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Stressor Identification (SI) at Contaminated Sites: Upper Arkansas River, Colorado

National Center for Environmental Assessment
Office of Research and Development, Washington DC 20460

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National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

NOTICE

ABSTRACT

During the performance of an ecological risk assessment, it is often unclear whether observed impairments are due to the contaminants of concern, to other contaminants or to other factors such as habitat disruption. In 2000, the U.S. Environmental Protection Agency developed a methodology for determining the causes of biological impairments termed Stressor Identification (SI), and the utility of the methodology is being demonstrated in the context of case studies for the Clean Water Act. The Agency has not applied this methodology to a waste site assessment. The purpose of this project was to apply the SI process at a terrestrial contaminated site to shed light on its utility in such an environment. The site chosen is in a highly mineralized area of the Colorado Rocky Mountains, consisting of the 500-year floodplain and adjacent irrigated lands of the Upper Arkansas River from the confluence of California Gulch to approximately 11 miles downstream. Impairments evaluated were barren areas in the floodplain (reduced vegetation), and reduced plant growth and plant species richness in meadows irrigated with water from the Upper Arkansas River. After a number of candidate causes were considered, the various lines of evidence support the interaction of elevated levels of extrinsic metal with decreased pH as the cause of the barren areas in the floodplain. Similar evaluation of reduced plant growth and plant species richness in the irrigated meadows leads to the conclusion that elevated levels of extrinsic metal is the cause. Aspects of the assessment process that may differ between aquatic and terrestrial systems include the critical variables that are measured, degree of development of bioassessment criteria, spatial heterogeneity and linearity of physico-chemical factors, and management practices. This project demonstrates the usefulness of the SI methodology for terrestrial systems.

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United States Geological Survey (http://toxics.usgs.gov/photo_gallery/photos/upper_ark/upper_ark_river_lg.jpg). A view of the Arkansas River, Colorado.

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LIST OF ABBREVIATIONS

ASTM	American Society for Testing and Materials
AVIRIS	Airborne Visible and Infra-Red Imaging Spectrometer
BA	Smith ranch
BLM	Biotic Ligand Model
$CaCO_3$	lime
CEC	cation-exchange capacity
CV	coefficient of variation
CVA	contaminated vegetated area
DIC	deviance information criterion
DTPA	diethylenetriaminepentaacetate
GLIM	generalized linear model
lpH	laboratory measured pH
NPL	National Priority List
NRCS	Natural Resources Conservation Service
RI/FS	Remedial Investigation/Feasibility Study
RipES	Riparian Evaluation System
SB	Seppi ranch
SC	Seppi ranch
SD	Seppi ranch
SI	stressor identification
TCLP	toxicity characteristic leaching procedure
TKN	total Kjeldahl-N
TP	Tennessee Park
TSP	triple-super-phosphate
U.S. EPA	U.S. Environmental Protection Agency
UAR	Upper Arkansas River
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
UUC	upstream uncontaminated reference site

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1. STEP 1: DEFINE THE CASE

1.1. INTRODUCTION

With the development of biological survey and bioassessment methods, it is increasingly common for ecological risk assessments for contaminated sites to include descriptions of the actual biological impairment. However, whether those observed impairments are due to the contaminants of concern, to other contaminants or to other factors such as habitat disruption is often unclear. Without a formal, defensible method to determine causes, the use of bioassessment results in baseline risk assessments may be challenged. The United States Environmental Protection Agency (U.S. EPA or the Agency) has developed a methodology for determining the causes of biological impairments termed Stressor Identification (SI; U.S. EPA, 2000), and the utility of the methodology is being demonstrated in the context of case studies for the Clean Water Act. While the methodology is being applied by consultants in waste site assessments, the Agency has not addressed this application. The purpose of this project is to apply the SI process at a terrestrial contaminated site to shed light on its utility in such an environment. Although there is no a priori reason that the methodology for performing a causal assessment should differ between aquatic and terrestrial systems, there are inherent differences between these environments. Aspects of the assessment process that may differ between aquatic and terrestrial systems include the critical variables that are measured, degree of development of bioassessment criteria, spatial heterogeneity and linearity of physico-chemical factors, and management practices. This causal analysis was performed using the procedure in the SI Guidance Document (U.S. EPA, 2000), and updated with information from U.S. EPA's current procedure for causal analysis at http://www.epa.gov/caddis. Another terrestrial case study, focusing on an oil field in California, is also available (see www.epa.gov/caddis, U.S. EPA, 2008).

The SI process follows five steps that conclude with the identification of a probable cause. These steps are (1) Define the case, (2) List the candidate causes, (3) Evaluate data from the case, (4) Evaluate data from elsewhere and (5) Identify probable cause. The first two steps are Chapters 1 and 2, respectively. Steps 3, 4 and 5 are contained within Chapter 3. The lessons learned from performing this case study are summarized in Chapter 4.

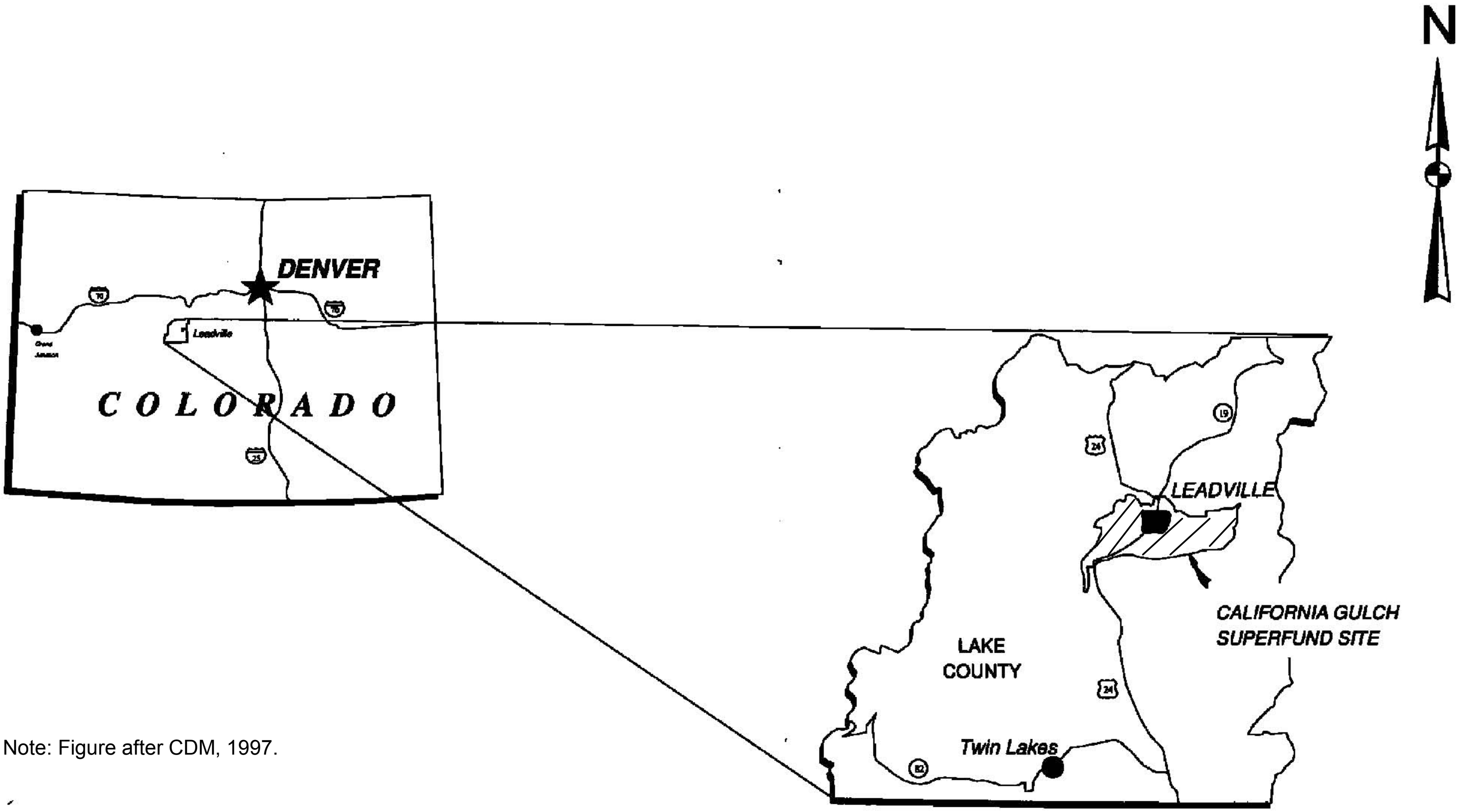
The site chosen for this case study is located in a highly mineralized area of the Colorado Rocky Mountains, approximately 100 miles southwest of Denver, CO (see Figure 1). It is comprised of the 500-year floodplain and adjacent irrigated lands of the Upper Arkansas River from the confluence of California Gulch to approximately 11 miles downstream (see Figures 2 and 3). Beyond this point, the Arkansas River is constricted into a canyon. Part of the 11 miles overlaps the California Gulch National Priority List (NPL) Site. The elevation at the confluence of California Gulch with the Arkansas River is 9515 feet above mean sea level. The impairments evaluated in this study were barren areas in the floodplain (reduced vegetation), and reduced plant growth and plant species richness in meadows irrigated with water from the Upper Arkansas River. Following consideration of a number of candidate causes and lines of evidence, the probable cause of each impairment was determined through a strength of evidence analysis that uses all the evidence generated in the analysis phases to examine the credibility of likely candidate causes. The causal considerations for the strength of evidence analyses use three types of evidence: case-specific evidence, evidence from other situations or biological knowledge, and evidence based on multiple lines of evidence.

1.1.1. Site History

The site is located in a highly mineralized area of the Colorado Rocky Mountains. The most prominent minerals in the ore deposits include iron (Fe), manganese (Mn), zinc (Zn), lead (Pb), copper (Cu), and small amounts of gold (Au) and silver (Ag) (U.S. EPA, 2004a). Mining, mineral processing and smelting activities have produced gold, silver, lead, manganese and zinc for more than 130 years. Mining began in the Leadville area in 1859 when prospectors working channels of Arkansas River tributaries discovered gold at the mouth of California Gulch. Initial activities consisted only of small-scale placer mining until 1868 when the first gold ore veins were discovered along California Gulch. However, by 1872, problems with water transportation and labor made ore removal so difficult that most miners left the area. In 1874, silver-bearing lead carbonate was discovered and mining in the Leadville district boomed (U.S. EPA, 2004a).

Extensive deposits of lead, silver and gold ores associated with fissure veins were discovered and mined. Zinc and manganese, which were of little value in the early days, later were mined extensively. As surface veins diminished, miners tunneled deeper into the mountains. As underground mines were established, groundwater had to be pumped out continuously. In 1889, the Yak Tunnel was constructed to drain water from the mines. Through the years, the Yak Tunnel was extended more than three miles eastward to drain other underground mines. Until 1992, untreated water from the Yak Tunnel drained directly into California Gulch (U.S. EPA, 2004a; U.S. EPA Region 8 and USFWS, 2003).

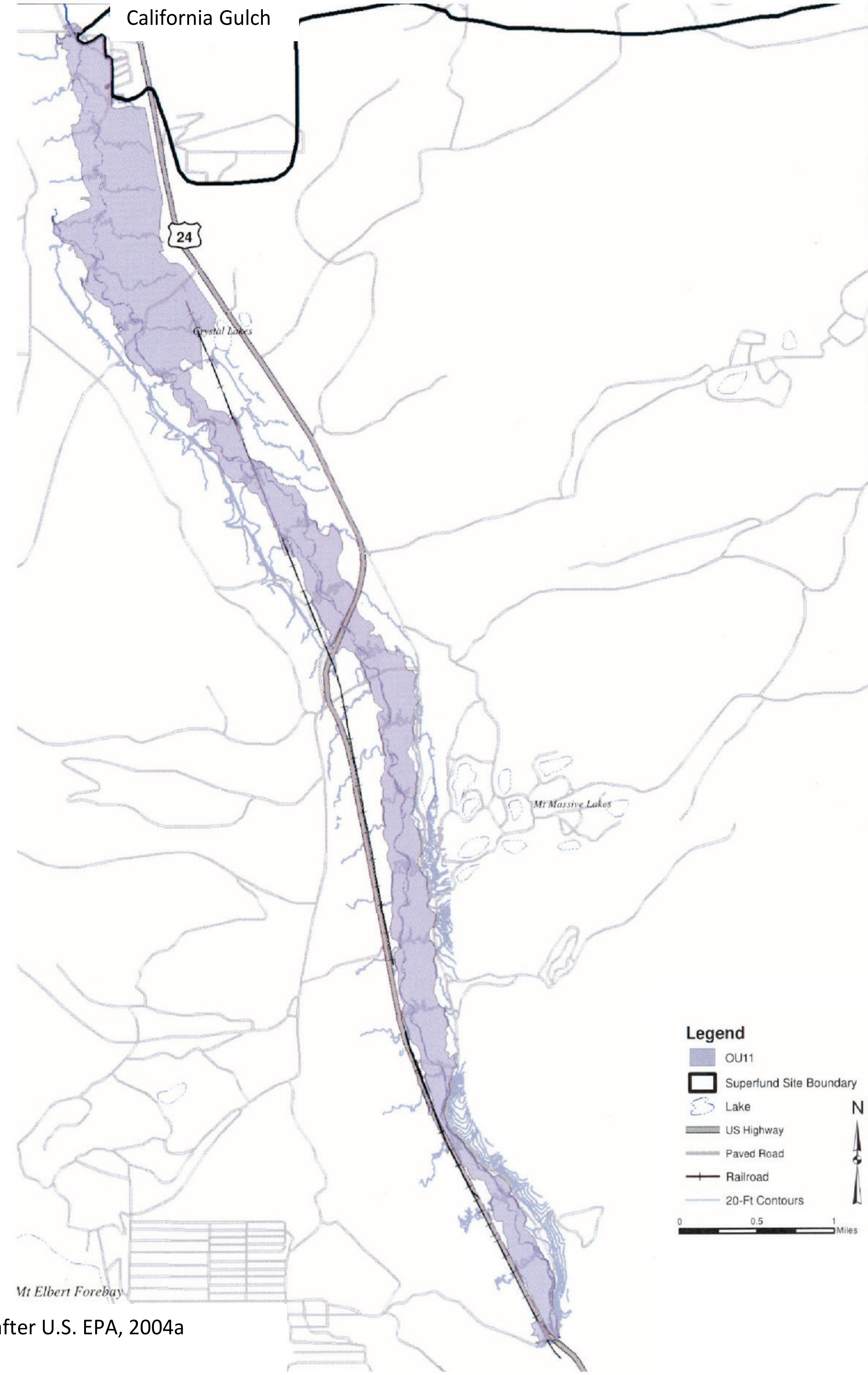
As mines were developed, waste rock was excavated along with the ore. The waste rock was placed near the mine entrance, and the ore was transported to the mill. At the mill, ores were crushed and separated into metallic concentrates and waste products by physical processes. The metallic concentrates were then shipped elsewhere or further processed at a smelter in the area. Forty-four different smelters are known to have operated in the California Gulch area at various times. Because of the crude methodology, the tailings from these operations had relatively high residual metal content and were coarse sand to gravel-sized particles. The lack of area for storage of milling wastes led to the release of these wastes directly into tributary drainages to California Gulch as a method for disposal. This resulted in downstream movement of mine waste during periods of normal flow and large transport events during flooding (U.S. EPA, 2004a; U.S. EPA Region 8 and USFWS, 2003). Mining activity occurred in most of the watersheds in the region, both upstream and downstream of the California Gulch confluence with the Arkansas River, but the greatest concentration of mining activity took place in the California Gulch watershed.



Note: Figure after CDM, 1997.

FIGURE 1. Location of the 11-Mile Reach of the Arkansas River

FIGURE 2. Study Area Terrain Map



Note: Figure after U.S. EPA, 2004a

FIGURE 3. The 500-Year Floodplain and Adjacent Irrigated Meadows Area (OU11) of the Upper Arkansas River

1.1.2. Riparian Areas

Releases of mine tailings into the upper Arkansas River floodplain have occurred and continue to occur as a result of releases from sources within the California Gulch drainage area in Leadville, CO (USFWS, 2002). Fluvial mine tailing deposits are found at 153 sites along the floodplain of the upper Arkansas River. They cover an area of 2,829,911 ft^2 (64 acres) and have an estimated volume of 2,698,514 ft^3 (see Figures 4 through 6).

An upstream area referred to as Reach 0 was evaluated as a relatively less-impacted reference area. The 11-mile reach of the upper Arkansas River floodplain was divided into four sub-reaches (Reach 1, 2, 3 and 4) based on the characteristics of the channel and of the fluvial mine-waste deposits (USFWS, 2002).

Reach 0. The upstream reference area, Reach 0, includes portions of Tennessee Creek and the east fork of the Arkansas River upstream from their confluence, and the Arkansas River extending downstream to just upstream of the confluence of California Gulch (see uppermost portion of Figure 4). Mining also occurred in the watersheds of both Tennessee Creek and the east fork of the Arkansas River. Consequently, Reach 0 has been influenced by mining activity. The primary mining activity in the Tennessee Creek drainage occurred in St. Kevin's Gulch, but there are also other abandoned mines in the Tennessee Creek drainage. Although mining has occurred at numerous locations in the east fork Arkansas River drainage, the primary mining influence has likely been the discharge of water from the Leadville Mine Drainage Tunnel. The upstream reach (Reach 0) was characterized as a point of comparison to establish baseline conditions for the 11-mile reach, with the realization that mine wastes have affected this area. Despite the mining influences upstream of California Gulch, no fluvial mine-waste deposits were found within Reach 0 (Keammerer, 1987).

Vegetation in Reach 0 is dominated by a riparian shrub community consisting primarily of willow species, interspersed with open water wetlands and grasses. The uplands are dominated by herbaceous riparian vegetation consisting of sedges, rushes and mesic grasses representative of moist soils. These areas are interspersed with upland grasses (CDOW, 1988).

Downstream Reaches. Fluvial deposits in the first few miles downstream of California Gulch appear to be older, coarser mine wastes than those found further downstream in the 11-mile reach (see Reach 1 in Figure 4). Approximately three miles downstream of California Gulch, Lake Fork Creek joins the Arkansas River. The flow from Lake Fork Creek is augmented by large volumes of water diverted from the western slope of the Rocky Mountains. The dilution effects of the augmented flow are significant, resulting in reductions of metal concentrations in the Arkansas River. Water quality continues to improve downstream as the flow from more tributaries dilutes the contaminated water of the Arkansas River (USFWS, 2002).

For the next several miles downstream from the Lake Fork Creek confluence, the floodplain broadens (see Reach 2 in Figure 4). The volume of tailings deposits per stream length is less than that upstream of Lake Fork, probably due to the increased flow capacity of the channel in this area, which would reduce the frequency of overbank flow conditions. Further downstream, however, the number of deposits increases as the channel becomes shallower, making the creek more susceptible to overbank flow (see Reach 3 in Figures 5 and 6). Over the remaining length of the 11-mile reach, the floodplain generally narrows, resulting in increased flow velocity and less deposition of mine waste sediments (see Reach 4 in Figure 6). Only a few, small deposits are present along this section of the 11-mile reach (USFWS, 2002). Details on the vegetation of the downstream reaches of the upper Arkansas River floodplain are contained in Appendix A.

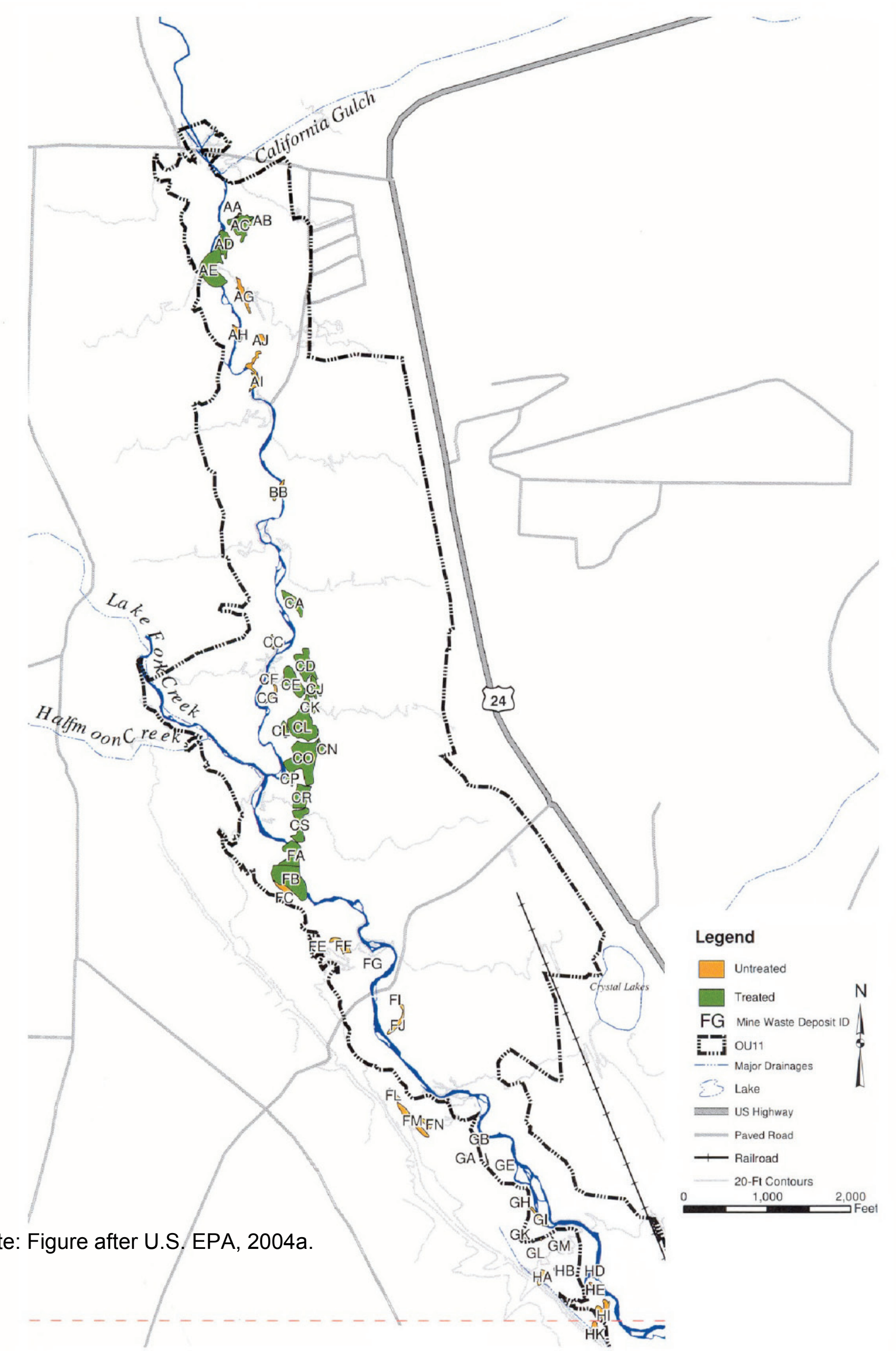
1.1.3. Irrigated Meadows

Soils suitable for irrigated pasture and hay production are found just outside the 500-year floodplain of the Upper Arkansas River in several locations along the upper, middle and lower parts of the 11-mile reach (Fletcher, 1975). Areas of Newfork gravelly sandy loam and Rosane loam typically are found in association with each other. Small areas of marsh are included in the areas of Newfork and Rosane soils. Several areas of Newfork-Marsh-Rosane soil association have been irrigated with water from California Gulch, the Upper Arkansas River and/or from other tributaries. Nearly all areas of Rosane loam soil are flood irrigated and used as pasture grazed by cattle. Newfork gravelly sandy loam soil occurs on low terraces in Lake County. Most areas of this soil are in irrigated meadow that is cut for hay and grazed early and late in the growing season. The marsh soil areas are of little grazing value and are used by wildlife for food and protection.

1.2. EVIDENCE OF IMPAIRMENT

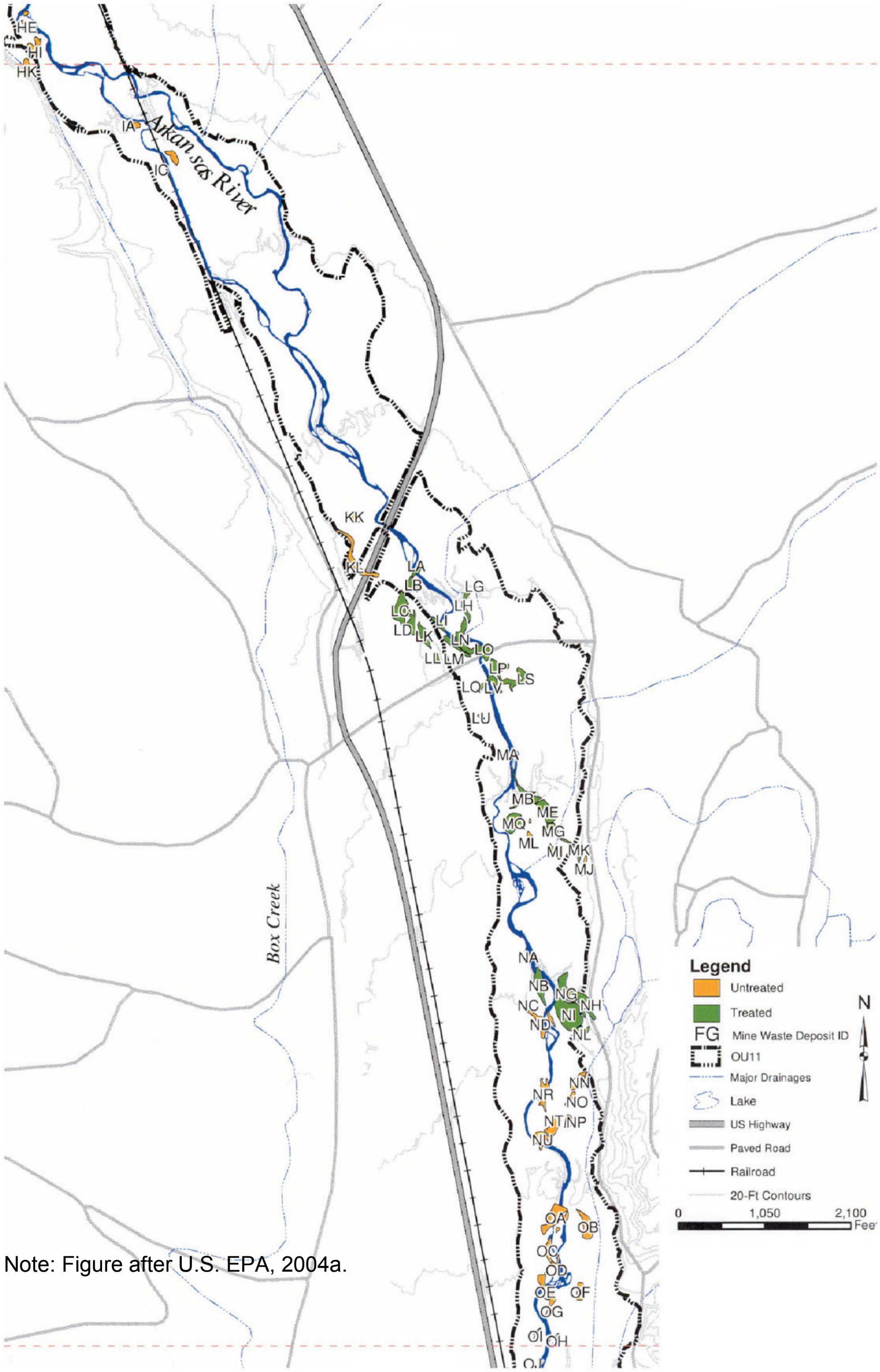
1.2.1. Impairment 1: Barren Areas in the Arkansas River Floodplain

A total of 153 separate fluvial mine waste deposits with an overall area of 64 acres have been delineated in the 500-year floodplain of the upper Arkansas River along the 11-mile reach (USFWS, 2002). Growth of vegetation on these deposits is impaired. The vegetative cover is less than 10% on 42 of the deposits, between 10−50% on 84 deposits, and greater than 50% on 27 deposits (Table 1 in Chapter 3). Outside the fluvial mine waste deposits, the vegetative cover, biomass and number of plant species on riparian (floodplain) soils below the confluence of California Gulch are equal to or greater than those upstream of California Gulch (USFWS, 2002).



Note: Figure after U.S. EPA, 2004a.

FIGURE 4. Fluvial Mine-Waste Deposit Locations in Reaches 1 and 2 Along the Arkansas River Floodplain. The confluence of Lake Fork Creek and the Arkansas River marks the boundary between Reach 1 (upstream, or North) and Reach 2 (downstream, or South). Reach 0, the upstream reference area, is North of California Gulch



Note: Figure after U.S. EPA, 2004a.

FIGURE 5. Fluvial Mine-Waste Deposit Locations in Reaches 2 and 3 Along the Arkansas River Floodplain. The highway (24) crossing the Arkansas River marks the boundary between Reach 2 (upstream, or North) and Reach 3 (downstream, or South)

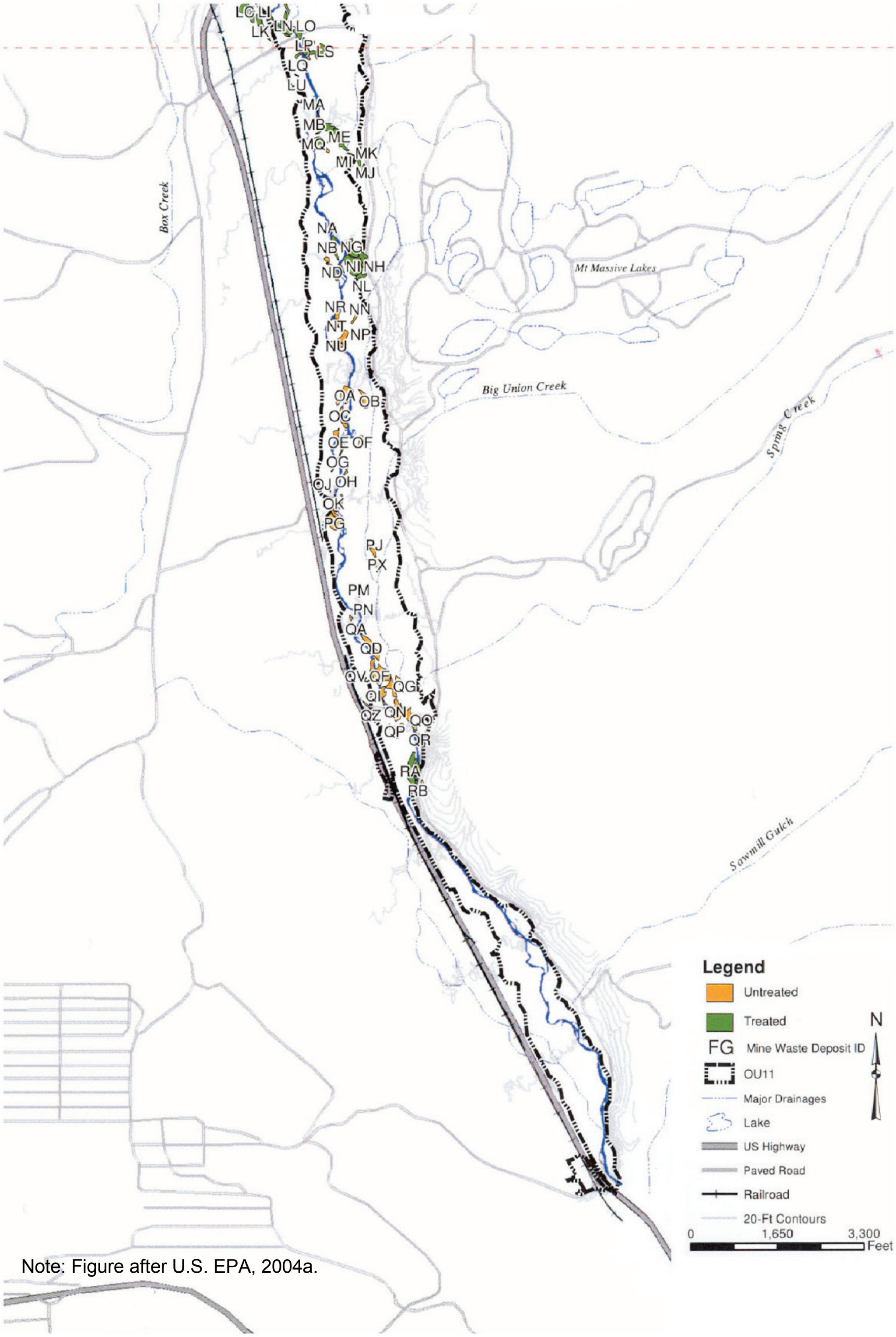


FIGURE 6. Fluvial Mine-Waste Deposit Locations in Reach 3 Along the Arkansas River Floodplain. Reach 4 begins just downstream, or South, of the confluence of Spring Creek and the Arkansas River where the floodplain narrows

1.2.2. Impairment 2: Reduced Plant Growth and Reduced Plant Species Richness in Irrigated Meadows

Plant growth in the irrigated meadows has also been affected. One ranch shows evidence of severely chlorotic plants and barren sections of ground (Levy et al., 1992; Swyers, 1990). Aerial photographs of the most affected meadows show areas of barren ground intermingled with vegetated areas. The affected area is approximately 354 contiguous acres. Levy et al. (1992) stated that forage yield in the affected irrigated meadow has decreased from 4.48 megagrams/hectare (Mg/ha) (3990 lbs/acre) in 1874 to 1.68 Mg/ha (1496 lbs/acre) in the early 1990s. Swyers (1990) quoted Dr. Bernard Smith, a local veterinarian and rancher, as stating that ranchers had noted decreases in hay yield after mining and smelting became widespread in the late 1880s. Swyers (1990) conducted an investigation of the extent of metal contamination in the soils of a large irrigated meadow on the property of E. Seppi. He reported barren areas that were located near an old irrigation ditch. He also reported an area described as "a barren, gray and white, crusted wasteland." This area, however, was located in the flood basin near the Arkansas River and may be in a fluvial mine waste deposit area.

