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Tri-State Mining District Natural Resource Damage  
 Assessment and Restoration

Migratory Bird Injury Confirmation

Project Proposal and Study Design

Title: Survey of Potentially Toxic Metal Concentrations in Waterfowl, Water, and Soil in the Tri-State Mining District (Kansas, Oklahoma, Missouri)

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## Background

The Tri-State Mining District of Oklahoma, Kansas, and Missouri was once one of the world's greatest producers of lead-zinc ores (USGS 1998). The District covers approximately 3000 km<sup>2</sup> and is contaminated with potentially toxic metals including lead, cadmium, and zinc from mining, milling, and smelting. The District extends from the northwestern edge of the Ozark Uplift across rolling prairie west to the Neosho River (Gibson 1972). Metals have been dispersed throughout the District in the form of milled mine waste ("chat"), as flotation tailings and from smelters as aerial deposition or slag. The chat is composed mainly of chert, a siliceous rock mined with the ores (Dames and Moore 1993) and contains lead, zinc, cadmium, and other elements in the ores that were not separated from the chert. Much of the chat was processed more than once and it was disposed of in massive piles covering thousands of hectares (Magoo 1996).

Migratory birds and their habitats have been adversely affected as a result of mining and mining-related activities. Potential hazards to wildlife feeding on the edges of these highly disturbed areas or in associated contaminated aquatic habitats have not been well documented. Soils surrounding the mining-impacted areas have been severely contaminated. The landscape is marked with mining refuse and sinkholes where ground has given way and fallen into subterranean mines. The most contaminated areas tend to be poor wildlife habitat, which are either barren or sparsely populated with invasive plant species that have replaced native vegetation. Although the mining refuse and adjacent soils contain high concentrations of metals, much of the contamination is in the form of primary ores, which are likely to have low bioavailability to animals.

Much of the area is drained by the Spring River. Metals associated with the mining process have contaminated waters (KDHE 2005) and sediment (Pope 2005; Juracek 2005), thus causing toxic effects in fish (Schmitt et al. 1993) and limiting the population (Wildhaber et al. 2000) of the Neosho madtom (*Noturus placidus*), a species of fish federally listed as threatened (55 Federal Register 21148, May 22, 1990). Angelo et al. (2007) associated depauperate Unionid mussel communities with increasing metal concentrations in the Spring River downstream of the confluence with Turkey Creek.

A textbook on waterfowl (Phillips and Lincoln 1930) reported deaths of many mallards (*Anas platyrhynchos*), pintails (*A. acuta*), and teal (*A. crecca*) on Spring River, near Riverton, KS, in 1923. The deaths were attributed to lead poisoning from sediments contaminated with mining waste. Recent work has demonstrated zinc poisoning in individual waterfowl from the District. Sileo et al. (2003) diagnosed zinc poisoning in three Canada geese (*Branta canadensis*) and a mallard from the District. Diagnoses were based on the finding of mild to severe degenerative abnormalities of the exocrine pancreas associated with hepatic and pancreatic zinc concentrations known to be toxic. Carpenter et al. (2004) diagnosed zinc poisoning in a trumpeter swan (*Cygnus buccinator*) after it had been observed for 4 weeks on a millpond approximately 5 km east of Picher. The postmortem diagnosis was based on highly increased concentrations of zinc in its blood, liver, and kidneys and on pancreatic lesions. Beyer et al. (2004) also described lead and zinc poisoning in several species of wild birds collected from the

Tri-State Mining District. Lead poisoning was diagnosed in American robins (*Turdus migratorius*), northern cardinals (*Cardinalis cardinalis*), and waterfowl based on increased lead concentrations in tissues as compared with reference birds. Several birds had tissue concentrations of lead that have been associated with impaired biological functions and external signs of poisoning. In addition, mean activities of the lead-sensitive enzyme delta-aminolevulinic acid dehydratase (ALAD) were decreased by >50% in red blood cells in these birds. Zinc concentrations in liver, pancreas, and kidney of waterfowl were significantly higher than reference values, and consistent with those associated with death in two experimental studies on mallards (Gasaway and Buss 1972; Levengood et al. 1999).

In summary, contamination resulting from mining and mining-related activities in the District has impacted migratory birds. Several species of birds have been shown to be injured from the exposure to lead, as determined by death or the inhibition of ALAD. A total of five (5) waterfowl collected from a limited area within the District were also shown to be injured by exposure to excessive quantities of zinc. This study is intended to determine if the previous results of zinc poisoning were limited to the birds that were previously collected or if lead and zinc toxicosis is an on-going and more widespread phenomenon.

## **Methods**

### Collection

#### *Soils/sediments*

Surface (upper 10 cm) soil and sediment samples (500 g) will be collected from at least four disturbed sites and four sites without apparent disturbance in the study area. Sampling will be systematic, using a standard scoop (containing about 50 g per scoop), every 5 m along a central transect traversing the core of the sampled area until 500 g or more combined sample is collected, away from the margins and boundaries of identified disturbed and undisturbed areas. Aquatic sediment samples will be collected from depths less than 60 cm to coincide with typical waterfowl foraging depths.

