

GECKOS AS INDICATORS OF URBAN POLLUTION

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Abstract — Geckos are common inhabitants of urban areas where they reside on manmade structures (e.g., building walls). Although their diets are generally opportunistic and include a variety of arthropods, foraging often occurs around artificial lights. The gecko's carnivorous diet and ability to adapt to urbanized conditions make them excellent organisms to study uptake and effects of urban pollution. We used the Moorish Wall Gecko (*Tarentola mauritanica*) to detect accumulation of trace elements in urban areas. Whole body accumulation was assessed for 15 elements (Be, Al, V, Mn, Ni, Cu, As, Se, Mo, Cd, Sb, Cs, Pb, U, and Tl). Accumulation was compared among three locations in southern Spain that represent a gradient in urbanization. Locations included a large city (Córdoba), a smaller town (Villaviciosa de Córdoba), and a rural area (Guadalmellato Reservoir). Multivariate analyses of element concentrations identified contaminants that increased with urbanization. This pattern was evident for Cs, V, Se, As, Ni, and Cd. However, Pb showed the most striking elevation in urban areas. Lead concentrations in geckos from the city were 15 times that of those from the rural site, exceeding those known to impose toxicological effects on wildlife. Comparison of element accumulation in the tails of geckos with whole body concentrations also revealed the utility of tail clips as a nondestructive index of contaminant uptake. We conclude that in areas where abundant, geckos represent useful taxa to study the bioavailability and effects of several environmentally important urban pollutants.

Key Words — Bioindicators, Gekkonidae, Reptile, Trace Elements, Urban Pollution

Tissue residues from wildlife are frequently used as indicators of environmental contamination because they offer an integrative measure of bioavailable pollutants (Hopkins 2006). Numerous criteria influence which wildlife species to target for monitoring efforts, including their abundance, ease of collection, and life history and/or ecological characteristics that make the species likely to accumulate contaminants of interest. For example, trophic level, longevity, and home range can all influence the tractability of wildlife for contaminant assessments.

Although reptiles are the most poorly studied vertebrates in ecotoxicology (Hopkins 2000), they have occasionally been

used as indicators of habitat quality. Tissue residues from turtles have been the most frequently examined, and have proven useful as sentinels of habitat degradation. For example, Snapping Turtles (*Chelydra serpentina*) are long-lived, high trophic level predators that bioaccumulate lipophilic contaminants in aquatic food chains (Meyers-Schöne and Walton 1994). In addition, turtles appear quite tolerant of certain contaminants (e.g., radionuclides, Hinton and Scott 1990) allowing them to persist in habitats where abundance of other wildlife species might be reduced. However, this insensitivity may also diminish how representative turtles are of other taxa.

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Although reptiles have been useful as indicators of habitat contamination (Hopkins 2006), they have seldom been used specifically as indicators of urban pollution. In many cases, the utility of sampling reptiles in urban environments is limited due to constraints on habitat which influence their abundance. For example, aquatic turtles may only be collected in sufficient sample sizes where aquatic habitat is plentiful enough to support a substantial urban population. However, in tropical and subtropical habitats, anthropogenic structures within the urban landscape are actually attractive to certain squamate reptiles, particularly geckos (Family Gekkonidae). Geckos use vertical structures (e.g., building walls) that are readily abundant in urbanized areas, and are often found near lights where their invertebrate prey congregate at night (Gil et al. 1994; Pérez-Mellado 1994). This latter characteristic makes sampling geckos in urban areas practical because collection sites can be easily targeted. Moreover, gecko populations have demonstrated sensitivity to industrial contaminants (Read 1998; Read and Pickering 1999). Despite the frequency at which these organisms are encountered in urban environments, no studies have explicitly examined their utility as bioindicators of urban pollution.

In this study, we examine the feasibility of using Moorish Wall Geckos (*Tarentola mauritanica*) as indicators of trace element contamination in urbanized areas. *Tarentola mauritanica* is a rather small gecko, reported up to 86 mm SVL (Barbadillo and Martínez-Solano 2000), with a widespread distribution extending around the Mediterranean Basin where it is common and frequently abundant across southern Europe and North Africa from the Mediterranean Sea to the Sahara Desert. Recent genetic investigations suggest that high genetic variability across its range is indicative of a species complex (Harris et al. 2004). *Tarentola mauritanica* occupies diverse habitats ranging from wet woodlands to desert areas, but thrives in urban environments (Pérez-Mellado 1994; Hódar and Pleguezuelos 1999; Luiselli and Capizzi 1999). It is primarily nocturnal, but occasionally exhibits diurnal activity (Capula and Luiselli 1994; Pérez-Mellado 1994).

To determine whether geckos can be used as indicators of urban pollution, we measured concentrations of 15 metals and metalloids in geckos sampled from three locations in southern Spain representing a gradient in urbanization ranging from a large city to a small village to a rural area. As a second objective, we sought to determine whether tail tissue could be sampled from geckos as a nonlethal indication of whole-body contaminant concentrations. Similar techniques have been developed for snakes (Hopkins et al. 2001, 2005a; Jackson et al. 2003) and lizards (Hopkins et al. 2005b) and show promise as a more conservation-minded approach to using reptiles as bioindicators of pollution. Finally, we describe concentrations of contaminants in the stomach contents of geckos as an indication of trophic transfer potential in these various habitat types.

METHODS AND MATERIALS

Study Sites — Geckos were collected from three locations in southern Spain. Our reference site was in a rural area near the Guadalmellato Reservoir, a 774 ha reservoir surrounded by Mediterranean forest. Villaviciosa de Córdoba, a relatively small town, has a population of fewer than 3,700. Our upper level of urbanization is represented by Córdoba, a large densely-populated city with a population of over 360,000 people — nearly two orders of magnitude more than Villaviciosa de Córdoba. Córdoba is also a center of industrial activity as indicated by the amount of electricity consumed by local industries. When considering industrial usage alone, Córdoba used 277,386 mega watts in 2001 compared to only 1,975 used in Villaviciosa de Córdoba.

