Bull Environ Contam Toxicol (2016) 97:44–49
DOI 10.1007/s00128-016-1826-3

Assessment of Trace Metals in Soil, Vegetation and Rodents in Relation to Metal Mining Activities in an Arid Environment

Lia C. Méndez-Rodríguez[1] · Sergio Ticul Alvarez-Castañeda[1]

Received: 16 October 2015 / Accepted: 11 May 2016 / Published online: 20 May 2016

Abstract Areas where abandoned metal-extraction mines are located contain large quantities of mineral wastes derived from environmentally unsafe mining practices. These wastes contain many pollutants, such as heavy metals, which could be released to the environment through weathering and leaching, hence becoming an important source of environmental metal pollution. This study evaluates differences in the levels of lead, iron, nickel, manganese, copper and cadmium in rodents sharing the same type of diet under different microhabitat use in arid areas with past mining activities. Samples of soil, roots, branches and seeds of *Palo Adán* (*Fouquieria diguetii*) and specimens of two rodent species (*Chaetodipus arenarius* and *C. spinatus*) were collected in areas with impact from past metal mining activities as well as from areas with no mining impact. Both rodent species mirrored nickel and iron levels in soil and seeds, as well as lead levels in soil; however, *C. arenarius* accumulated higher levels of manganese, copper and cadmium.

Keywords Heavy metals · Rodent · *Fouquieria diguetii* · *Chaetodipus arenarius* · *Chaetodipus spinatus*

A key route of exposure of animals (including humans) to metals involves the uptake of these chemicals from soil by plants, with the subsequent transfer to higher trophic levels. Areas where abandoned metal-extraction mines are located contain large quantities of mineral wastes derived from environmentally unsafe mining practices conducted over a long period of time. These wastes include many pollutants that are released to the environment through weathering and leaching, becoming an important source of environmental metal pollution (Marguí et al. 2007).

The toxicity of metals will depend on their concentration and bioavailability, which in turn will depend on the chemical moieties present—free metal ions, soluble metal complexes (sequestered to ligands), exchangeable metal ions, organically bound metals, precipitated or insoluble compounds such as oxides, carbonates, hydroxides, among others—in soil (Leyval et al. 1997). The complexity involved in evaluating the bioavailability of metals in soil makes it easier to assess metal bioavailability through analyses of metal content in plant and animal tissues. Therefore, while the analysis of metals in soil allows assessing the history of an area, testing for metals in living organisms provides information on metal bioavailability. In the case of plants, they give information on the bioavailability of elements in the soil of their rooting zone whereas for small mammals, they are useful for a larger area (Mertens et al. 2001). In addition, metal toxicity depends on biological traits of plants and animals (e.g. biosorption, bioaccumulation, and even physiological features). In organisms, the concentration of a given metal may be insufficient to cause death (i.e., sublethal) but sufficiently high to disrupt physiological pathways associated with reproduction or susceptibility to disease (Matzuk and Lamb 2002).

This study aims to assess the differences in trace metal buildup rates between two rodent species that share a similar diet under different conditions of microhabitat use in arid areas. The differences in concentrations levels of trace metals between soil and various parts of plants (root, branches and seeds) that are a source of trace metals for

✉ Sergio Ticul Alvarez-Castañeda
sticul@cibnor.mx

1 Centro de Investigaciones Biológicas del Noroeste, S.C. Instituto Politécnico Nacional, 195, Playa Palo de Santa Rita, 23096 La Paz, Baja California Sur, Mexico

both species of rodents were also evaluated. Both aspects were investigated within the same arid areas, in sites with and without records of previous mining activities.

