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Heavy metals accumulation in the subterranean rodent *Ctenomys talarum* (Rodentia: Ctenomyidae) from areas with different risk of contamination

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Abstract. The objective of this work was to determine the concentrations of four heavy metals (Pb, Zn, Fe and Cu) in muscle and liver from the solitary subterranean rodent *Ctenomys talarum* from natural dunes, cultivated area and military area with the purpose of assessing their levels and their possible effect on the biology of this small mammal. Only Pb in tissues of *C. talarum* from the agricultural zone indicates a risk of toxic effects on the organism. Overall concentrations of heavy metals in soils and tissues of *C. talarum* from all areas are consistent with lightly polluted habitats, although the increments observed in areas under anthropic use suggest for the possibility that carrying out these human activities for long periods of time, or in an intensive way, could exert an evident impact on ecosystems.

Key words: anthropic activities, bioaccumulation

Introduction

Sensing pollution through the accumulation of contaminants and their effects on wild animals is crucial for the evaluation of environmental quality and to progress in the understanding of the tolerance capacity of natural populations to contamination (Sánchez-Chardi et al. 2007).

Several studies have shown that the concentrations of heavy metals in vegetation and natural rodent populations are generally correlated with environmental pollution (Hunter et al. 1989, Ma et al. 1991, Mertens et al. 2001, Mažeikytė & Balčiauskas 2003, Sánchez-Chardi et al. 2007). However, the information about environmental contamination provided by both of these sources differs. While plant analyses give primarily information about the presence of contaminants in the rooting zone (although contaminants are also found on the surface of leaves), small mammals respond to larger areas because of their high mobility (Mertens et al. 2001). Therefore, rodents are usually used as indicators of pollution, with elements being determined in either whole body or in specific organs, generally the liver or muscles (Talmage & Walton 1991, Sawicka-Kapusta et al. 1990, 1995, Mertens et al. 2001).

Many terrestrial ecosystems, which include wild populations of small mammals, are usually contaminated with potentially toxic trace elements from the accumulation of agricultural pesticides or fertilizers, or from the disposal on land of industrial or military wastes (Cook et al. 1990). These wastes are usually high in heavy metals which can be taken up by plants and later found in high concentrations in animal tissues, being hazardous for small herbivores, their predators and finally humans. Therefore, knowing the impact of different human activities on natural populations of plants and animals in general, and in small mammals in particular, is of extreme importance for wildlife conservation as well as for site management objectives.

Recent works have focused their attention on the accumulation of heavy metals in some species of

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herbivorous subterranean rodents (da Silva et al. 2000, Šumbera et al. 2003). Since they maintain the same territory during long periods and consume large amounts of vegetation, they become an interesting model to test the contamination levels present in their natural habitats through the determination of heavy metals concentrations in different body tissues.

The genus *Ctenomys* comprises around 60 species of subterranean herbivorous rodents that are distributed in the southern parts of South America; within this genus, one of the best-studied species is *Ctenomys talarum* (tuco-tucos), which inhabits sand dune belts along the coast of Buenos Aires Province, Argentina (Busch et al. 2000). Individuals of this species are solitary and maintain exclusive territories throughout their life, with male mean home-range size of 70 m^2 (Cutrera et al. 2006). These organisms behave as generalists and opportunists because they consume most of the plant species present in their habitat but also change their diet in relation to the availability of them in their microhabitat. In the coastal region of Mar Chiquita (Buenos Aires Province, Argentina) this species inhabits natural dunes and a military area. This military region (CELPA; 37°43'4"S-57°25'35"W, Fig. 1), comprising an area of 1740 ha, was first used in meteorological studies but later reconditioned as a center for experimental rocket launches and bombing zone, and contains residues of military activity. It is known that several heavy metals, including Fe, Zn, Cu and Pb, are used in the production of military equipment, like explosives and munitions (García-Martínez et al. 2001). These metallic residues often persist in the soil for decades. In the surroundings of Necochea (Buenos Aires Province, Argentina, 38°36'46"S-58°49'26"W, Fig. 1), *C. talarum* is found in natural dunes and in cultivated fields. The presence of heavy metals in inorganic fertilizers and pesticides commonly used in agricultural activities is well established (Gimeno-García et al. 1996, de López-Carnelo et al. 1997, Ju et al. 2007).

