EFFECT OF APPLIED SEWAGE SLUDGE, AMMONIUM NITRATE AND PHOSPHORUS ON YIELD OF WINTER WHEAT, SOIL PROFILE INORGANIC NITROGEN ACCUMULATION AND CADMIUM UPTAKE

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INTRODUCTION

This document consists of two chapters, each reporting separated studies conducted during my doctoral program. Both chapters are presented in formats suitable for publication in professional journals.

CHAPTER I

EFFECT OF APPLIED SEWAGE SLUDGE AND AMMONIUM NITRATE ON WHEAT YIELD AND SOIL PROFILE INORGANIC NITROGEN ACCUMULATION

ABSTRACT

The beneficial effect of sewage sludge in crop production has been demonstrated, but there is concern regarding its contribution to NO_3 leaching. The objectives of this study were to compare N rates of sewage sludge and ammonium nitrate on soil profile (0-180 cm) inorganic N (NH_4-N and NO_3-N) accumulation, yields, N uptake and soil-plant inorganic N buffering in winter wheat (*Triticum aestivum L.*). One field experiment was established in 1993 that evaluated six N rates (0-540 kg ha^{-1} yr^{-1}) as dry anaerobically digested sewage sludge and ammonium nitrate. Lime application in 1993 (4.48 Mg ha^{-1}) with 540 kg N ha^{-1} yr^{-1} was also evaluated. An incubation study was included to simulate N mineralization from sewage sludge applied at rates of 45, 180 and 270 kg N ha^{-1} yr^{-1}. Total N in sewage sludge was 2.2%. Treatments did not affect surface soil (0-30 cm) pH, organic C and total N in samples taken following the second year harvest (1995). Soil-plant inorganic N buffering, only evaluated following

the second year harvest, was present at rates $\leq$ 173 and $\leq$ 540 kg N ha^{-1} as ammonium nitrate and sewage sludge, respectively, since soil profile inorganic N accumulation increased only when 270 and 540 kg N ha^{-1} were applied as ammonium nitrate. Less soil profile inorganic N accumulation was detected when lime was applied. In general, wheat yields and N uptake increased linearly with applied N as sewage sludge, while wheat yields and N uptake peaked at 270 kg N ha^{-1}, when N was applied as ammonium nitrate. Lime did not affect yields or N uptake. Sewage sludge estimated N use efficiency in grain production was 20% (average of two harvests) with respect to ammonium nitrate. Estimated plant N recovery ranged between 6 to 17% for sewage sludge and from 11 to 27% for ammonium nitrate in 1995. The high N rates required for maximum yields are explained by a net N immobilization found in a simulation study with sewage sludge at N rates of 45 to 180 kg ha^{-1} during the first crop cycle and for the 45 kg N ha^{-1} in the second crop cycle.

INTRODUCTION

Although various researchers have evaluated the effects of applied sewage sludge on heavy metal accumulation in grain crops and soils, little work has targeted soil profile accumulation of NH_4-N and NO_3-N as a result of applying these materials. In addition, there has been little work conducted that evaluates the effects of increasing total N in organic pools as result of applying sewage sludge and the potential for NO_3-N leaching.

Most of the N in sewage sludge is in organic forms (> 1%), with smaller amounts (< 0.5%) present as ammonium, and only trace amounts present as nitrate (Basta, 1995). Under laboratory conditions, N mineralized ranges from 10 to 40% (U.S. EPA, 1983), but under field conditions it is more variable and ranges from 7 to 55% of the organic N mineralized (Fox and Axley, 1985). Considering these values of N mineralization, federal regulations recommend sewage sludge land application based on the "agronomic rate", defined as the amount of sludge that will meet the crop N requirement (U.S. EPA, 1983). More field studies that estimate nitrogen mineralized under specific environments should provide better guidelines for sewage sludge land application.

Available N from inorganic nitrogen or sewage sludge applied at recommended plant-available N rates for durum wheat have been studied under field conditions. One study concluded that grain yield and heavy metal (Cd, Cu, Ni, Pb and Zn) content in grain and straw were not affected by N source: inorganic N or fresh anaerobically digested sewage sludge (Day et al., 1988; Day et al., 1987a). The same conclusion was drawn when dried sewage sludge was applied in a wheat hay system (Day et al, 1987b). However, another study with six wheat varieties showed that dry sewage sludge applied at recommended N rates increased Zn, Cu, Pb and Ni contents in grain compared to inorganic N-P-K (Day and Thompson, 1986).

Nitrate from sewage sludge or inorganic fertilizer that is not taken up by plants, volatilized from plants, immobilized, or denitrified has the potential to move with the soil water and eventually enter the groundwater (Raun and

Johnson, 1995; Hue, 1995). Application of sewage sludge at agronomic N rates minimizes contamination risk (U.S. EPA, 1993), and excessive sludge applications increases the risk of nitrate leaching and potential groundwater contamination. After 2 yr, N rates greater than 2600 kg ha^{-1} yr^{-1} as liquid anaerobically digested sewage sludge resulted in nitrate accumulation in the 0-120 cm soil profile in a bermudagrass (*Cynodon dactylon* L.) system (King and Morris, 1972). A 12-yr field study indicated that liquid anaerobically digested sewage sludge at a total N rate of 100 kg ha^{-1} every 2 yr should be used in order to avoid soil accumulation of nitrate in a continuous dryland winter wheat production system (Ippolito et al., 1994). In a simulation study, 225 kg N ha^{-1} applied as anaerobically digested sewage sludge every 3 yr did not result in adverse effects on drinking water standards for N in 99% of the years in a 31-yr-old forest site (Chron and Haith, 1994). Although soil nitrate accumulation can take place, it may not represent ground water contamination. Nitrogen rates as sewage sludge greater than the agronomic rate resulted in high levels of nitrate in soil water, but not deep groundwater in a 20-yr field study (Clapp et al., 1994). In this study it was also observed that nitrate levels in the shallow water decreased with reduction in the sewage sludge rates, without reducing grain yields. The objectives of this study were to compare N rates of sewage sludge and ammonium nitrate on soil profile (0-180 cm) inorganic N (NH_4-N and NO_3-N) accumulation, yields, N uptake and soil-plant inorganic N buffering in winter wheat.

MATERIALS AND METHODS

One winter wheat field experiment was established at the Efaw Experiment Station (Oklahoma State University, Stillwater, OK) in the fall of 1993 and 1994. For more than 10 yr before beginning this experiment, bermudagrass was grown at this site without any fertilization or tillage. This is reflected in the high soil organic C levels (Table 1). A complete factorial arrangement of treatments consisting of six N rates (0, 45, 90, 180, 270 and 540 kg N ha^{-1} yr^{-1}) and two N sources (ammonium nitrate, 34-0-0, and dried anaerobically digested sewage sludge) was evaluated. Two added treatments outside the factorial included lime applied in 1993 (4.48 Mg ha^{-1}) to the high N rate (540 kg N ha^{-1} yr^{-1}) for both N sources. The experimental design was a randomized complete block with three replications. The soil at this location is a Norge loam (fine mixed, thermic Udertic Paleustoll). Baseline soil test levels, elemental analysis and pH of sewage sludge used is reported in Table 1. Wheat varieties and seeding rates, treatment application, soil sampling and harvest dates are reported in Table 2.

Surface soil samples (0-15 and 15-30 cm) were collected from all plots following harvest in 1994 and 1995. These samples were analyzed for pH, total N and organic C. In addition, following harvest in 1995, soil cores (4.5 cm diam.) were taken to a depth of 180 cm (0-30, 30-60, 60-90, 90-120, 120-150 and 150-180 cm) and analyzed for NH_4-N and NO_3-N. Inorganic soil profile N accumulation was determined using inorganic N concentration and measured bulk density to a depth of 180 cm. Soil pH was determined in 1:1 soil:deionized water. Total N and organic C was determined using dry combustion (Schepers et al., 1989). Soil analysis for NH_4-N and NO_3-N was accomplished by extracting 2-g samples with 2 M KCl and concentration determined using an automated flow injection system (Lachat 1989, 1990). Sewage sludge samples were digested with nitric and perchloric acid for determination of total heavy metal content (U.S. EPA, 1986). These extracts were analyzed for total Pb, Cd, Mo, As, Se, Ni, Cu and Zn using inductively coupled argon plasma atomic emission spectroscopy. Grain yield was determined by harvesting the center 3.05 m of each plot (10 m in length) using a self propelled combine. Straw yields were determined by collecting all the residue from the same area used for grain yield. Wheat straw was uniformly distributed in all plots after harvest. Grain and straw subsamples were collected for moisture determination. Grain and straw samples were analyzed for total N using dry combustion (Schepers et al., 1989).

Analysis of variance for surface soil analysis and soil profile inorganic N accumulation following the second year harvest is reported in Table 3. A split-plot in space analysis of variance model was used to evaluate soil profile inorganic N accumulation. Analysis of variance was performed on grain and straw yields, N uptake in grain and N uptake in straw (Table 4). Single degree of freedom non-orthogonal contrasts were used to detect treatment differences. Linear plateau and linear models for grain yield were evaluated on N rates as ammonium nitrate and sewage sludge, respectively (Table 5).

An additional study was conducted in a controlled environment to determine the potential N mineralization of the soil and sewage sludge used. This was conducted using soil collected from the field experiment and using the same rates for selected treatments (Table 6). A modification of the aerobic incubation procedure described by Stanford and Smith (1972) and Douglas and Magdoff (1991) was used to determine the potentially mineralizable N. Ten g of soil (20 mesh) was mixed with perlite at a 1:1 ratio by volume and placed in a 2 by 5 cm tube. Glass wool pads were placed above and below the sludge-soil-perlite mixture, to minimize the movement of particles during the leaching process. Three replications of each mixture were incubated at 30^0 C for 22 weeks. Each column was leached with 30 mL of 0.01 M $CaCl_2$ at the beginning of the incubation and at 2, 4, 6, 8, 10, 14, 18, 22 weeks. Leachate from each tube was analyzed for NH_4-N and NO_3-N using an automated flow injection analysis system (Lachat 1989, 1990). Potentially mineralizable N (N_o) for each treatment was estimated using an exponential model, $Nm=N_o(1-e^{b1*week})$. Nm was the accumulated inorganic N (NH_4-N + NO_3-N) with time. Estimates of N_o, b1 and their respective lower and upper confidence intervals (p=0.05) were obtained using PROC NLIN of SAS (SAS Institute, 1988).

For the 1995 crop year fertilizer plant N uptake was estimated by subtracting grain+straw N uptake in the check from the fertilized treatment. These values were used to estimate plant N recovery in % (fertilizer N uptake times 100 divided by N rate applied). Because straw yield was not determined in 1994, fertilizer plant N uptake was not estimated for this year.

Soil-plant inorganic N buffering when N was applied as ammonium nitrate was assessed using non-linear regression models in 1995. A linear-plateau model was determined for grain yield on N rate and a plateau-linear model was evaluated for soil profile inorganic N accumulation on N rate. “Jointing” points and confidence intervals for each model were calculated. Grain yield on N rate and soil profile inorganic N accumulation on N rate models were compared. When the N rate needed to significantly increase soil profile inorganic N accumulation is greater than the N rate required for maximum grain yield, a soil-plant inorganic N buffering mechanism is implied (Raun and Johnson, 1995). Soil-plant inorganic N buffering could not be evaluated using non-linear regression for the sewage sludge N source, since increased soil profile inorganic N accumulation could not be detected at the highest N rate while grain yield continued to increase at the high N rate. The coefficient of determination of non-linear models was calculated by subtracting the residual sums of squares from the corrected total sums of squares and the product divided by the corrected total sums of squares.

RESULTS AND DISCUSSION

Soil Characteristics

The effect of treatment on surface soil analysis (pH, total N and organic C) and soil profile inorganic N accumulation from 1995 is reported in Table 3. Over this relatively short time period (2 yr) differences in surface soil analysis were not significant as was expected. It was interesting to observe that, for this soil with pH 6.4 and high pH buffer index (7.1), lime application did not affect pH measured following the second harvest (Table 3).

