Molybdenum Uptake by Forage Crops Grown on Sewage Sludge-Amended Soils in the Field and Greenhouse

M. B. McBride,* B. K. Richards, T. Steenhuis, and G. Spiers

ABSTRACT

Molybdenum (Mo) is a plant-available element in soils that can adversely affect the health of farm animals. There is a need for more information on its uptake into forage crops from waste materials, such as sewage sludge, applied to agricultural land. Field and greenhouse experiments with several crops grown on long-term sewage sludge-amended soils as well as soils recently amended with dewatered (DW) and alkaline-stabilized (ALK) sludges indicated that Mo supplied from sludge is readily taken up by legumes in particular. Excessive uptake into red clover (Trifolium pratense L.) (>30 mg/kg) was seen in a soil that had been heavily amended with sewage sludge 20 yr earlier, where the soil contained about 3 mg Mo/kg soil, three times the background soil concentration. The greenhouse and field studies indicated that Mo can have a long residual availability in sludge-amended soils. The effect of sludge application was to decrease Cu to Mo ratios in legume forages, canola (Brassica napus var. napus) and soybeans [Glycine max (L.) Merr.] below the recommended limit of 2:1 for ruminant diets, a consequence of high bioavailability of Mo and low uptake of Cu added in sludge. Molybdenum uptake coefficients (UCs) for ALK sludge were higher than for DW sludge, presumably due to the greater solubility of Mo measured in the more alkaline sludges and soils. Based on these UCs, it is tentatively recommended that cumulative Mo loadings on forages grown on nonacid soils should not exceed 1.0 kg/ha from ALK sludge or 4.0 kg/ha from DW sludge.

Molybdenum is a trace element required in very small amounts by animals and consequently has a narrow range between deficiency, sufficiency, and toxicity. Normal total Mo concentrations in agricultural soils of the Northeast are in the range of 0.5 to 2 mg/kg, but higher concentrations are commonly found in western North America, where Mo uptake into forages can lead to molybdenosis (induced Cu deficiency) (Kubota, 1980). Ruminants have a high sensitivity to dietary Mo, which causes molybdenosis in the animals if the Cu to Mo ratio in the feed that makes up a high percentage of the diet falls below 2:1 (McDowell, 1985; Miltimore and Mason, 1971). A higher ratio of 3:1 to 5:1 has been advised as a minimum in pastures, forages, and animal feeds (Alloway, 1973; Suttle, 1986; Radostits et al., 1994, p. 1493-1495). In certain circumstances, total soil Mo concentrations as low as 2 to 3 mg/kg can lead to serious disease in cattle grazing on pastures (Walsh et al., 1953; Fleming, 1980) if a substantial fraction of the total is easily extractable by soil test, and pastures in many parts of the UK are recognized to cause Mo-induced Cu deficiency (Alloway, 1973). Relatively small increases of a few mg/kg of Mo in herbage cause substantial reductions in Cu availability to ruminants (Suttle, 1986) with 5 to 6 mg/kg of Mo in forage inhibiting Cu storage and producing molybdenosis (National Research Council, 1980). High forage Mo has been implicated in predisposing horses to bone development diseases (Walsh and O’Moore, 1953, Bridges et al., 1984). Sulfur interacts strongly with Cu and Mo, and dietary S above 3 to 4 g/kg can be toxic to ruminants (McDowell, 1985). Beside sulfur, other elements in feed, such as excessive zinc, cadmium or iron, depress copper absorption by animals and can cause or exacerbate copper deficiency (Davis and Mertz, 1987). It is therefore important that all of the elements likely to interfere with Cu absorption by animals be analyzed in the feeds grown on waste-amended soils where substantial loading of these elements has occurred.

Sewage sludges, which are commonly applied to farmlands, can contain relatively high amounts of Mo, 5 to 50 mg Mo/kg being a common range. The USEPA conducted a risk assessment (Part 503) (USEPA, 1992) for agricultural application of sewage sludges in which the transfer of Mo to forage crops was evaluated. The determination of the potential for Mo to be taken up by forage crops was estimated by the USEPA on the basis of two field experiments with sewage sludge-amended soils (Soon and Bates, 1985; Pierzynski and Jacobs, 1986a) and a greenhouse study (Pierzynski and Jacobs, 1986b). The value of these data is limited by the extreme contamination of the sludge used in the latter studies, with forage Mo exceeding 700 mg Mo/kg in some cases, and the fact that forage legumes were not included in the Soon and Bates experiment. In that experiment, Mo uptake was measured into corn (Zea mays L.) and bromegrass forage after applying three chemically different sewage sludges, with the alkaline (lime-stabilized) sludge producing the greatest uptake of Mo. Since the USEPA averaged the Mo UCs for these different crops, soils, and sludges by taking the geometric mean of all the measured UCs, the proposed soil Mo loading limit of 18 kg/ha (in the 503 risk assessment support document) does not represent reasonable

Abbreviations: UC, uptake coefficient; USS, upper sludge site; LSS, lower sludge site; DW, dewatered; ALK, alkaline-stabilized; ICP, inductively coupled plasma.