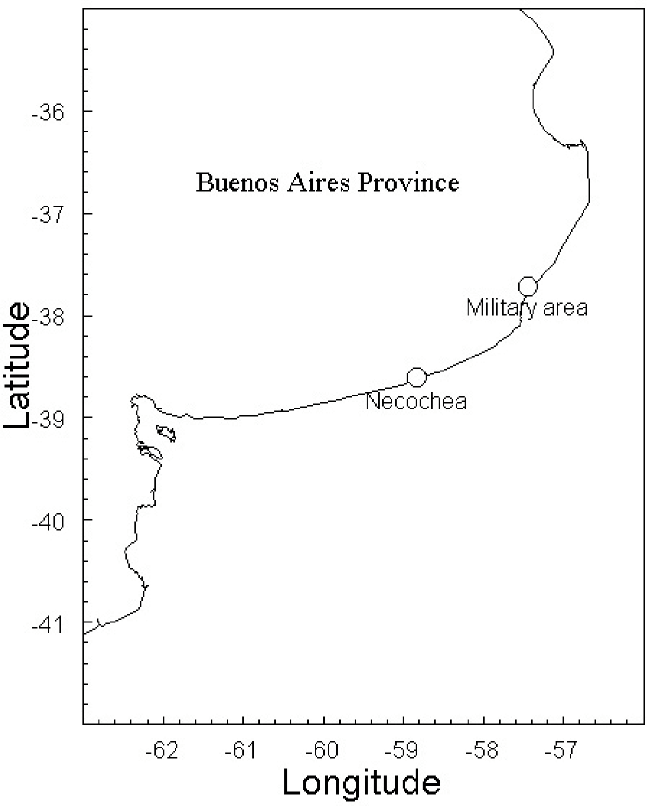

Fig. 1. Map of Buenos Aires Province (Argentina), showing the study places.

Therefore, the objective of this work was to analyze the concentration of heavy metals in the solitary subterranean rodent *C. talarum* from areas which differ in the origin and probably in the levels of these elements. In particular, we determined the concentrations of four heavy metals: Pb, Zn, Fe and Cu in muscle and liver of *C. talarum* from natural dunes, a cultivated area and a military area with the purpose of assessing their concentrations in these regions that differ in the kind of human activity that is carried out, and the possible effect, through the bio-accumulation in different tissues, on the biology and conservation of this species of subterranean rodent. Additionally, the possible role of this genus of subterranean rodent as a widespread environmental pollution indicator is discussed.

Material and Methods

Eighteen adult males from two different populations of *C. talarum* were captured in a natural dune (control; n = 4), a cultivated area (potential source of contamination due to the use of fertilizers and agro-chemicals, n = 3) in Necochea (38º36'S, 58º48'W, Buenos Aires Province, Argentina); and in a natural dune (control, n = 5) and a military area (which presents metal residues, n = 6) situated in the region of Mar de Cobo (37º45'S, 57º56'W, Buenos Aires Province, Argentina) during February-March 2008. It is known that bioaccumulation of heavy metals vary according the sex, size and/or age of the animals (Hunter et al. 1989, Sawicka-Kapusta et al. 1995, Damek-Poprawa & Sawicka-Kapusta 2004). Therefore, we decided to use only adult males that weighed around 140-160 g to avoid differences caused by any of these factors. In the agricultural field, tuco-tucos were only found in the edges of the cultivated area and in very low density. For that reason only three adult males were obtained from this area for heavy metals determination. Both control areas (natural undisturbed dunes) were located near the cultivated and military areas respectively in order to maintain the same environmental conditions between compared zones. Moreover, soil granulometry parameters were similar among contiguous areas. Samples of soil (three from each location, obtained inside the tuco-tucos burrows' area) from the upper layer (0-15 cm) and herbaceous vegetation (samples of the two most abundant plant species at each site that are part of the diet of this subterranean rodent) were also obtained from these areas.