Materials and Methods

The study was conducted in the southern part of the Baja California peninsula, in two adjacent areas separated by a mountain range 20 km wide (Fig. 1). One study area was located in Los Planes basin. There mining operations dealt mainly with gold and silver extraction, and were undertaken since the second half of the 18th century (Carrillo-Chávez et al. 2000). Minerals were extracted mainly through burning and smelting that produced approximately 2500 tons of lead, among other elements (Consejo de Recursos Minerales 1999). As byproducts of these operations, between 800,000 and 1,000,000 tons of mining waste are estimated to be scattered across 350–400 km^2 in Los Planes basin (Carrillo-Chávez et al. 2000). These wastes are spread by wind and rain and then covered with vegetation that is consumed as food by the local fauna. In Los Planes basin, three stations were sampled: two, in sites with records of past gold and silver mining [San Antonio (A; 23.7875°, −110.0539°) and Ensenada de Muertos (E; 23.9911°, −109.8348°)], and one located about 10 km apart from the others, in a site with no records of mining activities (El Sargento, S; 24.0962°, −110.005°). The second study area was located in La Paz Basin, 40 km north, in a site with no previous metal mining activities (Brisamar, B; 24.1755°, −110.5131°).

Samples of soil, roots, branches and seeds of *Palo Adán* (*Fouquieria diguetii*)— an abundant plant species found in the four sites studied and used as food by the local fauna (Armenta-Quintana et al. 2011) —were collected. Soil samples were taken from the area surrounding the roots of each plant at a maximum sampling depth of 20 cm using plastic tools. All samples were collected in new plastic bags to avoid any potential contamination.

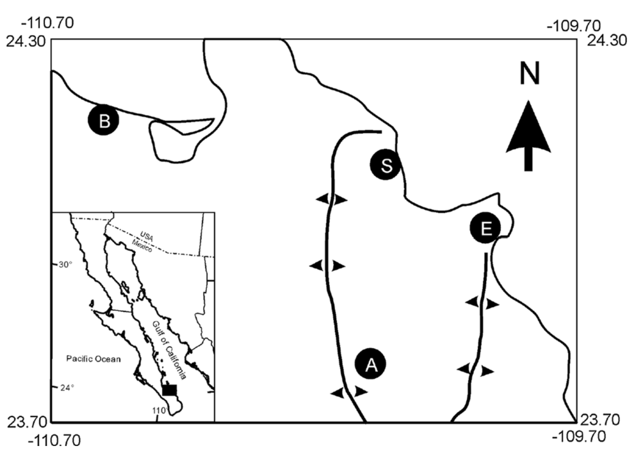

Fig. 1 Sites sampled in Baja California Sur, México. *B* Brisamar, *E* Ensenada de Muertos, *S* El Sargento, *A* San Antonio

Rodent specimens (n = 79) were captured using standard Sherman live traps placed in the same area where plant samples were collected. The study focused on two rodent species, namely *C. arenarius* and *C. spinatus*, because of the heterogeneous soil texture in the areas studied. Although both species belong to the same genus and have similar habitat restrictions, *C. arenarius* is mainly associated with sandy areas and *C. spinatus* with stony areas. The average age of specimens collected ranged between 6 and 8 months, based on molar wear. Specimens of *C. spinatus* (n = 45) were collected in Ensenada de Muertos (n = 18); San Antonio (n = 16) and in El Sargento (n = 11). Specimens of *C. arenarius* (n = 34) were collected in Ensenada de Muertos (n = 18); El Sargento (n = 6) and Brisamar (n = 10). No specimens of *C. arenarius* were collected in El Sargento because the area is mostly stony and this species is only found in sandy areas.

