Evaluating the Influence of Soil Geochemical Characteristics on Metal Bioavailability

James Ranville, Ph.D.
Department of Chemistry and Geochemistry
Colorado School of Mines
Golden, CO 80401
Telephone: 303-273-3004
FAX: 303-273-3629
E-mail: jranvill@mines.edu

Introduction

The presence of toxic metals in mineral soils and in soils impacted by mining, industry, agriculture and urbanization, is a major concern for both human health and ecotoxicology. The overall research objective of this project was to obtain further understanding of the relationship between soil chemical characteristics and metal ecotoxicity. Over recent years, considerable research effort has gone into describing the toxicological effects of metals on aquatic organisms. This has lead to the realization that water composition plays a significant role. Our goal was to extend this view to the soil pore water system. We also wished to examine if a relationship between soil pore water composition and bulk soil chemical characteristics can be discerned. A large database exists on soil chemistry but little data is available on soil pore water chemistry. Another goal was to evaluate a microbial bioassay as an alternative to traditional toxicity tests that rely on cultured organisms, such as macroinvertebrates.

Background and Rationale

The molecular form of metals and metalloids, particularly the non-complexed (free) form, controls their bioavailability (binding to a biological receptor site) and toxicity in solution. The free ion activity model (FIAM) has been successfully applied to describe metal toxicity data for a wide range of organisms (Brown and Markich, 2000). Several exceptions to the FIAM approach have been observed (Parker and Pedler, 1997: Meyer et al., 1999). An alternative model, the biotic ligand model (BLM) takes into account not only metal complexation by ligands such as dissolved organic matter (DOM) and alkalinity, but also competitive binding of hardness cations (Ca^{2+}, Mg^{2+}) and H^+ to biological receptor sites (DiToro et al., 2001). This view is illustrated in Figure 1.
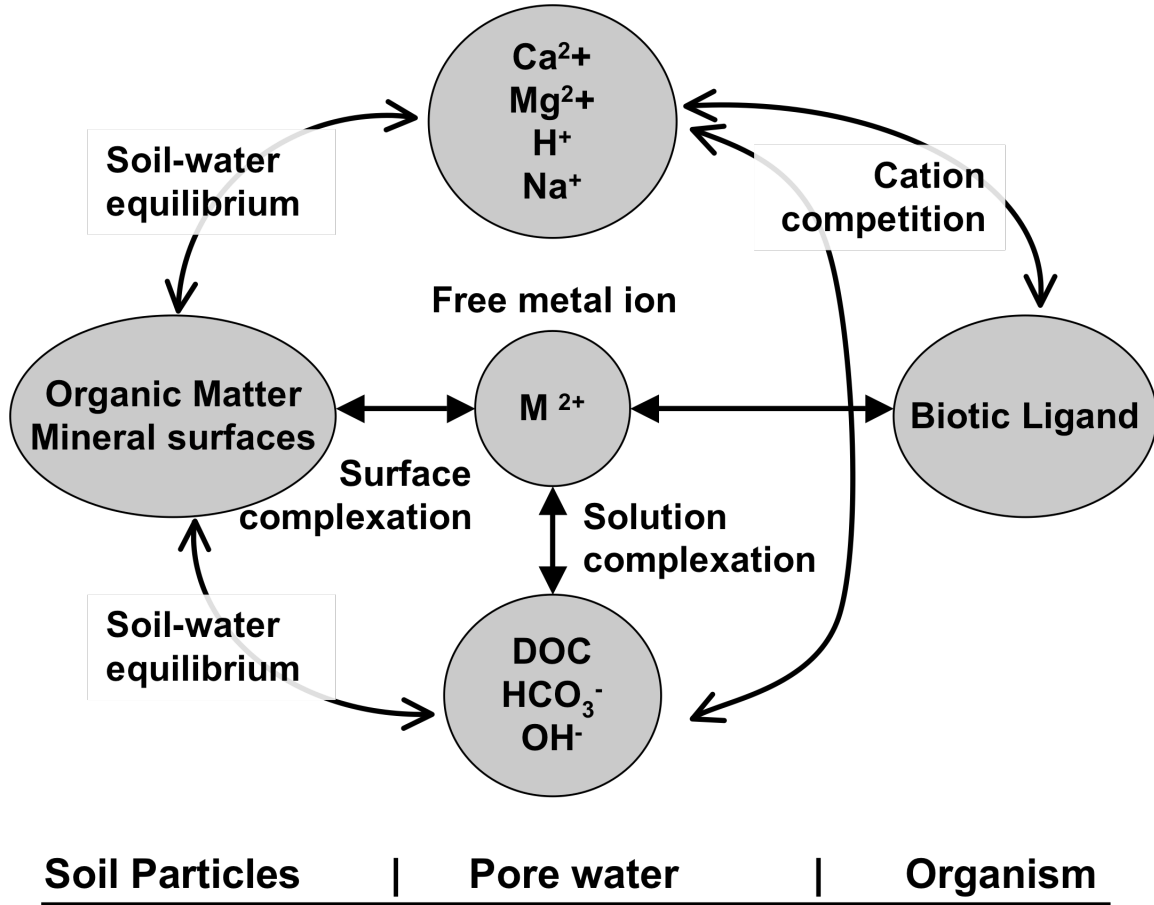
Figure 1. Conceptual model of the chemical processes controlling metal uptake at metal binding sites (biotic ligand) on soil organisms. Important soil constituents include porewater dissolved organic carbon (DOC), hardness cations (Ca^{2+}, Mg^{2+}), alkalinity, pH, and soil organic matter. (modified from DiToro et al., 2005)

