Effect of Using High Molybdenum Biosolids (Sludge) as Fertilizers on Copper Status of Beef Cattle

L. R. McDowell, G. A. O'Connor†, M. E. Tiffany*, N.S. Wilkinson*, H. Nguyen, and E. C. Cardoso††*
**Departments of Animal Sciences and †Soil and Water Science, University of Florida, Gainesville and ††Faculdade de Ciências Agrárias do Para, Belém, Pa. Brazil*

Introduction

With the exception of phosphorus, deficiency of copper is the most severe mineral limitation to grazing livestock throughout extensive world regions (McDowell, 1992, 1997). Copper deficiencies in ruminants occur mainly under grazing conditions, with gross signs of the deficiency being rare when concentrate feeds are fed. The majority of world reports are concerned with a "conditioned" copper deficiency where normal amounts of copper (6-16 ppm) are inadequate due to higher than normal amounts of other elements such as molybdenum, sulfur, and other factors which block the utilization of copper by the body. Copper deficiencies usually occur when forage molybdenum exceeds 3 ppm and the copper level is below 5 ppm. Clinical signs of molybdenum or sulfur-induced copper deficiency in ruminants include anemia, loss of hair color, and neonatal ataxia (McDowell, 1992).

Copper intake is the primary interacting factor in molybdenum toxicity because sufficient copper supplementation can counteract almost all disorders associated with high molybdenum intakes (Clawson et al., 1972). In addition to molybdenum intake and availability, Ward (1994) identified dietary factors clearly related to molybdenosis, or copper deficiency, as: 1) copper intake, 2) copper availability, 3) sulfur intake, 4) iron intake, and 5) the physical form of the feed.

In the presence of sulfur, high intakes of molybdenum can induce a copper deficiency due to formation of insoluble copper-molybdenum-sulfur complexes (e.g., tetrathiomolybdate) in the digestive tract that reduce the absorption of copper (McDowell, 1992). Several pathways exist by which copper H molybdenum H sulfur interactions mediate copper deficiency. High sulfur intake can also decrease copper status independent of molybdenum status (Smart et al., 1986). The effect of sulfur alone may be greater than the sulfur-dependent effects of molybdenum (Underwood and Suttle, 1999).

Most municipal wastewater biosolids (sewage sludge) are disposed via land application, landfilling, or incineration (U.S. EPA, 1995). Applying biosolids on agricultural land is the most common beneficial use of biosolids today (NRC, 1996a), with pastures frequently representing attractive applications sites. Pastures, which usually comprise large acreage, normally are under fertilized. For example, approximately 5×10^6 ha of grassland in Florida require fertilization to achieve acceptable forage production (Muchovej and Rechcigl, 1997). Of these grasslands, approximately 2×10^6 ha are planted with bahiagrass (*Paspalum notatum Flugge*) that has been well documented (Sveda et al., 1992) to respond to additions of nitrogen, sulfur, and iron, which are readily supplied by biosolids (Muchovej and Rechcigl, 1997;Nguyen, 1998).

Application of biosolids to pasture is of interest to the scientific community, since some sources contain high concentrations of molybdenum and other metals, which could be absorbed by plants and ingested by grazing ruminants, to promote toxicosis. Molybdenum can be high in biosolids because molybdate systems are used in about 40-50% of the smaller comfort cooling tower units, and in about 10-20% of the larger units (Bastain and Brobst, 1993). However, the mean concentration of total Mo in biosolids nationwide is 20 ppm (± 100 SD). The use of biosolids as a soil amendment fertilizer would be of benefit if it increased forage yields without causing toxicity. The process would also provide a means for safe recycling of biosolids to land. The objective of three years of experiments was to evaluate the performance and copper status of yearling cattle grazing forage subjected to applications of large quantities of biosolids containing molybdenum in successive years or applied the previous (residual) year. These biosolids were classified as exceptional quality (U.S. EPA, 1993), with varying amounts of molybdenum (12-60 mg/kg). This report will limit observation only to copper status of animals as it is affected by molybdenum and sulfur.

Materials and Methods

The field study of biosolids-molybdenum transfer to bahiagrass and then to cattle began in April 1996, and ended in November 1998. Plots were established at the University of Florida Santa Fe Beef Unit, which is located approximately 50 km north of the main University of Florida campus in Gainesville. Each experimental unit (plot) is approximately 0.8 ha (2A) and is permanently fenced. The predominant soil series in the experimental area is Millhopper sand (Grossarenic Paleudult), with significant inclusions of other well-drained sands. During the 3-year study, soil pH remained in the 5.0 to 5.8 range. Biosolids were applied by trucks equipped with flotation tires. All materials were heat-dried, anaerobically digested biosolids in the form of granules greater than or equal to 2 mm in diameter.

