Purification and Characterization of Molybdenum Species from *Medicago sativa* (Alfalfa) Grown on Mine Tailings

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Extraction, purification, and identification procedures were developed for the chemical investigation of molybdenum (Mo) in freeze-dried aerial portions of alfalfa (*Medicago sativa* L.) collected from a reclaimed tailings pond at the Highland Valley Copper mine in British Columbia. The purification procedures were guided by ICP and colorimetric analyses. The methods included development of an efficient aqueous extraction protocol, sample cleanup by partitioning against *n*-butanol, and filtration through diatomaceous earth. Further purification was achieved by anion-exchange chromatography, elution with aqueous NaCl, and desalting by gel filtration. Final purification of the Mo-containing fraction was carried out using preparative anion-exchange HPLC. Molybdenum was found to be present in its purified form as the molybdate anion (MoO_4^{2-}) primarily on the basis of multinuclear (^1H, ^{13}C, and ^{95}Mo) NMR studies.

Keywords: *Medicago sativa; alfalfa; molybdenum; ion exchange chromatography*

INTRODUCTION

The Highland Valley Copper (HVC) mine, one of the world's largest open-pit copper mines, is located in the southern interior of British Columbia near the city of Kamloops. Approximately 247000 tons of rocks are mined daily with the ore averaging 0.40% copper (Cu) and 0.006% molybdenum (Mo) (*1*). Average recovery for the mining is 91% for Cu and 55% for Mo. Reclamation of disturbed lands is ongoing, but the residual mineral content from the extraction process is of concern for reclamation. The planned agricultural uses of the reclaimed land sites are cattle grazing and hay production.

The reclamation program largely consists of establishing domestic plant species with the aid of fertilizers (*2*, *3*). However, the established vegetation contains elevated levels of residual Mo (*4*). Determination of the Mo levels has shown that the Highmont site at HVC is of primary concern, because the Mo levels in forages there are among the highest in the area (>100 ppm). In grazing animals, elevated Mo levels can reduce the availability of Cu. Current theory suggests that Mo can interact with Cu in ruminant systems through the formation of thiomolybdates to form insoluble Cu thiomolybdate complexes leading to Cu deficiency (*5*). Animal health guidelines (*6*) recommend a maximum level of 3.5 ppm Mo in feed for beef cattle and maintenance of a Cu/Mo ratio of 2:1 to avoid Cu deficiency in grazing ruminants (*7*).

To address these concerns, recent reclamation studies have included grazing trials to determine the effects of elevated levels of Mo (120−620 ppm) on animal health and performance (*4*). The results of the grazing studies, which showed no major signs of poisoning during 1998 (*8*) and 1999 (*4*), prompted a chemical investigation of the form of the Mo as it occurs in alfalfa, which is one of the predominant plant species at the Highmont site. A possible explanation for the lack of Mo toxicity in grazing livestock was hypothesized to be the result of a stable organometallic Mo complex of plant origin. This complex might be excreted from the gastrointestinal system of ruminants.

Accordingly, a chemical investigation of the form of the Mo in alfalfa grown on the Highmont tailing site was initiated. The present objectives were to develop a purification protocol that would give an acceptable yield of the Mo species and to characterize the isolated Mo species through spectroscopic analysis.

MATERIALS AND METHODS

Plant Material. All experimental procedures were carried out using aerial portions of *Medicago sativa* L. (cv. Rangelander) collected in the vegetative to bud stage from the Highmont tailings site in August 1996. The composite plant sample was freeze-dried and then ground with a Wiley mill to pass through a 1-mm steel sieve.

Molybdenum Analysis by ICP and Dithiol Method. A Thermo Jarrell Ash Atomscan 25 sequential inductively coupled argon plasma atomic emission spectrophotometer (ICP) was used for Mo analysis. The Mo emission spectrum was monitored at $\lambda = 203.844$ nm. A 2-kW crystal controlled radio frequency operating at 27.12 MHz powered the emission source. The peristaltic pump was set to 100 rpm (1.3 mL/min).

A 10-ppm Mo calibration standard was prepared from $Na_2MoO_4 \cdot 2H_2O$ and was used as an ICP reference standard for screening chromatographic fractions. Additional standards were employed for the determination of total Mo in plant samples and extracts. ICP analysis was carried out directly on diluted chromatography fractions. Plant sample preparation required overnight ashing in a muffle furnace at 475 °C followed by solubilization in 0.5 N HCl for 3 h at room temperature and further dilution (× 100) with water for quantitative ICP.

