

## Effect of Long-Term Application of Biosolids on Molybdenum Content and Quality of Winter Wheat Forage

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### ABSTRACT

Forages that accumulate excessive molybdenum (Mo) from excessive land application of Mo can cause molybdenosis, a copper (Cu) induced deficiency in ruminants. Limited information is available on the effect of land-applied biosolids, Mo content, and quality of winter wheat forage (*Triticum aestivum* L.). The objectives of this study were (1) to determine the effect of biosolids application on tissue molybdenum and tissue copper (Cu):Mo of winter wheat forage and, (2) to measure the Mo uptake coefficient value (UC) for winter wheat under field conditions and compare it with the U.S. EPA Part 503 risk-based UC value of 0.42. Two nitrogen sources, anaerobically digested biosolids and ammonium nitrate, were applied annually from 1993–2001 to continuous winter wheat. The experimental design was a complete factorial arrangement of treatments composed of six nitrogen (N) rates (0, 45, 90, 180, 269, and 539 kg N ha<sup>-1</sup> yr<sup>-1</sup>) and two N sources (anaerobically-digested biosolids and ammonium nitrate, 34-0-0). Application of biosolids did not significantly alter soil pH, which ranged from 6.6 to 7.2. Biosolids application increased soil Cu from 7.19 to 19.6 mg kg<sup>-1</sup> and soil Mo from 0.44 to 1.02 mg/kg. Forage uptake of Mo and Cu showed temporal variation between years but increased with biosolids application rate. The Cu:Mo ratio of washed forage was >2.9 and was inversely related to biosolids application rate. Forage Mo was <2 mg kg<sup>-1</sup>. Unwashed forage had a larger Cu:Mo ratio than washed forage. Forage Mo increased with biosolids application for unwashed forage. However, the Cu:Mo ratio of unwashed forage decreased with increased biosolids application and the unwashed forage Cu:Mo ratio was >10. Biosolids application had no effect on forage sulfur (S) content that was <3000 mg kg<sup>-1</sup>. The calculated UC of Mo for winter wheat in Oklahoma of 0.24 (washed forage) and 0.36 (unwashed forage) is less than the UC of 0.42 used by U.S. EPA in its risk assessment governing land application of biosolids. These results suggest

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winter wheat forage produced using biosolids presents minimal risk of molybdenosis to livestock. Ingestion of soil on unwashed forage increases forage Cu:Mo and offers more protection from the risk of molybdenosis.

**Keywords:** biosolids, heavy metals, molybdenum, sewage sludge, winter wheat

## INTRODUCTION

Almost 40% of municipal wastewater biosolids are land applied to cropland in the United States (U.S. EPA, 1995b). In 1993, the U.S. EPA promulgated federal regulations governing land application of biosolids (U.S. EPA, 1993). One major concern addressed in Part 503 is the accumulation of heavy metal contaminants in land receiving biosolids. Fourteen risk-based exposure pathways to humans and ecological receptors were used to calculate acceptable cumulative limits on heavy metal contaminants via land application of biosolids. Cumulative metal loadings were based on the pathways that posed the most risk (i.e. the limiting pathway). The limiting pathway for molybdenum (Mo) was the biosolids-Mo → soil → forage → animal pathway, which represented livestock grazing on forage grown on soil treated with biosolids. A cumulative Mo loading rate of 18 kg/ha from biosolids application was calculated using an algorithm that assumed an uptake coefficient (UC) of 0.42 (O'Connor and McDowell, 1999). The UC is the ratio of forage Mo to soil Mo from biosolids application. Excessive land application of Mo may cause molybdenosis, a copper (Cu) induced deficiency in ruminants.

Biosolids commonly applied to farmland contain Mo concentrations ranging from 5 to 50 mg kg<sup>-1</sup> (McBride et al., 2000). Currently, biosolid material containing more than 75 mg Mo kg<sup>-1</sup> is prohibited from being land applied (U.S. EPA, 1993). Plant uptake of Mo has been well correlated with the amount of Mo in soil solution under field conditions (Adriano, 2001). Excessive land application of Mo in biosolids may increase soil Mo concentrations and result in increased Mo in plants (Adriano, 2001). Consumption of forages containing elevated levels of Mo (e.g., 10 mg Mo kg<sup>-1</sup> forage) may result in a Mo induced Cu deficiency known as molybdenosis in cattle and sheep that may be fatal (Adriano, 2001). More specifically, consumption of forages with a Cu:Mo ratio of < 2:1 has been associated with increased susceptibility to molybdenosis (National Research Council, 1996). Gupta and Gupta (1998) identified a Cu:Mo ratio of 6:1 to be critical. Other ratios have been proposed when dealing with pastures and forages, suggesting Cu:Mo ratios of 4:1 (Alloway, 1973) and 5:1 (Suttle, 1991). Recently, O'Connor and McDowell (1999), who conducted an extensive study on land-applied Mo from biosolids and performed a comprehensive review of forage quality and molybdenosis, recommended that 10 mg kg<sup>-1</sup> Mo in forage is "an oversimplification of animal response to forage molybdenum exposure, but is reasonable

for a national risk assessment if practical aspects of animal management (for example, mineral supplementation and total diet considerations) are recognized.”

