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Metal and metalloid accumulation in shrews (Soricomorpha, Mammalia) from two protected Mediterranean coastal sites

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Sr, Ba, Fe, Mn, Mo and B were bioaccumulated in bones of shrews from the Ebro Delta area and Cu in Medas Islands, whereas Cr and Zn showed similar levels at both sites.

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ABSTRACT

Although ecotoxicological data on heavy metals are abundant, information on other potentially toxic elements with attributed deficiency and/or toxic disturbances is scarce. Here we quantify zinc, copper, iron, manganese, chromium, molybdenum, strontium, barium, and boron in bones of greater white-toothed shrews, *Crocidura russula*, inhabiting two protected Mediterranean coastal sites: the Ebro Delta, a wetland impacted by human activities, and the Medas Islands, a reference site. Natural and anthropogenic inputs significantly increase Fe, Mn, Mo, Sr, Ba, and B in specimens from the Ebro Delta, whereas Cu and Cr were higher in Medas' shrews. Principal component analysis allowed complete separation between sites along the first two axes in particular due to B, Sr, and Cu. This study provides metal reference values in bones of insectivores, explores their variability and bioaccumulation patterns in depth, and assesses the potential environmental risk and toxicity for biota exposed to the above elements.

1. Introduction

Non-essential metals, such as lead (Pb), mercury (Hg), and cadmium (Cd), and essential elements, such as zinc (Zn) and copper (Cu), are common pollutants widely distributed throughout the ecosystems. They have been extensively quantified and their toxic effects for biota are well-known (e.g. Goyer, 1997; Mañosa et al., 2001; Nriagu, 1988; Sánchez-Chardi et al., 2007a). In contrast, environmental information concerning other metals and metalloids, like iron (Fe), manganese (Mn), molybdenum (Mo), strontium (Sr), barium (Ba), and boron (B), is less readily available. All these elements are natural constituents of soils and sediments (Fernández-Turiel et al., 2003; He et al., 2005; Nriagu, 1988; West et al., 2001) and their levels in mammalian tissues may depend on season, age and/or sex, as well as physiological, pathological, and ecological conditions (Komarnicki, 2000; Lopes et al., 2002; Pankakoski et al., 1993, 1994; Sánchez-Chardi and Nadal, 2007; Sánchez-Chardi et al., 2007a,b). Moreover, their background levels and/or bioavailability may increase due to industrial, agricultural, and domestic pollution, and other human activities such as hunting. Thus, lead shot pellets may contain traces of Zn and Cu (Mozafar et al., 2002), fertilizers are a source of Zn, Cu, Fe, Mn, Cr, Mo, and B (He et al., 2005; Otero et al., 2005), and plaguicides may have traces of Zn, Cu, Fe, Mn, Ba, and B (He et al., 2005; Mañosa et al., 2001). Additionally, effluents and atmospheric deposition from industries may contain virtually all elements, and domestic effluents may be an important input of Zn, Cu, Fe, Mn, Cr, and B (e.g. Lucho-Constantino et al., 2005; Outridge and Scheuhammer, 1993). Generally, data on some of these metals and metalloid in wild populations are scarce or absent, probably due to their low toxicity compared with those of widely distributed non-essential elements and the complexity of behaviour of metal mixtures at real conditions. This lack of data hinders our understanding of metal and metalloid migration through food chains.

Deltaic environments are fragile ecosystems often affected by heavy metals and other pollutants, especially in developed countries (e.g. Oliveira Ribeiro et al., 2005; Sánchez-Chardi et al., 2007a) and one of the main goals when analysing heavy metal concentrations is to distinguish between natural background levels and those originating from anthropogenic contaminant sources (Rodríguez Martín et al., 2006). For many decades, the Ebro Delta