Nitrogen applied as sewage sludge for 2 yr had no effect on soil profile (0-180 cm) inorganic N accumulation (Figure 1). Alternatively, soil profile inorganic N accumulation increased significatively when N rates were at least 270 kg N ha^{-1} as ammonium nitrate. Because no differences in the sewage sludge treatments were noted at any rate, this suggest that the mineralization rate of the sludge applied was $\leq$ 50 %. Nitrogen as ammonium nitrate applied at an N rate of 270 kg ha^{-1} resulted in a significant bulge at 30-60 cm layer with 270 kg N ha^{-1}, and at 90-120 cm layer with 540 kg N ha^{-1} (Figure 2). Because the bulge at the 270 kg ha^{-1} N rate was at a depth less than that noted at the 540 kg N ha^{-1}, it suggests that the plant-soil system was able to buffer against NO_3-N leaching. Had buffering not been taking place, the bulges would have been present at the same depth. Lime applied with ammonium nitrate at the 540 kg N ha^{-1} rate resulted in lower inorganic N accumulation in the soil profile. Because no differences were found in grain and straw yield (540 N kg ha^{-1} + lime vs 540 kg N ha^{-1} with no lime), and more data was not obtained, this lime effect does not have a straight explanation. It could be that lime temporally raised the soil pH allowing ammonia volatilization on one hand. On the other hand, lime could enhance microbial activity which in turn immobilized N to decompose the accumulated residue of undecomposed grass and the straw incorporated after harvest. Denitrification could also have been increased as a result of lime application, since bacteria responsible for this process have optimum activity at pH from 6.0 to 8.0 (Sims, 1995).

Wheat Yields and N Uptake

Grain and straw yield and N uptake increased with increasing applied N in both years and for both N sources (Table 4). However, grain yield and grain N uptake peaked at the 270 kg N ha^{-1} rate using ammonium nitrate, except grain N uptake in 1994 which continued to increase only at the highest N rate, while grain yield and grain N uptake continued to increase at the higher 540 kg N ha^{-1} rate using sewage sludge. Straw yield and straw N uptake peaked at 270 kg N ha^{-1} rate using either N source, except straw N uptake which increased at the highest N rate as ammonium nitrate. Although grain yield maximums were expected to take place at much higher N loading rates using sewage sludge, the 540 kg N ha^{-1} rate was presumed to exceed the rate required for maximum yield. Similarly, grain yields peaked at 270 kg N ha^{-1} using ammonium nitrate which would be 203 kg N ha^{-1} more than what would be normally recommended (if 1 kg N ha^{-1} would produce 30 kg grain ha^{-1} then 67 kg N ha^{-1} would have been needed to produce 2004 kg grain ha^{-1}, which is the average grain yield of both years). The high rate of applied N as either ammonium nitrate or sewage sludge for maximum yields was likely due to a very large N-poor organic matter pool. Its decomposition implied a soil-solution N depletion by the microbial population growth. In 1994 the organic matter was from the bermudagrass (*Cynodon* *dactylon* L.) pasture, and in 1995, from bermudagrass leftover plus the straw incorporated after harvest 1994. Additional time will be needed to evaluate this effect. Low available N from sewage sludge also contributed to low yields with this N source. This fact will be discussed later. Lime with either N source did not affect wheat yields and N uptake (Table 4). In general maximum grain yields were higher in 1994 than in 1995. The low grain yields observed in 1995 were due to excessive rain and increased disease pressure.

The relationship between grain yield and N rates as ammonium nitrate was better explained by a linear-plateau model than by a linear model (Table 5). These linear-plateau models estimated maximum grain yields at 225 ($\pm$ 115) and 216 ($\pm$ 73) kg N ha^{-1} in 1994 and 1995, respectively. When comparing the linear segment of these models with the linear one obtained for grain on N rates as sewage sludge by year (Table 7), this N source (from 0 to 540 kg N ha^{-1}) had an estimated relative efficiency of 17% (in 1994) and 22% (in 1995) with respect to ammonium nitrate from 0 to 90 (in 1994) and 0 to 117 kg N ha^{-1} (in 1995). These estimates imply that 1324 (in 1994) and 982 kg of actual N ha^{-1} (in 1995) as sewage sludge would have been needed to obtain maximum grain yields, which were reached with ammonium nitrate at a rate of 225 and 216 kg N ha^{-1} in 1994 and 1995, respectively. These estimated efficiencies followed the same trend as those reported for anaerobically digested sewage sludge applied to a perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens* L.) sward when considering dry matter yield over a 2-yr period (O'Riordan et al., 1987). In this study sewage sludge as N source was 20 (first year) and 31% (second year) efficient with respect to calcium ammonium nitrate. Our results are also in agreement with the 20 % mineralization suggested by EPA when anaerobically digested sewage sludge is land applied for crop production (U.S. EPA, 1983). Since the 20% mineralizable N (based on incubation studies conducted in the laboratory) suggested by EPA is an estimate of the plant available N from sewage sludge, the relative N use efficiency for grain (our study) or dry matter yield (O'Riordan et al., 1987) also estimates the plant available N under field conditions.

N Available from Sewage Sludge and Fertilizer N Uptake

A controlled mineralization study including selected rates of N as sewage sludge applied in the field was conducted. Using cumulative data of inorganic N ($Nm=NH_4\text{-}N + NO_3\text{-}N$) the N potentially mineralizable (N_o) for each treatment incubated was estimated using an exponential model, $Nm=N_o(1\text{-}e^{b1*week})$. Parameters of the model and their respective 95% confidence intervals are reported in Table 6. Using the estimated N_o, available N derived from sewage sludge was estimated by the difference method. Although these results are presented in kg ha^{-1}, these equations cannot be extrapolated to field conditions due to the altered temperature and soil moisture. Therefore these equations can only be used as an index. Treatments 1 to 4 (Table 6) simulated the fate of N as sewage sludge (1993) applied to a soil previously maintained as a bermudagrass pasture which was subsequently plowed (year 1). N_0 from treatments 1 to 4 (Table 6) shows a net immobilization of N when 45 to 180 kg N ha^{-1} were applied and only 63 kg N from the 540 kg N ha^{-1} applied would have been mineralized in the 1993-1994 crop cycle. Treatments 5 to 8 simulated the fate of N as sewage sludge applied to the same soil which had been cropped for 1 yr without fertilization (year 2, Table 6). Estimated N_o from these treatments was much greater, especially at rates > 45 kg N ha^{-1}. These results can be explained by the higher N demand during organic matter decomposition in the first year (year 1) than in the soil that had been cropped for 1 yr (year 2). The remaining treatments (9 to 14, Table 6) simulated the amount of N released from sewage sludge following one cropping cycle. If no additional N was applied other than that from 1993, only the 540 kg N ha^{-1} rate will continue to show net N mineralized during the crop cycle 1994-1995 (year 2r, Table 6). When the same sewage sludge N rates were applied again in 1994, mineralized N rates were similar to that noted for year 2 results (6, 7, 8 vs 12, 13, 14). Another mineralization study using four types of sewage sludge at a rate of 250 to 398 kg N ha^{-1} showed N immobilization during a period of 22 weeks (Sims, 1990). In this study, wheat plants that had received N rates from 0 to about 800 kg ha^{-1} as sewage sludge, exhibited N deficiency symptoms, reduced dry matter and low plant N content, although complementary ammonium nitrate had been added.

Incubation results were compared with fertilizer N uptake in grain and fertilizer N uptake in grain+straw (Table 7). Maximum potentially mineralizable N from sewage sludge (potentially available N times 100 and divided by total N rate applied) was 12% in the 1993-1994 and 29% in the 1994-1995 crop cycle .

Although there is only one potential mineralizable N value for a specific sewage sludge, during the incubation the interaction of this N source at different rates with soil did not allow to determine such value. However, the potentially mineralizable N average value (21%) of this study is in the range of values (13-49%) determined by incubation for a wide variety of anaerobically digested sewage sludges (Sims, 1995). Relatively low fertilizer grain N uptake was recorded in 1994 with N rates as sewage sludge from 45 to 180 kg ha^{-1}. This reflects net N immobilization in 1993-1994, accordingly to the incubation study. Nitrogen rates as sewage sludge of 540 kg N ha^{-1} were associated with fertilizer grain N uptake values higher than those obtained with equal or less than 180 kg of N ha^{-1}, which is in agreement with the N release from 540 kg N ha^{-1} as sewage sludge in the 1993-1994 crop cycle simulated in the laboratory. In 1995, fertilizer N uptake in grain+straw was compared with the values of N released in the incubation study. Low fertilizer grain+straw N uptake was observed for N rates as sewage sludge from 45 to 180 kg ha^{-1}, but as N rates increased, N uptake increased, which is in agreement with the release of N found in the incubation study when the N rate was 180 and 270 kg N ha^{-1} as sewage sludge. The same trend of low fertilizer N uptake in grain (1994) and grain+straw (1995) was observed when a low rate of N was applied as ammonium nitrate, but no equivalent treatment in the incubation study was available for comparison.

Lime application with either N source did not affect fertilizer N uptake in grain and grain+straw.

Considering the fertilizer grain+straw N uptake in 1995, wheat N recovery ranged from 15 to 6% with rates from 45 to 540 kg ha^{-1} as sewage sludge, and from 27 to 11% with rates from 90 to 540 kg ha^{-1} as ammonium nitrate (18% of 45 kg N ha^{-1} was recovered by wheat). These figures show that wheat takes up more N from ammonium nitrate than sewage sludge. These values for sewage sludge N uptake (recovery) by wheat are in agreement with findings for other grain and vegetables crops: 8-14% of N applied (Larsen and Petersen, 1993). However, ammonium nitrate N uptake (recovery) by wheat was small compared to the 44-57% fertilizer use efficiency for ammonium sulfate (50 and 100 kg N ha^{-1}) applied to winter wheat (Olson et al., 1979).

In general N immobilization found in the incubation study and the low fertilizer N uptake in grain and grain+straw are in agreement with soil profile inorganic N found after harvest in 1995. None of the N rates as sewage sludge resulted in soil profile inorganic N accumulation, which indicates a net N immobilization (Figure 1). Nitrogen rates as ammonium nitrate from 45 to 180 kg ha^{-1} did not result in residual accumulation of inorganic N, but with 270 and 540 kg N ha^{-1} significant amounts of soil profile inorganic N accumulation were observed. Leaching was unlikely in these plots as was observed in the soil core analysis performed after harvest 1995 (Figure 2). At depths $\geq$ 120 cm, soil profile inorganic N was reduced to about the same levels observed in the check plot. It seems that N loss as gas in this study could be low compared to the N rate applied. Nitrogen applications of 56 to 224 kg N ha^{-1} as ammonium nitrate to barley (*Hordeoum vulgare*) resulted in 0.93 to 1.43 kg N ha^{-1} lost as N_2O, representing only 0.05% of the N applied, while addition of 16.7 and 83.5 ton

ha^{-1} of sewage sludge resulted in 1.09 and 4.19 kg lost as N_2O ha^{-1} and emitted to the atmosphere (Mosier et al., 1982).

Soil-Plant Inorganic N Buffering

Following harvest in 1995, only N rates of 270 and 540 kg ha^{-1} as ammonium nitrate resulted in significant soil profile inorganic N accumulation. Because grain yields peaked at such high N rates soil-plant buffering was not observed (Figure 3) as has been reported by Raun and Johnson (1995). In our study a previously stable bermudagrass pasture apparently led to N immobilization over this short period of time at N rates < 270 kg ha^{-1}. Nitrogen immobilization detected in the simulation study for sewage sludge besides wheat N uptake and possible loss of N as gas from soil and plant explain the soil-plant inorganic N buffering found for this winter wheat system at N rates $\leq$ 173 kg ha^{-1} as ammonium nitrate (Figure 3) and at all N rates as sewage sludge.

CONCLUSIONS

Nitrogen applied as sewage sludge or ammonium nitrate did not affect the soil surface (0-30 cm) pH, organic C and total N after the second winter wheat harvest. Lime addition with either source of N did not affect these soil surface variables.

Soil profile inorganic N accumulation (0-180 cm) was present only for N rates of 270 and 540 kg ha^{-1} as ammonium nitrate. Less soil profile inorganic N accumulation was detected with lime application. Lime could have increased NH_3 volatilization, denitrification and N immobilization by microbial activity to decompose organic matter, but wheat yields and N uptake were not affected.

