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Bioaccumulation of Heavy Metals in the Lizard *Psammodromus algirus* After a Tailing-Dam Collapse in Aznalcóllar (Southwest Spain)

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Abstract Quantification of heavy metal concentrations in biota is a common technique that helps environmental managers measure the level of pollutants circulating in ecosystems. Despite interest in heavy metals as indicators of localized pollution, few studies have assessed these pollutants in reptiles. In 1998, the tailing pond of a pyrite mine near Aznalcóllar (southwestern Spain), containing mud with high heavy metal concentrations, collapsed, releasing 6 million m^3 of toxic sludge into the Guadiamar Basin. Here we analyze heavy metal concentrations in the most common reptile in the area, the large psammodromus, *Psammodromus algirus*, a rather small lizard. We quantified levels of several elements (Hg, Sb, Cd, Cr, Tl, Sn, Ba, Cu, Pb, Sr, Mn, Rb, As, and Zn) in lizard tail clips collected in and around the affected area during the springs of 2005 and 2006. Samples were collected from two contaminated localities, one directly affected by the spill, and another adjacent to the tailing pond, but not covered by toxic mud. We also collected samples from a nonpolluted control site in the same basin. We found higher concentrations of As, Tl, Sn, Pb, Cd, and Cu in lizards from the affected area than in lizards from the control site, indicating the continued presence of heavy metal pollutants in the terrestrial food chain 8 years after the mine accident. We did not uncover sexual or annual differences in heavy metal concentrations, although concentrations increased with lizard size. We discuss how heavy metals moved across the food chain to lizards, despite intensive restoration efforts after the accident, and suggest that reptiles to be included in biomonitoring programs of heavy metals pollution in terrestrial habitats.

Heavy metals contamination associated with mining activities has caused environmental problems in several countries (Hsu et al. 2006), hence, environmental managers are particularly interested in developing methods to detect heavy metals loads in biota (Lambert et al. 1996; Loumbourdis, 1997; Meharg et al. 1999; Burger et al. 2006). The events occurring in the Guadiamar Basin, on the southwestern Iberian Peninsula (Spain), provides resource managers with a model case study for biomonitoring heavy metals in the ecosystems. On April 1998, the wall of a large pond containing sulfide ore deposits collapsed, spilling more than 6 million m^3 of acidic water and toxic sludge directly into the Agrio and Guadiamar rivers (Gallart et al. 1999; Grimalt et al. 1999; Dorronsoro et al. 2002). The main toxic metals spilled were Pb, Zn, As, Cu, and Cd (Alastuey et al. 1999; Cabrera et al. 1999). Tailing materials reached an area 40 km long and 0.5 km wide in the Guadiamar Basin, with sludge covering the ground in a layer 0.3–3.0 m thick, depending on the distance from the collapsed dam. Environmental managers first (year 1998) attempted to clean up the pyrite slurry by mechanically removing the mud and 10 cm of the underlying soil (Simón et al. 1999). However, characteristics of the Guadiamar Basin, including the low-profile topography and mosaic

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pattern of contamination, complicated soil recovery. Thus, a second cleanup activity was undertaken (year 1999), adding different amendments to the soil to immobilize pollutants (Querol et al. 2006; Aguilar et al. 2007). These actions helped decrease contaminants in upper soil layers, however, pollutant levels increased greatly in deeper horizons of the soil, enhancing the risk of groundwater contamination (Kraus and Wiegand 2006; Ordóñez et al. 2006). After these cleanup operations in the spill-affected area, a reforestation program was initiated (1999–2001) as part of an effort to designate the area as a natural space, the Guadiamar Green Corridor.

Between 1999 and 2006, several research teams monitored the accumulation of heavy metals in soils, running waters, and organisms, including aquatic macroinvertebrates, fish, amphibians, mammals, reptiles, birds, shrubs, and trees, to examine the transfer of heavy metals through the food chain and to assess environmental managing tasks (Benito et al. 1999; Cabrera et al. 1999; PICOVER 2003; Madejón et al. 2004; Solá et al. 2004; Alcorlo et al. 2006; Olías et al. 2006; Taggart et al. 2006). In the polluted area, only one reptile species, rather marginal in the ecosystem because of its tree-dwelling habits, the Moorish gecko (*Tarentola mauritanica*), has been included in analyses of heavy metal bioaccumulation (Fletcher et al. 2006). Indeed, ecotoxicological studies on reptiles have been scarce, a trend that has only recently begun to change (Avery et al. 1983; Hopkins et al. 2000; Linder and Grillitsch 2000; Campbell and Campbell 2002; Mann et al. 2006). However, terrestrial reptiles are good bioindicators of high metals concentrations, because they occupy intermediate or high levels in food chains, frequently have a generalist diet, and have low vagility (Loumbordis 1997; Campbell and Campbell 2002; Burger et al. 2004).

