PLANT GROWTH AND SOIL METAL CONCENTRATIONS
A SPATIAL EFFECTS MODEL

D.R. Neuman, S.R. Jennings, and M.K. Reeves

Abstract. In situ reclamation techniques are proposed for remediation of areas affected by metal mine wastes in Montana’s Clark Fork River Basin. In 1990, an in situ reclamation project was implemented on a fluvially-deposited tailing areas adjacent to the Clark Fork River. As part of this project, a 2.3 ha field containing tailing deposits was tilled to 1.2 m, lime was incorporated into the soil, and rangeland grasses were initially planted. In June of 2000, the field was plowed and seeded with six-row barley (Hordeum vulgare). Variable growth of this species was observed. It was hypothesized that plant growth was negatively correlated with metal and arsenic concentrations in the soil. Variability in barley growth attributable to other measurable soil characteristics was statistically quantified and modeled to account for the effects of landscape spatial heterogeneity. A stratified sampling method was employed for plot selection, with plots selected based on plant height. Three short, five medium, and four tall plant plots were selected. Within each plot, two vegetation and co-located soil samples (0-15 cm) were collected. The <2 mm fraction of the each soil was analyzed for factors related to plant growth and total elemental concentrations of: arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn). Elemental data were first reduced using Principal Components Analysis (PCA) to provide a primary “metals” predictor variable for subsequent regressions. All subset regression analyses were used on the principal components to determine which component was the best predictor of plant biomass. Spatial regression analyses were then performed to assess whether a model that accounts for spatial heterogeneity in the landscape was necessary, or whether ordinary multiple regression techniques adequately modeled plant response to soil metal concentrations adequately. Metal concentrations in the soil were the only statistically significant predictors of plant biomass among all factors tested. No spatial autocorrelation was found in the residuals of the ordinary least squares model used. Therefore, a spatial regression model was not required to explain the relationships.

Additional Keywords: Principal Components Analysis, barley, spatial regression, mine tailings.


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Proceedings America Society of Mining and Reclamation pp 194-211
DOI: 10.21000/JASMR02010194
Introduction

The Clark Fork River is one of several Environmental Protection Agency (EPA) Superfund Sites in Montana’s Clark Fork River Basin. Fluvially-deposited acid metalliferous tailings are present on the banks of the river, and have contaminated adjacent and underlying soil, ground and surface waters. Mine tailing impacts include phytotoxicity, reduced agricultural production, unstable river banks, historic fish kills, and human health risks (ARCO, 1998). The EPA is currently assessing remedial alternatives (ARCO, 2000a) for the cleanup of this large (greater than 160 km long) site. Remedial strategies under consideration include removal of metal contaminants, in-situ treatment using phytostabilization techniques, bank stabilization methods, and various combinations of these technologies.

The model for in situ treatment of the mine tailings is a phytostabilization demonstration implemented along 2.4 km of the river in 1990. This technology demonstration was implemented (Schafer and Associates, 1991) after a temporary elevation of metal levels in the Clark Fork River was determined to be the causative factor in a large fish kill (Phillips and Lipton (1995)). Neutralizing materials (lime) were incorporated into mine tailings to depths of 45 to 120 cm followed by seeding with rangeland grasses and legumes. A total of 837 Mg of CaO and 5148 Mg of CaCO₃ were incorporated to control acidity and to reduce solubility of metals in the rootzone. Vegetation established following treatment stabilized the landscape, harvested water in the rootzone, and reduced surface water runoff and leachate to groundwater. A limited amount of streambank stabilization work was also conducted within the demonstration area. Six years of monitoring of the resulting vegetation, treated soil, and hydrology was conducted (ARCO, 2000b). Since phytostabilization does not remove metal contaminants, the permanence of this remedial alternative has received significant scrutiny (CH2M Hill, 2001). Continued vegetation monitoring within the demonstration area over time and through changes in land use is crucial for an understanding of the effectiveness and permanence of in situ remedies. One of the goals of this study was to examine the permanence of the phytostabilization remedy.