In general, wheat yields and N uptake increased linearly with applied N as sewage sludge while wheat yields and N uptake peaked at 220 kg N ha^{-1}, when N was applied as ammonium nitrate. Sewage sludge application had an estimated relative N use efficiency in grain production of 20% (average of two harvests) with respect to ammonium nitrate, so five-fold more total N as sewage sludge needs to be applied with respect to the N rate as inorganic N required to meet the plant N requirement. This field observation agrees with 20% mineralizable N suggested by EPA for land application of anaerobically digested sewage sludge. Maximum plant N recoveries was 16% for sewage sludge and from 27% for ammonium nitrate in 1995.

Soil-plant N buffering evaluated after the second winter wheat harvest was present at N rates $\leq$ 173 kg ha^{-1} as ammonium nitrate (Figure 3) and at all N rates as sewage sludge.

Net N immobilization from sewage sludge was found under controlled conditions for N rates of 45 to 180 kg ha^{-1} during the first crop cycle and for 45 kg N ha^{-1} in the second crop cycle. This was consistent with detected low fertilizer grain N uptake (1994), fertilizer grain+straw N uptake (1995) and soil profile inorganic N accumulation (following harvest in 1995). Nitrogen immobilization was attributed to high N demand to decompose the accumulated residue of undecomposed grass from several years growth and the straw incorporated after harvest.

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Table 1. Chemical characteristics† of the experimental site prior to treatment application and elemental analysis of sewage sludge.

Soil (0-15 cm)

pH	Organic C	Total N	NH_4-N	NO_3-N	Ca	Mg	K	Na	P	CEC
	g kg^{-1}		mg kg^{-1}							cmol kg^{-1}
6.4	11.9	1.1	7.0	5.0	88.7	23.2	25.3	0.27	30.2	13.05

Sewage sludge

Year	pH	Total N	As	Cd	Cu	Mo	Ni	Pb	Se	Zn
		g kg^{-1}	mg kg^{-1}							
1993	6.4	22	1.93	3.7	976	44.5	30.2	916	0.55	1115
1994	6.9	23	2.08	4.2	752	34.2	25.6	807	0.79	928

† pH, soil:deionized water 1:1 and sewage sludge:deionized water 1:2; organic C and total N, dry combustion; NH_4-N and NO_3-N from KCl extract; Ca, Mg, K, Na and P, Mehlich-3; CEC, 1 N NH_4OAc extract; heavy metals extracted with HNO_3 and $HClO_4$.

Table 2. Treatment application, soil sampling and planting dates, wheat varieties and rates, Stillwater, OK.

Treatment application	Planting	Variety and seed rate	Harvest	Soil sampling and depth (cm)
6 Oct. 93	7 Oct. 93	Karl, 67 kg ha^{-1}	8 June 94	11 Oct. 94, 0-30
13 Oct. 94	14 Oct. 94	Tonkawa, 84 kg ha^{-1}	15 June 95	21 June 95, 0-180
				16 Aug. 95, 0-30

Table 3. Treatment means and analysis of variance for soil surface (0-30 cm) pH, organic C and total N, and soil profile (0-180 cm) inorganic N accumulation, Stillwater, OK, 1995.

				Surface			Profile
				pH	Organic C	Total N	Inorganic N
					------- g kg^{-1} -------		kg ha^{-1}
Treatment means:							
No.	kg N ha^{-1}	N source†					
1.	0	-		6.65	8.20	0.76	163
2.	45	SS		6.60	7.13	0.70	134
3.	90	SS		7.10	7.07	0.67	92
4.	180	SS		6.41	7.98	0.72	167
5.	270	SS		6.97	8.13	0.77	183
6.	540	SS		6.82	8.46	0.79	139
7.	45	AN		7.22	7.09	0.67	154
8.	90	AN		7.23	7.58	0.68	135
9.	180	AN		6.81	8.37	0.75	165
10.	270	AN		6.50	7.23	0.65	303
11.	540	AN		6.58	7.66	0.72	750
12.	540	SS + L		7.05	9.43	0.77	149
13.	540	AN + L		6.99	9.41	0.80	481
SED‡				0.47	0.90	0.06	46
Analysis of variance:							
------ Source -------			df	-------------	Mean	squares	-----------
Replication			2	0.97	5.19*	0.048**	31690**
Treatment			12	0.24	1.94	0.007	824445**
N rate linear (SS)			1	0.07	1.16	0.007	305
N rate quad (SS)			1	0.00	2.18	0.018	586
N rate linear (AN)			1	0.38	0.01	0.001	333960**
N rate quad (AN)			1	0.56	0.05	0.004	201726**
Lime effect (SS)			1	0.08	1.43	0.001	114
Lime effect (AN)			1	0.26	4.59	0.008	72665**
Residual			24	0.34	1.22	0.006	2139
CV, %				8.48	13.83	10.30	20

† SS, sewage sludge; AN, ammonium nitrate; L, lime applied in 1993.
‡ SED, standard error of the difference between two equally replicated means.
*,** Significant at the 0.05 and 0.01 probability levels, respectively.

Table 4. Treatment means and analysis of variance for grain and straw yields and N uptake in grain and straw, Stillwater, OK, 1994-1995.

			Grain				Straw	
			Yield 94	Yield 95	N uptake 94	N uptake 95	Yield 95	N uptake 95
			kg ha^{-1}					
Treatment means:								
No.	kg N ha^{-1}	N source†						
1.	0	-	422	510	9	14	1194	7
2.	45	SS	527	571	11	16	2101	12
3.	90	SS	628	574	13	16	2270	14
4.	180	SS	886	569	19	17	2571	16
5.	270	SS	1038	611	24	19	2973	22
6.	540	SS	1505	1027	31	32	2712	21
7.	45	AN	1782	621	39	17	1917	13
8.	90	AN	1739	906	38	28	2570	18
9.	180	AN	1921	1196	47	36	2361	28
10.	270	AN	2588	1419	68	44	3089	33
11.	540	AN	2337	1282	69	40	2759	40
12.	540	SS + L	1669	1052	38	32	3011	23
13.	540	AN + L	2187	1326	62	41	3172	42
SED‡			174	179	5	6	380	4

Analysis of variance: Source	df	Yield 94	Yield 95	N uptake 94	N uptake 95	Yield 95	N uptake 95
		Mean squares					
Replication	2	168608*	15554	104	14	1889130**	205**
Treatment	12	1543352**	340561**	1309**	372**	919882**	349**
N rate linear (SS)	1	2230908**	312102*	960**	419**	4730073**	431**
N rate quad (SS)	1	151049	132748	56	144	859911*	6
N rate linear (AN)	1	6355922**	1838633**	6634**	2090**	5308459**	2299**
N rate quad (AN)	1	971096**	79124	120	71	880753*	1
Lime effect (SS)	1	40628	954	76	0	134374	8
Lime effect (AN)	1	33810	2865	66	2	256587	12
Residual	24	45554	47833	37	46	216160	27
CV, %		14	24	17	25	18	24

† SS, sewage sludge; AN, ammonium nitrate; L, lime applied in 1993.
‡ SED, standard error of the difference between two equally replicated means.
*,** Significant at the 0.05 and 0.01 probability levels, respectively.

Table 5. Linear and linear-plateau models for grain yield (Y) on N rate as sewage sludge and ammonium nitrate, respectively, Stillwater, OK, 1994-1995.

Year	N source	Equation		Joint (N rate)	r^2
		kg ha^{-1}		kg ha^{-1}	
1994	Ammonium nitrate	Y = 929.461 + 6.811(N rate) Y = 2462	if N rate < 225 if N rate $\geq$ 225	225 $\pm$ 115†	0.67**
	Sewage sludge	Y = 454.609 + 2.0145 (N rate)		-	0.95**
1995	Ammonium nitrate	Y = 495.836 + 3.965 (N rate) Y = 1352	if N rate < 216 if N rate $\geq$ 216	216 $\pm$ 73†	0.81**
	Sewage sludge	Y = 476.053 + 0.893 (N rate)		-	0.49**

† 95% confidence intervals.
** Significant at the 0.01 probability level.

Table 6. Estimates of potentially mineralizable N (N_o) as affected by time (0 to 22 weeks), N rate and cropping sequence.

Treat-ment	Origin of soil†	Cropping sequence‡	N rate§	Sewage sludge	N available from sewage sludge	N_o¶	b1#	r^2
			kg ha^{-1}		--------- kg ha^{-1} ----------			
1	Check, 93	year 1	0	-		441 $\pm$ 35	0.106 $\pm$ 0.018	0.97
2	"		45	1993	-25	416 $\pm$ 35	0.120 $\pm$ 0.023	0.95
3	"		180	1993	0	441 $\pm$ 24	0.123 $\pm$ 0.010	0.98
4	"		540	1993	63	504 $\pm$ 27	0.144 $\pm$ 0.020	0.97
5	Check, 94	year 2	0	-		288 $\pm$ 15	0.145 $\pm$ 0.019	0.97
6	"		45	1994	- 2	286 $\pm$ 15	0.165 $\pm$ 0.043	0.96
7	"		180	1994	48	336 $\pm$ 17	0.155 $\pm$ 0.021	0.97
8	"		540	1994	133	421 $\pm$ 19	0.173 $\pm$ 0.021	0.97
9	Trt 2, 94	year 2r	45	-	-36	252 $\pm$ 15	0.130 $\pm$ 0.019	0.97
10	Trt 4, 94		180	-	1	289 $\pm$ 15	0.139 $\pm$ 0.018	0.97
11	Trt 6, 94		540	-	30	318 $\pm$ 23	0.139 $\pm$ 0.025	0.95
12	Trt 2, 94		45	1994	36	288 $\pm$ 23	0.140 $\pm$ 0.028	0.93
13	Trt 4, 94		180	1994	45	334 $\pm$ 17	0.151 $\pm$ 0.020	0.97
14	Trt 6, 94		540	1994	129	447 $\pm$ 25	0.177 $\pm$ 0.027	0.95

† From field study, before planting.
‡ year 1, native bermudagrass sod without fertilization tilled for the first time;
year 2, native bermudagrass sod that had been tilled and cropped to wheat without fertilization for 1 yr;
year 2r, native bermudagrass sod that had been tilled and cropped to wheat applied with sewage sludge for 1 yr.
§ Rates were applied considering a soil furrow slice of 15 cm depth, so the N_o is expressed in kg N ha^{-1} for the 0-15 cm soil layer.
¶ Mineralizable N = $N_o(1-e^{b1*week})$ and associated 95% confidence interval.
# Slope and 95% confidence interval.

Table 7. Treatment means and analysis of variance for fertilizer N uptake in grain (1994) and grain+straw (1995), Stillwater, OK.

			N_o†		Fertilizer	
			93-94	94-95	Grain N uptake 94	Grain+straw N uptake 95
			----------	----------	kg ha^{-1}	----------
Treatment means:						
No.	kg N ha^{-1}	N source‡				
2.	45	SS	-25	0	2	7
3.	90	SS			3	9
4.	180	SS	0	46	9	12
5.	270	SS			15	20
6.	540	SS	63	159	21	32
7.	45	AN			29	8
8.	90	AN			29	24
9.	180	AN			38	43
10.	270	AN			59	55
11.	540	AN			59	58
12.	540	SS + L			28	34
13.	540	AN + L			53	62
SED§					5	8
Analysis of variance:						
------ Source ------	df				---- Mean	squares ----
Replication	2				66	20
Treatment	11				1220**	1263**
N rate linear (SS)	1				738**	1095**
N rate quad (SS)	1				23	140
N rate linear (AN)	1				2421**	5214**
N rate quad (AN)	1				42	220
Lime effect (SS)	1				76	9
Lime effect (AN)	1				66	24
Residual	22				40	98
CV, %					22	33

† Potentially mineralizable N from incubation study.
‡ SS, sewage sludge; AN, ammonium nitrate; L, lime applied in 1993.
§ SED, standard error of the difference between two equally replicated means.
**,* Significant at the 0.01 and 0.05 probability levels, respectively.

