Research Study Plan Title: Heavy metal effects on growth and survival of native floodplain species

USGS Center: Columbia Environmental Research Center, Columbia, MO

Facility Contact: Mr. Rip S. Shively: 573-876-1900; rsshively@usgs.gov

Project Contact: Dr. Esther D. Stroh: 573-441-2944; estroh@usgs.gov

Introduction

In response to a request from the U.S. Fish and Wildlife Service (USFWS), in collaboration with Missouri Department of Natural Resources (MDNR) as the Missouri Trustee Council, the Columbia Environmental Research Center will conduct an assessment of the effects of contamination from mining-derived metals on early growth of herbaceous and woody species native to southeast Missouri bottomland forests. This study is part of the ongoing Southeast Missouri Natural Resource Damage Assessment for the Big River Mine Tailings Superfund Site and Surrounding Area.

Southeast Missouri contains some of the highest concentrations of lead ore deposits in the world. A region of the Southeast Missouri Lead Mining District known as the "Old Lead Belt" was a globally important producer of lead from the mid nineteenth century until the early 1970's. Early on, mine wastes were released directly into waterways or on the landscape; after World War II, mine wastes were kept in piles and impoundments that continued to erode or experience dam failures Although tailings piles are now stabilized or in the process of being stabilized, past and ongoing releases of mine waste have resulted in high levels of Pb concentrations in aquatic sediments and floodplain soils along the Big River. Floodplain soils contain Pb concentrations from 400 ppm to greater than 2000 ppm at depths of one to 4 meters from Leadwood to the mouth (Pavlowsky and others 2010). In particular, overbank floodplain soils along the Big River are estimated to contain 86,800,000 m^3 of contaminated deposits (Pavlowsky and others 2010).

Lead and zinc contaminated aquatic sediments are known to affect aquatic food chains, contributing to loss of mussels and crayfish (Schmitt and others, 2007), but little is known about effects of contaminated floodplain soils on native floodplain vegetation. Effects of mining-derived metals contamination have been demonstrated on numerous agricultural plants in the field and in laboratory experiments (Andersson, 1988; Balsberg, 1989), but heavy-metal sensitivity of most native plants are unknown (Leyval and others, 1997). Plants grown in native soil from mine-waste contaminated sites produced 40% less biomass than plants grown in native soil from uncontaminated sites (Grabner and others 2009). Although zinc is an essential micronutrient for plants, elevated soil Zn concentrations resulting from anthropogenic sources including mining and smelting activities can have toxic effects in plants (Chaney, 1993). At the plant community level, Pierzynski and Fick (2007) demonstrated decreasing plant species richness with increasing total and bioavailable Zn concentration. Stroh and others (2009) demonstrated a negative effect of metals, especially soil zinc concentrations, on the floristic quality of native plant communities. The negative effects of metals were more pronounced on native soil plots in relatively undisturbed conditions, indicating that elevated Pb and Zn concentrations affect floristic quality where no other human disturbance is evident. Sites located

on native soils in the Big River floodplain exhibited much lower floristic quality than more upland sites on native soil (Stroh and others, 2009). These floodplain sites were located downstream and downslope from mine waste and exhibited much higher soil PB and ZN concentrations than other native soil sites in the study. These sites supported a suite of native but flood disturbance-adapted species, which may also partly explain the lower floristic quality. The combination of flooding disturbance and increased metals concentrations suggested that more research is needed to characterize the impact of Pb and Zn concentrations on native floodplain species.

Purpose

The purpose of this study is to measure early growth and survival of native floodplain herbaceous and woody species grown as young seedlings in homogeneous native soils exhibiting a wide range of Pb and Zn concentrations. We will employ a 56+ day outdoor growth study in texturally similar native soils collected from the Big River flood plain that exhibit an in-situ range of Pb and Zn concentrations.

Methods

Because plant community floristic quality was more closely related to soil Zn concentration than to soil Pb concentration (Stroh and others 2009), we will use soil Zn concentration to identify collection sites for floodplain soils. Soils representing a broad range of Zn concentrations (based on field readings made with a portable X-Ray Fluorometer, XRF) will be collected from locations in the Big River floodplain, including sites with Pb and Zn concentrations representing background levels of the area (control site). Soils will be collected from several locations along the Big River and its tributary Flat River Creek. Five 5 gallon (22 L) plastic buckets of soil will be excavated from a small area at each location.