Biosolids

A summary of biosolids treatments over the 3-year study is presented in Table 1. Biosolids application rates are multiples (X, 2X, 3X, and so on) of the "high-N [nitrogen] option" (179 kg nitrogen per hectare) nitrogen fertilization rate described by Chambliss (1996) that allows bahiagrass to achieve well-above-average production. The study authors assumed that 40% of the total nitrogen of biosolids would be available in the first years, based on experience with similar biosolids elsewhere in Florida. Nitrogen was supplied at the "X" rate as NH_4NO_3 in the control treatments; half of the fertilizer was applied at the beginning of each season, and the remainder applied mid-season (approximately July 1).

Year 1 treatments involved only Baltimore and Tampa biosolids applied at X and 2X rates, which were replicated five or six times (Table 1). In Year 2, a third material

(Largo, Fla.) was added and applied at high rates (56 t/ha and 112 t/ha) to dramatically increase soil molybdenum loads. In year 2, the study authors also reapplied the other biosolids treatments to half of the replicate plots and studied residual effects (no additional material) in the remaining replicate plots. The simplistic experimental design of Year 1 was sacrificed in Year 2, and replication was reduced as treatment numbers increased. In most cases, however, three replicates of each treatment were maintained. In Year 3, the study authors attempted to regain statistical power by adding sufficient Baltimore and Tampa biosolids to equalize residual and reamended treatments (while further increasing overall soil molybdenum loads), and increasing replicate numbers of most treatments. Modest amounts of agricultural limestone were added to Year 2 (2 t/ha) and Year 3 (5.6 t/ha) to restrict soil pH decreases associated with heavy biosolids loads.

Animals

Two yearling cattle (200 kg to 300 kg initial weight) of Angus and Hereford cross (Year 1) or Angus predominate (Year 2) breeding were assigned to each plot. In Year 3, three Angus yearlings were assigned to each plot, with one animal per plot receiving a 3-ml subcutaneous injection of copper glycinate (a copper concentration of 60 mg/ml) to counteract copper deficiencies. Heifers were utilized in Year 1, whereas steers were used for Years 2 and 3. Seventy (Year 1 and 2) or 100 (Year 3) yearlings were initially purchased for the experiments. The final number of animals (60 in Year 1 and 2; 96 in Year 3) were selected on the basis of initial weight uniformity, lack of disease, disposition, and, where possible, uniformity in initial liver copper status for a random assignment to the plots.

Analyses

Blood was collected monthly and liver biopsy three times from May to October. Forage samples were collected six times, once every four weeks, beginning on June 14, 1996, with a transect technique. Two composite (three subsamples per composite) forage samples were taken from each pasture. The three subsamples were taken from the beginning, middle, and end of the pasture, the left three making composite one, and the right three making composite two. Samples were never taken closer than 20 m from fences.

Plasma and liver copper was determined by atomic absorption (AA) spectrophotometry (Perkin-Elmer, 1980). Liver molybdenum was determined by flameless atomic absorption spectrophotometry with Zeeman background correction (Perkin-Elmer Corp., 1984). Forage molybdenum was determined by the dithiol method of analysis (Clark and Axley, 1955).

Results and Discussion

Year 1

Animals on pastures receiving some biosolids treatments gained more ($P < 0.05$) than control animals; however, weight gains for all treatments were similar and within expected ranges. Biosolids treatments had little effect on animal mineral status, with the exception of copper.

Forage copper means (Table 2) were low for all treatments (including the control pastures) at each of the samplings, and below the 10 ppm concentration considered adequate for beef cattle (NRC, 1996b). Biosolids-borne copper did little to improve the low copper concentrations of the bahiagrass.

Forage molybdenum means were variable among treatments, but generally low throughout all sampling periods (Table 3). Tampa 1X and Tampa 2X pastures had molybdenum concentrations consistently higher ($P < 0.05$) than the control in June through September, while Baltimore-1X and Baltimore -2X were higher generally than the control in July and August, respectively. While some treatments produced forage with higher ($P < 0.05$) molybdenum concentrations than the control, at no time did they approach the level of 5.0 ppm considered toxic (McDowell, 1997) based on molybdenum interference with copper absorption and resultant copper deficiency diseases. Forage sulfur concentrations were high and ranged from 0.30 to 0.47%.

