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Trace metal concentrations in tissues of two tinamou species in mining areas of Bolivia and their potential as environmental sentinels

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Abstract Mining has a long history in the Bolivian Andes and has left many tailing piles, from which trace metals may reach surface waters, soils, and biota. The potential of tinamous (Birds: Tinamidae) as sentinels has never been tested before, although their biological and ecological characteristics mean they could well be appropriate bioindicators. We captured 13 and nine individuals of the Ornate Tinamou (*Nothoprocta ornata*) from two polluted sites (P1 and P2) and 10 and five from control unpolluted sites (NP1 and NP2) and used, for comparative purposes, four specimens bred in captivity. We also captured six specimens of Darwin's Nothura (*Nothura darwinii*) from the polluted site, P2. We determined the concentration of As, Cd, Pb, and Sb in feathers, liver, and kidney and conducted histological analyses of liver and kidney. For the Ornate Tinamou, a site effect was found for all trace metals in all tissues, with the highest concentrations at polluted sites. At the P2 site, no differences between the two tinamou species were detected except in some cases where Darwin's Nothura shows near-double concentrations. In some cases, mean and/or individual values of trace metal concentrations reached toxicity levels at the polluted sites. Thesaurismosis in proximal convoluted renal tubules, probably related to Cd exposure, was observed in 30% of the samples from the P1 site. Significant correlations were observed between all tissues for all trace metals and also for all trace metals in each tissue. Because the species studied are ubiquitous and relatively abundant, we recommend monitoring programs based on feather analysis.

Keywords Mining activity · Andes · Antimony · Ornate Tinamou · Darwin's Nothura

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Introduction

Anthropogenic mining has taken place in the Bolivian high Andes since at least the fifteenth century (Sanabria 2000). Particularly in the East of Oruro Department in the Eastern Andean Cordillera, there are extensive polymetallic deposits (SERGEOMIN 1999). In the past, extraction of gold and silver (associated with sulfurs of Fe, Cu, Zn, Pb, As, Sb, etc.) was the main activity, while at present, extraction of tin and the Zn–Ag–Pb complex predominates (Rios 2002). These mining activities were characterized by the deposition of large tailing piles, which accumulated in abandoned and active mines, where trace metals, such as lead, arsenic, cadmium, and antimony could reach surface waters and soils, and so the biota. For example, high Cd levels were found in potato tubers cultivated in agrosystems irrigated by the Chayanta River, which receives mineral residuals from the principal tin Bolivian mines, situated 60 Km away (Oporto et al. 2007; Rojas and Vandecasteele 2007).

Contamination of this region through the centuries has polluted the principal water body, the Poopó Lake. Not only does its water have higher metal concentrations than the permissible values for human consumption (UNEP/OEA 1996; Van Ryckeghem 1997), but the fish that live in the lake do too (Beveridge et al. 1985).

Interest in sentinel organisms as environmental biomonitors is rising (Borràs and Nadal 2004; Burger 1993; Burger and Gochfeld 2000a, 2009; de Lapuente et al. 2008; Furness et al. 1993; Grove et al. 2009; Llacuna et al. 1995; Lounsbury-Billie et al. 2008; Nam et al. 2004a; Sanchez-Chardi et al. 2007).

Tinamous are birds that have been studied little (Cabot 1992; Davies 2002); their potential as sentinels has never been tested before. The Ornate Tinamou (*N. ornata*) and Darwin's Nothura (*N. darwinii*) live in the Bolivian highlands between 3,700 and 4,200 m asl (Cabot 1992; Davies 2002; Garitano-Zavala et al. 2003), height which includes mining sites; both species are relatively common and major hunting targets (Garitano-Zavala 2002).

Highland tinamou species possess characteristics that mean they could be appropriate bioindicators, such as a sedentary lifestyle and diet. Pearson and Pearson (1955) determined that Ornate Tinamou individual home ranges are 2.43 ha. Moreover, the feeding habits of the Ornate Tinamou and Darwin's Nothura are generalist and opportunist, consisting of a wide variety of seeds, leaves, and fruits from crops and weeds as well as invertebrates (Garitano-Zavala et al. 2003). Although their total life span in the wild has not been studied, it is known that Ornate Tinamou live at least 6 years in captivity (Garitano-Zavala pers. obs.). Therefore, trace metal levels in tinamou tissues from a polluted area should reflect local exposure.

In this study, we determine the differential concentration of arsenic (As), cadmium (Cd), lead (Pb), and antimony (Sb) in feathers, liver, and kidney of tinamous collected at mining and control sites and the histopathological status of liver and kidney, with the aim of evaluating the potential of these species as trace metal pollution sentinels.

Methods

Study area

Four sites were included: two mining sites with trace metal contamination reported in soils and water and two sites without any mining activity (control sites). The mining sites are in the Eastern Andean Cordillera, Southeast of Oruro city and East of the Poopó Lake. They are called Antequera or Polluted 1 (P1) (4,000 m, 18°28′ S lat; 66°52′ W long), and Poopó or Polluted 2 (P2) (3,800 m, 18°23′ S lat; 66°58′ W long). There are 13 linear kilometers between these two sites, whose waters drain in two distinct hydrographic sub-basins, which finally drain into Lake Poopó (Fig. 1).

In Antequera, the zinc–silver–lead complex is industrially exploited and all the residuals are deposited in a storage pond that closes a little valley near the Antequera river course (Rios 2002), where there are several old tailing piles scattered. All this mining activity has polluted the river soils, with concentrations several times above permissible values for As, Sb, Cd, Cu, Cr, Sn, Fe, Hg, Pb, and Zn (MEDMIN 2001). At Poopó, tin and secondarily the zinc–silver–lead complex are exploited and concentrated. Numerous tailing piles are scattered across the landscape (Rios 2002), and metals and other elements are easily dispersed by water and wind to soils and water