M.B. McBride, Dep. of Crop and Soil Sciences, Cornell Univ., Ithaca, NY 14853; B.K. Richards and T. Steenhuis, Dep. of Agricultural and Biological Engineering, Cornell Univ., Ithaca, NY 14853; and G. Spiers, Research and Productivity Council, Fredericton, New Brunswick. Received 15 Mar. 1999. *Corresponding author (mbm7@cornell.edu).

worse-case situations such as legume-based forages or lime-stabilized sludges. Furthermore, despite the 18 kg/ha Mo loading limit that appears in the USEPA 503 support document, the USEPA has not to date set a cumulative loading limit for Mo on agricultural land. This situation is of particular concern for farms with ruminant animals because Mo is readily accumulated by forages, particularly legumes, from soils containing as little as a few kg/ha of added Mo (Davis, 1981; Williams and Gogna, 1981; Nguyen and O’Connor, 1997).

Availability of soil Mo to crops is greatest in nonacid soils (pH 6.5 or higher), particularly if the soils have high phosphate and organic matter content (Jones et al., 1990; Karimian and Cox, 1978; Gupta and Lipsett, 1981). High soil pH inhibits molybdate adsorption by clays (Goldberg and Forster, 1998), which may explain the effect of liming soils in enhancing Mo uptake by crops. Existing evidence indicates that Mo availability to crops from alkaline sludges is likely to be higher than that from acid or near-neutral sludges. In alkaline sludges, a substantial fraction of the total Mo is immediately water-soluble or easily extractable (Richards et al., 1997; McBride, 1998). Molybdenum availability to crops from sewage sludge may be at least as high as from the salt Na molybdate (Williams and Gogna, 1981), possibly because part of the Mo leaches out of the root zone when applied in the soluble molybdate form. This is in accordance with greenhouse studies that show a slow release of Mo from soil columns into drainage water for several years following application of sludges (B.K. Richards, personal comm.).

Based on the potential for Mo transfer into forages described here, and the paucity of data for Mo uptake in the economically important forage legumes grown in the Northeast, we have undertaken field and greenhouse studies of Mo uptake into legumes and other crops. Greenhouse results for red clover and field results for soybeans and corn are reported here, as well as the results from a limited sampling of forages in farm fields. These results may allow a soil loading or concentration limit to be proposed for Mo, as no limit presently exists in the USA or most European countries, although Ontario, Canada (Ministry of Agriculture and Food and Ministry of the Environment, 1992) and the UK (Alloway, 1990, p. 324) have set limits of 4 mg/kg total Mo in sludge-amended soils.

MATERIALS AND METHODS

Greenhouse Experiment (Red Clover)

Sewage sludge, reported to contain 7 mg/kg of Mo based on neutron activation analysis (Furr et al., 1981; Heffron et al., 1980), had been applied heavily and tilled into the soil at two sites in the Cornell Orchard (Ithaca, NY) in 1977-1978. Corn grown on this sludge at that time had no apparent abnormal uptake of Mo (Heffron et al. 1980). These previously amended sites are referred to hereafter as the upper and lower sludge sites, USS and LSS. Twelve undisturbed soil columns (28 cm. diameter, 35 cm. deep) from the USS and further described by Richards et al. (1998), as well as a number of columns from the nearby control site, were set up in the greenhouse in 1993. A series of six successive crops was initially grown on the USS columns (12 replicates), control soil columns amended with DW sewage sludge (three replicates), control soil columns amended with N-Viro (Manchester, UK) ALK product (three replicates), and control soil columns with no amendments (three replicates). The DW and ALK applications totaling 215 t/ha DW sludge and 663 t/ha ALK sludge had been made during these cropping cycles conducted over several years prior to planting clover. Based on the measured Mo concentrations in these materials, these loadings corresponded to about 5.8 and 4.2 kg/ha of Mo applied from the two sludges, respectively. All soil columns were Hudson silt loam (fine, illitic, mesic, Glossaquic Hapludalf), with pH adjusted initially to the 6.5 to 7.0 range. Because the soil columns were much larger than pots typically used in greenhouse studies and included the much less contaminated subsoil, metal uptake by plants was not expected to be exaggerated substantially relative to uptake in the field. Some studies have reported much-enhanced uptake of heavy metals into crops grown in pots (e.g., de Vries and Tiller, 1978), but under the soil and climate conditions relevant to the study reported here, uptake of metals into crops has not been found to be markedly higher from greenhouse pots (Barmasse, 1981).

