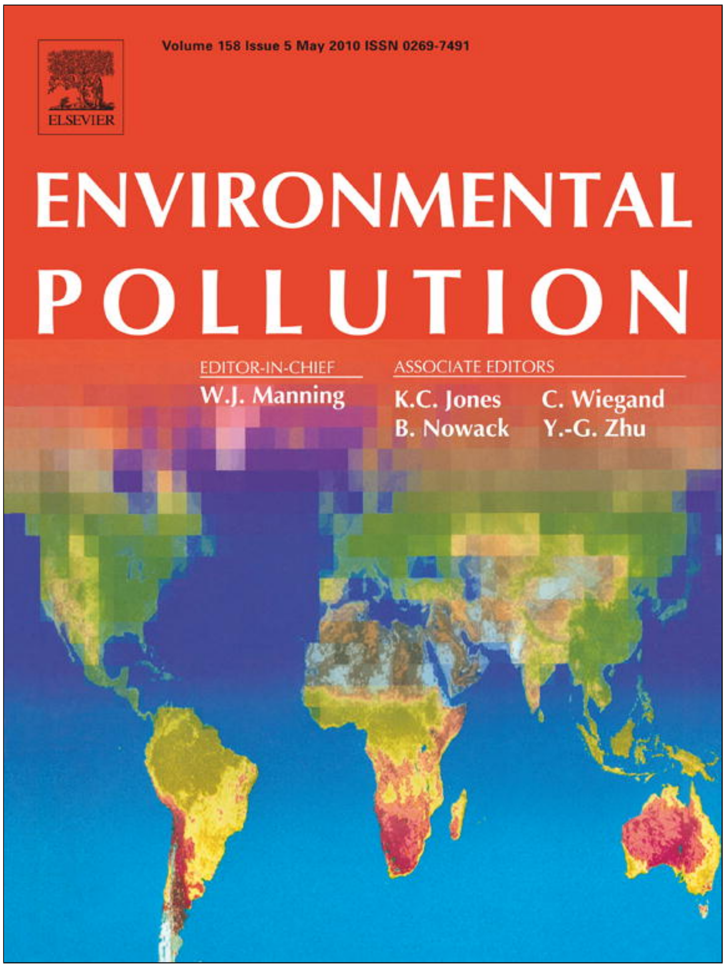
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Heavy metal distribution and bioaccumulation in Chihuahuan Desert Rough Harvester ant (*Pogonomyrmex rugosus*) populations

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Heavy metals bioaccumulate in desert ants.

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A B S T R A C T

Heavy metal contamination can negatively impact arid ecosystems; however a thorough examination of bioaccumulation patterns has not been completed. We analyzed the distribution of As, Cd, Cu, Pb and Zn in soils, seeds and ant (*Pogonomyrmex rugosus*) populations of the Chihuahuan Desert near El Paso, TX, USA. Concentrations of As, Cd, Cu, and Pb in soils, seeds and ants declined as a function of distance from a now inactive Cu and Pb smelter and all five metals bioaccumulated in the granivorous ants. The average bioaccumulation factors for the metals from seeds to ants ranged from 1.04× (As) to 8.12× (Cd). The findings show bioaccumulation trends in linked trophic levels in an arid ecosystem and further investigation should focus on the impacts of heavy metal contamination at the community level.

1. Introduction

Metals such as arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) have been released into the Chihuahuan Desert ecosystem near El Paso, TX from smelting activities (as well as other urban sources) for nearly a century (Pingitore et al., 2005). Previous studies have shown that these metals have accumulated in some of the plants and invertebrates of the region (Mackay et al., 1998, 1999; Calderon et al., 1999). Although it is clear that metal contamination can negatively impact ecosystem structure and function (Worthington, 1989; Calderon et al., 1999; Creamer et al., 2008), a thorough examination of the distribution and accumulation patterns of heavy metals in multiple desert trophic levels has not been completed. To understand the community-level effects of metal pollution in desert ecosystems, it is essential to understand the distributions of these metals in soil–plant–invertebrate environments.

