

## Ecosystem Function in Alluvial Tailings after Biosolids and Lime Addition

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### ABSTRACT

Municipal biosolids and agricultural limestone were incorporated into the surface of alluvial highly acidic, metal-contaminated mine tailings in Leadville, CO, in 1998. Amended sites were seeded and a plant cover was subsequently established. A range of chemical and biological parameters were measured over time to determine if treatment was sufficient to restore ecosystem function. An uncontaminated upstream control (UUC), a contaminated vegetated area (CVA), and soils collected from the tailings deposits before amendment addition were used for comparison. Standard soil extracts showed decreases in extractable Pb, Zn, and Cd in the amended soils. Increased CO<sub>2</sub> evolution, reduced N<sub>2</sub>O, and elevated NO<sub>3</sub><sup>-</sup> in the amended tailings indicated an active microbial community. Levels of CO<sub>2</sub> and NO<sub>3</sub><sup>-</sup> were elevated in comparison with the CVA and UUC. Ryegrass (*Lolium perenne* L.) and earthworm (*Eisenia foetida*) survival and metal uptake values were similar in amended tailings to a laboratory control soil. Ryegrass and worms in unamended tailings died. Field plant diversity was lower in amended areas than in CVA or UUC, with a higher percentage of the vegetative cover consisting of grasses. Small mammal analysis showed a low potential for elevated body Cd and Pb in the amended tailings. A re-entrainment study using fathead minnows (*Pimephales promelas*) showed no danger for resuspended amended tailings, as survival of fish was similar to the laboratory control. Data suggest that ecosystem function has been restored to the amended tailings, but that these systems are not yet in equilibrium.

LEADVILLE, COLORADO, was a site of historic metal mining for close to 100 years. The last mining operation in the area closed in 2001. For most of the active mining period, there were no regulations in place concerning the treatment or disposal of mine wastes. Mine wastes were generally stockpiled or dumped directly into the Upper Arkansas River, resulting in concentrated deposits of mine tailings along a 22-km stretch of the river. The tailings have low soil pH (2.0–4.5) and elevated concentrations of Pb (1550–3450 mg kg<sup>-1</sup>), Zn (1400–3400 mg kg<sup>-1</sup>), and Cd (9–27 mg kg<sup>-1</sup>) and are phytotoxic (URS Operating Services, 1997). Without vegetation for stabilization, tailings are prone to erosion and are frequently re-entrained into the river following high water events. The alluvial tailings deposits are listed as part of a removal action under the USEPA CERCLA National Priorities List (Superfund). The standard action for this type of material within the Superfund program is removal of the contaminated tailings. At the Leadville site, an alternative technology that amends the tailings with municipal biosolids and biosolids compost + limestone is being evaluated. As the primary threat posed by the

contaminants is to an ecosystem rather than human health, this site requires an alternative assessment methodology. This paper discusses both the range of evaluation techniques used and the performance of the in situ amendment.

The conventional remedial approach to metal-contaminated soils within the USEPA Superfund program involves removal and replacement of the soil with clean material or capping the soil with an impermeable material to reduce potential exposure to the contaminants. Standardized tests exist to evaluate the contaminated soils as well as to measure the success of the remedial action, but the tests are largely engineering based and do not consider ecosystem function. Tests commonly used include measures of total metal concentrations and of the potential for metals to leach into ground water (i.e., toxicity characteristic leaching procedure and multiple extraction procedure; USEPA, 1995c, 1995f). The tests generally involve single or multiple extractions with different molar acid solutions. Human exposure to contaminated ground water is the driving factor both in identifying contaminants of concern as well as in setting appropriate concentration limits (National Research Council, 2003).

Alternative remedial technologies are currently being developed that involve leaving the contaminated materials in place and using soil amendments to reduce the bioavailability of the contaminants in situ. One example involves the addition of P to soils to limit Pb bioavailability to humans (Brown et al., 2004; Ryan et al., 2004). Another involves amending soils with municipal biosolids and lime to reduce the bioavailability of contaminants and to restore a vegetative cover to large-scale metal-contaminated sites (Basta et al., 2001; Brown et al., 2003a, 2003b).

The use of municipal biosolids for restoration of disturbed lands is well documented (e.g., Sopper, 1993). Recent work suggests that application of biosolids in combination with limestone or other high calcium carbonate effectively restores ecological function to metal-contaminated soils (Brown et al., 2003a, 2003b; Conder et al., 2001; Li et al., 2000).

While conventional extraction tests have been used to evaluate the success of in situ technologies, additional assays are necessary to measure restoration of ecosystem function. In particular, bioavailability has to be more broadly considered to include a range of ecological receptors and relevant pathways (National Research Council, 2003).

Several procedures have been developed to measure the bioavailable, rather than total, fraction of contaminants in soils and sediments and these are generally based on the exposure pathway for the most sensitive ecological receptor. In many cases, toxicity is the defined

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Published in J. Environ. Qual. 34:139–148 (2005).

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**Abbreviations:** CVA, contaminated vegetated area; UUC, uncontaminated upstream control.

endpoint. For example, soil extracts are routinely used to determine the phytoavailable fraction of total nutrient concentrations in soils (McLaughlin et al., 2000; Sparks, 1996). In cases of contaminated soils, extracts have been altered to better mimic the behavior of plants in these environments. For example, more passive extracts or measures of soluble metals are used to assess the potential for phytotoxicity (Sauvé, 2002; Zhang et al., 2001). Extracts have also been correlated with reductions in microbial activity, as measured by microbial lux biosensors (Shaw et al., 2000; Vulkan et al., 2000).

Direct toxicity tests and animal feeding trials are also used. Earthworm mortality has been used as a measure of the effectiveness of soil amendments to reduce bioavailability in mine tailings (Conder et al., 2001). Both in vivo and in vitro extracts have been used to predict the bioavailability of soil Pb to humans (Ruby et al., 1999). In each case, the test was developed to focus on a particular endpoint or receptor. None of the tests attempts to evaluate the collective ecosystem function.

