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Abstract Mining and smelting in the Southeast Missouri Lead Mining District has caused widespread contamination of soils with lead (Pb) and other metals. Soils from three study sites sampled in the district contained from approximately 1,000–3,200 mg Pb/kg. Analyses of earthworms [33–4,600 mg Pb/kg dry weight (dw)] collected in the district showed likely high Pb exposure of songbirds preying on soil organisms. Mean tissue Pb concentrations in songbirds collected from the contaminated sites were greater ($p < 0.05$) than those in songbirds from reference sites by factors of 8 in blood, 13 in liver, and 23 in kidney. Ranges of Pb concentrations in livers (mg Pb/kg dw) were as follows: northern cardinal (*Cardinalis cardinalis*) = 0.11–3.0 (reference) and 1.3–30 (contaminated) and American robin (*Turdus migratorius*) = 0.43–8.5 (reference) and 7.6–72 (contaminated). Of 34 adult and juvenile songbirds collected from contaminated sites, 11 (32 %) had hepatic Pb concentrations that were consistent with adverse physiological effects, 3 (9 %) with systemic toxic effects, and 4 (12 %) with life-threatening toxic effects. Acid-fast renal intranuclear inclusion bodies, which are indicative of Pb poisoning, were detected in kidneys of two robins that had the greatest renal Pb concentrations (952 and 1,030 mg/kg dw). Mean activity of the enzyme delta-aminolevulinic acid dehydratase (ALAD) in red blood cells, a well-established bioindicator of Pb poisoning in birds, was decreased by 58–82 % in songbirds from the mining sites. We conclude that habitats within the mining district with soil Pb concentrations of ≥1,000 mg Pb/kg are contaminated to the extent that they are exposing ground-feeding songbirds to toxic concentrations of Pb.

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The world's largest deposit of the mineral galena (lead sulfide), mined since 1721, lies in the Southeast Missouri Lead Mining District. As part of a Natural Resource Damage Assessment by the United States Department of the Interior, we investigated whether terrestrial habitat within the district is contaminated to the extent that wildlife might be poisoned. We selected ground-feeding songbirds for study, expecting them to have a greater dietary exposure to lead (Pb) than songbirds feeding in shrubs or in the canopy (Grue et al. 1986; Roux and Marra 2007). Our study combined modeling the potential exposure of songbirds to Pb through ingestion of earthworms with an examination of Pb tissue concentrations and associated toxicological effects.

Pb from tailings, milled mine waste (chat), smelting, and dust have contaminated areas surrounding mines in the district (Seeger 2008). Episodic dam failures, routine operational releases from mining and milling facilities, and ongoing erosion of tailings have contaminated stream sediments and floodplain soils with Pb and other metals. For example, a dam near the Desloge tailings pile was breached in 1977, releasing approximately 38,000 m^3 of tailings into the Big River (Niethammer et al. 1985). As a result, mean sediment Pb concentrations in Big River and Flat River Creek (Fig. 1) increased to approximately 3,200 mg/kg (Gale et al. 2004). Recent studies by Roberts et al. (2009) found high levels of Pb contamination in-stream sediment along more than 150 river km of the Big River. Pavlowsky et al. (2010) showed floodplain soil contamination of >400 mg/kg over 170 km of the Big River. Fine sediments from streams in the Viburnum Trend were found to have concentrations that were enriched in metals compared with reference values, as high as 95 times for Pb, 150 times for zinc (Zn), and 75 times for cadmium (Cd) (Brumbaugh et al. 2007). Fish from the area have increased concentrations of Pb in fillets (Czarnezki 1985), liver, and blood (Schmitt et al. 2007) and decreased activity of the enzyme ALAD (Schmitt et al. 1993). This enzyme is a biomarker for Pb toxicity in humans (Grandjean 1978; Gurer-Orhan et al. 2004). Elevated Pb concentrations have been detected in bullfrogs (*Rana catesbeiana*), muskrats (*Ondatra zibethicus*), green-backed herons (*Butorides striatus*), and bank swallows (*Riparia riparia*) in areas contaminated by mining (Niethammer et al. 1985). In 1970, emissions containing sulfur and Pb from the Buick Smelter in the Viburnum Trend were phytotoxic to the adjacent forest (Seeger 2008).

Mining and smelting may also have affected songbirds in the Southeast Missouri Lead Mining District, as it has at other sites. In Colorado, for example, sediment contamination of the Arkansas River from mining increased tissue concentrations of Pb in tree swallows (*Tachycineta bicolor*) feeding along the shore (Custer et al. 2003). Chapa-Vargas et al. (2010) found greater blood Pb concentrations in wild birds from a mining area in central Mexico than in wild birds from a reference area. In addition, Pb contamination from mining and smelting has been shown to be toxic to songbirds in the Coeur d'Alene River Basin in Idaho (Hansen et al. 2011), in the Tri-State Mining District (Oklahoma, Kansas, Missouri) (Beyer et al. 2005), and near the Palmerton smelters in Pennsylvania (Beyer et al. 1985). Pathologies associated with Pb poisoning in birds include abnormal neuromuscular function, kidney failure, liver failure, decreased fertility, anemia, and inhibition of heme-biosynthesis enzymes, such as ALAD (Eisler 2000; Berglund et al. 2010). Pb-poisoned songbirds do not exhibit the gross lesions typically observed in Pb-poisoned waterfowl, and consequently assessing toxicity must be based mainly on tissue concentrations (Beyer et al. 1988). Based on results from the Coeur d'Alene River Basin (Hansen et al. 2011), we expected that ground-feeding songbirds, which inadvertently ingest soil while feeding, would be exposed to especially high concentrations of Pb. Our aim is to determine whether soil Pb concentrations at contaminated sites within the mining district are causing toxicological injury to ground-feeding songbirds.

