HEAVY METAL CONCENTRATION IN FORAGE GRASSES AND EXTRACTABILITY FROM SOME ACID MINE SPOILS

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Abstract. Laboratory and greenhouse studies were conducted on several forage grasses, bermudagrass (*Cynodon dactylon*), creeping red fescue (*Festuca rubra*), Kentucky 31-tall fescue (*Festuca arundinacea*), oat (*Avena sativa*), orchardgrass (*Dactylis glomerata*), perennial ryegrass (*Lolium perenne*), sorghum (*Sorghum bicolor*), triticale (*X. triticosecale Wittmack*), and winter wheat (*Triticum aestivum*) grown on three Alabama acid mine spoils to study heavy metal accumulation, dry matter yield and spoil metal extractability by three chemical extractants (Mehlich 1, DTPA, and 0.1 M HCl). Heavy metals removed by these extractants were correlated with their accumulation by several forage grasses. Among the forages tested, creeping red fescue did not survive the stressful conditions of any of the spoils, while orchard grass and Kentucky 31-tall fescue did not grow in Mulberry spoil. Sorghum followed by bermudagrass generally produced the highest dry matter yield. However, the high yielding bermudagrass was most effective in accumulating high tissue levels of Mn and Zn from all spoils (compared to the other grasses) but did not remove Ni. On the average, higher levels of metals were extracted from spoils in the order of 0.1 M HCl $>$ Mehlich 1 $>$ DTPA. However, DTPA extracted all the metals from spoils while Mehlich 1 did not extract Pb and 0.1 M HCl did not extract detectable levels of Ni. All of the extractants were quite effective in determining plant available Zn from the spoils. For the other metals, the effective determination of plant availability depended on the crop, the extractant, and the metal in concert.

1. Introduction

Each year large areas in the southeastern USA (in Alabama alone more than 34 000 ha have been permitted for surface coal mining during 1982–1988) are drastically disturbed by strip-surface mining for coal, limiting land revegetation due to high acidity, toxic seepage and heavy metals toxicity. The destruction of soil chemical and physical properties may result in a permanent reduction in soil productivity (Toy and Shay, 1987; Seaker and Sopper, 1988). Some of these mine spoils have no profile development and contain a mixture of broken parent rock material, S bearing pyritic coal fragments, shales, sandstones, and original soil. (Lacey and Lawson, 1970; Ivarson *et al.*, 1982). Oxidation of the pyritic materials after removal of surface substances results in the development of acidic conditions which may create heavy metal toxicity in spoils (Evangelou *et al.*, 1985; Taylor and Shuman, 1988; Pietz *et al.*, 1989). Heavy metals are potential pollutants for surface and ground water and also prevent natural revegetation of the mined land. Nevertheless, some researchers are using acid tolerant plants to remove heavy metals from the spoils while revegetating the sites. (Fleming *et al.*, 1974; Armiger *et al.*, 1976; Barnhished, 1977).

Several extractants have been proposed for heavy metals determination, but many

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of them are so condition-specific that blanket adoption of them without proper assessment may not be advisable (Singh and Narwal, 1984). Also, knowledge gained from previous studies may or may not be valid under different soil metal concentrations and pH. The objectives of this study were: (1) to identify cover crop forage grasses suitable for reclamation of mined sites based on their tolerance to acid infertility conditions; and (2) to identify chemical extractants suitable for determining plant availability of heavy metals in the spoils.

