Copper and Molybdenum Uptake by Forages Grown on Coal Mine Soils

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Abstract

Coal mine soils have shown a tendency to produce leguminous vegetation containing elevated concentrations of molybdenum (Mo). The potential for cattle developing copper (Cu) deficiency by grazing vegetated areas is increased at one mine where a shale interburden material contains elevated Mo levels. The purpose of this study was to determine if mixing or dilution of the interburden with low-Mo sandy overburden would produce vegetation with undesirably high Mo levels or low Cu/Mo ratios.

Concentrations of Cu, Mo, sulfur, and Cu/Mo ratios of several legumes and one grass species grown on these alkaline coal mine soils suggest that, with the exception of white sweetclover, mixing of the Mo-bearing interburden material with sandy overburden resulted in desirable elemental levels and ratios for grazing cattle if the mine soils were covered with an adequate depth (0.6 m) of suitable topsoil. Vegetation uptake of Mo was species and site specific.

In 1976, an investigation of copper (Cu) and molybdenum (Mo) levels in 2 leguminous species, yellow and white sweetclover (*Melilotus officinalis* and *M. alba*), grown on revegetated mine soils of the Northern Great Plains was initiated by Erdman and his co-workers (1978). They reported a Cu/Mo ratio of 0.44 and a Mo level of 13 $\mu\text{g/g}$ for white sweetclover collected at the Big Sky Mine in southeastern Montana and concluded that cattle grazing predominantly on this vegetation could display subclinical or acute symptoms of molybdenosis. Legumes growing on revegetated mine areas have exhibited a tendency to accumulate higher concentrations of Mo than those growing on native range or cultivated fields (Munshower and Neuman 1978a). As a complicating factor the Cu content of vegetation on the Northern Great Plains in southeastern Montana is generally marginal in terms of required cattle nutritional needs. This low Cu status is common to both native range vegetation and established plants on revegetated coal

mine soils (Munshower and Neuman 1978b and 1980).

At the Big Sky Mine the possibility of elevated plant available Mo in spoil material is increased by the high levels of extractable Mo (1 to 4 $\mu\text{g Mo/g}$) in the blue-grey shale interburden material located between the Rosebud and McKay coal seams. The mining company has been required to selectively handle the shale and bury it below 2.4 m of sandy overburden in the reclamation process. The ultimate goal of this study was to determine if mixing or dilution of the shale interburden with sandy overburden would produce vegetation with undesirably high Mo levels or low Cu/Mo ratios. A corollary to this goal was the investigation of the impact of topsoiling on these plant Mo concentrations and Cu/Mo ratios.

Leguminous plants are known to be accumulators of Mo. This element is an integral part of the nitrogenase enzyme system responsible for the reduction of atmospheric nitrogen to ammonia and hence is important to the symbiotic bacteria in root nodules of nitrogen-fixing legumes.

Although elevated levels of Mo in vegetation are not injurious to the plants themselves, animals are very sensitive to high Mo levels. The required dietary level for grazing cattle and sheep is small and concentrations in the total diet as low as 5 $\mu\text{g Mo/g}$ may be toxic under certain conditions (Webb and Atkinson 1965). Dietary levels of 10-20 $\mu\text{g/g}$ are nearly always associated with disturbed Cu metabolism because of the antagonistic action of these elements (Allaway 1977). The ratio of these 2 elements in vegetation is of prime concern when discussing the potential for induced Cu deficiency in grazing animals. A Cu/Mo ratio of 2.0 is considered the lowest acceptable value in ruminant feeds (Miltmore and Mason 1971).

The sulfur (S) intake of the ruminant animal exerts a significant influence on the Cu-Mo interrelationship (Suttle 1975). Ingested Mo plus S can either increase or decrease the absorption of Cu, depending on their ingestion relative to Cu (Underwood 1977). Suggested dietary intake of S is 0.1% for beef cattle (National Research Council 1976) and 0.2% for dairy cattle (National Research Council 1971).

