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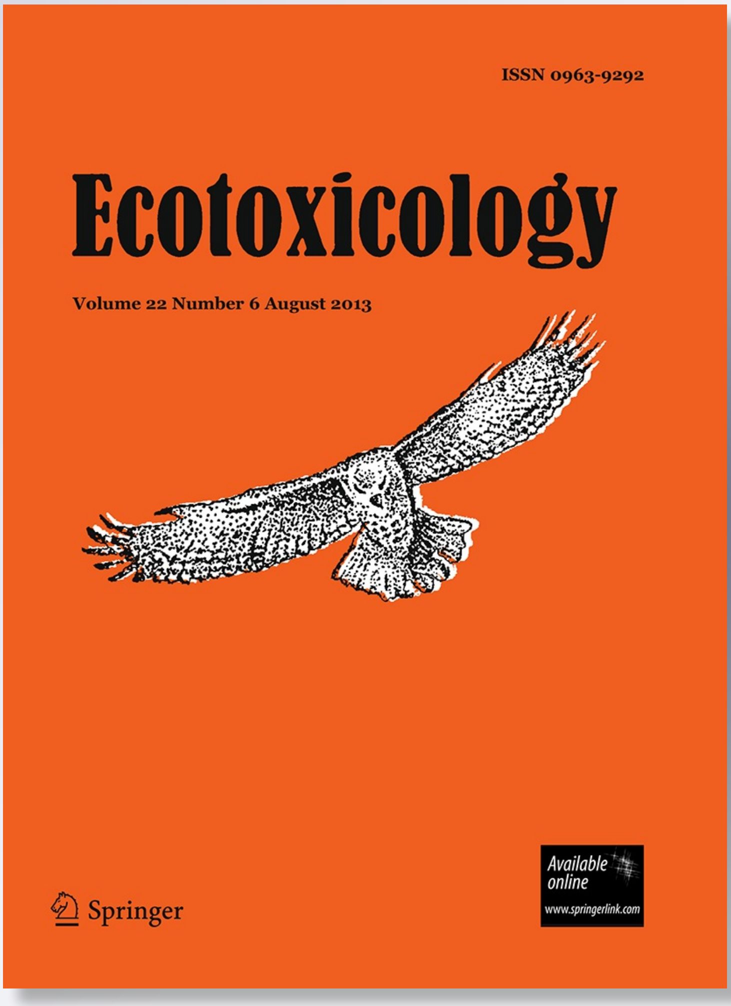

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Abstract Nine-banded armadillos (*Dasypus novemcinctus*) are widespread and abundant New World mammals with a lifestyle that entails prolonged, intimate contact with soils. Thus, armadillos would seem a promising candidate as a sentinel species to monitor chemical contamination in terrestrial ecosystems. Surprisingly, there have been virtually no toxicology studies on armadillos. Here, we provide the first analysis of metal contaminants for wild armadillos. Liver tissues were obtained from 302 armadillos collected at 6 sites in Georgia and Florida, USA that varied in their extent of human disturbance, from rural pine plantations to highly modified military/space installations. Data were stratified by age (juvenile and adult), sex, and site. Temporal (yearly) variation was examined at two of the sites that were sampled over three consecutive years. Concentrations of aluminum, cadmium, copper, nickel, lead, and zinc were measured in liver samples from each site. Although reference levels are not available for armadillos, accumulated metal concentrations were comparable to those reported for other mammals. We found no evidence of sex or age differences in the concentrations of any metal, except for Cd (age) and Pb (sex and age). However, concentrations of most metals varied substantially across sites and over time. Finally, concentrations of many metals were positively correlated with one another, suggesting that they likely co-occurred in some areas. Collectively, this study indicates the utility of armadillos as a sentinel species for studies of metal contamination in terrestrial systems, and highlights the need for further studies of other toxicants in these animals.

Keywords Aluminum · Armadillo · Cadmium · Copper · *Dasypus novemcinctus* · Nickel · Lead · Zinc

Introduction

There is continuing concern about how anthropogenic disturbance of natural habitats impacts a variety of wildlife (Pyati et al. 2012). However, it is virtually impossible, as well as impractical, to test every animal for exposure to every possible toxicant. Thus, a more reasonable approach has been to identify certain sentinel species that might serve as proxies for multiple members of particular ecosystems (Wren 1986).

Among mammals, the nine-banded armadillo (*Dasypus novemcinctus*; hereafter referred to as "armadillo") possesses many attributes that might make it an attractive choice as a sentinel species for terrestrial toxicology studies. This species has the broadest geographic distribution of any armadillo, ranging from northern Argentina to the southern United States (Abba and Superina 2010). Particularly within the southern United States, armadillos are widespread and abundant (Loughry and McDonough 2013). Although they seem to favor areas comprised of bottomland hardwoods and in close proximity to water (Loughry and McDonough 2013), armadillos can occupy a wide range of terrestrial habitats. Notably, armadillos seem quite tolerant of human disturbance, and can be found even in highly altered sites.

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The lifestyle of armadillos further contributes to their potential utility as sentinels of environmental contamination in terrestrial systems. Armadillos are medium-sized (adult body weight ~ 4 kg), fossorial mammals (McBee and Baker 1982) that are in constant contact with soils through construction of burrows, and while foraging for various invertebrate prey. Whether incidental or intentional, armadillos ingest large quantities of soil while foraging (McDonough and Loughry 2008). Thus, armadillos would seem likely to experience high exposure to any soil-borne contaminants, either directly from their burrowing and foraging activities, or indirectly via consumption of their soil-dwelling prey.