A modified version of the dithiol procedure (*9*) was used as a qualitative visual assay of Mo in chromatographic fractions. The visualization procedure requires placing 3−5 drops of sample in a spotting dish, followed by acidification with 6 N HCl, and then addition of 3−5 drops of the dithiol solution. A

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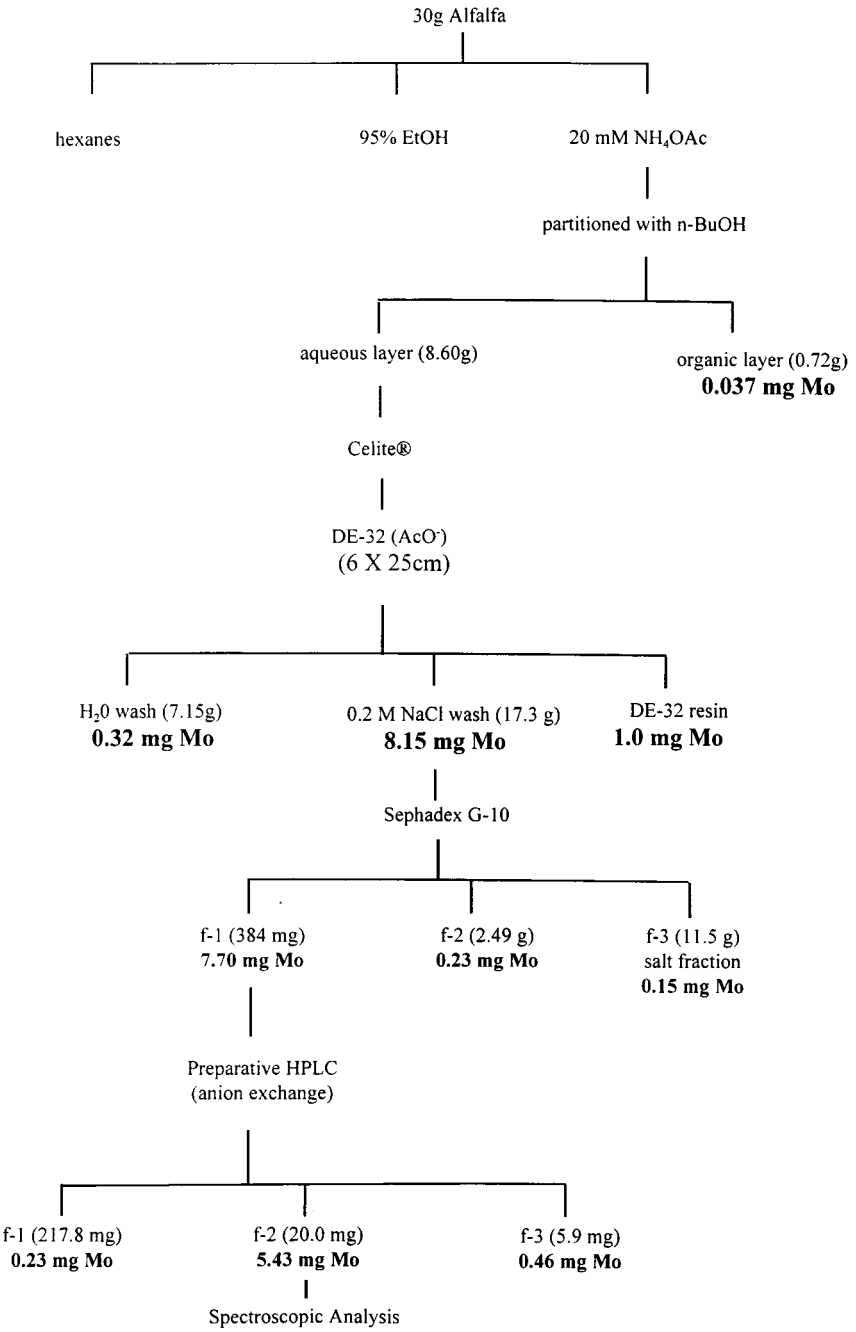

Figure 1. Purification procedure for Mo species from alfalfa.

green precipitate is indicative of Mo. The detection limit is approximately 1 ppm Mo.