Contaminants that accumulate in the plants of the region can eventually be passed on to higher desert trophic levels such as the arthropods. The Rough Harvester ant (*Pogonomyrmex rugosus*) is a dominant granivorous species in the Chihuahuan Desert. As with most terrestrial insects, these ants are primarily exposed to heavy metal contaminants through their diet (Hopkin, 1989; Rabitsch, 1995). When seeds with elevated metal concentrations are consumed by harvester ants, metals accumulate in their hindgut tissues (Rabitsch, 1997). High contamination levels have resulted in reduced reproductive success and smaller colony size (Eeva et al., 2004), which could lead to microevolutionary changes as populations become locally adapted to elevated levels of heavy metals (Holloway et al., 1990). Additionally, heavy metals accumulated in harvester ants can impact multiple desert trophic levels because a variety of myrmecophagous predators depend heavily on harvester ant communities. An example is that of the horned lizards, one of which (*Phrynosoma cornutum*) is currently listed as threatened in Texas and mainly preys on desert harvester ants and termites (Whiting et al., 1993). In predatory vertebrates heavy metals are often bioaccumulated via direct exposure, drinking contaminated water or ingesting prey which have stored metals (in this case *P. rugosus*) (Smith et al., 2007). To date we know of no works which identify critical metal concentrations in harvester ant predators.

In this study we analyzed the distribution and bioaccumulation of As, Cd, Cu, Pb, and Zn in the soils, seeds of two grasses (*Dasyochola pulchela* and *Bouteloua curtipendula*) and *P. rugosus* populations of the northern Chihuahuan Desert near El Paso, TX. Our objectives were (1) to survey the toxic metal distribution in ants of

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the region, and (2) to evaluate the bioaccumulation factors (BAF) of As, Cd, Cu, Pb and Zn at multiple trophic levels.

2. Materials and methods

2.1. Study sites

We collected ants at 17 sites in the Chihuahuan Desert between September and October of 2007 (Fig. 1). Twelve of the study sites were located on the western slope of the Franklin Mountains and five sites were in the Hueco Mountains, 50–60 km east of the Franklins and well outside the urban footprint of El Paso (i.e. far enough from the urban setting such that there exist minimal sources of anthropogenic metal contaminants). The Franklin Mountain sites were in close proximity to a former copper smelter (0.5–10.0 km) while the Hueco Mountain sites were assumed to be uncontaminated, because they were more than 40 km from suspected sources of heavy metals, and were thus treated as reference sites. Sampling sites were restricted to mountainous areas within the study region since *P. rugosus* nests occur in higher densities in rocky soils as compared to sandy soils (Taber, 1998).

Based on the results from 2007, a more intensive sampling campaign was conducted in 2008. At this time soils, seeds and ants were collected at three sites that were identified as having high (site 1), medium (site 2) and low (site 3) levels of heavy metal contamination (Fig. 1, closed stars). The high contamination site was located approximately 1 km east of the former Cu and Pb smelter, on the campus of the University of Texas at El Paso (Fig. 1). Foraging observations were conducted at each sampled nest for a period of 5 min to determine the primary foraging preference at each site. Rough Harvester ants at this site were observed foraging primarily for seeds of desert fluff grass (*D. pulchela*). The medium contamination site is located 8.2 km northeast of the smelter along the western slopes of the Franklin Mountains (Fig. 1). Rough Harvester ants at this site were observed foraging primarily for seeds of sideoats grama (*B. curtipendula*). The low contamination site was located 47 km northeast of the smelter in Hueco Mountains State Park. Rough Harvester ants at this site were observed collecting seeds of both fluff grass and sideoats grama. Although there were differences in the bedrock geology of the sampled areas, none of the rocks contain naturally-elevated concentrations of heavy metals, suggesting that the accumulations of elements like As, Cd, Cu, and Pb were mainly anthropogenic in origin.

2.2. Field sampling

In the 2007 extensive sample, Rough Harvester ant foragers were sampled by randomly selecting three nests at each site (Fig. 1) and collecting approximately 60 individuals per nest, each nest was analyzed independently. The mean metal concentration in ants per site was determined by taking the average of the three individual nests at each site resulting in a final $n = 17$. The ants were collected using plastic forceps and stored in acid washed vials.

For the 2008 intensive sampling, a 30 m linear transect was laid along the main foraging trail, originating from the nest entrance and approximately 60 ants were collected as previously described. Five nests were sampled in site one and three, and four nests were sampled in site two ($n = 14$), each transect was treated as an independent unit. This distance was used because it is the maximum forging range identified for *P. rugosus* (Taber, 1998). Seeds from the plants of interest and soils were collected along the 30 m transect at 10 m intervals. Therefore each transect consisted of 4 seed and soil subsamples, which were averaged to determine the mean metal concentration. Harvester ants are generally not dependent on specific plant species for seeds; however, there can be a slight preference for grass seeds (Taber, 1998; Whitford, 1978). Only *D. pulchela* and *B. curtipendula* grass seeds were collected, as these appeared to be the main seeds that were actively foraged by the ants. Soil and seed samples were stored in individual quart sized plastic bags and taken to the laboratory for analysis. Surface soils were collected from the immediate vicinity of each plant being sampled. Soils were collected at a depth of about 2 cm using plastic spoons and stored in individual quart sized plastic bags. These methods were originally used by Barnes (1993) to evaluate the amount of heavy metals in surface soils of the region. Due to the mountainous terrain of the region, soil samples consisted mostly of weathered rock debris, with little organic matter content.