Methods to assess ecosystem function are rare. Techniques have been developed to assess the health of the soil microbial population, including measures of soil function through respiration, N cycling, and ability to utilize added substrates (Chang and Broadbent, 1982; Cela and Sumner, 2002; McGrath, 2002; Sauvé et al., 1998). One example is the Biolog extraction (Kelly and Tate, 1998), which attempts to evaluate the functionality of the soil microbial population through its ability to utilize a range of carbon sources. The procedure has been criticized for difficulty of interpretation, and the ability of certain groups of microbes to utilize a range of substrates (National Research Council, 2003). Organism presence can falsely suggest a robust microbial community. Other tests assess bioavailability of contaminants by measuring the reactions of single types of organisms to exposure to remediated soils (Geebelen et al., 2003).

For the amended tailings in Leadville, an alternative testing protocol was developed. In addition to conventional engineering criteria, standard principles of ecological risk were used to develop a series of tests to assess amendment effects on ecosystem function. For example, the ability of the system to decompose organic matter and recycle nutrients can indicate the stability of the restored system. In addition, examining the health of, and contaminant uptake by, a select range of receptors can assess of the potential for transfer of contaminants from the amended soil through the food chain.

We hypothesized that results from conventional tests, as well as those used to measure ecosystem function and the potential for contaminant transfer through the food chain, are appropriate to evaluate the ability of in situ amendment to restore ecosystem function to metal-contaminated mine tailings. This study measured the function of the amended tailings in Leadville, CO, using a range of indices and potential receptors.

## MATERIALS AND METHODS

### Site Characterization

Alluvial tailings deposits along the Upper Arkansas River in Lake County, outside of Leadville, CO, were identified as

part of a USEPA CERCLA removal action in 1983 (URS Operating Services, 1997). This portion of the river is situated between the Mosquito Range to the east and the Sawatch Range to the west, and elevation ranges from 2790 to 2900 m (USGS, 1967, 1969, 1970). Tailings originated upriver in the historic mining district and were deposited downstream over the course of several high water events during the last century (Levy, 1990). The tailings were included as a part of the California Gulch Superfund Site, and the site was placed on the National Priorities List in 1983. Work on the site before amendment addition generally involved site characterization and feasibility studies of standard removal actions (URS Operating Services, 1997). The tailings deposits were surveyed before amendment addition. Samples were taken from each deposit and total metals, depth of the tailings, and pH were measured on random samples from the contaminated areas. Each tailings deposit was also given an alphabetic name (URS Operating Services, 1997).

### Amendment Addition

An amendment mixture was applied to portions of the tailings deposits as part of a remedial action in the summer of 1998. The amendment consisted of 224 Mg ha<sup>-1</sup> municipal biosolids from the Denver wastewater treatment facility and 224 Mg ha<sup>-1</sup> agricultural limestone (Calco, Salida, CO). The biosolids were anaerobic digested cake with a solids content of approximately 17% and met Class B criteria for pathogen reduction (USEPA, 1993). Materials were mixed on a volume basis using a front-end loader before application. The amendment mixture was surface-applied using a rear throw spreader with floatation tires and incorporated to a depth of 20 cm using a ripper.

Samples were collected from four areas that received biosolids and lime addition in 1998. The areas ranged in size from 72 000 to 123 400 m<sup>2</sup>. Samples were collected before amendment addition in 1998 and for two years (1999 and 2000) following amendment addition. Samples were also taken from unvegetated tailings deposits in 1999 that ranged in size from 3400 to 79 700 m<sup>2</sup>. In 2000, all tailings sites except one had been amended with biosolids mixtures, so baseline measurements were only taken from the single unamended tailings deposit. Samples taken from two nearby vegetated areas served as a measure of typical ecosystems in this environment; samples were collected in 1999 and 2000 from an upstream uncontaminated control (UUC) north of Leadville and upstream from the Mine Waste Treatment Plant. The areas were heavily vegetated with grasses and willows. Samples were also collected from a contaminated vegetated area (CVA) that had elevated total metal content, but supported a dense vegetative stand with grasses and willows.

### Sampling Procedure

Surface soil samples for physical and chemical characterization and soil community measures were collected according to procedures outlined in the ERTC/Response, Engineering, and Analytical Contract (REAC) Standard Operating Procedure (SOP) #2012, *Soil Sampling*. Unless otherwise noted, all samples were shipped within 24 h of collection to REAC in Edison, NJ, by overnight courier and stored at 4°C before analysis. Four liters of soil was collected from each location in 1998 from random locations in the plot area. Soil was collected from each site in 1999 and 2000 from a minimum of three separate areas within each location. A minimum of one 4-L bucket of soil was collected from each area within the plot. These samples were used for chemical and physical property

analysis and for toxicity testing. Samples for soil function analysis were collected simultaneously and were packed in ice before shipping to McGill University for analysis.

Soil samples were analyzed to evaluate changes in chemical and physical properties, biological activity, and toxicity. Changes in chemical properties of the soil were evaluated using a range of assays. These included total metals (USEPA, 1995a), water-extractable metals (Council on Soil Testing and Plant Analysis, 1992), exchangeable metals (Suarez, 1996) and weak acid-extractable metals (Wolf and Beegle, 1995), toxicity characteristics leaching procedure (TCLP) (USEPA, 1995f), multiple extraction procedure (MEP) metals (USEPA, 1995c), and pH (USEPA, 1995d). Measured physical properties of the soils included texture and water-holding capacity (Gee and Bauder, 1986; American Society for Testing and Materials, 1996). Fertility of the soil was measured using total organic carbon (USEPA, 1995e), nitrogen as total Kjeldahl N, Mehlich 3–available P (Mehlich, 1984), and cation exchange capacity (CEC) (USEPA, 1995b) as indices.

