POULTRY LITTER AS A NITROGEN SOURCE FOR DEGRADATION OF HYDROCARBONS IN CONTAMINATED SOILS

By

RICK KOCHENOWER

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1996

Submitted to the Faculty of the
Graduate College of
Oklahoma State University
In partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1998

OKLAHOMA STATE UNIVERSITY

POULTRY LITTER AS A NITROGEN SOURCE FOR

DEGRADATION OF HYDROCARBONS IN

CONTAMINATED SOILS

Thesis Approved:

Jeffrey A. Hattey
Thesis Adviser

Brian J. Carter

Neil Sarta

Doug Hamilt—

Wayne B. Powell
Dean of the Graduate College

ACKNOWLEDGMENTS

I am very grateful to Dr. Jeff Hattey my major advisor for his patience, leadership, guidance, and the opportunity to work on this project. Doc I could not have done it with out you. Also my other committee members, Drs. Nick Basta, Brian Carter, and Doug Hamilton for their suggestions and help when needed the most. I appreciate Oklahoma State University and the Department of Plant and Soil Sciences for allowing me the use of their equipment, facilities, and personnel.

A very special thank you goes to Jackie Schroder and Dave Kidd, without your help I would still be here trying to figure out how the GC ran. I could never have gotten done with out your late nights when things were acting up.

To my family Mom, Dad and my brothers Jeff and Hank it would never have been possible with out your encouragement, love and support. Especially to Hank you left us way too early, and you did not get to see the end, but not a day went by you were not in my thoughts. I owe a big part of this to you.

Finally to all the graduate students I have had discussions and arguments with, that is the part I will always remember. Dale, Curt, Todd, Mark, Matt, Mick, Lance, and Renee, the exchange of ideas was wonderful and a great learning experience.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Oil exploration and production has been an important part of Oklahoma's economy since the first well was drilled in 1889. Spills of crude oil during the drilling, production, transportation, storage, and refining processes have occurred. The Natural Resource Conservation Service classifies these sites as oil-waste land with approximately 4812 ha found in Oklahoma. Petroleum hydrocarbons are low in nutrients therefore, nutrient additions are required for remediation of contaminated spill sites by the indigenous microbial population. Poultry litter, being rich in nutrients, could supply the required nutrients for remediation of contaminated spill sites. Crude oil spill sites were located and sampled in Logan and Creek counties of Oklahoma. The Creek county site has PH's concentration ranging from 9640 to 13,400 mg kg^{-1} soil and 36,500 to 43,300 for the Logan county site. The objective of this research was to determine and compare percent degradation and degradation rates of hydrocarbons in soils with long term contamination when treated with selected N sources. Composted poultry litter was the best N source for percent degradation and degradation rate for both sites at day 30 possibly due to an increased microbial population associated with litter additions. When N is not limiting as in the Creek county site both composted and fresh poultry litter were better N source at last sampling date. When N is limiting as in the Logan county site after day 60 of incubation no differences observed between N sources. The optimum C:N ratio for remediation of PH's in soil is between 32:1 and 122:1. Nitrification was inhibited by short-chained hydrocarbons, salt content of contaminated areas, or a possible reduction in ammonium oxidizer population.

INTRODUCTION

Mapping

Oil exploration and production has been an important part of Oklahoma's economy since the first well was drilled in 1889 (Owen, 1975). Until the 1940's production was not closely regulated or environmentally sound. This resulted in spills of crude oil during the drilling, production, transportation, storage, and refining processes. Crude oil spills are devastating to the soil ecosystems, destroying plants and reducing microbial activity. Loss of vegetative cover and biological activity makes remediation and revegetation of these sites difficult. Natural Resource Conservation Service (NRCS) classifies sites as oil-waste land (with approximately 4812 ha found in Oklahoma). The largest amount is found in Osage county with 788 ha (Table 1). These sites can be found in many locations throughout Oklahoma ranging from an area of a few square meters to many hectares. The total hectares classified by NRCS is an under estimation of actual contaminated area, since minimal mapping unit is approximately 2 ha. When NRCS digitized soil surveys to raster data sets, additional sites were lost as the resolution was increased to 4 ha (Table 1). One site in this study is not mapped as oil-waste land.

Plants

The effect of crude oil on soil has been studied for many years; early research examined its effect on germination and yield of crops. Murphy (1929) studied the effect of crude oil on germination of wheat. Murphy's research showed that as little as 4,675 L ha^{-1} crude delayed germination and a spill of 46,750 L ha^{-1} reduced germination by 77 %. The effect of crude oil on germination and yield was studied further by Udo and Fayemi, (1975) who found that crude concentrations at 4.2% (wt/wt) of soil reduced germination

by 50% and yield by up to 92%. Reduction of germination was attributed to oil entering seed and destroying the embryo. After stand establishment, yield reductions were not due to toxicity of crude oil, but decreased uptake of water and nutrients (Udo and Fayemi, 1975). Interference is most likely due to salt content of brine increasing soil osmotic potential and lowering amount of plant available water. Brine is a part of the production process and generally is found with crude oil spills. Most brine is removed at the well site by separators and stored in separate tanks, but is not completely removed from crude oil until the refining process. Thus spill sites are a combination of crude oil and brine.

Remediation

At spill sites there are two sources of contamination to be remediated, brine and crude oil which is the focus of this study. This study was to enhance degradation of oil in the soil by the indigenous microbial population. Over 100 species of indigenous microbes representing 31 genera have been identified as capable of oxidizing petroleum hydrocarbons (PH's) and incorporating intermediates into their biomass (Ellis and Adams, 1961; Dobson and Wilson, 1964). Overcash and Pal (1979) identified four major products of hydrocarbon degradation by soil microbes as CO_2, H_2O, a variety of end products (including soil organic matter), and microbial biomass. Bacterial counts of a soil prior to saturation with oil were 8.1 million g^{-1} soil, but after 2 years of oil saturation counts increase to 110 million g^{-1} soil in uncultivated soil (Ellis and Adams, 1961). The indigenous microbial population is capable of degrading hydrocarbons, but growth can be limited by environmental factors.

Environmental Factors

Four primary factors which limit microbial activity are nutrient supply (N and P), oxygen, water, and temperature. Hydrocarbons do not contain enough N or P for synthesis of microbial biomass therefore, nutrient additions are used to enhance biodegradation of hydrocarbons (Dibble and Bartha, 1979a). Amount of N required for degradation can be estimated based on C:N ratios, but at what ratio? The USEPA recommends supplemental N additions to achieve C:N ratios of 10:1-100:1 (Braddock et al., 1997). Most research has utilized inorganic N sources to lower the C:N (Dibble and Bartha, 1979b; Loynachan, 1978; Wrenn et al., 1994). Dibble and Bartha (1979b) reported at C:N of 15:1 no difference observed in CO_2 evolution between control and added N, while ratios of 300:1 and 60:1 had the highest CO_2 evolution. They concluded that a C:N ratio of 60:1 was optimum for hydrocarbon degradation.