After killing the animals by exposure to halothane, samples of femoral muscles and liver were extracted carefully. Plant samples were thoroughly cleaned with fresh water and then distilled water, for the quantitative removal of soil and strange particles. All bioclasts were removed from the sediment's samples. Before analysis, all samples were dried to constant weight at 60ºC for heavy metal (Pb, Zn, Fe and Cu) determinations. We chose these metals (among others, like Cd and Mn – not detected, and Cr and Ni – detected in very low levels in animal tissues: 0.9-1.2 $\mu g.g^{-1}$ and 2.7-4 $\mu g.g^{-1}$, respectively) because in a preliminary analysis they were found to be present in soils and tissues of tuco-tucos from all studied areas.

Homogenization of each sample was done by crushing in a porcelain mortar. Samples were digested in a mixture of concentrated acids, according to the method described by Dalziel & Baker (1983) and modified by Marcovecchio et al. (1988). Subsamples of 300 ± 50 mg (animals and plants) and 500 ± 50 mg (sediments) were removed, and mineralized with a 1 : 3 perchloric-nitric acid mixture in a thermostatted bath (at 110 ± 10ºC) to minimum volume. Solutions were made up to 10 ml with 0.7% nitric acid. Samples´ digestion was carried out in duplicate to ensure the reproducibility of the method. Heavy metal (Fe, Pb, Zn, Cu) concentrations were determined with a Perkin-Elmer Model 2380 atomic absorption spectrophotometer with an air/acetylene flame. Analytical grade reagents were used to make up the relevant blanks and calibration curves, and the analytical quality (AQ) were tested against reference materials "pond sediments, R.M. Nº2" and "mussel tissue flour, R.M. Nº6" provided by The National Institute for Environmental Studies (NIES) from Tsukuba (Japan). Recoveries were above 90% for all the trace metals measured. The method detection limits (in $\mu g.g^{-1}$) are Pb: 2.15, Zn: 0.88, Cu: 0.77 and Fe: 2.73 for each metal.

Student t-test, paired t-test and Wilcoxon signed rank test (when assumptions were not met), were used to test the null hypothesis of no differences in heavy metal concentrations among individuals from locations under anthropogenic use and their controls, and among different tissues from animals from the same areas. Data are showed as means ± standard deviations, with exception of the vegetation, which is described as a range of the lower and higher values of heavy metals present in the different plant species obtained from each area.

Results

The soils of the analyzed areas differ from their respective controls in the concentration of heavy metals, with Fe concentrations higher in the military and cultivated areas, and Pb – Cu concentrations moderately elevated with respect to their controls (undisturbed natural dunes).

Values of heavy metals obtained after the study of *C. talarum* from control, military and cultivated areas are shown in Fig. 2. Concentrations of heavy metals in *C. talarum´s* tissues from all the areas followed this order: Fe > Zn > Pb ≈ Cu. This pattern was nearly similar to the one observed in the soils' samples, although the concentrations of Zn in soils were lower than in *C. talarum's* tissues and similar to the ones obtained for Pb and Cu in the soils.

Heavy metals concentrations also varied among individuals from different areas. Concentrations of Zn and Cu in tuco-tucos from the military area were higher than the ones obtained from the natural dune (Student's t-test, $p = 0.004$ and $p = 0.006$ for Zn and Cu liver concentrations, $p = 0.006$ and $p = 0.001$ for Zn and Cu muscle concentrations). Concentrations of Pb were lower in the military area (Student's t-test, $p = 0.032$ and $p = 0.008$ liver and muscle concentrations respectively) while there were no significant differences among Fe tissue values in both areas. The situation was different when comparing individuals from the agricultural field against the natural dune. Individuals from the agricultural area presented higher concentrations of Pb and Cu (Student's t-test, $p = 0.05$ and $p = 0.057$ for Pb and Cu liver concentrations, $p = 0.001$ and $p = 0.05$ for Pb and Cu muscle concentrations respectively). Zn and Fe concentrations were also higher in the tuco-tucos from the agricultural area, but only in the liver (Student's t-test, $p = 0.001$ and $p = 0.05$ for Zn and Fe respectively).