High Mo forages that may be toxic to grazing animals in the spring have been shown to lose their toxicity when cut as hay or when dry and cured in the field (Ferguson et al. 1943). However, Barshad (1948) reported large increases in Mo concentrations for several plants as they aged. Furthermore, a seasonal decrease in the

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Cu content of native grasses has been reported (Munshower and Neuman 1978b).

Methods and Materials

Site Selection

The Big Sky Mine, owned and operated by Peabody Coal Co., is located in Rosebud County 5 miles south of Colstrip, Mont. Four study sites were established at this mine. Site 1 consisted of old mine spoils of mixed interburden and overburden devoid of any topsoil. Site 2 was mixed interburden and overburden covered with 0.6 m of topsoil. At Site 3 the mixed interburden and overburden was buried below 2.4 m of overburden material and 0.6 m of topsoil. An undisturbed, native range site was used as a control plot.

Plot Design and Seeded Species

Yellow sweetclover (*Melilotus officinalis* Lam.), white sweetclover (*M. alba* Desr.), alfalfa (*Medicago sativa* L.), white prairieclover (*Petalostemon candidum* Michx.), purple prairieclover (*P. purpureum* Rydb.), cicer milkvetch (*Astragalus cicer* L.) and green needlegrass (*Stipa viridula* Trin.) were seeded in 1.2 × 1.5-m plots that were replicated 3x at each of the 4 study sites in fall 1980. Straw mulch was used as an amendment except on the native range site where native vegetation provided a natural mulch. Germination and emergence of most species were complete in spring 1981.

Soil Determinations

Soils were collected on 2 occasions, at vegetation seeding (fall 1980) and in fall 1981. Soil cores were augered at the corners of each study site and the surface, 20, 40, 80, and 120 cm fractions were air-dried and gently crushed to pass a 2-mm sieve. Several physical and chemical determinations were made including pH of the saturated soil paste, DTPA extractable Cu, and ammonium oxalate extractable Mo.

Vegetation Determinations

Several grams of vegetation were collected from each plot at each site on 4 occasions: after 1 growing season in September 1981, and on June 1, July 14 and September 9, 1982. Short plants were clipped approximately 5 cm above ground level to avoid soil contamination, while only the top 22 cm of taller plants, sweetclovers and alfalfa, were collected. The vegetation was oven dried, ground to pass a 40-mesh screen and analyzed for Cu and Mo concentrations by electrothermal atomic absorption spectroscopy (AAS) after acid digestion (Neuman and Munshower 1981). Total S concentration was indirectly determined by analysis of excess barium (Ba) by flame AAS after formation of insoluble barium sulfate in the dissolved ash of plant samples (Horwitz 1975).

Results and Discussion

The question of burial of the Mo bearing interburden material or dilution with sandy overburden may be resolved by examining the following: plant available Cu and Mo within the root zone; vegetation Mo, Cu, and S concentrations; and the subsequent Cu/Mo ratios.

Copper and Molybdenum in Soils

Soil pH is one of the most important single factors affecting the uptake of Mo and Cu in plants. In this study the soil pH levels ranged from 7.0 to 7.9 with little intersite variation. Molybdenum is readily available to vegetation in this pH range and Cu less available. The concentrations of extractable Mo in the study site soils are shown in Table 1 with the data arranged by soil profile depth. The overall mean extractable Mo level for each site is also given. Non-topsoiled mine soils from Site 1 exhibited the highest overall mean level of 0.46 µg Mo/g. Of the topsoiled study sites, Site 3 (segregated interburden) and Site 2 (mixed interburden) revealed similar extractable Mo concentrations. However, when intersite variation by profile depth was evaluated, Site 3 contained higher ($p \leq 0.05$) extractable Mo at the 80-cm level than soils from Site 2. Both of these sites exhibited elevated Mo at the lower soil

Table 1. Mean values of extractable molybdenum (µg/g) and copper µg/g) in study site soils.

