A METHOD TO EVALUATE IN-SITU TREATMENT OF LARGE PESTICIDE CONCENTRATIONS IN SOIL

By

LADONNA CECILE MCCOWAN

Bachelor Of Science

Oklahoma State University

Stillwater, Oklahoma

1977

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1997

A METHOD TO EVALUATE IN-SITU TREATMENT

OF LARGE PESTICIDE CONCENTRATIONS IN

SOIL

Thesis Approved:

Thesis Advisor

Dean of the Graduate College

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to my advisor, Dr. Glenn Brown for his constructive guidance, especially his vigilance in ensuring that research and education is rewarding. I would like to thank my other committee members Dr. Billy Barfield, Dr. Nick Basta, Dr. Jim Criswell, and Mr. Ronald Noyes, who lent their time and expertise for this study. Special thanks are also given to Mr. Mark Payton for his statistical support for this study. My sincere appreciation extends to the Department of Biosystems and Agricultural Engineering for encouragement and support in the completion of this research. Funding for this project was provided by the Oklahoma Agricultural Experiment Station, Targeted Research Initiative.

Finally I would like to thank my Mother (Genevee Moore), children: LaCosha, Genaura, Mitchel, friend (Diana), and other precious family members for their prayers, unconditional love, belief, and patience throughout the achievement of this study.

DISCLAIMER

The use of trade names os for informational purposes only and does not constitute endorsement by Oklahoma State University or the Oklahoma Agricultural Experiment Station.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure

CHAPTER I

INTRODUCTION

Background

Detection of agricultural chemicals in surface and ground water causes major environmental concerns. Immense utilization of pesticides exists in the United States. Approximately 10 million pounds of 2,4-D were used in the United States in 1989 (WHO, 1989). Repercussions of high concentrations of pesticides in the unsaturated zone have been a topic of active research in recent years. Contingent agricultural practices that cause soil contamination are mixing and loading pesticides without a protective pad, chemical spills, and leaks from field equipment. Contamination of soil may adversely affect groundwater. While researchers differ on the sources of ground water contamination, the general public perceives the contamination mechanism as leaching of chemicals from normal field application rates. Mass (1989) stated that groundwater contamination is not originating from field application at label rates but from point sources, where high pesticide concentrations exist. Point source pollution applies to any discernible, confined and discrete effluent conveyance from which pollutants may be discharged. Chemical storage, rinse pads, and spills during pesticide mixing are possible point sources. Nonpoint source pertains to other pollutants that are

not tangible, confined or have a distinct transmission. Davidson et al.(1980) demonstrated that normal application of pesticides for agronomic doesn't hinder soil microbial populations or bioactivity. Effective treatment methods for point source pollution should permit manageable alternatives for contamination remediation.

There are a number of methods currently being used to remediate soils. Treatment by incineration and landfilling requires soil excavation, possible transportation and either soil burning or burial. Chemical dispersion to soils, in-situ, is another remedial process. These treatment alternatives are costly and unless properly managed, could pose additional short and long term dangers to health and the environment.

Bioremediation is an option to the more intensive methods. It uses biological processes to enhance natural degradation of pesticides. Bioremediation converts organic pesticides into biomass and harmless by-products of metabolisms such as carbon dioxide, water and inorganic salts. While attractive, bioremediation's effectiveness is poorly understood. The complex variables required to assess degradation of pesticide spills has led to contradictory results. Biological treatment methods have been successful for some site remediations; however, scientifically based recommendations are not available.

Herbicide contamination resulting from multiple spills of 2,4-D, 2,4-T, Dibenzofuran, and Silvex resulted from a commercial pesticide operation located at the Stilllwater Municipal airport. The location consisted of a fairly level area of approximately 120 ft by 45 ft. Grassed areas surrounded the site. The vegetation varied from barren soils, to areas of only Bermudagrass, and finally to areas with several species of native plants.

One year after the site was last used by the applicator, a commercial bioremediation product was applied to the area. This commercial compound is a microbial product that consists of selective microbes used to safely dispose of insecticides, fungicides, herbicides, and petroleum products such as, oil, diesel fuel, gasoline, and solvents. The compound was manufactured and tested on sandy soils in Cameron Park, California; therefore, no documentation exists of its effectiveness to clay type soils in Oklahoma. The compound is composed of a superfactant and OR 888 fish & seaweed foliar fertilizer, supplied by the manufacturer (Chemical Specialties). The compound distribution followed the recommended label application rate. Additional enhancements to the soil included 18" deep cultivation and watering once a week. After eight months 2,4 D, was reduced from 447,000 ug/kg to 4,690 ug/kg, a 98% reduction using this treatment. Within two years the entire area regained all vegetative growth and complied with the Department of Environmental Quality standards (Noyes, 1996).

An expeditious recommendation of a simple treatment method would enable immediate implementation of an effective contingency plan, but there is no substantial evidence that the commercial compound warrants credit for the complete recovery at the Stillwater site. Naturally occuring microbes from straw used to reduce evaporation and local soil microbes may have been the major bioremediation factors.

Remediation studies do not generally indicate significant contaminant degradation accomplishment by introducing single microbes although King et al.(1992) justified limited successes of this method. Native microbes can become accustomed to the existing environment and use the pollutant as a food source. Introduced species may not

desire the environment nor the specific pollutant as a food source. Thus, the introduced species may decrease rapidly in numbers within a short time. Effective simple treatment practices for soil degradation would encourage microbial use and enhance trends in bioremedial technology. The lack of data and knowledge hinder the remediation of simple effective treatment methods of pesticide spills.

One technique developed in this research study to evaluate soil pesticide cleanup potential uses large core in-situ bioremediation. This unique method of testing better represents field conditions and does not disrupt soil structure or microbe activity. Intact undisturbed soil cores are common-tools used when evaluating in-situ water and chemical movement in soil. Using large undisturbed soil columns to test pesticide degradation preserves the natural soil structure with existing macropores and flow channels. Studies have shown that core size will significantly affect test results. Cores larger than 17 cm demonstrated less variable biomass weight, however cost and extraction problems prohibits larger core use (Zwick et al., 1984). Most studies conducted use small cores (less than 17 cm diameter). Small cores have the advantage of being less expensive to obtain soil samples, therefore, allowing the use of multiple treatments to document the results.

Objectives

There were two objectives of this study. The first objective was to develop equipment required to conduct in-situ remediation evaluation testing and establish test procedures for large (>17 cm) core columns. The second objective was to test simple

treatment techniques to biodegrade high concentrations of 2,4-D. Objective 2 was designed to provide data for scientifically based recommendations for pesticide treatment.

Five treatment alternatives used for the study were: 1) nothing (control); 2) fertilize; 3) innoculant / Superbug; 4) fertilize and irrigate; and 5) irrigate /innoculant/Superbug. A total of 20 large soil columns were collected. Ten large undisturbed soil columns (46 cm diameter and 75 cm in length) were extracted from loam soil, and ten from sandy soil. These were local soils whose physical and chemical properties have been well defined by previous research. The soil columns were exposed to local weather while the bottoms of each column were fitted with drains. The drains serve as an effluent collection device and a containment vessel should contamination exist. The common herbicide pesticide 2,4-D was used for the analysis because of its widespread use.

Two soil samples were taken from each column to determine the pesticide concentrations. Collected bottom drainage was analyzed for pesticide concentration. Elisha immuno-assay test procedures were used for the chemical analysis of the soil and water samples collected. High performance liquid chromatography were used to verify the assay results.

CHAPTER II

LITERATURE REVIEW

This chapter will review the literature in microbial degradation of hydrocarbons and general principles pertaining to microbial activity. It will also encompass 2,4-D degradation studies and discuss effective column studies.

Microbial Activity

Hydrocarbon degrading microbes are essential for bioremediation. Essentially all ground water and soil maintain a population of viable bacteria. Bacteria function at depths up to 1,500 m below ground surface in deep soils and ground water (Zobell, 1958). Soils and ground waters contain many kinds of microbes including fungi (molds and yeast), protozoans, and bacteria. Of these common native (indigenous) microorganisms, it is the bacteria and fungi that account for the degradation of practically all hydrocarbon contamination entering the natural environment. Previous research shows certain naturally occurring marine bacteria can quickly biodegrade petroleum entering the ocean environment (Anon, 1990) as can terrestrial soil bacteria (Jones and Edington, 1980).

Fundamental elements for all life forms are food, water, and a suitable environment in which to live, grow, and multiply. Nutritional substrate for microbes must provide a source of carbon for synthesis of essential biochemical and cellular

components. The substrate may consist of organic carbon of many varieties including hydrocarbons. Total CO_2 evolution is a good indicator of microbial activity in soils. This procedure was used by Stojanovic et al. (1972) to study soils systems receiving high pesticide degradation. The bacteria used in bioremediation are the hydrocarbon microbes which utilize only organic carbon substrate combined with bacteria, which can process inorganic carbon and salts. Just having a suitable substrate is not enough to drive bioremediation. Microbes require a source of energy to initiate metabolic reactions. Heterophic bacteria and fungi are capable of metabolizing in darkness. This requires chemical energy derived wholly from oxidation-reduction reactions, which enables the degradation of hydrocarbons.

Major nutritional factors that are essential in microbial nutrition are: (1) Nitrogen (as nitrate or ammonia), and (2) Phosphorus (as phosphate). Either nitrogen or phosphate may become the limiting factor in sustaining microbial growth. King et al. (1992) through practical experience established a general rule: Nitrogen must be present in the form of nitrates, but certain organisms can utilize ammonia or nitrogen gas. Usually, ortho-phosphate must also be available as the source of phosphorus. Minor trace elements such as sulfur, potassium, magnesium, calcium, manganese, iron, cobalt, copper, molybdenum, and zinc are usually present in sufficient amounts in the natural environment.

Microbial growth and metabolism are greatly affected by the chemical and physical environment. Moisture is essential for growth and multiplication of microbes. Biodegradation of organic chemicals is often assumed to occur in the liquid phase of the

soil environment. Rao and Davidson (1979) demonstrated that adsorbed phase solutes are generally not available for degradation. Certain thermophilic bacteria can metabolize and proliferate at temperatures up to 250° C. Dissolved solutes determine the osmotic pressure affecting the microbial environment. As environmental conditions change, so does the potential for active metabolism by specific groups of microbes; certain groups become favored and certain others slow or cease metabolism (King, 1992).

2,4-D Degradation

The most commonly used location for the disposal of pesticide residues is soil. The ability of the soil to degrade pesticides is well established at low concentrations (<50 ug/g of soil), but not, however, at high concentrations (>1,000 ug/g of soil). Pesticide use has generated many studies using concentrations equivalent to field application rates, or < 100 ug/g of soil (ppm).

The persistence of the herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) in soils has been the focus of studies under laboratory conditions for many years. A generally accepted belief is that 2,4-D does not persist in the soil beyond one growing season at low field application rates. The half-life for these herbicide concentrations in the soil ranges from 4 to 31 days, depending upon environmental conditions and soil type (Altom and Stritzke, 1973; Foster and McKercher, 1973; Norris, 1966). The degradation pathway of 2,4-D follows microbiological and enzymatical means (Kearny and Kaufman, 1972; Loos, 1969). Most studies conducted used concentrations equivalent to field application rates, or < 100 ug/g of soil ppm (Altom and Stritzke, 1973; Foster and McKercher,

1973). Their results showed slow movement of contaminated water through porous soils with large surface areas on which sorption and biodegradation reactions occur. This often removes contaminants from the infiltrating water and prevents the contamination of deeper ground water.

Ou et al (1978) determined the persistence and effect on microbial activity of high 2,4-D concentrations in the soil (>50 ppm) under laboratory conditions. Degradation rates of 2,4-D were determined by measuring CO_2 evolution resulting from oxidation of uniformly ring labeled carbon. Some pesticides enhance soil microbial activity and others exhibit adverse effects (Grossbard and Davis, 1976; Stojanovic et al, 1972).

Degradation rates at low concentrations (1-100 ug/g of soil) do not agree with degradation rates for extremely low concentrations (ng/ml or ng/g and pg/ml or pg/g). Dzantor and Felsot (1991) examined microbial responses of large concentrations (10,000 mg/kg) of formulated alachlor alone and as a mixture with formulated atrazine, metolachlor, and trifluralin. After one year, the concentrations were negligible. Wolfe et al (1992) formulated parathion at soil concentrations of 0.5 and 5% (w/w) and found significant inhibition of microbial numbers. On the other hand, various microbial groups showed both selective stimulations and inhibition attributable to applying single elevated concentrations of atrazine, trifluralin, and 2,4-D (Davidson, 1980). Following large application rates or spills, some pesticides persist in soils. After 5 years of parathion (*O*,O-diethyl O-*p*-nitrophenyl phosphorothioate) application to fields at rates of (30,000 to 95,000 ppm), the lowest residue level was 13,800 ppm (Wolf et al, 1992). Extrapolation of low concentration degradation rates to extremely low concentrations can

over-predict actual degradation rates (Alexander, 1974). Lesikar et al (1990) investigated the effect of concentration on pesticide movement and degradation in disturbed soil columns subjected to simulated rainfall. Disturbed soil columns were contaminated with alachlor (Lasso 4EC) at three applications rates (5, 50 and 500 ppm). Degradation in the 5 and 50 ppm columns was not significantly different.

