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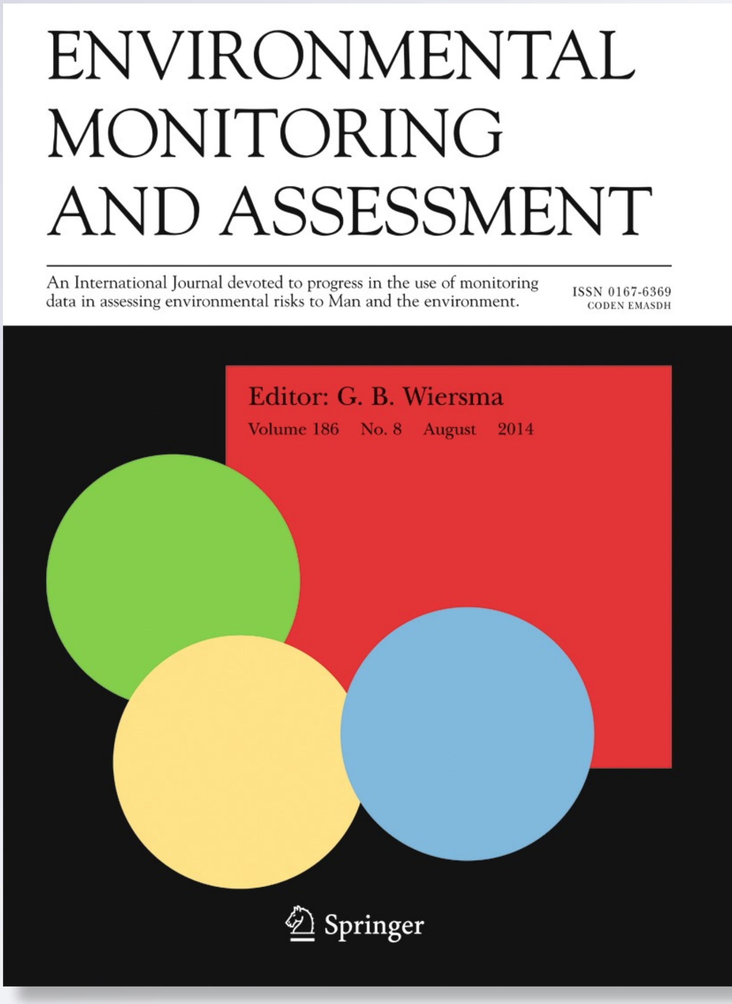

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The distribution and extent of heavy metal accumulation in song sparrows along Arizona's upper Santa Cruz River

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Abstract Heavy metals are persistent environmental contaminants, and transport of metals into the environment poses a threat to ecosystems, as plants and wildlife are susceptible to long-term exposure, bioaccumulation, and potential toxicity. We investigated the distribution and cascading extent of heavy metal accumulation in southwestern song sparrows (*Melospiza melodia fallax*), a resident riparian bird species that occurs along the US/Mexico border in Arizona's upper Santa Cruz River watershed. This study had three goals: (1) quantify the degree of heavy metal accumulation in sparrows and determine the distributional patterns among study sites, (2) compare concentrations of metals found in this study to those found in studies performed prior to a 2009 international wastewater facility upgrade, and (3) assess the condition of song sparrows among sites with differing potential levels of exposure. We examined five study sites along with a reference site that reflect different potential sources of contamination. Body mass residuals and leukocyte counts were used to assess sparrow condition. Birds at our study sites typically had higher metal concentrations than birds at the reference site. Copper, mercury, nickel, and selenium in song sparrows did exceed background levels, although most metals were below background concentrations determined from previous studies. Song sparrows generally showed lower heavy metal concentrations compared to studies conducted prior to the 2009 wastewater facility upgrade. We found no cascading effects as a result of metal exposure.

Keywords Heavy metals · Song sparrow · Santa Cruz River · Wastewater treatment · Mining · Body condition

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Introduction

Along the US/Mexico border in southern Arizona, the upper Santa Cruz River receives contaminants from a variety of natural and anthropogenic sources, including runoff from urban areas, active and abandoned mines, as well as effluent from the Nogales International Wastewater Treatment Plant. The effluent provides a beneficial source of water to riparian vegetation within the ecosystem, but studies prior to a 2009 facility upgrade found elevated levels of organic and inorganic contaminants in water, sediment, insects, fish, and birds (King et al. 1999; Kirkpatrick et al. 2010).

Birds represent good subjects for contaminant studies because they occupy a high trophic level, are well studied, are easy to detect, and are indicators of environmental health. Heavy metals, although naturally occurring, are often mobilized into birds and the environment at faster-than-normal rates due to anthropogenic activities (Sharma and Agrawal 2005). Once in the environment, metals may persist, potentially bioaccumulating and cascading through food webs (Webb and Leake 2006). In particular, riparian areas are often vulnerable as they concentrate water and may collect high levels of heavy metals from industrial and natural sources via runoff. Consequently, organisms living in riparian environments are at risk of accumulating metals to concentrations that may cause harmful effects.

Exposure to high concentrations of contaminants, such as heavy metals, can result in adverse health effects, and organisms that are higher on the food chain are especially susceptible to bioaccumulative effects (Winter and Streit 1992). For example, lead exposure in birds has correlated with *Trichomonas gallinae* (protozoan parasite) in mourning doves (*Zenaida macroura*; Locke and Bagley 1967), while Rocke and Samuel (1991) found aspergillosis (a fungal disease) and avian cholera in waterfowl. Body condition is also negatively correlated with disease condition and positively correlated with immune function (Møller et al. 1998). A reduced body condition may lead to lower reproductive output (Chastel et al. 1995). Understanding how birds accumulate metals and respond to living in a polluted environment is important for making informed management decisions about birds that inhabit southwestern riparian communities.

