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Zinc and Lead Poisoning in Wild Birds in the Tri-State Mining District (Oklahoma, Kansas, and Missouri)

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Abstract. The Tri-State Mining District (Oklahoma, Kansas, and Missouri) is contaminated with Pb, Cd, and Zn from mining, milling and smelting. Metals have been dispersed heterogeneously throughout the District in the form of milled mine waste (“chat”), as flotation tailings and from smelters as aerial deposition or slag. This study was conducted to determine if the habitat has been contaminated to the extent that the assessment populations of wild birds are exposed to toxic concentrations of metals. American robins (*Turdus migratorius*), northern cardinals (*Cardinalis cardinalis*), and waterfowl had increased Pb tissue concentrations ($p < 0.05$) compared with Pb tissue concentrations from reference birds, and the exposure of songbirds to Pb was comparable with that of birds observed at other sites severely contaminated with Pb. Mean activities of the Pb-sensitive enzyme delta-aminolevulinic acid dehydratase (ALAD) were decreased by >50% in red blood cells in these birds ($p < 0.05$). Several birds had tissue concentrations of Pb that have been associated with impaired biological functions and external signs of poisoning. Cadmium was increased in kidneys of songbirds ($p < 0.05$), but no proximal tubule cell necrosis associated with Cd poisoning was observed. Zinc concentrations in liver and kidney of waterfowl were significantly higher ($p < 0.05$) than reference values. The increased environmental concentrations of Zn associated with mining in the District accounted for the pancreatitis previously observed in five waterfowl from the District. The District is the first site at which free-flying wild birds have been found to be suffering severe effects of Zn poisoning.

The District was once the world’s greatest producer of Pb–Zn ores. Metals associated with the mining process have contaminated waters and sediment, thus causing toxic effects in fish (Schmitt *et al*. 1993) and limiting the population (Wildhaber *et al*. 2000) of the Neosho madtom (*Noturus placidus*), a species of fish federally listed as threatened (55 Federal Register 21148, May 22, 1990). Soils have also been severely contaminated. The landscape is marked with mining refuse and sinkholes where ground has given way and fallen into subterranean mines. The most contaminated areas tend to be poor wildlife habitat, which are either barren or sparsely populated with invasive plant species that have replaced native vegetation. Potential hazards to wildlife feeding on the edges of these highly disturbed areas or in associated contaminated aquatic habitats have not been well documented. Although the mining refuse and adjacent soils contain high concentrations of metals, much of the contamination is in the form of primary ores, which are likely to have low bioavailability to animals. We undertook this study to evaluate exposure to metals and potential poisoning of assessment populations of wild birds in the District.

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History of Mining and Smelting in the District

The District covers approximately 3000 km and includes parts of Ottawa County, OK; Cherokee County, KS; and Jasper and Newton Counties, MO (Gibson 1972; Fig. 1). The District extends from the northwestern edge of the Ozark Uplift across rolling prairie west to the Neosho River (Gibson 1972). Much of the area is drained by the Spring River. The area has been mined for the sulfide forms of Pb (galena) and Zn (sphalerite) and to a lesser extent for Zn carbonate (smithsonite), Pb carbonate, Pb phosphate (pyromorphite), and other less abundant ores. Lead and Zn deposits in the District were generally associated with each other, and milling processes were generally designed to recover both metals. Shallow mines were dug in southwestern Missouri by approximately 1850, and mining expanded westward with discoveries of ore in Galena, KS, in 1876 (Gibson 1972) and in Picher, OK, in 1904 (Magoo 1996). Weidman (1932) wrote a detailed history of mining in the Oklahoma fields.

Ores were at first smelted locally at the mining sites on log piles or furnaces (Magoo 1996). Zinc smelting became more