Despite multiple attributes that might make armadillos a useful species in which to examine environmental contamination in terrestrial systems, there have been very few studies investigating the accumulation and effects of contaminants in these animals. Wheeler et al. (1975) reported the presence of the organochlorine pesticide, dichlorodiphenyltrichloroethane (DDT), and its metabolites in the stomach contents of armadillos, presumably the result of animals consuming insects exposed to the pesticide. However, tissue accumulation of the contaminants was not examined. Wheeler et al. (1977) sampled a total of 26 armadillos over a 2-year period following an application of mirex to control fire ants (*Solenopsis richteri*), and showed that the pesticide did accumulate at high levels in various tissues, at least up to 18 months following application. Related to both studies, Herbst et al. (1989) reported cholinesterase values for armadillos in the hope that these would be used to evaluate exposure of animals to organophosphate-based (anti-cholinesterase) pesticides. However, there has been no follow-up work on any of these studies in the intervening decades. Additionally, the value of these early studies is somewhat limited because each utilized only a small number of animals from a single population.

Monitoring bioaccumulation of contaminants is an important aspect of wildlife exposure assessments and may provide insight into toxic effects exerted by contaminants detected (Eisler 1985, 1988, 1993, 1998). In this paper, we present the first analysis of metal contaminants in armadillos. A large number of individuals were screened from multiple sites and for multiple metals to better understand metal transfer and accumulation in areas with varying land use. Metals were selected because they are commonly used in many facets of modern society and, as such, are frequently released into the environment, often with adverse consequences for wildlife (Domingo 1994; Shore and Rattner 2001; Pyati et al. 2012). Beyond documenting exposure of armadillos to metals, our sampling design allowed us to examine differences in metal concentrations due to age (juvenile versus adult), sex, and time (yearly variation). In addition, we were able to assess differences among sites because we sampled areas that differed substantially in their degree of human disturbance. In total, this study provides the first extensive data for metal accumulation in armadillos, and highlights their potential use as a sentinel species for chemical contaminants in many terrestrial habitats.

Methods

Sample collection

Armadillos were collected from six locations in Georgia and Florida, USA between 2003 and 2012 (Fig. 1). As part of an experiment to eliminate nest predators of northern bobwhite (*Colinus virginianus*), armadillos were removed and samples collected from the eastern portion of Pinebloom Plantation, near Albany, Georgia, and Pebble Hill Plantation, near Thomasville, Georgia in 2003 (McDonough et al. 2007). Subsequently, this experiment was repeated over the next 3 years (2004–2006) on the western portion of Pinebloom Plantation, and at Tall Timbers Research Station, located near Tallahassee, Florida. Armadillos at all of these sites were either trapped or shot by technicians working for the United States Department of

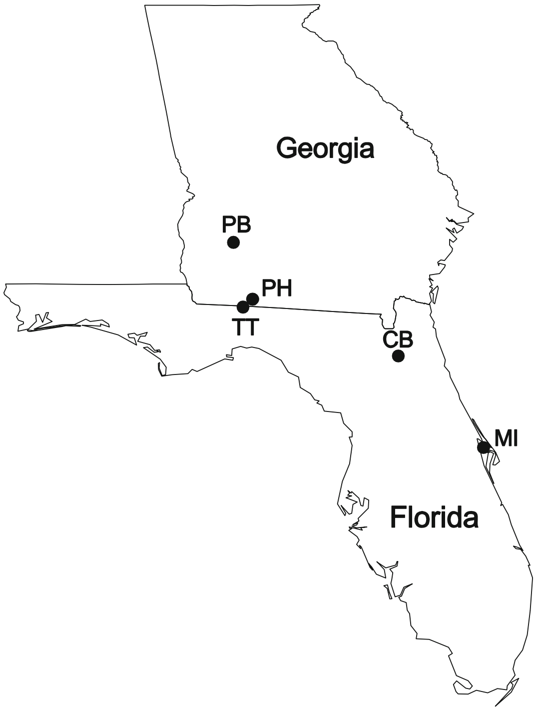

Fig. 1 Map showing locations where armadillos were collected in Georgia and Florida, USA. *CB* Camp Blanding Joint Training Center, *MI* Merritt Island National Wildlife Refuge, *PB* Pinebloom Plantation, *PH* Pebble Hill Plantation, *TT* Tall Timbers Research Station

Agriculture between 1 March and 30 September of each year. The number and demographic composition of individuals collected per month each year at each site were very similar (Lockhart, unpublished data).

