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# ADVERSE HEALTH EFFECTS IN CANADA GEESE (*BRANTA CANADENSIS*) ASSOCIATED WITH WASTE FROM ZINC AND LEAD MINES IN THE TRI-STATE MINING DISTRICT (KANSAS, OKLAHOMA, AND MISSOURI, USA)

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ABSTRACT: Lead and zinc poisoning have been recorded in a variety of bird species, including migrating waterfowl such as Canada Geese (*Branta canadensis*), at sites contaminated with mine waste from lead and zinc mines in the Tri-State Mining District, Kansas, Oklahoma, and Missouri, USA. The adverse health impacts from mine waste on these birds may, however, be more extensive than is apparent from incidental reports of clinical disease. To characterize health impacts from mine waste on Canada Geese that do not have observable signs of poisoning, four to eight apparently healthy birds per site were collected from four contaminated sites and an uncontaminated reference site, and examined for physical and physiologic evidence of metals poisoning. Tissue concentrations of silver, aluminum, arsenic, barium, cadmium, cobalt, chromium, copper, iron, magnesium, manganese, molybdenum, nickel, lead, selenium, thallium, vanadium, and zinc were determined by inductively coupled plasma mass spectroscopy. Adverse health effects due to lead were characterized by assessing blood δ-aminolevulinic acid dehydratase (ALAD) enzyme activity. Adverse effects associated with zinc poisoning were determined from histologic examination of pancreas tissues. Elevated tissue lead concentrations and inhibited blood ALAD enzyme activities were consistently found in birds at all contaminated sites. Histopathologic signs of zinc poisoning, including fibrosis and vacuolization, were associated with elevated pancreatic zinc concentrations at one of the study sites. Adverse health effects associated with other analyzed elements, or tissue concentrations indicating potentially toxic exposure levels to these elements, were not observed.

*Key words:* *Branta canadensis*, Canada Goose, lead poisoning, mine waste, Tri-State Mining District, zinc poisoning.

## INTRODUCTION

Lead and zinc ores are unevenly distributed in the Tri-State Mining District over a 6,500 $km^2$ area in a southwest to northeast arc through northern Ottawa County in Oklahoma, southeast Cherokee County in Kansas, and multiple counties in southwest Missouri, USA (Pope, 2005). Major mining activities in the Tri-State Mining District ceased during the 1970s, but hazardous metals, including lead, zinc, and cadmium, are still present at high concentrations in mine wastes and stream sediments (Pope, 2005). Lead and zinc poisoning in wild birds has been recorded in the area (Sileo et al., 2003). A survey of wild birds in the Tri-State Mining District in 2001, which included species of waterfowl, upland game birds, and passerines, revealed high tissue concentrations of lead. There were also physiologic indications of lead exposure in the form of inhibited blood δ-aminolevulinic acid dehydratase (ALAD) activity. Zinc concentrations were elevated in waterfowl tissues, and cadmium concentrations were elevated in songbirds (Beyer et al., 2004). Elevated tissue lead and adverse health effects of lead exposure have been recorded in a variety of other species in the Tri-State Mining District, including fish (Wildhaber et al., 2000), mussels and clams (Mytilidae and Corbiculidae; 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 (Chironomidae; Reynolds and Ferrington, 2002).

The landscape at old mine sites in the Tri-State Mining District is characterized by sparsely vegetated soil and mine waste piles as much as 800 m in diameter. The ground surface in these areas consists of mine and mill wastes including fragmented rock, fine gravel (chat), and tailings of sand and silt (Xiao and Ji, 2007). The region has a generally flat topography, occasionally broken by rivers and streams. Water-filled subsidences have formed over collapsed underground caverns. Acid mine drainage and contaminated groundwater enter creeks close to mine sites (CH2MHILL, 2005). Between mine sites, forested areas are interspersed with other forms of land cover typical of agricultural landscapes, including pasture and crops such as corn, wheat, and soybeans. Some mined areas have been reclaimed for residential and industrial use (Murgueytio et al., 1996). Most mine waste surface drainage flows into the Spring River, which meanders southwesterly through the central region of the Tri-State Mining District and merges with the Neosho River in central Ottawa County, Oklahoma. Smaller streams draining mine sites flow directly into the Neosho River in northern Ottawa County. Habitat elements that attract Canada Geese (*Branta canadensis*) are present in the Tri-State Mining District, including shelter, open water, and food sources. Canada Geese tolerate human activity and often occur in suburban and agricultural environments (Conover and Chasko, 1985).