Differences in plant species richness in irrigated pastures above and below the confluence of California Gulch with the Arkansas River have also been reported (Levy et al., 1992). Nine plant species were observed in an irrigated meadow on a similar soil type located upstream of the California Gulch confluence. This upstream meadow had received relatively "clean" irrigation water from Tennessee Creek. In contrast, at three locations in irrigated meadows just downstream of the California Gulch confluence, only three or four plant species were observed at each sampling location. Similar low plant species abundance (four species) was observed in a nonirrigated, riparian, floodplain area used for grazing, just downstream of the California Gulch confluence (Levy et al., 1992). Several of the plant species at the upstream location were also present in at least one of the downstream locations, but three species, Alpine timothy (*Phleum alpinium*), dandelion (*Taraxacum* spp.) and clover (*Trifolium* spp.), were not reported at any of the four downstream locations.

1.2.3. Impairment 3: Effects on Livestock

Area ranchers recognized the poor health of livestock raised downstream from the California Gulch confluence. The most affected areas were the ranches of the Seppi and Smith families, located from the California Gulch confluence to 5.5 miles downstream. Livestock grazed on these irrigated meadows suffered from swelling of the joints, coat discoloration, and scanty weight gain (Levy et al., 1989). Young colts and calves were most likely to develop symptoms of trace metal toxicity through ingestion of contaminated forages, but would recover within a few weeks when moved to uncontaminated meadows upstream from California Gulch (Levy et al., 1992). Due to a paucity of more specific data on this impairment, effects on cattle are not evaluated further in this report.

1.3. REGULATORY AUTHORITY

The Colorado Water Quality Control Act of 1981, CRS 1973, 25-8-101 et seq. (“Act”) designates stream classifications and water quality standards for state waters of the Arkansas River Basin. It also contains provisions for establishing basic standards, and an antidegradation rule. The basic regulations establish a system for classification of state waters according to “the beneficial uses for which they are suitable or are to become suitable, and for assigning specific numerical water quality standards according to such classifications” (CDPHE, 2004).

Streams and water bodies throughout Colorado have been designated as “High Quality—Class 1” and “High Quality—Class 2” where evidence indicates that the requirements of Section 3.1.13(1)(e) of the basic regulations contained in the Act have been met. The Arkansas River Basin is designated as High Quality—Class 2; however, California Gulch was determined to be one of the most degraded streams in Colorado. As a result, the State of Colorado Water Quality Commission has adopted only a limited set of numeric water quality standards for compliance within the California Gulch. Under the State’s *Mined Land Program*, mining discharge to California Gulch is to be eliminated. Goals have been established for cleanup of the site to allow for eventual agricultural redesignation.

For existing mining, 40 CFR, Section 440—ore mining and dressing—regulates the runoff from mining sites, provides guidelines for reestablishing vegetation on exposed soils, and provides guidelines for minimization of runoff. A *Metal Mining Stormwater Management Plan* is required for any operations that may result in direct discharge or stormwater runoff from a mining site. This plan must establish procedures to determine that both state and federal water quality standards are met.

2. STEP 2: LIST CANDIDATE CAUSES

2.1. IMPAIRMENTS 1 AND 2

Seven candidate causes for the observed impairments of plant growth and plant species richness in the riparian and irrigated meadows were considered. (Cause #7 is only applicable to the irrigated meadows.) The candidate causes are shown in terms of a conceptual model (see Figure 7).

1. **Increased concentrations of extrinsic metals** in the soil may result in increased levels of soluble metals and their transport throughout the environment. Increased metals in solution may also become bioavailable and lead to the increased uptake of metals by plants, resulting in phytotoxicity. Mine wastes and/or smelter emissions may have resulted in metal concentrations high enough to be phytotoxic, even at neutral pH levels.
2. Interaction of **decreased pH and increased extrinsic metals** in the soil from mining operations may result in increased levels of metals in soil solution (Arienzo, 2005). Increased metals in solution may lead to increased uptake of metals by plants, resulting in phytotoxicity. Solubility of many metals is increased at lower pH levels. Increased soil metal concentrations may interact with reduced pH levels due to acid mine drainage or smelter emissions (which are also acidic), resulting in bioavailable metal concentrations that are phytotoxic.
3. Interaction of **decreased pH and intrinsic (background) metals** in the soil may result in increased levels of metals in soil solution, causing phytotoxicity. Solubility of many metals is increased at lower pH levels. The upper Arkansas River area is highly mineralized. Soils derived from these parent materials may have high background concentrations of metals. Acid mine drainage or smelter emissions could result in decreased pH levels that could result in phytotoxicity at background soil metal concentrations. Soil pH levels below 5.5 result in increased solubility of aluminum (Al) and Mn that can be toxic to plants at background concentrations (Rengel, 2002). (Below pH 5.5, Mn oxides solubilize and release Mn^{2+} into soil solution. At soil pH levels <4.2, Al ions dominate the soil solution. At soil pH levels <3.8, Fe^{2+} becomes the dominant ion.)
4. **Decreased soil organic matter** results in higher metal bioavailability. Soil organic matter binds metal cations, removes them from solution and reduces metal uptake (Arienzo, 2005). Decreased soil organic matter, either in mine waste deposits or resulting from agricultural tillage, could result in reduced soil microbial activity and potentially reduced mycorrhizal colonization of plant roots. Mycorrhizal colonization of plant roots enhances plant growth by increasing uptake of phosphorus. The low organic matter content in mine waste deposits could result in the increased uptake of metals by plants and phytotoxicity. There also is evidence that mycorrhizae may reduce metal uptake due to immobilization of metals in fungal cell walls (Punshon et al., 2005). Agricultural tillage over a

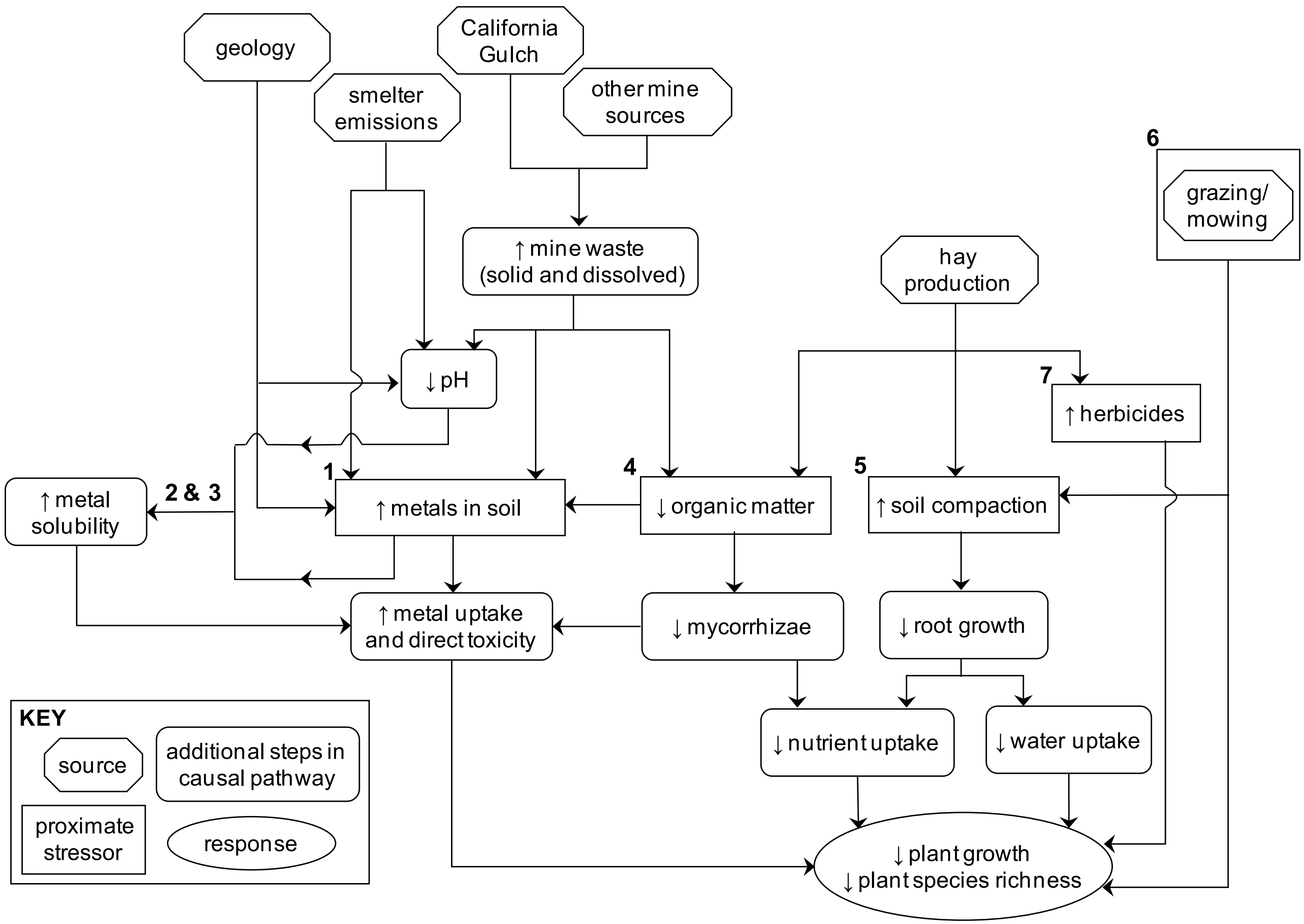


FIGURE 7. Conceptual Model for Seven Candidate Causes for Impairments of Plant Growth and Plant Species Richness

Candidate causes are identified by number.

period of years results in more rapid oxidation of soil organic matter and also results in lower organic matter. Lowered organic matter content could result in increased metal bioavailability and phytotoxicity.

5. **Increased soil compaction** can reduce plant growth. Cattle grazing and, to a lesser extent, hay production, particularly when soils are saturated, can result in soil compaction. Cattle grazing in a montane riparian ecosystem in northern Colorado caused a decrease in infiltration rates and an increase in bulk density at 5−10 cm and 10−15 cm depths, though conditions returned to predisturbed values within 1 year after grazing events (Wheeler et al., 2002). Normal farming operations such as tilling or harvesting can also cause soil compaction (USDA, 1996). Lastly, soil compaction can arise from differences in soil origin or type, e.g., deposited fines. Soil compaction reduces the permeability of the soil surface layers, resulting in reduced water availability, reduced nutrient movement and reduced availability of oxygen to roots (USDA, 1996). This may reduce root growth and thus, lower the availability of nutrients for plant growth. Decreased root growth due to soil compaction would reduce water availability for plant growth (Unger and Kaspar, 1994).
6. **Increased grazing and mowing** (for hay production) can affect plant growth or species richness (Hickman et al., 2004; Williams et al., 2007). Cattle preferentially graze certain plant species (Uresk, 1986), which may reduce their abundance. Some plant species are more able to withstand grazing pressure, which allows them to increase in abundance in grazed areas (e.g., Kentucky bluegrass, *Poa pratensis* [Schulz and Leininger, 1990]). Similar effects may also occur as a result of mowing.
7. **Increased herbicide usage** to enhance hay production may affect plant growth or species composition (Tomkins and Grant, 1977). Repeated applications of selective herbicides over a period of years could eliminate some plant species and preferentially select for others. High application rates of persistent herbicides can also reduce plant growth. This candidate cause is only relevant in the irrigated meadows and hence was considered for Impairment 2.

3. IMPAIRMENTS 1 AND 2: EVALUATE DATA AND IDENTIFY PROBABLE CAUSES

3.1. IMPAIRMENT 1: ARKANSAS RIVER FLOODPLAIN BARREN AREAS

Data on soil metal concentrations, pH, organic matter content and plant growth were used to evaluate associations among the candidate causes and areas of impaired plant growth in the Arkansas River floodplain. Information from reports published in peer-reviewed journals, U.S. Geological Survey (USGS) reports, Remedial Investigation/Feasibility Study (RI/FS) reports, graduate theses and undergraduate research reports were used.

Step 3: Evaluate Data from the Case

Several investigations provide evidence on the potential causes of impaired plant growth in the upper Arkansas River floodplain. At the request of EPA, URS (1997, 1998) identified and selected mine-waste deposits from landowner's input, as well as from observations of tailing material on cut banks, dead vegetation and/or metal salts on the soil surface. The relative vegetation cover of the mine-waste deposits was evaluated on a qualitative scale of 1 to 3 and compared with average metal concentrations along the 11-mile reach of the Upper Arkansas River (USFWS, 2003). The significance of trends in metal concentrations with distance from the confluence of California Gulch was tested statistically. Walton-Day et al. (2000) compared concentrations of metals in 11 barren fluvial mine waste deposits to concentrations in two nearby vegetated areas. A number of studies that performed experimental manipulation of exposure in the lab and field provided particularly strong evidence for determining the cause of impaired plant growth (Brown et al., 2005; Fisher, 1999; Fisher et al, 2000). Brown et al. (2005) added lime and municipal biosolids to a depth of 20 cm into fluvial mine-waste deposits at four sites, and examined resulting metals concentrations, pH, organic carbon, total Kjeldahl-N (TKN), and phosphorus. Properties of the amended deposits were compared to those from the unamended deposits, an upstream uncontaminated reference site (UUC), and a contaminated vegetated area (CVA). Brown et al. also conducted phytotoxicity tests using rye grass emergence and early growth protocols, measured above-ground plant tissue concentrations, and evaluated plant species diversity. Fisher (1999; Fisher et al., 2000) conducted greenhouse studies with fluvial mine-waste tailing from the Arkansas River floodplain amended with lime, biosolids or lime plus biosolids with Geyer willow cuttings. Fisher (1999) also conducted field experiments on effects of lime, biosolids and phosphorus on establishment of plant cover (tufted hairgrass and creeping bentgrass) and Geyer willow. The results of the above studies are combined to evaluate the strength of evidence.

A. *Fluvial Mine-Waste Deposit Areas*

U.S. EPA charged the Superfund Technical Assessment and Response Team with locating and characterizing fluvial mine-waste deposits along 10 miles of the

Arkansas River, with the confluence of California Gulch and the Arkansas River being the northern boundary (URS, 1997, 1998). The study focused on fluvial mine-waste deposits within 100 feet of the river. Data were collected on location, depth and area of each deposit. Samples were analyzed for total metal concentrations. Using these data, location information was paired with aerial photographs obtained from geographic information system (GIS) to obtain a more accurate estimate of the area of the deposits.

Mine-waste deposits were identified from landowner's input, as well as from observations of tailing material on cut banks, dead vegetation and/or metal salts on the soil surface. Some deposits were observed but not studied because they were small, had vegetation cover and/or were away from the riverbank (URS, 1997, 1998).

The relative vegetation cover of the mine-waste deposits was evaluated on a qualitative scale of 1 to 3. Mine-waste deposits with >50% vegetative cover were rated as good and assigned a rating of 1, deposits with 10−50% vegetative cover were rated as fair and assigned a rating of 2 and deposits with <10% vegetative cover were rated as poor and assigned a rating of 3 (see Tables 1 and 2).

Reach 1. Twenty-four mine-waste deposits were selected for study in Reach 1. Among these deposits, there was a total of approximately 887,000 ft^3 of mine waste, covering a surface area of approximately 785,364 ft^2. The average depth of the deposits was 1.1 ft; no deposits averaging greater than 2.0 ft in depth were found. Total metal concentrations in the deposits averaged 177 mg/kg for cadmium (Cd), 446 mg/kg for Cu, 4228 mg/kg for Pb and 7271 mg/kg for Zn (see Table 2).

Reach 2. Thirty-five mine-waste deposits were identified and sampled in Reach 2 (URS, 1998). Among these deposits, there was a total of approximately 233,389 ft^3 of mine waste, covering a surface area of approximately 405,936 ft^2. The average depth of the deposits was 0.6 ft, and no deposits averaging greater than 1.5 ft in depth were found. Total metal concentrations in the deposits averaged 153 mg/kg for Cd, 200 mg/kg for Cu, 3266 mg/kg for Pb and 3438 mg/kg for Zn (see Table 2).

Reach 3. Ninety-four mine-waste deposits were identified and sampled in Reach-3 (URS, 1998). Among these deposits, there was a total of approximately 1,578,311 ft^3 of mine-waste, covering a surface area of approximately 1,639,612 ft^2 in Reach 3. The average depth of the deposits was 1 foot. Seven deposits averaging over 2 ft in depth were found, with one deposit having an average depth of 3 ft. Total metal concentrations in the deposits averaged 129 mg/kg for Cd, 258 mg/kg for Cu, 3059 mg/kg for Pb and 4926 mg/kg for Zn (see Table 2).

In Reach 3 (not in other reaches), pH values were reported for several of the fluvial mine-waste deposits. The pH values in the fluvial deposits in Reach 3 ranged from 1.26−5.80 (USFWS, 2002).

TABLE 1. Summary of Vegetation Coverage of Fluvial Mine-Waste Deposits in the Upper Arkansas River Floodplain				
Reach Distance[a]	Vegetative Cover Evaluation (Categories defined by percentage of mine-waste deposit with vegetative cover)			Total
	Poor ≤10%	Fair 10−50%	Good >50%	
1 (1.8 miles)	14 (58)[b]	9 (38)	1 (4)	24
2 (1.8−5.6 miles)	2 (6)	19 (54)	14 (40)	35
3 (5.6−9.5 miles)	26 (28)	56 (60)	12 (13)	94

[a]Arkansas River miles downstream from confluence with California Gulch.
[b]Percent of fluvial mine-waste deposits in each reach.
Table adapted from USFWS (2002).

TABLE 2. Comparison of Vegetation Coverage and Average Metal Concentration in Fluvial Mine-Waste Deposits					
Reach #	Avg Veg Class*	Avg Cd mg/kg dry wt	Avg Cu mg/kg dry wt	Avg Pb mg/kg dry wt	Avg Zn mg/kg dry wt
0	NA	3.3	29.9	238	428
1	2.54	177	446	4228	7271
2	1.66	153	200	3266	3438
3	2.14	129	258	3059	4926

*Vegetation coverage class: good (1, >50%); fair (2, 10−50%); poor (3, <10%).
Table adapted from USFWS (2002).

Reach 4. Small areas of fluvial mine-waste deposits occurred along Reach 4, but there were no data available to characterize the physical or chemical characteristics of these deposits.

Summary. Average metal concentrations in the fluvial mine-waste deposits declined with distance from the confluence of California Gulch with the Arkansas River. The concentrations furthest downstream in Reach 3 were still highly elevated in comparison to the upstream floodplain soil concentrations (see Table 2). The average concentrations of Zn and Cu in the fluvial mine-waste deposits declined from Reach 1 to Reach 2 and increased in Reach 3, although they were still lower than in Reach 1. The decline in concentration with distance from the California Gulch was significant only for Cu. Cu concentrations in Reach 1 were significantly greater than in Reach 2 or Reach 3, and there was no significant difference in Cu concentration between Reaches 2 and 3 ($p < 0.05$; General Linear Model Regression, Tukey's Studentized Range [HSD] Test for average Cu concentration using data in U.S. Fish and Wildlife Service [USFWS, 2002]).

The evaluation of vegetative cover of the mine-waste deposits suggested that vegetative cover improved with distance from the confluence of California Gulch (see Table 1; also note Avg Veg Class in Table 2). Only 4% of the mine-waste deposits had good vegetative cover within Reach 1, 40% in Reach 2 and 13% in Reach 3. The rating, however, is subjective, and interpretation of these data on a quantitative basis is complicated by the fact that the definition of a mine-waste deposit was based at least in part on the presence of dead vegetation or lack of vegetation. Also, some fluvial mine-waste deposits were not included in the URS study because they were too small or had good vegetative cover. This situation suggests that the selection of the sites included in this evaluation may have been biased toward poor vegetation. The apparent (qualitative) improvement in vegetative cover of mine-waste deposits with distance from the confluence of California Gulch was correlated with a decrease in metal concentrations. This decrease was statistically significant only for Cu.

B. *Comparison of Barren Areas with Nearby Vegetated Areas*

In a study on the potential effects of fluvial mine waste deposits on ground water and surface water, Walton-Day et al. (2000) compared concentrations of 13 metals in 11 fluvial mine waste deposits to concentrations in two nearby vegetated areas (see Table 3). Sampling locations were along a 3-mile reach of the east side of the Arkansas River floodplain, south of Leadville. The 3-mile reach sampled by Walton-Day et al. (2000) corresponded approximately to Reach 3 shown in Figure 5. Samples were collected from the 6−12 inch (15−30 cm) depth interval. Of 13 samples analyzed for metals, 11 were collected from areas where elevated metal concentrations were anticipated, based on lack of vegetation and the presence of iron-oxide staining or other indicators. Examples of other indicators of mine waste include the presence of pyrite or the odor of sulfur. The two additional samples were collected on or topographically above the floodplain in apparently uncontaminated areas. These two samples provided an estimate of background metal concentrations. The soil sample locations in the vegetated areas were within 200 m of the Arkansas River channel and within the same 3-mile reach.

Elevated concentrations of arsenic (As), Ag, Cd, Cu, Fe, mercury (Hg), Pb and Zn were observed in the barren fluvial mine-waste deposits, when compared to concentrations in nearby vegetated areas in the floodplain (see Table 3). Elevated metal concentrations were found in all 11 areas that had been selected based on lack of vegetation. In contrast, the metal concentrations in the vegetated area soils were much closer to concentrations typically found in the western United States (Schacklette and Boerngen, 1984). Nonetheless, the observed metal concentration ranges for As, Cd, Mn, Pb and Zn were still higher than the background concentrations typically found in the western United States.

TABLE 3. Concentration Ranges of Selected Elements from Eleven Barren Fluvial Tailings Deposits and Two Nearby Vegetated Area Background Soil Samples from the Upper Arkansas River Floodplain and Mean Background Concentrations for Soils in the Western United States

Element (concentration units in dry wt)	Western United States[a]	Arkansas River Floodplain, Vegetated Areas[b]	Arkansas River Fluvial Tailings Areas Devoid of Vegetation[c]
Iron (percent)	2.1	1.9−2.6	4.7−30
Arsenic (mg/kg)	5.5	10−10	85−440
Cadmium (mg/kg)	--	<2−5	7−91
Chromium (mg/kg)	41	23−50	6−48
Cobalt (mg/kg)	7.1	7−11	3−8
Copper (mg/kg)	21	31−34	37−390
Lead (mg/kg)	17	180−200	1300−6500
Manganese (mg/kg)	380	450−610	120−720
Mercury (mg/kg)	0.05	0.07−0.6	0.2−2.5
Nickel (mg/kg)	15	9−18	2−11
Selenium (mg/kg)	0.23	0.1−0.5	0.4−2.0
Silver (mg/kg)	--	<2−<2	8−51
Zinc (mg/kg)	55	130−230	1100−12,000

[a]Table 2 in Shacklette and Boerngen (1984).
[b]*n* = 2.
[c]*n* = 11.

Table adapted from Walton-Day et al. (2000).

Although Walton-Day et al. (2000) did not report pH values for the fluvial mine-waste deposits, the pH values were likely acidic. Shallow ground water in four wells in a 12-acre area (see Mine Waste Deposit IDs NB, NG, NH and NI in

Figures 5 and 6) of fluvial mine-waste deposits had pH values lower than 3 (Walton-Day et al., 2000). In contrast, shallow groundwater pH values from wells outside the area ranged from 6−7. Thus, acidic drainage from the fluvial deposits apparently has caused acidification of ground water. Pyrite and some of its weathering products were identified in the fluvial mine-waste deposit samples. In a separate study (summarized by USFWS, 2002), pH values in the N group of fluvial deposits ranged from 2.1−4.5. The pH values in the barren fluvial mine-waste deposits examined by Walton-Day et al. (2000) probably were similar.

C. *Field and Greenhouse—Manipulation of Exposure*

Brown et al. (2005) Field Experiment with Lime/Biosolids Amendment—Soil Properties. Brown et al. (2005) conducted field experiments to characterize fluvial mine waste deposits in the Arkansas River floodplain. Lime and municipal biosolids (224 kg/ha each) from the Denver wastewater treatment facility were incorporated to a depth of 20 cm into fluvial mine-waste deposits at four sites in the C, M and R groups of deposits in the summer of 1998. The unamended fluvial deposits lacked plant cover. The C group fluvial deposits were located just above the confluence of Lake Fork Creek in Reach 1 as described by USFWS (2002) (see Figure 4). The M and R groups were located in Reach 3 (see Figure 6). Brown et al. (2005) also characterized soil from a contaminated vegetated area (CVA) and an upstream uncontaminated reference site (UUC). The amended areas were seeded with a native seed mix with a composition of species that was similar to the vegetation at the UUC site (Sally Brown, personal communication to David Eskew, August 15, 2005).

Soil concentrations of Cd, Pb and Zn observed by Brown et al. (2005) in the UUC area were typical of other background samples in the Arkansas River floodplain, and were similar to background concentrations reported by Walton-Day et al. (2000) in two vegetated downstream areas (see Tables 3 and 4). Average concentrations of Cd, Pb and Zn in the fluvial mine waste deposits were highly elevated, but the highest concentrations were observed at the CVA. The CVA supported a dense vegetative stand of grasses and willows despite high metal concentrations. Plant cover was established on all the amended areas. Plant sample collection was limited to two of the amended areas, because the property owners harvested hay from the experimental plots and allowed cattle to graze the area.

Adding lime and biosolids increased soil pH and organic carbon. Lime addition raised the pH of the fluvial mine waste deposits from 3.5 to approximately 6.7 (see Table 4) (Brown et al., 2005). Total organic carbon increased from a range of 17−26 g/kg before treatment, to 42−76 g/kg following treatment. In comparison, the organic matter concentration at the UUC site was 106 g/kg. Amendment also was successful in reducing soluble concentrations of Cd, Pb and Zn. This was determined by four methods: water extraction, exchangeable, weak acid-extractable, and toxicity characteristic leaching procedure (TCLP). TCLP concentrations were reduced by approximately an order of magnitude in fluvial mine-waste deposits where lime was added.

TABLE 4. Total Metals (mg/kg dry wt) and pH of Amended Fluvial Mine-Waste Deposits (1−4) Along the Upper Arkansas River, Leadville, CO, After Adding a Mixture of Municipal Biosolids (224 Mg/ha) and Agricultural Limestone (224 Mg/ha) in 1998				
Area	Cd mg/kg	Pb mg/kg	Zn mg/kg	pH[a]
1	9.5 ± 2.9[b]	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
Unamended Mine-waste	15.9 ± 3.1	3170 ± 490	1730 ± 350	3.53
UUC	2.3 ± 0.1	100 ± 12	210 ± 40	7.05
CVA	27 ± 5.7	3450 ± 2050	3400 ± 1415	7.47

[a]Before amendment, pH in all fluvial mine-waste deposits averaged 3.44.
[b]Means ± SE for samples collected annually from 1998–2000.

Table adapted from Brown et al. (2005).
Also included are an UUC and a CVA, and unamended fluvial mine-waste material.

Addition of lime and biosolids also affected other soil properties that could potentially affect plant growth. Total Kjeldahl-N (TKN) increased from a range of 1−1.6 g/kg in the untreated fluvial mine-waste deposits to 4.6 to 8.9 g/kg in the amended areas. The TKN concentration was 4.5 g/kg in the UUC soil and 2.9 g/kg in the CVA soil. Available phosphorus concentrations increased greatly also as a result of the lime and biosolids addition. Before amendment, the available phosphorus in the fluvial mine-waste deposits ranged from 1−12 mg/kg, and after amendment concentrations ranged from 333−433 mg/kg. Available phosphorus in the UUC soil was 23 mg/kg. Both the unamended deposits and UUC soil would be considered potentially deficient in available phosphorus for plant growth (Pote et al., 1996). Brown et al. (2005) did not report available phosphorus concentrations in the CVA soil.

Brown et al. (2005) Lab Experiments with Lime/Biosolids Amendment. Brown et al. (2005) conducted phytotoxicity tests using rye grass emergence and early growth following protocols established by the American Society for Testing and Materials (ASTM, 2003). Plant emergence, shoot length, biomass and tissue metal concentrations were determined. No emergence occurred in unamended fluvial mine waste deposit samples with a pH of 3.5 (see Table 5) (Brown et al., 2005). After liming to pH 6.7 and adding biosolids, however, rye grass emergence in fluvial mine-waste material was similar to that of the laboratory control soil. Similar emergence rates were observed in soil collected from the UUC and CVA areas. The elevated Cd, Pb and Zn concentrations in the CVA soil and the amended mine-waste deposits did not inhibit rye grass emergence when compared to the laboratory control soil or the UUC site soil.

TABLE 5. Emergence and Growth of Rye Grass in a Greenhouse in Soil Collected from the Field

Soil Source	Emergence %	Aboveground Biomass mg dry wt/pot
Unamended Fluvial Deposits	0	0
Amended Fluvial Deposits	71.4−88	84
CVA	Similar to laboratory control*	12.8
UUC	Similar to laboratory control*	24.4
Laboratory Control Soil	87	42

*Numerical data were not presented.

Data from Brown et al. (2005).

Rye grass shoot length was comparable among the four treatments (Brown et al., 2005). Aboveground biomass accumulation in the amended tailing was about twice that observed in the lab control soils and 6.5 times that in the CVA soil (see Table 5). Growth in both the UUC soil and CVA soil was reduced compared to that of the laboratory control soil. However, Brown et al. (2005) did not present data allowing statistical evaluation of the aboveground biomass accumulation. The increased aboveground growth in the amended tailings may have been due to additions of N in the biosolids. Total Kjeldahl-N increased approximately 4-fold from 1−1.6 g/kg to 4.6−8.9 g/kg after biosolid amendment of fluvial mine-waste material. Measured NH_4-N and NO_3-N were over an order of magnitude higher in the amended fluvial mine-waste material, when compared to the UUC, CVA or unamended fluvial mine-waste material (Brown et al., 2005).

Metal concentrations in aboveground tissue of rye grass grown in a greenhouse in pots containing amended fluvial mine-waste material collected from the field were determined one and two years (1999 and 2000) after amendment incorporation (see Table 6) (Brown et al., 2005). Metal concentrations in rye grass tissue in soils collected from the UUC and CVA sites were also tested. Metal concentrations—Cu, Pb, Mn and Zn—were clearly elevated in rye grass grown in amended fluvial mine-waste material

TABLE 6. Metal Concentration (mg/kg dry wt) in Aboveground Tissue of Ryegrass Grown in a Greenhouse in Plots Containing (1) Amended Fluvial Mine-Waste Material Collected from the Field, (2) Soils from a UUC and a CVA in 1999 and 2000 (one and two years after amendment incorporation)[a]

Location, Year	Cd	Cu	Pb	Mn	Zn
Amended Tailings					
1999	3.4 ± 1.4[b]	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

[a]Plants in the unamended fluvial tailings did not germinate.
[b]Means followed by SE (n = 6).

Table adapted from Brown et al. (2005).

when compared to UUC site soil (see Table 6). Elevated levels of Pb and Zn also were observed in rye grass plants grown in soil from the CVA site. The enhanced growth of rye grass seedlings in the amended fluvial mine-waste material suggests that these concentrations were not phytotoxic at pH 6.7.

Brown et al. (2005) Field Experiment with Lime/Biosolids Amendment—Plant Tissue Concentrations. Aboveground plant tissue metal concentrations were also measured in plants growing in the field in 2000 and 2001 (Brown et al., 2005). Plant tissue Zn concentrations from the amended fluvial mine-waste plots were elevated relative to those at the UUC area in both years (see Table 7). Tissue Zn concentrations were intermediate at the CVA site. Tissue Pb concentrations were elevated in plants from the amended fluvial mine-waste area in 2000, but were much lower in 2001. Plant tissue Cd concentrations were somewhat higher in plants from the amended fluvial mine-waste and CVA sites than the UUC site in both years. No plant biomass data were reported from the field plots. Therefore, it is not possible to draw conclusions about whether the elevated plant tissue concentrations were phytotoxic.

TABLE 7. Means (± SE) of Cd, Pb and Zn (mg/kg dry wt) in Aboveground Tissue of Plant Samples Collected in 2000 and 2001 from UUC, CVA and Amended Areas in Leadville, CO[a]			
Location, Year	Cd mg/kg	Pb mg/kg	Zn mg/kg
UUC			
2000[b]	0.1 ± 0.1	1.4 ± 0.7	23 ± 7.6
2001[c]	0.07 ± 0.03	0.3 ± 0.15	8.8 ± 1.4
CVA			
2000	0.6 ± 0.2	3.3 ± 3.3	77 ± 12
2001	1.2 ± 0.4	2.2 ± 0.8	69 ± 20
Amended Tailings			
2000	4.5 ± 2.4	400 ± 282	546 ± 264
2001	0.9 ± 0.3	3.1 ± 0.6	121 ± 42

[a]Each collection included a range of plant 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].
[b]In 2000, $n \geq 2$.
[c]In 2001, $n \geq 7$.

Table adapted from Brown et al. (2005).

Brown et al. (2005) Field Experiment with Lime/Biosolids Amendment—Plant Species Diversity. Brown et al. (2005) evaluated the plant communities at the UUC, CVA and an amended fluvial mine-waste deposit. Fourteen 20 × 20-cm quadrants were randomly placed in one of the amended deposits and both (contaminated and uncontaminated) reference areas (CVA and UUC). Greater plant species biodiversity was observed at the CVA reference area than at the UUC reference area (see Table 8). A total of 27 plant species from 12 different families were identified at the CVA in comparison to 16 species from 8 different families at the UUC. The amended fluvial mine-waste deposit was disturbed by the incorporation of lime and biosolids two years before plant species observations were made. The amended deposit was also seeded with a mixture of native plant species. Therefore, a species biodiversity comparison between the amended deposit and the undisturbed CVA and UUC areas cannot be justified. More grass species (Poacae) were observed in the amended deposit than at either undisturbed reference area, but only four families were identified. The family Brassicaceae, found at the amended deposit and CVA but not the UUC, is known to contain a number of hyperaccumulators of metals (Baker et al., 1994).

Plant Family	UUC	CVA	Amended Plots
Poaceae	4	4	14
Rosaceae	2	2	0
Asteraceae	5	6	0
Ranunculaceae	1	1	0
Scrophulariaceae	1	4	0
Fabaceae	1	2	0
Campanulaceae	1	1	0
Onagraceae	1	0	0
Cistaceae	0	1	0
Polygalaceae	0	1	1
Boraginaceae	0	1	1
Brassicaceae	0	3	3
Gentianaceae	0	1	0

TABLE 8. Plant Families and Number of Species Identified at the UUC, CVA and an Amended Fluvial Mine-Waste Deposit, Two Years after Amendment Addition and Seeding with a Mixture of Native Plant Species

Data from Brown et al. (2005).