Ion-Exchange Chromatography. A weak anion exchanger, diethylaminoethyl cellulose (Whatman, DE-32, microgranular form), was prepared as follows. The required amount of resin was allowed to equilibrate in 0.5 N NaOH for 30 min and then washed with water on a Büchner funnel to remove excess alkali. It was converted to the acetate form by treatment with 10% acetic acid and then washed with water to remove excess acid. The sample was dissolved in a minimal amount of water, applied to the resin under slight vacuum, and washed with distilled water. Elution of the Mo species was carried out with 0.2 M NaCl. The qualitative dithiol Mo assay was used to determine Mo elution.

The cation-exchange resin (Whatman, CM-32, microgranular form) was prepared by equilibration in 10% acetic acid and then washing with water to remove excess acid.

HPLC. Preparative HPLC was carried out using a Varian 9012 liquid chromatograph coupled to a Varian 2550 UV/visible detector. A Hamilton PRP-X100 analytical anion-exchange column (4.1 mm × 250 mm) was used for method development. A Hamilton PRP-X100 semipreparative anion-exchange column (7.1 mm × 305 mm) was used for preparative isolations. The mobile phase consisted of 100 mM NH_4OAc containing 10% CH_3CN with a flow rate of 2.5 mL/min and UV detection at 246 nm. Samples were passed through a 0.45-μm filter before HPLC. Sample concentrations ranged from 0.12 mg Mo/mL to 0.2 mg Mo/mL with a maximum injection volume of 500 μL.

NMR Spectroscopy. ^{95}Mo NMR spectra were obtained at 19.56 or 26.07 MHz using a Bruker AMX 2 300 or a Bruker AM-400 spectrometer, respectively. The spectra were all collected in 99.9% D_2O solutions at room temperature with 0.1 M Na_2MoO_4 as an external standard. The chemical shifts are reported in ppm and the line widths at half-height ($\nu_{1/2}$) are in Hz. For purified samples, pH was measured directly in the NMR sample in a 5-mm tube. The pH was adjusted directly

with a 30% NH_4OH solution in D_2O using a Corning 140 pH meter equipped with a 2-mm micro glass capillary probe.

^1H NMR spectra were obtained at 200 or 400 MHz with Bruker ACE-200 or Bruker AM-400 spectrometers, respectively. The chemical shifts (δ) are reported in ppm and the coupling constants (J) are given in Hz. Spectra were recorded in D_2O with an internal reference signal of δ 4.80 ppm (residual HOD). The multiplicities of signals are indicated by br d (broad doublet), d (doublet), t (triplet), or dd (doublet of a doublet).

^{13}C NMR spectra were determined at 50.3 or 100.6 MHz with Bruker ACE-200 or Bruker AM-400 spectrometers. The chemical shifts (δ) of carbon were obtained in proton-decoupled mode, and are reported in ppm. Spectra were collected in D_2O using methanol (δ 49.0 ppm) as an internal reference.

Isolation and Purification. Freeze-dried plant material (30 g) was washed on a sintered glass funnel with 0.5 L of hexanes and 0.5 L of boiling 95% ethanol before extraction (Figure 1). The plant material was transferred to a mortar and pestle and ground to a slurry with 20 mM NH_4OAc. The slurry was transferred to a glass column and extracted with 2 L of NH_4OAc. The aqueous extract was partitioned with 2 × 300 mL of *n*-butanol. Both the aqueous and organic layers were concentrated to dryness at 40 °C. The aqueous extract was redissolved and clarified by filtration through Celite diatomaceous earth. The filtrate was applied to a 6 × 2.5 cm DE-32 column under slight vacuum. The column was washed with 0.5 L of water. Elution of the Mo species was carried out using 1.5 L of 0.2 M NaCl. The DE-32 anion-exchange resin was ashed at 475 °C for 24 h and solubilized in 0.5 N HCl for Mo analysis by ICP. The fraction eluted with NaCl was concentrated on a flash evaporator at 40°. Gel filtration on Sephadex G-10 (5 × 46 cm) equilibrated in 20 mM NH_4OAc was used for desalting. The sample was dissolved in a minimal amount of 20 mM NH_4OAc, applied to the bed, and eluted with 20 mM NH_4OAc (5 × 40 mL), and the Mo was detected immediately after the exclusion volume. The Mo-containing fractions, free of NaCl, were pooled, concentrated, and dissolved in 1 mL of mobile phase for purification by HPLC. Three fractions were collected during each preparative run. Typical retention times were 4−33 min, 33−66 min, and 66−90 min. Samples were pooled and concentrated to dryness, dissolved in a minimal amount of H_2O, and freeze-dried to remove residual NH_4OAc. The theoretical amount of 12 mg of Mo in 30 g of plant material was based on an average concentration of 400 ppm Mo in the alfalfa sample. Acceptable recoveries of Mo were obtained at all stages, ranging from 79 to 99%. The optimized purification procedure (Figure 1) yielded 5.43 mg of Mo in 20 mg of purified sample.