Results of P additions on degradation rate have been mixed on whether an increase occurs. Dibble and Bartha (1979b) reported an increase in percent degradation when C:P ratio is 800:1. Other studies have not shown an increase in degradation rate with P additions. Toccalino et al. (1993) observed no increase in degradation of propane in soil with additions of P and trace elements. Crude oil degradation was not enhanced by additions of P or K at any rate even with N additions (Huntjens et al., 1985). In an experiment of a crude spilled on an Alaska soil, additions of N and P reduced half-life of oil in soil by 40 % (Loynachan, 1978). With inconclusive results from P studies only N additions were compared in this study. These nutrient additions increase mineralization of PH's, thus an increase in oxygen (O_2) demand.

Oxygen is difficult to supply to microbes in the soil, diffusion typically cannot supply enough for microbes when an easily degradable C source is available. The importance of O_2 in degradation of PH's is microbial requirement for an electron acceptor during the mineralization process. Tillage of contaminated soil is the most common form of aeration and increasing O_2 supply during remediation of contaminated sites. When an oil saturated soil is cultivated, degradation is accelerated and plant life is supported (Plice, 1948). Plice (1948) saturated a soil to a depth of 1.2m with crude oil to simulate a pipeline break. Plots were split in half where one was cultivated and the other uncultivated. Cultivated soil was revegetated within 5 years while uncultivated soil still had no vegetation. Mitchell et al. (1979) found tillage combined with proper fertilization reduced half-life of oil in soil from 20-30 to 1 year in a wet Alberta Canada soil. A respiration study measuring O_2 consumption conducted on an oil soaked soil using intact cores and disturbed-mixed samples found the disturbed samples showed an increase in respiration of 1.46 to 2.46 mL hr^{-1} (Dobson and Wilson, 1964). They also reported that microbes (bacteria, fungi, and actinomycetes) capable of oxidizing hydrocarbons were widely distributed in nature. These organisms are found in soil around producing oil fields, to garden soils found in many backyards.

Microbial populations require water during the degradation process, but not a specific level. Dibble and Bartha (1979b) stated that aerobic degradation of simple or complex organic material in soil is commonly greatest at 50 to 70% of water holding capacity. They reported no change in CO_2 evolution on soils contaminated with oil sludge at a range of 30 to 90% of water holding capacity. Marshall (1988) reported the optimum gravimetric moisture content at 14 to 15%. Moisture content could have an effect on soil

temperature, where temperature of a dry soils could be to high for optimum microbial activity.

Temperature affects hydrocarbon degradation both physically and chemically. Biodegradation rates increase as temperature approaches an optimum temperature range of 30-40° C (Atlas, 1991). As temperature increases, viscosity of oil decreases, exposing more surface area for microbes to attack (Atlas, 1991). Lower temperatures also decrease rates of enzyme activity due to Q_{10} effect. Kerosene degradation rates were the greatest when temperatures were > 20° C in a New Jersey wheat field contaminated by a pipeline break (Dibble and Bartha, 1979c).

Petroleum Hydrocarbons

Crude oil composition varies from one oil well to the next, composition is separated into volatiles (vp > 0.1 mm Hg, BP < 300° C) and semi-volatiles (vp 10^{-1}mm to 10^{-7}mm, BP 300-600° C) (Smith, 1994). Petroleum hydrocarbons are difficult to separate into individual compounds, Petrov (1987) identified over 700 complexes found in crude oil between the ${}_nC_1$ and ${}_nC_{40}$ chains. He also identified the main fraction of crude oil as that between ${}_nC_{11}$ and ${}_nC_{27}$ in size, which have a boiling point between 200 and 430° C. The most abundant element in crude oil is carbon (C) with 75-87%, hydrogen (H) is the other major element at 11-14%, and 2-3% can be oxygen (O), nitrogen (N), and/or sulfur (S) (Brooks et al., 1954; Deuel and Holliday, 1994). This study looked at only the semi-volatile fraction. Three primary classes of crude oil are (1) alkanes (straight, branched, and cyclic chains), (2) aromatics BTEX's (benzene, toluene, ethylbenzene, and xylene), and (3) asphaltenes which have complex ring structures that are poorly defined

(Frankenberger and Johanson, 1982). Generally ease of degradation is alkanes > aromatic > asphaltenes.

Poultry litter

In recent years poultry production has increased all across the United States. In the eastern half of Oklahoma there has been an increase from 80 million broilers in 1986 to 200 million in 1996 (Oklahoma Ag Statistics, 1996). Production practices concentrate animals on smaller parcels of land than historically observed, resulting in an accumulation of poultry litter. These organic N and P rich materials are accumulating in eastern Oklahoma counties, in quantities greater than can be agronomically utilized. This accumulation of litter has led to water quality concern for these areas. Moving the nutrient rich poultry litter to a nutrient deficient crude oil spill site would reduce the amount of litter in these local watersheds and alleviate water quality concerns. Sites contaminated by crude oil, being deficient of plant nutrients and biological activity, are possible locations for application of the litter.

The addition of poultry litter could stimulate microbial growth by providing an easily assessable and degradable carbon source, N, and P. A study on a gasoline contaminated soil treated with poultry litter reported an 80% increase in microbial population, when compared to only gasoline contamination (Gupta and Tao, 1996). They attributed 50% of the increased microbial population to the addition of poultry litter. The increased microbial population associated with the litter additions will rapidly degrade the litter and require another C source that and PH's could provide. Also as litter is degraded N and P would be mineralized and available for microbial use. This would have a priming effect, for the degradation of crude oil, which is low in N and P. Additions of litter could also

increase soil aeration by decreasing bulk density. This increase in pore space could supply more O_2 for microbial respiration. The additional O_2 supplied by poultry litter additions could stimulate the indigenous microbial population to reduce levels of the semi-volatile fraction of PH's. A solution to the problems of excess poultry litter and contaminated spill sites would be to use poultry litter as an organic material and nutrient to stimulate microbial activity at contaminated oilfield sites. The objective of this research was to determine and compare percent degradation and degradation rates of hydrocarbons in soils with long term contamination when treated with selected N sources.

MATERIALS AND METHODS

Sample Collection

Crude oil spill sites were located and sampled in Creek and Logan counties of Oklahoma. Both counties had significant oil exploration and production in the past, with 532 ha and 32 ha respectively classified as oil-waste land (Table 1). Little or no vegetation was present at both sites and they are low in plant nutrients (Table 2). These sites are abandoned sites and are under the Oklahoma Energy Resource Board cleanup and restoration program. The Creek county site is a silt loam with PH's concentration from 9640 to 13,400 and mean concentration of 10,300 mg kg^{-1} soil, other properties are listed (Table 2). The site is mapped as oil-wasteland, but is surrounded by Dennis (Fine, mixed, thermic Aquic Paleudolls) and Okemah (Fine, mixed, thermic Aquic Paleudolls) series. The Logan county site is a silty clay loam classified as Port (Fine-silty, mixed thermic Cumulic Halpustolls) series, with PH's a ranging from 36,500 to 43,300 mg kg^{-1} soil and a mean concentration of 39,100 mg kg^{-1} soil, other properties are listed (Table 2). The specific date of contamination at both sites was difficult to determine. However, it was prior to present landowners, which have owned the property for the last 40 years.