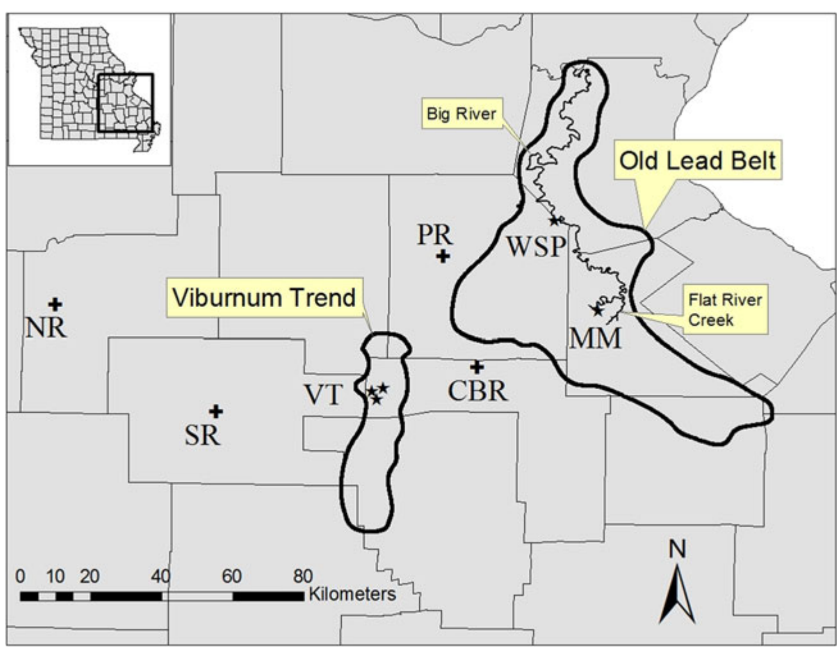

Fig. 1 Map of the two mining areas in the Southeast Missouri Lead Mining District and reference sites. The Viburnum Trend (VT) study site, in the New Lead Belt, and Missouri Mines (MM) in the Old Lead Belt were contaminated by smelting and mining. The Washington State Park (WSP) study site was contaminated by sediments deposited in the flood plain of the Big River. Reference sites in Missouri were located in Salem (SR), near Potosi (PR), and in the Mark Twain National Forest, near Newburg (NR) and at Council Bluff Lake Recreation Area (CBR)

Methods

Ores in the Mining District

Boundaries of the Southeast Missouri Lead Mining District are delineated as the geologic area within Mississippi Valley-Type deposits of sulfides of Pb, Zn, and copper (Cu) in Cambrian rocks of the St. Francois Mountains (Seeger 2008). Other elements associated with these sulfides include arsenic (As), Cd, cobalt (Co), and nickel (Ni). The mining district encompasses mainly the Old Lead Belt, to the east, and the New Lead Belt, to the west, also known as the Viburnum Trend (Fig. 1). Underground mining started in the Old Lead Belt in the 1860s and, by the early 1900s, approximately 15 companies were operating in the area. By 1960, eight and a half million tons of Pb had been mined in the Old Lead Belt located around Park Hills in St. Francois County. Mining activity decreased in the 1950s as ore bodies in the Old Lead Belt were depleted. Pb and Zn mining then began 80 km to the west in 1955, in the New Lead Belt, with the discovery of higher-grade ores in the counties of Reynolds, Iron, Dent, and Crawford (Seeger 2008). The Viburnum Trend refers to a geological area in the Ozarks marked by a series of Pb mines that run south from the city of Viburnum for approximately 40 km.

Earthworm Collections

To estimate the potential exposure of songbirds to Pb, we collected earthworms together with soil across a range of soil Pb concentrations in the mining district. Songbirds feeding on earthworms incidentally ingest internal and external soil associated with earthworms. Although earthworm tissue contains some Pb, the amount of Pb in earthworm tissue is generally far less than in the soil contained in their intestines. In June and October, 2009, earthworms and associated soils were collected from holes dug with a spade in 13 sampling plots at 5 locations in the district (Table 1). We rinsed the earthworms with distilled water and then froze them before they depurated their ingested soil. Earthworm and soil samples were sent to the United States Geological Survey Columbia Environmental Research Center to be analyzed for Pb, iron (Fe), Cu, Ni, Zn, Cd and aluminum (Al) (a marker for soil) by inductively coupled plasma-mass spectrometry (ICP-MS).

Avian Study Sites

Southeastern Missouri contains many small and a few large mining and smelting operations. Three contaminated sites within the mining district were selected for collecting songbirds, two in the Old Lead Belt and one in the Viburnum Trend. Our Missouri Mines site is adjacent to St. Joe State Park, where shallow pits were worked as early as 1742. The Federal Mine-Mill Complex at Missouri Mines Historic Site and adjacent to St. Joe State Park operated from 1906 to 1972 (Barr Engineering Co 2009). We selected for study a disturbed, forested habitat of American elm (*Ulmus americana* L.), chestnut oak (*Quercus prinus* L.), and sugar maple (*Acer saccharum* Marsh) located below the main tailings dam of Federal Mill and 0.4 km west of the buildings of the complex and a former chat pile.

Washington State Park, our second site, is in a riparian hardwood forest of American elm, sycamore (*Platanus occidentalis* L.) and box elder (*Acer negundo* L.) located on the Big River floodplain. Lead-contaminated sediments have been repeatedly deposited on the site's flood plain. Pavlowsky et al. (2010) found that eight cores of surface soil on the site's flood plain contained approximately 2,000 mg/kg Pb overlaying 6,000–8,000 mg/kg of Pb at a depth of approximately 1 m.

Our third site, Viburnum Trend, refers to three subsites within 2.5 km of Buick smelter and the Magmont Mine–Mill Complex, which are located in an area of active mining and smelting within Mark Twain National Forest (MTNF) and approximately 8 km south of Viburnum. The Buick Smelter, the first primary Pb smelter in the Viburnum Trend, is now a secondary smelter for recycled Pb. Sampled subsites include both a grassy area and parcels of mixed oak (*Quercus* spp.) and shortleaf pine (*Pinus echinata*) forest. Historic smelting, dust from tailings, and spillage from trucks have contaminated the general area, especially along roadsides. Current aerial emissions of Pb from recycling may also be contaminating avian diets.

"Reference songbirds" in this study refer to those expected to be exposed to usual background concentrations of metals in the absence of mining or smelting in the immediate vicinity. We selected multiple reference sites in Missouri to collect the same species of songbirds as from the three mining sites as follows: (1) MTNF Council Bluff Lake Recreation Area (approximately 14 km east of the Viburnum Trend and 18 km south-southeast of the Old Lead Belt); (2) MTNF south of Newburg near Mill Creek (approximately 70 km west-northwest from the Viburnum Trend); (3) a residential lawn near Potosi (30 km northeast of the Viburnum Trend and 4 km northwest of the mining sites within the Old Lead Belt); and (4) MTNF District Office in Salem (35 km west of the Viburnum Trend). In addition, we collected songbirds from an upland mixed hardwood and pine forest reference area on the grounds of the Patuxent Wildlife Research Center in Maryland that is remote from Pb mining.

