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ABSTRACT: A variety of extractants has been used to assess the availability of molybdenum (Mo) in soils. Most of the extractants have been studied from a deficiency aspect rather than for soils with Mo toxicity, and none of them have been used to extract available Mo from mine spoils. The purpose of this study was to examine the potential of different chemical extractants for assessing the availability of Mo in mine spoils and soils. One mine spoil and three soils were treated with sodium molybdate and then subjected to wetting and drying cycles for two months. These spoil/soils were extracted with ammonium bicarbonate DTPA (AB-DTPA), ammonium carbonate, and ammonium oxalate solution for available Mo. Crested wheatgrass (*Agropyron cristatum*) and alfalfa (*Medicago sativa*) were grown in the spoil/soils in a greenhouse to determine plant uptake of Mo. Additionally, four mine spoils and six soils were extracted and analyzed for available Mo as mentioned above. The results obtained by these three extractants were highly correlated. It was found that ammonium oxalate extracts the greatest amount of Mo among the three extractants from spoil/soils since it dissolves some adsorbed Mo from Fe-oxide and Al-oxide. The changes in pH of spoil/soils did not have a significant effect on the amount of Mo extracted by any of these methods. The relationships between Mo uptake and Mo extracted by each method were all significant at 1% level. None of the extractants were clearly better or worse than the others. All three methods can be used to assess Mo availability, and potential toxicity from plant uptake of Mo from reclaimed spoils.

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INTRODUCTION

Elevated Mo concentrations have been reported in reclaimed coal mine spoils and soils adjacent to mine sites in the Northern Great Plains (Erdman et al., 1978; Stone et al., 1983). The primary hazard of high levels of Mo in mine spoils and soils comes from the potential to produce forage high in Mo that would induce molybdenosis in ruminants. Molybdenosis has been reported as a potential problem in cattle and, possibly, sheep on coal mine spoils in the Hidden Water and Dave Johnston coal mine areas, Wyoming (Erdamn et al., 1978).

Molybdenosis disease is basically an Mo induced copper (Cu) deficiency in the ruminants diet (Jarrell et al., 1980; Underwood, 1977). The accumulation of Mo in plants is closely correlated with the available Mo in the soils. The ability of plants to extract Mo from soils depends upon physical, chemical, and environmental factors. To date, a variety of extractants have been used in attempts to extract available Mo. These include ammonium oxalate (Grigg, 1953), water (Gupta, 1969; Lavy and Barber, 1964), hotwater (Pathak et al., 1969), anion exchange resin (Bhella and Dawson, 1972), ammonium bicarbonate DTPA (Soltanpour et al. 1979), anmonium carbonate (Vlek and Lindsay, 1977), ammonium acetate and EDTA (Williams and Throton, 1972). The ammonium oxalate extraction method is the only method that has been extensively field calibrated with plant response (Neuman et al., 1987).

Although a number of extractants have been employed for the assessment of Mo availability, most appear to have been studied from the deficiency aspect rather than from consideration of toxic effects (Davies, 1980). Recently, Pierzynski and Jacobs (1986) used AB-DTPA extractant to study the extractability and plant availability of Mo from inorganic and sewage sludge treated soils. They concluded that the AB-DTPA extractant was more effective in predicting availability of Mo than the ammonium oxalate extractant. Extraction with 1M (ammonium carbonate [$(NH_4)_2CO_3$] was found to be an effective method for neutral and alkaline soils (Vlek and Lindsay, 1977). It produced good correlations between extractable Mo levels and Mo uptake by alfalfa. Ammonium oxalate has been employed as an extractant in studies on Mo toxicity. Walsh et al. (1952) found that ammonium oxalate was useful in identifying toxic pasture in Ireland. None of the above extractants have been used to extract the available Mo from mine spoils. The objective of this study was to examine AB-DTPA, $(NH_4)_2CO_3$, and ammonium oxalate as extractant for assessing Mo availability of mine spoils and soils. Crested wheat grass (*Agropyron cristatum*) and alfalfa (*Medicago sativa*) were used as test plants. These plants are often used for both coal mine spoil reclamation as well as forage for livestock in the western United States.

