The Effect of Growing Native and Exotic Missouri Legumes in Mining Waste from the Tri-State Region and the Old Lead Belt of Missouri.

Administrative Report for the United States Fish and Wildlife Service

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The Effect of Growing Native and Exotic Missouri Legumes in Mining Waste from the Tri-State Region and the Old Lead Belt of Missouri.

Keith Grabner, Esther Stroh, Matthew Struckhoff, and James Fairchild

Abstract

A 28-day greenhouse experiment was conducted to compare growth characteristics, seed germination, seedling survival, and seedling biomass, of native (*Desmodium paniculatum*), exotic (*Melilotus officinalis, Vicia sativa*) legumes and a standard phytotoxicity test species (*Lactuca sativa*) grown in waste from lead/zinc mining, native soil contaminated with lead and zinc, uncontaminated soil, and silica sand. Mine waste included chat from five sites in the Tri-State Mining District in southwestern Missouri, and tailings from four sites in the Old Lead Belt located in southeastern Missouri. Seed germination rates and seedling survival for plants grown on chat from the Center Creek Chat pile, from the Tri-State Mining District were the lowest for all growing media tested. Center Creek Chat also had the highest lead and zinc concentrations of mine waste for Tri-State mine waste. Plant biomass production was affected most in plants grown on mine waste, regardless of the origin of the waste. All tested plant species produced the most biomass on uncontaminated soil followed by soil contaminated with metals; there was no difference in biomass between plants grown on chat or grown on tailings. Plants grown on contaminated soil produced 40% less biomass than plants grown on uncontaminated soil. Plants grown on mine waste from Tri-State and the Old Lead Belt produced 10% and 21% of the biomass of plants grown on uncontaminated soil, respectively. Over the 28-day period, growing media texture had an effect on plant biomass production. When soil textural variables were accounted for, plant biomass production was negatively affected by zinc concentrations in mine waste. The results of this study demonstrate that metal contamination and the textural properties of mine waste affect legume growth, but additional research is needed to further separate the effects of soil texture and metal contamination on plant growth.

I. Introduction

Lead/Zinc mining has occurred in Southern Missouri for more than 250 years. Mining has been concentrated in two regions of Missouri: the Tri-State Region of southwest Missouri and the Old Lead Belt in southeast Missouri. Mining started in these regions in the mid-1800's and mid-1700's respectively, and the mines and mills were closed in these areas by the 1970's (Schmitt and others 2007).

The primary target metals of mining in Missouri have been lead and zinc. Secondarily extracted metals include copper, cadmium, cobalt, nickel, and silver. The impacts of contamination from mine and mill waste on environmental (Industrial Economics Incorporated 2008, Schmitt and others 2006, Brumbaugh and others 2005) and human health (ATSDR 2004) have been an ongoing concern for State and Federal officials in both the Tri-State Mining District and in the Old Lead Belt. In addition to the direct impacts (tailings ponds and chat piles) evident at mining sites, contamination of natural systems can occur through a variety of methods; Metals from mine waste can enter natural systems through erosion of tailings ponds and chat piles (physical transportation of waste), wind dispersal from waste

sites and during ore transport (dust transport), and leaching into runoff and ground water. Revegetating mine waste is one method for physically stabilizing mine waste (Tordoff and others 2000). However, establishing vegetation on mine waste is difficult due to the high levels of metals, nutrient deficiencies, and the physical properties of the waste materials (Ye and others 2003, Tordoff and others 2000).

The purpose of this study was to evaluate native and non-native legume seed germination and growth on mine waste from the Tri-State and Old Lead Belt regions and soil contaminated with metals. Legumes were selected for this study because their nitrogen fixing capabilities. This faculty makes legumes an important component for revegetating mine wastes, which are typically low in nitrogen (Ye and others 2001, Bradshaw 1984). Four species were selected for study: *Desmodium paniculatum* (Panicledleaf ticktrefoil), *Melilotus officinalis* (Yellow Sweetclover), *Vicia sativa* (Garden Vetch), and *Lactuca sativa* (Lettuce variety Buttercrunch). We tested seed germination and seedling growth for each species on 1) mine and mill waste, 2) mine waste treated with a low-phosphorous nutrient solution, 3) metals-contaminated soils and 4) uncontaminated soils.

II. Methods

A. Study Design

This study was conducted at the U.S. Geological Survey's Columbia Environmental Research Center in a small greenhouse (2.8 m wide x 2.2 m high x 3.7 m long) that relied on natural ventilation and had no supplemental lighting. Twelve different growing media were collected for this green house study. Growing media consisted of mine/mill waste from nine different sites (five from Tri-State and four from the Old Lead Belt areas), soil with elevated metals concentrations collected adjacent to mine waste sites in the Tri-State region, uncontaminated soil from the Tri-State region, and silica sand (Figure 1). Treatments included growing each test species in each of the of the 12 growing media; all were watered using deionized water. Additionally, to test for differences in germination and growth between mine waste with and without added nutrients, we watered an additional set of pots of mine waste from one site with a low-phosphorus Hoaglands nutrient solution (Arnon and Hoagland 1940, Hewitt 1966). The duration of the study was 28 days (August 27-September 25, 2008). The test species were *Desmodium paniculatum, Melilotus officinalis, Vicia sativa*, and *Lactuca sativa. Desmodium paniculatum* is a legume native to both the Tri-State Region and the Old Lead Belt. *Melilotus officinalis* and *Vicia sativa* are two non-native legumes that occur in both the Tri-State Region and the Old Lead Belt. *Lactuca sativa* and *Vicia sativa* are standard test species (ASTM 2002, OCED 2006). All of the tests were conducted in four inch pots filled with growing media. Within a growing medium type, pots were randomly assigned a test species. All four test species were replicated six times within each growing medium for a total of 24 pots for each growing medium. There were a total of 13 treatments, for a total of 312 pots or experimental units. Growing media were randomly located within the greenhouse.

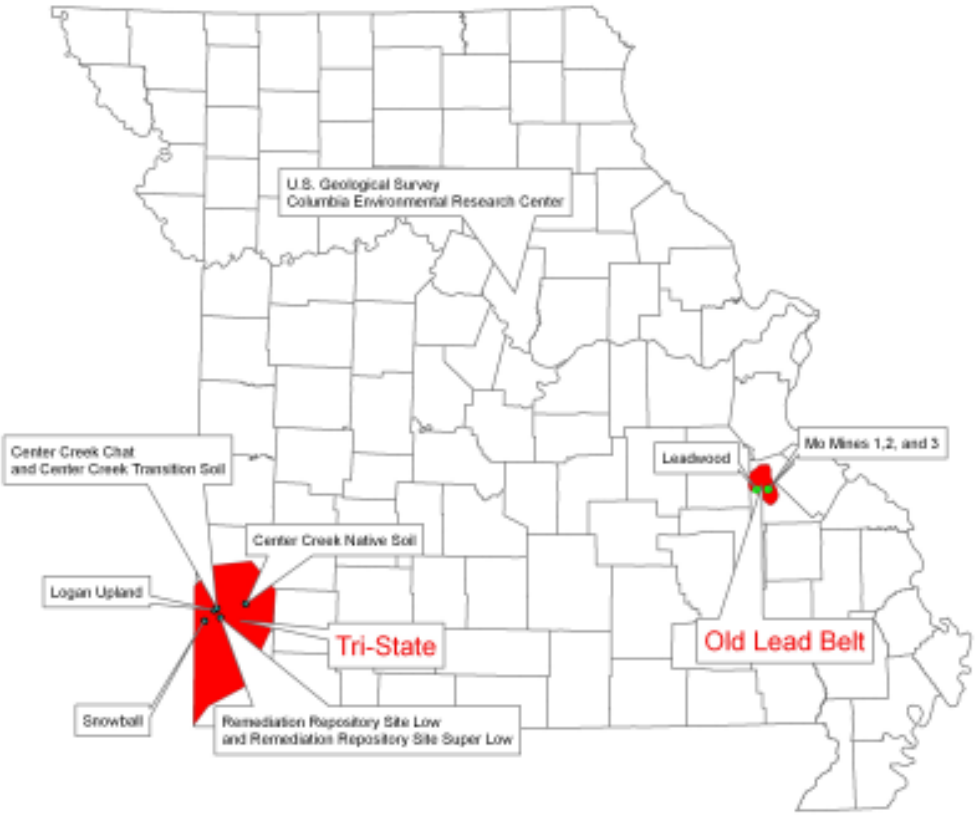

Figure 1. Map of Missouri with mining regions identified and locations where growing media were collected.

B. Growing Media Collection

Twelve different growing media were collected for this green house study. We used a Niton X-Ray Fluorescences (XRF) analyzer to measure the lead and zinc concentrations of the growing media *in situ* and to ensure that the growing media represented a wide range of lead and zinc concentrations. Growing media consisted of mine/mill waste from nine different sites (five from Tri-State and four from the Old Lead Belt areas), soil with elevated metals concentrations collected adjacent to mine waste in the Tri-State area, uncontaminated soil collected from the Tri-State area, and silica sand as a control, for a total of 12 growing media treatments (Figure 1).

U.S. Fish and Wildlife officials helped identify collection locations in the Tri-State area: Center Creek Chat Pile and Logan Upland Site (Figure 2), Remediation Repository Site (Figure 3), Snowball (Figure 4), and the Center Creek Native Soil site (Figure 5). Two growing media collected at the Center Creek Chat Pile include chat and contaminated soil. Contaminated soil was collected adjacent to a chat pile and contained elevated metals concentrations *in situ*. We collected two chat growing media from the Remediation Repository site, representing two concentrations of Zn (high and low). Mine waste growing media from the Old Lead Belt were collected from two locations. Three samples of growing

Figure 2. Growing media collection locations at the Center Creek and Logan Upland Sites in the Tri-State Mining District.

Figure 3. Growing media collection locations at the Remediation Repository Site in the Tri-State Mining District.