In the laboratory, soil samples were air-dried at room temperature for one week and then screened through a non-metallic sieve with 2-mm mesh size to remove stones, plants and animal debris. Roots, branches and seeds were carefully washed with deionized water and oven-dried at 70°C. In rodents, the liver was tested for trace metals, since this organ has proved to reflect differences in metal content between small mammals living in different habitats (Wren 1986). Individual livers were dissected, weighed and oven-dried at 70°C. Then, each soil sample (0.5 g) was subjected to acid digestion with 10 mL deionized water, 6 mL nitric acid and 3 mL hydrofluoric acid, while plant and rodent samples were separately transferred to acid-washed Teflon tubes and digested with concentrated HNO_3:$HClO_4$ (3:1) (both acids, TJ Baker analytical grade). All digestions were performed in a microwave oven (CEM model Mars5X, Matthews, NC). Afterwards, all samples were dissolved in 1 mL concentrated HCl and 24 mL deionized water in a volumetric flask (Méndez and Alvarez-Castañeda 2000). Levels of lead (Pb), iron (Fe), nickel (Ni), manganese (Mn), copper (Cu) and cadmium (Cd) were analyzed in samples using an atomic absorption spectrophotometer (GBC Scientific equipment, model AVANTA, Dandenong, Australia) with an air-acetylene flame. Certified standard reference materials TORT-2, MESS2 and ALGAE (National Research Council of Canada, Ottawa) were used to check accuracy. Analytical values were within the range of certified values. Recovery of metals was greater than 95 %. Metal concentrations were expressed as $\mu g\ g^{-1}$ dry weight. Metal concentrations reported in other studies in wet weight (ww) basis, were converted to dry weight multiplying them by 3.5 (Talmage and Walton 1991). Detection limits were Pb, 0.074 $\mu g\ g^{-1}$; Fe, 0.65 $\mu g\ g^{-1}$; Cu, 0.017 μg g^{-1}; Mn, 0.020 μg g^{-1}; Ni, 0.030 μg g^{-1}; Cd, 0.017 μg g^{-1}.

Normality and homogeneity of variances were determined using the Shapiro–Wilk and Barlett tests, respectively. Due the metal concentrations show a non-normal distribution and non-homogeneity of variance, non-parametric statistics were employed. Differences between each type of sample or across sites were estimated using Kruskal–Wallis tests using multiple comparisons of mean ranks for all groups (Zar 1999). The analyses were carried out using STATISTICA 8.0 (StatSoft Inc). Differences were considered significant when $p \leq 0.05$.

Results and Discussion

Metal bioavailability depends on several soil properties (pH, organic matter content) (Zhang et al. 2014). These properties influence the chemical moieties available and their accumulation and transfer along the trophic web. Soil in the study area showed a pH > 7 and predominantly sandy-loam and loamy-sand textures (Carrillo and Drever 1998). For soil of this pH value, the European Commission (EC) recommends the following maximum limits: Pb, 100 μg g^{-1}; Ni, 70 μg g^{-1}; Cu, 100 μg g^{-1}, and Cd, 1.5 μg g^{-1} (Gawlik and Bidoglio 2006). The levels recorded in this study for all soil samples were below the EC limits (Table 1). In general, Fe and Mn are not reported in any guidelines or studies on heavy metal content in soil, as these two elements are not deemed pollutants. Instead, these are classified as essential crop micronutrients due to their importance in plant nutrition (Micó et al. 2006). Normal levels in soil are Mn concentrations of 850 μg g^{-1} and Fe levels ranging from 1000 to 100,000 μg g^{-1} (Alloway 2012). Manganese and Fe levels of 225 and 1700 μg g^{-1} (45 and 340 μg·g^{-1}, fresh weight), respectively, are found in edible parts of plants growing in areas with pollutant-free soil and water (Arora et al. 2008). Besides their direct importance as nutrients, Fe and Mn oxides, reduce Cd, Ni and Pb uptake from soil to plants, hence restraining the entry of the latter into the food chain (Mench et al. 1994; Alloway 2012). This fact is important in this study because Los Planes basin is an area with high natural iron levels (ferrihydrite), associated with other elements in several minerals (Carrillo and Drever 1998).

The San Antonio mining area showed the highest Pb and Cd concentrations in soil (Table 1). Reports of trace metal content in ashes from the walls of old roasters where ores were burned for gold extraction in the study area reveal Pb and Cd levels of up to 29,363 and 203 μg g^{-1}, respectively (Acosta et al. 2001), but the amount of ashes that have been dispersed by wind or rain is unknown.