MATERIALS AND METHODS

Selection of Extractation Method

There were three extraction methods used in this study: AB-DTPA (Soltanpour and Workman, 1979), $(NH_4)_2CO_3$ at pH 9.0 (Vlek, 1975), and ammonium oxalate (Grigg, 1953). The AB-DTPA soil test extracts phosphorus (P), potassium (K), zinc (Zn), iron (Fe), Cu, and manganese (Mn) simultaneously from soils. It is obvious that monitoring the plant availability of multiple elements with the above soil test will make soil testing more efficient. This is especially true for laboratories that are equipped with inductively coupled plasma emission spectrometers (ICP-AES) which have the capability to determine many elements simultaneously. The $(NH_4)_2CO_3$ method was chosen because it has a good correlation of plant uptake of Mo and extractable Mo, especially for soils which have a Mo toxicity problem. In addition, $(NH_4)_2CO_3$ has the advantage of being an excellent buffer, volatilizes during digestion, and leaves a decolorized extract when treated with hydrogen peroxide (H_2O_2) prior to the colorimetric Mo analyses. This is particularly useful for laboratories which do not have atomic absorption spectrometry (AAS) or ICP but have a visible-ultraviolet spectrometer. The acid oxalate extraction method is the only test that has been adequately checked with field trials. Due to problems with its performance, there is a need for a versatile soil test that is accurate over a wide range of soil, and that has been evaluated for both greenhouse and field use.

The general principles of three extraction methods are: In the AB-DTPA method, ammonium bicarbonate is a buffer and DTPA acts as the extractant and stabilizes the solution for Mo. For the $(NH_4)_2CO_3$ method, ammonium carbonate is a buffer and, since the extractant is at pH 9.0, both hydroxyl (OH^-) and carbonate (CO_3^{2-}) anions can replace exchangeable Mo from exchange sites. This fraction of soil Mo is available for plant uptake. Ammonium oxalate solution is believed to bring Mo, Fe, Al, and Mn ions into solution and stabilize them by formation of a complex with oxalate.

Greenhouse and Laboratory Experiments

Spoil samples were collected from the Dave Johnston coal mine at the southern edge of the Powder River Basin, Wyoming. Soil 1 was collected from a ranch adjacent to the coal mine. Samples from two native soils (Soils 2 and 3) were also included in this study for comparison. These were selected based on the physical and chemical properties of the soils (*e.g.* soil texture, pH, EC, and organic matter content). Soil 2 was collected from a draw in the Laramie Range, Wyoming and Soil 3 was collected from the Stockfarm of the University of Wyoming. The reclaimed spoil and Soil 1 near the coal mine site are classified as sandy, mixed, Ustic Torriorthents. Soil 2 is classified as a coarse-loamy, mixed Cumulic Haploboroll and Soil 3 is classified as a sandy-skeletal, mixed Borollic Haplargid.

The spoil/soils samples were ground and sieved through a 2-mm screen. Sieved samples were used for subsequent experiments. The texture, pH, electrical conductivity (EC), organic matter (OM), calcium carbonate content, and total Mo of samples were determined for initial characterization. For greenhouse studies, solutions of sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) were added to samples to achieve the rate of 1, 3, and 5 mg Mo/kg of spoil/soil. Hoagland nutrient solution, without Mo, was used to insure adequate plant growth (Table 1). Three hundred milliliters of Hoagland solution were added to the nutrient treated pots. The overall design of the experiment was a randomized complete-block. The following conditions were used:

1. Control.
2. Hoagland nutrient solution.
3. One mg Mo/kg of spoil/soil plus nutrient solution.
4. Three mg Mo/kg of spoil/soil plus nutrient solution.
5. Five mg Mo/kg of spoil/soil plus nutrient solution.