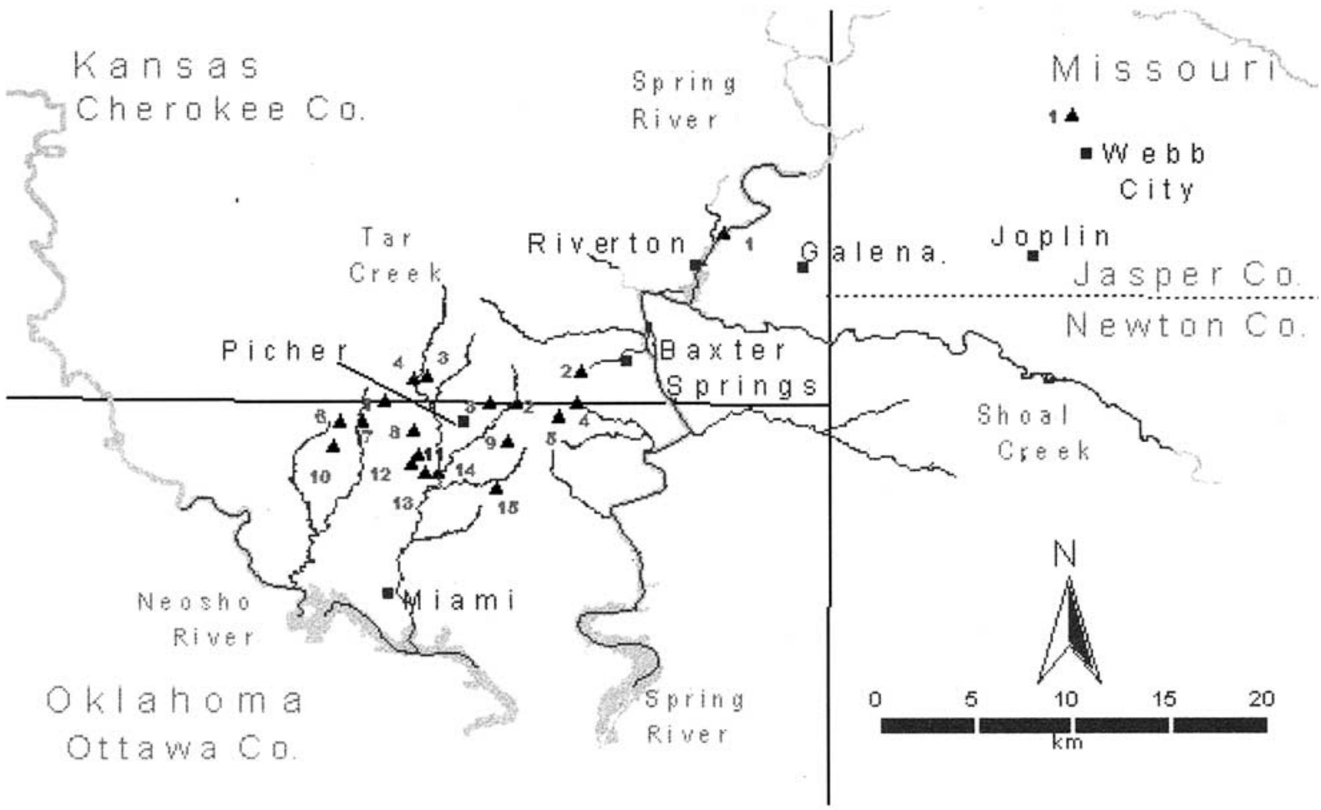

Fig. 1. Locations of collection sites within the Tri-State Mining District, which is irregularly contaminated with Pb, Cd, and Zn from mining, milling, and smelting. Large smelters were located at Joplin, MO; Galena, KS; and east of Picher, OK. Most of the remaining large chat piles (milled mine waste) are in the Picher area. In 1923, waterfowl were reported to be poisoned from metals in the Spring River near Riverton (Phillips and Lincoln 1930).

centralized by 1873, first with the construction of Zn smelters in southeastern Kansas. These smelters were not successful economically, however, and were soon abandoned. In later years, Pb smelting was consolidated, and smelters were operated in Joplin (1881) and Galena (1893). Another Pb smelter was constructed in 1918 in Hockerville, OK, 3 miles east of Picher, and operated until the early 1930s (Magoo 1996).

Mining and smelting produced several sources of contamination. The milled mine waste, or chat, is composed mainly of chert, a siliceous rock mined with the ores (Dames and Moore 1993). Chat contains Pb, Zn, Cd, and other elements in the ores that were not separated from the chert. Much of the chat had been processed more than once and it had been disposed of in massive piles covering thousands of hectares. Many of the piles have been dispersed for various uses such as in local road construction, but some large piles remain, mainly in Oklahoma (Magoo 1996). Three samples of chat from Oklahoma contained 270 to 732 mg of Pb, 41 to 57 mg of Cd, and 8,266 to 11,086 mg of Zn/kg (Oklahoma Department of Environmental Quality 2000). Flotation tailings are fine-grained materials derived from the froth-flotation milling process (Dames and Moore 1993) that were deposited in settling impoundments. They may be highly contaminated with Pb or Zn. Slag, which is a residue from smelting, is an additional source of contamination. Smelting also dispersed metals by air, thus contaminating local soils. An examination of the mineralogy of the residential soils in Picher suggested that the Pb detected was mainly from smelting rather than from chat (Magoo 1996). Sediments may contain metals as they come from the ores or as secondary minerals. Metals may precipitate with iron oxyhydroxides and form highly contaminated surface crusts (Parkhurst *et al*. 1988), a possible but unproven source of contamination for terrestrial wildlife. Tar Creek, south of Picher, OK, is especially contaminated with mine-water discharge and runoff from chat piles. Surface deposits on sediments along this creek contain as much 35,000 mg of Zn, 8700 mg of Pb, and 130 mg of Cd/kg (Parkhurst *et al*. 1988).

Contamination across the District is extremely irregular, which means that average soil metal concentrations and estimates of average exposure of birds to metals provide only a partial picture of risk to birds. Characterizing soil concentrations is further complicated by emissions from smelters and by the association of ore metals with particular size fractions of soil particles, which may vary from site to site. Soil contamination associated with chat piles is quite variable and decreases within a few meters of the piles. Given the extreme heterogeneity of the soil metal concentrations in the District, we did not try to estimate average soil concentrations through soil sampling.

Evidence of Toxic Effects on Wild and Domestic Animals in the District

A textbook on waterfowl (Phillips and Lincoln 1930) reported deaths of many mallards (*Anas platyrhynchos*), pintails (*A. acuta*) and teal (*A. crecca*) on Spring River, near Riverton, KS, in 1923 (Fig. 1). The deaths were attributed to Pb poisoning from sediments contaminated with mining waste. Residents have described several instances of deaths and crippling of foals in the area that were caused by Zn poisoning (Beyer *et al.* 2003). A local farrier developed an orthopedic horseshoe designed to alleviate the strain on the shortened tendons in the foals' feet (Beyer *et al.* 2003). A veterinarian diagnosed and treated Zn poisoning in ostriches on local ranches that were poisoned by ingesting chat in their pens (Beyer *et al.* 2003). Based on analyses of deer mandibles collected from hunters, Conder and Lanno (1999) concluded that white-tailed deer (*Odocoileus virginianus*) from the District were exposed to increased concentrations of Pb.