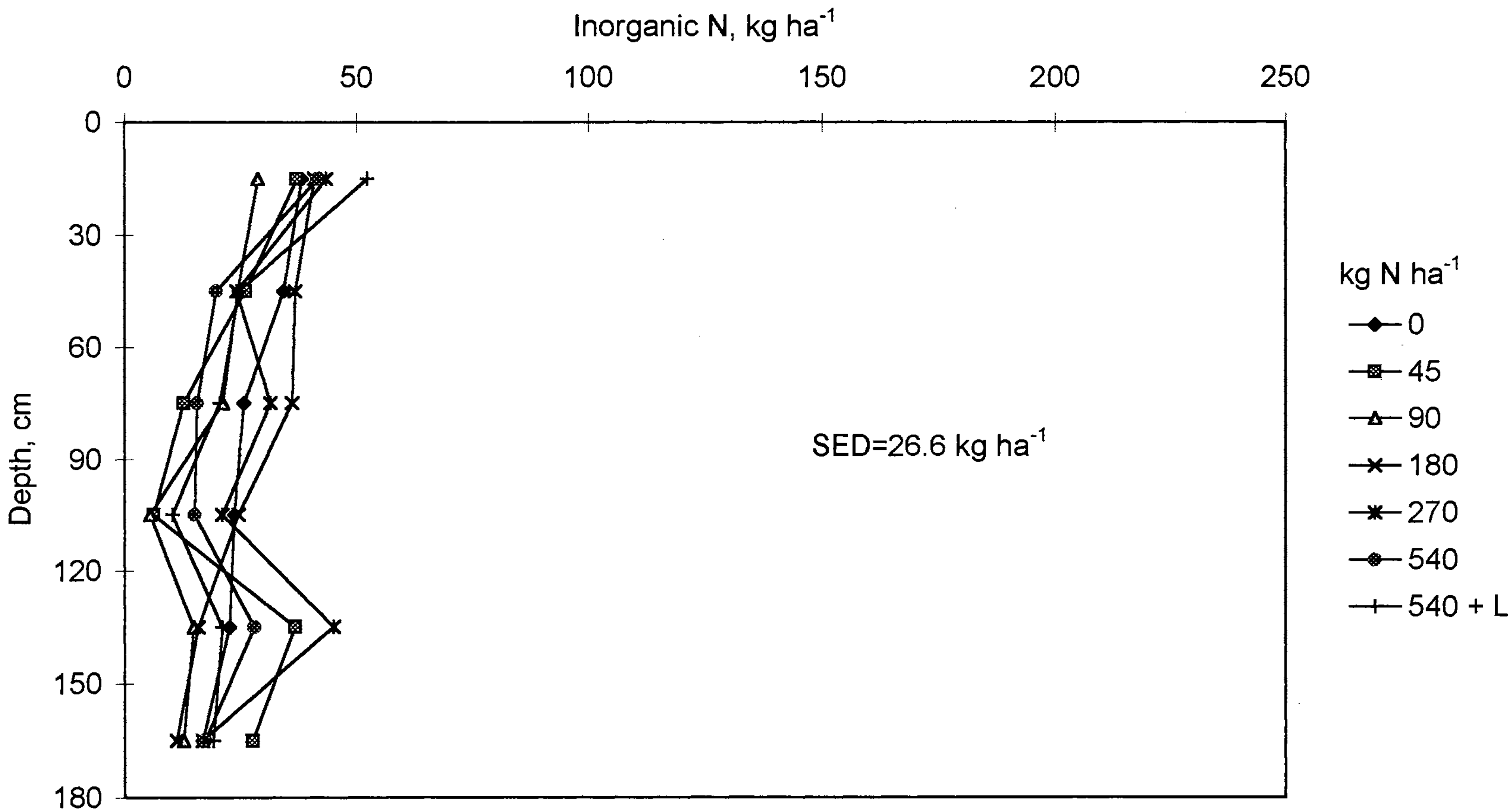

Figure 1. Soil profile inorganic N accumulation as affected by applied N as sewage sludge following two years of winter wheat production (L, lime)

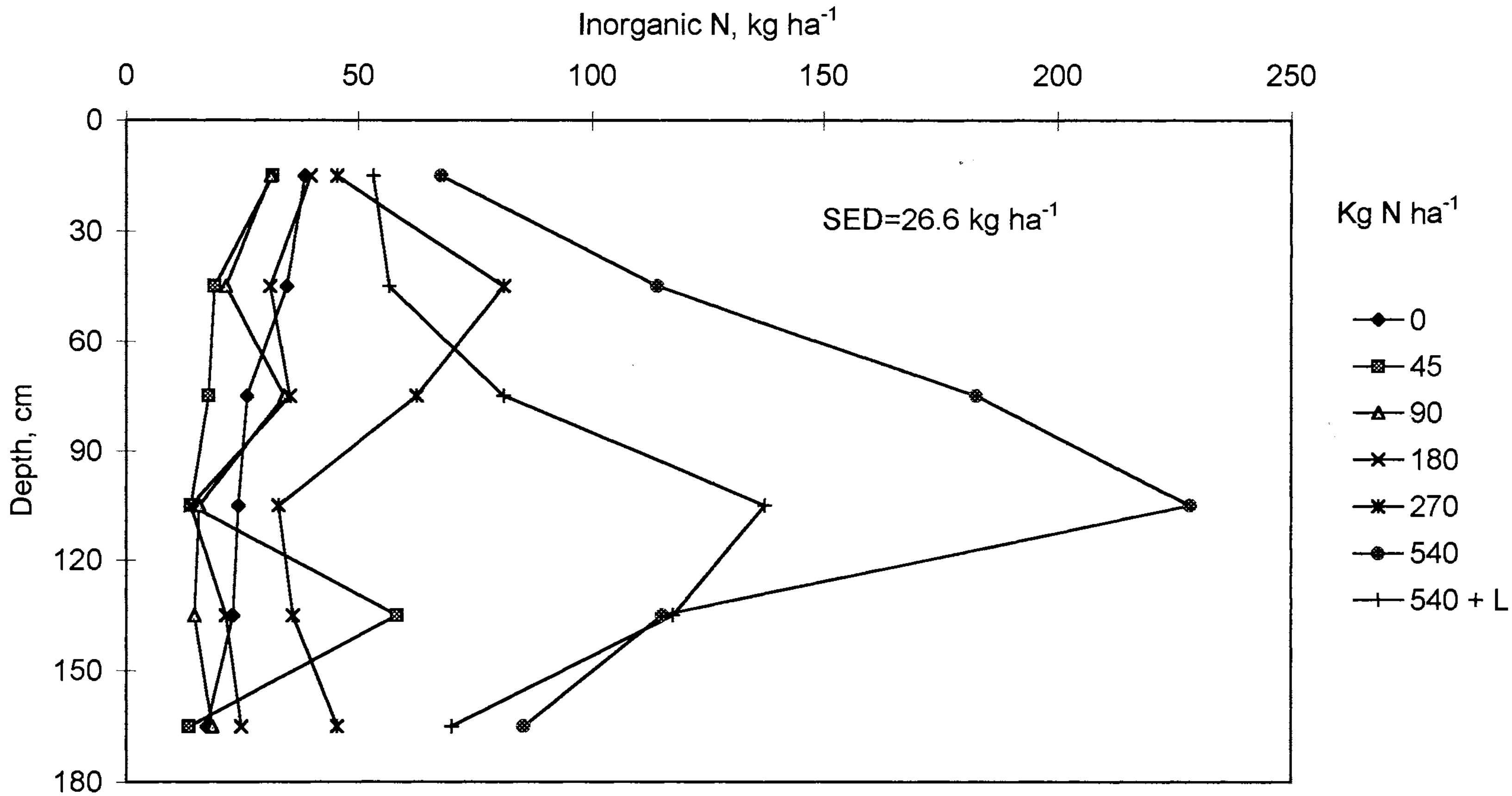

Figure 2. Soil profile inorganic N accumulation as affected by applied N as ammonium nitrate following two years of winter wheat production (L, lime)

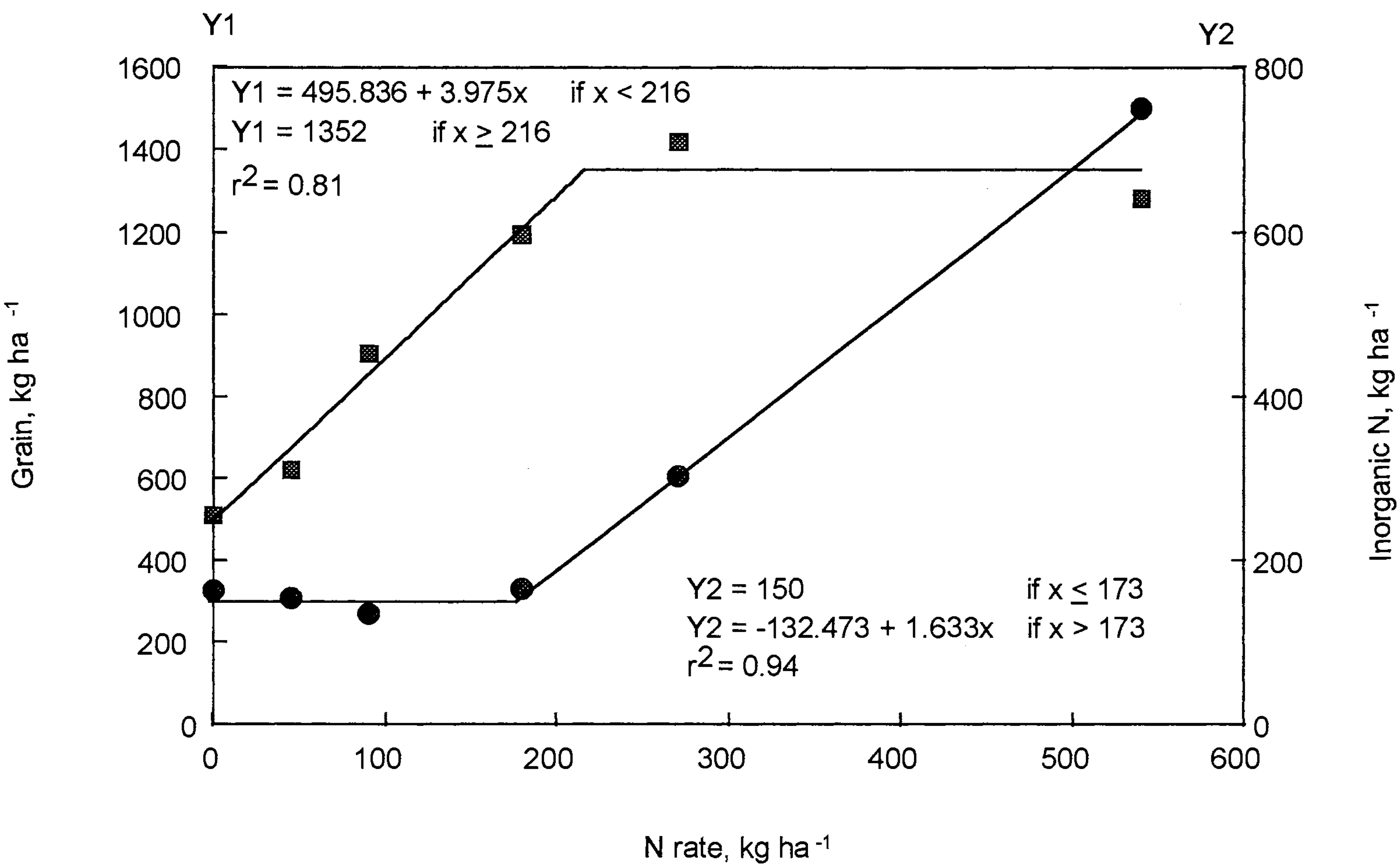

Figure 3. Soil-winter wheat inorganic N buffering. Grain yield and soil profile (0-180 cm) inorganic N accumulation on N rate as ammonium nitrate, Stillwater, OK, 1995

CHAPTER II

CADMIUM ACCUMULATION IN WHEAT GRAIN AS AFFECTED BY LONG-TERM N AND P FERTILIZATION AND SOIL ACIDITY

ABSTRACT

Earlier studies show that phosphorus fertilization increases soil Cd and that nitrogen use may increase Cd availability through soil acidification. The presence of acid soils has increased concerns associated with Cd uptake by wheat (*Triticum aestivum* L.). The objective of this study was to determine Cd accumulation in winter wheat grain under long-term N-P fertilization and different soil pH. Grain and soil samples were collected in 1993 from seven long-term experiments ranging from 16 to 64 yr of fertilization. Fertilizer N and P_2O_5 rates studied were up to 112 and 134 kg ha^{-1} yr^{-1}, respectively. In general, soil Cd, soil Zn and grain Zn contents were not affected by phosphate fertilization. Only small change in grain Cd (9 μg kg^{-1}) was found at one of the seven locations due to P fertilization, but this increase in Cd is not of health concern. No relationship between N fertilization, soil pH and Cd in grain was found within experiments. Correlation between soil pH and grain Cd, grain Zn, soil Cd or soil Zn presented low r^2 (< 0.39) within each experiment. Soil pH was also related to Cd (r^2 = 0.37)

and Zn (r^2 = 0.62) across all experiments. Total soil Cd increased linearly from 61 to 120 $\mu g\ kg^{-1}$ and total soil Zn from 11 to 24 $\mu g\ kg^{-1}$ in the range of soil pH from 3.8 to 6.8, however, grain Cd and Zn contents were higher at the more acidic soil pH. Across all experiments, accumulation of Cd in grain was directly related to soil acidity, not to N or P fertilizer applications. This relationship between soil pH (3.8 to 6.8) and grain Cd (9.7 to 45.2 $\mu g\ kg^{-1}$) was described by a plateau-linear-plateau model with a negative slope from soil pH 4.95 to 6.4. The maximum grain Cd content (45 $\mu g\ kg^{-1}$) determined by regression in this study is less than the maximum limits of 50-100 μg Cd kg^{-1} in wheat grain established in other countries, and lower than the grain Cd values reported in other long-term phosphate application studies.