Two kg of each treated spoil/soil sample were placed in plastic pots and each treatment was replicated four times. Two days after the first watering, three replications of each treatment were planted with alfalfa and crested wheatgrass. One replication was maintained, without plants, for analyzing of available Mo by the different chemical extractants. The spoil/soils were brought to field capacity with distilled-deionized water and covered with filter paper. They were kept near field capacity by daily addition of water to bring the pots to a prerecorded weight during the entire growing period. During the third and sixth week of plant growth, 100 mL of Hoagland solution was added to each nutrient treated pot. Two months after planting, the above ground parts of the plants were harvested, rinsed with distilled-deionized water, and dried in an oven at 70°C. The oven dry weight of plant material per pot was recorded. The plant material was ground, sieved through a 0.25-mm screen and stored in plastic bags for analyzing Mo.

TABLE 1. Composition of Hoagland's Solution Used for Green house Study.

Compound	Concentration (mg L^{-1})
KH_2PO_4	136
KNO_3	505
$Ca(NO_3)_2 \cdot 4H_2O$	1180
$MgSO_4 \cdot 7H_2O$	492
Micronutrient solution	
H_3BO_3	2.86
$MnCl_2 \cdot 4H_2O$	1.81
$ZnSO_4 \cdot 7H_2O$	0.22
$CuSO_4 \cdot 5H_2O$	0.08
$CoCl_2 \cdot 6H_2O$	0.025
Chelated iron solution	
$FeSO_4 \cdot 7H_2O$	27.8

The pH and EC were measured in 1:1 (solid:water) suspensions using an Orion combination electrode and conductivity meter, respectively. Texture of the samples was determined by the pipet method (Gee and Bauder, 1986). Organic matter and calcium carbonate equivalent were determined using the methods of Chapman and Pratt (1961) and Richards (1969), respectively. Samples were digested with lithium metaborate ($LiBO_2$ anhydrous) and analyzed for total concentration of Mo with graphite furnace atomic absorption (GFAA). Plant tissue was digested with HNO_3 using a CEM (CEM Corporation, Mathews, NC) microwave digester (Buckley and Cranston, 1971). The Mo and Cu in the plant digests were analyzed by ICP-AES. Spoils/soils from the pots with no plants were air dried after being subjected to wetting and drying cycles for two months.

The treated spoil/soil samples were extracted with AB-DTPA (Soltanpour et al., 1979), $(NH_4)_2CO_3$ at pH 9.0 (Vlek, 1977), and ammonium oxalate at pH 3.3 (Grigg, 1953). Molybdenum concentrations in the spoil/soils extracts were determined with graphite furnace atomic absorption.

In order to test the pH affect and the reproducibility of the correlation of three extractants, samples of four coal mine spoils and six soils were collected from the Dave Johnston coal mine and a nearby ranch. All of these samples were analyzed for AB-DTPA, $(NH_4)_2CO_3$, and ammonium oxalate extractable Mo.

RESULTS AND DISCUSSION

Analysis of selected properties of spoil and soils (Table 2) showed that the pH ranged from 6.95 to 8.39. The EC in Soil 3 is about 10 to 30 times higher than EC of the spoil and Soils 1and 2. The OM and calcium carbonate content of spoil and Soil 1 were considerably lower than those of Soils 2 and 3. Total Mo concentration in mine spoil/soil samples were higher than an average total Mo concentration of soil (Table 2).

Molybdenum concentration in the three extractants increased with each Mo addition (Table 3). The correlation coefficients, show a high degree of correlation between these three extractants (Table 4). While not as strongly correlated, results from tests of three extractants upon field spoil/soil samples were also significant.

Ammonium oxalate showed the greatest ability to remove Mo from the spoils/soils (Tables 3, 5, and 6). Molybdate in the soil can be adsorbed by various Fe and Al compounds. Ammonium oxalate can dissolve many of these Fe and Al compounds, releasing adsorbed Mo, and making it available for chelation with the oxalate anion. The AB-DTPA and $(NH_4)_2CO_3$ extractants did not show consistent differences in their ability to extract Mo from spoils/soils. It is likely that the same form of Mo was extracted by both AB-DTPA and $(NH_4)_2CO_3$ extractants.

pH is a factor that may affect the availability of Mo. pH of the spoils/soils did not correlate significantly with the extractable Mo as determined by any of the chemical reagents (Table 7). An increase in extractable Mo with increasing soil pH would be expected based on previous work in which the activity of molybdate was shown to increase in the water extract of soils after amendment with CaO (Follett and Barber, 1967). Sultana and Rahman (1982) also reported that the extractability of Mo increased with an increase in pH. However, ammonium oxalate often extracts more Mo as pH decreases due to release of Fe-bound Mo which is not

TABLE 2. Selected Properties of Spoil and Soils Used in Mo Extraction Study.