Recent work has demonstrated Zn poisoning in individual waterfowl from the District. Sileo *et al.* (2003) diagnosed Zn poisoning in three Canada geese (*Branta canadensis*) and a mallard from the District. Diagnoses were based on the finding of mild to severe degenerative abnormalities of the exocrine pancreas associated with hepatic and pancreatic Zn concentrations known to be toxic. Carpenter *et al.* (in press) tried unsuccessfully to rehabilitate a Zn-poisoned trumpeter swan (*Cygnus buccinator*) that appeared weak after it had been observed for 4 weeks on a millpond approximately 5 km east of Picher. The postmortem diagnosis was based on highly increased concentrations of Zn in its blood, liver, and kidneys and on pancreatic lesions. These examples demonstrate that wild birds are being poisoned in the District, but they fail to provide an overall picture of the extent of the contamination. Our study was designed to estimate the exposure of sampled populations of wild birds in the District to metals—emphasizing Pb, Zn and Cd—and to interpret the toxicity of the tissue concentrations detected.

Materials and Methods

Collection of Birds

We selected several species of birds having different routes of exposure to metals in this screening study. Northern bobwhites (*Colinus virginianus*) and mourning doves (*Zenaida macroura*) are ground gleaners and ingest some grit. Doves eat almost entirely seeds, whereas bobwhites eat some fruit, greens, and insects as well as seeds (Martin *et al.* 1951). American robins (*Turdus migratorius*) eat earthworms and insects and, as the summer progresses, shift their preference to include fruits and berries (Martin *et al.* 1951). Northern cardinals (*Cardinalis cardinalis*) eat mainly fruits, seeds, grains, and insects (Martin *et al.* 1951). Bank swallows (*Riparia riparia*) and rough-winged swallows (*Stelgidopteryx ruficollis*) are insectivorous and capture prey on the wing (Martin *et al.* 1951). They dig burrows (0.3 to 1.3 m) for nests directly in chat piles, thus exposing the young to metals through inhalation of dust and during preening (Niethammer *et al.* 1985). Only nestling swallows were collected. We collected a single brown thrasher (*Toxostoma rufum*), a species known to feed on soil and litter organisms. Health of the assessment populations was evaluated on the basis of residues in tissues, histopathologic lesions, and the activity of ALAD, an enzyme that serves as a marker of exposure to Pb (Hoffman *et al.* 2000).

We selected sites in the District where we believed collected birds would have been exposed to relatively high concentrations of metals. Soils from some of the potential sites were examined in advance by x-ray diffraction to verify that the soils were contaminated. Birds belonging to 13 species were collected between December 2000 and August 2001 with steel shot, decoy traps, mist nets, and by hand (Table 1) with collection permits from the United States Fish and Wildlife Service and the relevant states. Collections were timed to capture birds that would have been expected to have been in the area for at least a few weeks. Although the doves were collected only a little later in July than the passerines, they had formed flocks and were moving about. The doves were collected within the boundaries of District, but they were not from the sites that were most disturbed by mining. Reference birds (mallards, doves, swallows, robins, cardinals) were collected from uncontaminated sites (Table 1). Reference waterfowl were collected from the Neosho Wildlife Area, St. Paul, KS, and reference bank swallows were collected from cliffs on Chesapeake Bay, Chestertown, MD. Blood samples were collected from bobwhites in a captive colony at the University of Maryland for estimating a reference value of ALAD activity; no additional tissue samples were taken from these bobwhites.

Only one of the birds we planned to collect was noticeably unhealthy before it was collected. This was a Canada goose that appeared weak when observed and was retrieved by a dog and then examined by a veterinarian (Sileo *et al.* 2003). In addition to the planned collections, biologists from the Miami, OK, office of Bureau of Indian Affairs collected three mallards that appeared to be ill from Spring River, KS, in January 2001. These mallards were examined at necropsy at the National Wildlife Health Center, in Madison, WI. Analyses of the livers, kidneys, and pancreases are reported here.

Blood samples were collected through a needle into a vacutainer (both treated with lithium heparin) from either the jugular vein or from the heart of those birds that had just died. Hematocrits were read from a pair of centrifuged microhematocrit tubes. Remaining blood was split into two cryotubes, one frozen in liquid nitrogen for measuring ALAD activity and the second frozen for chemical analysis. Birds that had not died from shot wounds were killed with halothane or carbon dioxide. A portion of each liver and kidney was saved in buffered formalin for later sectioning and histopathologic examination, and a second portion was frozen for chemical analysis. We saved lungs from the swallows for histopathologic examination because they had been living directly in the chat piles and may have been exposed to inhaled dust contaminated with metals. We had adequate pancreatic tissue from waterfowl and bobwhites for both histopathology and chemical analyses but only enough pancreatic tissue from the other birds for histopathologic examination. Samples of digesta were collected from the large intestines and from the most distal portion of the small intestines of waterfowl, were frozen, and were saved for chemical analysis. Bobwhites and most of the waterfowl were examined by a veterinarian at necropsy. No lead shot was found in any of their gizzards.

Chemical and Histopathologic Methods

Samples of tissues were analyzed for elements at Research Triangle Institute in Research Triangle Park, NC. They were homogenized, freeze dried, digested in nitric acid in a microwave oven, and diluted. Lead was quantified by inductively coupled plasma–mass spectrometry and other elements (arsenic, cadmium, chromium, copper, iron, magnesium, manganese, nickel, selenium, strontium, vanadium, and zinc) by inductively coupled plasma–emissions spectroscopy on a Leeman Labs Plasma Spec 1. Values were reported on a dry weight (dw) basis. Results of quality-control procedures (reference materials, spikes, duplicates) were within acceptable bounds set by the Patuxent Analytical Control Facility (United States Fish and Wildlife Service, Laurel, MD).