Samples were collected from one square meter to a depth of 60 cm to keep variation in PH's concentration to a minimum. Even in a small sampling area there was considerable variation in PH's concentration (Table 3). Samples were immediately returned to the laboratory and stored at 4° C to retard biological activity until time of incubation. Experimental design was randomized complete block design.

Incubation

To determine if N sources would affect PH's degradation, 200 g soil on a dry weight basis (DWB) from Creek and Logan county site was placed in 950 mL mason jars. Nitrogen addition was 250 mg kg^{-1} soil, similar to what many poultry producers would field apply. Nitrogen additions were equivalent to a C:N ratios of 32:1 and 122:1 for Creek and Logan county sites respectively. Nitrogen sources used were ammonium nitrate (AN), fresh poultry litter (FPL), and composted poultry litter (CPL). Phosphorus and micronutrients for the AN treatment were supplied by addition of a complete + micronutrients fertilizer at P_2O_5 amounts of 72.8 kg ha^{-1}. Poultry litter was collected from production facilities, immediately returned to the laboratory and stored at 4° C until used. Litter was analyzed for total N content by near infrared reflectance spectroscopy (O'dell and Hattey, 1996). Nitrogen contents for FPL and CPL were 3.17% and 4.01% respectively. The mass of litter added was adjusted for moisture content. Incubations were conducted at a constant temperature of 30° C and moisture content of 16% on a gravimetric basis, adjusted weekly. During the moisture adjustments the samples were mixed which would simulate mixing with field implements. Incubation period was 240 days with samples collected at 0, 60, 120, and 240 days.

Analysis

Analysis for semi-volatile fraction of PH's was done utilizing the modified Wisconsin method (Wisconsin DNR, 1993; C_{10}-C_{28}). One gram of soil was extracted with 10 mL of methylene chloride and shaken for 1 hour with Eberbach 60 cycle shaker. Samples were centrifuged prior to analysis and a 1 mL aliquot was analyzed. Petroleum hydrocarbon

content was determined by gas chromatography (Hewlett Packard Series II Model 5890) with a mass selective spectrophotometer detector (Model 5971).

Percent degradation at sampling dates was determined by (concentration day 0 - concentration sampling date / concentration day 0) * 100, samples were removed from same jar through out incubation. Degradation rate was determined by (amount of degradation / number of days in sampling period). Extractable soil NH_4^+ and NO_3^- was determined by extracting 2 grams of soil with 20 mL of 2M KCl, shaken for one hour and filtered. Nitrogen analysis for all sampling dates was done by flow injection analysis utilizing indophenol blue for NH_4^+ and cadmium reduction for NO_3^- (Keeny and Nelson, 1982).

RESULTS AND DISCUSSION

Creek County Site

With an N applications of 250 mg kg^{-1} soil, all N additions were better than control for percent degradation at day 30 at $P < 0.10$ (Fig. 1). Composted poultry litter was better than AN and FPL among N additions with 30% degradation (Fig. 1). Composted poultry litter was a better treatment than control throughout the incubation (Fig. 1). This could be attributed to an increased microbial population associated with the composted poultry litter. Composted poultry litter will have an established microbial population because of the composting process. Throughout the incubation period no difference was observed between control and AN treatments except at day 30. This would indicate that N was not limiting at this site (Fig. 1). If N was the limiting factor then AN treatment should have a higher percent degradation than control at other sampling dates (Fig. 1) and applied N would have been utilized (Table 4), this was not observed. The difference between AN and control at day 30 could be attributed to N from AN being available for microbial utilization when applied, while N from control had to be mineralized before utilization (Table 4). At day 60 and 120 no difference was observed among added N sources. Fresh poultry litter and CPL were a better treatment than control at both day 120 and 240. The difference between control and litter treatments as incubation proceeded could possibly be attributed to an increased microbial population as found by (Gupta and Tao, 1996). The increased microbial population from litter treatments could have a higher percent degradation of PH's than control or AN as was observed in this study. Fresh and composted poultry litter treatments were better than control and AN with 62.9 and 59.1 % degradation respectively at day 240. The mean % degradation for all treatments was

53.4 % while control and AN were 43.8 and 47.8 % respectively. The poultry litter treatments appears to be a better N source than does AN possibly due to increased microbial population at this site. If N is not the limiting factor an increased microbial population associated with poultry litter treatments would explain the difference between AN and litter treatments at day 30 and 240. Increased aeration and reduced bulk density due to litter additions could also be a possible explanation for litter being a better treatment. But the addition of one gram or less should not create a large enough change in bulk density and/or pore space to have an impact on O_2 levels in the soil.

When comparing degradation rates among N sources the results were different than percent degradation. The only difference observed in degradation rates during the incubation was at day 30, where CPL with 109 mg kg^{-1} day^{-1} was higher when compared to mean for all other treatments of 69.7 mg kg^{-1} day^{-1} (Table 5). Again possibly this could be attributed to an increased microbial population. After day 60 a reduction in degradation rates was observed for all treatments (Fig. 2). This indicates something other than N is limiting degradation in this system.

Gupta and Tao (1996) reported an increased in microbial population when gasoline contaminated soil (200 mg kg^{-1}) was treated with poultry litter. They found the increase to be a short-term effect where no difference between gasoline contaminated soil and gasoline contaminated soil treated with poultry litter at day 90. It is possible that additions of an easier degradable C source like poultry litter could stimulate the degradation PH's. Therefore, repeated applications of poultry litter may be needed to stimulate degradation at this site. This could especially be true for these sites contaminated with crude oil which contain many compounds more difficult to degrade

than gasoline and at contamination levels 5-20 times greater than observed by (Gupta and Tao, 1996). With degradation rates observed in this study the gasoline would be oxidized in 10-20 days. Since large concentrations of N are found for the Creek county site throughout the incubation, it appears at C:N of 32:1 is below an optimum ratio. Reducing the amount of N added to a more optimum C:N or splitting the litter treatments with a second application at day 60 are options to be considered.

Logan County Site

At the Logan county site CPL again had a higher percent degradation than control and AN at day 30. Although no difference was observed between CPL and FPL with 14.9 and 15.7% degradation respectively and a mean of all sources of 11.2% (Fig. 3). This again could be explained by an increase in microbial population associated with poultry litter additions. After day 30 no difference in percent degradation was observed among added N sources. Fresh poultry litter was the only source significantly better than the control at day 60. All N sources were significantly better than the control at days 120 and 240 with a 10 to 11% increase in percent degradation (Fig. 3). At the Logan county site the cumulative amount of degradation continued to increase, whereas degradation for the Creek county site had reached a plateau from day 120 to 240 (Figs. 1 and 2).