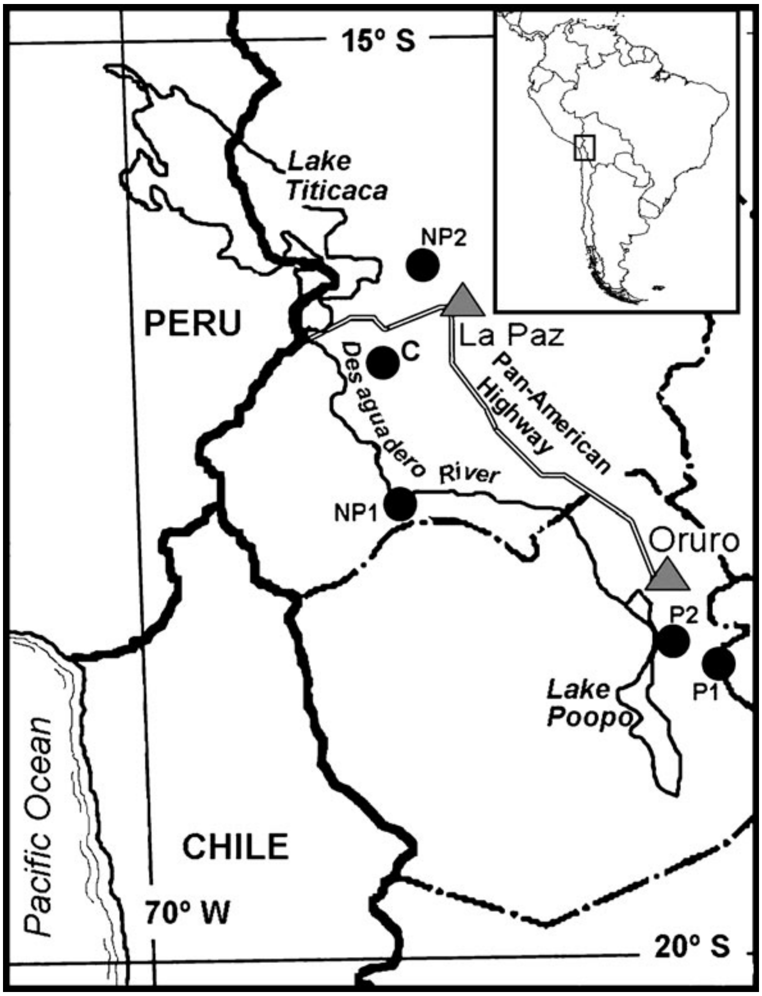

Fig. 1 Study sites on the Bolivian high plateau of the Central Andes. The control sites are called San Pedro de Ulloma or nonpolluted 1 (*NP1*) and Chuñavi or nonpolluted 2 (*NP2*): the mining sites are Antequera or Polluted 1 (*P1*) and Poopó or Polluted 2 (*P2*). Individuals of Ornate Tinamou (*N. ornata*) from captivity were obtained from a rural experimental breeding center (C)

bodies, especially to the Poopó River that drains into Lake Poopó. The soils at bends on the Poopó River have high concentrations of Fe, Zn, Cd, Cu, Pb, and Hg (MEDMIN 2001).

The control sites are called San Pedro de Ulloma or Non-polluted 1 (NP1) (3,850 m, 17°30′ S lat; 68°32′ W long), located to the West of the Desaguadero River, and Chuñavi or Non-polluted 2 (NP2) (4,100 m, 16°17′ S lat; 68°20′ W long), to the northwest of the city of La Paz and East of Lake Titicaca. The former is 200 linear kilometers from the mining localities; and the latter, 280 linear kilometers (Fig. 1).

All these sites are on the Bolivian high plateau and adjacent hills of the Eastern Andean Cordillera. The vegetation of the four sampling sites is dominated by spiny and resinous bushes (i.e., *Baccharis* spp., *Parastrephia* spp., and *Adesmia* spp.) and bunch grasses (i.e., *Festuca* spp. and *Stipa* spp.). Agricultural and livestock activities take place at the four sites, with the principal crops being potato tubers and barley, and extensive sheep, cattle, and llama breeding.

For comparative purposes, four Ornate Tinamou bred in captivity were analyzed. The birds were 18 months old and bred at an experimental rural captivity breeding center (C) (3,850 m, 16°40′ S lat; 68°51′ W long). The center is also on the Bolivian high plateau, to the East of the Desaguadero River (Fig. 1). These specimens descend from birds captured in the wild around the center.

Specimen collection

All specimens were captured by hunters with shotguns (helped by Pointers) between August and September 2006 and July and September 2007, during the dry season (southern winter) of the Bolivian highlands. In order to prevent any kind of contamination by residues of lead pellets or powder, in all cases, samples were collected from inside the tissue in which there was no sign of shot wound.

Ornate Tinamou were collected at the four study sites (13 at NP1, nine at NP2, 10 at P1, and five at P2), but only six specimens of Darwin's Nothura were captured at the P2 site. They were compared with Ornate Tinamou at this site.

Plumage characteristics led to all specimens being classified as adults; Ornate Tinamou reach adult plumage aged 9 to 10 months (Molina 2005) and, as Darwin's Nothura is a closely related species, it probably follows the same molting pattern. It was not possible to determine age accurately (years of life) because of the lack of a trustworthy method. A field necropsy was performed a few minutes after the death of the birds. Sex was determined from the gonads. Two tissue samples of liver and kidney (approx. 2 g wet weight) were taken from each bird with a steel scalpel, one for trace metal determination, and one for histological studies. Samples for trace metal determination were placed in individually marked plastic bags and conserved in a container with dry ice. Once in the laboratory these tissues were dried to constant weight at 60°C and conserved in individually marked disposable conical plastic tubes (NUNC^{TM}). Ten wing feathers (primaries and secondaries) were obtained in the field and placed in individually marked plastic bags, also for trace metal determination.

For histological analysis, liver and kidney samples were conserved in disposable conical plastic tubes with 10% formol. Dry tissues, tissues in 10% formol in plastic tubes and plastic bags with feathers, were shipped to the University of Barcelona for analysis.

The same process was used with the Ornate Tinamou from the experimental breeding center, but only liver and kidney samples for trace metal determination were taken.

Trace metal determination

Feathers were cleaned with a 0.1-M NaOH solution and dried out at 50°C prior to chemical determination of arsenic, cadmium, lead, and antimony by means of ICP-MS Perkin Elmer ELAN 6000. Before chemical determination, 100 mg of feather, liver, and kidney samples were digested according to the acid digestion protocol with Savilles Teflon digestion vessels, using H_2NO_3 (2 ml) and H_2O_2 (1 ml) for feathers and H_2NO_3 (3 ml) and H_2O_2 (2 ml) for liver and kidney (due to their high lipid content) for 12 h at 100°C. Accuracy of analysis was checked by measuring certified reference materials (Human Hair CRM 397—Community Bureau of Reference—and Lobster Hepatopancreas Tort-2—National Research Council Canada). Mean recoveries ranged from 98% to 100% for total arsenic, cadmium, lead, and antimony, and no corrections were done.

Quantification limits for the trace elements were 0.1 ppb. All trace element concentrations were expressed on a dry weight basis (ng/g, i.e., parts per billion). Trace element analyses were performed at the Serveis Científico-Tècnics (University of Barcelona).

Histological analyses

Tissue samples, embedded in Histocomp (Vogel), 5 μm thick, Hematoxylin–Eosin-stained and mounted in DPX were examined with a Nikon E400 microscope. Readings were performed blind.