#### *Birds*

Canada geese and mallard ducks will be collected in late winter before the onset of spring migration. Birds will be collected either by shotgun using non-toxic steel shot, or by rocket net.

### Sample preparation

#### *Soils/sediments*

Since not all soil and sediment constituents are bioavailable, soil and sediment samples will be subjected to serial extractions to obtain elemental concentrations that are biologically relevant (Milton et al. 2002). Dried soil and sediment samples will be thoroughly mixed, gently crushed to reduce large clumps, and then sieved through a 1 mm sieve to screen out large particles. 0.5 g of the samples will then be sequentially extracted in 10 ml of ultrapure water, 0.1 M CaCl<sub>2</sub> and

70 % nitric acid (adapted from Milton et al. 2002). Extractions will be done as follows: suspensions of soil/sediment will be agitated in water for 1 h, then centrifuged for 30 min at 10,000 rpm. The supernatant will then be drawn off and diluted in 1 % nitric acid up to a volume of 25 ml (or more if necessary to reduce total dissolved solids to <1 %). The procedure will be repeated for the next sequential solvent until all three sequential extractions are completed. A separate 0.5 g soil/sediment sample will be acid digested according to a method adapted from the Kansas State Veterinary Diagnostic Laboratory (KSVDL) SOP # 2075. In short, the sample will be placed into a 50 ml polypropylene tube, mixed with 3 ml of ultrapure water and 4 ml of 70 % nitric acid, capped and heated (HotBlock™, Environmental Express, Mt. Pleasant, SC) at 105 °C for 3 hours, then diluted in 1 % nitric acid up to a volume of 25 ml (or more if necessary to reduce total dissolved solids to <1 %).

#### *Birds-Tissue for chemical analysis*

Tissue samples (5 g or more of liver, kidney, blood, pancreas, muscle, femur, brain, and possibly breast feathers) will be harvested during necropsy, placed into specially cleaned glass containers with teflon-lined lids, and frozen at -20 °C until further processing. Tissues will be acid digested using standard operating procedures used in the KSVDL for metals analysis in tissues (KSVDL SOP # 2075). One gram of homogenized sample tissue will be placed into a 50 ml polypropylene tube, mixed with 3 ml of ultrapure water and 4 ml of 70 % nitric acid, capped, and heated (HotBlock™, Environmental Express, Mt. Pleasant, SC) at 105 °C for 3 hours, then diluted in 1 % nitric acid up to a volume of 25 ml (or more if necessary to reduce total dissolved solids to <1 %).

Blood samples for the determination of delta-aminolevulinic acid dehydratase (ALAD) activity will be collected in lithium-heparin-lined syringes and frozen in liquid nitrogen (Burch and Siegel 1971). Hematocrit samples will also be collected.

#### Sample analysis

##### *Birds-General health at necropsy*

The birds will be necropsied following the procedure outlined in the Avian Disease Manual (Charlton et al. 2000). Their body condition and any macroscopic lesions will be recorded. Samples of all abnormal tissues and organs will be fixed in 10% buffered neutral formalin for histologic examination. In addition, samples of the following tissues will be collected and fixed in formalin, regardless of the presence or absence of lesions: all parts of the gastrointestinal tract from the esophagus through the cloaca, trachea, lungs, heart, pancreas, liver, spleen, kidney, adrenal glands, gonads, breast muscle, brain, and bursa of Fabricius.

Samples of feathers, skeletal muscles, kidney, liver, pancreas, and a femur will be collected for chemical analysis as described above.

##### *Tissue-Chemical residue*

ICP-MS analysis (Agilent 7500cx ICP-MS, Agilent Technologies, Wilmington, DE) will follow standard operating procedures developed for multi-element analyses (KSVDL SOP # 2012-2016). Concentrations of environmentally relevant elements, including Fe, K, Ca, Na, Mg, Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, Th, and U, will be estimated in no-gas, hydrogen reaction and helium collision modes as appropriate for each element. Li6, Sc, Ge, Rh, In, Tb, Lu, and Bi will be used as internal standards.

### *Tissue-Histology*

The formalin-fixed tissues will be processed, embedded into paraffin blocks, sections cut and mounted on glass slides, and stained with hematoxylin and eosin by following the standard operating procedure of the KSVDL. The stained tissue sections will be examined and the results recorded by the same pathologist that performs the necropsies.

### *Soil/sediments-Chemical*

ICP-MS analysis will follow the same standard operating procedures used for the analyses on tissue samples.

### **Budget**

Analytical	\$13,680
Histopathology	\$1,500
Materials and supplies	\$1,000
Laboratory assistant	\$3,000
Student assistant	\$1,750
Publication costs	\$500
Misc. costs/contingency	\$1,500
Salary (DCS)	\$1,675
Travel	\$1,070
Subtotal:	\$25,675
University Overhead (48%)	\$12,325
TOTAL requested	\$38,000

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