Canada Geese experimentally exposed to toxic concentrations of lead in the form of lead shot pellets exhibit a poor correlation between the exposure and the appearance of overt clinical signs (Cook and Trainer, 1966). We hypothesized that although credible evidence for the occurrence of lead and zinc poisoning in Canada Geese in the Tri-State Mining District is available, the true extent of adverse health impacts are not apparent from reports of clinical poisoning. To test this hypothesis, we collected Canada Geese with no obvious signs of ill health associated with lead or zinc poisoning, such as abnormal movement or behavior or loss of body condition, at mine waste-contaminated sites and at a reference site to assess the presence of preclinical lesions, metals accumulation in tissues, and physiologic markers of adverse health effects associated with metals exposure.

## MATERIALS AND METHODS

### Sample collection

From 2 June 2009 to 4 June 2009, birds were collected by US Fish and Wildlife Service personnel under permits from the US Fish and Wildlife Service, the Kansas Department of Wildlife and Parks, the Missouri Department of Conservation, and the Oklahoma Department of Wildlife Conservation. Most birds were collected by shooting with a shotgun using steel shot, while birds at the reference site (hereafter referred to as CON) were collected by entrapment in a temporary enclosure. No birds displayed obvious signs of lead or zinc poisoning, such as abnormal movement or behavior, or loss of body condition. At this time of year, Canada Geese that hatched in the spring are preflight juveniles. Although we targeted preflight juveniles, the birds' tight flocking behavior resulted in the capture of some adults. Based on the protective behavior of the adult birds toward the preflight juveniles, the adult birds were presumed to be members of the rearing groups of the preflight juvenile birds. Four to eight birds per site were collected at four mine waste–contaminated sites and at a reference site outside the Tri-State Mining District that does not receive drainage from contaminated areas. The locations of the contaminated sites (Fig. 1) were as follows: three juveniles and two adults were collected from a subsidence pond surrounded by sparsely vegetated mine chat (37°9′36.36″N, 94°27′28.08″W), in a northern suburb of Webb City, Missouri, hereafter referred to as MO; five juveniles were collected from a farm pond next to a mine waste pile (36°59′15.00″N, 94°51′12.96″W), 2 km west of Picher, Oklahoma, hereafter referred to as OK; six juveniles and two adults were collected from the confluence between the Spring River and Short Creek where mine waste was deposited in the streambed of Spring Creek (37°5′36.96″N, 94°40′57″W), 4.2 km northwest of Galena, Kansas, hereafter referred to as KSE; and three juveniles and one adult were collected from the northern bank of Shoal Creek (37°2′32.28″N, 94°40′45.84″W) in an area without visible mine waste that receives