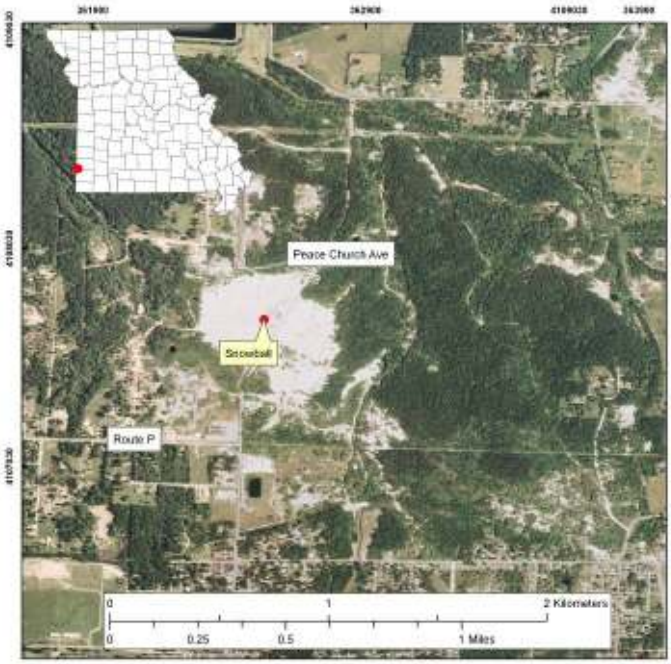

Figure 4. Growing medium collection location at the Snowball Site in the Tri-State Mining District.

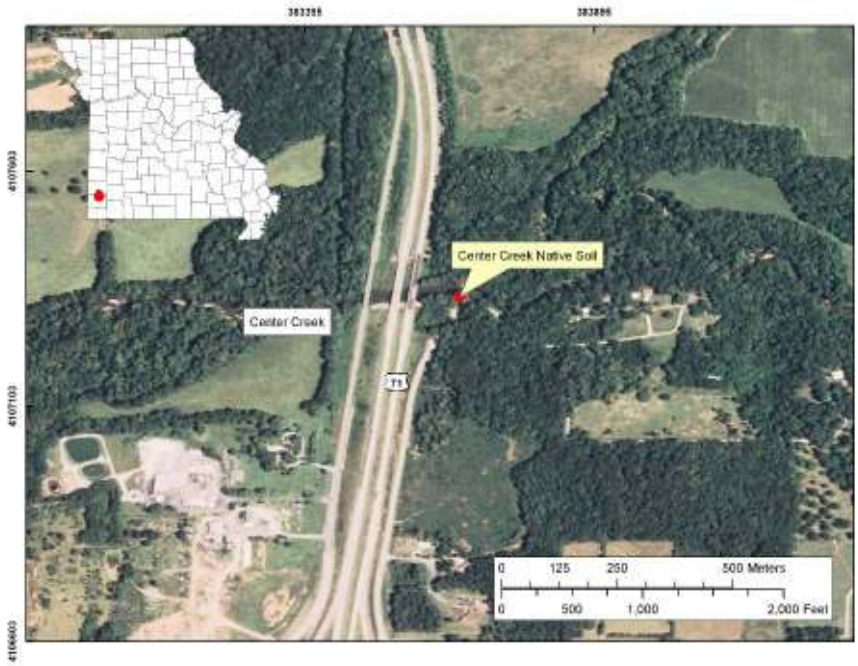

Figure 5. Growing medium collection location for the Center Creek Native Soil Site in the Tri-State Mining District.

media representing three Pb concentrations were collected at Missouri Mines State Historic Site (Figure 6). One tailings sample was collected from the Leadwood tailings pond (Figure 7). No uncontaminated native soil was collected from the Old Lead Belt. There was not enough room in the greenhouse for an additional growing medium, therefore plants grown on mine waste from the Old Lead Belt were compared to plants grown on Center Creek Native Soil. A neutral growing medium of silica sand (Silurian Filter Sand manufactured by U.S. Silica) was purchased at a local swimming pool supply store. A final growing medium was created by watering low-Zn concentration mine waste from the Remediation Repository site with a low-phosphorous Hoagland's solution (Arnon and Hoagland 1940, Hewitt 1966). The other 12 growth media were watered with deionized water and were otherwise unmodified (neither spiked with metals or organic matter nor modified texturally).

C. Greenhouse Operation

Prior to planting seeds were stratified following recommendations from the seed suppliers. *Desmodium panniculatum* and *Melilotus officinalis* were placed in moist silica sand and stored in a refrigerator for 10 days prior to planting. *Vicia sativa* seeds were soaked for 24 hours in deionized water prior. *Lactuca sativa* seeds required no stratification.

Growing media were homogenized prior to use in the greenhouse study but were not sieved or amended. Four inch pots were filled with the appropriate growing medium and 10 seeds of a randomly selected species (5 for *Vicia sativa*) were planted to a depth of approximately 0.25 cm. Each species (4) was planted in six (6) pots for each growth medium (13), for a total of 312 experimental units. Pots were placed in the greenhouse on August 27th, 2008. The greenhouse was covered with a 25% shade cloth and was vented naturally, through roof vents. Temperature at each table was monitored within the greenhouse using six Hobo® temperature and humidity sensors. Growing media treatments were randomly located in the greenhouse and were moved every three days to a new random location to account for potential temperature and light variation within the greenhouse. Plants were grown in natural light; no supplemental lighting was provided. All pots were thoroughly watered once daily with deionized water obtained using reverse osmosis (RO), except for one treatment that was watered with a low phosphorus Hoagland's nutrient solution (Arnon and Hoagland 1940, Hewitt 1966). Pots were watered from above until water began to seep out the bottom of the pots. After 10 minutes pots were watered again until water began to seep out the bottom of the pot. A representative sample of each growth medium was withheld in order to characterize the physical (particle size) and chemical (nutrient content and metals concentration) properties of each medium (see section that follows).

D. Data Collection

1. Physico-chemical Characterization of Growing Media

i. Particle Size Determination:

Growing medium particle size analysis was conducted at the Columbia Environmental Research Center using a Bouyoucos hydrometer (ASTM 2004). Air dried soil was sieved through a 2mm sieve to remove any particles larger than coarse sand, then dried at 60°C using a convection drying oven. One hundred grams of sieved dried soil was mixed with 250 mL of deionized water and 100 mL of a 50 g/L sodium hexametaphosphate solution and allowed to stand overnight. A stir bar was added and the mixture was stirred on a magnetic stirring plate. After calibration of the hydrometer, the suspended soil mixture was transferred to a sedimentation cylinder and the volume adjusted to 1000 mL with deionized water. After allowing for thermal equilibration, the temperature was recorded. Cylinder contents were

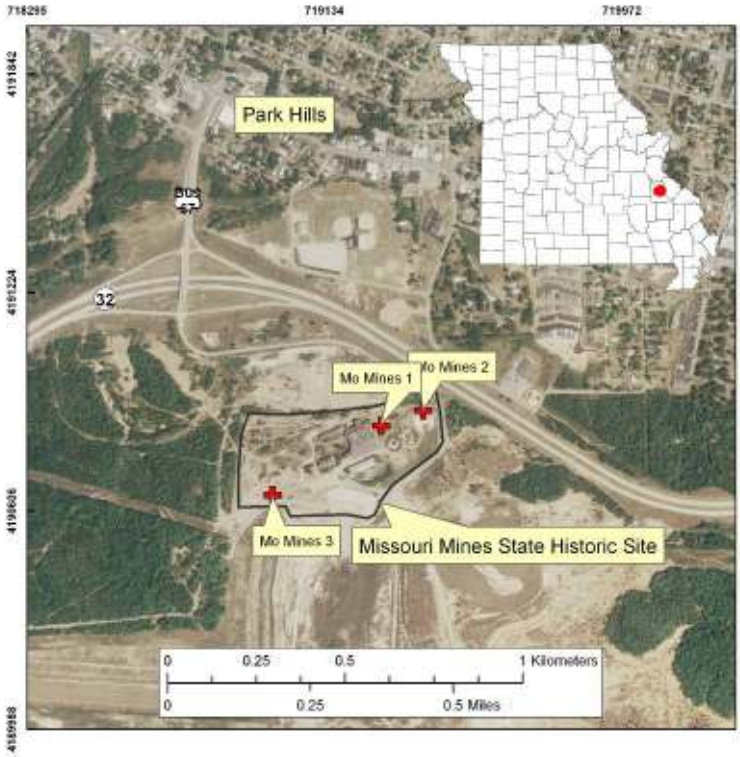

Figure 6. Growing media collection locations for the Missouri Mines Site in the Old Lead Belt.

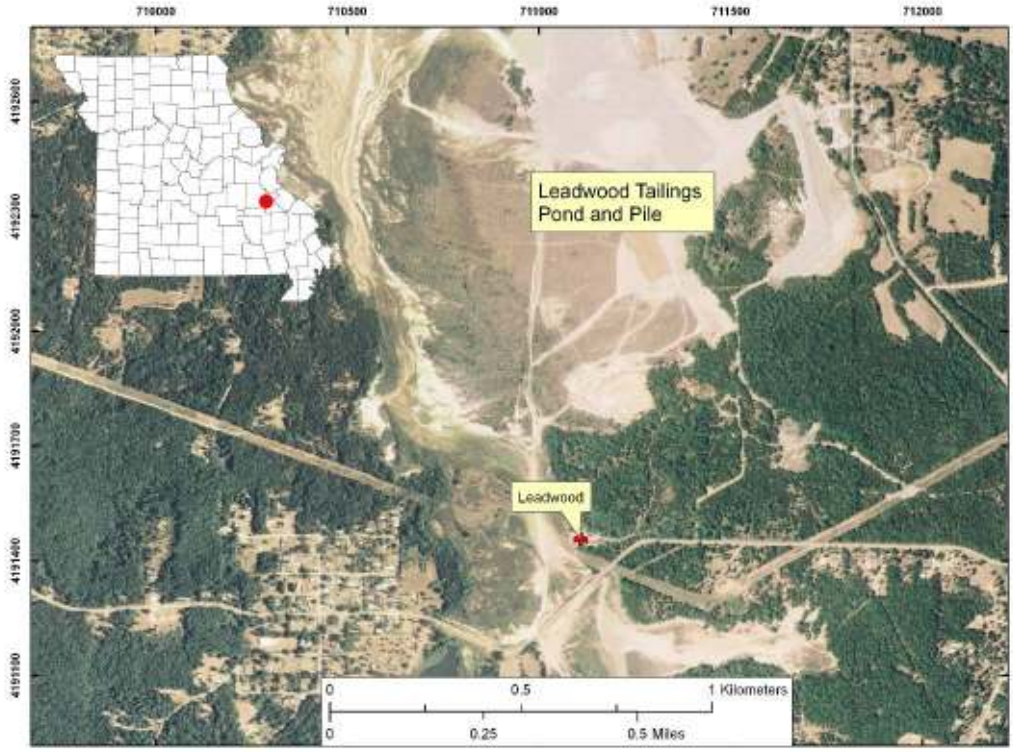

Figure 7. Growing medium collection location for the Leadwood site in the Old Lead Belt.

