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GECKOS AS INDICATORS OF MINING POLLUTION

DEAN E. FLETCHER,*† WILLIAM A. HOPKINS,‡ TERESA SALDAÑA,§ JENNIFER A. BAIONNO,† CARMEN ARRIBAS,§ MICHELLE M. STANDORA,† and CARLOS FERNÁNDEZ-DELGADO§
†Savannah River Ecology Laboratory, University of Georgia, PO Drawer E, Aiken, South Carolina 29803, USA
‡Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg Virginia 24061, USA
§Departamento de Zoología, Edificio Charles Darwin, 3 Planta, Campus Universitario de Rabanales, Universidad de Córdoba, Córdoba 14071, España Spain

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Abstract—Catastrophic collapse of a mine tailings dam released several million cubic meters of toxic mud and acidic water into the Guadiamar River valley, southern Spain, in 1998. Remediation efforts removed most of the sludge from the floodplain, but contamination persists. Clean-up activities also produced clouds of aerosolized materials that further contaminated the surrounding landscape. Whole-body concentrations of 21 elements in the Moorish wall gecko, *Tarentola mauritanica*, a common inhabitant of both rural and urban areas, were compared among seven locations. Locations spanned an expected contamination gradient and included a rural and an urban non–mine-affected location, two mine-affected towns, and three locations on the contaminated floodplain. Multivariate analyses of whole-body concentrations identified pollutants that increased across the expected contamination gradient, a trend particularly evident for As, Pb, and Cd. Additionally, higher contaminant concentrations occurred in prey items eaten by geckos from mine-affected areas. Comparison of element concentrations in tails and whole bodies suggests that tail clips are a viable nondestructive index of contaminant accumulation. Our results indicate that areas polluted by the mine continue to experience contamination of the terrestrial food chain. Where abundant, geckos represent useful taxa to study the bioavailability of some hazardous pollutants.

Keywords—Gecko Bioindicator Trace element Mine pollution Guadiamar River

INTRODUCTION

Pollution of surface waters from mining and the processing of mined materials is a pervasive problem in mining areas around the globe. In the continental United States alone, more than 19,300 km of rivers and more than 72,500 ha of lakes and reservoirs have been damaged by acid mine drainage [1]. Subsequent to removal from the mine, ore is crushed, ground, washed, and, after treatment, the valuable metal sulfides are separated by flotation from the undesirable sulfides [2,3]. This procedure produces enormous amounts of acidic waste water and fine-grain material (tailings), which are often stored behind dams. Oxidation of pyrite and other sulfidic minerals in these processing wastes produce materials with characteristically low pH (2–4) and high concentrations of toxic elements such as Cu, Zn, Pb, Cd, and As [4]. Accidents at mines involving stored tailings have produced catastrophic environmental damage on top of the chronic contaminant releases associated with mining activities. Recent accidents include 1.6 million cubic meters of mine tailings spilling into the Makulapnit–Boac River system of Marinduque Island, Philippines, in 1996 [5]. Similarly dam breakage in Baia Mare, Romania, released 100,000 cubic meters of mud containing cyanide and metals into the surrounding landscape, including the Tisza–Danube River system [6].

One of the most disastrous releases of mine tailings in European history occurred at the Boliden-Apirsa mine at Aznalcollar, Spain [3]. The Aznalcollar Zn, Ag, Pb, and Cu mine is located 30 km northwest of Seville (Fig. 1). Annual production of the Los Frailes mine included: 65 tons of Ag; 13,800 tons of Cu, 18,000 tons of Pb, and 47,800 tons of Zn [7]. It lies on the southeast edge of a rich mining region, the Iberian Pyrite Belt, within which some mines have operated since before Roman times [3,8,9]. Before the accident, the Guadiamar River Valley had a relatively long history of chronic contamination from Aznalcollar mining activities. Contamination of river water and sediments increased with proximity to the mine and pH decreased [10,11]. In fact, pollution was sufficient that contaminants in stream sediments oxidized to form white crusts of highly soluble salts when exposed to the air [10]. Enhanced mobilization of contaminants in the river by flooding [10] and irrigation of croplands by a contaminated aquifer [12] resulted in elevated concentrations of mine contaminants in riparian soils as well as aquatic and bank vegetation.

The Aznalcollar tailings dam was constructed in 1974 on the bank of the Agrio River 3.27 km above its confluence with the Guadiamar River, Spain. Waste materials from the Aznalcollar mine were stored in this reservoir until 1995 [3,8], after which it was used to store wastes from the new Los Frailes deposit. By the time of the accident, the dam had been heightened to approximately 25 m with over 30 million cubic meters of acidic water and toxic mud stored behind it [3]. On April 25, 1998, a 50-m section of the dam collapsed allowing 4 million cubic meters of acidic water (pH near 3) and 2 million cubic meters of toxic mud to be released into the Agrio and subsequently the Guadiamar Rivers [3]. The floodplain was devastated by an over 0.5-km-wide flow of toxic mud that reached 40 km downstream of the tailings reservoir and covered over 40 km^2 [3,9]. Acidic waters flowed 60 km downstream and were retained by dams constructed in the Entremuros area, where the water was treated before release.

The metal-rich tailings released from the Aznalcollar mine contained massive amounts of Fe, S, Zn, Pb, As, Cu, Sb, Co,

* To whom correspondence may be addressed (fletcher@srel.edu).

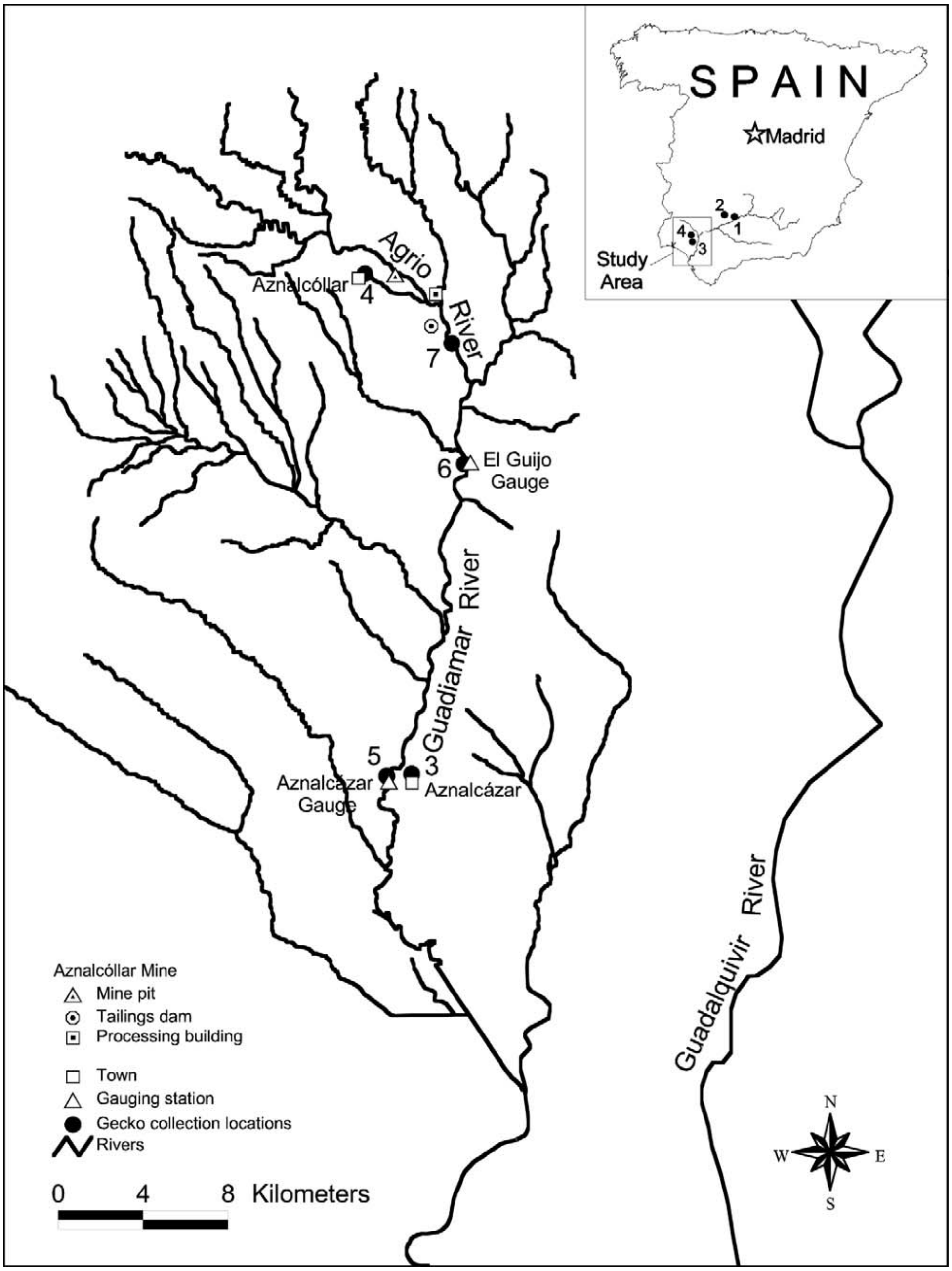

Fig. 1. Location of gecko collection sites in southern Spain. Guadalmellato Reservoir (site 1), Villaviciosa de Córdoba (site 2), Aznalcázar (site 3), Aznalcollar (site 4), Guadiamar River floodplain near the Aznalcázar (site 5) and Guijo (site 6) gauge stations, and the Agrio River floodplain (site 7).