Preparation of Na_2MoO_2(malate)$_2$. Literature precedence for the preparation of the NH_4^+ salt is available (*10*). The sodium (Na) salt was prepared as follows. (L)-Malic acid (2.68 g) was dissolved in 20 mL of water, 2.41 g of $Na_2MoO_4 \cdot 2H_2O$ was added, and the mixture was stirred. The pH was immediately adjusted to 5−6 using 3 N NaOH, and the solution was allowed to evaporate overnight to produce a viscous liquid. Ethanol was added until cloudiness persisted, and H_2O was then added dropwise until the cloudiness faded. The solution was refrigerated overnight. Fine crystals that formed were collected and washed with cold 90% ethanol. Yield 3.19 g, (73%). ^1H NMR: δ 2.875 (d, 4H, 5.74 Hz), 5.12 (br d, 2H). ^{13}C NMR: 38.84 (CH_2), 80.70 (CH), 175.30 (C═O), 184.00 (C═O). ^{95}Mo NMR: 83.50 ($\nu_{1/2}$ = 113 Hz).

RESULTS AND DISCUSSION

Extraction Protocol. It was evident that the purification of Mo would require a quantitative approach to determine the Mo yields at each step. The qualitative Mo assay was used for quick checks to follow the Mo through chromatographic fractionation. To our knowledge, methods for the purification of low-molecular-weight Mo components from a natural source have not been reported in the literature.

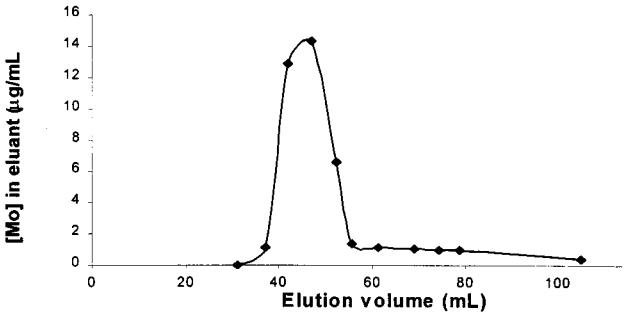

Figure 2. Elution profile of alfalfa (1 g) water extract on Sephadex G-10.

Solvents of increasing polarities were tested for efficiency of Mo extraction from the plant material. The Mo species appeared to be polar and very water-soluble. Nonpolar solvents such as hexanes and ethanol were unsuitable for use as extracting solvents for the Mo species. However, defatting with a lipophilic solvent might aid in the subsequent aqueous extraction, allowing for increased wetting and solublization of polar constituents. The hexane wash was followed by washing with ethanol, which could remove water-soluble interferences. Only trace amounts of Mo were found in the hexanes and ethanol washes. The major portion of the Mo was extracted in the initial aqueous fraction. A total of 9.47 mg of Mo was found in the combined H_2O extracts, representing 79% of the total Mo (Figure 1). The amount of Mo remaining in the residue was 21% of the total. Attempts were made to access the nonextractable Mo remaining in the residue but significant increases in extractable Mo were not achieved. It appears that a portion of the Mo is bound, as it could not be recovered from the plant material by conventional extraction procedures.

Gel Filtration and Dialysis. Initial studies were conducted with 1 g samples of alfalfa. Gel filtration experiments were carried out using Sephadex G-10, which has a fractionation range of <700 g/mol. Repeated experiments found the Mo species in the fractions following the exclusion volume (Figure 1). Therefore, the Mo species probably had a molecular weight of <700 g/mol. Elution was carried out with water, and the elution profile is shown in Figure 2. From the profile, it appears that the Mo species chromatographs as a single species on Sephadex G-10.