then thoroughly mixed followed by insertion of the hydrometer into the suspension. The meniscus reading was taken after 40 seconds. The hydrometer was removed and dried. After 120 minutes, the hydrometer was reinserted and the meniscus read again. Hydrometer meniscus readings were corrected by adjusting +0.25 percent for each degree above 18°C and -0.25 percent for each degree below 18°C. Percent fractions were determined as follows:

g sand = soil dry weight – (corrected 40 second reading – corrected calibration);
% sand = g sand/soil dry weight x 100;

g clay = soil dry weight – (corrected 120 minute reading – corrected calibration);
% clay = g clay/soil dry weight x 100;

% silt = 100 – (% sand + % clay).

ii. Nutrient Analysis

Nutrient analysis was performed at the Soil Characterization Laboratory (SCL) at the University of Missouri. The SCL follows standard soil survey laboratory analysis methods (Burt 2004).

a Cation Exchange Capacity

The Cation Exchange Capacity (CEC) was determined using a displacement after washing technique. The CEC is determined by saturating the exchange sites with an index cation (NH^{4+}); washing the soil free of excess saturated salt; displacing the index cation (NH^{4+}) adsorbed by the soil; and measuring the amount of the index cation (NH^{4+}) (Burt 2004). CEC is measured in units of milliequivalents per 100gm of soil (meq/100gm). Typical CEC ranges from 200 to 400 meg/100gm for organic matter to 2 to 6 meg/100gm for Kaolinite (Burt 2004).

b Potassium

The extract from the CEC procedure was diluted with an ionization suppressant and the extract was measured by an atomic absorption spectrophotometer for the following cations, Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}. Extracted potassium was reported as meq/100gm soil (Burt 2004).

c Phosphorus

A 2.5-g soil sample was shaken with 25 mL of Bray P-1 extracting solution for 15 min. The sample was centrifuged until solution was free of soil mineral particles, and then filtered until clear extracts were obtained. A 2 mL aliquot was diluted with 8 mL of ascorbic acid molybdate solution. Absorbance of the solution was read using a spectrophotometer at 882 nm. Data were reported as mg/kg soil (Burt 2004).

d Nitrogen

A soil sample was combusted at high temperature with oxygen to release NO_x. The gases released were scrubbed to remove interferences (e.g., CO_2 and H_2O), and the NO_x is reduced to N_2. The N_2 is measured by thermal conductivity detection and reported as percent N. The total N that was measured by the combustion method does not distinguish among the types of N that are present in the soil (Burt 2004).

e Organic Carbon

Percent organic carbon was determined through dry combustion (Burt 2004).

f pH

Twenty grams of soil were mixed with 20 mL of RO water. The water and soil were mixed for one hour. Once mixing was stopped, the pH of the water soil mixture was measured using a pH meter (Burt 2004).

iii. Metals Analysis

Metals analysis was conducted at the Columbia Environmental Research Center using a Niton XFR and an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Each of the 312 experimental units was sampled for metals concentration using a Niton XRF with a sample time of 90 seconds per pot (60 seconds on filter one and 30 seconds on filter two). XRF data were collected on unsieved growing media. Additionally, a 2-mm sieved portion of the reserved sample from each growing media was analyzed for total-recoverable (TR) zinc and lead concentrations using ICP-MS after microwave heating of a 0.25g sample with 5.5 mL nitric acid and 0.5 mL hydrochloric acid (Brumbaugh and others 2007).

2. Seed Germination Data

Pots were checked daily and the number of seedlings that emerged were recorded. We tracked seed germination, seedling survival, and seedling development daily for 28 days. Growing media treatments were moved every three days to a random location within the greenhouse to account for thermal and light intensity variation within the greenhouse.

3. Plant Biomass Data

After 28 days, all seedlings were removed from pots and prepared for biomass measurements. Seedlings were carefully removed from the growing media and the above and below ground portions of the plant were washed with RO water. The seedlings were cut with a razor blade into above- and below-ground parts at the root collar. The above- and below-ground parts were placed in paper envelops and dried in a 60°C convection drying oven. Above- and below-ground plant parts were weighed on a Mettler AT261 analytical balance (±0.00001 gm). For each pot, we calculated the mean per-plant biomass of plants that had germinated and survived for 28 days (pot biomass/number surviving plants). This was performed for above-ground and below-ground biomass. We then calculated the mean above-ground and below-ground biomass for each treatment by averaging the per-plant biomass calculations from the six pots for that treatment and species.

E. Data Analysis

Data were analyzed using analysis of variance procedures within SYSTAT v. 11 (SYSTAT Software. Inc. 2004b). Significant differences among individual treatment means were tested using Tukey-HSD tests (α=0.05; Ye and others 2002, Ye and others 2002). Metals data, XRF and ICP-MS metals concentrations, were log-transformed, an appropriate technique to make data sets with asymmetrically high values more symmetric (Moore and McCabe 1989). Univariate least squares linear regression of Mean Total Per-Plant Biomass (Mean Per-Plant Above Ground Biomass + Mean Per-Plant Below Ground Biomass) against Log transformed Pb and Zn concentrations from XRF and ICP-MS analysis, and soil textural variables of % Fine (the percent of material that passed through a 2mm sieve), % Sand, % Clay, and % Silt using SYSTAT v. 11 (SYSTAT Software, Inc. 2004a).

III. Results and Discussion

A. Greenhouse Conditions

Plots were placed in the greenhouse on August 27th, 2008 and the study was conducted for 28 days. Mean temperature in the greenhouse was 21.6^{o}C. Mean daytime temperature was 24.8^{o}C and

mean nighttime temperature was 18.4°C. There was some thermal variability within the greenhouse (Table 1), but growing media treatments were moved every three days to a random location within the greenhouse to account for thermal and light intensity variation.

Table 1. Temperature conditions within the Greenhouse during the 28-day study (August 27 - September 25, 2008).

Location in Greenhouse	Mean Temperature °C (Standard Deviation)	
	Day Time Temp	Night Time Temp
NW Corner	24.3 (6.4)	18.1 (3.5)
NE Corner	24.7 (6.5)	18.5 (3.4)
Center E	24.1 (5.9)	18.4 (3.3)
Center W	24.2 (6.1)	18.8 (3.4)
SW Corner	25.2 (7.0)	18.2 (3.6)
SE Corner	26.6 (8.3)	18.3 (3.7)

B. Physico-Chemical Properties of Growing Media

The physical properties of the growing media are summarized in Table 2. Based on particle size analysis, mine waste from the Tri-State and Old Lead Belt areas were categorized as either a sand or loamy sand. Most particles less than 2 mm were sand (0.05 to 2.0 mm), with lesser amounts of silt (0.002 to 0.05 mm) and clay (< 2 μm). Mine waste from Tri-State had a higher percentage of coarse material (>2mm) than waste from the Old Lead Belt. The Center Creek transition soil was identified as a loam and the Center Creek native soil was identified as a silt loam. Both of these soils contained a higher percentage of finer sized clay and silt particles than the mine waste (Table 2).

The amount of potassium, phosphorus, and nitrogen found in the mine waste from the Tri-State and Old Lead Belt regions were similar to values found in tailings in others studies (Ye and others 2002, Ye and others 2000, Levy and others 1999, Yang and others 1997, Sudhakar and others 1992, Day and Ludeke 1981; Table 2). The amount of phosphorus in the growing media ranged from a high of 36.7 mg/kg in Center Creek chat to a low of 0.4 mg/kg in Missouri Mines 2. By comparison, the native soil and transition soil had phosphorus values in of 8.0 mg/kg and 2.1 mg/kg respectively. The percent nitrogen in the mine waste samples ranged from 0.011% to 0.058%, which was lower than the percent nitrogen found in the native and transition soils (0.247% and 0.589%, respectively). Percent nitrogen found in typical cultivated soils ranges from 0.06% to 0.5% in the surface layers (Bremmer and Mulvaney 1982). The percent organic carbon found in mine waste samples and soil samples was within the range of values found by Ye and others (2000) in China, Day and Ludeke (1981) in Arizona, and Levy and others (1999) in the Tri-State area. Percent organic carbon was 2.6% in native soils; 8.0% in transition soils; and ranged from 0.0% to 3.7% in mine waste. Cation exchange capacity of the growing media varied with the texture of the growing media. Growing media with a higher percentage of sand had a lower CEC than growing media with a higher percentage of silt and clays. The properties of the growing media were within a range of physical and chemical properties of mine waste used in other seed germination and seedling survival studies in the United States and in other countries and are considered low in nutrients (Ye and others 2002, Ye and others 2001, Ye and others 2000, Sudhakar and others 1992).

Table 2. Particle size and nutrient analysis for growing media.

Growing Medium	# 10 Sieve (2mm)		Particle Size Distribution (<2mm)					Available Nutrient Analysis				
	% Passing	% Retained	% Sand	% Clay	% Silt	Soil Type	Organic Carbon %	K (mg/kg)	P (mg/kg)	N (%)	CEC	pH
Center Creek Chat Pile	39.8	60.2	81	3	16	Loamy Sand	0.3	<0.1	36.7	0.058	1.1	6.5
Logan Chat Pile	33.1	66.9	96	1	3	Sand	0.0	<0.1	1.9	0.013	0.7	7.5
Remediation Site Low	34.3	65.7	94	2	4	Sand	0.2	<0.1	0.7	0.033	1.4	7.0
Remediation Site Super Low	31.9	68.1	95	1	4	Sand	0.1	<0.1	11.2	0.027	1.0	6.6
Snow Ball Chat	56.5	43.5	84	3	13	Loamy Sand	0.3	<0.1	51.4	0.023	1.0	7.6
Center Creek Transition Soil	77.4	22.6	38	10	52	Loam	8.0	0.9	2.1	0.589	39.5	6.4
Center Creek Native Soil	94.3	5.6	18	8	74	Silt Loam	2.6	0.4	8.0	0.247	18.9	6.7
Missouri Mines 1	82.6	17.4	96	1	3	Sand	3.7	<0.1	5.2	0.021	0.6	7.7
Missouri Mines 2	67.9	32.1	83	4	13	Loamy Sand	0.9	<0.1	0.4	0.025	1.8	7.6
Missouri Mines 3	89.1	10.9	94	3	3	Sand	0.4	<0.1	3.4	0.011	0.7	8.0
Leadwood	99.3	0.7	85	2	13	Loamy Sand	0.8	<0.1	2.4	0.012	0.8	8.0
Silica Sand	100	0	100	0	0	Sand	0.0	<0.1	0.2	0.014	0.4	5.6

Lead and zinc concentrations determined by ICP-MS showed that the Center Creek native soil contained 27 mg/kg lead and 184 mg/kg zinc (Table 3). The transition soil contained 617 mg/kg lead and 2,440 mg/kg zinc, a 22- and 13- fold increase in metals over the native soil. Mine waste metals concentrations ranged from a high zinc ICP-MS value of 33,000 mg/kg for the growing medium from the Center Creek Chat Pile to a low of 184 mg/kg for the growing medium from Missouri Mines 1. Lead concentrations ranged from a high of 48,000 mg/kg for the growing medium from Missouri Mines 3 and a low lead value of 140 mg/kg for the growing medium from the Remediation Repository Site Low. Growing media metal concentrations measured by XRF were different from ICP-MS values for the same growing media. It is important to note that the samples that were measured using the XRF were not sieved prior to sampling while the ICP-MS samples were sieved. Center Creek Chat contained the highest metal content when sampled with XRF (3800 ppm lead and 30260 ppm zinc). Center Creek Transition Soil and Native Soil metals concentrations were similar between XRF and ICP-MS samples. Growing media from the Old Lead Belt did show some differences between XRF and ICP-MS samples (Table 3). Missouri Mines 2 had the highest XRF lead concentration (18920 ppm) which was seven times higher than the ICP-MS sample. Missouri Mines 3 XRF lead concentration was 1260 ppm which was 17 times lower than the ICP-MS reading of 48000ppm. Zinc readings were also different between XRF and ICP-MS readings. Differences between XRF and ICP-MS readings and the variability within XRF readings demonstrates how heterogeneous the metals concentrations are within the growing media.