Tissue Collection and Handling — A total of 30 geckos were collected from building walls at night between 31 May and 18 June 2001: Guadalmellato Reservoir ($n = 9$), Villaviciosa de Córdoba ($n = 13$), and Córdoba ($n = 8$). 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 (SVL) measured to the nearest mm. Subsequently the liver, gonads and gut (stomach and intestine) were removed. The stomach and intestine were opened, their contents removed and the interior surface gently rinsed with 18 M Ω deionized water. Food items were refrozen for later analyses. Cleaned guts were placed back into the carcass. Since *T. mauritanica* is not clearly outwardly sexually dimorphic (Luiselli and Capizzi 1999), sexes were recorded based on gonad examination, and individuals too small to possess visible gonads categorized as juveniles. Detachable portions of the tail, generally about 95% of the tail's total length, were broken from the carcasses. To ensure that analyses reflected accumulation of metals incorporated into body tissue, tails and carcasses were rinsed with deionized water before oven drying to remove surface contamination using a procedure similar to Goede and DeBruin (1984).

Separate dry weights were acquired for the liver, gonads, carcass, and tails of each individual. Samples were dried for three days in a drying oven at an average of 55°C (range 52 – 60°C). After oven drying, samples spent three days in a desiccator followed by three days in a dessicated glove box before being weighed to the nearest 0.1 mg. Gonasomatic index [GSI; (dry gonad mass/dry body mass) \times 100] and hepatosomatic index [HSI; (dry liver mass/dry body mass) \times 100] were calculated. We calculated a body condition index after that used by Romero and Wikelski (2001): (total wet body mass / snout vent length³) \times 10⁶.

Trace Element Analyses — The relatively small size of the geckos precluded element analysis of individual tissues. Thus, after weighing, the liver, emptied gut, and gonads were all returned to the carcass to allow analysis of whole body (minus the tail) accumulation employing ICP-MS. 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 15 elements were analyzed (Be, Al, V, Mn, Ni, Cu, As, Se, Mo, Cd, Sb, Cs, Pb, U, 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 25–250 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) with heating steps of 60, 60, 70, and 80 microwave power for 10, 10, 15, and 20 min, respectively. After digestion with HNO_3 , 0.5–1.0 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 10.0–25.0 ml (depending on tissue mass) with deionized water. Trace element analysis was performed by ICP-MS (Perkin Elmer, Norwalk, CT) on samples diluted 1:1 with deionized water. External calibration standards covering a range of 1–500 $\mu\text{g/L}$ were prepared daily by serial dilution of NIST traceable primary standards. Matrix-matched standard addition curves ranging from 10–500 $\mu\text{g/L}$ (from NIST traceable primary standards) were also included in the calibration for each type of sample analyzed. Certified reference material (TORT 2; NRC, Ottawa, 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 79–119%. Data were not corrected for percent recovery. Mean instrument detection limits among the different elements in the carcass, tail tissue, and stomach contents varied from 1.0–400.0 ng/g dry mass. Statistical treatment of concentrations registering below detection limits are discussed below. All element concentrations are presented on a dry mass basis.

Statistical Analyses, Bodies — Concentrations of Be, U and Sb in the carcasses of all individuals were below the detection limit (BDL), as were Tl concentrations in 90% of the geckos examined. Thus, we excluded these elements from further investigation. Of the 30 geckos analyzed, concentrations BDL also occurred in As (8 individuals, 27%), Mo (9 individuals, 30%), and Cs (1 individual, 3%). For statistical analyses, we replaced concentrations of elements below the detection limit with 50% of the mean detection limit (MDL). Distributions of element concentrations were improved by Log_n transformation.

We produced a Pearson correlation coefficient matrix (Table 1) from the element concentrations and dry soma mass as a measure of body size to investigate patterns of accumulation among trace elements and to evaluate potentially confounding effects of body size. All statistical comparisons were conducted with SYSTAT 10 statistical package. Because of the large number of elements examined and the similarities in accumulation among some elements revealed in the cor-

relation matrix, we employed Principal Components Analysis (PCA) to summarize the element concentration data. 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 11 elements (Al, V, Mn, Ni, Cu, As, Se, Mo, Cd, Cs, and Pb), we included dry soma mass in the PCA to account for allometric accumulations with body size. Since components by definition are orthogonal, the influence of body size can be isolated on components upon which body size loads leaving the remaining components free of the influence of size. We determined the number of interpretable principal components from scree plots and component eigenvalues. We employed a varimax rotation of the axes 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 (Wilkinson et al. 1996). Finally, we saved factor scores for each component in the PCA.

An ANOVA model using the PCA factor scores compared accumulation patterns between sexes and among levels of urbanization for each component that was independent of body size influences. The only juvenile was excluded from sexual comparisons. An ANOVA model was followed with a Tukey pairwise comparison as appropriate. Figure 1 was composed using the least squares means from the associated ANOVA model to illustrate differences among locations with the effects of sex controlled.

Small sample sizes precluded comparison of gecko body sizes and physical condition among sites within sexes separately. Alternatively, comparisons were made between sexes by including sex as a term in the ANOVA models along with site and the associated interaction term.

Statistical Analyses, 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 over one-half of the U, Sb and Mo concentrations were below or at the detection limit, these elements were excluded from further analyses. Concentrations BDL were also encountered in As (10 individuals, 33%), Tl (2 individuals, 7%), and Cd (3 individuals, 10%) and treated as described above. We compared the relationship between whole body and tail concentrations for each element with linear regression to establish similarity of trace element accumulation in these tissue sources.

Similar to the carcass analyses, PCA was conducted on Log_n transformed concentrations of 11 elements (Al, Mn, Ni, Tl, Cs, As, Se, Cd, Pb, V, and Cu) that remained after those elements with high rates of BDL concentrations were excluded. We again included dry soma mass to account for allometric accumulations with body size and we employed a varimax rotation of the axes to aid interpretation. Factor scores of each component

Table 1. Pearson correlation coefficient matrix of dry soma mass and whole body concentrations of 11 trace elements with all three sample locations combined. For clarity, we included only correlations significant at $P = 0.10$ and * marks those significant at $P = 0.05$, † marks those significant at $P = 0.01$, $n = 30$.

	Soma	Cs	V	Se	As	Pb	Ni	Cd	Al	Mo	Cu
V		0.616†									
Se	-0.491†	0.592†	0.608†								
As	-0.443*	0.370*	0.342	0.562†							
Pb		0.508†	0.669†	0.468†							
Ni		0.310	0.373*			0.564†					
Cd		0.357	0.479†	0.405*		0.556†	0.480†				
Al					0.307		0.311				
Mo	-0.406*		0.337	0.487†		0.315		0.327			
Cu	-0.327	0.378*		0.514†	0.332		0.375*			0.567†	
Mn	-0.369*						0.318		0.365*		0.514†

were compared with ANOVA. Because the sex of individuals and the consequential statistical problems arising from sex ratios would be unknown if only tail clips were collected, we did not include sex in the initial set of ANOVA models used to investigate spatial patterns of accumulation. Also because the single juvenile overlapped in size with other individuals and was thus not outwardly identifiable, it was included in the analysis of tail accumulation. Figure 2 was composed using least squares (LS) means from the associated ANOVA model. To examine whether the inability to identify the sex of a gecko weakened the analysis, additional ANOVA models which included sex and the sex*location interaction are presented for the components TPC-II and TPC-IV. Addition of sex to the model required dropping the juvenile from these comparisons.

