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Short communication

Considerations on the sample size of wood mice used to biomonitor metals

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Abstract

The concentrations of various metals (Zn, Cu, Mn and Cr) in liver, kidneys and brain from specimens of the wood mouse *Apodemus sylvaticus* captured from 5 sites were measured. Two of the sites were in a restored mine dump, another in an area characterized by serpentized soils and the remaining two were control sites. The sample size required for statistical differentiation of the sampling sites was calculated from the mean values and the variability in bioaccumulation corresponding to each of the sites. The relationship between the sample size and the results of the statistical test used to reveal significant differences between the mean concentrations of metals in organs from *A. sylvaticus* is demonstrated. The homeostatic control exerted by the wood mice on the tissue levels of metals reduced the interpopulational variability, thereby homogenizing the mean bioaccumulation corresponding to the different sampling stations. The sample size, tissue regulation of the levels of heavy metals and the patterns of variability are of vital importance in evaluating the usefulness of *A. sylvaticus* as a biomonitor of heavy metals.

Keywords: Trace elements; Homeostasis; *Apodemus sylvaticus*; Sample size

1. Introduction

Use of small mammals as biomonitors of heavy metals has been widely reported in the scientific literature (see e.g. Talmage and Walton, 1991; Milton and Johnson, 2002), and the wood mouse *Apodemus sylvaticus* has been one of the most commonly used species. One important aim of most of these types of studies is the differentiation of sampling stations (SS) on the basis of tissue concentrations of metals (Cooke et al., 1990a; Erry et al., 2000). Often no significant differences between control and polluted SS are found in these studies, especially when essential trace elements such as Zn, Cu or Mn are under study. Many researchers explain the absence of any difference as being due to the homeostatic control that *A. sylvaticus*, and small mammals in general, exert on the tissue levels of these elements (Cooke et al., 1990a,b; Talmage and Walton, 1991; Alloway and Ayers, 1997; Milton and Johnson, 2002); homeostatic control has also been described for the non-essential element Cr (Beardsley et al., 1978; Anthony and Kozlowski, 1982). Small mammals from control sites have even been found to contain higher concentrations of these elements than their counterparts from polluted SS (Hunter et al., 1989; Cooke et al., 1990a,b). Homeostatic control acts in reducing the interpopulational variability, but at the same time there exists the possibility that the lack of differences between SS may be due to high intrapopulational variability.

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Because of intrapopulational variability, the capacity to detect statistically significant differences between SS is directly related to the sample size used (Zar, 1984). In light of the effective homeostatic control exerted by *A. sylvaticus* on the tissue levels of some metals, the question arises as to the sample size required to detect significant differences in the concentrations of different elements in the organs on comparing two different SS.

The aims of the present study were, therefore: i) to calculate the number of individuals required to differentiate pairs of SS on the basis of element concentrations in organs of *A. sylvaticus*; and ii) to study the effect of variability and different mean concentrations on the number of samples required.

2. Materials and methods

Specimens of *Apodemus sylvaticus* L. (1758) were collected from: SS1 and SS2 located in restored mine dumps close to a coal-fired power plant; SS3 in an area of serpentinized rocks; SS4 and SS5, which were considered as control sites. At each SS traps were arranged on a regular sampling grid spaced at 10-m intervals (Erry et al., 2000). Captured wood mice were identified, sexed and killed in the field.

Liver, kidneys and brain were removed, dried to constant weight (45 °C), and finally homogenized. Each tissue sample was individually digested with HNO_3 in a microwave oven. The concentrations of Zn, Cu, Mn and Cr were determined by flame absorption spectrophotometry or graphite furnace spectrophotometry. Quality control was achieved by analysis of certified reference materials (BCR No. 186 and NIST 1577b); the recoveries ranged between 83% and 100%. The same data were also used to calculate the variability associated with the extraction process and sample analysis: 16% for Cr (liver only), 8% and 13% for Cu, 8% and 9% for Mn, and 9% and 9% for Zn in liver and kidneys, respectively (expressed as coefficient of variation (CV)). The total variability of the concentrations (CV_T) was calculated as: $CV_T = CV_A + CV_I$, where CV_A and CV_I correspond to analytical and interindividual variability, respectively.

The hypothesis tested was whether $\mu_1 = \mu_2$, where μ_1 and μ_2 represent the mean concentrations of the contaminant in the organs corresponding to the different SS. For this, the calculations for the power of the test described by Zar (1984) for normal distributions, and by Aboal et al. (2005) for a non-normal distribution, were applied. The significance level used in the test for normal distributions test was $\alpha = 0.05$ and $\beta = 0.1$, and that for non-normal distributions was $\alpha = 0.05$. A Box–Cox transformation was used to normalize the data by calculation of λ, which minimizes the associated variance. Normality was tested by means of Lilliefor's modification of the Kolmogorov–Smirnov test. The sample size required to detect significant differences between the mean values in bioaccumulation of different SS reported in previous similar studies was calculated. For this, it was assumed that the distributions observed by these authors were normal and we used, for a given element and organ, the values of CV obtained in the present study.

3. Results

The descriptive statistics for the elements determined is shown in Table 1. The highest mean concen-

Table 1
Descriptive statistics for the tissue concentrations (μg g^{-1} d.w.) of Zn in the liver (L), kidneys (K) and brain (B) from wood mice (*Apodemus sylvaticus*) caught at the sampling sites (SS) under study

Tissue		SS1			SS2			SS3			SS4			SS5		
		L	K	B	L	K	B	L	K	B	L	K	B	L	K	B
Zn	Mean	234.8	190.6	60.45	215.8	168.3	62.1	163	96.9	63.6	158.3	110	59.0	163.7	126.3	61.8
	Median	231.3	167.5	59.96	195	178.8	60.2	142.1	88.8	63.6	140.6	105	55.8	155.6	116.7	61.5
	CV	24	34	8	41	36	15	48	38	20	44	39	17	36	65	15
Cu	Mean	17.6	18.9	14.1	16.8	16.4	12.0	19.9	14.1	13.8	18.5	14.3	13.3	20.3	15.2	12.1
	Median	17.2	19.3	5.4	16.7	17.6	12.7	20.1	16.5	15.7	18.3	15.8	12.1	20.1	17.7	13.4
	CV	19	9	38	23	27	50	26	47	41	29	42	28	26	45	50
Mn	Mean	5.80	5.15	1.59	7.28	6.76	2.19	5.22	5.60	2.12	6.05	5.84	2.02	6.08	5.69	2.02
	Median	5.66	5.04	1.54	6.68	6.82	2.07	5.18	5.74	2.05	6.00	5.87	1.96	5.84	5.44	2.00
	CV	30	23	25	28	29	25	23	26	25	26	18	19	26	32	24
Cr	Mean	0.83			0.93			1.15			0.98			0.90		
	Median	0.71			0.61			0.52			0.57			0.55		
	CV	48			92			120			99			29		

CV: coefficient of variation, expressed as a percentage. Sample sizes for each sampling station (SS1 to SS5) were 25, 44, 40, 19 and 61, respectively. Levels of Cr in kidneys and brain were below the limit of quantification.