This study took place as part of an integrated research effort to quantify the levels of contaminants in water, sediments, plants, insects, fish, and birds of the upper Santa Cruz River ecosystem (Norman et al. 2010). A priori, our expectation was that the pattern of metal concentration in birds would reflect sources of metals in the environment and that birds associated with urban and mining areas would have higher burdens than birds found at an uncontaminated reference site. Our objectives were to (1) determine if birds were accumulating metals over time and in patterns consistent with their source, (2) examine how metal concentrations have changed over time, and (3) investigate the condition and response of birds living in a polluted environment.

Materials and methods

Study area

The Santa Cruz River flows south from its headwaters in the San Rafael grasslands of southeastern Arizona, USA, into Sonora, Mexico, then skirts around the Sierra San Antonio, where it turns northward and reenters the USA just east of Nogales, AZ. This study took place at a reference site and five study sites within the US portion of the Santa Cruz watershed, each with distinct potential sources of contamination (Fig. 1).

Our reference site was located along the headwaters of the Santa Cruz River in San Rafael State Natural Area, east of Lochiel, AZ. This site was upstream from all other locations and was expected to have the lowest contaminant concentration because the area is grazing land that is closed to the general public and experiences the least amount of human disturbance. The first study site was Nogales Wash, a tributary of the Santa Cruz River. Nogales Wash receives water from leaking pipes and in the form of runoff from the urban cities of Nogales, Arizona, and Mexico. The second and third study sites were on Sonoita Creek, also a tributary of the Santa Cruz River that receives runoff from abandoned mining areas in the Patagonia Mountains (Norman et al. 2008). Sonoita Creek is dammed to create Patagonia Lake, a popular recreational state park, before continuing toward the Santa Cruz River. Because contaminants carried by water may settle once reaching the lake, we established one study site upstream (PLSP) and another downstream (SOCR) from the lake. The fourth study site was located downstream from the Nogales International Wastewater Treatment Plant (NIWWTP), at the plant's outfall. This facility treats approximately 15–17 million gal sewage/day from the cities of Nogales, Sonora, and Nogales, Arizona, and the treated effluent is discharged into the Santa Cruz River. Our final study site was located along the Santa Cruz River at Tumacacori National Historical Park, approximately 17 km downstream of NIWWTP.

The bird

Southwestern song sparrows (*Melospiza melodia fallax*) are nonmigratory (Davis and Arcese 1999), thus are exposed only to contaminants from one location. Moreover, southwestern song sparrows primarily feed on riparian insects in the river channel or adjacent vegetation, especially during the breeding season (March–August; Rosenberg et al. 1982; Aldrich 1985). Birds are consequently exposed to metals through their foraging

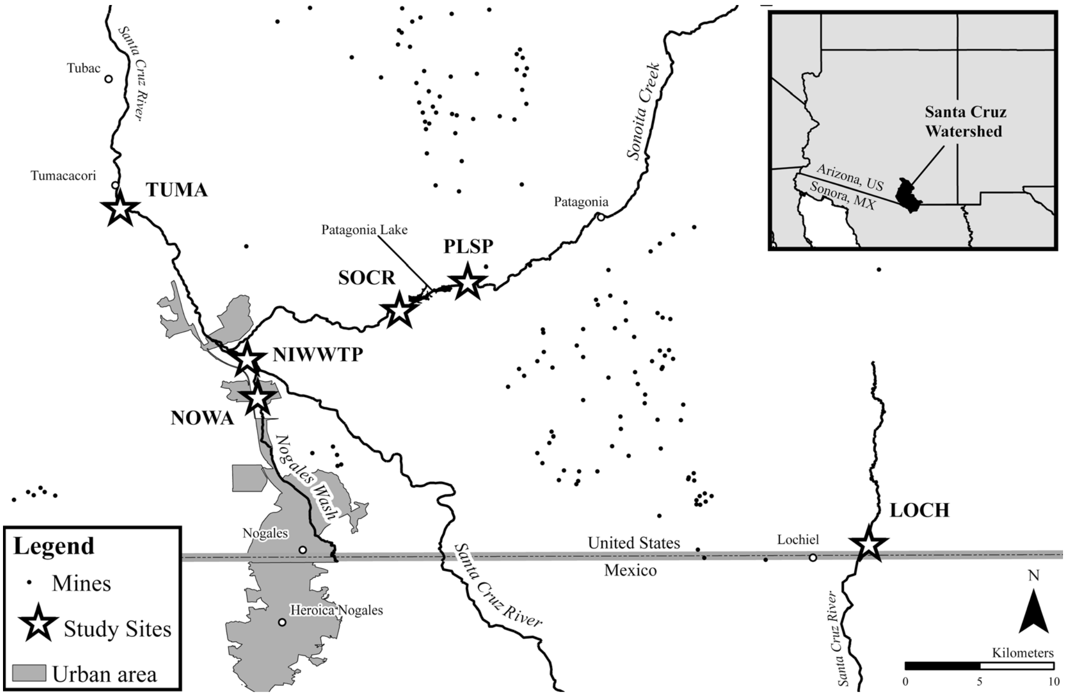

Fig. 1 Locations of six study sites sampled in 2011 and 2012 within Arizona's upper Santa Cruz River watershed: *LOCH* Lochiel (San Rafael Grasslands=reference site), *NOWA* Nogales Wash, *PLSP* Sonoita Creek (above Patagonia Lake), *SOCR* Sonoita Creek (below Patagonia Lake), *NIWWTP* Nogales International Wastewater Treatment Plant, *TUMA* Tumacacori National Historical Park. Source: USGS

behavior. Sparrows may also accumulate contaminants directly by drinking water from the river.

Song sparrows were captured at all sites between 1 April and 30 June 2011 and 2012 and in July and August at Tumacacori National Historical Park. We banded, aged, sexed, and measured all birds (Ralph et al. 1993). Outer tail feathers and blood samples were collected at capture.