Liver copper declined throughout the experiment, particularly the biosolids treatments (Figure 1). Biosolids treatment animals had especially low liver copper levels by season=s end, suggesting borderline copper deficiencies (Table 4). By Day 99, animals receiving the two Baltimore treatments and the lowest Tampa application rate had lower ($P < 0.05$) liver copper than the control; all treatments were lower at Day 176. Approximately 85% of the animals on biosolids-treated pastures had liver copper levels less than 25 ppm, which is a strong indication of copper deficiency. The decline of copper status (liver and plasma) reflects the low copper status of bahiagrass and the possibility of high forage sulfur (0.30% to 0.47%) interference with copper metabolism. Liver molybdenum levels suggested a slight biosolids (molybdenum load) effect as the season-end sampling, but molybdenum levels remained below the normal range of 2 to 4 ppm throughout the study. There is no evidence of molybdenum accumulation to levels indicative of toxicity (approximately 5 ppm), nor of biosolids-borne molybdenum-induced copper deficiency.

Blood plasma copper levels generally declined with time throughout the season, reflecting copper depletion in liver (Table 5). Biosolids treatment animals had lower plasma copper levels than the fertilized control animals. All treatment copper means remained above the critical level of 0.65 µg/ml, but, by the end of the season, greater than 25% of the animals had plasma copper levels less than 0.65 µg/ml. Had the animals continued on the pastures longer, study authors suspect that there would have been greater evidence of liver copper reserve depletion and further reduced plasma copper levels. The 6-month grazing period (season) is, however, typical, and grazed animals normally would be offered supplements containing copper (omitted in the study's supplements).

<u>Year 2</u>

Animals from all treatments had clinical signs of copper deficiency in August, based on loss of hair color, low plasma copper concentrations, and declining liver copper stores. The extent to which observed lower weight gains reflect the copper deficiency and/or the high forage sulfur concentrations remains unclear. Average daily gains (ADGs) were low for the first half of the grazing season, but by the end of the experiment, weight gains improved, plasma copper concentrations rebounded, and normal hair coat color returned. Seasonal ADGs ranged from 0.34 kg/d to 0.56 kg/d across all treatments, generally lower than those of Year 1 (0.45 kg/d to 0.59 kg/d) for heifers. However, Year 1 heifers arrived thin, and likely experienced compensatory gain.

The majority of forage samples in Year 2 were again copper deficient relative to beef cattle requirements (NRC, 1996b). Forages fertilized with the Largo 2 (L2) treatment were, however, substantially higher ($P < 0.05$) in copper than the control for four of the last five sampling times, with concentrations close to adequate for growing beef cattle (10 mg/kg, NRC, 1996b). There was a general trend for forage copper in most treatments to decline from September to November, when low concentrations were seen for all treatments (data not shown). Alloway (1973) suggested that copper:molybdenum ratios above 4:1 are adequate to prevent copper deficiencies in cattle associated with high forage molybdenum. Even though forage copper was low in most treatments, copper:molybdenum ratios exceeded 4:1 throughout the grazing season. This region is known to have forage deficient in copper (Espinoza et al., 1991; Cuesta et al., 1993) and, with the exception of treatment L2, biosolids application or reapplication did little to improve the problem in successive years.

Similar to Year 1 results, Year 2 forage molybdenum concentrations (date not shown) in all treatments were low at each sampling time, with all treatments well below the 5-10 ppm level considered toxic (McDowell, 1997). The low forage molybdenum concentrations observed in this experiment are consistent with concentrations found in bahiagrass grown in the acid soils (pH less than 6) of this region. Cuesta et al. (1993) reported low forage molybdenum concentrations similar to those of the study's control in north Florida, and similar data were also reported by Espinoza et al. (1991) in central Florida bahiagrass grown in soils with pH ranging from 5.4 to 5.8. Likewise, similar to Year 1, sulfur concentrations for biosolids treated forages were high (0.26-0.52%), thus having a detrimental effect on copper status. There is little evidence to suggest that molybdenum at these low concentrations would have much effect on copper status or thiomolybdate formation.