For the clover study, surface soil samples were collected from each of the 12 USS columns and from the three control soil columns, three DW-treated columns, and three ALK-treated columns at the time of planting a commercial variety (inoculated) of red clover seed. The clover was grown to maturity (full flowering) in the greenhouse with supplemental lighting and wick watering, but no additional fertilizers, over a period of 11 wk. The whole plants were then harvested by cutting several centimeters above the soil surface, oven-dried, weighed, and acid-digested prior to analysis for total trace metals by inductively coupled plasma (ICP)-emission and ICP-mass spectrometry. The greater sensitivity of the latter method resulted in lower, more accurate, determinations of a number of the trace elements in the plant tissues (i.e., Mo, Cd, Pb, As, and Cr). This difference in sensitivity and accuracy was most evident in the least contaminated samples with the lowest concentrations of trace elements. However, for most trace elements, there was good agreement between the two analytical methods.

The ICP-emission analysis (radial-view instrument without background correction) of acid digests of the soils, used to estimate total trace metals content, was found to give unreliable (too high) measurements of soil Mo based on comparisons with ICP-mass spectrometry analysis of the same soil, and by analyzing certified samples. The detection limit for standard ICP-emission analysis of pure aqueous solutions is about 0.01 mg/L, but Fe and Al in soil digests produce overlapping emissions that degrade the detection limit, so that the practical limit for analysis of soils and geological materials is 5 to 10 mg Mo/kg (Thompson and Zo, 1985). Because this detection limit is substantially higher than the normal Mo concentration in most soils, it was necessary to use a more sensitive analytical method, ICP-mass spectrometry.

After digesting the soils by a standard HF-HNO₃-HCl procedure (Ure, 1990), the digests were analyzed for total metals by ICP-mass spectrometry. By the same procedure, a sample of the archived sewage sludge that had been applied on these soils in 1978 was analyzed for total metals, along with the DW and ALK product. These total element analyses for the soils and sludges are reported in Table 1. The metal analyses reported in Table 1 for sludges have been verified for the heavy metals by digesting a separate sample using standard nitric perchloric acid and analyzing by ICP-emission spectrometry.
Table 1. Total trace metal concentrations (mg/kg) based on HF digestion of surface soil columns excavated from the upper sludge site (US) (average of 12 columns) alkaline-stabilized sludge (ALK)-treated soil columns (average of 3), dewatered sludge (DW)-treated soil columns (average of 3), and adjacent control soils (3 columns). Compositions of the archived sludge that was applied at the US as well as DW and ALK sludges recently applied to the soil columns are also reported. Values in parentheses are standard deviations of the means.

<table>
<thead>
<tr>
<th>Soils</th>
<th>US</th>
<th>ALK</th>
<th>DW</th>
<th>Control</th>
<th>Archived DW ALK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>US</td>
<td>ALK</td>
<td>DW</td>
<td>Control</td>
<td>Archived DW ALK</td>
</tr>
<tr>
<td></td>
<td>3.34 (0.59)</td>
<td>1.3 (0.2)</td>
<td>5.6 (0.5)</td>
<td>1.1 (0.3)</td>
<td>12.4 (27.2)</td>
</tr>
<tr>
<td>321 (49)</td>
<td>68.7 (3.2)</td>
<td>159 (16)</td>
<td>20 (2)</td>
<td>542 (658)</td>
<td>120 (240)</td>
</tr>
<tr>
<td>92.1 (9.7)</td>
<td>24.0 (1.7)</td>
<td>27.5 (0.7)</td>
<td>24.7 (1.5)</td>
<td>131 (38)</td>
<td>19 (12)</td>
</tr>
<tr>
<td>34.8 (4.6)</td>
<td>0.4 (0.0)</td>
<td>1.4 (0.1)</td>
<td>0.2 (0.0)</td>
<td>73.6 (5.3)</td>
<td>1.0 (1.0)</td>
</tr>
<tr>
<td>1600 (240)</td>
<td>119 (2)</td>
<td>204 (21)</td>
<td>98.3 (12.2)</td>
<td>2320 (598)</td>
<td>116 (20)</td>
</tr>
<tr>
<td>490 (71)</td>
<td>26 (6)</td>
<td>46 (2.8)</td>
<td>47 (1)</td>
<td>514 (168)</td>
<td>38 (14)</td>
</tr>
<tr>
<td>392 (24)</td>
<td>37.9 (2.6)</td>
<td>56.1 (2.5)</td>
<td>36.7 (0.6)</td>
<td>576 (141)</td>
<td>31.9 (19)</td>
</tr>
</tbody>
</table>