The increased degradation from added N suggests that N was limiting at this site. This can be seen by the concentration of extractable N for all sampling dates (Table 4). By day 120 no difference in total N concentrations for control and treatments was observed at 8.5 to 10 mg kg^{-1} soil (Table 4). When N concentrations for treatments and control was the same , it was considered limiting. This indicates that for this site after day 60 N had become the limiting factor in degradation and a C:N of 122:1 was to high

for optimum degradation to occur. When N is limiting a difference in percent degradation and/or degradation rates among N sources was not observed. For this site more N could have been added at day 60 or 120 to determine if a difference in degradation rate and/or percent degradation would be observed from a repeated application.

The Logan county site had the highest degradation rate of 249 mg kg^{-1} day^{-1} for the AN treatment between day 30-60 although no difference among N treatments was observed (Fig. 4). As with the Creek county site day 30 only date a difference was observed among N sources. Both FPL and CPL treatments with 192 and 202 mg kg^{-1} day^{-1} respectively, where different from control and AN with rates of 85.0 and 96.1 mg kg^{-1} day^{-1} respectively (Table 5). These degradation rates were higher than the Creek county site possibly due to higher concentration of easily degradable PH's for the Logan county site. This could be expected due to the higher concentration of total PH's and assuming the same percentage of total PH's at each site is easily degradable. If this were true the Logan county site could have contained a larger concentration of easily degradable PH's than the Creek county site. This higher concentration of easily degradable C could lead to higher degradation rates for the Logan county site when compared to the Creek county site. Again the difference in degradation rates between litter, AN and control treatments at day 30 could be attributed to an increase in microbial population from litter treatments. After day 30 no differences were observed in degradation rates among N sources (Table 5).

Nitrogen Availability

The amount of N mineralized in the control treatments for both sites was significant and possibly led to a greater percent degradation in the control (Table 4). Total extractable N was determined at each sampling date to measure N availability during the study (Figs. 5 and 6). Available N at day 0 was 10.9 and 12.1 mg N kg^{-1} soil for Creek and Logan county soils respectively. At day 30, 63.7 and 23.7 mg N was found, so 52.8 and 11.6 mg N was mineralized from Creek and Logan county soils respectively (Table 4). This probably led to the higher than expected percent degradation for the control treatment. The amount of NH_4^+ found in each treatment was unexpected for an aerobic system where the primary form of N found is generally expected to be NO_3^-.

Ammonium was the predominant form of mineralized N found in the control and litter treatments (Table 4). An initial concern was the system had become anaerobic. To determine if this occurred, lids were removed from jars on two blocks. At the next two sampling dates no difference in percent degradation, NH_4^+, or NO_3^- was observed, so lids were placed back on the jars to help reduce evaporation. Ammonium as the predominant form of N mineralized from control and litter treatments remained true throughout the study for the Creek county site where N appeared to not be the limiting factor. This was also true for Logan county site until day 60 after which time N appeared to be limiting. Since system was not anaerobic it was determined that inhibition of nitrification was occurring.

Nitrification Inhibition

Several factors that could inhibit nitrification are short-chained hydrocarbons, high salt content, and a lack of ammonium oxidizing organisms present. Short-chained

hydrocarbons (alkanes, alkenes, and alkynes) usually associated with crude oil have been reported to inhibit nitrification by nitrosomonas (Hyman et al., 1988). These short-chained hydrocarbons compete for binding sites on the ammonium monooxygenase enzyme that catalyzes oxidation of ammonium. Since these short-chained hydrocarbons are generally associated with crude oil it they possibly could have interfered with the nitrification process.

Sodium chloride content has been reported to inhibit the nitrification process as well as microbial activity (Westerman and Tucker, 1974). They observed inhibition of nitrification, due to effects of salt on N transformations at concentrations of 0.1M. The Na and Cl concentrations were almost identical for Creek county site at 0.11M and 0.10M respectively. The Logan county site concentrations were higher at 0.26M and 0.31M for Na and Cl respectively. For both sites, concentrations were at or above those reported to inhibit nitrification. As previously stated, brine associated with spills can be as much of a problem as the crude oil itself.

The long-term effect of crude oil spills on ammonium oxidizers is not well understood, there is a possibility of a large reduction in population at contaminated sites (Data not shown). For the Creek county site with NH_4^+ as predominant form of N found at all sampling dates, nitrification appears to have been inhibited for the entire incubation. For the Logan county site nitrification appears to have been inhibited until day 60 after which time the ammonium oxidizers converted most of the NH_4^+ to NO_3^-. This supports a reduction in ammonium oxidizer numbers, were mineralization of NH_4^+ is occurring faster than the small number of ammonium oxidizers can accomplish nitrification.

CONCLUSIONS

The use of poultry litter in remediation of petroleum hydrocarbon contaminated soil appears to be a good alternative to the use of commercial fertilizers. During this study when N was not the limiting factor as in the Creek county site CPL was significantly better at day 30 and 240, therefore something besides N in the litter was stimulating degradation. This could be attributed to low indigenous microbial population and the addition of litter increases the microbial population. In a system where N is the limiting factor differences in N sources was not be observed because N concentrations will not support an increased microbial activity associated with litter additions. The optimum C:N for degradation of PH's may be the 60:1 as previously stated. This study was conducted at ratios of 32:1 and 122:1 for Creek and Logan county sites respectively and appeared to be below and above the optimum C:N. The use of split applications of poultry litter could possibly stimulate degradation in both systems. When N is not the limiting factor as in the Creek county site an easily degradable C source could stimulate microbial population after day 60 when degradation rates are reduced. For a system like Logan county site where N is limiting after day 60 the N additions are needed for oxidation of PH's. Both litter treatments were as good or better than was AN in percent degradation as well as degradation rate at all sampling dates.

Nitrification was inhibited during the remediation process by short-chained hydrocarbons, the salt usually found on these sites, or reduction in number of ammonium oxidizers found at contaminated sites. It is possible any or all three of these were occurring in these systems. Future work could include determining if repeated application of poultry litter could extend period of increased microbial activity that

appears to be associated with litter treatments thus decreasing the period of remediation. Also work to determine if C:N should be utilized to determine amount of N additions. This resource would help reduce the levels of litter in high poultry producing areas. Beneficial results of moving the litter would be a reduction in water quality concerns associated with litter accumulation and the addition of nutrients to the nutrient deficient contaminated sites.

REFERENCES

Atlas, R.M., 1991. Microbial hydrocarbon degradation-bioremediation of oil spills. J. Chem. Technol. Biotechnol. 52(2):149-156.

Braddock, J.F., M.L. Ruth, and P.H. Catterall. 1997. Enhancement and inhibition of microbial activity in hydrocarbon-contaminated arctic soils: Nutrient-amended bioremediation. Environ. Sci. Technol. 31(7):2078-2084.

Brooks, B.T., S.S. Kurtz Jr., C.E. Boord, and L. Schmerling. (ed.) 1954-55. The chemistry of petroleum hydrocarbons. Reinhold Publishing. New York, NY.

Deuel, L.E., and G.H. Holliday. 1994. Soil remediation for the petroleum extraction industry. Penwell Publishing. Tulsa, OK.

Dibble, J.T., and R. Bartha. 1979a. Leaching aspects of oil sludge biodegradation in soil. Soil Sci. 127(6):365-370.

