EFFECT OF LONG-TERM APPLICATION OF BIOSOLIDS AND AMMONIUM NITRATE ON WINTER WHEAT YIELD, NITROGEN UPTAKE, AND HEAVY METAL ACCUMULATION

By

KATHIE JUNE WYNN

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1998

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
August, 2001

EFFECT OF LONG-TERM APPLICATION OF BIOSOLIDS AND AMMONIUM NITRATE ON WINTER WHEAT YIELD, NITROGEN UPTAKE, AND HEAVY METAL ACCUMULATION

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

ACKNOWLEDGEMENTS

First and foremost, I thank God for giving me the strength and wisdom to complete this degree, through him all things are possible. I am also very grateful to the Department of Plant and Soil Sciences for the opportunity to work and study at Oklahoma State University. Special thanks to the Soil Fertility Project for their continued support and assistance in accomplishing my goals and most of all for their friendship. Specifically, I would like to thank Wade Thomason, Billy Mullen and Kyle Freeman for there technical advice and field assistance. To my committee members, Dr. Gordon Johnson and Dr. Nick Basta, I thank you for your encouragement, time, and guidance I received throughout my pursuit of this degree. Much appreciation must be given to my major adviser, Dr. Bill Raun, for allowing me the opportunity to work in the Soil Fertility Project and obtain a graduate degree. Finally, I would like thank my parents, Dwayne and Karen Wynn, for the continued emotional and financial support throughout my collegiate career.

TABLE OF CONTENTS

LIST OF TABLES

(*Triticum aestivum* L.), grain N uptake, and grain and forage heavy metal uptake. The effect of these N sources was investigated at the Agronomy Experiment Station (Oklahoma State University, Stillwater, OK) on a Norge loam (fine mixed, thermic Udertic Paleustoll). Previous yield results show that the ammonium nitrate treatments produced significantly higher yields than the biosolid treated plots, which could be due to the low N mineralization rates (21%) noted in the biosolids (Lukina et al., 2000). Initial results show that the concentration of molybdenum (Mo) in wheat forage is significantly higher in plots that are treated with biosolids (Wynn et al., 2000).

As our cities grow there is an increasing need to find a safe method for disposing of biosolids from wastewater treatment plants. According to Sabey et al. (1975) a community with a population of 10,000 can produce about 1 metric ton of treated biosolids per day (dry weight). Biosolids contain significant amounts of plant nutrients that include 0.5-10 % N, 1-6 % phosphorus (P), 0.5-1.5% sulfur (S), 1-20% calcium (Ca), and 0.3-2% magnesium (Mg), 0.1% iron (Fe), <0.25 copper (Cu), manganese (Mn), and zinc (Zn), and <0.05% nickel (Ni), boron (B), cobalt (Co) and Mo (Furr et al., 1976; Sommers, 1977). Application of biosolids to agricultural fields can be beneficial to the producer and the wastewater treatment plants. The addition of biosolids to cropland has been found by Lindsay and Logan (1998) to have a beneficial effect on total porosity, moisture retention, and water stable aggregates. These beneficial effects can be attributed to the amount of organic matter that is found in the biosolids. Menelik et al. (1991) found that when biosolids were applied as the N source there was increased grain yield (20% increase) and N uptake (39% increase) compared with an inorganic N source. Their research also showed that concentrations of N, P, Mg, S, Zn, and Cu in wheat grain were increased when biosolids were used as the N source compared with an inorganic N source. They also reported higher concentrations of N present in the wheat grain where biosolids were applied. These higher concentrations of N could reflect a higher concentration of N present in the root zone at grain fill because of the slow release characteristics of biosolids. They also noted that wheat treated with biosolids was more aesthetically appealing than the wheat that was treated with inorganic N. Berti (1996) found that biosolid applications that result in high trace-element loadings could depress crop yields in corn, soybeans, and sorghum-sudangrass. The level of Zn found in the harvested plant tissue was well above sufficiency levels.

Day et al. (1987) reported that wheat fertilized with biosolids produced more vegetative growth than wheat grown with conventional fertilizers. This study was conducted under hot-dry conditions (with irrigation) and where rapid mineralization of the biosolids was expected. They also reported that wheat fertilized with biosolids produced grain with the same yield and volume-weight as wheat fertilized with suggested N, P, and K from commercial fertilizer. They also noted that grain grown with biosolids contained more Zn, Cu, Pb and Ni than grain produced with N, P, and K from commercial fertilizer. Protein concentration increased from 14 g kg^{-1} with commercial fertilizer to 38 g kg^{-1} where biosolids were applied. This 6-year study showed that long-term fertilization with biosolids did not decrease wheat grain and straw yields or result in any adverse effects on the soil that could not be corrected with minor changes in cropping practices. Research by Hooda et al. (1997) reports that wheat grain concentrations of Cd and Ni on biosolid amended soils were much greater than concentrations of wheat grain grown without biosolids. Lead and Zn concentrations were found to be only marginally higher. They also reported that plants that are grown on soils with pH values in the neutral range and clayey texture tend to accumulate less metals than those grown on soils with an acidic pH and a coarse texture. They reported that when the soil was limed to reach a pH of 6.5 it had no effect on the concentrations in the wheat grain for Cd, Cu, Ni and Zn but that there was a decrease in the concentration of Pb. Gavi et al. (1997a) reports that when soil pH falls below 5.0 the amount of Cd that is found in the grain ranges from 45 to 50 ug kg^{-1}. Above a soil pH of 6.0, grain Cd dropped to 10 μg kg^{-1}.

