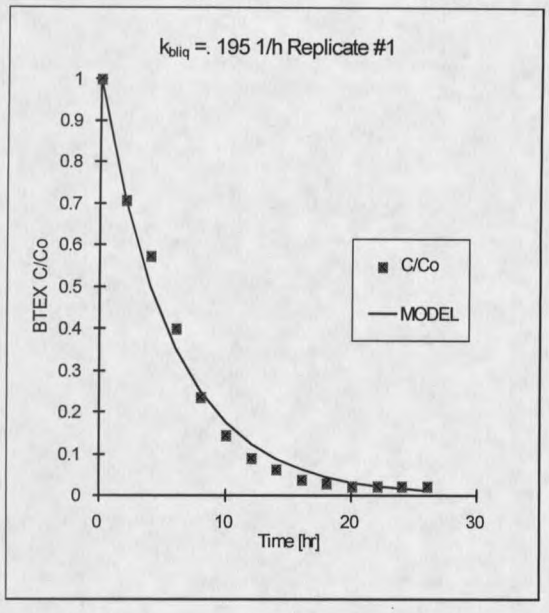


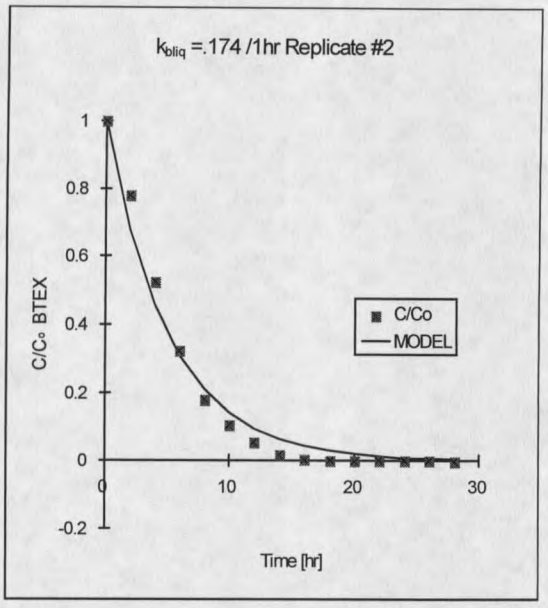
Figure 28. A comparison of bioavailability factors.

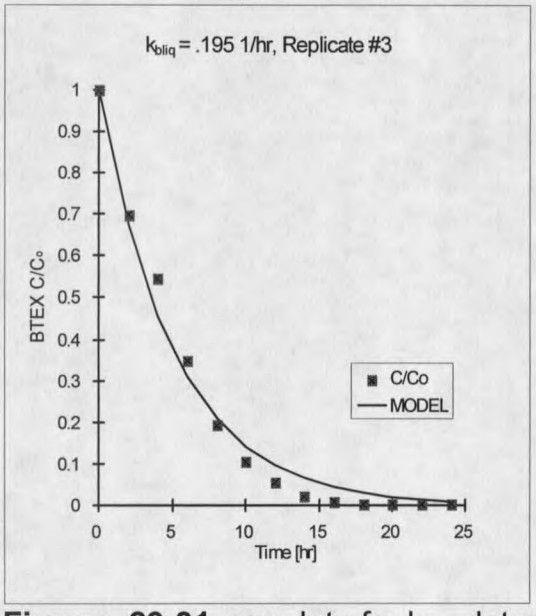
This model agreement is close of low concentrations. The agreement diminishes , however, as the concentration increases. Agreement would be closer if decay was incorporated into equation 25.