INTRODUCTION

Problems associated with Cd accumulation in humans have been identified and include bone disease, lung edema, renal dysfunction, liver damage, anemia and hypertension (Nordberg, 1974; Nath et al., 1984). One of the pathways for Cd intake by humans is through various food products. Grain and cereal products are primary sources of this nonessential and toxic metal, accounting for about 20% of the daily intake of Cd in the U.S. adult population (Wagner et al., 1984).

Cadmium and other heavy metals in wheat grain have been documented in major U.S. growing areas uncontaminated by human activities other than normal agricultural practices (Wolnik et al., 1983). However, there is little information concerning the effect of agricultural practices (e.g., the use of fertilizers) on content of Cd in wheat grain under long-term continuous production systems. Because Cd is more mobile and bioavailable than most heavy metals, its concentration in phosphate fertilizers is of concern when applied to agricultural land (Andersson, 1977; McBride et al., 1981; Sánchez-Martín and Sánchez-Camazano, 1993).

Cadmium in winter wheat grain, from long-term experiments under continuous wheat increased from 5-25 $\mu g\ kg^{-1}$ in the 1910's to 56-76 $\mu g\ kg^{-1}$ in the 1970's in Sweden (Anderson and Bingefors, 1985). The same trend was also observed by Kjellstrom et al. (1975) and was attributed to fertilization, soil acidification and atmospheric deposition in both studies. Long-term experiments conducted at Rothamsted, UK from 1880 to 1980, demonstrated that inorganic N-P-K fertilization (144-35-90 kg $ha^{-1}\ yr^{-1}$) increased the Cd content in wheat grain from 50 to 80 $\mu g\ kg^{-1}$ (Jones and Johnston, 1989). One of the main concerns in the above studies was Cd input to winter crop systems from phosphate fertilization. The specific effect of P-fertilizer and soil acidification on grain Cd could not be tested because these experiments were not designed to measure these effects.

Soil Cd and grain Cd content changes over time have been studied in fields with a long-term history of phosphate fertilization. Comparison of wheat-cropped plots for > 30 yr (old) with < 4 yr plots (new) indicated soil Cd contents were higher in the old plots than in new plots, but grain Cd presented an opposite trend (Baerug and Singh, 1990). Cd accumulation in wheat due to P-fertilization was not observed in long-term experiments in the Netherlands (Smilde and van Luit, 1983; Mortvedt, 1987). Wheat from Australian fields with long histories of superphosphate usage were associated with low Cd content (22 $\mu g\ kg^{-1}$) in grain, (Williams and David, 1973). In two long-term (40 and 49 yr) experiments under continuous winter wheat in USA, long-term application of triple superphosphate did not result in Cd accumulation in wheat grain (Mortvedt, 1987). Except for this last study, the other studies were not designed to test the specific effect of P application on grain Cd accumulation, so the effect of long-term P fertilization on wheat grain Cd accumulation is not clear.

In relatively short-term experiments (< 5 yr), phosphate fertilization has been shown to increase Cd content in wheat grain. A positive rectilinear relationship between P rates and Cd in wheat grain was established for wheat in a 5-yr trial of continuous winter wheat (Williams and David, 1976).

Soil acidity increases available Cd and Cd uptake by crops (Lindsay, 1979; McLean and Bledsoe, 1992; Williams and David, 1976). Mortvedt et al. (1981) found different amounts of Cd accumulation in wheat grain depending upon the soil pH. Diammonium phosphate (DAP) artificially enriched with Cd (153 $\mu g\ kg^{-1}$) increased the Cd content of wheat grain at soil pH of 5.0, but the same DAP did not affect grain Cd content at soil pH of 5.8 (Mortvedt et al., 1981). Ammonium sulfate or ammonium nitrate increased Cd uptake by spinach (*Spinacia oleracea* L.) (Willaert and Verloo, 1992) and lettuce (*Lactuca sativa* L.) (Singh et al., 1988). This effect on spinach was attributed to the acidic effect of ammonium sulfate on soil, but the effect of ammonium nitrate on soil pH and Cd accumulation in lettuce was not investigated by the authors. A 5-yr field experiment in Australia demonstrated that the Cd content of wheat grain was slightly higher in a superphosphate plus ammonium nitrate treatment than from superphosphate alone (Williams and David, 1976). This higher grain Cd content may be due to the acidic effect of ammonium nitrate. After 16 yr of wheat-fallow rotation with 45 kg N ha^{-1} as ammonium nitrate every other year, reduction in soil pH and increased extractable Cd was detected (Follet and Peterson, 1988). In a nine-year continuous wheat experiment, N fertilization increased grain Cd content and this was attributed to the acidic effect of the ammonium nitrate on soil (Oliver et al., 1993), but soil acidity and N fertilization effects on grain Cd accumulation on continuous winter wheat crop systems are still in need of more explicit studies.

The objective of this study was to evaluate the effect of long-term P and N fertilization on Cd accumulation in wheat grain under continuous cultivation over a wide range of soil pH.

MATERIALS AND METHODS

Soil and wheat grain from eight long-term (16 to 101 yr) continuous wheat experiments were collected in June, 1993. Selected treatments with their respective 4 or 5 replications were sampled except Magruder. Magruder plots were established only with one replication. The wheat variety 'Karl' was planted at all locations. Each long-term experiment was designed to study the effect of annual N, P and K fertilization on winter wheat grain yield and soil characteristics. Soils in this study were Kirkland silt loam (fine, mixed, thermic Udic Paleustoll) in experiment 222 and Magruder plots, Tillman-Hollister clay loam (fine mixed, thermic Typic Paleustoll) in experiment 407, Grant silt loam (fine-silty, mixed, thermic Udic Argiustoll) in experiments 502 and 503 and Taloka silt loam (fine-mixed, thermic Mollic Albaqualf) in experiments 801 and 802. Additional site information and soil properties is reported in Table 1. The Magruder plots studied in this work were initially established to evaluate wheat production on native prairie soils without fertilization from 1893 to 1929. In 1930 ten fertilization treatments were established on these plots. The remaining trials reported in Table 1 are all continuous wheat experiments with variable N, P and K treatments. Specific treatments were selected for soil and grain analysis that included variable N and P rates for each long-term experiment.

Soil samples were air dried and ground to pass a 20 mesh screen. Soil pH was measured in 1:1 soil:deionized water (McLean, 1982). Total soil Cd and Zn content were determined by wet digestion with HNO_3 and $HClO_4$ and subsequent analysis by inductively coupled plasma atomic emission spectroscopy (ICP). Cation exchange capacity using NaOAc, pH 8.2 (Rhoades, 1982); organic carbon (Yeomans and Bremner, 1988) and iron oxides using citrate bicarbonate dithionate (Jackson, 1969) were determined for soils from check plots (experiments: Magruder, 222, 407, 502 and 801) or soils from plots with the lowest rate of P fertilizer (experiments: 503 and 802). Grain samples were oven dried at 70^0 C for 48 hours and ground with a stainless mill to pass a 100 mesh screen. Grain samples were dissolved by HNO_3 wet digestion as described by Zarcinas et al. (1987). Cadmium and Zn in grain digests were determined using graphite furnace atomic absorption spectrophotometry and ICP, respectively.

Analysis of variance using a randomized complete block design, was performed for each experiment (Table 2) to determine the long-term effects of P and N fertilization on soil pH and grain Cd (Table 3). Linear and quadratic effects of P rates on soil pH, grain Cd and Zn, soil Cd and Zn were evaluated using contrasts. The least significant difference test ($p < 0.05$) was used to detect effects of N rates and P rates where less than three rates were available. A three segmented regression model (including all locations) of grain Cd on soil pH was also evaluated. This regression model was of the form plateau-linear-plateau (Anderson and Nelson, 1975). The coefficient of determination of this non-linear model was calculated by subtracting the residual sums of squares from the corrected total sums of squares and the product divided by the corrected total sums of squares. A program which uses the NLIN procedure of the Statistical Analysis System (SAS, 1988) was developed to estimate the plateau-linear-plateau model with the smallest residual mean square. The SAS program to obtain this equation is presented in the Appendix. Correlation analysis between grain Cd versus soil Cd, soil Zn, grain Zn and soil Zn:Cd ratio were performed.

RESULTS AND DISCUSSION

Soil pH, soil and wheat grain Cd and Zn content for each experiment and fertilizer treatment are presented in Table 3. The Standard Error of the Difference (SED) was used to determine significant differences between two treatment means. Statistical significance ($P < 0.05$) between two treatments can be calculated by multiplying SED by appropriate t values (approximately t value of 1.8 for this study). Potassium was applied at the same rate in each experiment to prevent crop deficiencies. Therefore, only N and P fertilizer effects will be discussed. Soil and grain Zn were included in this study, because Zn and Cd interaction in nutritive solution can modify Cd accumulation in plants (McKenna et al, 1993).

The effect of N fertilization was significant on soil pH only in experiments 222 and 502. In these experiments 90 and 67 kg of N ha^{-1} yr^{-1} decreased the soil pH 0.27 and 0.36 units, respectively. The Magruder plots data suggest a decrease of half unit of pH due to 67 kg of N ha^{-1} yr^{-1}. Cadmium content of grain and soil, and Zn content of soil were not affected by N fertilization in most of the experiments. Nitrogen fertilization increased the Zn concentration in wheat grain only in experiment 801. Although nitrogen fertilizer reduced soil pH in some experiments, the same N rates did not affect Cd and Zn in grain and Cd and Zn in soil. Continuous application of ammonium nitrate and diammonium phosphate may decrease soil pH, but this decrease did not result in Cd and Zn accumulation in wheat grain. Other studies have reported N fertilizer increased uptake of Cd by plants. Cadmium uptake by spinach (Willaert and Verloo, 1992) or lettuce (Singh et al., 1988) was increased by nitrogen application as ammonium sulfate or ammonium nitrate, respectively. The increase in Cd in spinach was attributed to the acidic effect of N fertilizer on soil (Willaert and Verloo, 1992), but in the case of lettuce the soil pH was not measured. In a 9-yr continuous wheat, N fertilization increased grain Cd content and this was attributed to the acidic effect of the ammonium nitrate on soil (Oliver et al., 1993). A 5-yr field experiment in Australia found the Cd content of wheat grain was slightly higher in a superphosphate plus ammonium nitrate (290 kg ha^{-1}) treatment than from superphosphate alone (Williams and David, 1976). In these two cases, perhaps the higher grain Cd content may be due to the acidic effect of ammonium nitrate, but the effect of nitrogen fertilizer on soil pH was not verified. In another study, after 16 yr of wheat-fallow rotation with 45 kg N ha^{-1} as ammonium nitrate every other year, reduction in soil pH and increased extractable Cd in soil was detected (Follet and Peterson, 1988), but Cd in grain was not evaluated.

When comparing specific treatments effect of P rates on soil pH were not detected. No linear or quadratic relationship between P_2O_5 and Cd in grain were found in experiments with at least three rates of P fertilizer (Table 2). In general, P fertilizer did not affect grain Cd (Table 3). Grain Cd content was increased by 8 $\mu g\ kg^{-1}$ with 45 kg of P_2O_5 ha^{-1} yr^{-1} only in experiment 407. However, the grain Cd (15 $\mu g\ kg^{-1}$) content obtained with 45 kg of P_2O_5 ha^{-1} yr^{-1} is one of the lowest found in this work and long-term studies reported elsewhere (Table 5). Wheat grain was not harvested in experiment 802 because of the strong soil acidity and lack of yield; only one plot was harvested. The lack of yield was due to Al toxicity at soil pH of 4.0 (Johnson et al., 1991).