Ippolito et al. (1999) shows that biosolids contain organic N which acts as a slow release N source and provides a more constant supply of N during the critical grain filling period of wheat versus commercial N sources. This sixteen year study at two sites showed no significant differences in yield between inorganic N and biosolids application. Their research also showed that there was no significant difference in wheat grain protein between the two N sources. Winter wheat grain and straw from plots that received biosolids did show significant increases in Zn concentrations, while Cu, Ni, Cd, Pb, and percent N were not significantly different among N sources. This study also showed that a significant amount of nitrate-N accumulates in the 0 to 150 cm depth when biosolids were applied compared to inorganic N sources. Research by Barbarick et al. (1995) indicated that when P is at adequate levels for plant growth, further additions of P through biosolids applications did not increase the plant absorption of P. They also noted that there was no significant change in grain Mo when biosolids were applied for 11 years. They note that this could be due to the antagonistic effects that Cu has on Mo. According to federal regulations governing land application of biosolids, the maximum amount of an inorganic pollutant that can be applied to an area of land is listed in Table 1 (Basta, 1995). When the USEPA regulatory cumulative pollutant loading rate is reached for a particular land site, no more of that pollutant can be applied to the site in bulk biosolids (Basta,1995).

Copper and Mo are two metals that are of concern when beef cattle are allowed to graze on winter wheat pasture where biosolids have been applied. Copper and Mo can become toxic to cattle when forage concentrations reach 100 mg kg^{-1} and 5 mg kg^{-1}, respectively. A critical winter wheat forage Cu:Mo ratio of 2:1 was identified by the National Research Council (1996). O'Connor and McDowell (1999) state that when there are excessive amounts of Mo, a chronic disease called molybdenosis can occur, or a Mo induced Cu deficiency. The ingested Mo is transformed in the rumen to a tetrathiomolybdate complex with Cu, reducing Cu availability to the animal. Copper deficiencies can cause anemia, reduced growth, depigmentation of hair and cardiac failure. The objective of this study is to determine long-term effects of biosolids versus ammonium nitrate application on grain and forage yield, and metal accumulation in wheat grain and forage.

MATERIALS AND METHODS

One winter wheat (*Triticum aestivum L.*) field experiment was established at the Agronomy Research Station (Stillwater, OK) in the fall of 1993. A complete factorial arrangement of treatments composed of six N rates (0, 45, 90, 180, 270 and 540 kg N ha^{-1} yr^{-1}) and two N sources (anaerobically-digested biosolids and ammonium nitrate, 34-0-0) was evaluated. Total N content and moisture content of the Stillwater, OK biosolids are listed in Table 2. Each nitrogen source was applied preplant and disk incorporated in the fall. Two added treatments outside of the factorial included lime applied in 1993 (8.96 Mg ha^{-1}), 1999 (8.96 Mg ha^{-1}) and 2000 (12.99 Mg ha^{-1}) to the high N rate plots (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 site is a Norge loam (fine mixed, thermic Udertic Paleustoll). Forage was hand harvested from a $1m^2$ area in each plot for metal analysis on January 5, 1999 , March 31, 2000 and April 19, 2001(Feekes growth stages 4, 6 and 5 respectively) (Large, 1954). Grain yield was determined by harvesting the center 2 m of each plot (10 m in length) using a self-propelled Massey Ferguson 8XP combine. Grain subsamples were collected and analyzed for total N and heavy metal content. Total N in grain and forage samples was determined using a Carlo-Erba NA 1500 dry combustion analyzer (Schepers et al., 1989). Soil samples (10 cores/plot, 0-15 cm) were collected from all plots in

December 1998. Copper, Mo, Pb and Cd content were determined by microwave wet digestion of soil using HNO_3, H_2O_2 (U.S. EPA Method 3051) and wet digestion of forage samples using HNO_3 ,$HClO_4$ (Jones and Case, 1990) followed by analysis using a high-resolution inductively coupled plasma spectrophotometer (Thermo-Jarrell Ash IRIS ICP). Analysis of variance was performed by year for all variables analyzed. Single degree of freedom contrasts (non-orthogonal) were used to evaluate specific treatment effects (SAS, 1989)

RESULTS AND DISCUSSION

Grain Yield

Treatment means and single degree of freedom contrasts for grain yield from 1994 to 2000 are reported in Table 3. A linear increase in wheat grain yield with increasing N rate for the ammonium nitrate source was found for almost every year excluding 1996 and 2000. Similarly, a quadratic trend for grain yield was noted for the ammonium nitrate source, excluding 1996. A linear trend in grain yield with increasing N rate of the biosolid source was observed for most years excluding 1996 and 1997. Quadratic trends in wheat grain yield from the biosolid N source were found only in 1996 and 2001. In 1996 there was not a response to added N in the AN or the BS plots. This was due to a poor stand, severe weed pressure, and adverse growing conditions encountered throughout the growing season. In 1997 there were no differences in the BS plots but there was a linear increase with the application of AN. In 1998 grain yields of the BS plots were comparable to that obtained using AN, at the same N rates. This is thought to have come from the mineralization of the biosolid and soil N. It is likely that conditions for mineralization of soil organic N and biosolid N were favorable since the yield of the check plot (1770 kg ha^{-1}) was the highest observed over the course of this eight year study. Gavi et al., (1997b) and U.S. Environmental Protection Agency (1983) reported that N mineralization in biosolid sources averaged 20 %. In the fifth year, the amount of N in easily mineralizable pools provided enough N to the wheat to be comparable with the AN plots. However, from 1999 to 2001, significantly lower yields were again observed from BS plots when compared to AN.

Grain N Uptake

A quadratic trend for grain N uptake from the ammonium nitrate source was observed in most years except 1996 (Table 4). A quadratic trend for grain N uptake from the biosolid source was found for N uptake in most years excluding 1997, 1999, and 2000. In every year but one (1996 being the exception), N applied as ammonium nitrate resulted in increased grain N uptake over that of the biosolid source. This difference is attributed in part to the slow N mineralization rate of the biosolids. Decreased grain N uptake in the biosolid source compared to ammonium nitrate was likely a result of decreased N mineralization in our study due to a cooler more temperate environment when compared to results from Day et al., (1987) in Arizona. A quadratic trend for grain N uptake efficiency from the ammonium nitrate was observed for most years except for 1994 and1996 (Table 5). There were no significant differences between the biosolid and ammonium nitrate treatments in 1996, 1998 and 2001.