2. Material and Methods

Spoil samples for the greenhouse study were collected from three North Alabama, USA, strip-coal mine sites, Mulberry Fork gage, Walker County; West Prong gage, Winston County; and Van Zandt Hollow gage, Etowah County. The spoil samples were air dried, mixed, passed through a 2 mm mesh stainless steel sieve, and initially analyzed for pH, available nutrients, and some heavy metal concentrations. The following extraction procedures were used for elemental determinations of spoil material: (1) Mehlich 1 solution - A 1:4 soil/solution ratio was used to extract soil soluble Ca, Mg, K, P, and Heavy metals Zn, Fe, Cu, Mn, Pb, and Ni. The extraction solution was made of 0.05M HCl and 0.025M H_2SO_4 (Page *et al.*, 1982). (2) DTPA (diethylenetriamine pentaacetic acid) - A 1:2 soil/solution ratio was used to extract available heavy metals, Zn, Fe, Cu, Mn, Pb, and Ni. The DTPA solution contained 0.005M DTPA, 0.01M $CaCl_2$, and 0.1M TEA adjusted to pH 7.3 (Lindsay and Norvell, 1978). (3) 0.1M HCl - A 1:10 soil/solution ratio was used to extract available Zn, Fe, Cu, Mn, Pb, and Ni (Page *et al.*, 1982). (4) 1M KCl - A 1:10 soil/solution ratio was used to extract exchangeable Al (Page *et al.*, 1982). All the metals, in both plant tissue and spoil were determined by atomic absorption spectroscopy. Phosphorus was determined by the Murphy-Riley (1962) method and total N by micro-Kjeldahl digestion (Bremner and Molvaney, 1982).

Greenhouse experiment was conducted using a randomized complete block design with 9 treatment applications of cover crop forage grasses, bermudagrass (*Cynodon dactylon*), creeping red fescue (*Festuca rubra* L.), Kentucky 31-tall fescue (*Festuca arundinacea*), oat (*Avena sativa*), orchardgrass (*Dactylis glomerata*), perennial ryegrass (*Lolium perenne*), sorghum (*Sorghum bicolor*), triticale (*X. Triticosecale Wittmack*), and winter wheat (*Triticum aestivum*) which were grown on the spoils. The treatments were replicated four times for each spoil type. Each pot containing 11 kg of spoil was fertilized according to spoil test recommended levels of 336 kg N ha^{-1}, 448 kg P ha^{-1}, 564 kg K ha^{-1}, 461 kg Ca ha^{-1}, and 224 kg Mg ha^{-1} as amendments to enhance growth of the plant species. All the forage grasses were harvested at maturity (83 days) and the harvested plant shoots were oven-dried at 65 °C for 48 h, weighed for dry matter yield, ground in a stainless steel Wiley mill, and stored in glass vials prior to analysis. Plant tissue levels of metals were determined by H_2SO_4-H_2O_2 digestion procedure (Allen *et al.*, 1974).

After harvest, spoil cores were taken from each pot, air-dried, mixed, sieved (to pass through a 2 mm mesh stainless steel sieve) and analyzed for heavy metal concentrations. Heavy metal accumulation and concentrations in the plant and spoil samples were statistically evaluated by analysis of variance for randomized complete block design. Duncan's Multiple Range test was applied to treatment means at 0.05 probability level. The correlation coefficient between metals extracted by each extractant and the accumulation of these metals in the shoot of grasses was determined.

3. Results and Discussion

Initial chemical analyses of the three spoils are presented in Table I. West Prong spoil had higher pH, Ca, Mg, K, and P than the others. Van Zandt followed by Mulberry had the highest Al content. All three spoils were very low in N (<0.05 g kg^{-1}) while Van Zandt did not have detectable levels of P. Table II shows the intitial heavy metal concentrations of the spoils as measured by three extractants. Metal extractability from the spoils generally followed the pattern of 0.1 M HCl $>$ Mehlich 1 $>$ DTPA. The exceptions were Fe in Mulberry spoil, Mn and Zn in Van Zandt spoil. Mehlich 1 did not extract Pb and 0.1M HCl did not extract Ni from any of the spoils, while DTPA extracted all the metals from all three spoils in smaller quantities. Norvell (1984) reported that 0.1 M HCl extractant removed greater quantities of most of the metals compared with other extractants which is generally in agreement with our results.