Complementary evidence was required to verify the molecular-weight range. The above experiment assumes the Mo species to be stable to the extraction and concentration steps prior to gel filtration. To avoid the concentration process, dialysis seemed a suitable alternative to gel filtration. It was possible to use a crude water extract directly in a dialysis experiment without subjecting the sample to heat and solvents. The water extract was transferred directly to a 20-cm Spectrum molecular porous-membrane tubing with a molecular cutoff of 3500 g/mol and suspended in the dialysis medium (300 mL of 0.1 mM NH_4OAc) with continuous mixing at 4 °C for 28 h. The extract was then dialyzed against water. The first dialysate contained 70% of the soluble Mo species and the second contained 12%, which indicated that the major Mo component in alfalfa had a molecular weight of less than 3500 g/mol. These results supported the earlier evidence obtained by gel filtration, and it was concluded that the Mo exists mostly as a low-molecular-weight species.

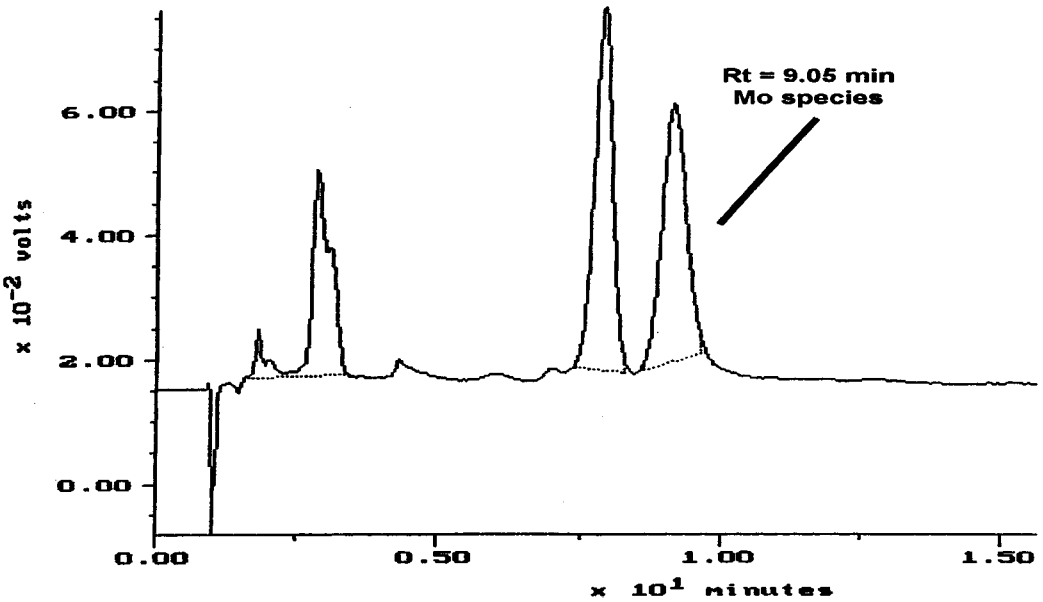

Figure 3. HPLC Chromatogram of semipurified Mo fraction from alfalfa.

Ion-Exchange Chromatography. Molybdenum, as it is found most commonly in the environment, exists in its highest oxidation state [Mo(VI)] as the MoO_4^{2-} oxy anion. Therefore, the ionic properties of the Mo species in alfalfa were screened on cellulose-based anion- and cation-exchange resins. Water extracts were applied to the resins. The Mo was retained on the anion resin when the resin was eluted with water but it was not retained on the cation resin, which clearly demonstrated the anionic property of the Mo species in alfalfa. Therefore, an anion exchange system was the method of choice for HPLC. For the purposes of purification, the buffer for the mobile phase had to be volatile. Ammonium acetate and acetonitrile were used to develop optimal HPLC conditions. A 50-μL injection of a 50 ppm Mo solution (as Na_2MoO_4) yielded a retention time (R_t) of 8.57 min when the mobile phase was 10% acetonitrile in 100 mM NH_4OAc. Examination of the chromatogram of a semi-purified DE-32 Mo-fraction (Figure 3) indicated the presence of the Mo species at $R_t = 9.05$ min. The preliminary results with the HPLC system suggested that the Mo species in alfalfa might be the MoO_4^{2-} anion.