Molybdenum availability to plants is strongly affected by soil pH, increasing with increased pH (Karimian and Cox, 1978). Alkaline-stabilized biosolids have been shown to increase Mo availability, resulting in low Cu:Mo ratios in red clover (McBride et al., 2000). Excessive uptake of Mo resulting in molybdenosis from biosolid-amended soils is specific to legume crops. In a study monitoring Cu and Mo uptake by peas, soybeans, and corn, legumes grown in biosolid-amended soils that contained 2 to 3 mg Mo kg<sup>-1</sup> showed a marked increase in Mo tissue concentration above that of non-legumes (McBride et al., 2000). Similarly, a study conducted using soybeans showed high Mo accumulation (>10 mg kg<sup>-1</sup>) in the grain from plants grown on biosolid-amended soils (O'Connor et al., 2001a). Conversely, corn grown in a biosolid-amended soil showed little to no increase in stover Mo concentration, even at high soil Mo loading rates (>18 kg ha<sup>-1</sup>) (O'Connor et al., 2001b). Another study conducted on bahiagrass showed a slight accumulation of Mo from pastures treated with biosolids compared with a control, but the concentrations never approached toxic levels (Tiffany et al., 2000). Winter wheat is commonly used as forage in the southern plains of the United States. Limited information is available on the effect of land-applied biosolids on winter wheat forage quality. The objectives of this study were (1) to determine the effect of biosolids application on tissue molybdenum and tissue Cu:Mo of winter wheat forage and, (2) to measure the Mo UC for winter wheat under field conditions and compare it with the U.S. EPA Part 503 risk-based UC value of 0.42.

## MATERIALS AND METHODS

A winter wheat (*Triticum aestivum* L.) field experiment was established at the Agronomy Research Station (Stillwater, Oklahoma) in the fall of 1993. A complete factorial arrangement of treatments composed of six nitrogen (N) rates (0, 45, 90, 180, 269, and 539 kg N ha<sup>-1</sup> yr<sup>-1</sup>) and two nitrogen (N) sources (anaerobically-digested biosolids and ammonium nitrate, 34-0-0) were evaluated within a randomized complete block experimental design with three replications. Biosolids were applied based on total N rate and on a dry basis to supply plant available N. Plant available N (PAN) of biosolids was calculated as NH<sub>4</sub>-N + NO<sub>3</sub>-N + 0.20 (organic N) (U.S. EPA, 1995b). This calculation assumes organic N in the biosolids had a nitrogen mineralization rate of 20%. This mineralization rate is recommended for anaerobically-digested biosolids to calculate PAN in biosolids (U.S. EPA, 1995b). Total N content, moisture content, and an example of application rate (90 kg N ha<sup>-1</sup>) of the biosolids are listed in Table 1. Each nitrogen source was applied preplant and disk incorporated in

Table 1  
Nitrogen content, moisture percentage, and biosolids application rates for the 90 kg N ha<sup>-1</sup> rate, 1993–2000

Year	Nitrogen, g kg <sup>-1</sup>	Moisture, %	Biosolids, kg ha <sup>-1</sup> †
1993	20.2	60	11 139
1994	17.4	35	7 958
1995	19.7	59	11 143
1996	27.3	55	7 326
1997	24.2	46	6 887
1998	24.3	50	7 407
1999	20.2	45	8 101
2000	24.4	17	4 444

† Applied to deliver 90 kg N ha<sup>-1</sup> plant available N.

the fall. Two added treatments outside of the factorial included lime applied in 1993 (8.96 Mg ha<sup>-1</sup>), 1999 (8.96 Mg ha<sup>-1</sup>), and 2000 (13.0 Mg ha<sup>-1</sup>) to the high N-rate plots (540 kg N ha<sup>-1</sup>yr<sup>-1</sup>) for both N sources. The soil at this site is a Norge loam (fine mixed, thermic Udertic Paleustoll). Forage was hand harvested from a 1 m<sup>2</sup> 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). Each year, 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 August 2000. Copper and Mo content of soil, biosolids, and plant material were determined by wet digestion using HNO<sub>3</sub>, HClO<sub>4</sub> (Jones and Case, 1990) followed by analysis using a high-resolution inductively coupled plasma spectrophotometer (Thermo-Jarrell Ash IRIS ICP). Analytical emission wavelengths used were 202.0 nm and 204.6 nm for Mo, 324.8 for Cu, and 182.0 nm for sulfur (S). Total N in soil and biosolids samples was determined using a Carlo-Erba NA 1500 dry combustion analyzer.