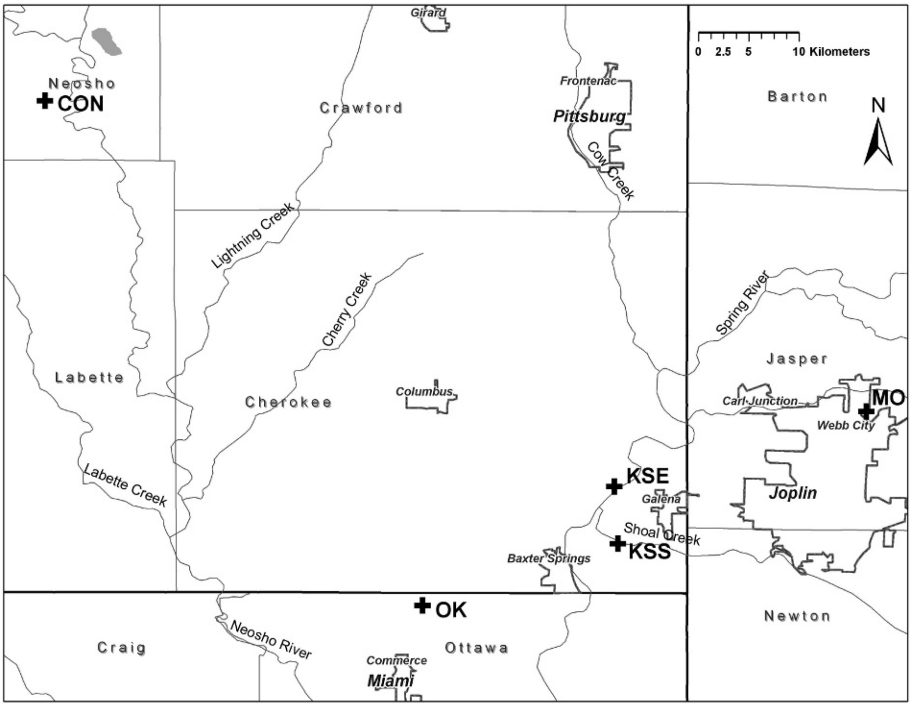


FIGURE 1. Study region showing Canada Geese (*Branta canadensis*) collection sites for determining lead- and zinc-associated adverse health effects during June 2009, including four mine waste–contaminated sites (MO, OK, KSE, KSS) and an uncontaminated reference site (CON) in Kansas, Oklahoma, and Missouri, USA.

drainage from mine waste, 5.1 km southwest of Galena, Kansas, hereafter referred to as KSS. Six juveniles were collected from the reference site (CON), which was situated at a farm pond (37°26′13.20″N, 95°11′26.16″W) 1.6 km northeast of Neosho State Fishing Lake, Kansas.

Birds captured alive were humanely euthanized by an overdose of the anesthetic gas isoflurane delivered via a facemask. Ages were estimated using guidelines developed for use in this species based on emergence, distribution, length, and color characteristics of down and feathers (Yocom and Harris, 1965). The midpoints of 7-day intervals were used when plumage development matched descriptions and were adjusted toward interval boundaries when plumage development was intermediate. Birds were weighed, and blood samples were collected shortly after capture from the jugular vein, the basilic vein, or the medial metatarsal vein using heparinized 3-ml syringes and 25-gauge needles. A 0.5-ml volume of each sample was placed into a 2-ml cryogenic vial (Fisherbrand, Fisher Scientific, Pittsburgh, Pennsylvania, USA), flash-frozen in liquid nitrogen (−196 C), and placed in an ultralow freezer (−80 C). Samples were shipped overnight on dry-ice (−78.5 C) to the Division of Comparative Pathology, Miller School of Medicine, University of Miami, Miami, Florida, USA, for ALAD analysis. The remaining blood from each sample was placed into a trace element blood collection tube (BD Vacutainer, BD, Franklin Lakes, New York, USA) for assays of packed cell volume (PCV) and element concentrations. The PCVs were measured in duplicate by the microhematocrit method using a microcapillary centrifuge at 5,000 × G for 5 min. The average PCV for each bird was then determined. No PCVs were obtained for some birds because the blood clotted prior to centrifugation.

Birds were necropsied following the procedure outlined in the Avian Disease Manual (Charlton et al., 2000). Their body condition and any macroscopic lesions were recorded. Samples of trachea, lungs, heart, pancreas, liver, spleen, kidney, adrenal glands, gonads, breast muscle, brain, bone marrow, and bursa of Fabricius were collected, regardless of the presence or absence of lesions, and fixed in 10% neutral-buffered formalin. 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, Manhattan, Kansas. The slides were examined by one pathologist and any abnormal findings were recorded. Samples for element analysis of brain, liver, kidney, pancreas, skeletal muscles, and a long bone (femur or metatarsus) were collected individually in digestion cups (SC475, Environmental Express, Mt. Pleasant, South Carolina, USA), without the addition of preservatives.

### Sample analysis

Blood ALAD activity was measured according to an established method (Burch and Siegel, 1971). A unit of ALAD activity was defined as an increase in corrected absorbance at 555 nm of 0.100 per hour, with a 1.0-cm light path length and 1-ml red blood cell volume, at 38 C. Activity was normalized for each sample's PCV. A duplicate KSE3 blood sample was analyzed for quality control and generated similar results to the first sample. Since there was no significant difference between the PCVs of any groups based on Kruskal-Wallis one-way analysis of variance on ranks, ALAD activity for samples without measured PCV values was estimated using the mean PCV value from the respective site. One sample was severely clotted and could not be analyzed (KSS1).