Some microorganisms are capable of using 2,4-D as their sole carbon source. More often, 2,4-D is co-metabolized with another carbon source. O'Connor et al (1981) found that 2,4-D applied at about 1.5 mg/kg readily degrades in soil. Adding extra carbon in the form of dried digested sewage sludge has a short-term effect in enhancing degradation of the compound. Regular treatment of 2,4-D stimulates the number of organisms which are capable of degrading the compound. Treatment with other phenoxy herbicides can also lead to an increase in organisms capable of degrading 2,4-D. Butler et al. (1975) exposed species of freshwater algae isolated from natural lake water to 2,4-D butoxyethanol ester, at a concentration of 0.01 mg/l, and looked for degrading ability. A single culture retained 64% of the added 2,4-D, while seven isolates reduced 2,4-D to less than 20% of the amount added. The remaining isolates showed 2,4-D recoveries ranging from 22% to 53%. Li-Tse (1984) investigated the breakdown of 2,4-D in two types of soil under dry and moist conditions, at two different temperatures. Numbers of microorganisms degrading 2,4-D were estimated. Generally, 2,4-D disappeared more rapidly from moist soil; after 14 days of a slow rate of disappearance, however the removal rate from dry, sandy soil increased. Numbers of organisms degrading 2,4-D were initially much lower in sandy than in clay loam soil. However, numbers increased

rapidly in sandy soils after the addition of the herbicide and, as a result, 2,4-D eventually degraded more rapidly in dry sandy soil than in moist clay loam soil. Research by Sattar and Paasivirta (1980) showed slower degradation of 2,4-D in acid soils than alkaline soil. It took 6 weeks for 50% of the 2,4-D to degrade from the acid soil, and more than 24 weeks to degrade 70%. In water logged soil, degradation of the 6 week residual herbicide was reduced. Lewis et al (1984) evaluated the breakdown of 2,4-D butoxyethyl ester and the effects of adding various extra components to the medium. The addition of unfiltered spent fungal medium, from which the majority of the fungus settled out, could be either stimulatory or inhibitory to herbicide degradation rates, depending on the particular fungus species cultured. Further analysis showed that effects were primarily due to differences in pH. Reduction of the pH below 6 inhibited bacterial transformation of the compound. Fungi commonly release large amounts of organic acids. The addition of spent fungi inhibited the breakdown of 2,4-D. Buffering the added fungal partially reduced this inhibitory effect. The addition of nutrients, or other bacteria which did not transform 2,4-D, stimulated the transformation of the herbicide. One explanation of the phenomenon is selection of other transforming enzymes. With increasing substrate concentration, enzyme systems are stimulated in bacteria. The presence of other organisms may stimulate the selection of these other enzymes at lower substrate concentrations than would normally select them. Increased biomass of transforming bacteria in the presence of competing organisms contributes to increased transformation rates.

Disparities exist between degradation efficiencies observed in laboratories and those encountered in the field. Variability in environment, organic mixtures, and active organisms affects the efficiency and rate of degradation. As a result, adding foreign microbes to a community may have no effect on the biodegradation rate. Fournier (1980) showed that while 2,4-D treatment increased the number of soil microorganisms capable of metabolizing 2,4-D as the sole carbon source and those capable of co-metabolizing the herbicide, this increase was dependent on the concentration of 2,4-D used. At concentrations of 2,4-D between 5 and 50 mg/l, there was a significant increase in the number of organisms metabolizing 2,4-D, and at 5 mg/l a very pronounced increased in organisms co-metabolizing the compound. At much higher (500 mg/l) or much lower (1.2 mg/l) 2,4-D concentrations, no increase in the numbers of either metabolizing or co-metabolizing organisms existed.

Core Extraction Techniques

Intact cores are useful tools for examining physiochemical, hydrogeological and microbiological factors controlling not only the biodegradation of the contaminant but also the mass transport of nutrients and oxygen (Nelson, 1993). Small intact soil cores, 17 cm diameter, have been used to predict accurate biomass reduction, nutrient loss and trace element enrichment in field grown crops (Zwick et al., 1984). Malanchuk (1980) used intact cores to assess the potential use of fly ash in agricultural systems and suggested its use for determining rates of metal uptake by plants.

Undisturbed, naturally structured soil with existing macropores and flow channels is a common tool used to address questions involving characterization of water movement in soils. The soil core size affords a significant effect on the result. Field variability diminishes considerably with core sizes of 17 cm diameter and larger. Intact subsoil of 20 cm insures adequate rooting zones. Previous methods examine extracting and analyzing large intact soil cores. Zwick et al (1984) evaluated five diameters (11, 17, 25, 33, and 48 cm) and depths of intact soil core to predict biomass reduction, nutrient loss and trace element enrichment in field-grown crops, amended with acidic precipitator fly ash. Findings from this study recommend an intact soil core depth of at least 60 cm with 20 cm of intact subsoil to ensure an adequate rooting zone. Soil core diameters of at least 17 cm were able to sustain agricultural crops.

The shortcoming of large intact core methods is the cumbersome and expensive field extraction. Another obstacle is the inability to simulate field conditions. One method used to extract cores from stony soils involved pouring concrete around the sample in the field. Problems associated with this method was the ability to simulate field conditions (Buchter et al., 1984). A method unsuitable for laboratory transport, due to its cubical shape and difficult field collection, was performed by Murphy et al. (1981). The method of Kluitenberg and Horton (1990) was very awkward and time consuming, requiring transport of a large sample back to the laboratory and trimming to a suitable size before encasement.

For this research, modification were made to a collection device designed by Hutton (1992). The collection device was designed to collect large-diameter soil cores in

a relatively quick manner, while minimizing compressional disturbances of the sample. Minimizing labor requirements and improving cost effectiveness while providing undisturbed soil cores for research added quality to this sample collection method.

Summary

Bioremediation requires microbes that are either artificially introduced into the soil or dependent on native organisms. Natural soils and organic matter contain a wide variety of native populations of microbes which contain some microbes which can adapt to the existing environment and learn to utilize pollutants present as a food source. Introduced microbes which are narrowly focused, find it difficult to adapt to the available food sources. Carbon sources as food may consist of organic carbon or inorganic carbon. Bioremediation requires microbes that utilize hydrocarbons (organic carbon) and those which can use inorganic carbon and salts.

Microbe optimal growth rate requires minimum moisture levels. Soil in the unsaturated zone generally provides sufficient liquid moisture within the soil pore spaces for cellular metabolism. Nitrogen (N) (as nitrate or ammonia) and orthophosphate (P)as a source of phosphorus are major microbial nutrition sources. Insufficient amounts of either N or P may limit microbial growth.

Bioremediation technologies may offer complete destruction of chemical contaminants and can be applied at lower total cost than other alternatives. Simple bioremediation methods have not been thoroughly researched and reported. 2,4-D degradation patterns are quantity dependent. Large concentrations of 2,4-D, representative of pesticide spills, need more research. Degradation studies using soil

cores has been successful. Previous large core extraction technology procedures for intact experiments have shortcomings. Soil sheer penetration is very difficult for some soil types and proposes difficulty for large core extraction methods; thus, making the extraction expensive. Most laboratory apparatus has difficulty keeping soil cores undisturbed to emulate unsaturated flow conditions in laboratories. Minimal usuable data limits treatment relationships and their relative effects on high pesticide concentrations in soil.

CHAPTER III

MATERIALS AND METHODS

This chapter presents materials and methods used to extract large soil cores, perform 2,4-D chemical analysis, and conduct the statistical analysis of research data. Because of the number of procedures used and a desire to keep this thesis concise, each description is relatively brief.

Soil Core Columns

Soils

Field soil selection sites were located near the Hydrological Experimental Research Laboratory in Stillwater, Oklahoma. Soils were chosen from that location because they were representative of Oklahoma soils and were free of prior pesticide application. The two soil types comprised physical and chemical properties well defined by previous research (Table 1) (USDA-SCS, 1987). Soil in site-1 was Pulaski fine sandy loam (Typic Ustifluvent). The area consisted of a low floodplain and prairie grass. The surface layer is reddish brown fine sandy loam about 8 inches thick. The underlying material, reddish brown fine sandy loam stratified with fine sand, typically extends to a depth of 72 inches or more. Natural fertility is medium and organic matter content is low < 1% oc. Permeability is moderately rapid and surface runoff is slow. The root zone is deep and can be easily penetrated by plant roots.

Soil in site-2 was Easpur loam (Arenic Haplustalf). This area occupied a slightly higher position on the flood plain and was covered by prairie grass. The surface layer was reddish brown loam about 11 inches thick. The underlying material, to a depth of 41 inches or more is stratified reddish brown loam, yellowish red fine sandy loam, and reddish brown clay loam. Natural fertility and organic matter are high. Soil pH of the surface layer ranges from slightly acid to moderately alkaline. Available water capacity is high, permeability is moderate, and surface runoff is slow. The soil can be worked throughout a wide range of moisture levels. The root zone is deep and can be easily penetrated by plant roots. Particle size analysis was performed on two samples of each soil and are listed in Table 2. Size was determined from surface samples and samples at 40 cm depth.

Large Core Extractor

Twenty (46 cm diameter by 75 cm long and 9 mm wall thickness) PVC hollow cylinders were inserted into the soil profile to extract undisturbed soil cores. The vegetation was not removed from the area prioe to soil core extraction The top of the PVC cylinders were cut square, while the bottom end of the cylinder was sharpened by filing to a 3 mm bevel to reduce friction. Two 11 mm diameter holes were drilled into cylinder sidewalls 1 cm below the top of each cylinder to assist lifting the soil columns from the ground to a trailer for transport.

Hutton's (1992) hydraulic ram technique was modified and used to insert the PVC columns into the soil to a depth of 61 cm. The machine consist of a remote controlled

hydraulic cylinder mounted on a free standing frame (Figure 1). The hydraulic ram stroke of 91 cm provided safety and increased the allowable column depths. The three cornered frame provided smooth steering for the guide plate, while the support anchor chain attachments located on each vertical member provided stability. Heavy earth screw chain anchors provided resistance against hydraulic forces required to push the cylinders into the soil. Six helical auger earth anchors were screwed into the soil profile and removed with a low-speed hydraulic motor. A 61 cm diameter guideplate was mounted on the end of the hydraulic arm to stabilize the insertion process and prevent damage to the hydraulic cylinder should asymmetric loading occur from the PVC sample cylinder.

Hydraulic power was furnished by a John Deere 2520 tractor. The closed-center hydraulic system of the tractor controls hydraulic fluid at high pressures, thus it minimizes excessive heating. Using the tractor's sustem fluid power simplified operating characteristics and incorporated a three point hitch transport system to move the insertion tool to each sample point.

After inserting each PVC cylinder 61 cm into the ground, a backhoe removed soil from the area outside the PVC columns. This relieved the pressure from the outside perimeter of the cylinders, and allowed the soil cores to be manually tilted and broken free at their base. A pre-fabricated base cap was then fastened to the bottom of each core. Soil cores were attached to a backhoe by chains and the entire unit was lifted to a trailer bed for transportation to the experimental field test site. After the cores were secured on the trailor, a plastic cover was sealed over the top of each cylinder to minimize evaporation.

The pre-fabricated PVC base caps were placed over the bottom of the columns immediately after shearing. The caps prevented soil from sliding out and contained fiberglass wicks. These plates were constructed by drilling four 9.5 cm diameter holes into a round aluminum plate, 46 cm in diameter and 9 mm thick. Fiberglass wicks (Figure 2) were attached to the plate. The wick was intended to draw water from saturated soil and provide drainage without applying external suction (Boll et al., 1992). The top end of the wicks were frayed and spread over the face of the plate to insure soil contact. The remainder of the wick remained braided and was inserted through the plate and placed in a glass drainage container (Figure 2).