### Soil Community Analysis

Soil function was assessed using several indices. For N, these included  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and microbial biomass N. For C, these included microbial biomass C and  $\text{CO}_2\text{-C}$  analysis. These analyses were performed by Dr. J. Whalen, Department of Natural Resource Science, McGill University, Quebec, Canada, as outlined below.

For  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ , field-moist soils were sieved to <2 mm and 10 g of the sieved soil was extracted with 40 mL of 0.5 M  $\text{K}_2\text{SO}_4$  for 45 min. Samples were then filtered through Whatman (Maidstone, UK) 42 filter paper and the filtrate was stored at 4°C until analysis. Values were determined colorimetrically using phenate for  $\text{NH}_4\text{-N}$  and cadmium reduction for  $\text{NO}_3\text{-N}$  (Mulvaney, 1996) using a Quik Chem AE flow-injection auto-analyzer (Lachat Instruments, Milwaukee, WI). Microbial biomass carbon and nitrogen were determined using the chloroform fumigation–direct extraction method (Brookes et al., 1985). Chloroform was added to a desiccator in a 50-mL beaker. Ten grams of field-moist soil was placed in a separate beaker in the same desiccator and the chloroform was distributed by evacuating the desiccator until the chloroform bubbled vigorously. This was repeated once per day for 4 d and then the soils were extracted with 40 mL of 0.5 M  $\text{K}_2\text{SO}_4$ . Microbial biomass N was determined as the difference between extractable  $\text{NO}_3$  before fumigation and following fumigation (Brookes et al., 1985).

Carbon mineralization was determined by measuring  $\text{CO}_2$  evolution from moist incubating soils, once per week during the course of a 42-d incubation (Zibilske, 1994). The incubation was performed on 20 g of field-moist soil. A 12-mL sample of gas was collected from the headspace of the jar containing the soil and the jar was left open for 15 min to assure adequate aeration (Zibilske, 1994). Carbon dioxide in the sample was determined using a Hewlett-Packard (Palo Alto, CA) 5890 Series II gas chromatograph equipped with a thermal conductivity detector. Results are expressed as the average value for each treatment minus the control value for all sampling times.

Toxicity was evaluated using ryegrass and earthworm assays; both lethal and sublethal endpoints were measured. Assays were conducted according to protocols established by the American Society for Testing and Materials. Plant germination, height and biomass, and metal concentration in aboveground biomass were measured (American Society for Testing and Materials, 2003c). Earthworm survival, biomass, and metal accumulation in expurgated tissue were analyzed (American Society for Testing and Materials, 2003b).

In addition to soil samples, plant tissue samples and small mammals were collected from both reference locations (UUC and CVA) and one treated area in 2000 and 2001. Collection procedures followed the protocol outlined in ERTC/REAC SOP #2037, *Terrestrial Plant Community Sampling* and #2029, *Small Mammal Sampling and Processing*. Fifty traps were set at each sampling location. Traps were checked in both the morning and evening after setting, and any animals were removed for processing. Mammal body samples were placed on dry ice and shipped within 24 h after collection for analysis. Whole-body analysis was performed on the animals (USEPA, 1995a). A minimum of three plant samples of a grass and an herb species were also collected in 2000. These were packed on ice and sent for analysis within 24 h after collection (USEPA, 1995a). Plant community was evaluated as follows. Fourteen 20- × 20-cm quadrats were randomly placed at one of the treated areas and both reference areas. There was no vegetation in untreated tailings. Plant species were keyed on site.

The potential for amended tailings to damage the river system on re-entrainment was also evaluated. Soils from four sites that had been amended with biosolids and lime, the UUC, the CVA, and untreated tailings were used. Tailings samples were shaken with site water in a 1:4 ratio for 30 min, the suspension was allowed to settle for 1 h and the eluate was tested for toxicity to fathead minnows in a 7-d assay (American Society for Testing and Materials, 2003a). The endpoints assessed were mortality and fish growth.

### Statistical Analysis

Data were analyzed using SPSS Version 11 (SPSS, 2001). Following analysis of treatment effects using a general linear model, means were separated using the Duncan–Waller means separation procedure. For soil extractions, statistics were performed on the log-transformed data.

## RESULTS

### Soil Chemical Properties

As is characteristic of mine tailings deposits, total metal concentrations were variable across the sites. Average metal concentrations after amendment addition are reported in Table 1. Total metals in the CVA were similar to those in the treated areas, whereas concentrations in the UUC were significantly lower than in the treated areas.

Extracts were done on the soils for samples collected in 1998 (pre-treatment) as well as on samples collected in 1999 and 2000 (post-treatment) (Fig. 1). For all ex-

**Table 1. Total metals and pH of tailings deposits along the Upper Arkansas River, Leadville, CO, that were amended with a mixture of municipal biosolids (224 Mg ha<sup>-1</sup>) and agricultural limestone (224 Mg ha<sup>-1</sup>) in 1998. Also included are an upstream uncontaminated control (UUC) and a contaminated vegetated area (CVA).**

Area	Cd	Pb	Zn	pH†
	mg kg <sup>-1</sup>			
1	9.5 ± 2.9‡	2490 ± 640	1950 ± 610	6.75
2	24.3 ± 12	2730 ± 370	2520 ± 580	6.7
3	16.9 ± 9.1	1560 ± 285	1440 ± 310	6.65
4	12.1 ± 2.8	1390 ± 610	1400 ± 425	6.84
Tailings	15.9 ± 3.1	3170 ± 490	1730 ± 350	3.53
UUC	2.3 ± 0.1	100 ± 13	210 ± 40	7.05
CVA	27 ± 5.7	3450 ± 2050	3400 ± 1415	7.47

† Before amendment addition, pH in all tailings deposits averaged 3.44.  
‡ Means ± standard error for samples collected annually from 1998–2000.