Songbird Collection and Processing

We selected American robins (*Turdus migratorius*) for study because they are ground-feeding songbirds and were abundant at the sites. Robins eat earthworms and insects

Table 1 Metal concentrations (mg/kg, dw) in soils and earthworms from southeastern Missouri

Collection site (earthworms)	Species	Al	Ni	Fe	Cu	Zn	Cd	Pb
MTNF	*O. tyrtaeum, P. posthuma*	10,200	9.48	6,590	14.6	415	7.56	64
St. Francois State Park A	*L. terrestris*	8,800	15.8	10,100	41	610	160	683
St. Francois State Park B	*L. rubellus*	7,860	9.02	6,100	20.3	230	9.99	33.4
St. Francois State Park C	*P. posthuma*	13,100	15.4	12,100	29.8	411	48.2	618
St. Francois State Park D	*P. posthuma*	13,600	18.4	14,000	47.3	532	79	1,010
Washington State Park A	*P. posthuma*	7,700	12.3	9,120	33.8	1,110	117	1,100
Washington State Park B	*P. posthuma*	11,000	17.7	14,300	55.9	482	65.6	1,610
Washington State Park C	*P. posthuma*	15,500	19.4	15,500	51.1	645	73.6	1,260
Washington State Park D	*P. posthuma*	8,420	12.7	11,000	39.8	982	111	1,340
Missouri Mines A	*L. rubellus, P. posthuma*	3,910	9.84	5,310	42.6	881	40.6	1,210
Missouri Mines B	*L. rubellus*	830	12.8	8,180	61.7	968	42	576
Flat Rock Creek A	*P. posthuma*	8,830	30.2	15,400	92.5	395	50.4	1,320
Flat Rock Creek B	*P. posthuma*	6,490	34.5	19,500	174	618	62.9	2,890
Collection site (soils)	**Location**	**Al**	**Ni**	**Fe**	**Cu**	**Zn**	**Cd**	**Pb**
MTNF	37°44.075 N 90°54.899 W	27,800	25.6	19,300	27.5	166	0.86	136
St. Francois State Park A	37°57.262 N 90°32.225 W	18,200	27.8	21,900	59.2	661	10.9	1,550
St. Francois State Park B	37°57.724 N 90°32.059 W	22,800	20.9	15,800	25.9	280	2.21	104
St. Francois State Park C	37°57.842 N 90°32.050 W	20,600	29.2	22,200	47.4	544	8.74	1,350
St. Francois State Park D	37°57.811 N 90°32.073 W	12,100	32	24,000	68.5	736	12.9	1,850
Washington State Park A	38°5.255 N 90°40.509 W	17,500	27.3	20,100	61.1	643	8.65	2,030
Washington State Park B	38°5.186 N 90°40.937 W	16,000	28.7	22,500	68.4	656	9.19	2,290
Washington State Park C	38°5.142 N 90°40.924 W	26,100	33	24,800	64.5	737	10.5	2,030
Washington State Park D	38°5.158 N 90°40.564 W	15,900	27.2	22,300	60	572	7.83	1,990
Missouri Mines A	37°50.380 N 90°30.882 W	13,200	41.8	27,900	183	676	5.83	4,600
Missouri Mines B	37°50.300 N 90°30.914 W	2,470	57.4	37,700	234	570	9.24	2,240
Flat River Creek A	37°53.216 N 90°30.055 W	15,400	49.4	28,900	143	484	7.05	2,410
Flat River Creek B	37°53.202 N 90°30.047 W	8,170	49.5	30,000	231	678	14	3,730
Summary		**Al**	**Ni**	**Fe**	**Cu**	**Zn**	**Cd**	**Pb**
Soil means		16,600	35	24,400	98	569	8.3	2,020
Earthworm means		8,570	17	10,800	57	611	63	1,040
Ratio of metals in earthworms/soil[a]		0.52	0.49	0.44	0.58	1.07	7.64	0.51

[a] Geometric mean of ratios of metal concentrations in earthworms to metal concentrations in soil

early in the breeding season (Martin et al. 1951) and would be expected to consume more soil than most other species. Their dietary preference shifts to include fruits and berries in the summer. We also selected northern cardinals (*Cardinalis cardinalis*), which were abundant and eat mainly fruits, seeds, grains, and insects (Martin et al. 1951). Cardinals do not depend on soil and litter organisms to the same extent as robins, but they do feed on the ground in the early spring (Halkin and Linville 1999) and are likely to ingest soil associated with fallen seeds and insects on the ground. Cardinals have been reported to have an average home range of 1.18 ha in Tennessee (Dow 1969) and robins have been reported to forage an average distance of 93 m from their nest sites in New York (Knupp et al. 1977). We collected a few additional species for comparison with robins and cardinals. Sampling occurred during the breeding season when the assessment population would have been in the area for at least a few weeks and when their foraging area would have been small.

Birds were captured and handled under procedures approved by the Patuxent Animal Care and Use Committee. Songbirds were collected at least 4 weeks after spring migration and during their nesting season when they were expected to be on breeding territories. Songbirds were collected from reference sites and contaminated sites in Missouri from June 16 to 25, 2009, and May 12 to 21, 2010, and from the Patuxent Wildlife Research Center from July 22 to August 5, 2009. Collections were performed under permits from the United States Fish and Wildlife Service and the State of Missouri. We set up mist nets at sites where songbirds were expected to be abundant and checked them approximately every 40 min, but we checked more frequently when ambient temperatures were high. Nets were closed in rainy or very hot weather. Recorded bird calls were played to lure songbirds into the nets. Several robins, but few other songbirds, were caught in traps designed for doves when baited with mealworms.

Once captured, target songbirds were identified and weighed. We drew blood (approximately 1 % of body weight) from the jugular vein through a 26-gauge hypodermic needle (rinsed with sodium heparin) into a 1-ml syringe. Blood was apportioned to provide a minimum of the following: 200 µl for metal analysis, frozen in acid-washed, preweighed test tubes; 100 µl for ALAD in cryotubes frozen in liquid nitrogen; and 30 µl in microhematocrit tubes that were centrifuged to obtain hematocrits. Red-blood cell ALAD activity was measured as described in Pain (1987), except that ALAD 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 according to Burch and Siegel (1971), and the sodium phosphate buffer was adjusted to pH 6.65. Songbirds were killed immediately after blood was collected. Portions of the liver, kidney, and pancreas of each study bird were fixed in 10 % buffered formalin and submitted to the Wisconsin Veterinary Diagnostic Laboratory in Madison, Wisconsin, to be processed for microscopic examination. Tissue sections were stained by the hematoxylin and eosin, Fite's acid-fast, and Perls iron methods and examined by light microscopy at the United States Geological Survey (USGS) National Wildlife Health Center in Madison, Wisconsin. The pathologist examining the slides was blind to the geographic origin and Pb concentrations of the samples.

Remaining portions of liver, kidney, and blood were shipped to the USGS Columbia Environmental Research Center for analysis of Pb, Zn, Cd, and Cu, expressed on a dry-weight (dw) basis. When discussing tissue concentrations reported as wet weight (ww) in the scientific literature, we converted to dry weights by multiplying concentrations in the liver (66 % moisture) by 2.94 and concentrations in the blood (81.7 % moisture) by 5.46 (Hansen et al. 2011).

Soil Sample Collection

One composite sample of eight soil cores was collected at each site, with each core being taken at a depth of 7.5 cm and 3 m from an avian mist net. These samples were sent to the USGS Columbia Environmental Research Center where concentrations of Pb, Zn, Cd, Cu, Al, Fe, and Ni were quantified.