In addition to the specimens just described, animals were also collected at two sites in Florida in 2012 as part of a survey to determine the prevalence of leprosy infection in wild armadillo populations. Armadillos were collected from Merritt Island National Wildlife Refuge, near Titusville, Florida in May, and from Camp Blanding Joint Training Center, near Starke, Florida, in June. Merritt Island is largely comprised of grounds occupied by the Kennedy Space Center; Camp Blanding is the primary training center for the Florida National Guard. As such, both of these sites have experienced considerable human disturbance, e.g., at Camp Blanding most notably due to the large amounts of live ammunition used during troop training, and at Merritt Island because of the disposal of chemicals and other potentially toxic materials used in the space industry. Consequently, our expectation was that armadillos at these two sites would be at high risk of exposure to various metals. In contrast, Pebble Hill, Pinebloom and Tall Timbers are managed for timber and wildlife, and are situated in relatively rural areas. Although various land management practices, such as prescribed burning and the use of pesticides, do occur (McDonough and Loughry 2005), we predicted that armadillos at these three sites would exhibit lower tissue concentrations of metals than those from Merritt Island and Camp Blanding.

At all sites, a portion of liver tissue was obtained from each animal collected. Liver was chosen as the target tissue because it is the major storage and detoxification organ in most organisms and has been shown to accumulate metals (Casarett et al. 2008). Liver samples were placed in vials (with no preservative), and stored frozen until analyzed. Prior to dissection, each animal was weighed, and weights were used to assign individuals to the following age categories: <2 kg = juveniles (young of the year); 2–3 kg = yearlings; and >3 kg = adults (Loughry and McDonough 1996). There can be some ambiguity in identifying yearlings versus adults because weights sometimes overlap (McDonough et al. 1998). Consequently, we opted to focus on the clear distinction between juveniles and adults, and did not screen any samples from yearlings.

Table 1 provides information on the number of individuals sampled at each site. Because the number of individuals collected at Camp Blanding, Merritt Island, Pebble Hill and Pinebloom East was small, we analyzed all samples taken from those sites. However, several hundred animals were collected at both Pinebloom West and Tall Timbers (McDonough et al. 2007). Consequently, we used a random number table to select individuals for screening. At both sites, ten adult males and ten adult females were screened from each year of sampling (see Table 1; note that an extra adult male was sampled at Tall Timbers in 2006 and likewise in 2004 at Pinebloom West because of inclusion of animals from a preliminary screen that was conducted). Because fewer were captured, we opted to select three juvenile males and three juvenile females from each year and each site for screening, but even this was not always possible due to the scarcity of juveniles at certain sites (Table 1).

Metal analyses

We initially screened ten adult armadillos (3 each from Pebble Hill and Tall Timbers, plus 2 each from Pinebloom East and Pinebloom West) for the presence of a number of different metals. Based on those results, we opted to focus on the six metals that were most elevated and of greatest environmental concern: aluminum (Al), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn).

Table 1 Numbers of nine-banded armadillos sampled in this study

Site	Adults		Juveniles	
	Males	Females	Males	Females
Camp Blanding (2012)	17	14	0	0
Merritt Island (2012)	17	42	3	2
Pebble Hill (2003)	8	5	5	3
Pinebloom East (2003)	15	16	1	3
Pinebloom West				
2004	11	10	3	3
2005	10	10	1	0
2006	10	10	3	2
Tall timbers				
2004	10	10	3	3
2005	10	10	3	2
2006	11	10	3	3

For each site, the year(s) samples were collected is provided

Liver samples were weighed, dried at 80 °C for at least 24 h, and then weighed again to determine both wet (ww) and dry (dw) weights. The means ± standard deviations of the dw and ww were 0.27 ± 0.09 and 0.94 ± 0.38 g, respectively, yielding a dw/ww ratio of approximately 3.5. The data below are reported as metal accumulated per dw tissue. Trace metal grade nitric acid (Fisher Scientific, Pittsburgh, PA, USA) was added to the dried liver samples in 15 ml polypropylene centrifuge tubes, and the samples were digested in a water bath at 60 °C for at least 24 h. The digested samples were diluted with 18 mΩ Milli-Q® water and analyzed for metals using atomic absorption spectrophotometry (GFAAS; Perkin Elmer AAnalysts 800, Norwalk, CT, USA) with graphite furnace detection (detection limit = 1 ppb). Certified 1 g/mL metal standards dissolved in 2 % HCl (Fisher Chemical, Fairlawn, NJ, USA) were used for each metal and samples were analyzed in triplicate. The blank and standards were used for re-calibration every 40 samples and they were also analyzed as samples periodically throughout each cycle. Three replicates of a certified reference material (LUTS-1, lobster hepatopancreas) were treated as the samples to determine average extraction efficiencies, which were 94, 91, 106, 105, and 66 % for Cd, Cu, Ni, Pb, and Zn, respectively. This method of digestion and metal analysis has been used in several other studies in our laboratory and has been effective (Main et al. 2010; Shyn et al. 2012).

Statistical analyses

Comparisons of metal concentrations in armadillo liver samples were performed for dw only, using Statview 4.01. Information on ww concentrations is provided in the Supplementary Material (Figs. S1–S3). To insure normality, all