During the years 2000–2006, we monitored recolonization of the restored Guadiamar Green Corridor by the reptile community. The first colonizer, and most abundant reptile, was the large psammodromus, *Psammodromus algirus* (Márquez-Ferrando et al. 2008). This small and opportunistic lizard is a generalist feeder of arthropods, exhibits fast-growing populations, has a short lifespan (mean lifespan, 2 years), and has a low vagility (Diaz and Carrascal 1990; Carretero and Llorente 1997; Salvador 1998). These biological traits make this species a suitable model for monitoring localized bioaccumulation of heavy metals in contaminated Mediterranean terrestrial habitats.

The objectives of this study are (i) to assess the quantity of heavy metals accumulated by *P. algirus* in the Guadiamar Green Corridor, 7–8 years after the mine spill; (ii) determine sexual, size-related, and interannual differences in heavy metal accumulation; (iii) detect simultaneous accumulation patterns of different metals; and (iv) contribute to the biomonitoring program of the Guadiamar Green Corridor.

Materials and Methods

Study Site

The Guadiamar River is situated in the southwest of the Iberian Peninsula (Fig. 1). Within the Guadiamar Basin, several studies have detected heavy metal accumulation in organisms from sites directly affected by the Aznalcóllar mine spill (Solá et al. 2004; Alcorco et al. 2006) and in nearby sites that were not covered by the toxic mud but were impacted by atmospheric pollution (Madejón et al. 2006). Thus, we collected *P. algirus* from three localities differently impacted by the mine spill. Two study sites are located within the Guadiamar floodplain (the Las Doblas bridge [site A] and the Agrio-Guadiamar confluence [site B]). The third site is located just outside the floodplain (Villamanrrique Pinewood [site C]). Site A, situated in the middle of the Green Corridor and 11 km downstream from the mine (Fig. 1), was severely affected by the spill and restored following the procedures described above. Site B was not covered by toxic mud, although it is located very close to the affected area (0.1 km). Site C, an unpolluted control site, is located 25 km downstream of the mine and 2 km outside of the Guadiamar floodplain.

Lizard Handling

We collected lizards by hand during May–June of 2005 and 2006. Thirty lizards from site A, 15 from site B, and 20 from site C were captured. We measured (snout-vent length [SVL], to the nearest millimeter) and sexed lizards (by color pattern and morphology of the femoral pores) to check for the effect of size and sex in metal accumulation, as has been reported in other reptile species (Linder and Grillitsch 2000). Nonlethal measurements can be used to assess pollutant levels in squamate reptiles (Hopkins et al. 2001; Burger et al. 2005; Fletcher et al. 2006), and the use of such methods here is advisory to reduce human-induced alterations, as the Guadiamar Green Corridor is currently a protected area. Thus, we collected a tail clip (<30 mm) from each individual to assess levels of heavy metals. Each lizard was later released at its capture site. We assumed that the probability of recapture in the second sampling year in the polluted site was minimal due to the high population density (Márquez-Ferrando et al. 2008); furthermore, during the 2006 sampling period, we did not capture individuals with regenerated tails.