Soils will be sieved to remove organic and mineral particles above 6 mm. A sample from each collection bucket will be oven dried and sieved to remove particles above 2 mm.
These samples (five from each sampling location) will be analyzed to determine the percent mass of particles above 2 mm; if 2 mm fraction (mass) is less than ten percent of the total sample, no further sieving will be performed, so that soils will best represent field conditions. If the mass fraction above 2mm exceeds ten percent for a given soil, that soil will be sieved with 2 mm screen to remove that fraction. We will then conduct particle size analysis (ASTM International, 2004) to determine that soil texture (percentages of sand, silt and clay) identifies all soils to similar locations on the USDA soil texture triangle (USDA 1987).

Lead and zinc concentrations of dried soils from all buckets from each site will be determined using standard operating procedures for laboratory analysis of soils using XRF (Missouri Department of Natural Resources [MDNR] 2008). If needed, soils from different sites will be mixed to obtain intermediate Zn concentration ranges. This process will yield greenhouse soils for use in the growth experiment. Each of these soils will be analyzed for nutrients (P, N, K), cation exchange capacity, pH, and organic carbon content following standard soil analysis methods (Burt, 2004).

Greenhouse soils will be distributed into four-inch plastic pots (approximately 500 ml volume) with an even distribution across the range of Zn concentrations. Soil samples will be taken from each pot and measured for elemental concentrations of Pb and Zn using XRF. For quality control purposes, samples from ten percent of the pots will be analyzed for total-recoverable Pb and Zn concentrations using ICP-MS (Brumbaugh and others 2007).

We will plant six to ten seeds (depending on seed size) of one native floodplain species into each pot. Each species will be planted into at least 50 pots representing the range of Zn concentrations. Seeds will be randomly assigned to pots. Criteria used to select four species for this study will include, but not be limited to: a native component of southeast Missouri floodplains, used as food or cover by native wildlife, small seed size (to minimize effect of embryo as a nutrient source), and seed availability. Potential woody species include ash (*Fraxinus* spp.), red maple (*Acer rubrum*), river birch (*Betula nigra*), sycamore (*Platanus occidentalis*) and elm (*Ulmus* spp.). Potential herbaceous species include blue star (*Amsonia illustrus*), cardinal flower (*Lobelia cardinalis*), downy skullcap (*Scutellaria incana*), and bur marigold (*Bidens aristosa*).

We do not plan to compare survival or biomass production between species. Depending on availability of seeds, each species may be started on a slightly different date in May or June. The experiments will be conducted outdoors in screen shelters. To control for any microclimatic variation within the screen shelters, pots will be arranged in a completely random order. Temperature and humidity will be recorded in the center of each screen shelter using Hobo® data loggers. To reduce leaching of metals and nutrients from soils, pots will be in individual trays; plants will be watered once or twice daily to soil saturation (until water first appears in tray at base of pot).

Pots will be monitored for germination and number of emerged seedlings per pot will be recorded. After 14-28 days (depending on individual species germination requirements), final germination percent per pot will be calculated. Three or four (depending on species size) remaining seedlings in each pot will be selected (using a random number generator) to continue the growth experiment; other seedlings in the pot will be removed.

After 56 – 120 days, depending on species (approximately early July to mid-September), the number of surviving seedlings in each pot will be recorded and mean percent survival per pot will be calculated. All seedlings will be removed and washed with water to remove soil from roots. Seedlings will be cut at the root collar with a razor blade into above- and below-ground parts, placed in paper envelopes and dried in a 60°C convection oven. Dried above- and below-ground plant parts from each pot will be weighed to the nearest 0.0001 gm on a Mettler AT261 analytical balance (±0.00001 gm). We will calculate the mean above-ground and below-ground biomass for each pot.

We will use standard statistical procedures to test for significant relationships between Pb and Zn concentrations and plant germination, survival and above-and below-ground.

