Adverse health effects in Canada geese (*Branta canadensis*) associated with waste from zinc and lead mines in the Tri-State Mining District

Prepared for the United States Fish and Wildlife Service by

Deon van der Merwe, James W. Carpenter, and Jerome C. Nietfeld

College of Veterinary Medicine, Kansas State University, 1800 Denison Ave, Manhattan, Kansas 66506, USA

ABSTRACT

Cases of lead (Pb) and zinc (Zn) poisoning in birds, including migrating waterfowl, have been reported in the Tri-State Mining District (TSMD), which includes areas of southeast Kansas, northeast Oklahoma, and southwest Missouri. It was hypothesized that the health effects on migrating waterfowl may be more widespread than the effects that are apparent from case reports alone. To characterize the health impact of elements from mine waste on Canada geese (*Branta canadensis*), 28 birds from four contaminated sites and an uncontaminated control site were harvested and examined for physical and physiological evidence of metals poisoning. Environmental samples, including plants, soil, and water were also included in the study. Adverse health effects due to Pb were determined by assessing blood δ-aminolevulinic acid dehydratase (ALAD) enzyme activity. Adverse effects associated with Zn poisoning were determined from histological examinations of pancreas tissues. Detailed necropsies and histopathological examinations were performed on the birds. The concentrations of silver (Ag), aluminum (Al), arsenic (As), barium (Ba), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), Pb, selenium (Se), thallium (Tl), vanadium (V), and Zn were determined by inductively coupled plasma mass spectroscopy (ICP-MS) in bird tissues and environmental samples. Elevated tissue Pb concentrations and inhibited ALAD enzyme activities were consistently found in birds at all mine waste-contaminated sites. Histopathological signs of Zn poisoning, associated with elevated pancreatic Zn concentrations, were found at one of the study sites where relatively high Zn concentrations were present in environmental samples. It is concluded that Canada geese at investigated mine waste-contaminated sites in the TSMD consistently suffer adverse effects due to Pb exposure, while adverse effects due to Zn exposure is present where environmental Zn concentrations are high. These results support the hypothesis that the adverse health effects from Pb and Zn exposure in the TSMD are more widespread than the effects that can be deduced from clinical case reports alone.

CONTENTS **Page**

LIST of TABLES

LIST of FIGURES

INTRODUCTION

The Tri-State Mining District (TSMD) stretches from northern Ottawa County in Oklahoma, through southeast Cherokee County in Kansas, and through southwestern counties in Missouri. The region was heavily mined for lead (Pb) and zinc (Zn) from the late 1800s to the 1970s. Lead and/or Zn poisoning have been recorded in the TSMD in a variety of wild bird species, including mallards (*Anas platyrhynchos*), pintails (*Anas acuta*) and teal (*Anas crecca*), since the early 1900s (Phillips and Lincoln 1930). Recent examples include cases of poisoning in Canada geese (*Branta canadensis*) (Sileo et al. 2003) and a trumpeter swan (Carpenter et al. 2004). Elevated tissue concentrations of Pb have been recorded in species other than birds in the TSMD, including fish (Schmitt et al. 1993, Wildhaber et al. 2000), mussels (Angelo et al. 2007), red-eared slider turtles (*Trachemys scripta*) (Hays and McBee 2007), white-tailed deer (*Odocoileus virginianus*) (Conder and Lanno 1999) and midges (Reynolds and Ferrington 2002).

Lead is associated with a wide range of adverse health effects in birds, and excessive exposures to Pb are frequently lethal to waterfowl (Beyer et al. 1998b). Young birds are most vulnerable (Pinowski et al. 1994). A survey of wild birds in 2001, which included species of waterfowl, upland game birds, and passerines from the TSMD compared to birds from areas not affected by mining activities, revealed significant increases in tissue concentrations of Pb. There were also physiological indications of Pb toxicity in the form of blood ALAD (δ-aminolevulinic acid dehydratase) enzyme inhibition. Other potentially poisonous metals that were elevated in birds within the TSMD included Zn, which was elevated in waterfowl, and cadmium, which was elevated in songbirds (Beyer et al. 2004). Histological lesions associated with Zn-poisoning were observed in three Zn-exposed Canada geese in the TSMD (Sileo et al. 2003). Excessive exposure to cadmium may cause anemia, poor weight gain, and increased mortality rates in birds (Jacobs et al. 1969). Although higher than normal cadmium concentrations are associated with TSMD mine wastes (Perry et al. 2005), and elevated tissue concentrations have been recorded in songbirds during the study by Beyer et al. (2004), adverse health effects in birds have not been specifically associated with cadmium exposure in the TSMD.

The Canada goose is common in the TSMD region and was chosen as a model species for assessing the potential health impacts of elements from mine waste, in particular Pb and Zn, on waterfowl in the TSMD. The area has ample open water, and also has some shelter and food sources,

providing habitat components that attract waterfowl. They tolerate human disturbance and therefore are often found in suburban and agricultural environments such as the TSMD (Conover and Chasko 1985). In the TSMD, Canada geese are found at several accessible surface bodies of water that receive drainage from mine waste-contaminated sites, including the Spring River, farm ponds, and subsidence ponds.

In Canada geese experimentally exposed to lethal concentrations of Pb in the form of Pb shot pellets, clinical signs may be absent before the final stages of poisoning (Cook and Trainer 1966). This observation suggests that the absence of obvious clinical signs is a poor predictor of Pb poisoning in Canada geese. Dependence on the observation of clinical signs to assess the incidence of Pb poisoning in Canada geese will therefore underestimate the true incidence of poisoning. Although credible evidence for the occurrence of Pb poisoning in geese in the TSMD is available, it was hypothesized that the true extent of the poisoning risk was poorly characterized by occasional reports of clinical poisoning. Testing this hypothesis required the sampling of apparently healthy birds to assess the presence of preclinical lesions and other signs of adverse health effects that are not discernible by visual surveillance.

MATERIALS AND METHODS

Sample collection

Birds were collected on the second, third and fourth of June, 2009. A combination of shooting by shotgun with non-toxic steel shot, and entrapment in a temporary enclosure was used. To increase site fidelity, pre-flight juvenile birds were targeted. The sampling of some adult birds was unavoidable due to the tight flocking behavior within family groups (Table 1). Based on the protective behavior of the adult birds toward the pre-flight juveniles, the collected adult birds were presumed to be members of the rearing group of the pre-flight juvenile birds. Four to eight birds per site were collected at four mine waste-contaminated sites, and at a control site outside the TSMD that does not receive drainage from contaminated areas. All collected birds displayed normal behavior at the time of collection. The specific locations of the contaminated sites were as follows (Figure 1):

- A subsidence pond (37.1601˚N; 94.4578˚W), Webb City, MO, hereafter referred to as MO. Three pre-flight juveniles and two adults were collected at this site.
- A pond (36.9875˚N; 94.8536˚W), 2 km west of Picher, OK, hereafter referred to as OK. Five pre-flight juveniles were collected at this site.
- The confluence between the Spring River and Short Creek (37.0936˚N; 94.6825˚W), 4.2 km northwest of Galena, KS, hereafter referred to as KSE. Six pre-flight juveniles and two adults were collected at this site.
- The northern bank of Shoal Creek (37.0423˚N; 94.6794˚W), 5.1 km southwest of Galena, KS, hereafter referred to as KSS. Three pre-flight juveniles and one adult were collected at this site.
- The control site was situated at a farm pond (37.4370˚N; 95.1906˚W), 1.6 km northeast of Neosho State Fishing Lake, KS, hereafter referred to as CON. Six pre-flight juveniles were collected at this site.

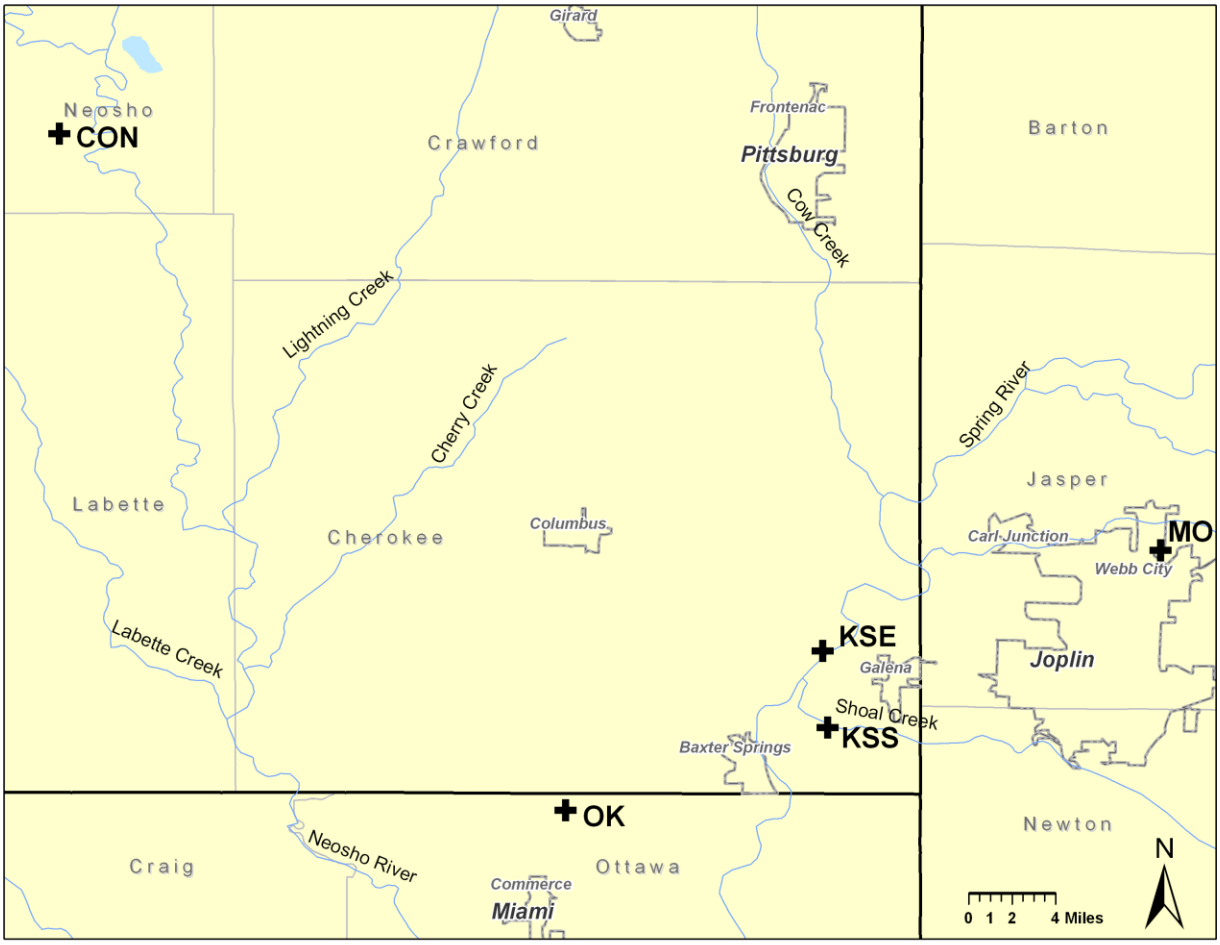

Figure 1. Sample collection locations, including four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON).

Live-captured birds were euthanized by isoflurane overdose, delivered via a facemask. Juvenile ages were estimated using plumage development criteria (Yocom and Harris 1965). The method is based on the timing of the development of down and feathers in terms of color, distribution, feather sheath-break, and feather length. The method's resolution is 7-day intervals. The midpoints of 7-day intervals were used where plumage development completely matched interval descriptions, or were adjusted towards interval boundaries where plumage development was intermediate. Birds were weighed, and blood samples were collected shortly after capture by venipuncture of the jugular vein, the basilica vein, or the medial metatarsal vein using heparinized 3-ml syringes and 25-g needles. A fraction of blood from

each sample was placed into a trace element blood collection tube for assays of packed cell volume (PCV) and element concentrations. The PCVs were measured in duplicate by the microhematocrit method. Microcapillary tubes were filled with blood using capillary action, and then one end of the tubes were plugged with clay. The tubes were centrifuged using a micro-capillary centrifuge at 7,700 rpm for 5 minutes. PCVs were read using a reference chart to estimate the volume of cells as a percentage of blood volume. PCVs were not obtained for some birds due to blood clotting prior to loading onto the centrifuge. The most likely cause for poor blood clotting in some samples was delayed or incomplete mixing between blood and heparin in the collection tubes. A 0.5 ml volume of each sample was placed into a 2-ml cryogenic vial (Fisherbrand, Fisher Scientific), flash-frozen in liquid nitrogen (-196 degees C), and placed in an ultralow freezer (-80 degrees C). Frozen samples were shipped overnight on dry-ice (-78.5^{o}C) to the Miller School of Medicine, University of Miami, Florida, and stored at -70^{o}C until further processing for ALAD analysis.

Birds were necropsied following the procedure outlined in the Avian Disease Manual (Charlton et al. 2000). Body condition and any macroscopic lesions were recorded. Regardless of the presence or absence of lesions samples of the following were collected and fixed in 10% buffered neutral formalin: tongue, esophagus, proventriculus, ventriculus, duodenum, jejunum, ileum, cecum, colon, cloaca, trachea, lungs, heart, pancreas, liver, spleen, kidney, adrenal glands, gonads, breast muscle, brain, bone marrow and bursa of Fabricius. Formalin-fixed tissues were routinely processed, embedded in paraffin, sectioned at 3 µm, mounted on glass slides, and stained with hematoxylin and eosin at the Kansas State Veterinary Diagnostic Laboratory, Kansas State University. The slides were examined by one pathologist and any abnormal findings recorded.

Samples for elemental analysis of the liver, kidney, pancreas, brain, blood, skeletal muscles, a long bone (femur or metatarsus), and the contents of the proventriculus were collected individually in digestion cups (SC475, Environmental Express, Mt. Pleasant, SC), without the addition of preservatives.

Three 50 ml surface water samples per site were collected in digestion cups, at 5 m intervals along the shore, centered at the shore point nearest to the bird collection site. Surface water conductivity, estimated dissolved salt concentration, pH and temperature were recorded at the same sites using a portable pH/conductivity meter (Accumet AP85, Fisher Scientific, Pittsburgh, PA). At each collection site, 20 surface soil samples of equal volume were collected at 1 m intervals at the shoreline, centered at the shore point nearest to the bird collection site, using a hand trowel and combined into a 1-gallon sealable plastic bag (Ziploc™, SC Johnson, Racine, WI).

Three samples of each of the two most dominant plant species at each site, except for trees and woody shrubs, were collected. All above-ground plant parts were included. To ensure that plant samples reflected dietary exposure to geese, including soil or dust accumulated on plant surfaces, plant samples were not washed prior to analysis. Reference specimens of sampled plant species were collected in a plant press for later identification at the Kansas State University (KSU) Herbarium, Division of Biology.