Interaction between the soil matrix and the soil pore water will control the soil water composition. Dissolved ion concentrations will be influenced by abiotic and biotic dissolution and precipitation reactions. For non-redox active metals, the most important process is complexation to mineral surface and soil organic matter. Soil pH plays a major role in metal toxicity. Complexation reactions between metals and mineral surfaces, soil organic matter, and solution-phase ligands are pH-dependant, as is mineral solubility. Protons also compete with metals for binding to the biotic ligand. Thus in several ways, pH plays a major role in determining free metal ion activity in solution. The geographical distribution of soil pH is shown in Figure 2. Although local pH conditions may vary, the overall distribution of pH is related primarily to climate (rainfall and temperature), and ranges from about 4.5 to 8.5.
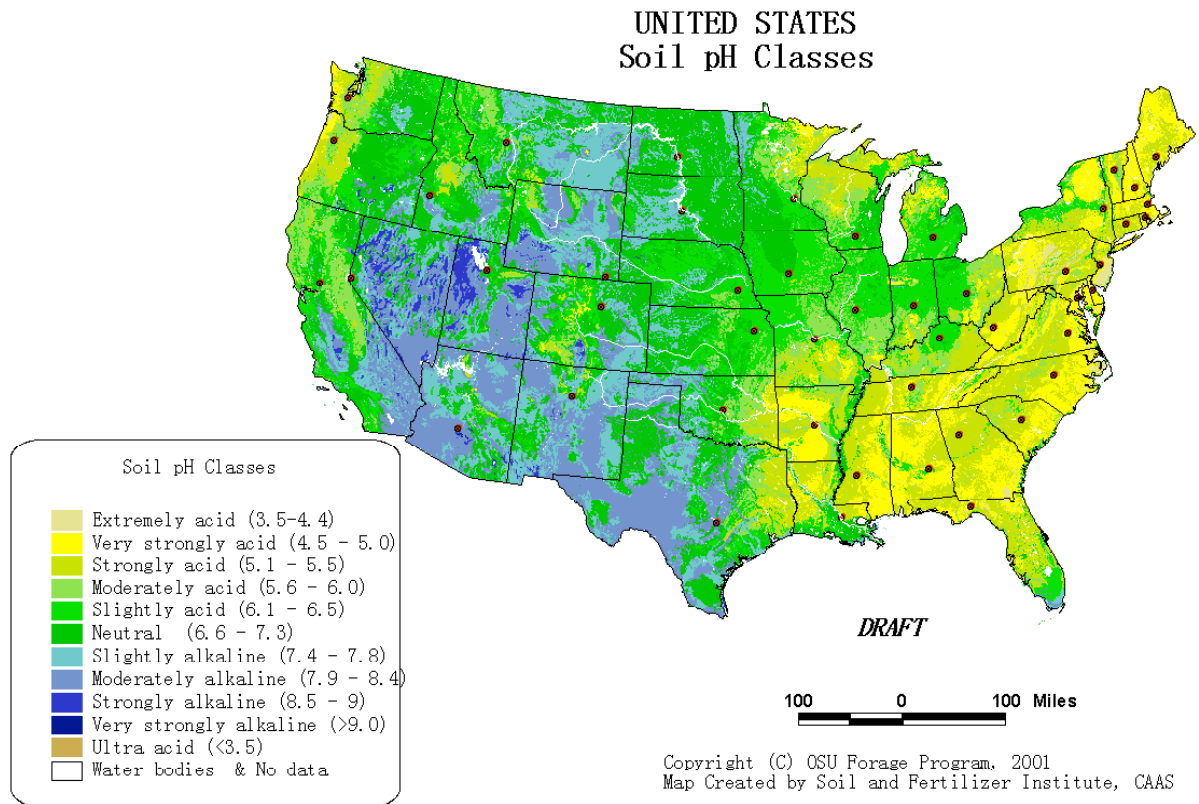
Figure 2. Geographical distribution of soil pH.

The primary route of exposure for terrestrial organisms such as plants and bacteria is through uptake of dissolved metals in the porewater. Soil invertebrates are also exposed to dissolved metals through their external membranes (dermal uptake) or through direct ingestion of metal-contaminated soil particles. The ingestion route is not well understood and may not be as significant as dermal uptake (Vijver et al., 2003). This study focused solely on the role of soil water composition in copper and zinc toxicity and utilized bacteria as the toxicity test organism.

Experimental Approach

The project involved two sets of experimental investigation: 1.) simulation of soil pore water using a short-term deionzed water leach, and 2.) microbial bioassays using copper and zinc in laboratory-prepared solutions of variable composition.

Pore water experiments
Soils were obtained from USGS that were part of a series collected from an east to west transect across California (Morrison et al., in prep). These soils are under investigation

as part of the USGS Soil Geochemical Landscapes Project. To simulate pore water composition a short-term (5-minute) water leach developed by USGS (Hageman, 2004) was used. This leaching procedure has been used extensively to characterize mine wastes and has been shown to give results similar to longer leaching procedures. This approach simulates short-term infiltration events that will produce pore water in the near surface soil environment. Following leaching, the suspension was filtered (0.45 μm) and analyzed for pH, alkalinity, DOC, and dissolved metals by ICP-MS. Data were obtained from USGS on the bulk composition of the soil including percent carbon (organic and inorganic) and elemental composition of a four-acid digestate using ICP-MS (Briggs and Meier, 2002). Results of the water leachate analyses were compared to data on the chemical constituents of the solid matrix, the goal being to determine if soil pore water chemistry can be determined from bulk soil chemistry.

Microbial enzyme inhibition bioassays: METPlate™
The METPlate™ toxicity test procedure uses a 96 well plate in which *E.coli* are used as the indicator species (Bitton, et al., 1994). The test is based on the inhibition of the β-glactosidase enzyme in the bacteria, and has been shown to be very sensitive to metal toxicity. The reaction is shown in Figure 3.

Chlorophenol-red
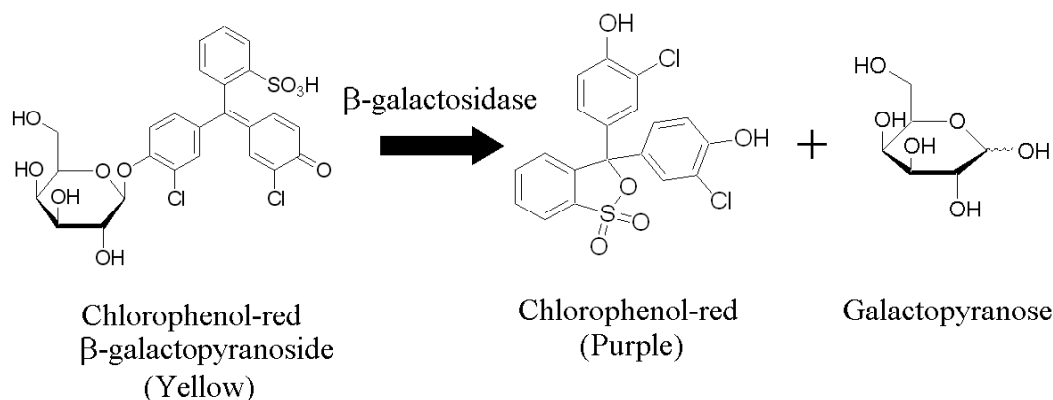β-galactopyranoside
(Yellow)

Chlorophenol-red
(Purple)

Galactopyranose

Figure 3. The transformation of the chromogenic indicator by the β-galactosidase reaction of healthy *E.coli*. The inhibition of this reaction by metal toxicity forms the basis of the test.

In the procedure the bacteria are first rehydrated in a buffer solution for 15 minutes at room temperature. The bacteria are then added to the test sample, in this case a series of copper and zinc solutions that were diluted in waters of variable hardness, alkalinity, DOC, and pH. The samples are incubated at 37° C for 90 minutes to allow any toxic action to occur. A chromophore (chlorophenol-red) is added, the samples transferred to the 96-well plate, and incubated up to 120 minutes at 37° C. In this case all tests involved transfer of duplicate aliquots. A purple color, developed according to the hydrolysis reaction shown in Figure 3, indicates healthy *E. coli*. Whereas a resulting yellow color indicate enzymes are inhibited, and this implies metal toxicity. The degree of color development was measured every 15 minutes over the 120-minute incubation period. Measurements were made at a wavelength of 575nm using a PowerWaveX 340

4

microplate spectrophotometer. Both positive (fully toxic) and negative (non-toxic) controls were utilized.