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

RESULTS

Whole Body Accumulation— Several elements (Se, As, Mo, and Mn) were negatively correlated with dry soma mass (Table 1). Additionally, Cu had a possible weak but insignificant correlation with body size. In total, 16 pairs of element concentrations were correlated at $P = 0.01$ and several others had weaker correlations. Lead and selenium concentrations showed strong correlations with five and six elements, respectively. Elements correlated with Pb include Cs, V, Se, Ni and Cd, whereas Cs, V, As, Pb, Mo, and Cu were correlated with Se.

Principal components analysis produced four components

(designated as PC-I to PC-IV) that together accounted for 76% of the variation in the data (Table 2). Dry soma mass loaded strongly on a single component, PC-II, which revealed a negative relationship between body size and several elements (Mo, Cu, Se, Mn, and As). Because of the confounding effect caused by the correlation of body size to this component on elemental relationships, it was excluded from further analysis. The lack of correlation between body size and principal components I, III, and IV allows an assessment of the relationships among element concentrations that load on these axes that is independent of body size.

Principal component I (PC-I) explained nearly a quarter of the variation in the PCA and depicts a positive relationship

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

	PC-I	PC-II	PC-III	PC-IV
Cs	0.825			
V	0.785		0.351	
Se	0.689	0.556		
As	0.547	0.405	-0.312	0.479
Pb	0.545		0.660	
Soma		-0.842		
Mo		0.728	0.370	
Cu		0.654	0.341	
Mn	-0.350	0.548		0.668
Ni			0.843	0.331
Cd	0.370		0.628	
Al				0.805
Eigenvalues	2.737	2.521	2.221	1.567
Percent total variance explained	23	21	19	13

Table 3. ANOVA results examining the effects of sex and site on each of the four principal components derived from whole body concentrations that were independent of body size. Elements with loadings with an absolute value greater than 0.4 are listed beside the component heading.

Source	df	MS	F	p
PC-I (Cs, V, Se, As, Pb) R²=0.60				
Sex	1	0.131	0.265	0.61
Site	2	7.970	16.096	<0.01
Site*Sex	2	0.717	1.447	0.26
Error	23	0.495		
PC-III (Ni, Pb, Cd) R²=0.40				
Sex	1	0.936	1.308	0.27
Site	2	2.384	3.332	0.05
Site*Sex	2	1.848	2.583	0.10
Error	23	0.715		
PC-IV (Al, Mn, As) R²=0.17				
Sex	1	1.916	1.829	0.19
Site	2	0.374	0.357	0.70
Site*Sex	2	1.546	1.476	0.25
Error	23	1.047		

among Cs, V, Se, As, and Pb (Table 2). This component is of particular interest because of the known toxicological properties of several of these elements. An ANOVA model comparing PC-I scores between sexes and among sites explained 60% of the variation (Table 3). Absence of a significant difference in PC-I scores between sexes and a non significant site*sex inter-

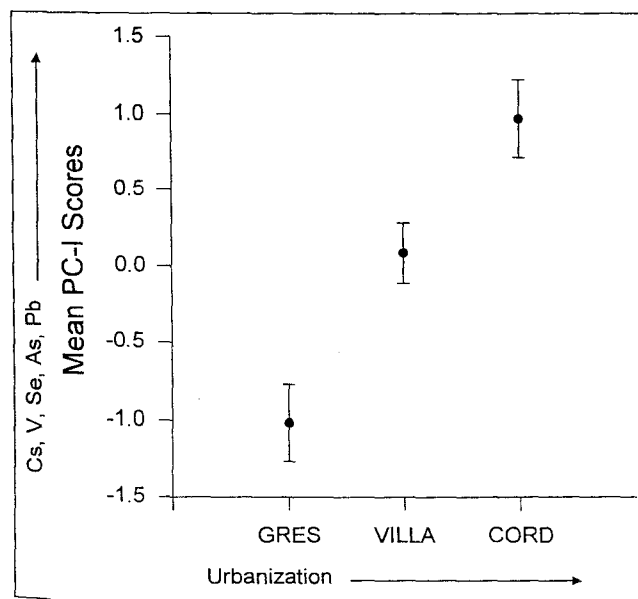


Fig. 1. Mean PC-I scores from the whole-body trace element accumulation analysis compared among three locations that represent a gradient in urbanization. Error bars represent ± 1 SE.

action term indicates a similar pattern of accumulation of these elements between sexes. In contrast, PC-I scores differed significantly among sites. Scores positively followed the urbanization gradient being lowest at the rural location (GRES), intermediate in the town (VILLA), and highest in the large city (CORD) (Fig. 1). Tukey pairwise comparison confirmed Córdoba PC-I scores to be higher than both VILLA and GRES ($P = 0.03$ and <0.01 , respectively). Corresponding VILLA scores were significantly higher than those for GRES ($P = 0.01$).

As expected, concentrations of elements loading on PC-I also followed the urbanization gradient exposed in the PC-I scores. This pattern of an accumulation gradient from GRES through VILLA to CORD was evident in Cs, V, Se and Pb (Table 4). The gradient was most striking in Pb with gecko tissue concentrations in VILLA and CORD on average being over 8 and 14 times higher, respectively, than at the rural location. Lead concentrations were also more variable in urban habitats as indicated by the wide ranges and high standard deviations (Table 4). Even though extremely variable, the lowest Pb concentration recorded in Córdoba was still higher than the high-

Table 4. Mean, standard deviation, and ranges of trace element concentrations (ppm) found in the whole bodies of individuals collected from each of the three locations.