Forage Metal Accumulation

As mentioned previously, the greatest concern regarding forage content of heavy metals is specifically Cu and Mo. As long as the ratio of copper and molybdenum remains above two, molybdenosis should not be a concern. The Cu:Mo ratio never fell below two in the three years the forage was analyzed, thus the threat of molybdenosis is considered to be small. The average Cu:Mo ratio for the BS treatments was 10:1 in 1999 and 3:1 in 2000. The average Cu:Mo ratio for the AN treatments was 32:1 in 1999 and 6:1 in 2000. In 1999 a linear increase for Mo was found with increasing N rate in the BS treated plots (Table 6). In 2000 there was a linear increase in forage Cu with increasing N rate in the AN plots (Table 7). In 2001 there was a significant difference between the AN treatments and the BS treatments (Table 8). There was not an observable difference by source or rate for the content of Cd, Mo or Pb in the wheat forage in 2001.

Grain Metal Accumulation

A linear trend for increased Mo content with increasing N rates was realized for the biosolid treatments in 1999 while the opposite was seen for ammonium nitrate treatments (Table 9). Similar results were found in 2000, although at lower significance levels (Table 10). In 2001 there was an observed linear decrease for Cu and Mo grain content with increasing N rate for the AN treatments (Table 11). Alternatively, grain Mo increased with increasing rates of biosolids. Cadmium, Cu and Pb in wheat grain did not provide consistent results for the three years analyzed. Forage Mo levels were some what higher in the biosolid treatments when compared to ammonium nitrate treatments. However, this was not observed in the grain suggesting that since Mo is an essential component of the enzyme NO_3^- reductase its greatest concentration will occur in the chloroplasts in leaves (Havelin et al. 1999).

Soil Metal

In general soil metal contents increased with increasing N applied as biosolids (Table 12). When N was applied as ammonium nitrate soil metal contents were unaffected. As biosolid loading rates increased, soil metal contents will need to be monitored more closely.

CONCLUSIONS

Nitrogen applied as ammonium nitrate resulted in increased yields above that of biosolids in every year of the study excluding 1998. Similarly, N uptake was consistently increased due to application of ammonium nitrate versus biosolids. It should be pointed out that biosolids were applied on a total N basis without considering mineralization rates. Thus at the same N rates, grain yields and N uptake were expected to be lower for the biosolid treated plots compared to ammonium nitrate treatments especially in the early years of the study. Although biosolid N rates resulted in equivalent grain yields (at comparable N rates) as that of ammonium nitrate in the fifth year of the study, yields continued lower for the biosolids in years 6, 7, and 8. Further evaluation of soil N (organic, inorganic, lignin-N, etc.) is needed to explain these results. In general, wheat grain Mo levels increased with increasing N rates with the biosolid source, while the opposite was observed with ammonium nitrate. Results for grain Cd, Cu, and Pb were inconclusive as a function of N rate and source. Forage accumulation of molybdenum did not reach a level where molybdenosis would be a concern when either ammonium nitrate or biosolids were applied as the N source. When lime was applied at the high N rate, forage Mo levels were increased when compared to ammonium nitrate. Forage Cu:Mo levels were found to decrease from 1999 to 2001, a result of increased Mo levels in the forage with time (Tables 5-7).

REFERENCES

Barbarick, K.A., J.A. Ippolito, and D.G. Westfall. 1995. Biosolids effect on phosphorus, copper, zinc, nickel, and molybdenum concentrations in dryland winter wheat. J. Environ. Qual. 24:608-611.

Basta, N.T. 1995. Regulations governing land application of sewage sludge. *In N.T. Basta* Land application of biosolids: A review of research concerning benefits, environmental impacts, and regulations of applying treated sewage sludge. Okla. Agric. Exp. Sta. B-808. p. 41-46.

Berti, W.R., and L. W. Jacobs. 1996. Chemistry and phytotoxicity of soil trace elements from repeated sewage sludge applications. J. Environ. Qual. 25:1025-1032.

Day, A.D., R.K. Thompson, and R.S. Swingle. 1987. Effects of sewage sludge on field and quality of wheat grain and straw in an arid environment. Desert Plants. 8(3). 104-105,142-143.

Furr, A.K., A.W. Lawrence, S.S.C. Tong, M.C. Grandolfo, R.A. Holfstader, C.A. Bache, W.H. Gutenmann, and D.J. Lisk. 1976. Multielement and chlorinated hydrocarbon analysis of sewage sludge of American cities. Environ. Sci. Technol. 10:683-687.

Gavi, F., N.T. Basta, and W.R. Raun. 1997a. Wheat grain cadmium as affected by long-term fertilization and soil acidity. J. Environ. Qual. 26:265-271.

Gavi, F., W.R. Raun, N.T. Basta, and G.V. Johnson. 1997b. Effect of sewage sludge and ammonium nitrate on wheat yield and soil profile inorganic nitrogen accumulation.. J. Plnt. Nutr. 20 (2&3). 203-218.

Havelin, J.L., J.D. Beaton, S.L. Tisdale, W. L. Nelson. 1999. Soil Fertility and Fertilizers. 6th edition. Prentice Hall. Upper Saddle River, New Jersey.

Hooda, P.S., D. McNulty, B.J. Alloway, M.N. Aitken. 1997. Plant availability of heavy metals in soil previously amended with heavy applications of sewage sludge. J. Sci. Food Agric. 73. 446-454.

Ippolito. J.A., K.A. Barbarick, and R. Jepson. 1998. Application of anaerobically digested biosolids to dryland winter wheat. Colorado Agric. Exp. Stn. TR98-8.

Large. E.C.1954. Growth stages in cereals. Plant Pathol. 3:128-129.

Lindsay, B.J., and T.J. Logan. 1998. Field response of soil physical properties to sewage sludge. J. Environ. Qual.. 27:534-542.