Tl, Bi, Cd, Ag, Hg, and Se [2,3,8]. After the spill, the toxic mud and upper layer of soil was mechanically removed and dumped into the Aznalcollar mine pit [3]. After reaching pit capacity, additional sludge was buried on the floodplain. The sludge excavation also removed all floodplain vegetation and most of the seed bank and reworked much of the river channel. Because of the relatively long time (May 1998–January 1999) required to remove such extensive mud deposits and the small particle size of the material [3], much of the mud material had dried; thus, cleanup produced enormous clouds of toxic aerosols. Cities, villages, and farmland in close proximity to the contaminated floodplain were at risk of contamination from the mine spill and remediation activities. In addition, critical ecological habitats such as the Doñana National Wildlife Refuge are downstream from the site, and contamination of this system could affect a variety of organisms, especially migratory birds [3]. Much work in the Guadiamar Valley has concentrated on pollution of water, river sediments, and riparian soils (e.g., [13,14]). Although work has been conducted on contaminant accumulation in natural and agricultural vegetation growing on the contaminated riparian zone (e.g., [15,16]), less is known about the fate and transport of toxic metals and metalloids through terrestrial food chains.

The objectives of this study were to assess whether the historical mining activities and the Aznalcollar disaster contaminated terrestrial food webs in the floodplain as well as in nearby towns and villages, determine the usefulness of gecko tail clips as an indicator of element accumulations, and explore dietary exposure by examining gecko gut contents. To address this question, we measured tissue levels of contaminants in the bodies, tail clips, and diet items of Moorish wall geckos (*Tarentola mauritanica*) collected along a suspected contamination gradient. This species is widespread and frequently abundant across southern Europe and North Africa from the Mediterranean Sea to the Sahara Desert. We postulated that *T. mauritanica* might be a particularly good model for studying the fate and transport of contaminants into the terrestrial landscape for several reasons. First, *T. mauritanica* is a fairly opportunistic predator but primarily feeds on invertebrates [17]. This trophic position renders it a good integrator of bioavailable contaminants that have entered terrestrial food webs. Second, the species has a relatively small home range [18], making it potentially useful for resolving problems related to the spatial distribution of contaminants. Finally, the species occupies diverse habitats ranging from wet woodlands to desert areas, but unlike many other types of wildlife, it also thrives in urban environments [18,19]. This latter characteristic makes them useful for assessing contaminant transport into human-dominated landscapes and could shed some insight into potential human health risks in these areas. Indeed, a recent study clearly demonstrated the utility of monitoring *T. mauritanica* to understand the extent of metal pollution (particularly Pb) across an urbanization gradient [20].

MATERIALS AND METHODS

Study site descriptions

Geckos were collected from seven southern Spain locations, including a rural and an urban location not affected by the mine, two towns affected by the mine, and three locations on the contaminated floodplain (Fig. 1). Our rural reference site was located near the Guadalmellato Reservoir (site 1), a 774-ha reservoir surrounded by Mediterranean forest. This location, most isolated from urban and mining pollution, was expected to be the most pristine. Villaviciosa de Córdoba (site 2), a relatively small town with a population of 3,679, represented our urban site not affected by the mine. Although not influenced by mining activities, geckos here were exposed to urban contaminants—particularly Pb [20]. Because of the influence of urbanization on trace element accumulation, use of a town similar in size to those in the affected area was crucial. Aznalcázar (site 3), population 3,581, is located 21.5 km downstream of the mine but is located within 1 km of the floodplain that was inundated with sludge from the spill. Consequently, aerosolized contaminants engulfed the town during cleanup activities, resulting in elevated concentrations of As, Cd, Co, Mn, Ni, Pb, Zn, Sb, and Tl [21,22]. Additionally, the proximity to the floodplain could present a risk of contaminated prey (e.g., flying insects) entering the town. We ranked the town Aznalcollar (site 4), population 5,845, next along our expected contamination gradient because of its proximity to the mine pit, processing plant, and tailings reservoir (Fig. 1). Located only 1.8 km from the mine and 4.1 km from the tailings dam, this location could be contaminated by normal mine operations or the disaster and subsequent remediation efforts. Tremendous plumes of contaminants were discharged into the air as truck loads of the dried sludge were dumped into the pit.

Three locations on the contaminated floodplain were selected along a downstream longitudinal contamination gradient. Geckos were collected from the Agrio River floodplain (site 7) only 4.4 km below the tailings dam that ruptured as well as from two sites on the Guadiamar River floodplain located near the Guijo (site 6) and Aznalcazar (site 5) water discharge gauging stations about 7.4 and 24.8 km below the accident site, respectively. All of the floodplain sites had been inundated by sludge discharged during the spill, but thickness of the deposited sludge decreased downstream [8]. Additionally, composition of the sludge changed downstream from the tailings dam to Aznalcázar gauge because particle size decreased as larger particles settled out [9]. In addition to contamination by the accident, these areas were chronically polluted by mining activities before the spill, as described above.

Tissue collection and handling

A total of 52 geckos were collected from building walls at night between May 31 and June 18, 2001: Guadalmellato Reservoir ($n = 9$), Villaviciosa de Córdoba ($n = 13$), Aznalcázar ($n = 8$), Aznalcollar ($n = 8$), Guadiamar River floodplain near the Aznalcázar ($n = 5$) and Guijo ($n = 5$) gauge stations, and the Agrio River floodplain ($n = 4$). Geckos were kept frozen at −70°C until dissection. After thawing, each individual was weighed to the nearest 0.1 mg and snout–vent length measured to the nearest millimeter. Subsequently, the gut (stomach and intestine) was removed, opened, contents emptied, and interior surface gently rinsed with 18 MΩ deionized water. Food items were refrozen for later analyses. Cleaned guts were placed back into the carcass. Because *T. mauritanica* is not clearly outwardly sexually dimorphic [18], sexes were recorded on the basis of gonad examination, and individuals too small to possess visible gonads were categorized as juveniles. The unfeasibility of sex determination in the field and the relatively low sample sizes at some sites resulted in skewed sex ratios. Thus the sex of individuals could not be rigorously considered in our subsequent statistical comparisons. Detachable portions of the tail, generally about 95% of the tail's total length, were broken from the carcasses. Before oven drying, the tails and carcasses were rinsed with deionized water to remove surface contamination. To obtain accurate dry weights for the tails and bodies, samples were dried for 3 days in a drying oven at approximately 55°C, desiccated for 3 d, and allowed to stabilize in a desiccated glove box before weighing to the nearest 0.1 mg.

Trace element analyses

Element concentrations in the carcass (including the rinsed gut), tail, and stomach contents were determined separately. Because gecko stomach contents represented little mass, all samples were pooled within each site to form one composite dietary sample for analyses. A total of 21 elements were analyzed (Be, Al, V, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Cd, Sb, Cs, Ba, Pb, U, and Tl) in the carcasses, tails, and stomach contents. Gecko tissues and stomach contents were lyophilized and homogenized before being digested and analyzed for trace element concentrations. Approximately 25 to 250 mg of dry sample was used for digestion; sample masses varied because large differences existed in dry masses among tissue types. Trace metal–grade nitric acid (HNO_3; 2.5–5.0 ml) was added to samples before digestion in a microwave (MDS 2000, CEM Corporation, Matthews, NC, USA) with heating steps at 60, 60, 70, and 80 microwave power for 10, 10, 15, and 20 min, respectively. After digestion with HNO_3, 0.5 to 1.0 ml of trace metal–grade hydrogen peroxide (H_2O_2) was added to the samples and microwaved at the same power and duration as the HNO_3 digestion. After digestion, samples were brought to a final volume of 10.0 to 25.0 ml (depending on tissue mass) with deionized water. Trace element analysis was performed by inductively coupled plasma mass spectroscopy

(ICP-MS; Perkin Elmer, Norwalk, CT, USA) on samples diluted 1:1 with deionized water. External calibration standards covering a range of 1 to 500 μg/L were prepared daily by serial dilution of National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) traceable primary standards. Matrix-matched standard addition curves ranging from 10 to 500 μg/L (from NIST traceable primary standards) were also included in the calibration for each type of sample analyzed. Certified reference material (Tort 2; National Research Council, Ottawa, ON, Canada) and blanks were included in the digestion and analysis procedure for quality control purposes. Mean percent recovery for elements in certified reference materials ranged between 79 and 119%. Data were not corrected for percent recovery. Mean instrument detection limits among the different elements in carcass, tail tissue, and stomach contents varied from 1.0 to 400 ng/g dry mass. Statistical treatment of concentrations registering below detection limits are discussed in the next section. All element concentrations are presented on a dry mass basis.

Statistical analyses

Bodies. Concentrations of Be and U in the carcasses of all individuals registered below the detection limit (BDL) as did Sb and Tl concentrations in 91 and 67% of the geckos examined, respectively. Thus these four elements were excluded from further statistical analyses. Of the 52 gecko carcasses analyzed, concentrations BDL also occurred in As (seven individuals, 13%), Mo (12 individuals, 29%), and Cs (one individual, 2%). For statistical analyses, concentrations of As, Mo, and Cs BDL were replaced with 50% of the mean detection limit (MDL). Distributions of element concentrations were improved by natural log transformation.