2.3. Sample preparation and analysis

All field samples were frozen at −4 °C within 4 h of sampling and stored for 72 h. Freezing preserved seeds and soil integrity by preventing microbial growth and sacrificed the ants. Samples were then weighed and soil particles attached to ants and seeds were removed using brushes and ultrapure water. Samples were oven dried to constant mass at 40 °C for 48–96 h. Dry masses were recorded and samples were prepared for analyses.

Ants and seeds were prepared for analyses by heat digesting 0.15 g of oven-dried biomass at 50 °C in Teflon bombs with covers using a 2:1 ml mixture of ultrapure HNO_3 and H_2O_2. The remaining residue was then diluted in 10 ml of 2% ultrapure HNO_3 and analyzed for metal concentrations using a Perkin–Elmer Optima 4300 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Perkin Elmer, Shelton, CT). Matrix-matched standards were prepared by diluting multi-element ICP standards and standard reference water (U.S. Geological Survey T-143) was used to check accuracy. Analytical uncertainties using this method were ±5%. The detection limits for each of the metals was 3 ppb, the reporting limits (about 3× the detection limits) were 10 ppb. Hence, values below this cutoff were not reported.

Moisture content of the soil samples was determined by recording wet soil weight (before any drying) and the weight of the soil after the oven drying treatment. Soil samples were digested in triplicate by microwave acid digestion (CEM MarsX, CEM Corporation, Mathews, NC) following a modification of EPA method 3051 (U.S. Government, SW-846). We added 0.25 g of soil to 5 ml of ultrapure HNO_3, and the solution was heated for 20 min at 180 °C. Samples were diluted to 50 ml

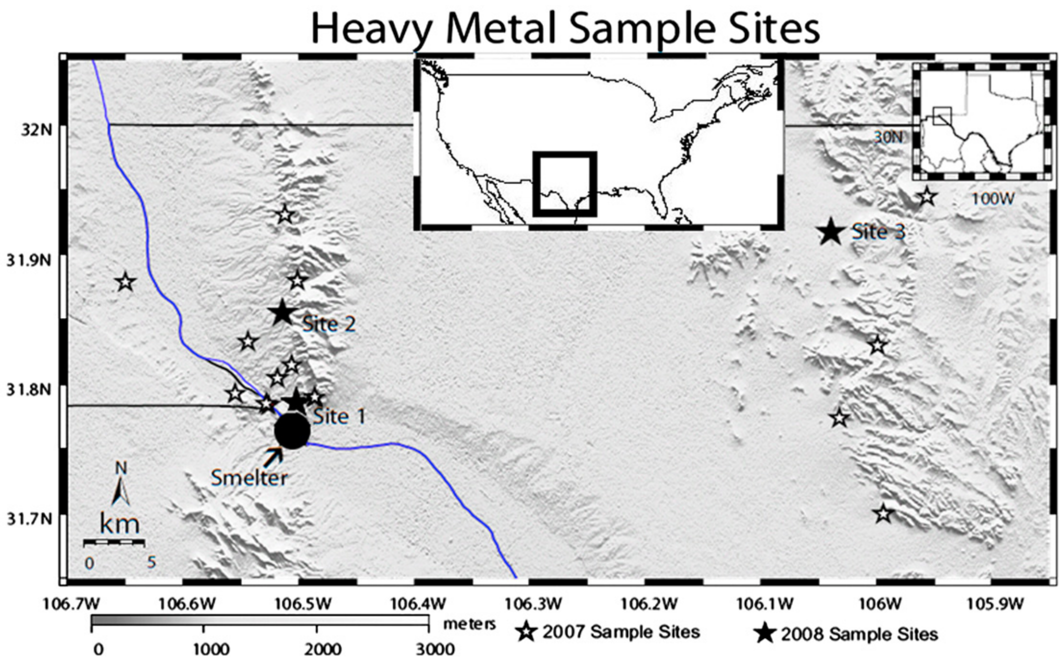

Fig. 1. Distribution of study sites. Open stars represent the 2007 sample sites and closed star symbols are the three sites revisited in 2008 (i.e. sites that were also sampled in 2007) for bioaccumulation analysis. Site 1 = high contamination site, site 2 = medium contamination site and site 3 = low contamination site. The circle is the site of the ASARCO Cu/Pb smelter which operated from 1911 to 1999. The scale shows altitudinal relief in meters of the study region.