General trends in the metals data showed that growing media from Tri-State had a higher zinc concentration than lead concentration (Table 3). One site from the Old Lead Belt, Leadwood, had a higher zinc value (5980 mg/kg) than lead (304 mg/kg). The other growing media from the Old Lead

Table 3. Origin of growing medium, water treatments, and metals concentration.

Growing Medium	Mining Region*	Watering Treatment	Mean XRF Readings† of Unsieved Growing Media (Standard deviation) Lead	Zinc	ICP-MS Readings‡ of Seived Growing Media (<2mm) Lead	Zinc
			mg/kg		mg/kg	
Center Creek Chat Pile	Tri-States	Reverse Osmosis H_20	3800 (960)	30260 (9670)	11800	33000
Logan Chat Pile	Tri-States	Reverse Osmosis H_20	120 (35)	4660 (2670)	407	18300
Remediation Site Low	Tri-States	Low-P Hoagland Solution	160 (37)	990 (300)	255	3360
Remediation Site Super Low	Tri-States	Reverse Osmosis H_20	120 (42)	340 (490)	140	10300
Snow Ball Chat	Tri-States	Reverse Osmosis H_20	290 (84)	7570 (2200)	304	5980
Tri-States Mine Waste Mean			898	8764	2581	14188
Center Creek Transition Soil	Tri-States	Reverse Osmosis H_20	440 (42)	1670 (180)	617	2440
Center Creek Native Soil	Tri-States	Reverse Osmosis H_20	22 (15)	140 (23)	27	184
Missouri Mines 1	Old Lead Belt	Reverse Osmosis H_20	2940 (830)	790 (170)	3240	184
Missouri Mines 2	Old Lead Belt	Reverse Osmosis H_20	18920 (6000)	2670 (680)	2810	530
Missouri Mines 3	Old Lead Belt	Reverse Osmosis H_20	1260 (150)	170 (38)	48000	2270
Leadwood	Old Lead Belt	Reverse Osmosis H_20	740 (250)	450 (140)	524	2480
Old Lead Belt Mine Waste Mean			5303	1020	13644	1366
Silica Sand	Old Lead Belt	Reverse Osmosis H_20	BDL*	BDL	1.41	2.02

† X-Ray Fluorescence elemental analyzer readings of growing media in plots prior to seeding
‡ Inductively Coupled Plasma Mass Spectroscopy analysis of a subsample for each growing medium.
*BDL = Below Detection Limits of XRF

Belt had higher lead values than zinc. Regardless of analytical method used (XRF or ICP-MS), mean lead concentrations in the Old Lead Belt were nearly an order of magnitude higher than concentrations in the Tri-States district. Similarly, mean zinc concentrations in the Tri-States district were about an order of magnitude higher than concentrations in the Old Lead Belt.

C. Tri-State Mining Waste

1. Seed Germination and Survival

Seed germination and survival were analyzed in two ways: 1) percent seed germination for each growing medium; and 2) percentage of germinated seeds that survived until the end of the study.

There were no significant differences in percent seed germination among Tri-State growing media for *Desmodium paniculatum* and *Lactuca sativa,* but there were significant differences for *Melilotus officinalis* and *Vicia sativa* among some Tri-State growing media (Table 4). The germination rate of *Melilotus officinalis* was significantly higher ($\alpha = 0.05$) in the silica sand (65 percent) than in mine waste from both the Center Creek Chat pile (18 percent) and the Remediation Repository Site

Super Low Chat (25 percent), which had the two lowest germination rates (Table 4). Germination for *Vicia sativa* in chat from Center Creek Chat pile (50 percent) was significantly lower than germination on chat from the Logan Creek (97 percent) and Snow Ball chat piles (100 percent; Table 4). The chat from these three sites had very different metals concentrations (Table 3), but Center Creek chat had the highest Pb and Zn concentrations of all sites and the lowest percent germination (Table 4). Others have shown seed germination success to be unaffected by chat or tailings (Ye and others 2002, Levy and others 1999, Yang and others 1999, Yang and others 1997) or metals contaminated solution (Peralta and others 2001). Påhlsson (1989) concluded from an extensive literature review that zinc and lead did not have much effect on seed germination. However, for *Melilotus officinalis* and *Vicia sativa*, our results are similar to those of Pierzynski and Fick (2007), who found that chat from Tri-State significantly affect seed germination of native grasses *Andropogon gerardii* (big bluestem) and *Panicum virgatum* (switch grass).

Correlation analysis was used to investigate if percent seed germination was correlated with soil texture or soil metals concentration. Percent seed germination did not correlate significantly with soil texture variables, all Pearson correlation coefficients for soil textural variables were less than 0.038, or soil metals concentration measured by either XRF or ICP-MS, person correlation coefficients for soil metals variables were between -0.183 to -0.245..

Plants that germinated had poorer survival on Center Creek Chat than any other growing medium (Table 4). Percent seed germination survival for *Desmodium paniculatum* on Center Creek Chat (71 percent) was significantly lower than survival rates on Logan, Center Creek Native Soil, Center Creek Transition Soil, and the Remediation Repository Site Super Low, all of which had 100 percent survival in all pots. Percent germinated seedling survival for *Lactuca sativa* and *Melilotus officinalis* was significantly lower on mine waste from Center Creek than on all other media.

In all but one instance (percent survival of germinated *Vicia sativa* seeds), germination and survival was lowest on Center Creek Chat. Center Creek Chat had the highest lead and zinc concentrations for the Tri-State mining waste, regardless of metals analysis methods used (Table 3). Zinc phytotoxicity has been previously demonstrated on chat from Tri-State (Pierzynski and Fick 2008). When the legume *Trifolium repens* and grasses *Cynodon dactylon*, *Lolium multiflorum*, and *Agropyron elongatum* were grown for four weeks on lead/zinc tailings from China, mortality was 100%; these mining waste had similar metals and nutrient concentrations to the chat we tested from Tri-State (Ye and others 2000). Center Creek Chat was low in nutrients, but there were other growing media from the Tri-State area we tested that had higher seedling survival and biomass production but had less nutrients than Center Creek Chat (Table 2).

2. Biomass Data

To compare biomass production among all growing media, we calculated mean per plant above and below ground biomass. Analyzing biomass on a per plant basis allowed us to compare the biomass produced by plants grown in different growing media that had different seed germination rates or seedling survival rates. Patterns in mean per plant biomass production, both above-ground and below-ground, was similar regardless of test species (Figure 8). For all species, mean above-ground biomass on native soils was significantly higher than on mine waste. *Desmodium paniculatum* had significantly lower above-ground biomass on transitions soils than on uncontaminated native soil. Center Creek Native Soil had significantly higher mean below-ground biomass than all other test media for all species. Center Creek Transition Soil had significantly higher below-ground biomass than all mine waste for all species, except *Vicia sativa* on Center Creek chat. Silica sand had significantly higher below-ground biomass than Center Creek Chat for *Desmodium paniculatum* and *Melilotus officinalis*

There was no significant difference between chat sources in either mean above-ground biomass or mean below-ground biomass (Figure 8, Table 4).

We were unable to determine to what extent the difference between biomass produced was due to soil type or to differences in metal concentrations for all Tri-State growing media. Correlation analysis was used to identify patterns in biomass production and physico-chemical characteristics of the growing media. Above- and below- ground per plant biomass was correlated with growing media texture, when correlation analysis was conducted on soil and mine waste growing media together. Per plant above ground biomass was negatively correlated to percent sand (-0. 739) but positively correlated with percent clay (0.686) and percent silt (0.740). Metals variables of XRF zinc (-0.257) and lead (-0.203) were not significantly correlated with above ground biomass. Per plant below ground biomass was also negatively correlated to soil textural variable percent sand (-0.670) but positively correlated with percent clay (0.573) and percent silt (0.677). Metals variables of XRF zinc (-0.250) and lead (-0.196) were not significantly correlated with total below ground biomass on soil and Tri-State mine waste. Plant biomass increased as the percentage of sand decreased and the percentage of clay and silt increased. Thus, soil texture was an important factor in per plant biomass. This analysis included all growing media and was for the Tri-State data only. Metal concentrations may have influenced per plant biomass but apparently to a lesser degree than soil texture, when all Tri-State growing media were tested. Test species consistently grew poorly on Center Creek Chat, which had the highest zinc concentration (33,000 µg/gm) in chat less than 2mm in size; however biomass was poorly correlated with XRF or ICP-MS metal concentrations.

Plants grown in native soil had significantly higher biomass than plants grown in mining waste. Contaminated transition soil and uncontaminated native soil had higher water holding capacity, nutrients, and organic matter when compared to the mining chat. There were two differences between the transition soil and the native soil: 1) the transition soil had a different particle size distribution and was classified as a loam soil compared to a silt loam for the native soil and 2) the transition soil contained higher concentrations of lead (617 mg/kg) and zinc (2440 mg/kg) compared to the native soil (lead 27 mg/kg and zinc 184 mg/kg; Tables 2 and 3). Plant biomass production was significantly greater on native soil than transition soil. On average transition soil produced 60% of the biomass that was produced on native soil.