Lukina, E.V., K.W. Freeman, R.W. Mullen, K.J. Wynn, W.E. Thomason, R.K. Teal, J. Mosali, T. Feng, D.E. Needham, C.N. Washmon, J.B. Solie, M.L. Stone, N.T. Basta, J.A. Hattey, H. Zhang, S. Deng, J.M. Shaver, R.L. Westerman, and W.R. Raun. 2000. Soil Fertility Research Highlights 2000. Oklahoma State University.

Menelik, G., R.B. Reneau,Jr., D.C. Martens, and T.W. Simpson. 1991. Yield and elemental composition of wheat grain as influenced by source and rate of nitrogen. J. Plant Nutr. 14(2). 205-217.

National Research Council (NRC). 1996. "Nutrient requirement of domestic animals". 7th Ed. National Academy of Sci. National Research Council. Wash. D.C.

O'Conner, G.A. and L.R. McDowell.1999. Understanding fate, transport, bioavailability, and cycling of metals in land-applied biosolids. Joint Residuals and Biosolids Management Conference. January 27, 1999, Charlotte, North Carolina.

Sabey, B.R. and W.E. Hart. 1975. Land application of sewage sludge:I. Effect on growth and chemical composition of plants. J. Environ. Qual. 4(2). 252-256.

Schepers, J.S., D.D. Francis, and M.T. Thompson. 1989. Simultaneous determination of total C, total N, and ^{15}N on soil and plant material Commin. in Soil Sci. Plant Anal 20:949-959.

SAS Institute. 1989. SAS/STAT user's guide. Version 6. 4th ed. Vol. 2. SAS Inst., Cary. NC

Sommers, L.E. 1977. Chemical composition of sewage sludges and analysis of their potential as fertilizers. J. Environ. Qual. 6:225-229.

U.S. Environmental Protection Agency. 1993a. 40 CFR Part 503. Standards for the use or disposal of sewage sludge. Fed. Reg. 58:9248-9415.

Wynn, K.J., N.T. Basta, W.E. Thomason, E.V. Lukina, G.V Johnson, K.W. Freeman, R.L. Westerman, and W.R. Raun. 2000. Heavy metal accumulation in wheat forage and grain in a continuous biosolid experiment. p. 290. *In* Agronomy Abstracts. ASA, Minneapolis, Mn.

TABLE 1. Cumulative pollutant loading rates (CPLR) governing the application of biosolids in U.S. EPA regulations (40 CFR Part 503 Table 3).

Pollutant	CPLR kg ha^{-1}
Arsenic	41
Cadmium	39
Chromium	3000
Copper	1500
Lead	300
Mercury	17
Molybdenum	18
Nickel	420
Selenium	100
Zinc	2800

Table 2. Total nitrogen and moisture content of Stillwater, OK biosolids. 1993 to 2000

1993	1994	1995	1996	1997	1998	1999	2000	Avg.
----------------Total N, %----------------								
2.02	1.74	1.97	2.73	2.42	2.43	2.02	2.44	2.22
----------------Moisture, %----------------								
60	35	59	na	55	46	45	17	45

na - not available

Avg. - average

TABLE 3. Winter wheat grain yield treatment means as influenced by N source and rate, Stillwater, OK 1994 to 2001.

N Source	N Rate	1994	1995	1996	1997	1998	1999	2000	2001	Average
	kg ha^{-1}	---Yield, kg ha^{-1}---								
-	0	463.6	498.6	612.3	1127.3	1932.9	907.7	922.9	1244.6	963.7
-	0	377.3	519.6	574.6	1286.8	1609.4	902.4	974.6	1074.3	914.9
BS	45	526.9	570.7	726.9	1557.1	2377.8	947.2	1309.3	1684.0	1212.5
BS	90	616.3	573.6	656.2	1514.0	2241.7	1067.8	1497.0	1572.8	1217.4
BS	180	885.3	568.3	1233.5	1398.5	3047.6	1361.2	1574.6	1980.8	1506.2
BS	270	1036.7	610.3	918.3	1388.5	2830.3	964.0	1233.0	1777.3	1344.8
BS	540	1503.6	1025.8	821.3	1457.0	3601.4	1833.1	1921.2	1541.2	1713.1
AN	45	1780.8	620.1	338.5	1367.9	2053.1	903.6	1717.7	1386.2	1270.9
AN	90	1737.4	904.9	511.5	1762.2	2860.7	1606.6	2884.2	1841.8	1763.7
AN	180	1919.3	1195.5	665.1	2175.6	3438.9	2366.9	3301.4	2269.2	2166.5
AN	270	2586.4	1418.1	535.0	2349.1	3038.6	2887.6	2783.4	2437.9	2254.5
AN	540	2335.4	1281.3	555.9	2555.2	3103.7	2475.3	2050.2	1789.4	2018.3
BS+L	540	1668.1	1051.0	880.1	1628.9	3726.7	2600.1	1568.4	1985.2	1738.7
AN+L	540	2185.3	1324.9	781.1	2840.7	3708.4	2857.8	2210.2	1823.6	2216.5
BS mean †		913.8	669.7	871.2	1463.1	2819.8	1234.7	1507.0	1711.2	1398.8
AN mean ‡		2071.9	1083.9	521.2	2042.0	2899.0	2048.0	2547.4	1944.9	1894.8
RI Harvest		6.2	2.8	2.1	2.4	2.1	3.2	3.5	2.1	
SED		176.2	172.1	139.5	225.2	208.9	236.9	298.6	220.2	
CV		15.4	24.3	34.5	22.4	12.8	24.3	27.6	22.1	
Contrasts										
AN linear		***	***	NS	***	***	***	NS	**	
AN quadratic		***	NS	NS	**	***	***	***	**	
BS linear		***	**	NS	NS	***	**	*	NS	
BS quadratic		NS	NS	**	NS	*	NS	NS	**	
AN vs BS		***	***	***	**	NS	***	***	NS	

*, **, *** - significant at the 0.05, 0.01, and 0.001 probability levels, respectively
NS- not significant
BS – biosolids obtained from the City of Stillwater
AN – ammonium nitrate (34-0-0)
L – lime applied at a rate of 9, 9, and 13 Mg ha^{-1} in the fall of 1993, 1999, and 2000, respectively
†,‡ treatment means for all rates for BS and AN, respectively (excluding lime treatments)
RI Harvest -response index determined by dividing the maximum yield of plots receiving N by the zero-N check.