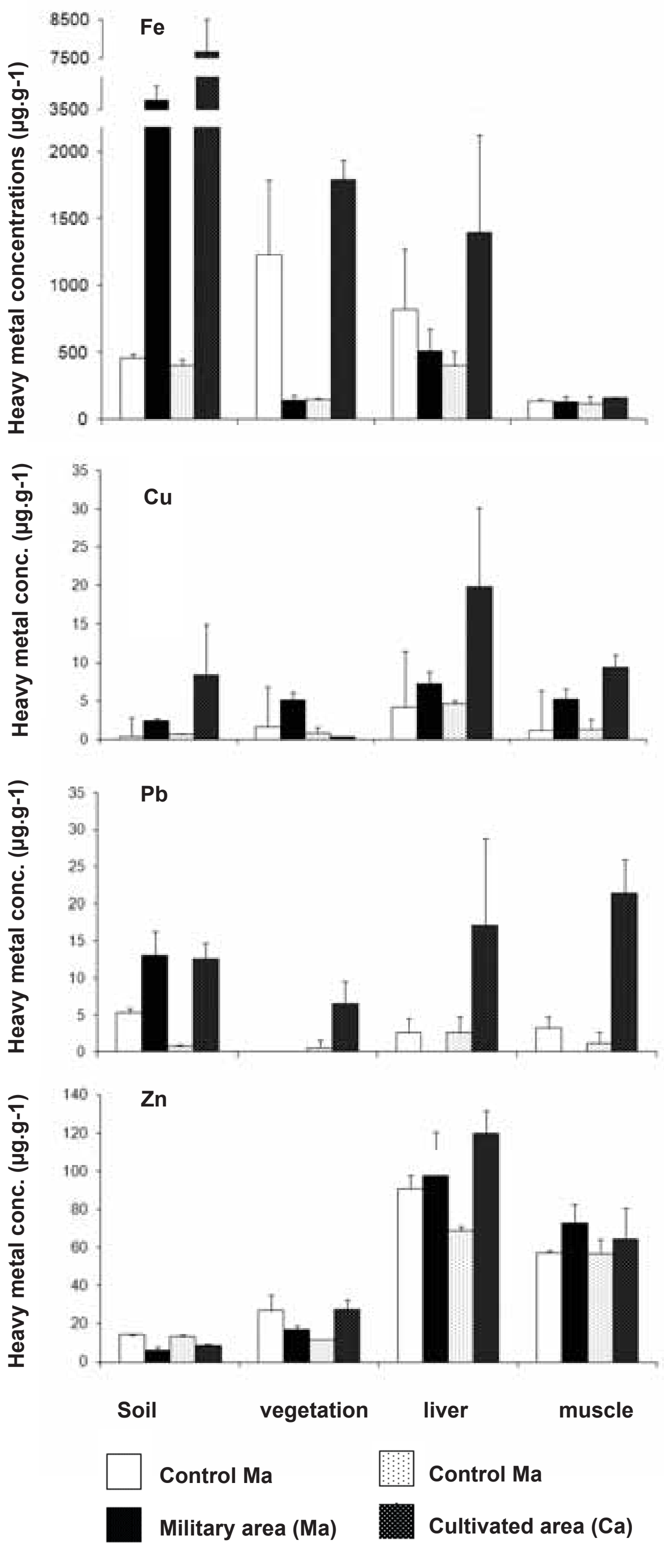

Fig. 2. *Concentrations of heavy metals: Pb, Zn, Fe and Cu, obtained from* C. talarum´s *tissues (liver and femoral muscle), soil sediments and plant tissues from the military and agricultural areas and their respective controls. Data are shown as μg of heavy metal per g of sample (mean ± standard deviation). Heavy metals concentrations in plants are shown as a range of minimum and maximum values.*