Field Experiment

The soil at the LSS (old site) is a calcareous silty-clay loam with pH 7.0 to 7.3 in both the sludge-treated and control plots. Corn and soybeans, planted in mid-May 1998, were sampled (whole plant tops) on 20 July from the sludge-treated and control areas. Canola and pea (Pisum sativum L.), planted in the spring of 1999, were sampled at flowering (June 15) for trace element analysis. All plant tissues were sampled in duplicate, oven-dried, acid-digested, and analyzed for trace metals by ICP-emission spectrophotometry. Corn and soybean seed was harvested in the fall (two composite samples from both the sludge-treated and control areas) and analyzed by ICP-mass spectrometry for trace elements (including Cu and Mo). Surface soil samples were collected from the locations in the plots where plants were sampled and analyzed for total trace elements by ICP-mass spectrometry following digestion of the homogenized samples by a standard HF–HNO3–HCl procedure. These analyses were confirmed by analysis of the same samples using nitric-perchloric acid digestion and ICP-emission spectrometry (data not reported).

Forage Sampling

Composite samples of alfalfa (Medicago sativa L.) and grass (mixed species) forages, each considered to represent one field, were collected in 1998 on several farms in New York that had applied an ALK sludge as lime (one application) on some fields in the period 1993–1997. These forage samples were analyzed for trace elements, including Mo and Cu, by ICP-emission as described above. Where available, forages from fields on the same farms where the sludge product had not been applied were used as controls.

Quality Control on Molybdenum Analysis

Available National Institute of Standards and Technology (NIST) standard reference plant materials were of limited value in determining accuracy of Mo analyses in forage legumes because the low certified concentrations (<0.10 mg/kg) in these standards are well below the range of concern (0.5–20 mg/kg) in forages. It was necessary, therefore, to spike laboratory plant reference standard samples (Mo determined to be 0.31, 0.33, and 0.28 mg/kg in separate analyses) with sufficient molybdate to increase the final concentration into the range of concern. In duplicated reference samples spiked to add 10 mg/kg Mo and acid digested, the recovery of the added Mo was 94 and 95%. Thus, the ICP-emission instrument used for the plant tissue analyses was reliable at higher tissue

Mo, although it overestimated Mo in samples with Mo <0.5 mg/kg.

Further quality control testing of the plant tissue Mo analyses was done by inter-laboratory comparison of ICP-mass spectrometry (Research and Productivity Council, Fredericton, New Brunswick, Canada) and ICP-emission (Cornell Fruit and Vegetable Science ICP laboratory, Ithaca, NY) of several alfalfa forage and other plant tissue samples. This showed very good agreement between the two laboratories for 15 samples, covering a range of Mo concentration from 0.5 to 40 mg/kg (r = 0.998), but ICP-emission overestimated Mo at concentrations below 0.5 mg/kg. Analysis of NIST standard reference 1547 (peach leaves) by ICP-mass spectrometry gave 0.056 ± 0.005 mg/kg for Mo, in agreement with the certified value of 0.060 ± 0.008.

Only ICP-mass spectrometry was used for analysis of total Mo in soils and sewage sludges, as preliminary studies had shown ICP-emission without background correction to report erroneously high values on acid digests of soils. The accuracy of ICP-mass spectrometry was checked using a certified sediment reference material (National Research Council of Canada, reference material MESS-2) with a certified total Mo concentration of 2.85 ± 0.12 mg/kg. After digesting this reference by the same HF method used for all soils and sludges, the measured Mo concentration was 2.9 mg/kg, in agreement with the certified value.