Chemical Methods

Earthworms, avian tissues, and sieved soils (2 mm mesh) were analyzed at the Columbia Environmental Research Center in Columbia, Missouri. Digestion methods depended on the matrices. Concentrated HNO_3 and HCl were added to subsamples of freeze-dried earthworms and soils, the mixture was heated, and "total recoverable" metals were extracted using a method similar to United States Environmental Protection Agency (USEPA) method 3051 (Brumbaugh et al. 2007). Freeze-dried blood samples were digested in capped tubes, to which concentrated HNO_3 was added. The bottoms of the tubes were placed in a dry-block heater set to 110 °C, and the tops were cooled by air flow to condense the volatilized acid (Brumbaugh et al. 2005). Freeze-dried liver and kidney tissues were digested in concentrated HNO_3 and 40 % high-purity H_2O_2 in tetrafluoroethylene-lined vessels heated in a microwave oven to 200 °C (Brumbaugh et al. 2005). After digestion, all samples were diluted with ultrapure H_2O and analyzed by ICP-MS. Avian tissues were analyzed for Pb, Zn, Cu and Cd. Soil and earthworms were analyzed for those metals and for Al, Ni, and Fe. Blanks, certified reference materials, spikes, and duplicate digestate analyses in all runs were within the quality-control guidelines of the Columbia

Environmental Research Center. With the exception of Cd concentrations in three blood samples from reference birds, measured values were greater than the method detection limits. Those values lower than the detection limit were approximated as one-half of the method detection limit for use in statistical tests. All analytical results are expressed as dw.

Statistics

Pb concentrations in earthworms were linearly regressed by least squares on concentrations of Pb in soil where the earthworms were collected. We compared tissue metal concentrations and ALAD activities in songbirds from mining study sites with those measured in reference birds with Dunn's method following a Kruskal–Wallis analysis of variance (ANOVA) based on ranks. Concentrations of Pb in livers were related to concentrations of Pb in soil by Spearman rank order correlations. The probability of a lesion randomly occurring in 2 of 13 kidneys also having the highest Pb concentrations was calculated as $1/13 \times 1/12 \times 2$ or $p = 1.28$ %. Pearson's Chi square test was performed to determine whether the greater frequency of elevated blood Pb concentrations in songbirds from mining study sites than that in reference birds might have occurred by chance. Liver Pb concentrations in cardinals were linearly regressed by least squares on soil Pb concentrations. However, because the variances were not homogeneous, but rather increased with soil Pb concentration, the regression was recalculated with data weighted by the reciprocal of the predicted liver Pb concentration. Statistics were calculated using SigmaPlot 12.5 (Systat Software, San Jose, CA, USA).

Results

Soils

Concentrations of Pb, Zn, and Cd detected in soils from our reference sites were generally consistent with published background concentrations of those metals in St. Francois County, where Missouri Mines is located, and in Missouri as a whole (Table 2). Soil Pb, Zn, and Cd concentrations from the Potosi reference site were slightly greater than background concentrations shown. We consider Pb, detected at 1,000 to 3,200 mg Pb/kg at the three mining sites, to be the most important terrestrial contaminant to birds in the mining

Table 2 Soil metal concentrations from southeastern Missouri compared with published values

Locations[a]	Pb (mg/kg)	Zn (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Ni (mg/kg)
Reference sites					
MTNW (Newburg)	51	60	0.19	14	18
MTNW (Council Bluff)	35	48	0.15	10	13
Salem	31	39	0.13	8	8.8
Potosi	85	149	0.69	19	19
Patuxent Wildlife Research Center	51	44	0.09	10	11
Mean	51	68	0.25	12	14
Mining sites					
Missouri Mines	3,200	1,020	9.7	250	58
Washington State Park	1,700	53	7.3	53	27
Viburnum Trend	1,000	70	1.7	29	10
Mean	2,000	540	6.2	110	31
Published values					
Background Missouri[b]	22	55	0.53		
Background St. Francois County[b]	62	71	0.63		
Missouri agricultural soils[c]	20	60	0.27	18	24.3
Ratio of mean mining/mean reference	39	7.9	25	9.0	2.3

[a] Locations: (1) MTNW (Newburg) = 37°43′52.292″N, 90°55′45.707″W; (2) MTNW (Council Bluff) = 37°44′2.632″N, 90°55′1.097″W; (3) Salem = 37°38′0.262″N, 91°32′10.175″W; (4) Potosi = 37°58′7.137″N, 90°54′32.537″W; (5) Patuxent Wildlife Research Center = 39°03′17.730″N, 76°49′7.830″W; (6) Missouri Mines = 37°50″23.378″N, 90°30′53.638″W; (7) Washington State Park = 38°05′12.229″N, 90°40′26.292″W; and (8) Viburnum Trend = 37°39′21.330″N, 91°07′15.583W; 37°37′42.991″N, 91°08′0.444W, and 37°38′43.006N, 91°9′8.061″W

[b] NewFields 2006 (geometric means)

[c] Holmgren et al. 1993 (arithmetic means)

district, although concentrations of Zn and Cd in the mining soils were greater than the USEPA Ecological Soil Screening Levels (46 mg Zn/kg and 0.77 mg Cd/kg, respectively [http://www.epa.gov/ecotox/ecossl/]) meant to protect biota. Ratios of mean soil metal concentrations from mining study sites to those from reference sites were 39 for Pb, 7.9 for Zn, 25 for Cd, 9.0 for Cu, and 2.3 for Ni (Table 2). Each of these metals is associated with Mississippi Valley Type deposits of sulfides (Seeger 2008).

Earthworms

Three members of the Lumbricidae (*Octolaseum tyrtaeum, Lumbricus terrestris*, and *L. rubellus*) and one member of the Megascolidae *(Pheretima posthuma)* family were collected at the sites. *P. posthuma* is a peregrine species native to the Orient, and, although abundant at the sites, seems not to have been reported in Missouri previously.

Pb concentrations in earthworms from the mining district may be predicted from Pb concentrations in soil at the collecting site. The regression of earthworm Pb concentration on soil Pb concentration [worm Pb = 146 + (0.449 × soil Pb)] was significant at $p = 0.05$ with an adjusted r^2 of 0.51 (Fig. 2). Al may be used as a marker for soil in earthworms because earthworm tissue contains only very low concentrations of this metal (Beyer and Stafford 1993). The geometric mean of the ratios of Al concentration in earthworms to Al concentration in soil was 0.52 (Table 1) indicating that the dry weight of the ingested soil was approximately half that of the total earthworm dry weight. Comparable geometric means of the ratios for Ni and Fe, additional potential soil markers, were slightly lower (0.46, 0.44) but also close to 0.5 (Table 1). Cd, in contrast, was concentrated in earthworm tissue and had a geometric mean of 7.4. The geometric mean of the Pb ratios was 0.48, which is comparable with the estimated proportion of soil in the earthworms. This means that most of the Pb in the earthworms could be attributed to soil in their intestines. Some samples of earthworms contained a higher fraction of soil than did others and, when we corrected for these differences (using the Al concentration detected in earthworms and in matched soils), the adjusted r^2 of the regression increased to 0.88. As might be expected, the Pb concentration in the whole earthworms depends mainly on two variables: the soil Pb concentration and the amount of soil in the earthworms. Even without the correction for the amount of soil ingested, however, the regression relating earthworm Pb concentrations to soil Pb concentrations provides us with a straightforward means to predict the exposure to Pb of a robin eating earthworms in southeastern Missouri for a given soil Pb concentration.