Bioaccumulation of these elements was different among *C. talarum's* tissues. While there were no significant differences among the concentrations of Pb in liver and muscle within each area (Wilcoxon signed rank test, $p = 0.118$ and $p = 1$ for control and military areas; paired t-test, $p = 0.261$ and $p = 0.682$ for control and agricultural areas respectively), there were higher concentrations of Zn, Fe and Cu in livers than in femoral muscle within areas (Zn: paired t-test, $p = 0.001$ for military and control area, $p = 0.032$ and $p = 0.03$ for control and agricultural area respectively; Fe: paired t-test and Wilcoxon signed rank test, $p = 0.026$ and $p = 0.031$ for control and military areas; $p = 0.001$ and $p = 0.009$ for control and agricultural areas respectively; Cu: paired t-test, $p = 0.01$ and $p = 0.037$ for control and military areas; $p = 0.025$ for control area of Necochea), except for Cu in the cultivated area (paired t-test, $p = 0.261$).

Discussion

Heavy metals are naturally occurring elements. However, their concentrations in different environments vary, among other things, as a consequence of diverse human activities (Pereira et al. 2006). These activities are responsible for serious impacts on the environment, which most of the times continue for long periods.

Along the Atlantic coast of Argentina, two populations of the subterranean herbivorous rodent *C. talarum* inhabit regions affected by two different human activities (agriculture and military exercises) which were shown to affect the heavy metals' content of soils and vegetation from different locations (Gimeno-García et al. 1996, de López-Carnelo et al. 1997, García-Martínez et al. 2001, Ju et al. 2007). In coincidence with these works, this study showed that the areas under anthropic exploit presented higher contents of heavy metals, mainly Fe and Pb, and, in a lesser degree, Cu. Unexpectedly, these increments were more marked in the agricultural area than in the military area, suggesting a higher level of contamination in this habitat most likely caused by the use of fertilizers and pesticides, which present elevated contents of several heavy metals (including Cu, Zn, Pb, and Fe; Gimeno-García et al. 1996, de López Camelo et al. 1997, Ju et al. 2007). The lower increments in the concentrations of the heavy metals analyzed in the military area could be due to a decline in the number of military tests made in the last decade. Although geographically distant, the heavy metal profiles of both natural dunes used as controls were similar.

High concentrations of heavy metals have been found in wild mammals inhabiting polluted areas (Ma et al. 1991, Mertens et al. 2001, Świergosz-Kowalewska et al. 2005, Sánchez-Chardi & Nadal 2007, Sánchez-Chardi et al. 2007). The main route of exposure of small mammals to heavy metals in a contaminated environment is through the consumption of food (Ma et al. 1991). Irrespective of their presence in the environment and/or in the food, the concentration of heavy metals in animal tissues also depends on several factors like the age, physiological state and homeostatic mechanisms of the individual (Sawicka-Kapusta et al. 1995). Nevertheless, it has been shown that the concentration of the different heavy metals in small mammals follow a regular sequence, Fe > Zn > Cu > Pb, with small differences according to the species' lifestyle (Šumbera et al. 2003, Damek-Poprawa & Sawicka-Kapusta 2004, Sánchez-Chardi & Nadal 2007, Sánchez-Chardi et al. 2007). The results of this work showed that bioaccumulation of the analyzed heavy metals in *C. talarum* from all areas followed the same order. Moreover, and similar to what observed in the silvery mole rat *Heliophobius argenteocinereus* (Šumbera et al. 2003), nearly all of the metals (with exception of Pb) were found at higher levels in the liver than in the femoral muscle.