Heavy metal analysis

Feathers are often used to monitor exposure to heavy metals because they remain connected to the blood source until fully grown. As a result, metals in feathers can accumulate over a longer window of time than in blood (Braune and Gaskin 1987; Burger 1993). Blood samples represent approximately 2 weeks of a given bird's diet (Craighead and Bedrosian 2008).

Blood and feather samples were sent to Activation Laboratories (Ontario, Canada) for preparation and analysis. Samples were analyzed for 21 metals with eight selected for comparison with previous regional studies (King et al. 1999; Kirkpatrick et al. 2010): cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se),[1] and zinc (Zn).

Feathers

To remove external contamination, feathers were washed twice with deionized water followed by rinsing in 1 mol/L acetone (Optima grade, Fisher Scientific; Burger et al. 1992). Samples were placed in a 45 °C oven for 4–5 h until completely dry and then weighed. Samples were digested with HNO_3 (Optima grade, Fisher Scientific) and H_2O_2 (Ultra grade, Fisher Scientific) and twice heated at 85 °C in a JULABO hot water bath (Allentown, PA). The ratio of HNO_3 to H_2O_2 was approximately 3:1. We diluted all samples to 5 mL with deionized water, and concentrations were measured with a Thermo Scientific Finnigan™ Element2 (Waltham, MA) high-performance high-resolution inductively coupled plasma-mass spectrometer (HR ICP-MS). Samples were spiked with internal

[1] Although Se is not truly a heavy metal, for simplicity, it will be referred to as such throughout this paper.

standards and analyzed in batches with certified reference material (SRM 1575a) from the National Institute of Standards and Technology (Gaithersburg, MD). Recoveries ranged from 85 to 110 %.

Blood

Approximately 100 μL of blood was collected from the brachial vein of each sparrow into ammonium-heparinized plastic capillary tubes. Samples were stored on ice packs in the field and then transferred to a −10 °C freezer. Digestion and analysis of blood followed the same procedure as for feathers although the ratio of reagents used to digest the blood was 1:1 instead of 3:1.

Comparison to previous studies

In 1997, the US Fish and Wildlife Service (USFWS) measured concentrations of Cd, Cu, Hg, Se, and Zn in a small composite sample of killdeer (*Charadrius vociferus*) livers from Rio Rico North, which is located midway between the NIWWTP and Tumacacori National Historical Park (King et al. 1999). Since we did not collect song sparrow liver samples, we used comparative values of reference species in similar feeding guilds (Llacuna et al. 1995; Agusa et al. 2005; Nam et al. 2005; Deng et al. 2007; Pan et al. 2008; Abduljaleel et al. 2012) to develop an average feather to liver ratio for each metal, which we then used to estimate sparrow liver concentrations. Estimated liver concentrations for our song sparrows were then compared to killdeer liver concentrations reported by King et al. (1999).

In 2008 and 2009, Kirkpatrick et al. (2010) measured concentrations of Cd, Cr, Cu, Hg, Ni, Pb, Se, and Zn in blood of Abert's towhees (*Melozone aberti*) and yellow-breasted chats (*Icteria virens*) at Tumacacori National Historical Park. Our song sparrow blood metal concentrations were compared directly to the average blood metal concentrations that they found for towhees and chats.

Leukocyte count and body condition

Thin blood smears were prepared in the field, air-dried, fixed in 200-proof ethanol for 30–60 s, and then stained with a Giemsa solution for 60 min. We utilized a light microscope (×1,000 magnification; Leica Microsystems, Buffalo Grove, IL) to enumerate leukocytes (Bennett 1970; Morishita et al. 1999; Houwen 2000). Each slide was read until approximately 30,000 red blood cells were examined. All white blood cells were pooled into one category and expressed as a proportion of the number of leukocytes per total cells. Bird body condition was determined based on body mass residuals, calculated from mass and tarsus–metatarsus length.

Statistical analysis

We used JMP 9.0 (SAS Institute Inc. 2010) to perform all statistical analyses. For individuals with metal concentrations below detection limits (3 % of samples), we assigned a value equal to half the detection limit, as suggested by Wong et al. (2002) and Agusa et al. (2005), in order to minimize potential biases in overall sample analyses. All concentrations were converted to parts per million (ppm; dry wt.) and natural log-transformed for analysis (because data were not normally distributed), with values presented in tables untransformed.

We used two- and three-way ANOVA tests to determine if differences existed in mean concentrations of each metal among sites for blood and feathers, respectively. Sex was considered a main factor; feather age was included as a main factor only for feather analysis. This was done to account for the possibility of increasing metal concentrations as a result of external deposition (prior to collection) that could not be removed by washing. Feather age was entered into the model as the number of days from the first date of feather collection for each year. All possible interactions were included in both models with the exception of feather age × sex × site interaction, so as to avoid over-fitting the models. LSMeans Student's *t* tests were then used post hoc for site pairwise comparisons. We used matched-pair *t* tests for feather samples of 37 individuals and blood samples of 33 individuals captured in both 2011 and 2012 in order to determine if heavy metal concentrations differed between years within the same individuals. Student's *t* tests were used to test for differences in metal concentrations between years.

White blood cells were analyzed as a proportion of total number of cells read for each blood smear. Proportions were natural log-transformed. A two-way ANOVA with LSMeans Student's *t* test was used to look for differences in white blood cell proportions among sites and between years.

A one-way ANOVA with LSMeans Student's *t* test was used to test for differences in means of body mass residual values among sites. Body mass residuals were used as an index for body condition and were calculated after fitting a linear regression to body mass vs. tarsus–metatarsus length for males and females separately. A residual above or below the regression line indicated if an individual was above or below average body condition, respectively (Ots et al. 1998; Snoeijs et al. 2004). All analyses were considered significant at or below the $p=0.05$ level.