Sulfur content of the plant material was determined by nitric digestion (Jones and Case, 1990) followed by analysis using a high-resolution 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, 2001). The standard error of the difference (SED) between two equally replicated means is reported in Figures 1–6.

Standard reference materials from the National Institute of Standards and Technology rice flour (SRM 1568a) and tomato leaf (SRM 1573a) were used for

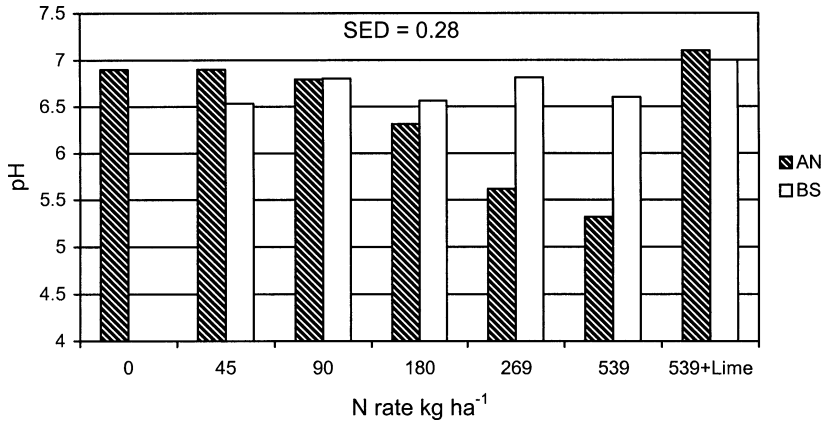


Figure 1. Effect of biosolid (BS) and ammonium nitrate (AN) application on soil pH, samples collected in August 2000.

quality assurance/quality control (QA/QC) of plant tissue analysis. Recoveries of Cu and Mo were 97% and 95%, respectively. A biosolids-treated soil from Resource Technology Corporation Certified Reference Material (CRM 005-050) was used to evaluate QA/QC for soil analysis. Recoveries of 86% were obtained for Cu and Mo analysis. A biosolids CRM 011-100 from Resource