APPENDIX B

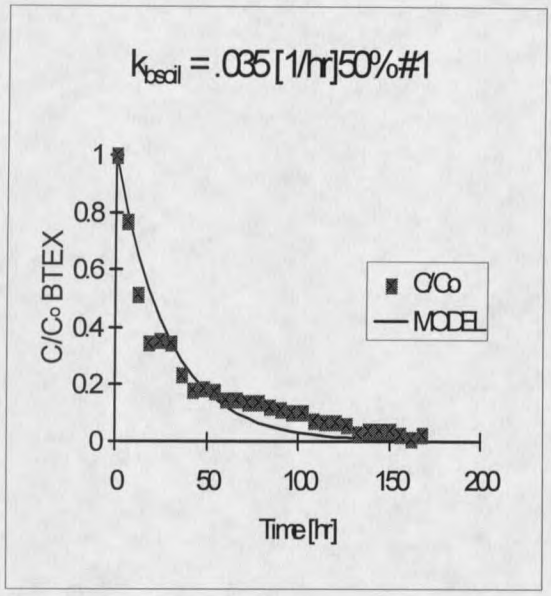
Data for Degradation Coefficients

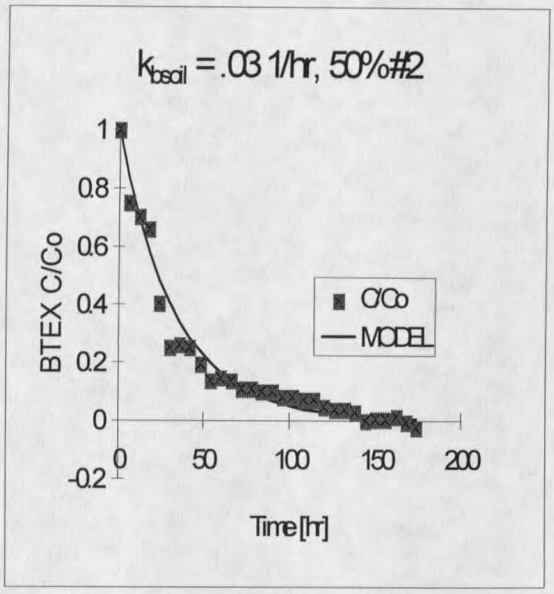


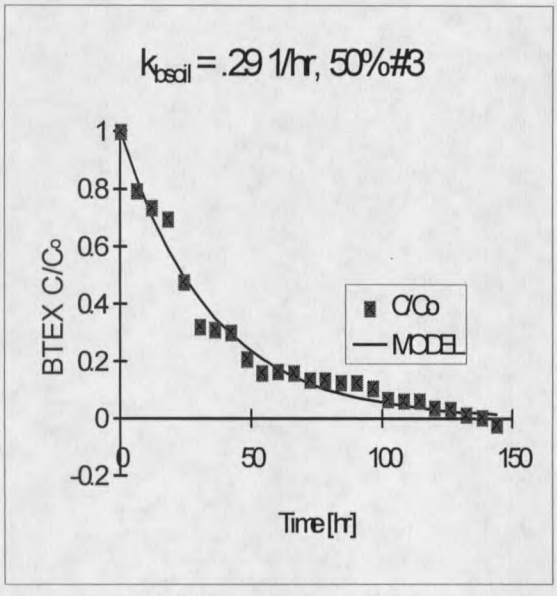


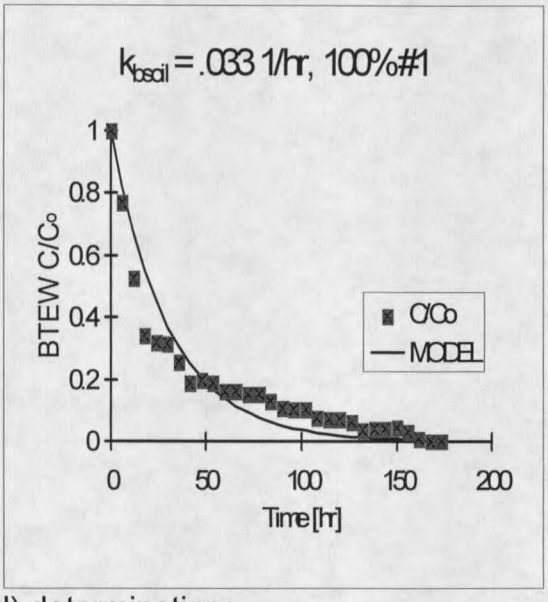


Figures 29-31. raw data for k_{bliq} determination

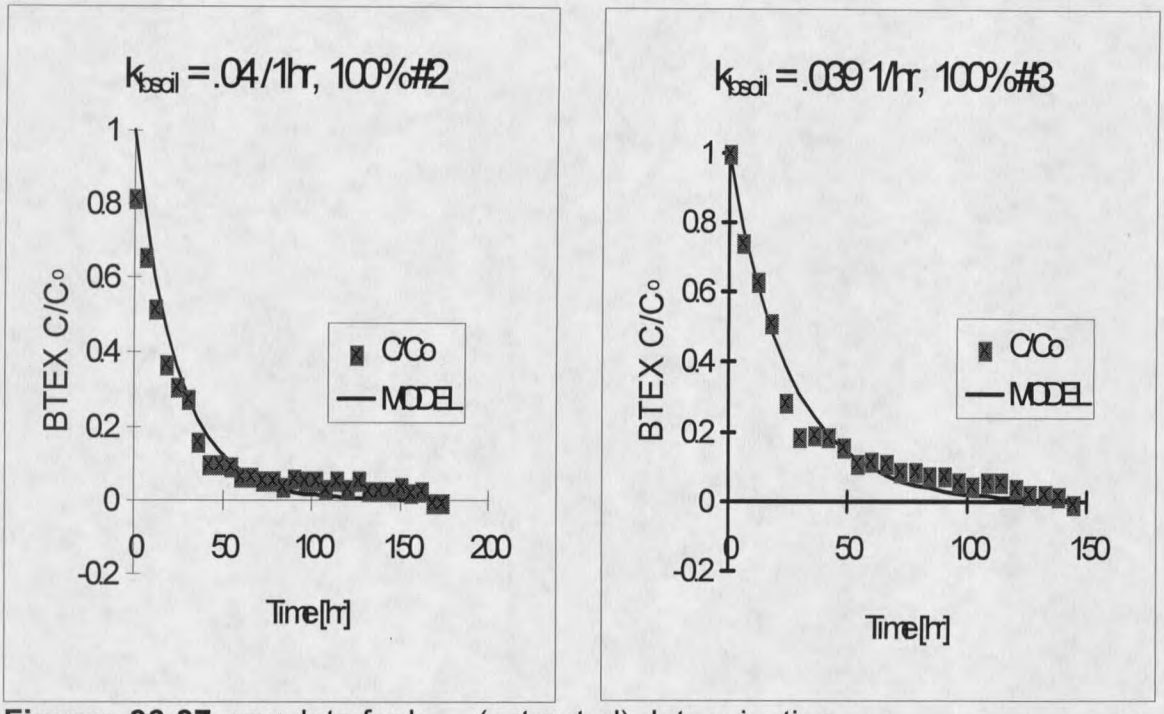




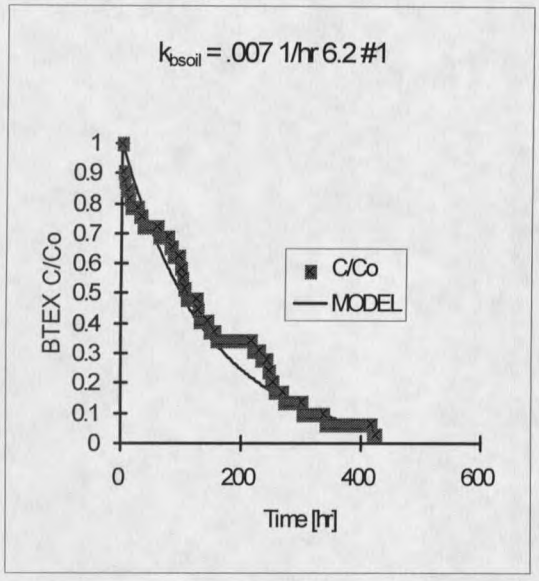


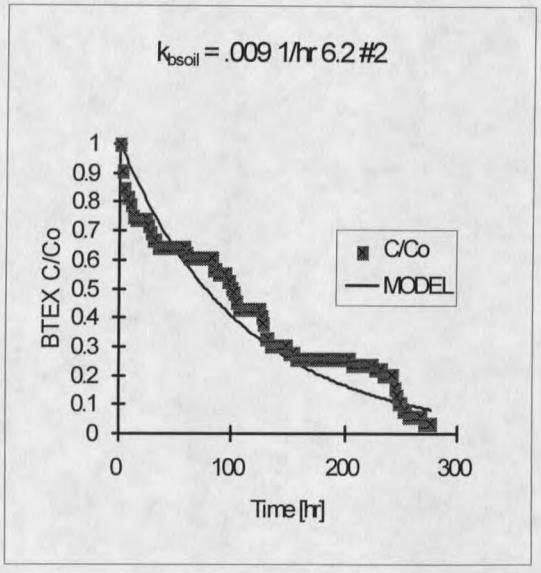


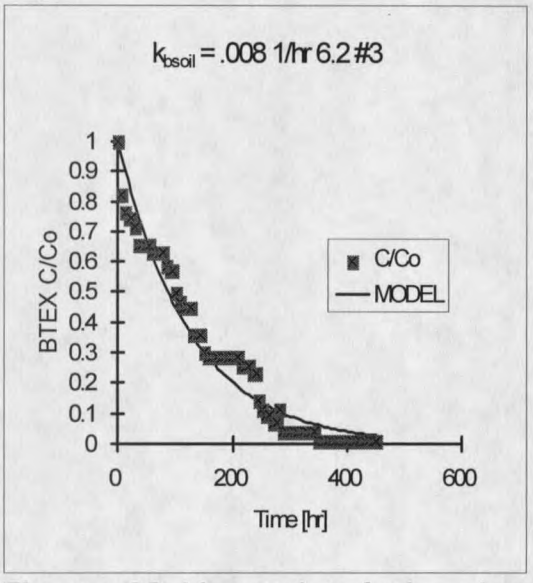
Figures 32-35. raw data for k_{bsoil} (saturated) determinations