Results

Distributional patterns among sites

Feathers

We analyzed a total of 201 outer tail feather samples from sparrows in 2011 and 2012. Sex was only a significant factor for Cr (three-way ANOVA, $F_{(1,\ 195)}=3.96$, $p=0.0482$), whereas the site×feather age interaction was significant for Cr, Pb, Se, and Zn (three-way ANOVA, Cr: $F_{(5,\ 195)}=2.45$, $p=0.0351$; Pb: $F_{(5,\ 195)}=3.23$, $p=0.0081$; Se: $F_{(5,\ 195)}=2.29$, $p=0.0473$; Zn: $F_{(5,\ 195)}=3.00$, $p=0.0125$).

Birds at all our field sites had feather concentrations greater than or not significantly different from birds at our reference site, Lochiel, for Cd, Cu, Hg, Pb, Se, or Zn (Table 1).

Cd, Cr, and Ni tended to be significantly highest in song sparrows at NIWWTP, whereas sparrows at Nogales Wash had significantly higher concentrations of Cu compared to all other sites. Concentrations of Pb were highest in feathers collected from our two Sonoita Creek sites, SOCR and PLSP, whereas feathers from NIWWTP and Nogales Wash had the highest Se feather concentrations.

Thirty-seven individuals were recaptured during the second year of this study, providing an opportunity to investigate if heavy metals differed within the same individual between years. Of the eight metals examined, Cd and Cr showed a significant increase from 2011 to 2012, irrespective of site (matched-pair *t* test, Cd: $t_{(36)}=2.87$, $p=0.0034$; Cr: $t_{(36)}=1.75$, $p=0.0441$). When all birds were considered, as opposed to just recaptured individuals, Cd showed a significant increase from 2011 to 2012, whereas Cu, Hg, and Ni showed significant decreases (*t* test, Cd: $t_{(199)}=2.51$, $p=0.0065$; Cu: $t_{(199)}=-2.44$, $p=0.0079$; Hg: $t_{(199)}=-3.61$, $p=0.0002$; Ni: $t_{(199)}=-3.69$, $p=0.0001$).

Blood

We collected 208 blood samples and sex was not a significant factor for any metal. Blood concentrations of Cr, Cu, Ni, Pb, and Zn did not differ significantly among sites (Table 1). Song sparrows at all field sites showed higher blood Cd levels compared to birds at our reference site. Birds at SOCR had the highest mean blood concentration of Hg, whereas those at Nogales Wash had the highest Se levels.

Thirty-three individuals provided blood samples in both 2011 and 2012, providing an opportunity to investigate if heavy metals were accumulating within the same individuals over multiple years. Of the eight metals examined, Cd, Cu, Hg, Pb, and Zn were significantly higher in 2012 than 2011, and Ni was significantly lower in 2012 than 2011. Conversely, when all adult birds were considered, Cd, Cu, Hg, Pb, and Zn showed significantly lower concentrations in 2012 than 2011, whereas Ni was significantly higher in 2012 than 2011 (Table 2).

Comparison to previous studies

We utilized a conversion factor to estimate song sparrow liver concentrations for Cu, Cd, Se, and Hg at NIWWTP and Tumacacori National Historical Park (Table 3). Estimated sparrow liver concentrations for these metals were all lower than concentrations reported by King et al. (1999) for killdeer livers at Rio Rico North. Zinc was the only metal higher in song sparrows than in killdeer (Fig. 2).

Blood concentrations of Cr and Ni for song sparrows at Tumacacori National Historical Park were lower than concentrations of these metals reported by Kirkpatrick et al. (2010) for Abert's towhees and yellow-breasted chats, with Cr the only significant decrease (Wilcox test=142; $p\leq0.01$). Cadmium showed similar concentrations between towhees and sparrows, but chats were below detection limits. Lead, mercury, and zinc concentrations were not significantly higher in song sparrows, whereas Cu (Wilcox test=28; $p\leq0.01$) and Se (Wilcox test=0; $p\leq0.01$) were significantly higher in song sparrows compared to towhees and chats (Fig. 3).

Table 1 Mean (ppm) and standard error (SE) of eight heavy metals found in tail feathers and blood of adult song sparrows at six sites within the upper Santa Cruz River watershed, AZ in 2011 and 2012. Background levels found in literature are also presented as a reference range with the number of publications as sample size