Within the phytostabilization demonstration, an easily accessible 2.3 ha portion is privately owned and has undergone several changes in land use since implementation of the demonstration in 1990. The treated field has functioned as an open space area, a forage pasture, an alfalfa field, a
pasture for bulls, and most recently, a field for barley. In June of 2000, the field was plowed with a three bottom disc plow, fertilized with a 16-16-16 fertilizer, and seeded with six-row barley, *Hordeum vulgare*, at 112 kg/ha (100 pounds/acre). The field was irrigated with an agricultural sprinkler system 2 or 3 times during the growing season; irrigation was supplied uniformly across the field.

Growth of barley in the field was variable. Some areas supported substantial plant growth; the average plant height was greater than 90 cm tall, and most plants bore large seed heads. Other areas contained plants that grew no more than 60 cm tall; the average plant height was slightly over 30 cm, seed head production was low to moderate. Still different areas supported few plants. The plants on these low-growth areas were less than 30 cm in height, chlorotic and stunted in appearance, and seedless. Although variability in plant response within any field is common, the cause for this field may be related to some metal-induced phytotoxic effect. Other researchers have found evidence for metal-induced phytotoxicity in controlled experiments. For example, metal-induced changes in plants can cause reductions in whole plant growth (Baker, 1981, Brun *et al.*, (2001), Porter and Peterson (1977), Nanda Kumar *et al.*, (1995), Retana *et al.*, (1993), Sharma *et al.*, (1999), and Sneller *et al.*, (1999)) or reductions in water use (Pahlsson, 1989 and Wierzbicka and Panufnik (1998)).

Spatial structure or autocorrelation is often present in ecological data (Legendre and Fortin (1989) and Urban, 2001). Spatial structure may violate the parametric statistical assumption of independence of errors, resulting in inappropriate conclusions. The primary purpose of the current study is to determine and describe the relationship between plant growth and metal concentrations in the soil. An assessment of spatial structure and its relationship to the plant-soil model is incorporated into the data analysis.

**Objectives**

**Experimental Hypotheses:**
1. Soil metal concentrations in the rooting zone are negatively correlated with barley growth, once the variability in growth attributable to other measurable soil characteristics has been statistically quantified.
2. A model that accounts for the effects of spatial heterogeneity in the landscape while modeling effects of metal concentrations on plant growth, will be significantly better parameterized than an ordinary multiple regression model.

**Methods and Materials**

**Vegetation Sampling**

A stratified sampling method was employed for plot selection. Stratified designs divide a single population (such as all plants in a field) into subpopulations, which are based on a specific characteristic such as height. The larger study population of all the plants in the field was divided into three sampling units of short, intermediate, and tall plants. This stratification of the sampling effort ascribes a defined characteristic to population variability and thereby ensures that all subpopulations within the larger population are adequately sampled. In a purely random sampling effort, one of the subpopulations might be over or under represented. For example, more small plants than large ones might be randomly chosen for sampling. Such a biased representation of subpopulations would limit exploration of the experimental hypothesis regarding the effects of soil metal concentration on plant growth. Thus, plants were stratified into different height classes and randomly sampled within each plot selected by size class.

A total of 12 sample locations were chosen and mapped using a compass and tape method. Locations were rated as short, intermediate, or tall vegetation by average plant height (0-30 cm, 30-60 cm, and greater than 60 cm) in the plot. Three locations within the 2.3 ha area were classed as short, five as intermediate, and four as tall.

A replicated strategy was integrated into the sampling design to examine the possibility of small-scale spatial autocorrelation in the data. At each sample location, two vegetation samples were collected. Samples were collected within 7 m of the measured station location, in a representative area within a randomly selected quadrant (NE, NW, SE, or SW). All replicate samples were located within 10 m of each other. A 20 x 50 cm frame was placed on the ground, the maximum height of the tallest barley plant in the frame measured, and all barley plants within the frame clipped between 2 and 5 cm above the ground surface. Vegetation samples were placed in labeled bags and returned...
to the laboratory for processing. Presence and species of other vegetation in the frame were noted, but plant species other than barley were not collected.