	Guadalmellato Reservoir (n=9)	Villaviciosa de Córdoba (n=13)	Córdoba (n=8)
Cs	0.017 \pm 0.006 (0.005-0.025)	0.022 \pm 0.007 (0.012-0.040)	0.043 \pm 0.013 (0.022-0.061)
V	0.360 \pm 0.047 (0.283-0.439)	0.514 \pm 0.196 (0.277-0.959)	0.706 \pm 0.224 (0.474-1.046)
Se	0.583 \pm 0.119 (0.419-0.791)	0.820 \pm 0.148 (0.573-1.010)	0.970 \pm 0.256 (0.688-1.326)
As	0.066 \pm 0.031 (0.046-0.120)	0.186 \pm 0.123 (0.046-0.507)	0.140 \pm 0.053 (0.046-0.203)
Pb	2.036 \pm 0.883 (0.782-3.145)	17.316 \pm 40.502 (1.489-150.521)	30.140 \pm 36.434 (6.957-114.144)
Ni	0.370 \pm 0.126 (0.236-0.630)	0.371 \pm 0.124 (0.214-0.555)	0.505 \pm 0.282 (0.294-1.117)
Cd	0.062 \pm 0.028 (0.038-0.133)	0.081 \pm 0.058 (0.023-0.186)	0.122 \pm 0.063 (0.039-0.242)
Al	23.020 \pm 11.623 (10.857-46.876)	23.012 \pm 14.235 (11.100-62.992)	26.702 \pm 15.950 (6.477-56.946)
Mo	0.132 \pm 0.051 (0.071-0.217)	0.142 \pm 0.063 (0.071-0.258)	0.166 \pm 0.075 (0.071-0.300)
Cu	2.757 \pm 0.781 (1.943-4.300)	3.731 \pm 1.551 (2.016-7.226)	8.409 \pm 11.456 (2.599-36.536)
Mn	1.299 \pm 0.333 (0.872-2.000)	1.770 \pm 0.865 (0.840-3.561)	1.293 \pm 0.645 (0.596-2.579)

est concentration found at the rural location. In contrast to the other four elements, As concentrations were elevated in the urban areas, but were more similar in VILLA and CORD: 2.8 and 2.1 times higher, respectively, than those from GRES.

Principal component III (PC-III) illustrated a positive relationship among Ni, Pb and Cd and explained 19% of the variation in the PCA values (Table 2). Factor scores of PC-III did not differ between sexes, but did differ among sites (Table 3). The significance of the site*sex interaction term is less clear (P = 0.10). Examination of the least squares means from this ANOVA indicates an increase in scores with urbanization in females. However, scores in males were similar at CORD and GRES and lower at VILLA. On average, concentrations of Ni, Pb, and Cd were highest in CORD. Nickel concentrations were about 36% higher in CORD than both VILLA and GRES which had similar mean concentrations (Table 4). Similarly, average Cd concentrations in CORD were nearly twice that of those in GRES, but VILLA geckos had only slightly higher concentrations than at GRES. As indicated in the discussion of PC-I, Pb concentrations increased with urbanization.

Aluminum and Mn strongly, and As more weakly, loaded on PC-IV that explained 13% of the variation in the PCA (Table 2). The ANOVA model comparing scores of PC-IV between sexes and among sites explained only 17% of the variation. Concurrently PC-IV scores did not differ between sexes or among sites and the sex*location interaction was not significant (Table 3).

Nondestructive Element Analyses — Concentrations of 10 of the 16 examined elements had sufficient levels of accumulation in both the tails and bodies to warrant direct comparison of the two tissue sources: Cs, V, Se, As, Pb, Cu, Mn, Ni, Cd, and Al. In 6 of these 10 elements (Cs, V, Se, Pb, Mn, and Al), concentrations recorded in the tail were linearly related at P = 0.01 with those occurring in the body (Table 5). At least 40% of the variation was explained by the regression of five

Table 5. Regression statistics summarizing linear relationship between log₁₀ transformed whole body concentrations with tail concentrations for 10 elements.

Element	Regression Coefficient	R ²	P
Cs	0.65	0.58	<0.01
V	0.73	0.43	<0.01
Se	0.52	0.56	<0.01
As	0.19	0.15	0.03
Pb	0.86	0.93	<0.01
Cu	0.07	0.02	0.51
Mn	0.32	0.26	<0.01
Ni	0.24	0.12	0.07
Cd	0.19	0.15	0.03
Al	0.37	0.40	<0.01

Table 6. Loadings of dry soma mass and tail concentrations of trace elements on the first four principal components. For clarity, we include only loadings > 0.3 or < -0.3.

	TPC-I	TPC-II	TPC-III	TPC-IV
Al	0.945			
Mn	0.915			
Ni	0.875		0.300	
Tl		0.883		
Cs		0.856		
As		0.758		0.302
Se	0.455	0.676		
Cd			0.866	
Pb		0.452	0.701	
V				0.860
Cu	0.341	0.359	0.400	-0.614
Soma	-0.357		-0.415	
Eigenvalues	3.200	3.072	1.804	1.319
Percent total variance explained	27	26	15	11

of these elements indicating that the tail clips most effectively indicated whole body concentrations in Cs, V, Se, Pb and Al. Lead (Pb) was most notable with over 90% of the variance explained and a slope of 0.86. Regressions of two more elements (As and Cd) were significant at P < 0.05, but less than 20% of the variation was explained. Linear relationships between concentrations in the whole body and tail were not significant for only Cu and Ni.

Principal components analyses in the assessment of element accumulation in tails also produced four components (designated as TPC-I to TPC-IV) that explained a total of 79% of the variation (Table 6). In contrast to the analyses of the body element accumulation, dry soma mass did not load strongly on a single component but instead loaded moderately to lightly on two components (TPC-I and TPC-III). In addition to a positive loading of Al, Mn, Ni and Se, dry soma mass loaded at -0.357 on TPC-I. While not a particularly strong correlation, it could be strong enough to bias comparisons using scores from this component. Similar to that of PC-III of the body analyses, Cd and Pb loaded strongly on TPC-III, but was confounded by soma mass also loading negatively on this component. Copper also loaded positively on TPC-III. Because of the potential influence of body size on TPC-I and TPC-III, these components were excluded from further analyses.

Tail principal component II explained over a quarter of the variation and was analogous to PC-I from the whole body analyses. Four elements (Cs, As, Se, and Pb) loaded on both of these components, with two differences: Tl loaded on TPC-II and V on PC-I. When sex was excluded from the spatial analyses to conduct a realistic test of a nondestructive trace ele-

Table 7. ANOVA results first examining the effects of site alone and then with sex and site included on each of the two principal components derived from tail concentrations that were independent of body size. Elements with loadings of absolute value greater than 0.4 are listed with the component heading.