Metal	LOCH			NOWA		PLSP			SOCR			NIWWTP			TUMA			F value	p value	Reference range (n)[a]
	n=39	n=40	SE	n=41	SE	n=33	n=36	SE	n=11	n=12	SE	n=38	n=42	SE	n=39	n=37	SE			
Feathers																				
Cd	0.109 (A)		0.012	0.362 (B)	0.069	0.366 (B)		0.027	0.127 (A)		0.018	1.022 (C)		0.193	0.422 (B)		0.035	39.9	<0.0001	0.10–1.17 (8)[a]
Cr	0.418 (A)		0.108	0.431 (AB)	0.047	0.238 (C)		0.049	0.387 (ABC)		0.096	0.763 (B)		0.282	0.364 (AB)		0.027	9.84	<0.0001	0.18–2.38 (4)[a]
Cu	11.69 (A)		0.860	15.35 (B)	0.963	11.40 (A)		0.372	10.86 (AB)		0.790	11.87 (A)		0.451	10.73 (A)		0.422	4.85	0.0003	6.47–14.45 (9)[a]
Hg	0.251 (A)		0.089	0.386 (B)	0.061	0.316 (B)		0.086	2.816 (B)		0.864	0.326 (B)		0.089	0.221 (B)		0.041	3.48	0.0050	0.50–2.11 (6)[a]
Ni	1.223 (AB)		0.231	1.144 (A)	0.169	0.752 (B)		0.129	0.736 (ABC)		0.055	3.457 (C)		0.457	1.323 (A)		0.213	21.3	<0.0001	0.31–1.41 (5)[a]
Pb	0.706 (A)		0.061	1.191 (B)	0.086	2.283 (C)		0.130	2.273 (C)		0.181	1.069 (B)		0.078	1.050 (B)		0.070	31.0	<0.0001	0.73–17.30 (9)[a]
Se	0.984 (A)		0.095	1.958 (B)	0.230	0.989 (A)		0.165	1.257 (AB)		0.167	1.378 (B)		0.096	0.648 (A)		0.078	5.20	0.0002	0.83–6.04 (4)[a]
Zn	195.1 (A)		11.52	215.6 (A)	7.256	196.3 (A)		5.334	189.0 (A)		7.788	206.3 (A)		7.461	171.6 (A)		5.488	1.52	0.1838	119.5- 291.1 (9)[a]
Blood																				
Cd		0.002 (A)	0.0002	0.005 (B)	0.0005		0.004 (BC)	0.0003		0.002 (A)	0.0007		0.004 (BC)	0.002		0.004 (C)	0.0004	9.97	<0.0001	0.002–0.47 (4)[b]
Cr		0.035 (A)	0.009	0.047 (A)	0.012		0.023 (A)	0.009		2.145 (A)	2.088		0.120 (A)	0.090		0.018 (A)	0.002	1.89	0.0982	0.94–14.37 (3)[b]
Cu		0.672 (A)	0.050	0.811 (A)	0.082		1.118 (A)	0.549		0.578 (A)	0.039		0.699 (A)	0.056		0.740 (A)	0.072	0.70	0.6231	0.13–5.13 (5)[b]
Hg		0.058 (A)	0.015	0.080 (B)	0.011		0.046 (A)	0.005		0.072 (B)	0.007		0.035 (A)	0.006		0.065 (A)	0.014	4.86	0.0003	0.04–.92 (6)[b]
Ni		0.136 (A)	0.120	0.033 (A)	0.009		0.043 (A)	0.021		0.035 (A)	0.014		0.083 (A)	0.042		0.025 (A)	0.007	0.39	0.8584	0.93–10.84 (2)[b]
Pb		0.041 (A)	0.121	0.031 (A)	0.004		0.039 (A)	0.004		0.031 (A)	0.005		0.108 (A)	0.065		0.027 (A)	0.004	1.66	0.1465	0.05–5.76 (4)[b]
Se		2.493 (A)	0.190	15.122 (B)	1.672		2.800 (A)	0.386		5.794 (C)	0.598		2.883 (A)	0.230		3.364 (A)	0.424	45.1	<0.0001	0.499–7.97 (4)[b]
Zn		7.132 (A)	0.540	9.606 (A)	1.091		6.425 (A)	0.490		6.831 (A)	0.552		7.509 (A)	0.732		8.219 (A)	0.803	1.53	0.1809	1.77–26.83 (5)[b]

Sites sharing the same parenthetical letter do not differ significantly

LOCH Lochiel (San Rafael Grasslands—local reference site), *NOWA* Nogales Wash, *PLSP* Sonoita Creek (above Patagonia Lake), *SOCR* Sonoita Creek (below Patagonia Lake), *NIWWTP* Nogales International Wastewater Treatment Plant, *TUMA* Tumacacori National Historical Park

[a] Llacuna et al. (1995), Eens et al. (1999), Dmowski (2000), Janssens et al. (2001), Dauwe et al. (2002), Morrissey et al. (2005), Deng et al. (2007), Costa et al. (2011), and Lane et al. (2012)

[b] Burger and Gochfeld (1997), Evers et al. (1998), Kahle and Becker (1999), Thompson and Dowding (1999), Osofsky et al. (2001), van Wyk et al. (2001), Wayland et al. (2001), Golden et al. (2003), Martinez-Lopez et al. (2005), Matz and Rocque (2007), Rattner et al. (2008), and Abduljaleel et al. (2012)

Table 2 Mean difference in log-transformed heavy metal concentrations between 2011 and 2012 for blood samples of **a**) 33 recaptured song sparrows (*Melospiza melodia*) and **b**) all song sparrows captured in the upper Santa Cruz River watershed, AZ. Only significant metals from a matched-pair *t* test are reported along with standard error, *t* ratio (df), and *p* value

Metal	Mean difference (Ln(2012)–Ln(2011))	SE	a) $t_{(32)}$	b) $t_{(205)}$	*p* value
Cd	0.3707	0.0813	4.56		<0.0001
Cu	0.5355	0.1214	4.41		<0.0001
Hg	0.8785	0.1642	5.35		<0.0001
Ni	−1.3706	0.2687	−5.10		<0.0001
Pb	0.3667	0.1886	1.94		0.0304
Zn	0.5715	0.0970	5.89		<0.0001
Cd	−0.3404	0.07		−4.66	<0.0001
Cu	−0.4609	0.06		−7.23	<0.0001
Hg	−0.8075	0.11		−7.32	<0.0001
Ni	1.1338	0.19		5.93	<0.0001
Pb	−0.5286	0.10		−5.11	<0.0001
Zn	−0.4827	0.07		−7.26	<0.0001

Sparrow condition

Leukocyte count

Leukocytes were quantified in 102 song sparrow blood smears from all field sites. Birds at SOCR and Nogales Wash had significantly higher mean white blood cell counts than Lochiel, PLSP, NIWWTP, and Tumacacori National Historical Park in 2011 only (two-way ANOVA, $F_{(5,\ 82)}$=5.12, p=0.0004). There was no significant difference among sites in 2012 (two-way ANOVA, $F_{(5,\ 109)}$=1.01, p=0.4142; Fig. 4).