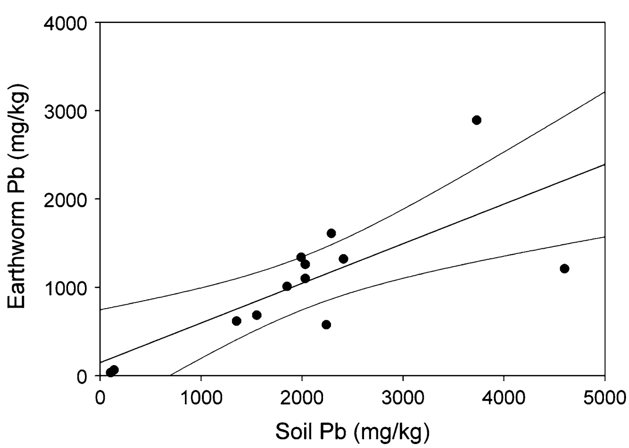

Fig. 2 Regression of Pb concentrations (with 95 % confidence intervals) in earthworms (dw) on soil Pb concentrations from 13 locations in southeastern Missouri. The regression [worm Pb = 146 + (0.449 × soil Pb)] is significant at $p < 0.01$ with an adjusted r^2 of 0.51. Because the earthworms were not purged of soil, Pb in the earthworms is mainly from the soil in the earthworms' intestines

Songbirds

Our assessment population contained 34 adult and juvenile songbirds captured from Missouri Mines, Washington State Park, and Viburnum Trend: 15 northern cardinals, 6 American robins, 8 blue jays (*Cyanocitta cristata*), 3 eastern towhees (*Pipilo erythrophthalmus*), 1 wood thrush (*Hylocichla mustelina*), and 1 Carolina wren (*Thryothorus ludovicianus*). Thirty-nine songbirds were captured from the reference sites: 18 northern cardinals, 7 American robins, 5 brown-headed cowbirds (*Molothrus ater*), 2 eastern towhees, 4 gray catbirds (*Dumetella carolinensis*), 1 eastern phoebe (*Sayornis phoebe*), 1 wood thrush, and 1 purple martin (*Progne subis*). Cardinals accounted for 44 % of the birds collected at the contaminated sites and 46 % of those collected in the reference sites, and robins accounted for 18 % of the total at both contaminated and reference sites.

Pb concentrations in blood, liver, and kidney tissues were markedly greater in songbirds from the three mining study sites than in songbirds from reference sites (Fig. 3) by a factor of 8 in blood, 13 in liver, and 23 in kidney ($p < 0.05$, Table 3). Mean Pb concentrations in all tissues of all songbirds combined from each of the three mining study sites (analyzed separately) were also significantly greater than the reference means of the three tissues ($p < 0.05$, Table 4). (Data on individual songbirds are shown in Supplementary material Table 1) Concentrations of Pb were greater in robins than in cardinals, especially in kidneys (Fig. 3; Table 3). Concentrations of Pb in kidneys of two robins from the Viburnum Trend site were approximately a thousand mg/kg (952, 1,030). Tissue Pb concentrations in cardinals were generally greater than in other songbirds (Fig. 3). Cd concentrations in tissues of songbirds from mining study sites were several times those

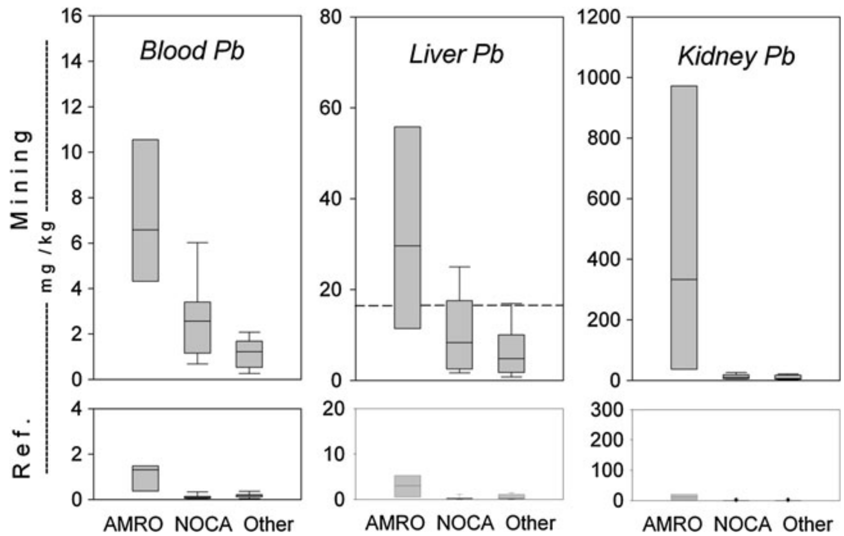

Fig. 3 *Box plots* (median and 5th, 25th, 75th, and 95th percentiles) illustrating tissue Pb concentrations (dw) in *AMRO* American robins (only median and 25th and 75th percentiles), *NOCA* northern cardinals, and *other* other songbirds. Note the much greater Pb concentrations in songbirds from the Southeast Missouri Lead Mining District than in reference songbirds. Pb concentrations are highest in robins, followed by cardinals, and then other songbirds. Most of the robins and approximately one quarter of the cardinals from the mining sites have liver Pb concentrations greater than the systemic toxic effects threshold (17.6 mg Pb/kg), which is illustrated with a *dotted line*. Robins from both mining and reference sites store a much greater fraction of their Pb in kidney than in liver tissue compared with cardinals

Table 3 Mean metal concentrations [±SEM (mg/kg dw)] in tissues of birds from the Southeast Missouri Lead Mining District and reference areas