Sample	Texture	pH	EC	OM	$CaCO_3$ EQ.	Mo
			ds m^{-1}	----------------	g kg^{-1} ----------------	
Spoil 1	Sandy loam	8.39	0.19	3.90	7.80	5.01
Soil 1	Loamy sand	6.95	0.11	9.40	8.60	4.94
Soil 2	Sandy clay	8.18	0.27	16.10	13.40	3.57
Soil 3	Sandy loam	7.88	3.34	14.40	41.60	5.14

Table 3. Plant Uptake of Mo and Extractable Mo by Three Different Methods.

Treatment†	Mo Uptake		xtractable Mo		
	Crested wheatgrass	Alfalfa	AB-DTPA	$(NH_4)_2CO_3$	OXALATE
	--------- kg^{-1}--------		------------μg kg^{-1}-----------		
		Spoil 1			
0	38.8	105.2	13	30	30
Nutrients	7.5	20.6	9	12	60
Nutrients + Mo-1	105.7	156.2	400	410	850
Nutrients + Mo-3	190.8	374.2	2190	2320	2450
Nutrients + Mo-5	334.2	625.4	4150	4010	4500
		Soil 1			
0	42.7	6.5	10	20	100
Nutrients	12.0	6.9	13	27	120
Nutrients + Mo-1	43.76	92.6	430	390	700
Nutrients + Mo-3	117.1	348.7	2350	2430	2700
Nutrients + Mo-5	176.0	575.0	4607	4560	4800
		Soil 2			
0	20.7	14.4	3	15	320
Nutrients	5.1	12.9	3	15	300
Nutrients + Mo-1	34.8	110.0	440	1020	1250
Nutrients + Mo-3	139.9	357.6	2340	2980	3100
Nutrients + Mo-5	251.6	593.9	4700	4620	4890
		Soil 3‡			
0	6.1	-----	50	162	600
Nutrients	2.0	-----	73	120	600
Nutrients + Mo-1	23.4	-----	660	570	1600
Nutrients + Mo-3	39.7	-----	2360	2900	2900
Nutrients + Mo-5	65.2	-----	4590	4790	4900

†0, Nutrients, Mo-1, Mo-3, and Mo-5, are controk, Hoagland's nutrient solution and 1, 3, 5 mg Mo kg^{-1} spoil/soils, respectively.

‡Alfalfa did not survive in soil 3 due to high salt concentration.

TABLE 4. Regressions Between Mo-contents in AB-DTPA, $(NH_4)_2CO_3$ and Ammonium Oxalate Extracts from Greenhouse Study Samples.

Y	X	Number of observation n	Correlation coefficient r	Regression
AB-DTPA	Oxalate	20	0.98**	$y = -344.4 + 0.98X$
AB-DTPA	$(NH_4)_2CO_3$	20	0.99**	$y = -69.72 + 0.98S$
$(NH_4)CO_3$	Oxalate	20	0.99**	$y = -268.0 + 1.00X$

**Relationship between two extractants is significant at 1% level.

TABLE 5. Extractable Mo as Determined by Three Methods in Spoils/Soils from Dave Johnston Coal Mine, Wyoming.

Samples	pH	Extractable Mo		
		AB-DTPA	$(NH_4)_2CO_3$	Oxalate
		------------------μg kg-1------------------		
Spoil 1	7.87	82.3	80.0	371.3
Spoil 2	7.84	70.0	61.2	291.6
Spoil 3	7.13	120.1	108.7	398
Spoil 4	5.31	82.7	113.2	377.2
Soil 1	8.44	81.7	71.6	376.2
Soil 2	8.17	82.5	75.4	277.5
Soil 3	8.15	57.4	43.9	299.5
Soil 4	8.12	74.0	80.0	314.8
Soil 5	7.86	46.6	57.9	241.8
Soil 6	6.43	69.7	54.9	259

TABLE 6. Regressions Between Mo-contents in AB-DTPA, $(NH_4)_2CO_3$ and Ammonium Oxalate Extracts of Spoils/Soils from Dave Johnston Coal Mine, Wyoming.