Overall, birds in 2011 showed a significantly higher proportion of white blood cells than they did in 2012 (two-way ANOVA, $F_{(1,\ 201)}$=8.98, p=0.0031). This trend is confirmed by recaptured song sparrows, as these individuals also had a higher proportion of white blood cells in 2011, compared to 2012 (matched-pair *t* test, $t_{(32)}$=−4.18, p=0.0002).

Body condition

Site was only significant in 2011 (two-way ANOVA, $F_{(5,\ 93)}$=4.07, p=0.0023). SOCR had significantly higher body mass residuals than the Nogales International Wastewater Treatment Plant, and Tumacacori National Historical Park had significantly lower body condition than all other sites, except the wastewater treatment plant (Fig. 5). There was no significant difference in body mass residuals between 2011 and 2012 (two-way ANOVA, $F_{(1,\ 191)}$=0.49, p=0.4861).

Discussion

Distributional patterns among sites

For all metals, birds at the reference site, Lochiel, had the lowest mean concentrations. This corroborates our a priori prediction that song sparrows at this site would have the lowest heavy metal concentrations, given that

Table 3 Mean feather concentration (ppm), conversion factor, and estimated liver concentration (ppm) for five heavy metals found in song sparrows at the Nogales International Wastewater Treatment Plant (NIWWTP) and Tumacacori National Historical Park (TUMA), AZ, in 2011 and 2012. Feather concentration is an average of the mean concentration for each metal in 2011 and 2012. Conversion factor was determined based on the ratio of feather concentrations to liver concentrations for reference species from previous studies (Llacuna et al. 1995; Agusa et al. 2005; Nam et al. 2005; Deng et al. 2007; Pan et al. 2008; Abduljaleel et al. 2012). Estimated liver concentration is determined by multiplying the feather concentration by the conversion factor

Metal	NIWWTP			TUMA		
	Feather concentration (ppm)	Conversion factor (multiply)	Estimated liver concentration (ppm)	Feather concentration (ppm)	Conversion factor (multiply)	Estimated liver concentration (ppm)
Cd	0.728	1.463	1.064	0.545	1.463	0.545
Cu	11.619	1.349	15.671	10.292	1.349	13.783
Hg	0.201	0.196	0.039	0.149	0.196	0.029
Se	1.253	2.732	3.423	0.394	2.732	1.076
Zn	201.979	0.604	121.980	165.887	0.604	100.183

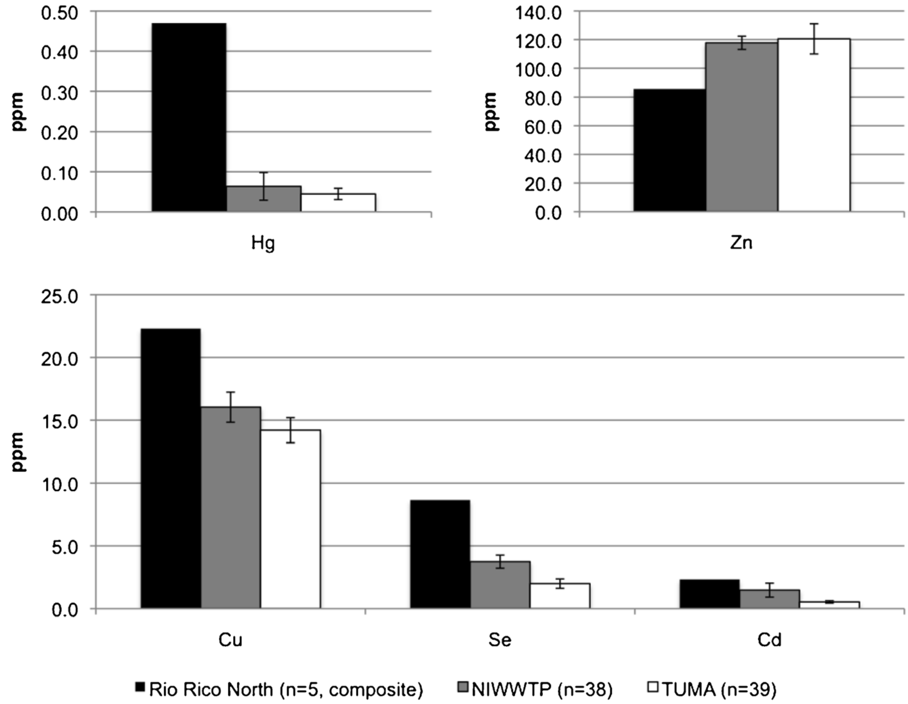

Fig. 2 Comparison of the 1997 USFWS study (Rio Rico North; King et al. 1999) with the present study (NIWWTP and TUMA). Presented are mean concentrations (ppm) of Hg, Zn, Cu, Se, and Cd in livers of killdeer (*Charadrius vociferous*) at Rio Rico North and song sparrows (*Melospiza melodia*) at NIWWTP and TUMA. Concentrations for killdeer are observed, whereas concentrations for song sparrows are estimated based on observed feather concentrations and values found in literature. *Error bars* represent 95 % confidence interval of estimated values. *NIWWTP* Nogales International Wastewater Treatment Plant, *TUMA* Tumacacori National Historical Park

its location at the headwaters in the rural San Rafael Grasslands is upstream from any industry. In general, the distribution of tissue concentrations among field sites reflected the environmental concentrations, indicating that birds accumulated heavy metals in relation to their sources of pollution.