Fisher Lab and Field Experiments with Amendments. Fisher (1999; Fisher et al., 2000) conducted greenhouse studies with fluvial mine-waste tailings from the Arkansas River floodplain amended with lime, biosolids or lime plus biosolids with Geyer willow cuttings (*Salix geyeriana* Anderson). Fisher (1999) also conducted field experiments on effects of lime, biosolids and phosphorus on establishment of plant cover (tufted hairgrass [*Deschampsia caespitosa* L. Beauv.] and creeping bentgrass [*Agrostis stolonifera* L.]) and establishment of Geyer willow. Willows are a key species for ecosystem function in riparian areas. However, they can readily colonize metal-enriched soils too (Punshon, 1996). Willows and other shrubs account for 40 −50% of the annual primary production on undisturbed soils near the field site (NRCS, 1997).

Fisher Lab Experiments. Fisher (1999; Fisher et al., 2000) collected material from a fluvial mine-waste deposit on the east bank of the upper Arkansas River approximately 8 km south of Leadville, CO (39.20 latitude, 106.35 longitude). Table 9 summarizes characteristics of the tailings material. The tailings were amended with lime, organic matter, or lime plus organic matter. Lime, added at the rate of 1 kg lime ($CaCO_3$) per 32.5 kg dry weight tailings, increased its pH from 3.8−7.3. The organic matter source was a composted biosolids product from Summit County, CO (see Table 9). Organic matter was added at a rate of 1 kg per 32.5 kg tailings. Tailings material was placed in 15-cm diameter polyvinyl chloride tubes to a depth of 60 cm over a 20-cm layer of sand. Willow cuttings, pruned to a length of 90 cm, were placed in the tailings and sand material to a depth of 70 cm. Plant material was harvested after 4 months.

TABLE 9. Characterization of Fluvial Mine-Waste Deposits Collected from a Deposit on the Bank of the Arkansas River and Composted Biosolids (Summit-Grow™) from Summit County, CO

Parameter	Units	Mine Tailing	Biosolids[a]
Texture	-	Sandy Loam	NA[b]
pH	-	4.0	6.2
EC[c]	Siemen	1.4	2.2
Cd	mg/kg dry wt	11.0	1.6
Cu	mg/kg dry wt	413.5	69.0
Pb	mg/kg dry wt	3062.8	35.0
Zn	mg/kg dry wt	1470.0	NA
Organic Matter	%	3.0	35.7
SMP Buffer Capacity	Mg/ha	46.95	NA
Total Sulfur	%	0.95	0.29
Pyritic Sulfur	%	0.78	NA
Acid: Base Potential[d]	-	24.4	NA

[a]Characterization performed by ACZ Laboratories, Steamboat Springs, CO.
[b]NA = Not applicable; not analyzed.
[c]Electrical conductivity.
[d]Calculated as: 31.25 (% Pyritic S) = Mg $CaCO_3$ per 907.2 Mg of material.

Table adapted from Fisher et al. (2000).

Increasing the pH by adding lime greatly increased plant growth, but organic matter alone had no significant effect. Adding lime alone increased growth of willow cuttings by 12-fold from 0.7 g/container in unamended tailings to 8.2 g/container. Adding organic matter alone increased willow growth to 1.7 g/container, but the increase was not statistically significant ($p > 0.05$). Similarly, adding organic matter plus lime did not significantly increase plant growth (8.8 g/container) over addition of lime alone.

Plant available metal concentrations were estimated by shaking a soil suspension with metal-chelating cation exchange resin for 24 hours. The resin was contained in a mesh bag and was subsequently extracted with 0.12 M hydrochloric acid to recover metals for analysis. Liming significantly ($p < 0.05$) decreased the chelating cation exchange resin-extractable concentrations of Cd, Cu, Pb and Zn in the tailings material (see Table 10). Amendment with organic matter also significantly reduced extractable concentrations of Cu, Pb and Zn; however, no significant reduction in extractable Cd was observed.

TABLE 10. Metal-Chelating Cation Exchange Resin-Extractable Metal Concentrations (mg/kg dry wt) as Affected by Lime, Lime Plus Organic Matter and Organic Matter Soil Amendments

Treatment	Cd	Cu	Pb	Zn
Lime	1.29b[a]	1.13c	7.97c	33.62c
Lime Plus Organic Matter	1.44b	1.45c	6.99c	43.81c
Organic Matter	2.62a	5.57b	32.19b	252.93b
Control[b]	3.15a	6.89a	51.37a	326.23a

[a]Different lower case letters in columns indicates significant differences among means at $p \leq 0.05$.
[b]Unamended fluvial mine-waste material collected from the Arkansas River floodplain.

Table adapted from Fisher et al. (2000).

Aboveground plant tissue concentrations of Zn, Cd, Cu and Pb were significantly lower in plants grown in tailings amended with lime (see Table 11). Aboveground plant tissue concentrations of Cd, Cu and Pb were significantly lower in plants grown in tailings amended with organic matter, but the plant Zn concentrations were not significantly affected. The response of plant Zn concentrations to liming and organic matter addition compared to the respective responses in plant growth suggest that elevated Zn limited plant growth in mine tailings (see Table 12).

TABLE 11. Metal Concentrations (mg/kg dry wt) in Aboveground Plant Tissue from Geyer Willow (*Salix geyeriana* Andersson) as Affected by Lime, Lime Plus Organic Matter and Organic Matter Soil Amendments Incorporated into Fluvial Mine-Waste Deposits				
Treatment	Cd	Cu	Pb	Zn
Lime	23.75abc[a]	8.88bc	8.05b	1130.0b
Lime Plus Organic Matter	29.69ab	8.99bc	7.03b	1233.8b
Organic Matter	24.45abc	9.57b	5.47b	2847.0a
Control[b]	28.70ab	24.90a	28.52a	2328.0a

[a]Different lower case letters in columns indicate significant differences in means at $p \leq 0.05$.
[b]Unamended fluvial mine-waste material collected from the Arkansas River floodplain.

Table adapted from Fisher et al. (2000).

TABLE 12. Trends in Willow Tissue Metal Concentrations and Growth as a Result of Lime and Organic Matter Soil Amendments Incorporated into Fluvial Mine Waste Deposits. (Lack of a trend is denoted by "−".)					
Treatment	Zn	Cd	Cu	Pb	Plant Growth
Lime	↓	↓	↓	↓	↑
Organic Matter	−	↓	↓	↓	−

Data from Table 11 and Fisher et al. (2000).

***Fisher Field Experiment*.** Fisher (1999) also conducted a field experiment on the effects of lime, organic matter and triple-super-phosphate amendments on *in situ* revegetation of fluvial mine tailing deposits. Lime was added at a rate of 200 Mg/ha, and organic matter at a rate of 100 Mg/ha. These materials were incorporated to a depth of 60 cm. Triple-super-phosphate was incorporated at a rate of 120 kg phosphorus/ha. Specifically, he tested the effects of lime, organic matter, lime plus organic matter, and triple-super-phosphate soil amendments on the establishment of Geyer willow cuttings and establishment of plant cover in plots seeded with a mixture of 67% tufted hair-grass (*Deschampsia caespitosa* L. Beauv) and 33% creeping bentgrass (*Agrostis stolonifera* L.). Because this experiment was planned as a multi-year study, the plants could not be sacrificed after the first season to determine growth. As surrogate measurements of growth, willow cuttings were assessed for survival, number of leaders and total leader length (sum of all leader lengths on each cutting). Establishment of grass cover was evaluated by counting the number of individual plants and estimating the percent plant cover within a 10-cm diameter quadrant at each sample point.

Liming was effective in neutralizing acidity (Fisher, 1999). Soil pH increased from 4.83 to approximately 7.0 (see Table 13). Exchangeable concentrations (1.0 M KCl) of Cd, Cu, Pb and Zn were significantly reduced in treatments that received lime, but organic matter addition had no significant effect. Addition of triple-super-phosphate did not increase the available phosphorus in the soil samples.

TABLE 13. Soil pH, 1.0 M KCl Exchangeable Metal Concentrations (mg/kg dry wt), and Available Phosphorus (P) Concentrations as Affected by Lime, Organic Matter (OM), Lime Plus Organic Matter (LOM) and Triple-Super-Phosphate (TSP) Soil Amendments

Treatment	pH	KCl Exchangeable				Available
		Cd	Cu	Pb	Zn	P
Control[a]	4.83b[b]	23.48a[c]	16.83a	292.90a	1992.25a	2.32a
Control + TSP	4.39a	23.41a	30.25a	329.30a	2214.75a	3.45a
OM	4.71ab	20.20a	20.43a	279.59a	2016.75a	4.42a
OM + TSP	4.74ab	24.06a	15.72a	233.48a	2361.50a	4.62a
Lime	7.05c	9.09b	0.68b	2.25b	71.38b	5.53a[d]
Lime + TSP	7.01c	8.50b	0.63b	1.23b	46.06b	6.48a[d]
LOM	6.95c	8.32b	0.54b	0.93b	27.14b	8.83a[d]
LOM + TSP	7.01c	9.59b	0.61b	2.29b	68.06b	6.86a[d]

[a]Unamended fluvial mine-waste material collected from the Arkansas River floodplain.
[b]Different lower case letters in columns indicate significant differences among means at $p \leq 0.05$.
[c]Statistical comparisons were based on transformed values, but data are reported as nontransformed means for ease of interpretation.
[d]Statistical comparisons for available phosphorus were separated for lime and nonlime treatments because analysis procedures were different.

Table adapted from Fisher (1999).

Survival of Geyer willow was 24% greater in treatments that received lime (see Table 14) (Fisher, 1999). Lime addition was the only variable that significantly increased the number of leaders per cutting. Liming also increased total leader length per surviving cutting. Organic matter or triple-super-phosphate addition alone did not significantly affect survival or leader growth, but addition of organic matter or triple-super-phosphate to limed treatments resulted in a significant increase in total leader length. The density of grasses increased from 35/m^2 to approximately 1100/m^2 in treatments that received lime. Similarly, plant cover increased from 0.3% to approximately 3.8%. Plant establishment and growth in the first year after planting was probably limited by the brief growing season (approximately 6 weeks) at the altitude of the study location.

TABLE 14. Survival and Growth Indices from Field Plots for Geyer Willow (*Salix geyeriana* Andersson) and Grass Species—Tufted Hairgrass (*Deschampsia caespitosa* L. Beauv.) and Creeping Bentgrass (*Agrostis stolonifera* L.)—as Affected by Lime, Organic Matter (OM), Lime Plus Organic Matter (LOM) and Triple-Super-Phosphate (TSP) Soil Amendments

Treatment	Willows			Grasses	
	Survival (%)	Number of Leaders (#/cutting)	Total Leader Length (mm/cutting)	Density (plants/m^2)	Cover (%)
Control[a]	50.0bcd[b]	1.90ab	158.17a	35.03a	0.28a
Control + TSP	32.5d	1.33a	101.90a	0.00a	0.0a
OM	45.0bcd	1.54ab	138.32a	6.37a	0.3a
OM + TSP	42.5cd	1.70ab	183.92a	31.85a	0.05a
Lime	60.0abc	2.11bc	215.86ab	1050.89b	5.18b
Lime + TSP	57.5abc	2.58bc	280.27bc	1445.76b	4.13b
LOM	80.0a	2.84c	370.21c	1108.21b	2.53b
LOM + TSP	67.5ab	2.84c	332.13c	901.21b	3.43b

[a]Unamended fluvial mine-waste material collected from the Arkansas River floodplain.
[b]Different lower cases in columns indicate significant differences among means at $p \leq 0.05$.

Table adapted from Fisher (1999).

Step 4: Evaluate Data from Elsewhere

Similar conditions to those occurring in OU 11 in the floodplain of the upper Arkansas River (see Figure 3) also occur along the Clark Fork River near the city of Deer Lodge, MT. Large-scale mining and smelting operations occurred upstream from 1880 to 1980 in Butte and Anaconda, MT. Large quantities of mine waste from the mining and smelting operations have moved into the river and have been deposited throughout the floodplain of the Clark Fork River. Barren areas devoid of vegetation occur frequently in the floodplain along the Clark Fork River. Soils or tailings at the sites contain acid producing materials and are elevated in metal and As concentrations. Amendments were added so that the soil reached a target pH level of 7. At some sites, organic matter amendments also were added. The calcium carbonate amendments (ground limestone or industrial waste) have successfully raised pH levels and are still effective in several test areas after 6−19 years. There are indications that once vegetation is established on the waste, the plant root mass complexes the metal ions and renders them less bioavailable. This permits further root proliferation into nonamended areas and initiates a self-perpetuating cycle. Over a period of years, successional changes in vegetation have been observed in several areas (Neuman et al., 2005).

Detailed phytotoxicity studies of the fluvial mine-waste deposits along the floodplain of the Clark Fork River also demonstrated that decreased pH and increased extrinsic metals were invariably associated with barren areas (Rader et al., 1997; Brown et al., 2005). Studies along the Clark Fork River showed that vegetated areas could have metal concentrations equal to or higher than those found in barren areas. Soils adjacent to the barren areas had higher, often near neutral pH values, but did not inhibit emergence of *Echinochloa crusgalli* (barnyard grass) seeds in 5-day emergence tests, despite the high metal concentrations. Rader et al. (1997) collected samples along a 7.6-m transect starting from a vegetated area and extending into one of the barren areas. Soil samples from within the barren areas were acidic, with pH values between 4.4 and 4.9, in contrast to more neutral pH values, between 5.8 and 6.2, in the vegetated area (see Table 15). Emergence of *E. crusgalli* decreased from over 80% (versus 90% in control soil) in soil from the vegetated area, to 7% in soil collected just 1.5 m away within the barren area. Concentrations of Cu, Cd, Pb and Zn were comparable along the transect.

Similar results also were reported for samples collected from seven barren areas and compared to paired sample points selected at random within vegetated areas less than 20 m away. Liming the acidic mine-waste deposit material to near pH 7 improved emergence of *E. crusgalli* to control levels, but root growth was still inhibited (Rader et al., 1997). As observed by Brown et al. (2005), areas with high metal concentrations but near neutral pH values can support healthy plant communities.

Metal and As concentrations observed in the fluvial mine-waste deposit areas in the upper Arkansas River floodplain, along with soil concentrations that are considered to be potentially phytotoxic, are summarized in Table 16. The chemicals listed in the

TABLE 15. Results of Metal Analyses, pH Measurements and *Echinochloa crusgalli* Emergence Performed on Soils Collected Along a Transect Perpendicular to a Barren Area Boundary on the Clark Fork River								
Distance Along Transect (m)[a]	Location[b]		Acid-Extracted (mg/kg dry wt)					
		pH	Cu	Zn	As	Cd	Pb	Emergence[c]
0	Outside	6.2	3970	2190	433	12	368	86
1.5	Outside	5.8	4640	2140	293	13	256	81
3.0	Outside	6.1	3440	1670	292	8	262	78
4.6	Inside	4.4	2880	1380	272	5	270	7
6.1	Inside	4.9	5040	2340	233	8	260	0
7.6	Inside	4.8	6050	2070	210	8	213	0

[a]Samples were collected at 1.5-m intervals along a 7.6-m transect drawn across and perpendicular to the barren area boundary.
[b]Location refers to whether the sample was collected outside or inside the barren area.
[c]Emergence data were estimated from Figure 3 in Rader et al. (1997).

table are those found at elevated concentrations in the barren fluvial mine-waste deposits (Walton-Day et al., 2000). Concentrations of Cd, Pb and Zn reported by Brown et al. (2005) in the CVA, a vegetated area with high metal concentrations within the floodplain that supported a dense growth of a diverse native plant community, are also listed for comparison. The upper end of the concentrations reported by Walton-Day et al. (2000) for Ag, As, Cd, Cu, Pb and Zn were all well above the critical soil concentrations where phytotoxicity may be possible (Alloway, 1990; Efroymson et al., 1997; Kapustka et al., 2004). The upper end of the reported Pb and Zn concentration ranges exceed the upper end of the critical ranges by 16 and 30-fold, respectively. The reported concentration ranges of several chemicals are sufficient reason to expect that fluvial mine-waste deposits could be toxic to plants. Soil concentrations in the CVA, however, also exceeded the soil critical concentrations for Cd, Pb and Zn, but this area supported a dense growth of willows and grasses. The plant community was more diverse than that found at the UUC area, where soil concentrations of metals were near background levels (Brown et al., 2005).

TABLE 16. Comparison of Soil Concentrations of Metals and Arsenic Observed in the Floodplain of the Upper Arkansas River to Published Phytotoxicity Thresholds[a]

Chemical	Concentrations Observed		Phytotoxicity Thresholds		
	Walton-Day et al. (2000)[b]	Brown et al. (2005)[c]	Alloway (1990)[d]	Kapustka et al. (2004)	Efroymson et al. (1997)
Ag	8−51	nd[e]	2	nd	2
As	85−440	nd	20−50	31	nd
Cd	7−91	27	3−8	28	4
Cu	37−390	nd	60−125	95	100
Fe	4.7−30	nd	nd	nd	nd
Hg	0.2−2.5	nd	0.3−5	nd	0.3
Pb	1300−6500	3450	100−400	210	50
Zn	1100−12,000	3400	70−400	130	50

[a]Concentrations are in mg/kg dry wt except for Fe, which is in percent.
[b]Range of concentrations reported by Walton-Day et al. (2000) from 13 barren areas selected as fluvial mine-waste deposits in the Upper Arkansas River floodplain.
[c]Concentrations reported by Brown et al. (2005) from a contaminated vegetated area adjacent to a barren area of fluvial mine-waste deposit.
[d]Critical range of soil concentrations above which toxicity is considered to be possible. Data from Kabata-Pendias and Pendias (1984).
[e]nd = no data on this chemical.

Step 5: Identify Probable Cause

Step 5—identifying the probable cause—is the last step in the Stressor Identification (SI) process. Based on available evidence organized in Steps 3 and 4, the most probable cause(s) is distinguished from a set of less probable causes. Step 5 consists of three components discussed below.

A. *Characterize Causes: Eliminate*

Considerations for determining whether or not candidate causes should be eliminated are summarized in this section and in Table 17. Table 17 addresses four questions for each candidate cause. These questions are:

TABLE 17. Considerations for Determining Whether or not Candidate Causes Should be Eliminated for Impairment 1					
Candidate Cause	Impairments Occur Same Place as Exposure?	Exposure Increased Over Reference Site?	Gradient of Recovery at Reduced Exposure?	Exposure Pathway Complete?	Candidate Causes Remaining
1. Increased extrinsic metals	Yes	Yes/No[a]	?	Yes	Yes
2. Decreased pH with increased extrinsic metals	Yes	Yes	Yes	Yes	Yes
3. Decreased pH with intrinsic (background) metals	Yes	Yes	Yes	Yes	Yes
4. Decreased organic matter	Yes	Yes	No	Yes	No
5. Soil compaction	NE[b]	No	NE	NE	No
6. Grazing and mowing	Yes	No	No	Yes	No

[a]Soil concentrations of extrinsic metals were increased in the impaired areas in comparison to an upstream uncontaminated background site, but were not increased in comparison to a nearby contaminated vegetated site where good plant growth and species diversity was observed.
[b]NE = no evidence.

- Do impairments occur in the same place as exposure?
- Is exposure increased over that at the reference site?
- Is there a gradient of recovery with reduced exposure?
- Is the exposure pathway complete?

If any of the answers for a candidate cause are clearly “no,” then that cause can be eliminated. Otherwise, including cases where insufficient data are available, the candidate cause is carried forward to the *strength of evidence* analysis (i.e., the candidate cause is not eliminated, or is remaining).

1. Increased Concentrations of Extrinsic Metals in the Soil. Total plant cover was approximately 52% in Reach 0, upstream of the confluence of California Gulch with the Arkansas River. Soil metal concentrations in Reach 0 were roughly comparable to background soil concentrations typical of the western United States

(Schacklette and Boerngen, 1984). The vegetation cover values for Reach 0 also reflect land use impacts of cattle and horses grazing at a number of the sample locations. Downstream of the confluence of California Gulch, numerous areas were identified as fluvial mine-waste deposits that had less than 50% vegetative cover, and many additional areas had less than 10% vegetative cover (see Tables 1 and 2). The concentrations of Cd, Cu, Pb and Zn in the fluvial mine-waste deposits were elevated in comparison to average soil concentrations upstream of the confluence with California Gulch (see Table 2). Areas with poor vegetative cover were associated with elevated metal concentrations.

The evaluation of vegetative cover of the mine-waste deposits suggested that vegetative cover improved with distance from the confluence of California Gulch (see Table 1). The rating, however, is subjective, and interpretation of the data on a quantitative basis is complicated by the fact that the definition of a fluvial mine-waste deposit was based at least in part on the presence of dead vegetation or lack of vegetation. The apparent (qualitative) improvement in vegetative cover of mine-waste deposits with distance from the confluence of California Gulch was correlated with a decrease in metal concentrations. This decrease was statistically significant only for Cu.

Brown et al. (2005) also found that concentrations of Cd, Pb and Zn were elevated in four barren fluvial mine-waste deposits in comparison to the UUC upstream reference area (see Table 4). However, even higher Cd, Pb and Zn concentrations were found in another location, CVA, in the Arkansas River floodplain, which supported a dense vegetative stand with grasses and willows. Based on this evidence, elevated metal concentrations were frequently associated with barren areas in the Arkansas River floodplain, but elevated metals alone were not a sufficient cause to prevent establishment of vegetative cover.

There are very limited data that can be used to evaluate whether the pathway for exposure of plants to metals in the fluvial mine-waste deposits is complete. Intuitively, because plant roots would extend into the mine-waste deposit during growth, the exposure pathway should be complete. Interpretation, however, is complicated by the fact that these areas were selected on the basis of lack of vegetation, and seeds planted in the fluvial mine-waste deposits failed to germinate (Brown et al., 2005; Fisher, 1999). Therefore, no plant tissue was available to analyze for metal uptake. The data of Fisher (1999) for Geyer willow cuttings grown in a greenhouse in unamended mine-waste deposit material is the only applicable data found. The plant tissue concentrations of Cu, Pb and Zn were elevated in unamended mine-waste deposit material in comparison to cuttings growing in mine-waste amended with lime. This provides evidence that the pathway for exposure of plants to elevated metal concentrations in the fluvial mine-waste deposits is complete.

Increased concentrations of extrinsic metals in the fluvial mine-waste deposits have been frequently observed in the field in association with impaired plant growth. There is evidence suggesting that a gradient of declining metal concentrations coincides with an improvement in the vegetation cover of fluvial mine-waste deposits. Therefore, Candidate Cause #1 (increased extrinsic metals) could not be eliminated.

2. Interaction of Decreased pH and Increased Extrinsic Metals in the Soil. Low soil pH, in combination with increased extrinsic metal concentrations, occurred consistently in fluvial mine-waste deposits in association with reduced plant growth, both in the field and in greenhouse studies (Brown et al., 2005; Fisher, 1999; URS, 1997, 1998; USFWS, 2002; Walton-Day et al., 2000). Amending the fluvial mine-waste deposits with lime and biosolids to increase the soil pH from 3.5 to 6.7 was accompanied by an increase in rye grass emergence (Brown et al., 2005). These amendments also reduced soluble and/or extractable concentrations of Cd, Pb and Zn. Aboveground tissue concentrations of Cd, Cu, Pb and Zn in plants grown in amended fluvial mine-waste material, however, were still elevated when compared to plants from an upstream uncontaminated reference soil, both in greenhouse and field experiments. Also, plant tissue metal concentrations in plants from the CVA were elevated in comparison to those from UUC.

Fisher (1999) reported that adding lime to raise the pH of fluvial mine-waste deposits from 4.8 to 7.0 decreased exchangeable/extractable metal concentrations and increased growth of willow cuttings, both in greenhouse and field experiments. Adding lime also reduced plant tissue concentrations of Cd, Cu, Pb and Zn.

Observations of decreased soluble and/or extractable metal concentrations after adding lime, coupled with decreased aboveground plant tissue concentrations, are consistent with the impairment of plant growth being caused by the interaction of low pH with elevated extrinsic metals. Therefore, Candidate Cause #2 (interaction of decreased pH with extrinsic metals) could not be eliminated.

3. Interaction of Decreased pH and Intrinsic (Background) Metals in the Soil. Interaction of decreased pH and intrinsic (background) metals in the soil may result in increased levels of metals in solution, causing phytotoxicity (see Section 2.1 #3). However, in the barren areas of the UAR Floodplain, low pH values were invariably associated with high *extrinsic* metal concentrations.

4. Decreased Soil Organic Matter Results in Decreased Plant Growth. Total organic carbon in barren fluvial mine-waste deposits was reported to range from 17−26 g/kg, in comparison to 106 g/kg at the vegetated UUC site (Brown et al., 2005). No other quantitative data on organic carbon content of the fluvial mine-waste deposits were found. No mycorrhizae data were available. Fisher (1999), however, reported that adding organic matter alone at a rate of 225 kg/ha did not significantly increase growth of willow cuttings in either greenhouse or field experiments. Adding organic matter together with lime also did not further increase plant growth over lime alone. **Because adding organic matter did not improve willow growth, either with or without lime, Candidate Cause #4 (decreased soil organic matter) was eliminated from further consideration.**

5. Soil Compaction Reduces the Permeability of the Soil Surface Layers and Root Growth. Soil compaction is a common problem that restricts plant growth. Soil compaction is particularly severe under the saturated soil conditions that predominate in the upper Arkansas River area. Grazing cattle can compact soil (Wheeler et al., 2002). However, grazing occurs both upstream and downstream of the confluence of California Gulch with the Arkansas River (see below), and barren areas were not reported upstream. Soil compaction can also arise from differences in soil origin or type, e.g., deposited fines, but evidence of substantially differing soil types above and below the confluence of California Gulch is lacking. **Soil compaction does not appear to be a cause of the barren areas.**

6. Grazing by Cattle and Mowing (for hay production) can Affect Plant Growth or Species Richness. The available data do not suggest that grazing or mowing were notably different for the study area—which includes the floodplain barren areas—compared to surrounding areas. According to data from 1997 and 2002 Cattle and Calves Inventories (USDA, 2004), the study area county (Lake) contained 1858 and 287 head of cattle in 1997 and 2002, respectively, compared to 12,803 and 5964 head of cattle (1997 and 2002) in the county (Eagle) north of the study area and 11,167 and 6590 head of cattle (1997 and 2002) in the county (Chaffee) south of the study area. Data on mowing per se is not available, but according to harvested hay data from a 2002 census (USDA, 2004), 220 acres, yielding 141 tons, was harvested from the study area county (Lake), compared to 6391 acres, yielding 7021 tons in Eagle County, and 7198 acres in Chaffee County. Based on the above data, there does not appear to be evidence for a relationship between either grazing by cattle or mowing and plant growth or species richness. **Because cattle grazing and mowing occur both upstream and downstream, they are not likely to be causes of Impairment 1.**

B. *Characterize Causes: Strength of Evidence*

Strength of evidence analysis uses all the evidence generated in the analysis phase to examine the credibility of the remaining candidate causes. The causal considerations for the strength of evidence analysis use three types of evidence: case-specific evidence, evidence from other situations or biological knowledge, and evidence based on multiple lines of evidence. All evidence was evaluated for consistency with the hypothesized causes, and coherency where necessary (i.e., to explain inconsistent evidence). The results of the strength of evidence analysis are summarized in Table 18. In the table, each line of evidence is given a score as follows:

NE	= no evidence.
+ + +	= convincing evidence as cause.
+ +	= strong evidence as cause.
+	= weak evidence as cause.
0	= unclear.
–	= weak evidence as not cause.
– –	= strong evidence as not cause.
– – –	= convincing evidence as not cause.

TABLE 18. Strength of Evidence for Specific Causes and Considerations for Barren Areas in the Upper Arkansas River Floodplain

	Consideration	Extrinsic Metals		Extrinsic Metals with Decreased pH		Intrinsic Metals with Decreased pH	
		Results	Score	Results	Score	Results	Score
Case-Specific Evidence	Spatial co-occurrence	Incompatible: Area with elevated extrinsic metals but neutral pH values downstream of California Gulch (CVA) supported dense diverse vegetation, compared with relatively uncontaminated site above California Gulch (UUC) (3.1 Step 3 C).	– – –	Compatible: Barren areas—only found downstream of California Gulch—had low pH values and elevated extrinsic metals (3.1 Step 3 B).	+	Incompatible: In the barren areas, low pH values were invariably associated with high *extrinsic* metal concentrations (3.1 Step 5 A).	– – –
	Temporality	Compatible: Barren areas were not observed prior to mining activity in California Gulch.	+	Compatible: Barren areas were not observed prior to mining activity in California Gulch.	+		
	Consistency of association	Inconsistent: Areas with elevated extrinsic metals supported dense diverse vegetation (3.1 Step 3 C).	–	Consistent: Barren areas had low pH values and elevated levels of extrinsic metals (3.1 Step 3 B).	+		
	Biological gradient	Moderate: There was evidence for decreased metal concentrations and improved vegetation with distance from California Gulch (3.1 Step 3 A).	+ +	No evidence. Data for pH was available only in Reach 3.	NE		
	Complete exposure pathway	Increased extrinsic metal concentrations were observed in plants both in the field and greenhouse (3.1 Step 3 C).	+ +	Increased extrinsic metal concentrations were observed in plants both in the field and greenhouse (3.1 Step 3 C).	+ +		

TABLE 18 cont.

	Consideration	Extrinsic Metals		Extrinsic Metals with Decreased pH		Intrinsic Metals with Decreased pH	
		Results	Score	Results	Score	Results	Score
Case-Specific Evidence cont.	Experiment	Inconcordant: Field and greenhouse studies with lime to raise pH to neutrality allowed vegetation to establish, although soil metal concentrations were not changed (3.1 Step 3 C).	–	Concordant: Field and greenhouse studies with lime to raise pH to neutrality allowed vegetation to establish. Extractable soil metal concentrations and plant tissue metal concentrations were reduced by liming (3.1 Step 3 C).	+ + +	No evidence.	NE
Information From Other Situations or Biological Knowledge	Mechanism	Plausible: Plants are sensitive to elevated metal concentrations (2.1).	+	Plausible: Plants are sensitive to elevated metal concentrations and metal solubility increases when pH declines (2.1).	+	Plausible: Plants are sensitive to soluble concentrations of intrinsic metals (Al, Mn and Fe) that occur in acidic soils (2.1).	+
	Stressor-response	Concordant: Concentrations of extrinsic metals in soil are sufficient to potentially cause phytotoxicity (3.2 Step 4)	+	Concordant: pH values were low enough to make metals bioavailable, and concentrations of extrinsic metals in soil are sufficient to be potentially phytotoxic (3.2 Step 4).	+	No evidence.	NE
	Consistency of association	Inconsistent: Many areas with high concentrations of extrinsic metals supported vegetation in the Clark Fork River floodplain (3.2 Step 4).	–	Invariant: Barren areas in the Clark Fork River floodplain consistently had high extrinsic metal concentrations and low pH (3.2 Step 4).	+ + +	No evidence.	NE

TABLE 18 cont.

	Consideration	Extrinsic Metals		Extrinsic Metals with Decreased pH		Intrinsic Metals with Decreased pH	
		Results	Score	Results	Score	Results	Score
Information From Other Situations or Biological Knowledge cont.	Specificity of cause	Other causes result in barren areas.	0	Other causes result in barren areas.	0	Other causes result in barren areas.	0
	Analogy	No evidence	NE	No evidence	NE	A number of metals in well-drained organic soils are differentially leached at an accelerated rate as precipitation pH decreases (i.e., acid deposition) (Hanson et al., 1982). Soil pH levels below 5.5 result in increased solubility of Al and Mn that can be toxic to plants at background concentrations (Rengel, 2002) (2.1).	+

TABLE 18 cont.

	Consideration	Extrinsic Metals		Extrinsic Metals with Decreased pH		Intrinsic Metals with Decreased pH	
		Results	Score	Results	Score	Results	Score
Information From other Situations or Biological Knowledge cont.	Experiment	Inconcordant: - In soil samples from a 7.2 m transect extending from a vegetated area into a barren area, emergence of *E. crusgalli* decreased from over 80% in the vegetated area to 7% within the barren area. Concentrations of metals were high, and comparable, along the entire transect, but pH was low in the barren area. - Liming acidic, metals-contaminated mine-waste samples from a barren area to near pH 7 improved emergence of *E. crusgalli* to control levels (root growth was still inhibited) (3.1 Step 4).	– – –	Concordant: - In soil samples from a 7.2 m transect extending from a vegetated area into a barren area, emergence of *E. crusgalli* decreased from over 80% in the vegetated area to 7% within the barren area. Concentrations of metals were high, and comparable, along the entire transect, but pH was low in the barren area. - Liming acidic, metals-contaminated mine-waste samples from a barren area to near pH 7 improved emergence of *E. crusgalli* to control levels (root growth was still inhibited) (3.1 Step 4).	+ + +	No evidence	NE
	Predictive Performance	No evidence	NE	No evidence	NE	No evidence	NE

TABLE 18 cont.							
	Consideration	Extrinsic Metals		Extrinsic Metals with Decreased pH		Intrinsic Metals with Decreased pH	
		Results	Score	Results	Score	Results	Score
Considerations Based on Multiple Lines of Evidence	Consistency of Evidence	Not consistent: Dense diverse plant communities exist and seeds emerge in areas with high metal concentrations and near neutral pH values.	– – –	All lines of evidence are consistent with this candidate cause.	+ + +	Consistency of evidence is limited. Low pH levels can be toxic to plants at background metal concentrations, but in the study area, low pH values were invariably associated with high *extrinsic* metal concentrations.	–
	Coherence of evidence	Inconsistency may be explained by low bioavailability of metals at neutral pH.	+				

Increased extrinsic metal concentrations were observed in both well vegetated areas with a diverse collection of plant species and in barren areas in the floodplain of the 11-mile reach of the Upper Arkansas River. Soil pH values below 5.5 combined with increased extrinsic metals were invariably observed in the barren areas, or areas with impaired vegetation. Similar observations were reported in the Clark Fork River floodplain downstream from the Anaconda mining area in Montana. Barren areas there were also observed only in conjunction with both low pH values and increased extrinsic metal concentrations. Increased extrinsic metal concentrations alone were not sufficient to cause impairment of plant cover and species diversity. The extrinsic metals associated with the fluvial mine-waste deposits are all potentially phytotoxic (the upper end of the concentration range exceeded phytotoxicity screening levels). The bioavailability and potential phytotoxicity of these metals depends on pH. Increased plant tissue metal concentrations were also observed with increased soil metal concentrations.