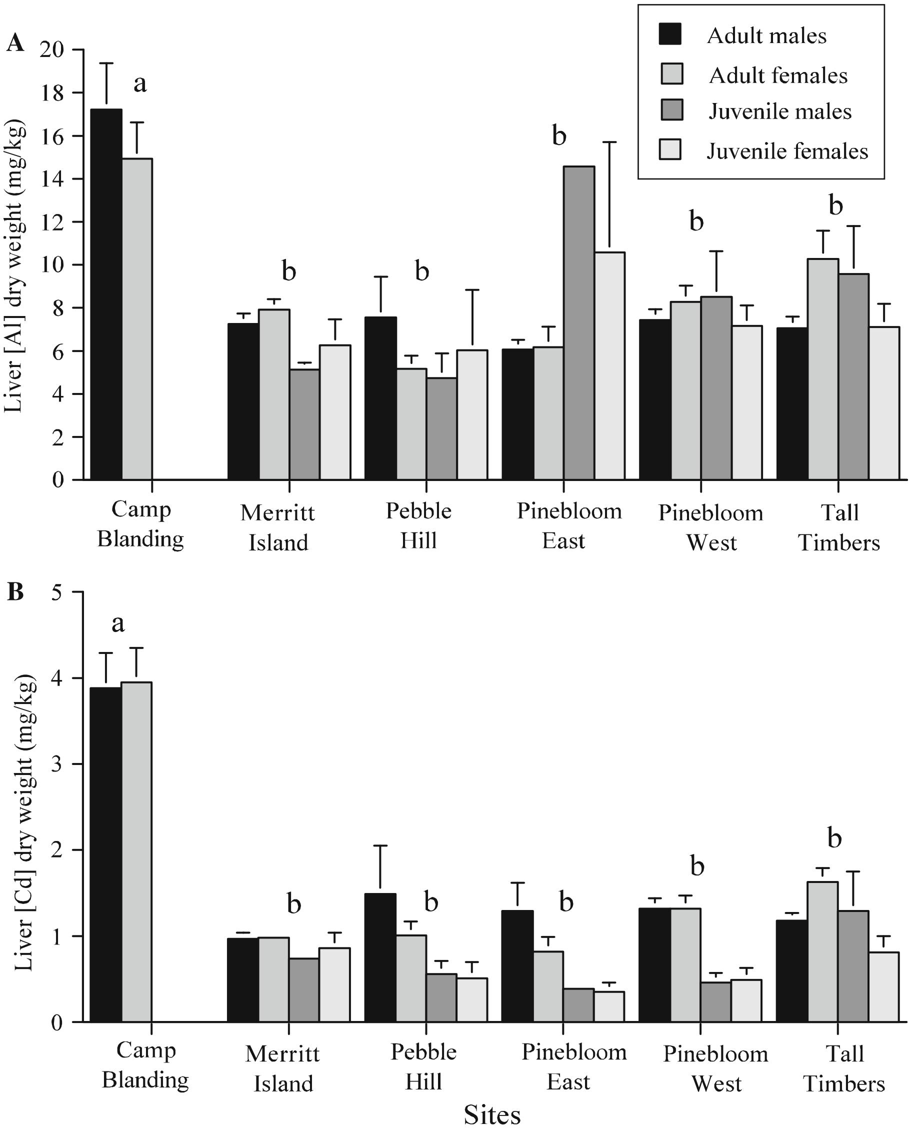

Fig. 2 Mean ± SE dw (mg/kg) metal concentration in liver tissue of Al (**a**) and Cd (**b**) for each age/sex class of nine-banded armadillos collected at six sites. There was an overall main effect for site differences for both metals ($F = 16.56$ for Al and 19.17 for Cd, both $P < 0.0001$); *letters* indicate sites that did not differ from one another using Bonferroni–Dunn post hoc pair-wise comparisons. For Cd, there was also a significant main effect of age (across all sites; see "Results" section)