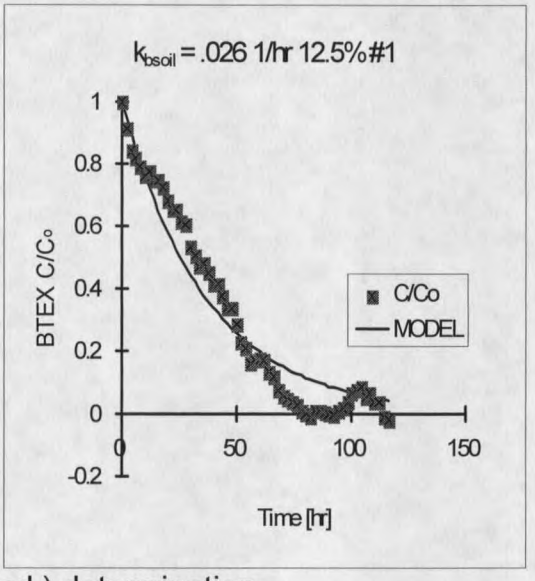


Figures 36-37. raw data for k_{bsoil} (saturated) determination

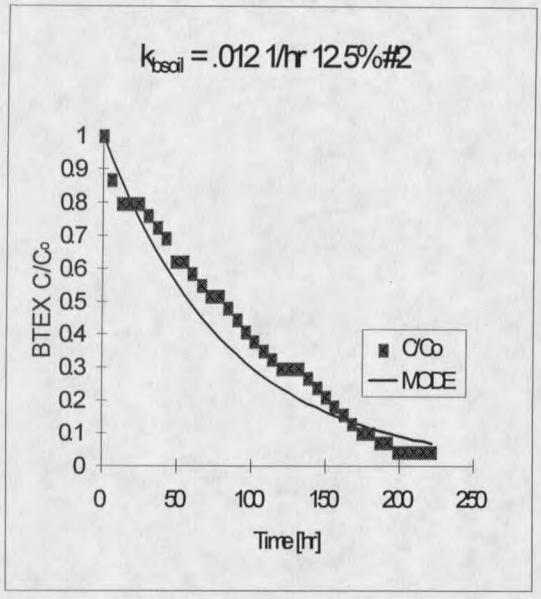


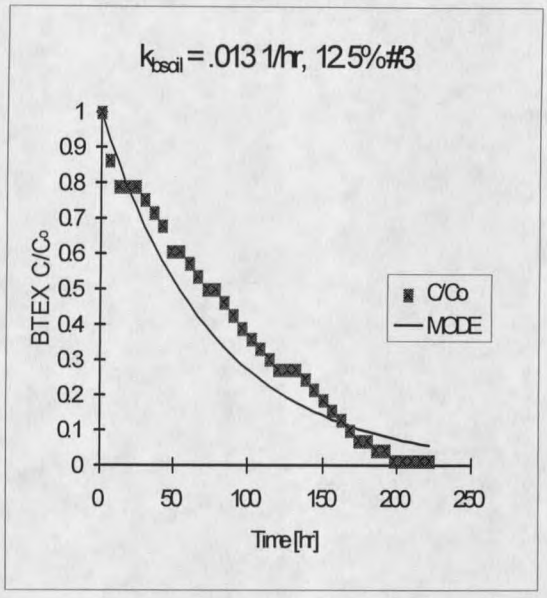


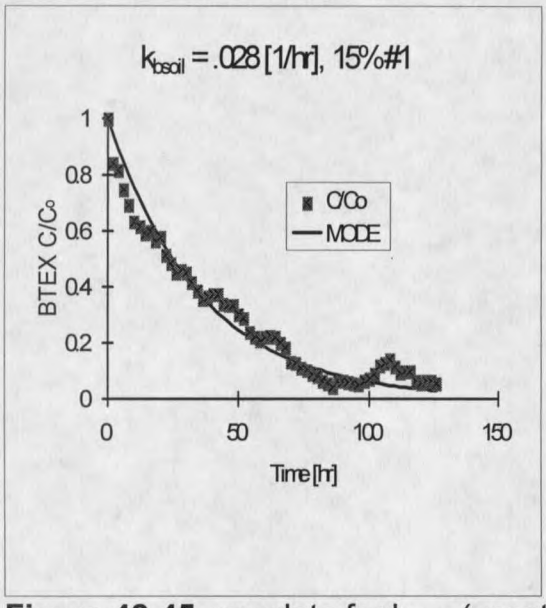




Figures 38-41, raw data for k_{bsoil} (unsaturated) determination







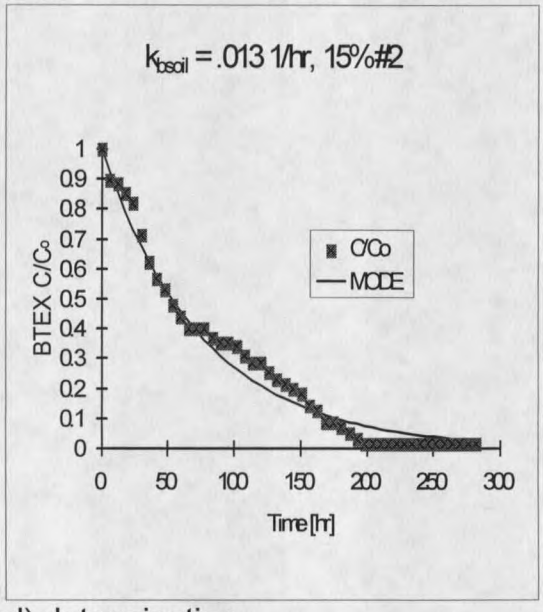


Figure 42-45. raw data for k_{bsoil} (unsaturated) determination