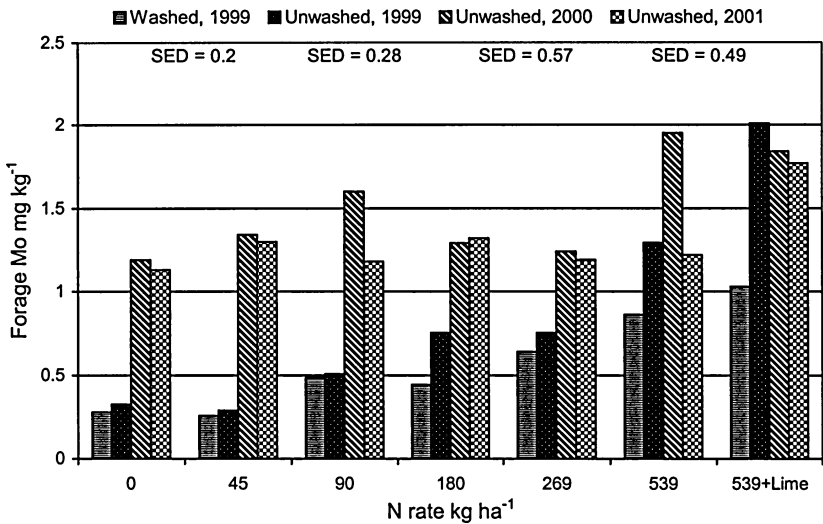
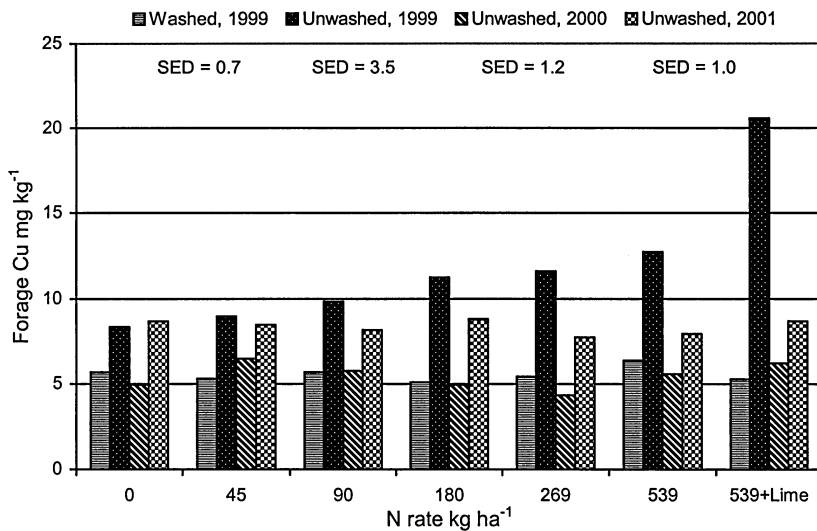
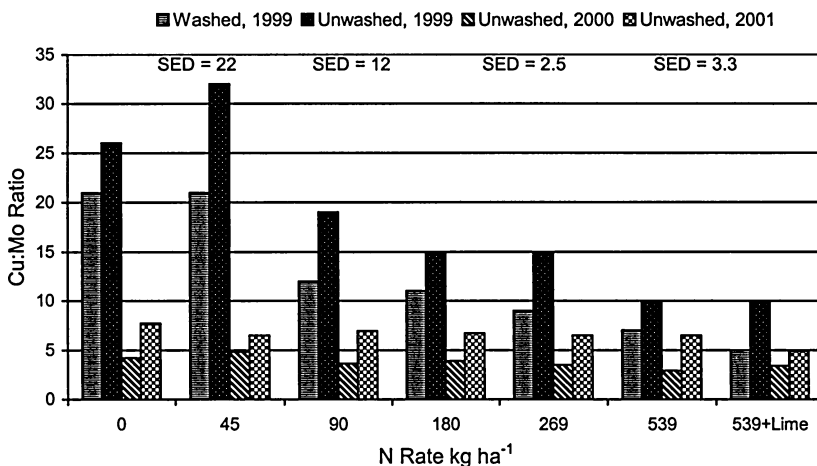


Figure 2. Effect of biosolid application on wheat forage uptake of Mo for washed and unwashed samples, 1999–2001.



**Figure 3.** Effect of biosolid application on wheat forage uptake of Cu for washed and unwashed samples, 1999–2001.

Technology Corporation was used to evaluate the biosolids QA/QC, which resulted in Cu and Mo recoveries of 87%. An apple leaf SRM from the National Institute of Standards and Technology (SRM 1515) was used to evaluate QA/QC for plant tissue S. Recovery of 97% S was obtained.



**Figure 4.** Effect of biosolid application on Cu:Mo ratio of wheat forage, 1999–2001.

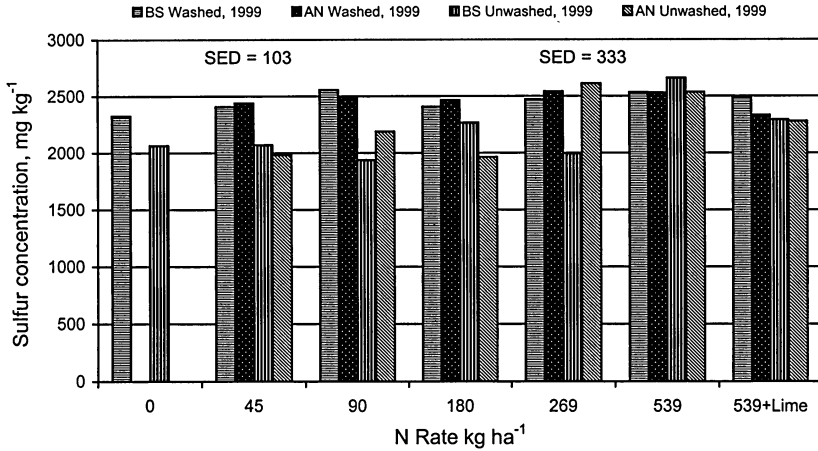


Figure 5. Effect of biosolid (BS) and ammonium nitrate (AN) application on sulfur concentration of washed and unwashed wheat forage, 1999.