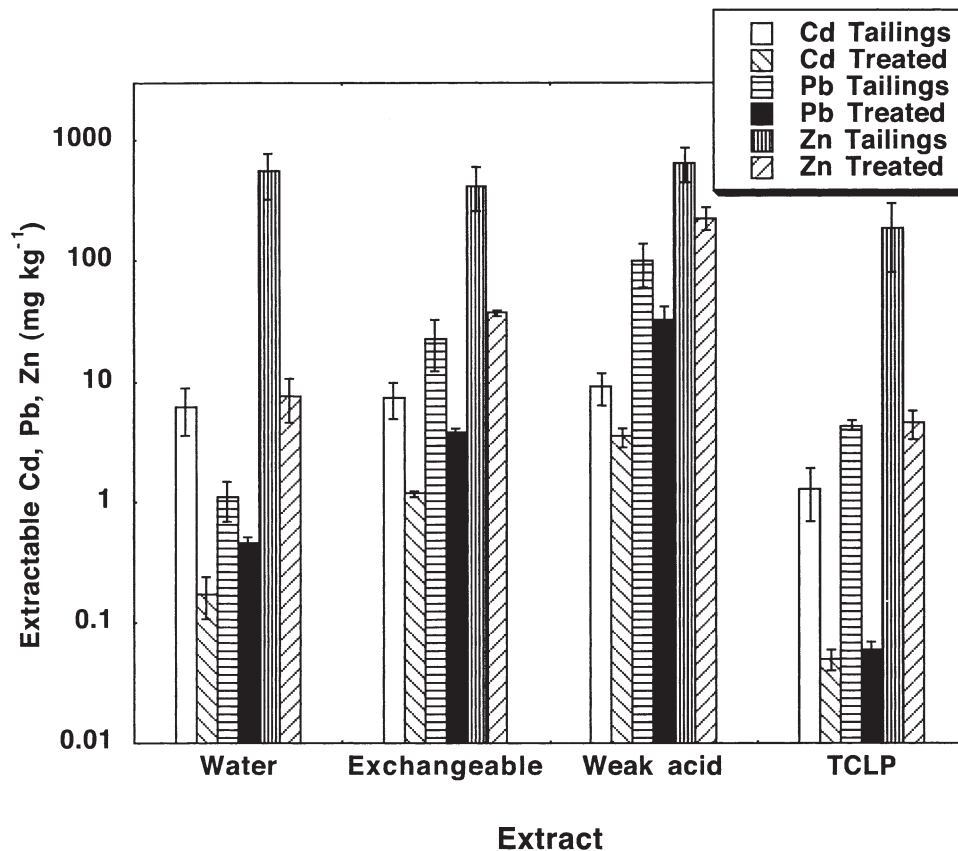


Fig. 1. Water-extractable, exchangeable, weak acid-extractable, and toxicity characteristic leaching procedure (TCLP) Cd, Pb, and Zn from alluvial tailings deposits along the Upper Arkansas River, Leadville, CO, before and for two years after amendment with municipal biosolids ( $224 \text{ Mg ha}^{-1}$ ) and agricultural limestone ( $224 \text{ Mg ha}^{-1}$ ). Bars represent standard error of the means.

tracts, amendment addition significantly reduced extractable Cd, Pb, and Zn over unamended soils. Reduction was most pronounced for the samples where extractable metal before amendment addition was highest. Water-leachable metals were measured to assess changes in the portion of total metal that is immediately available for biological action. Cadmium by this measure was reduced from  $0.4\text{--}25$  to  $0.01\text{--}0.1 \text{ mg kg}^{-1}$  following amendment addition. Similar amendment affects were observed on water-leachable Zn and Pb, although the decrease for Pb was less pronounced (Fig. 1). Water-leachable metal concentrations in the treated tailings were similar to those found in the CVA and were similar (Cd, Pb) or higher (Zn) than those in the UUC (Fig. 1).

Changes in exchangeable metal concentrations followed a similar pattern as changes in water-leachable metals for Cd, Pb, and Zn (Fig. 1). This is a more aggressive extract and is used by the USEPA to measure metals that may be phytoavailable over a growing season. Weak acid-extractable metals (thought to be indicative of the portion of total metals that may be available to biota as a result of natural processes such as ingestion [weak stomach acids] or rhizosphere acidification) also decreased with amendment addition. Values for the CVA were higher than for all amended areas, and values for the UUC were lower than those observed in the amended areas.

The toxicity characteristic leaching procedure (TCLP),

generally done to predict leachability of metals to ground water, also showed decreases for Cd, Zn, and Pb in amended versus unamended tailings (Fig. 1). Repeated extractions (done only on samples collected 8 and 14 mo following amendment addition) showed decreasing extractability for Zn and Cd over time. The TCLP-extractable Cd was  $1.32 \pm 0.63 \text{ mg kg}^{-1}$  before amendment addition and  $0.06 \pm 0.03 \text{ mg kg}^{-1}$  two years after addition. Similar trends were observed for Pb ( $4.3 \pm 0.43$  versus  $0.06 \pm 0.01 \text{ mg kg}^{-1}$ ) and for Zn ( $190 \pm 108$  versus  $5 \pm 3.5 \text{ mg kg}^{-1}$ ). Values for Cd and Pb exceeded regulatory limits ( $1$  and  $5 \text{ mg kg}^{-1}$ , respectively) before amendment addition (USEPA, 1993).