Results

Pore Water Chemistry
One of the project goals was to investigate the relationship between soil composition and pore water composition. The soils tested were collected from a transect east to west across California and represent soils having undergone variable amounts of weathering from the parent material. Although conclusions can be drawn from this dataset, it is the case with all the following discussions that further investigation is required to generalize the results or to extrapolate to other soils.

Figure 4 shows the relationship between soil organic carbon (SOC) and dissolved organic carbon (DOC) in the leachate. A strong linear correlation (slope = 2.63, r^2=0.80) is seen between the variables. This implies that a labile organic carbon pool exists in the soil and that it forms a relatively constant fraction of the total SOC. Furthermore it would seem that pore water DOC might be predictable from the SOC content of a given soil. Considerable data exist for SOC (see Figure A-1 in the Appendix) whereas little pore water DOC data exists. DOC is an important parameter in understanding metal toxicity (Figure 1) as it reduces the bioavailable metal fraction. Measurements of metal content of pore waters could be adjusted to the "free" concentration by application of organic matter complexation models such as WHAM (Tipping, 1994).
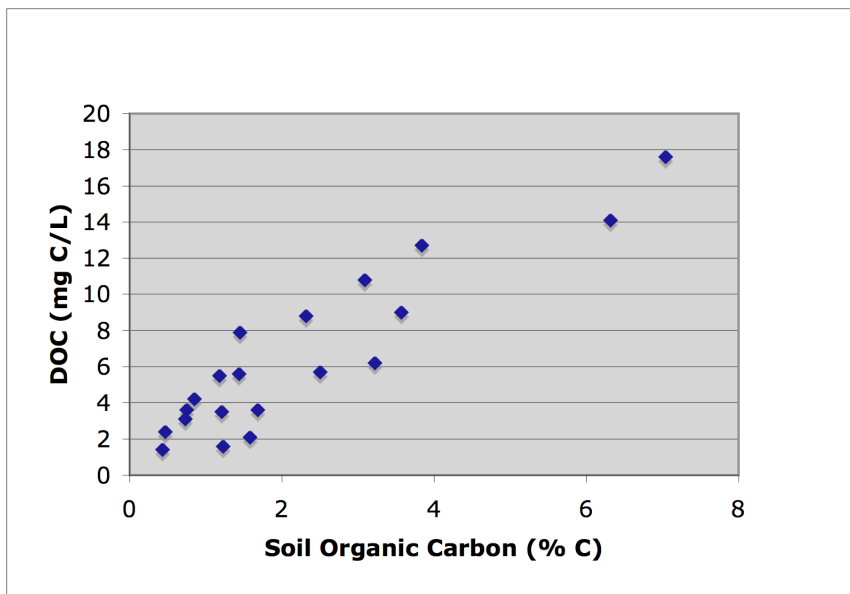

Figure 4. Relationship between soil organic carbon content (% C) and leachate dissolved organic carbon (mg C/L).

Figure 5 shows the relationship between soil inorganic carbon content and the leachate alkalinity. Although there is a general trend of increasing alkalinity with increasing inorganic carbon content, the relationship is fairly weak, suggesting the importance of other soil factors in influencing leachate alkalinity. Alkalinity expresses the dissolved carbonate species in the water, as well as the hydroxide ion (OH⁻) content. The inorganic carbon content of the soil represents primarily carbonate minerals in the soil. These data are obtainable on the regional scale (see Figure A-2 in the appendix). The results may suggest that equilibrium with the carbonate minerals may not completely explain the alkalinity. Several possibilities exist for the lack of a strong correlation. Microbial respiration may cause a non-equilibrium situation with respect to carbonate mineral equilibrium. The short period of the leaching procedure might limit the ability of some samples to release carbonate to solution. Although the correlation is weak the results suggest some ability to predict pore water alkalinity from soil inorganic carbon content exits.
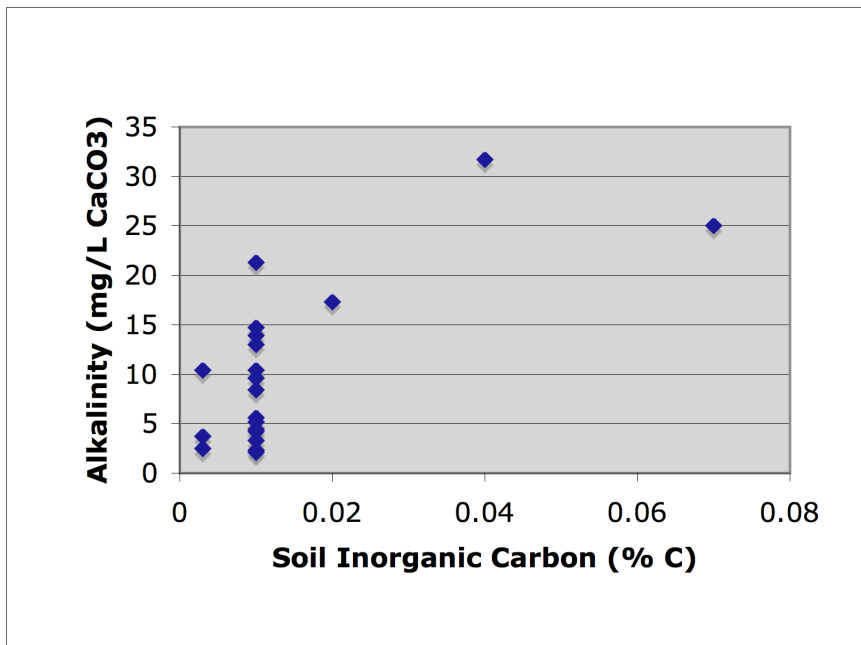

Figure 5. Relationship between soil inorganic carbon content (% C) and leachate alkalinity (mg $CaCO_3$/L).

The leaching procedures used on the soils investigated in this study resulted in pH values ranging from about 5.6 to 8.4. This represents a considerable portion of the pH range displayed for continental USA soils (Figure 2). In Figure 6 the relationship between alkalinity and pH is shown. The observed relationship is what is expected in that as pH increases alkalinity should increase due to the conversion of dissolved carbon dioxide to HCO_3^- and $CO_3^=$ ions. With respect to metal toxicity the pH-alkalinity relationship results in considerable complexity. As pH drops, the ligands that are capable of binding metals, -COOH on DOC and HCO_3^- and $CO_3^=$ become protonated. The result is more un-complexed, and thus toxic, metal. However the increased protons present at low pH