The evidence presented in Table 18 is consistent with increased extrinsic metal concentrations interacting with pH values below 5.5 as the cause of barren areas in the Arkansas River floodplain. Soil pH values as low as 3.5 were observed in the barren areas. At these pH levels Al, Mn and Fe can all become soluble in soil, bioavailable to plants and phytotoxic at concentrations measured in background soil. Therefore, the combination of intrinsic metals and decreased pH from mining and smelting also could have caused phytotoxicity. In the barren areas of the Arkansas River floodplain, however, low pH values were invariably associated with high *extrinsic* metal concentrations.

C. *Characterize Cause: Probable Cause*

After three candidate causes were eliminated in Step 5A, three candidate causes remained. These remaining scenarios were then evaluated on a strength of evidence basis. These scenarios were #1, increased extrinsic metals; #2, interaction of extrinsic metals with decreased pH; and #3, interaction of decreased pH with intrinsic metals. Experimental manipulation of exposure in both the lab and field was a particularly strong line of evidence for determining the cause of impaired plant growth in the upper Arkansas River floodplain.

The evidence for Candidate Cause #1, increased extrinsic metal concentrations, was inconsistent. Both in the upper Arkansas River floodplain and in the Clark Fork River floodplain, areas with dense vegetation and a high diversity of plant species occurred where metal concentrations were as high as, and in many cases higher than, those observed in the barren areas. The areas with vegetation consistently had pH values ranging from 5.8−7.6. Barren areas, in contrast, consistently had pH values below 5.5. Adding lime to the material in the barren fluvial mine-waste deposit materials to raise the pH to near neutrality allowed seed emergence and permitted vegetative cover to become established despite high metal concentrations.

The evidence supporting Candidate Cause #2, impairment of plant growth due to interaction of elevated levels of extrinsic metal with decreased pH, was consistent throughout the lines of evidence. The strength of association, spatial co-occurrence, plausible stressor response, and experimental lines of evidence strongly supported this candidate cause. The quality of the data are adequate for this conclusion, and confidence is high (see Table 18).

Site-specific evidence for Candidate Cause #3, interaction of low pH with intrinsic metals such as Al, Mn and Fe, was limited. In the barren areas, low pH values were invariably associated with high *extrinsic* metal concentrations.

3.2. IMPAIRMENT 2: REDUCED PLANT GROWTH AND PLANT SPECIES RICHNESS IN IRRIGATED MEADOWS

Soil and vegetation data and the results of laboratory phytotoxicity tests were used to evaluate associations among the candidate causes and reduced plant growth and plant species richness in the irrigated meadows areas. Information from reports published in peer-reviewed journals, USGS reports, RI/FS reports, graduate theses and undergraduate research reports were used.

Step 3: Evaluate Data from the Case

Several investigations provide evidence on the potential causes of the reduced forage yields and reductions in plant species richness in the irrigated meadows areas. Levy et al. (1992) examined the depth profile of metals concentrations at an upstream site in comparison to several irrigated meadow sites just below the confluence of California Gulch and the Arkansas River. Swyers (1990) investigated the soil metals concentrations in an irrigated meadow where chlorosis and barren areas had been observed on the Seppi Ranch, the first ranch below the confluence of California Gulch. Keammerer (1987) collected plant data and soils samples from 40 locations along the Arkansas River, Tennessee Creek, and in California Gulch. U.S. EPA Region 8 and USFWS (2003) collected soil samples from 120 locations over the 11-mile reach and determined soil chemical properties and conducted laboratory phytotoxicity tests using soil samples collected from a subset of 20 of these locations. The USDA Natural Resources Conservation Service (NRCS, 2001) collected forage samples from 16 locations in the irrigated pastures area. The results of these studies are combined to evaluate the strength of evidence.

A. *Soil Characterizations and Laboratory Phytotoxicity Testing*

U.S. EPA Region 8 and USFWS (2003) conducted a study designed to characterize the soil properties and develop a method to predict phytotoxicity along the 11-mile reach of the Arkansas River (see Figure 8). In Phase I of this study, soil samples (15-cm depth) were collected from 126 stations at systematic locations throughout the riparian zone and the irrigated meadows (U.S. EPA Region 8 and USFWS, 2003). These samples were analyzed for 23 metals, pH, total organic carbon and nutrients (N, phosphorus and Ca). In Phase II of this study, a subset of 20 sampling stations were selected for more intensive studies. The Phase II sampling locations were selected to be representative of the range of soil characteristics observed in Phase I. Statistical analysis of laboratory phytotoxicity tests on Phase II

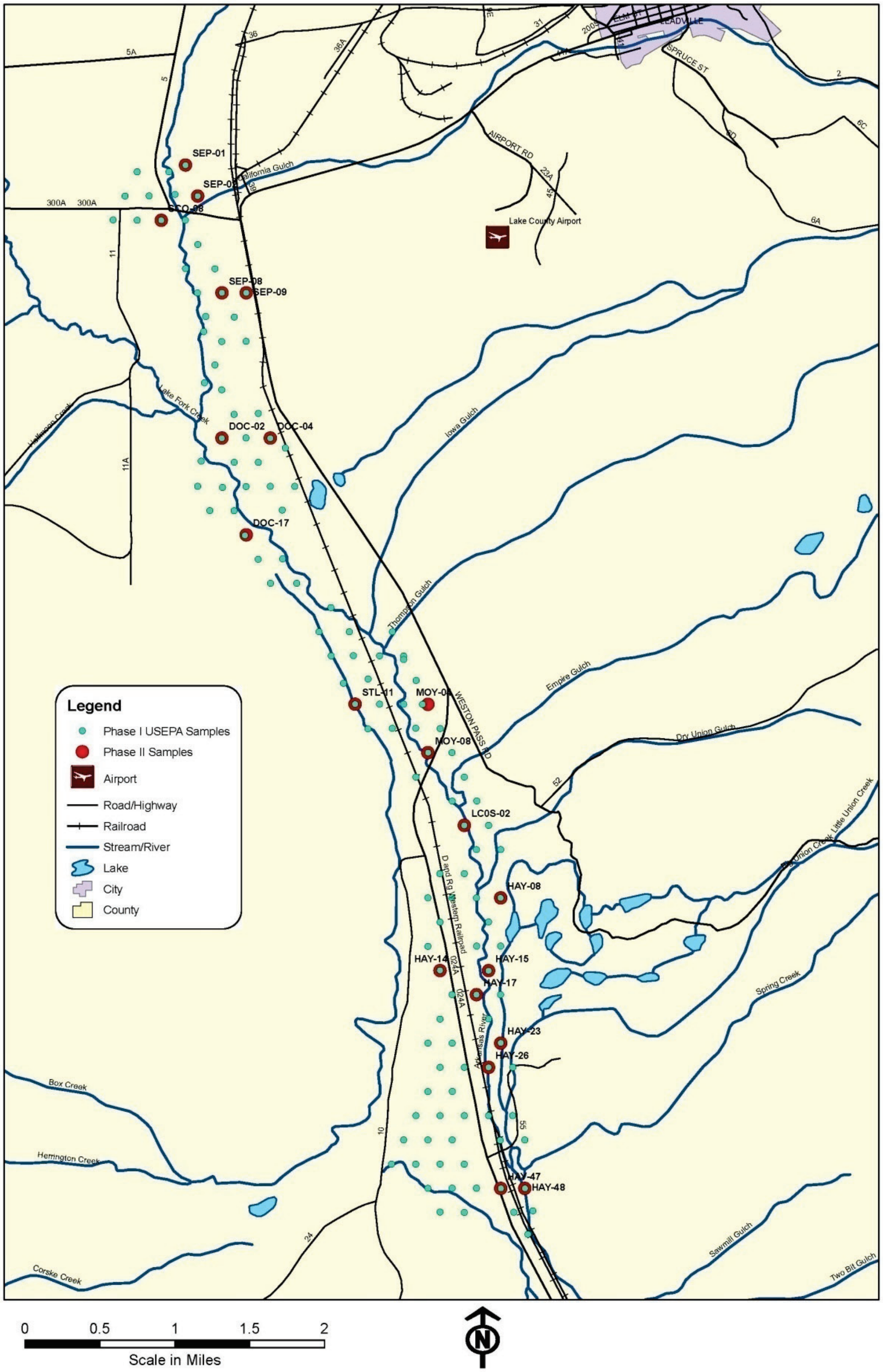


FIGURE 8. Phase I and Phase II Soil Sampling Locations (U.S. EPA Region 8 and USFWS, 2003)

samples (see Section 3.2 Step 3 B) indicated that Zn and Cu concentrations, in that order, appear to be reasonable measures of exposure for investigating the relationship between soil properties and phytotoxicity in the upper Arkansas River valley.

A wide range of soil characteristics were found among the 126 soil sampling stations (see Table 19). The distribution of Zn and pH is shown in Figure 9. As seen in Figure 9, the highest concentrations of Zn occurred in the irrigated meadows area about 1.5 miles below California Gulch. The area of highest Zn concentrations was also characterized by near-neutral pH values (range: 6.1−7.1). Other areas with Zn concentrations up to 2599 mg/kg occur further downstream, with some isolated “hot spots” of Zn (concentrations up to 5562 mg/kg) up to 5 miles downstream.

TABLE 19. Range of Element Concentrations, pH and Total Organic Carbon Found at the 126 Phase I Soil Sampling Stations

Parameter	Units in dry wt	Concentration		
		Minimum	Maximum	Average
Al	mg/kg	926	15,700	7965
As	mg/kg	1.5	270	31
Cd	mg/kg	0.11	73	10
Ca	mg/kg	593	204,000	19,311
Cu	mg/kg	7.0	709	86
Fe	mg/kg	3040	72,100	20,027
Pb	mg/kg	4.2	24,200	1075
Mn	mg/kg	20	4910	770
Hg	mg/kg	0.03	122	2.2
Ag	mg/kg	0.05	64	5.7
Zn	mg/kg	56	13,500	1133
pH	–	3.3	9.0	6.6
Total Organic Carbon	g/kg	0.48	444	50

Ca = calcium.

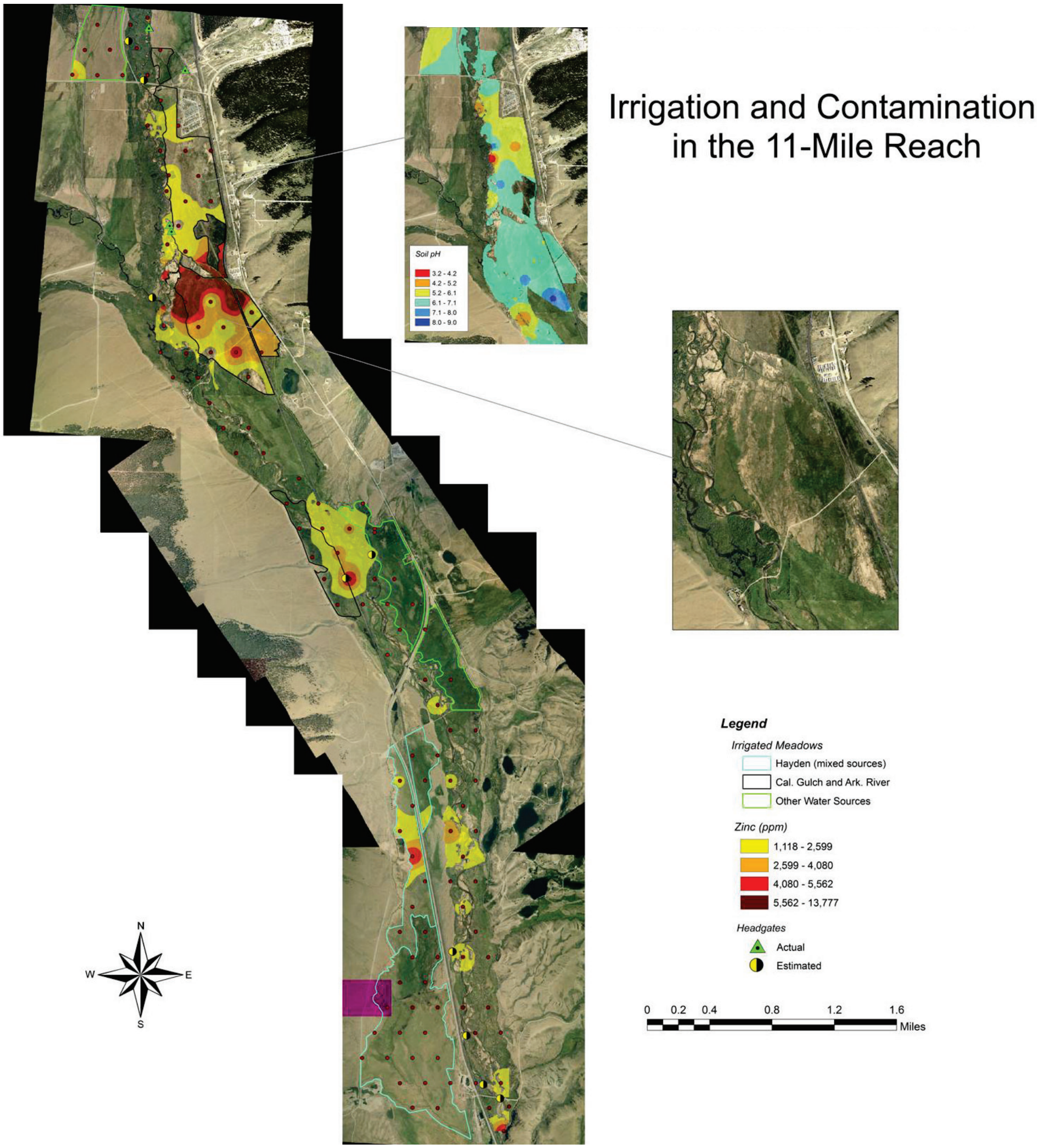


FIGURE 9. Distribution of Zinc and Soil pH in the 11-Mile Reach of the Arkansas River Valley and Sources of Irrigation Water

In Phase II sampling, soil samples collected at 20 stations were taken to a laboratory where the ability of the soils to support the growth of plants was measured (EP&T, 2002). Standard artificial soil consisting of 70% sand, 20% kaolinite and 10% peat moss, adjusted to pH 7 with calcium carbonate, was used as laboratory control soil. Three species of plants were tested: alfalfa (*Medicago sativum*), tall wheatgrass (*Elymus elongatum*) and yarrow (*Achillea millefolium* L. var. *occidentalis*). For each species, up to 26 indices of growth and phytotoxicity were measured (23 for alfalfa, 22 for wheatgrass and 26 for yarrow). A phytotoxicity score combining information from the indices of growth/phytotoxicity for each species was developed and assigned to each of the 20 stations (U.S. EPA Region 8 and USFWS, 2003). The phytotoxicity score ranged from 1−4. The score is interpreted as shown in Table 20.

TABLE 20. Scheme for Scoring Results of Laboratory Phytotoxicity Tests

Magnitude of Endpoint Response (% of control)	Phytotoxicity Score	Description
>90%	0	Nonphytotoxic
>75−90%	0.5	Mildly phytotoxic
>50−75%	1.0	Moderately phytotoxic
>25−50%	2.0	Highly phytotoxic
0−25%	4.0	Severely phytotoxic

EPA Region 8 and USFWS (2003) developed a site-specific model using principal components analysis based on the results of the laboratory phytotoxicity test to predict the potential distribution of phytotoxic conditions in the field. Because most of the mining-related metals observed in the soils were highly correlated with each other, the set of metal concentration values were reduced to a smaller number of variables by principal components analysis. Based on data for Zn, Cd, Cu and Pb at the 20 Phase II sampling sites, the first principal component for metals in bulk soil explained a large fraction (93.1%) of the total variability in the soil concentrations of mining-related metals. Thus, only the first principal component was used to develop site-specific models. The bulk soil variables giving the best prediction of laboratory mean phytotoxicity score were the first principal component for metals, pH and Ca concentration. The best-fit model was

$$MPS = 2.07 + 0.025 \bullet PC1 - 0.235 \bullet pH + 0.00001 \bullet Ca \text{ (Adj } R^2 = 0.69) \qquad (1)$$

where

MPS = Mean Phytotoxicity Score

PC1 = First principal component based on log-soil concentrations of metals in bulk soil

pH = Bulk soil pH

Ca = Concentration of calcium in bulk soil

U.S. EPA Region 8 and USFWS (2003) concluded that:

> …the primary contributors to phytotoxicity in the upper portions of the 11-mile reach area are usually metals or metals plus pH, while the lower portions of the reach include a number of stations where calcium is the main driver. Those stations for which calcium was the primary contributor to phytotoxicity may represent locations where over-liming has occurred.

An alternative interpretation, however, is that the high Ca concentrations observed are also a result of mining activity. The Zn, Pb and Ag deposits mined in the Leadville area are found as bedded replacement deposits in limestone, in particular the Leadville Dolomite. The lowest 20 to 30 ft of Leadville Limestone is a very sandy dolomite that is finely crystalline, gray and thought to be equivalent to the Gilman Sandstone Member of Leadville. This unit is useful for recognizing the Chaffee-Leadville contact. It is overlain by unnamed gray limestone typical of Leadville (USGS, 2005). Although the geology of the Leadville district is complex, the majority of the metal production came from manto deposits in the Leadville dolomite that followed pre-existing paleokarst features (Maslyn, 1996). The characteristic paleokarst caves are described as "upper portion is calcite lined, the middle is filled with stratified dolomite sand and the lower portion is mineralized (in part with detrital sulfide fragments)." Thus, it is possible that the high Ca concentrations observed in some of the irrigated meadows soils are due to dolomitic limestone transported to regions of California Gulch and Upper Arkansas River from which irrigation water was obtained.

B. *Statistical Analysis of Laboratory Phytotoxicity Tests*

A Microsoft® Office Excel file containing the site-specific laboratory measurements and phytotoxicity scores discussed in U.S. EPA Region 8 and USFWS (2003) was obtained from U.S. EPA Region 8 (EP&T, 2002). Data included concentrations of As, Cd, Cu, Pb, Hg and Zn. Measurements of field pH and laboratory pH (lpH) also were available for each sample (see Table 21). Phytotoxicity scores and dry weight per sample were available for alfalfa, wheatgrass and yarrow.

The raw data indicated that the phytotoxicity score could be simplified into a binary variable, representing nontoxic sites (assigned a value of zero) or toxic sites (assigned a value of one). The binary value simplifies statistical analysis of the data and reduces uncertainty in the classification of toxicity at each site. Photographs showing all 5 replicate pots for each test plant species, taken just before harvest, were obtained from U.S. EPA Region 8. To develop a binary classification, the photographs and the raw laboratory toxicity test results for each plant species were examined and contrasted with control plants grown in the standard artificial test soil. Based on visual evaluation of the photographs, each soil sample was classified as either nontoxic or toxic for each of the three test plant species. From this visual assessment, it seemed

TABLE 21. Site-Specific Laboratory Data													
Site	Area	As	Cd	Cu	Pb	Hg	Zn	pH	IpH	Alfalfa	Wheatgrass	Yarrow	Score
HAY-17	Meadow	9.7	2.6	18.0	91.6	0.05	98.2	5.38	6.92	0.194	0.290	0.267	0.18
SEP-02	Meadow	23.9	4.2	26.4	572.0	0.11	674.0	6.88	7.57	0.179	0.279	0.147	0.33
MOY-08	Riparian	216.0	19.5	113.0	5120.0	66.10	2150.0	7.73	8.71	0.116	0.291	0.119	0.39
STL-11	Meadow	6.9	2.0	18.7	119.0	0.06	99.9	8.44	9.40	0.143	0.261	0.085	0.48
SEP-09	Meadow	25.1	9.6	43.8	761.0	0.16	579.0	5.50	6.50	0.096	0.337	0.149	0.61
MOY-04	Meadow	4.5	0.8	14.3	20.1	0.07	79.1	7.51	8.58	0.142	0.247	0.037	0.80
HAY-47	Riparian	18.8	14.4	36.8	565.0	0.20	744.0	8.25	9.39	0.160	0.230	0.038	0.82
SEP-08	Meadow	17.4	9.3	86.7	368.0	0.14	966.0	6.07	6.66	0.097	0.192	0.071	0.90
HAY-23	Riparian	12.3	4.6	56.4	242.0	0.18	594.0	5.16	5.49	0.073	0.325	0.066	0.96
HAY-08	Riparian	11.3	3.1	20.7	126.0	0.06	131.0	8.69	9.86	0.101	0.258	0.022	1.01
HAY-14	Meadow	19.9	17.5	119.0	10.6	0.37	1960.0	6.34	6.54	0.069	0.176	0.028	1.32
HAY-26	Riparian	36.0	13.2	117.0	751.0	0.59	1880.0	5.81	7.43	0.076	0.170	0.031	1.35
DOC-17	Riparian	95.7	8.6	164.0	3520.0	4.80	1400.0	5.69	6.38	0.077	0.208	0.016	1.38
SEP-01	Riparian	19.8	22.2	222.0	367.0	0.14	2920.0	5.56	5.78	0.071	0.176	0.028	1.39
SCO-08	Meadow	3.9	2.8	11.9	43.9	0.05	468.0	7.82	7.61	0.000	0.539	0.050	1.48
DOC-04	Meadow	293.0	113.0	806.0	56,200.0	2.40	17,100.0	6.40	6.84	0.056	0.239	0.014	1.49
HAY-15	Riparian	34.8	6.9	89.8	70.6	0.41	843.0	5.24	5.88	0.056	0.212	0.015	1.59
HAY-48	Riparian	125.0	24.8	350.0	4360.0	2.30	2920.0	4.83	4.86	0.062	0.124	0.015	1.63
DOC-02	Meadow	273.0	48.1	687.0	22,000.0	4.50	11,800.0	6.70	6.44	0.019	0.093	0.008	2.23
LCOS-02	Riparian	153.0	8.8	173.0	4940.0	4.80	1430.0	3.52	3.11	0.000	0.003	0.000	2.32

Units are mg/kg dry wt for metals, standard units for pH, and g dry wt for plant species.

reasonable to interpret those sites with an average phytotoxicity score (for the 23−26 growth/phytotoxicity indices for the three species) of 0.82 and smaller as nontoxic. Similarly, sites with a larger score were interpreted as toxic. The phytotoxicity score of 0.82 selected by this process as the binary break point between nontoxic and toxic falls between the designations of mildly phytotoxic and moderately phytotoxic (see Table 20). The binary assignment of toxicity described here resulted in 13 “toxic” sites and 7 “nontoxic” sites.

Box-and-whisker plots were generated for each of the measurement endpoints using data from the 20 sites (see Appendix B). The plots were developed using the data in the original units and natural logarithm transformed data. The box-and-whisker plots display the distribution of each available laboratory and field endpoint for the toxic and nontoxic site data (i.e., the nontoxic site data consist of 7 values, and the toxic site data consist of 13 values for each endpoint). Examining the graphics (see Appendix B) indicated the following:

- Of the metals, a clear difference in the distribution of Zn and Cu between the toxic and nontoxic sites is evident. Some separation among the toxic and nontoxic site distributions is evident based on As data, but little separation is evident for Pb or Hg data.
- For all metals, log transformation of the data reduces the effect of high extreme values. This also normalized the data and increased the separation among the toxic and nontoxic site distributions.
- Little overlap among the toxic and nontoxic site distributions based on lpH is evident. However, a much larger degree of overlap is evident from distributions based on field measured pH.
- The distributions of the alfalfa, wheatgrass and yarrow average dry weight values (per plant) are dissimilar among the toxic and nontoxic sites.
- Cd distributions among the toxic and nontoxic sites have a large overlap.

Based on the box-and-whisker plots, Zn and Cu appear to be reasonable measures of exposure for investigating the relationship between soil properties and phytotoxicity in the Upper Arkansas River Valley. The separation between toxic and nontoxic site distributions based on laboratory pH suggests that the laboratory pH influenced toxicity.

Development of a Generalized Linear (GLIM) Model of Phytotoxicity. Various methods and approaches are available for building phytotoxicity models. In this project, Bayesian inference and graphical evidence are used to select a final model. Initially, the correlation among the available measurement endpoints was examined (see Table 22). A high ($r > 0.8$) linear correlation is found among many of the metals (e.g., Zn and Cu, As and Cu). The high correlation among the metals presents a number of problems from a model-building perspective, including the following:

TABLE 22. Correlation Among Candidate Measurement Endpoints							
	ln(Zn)	ln(Pb)	ln(Hg)	Lab pH	ln(Cu)	ln(Cd)	ln(As)
ln(Zn)	1.0	0.7	0.7	−0.5	0.9	0.9	0.8
ln(Pb)	0.7	1.0	0.7	−0.3	0.8	0.7	0.9
ln(Hg)	0.7	0.7	1.0	−0.3	0.7	0.6	0.9
lab pH	−0.5	−0.3	−0.3	1.0	−0.6	−0.3	−0.4
ln(Cu)	0.9	0.8	0.7	−0.6	1.0	0.9	0.9
ln(Cd)	0.9	0.7	0.6	−0.3	0.9	1.0	0.8
ln(As)	0.8	0.9	0.9	−0.4	0.9	0.8	1.0

- High correlation among the measurement endpoints significantly reduces the ability to distinguish the relative contribution of each endpoint to site toxicity. Therefore, statistical approaches for selecting from among a set of candidate predictor variables (e.g., stepwise variable selection procedures) are compromised. The outputs from these procedures can be used as general guidance, but should not be used exclusively to select the final model.
- Nonlinear models may have convergence issues under conditions of high correlation. This is particularly true when building multiple parameter models.
- The signs (positive or negative) on the resulting model parameters may not reflect biological reality. For example, we expect higher metal concentrations to be associated with greater toxicity, therefore, the sign on the model parameter should be positive. Multi-parameter models under conditions of high correlation frequently result in inverted parameter signs. Therefore, the predictive ability of the models on future data sets is compromised.

Mindful of the above issues, we developed models based on (1) a conceptual model of the relationships between toxicity, metals concentration, and metals solubility and bioavailability, and (2) support available through the examination of graphics. A complete discussion of the relationship between phytotoxicity and metals concentrations is available in the previous section of this report. Box-and-whisker plots are presented in Appendix B and discussed earlier in this section. In addition, we considered the findings of other researchers (see EP&T, 2002). Relative to the other metals, Zn and Cu were shown to be reasonable predictors of site toxicity (see Appendix B). Laboratory pH was shown to be superior to field pH, and Cd was shown to be a poor indicator of toxicity. A model consisting of a metals concentration and pH as predictor variables is consistent with the conceptual understanding of metals toxicity presented in the previous section.

Based on toxicological evidence, and the results of assessments by earlier investigators, two models were selected for detailed study. The final models were developed using the Bayesian software WinBugs (Lunn et al., 2000). Advantages of the Bayesian approach and WinBugs software, in the present study, include the following:

- The sampling routines used in the procedure result in informative graphical presentations of the model outputs.
- The Bayesian framework allows specification of a probability model matching the biological and toxicological concepts.
- The resulting model can be used as an aid for examining future analyses of field-based toxicity data.

For the simple models examined below, a standard classical statistics approach would result in very similar estimates of the model parameters. However, the flexible probability model used in the Bayesian approach is difficult to solve using classical statistics, and the graphical outputs are difficult to create.

A generalized linear model (GLIM) is described in the following text. For each site (i), a binary measure of toxicity (y_i) is assigned. The distribution of y is assumed to be Bernoulli (Evans et al., 2000) with random parameter P. P_i is the probability of toxicity at site i:

$$y_i \sim \text{Bernoulli}\ (P_i) \qquad (2)$$

In the second step, P is linked to the stressor concentrations using a logistic function:

$$\text{logit}(P_i) = \beta_0 + \beta_1 \ln(C_i) + \beta_2 \, \text{lpH} \qquad (3)$$

Consistent with the exploratory statistical analyses (see above), C is the concentration of either Cu or Zn at site i. Therefore, there are two competing models, one based on Zn and one based on Cu. Laboratory measured pH was selected as the covariable based on the evidence discussed above. The random parameters β_0, β_1 and β_2 are assumed to be normally distributed.[1] While these models are not the only available models, they should provide reasonable estimations of site-specific toxicity. Additional models could be explored in future studies.

[1] In the Bayesian framework, the model parameters are assumed to be random and assigned a distribution. The final sufficient statistics of these distributions (termed a posterior estimate) are generated from a function of the data (termed a likelihood function) and prior information on the random parameters. In this study, prior distributions on the model parameters did not exist, so noninformative prior distributions are used. The noninformative prior distributions have no effect on the final result. Model predictions utilize the posterior estimates of the model parameters.

The Cu and Zn model parameter estimates are shown in Table 23.[2] The signs on the parameters match the toxicological expectations. As larger metal concentration results in increased toxicity and a smaller pH results in increased toxicity, WinBugs produces a measure of fit called the deviance information criterion (DIC), with smaller values indicating a better fit. The models had almost identical values (DIC Cu = 23.7; DIC Zn = 22.9), indicating that the models had very similar fits. Notice that the coefficients of variation (CVs) of the Cu model parameters are larger than those found in the Zn model, indicating increased uncertainty in the parameter distributions. In both models, β_0 has the largest CV of all model parameters, indicating relatively high uncertainty in the general toxicity at all sites. The CV of β_1 in the Zn model is much smaller than the CV of β_1 in the Cu model. This finding indicates that across all sites, the relationship between toxicity and Zn is stronger than the relationship between Cu and toxicity.

TABLE 23. Posterior Estimates of the Model Parameters

Parameter	Zinc Model			Copper Model		
	Median	Std.	CV	Median	Std.	CV
β_0	12.53	11.75	0.94	8.93	11.1	1.24
β_1	9.80	0.61	0.06	1.14	0.78	0.68
β_2	−8.67	5.09	0.59	−6.46	4.82	0.75

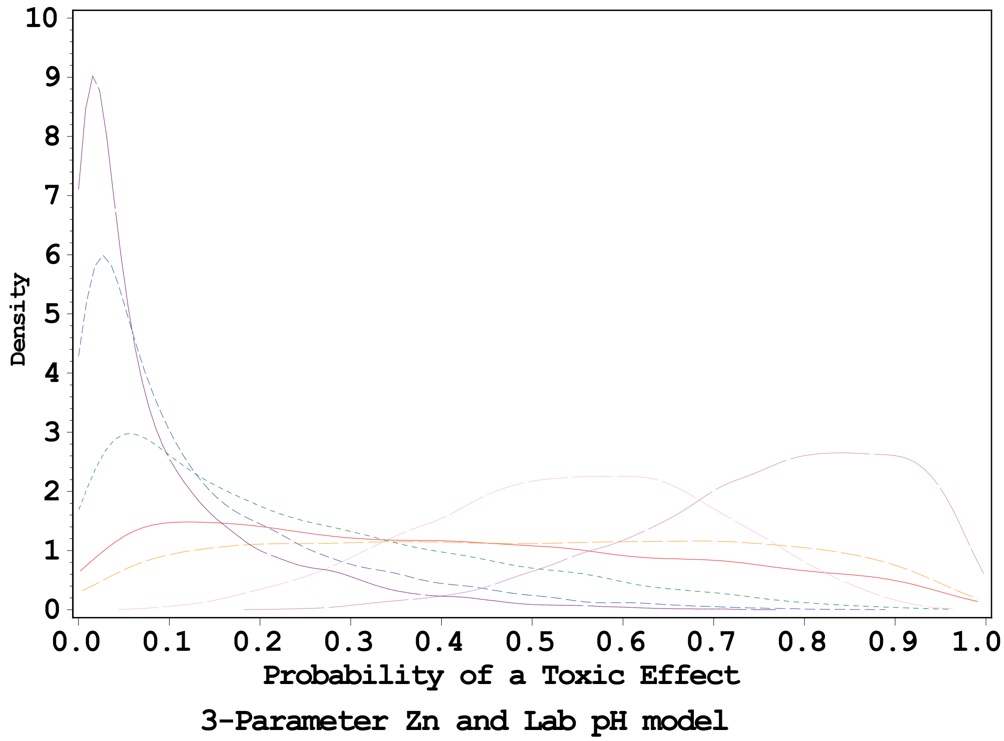
Std. = standard deviation.

The median and standard deviation of P_i at each site based on the Zn and Cu models are shown in Table 24. The distribution of P_i for each site is shown in Figures 10 and 11. Figure 10 displays the distribution of P_i for the seven nontoxic sites. Figure 11 displays the distribution of P_i for the toxic sites. When examining the figures, one should note the relative height and breadth of each distribution. Tall and narrow distributions indicate a relatively small amount of uncertainty in the probability of a toxic effect. Those sites with a low and broad distribution of P_i have a higher degree of uncertainty in the probability of site-specific toxicity. One should also note the position (on the abscissa) of the peak of each distribution. Those sites with a low expected toxicity are shifted to the left. Those sites with relatively high expected toxicity are shifted to the right. Examination of the table and figures provides the following findings:

[2] Winbugs uses a sampling procedure to solve for the parameter estimates. Extreme estimates of the parameters can occur. To minimize the effect of these values, a median is used to represent the center of the distribution.