Tissue samples for element analyses were processed at the Kansas State Veterinary Diagnostic Laboratory. Samples were digested in nitric acid, and analyzed on a wet weight basis. One gram of sample (1 ml for blood) was mixed in a digestion cup (SC475, Environmental Express) with 3 ml of ultrapure water (Milli-Q Biocel, Millipore, Bedford, Massachusetts, USA) and 4 ml of 70% nitric acid (TraceMetal Grade, Fisher Scientific), capped and heated (HotBlock™, Environmental Express) at 105 C for 3 hr, then diluted with 18 ml of water. Samples that contained visible particulates after digestion were filtered using 2-µm filters (Filtermate™, Environmental Express). Prepared samples were analyzed by inductively coupled plasma mass spectroscopy (ICP-MS; Agilent ICP-MS 7500cx, Agilent Technologies, Wilmington, Delaware, USA) for a panel of elements including magnesium, aluminum, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, selenium, molybdenum, silver, cadmium, barium, thallium, and lead. Hydrogen reaction and helium collision were used for interference removal and an argon dilution system was used to extend the range of acceptable total dissolved solids concentrations up to 1%. Standards for element analyses by ICP-MS were obtained from Environmental Express. To account for sample matrix effects, scandium, rhodium, indium, lutetium, and bismuth were used as fixed concentration internal standards and quantitation was achieved by measurements of the ratios between internal standards and the elements of interest. Limits of quantitation varied by matrix because of different dilution requirements and were defined as the lowest quantifiable standard multiplied by the sample dilution factor. Reference samples containing element concentrations of 1 mg/l, prepared from commercially available standards (Environmental Express), were used for data quality assurance. Acceptable quality was defined as a measured concentration between 0.8 and 1.2 times the actual concentration. Each batch of samples included digestion or processing blanks. Element concentrations in blanks were used to correct results for background contaminants.

### Statistical analyses

Statistical analyses were performed in SigmaPlot Version 11.2, Build 11.2.0.5 (Systat Software Inc., Chicago, Illinois, USA). Statistical significance was set at alpha = 0.05. Multiple group comparisons by one-way analysis of variance were performed on data that passed normality tests. Kruskal-Wallis one-way analysis of variance on ranks was used on data that failed normality tests. Lead in liver, brain, and muscle was excluded from these analyses because lead concentrations in the reference samples were below the limit of detection and variability could therefore not be estimated. Where group differences were significant ($P<0.05$), multiple comparisons were performed using Dunnett's method for normally distributed data, or Dunn's method for data that were not normally distributed. The statistical analyses were repeated on preflight juveniles only. Pearson product moment correlation tests were performed to identify and characterize correlations.

## RESULTS

Necropsies revealed no significant gross pathology. All birds were in good body condition with normal muscling and adequate subcutaneous and internal body fat. There were no consistent differences in microscopic lesions between juvenile and adult birds. The most common histologic changes in organs other than the pancreas were associated with parasitism. Parasit-

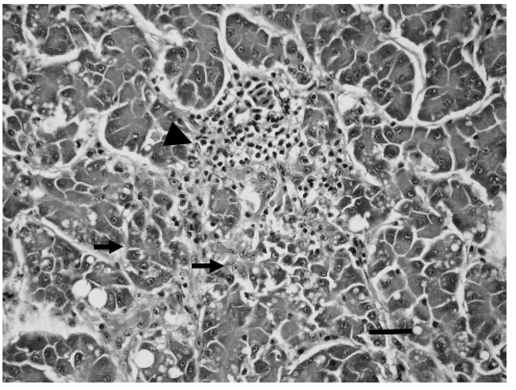

FIGURE 2. Photomicrograph of the pancreas from a Canada Goose (*Branta canadensis*) collected at a mine waste contaminated site in the Tri-State Mining District during June 2009 (Kansas, Oklahoma, and Missouri, USA), showing lesions associated with zinc poisoning, including vacuolation of exocrine epithelial 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.