Table 1. Collection data on birds from the Tri-State Mining District of Kansas, Missouri, and Oklahoma and reference sites

Species (sample size)	Method	Site (date)[a]
Birds from Tri-State Mining District		
Waterfowl		
Canada goose (*Branta canadensis*) (3)	Steel shot	2-KS (2/8/01), 14-OK (2/8/01)
Mallard (*Anas platyrhynchos*) (8)	Steel shot	5-OK (2/6/01), 2-OK (2/8/01), 3-OK (2/8/01), 9-OK (2/9/01), (3/3/01)
Common pintail (*A. acuta*) (2)	Steel shot	8-OK (3/3/01)
Green-winged teal (*A. crecca*) (1)	Steel shot	4-OK (2/9/01)
Ring-necked duck (*Aythya collaris*) (2)	Steel shot	7-OK (3/3/01)
Lesser scaup (*A. affinis*) (1)	Steel shot	1-KS (2/7/01)
Upland game birds		
Common bobwhite (*Colinus virginianus*) (7)	Decoy trap	3-KS (12/7/00), 8-OK (12/9/00, 7/12/01)
Mourning dove (*Zenaida macroura*) (12)	Steel shot	1-OK, 6-OK, 7-OK, 10-OK, 11-OK, 13-OK, 15-OK (7/15/01, 7/16/01)
Passerines		
Bank swallow (*Riparia riparia*) (9)	Taken from nests	12-OK (6/6/01, 6/8/01)
Rough-winged swallow (*Stelgidopteryx ruficollis*) (6)	Taken from nests	4-KS (6/7/01)
Brown thrasher (*Toxostoma rufum*) (1)	Mist nets	1-MO (7/12/01)
American robin (*Turdus migratorius*) (10)	Mist nets	1-MO (7/10/01, 7/11/01)
Northern cardinal (*Cardinalis cardinalis*) (8)	Mist nets	14-OK (7/10/01), 1-MO (7/11/01, 7/12/01), 8-OK (7/13/01)
Reference birds		
Waterfowl		
Mallard (*A. platyrhynchos*) (5)	Steel shot	3-Ref (2/9/01)
Upland game birds		
Mourning dove (*Zenaida macroura*) (4)	Steel shot	2-Ref (8/22/01)
Passerines		
Bank swallow (*Riparia riparia*) (7)		1-Ref (6/21/01)
American robin (*T. migratorius*) (6)	Mist nets	2-Ref (8/7/01, 8/19/01)
Northern cardinal (*Cardinalis cardinalis*)	Mist nets	2-Ref(8/2/01-8/16/01), 4-Ref(7/15/01)
Birds from district collected by Bureau of Indian Affairs		
Waterfowl		
Mallard (*A. platyrhynchos*) (3)	Found dead or injured	Spring River, KS (1/4/01, 1/5/01)

[a] Longitude and latitude of sites: site in Missouri: 1-MO (37°09.86′N, 94°28.78′W); sites in Kansas: 1-KS (37°05.73′N, 94°40.84′W), 2-KS (37°00.74′N, 94°45.80′W), 3-KS (37°00.79′N, 94°51.14′W), 4-KS (37°00.74′N, 94°51.63′W); sites in Oklahoma: 1-OK (36°59.98′N, 94°52.63′W), 2-OK (36°59.90′N, 94°48.08′W), 3-OK (36°59.89′N, 94°49.02′W), 4-OK (36°59.88′N, 94°46.01′W), 5-OK (36°59.44′N, 94°46.58′W), 6-OK (36°59.25′N, 94°54.15′W), 7-OK (36°59.25′N, 94°53.40′W), 8-OK (36°58.93′N, 94°51.66′W), 9-OK (36°58.56′N, 94°48.40′W), 10-OK (36°58.39′N, 94°54.43′W), 11-OK (36°58.08′N, 94°51.47′W), 12-OK (36°57.80′N, 94°51.71′W), 13-OK (36°57.48′N, 94°51.20′W), 14-OK (36°57.49′N, 94°50.75′W), 15-OK (36°56.97′N, 94°48.75′W); reference sites: 1-Ref (39°22′N, 76°04′W, in MD), 2-Ref (39°03.24′N, 76°48.82′W, in MD), 3-Ref (37°30.20′N, 95°09.11′W, in KS), 4-Ref (36°56.15′N, 95°10.89′W, in OK).

Blood samples were analyzed for ALAD activity as in Pain (1987), except that the sodium phosphate buffer was adjusted to a pH of 6.65, and the units (increase in absorbance of 0.100 at 555 nm with a 1.0-cm light path/ml of erythrocytes/h at 38°C) were expressed as in Burch and Siegel (1971).

Formalinized tissues were processed for histologic examination at the College of Veterinary Medicine, University of Wisconsin. All tissues were stained with hematoxylin and eosin and examined by light microscope without knowledge of tissue concentrations or collection sites. After metal tissue concentrations were estimated, however, those kidneys having the highest concentrations of Pb and a sample of kidneys from reference birds were stained with the Ziehl-Neelsen acid-fast method.

Results and Discussion

Lead

The histologic examination of the avian tissues revealed various lesions that are sometimes present in wild birds but none that we would attribute unequivocally to Pb toxicity. Based on tissue concentrations, however, we concluded that several birds collected from the District should be considered Pb poisoned. Pain (1996) developed criteria for evaluating Pb concentrations in tissues of individual waterfowl and concluded that biological functions may be impaired and that external signs of poisoning may be present when blood Pb is between 0.5 and 1.0 μg/ml or when hepatic Pb is between 6 and 15 mg/kg wet weight (ww) (approximately 20 and 50 mg/kg dw, respectively). Because avian blood weighs approximately 1.05 g/ml (Sturkie 1986), Pain's criterion for blood is equivalent to approximately 2.6 to 5.2 mg/kg dw. A female mallard collected in February 2000 at site 9-OK had Pb concentrations (dw) of 5.9 mg/kg in blood, 3.8 mg/kg in liver, and 51 mg/kg in kidneys. ALAD activity of the blood was 5 U compared with a mean of 270 U for reference mallards. A male ring-necked duck (*Athya collaris*) collected in March at site 7-OK had Pb concentrations of 41 mg/kg in liver and 43 mg/kg in kidneys. No blood was collected from this duck.