### Soil Properties

Total metal concentrations in the amended areas were similar to the untreated areas and the CVA (Table 1). Soil pH increased from  $3.5 \pm 0.3$  in the untreated tailings to  $6.75 \pm 0.1$  in the amended tailings (Table 1). Texture was not changed after amendment addition with the silt fraction of the soil ranging from 15 to 30%, sand from 63 to 80%, and clay from 5 to 7%. Total organic carbon increased from 17 to  $26 \text{ g kg}^{-1}$  before treatment to  $42$  to  $76 \text{ g kg}^{-1}$  following treatment. Organic matter concentrations in the UUC were  $102 \text{ g kg}^{-1}$ . Potentially, as a result of added organic matter from the biosolids, water-holding capacity increased after amendment ad-

**Table 2. Measures of soil function in remediated ( $n = 4$ ) and control ( $n = 6$ ) tailings deposits along the Upper Arkansas River, Leadville, CO, for samples collected in 1999 and 2000 following amendment addition in 1998. Measurements are also presented for soils collected from an upstream uncontaminated control (UUC) and a contaminated vegetated area (CVA) along the Arkansas River.**

Treatment	Biomass C	Biomass N	CO <sub>2</sub> -C respiration	NH <sub>4</sub> -N	NO <sub>3</sub> -N	NH <sub>4</sub> to NO <sub>3</sub> ratio
	mg kg <sup>-1</sup>					
	1999					
Tailings	233c†	2.52c	454c	45.8b	5.33b	8.3
Amended tailings	1224a	172a	4533a	174a	271a	0.9
UUC	935ab	97b	1073b	0.7c	0.8c	0.9
CVA	562b	61.3b	930b	0.1c	3c	0.03
	2000					
Tailings	42.7c	46.3c	4.7b	16b	0.2c	100
Amended tailings	120b	373a	28.2a	41a	519a	0.08
UUC	418a	147b	16.9a	4.2c	4.5b	0.9
CVA	195ab	100b	19a	0.3c	13bc	0.23

† Means within year followed by the same letter are not significantly different at the 0.05 probability level.

dition. Average water-holding capacity before treatment ranged from 11.9 to 24.5% and was 43.1 to 73.3% (by volume) following amendment addition.

Total Kjeldahl N increased from 1 to 1.6 g kg<sup>-1</sup> in the untreated tailings to 4.6 to 8.9 g kg<sup>-1</sup> in the amended areas. This was similar to the N concentrations in the UUC (4.5 g kg<sup>-1</sup>) and higher than those in the CVA (2.9 g kg<sup>-1</sup>). Available P was also increased by amendment addition. Tailings contained 1 to 12 mg kg<sup>-1</sup> available P before amendment, which is similar to the concentrations in the UUC (23 mg P kg<sup>-1</sup>); both sites would be considered potentially deficient in P for plant growth (Pote et al., 1996). After amendment, available P increased to 333 to 433 mg kg<sup>-1</sup>. Cation exchange capacity (CEC) of the soils did not change after amendment addition with values ranging from 12.4 to 24.7 cmol kg<sup>-1</sup> soils after amendment addition. However, percent saturation of exchange sites increased for Ca, Mg, and K.

### Soil Function Measures

Soil microbial toxicology is a relatively new field and no standardized measures exist to evaluate soil health (McGrath, 2002). Soil organisms, as they are in direct contact with contaminants, may be the most sensitive to changes in contaminant bioavailability (Giller et al., 1998). For this study, function measures included extractable NH<sub>4</sub> and NO<sub>3</sub>-N, biomass N and C, and CO<sub>2</sub> production. The ability of the soil microorganisms to utilize added carbon and to recycle nutrients may provide insights into the ability of the restored system to be self sustaining.

Carbon mineralization or basal respiration (CO<sub>2</sub>-C; Table 2) has been used to evaluate the health of the microbial community in metal-contaminated soils (Chander et al., 2001; Scott-Fordsmand and Pedersen, 1995). Evolution of CO<sub>2</sub> was reduced 50% in 1999 and 75% in 2000 in the unamended tailings compared with both the CVA and UUC, indicating that microbial activity was reduced in the tailings. In contrast, CO<sub>2</sub> evolution was increased in the amended tailings compared with the CVA and UUC, probably a response to added organic matter in the biosolids. The increased CO<sub>2</sub> evolution suggests that a microbial population has been restored to the tailings.

The rate of conversion of NH<sub>4</sub> to NO<sub>3</sub> has also been used as a measure of microbial health in Pb- and Cu-

contaminated soils (Sauvé et al., 1998) and metal spiked soils (Chang and Broadbent, 1982; Cela and Sumner, 2002). An accumulation of NH<sub>4</sub> in soils indicates the inability of soil microorganisms to oxidize N and that soil biota are incapable of carrying out the N transformations required for an ecosystem to be self sustaining. In the unamended tailings, the ratio of NH<sub>4</sub> to NO<sub>3</sub>-N exceeded 1 for both years (range = 8.3 to 100:1), suggesting that the soil microorganisms had been compromised by the elevated soil metal concentrations or the acidic pH (Ibekwe et al., 1995). In the amended soils, the NH<sub>4</sub> to NO<sub>3</sub>-N ratio was ≤1. This was also the case in both the UUC and the CVA, both of which have self-sustaining plant covers (Table 2). In addition, the biomass C and N measures in the amended soils were greater than the unamended tailings, the CVA, and the UUC. The soil function measures indicate that a functioning microbial community has been restored to the amended tailings.

### Earthworm Bioaccumulation Assay

Soil samples were collected for earthworm bioaccumulation assays from four untreated tailings deposits in 1998 and from the same areas after amendment addition in 1999 and 2000. Mortality in the untreated tailings collected in 1998 was 100%; all worms died within 48 h of exposure to the soils. After amendment addition, replicate assays were conducted on soils collected from the same areas in both 1999 and 2000 (Table 3). Earth-

**Table 3. Earthworm survival and biomass for worms placed in tailings before (1998) biosolids and limestone amendment and at one and two years after amendment addition (1999 and 2000). Measurements are also presented for worms in an upstream uncontaminated control (UUC) and a contaminated vegetated area (CVA), and a laboratory control soil.**

Site	Survival			Biomass
	1998	1999	2000	1999
	%			mg wet weight
1	0b†	100a	83b	11a
2	0b	99a	92a	10.9a
3	0b	90b	80b	13.9a
4	0b	10c	78b	—
CVA		98a	83b	8.3b
UUC		97a	98a	6.8b
Lab control	100a	100a	98a	8.6b

† Means followed by the same letter are not significantly different at the 0.05 probability level.