with deionized water and centrifuged at 5000 g for 5 min to pellet undigested soil (primarily silicates from sandy soils common to the study sites). Approximately 10 ml of the supernatant was transferred to a clean centrifuge tube and analyzed directly on the ICP-OES. To determine concentrations of metals below the detection limit of the ICP-OES, an additional ten-fold dilution of the supernatant was made for analysis with a Perkin–Elmer Inductively Coupled Plasma Mass Spectrometer (ICP-MS Perkin Elmer ELAN DRC II, Shelton CT). Additionally, we used the USGS Field Leach Test (USGS FLT) (Hageman, 2007) to evaluate the quantities of easily leachable metals in the soils at each site and evaluate pH. Extractions were analyzed directly using the ICP-MS. For each batch of soil samples processed, at least two method blanks were carried through the entire sample preparation and analytical procedures.

Internal standards and laboratory blanks were added to check for instrument performance and to maintain quality control. Analytical uncertainties on the ICP-MS were also ±5%. The soil samples digested in triplicate showed consistent results, with variability between replicates generally <10%. The variation between the field sites was much greater than the variation between the replicate digests (the analytical uncertainties). In all tables and figures we report the mean metal concentration of the replicate field samples. Standard deviation bars are based on these replicated field samples and not on the analytical uncertainties.

We determined that the data was normally distributed for each metal using a D'Agostino–Pearson test for normal distribution. Using the 2007 metal concentrations from ants, logarithmic regression analyses were used to explore the relationship between mean metal concentration in ants and the distance from the smelter (Fig. 2). Mean metal concentrations in ants, seeds, and soils were each compared using a single factor ANOVA. For all ANOVAs used to analyze the 2007 ant data, degrees of freedom were always 16 ($n = 17$). For the ANOVAs used to analyze the 2008 data for soils, seeds and ants, degrees of freedom were always 13 ($n = 14$) A significant difference of means value was established at $p \leq 0.05$. The Z-test was used to derive 95% confidence intervals for all bioaccumulation factors. Additionally, Tukey tests along with every ANOVA were performed to determine specific differences in metal concentrations between site means.

Bioaccumulation factors (BAFs) were calculated as the ratio of metal concentrations in ants to the metal concentrations in seeds (compared on a dry mass basis). All metal concentrations in ants were compared to metal concentrations in seeds using a single factor ANOVA to determine if a significant difference existed between producers and consumers. Site specific BAFs were calculated individually for each metal of interest at sites 1, 2 and 3. The BAFs reported in this work are simply used to quantify the bioaccumulation trend across two trophic levels and varying degrees of environmental contamination and do not have implications for hazard assessment of each metal.

3. Results and discussion

3.1. Heavy metal distribution Rough Harvester ants

We observed a clear contamination gradient for As, Cd, Cu, and Pb in Rough Harvester ants, with the concentrations of these elements decreasing logarithmically with distance from the former smelter (Fig. 2) in 2007. All metal concentrations of ants sampled in the 2008 field season were not significantly ($p > 0.05$) different than those identified in 2007. Metal concentrations from highest to lowest were typically Zn > Cu > Pb > As > Cd. Some of the variation in metal concentrations of the Franklin Mountain sites may be attributable to their proximity to the urban areas of the city of El Paso where a variety of industrial and vehicle metal sources exist. Zn concentrations were not correlated with increasing distance from the smelter. Previous works suggest that Zn is often regulated within narrow ranges in ants (Grzes, in press) and typically Zn bioaccumulation shows an inverse relationship with exposure level (reviewed in McGeer et al., 2003). Therefore the Zn BAFs presented in Table 1 may only relate to the range over which internal Zn concentrations are regulated in *P. rugosus* and do not reflect implications for hazard assessment.

Contamination gradients (away from point sources of contamination) in ants have been observed in temperate forested regions