**Soil Sampling**

At each vegetation sample location, a soil sample was collected directly beneath the clipped vegetation, in the rooting zone (0 to 15 cm). Empirical observations in the field suggested that the majority of the barley roots were contained in the top 15 cm of soil. Soil samples were dug by hand with a sharpshooter shovel, placed into labeled bags, and transported to the laboratory for processing. Decontamination of sampling equipment was accomplished using a dry nylon bristle brush. An National Institute of Standards & Technology (NIST) standard reference soil (SRM 2711) was inserted as a quality control sample for laboratory determinations of soil metal concentrations. Appropriate sample documentation and chain of custody protocols were followed.

**Laboratory Analyses**

**Vegetation.** Vegetation was oven-dried at 60°C until the samples reached a constant weight. The total dry mass of all aboveground plant parts in each 20 x 50 cm plot was measured (Table 1). Seed heads were then separated from other above-ground vegetative tissue, and the mass of seed heads was also determined by weighing (Table 1). Dried plant tissues have been retained for future determinations of As, Cd, Cu, Pb, and Zn concentrations.

**Soil.** Soil samples were air-dried and passed through a 2 mm sieve. The <2 mm fraction of the material was analyzed (EPA, 1995) for the total concentrations of the five elements considered to be contaminants of concern at the Clark Fork River Superfund Site: Cd, Pb, As, Zn, and Cu (Table 1). In addition to the metal concentrations in soil, the following factors are known to affect plant growth and were quantified, using standard analytical methods, to account for variations in growth that might be correlated with these other measurable soil characteristics. These included cation exchange capacity (CEC), electrical conductivity (EC), total organic carbon, pH, acid and neutralization potentials, fertility (available nitrogen (N), phosphorus (P), and potassium (K)), and other essential plant nutrients (sulfur (S), calcium (Ca), magnesium (Mg), molybdenum (Mo), iron (Fe), sodium (Na), chlorine (Cl), and manganese (Mn)). Results for some of these parameters are provided in Table 1.
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† T, M, S indicate barley plants of tall, medium, and short stature respectively; a and b are replicate samples of both vegetation and soil at small (< 10 m) scales.

‡ NP:AP ratio is the ratio of the neutralization potential to the acid potential of the soil sample.
**Statistical Analysis**

Various methods exist to describe and statistically test for spatial structure. Moran's I and Geary's $c$ both allow for tests of significant spatial structure at predetermined distances (lags) between data points (Legendre and Fortin (1989) and Houle, 1998). Moran's I ranges between -1 and 1, with 0 being no autocorrelation, 1 representing positive and -1 representing negative autocorrelation. Geary's $c$ holds a similar concept, except that the ranges are different, and the statistic may be more difficult to interpret (Urban, 2001). Development of a correlogram with Moran's I on the vertical axis and lag distance on the horizontal allows for a visual inspection and formal testing of significance of autocorrelation at different lag intervals. Three data sets were initially evaluated as described below.

**Plant Biomass and Spatial Coordinates of Sample Location.** The above ground biomass data were transformed ($\log_{10}$) to achieve a normal distribution. Ordinary least squares (OLS) regressions were calculated, with spatial coordinates as the predictor variables to meet the variogram assumption of intrinsic stationarity. Residuals of the regression model for space were assessed for spatial autocorrelation using correlogram and the variogram. A spatial neighbor function was created using the sampling design. This function was simple: replicates from individual plots were neighbors, and non-replicates were not.