## RESULTS AND DISCUSSION

### Biosolids Quality

The biosolids evaluated in this study contained relatively low amounts of heavy metals compared with U.S. EPA limits (Table 2). Most metal levels were below

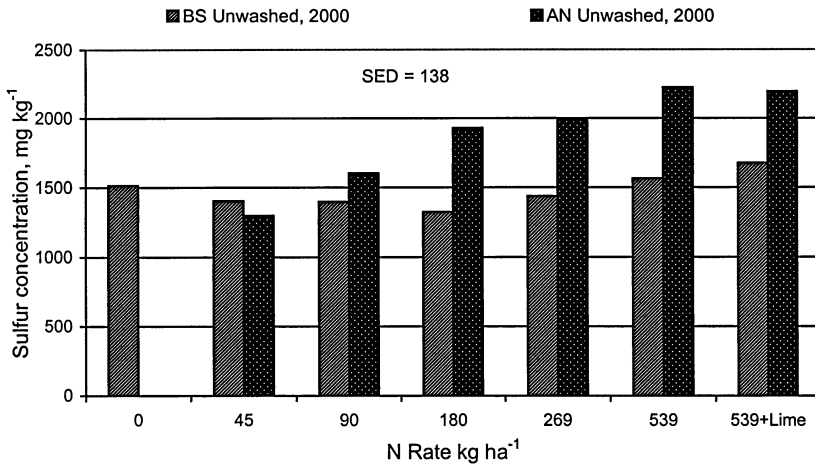


Figure 6. Effect of biosolid (BS) and ammonium nitrate (AN) application on sulfur concentration of unwashed wheat forage, 2000.

Table 2

Summary statistics of biosolids evaluated compared with ceiling concentrations and pollutant concentrations defined by the U.S. EPA (40 CFR Part 503)<sup>†</sup>

Pollutant	Pollutants in biosolids				“EQ” pollutant concentrations <sup>‡</sup>	Ceiling pollutant concentrations <sup>§</sup>
	Min	Max	Mean	Median		
	mg kg <sup>-1</sup>					
Arsenic	0	2.5	1.3	1.4	41	75
Cadmium	0	3.5	1.9	2.3	39	85
Chromium	38	177	98	101	1200	3000
Copper	233	898	461	384	1500	4300
Lead	153	1429	510	273	300	840
Molybdenum	20	49	29	24	18	75
Nickel	21	79	37	26	420	420
Zinc	942	1222	1043	1000	2800	7500

<sup>†</sup>U.S. EPA (1993).

<sup>‡</sup>Exceptional Quality, Table 3 of U.S. EPA 40 CFR Part 503 (U.S. EPA, 1993).

<sup>§</sup>Table 1 of Table 3 of U.S. EPA 40 CFR Part 503 (U.S. EPA, 1993).

“Exceptional Quality” or EQ pollutant concentrations except for Mo (all years) and lead (Pb) (for two of the study years). Biosolids pollutant contents were well below the U.S. EPA Part 503 ceiling pollutant concentrations and were allowed to be land applied for beneficial use.

### Effect of Biosolids Application on Soil Characteristics

Application of biosolids did not significantly alter soil pH, but ammonium nitrate application decreased soil pH, specifically at the high N rates (Figure 1). Application of biosolids significantly increased several soil metal levels after eight years (Table 3). Soil Cu and Mo levels more than doubled when biosolids were applied at a rate of 539 kg N ha<sup>-1</sup>, and there was a linear trend of increasing soil Cu, Mo, Pb, and Zn as the rate of biosolids applied increased (Table 3). Soil Cr and Ni levels were also increased with increasing biosolid rate, but reached a maximum at the 180 kg N ha<sup>-1</sup> rate. However, application of biosolids did not increase soil Cr or Ni levels above values at comparable N rates of ammonium nitrate. Cadmium levels were below detectable limits (~0.500 mg kg<sup>-1</sup>) for all treatments (Table 3). Soil Pb levels were increased by a factor of 4 at the highest biosolids rate compared with the plot that received no biosolids.

### Effect of Biosolids Application on Forage Quality

Forage uptake of Mo and Cu showed temporal variation between years. In 1999, uptake of Mo was relatively low but did increase with increasing biosolid rate



Table 3

Pollutant concentration of heavy metals in soils following eight years of biosolids application

N rate, kg ha <sup>-1</sup>	Pollutant concentration in soil								
	Pollutant								
	As	Cd	Cr	Cu	Pb	Mo	Ni	Zn	
				mg kg <sup>-1</sup>					
0	1.25	<0.5	45.18	7.19	4.86	0.44	26.17	35.18	
45	1.60	<0.5	49.68	10.42	7.12	0.49	28.17	40.05	
90	1.78	<0.5	49.22	10.93	8.43	0.54	28.56	47.64	
180	1.58	<0.5	56.65	15.42	12.66	0.83	31.21	46.16	
269	1.83	<0.5	51.84	15.26	13.87	0.77	28.80	50.86	
539	1.52	<0.5	49.82	19.57	19.06	1.02	27.34	56.27	
Significance									
Linear	NS	NS	NS	***	***	***	NS	***	
Quadratic	NS	NS	**	*	*	*	**	NS	

NS, \*, \*\*, \*\*\* - Non-significant (NS) or significant at 5% (\*), 1% (\*\*), or 0.1% (\*\*\*).