Products

An electronic copy of a draft manuscript will be submitted to USFWS and MDNR for review and comment. Results will be published in as an Administrative Report to the Missouri Trustee Council and (with the prior consent of the Missouri Trustee Council) in a peer-reviewed journal.

Proposed Schedule

Calendar Year	2011	2011	2011	2011	2011	2011
Month	April	May	June-Aug	Sept	Oct-Nov	Dec
Collect and process soils	X	X	X			
Germination and growth studies		X	X	X		
Soils data analyses		X	X			
Plant data analyses			X	X		
Manuscript prep/review				X	X	
Deliver manuscript						X

Relationship to Cooperator Needs

The assessment conducted within this study plan has been specifically requested by the U.S. Fish and Wildlife Service in collaboration with Missouri Department of Natural Resources, as the Missouri Trustee Council, as part of the ongoing Natural Resource Damage Big River Mine Tailings Superfund Site and Surround Area.

Literature Cited

Andersson, M. 1988. Toxicity and tolerance of aluminum in vascular plants. Water, Air, and Soil Pollution 39:439-462.

ASTM International, 2003. Standard test method for particle-size analysis of soils (Standard D422-63[2002]). In Annual Book of ASTM Standards (Vol. 04.08): Soil and Rock I). West Conshohocken, PA (Am. Soc. Testing and Mater.), p. 10-17.

Balsberg, A-M. 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants.

Brumbaugh, W.G., May, T.W., Besser, J.M., Allert, A.L., Schmitt, C.J., 2007. Assessment of elemental concentrations in streams of the New Lead Belt in southeastern Missouri, 2002–2005. US Geological Survey Scientific Investigations Report 2007–5057.

Burt, R (editor). 2004. Soil Survey Laboratory Methods Manual. U.S. Department of Agriculture, Natural Resource Conservation Service. Soil Survey Investigations Report No. 42 Version 4.0. November 2004. 700p.

Chaney, R.L. 1993. Zinc phytotoxicity. In A.D. Robson (ed.), Zinc in Soils and Plants, Kluwer Academic Publishers, London, pp. 135–150.

Grabner, K.W., Stroh, E.D., Struckhoff, M.A., and Fairchild, J.F., 2009, The Effect of Growing Native and Exotic Missouri Legumes in Mining Waste from the Tri-State Region and the Old Lead Belt of Missouri: USGS Administrative Report to the United States Fish and Wildlife Service. 29p.

Leyval, C., K. Turnau, and K. Haselwandter, 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7:139-154.

Missouri Department of Natural Resources (MDNR), 2008. Standard Operating Procedure for Innov-X α 400sl XRF Analyzers. Division of Environmental Quality, Hazardous Waste Program, Superfund Section. Jefferson City, MO. September 17, 2008. 3 pp.

Pavlowsky, R. T., M.R. Owen, and D.J. Martin, 2010. Distribution, geochemistry, and storage of mining sediment in channel and floodplain deposits of the Big River system in St. Francois, Washington, and Jefferson Counties, Missouri. OEWRI-10-002m The Ozarks Environmental and Water Resources Institute, Missouri State University, Springfield, MO, 141 p. with appendix.

Pierzynski, G.M. and W. Fick. 2007. Zinc phytotoxicity in the Tri-State Mining Region: Field and greenhouse studies. Final Report. Funding provided by the United States Department of Interior, Fish and Wildlife Service. 41 pp. plus appendix.

Schmitt, C.J., Brumbaugh, W.G, Besser, J.M., and May, T.W., 2007, Concentrations of metals in aquatic Invertebrates from the Ozark National Scenic Riverways, Missouri: U.S. Geological Survey Open-File Report 2007–1435, 23 p. with appendices.

Stroh, E.D., M.A. Struckhoff and K.W. Grabner. 2009. Effects of Mining-Derived Metals Contamination on Native Floristic Quality. USGS Administrative Report. USGS Columbia Environmental Research Center, Columbia, MO. 85p. plus Appendices.

USDA. 1987. Soils mechanics level 1, Module 3; USDA textural soil classification study guide. National Employee Development Staff, Soil Conservation Service, United States Department of Agriculture. Washington, D.C.