can compete for binding sites on the biotic ligand, thus reducing metal toxicity. Further work is needed to more fully understand the role of pH in metal toxicity in soils.
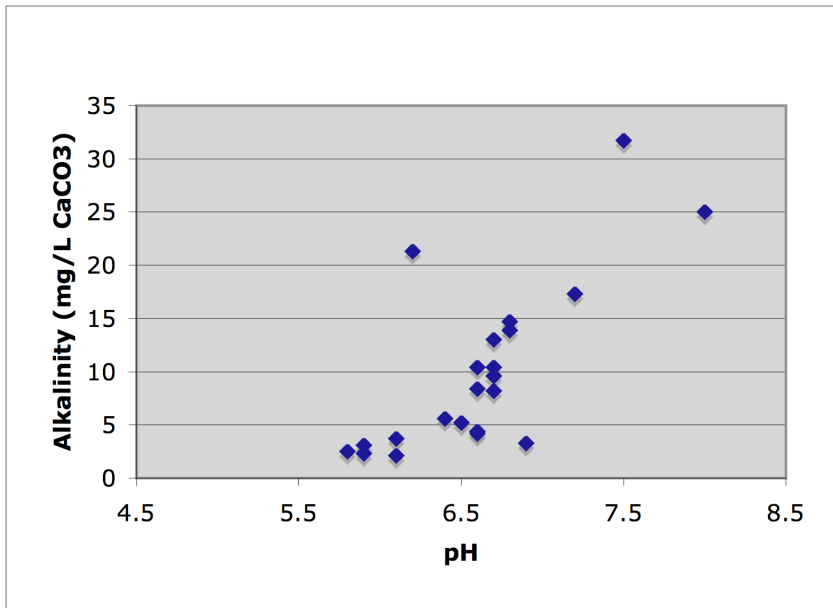
Figure 6. Relationship between pH and leachate alkalinity (mg $CaCO_3$/L).

The hardness of the leachate was dominated by Ca^{2+} whereas the bulk chemical analysis showed more Mg than Ca in the soils. There is no correlation between total soil calcium and leachate hardness, as shown in Figure 7. Hardness cations, are likely released to
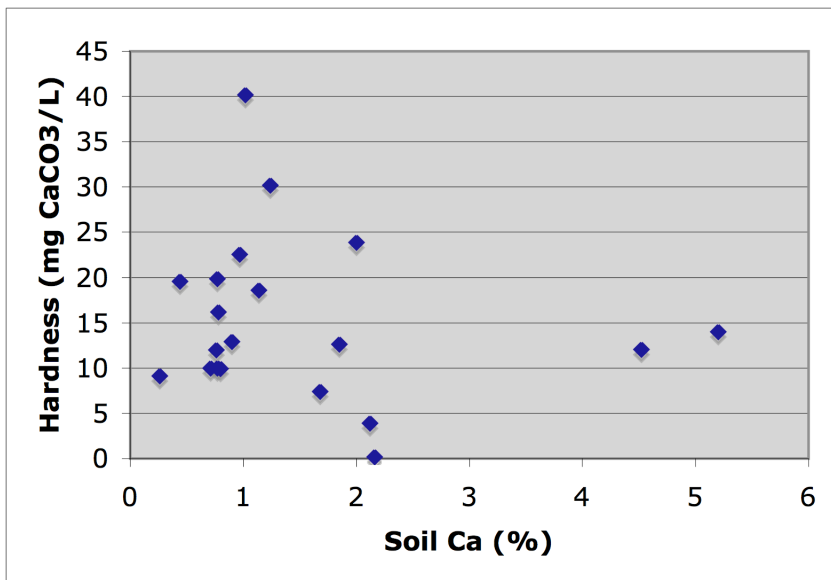

Figure 7. Relationship between soil calcium content and leachate hardness (mg $CaCO_3$/L).

solution by equilibrium with carbonate mineral phases, ion exchange reactions, and weathering of other minerals. The lack of correlation suggests that the 4-acid extraction is releasing Ca and Mg from phases that are not in equilibrium with the solution. Improvement in predicting hardness could come from the use of a sequential extraction procedure. However the goal is to use readily available soil data, such as total elemental analysis, to estimate the pore water composition in the associated soil.

The relationship between hardness and alkalinity is shown in Figure 8. Although the correlation between soil inorganic carbon and alkalinity was weak (Figure 6), a strong
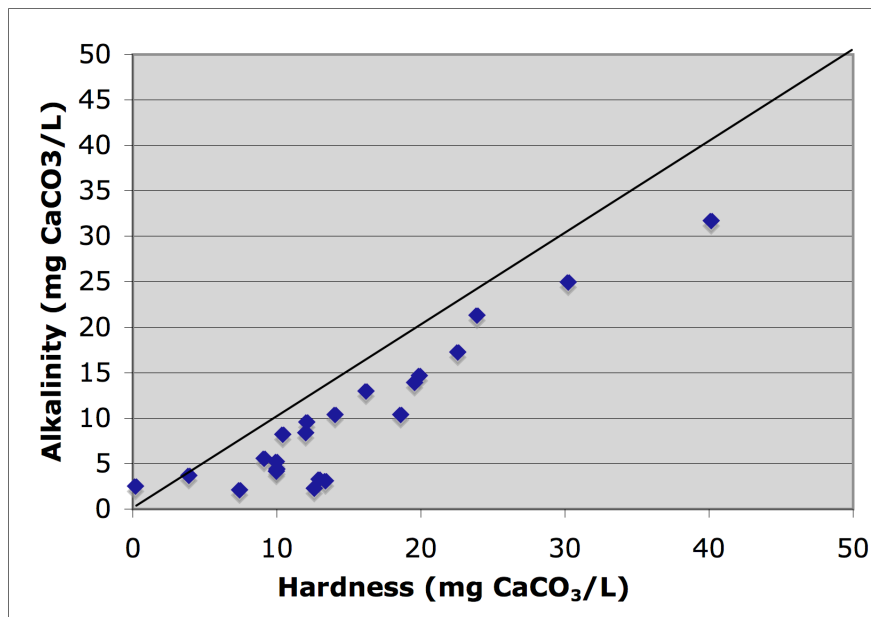

Figure 8. Relationship between leachate hardness (mg $CaCO_3$/L) and alkalinity (mg $CaCO_3$/L).

correlation is seen between hardness and alkalinity. This demonstrates that the major counter ions for the hardness cations are bicarbonate and carbonate. The observed correlation means that metal toxicity can be simultaneously influenced both by competitive hardness cations and complexation by carbonate species. Thus it becomes difficult to isolate the relative importance of each process. In some systems, such as mining impacted soils where significant acidity can be generated by sulfide oxidation, hardness cations can be released by acid weathering without alkalinity generation. For the afore-mentioned reasons the toxicity experiments were performed under conditions where both parameters co-varied and where only one varied while the other was held constant.

Microbial Enzyme Bioassay

The METPlate™ bioassay provides a toxicity test that is inexpensive, rapid, and sensitive to metals. It is experimentally less complex that other toxicity tests such as earthworm