**Table 4. Metal concentrations in earthworms exposed to amended mine tailings for 28 d in toxicity tests conducted in 1999 and 2000 in Leadville, CO. Worms were also exposed to a laboratory control soil and soils from upstream uncontaminated control (UUC) and a contaminated vegetated area (CVA).**

Site	Cd	Pb	Zn
mg kg <sup>-1</sup> dry wt.			
Amended tailings	18.1b <sup>†</sup>	270b	432b
UUC	12.1c	152b	244b
CVA	30.6a	786a	782a
Laboratory control	2.87d	1.36c	95.7c

<sup>†</sup> Means followed by the same letter are not significantly different at the 0.05 probability level.

worm tissue was also analyzed for metal accumulation after 28 d of exposure to the soils (Table 4). Metal concentrations (Cd, Pb, and Zn) in the worms in the treated soils were elevated in comparison with those in the UUC, but were lower than those in the CVA for all elements. Bioaccumulation factors (BAF: ratio of metal in the organism to metal in the soil) were also calculated for worms in the amended tailings and the UUC and CVA soils (data not shown). The BAF values for Cd, Pb, and Zn for worms in the amended areas were similar to BAF values in the CVA and lower than those observed in the UUC. Thus, amendment addition apparently reduced the bioavailability of contaminants in the tailings. Studies have shown that the pH in the earthworm intestines mediates uptake, and that soil adsorption is more important than soil pH in limiting Cd uptake (Oste et al., 2001a, 2001b). Similar results on biosolids-amended mine wastes have also been reported by Conder et al. (2001). It is unlikely that earthworms are naturally occurring in Leadville (due to the high altitude and low soil temperature). Nevertheless, this assay suggests that the amended tailings are capable of supporting an earthworm population, which is an important component of many self-sustaining ecosystems.

### Plant Growth

Soil samples were collected before amendment addition from two tailings deposits and refrigerated until use. Seedlings of perennial ryegrass were planted and germination and growth were measured. Amended tailings were also collected from the same areas in 1999 and 2000, one and two years after amendment addition. The same assay was performed. Before amendment addition, no germination was observed in the tailings materials compared with 87% germination in a laboratory control soil. After amendment addition, the germination rate was 71.4% in 1999 and 88% in 2000 (data not presented). Both rates were statistically similar to the germination rate attained in the laboratory control soil and the CVA and UUC materials. For the tests conducted in 1999 and 2000, both shoot length and biomass were measured. Shoot length in the amended tailings was similar to that observed in all other treatments for both years (data not presented). However, aboveground biomass in the amended tailings was approximately twice that observed in the lab control (84 versus 42 mg dry wt. per pot). Biomass in the amended soils also

**Table 5. Metal concentrations in aboveground tissue of ryegrass grown in a greenhouse in pots containing soils collected in the field from biosolids- and limestone-amended soils and an upstream uncontaminated control (UUC) and a contaminated vegetated area (CVA) in 1999 and 2000.<sup>†</sup>**

Location, year	Cadmium	Copper	Lead	Manganese	Zinc
mg kg <sup>-1</sup>					
Amended tailings					
1999	3.4 ± 1.4 <sup>‡</sup>	75 ± 31	313 ± 247	2028 ± 1800	670 ± 290
2000	1.9 ± 0.5	49.3 ± 5.8	88 ± 37	360 ± 113	385 ± 96
UUC					
1999	1.5	20	17	100	150
2000	0.5	12.5	3.3	34.2	62.5
CVA					
1999	8.9	30.8	343	161	920
2000	4.1	28	390	200	630

<sup>†</sup> Plants in the untreated control soils did not germinate.

<sup>‡</sup> Means followed by standard error ( $n = 6$ ).

exceeded that in the CVA (12.8 mg dry wt. per pot) and the UUC (24.4 mg dry wt. per pot).

Metal concentrations in aboveground biomass were also measured (Table 5). Both plant Zn and Pb concentrations were elevated in plants grown in soils collected in 1999, indicating high bioavailability of metals in the amended soils. In one sample from the 1999 study, Zn (1400 mg Zn kg<sup>-1</sup>) and Pb (930 mg Pb kg<sup>-1</sup>) concentrations in plant tissue were sufficient to indicate phytotoxicity (Chaney, 1993), but growth was not suppressed. Tissue metal concentrations decreased for plants grown in soils collected in 2000, suggesting stabilization of the amendment. Plant uptake of metals in soils receiving high rates of biosolids is often greatest in the first year after amendment addition and decreases thereafter (Brown et al., 1998).

### Field Samples

Field plant and small mammal samples were collected from the treated areas after amendment addition. Plant sample collection was limited to two of the treated areas, as two of the amended areas were used as pasture after a plant cover had been restored. The landowner harvested hay from the field and allowed cows to graze directly on the grass, limiting the plant material available for sampling. Plant species diversity and metal concentrations in aboveground tissue were measured. The treated areas had a higher occurrence of grass species and lower diversity than the CVA and the UUC (Fig. 2). The greater N content of amended soils may have permitted grasses to out-compete forbs. Species evolution and changes in plant community are important variables that may change over time. Future land use and populations in neighboring areas will influence species diversity on the sites over time.