Sample analysis

ALAD was measured as described by Burch and Siegel (Burch and Siegel 1971) and as previously implemented by the University of Miami, FL, in two other published investigations (Kelly et al. 1998, Mitchell et al. 2001). Briefly, the hemolysate was mixed with a buffered ALA substrate and incubated for 1 hour at 38^{o}C. After addition of a reagent containing N-ethylmaleimide, the resultant mixture was centrifuged and the supernatant was removed for the final color reaction. The final reaction involved mixing the supernatant and Ehrlich's reagent. After 13 minutes, the absorbance was read at 555 nm. The corrected absorbance was determined as the absorbance of the test sample minus that of the blank (the same mixture not subject to the 1 hour incubation). A unit of ALAD activity was an increase in corrected absorbance at 555 nm of 0.100, with a 1.0 cm light path length, and 1 ml red blood cell volume, per hour at 38^{o}C. Activity was normalized for each sample's hematocrit. A duplicate tube of KSE3 was analyzed for quality control purposes and generated similar results to the first tube. Since there was no statistically significant difference between the PCVs of any groups based on Kruskal-Wallis one way analysis of variance on ranks, ALAD activity for samples without specific PCV values was estimated using the mean PCV value from the respective site. One sample was severely clotted and could not be run (KSS1).

Samples for elemental analyses were processed at the Kansas State Veterinary Diagnostic Laboratory. Bird tissues and plant samples were digested in nitric acid. Tissues were analyzed on a wet weight basis, while soil and plants were analyzed on a dry weight basis because the water content of soil and plants are highly variable depending on weather conditions and plant growth stage. The water content of fresh tissues, on the other hand, varies within narrow bounds and wet-weight reference concentrations for waterfowl are available for a variety of relevant elements (Puls 1994). Plants were dried at 100^{o}C for 12 hours prior to preparation. One gram of sample was mixed in a digestion cup (SC475, Environmental Express) with 3 ml ultrapure water (Milli-Q Biocel, Millipore, Bedford, MA) and 4 ml 70% nitric acid (TraceMetal Grade, Fisher Scientific, Pittsburgh, PA), capped and heated (HotBlock™, Environmental Express) at 105 ˚C for 3 hours, then diluted by the addition of 18 ml water (or more if

necessary to reduce total dissolved solids to <1%). Each batch of samples included a blank that was processed using the same method, equipment and reagents as the samples to account for background elemental concentrations.

To obtain an index of plant-bioavailable soil elements, a rhizosphere-based extraction method was used (Feng et al. 2005). Soil samples were dried at 60 ˚C for 48 hours, followed by 100 ˚C for 12 hours, and then sieved through a 1 mm sieve to remove large particles. A rhizoshere extraction solution was prepared by mixing acetic acid (TraceMetal Grade, Fisher Scientific), lactic acid (Certified ACS, Fisher Scientific), citric acid (Certified ACS, Fisher Scientific), malic acid (99 %, Acros Organics, Geel, Belgium) and formic acid (98 %+, Acros Organics) at a 4:2:1:1:1 molar ratio and a total concentration of 10 mM. Soil samples (2 g) were placed into digestion cups and mixed with 20 ml extraction solution on a wrist action shaker (Burrell Model 75, Burrell Corporation, Pittsburgh, PA) on an amplitude setting of 5, for 16 hours. Extracts were then centrifuged at 1,500 rpm for 20 minutes and filtered using 2 µm filters (Filtermate™, Environmental Express). Five ml of supernatant was removed and mixed with 5 ml of 2% nitric acid.

Prepared samples were analyzed by inductively coupled plasma mass spectroscopy (ICP-MS) (Agilent ICP-MS 7500cx, Agilent Technologies, Wilmington, DE) for a panel of elements including magnesium (Mg), aluminum (Al), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), Zn, arsenic (As), selenium (Se), molybdenum (Mo), silver (Ag), cadmium (Cd), barium (Ba), thallium (Tl), and Pb. Hydrogen reaction and helium collision were used for interference removal and argon dilution was used to extend the range of acceptable total dissolved solids concentrations up to 1 %. Standards for elemental analyses by ICP-MS were obtained from Environmental Express (Mt. Pleasant, SC). High molecular weight elements, including Ag, Cd, Ba, Tl, and Pb were analyzed without the use of reaction cell interference removal. Selenium was analyzed in the hydrogen reaction mode, while the rest of the elements were analyzed in the helium collision mode. To account for sample matrix effects, scandium (Sc), rhodium (Rh), indium (In), lutetium (Lu) and bismuth (Bi) were used as fixed concentration internal standards in all samples and calibration standards. Quantification was achieved by measurements of the count rate ratios between internal standards and the elements of interest, and based on linear regression models derived from a series of seven standards ranging in element concentrations from 0 µg/L to 1 mg/L. Element concentrations in blanks were used to correct sample-measured concentrations for background contaminants associated with analytical reagents and processes. System stability was verified by analysis of 10 µg/L and 100 µg/L

standards at the end of each batch of samples. Acceptable stability was defined as <20% variation between initial and end samples. Limits of detection were defined as three times the background standard deviation of a 10 µg/L standard, derived from seven independent analyses. Limits of quantification were defined as ten times the background standard deviation of a 10 µg/L standard, derived from seven independent analyses.

Soil extracts were associated with very high total dissolved solids. The high degree of dilution needed to prepare such samples for ICP-MS could mask low concentration elements. The elements of primary interest in soil extracts, Pb, Zn and Cd, were therefore analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) at the Kansas State University Soil Testing Laboratory using a Varian 720-es (Varian, Palo Alto, CA). Standards for ICP-OES were obtained from Exaxol Chemical Corporation (Clearwater, FL). Reference samples containing element concentrations of 1 mg/kg, prepared from commercially available standards (Environmental Express), were used for data quality assurance. As for ICP-MS analysis, each batch of samples included digestion/processing blanks, used to correct for background concentrations.

Statistical analysis

Statistical analyses were performed using SigmaPlot Version 11.2, Build 11.2.0.5 (Systat Software Inc., Chicago, IL). Significant differences between multiple groups that passed normality tests were analyzed by one way analysis of variance, except for lead in liver, brain, and muscle because lead concentrations in the control samples of these tissues were below the limit of detection and, therefore, variability around the means could not be estimated for these groups. Where group differences were significant (p-values < 0.05) multiple comparisons were performed using Dunnett's method to identify significant differences of each group versus the control group (Dunnett 1955). Significant differences between groups that failed normality were analyzed by Kruskall-Wallis one way analysis of variance on ranks (Kruskal and Wallis 1952). Where group differences were significant (p-values < 0.05) multiple comparisons were performed using Dunn's method to identify significant differences of each group versus the control group (Dunn 1964). A separate statistical analysis was performed on pre-flight juveniles only, using the same statistical methods. These methods were also used to compare Pb and Zn concentrations in plants. The significance of correlations was quantified using Pearson product moment correlations (PPMC). Error bars in figures indicate standard errors.

RESULTS

The analysis of element concentrations in bird and environmental samples was targeted at Pb and Zn because the existing evidence for adverse health effects was limited to effects attributable to Pb and Zn. Although various other elements, including Cd, Cr, Ag, Ni, Al, As, Tl, Co, Mo, and V were inconsistently elevated depending on the site and tissue, these elements were not associated with tissue concentrations generally associated with toxicity. The concentrations of elements other than Pb and Zn in bird tissues were summarized by population, and are presented in the Appendix (Tables A1-A8). The limits of detection for Pb and Zn were 0.024 mg/kg and 0.121 mg/kg, respectively. The limits of quantification for Pb and Zn were 0.079 mg/kg and 0.402 mg/kg, respectively. The limits of detection and limits of quantification for all reported elements are presented in the Appendix (Table A9).

At necropsy, all birds were in good body condition with normal muscling and adequate subcutaneous and internal body fat. They appeared healthy and none contained significant gross pathology. Microscopic lesions are summarized in Table 1. There were no consistent differences in microscopic lesions between pre-flight juvenile and adult birds. Pancreases of five of the eight birds from the KSE site, including two adults and three juveniles, contained multiple areas where exocrine epithelial cells displayed degenerative changes. In affected areas, exocrine cells contained variably-sized, clear vacuoles, and many cells were smaller, disorganized, and lacked or contained decreased quantities of zymogen granules in comparison to normal pancreas. Individual and small groups of pyknotic cells were also present (Figures 2-3). In geese KSE3, KSE4 (Figure 3), and KSE7 the lesions involved large coalescing areas of pancreatic tissue. Pancreases from geese KSE4 and KSE7 also contained areas of interstitial fibrosis associated with the degenerative changes. The pancreas from goose KSE1 contained a few scattered small areas with vacuolated exocrine cells but no other significant changes. The pancreases of geese from all the other sites were normal. The most common histologic changes in organs other than the pancreas were associated with parasitism. Parasitism was present in mine waste-exposed birds and control birds. The patterns appeared to be random, indicating that parasitism was not correlated with exposure to mine waste. Kidneys of 14 juveniles had multifocal tubulointerstitial inflammation containing developing stages of coccidia compatible with *Eimeria truncate* (T. P. Brown, 1996). One adult and 12 juveniles had developing stages of coccidia in the small intestine. Cross sections of trematodes were present in vessels in adrenal glands or the connective tissue surrounding the adrenals and the gonads of two adults and one juvenile. Trematode ova

surrounded by granulomatous inflammation were present in the liver of one juvenile. Viable and mineralized trematode ova surrounded by granulomatous inflammation were present in the mucosal, submucosal, and muscular layers, and occasionally on the serosa, of the large intestine of five juveniles. Within the muscular wall of the ventriculus of two juveniles, and one adult, were areas of necrosis and chronic inflammation containing cross sections of nematodes. Although nematodes were not found, the ventriculi of two additional juveniles contained foci of inflammation and necrosis that were similar to the lesions in the birds with nematodes. Other lesions consisted of multifocal, mild, chronic inflammation of various organs of several birds.

Bird weight, sex, age, PCV, blood ALAD activity, and ALAD activity as a percentage of the control average are summarized in Table 2. The average blood ALAD activity in control birds was 31.0 units (range 22.8-40.6, SE 3.09, n = 6), while the average for mine waste site birds was 11.8 units (range 0.1-14.7, SE 1.03, n = 20). The degree of inhibition of ALAD activity at the mine waste sites, expressed as a percentage derived from the equation: control average – population average)*100, was 97.1 % (SE 0.8%) at the MO site, 78.2% (SE 2.1%) at the OK site, 70.7% (SE 5.8%) at the KSE site, and 82.9% (SE 3.2%) at the KSS site. ALAD inhibition at mine waste sites was not correlated with age in juvenile birds (PPMC coefficient: -0.0683; p-value: 0.809). When analyzed as a group, the degree of inhibition of ALAD activity in pre-flight juveniles (excluding adult birds) at the mine waste sites was 97.2 % (SE 1.3%) at the MO site, 78.2% (SE 2.1%) at the OK site, 66.1% (SE 6.7%) at the KSE site, and 79.7% (no SE; n=1) at the KSS site. The pattern of ALAD inhibition, therefore, remained the same when adults were included or excluded. Decreasing blood ALAD activities were correlated with increasing tissue Pb concentrations in all tissues except muscle, when all birds were included in the analysis, and when pre-flight juveniles were analyzed separately (Table 2). The highest degree of correlation occurred between blood ALAD and bone Pb. The relationship between blood Pb and ALAD activity, including all birds, could be described using a power equation with an R^2 value of 0.627:

$$y = 3.0018x^{-1.113}$$

where *y* is the ALAD activity expressed as a percentage of the average control activity, and *x* is the blood Pb concentration in μg/ml (Figure 4).

Individual Pb and Zn tissue concentrations are presented in Tables 3 and 4, and the statistical significance of differences between site means, compared to the control site birds, are presented in Table 5. Pb and Zn tissue concentrations in different tissues are also graphically summarized in the Appendix (Figures A1-A8).

Table 1. Histological pathology in Canada geese collected at four mine waste-contaminated sites (MO1-5; OK1-5; KSE1-8; KSS1-4) and birds collected at an unexposed control site (CON1-6).

ID	Pancreatic lesions	Coccidia		Flukes	Other pathology and comments
		Renal	Intestinal		
MO1	No	No	No	Yes	Trematode in vessel adjacent to adrenal; chronic orchitis
MO2	No	No	No	Yes	Mild, chronic inflammation in kidney, adrenal, thyroid, and ventriculus; trematode ova in wall of large intestine
MO3	No	No	No	Yes	Mild chronic inflammation in thyroid, adrenal, tongue, and kidney; trematode ova in large intestine
MO4	No	No	Yes	Yes	Mild, chronic inflammation in thyroid, adrenal, lung, and liver; cestode in small intestine; trematodes in colon
MO5	No	No	No	No	Neutrophilic inflammation in lung and tongue
OK1	No	No	No	No	Multifocal heterophilic inflammation in small intestine
OK2	No	Yes	No	No	
OK3	No	Yes	No	Yes	Trematode ova in large intestine
OK4	No	No	No	No	Mild chronic inflammation in lung and kidney
OK5	No	Yes	No	No	Nematode in ventriculus
KSE1	Yes	No	Yes	No	Small foci of vacuolation
KSE2	No	No	Yes	No	
KSE3	Yes	Yes	Yes	No	Large, coalescing areas of vacuolation in pancreatic exocrine cells; pancreatic epithelial cells smaller than normal and multifocally disorganized; and scatttered small groups off mononuclear cells
KSE4	Yes	No	No	No	Nonsuppurative encephalitis; and vacuoles in pancreas exocrine cells and interstitial pancreatic fibrosis
KSE5	No	Yes	Yes	No	Mild, lymphocytic nephritis, meningitis, and encephalitis
KSE6	No	Yes	Yes	No	Nematode in wall of ventriculus
KSE7	Yes	No	No	Yes	Mild inflammation in kidneys, adrenals, testes, and liver; trematode in vessel in adrenal; and multifocal areas of interstitial fibrosis with mature collagen in the pancreas
KSE8	Yes	No	Yes	No	Nematode in wall of ventriculus; multiple areas where pancreatic exocrine cells contain vacuoles
KSS1	No	Yes	Yes	Yes	Trematode in vessel adjacent to adrenal gland; inflammation in wall of ventriculus
KSS2	No	Yes	No	No	
KSS3	No	No	No	No	Infrequent *Sarcocystis* sp. in muscle
KSS4	No	Yes	No	No	Neutrophilic inflammation in the lungs
CON1	No	Yes	Yes	Yes	Trematode ova in liver
CON2	No	Yes	No	No	
CON3	No	No	Yes	No	
CON4	No	Yes	Yes	No	Inflammation and necrosis in ventriculus
CON5	No	Yes	Yes	Yes	Trematode ova in large intestine
CON6	No	Yes	Yes	No	

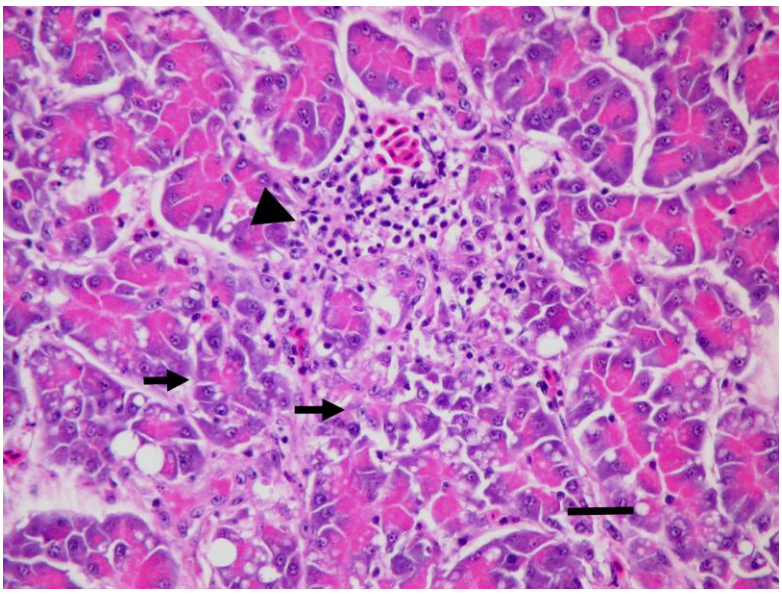

Figure 2. Photomicrograph of pancreas from KSE8 with vacuolation of exocrine epithelial cells, small epithelial cells that lack or have decreased zymogen granules (arrows), and scattered small groups of pyknotic cells (arrowhead). The bar indicates 100 µm.