Extractants Y	 X	Number of observations n	Correlation coefficient r	Regression equations
AB-DTPA	Oxalate	10	0.76*	Y = -8.22 + 0.26X
AB-DTPA	$(NH_4)_2CO_3$	10	0.78**	y = 26.39 + 0.68X
$(NH_4)CO_3$	Oxalate	10	0.75*	Y = -21.77 + 0.30X

* Relationship between two extractants is significant at 5% level.
**Relationship between two extractants is significant at 1% level.

Table 7. Regressions of pH vs. Extractable Mo in Spoils/Soils from Dave Johnston, Wyoming.

Extractants	Number of Observations n	Correlation Coefficient r	Regression equations
AB-DTPA	10	-0.22	Y = 8.40 - 11.33(pH)
$(NH_4)_2CO_3$	10	-0.56	Y = 8.41 - 14.09(pH)
Oxalate	10	0.20	Y = 408.5 - 11.65(pH)

available to plants (Karimian and Cox, 1978). Other studies have also failed to find significant differences in the extractable Mo with changes in soil pH (Gupta, 1970; Vlek, 1975; Pierzynski, 1986). In acid soils, the $(NH_4)_2CO_3$ soil test has been found to overestimate the amount of Mo available to plants (Vlek, 1975).

Correlation coefficients from simple regression of plant uptake Mo and extractable Mo indicated that the relationships of plant uptake Mo to extractable Mo by each reagent were all significant at 1% level (Figures 1 and 2). None of the three extractants was clearly better or worse than the others. Similar correlations between plant uptake Mo and extractable Mo, have also been reported by Reisenauer (1967), Pierzynski (1986), and Vlek and Lindsay (1977). The relationship between plant uptake Mo and extractable Mo suggests that all three reagents can be used to assess Mo availability. However, the value of these three extractants as an index of Mo availability and toxicity cannot be determined on the basis of results for only two plant species in this greenhouse study. Further testing is needed, because Mo uptake is species specific. Considerable evidence indicates that legumes accumulate more Mo than grasses growing in the same field or in solution culture (Allaway, 1977). In addition, Mo accumulation is dependent on