To investigate patterns of accumulation among trace elements and to evaluate potentially confounding effects of body size, element concentrations and dry soma mass were used to produce a Pearson correlation coefficient matrix, SETAC Supplemental Data Archive, Item ETC-25-09-003; http://etc.allenpress.com. All statistical comparisons were conducted with SYSTAT® 10 statistical package (SPSS, Chicago, IL, USA). Because of the large number of elements examined and the similarities in accumulation among some elements revealed in the correlation matrix, principal component analysis (PCA) was employed to summarize the element concentration data. The PCA produces orthogonal summary components consisting of linear combinations of the original variables. Condensation of the data into interpretable components simplifies the comparison of element accumulation among sites. In addition to the concentrations of 17 elements (Al, V, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Cd, Cs, Ba, and Pb), dry soma mass was included in the PCA to account for allometric accumulations with body size. Components produced by PCA are by definition orthogonal; thus, the influence of body size can be isolated on components on which body size loads. Although ignoring components on which soma mass loads might discard some spatial variability in the data, the remaining components can be analyzed free of body size influences. The number of interpretable principal components was determined from scree plots and component eigenvalues. A varimax rotation of the axes was employed to aid in interpretation of the components. This rotation tends to distribute the primary loadings of different variables across more components, consequently distributing the amount of variation explained more evenly across the set of components. Factor scores were saved for each component in the PCA.

To compare accumulation patterns among locations, we used an analysis of variance (ANOVA) model that uses the PCA factor scores of each component that was independent of the influences of body size. Each ANOVA model was followed with a Tukey pairwise comparison as appropriate. Least squares (LS) means and associated standard errors from each ANOVA model were used to graphically compare factor scores among sites.

Tails. In an effort to present a realistic assessment of a less intrusive data collection technique, we structured our tail clip element accumulation analysis independent of results of the whole-body analysis. Because all Be and more than 80% of the Mo concentrations were below or near the detection limit, these elements were excluded from further analyses in the tail tissues. Concentrations BDL were also encountered in As (13 individuals, 27%), Sb (24 individuals, 49%), Tl (two individuals, 2%), and U (18 individuals, 37%). For statistical analyses, BDL concentrations in these four elements were replaced with 50% of the MDL. Linear regression compared whole-body and tail concentrations for each element to examine similarity of trace element accumulation in these tissue sources. A Spearman rank correlation coefficient (r_s) compared whole-body and tail concentrations for Mo, Sb, and Tl for which a large number of individuals had BDL concentrations.

Similar to the carcass analyses, PCA was conducted on the natural log–transformed concentrations of 19 elements (Sr, Rb, Cs, Co, U, Ba, Fe, Zn, Tl, As, Se, Sb, Al, Mn, Ni, V, Pb, Cd, and Cu) that remained after those elements with high rates of BDL concentrations were excluded. Dry soma mass was again included to control for allometric accumulations with body size, and a varimax rotation of the axes was employed to aid interpretation. Factor scores of each component were compared with ANOVA. Additionally, we graphically compared the LS means and associated standard errors from these ANOVA models.

Diet. Sample sizes were not sufficient to conduct a thorough diet analysis, but gut contents were sorted and, in most cases, identified taxonomically to the level of order to provide a general indicator of the collected geckos' diet. For each gecko, the relative volumetric rank of each prey taxonomic category was visually estimated and recorded, with the dominant item scored at 15. Also, the proportion of geckos collected from a location that had eaten each prey category was calculated.

RESULTS

Whole-body accumulation

Concentrations of several elements correlated positively, indicating a similarity in accumulation among these elements. In total, 38 pairs of element concentrations were positively correlated at $p < 0.01$, and 17 additional pairs had weaker correlations ($p < 0.05$). Arsenic and cadmium concentrations showed correlations with the most elements: nine and 10, respectively. Along with As and Cd being correlated to each other, both were correlated with Al, Cu, Mn, Pb, Sb, Se, and Tl. Arsenic was also correlated with Cs, whereas Cd correlations included Ni and Mo. Of these 12 elements, As, Cd, Cu, Pb, Sb, Ni, Se, and Tl were of particular concern in the sludge [2,9,12]. The sludge also contained large amounts of Al and Mn, and Mo was present [2]. Negative correlations among elements were comparatively rare but did exist among some element pairs, all of which involved Sr and Ba. Strontium and barium were positively correlated to each other, but both were negatively correlated with As, Cs, and Tl ($p < 0.05$). Additionally, concentrations of several elements (Mn, Cu, Se, and Mo) were negatively correlated with dry soma mass (Supplemental Data 1), indicating lower concentrations in larger geckos. Thus gecko size could be a confounding factor in comparisons involving some elements.

Antimony and Tl were important constituents of the mine slurry and subsequent contamination [2,3,9]. Thallium is of particular environmental concern because of its extreme toxicity. Only carcasses of four individuals contained detectable levels of Sb, but all four were collected on the Agrio River floodplain (site 7), the floodplain site nearest to the tailings dam and mine. Similarly, all individuals from the rural reference site (site 1) and Aznalcázar (site 3) were BDL for Tl, as were 92% of the gecko carcasses from the non–mine-affected town (site 2) and 63% from Aznalcollar (site 4). However, Tl concentrations were only BDL in one of 14 geckos collected from the contaminated floodplain (sites 5–7), with concentrations highest in geckos from the Agrio (site 7) and upper Guadiamar (site 6) Rivers floodplain locations and decreasing by over 50% at the downstream Guadiamar River site (site 5; Table 1).

Principal component analysis condensed the element concentrations into five components (PC-1 to PC-5) that together accounted for 71% of the variation in the data (Table 2). Dry soma mass loaded strongly on a single component, PC-1, revealing a negative relationship between body size and several elements (Mn, Se, Mo, Al, and Cd). Because of this confounding effect of body size on PC-1, it was excluded from further analysis. Lack of a correlation between body size and PC-2, -3, -4, and -5 allows assessment of the relationships among element concentrations that is independent of body size.

Six elements (Co, Fe, Sr, Ba, Zn, and Ni) strongly correlated with PC-2, which explained 22% of the variation in the PCA (Table 2). The ANOVA model comparing scores saved from PC-2 indicated a significant difference among sites, but explained only 27% of the variation (Table 3). Pairwise comparison indicated that only sites with the most extreme low and high scores differed significantly (Fig. 2). Scores for geckos from Aznalcollar (site 4; $p = 0.02$) and possibly the upper Guadiamar floodplain site (site 6; $p = 0.06$) were significantly lower than those from the non–mine-affected town (site 2). No other pairs differed significantly ($p > 0.40$).

Two elements loaded strongly (Mo and V) and two moderately (Pb and Cs) on PC-3, which explained 9% of the variation in the PCA. The associated ANOVA explained only 14% of the variation and indicated no significant difference among locations.

Of particular interest, concentrations of several potentially toxic elements known to be constituents of mining pollution were positively correlated to PC-4. Principal component 4 explained 13% of the variation in the PCA and depicts a positive relationship among Cd, As, Pb, and Se (weakly Cs and Ni). Additionally Ba had a moderate negative correlation. Mean factor scores illustrated an obvious gradient among the seven locations (Fig. 2), with scores increasing along the expected contamination gradient (see *Discussion* section for details of expected gradient). An ANOVA model that explained 71% of the variation, confirmed a significant difference among sites (Table 3). Principal component 4 scores did not significantly differ between pairs of locations with similar disturbance histories (e.g., the two non–mine-affected sites [sites 1 and 2], two mine-affected towns [sites 3 and 4], and two Guadiamar floodplain locations [sites 5 and 6] did not differ at $p > 0.90$). However, pairwise comparison confirmed a general gradient, with PC-4 scores for geckos from the non–mine-affected locations (sites 1 and 2) lowest, the mine-affected towns (sites 3 and 4) intermediate, and heavily contaminated floodplain (sites 5–7) highest (Fig. 2). The comparison of the Agrio River floodplain (site 7) scores to those from the lower Guadiamar floodplain site (site 5), as well as the comparisons of Aznalcollar (site 4) to the rural reference site (site 1) and the lower floodplain location (site 5) were not well resolved ($p = 0.08$). As expected, concentrations of elements that loaded on PC-4 (As, Cd, Se, and Pb) were elevated in geckos from more contaminated locations corresponding to the pattern revealed by the PCA scores (Table 1). Highly elevated Pb concentrations in the non–mine-affected town (site 2) were the notable exception.

Principal component 5 illustrated a positive relationship among Rb, Cs, Se, and V (weakly As) and explained 10% of the variation in the PCA (Table 2). An ANOVA model explaining 41% of the variation indicated a significant difference in PC-5 scores among sites. Mean scores from the upper two floodplain locations (sites 6 and 7) and the three towns (sites 2, 3, and 4) appeared very similar ($p > 0.98$; Fig. 2), as did the rural reference location (site 1) and the downstream Guadiamar floodplain (site 5; $p = 0.90$). The rural reference site (site 1) scores were significantly lower than four of the five former locations (sites 2, 3, 4, and 6; $p < 0.02$) and possibly the fifth (site 7; $p = 0.06$). Although scores from the lower Guadiamar floodplain location (site 5) graphically also appeared lower in Figure 2, they did not significantly differ from the towns and upper floodplain locations ($p > 0.13$; Fig. 2).