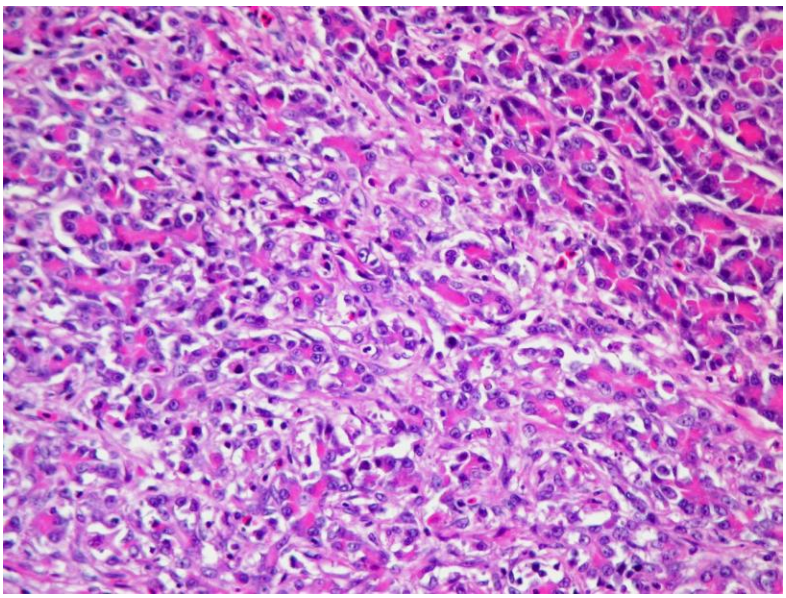

Figure 3. Area of interstitial fibrosis with loss of exocrine glands in the pancreas of goose KSE4.

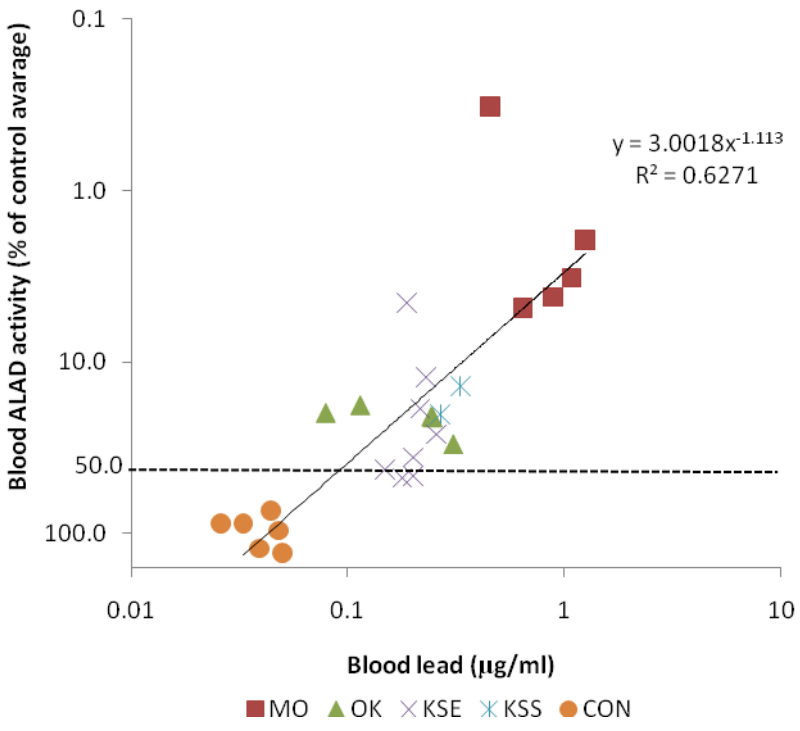

Figure 4. The relationship between blood ALAD activity and blood lead (Pb) concentrations, including all birds.

Table 2. Bird weight, sex, age, packed cell volume (PCV), δ-aminolevulinic acid dehydratase (ALAD) activity units, and ALAD activity as a percentage of the control average in Canada geese collected at four mine waste-contaminated sites (MO1-5; OK1-5; KSE1-8; KSS1-4) and birds collected at an unexposed control site (CON1-6).

ID	Weight (kg)	Sex	Age (days)	PCV (%)	ALAD (units)	ALAD (% of control)
MO1	4.75	Male	Adult	38*	1.3	4.2
MO2	2.32	Male	40	37	1	3.2
MO3	2.38	Male	41	37	1.5	4.8
MO4	2.08	No data	37	38*	0.1	0.3
MO5	4.25	Female	Adult	39	0.6	1.9
OK1	2.57	Male	43	34	9.3	30.0
OK2	3.17	Male	46	38*	6.5	21.0
OK3	3.23	Male	43	35	6.1	19.7
OK4	2.43	Female	46	41	6.4	20.7
OK5	1.51	Female	37	38*	5.5	17.8
KSE1	2.87	No data	47	49	8.2	26.5
KSE2	2.49	Male	43	36	11.2	36.2
KSE3	2.41	Male	40	34	13.2	42.6
KSE4	2.42	Male	37	38	14.4	46.5
KSE5	2.24	Male	41	37	1.4	4.5
KSE6	1.48	No data	33	25	14.7	47.4
KSE7	4.68	Male	Adult	38*	5.8	18.7
KSE8	3.75	Female	Adult	38*	3.8	12.3
KSS1	1.07	Male	25	43	No data	No data
KSS2	1.03	Female	21	31	6.3	20.3
KSS3	4.38	Female	Adult	35	4.3	13.9
KSS4	0.95	No data	21	38*	No data	No data
CON1	2.01	Female	43	43	22.8	73.6
CON2	1.45	Female	36	41	40.6	131.1
CON3	2.29	Male	43	43	27.3	88.1
CON4	2.37	Male	43	41	29.9	96.5
CON5	1.39	Female	34	39	38.1	123.0
CON6	2.18	Male	42	42	27.2	87.8

* Measured PCV not available; average PCV was used

Table 3. Tissue Pb concentrations in Canada geese collected at four mine waste-contaminated sites (MO1-5; OK1-5; KSE1-8; KSS1-4) and birds collected at an unexposed control site (CON1-6). ND values were below the limit of detection (<0.024 mg/kg or <0.024 μg/ml).

ID	Liver (mg/kg)	Kidney (mg/kg)	Pancreas (mg/kg)	Brain (mg/kg)	Blood (μg/ml)	Muscle (mg/kg)	Bone (mg/kg)	Proventriculus contents (mg/kg)
MO1	1.638	6.524	4.536	0.447	0.886	0.051	38.784	11.080
MO2	1.632	6.333	3.442	0.542	1.081	0.381	75.754	5.042
MO3	1.131	5.551	2.167	0.501	0.643	0.112	91.934	7.244
MO4	1.427	8.016	3.398	0.475	0.453	0.143	119.204	8.806
MO5	2.794	8.714	6.872	0.783	1.249	0.098	38.624	No data
OK1	0.350	1.324	0.782	0.235	0.306	0.040	26.094	36.100
OK2	0.400	1.153	0.885	0.205	0.246	<0.024	31.874	38.300
OK3	0.418	1.651	1.191	0.162	0.079	0.024	32.004	49.570
OK4	0.442	1.633	0.869	0.163	0.242	<0.024	29.024	60.180
OK5	0.299	1.305	0.633	0.204	0.114	<0.024	29.544	35.760
KSE1	0.594	0.961	1.088	0.126	0.257	<0.024	16.734	68.450
KSE2	0.263	1.080	0.725	0.796	0.201	<0.024	15.364	54.190
KSE3	0.238	1.214	0.486	0.087	0.149	<0.024	17.464	62.800
KSE4	0.241	1.097	0.456	0.120	0.200	<0.024	24.564	48.580
KSE5	0.025	0.025	0.797	0.085	0.187	<0.024	19.644	63.080
KSE6	0.233	1.055	0.908	0.134	0.179	<0.024	25.674	20.300
KSE7	0.283	1.304	2.444	0.155	0.216	<0.024	21.254	No data
KSE8	0.795	2.704	2.929	0.242	0.230	0.031	47.164	No data
KSS1	0.381	1.524	0.638	0.097	0.588	0.427	22.174	14.700
KSS2	0.554	2.399	1.090	0.281	0.268	<0.024	39.884	18.340
KSS3	1.297	2.604	1.666	0.363	0.331	<0.024	28.244	2.723
KSS4	0.438	1.401	0.585	0.077	No data	<0.024	24.174	No data
CON1	0.213	0.070	0.049	<0.024	0.044	<0.024	0.644	No data
CON2	<0.024	0.098	0.062	<0.024	0.050	<0.024	1.345	No data
CON3	<0.024	0.064	0.031	<0.024	0.033	<0.024	0.505	1.614
CON4	<0.024	0.79	0.040	<0.024	0.048	<0.024	0.733	No data
CON5	0.028	0.105	0.051	<0.024	0.039	<0.024	1.434	No data
CON6	<0.024	0.058	0.031	<0.024	0.026	<0.024	0.621	No data

Table 4. Tissue Zn concentrations in Canada geese collected at four mine waste-contaminated sites (MO1-5; OK1-5; KSE1-8; KSS1-4) and birds collected at an unexposed control site (CON1-6).

ID	Liver (mg/kg)	Kidney (mg/kg)	Pancreas (mg/kg)	Brain (mg/kg)	Blood (µg/ml)	Muscle (mg/kg)	Bone (mg/kg)	Proventriculus contents (mg/kg)
MO1	38.9	34.4	33.2	10.5	6.2	8.8	234.3	131.6
MO2	35.8	17.1	31.6	9.1	7.7	11.9	113.9	246.9
MO3	47.8	19.9	34.2	10.7	7.6	14.9	250.2	475.5
MO4	41.9	21.3	32.7	9.7	7.0	29.8	207.3	413.8
MO5	96.1	38.6	60.7	10.3	6.6	11.9	599.7	No data
OK1	100.6	26.1	44.0	8.4	8.5	11.1	365.0	209.2
OK2	94.3	22.3	49.3	9.9	6.9	23.5	302.6	278.1
OK3	88.2	24.0	43.0	9.5	22.0	10.2	330.1	242.8
OK4	71.6	22.5	41.1	9.6	5.9	11.2	337.1	297.4
OK5	61.6	19.2	36.0	9.2	5.9	21.6	356.7	229.1
KSE1	147.8	83.2	142.6	10.0	7.8	11.4	466.2	264.5
KSE2	172.8	32.0	212.0	22.3	6.2	17.8	389.7	266.3
KSE3	243.8	33.3	349.5	9.7	6.4	10.5	641.2	500.6
KSE4	154.0	51.8	222.6	17.6	6.4	13.4	609.2	286.8
KSE5	148.0	31.3	262.6	10.5	7.0	13.3	520.7	415.2
KSE6	126.5	47.7	134.7	9.8	6.4	14.8	419.2	230
KSE7	240.3	44.0	182.4	11.1	5.1	11.0	178.1	No data
KSE8	194.3	66.0	193.5	10.3	6.5	12.5	387.0	No data
KSS1	93.3	24.8	46.9	10.1	21.0	24.3	261.8	141.9
KSS2	88.1	27.0	48.0	9.3	6.7	19.6	237.3	176.9
KSS3	63.6	26.5	133.1	10.5	5.8	10.7	233.5	137.2
KSS4	135.2	26.5	47.5	9.9	No data	17.0	342.7	No data
CON1	76.3	26.7	37.6	10.1	6.7	11.9	126.7	No data
CON2	63.5	21.4	35.8	9.4	7.0	14.7	154.7	No data
CON3	66.1	25.3	37.4	10.2	6.8	18.5	131.6	8.5
CON4	68.5	27.3	34.4	10.3	7.3	11.8	119.5	No data
CON5	61.0	21.9	38.6	9.6	6.5	11.3	150.8	No data
CON6	72.6	28.7	39.4	9.5	4.9	13.5	124.83	No data

Table 5. Statistical significance of multiple comparisons of differences between lead and zinc concentrations in bird tissues from mine waste-exposed sites compared to the control site, including all birds, and pre-flight juveniles only, at a confidence level of 95%.