The highest tissue concentrations of Pb and associated ALAD activities measured in passerines from the District

Table 2. Highest concentrations of Pb in tissues of songbirds from the Tri-State Mining District (2001) compared with 10% quantiles of Pb in songbirds experimentally poisoned

Song birds	mg Pb/kg dw			ALAD activity (%)[a]
	Liver	Kidney	Blood	
Wild from Tri-State Mining District				
Robin 1 (immature from 1-MO)	12	34	2.2	7
Robin 2 (immature from 1-MO)	25	45	2.7	5
Cardinal (female from 1-MO)	13	25	3.6	9
Brown thrasher (male from 1-MO)	94	150	4.0	8
Swallow 1 (juvenile from 12-OK)	14	19	1.3	
Swallow 2 (juvenile from 12-OK)	7.5	11	1.4	
10% quantiles of lethally dosed songbirds[b]				
Red-winged blackbirds	27	33		
Brown-headed cowbirds	33	87		
Common grackle	67	220		

[a] Estimated as the ALAD activity of the bird divided by the mean for reference birds of the same species. The thrasher value was divided by the mean for reference robins.
[b] Beyer *et al.* (1988). Published concentrations expressed as ww were converted to dw assuming 70% moisture.
ALAD = δ-aminolevulinic acid dehydratase.

are listed in Table 2. The decrease in ALAD activity to <10% of reference values in four songbirds demonstrated increased exposure to Pb. Tissue concentrations may be compared with 10% quantiles of Pb concentrations in tissues of lethally poisoned songbirds, which are the concentrations at which sensitive birds in a population would be expected to die from Pb poisoning. Four songbirds (two robins, one cardinal, and one thrasher) had hepatic Pb concentrations comparable with the 10% quantiles for blackbird (*Agelaius phoeniceus*) livers or kidneys. Susceptibility among songbird species varies, however, as shown by the higher 10% quantiles for grackles (*Quiscalus quisqula*; Table 2). The single brown thrasher (*T. rufum*) had the highest hepatic (94 mg/kg) and renal (150 mg/kg) Pb concentrations of any bird collected in this study.

Red blood cell ALAD activities in blood of birds from the District verified that the sampled populations were exposed to increased concentrations of Pb (Table 3). ALAD activity was decreased by >50% in robins, cardinals, and waterfowl, and activity was decreased by slightly <50% in bobwhites. ALAD activity was not measured in swallows. We found highest mean Pb concentrations in livers and kidneys of robins and significantly increased Pb concentrations in the same tissues of waterfowl, swallows, and cardinals (Table 4). The low Pb tissue concentrations and normal ALAD activity of the doves collected may have been a result of their preference for agricultural grains (Mirarchi and Baskett 1994) to the vegetation present on the most contaminated sites or an artifact of our collecting. Because the doves were moving about, they may not have been feeding at the relatively contaminated sites.

The Pb concentrations detected in the swallows (Table 4) were noteworthy because the birds were nestlings and thus exposed to Pb for only a few weeks. We do not know if the higher mean hepatic Pb concentration in bank swallows (5.4 mg/kg vs. 2.8 mg/kg, Mann–Whitney U $p < 0.05$) than in rough-winged swallows was attributable to differences between the species or between collection sites. Custer *et al.* (2002) measured Pb in liver of nestling tree swallows

Table 3. Activity of the Pb-sensitive enzyme ALAD in blood of birds collected from the Tri-State Mining District and from reference sites (2001)

Birds	ALAD activity Tri-State		Mean (SD) [N[a]] reference sites		Relative activity (%)
Robins	65	(52) [6]	188	(106) [4]	35*
Cardinals	137	(67) [6]	343	(59) [6]	40*
Waterfowl	89	(63) [10]	217	(63) [5]	41*
Bobwhite	166	(48) [7]	297	(69) [6]	56*
Doves	167	(98) [5]	133	(37) [4]	126

[a] N = Sample size.
* Significant at $p < 0.05$ by Mann-Whitney U test.

(*Tachycineta bicolor*) at several Pb-contaminated sites along the Upper Arkansas River near Leadville, CO. They found a maximum mean of 0.54 mg/kg dw and concluded that the Pb was physiologically affecting the swallows (decreased ALAD activity). The mean of 4.2 mg of Pb/kg in liver of bank and rough-winged swallows collected in the District is approximately eight times that in swallows from the Leadville study.

The mean concentrations of Pb in livers of robins (9.3 mg/kg) and cardinals (4.7 mg/kg) from the District (Table 4) were similar to those reported in the livers (6.73 mg/kg dw) of song sparrows (*Melospiza melodia*) from the Coeur d' Alene River Basin, ID, which is known to be severely contaminated with Pb and Zn from mining wastes (Johnson *et al.* 1999). The mean inhibition of ALAD activity in songbirds from the District (65% in robins and 60% in cardinals) was similar to that detected in songbirds from the Coeur d'Alene River Basin (51% in song sparrows and 75% in robins) and slightly greater than that in songbirds collected near the Palmerton Zn smelters (47%) in Pennsylvania (Beyer *et al.* 1985). The United States Environmental Protection Agency concluded that both the Coeur d'Alene River Basin and the Palmerton Superfund sites should be remediated because of high concentrations of Pb.