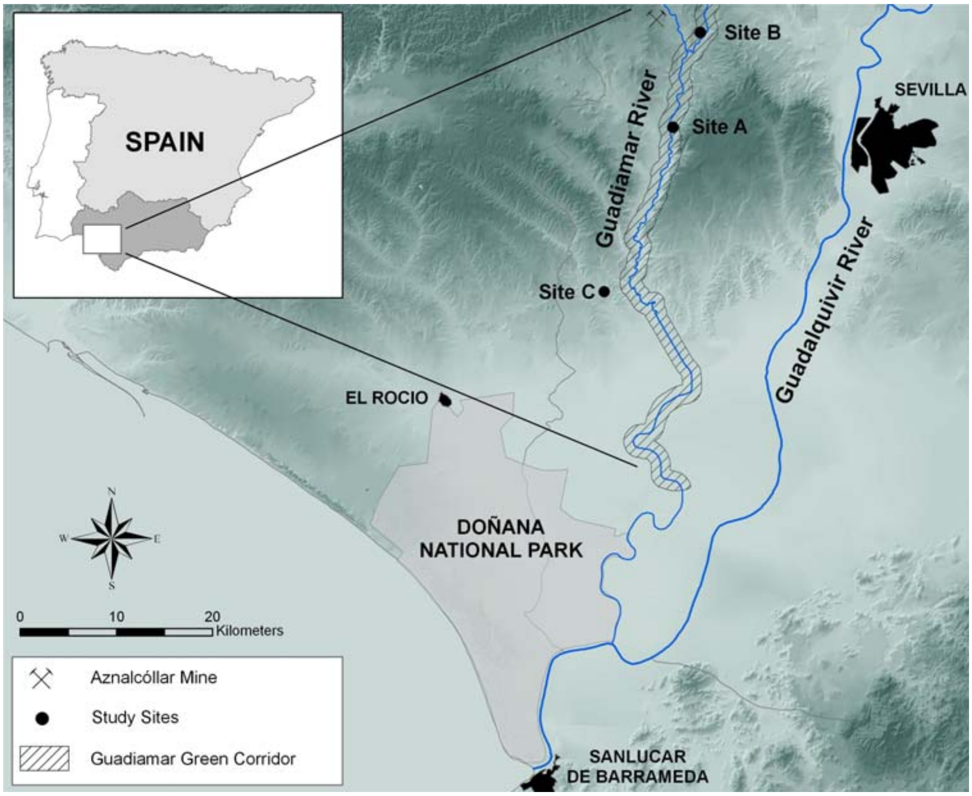

Fig. 1 Study area. Maps showing the location of the Guadiamar Green Corridor in the Iberian Peninsula and the locations of the three study sites within the Guadiamar Basin

Laboratory Procedures

We analyzed concentrations of 14 metals (Hg, Sb, Cd, Cr, Tl, Sn, Ba, Cu, Pb, Sr, Mn, Rb, As, and Zn). Of these, Pb, Zn, As, Cu, and Cd were abundant in the toxic mud (Alastuey et al. 1999), whereas the other metals, although less abundant in the mud, have been analyzed previously in several organisms of the Guadiamar Basin and, hence, contribute to a wider overview of heavy metal mobilization along the food chain (Solá et al. 2004; Fletcher et al. 2006). After collection, tail clips were cleaned with deionized water and freeze-dried. Samples were oven-dried at 60°C until they attained constant weights and digested for 8 h with 2 ml HNO_3 and 1 ml H_2O_2 in Teflon vessels. Samples were brought to a final volume with deionized water. Metal concentrations were measured by mass spectroscopy (Perkin-Elmer ELAN-6000) by the Scientific-Technical Services at the University of Barcelona. We included 10 blanks in digestion and analysis procedures as controls. Results of element levels are expressed as micrograms per gram on a dry weight basis. For lizards collected in the control population we found concentrations below the detection limits for several metals. In these cases, we used one-half the detection limits as surrogate values for nondetects (Hesel 1990).

Statistical Analyses

We used nonparametric tests when data did not fit normality after log transformation and compared heavy metal concentrations among the three localities with a two-way ANOVA with rank-transformed dependent variables, considering sex, location, and their interaction, as factors. Differences between pairs of localities were tested using Tukey posthoc tests. We checked the relationship between body size and heavy metal levels in lizards from site A using Spearman rank correlations. This analysis was also used to investigate patterns of accumulation in pairs of heavy metals. Scores of the correlation matrix were used to create a cluster tree of similarities among metals according to Euclidean distances. Thus, metals were organized as functions of the linkage distances between them, using single linkage as the aggregation algorithm. In all tests, significant differences were assumed at $p < 0.05$.

Results

Differences Among Localities

We failed to detect difference in lizard size among localities (mean SVL = 66.6 ± 1.6 mm [site A], 67.0 ± 2.1 mm

Table 1 Means and standard errors of metal concentrations (μg g^{-1} dry weight) found in tails of the lizard *Psammodromus algirus* collected at three locations in the Guadiamar Basin (southwestern Spain)

	Site A ($n = 30$)	Site B ($n = 15$)	Site C ($n = 20$)	F (p)	
Hg	0.17 ± 0.02	0.24 ± 0.03	0.09 ± 0.01	12.73 (<0.01)	B and A > C
Sb	0.38 ± 0.06	0.08 ± 0.02	0.17 ± 0.14	19.47 (<0.01)	A > C > B
Cd	0.14 ± 0.02	0.05 ± 0.02	0.03 ± 0.02	10.85 (< 0.01)	A and B > C
Cr	2.05 ± 0.16	1.43 ± 0.08	1.41 ± 0.07	1.85 (0.17)	–
Tl	0.08 ± 0.01	0.05 ± 0.02	0.00 ± 0.00	88.52 (<0.01)	A > B > C
Sn	0.20 ± 0.06	0.02 ± 0.00	0.03 ± 0.01	4.09 (0.02)	A > C
Ba	2.88 ± 0.33	1.76 ± 0.38	4.38 ± 0.50	6.45 (<0.01)	C > B
Cu	10.54 ± 1.55	4.25 ± 0.38	3.03 ± 0.17	24.29 (<0.01)	A > B and C
Pb	10.18 ± 1.94	2.60 ± 0.69	2.17 ± 1.00	10.82 (<0.01)	A > B > C
Sr	10.56 ± 0.89	6.30 ± 1.36	8.86 ± 0.88	1.35 (0.27)	–
Mn	13.71 ± 1.90	6.72 ± 0.98	8.32 ± 1.01	1.88 (0.17)	–
Rb	7.60 ± 0.81	6.96 ± 0.52	3.38 ± 0.25	29.64 (<0.01)	A and B > C
As	6.26 ± 1.05	1.20 ± 0.23	0.30 ± 0.03	62.72 (<0.01)	A > B > C
Zn	106.76 ± 4.20	86.92 ± 5.61	85.08 ± 8.45	1.91 (0.16)	A > B > C