Metal concentrations in plants from the amended tailings were greater in 2000 than 2001 (Table 6). Both Zn and Pb tissue concentrations in the 2000 sampling were sufficiently high to suggest potential phytotoxicity. Data variability in 2000 was greater than in 2001 suggesting uneven amendment addition or sampling in more contaminated areas. For both years, Zn, Cd, and Pb tissue concentrations were higher in the amended tailings than in the UUC. In 2001, tissue Cd and Pb concentrations

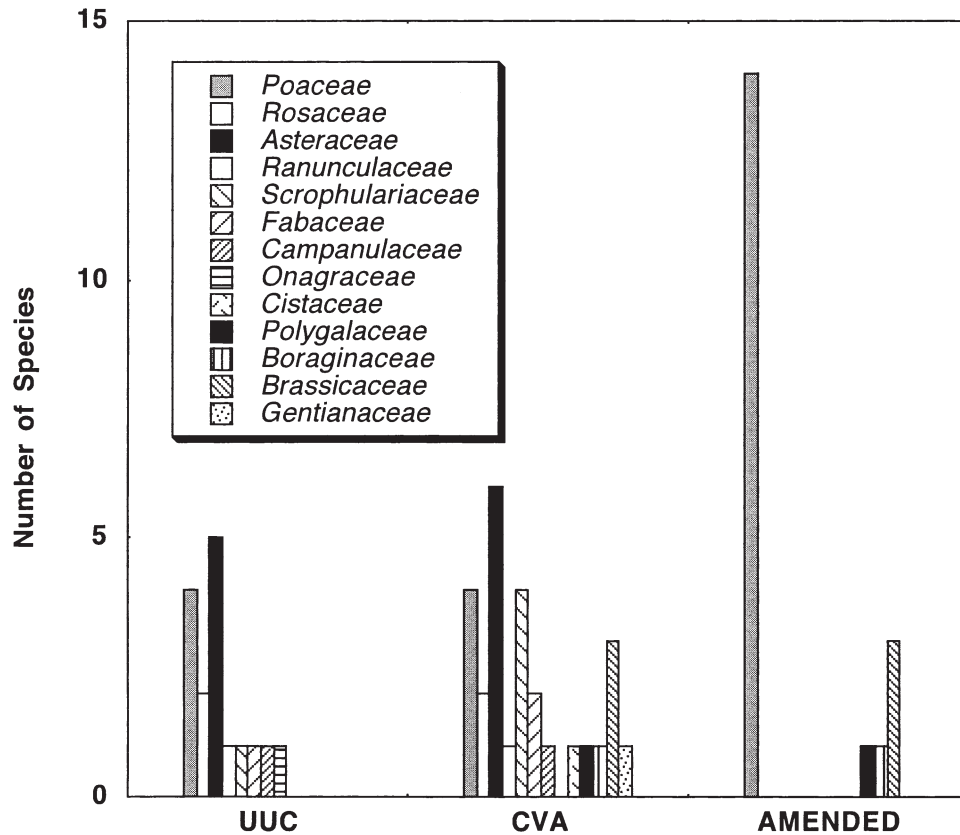


Fig. 2. Family and number of species of plants collected from the upstream uncontaminated control (UUC), contaminated vegetated area (CVA), and a biosolids- and lime-amended tailings deposit in 2000, Leadville, CO.

of plants from the amended tailings were similar to values for plants in the CVA. In 2001, tissue Zn concentrations were greater in the treated tailings than in the CVA but below levels associated with phytotoxicity for most species (Chaney, 1993).

### Small Mammals

As with the other field sampling portions of the study, no mammal samples were collected from the unamended tailings. There was no plant cover on these areas and no habitat for small mammals. Any mammals that might have been captured in these sections would have been passing through the area, and measures made on these animals would not reflect metal availability at the site. Mammals captured at the UUC, CVA, and the amended areas included voles (*Microtus* spp.), shrew (*Sorex*, unidentified species), short-tailed shrew (*Blarina brevicauda*), western jumping mouse (*Zapus princeps*), and deer mouse (*Peromyscus maniculatus*). Whole bodies of the animals were digested and analyzed for Zn, Pb, and Cd (Table 7). Food consumption patterns of the animals collected in this study differ: a higher portion of the diet of the shrews consists of insects, whereas the majority of the vole diet is plant based (Anderson et al., 1982; Andrews et al., 1984; Beardsley et al., 1978; Churchfield, 1982). Shrew diets frequently consist, in large part, of earthworms (Pankakoski et al., 1994; Read and Martin, 1993). As earthworms in the field contain large amounts of soil, shrews feeding on worms will inad-

vertently ingest soil. Consumption patterns also differ over the course of a year and with the age and reproductive cycle of the animal (Hunter et al., 1987). The mammal kidney and liver are usually targeted for metal analysis to assess if function has been impaired as a result of elevated metal burden (Beyer, 2000).

The elevated Zn at the site was not reflected in increased Zn body burden, because animals do not retain significant fractions of excess Zn in diets (Andrews et al., 1989; Cooke et al., 1990). Increased body burden of both Cd and Pb was observed in the amended and CVA

Table 6. Means ( $\pm$ standard errors) of Cd, Pb, and Zn in aboveground tissue of grass samples collected in 2000 and 2001 from uncontaminated (UUC), contaminated vegetated (CVA), and amended areas in Leadville, CO. Each collection included a range of grass species potentially including bluegrass (*Poa compressa* L.), tufted hairgrass [*Deschampsia cespitosa* (L.) P. Beauv.], horsetail (*Equisetum arvense* L.), and crested wheatgrass [*Agropyron cristatum* (L.) Gaertn.].

Location, year	Cadmium	Lead	Zinc
	mg kg <sup>-1</sup>		
UUC			
2000†	0.1 $\pm$ 0.1	1.4 $\pm$ 0.7	23 $\pm$ 7.6
2001‡	0.07 $\pm$ 0.03	0.3 $\pm$ 0.15	8.8 $\pm$ 1.4
CVA			
2000	0.6 $\pm$ 0.2	3.3 $\pm$ 3.3	77 $\pm$ 12
2001	1.2 $\pm$ 0.4	2.2 $\pm$ 0.8	69 $\pm$ 20
Amended tailings			
2000	4.5 $\pm$ 2.4	400 $\pm$ 282	546 $\pm$ 264
2001	0.9 $\pm$ 0.3	3.1 $\pm$ 0.6	121 $\pm$ 42

† In 2000,  $n \geq 2$ .

‡ In 2001,  $n \geq 7$ .