Zinc and lead in solution have been shown to negatively affect plants grown at various concentrations. Godbold and Huttermann (1985) documented that Norway spruce (*Picea abies*) root elongation was inhibited when zinc concentrations reached 30 and 60 mg/L. Legumes tested by Carroll and Loneragan (1968) had maximum yields when grown in solution with zinc at 0.05 mg/L, but began to show signs of zinc toxicity at concentrations between 1-3 mg/L. Shoot and root length of corn and vetch grown in zinc contaminated soil (806 mg/kg and 929 mg/kg) were negatively affected (Pečiulytė et al. 2006). *Andropogon gerardii* and *Panicum virgatum* seedlings grown in mining chat with a range of zinc concentrations (5 to 100 mg/kg Zn) did not show a significant reduction in survival, biomass, and growth (Pierzynski and Fick 2007). Lead has been shown to affect radish seedling growth at 1000 mg/L and a significant reduction in total plant growth was observed for radish plants grown in lead concentrations of 500 mg/L and 1000 mg/L contaminated soil (Zaman and Zereen 1998). Hooper (1937) found no effect on French bean growth in lead contaminated nutrient solution with a range of lead from 3-30 mg/L. Corn and vetch grown in lead contaminated soil showed a negative effect on shoot and root length at concentrations of 560 and 545 mg/kg (Pečiulytė et al. 2006).

Table 4. Mean percent germination and dry weight biomass data for plants grown on Tri-State Growing Medium, standard deviation in parentheses. Different letters in the same column indicate a significant difference at $p \leq 0.05$ using Tukey-HSD test.

Growing Medium	Watering Solution	Mean % Germination	Mean % Survival	Mean Per Plant	
				Above Ground Biomass (mg)	Below Ground Biomass (mg)
		Desmodium paniculatum			
Center Creek Native Soil	RO	46.7 (19.7)a	100.0 (0.0)a	36.8 (8.4)a	12.9 (4.2)a
Center Creek Transition Soil	RO	60.0 (16.7)a	100.0 (0.0)a	17.3 (2.9)b	5.4 (0.7)b
Center Creek Chat	RO	45.0 (13.7)a	71.4 (36.2)b	7.0 (4.4)c	0.8 (0.3)c
Logan Chat	RO	63.3 (21.6)a	100.0 (0.0)a	6.8 (0.9)c	1.0 (0.3)c
Remediation Site Low	RO	60.0 (6.3)a	94.4 (8.6)ab	9.3 (1.9)c	2.2 (0.5)bc
Remediation Site Super Low	RO	68.3 (14.7)a	100.0 (0.0)a	7.7 (0.8)c	2.7 (0.6)bc
Snow Ball Chat	RO	60.0 (30.3)a	96.0 (6.1)ab	8.4 (0.8)c	2.5 (1.4)bc
Silica Sand	RO	51.7 (27.9)a	96.7 (8.2)a	7.6 (3.3)c	6.7 (3.8)b
		Lactuca sativa			
Center Creek Native Soil	RO	76.7 (15.0)a	97.2 (6.8)b	54.4 (15.7)a	25.4 (6.9)a
Center Creek Transition Soil	RO	65.0 (33.9)a	83.3 (40.8)b	40.5 (20.9)a	13.7 (7.6)b
Center Creek Chat	RO	55.0 (19.8)a	40.7 (42.0)a	0.8 (0.6)b	0.2 (0.3)c
Logan Chat	RO	68.3 (9.8)a	94.4 (8.6)b	1.6 (0.5)b	0.7 (0.3)c
Remediation Site Low	RO	65.0 (20.7)a	87.9 (10.4)b	1.7 (0.2)b	0.8 (0.2)c
Remediation Site Super Low	RO	55.0 (24.3)a	80.8 (22.4)b	0.9 (0.4)b	1.5 (1.8)c
Snow Ball Chat	RO	68.3 (7.5)a	100.0 (0.0)b	3.2 (1.0)b	1.0 (0.3)c
Silica Sand	RO	58.3 (20.4)a	97.9 (5.1)b	3.1 (1.2)b	1.7 (1.2)c
		Melilotus officinalis			
Center Creek Native Soil	RO	46.7 (26.6)ab	100.0 (0.0)b	34.2 (15.7)a	16.5 (7.6)a
Center Creek Transition Soil	RO	38.3 (23.2)ab	100.0 (0.0)b	23.8 (5.9)a	8.6 (1.9)b
Center Creek Chat	RO	18.3 (17.2)a	16.7 (40.8)a	0.2 (0.6)b	0.1 (0.2)c
Logan Chat	RO	38.3 (20.4)ab	79.2 (40.0)b	1.6 (0.8)b	0.8 (0.5)cd
Remediation Site Low	RO	31.7 (13.3)ab	89.9 (14.9)b	2.6 (0.2)b	1.7 (0.4)cd
Remediation Site Super Low	RO	25.0 (15.2)a	100.0 (0.0)b	2.1 (0.6)b	1.2 (0.3)cd
Snow Ball Chat	RO	55.0 (23.4)ab	79.5 (12.6)b	2.6 (0.6)b	1.3 (0.5)cd
Silica Sand	RO	65.0 (25.9)b	96.7 (8.2)b	2.7 (0.2)b	5.7 (2.5)bd
		Vicia sativa			
Center Creek Native Soil	RO	66.7 (32.7)ab	100.0 (0.0)a	60.8 (20.4)a	51.7 (22.9)a
Center Creek Transition Soil	RO	80.0 (25.3)ab	100.0 (0.0)a	47.7 (6.8)a	26.2 (6.4)b
Center Creek Chat	RO	50.0 (21.0)a	91.7 (20.4)a	1.3 (0.9)b	14.0 (9.6)bc
Logan Chat	RO	96.7 (8.2)b	100.0 (0.0)a	5.2 (1.5)b	6.4 (0.7)c
Remediation Site Low	RO	93.3 (10.3)ab	100.0 (0.0)a	8.6 (1.0)b	7.6 (3.1)c
Remediation Site Super Low	RO	76.7 (38.8)ab	80.0 (40.0)a	5.5 (2.8)b	5.4 (2.8)c
Snow Ball Chat	RO	100.0 (21.9)b	100.0 (0.0)a	7.0 (1.0)b	5.9 (0.8)c
Silica Sand	RO	70.0 (27.6)ab	100.0 (0.0)a	10.0 (6.5)b	13.0 (6.4)bc

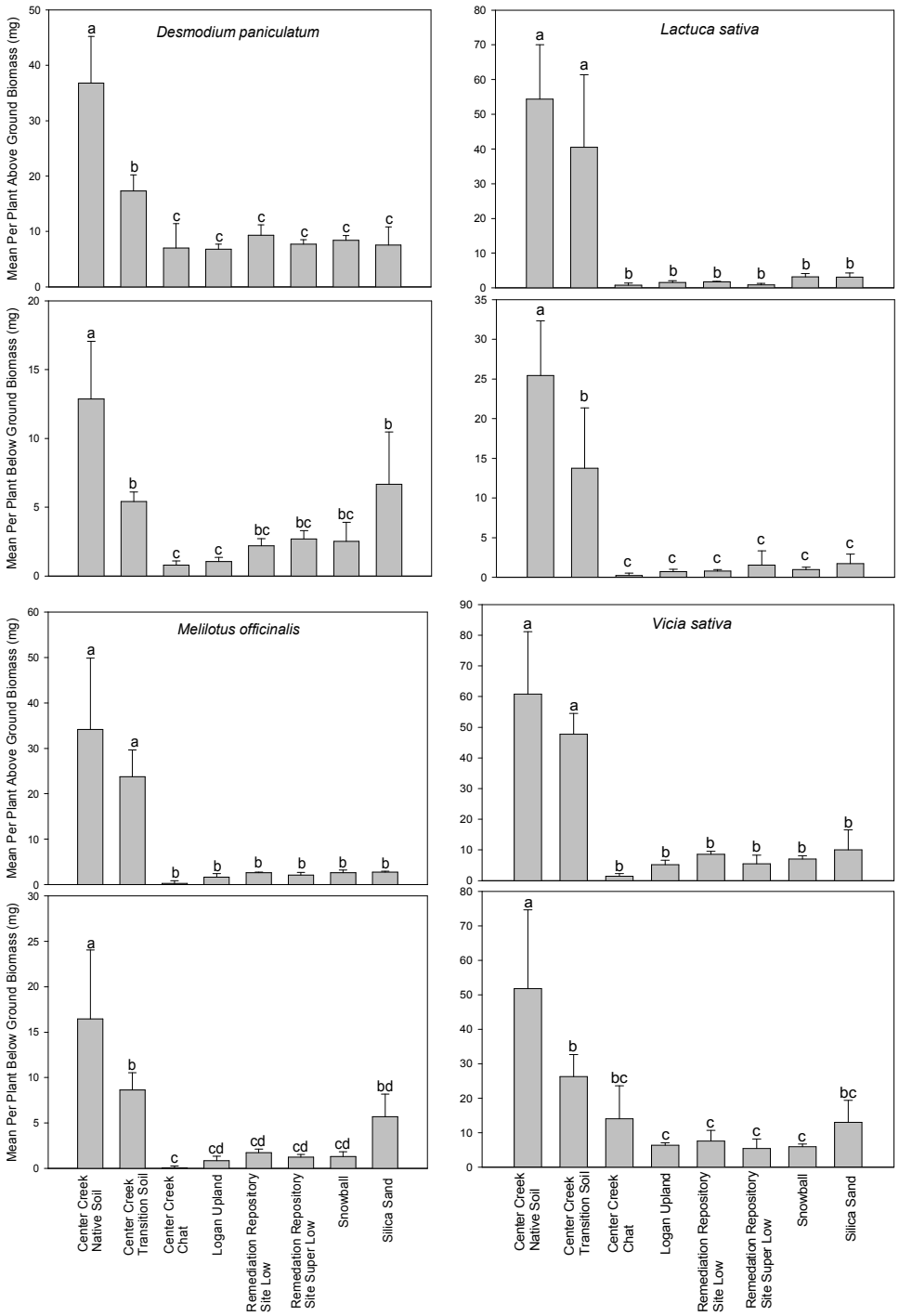

Figure 8. Mean per plant above and below ground dry weight biomass by species and growing media from Tri-States Mining District. Error bars equal +/- 1 standard deviation n=6 for each growing media test species combination. Columns with different letters indicate a significant difference using Tukey's-HSD (α=0.05).