For plants, the European Commission (2006) has established different maximum levels according to the plant structure (root, fruit or leaf): Cd, between 0.050 and 0.20 μg g^{-1} wet weight (0.25 and 1.0 μg g^{-1} dry weight); and Pb, between 0.10 and 0.30 μg g^{-1} wet weight (0.50 and 1.5 μg g^{-1} dry weight). Concentrations considered as normal in organ plants including seeds are for Pb: 0.5–10 μg g^{-1}; Fe: 30–300 μg g^{-1}; Ni: 0.5–5 μg g^{-1}; Mn: 40–200 μg g^{-1}; Cu: 4–15 μg g^{-1}; Cd: 0.05–2 μg g^{-1} (Vondráčková et al. 2014, Jabeen et al. 2010). For edible plants, WHO/FAO have published maximum recommended limits for Cu and Ni of 15 and 8.15 μg g^{-1} (3.0 and 1.63 μg g^{-1} fresh weight), respectively, (Jabeen et al. 2010). According to our findings, some of these recommended upper limits were exceeded in roots, especially in plants from Los Planes basin (Table 2).

In plants, complex physiological mechanisms regulate the uptake and transport of metals. An example is the performance of chemicals that serve as metal carriers (ZRT1/IRT1-like proteins, also called SLC39) (Guerinot 2000; Eide 2004), which are essential in the uptake of Fe and, to a lesser extent, of other elements such as Zn, Mn, Co and Cd (Korshunova et al. 1999). This system is complex because the uptake of Zn and other non-essential elements such as Cd, enter to plant cells through the same uptake systems used for essential cations such as Fe (Clemens 2006). Based on the current analyses in *F. diguetii,* the general concentration pattern observed for all the metals studied was: roots > branches > seeds (Table 2). The largest reduction in metal concentration along the plant was observed between roots and branches, especially for Fe, which showed a more-than-tenfold decrease, followed by smaller reductions in Pb, Mn and Cd levels; Ni and Cu remained within the same concentration

Table 1 Trace metal levels (μg g^{-1} dry weight, mean ± error standard) in soil from four sampling sites in the southern Baja California peninsula

Site	Pb	Fe	Ni	Mn	Cu	Cd
B	0.41 ± 0.41[a]	16,433 ± 2499[a]	7.0 ± 2.4[b]	294 ± 58[a]	12.02 ± 0.29[b]	<0.017
E	3.98 ± 0.23[b]	22,412 ± 763[ab]	10.0 ± 0.6[c]	143 ± 3[a]	21.30 ± 4.30[c]	0.13 ± 0.60[a]
S	5.24 ± 0.56 [b]	18,579 ± 3866[ab]	2.7 ± 0.3[a]	162 ± 12[a]	0.72 ± 0.12[a]	0.05 ± 0.02[a]
A	7.12 ± 1.94[b]	33,289 ± 5855 [b]	11.5 ± 0.9[c]	355 ± 84[a]	17.62 ± 2[bc]	0.32 ± 0.31[a]

B Brisamar, *E* Ensenada de Muertos, *S* El Sargento, *A* San Antonio. Values followed by different letters within columns indicate significant difference ($p \leq 0.05$)

Table 2 Trace metal levels (μg g^{-1} dry weight, mean ± error standard) in roots, branches and seeds of *Palo Adán* from four sampling sites in southern Baja California peninsula