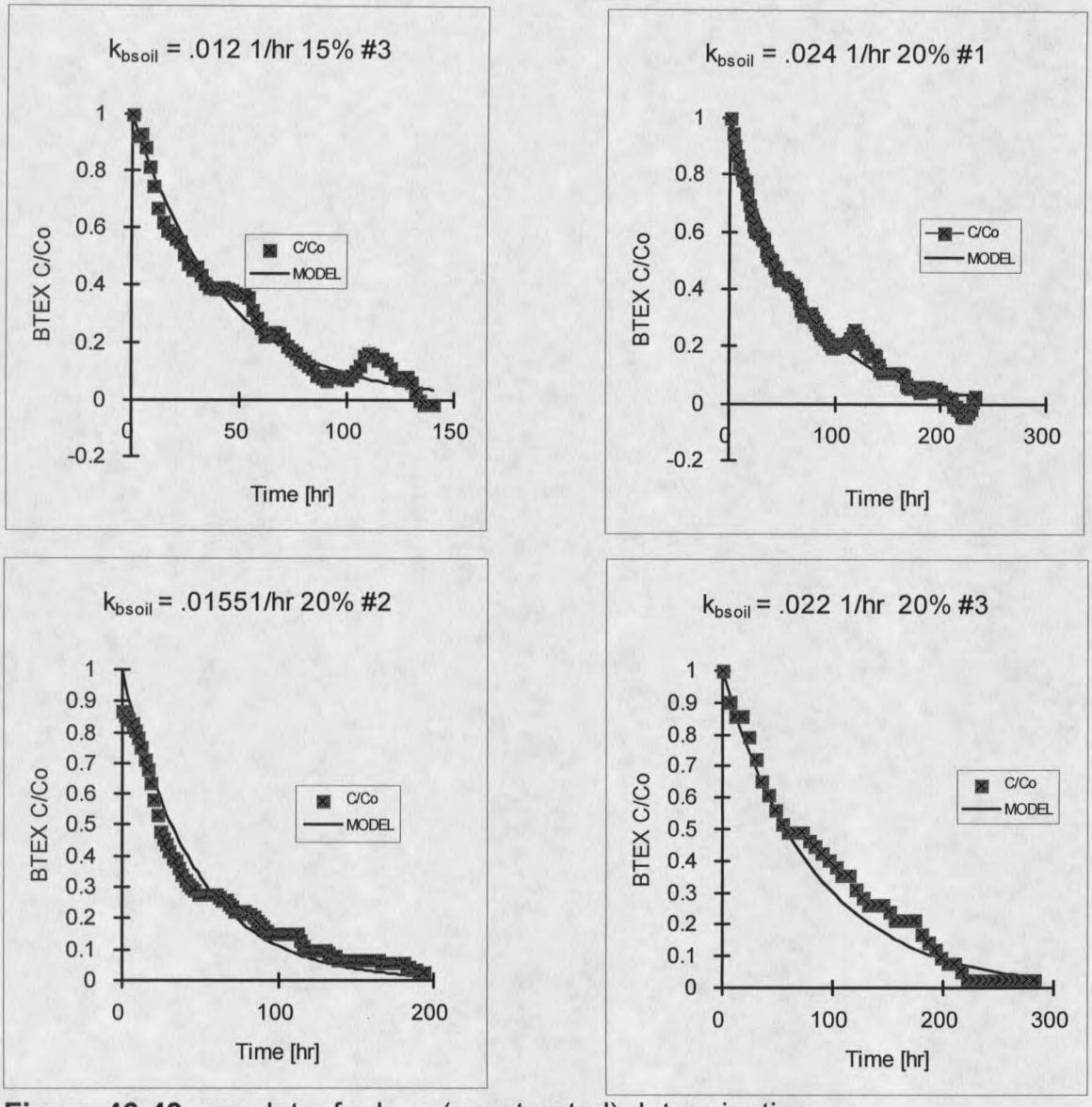
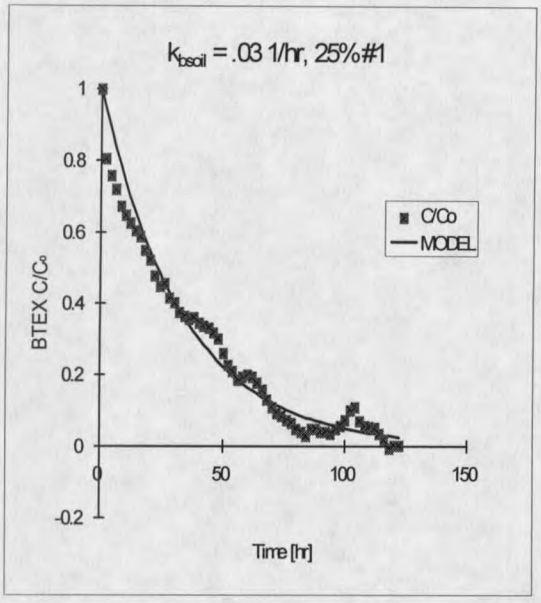
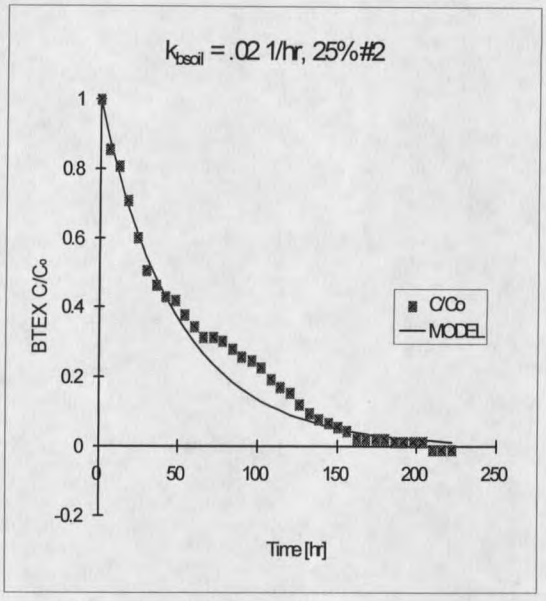
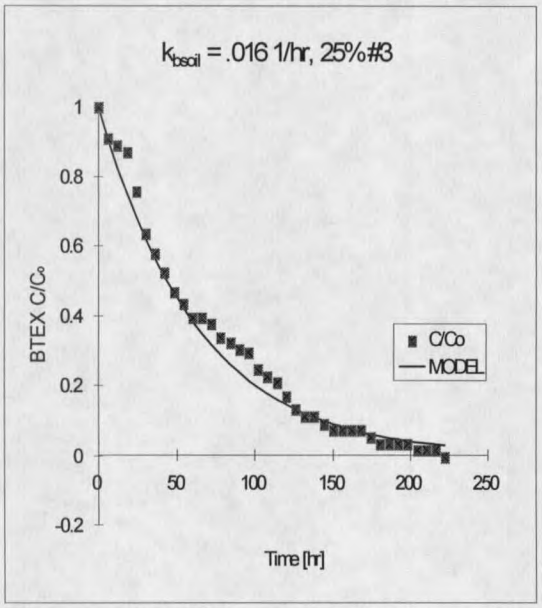


Figure 46-49. raw data for k_{bsoil} (unsaturated) determination



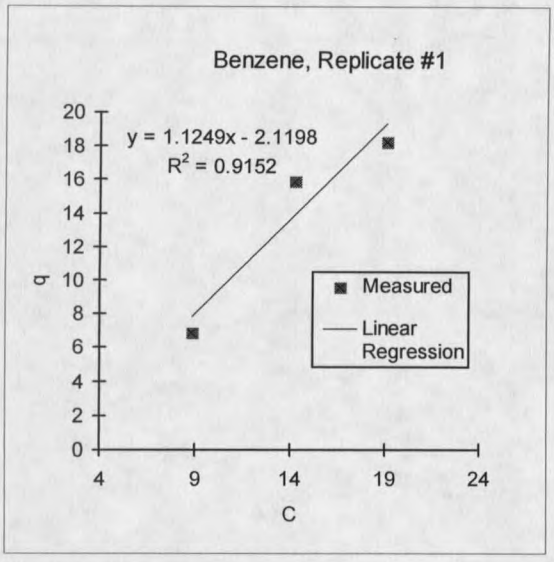


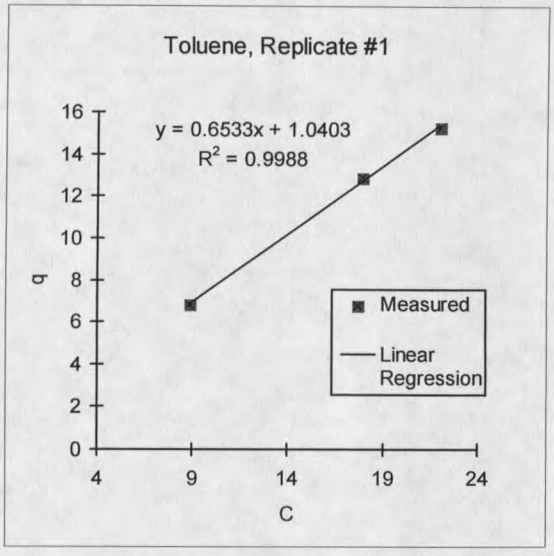


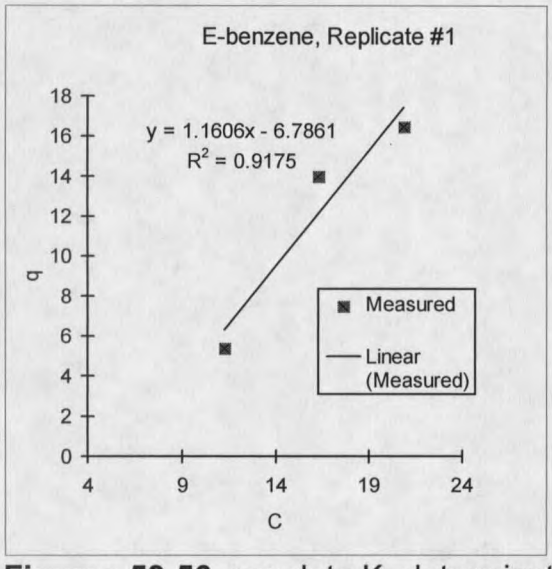
Figures 50-52. raw data for k_{bsoil} determination