Experimental Setup

The twenty large soil cores encased in the 46 cm x 75cm PVC tubes were buried in two trenches, with ten samples per trench, as shown in Figure 3. Each trench was prepared with a positive bottom gradient and a drain pipe to minimize flooding potential. Figure 4 illustrates the stand design. Each stand was designed to hold 5 large soil cores. The stand height of 20 cm provided access and protection to the bottom core apparatus. The braided fiberglass wicks extended into the glass jar, but did not contact the bottom of the jars, effluent collection. The collection system was protected from soil, water, and other environmental factors. A tube extending from the bottom of the glass jar to the top of the column was used to extract effluent. The columns and stands were backfilled with the excavated soil from the trench. The top 14 cm of each PVC column remained above the top of the trenches. During the first 6 months a plastic cover was placed over the

columns (approximately 4 ft) to control weather parameters. The plastic cover was removed during the last 10 months of the research.

2,4-D Application

The test herbicide was AMINE 4 2,4-D Weed killer. The active ingredients consisted of 46.5% Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid. The inert ingredients of the AMINE 4 compound were 53.5%. Prior to 2,4-D application, the soil was disked slightly approximately 5 cm deep. The 2,4-D mixture (4750 ml of water and 250 ml of 2,4-D) was evenly distributed on the 20 soil cores. Recommended 2,4-D application rate was 1.21×10^{-3} oz/core. Spill simulation of 1000 times normal applied rate required an application of 1.2 oz/core of 2,4-D mixture. A total volume of 1500 ml of 2,4-D mixture was applied to all cores. The initial vegetation remained inside the soil columns.

Treatments

Five treatments were selected (Figure 5), with four columns for each treatment. Treatment 1, the control, was considered an alternative and used to compare other treatment alternatives. These columns did not receive any additional treatment and represent a "no action" option.

Treatment alternative 2 used fertilizer only. Granular fertilizer, 10% nitrogen-20% phosphorus-10% potassium, was applied to the soil columns according to the manufacturer label application rate of one pound per 1000 square feet. Therefore, 812 grams of 10-20-10 fertilizer was applied to the columns for treatment alternative 2.

Treatment 3 combined seaweed fertilization, 8% nitrogen, 8% available phosphoric acid, and 8% soluble potash as an innoculant and Superbug. The Superbug solution required 40 ml/column added to one liter of seaweed fertilizer solution. The fertilizer solution specifically for the superbug application consisted of 2.5 ml of surfactant, 2.5 ml of seaweed fertilizer, and one liter of water.

Treatment 4 was to fertilize and irrigate. Label application rate, 821 grams/column, of granular fertilizer, 10-20-10, was applied to each column for treatment 4. Irrigation amounts were based on the column area. A field would normally require 3.8 cm of water to insure proper water intake. Irrigation for the soil cores based on the surface area of each column was 1500 ml of water. The irrigation water was evenly distributed over the column area once a week for 20 weeks.

Treatment 5 combined three previous treatment practices, seaweed fertilize (innoculant), irrigate, and Superbug. Application to these columns were one liter of innoculant solution, 40 ml of Superbug solution applied initially, and 1500 ml of water for irrigation added weekly for 20 weeks.

2,4-D Analysis

Small Core Extractor

Small sample cores (2 cm diameter by 55 cm long) were extracted from the large cores to perform the soil analysis. Polycarbonate (Lexan) tubing (2 cm by 70 cm) was placed inside a stainless steel core probe tube (3 cm by 70 cm long) (Figure 6). A hydraulic arm mounted to a John Deere 550 front end loader was used to push the core to

a depth of 61 cm. The bottom of the probe had inside threads for a beveled tip. The beveled tip prevented the soil from falling out during retraction and minimized the forces exerted on the walls of the plastic tube. Prior to each sample collection the tip was removed and cleaned to prevent soil and treatment cross-contamination. Following each soil sample taken from the cores bentonite clay was used to backfill as a measure to control contamination to later soil samples. Soil contained in the plastic tubes was removed from the steel probe tube and immediately capped on both ends.

Sample Preparation

The soil sample cores extracted and stored in plastic tubing, were further divided for analysis. Figure 7 displays a soil sample verticle profile. The top 10 cm of soil was removed to obtain a 2.5 cm section representing the top soil. A 2.5 cm section obtained at the interval of 40 to 42.5 cm represented the bottom soil. Each 2.5 cm section was labeled and wrapped in aluminum foil. The soil samples were taken to the laboratory and frozen until analysis.

Chemicals

Superbug was purchased from Chemical Specialties International located in Cameron Park, California. The surfactant and Fish & Seaweed Foliar fertilizer which was applied with Superbug, was also purchased from chemical specialties (Chemicals Specialties International, 1990). The fertilizer was derived from fish, seaweed, phosphoric acid, potassium, hydroxide, and urea. Its contents were 8% total nitrogen, 7%

urea, and 1% water insoluble. Available phosphoric acid was 8% with 8% soluble potash.

Ohmicron 2,4-D RaPID Assay kit and sample diluent were used for quantification of 2,4-D in soil samples. Other reagents provided in the RaPID Assay kit to detect pesticides were: 1) 2,4-D antibody coupled paramagnetic particles. The 2,4-D antibody (rabbit anti-2,4-D) is covalently bound to paramagnetic particles, which are suspended in tris buffered salie containing bovine serum albumin with preservative and stabilizers. 2) 2,4-D enzyme conjugate and 2,4-D standards (1,10,35, and 50 ppb). 3) Peroxide solution. Hydrogen peroxide (0.02%) in a citric acid buffer. 4) Chromogen (TMB) solution. 3,3',5,5'-Tetramethylbenzidine (0.4) g/l) in an organic base. 5) Stopping solution. A solution of sulfuric acid (2M).

2,4-D Extraction

Soil samples were broken down or crushed to small aggregates and thoroughly mixed for uniformity. Moist samples were air dried prior to mixing. In order to extract 2,4-D from soil, a 30 ml solution of 75% methanol, 23% water, and 2% acetic acid, mixture was added to 3 grams of soil in a glass bottle (Chesters et al. 1974). The soil solution was vigorously shaken for 30 minutes on a custom rotary shaker. They were then placed in a Lab-line shaker bath, with the speed set to 50 rpm for a 12 hour duration. The extract was then filtered through a filter pore size of 0.45 micron. This procedure provided a transparent clean extract for the soil chemical analysis.

Immuno Assay

Soil analysis was performed with immuno-assays to determine the 2,4-D concentration from the columns. Ohmicron's RaPID pesticide immuno detection (RaPID) assays were used for detection of 2,4-D in the soil samples. Ohmicron's technical concept is based on the use of novel magnetic particles as the solid support and means of separation in an enzyme linked immunosorbent assay (ELISA) system. The soil analysis procedure was followed according to the instructions presented in Ohmicron's startup manual (Ohmicron, 1992). RaPID assays range of detection for 2,4-D is 150 ppb to 7.5 ppm. The sample to be tested is added along with an enzyme conjugate, to a disposable test tube, followed by paramagnetic particles with antibodies specific to chlorophenoxy herbicides attached. Both the 2,4-D (which may be in the sample) and the enzyme labeled 2,4-D in the enzyme conjugate compete for antibody sites on the magnetic particles. At the end of an incubation period, a magnetic field is applied to hold the paramagnetic particles, with 2,4-D and labeled 2,4-D analog bound to the antibodies on the particles, in proportion to their original concentration, in the tube, which allows the unbound reagents to be decanted. After decanting, the particles are rinsed with washing solution.

The presence of 2,4-D is detected by adding the enzyme substrate, hydrogen peroxide, and chromogen, 3, 3',5, 5'-tetramethylbenzidine, to generate a colored product. After an incubation period, the reaction is stopped and stabilized by the addition of a sulfuric acid solution. Since the 2,4-D was in competition with the unlabeled 2,4-D for

the antibody sites, the color developed is inversely proportional to the concentration of 2,4-D in the sample.

Concentration of 2,4-D were determined with a Hitachi U-1100 spectrometer. The target setting for the spectrometer, 450 nm, was set according the recommended parameters from the 2,4-D RaPID Assay. If the concentrations of the sample exceeded 50 ppb, the sample was further diluted by ten-fold dilution.

Data

Absorbency readings for 2,4-D were made with the Hitachi spectrometer determined at the 450 NM wavelength. Data were interpreted by constructing a best fit to the calibration:

$$\log (abs) = a + b \log(c) \qquad (1)$$

where abs = absorbency values from the spectrometer readings of 2,4-D standards, and a, b, and c were calculated by substituting standard absorbencies for 1 ppb and 50 ppb.

The 2,4-D RaPID Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 0.70 ppb. The mean absorbency value for a clean, (no 2,4-D), standard is the B_o. The mean absorbency value for the other calibrators is the B value. To obtain the B/B_o., divide the absorbency of the standard or sample by the zero absorbency and multiply by 100

A calibration curve was constructed by plotting the B/B_o values. Using standards, interpolation was done to obtain values for 2,4-D concentrations in ppb. The absorbency range of 2,4-D detection from RaPID assay was 150 ppb to 7.5 ppm. Sample

concentrations outside of this range were diluted. Dilution factors (D.F.) were calculated and multiplied by the assay results to obtain the concentration of 2,4-D in soil (ppb) (Appendix I),

$$D.F. = \frac{Vm}{Ws} \frac{Ve + Vd}{Ve} \quad (2)$$

where Vm = volume of methanol (ml),

Ws = weight of soil (g),

Ve = volumn of extact (ml), and

Vd = volumn of dilutent (ml).

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) Dionex Dx-300 Gradient Chromatography system combined with an AI-450 Chromatography software program and a variable wavelength Dector-II was used to confirm the 2,4-D concentration of the soil samples. HPLC is a highly versatile nondestructive technique that allows the separation and identification of a wide variety of volatile and nonvolatile organic compounds. The acidic compound and methanol solution allowed direct analyzation by HPLC. The goal of chromatography is to separate mixtures of compounds into separate bands or peaks of individual compounds within a reasonable period of time. Retention time is used to identify the chemical 2,4-D. The area under the curve determines the concentration of the chemical compound. Figure 8a and Figure 8b illustrates the HPLC analysis of sample 8 and sample 12. A retention time of 9.55 minutes identifies the chemical compound 2,4-D for both samples. In Figure 8a, 2,4-D concentration for one dilution is 0.18 ppm.

Statistical Analyses

Statistical analysis were performed at the 95-percent level of probability for statistical significance among soil type, location and treatment method for each dependent variable. Statistical Analysis System (SAS) software in conjunction with a Leveen's test performed the analysis of varaiance (ANOVA) (SAS Institute Inc. 1984). The statistical analysis was performed with the assistance of Dr. Mark Payton at Oklahoma State University. The results of this analysis is listed in Appendix II. Each treatment was tested separately with two replications performed on each treatment. Within each replication, a control sample (not treated) and four other samples (1) treated with fertilize, 2) innoculant / Superbug, 3) fertilize / irrigate, and 4) innoculant / irrigate / Superbug) were tested at random points for each soil type. Two-tailed t tests were performed on the 2,4-D concentrations at the 95-percent level of probability.

Test Design

The number of samples and method of extraction was designed to obtain random and replicate samples for each option. Two soils were used, loam and sand, with two locations within each soil, top and bottom. Ten large soil cores were extracted from each soil type. The analysis was performed with two soil samples from both locations of each soil core. A total of 80 soil samples were used for the soil analysis of each test.

CHAPTER IV

RESULTS AND DISCUSSION

Comparison of Immuno Assay and HPLC

Forty sand samples were chosen to compare HPLC and RaPID Assay (Table 3). Their concentrations were within the limits of the HPLC method, but required dilution into the detection range of the RaPID Assay. A positive correlation, $r = 0.821$, exist between 2,4-D concentrations determined by the two methods. Figure 9 presents a plot of HPLC vs. RaPID Assay. Prior to commercialization Ohmicron compared 2,4-D pesticide immuno-assay to HPLC methods. Ohmicron's results displayed a positive correlation, $r=0.9908$ between 2,4-D concentrations in 15 samples. The results in figure 9 do not coincide with the correlation found by Ohmicron (Corcia and Marchetti, 1992). Some factors influencing this variation are: possible motabolites in the 2,4-D AMINE product used causing false positive readings, the difference in the detection range for the ELISA kits (0.7 - 50 ppb)and HPLC (.003 - 0.07 ug/L), and the number of dilutions performed on the extractant. Some dilutions were1,000 fold.

Statistics

A total of 80 soil samples were analyzed at 7 days, 60 days and 16 month intervals. Statistical procedures performed by a Leveen's test were used to analyze

homogeneity among the experimental units. The significance level analyzed was at the 95-percent level of probability. Input parameters for the statistical analysis were soil column location (top or bottom), treatment (control, fertilize, innoculant and Superbug, fertilize and irrigate, and innoculant, irrigate, and Superbug), and soil type (sand or loam).