assays or greenhouse plant growth tests.   The test can be performed in less than 4 hours, and depending on the experimental design can either be used to provide a toxicity screening for up to 90+ samples, or an EC50 test for 5 samples in duplicate. In the EC50 test serial dilutions are used to determine the concentration of metal that causes 50% inhibition of the bacterial activity. A typical EC50 test is shown in Figure 9. In the
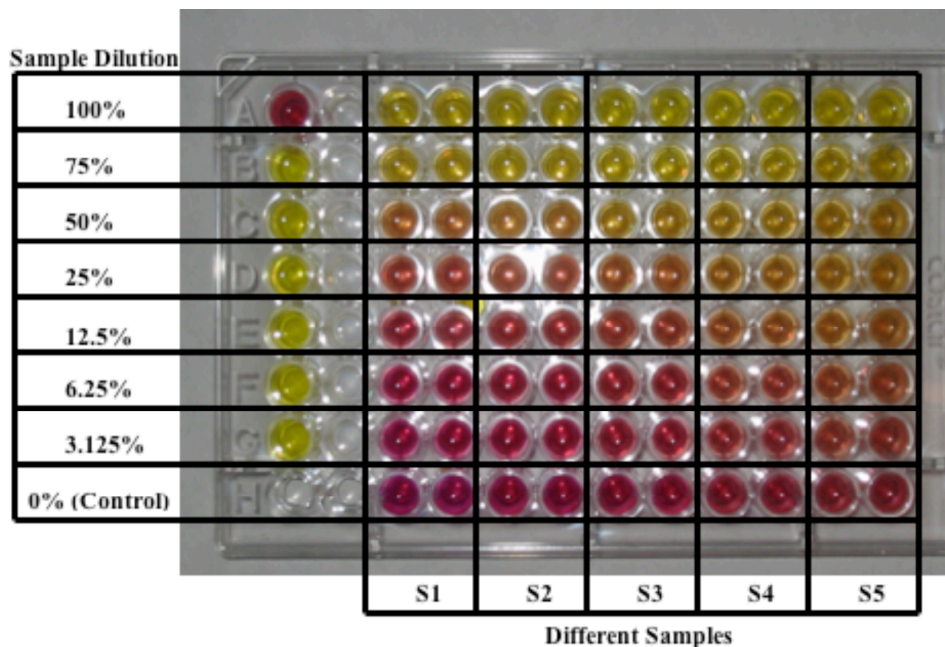

Figure 9. The METPlate™ microbial bioassay illustrating the use of serial dilutions and controls.

specific test illustrated in Figure 9, each row represents a dilution of the test sample starting at 100% sample and ending at 3.125 %. A row of diluents (0%) serves as a control. Other controls include a positive control (complete toxicity) containing 1000 μg/L copper sulfate. The figure also illustrates that five samples were performed in duplicate. If instead the goal was to perform a site toxicity screening then each well could be filled with sample, either a water sample or a soil leachate sample. Both positive and negative controls should be run as well.

Based on our understanding of the effects of water composition on toxicity, the microbial bioassay was performed using a series of different EPA laboratory test waters (USEPA, 2002). The results of experiments performed with copper, where both alkalinity and hardness varied (see Table 1) are given in Figure 10. The measured absorbance is plotted versus the concentration of metal, in this case copper. The overall trend is that as copper concentration increases, the absorbance decreases. This is indicative of the inhibition of the bacteria as metal toxicity becomes greater. Another effect is seen in that for the negative controls, no added copper, a different absorbance is seen for each water type. In Figure 11 the results are shown after normalizing all the measured absorbance values to the control absorbance and expressing the results as percent inhibition (decrease from control absorbance). For copper little effect of solution composition on toxicity is seen
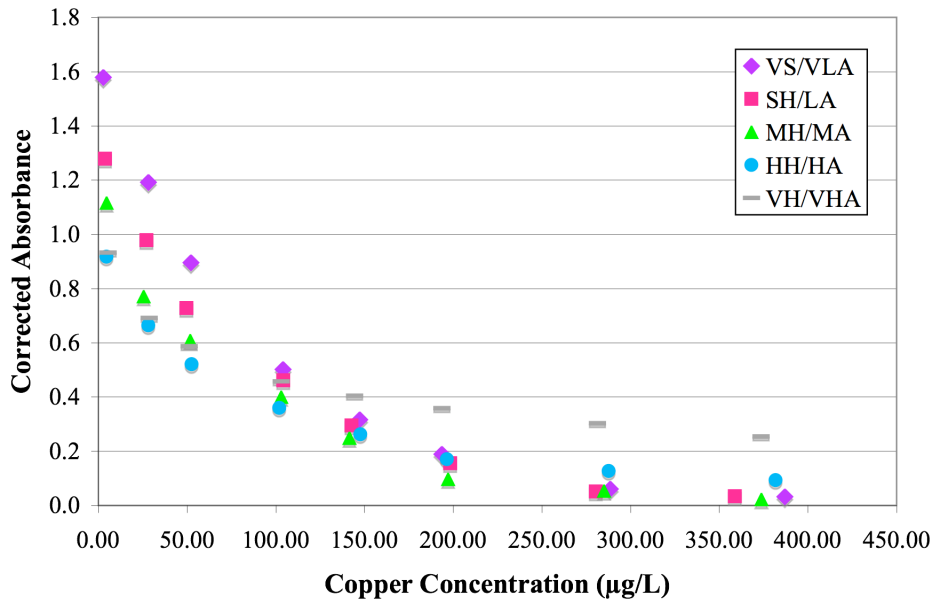
Figure 10. METPlate™ results for copper in waters where both alkalinity and hardness varied. Alkalinity: VLA-very low alkalinity, LA-low alkalinity, MA-moderate alkalinity, HA-high alkalinity, VHA-very high alkalinity. Hardness: VS-very soft, SH-soft, MH-moderate hardness, HH-high hardness, VH-very high hardness.
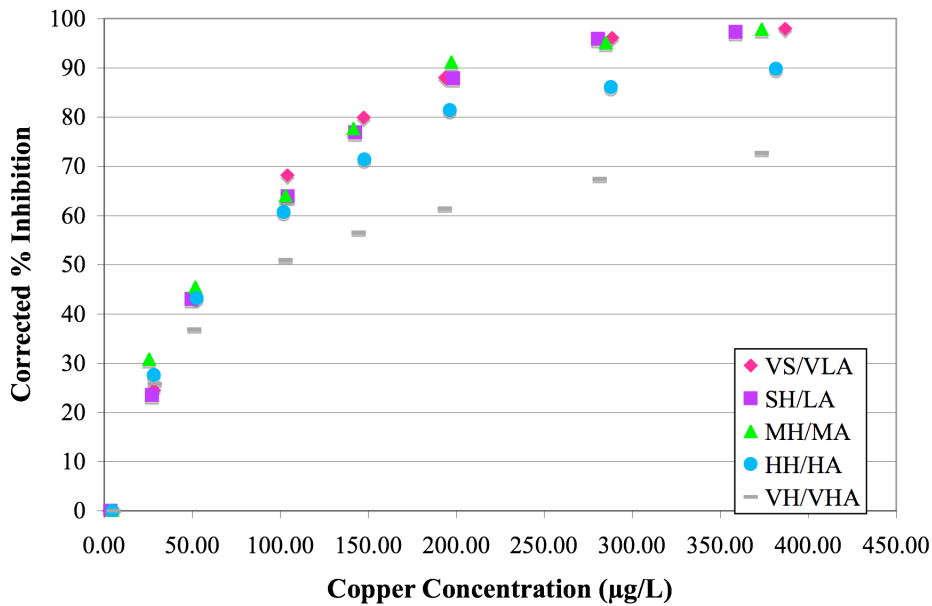

Figure 11. METPlate™ results for copper corrected for matrix effects and presented as percent inhibition of the bacteria.

except in the case of high hardness and high alkalinity. Possible mechanisms include the formation of non-bioavailable copper carbonate complexes or competition between copper and the hardness cations (Ca^{2+}, Mg^{2+}) and metal binding sites on the bacteria.