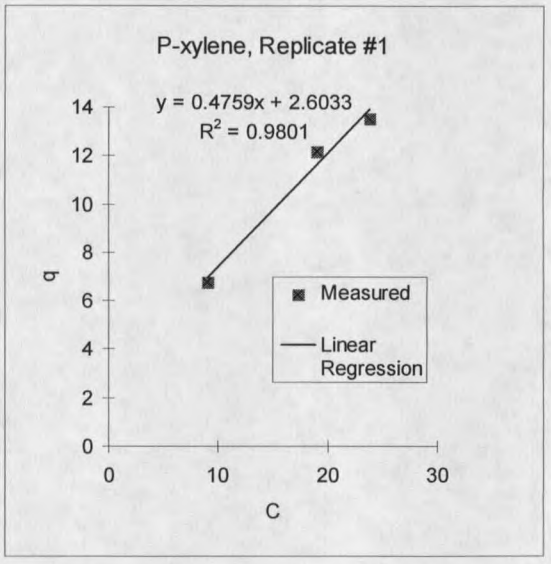
APPENDIX C

Data for Bioavailability Parameters









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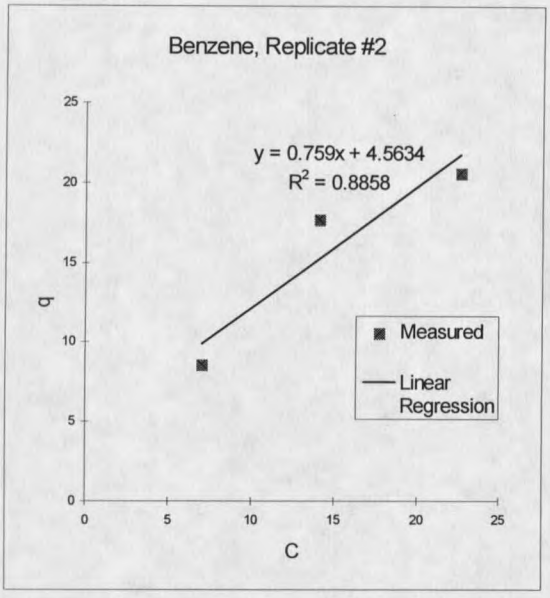

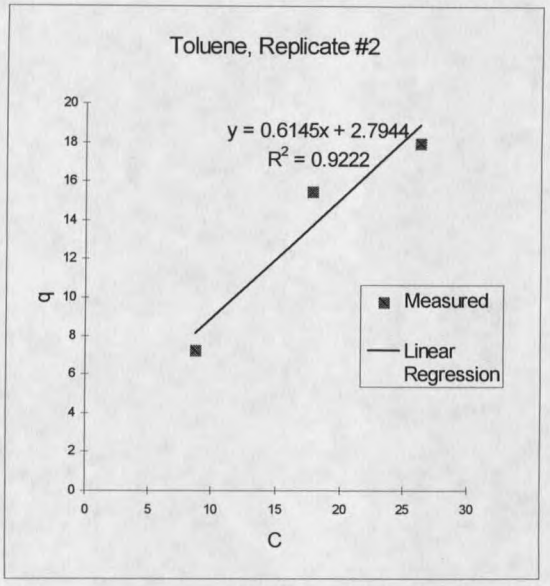

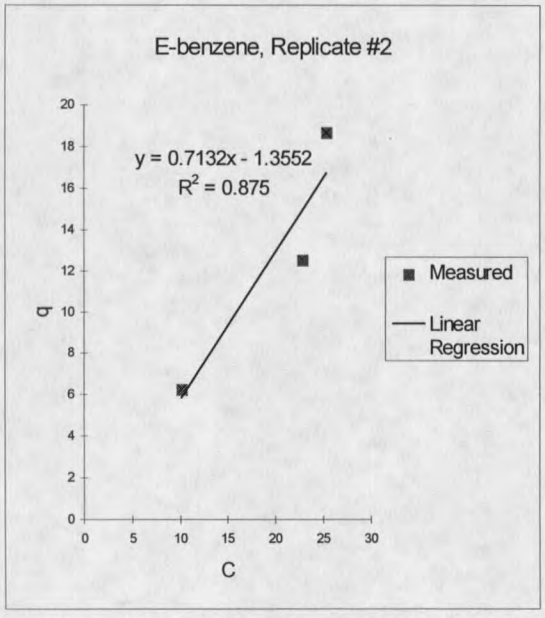

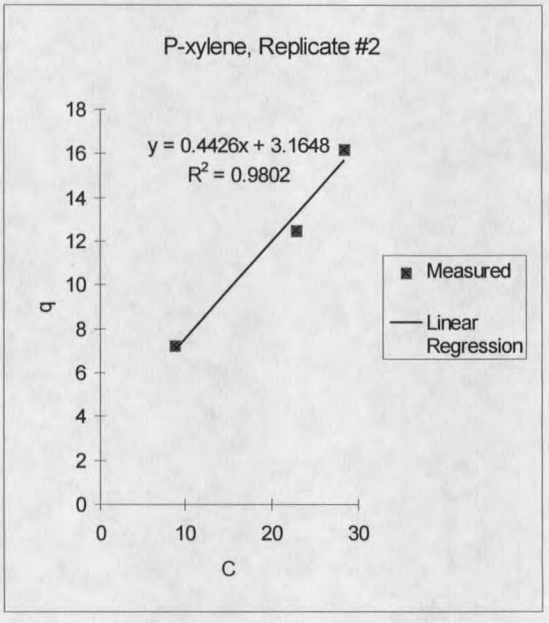

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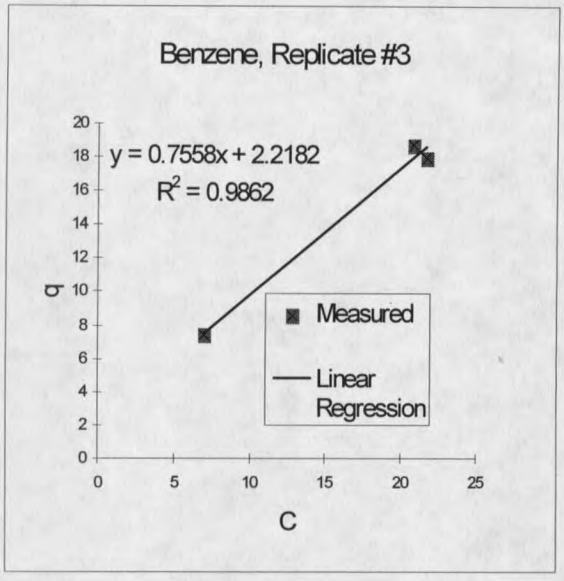
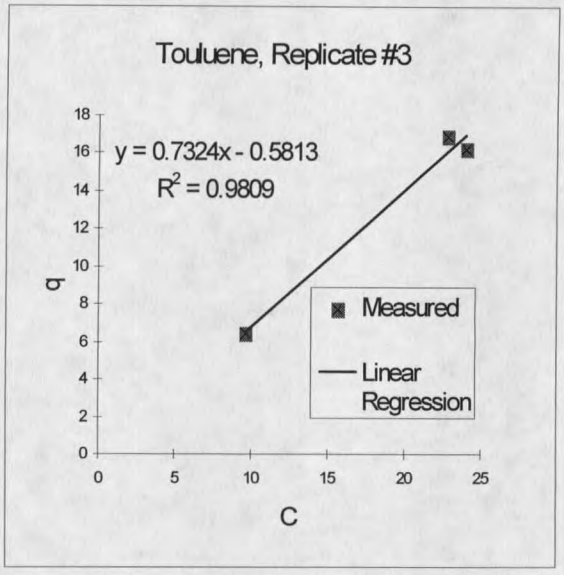
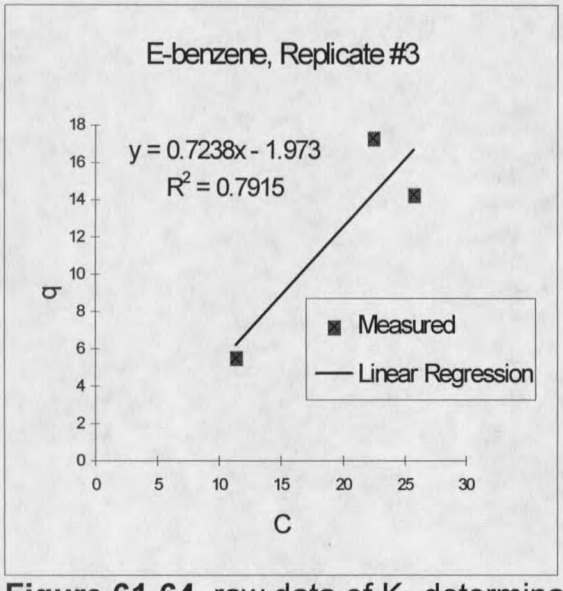