Metal and tissue	All songbirds		Cardinals		Robins	
	$N = 39$ Reference	$N = 34$ Mining	$N = 18$ Reference	$N = 15$ Mining	$N = 7$ Reference	$N = 6$ Mining
Pb						
Blood	0.38 ± 0.12	2.91 ± 0.56*	0.12 ± 0.026	2.50 ± 0.51*	1.42 ± 0.53	7.36 ± 1.74*
Liver	1.04 ± 0.27	13.8 ± 2.90*	0.45 ± 0.16	10.5 ± 2.33*	3.41 ± 1.10	33.6 ± 10.04*
Kidney	4.24 ± 2.25	99.6 ± 46.8*	0.68 ± 0.23	12.6 ± 2.23*	20.1 ± 11.3	453 ± 180*
Zn						
Blood	21.8 ± 0.76	22.4 ± 1.50	22.5 ± 1.00	22.0 ± 1.34	20.7 ± 1.06	19.5 ± 0.50
Liver	99.6 ± 2.83	95.9 ± 3.40	103 ± 3.84	96.9 ± 4.99	91.6 ± 2.70	96.0 ± 6.15
Kidney	92.7 ± 2.30	94.3 ± 2.44	89.4 ± 2.70	94.2 ± 4.0	110 ± 3.96	101 ± 4.27
Cu						
Blood	1.54 ± 0.14	1.53 ± 0.10	1.72 ± 0.27	1.40 ± 0.045	1.34 ± 0.074	1.71 ± 0.44
Liver	20.8 ± 1.01	19.4 ± 0.91	23.7 ± 1.84	21.5 ± 1.56	17.6 ± 0.65	16.0 ± 0.84
Kidney	23.2 ± 2.33	21.7 ± 1.45	16.9 ± 0.64	17.1 ± 0.53	48.3 ± 7.58	35.6 ± 2.64
Cd						
Blood	0.0063 ± 0.00086	0.016 ± 0.0024*	0.0063 ± 0.00094	0.017 ± 0.0045*	0.0037 ± 0.00068	0.028 ± 0.0031*
Liver	2.06 ± 0.32	7.97 ± 1.35*	1.20 ± 0.203	5.50 ± 1.09*	4.03 ± 0.73	19.9 ± 2.64*
Kidney	13.2 ± 2.40	33.0 ± 5.23*	7.16 ± 1.63	32.2 ± 8.27*	28.4 ± 7.20	52.8 ± 12.0

* Comparison of mining with its reference value was significant at $p < 0.05$ (Dunn's method following Kruskal–Wallis ANOVA on ranks)

in reference songbirds ($p < 0.05$), but Zn and Cu concentrations showed no difference (Table 3).

Tissue Pb concentrations varied considerably among songbirds even within a site. The Spearman rank correlation coefficient relating liver Pb concentrations to soil Pb concentrations was 0.74 ($p < 0.01$) for all species of songbirds combined. For cardinals alone, the main species collected, the correlation coefficient was 0.85 ($p < 0.01$). We calculated the linear regression of cardinal liver Pb concentrations on soil Pb concentrations as: liver $\text{Pb} = 0.14 + 0.0057 \times \text{soil Pb}$;

Table 4 Mean [±SEM (mg/kg dw), *N*] tissue Pb concentrations and ALAD activity in songbirds collected from contaminated sites in the Southeast Missouri Lead Mining District and from reference sites

Site	Missouri Mines	Washington State Park	Viburnum Trend	Reference
Soil Pb (mg/kg)[a]	3,200	1,700	1,000	30.9–85.2
Blood Pb (mg/kg)	3.21 ± 0.56 (10)*	2.05 ± 0.61 (12)*	3.50 ± 1.30 (12)*	0.38 ± 0.12 (39)
Liver Pb (mg/kg)	12.7 ± 2.52 (10)*	10.3 ± 3.04 (12)*	17.1 ± 6.73 (12)*	1.04 ± .27 (39)
Kidney Pb (mg/kg)	46.6 ± 28.8 (10)*	13.2 ± 2.96 (12)*	204 ± 111 (12)*	4.24 ± 2.25 (39)
ALAD (U)	53.5 ± 35.5 (10)*	123 ± 31.5 (12)*	87.5 ± 22.0 (12)*	295 ± 25.0 (39)

An asterisk indicates that comparison of a value with its reference was significant at $p < 0.05$ (Dunn's method following a Kruskal–Wallis ANOVA on ranks)

[a] The reference value shows a range of composite samples from five sites; the values from the contaminated sites were measured in a single composite sample

because the variances increased with soil Pb concentrations, however, we could not calculate confidence limits for this regression. Instead, by assuming that the variance increased proportionately with soil Pb concentrations, and by weighting the data by dividing by the predicted means (SigmaPlot 12.5), we calculated the regression as: liver Pb = 0.15 + 0.0057 × soil Pb, with confidence intervals (Fig. 4). This graph illustrates both the variability in tissue Pb concentrations among birds at a site as well as the dependence of mean tissue Pb concentrations on soil Pb concentrations.

The mean hematocrit of songbirds from the mining study sites (43.7 %) was the same as the mean hematocrit (43.7 %) of reference songbirds. The mean activity of red-blood cell ALAD in songbirds (92 units) from mining study sites was 31 % of its mean activity in songbirds (295 units) from reference sites ($p < 0.05$ by Mann–Whitney rank sum test), and the inhibition was also significant for robins (11 *vs*. 116 units; $p < 0.05$), for cardinals (94 *vs*. 347 units; $p < 0.05$) and for all other species (132 *vs*. 334 units; $p < 0.05$), when tested separately. Activity of ALAD was decreased ($p < 0.05$) by an average of 58 % at Washington State Park, 70 % at the Viburnum Trend, and 82 % at Missouri Mines.

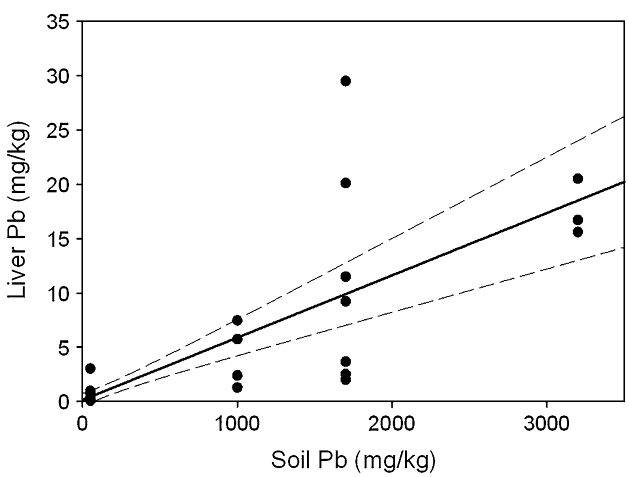

Fig. 4 Weighted least squares regression of cardinal liver Pb concentrations (mg/kg) on soil Pb concentrations (mg/kg) with 95 % confidence limits of the slope (liver Pb = 0.14 + 0.0057 [soil Pb], $p < 0.01$). Note the strong linear relation of liver Pb concentrations to soil Pb concentrations despite the considerable variability of liver Pb concentrations among cardinals at a site. The points were weighted as the reciprocal of the predicted liver Pb concentration

Acid-fast intranuclear inclusion bodies were found in proximal tubular epithelial cells on histopathological examination of kidneys from 2 robins collected from the Viburnum Trend study site. These two robins had the highest concentrations of renal Pb (952 and 1,030 mg Pb/kg dw) in the study, well above the mean for all birds from mining study sites (99.6 mg/kg dw) and those from reference sites (4.24 mg/kg dw) (Table 3). The probability of 2 of 13 robins having those lesions and also the 2 highest renal Pb concentrations would be 1.3 % ($p < 0.05$ %) if the events happened by chance. No other Pb-associated abnormalities were observed in liver, pancreas, or kidney tissues of birds from the mining study sites or the reference sites.