In agreement with Year 1 data (liver copper), all treatments declined ($P < 0.05$) from Day 1 to Day 180 (Table 6). In addition, most treatments declined ($P < 0.05$) between Day 1 and Day 95. Steers from several biosolids treatments showed a strong tendency to have lower liver copper concentrations than the control at Day 180. At the end of the experiment, liver copper treatment means for a few biosolids treatments were below the value (75 mg/kg), suggesting borderline deficiency; several other biosolids treatments also approached this concentration. Also in agreement with Year 1, liver molybdenum concentrations were low (Table 6)

Copper concentrations of plasma decreased dramatically ($P < 0.01$) in all treatments by Day 95 (data not shown), and evidence of copper deficiency was apparent in clinical signs of loss of hair coat color for many animals. The normal black pigment of the Angus' hair coat became red. The degree to which low forage copper and/or high forage sulfur aggravated the situation is unclear. In addition, although there was a possibility of direct consumption of biosolids early in the season, this would not explain the decline in plasma copper of control steers. At the end of the experiment, plasma copper concentrations increased ($P < 0.05$) to concentrations above the critical levels for all treatments. Even though liver copper concentrations continued to decline to Day 180, there was no difference ($P < 0.05$) in plasma copper between the control and treatment animals.

Smart et al. (1996) reported that plasma and liver copper concentrations (initially deficient) in pregnant heifers declined at calving for two treatment groups: one receiving desulfated water (0.20% sulfur in the diet) and the other receiving sulfated water (0.35% sulfur in the diet), although the decrease was greater for animals in the sulfated water group. Liver copper was lower for the cows receiving high sulfur as well. After calving, plasma and liver copper increased for both groups; however, the low-sulfur treatment group increased more than the high-sulfur group. It was concluded that decreased sulfur intake positively affected the copper status of beef cows, and that 10 ppm dietary copper was not enough to improve the deficient status of these cattle.

Year 3

Because of the lack of rainfall, animals were not assigned to pasture until June 11 and were removed on November 9, for a total of 151 days on pasture. On Day 117, 12 animals were removed from the experiment because of inadequate forage. Weight gains were independent of treatments, and did not reflect potential copper deficiencies. Some Year-3 animals initially demonstrated hair discoloration indicative of copper deficiency, but most animals' coats were normal by Day 57, when the animals were finally introduced to the plots. Observation of the animals in the week prior to the second plasma collection revealed that one-third of the animals had some hair discoloration, but it was much less evident, both in frequency and severity, than what was observed in Year 2. Across treatments, there were still hair discolorations recorded on the 117th day on pasture (Day 174), specifically moderate-to-severe hair discoloration in 22 animals and slight discoloration in 10 animals. At the end of the experiment, one-third (30 out of 88) of the animals across treatments had slight-to-moderate hair discoloration. Slight discoloration was evident even for six animals that had received copper injections.

Forage copper and molybdenum (data not shown) were low and sulfur was high (0.40 to 0.45%) (data not shown). Copper was depleted from livers of animals grazing biosolids-treated pastures by experimental termination (Table 7). The mean liver concentration for control animals was 110 ppm, compared to a mean range of 29 to 83.4 ppm for biosolids treatments. For all biosolids treatments, plasma copper was low (less than 0.65 μg/ml), and values were less than the control treatment value of 0.68 μg/ml (Table 8). At the end of the experiment, plasma copper ranged from 0.37 μg/ml to 0.55 μg/ml for biosolids treatments; however, because of animal variability, only the lowest value was significantly ($P < 0.05$) lower. The impact of the copper glycinate injections on liver concentrations was dramatic; for all pens, the copper-injected animal had higher liver copper than the two corresponding pen mates.

Molybdenum derived from biosolids applications apparently had little or no effect on declining copper stores of cattle grazing biosolids-treated pastures. All bahiagrass molybdenum concentrations were less than 5 ppm. Liver molybdenum concentrations were low in all treatments, reflecting the low dietary consumption of molybdenum. As in Years 1 and 2, bahiagrass was low in copper in Year 3. With the exception of the L2X treatment (heavily infested by bermudagrass that later became infected with disease and died), all treatments had low forage copper concentrations (< 10 ppm) in relation to cattle requirements (NRC, 1996b). Forage sulfur was in the 0.4% to 0.45% range, and could have affected copper metabolism. Not only is high sulfur detrimental to copper, it is also detrimental to molybdenum, thereby further reducing a detrimental effect of molybdenum on copper metabolism.

Summary and Implications

Risk of Copper deficiency from high molybdenum biosolids.

Data from the 3-year experiment indicated that the high molybdenum biosolids applications had little effect on forage molybdenum. Forage molybdenum concentrations were generally less than 1 ppm and never exceeded 3 ppm (well below the molybdenum level of 10 ppm considered excessive), with the critical copper:molybdenum ratio never breached. The tolerable risk threshold of copper:molybdenum ratio in feed is not fixed, but declines from 5:1 to 2:1 as pasture molybdenum concentrations increase from 2 to 10 ppm (Suttle, 1991). Alloway (1973) suggests that the critical copper:molybdenum ratio is 4:1, whereas Miltimore and Mason (1971) suggest a narrower ration of 2:1.