The apparent lower scores in the rural reference site (site 1) and the least affected floodplain site (site 5) might indicate an interaction of mining and urban pollution. Figure 3 plots the factor scores from PC-4 (mining contaminants) with those from PC-5. The separation among polygons encircling points from each location illustrates spatial variation in accumulation among these elements. Principal component 4 causes horizontal separation of the polygons, with mining contamination increasing to the right. The two non–mine-affected sites (sites 1 and 2) are located in a similar position in relation to this axis. In contrast, PC-5 produces vertical separation among the non–mine-affected polygons, as well as an elevation of both urban habitat types (site 2 vs sites 3 and 4), with the mine-affected urban polygon (sites 3 and 4) the highest.

Nondestructive element analyses

As described separately for the tails and bodies, concentrations of 16 of the 21 examined elements (Co, Fe, Sr, Ba, Zn, Ni, Cu, Mn, V, Al, Cs, Rb, Pb, As, Cd, and Se) had sufficient levels of accumulation in both the tails and bodies to warrant direct comparison of the two tissue sources by linear regression. Concentrations in these two tissue sources were linearly related at $p < 0.01$ (Table 4) in nine of these 16 elements (Co, Sr, Ba, Al, Cs, Rb, Pb, As, and Se). More than 50% of the variation was explained by the regression of six of these elements, indicating that the tail clips most effectively indicated whole-body concentrations in Ba, Cs, Rb, Pb, As, and Se. Lead was most notable, with nearly 90% of the variance explained. Regressions of three elements (Fe, Mn, and V) were significant at $p < 0.05$, but less than 20% of the variation was explained. Tail and whole-body concentrations

Table 1. Trace metal concentrations found in the whole bodies of individuals collected from each of the seven locations in southern Spain[a]

	Concentration (mean ± SD and range) (ppm)						
Metal	Site 1 (n = 9)	Site 2 (n = 13)	Site 3 (n = 8)	Site 4 (n = 8)	Site 5 (n = 5)	Site 6 (n = 5)	Site 7 (n = 4)
Al	23.020 ± 11.623 (10.857–46.876)	23.012 ± 14.235 (11.100–62.992)	21.238 ± 11.045 (14.037–45.734)	26.659 ± 13.715 (11.250–46.182)	20.865 ± 8.347 (14.369–35.299)	23.553 ± 18.075 (10.349–54.887)	37.997 ± 6.723 (30.302–46.474)
V	0.360 ± 0.047 (0.283–0.439)	0.514 ± 0.196 (0.277–0.959)	0.532 ± 0.169 (0.268–0.796)	0.586 ± 0.261 (0.353–1.068)	0.415 ± 0.129 (0.316–0.588)	0.574 ± 0.439 (0.331–1.355)	0.530 ± 0.160 (0.417–0.763)
Mn	1.299 ± 0.333 (0.872–2.000)	1.770 ± 0.865 (0.840–3.561)	1.057 ± 0.385 (0.750–1.676)	1.679 ± 0.751 (0.672–2.761)	1.549 ± 0.837 (0.761–2.567)	1.423 ± 0.520 (0.946–2.206)	2.466 ± 0.512 (1.837–2.889)
Fe	442.934 ± 71.347 (336.941–588.338)	550.867 ± 29.722 (360.589–233.659)	562.816 ± 171.820 (378.012–808.278)	418.296 ± 80.565 (283.870–527.399)	563.918 ± 151.553 (365.317–747.608)	437.151 ± 86.568 (353.842–553.346)	474.287 ± 63.556 (390.687–540.161)
Co	0.284 ± 0.081 (0.183–0.401)	0.271 ± 0.084 (0.179–0.446)	0.238 ± 0.045 (0.170–0.286)	0.190 ± 0.050 (0.119–0.290)	0.298 ± 0.106 (0.158–0.418)	0.197 ± 0.044 (0.151–0.254)	0.234 ± 0.074 (0.160–0.336)
Ni	0.370 ± 0.126 (0.236–0.630)	0.371 ± 0.124 (0.214–0.555)	0.367 ± 0.074 (0.261–0.465)	0.353 ± 0.138 (0.226–0.663)	0.512 ± 0.201 (0.265–0.685)	0.317 ± 0.065 (0.228–0.394)	0.330 ± 0.047 (0.288–0.397)
Cu	2.757 ± 0.781 (1.943–4.300)	3.731 ± 1.551 (2.016–7.226)	2.732 ± 0.491 (2.106–3.304)	4.072 ± 1.789 (1.948–6.599)	4.522 ± 1.296 (3.443–6.682)	4.372 ± 1.928 (2.417–6.873)	3.879 ± 1.207 (2.510–5.321)
Zn	114.408 ± 19.626 (91.161–158.649)	127.192 ± 31.195 (90.991–191.892)	127.552 ± 18.502 (106.272–161.173)	108.479 ± 15.118 (83.605–126.117)	109.689 ± 31.178 (71.364–150.149)	106.352 ± 12.671 (91.681–126.341)	153.008 ± 13.050 (141.309–166.442)
As	0.066 ± 0.031 (0.046–0.120)	0.186 ± 0.123 (0.046–0.507)	0.284 ± 0.237 (0.112–0.854)	0.542 ± 0.303 (0.283–1.161)	0.493 ± 0.122 (0.358–0.691)	0.928 ± 0.664 (0.253–1.999)	3.436 ± 1.104 (2.520–4.814)
Se	0.583 ± 0.119 (0.419–0.791)	0.820 ± 0.148 (0.573–1.010)	0.789 ± 0.120 (0.656–0.958)	0.912 ± 0.250 (0.525–1.242)	0.919 ± 0.367 (0.504–1.474)	1.465 ± 0.309 (0.940–1.761)	1.003 ± 0.181 (0.830–1.252)
Rb	5.104 ± 1.246 (3.794–7.963)	8.645 ± 5.113 (5.000–24.053)	7.853 ± 1.473 (5.131–9.461)	8.899 ± 3.979 (5.186–16.034)	7.395 ± 1.207 (6.078–9.119)	7.199 ± 2.209 (4.661–9.680)	6.299 ± 2.067 (4.121–8.927)
Sr	97.764 ± 30.799 (52.363–145.582)	103.923 ± 34.532 (58.008–174.609)	77.397 ± 21.835 (45.724–120.103)	59.640 ± 19.478 (39.080–93.112)	94.039 ± 31.439 (56.376–141.967)	66.995 ± 31.007 (26.554–99.786)	71.092 ± 37.436 (31.063–113.816)
Mo	0.132 ± 0.051 (0.071–0.217)	0.142 ± 0.063 (0.071–0.258)	0.152 ± 0.081 (0.071–0.288)	0.184 ± 0.056 (0.071–0.229)	0.161 ± 0.046 (0.101–0.208)	0.137 ± 0.064 (0.071–0.205)	0.114 ± 0.051 (0.071–0.171)
Cd	0.062 ± 0.028 (0.038–0.133)	0.081 ± 0.058 (0.023–0.186)	0.069 ± 0.039 (0.035–0.145)	0.114 ± 0.071 (0.032–0.239)	0.313 ± 0.156 (0.106–0.488)	0.179 ± 0.087 (0.079–0.262)	0.311 ± 0.162 (0.074–0.435)
Sb	0.044 ± 0.000 (0.044–0.044)	0.044 ± 0.000 (0.044–0.044)	0.044 ± 0.000 (0.044–0.044)	0.044 ± 0.000 (0.044–0.044)	0.044 ± 0.000 (0.044–0.044)	0.044 ± 0.000 (0.044–0.044)	0.258 ± 0.058 (0.209–0.336)
Cs	0.017 ± 0.006 (0.005–0.025)	0.022 ± 0.007 (0.012–0.040)	0.029 ± 0.010 (0.016–0.042)	0.054 ± 0.025 (0.026–0.098)	0.023 ± 0.002 (0.021–0.026)	0.032 ± 0.017 (0.014–0.057)	0.035 ± 0.004 (0.029–0.038)
Ba	32.934 ± 15.158 (14.005–58.444)	88.120 ± 57.835 (37.152–197.913)	20.712 ± 9.735 (9.819–39.089)	15.281 ± 5.816 (8.878–26.762)	23.333 ± 11.733 (10.669–39.102)	10.902 ± 6.266 (4.120–19.104)	18.223 ± 12.569 (5.637–30.501)
Pb	2.036 ± 0.883 (0.782–3.145)	17.316 ± 40.502 (1.489–150.521)	9.712 ± 6.882 (4.474–23.378)	8.376 ± 5.179 (4.852–19.829)	7.936 ± 8.172 (1.866–20.217)	3.840 ± 1.839 (1.804–5.666)	19.199 ± 7.733 (8.863–27.431)
Tl	0.004 ± 0.000 (0.004–0.004)	0.004 ± 0.002 (0.004–0.010)	0.009 ± 0.014 (0.004–0.043)	0.006 ± 0.003 (0.004–0.012)	0.017 ± 0.005 (0.011–0.021)	0.041 ± 0.028 (0.004–0.076)	0.048 ± 0.020 (0.018–0.060)

[a] Site 1 = Guadalmellato Reservoir; site 2 = Villaviciosa de Córdoba; site 3 = Aznalcázar; site 4 = Aznalcollar; site 5 = Guadiamar River floodplain near the Aznalcázar gauge station; site 6 = Guadiamar River floodplain near the Guijo gauge station; site 7 = Agrio River floodplain.