Table 4. Mean concentrations of Pb, Cd, and Zn in tissues of an assessment population of birds from the Tri-State Mining District and reference sites (2001)

Species and tissue (site, sample size)	mg/kg dw					
	Pb		Cd		Zn	
	Mean	SD	Mean	SD	Mean	SD
Waterfowl						
Liver (Tri, 17)	4.1*	9.6	2.1	3.1	440**	710
Liver (ref, 5)	0.57	0.36	0.92	0.59	93	30
Kidney (Tri, 17)	11**	14	17	30	210*	240
Kidney (ref, 5)	0.86	0.42	7.4	4.6	80	8.3
Blood (Tri, 11)	2.1**	1.5	<0.04	—	41	40
Blood (ref, 5)	0.35	0.23	<0.04	—	22	2.5
Pancreas (Tri, 17)	2.9**	3.8	0.81	1.0	530	700
Pancreas (ref, 5)	0.18	0.078	0.49	0.30	210	59
Bobwhite						
Liver (Tri, 7)	0.16	0.12	8.5	11	91	20
Kidney (Tri, 7)	2.5	1.4	89	170	97	22
Blood (Tri, 7)	0.90	0.52	<0.02	—	35	3.7
Pancreas (Tri, 7)	0.41	0.18	1.8	2.5	130	26
Doves						
Liver (Tri, 12)	1.2	0.75	4.3	4.3	100	34
Liver (ref, 4)	1.3	1.0	2.8	1.4	82	17
Kidney (Tri, 11)	7.2	5.5	33	46	140	40
Kidney (ref, 4)	7.0	4.0	90	5.9	99	11
Blood (Tri, 5)	0.91	0.46	0.18	0.074	24	2.2
Blood (ref, 4)	0.77	0.41	0.20	0.040	23	0.92
Swallows						
Liver(Tri, 14)	4.2**	3.2	0.91**	0.33	140	80
Liver (ref, 7)	<0.02	0.014	0.33	0.086	110	22
Kidney (Tri, 14)	6.2**	4.5	0.65**	0.32	86	22
Kidney (ref, 7)	0.081	0.032	0.066	0.042	79	5.2
Blood (Tri, 14)	1.1**	0.71	0.18	0.030	28	9.3
Blood (ref, 6)	<0.04	0.067	0.18	0.049	29	3.0
Robins						
Liver (Tri, 10)	9.3	7.2	5.9**	3.3	92	31
Lliver (ref, 6)	5.1	3.8	1.3	0.42	120	16
Kidney (Tri, 10)	20*	12	21**	9.7	110**	9.9
Kidney (ref, 6)	8.9	7.1	3.0	1.2	88	5.7
Blood (Tri, 10)	1.7*	0.051	<0.1	—	21	1.7
Blood (ref, 5)	0.86	0.31	0.18**	0.032	22	1.6
Cardinals						
Liver (Tri, 8)	4.7**	4.3	2.8*	2.5	86	13
Liver (ref, 8)	0.48	0.59	0.49	0.24	100*	15
Kidney (Tri, 8)	9.4**	7.5	20**	21	100**	14
Kidney (ref, 8)	0.41	0.40	0.80	0.50	81	3.9
Blood (Tri, 7)	1.4**	1.1	<0.1*	—	23	2.1
Blood (ref, 7)	0.10	0.068	0.15	0.071	24	2.7

* Mean is significantly greater than paired mean (Tri-State versus reference) by Mann-Whitney U test at $p < 0.05$.
** Mean is significantly greater than paired mean by Mann-Whitney U test at $p < 0.01$.
Ref = reference site; Tri = Tri-State Mining District.

Cadmium and Other Elements

Concentrations of Cd in livers and kidneys of swallows, cardinals, and robins were significantly higher in birds from the District than in birds from reference sites (Table 4). The highest mean renal Cd concentration, 89 mg/kg, was detected in bobwhites. Furness (1996) concluded that threshold concentrations of Cd poisoning in birds might be expected at 40 mg/kg ww (approximately 130 dw) in liver and at 100 mg/kg ww (approximately 330 dw) in kidneys. One bobwhite collected from 8-OK had Cd concentrations of 32 mg/kg in liver and 470 mg/kg in kidneys. This renal Cd concentration is higher than the threshold Furness associated with nephropathy. This bobwhite's kidneys showed subtle changes (occasional karyomegaly and presumed hemosiderin in a few scattered proximal tubule epithelial cells and regenerating epithelium in one tubule) that may have been within normal limits for free-ranging bobwhites. Neither this bobwhite nor any of the other birds in this study had the proximal tubule cell necrosis associated with Cd poisoning. Mean hepatic concentrations of arsenic, chromium, copper, iron, magnesium, manganese, nickel, selenium, strontium, and vanadium in birds from the District were similar to those in reference birds (Table 5).

Zinc

Waterfowl were the only birds from the District that had significantly increased Zn concentrations in both livers and kidneys (Table 4). Tissue concentrations of Zn, however, have been shown to be imperfect indicators of exposure in birds. Songbirds from a site severely contaminated with Zn from smelting, for example, had whole-body Zn concentrations that were increased only 20% compared with concentrations in songbirds from a reference site, although there was a >10-fold difference in soil Zn concentrations (Beyer *et al.* 1985). Birds regulate Zn effectively within a wide range of exposure. In experimental studies on chickens, hepatic Zn concentrations remained constant as dietary concentration was increased from 37 to approximately 110 mg/kg but then increased >10-fold when the dietary concentration was increased to approximately 2200 mg/kg (Stahl *et al.* 1989). The proportional increase in Zn concentrations in kidneys and pancreases has been shown to be greater than that in livers of dosed birds (Gasaway and Buss 1972; Levengood *et al.* 1999). Day *et al.* (2003) found that mute swans (*C. olor*) fed a contaminated and suboptimal diet (rice) accumulated three times the concentration of hepatic Zn as did mute swans fed a commercial waterfowl maintenance diet with the same level of Zn and other metals.