TABLE 24. Distribution of P_i								
Site	Area	Zn Model		Cu Model		Zn mg/kg	Cu mg/kg	Lab pH
		Median P_i	Std	Median P_i	Std			
HAY-17	Meadow	0.37	0.26	0.42	0.23	98.2	18.0	6.92
SEP-02	Meadow	0.55	0.16	0.40	0.17	674.0	26.4	7.57
MOY-08	Riparian	0.48	0.26	0.59	0.26	2150.0	113.0	8.71
STL-11	Meadow	0.04	0.11	0.11	0.13	99.9	18.7	9.40
SEP-09	Meadow	0.78	0.15	0.74	0.16	579.0	43.8	6.50
MOY-04	Meadow	0.08	0.14	0.14	0.14	79.1	14.3	8.58
HAY-47	Riparian	0.18	0.20	0.21	0.18	744.0	36.8	9.39
SEP-08	Meadow	0.82	0.12	0.84	0.11	966.0	86.7	6.66
HAY-23	Riparian	0.76	0.16	0.79	0.15	594.0	56.4	5.49
HAY-08	Riparian	0.04	0.11	0.09	0.14	131.0	20.7	9.86
HAY-14	Meadow	0.90	0.01	0.89	0.10	1960.0	119.0	6.54
HAY-26	Riparian	0.99	0.04	0.99	0.04	1880.0	117.0	7.43
DOC-17	Riparian	0.90	0.10	0.93	0.09	1400.0	164.0	6.38
SEP-01	Riparian	0.99	0.06	0.99	0.06	2920.0	222.0	5.78
SCO-08	Meadow	0.47	0.17	0.21	0.19	468.0	11.9	7.61
DOC-04	Meadow	0.97	0.12	0.98	0.12	17,100.0	806.0	6.84
HAY-15	Riparian	0.92	0.11	0.92	0.10	843.0	89.8	5.88
HAY-48	Riparian	0.97	0.06	0.97	0.06	2920.0	350.0	4.86
DOC-02	Meadow	0.98	0.09	0.98	0.09	11,800.0	687.0	6.44
LCOS-02	Riparian	0.99	0.06	0.99	0.06	1430.0	173.0	3.11

Std. = standard deviation.



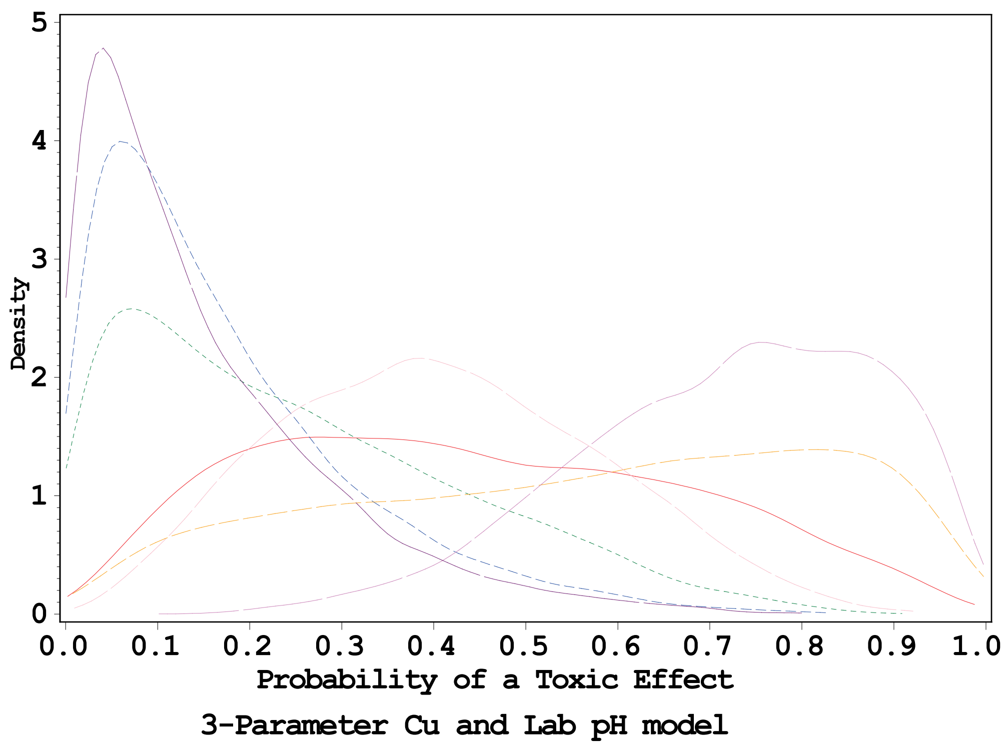
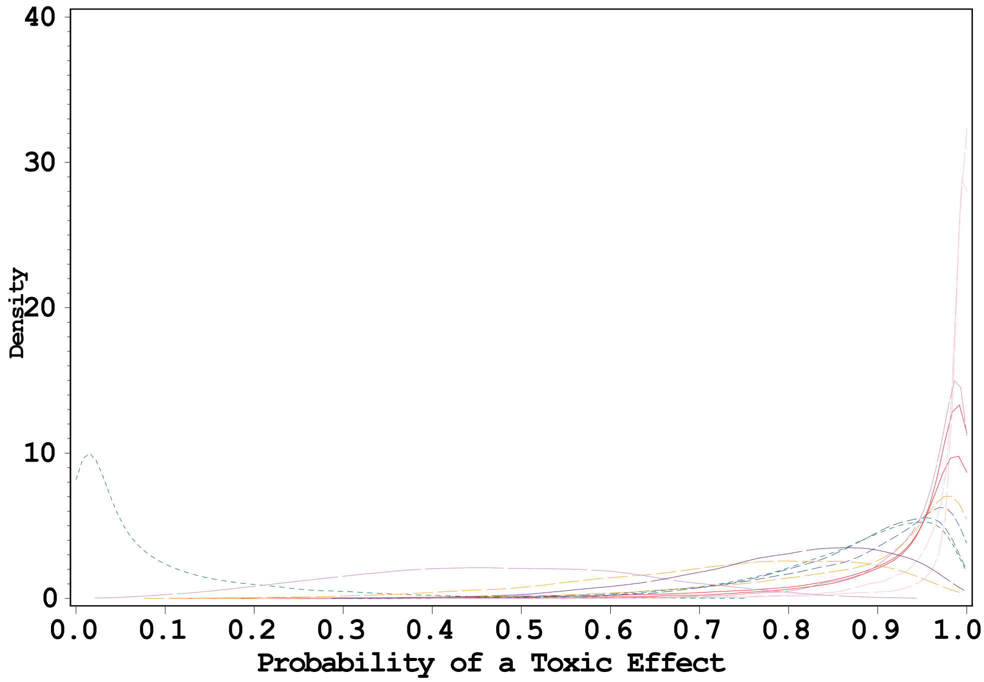
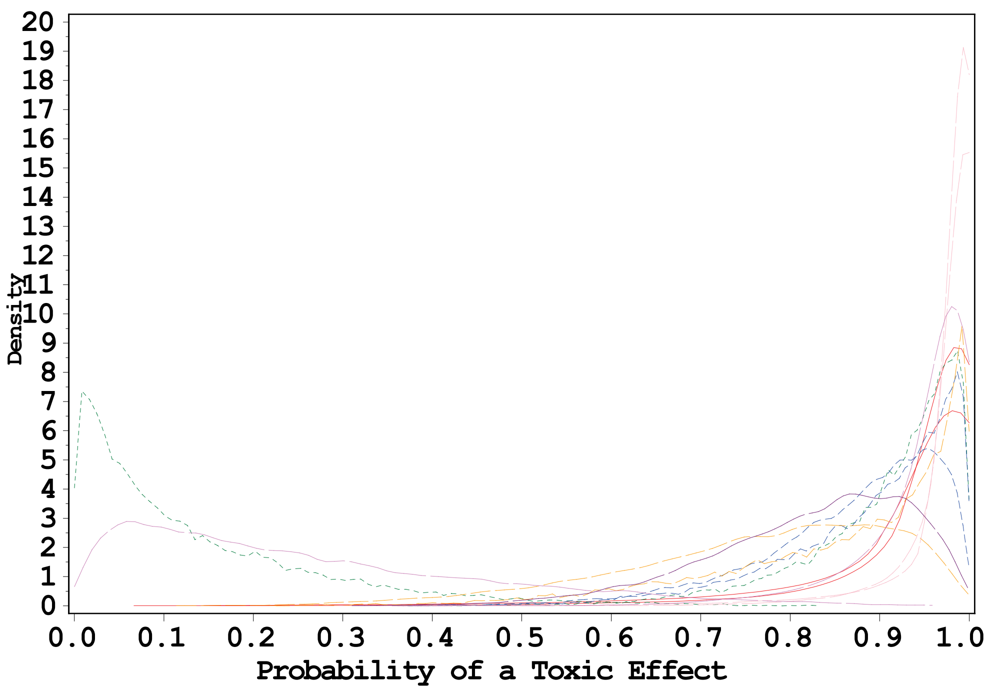


FIGURE 10. Distribution of P_i for Zinc and Copper Models for the Seven Upper Arkansas River Valley Sites Considered to be Nonphytotoxic



3-Parameter Zn and Lab pH model



3-Parameter Cu and Lab pH model

FIGURE 11. Distribution of P_i for Zinc and Copper Models for the 13 Upper Arkansas River Valley Sites Considered to be Phytotoxic

- Sites STL-11 and MOY-04 have a very low probability of toxicity. These sites also have low metals concentrations. A third site, HAY-47, also has a low probability of toxicity, but the distribution of toxicity is relatively broad indicating some uncertainty in the expected toxicity at this site.
- Site SEP-09 was interpreted as a nontoxic site (phytotoxicity score <0.82). However, based on either model the expected probability of toxicity is high (Zn model P_i = 0.78, Cu model P_i = 0.74). This site has moderately high metals concentrations at a pH of 6.5.
- The distributions of P_i for nontoxic sites are similar for both models. However, the distributions associated with the Cu model are more uncertain than their respective distributions generated by the Zn model. The Zn model is found to have a smaller model prediction error than the Cu model.
- Of the toxic sites, both models indicate that site HAY-08 may be misclassified. The median P_i at this site indicates a small chance the site is toxic.
- The Cu model and Zn model treat site SCO-08 somewhat differently. The Cu model indicates a low chance of toxicity (P_i = 0.21) and the Zn model indicates a moderate chance of toxicity (P_i = 0.47). However, the distribution of P_i based on each model is relatively broad, indicating uncertainty in the model predictions. The Zn model estimate of toxicity is more consistent with the original interpretation of toxicity at this site than the Cu model.

Overall, the Zn model is shown to be moderately superior to the Cu model for predicting site phytotoxicity, although both models seem viable.

C. *Soil and Vegetation Data in Irrigated Meadow Areas: Field Studies*

Plant growth is impaired in the irrigated meadows below California Gulch. On one ranch, severely chlorotic plants and barren sections of ground have been observed and forage yield in the affected meadow has decreased from 4.48 Mg/ha (3990 lbs/acre) in 1874 to 1.68 Mg/ha (1496 lbs/acre) in the early 1990s (Levy et al., 1992). Levy et al. (1992) collected soil samples at six depth intervals to a total depth of 30 cm from two ranches just below the confluence of California Gulch with the Arkansas River. This sampling was replicated six times at each study site. Replicates for the reference meadow (slope 5%) were collected along a 200-m transect from the summit to the toe position. Replicates for all other locations (0−3% slope) were sampled within a 5-m radius of the initial excavation. The reference site used in this study for background metal levels in the Newfork-Marsh-Rosane Association soil series was located in Tennessee Park (TP), upstream from the East Fork of the Arkansas River (see Figure 12). The other four study sites were selected to represent areas of distinct vegetation, landscape position and irrigation history located on the Seppi (SB, SC, SD) or Smith (BA) ranches. The reference site received “clean” irrigation water from East Tennessee Creek. Water from California Gulch was used to irrigate meadows on local ranches from 1874 until the 1920s (Levy et al., 1992) before the detrimental effects on animal health and forage quality in the meadows were noted in 1939. Since the 1920s,

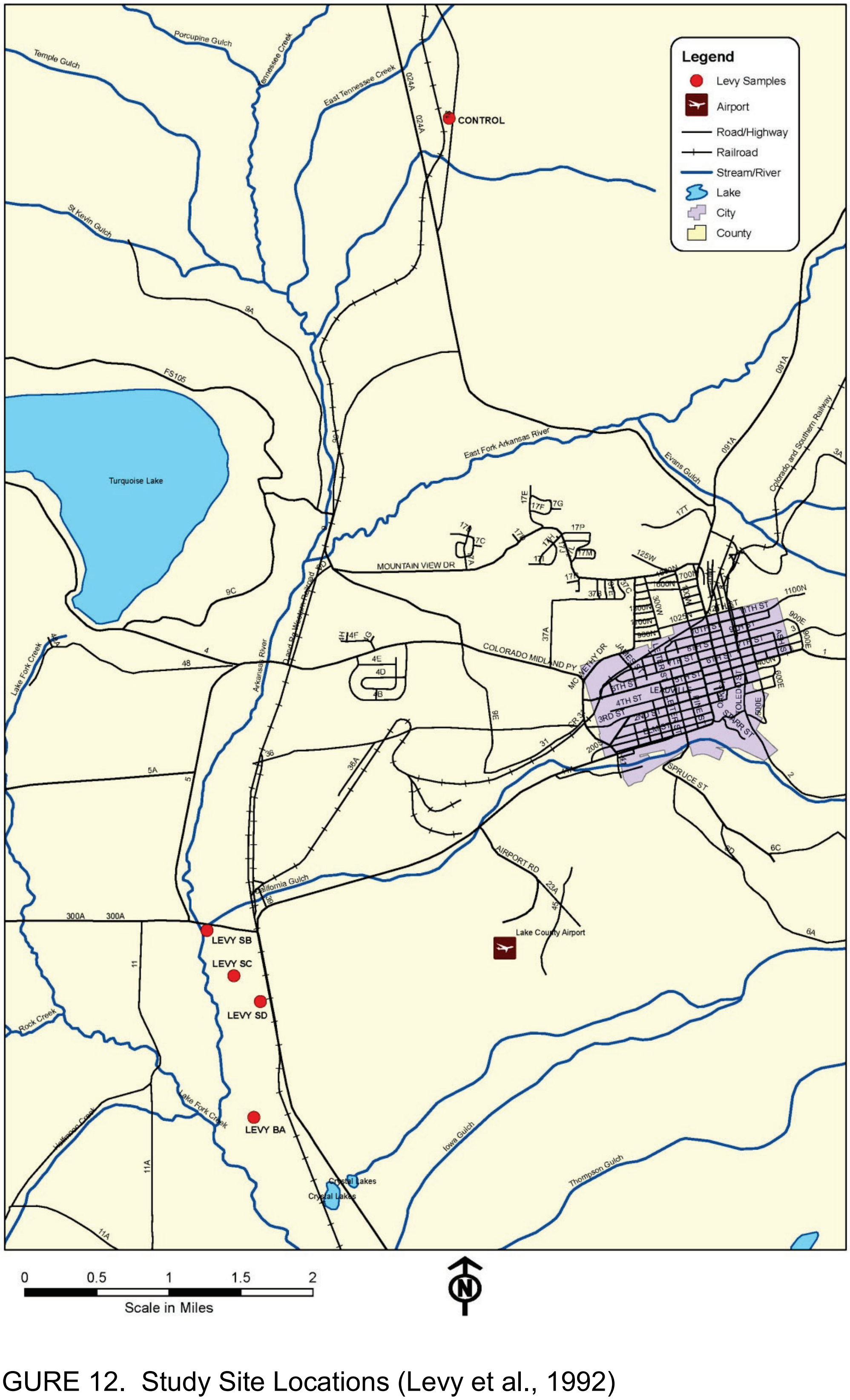


FIGURE 12. Study Site Locations (Levy et al., 1992)

the meadows have been irrigated with water diverted from the Arkansas River above and below California Gulch.

Weighted-average soil concentrations of Cd, Cu, Pb and Zn were elevated at all four locations sampled in the impaired meadows compared to the upstream reference site (see Table 25). The highest concentrations of Pb and Zn were found at a depth of 15 cm below the soil surface at sites BA and SB (Levy et al., 1992). This finding correlated with the presence of a 10-cm layer of sand uncovered during the sampling. The sand layer is not described in the Chafee-Lake Area Soil Survey, and probably washed into the valley from hydraulic mining activities. It is said that at one time the sands blanketed this area of the Upper Eastern Arkansas Valley (Levy et al., 1992).

TABLE 25. Weighted-Average Total Metals (g/m^3), pH and Organic Carbon (g/kg) to a Depth of 30 cm at the Reference (TP) and Impaired Meadow Sites (SB, SC, SD, BA)

Parameter	Location				
	TP	SB	SC	SD	BA
Cd	4	15	9	14	39
Cu	16	162	103	81	431
Pb	82	870	301	308	18,000
Zn	62	1400	706	699	7650
pH*	4.5−4.8	6.1−7.0	4.7−5.3	2.8−4.6	5.6−5.9
Organic C	9−288	58−243	19−304	53−403	18−183

*Soil pH values and organic C concentrations are the range of values observed over the six soil depth intervals sampled at each site.

Adapted from Levy et al. (1992).

Neither soil pH values nor soil organic C concentrations were consistently different between the upstream reference site and the downstream sites in the impaired meadows (see Table 25). The soil pH was acidic at the upstream reference site and at two of the impaired meadow sites. In contrast, pH at the impaired meadow sites SB and BA were more alkaline than the upstream reference site. The range of organic C concentrations was similar to the upstream reference site at two of the impaired meadow sites (SB and SC), lower at one impaired meadow site (BA) and higher at the fourth impaired meadow site (SD). The impaired meadow site SD was in a peat bog; at this site there was no indication of a hydraulically deposited sediment layer, although the site has been irrigated with water from California Gulch. Differences in pH and organic carbon are not consistent with either of these parameters being the primary cause of impairment in the downstream irrigated meadows areas.

Differences in plant species richness in irrigated pastures above and below the confluence of California Gulch with the Arkansas River have also been reported (Levy et al., 1992). Nine plant species were observed in an irrigated meadow on a similar soil type located upstream of the California Gulch (see Table 26). This upstream meadow had received relatively "clean" irrigation water from Tennessee Creek. In contrast, at four locations in irrigated meadows just downstream of California Gulch, only three or four plant species were observed at each sampling location. Several of the plant species observed at the upstream location also were observed in at least one of the downstream locations, but three species—Alpine timothy (*Phleum alpinum*), dandelion (*Taraxacum* spp.), and clover (*Trifolium* spp.)—were not reported at any of the four impaired meadow locations. However, the sampling method at the reference site differed from the method employed at the four study sites. Samples were collected along a 200-m transect from the summit to the toe of a slope at the reference site but all samples were collected within a 5-m radius at the impaired meadow sites. Therefore, a wider range of potential habitats was sampled at the reference site. As a result, no conclusion can be supported based on these limited observations.

Above-ground plant metal (Cd, Cu, Pb or Zn) concentrations were not elevated greatly at sites SB and SC relative to the upstream reference site (see Table 26). The concentration of Zn, however, clearly was elevated over the reference site at impaired meadow sites SD and BA. Cd, Cu and Pb also clearly were elevated at site BA.

Swyers (1990) conducted an investigation of the extent of metal contamination in the soils of a large irrigated meadow on the property of E. Seppi. He collected soil samples from 36 locations along a series of transects spaced approximately 91 m apart. Soil metal concentrations were highly variable, but elevated concentrations frequently were observed (see Table 27). Swyers (1990) reported barren areas with exceptionally high metal concentrations at several points near an old irrigation ditch. He described sampling point M5 as an area of extremely sparse growth just south of the old irrigation ditch. Soil concentrations at M5 were 113 mg/kg Cd; 177 mg/kg Cu; 1096 mg/kg Pb; and 8722 mg/kg Zn. He also reported an area described as "a barren, gray and white, crusted wasteland." This area, however, was located in the floodplain near the Arkansas River and may be in a fluvial mine-waste deposit area. For this reason, the results from this sampling site were omitted from Table 27. The results described in the impaired meadow on the Seppi property are consistent with elevated metal concentrations as the cause of sparse plant growth.

TABLE 26. Plant Species, Percent Ground Cover and Plant Metal Concentrations (mg/kg dry wt) from Reference and Impaired Meadow Sites							
Location	Species	Common Name	Percent Cover	Cu	Cd	Pb	Zn
Reference	*Achillea lanulosa*	Yarrow	10	7.9	2.23	1.85	30
	Carex spp.	Sedge, sloughgrass	15	6.4	0.55	0.92	27
	Juncus spp.	Rush, wiregrass	7	6.1	0.77	0.25	35
	Pedicularis spp.	Lousewort	1	9.4	0.63	0.98	42
	Phleum alpinum	Alpine timothy	8	4.2	0.11	0.74	39
	Poa spp.	Bluegrass	3	6.3	0.22	0.81	26
	Potentilla spp.	Cinquefoil	20	6.4	1.52	1.39	52
	Taraxacum spp.	Dandelion	15	12.5	2.01	1.45	47
	Trifolium spp.	Clover	10	9.8	0.35	1.3	109
SB	*Agropyron* spp.	Wheatgrass	60	8.1	0.27	1.06	61
	Poa spp.	Bluegrass	30	4.5	0.25	1.20	28
	Potentilla spp.	Cinquefoil	10	6.2	0.89	2.79	100
SC	*Carex* spp.	Sedge, sloughgrass	4	8.3	0.98	1.27	74
	Juncus spp.	Rush, wiregrass	8	6.7	1.25	0.52	150
	Muhlenbergia spp.	Muhly	88	5.3	0.84	1.16	102
SD	*Carex* spp.	Sedge, sloughgrass	44	13.3	3.04	1.76	263
	Juncus spp.	Rush, wiregrass	45	4.5	3.55	0.81	306
	Pedicularis spp.	Lousewort	1	4.4	3.80	3.31	634
	Salix spp.	Willow shrub	10	4.4	13.2	2.24	588
BA	*Achillea lanulosa*	Yarrow	10	11.2	7.27	52.0	517
	Carex spp.	Sedge, sloughgrass	62	21.1	2.12	26.4	593
	Iris missouriensis	Iris	1	4.7	21.0	23.4	403
	Juncus spp.	Rush, wiregrass	2	6.8	3.5	12.7	443
	Poa spp.	Bluegrass	25	11.6	1.9	43.1	570

Adapted from Levy et al. (1992).

TABLE 27. Total Soil Metal Concentrations (mg/kg dry wt) Observed in Impaired Meadow on E. Seppi Property		
Metal	Concentration Range	Mean (± SE)
Cd	5−113	19 ± 4
Cu	21−409	106 ± 12
Pb	46−1112	292 ± 41
Zn	294−8722	1842 ± 311

Data from Swyers (1990).

Keammerer (1987) conducted a survey of plant growth and species composition at 40 locations, including 13 locations upstream of the confluence of California Gulch, 4 locations along California Gulch and 23 locations in irrigated meadows along the Arkansas River Valley within the 11-mile study reach. Two of these samples were collected 2.5 miles upstream near the reference location sampled by Levy et al. (1992); another was collected 2.3 miles upstream in the floodplain of the east fork of the Arkansas River. These three sampling locations are not illustrated. However, the other 37 sampling locations are shown in Figure 13.

Keammerer (1987) collected soil samples (0−15 cm) at the same locations. The soil samples were analyzed for total and plant-available (ammonium bicarbonate diethylenetriaminepentaacetate [DTPA] extractable) As, Cd, Cu, Pb and Zn concentrations. Extracting with the chelate, DTPA, is a standard method for estimating bioavailable trace element concentrations in soils (Soltanpour, 1985). Above-ground plant tissue samples also were analyzed for As, Cd, Cu, Pb and Zn. Data on plant-tissue metal concentrations were grouped by plant type, such as grasses and forbs. In this study, grass-like species such as sedges and reeds were included with the true grass species for metal analysis.

The Keammerer (1987) data are difficult to interpret for several reasons. Because the study was unpublished, copies are not available and few methodological details are known. For example, the basis for selecting the sampling locations, the size of the plots sampled for plant species richness, and date of sample collection are not reported. The Upper Arkansas River Valley is located at approximately 9500 ft elevation and has a frost-free growing season of 30−40 days. Thus, sampling date can make a large difference in plant biomass. The areas sampled are known to be grazed by cattle, but little information is available on grazing intensity. As summarized in USFWS (2002), Keammerer (1987) reported that grazing ranged from 0−35% forage utilization within the upstream locations. However, no similar information was provided for locations below California Gulch.

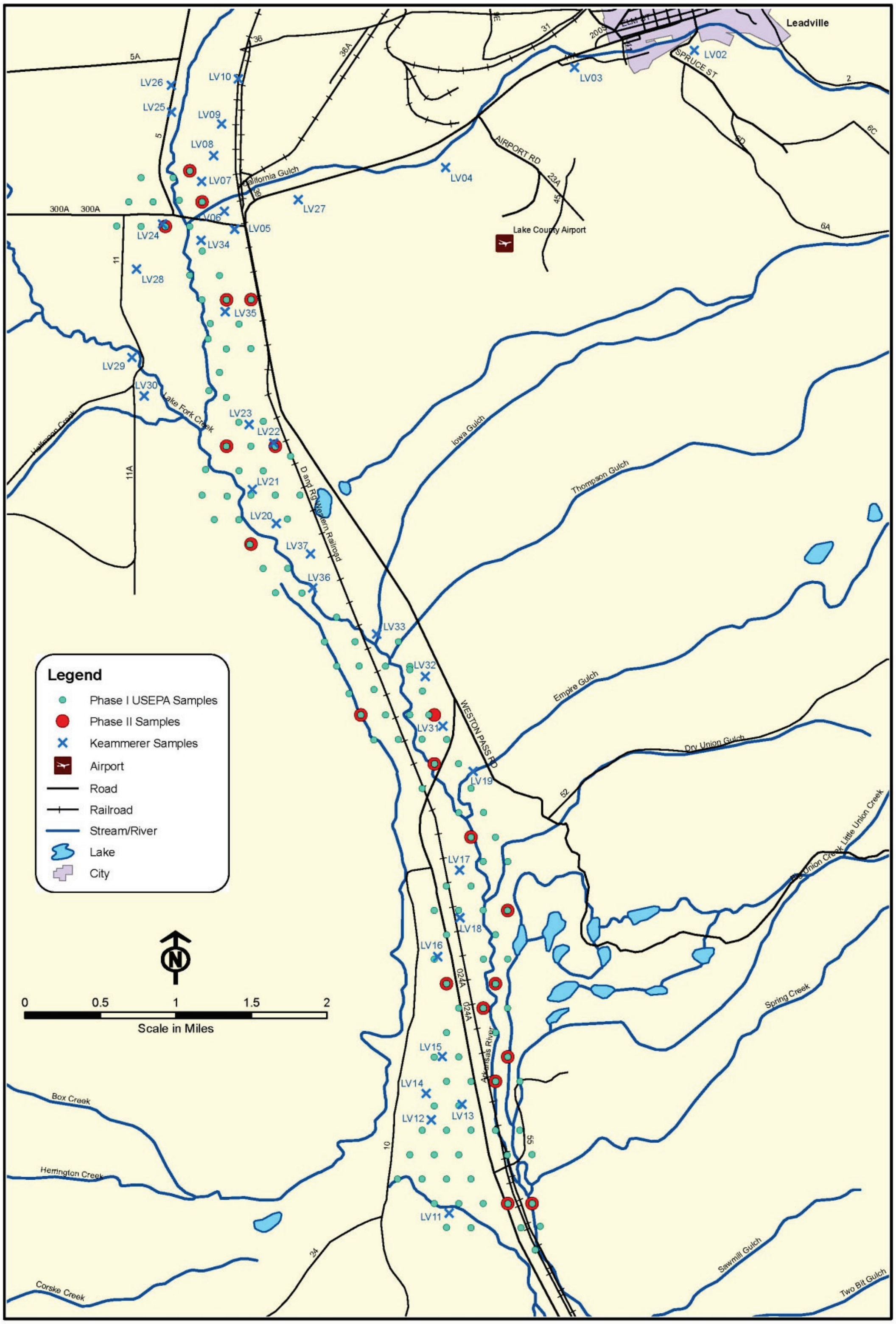


FIGURE 13. Soil and Plant Sampling Locations (Keammerer, 1987)

Factors other than grazing could account for the differences in plant growth at a given location and time. The sample locations within the irrigated meadows areas include some areas of higher ground that have not been irrigated due to the topography. Due to the limited rainfall in this area, plant growth could well be limited by available moisture. Plant growth may also be affected by agricultural practices such as fertilization and herbicide use, and these applications may differ among the ranches in the study area. Therefore, a range of plant growth rates may be expected even in the absence of phytotoxicity. However, if phytotoxic conditions are present, the upper limit of plant growth could be limited.

Several of the samples from areas just below California Gulch are west of the Arkansas River. The soils on these ranches have soil total metal concentrations that are typical of the background in the Arkansas River Valley. Therefore, plotting the plant growth and species data against distance from the confluence of California Gulch is not meaningful. Instead, the plant biomass data were plotted versus soil total Zn concentration and against DTPA-extractable soil Zn. Similar plots of plant growth and species richness versus concentrations of As, Cd, Cu or Pb could also be prepared. Statistical analysis of the laboratory plant growth tests suggested that Zn was the strongest determinant of phytotoxicity. In addition, concentrations of these elements are highly correlated with Zn concentration (see Table 21). For this reason, only figures for Zn concentrations are presented in this report.

In the present case study, as in other situations, a number of different stressors can account for a given biological response such as differences in plant growth. Quantile regression is a technique that can be used to help describe stressor-response relationships (see data analysis section at http://www.epa.gov/caddis). It models the relationship between a specified conditional quantile (or percentile) of a dependent (response) variable and one or more independent (explanatory) variables (Cade and Noon, 2003). As with mean regression, the relationship is often assumed to be a straight line.

In this case study, quantile regression is used to estimate the location of the upper boundary of a scatter plot (e.g., the 95th percentile line). An assumption for using this upper boundary is that the wedge shape often observed in scatter plots of biological metrics results from the effects of other stressors co-occurring with the modeled stressor that cause additional negative effects on the biological response. Hence, it is assumed that an upper quantile line represents the best possible performance of the plants given the concentration of the metal.

Figure 14 shows a possible relationship between maximum plant biomass and total soil Zn concentration, i.e., decreasing maximum plant biomass with increasing Zn. Plant biomass at the two locations with the highest soil Zn concentrations was dominated by plant species that are known to be tolerant of high soil metal concentrations. If these two points are treated as outliers, the relationship between maximum plant biomass and soil total Zn concentration is strengthened but is still not significant (SAS quantile regression [QUANTREG Procedure] at 0.9 and 0.95 quantiles [Figure 15] tested with Likelihood Ratio).

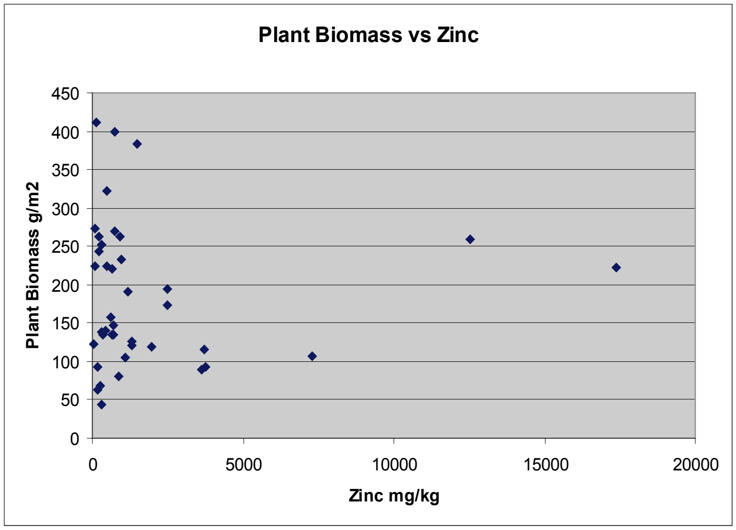


FIGURE 14. Plant Aboveground Biomass Versus Soil Total Zinc (all data points)

Data from Keammerer (1987).

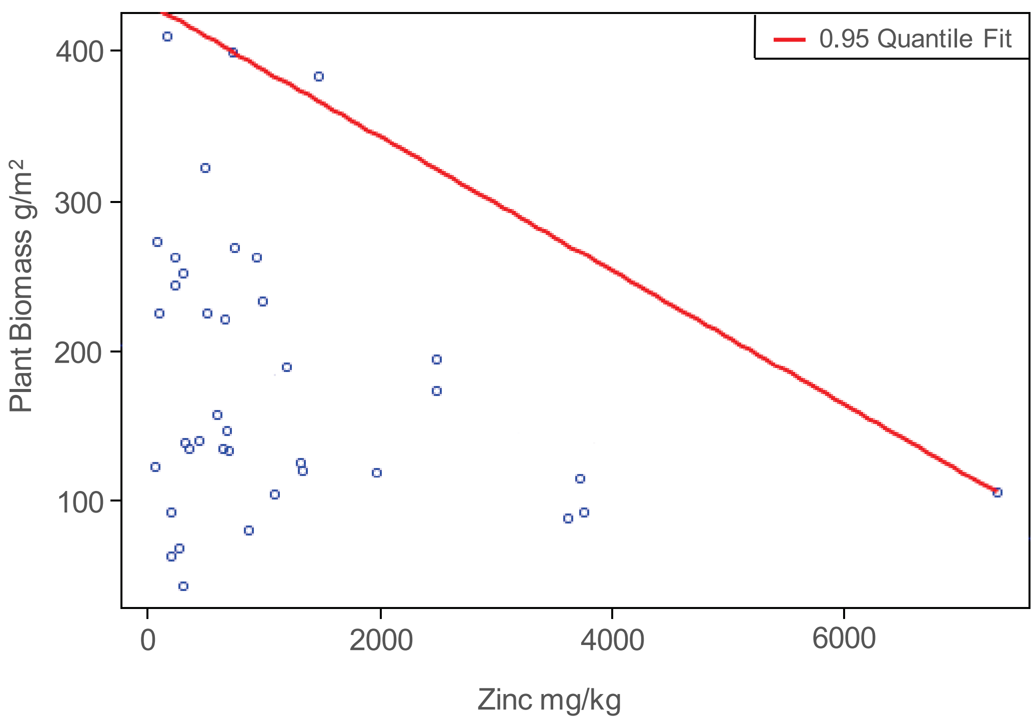


FIGURE 15. Plant Aboveground Biomass Versus Soil Total Zinc (minus two outliers; see text)

Data from Keammerer (1987).

At the location where Zn concentration was highest (LV22; 17,400 mg/kg), four plant species were reported (Keammerer, 1987). In terms of plant dry weight, *Deschampsia caespitosa* (tufted hairgrass) accounted for 58%, *Carex* spp. (sedge, sloughgrass) accounted for 15%, *Juncus arcticus* spp. *Ater* (wiregrass) accounted for 14% and *Phelum pratense* (timothy) accounted for 13%. Keammerer (1987) reported three plant species as present at the site with the second highest Zn concentration (LV03; 12,550 mg/kg); but only one species, *Carex aquatilis*, accounted for 100% of the plant dry weight. Both tufted hairgrass and *Carex* species are considered indicator plants for metal contamination (Brown et al., 1988; Cooper and Emerick, 1989).