3.1. Dry matter yield and heavy metal accumulation

Among the forage grasses investigated, creeping red fescue in all spoils and orchardgrass and Kentucky 31-tall fescue in Mulberry spoil did not survive the stressfull conditions of the spoils. Dry matter production of different grass species

TABLE I

Initial chemical analyses for pH, Al and available nutrient concentrations in three Central Alabama strip-coal mine spoils

Spoil type	Extractant	pH	Ca	Mg	K	P	Al[a]	N[b]
			mg kg^{-1}					g kg^{-1}
Mulberry Spoil	Mehlich 1	3.86	93.3	162.7	120.0	0.92	241.9	0.02
West Prong Spoil	Mehlich 1	4.92	378.0	435.0	172.0	8.40	66.0	0.02
Van Zandt Spoil	Mehlich 1	4.06	41.9	231.4	152.0	-	518.6	0.01

[a] Aluminium – was extracted by 1M KCl.

[b] Nitrogen was determined by micro-Kjeldahl procedure.

TABLE II

Inital heavy metal concentrations of selected Central Alabama strip-coil mine spoils

Spoil type	Extractant	Mn	Fe	Zn	Pb	Cu	Ni
		mg kg^{-1}					
Mulberry Spoil	Mehlich 1	66.0	128.0	37.20	–	16.6	2.8
	DTPA	106.0	187.0	26.60	1.4	7.7	2.3
	0.1M HCl	121.0	162.0	33.0	44.0	22.5	–
West Prong Spoil	Mehlich 1	104.0	168.0	19.20	–	15.4	4.2
	DTPA	97.0	92.0	10.20	1.5	10.8	3.5
	0.1M HCl	145.0	200.0	29.00	63.0	25.0	–
Van Zandt Spoil	Mehlich 1	52.0	49.0	44.40	–	21.0	4.2
	DTPA	62.0	31.0	36.00	3.9	12.0	3.6
	0.1M HCl	66.0	210.0	41.00	149.0	22.5	–

TABLE III

Dry matter and metal concentration in forage shoot tissue harvested from Mulberry Fork gage spoil

Plant Species	Total[a] dry matter g pot^{-1}	Al	Mn	Fe	Zn	Pb	Cu	Ni
				mg kg^{-1}				
Bermudagrass	49.7a[b]	138.1b	701.3a	92.7cd	48.1a	–	23.1b	–
Sorghum	53.0a	99.4b	134.1d	62.5d	26.9b	–	28.3ab	–
Oat	9.2d	165.0b	360.6c	111.6bcd	45.9a	11.8c	20.3b	0.22a
Triticale	12.4cd	157.8b	498.4b	162.8ab	32.5b	153.3b	35.6a	–
Winter wheat	17.4bc	158.8b	416.3bc	137.5bc	30.0b	226.5a	36.2a	0.19a
P. ryegrass	18.1b	422.8a	360.0c	213.4a	32.8b	165.6b	25.0b	–
R. Fescue	0.0							
K-31 Tall Fescue	0.0							
Orchardgrass	0.0							

[a] Average of four replicates, respectively.
[b] Means followed by the same letter within a column are not significantly different (DMRT, $P<0.05$).
– Metals not detected.

grown on the spoils was quite different. However, the highest dry matter yields were generally obtained from sorghum followed by bermudagrass except for Van Zandt spoil (Tables III, IV, and V). Significant yield reduction occurred in plants that accumulated largest concentration of Al and showed visual Al toxicity symptoms compared to those grasses which grew well and did not show toxicity symptoms (i.e. bermudagrass). Toxicity of Al, Mn and other metals in acid soils is well documented (Kamprath and Toy, 1971). For other metals, asccumulation was determined by forage grass species and spoil types.