(Figure 2). Copper in unwashed forage in 1999 also increased with increasing biosolids rate but levels were lower in subsequent years (Figure 3). Washed and unwashed forage samples were analyzed to determine the effect of pretreatment on removal of soil contamination for the 1999 season. In a grazing situation, analysis of the unwashed forage may provide a more accurate representation of metal uptake than washed samples. Measured Mo for washed forage samples was equal to or less than unwashed samples in 1999. Measured Cu for unwashed forage samples was greater than in washed forage samples in 1999 (Figure 3). The increase in forage Cu was greater than forage Mo for unwashed samples because the soil covering the forage contained much greater amounts of Cu than Mo (Table 3). Unwashed forage had a larger Cu:Mo ratio than washed forage in 1999 (Figure 4). Despite the increasing Mo uptake as biosolid rate increased, the Cu:Mo ratio of the unwashed forage never dropped below 10:1 in 1999 (Figure 4). The Cu:Mo ratio of the washed forage reached 7:1 at the highest biosolid rate. Application of lime at the highest N rate increased Mo tissue levels of washed and unwashed forage, but Cu:Mo ratios were still greater than 5:1.

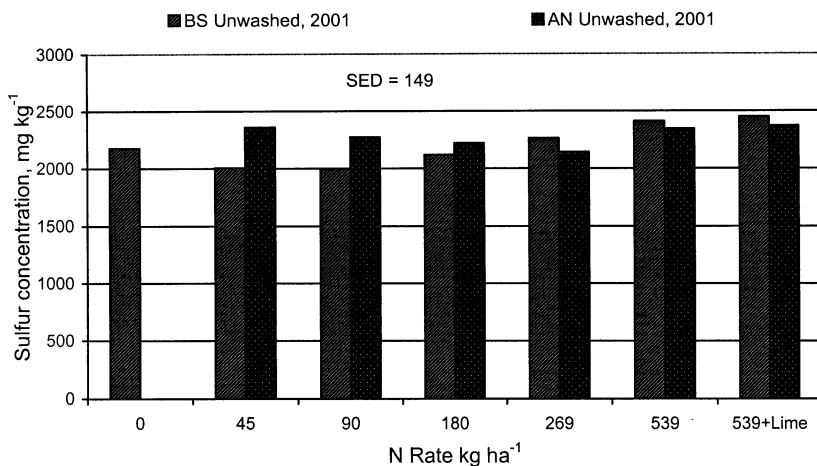
In general, forage uptake of Mo was significantly higher in 2000 than in the previous year (Figure 2). Although differences were statistically insignificant, Mo tissue levels tended to increase linearly up to 90 kg N ha<sup>-1</sup> and were maximized at the 539 kg N ha<sup>-1</sup> rate (Figure 2). The highest level was around 2 mg Mo kg<sup>-1</sup> at the highest biosolid rate. Copper:Mo ratios were significantly lower in 2000 with the lowest being 2.9:1 at the highest biosolid rate (Figure 4).

It is interesting to note that the Cu:Mo ratio of the check plot was 4.2:1, the lowest of any year of the study, suggesting that growing season environmental conditions may play a strong role in determining Cu and Mo availability and uptake.

Molybdenum uptake in 2001 was not as high as in 2000, and no significant differences between Mo uptake of the control and biosolid treatments existed (Figure 2). Copper:molybdenum ratios were all near 6:1 for all treatments (Figure 4). Liming did increase Mo uptake at the highest N rate, but the Cu:Mo ratio was still relatively high (~5:1).

Plant tissue S concentration is also a concern for grazing livestock with a critical level set at 0.4% to 0.5% (National Research Council, 1996). High forage sulfur may offset the Cu:Mo ratio even if the ratio is greater than 2 and the threat of molybdenosis is considered small (National Research Council, 1989). In the rumen, S may react with Mo to form thiomolybdates (Suttle, 1991), which may react with Cu to form insoluble complexes that are poorly absorbed, causing Cu deficiency signs to occur (O'Connor and McDowell, 1999). Biosolids contain moderate amounts of sulfur (approximately 1% dry weight); if biosolids are applied to meet crop N requirements, its presence can result in high amounts of S applied to agricultural soils (O'Connor and McDowell, 1999).