A plot of plant biomass versus soil DTPA-extractable Zn also shows a trend of decreasing maximum plant biomass with increasing DTPA-Zn (see Figure 16). This trend is significant (SAS quantile regression [QUANTREG Procedure] at 0.95 quantile tested with Likelihood Ratio, $p = 0.0281$). These results offer at least a partial explanation for the relatively high plant biomass observed at the highest bulk soil Zn concentrations. The points with the highest bulk soil Zn values had lower DTPA-Zn concentrations (LVO3, 341 mg/kg; LV22, 544 m/kg) than some points with lower bulk soil Zn values. As was the case for the bulk soil concentrations of Zn, the relationship between maximum plant biomass and DTPA-Zn is complicated by the limited number of samples at high DTPA-Zn concentrations and the variability exhibited at lower concentrations of DTPA-Zn.

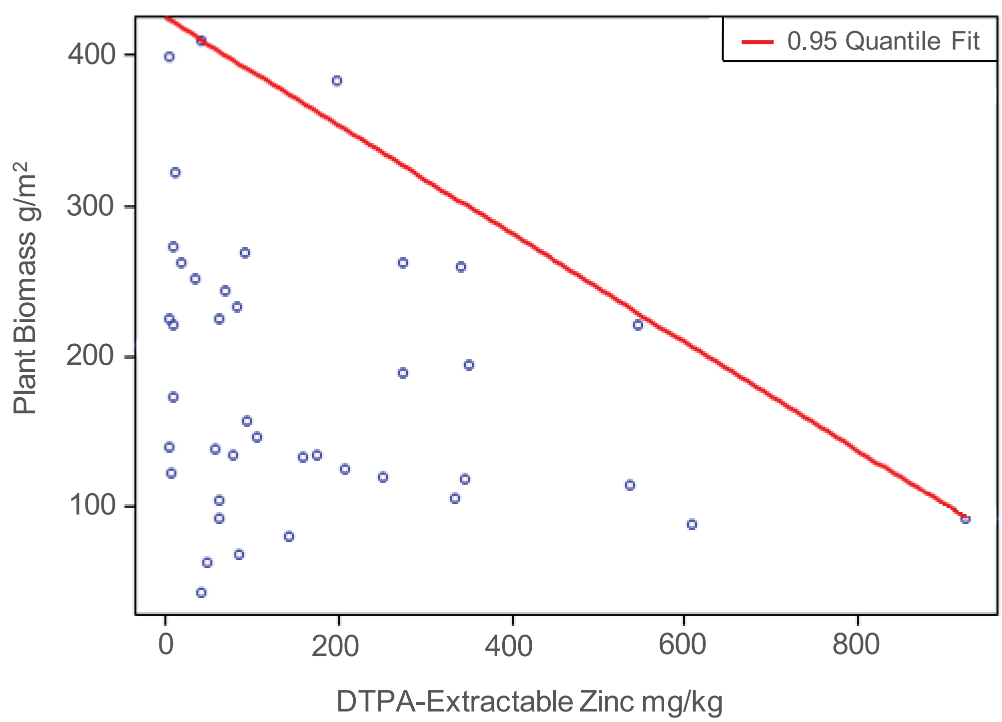


FIGURE 16. Plant Aboveground Biomass Versus Soil Ammonium Bicarbonate DTPA Extractable Zinc

Data from Keammerer (1987).

The number of plant species - or species richness - observed by Keammerer (1987) at low soil Zn concentrations was highly variable (see Figure 17). Up to nine plant species were observed at four locations with soil Zn concentrations up to 1185 mg/kg soil. The number of plant species observed at each sampling point ranged from 2−9 at soil Zn concentrations up to 1500 mg/kg and from 1−5 at higher concentrations. Interpretation of the number of species observed at high soil Zn concentrations is difficult because there were only a few sample points. Nonetheless, the observations are consistent with a decline in maximum species richness with increasing soil Zn concentrations. This trend is significant (SAS quantile regression [QUANTREG Procedure] at 0.9 and 0.95 quantiles tested with Likelihood Ratio, $p = 0.0031$ and 0.0029 respectively).

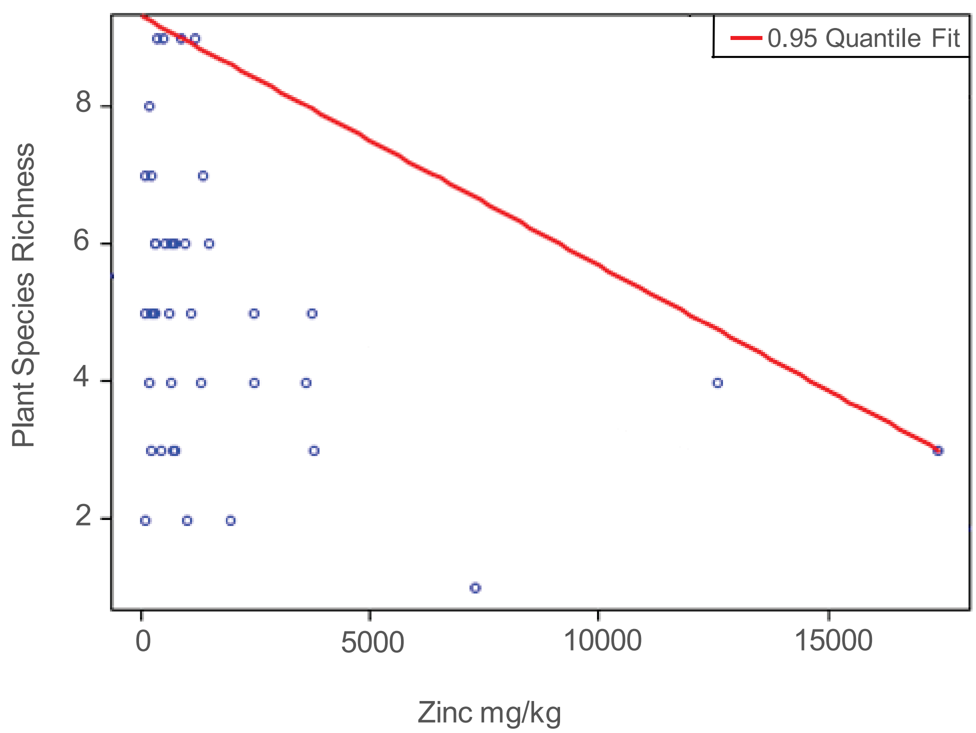


FIGURE 17. Plant Species Richness Versus Soil Total Zinc

Data from Keammerer (1987).

Figure 18 presents a plot of the number of plant species—or species richness—observed by Keammerer (1987) against DTPA-Zn concentration. The number of plant species observed was highly variable, but all four locations with nine species observed occurred at DTPA-Zn concentrations at or below 274 mg/kg. Interpretation of the number of species observed at high DTPA-Zn concentrations is difficult because there were only a few sample points. Nonetheless, the observations are consistent with a decline in maximum species richness with increasing DTPA-Zn concentrations. This trend is significant (SAS quantile regression [QUANTREG Procedure] at 0.9 quantile tested with Likelihood Ratio, $p = 0.0135$).

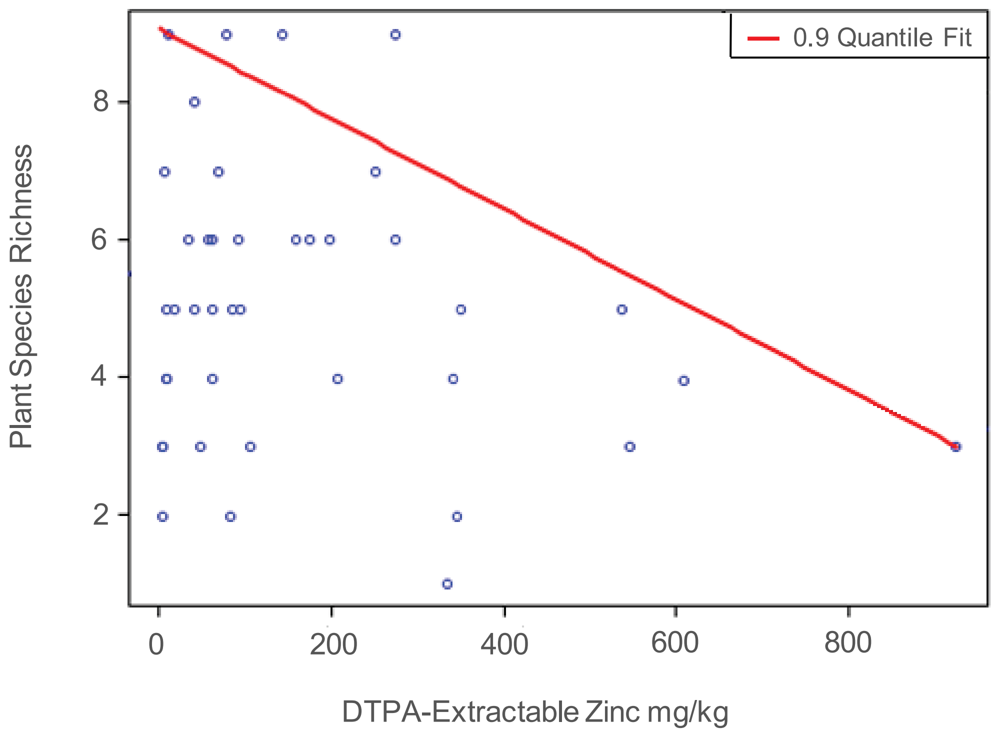


FIGURE 18. Plant Species Richness Versus Soil Ammonium Bicarbonate DTPA Extractable Zinc

Data from Keammerer (1987).

The exposure pathway is complete, as demonstrated by increasing Zn concentrations in the above-ground biomass of grasses and grass-like species with increasing soil total Zn and with increasing DTPA-Zn (see Figures 19 and 20). Although plants can accumulate metals in vacuoles without expressing toxic effects (Kapustka et al., 2004), the increasing above-ground plant concentrations demonstrate that the Zn is in a biologically available form and is taken up and translocated to the stems and leaves. Grasses and grass-like species are the dominant plant types present in the native vegetation in the irrigated meadows, and no forbs were observed at several of the sampling points. Therefore, only the data for grasses and grass-like species were plotted. The station with the highest DTPA-Zn concentration (924 mg/kg) appears to be an anomaly in that the above-ground grass Zn concentration was only 158 mg/kg. This station consisted almost exclusively of Western wheatgrass, *Agropyron smithii*.

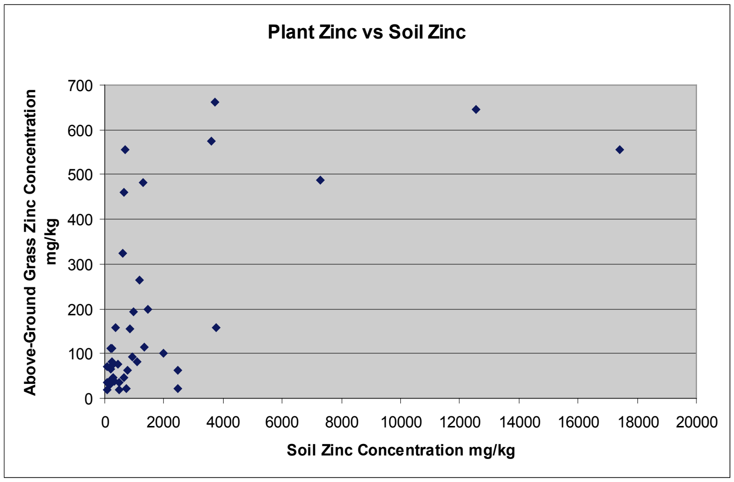


FIGURE 19. Zinc Concentration in Above-Ground Plant Parts of Grasses and Grass-Like Species Versus Soil Total Zinc

Data from Keammerer (1987).

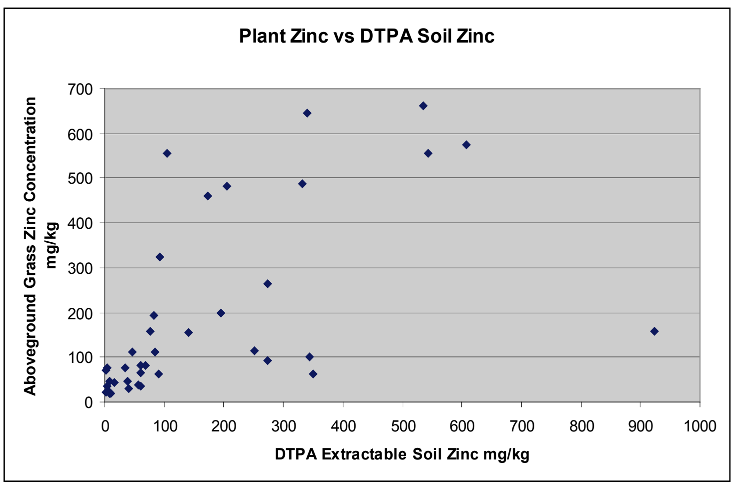


FIGURE 20. Zinc Concentration in Above-Ground Plant Parts of Grasses and Grass-Like Species Versus Soil Ammonium Bicarbonate DTPA Extractable Zinc

Data from Keammerer (1987).

Step 4: Evaluate Data from Elsewhere

A. *Verification of Prediction*

Forage yield samples (n = 16) were collected by the Lake County USDA Natural Resources Conservation Service agent on August 11, 2000 (NRCS, 2001). The sample points were randomly selected, and only the current year's plant growth was harvested. These forage yield data were collected independently from other sampling efforts and thus allow testing of the prediction that plant growth along the 11-mile reach is limited by elevated concentrations of extrinsic metals. Although the Natural Resources Conservation Service (NRCS, 2001) did not collect soil samples, the U.S. EPA Phase I data set was used to estimate the soil Zn concentration at the forage sampling points. One forage data point was excluded because it was too far from the nearest Phase I sampling point. Locations of the forage sampling points are shown in Figure 21. No information was provided in the NRCS (2001) about grazing intensity, irrigation or any other factor that might affect plant growth. Forage yield declined with increasing estimated levels of soil Zn (see Figure 22). This trend is significant (SAS quantile regression [QUANTREG Procedure] at 0.95 quantile tested with Likelihood Ratio, $p < 0.0001$). The limited number of sampling points in the highest Zn concentration areas makes interpretation difficult. Nonetheless, the lower yields observed at the highest Zn concentrations are consistent with Zn (or other correlated extrinsic metals) phytotoxicity.

B. *Comparison to Phytotoxicity Screening Levels*

Metal and As concentrations in irrigated meadows areas, along with soil concentrations considered to be potentially phytotoxic, are summarized in Table 28. The elements listed in Table 28 are those that are found at elevated concentrations in the irrigated meadows areas (Levy et al., 1992; Swyers, 1990; U.S. EPA Region 8 and USFWS, 2003). The upper end of the observed total concentrations for Ag, As, Cd, Cu, Pb and Zn were all well above the concentrations capable of causing phytotoxicity (Alloway, 1990; Efroymson et al., 1997; Kapustka et al., 2004). The upper end of the reported concentration ranges for Pb and Zn exceed the upper end of the critical ranges by 60- and 34-fold, respectively. The reported concentration ranges of several elements support the hypothesis that meadows irrigated with water from California Gulch are toxic to plants.

C. *Indicator Species*

Plant biomass at the two locations with the highest total soil concentrations of Zn was dominated by plant species that are known to be resistant to metal contamination and are considered indicator species. The plant biomass at the LV22 location with the highest Zn concentrations in the Keammerer (1987) study was dominated by *Deschampsia caespitosa* (tufted hairgrass) and *Carex* spp. At LV03, with the second highest soil total Zn concentration, *Carex aquatilis* accounted for 100% of the plant biomass. Both tufted hairgrass and *Carex* species are considered indicator plants for metal contamination.

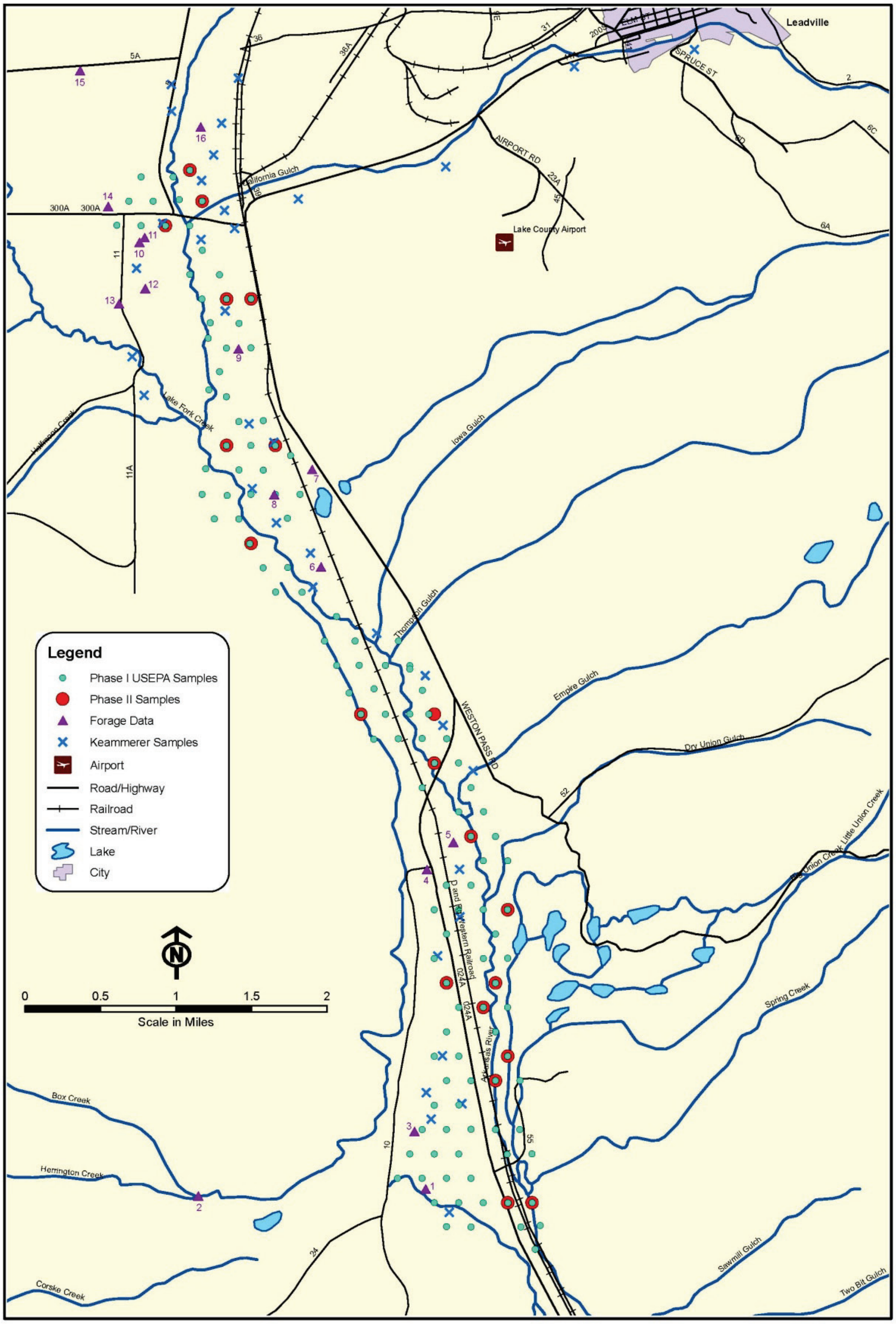


FIGURE 21. Forage Yield Sampling Locations (NRCS, 2001)

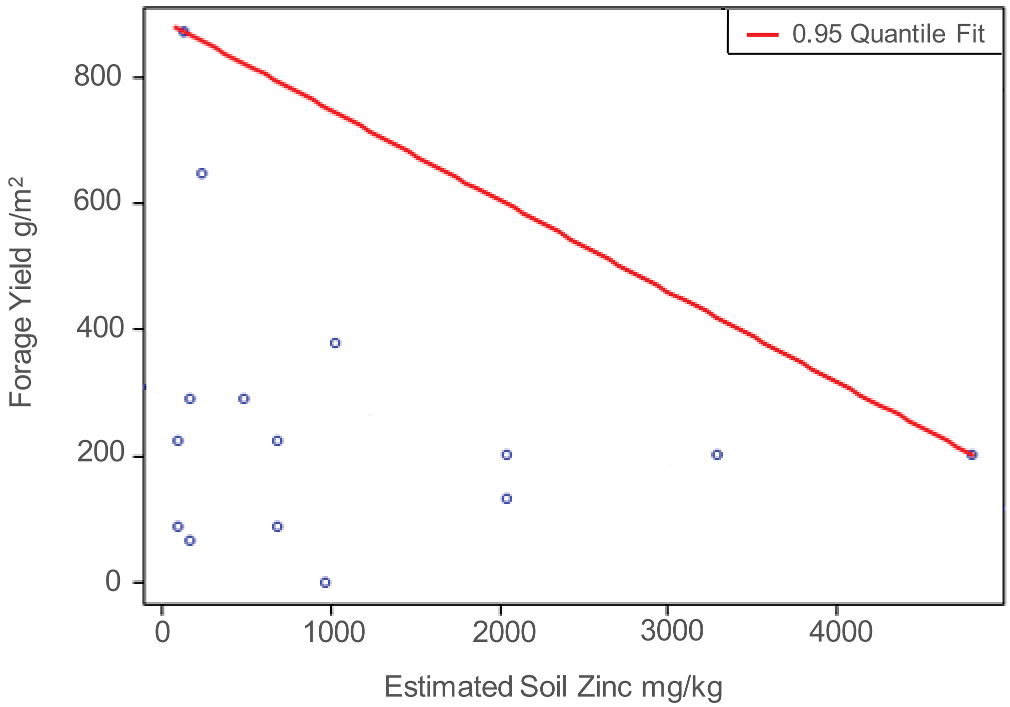


FIGURE 22. Comparison of Measured Forage Yield (g/m^2) with Estimated Soil Zinc Concentrations (mg/kg dry wt)*

*Forage yield data were from random locations in the upper Arkansas River valley collected by the USDA NRCS (2001). Soil zinc concentrations were estimated independently based on the nearest soil sample from the U.S. EPA Region 8 Phase I study (U.S. EPA Region 8 and USFWS, 2003).

TABLE 28. Comparison of Soil Concentrations (mg/kg dry wt) of Metals and Arsenic Observed in Irrigated Meadows of the Upper Arkansas River to Published Thresholds for Phytotoxicity[a]

Chemical	Concentrations Observed			Phytotoxicity Thresholds		
	Levy et al. (1992)[b]	Swyers (1990)[c]	U.S. EPA Region 8 and USFWS (2003)[d]	Alloway (1990)[e]	Kapustka et al. (2004)	Efroymson et al. (1997)
Ag	nd[f]	nd	0.05−64	2	nd	2
As	nd	nd	1.5−270	20−50	31	
Cd	9−39	5−113	0.11−73	3−8	28	4
Cu	81−431	21−409	7−709	60−125	95	100
Hg	nd	nd	0.03−122	0.3−5	nd	0.3
Pb	301−18,000	46−1112	4.2−24,200	100−400	210	50
Zn	699−7650	294−8722	56−13,500	70−400	130	50

[a]Concentration units are mg/kg.
[b]Range of concentrations reported by Levy et al. (1992) from the impaired meadows area.
[c]Concentrations reported by Swyers (1990) from an impaired meadow area.
[d]Concentration range reported by U.S. EPA in the 11-mile reach.
[e]Critical range of soil concentrations above which toxicity is considered to be possible. Data from Kabata-Pendias and Pendias (1984).
[f]nd = no data on this element.

Tufted hairgrass is a metals-tolerant species and occurs on acidic or pyritic mine spoils at high elevations throughout the western United States (Brown et al., 1988). This grass also is good to excellent forage for all types of livestock and wildlife, and is commonly planted on mine spoil sites as a restoration technique (Morris et al., 1950; Stubbendieck et al., 1992). Tufted hairgrass also was the dominant plant species at other sampling locations where high concentrations of metals were not present. Thus, the presence of tufted hairgrass alone is not sufficient to reliably indicate metal toxicity. Because tufted hairgrass is seeded in high altitude meadows as a desirable forage grass, its distributions also are affected by agricultural management of the meadows.

Some herbaceous plants are quite sensitive to heavy metals and other contaminants. Thus, contamination of soils with metals can alter plant species composition and decrease species richness, canopy coverage and net annual productivity of wetland communities (e.g., Cooper and Emerick, 1989; Olson, 1979).

Based on studies of eight Colorado wetlands exposed to varying degrees of heavy metal-contaminated runoff, Cooper and Emerick (1989) noted

> Subalpine fen wetlands in the Colorado Front Range that have less than three vascular plant species growing in the main part of the wetland (not the edges) and have less than 50 percent total canopy coverage and less than 100 g/m^2 total annual primary production, are likely to indicate impact from heavy metal toxicity. An exception is areas that are flooded or have ponded water for much of the growing season.

Forbs (herbaceous dicots in the Cooper and Emerick [1989] study) seemed particularly uncommon in polluted wetlands. These authors noted no species that occurred only at contaminated sites, but found that the sedges *Carex aquatilis*, *C. utricularia* and/or *C. scopulorum* dominated those areas (Cooper and Emerick, 1989).

Step 5: Identify Probable Cause

Step 5—identifying the probable cause—is the last step in the Stressor Identification (SI) process. Based on available evidence organized in Steps 3 and 4, the most probable cause(s) is distinguished from a set of less probable causes. Step 5 consists of three components discussed below.

A. *Characterize Causes: Eliminate*

Considerations for determining whether or not candidate causes should be eliminated are summarized in this section and in Table 29. Table 29 addresses four questions for each candidate cause. These questions are:

- Do impairments occur in the same place as exposure?
- Is exposure increased over that at the reference site?
- Is there a gradient of recovery with reduced exposure?
- Is the exposure pathway complete?

If any of the answers for a candidate cause are clearly "no," then that cause can be eliminated. Otherwise, the candidate cause is carried forward to the *strength of evidence* analysis (i.e., the candidate cause is not eliminated, or is remaining).

1. Increased Concentrations of Extrinsic Metals in the Soil. Concentrations of As, Cd, Cu, Pb and Zn were higher in the impaired meadows that were irrigated with water from California Gulch from 1874 until the 1920s, compared to either upstream meadows or meadows further downstream, which were irrigated with water from other sources. Forage yield in the affected meadow has decreased from 4.48 Mg/ha (3990 lbs/acre) in 1874 to 1.68 Mg/ha (1496 lbs/acre) in the early 1990s (Levy et al., 1992). Swyers (1990) quoted Dr. Bernard Smith, a local veterinarian and rancher, as stating that ranchers had noted decreases in hay yield after mining and smelting became

TABLE 29. Considerations for Determining Whether or not Candidate Causes Should be Eliminated for Impairment 2					
Candidate Cause	Impairments Occur Same Place as Exposure?	Exposure Increased over Reference Site?	Gradient of Recovery at Reduced Exposure?	Exposure Pathway Complete?	Candidate Causes Remaining
1. Increased extrinsic metals	Yes	Yes	Yes	Yes	Yes
2. Decreased pH with increased extrinsic metals	NE[a]	Yes	Yes	Yes	Yes
3. Decreased pH with intrinsic (background) metals	No	No	No	No	No
4. Decreased organic matter	No	No	No	No	No
5. Soil compaction	NE	No	No	Yes	No
6. Grazing and mowing	Yes	No	NE	Yes	No
7. Herbicides	NE	No	No	NE	No

[a]NE = no evidence.

widespread in the late 1880s. Statistical models based on soil concentrations of either Zn or Cu both provided good fits with distribution of phytotoxicity in laboratory tests (EP&T, 2002; U.S. EPA Region 8 and USFWS, 2003). Although the data were variable and there were few sampling points at the highest Zn concentrations, plots of plant biomass and plant species richness versus either total soil Zn concentration or DTPA-extractable Zn were consistent with decreasing maximum plant biomass and species richness with increasing Zn concentrations (Keammerer, 1987). Intermediate levels of plant biomass observed at the highest concentrations of total soil Zn were dominated by plant species that are considered to be indicators of metal contamination (Brown et al., 1988; Cooper and Emerick, 1989). Forage yields based on data collected independently by the USDA NRCS (2001) declined with increasing estimated concentrations of soil Zn. Because increased concentrations of extrinsic metals consistently were observed in association with decreased plant growth, decreased plant species richness and presence of metal-tolerant indicator species, Candidate Cause #1 (increased extrinsic metals) cannot be eliminated.

2. Interaction of Decreased pH and Increased Extrinsic Metals in the Soil. No data are available on change in soil pH levels over time in the irrigated meadows. Data on upstream pH levels are very limited (Levy et al., 1992). Statistical analyses of laboratory phytotoxicity tests indicate that low soil pH is a contributing factor to phytotoxicity (U.S. EPA Region 8 and USFWS, 2003). Based on the laboratory phytotoxicity testing, Candidate Cause #2 (interaction of decreased pH and increased extrinsic metals) cannot be eliminated.

3. Interaction of Decreased pH and Intrinsic (Background) Metals in the Soil. Interaction of decreased pH and intrinsic (background) metals in the soil may result in increased levels of metals in solution, causing phytotoxicity (see Section 2.1 #3).

The pH values found in areas where impairment was observed ranged from 5.2−7.1 (see Figure 9). The pH value in an unimpaired, upstream meadow was lower than the pH values for the impaired meadow areas (Levy et al., 1992). **Based on the absence of colocation of decreased pH with the observed impairment, Candidate Cause #3 (interaction of decreased pH with intrinsic metals) was eliminated from further consideration**.

4. Decreased Soil Organic Matter Results in Higher Metal Bioavailability. Data presented by Levy et al. (1992) showed no consistent difference in soil organic carbon between an upstream meadow and the impaired meadows. Statistical analyses also did not reveal a significant correlation between soil organic carbon and phytotoxicity in laboratory tests. **Based on the absence of colocation of decreased soil organic carbon with the observed impairment, Candidate Cause #4 (decreased soil organic matter) was eliminated from further consideration**.

5. Soil Compaction Reduces the Permeability of the Soil Surface Layers and Root Growth. Soil compaction is a common problem that restricts plant growth. Soil compaction is particularly severe under the saturated soil conditions that predominate in the upper Arkansas River area. Grazing cattle can compact soil (Wheeler et al., 2002). However, grazing occurs both upstream and downstream of the confluence of California Gulch with the Arkansas River (see below), and chlorosis and barren areas in irrigated meadows were not reported upstream of California Gulch. Soil compaction can also arise from differences in soil origin or type, e.g., deposited fines, but evidence supporting this is lacking. **Soil compaction does not appear to be a cause of impairment in the irrigated meadows.**

6. Grazing by Cattle and Mowing (for Hay Production) can Affect Plant Growth or Species Richness. The available data do not suggest that grazing or mowing were different for the study area—which includes the impaired meadows—compared to surrounding areas. According to data from 1997 and 2002 Cattle and Calves Inventories (USDA, 2004), the study area county (Lake) contained 1858 and 287 head of cattle in 1997 and 2002, respectively, compared to 12,803 and 5964 head of cattle (1997 and 2002) in the county (Eagle) north of the study area and 11,167 and

6590 head of cattle (1997 and 2002) in the county (Chaffee) south of the study area. Data on mowing per se is not available, but according to harvested hay data from a 2002 census (USDA, 2004), 220 acres, yielding 141 tons, was harvested from the study area county (Lake), compared to 6391 acres, yielding 7021 tons in Eagle County, and 7198 acres in Chaffee County. Based on the above data, there does not appear to be evidence for a relationship between either grazing by cattle or mowing and plant growth or species richness. **Because cattle grazing and mowing occur both upstream and downstream, they are not likely to be causes of the impairment.**

7. Increased Herbicide Usage to Enhance Hay Production May Reduce Plant Growth or Species Richness. Selective herbicides sometimes are used to favor the growth of grass species at the expense of other plant species. Repeated applications of selective herbicides over a period of years could eliminate or reduce the growth of some plant species. The limited available data do not suggest an increased use of herbicides in the study area—which includes the impaired meadows—compared to surrounding areas. According to data from a 1997 census (USDA, 2004), herbicides were applied at 3 farms in the study area county (Lake) compared to 33 and 24 farms, respectively, in counties north (Eagle) and south (Chaffee) of the study area. The data were similar for the 2002 census, where herbicides were applied to 2 (Lake), 30 (Eagle) and 7 (Chaffee) farms. Hence, there does not appear to be evidence for a relationship between herbicide usage and plant growth or species richness. **Because herbicide usage for hay production occurs both upstream and downstream, it is not likely to be a cause of the impairment.**

B. *Characterize Causes: Strength of Evidence*

Strength of evidence analysis uses all the evidence generated in the analysis phase to examine the credibility of the remaining candidate causes. The causal considerations for the strength of evidence analysis use three types of evidence: case-specific evidence, evidence from other situations or biological knowledge, and evidence based on multiple lines of evidence. All evidence was evaluated for consistency or coherence with the hypothesized causes. The results of the strength of evidence analysis are summarized in Table 30. In the table, each line of evidence is given a score as follows:

NE	= no evidence.
+ + +	= convincing evidence as cause.
+ +	= strong evidence as cause.
+	= weak evidence as cause.
0	= unclear.
–	= weak evidence as not cause.
– –	= strong evidence as not cause.
– – –	= convincing evidence as not cause.