RESULTS

Soil Molybdenum

As Table 1 shows, total Mo in the sludge-amended topsoil from the field site US averaged 3.3 mg/kg, about three times greater than the background concentration in soils collected adjacent to the sludge application site. The Mo concentration of 12.4 mg/kg in the sludge applied at the site (see Table 1 for sludge composition) more than accounts for this increase; in fact, nearly 80% of the Mo applied at this site appears to have been lost from the topsoil over the nearly 20 yr since the sludge was applied (McBride et al., 1999). Rapidly water-extractable (3:1 water to soil ratio) Mo averaged 13 (±4) µg/L in soils from the old sludge site, compared with 1 (±1) µg/L in the control soils.

Total Mo in the topsoil at the LSS averaged 2.5 mg/kg, several times greater than the Mo concentration in adjacent control soils.

Metal Uptake in Clover (Greenhouse)

Table 2 provides data for selected trace element composition of clover grown on the US soil columns and recently sludge-treated and control soil columns in the
Clover grown on the USS columns and on the recently DW sludge-amended columns had markedly elevated concentrations of Mo and Ni, with small (if any) increases in tissue Cu relative to controls. For clover grown on the ALK sludge-treated soil, no increase in Ni was detected. The ALK sludge treatment resulted in a larger increase in clover Mo concentration than the DW sludge, but with a relatively small increase in clover Cu. This is probably a result of the higher soil pH maintained by the ALK sludge treatment, favorable to increasing Mo solubility. Although the high pH also increases Cu solubility in alkaline sludge materials and soils, bioavailability may be limited by most of the soluble Cu being in a strongly complexed form (McBride, 1998).

All sludge-treated soils produced clover forage with low Cu to Mo ratios, well under the threshold value of about 2:1 that could induce molybdenosis in ruminant animals on unamended diets. The Cu added to the soils from sludge did not increase forage Cu sufficiently to offset the Mo bioaccumulated by the clover. This was the case whether the sludge had been applied recently or decades earlier and is evidently the result of the weak tendency of Cu to be translocated from roots to tops. Red clover grown on the highly Zn- and Cd-contaminated old sludge site (USS) columns had much higher Zn and Cd contents than the clover on the control columns (Table 2). Lettuce (Lactuca sativa L.), grown earlier on these same USS columns, was a stronger accumulator of Cd than red clover, but clover accumulated higher concentrations of Ni and Mo than lettuce (data not shown). Dewatered sludge application to the columns increased Zn and Cd in the clover, while ALK sludge, by raising the soil pH to about 8, decreased Zn and Cd in the clover (Table 2). On the USS columns, there was substantial dry matter yield reduction relative to controls (about 30–50%) attributable to Zn phytotoxicity. Thus, Mo concentrations in tissue may have been enhanced by stunting of growth; however, there was no relationship between clover tissue Zn and Mo, suggesting that growth stunting had little effect on Mo concentrations in the clover.

### Field Tests of Molybdenum Uptake

It was possible to test whether Mo uptake by red clover in the greenhouse from the USS columns is indicative of Mo uptake by legumes in the field, as these soil columns had been excavated from one of two field sites, LSS and USS, where the same sewage sludge had been applied in 1977–1978. Thus, peas, canola, soybeans, and corn grown at the LSS were analyzed for trace metals and the Mo and Cu contents are presented in Table 3. Topsoil at this site had metal concentrations generally much higher than in the nearby control site, as shown in Table 4. The soil Mo concentration at LSS averaged 2.5 mg/kg, about three times higher than the Mo concentration in the control site. The Mo concentrations were more markedly elevated in the legumes (peas, soybean) and canola than in the corn grown at this site. Therefore, it appears that the high Mo uptake that occurs with red clover in the greenhouse also occurs with legumes (and canola) in the field at soil concentrations of Mo of 2 to 3 mg/kg. Furthermore, Mo transferred into the soybean seed in substantial concentrations, while this was not the case for corn grain (see Table 3). Because growth reduction of soybeans at the LSS site, attributed to Zn toxicity, did occur, the high concentration of Mo in the leaves and seed might be due in part to stunting of growth.

Uptake of sulfur into legumes at the LSS also was markedly higher than at the control site. Although the sulfur content of corn grain (0.8–1.0 g/kg S) and soybean seed (2.3–2.9 g/kg S) was not significantly different in the control and LSS crops, canola and pea plants averaged 8.9 and 3.2 g/kg at the sludge site, respectively, compared with 3.9 and 1.8 g/kg at the control site. The total sulfur content of soil at LSS averaged 830 mg/kg, compared with 527 mg/kg at the control site (Table 4).