Lead (all birds)

Site	Liver	Kidney †*	Pancreas †*	Brain	Blood †*	Muscle	Bone †*
MO	No test	+	+	No test	+	No test	+
OK	No test	-	-	No test	-	No test	+
KSE	No test	-	+	No test	-	No test	-
KSS	No test	-	-	No test	-	No test	-

Zinc (all birds)

Site	Liver ‡•	Kidney †*	Pancreas †*	Brain †	Blood †	Muscle ‡	Bone †*
MO	-	-	-	-	-	-	-
OK	-	-	-	-	-	-	+
KSE	+	+	+	-	-	-	+
KSS	-	-	-	-	-	-	-

Lead (juveniles)

Site	Liver	Kidney †*	Pancreas †*	Brain	Blood †*	Muscle †*	Bone †*
MO	No test	+	+	No test	+	No test	+
OK	No test	+	-	No test	-	No test	+
KSE	No test	-	+	No test	-	No test	-
KSS	No test	-	-	No test	-	No test	-

Zinc (juveniles)

Site	Liver ‡ •	Kidney †*Δ	Pancreas †*	Brain †	Blood †	Muscle ‡	Bone †*
MO	-	-	-	-	-	-	-
OK	-	-	-	-	-	-	-
KSE	+	-	+	-	-	-	+
KSS	-	-	-	-	-	-	-

† = Kruskal-Wallis one way analysis of variance on Ranks

* = Multiple Comparisons versus Control Group (Dunn's Method)

‡ = Parametric One Way ANOVA

• = Multiple Comparisons versus Control Group (Dunnett's Method)

Δ = Difference greater than expected by chance using Kruskal-Wallis one way analysis of variance on Ranks, but the group(s) responsible for the difference compared to control is (are) not identifiable by Dunn's Method

Pb concentrations were consistently elevated in tissues from birds at mine waste-contaminated sites compared to the control site. Statistical significance was demonstrated the MO, OK, and KSE sites (Table 5). Statistical comparisons of group means of Pb concentrations with controls could not be performed in liver, brain, and muscle because the Pb concentrations in the control samples were below the limit of detection. However, Pb concentrations in liver and brain samples from mine waste sites were one to two orders of magnitude higher than the limit of detection (Table 3), indicating considerable Pb accumulation in these tissues compared to the controls. Tissue Pb concentration elevations at the KSS site, compared to control, was consistent across tissues except for muscle. Lack of statistical significance of tissue Pb concentration elevations at the KSS site compared to the control site should be interpreted with caution because the power of statistical tests to resolve differences between groups was limited by relatively low sample numbers at the KSS site (n=4).

Blood Pb concentrations were not correlated with PCVs (PPMC coefficient: -0.0165; p-value: 0.943).

Average pancreas Pb concentrations from the KSE site ranked second highest amongst the four exposure sites, while it ranked third highest in most other tissues. Moreover, Pb concentration elevations in pancreas tissues were consistently significant at the KSE site, whether all birds or pre-flight juveniles were included in the analysis (Table 5). This pattern occurred in association with high Zn concentrations and Zn-associated pancreatic lesions at the KSE site (Figures 2-3; Tables 1 and 4).

Pancreas, liver, and bone Zn concentrations from the KSE site were consistently and significantly elevated compared to the control site, and also in kidney when all birds were included in the analysis (Table 5). The only other site where significant Zn elevations occurred in tissues was the OK site, where Zn elevation was significant in bone when all birds were included in the analysis. Two birds from the KSE site (KSE3 and KSE7) had liver Zn concentrations in excess of 200 mg/kg, which is the reference concentration used to confirm acute Zn poisoning in domestic poultry (Puls 1994).

Proventriculus contents were absent in the following birds: MO5, KSE7, KSE8, KSS4, CON1, CON2, CON4, CON5, and CON6. Proventriculus Pb and Zn concentrations were not positively correlated with tissue concentrations, as illustrated by PPMC coefficients for proventriculus and liver concentrations, including all birds, of -0.472 (p-value: 0.0415) for Pb, and 0.369 (p-value: 0.132) for Zn.

As illustrated by the covariance between tissues (Table 6), relative Pb concentrations in bird tissues, with the exception of muscle, were consistent. Moreover, tissue concentrations (again with the exception of muscle) were negatively correlated with blood ALAD activity. These covariance patterns remained similar when adults were excluded from the analysis.

Table 6. Pearson product moment correlation coefficients (PPMC), and their associated p-values, for correlations between tissue lead concentrations and blood ALAD activity.

ALL BIRDS								
Muscle	PPMC	0.511						
	p-value	0.00759						
Blood	PPMC	0.57	0.579					
	p-value	0.00238	0.00196					
Brain	PPMC	0.556	0.375	0.755				
	p-value	0.00322	0.059	8.17E-06				
Pancreas	PPMC	0.582	0.347	0.891	0.718			
	p-value	0.0018	0.082	1.04E-09	3.66E-05			
Kidney	PPMC	0.798	0.486	0.892	0.762	0.909		
	p-value	1.02E-06	0.0118	9.1E-10	6.2E-06	1.26E-10		
Liver	PPMC	0.628	0.447	0.944	0.758	0.953	0.954	
	p-value	0.000598	0.0219	5.09E-13	7.26E-06	5.86E-14	4.37E-14	
ALAD	PPMC	-0.677	-0.258	-0.553	-0.539	-0.625	-0.61	-0.564
	p-value	0.000146	0.203	0.00337	0.00453	0.000644	0.000942	0.00267
		Bone	Muscle	Blood	Brain	Pancreas	Kidney	Liver
JUVENILES								
Muscle	PPMC	0.53						
	p-value	0.0136						
Blood	PPMC	0.753	0.883					
	p-value	8.05E-05	1.17E-07					
Brain	PPMC	0.621	0.472	0.633				
	p-value	0.00266	0.0307	0.00208				
Pancreas	PPMC	0.936	0.74	0.867	0.653			
	p-value	4.48E-10	0.000126	3.68E-07	0.00134			
Kidney	PPMC	0.967	0.667	0.817	0.654	0.957		
	p-value	9.17E-13	0.000958	6.27E-06	0.00132	1.24E-11		
Liver	PPMC	0.905	0.772	0.899	0.641	0.965	0.963	
	p-value	1.67E-08	4.12E-05	3.09E-08	0.00175	1.52E-12	2.90E-12	
ALAD	PPMC	-0.698	-0.317	-0.561	-0.505	-0.697	-0.595	-0.607
	p-value	0.000437	0.161	0.00811	0.0197	0.000445	0.00447	0.00355
		Bone	Muscle	Blood	Brain	Pancreas	Kidney	Liver

Water quality parameters are summarized in Table 7. Parameter values from the Spring River and Short Creek, which are both at the KSE site, are reported separately. Water pH values were alkaline at all sites. Among the elements of greatest toxicological interest, Pb (limit of quantification: 0.079 μg/ml) was not detectable in the water at any site. Zinc (limit of quantification: 0.402 μg/ml) was detectable in water at the KSE site at an average concentration of 3.52 μg/ml (SE 0.23). All other elements in water samples were not detectable, or present at concentrations that do not pose a known toxicity risk.

Lead and Zn concentrations in nitric acid and rhizosphere soil extractions are summarized in Table 8.

Table 7. Water quality parameters including conductivity, total dissolved solids (TDS), pH and temperature collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an unexposed control site (CON). Water at the KSE site was collected from the Spring River (Spring) and Short Creek (Short).

	KSE					
Parameter	(Spring)	(Short)	KSS	OK	MO	CON
Conductivity (μS)	395	365	341	196	153.8	251
TDS (ppm)	196	183	168	98	77	124
pH	7.92	8.17	7.95	9.58	8.60	10.67
Temp (˚C)	24.7	25.1	24.8	22.4	24.0	29.7

Table 8. Lead and zinc concentrations in rhizosphere (Rhiz) and nitric acid (Nitric) soil/sediment extracts at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an unexposed control site (CON).

	MO		OK		KSE		KSS		CON	
Element	Rhiz	Nitric	Rhiz	Nitric	Rhiz	Nitric	Rhiz	Nitric	Rhiz	Nitric
Pb (mg/kg)	20.9	22.6	0.5	3.1	2.3	29.6	0.9	6.4	0.3	0.8
Zn (mg/kg)	188.5	233.5	21.4	24.4	516.2	427.8	75.3	81.5	0.6	2.4

Lead and Zn concentrations, using both extraction methods, were higher at all mine waste sites compared to the control site. While the nitric acid extraction method consistently resulted in greater concentrations of Pb compared to the rhizosphere extraction method, the magnitude of the difference was inconsistent, with a PPMC coefficient of 0.518, and a p-value of 0.372, indicating that there was no

significant correlation between the extraction methods across sites. The extracted Zn concentrations were, however, more similar between the two extraction methods. The PPMC coefficient was 0.983, and the p-value was 0.00258, indicating that there was significant correlation between the extraction methods across sites. If, based on an assumption that nitric acid extraction is a closer reflection of total concentrations compared to rhizosphere extraction, the difference between nitric acid extraction and rhizosphere extraction is used as an indicator of bioavailable concentrations, the bioavailability of Pb ranged from 8 % (KSE) to 92% (MO), while the bioavailability of Zn ranged from 25% (CON) to 124% (MO). Results from the nitric acid extraction method were not correlated with tissue concentrations of Pb. The PPMC coefficient between the nitric acid extracted Pb, and kidney Pb, for example, was 0.346; p-value: 0.0711). Nitric acid extraction were, however, correlated with pancreas Zn (PPMC coefficient: 0.797; p-value: <0.0000). The rhizosphere extraction method, however, was significantly correlated with kidney concentrations of Pb (PPMC coefficient: 0.936; p-value: <0.0000) and pancreas Zn (PPMC coefficient: 0.849; p-value: <0.0000). Linear regressions demonstrating the correlations in these examples are presented in Figure 5.

Lead concentrations in plants were highly variable, both within species at the same site and between species and sites. Plant samples with high Pb concentrations (up to 33 mg/kg in a sample from the KSE site) were found at the MO, OK and KSE sites, but due to high variability the differences compared to control were not statistically significant (Figure 6). Average Pb concentrations in plants at each site were, however, correlated with nitric acid soil extracts, but not with rhizosphere soil extracts. Nitric acid extraction and plant Pb had a PPMC coefficient of 0.911 (p-value: 0.0313), while rhizosphere extraction and plant Pb had a PPMC coefficient of 0.180 (p-value: 0.772). Zinc concentrations in plants were less variable within species and between species at the same site. Statistically significant elevations in plant Zn compared to control were found at the KSE site (Figure 7). In contrast to Pb, both nitric acid and rhizosphere soil extraction methods resulted in Zn concentrations that were correlated with average plant concentrations at each site. The strongest correlation score was associated with rhizosphere extraction and plant Zn. Nitric acid extraction and plant Zn had a PPMC coefficient of 0.908 (p-value: 0.0328), while rhizosphere extraction and plant Pb had a PPMC coefficient of 0.969 (p-value: 0.00656).

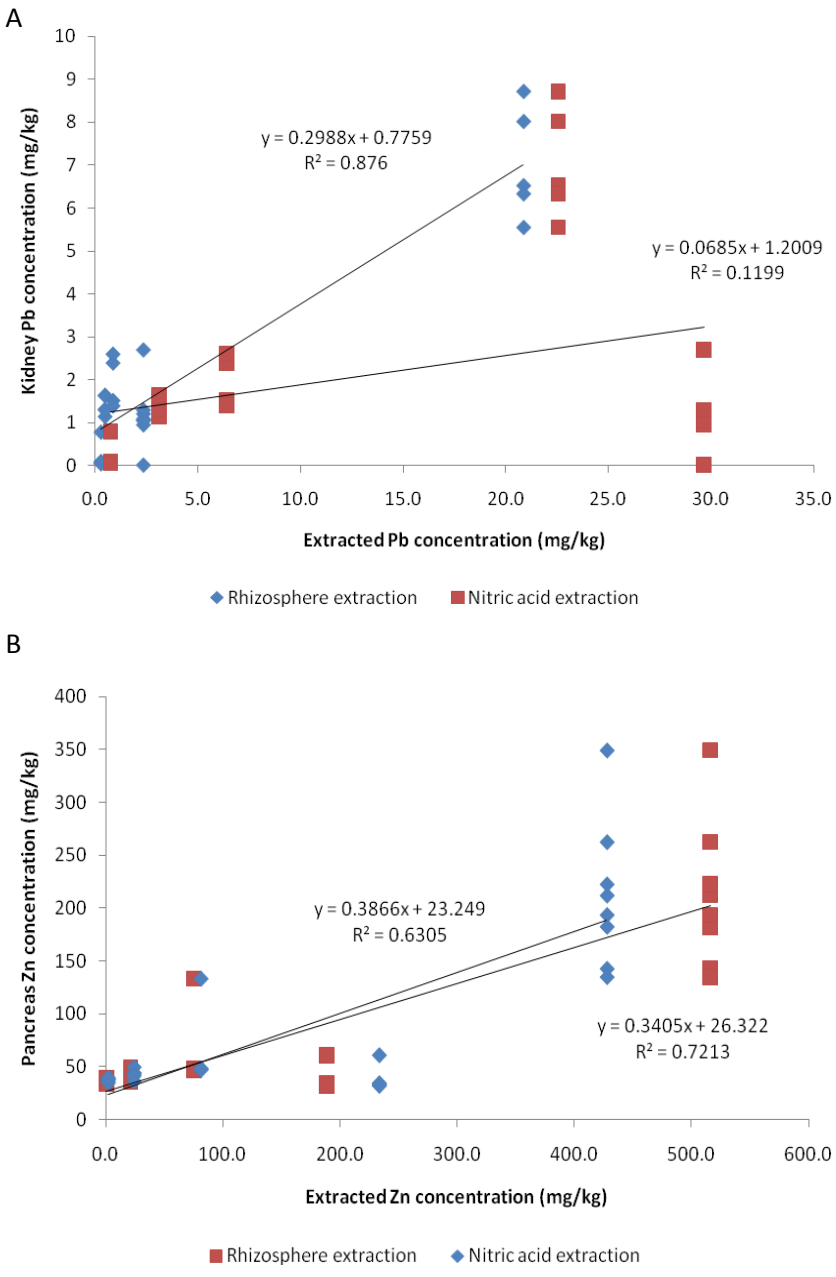

Figure 5. Linear regressions between kidney Pb and soil-extracte Pb (A), and between pancreas Zn and soil-extracted Zn (B), using the rhizosphere and nitric acid extraction methods.