Data analysis

Non-normally distributed and heteroscedastic data were log transformed (Log_{10} [trace element concentration in ppb d-w + 1]). Main site and sex effect and their interactions were analyzed by an ANOVA bifactorial model for the Ornate Tinamou specimens from the five sites. The Scheffé post hoc test was run. For the P2 site, an ANOVA bifactorial model was used to analyze the principal species (Ornate Tinamou and Darwin's Nothura) and sex effect, together with their interaction.

Table 1 Mean and Standard Deviation (SD) of trace metal levels (ppb d-w) (ng/g dry weight), in Ornate Tinamou feathers, liver, and kidney from the wild at nonpolluted sites (NP1 and NP2) and polluted sites (P1 and P2) and from captivity (C)

Site	N	Feathers		Liver		Kidney	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
As							
C	4			127.63 ± 53.58	84.73–205.20	339.77 ± 79.32	268.68–437.48
NP1	13	315.09 ± 76.58	169.18–444.39	98.22 ± 31.83	45.93–142.25	328.41 ± 174.19	151.69–823.69
NP2	9	467.85 ± 74.09	414.98–658.06	200.06 ± 66.16	101.71–287.60	367.12 ± 66.24	248.14–447.56
P1	10	2,270.67 ± 1,140.83	1,148.28–5,084.35	580.25 ± 260.36	335.06–1,081.83	1,104.25 ± 324.69	622.43–1,804.27
P2	5	787.94 ± 291.78	497.31–1,256.45	228.52 ± 85.76	163.63–374.86	417.78± 204.97	212.25–737.97
F(*df*); *p* value		47.80 (3, 29); <0.001		23.65 (4, 31); <0.001		14.09 (4, 31); <0.001	
Subgroups		(NP1–NP2) (P2) (P1)		(NP1–C) (C–NP2–P2) (P1)		(NP1–C–NP2–P2) (P1)	
Cd							
C	4			122.04 ± 23.44	97.58–154.00	821.84 ± 160.30	592.63–946.83
NP1	13	9.50 ± 7.04	3.01–26.94	786.76 ± 630.40	230.89–2,350.91	7,137.19 ± 8,937.02	715.90–26,631.65
NP2	9	4.82 ± 2.48	2.73–10.63	2,793.80 ± 2,778.24	468.63–8,860.29	26,059.04± 26,602.53	1,454.02–86,120.05
P1	10	248.89 ± 295.76	46.25–1,023.57	14,822.90 ±9,498.30	2,876.10–28,313.77	62,032.22 ± 63,430.86	7,655.68–207,331.50
P2	5	52.32 ± 14.82	33.22–71.74	9,328.85 ± 9,589.63	1,548.72–24,146.77	59,104.64 ± 73,342.39	6,452.04–180,590.43
F(*df*); *p* value		40.66 (3, 29); <0.001		26.92 (4, 31); <0.001		8.89 (4, 31); <0.001	
Subgroups		(NP2–NP1) (P2) (P1)		(C) (NP1–NP2) (NP2–P2) (P2–P1)		(C–NP1) (NP1–NP2–P2) (NP2–P2–P1)	
Pb							
C	4			64.78 ± 57.94	22.19–146.10	246.18 ± 80.52	166.27–353.81
NP1	13	1,335.24 ± 2,149.21	173.81–7,817.12	104.80 ± 173.32	6.54–617.23	489.36 ± 621.29	82.05–2,455.59
NP2	9	1,643.05 ± 2,005.29	264.56–5,716.57	100.27 ± 60.09	36.99–231.89	392.43 ± 106.06	266.78–555.95
P1	10	7,769.48 ± 8,947.08	1,701.58–25,273.0	757.83 ± 1,195.88	192.66–4,097.68	1,754.26 ± 652.63	823.31–2,584.24
P2	5	3,969.70 ± 2,001.57	1,510.17–6,513.48	1,928.44 ± 3,808.35	121.60–8,738.12	1,484.53 ± 612.39	903.51–2,502.37
F(*df*); *p* value		6.44 (3, 29); 0.002		4.54 (4, 31); 0.005		10.79 (4, 31); <0.001	
Subgroups		(NP1–NP2) (NP2–P2) (P2–P1)		(NP1–C–NP2) (NP2–P1–P2)		(C–NP1–NP2) (P2–P1)	
Sb							
C	4			2.47 ± 1.01	1.55–3.90	3.96 ± 1.12	2.58–4.92
NP1	13	29.00 ± 27.77	11.49–117.41	9.97 ± 9.85	3.93–35.94	17.06 ± 21.29	3.77–85.83
NP2	9	54.81 ± 40.81	18.74–142.74	29.45 ± 14.96	7.57–59.71	22.46 ± 15.37	6.83–50.57
P1	10	442.94 ± 286.73	193.72–997.36	64.86 ± 51.11	32.00–202.98	259.90 ± 504.54	38.08–1,689.31
P2	5	499.23 ± 161.59	371.46–761.61	58.93 ± 66.66	16.78–175.71	27.36 ± 17.67	7.98–55.19
F(*df*); *p* value		37.53 (3, 29); <0.001		17.69 (4, 31); <0.001		21.12 (4, 31); <0.001	
Subgroups		(NP1–NP2) (P1–P2)		(C–NP1) (NP2–P2–P1)		(C–NP1) (NP1–NP2–P2) (P1)	

The *F* value, degree of freedom (*df*), and *p* value from the ANOVA analysis are only shown for the principal factor "site"; the subgroups were formed with the Schefeé post hoc test

Table 2 Mean and Standard Deviation (SD) of trace metal levels (ppb d-w) (ng/g dry weight) in Ornate Tinamou (*Nothoprocta ornate*) and Darwin's Nothura (*Nothura darwinii*) feathers, liver, and kidney from polluted site P2