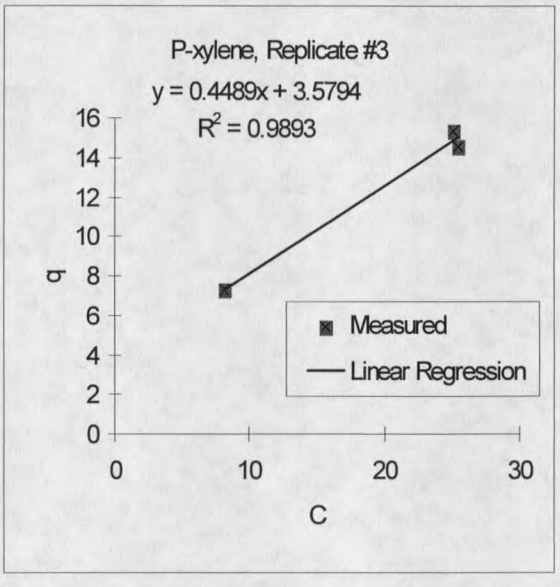
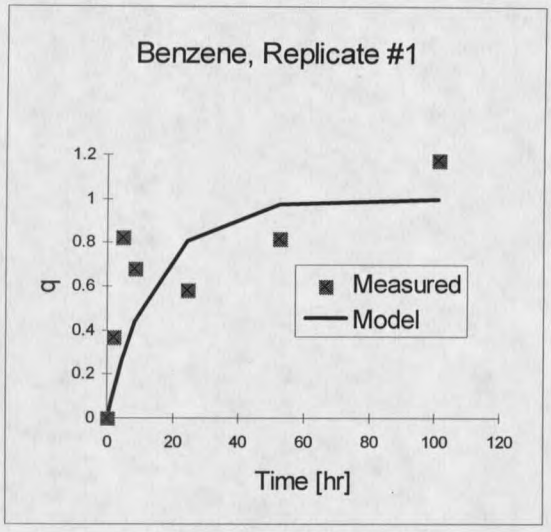
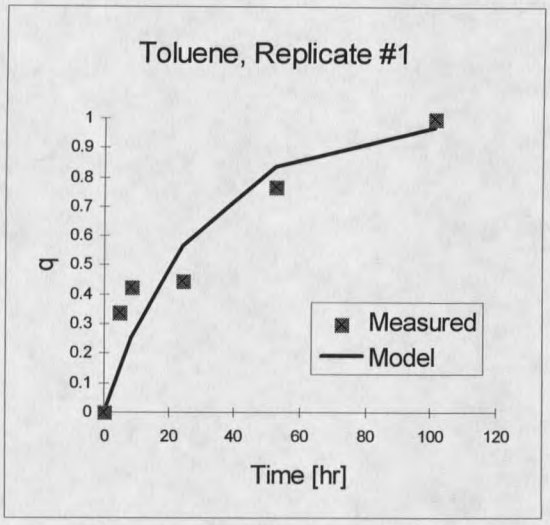
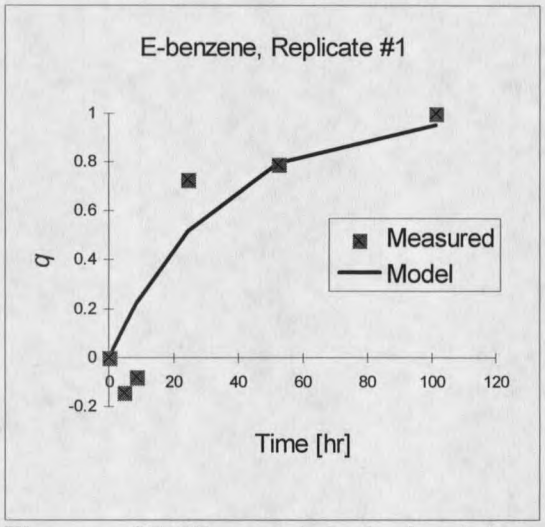
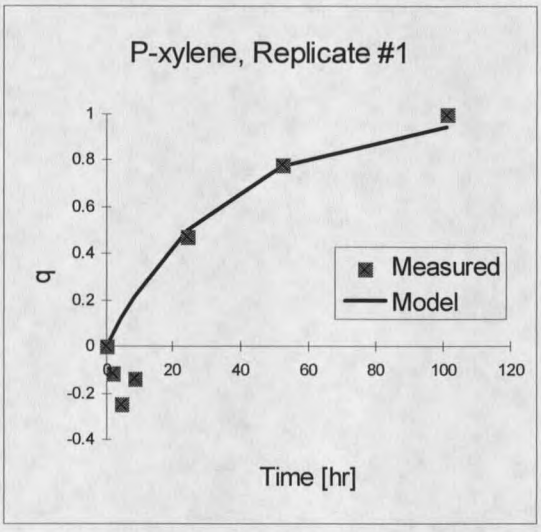