Discussion

Earthworms

The regression equation relating Pb concentrations in earthworms to soil Pb concentrations may be used to estimate the site-specific exposure of earthworm-eating songbirds to Pb. American robins were found to have ingested a mean of approximately 20 % soil in their diets, by dry weight, in the Coeur d'Alene River Basin (Hansen et al. 2011). Because the earthworms we analyzed in this study contained approximately 50 % soil, this 20 % soil in the diet would be equivalent to ingesting 40 % earthworms by weight in the diet. (Alternately, we could think of the robins as ingesting <40 % earthworms, but some additional soil associated with other items in their diet, to bring the total soil content to 20 % of the diet.) To illustrate a robin's estimated exposure to Pb, we assume that a soil from the mining district contained 1,000 mg Pb/kg dw. Then, from our equation [worm Pb = 146 + (0.449 × soil Pb)], we would estimate that earthworms found in that soil would contain 595 mg/kg of Pb. If we ignore additional Pb in the diet, then a robin ingesting a diet that was 40 % earthworms would be ingesting an estimated 238 mg/kg dw of Pb (40 % of 595) along with the 20 % soil. This value is well above the National Research Council's (2005) estimate of 10 mg/kg of Pb as a maximum tolerable level in the diet of poultry (as well as in diets of rodents, swine, and horses). Bioavailability of Pb in the district may be considered. Mosby (2000) estimated that the relative bioavailability of Pb in mining soil from Joplin, Missouri, was approximately 0.59 to 0.67 of the bioavailability of Pb acetate, which is considered to have a bioavailability of 100 %. If we estimate relative bioavailability as 0.63, i.e., the average of the two values, then a robin eating a diet of 40 % earthworms would ingest the equivalent of 150 mg Pb/kg, which is 15 times the maximum tolerable level set by the National Research Council.

Soils

Soil Pb concentrations (1,000– 3,200 mg/kg dw Pb) at the three mining study sites may be compared with target cleanup levels typically derived in ecological risk assessments that estimate Pb exposure in wildlife. Four examples of these levels published in the Records of Decision from the USEPA are: (1) 380 mg of Pb/kg as a preliminary goal based on robins (Jacobville Neighborhood Soil Contamination Site, USEPA, Region 5, September 2009, http://www.epa.gov/region5/cleanup/jacobsville/pdfs/jacobsville_rod_200909.pdf; accessed May 7, 2013); (2) 400 mg/kg of Pb as a cleanup level based on wildlife feeding on earthworms in the Tri-State Mining District (OU3 and OU4, Cherokee County, KS, Superfund Site, USEPA, September, 29, 2006, http://www.epa.gov/superfund/sites/rods/fulltext/a2006070001149.pdf; accessed May 7, 2012); (3) 500 mg of Pb/kg as a cleanup level based on exposure of ground-feeding insectivores (OU4, Tar Creek Superfund Site, USEPA, Region 6, February 20, 2008, http://www.epa.gov/region6/6sf/oklahoma/tar_creek/ok_tar_creek_ou4_rod_200802.pdf; accessed May 7, 2012); and (4) 605 mg of Pb/kg as the recommended remedial goal based on exposure of shrews and woodcock (Raleigh Street Dump Site, USEPA, Region 4, June 2009, http://www.epa.gov/superfund/sites/rods/fulltext/r2009040003099.pdf; accessed May 7, 2013).

In an ecological assessment of the risks of Pb to songbirds in the Coeur d'Alene River Basin, Sample et al. (2011) regressed Pb concentrations measured in blood on Pb in soil to set a preliminary remedial goal of 490 mg/kg of Pb in soil to protect robins. We conclude that soil Pb concentrations at the three mining study sites in Missouri are well above those that would be considered hazardous based on ecological risk assessment guidelines.

Songbirds

From the earthworm analyses (Table 1), we know that songbirds feeding on earthworms or other dietary items contaminated with soil would be exposed to increased concentrations of Pb, Cu, Zn, and Cd. Average concentrations of Cu and Zn in songbird tissues, however, were not significantly greater in songbirds from the mining district than in reference songbirds. As has been shown in other studies of Zn or Cu in wildlife (Beyer et al. 2005; Reynolds et al. 2006), homeostatic regulation of these metals in tissues prevents accumulation of these metals at moderate exposures. Cd concentrations in tissues of songbirds collected from the mining study sites were greater than those in songbirds from reference sites but lower than the threshold concentrations associated with injury to birds (0.26 mg/kg ww in blood, 45 mg/kg ww in liver, and 65 mg/kg ww in kidney) suggested by Wayland and Scheuhammer (2011).

Mean tissue Pb concentrations detected in songbirds from the Southeast Missouri Lead Mining District were greater than those reported from the Tri-State Mining District (Beyer et al. 2005) and the Coeur d'Alene River Basin (Hansen et al. 2011), two sites at which toxic concentrations of Pb have been reported in songbirds. Our results may also be compared with those of Roux and Marra (2007), who collected robins and cardinals from urban sites (approximately 300 mg of Pb/kg soil) near Washington, District of Columbia, and Baltimore, Maryland, USA. Mean blood Pb concentrations in robins (7.36 mg/kg dw) and cardinals (2.50 mg/kg dw) from our contaminated sites were approximately 4-5 times as high as in those species from these urban areas [robins = 0.264 mg/kg ww (approximately 1.44 mg/kg dw); cardinals = 0.112 mg/kg ww (approximately 0.61 mg/kg dw)].

Franson and Pain (2011) concluded that 1.04 mg/kg of Pb in blood (dw; converted from 0.2 mg/kg ww) was the upper limit of what would be considered a background concentration of Pb in birds. Pb concentrations in blood samples of 4 robins of 38 reference songbirds exceeded that value. In contrast, 24 of 31 of blood samples from songbirds collected from the mining study sites had Pb concentrations >1.04 mg Pb/kg dw. This difference was significant ($p < 0.05$) when examined with Pearson's Chi square test. Buekers et al. (2009) estimated a "critical" concentration for protecting birds of 71 µg Pb/dl blood (approximately 3.9 mg/kg Pb dw) from the fifth percentile of blood Pb concentrations associated with no effect levels in avian toxicity studies measuring growth, reproduction, hematological, or physiological variables. One robin from a reference site and 5 robins and 2 cardinals from mining study sites had blood Pb concentrations exceeding that value. Analyses of tissues in addition to blood corroborated the high exposure to Pb of songbirds from the mining study sites. Sixteen of 30 songbirds from the mining study sites had hepatic Pb concentrations (8.73–71.6 mg/kg dw (Supplementary material Table 1) >8.54 mg/kg dw (Supplementary material Table 1), which is the maximum hepatic Pb concentration detected in reference songbirds. Ten songbirds from the mining study sites had renal Pb concentrations (20.3–1,030 mg/kg) >20 mg/kg, which is the maximum renal concentration detected in reference songbirds. The moderately increased Pb tissue concentrations in 1 reference robin (4.38 mg/kg in blood, 8.54 mg/kg in liver, and 86 mg/kg in kidneys) suggest a source of Pb in addition to mining. However, the contrast between the mean Pb concentrations in robins from the mining district (fivefold greater in blood, tenfold greater in liver, and 23-fold greater in kidney) and the mean Pb concentration in reference robins suggests that other possible sources of Pb to robins are minor compared with mining.