TABLE IV

Dry matter and metal concentration in shoot tissue harvested from West Prong gage spoil

Plant Species	Total dry matter[a] g pot^{-1}	Al	Mn	Fe	Zn	Pb	Cu	Ni
				mg kg^{-1}				
Bermudagrass	56.6a[b]	88.8d	231.7ab	79.1c	22.5b	138.2a	21.6cd	–
Sorghum	102.0a	74.4d	40.3d	29.7d	8.8d	–	14.4e	–
Oat	20.1d	184.8b	181.3c	89.4c	24.4ab	24.4c	17.8de	1.04a
Triticale	28.7d	165.3c	247.6a	104.6bc	15.6c	–	26.3c	–
Winter wheat	43.2c	670.5a	261.9a	350.9a	27.5a	43.8b	53.8a	0.88a
Orchardgrass	20.4d	160.3c	248.4a	103.4bc	23.1b	20.7c	34.7b	–
K-312 tall fescue	19.9d	205.4c	204.1bc	119.6bc	14.4c	–	17.9de	–
P. ryegrass	21.9d	312.5b	231.6ab	149.4b	21.9b	–	22.5cd	–
R. Fescue	0.0							

[a] Mean of four replicates, respectively.
[b] Means followed by the same letter within a column are not significantly different (DMRT, $P<0.05$).

TABLE V

Dry matter and metal concentration in forage shoot tissue harvested from Van Zandt Hollow gage spoil

Plant Species	Total dry matter[a] g pot^{-1}	Al	Mn	Fe	Zn	Pb	Cu	Ni
				mg kg^{-1}				
Bermudagrass	50.9a[b]	72.8d	536.3a	62.7de	72.2b	32.1c	20.9cde	–
Sorghum	29.5b	108.1d	80.0e	52.8e	36.6cd	–	17.2def	0.84c
Oat	10.1d	205.9bc	190.3c	103.4bc	79.7ab	204.6a	13.7ef	1.60ab
Triticale	21.4c	178.4c	312.5c	109.4bc	43.1c	–	36.9a	0.22d
Winter wheat	13.0d	228.1bc	175.3d	89.4cd	21.9d	77.0b	22.5cd	–
Orchardgrass	20.7c	209.1bc	288.4c	84.4cd	65.6b	69.2b	33.1ab	2.08a
K-312 tall fescue	13.2d	257.1b	332.5c	119.6b	71.9b	36.5c	11.9f	1.38bc
P. ryegrass	11.4d	507.8a	398.4b	198.4a	96.3a	–	28.1bc	1.33bc
R. Fescue	0.0							

[a] Mean of four replicates, respectively.
[b] Means followed by the same letter within a column are not significantly different (DMRT, $P<0.05$).

3.2. Mulberry Spoil

Sorghum followed by bermudagrass, which had the highest dry matter yield, generally had the lowest tissue concentrations of Al in Mulberry spoil (Table III). However, while perennial ryegrass had significantly the highest concentration of Al, there were not significant differences among other crops with regard to Al concentration in their shoots. According to Kabata-Pendias and Pendias (1985), the range of toxicity levels for some metals in mature leaf tissue for various species are as follow: Mn 300 to 500, Zn 100 to 400, Pb 30 to 300, Cu 20 to 100, and Ni 10 to 100 (mg kg^{-1} DW). Jones *et al.* (1991) also reported the following ranges as high level of Mn, Zn and Cu for most of the grasses included in our study:

	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
Sorghum[a]	>150	>60	>15
Oat[a]	>100	>70	>25
Winter Wheat	201–350	71–150	51–70
P. Ryegrass	>60	>20	>8
Orchard grass	>150	>50	>5
Bermuda grass[a]	>300	>50	>25

[a] Above ground portion.

Based on the above, with the exception of sorghum, all grasses accumulated Mn in what may be toxic range, notably bermudagrass which produced second highest dry matter yield. This was also the case for Pb in triticale, winter wheat, and ryegrass. Copper concentrations in bermudagrass, sorghum and perennial ryegrass was in the high range. Based on dry matter production, crops were ranked as follows: Sorghum > bermudagrass > ryegrass > winter wheat > triticale > oat. Bermudagrass had the highest concentrations of Mn and Zn while winter wheat had the highest Pb and Cu tissue concentrations. Bermudagrass and sorghum did not remove Pb and Ni while triticale and ryegrass did not have detectable tissue levels of Ni (Table III). Creeping red fescue, Kentucky-31 tall fescue and orchardgrass were unable to survive the stressful conditions of the Mulberry spoil and did not grow.