Phosphorus rates were related to soil Cd in experiments 502 and 802 (Table 2). In experiment 802, increasing rates of P_2O_5 from 0 to 269 kg ha^{-1} yr^{-1} were negatively correlated with soil Cd, but soil Cd was increased to 67 mg kg^{-1} (Table 3) with the highest P rate studied: 672 kg P_2O_5 ha^{-1} yr^{-1} (soil Cd = $54.7126-0.1009P+0.0002P^2$, $r^2 = 0.42$). In the another experiment (502), a quadratic relationship between soil Cd and P rates was found, but none of the soil Cd values was different from the soil Cd of the check plot (Table 3). Except for the high P rate of experiment 802, P fertilizer did not increase soil Cd.

Several studies have documented Cd accumulation in wheat grain (Table 4). Although several of these studies suggest fertilizer may be a source of grain Cd accumulation, only two studies (Mortvedt, 1987; Williams and David, 1976) in Table 5 were designed to investigate the effect of P fertilizer on Cd accumulation in grain. Accumulation of Cd in grain from P fertilizer was studied for two long-term experiments; Sanborn field at the University of Missouri and the Magruder plots at Oklahoma State University.

After 40 yr of continuous application of phosphorous fertilizer in Sanborn field, levels of Cd in soil were increased from 290 to 426 $\mu g\ kg^{-1}$, but Cd in grain was lower in the fertilized plot compared to the check. Apparently continuous phosphorous application in wheat systems result in lower wheat grain Cd (Mortvedt, 1987). Changes in soil Cd contents were not observed after 49 yr of continuous P application in winter wheat in the Magruder plots, and grain Cd content were lower in the plot with phosphorus (30 $\mu g\ kg^{-1}$) compared to the check (58 $\mu g\ kg^{-1}$) (Mortvedt, 1987). These findings are consistent with our grain Cd findings of the Magruder plots after 64 yr of continuous application. Grain Cd was lower in P-applied plot (22 $\mu g\ kg^{-1}$) compared to the check (32 $\mu g\ kg^{-1}$), but soil Cd followed an opposite trend compared to the results for Magruder plots reported by Mortvedt (1987). In our study, the check plot contained more Cd than the fertilized plot.

However after 5 yr of phosphate fertilization, P rates (0 to 138 kg P_2O_5 $ha^{-1}\ yr^{-1}$) were linearly related to wheat grain Cd (30 to 60 $\mu g\ kg^{-1}$) and soil Cd (8 to 28 $\mu g\ kg^{-1}$) accumulation (Willams and David, 1976).

In other long term studies, increase in wheat grain Cd have been observed when studying long-term continuous wheat (> 56 yr). Kjellstrom et al. (1975), analyzed wheat grain samples taken between the years 1916 and 1972, and found an increase of grain Cd with time. Andersson and Bingefors (1985) analyzed grain samples from fields within the same limited area as Kjellstrom et al. (1975) and also found a systematic increase of grain Cd (25 to 56 $\mu g\ kg^{-1}$) with time in a 62-yr period. Both authors suggest that the observed grain Cd increase may be due to fertilizers, and atmospheric deposition. However, these studies were not designed to study P and it is uncertain whether Cd increases were due to P application. Our study does not show accumulation of Cd in grain due to P.

No relationship between grain Zn and P rates was found in any of the experiments (Table 2). In general, P fertilizer did not increase grain Zn (Table 3). Only when the rate of P (134 kg ha^{-1} yr^{-1}) was higher than the recommended for maximum wheat yield and in the most acidic soil studied (experiment 801), a significant increment of Zn in grain was observed.

Although a significant quadratic relationship was found between soil Zn and P rates in experiment 801, the range of values found was very narrow (0.9 mg kg^{-1}). Phosphorus fertilizer had little effect on soil Zn content.

Fertilizer was applied only once at the beginning of a 16-yr period in experiment 802. The P rate applied was 1344 kg of P_2O_5 ha^{-1} which would be equivalent to 84 kg of P_2O_5 ha^{-1} yr^{-1} applied annually over a 16-yr period. Comparison of treatment 11 of experiment 801 with treatment 6 of experiment 802 (Table 3) suggests applying similar amounts of fertilizer all at once or over a 16-yr period had the same effect on soil pH, grain Cd and Zn, and soil Cd and Zn.

Although a statistical analysis cannot be performed for the Magruder plots in the present study, results from these plots suggest long-term fertilization did not increase grain Cd or Zn and soil Cd and Zn (Table 3). In fact, soil Cd is higher in the check plot than in the fertilized plots. Similar results were reported by Mortvedt (1987) for grain and soil samples collected from the same check and phosphate-treated Magruder plots (Table 4). These observations strongly suggest that long-term continuous P fertilization does not result in elevated grain Cd.

In general, soil pH values were not or low correlated with Cd and Zn in grain or Cd and Zn in soil contents in each experiment. In the cases where pH was significant in explaining the variation of some response variables, the r^2 of the regression models were low (Table 5). However, many studies have shown that grain Cd is inversely related to soil pH (Bingham et al. 1979; Logan and Chaney, 1983; Meyer et al., 1982). The lack of a strong relationship between soil pH and grain Cd in this study may be attributed to the narrow ranges of soil pH and grain Cd observed within each experiment. However, a wide range of soil pH and grain Cd were observed across all experiments (Table 3). Grain Cd and soil pH showed a plateau-linear-plateau relationship across all experiments (Figure 1). The sign of the regression slopes detected in each experiment (Table 5) were consistent with their respective segments in the regressions across all experiments. Cadmium solubility and bioavailability is increased under acidic conditions (Lindsay, 1979; McLean and Bledsoe, 1992). A negative linear relationship between soil pH and grain Cd existed between soil pH 4.95 to 6.24. At soil pH< 4.95 Cd grain contents remained constant at 45 $\mu g\ kg^{-1}$ while at soil pH> 6.24 remained constant at 9.9 $\mu g\ kg^{-1}$.

Regarding grain Cd accumulation, the 45 μg Cd kg^{-1} grain content represents the worst case scenario for the wheat cultivar used, since wheat can not grow in soil that are more acidic than pH 3.7. This grain Cd content is about a half the safety limit for Cd in wheat grain (100 $\mu g\ kg^{-1}$) established in European countries (Chaney, 1994) and less than the maximum limit (50 $\mu g\ kg^{-1}$) for unspecified foods in Australia (Oliver et al., 1993). In USA maximum limit of Cd in wheat has not been established because Cd risk has not been identified from normal wheat grain production (Chaney, 1994). Major U.S. growing areas produce grain wheat with 43 μg Cd kg^{-1} in average (Wolnik et al., 1983), which is comparable to average values of Cd found in major growing regions of Britain: 52 (1982), 42 (1992) and 37 (1993) μg Cd kg^{-1} (Chaudry et al., 1995).

Total Cd and Zn in soil were positively and linearly related to soil pH across all experiments (Fig. 2 and 3). The largest soil Cd and Zn contents were associated with high pH values. However, the smallest grain Cd and Zn values were found for soils that contained the largest amount of soil Cd and Zn. Therefore, grain Cd content was not affected by soil Cd and Zn contents.

Several studies have shown that Cd, Zn and the ratio Zn:Cd in solution can affect accumulation of grain Cd (Adriano, 1986). In general a strong antagonistic Zn effect on Cd accumulation in crops at low solution Cd but not at high Cd level is found (McKenna et al., 1993; Adriano, 1986). Grain Cd as affected by soil Cd and Zn and grain Zn was determined by correlation analysis. Correlation was performed between Cd in grain versus soil Cd, soil Zn, Zn:Cd in soil and grain Zn. There was a significant positive linear relationship between grain Cd and grain Zn in experiment 503 ($r=0.71234$, $p=0.0004$). Across all experiments a significant negative linear relationship was found between grain Cd and soil Zn ($r=-0.42543$, $p=0.0001$). Soil Cd and Zn:Cd were not correlated to grain Cd across experiments.

CONCLUSIONS

Nitrogen fertilizer decreased soil pH in two experiments but these decreases were small and did not affect grain Cd. In general, nitrogen fertilization did not affect Cd and Zn in grain or Cd and Zn in soil. In general phosphorous fertilization did not affect grain and soil Cd contents or grain and soil Zn contents. Correlation between soil pH and grain Cd, grain Zn, soil Cd or soil Zn presented low r^2 (< 0.39) within each experiment.

Across all experiments, soil pH was strongly related to Cd and Zn availability. Total soil Cd and Zn increased with soil pH. However, grain Cd and Zn contents were higher at the more acidic soil pH. Soil pH is a more important factor than N or P fertilizer in determining accumulation of Cd in wheat grain. Accumulation of Cd in grain was directly related to soil acidity, not to N or P fertilizer applications.

The maximum grain Cd contents (45 $\mu g\ kg^{-1}$) determined by regression in this study is less than half the maximum limits of 50 and 100 μg Cd kg^{-1} in wheat grain established in other countries, and lower than the values reported in some long-term phosphate application studies. This figure (45 μg Cd kg^{-1}) represents a worst case scenario for this cultivar of hard red winter since wheat cannot grow in soils that are more acidic. Accumulation of Cd in wheat grain can be minimized by management practices that prevent strongly acidic conditions.

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Table 1. Description of long-term continuous wheat experiments and soil characteristics.

Location	Experiment	Year	Soil series	Texture	CEC	Organic C	Fe_2O_3†
					cmol kg^{-1}	g kg^{-1}	
Altus	407	1966	Tillman-Hollister	Clay loam	17.90	10.4	0.13
Haskell	801	1977	Taloka	Silt loam	5.60	8.6	0.12
Haskell	802	1977	Taloka	Silt loam	5.95	12.2	0.12
Lahoma	502	1970	Grant	Silt loam	10.4	9.6	0.12
Lahoma	503	1970	Grant	Silt loam	11.5	9.9	0.12
Stillwater	Magruder	1892 (1929)‡	Kirkland	Silt loam	13.25	8.1	0.19
Stillwater	222	1969	Kirkland	Silt loam	16.95	9.7	0.17

† Fe_2O_3 content expressed as Fe on elemental basis.
‡ Specific year when inorganic P fertilization was started.

Table 2. Analysis of variance for soil surface (0-15 cm) and wheat grain analysis at harvest of long-term fertilized winter wheat systems, Oklahoma, 1993.

Experiment	Source†	df	Mean squares				
			Soil			Grain	
			pH	Cd	Zn	Cd	Zn
222	Replication	3	0.013	867.23	2.07	197.15**	18.84
	Treatment	5	0.121**	1101.89	4.17	22.98	9.67
	P	1	0.008	12.01	5.51	20.16	3.69
	P^2	1	0.025	1463.06	12.96	1.32	5.09
	Error	15	0.017	1039.59	9.53	13.99	11.07
	CV		2.33	35.52	16.56	24.06	14.74
407	Rep	5	0.341*	679.78	48.12	18.48	77.63
	Treatment	2	0.028	307.25	20.07	118.57*	0.84
	Error	9	0.056	541.87	19.94	16.47	49.07
	CV		3.53	18.43	19.00	42.79	21.70
502	Replication	3	0.022	79.92	6.80	53.96	36.75*
	Treatment	4	0.136*	417.83	5.29	36.47	11.49
	P	1	0.006	114.50	0.50	0.00	0.00
	P^2	1	0.001	1120.67*	18.03	75.07	0.30
	Error	12	0.023	221.13	8.24	42.87	8.15
	CV		2.80	15.47	16.97	14.65	13.50
503	Replication	3	0.024*	517.13	11.19	28.23	40.70
	Treatment	4	0.009	121.57	1.34	8.57	12.24
	P (map)	1	0.000	1.13	1.53	1.64	41.41
	P^2 (map)	1	0.002	30.38	3.01	30.66	0.14
	P (dap)	1	0.000	128.00	1.28	0.01	1.20
	P^2 (dap)	1	0.029	322.67	2.16	1.90	1.76
	Error	12	0.005	537.18	5.26	24.34	16.24
	CV		1.40	21.05	11.69	11.12	18.73
801	Replication	3	0.307	590.51	0.77	13.43	40.59
	Treatment	5	0.278	345.03	0.75**	53.20	158.28**
	P	1	0.470	1193.52	0.38	81.99	54.12
	P^2	1	0.080	18.10	0.68*	61.32	2.40
	Error	15	0.126	673.62	0.11	25.48	18.47
	CV		8.37	36.95	2.68	11.18	10.64
802	Replication	3	0.011	349.78	0.33	-	-
	Treatment	5	0.232	373.46*	0.23	-	-
	P	1	0.003	525.04*	0.00	-	-
	P^2	1	0.013	878.89*	0.00	-	-
	Error	15	0.005	102.66	0.32	-	-
	CV		1.78	18.85	5.91	-	-

† P, P rate linear effect; P^2, P rate quadratic effect; map, monoammonium phosphate; dap, diammonium phosphate; CV, coefficient of variation in %.
*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

Table 3. Treatment means for soil surface (0-15 cm) and wheat grain analysis at harvest of long-term fertilized winter wheat systems, Oklahoma, 1993.