Figure 61-64. raw data of K_d determination

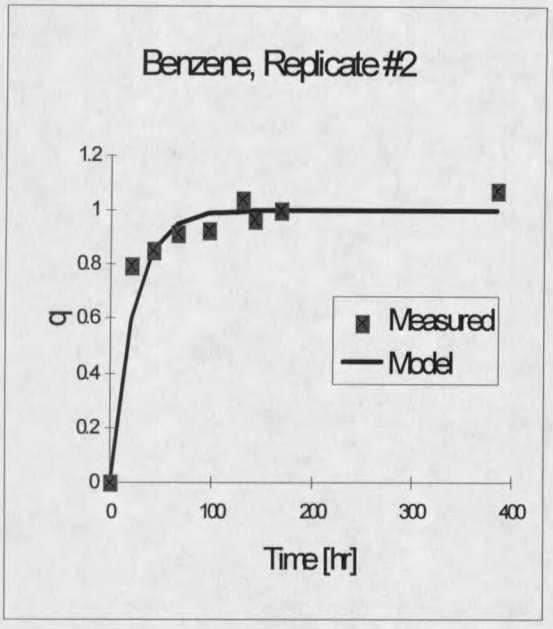


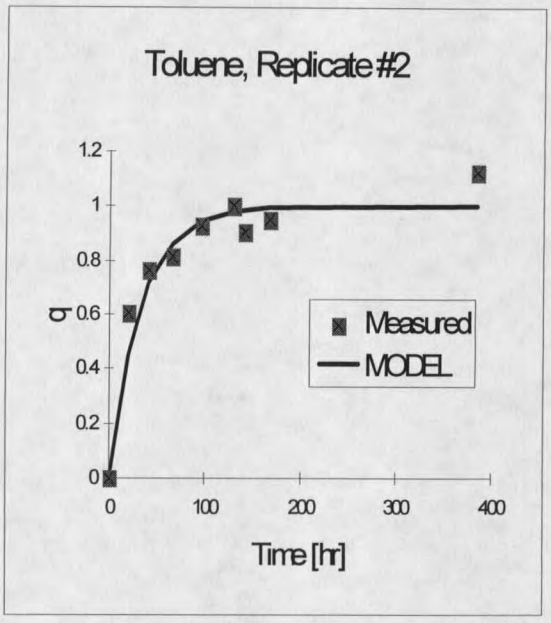


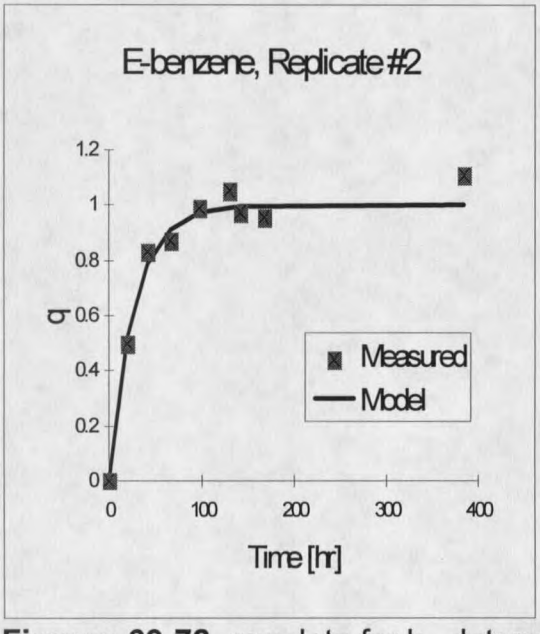


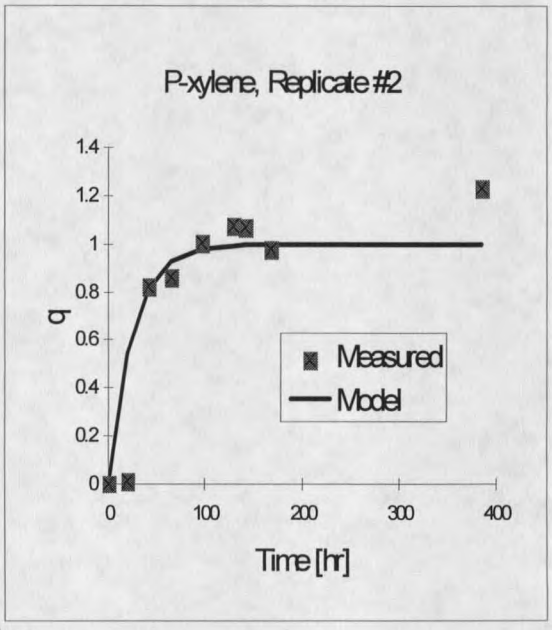


Figures 65-68. raw data for k_m determination

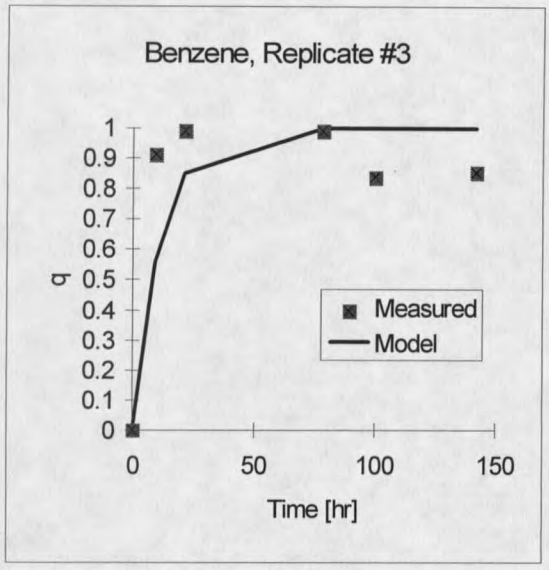


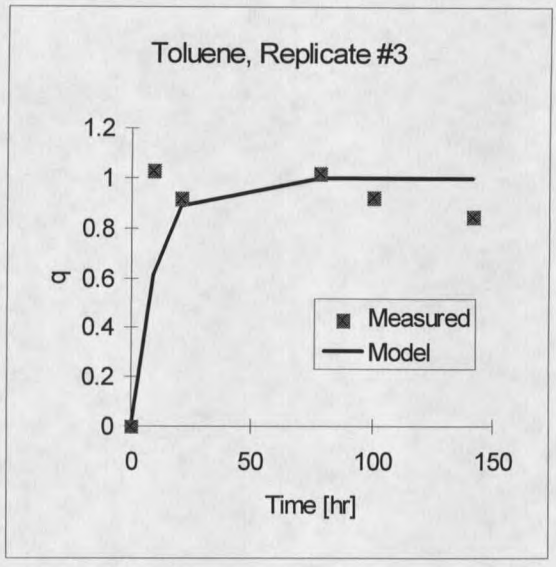


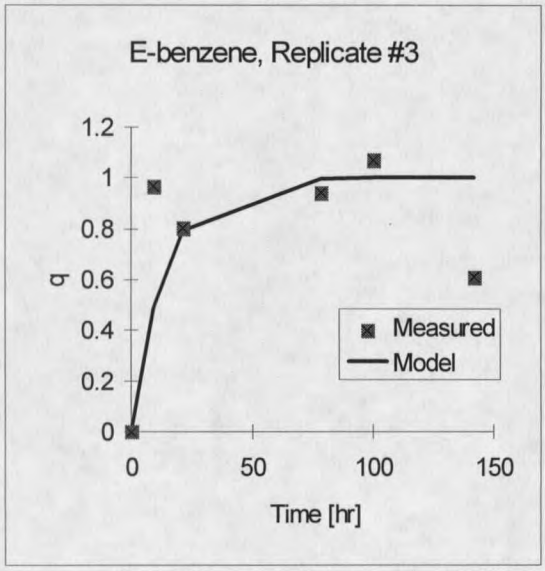


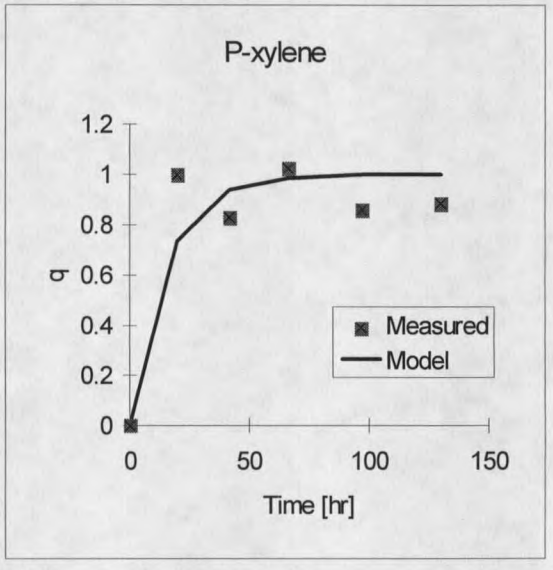


Figures 69-72. raw data for k_m determination

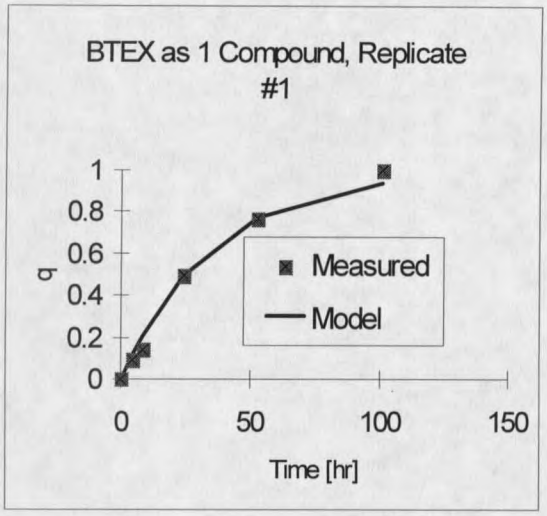


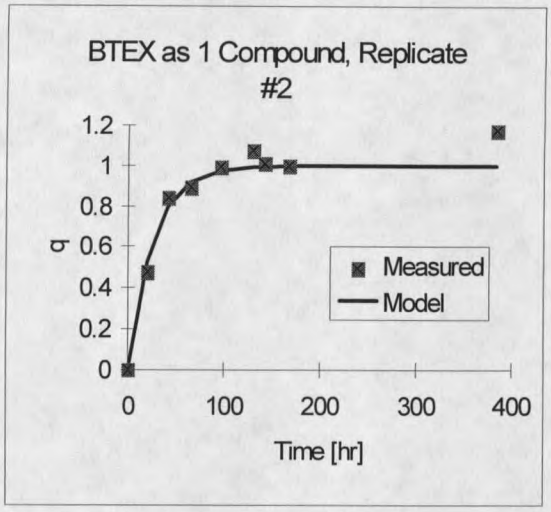






Figures 73-76. raw data for k_m determination





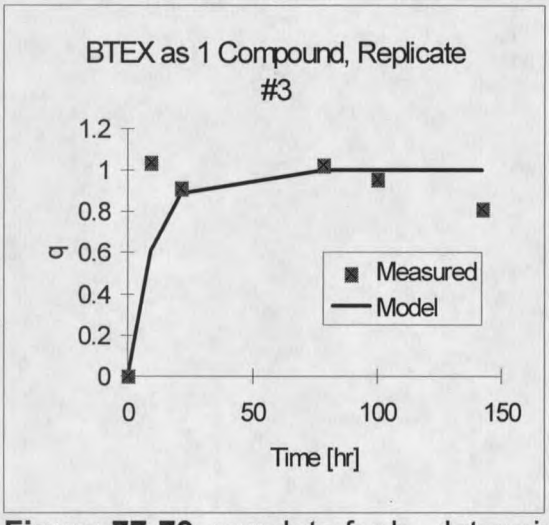