Site	N	Feathers		Liver		Kidney	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
As							
ND	6	1,045.24 ± 383.49	568.90–1,561.66	353.12 ± 184.33	193.98–590.64	731.15 ± 253.37	438.58–1,103.77
OT	5	787.94 ± 291.78	497.31–1,256.45	228.52 ± 85.77	163.63–374.86	417.78 ± 204.97	212.25–737.97
F(*df*); *p* value		3.54 (1, 7); 0.102		0.96 (1, 7); 0.359		6.45 (1, 7); 0.039	
Cd							
DN	6	142.05 ± 85.03	52.08–262.53	5,514.64 ± 3,029.15	2,816.16–10,200.12	28,721.07 ± 13,596.07	14,623.46–48,481.37
OT	5	52.32 ± 14.82	33.22–71.74	9,328.85 ± 9,589.63	1,548.72–24,146.77	59,104.64 ± 73,342.39	6,452.04–180,590.43
F(*df*); *p* value		17.34 (1, 7); 0.004		0.06 (1, 7); 0.817		0.055 (1, 7); 0.821	
Pb							
DN	6	16,528.24 ± 24,960.86	3,132.1–67,265.59	295.98 ± 235.08	69.68–724.31	10,713.13 ± 19,660.47	1,032.68–50,647.36
OT	5	3,969.70 ± 2,001.57	1,510.17–6,513.48	1,928.44 ± 3,808.35	121.60–8,738.12	1,484.53 ± 612.39	903.51–2,502.37
F(*df*); *p* value		9.30 (1, 7); 0.019		0.17 (1, 7); 0.689		4.09 (1, 7); 0.083	
Sb							
ND	6	1,023.62 ± 721.26	474.17–2,333.70	46.05 ± 22.25	24.49–83.10	247.24 ± 433.54	8.93–1,125.55
OT	5	499.23 ± 161.59	371.46–761.61	58.93 ± 66.66	16.78–175.71	27.36 ± 17.67	7.98–55.19
F(*df*); *p* value		10.13 (1, 7); 0.015		0.21 (1, 7); 0.662		4.00 (1, 7); 0.086	

The *F* value, degree of freedom (*df*), and *p* value from the ANOVA analysis are only shown for the principal factor "species"

Nonparametric Spearman correlations evaluated trace metal relationships between and within tissues. Analyses were performed using SPSS® v.15. 0.05 probability level, but due to sample size restrictions, all probability values are shown to allow the reader to assess significance.

Results

Trace metals means, Standard Deviation and ranges of As, Cd, Pb, and Sb for Ornate Tinamou are shown in Table 1. Values for Ornate Tinamou and Darwin's Nothura from the P2 site are shown in Table 2. No significant interactions ($p > 0.05$) or sex effects ($p > 0.05$) were found in the analysis.

For the Ornate Tinamou, site effects were found for all trace metals in all tissues. Arsenic concentrations in feathers, liver, and kidney were significantly higher at the P1 site. Feathers from the polluted sites formed two distinct subgroups. In relative terms, arsenic concentrations were higher in feathers and kidney, nearly doubling the liver concentrations (Table 1, Fig. 2). Liver and kidney concentrations of captive birds ranged within the range of unpolluted sites.

Cadmium concentrations in feathers and liver were significantly higher at the P1 site, but for kidney, the difference with the unpolluted NP2 site was less clear; as with arsenic, differences between

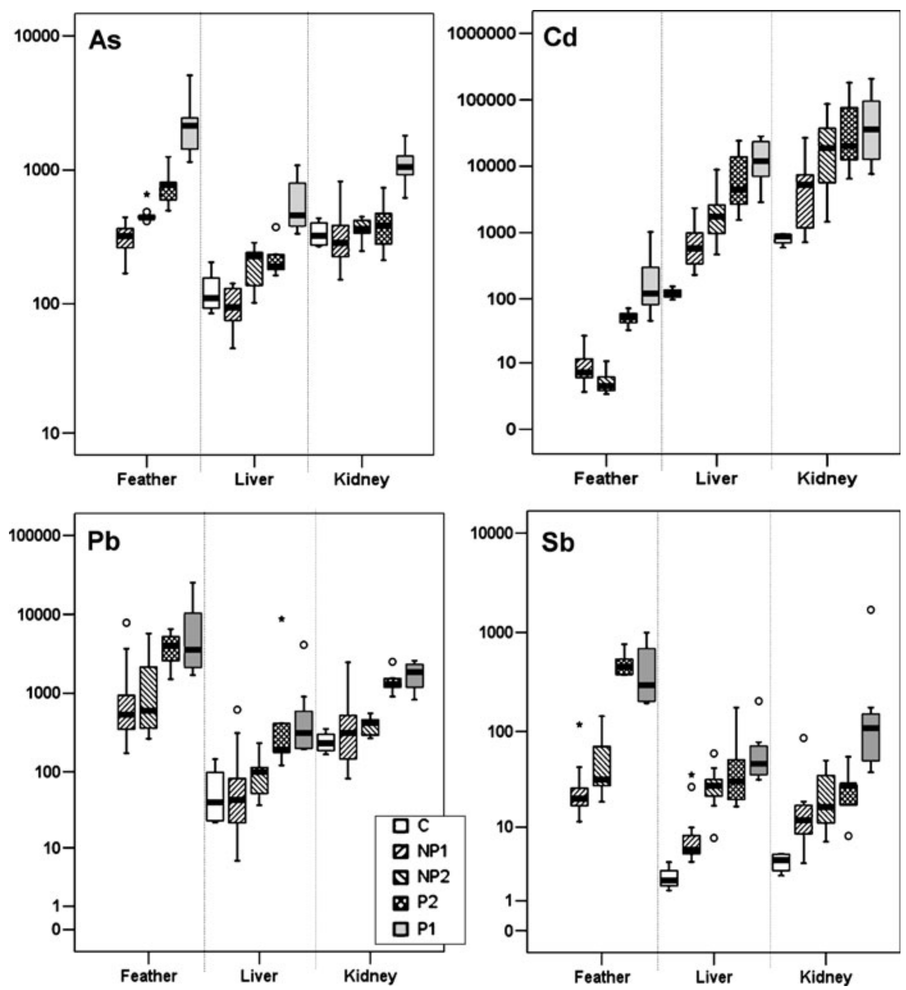

Fig. 2 *Boxplots* for each trace metal concentration in feather, liver, and kidney of Ornate Tinamou (*N. ornata*), from captivity (C), control sites San Pedro de Ulloma (NP1) and Chuñavi (NP2) and mining sites Antequera (P1) and Poopó (P2)

polluted and nonpolluted sites were more noticeable in feathers (Fig. 2). Liver and kidney Cd concentrations of the captive birds were around seven times lower than the minimal media in the wild. Relatively, cadmium was much higher in kidney, with five to ten times higher concentrations than in liver and 250 to 5,000 times higher than in feathers (Table 1).

Lead was clearly higher in feathers (around three times higher concentrations than in kidney and ten times higher than in liver). Polluted sites showed the highest concentrations, especially for kidney and at least for the P1 site in feathers (Table 1, Fig. 2). Liver and kidney Pb concentrations in captive birds were within the range of unpolluted sites.

Antimony concentrations were higher at the polluted sites, with this clearer in feathers, and for the P1 site in kidney. Feathers accumulated Sb relatively more than the other tissues (Table 1, Fig. 2). Liver and kidney Sb concentrations of captive birds were at least four times lower than the minimum mean value in the wild birds.