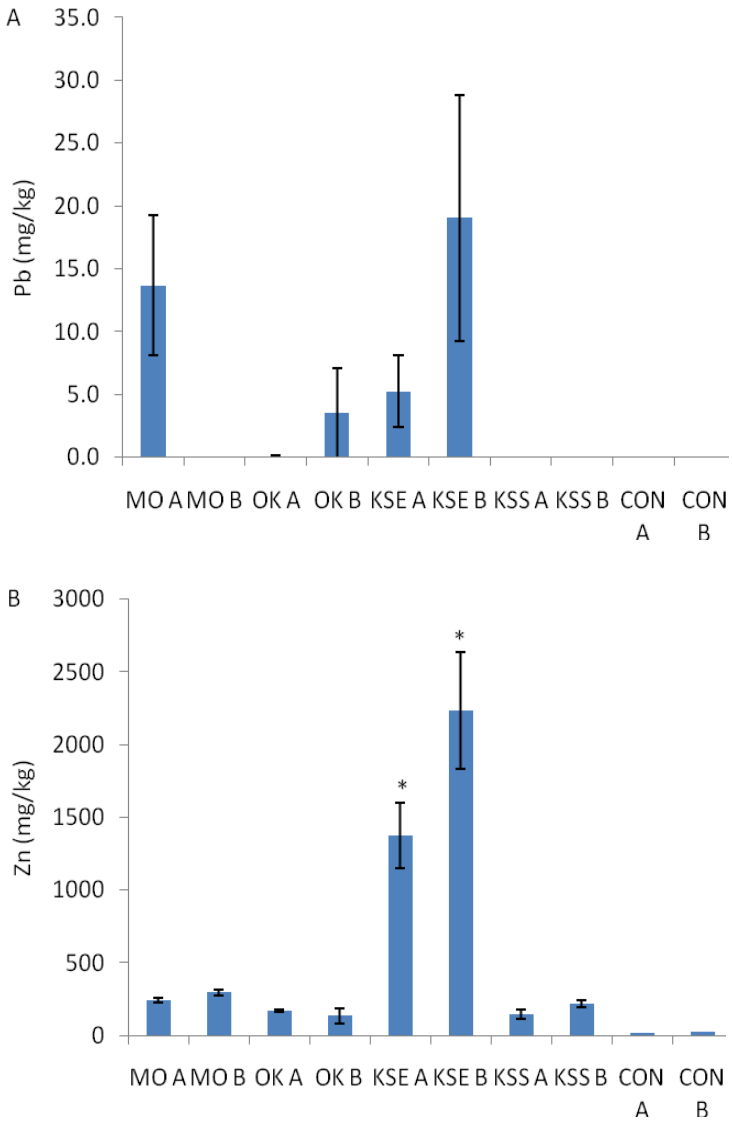

Figure 6. Pb (A) and Zn (B) concentrations in above-ground plant tissues collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) in the Tri-State Mining District and an unexposed control site (CON) (The plants are *Eleocharis palustris* (MO A), *Persicaria hydropiperoides* (MO B), *Trifolium repens* (OK A), *Rorippa palustris* (OK B), *Justicia americana* (KSE A), *Elymus virginicus* (KSE B), *Rudbeckia luciniata* (KSS A), *Elymus virginicus* (KSS B), *Persicaria hydropiperoides* (CON A) and *Eleocharis macrostachya* (CON B).

DISCUSSION

Lead poisoning in wild birds is a common and ongoing problem, often associated with ingestion of Pb shot, as well as urban and industrial environmental contamination (Hutton and Goodman 1980, Mudge 1983, Berglund et al. 2010, Chapa-Vargas et al. 2010, Guitart et al. 2010). Common lesions associated with Pb poisoning in wild waterfowl include impactions of the alimentary tract, submandibular edema, myocardial necrosis, and biliary discoloration in the liver, yet none of these lesions are considered to be pathognomonic (Beyer et al. 1998b). Functional deficits associated with Pb poisoning in birds include abnormal neuromuscular function, kidney failure, liver failure, decreased fertility, and inhibition of heme-biosynthesis enzymes such as ALAD (Eisler 1988, Berglund et al. 2010). Blood ALAD enzyme inhibition is consistently associated with excessive Pb exposure in birds, and the degree of inhibition is correlated with the level of exposure (Hoffman et al. 1981, Eastin et al. 1983, Hirai et al. 1991, Blus et al. 1993, Henny et al. 1994, Scheuhammer 1996, Vanparys et al. 2008). Greater than 50% inhibition in blood ALAD activity is generally regarded as a reliable indicator of Pb exposure (Beyer et al. 2004). Federal regulations (NRDA regulation 43 CFR11.62) define Pb-poisoning injury in wildlife as >50% inhibition in blood ALAD activity of an exposed population compared to an unexposed reference population. Average inhibition rates for exposed populations in the study all exceeded 50%. The correlation pattern observed in the current study, which was an inverse exponential relationship between ALAD enzyme activity and blood Pb concentrations (Figure 1) is similar to correlation patterns observed in previous studies in birds (Vanparys et al. 2008). ALAD inhibition at fine temporal resolutions (days or weeks) that would indicate trends depending on bird age could not be identified. However, when adults are excluded, ALAD inhibition remains >50% in exposed populations. It indicates that birds (pre-flight juveniles), which could not have been exposed to Pb from areas other than the study sites, developed physiological manifestations of Pb-poisoning within their lifetimes of 25-47 days. All Canada geese, adults and juveniles, suffered from adverse physiological effects in the form of blood ALAD inhibition, associated with exposure to Pb at all four mine waste-contaminated sites where birds were collected.

Zn concentrations in tissues from birds at mine waste-contaminated sites were not consistently elevated compared to controls. Significant elevations in Zn concentrations were found only in pancreas samples from the KSE site, and in bone samples from the KSE site (where it was the highest) and the OK

site. Pancreatic lesions consistent with Zn poisoning were observed at the KSE site only (Table 2). In addition, two birds from the KSE site had liver Zn concentrations above concentrations accepted as confirmatory for Zn poisoning in domestic poultry. The relevance of reference concentrations developed for domestic poultry to Canada geese is, however, not established. Average kidney Zn concentrations were highest at the KSE site, but the average was not statistically different from the control average. Zn concentrations were also highest at the KSE site in water, plants, and soil. Cd may increase liver and kidney Zn concentrations in poultry (Puls 1994), and at the study sites cadmium was elevated in liver, kidney, pancreas, and bone compared to control, with the highest elevations found at the KSE, KSS, and MO sites. Zn is an essential nutrient with identified involvement in the functioning of over 300 enzymes. It is effectively regulated in the body, partially through well described interactions with metallothioneins (Lindh 2005). Given the regulation potential for Zn, it can be hypothesized that significant Zn accumulation in tissues indicate extremely high levels of exposure to Zn.

Microscopic pancreatic lesions, which were evident in birds from the KSE site, were indicative of Zn poisoning. Lesions including vacuolation, disorganization and lack of zymogen granules in the exocrine cells, and interstitial fibrosis, similar to that found in 4 of 8 birds from the KSE site, were previously reported in waterfowl and other wild birds from this area with Zn toxicity (Sileo et al. 2003, Beyer et al. 2004). Similar pancreatic changes occur in poultry with selenium deficiency (Goodwin 1996), but tissue selenium concentration of birds at the KSE site were not significantly different from those in birds in the other groups. A fifth bird, KSE1 had small, widely scattered foci where exocrine cells were vacuolated but otherwise normal. This could be the result of early or borderline Zn toxicity, but it could also be incidental because pancreatic vacuolation without other degenerative changes is a common finding in healthy poultry (Goodwin 1996). Overall, the co-occurring of elevated Zn with pancreatic lesions is indicative of Zn poisoning and is consistent with injury documented in other studies in the TSMD (Sileo et al. 2003). No other causes for pancreas fibrosis were apparent.

Microscopic examination also revealed that many birds were infected with one or more parasites. The pathologic changes were minor, in that they did not result in grossly visible pathology, and were unlikely to have caused overt clinical problems in any of the birds. Intestinal and renal infections with coccidia are common in waterfowl, but infection severe enough to cause clinical disease and/or death is uncommon (Anonymous 1999b, 1999c). Evidence of trematode infection was found in nine birds. The location of adult trematodes in blood vessels and ova in the liver and all layers of the

large intestine are consistent with typical findings associated with infection by shistosoma genera. Shistosomes are common in waterfowl, but because of their small size, location in blood vessels, and lack of visible pathology, they are not often seen at necropsy and are only rarely associated with clinical disease (Wojcinski et al. 1987). The nematodes in the wall of the ventriculus of three geese were most likely either *Amidostomum* sp or *Epomidiostomum* sp, which are the two most common gizzard worms affecting waterfowl (Anonymous 1999a). Both can cause debilitation and even death with severe infections, but in histologic sections from all three birds only a single nematode was found, suggesting that infections were mild. Although there did not appear to be any association between parasitism and exposure to mine waste, relatively weak associations between mine waste-exposure and parasites would not be resolved in this study because of low sample numbers and the qualitative nature of parasite-associated lesions.

Canada geese are selective grazers. Their diet includes a variety of low-growing plant types, with a preference for grass. Nutritional value and secondary metabolite concentrations are factors in plant selection by both juveniles and adults (Buchsbaum et al. 1984, Buchsbaum and Valiela 1987). Goose feeding habits result in incidental soil ingestion, which can constitute up to 9% of the diet (Beyer et al. 1998a). Apart from exposure to Pb and Zn in food plants and water, soil ingestion may therefore be a significant risk factor in birds that reside on contaminated substrates. The effects of soil characteristics on metal bioavailability can be highly significant, to the extent that total soil metals are not necessarily predictive of exposure (Davis 1993, Pierzynski and Schwab 1993, Gulson et al. 1994, Bradham et al. 2006). Bioavailability from food plants is further complicated by the fact that plants differ in the rates at which they accumulate soil metals (Pierzynski and Schwab 1993, Pichtel et al. 2000, Kim et al. 2010). The bioavailability of soil metals to plants is related to the solubility of metals in the rhizosphere, which is influenced by organic acids secreted into the rhizosphere by plant roots (Feng et al. 2005, Kim et al. 2010). Different plant species produce different rhizosphere characteristics, which are related to differences in the bioavailability of soil metals between plant species (Kim et al. 2010). Consequently, there is no universally suitable extraction method for predicting bioavailability and different extraction methods have relevance to different situations and predictive goals (Feng et al. 2005). There are, however, important similarities between plants due to similarities in rhizosphere acids (Kim et al. 2010). Thus, it can be argued, rhizosphere extraction methods such as the one used in this study can be generally predictive of bioavailability for groups of plants. The best, statistically significant correlation

was observed between rhizosphere-extracted soil Zn and plant Zn. The rhizosphere-extracted Pb was, however, not correlated with plant Pb, which indicates larger inter-species differences in Pb accumulation by plants from the rhizosphere. Since geese consume a variety of plants, a rhizosphere extraction method can provide an approximation of Pb and Zn exposure through plant ingestion, although the success of this method of prediction is expected to be more successful for Zn than for Pb. Nevertheless, the most accurate predictions of risk from plant ingestion will be derived from studies on specific food plants. To achieve this goal the feeding behavior of Canada geese in the Tri-State District needs to be thoroughly characterized to identify the most important food plants at different time periods and life stages of the birds.

The lack of correlation between proventriculus content element concentrations and tissue concentrations indicates that proventriculus contents did not represent exposure over time periods of several weeks or more. This observation is expected when the potential variability of the diet and the variability in element uptake between plants are considered. The nitric acid extraction method used in this study is not a true total digestion method, and comparisons between nitric acid extraction and rhizosphere extraction should therefore not be used as a true bioavailability index, but only as a comparative index of bioavailability.

Since the environmental contamination in the study area is associated with substrates contaminated with mine wastes, an index of bioavailability from substrates that is correlated with goose tissue concentrations is potentially useful. In the context of this study, the most directly relevant index of bioavailable elements in the environment is bird tissue concentrations because tissue concentrations reflect the true bioavailability to geese from all sources of exposure. To create such an index, extraction methods were compared to average kidney Pb and average pancreas Zn concentrations from each site. Kidney was chosen because it is commonly used to derive clinically relevant Pb concentrations in birds and other animals, while the pancreas is a clinically useful tissue for determining Zn status (Puls 1994). The rhizosphere extraction method, which has been used successfully to predict elemental bioavailability in barley (Feng et al. 2005), was used because Canada geese have a preference for grasses (Conover and Chasko 1985). The rhizosphere extraction patterns for Pb and Zn were significantly correlated with Pb and Zn concentrations found in bird tissues, indicating that the rhizosphere extraction method may serve as an indicator of goose-bioavailable Pb and Zn in soil in the study area Figure 5). As in the case of soil metal bioavailability in plants, it should be noted that the potential

effects of differences in soil chemistry between sites may affect the relevance of extraction methods for predicting bioavailability to Canada geese. Further studies need to be conducted on a variety of sites before it can be assumed that this method has general applicability.

In conclusion: Canada geese, including adult birds and pre-flight juveniles exposed to varying amounts and forms of Pb and Zn at four mine waste-contaminated sites in the TSMD almost universally suffer adverse health effects associated with Pb exposure, and, at one of the sites where environmental Zn concentrations are high, adverse health effects due to Zn exposure. The adverse health impacts of exposure to mine waste are more widespread than can be deduced from reports of clinical poisoning cases, because signs of adverse health effects due to Pb and Zn are not apparent in affected birds except during the final phases of severe poisoning. The direct relationship between blood Pb concentration and blood ALAD inhibition suggests that bird health will improve if exposure to environmental Pb is reduced.

ACKNOWLEDGEMENTS

We thank John Miesner and Gibran Suleiman of the United States Fish and Wildlife Service for invaluable assistance in planning and sample collection. We thank Snehal Tawde, Shiva Mohandass, Lori Blevins, Ashley Smit, and Elizabeth Prigge of Kansas State University for assistance with sample collection, sample processing and sample analysis. We thank Carolyn Cray of the Division of Clinical Pathology, University of Miami Miller School of Medicine, FL, for assistance with blood ALAD activity assays. Major funding was provided by the Unites States Fish and Wildlife Service. Funding assistance was also provided by the National Institutes of Health (NIH T35 RR007064), the Merck-Merial Veterinary Scholar Program, and Kansas State University College of Veterinary Medicine.

LITERATURE CITED

ANGELO, R. T., M. S. CRINGAN, D. L. CHAMBERLAIN, A. J. STAHL, S. G. HASLOUER, AND C. A. GOODRICH. 2007. Residual effects of lead and zinc mining on freshwater mussels in the Spring River Basin (Kansas, Missouri, and Oklahoma, USA). Science of The Total Environment 384: 467-496.

ANONYMOUS. 1999a. Gizzard worms.*In* Field manual of wildlife diseases, E. A. Ciganovich, (ed.). U.S. Department of the Interior, U. S. Geological Survey, Washington D.C. pp. 235-239.

ANONYMOUS. 1999b. Intestinal coccidiosis.*In* Field manual of wildlife diseases, E. A. Ciganovich, (ed.). U.S. Department of the Interior, U. S. Geological Survey, Washington D.C. pp. 207-213.

ANONYMOUS. 1999c. Renal coccidiosis.*In* Field manual of wildlife diseases, E. A. Ciganovich, (ed.). U.S. Department of the Interior, U. S. Geological Survey, Washington D.C. pp. 215-218.

BERGLUND, Å. M. M., P. K. INGVARSSON, H. DANIELSSON, AND N. E. I. NYHOLM. 2010. Lead exposure and biological effects in pied flycatchers (Ficedula hypoleuca) before and after the closure of a lead mine in northern Sweden. Environmental Pollution 158: 1368-1375.

BEYER, W. N., D. J. AUDET, A. MORTON, J. K. CAMPBELL, AND L. LECAPTAIN. 1998a. Lead exposure of waterfowl ingesting Coeur d'Alene River Basin sediments. Journal of Environmental Quality 27: 1533-1538.

BEYER, W. N., J. DALGARN, S. DUDDING, J. B. FRENCH, R. MATEO, J. MIESNER, L. SILEO, AND J. SPANN. 2004. Zinc and lead poisoning in wild birds in the Tri-State Mining District (Oklahoma, Kansas, and Missouri). Archives of Environmental Contamination and Toxicology 48: 108-117.