^{95}Mo NMR Optimization and Spectroscopic Characterization. Molybdenum has two NMR active isotopes (*11*). Both ^{95}Mo and ^{97}Mo have a spin $I = {}^5/_2$ and low detection sensitivities. Comparatively, ^{95}Mo is more readily detected with a relative receptivity to ^{13}C of 2.9 (*12*). The quadrupole moment for ^{95}Mo is 0.12×10^{-24} cm^2, and, in light of this, very wide lines are expected for all but the most symmetric environments. Information from ^{95}Mo NMR is obtained from the chemical shift (using Na_2MoO_4 as external reference), the line width, and less commonly spin−spin coupling. The abbreviations used for NMR analysis are noted in Table 1. Increasing SWH decreases AQ, however a larger SWH can require a larger TD to adequately define peak shapes (particularly narrow line-widths), and a compromise set of parameters usually needs to be chosen. Not knowing where the ^{95}Mo signal might resonate, initial SWH values were large so as to cover a wide chemical shift range. For later cases where chemical shifts were better known, a decreased SWH value was used. Typical T_1 relaxation times for ^{95}Mo range from 0.2 to 0.6×10^{-3} sec (*13*), which is largely determined by the symmetry of the Mo species being measured. Implications of this were that short recycle delays could be employed given these inherently short relaxation times. The pre-scan delay (DE) and the delay time (D1) were both set to 50 μs.

Table 1. Important NMR Parameters

parameter	abbreviation	optimized value
spectral width	SWH	25000 Hz
acquisition time	AQ	0.0817 s
pre-scan delay	DE	50 μs
delay time	DI	50 μs
no. of data points	TD	4.0 K

The Na_2MoO_4 ^{95}Mo NMR standard (0.5 mg of Mo as Na_2MoO_4 in 0.6 mL of D_2O) was found to be relatively sensitive, and only 6000 scans were required to adequately detect the Mo signal at 0.0 ppm. The increased sensitivity in this experiment is a direct consequence of this narrow line-width (9.04 Hz), which reflects the high symmetry of MoO_4^{2-} (longer T_2). These results are in agreement with literature findings (*12*), in which structural changes have been correlated with line width. Initially, the above results were promising for us because if a purified Mo sample contained 0.5 mg of Mo as a MoO_4^{2-} salt then ^{95}Mo NMR should be able to detect the Mo signal. On the other hand, no signal detection would provide negative evidence that the Mo was not occurring as free MoO_4^{2-}, but as a Mo species with a wider peak width (and perhaps different chemical shift).

In fact, ^{95}Mo NMR on purified material containing up to 5 mg of Mo (Figure 1), produced no detectable NMR signal after 6000 scans or even after much larger scan numbers. These spectra were run by simply dissolving the Mo isolate in 0.6 mL of D_2O. This result seemed at first sight to rule out MoO_4^{2-} as the form of Mo being isolated.

A possible complex of MoO_4^{2-}, which would still be an anion, might be formed from an α-hydroxy acid (e.g., malic or citric acid) which are common constituents of many plants. Indeed, malic acid was observed in the ^{1}H

NMR spectrum of some of the early alfalfa chromatographic fractions, and molybdate−malate complexes have been described (*10*).

To assess the ^{95}Mo NMR properties of such complexes we synthesized $Na_2MoO_2(malate)_2$. The results showed that a sample of the synthetic containing 1 mg of Mo required 3 h of collection (100000 scans) to detect a signal at 84 ppm, $\nu_{1/2}$ = 113 Hz. This significantly broader signal can be correlated with a less symmetrical environment around the Mo nucleus. However, the Mo amounts in our unknown samples were in excess of 1 mg (Figure 1), so it appeared that other factors were responsible for the nondetection of a ^{95}Mo signal.

Other factors besides complexation that can influence the ^{95}Mo NMR behavior of MoO_4^{2-} include the counterion, solvent, and pH (*11*). Of these, pH was considered the most important. A previous study reported ^{95}Mo T_1 relaxation times as a function of solution pH (*12*). The study involved measuring the rate of polymerization of MoO_4^{2-} as a function of a decreasing solution pH. These line-width measurements reflect structural differences at the different pH values. It was noted that between pH 9 and 12, ^{95}Mo relaxation times were independent of hydrogen ion concentration. However, below pH 9 protonation of the Mo species caused line broadening due to the formation of less symmetrical polymolybdates. In light of this, pH was considered to be an important factor concerning the nondetection of a ^{95}Mo signal.