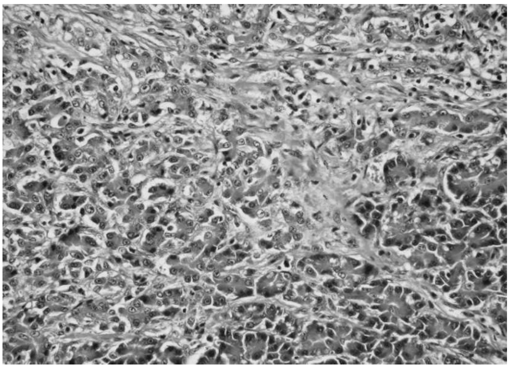

FIGURE 3. Photomicrograph of the pancreas from a Canada Goose (*Branta canadensis*) collected at a mine waste–contaminated site in the Tri-State Mining District during June 2009 (Kansas, Oklahoma, and Missouri, USA), showing lesions associated with zinc poisoning, including an area of interstitial fibrosis with loss of exocrine glands.

ism was present in mine waste–exposed birds and reference birds in an apparently random pattern. There was some differentiation between parasitism in juveniles and adults. 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. The kidneys of 14 juveniles had multifocal tubulointerstitial inflammation associated with developing stages of coccidia. Two species of nematodes were observed in the wall of the ventriculus of three geese.

Multifocal degenerative changes were present in the pancreas of five of eight birds (three juveniles and two adults) from the KSE site (Figs. 2 and 3). In affected areas, pancreatic exocrine cells were disorganized and smaller than normal with variably sized, clear cytoplasmic vacuoles and decreased quantities of zymogen granules. There were individual and small groups of shrunken, eosinophilic cells with pyknotic nuclei. The cellular degeneration was accompanied by interstitial fibrosis in one juvenile and one adult. The degenerative changes occurred as large coalescing areas in two juveniles and one adult, and were lightly scattered throughout the pancreas of one juvenile and one adult.

Bird weights averaged 2.1 kg (median 2.29 kg, range 0.95–3.23 kg, SE 0.134 kg) for juveniles and 4.36 kg (median 4.38 kg, range 3.75–4.75 kg, SE 0.179 kg) for adults. Juvenile bird ages averaged 38 days (median 41 days, range 21–47 days, SE 1.54 days). Packed cell volumes of mine waste-exposed birds averaged 36.7% (median 36.7%, range 25.0–48.7%, SE 1.39%) and were significantly lower than reference PCVs ($P=0.046$), which averaged 41.5% (median 41.6%, range 39.4–43.1%, SE 0.588%). Blood ALAD activities of mine waste–exposed birds averaged 6.1 units (median 6.0, range 0.1–14.7, SE 1.03) and were significantly lower than reference blood ALAD activities ($P<0.001$), which averaged 30.1 units (median 28.6, range 22.8–40.6, SE 2.82). Blood ALAD inhibition at the mine waste sites, expressed as a percentage derived from the equation: (reference 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

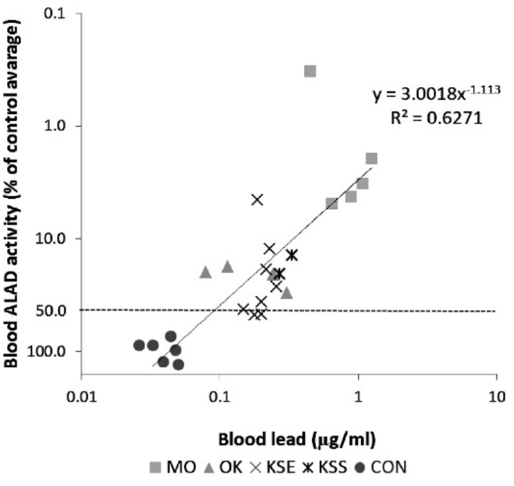


FIGURE 4. Relationship between blood δ-aminolevulinic acid dehydratase (ALAD) enzyme activity and blood lead concentrations in Canada Geese (*Branta canadensis*) collected at a mine waste–contaminated sites in the Tri-State Mining District during June 2009 (Kansas, Oklahoma, and Missouri, USA).

site, and 82.9% (SE 3.2%) at the KSS site. Blood ALAD inhibition was not correlated with age in juvenile birds ($R=-0.0683$; $P=0.809$). Blood ALAD inhibition in preflight 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% ($n=1$) at the KSS site. The pattern of ALAD inhibition remained the same whether adults were included or excluded. Blood ALAD activities were inversely correlated with tissue lead concentrations in all tissues except muscle. The quantitative relationship between blood lead and ALAD activity, derived using all birds, could be mathematically modeled using a power equation with an $R^2$ of 0.627: $y=3.0018x^{-1.113}$, where $y$ is the ALAD activity expressed as a percentage of the average reference activity, and $x$ is the blood lead concentration in micrograms per milliliters (Fig. 4).