Table 2. Loadings of dry soma mass and whole-body concentrations of trace elements on the first five principal components (PC). For clarity, we include only loadings >0.3 or <−0.3

Element	PC-1	PC-2	PC-3	PC-4	PC-5
Cu	0.844				
Soma	−0.833				
Mn	0.832				
Se	0.511			0.415	0.533
Al	0.490				
Co		0.886			
Fe		0.882			
Sr		0.864			
Ba		0.803		−0.410	
Zn		0.695			
Ni		0.505		0.369	
Mo	0.448		0.759		
V			0.748		0.408
Cd	0.392			0.790	
As				0.734	0.395
Pb		0.337	0.412	0.478	
Rb					0.827
Cs			0.397	0.399	0.568
Eigenvalues	3.121	3.929	1.710	2.418	1.856
Total variance explained (%)	17	22	9	13	10

appeared to be correlated in Sb and Tl ($r_s = 0.44$ and 0.76, respectively; $p < 0.01$), whereas Mo concentrations in these tissues were not correlated ($r_s = 0.21$; $p > 0.10$).

Principal component analyses summarizing the tail element concentrations and dry soma mass produced five components (TPC-1 to TPC-5) that explained a total of 72% of the variation (Table 5). Dry soma mass loaded relatively strongly on PC-3, which revealed a negative relationship between body size and several elements (Sb, Al, Mn, Ni, and Cu). In contrast to the analyses of the body element accumulation, dry soma mass loaded lightly on a second component. In addition to a positive loading of Fe, Zn, Ni, and Cu, and a negative loading of V, dry soma mass loaded at −0.378 on TPC-4. Although not a particularly strong correlation, it could be strong enough to bias comparisons with the use of scores from this component. Thus, to avoid this potential confounding effect caused by the correlation of body size, TPC-3 and TPC-4 were excluded from further analysis.

Table 3. Analysis of variance results comparing principal component (PC) scores among sites for each of the four PCs that were independent of body size. Elements with loadings with an absolute value greater than 0.4 are listed beside the component heading[a]

Source	*df*	MS	*F*	*p*
PC-2 (Co, Fe, Sr, Ba, Zn, Ni)				$r^2 = 0.27$
Site	6	2.258	2.714	0.03
Error	45	0.832		
PC-3 (Mo, V, Pb Cs)				$r^2 = 0.14$
Site	6	1.196	1.228	0.31
Error	45	0.974		
PC-4 (Cd, As, Pb, Se)				$r^2 = 0.71$
Site	6	6.042	18.439	<0.01
Error	45	0.328		
PC-5 (Rb, Cs, Se, V)				$r^2 = 0.41$
Site	6	3.460	5.149	<0.01
Error	45	0.672		

[a] *df* = degrees of freedom; MS = mean square; *F* = *F* ratio; *p* = probability.

Concentrations of 10 elements were correlated with TPC-1 (Sr, Rb, Cs, Co, U, Ba, Fe, Zn, Ni, and Se), which consequently explained 21% of the variation in the PCA (Table 5). Although the ANOVA comparing TPC-1 scores accounted for a relatively small amount of the variation (30%), a significant difference among locations was indicated (Table 6). Examination of LS means showed the highest and quite similar ($p > 0.95$) scores occurred at the three towns (sites 2–4) and the floodplain location near Aznalcázar (site 5; Fig. 4) gauge station. Only scores from the extreme locations differed significantly (i.e., site 7 vs the two towns with highest scores, sites 2 and 3).

Tail principal component 2 accounted for 13% of the variation and appeared analogous to PC-4 from the whole-body analyses. Concentrations of elements known to be constituents of mine pollution (As, Se, Pb) loaded on both of these components derived from different tissue sources. Additionally, Tl and Sb loaded on TPC-2, whereas Cd loaded on PC-4. The ANOVA comparing TPC-2 scores among sites explained 76% of the variation and indicated a significant difference among sites (Table 6). Mean TPC-2 scores illustrated a remarkable gradient across sites that corresponded to the expected contamination gradient (Fig. 4). Pairwise comparison, as noted in Figure 4, confirmed this gradient.

Elements loading positively on TPC-5 that explained 11% of the variation included Pb, Cd, Ba, Cu, Sr, and U (Table 5). The ANOVA model comparing scores of TPC-5 among sites explained only 16% of the variation and did not detect spatial variation (Table 6).

Diet

The location mean of the volumetric ranks for each taxon indicates how prevalent the prey item was in the gut contents of geckos from each location. Importance of a prey taxon is also indicated by the proportion of individuals from a site that had eaten the prey item (Supplemental Data 2, SETAC Supplemental Data Archive, Item ETC-25-09-003; http://etc.allenpress.com). Four prey taxa, including hemipterans, homopterans, adult coleopterans, and spiders were common prey items and were eaten at all seven locations. Additionally, lepidopteran adults and larvae, dipterans, hymenopterans (nonformicids), and formicids had been eaten at six of the seven sites. Spatial variation in the gecko diets was apparent, but samples sizes precluded definitive comparisons among sites. Dipterans were dominant items at the three towns (sites 2–4) and the lower Guadiamar floodplain site (site 5); dipterans were absent from the rural reference site (site 1) and less prevalent in the upper two floodplain sites (sites 6 and 7; Supplemental Data 2). Ants were found in the diet of geckos from all sites except the Agrio River floodplain (site 7). Adult beetles were eaten by more than 50% of the individuals at all sites except the rural reference site (site 1) and the Agrio River floodplain (site 7). Hymenoptera were dominant prey items at the mine-affected locations (sites 3–7), being found in at least 60% of the geckos in these habitats. Geckos from the rural reference site (site 1) and the Agrio River floodplain had eaten the fewest numbers of prey taxa (mean number of prey taxa eaten by individuals followed by the standard deviation and range: site 1, 2.9 ± 1.76, 1–7; site 2, 5.0 ± 1.68, 2–8; site 3, 5.4 ± 2.45, 3–10; site 4, 6.8 ± 1.67, 5–10; site 5, 6.0 ± 2.45, 3–9; site 6, 5.0 ± 1.41, 3–6; site 7, 3.0 ± 1.41, 1–4). Because

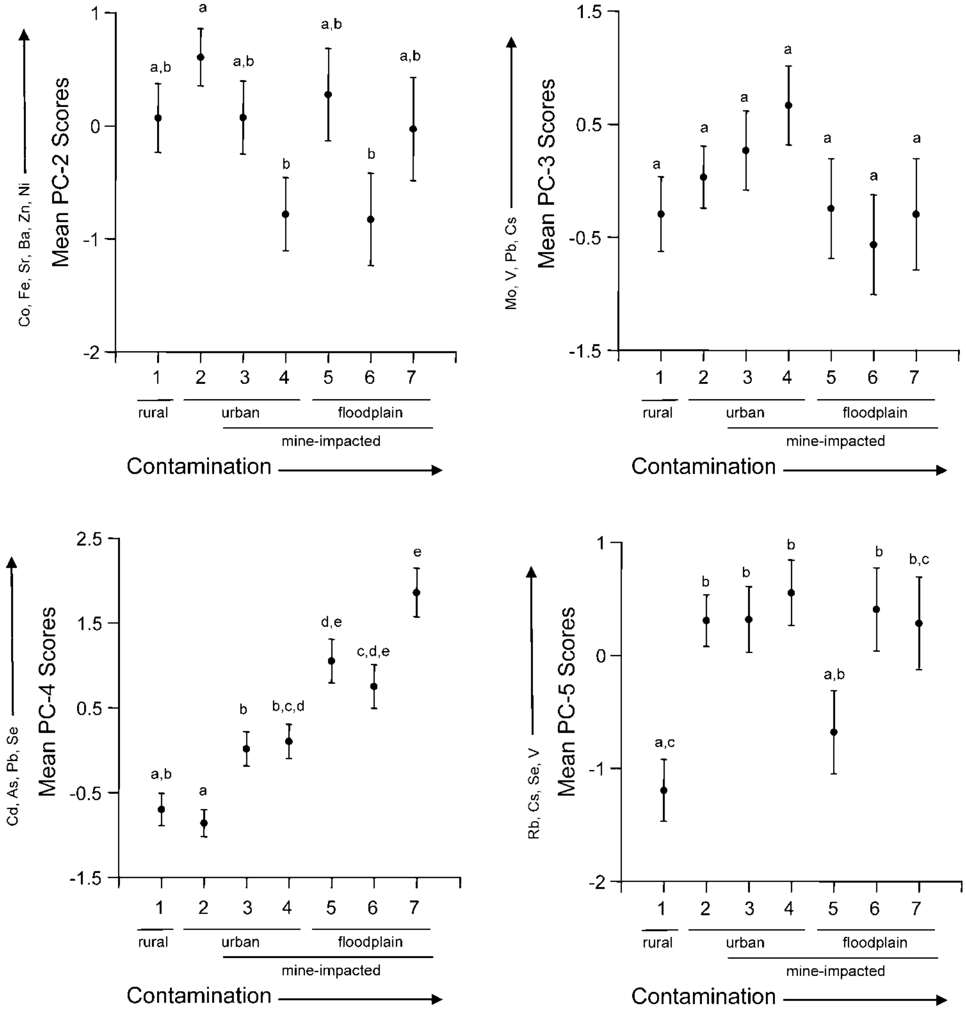

Fig. 2. Mean principal component (PC) analysis scores from the whole-body trace element concentration analysis compared among the seven collection locations. Error bars represent ±1 standard error. Means with different letters above them are significantly different (Tukey pairwise comparison, $p < 0.05$). Locations in southern Spain: Guadalmellato Reservoir (site 1), Villaviciosa de Córdoba (site 2), Aznalcázar (site 3), Aznalcollar (site 4), Guadiamar River floodplain near the Aznalcázar (site 5) and Guijo (site 6) gauge stations, and the Agrio River floodplain (site 7).

sediment ingestion could represent a vector of contaminant ingestion, it is also important to note that 17 of 52 geckos had visible amounts of sediment in their guts.