Sileo *et al.* (2003) diagnosed Zn poisoning in all three Canada geese collected and in one of the mallards collected in the District. These four waterfowl had mild to severe degenerative abnormalities of the exocrine pancreas with Zn concentrations in livers and pancreases that were consistent with the tissue concentrations detected in waterfowl experimentally poisoned with Zn. The pancreatitis described has been widely used to diagnose Zn poisoning in pet or captive birds that have swallowed hardware or other items containing Zn (Droual *et al.* 1991; Zdziarski *et al.* 1994). One of the geese was emaciated and moribund when collected and had decreased motor function in its legs, a clinical sign of Zn poisoning (Gasaway and Buss 1972; Zdziarski *et al.* 1994; Grandy *et al.* 1968). Based on pancreatic lesions and increased tissue concentrations, Carpenter *et al.* (in press) diagnosed Zn poisoning in a trumpeter swan that had been observed on a mill pond for 4 weeks in February and March 2003, about 5 km east of Picher. This swan was weak, stumbled, and was taken to the College of Veterinary Medicine, Kansas State University, Manhattan, KS, for rehabilitation, but it died within 1 day.

The three mallards (National Wildlife Health Center Necropsy Report, Case 17088, ACC: 001, 002, 003) collected on Spring River by the Bureau of Indian Affairs also had increased tissue concentrations of Zn (Table 6). Mallards no. 1 and 2 were diagnosed with trauma and mallard no. 3 with caseous typhlitis (inflammation of the caeca). Although the etiology of the typhlitis remains uncertain, we note that Levengood *et al.* (1999) found typhlitis to be the most consistently observed lesion in mallards experimentally poisoned with zinc shot. The tissue had significantly autolyzed by the time the pancreases were fixed and no pancreatic lesions were noted in the necropsy report. Zinc concentrations in liver and pancreas of mallard no. 3 were consistent with those associated with death in two experimental studies on mallards (Table 6). Mallard no. 3 also had a highly increased hepatic concentration of Pb (7.3 mg/kg ww) and hemosiderosis of the liver and spleen but none of the gross lesions associated with Pb poisoning.

Although previous researchers have reported birds poisoned from ingesting galvanized hardware and other items containing Zn, the District is the first site at which severe effects of poisoning from environmental Zn have been demonstrated in wild birds. We recognize, however, that Zn poisoning may have been overlooked at other sites by pathologists unfamiliar with the signs of Zn poisoning and also that few mining sites are as severely contaminated with Zn as the District.

Routes of Exposure

Analyses of the intestinal digesta provide estimates of exposure of waterfowl to metals. Mean metal concentrations in milligrams per kilogram dw (SDs in parentheses) were higher (Mann-Whitney U test $p < 0.01$) in digesta from the District (N = 13) than in digesta from the reference site (N = 5) as follows: Pb 60 (68) versus 1.6 (1.4); Cd 7.5 (9.3) versus 0.89 (1.6); and Zn 1100 (900) versus 130 (38). The maximum concentrations in digesta of waterfowl from the District were 180 mg of Pb/kg, 35 mg/kg of Cd, and 3200 mg/kg of Zn. Concentrations of Pb, Cd, and Zn were closely correlated with each other (Spearman's rho = 0.70 for Cd vs. Pb, 0. 85 for Zn vs. Pb, and 0.85 for Zn vs. Cd; $p < 0.05$). The digesta are presumably a mixture of plant and invertebrate material with some sediment or soil. High concentrations of Zn interfere with the absorption of Cd and the high Zn-to-Cd ratio (approximately 150 to 1) and the close correlation between these two elements probably protects terrestrial food chains somewhat from Cd toxicity (Chaney *et al.* 2001). Average metal concentrations reported in aquatic invertebrates from Turkey and Center Creeks within the District in Missouri were 80 mg of Pb/kg, 3.6 mg of Cd/kg, and 460 mg of Zn/kg dw (Wildhaber *et al.* 2000).

The nestling swallows collected from the District were living in the most disturbed sites and were fed insects caught on the wing by adults. They may have been exposed to additional Pb from chat dust inhaled or ingested while preening. The brown thrasher, robins, and cardinals lived in less-disturbed habitats than did the swallows, but presumably they were exposed to Pb in soil ingested incidentally as well as to Pb in their food items. Brown thrashers are known for sweeping soil and litter with side-to-side motions of the bill to pick out food items, especially insects such as beetles (Cavitt and Haas 2000). Because they sometimes peck or hammer the soil with their bills, they may be especially likely to ingest soil and associated Pb. Ingestion of grit remains a potential source of Pb, although the two species most likely to be contaminated by this route of exposure, bobwhites and mourning doves, did not have high Pb tissue concentrations compared with the other species.

We are uncertain whether increased Pb levels in songbirds zinc should be attributed to historic smelting or to chat and

Table 5. Concentrations of elements in livers of birds from Tri-State Mining District and reference sites (2001)