The nutrient and physical characteristics of all the growing media were very different. These differences may have confounded our ability to differentiate the role metals and soil texture had on per plant biomass. The results of this study show that native and exotic legumes can germinate, survive and grow on chat from the Tri-State area, but that growth is significantly inhibited compared to transition and native soils. In a 28-day greenhouse study growing media texture can have a significant effect on seedling survival and growth. This may be due to water stress, which can be a significant problem in a greenhouse environment (Nemali and van Iersel 2006, Linker and Seginer 2003). The metal concentrations in the growing media we studied may begin to affect plant survival and growth over a longer period of time than 28 days.

3. Nutrient Solution vs Water Only

It has been well established that mining waste has many confounding factors that affect plant growth, including high metals concentrations, poor soil structure, salinity, low water holding capacity, lack of organic matter, and poor nutrient levels (Ye and others 2002, Tordoff and others 2000, Yang and others 1997). To assess the impact of increased nutrient levels, we compared the germination rate and growth of test species in chat from the Remediation Site Low site between pots that were watered with 1) RO water and 2) a low phosphorus Hoagland's solution. Seeds planted in the Remediation Site Low Chat and watered with RO water had a significantly higher percent germination than seeds watered with nutrient solution for all test species except *Lactuca sativa* (Table 5). *Desmodium paniculatum* and *Lactuca sativa* plants watered with the nutrient solution had a significantly higher mean per plant above-ground biomass (16.4 mg and 8.8 mg respectively) and below-ground biomass (8.8 mg and 2.8 mg respectively) than plants watered with RO water only. The addition of nutrients did improve plant growth for these two species, but *Melilotus officinalis* and *Vicia sativa* mean per plant biomass was not significantly different between the two treatments. Results may have been influenced by the fact that nutrient solution may not have been retained in the chat and available to the plants growing in the chat. This conclusion was supported by Yang and others (1997) who found that the application of inorganic fertilizer did not appear to improve plant growth on tailings.

Table 5. Comparison of mean percent seed germination and plant dry weight biomass between plants watered with reverse osmosis filtered water and a low phosphorus nutrient solution. One standard deviation is in parentheses.

Growing Medium	*Watering Solution*	*% Germination*	*Mean Per Plant*	
			Above Ground Biomass (mg)	*Below Ground Biomass (mg)*
		Desmodium paniculatum		
Remediation Site Low	RO	60.0 (6.3)*	9.3 (1.9)*	2.2 (0.5)*
Remediation Site Low	NS	33.3 (8.2)*	16.4 (5.4)*	8.8 (5.9)*
		Lactuca sativa		
Remediation Site Low	RO	65.0 (20.7)	1.7 (0.2)*	0.8 (0.2)*
Remediation Site Low	NS	66.7 (10.3)	8.8 (4.7)*	2.8 (1.1)*
		Melilotus officinalis		
Remediation Site Low	RO	31.7 (13.3)*	2.6 (0.2)	1.7 (0.4)
Remediation Site Low	NS	11.7 (11.7)*	4.9 (4.7)	2.4 (2.5)
		Vicia sativa		
Remediation Site Low	RO	93.3 (10.3)*	8.6 (1.0)	7.6 (3.1)
Remediation Site Low	NS	36.7 (36.6)*	6.6 (4.0)	8.2 (5.5)

* Indicates a significant difference between watering solution treatements $\alpha=0.05$

D. Old Lead Belt

1. Seed Germination

Seed germination and survival were analyzed in two ways: 1) percent seed germination for each growing medium; and 2) percentage of germinated seeds that survived until the end of the study.

Desmodium paniculatum, *Melilotus officinalis*, and *Vicia sativa* had significant differences in percent seed germination between growing media. The percentage of *Desmodium paniculatum* seeds that germinated was significantly lower on mine waste from Leadwood compared to all mine waste from Missouri Mines (Table 6). The percentage of *Melilotus officinalis* seed germination was significantly lower on Leadwood waste than on Missouri Mines 2 waste and silica sand. Finally, *Vicia sativa* seed germination rates were significantly lower on Leadwood waste than on Missouri Mines 1 and 2 (Table 6). Seed germination rates on Leadwood growing medium were the lowest for *Melilotus officinalis* (Table 6). Germination rates for all test species across all mine waste growing media were not different from the Center Creek Native Soil, except for *Lactuca sativa* on Leadwood waste (Table 6). An additional finding was that seed germination rates for all species were lowest on the Leadwood growing media, which had the highest zinc concentrations and lowest lead concentrations of all Old Lead Belt growing media.

Our results are consistent with other studies that have found seed germination to be unaffected by mining waste (Ye and others 2002, Peralta and others 2001, Levy and others 1999, Yang and others 1999, Påhlsson 1989). Notably, fine tailings particles in the mine waste from the Old Lead Belt were observed to be very mobile when watered. Correlation analysis of the seed germination data with growing media texture was performed to determine if germination rates were related to the physical properties of the growing media. Correlations between seed germination and texture data were weak and non-significant. Percent fine, the percentage of material passing through a 2mm sieve had the highest correlation (0.142) with percent germination. Regardless of the correlation results we observed that the sandy nature of the mine waste permitted a lot of movement of tailings when pots were watered from above, which may have affected the observed seed germination rates.

2. Biomass Data

Because of the variability in seed germination and seedling survival, biomass comparisons were made using the mean per plant above- and below-ground biomass. Mean per plant above- and below-ground biomass describes how productive a plant was over 28 days grown on each growing medium.

Mean per plant above- and below- ground biomass was significantly higher on Center Creek Native Soil than on all other growing media. Soil has more nutrients, better water holding capacity, and lower levels of metals (Ye and others 2002, Tordoff and others 2000, Yang and others 1997). Correlation analysis was used to identify patterns in biomass production and physico-chemical characteristics of the growing media. Correlation analysis of per plant total biomass on Old Lead Belt growing media and Center Creek native soil showed that per plant above-ground biomass was negatively correlated to the soil texture variable percent sand (-0.747) but positively correlated to percent clay (0.682) and percent silt (0.747). XRF zinc (-0.147) and lead (-0.132) variables were correlated poorly with above-ground biomass production on Old Lead Belt mine waste and uncontaminated soil. Below-ground biomass also negatively correlated to the soil textural variable of percent sand (-0.622) but positively correlated with percent clay (0.566) and percent silt (0.622). XRF zinc (-0.151) and lead (-0.137) did not correlate well with below-ground plant biomass. The stronger correlation between biomass and soil textural variables leads to the conclusion that soil physical properties were important factors affecting per plant biomass. The growing media metal concentration

Table 6. Mean percent germination and dry weight biomass data for plants grown on Old Lead Belt Growing Medium, one standard deviation in parentheses. n=6 for each test species growing media combination. Different letters in the same column indicate a significant difference at $p \leq 0.05$ using Tukey-HSD test.

Growing Medium	Water	% Germination	% Survival	Per Plant	
				Above Ground Biomass (mg)	Below Ground Biomass (mg)
Desmodium paniculatum					
Center Creek Native Soil	RO	46.7 (19.7)ab	100.0 (0.0)a	36.8 (8.4)a	12.9 (4.2)a
Missouri Mines 1	RO	65.0 (12.2)b	97.6 (5.8)a	12.4 (1.2)b	6.7 (0.4)b
Missouri Mines 2	RO	63.3 (24.2)b	95.8 (10.2)a	11.2 (2.3)b	13.1 (4.0)b
Missouri Mines 3	RO	58.3 (14.7)b	100.0 (0.0)a	12.7 (3.1)b	6.3 (1.9)b
Leadwood	RO	23.3 (13.7)a	100.0 (0.0)a	8.7 (4.5)b	4.9 (3.0)b
Silica Sand	RO	51.7 (27.9)ab	96.7 (8.2)a	7.6 (3.3)b	6.7 (3.8)b
Lactuca sativa					
Center Creek Native Soil	RO	76.7 (15.1)a	97.2 (6.8)a	54.4 (15.7)a	25.4 (6.9)a
Missouri Mines 1	RO	73.3 (12.1)ab	100.0 (0.0)a	6.1 (0.9)b	3.7 (1.0)b
Missouri Mines 2	RO	63.3 (16.3)ab	97.6 (5.8)a	5.5 (2.6)b	3.1 (0.6)b
Missouri Mines 3	RO	68.3 (22.3)ab	86.4 (16.7)a	5.1 (1.1)b	2.6 (5.9)b
Leadwood	RO	46.7 (15.1)b	92.1 (13.7)a	6.1 (1.9)b	3.6 (1.5)b
Silica Sand	RO	58.3 (20.4)ab	97.9 (5.1)a	3.1 (1.2)b	1.7 (1.2)b
Melilotus officinalis					
Center Creek Native Soil	RO	46.7 (26.6)ab	100.0 (0.0)a	34.2 (15.7)a	16.5 (7.6)a
Missouri Mines 1	RO	43.3 (8.2)ab	100.0 (0.0)a	4.5 (0.7)b	4.1 (1.3)b
Missouri Mines 2	RO	61.7 (23.2)a	96.7 (8.2)a	4.0 (0.6)b	2.8 (0.8)b
Missouri Mines 3	RO	30.0 (19.0)ab	100.0 (0.0)a	4.3 (2.3)b	3.7 (2.4)b
Leadwood	RO	20.0 (14.1)b	83.3 (23.6)a	3.6 (2.4)b	1.9 (1.4)b
Silica Sand	RO	65.0 (25.9)a	96.7 (8.2)a	2.7 (0.2)b	5.7 (2.5)b
Vicia sativa					
Center Creek Native Soil	RO	66.7 (32.7)ab	100.0 (0.0)a	60.8 (20.4)a	51.7 (22.9)a
Missouri Mines 1	RO	93.3 (10.3)a	100.0 (0.0)a	11.2 (2.3)b	13.0 (4.0)b
Missouri Mines 2	RO	90.0 (21.0)a	100.0 (0.0)a	11.4 (3.6)b	13.5 (3.8)b
Missouri Mines 3	RO	63.3 (19.7)ab	100.0 (0.0)a	11.8 (2.0)b	23.9 (8.1)b
Leadwood	RO	40.0 (12.6)b	100.0 (0.0)a	10.2 (3.6)b	12.7 (7.8)b
Silica Sand	RO	70.0 (27.6)ab	100.0 (0.0)a	10.0 (6.5)b	13.0 (6.4)b