An analysis of variance (ANOVA) test determined how much variance existed at the top and bottom of the soil cores. Two soil samples were extracted from twenty sand and twenty loam soil cores. The analysis contained samples located about 10cm and 40 cm levels near the top and bottom of each soil sample. A total of 80 samples were input for the statistical analysis. The 2,4-D concentration in the top soils, sand and loam, were not significantly different within the treatment methods (Table 4). The depth at which the samples were taken shows a significant difference. A comparison of the data for 2,4-D concentration indicates a reduction in 2,4-D concentration for all the different methods. The bottom location for sand indicates three combinations of method with no significant difference. One of these three methods is the control. The bottom location for the loam soil indicate four combinations of methods with no significant difference. Again, one of the four methods is the control.

Comparison of Treatments

Initially 1.46 kg/cm^2 of 2,4-D was applied to each soil column. Soil analysis were measured by ELISA at different intervals as shown in Table 5. Average 2,4-D concentrations (ppm) are presented for each treatment. The 2,4-D compound was applied to the twenty soil columns July 3, 1993. Soil samples were extracted for soil analysis 7

days, 60 days, and 16 months after application. Effluent was analyzed from the bottom of the columns after 16 months of the application.

The first soil analysis in Table 5, after 7 days, showed a larger average 2,4-D concentration difference of 0.7 ppm in the top loam soil. The average 2,4-D concentration was calculated between treatment 5 and treatment 3. The largest difference in concentrations for the top sand soil was 0.2 ppm between treatment 3 and treatment 2 or treatment 4. The top and bottom locations were analyzed 60 days after applying 2,4-D. The top 2,4-D concentrations for loam and sand soils indicated a difference of 1.3 ppm between treatment 2 and treatment 5 and 0.77 ppm between treatment 1 and treatment 5. The bottom soil for loam and sand soils after 60 days showed a difference of 1.6 ppm between treatment 2 and treatment 5 and 1.4 ppm between treatment 3 and treatment 5. The top and bottom location of loam and sand soils were compared 16 months after the 2,4-D application. Top soil for loam and sand differed in 2,4-D concentration by 1.7 ppm between treatment 2 and treatment 3 or treatment 5 and .15 ppm between treatment 4 and treatment 2. Bottom location 2,4-D concentrations differed by .01 ppm between either treatment 1, treatment 3, treatment 4, or 5 and treatment 2 and .13 ppm between treatment 3 or treatment 4 and treatment 1 for loam and sand soils. Effluent, via fiber glass wicks, was extracted from the glass containers located at the bottom of the soil cores after 16 months of pesticide application. The 2,4-D concentration ranged from 0.002 ppm to 0.3 ppm. These amounts shown in Table 6 were significantly small compared the the soil analysis. Only one 2,4-D test was conducted on the effluent. Due to insufficient data the effluent values were eliminated for this study.

Comparing the two soil types, loam and sand, there is less difference in the sand 2,4-D concentrations than loam 2,4-D concentrations among the five treatment methods. This comparison is graphically demonstrated in Figure 10 for the top loam and sand soil for each test. The sand soil columns had less 2,4-D present than loam for each test. All treatments were within 2 ppm of 2,4-D remaining in the soil. Figure 11 demonstrates this comparison for the bottom location of the soil column 60 days and 16 months after 2,4-D apllication. After 60 days, treatment 5 contained less 2,4-D than the other treatments, however after 16 months there was no significant difference in treatments or soil type. Lower cohesion forces of sand may contribute to this difference. The smaller 2,4-D concentration difference indicates less variability between treatment methods.

The percent of 2,4-D remaining in the top and bottom of the soil columns is shown in Table 7a and Table 7b. The comparisons are for the time interval of 60 days and 16 months following the 2,4-D application. Computation for soil mass was based on the assumption of soil density for sand and loam of 1.6 kg/l. Based on the volume of the soil columns, 1.46 mg/cm2 of 2,4-D was applied to each soil core. Comparison of data among treatments for the top location of loam and sand soil reveals a minimum 3.3% and 3.2% of the initial 2,4-D remained in the columns after 60 days. The bottom location of loam and sand soil showed a minimum of 5.7% and 3.2% after 60 days of the 2,4-D application. The top location of loam and sand soil showed a minimum of .5% and 1.3% of initial 2,4-D remaining in the columns after 16 months. The bottom location of the loam and sand soil showed a minimum of 006% and 0.05% of initial 2,4-D remaining in the soil columns after 16 months.

Alternative treatment 5 (innoculant/irrigate/Superbug) showed a higher percent reduction of initial 2,4-D concentration for the first 60 days in loam and sand soil. After 16 months of the 2,4-D application, the bottom location for both loam and sand soils contained considerably less 2,4-D concentration in treatment 1 (control). The top location after the 16 month period contained minimum values in more than one treatment method. Thus, the relationship between treatment methods and pesticide degradation depends on the amount of time the pesticide is in the soil and the soil type.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

Collecting large intact soil columns combined with the proper experimental setup was a feasibnle method to evaluate biodegradation of pesticides. The method is an inexpensive way to collect useful undisturbed soil columns for many soil hydraulic and biodegradation studies. Another positive aspect about the collection device is the repeatability and insertion rate. Ten PVC cylinders were inserted per day. The average insertion rate was approximately 5 cm/min.

Soil columns treated with 2,4-D to represent pesticides spills were subjected to five simple treatments. 2,4-D concentration values from treatment 5 were significantly lower after 7-days of pesticide application. However, 2,4-D concentrations after 16-months denote insignificant differences between treatments. Previous studies have shown an initial increase in degradation rate when microbes are introduced to a soil substrate. The rate eventually will decrease due to the slow adaptability of the microbes. This process may have ocurred here.

Recommendations

Soil samples were taken from the large cores and analyzed to determine the 2,4-D concentration. Obtaining the samples vertically may have influenced or affected transport of some of the pesticide from the top soil to the bottom soil

ELISA test were used to obtain reliable and quick 2,4-D concentration readings. The assay procedure is fast, but results are not as precise as using chromatography. Analyzing a smaller quantity of samples by Elisha and a larger quantity by chromatography will enhance the reliability of the assay results.

Testing a more stable chemical other than 2,4-D would enable a more complete evaluation of adverse affects pertaining to temperature and evaporation.

The test procedure is suitable to test other pesticide biodegradation patterns and the ecological effects of these chemicals. Further research is needed to incorporate important microbial parameters, such as burial depth, temperature, and herbicide mobility, to degradation of 2,4-D under field conditions.

Table 1

Soil properties for Pulaski and Easpur soil

Properties	Pulaski	Easpur	
Texture	Sandy Loam	Loam	Clay Loam
Depth (cm)	0-94*	0-28	28-94*
Moist bulk density (g/cc)	1.3-1.7	1.3-1.55	1.4-1.70
Organic matter content (%) Surface layer	.5-1	1-3	---
Surface layer reaction (pH)	5.6-7.3	5.6-8.4	6.1-8.4
Available water capacity (in/in)	0.11-0.20	0.15-0.24	0.11-0.20
Permeability (in/hr)	2.0-6.0	0.6-2.0	0.6-2.0
Clay (%)	10-18	12-26	18-35

* Average depth of the horizon
(USDA-SCS, 1987)

Table 2

Particle Size Analysis

USDA Classification System:

	Sand Top	Sand Bottom	Loam Top	Loam Bottom
% sand (0.05 - 2 mm)	54.09983	51.83273	22.37154	34.0631
% silt (0.002 - 0.05 mm)	32.25875	31.99814	59.8768	50.42094
% clay (< 0.002 mm)	13.64143	16.16913	17.75166	15.51596
% total fine material	100	100	100	100
Classification	Sandy Loam	Sandy Loam	Silt Loam	Silt Loam

Table 3

HPLC and ELISA Assay 2, 4-D Analysis (ppm)

Treatment	Soil	Top Soil Sample No.	ELISA Assay 11-04-94	HPLC 6-15-95	Bottom Soil Sample No.	ELISA Assay 11-04-94	HPLC 6-15-95
1	sand	1	.42	.10	21	.01	.21
1	sand	2	.11	.00	22	--	.00
2	sand	3	.18	.00	23	.10	.06
2	sand	4	.18	--	24	--	.00
3	sand	5	4.3	.07	25	.11	.21
3	sand	6	.30	.00	26	--	.38
4	sand	7	.62	.00	27	.09	.12
4	sand	8	.23	.18	28	--	.00
5	sand	9	.33	.00	29	.08	.00
5	sand	10	.31	.00	30	--	.00
1	sand	11	.61	.00	31	.07	.25
1	sand	12	.35	.83	32	--	.08
2	sand	13	.27	.67	33	.07	.17
2	sand	14	.34	.17	34	--	.43
3	sand	15	.22	.45	35	.17	.71
3	sand	16	.57	.97	36	--	2.2
4	sand	17	.51	.00	37	.18	4.2
4	sand	18	.20	.00	38	--	.00
5	sand	19	.32	.12	39	.15	.00
5	sand	20	.38	.00	40		.00

Alternative Treatments
1.Control
2.Fertilize
3.Inoculant/Superbug
4.Fertilize/Irrigate
5.Inoculant/Irrigate/Superbug

Table 4

Mean 2,4-D concentrations (ppm) detected as a function of soil depth and soil treatment for 2,4-D soil analysis 60 days after application

2a) Mean 2,4-D concentrations for loam type soil

Loam Soil

Top			Bottom		
Treatment	Mean concentration ppm	T-Grouping	Treatment	Mean concentration ppm	T-Grouping
2	.59	A	2	.74	A
3	.48	A	1	.69	A-B
1	.47	A	4	.57	A-B
4	.37	A	3	.47	A-B
5	.19	A	5	.30	B

Means with the same letter are not significantly different at P=.05.

2b) Mean 2,4-D concentrations for sand type soil

Sand Soil

Top			Bottom		
Treatment	Mean concentration ppm	T-Grouping	Treatment	Mean concentration ppm	T-Grouping
1	.43	A	3	.56	A
2	.34	A	2	.48	A-B
3	.31	A	1	.30	A-B-C
4	.24	A	4	.26	B-C
5	.18	A	5	.17	C

Means with the same letter are not significantly different at P=.05.

Alternative Treatments
1. Control
2. Fertilize
3. Inoculant/Superbug
4. Fertilize/Irrigate
5. Inoculant/Irrigate/Superbug

Table 5

2,4-D Average Concentrations (ppm) for Loam and Sand Soil with Alternative Treatments

	Loam Soil					Sand Soil				
Time	(1)	(2)	(3)	(4)	(5)	(1)	(2)	(3)	(4)	(5)
		Loam Top					Sand Top			
7day	3.7	3.9	3.4	3.8	4.1	3.3	3.2	3.4	3.2	3.3
60day	1.5	1.9	1.6	1.2	0.62	1.4	1.1	1.1	0.85	0.63
16mon	0.6	1.8	0.1	1.7	0.1	0.37	0.24	0.36	0.39	0.34
		Loam Bottom					Sand Bottom			
60day	2.4	2.6	1.6	2.0	1.0	1.0	1.7	2.0	0.91	0.59
16mon	0.01	0.09	0.1	0.1	0.1	0.01	0.08	0.14	0.14	0.11
		Loam Effluent					Sand Effluent			
16mon	0.002	0.009	0.003	0.06	0.05	0.004	0.01	0.23	0.22	0.08

Alternative Treatments

1.Control

2.Fertilize

3.Inoculant/Superbug

4.Fertilize/Irrigate

5.Inoculant/Irrigate/Superbug

Table 6

Effluent 2,4-D Concentrations 16 months after Pesticide Application

Sand Treatment No.	Concentration (ppm)	Loam Treatment No.	Concentration (ppm)
30	0.01	10	0.00
31	0.01	11	0.01
32	0.16	12	0.02
33	0.03	13	0.09
34	--	14	0.03
40	0.00	20	0.00
41	0.01	21	0.09
42	0.30	22	0.05
43	0.01	23	0.03
44	0.08	24	0.08

Table 7

Percent of initial 2,4-D remaining in loam and sand soil columns 60 days and 16 months after application

a) Top location in loam and sand soil type

Treatment	Loam Top		Sand Top	
	60 Days	16 Months	60 Days	16 Months
1	8.1	3.3	8.1	2.0
2	10.3	9.7	5.9	1.3
3	8.7	0.5	5.9	1.9
4	6.5	9.2	4.6	2.1
5	3.3*	0.5	3.2	1.8

b) Bottom location in loam and sand soil type

Treatment	Loam Bottom		Sand Bottom	
	60 Days	16 Months	60 Days	16 Months
1	13.1	0.06	5.5	0.05
2	14.1	0.48	9.0	0.44
3	7.4	0.69	10.6	0.78
4	10.8	0.69	4.9	0.74
5	5.7	0.71	3.2	0.62

% 2,4-D remaining was calculated using the top 30.5 cm of soil, 1500 mg of 2,4-D application, soil density of 1.6 kg/L, and the 2,4-D average concentrations from Table 5. The volume use is 50.7 L. Example: From Table 5 the the loam soil 60 day average 2,4-D concentration for treatment 5 is 0.62 ppm. The % remaining is thus:
[[0.62 mg/kg (1.6 kg/L) (50.7 L)]/1500mg] (100) = 3.3 % of initial 2,4-D remaining in soil.