Source	df	MS	F	p
TPC-II (Tl, Cs, As, Se, Pb) R²=0.26				
Site	2	3.700	4.625	0.02
Error	27	0.800		
TPC-IV (V,-Cu) R²=0.09				
Site	2	1.365	1.403	0.26
Error	27	0.973		
TPC-II (Tl, Cs, As, Se, Pb) R²=0.50				
Sex	1	1.534	2.610	0.12
Site	2	3.004	5.110	0.02
Site*Sex	2	3.478	5.916	0.01
Error	23	0.588		
TPC-IV(V,-Cu) R²=0.26				
Sex	1	2.556	2.842	0.11
Site	2	1.124	1.249	0.31
Site*Sex	2	0.777	0.864	0.44
Error	23	0.900		

ment analysis, locations differed significantly, but the model explained only 26% of the variation (Table 7). Variation in TPC-II scores constructed using LS means from ANOVA (Fig. 2) illustrates the same gradient of scores in relation to urbanization (GRES lowest, VILLA intermediate, CORD highest) as was observed for PC-1 (Fig. 1). Tukey pairwise comparisons indicated that the ends of the gradient differed significantly ($P = 0.01$), but VILLA did not differ from the other two sites ($P \geq 0.20$). Inclusion of sex and the site*sex interaction into the ANOVA model nearly doubled the variation explained (Table 7). Note that the juvenile had to be dropped from the analysis when sex was added. Sexes did not differ significantly, but along with site, the site*sex interaction term was significant. Examination of least squares means showed scores for males to be elevated in CORD while scores of females were elevated in CORD and VILLA. Pairwise comparisons following this model yielded nearly identical results as the model with sex excluded. For both PC-I generated from the body concentrations and TPC-II generated from the tail concentrations, urban sites had significantly higher factor scores than the rural site ($n = 29, U = 155.0, P < 0.01$; $n = 30, U = 143.0, P = 0.03$).

Although V loaded positively and Cu negatively on TPC-IV, no spatial pattern was evident (Tables 6, 7). Inclusion of sex and the site*sex interaction into the ANOVA explained more of the variation but had little effect on the spatial comparison (Table 7).

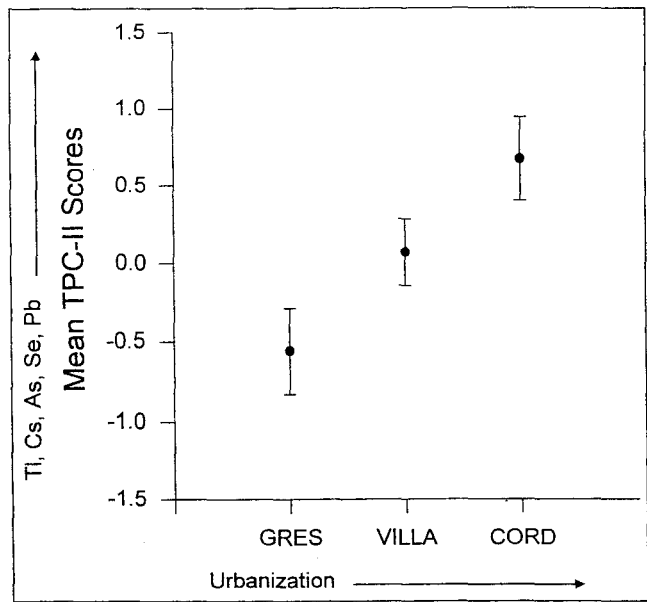


Fig. 2. Mean TPC-II scores from the tail clip analysis compared among three locations that represent a gradient in urbanization. Error bars represent ± 1 SE.

Body Size and Physical Condition — Males and females did not differ in size ($P = 0.23$), but total wet body mass did differ among sites ($P < 0.01$) and the sex*site interaction was significant ($n = 29$; $R^2 = 0.58, df = 1,2,2,23, P = 0.03$). Tukey pairwise comparisons indicated that geckos from the rural site, GRES, were larger than those in both urban sites (VILLA $P < 0.01$; CORD $P = 0.03$), but gecko size was similar in the two urban habitats ($P = 0.22$) (Fig. 3). Based on examination of least squares means, the significant interaction term appeared to largely be driven by males being larger than females at GRES.

Based on the condition index, physical condition was simi-

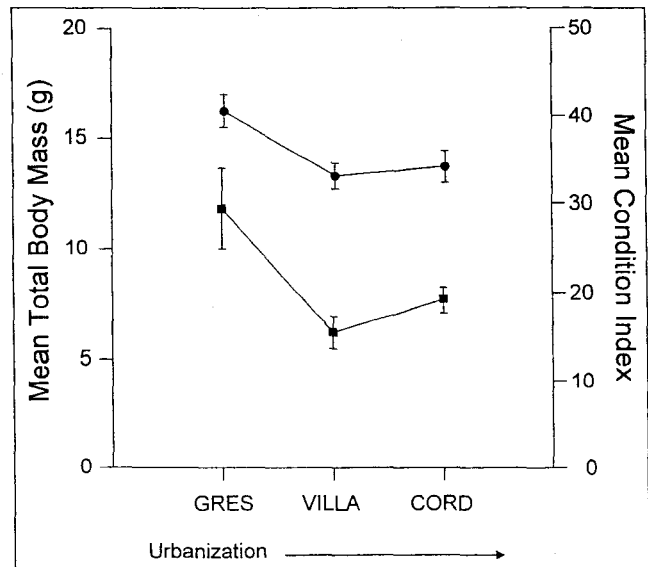


Fig. 3. Mean total wet body mass (■) and condition index (●) compared among locations. Error bars represent ± 1 SE.

lar between sexes ($P = 0.76$), differed among sites ($P = 0.01$), and the site*sex interaction was not significant ($P = 0.32$; $n = 29$, $R^2 = 0.39$, $df = 1,2,2,23$) (Fig. 3). Pairwise comparisons further indicated that similar to body size, the condition index was highest in the rural site (VILLA $P = 0.01$; CORD $P = 0.04$) and gecko physical condition did not differ between the two urban sites ($P = 0.90$).

Analysis of variance ($n = 29$, $R^2 = 0.73$, $df = 1,2,2,23$) indicated that the hepatosomatic index (HSI) was higher in females ($P < 0.01$) and differed among sites ($P < 0.01$). The interaction approached significant ($P = 0.08$) which should be considered in the following comparisons. Pairwise comparisons indicated that HSI in geckos from the largest urban area, CORD, was lower than both VILLA ($P = 0.02$) and GRES ($P < 0.01$). As expected, females had a larger GSI than males ($P < 0.01$), but again sites differed ($P = 0.01$) and the interaction was significant ($P = 0.01$). Similar to HSI, GSI was lowest in CORD (VILLA $P = 0.01$; GRES $P = 0.03$) and, VILLA and GRES did not differ ($P = 0.77$). However again, the significant interaction term indicates that the effects on sexes may have differed among locations.