### Sampling of Farm Forages


### Table 3. Molybdenum and copper concentrations (mg/kg, dry wt.) in canola, peas, field corn and soybeans grown at the lower sludge site (LSS) and an adjacent control area. (Numbers after sample names are the number of samples analyzed, while values in parentheses are standard deviations. Ratios in italic type fall below the 2:1 ratio minimum recommended for the whole ration.)

<table>
<thead>
<tr>
<th>Plant sample</th>
<th>Mo</th>
<th>Cu</th>
<th>Ca/Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LSS (n = 3)</td>
<td>Control (n = 1)</td>
<td></td>
</tr>
<tr>
<td>Canola (tops) (2)</td>
<td>8.39 (1.22)</td>
<td>1.48 (0.99)</td>
<td>14.7 (0.86)</td>
</tr>
<tr>
<td>Pea (tops) (2)</td>
<td>5.64 (1.09)</td>
<td>2.49 (0.43)</td>
<td>8.59 (0.36)</td>
</tr>
<tr>
<td>Soybean leaves (3)</td>
<td>13.1 (5.4)</td>
<td>1.8 (0.3)</td>
<td>8.95 (0.61)</td>
</tr>
<tr>
<td>Soybean seed (2)</td>
<td>25.5 (8.8)</td>
<td>7.4 (1.3)</td>
<td>24.0 (2.8)</td>
</tr>
<tr>
<td>Corn leaves (3)</td>
<td>2.6 (0.9)</td>
<td>1.2 (0.1)</td>
<td>11.2 (1.3)</td>
</tr>
<tr>
<td>Corn seed (2)</td>
<td>0.45 (0.07)</td>
<td>0.40 (0.14)</td>
<td>37.5 (17.7)</td>
</tr>
</tbody>
</table>

### Table 4. Total metal concentrations (mg/kg) based on HF digestion of soils from the lower sludge site (LSS) and an adjacent control area.

<table>
<thead>
<tr>
<th>Metal</th>
<th>LSS (n = 3)</th>
<th>Control (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo</td>
<td>2.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Cu</td>
<td>14.7</td>
<td>11.2</td>
</tr>
</tbody>
</table>
Table 5. Molybdenum concentrations (mg/kg, dry wt.) of forage samples collected from five dairy farms where lime-stabilized sewage sludge had been applied.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Forage type</th>
<th>sludge</th>
<th>no sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm E</td>
<td>Cornell Research Station</td>
<td>NA</td>
<td>no control sample available on this farm</td>
</tr>
</tbody>
</table>

that had applied an ALK sludge product (used as lime) one time at an estimated 20 t/ha suggested that the amendment may have affected the Mo content of forages. The Mo concentrations in forages from these farms are reported in Table 5. Because fields not treated with sludge were not available on some farms, direct comparisons of Mo in grass or alfalfa grown on the same farm were only possible in three cases, but in all three cases, the Mo in forages grown on the sludge-treated fields was higher than on the untreated fields. The highest value of Mo, reported to be 10.1 mg/kg for alfalfa on Farm C, is very unusual. By comparison, the average value reported for more than 100 alfalfa samples grown at the Cornell Research Station (Aurora, NY) was 0.49 mg/kg. A subsequent larger survey in 1999 of forages on other farms in this area that had applied the same alkaline sludge product confirmed high Mo in alfalfa and clover in some fields (5-15 mg/kg), with forage Cu tending to be relatively constant in the 8 to 12 mg/kg range (data not shown).

A field-collected sample of the sludge product applied to the forages represented in Table 5 was analyzed for total Mo by ICP-mass spectrometry, and found to contain 45 mg/kg. Assuming a 20 t/ha (reported) application rate, and if the Mo content of the sample analyzed is representative, then the Mo loading on these fields was about 1.0 kg/ha.

Table 6. Uptake coefficients (UC) of Mo by animal feed crops grown in the field.