At the P2 site, the tissues of Darwin's Nothura had concentrations of the four trace metals analyzed that were similar to the Ornate Tinamou with, in most cases, no significant differences between the two species detected (Table 2). Exceptions were observed for all trace metals except As in feathers and As in kidney, in which cases Darwin's Nothura concentrations nearly doubled Ornate Tinamou ones.

Concentrations of the four trace metals analyzed correlated significantly in each tissue (Figs. 3, 4, and 5). As significant correlations were also observed between the four trace metal

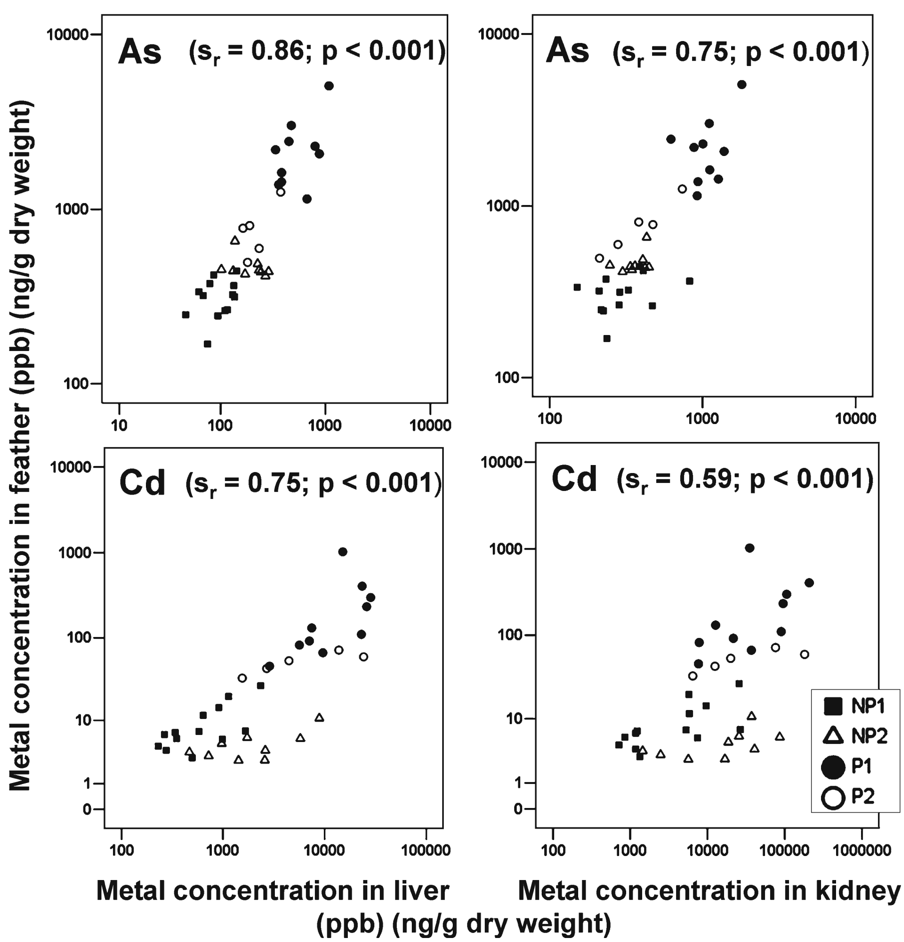

Fig. 3 *Scatter plots* of arsenic and cadmium concentrations in feathers versus liver (*left*) and kidney (*right*) in Ornate Tinamou (*N. ornata*) from control sites San Pedro de Ulloma (NP1) and Chuñavi (NP2), and mining sites Antequera (P1) and Poopó (P2). The Spearman correlation value (s_r) and p value are included

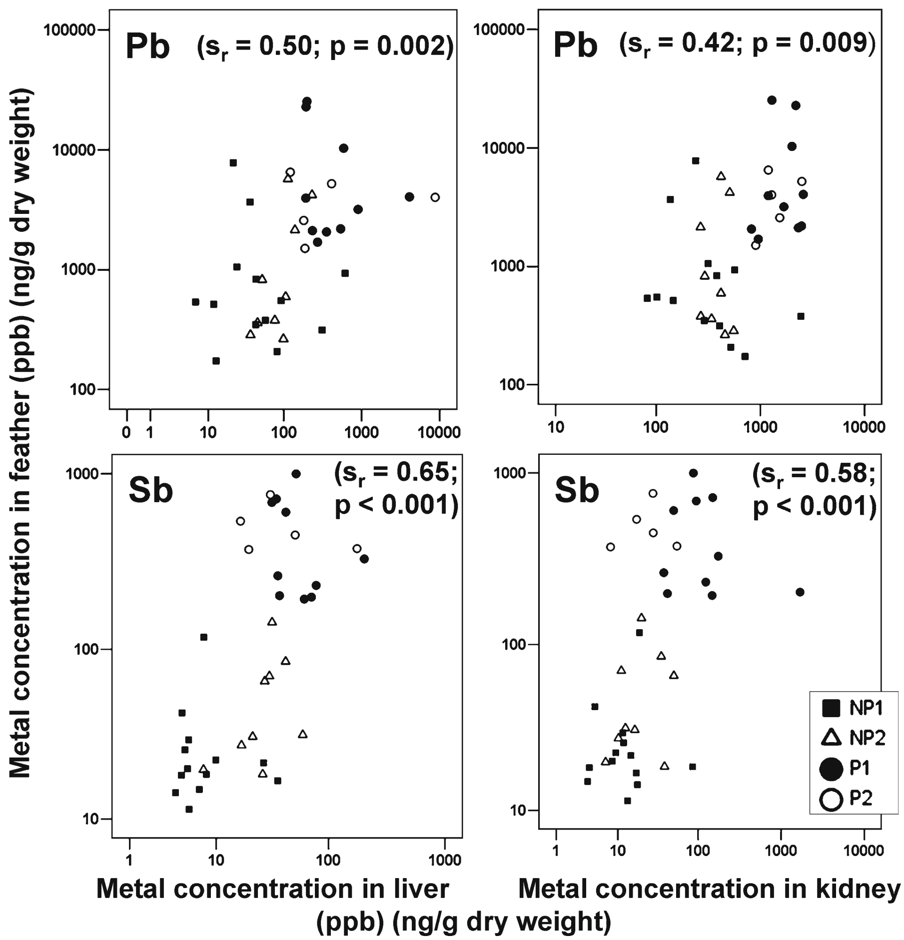

Fig. 4 *Scatter plots* of lead and antimony concentrations in feathers versus liver (*left*) and kidney (*right*) in Ornate Tinamou (*N. ornata*) from control sites San Pedro de Ulloma (NP1) and Chuñavi (NP2), and mining sites Antequera (P1) and Poopó (P2). The Spearman correlation value (s_r) and *p* value are included

concentrations in each tissue (Table 3), we can say that pollution of the four trace metals is similar at each site.