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has been impacted by several human activities such as lead shot pellets from hunting, fertilizers and pesticides from agricultural processes, industrial poles, and domestic sewage (Lacorte et al., 2006; Lavado et al., 2006; Mañosa et al., 2001; Ocampo-Duque et al., 2008; Schuhmacher et al., 1993). However, most studies focusing on the Ebro area have reported fragmentary, not always consistent, information about elements at both biotic and abiotic levels (e.g. Grimalt and Albaigés, 1990; Lavado et al., 2006; Mañosa et al., 2001; Navas and Machín, 2002; Ocampo-Duque et al., 2008). The Medas Islands are a nature reserve that is barely impacted by pollution sources and considered a pristine site with background levels of toxic metals and other pollutants (Pastor et al., 1995; Sánchez-Chardi et al., 2007a).

Small mammals, especially shrews, are reliable bioindicators of environmental pollution (bioaccumulation of metals and physiological effects) because they show high food requirements and their metabolic rate is high (e.g. Komarnicki, 2000; Pankakoski et al., 1993, 1994; Sánchez-Chardi et al., 2007a,b; Talmage and Walton, 1991). Likewise, bones accumulate several metals, especially bivalent elements that mimic calcium (e.g. EPA, 1998; Nielsen, 2004). In addition, bones from zoological collections have been used successfully to study contamination levels in small mammals (e.g. Sánchez-Chardi et al., 2007a). However, there is little information on the accumulation of several elements in the hard tissues in wild mammals.

With the above considerations, the aims of this study were: (i) to determine levels of those elements scarcely quantified in previous ecotoxicological studies; (ii) to analyse site, age-, and sex-dependent variation in the bioaccumulation patterns; (iii) to provide reference values for metal and metalloid contents in hard tissues of shrews; and (iv) to assess the use of these parameters as biomarkers in Mediterranean sites.

2. Materials and methods

We analysed the element content in large bones of 105 greater white-toothed shrews, *C. russula*. Animals were collected from1976 to 1981 in the Ebro Delta ($n = 73$) and the Medas Islands ($n = 32$), two partially protected coastal areas in north-eastern Spain. Specimens were sexed and aged: (1. Juveniles: immature shrews in their first year of life; 2. Adults: mature shrews in their first year of life; 3. Seniles: shrews in their second year of life) according to toothwear (Vesmanis and Vesmanis, 1979) and reproductive condition (López-Fuster et al., 1985). Bones were cleaned by exposure to dermestid larvae (Dermestidae, Coleoptera), kept in single metal-free paperbags and stored in the zoological collection of the Animal Biology Department (University of Barcelona). Initially, this material constituted the basis of several investigations on morphometric and biological aspects of the species (López-Fuster, 1985; López-Fuster and Ventura, 1992; López-Fuster et al., 1985, 1986).

For chemical quantification, dried samples were placed in Teflon vessels and digested by nitric acid and hydrogen peroxide (Instra, Baker Analyzed). Samples were diluted 1:5 in deionized (Milli-Q©) water with 1% nitric acid. Rhodium was used as internal standard and bovine liver (SRM-1577a), certified by the National Bureau of Standards, was included as reference material in the analysis. Concentrations of Fe were quantified by a Perkin-Elmer OPTIMA-3200RL inductively coupled plasma optical spectrometer (ICP-OES) whereas the rest of the metals (Zn, Cu, Mn, Cr, Mo, Sr, Ba, Ni, and Co) and a metalloid (B) were determined by a Perkin-Elmer ELAN-6000 inductively coupled plasma mass spectrometer (ICP-MS). The results of element concentrations were expressed as mean ± standard error of the mean ($M \pm$ SEM) in micrograms/gram (μg/g) on a dry weight basis. Detailed methodological information is described by Sánchez-Chardi et al. (2007a,b).