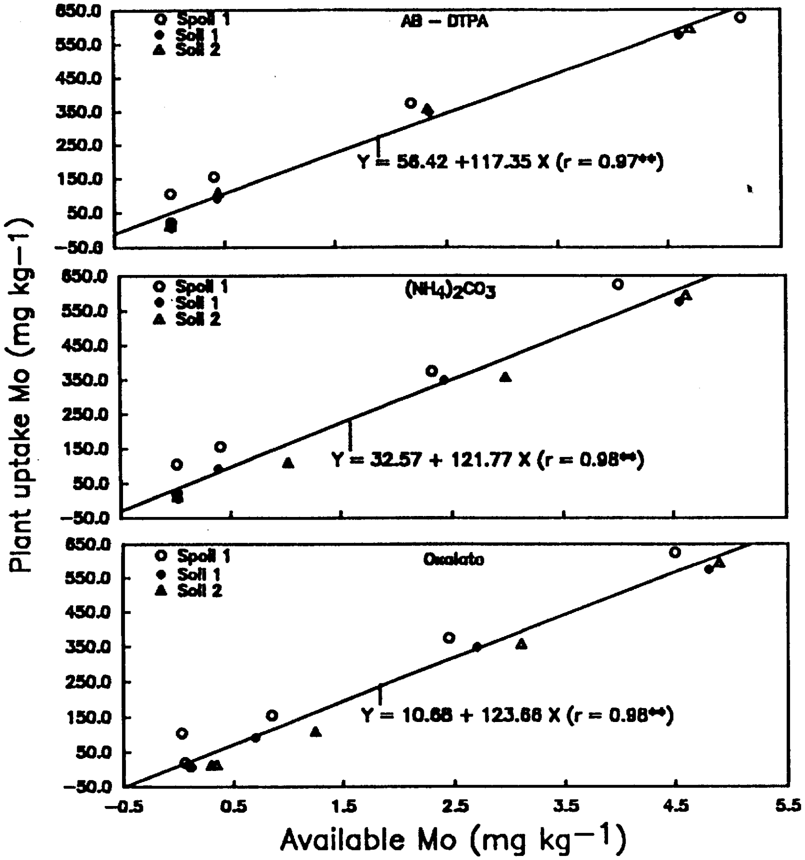

FIGURE 1. Molybdenum uptake by alfalfa as a function of extractable Mo in spoils and soils.

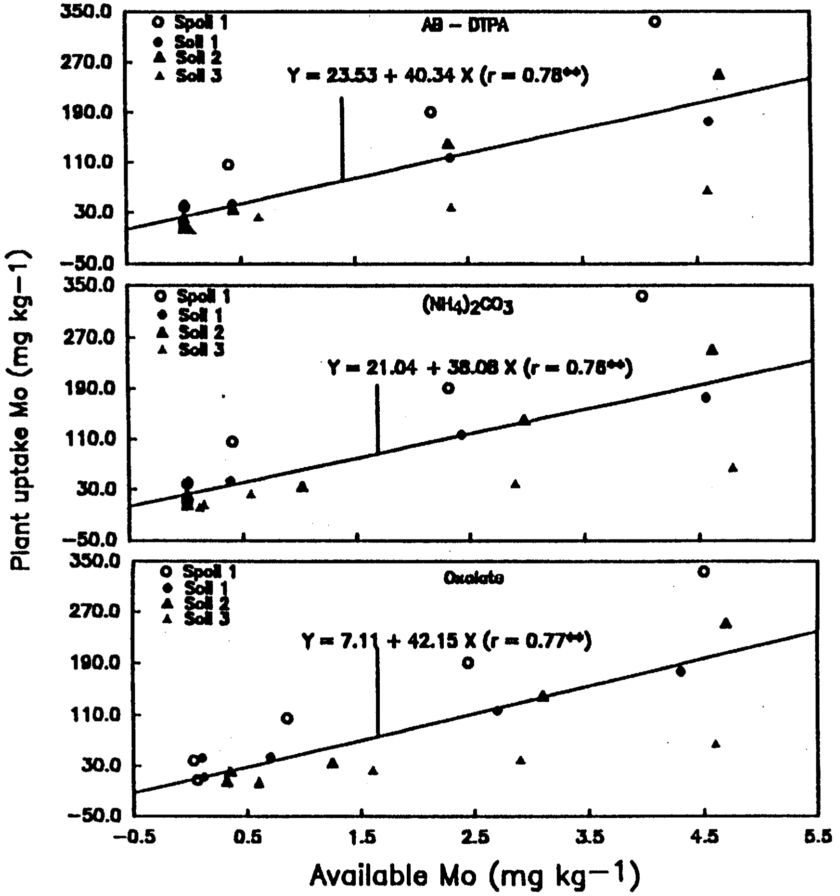

FIGURE 2. Molybdenum uptake by crested wheatgrass as a function of extractable Mo in spoils and soils.

water regimes. It was found that Mo accumulation by alfalfa in the field was significantly lower than the rate of accumulation in the greenhouse (Neuman et al., 1987). This difference was attributed to differences in water regimes. The spoil/soils in this greenhouse study were watered daily, and uptake of Mo is known to increase with greater water availability. Water availability in the field in a dry climate, such as Wyoming's, will be much lower. Thus, a field test of these methods to determine their suitability as test of Mo availability and use as a toxicity index is necessary.

CONCLUSIONS

The results obtained by the three extractants are highly correlated. The ammonium oxalate reagent extracts the greatest amount of Mo from spoil/soil samples since it dissolves some adsorbed Mo from Fe-oxide and Al-oxide. Significant correlations were found between plant uptake of Mo and extractable Mo in the greenhouse. The results suggest that all three reagent can be used to assess Mo availability. Further studies are needed to correlate plant uptake of Mo and extractable Mo in a field setting, using additional plant species.

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