TABLE 30. Strength of Evidence for Specific Causes and Considerations for Impaired Irrigated Meadows

	Consideration	Extrinsic Metals		Extrinsic Metals with Decreased pH	
		Results	Score	Results	Score
Case-Specific Evidence	Spatial co-occurrence	Compatible: Concentrations of extrinsic metals were consistently elevated in the impaired meadows downstream of California Gulch (3.2 Step 3 A, C). Concentrations of metals were elevated at impaired meadow sites on two ranches that received irrigation water from California Gulch, compared to an upstream reference site (3.2 Step 3 C).	+	Incompatible: pH values in most of the impaired areas were near neutral (3.2 Step 3 A).	– –
	Temporality	Compatible: Decreased forage yields were reported after mining and smelting became widespread in the area (3.2 Step 3 C).	+	NE	NE
	Consistency of association	Consistent: Decreased plant biomass and species richness was observed consistently with increased Zn (3.2 Step 3 C).	+ +	Inconsistent: Impaired plant growth occurred in areas with near-neutral pH (3.2 Step 3 C).	–
	Biological Gradient	Moderate: There is a general trend towards less plant biomass and species richness with increasing Zn. However, intermediate plant biomass occurred at two locations where Zn concentrations were highest, due to the dominance of metals-tolerant indicator species at those locations (3.2 Step 3 C).	+ +	Inconsistent: Areas of impaired vegetation often had near-neutral or alkaline pH values (3.2 Step 3 C).	0
	Complete exposure pathway	Complete: Increasing Zn concentrations were found in plants with increasing soil total Zn and with increasing DTPA-Zn (3.2 Step 3 C).	+ +	NE	0

TABLE 30 cont.					
	Consideration	Extrinsic Metals		Extrinsic Metals with Decreased pH	
		Results	Score	Results	Score
Case-Specific Evidence cont.	Experiment	Concordant: Phytotoxicity tests and follow-up models indicate that Zn or Cu concentrations are reasonable predictors of site phytotoxicity (3.2 Step 3 A; B).	+ +	Concordant: Phytotoxicity tests and follow-up models indicate that Zn or Cu concentrations are reasonable predictors of site phytotoxicity. Low soil pH is a contributing factor (3.2 Step 3 A; B).	+
Information From Other Situations or Biological Knowledge	Mechanism	Plausible: Ag, As, Cd, Cu, Hg, Pb, Zn are known to cause the types of effects seen in the meadows (2.1).	+	Plausible: Plants are sensitive to elevated metal concentrations and metal solubility increases when pH declines (2.1).	+
	Stressor-response	Concordant: Ag, As, Cd, Cu, Hg, Pb, Zn were present at concentrations that exceed phytotoxicity screening thresholds (3.2 Step 4 B).	+	Inconcordant: Increased extrinsic metals alone was sufficient to cause impairment (3.2 Step 4 B).	–
	Consistency of association	Consistent: Contamination of soils with metals altered plant species composition, and decreased species richness, canopy coverage and net annual productivity of subalpine wetland communities (3.2 Step 4 C).	+ +	Inconsistent: Reported concentration ranges of several elements, alone, were sufficient to cause impairment (3.2 Step 4 B).	–
	Specificity of cause	Many other stressors can impair vegetation; nonetheless, locations with the highest Zn concentrations were associated with few plant species, two of which are indicator species for metal contamination (3.2 Step 3 C; 3.2 Step 4 C).	+	Many other stressors can impair vegetation.	0

TABLE 30 cont.					
	Consideration	Extrinsic Metals		Extrinsic Metals with Decreased pH	
		Results	Score	Results	Score
Information From Other Situations or Biological Knowledge cont.	Analogy	Dominance of tolerant species including *Deschampsia caespitosa* and *Carex* species at metal-contaminated sites has been reported in many other locations (3.2 Step 4 C).	+	NE	
	Experiment	NE		NE	
	Predictive Performance	Confirmed: Forage yield measured at random locations declined with estimated concentrations of Zn (3.2 Step 4 A).	+ +	NE	
Considerations Based on Multiple Lines of Evidence	Consistency of Evidence	All lines of evidence are consistent with increased levels of extrinsic metals as a cause of impaired vegetation.	+ + +	Some lines of evidence were consistent with decreased pH as a contributing cause, but neutral to alkaline areas were also impaired.	+
	Coherence of evidence			Inconsistency may be explained by the fact that concentrations of metals greatly exceeded concentrations known to be phytotoxic and would be expected to cause toxicity even at circum-neutral pHs.	+

Impaired areas of irrigated meadows occur on the Seppi and Smith ranches just below the confluence of California Gulch. Plant growth in these areas was impaired relative to upstream reaches and to meadows further downstream. Elevated concentrations of extrinsic metals were consistently observed in the impaired areas (Levy et al., 1992; Swyers, 1990; U.S. EPA Region 8 and USFWS, 2003). A layer of buried sand with elevated levels of extrinsic metals was reported in several locations in the meadows on the Seppi ranch (Levy et al., 1992; Swyers, 1990). Soil pH values in the impaired meadows on the Seppi ranch were slightly acidic: this condition is consistent with increased availability of extrinsic metals to plants. However, the pH levels were not acidic enough to be consistent with known effects of acidity on solubility of Al, Fe and Mn, intrinsic metals that are phytotoxic at low pH. The highest concentrations of metals were observed on the Smith ranch, accompanied by neutral to slightly alkaline pH. In general, plant biomass and species richness declined with increasing concentrations of Zn and other extrinsic metals (see Figures 14 through 17). However, intermediate plant biomass occurred at the two locations where Zn concentrations were highest (see Figure 14). The plant biomass at these two locations was dominated by *Deschampsia caespitosa, Carex aquatilis* and *Carex* spp. These plant species can tolerate high concentrations of metals and are considered to be indicator species for metal contamination.

The results of laboratory phytotoxicity tests were consistent with increased concentrations of extrinsic metals as the cause of impaired plant growth in the irrigated meadows on the Seppi and Smith ranches (U.S. EPA Region 8 and USFWS, 2003). Soil samples collected at 20 representative locations were tested in the laboratory using alfalfa, yarrow and wheat grass (EP&T, 2002). The bulk soil variable giving the best prediction of laboratory mean phytotoxicity score was the first principal component, which was dominated by metals, pH and Ca concentration.

Additional examination of the laboratory phytotoxicity test results was performed by dividing the data into a binary variable: phytotoxic versus nontoxic locations. Bayesian models developed for Zn and Cu had almost identical DIC values (DIC Cu = 23.7; DIC Zn = 22.9). This result shows that the models had very similar fits. The CVs of the Cu model parameters were larger than those found in the Zn model, indicating greater uncertainty in the parameter distributions. The CV of β_1 in the Zn model is much smaller than the CV of β_1 in the Cu model. This finding indicates that, across all sites, the relationship between toxicity and Zn is stronger than the relationship between Cu and toxicity.

We used forage yield data collected independently by the USDA NRCS (2001) to test the prediction that increased concentrations of extrinsic metals caused decreased plant growth. Although metal concentrations were not measured at the locations of the forage sample collection, we used the data from 126 sample locations (U.S. EPA Region 8 and USFWS, 2003) to estimate Zn concentrations at the forage-yield locations. As predicted, the upper limit of forage yield declined monotonically as the estimated concentration of Zn increased (see Figure 21). High variability at low Zn concentrations and relatively few observations decrease the strength of this evidence. The relationship between forage yield and Zn concentrations was consistent with the predictions from models of laboratory studies.

C. *Characterize Cause: Probable Cause*

After five candidate causes were deemed improbable, two candidate causes remained. These were compared on the basis of strength of evidence. The two candidate causes were: #1 (increased extrinsic metals) and #2 (interaction of extrinsic metals with decreased pH).

The evidence supporting Candidate Cause #1 (impairment of plant growth due to elevated levels of extrinsic metal) was consistent throughout the lines of evidence. The strength of association, spatial co-occurrence, plausible stressor response and experimental lines of evidence strongly supported this candidate cause. The quality of the data is adequate for this conclusion, and confidence is high (see Table 30).

In contrast, the evidence for Candidate Cause #2 (interaction of decreased pH with increased extrinsic metal concentrations) was inconsistent. Impaired plant growth occurred in meadow areas with near-neutral to alkaline pH values. Hence, it appears that increased extrinsic metals alone were sufficient to cause impairment of plant growth in the irrigated meadows.

4. LESSONS LEARNED

4.1. INTRODUCTION

The Stressor Identification methodology (U.S. EPA, 2000) has been demonstrated in the context of case studies for the Clean Water Act. The present case study applies the SI methodology at a terrestrial contaminated site, the highly mineralized area of the Colorado Rocky Mountains, to shed light on its utility in such an environment. This section summarizes lessons learned during application of the SI process to a terrestrial hazardous waste site relative to application at aquatic sites. It also highlights what, if any, aspects of the SI process (as described in U.S. EPA, 2000) are different, and provides suggestions for further development of the SI process at terrestrial sites.

The greatest difference between aquatic and terrestrial systems is the uncertainty associated with measuring environmental parameters. In aquatic systems, a certain degree of homogeneity can be assumed for water column exposures at a local level due to the mixing effects of water currents. Sediments are less homogeneous, but soil systems present a difficult problem with the great heterogeneity in environmental parameters, both horizontally and vertically within the soil profile. Such is the case in this study.

4.2. CRITICAL VARIABLES

Dominant soil chemical/physical properties known to affect the bioavailability of contaminants are soil pH, organic carbon, cation-exchange capacity (CEC), clay content, and reactive iron, aluminum and manganese oxides (Basta et al., 2005; Fairbrother et al., 2007). These parameters are very important factors affecting the bioavailability of both intrinsic and extrinsic metals to plants. Concentrations of Al, Mn and Fe present in all soils can be phytotoxic at low pH. Conversely, extrinsic metals that have been introduced by human activity may not be phytotoxic even at elevated concentrations if the pH is high, because the solubility of many metals is reduced at high pH levels. The contaminated, vegetated area in the Upper Arkansas River (UAR) illustrates this, with its abundant, diverse vegetation despite As, Cu, Cd, Pb and Zn levels that were as high as those in barren fluvial mine-waste deposit areas. The critical difference was the difference in pH. Thus, pH must be known in order to determine if impairment is due to elevated metal concentrations.

Soil compaction can reduce plant growth. In the present study, the known causes of soil compaction did not appear to be associated with impaired areas. Nonetheless, *measurements* of soil compaction would lend more credibility to the analysis.

In the UAR case study, organic matter appeared to be less critical than soil pH. This may not be true in other cases, and both soil organic matter and CEC are important determinants of how metals “operate” on plants. Brown et al. (1998) suggests that the effects of added organic matter are more important after a period of aging. (To date, there are no models available that incorporate an aging term linked to chemical bioavailability.) When designing investigations of terrestrial sites, these soil parameters should be included. If the investigator is working with available information and data for these parameters are not available, then these data gaps should be pointed out in an uncertainty section.

Although metals and low pH are the cause of the barren areas surrounding mine tailing deposits, low nutrient levels may be a contributing factor. TKN ranged from 1−1.6 g/kg in untreated fluvial mine-waste deposits, compared to 4.5 g/kg in the upland reference and 2.9 g/kg in the contaminated vegetated area soils (Brown et al., 2005). Available phosphorus concentrations ranged from 1−12 mg/kg in the untreated fluvial mine-waste deposits, compared to 23 mg/kg in the upland reference soil (though both of these soils would be considered potentially deficient in available phosphorus for plant growth). The addition of lime and biosolids to fluvial mine-waste deposits (that resulted in the establishment of plant cover) increased soil pH, but also increased TKN and phosphorus concentrations (Brown et al., 2005). In other field experiments (Fisher, 1999), adding triple-super-phosphate (TSP) alone did not increase the available phosphorus in soil samples and did not significantly affect survival or leader growth of willow cuttings. However, the addition of TSP to limed treatments resulted in a significant increase in total leader length. This indicates that phosphorus may have been a factor contributing to decreased plant growth. Elucidation of the contribution of nutrients to the effects of metals would require more and better nutrient data.

It is essential that critical soil chemical/physical characteristics are measured in association with chemical levels at terrestrial sites to establish the effects of these modifying factors on bioavailability. In aquatic systems, the Biotic Ligand Model (BLM) can be used to examine the effects of environmental modifying factors (i.e., pH, water hardness) on metal speciation and subsequent binding to biotic ligand (fish gill). BLMs exist for Cu and Ag in aquatic systems (U.S. EPA, 2003), and a few examples exist for terrestrial systems (Thakali et al., 2006a,b; Koster et al., 2006). Also, regression equations, similar to the one developed in this document, have been developed to describe the effects of soil properties on bioavailability and toxicity. These regression-type models are somewhat limited in their range of application over different soils, but are useful in getting a rough idea of the most important factors determining metal bioavailability at a given site (reviewed by Smolders et al., 2009). Use of Path Analysis, another statistical tool, can augment regression analysis and provide a measure of the relative contribution of specific soil properties in modifying metal bioavailability and toxicity (Dayton et al., 2006).

4.3. BIOASSESSMENT CRITERIA ARE NOT WELL DEVELOPED FOR PLANTS

Bioassessment criteria for terrestrial systems are not well developed. For plants, the Riparian Evaluation System (RipES) has been developed, but the criteria are very general (U.S. EPA, 2004b). Basically the RipES method evaluates vegetation as present or absent, and indicates that *Deschampsia caespitosa* (tufted hairgrass) is an indicator of metal contamination. There is also some limited information for indicator species for metal contamination in wetlands (U.S. EPA, 1990).

Forbs, and probably legumes in particular, may be uniquely sensitive to metal contamination, whereas grasses generally tend to be more resistant (U.S. EPA Region 8 and USFWS, 2003). This generality is similar to the situation in streams, where the Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) (EPT) species are intolerant of pollutants and will not be found in polluted waters (Carlisle and Clements, 1999). The greater the pollution, the lower the species richness expected, as only a few species are pollutant tolerant. The metal resistant grass species, *D. caespitosa*, is, however, a palatable, nutritious forage grass for both domestic animals and wildlife species (Morris et al., 1950; Stubbendieck et al., 1992). *Carex aquatilis*, a wetland plant recognized as an indicator species for metal contamination, is also considered a good source of forage. In the presence of high soil metal concentrations these species were observed to be dominant, almost monocultures. Thus, the biodiversity of the habitat became very limited. However, without data on metal uptake by (and toxicity to) domestic animals/wildlife species, it is not clear that these plant species are undesirable or incapable of supporting a healthy ecosystem.

There is a potential physiological basis for the tendency for grasses to be more metal tolerant. There are two basic strategies among plant families for uptake of Fe and other metal micronutrients (Welch, 1995). Dicots and nongrass monocots, reduce Fe(III)-chelates to Fe(II) outside the cell membrane in the apoplasm prior to uptake. Actual uptake of Fe(II) into the cytoplasm is driven by active export of H^+ ions out of the plant cell. The membrane potential generated by H^+ export drives uptake of divalent cations (Ca^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+} and Ni^{2+}) across the plasmalemma. Divalent cation uptake is thought to be mediated by inward directed divalent cation channels. In contrast, grasses have not been shown to reduce Fe(III)-chelates. Also, these plants do not acidify the rhizosphere in response to Fe-deficient conditions. Instead, these plants apparently depend on specific transport proteins in the plasmalemma with high specificity Fe(III)-phytometallophores (aka phytosiderophores). These transport proteins specifically bind the Fe(III)-phytometallophores and transport the intact complex across the plasmalemma. This type of mechanism could drive the uptake of other micronutrient metals (e.g., Zn^{2+}, Mn^{2+}, Cu^{2+} and Ni^{2+}) phytometallophore chelates into plant cells. These apparent fundamentally different multivalent metal uptake mechanisms could help explain why grasses are apparently less sensitive to metal toxicity than dicots and nongrass monocots. That is, the nongrass plant species acidify the rhizosphere, which would increase metal solubility near the root surface, and divalent ions are taken up through relatively nonspecific cation channels. Both of these factors would increase indiscriminant uptake of potentially toxic metals. This concept, however, does not offer an explanation for the metal tolerance of *C. aquatilis*, which is a sedge species, not a grass species.

Loss of legumes from the plant community could also be a critical parameter. Biological nitrogen fixation by legume symbiosis provides critical inputs of nitrogen to soil in a form needed for plant growth. Alfalfa was more sensitive to the phytotoxic effects of the soils from the UAR valley in laboratory studies (EP&T, 2002). Also, Siemer (1999) reported that clovers were abundant in the irrigated meadows of the UAR valley on ranches where metal concentrations were not elevated. In contrast, few legumes were found in areas with elevated metal concentrations (Keammerer, 1987). In general nodulation is more affected than host plant growth (Crowley and Alvey, 2002). Loss of legumes and biological nitrogen fixation could affect overall plant community growth.

Additional research on plant species metal tolerance, may help identify shifts in plant species composition that are indicative of specific stressors. Systematic evaluation of existing data from metal contaminated sites may define patterns in species shifts that are indicative of metal contamination.

4.4. SPATIAL SCALE ISSUES

Small scale changes in soil characteristics and moisture availability can confound interpretation of evidence of impairment. The plant species composition varies greatly between wetland and upland ecosystems. Heterogeneity in soil characteristics can affect plant growth and species composition. The effects of these factors need to be separated from effects due to stressors when assessing the cause of an impairment.

Wetlands are the transition from the aquatic ecosystems to purely upland terrestrial ecosystems. The plant species composition differs for the wetland areas in contrast to the completely upland areas. This is particularly problematic in the UAR valley due to its semi-arid climate. Near the river, wetlands predominated and the soil is saturated most of the year. Along a river-to-upland transect, much of the first bench level that was included in the study was irrigated. This situation limited differences in moisture availability. Flood irrigation could not reach the few elevated areas within the meadows. These elevated, upland areas were very different in plant species composition. In the application of SI principles to terrestrial ecosystems, it is important to understand that plant community species composition is strongly influenced by the hydrology of the site.

Soil is very heterogeneous, and soil properties can change greatly over a distance of a few meters. This can result in changes in plant growth and species composition that are unrelated to any changes in anthropogenic stressors. This is reflected in the large variability in plant growth that was observed at low metal concentrations in the UAR. These variations can result from differences in water availability, soil fertility and agricultural management practices. One method to control for differences caused by soil heterogeneity is to obtain available information on soil properties. In the UAR we first consulted the Chafee-Lake Area Soil Survey (Fletcher, 1975) to check that the areas included in the irrigated meadows areas were of a similar soil type, to limit the possibility that differences in plant growth and species compositions were due to natural soil differences as opposed to introduced stressors.

4.5. MULTIDIMENSIONAL

The SI process for streams assumes that the systems are linear, with upstream versus downstream being very clear delineations. This concept may not work very well for most terrestrial systems. Where the contaminants are transported by stream processes, such as the fluvial mine-waste deposit areas in the UAR case study, the upstream versus downstream comparison can work. In the irrigated meadows areas, the source of the irrigation water became very important. In this case study, we addressed this problem by plotting plant growth and plant species richness data as a function of measured soil metal concentrations, rather than as a function of distance from the assumed source.

The distribution of contaminants as a function of soil depth is also important to consider. This was illustrated by the data of Levy et al. (1992) that indicated the highest concentrations of Pb and Zn were found 15 cm below the soil surface at two sites. We also had access to data from the UAR site from soil samples collected from a depth of 5 cm. These data would have missed the highest concentrations observed by Levy et al. (1992) and could have resulted in incorrect assessment of the cause of impairment. Soil sampling for terrestrial SI should occur over a range of soil depths appropriate to the plant community being examined. For example grasses tend to have shallow root systems, and soil samples from the top 30 cm of soil should be adequate. Many shrub and tree species have deeper roots systems.

4.6. HUMAN MANAGEMENT

Plant species distribution and plant growth can be strongly affected by agricultural management practices. In the UAR area, the resistant plant species *D. caespitosa*—tufted hairgrass—may have been purposely planted in the areas of high metal concentrations to provide some forage yield in the metal contaminated meadows. *D. caespitosa* is a palatable forage grass and is grazed readily by wildlife. Fertilization, mowing practices, grazing, irrigation and herbicide use all can affect plant growth. Information on these factors should be collected to allow factors that affect plant growth and species composition to be properly evaluated.

4.7. SOIL MICROBIAL PROCESSES

Soil microbial processes can affect plant growth. Mycorrhizal symbioses, both ectomycorrhizal and endomycorrhizal, can affect nutrient uptake and metal uptake by plants. Mycorrhizal fungal hyphae greatly increase the absorptive surface area of plant roots interacting with the soil. Mycorrhizae also can reduce metal uptake in plants by immobilizing metals in fungal cell walls (Punshon et al., 2005).

Biological nitrogen fixation is a key process in many nutrient-poor terrestrial areas. As evidenced by the greater phytotoxic effects with alfalfa observed in the laboratory, legumes may be more sensitive to metal phytotoxicity (U.S. EPA Region 8 and USFWS, 2003). Observations by a local agronomist suggest that legumes, clover in particular, are abundant on ranches without high metal concentrations; in the samples reported by Keammerer (1987) there were few legumes present. Both nodulation and nitrogen fixation are more sensitive to high metal concentrations and lower pH than is legume growth (Crowely and Alvey, 2002). Additional data presented by Brown et al. (2005) indicate that nitrification was inhibited in the barren fluvial mine-waste deposits. This effect was ameliorated when lime was added to raise the soil pH. The form of nitrogen present in the soil also affects plant species composition.

Although methods for assessing effects of soil microbes on plant growth are not highly developed, researchers should keep these processes in mind when designing investigations.

Nitrogen deficiency can be diagnosed rapidly over large areas using remote sensing data. This would provide a rapid method for assessing nitrogen nutrition of plants. Legumes can readily be analyzed for differences in ^{15}N natural abundance variation as a measure of their dependence on biological nitrogen fixation versus soil nitrogen (Eskew et al., 1992). This in turn may provide an indication of the amount of bioavailable nitrogen in the soil.

4.8. BINARY DISTRIBUTION OF PHYTOTOXICITY

Reducing the evaluation of phytotoxicity to a simple binary distribution of toxic versus nontoxic allowed the statistical analysis to distinguish more subtle differences in the correlations between soil chemical concentrations and observed phytotoxicity. The variability inherent in continuous measures of phytotoxicity obscured the relationship between soil metal concentrations and phytotoxicity.

4.8.1. Statistical Analysis of Vegetation Data

Various follow-up statistical analyses can be used to provide additional information:

- Exploration of laboratory models with additional predictor variables, including combinations of metals such as Zn, Cu and As.
- Examination of the role of pH in estimating toxicity. Examination of the relative change in the log-likelihood statistic based on pH alone (called a slice function) may provide useful insights into the relative contribution of multiple metals at fixed pH intervals.
- Exploration of models for plant species presence/absence based on field observations.
- Comparative analysis of laboratory-derived models and field-derived models. Any differing relationships found in the lab and field data could be modeled and explored.
- Investigation of statistical methods for overcoming the effect of correlation among candidate predictor variables.

- Examination of additional response variables measured in the laboratory and field, such as plant dry weight or root growth.

4.9. REMOTE SENSING

Remote sensing techniques can be a powerful tool for obtaining fine-scale spatial data on plant abundance and near-surface concentrations of metals. These data would be limited to surface soil metal concentrations. Remote sensing data produced by the USGS using information from National Aeronautics and Space Administration's Airborne Visible and Infra-Red Imaging Spectrometer (AVIRIS) are available for the area immediately around Leadville (Swayze et al., 1998). Combining the AVIRIS hyperspectral data with other available remote sensing data may allow development of maps that correlate soil metal concentrations with plant stress levels. Remote sensing data also could be used to evaluate grazing intensity or land use practices.

4.10. CONCLUSION

There is no a priori reason that the methodology for performing a causal assessment should differ between aquatic and terrestrial systems. Yet, there are inherent differences between these environments. Aspects of the assessment process that may differ between aquatic and terrestrial systems include the critical variables that are measured, degree of development of bioassessment criteria, spatial heterogeneity and linearity of physico-chemical factors, and management practices. The causal analysis described here would have yielded conclusions with a higher degree of certainty if more data were available, e.g., data on grazing intensity and agricultural practices. Nonetheless, this exercise, along with the causal assessment for kit foxes on the Elk Hills, CA (U.S. EPA, 2008), demonstrates the usefulness of the Stressor Identification methodology for terrestrial systems. Additional case studies should shed light on whether the SI methodology is sufficiently robust to be applied to a greater variety of terrestrial systems having other potential stressors such as organic contaminants.

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APPENDIX A

DESCRIPTION OF DOWNSTREAM REACHES ALONG THE UPPER ARKANSAS RIVER FLOODPLAIN

Reach 1. This reach, about 8850 feet in length, extends from California Gulch to the confluence of Lake Fork Creek and contains numerous fluvial mine-waste deposits (see Figure 4). In the lower 3600 feet of this reach, before the confluence of Lake Fork Creek, there are numerous irrigation ditches that may have diverted water containing elevated concentrations of metals to the floodplain and adjacent meadows.

In 1997, the Natural Resources Conservation Service (NRCS) completed vegetation mapping of the 11-mile reach of the Arkansas River basin. The assessment was designed to map vegetation communities adjacent to the river. Five major plant community types were identified along the 11-mile reach (NRCS, 1997). Three of these community types were mapped within Reach 1 including meadow, subirrigated and riparian subirrigated community types. Vegetation in Reach 1 is dominated by a riparian shrub community consisting primarily of willow species. It is interspersed with open water wetlands and herbaceous riparian vegetation consisting of sedges and rushes representative of saturated soils. Areas of unvegetated fluvial mine-waste deposits and unvegetated sandbars occur throughout. The uplands are dominated by herbaceous riparian vegetation consisting of sedges, rushes, and mesic grasses representative of moist soils. These areas are interspersed with upland grasses. Agricultural activities influence the composition and productivity of vegetation in Reach 1 (CDOW, 1988).

Reach 2. This reach, about 18,000 feet in length, extends from the confluence of Lake Fork Creek downstream to the Highway 24 bridge across the Arkansas River (see Figures 4 and 5). Tributaries in Reach 2 include Lake Fork Creek, Halfmoon Creek, Iowa Gulch and Thompson Gulch (see Figure 8). One large agricultural diversion ditch (Derry Ditch No. 1) collects water on the western side of the river and conveys the water to the floodplain. Other agricultural irrigation ditches are also present.

Vegetation mapping conducted by NRCS (1997) identified five plant community types along Reach 2: wet meadow, subirrigated, riparian subirrigated, irrigated pasture and upland. Vegetation in the upper half of Reach 2 is dominated by riparian shrub community consisting of willow species, and herbaceous vegetation consisting of sedges and rushes, interspersed with areas of open standing water. The area contains unvegetated mine-waste deposits and unvegetated sandbars. The lower half of Reach 2 is dominated by riparian herbaceous vegetation consisting primarily of sedges and rushes indicative of saturated soils. The uplands are dominated by herbaceous riparian vegetation consisting of sedges, rushes and mesic grasses representative of moist soils. The area is interspersed with unvegetated fluvial mine-waste deposits and unvegetated sandbars (CDOW, 1988).

Reach 3. This reach, about 19,000 ft in length, extends from the Highway 24 bridge across the Arkansas River to a short distance downstream of County Road 55 (Spring Creek) (see Figures 5 and 6). Tributaries in Reach 3 include Empire Gulch, Dry Union Gulch, drainage from the Mount Massive Lakes area located downstream of Dry Union Gulch, and Spring Creek (see Figure 8).

Vegetation mapping identified four plant community types along Reach 3: wet meadow, subirrigated, riparian subirrigated, and upland (NRCS, 1997). Vegetation in Reach 3 is dominated by riparian herbaceous vegetation consisting primarily of sedges and rushes indicative of saturated soils with areas of open standing water. The area is interspersed with riparian shrub vegetation consisting of willow species. There are large areas of unvegetated fluvial mine-waste deposits and unvegetated sandbars (CDOW, 1988).

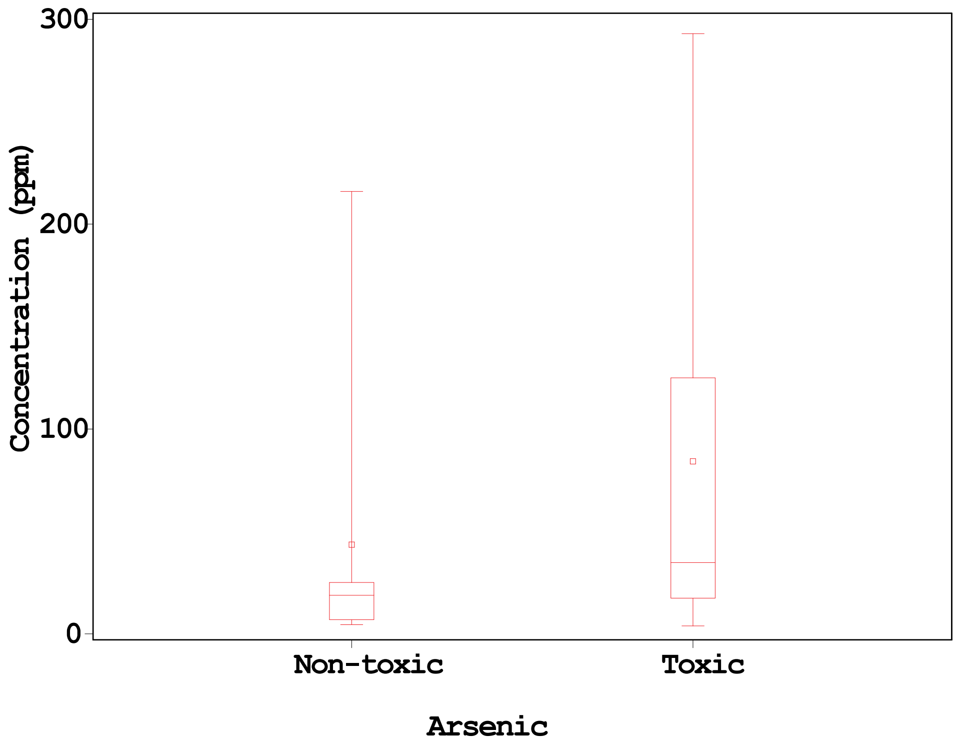
Reach 4. This reach, about 9400 ft in length, extends from the narrows below County Road 55 downstream to approximately 700 ft above the confluence of Two-Bit Gulch. At the downstream end of Reach 4, the channel enters a bedrock canyon (see Figure 6).

Vegetation mapping identified three plant community types along Reach 4: wet meadow, subirrigated, and riparian subirrigated (NRCS, 1997). Vegetation in Reach 4 is dominated by riparian herbaceous vegetation consisting primarily of sedges and rushes characteristic of waterlogged soils. The reach is interspersed with riparian shrub vegetation, small areas of unvegetated fluvial mine-waste deposits and small areas of unvegetated sandbar (CDOW, 1988).

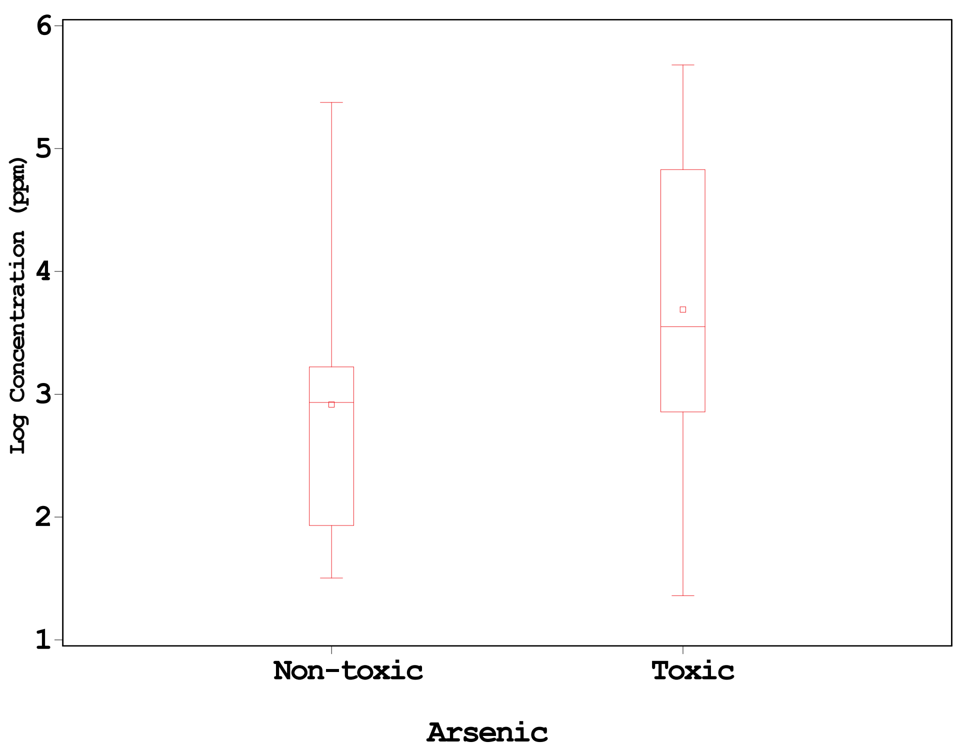
APPENDIX B

BOX-AND-WHISKER PLOTS

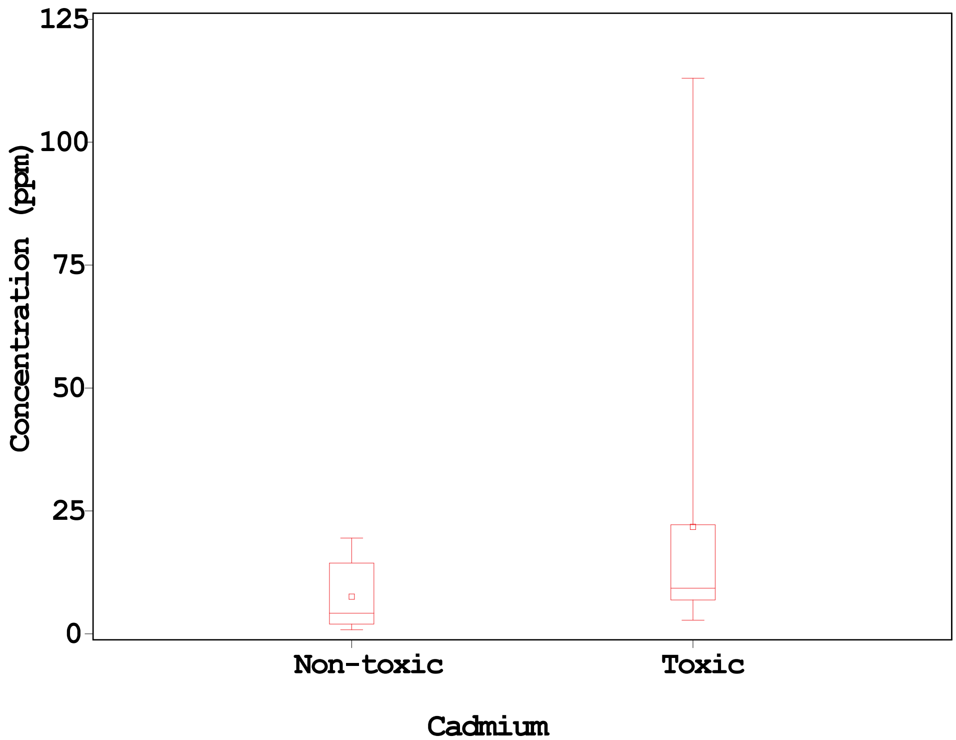
Box-and-whisker plots for measurement endpoints using laboratory phytotoxicity test data (site-specific laboratory measurements and phytotoxicity scores) from 20 sites (see Section 3.2 Step 3 B). The small square represents the mean of the toxic or nontoxic site data.