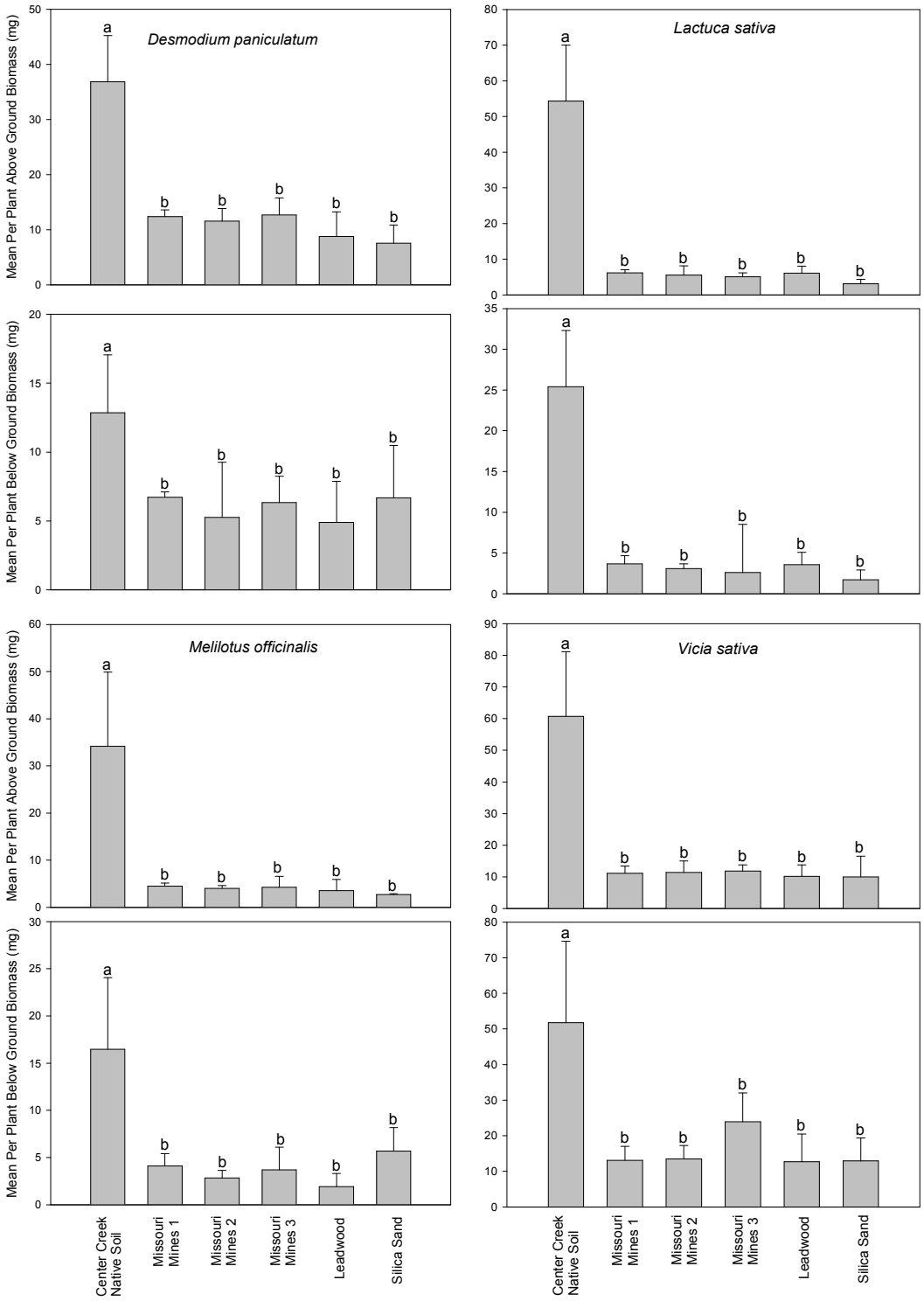

Figure 9. Mean per plant above and below ground dry weight biomass by species and growing media from the Old Lead Belt. Error bars equal 1 standard deviation and n=6 for each test species growing media combination. Columns with different letters indicate a significant difference using Tukey's-HSD (α=0.05).

may have been important but when comparing plant biomass production on soil and mine waste together soil physical properties were important. Also supporting this conclusion was the fact that plants produced a similar amount of biomass on the inert growing medium silica sand as was produced on Old Lead Belt tailings. During a 28-day study plants will respond to the first stressor, which in this case may have been water availability. Water stress can be a significant problem in a greenhouse environment (Nemali and van Iersel 2006, Linker and Seginer 2003) and may be a more immediate survival stressor than metals, for plants grown in a short duration greenhouse study.

Native soil had superior water holding capacity, nutrients, and organic matter when compared to the mining chat. These facts would permit more water to be available to plants grown in native soil. The results of this study show that native and exotic legumes can germinate, survive and grow on tailing from the Old Lead Belt, but that growth is significantly reduced compared to native soils. In a 28-day greenhouse study, growing media texture can have a significant effect on seedling survival and growth. Water stress can be a significant problem in a greenhouse environment (Nemali and van Iersel 2006, Linker and Seginer 2003) and may be a more immediate survival stressor than metals. Additionally, the short duration of this study will only detect factors that immediately affect seedling growth and survival. The metal concentrations in the growing media we studied may begin to affect plant survival and growth over a longer period of time than 28 days.

E. Biomass Production on Mine Waste Only

Analysis of Tri-State and Old Lead Belt biomass data showed that plants grown on native soil produced greater biomass than plants grown on metals-contaminated transition soil. Plants grown on transition soil produced more biomass than plants grown on mine waste. Correlation analysis of soil and mine waste growing media together showed a strong correlation between soil texture and plant biomass production regardless of mining region. We investigated the effects of texture and metals on biomass production by analyzing biomass production within similar soil textural classes across both Tri-State and Old Lead Belt growing media. Mine waste data were selected for this analysis because there was a large sample size. Mine waste was classified into two soil classes; 1) sand at three sites from Tri-State and two sites from the Old Lead Belt, and 2) loamy sand at two sites from Tri-State and two from the Old Lead Belt (Table 2). We tested biomass production between soil classes and found that biomass production was not significantly different between sand or loamy sand mine waste for any test species (*Desmodium paniculatum* $p=0.384$; *Lactuca sativa* $p=0.386$; *Melilotus officinalis* $p=0.544$; *Vicia sativa* $p=0.803$). Therefore, biomass data were pooled for all sand and loamy sand mine waste. Previous analysis showed that plant biomass increased as soil texture acquired more fine material (less than 2mm in size) and this was true for loamy sand and sandy growing media (Figure 10). Lead and zinc data appeared to be related to plant biomass in loamy sand and sandy growing media as well, with plant biomass decreasing as zinc concentrations increased (Figure 11).

Least squares linear regression showed that all test species exhibited a negative relationship between total biomass and Zn concentration and a positive relationship between total biomass and the percent growing media passing through a 2 mm sieve (Figure12.; Table 7). These relationships were significant ($p \leq 0.05$) between both independent variables and all species, except when examining the relationship between *Vicia sativa* and zinc concentration. *Vicia sativa* was the only species for which biomass had a significant negative relationship with lead concentration. Based upon the r-squared values derived from linear regression, the strength of the relationship varied by species. *Desmodium paniculatum* and *Melilotus officinalis* had a stronger relationship between total biomass and zinc concentration than between total biomass and percent passing though a 2 mm sieve (Table 7). *Lactuca sativa* and *Vicia sativa* had a stronger relationship between the percent passing though a 2mm sieve than to zinc concentration (Table 7).

The results of this analysis demonstrate that metals are affecting biomass production in all test species grown in mine waste growing media; however, this study was not designed to quantify the relative contribution of metals or soil texture to changes in biomass production. Additional research would be needed to quantify the impacts of metals on biomass production. Biomass production of individual plants on mine waste provide a means to demonstrate how individual species respond to mine waste as a growing media, but the application of one greenhouse study to the landscape level is complicated by the fact that metal concentration effects varied by species. Entire plant communities may be responding to environmental and metal contamination in ways that are not addressed by a greenhouse study evaluating the growth of four species in pots on mine waste.

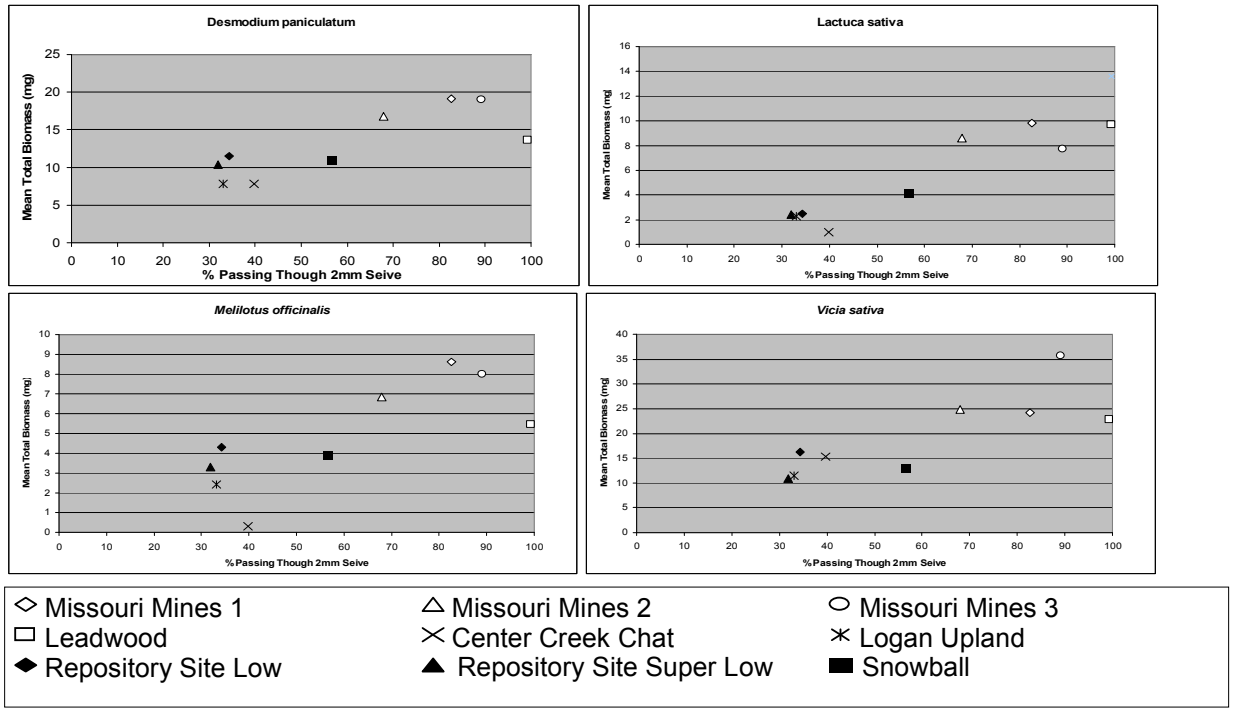

Figure 10. Total biomass production by percent soil passing through a 2mm sieve.