Biosolids application had no effect on forage S content (Figures 5–7). Except for data from the 2000 season, forage S was similar for both nitrogen fertilizer sources (e.g., biosolids and ammonium nitrate). In 2000, forage S tended to be higher in ammonium nitrate than in biosolids-treated soil (Figure 6). Forage S concentrations did not approach 3000 mg S kg<sup>-1</sup> in any year of the trial

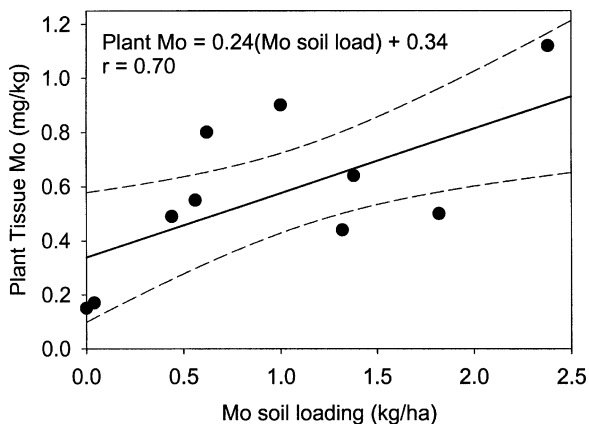


**Figure 7.** Effect of biosolid (BS) and ammonium nitrate (AN) application on sulfur concentration of unwashed wheat forage, 2001.

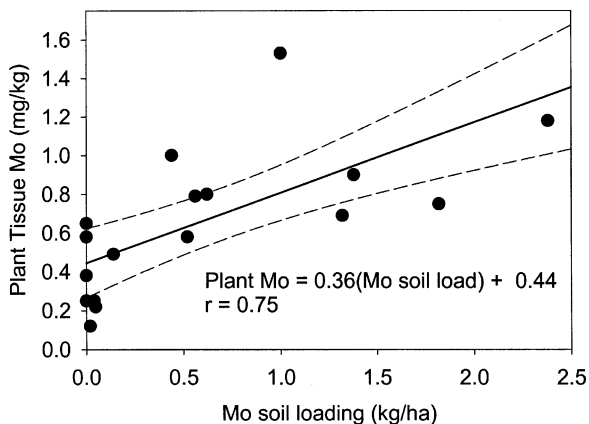
(Figures 5, 6, and 7), which is below the 4000 to 5000 mg S kg<sup>-1</sup> critical level set by the NRC (National Research Council, 1996). Biosolids would not be expected to increase the potential for molybdenosis for cattle grazing winter wheat forage in this experiment because biosolids did not increase forage S.

### Effect of Biosolids Application on U.S. EPA Part 503 Risk-Based Uptake Coefficients

There were linear relationships between unwashed and washed forage (plant) tissue Mo and Mo soil loading for the 1998–1999 season (Figures 8 and 9). However, forage Mo was not related to Mo soil loading for the 1999–2000 season ( $r = 0.01$ ). The slope of the forage Mo vs. Mo soil loading plot is referred to as the uptake coefficient (UC) (U.S. EPA, 1995a). The Mo UC of washed winter wheat forage 1998–1999 was 0.24 (Figure 8). This UC is below the Mo UC of 0.42 used by U.S. EPA in its risk assessment used for governing land application of biosolids (e.g., Part 503; 2) (Table 4). These results suggest washed winter wheat forage in our study presents minimal risk of molybdenosis to livestock. The Mo UC of unwashed winter wheat forage 1998–1999 was 0.36 (Figure 8), 50% larger than the UC of the same washed forage (Figure 8). Dust on forage increased the forage Mo by a factor of 1.5. These results may initially suggest ingestion of unwashed forage by livestock may present more risk than washed forage. However, unwashed forage had a smaller Cu:Mo ratio than washed forage (Table 3). The Cu:Mo ratio suggests less risk associated with ingestion of unwashed forage. The UC of 0.36 in unwashed forage (Figure 9)



**Figure 8.** Linear model of washed wheat forage Mo vs. soil loading for 1999. Dashed lines represent the 95% confident interval.



**Figure 9.** Linear model of unwashed wheat forage Mo vs. soil Mo loading for 1999. Dashed lines represent the 95% confident interval.

was less than the UC of 0.42 used by U.S. EPA in Part 503. These results suggest unwashed winter wheat forage in our study presents minimal risk of molybdenosis to livestock.