Site	Tissue	Pb	Fe	Ni	Mn	Cu	Cd
B	Root	0.58 ± 0.57[a]	1018 ± 62[b]	3.99 ± 0.66[a]	58.9 ± 11.4[a]	5.22 ± 1.01[a]	0.34 ± 0.21[a]
E	Root	1.62 ± 0.67[a]	2015 ± 293[b]	1.95 ± 0.85 [ab]	74.0 ± 8.7[a]	6.78 ± 0.95[a]	1.29 ± 0.45[ab]
S	Root	4.28 ± 2.08[a]	2588 ± 1007[b]	<0.05	221.5 ± 59[b]	3.92 ± 1.08[a]	3.14 ± 1.36[b]
A	Root	0.34 ± 0.23[a]	2034 ± 705[b]	2.98 ± 0.66[ab]	50.2 ± 13.0[a]	6.56 ± 1.28[a]	0.92 ± 0.64[a]
B	Branch	<0.074	99 ± 18[a]	3.99 ± 1.72[ab]	15.9 ± 1.52[a]	5.31 ± 1.13[a]	<0.017
E	Branch	0.90 ± 0.43[a]	234 ± 53[a]	1.06 ± 0.69[ab]	23.4 ± 5.35[a]	6.75 ± 0.85[a]	<0.017
S	Branch	0.26 ± 0.22[a]	58 ± 19[a]	1.02 ± 0.51[ab]	15.6 ± 3.26[a]	3.34 ± 1.57[a]	0.13 ± 0.05[a]
A	Branch	2.59 ± 2.08[a]	94 ± 19[a]	0.54 ± 0.21[ab]	11.4 ± 0.76[a]	13.22 ± 2.64[b]	<0.017
B	Seed	0.10 ± 0.10[a]	84 ± 20[a]	2.43 ± 1.14[ab]	22.7 ± 3.3[a]	4.06 ± 0.38[a]	0.12 ± 0.12[a]
E	Seed	3.31 ± 2.50[a]	145 ± 39[a]	0.88 ± 0.41[ab]	19.5 ± 4.0[a]	5.22 ± 0.51[a]	<0.017
S	Seed	1.31 ± 0.50[a]	48 ± 7[a]	0.08 ± 0.08[b]	21.7 ± 3.0[a]	1.35 ± 0.37[a]	0.02 ± 0.02[a]
A	Seed	0.84 ± 0.74[a]	46 ± 8[a]	1.52 ± 1.39[ab]	15.6 ± 1.8[a]	10.0 ± 1.6[ab]	<0.017

B Brisamar, *E* Ensenada de Muertos, *S* El Sargento, *A* San Antonio. Values followed by different letters within columns indicate significant difference ($p \leq 0.05$)

range. This pattern is in agreement with the trend observed for many metals to become immobilized in roots and other underground storage tissues and undergo limited translocation to aboveground structures (Korshunova et al. 1999; Gall et al. 2015).

Rodents of the genus *Chaetodipus* are found in underground burrows in arid chaparral areas, where they are able to survive without drinking water because they can metabolize water from food (MacMillen and Hinds 1983). Therefore, metal levels in their tissues reflect the content of these elements in food rather than in the water they drink.

In mammals, lead concentrations in excess of 4.2 μg g^{-1} (1.4 μg g^{-1}, fresh weight) are considered toxic (Ma 1996). Lead is related to chromosome aberrations (Sang and Li 2005), detrimental effects during embryonic and fetal development, and lower fertility (Domingo 1994). The percentage of rodents collected that recorded Pb levels above this reference limit were Ensenada de Muertos, 47 %; San Antonio, 31 %; El Sargento, 23 %; and Brisamar, 0 %. From these, only in Ensenada de Muertos Pb concentrations over 8 μg g^{-1}—twice the level established as toxic—occurred in 26 % of the rodents tested, with no significant differences between both species. Today, Ensenada de Muertos is an industry-free area, with no current mining activity. Therefore, the sources of lead are unknown. Although the bioavailable fraction of lead could be higher in food consumed by rodents in this area in relation to the other three sites, this element could also be assimilated by rodents through inhalation when digging their borrows. Kålås et al. (2000) found that small herbivorous species can accumulate significant Pb levels through the continued intake of relatively low doses from air pollution; in this case, this could occur when rodents are digging.

Cu levels observed in this work were similar to concentrations in the liver of small mammals (12.9 to 23 μg g^{-1}) from unpolluted areas (Talmage and Walton 1991). Since Cu is well regulated by the body, it is not a candidate for monitoring in small mammals, except at extremely polluted sites, which was not the case in this study (Table 1). Ni, Mn and Cd (Table 3) were also found within the concentration range considered normal in mammals: Ni, 2.00–4.80 (0.50–1.20 μg g^{-1} ww, Torres and Johnson 2001); Mn, 2.92–13.20 μg g^{-1} (0.73–3.30 μg g^{-1} ww, Lewis et al. 2001); Cd, 0.12–1.20 μg g^{-1} (0.03–0.30 μg g^{-1} ww, Torres and Johnson 2001).