A pH study with MoO_4^{2-} was then carried out to examine the effect of pH on the ^{95}Mo line-width and results were in agreement with previous work (*12*). The goal was to determine the pH at which Mo could no longer be detected because of NMR line broadening. It was concluded that major structural changes caused by polymerization take place below pH 6, and below pH 5 no signal was detected.

Spectroscopic Studies on the Purified Mo Fraction. The Mo-containing fraction from prep HPLC (Figure 3) was dissolved in D_2O, and the pH was determined to be 5.6. The sample was subjected to ^{1}H, ^{13}C, and ^{95}Mo NMR. The ^{1}H NMR spectra revealed three single peaks in the 1.8−2.5 ppm range as well as the solvent HOD peak, which was used as the reference signal (4.80 ppm). The methyl-group signal from acetate occurs in this region and therefore was a likely candidate as it was a component of the mobile phase for HPLC. Other minor ^{1}H peaks were also detected. After acquiring 34000 scans no signals were detected by ^{13}C NMR. After acquiring 103000 scans no signals were detected by ^{95}Mo NMR. The pH of the sample was then adjusted with 50% NH_4OH in D_2O to pH 9.2, and a strong signal was found after collecting 492000 scans (Figure 4). The signal occurred at a chemical shift of 0.1 ppm, which suggests that the Mo is in a chemical environment similar to that of MoO_4^{2-}, but the line width of 109 Hz is much larger than that of Na_2MoO_4 at this pH. We therefore concluded that some other sample component must be interacting with the MoO_4^{2-} anion, to alter the chemical environment.

One possibility for a broad line ^{95}Mo peak would be a mobile equilibrium between a small amount of an organic compound and MoO_4^{2-}. The magnitude of such an effect would depend on the concentration of the organic compound and the symmetry of the complex. ^{13}C NMR signals were not detected in the isolate, but ^{1}H signals were seen, and a quantitative calibration of

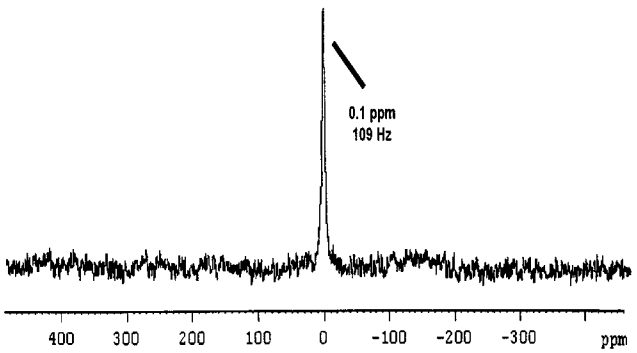

Figure 4. ^{95}Mo NMR Spectrum of the purified Mo species.

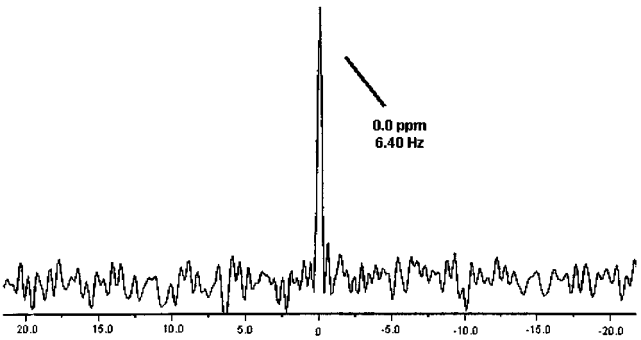

Figure 5. ^{95}Mo NMR Spectrum of $(NH_4)_6Mo_7O_{24}$, pH adjusted to 10.2 with 50% NH_4OH.

the signals was carried out by spiking the solution with a known amount of methanol (2.0 mg). The most likely source of the ^{1}H NMR peaks is simple CH_3-X compounds (X = quaternary carbon), but there was considerably less material (<1 mg) than that necessary for a 1:1 complex with MoO_4^{2-}, because the ICP analysis showed that 5.4 mg of Mo is present in a total isolate of 20 mg (Figure 1).