Bioaccumulation of Pb in the liver of small mammals in contaminated areas has been reported (Torres et al. 2006, Sánchez-Chardi & Nadal 2007). This metal is highly toxic and causes renal dysfunction, liver cirrhosis, and affects the nervous and reproductive systems (Shore & Douben 1994, Milton et al. 2003). Moreover, Pb has clastogenic and/or genotoxic effects (Tull-Singleton et al. 1994). Critical liver concentrations of Pb are considered to be around 25-35 $\mu g.g^{-1}$ dry weight (Scheuhammer 1991, Shore & Douben 1994). While individuals of *C. talarum* from the military area did not differ in the content of Pb with respect to individuals from the control area, the concentration of this element was closer to the critical level in tuco-tucos from the agricultural region, showing the potentially toxic impact of this activity on wildlife and ultimately on human health. However, due to the low number of samples obtained from the cultivated field, results from this area must be interpreted with caution, although the values obtained were similar amongst examined animals.

While Pb is a toxic element, Zn, Fe and Cu are essential for the normal development and function of the organism (Topolska et al. 2004). Moreover, Zn and Cu also participate in detoxification processes, as part of the enzymes of the antioxidant systems that impede the formation of reactive oxygen species (Sánchez-Chardi & Nadal 2007) and they also bind to some metallothioneins (Włostowski et al. 2003, Pereira et al. 2006). Likewise, Fe concentrations reduce the toxic effects of pollutants like cadmium. Besides the importance of these elements for the correct functioning of living mammals, high concentrations or deficiencies of heavy metals can also be dangerous to organisms.

High levels of Zn and Cu in the liver or other tissues of several mammalian species in contaminated sites have been reported (Sánchez-Chardi et al. 2007). Increments in these elements are sometimes related to protective and/or detoxification regulation, though large variations can also be harmful for the organisms. Although Zn concentration in tissues of *C. talarum* in both military or agricultural areas were generally higher than in the control areas, the values were within the mammal average, and below the critical upper level situated above 274 and 465 $\mu g.g^{-1}$ dry weight for kidney and liver respectively (Cook et al. 1990, Eisler 1997, Świergosz-Kowalewska et al. 2005). Regarding Cu, the concentrations of this metal were also higher in animals from both military and agricultural areas. However, only the values observed in the liver of individuals from the agricultural zone were inside the range of concentrations considered potentially dangerous for mammals and closer to the upper range limit described for the liver of other rodent species. Similar trend and values were also observed in the silvery mole-rat (*Heliophobius argenteocinereus*) from cultivated areas; although the possible origin of this metal in the habitat of this subterranean species was not clearly stated (Šumbera et al. 2003).

In most cases Fe concentrations in *C. talarum* tissues were at physiological levels and did not differ significantly between areas. Only liver values of individuals from the agricultural area were significantly higher and above concentrations observed in other mammals (Damek-Poprawa & Sawicka-Kapusta 2004, Topolska et al. 2004), although still lower than the ones obtained in tissues of the greater white-toothed (*Crocidura russula*) from polluted areas (Sánchez-Chiardi & Nadal 2007).

In conclusion, despite the fact that almost all values of heavy metals in soils and tissues of *C. talarum* from the analyzed areas may not indicate a severe risk of toxic effects on wildlife and are typical for lightly polluted habitats, the high levels of the extremely toxic Pb in the cultivated field suggests for the possibility that carrying out this activity for long periods of time or in an intensive way could exert an impact on individuals, communities and ecosystems. Although the bioaccumulation of the analyzed heavy metals in *C. talarum* approximately follows variations of these elements in the different areas, discrepancies in some values like the lower concentration of Zn in the military area but higher in the tuco-tucos tissues in comparison with the control area, suggests that this kind of study must be extended in time and to other habitats, increasing both the number of individuals and the diversity of heavy metals analyzed in order to finally establish *C.talarum* as an effective bioindicator of environmental pollution.

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