Concentrations of 12 elements (As, Zn, Mn, Cd, Co, Cu, Cs, Al, Fe, V, Ni, and Mo) were consistently higher in the gecko's prey items than in their bodies at all sites (Fig. 5). Only Rb and Sr concentrations were consistently higher in their bodies than diets. As evidenced by the crossing lines in Figure 5, the relationship between concentrations of Pb, Ba, and Se in their body and diet varied among sites; of these three elements, Pb is notable in being elevated in the diet of floodplain (sites 5–7) geckos.

Several groups of elements showed similar spatial patterns of element concentrations in prey items. For example, six elements (As, Zn, Mn, Cd, Pb, and Co) were not only elevated in the floodplain but increased up the mine contamination gradient (Fig. 5). Concentrations of Zn, Mn, and Cd were more than five times higher in prey items from geckos collected from the Agrio River floodplain (site 7) than those from the rural reference site (site 1). Similarly, Pb concentrations were 11 times higher, and As was the most extreme at more than 68 times higher in prey items of geckos from the Agrio River floodplain than in those from site 1. Most of these six elements also showed a minor peak at the non–mine-affected town (site 2). More pronounced elevations in accumulation at site 2 are illustrated for Al, Fe, V, and Ni by the U-shaped curves in Figure 5 formed by concentrations being highest in the diet items of geckos from site 2 and the two upstream floodplain locations (sites 6 and 7).

Similar to the concentrations in their bodies, Se dietary concentrations were elevated in the geckos from the floodplain, but concentrations peaked at the upper Guadiamar River (site 6) rather than along the Agrio River (site 7). Rubidium dietary concentrations almost mirrored their body concentrations. It is worthy to note that Sb concentration in prey items were BDL at all sites except the upper two floodplain sites (site 7, 1.417 ppm; site 6, 0.352 ppm) and Aznalcollar (site 4, 0.382 ppm), whereas body concentrations were BDL at all sites except site 7 where body concentrations averaged only 0.258 ppm. Similarly Tl concentrations were BDL in the prey items of geckos from all locations except the Agrio River floodplain, where prey items contained 0.208 ppm Tl, four times their whole-body concentrations.

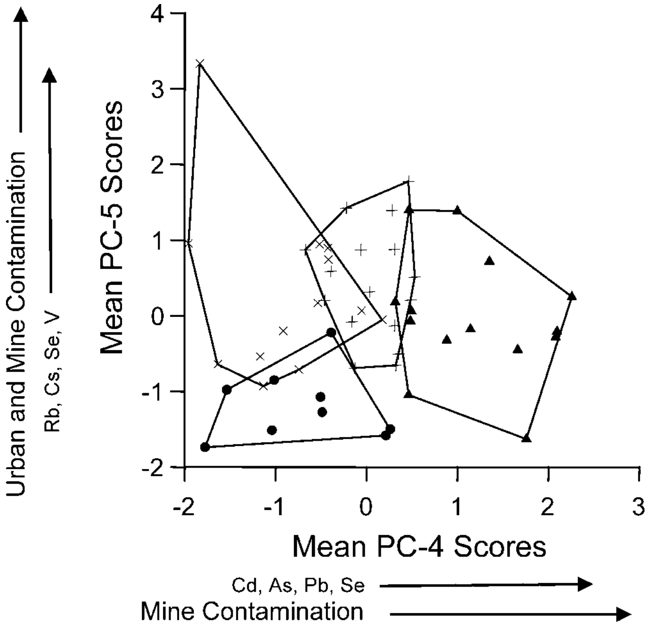

Fig. 3. Plot of principal component 4 and principal component 5 (PC-4 and PC-5) scores from the whole-body trace element concentration analysis illustrating spatial variation in mining and urban contaminants. Locations in southern Spain: Guadalmellato Reservoir (site 1), Villaviciosa de Córdoba (site 2), Aznalcázar (site 3), Aznalcollar (site 4), Guadiamar River floodplain near the Aznalcázar (site 5) and Guijo (site 6) gauge stations, and Agrio River floodplain (site 7). (●) Rural reference (site 1), (×) non–mine-affected urban (site 2), (+) mine-affected urban (sites 3 and 4), (▲) mine-affected floodplain (sites 5–7).

DISCUSSION

Contaminant accumulation in geckos

Derivation of ores from the polymetallic deposits from the Aznalcollar and Los Frailes area has produced pollution containing a broad array of metals and metalloids. This complex assortment of contaminants likely explains the large number of elements in our study in which concentrations in gecko bodies were correlated—55 pairs of elements in total were correlated (Supplemental Data 1). Indeed, the mine is known to have contributed several potentially toxic materials to the Guadiamar Valley: Fe, Zn, Pb, Cu, As, Sb, Bi, Cd, and Tl among other elements [7–9,12,13]. In our data, As and Cd, two important components of the mining contaminants, were correlated with the most elements.

Although not all elements occurring in the mine tailings were elevated in the current analyses, our study clearly demonstrated the accumulation of some environmentally hazardous mine-related contaminants in geckos. Principal component analyses (PC-4 of whole-body concentrations) identified several elements (Cd, As, Pb, and Se) elevated in the bodies of geckos from mine-affected habitats. More convincing, patterns in the PCA scores from this component indicated increased contaminant bioaccumulation across the expected contamination gradient, being highest on the contaminated floodplain (sites 5–7), particularly areas closest to the ruptured tailings dam and the historical source of contamination. Scores were intermediate in mine-affected towns (sites 3 and 4), and lowest in the urban (site 2) and rural (site 1) non–mine-affected habitats.

Table 4. Regression statistics summarizing linear relationship between natural log–transformed whole-body concentrations with tail concentrations for 16 elements

Element	Regression coefficient	r^2	p
Co	0.30	0.19	<0.01
Fe	0.09	0.08	0.05
Sr	0.32	0.31	<0.01
Ba	0.76	0.78	<0.01
Zn	0.09	0.06	0.10
Ni	0.09	0.03	0.26
Cu	0.06	0.01	0.43
Mn	0.28	0.12	0.02
V	0.29	0.12	0.02
Al	0.34	0.33	<0.01
Cs	0.62	0.52	<0.01
Rb	0.61	0.62	<0.01
Pb	0.84	0.87	<0.01
As	0.48	0.51	<0.01
Cd	0.05	0.01	0.58
Se	0.70	0.50	<0.01

Table 5. Loadings of dry soma mass and tail concentrations of trace elements on the first five principal components from tail analyses (TPC). For clarity, we include only loadings >0.3 or <−0.3

Element	TPC-1	TPC-2	TPC-3	TPC-4	TPC-5
Sr	0.739				0.470
Rb	0.731				
Cs	0.674	0.364			
Co	0.665				
U	0.637		0.301		0.422
Ba	0.609	−0.310			0.524
Fe	0.599			0.660	
Zn	0.575			0.708	
Tl		0.907			
As		0.793		−0.371	
Se	0.404	0.678		0.365	
Sb		0.517	0.628		
Al	0.373		0.829		
Mn	0.398		0.790		
Ni	0.411		0.607	0.462	
Soma			−0.591	−0.378	
V				−0.765	
Pb		0.422			0.723
Cd					0.707
Cu			0.489	0.434	0.519
Eigenvalues	4.163	2.672	3.043	2.490	2.197
Total variance explained (%)	21	13	15	12	11

Table 6. Analysis of variance results examining the effects of site on each of the four principal components that were independent of body size. Elements with loadings of absolute value greater than 0.4 are listed with the component heading[a]

Source	df	MS	F	p
TPC-1 (Sr, Rb, Cs, Co, U, Ba, Fe, Zn, Ni, Se)				$r^2 = 0.30$
Site	6	2.396	2.993	0.02
Error	42	0.801		
TPC-2 (Tl, As, Se, Sb, Pb)				$r^2 = 0.76$
Site	6	6.074	22.077	<0.01
Error	42	0.275		
TPC-5 (Pb, Cd, Ba, Cu, Sr, U)				$r^2 = 0.16$
Site	6	1.289	1.344	0.26
Error	42	0.959		

[a] df = degrees of freedom; MS = mean square; F = F ratio; p = probability; TPC = principal component from tail analyses.