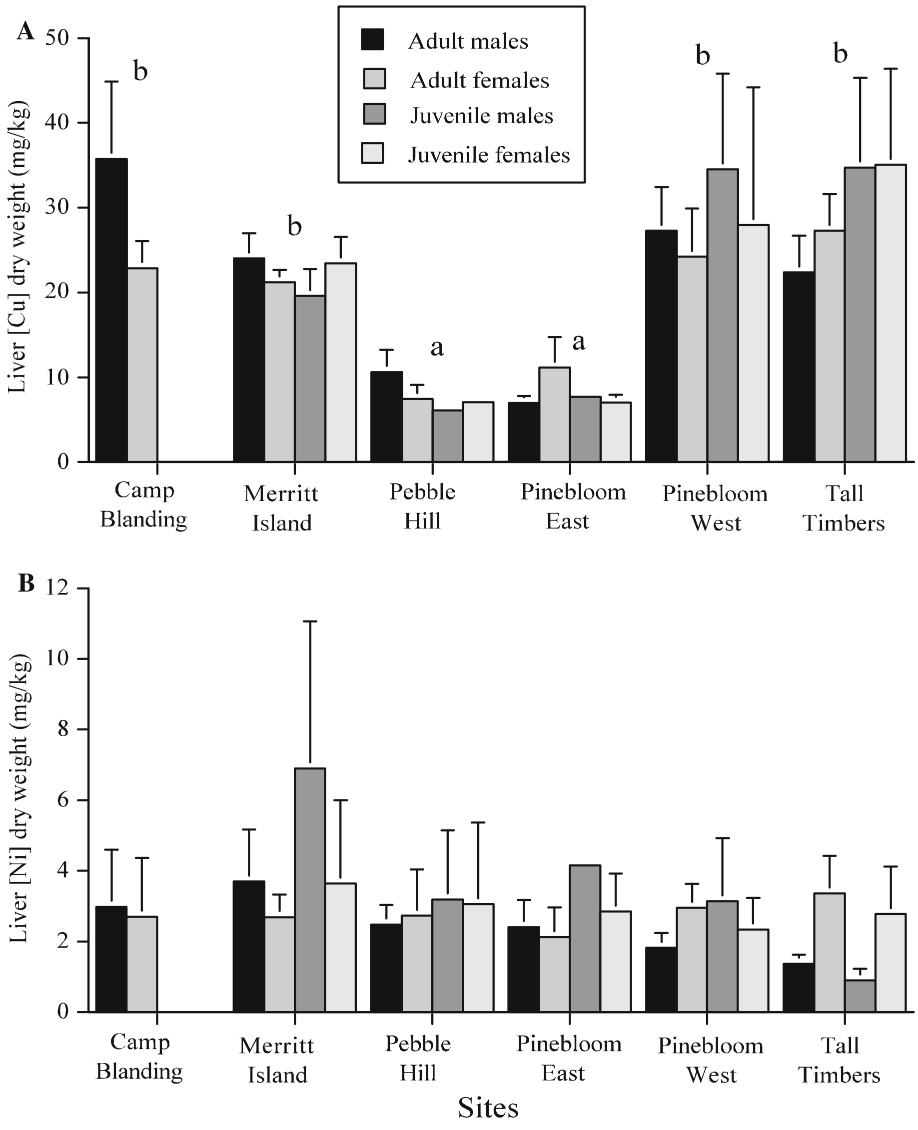

Fig. 3 Mean ± SE dw (mg/kg) metal concentration in liver tissue of Cu (**a**) and Ni (**b**) for each age/sex class of nine-banded armadillos collected at six sites. For Cu, there was an overall main effect for site differences ($F = 18.49$, $P < 0.0001$); *letters* indicate sites that did not differ from one another using Bonferroni–Dunn post hoc pair-wise comparisons. There were no significant differences in concentrations of Ni for any main effect

data were log-transformed prior to analysis. However, untransformed values are provided in the tables and figures.

We used ANOVA to examine age, sex, and site differences in the dw concentration of each metal. Ideally, this would have been done using a three-way factorial design. However, because no juveniles were sampled at Camp Blanding, and very few at some other sites (see Table 1), this was not possible. Instead, we initially ran one-way ANOVAs for each main effect variable. In order to detect any potential interactive effects, we also ran two-way ANOVAs that included sex and site, and age and sex as the two factors. Because the F-ratios for each main effect obtained in the one-way and two-way analyses did not differ substantially, we report the results from just the one-way tests. In all these analyses data were pooled across years for the Tall Timbers and Pinebloom West sites, and Bonferroni–Dunn tests were used for post hoc pair-wise comparisons.

We used a two-way ANOVA to examine yearly variation in the concentration of each metal, with site (Tall Timbers and Pinebloom West) and year (2004, 2005 and 2006) as the two factors. Data were pooled across all individuals for these analyses. Finally, we performed linear Pearson correlations, using data from all sites, to determine whether there were relationships between the tissue concentrations of different metals found in each animal.

Results

Samples from a total of 302 armadillos were analyzed. With the exception of Ni (Fig. 3b), for which we found no significant differences, concentrations of every other metal varied significantly among sites, but there was less evidence of age or sex differences (Figs. 2, 3, 4). There were

