Plant Bioassay

Plant biological endpoints (germination, tissue contaminant content, dry matter growth) are often used as indicators of soil phytotoxicity. Estimates of the bioavailability or toxicity of soil contaminants are important for making remedial decisions and for evaluating remedial success. Phytoaccumulation and phytotoxicity are often poorly related to total soil contaminant content. Contaminant bioavailability may be a better predictor. Soil properties affect metal bioavailability to ecological receptors. Therefore contaminant bioavailability varies among soil types and may depend on one or a combination of soil properties. A single total contaminant level can result in multiple contaminant exposure doses across different soils due to modification by soil properties or modification due to *in situ* remediation.

Plant biological endpoints (germination, tissue contaminant content, dry matter growth) are used to measure soil contaminant bioavailability and toxicity. It is important to ensure, as far as possible, that variation in plant response is due to contaminant bioavailability and not due to uncontrolled variables such as problems with soil quality (extremes of pH or salinity, water availability etc.) or plant nutrient deficiencies. If these variables are not controlled, it will be impossible to assess the effect of contaminant bioavailability on the plant endpoint. Thus, control of soil quality and soil fertility is essential to make meaningful comparisons between or among different soils or even within a single soil before and after remediation comparisons. Therefore, before the bioassay is conducted:

- Remove excess salt and/or adjust soil pH of the contaminated soil
- Ensure soil has adequate moisture
- Ensure sufficient plant nutrients
- For heavy or compacted soils add inert bulking agent (i.e., vermiculite, perlite) to improve soil aeration and drainage.

Calibrated *In Vitro* Surrogate Methods for Plant Bioassay

Several tests that measure direct exposure include both *in vivo* bioassays (i.e., living plant, invertebrate, or swine—human surrogate) and *in vitro* soil tests (i.e., laboratory soil chemical methods correlated with *in vivo* models). *In vivo* bioassay tests are more expensive and require more time to conduct (i.e., weeks to months) than *in vitro* soil tests. However, a critical prerequisite for use of *in vitro* methods is documentation demonstrating the *in vitro* method is strongly correlated with the *in vivo* endpoint. On a limited budget, more soil samples can be tested by *in vitro* soil tests than *in vivo* bioassays. Analysis of greater numbers of samples allows a more thorough characterization of the site's potential bioavailability/toxicity. Grid sampling for bioavailability/toxicity testing is possible using *In vitro* methods but not feasible using bioassays. A strong relationship between *in vitro* and *in vivo* is a prerequisite for using *in vitro* to predict *in vivo* (as a surrogate method) while limiting the onus and expense of multiple bioassays.

Examples of Calibrated Bioassay

A "calibrated bioassay" is a test where soils have paired *in vivo* and *in vitro* measures in order to determine if a robust relationship between them can be established. Examples of robust relationships (i.e. calibration) between *in vivo* plant ecotoxicological endpoints and *in vitro* measurements for Cd (Fig. 1), As (Fig. 2) and Zn (Fig. 3) are shown below. A weak salt extraction such as 0.01 M $CaCl_2$, KNO_3, or NH_4NO_3 can be used to mimic the soil solution (pore water) contaminant concentration. This may provide a measure of the contaminant dose the plant is directly exposed to and is often highly related to plant response. Using the regression equation, contaminant bioavailability/toxicity can be predicted across a site.