TABLE 4. Grain N uptake treatment means as influenced by N source and rate, Stillwater, OK, 1994 to 2001.

Source	N Rate	1994	1995	1996	1997	1998	1999	2000	2001	Average
	kg ha^{-1}	N uptake, kg ha^{-1}								
-	0	10.6	13.9	7.6	31.0	59.9	19.6	18.9	23.7	23.2
-	0	8.1	14.0	5.9	34.1	50.7	19.4	19.4	19.7	21.4
BS	45	11.4	16.2	8.3	44.9	74.9	20.5	28.9	31.9	29.6
BS	90	12.8	16.4	7.5	44.6	72.8	23.9	33.3	33.2	30.6
BS	180	18.5	16.8	16.2	44.1	95.6	31.2	35.3	39.6	37.2
BS	270	23.9	18.7	13.9	43.5	93.0	24.6	25.9	38.8	35.3
BS	540	30.6	32.2	12.5	48.1	116.1	45.5	44.9	35.8	45.7
AN	45	38.6	17.1	4.7	42.7	67.9	21.5	34.9	29.7	32.1
AN	90	38.2	27.6	7.4	57.7	94.1	44.5	75.2	43.0	48.5
AN	180	47.2	36.1	8.9	74.3	110.1	68.4	92.5	48.2	60.7
AN	270	67.9	44.1	7.0	74.8	98.5	86.1	80.9	49.9	63.7
AN	540	68.7	40.3	8.9	83.4	102.9	75.1	61.5	49.3	61.3
BS+L	540	37.8	32.4	12.4	52.4	120.4	69.8	43.1	47.2	51.9
AN+L	540	62.0	41.4	9.2	93.2	124.6	87.3	60.1	38.7	64.6
BS mean †		19.4	20.1	11.7	45.0	90.5	29.1	33.7	35.9	35.7
AN mean ‡		52.1	33.1	7.4	66.6	94.7	59.1	69.0	44.0	53.3
SED		4.9	5.3	2.3	6.4	6.4	7.1	8.1	5.9	
CV		17.6	24.9	42.1	20.1	12.2	27.2	29.5	19.5	
Contrasts										
AN linear		***	***	*	***	***	***	**	***	
AN quad		***	**	NS	**	***	***	***	**	
BS linear		***	**	**	NS	***	**	*	*	
BS quadratic		NS	NS	**	NS	**	NS	NS	**	
AN vs BS		***	***	***	***	NS	***	***	**	

*, **, *** - significant at the 0.05, 0.01, and 0.001 probability levels, respectively
NS- not significant
BS – biosolids obtained from the City of Stillwater
AN – ammonium nitrate (34-0-0)
†,‡ treatment means for all rates for BS and AN, respectively (excluding lime treatment

Table 5. Nitrogen uptake efficiency means as influenced by N source and rate, Stillwater, OK, 1994-2001.

Source	N Rate	1994	1995	1996	1997	1998	1999	2000	2001	Average
	kg ha^{-1}	--------------------	--------------------	----------	N uptake efficiency %	----------	--------------------	--------------------	--------------------	
BS	45	1.79	4.93	1.66	30.88	33.15	9.19	22.22	18.15	15.25
BS	90	2.39	2.69	-0.01	15.13	14.24	8.40	16.06	10.48	8.67
BS	180	4.37	1.58	4.81	7.27	19.78	8.21	10.05	8.82	8.11
BS	270	4.93	1.74	2.37	4.62	12.23	3.04	4.19	5.58	4.84
BS	540	3.70	3.38	0.91	3.17	10.39	5.38	4.81	2.23	4.25
AN	45	62.12	18.07	-6.39	26.03	17.82	11.39	43.92	13.26	23.28
AN	90	30.69	15.19	-0.19	29.71	37.88	31.25	62.62	21.45	28.58
AN	180	20.29	12.27	0.78	24.05	27.86	28.89	40.91	13.56	21.08
AN	270	21.20	11.16	-0.19	16.21	14.27	25.81	22.99	9.55	15.13
AN	540	10.75	4.87	0.25	9.70	7.96	10.88	7.89	2.87	6.89
BS+L	540	2.03	3.41	0.89	3.97	11.19	9.89	4.49	4.33	5.03
AN+L	540	9.52	5.08	0.30	11.52	11.98	13.13	7.64	2.78	7.74
BS mean†		3.44	2.86	1.95	12.21	17.96	6.84	11.47	9.05	8.22
AN mean‡		29.01	12.31	1.15	21.14	21.16	21.64	35.67	12.14	18.99
SED		48.11	16.12	12.88	50.01	120.29	18.30	43.17	25.24	
CV		67.3	81.6	1184.1	66.5	85.9	44.3	45.8	76.5	
Contrasts										
AN linear		**	NS	NS	NS	NS	NS	**	NS	
AN quadratic		NS	**	NS	*	*	***	***	*	
BS linear		NS	NS	NS	NS	NS	NS	NS	NS	
BS quadratic		NS	NS	NS	NS	NS	NS	NS	NS	
AN vs BS		***	***	NS	**	NS	***	***	NS	

*, **, *** - significant at the 0.05, 0.01, and 0.001 probability levels, respectively

NS- not significant

BS – biosolids obtained from the City of Stillwater

AN – ammonium nitrate (34-0-0)

L – lime applied at a rate of 9, 9, and 13 Mg ha^{-1} in the fall of 1993, 1999, and 2000, respectively

†,‡ treatment means for all rates for BS and AN, respectively (excluding lime treatment