For plant uptake of molybdenum to be sufficient to result in ruminant copper deficiencies, alkaline soil pH and poor drainage conditions are generally required (Kubota et al., 1961; McDowell, 1992). For the experimental site, soils did not encourage high plant uptake of molybdenum since they were both well drained and acidic.

Although biosolids molybdenum had little effect on cattle copper status, copper deficiency was evident each of the three years based on low liver and plasma concentrations and the clinical signs of faded hair coat in Years 2 and 3. Copper deficiency resulted because forage copper concentrations were inadequate (< 10 ppm) and there was elevated forage sulfur (< 0.4%) as a result of the biosolids treatments. Forages grown on biosolids-amended soil frequently have increased sulfur contents (Nguyen, 1998; O'Connor and McDowell, 1999; McBride et al., 2000). For cattle receiving low forage copper and copper status aggravated by high forage sulfur, copper supplementation is definitely warranted.

Risk of high molybdenum biosolids on copper deficiency.

There is little or no risk of using high molybdenum biosolids as a fertilizer on copper status in cattle in regions where soils are acidic and/or are well drained. However, in world regions where molybdenosis is a problem, precautions need to be in place.

For grazing bovines, the problem of copper deficiency due to low forage copper or a conditioned copper deficiency (e.g., high forage molybdenum and/or sulfur) is seasonally restricted to the usual six months grazing of green forages. The condition is rarely seen during the feeding of stored forages in either beef or dairy cattle. Copper deficiency can be highly detrimental for cattle grazing fresh forage in some regions, but when this same forage is dried as hay, there is no copper deficiency (Huber et al., 1971; Allaway, 1977). These authors suggested that drying forage makes copper more available for absorption and reduces the availability of molybdenum.

Suttle (1980) evaluated copper bioavailability of grazed pastures, dried grass, hay and silage by responses in plasma copper during repletion of hypocupremic ewes. Copper in cut hay and grass was more bioavailable than copper in fresh grass and silage from the same field. Copper absorption in fresh grass ranged from 0.5 to 2.8% in three of the four grasses. Copper absorption was 0.9 to 1.9% for grass silage, 3.1 to 4.9% for dried grass, and 5.2 to 7.2% for hay.

Dietary copper is poorly absorbed in most animal species, although absorption is greater in young than mature animals and in copper-deficient than copper-sufficient animals. Mature sheep absorb less than 10% of the copper ingested (Suttle, 1973). Often, only 1 to 3% of dietary copper is absorbed in ruminants. The copper availability in cereal grains may be 10 times greater than in forages (Suttle, 1986). This partially explains why copper deficiency can be a problem with grazing bovines, but usually not with dairy cattle or finishing cattle that receive high amounts of concentrates in their diets.

The cattle groups perhaps at greatest risk of molybdenum-induced copper deficiency are beef cows, growing beef calves and pregnant beef and dairy heifers because of the dominance of fresh forages in their diets (O'Connor et al., 2000). Regardless of the pasture forage species, the entire ration of the dairy cow rarely consists of more than 60% fresh forages because of the need to incorporate other feed ingredients into their diets to maximize milk production. Most large herds of dairy cattle do not graze pastures, and their diets remain fairly constant during all seasons of the year.

The risk of molybdenum-induced copper deficiency is greatest during the period of active growing forage, which is only 5 to 6 months in many areas of the country. Also, the risk is greater for legumes (e.g., alfalfa and clover) than grasses. Legumes can accumulate much greater concentrations (2 to 40 ppm) under natural conditions, but the literature (e.g., Miltimore and Mason, 1971) suggest wide variations in legume forage molybdenum contents. Molybdenum-induced copper deficiency is not a problem for ruminants receiving stored forages apparently because of increased availability of copper and reduced availability of molybdenum in these feeds (Underwood and Suttle, 1999). Land fertilization using exceptional quality biosolids provides safe sources of plant nutrients (e.g., nitrogen) at a low cost (O'Connor et al., 2000). Most important, molybdenum-induced copper deficiency regions in the U.S. are well known and farmers compensate by providing adequate copper in mineral supplements. Copper supplementation will be even more important to counteract any adverse effects of high forage molybdenum and/or sulfur that resulted from biosolids use.