**Metals Data.** Because multicolinearity among the individual soil metal concentrations was evident, Principal Component Analysis (PCA) was used to reduce the Superfund Site contaminants As, Cu, Pb, and Zn to a single vector for the regression analysis. In a second PCA hydrogen ion concentration (calculated from soil pH values) was added to the Superfund contaminant list. Although Cd is a contaminant of concern at the Superfund site, it was excluded from the PCA because it was not significantly correlated with plant mass in a univariate analysis. The concentration at which any of the individual elements may become phytotoxic to the barley in this investigation is not known, nor was it possible to investigate this aspect in this field study. It is assumed that even low concentrations of the non-essential elements As and Pb may have deleterious effects on plant growth, and that these elements vary in their intrinsic toxicity. Copper and Zn are required for normal plant metabolism, yet at some higher concentrations, they too can negatively
affect plant growth. In addition, any potential antagonistic or synergistic interactions among these elements may affect phytotoxic responses. These factors can not be segregated in this study, but an approach was used to standardize the metal concentrations by reducing inter-element variability and by combining the resulting indices for all four elements. Variability was reduced by calculating the difference between an individual elemental concentration and the mean of the data set and then dividing the result by a measure of the data’s dispersion as quantified by the standard deviation. These indices were then summed to achieve a “standardized metals vector”. There is some precedence for summing contaminant elemental concentrations to assess phytotoxicity of Clark Fork River tailings (ARCO, 1994 and EPA, 1999). All soil pH values were converted to hydrogen ion concentrations for the Principal Component Analysis.

Other Predictor Variables. Mechanistic and statistical assessments of the potential effects of each variable on plant growth were used to select variables from this data set. The other predictor variables included spatial coordinates, CEC, organic carbon, acidity, and essential plant nutrients N, P, K, S, Ca, Mg and Cl. Interactions and colinearity among these variables were evaluated. All subsets regression, forward stepwise regression, and reverse stepwise regression were used to select appropriate variables for inclusion in the best-fit OLS regression.

Final Model Selection. Data from the three sets described above were combined and used for the OLS regression model. All subsets regression and mechanistic considerations were used as guides in the variable selection process and in the selection of the best model. The spatial neighbor function and the best OLS model were used to perform a spatial regression using all available data. The first hypothesis was addressed by testing for significance of the “standardized metals vector” once the variability due to other factors had been statistically quantified. Moran’s I and Geary’s c test statistics were used to determine whether significant spatial autocorrelation remained in the residuals from the OLS regression.

Results

Principal Components Analysis (PCA) yielded eigenvector values that were consistent and fairly
straightforward to interpret (Table 2). The first component was interpreted as a “tailings” or “metals” vector, in which all metals were positive and weighted roughly equally. The second component was the difference of Cu, Zn, (and acidity) from Pb and As. The third (only present in the metals and acidity PCA) appeared to be a solubility index, in which the metals aligned in PCA-space according to differential solubilities in soil solution. The fourth component (third in the metals-only vector) was the difference between Cu and Zn. And the fifth component (fourth in metals-only) was the difference between As and Pb. Standardized metals vectors associated with two of these components, the “metals” component and the “Pb versus As” component were found to be significant predictors of plant biomass in the OLS regression.

The study provided evidence to reject the null of hypothesis 1; significant correlations were found between total metal concentrations in soil (as standardized metals vectors) and the plant mass ($\log_{10}$). Metals were the only predictors found to be statistically significant in the variable selection process. The two variables found significant were the standardized metals vector ($p < 0.0001$) without acidity, and the difference between standardized Pb and standardized As ($p = 0.02$). The best fit OLS model follows:

$$\log_{10} \text{PlantWeight} = 1.05 - 0.18 \text{(Standardized Metals Vector)} - 0.81 \text{ (standardized Pb minus standardized As)};$$

Model p value $= <0.0001$, Multiple $R^2 = 0.78$

The meaning of the Pb minus As predictor variable is unclear. It was tested during variable selection because it was one of the components found in the PCA and was the only other significant predictor of plant mass found during variable selection for the OLS model. This variable is not correlated with plant biomass in the univariate analysis. Nonetheless, it adds 6% to the adjusted $R^2$ value for the standardized metals vector model, and thus seems appropriate to report for further consideration about its potential meaning in the scientific community. The best fit regression model (Figure 1) without the standardized Pb minus As predictor follows:

$$\log_{10} \text{PlantWeight} = 1.05 - 0.18 \text{(Standardized Metals Vector)};$$

Model p value $= <0.0001$, Multiple $R^2 = 0.71$
Regression assumptions of normally distributed residuals and equal variance were adequately met.