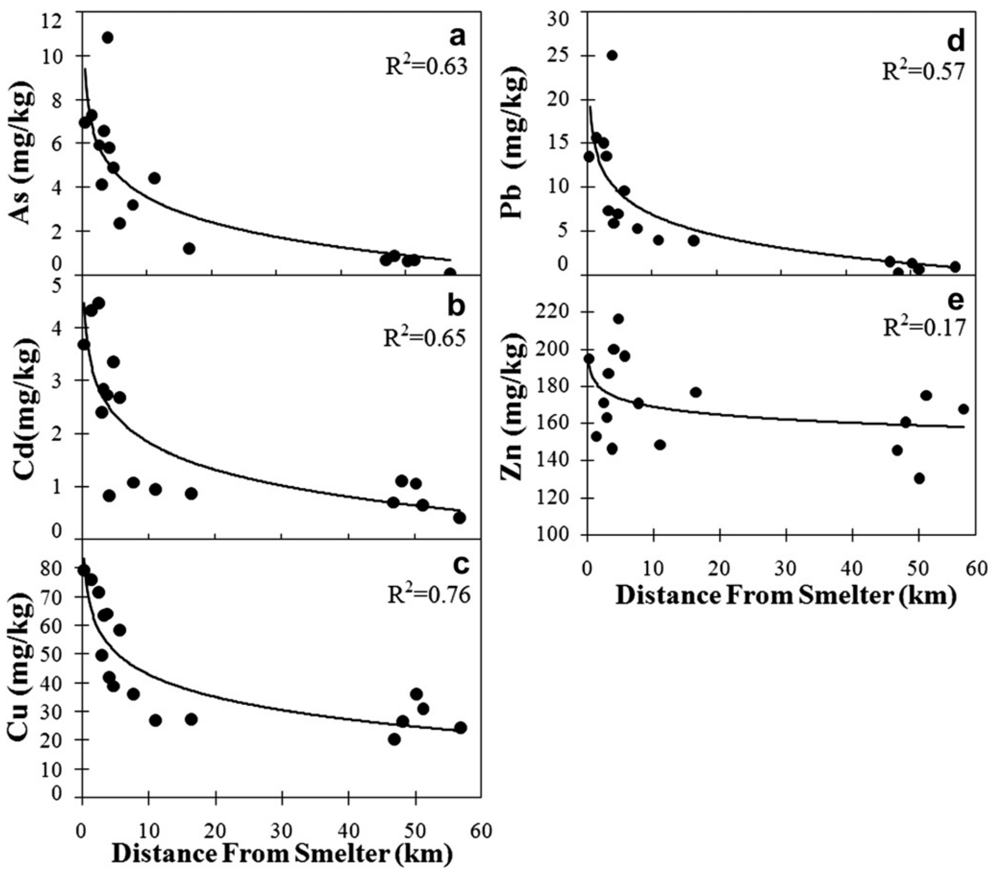

Fig. 2. Concentration of As (a), Cd (b), Cu (c), Pb (d) and Zn (e) in Rough Harvester ants (*Pogonomyrmex rugosus*) as a function of distance from the site of the former Cu/Pb smelter determined from the 2007 samples ($n = 17$). Line show is the best-fit non-linear (logarithmic) regression.

Table 1
Bioaccumulation factors (BAFs) for Rough Harvester Ants in desert soils and 95% confidence intervals at high, medium and low Contamination sites. *Note that a BAF was not calculated for As in Medium and Low Contamination sites, as As concentrations were below detection limits in these samples. BAF are determined as the ratio of metal concentration in ants to the metal concentrations in seeds.

Individual Site BAF	As	Cd	Cu	Pb	Zn
Site 1 Mean	1.04	11.74	2.85	2.85	3.84
Site 1 95% C.I.	0.35–1.73	0.83–22.65	1.62–5.72	0.58–5.11	1.90–5.79
Site 2 Mean	–	6.80	3.75	5.53	2.92
Site 2 95% C.I.	–	6.38–7.22	3.02–4.47	3.75–7.31	1.85–3.98
Site 3 Mean	–	6.28	4.01	4.77	5.20
Site 3 95% C.I.	–	2.35–10.21	2.75–5.27	−1.11 to 10.64	2.44–7.95

for As, Cd, Cu, and Pb (Eeva et al., 2004; Rabitsch, 1995); we identify a similar pattern with Rough Harvester ants in a desert region. However, most studies with ants have only considered the impact of metal pollution on their abundances and diversity and little is known about how the metals might impact higher-level trophic systems which may be a topic of critical future investigation. For example, Bengtsson and Rundgren (1984) report an inverse relationship between the abundance of *Myrmica ruginodes*, a secondary consumer in temperate ecosystems, and soil metal concentrations. In Bengtsson and Tranvik (1989) critical levels of Cu, Pb and Zn that impact ant abundance are identified for ants from the Bengtsson and Rundgren (1984) study. A comparison of our results with these values shows that metal concentrations in Rough Harvester ants are an order of magnitude lower than the minimum critical concentration needed in ants to impact their abundances.