BEYER, W. N., J. C. FRANSON, L. N. LOCKE, R. K. STROUD, AND L. SILEO. 1998b. Retrospective study of the diagnostic criteria in a lead-poisoning survey of waterfowl. Archives of Environmental Contamination and Toxicology 35: 506-512.

BLUS, L. J., C. J. HENNY, D. J. HOFFMAN, AND R. A. GROVE. 1993. Accumulation and effects of lead and cadmium on wood ducks near a mining and smelting complex in Idaho. Ecotoxicology 2: 139-154.

BRADHAM, K. D., E. A. DAYTON, N. T. BASTA, J. SCHRODER, M. PAYTON, AND R. P. LANNO. 2006. Effect of soil properties on lead bioavailability and toxicity to earthworms. Environmental Toxicology and Chemistry 25: 769-775.

BUCHSBAUM, R., AND I. VALIELA. 1987. Variability in the chemistry of estuarine plants and its effect on feeding by Canada geese. Oecologia 73: 146-153.

BUCHSBAUM, R., I. VALIELA, AND T. SWAIN. 1984. The role of phenolic compounds and other plant constituents in feeding by Canada geese in a coastal marsh. Oecologia 63: 343-349.

BURCH, H. B., AND A. L. SIEGEL. 1971. Improved method for measurement of delta-aminolevulinic acid dehyratase activity of human erythrocytes. Clinical Chemistry 17: 1038-1041.

CARPENTER, J. W., G. A. ANDREWS, AND W. N. BEYER. 2004. Zinc toxicosis in a free-flying trumpeter swan (*Cygnus buccinator*). Journal of Wildlife Diseases 40: 769-774.

CHAPA-VARGAS, L., J. J. MEJIA-SAAVEDRA, K. MONZALVO-SANTOS, AND F. PUEBLA-OLIVARES. 2010. Blood lead concentrations in wild birds from a polluted mining region at Villa de La Paz, San Luis PotosÃ, Mexico. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering 45: 90 - 98.

CHARLTON, B. R., A. J. BERMUDEZ, M. BOULIANNE, D. A. HALVORSON, J. S. SCHRADER, L. J. NEWMAN, J. E. SANDER, AND P. S. WAKENELL. 2000. Necropsy of the Fowl.*In* Avian Disease Manual. The American Association of Avian Pathologists, Kennett Square, Pennsylvania. pp. 37-38.

CONDER, J. M., AND R. P. LANNO. 1999. Heavy metal concentrations in mandibles of white-tailed deer living in the Picher Mining District. Bulletin of Environmental Contamination and Toxicology 63: 80-86.

CONOVER, M. R., AND G. G. CHASKO. 1985. Nuisance Canada goose problems in the eastern United States. Wildlife Society Bulletin 13: 228-233.

COOK, R. S., AND D. O. TRAINER. 1966. Experimental lead poisoning of Canada geese. The Journal of Wildlife Management 30: 1-8.

DAVIS, A. 1993. Micromineralogy of mine wastes in relation to lead bioavailability, Butte, Montana. Environmental science & technology 27: 1415.

DUNN, O. J. 1964. Multiple comparisons using rank sums. Technometrics 6: 241-252.

DUNNETT, C. W. 1955. A multiple comparison procedure for comparing several treatments with a control. Journal of the American Statistical Association 50: 1096-1121.

EASTIN, W. C., D. J. HOFFMAN, AND C. T. O'LEARY. 1983. Lead accumulation and depression of δ-aminolevulinic acid dehydratase (ALAD) in young birds fed automotive waste oil. Archives of Environmental Contamination and Toxicology 12: 31-35.

EISLER, R. 1988. Lead hazards to fish, wildlife, and invertebrates: a synoptic review. U. S. Fish and Wildlife Service. Biological Report 85: 1-14.

FENG, M.-H., X.-Q. SHAN, S. ZHANG, AND B. WEN. 2005. A comparison of the rhizosphere-based method with DTPA, EDTA, $CaCl_2$, and $NaNO_3$ extraction methods for prediction of bioavailability of metals in soil to barley. Environmental Pollution 137: 231-240.

GOODWIN, M. A. 1996. Chapter 7 Alimentary system.*In* Avian histopathology, C. Riddell, (ed.), Kennett Square, Pennsylvania. pp. 111-141.

GUITART, R., M. SACHANA, F. CALONI, S. CROUBELS, V. VANDENBROUCKE, AND P. BERNY. 2010. Animal poisoning in Europe. Part 3: Wildlife. The Veterinary Journal 183: 260-265.

GULSON, B. L., J. J. DAVIS, K. J. MIZON, M. J. KORSCH, A. J. LAW, AND D. HOWARTH. 1994. Lead bioavailability in the environment of children: blood lead levels in children can be elevated in a mining community. Archives of environmental health 49: 326-331.

HAYS, K., AND K. MCBEE. 2007. Flow cytometric analysis of red-eared slider turtles (*Trachemys scripta*) from Tar Creek Superfund Site. Ecotoxicology 16: 353-361.

HENNY, C. J., L. J. BLUS, D. J. HOFFMAN, AND R. A. GROVE. 1994. Lead in hawks, falcons and owls downstream from a mining site on the Coeur d'Alene River, Idaho. Environmental Monitoring and Assessment 29: 267-288.

HIRAI, M., T. KAWAMOTO, AND Y. KODAMA. 1991. Toxic effects of ingested lead shots in domestic fowls. Biological Trace Element Research 30: 291-307.

HOFFMAN, D. J., O. H. PATTEE, S. N. WIEMEYER, AND B. MULHERN. 1981. Effects of lead shot ingestion on delta-aminolevulinic acid dehydratase activity, hemoglobin concentration, and serum chemistry in bald eagles. Journal of Wildlife Diseases 17: 423-431.

HUTTON, M., AND G. T. GOODMAN. 1980. Metal contamination of feral pigeons Columba livia from the London area: Part 1--tissue accumulation of lead, cadmium and zinc. Environmental Pollution Series A, Ecological and Biological 22: 207-217.

JACOBS, R. M., M. R. S. FOX, AND M. H. ALDRIDGE. 1969. Changes in plasma proteins associated with the anemia produced by dietary cadmium in Japanese quail. The Journal of Nutrition 99: 119-128.

KELLY, M. E., S. D. FITZGERALD, R. J. AULERICH, R. J. BALANDER, D. C. POWELL, R. L. STICKLE, W. STEVENS, C. CRAY, R. J. TEMPELMAN, AND S. J. BURSIAN. 1998. Acute effects of lead, steel,

tungsten-iron, and tungsten-polymer shot administered to game-farm mallards. Journal of Wildlife Diseases 34: 673-687.

KIM, K.-R., G. OWENS, R. NAIDU, AND S.-L. KWON. 2010. Influence of plant roots on rhizosphere soil solution composition of long-term contaminated soils. Geoderma 155: 86-92.

KRUSKAL, W. H., AND W. A. WALLIS. 1952. Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association 47: 583-621.

LINDH, U. 2005. Biological functions of the elements.*In* Essentials of medical geology. , O. Selinus, B. Alloway, J. A. Centeno, R. B. Finkelman, R. Fuge, U. Lindh, AND P. Smedley, (eds.). Elsevier Academic Press, Burlington. pp. 115-160.

MITCHELL, R. R., S. D. FITZGERALD, R. J. AULERICH, R. J. BALANDER, D. C. POWELL, R. J. TEMPELMAN, C. CRAY, W. STEVENS, AND S. J. BURSIAN. 2001. Hematological effects and metal residue concentrations following chronic dosing with tungsten-iron and tungsten-polymer shot in adult game-farm mallards. Journal of Wildlife Diseases 37: 459-467.

MUDGE, G. P. 1983. The incidence and significance of ingested lead pellet poisoning in British Wildfowl. Biological Conservation 27: 333-372.

PERRY, P. M., J. W. PAVLIK, R. W. SHEETS, AND R. N. BIAGIONI. 2005. Lead, cadmium, and zinc concentrations in plaster and mortar from structures in Jasper and Newton Counties, Missouri (Tri-State Mining District). Science of The Total Environment 336: 275-281.

PHILLIPS, J. C., AND F. C. LINCOLN. 1930. American waterfowl: their present situation and the outlook for their future. Houghton Mifflin, Boston, MA.

PICHTEL, J., K. KUROIWA, AND H. T. SAWYERR. 2000. Distribution of Pb, Cd and Ba in soils and plants of two contaminated sites. Environmental Pollution 110: 171-178.

PIERZYNSKI, G. M., AND A. P. SCHWAB. 1993. Bioavailability of zinc, cadmium, and lead in a metal-contaminated alluvial soil. Journal of Environmental Quality 22: 247-254.

PINOWSKI, J., M. BARKOWSKA, A. KRUSZEWICZ, AND A. KRUSZEWICZ. 1994. The causes of the mortality of eggs and nestlings of *Passer* sp. Journal of Biosciences 19: 441-451.

PULS, R. 1994. Mineral levels in animal health. 2nd edition. Sherpa International, Aldergrove, British Columbia.

REYNOLDS, S. K., JR., AND L. C. FERRINGTON, JR. 2002. Differential morphological responses of chironomid larvae to severe heavy metal exposure (Diptera: Chironomidae). Journal of the Kansas Entomological Society 75: 172-184.

SCHEUHAMMER, A. M. 1996. Influence of reduced dietary calcium on the accumulation and effects of lead, cadmium, and aluminum in birds. Environmental Pollution 94: 337-343.

SCHMITT, C. J., M. L. WILDHABER, J. B. HUNN, T. NASH, M. N. TIEGER, AND B. L. STEADMAN. 1993. Biomonitoring of lead-contaminated Missouri streams with an assay for erythrocyte δ-aminolevulinic acid dehydratase activity in fish blood. Archives of Environmental Contamination and Toxicology 25: 464-475.

SILEO, L., N. W. BEYER, AND R. MATEO. 2003. Pancreatitis in wild zinc-poisoned waterfowl. Avian Pathology 32: 655-660.

VANPARYS, C., T. DAUWE, K. VAN CAMPENHOUT, L. BERVOETS, W. DE COEN, R. BLUST, AND M. EENS. 2008. Metallothioneins (MTs) and [delta]-aminolevulinic acid dehydratase (ALAD) as biomarkers of metal pollution in great tits (*Parus major*) along a pollution gradient. Science of The Total Environment 401: 184-193.

WILDHABER, M. L., A. L. ALLERT, C. J. SCHMITT, V. M. TABOR, D. MULHERN, K. L. POWELL, AND S. P. SOWA. 2000. Natural and anthropogenic influences on the distribution of the threatened

Neosho madtom in a midwestern warmwater stream. Transactions of the American Fisheries Society 129: 243-261.

WOJCINSKI, Z. W., I. K. BARKER, D. B. HUNTER, AND H. LUMSDEN. 1987. An outbreak of schistosomiasis in Atlantic Brant geese, *Branta bernicla hrota*. Journal of Wildlife Diseases 23: 248-255.

YOCOM, C. F., AND S. W. HARRIS. 1965. Plumage descriptions and age data for Canada goose goslings. The Journal of Wildlife Management 29: 874-877.

APPENDIX

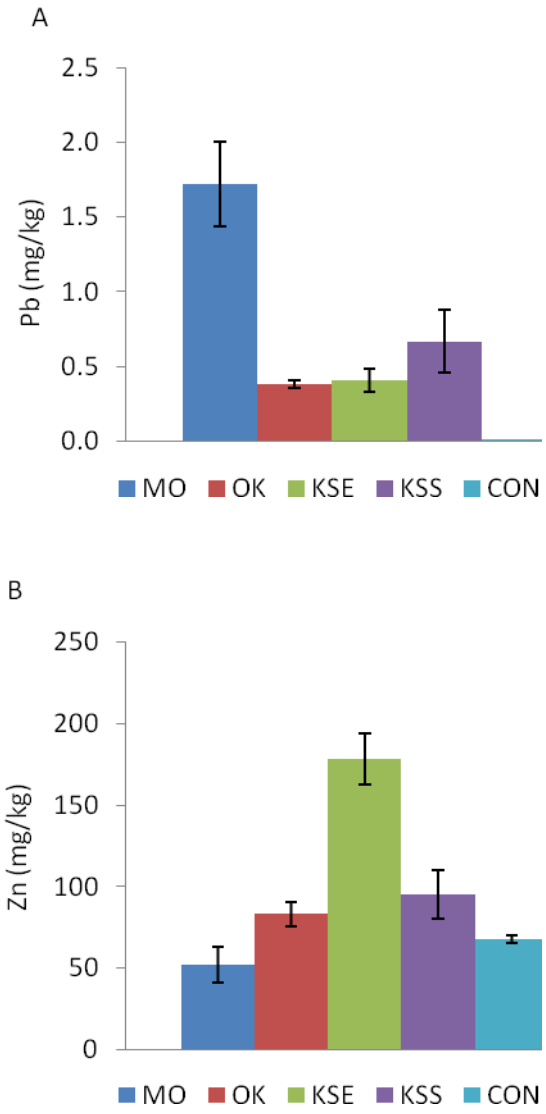

Figure A1. Average liver Pb (A) and Zn (B) concentrations in Canada geese collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON).

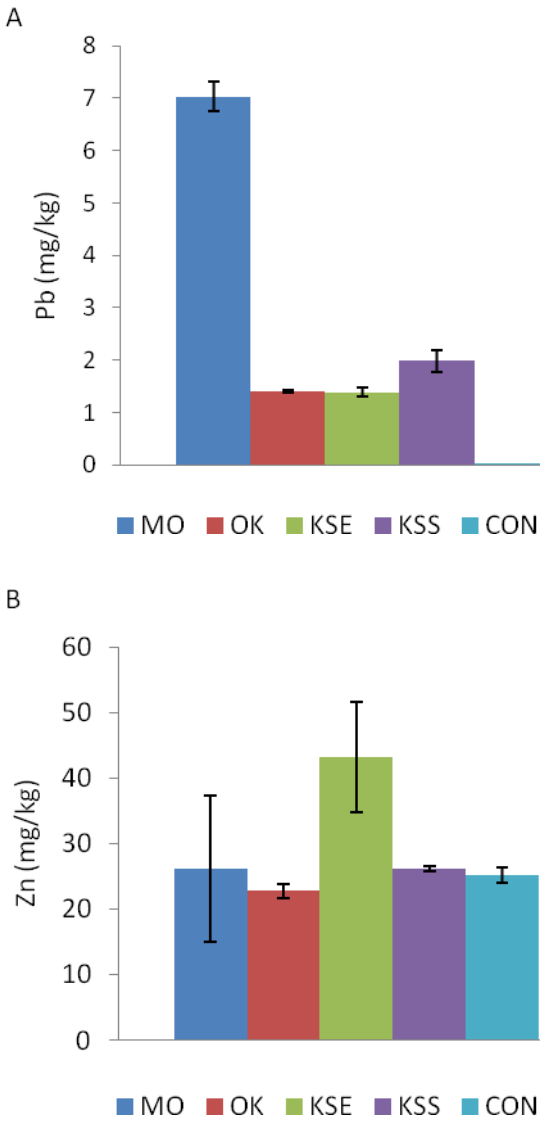

Figure A2. Average kidney Pb (A) and Zn (B) concentrations in Canada geese collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON).