ALAD activity in blood is used as a measure of the degree of Pb poisoning (Konuk et al. 2010) and has been well established as a marker of exposure and injury in studies of birds exposed to Pb (Hoffman et al. 2000). The decrease of ALAD activity in blood of songbirds at each of the Missouri study sites, ranging from 58 to 82 % (Table 4), is consistent with the increased blood Pb concentrations observed. These values are somewhat lower than those associated with 50 % mortality (81 % in brown-headed cowbirds, 89 % in red-wing blackbirds, and 93 % in grackles) in songbirds (Beyer et al. 1988). ALAD activity in other tissues is inhibited concurrently with its activity in blood. The inhibition of ALAD leads to a buildup of aminolevulinc acid in tissues, including the brain, where it causes neuropathogenic effects probably by interfering with delta-aminobutyric acid (Scinicariello et al. 2007). In a Pb-dosing study on mallards, Dieter and Finley (1979) associated a 75 % inhibition of ALAD activity in the blood with concurrent inhibitions (35 % in the cerebral hemisphere and 50 % in the cerebellum) in brain tissue, which they concluded showed evidence of pathology. In a study of nestling American kestrels (*Falco sparverius*) dosed with Pb (125 mg/kg bw/day), Hoffman et al. (1985) found that a 59 % inhibition of red blood cell ALAD activity was associated with a significant detrimental effect on growth. Decreases of ALAD activity in brain and kidney of these nestlings were also observed. Hoffman et al. (1985) concluded that altricial young, which include songbirds, seem to be especially sensitive to Pb. Grue et al. (1986) found that adult starlings exposed to elevated Pb concentrations from a highway had red blood cell ALAD activity decreased by from 46 to 60 % and that their young had slightly but statistically significant decreases in brain weight and decreased haemoglobin concentrations in blood compared with reference juveniles.

Tissue concentrations of Pb, especially in liver, are probably the most reliable measure for assessing possible toxic effects in songbirds. Pb-poisoned songbirds failed to show the gross pathological lesions associated with Pb that are typical of waterfowl (Beyer et al. 1988). The criteria for evaluating Pb concentrations in livers in songbirds, however, should be similar to those developed for waterfowl. When mallards and songbirds of three species were similarly dosed with Pb at dietary concentrations increasing to 50 % lethality, songbirds died at lower dietary concentrations than mallards but had similar median concentrations of Pb in liver and kidneys (Beyer et al. 1988); the average of the median concentration of Pb in livers in those songbirds was 31 mg Pb/kg dw compared with a median value of 30 mg Pb/kg in mallards. Franson and Pain (2011) developed criteria for waterfowl that we adopt here for songbirds (expressed as mg/kg dw). Adverse physiological effects (referred to as subclinical by Franson and Pain), such decreased ALAD activity, occur between 5.9 and 17.6 mg/kg dw (2 and 6 mg/kg ww). Systemic toxic effects (clinical poisoning), such as weight loss, incoordination, anemia, histopathological lesions, and anorexia, occur at 17.6–29.4 mg/kg (6–10 mg/kg ww). Pb concentrations in this range could lead to death if the exposure were to continue (Franson and Pain 2011). Concentrations >29.4 mg/kg are considered directly life threatening (Franson and Pain 2011). This concentration is consistent with thresholds of lethality observed in three species of songbirds—24 to 59 mg Pb/kg dw—estimated as the 10 % quantiles at a 50 % lethal dose (Beyer et al. 1988). Hepatic Pb concentrations of individual songbirds are summarized in Supplementary material Table 1. Based on the criteria previously mentioned, 1 robin of 39 birds (38 livers analyzed) from the reference area had a hepatic Pb concentration that was consistent with adverse physiological effects. In contrast, of 34 birds (30 livers analyzed) from the contaminated study sites, 11 had hepatic Pb concentrations that were consistent with adverse physiological effects, 3 with systemic toxic effects, and 4 with life-threatening toxic effects. The dashed line in Fig. 3 shows the threshold for systemic toxic effects (17.6 mg/kg) in the liver. Four of 6 robins, 3 of 14 cardinals, and 1 of 8 blue jays collected from mining study sites had hepatic Pb concentrations greater than this threshold. The presence of acid-fast intranuclear inclusion bodies in the proximal tubular epithelial cells of the kidneys of 2 robins, containing approximately 1,000 mg Pb/kg each, corroborates the severity of the exposures. Their presence is indicative of Pb poisoning (Sileo et al. 2001; Franson and Pain 2011) and they have previously been detected in songbirds experimentally exposed to lethal concentrations of Pb (Beyer et al. 1988). They are not present, however, in all cases of Pb-poisoned songbirds (Franson and Pain 2011), and thus it is not surprising that inclusion bodies were not seen in the two other robins with hepatic Pb concentrations associated with systemic toxic effects.

Conclusion

1. Concentrations of ore-related metals in soils at three contaminated sites in the Southeast Missouri Lead Mining District were greater (Pb = 39-fold; Zn = 7.9-fold; Cd = 9.0-fold; Cu = 25-fold) than background concentrations in the region. Based on published ecological risk assessments, the 1,000 to 3,200 mg Pb/kg detected in soil at the sites would likely be toxic to songbirds.
2. Concentrations of Pb detected in earthworms from the mining district were directly related to the soil Pb concentrations where the earthworms were collected, and they showed the importance of Pb-contaminated soil within the earthworms as an important pathway of exposure to songbirds. The dietary Pb exposure predicted for robins feeding on earthworms in the mining district was much greater than the exposure that would be considered toxic to birds in a typical ecological risk assessment.
3. The ALAD activity in blood is a well-established biomarker of Pb poisoning in wild and captive birds. The activity of this enzyme was decreased by 58 % at Washington State Park, 70 % at Viburnum Trend, and 82 % at Missouri Mines compared with reference birds.
4. Many of the songbirds in the assessment population collected from the mining district had Pb concentrations in their blood, liver, and kidneys that were in the ranges associated with toxicity (Franson and Pain 2011). Four of 6 robins, 3 of 14 cardinals, and 1 of 8 blue jays collected in the mining district had hepatic Pb concentrations above the threshold for systemic toxic effects. This finding helps explain the observed inhibition of ALAD activity and confirms the predictions of toxicity based on the analyses of earthworms.
5. Acid-fast renal intranuclear inclusion bodies, which are histologic lesions indicative of Pb poisoning in birds, were observed in kidneys of two robins from the mining district having the highest renal Pb concentrations (952 and 1,030 mg Pb/kg).
6. The terrestrial habitat examined in the Southeast Missouri Lead Mining District is contaminated by mining and smelting to the extent (>1,000 mg Pb/kg soil) that ground-feeding songbirds living there are being poisoned by Pb.

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