<table>
<thead>
<tr>
<th>Crop</th>
<th>UC</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn (whole plant) (3rd year crop)</td>
<td>Pierzynski &amp; Jacobs (1986a)</td>
<td>Sludge had extremely high Mo content</td>
<td></td>
</tr>
<tr>
<td>Corn (leaf) (3rd year crop)</td>
<td>Soon and Bates (1985)</td>
<td>Ca-sludge (12 mg Mo/kg) on Conestoga loam</td>
<td></td>
</tr>
<tr>
<td>Soybean (whole plant) (3rd year crop)</td>
<td>Soon and Bates (1985)</td>
<td>Fe-sludge (43 mg Mo/kg) on Conestoga loam</td>
<td></td>
</tr>
<tr>
<td>Soybean (leaf) (3rd year crop)</td>
<td>Soon and Bates (1985)</td>
<td>Al-sludge (39 mg Mo/kg) on Conestoga loam</td>
<td></td>
</tr>
<tr>
<td>Soybean (grain) (3rd year crop)</td>
<td>Soon and Bates (1985)</td>
<td>Ca-sludge (12 mg Mo/kg) on Conestoga loam</td>
<td></td>
</tr>
<tr>
<td>Alfalfa</td>
<td>Soon and Bates (1985)</td>
<td>Fe-sludge (43 mg Mo/kg) on Conestoga loam</td>
<td></td>
</tr>
<tr>
<td>Alfalfa</td>
<td>Soon and Bates (1985)</td>
<td>Al-sludge (39 mg Mo/kg) on Conestoga loam</td>
<td></td>
</tr>
<tr>
<td>Bromegrass</td>
<td>Soon and Bates (1985)</td>
<td>Ca-sludge (12 mg Mo/kg) on Caledon loamy sand</td>
<td></td>
</tr>
<tr>
<td>Corn (stover) (last crop)</td>
<td>Soon and Bates (1985)</td>
<td>Fe-sludge (43 mg Mo/kg) on Caledon loamy sand</td>
<td></td>
</tr>
<tr>
<td>Corn (stover) (last crop)</td>
<td>Soon and Bates (1985)</td>
<td>Al-sludge (39 mg Mo/kg) on Caledon loamy sand</td>
<td></td>
</tr>
<tr>
<td>Corn (stover) (last crop)</td>
<td>Soon and Bates (1985)</td>
<td>Soil contained 19.2 mg/kg of Mo</td>
<td></td>
</tr>
<tr>
<td>Corn (stover) (last crop)</td>
<td>Soon and Bates (1985)</td>
<td>Sludge contained 21 mg/kg Mo</td>
<td></td>
</tr>
<tr>
<td>Corn (stover) (last crop)</td>
<td>Soon and Bates (1985)</td>
<td>Sludge contained 36 mg/kg Mo</td>
<td></td>
</tr>
<tr>
<td>Corn (leaf)</td>
<td>Webber et al. (1983)</td>
<td>Sludge had extremely high Mo content</td>
<td></td>
</tr>
<tr>
<td>Bahia grass</td>
<td>Nguyen and O'Connor (1997)</td>
<td>Sludge had extremely high Mo content</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION
Measurement of Molybdenum Uptake into Forages in Earlier Studies

The USEPA 503 rule set limits for toxic metal loadings on agricultural soils from sewage sludge use, based on a detailed risk assessment, although no loading limit exists in the rule at present for Mo. In the support document, the degree of Mo transfer into animal feed was estimated from two field experiments with sewage sludge-amended soils reported by Soon and Bates (1985) and Pierzynski and Jacobs (1986a), and a greenhouse experiment described by Pierzynski and Jacobs (1986b). The Pierzynski and Jacobs studies used a sludge severely contaminated with Mo (1500 mg/kg); consequently the soil Mo loadings and forage Mo concentrations were very high (forages exceeded 700 mg Mo/kg in some cases). The Soon and Bates study involved no legumes, only corn and bromegrass, and two of the three sludges were nonalkaline, high-Fe and -Al materials. Crop UCs for the alkaline (high Ca) sludge were greater than for the nonalkaline sludges (see UC values summarized in Table 6), although lower than other reports of Mo into grasses from alkaline sludge (e.g., Nguyen and O'Connor, 1997). The UC, as defined in the USEPA 503 risk assessment, is the increase of metal concentration in the plant sample (mg/kg) per unit increase of metal loading in the soil (kg/ha).

Of the experimental data used by the USEPA to estimate an average UC for Mo in forages, the only forage legume data included were from the greenhouse study of alfalfa by Pierzynski and Jacobs (1986b). In that study, because of the severe contamination of the sludge, Mo in the alfalfa approached 1000 mg/kg at a soil loading of 188 kg Mo/ha and the UC was in the range of 2.4 to 2.9. It is probably questionable to use UCs from such an extreme loading to extrapolate to a more acceptable range; nevertheless, a linear extrapolation to a tolerable Mo concentration in forage (5 mg Mo/kg, assuming about 10 mg Cu/kg in the animal feed) suggests that Mo loading on neutral or alkaline soils should not exceed 1.0 kg/ha. In contrast, because the USEPA selected a less cautious forage concentration
limit of 10 mg Mo/kg and used a UC for Mo of 0.423 (based in the geometric mean of the UC's from the two studies reported above), a much higher loading limit of 18 kg Mo/ha was calculated (but not entered in the final 503 rule). By averaging the data in this way, the USEPA largely discounted the high availability of Mo in alkaline sludges and the greater UC's of forage legumes compared with grasses. Inspection of the UC data compiled by the USEPA for Mo in forages (USEPA, 1992) shows that the geometric mean UC of 0.423 underestimates potential for Mo uptake for forage legumes where high-lime sludges are applied (Table 6). In fact, very large UC's have been measured for some grass and clover species in the greenhouse (Table 7), although pot studies can exaggerate the actual uptake expected in the field.