Alternative Treatments

1. Control
2. Fertilize
3. Inoculant/Superbug
4. Fertilize/Irrigate
5. Inoculant/Irrigate/Superbug

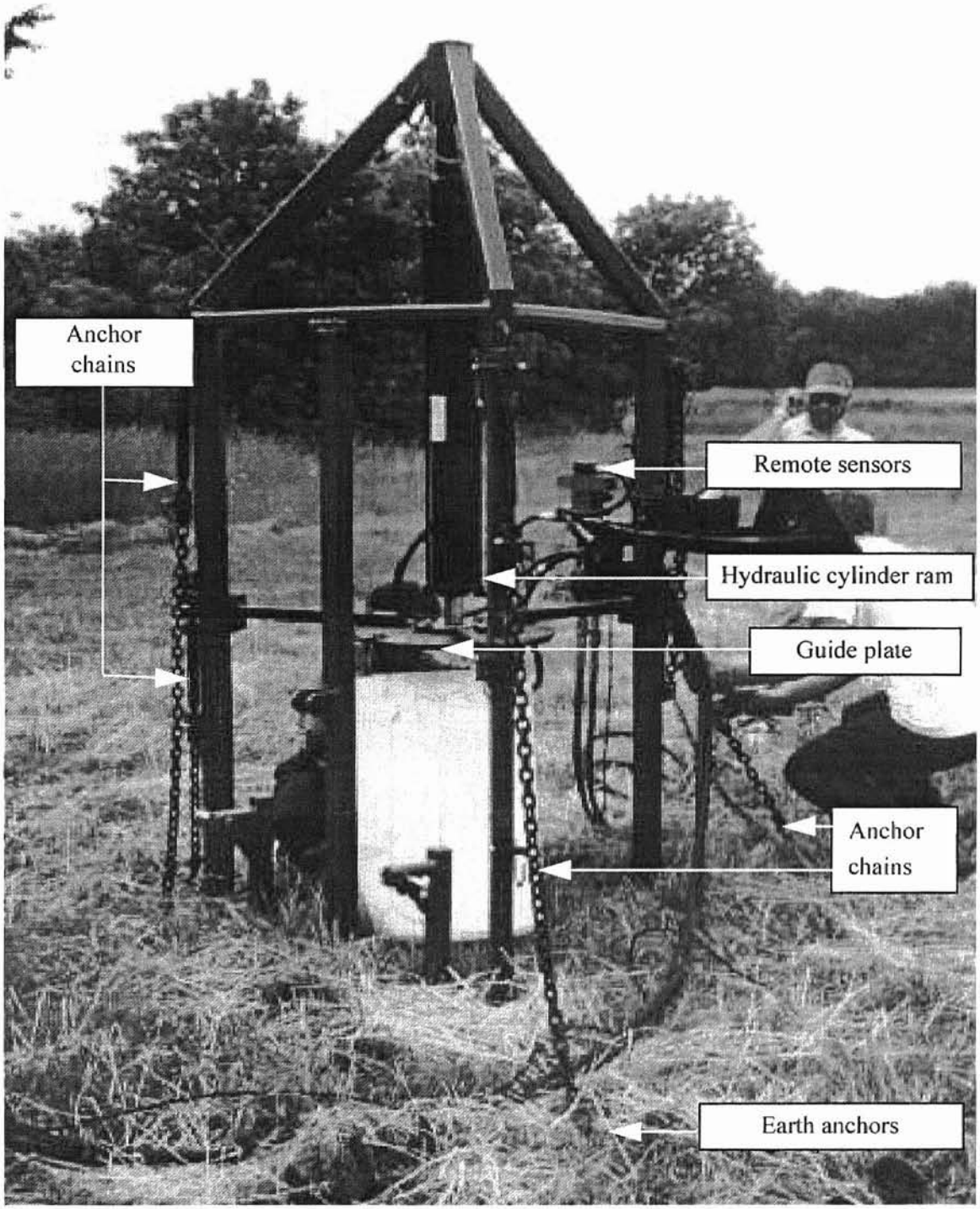


Figure 1. Free standing soil core extractor.

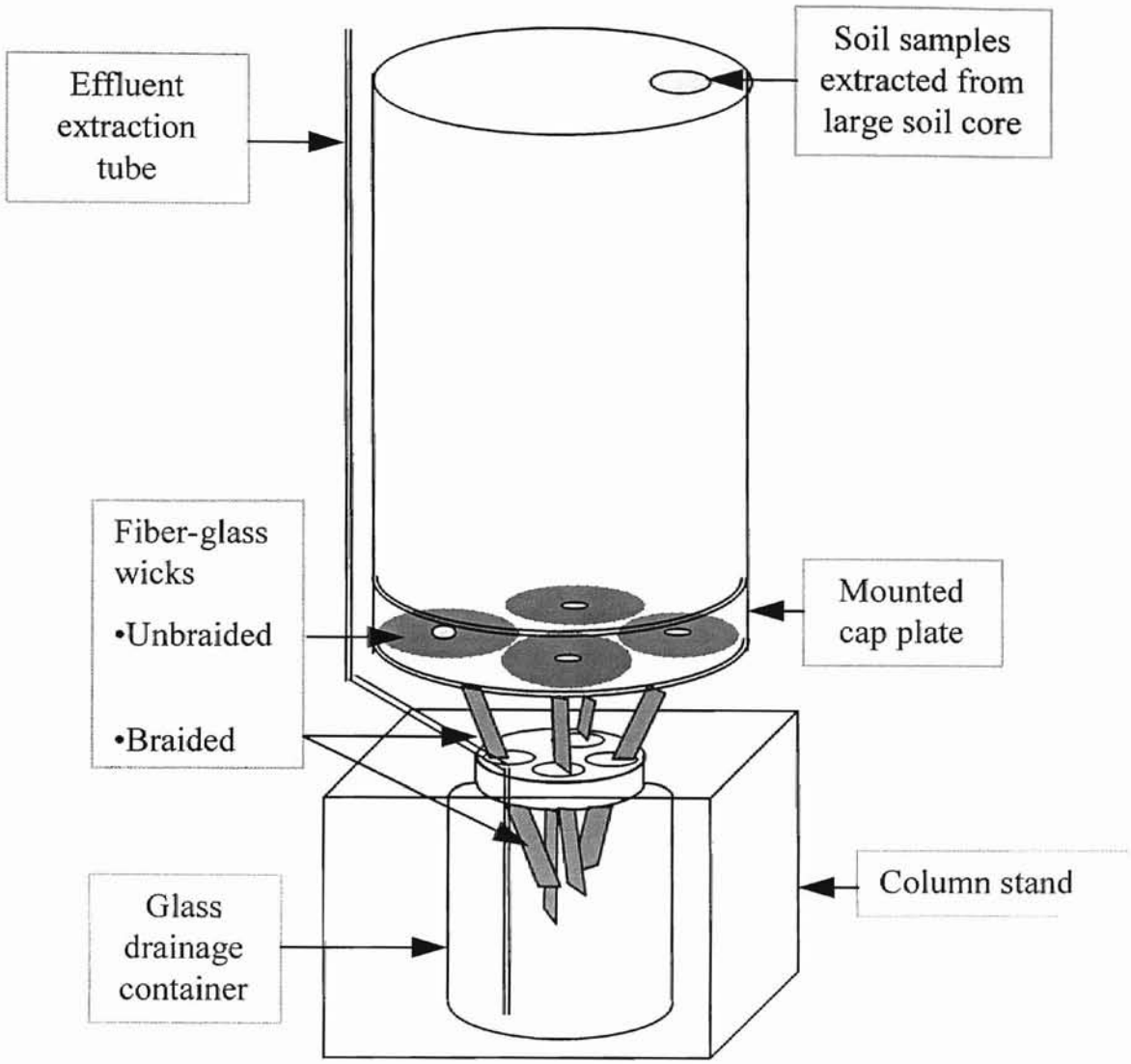


Figure 2. Schematic of soil cores on stand, effluent extraction tube and fiberglass wicks mounted through a PVC cap into a glass container.

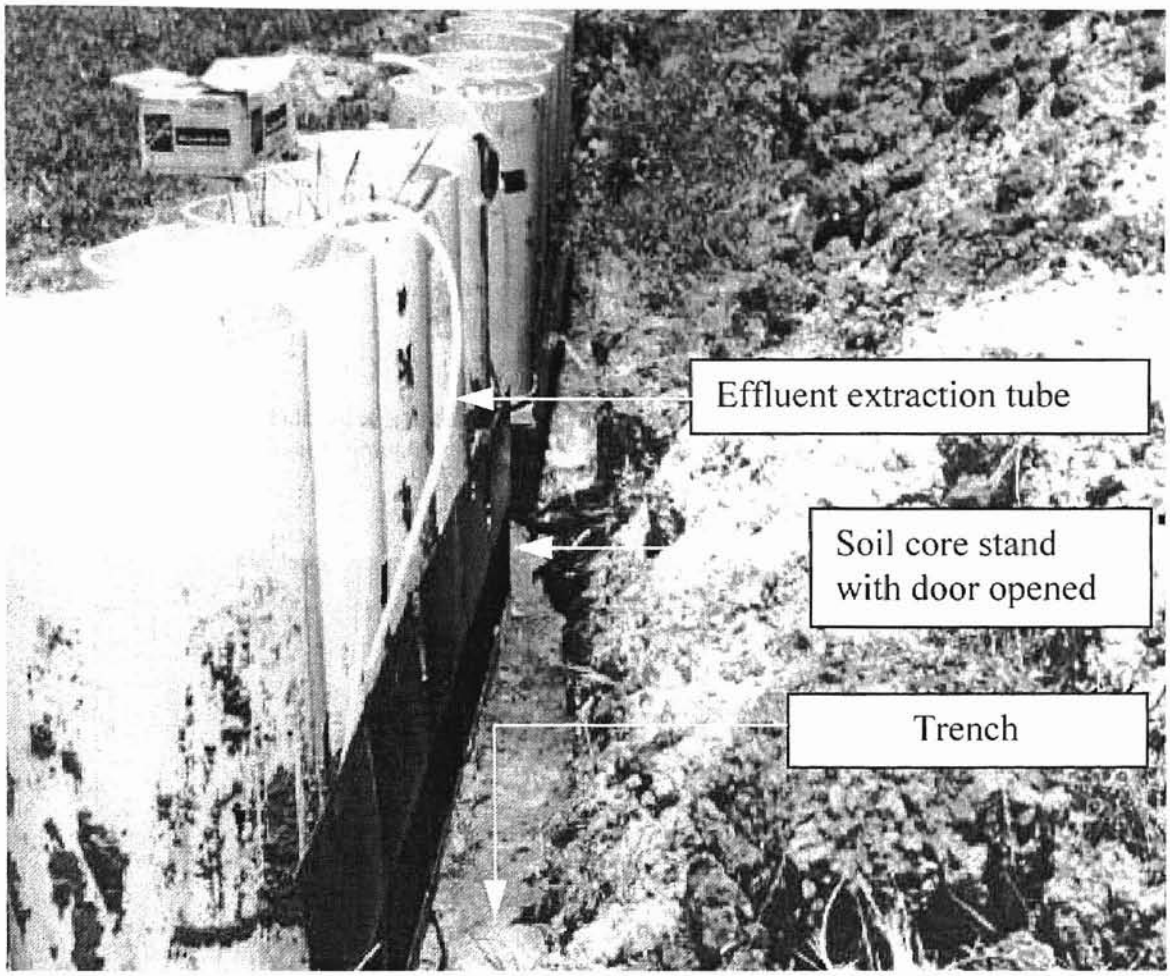
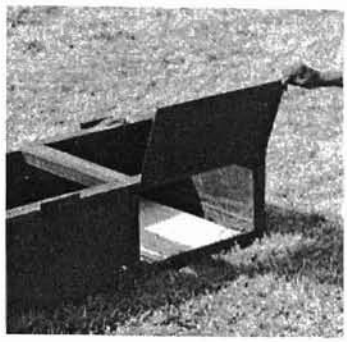
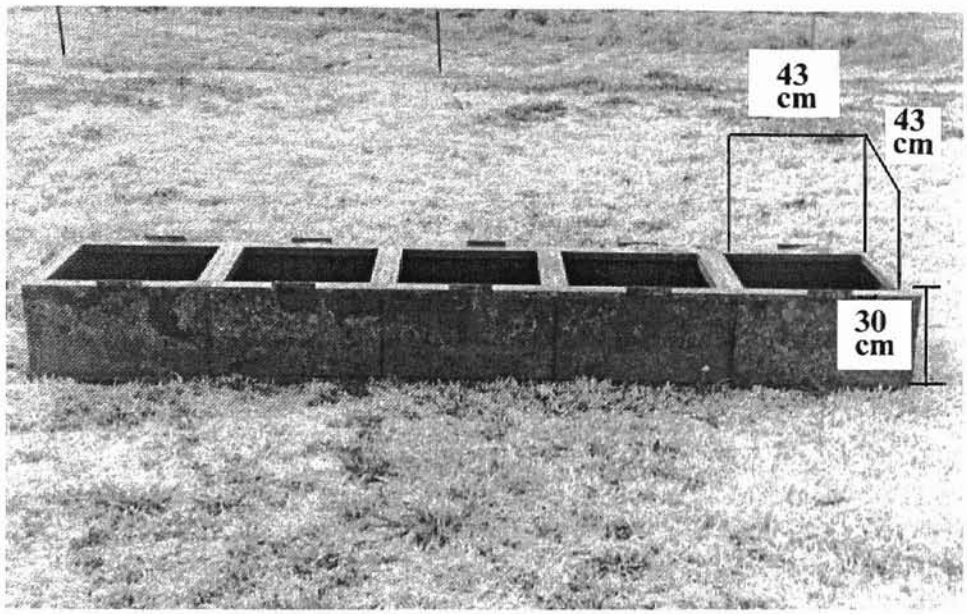