Soil depth	Bare spoils #1	Mixed interburden #2	Segregated interburden #3	Native range #4
Molybdenum Concentrations				
Surface	.53 aA ¹	.17 bA	.17 bA	.23 bA
20 cm	.54 aA	.19 bA	.16 bA	.22 bAB
40 cm	.45 aA	.23 bB	.24 bA	.14 bC
80 cm	.32 aA	.28 aB	.62 bB	.17 aBC
120 cm	.45 aA	.32 aB	.46 aB	.23 aA
overall Mo means	.46 a	.23 ab	.31 ab	.19 b
Copper Concentrations				
Surface	1.2 aA	0.6 cA	0.5 cA	0.8 bAB
20 cm	1.4 aA	0.8 aA	0.6 aA	1.1 aA
40 cm	1.6 aA	1.0 abA	0.5 cA	0.8 cAB
80 cm	1.6 aA	1.2 bA	0.3 cB	0.5 cC
120 cm	1.2 aA	1.9 aA	0.3 bB	0.7 bBC
overall Cu means	1.4 a	1.0 b	0.5 c	0.8 b

¹Means followed by same lower-case letter in rows and same capital letter in columns are not different at $p \leq 0.05$ N=8 for all depths except 120 cm where N=4, N=36 for overall mean values.

depths which are within the root zone of the legume species and below the 60 cm of applied topsoil.

Table 1 shows the mean extractable Cu concentrations for each profile depth and overall mean values for each study site. The topsoiled spoils at Site 3 exhibited a significant ($p \leq 0.5$) depression in available Cu at the 80 and 120 cm depths. This is in sharp contrast to the extractable Mo levels which were elevated in soils from this site at these depths.

Seasonal Variation of Elemental Levels and Ratios

Plant samples were collected throughout the growing season to evaluate elemental concentrations and subsequent Cu/Mo ratios from early growth through senescence.

The single grass species evaluated in this Cu/Mo uptake study revealed a relatively constant Mo level at each site throughout the growing season. Grass Cu concentrations decreased at 3 of the 4 sites between spring and fall collections. Alfalfa showed significantly higher levels of Mo in spring samples collected from Sites 2, 3, and 4. The other legumes did not demonstrate any change in Cu or Mo concentrations throughout the growing season or only minor fluctuations at one site. The effects of the few seasonal variations in the elemental status of vegetation on the Cu/Mo ratios resulted only in a lower ratio for alfalfa collected in the spring at Site 4 and an elevated ratio for cicer milkvetch in the summer at Site 1.

Since seasonal variations appeared to be relatively unimportant, the data were combined for the 4 sampling periods with mean values for Cu, Mo, and S concentrations and Cu/Mo ratios exhibited in Table 2. For most species the mean values are based on N=12. The prairieclovers were generally not available for collection in the spring from all plots at all the sites.

Molybdenum Status of Seeded Species

Most of the legumes from Site 1 demonstrated elevated Mo levels ($p \leq 0.05$) when compared with the same species grown on the 2 topsoiled mine soil sites or the native range site (Table 2). Legumes grown on the segregated interburden at Site 3 contained higher Mo levels than those grown on the mixed interburden at Site 2, although these differences were not significant. White sweetclover demonstrated the highest mean Mo concentration of all the species at Sites 1, 2, and 3.

The accumulation of Mo was species specific with white sweetclover and white prairieclover accumulating more of the metal

Table 2. Mean elemental concentrations and ratios in seeded species.