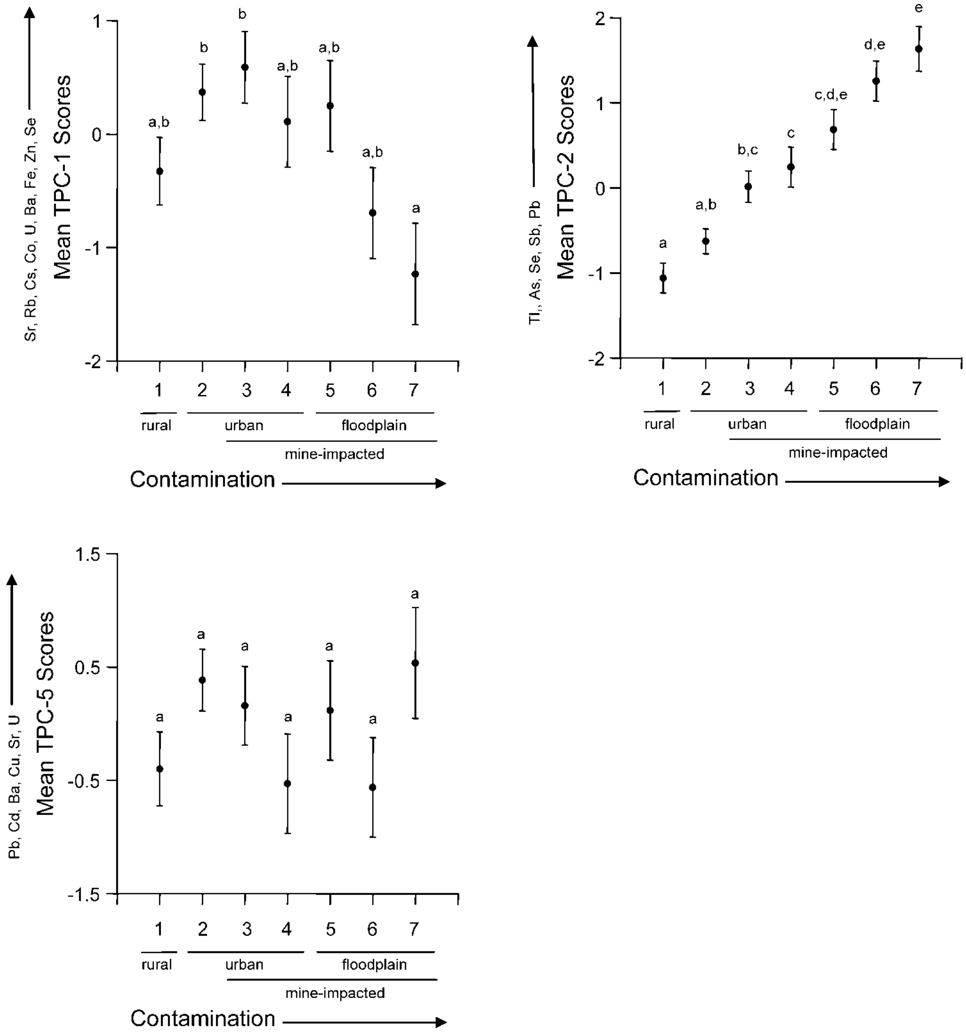

Fig. 4. Mean principal component (PC) analysis scores from the nondestructive trace element analysis (tail clip) compared among the seven collection locations. Error bars represent ±1 standard error. Means with different letters above them are significantly different (Tukey pairwise comparison, $p < 0.05$). Locations in southern Spain: Guadalmellato Reservoir (site 1), Villaviciosa de Córdoba (site 2), Aznalcázar (site 3), Aznalcollar (site 4), Guadiamar River floodplain near the Aznalcázar (site 5) and Guijo (site 6) gauge stations, and the Agrio River floodplain (site 7).

The elevation of element concentrations that loaded on PC-4 in mine-affected areas is most evident by comparing extremes of the gradient, the Agrio River floodplain (site 7), and non–mine-affected locations (sites 1 and 2). Concentrations of As, Cd, and Se on average were 52, 5, and 2.5 times higher, respectively, in geckos from the Agrio River floodplain (site 7) than in those from the rural reference location (site 1). Moderate contamination of the urban habitat reduced the difference in As, Cd, and Se concentrations between the Agrio River floodplain and the urban non–mine-affected town (site 2) to 18, 3.8, and 1.8 times higher, respectively. Notably, average Pb concentrations were similar in geckos from the Agrio River floodplain and the non–mine-affected town (site 2), both about nine times higher than the rural reference location (site 1). However, geckos from the urban habitat had more variable Pb concentrations, with some individuals possessing extremely high body burdens (site 2, 1.489–150.521 ± 10.502; site 7, 8.863–27.431 ± 7.733). A previous analysis that included a larger city indicated both greater contaminant accumulation and higher variability along an urbanization gradient [20].

Comparison to other biota

Consequent to the historical contamination by mining activities and the accident, and despite remediation efforts, organisms in the Guadiamar Valley continue to be exposed to mine-derived contaminants. Waters of the Agrio and Guadiamar Rivers remained polluted in 2000 to 2001, with contaminant concentrations decreasing downstream away from the mine in the upper reaches of the Guadiamar River [14]. Contaminant distributions became more complicated further downstream as the river received additional pollutants from urban and industrial wastes. The contamination of the river water and sediments degraded algae and macroinvertebrate communities while elevating contaminant accumulation in biofilm, suspended particles, and macroinvertebrates; in July 2000, some samples of caddisfly nymphs exceeded concentrations of greater than 50 ppm Pb, 10 ppm As, 6 ppm Cd, 0.5 ppm Tl, and 0.61 ppm Sb [23–25]. Red crayfish (*Procambarus clarkii*) also displayed elevated metal concentration in the affected zones within our study area: in 2000, mean concentra-

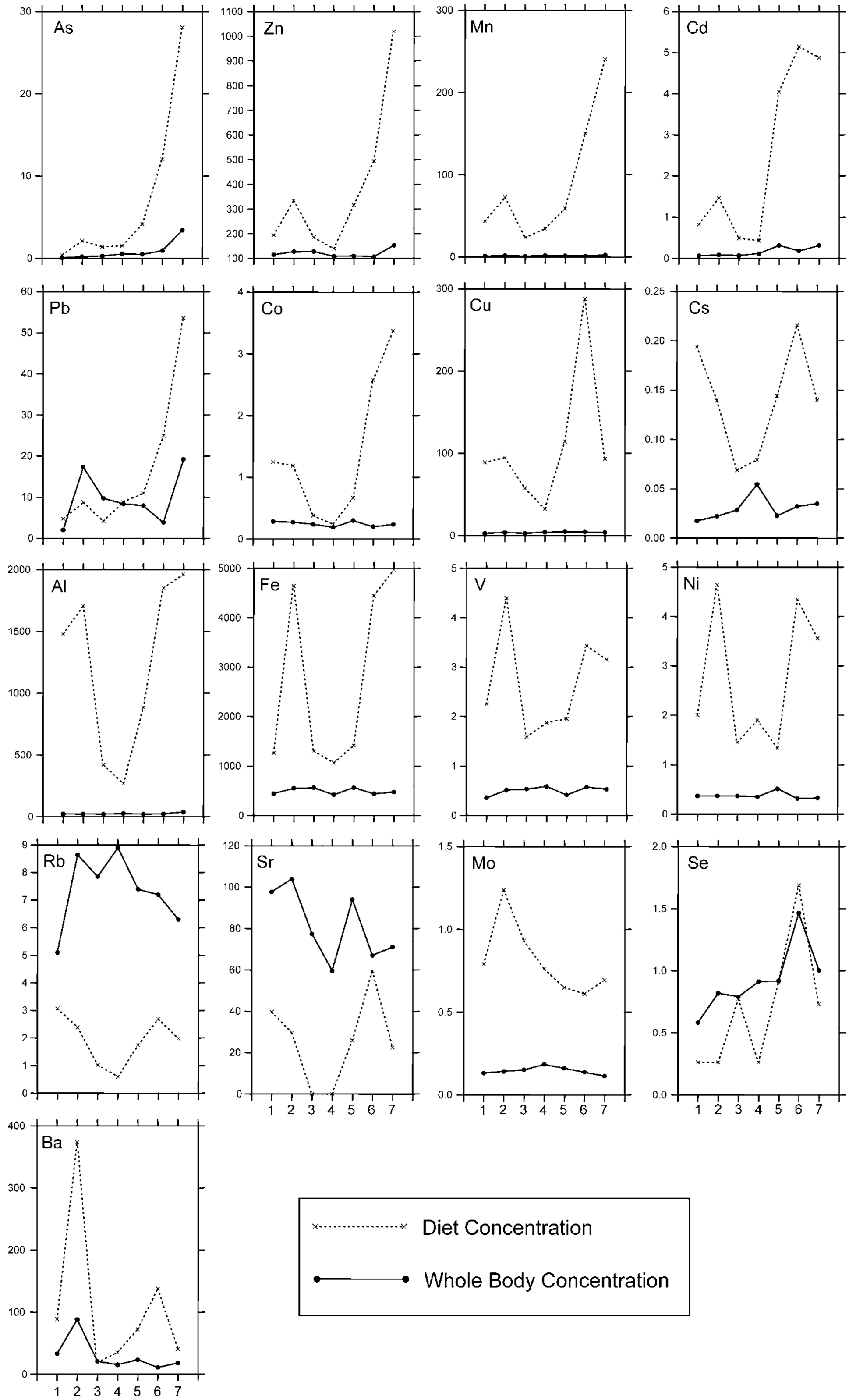

Fig. 5. Comparison of gecko whole-body and dietary trace element concentrations (ppm). Locations in southern Spain: Guadalmellato Reservoir (site 1), Villaviciosa de Córdoba (site 2), Aznalcázar (site 3), Aznalcollar (site 4), Guadiamar River floodplain near the Aznalcázar (site 5) and Guijo (site 6) gauge stations, and the Agrio River floodplain (site 7).

tions for some samples exceeded 50 ppm Zn and Cu, 12 ppm Pb, and 0.14 ppm Cd [26]. Concentrations of contaminants in these aquatic organisms generally exceeded those found in terrestrial geckos on the Guadiamar River floodplain. As a result of disturbance, few aquatic animals live in the Agrio River downstream of the mine.