Dibble, J.T. and R Bartha. 1979b. Effect of environmental parameters on the biodegradation of soil sludge. Appl. Environ. Microbiol. 37(4):729-739

Dibble, J.T., and R. Bartha. 1979c. Rehabilitation of oil-inundated agricultural land: A case history. Soil Sci. 128(1):56-60.

Dobson, A.L., and H.A. Wilson. 1964. Respiration studies on soil treated with some hydrocarbons. Soil Sci. Soc. Am. Proc. 28(4):536-539.

Ellis, R. Jr., and R.S. Adams Jr. 1961. Contamination of soils by petroleum hydrocarbons. Adv. in Agron. 13:197-216.

Frankenberger, W.T. Jr., and J.B. Johanson. 1982. Influence of crude oil and refined petroleum products on soil dehydrogenase activity. J. Envir. Qual. 11(4): 602-607.

Gupta, G. and J. Tao. 1996. Bioremediation of gasoline-contaminated soil using poultry litter. J. Environ. Sci. Health. A31(9):2395-2407.

Huntjens, J.L.M., H.D. Potter, and J. Barendrecht. 1985. The degradation of oil in soil. Contaminated soil: First International TNO Conference on Contaminated Soil, 11-15 November, Utrecht, The Netherlands. Kluwer Academic Publishers. Hingham, MA.

Hyman, M.R., J.B. Burton, and D.J. Arp. 1988. Interaction of ammonia monooxygenase from nitrosomonas europaea with alkanes, alkenes, and alkynes. Appl. Environ. Microbiol. 54(12):3187-3190.

Keeney, D.R. and D.W. Nelson. 1982. Methods of Soil Analysis, 2nd ed.; Page, A.L. ed.; ASA and SSSA. Madison, WI.

Loynachan, T.E., 1978. Low-temperature mineralization of crude oil in soil. J. Envir. Qual. 7(4): 494-500.

Marshall, T.R. 1988. Biodegradation of petroleum waste in soil: The microbial ecosystem and optimization of a treatment process. Dissertation. University of Southern California.

MIADS. 1985. Landuse data set. NRCS-USDA.

Mitchell, W.W., T.E. Loynachan, and J.D. McKendrick. 1979. Effects of tillage and fertilization on persistence of crude oil contamination in an Alaskan soil. J. Environ. Qual. 8(4)525-532.

Murphy, H.F., 1929. Some effects of crude petroleum on nitrate production, seed germination, and growth. Soil Sci. 27:117-120.

O'Dell, A.W. and J.A. Hattey. 1996. Near infrared reflectance spectroscopy analysis of poultry and swine waste. P. 310. Agronomy Abstracts. ASA, CSSA, and SSSA, Madison, WI.

Oklahoma Ag Statistics. 1996. Oklahoma agricultural statistics service. Oklahoma City, OK.

Overcash, M.R., and D. Pal. 1979. Plant-Soil assimilative capacity for oils. Water Am. Inst. Chem. Eng. 75(190):357-365.

Owen, E.P. 1975. Trek of the oil finders: History of exploration for petroleum. Amer. Ass. Pet. Geologist. Tulsa, Oklahoma.

Petrov, A.A. 1987. Petroleum hydrocarbons. Springer-Verlag. New York, NY.

Plice, M.J. 1948. Some effects of crude petroleum on soil fertility. Soil Sci. Soc. Amer. Proc. 13:413-416.

Smith, R.K. 1994. Handbook of Environmental Analysis 2nd. Genium Publishing, Schenectady, NY.

Toccalino, P.L., R.L. Johnson, and D.R. Boone. 1993. Nitrogen limitation and nitrogen fixation during alkane biodegradation in a sandy soil. Appl. Environ. Microbiol. 59(9):2977-2983.

Udo, E.J., and A.A.A. Fayemi. 1975. The effect of oil pollution of soil on germination, growth, and nutrient uptake of Corn. J. Environ. Qual. 4(4):537-540.

Westerman, R.L., and T.C. Tucker. 1974. Effect of salts and salts plus nitrogen-15-labeled ammonium chloride on mineralization of soil nitrogen, nitrification, and immobilization. Soil. Sci. Soc. Amer. Proc. 38:602-605.

Wisconsin, DNR (1993) Method for determining diesel range organics. Publ-SW_141, Dept. Nat. Res., Madison, WI.

Wrenn, B.A., J.R. Haines, A.D. Venosa, M. Kadkhodayan, and M.T. Suidan. 1994. Effects of nitrogen source on crude oil degradation. J. Indust. Microbiol. 13:279-286.

APPENDICES

APPENDIX A
TABLES

Table 1. Land area in Oklahoma counties classified as oil-waste land by Natural Resource Conservation Service.

County	ha †	% of total
Beckham	88	1.83
Blaine	28	0.58
Caddo	424	8.81
Canadian	60	1.25
Carter	320	6.65
Comanche	212	4.41
Cotton	8	0.17
Creek	532	11.06
Custer	8	0.17
Garfield	32	0.67
Grady	64	1.33
Hughes	92	1.91
Jefferson	108	2.24
Kay	392	8.15
Latimer	4	0.08
Lincoln	204	4.24
Logan	32	0.67
McClain	8	0.17
Major	12	0.25
Muskogee	24	0.50
Nowata	200	4.16
Okfuskee	244	5.07
Okmulgee	28	0.58
Osage	788	16.38
Pontotoc	88	1.83
Pottawatomie	12	0.25
Roger Mills	148	3.08
Seminole	92	1.91
Stephens	64	1.33
Tulsa	4	0.08
Washington	152	3.16
Washita	340	7.07
Total	**4812**	**100**

† From Landuse Data Set resolution 4 ha (MIADS, 1985).

Table 2. Soil physical and chemical properties of contaminated soil from Creek and Logan county sites.

Soil Properties	Site	
	Creek county	Logan county
Texture	SiL	SiCL
Initial pH†	6.4	6.8
Final pH†	6.0	7.1
EC (dS m^{-1}) ‡	10.2	25.5
SAR ‡	41.7	46.9
Na^+ (mol L^{-1})‡	0.11	0.26
Cl^- (mol L^{-1})‡	0.10	0.31
NH_4^+ (mg kg^{-1})#	10.3	11.2
NO_3^- (mg kg^{-1})#	0.45	0.87
P_2O_5 (mg kg^{-1})††	16.0	4.00

† 2:1 water
‡ Saturated paste
# 2 M KCl
†† Mehlich 3

Table 3. Petroleum hydrocarbon concentration at day 0 for each treatment block.

	Creek County				Logan County			
N source	Control	AN†	FPL	CPL	Control	AN	FPL	CPL
Block	mg kg^{-1}							
1	9720	9230	9640	9650	36500	37500	36900	36900
2	10000	10000	9950	9830	37900	37700	39500	37300
3	10600	10500	10400	11200	41000	40500	38400	39800
4	10600	13400	11400	10900	40200	41700	43300	40600

† AN = Ammonium nitrate; FPL = Fresh poultry litter; CPL = Composted poultry litter.

Table 4. Nitrate and ammonium concentrations for all treatments by sampling date for Creek and Logan county sites extracted with 2 M KCl.