In order to more fully characterize the toxic response of the bacteria to metals the terms EC25 (25% inhibition) and EC75 (75% inhibition) are defined in addition to EC50. The results for copper and zinc in waters of co-varying hardness and alkalinity are given in Tables 1 and 2. A clear toxicity effect of increasing metals concentration is seen for both

Sample Name	µg/L			mg/L				
	Cu EC$_{25}$	Cu EC$_{50}$	Cu EC$_{75}$	Hardness	Alkalinity	Sulfate	DOC	pH
VS/VLA	29	66	123	10.2	7.0	15.2	0.0	7.1
SH/LA	29	68	137	40.3	30.8	59.9	0.0	7.5
MH/MA	21	64	134	80.8	57.3	121.4	0.0	7.6
HH/HA	26	72	165	156.3	115.2	245.4	0.0	7.7
VH/VHA	27	98	413	307.6	222.8	489.9	0.0	7.9

Table 1. Copper effect concentration values (EC25, EC50, EC75) for METPlate™ under variable hardness, alkalinity, and pH values. Highlighted cell represents an extrapolated value that was above the measured response range.

Sample Name	µg/L			mg/L				
	Zn EC$_{25}$	Zn EC$_{50}$	Zn EC$_{75}$	Hardness	Alkalinity	Sulfate	DOC	pH
VS/VLA	44	97	200	10.2	7.0	11.7	0.0	7.1
SH/LA	47	99	233	40.3	30.8	49.8	0.0	7.5
MH/MA	39	79	201	80.8	57.3	108.1	0.0	7.6
HH/HA	31	70	536	156.3	115.2	230.2	0.0	7.7
VH/VHA	28	95	1,011	307.6	222.8	464.6	0.0	7.9

Table 2. Zinc effect concentration values (EC25, EC50, EC75) for METPlate™ under variable hardness, alkalinity, and pH values. Highlighted cells represent extrapolated values that were above the measured response range.

metals. Hardness and alkalinity effects are minimal except for the EC75 values, suggesting that only for heavily contaminated soils will a protective effect of hardness and alkalinity be observed.

In order to determine if an individual effect could be discerned for either hardness or alkalinity, another set of experiments were performed. In these tests sodium and chloride salts were used in addition to the carbonate and sulfate salts to allow individual variations in hardness and alkalinity. Results of these tests run in duplicate are given in Table 3-6.

	μg Cu/L						mg/L			
Sample	EC25	Stand. Err.	EC50	Stand. Err.	EC75	Stand. Err.	Hardness	Alkalinity	DOC	pH
MH/MA	51	10	105	12	199	20	88.3	64.5	-	7.7
HH/MA	44	1	100	2	195	7	158	64.5	-	8.0
VH/MA	40	5	101	6	280	34	319	63.3	-	8.0

Table 3. Copper effect concentration values (EC25, EC50, EC75) and their standard error for METPlate™ under variable hardness and constant alkalinity.

	μg Cu/L						mg/L			
Sample	EC25	Stand. Err.	EC50	Stand. Err.	EC75	Stand. Err.	Hardness	Alkalinity	DOC	pH
MH/VLA	46	4	120	21	249	59	83.7	11.0	-	7.4
MH/LA	45	2	116	9	227	34	88.2	53.7	-	7.6
MH/MA*	51	10	105	12	199	20	85.7	64.5	-	7.7

Table 4. Copper effect concentration values (EC25, EC50, EC75) and their standard error for METPlate™ under variable alkalinity and constant hardness.

	μg Zn/L						mg/L			
Sample	EC25	Stand. Err.	EC50	Stand. Err.	EC75	Stand. Err.	Hardness	Alkalinity	DOC	pH
MH/MA	47	10	117	19	319	75	85.9	64.5	-	7.7
HH/MA	33	6	115	37	326	96	168	64.5	-	8.0
VH/MA	48	17	239	136	1415	310	330	63.3	-	8.0

Table 5. Zinc effect concentration values (EC25, EC50, EC75) and their standard error for METPlate™ under variable hardness and constant alkalinity.

	μg Zn/L						mg/L			
Sample	EC25	Stand. Err.	EC50	Stand. Err.	EC75	Stand. Err.	Hardness	Alkalinity	DOC	pH
MH/VLA	62	12	138	15	598	59	82.9	11.0	-	7.4
MH/LA	64	18	159	33	407	34	86.0	53.7	-	7.6
MH/MA*	47	10	117	19	319	75	85.9	64.5	-	7.7

Table 6. Zinc effect concentration values (EC25, EC50, EC75) and their standard error for METPlate™ under variable alkalinity and constant hardness.

For copper under variable hardness, only the high hardness condition demonstrates a decrease in toxicity, as seen in the EC75 result. No statistically significant effect of alkalinity is observed for copper.

For zinc, under variable hardness, both the average EC 50 and EC75 values were considerably higher for the high hardness waters. However these results also displayed very large uncertainties, making it difficult to draw any significant conclusions. Zinc showed the unusual result that increasing alkalinity resulted in increasing toxicity, especially for the EC75 results. This result cannot be explained by zinc complexation in solution, unless zinc carbonate complexes are more bioavailable. Other biochemical effects of alkalinity on the test organisms might explain this result.

The final parameter investigated was dissolved organic carbon (DOC). Solutions of moderate hardness and alkalinity were amended with DOC using Deer Creek fulvic acid. This DOC was isolated using XAD-8 resin from a creek in the Snake River system just west of the Continental Divide in central Colorado (Blumenstein, 2006). For copper concentrations of approximately 1 and 5 mg DOC/L were used. For zinc only 5 mg DOC/L was used. The results are shown in Tables 7 and 8.

	μg/L			mg/L				
Sample Name	Cu EC$_{25}$	Cu EC$_{50}$	Cu EC$_{75}$	Hardness	Alkalinity	Sulfate	DOC	pH
0 DOC (Control)	51	105	199	94.0	67.0	84.5	0.0	7.7
DC1 DOC	46	128	251	95.3	67.0	85.7	1.1	7.7
DC5 DOC	77	165	331	95.7	67.0	84.8	5.3	7.7

Table 7. Copper effect concentration values (EC25, EC50, EC75) for METPlate™ under variable DOC and constant alkalinity and hardness.

	μg/L			mg/L				
Sample Name	Zn EC$_{25}$	Zn EC$_{50}$	Zn EC$_{75}$	Hardness	Alkalinity	Sulfate	DOC	pH
0 DOC (Control)	47	117	319	85.9	64.5	85.4	0.0	7.7
DC5 DOC	68	125	300	98.3	67.0	83.0	5.3	7.8

Table 8. Zinc effect concentration values (EC25, EC50, EC75) for METPlate™ under variable DOC and constant alkalinity and hardness.

Increasing DOC lead to decreasing toxicity for copper and no significant effect for zinc. This is in keeping with the fact that DOC forms stronger, and less bioavailable complexes with copper as compared to zinc.

Summary

From this study we hope to drawn some preliminary general conclusions about the role of soil properties in influencing metal toxicity when occurring through pore water exposure.