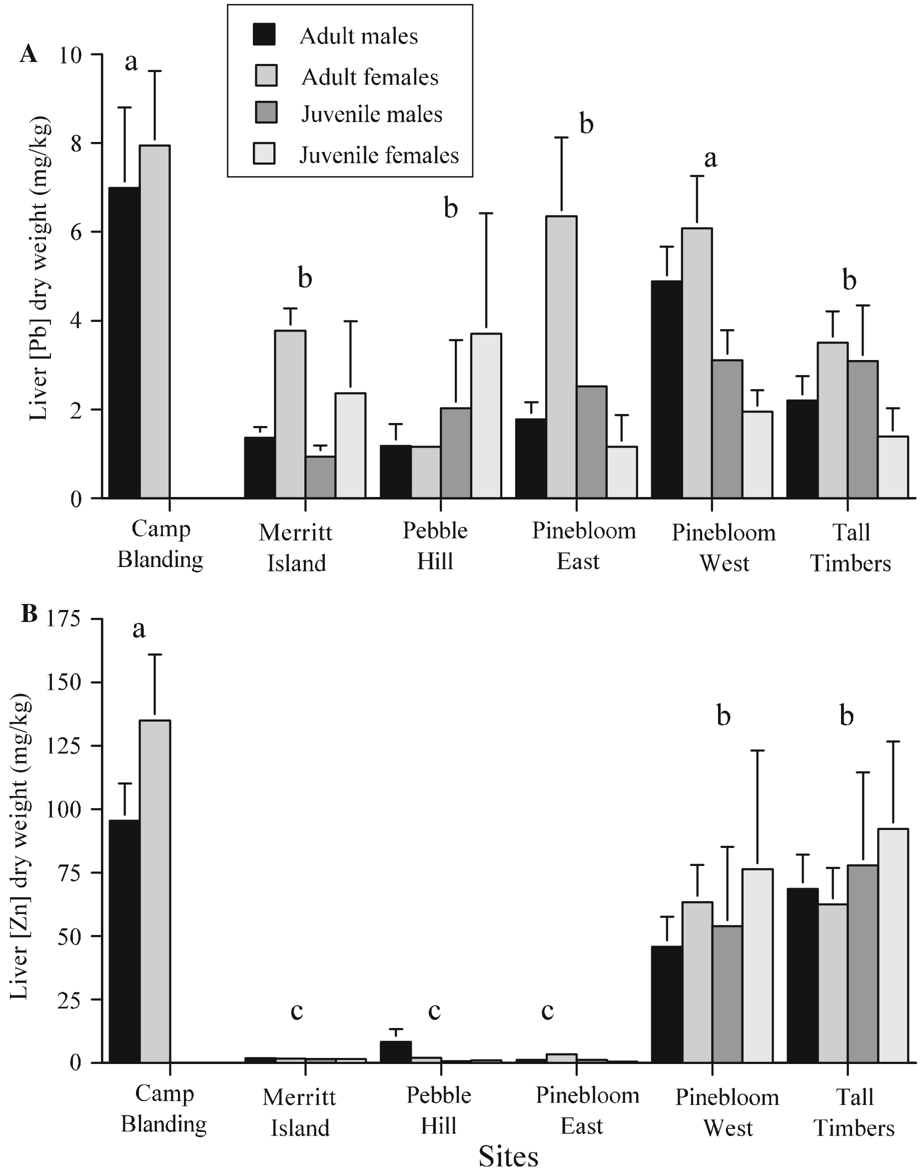

Fig. 4 Mean ± SE dw (mg/kg) metal concentration in liver tissue of Pb (**a**) and Zn (**b**) for each age/sex class of nine-banded armadillos collected at six sites. There was an overall main effect for site differences for both metals ($F = 11.06$ for Pb and 52.10 for Zn, both $P < 0.0001$); *letters* indicate sites that did not differ from one another using Bonferroni–Dunn post hoc pair-wise comparisons. There were also significant main effects for both age and sex for Pb (across all sites; see "Results" section)

no significant interaction terms (age × sex or sex × site) for any metal (all F ratios <2.44, $P > 0.12$).

For Al (Fig. 2a) and Cd (Fig. 2b), animals from Camp Blanding had higher concentrations than at any other site (Bonferroni–Dunn tests, all $P < 0.0001$). For Cd there was also a main effect of age ($F = 48.90$, $P < 0.0001$), with adults averaging higher concentrations than juveniles.

Concentrations of Cu (Fig. 3a) clustered into two groups: armadillos from Pinebloom East and Pebble Hill had significantly lower values than those found at the other four sites (all pair-wise comparisons, $P < 0.0001$; there were no differences between Pinebloom East and Pebble Hill, or among the other four sites).

Lead accumulation was unusual because, in addition to site differences, it was the only metal in which we found evidence of both age and sex differences (Fig. 4a). Specifically, adults exhibited higher concentrations than did juveniles ($F = 8.62$, $P = 0.004$), and females had higher levels than males ($F = 6.55$, $P = 0.011$). However, the lack of any significant interaction terms suggests each main effect operated independently. Site differences consisted of higher values at Camp Blanding than at Merritt Island, Pebble Hill, Pinebloom East and Tall Timbers (pair-wise comparisons, all $P < 0.0001$). In addition, concentrations were higher at Pinebloom West than at Pebble Hill and Tall Timbers (both $P < 0.0001$).

Liver Zn concentrations fell into three groups (Fig. 4b). Animals from Camp Blanding had significantly higher Zn liver burdens than at any other site (pair-wise comparisons, all $P < 0.0001$). Armadillos at Pinebloom West and Tall Timbers comprised an intermediate group with levels that did not differ from one another, but which were

Table 2 Mean (±SE) dw concentration of metals in the liver tissue of nine-banded armadillos sampled over three consecutive years at two sites

Metal	Tall Timbers			Pinebloom West		
	2004	2005	2006	2004	2005	2006
[Al] (mg/kg)	10.22 (1.05)	8.35 (1.05)	7.26 (1.11)	8.68 (0.40)	7.96 (0.77)	6.92 (0.95)
[Cd] (mg/kg)	1.11 (0.15)	1.50 (0.16)	1.74 (0.39)	0.90 (0.06)	2.47 (0.89)	1.15 (0.14)
[Cu] (mg/kg)	12.38 (0.88)	40.99 (6.20)	28.07 (4.85)	9.12 (0.88)	34.27 (4.95)	39.48 (8.21)
[Ni] (mg/kg)	2.30 (0.53)	3.22 (1.21)	1.23 (0.39)	3.39 (0.74)	2.62 (0.76)	1.28 (0.32)
[Pb] (mg/kg)	1.65 (0.31)	5.29 (0.96)	1.36 (0.24)	7.29 (1.24)	4.67 (0.85)	2.83 (0.66)
[Zn] (mg/kg)	1.50 (0.27)	39.13 (6.95)	163.58 (12.39)	1.95 (0.20)	32.38 (8.82)	168.04 (33.82)

Data were pooled for adults and juveniles, and males and females. See Table 1 for samples sizes at each site each year, and Table 4 for results of statistical analyses

significantly higher than those found at the remaining three sites (pair-wise comparisons, all $P < 0.0001$; there were no significant differences in concentrations among the latter three sites).