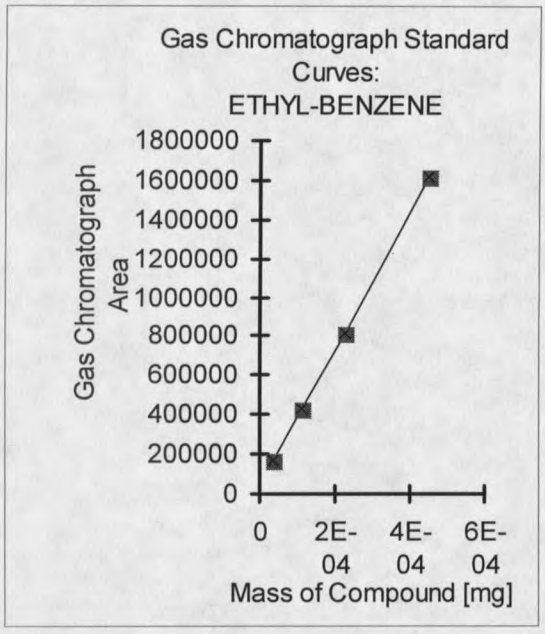
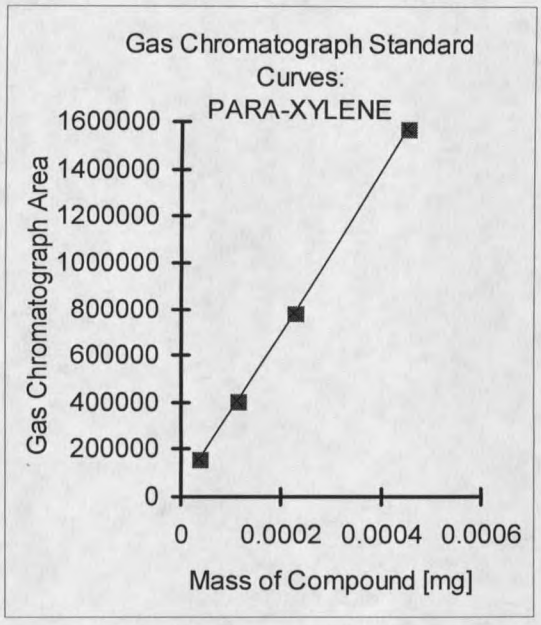


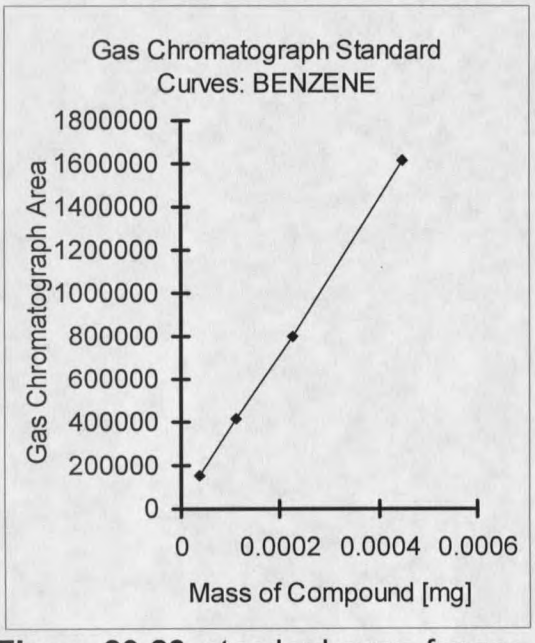
Figure 77-79. raw data for k_m determinations

APPENDIX D

Gas Chromatography Standards and Media Recipe







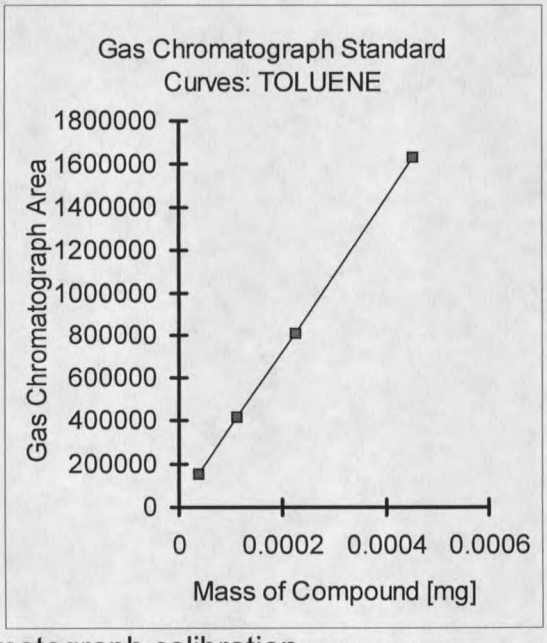


Figure 80-83. standard curve for gas chromatograph calibration

Recipe for msmx media per liter of distilled water

.7g KH_2PO_4

.7g K_2HPO_4

.35g $MgSO_4\,7H_20$ (autoclave separately)

.5 g NH_4SO_4

Augmented with micronutrients to:

10ppm $FeSO_4$ (filter sterilized)
3.5 ppm CaCl (autoclaved separately)
3.5 ppm $MnSO_4$ (autoclaved separately)
3.5 ppm NaMo (autoclaved separately)