### Effect on Grain Yield

Yield results for all years of the trial are presented in Table 5. In five out of eight years, N supplied as ammonium nitrate resulted in higher average yields than biosolid-treated plots. There was good response to N applied as biosolids

Table 4  
Comparison of uptake coefficients of Mo for wheat, corn, red clover, and soybean from land application of biosolids

Crop	UC	Reference
Wheat (Unwashed)	0.36	This study
Wheat (Washed)	0.24	This study
Corn	0.001	O'Connor et al. (2001)
Red Clover	1.06	McBride et al. (2000)
Soybean	1.66	O'Connor et al. (2001)
Alfalfa	2.9	Pierzynski and Jacobs (1986)
Bromegrass	0.11	Soon and Bates (1985)
Bahiagrass	0.81	O'Connor et al. (2001)
USEPA	0.42	USEPA (1993)

Table 5

Effect of biosolids application on wheat grain yield at Stillwater, OK, 1994–2001

N Source	N Rate	1994	1995	1996	1997	1998	1999	2000	2001
—	0	464	499	612	1127	1933	908	923	1245
—	0	377	520	575	1287	1609	902	975	1074
BS	45	527	571	727	1557	2378	947	1309	1684
BS	90	616	574	656	1514	2242	1068	1497	1573
BS	180	885	568	1234	1399	3048	1361	1575	1981
BS	269	1037	610	918	1389	2830	964	1233	1777
BS	539	1504	1026	821	1457	3601	1833	1921	1541
AN	45	1781	620	339	1368	2053	904	1717	1386
AN	90	1737	905	512	1762	2861	1607	2884	1842
AN	180	1919	1196	665	2176	3439	2367	3301	2269
AN	269	2586	1418	535	2349	3039	2888	2783	2438
AN	539	2335	1281	556	2555	3104	2475	2050	1789
BS + L	539	1668	1051	880	1629	3727	2600	1568	1985
AN + L	539	2185	1325	781	2841	3708	2858	2210	1824
BS means <sup>†</sup>		957	700	836	1439	2823	1383	1264	1684
AN means <sup>‡</sup>		1846	1038	562	2048	2830	2000	2275	1803
SED		176	172	139	225	209	237	299	220
CV, %		15	24	35	22	13	24	28	22
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.

AN – ammonium nitrate (34-0-0).

L – lime applied at a rate of 9, 9, and 13 Mg ha<sup>-1</sup> in the fall of 1993, 1999, and 2000, respectively.

<sup>†</sup>, <sup>‡</sup> Treatment means for all rates for BS and AN, respectively.

CV – coefficient of variation.

in five out of eight years, indicated by a linear response to applied biosolids. In 1996, application of biosolids actually resulted in higher average yields than did ammonium nitrate treatments, but yield levels were relatively low. Following eight years of continuous N application, N applied as ammonium nitrate resulted in increased yields when compared with the biosolid N source, even in the later years of the experiment. The amount of biosolids land applied

was based on plant available N. Plant available N (PAN) of biosolids was calculated assuming a N mineralization rate of 20%. This mineralization rate is recommended for anaerobic-digested biosolids to calculate PAN in biosolids (U.S. EPA, 1995b). These results suggest the anaerobic biosolids used in our study had a nitrogen mineralization of <20%, resulting in less than optimum crop yield. The anaerobic biosolids used in this study are commonly stockpiled for several months after dewatering at the wastewater treatment facility. It is possible that PAN decreased during storage of biosolids, resulting in an N mineralization rate of <20%.

## CONCLUSIONS

Land application of biosolids for winter wheat production did not result in forage Cu:Mo ratios less than 2:1 in any of the three years analyzed. As expected, lime application in conjunction with biosolid application did increase Mo uptake, but Cu:Mo ratios were still greater than 2:1. Soil Mo levels were increased over the eight years of the study, but concentrations were still quite low. Washing of forage samples taken for tissue analysis may result in lower Cu:Mo ratios, specifically if the forage is grazed. Not washing forage samples is a more accurate representation of forage that cattle may consume when grazing. Cattle may ingest enough soil with the forage to maintain protective Cu:Mo ratios, thus minimizing the risk of molybdenosis. The calculated UC of Mo for winter wheat in Oklahoma of 0.24 (washed forage) and 0.36 (unwashed forage) is less than the UC of 0.42 used by U.S. EPA in its risk assessment used for governing land application of biosolids (Part 503; U.S. EPA, 1993). These results suggest winter wheat forage presents minimal risk of molybdenosis to livestock. Ingestion of soil on unwashed forage increases forage Cu:Mo and offers more protection from the risk of molybdenosis.

## ACKNOWLEDGMENTS

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