Figure 3. Ten large intact soil cores on stands positioned in trench. The excavated soil was used to bury the stand.

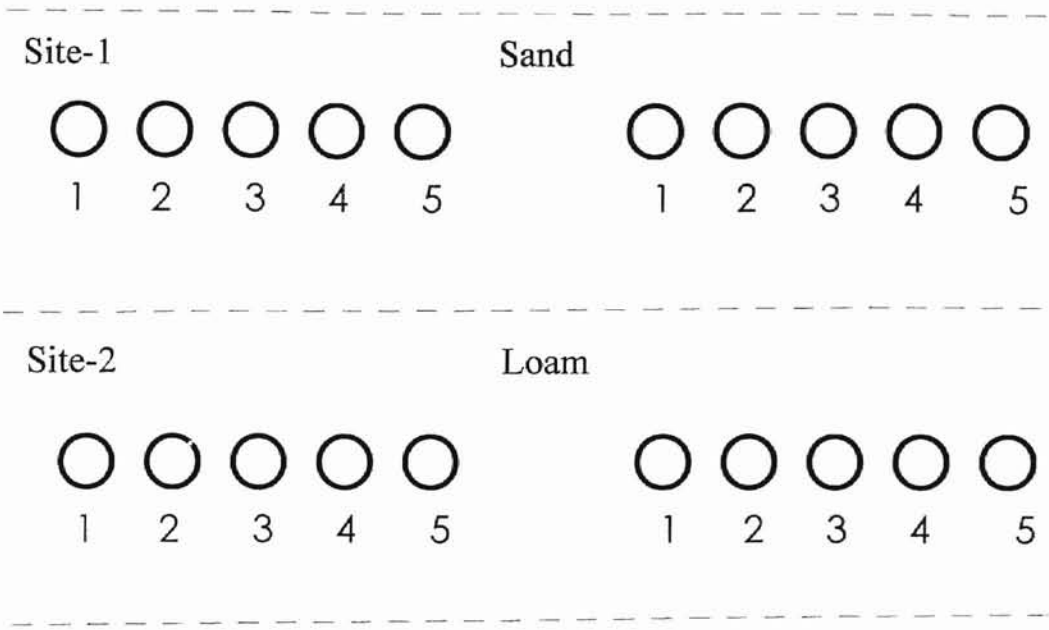


4a) The swing door provides access to the bottom of the soil core and protection to the glass container should contaminants seep through.



4b) The openings at the top, 43 cm x 43 cm, will accomodate five 46 cm diameter soil cores. The height of 30 cm provides space to assemble effluent drainage from the bottom of the cores.

Figure 4. Stand for large soil cores. The capacity for each stand is five 46 cm diameter soil cores.



Treatment Alternatives:

1.Control

2.Fertilize only

3.Fertilize and innoculate

4.Fertilize and irrigate

5.Fertilize, irrigate and innoculate

Figure 5. Sand and loam soils with 2,4-D treatment alternatives located on-site.

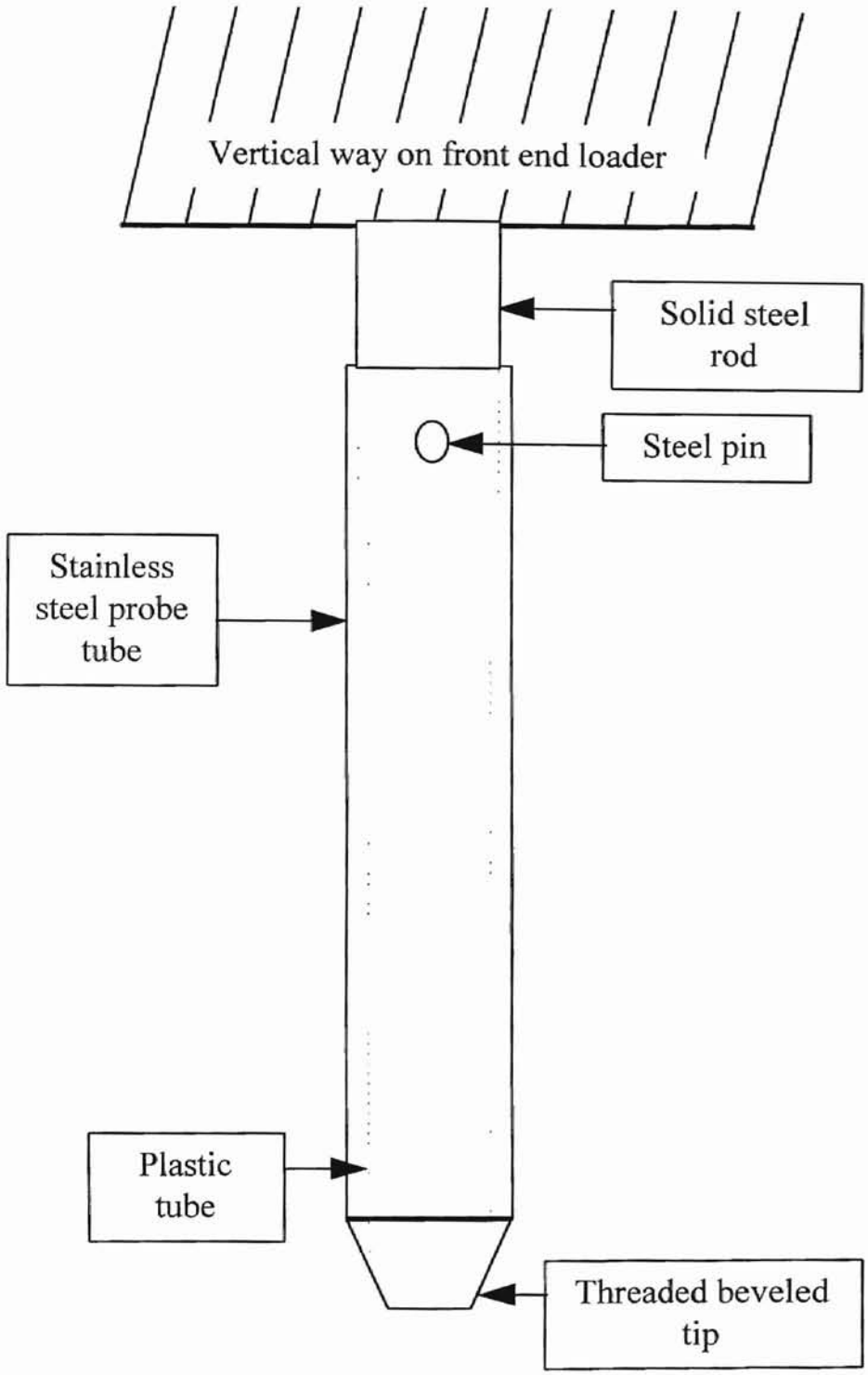


Figure 6. Small core extracter mounted to front end loader.

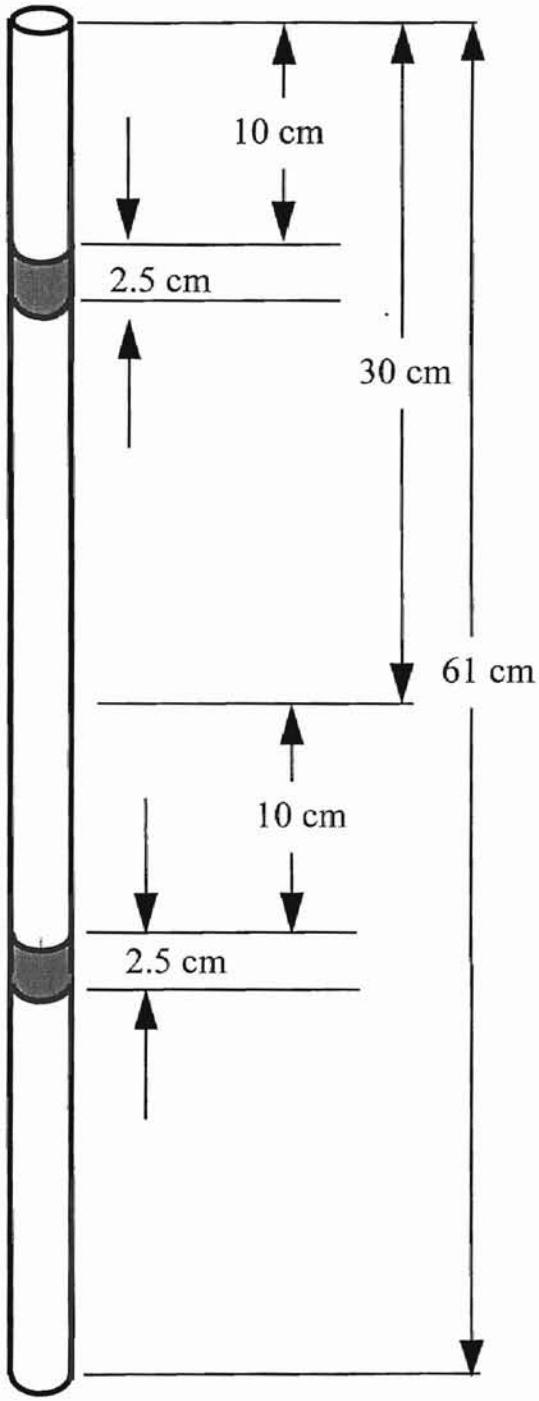


Figure 7. Vertical profile of soil samples 61 cm long x 2.5 cm diameter core.

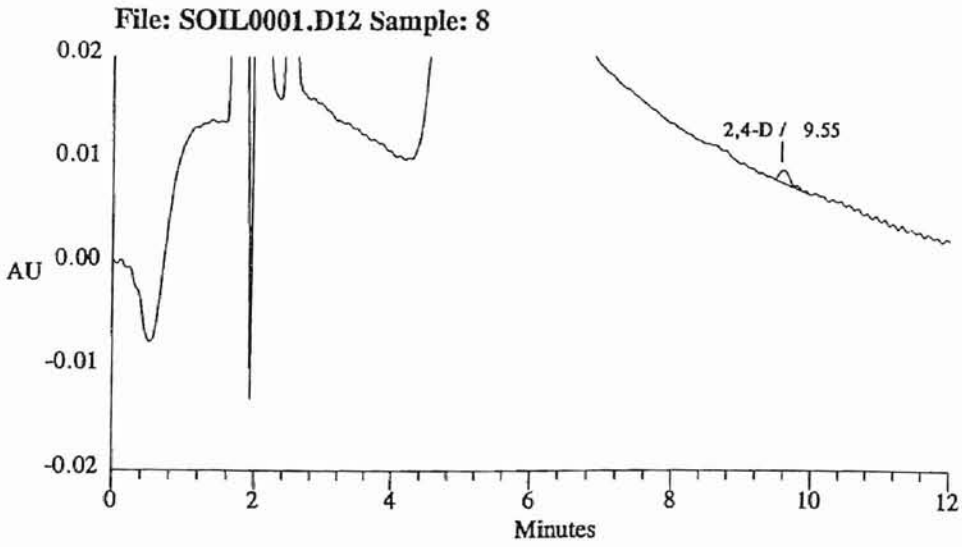


Figure 8a) Sand soil sample with HPLC results 0.178 ppm 2,4-D concentration.