Just as with site differences, we found substantial evidence of yearly variation in metal concentrations at the Pinebloom West and Tall Timbers sites (Tables 2 and 3). This variation did not appear to be site-specific, because there were few differences found between sites, nor were there many significant year × site interactions (Table 3). However, patterns were not consistent across metals. For example, concentrations of Cd, Cu and Zn increased over time (dramatically so in the case of Zn), but those of Al, Ni and Pb declined.

From a total of 15 possible pair-wise associations, concentrations of the metals were significantly positively correlated with one another in 12 instances; there was also one significant negative correlation between Ni and Zn (Table 4).

Discussion

To our knowledge, this is the first documentation of metal accumulation in nine-banded armadillos. An immediate question raised by our analyses is whether any of the levels we found were high enough to be toxic to the animals. Unfortunately, this is a difficult question to answer because no reference values are available for armadillos. However, comparisons of our data with reports of tissue metal concentrations in other mammals (Shore and Rattner 2001) suggest that metal accumulation may have been high enough in some cases to cause deleterious effects.

Taking each metal in turn, studies documenting concentrations of Al in wildlife are scarce. Consequently, we cannot evaluate how our data compare with values reported in other species of mammals. It does seem, however, that armadillos collected in areas that were presumably more contaminated had more accumulated Al. Although Cd accumulation patterns can vary by species, gender, and age, residues in vertebrate liver tissues exceeding 10 mg/kg ww are typically indicative of Cd contamination (Eisler 1985). In our study, liver Cd concentrations in armadillos collected from Camp Blanding (the presumed most polluted site) were approximately fourfold higher than those from the other sites; however, they did not exceed 1.25 mg/kg ww (4.5 mg/kg dw). Eisler (1985) reported that Cd concentrations are generally higher in older individuals than in younger ones; this is consistent with our findings.

Cu is abundant in the environment and because of its essentiality it accumulates, at least to some extent, in most biota. The Cu concentrations we found in armadillos are similar to those reported for other terrestrial mammals (Shore and Rattner 2001; Eisler 1998). Cu concentrations in animals are generally elevated in areas with more anthropogenic impact, such as those treated with Cu-containing herbicides, near smelters, and from heavily urbanized and industrialized areas (Eisler 1998). From the data collected in this study, all sites other than Pinebloom East and Pebble Hill were somewhat impacted by Cu. However, the reason for this is unknown.

Generally, Ni concentrations in the tissues of mammals are <0.1–5 mg/kg dw in uncontaminated areas and 0.5–10 mg/kg dw in Ni-contaminated areas (Eisler 1998). The extent of Ni accumulation in biota can vary greatly among species. The liver Ni concentrations (~1–10 mg/kg dw) of armadillos in our study did not significantly differ among sites and were within the range of other values reported in the literature (Shore and Rattner 2001). We hypothesized that Ni concentrations would be higher in armadillos collected from sites closer to industrial areas; however, our data did not support this. It is possible that armadillos are able to efficiently regulate internalized Ni or that those collected were not exposed to substantially elevated levels of Ni at any one site. Additionally, atmospheric Ni may travel substantial distances before settling, thus making point source contributions of Ni difficult to determine.

Table 3 F-ratios from two-way ANOVAs examining yearly variation in dw metal concentrations in the liver tissue of nine-banded armadillos collected at two sites

Metal	Year (df = 2, 145)	Site (df = 1, 145)	Year × site (df = 2, 145)	Post-hoc pair-wise comparisons for year
[Al] (mg/kg)	9.06***	0.22	0.20	a
[Cd] (mg/kg)	3.51*	0.41	0.85	a
[Cu] (mg/kg)	35.34***	0.09	2.47	a, b
[Ni] (mg/kg)	15.34***	1.67	0.47	b, c
[Pb] (mg/kg)	10.01***	22.44***	7.59***	b, c
[Zn] (mg/kg)	299.24***	1.26	3.58*	a, b, c

See Table 1 for sample sizes and Table 2 for means

Significant post hoc pair-wise comparisons for year designated as: a = 2004 versus 2005, b = 2004 versus 2006, c = 2005 versus 2006

* $P \leq 0.05$

** $P \leq 0.01$

*** $P \leq 0.001$

Table 4 Pearson correlations (*r* values) among the dw concentrations (mg/kg) of metals in the liver tissue of nine-banded armadillos across all collecting sites (*n* = 293 individuals)

	[Al]	[Cd]	[Cu]	[Ni]	[Pb]	[Zn]
[Al]	–	0.47***	0.15**	0.23***	0.33***	0.14*
[Cd]		–	0.20***	0.02	0.30***	0.34***
[Cu]			–	−0.07	0.19**	0.54***
[Ni]				–	0.16**	−0.26***
[Pb]					–	0.15**
[Zn]						–

* $P \leq 0.05$

** $P \leq 0.01$

*** $P \leq 0.001$

Pb can exist as an organometal and has a higher partition coefficient than the other metals in this study; therefore, Pb would be preferentially distributed in more hydrophobic compartments (Eisler 1988). The higher Pb accumulation we observed in female armadillos could have been related to the chemical properties of organolead (Eisler 1988). More generally, the Pb residues observed in our samples were within the range of those reported for other mammals (Shore and Rattner 2001; see also Eisler 1988). Because Camp Blanding is used as a military facility, it seems likely that Pb contamination is greater in this area, thus accounting for the higher Pb concentrations observed in the livers of armadillos collected there. More frequent vehicular traffic could have also contributed to the increased Pb contamination.