Normal distribution and homogeneity of variance of the log transformed data were assessed by the Kolmogorov–Smirnov test and the Levene *F*-test, respectively. Initially, a three-way multivariate analysis of variance (MANOVA) was performed to obtain an overall estimation of the effects of site, age, and sex and their interactions on the element levels. When element concentrations did not differ according to age and/or sex, data were combined to increase the sample size of each site. Depending on this previous procedure, intra- and interpopulational comparisons were evaluated either by Student's *t*-test or by one-way analysis of variance (ANOVA). When ANOVA was applied, pair-wise comparisons of sample means were conducted by Scheffe's method. In an attempt to visualize the degree of divergence between both populations considering all elements analysed, a principal component analysis was performed. This statistical technique reduces multidimensional data sets to lower dimensions while retaining those characteristics of the data set that contribute most to its variance. After factor extraction, a Varimax rotation was employed to aid interpretability of the low-variance principal components. Pearson's correlation coefficients were calculated to evaluate the relationship between the elements analysed in shrews from the polluted site. For all sequential tests, *p*-values were corrected by the Bonferroni adjustment (Rice, 1989), as modified by Chandler (1995). For all statistical analyses, SPSS 14.0 (2005) was used.

3. Results

Nickel and cobalt concentrations were under threshold limit of ICP-MS (in μg/kg in diluted acidic solution: Ni 0.20, Co 0.05) and therefore were not included in the analyses. The rest of elements were above the detection threshold in all specimens (in μg/kg in diluted acidic solution: Fe 10, Zn 0.50, Cu 0.10, Mn 0.10, Cr 0.50, Mo 0.05, Sr 0.10, Ba 0.05, B 0.05).

MANOVA showed significant differences in the element concentrations by site ($F = 103.487$, $p = 0.001$), age ($F = 2.528$, $p = 0.001$), and their interaction ($F = 2.434$, $p = 0.002$). Since sexual divergence was not observed, sexes were pooled in subsequent analyses. Descriptive statistics of the elements quantified according to site and age are shown in Table 1.

Significant age-dependent variation was only observed in Zn levels in the Medas Islands ($F = 8.226$, $p < 0.001$), with statistical divergences between juveniles and both adults ($p = 0.009$) and seniles ($p = 0.003$). Mean values of this metal increased with age in the two capture sites although in the Ebro Delta differences were not significant.

Element concentrations varied significantly between sites (in all cases $p < 0.001$), except for Zn (differences evaluated for each age-class) and Cr (Table 1). Globally, shrews from the polluted area showed the highest mean values, with the exception of Cu.

Principal component analysis was performed on the log transformed data excluded Zn due to the age-dependent variation observed in the Medas' animals and the absence of interpopulational differences. Two rotated principal components with eigenvalues greater than 1 were extracted, which accounted for 61.46% of the total variance. Variables with higher positive loadings were B and Sr on the first factor (PC I) and Cu on the second factor (PC II; Table 2). Projection of the specimens onto the first two axes showed a complete separation along PC I, due to the lower scores exhibited by the Medas' shrews (Fig. 1).

Significant Pearson's correlation coefficients and the corresponding *p*-values evaluated in shrews from the Ebro Delta are shown in Table 3. In general, the highest number of correlations appeared between Cu and Fe and the rest of elements (7 and 6 cases, respectively), whereas Cr correlated with none of the elements analysed.

4. Discussion

4.1. Bioaccumulation by site, age, and sex

Levels of Zn found in *C. russula* agreed with those reported in the bones of other Soricomorpha (Andrews et al., 1989; Komarnicki, 2000; Topashka-Ancheva and Metcheva, 1999). Similar concentrations observed between the sites studied might be mainly due to the good homeostatic mechanisms of this metal in mammalian tissues (Goyer, 1997).

Copper levels found in bones of *C. russula* from Medas Islands were in agreement with data reported for the common shrew, *Sorex araneus*, from reference sites and lower than those observed in *S. araneus* and *C. leucodon* from polluted sites (Hunter and Johnson, 1982; Hunter et al., 1989; Topashka-Ancheva and Metcheva, 1999). In the Delta shrews, Cu levels were significantly low, a circumstance that has also been reported in fish and eggs of seabirds from the same area (Lavado et al., 2006; Morera et al., 1997; Sanpera et al.,