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Table 1. Total Elemental Analyses of Biosolids

	Metal Content, mg/kg						
Biosolids	Mo	Cu	Cd	Ni	Zn	Pb	Fe & Al
Largo	60	989	2.7	21	516	69	12,600
Baltimore	12	431	5	42	730	90	114,000
Tampa	36	733	10	80	1175	80	21,000

Table 2. Treatments of the 3-Year Project

Treatment	Biosolids (t/ha)	Mo (kg/ha)	No. Replicates
Year 1			
Absolute control	-	-	2
NH_4NO_3	-	-	6
Baltimore 1X	22.4	0.27	5
Baltimore 2X	44.8	0.54	5
Tampa 1X	16.8	0.6	6
Tampa 2X	33.6	1.2	6
Year 2			
NH_4NO_3	-	-	2
Largo 1	56.0	1.30	3
Largo 2	112.0	2.56	3
Baltimore 1X	22.4	0.22	2
Baltimore 1X-R[a]	44.8	0.44	3
Baltimore 2X	44.8	0.66	2
Baltimore 2X-R	89.6	1.28	3
Tampa 1X	16.8	0.52	3
Tampa 1X-R	33.6	0.88	3
Tampa 2X	33.6	0.88	3
Tampa 2X-R	67.2	1.68	3
Year 3			
NH_4NO_3	-	-	4
Largo 1	56.0	1.32	3
Largo 2	112.0	2.20	3
Baltimore 3X	67.2	0.46	5
Baltimore 6X	134.4	1.78	5
Tampa 3X	50.4	1.25	6
Tampa 6X	100.8	1.98	6

[a]Reapplied biosolids

Table 3. Forage Cu and Mo concentrations (ppm DM) by month as affected by biosolids treatments in Year 1.[a]

	June		July		August		September		October		November	
	Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo
NH_4NO_3	4.66[d]	0.28[d]	7.79	0.12[d]	4.56	0.23[d]	3.67	0.20[d]	4.53[d]	-	3.61	-
Tampa 1X	5.13[de]	0.88[c]	5.23	0.57[f]	4.22	1.00[g]	3.17	1.07[c]	4.92[de]	-	4.03	-
Tampa 2X	5.25[de]	0.99	6.86	0.70[g]	4.54	0.82[fg]	3.52	1.23[f]	6.05[c]	-	4.05	-
Baltimore 1X	5.86[c]	0.47[d]	6.41	0.26[c]	4.84	0.38[de]	3.74	0.40[d]	4.16[d]	-	3.60	-
Baltimore 2X	5.10[de]	0.44[d]	6.15	0.24[de]	4.67	0.56[ef]	3.03	0.45[d]	4.88[de]	-	4.51	-
SE[c]	0.43	0.12	0.92	0.41	0.50	0.12	0.26	0.10	0.57	-	0.41	-

[a] Critical concentrations (mg kg^{-1}) are: Cu, 10 and for excess Mo, 5 (NRC, 1996)
[b] Biosolids load rates (t ha^{-1}) are: Tampa 1X, 16.8; Tampa 2X, 33.6; Baltimore 1X, 22.4; and Baltimore 2X, 44.8.
[c] Standard error.
[defg] Least square means with different superscripts within a column differ (P<0.05).

Table 4. Liver Cu and Mo concentrations (mg/kg DM) by month as affected by biosolids treatments in Year 1.[a]

	Sludge load rate, t/ha	July		August		October	
		Cu	Mo	Cu	Mo	Cu	Mo
NH_4NO_3	-	69.4	0.95[c]	57.8[d]	1.87[cd]	45.2[d]	1.38
Baltimore 1X	22.4	59.6	1.23[cd]	27.4[c]	2.18[d]	18.7[c]	1.81
Baltimore 2X	44.8	67.9	1.25[cd]	32.3[c]	1.67[c]	16.2[c]	.67
Tampa 1X	16.8	69.0	1.52[d]	32.6[c]	1.87[cd]	12.9[c]	1.78
Tampa 2X	33.6	72.2	1.26[cd]	36.8[cd]	2.00[cd]	14.6[c]	1.68

[a] Critical concentrations (mg kg^{-1}) are: Cu <75 is borderline to deficient and Cu < 25 is seriously deficient. Over 5.0 is considered critical Mo as it relates to influencing Cu metabolism.
[b] Standard error for least squares means are 7.9 and 18 for Cu and Mo, respectively.
[cd] Means lacking common superscripts within a column differ (P < .05).