The apparent accumulation of mine-related contaminants in various species of aquatic birds after the spill suggested the movement of mine-related contaminants up the food chain [27] (e.g., gadwall [*Anas strepera*] liver from the Doñana area contained means of 3.8 ppm Pb, 0.17 ppm As, and 1.27 ppm Cd; pochard [*Aythya ferina*] liver tissue contained 6.5 ppm Pb, 0.31 ppm As, and 0.54 ppm Cd). Collection of these bird samples much further downstream than our geckos makes direct comparison difficult. One year after the accident, comparison of lead isotope ratios in white stork chicks (*Ciconia ciconia*) confirmed accumulation of Pb derived from the mine sludge [28]. Accordingly, the Comet assay identified an increase in genetic damage in white storks born in 1999 from a population near the lower Guadiamar River [29].

Remediation efforts reduced contaminants in soil of the terrestrial habitats along the river, but two years after the accident, elevated levels of contaminants persisted [16,30]. Three years after the accident (2001), soils remained highly contaminated with As in the upper corridor and moderately contaminated in the lower portions; levels of Cd, Zn, and Cu were also higher in the upper corridor nearer to the mine [13]. Pollution remaining in the soils after remediation continued to elevate contaminant concentrations in trees [31], crops [16,32], and grass [30]. Phytoavailability of elements in the sludge varied greatly [33], as did the accumulation rates among 99 naturally colonizing plant species [15]. This variability among primary producers complicates assessment of contaminant trophic transfer throughout the terrestrial food web and illustrates the need of a bioindicator species that integrates food resources in a local area.

Dietary uptake

Dietary concentrations for several elements associated with mine contamination were highly elevated in geckos collected from the mine-affected floodplain. Concentrations of five elements (As, Zn, Mn, Pb, Co) followed the longitudinal contamination gradient downstream of the tailings dam and mine. It should be noted that three of these, Zn, Mn, and Co, only appeared elevated in tissue of geckos from the uppermost floodplain locations (site 7). Cadmium dietary concentrations were exclusively elevated in the floodplain (sites 5–7), but not in a distinct gradient. Selenium was elevated on the floodplain and Aznalcázar (site 3).

Dietary Pb concentrations exceeded gecko body concentrations at all three floodplain locations (sites 5–7). Moreover, on the floodplain, dietary Pb concentrations followed the mining contaminant gradient more clearly than did body concentrations. Diet concentrations also exceeded body concentrations at the rural reference site (site 1). However, a different pattern emerged in urban habitats. Intriguingly, dietary Pb concentrations were lower than gecko body concentrations at the urban non–mine-affected site (site 2) and Aznalcázar (site 3). This pattern was also observed in a large city, Cordoba [20]. Diet and body concentrations were similar at Aznalcollar (site 4) near the mine. These contrasting patterns could be indicative of differences in Pb bioavailability from urban versus mining sources.

The diet of *T. mauritanica* varies seasonally [17,19], so caution should be exercised when interpreting the relationships between element concentrations in stomach contents (a snapshot in time) and gecko tissues (an integrated measure across time) because dietary concentrations can vary seasonally and among prey types. However, our data still show an obvious elevation of contaminants in the diet of *T. mauritanica* on the mine-contaminated floodplain. It is also important to note, that on the basis of this dietary data, terrestrial invertebrates might be useful indicators of mining pollution. Although logistic challenges and home range sizes must be addressed, the higher element concentrations found in the diet items indicates that, in some instances, terrestrial insects might be more sensitive indicators of some mine pollutants. However, individual invertebrate species, which often have narrower diets, might not integrate contaminant sources as well as predatory geckos.

Ingestion of contaminated prey is likely the primary route of contaminant exposure for geckos, as has been shown for other reptiles [34,35], but ingestion of other materials could also contribute. For example, ingestion of contaminated soil is an important route of exposure in some wildlife [see citations in 20,34]. Visible sediments were found in the guts from 17 of our 52 geckos. Additional fine particulates probably existed but would not have been identifiable in our dietary analysis. Mechanisms of sediment ingestion for gekkonids include the cleaning of dust from their eyes, which have immobile eyelids [36], or the frequent licking of their faces before or after feeding [37]. Inhabiting dry dusty habitats with contaminated soils might enhance contaminant ingestion via sediments. This could be applicable to the seasonally arid climate of the Guadiamar Valley, in which soils are contaminated [9], particularly the upper layer that is exposed to surface-dwelling animals. Another likely mechanism of sediment ingestion in these habitats arises via sediments occurring on prey items. This risk was apparent in the comparison of contaminants on washed and unwashed grasses grown on the Guadiamar floodplain [30]. Although trophic exposure is likely the most important mechanism of uptake, the additional contribution of dermal exposure cannot be dismissed given that some geckos have relatively soft skin and large eyes compared with many other lizards [37].

Nondestructive sampling

Subsamples of tail tissue retrieved from geckos showed promise as a nondestructive measure of contaminant exposure and accumulation. Because tail tissue represents a composite of muscle, blood, bone, and skin, it has a higher probability of including a variety of elements than single tissue types [38–40]. Indeed, of the 16 trace elements compared, concentrations of 12 elements showed a significant linear relationship between concentrations in the tail tissue and those in the remaining carcass. However, because several of the regressions explained a relatively small proportion of the variation, tail tissue appeared to be a particularly good indicator for six elements (Ba, Cs, Rb, Pb, As, and Se). This includes three of the four elements (As, Pb, and Se, but not Cd) that showed a strong accumulation gradient among sites in the whole-body analysis (PC-4). This observation, coupled with the heavy loading of As, Pb, and Se on the tail principal component (TPC-2) which also followed the same contamination gradient among sites, suggests that tail tissue might be a powerful indicator of these environmentally hazardous metals and metalloids.

Although other studies have examined the utility of the use of nondestructive tissues such as blood, tail, or skin for assessing contaminant exposure in squamate reptiles, few have examined such a large suite of elements. The notable exception is the recent study by Fletcher et al. [20], who described elemental accumulation patterns in geckos along a gradient of urbanization in southern Spain. In that study, concentrations of 8 of 10 elements examined in tail tissue correlated with the remaining carcass. Fletcher et al. [20] found that Cu and Ni were the only two elements that were not correlated between tissue types, a finding corroborated by our results. Studies focused on a more limited subset of elements also support the findings of these recent studies. Semiaquatic snakes, fed controlled diets of metal-contaminated fish, accumulated As, Cd, Se, Sr, and V in their target tissues (e.g., liver), and concentrations of Se, Sr, and As in nondestructive tissues (skin, tail, and/or blood) could be used to make reliable predictions about previous exposure [38]. Hopkins et al. [38] also determined that tail tissue was a more predictive index of exposure history than blood or shed skin, supporting our assertion that the integrative nature of tail tissue could make it particularly useful for biomonitoring [38]. Similar studies evaluating lizards and snakes fed diets containing various levels of Se support the use of tail tissue [35,39], and mathematical models describing the functional relationships among diet, target tissue, and nondestructive tissues are now available [39]. With the advent of technologies that minimize the amount of tail tissue needed for contaminant analysis (e.g., [40]), use of tail tissue might even be feasible with species that are sensitive to tail loss because of a variety of important functional roles.

Taken together, the results of this study and several others provide mounting evidence that nondestructive measures could be a viable alternative to killing reptiles for exposure assessments of some pollutants. Although method validation will likely be needed in most cases, the ability to repeatedly sample individuals over time has enormous implications for study design and might be sustainable with little detrimental effect on study populations. Given the global plight of reptiles, such methods are critically important for approaching important ecotoxicologic questions with a conservation mindset.

CONCLUSION

Tarentola mauritanica appears to be a useful bioinidicator of a variety of pollutants, including some elements originating from mining (this study) and urban sources [20]. High site-fidelity and relatively small home ranges [18] make *T. mauritanica* suitable for characterizing bioavailability of local pollutants by integrating the local area temporally and spatially. This could be of critical importance in areas such as the Guadiamar River floodplain, where contaminants are heterogeneously distributed in the soil and hotspots exist where sludge particles were missed by remediation efforts. Additionally, contaminant accumulation in geckos represents an integration of highly variable lower trophic levels. Geckos were shown to transfer mine pollutants in the terrestrial food chain by accumulating elements from contaminated invertebrates and sediment, consequently providing a potential trophic link between invertebrates and higher level predators such as birds, snakes, mammals, and larger lizards that might prey on geckos. This could also be true for other reptiles inhabiting the affected habitats. Overall, our study revealed that the floodplain and urban areas affected by the disaster and mining activities remained sufficiently polluted for contaminants to enter the terrestrial food chain. In areas in which abundant, geckos represent useful taxa to study the bioavailability of several environmentally important pollutants.

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