Paramagnetic metals can also affect NMR line widths (*14*). Of the possible metals that can cause this effect, copper was deemed most likely because it was found in slightly elevated levels in the plant material. To test this hypothesis, an NMR solution containing Na_2MoO_4 (13.5 mg) was spiked with 9 μL of aqueous $CuSO_4$ (1μg/μL). A ^{95}Mo signal at 1.7 ppm was detected with a line width of 85 Hz. Thus, a very small molar ratio addition of $CuSO_4$ caused a significant broadening of the NMR signal. The next step was to obtain an accurate measurement of Cu in the purified Mo sample to see whether this could affect the ^{95}Mo NMR spectrum. A Cu analysis (by AAS) found 62 μg in our isolate versus 4.57 mg of Mo.

To mimic the isolate, 4.57 mg of Mo as $(NH_4)_6Mo_7O_{24}$ (8.43 mg) was dissolved in 0.5 mL of D_2O and transferred to an NMR tube. The pH was then adjusted to 10.2 so as to eliminate formation of polymolybdates, the ^{95}Mo NMR spectrum was obtained, and a sharp signal at 0.0 ppm was observed (Figure 5). The sample was then spiked with 155 μL of $CuSO_4$ (1 μg/μL, 62 μg Cu) and the spectrum was again recorded (Figure 6). The NMR solution contained the same amount of Mo and Cu as the purified Mo isolate, and $(NH_4)_6Mo_7O_{24}$ was used because the Mo species in our isolate should mostly exist as the NH_4^+ salt after HPLC. The addition of the $CuSO_4$ caused an increase in the line width from 6 to 100 Hz, which was similar to the line width of the Mo isolate. The chemical shift of the reference solution was

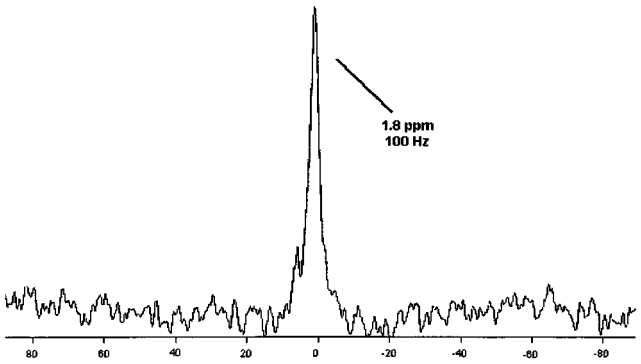

Figure 6. ^{95}Mo NMR Spectrum of $(NH_4)_6Mo_7O_{24}$, pH adjusted to 10.2 with NH_4OH, 155 μg of $CuSO_4$ (reference solution).

1.8 ppm, which, within experimental error, was the same as that of the Mo isolate. It was concluded that the purified Mo isolate was the MoO_4^{2-} anion and that the presence of 62 μg of Cu caused paramagnetic line broadening of the ^{95}Mo NMR signal.

In summary, with the intent of determining the chemical form of Mo in alfalfa grown on mine tailings, extraction, cleanup, and purification procedures were developed. Determination of optimal parameters for detecting Mo by ^{95}Mo NMR was also achieved. The Mo was found to be present in its purified form as the molybdate (MoO_4^{2-}) anion. This was primarily based on multinuclear (^{1}H, ^{13}C, and ^{95}Mo) NMR studies. A model Na_2MoO_2(malate)$_2$ complex was prepared to aid in the purification and identification studies, but it was not detected in purified Mo fractions from alfalfa.

Quantitative ^{1}H NMR and ^{13}C NMR indicated the absence of significant amounts of organic material. Trace amounts of copper in the isolate resulted in paramagnetic line broadening of the ^{95}Mo NMR signal, which was demonstrated using reference solutions of ammonium molybdate containing copper sulfate. All spectroscopic data obtained indicated the occurrence of inorganic MoO_4^{2-} anion in the final purified fraction from alfalfa. The Mo may exist as a variety of complexes in vivo; however, a complex of MoO_4^{2-} was not detected after purification using the above-described procedures. Care was taken to use mild extraction conditions and solvents so as to prevent altering the chemical form of the Mo species during purification.

An explanation for the absence of Mo toxicity or Cu deficiency in grazing livestock during 1998 and 1999 has yet to be determined. The present work on the chemical form of Mo in plants grown on the Highmont site does not provide an answer, and a different approach to the problem is required.

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