Although various elements, including cadmium, chromium, silver, nickel, aluminum, arsenic, thallium, cobalt, molybdenum, and vanadium were elevated at one or more contaminated sites compared to the reference site (data not shown), these elements were not associated with tissue concentrations known to be associated with toxicity in birds (Puls, 1994). The analysis, therefore, was focused on lead and zinc. The limits of detection for lead and zinc were 0.024 mg/kg and 0.121 mg/kg, respectively, while the limits of quantification for lead and zinc were 0.079 mg/kg and 0.402 mg/kg, respectively. Limits of detection and quantification values were the same in blood, but with units in micrograms per milliliters. Lead and zinc tissue concentrations are presented in Table 1, and the statistical significance of differences between site means, compared with the reference site birds, are summarized in Table 2. Lead concentrations were consistently elevated in tissues from contaminated sites compared with the reference site. Although statistical significance tests could not be performed in liver, brain, and muscle because the lead concentrations in the reference samples were below the limit of detection, lead concentrations in liver and brain samples from mine waste sites were one to two orders of magnitude higher than the limit of detection, which indicates substantial lead accumulation in these tissues compared to the references. Lack of statistical significance of tissue lead concentration elevations at the KSS site compared to the reference 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 lead concentrations were not correlated with PCVs ($R=-0.0165$; $P=0.943$). There was significant covariance between lead concentrations in tissues, with the exception of muscle. These covariance patterns remained similar when adults were excluded from the analysis. Pancreas lead concentrations from the KSE site ranked second highest among the four exposure sites, while they ranked third highest in most other tissues. This anomaly occurred in

TABLE 1. Tissue lead and zinc concentrations (±SE) in Canada geese (*Branta canadensis*) collected at four mine waste–contaminated sites (see Fig. 1) designated MO ($n=5$), OK ($n=5$), KSE ($n=8$), KSS ($n=4$), and an uncontaminated reference site, CON ($n=6$). Units are milligrams per kilogram in all tissues except blood (micrograms per milliliter). Limits of detection for lead and zinc were 0.024 mg/kg (μg/ml in blood) and 0.121 mg/kg (μg/ml in blood), respectively.

| | Tissue type[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Site[a] | Liver | Kidney | Pancreas | Brain | Blood | Muscle | Bone | Proventriculus contents |
| Lead | | | | | | | | |
| MO | 1.72±0.13 | 7.03±0.26 | 4.08±0.35 | 0.55±0.03 | 0.86± (0.06) | 0.16± (0.16) | 72.86 ± (6.97) | 8.04±0.51 |
| OK | 0.38±0.01 | 1.41±0.04 | 0.87±0.04 | 0.19±0.01 | 0.20±0.02 | 0.03±0.00 | 29.71±0.49 | 43.98±2.14 |
| KSE | 0.33±0.03 | 1.18±0.09 | 1.23±0.12 | 0.22±0.03 | 0.20±0.00 | BD | 23.48±1.28 | 52.90±2.18 |
| KSS | 0.67±0.11 | 1.98±0.15 | 1.00±0.13 | 0.21±0.04 | 0.40±0.04 | BD | 28.62±1.98 | 11.92±2.04 |
| CON | BD | 0.20±0.05 | 0.04±0.00 | BD | 0.04±0.00 | BD | 0.88±0.07 | ND |
| Zinc | | | | | | | | |
| MO | 52.10±5.00 | 26.26±1.92 | 38.48±2.49 | 10.06±0.13 | 7.02±0.13 | 15.46±1.66 | 281.08±37.16 | 316.95±39.21 |
| OK | 83.26±3.24 | 22.82±0.51 | 42.68±0.96 | 9.32±0.11 | 9.84±1.38 | 15.52±1.29 | 338.30±4.89 | 251.32±7.20 |
| KSE | 178.44±5.50 | 48.66±2.29 | 212.49±8.66 | 12.66±0.59 | 6.48±0.09 | 13.09±0.30 | 451.41±18.28 | 327.23±17.2 |
| KSS | 95.05±7.43 | 26.20±0.24 | 68.88±10.70 | 9.95±0.13 | 11.17±2.13 | 17.90±1.42 | 268.83±12.71 | 152.00±7.23 |
| CON | 68.00±0.95 | 25.22±0.50 | 37.20±0.31 | 9.85±0.07 | 6.53±0.14 | 13.62±0.45 | 134.69±2.43 | ND |