N source	Creek county								Logan county							
	Control		AN†		FPL		CPL		Control		AN		FPL		CPL	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
Day	mg kg^{-1}															
0	10.3	0.45	10.3	0.50	10.3	0.45	10.3	0.45	11.2	0.87	11.2	0.87	11.2	0.87	11.2	0.87
30	60.2	1.03	192.0	86.2	194.0	1.47	185.0	0.43	23.7	0.00	89.4	84.0	16.7	0.38	39.6	0.00
60	54.2	3.17	177.0	90.0	165.0	2.27	174.0	3.03	4.20	2.16	41.6	34.6	4.30	1.30	5.50	1.15
120	40.9	3.80	176.0	89.5	117.0	1.68	128.0	4.10	4.80	3.83	5.20	3.93	4.80	3.85	6.30	3.83
240	5.20	5.88	157.0	120.0	38.6	3.55	27.3	5.61	0.00	6.26	0.00	6.07	0.00	6.34	0.00	5.56

† AN = Ammonium nitrate; FPL = Fresh poultry litter; CPL = Composted poultry litter.

Table 5. Mean petroleum hydrocarbon degradation rates as affected by N sources at each sampling date in mg kg^{-1} day^{-1}.

	Creek county				Logan county			
N source	Control	AN†	FPL	CPL	Control	AN	FPL	CPL
Days	---------- mg kg^{-1} day^{-1} ----------							
0-30	55.5	75.3	78.3	109*	85.0	96.1	192*	202*
30-60	66.4	75.7	72.3	62.8	223	249	211	213
60-120	7.82	0.05	16.5	11.8	68.3	133	89.6	104
120-240	1.82	2.90	8.88	4.30	41.0	34.0	47.8	32.2

* Signifies difference between N sources at sampling date 30 for each site ($P < 0.05$) not between sampling dates and sites.

† AN = ammonium nitrate; FPL = Fresh poultry litter; CPL = Composted poultry litter.

APPENDIX B
FIGURES

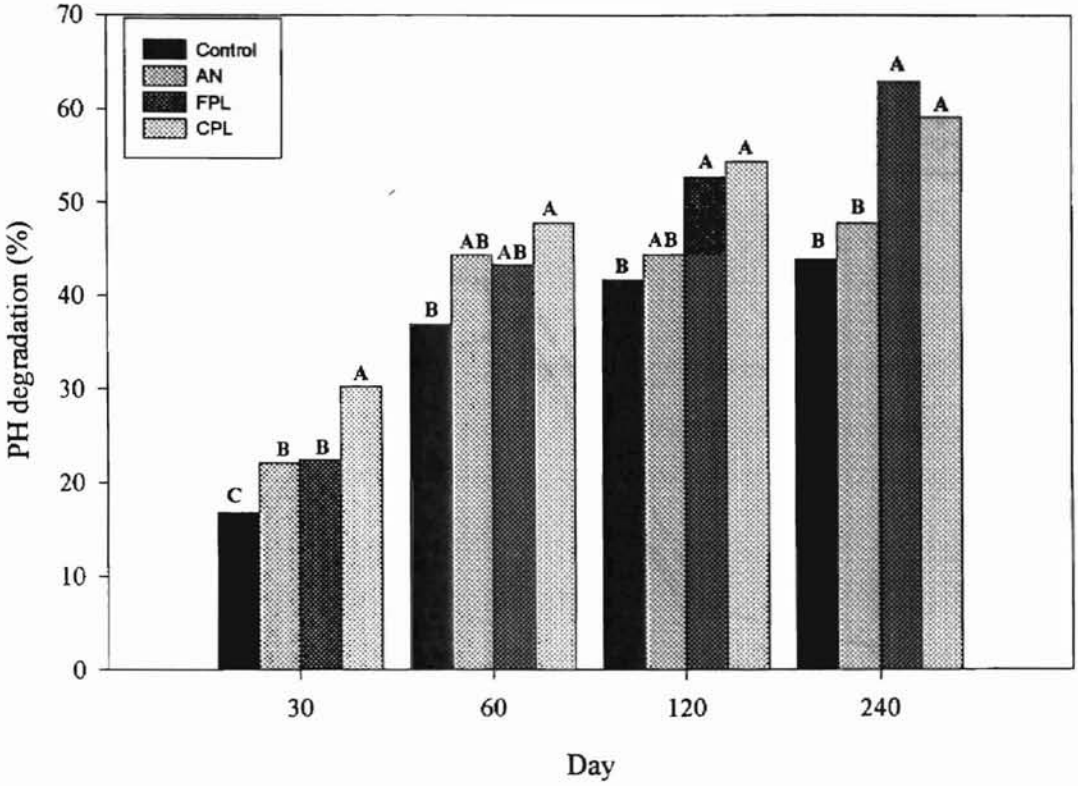

Fig. 1. Percent petroleum hydrocarbon degradation for Creek county site as affected by N source at 250 mg N kg^{-1} for all sampling dates. Treatments with the same letter within a sampling date are not significantly different ($P < 0.10$).

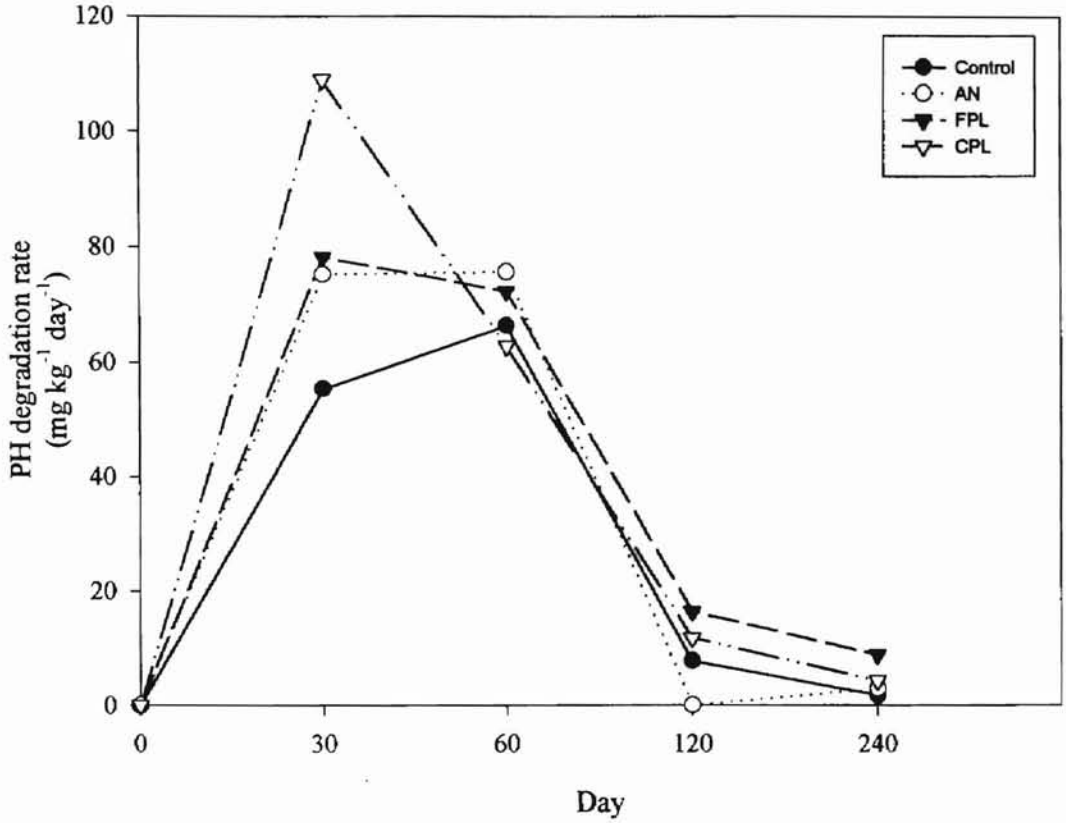

Fig. 2. Petroleum hydrocarbon degradation rate at the Creek county site as affected by N source.
Note degradation rate scale is different between figures 2 and 4.