Liver histopathological slides revealed that some birds trapped at the four wild sites had inflammatory cells: 7% at NP1, 22% at NP2, 20% at P1, and 27% at P2 (at this last site, both species were taken into account). Kidney histopathological slides showed proportionally greater interstitial nephritis in birds from polluted sites (30% at P1 and 27% at P2) than in those from nonpolluted sites (15% at NP1 and 0% at NP2). Thesaurismosis (a storage disease) in proximal convoluted tubules was observed in 30% of the samples only at the P1 site. Development of thesaurismosis is probably related to mineral concretions, like a phenomenon observed in a cadmium-controlled dose-dependent study of Common Quail (*Coturnix coturnix*; Richardson et al. 1973).

Discussion

Trace metal levels and their toxicity

Trace metal concentrations in tissues are not directly associated with adverse impacts on bird health. Although this situation depends on each element's effect, it also depends on the bird species itself and intrinsic factors such as age, sex, physiology, etc., making it difficult to predict possible effects. Laboratory studies are necessary to identify the metal levels that may result in death or adverse impacts on behavior, physiology

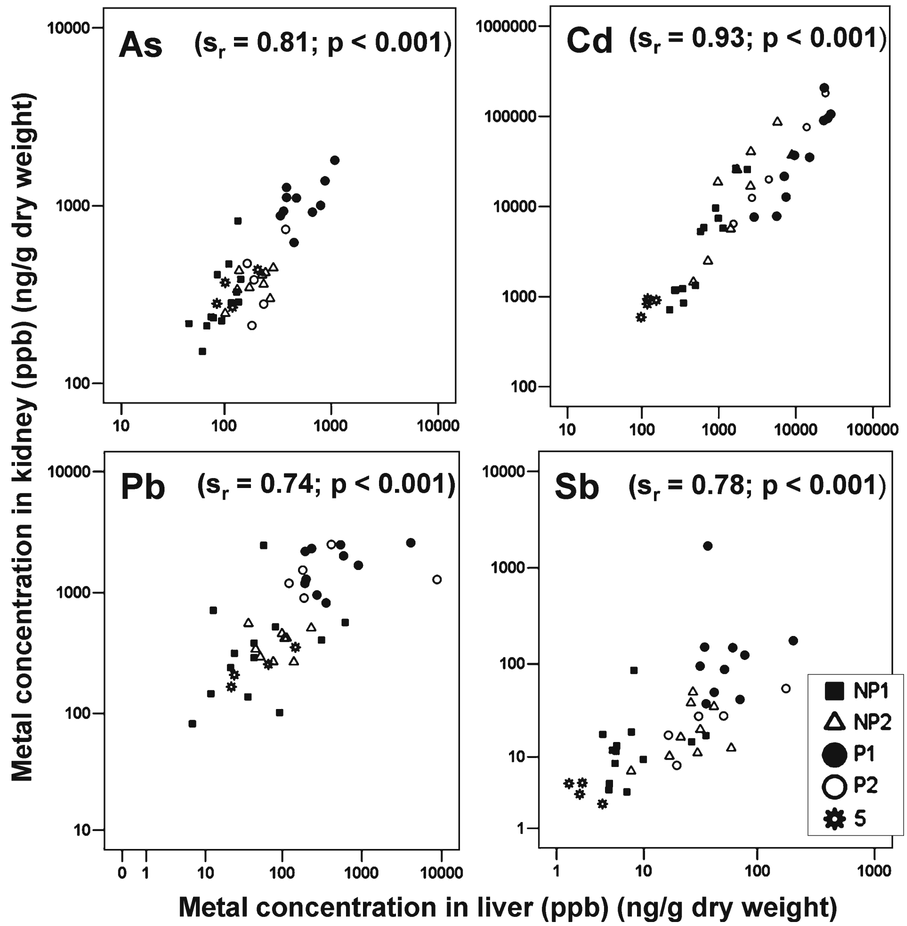

Fig. 5 *Scatter plots* of all trace metal concentrations in kidney versus liver in Ornate Tinamou (*N. ornata*) from control sites San Pedro de Ulloma (NP1) and Chuñavi (NP2), mining sites Antequera (P1) and Poopó (P2), and captivity (C). The Spearman correlation value (s_r) and *p* value are included

or reproductive success of birds. With the exception of Hg, Pb, and Cd, there are few controlled laboratory studies (Burger and Gochfeld 2009), which means that the comparison of our results with other bird species' reported values, with the background or poisoning threshold values proposed by several authors, is only a rough guide because of the lack of specific information for tinamous. In the following paragraphs, trace metal concentrations reported in tissues by the literature as wet weight (w-w) concentrations are multiplied by a factor of 3 to correspond to dry weight (d-w) concentrations (Clark and Scheuhammer 2003).

Arsenic

As arsenic is a micronutrient for some vertebrates (Eisler 1988a), and as it is rapidly metabolized by birds (Pendleton et al. 1995), it may not accumulate to toxic levels in tissues unless exposure is extreme (Custer et al. 2009). However, Burger and Gochfeld (2009) found that it biomagnifies in

Table 3 Nonparametric correlations between trace metal concentrations in each tissue type of the Ornate Tinamou (*Nothoprocta ornata*)

	Tissues	Feather	Liver
As	Liver	0.86 (<0.001)	
	Kidney	0.75 (<0.001)	0.81(<0.001)
Cd	Liver	0.75 (<0.001)	
	Kidney	0.59 (<0.001)	0.93 (<0.001)
Pb	Liver	0.50 (0.002)	
	Kidney	0.42 (0.009)	0.74 (<0.001)
Sb	Liver	0.65 (<0.001)	
	Kidney	0.58 (<0.001)	0.78 (<0.001)

The Spearman's Rho value is given in each box and the *p* value in brackets. $N = 37$ for feather and $N = 41$ for liver and kidney nature, with the highest values at the top of the trophic web.

Feather values for As reported for several bird species were between 604 to 13 ppb d-w (Burger and Gochfeld 2009; Burger et al. 2008; Lounsbury-Billie et al. 2008). The values we found in feathers are within this range for Ornate Tinamou at all sites, except for P1, with more than 2,200 ppb d-w; and except for Darwin's Nothura at P2, with values higher than 1,000 ppb d-w. Thus, tinamous feather values at mining sites are among the highest ever reported. Comparison with the control sites means that these values are related to the level of exposure.