Element	Site	mg/kg mean (SD) dw					
		Waterfowl	Bobwhite	Doves	Swallows	Robins	Cardinal
Arsenic	Tri	<0.2	<0.2	<0.25	<0.25	<0.25	<0.25
	Ref	<0.2		<0.25	<0.25	<0.25	<0.25
Chromium	Tri	<0 5	<0.5	<0.05	0.14 (0.018)	<0.05	<0.05
	Ref	<0.5		<0.05	0.17 (0.032)	<0.05	<0.05
Copper	Tri	85 (88)	18 (2.2)	14 (1.8)	18 (3.0)	17 (5.2)	20 (5.4)
	Ref	140 (90)		15 (3.3)	27 (13)	17 (2.7)	20 (7.4)
Iron	Tri	3400 (2300)	690 (260)	1200 (330)	1600 (470)	2300 (1500)	1600 (780)
	Ref	2800 (1800)		1400 (290)	690 (240)	2900 (700)	2200 (940)
Magnesium	Tri	660 (97)	620 (83)	690 (78)	750 (52)	670 (80)	640 (60)
	Ref	590 (130)		670 (62)	750 (39)	820 (49)	750 (26)
Manganese	Tri	13 (5.5)	15 (2.4)	12 (2.6)	5.2 (1.4)	5.3 (0.72)	6.9 (5.4)
	Ref	12 (4.6)		14 (2.6)	6.6 (1.0)	6.6 (1.3)	6.6 (1.4)
Nickel	Tri	<0.5	<0.5	0.64 (0.059)	0.44 (0.78)	2.3 (1.8)	1.3 (1.1)
	Ref	<0.5		1.7 (1.4)	<0.05	0.62 (0.20)	0.79 (0.27)
Selenium	Tri	3.5 (1.6)	1.1 (0.22)	1.9 (0.43)	4.5 (0.68)	1.9 (0.76)	2.2 (0.65)
	Ref	2.9 (1.1)		3.2 (0.30)	5.0 (0.66)	3.4 (1.1)	3.7 (0.86)
Strontium	Tri	<0.2	<0.2	<0.05	0.23 (0.098)	<0.05	<0.05
	Ref	<0.2		0.093 (0.046)	0.17 (0.033)	0.18 (0.12)	<0.05
Vanadium	Tri	1.1 (0.35)	0.52 (0.07)	0.31 (0.057)	<0.05	0.36 (0.036)	0.29 (0.045)
	Ref	0.83 (0.23)		0.24 (0.13)	<0.05	0.27 (0.11)	0.30 (0.071)

Table 6. Zinc concentrations in tissues of mallards collected from Spring River, Kansas, Tri-State Mining District (2001) compared with Zn concentrations in tissues of mallards dosed with Zn

Mallards	mg of Zn/kg dw[a]		
	Liver	Kidney	Pancreas
Mallards from Spring River			
Mallard 1	770	300	2400
Mallard 2	920	290	2000
Mallard 3	1100	620	7400
Laboratory references (means)			
Gasaway and Buss (1972)	180	90	300
Levengood *et al.* (1999)	210	90	290
Zinc-poisoned mallards (means)			
Gasaway and Buss (1972)[b]	1100–600	1000–1700	4200–8900
Levengood *et al.* (1999)	1300	1000	7300

[a] Ww in cited references was converted to dw by assuming 70% moisture.
[b] Means of treatment groups.
Data supplied by the Bureau of Indian Affairs, Miami, OK, Office; and the National Wildlife Center, case 17088, Accession Nos. 001, 002, 003.

other mining waste in the soil. The Pb from smelting in Joplin, MO, is definitely quite bioavailable to animals. Studying swine exposed to Pb in soil collected within approximately 0.3 km of the Joplin smelter, Mosby (2000) estimated the relative bioavailability of the Pb as 0.59 to 0.67 of Pb acetate, which is assumed to be 100% available. The Pb deposited from smelting is also concentrated at the soil surface where songbirds may feed. Although only a single steel pellet and no lead shot were found in waterfowl gizzards, spent lead shot is a potential source of Pb to waterfowl. In general, at least one lead shot has been found in gizzards of waterfowl diagnosed with Pb poisoning from lead shot (Beyer *et al*. 1998c).

Ingestion of contaminated sediments has been shown to be the most important route of exposure to Pb by waterfowl, other than to lead shot (Beyer *et al*. 1998a, 1998b). Beyer *et al.* (2000) concluded that some waterfowl mortality may occur in the Coeur d'Alene River Basin in wetlands with sediments contaminated with at least 1800 mg of Pb/kg. Sediment sampling in the District has provided a partial picture of contamination. Two sediment samples from Short Creek, KS, contained 1587 and 1607 mg of Pb/kg (Ferrington 1989). Sediment Pb concentrations detected in five creeks and rivers in mining areas in Jasper County, MO, were from 6.77 to 4290 mg of Pb/kg (Black and Veatch 1998).

The habitat in the District is contaminated to the extent that waterfowl are poisoned, but the route of exposure is uncertain. Zinc poisoning in the District seems to be hazardous primarily to waterfowl. Although captive ostriches were reported to suffer Zn poisoning from ingesting large amounts of chat (Beyer *et al*. 2003), this seems unlikely in free-flying birds. Canada geese and swans may be especially susceptible to metal poisoning because they ingest substantial amounts of sediment when they feed. In the Coeur d'Alene River Basin, Canada geese and tundra swans (*C. columbianus*) ingested an average of approximately 9% sediment in their diet (Beyer *et al*. 1998a). Some sediments in the District contain extremely high concentrations of Zn, as high as 22,000 and 25,000 mg/kg in Spring River at the mouth of Short Creek in Kansas (Ferrington 1989) and from 103 to 13,800 mg/kg in five creeks and rivers in mining areas in Jasper County, MO (Black and Veatch 1998).

Zinc is also taken up by freshwater vascular plants and has been reported at concentrations >1000 mg/kg dw at several polluted sites (Outridge and Noller 1991). Sequestration of Zn (and Pb) into iron plaque deposited on roots of aquatic plants (Hansel *et al*. 2001) is an additional potential route of exposure. Canada geese feed especially on rootstocks (Martin *et al*. 1951). We cannot rule out the possibility that geese are exposed to Zn through terrestrial food chains, however, because they feed also on agricultural grains (Mowbray *et al*. 2003). We do conclude, however, that the exposure of some birds to Zn in the District is high enough to cause serious toxic effects.

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