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 that location. In our samples, geckos from urban locations frequently had the same prey taxa ranked as the dominant prey items in their gut. Mean ranks of two taxa (Hymenoptera and adult Coleoptera) from CORD and three (adult Coleoptera, Diptera and Homoptera) from VILLA exceeded 10 (Table 8). Occurrence of these prey taxa in the diets of 77–92% of the individuals at the respective locations also indicates the regularity to which these taxa were preyed upon. In contrast, the more diverse diet among the rural collected individuals is indicated by only a single prey taxon (Homoptera) exceeding a mean rank greater than seven. The more varied diet among geckos from the rural habitat is also expressed by only Homopterans occurring in the guts of more than 50% of the individuals. Interestingly, even though geckos collected from the rural site lacked consistently dominant prey taxa among individuals, single individuals appeared to have eaten fewer prey types than those from the urban habitats [mean number of prey taxa eaten by individuals followed by the SD and

range: CORD 4.25 ± 0.71 (3–5); VILLA 5.00 ± 1.68 (2–8); GRES 2.89 ± 1.76 (1–7)].

As indicated by the consistently sloping lines in Fig. 4, with the exception of Pb, concentrations of each element were either consistently higher or lower in the diet than in the whole body at all three locations. Lead appeared elevated in the body at the

Table 8. Gecko gut contents expressed as the mean, SD and range of the volumetric ranks of each taxon eaten, and the percent of geckos at each location that had eaten the prey item. Highest possible volumetric rank = 15.

	GRES (n=9)		VILLA (n=13)		CORD (n=8)	
	rank	%	rank	%	rank	%
Hymenoptera	0.0 ± 0.00	0.0	4.8 ± 6.31	38.5	12.8 ± 5.20	87.5
(non-Formicids)			(0–14)		(0–15)	
Coleoptera	3.1 ± 6.17	22.2	12.5 ± 3.93	92.3	12.1 ± 5.00	87.5
(adult)	(0–14)		(0–15)		(0–15)	
Hemiptera	4.3 ± 6.52	33.3	7.1 ± 6.95	53.8	6.3 ± 6.69	50.0
	(0–14)		(0–15)		(0–13)	
Diptera	0.0 ± 0.00	0.0	10.0 ± 5.97	76.9	5.1 ± 7.08	37.5
			(0–15)		(0–14)	
Araneae	4.1 ± 6.25	33.3	5.7 ± 6.54	46.2	4.6 ± 6.39	37.5
	(0–14)		(0–15)		(0–13)	
Formicidae	1.4 ± 4.33	11.1	1.6 ± 3.99	15.4	4.5 ± 6.23	37.5
	(0–13)		(0–12)		(0–13)	
Homoptera	7.6 ± 7.21	55.6	10.2 ± 4.76	84.6	3.1 ± 5.84	25.0
	(0–15)		(0–15)		(0–14)	
Lepidoptera	5.0 ± 7.50	33.3	3.4 ± 6.44	23.1	1.9 ± 5.30	12.5
(adult)	(0–15)		(0–15)		(0–15)	
Orthoptera	3.3 ± 6.61	22.2	1.2 ± 4.16	7.7	1.7 ± 4.95	12.5
	(0–15)		(0–15)		(0–14)	
Lepidoptera	2.9 ± 5.82	22.2	1.8 ± 4.32	15.4	1.6 ± 4.60	12.5
(larva)	(0–15)		(0–12)		(0–13)	
Isopoda	0.0 ± 0.00	0.0	1.1 ± 3.88	7.7	1.5 ± 4.24	12.5
			(0–14)		(0–12)	
Acarina	0.0 ± 0.00	0.0	0.0 ± 0.00	0.0	1.4 ± 3.90	12.5
					(0–11)	
Coleoptera	1.4 ± 4.33	11.1	3.8 ± 6.03	30.8	0.0 ± 0.00	0.0
(larva)	(0–13)		(0–14)			
Tricoptera	0.0 ± 0.00	0.0	0.7 ± 2.50	7.7	0.0 ± 0.00	0.0
			(0–9)			
Chilopoda	1.6 ± 4.67	11.1	0.0 ± 0.00	0.0	0.0 ± 0.00	0.0
	(0–14)					
Scorpionida	1.7 ± 5.00	11.1	0.0 ± 0.00	0.0	0.0 ± 0.00	0.0
	(0–15)					
Gastropoda	2.7 ± 5.50	22.2	0.0 ± 0.00	0.0	0.0 ± 0.00	0.0
	(0–15)					