As in all ants, Rough Harvester ants have a complex social structure which provides the potential for further investigation on the impacts of heavy metals on the multiple castes of a colony. Larvae, pupae and reproductive castes all can occur simultaneously within a colony at various times of the years and each can be exposed to varying levels of heavy metals. Migula et al. (1993) show that metal concentrations in larvae can be significantly different than the foraging workers of the same colony. An analysis of metal concentrations in Rough Harvester ant larvae may reveal that toxic metals accumulate in different rates and may impact various physiological processes such as energy production (as observed in Migula et al., 1993).

3.2. Heavy metal distribution and bioavailability in soils

Among the three sites studied for bioaccumulation trends (all completed in the 2008 sample season), metal concentrations in the soil samples were significantly higher (Tukey test, $p < 0.01$ for all metals, $df = 13$) at site 1 (i.e. closest to the smelter) than those at site 2 (intermediate distance), and the lowest concentrations of metals were found in site 3 (Fig. 3). However, As levels at sites 2 and 3 did not statistically differ from each other ($p = 0.064$). At these three sites metal concentrations in soils were typically distributed from highest to lowest as Cu > Zn > Pb > As > Cd. This pattern differs slightly from the pattern found in the ants, in that Zn was present in the ants at higher concentrations than Cu. The pattern of our data

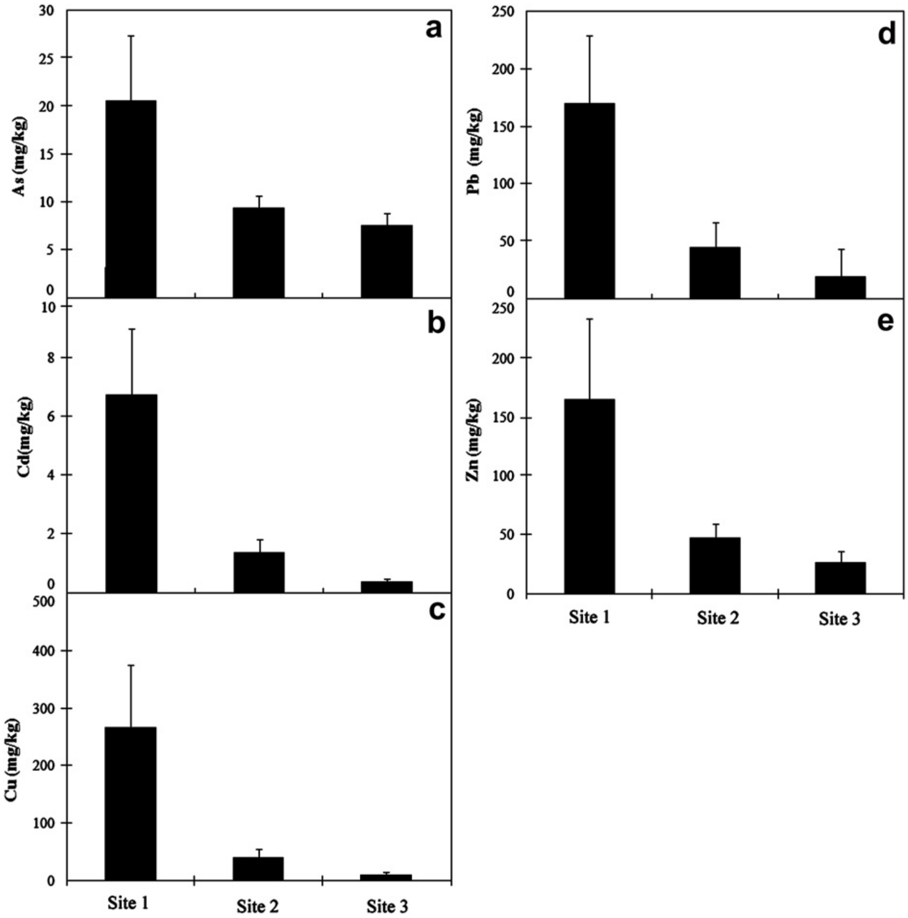

Fig. 3. Concentration of As (a), Cd (b), Cu (c), Pb (d) and Zn (e) in soils at high (Site 1), medium (Site 2), and low (Site 3) contamination sites determined from the 2008 samples ($n = 14$). Error bars indicate one standard deviation from the mean concentration.

supports the findings of McGeer et al. (2003) which suggest that Zn is a critical element for multiple organisms required for critical physiological processes and therefore is often regulated within very narrow ranges (typically by excreting excess metal concentrations). Furthermore, elevated Zn concentrations have been shown to influence the developmental processes of ant larvae by having larger larvae in more polluted sites (Grzes, in press). Other metals like Cu may have a similar regulatory mechanism in terrestrial arthropods but this trend is not strongly supported by our data.