Experiment	Treatment	Fertilizer†			Total P_2O_5 loading	Soil			Grain	
		N	P_2O_5	K_2O		pH	Cd	Zn	Cd	Zn
		kg ha^{-1} yr^{-1}			kg ha^{-1}		μg kg^{-1}	mg kg^{-1}	μg kg^{-1}	mg kg^{-1}
222	10	0	0	0	0	5.9	111	19	19	24
	1	0	67	45	1608	5.8	110	18	18	25
	5	90	0	45	0	5.5	96	19	15	22
	6	90	34	45	816	5.4	69	17	15	22
	3	90	67	45	1608	5.5	79	18	14	23
	9	90	101	45	2424	5.6	90	20	13	20
					SED‡	0.1	22.8	2.2	2.6	2.4
407	1	0	0	0	0	6.8	133	27	7	32
	3	45	0	0	0	6.6	123	21	7	33
	7	45	45	0	1215	6.7	124	24	15	32
					SED	0.2	13.4	2.6	2.9	4.0
502	1	0	0	0	0	5.6	103	17	44	23
	2	0	45	67	1035	5.7	100	18	42	22
	5	67	45	67	1035	5.4	90	16	43	20
	10	67	67	67	1541	5.3	106	18	50	20
	11	67	90	67	2160	5.3	82	16	44	20
					SED	0.1	10.5	2.0	4.6	2.0
503	1	90	0	45	0	5.0	109	21	45	20
	2§	90	45	45	1035	5.0	113	19	42	22
	3§	90	90	45	2160	5.0	110	20	46	24
	6¶	90	45	45	1035	4.9	102	19	44	21
	7¶	90	90	45	2160	5.0	117	20	45	21
					SED	0.1	16.4	1.6	3.5	2.9
801	1	0	0	0	0	4.3	78	12.2	44	37
	2	0	134	134	2244	4.6	68	12.8	43	30
	9	112	0	134	0	3.9	84	11.9	-	-
	10	112	45	134	720	4.0	67	12.1	43	39
	11	112	90	134	1440	4.4	70	11.6	41	44
	8	112	134	134	2144	4.4	57	12.5	52	49
					SED	0.3	18.4	0.23	3.6	3.0
802	1	112	0	112	0	3.8	54	10	-	-
	2	112	67	112	1072	3.8	50	10	-	-
	3	112	134	112	2144	3.8	47	10	-	-
	4	112	269	112	4304	3.9	41	10	-	-
	5	112	672	112	10752	5.0	67	10	-	-
	6	112	1344#	112	1344	4.4	63	9	44	34
					SED	0.5	7.2	0.4		
Magruder	2	0	0	0	0	5.7	152	22	32	30
	3††	0	34	0	2176	5.7	109	22	22	21
	4††	67	34	0	2176	5.2	124	21	31	26
	5††	67	34	34	2176	5.2	85	23	27	23

† Ammonium nitrate, superphosphate and potassium chloride, unless otherwise specified.
‡ SED, standard error of the difference between two equal-repeated means.
§ Monoammonium phosphate. ¶ Diammonium phosphate. # Applied once at start. Equivalent to 90 kg ha^{-1} yr^{-1}. †† From 1930 to 1945: Sodium nitrate and ordinary superphosphate.

Table 4. Grain and soil Cd content and soil pH in long-term fertilized winter wheat systems.

Reference	years of fertilization	Change in Grain Cd	Change in Soil Cd†	Change in soil pH	Comments
		.... $\mu g\ kg^{-1}$			
This study					
Stillwater (222)	24	15 to 13	96 to 90	5.5 to 5.6	All changes are from lowest to highest P rate of each experiment
Lahoma (502)	23	43 to 44	90 to 82	5.4 to 5.3	
Lahoma (503)	23 (map)	45 to 46	109 to 110	5,0 to 5.0	
Lahoma (503)	23 (dap)	45 to 45	109 to 117	5.0 to 5.0	
Haskell (801)	16	42 to 52	67 to 57	4.0 to 4.4	
Haskell (802)	16	ND‡	54 to 67	3.8 to 5.0	
Magruder	64	32 to 22	152 to 109	5.7 to 5.7	
Alltus (407)	27	7 to 15	123 to 124	6.6 to 6.7	
Andersson and Bingefors, 1985	62	25 to 56	Not reported	Not reported	Grain Cd increase with time attributed to management practices, fertilizers, and atmospheric deposition
	56	5 to 76	Not reported	Not reported	
Kjellstrom et al., 1975					
Jones and Johnston, 1989	107	50 to 90	330 to 430	Neutral to slightly calcareous	Grain and soil Cd increase with time in plots with NPK. Increase attributed to wheat varieties, fertilizers, soil acidification and atmospheric deposition.
Mortvedt, 1987.					
Sanborn field, Univ. of MO	40	186 to 80	290-426§	Not reported	All changes are from 0 to highest P rate.
Magruder plots, OSU	49	58 to 30	139 to 139§	Not reported	
Williams and David, 1976	5	8 to 28	30-60¶	5.5	All changes from 0 to highest P rate.

† Soil Cd measured in HNO_3, $HClO_4$ digest unless, specified.
‡ Not determined.
§ Soil Cd measured in HNO_3, $HClO_4$, HF digest.
¶ Soil Cd in HCl.

Table 5. Regression equations for Cd ($\mu g\ kg^{-1}$) and Zn ($mg\ kg^{-1}$) contents in grain and soil on soil pH by experiment.

Experiment	Model	r^2	P > F for slope
407	soil Zn = -38.75 + 9.27 pH	0.38	0.0425
502	grain Cd = 129.0038 - 15.423 pH	0.27	0.0236
503	soil Zn = - 45.0787 + 13.046 pH	0.27	0.0177
802	soil Cd = -46.7146 + 25.1622 pH	0.18	0.0457

Note: For the other experiments and variables regressed on soil pH, the models were not significant at $p < 0.05$.

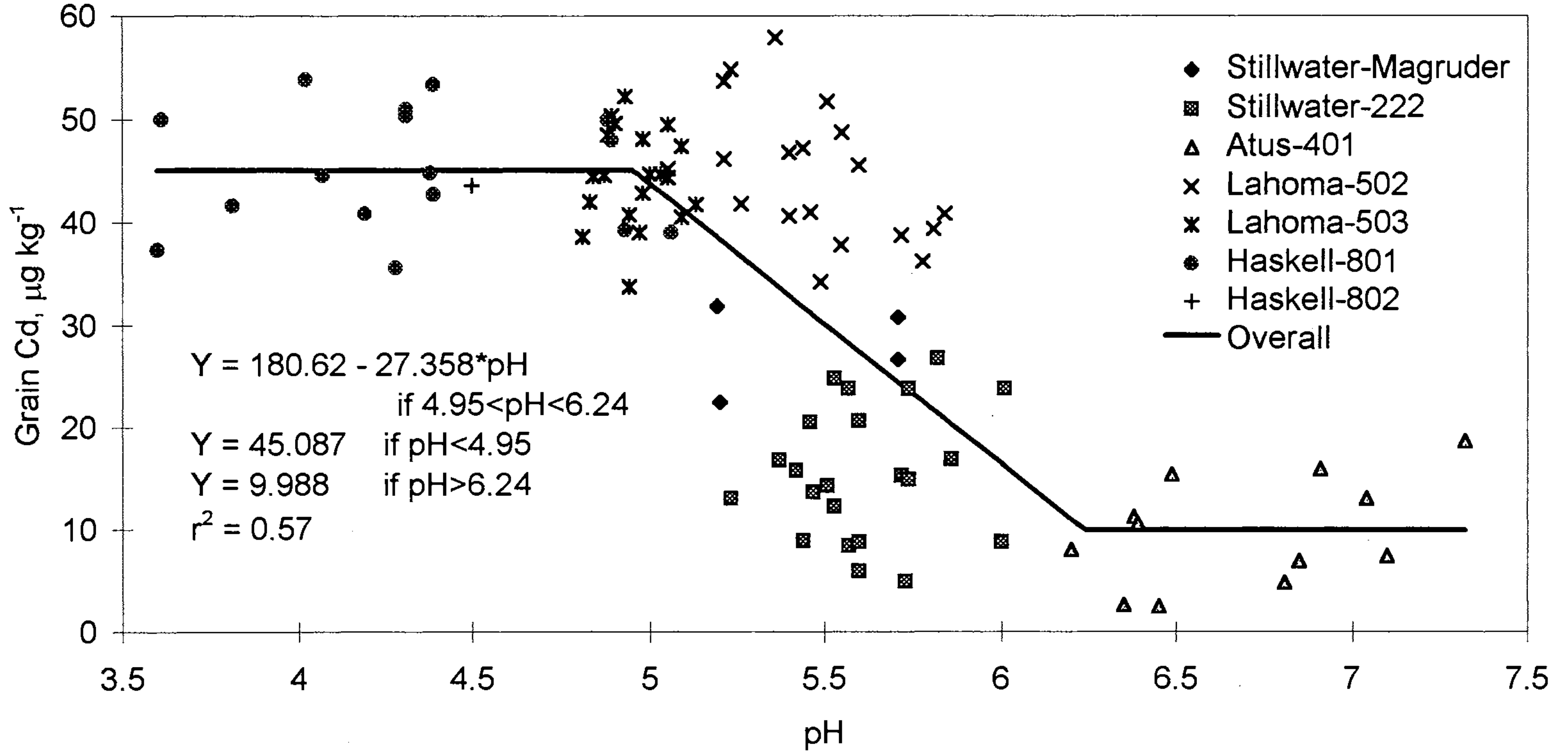

Figure 1. Relationship between soil pH and grain Cd in winter wheat continuously fertilized for 16 (Haskell), 23 (Lahoma), 24 (Stillwater-222), 27 (Altus) and 64 (Stillwater-Magruder) years, Oklahoma, 1993.