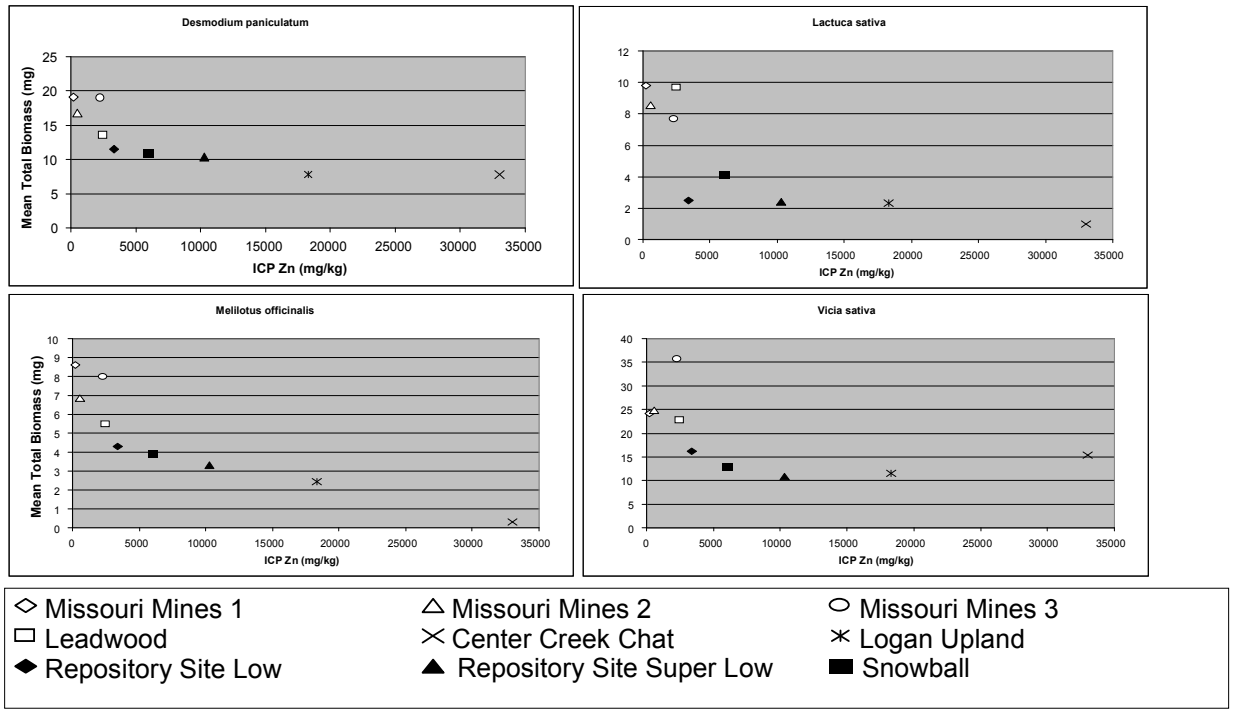

Figure 11. Figure 2.Total biomass production by ICP-MS Zn concentration.

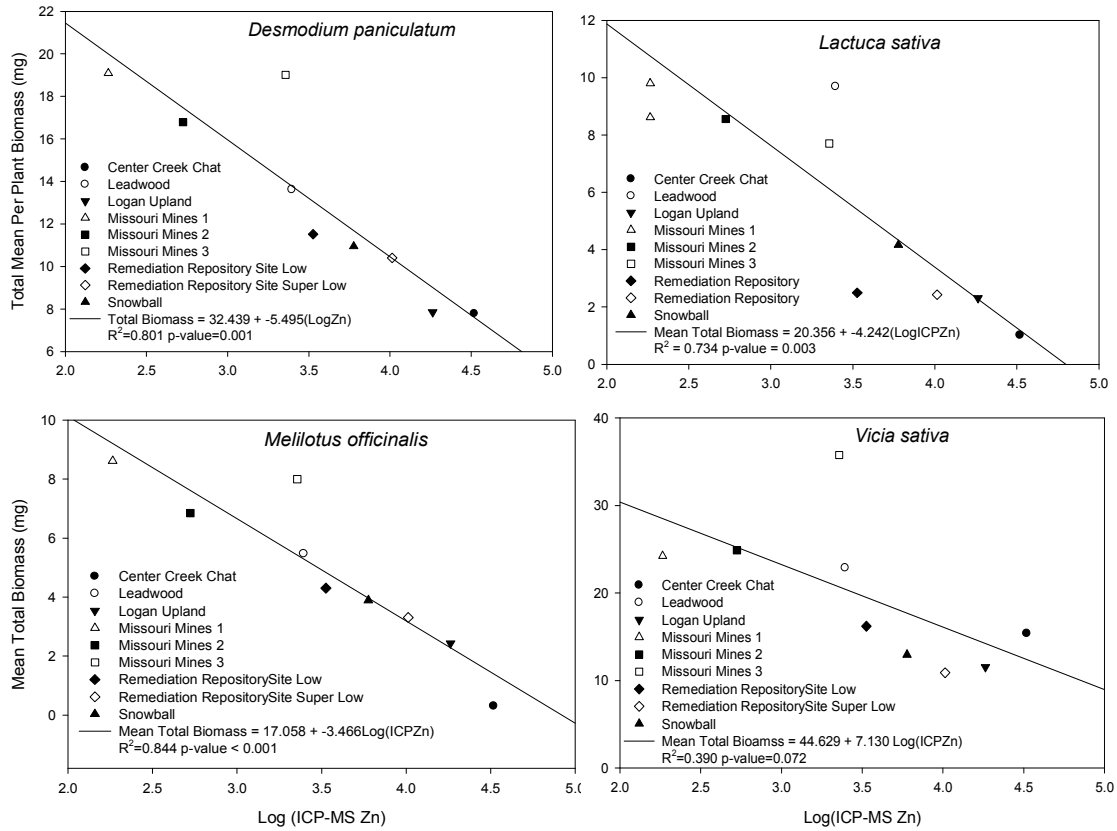

Figure 12. Least squares linear regression results for mean total biomass and Log (ICP-MS Zn) for each test species grown on mine waste only.

Table 7. Results of least squares linear regression of mean total biomass against soil textural variables and Log of metal concentrations by test species grown on mine waste only. Values with a p-value ≤ 0.05 are in bold.

Independent Variable	Intercept	Coefficient	R^2	p-value
Desmodium paniculatum				
Log(ICP-MS Zn)	**32.439**	**-5.495**	**0.801**	**0.001**
Log(ICP-MS Pb)	4.885	2.601	0.257	0.163
%Fine	**5.209**	**0.131**	**0.618**	**0.012**
%Sand	1.642	0.127	0.033	0.640
%Clay	11.144	0.835	0.043	0.592
%Silt	14.574	-0.197	0.062	0.520
Lactuca sativa				
Log(ICP-MS Zn)	**20.356**	**-4.242**	**0.734**	**0.003**
Log(ICP-MS Pb)	1.220	1.324	0.103	0.401
%Fine	**-2.070**	**0.125**	**0.862**	**<0.001**
%Sand	7.098	-0.019	0.001	0.929
%Clay	4.243	0.498	0.024	0.693
%Silt	5.303	0.006	0.000	0.981
Melilotus officinalis				
Log(ICP-MS Zn)	**17.058**	**-3.466**	**0.844**	**<0.001**
Log(ICP-MS Pb)	1.511	1.053	0.112	0.379
%Fine	**0.159**	**0.078**	**0.580**	**0.017**
%Sand	-6.494	0.126	0.087	0.442
%Clay	4.346	0.203	0.007	0.833
%Silt	6.168	-0.171	0.123	0.354
Vicia sativa				
Log(ICP-MS Zn)	44.629	-7.130	0.390	0.072
Log(ICP-MS Pb)	**-3.210**	**7.250**	**0.578**	**0.017**
%Fine	**4.424**	**0.252**	**0.661**	**0.008**
%Sand	16.319	0.034	0.001	0.946
%Clay	12.805	2.971	0.158	0.290
%Silt	20.688	-0.160	0.012	0.781

IV Conclusions

Over the course of a 28-day study growing native and exotic legumes on mine waste from the Tri-State region and Old Lead Belt, germination rates were significantly different between growing media and test species. Seedling survival was significantly lower on Center Creek Chat from the Tri-State region, and plant biomass was significantly higher for plants grown on uncontaminated soil. Additionally, plants grown on native soil produced significantly more biomass than plants grown on soil that was contaminated with lead and zinc. Plants grown on contaminated soil produced 60.8% of the biomass plants grown on uncontaminated soil. Plants grown on mine waste on average produced only 16.7% of the biomass that plants grown on uncontaminated soil produced. However, when comparing

plant growth on soil compared to mine waste, the texture and nutrient properties of mine waste may have affected plant growth more than metals concentrations. When sandy and sandy loam growing media were analyzed together both zinc concentration and the percentage of material less than 2mm in size were significant variables affecting biomass production. *Desmodium paniculatum*, the only native Missouri legume tested, and *Melilotus officinalis* had a strong negative relationship between total per plant biomass and zinc concentration. *Lactuca sativa* and *Vicia sativa* also had a significant negative relationships between total per-plant biomass and zinc concentration, but this relationship was not as strong as the relationship between these species and the percent of material smaller than 2mm in size in the growing media.

Similar findings have been reported (Ye and others 2001, Ye and others 2000, Yang and others 1997, Alverez and others 1974). Others have discussed the problems with mine waste and poor structure and nutrients (Ye and others 2003, Tordoff and others 2000, Yang and others 1997). Plant growth and legume nitrogen fixation rates have been improved through the addition of various type of organic matter to mine wastes (Ye and others 2002, Ye and others 2001, Zhang and others 2001, Tordoff and others 2000, Ye and others 2000, Yang and others 1997, Bradshaw 1984). Future research in revegetating mining waste in Tri-State and the Old Lead Belt should include investigating the effects of adding organic matter to mine wastes. Additionally more research is needed to document the effect of growning legumes on mine waste long-term. Long-term effects that should be investigated include growth and biomass for more than 28 days, flowering success, seed production, and seed viability for plants grown on mine waste. In addition, growing plants over a longer term in mine wastes may begin to demonstrate how the metals in mine waste will influence plant growth. Additionally, determination of metals accumulation in native plants grown on these mine wastes will help determine how the plants and growing media are interacting.

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