Sites	Bare spoils #1	Mixed interburden #2	Segregated interburden #3	Native range #4
Yellow Sweetclover				
Mo ¹	3.06 a ²	1.12 b	1.51 ab	0.86 b
Cu	7.8 a	6.9 a	6.5 a	6.4 a
S	0.47 a	0.28 b	0.37 ab	0.31 b
Cu/Mo	3.2 a	7.6 b	5.2 ab	7.5 b
White Sweetclover				
Mo	5.69 a	1.75 b	2.73 b	0.75 b
Cu	8.1 a	6.2 a	6.9 a	6.1 a
S	0.61 a	0.29 b	0.33 b	0.32 b
Cu/Mo	1.9 a	4.5 a	4.0 a	9.9 b
Alfalfa				
Mo	2.17 a	0.96 b	1.13 b	0.67 b
Cu	8.4 a	6.4 ab	6.7 ab	5.9 b
S	0.50 a	0.27 b	0.31 b	0.29 b
Cu/Mo	4.4 a	7.3 ab	7.3 ab	10.3 b
Green Needlegrass				
Mo	0.85 ab	1.00 a	0.70 ab	0.62 b
Cu	4.2 a	4.0 a	3.5 a	4.1 a
S	0.21 a	0.16 ab	0.15 b	0.17 ab
Cu/Mo	5.5 ab	4.0 a	5.4 ab	7.4 b
White Prairieclover				
Mo	4.32 a	0.68 b	1.27 b	0.62 b
Cu	10.3 a	7.0 ab	5.9 b	6.4 b
S	0.29 a	— ³	.26 a	—
Cu/Mo	2.7 a	10.3 b	4.9 a	11.3 b
Purple Prairieclover				
Mo	3.15 a	0.83 b	1.02 b	0.57 b
Cu	7.9 a	7.7 a	7.2 a	5.9 a
S	0.37 a	—	0.21 b	0.18 b
Cu/Mo	3.0 a	9.3 b	9.6 b	11.4 b
Cicer Milkvetch				
Mo	2.23 a	0.76 b	0.92 b	0.83 b
Cu	7.2 a	5.5 a	6.2 a	5.8 a
S	0.47 a	0.26 b	0.27 b	0.27 b
Cu/Mo	4.3 a	7.4 b	8.1 b	8.9 b

¹Mo and Cu concentrations in µg/g, S in %.²Means followed by same letter in rows are not different at $p \leq 0.05$. N=12 for most means; N varies from 6 to 10 for prairieclover mean values.³(—) indicates insufficient sample for analysis.

than the other legumes. Uptake of Mo was also site specific with the plants containing elevated levels of Mo in soils of higher extractable concentration. These findings are in agreement with Allaway (1977) who found that different plant species accumulated substantially different amounts of Mo from the same soil or culture, with leguminous vegetation containing more of the metal than grasses in the same area.

Table 3. Vegetation showing acceptable molybdenum concentrations and ratios when grown on each study site.

Vegetation species	Acceptable Mo content (90% of samples <5.0 µg/g)	Acceptable Cu:Mo ratio (90% of samples >2.0)	Overall acceptability of vegetation
	Sites	Sites	Sites
Yellow sweetclover	1 2 3 4	2 3 4	2 3 4
White sweetclover	2 3 4	2 4	2 4
Alfalfa	1 2 3 4	1 2 3 4	1 2 3 4
Green needlegrass	1 2 3 4	1 2 3 4	1 2 3 4
White prairie clover	2 3 4	2 3 4	2 3 4
Cicer milkvetch	1 2 3 4	1 2 3 4	1 2 3 4
Purple prairieclover	2 3 4	2 3 4	2 3 4

Copper Status of Seeded Species

Table 2 lists Cu concentrations in the 6 legumes and 1 grass species. The data indicate that vegetation grown at Site 1 contained the highest levels of Cu with alfalfa and white prairieclover concentrations being elevated ($p \leq 0.05$). Green needlegrass, cicer milkvetch, purple prairieclover, and both sweetclovers exhibited no variation in Cu levels among the 4 study areas. The Cu status of the vegetation appeared to be species specific, but not site specific. Only some species accumulated higher levels of Cu when grown on soil containing more available Cu.

Levels of this metal in vegetation grown on native range soils were statistically equivalent to levels of Cu in vegetation from the 2 topsoiled sites. Other studies (Kubota et al. 1967, Kubota 1975) have also confirmed that plants of the same species with widely different Mo concentrations may have similar levels of Cu.