TABLE 6. Winter wheat forage content of heavy metals as influenced by N source and rate, Stillwater, OK 1999.Δ

N Source	N Rate	Cadmium	Copper	Molybdenum	Lead	Cu:Mo
	kg ha^{-1}	----------mg kg^{-1}----------				
-	0	0.021	5.708	0.438	0.093	13:1
-	0	0.031	5.822	0.278	0.178	21:1
BS	45	0.041	5.315	0.255	0.138	20:1
BS	90	0.053	5.686	0.483	3.019	11:1
BS	180	0.052	5.097	0.444	0.285	11:1
BS	270	0.057	5.447	0.639	0.235	8:1
BS	540	0.073	6.393	0.859	0.209	7:1
AN	45	0.037	5.874	0.191	0.187	30:1
AN	90	0.048	5.819	0.239	0.169	24:1
AN	180	0.073	5.906	0.102	0.131	57:1
AN	270	0.064	6.044	0.077	0.184	78:1
AN	540	0.078	6.510	0.099	0.150	65:1
BS+L	540	0.024	6.487	1.031	0.166	6:1
AN+L	540	0.051	3.329	0.142	0.104	23:1
BS mean †		0.055	5.588	0.536	0.777	12:1
AN mean‡		0.060	6.031	0.142	0.164	50:1
SED		0.014	0.554	0.216	1.073	
CV		33.5	11.5	70.1	350.6	
Contrasts						
AN Linear		**	NS	NS	NS	
AN quadratic		NS	NS	NS	NS	
BS linear		**	NS	**	NS	
BS quadratic		NS	NS	NS	NS	
AN vs BS		NS	NS	**	NS	

*, **, *** - significant at the 0.05, 0.01, and 0.001 probability levels, respectively
NS- not significant
BS – biosolids obtained from the City of Stillwater
AN – ammonium nitrate (34-0-0)†
,‡ treatment means for all rates for BS and AN, respectively (excluding lme treatments)
Δ - forage samples rinsed in deionized water prior to being dried and ground.
Detection limits - 0.0008, 0.0075, 0.0033, 0.0130 ppm for Cd, Cu, Mo, and Pb , respectively

TABLE 7. Winter wheat forage content of heavy metals as influenced by N source and rate, Stillwater, OK, 2000.

N Source	N Rate	Cadmium	Copper	Molybdenum	Lead	Cu:Mo
	kg ha^{-1}	----------	----------mg kg^{-1}	----------	----------	
-	0	0.042	4.381	1.430	0.309	3:1
-	0	0.049	4.136	0.542	0.332	7:1
BS	45	0.005	5.409	1.345	0.109	4:1
BS	90	0.008	4.812	1.596	0.052	3:1
BS	180	0.041	4.138	1.294	0.243	3:1
BS	270	0.110	3.624	1.241	0.265	2:1
BS	540	0.044	4.678	1.281	0.239	3:1
AN	45	0.043	4.624	0.761	0.260	6:1
AN	90	0.021	4.102	0.415	0.156	9:1
AN	180	0.052	6.312	1.283	0.114	4:1
AN	270	0.037	6.143	0.824	0.191	7:1
AN	540	0.065	5.042	0.543	0.136	9:1
BS+L	540	0.042	5.211	1.845	0.239	2:1
AN+L	540	0.068	4.718	0.649	0.110	7:1
BS mean †		0.042	4.532	1.351	0.182	3:1
AN mean ‡		0.044	5.245	0.765	0.171	7:1
SED		0.1456	0.985	0.434	0.148	
CV		252.9	25.1	47.90	92.20	
Contrasts						
AN Linear		NS	NS	NS	NS	
AN quadratic		NS	*	NS	NS	
BS linear		NS	NS	NS	NS	
BS quadratic		NS	NS	NS	NS	
AN vs BS		NS	NS	*	NS	

*, **, *** - significant at the 0.05, 0.01, and 0.001 probability levels, respectively
NS- not significant
BS – biosolids obtained from the City of Stillwater
AN – ammonium nitrate (34-0-0)
†,‡ treatment means for all rates for BS and AN, respectively (excluding lime treatments)
Detection limits - 0.0008. 0.0075, 0.0033, 0.0130 ppm for Cd, Cu, Mo, and Pb , respectively

TABLE 8. Winter wheat forage content of heavy metals as influenced by N source and rate, Stillwater, OK 2001.

N Source	N Rate	Cadmium	Copper	Molybdenum	Lead	Cu:Mo
	kg ha^{-1}	----------	----------	mg kg^{-1}	----------	----------
-	0	0.0	8.875	1.426	0.130	6:1
-	0	0.0	8.158	1.014	0.130	8:1
BS	45	0.0	7.884	1.190	0.108	6:1
BS	90	0.0	7.555	1.111	0.161	6:1
BS	180	0.0	8.305	1.236	0.153	6:1
BS	270	0.014	7.214	1.052	0.175	6:1
BS	540	0.007	7.303	1.120	0.153	6:1
AN	45	0.0	9.898	0.948	0.145	10:1
AN	90	0.0	7.442	0.781	0.164	9:1
AN	180	0.0	7.973	0.623	0.167	12:1
AN	270	0.0	9.152	1.709	0.080	5:1
AN	540	0.0	9.406	1.163	0.087	8:1
BS+L	540	0.043	8.003	1.597	0.137	5:1
AN+L	540	0.0	8.023	1.041	0.142	7:1
BS mean †		0.004	7.652	1.142	0.150	6:1
AN mean‡		0.0	8.774	1.045	0.129	9:1
SED		0.018	0.970	0.469	0.068	
CV		467.8	14.45	50.27	60.2	
Contrasts						
AN Linear		NS	NS	NS	NS	
AN quadratic		NS	NS	NS	NS	
BS linear		NS	NS	NS	NS	
BS quadratic		NS	NS	NS	NS	
AN vs BS		NS	*	NS	NS	

*, **, *** - significant at the 0.05, 0.01, and 0.001 probability levels, respectively
NS- not significant
BS – biosolids obtained from the City of Stillwater
AN – ammonium nitrate (34-0-0)
†,‡ treatment means for all rates for BS and AN, respectively (excluding lime treatments)
Detection limits - 0.0008. 0.0075. 0.0033, 0.0130 ppm for Cd, Cu, Mo, and Pb , respectively

TABLE 9. Winter wheat grain content of heavy metals as influenced by N source and rate, Stillwater, OK, 1999.