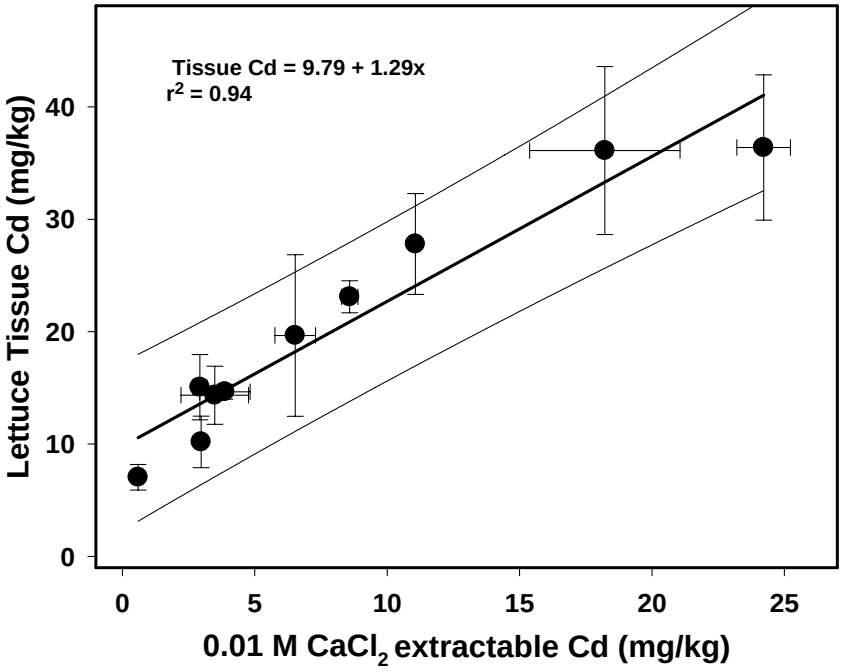

Figure 1. Relationship between lettuce tissue Cd and soil weak salt extractable Cd. Basta and Gradwohl (2000)

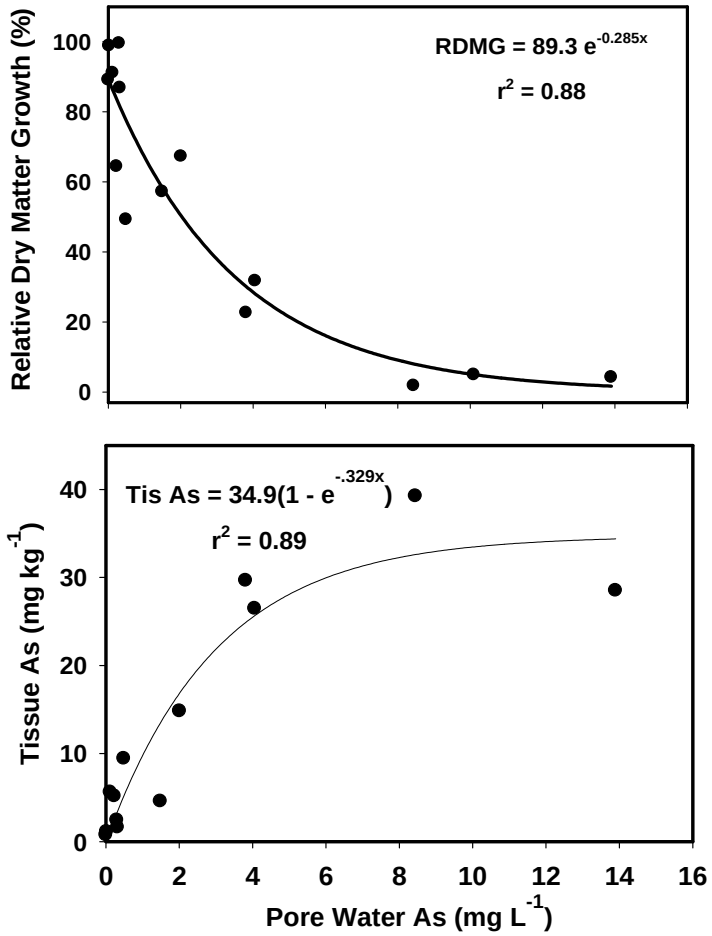

Figure 2. Relationship between Lettuce dry matter growth (relative to the control soils) and tissue As with soil pore water As (Dayton and Basta, 2005).