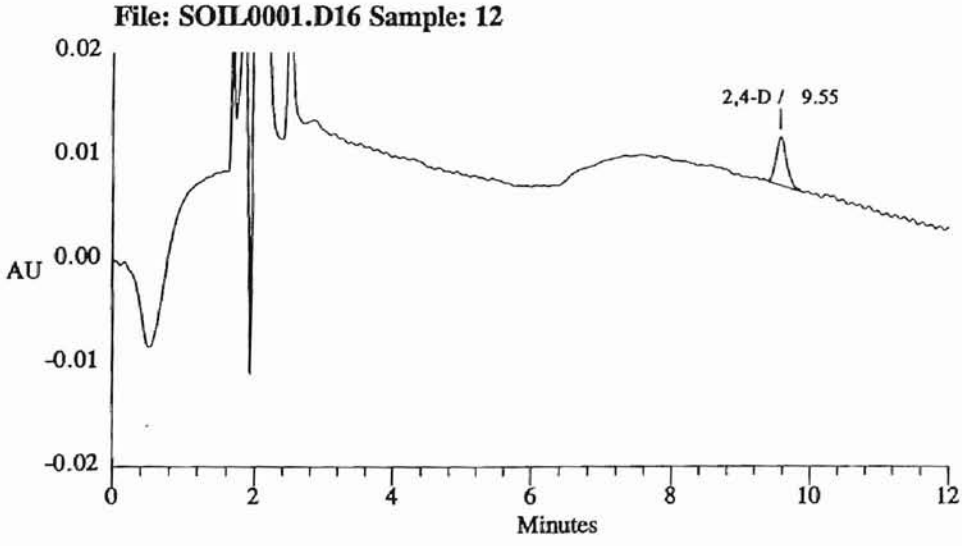


Figure 8b) Loam soil sample with HPLC results 0.831 ppm 2,4-D concentration.

Figure 8. Example of high performance liquid chromatography results.

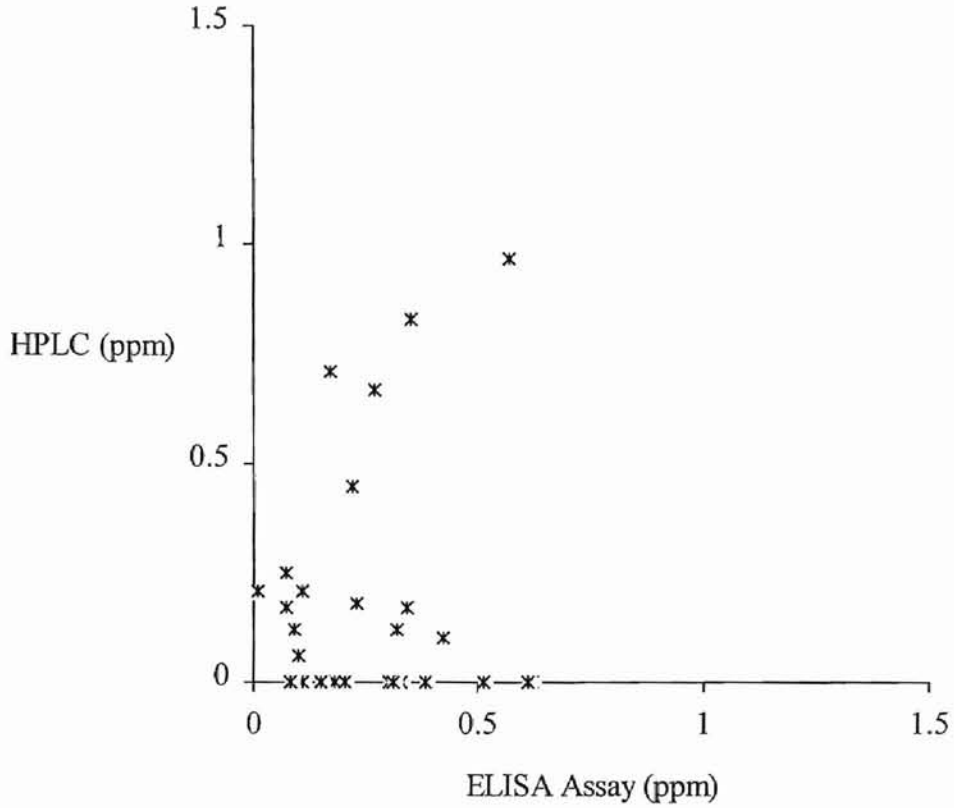
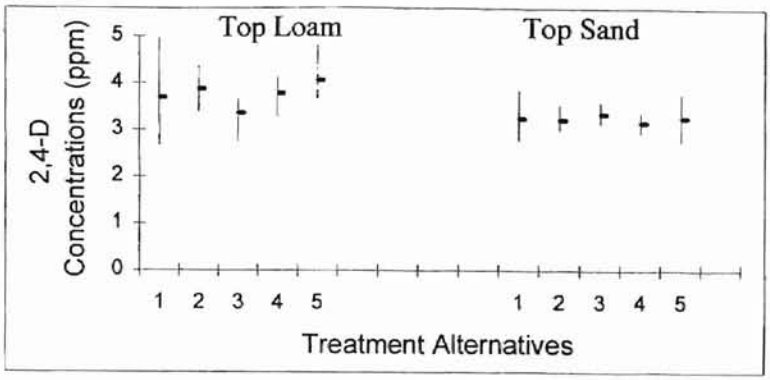
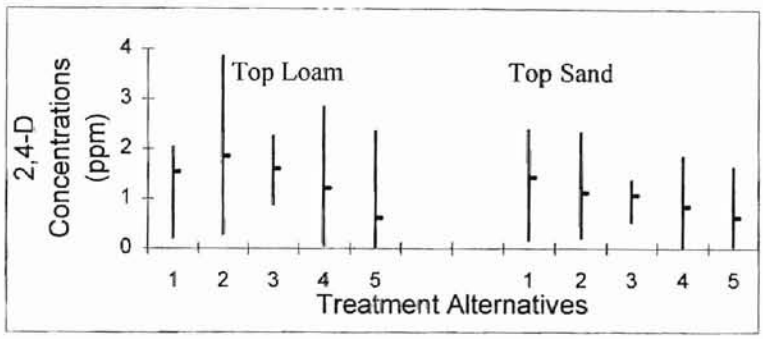


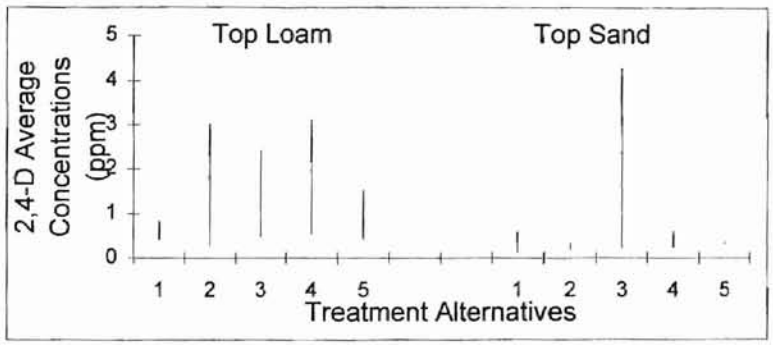
Figure 9. 2,4-D Correlation: ELISA vs HPLC.



10a) 7-days after 2,4-D application.



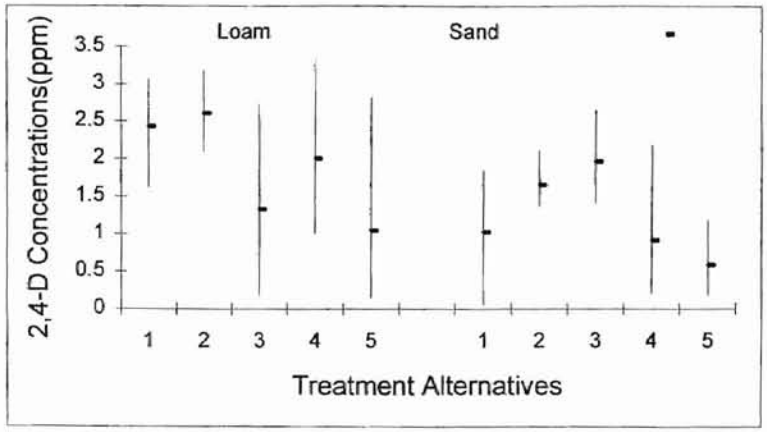
10b) 60-days after 2,4-D application.



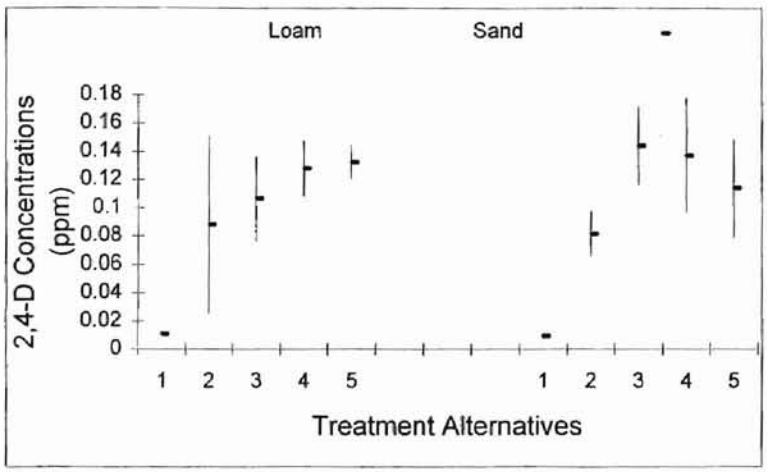
10c) 16-months after 2,4-D application.

Treatment Alternatives: 1) Control, 2) Fertilize, 3) Inoculant/Superbug, 4)Fertilize/Irrigate, 5) Inoculant/Irrigate/Superbug

Figure 10. Top sample of loam and sand soil 2,4-D average concentrations: 7-days, 60-days, and 16-months after 2,4-D application .



11a) 60-days after 2,4-D application



11b) 16-months after 2,4-D application.

Treatment Alternatives: 1) Control, 2) Fertilize, 3) Inoculant/Superbug, 4) Fertilize/Irrigate, 5) Inoculant Irrigate/Superbug

Figure 11. Bottom sample of loam and sand soil 2,4-D average concentrations: 60-days and 16-months after 2,4-D application.

BIBLIOGRAPHY

Altom, J.D., and J. H. Stritzke. 1973. Degradation of dicamba, picloram, and four phenoxy herbicides in soils. Weed Sci. 21:556-560.

Alexander, M. 1974. Microbial formation of environmental pollutants. Adv. Appl. Microbial. 18:1-73.

Anon. 1990. " Alaska's big spill; Can the wilderness survive," National Geographic, June, 177(1):5-43.

Boll, J., J. S. Selker, B. M. Nijssen, T. S. Steenhuis, J. Van Winkle, and E. Jolles. 1992. Water quality sampling under preferential flow conditions. Proceedings of American Society of Civil Engineers International Symposium on Lysimetry, Honolulu, HI.

Buchter, B. L., Leuenberger, P. J., Wierenga, and F. Richard. 1984. Preparation of large core samples from stony soils. Soil Sci. Soc. Am. J. 48:1460-1462.

Butler, G. L., T. R. Deason, and J. C. O'Kelley. 1975. The effect of Atrazine, 2,4-D, methoxychlor, carbonyl and diazinin on the growth of planktonic algae. Br. phycol. J. 10:371-376.

Chesters, G., H. B. Pionke, and T. C. Daniel. 1974. Extraction and analytical techniques for pesticides in soil, sediment, and water. In W. D. Guenzi (ed.), Pesticides in Soil and Water, pp. 451-550. Soil Sci. Soc. Am. Madison, WI, USA.

Chemical Specialties International. 1990. Don't wait 100 years to get rid of hazardous waste. Robinsona Research, Inc. Cameron Park, Ca.

Corcia, A. and M. Marchettii. 1992. Method development for monitoring pesticides in environmental waters: Liquid-Solid-Extraction followed by liquid chromatography. Environ. Sci. Technol. 26(1) 66-74.