Note: Comparisons were tested by two-way ANOVA with rank-transformed data. Site A, Guadiamar River floodplain, fully affected by the spill; site B, Guadiamar River floodplain, very near the affected area; site C, Villamanrrique pinewood, 2 km away from the affected area

[site B], 64.0 ± 2.1 mm [site C]; ANOVA test, $F_{2,48} = 0.69$, $p = 0.51$). We found differences among localities in all metal concentrations (Table 1). Results of two-way ANOVA showed differences between populations ($F_{2,48} = 7.36$, $p < 0.01$), between sexes ($F_{1,24} = 2.64$, $p = 0.02$), and in the interaction sex × location ($F_{2,48} = 1.93$, $p = 0.02$) for overall heavy metal concentration. Tukey posthoc test indicated a similar pattern in 10 of the 14 elements: lizards collected at site A showed higher levels than did lizards from the other two localities. Lizards from site A showed 21-, 8-, 7-, 5-, and 4-fold higher concentrations of As, Tl, Sn, Pb, Cd, and Cu, respectively, compared to lizards from site C. We also detected differences between lizards from site B and lizards from site C in Tl, As, Hg, Rb, Cu, Pb, and Zn. In each case, lizards from site B had higher metal concentrations than did lizards from site C.

Sexual, Interannual, and Size-Related Differences

There was no significant sexual difference in body size among individuals collected at site A (mean SVL, males 68.5 ± 7.8 mm; females 64.5 ± 4.5 mm; ANOVA, $F_{1,11} = 0.92$, $p = 0.36$). Sexes did not differ in heavy metal levels except for Cr (Table 2). For this reason, we did not separate sexes in further analyses. Likewise, there was no difference in body size in individuals collected in 2005 versus 2006 at site A (mean SVL: year 2005, 66.7 ± 9.0 mm; year 2006, 66.3 ± 1.9 mm; ANOVA test, $F_{1,20} = 0.01$, $p = 0.91$). We did not find significant differences in any metal concentrations, except for Hg and Sn, in lizards collected in 2005 versus 2006 at site A. Mercury level was higher in lizards collected during 2006 (Table 3), and Sn levels were lower in 2006. The relationship between metal concentration and lizard size approached statistical significance for several metals, but was significant only for Cd (Fig. 2).

Correlations Among Metal Concentrations

At site A, metal concentrations were positively correlated in 41 of 98 pairs of elements (Table 4), indicating that many elements shared similar accumulation trends (Fig. 3). The group of Sb, As, and Tl were all strongly associated, with Spearman coefficients >0.8. A second group was composed of Cd, Ba, Mn, Cu, and Pb, with correlation coefficients >0.7. The pair formed by Hg and Rb was positively correlated, with a value of 0.5, and exhibited sharp differences with respect to other elements. Finally, a

Table 2 Means and standard errors of metal concentrations (μg g^{-1} dry weight) in tail clips of male and female *Psammodromus algirus* collected in the most affected area by the Azanalcóllar mine spill (site A; see Materials and Methods) of the Guadiamar Basin (southwestern Spain)

	Male ($n = 9$)	Female ($n = 5$)	F (p)
Hg	0.16 ± 0.02	0.20 ± 0.05	0.82 (0.38)
Sb	0.47 ± 0.13	0.34 ± 0.10	0.45 (0.51)
Cd	0.19 ± 0.05	0.06 ± 0.03	2.88 (0.12)
Cr	2.32 ± 0.35	1.37 ± 0.08	5.81 (0.03)
Tl	0.09 ± 0.02	0.09 ± 0.04	0.13 (0.73)
Sn	0.24 ± 0.13	0.08 ± 0.04	0.62 (0.45)
Ba	3.06 ± 0.58	2.13 ± 0.54	0.63 (0.44)
Cu	14.17 ± 4.17	5.92 ± 2.34	2.25 (0.16)
Pb	12.59 ± 3.12	6.18 ± 1.06	1.77 (0.21)
Sr	10.53 ± 1.77	7.99 ± 1.05	1.61 (0.23)
Mn	12.76 ± 1.99	8.13 ± 1.60	4.58 (0.05)
Rb	7.10 ± 1.04	10.89 ± 2.78	2.73 (0.13)
As	8.19 ± 2.23	5.23 ± 1.99	0.42 (0.53)
Zn	102.77 ± 47.46	95.36 ± 11.92	0.68 (0.43)