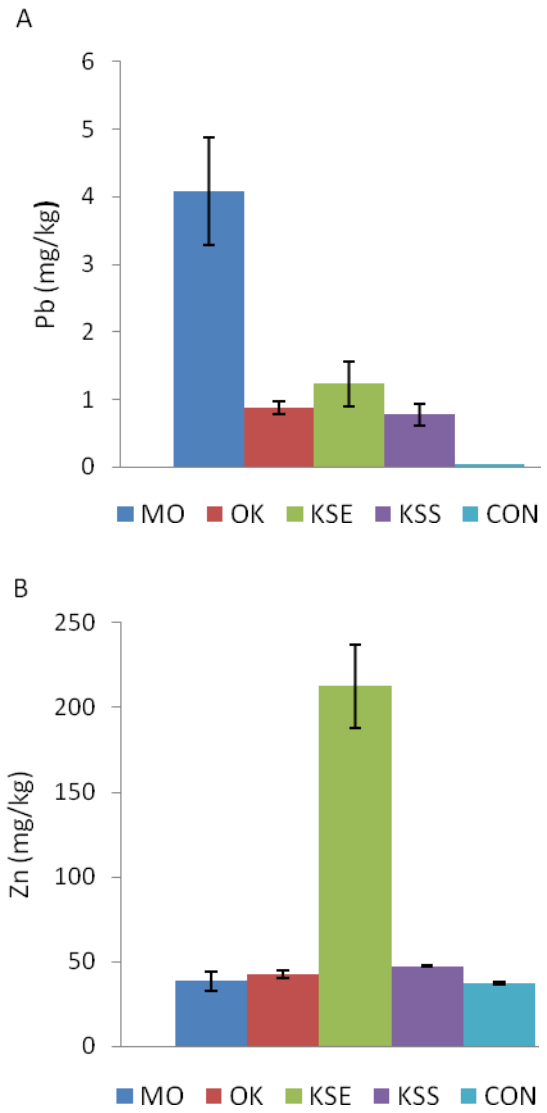

Figure A3. Average pancreas Pb (A) and Zn (B) concentrations in Canada geese collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON). No Pb was detectable at CON.

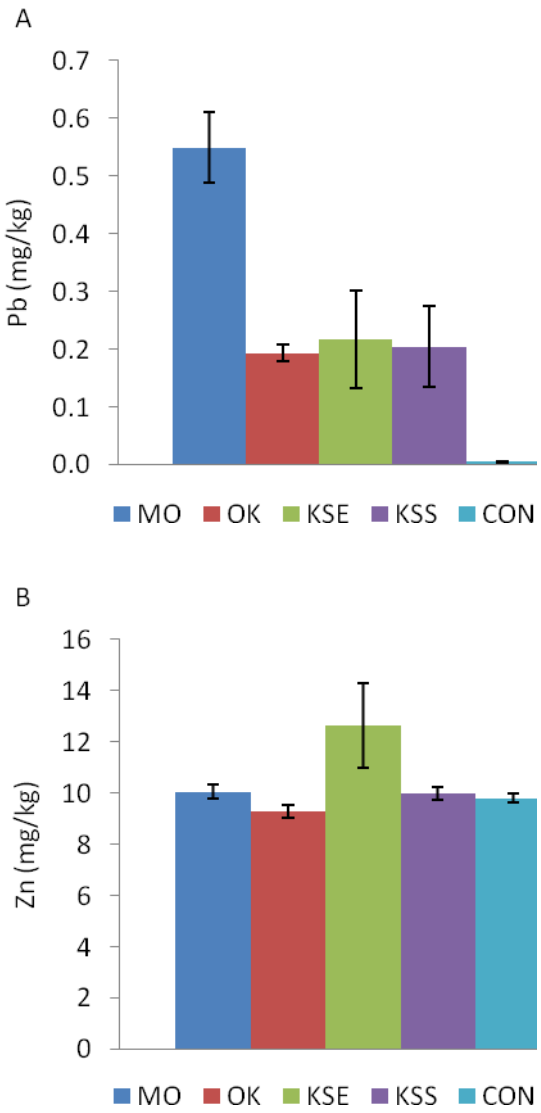

Figure A4. Average brain Pb (A) and Zn (B) concentrations in Canada geese collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON). No Pb was detectable at CON.

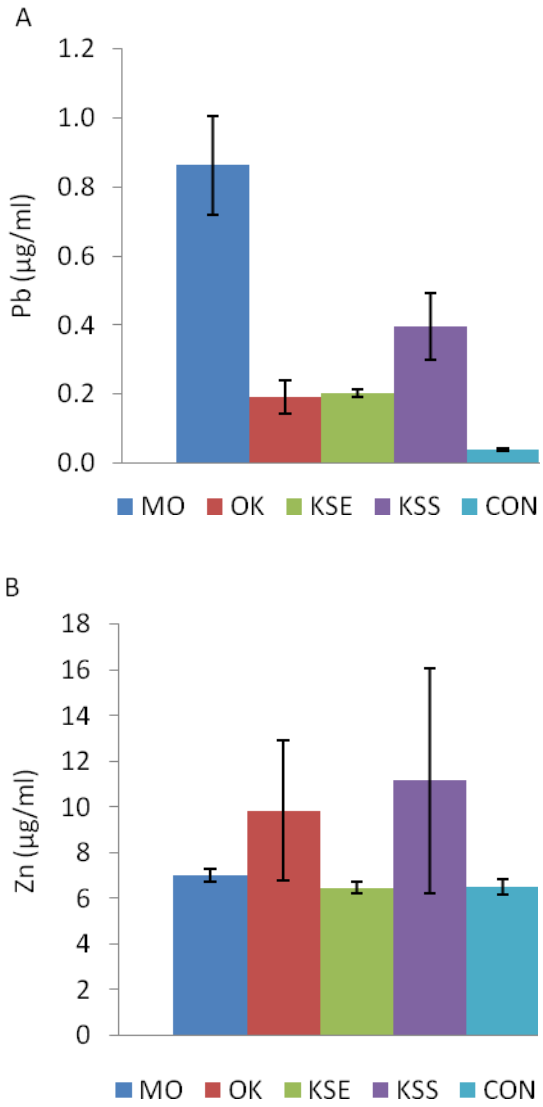

Figure A5. Average blood Pb (A) and Zn (B) concentrations in Canada geese collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON). No Pb was detectable at CON.

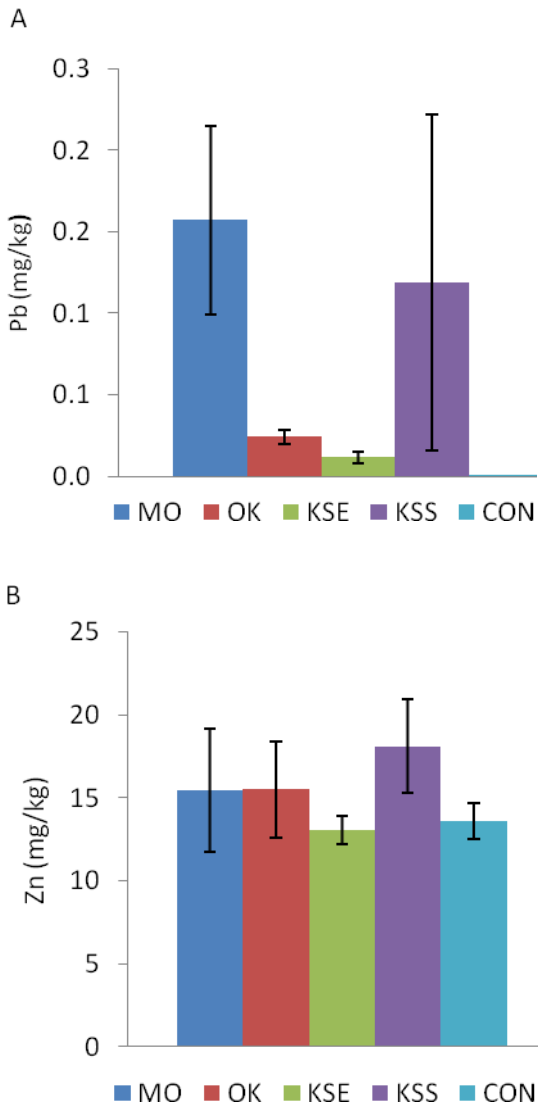

Figure A6. Average muscle Pb (A) and Zn (B) concentrations in Canada geese collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON). No Pb was detectable at CON.

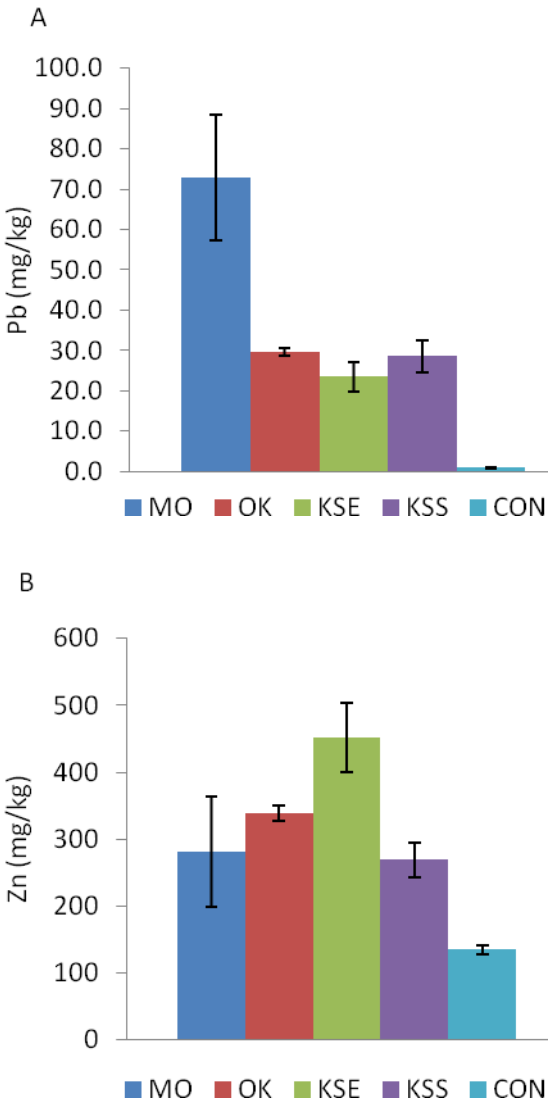

Figure A7. Average bone Pb (A) and Zn (B) concentrations in Canada geese collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON).

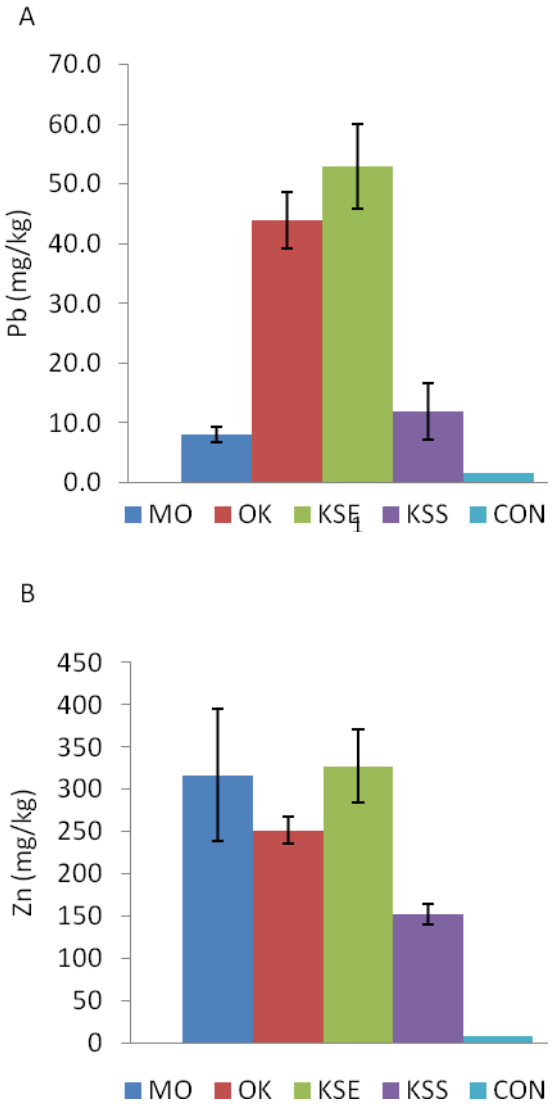

Figure A8. Average proventriculus Pb (A) and Zn (B) concentrations in Canada geese collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON).

Table A1. Element concentrations in Canada geese livers collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON). SE refers to standard errors, ND refers to undetectable concentrations and NA refers to SE calculations that are not applicable. All values are expressed in mg/kg.

	MO		OK		KSE		KSS		CON		1 mg/kg QC
Element	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg
Mg	176.33	8.35	181.05	5.48	199.29	7.35	225.06	12.21	213.07	5.19	0.96
Al	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	0.99
V	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	0.98
Cr	0.12	0.03	0.10	0.02	0.06	0.03	ND	NA	0.02	0.02	0.99
Mn	3.64	0.21	3.09	0.23	2.30	0.14	4.12	0.50	6.79	0.13	1.00
Fe	314.73	112.53	141.86	20.45	375.77	151.17	427.17	275.07	256.22	17.30	0.99
Co	0.01	0.01	0.01	0.01	0.02	0.01	0.06	0.01	0.05	0.00	0.98
Ni	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	0.98
Cu	27.10	6.00	23.70	2.35	19.34	2.79	22.25	3.88	38.25	4.04	0.96
Zn	52.11	11.18	83.26	7.26	178.44	15.55	95.05	14.86	67.99	2.33	1.00
As	ND	NA	ND	NA	ND	NA	ND	NA	0.00	0.00	0.98
Se	1.21	0.13	0.50	0.03	1.25	0.09	1.30	0.10	1.69	0.07	0.96
Mo	0.49	0.12	0.54	0.04	0.94	0.05	0.97	0.07	1.11	0.06	0.98
Ag	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	1.00
Cd	1.73	1.07	0.12	0.07	1.71	0.63	0.76	0.65	ND	NA	1.00
Ba	0.01	0.01	0.01	0.01	0.03	0.01	0.12	0.03	0.03	0.00	1.00
Tl	0.01	0.01	ND	NA	ND	NA	ND	NA	ND	NA	1.01
Pb	1.72	0.28	0.38	0.03	0.41	0.08	0.67	0.21	ND	NA	1.01

Table A2. Element concentrations in Canada geese kidneys collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON). SE refers to standard errors, ND refers to undetectable concentrations and NA refers to SE calculations that are not applicable. All values are expressed in mg/kg.