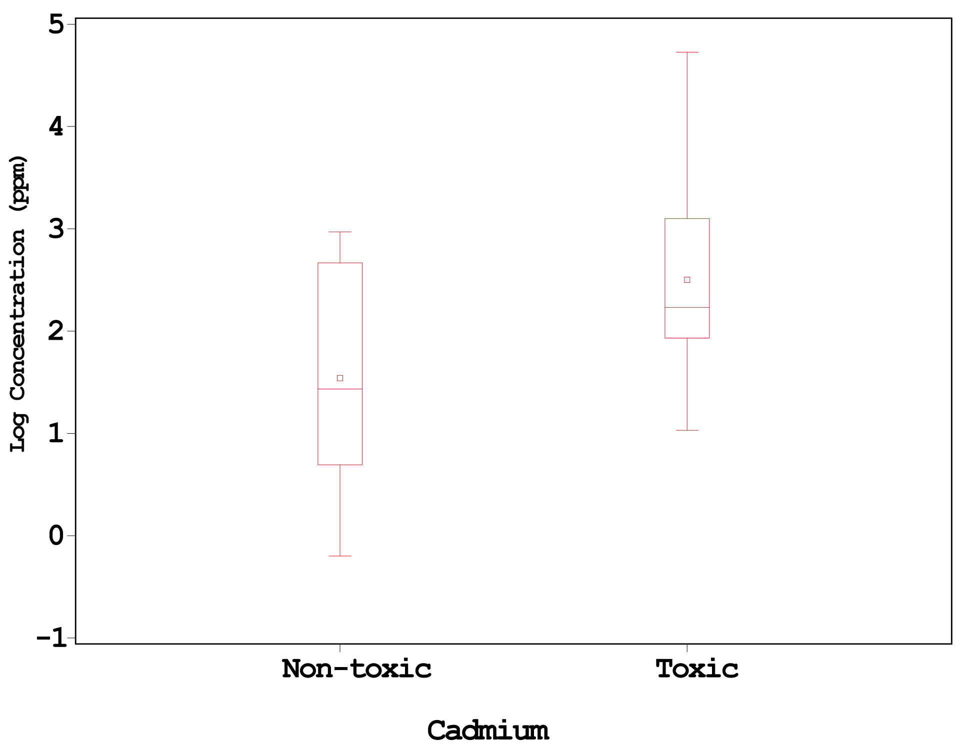


300
200
100
0
Concentration (ppm)
Non-toxic
Toxic
Arsenic



6
5
4
3
2
1
Log Concentration (ppm)
Non-toxic
Toxic
Arsenic


125
100
75
50
25
0
Concentration (ppm)
Non-toxic
Toxic
Cadmium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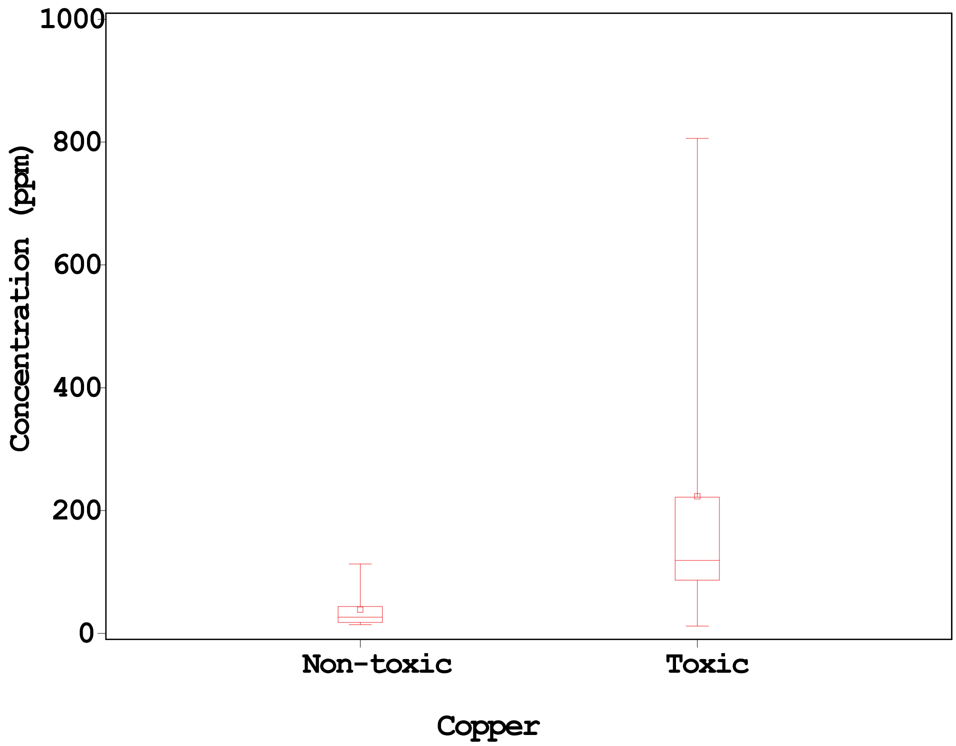
4
3
2
1
0
-1
Log Concentration (ppm)
Non-toxic
Toxic
Cadmium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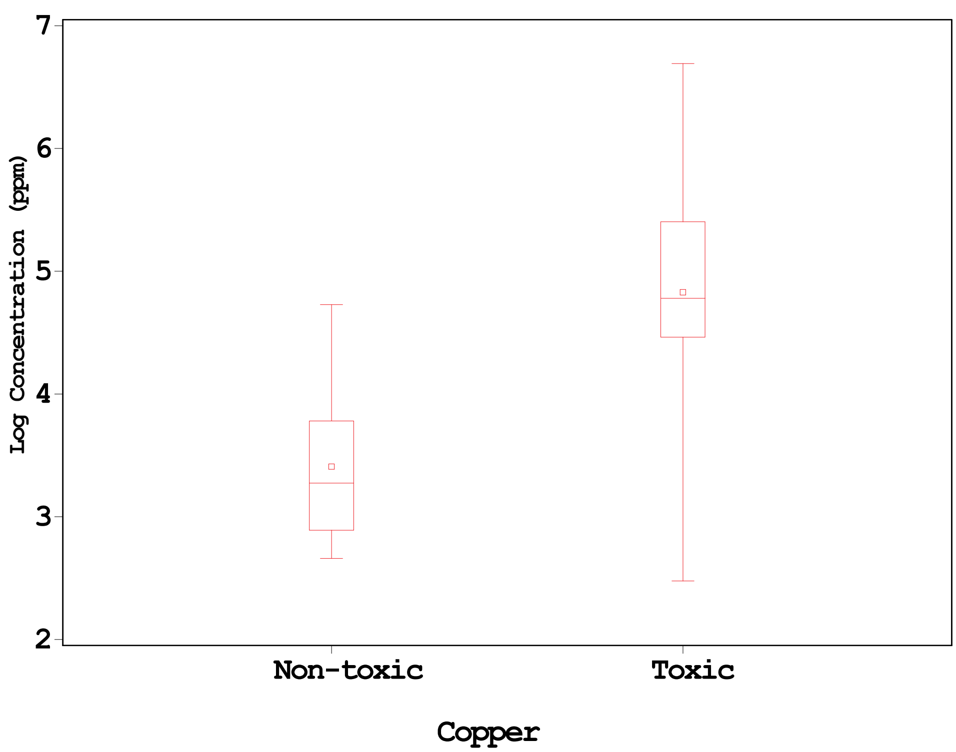
800
600
400
200
0
Concentration (ppm)
Non-toxic
Toxic
Copper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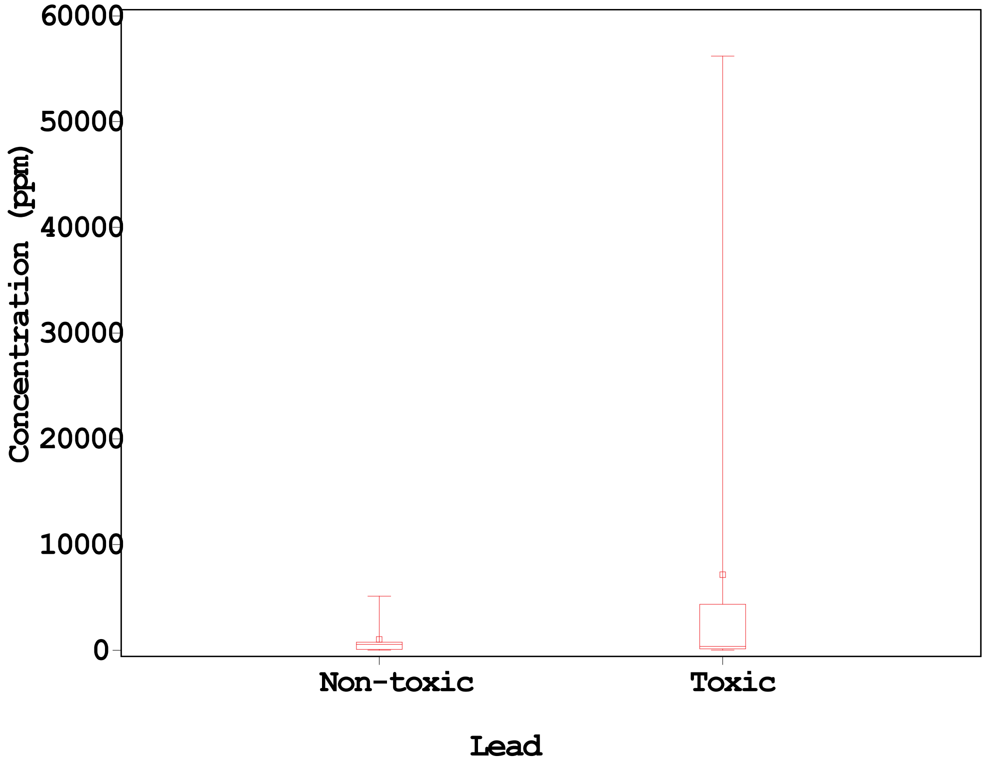
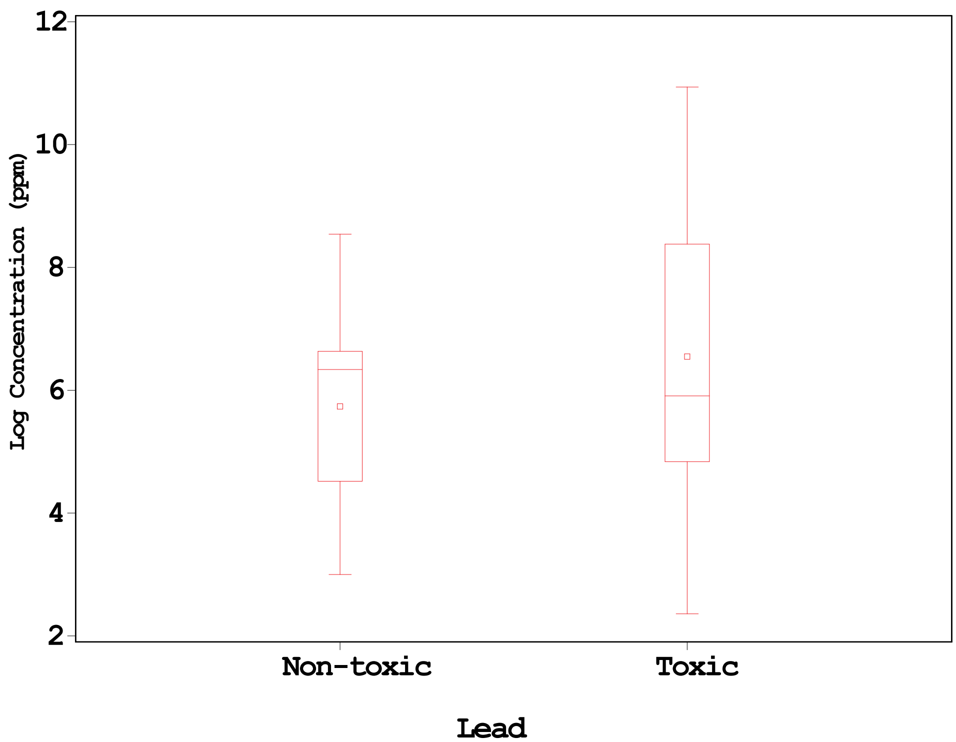
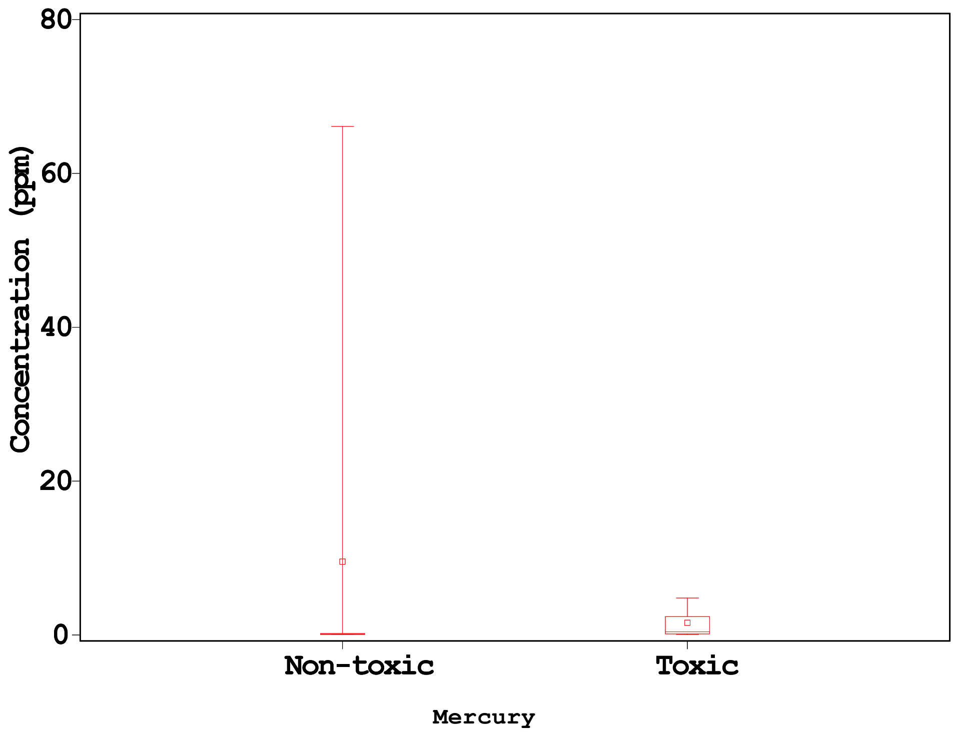
6
5
4
3
2
Log Concentration (ppm)
Non-toxic
Toxic
Copper

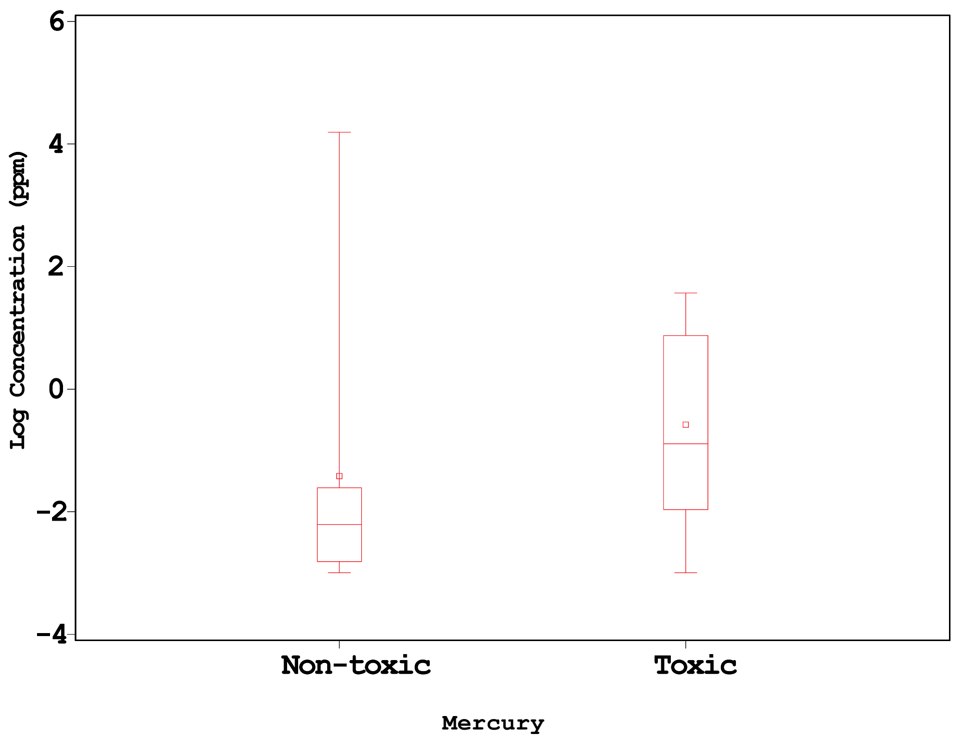


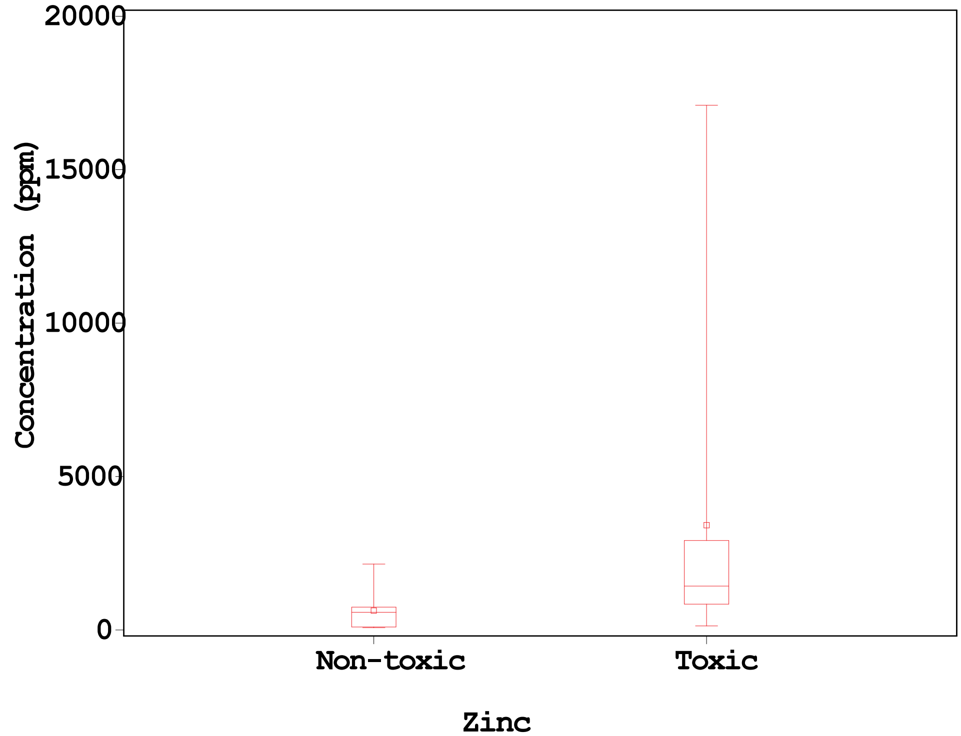
60000
50000
40000
30000
20000
10000
0
Concentration (ppm)
Non-toxic
Toxic
Lead

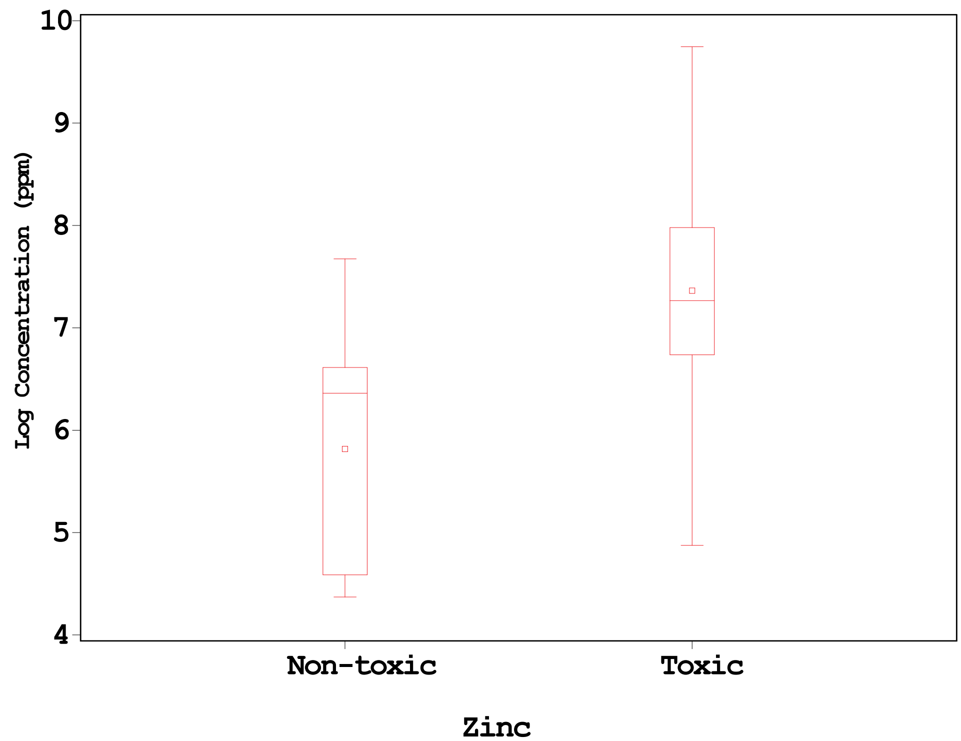


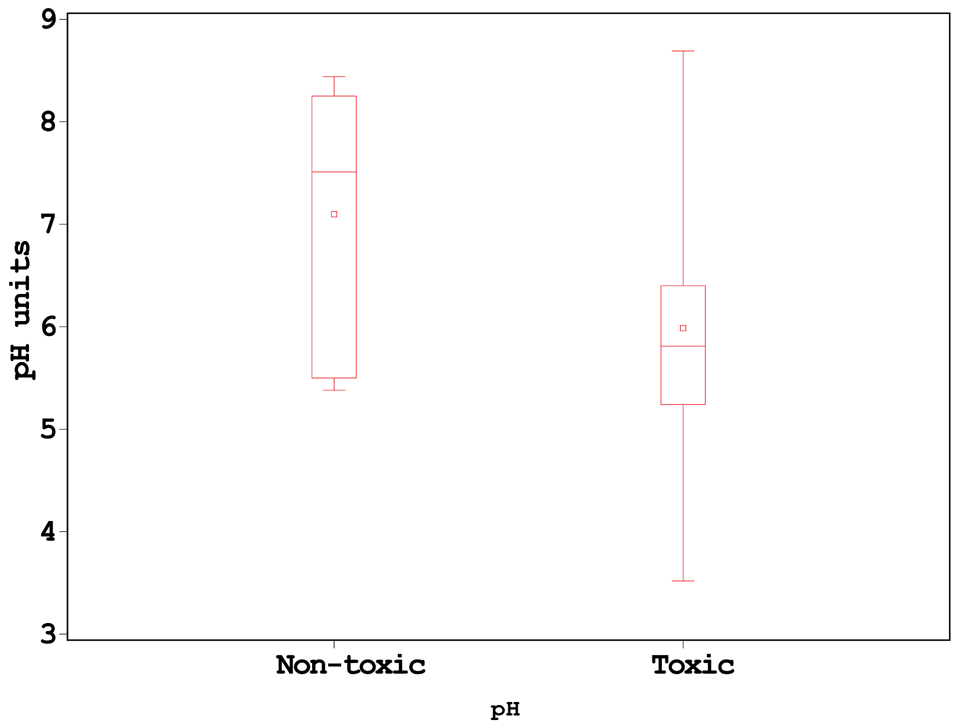
12
10
8
6
4
2
Log Concentration (ppm)
Non-toxic
Toxic
Lead

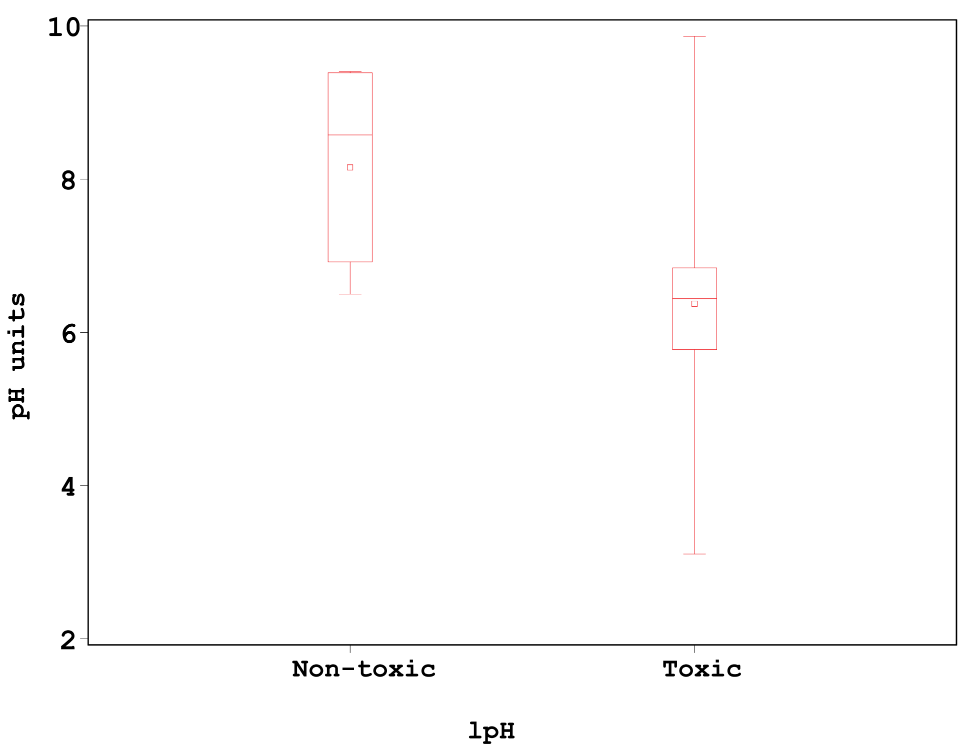

80
60
40
20
0
Concentration (ppm)
Non-toxic
Toxic
Mercury

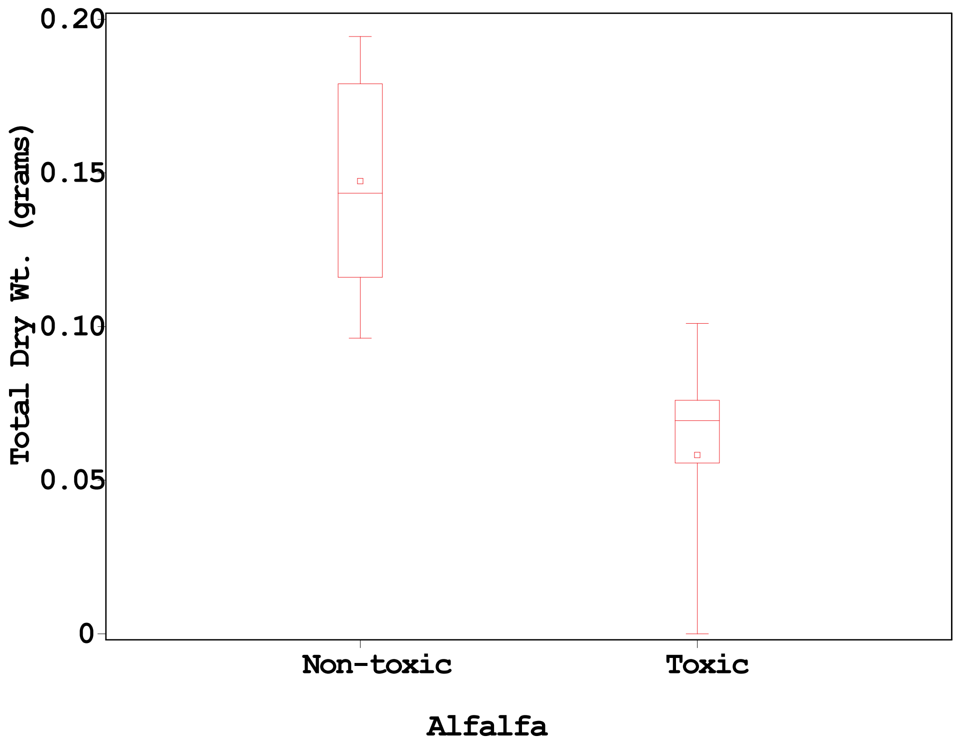

6
4
2
0
-2
-4
Log Concentration (ppm)
Non-toxic
Toxic
Mercury


20000
15000
10000
5000
0
Concentration (ppm)
Non-toxic
Toxic
Zinc


10
9
8
7
6
5
4
Log Concentration (ppm)
Non-toxic
Toxic
Zinc


9
8
7
6
5
4
3
pH units
Non-toxic
Toxic
pH


10
8
6
4
2
pH units
Non-toxic
Toxic
lpH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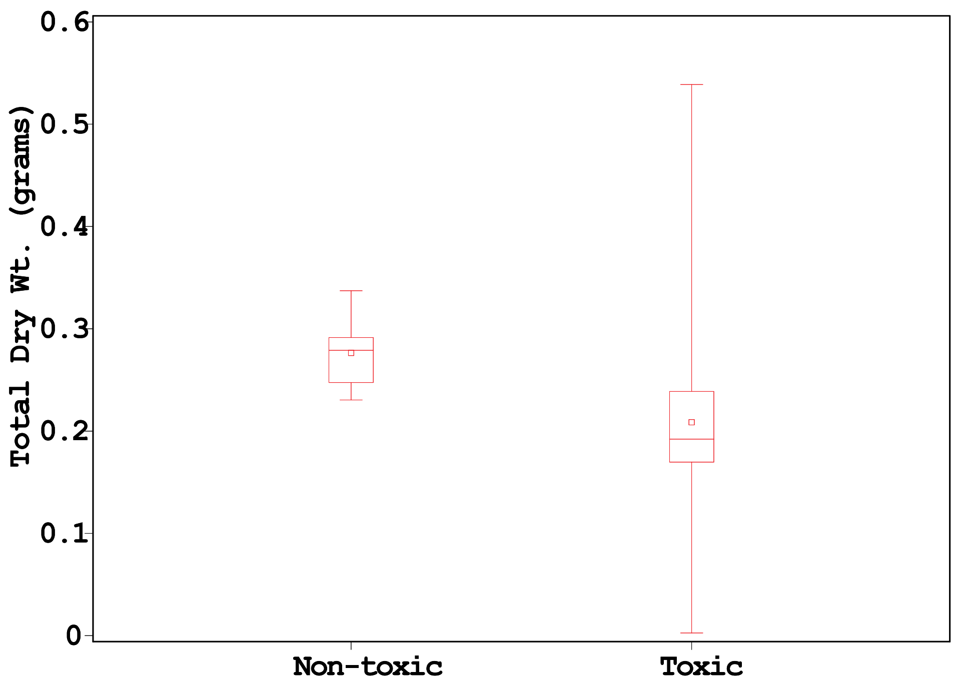
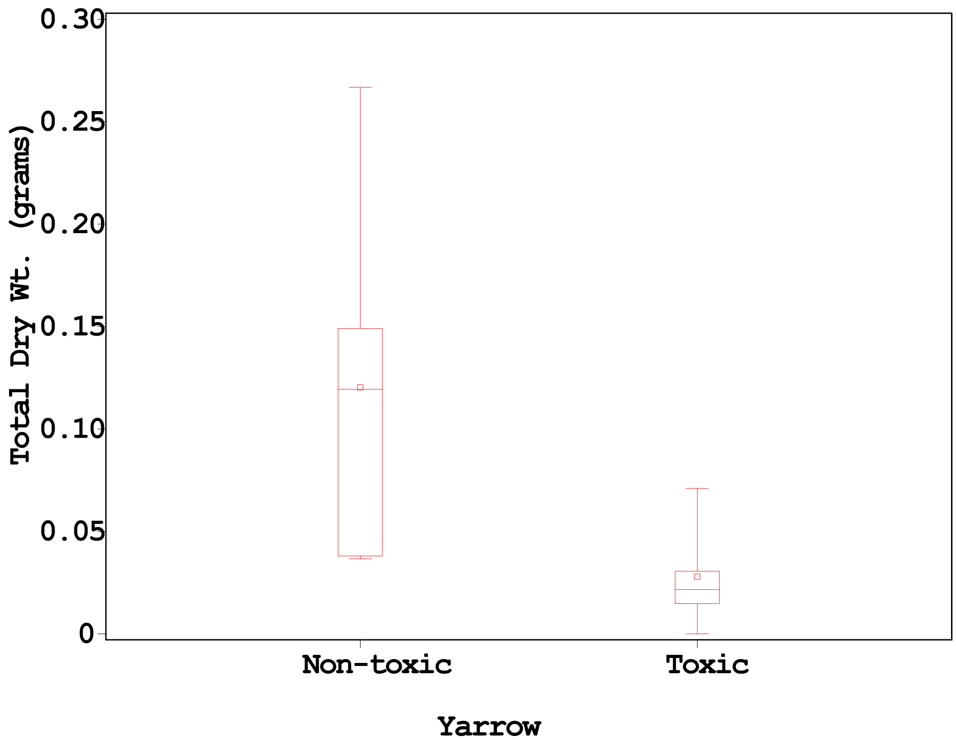
0.15
0.10
0.05
0
Total Dry Wt. (grams)
Non-toxic
Toxic
Alfalfa


0.6
0.5
0.4
0.3
0.2
0.1
0
Total Dry Wt. (grams)
Non-toxic
Toxic


0.30
0.25
0.20
0.15
0.10
0.05
0
Total Dry Wt. (grams)
Non-toxic
Toxic
Yarrow