Note: Differences between sexes were tested by one-way ANOVA with rank-transformed data

Table 3 Means and standard errors of metal concentrations (μg g^{-1} dry weight) in tail clips of the lizard *Psammodromus. algirus* collected in 2005 and 2006 in the area most affected by the Aznalcóllar mine spill (site A; see Materials and Methods) of the Guadiamar Basin (southwestern Spain)

	2005 ($n = 20$)	2006 ($n = 10$)	F (p)
Hg	0.12 ± 0.01	0.28 ± 0.03	29.95 (<0.01)
Sb	0.43 ± 0.08	0.29 ± 0.09	1.32 (0.26)
Cd	0.14 ± 0.03	0.14 ± 0.03	0.55 (0.46)
Cr	2.20 ± 0.21	1.74 ± 0.21	3.35 (0.08)
Tl	0.08 ± 0.02	0.07 ± 0.02	0.27 (0.61)
Sn	0.28 ± 0.09	0.04 ± 0.01	11.98 (<0.01)
Ba	2.82 ± 0.39	3.01 ± 0.62	0.03 (0.86)
Cu	9.74 ± 2.28	11.05 ± 3.78	0.07 (0.93)
Pb	11.21 ± 2.17	9.20 ± 1.77	0.15 (0.69)
Sr	11.82 ± 1.14	8.04 ± 1.05	3.94 (0.06)
Mn	12.09 ± 1.42	16.96 ± 4.96	0.55 (0.46)
Rb	6.55 ± 0.75	9.70 ± 1.77	3.94 (0.06)
As	6.76 ± 1.35	5.25 ± 1.67	0.37 (0.55)
Zn	109.34 ± 4.94	101.60 ± 7.91	0.93 (0.34)

Note: Differences between years were tested by one-way ANOVA with rank-transformed data

group consisting of Sr, Cr, Zn, and Sn exhibited very low correlations with the rest of metals.

Discussion

Differences Among Localities

This study describes heavy metal contamination of terrestrial organisms from the Guadiamar Green Corridor following the collapse of the Aznalcóllar mine tailing pond. Lizards from the most impacted site exhibited higher metal concentrations 8 years after the spill than did both lizards collected at a control site and lizards from a nearby locality not covered by toxic mud. Water quality in the Guadiamar River increased from 2002 onward (Olías et al. 2006), and consequentially, species richness and the community structure of freshwater organisms improved (Toja et al. 2003). In contrast, and despite extensive cleaning actions, the soils of the Doblas (site A) still exhibited high concentrations of heavy metals 4 years after the spill because they persist in deeper horizons (Kraus and Wiegand 2006). These contaminants are taken up by plants through their roots and, depending on the mobility of the chemicals, are moved to vegetative parts (in general to leaves) of the plants (Madejón et al. 2004). Leaves, as well as other plant parts, may be eaten by insects that are reptile prey. Several studies have shown that the principal avenue of pollutant acquisition by reptiles is through the ingestion of polluted prey (Hopkins et al. 2001, 2002; Fletcher et al. 2006; Mann et al. 2006). *Psammodromus algirus* is a generalist forager on epigeous invertebrates, Colepotera, Heteroptera, and Araneae being the main prey for populations of this lizard from southwestern Spain (Pérez Quintero and Rubio García 1997) which live in the upper levels of soils and can be exposed to several metals, such as Cd and Pb, in polluted areas (Jelaska et al. 2007). Furthermore, a complementary avenue for acquisition of contaminants in lizards is the accidental ingestion of sediment particles with food (Fletcher et al. 2006; Mann et al. 2006), very important in *P. algirus* due to its foraging habits (Carretero and Llorente 1993).

Arsenic was the toxic element that showed the highest difference between individuals dwelling in contaminated and control sites. This metal was very important in the toxic mud (Alastuey et al. 1999) and its monitoring in the food chain is potentially critical because of its neurotoxic effects on a variety of organism (Chang 1996), as well as its negative effects on the embryonic development in the Iberian rock lizard (*Lacerta monticola cyrenni* [Marco et al. 2004]).

We also detected highly significant differences in Tl among populations. Thalium persists for long periods in terrestrial ecosystems and is still detectable at high levels in the contaminated areas, indicating wide dispersal through the terrestrial food chain (Madejón et al. 2004; Sánchez-Chardi 2007), although the exact mechanism of toxicity is still unclear (Jon Peter and Viraraghavan 2005).