Table 2. Results of the Principal Components Analyses: Eigenvectors.

**PCA with Metals and Hydrogen Ion Concentration**

<table>
<thead>
<tr>
<th></th>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 3</th>
<th>Component 4</th>
<th>Component 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>H (moles/L)</td>
<td>0.375119</td>
<td>0.347171</td>
<td>0.816117</td>
<td>0.227567</td>
<td>0.144656</td>
</tr>
<tr>
<td>As (mg/kg)</td>
<td>0.461889</td>
<td>-0.538208</td>
<td>-0.020491</td>
<td>-0.278328</td>
<td>0.647383</td>
</tr>
<tr>
<td>Cu (mg/kg)</td>
<td>0.468252</td>
<td>0.4713</td>
<td>-0.182322</td>
<td>-0.68304</td>
<td>-0.241806</td>
</tr>
<tr>
<td>Pb (mg/kg)</td>
<td>0.466765</td>
<td>-0.52173</td>
<td>0.078281</td>
<td>0.181908</td>
<td>-0.686083</td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>0.456963</td>
<td>0.308996</td>
<td>-0.54237</td>
<td>0.608894</td>
<td>0.175471</td>
</tr>
</tbody>
</table>

**PCA with Metals Only**

<table>
<thead>
<tr>
<th></th>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 3</th>
<th>Component 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>As (mg/kg)</td>
<td>0.512191</td>
<td>-0.482615</td>
<td>0.068667</td>
<td>-0.707127</td>
</tr>
<tr>
<td>Cu (mg/kg)</td>
<td>0.479775</td>
<td>0.543186</td>
<td>0.687653</td>
<td>0.043564</td>
</tr>
<tr>
<td>Pb (mg/kg)</td>
<td>0.509231</td>
<td>-0.493727</td>
<td>-0.009943</td>
<td>0.704854</td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>0.498157</td>
<td>0.477771</td>
<td>-0.722716</td>
<td>-0.035432</td>
</tr>
</tbody>
</table>

Traditional diagnostics show that OLS regression assumptions of normally distributed residuals and equal variance were adequately met.
Figure 1. Standardized Metals vs. Log$_{10}$ Plant Mass.

No other variables were found to be significant predictors of plant mass. Statistical power was limited in this study because of the moderate sample size (n=24). Power calculations show that the detectable practically significant difference with this sample size was 0.573; whereas the range in plant mass values for this data set (Log$_{10}$) was -0.15 to 2.08. A larger sample size may have afforded the power to detect smaller practically significant differences in the response variable and may have rendered other predictor variables significant, if results were consistent with those collected already.

Two different PCA analyses were run because of colinearity between acidity and the metals in the data set. Based on the two PCAs, two different standardized metals vectors were calculated, one with and one without acidity. First, the metals-only vector and acidity were both included in the same regression equation. Acidity failed to be a significant predictor of log$_{10}$ plant mass (p = 0.304). The R$^2$ value for the first test equation was 0.71. Second, the standardized (metals and acidity) vector was regressed on log$_{10}$ plant mass. The vector was highly significant, but only improved the R$^2$ value of the equation by 1%. It was therefore decided that once the standardized metal vector was used as a predictor, acidity was no longer a potentially significant predictor of plant growth.

Hypothesis 2 was rejected in the current study. The residuals from the best fit OLS model
indicate no evidence of significant spatial autocorrelation. Although the log$_{10}$ plant biomass response variable suggested significant spatial autocorrelation prior to OLS analysis, the standardized metals predictors in the OLS regression seem to have accounted for all of the spatial autocorrelation in the response variable. The non-autocorrelated residuals indicate that the spatial structure in the response variable was explained by the standardized metals vectors in the predictive equation. A spatial regression equation was unnecessary in this study; the OLS regression assumption of independence of residuals was met.