	MO		OK		KSE		KSS		CON		1 mg/kg QC
Element	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg
Mg	165.83	4.99	170.53	7.11	160.30	21.45	199.36	8.62	182.57	4.76	0.96
Al	ND	NA	ND	NA	0.04	0.04	ND	NA	ND	NA	0.99
V	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	0.98
Cr	ND	NA	0.13	0.08	0.01	0.01	ND	NA	ND	NA	0.99
Mn	3.54	0.28	2.49	0.18	2.72	0.38	3.48	0.24	3.22	0.12	1.00
Fe	110.76	16.15	121.21	15.90	129.76	27.71	95.51	23.94	139.49	8.63	0.99
Co	0.05	0.00	0.04	0.01	0.05	0.01	0.09	0.00	0.06	0.01	0.98
Ni	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	0.98
Cu	5.66	1.24	4.30	0.30	9.95	1.54	5.23	0.93	4.50	0.37	0.96
Zn	26.26	4.29	22.79	1.14	43.24	8.34	26.19	0.46	25.19	1.23	1.00
As	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	0.98
Se	1.49	0.15	0.58	0.07	1.04	0.17	1.05	0.05	1.24	0.07	0.96
Mo	0.73	0.08	0.80	0.09	1.21	0.19	1.13	0.08	1.21	0.07	0.98
Ag	ND	NA	ND	NA	0.01	0.01	ND	NA	ND	NA	1.00
Cd	11.10	6.62	0.68	0.08	5.65	3.12	1.96	1.50	ND	NA	1.00
Ba	ND	NA	ND	NA	ND	NA	0.26	0.10	0.13	0.09	1.00
Tl	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	1.01
Pb	7.03	0.58	1.41	0.10	1.39	0.33	1.98	0.30	ND	NA	1.01

Table A3. Element concentrations in Canada geese pancreases collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON). SE refers to standard errors, ND refers to undetectable concentrations and NA refers to SE calculations that are not applicable. All values are expressed in mg/kg.

	MO		OK		KSE		KSS		CON		1 mg/kg QC
Element	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg
Mg	336.64	5.23	339.38	15.48	322.09	5.22	250.65	83.66	353.25	7.02	0.99
Al	ND	NA	ND	NA	ND	NA	0.63	0.24	0.23	0.04	1.04
V	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	0.97
Cr	ND	NA	ND	NA	ND	NA	0.02	0.01	0.01	0.01	0.95
Mn	3.43	0.25	2.73	0.07	2.67	0.43	2.56	0.93	6.47	0.36	1.00
Fe	36.87	7.73	29.42	2.43	36.04	3.45	25.07	9.72	40.23	2.71	0.97
Co	ND	NA	ND	NA	ND	NA	ND	NA	0.01	0.00	0.95
Ni	ND	NA	ND	NA	0.04	0.04	0.01	0.01	ND	NA	0.93
Cu	1.32	0.05	1.27	0.07	1.79	0.11	0.96	0.33	1.62	0.03	1.03
Zn	38.51	5.56	42.68	2.15	212.49	24.49	57.00	27.72	37.22	0.75	1.03
As	ND	NA	ND	NA	ND	NA	0.01	0.00	ND	NA	1.11
Se	0.84	0.10	0.28	0.07	0.63	0.02	0.49	0.18	0.88	0.04	0.93
Mo	ND	NA	ND	NA	ND	NA	0.14	0.05	0.17	0.01	1.04
Ag	ND	NA	0.01	0.01	ND	NA	ND	NA	ND	NA	1.02
Cd	0.41	0.31	ND	NA	0.58	0.12	0.14	0.07	0.01	0.00	1.01
Ba	ND	NA	ND	NA	ND	NA	0.26	0.11	0.04	0.00	1.02
Tl	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	1.06
Pb	4.08	0.79	0.87	0.09	1.23	0.33	0.85	0.35	0.04	0.01	1.05

Table A4. Element concentrations in Canada geese brains collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON). SE refers to standard errors, ND refers to undetectable concentrations and NA refers to SE calculations that are not applicable. All values are expressed in mg/kg.

	MO		OK		KSE		KSS		CON		1 mg/kg QC
Element	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg
Mg	156.46	4.10	155.98	2.58	172.13	12.52	166.76	0.90	168.65	5.28	1.12
Al	0.18	0.03	1.25	0.74	0.31	0.06	0.21	0.05	0.22	0.03	1.10
V	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	1.08
Cr	0.01	0.01	ND	NA	0.01	0.01	0.01	0.01	ND	NA	1.09
Mn	0.60	0.14	0.46*	0.01	0.53	0.08	0.44	0.02	0.54	0.03	1.09
Fe	33.06	10.04	42.30	14.69	21.76	4.35	23.02	5.80	15.82	0.88	1.10
Co	ND	NA	ND	NA	0.01	0.01	ND	NA	ND	NA	1.09
Ni	0.07	0.03	ND	NA	0.03	0.01	0.03	0.01	0.02	0.01	1.09
Cu	3.18	0.26	2.40	0.09	2.58	0.07	3.64	0.40	3.25	0.22	1.09
Zn	10.06	0.28	9.31	0.25	12.65	1.66	9.97	0.25	9.82	0.16	1.12
As	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	1.09
Se	0.32	0.03	0.19	0.01	0.23	0.02	0.24	0.00	0.31	0.02	0.78
Mo	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	1.09
Ag	0.01	0.00	ND	NA	ND	NA	ND	NA	ND	NA	1.01
Cd	0.01	0.00	ND	NA	ND	NA	ND	NA	ND	NA	1.02
Ba	0.08	0.02	0.10	0.01	0.47	0.24	0.17	0.05	0.29	0.10	1.01
Tl	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	1.07
Pb	0.55	0.06	0.19	0.01	0.22	0.08	0.20	0.07	0.01	0.00	1.04

Table A5. Element concentrations in Canada geese blood collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON). SE refers to standard errors, ND refers to undetectable concentrations and NA refers to SE calculations that are not applicable. All values are expressed in mg/kg.

	MO		OK		KSE		KSS		CON		1 mg/kg QC
Element	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg
Mg	76.34	6.38	153.91	72.72	76.84	3.84	95.19	16.22	93.88	3.20	1.04
Al	1.46	0.16	1.39	0.34	1.27	0.14	1.56	0.27	1.30	0.09	1.07
V	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	1.01
Cr	0.23	0.02	0.21	0.05	0.20	0.04	0.35	0.13	0.20	0.02	1.00
Mn	0.06	0.01	0.16	0.05	0.05	0.01	0.07	0.02	0.07	0.01	1.03
Fe	354.21	46.58	241.09	58.08	341.65	22.64	354.45	61.28	427.88	11.73	1.01
Co	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	0.99
Ni	0.05	0.01	1.06	1.04	0.30	0.29	6.45	6.44	0.18	0.10	0.99
Cu	0.37	0.03	1.43	0.98	0.24	0.03	0.37	0.05	0.41	0.03	1.05
Zn	7.01	0.29	9.83	3.07	6.47	0.27	11.15	4.92	6.51	0.34	1.05
As	0.02	0.00	0.02	0.00	0.02	0.00	0.02	0.01	0.01	0.00	1.06
Se	0.33	0.03	0.16	0.04	0.19	0.02	0.31	0.07	0.27	0.02	0.92
Mo	0.08	0.02	0.06	0.01	0.02	0.00	0.06	0.03	0.01	0.00	1.04
Ag	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	1.05
Cd	ND	NA	ND	NA	ND	NA	ND	NA	0.01	0.01	1.04
Ba	0.03	0.00	0.04	0.01	0.03	0.01	0.07	0.01	0.04	0.00	1.06
Tl	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	1.12
Pb	0.86	0.14	0.19	0.05	0.20	0.01	0.40	0.10	0.04	0.00	1.14

Table A6. Element concentrations in Canada geese muscle collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CONSE refers to standard errors, ND refers to undetectable concentrations and NA refers to SE calculations that are not applicable. All values are expressed in mg/kg.

	MO		OK		KSE		KSS		CON		1 mg/kg QC
Element	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg
Mg	242.70	21.01	214.30	4.99	253.29	11.91	265.29	10.80	239.01	14.48	1.01
Al	0.08	0.03	0.20	0.08	0.27	0.13	0.36	0.17	0.11	0.06	1.03
V	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	0.97
Cr	0.08	0.03	0.19	0.07	0.06	0.01	0.04	0.01	0.05	0.01	0.96
Mn	0.26	0.04	0.20	0.04	0.19	0.02	0.36	0.06	0.20	0.04	0.99
Fe	40.59	11.20	32.85	9.31	35.71	13.37	31.49	13.08	28.38	3.31	0.97
Co	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.95
Ni	0.01	0.01	0.01	0.01	0.02	0.01	ND	NA	ND	NA	0.95
Cu	4.12	0.82	3.11	0.26	4.25	1.24	4.60	1.44	3.73	0.55	0.97
Zn	15.45	3.72	15.52	2.89	13.07	0.84	18.12	2.83	13.61	1.11	1.01
As	ND	NA	ND	NA	ND	NA	ND	NA	0.00	0.00	1.03
Se	0.54	0.04	0.14	0.02	0.27	0.04	0.40	0.05	0.49	0.03	0.95
Mo	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	1.01
Ag	0.01	0.01	ND	NA	ND	NA	ND	NA	ND	NA	0.99
Cd	0.02	0.01	ND	NA	0.04	0.03	0.01	0.00	ND	NA	1.00
Ba	0.02	0.01	0.02	0.01	0.01	0.00	0.27	0.25	0.02	0.00	1.00
Tl	0.01	0.00	ND	NA	ND	NA	ND	NA	ND	NA	1.03
Pb	0.16	0.06	0.02	0.00	0.01	0.00	0.12	0.10	ND	NA	1.03

Table A7. Element concentrations in Canada geese bone collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON). SE refers to standard errors, ND refers to undetectable concentrations and NA refers to SE calculations that are not applicable. All values are expressed in mg/kg.

	MO		OK		KSE		KSS		CON		1 mg/kg QC
Element	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg
Mg	2221	219	2920	68	2785	157	2778	245	3234	73	1.00
Al	0.83	0.70	0.12	0.08	1.90	0.75	1.18	0.38	0.55	0.42	0.95
V	0.13	0.04	0.12	0.06	0.13	0.04	0.13	0.03	0.08	0.00	1.00
Cr	0.03	0.03	0.03	0.03	ND	NA	ND	NA	ND	NA	0.98
Mn	21.10	5.21	13.77	1.10	24.29	2.63	16.63	1.32	20.05	1.76	1.00
Fe	56.80	13.52	48.96	2.10	53.74	6.03	57.22	5.12	52.63	1.66	0.99
Co	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	0.96
Ni	0.52	0.33	2.19	0.53	2.53	0.88	1.02	0.09	1.08	0.30	1.00
Cu	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	0.94
Zn	281.11	83.08	338.33	10.94	451.44	51.69	268.85	25.41	134.71	5.95	0.98
As	0.55	0.12	0.45	0.08	0.38	0.01	0.39	0.03	0.38	0.01	1.01
Se	0.55	0.02	0.33*	0.01	0.38	0.01	0.43	0.01	0.40	0.01	1.02
Mo	2.66	1.38	0.44*	0.06	0.27	0.03	0.21	0.01	0.16	0.01	1.00
Ag	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	1.00
Cd	0.18	0.02	0.17	0.02	0.10	0.01	0.15	0.01	0.13	0.01	1.01
Ba	63.09	19.28	50.66	2.61	76.74	10.18	101.56	21.86	104.00	8.62	1.00
Tl	0.08	0.02	0.04	0.01	0.02	0.00	0.03	0.00	0.02	0.00	1.00
Pb	72.86	15.57	29.71	1.08	23.48	3.62	28.62	3.96	0.88	0.16	0.98

Table A8. Element concentrations in Canada geese proventriculus contents collected at four mine waste-contaminated sites (MO; OK; KSE; KSS) and an uncontaminated control site (CON). SE refers to standard errors, ND refers to undetectable concentrations and NA refers to SE calculations that are not applicable. All values are expressed in mg/kg.

	MO		OK		KSE		KSS		CON		1 mg/kg QC
Element	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg	SE	mg/kg
Mg	864.50	137.02	154.01	46.41	72.46	14.85	747.83	314.53	94.94	NA	0.98
Al	74.61	7.73	167.06	16.24	320.95	18.20	541.27	37.13	369.40	NA	1.07
V	0.21	0.02	1.74	0.24	10.02	0.57	6.56	2.90	7.52	NA	0.98
Cr	0.32	0.04	2.15	0.15	7.58	0.38	7.41	3.56	2.91	NA	0.96
Mn	476.08	100.54	9.33	0.95	17.54	0.97	58.46	19.68	18.82	NA	1.01
Fe	409.45	45.87	1572.20	108.86	4219.33	264.48	2707.17	1201.47	2147.00	NA	0.97
Co	1.51	0.24	0.17	0.01	1.57	0.15	0.84	0.34	0.76	NA	0.94
Ni	1.29	0.21	1.47	0.08	2.48	0.18	2.18	0.52	2.64	NA	0.94
Cu	4.28	0.43	4.04	0.59	7.30	1.09	7.46	1.18	2.73	NA	1.02
Zn	316.95	78.41	251.32	16.10	327.23	43.41	152.00	12.52	8.47	NA	1.03
As	0.47	0.02	1.87	0.13	3.60	0.24	3.44	1.43	1.93	NA	1.07
Se	1.09	0.13	0.34	0.02	0.15	0.02	0.21	0.03	0.16	NA	0.77
Mo	5.55	0.72	0.61	0.11	0.57	0.07	0.49	0.15	0.20	NA	1.05
Ag	ND	NA	0.02	0.00	0.03	0.00	0.03	0.02	0.12	NA	1.01
Cd	1.77	0.52	1.26	0.11	1.66	0.13	0.67	0.11	0.01	NA	0.99
Ba	24.36	3.73	7.41	0.61	12.46	0.30	75.58	29.51	23.72	NA	1.02
Tl	0.08	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.01	NA	1.12
Pb	8.04	1.27	43.98	4.77	52.90	7.13	11.92	4.72	1.61	NA	1.09

Table A9. Limits of quantification (LOQ) and limits of detection (LOD) for reported elements.

	LOQ	LOD
Element	mg/kg	mg/kg
Mg	0.147	0.044
Al	2.789	0.837
V	0.082	0.025
Cr	0.110	0.033
Mn	0.056	0.017
Fe	0.432	0.130
Co	0.108	0.032
Ni	0.152	0.046
Cu	0.103	0.031
Zn	0.402	0.121
As	0.095	0.029
Se	0.344	0.103
Mo	0.054	0.016
Ag	0.400	0.120
Cd	0.063	0.019
Ba	0.574	0.172
Tl	0.146	0.044
Pb	0.079	0.024