Among the metals analyzed, Zn showed the highest concentrations in lizard tails, reinforcing the pattern previously observed in *T. mauritanica* (Fletcher et al. 2006). Concentrations of Zn may remain higher than those of other metals because Zn can bind to specific metallothioneins in reptiles, interfering with the organism's detoxification processes (Linder and Grillitsch 2000; Lance et al. 2000). Other metals, such as Mn and Cr, were very scarce in the sludge and in the surrounding area (Cabrera et al. 1999; Simón et al. 1999), although we detected accumulations in lizards (Table 1). These elements are lithophilic and, therefore, despite occurring in low levels, became increasingly available to plants as a consequence of soil acidification following the spill (Madejón et al. 2006).

Although site B was not covered by the toxic sludge, lizard tails from this site showed intermediate metal concentrations (Table 1). Madejón et al. (2006) suggested that metals may spread from contamination sites via atmospheric transport and deposition of contaminants over surrounding areas. This process may have been exacerbated by the removing of affected soils during clean-up activities in the 1998–1999 (Querol et al. 2000). Consequently, deposition of aerosolized elements may explain

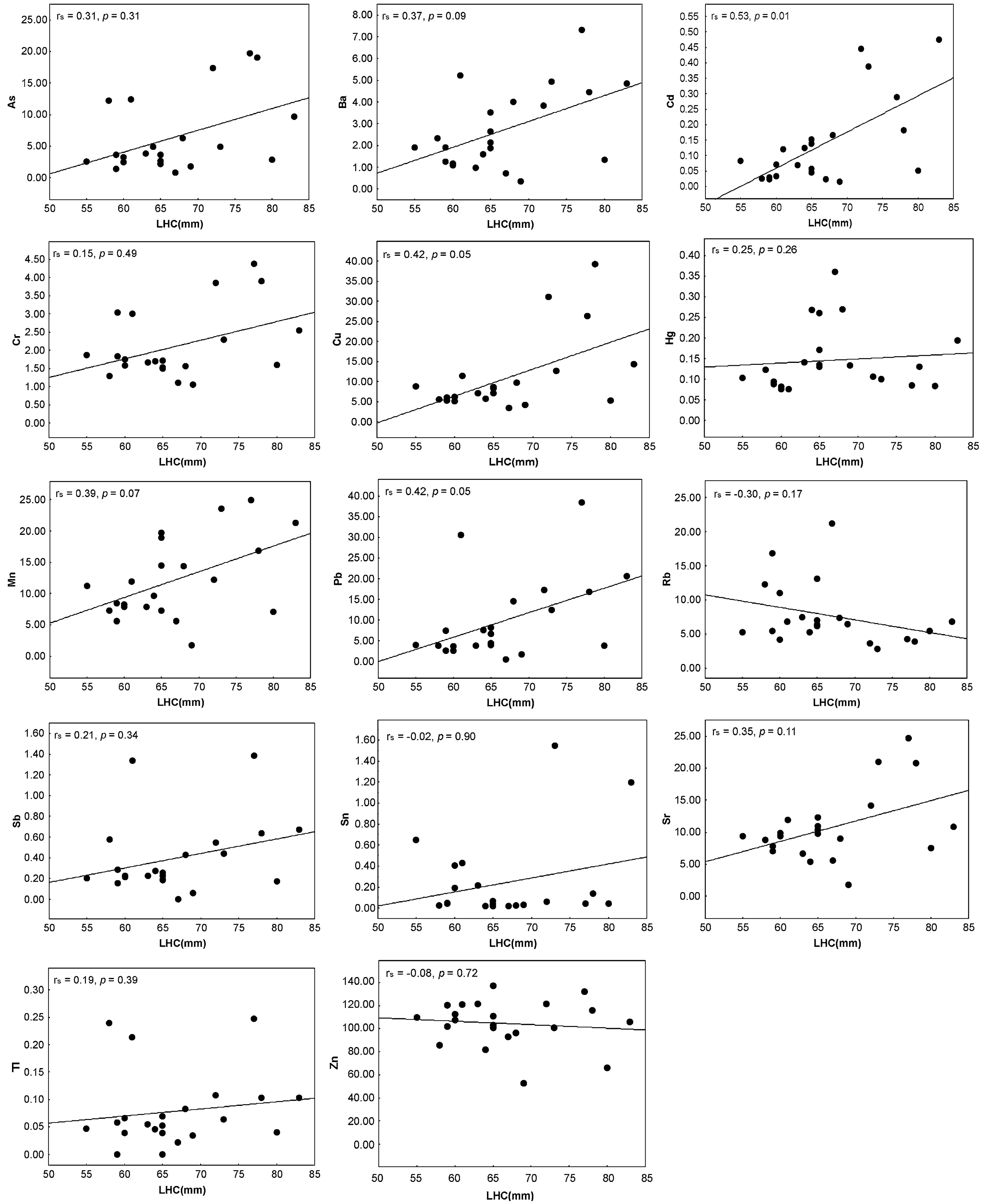

Fig. 2 Size-related differences in metal concentrations. Spearman correlations between metal concentrations (μg g^{-1} dry weight) in tail clips of *Psammodromus algirus* ($n = 22$) and lizard size (SVL), in the area most affected by the Azanalcóllar mine spill (Site A, see Materials and Methods) of the Guadiamar Basin (southwestern Spain)