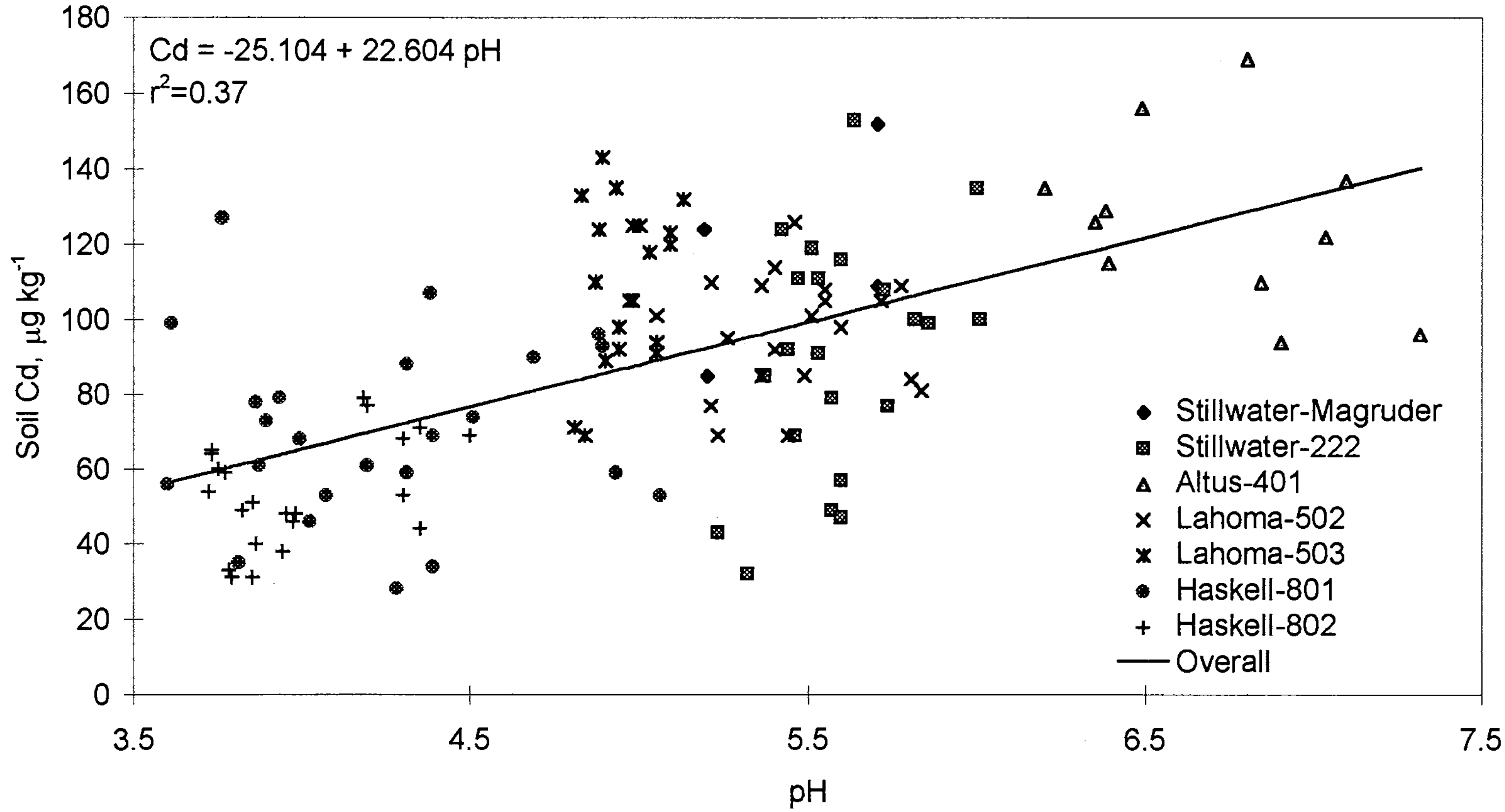

Figure 2. Relationship between soil pH and total Cd across all experiments, Oklahoma, 1993.

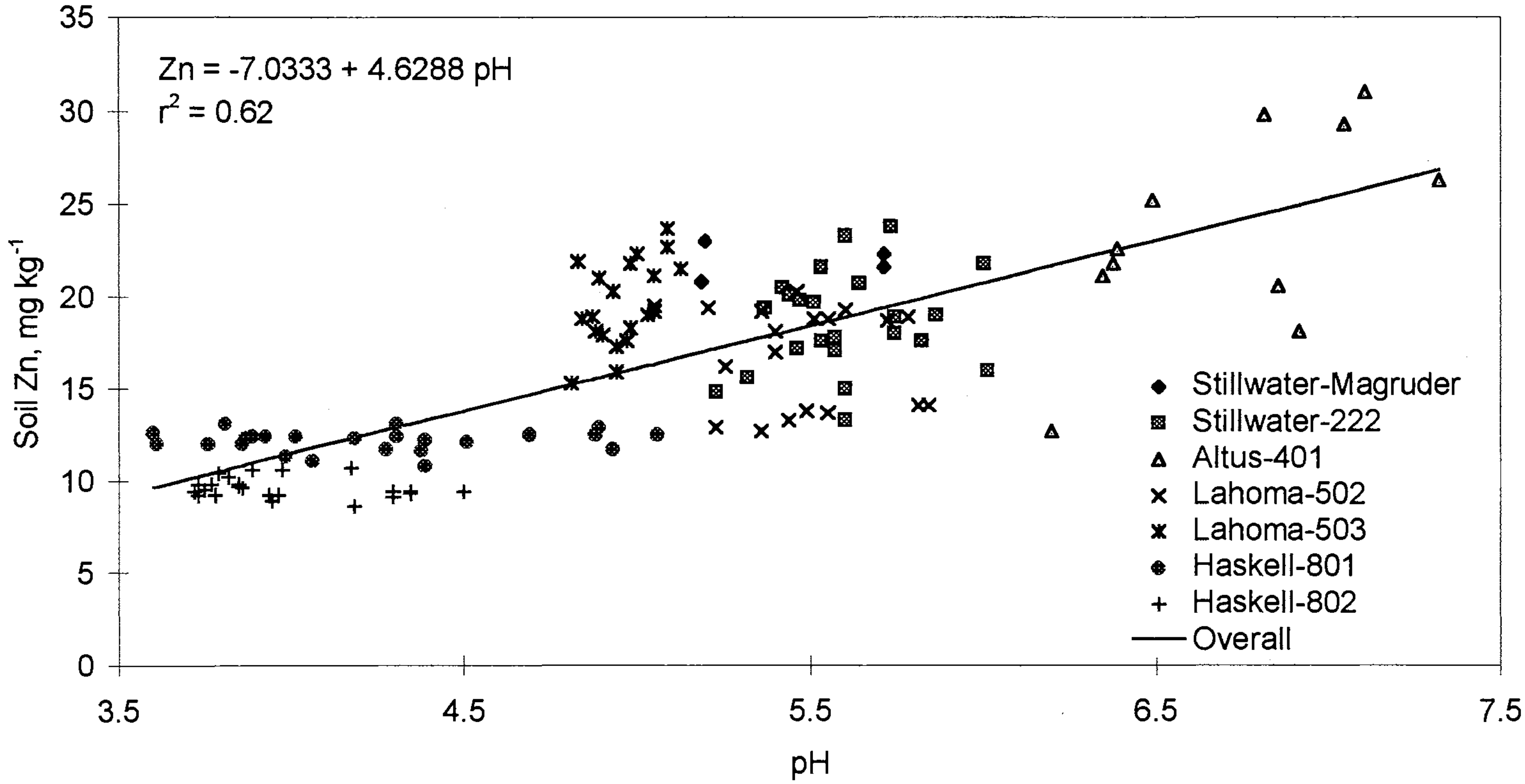

Figure 3. Relationship between soil pH and total Zn across all experiments, Oklahoma, 1993.

APPENDIX A

Table 1a. Split plot "in space" analysis of variance for soil profile (0-180 cm) inorganic N (kg ha^{-1}) following harvest. Stillwater, OK, 1995.

Source of variation	df	Mean squares
Replication (R)	1	5606**
Treatment (T)	12	11473**
R*T (Error a)	12	534
Depth	5	475
T*Depth	60	975
Residual	59	707
CV, %		66

** Significant at the 0.01 probability level.

Table 2a. Treatment means and analysis of variance for surface (0-30 cm) soil chemical analysis, Stillwater, OK, 1994-1995.

				1994		1994	1995
			pH	Organic C	Total N	Inorganic N†	
				------- $g\ kg^{-1}$ ------		---- $kg\ ha^{-1}$ ------	
Treatment means:							
No.	$kg\ N\ ha^{-1}$	N source‡					
1.	0	-	6.71	9.28	0.77	118	22
2.	45	SS	6.58	8.18	0.68	105	21
3.	90	SS	7.02	8.29	0.70	116	21
4.	180	SS	6.45	9.10	0.75	117	22
5.	270	SS	6.91	9.54	0.80	126	26
6.	540	SS	6.76	9.31	0.78	125	28
7.	45	AN	7.07	8.40	0.72	100	20
8.	90	AN	7.16	8.37	0.70	99	22
9.	180	AN	6.92	8.58	0.73	92	33
10.	270	AN	6.47	8.58	0.72	120	31
11.	540	AN	6.50	8.94	0.76	186	40
12.	540	SS + L	6.83	8.45	0.74	122	32
13.	540	AN + L	6.82	9.62	0.83	180	36
SED§			0.41	0.87	0.06	14	5

Analysis of variance:

------ Source -------	df	------------ Mean	squares	------------	
Replication	2	1.41**	17.50**	0.091**	207	47
Treatment	12	0.16	0.76	0.010	2453**	135**
N rate linear (SS)	1	0.02	1.10	0.009	444	87
N rate quad (SS)	1	0.00	1.16	0.000	106	37
N rate linear (AN)	1	0.41	0.04	0.008	6634**	793**
N rate quad (AN)	1	0.52	1.44	0.000	10309**	60
Lime effect (SS)	1	0.08	1.13	0.004	13	21
Lime effect (AN)	1	0.16	0.69	0.006	48	27
Residual	24	0.25	1.15	0.006	287	34
CV, %		7.41	12.03	10.26	14	22

† Samplig dates: 11 Oct. 94 and 16 Aug. 95.
‡ SS, sewage sludge; AN, ammonium nitrate; L, lime applied in 1993.
§ SED, standard error of the difference between two equal replicated means.
**, * Significant at the 0.01 and 0.05 probability levels, respectively.

APPENDIX B

SAS PROGRAM TO OBTAIN THE EQUATION PLATEAU-LINEAR-PLATEAU PRESENTED IN FIGURE 1 OF CHAPTER II

CDGPPB = Grain Cd in $\mu g\ kg^{-1}$
PH = soil pH

```
PROC NLIN DATA = ONE BEST = 2;
 PARMS B0=270 TO 280 BY 1 B1=-62 TO -64 BY -1 PJOINT1=5.1 TO 5.2 BY .1
 IF PH>PJOINT1 AND PH<PJOINT2 THEN DO;
  MODEL CDGPPB = B0 + B1*PH;
   DER.B0=1;
   DER.B1=PH;
   DER.PJOINT1=0;
   DER.PJOINT2=0;
 END;
 IF PH<PJOINT1 THEN DO;
   MODEL CDGPPB = B0+B1*PJOINT1;
   DER.B0=1;
   DER.B1=PJOINT1;
   DER.PJOINT1=B1;
   DER.PJOINT2=0;
 END;
 IF PH>PJOINT2 THEN DO;
   MODEL CDGPPB = B0 + B1*PJOINT2;
   DER.B0=1;
   DER.B1=PJOINT2;
   DER.PJOINT1=0;
   DER.PJOINT2=B1;
 END;
FILE PRINT;
  IF _obs_=1 AND MODEL = 0 THEN DO;
  PLATEAU1=B0+B1*PJOINT1;
  PLATEAU2=B0+B1*PJOINT2;
  PUT PLATEAU1=;
  PUT PLATEAU2=;
 END;
  PLATEAU1=B0+B1*PJOINT1;
  PLATEAU2=B0+B1*PJOINT2;
  ID PLATEAU1;
  ID PLATEAU2;
  OUTPUT OUT = NEW P=PCDGPPB PARMS=B0 B1 PJOINT1 PJOINT2 SSE=sse;

PROC PLOT;
 PLOT CDGPPB*PH=LOC PCDGPPB*PH='.'/OVERLAY
 VAXIS = 0 TO 60 BY 5 HAXIS = 3.5 TO 7.5 BY .5;

PROC MEANS NOPRINT;
 VAR CDGPPB SSE B0 B1 PJOINT1 PJOINT2 PLATEAU1 PLATEAU2;
 OUTPUT OUT = NEW2 N=TDF
   MEAN = CGPPB SSE B0 B1 PJOINT1 PJOINT2 PLATEAU1 PLATEAU2
CSS=CSST;

DATA NEW3; SET NEW2;
 INTERCPT = B0; SLOPE=B1; JOINT1=PJOINT1; JOINT2=PJOINT2;
 RSQ=(CSST-SSE)/CSST;
 EDF=TDF-4;
 SSR=CSST-SSE;
 MSR=SSR/3;
 MSE=SSE/EDF;
 F=MSR/MSE;
PROBF=1-(PROBF(F,3,EDF));
KEEP INTERCPT SLOPE JOINT1 JOINT2 PLATEAU1 PLATEAU2 RSQ F PROBF;
PROC PRINT;
RUN;
```

2

VITA

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Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECT OF APPLIED SEWAGE SLUDGE, AMMONIUM NITRATE AND PHOSPHORUS ON YIELD OF WINTER WHEAT, SOIL PROFILE INORGANIC NITROGEN ACCUMULATION AND CADMIUM UPTAKE

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