Pore water DOC and, to a lesser extent, alkalinity seemed to be directly related to soil organic and inorganic carbon content respectively. Previously measured geographical variations in these soil properties thus might be used to predict pore water composition for these species. Ongoing studies such as the USGS Geochemical Landscapes Project should collect data on pore water DOC and alkalinity to further expand on our understanding of this relationship. In particular the strong influence of DOC on metal toxicity suggest that SOM maybe a controlling factor in metal toxicity. Although not directly examined in this study, pH is likely to be a second controlling factor.

Since poor correlation between total soil calcium and pore water hardness, but good correlation between pore water alkalinity and hardness, were observed, more work should be done to determine if hardness could be predicted from soil inorganic carbon content. However, hardness and alkalinity did not affect the *E. coli* test species. This result suggests that primarily it is metal content that will affect the METPlate toxicity test. The METPlate test will be sensitive to the effect of porewater DOC on the bioavailablity of copper and zinc, whereas the other porewater constituents will not affect the test. While this makes the METPlate test more applicable across a wide range of water compositions, it may not capture the affects of water chemistry on modifying the toxicity of metals to other organisms. Data on these effects on other species is very limited.

References

Bitton, G., Jung, K., and Koopman, B. 1994. "Evaluation of a microplate assay specific for heavy metal toxicity." *Archives of Environmental Contamination and Toxicology*. Vol. 27, pp. 25-28.

Eric Blumenstein, 2006. CSM MS Thesis: " Evaluating Aquatic Bioassays for Copper and Zinc Toxicity in Mining Influenced Waters"

Briggs, P. H. and Meier. 2002. The determination of forty-two elements in geological materials be inductively coupled plasma-mass spectrometry. USGS Open File Report 02-223-I.

Brown, P.L. and Markich, S.J. , 2000. Evaluation of the free ion activity model of metal-organism interaction: extension of the conceptual model. Aquatic Toxicology 51, 177-194.

Di Toro, D. M,Allen, H. E., Bergman, H. L., Meyer, J. S., Paquin, P. R., and Santore, R. C., 2001. A biotic ligand model of the acute toxicity of metals I. Technical Basis. Environ. Toxicol. Chem. 20, 2383-2396.

Di Toro, D.M., McGrath, J.A., Hansen, D.J., Berry, W.J., Paquin, P. R., Mathew, R., Wu, K.B., and Santore, R. C. 2005. Predicting sediment metal toxicity using a sediment biotic ligand model: Methodology and initial application. Environ. Toxicol. Chem., 24,2410-2427.

Hageman, P. L., 2004, Use of short-term (5-minute) and long-term (18-hour) leaching tests to characterize, fingerprint, and rank mine waste material from historical mines in the Deer Creek, Snake River, and Clear Creek watersheds in and around the Montezuma Mining District, Colorado: U.S. Geological Survey Scientific Investigations Report 2004-5104, 41 p.

Hargrove, W.W. and Hoffman, F. M., 1999, Using multivariate clustering to characterize ecoregion borders. Computing in Sci. and Eng., 4, 18-25.

Meyer, J.S., Santore, R.C., Bobbitt, J.P., Debrey, L.D., Boese, C.J., Paquin, P.R., Allen, H.E., Bergman, H.L. and Ditoro, D.M. 1999. Binding of nickel and copper to fish bills predicts toxicity when water hardness varies, but free-ion activity does not. Environmental Science & Technology 33(6), 913-916.

Parker, D.R. and Pedler, J.F. 1997. Reevaluating the free-ion activity model of trace metal availability to higher plants. Plant and Soil 196(2), 223-228.

Tipping, E. 1994. "WHAM—A computer equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances." *Computers and Geosciences*. 1994. Vol. 20, pp. 973–1023.

USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Maringe Organisms. EPA-821-R-02-012. USEPA Office of Water. Washington, DC. 275 p.

Vijver, M. G., Vink, J.P.M., Miermans, J.H., and van Gestel, C.A.M. 2003. Oral sealing using glue:A new method to distinguish between intestinal and dermal uptake of metals in earthworms. Soil Biol. And Biochem. 35, 125-132.

Publications and Presentations from this Work
Two students and the PI were partially supported on this project and two manuscripts are in preparation for submission to the SETAC journal.

Eric Blumenstein, 2006. CSM MS Thesis: " Evaluating Aquatic Bioassays for Copper and Zinc Toxicity in Mining Influenced Waters"

Jessica (Moehle) Jeppson, 2007. CSM MS Thesis " Examining Metal Bioaccessability and Bioavailability of Contaminated Solids"

Wildeman, T.R., Smith, K.S., and Ranville, J.F., 2007, A simple scheme to determine potential aquatic metal toxicity from mining wastes: *Environmental Forensics*, v. 8, 119-128

Ranville, J.F., Blumenstein, E., Adams, M., Choate, L., Smith, K., and Wildeman, T. 2006. Integrating bioavailability assessments into waste rock evaluations. *In* Proceeding of 2006 7th ICARD, March 26-30, 2006, St. Louis, MO, ASMR, Lexington, KY.

Blumenstein, E.P., Ranville, J.F., Choate, L.M., Ross P.E., 2005. Using Enzyme Bioassays as a Rapid Screen for Metal Toxicity. *In* Proceedings of the 2005 National Meeting of the American Society of Mining and Reclamation, Breckenridge, Colorado, June 19-23, 2005. Published by ASMR, 3134 Montavesta Rd. Lexington, KY 40502

Ranville, J. F., Blumenstein, E. P., Ross, P., and Choate, L. M. 2006. Using an Enzyme Bioassay to Evaluate Metal Contaminated Soils: Influence of Water Chemistry on Bioavailability and Toxicity 9th International symposium on metal ions in biology and medicine. May 21-24, Lisboa, Portugal

Blumenstein, E.P., Ranville, J.F., and Choate, L.M., 2006, Evaluating an enzyme bioassay for the purpose of screening for metals contamination in mining impacted soils (MIS). 10th Billings Land Reclamation Symposium, Billings, Montana, June 4-8, 2006.

Blumenstein, E.P., Ranville, J.F., and Choate, L.M., 2006, Evaluating the effects of water chemistry variation on the MetPLATE enzyme bioassay, when used to screen for metals contamination in mining impacted soils and waters. Seventh International Conference on Acid Rock Drainage (ICARD 7), St. Louis, Missouri, March 26-30, 2006, p. 2634.

Blumenstein, E.P., Ranville, J.F., Choate, L.M., Smith, K.S., Wildeman, T., and Ross, P., 2006, Use of an enzyme bioassay in a simple decision tree for assessing aquatic and sediment leachate toxicity potential of mining wastes. U.S. EPA Hard Rock Mining 2006 Conference, Tucson, Arizona, November 14-16, 2006.

Blumenstein, E. P., Ranville, J.F., Choate, L.M., Ross P.E., 2005. Assessing the validity of enzyme bioassays for screening bioavailable metals in mining impacted soils. Abstracts from the 26th Annual North American Conference of the Society of Environmental Toxicology & Chemistry, Pensacola, FL.