Residual (Long-Term) Availability of Molybdenum

The long-term residual uptake of Mo in the field is substantial, despite the relatively high potential for Mo leaching out of the soil. Webber et al. (1983) reported a farm in Ontario that applied about 4.5 t/ha/yr of a high-Mo sewage sludge (about 200 mg/kg Mo) for 21 yr. The soil contained 19.2 mg/kg of Mo and leaves in corn forage sampled at this site contained 32 mg/kg of Mo, compared with 0.72 in the control crop. The Cu to Mo ratio in the leaves was 7.8:32.2 = 0.55. Although this sludge had a high Cu content, the Cu concentration in corn leaves was not increased by the sludge application; as observed in our greenhouse study with red clover, there was no significant protective effect of Cu against excess Mo in the forage.

Both field experiments used in the USEPA 503 risk assessment reported large residual effects, with Mo concentrations in successive crops either sustained or increasing for several years after sludge application (Pierzynski and Jacobs, 1986a; Soon and Bates, 1985). Therefore, cumulative effects of Mo in soils must be factored into any risk assessment of this element. The very high residual uptake into red clover 20 yr after sludge application (this study) indicates that a significant fraction of the Mo is not leached out of the rooting zone. In a separate study, it has been estimated that about 20 to 25% of the originally applied Mo in sludge remains in the topsoil (McBride et al., 1999). Davis (1981) measured high levels of Mo in forage legumes (18.3–51.5 mg/kg) grown in the greenhouse on an old sludge-amended soil containing 6.0 mg Mo/kg, but other forage crops, such as timothy and ryegrass, did not show a marked uptake. It appears, therefore, that sludge-applied Mo will affect forage legume quality for a long time. Retention of Mo by soil organic matter occurs by several mechanisms (Fleming, 1980; Bloomfield and Kelso, 1973) and this may provide a means of retaining a fraction of the molybdate against leaching. It also has been suggested that Mo reduction by organic matter could convert the metal to a more strongly bound form since Mo can accumulate in poorly drained peaty soils (Williams and Brown, 1971), perhaps by association with sulfides (Bloomfield and Kelso, 1973). Direct evidence for molybdate reduction to Mo(V) and Mo(III) by soil organic matter has been reported (Goodman and Cheshire, 1982).

Using Uptake Coefficients to Estimate Soil Loading Limits for Molybdenum

The UCs for Mo in red clover were, for DW and ALK sludge:

\[
\text{UC (DW)} = (6.69 - 0.57) \text{ mg Mo/kg}
\]

\[
\text{UC (ALK)} = (18.5 - 0.57) \text{ mg Mo/kg}
\]

Assuming a tolerable Mo concentration in forage to be 5 mg/kg, the acceptable loading limit for the ALK sludge material (if the UC is 4.27) would be about:

Acceptable Mo loading limit =

\[
(5.0 - 0.57)/4.27 = 1.0 \text{ kg/ha}
\]

For the DW sludge, a similar calculation gives a loading limit of about 4.2 kg/ha. The higher solubility of Mo in the alkaline sludge product (Richards et al., 1997) presumably accounts for the greater UC and the lower recommended loading limit. Since Mo loadings on the order of several kg/ha could only be achieved experimentally by very high applications of sludge products, these calculated limits need to be tested in long-term field experiments where applications are at agronomically reasonable rates. Nevertheless, based on these calculations, Mo could become the metal limiting farm application of some sewage sludges, and the lack of a cumulative loading limit for Mo in the USEPA 503 rule for livestock farm-applied sewage sludge appears to be a serious deficiency.

**ACKNOWLEDGMENTS**

Appreciation is expressed to Dr. Lucia Tyler for ICP-emission analysis of plant samples. Research was supported in part by the Agricultural Ecosystems Project.
REFERENCES