Song sparrows at Nogales Wash had the highest concentrations of Se and Cu, which are likely from anthropogenic sources (e.g., leaking pipes) and natural deposits (Long et al. 1998; Norman et al. 2008), respectively. However, the source of Se for song sparrows at Nogales Wash is unclear. Song sparrows at NIWWTP contained concentrations of Cr similar to those at Nogales Wash, and concentrations of Cd and Ni significantly higher than most other sites. Water pumped to the treatment plant includes runoff and wastewater from metal plating factories in Nogales, Sonora (Norman 2010). Song sparrows at Tumacacori National Historical Park had similar or significantly lower mean concentrations of metals compared to NIWWTP due to its downstream location. The two Sonoita Creek sites (PLSP and SOCR) were highest for Pb largely as a result of runoff from lead mines in the Patagonia Mountains (Norman et al. 2008). Interestingly, song sparrows at SOCR, but not PLSP, accumulated high levels of Hg, which was historically used in the sixteenth century by Spaniards to extract gold from ore in the Patagonia Mountains (Malm et al. 1990). The fact that these two closely located sites do not show similar concentrations may indicate that Hg in Patagonia Lake undergoes a biotransformation to methylmercury via photoreduction or microbial processes. Subsequently, bioaccumulation may occur downstream, such as at SOCR (Gardner et al. 1978, Morel et al. 1998). Further study is needed to determine the extent of mercury methylation in Patagonia Lake.

Whereas this study assumes diet to be the main route of metal exposure for song sparrows, it is important to note that one issue is the ability of feathers to accrue metals externally via environmental deposition or through preening (Jaspers et al. 2004). While this exogenous contamination could well reflect environmental pollution, it may skew results related to internal accumulation. Feathers were washed rigorously in this study

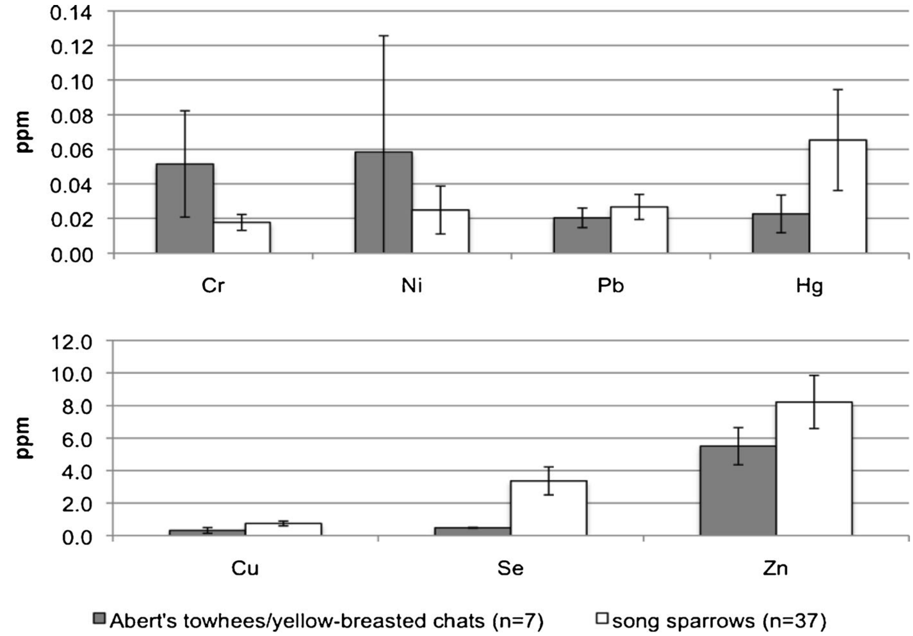

Fig. 3 Comparison of the 2008/2009 Kirkpatrick et al. (2010) study (Abert's towhees/yellow-breasted chats) with the present study (song sparrows). Presented are mean concentrations (ppm) of Cr, Ni, Pb, Hg, Se, Zn, and Cu in blood of birds at Tumacacori National Historical Park, AZ. *Error bars* represent 95 % confidence interval. Mean concentrations for song sparrows were averaged between 2011 and 2012

to remove as much external accumulation and mediate this issue as much as possible. However, this process is unlikely to remedy the problem completely. It is possible that exogenous accumulation explains significant interaction effects for Cr, Pb, Se, and Zn, since feathers no longer accumulate metals once they finish growing and are cut off from the blood supply (Braune and Gaskin 1987; Burger 1993). Although we did find that there were significant decreases in concentrations between 2011 and 2012 among all individuals, there were significant increases among recaptured birds, perhaps a result of bioaccumulation over time.

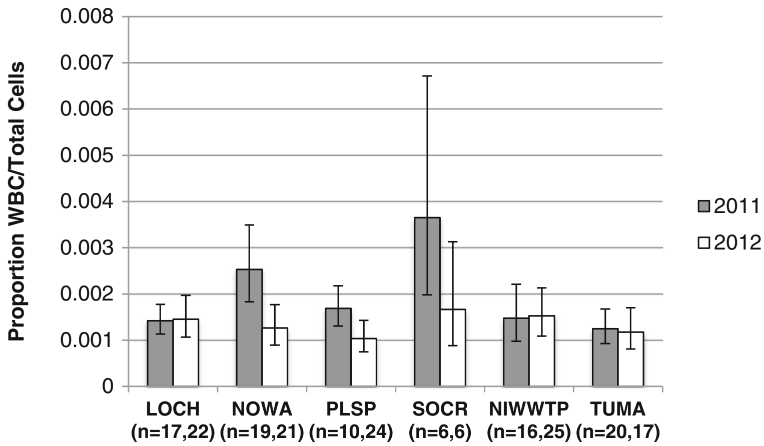

Fig. 4 Mean proportion of white blood cells per total number of cells read for song sparrows (*Melospiza melodia*) at six field sites within the upper Santa Cruz River watershed in 2011 and 2012. *Error bars* represent the 95 % confidence interval. Sample size read as (n=2011, 2012). *LOCH* Lochiel (San Rafael Grasslands—control site), *NOWA* Nogales Wash, *PLSP* Sonoita Creek (above Patagonia Lake), *SOCR* Sonoita Creek (below Patagonia Lake), *NIWWTP* Nogales International Wastewater Treatment Plant, *TUMA* Tumacacori National Historical Park