Wildeman, T., Ranville, J., Blumenstein, E., Smith, K., 2005. Advances in Simple Methods of Assessing the Toxicity of Mine Wastes. Presented at the 2005 Geological Society of America national Conference, Salt Lake City, UT, Nov. 16th-19th.

Moehle, J., Fox, S., Ranville, J.F., Wildeman, T.R., and Ross, P.E. 2005. Characterization and leach test assessment at the Tip Top Mine, a marginally impacted site . *in* Proceedings of the 2005 National Meeting of the American Society of Mining and Reclamation, Breckenridge, Colorado, June 19-23, 2005. ASMR, Lexington, KY.

Moehle, J., Ranville, J. F., Choate, L. M., Wildeman, R. and Ross, P. 2006. Leachate and enzyme bioassay assessment tests at the Tip Top mine, a marginally impacted site. *In* Proceeding of 2006 7th ICARD, March 26-30, 2006, St. Louis, MO, ASMR, Lexington, KY.

Jeppson, J., Ranville, J.F., Wildeman, T.R., and Machemer, S. 2006. Leachability study of sediments deposited in New Orleans by hurricane Katrina. 232nd ACS National Meeting, San Francisco, CA, Sept. 10-14, 2006.

Jeppson, J., Ranville, J.F., Smith, K.S., and Wildeman, T.R. 2006. Use of enzyme bioassays in a simple decision tree for assessing aquatic toxicity potential of mining wastes. U.S. EPA Hard Rock Mining 2006 Conference, Tucson, Arizona, November 14-16, 2006.

Jeppson, J., Ranville, J.F., and Wildeman, T.R. 2006. Using leaching studies to examine metal toxicity in contaminated soils. Society of Environmental Toxicology and Chemistry (SETAC) North America 27th Annual Meeting, Montréal, Québec, Canada, November 5-9, 2006.

Moehle, J., Ranville, J., Choate, L., Wildeman, T., and Ross, P. 2006. Leachate and enzyme bioassay toxicity assessment tests at the Tip Top Mine, a marginally impacted site. Seventh International Conference on Acid Rock Drainage (7th ICARD), St. Louis, Missouri, March 26-30, 2006.
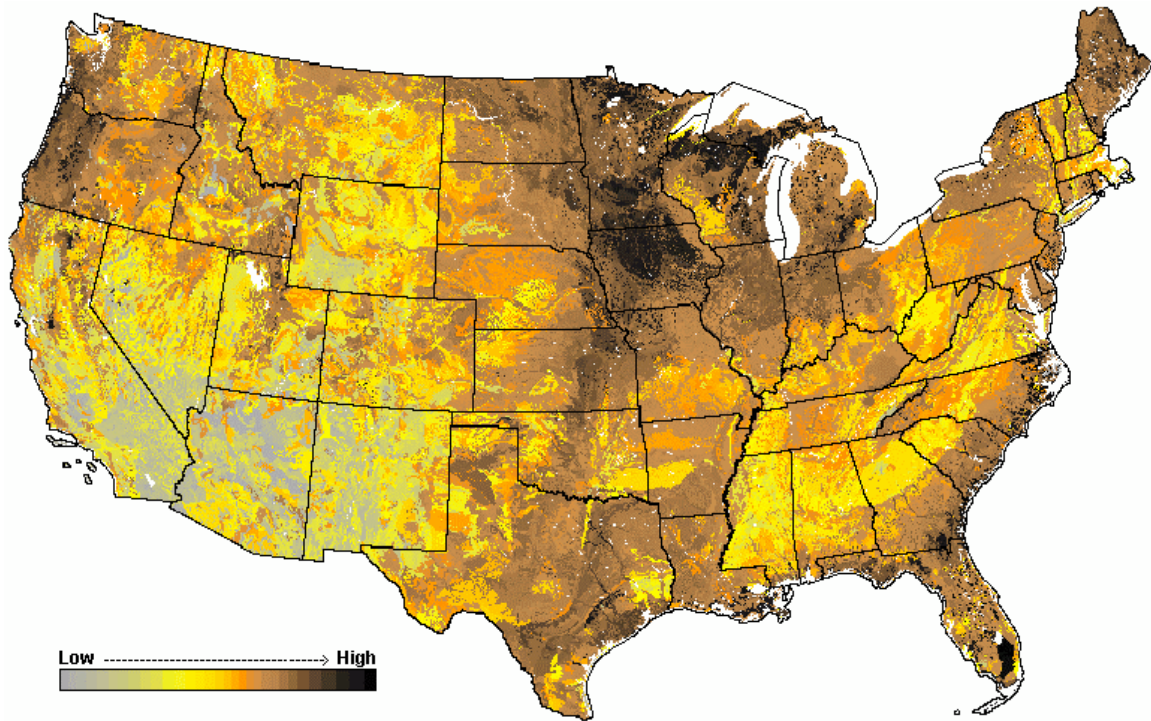
Acknowledgements

Appendix

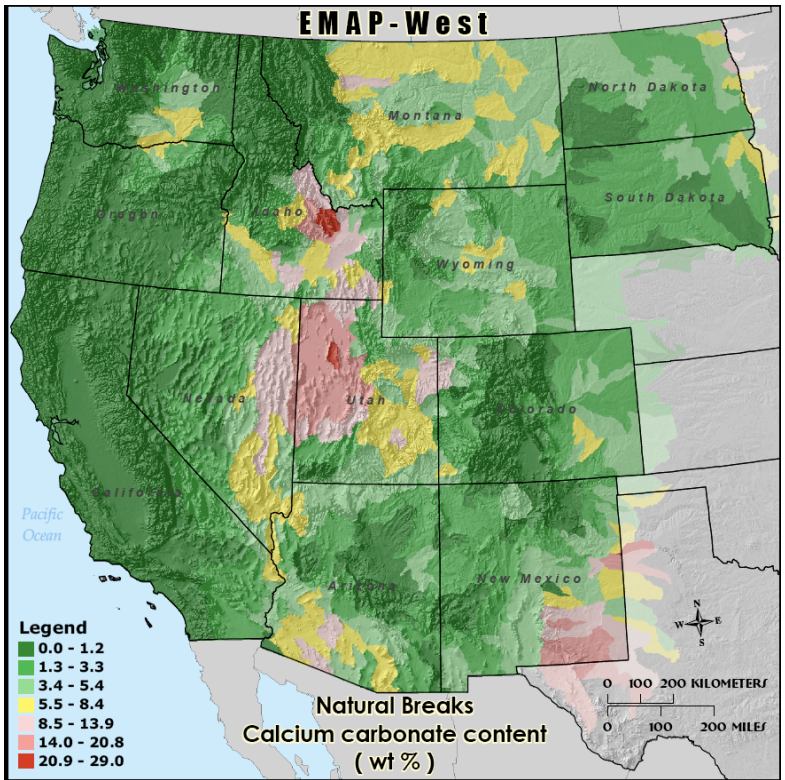Figure A-1. Soil organic matter content from Hargrove and Hoffman (1999)

Figure A-2. Soil calcite in the western United States (images from USEPA EMAP program (http://www.epa.gov/emap/).