Table 4 Spearman correlations among metal concentrations in tail clips of the lizard *Psammodromus algirus* ($n = 30$) collected in the area most affected by the mine spill of Aznalcóllar (site A; see Materials and Methods) of the Guadiamar Basin (southwestern Spain)

	Hg	Sb	Cd	Cr	Tl	Sn	Ba	Pb	Cu	Sr	Mn	Rb	As	Zn
Hg	1.00													
Sb	–0.09	1.00												
	(0.06)													
Cd	0.34	0.60	1.00											
	(0.06)	(<0.001)												
Cr	−0.26	0.68	0.59	1.00										
	(0.16)	(<0.001)	(<0.001)											
Tl	−0.02	0.84	0.59	0.53	1.00									
	(0.91)	(<0.001)	(<0.001)	(0.003)										
Sn	−0.38	0.32	0.26	0.45	0.25	1.00								
	(0.04)	(0.09)	(0.16)	(0.01)	(0.18)									
Ba	0.11	0.73	0.74	0.62	0.68	0.26	1.00							
	(0.55)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(0.17)								
Pb	0.19	0.82	0.85	0.71	0.76	0.34	0.86	1.00						
	(0.46)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(0.07)	(<0.001)							
Cu	0.14	0.65	0.81	0.69	0.62	0.39	0.84	0.85	1.00					
	(0.31)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(0.04)	(<0.001)	(<0.001)						
Sr	−0.19	0.39	0.50	0.50	0.39	0.31	0.68	0.45	0.67	1.00				
	(0.12)	(0.03)	(0.005)	(0.005)	(0.03)	(0.09)	(<0.001)	(0.01)	(<0.001)					
Mn	0.29	0.59	0.84	0.60	0.48	0.26	0.84	0.81	0.83	0.58 (<0.001)	1.00			
	(0.06)	(<0.001)	(<0.001)	(<0.001)	(0.008)	(0.17)	(<0.001)	(<0.001)	(<0.001)					
Rb	0.34	0.02	−0.26	−0.35	0.01	−0.32	−0.22	−0.11	−0.29	−0.58 (<0.001)	−0.16 (0.41)	1.00		
	(0.76)	(0.91)	(0.165)	(0.06)	(0.96)	(0.08)	(0.25)	(0.57)	(0.12)					
As	0.06	0.89	0.71	0.63	0.88	0.25	0.73	0.82	0.65	0.40 (0.027)	0.58 (<0.001)	−0.18 (0.35)	1.00	
	(0.85)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(0.18)	(<0.001)	(<0.001)	(<0.001)					
Zn	−0.04	0.29	0.35	0.56	0.27	0.32	0.45	0.41	0.61	0.60 (<0.001)	0.42 (0.02)	−0.25 (0.17)	0.23	1.00
	(0.64)	(0.12)	(0.06)	(<0.001)	(0.15)	(0.08)	(0.01)	(0.02)	(<0.001)				(0.22)	

Note: After sequential Bonferroni correction for multiple test, the p value was adjusted for 5% of the nominal level, $p < 0.001$

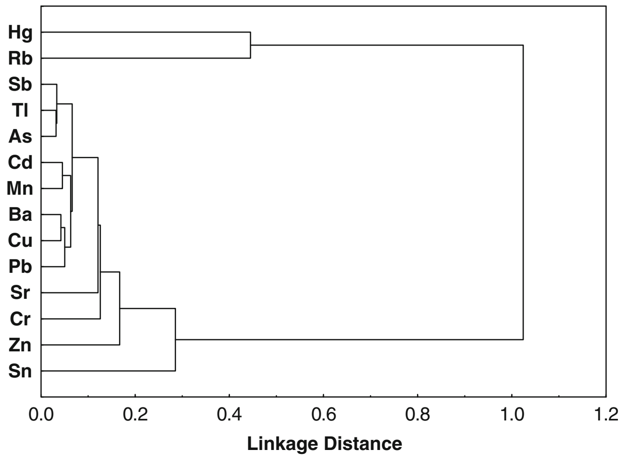

Fig. 3 Correlations among metal concentrations. Cluster tree that show the linkage (Euclidean distance) between metal concentrations by single linkage as aggregation algorithm in tail clips of the lizard *Psammodrolus algirus* in the area most affected by the Azanalcóllar mine spill (site A; see Materials and Methods) of the Guadiamar Basin (southwestern Spain)