Armadillos collected from three sites, including one with substantial anthropogenic influence (Camp Blanding) as well as two forested areas with intermediate human impact (Tall Timbers and Pinebloom West) all contained elevated Zn liver concentrations. Corrosion of Zn alloys and galvanized surfaces, and erosion of agricultural soils can substantially contribute to increased Zn levels in the environment (Eisler 1993). Increased Zn use and/or contamination may have occurred in the collection areas where armadillos had the highest tissue Zn levels, and also across years at Tall Timbers and Pinebloom West. However, the specific causes underlying these changes are currently unknown. Substantially elevated Zn tissue concentrations in invertebrates, particularly earthworms, have been reported in those collected from Zn-contaminated areas (Amiard et al. 1986; Andrews et al. 1989). Zn concentrations in earthworms range from 120 to 650 mg/kg dw in uncontaminated soils and 200–1,600 mg/kg dw from more industrialized sites (Beyer and Cromartie 1987). Armadillos feed on earthworms (McDonough and Loughry 2008); therefore, they may be more susceptible to Zn accumulation and possibly toxicity. Even so, values obtained for armadillos in this study were comparable to those reported for other mammals with very different diets (Shore and Rattner 2001). Zn homeostasis is generally well regulated in most mammals; however, some exceptions have been documented. The reported range of Zn concentrations in the liver of the Long-finned pilot whale (*Globicephala melaena)* and horse (*Equus caballus)* in control versus Zn-contaminated sites is relatively broad (at least tenfold; Gunson et al. 1982; Muir et al. 1988), similar to the range of Zn concentrations observed in the armadillos in this study. Variations in zinc content among species can be substantial, and intraspecies variations can also vary extensively with age, size, sex, season, and exposure to contamination (Eisler 1993).

Overall, and consistent with our expectations, armadillos collected from Camp Blanding, (one of the sites with substantial anthropogenic disturbance) had the highest tissue metal residues, suggesting increased exposure to metal pollutants in that area. However, because our samples were collected between 2003 and 2012 we cannot exclude the possibility that some of the other differences among sites reflect differences between years rather than sites. Nonetheless, the general pattern of differences conformed with our expectations based on history of land use. The one exception to this was Merritt Island where, contrary to our prediction, armadillos did not have particularly high concentrations of any metal. This leads us to conclude that, despite the intensive use of various chemicals at that site, preventive measures seem to have been successful at minimizing exposure of wildlife to these materials. For all sites, a fuller understanding of metal accumulation patterns requires knowledge of metal concentrations in the environment (e.g., in soil samples). Unfortunately, because of the retrospective nature of our study, in which animals were collected for purposes other than toxicology analyses, such information is not available. An important task for the future will be to examine how environmental concentrations of metals are reflected in tissue concentrations.

The general lack of sex differences in tissue metal accumulation (with the exception of Pb) is perhaps expected given previous findings that male and female armadillos—of all age groups—do not differ much physically, behaviorally or ecologically (Loughry and McDonough 2013). Thus, both sexes probably experience equivalent levels of exposure to metal contaminants. As for age differences, because the nonessential metals, Cd and Pb, bioaccumulate and persist to a greater extent than the other metals, it follows that liver tissue residues of these metals would be higher in adults than in juveniles. The other metals are likely metabolized and better regulated than Cd and Pb; therefore, accumulation in liver tissue does not continue to increase over time.

We found considerable fluctuations in metal concentrations between years at Pinebloom West and Tall Timbers (Tables 2 and 3). Most remarkable was the massive increase in Zn at both sites in 2006. The fact that this occurred at both sites suggests a common, widespread cause, but at present the nature of this cause is unknown. It should be noted that yearly variation in all metals may not have been due just to changes at the collecting sites. Because animals were being culled from each property, it seems likely that armadillos collected in later years (especially in 2006) were not residents that had somehow evaded capture, but instead were immigrants that moved into newly vacated areas (McDonough et al. 2007). Thus, yearly variation in metal concentrations may reflect conditions experienced in areas remote from the collecting sites. At present, the source(s) of immigrants is unknown.

Concentrations of most metals were positively correlated with one another, particularly Al and Cd, and Cu and Zn. Alternatively, Ni and Zn were negatively correlated. These results suggest similar uses for the metals and thus their concomitant release in the environment. In addition, the possibility cannot be discounted of varying patterns of metal accumulation as a consequence of interactions between the metals, or due to differences in the physiology of accumulation for each metal.

To summarize, our results show that metal accumulation occurs in the livers of armadillos, and that accumulation may be related, at least in part, to land use. Anthropogenic disturbance exposes wildlife to many potentially harmful contaminants. Our data indicate that armadillos might prove useful as a sentinel species for monitoring metal contamination in terrestrial ecosystems. However, additional research is needed to examine whether the accumulated metals exert toxic effects in the animals, and to determine how environmental levels of contaminants are related to tissue concentrations. It would also be worthwhile to examine metal accumulation patterns in other mammals collected at our study sites in order to assess the generality of our findings.

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Conflict of interest The authors declare that they have no conflict of interest.

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