Although Mn, Cu and Cd levels recorded are normal in rodents, they were almost twice as high in *C. arenarius* compared to *C. spinatus* (Table 3). Metal bioaccumulation, for example Cu, may also be affected by genetic factors and dietary levels of protein, Cd, Fe, Ag, Zn and Mo, among others, all of which are influenced by diet and life habits (Camakaris et al. 1999; Torres and Johnson 2001). *C. arenarius* lives in an extremely arid region, in habitats characterized by sparse vegetation and soil consisting almost entirely of sand; thus, seeds are this rodent species' main food source. Mounds and burrows of this species were found in streams with sandy substrates (Lackey 1991) where, during the rainy season, water might carry soil with trace metals from mining-waste areas. Moreover, *C. spinatus* usually inhabits rough desert landscapes of boulders, rocky slopes, coarse soil, and sparse vegetation, characteristic of the southern Sonora life area. This rodent's diet consists mostly of seeds, although it also

Table 3 Trace metal levels (μg g^{-1} dry weight, mean ± error standard) in rodents from four sampling sites in southern Baja California Peninsula

Specie	Site	Pb	Fe	Ni	Mn	Cu	Cd
C. arenarius	B	1.81 ± 0.34^{a}	279 ± 32^{a}	0.92 ± 0.22^{b}	6.02 ± 0.27^{a}	9.7 ± 0.65^{a}	0.87 ± 0.09^{a}
C. spinatus	E	5.33 ± 1.20^{b}	504 ± 55^{a}	0.54 ± 0.20^{a}	4.91 ± 0.68^{a}	9.8 ± 1.01^{a}	1.34 ± 0.29^{a}
C. arenarius	E	5.51 ± 1.17^{b}	522 ± 62^{a}	0.11 ± 0.05^{a}	4.76 ± 0.67^{a}	12.3 ± 2.17^{a}	0.98 ± 0.29^{a}
C. arenarius	S	4.92 ± 1.55^{ab}	743 ± 90^{a}	0.17 ± 0.08^{a}	10.8 ± 1.4^{b}	22.9 ± 4.00^{b}	1.72 ± 0.52^{b}
C. spinatus	S	2.73 ± 0.50^{ab}	555 ± 83^{a}	0.06 ± 0.02^{a}	6.41 ± 0.97^{a}	16.8 ± 2.25^{a}	0.32 ± 0.05^{a}
C. spinatus	A	3.11 ± 0.67^{ab}	765 ± 363^{a}	0.04 ± 0.0^{a}	5.06 ± 0.60^{a}	16.0 ± 2.46^{a}	0.76 ± 0.30^{a}

B Brisamar, *E* Ensenada de Muertos, *S* El Sargento, *A* San Antonio. Values followed by different letters within columns indicate significant difference ($p \leq 0.05$)

includes green vegetation when it is available following brief rainy periods. Since water is scarce in its habitat most of the time, this rodent uses water contained in food (Linzey et al. 2008). Therefore, it is less likely to take up trace elements form water or air when digging, relative to *C. arenarius*.

The present study demonstrates that *Fouquieria diguetii* is a suitable plant for assessing the mobility of metals from soil to plants and then to animals, although different trends in metal concentrations were recorded. Particularly, Fe showed a tenfold decrease between soil and roots; once in the plant, Fe again showed a tenfold decrease between roots and the rest of the plant. Similar to all other elements, Fe is found in branches and seeds at concentrations that were similar in almost all the areas studied. The two *Chaetodipus* species studied showed that, in spite of living in arid environments, they can display different metal accumulation patterns. *C. arenarius*, a rodent associated with sandy areas, tends to accumulate higher Mn, Cu and Cd levels than *C. spinatus,* which prefers stony habitats. The significantly higher Mn, Cu and Cd levels recorded in *C. arenarius* also coincide with the highest levels of these elements in roots. In arid areas where water is scarce, rodents of the genus *Chaetodipus* are suitable indicators of metal levels.

Acknowledgments The authors thanks to B. Acosta-Vargas and G. Peña-Armenta for their technical support, Ma. Elena Sánchez-Salazar for the English edition, and the Funding provided by the Centro de Investigaciones Biológicas del Noroeste, S.C (P.C.0.5) and the Consejo Nacional de Ciencia y Tecnología (CONACyT-151189).

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