Copper:Molybdenum Ratios in Seeded Species

Legume species from Site 1 revealed lower Cu/Mo ratios than the same vegetation grown on the native range study site (Table 2). White sweetclover from Site 1 had a mean ratio below the critical dietary ratio for cattle of 2.0. Vegetation grown on the two topsoiled sites exhibited similar Cu/Mo ratios except for white prairieclover. The mean ratios of all species at Sites 2, 3, and 4 were well above the critical dietary value with vegetation grown on the native soil displaying the highest ratios.

Sulfur Status of Seeded Species

High levels of dietary S and Mo restrict Cu utilization by depressing its solubility in the digestive tract through the precipitation of insoluble copper thiomolybdate, CuMoS_4 (Dick et al. 1975). Suttle (1975) demonstrated that total S rather than inorganic S is the more useful measurement in evaluating the Cu-Mo-S interrelationship.

Most legumes grown on Site 1 exhibited elevated S levels ($p \leq 0.05$) compared with vegetation from the other sites (Table 2). White sweetclover from Site 1 showed the highest mean S level (0.61%) and green needlegrass from Site 3 exhibited the lowest S concentration (0.15%). Martin and Matocha (1973) summarized the S status of alfalfa and described deficient, critical, adequate, and high nutrient concentrations. This species was considered S critical at <0.20-0.35%, adequate at 0.26-0.50%, and high at levels greater than 0.51%. In comparison to alfalfa, legumes in this mine soil study appear to be within low-normal limits for adequate vegetation S nutrition.

Limits of S concentration associated with disturbed Cu metabolism in animals have not been well defined in the literature. However, several investigators have indicated that levels above 0.35% are generally found in relation to abnormal Cu metabolism (Spais 1959, Whitehead and Jones 1969, Havre and Dishington 1962). Mean S concentrations in the legumes from Sites 2, 3, and 4 are below this level, while those from Site 1 exceed 0.35% (Table 2).

Summary and Conclusions

Statistical differences between specific parameters at Site 1 (no topsoil), Site 2 (mixed interburden and overburden) and Site 3 (segregated interburden) were of prime importance. These parameters included differences in extractable Mo within the plant root zones, differences in Cu and Mo levels in the seeded species, and differences in vegetation Cu/Mo ratios.

Extractable soil Mo concentrations in the topsoil applied at Sites 2 and 3 were similar. All species grown on the two topsoiled areas exhibited mean Mo levels below critical dietary concentrations for grazing cattle of 5 µg/g.

The uptake of Mo by the legumes was species and site specific. All of the legumes accumulated higher levels of Mo when grown on soils with increasing extractable Mo concentration.

Table 3 shows which of the seeded species evaluated may be planted on different sites and meet acceptable Mo levels and Cu/Mo ratios for grazing cattle. Acceptable performance was based upon 2 factors: 90% of the samples of any species must reveal Mo concentrations below 5 µg/g and Cu/Mo ratios must be greater than 2.0. Based on these criteria, 4 of the legumes should not be seeded on bare spoils of Site 1.

With the exception of white sweetclover, the legumes grown on the 2 topsoiled mine soils exhibited mean Cu, Mo, and S levels and Cu/Mo ratios within normal dietary limits for grazing cattle and were not statistically distinct. They could safely be seeded and consumed by livestock on these topsoiled mine soils.

Since leguminous species would not constitute the sole diet of grazing animals, the intake of Mo in the total diet would not be as high as levels provided by these legumes alone. The subsequent dietary Cu/Mo ratio of free ranging livestock or wildlife would probably be higher than values reported in this study.

Mixing or dilution of the Mo bearing shale interburden located between the Rosebud and McKay coal seams at the Big Sky Mine with sandy overburden in the reclamation process did not result in undesirable Mo levels or Cu/Mo ratios in the legumes evaluated as long as the site was covered with an adequate depth of suitable topsoil.

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