**Discussion**

Other models have been developed to predict metal toxicity to plants for contaminated (non-remediated) areas in the Clark Fork River Basin, but these model results disagree in part with the findings of the current study. Multiple linear regression models were developed as part of a regional ecorisk field investigation for the Upper Clark Fork River Basin (ARCO, 1994). These models relate plant biomass and the number of plant species to pH and the sum of total metal (As, Cd, Cu, Pd, and Zn) concentrations in non-remediated soils and tailings. Also evaluated in these models were soil factors, which were found to be insignificant. A simple sum of metal concentrations ignores the differential toxicity of these elements and unevenly weights the element with the greatest concentration. For example, ARCO models predicted that at pH greater than approximately 7.0, there is no detrimental response to biomass or species richness from increasing metal concentrations (ARCO 1994). The usefulness of ARCO models for remediated lands with pH levels greater than about 7 is questionable. The current study found fairly dramatic differences in plant biomass, all within a circumneutral pH range, and these differences in plant mass were significantly correlated with the metal concentrations in the remediated soil.

Acidity of the soil (pH) was not a significant predictor of plant biomass in this study, whereas numerous studies have shown pH to significantly affect plant growth (Moore, 1971). Nonetheless, the current study area was reclaimed using techniques that neutralized the actual and potential acidity of the soil. Measured pH of soil in the current study was below 7.0 in only one sample, and the ratio of the neutralization potential to the acid potential (NP:AP ratio) was rarely below 2.0 (Table 1). One of the goals of the current study was to evaluate potential metal toxicity, in the form of reduced
growth, to plants in a post-reclamation environment, while considering other factors known to affect plant growth. Although pH has been shown in other studies to affect plant growth, no relationship between plant growth and pH in this study was found. A highly significant relationship was found between the metals present in rooting zone soil and plant biomass (p<0.0001). No other predictor variables analyzed were found to be significant predictors of plant growth. If pH had varied farther from neutral, it is likely that it would also have been a significant predictor of plant biomass (Moore, 1971). The model presented in this paper is thus limited to situations in which pH is near neutral and fairly homogenous across the study site.

The current study found no spatial autocorrelation in the residuals from the OLS regression. Spatial autocorrelation is often present in field sampled data. If it is present, spatial autocorrelation violates parametric regression assumptions and can render use of parametric regression techniques inappropriate. It is therefore recommended that spatial structure be considered during development of other similar predictive models.

The current model sums the metal concentrations, but only after these concentrations are standardized. The standardization prevents over weighting of the elements with the highest concentrations. Nonetheless, the sum of metals in the model presented weights them equally in terms of their contribution to plant biomass reduction. The model therefore does not provide a mechanistic model of plant toxicity and cannot account for additive, synergistic, or antagonistic effects. The goal of the current model is to provide a means to predict potential for metal toxicity to more sensitive plant species in a post-remedial context.

The field was initially seeded with rangeland grasses and alfalfa, which provided abundant production (2730 kg/ha), adequate canopy cover (70%), and species richness (ten or more plant species contributing at least 1% to canopy cover) for open space and cattle grazing over a ten year period (ARCO, 2000). Redtop (Agrostis alba) invaded much of the field after it was used as a bull pasture. This species and others present have shown a tolerance to the chemical and climatic environment of the field for a decade of time.

It has been established that barley suffers decreased growth in response to exposure to Cu and Zn (Beckett and Davis (1977) and (1978)). The current study suggests that the barley in this field had a phytotoxic response to the metals in the soil. High levels of metals in the neutralized soil were correlated with lower barley production, while soil with moderate or low concentrations of metals
supported adequate barley production. The current study also suggests that phytostabilization of metal-contaminated soils may limit the establishment of some plant species. Phytostabilized areas with high metal levels may have limitations on the vegetation communities they can sustain and in some cases, the desired plant community for the given land use may not be achieved.

**Acknowledgments**

The authors thank US EPA, Region VIII and Montana Department of Environmental Quality for funding analytical work, and the rancher for allowing access and sampling.

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