**Table 7. Small mammal number and whole-body Cd, Pb, and Zn concentrations from animals trapped in the upstream uncontaminated control (UUC), contaminated vegetated area (CVA), and biosolids- and limestone-amended tailings in Leadville, CO, in 2001.**

Species	Location	n	Cadmium	Lead		Zinc
				mg kg <sup>-1</sup>		
Vole	UUC	1	0.26	BD†		75
	CVA	1	2.1	12		130
	amended tailings	3	0.64 ± 0.01‡	7.87 ± 3.3		109 ± 13
Shrew (unidentified species)	UUC	3	0.67 ± 0.3	2.1 ± 0.4		94 ± 8
	CVA	3	3 ± 1.5	4.7 ± 1.4		113 ± 9
Short-tailed shrew	UUC	2	0.68 ± 0.4	1.27 ± 0.6		92.5 ± 9
	CVA	2	2.35 ± 0.35	6.1 ± 0.4		104 ± 8.5
	amended tailings	1	3	7.4		110
Western jumping mouse	UUC	3	0.1	2.4 ± 0.8		102 ± 29
	amended tailings	2	0.55 ± 0.06	12.5 ± 2.1		104 ± 9
Deer mouse	UUC	1	BD	6.5		97
	amended tailings	8	0.53 ± 0.06	17 ± 3		105 ± 4

† Below detection.

‡ Means ± standard errors.

sites. However, the increase was generally below thresholds associated with injury or other compromised function (Beyer, 2000; Hunter et al., 1983; Larison et al., 2000; Ma, 1989). Target organ concentration is often used as an indication of whether animals have been exposed to potentially harmful concentrations of Cd and Pb. Ideally, evidence of injury to these organs is used to verify compromised function (Beyer, 2000; Larison et al., 2000). There have also been studies where both total body burden and specific organ concentrations of Cd and Pb have been measured (Andrews et al., 1984; Cooke et al., 1990). In these studies the ratio of kidney to total body Cd generally ranged from 2:1 to 3:1 for voles, mice, and shrews. If a 3:1 ratio is used to calculate potential kidney Cd for the animals collected in the CVA and biosolids- and limestone-amended soils, the highest observed burden would be about 9 mg kg<sup>-1</sup> kidney Cd for both species of shrews collected. This is below values generally associated with damage, but precise concentrations associated with impaired function are a matter of debate (Beyer, 2000).

Shrews collected from Leadville do not include earthworms as a staple in their diets as earthworm survival at this altitude is limited. Thus, the total body burden of Cd and Pb for shrews in Leadville may not reflect the potential accumulation at other biosolids-remediated sites where earthworm populations have been restored (Ma et al., 1991).

**Table 8. Survival and mean growth of fathead minnows kept in water for 7 d from an eluate of a 4:1 water to soil ratio to assess potential toxicity associated with re-entrained tailings material. A lab control, water collected from the Arkansas River, water incubated with soil from the upstream uncontaminated control (UUC), control vegetated area (CVA), biosolids- and lime-amended tailings, and control tailings were included in the incubation.**

Location	Mean survival	Mean growth
	%	mg
Laboratory control	95a†	0.41a
Arkansas River water	100a	0.42a
UUC	92.5a	0.36a
CVA	92.5a	0.35a
Amended tailings	93.8a	0.38a
Tailings	0b	-

† Means followed by the same letter are not significantly different at the 0.05 probability level.

### Re-Entrainment Study

Survival of fathead minnows in water derived from the amended sites was similar to survival in water derived from all other treatments, except the unamended tailings (Table 8). All fish in the water from the unamended tailings died. Survival in all other treatments was greater than 90%. Growth was not impaired in the amended tailings. Fathead minnows are a standard species for this type of aquatic toxicity testing. The behavior of native trout in the Arkansas River in Leadville may or may not be comparable with the minnow used in this assay. However, these results suggest that the likelihood of resuspension of the amended tailings causing harm to aquatic organisms is low. Previous studies have linked the inorganic components of biosolids (e.g., Fe and Mn oxides) with observed metal-binding capacity (Hettiarachchi et al., 2003). In an anaerobic environment, metal oxides can dissolve and release complexed metals that potentially cause aquatic toxicity. The Arkansas is a well-aerated stream, so reduction of Fe and Mn oxides in the suspended sediment is not likely.

### CONCLUSIONS

The untreated tailings have high metal availability and reduced function for the soil community. Toxicity tests on these tailings showed 100% mortality for all toxicity endpoints measured. Field sampling for plant and small mammal analysis was not performed on untreated tailings as there was no plant cover and, therefore, no habitat for small mammals. Clearly, the metals and acidity from the tailings are sufficient to severely disrupt ecosystem function on the untreated sites.

Biosolids and lime amendment reduced metal availability and increased soil fertility sufficiently to restore function to the ecosystem. Ecosystem function measures on the amended tailings were generally comparable with those from the CVA and, in many cases, were similar to measures made on both laboratory control soils and the UUC. Soil function measures indicated greater microbial activity in the amended tailings than in either the CVA or the UUC. As the organic matter added in the biosolids is stabilized, rates of microbial activity may become similar to the CVA and UUC. Toxicity test



results using ryegrass, earthworms, and fathead minnows of the amended tailings were similar to results in CVA, UUC, and lab controls. Field sampling data suggests that the plant community in the amended sites is not as diverse as the CVA and UUA and has a higher frequency of grass species. This is potentially the result of the high N loading rates associated with the biosolids application. Metal concentrations in plant tissue collected from the amended tailings were greater than the UUC for both years, and greater than the CVA for the first year. Small mammal whole-body metal concentrations from amended tailings sites were similar to those of the CVA and do not suggest negative effect on function as a result of elevated metal concentration.

The combined data suggest that biosolids and lime amendment has restored function to the alluvial tailings deposits, but that the systems are not yet in equilibrium. By many measures, the amended tailings were indistinguishable from the UUC. However, other measures indicate increased function over the UUC soils, and certain measures suggest elevated metal availability over both the UUC and the CVA. Continued monitoring over time is necessary to determine if the restored system is sustainable.

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