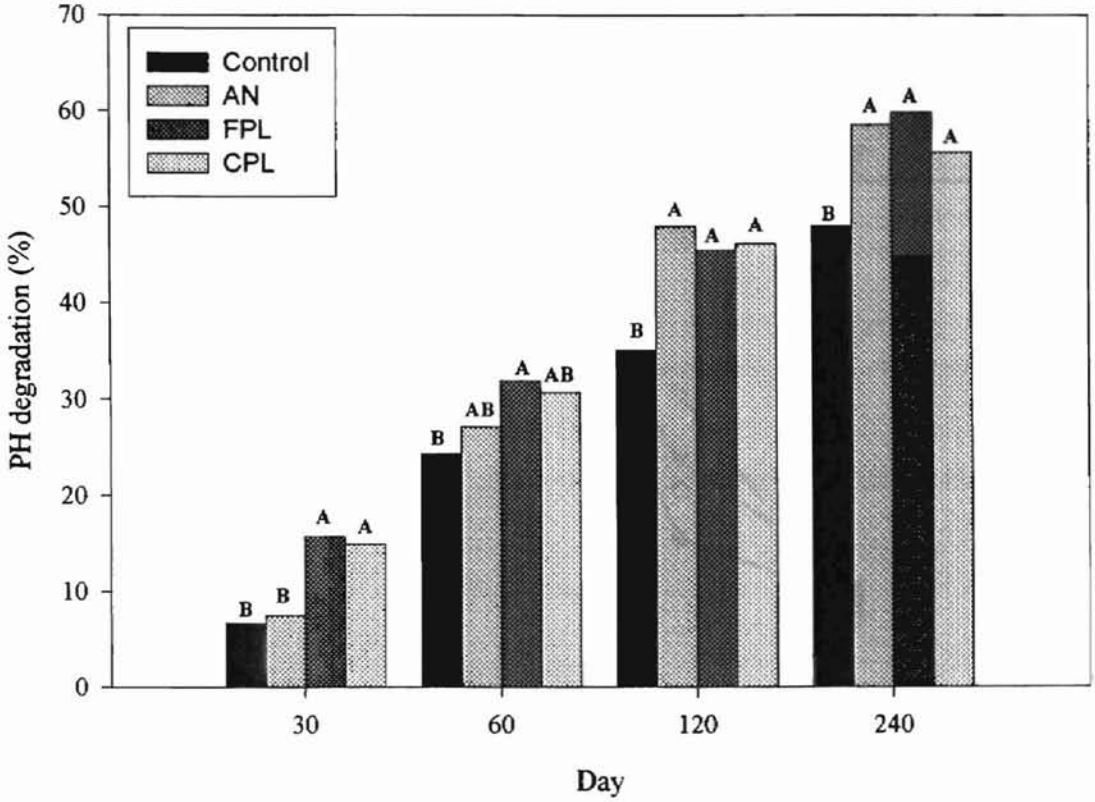

Fig. 3. Percent petroleum hydrocarbon degradation for Logan county site as affected by N source at 250 mg N kg^{-1} soil for all sampling dates. Treatments with the same letter within a sampling date are not significantly different ($P < 0.10$).

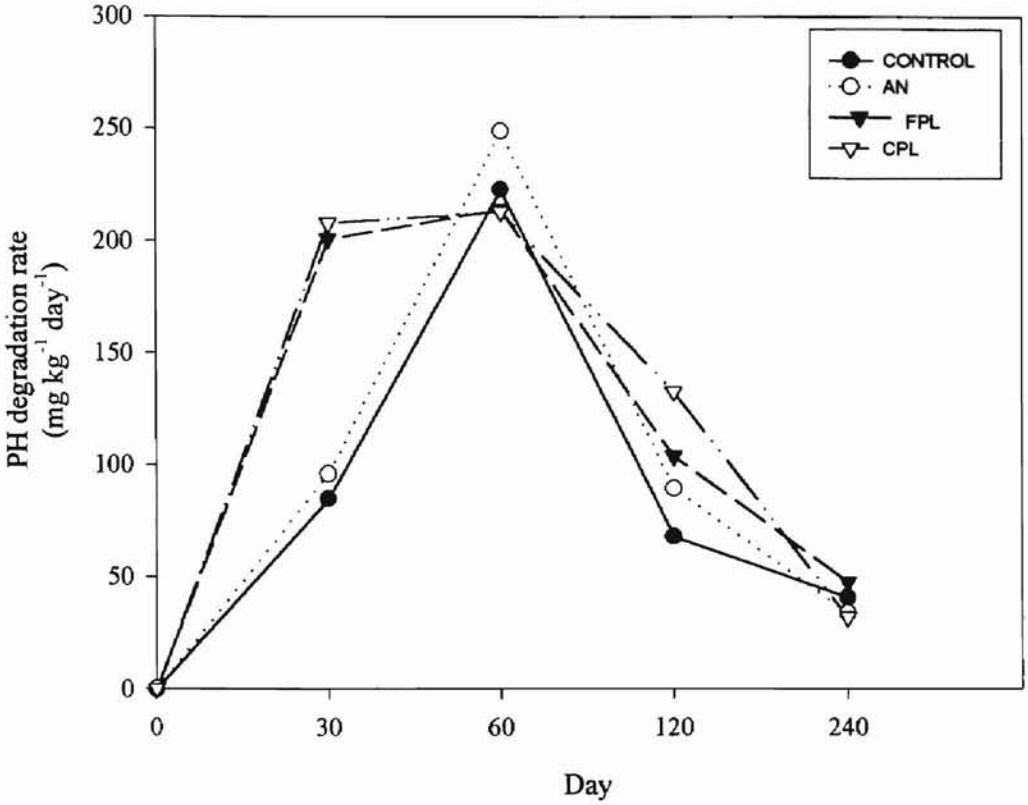

Fig. 4. Petroleum hydrocarbon degradation rate at the Logan county site as affected by N source.
Note degradation rate scale is different between figures 2 and 4.