3.3. West Prong Spoil

Sorghum followed by bermudagrass had the highest dry matter yield and lowest tissue concentrations of Al in West Prong spoil. The same results were found for Mulberry spoil. Winter wheat, which produced the third highest yield among the grasses, had the greatest tissue concentrations of Al, Mn, Fe, Zn, and Cu. Bermudagrass and winter wheat accumulated Pb in what may be the toxic range (Kabata-Pendias and Pendias, 1985) while producing the second and third highest dry matter yield. All grasses except sorghum, oat, and tall fescue accumulated high levels of Cu in shoot tissue. Among the grasses, only oat and winter wheat had detectable tissues levels of Ni, as was the case for Mulberry spoil. Bermudagrass accumulated the largest amount of Pb. Similar to Mulberry spoil, only oat and winter wheat had detectable tissue levels of all the metals in West Prong spoil. Triticale, tall fescue, and ryegrass did not have detectable amounts of Pb in tissues (Table IV). With regard to the dry matter production, crops were ranked as follows: Sorghum > bermudagrass > winter wheat > ryegrass > orchardgrass > oat > tall fescue. Dry matter yields of the grasses were greatest for West Prong spoil. This was probably due to the higher pH, Ca, Mg, K, P and lower Al levels of this spoil compared to the others (Table I). However creeping red fescue was the only grass that did not grow in this or any of the coal mined spoils.

3.4. Van Zandt spoil

Similar to Mulberry and West Prong spoils, bermudagrass and sorghum had the lowest concentration of Al while producing the greatest dry matter yield in Van Zandt spoil. Sorghum dry matter yield was significantly lower in this spoil (29.5 mg kg^{-1}) than the others (53.0 and 102.0 mg kg^{-1} for Mulberry and West Prong spoils, respectively). The reason for this is not apparent from our data (Table V). Ryegrass had the highest tissue concentrations of Al, Fe, and Zn. Triticale had significantly the highest tissue levels of Cu, and orchardgrass the highest tissue levels of Ni, while Mn concentration was the highest in bermudagrass. All grasses had high levels of Cu in their tissue except for sorghum, oat, and tall fescue. Bermudagrass followed by ryegrass, tall fescue, and triticale had tissue concentrations of Mn which may be in toxic range (Pendias and Pendias, 1985; Jones *et al.*, 1991). Oat shoot contained significantly the highest concentration of Pb, followed by winter wheat, orchardgrass, tall fescue, and bermudagrass which had high levels of Pb (Table V) while ryegrass and triticale did not contain any detectable level of Pb. Bermudagrass and winter wheat shoot did not contain any detectable amount of Ni.

Table VI presents the correlation coefficients (r) obtained between metals extracted by different extractant and total metals concentration in the forage grasses from the three spoils. Chemically-extracted available Zn by all three extractants showed highly significant positive correlation with Zn accumulated by forage grasses except for winter wheat which showed a negative correlation. It is not known why winter wheat behaved differently than other crops. Tall fescue's Mn content showed a highly significant negative correlation (− 0.91) with Mn extracted by all extractants, while the Pb content had highly significant positive correlation only with Pb extracted by 0.1 M HCl. Fe levels in bermudagrass and triticale shoot showed significant and highly significant correlation with the Fe extracted by DTPA. The quantity of Cu extracted by all extractants was significant by correlation with Cu level in triticale only while Fe extracted by Mehlich 1 showed highly significant correlation with Fe in winter wheat. Significantly high correlation was observed between Ni extracted by Mehlich 1 and Ni accumulated by oat. Orchardgrass, oat, and tall fescue showed highly significant correlation only with the Pb extracted by 0.1 M HCl. In general, the effectiveness of the extractants for removing plant available metals depended on the crop species, the spoil type and the extractant used. The data suggest that none of the extractants adequately and consistently predicted the plant availability of these metals (except Zn) in the spoils.