Our mean liver values for Ornate Tinamou have a maximum of 580 ppb d-w at P1 and a maximum individual value of 1,081 ppb d-w. These values are lower than the 5,000 ppb w-w (approximately 15,000 ppb d-w) threshold reported by Goede (1985) as the upper limit for background levels in waders. Fedynich et al. (2007) reported values of 6 to 220 ppb w-w in migratory ducks (approximately 18 to 660 ppb d-w). Although all our values for liver may be considered within background levels, they reflect that bioaccumulation of As in tinamous is related to their exposure level. For kidney, there are no previous reports.

Cadmium

Cadmium is a nonessential element ubiquitous in natural environments and one of the trace metals whose accumulation and toxic effects in terrestrial, fresh water, and marine birds have been studied most. Cd toxicity is more common among natural vertebrate populations than was previously known (Larison et al. 2000). In particular, cadmium damages kidneys (Furness 1996; Larison et al. 2000).

Burger (1993) suggests that Cd feather levels associated with adverse effects range from 100 ppb d-w (shearwaters) to 2,000 ppb d-w (terns). Our mean feather values ranged between 5 to 9.5 ppb d-w at control sites, which are comparatively very low, while at polluted sites, the values are several times higher for both tinamou species: at P1 (for Ornate Tinamou) and P2 (for Darwin's Nothura), at least, values are in the toxicity range proposed by Burger (1993). Other studies report higher mean Cd values in feathers (but from different origins: breast, down feathers, or wing feathers) of 307 ppb d-w for Bald Eagle (Burger and Gochfeld 2009), 203 ppb d-w for Wood Stork (*Mycteria americana*) from Florida (Burger et al. 1993), and between 70 and 139 ppb d-w in ospreys from the Florida Bay estuary (Lounsbury-Billie et al. 2008), but these studies lack analysis of the toxic effects that these Cd concentrations may produce.

Scheuhammer (1987) suggested that cadmium background levels in liver are <3,000 ppb d-w and Furness (1996) concluded that threshold concentrations for Cd poisoning in birds might be expected at around 40 mg/kg w-w (approximately 120,000 ppb d-w), but with a wide toxicity variation between species and age. Renal damage in water birds was found at over 30,000 ppb d-w in livers (Mateo and Guitart 2003). In the case of Willow Grouse (*Lagopus lagopus*), Cd liver concentrations of approximately 10,000 ppb d-w were related to high metallothionein levels, indicating a physiological response to Cd exposure (Pedersen and Hylland 2007). A geometrical mean with various insectivorous birds considers 100 ppb d-w as background levels, since kidney samples did not reveal changes related to toxic cadmium levels (Custer et al. 2009). Mean Cd values in livers from captive Ornate Tinamou showed very low concentrations, with both control sites situated within Scheuhammer's background levels, but at NP2, an individual reached 8,800 ppb d-w. At P1, the mean value for Ornate Tinamou was higher than 10,000 ppb d-w, and at both sites, several Ornate Tinamou individuals reached values higher than 20,000 ppb d-w, indicating a high Cd exposure in the field.

As other studies did, our results demonstrate that Cd concentrations are higher in kidney than in other tissues (e.g., Kim et al. 2007, who examined liver, muscle, and bone). Background Cd levels in kidney are suggested as <8,000 ppb d-w by Scheuhammer (1987), and Furness (1996) concluded that kidney threshold concentrations for Cd poisoning in birds might be expected at around 100 mg/kg w-w (approximately 300,000 ppb d-w). Renal damage in water birds was found at over 300,000 ppb d-w in kidney (Mateo and Guitart 2003). Larison et al. (2000) demonstrated that 57% of the adult Willow Grouse individuals at a polluted mining site (with values of Cd > 100,000 ppb d-w in kidney) showed irreversible renal tubular damage.

In our results, only Ornate Tinamou individuals from captivity had Cd values in kidney below Scheuhammer's background level. In the field, individual higher values were found at all sites for both tinamou species. One possible explanation is that Cd is a trace metal naturally common in the Andean soils.

No site had higher mean values than 100,000 ppb d-w, except in some Ornate Tinamou individuals from polluted sites. At polluted sites, interstitial nephritis was more frequent, and thesaurismosis was reported only at P1. This site has the highest mean and individual Cd values for all tissues. Tissue effects are not related directly to individual level, as might be expected. For example, the birds with the highest Cd concentration in kidney (>100,000 and >200,000 ppb d-w, respectively) had no tissue effects, although these were found in individuals with 95,200, 35,200, and 7,800 ppb d-w. Interstitial nephritis appears to be nonspecific or not directly associated with Cd concentrations, given that it was observed in individuals with Cd concentration values in a range from 850 to 105,700 ppb d-w.

Possible explanations of these cases are age effect, individual physiology, or time of exposure. Larison et al. (2000) showed that older birds accumulated substantial amounts in their kidneys during their lives and developed not only renal damage but also less calcium deposition in bones due to general renal failure. Nam et al. (2004b) observed age-dependent Cd accumulation in kidney in Rock Pigeon (*Columba livia*). However, Hindell et al. (1999) reported higher Cd concentrations in the kidneys of juvenile Shy Albatross (*Thalassarche cauta*) and Wandering Albatross (*Diomedea exulans*) than in adult birds and suggested that cadmium concentrations are regulated to some degree throughout the bird's life, for example by excretion in feathers. This contradiction probably depends on the total lifetime and diet habits of each species and may also be related to metal ingestion variation during the lifespan of birds. Anyway, age effect in our results is probably reflected by broad standard deviations. Though we considered all our specimens as adults, accurate individual ages are not known. Thus, data may reflect the different individual histories for trace metal exposure and accumulation.

Lead

Lead toxicity and poisoning effects, widely studied in field and laboratory experiments, were described several physiological and somatic effects and even death if exposure is high enough (see reviews in Eisler 1988b and Franson 1996). Lead biomagnifies through the trophic chain (Burger and Gochfeld 2009), and its levels may increase during a bird's lifetime, depending on exposure (Grue et al. 1984). Muscular exercise, excretion, elimination, and high protein consumption are factors that reduce the physiological effects of high lead concentrations in wild birds (Burger et al. 1997; Roux and Marra 2007).