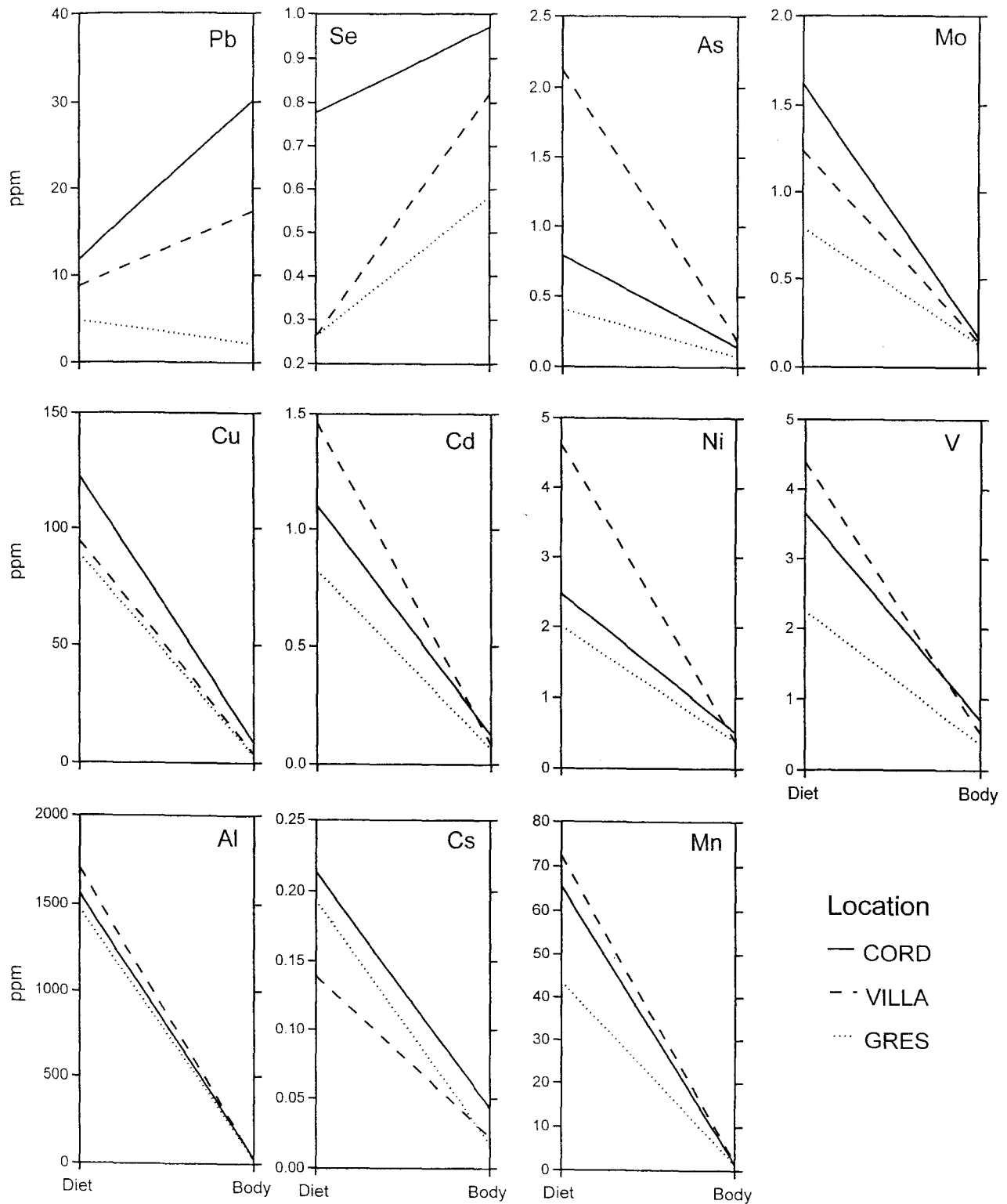


Fig. 4. Comparison of trace element concentrations in the gecko bodies and their prey items. Locations represent a gradient in urbanization ranging from a rural site (GRES) to a small town (VILLA) to a large city (CORD).

urban locations relative to the urban diet, but similar or lower in the body than diet at the rural location. Concentrations of nine elements (Cs, V, As, Ni, Cd, Al, Mo, Cu, and Mn) were elevated in the gut contents compared to the whole body concentrations, but whole body concentrations were higher than the diet for Se and Pb at the urban sites. As evident by lack of crossing lines in Fig. 4, for several elements (e.g. Pb, Se, As, Mo, and Cu), concentrations in the diet and body varied among sites in similar patterns (i.e., locations had similar rank orders). In some elements (Cd, Ni, and V), the urban locations exchanged rank orders, but the rural reference location remained at the lowest rank. Although Ni body accumulation appeared to be similar in geckos from VILLA and GRES, Ni concentrations in gut contents were notably elevated in VILLA (Fig. 4).

DISCUSSION

Geckos as Bioindicators — Our study demonstrated that geckos may be useful indicators of urban pollution. Principal component analyses identified several elements (Cs, V, Se, As, Ni, Cd, and Pb) that were elevated in the bodies of geckos from urban habitats. Importantly, concentrations of most of these elements increased with level of urbanization, suggesting the utility of using geckos to identify subtleties among urban habitats.

Ingestion of contaminated prey in urbanized landscapes is likely the primary route of contaminant exposure for geckos as has been shown for other reptiles (Loumbourdis 1997; Hopkins et al. 2004, 2005b), but ingestion of other materials may also contribute. For example, ingestion of contaminated soil is an important route of exposure in some wildlife (Sokol 1971; Sylber 1988; Beyer et al. 1994; Loumbourdis 1997). Sediments were found in the guts of six geckos: 1, 4, and 1 from CORD, VILLA and GRES, respectively. Many gekkonids that have immobile eyelids also wipe their tongue across their eyes to clean away dust or foreign materials (Bustard 1963). This and the practice of frequently licking their face before or after feeding could also promote contaminant ingestion (Read and Pickering 1999). Although trophic exposure is likely the most important mechanism of uptake, dermal exposure possibly contributes given that some geckos have relatively soft skin and large eyes compared to many other lizards (Read and Pickering 1999).

Although the gut content analysis of geckos only represented a snapshot in time of recent predation events, the findings were generally supportive of the importance of dietary exposure. Dietary concentrations for several elements (Pb, Se, As, Mo, and Cu) ranked in the same order among sites as in gecko tissue. Concentrations of Cd and V in their diet were also higher in urban locations as in the body tissues, but the two urban locations exchanged ranks. Lead and selenium concentrations were generally higher in gecko tissue than in their gut contents, whereas the other elements often showed the opposite trend. Nickel was interesting because its concentration in the diet of geckos from VILLA was nearly twice as high

as in the diet from other locations, but gecko tissue concentrations did not follow this pattern suggesting possible differences in bioavailability among sites. However it is also important to point out that *T. mauritanica* is a generalist predator (Capula and Luiselli 1994; Gil et al. 1994) and its diet varies seasonally (Gil et al. 1994; Hódar and Pleguezuelos 1999). Therefore, caution should be exercised when interpreting the relationships between element concentrations in stomach contents (a snapshot in time) and tissues (an integrated measure across time) since dietary concentrations may vary seasonally and among prey types.

Nondestructive Analysis — Overall, tails appeared to be a relatively good indicator of trace element contamination in the urban areas where our geckos were collected. Indeed, of the 10 elements compared, concentrations of eight (Cs, V, Se, As, Pb, Mn, Cd, and Al) showed a significant linear relationship between concentrations in the tail tissue and those in the remaining carcass. However because several of these regressions explained a relatively small proportion of the variation, tail tissue appeared to be a particularly good indicator for five elements (Cs, V, Se, Pb, and Al). Because of its prevalence in urban landscapes, it is important to note that the significant linear relationship between body and tail concentrations of Pb explained over 90% of the variation and had a slope approaching 0.9. In contrast, Cu and Ni were the only elements in which concentrations in the whole body and tail were not significantly related. The findings are in general agreement with other recent studies that suggest the utility of tail clips over some other nondestructive indices (e.g., blood) because tail tissue represents a composite of multiple tissues (e.g., muscle, blood, bone and skin) and thus may have a higher probability of including a variety of elements than single tissue types (Hopkins et al. 2001, 2005a,b; Jackson et al. 2003). For example, in a comparison of shed skins, tail clips, and blood collected from captive snakes reared on a metal-contaminated diet, tail clips were the most predictive index of previous exposure history to Se, Sr, and As (Hopkins et al. 2001).