Table 5. Plasma Copper concentrations (μg/ml) by month as affected by biosolids treatments in Year 1.[a]

	May (1 d)c	June (50 d)	August (99 d)	September (135 d)	October (176 d)
	Copper	Copper	Copper	Copper	Copper
NH_4NO_3	0.95	1.41^{d}	1.20^{e}	0.90	1.11^{f}
Baltimore 1X	0.90	1.36^{d}	1.05^{de}	0.84	0.92^{ef}
Baltimore 2X	0.92	1.53^{de}	1.06^{de}	0.83	0.80^{de}
Tampa 1X	0.92	1.45^{de}	0.88^{d}	0.76	0.63^{d}
Tampa 2X	0.92	1.74^{e}	0.99^{de}	0.91	0.84^{def}

[a] Critical concentrations (μg/ml) for copper is 0.65 μg/ml.
[b] Biosolids load rates (t/ha) are as follows: Baltimore 1X, 22.4; Baltimore 2X, 44.8; Tampa 1X, 16.8; and Tampa 2X, 33.6.
[c] Standard errors for least squares means are 0.11.
[def] Means lacking common superscripts within a column differ ($P < 0.05$).

Table 6. Liver Cu and Mo concentrations (mg/kg DM) by month as affected by biosolids treatments in Year 3.[a,b]

	Sludge load rate, t/ha	May (1 d)		August (95 d)		November (180 d)[f]	
		Cu	Mo	Cu	Mo	Cu	Mo
NH_4NO_3	-	198.6^{cde}	2.54^{cd}	164.6	1.85	132.8	2.51
Largo 1	56	217.8^{cde}	2.09^{c}	129.2	2.26	103.0	2.55
Largo 2	112	258.9^{de}	3.11	121.7	2.40	115.7	2.64
Baltimore 1X-RS	-	174.5^{c}	2.33^{cd}	89.6	2.59	51.0	2.74
Baltimore 1X-RA	22.4	264.5^{e}	2.67^{cd}	113.1	2.51	110.6	2.48
Baltimore 2X-RS	-	216.0^{cde}	2.18^{cd}	168.7	2.60	108.9	2.75
Baltimore 2X-RA	44.8	221.3^{cde}	2.01^{c}	121.6	2.66	76.6	2.88
Tampa 1X-RS	-	226.4^{cde}	2.40^{cd}	126.1	2.19	92.3	3.11
Tampa 1X-RA	16.8	219.8^{cde}	2.22^{cd}	129.3	2.10	81.7	2.78
Tampa 2X-RS	-	207.9^{cde}	2.95^{c}	106.4	2.35	65.3	2.72
Tampa 2X-RA	33.6	184.1^{cd}	2.50^{cd}	97.5	2.35	56.2	2.92

[a] Critical concentrations (mg/kg) are: Cu < 75 is borderline to deficient and < 25 is seriously deficient. Over 5.0 mg/kg Mo is considered critical as it relates to influencing Cu metabolism.
[b] Standard error for least squares means are: Cu 32.9 and Mo, .37 for the control and two residual Baltimore treatments. All other standard errors are Cu, 26.9 and Mo, .30.
[cde] Means with different superscripts within a column differ (P < .05).
[f] Treatments B1RS (P < .08), T2RA (P < .12) differed from the control in November.

Table 7. Liver Copper (mg/kg DM) concentrations as affected by biosolids treatments in Year 3.

Treatment[a]	Collection 1 (4/16/98) D1	Collection 2 (8/13/98) D120	Collection 3 (11/9/98) D208
$NH_4NO_3$1X	84.2 ± 37.9	110.4 ± 37.9^{bcd}	110.6 ± 33.6^{cde}
NH_4NO_3 1XC[1]	121.5 ± 37.9	222.8 ± 41.2^{e}	148.2 ± 33.6^{de}
Largo 1X	86.0 ± 43.8	114.8 ± 43.8^{bcd}	63.2 ± 38.8^{cd}
Largo 1XC	94.7 ± 43.8	176.2 ± 43.8^{bcd}	96.6 ± 38.8^{cd}
Largo 2X	80.9 ± 43.8	87.9 ± 43.8^{bcd}	83.4 ± 38.8^{cd}
Largo 2XC	97.9 ± 43.8	182.4 ± 43.8^{bcd}	164.7 ± 38.8^{cd}
Baltimore 3X	69.5 ± 33.9	115.7 ± 33.9^{bcde}	61.8 ± 30.1^{cd}
Baltimore 3XC	112.2 ± 33.9	215.3 ± 33.9^{e}	134.7 ± 30.1^{d}
Baltimore 6X	84.5 ± 33.9	83.1 ± 33.9^{bc}	40.3 ± 30.1^{bc}
Baltimore 6XC	113.7 ± 33.9	185.9 ± 33.9^{de}	106.4 ± 30.1^{cd}
Tampa 3X	105.3 ± 31.0	68.4 ± 31.0^{b}	29.0 ± 27.5^{b}
Tampa 3XC	11.6 ± 31.0	170.9 ± 31.0^{cde}	74.1 ± 27.5^{cd}
Tampa 6X	87.5 ± 31.0	96.3 ± 31.0^{bcd}	41.0 ± 27.5^{bc}
Tampa 6XC	119.1 ± 31.0	148.3 ± 31.0^{bcde}	75.7 ± 27.5^{cd}