[a] MO; OK; KSE; KSS; CON.

[b] BD = below detection limit; ND = no data.

TABLE 2. Statistical significance of multiple comparisons of differences between lead and zinc concentrations in bird tissues from four mine waste–exposed sites (see Fig. 1) compared with a reference site. Includes all birds and preflight juveniles, at a confidence level of 95%. "+" = significantly different from reference site; "−" = not significantly different.

| Site | Liver | Kidney[ab] | Pancreas[ab] | Brain | Blood[a] | Muscle | Bone[ab] |
|---|---|---|---|---|---|---|---|
| Lead (all birds) | | | | | | | |
| MO | No test | + | + | No test | +[b] | No test | + |
| OK | No test | − | − | No test | −[b] | No test | + |
| KSE | No test | − | + | No test | −[b] | No test | − |
| KSS | No test | − | − | No test | −[b] | No test | − |
| Zinc (all birds) | | | | | | | |
| MO | −[cd] | − | − | −[a] | − | −[c] | − |
| OK | −[cd] | − | − | −[a] | − | −[c] | + |
| KSE | +[cd] | + | + | −[a] | − | −[c] | + |
| KSS | −[cd] | − | − | −[a] | − | −[c] | − |
| Lead (juveniles) | | | | | | | |
| MO | No test | + | + | No test | +[b] | No test | + |
| OK | No test | + | − | No test | −[b] | No test | + |
| KSE | No test | − | + | No test | −[b] | No test | − |
| KSS | No test | − | − | No test | −[b] | No test | − |
| Zinc (juveniles) | | | | | | | |
| MO | −[cd] | −[e] | − | −[a] | − | −[c] | − |
| OK | −[cd] | −[e] | − | −[a] | − | −[c] | − |
| KSE | +[cd] | −[e] | + | −[a] | − | −[c] | + |
| KSS | −[cd] | −[e] | − | −[a] | − | −[c] | − |

[a] Kruskal-Wallis one-way analysis of variance on ranks.

[b] Multiple comparisons versus reference group (Dunn's method).

[c] Parametric one way analysis of variance.

[d] Multiple comparisons versus reference group (Dunnett's method).

[e] Difference greater than expected by chance using Kruskal-Wallis one-way analysis of variance on ranks, but the group(s) is (are) not identifiable by Dunn's method.

association with high zinc concentrations and zinc-associated pancreatic lesions at the KSE site. Zinc concentrations were elevated in pancreas, liver, and bone from the KSE site compared with the reference site when juveniles were analyzed separately, and also in kidney when all birds were included in the analysis. The only other site where significant zinc elevations occurred in tissues was the OK site, where zinc elevation was significant in bone when all birds were included in the analysis. Two birds from the KSE site had liver zinc concentrations in excess of 200 mg/kg, a concentration associated with zinc poisoning in domestic poultry (Puls, 1994).

All tissue lead concentrations, except muscle, were positively correlated with each other, and negatively correlated with blood ALAD activity. Muscle lead was not correlated with lead in brain and pancreas, or blood ALAD activity. The best correlation with blood ALAD activity ($R=-0.677$; $P=0.0001$) was obtained with bone lead. Proventriculus lead and zinc concentrations were not correlated with tissue concentrations or blood ALAD activity.