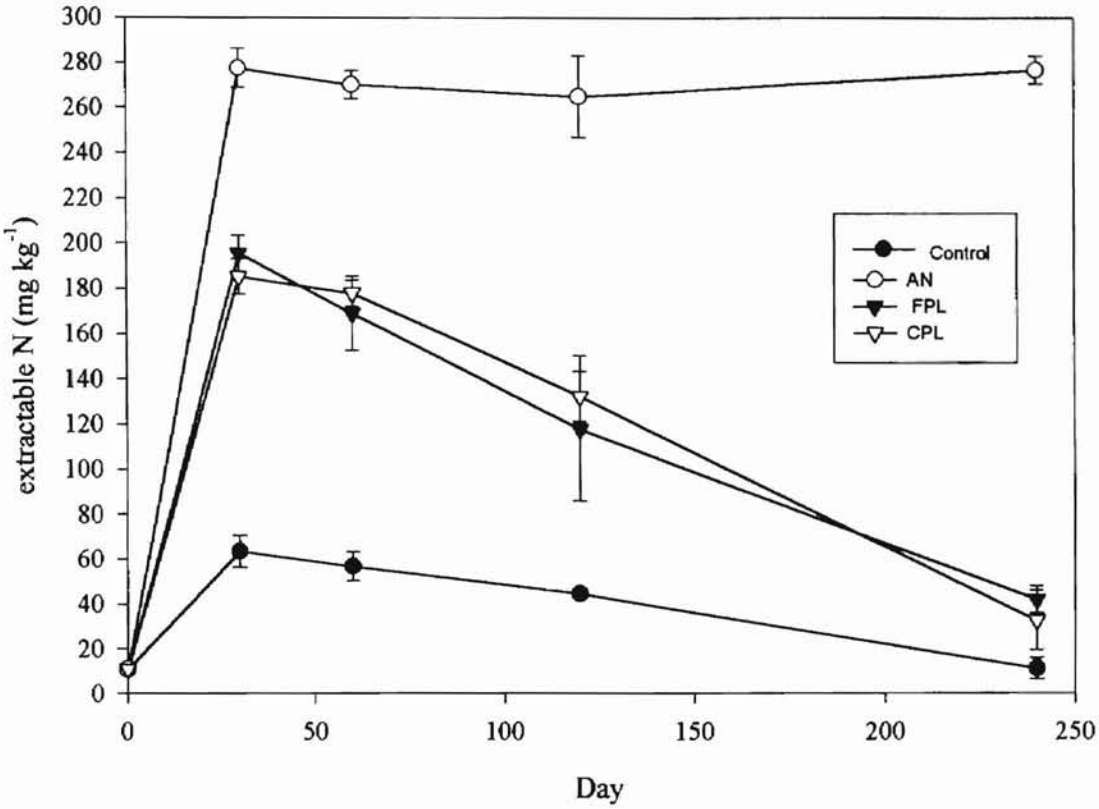

Fig. 5. Creek county site total extractable N for each N source.
Note extractable N scale different between figures 5 and 6.

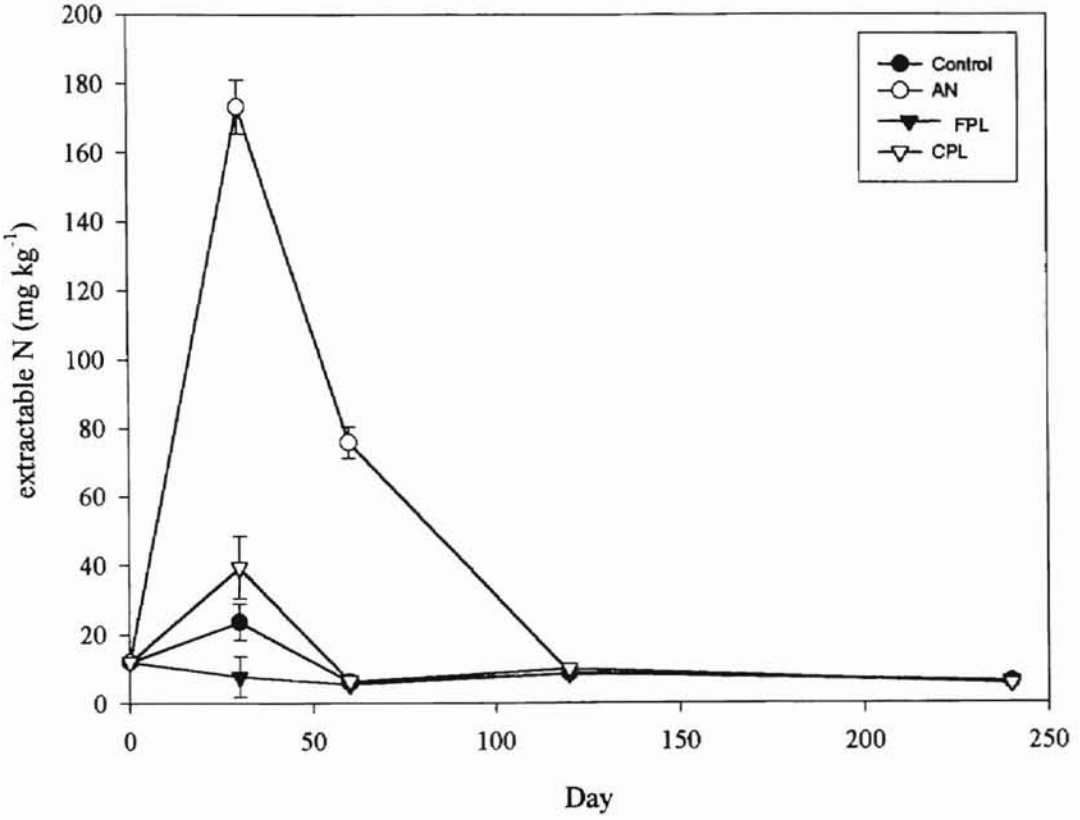

Fig. 6. Logan county site total extractable N for each N source. Note extractable N scale different between figures 5 and 6.

VITA

Ricky Don Kochenower

Candidate for the Degree of

Master of Science

Thesis: POULTRY LITTER AS A NITROGEN SOURCE FOR DEGRADATION OF HYDROCARBONS IN CONTAMINATED SOILS

Major Field: Agronomy

Biographical:

Personal Data: Born in Borger, Texas July 24, 1958.

Education: Graduated for Kingfisher High School, Kingfisher, Oklahoma, in May 1976; Received Bachelor of Science degree in Agronomy from Oklahoma State University, Stillwater, Oklahoma in May, 1996; Completed requirements of Master of Science degree in Agronomy Majoring in Soil Science at Oklahoma State University, Stillwater, Oklahoma in July, 1998

Professional Experience: Teaching Assistant, Department of Agronomy, Oklahoma State University, January 1995 to August 1996; Graduate Teaching and Research Assistant, Department of Plant and Soil Sciences, Oklahoma State University, August 1996 to July 1998.

Professional Organizations: American Society of Agronomy, Soil Science Society of America, Soil and Water Conservation Society, The Honor Society of Agriculture – Gamma Sigma Delta.