4. Summary and Conclusion

Generally, forage crops that produced the highest dry matter yield had the lowest

TABLE VI

Correlation coefficient (*r*) between extracted metals and total metals removed by each grass cover crop

Extractants	Correlated parameters for plant and soil	Bermudagrass	Sorghum	Oat	P. ryegrass	K-31 tall fescue	Triticale	W. wheat	Orchardgrass
Mehlich 1	Mn	-0.52	-0.60*	-0.53	-0.73**	-0.93**	-0.11	0.24	-0.25
	Fe	0.52	-0.59	-0.01	-0.19	0.03	-0.33	0.71**	0.35
	Zn	0.92**	0.96*	0.93**	0.75**	0.96**	0.90**	-0.36	0.89**
	Cu	-0.02	-0.73**	-0.60*	0.44	-0.51	0.84**	-0.86**	0.10
	Ni	-	-	0.82**	-0.14	0.23	0.58*	-0.32	0.41
DTPA	Mn	0.12	0.11	-0.12	-0.36	-.91**	0.44	0.82**	-0.26
	Fe	0.63*	0.29	0.16	0.19	0.05	0.73**	0.05	0.31
	Zn	0.92**	0.96**	0.97**	0.79**	0.95**	0.91**	-0.45	0.83**
	Pb	0.15	-	-0.16	0.37	0.64	-0.25	0.15	0.72*
	Cu	-0.23	-0.74**	-0.62*	0.45	-0.51	0.70**	-0.78**	0.26
	Ni	-	-0.17	0.38	0.14	0.51	-0.06	-0.51	0.62
0.1N HCl	Mn	0.14	-0.21	-0.05	-0.45	-0.91**	0.53	0.91**	-0.21
	Fe	-0.49	-0.08	0.01	-0.12	0.26	-0.68*	-0.87**	-0.28
	Zn	0.91**	0.85**	0.97**	0.85**	0.96**	0.88**	-0.62*	0.73
	Pb	-0.13	-	0.99**	-0.58*	0.96**	0.65*	0.49	0.89**
	Cu	0.44	-0.81**	0.19	0.26	-0.55	0.79**	-0.63*	0.50

**, * Significant at 0.01 and 0.05 probability level, respectively.

tissue concentration of Al from the spoils while the reverse was true for those that produced the lowest yields and exhibited Al toxicity based on visual observation. Sorghum followed by bermudagrass and winter wheat generally produced significantly higher dry matter yields. Orchardgrass and K-31 tall fescue did not grow on Mulberry spoil. It may be because of a very low pH coupled with relatively high Al concentration in this spoil.

Bermudagrass accumulated significantly higher tissue concentrations of Mn and Zn from spoils without apparent toxicity effects. Mn concentration in bermudagrass, triticale, and winter wheat tissue was very high in some spoils. Winter wheat had the highest tissue levels of Al, Mn, Fe, Zn, Cu and Ni from West Prong spoils with high levels of Pb and Cu for Mulberry spoils. Most of the forage crops accumulated tissue metal levels in what may be toxic ranges. Provided the dry matter yields are high they may be the right crop to use to scavenge for these metals on mine spoils. Such crops could be used to reduce toxic concentrations of these metals in spoils with time via many croppings followed by removal of crop biomass from the area.

The three extractants (Mehlich 1, DTPA, and 0.1 MHCl) used to assess available metal levels for forages grown on the mine spoils studies would be effective for Zn only. Depending on the extractant and the forage crop, available levels of Pb, Fe, and Cu may also be assessed for these mine spoils. However, one should be careful when using extractable levels of heavy metals from mine spoils as an index of metal availability, in general, to forage cover crops.

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