## DISCUSSION

Blood ALAD inhibition by >50% was found in all tested mine waste-exposed Canada Geese in the Tri-State Mining District. This finding is important because ALAD activity is characteristic of lead exposure in birds, and the degree of inhibition is positively correlated with the level of exposure. Blood ALAD inhibition following lead exposure is a consistent finding in birds and can be used as a biomonitoring tool for lead exposure (Vanparys et al., 2008). The inverse exponential relationship we found between ALAD inhibition and blood lead (Fig. 4) is in agreement with patterns observed in previous studies (Vanparys et al., 2008). A reduction in ALAD activity of >50% compared to unexposed references is regarded as a reliable indicator of adverse physiologic effects (Vanparys et al., 2008), and this level of ALAD inhibition is accepted as legal evidence of lead poisoning injury in the United States (US Department of the Interior, 1986).

Inhibition of ALAD remained >50% whether preflight juveniles or all birds were included in the analysis. If it is assumed that preflight juveniles spend all of their lives close to the collection sites, it indicates that the effect was caused by local exposure. The ALAD activities were not only correlated with blood lead, but also with lead concentrations in other tissues, except muscle. This has practical implications because blood may not be available postmortem. Other soft tissues such as liver and kidney, or bone, which is resistant to decay, could be useful indicators of potential ALAD inhibition.

With the exception of bone, lead concentrations in tissues were below published ranges associated with lead poisoning in waterfowl (Puls, 1994). The difference was up to 18-fold, as demonstrated by the average liver lead of 0.33 mg/kg at the KSE site, compared with the reference toxic concentration of >6 mg/kg. Other available toxic reference concentrations in geese (Puls, 1994) include kidney (>60 mg/kg), blood (>1 mg/kg), brain (>3 mg/kg), and bone (>20 mg/kg). This may be explained by the use of a different definition of lead poisoning in the current study, which was >50% blood ALAD suppression, compared with behavioral changes, lethargy, anorexia, anemia, and various macroscopic lesions observed at necropsy used to define lead poisoning in the reference data. The importance of this observation is that the legal requirement for defining lead poisoning injury may be reached at tissue concentrations substantially lower than the concentrations associated with visible clinical signs of lead poisoning.

Elevations in tissue zinc compared to the reference site were found only in tissue samples from the KSE site and the OK site (Table 2). Furthermore, two birds from the KSE site had liver zinc concentrations above concentrations accepted as confirmatory for zinc poisoning (>200 mg/kg) in domestic poultry. Tissue zinc concentrations did not exceed other available toxic ranges for poultry, including kidney (>300 mg/kg), bone (>1,500 mg/kg), and pancreas (>1,000 mg/kg; Puls, 1994). Pancreatic lesions associated with zinc poisoning were present in birds from the KSE site. Such lesions were previously described in waterfowl in the study area (Sileo et al., 2003). Pancreatic lesions resembling zinc poisoning can occur in poultry with selenium deficiency (Goodwin, 1996), but selenium concentrations (data not shown) were in the normal ranges (Puls, 1994) in birds at all study sites.

Zinc is an essential nutrient that plays a role in the functions of more than 300 enzymes (Lindh, 2005). Zinc is also very highly regulated, in part through interactions with metallothioneins (Lindh, 2005), and it can be hypothesized that high levels of tissue accumulation are associated with exceptionally high rates of exposure that overcome physiologic control mechanisms. Overall, the co-occurring of elevated zinc with pancreatic lesions was indicative of zinc poisoning and is consistent with injury documented in other studies in the Tri-State Mining District (Sileo et al., 2003).

In conclusion, our results confirm that Canada Geese at mine waste–contaminated sites in the Tri-State Mining District consistently suffer adverse health effects associated with lead exposure, and in some areas, adverse effects due to zinc exposure. These findings support the hypothesis that the health impacts of exposure to mine waste are often not obvious from casual observation of clinical signs and are more widespread than the impacts that can be determined from reports of poisoning cases involving observable signs of disease. The degree of adverse effects is correlated with the degree of exposure. Reducing exposure is, therefore, expected to result in a reduction of adverse health effects in birds. Key questions that remain unanswered include the relative importance of dietary components and how that varies over time, the relative importance of route of exposure and the absorption kinetics of metals from different materials and routes of exposure, the sphere of impact around contaminated sites, and differences in susceptibility between resident and migratory birds.

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