[1] Copper injections were not administered until animals were allotted to pasture on 6/11/98. D120 and D208 correspond to D64 and D152, respectively, for animals on pasture.
[a] Data represent treatment means and standard errors.
[cde] Means with different superscripts within a column differ ($P < 0.05$).

Table 8. Plasma Copper (µg/ml) Concentration by Time[1] as Affected by Biosolids Treatments in Year 3

Treatment[1]	Collection 1 (4/16/98) D1	Collection 2 (7/8/98) D84	Collection 3 (8/13/98) D120	Collection 4 (10/6/98) D174	Collection 5 (11/9/98) D208
$NH_4NO_3$1X	1.21 ± 0.08^{bc}	1.01 ± 0.08^{bcd}	0.73 ± 0.08^{bc}	0.78 ± 0.09^{cd}	0.68 ± 0.09^{c}
NH_4NO_3 1XC[1]	1.37 ± 0.08^{bcd}	1.10 ± 0.08^{cd}	0.78 ± 0.08^{bc}	0.88 ± 0.09^{d}	0.69 ± 0.09^{c}
Largo 1X	1.13 ± 0.09^{b}	1.03 ± 0.09^{bcd}	0.59 ± 0.09^{b}	0.64 ± 0.11^{bc}	0.55 ± 0.11^{bc}
Largo 1XC	1.43 ± 0.09^{cd}	1.08 ± 0.09^{bcd}	0.84 ± 0.09^{c}	0.75 ± 0.11^{cd}	0.60 ± 0.11^{bc}
Largo 2X	1.17 ± 0.09^{bc}	1.00 ± 0.09^{bcd}	0.77 ± 0.09^{bc}	0.62 ± 0.11^{bc}	---
Largo 2XC	1.52 ± 0.09^{d}	1.27 ± 0.09^{d}	0.77 ± 0.09^{bc}	0.62 ± 0.11^{bc}	---
Baltimore 3X	1.37 ± 0.07^{bcd}	0.98 ± 0.07^{bc}	0.76 ± 0.07^{cd}	0.71 ± 0.08^{cd}	0.47 ± 0.08^{bc}
Baltimore 3XC	1.31 ± 0.07^{bcd}	1.00 ± 0.07^{bcd}	0.72 ± 0.07^{bc}	0.73 ± 0.08^{cd}	0.57 ± 0.08^{bc}
Baltimore 6X	1.29 ± 0.07^{bcd}	0.92 ± 0.07^{bc}	0.61 ± 0.07^{b}	0.75 ± 0.08^{cd}	0.50 ± 0.08^{bc}
Baltimore 6XC	1.18 ± 0.07^{bc}	0.97 ± 0.07^{bc}	0.71 ± 0.07^{bc}	0.45 ± 0.08^{b}	0.63 ± 0.08^{c}
Tampa 3X	1.32 ± 0.07^{bcd}	0.84 ± 0.07^{b}	0.57 ± 0.07^{b}	0.67 ± 0.08^{c}	0.37 ± 0.08^{b}
Tampa 3XC	1.27 ± 0.07^{bc}	0.97 ± 0.07^{bc}	0.72 ± 0.07^{bc}	0.63 ± 0.08^{bc}	0.60 ± 0.08^{bc}
Tampa 6X	1.29 ± 0.07^{bcd}	0.94 ± 0.07^{bc}	0.61 ± 0.07^{b}	0.72 ± 0.08^{cd}	0.51 ± 0.08^{bc}
Tampa 6XC	1.36 ± 0.08^{bcd}	0.95 ± 0.07^{bc}	0.66 ± 0.07^{bc}	0.92 ± 0.08^{d}	0.58 ± 0.08^{bc}

[1] Animals were allotted to pasture on 6/11/98, at which time they received Cu injections. Days 84, 120, 174, and 208 correspond respectively to days 28, 64, 117, and 152 for animals on pasture.

[a] Data represent treatment means and standard errors.

[bcd] Means with different superscripts within a column differ ($P < 0.05$).