N Source	N Rate	Cadmium	Copper	Molybdenum	Lead
	kg ha^{-1}	----------------------------	mg kg^{-1}	----------------------------	
-	0	0.030	4.647	0.659	0.162
-	0	0.008	5.554	0.296	0.207
BS	45	0.015	3.831	0.342	0.081
BS	90	0.021	4.123	0.386	0.143
BS	180	0.017	3.852	0.496	0.102
BS	270	0.024	3.826	0.489	0.095
BS	540	0.033	3.798	0.605	0.406
AN	45	0.017	4.460	0.297	0.395
AN	90	0.024	5.023	0.343	0.201
AN	180	0.085	5.333	0.368	0.021
AN	270	0.069	3.999	0.184	0.185
AN	540	0.026	4.301	0.172	0.052
AN+L	540	0.018	3.849	0.899	0.170
BS+L	540	0.047	3.889	0.285	0.667
BS mean †		0.022	3.886	0.464	0.081
AN mean ‡		0.044	4.623	0.273	0.171
SED		0.018	0.704	0.111	0.169
CV		71.58	19.90	32.69	126.42
Contrasts					
AN linear		NS	NS	NS	NS
AN quadratic		***	NS	NS	NS
BS linear		NS	NS	**	NS
BS quadratic		NS	NS	NS	NS
AN vs BS		NS	NS	NS	NS

*, **, *** - significant at the 0.05, 0.01, and 0.001 probability levels, respectively
NS- not significant
BS – biosolids obtained from the City of Stillwater
AN – ammonium nitrate (34-0-0)
†,‡ treatment means for all rates for BS and AN, respectively (excluding lime treatments)
Detection limits - 0.0008, 0.0075, 0.0033, 0.0130 ppm for Cd, Cu, Mo, and Pb , respectively

TABLE 10. Winter wheat grain content of heavy metals as influenced by N source and rate, Stillwater, OK, 2000

N Source	N Rate	Cadmium	Copper	Molybdenum	Lead
	kg ha^{-1}	----------------------------	--mg kg^{-1}--	-------------------------------------	
-	0	0.028	5.031	0.641	0.414
-	0	0.063	4.780	0.577	0.175
BS	45	0.046	4.646	0.492	0.114
BS	90	0.027	5.094	0.843	0.119
BS	180	0.065	4.654	1.011	0.254
BS	270	0.042	4.602	1.255	0.163
BS	540	0.029	4.175	0.716	0.145
AN	45	0.016	5.100	0.562	0.507
AN	90	0.024	4.418	0.449	0.189
AN	180	0.026	4.503	0.493	0.223
AN	270	0.036	5.116	0.597	0.435
AN	540	0.033	4.947	0.303	0.270
BS+L	540	0.065	3.042	0.727	1.396
AN+L	540	0.117	6.615	1.438	0.200
BS mean †		0.042	4.634	0.863	0.159
AN mean ‡		0.027	4.817	0.481	0.325
SED		0.035	569	0.377	0.465
CV		98.56	14.62	64.01	173 15
Contrasts					
AN Linear		NS	NS	NS	NS
AN quadratic		NS	NS	NS	NS
BS linear		NS	NS	NS	NS
BS quadratic		NS	NS	*	NS
AN vs BS		NS	NS	NS	NS

*, **, *** - significant at the 0.05, 0.01, and 0.001 probability levels, respectively
NS- not significant
BS – biosolids obtained from the City of Stillwater
AN – ammonium nitrate (34-0-0)
†,‡ treatment means for all rates for BS and AN, respectively (excluding lime treatments)
Detection limits - 0.0008, 0.0075, 0.0033, 0.0130 ppm for Cd, Cu, Mo, and Pb , respectively

TABLE 11. Winter wheat grain content of heavy metals as influenced by N rate and source, Stillwater, OK, 2001

N Source	N Rate	Cadmium	Copper	Molybdenum	Lead
	--kg ha^{-1}--	-----------------------mg kg^{-1}-------------------------------			
-	0	0.018	5.484	0.636	0.102
-	0	0.017	6.021	0.433	0.078
BS	45	0.030	6.339	0.321	0.558
BS	90	0.018	5.069	0.339	0 135
BS	180	0.035	6.110	0.414	0.101
BS	270	0.027	6.058	0.494	0.161
BS	540	0.044	6 185	0.604	0.126
AN	45	0.012	6.562	0.270	0.126
AN	90	0.020	6.055	0.231	0.076
AN	180	0.019	5.415	0.197	0.138
AN	270	0.022	5.247	0.157	0.138
AN	540	0.029	5.449	0.137	0.077
BS+L	540	0.031	5.459	0.911	0.144
AN+L	540	0.026	6.046	0.279	0.167
BS mean †		0.031	5.952	0.434	0.216
AN mean ‡		0.020	5.746	0.198	0.111
SED		0.011	0.482	0 096	0.161
CV		55.5	10 1	29.9	129.1
Contrasts					
AN linear		NS	*	*	NS
AN quadratic		NS	NS	NS	NS
BS linear		*	NS	**	NS
BS quadratic		NS	NS	NS	NS
AN vs BS		NS	NS	***	NS

*, **, *** - significant at the 0.05, 0.01, and 0.001 probability levels, respectively

NS- not significant

BS – biosolids obtained from the City of Stillwater

AN – ammonium nitrate (34-0-0)

†,‡ treatment means for all rates for BS and AN, respectively (excluding lime treatments)

Detection limits - 0.0008, 0.0075, 0.0033, 0.0130 ppm for Cd, Cu, Mo, and Pb , respectively

Table 12. Chemical characteristics of Norge loam at initiation and 8 years after initiation, Stillwater, OK.