Easily leachable metal quantities derived from the USGS Field Leach Test ranged from 0.171 to 0.701 mg/kg for As, 0.00 to 0.005 mg/kg for Cd, 0.023 to 0.45 mg/kg for Cu, 0.003 to 0.117 mg/kg for Pb and 0.00 to 0.079 mg/kg for Zn. Arsenic, Cd, Cu and Pb were significantly higher at site 1, while Zn was significantly higher at site 3 ($p < 0.05$). At the investigated sites soil pH (measured using the USGS FLT) ranged from 7.6 to 8.9 and there were no significant differences in pH levels between sites 1, 2, and 3 when compared using a one-way ANOVA ($p > 0.05$, df = 13). Mean soil pH at site 1 and 2 was 8.5, at site 3 pH was a slightly lower (8.2). In all cases soil moisture was less than 3% and moisture levels were not significantly different between sites ($p > 0.05$, df = 13).

Metal concentrations for digested soil samples are an order of magnitude lower than those reported by Bengtsson and Rundgren (1984) for heavy metal concentrations in the polluted temperate forests of Gusum, Sweden; a region subjected to heavy metal contaminants as a result of brass milling activities. However, in the Bengtsson and Rundgren (1984) study, similar metal accumulation trends were observed in ants. We suggest that at least part of this difference can be attributed to the alkalinity and aridity of desert soils, which may reduce the mobility and bioavailability of heavy metals (Harter, 1983; Martinez and Motto, 2000). Additional uncertainty may be explained by variation in the dietary requirements and foraging preferences of Rough Harvester ants.

Less attention has been paid to heavy metal distributions in deserts than other ecosystems such as forests. Previous works completed in the Chihuahuan Desert (Barnes, 1993; Pingitore et al., 2005) sampled surface soils in the general vicinity (<1 km) of sample sites 1 and 2 of our current study. These investigations were conducted in 1991–1992, while the smelter was still operational (the smelter ceased all operations in 1999). A comparison of our results to the Barnes (1993) and Pingitore et al. (2005) studies, which used similar analytical methods, suggests that metal concentrations in near-surface soils closest to the smelter have decreased approximately one order of magnitude since the early 1990s. It is likely that heavy metal concentrations in the surface soils of the Franklin Mountain study area decreased during the decade-long period of inactivity of the smelter between these sampling events. Similar decreasing heavy metal concentrations in surface soils after periods of smelter inactivity in Austria were noted by Rabitsch (1995). We speculate that the metals were washed away to some extent during surface runoff but that they also may have migrated downward in the soils. This second possibility is a subject for further investigation.

3.3. Bioaccumulation of heavy metals in seeds and granivorous ants

Arsenic, Cd, Cu, Pb and Zn were present in higher concentrations in the primary consumer (ants) trophic level than in the producer (seeds) trophic level (Fig. 4). In the seeds, As, Cd, Cu and Pb concentrations were significantly higher at the high contamination site (site 1) when compared to the medium (site 2) and low (site 3) contamination sites ($p < 0.001$ for As, Cu and Pb and $p = 0.004$ for Cd). In ants, As, Cd, Cu and Pb were significantly higher at site 1 than in site 2 or 3 (all $p < 0.001$). Although Zn concentrations were significantly higher in the ants than in the seeds, Zn showed little differences between sites for either ants or seeds (Fig. 4). Results