Davidson, J. M., P. S. C. Rao, L. T. Ou, W. B. Wheeler and D. F. Rothwell. 1980. Adsorption, movement, and biological degradation of large concentrations of selected pesticides in soils. U. S. EPA report no. 600/2-80-124, 111 pp. Municipal Environmental Research Laboratory, Cincinnati, OH.

Dzantor, K. and A. Felsot. 1991. Microbial responses to large concentrations of herbicides in soil. Envir. Tox. and Chem. 10(5):649-655.

Foster, R. K. and R. B. McKercher. 1973. Laboratory incubation studies of chlorophenoxyacetic acids in Chernozenmic soils. Soil Biol. Biochem. 5:333-337.

Fournier, J. C. 1980. Enumeration of the soil micro-organisms able to degrade 2,4-D by metabolism or co-metabolism. Chemosphere 9:169-174.

Grossbard, E., and H. A. Davis. 1976. Specific microbial responses to herbicides. Weed Res. 16:163-169.

Hutton, K. J.. 1992. Large diameter soil core collection device. Presented at the 1992 International Summer Meeting, Paper No. 92-2069, Am. Soc. Ag. Eng., St. Joseph, MI.

Jones, J. G. and M. A. Edington 1980. An ecological survey of hydrocarbon-oxidizing microorganisms. J. Gen. Microbial. 52:381-390.

Kearney, P. C., and D. D. Kaufman. 1972. Microbial degradation to some chlorinated pesticides. p. 166-189. In Degradation of Synthetic Organic Molecules in the Biosphere. Natl. Acad. of Sci., Washington, D. C.

King, B. R., G. M. Long, and J. K. Sheldon. 1992. Practical Environmental Bioremediation. Lewis Publishers. Boca Raton, Florida.

Kluitenberg, G. J., and R. Horton. 1990. Effect of solute application method on preferential transport of solutes in soil. Geoderma 46:283-297.

Lesikar, B., M. Hirschi, A. Felsot, and J. Mitchell. 1990. Evaluation of concentration effects on transport and degradation of pesticides in soil. The American Society of Agricultural Engineers, International Winter Meeting "90". Presented at the American Society of Agricultural Engineers Conference, December 18-21. Paper No. 90-2552. Chicago, Ill.

Lewis, D. L., R. E. Hodson, and L. F. Freeman. 1984. Effects of microbial community interactions on transformation rates of xenobiotic chemicals. Appl. Environ. Macrobiol 48:561-565.

Li-Tse, O. 1984. 2,4-D degradation and 2,4-D degrading microorganisms in soils. Soil Sci. 137:100-107.

Loos, M. A. 1969. Phenoxyalkanoic acids. p. 1-49. In P. C. Kearney and D. D. Kaufman (eds.) Herbicides, Vol. 1. Marcel Dekker, New York.

Malanchuk, J. L. 1980. Microcosm evaluation of the agricultural potential of fly-ash-amended soils. In Microcosms in ecological research. Ed. J. Giesy. US DOE CONIF-781101, pp. 1034-1049, NTUS, U. S. Dept. Comm., Springfield, Va.

Mass, R. P. 1989. USDA's rural clean water program (RCWP) has been called the " cutting edge" of nonpoint source(NPS) pollution control. Water Newsletter, Water Information Center, 31(9).

Murphy, J. B., E. H. Grissinger, and W. C. Little. 1981. Fiberglass encasement of large, undisturbed, weakly cohesive soil samples. Soil Sci. 131:130-134.

Nelson, C. 1993. A natural cleanup. Civil Engineering 63(3) March.

Norris, L. A. 1966. Degradation of 2,4-D and 2,4-T in forest litter. J. For. 64:475-462.

Noyes, Ronald T. 1996. Personal Communication.

O'Connor, G. A., B. C. Fairbanks, and E. A. Doyle. 1981. Effects of sewage sludge on phoxy herbicide adsorption and degradation in soils. J. Environ. Qual. 10:510-515.

Ohmicron. 1992. Start-up Manual for RaPID Assay™ System. Part No. A00102, Newtown, Pa. Rev. 6/92.

Ou, L.T., D. F. Rothwell, W.B. Wheeler and J.M. Davidson. 1978. The effect of high 2,4-D concentrations on degradation and carbon dioxide evolution in soils. J. Environ. Qual. 7:241-246.

Rao, P. S. C. and J. M. Davidson. 1979. Adsorption and movement of selected pesticide at high concentrations in soils. Water research 13(4):375-380.

SAS Institute Inc. 1984. SAS/ETS™ User's Guide, Version 5 Edition. Cary, N.C.: SAS Institute Inc., 1984. 738 pp.

Sattar, M. A. and J. Paasivirta. 1980. Fate of chlorophenoxyacetic acids in acid soil. Chemosphere. 9:745-752.

Stojanovic, B. J., M. V. Kennedy, and H. L. Shuman, Jr. 1972. Edaphic aspects of the disposal of unused pesticide wastes, and pesticide containers. J. Environ. Qual. 1:54-62.

USDA-SCS. 1987. Soil Survey of Payne County, Oklahoma. April, 1987.

WHO. 1989. Environmental Health Criteria. 29: 2,4-dichlorophenoxyacetic acid (2,4-D), Geneva, World Health Organization, 151 pp.

Wolf, S. H., M. A. Cella, and K. M. Hess. 1992. Evaluation of hydraulic conductivities calculated on multiport-permeameter measurements. Ground Water 29(4):516-524.

Zobell, C. E. 1958. Ecology of sulfate reducing bacteria. Producers Monthly 22:12-39.

Zwick, T. C., M. F Arthur,. D. A. Tolle,. and P. Van Vorris. 1984. A unique laboratory method for evaluating agro-ecosystem effects of an industrial waste product. Plant and Soil 77:395-399.

APPENDIX

APPENDIX I

Raw Data
2, 4-D Concentration (ppm) from RaPID Assay Test

Date	Location in Column	Treatment	Soil Type	*Column ID	2,4,D Concentration ppm
7/13/93	Top	1	Loam	10A	3.097
				10B	4.973
				20A	2.672
				20B	4.058
			Sand	30A	2.770
				30B	3.282
				40A	3.862
				40B	3.117
		2	Loam	11A	3.704
				11B	4.059
				21A	4.367
				21B	3.392
			Sand	31A	3.304
				31B	3.039
				41A	3.001
				41B	3.555
		3	Loam	12A	2.737
				12B	3.678
				22A	3.415
				22B	3.628
			Sand	32A	3.137
				32B	3.604
				42A	3.531
				42B	3.137
		4	Loam	13A	4.148
				13B	4.030
				23A	3.282
				23B	3.678
			Sand	33A	2.982
				33B	3.370
				43A	2.928
				43B	3.370
		5	Loam	14A	3.704
				14B	---
				24A	4.820
				24B	3.653
			Sand	34A	2.770
				34B	3.438
				44A	3.078
				44B	3.782

APPENDIX I (CONT'D)

Raw Data
2, 4-D Concentration (ppm) from RaPID Assay Test

Date	Location in Column	Treatment	Soil Type	*Column ID	2,4,D Concentration ppm
9/07/93	Top	1	Loam	10A	.194
				10B	2.740
				20A	1.174
				20B	2.049
			Sand	30A	1.070
				30B	2.409
				40A	2.096
				40B	.147
		2	Loam	11A	.769
				11B	.273
				21A	3.868
				21B	2.510
			Sand	31A	.199
				31B	.348
				41A	2.355
				41B	1.581
		3	Loam	12A	.865
				12B	1.053
				22A	2.270
				22B	2.226
			Sand	33A	1.355
				33B	.516
				43A	1.037
				43B	1.392
		4	Loam	13A	2.861
				13B	1.515
				23A	.423
				23B	.041
			Sand	33A	.957
				33B	.531
				43A	1.877
				43B	.024
		5	Loam	14A	.033
				14B	.026
				24A	.035
				24B	2.375
			Sand	34A	1.663
				34B	.720
				44A	.083
				44B	.042

APPENDIX I (CONT'D)

Raw Data
2, 4-D Concentration (ppm) from RaPID Assay Test

Date	Location in Column	Treatment	Soil Type	*Column ID	2,4,D Concentration ppm
9/07/93	Bottom	1	Loam	10A	1.620
				10B	3.075
				20A	2.375
				20B	2.655
			Sand	30A	1.305
				30B	.906
				40A	1.847
				40B	.047
		2	Loam	11A	3.182
				11B	2.688
				21A	2.082
				21B	2.492
			Sand	31A	1.752
				31B	1.408
				41A	1.361
				41B	2.117
		3	Loam	12A	2.410
				12B	1.229
				22A	.168
				22B	2.739
			Sand	32A	2.262
				32B	2.655
				42A	1.545
				42B	1.408
		4	Loam	13A	3.331
				13B	2.375
				23A	1.007
				23B	1.305
			Sand	33A	.760
				33B	.507
				43A	.201
				43B	2.188
		5	Loam	14A	2.824
				14B	.635
				24A	.148
				24B	.573
			Sand	34A	.251
				34B	.180
				44A	.727
				44B	1.183

APPENDIX I (CONT'D)

Raw Data
2, 4-D Concentration (ppm) from RaPID Assay Test

Date	Location in Column	Treatment	Soil Type	*Column ID	2,4,D Concentration ppm
11/20/94	Top	1	Loam	10A	.749
				10B	.840
				20A	.547
				20B	.403
			Sand	30A	.419
				30B	.108
				40A	.605
				40B	.353
		2	Loam	11A	.251
				11B	.736
				21A	2.646
				21B	3.040
			Sand	31A	.180
				31B	.182
				41A	.269
				41B	.341
		3	Loam	12A	1.218
				12B	2.429
				22A	.553
				22B	.475
			Sand	32A	4.296
				32B	.296
				42A	.219
				42B	.573
		4	Loam	13A	3.131
				13B	.681
				23A	.527
				23B	2.378
			Sand	33A	.617
				33B	.227
				43A	.506
				43B	.197
		5	Loam	14A	1.426
				14B	1.551
				24A	.547
				24B	.411
			Sand	34A	.334
				34B	.306
				44A	.323
				44B	.380

APPENDIX I (CONT'D)

Raw Data
2, 4-D Concentration (ppm) from RaPID Assay Test

Date	Location in Column	Treatment	Soil Type	*Column ID	2,4,D Concentration ppm
11/20/94	Bottom	1	Loam	10	.013
				20	.009
			Sand	30	.012
				40	.007
		2	Loam	11	.025
				21	.151
			Sand	31	.098
				41	.065
		3	Loam	12	.076
				22	.137
			Sand	32	.116
				42	.172
		4	Loam	13	.148
				23	.108
			Sand	33	.096
				43	.178
		5	Loam	14	.120
				24	.145
			Sand	34	.079
				44	.149

APPENDIX II

Statistical Analysis of Variance for Treatment

Class	Degrees of Freedom	P-Value	Error	Total
Bottom Loam	4	0.1877	15	19
Bottom Sand	4	0.0459	15	19
Top Loam	4	0.6986	15	19
Top Sand	4	0.7122	15	19

Statistical Analysis of Variance for Top Location

ANOV	D.F.	P-Value
Treatment	4	0.4619
Soil	1	0.2710
Trt*Soil	4	0.9511
Model	9	0.7642
Error	30	
Total	39	

Statistical Analysis of Variance for Bottom Location

ANOV	D.F.	P-Value
Treatment	4	0.0338
Soil	1	0.0096
Trt*Soil	4	0.2577
Model	9	0.0161
Error	30	
Total	39	

Vita

LaDonna McCowan

Candidate for the Degree of

Master of Science

Thesis: METHOD TO EVALUATE IN-SITU TREATMENT OF LARGE PESTICIDE CONCENTRATIONS IN SOIL

Major Field: Biosystems Engineering

Biographical:

Personal Data: Born in Boley, Oklahoma, on March 5, 1955, the daughter of L. D. and Genevee Moore.

Education: Graduated from Bristow High School in Bristow, Oklahoma, May 1973. Received Bachelor of Science degree in Agricultural Engineering from Oklahoma State University, Stillwater, Oklahoma. Completed the requirements for the Master of Science degree with a major in Biosystem Engineering at Oklahoma State University in May 1997.

Experience: Employed with John Deere Tractor Works as Manufacturing Engineer from May 1977 to December 1980. Employed with United States Corps of Engineers as a Hydrologic Engineer from January 1981 to August 1986. Employed with Armstrong World Industries as a Production Coordinator from February 1988 to August 1991.

Professional Memberships: American Society of Agricultural Engineers.