N Source	N Rate	Cadmium	Copper	Molybdenum	Lead
Initialφ		0.179	-	0.406	12.24
	---kg ha^{-1}---	---------------------------	mg kg^{-1}	---------------------------	
-	0	0.247	7.465	0.097	2.32
-	0	0.235	6.377	0.072	0.935
BS	45	0.252	9.843	0.040	3.783
BS	90	0.274	10.260	0.203	4.800
BS	180	0.278	14.510	0.485	9.143
BS	270	0.283	14.412	0.346	9.992
BS	540	0.305	18.530	0.573	14.977
AN	45	0.274	6.363	0.037	1.575
AN	90	0.279	7.245	0.090	2.598
AN	180	0.248	6.257	0.111	3.415
AN	270	0.252	8.158	0.095	2.285
AN	540	0.257	6.467	0.060	2.228
BS+L	540	0.313	19.477	0.616	15.982
AN+L	540	0.233	5.575	0.223	1.665
BS mean †		0.278	13.511	0.329	8.539
AN mean ‡		0.262	6.898	0.079	2.420
SED		0.031	1.57	0.115	1.097
CV		14.2	19.1	65.0	35.1
Contrasts					
AN linear		NS	NS	NS	NS
AN quadratic		NS	NS	NS	NS
BS linear		*	***	***	***
BS quadratic		NS	NS	NS	*
AN vs BS		NS	***	***	***

*, **, *** - significant at the 0.05, 0.01, and 0.001 probability levels, respectively

NS- not significant

BS – biosolids obtained from the City of Stillwater

AN – ammonium nitrate (34-0-0)

†,‡ treatment means for all rates for BS and AN, respectively (excluding lime treatments)

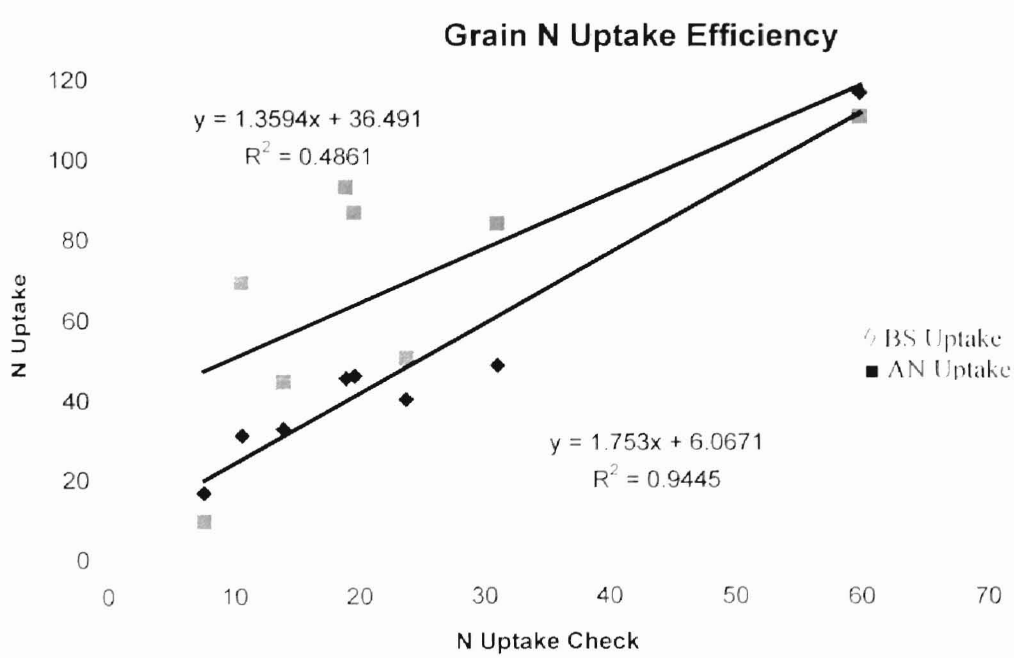
φ- Initial soil sample, 1993

APPENDIX

Figure 1 shows that both biosolid and check N treatments were more dependent upon the the environment and whether or not the environment was conducive to N mineralization since they were highly correlated (R^2=.94). Alternatively, N uptake in the check verses N uptake in the ammonium nitrate treatments was more variable (R^2= .48) since N availability was less dependent upon the N mineralization environment for this inorganic N source. Nitrogen uptake efficiency was calculated by the equation:

$$\frac{\text{(N uptake of treated plot - N uptake of 0N plot)}}{\text{N Rate}} * 100$$

Figure 1. Grain N uptake efficiency as influenced by N source and N rate, Stillwater, OK, 1994 to 2001.



VITA

Kathie June Wynn

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF LONG-TERM APPLICATION OF BIOSOLIDS AND AMMONIUM NITRATE ON WINTER WHEAT YIELD, NITROGEN UPTAKE, AND HEAVY METAL ACCUMULATION

Major Field: Plant and Soil Sciences

Biographical:

Personal Data: Born in Mangum, Oklahoma, on October 20, 1975.

Education: Graduated from Mangum High School, Mangum, Oklahoma in May 1994; received Associate of Science degree in Agriculture from Western Oklahoma State College, Altus, Oklahoma in May 1996; received Bachelor of Science degree in Animal Science from Oklahoma State University in May 1998. Completed the requirements for the Master of Science degree with a major in Plant and Soil Science at Oklahoma State University in August 2001.

Experience: Employed as an office assistant at the Mangum Star News, Mangum, Oklahoma, 1994-1996; employed by Oklahoma State University, Department of Plant and Soil Sciences as a hourly employee, 1996-1999; employed by Oklahoma State University, Department of Plant and Soil Sciences as a graduate research assistant, 1999-present.