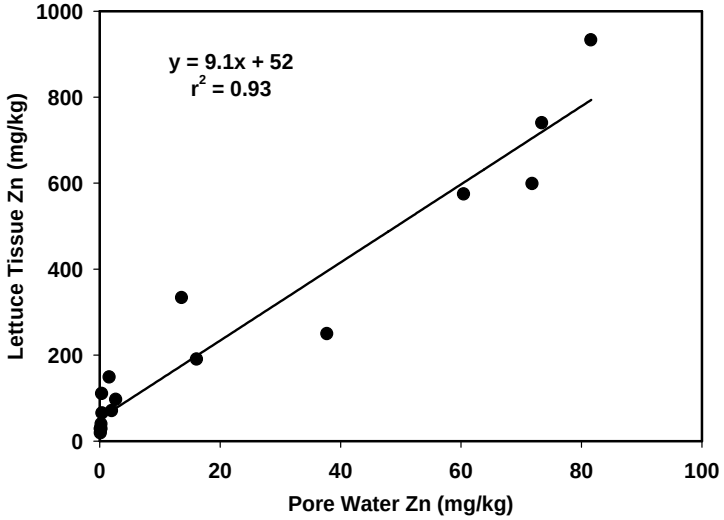

Figure 3. Relationship between soil pore water Zn and lettuce tissue Zn (Dayton et al., 2006).

Trace element phytoavailability estimated by soil extraction methods

Availability of trace elements and nutrients in soils has been measured using single extractions (*in vitro*) of soil or residual-treated soils. Good correlation between soil extractants and plant uptake has allowed use of the extractant to make reasonable predictions of plant available nutrients in soil and fertilizer recommendations (Amacher, 1996). One of the requirements of a good soil extractant (one where plant response is correlated with nutrient extracted by the soil test) is that the soil test measures "some or all of the (phyto) available pool" (Bray, 1948). Soil extraction methods correlated with plant uptake of trace element include those based on solutions containing chelates, such as diethylenetriaminepentaacetic acid (DTPA), and neutral salt solutions ($CaCl_2$). The extensive use of such methods to estimate trace element availability in residual-treated soils has been reviewed by McLaughlin (2002) and Pierzynski (1998).

References

Amacher, M.C. 1996. Nickel, Cadmium, and Lead. P. 739-768. *In* D.L. Sparks (ed.) Methods of Soil Analysis, Part 3. SSSA Book Ser. 5 SSSA, Madison, WI.

Basta, N.T., J.A. Ryan, and R.L. Chaney. 2005. Trace Element Chemistry in Residual-Treated Soil: Key Concepts and Metal Bioavailability.

Basta, N.T. and R. Gradwohl. 2000. Estimation of cadmium, Pb and zinc bioavailability in smelter-contaminated soils by a sequential extraction procedure. J. Soil Contam. 9:149-164.

Bray, R.H. 1948. Requirements for successful soil tests. Soil Sci. 66:83-89.

Dayton, E.A., and N.T. Basta. 2005. *In vitro* extraction methods and ecotoxicity endpoints of *Lactuca Sativa* in arsenic contaminated soils. 8th International Conference on the Biogeochemistry of Trace Elements, Adelaide, Australia, April 3-7, 2005.

Dayton, E.A, N.T. Basta, M.E. Payton, R.P. Lanno, J.L. Schroder, and K.D. Bradham. 2006. Evaluating the contribution of soil properties to modifying lead phytoavailability and phytotoxicity. Environ. Toxicol. Chem. 25:719-725.

McLaughlin, M.F. 2002. Bioavailability of metals to terrestrial plants. P. 39-68. In H.E. Allen (ed.) Bioavailability of Metals in Terrestrial Ecosystems: Importance of Partitioning for Bioavailability to Invertebrates, Microbes, and Plants. SETAC Press, Pensacola, FL.

Pierzynski, G.M. 1998. Past, present, and future approaches for testing metals for environmental concerns and regulatory approaches. Commun. Soil Sci. Plant Anal. 29:1523-1536