Burger and Gochfeld (2000b) considered that adverse reproductive effects in birds occur at levels of 4,000 ppb d-w in feathers; Burger and Gochfeld (1994) found that experimentally lead-injected 40-day-old Herring Gull chicks (*Larus argentatus*) had Pb concentrations in feathers of $4{,}790 \pm 1{,}693$ ppb d-w and showed several motor problems, while untreated chicks had lead values from 853 to 1,205 ppb d-w. In our study, Ornate Tinamou from control sites had mean Pb concentrations in feathers lower than 2,000 ppb d-w, but five individuals from both control sites had values over 4,000 ppb d-w. Ornate Tinamou and Darwin's Nothura from both polluted sites had mean values of 4,000 ppb d-w or higher, with the highest values at >25,000 ppb d-w (for Ornate Tinamou, P1 site) and >67,000 ppb d-w (for Darwin's Nothura, P2 site). With these values, many tinamous from the polluted sites would be considered within Pb toxic levels. According to other authors (e.g., Burger 1993), feathers had the highest lead concentrations of all the analyzed tissues. Burger (1993) suggested the feather/liver ratio of 0.42:1 as usual in several species. Ratios in our data are 0.02:1–0.1:1 (for both tinamou species), showing higher feather Pb concentrations. Only Ornate Tinamou at the P2 site had a 0.49:1 ratio.

When Pb concentrations in liver are 6 to 15 mg/kg w-w (approximately 18,000 and 45,000 ppb d-w), Pain (1996) concluded that waterfowl's biological functions may be disabled and external signs of poisoning may be observed. Franson (1996) found that 10 to 20 μg/g d-w liver Pb concentrations cause clinical signs of lead poisoning in other bird species. Clark and Scheuhammer (2003) considered that raptors have as "background" Pb exposure levels concentrations of <6,000 ppb d-w in liver and/or kidney, and so raptors with concentrations over 6,000 ppb d-w in liver or kidney have been lead-exposed. They are poisoned with concentrations over 20,000 ppb d-w. Following these criteria, all our individuals from captivity, control sites, and perhaps from the P1 site would be considered at the background level. Only at the P2 site were higher Pb concentrations found, with remarkable differences between species: one Ornate Tinamou had 8,700 ppb d-w in liver, but lower concentrations in kidney; whereas two Darwin's Nothura individuals showed higher concentrations in kidney, but lower ones in liver (6,500 and 50,600 ppb d-w, respectively). The last individual reaches the poisoning level cited above.

Kim et al. (2007) also found great variation of Pb concentrations between five shorebird species (individual liver concentration values from n.d. to 23,250 ppb d-w approximately and from n.d. to 141,600 ppb d-w in kidney). The tissue with the highest Pb concentration varied greatly between species.

All specimens were captured when flying, which indicates that if toxic levels do exist for these birds, at least they do not affect birds' movement skills. However, our sampling method may not adequately reflect Pb exposure and acquisition in the field, as Pb-poisoned birds from the polluted sites were not sampled because of their flight disability and, thus, were not included in calculations of the comparative bioaccumulation pattern between sites.

Antimony

Antimony is only potentially toxic, as it is not an essential trace element for plants and animals and has no known biological function (Fowler and Geering 1991). However, as there have been much fewer studies of antimony than of other potentially toxic metals, its environmental significance may have been underestimated (Shotyk et al. 2005).

In birds, Sb toxicity is cited because of the implications of the administration of antimony in the form of a potassium tartrate emetic (Carlisle and Holberton 2006), but very few studies have reported their bioaccumulation in tissues. Lounsbury-Billie et al. (2008) reported mean values of 4 to 66 ppb d-w in Osprey feathers, which they considered low. Our values from control sites were in that range, but at polluted sites, values became approximately ten times higher for Ornate Tinamou and 20 times higher for Darwin's Nothura. Although our liver and kidney values cannot be compared due to the lack of other studies, concentration increases at the polluted sites (particularly P1 for Ornate Tinamou and P2 for Darwin's Nothura) over the figures for captivity and control sites, which shows that birds exposed in the field to Sb probably accumulate this trace element in their tissues in direct proportion to their exposure, although nothing definite can be said about the possible risks of these concentrations. It is known that vertebrates excrete absorbed Sb rapidly via urine and feces, and only with exposure are high concentrations in thyroid, adrenals, liver, and kidney sometimes found (Hayes and Laws 1991).

The highland tinamou species as sentinels of the bioaccumulation of trace metals in agrosystems polluted by mining activities

Our analysis focuses on the four trace metals that were reported at high concentrations in soils and water at the two polluted sites studied. Trace metals are transferred from the environment to birds via multiple mechanisms. They move from soil to plants, where they are stored in leaves, seeds, or tubers (Olivares 2003; Oporto et al. 2007), then to phytophagous insects or soil invertebrates (Custer et al. 2009; Ma 1987). Birds also pick them up secondarily through casual soil consumption when feeding (Beyer et al. 1994; Roux and Marra 2007). Ornate Tinamou and Darwin's Nothura are species that forage a wide variety of vegetal and animal food items and have sedentary diet habits (Garitano-Zavala et al. 2003). Moreover, it is known that at least Ornate Tinamou is a resident species, and individuals have a relatively restricted home range during their life (Pearson and Pearson 1955). Thus, individuals living in agrosystems irrigated by polluted waters caused by mining activities and/or tailing piles are exposed to local pollutants throughout their lifespan.

Normally species at the top of the trophic chain are thought to be the best bioindicator organisms, especially if they are aquatic, due to the fast movement of pollution in water (Burger and Gochfeld 2009; Lounsbury-Billie et al. 2008). However, our results with the two tinamou species studied showed that different exposure levels of As, Cd, Pb, and Sb at different sites were clearly expressed in the bird tissues. High data dispersion observed in the standard deviations should be attributed to the age effect, which is very difficult to control in field experiments because of the lack of an accurate ageing method. Collection of more samples should help with this problem. Other characteristics in favor of tinamou species as bioindicators are their ubiquity and relatively high abundance in their distributional areas (Cabot 1992; Davies 2002): as both are hunted for sport, biological material can be obtained easily.

Molting is a useful bio-mechanism for toxic metal removal from the bird body through physiological transportation and deposition in feathers (Furness et al. 1986). It is also known that metal concentrations in feathers reflect the values of internal tissues (as well as potential adverse effects) in several bird species (Burger 1993; Burger and Gochfeld 2000a; Pilastro et al. 1993). High correlation values obtained between feather–liver–kidney in both tinamou species allowed us to generalize that situation to As and Sb, too.

We recommend that future monitoring programs at mining sites using highland tinamou species should be based on feathers, because they are easy to collect noninvasively (from living or dead specimens) and easy to store indefinitely. The body burden proportion found in feathers is relatively constant for each metal, and a high proportion of the body burden of certain metals is stored in the feathers due to their affinity to the sulfhydryl-rich keratin protein and melanin pigments (Burger et al. 2008; Burger and Gochfeld 2009). Use of feathers is also interesting because of the advances in analytical chemistry, which lets us use museum specimens or other ornithological pieces to generate historical or background data, if in the past they had been obtained from currently polluted sites (Lounsbury-Billie et al. 2008).

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