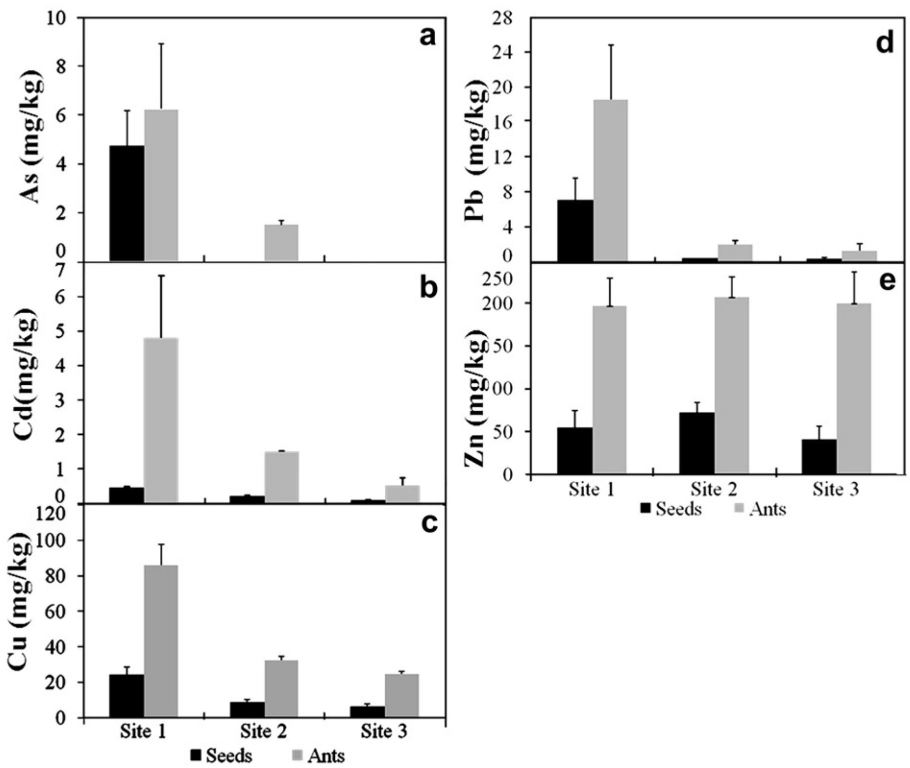

Fig. 4. Concentration of As (a), Cd (b), Cu (c), Pb (d) and Zn (e) in seeds (black bars, $n = 14$) and ants (grey bars, $n = 14$) at high (Site 1), medium (Site 2) and low (Site 3) contamination sites determined from the 2008 samples. Error bars indicate one standard deviation from the mean concentration.

from the present study establish a clear relationship between Harvester ants and seeds; however we cannot be sure this relationship holds for seeds other than those measured (*B. curtipendula* and *D. pulchela*). In fact, the variation in BAFs calculated for different sites (Table 1) may be partially attributed to the ant's dietary variation. It is important to note that *P. rugosus* is not limited to foraging on the two species of grasses that we sampled, so additional contaminated food sources remain unidentified. This suggestion is supported by earlier investigations where Calderon et al. (1999) and Mackay et al. (1999) analyzed the distribution of heavy metals in *B. curtipendula* and *Dasyochola pulchellum* in the El Paso region. They found that both species accumulated metals, but that *B. curtipendula* accumulated slightly lower concentrations of Cd, and Pb than *D. pulchellum*. Concentrations of heavy metals in the seeds collected in the present study are approximately one order of magnitude lower than the metal concentrations reported by Calderon et al. (1999) and Mackay et al. (1999) for As, Cd, Cu and Pb (neither study considered Zn). This same pattern was observed in the digested soil samples (discussed above) of the study region and supports our interpretation that near-surface metal concentrations have decreased over the last decade since the smelter closure in 1999.

Although a bioaccumulation trend does exist for all sites and all metals tested, there is variability in the BAFs among sites and metals (Table 1). It appears that Cd has the highest BAFs for all sites (average of 8.12×, Table 1), while Cu, Pb, and Zn show BAFs for each site of around 4×. Arsenic has the lowest BAF of approximately 1× (for the high contamination site only, as As was below detection in the other sites). Hunter et al. (1987) report similar heavy metal bioaccumulation factors in herbivorous insects (including ants) of 3–5× for Cd and 2–4× for Cu. In both cases Cd accumulates at higher rates than Cu, which may offer support for our suggestion that ants may have a physiological homeostatic control for the concentration of Cu (but not Cd). Elevated levels of Cd, Pb, and As are known to negatively influence physiological processes in plants (Mackay et al., 1999), but less is known about their impact on higher trophic levels of desert ecosystems. Additional work is needed to address this question and to assess the impact of metals on secondary consumers in the Chihuahuan Desert that are generally dependent on harvester ants as prey (e.g. horned lizards).

4. Conclusions

We show that granivorous ants, which are abundant in arid ecosystems, accumulate heavy metals from the seeds which they consume. In some cases the ants may be able to regulate the concentrations of metals like Zn in their bodies (a subject for further investigation). We also demonstrate that the distribution of As, Cd, Cu and Pb in soils, seeds and ants significantly correlates with the distance from a closed Cu and Pb smelter, the suspected point source for heavy metals in this study. We suggest that heavy metal concentrations have decreased an order of magnitude in near-surface soils, plants, and ants over the decade-long period of inactivity at the copper smelter. The consequences of these elevated metals on organisms in arid ecosystems are unclear; however, keystone species such as harvester ants are good indicators of multiple trophic level interactions and key ecosystem processes. Their abundance in arid environments makes Rough Harvester ants ideal biological indicators of heavy metals contamination in said ecosystems. This work can serve as a foundation for additional work focused on understanding the links among the metals, ants, and health of the larger-scale ecosystem. These links are necessary for a rigorous risk assessment of metal contamination in deserts.

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