Closer inspection of the data reveals that tail and whole body concentrations were correlated for all five elements (Cs, V, Se, As, Pb) that loaded highly on PC-I, the principal component that showed a strong accumulation gradient among sites in the whole body analysis. Of particular interest is the production of analogous principal components in the body (PC-I) and tail (TPC-II) analyses. Concentrations of Cs, As, Se and Pb were common to these two principal components. Remarkably, not only did the elements loading on these components overlap, but their factor scores showed the same pattern with respect to urbanization. Such a finding further illustrates the potential power of using tail tissue as a nondestructive indication of contamination resulting from urbanization.

Although the benefits of using nondestructive samples such as tails clips are clear, there are also limitations to this approach. For example, the variance associated with tail clip concentrations was greater than that in carcasses; larger standard errors

accompanied the LS means derived from the TPC-II ANOVA model compared to that using PC-I from the bodies. However, if using a less destructive tissue collection technique allowed greater numbers of individuals to be analyzed, the increased sample size may offset the greater variability and actually better characterize the population. This is especially true in sexually dimorphic species in which increasing sample size may allow analysis of sexes separately. However, if small sample sizes are to be used, whole body analysis may be needed to obtain the statistical power necessary to differentiate among sites.

Another consideration relates to whether or not tail autotomy has a negative effect on individuals sampled. Tails can be used for a variety of functions in squamates including storage of fat, crypsis, stabilization in parachuting, climbing, prehensile or adhesive organs, burrow plugging devices, ejectors of defensive secretions, sensory devices, signaling devices, and production of defensive noises (reviewed in Bauer and Russell 1994). As a result of these important functions, the consequences of tail autonomy in lizards have been the subject of much debate (e.g. Vitt et al. 1977; Vitt and Ballinger 1982; Bauer and Russell 1994; Niewiarowski et al. 1997). Clearly, sampling tail tissue from species with specialized tails (e.g., prehensile tails, adhesive pads; Bauer and Russell 1994; Bauer 1998) should be avoided when feasible. Speed of tail regeneration should also be considered as some smaller species can regenerate functional tails in only four weeks, whereas larger longer-lived species may take years or never regenerate a tail tip similar to the original (Bauer and Russell 1994). Reducing the size of the tail clip will generally decrease adverse effects, but gecko species with adhesive pads on their tail may be a unique case. Since the pad tends to be on the tip of the tail, removal of the tail sample will necessarily remove the caudal pad. Recent advances in laser-ablation technologies that allow analysis of minute portions (i.e., ~1–2 mg) of tissue may permit such species to be sampled with negligible consequences (Jackson et al. 2003), but such technologies are so specialized that their widespread use is not immediately probable. It should be noted that although we removed 95% of the tail from previously sacrificed animals, much smaller tail portions (~30% of the tail) would actually be necessary for field applications employing the same routine analytical procedures described here.

Effects — Although our analyses indicated that concentrations of several elements are elevated in geckos from urbanized areas, most do not appear to be high enough to be of great concern, except for Pb. Lead is toxic to a wide variety of wildlife, but its effects on reptiles remain poorly studied (Holem et al. 2006). In waterfowl, liver concentrations of Pb exceeding 15 ppm wet weight (approximately 75 ppm dry weight assuming 80% moisture) in liver and 20 ppm in bone are indicative of severe toxicosis (Pain 1996). In mammals, liver and kidney concentrations of Pb exceeding 30 and 90 ppm dry weight, respectively, are suggestive of Pb toxicity, but much lower concentrations of 10 and 25 ppm dry weight, respectively, are used to diagnose Pb toxicity in livestock (Ma 1996). Because Pb is

generally highest in liver, kidney and bone tissue (Pattee and Pain 2003), it is reasonable to suggest that the gecko whole body concentrations presented in this study are lower than the concentrations that would occur in each of these individual gecko tissues (i.e., other body components such as muscle dilute the concentration found in these target tissues). Thus, based on mean whole body concentrations of 30 ppm found in geckos in this study, we suggest that geckos from Córdoba exceed many of the above-mentioned toxicity thresholds for other vertebrates and are at substantial risk of adverse effects including hematological, neurobehavioral, and reproductive maladies. It is also important to note that Pb concentrations were highly variable among geckos in Córdoba, exceeding 114 ppm dry mass in one individual. Moreover, this individual possessed the lowest condition index of all geckos in the study. Further studies that identify the sources of this variability, the sources of Pb contamination in Córdoba, and the effects that Pb has on gecko physiological, behavioral, and life history traits would be of value not only from an ecological point of view, but also from a human health perspective.

Geckos in the rural habitat tended to be larger while also having relatively larger gonads and higher condition indices. Size could influence condition indices and GSI, but current sample sizes preclude detailed assessment. Larger sample sizes are needed to rigorously compare these traits among sites. In addition to contaminant stress, diverse environmental differences intrinsic to urban and rural living could produce the smaller sizes and lower condition indices observed in the urban areas. For example, the diet of *T. mauritanica* differs between natural and human-dominated habitat (Hóðar and Pleguezuelos 1999); not only did prey items change, but so did gecko hunting behavior. By triggering a change from hunting on the ground (Hóðar and Pleguezuelos 1999) or from perching in trees (Gramentz 2002) to residing primarily on buildings, structural changes to the environment may also influence a gecko's life history and/or element accumulation. Our study moderated this effect, to at least some degree, by collecting geckos from manmade structures in the rural as well as urban sites. However, dietary differences of geckos between the rural and urban settings were clearly evident in our study. Separating such dietary vs. chemical effects will require extensive study of the natural history of geckos in these populations.

Overall, geckos satisfy several criteria indicative of a useful target organism for detecting bioavailable pollutants. Geckos are broadly distributed and within their geographic range are often both common and relatively abundant in urban areas where they are easy to capture. Geckos such as *T. mauritanica* display high site fidelity and small home ranges (Luiselli and Capizzi 1999) allowing association of the animals to local pollutants. Furthermore, gecko populations have also demonstrated sensitivity to environmental contaminants (Read 1998; Read and Pickering 1999). The carnivorous diet of geckos also provides a trophic link between invertebrates and higher level predators such as birds, snakes, mammals, and larger lizards (Bauer 1990). These characters combined with our observed

patterns of trace element accumulation make geckos well suited as bioindicators of urban pollution.

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