the contamination of lizards in areas surrounding the affected site. Surprisingly, lizards from site B showed higher levels of Hg than lizards from site A. Mercury was present in low levels within the sludge (Alaustey et al. 1999; Cabrera et al. 1999), and was of great concern in aquatic ecosystems (Pain et al. 1998; Sanpera et al. 2000), but information concerning impacts and transport of this metal in terrestrial food chains is scarce.

Our results were similar to those reported in whole-body samples of *T. mauritanica* from the same study area (Fletcher et al. 2006). In this gecko, As, Tl, Cd, and Pb levels were also higher among individuals at contaminated sites than in control geckos. However, tail samples from *P. algirus* had higher concentrations of As, Tl, Mn, Sb, Pb, and Cu than did whole-body samples of *T. mauritanica*. Both studies suggest that reptile species are useful indicators of heavy metal levels in the Guadiamar Green Corridor.

Sexual, Annual, and Lizard Size Comparisons

In some reptiles, sex can influence metal accumulation due to differences in physiology, size, and diet (Linder and Grillitsch 2000; Van Straalen et al. 2001; Hopkins 2002; Burger et al. 2004; Hopkins et al. 2005). In site A, *P. algirus* did not exhibit sexual size dimorphism, in contrast to other Iberian populations (Mellado and Martínez 1974; Carretero and Kaliontzopoulou 1990), however, this could be because in our study a low number of males and females were compared. Furthermore, this lizard did not display sexual differences in diet and microhabitat use (Díaz and Carrascal 1990; Salvador 1998; Carretero et al. 2002). Accordingly, it is not surprising that we did not detect sexual differences in metal concentrations.

Likewise, we did not detect any differences in samples collected in 2005 versus 2006, even though several authors have found that metal levels in water, soil, and plants declined over time in the Agrio and Guadiamar river floodplains (Madejón et al. 2006; Olías et al. 2006; Querol et al. 2006). It is likely that a longer-term study would be needed to detect temporal changes in accumulation of metals by lizards.

Cadmium was the only metal that increased significantly with lizard size in *P. algirus*. Cadmium accumulation seems to be dependent on the duration of metal exposure (Mann et al. 2007). Correlations between metal concentrations and lizard size have been documented for Cd and Pb in *Podarcis muralis* and *Anolis sagrei*, and for Pb and Cu in *Zootoca vivipara* (Schmidt 1980, 1988; Burger et al. 2004). In general, larger individuals can accumulate higher amounts of pollutants, although considerable differences exist among species based, at least in part, on species longevity (Santos et al. 1999; Linder and Grillitsch 2000). *Psammodromus algirus* is a short-lived species and all individuals we captured were likely between 1 and 2 years old. Thus, the lack of correlation with lizard size for most heavy metals is not surprising, since the correlation between size and longevity in adults is very weak in *P. algirus* (Carretero, pers. commun.).

Relationships Among Elements

In general the presence of simultaneous heavy metals within an ecosystem favors the existence of interactions between them (Beyersmann 1991). In our study high, positive correlations among eight elements suggest that these metals were accumulated simultaneously. A similar pattern occurred in *T. mauritanica* from the Guadiamar River (Fletcher et al. 2006). Low correlations between Sr, Cr, Zn, and Sn and the rest of the heavy metals suggests that these elements may be acquired or taken up differently than the first group of metals are, although whole-body analyses of metal concentrations might yield different results and are necessary to clarify the pattern of correlation of heavy metal accumulated by reptiles.

Conclusion

Over recent years several studies have focused on bioaccumulation of heavy metals by wildlife in the Guadiamar Green Corridor following the release of mining by-products in this area. Our study adds to the knowledge of this issue in terrestrial animals and demonstrates that, even 8 years after a toxic spill and subsequent cleanup activities, the terrestrial food chain still demonstrates exposure to high levels of heavy metals. Lizards from contaminated sites showed significantly higher concentrations of several metals than did lizards from noncontaminated sites. Because of their biological traits (low vagility, middle and upper position in the trophic food chain, generalist diet, rapid population turnover, short lifespan), we suggest that small reptiles such as *P. algirus* are good bioindicators of local heavy metal contamination (for the same study area see also Fletcher et al. 2006). Long-term studies of trace-element accumulation in aquatic and terrestrial biota are necessary to understand how pollutants move across food chains, and to assess the continued impact of the mining spill on Guadiamar Green Corridor wildlife.

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