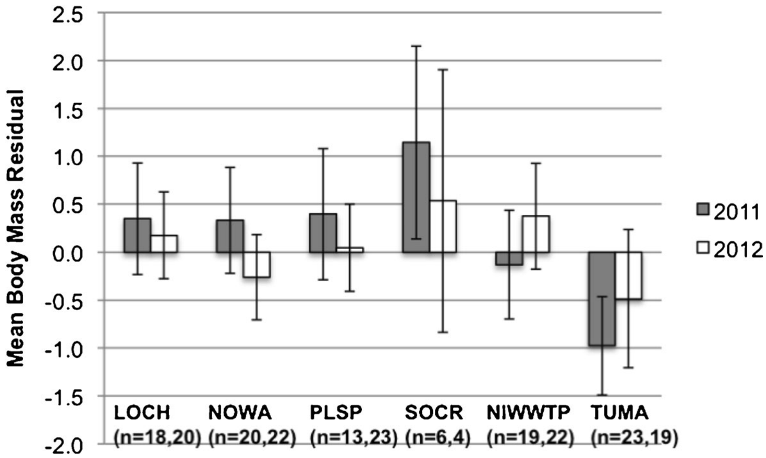

Fig. 5 Mean body mass residual score of adult song sparrows (*Melospiza melodia*) in 2011 and 2012 for six sites within the upper Santa Cruz River watershed, AZ. *Error bars* represent the 95 % confidence interval. Sample sizes read as (*n*=2011, 2012). *LOCH* Lochiel (San Rafael Grasslands—control site), *NIWWTP* Nogales International Wastewater Treatment Plant, *NOWA* Nogales Wash, *PLSP* Sonoita Creek (above Patagonia Lake), *SOCR* Sonoita Creek (below Patagonia Lake), *TUMA* Tumacacori National Historical Park

Comparison to previous studies

Song sparrows at NIWWTP and Tumacacori National Historical Park both showed lower estimated liver concentrations of Cd, Cu, Hg, and Se compared to what King et al. (1999) found in killdeer. Similarly, concentrations of Cr and Ni in blood of song sparrows were lower than concentrations in the blood of Abert's towhee and yellow-breasted chats collected by Kirkpatrick et al. (2010) in 2008 and 2009. Recognizing that unequal sample sizes reduced our ability to find significant differences, we argue that the pattern of lower concentrations for certain metals in song sparrows can potentially be attributed to an upgrade at the wastewater treatment plant in 2009. Zinc, however, has increased downstream from the wastewater plant since the upgrade. It could also be that song sparrows generally have lower inorganic constituent levels than other species in the region, but Pb, Hg, Se, and Zn levels that we recorded were higher than 2008/2009 concentrations for yellow-breasted chats and Abert's towhees. Given the increasing proposals of new mines that may affect the Santa Cruz River watershed (Myers 2010), our study provides an important source of baseline information of inorganic constituent levels in birds for the region.

Metals exceeding normal background concentrations

Cu, Hg, Ni, and Se are of most concern in the Santa Cruz River as they exceeded the reference range for feathers or blood, particularly at Nogales Wash, NIWWTP, and SOCR. Whereas these metals are not necessarily present at toxic levels in the upper Santa Cruz watershed (Kirkpatrick et al. 2010), they should be monitored in the future as they have the potential to cause harmful effects if they do accumulate to toxic levels. Furthermore, combinations of different contaminants can also amplify an individual's susceptibility to different health effects (Kakkar and Jaffery 2005), even if individual metals do not reach toxic concentrations.

Condition

The effects of heavy metal exposure on avian health have been well documented. Takekawa et al. (2002) found certain heavy metals to be associated with reduced body condition in wintering diving ducks off coastal California. Fairbrother and Fowles (1990) have shown that Se can reduce cell-mediated immunity and suppress immunocompetence at some concentrations in mallards (*Anas platyrhynchos*). Additionally, white blood cell count and antibody production were reduced in lead-dosed mallards (Trust et al. 1990; Rocke and Samuel 1991; Fairbrother et al. 2004). However, our results did not indicate any major effect of heavy metals on song sparrow condition in the upper Santa Cruz River watershed. There were no significant site differences in body mass residuals among sites in 2012, and there is no clear indication that heavy metal exposure caused Tumacacori National Historical Park's low body condition in 2011.

Mean leukocyte proportion was significantly higher for birds at SOCR and Nogales Wash compared to other sites in 2011, indicating a possible low-level immune response. Whereas exposure to moderate levels of heavy metals may result in a slightly enhanced immune response (Eeva et al. 2005), we cannot rule out other explanations for a heightened level of white blood cells at SOCR and Nogales Wash. Both of these areas are located downstream of human habitation and may be a result of bacteria or organic contaminants. Since the overall health of birds appears normal, we have no reason to believe that heavy metals are currently affecting avian well-being in the region.

Conclusions

We found that distributional patterns of heavy metal accumulation in birds reflected urban and mining sources of pollution among sites in the upper Santa Cruz River watershed. Certain potentially toxic metals such as Ni, Cu, Hg, and Se, did exceed background concentrations found in the literature, particularly at the Nogales International Wastewater Treatment Plant, Nogales Wash, and SOCR. However, we did not find any strong evidence suggesting present reduced immune or body condition in song sparrows due to heavy metal exposure. Furthermore, most metal levels seem to have decreased over time following an upgrade to the wastewater treatment plant, since we found concentrations at sites downstream from the treatment facility were lower than in 1997 and 2008/2009. Continued monitoring of avian exposure to heavy metals in the upper Santa Cruz River watershed is suggested in order to provide a better picture of long-term bioaccumulation trends and their effects on the region's wildlife. Future studies may also consider using our study as a baseline for comparison, especially given the potential for the development of new mines in the region.

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