Statistical Analysis of Eco-Toxicologic Data of Mammals from a Polluted Area

A. Sánchez-Chardi[1], **M. J. López-Fuster**[1], **and J.J. Egozcue**[2]
[1]Dep. Biologia Animal, Universitat de Barcelona, Barcelona, Spain;
asanchez@bio.ub.es; marialopez@ub.edu
[2]Dep. Matemàtica Aplicada III, Universitat Politècnica de Catalunya, Barcelona, Spain;
juan.jose.egozcue@upc.edu

Abstract

Several eco-toxicological studies have shown that insectivorous mammals, due to their feeding habits, easily accumulate high amounts of pollutants in relation to other mammal species. To assess the bio-accumulation levels of toxic metals and their influence on essential metals, we quantified the concentration of 19 elements (Ca, K, Fe, B, P, S, Na, Al, Zn, Ba, Rb, Sr, Cu, Mn, Hg, Cd, Mo, Cr and Pb) in bones of 105 greater white-toothed shrews (*Crocidura russula*) from a polluted (Ebro Delta) and a control (Medas Islands) area. Since chemical contents of a bio-indicator are mainly compositional data, conventional statistical analyses currently used in eco-toxicology can give misleading results. Therefore, to improve the interpretation of the data obtained, we used statistical techniques for compositional data analysis to define groups of metals and to evaluate the relationships between them, from an inter-population viewpoint. Hypothesis testing on the adequate balance-coordinates allow us to confirm intuition based hypothesis and some previous results. The main statistical goal was to test equal means of balance-coordinates for the two defined populations. After checking normality, one-way ANOVA or Mann-Whitney tests were carried out for the inter-group balances.

Key words: balance, compositional data, simplex, Aitchison geometry, composition, orthonormal basis, bio-indicator, environmental pollution, *Crocidura russula* Insectivora.

1 Introduction

Natural and anthropogenic activities are sources of heavy metals and other pollutants in the environment. On the last decades, industrial, agricultural and domestic processes have dispersed these potentially toxic elements virtually in all habitats. Some heavy metals such as cadmium (Cd), mercury (Hg), lead (Pb) and aluminium (Al), as well as organic compounds of tin (Sn) and hexavalent chromium (CrVI) have none metabolic function in biological systems, produce high toxic effects, and are considered non-essential elements. In contrast, essential metals such as zinc (Zn), copper (Cu), trivalent chromium (CrIII), niquel (Ni), manganese (Mn), sodium (Na), calcium (Ca), and iron (Fe) have generally lower toxicity rates than the non-essential metals, are dangerous only at high concentrations, and organisms have usually homeostatic mechanisms to control the levels of these metals in their tissues (e.g. Schuhmacher et al., 1993; Smith and Rongstad, 1982). Moreover, phosphorus (P) and sulphur (S) are essential, non-metallic elements, with known basic metabolic functions, which appear in organisms in high concentration. The biological impact of metals depends on the physical-chemical conditions of soil, as well as on the exposure route and their particular toxicity among others. Additionally, the toxic effects of non-essential metals may be mediated or enhanced by interactions or deficiencies of nutritionally essential elements (Goyer, 1997).

The chemical content in the different webs from trophic chain is a direct measure to evaluate the presence and levels of pollutants in organisms. An approach to assess the environmental quality for humans and wildlife is the use of autochthonous and common species of small mammals (insectivores and rodents) as bio-indicator (e.g. Talmage and Walton, 1991). Several eco-toxicologic studies have shown that representatives of the Order Insectivora, such as the common shrew, *Sorex araneus*, the pigmy shrew, *S. minutus*, and the northern short-tailed shrew, *Blarina brevicauda* (Read and Martin, 1993; Ma, 1989, 1996; Talmage and Walton, 1991) are suitable bio-indicators for toxic compounds. This fact is basically due to their high metabolic rate and high position in the trophic chain, and because their tissues accumulate metals in relation with the environment (Andrews et al., 1984; Talmage and Walton, 1991; Komarnicki, 2000; Pankakoski et al., 1994).

A crucial question is how to treat the data obtained from a toxicologic analysis, in order to interpret the effects of contamination. When observing chemical contents of a bio-indicator species, data are mainly compositional. This means that the observed amount of a particular chemical element is irrelevant; the important information is just relative to the amounts of other elements. In this situation, standard multivariate statistical methods applied to the raw concentrations can give misleading results. Compositional methods (Aitchison, 1986) deal with log-ratios of parts of a composition, thus taking into account the relevant information: the ratios of parts. However, the use of log-ratios introduces some interpretation difficulties, especially when the log-ratio involves many parts. In order to compare populations represented by compositional vectors, a standard practice has been to compare some simple log-ratios which interpretation appears clear to the analyst. When using more complicated log-ratios comparisons may give some conclusions but the interpretation is normally difficult.

Recently, the development of the Aitchison geometry on the simplex (Aitchison et al., 2002; Pawlowsky-Glahn and Egozcue, 2001, 2002; Billheimer et al., 2001; Egozcue et al., 2003) permits the use of log-ratios as coordinates of the compositional vectors with respect to an orthonormal basis. A first consequence is that using orthonormal coordinates compositional computations are easier and simpler. However, interpretation of such coordinates may be difficult. A step ahead has been the definition of balance between groups of parts (Egozcue et al., 2003; Egozcue and Pawlowsky-Glahn, 2005b), a particular kind of orthogonal coordinates for compositions. Balances are interpretable and are closely related to partitions of the compositional vectors into groups of parts. Comparison of two populations can be carried out using orthonormal balances designed to represent interpretable features of the populations. This strategy avoids testing of redundant hypothesis and allows working with large compositional vectors with an improved interpretation of the results.

The goal of the present study is two fold: to contribute to the knowledge of the greater white-toothed shrew (*Crocidura russula*) as bio-indicator of contamination; and to show how balances may improve interpretation of statistical results when comparing mean values and variabilities in different populations.

2 Materials and methods

Study areas and sampling

Deltaic areas are important and fragile ecosystems, habitat of endangered and protected species, and frequently remain as "hot-spots" of pollutants in developed countries. Our selected case-study area, the Ebro Delta is not an exception: located in NE Spain is a partially protected area direct and indirectly impacted by a wide range of human activities (e.g. Mañosa et al., 2001). The industrial and domestic effluents that spill in the Ebro river and the agricultural and hunting activities in the Ebro Delta, load considerable amounts of heavy metals in this wetland. As control or reference site, we used material from the Medas Islands, a small archipelago located at the Mediterranean Sea (less than a km out L'Estartit), uninhabited and protected by law.

Sample analyzed consisted of large bones from the greater white-toothed shrew, *Crocidura russula* (Insectivora, Mammalia). Specimens were caught from 1976 to 1981 in the Ebro Delta

(L'Encanyissada, n=73) and the Medas Islands (Meda Gran Island, n=32).

Chemical analyses

The bones were rinsed with high purity grade water (Milli-Q system, 18.2 MΩcm^2) and dried until constant weight (50^{o}C, 48h). Tissue of each animal (80-100 μg) was placed on Teflon vessels and digested (120^{o}C, 12h) with 2 ml of nitric acid 70% instra quality (Baker) and 1 ml oxygen peroxide 30% instra quality (Baker). Samples were diluted 1:5 in Milli-Q water with 1% HNO3 with Rhodium as internal standard. Concentrations of calcium (Ca), potassium (K), iron (Fe), phosphorus (P) and sulphur (S) were quantified by a Perkin-Elmer OPTIMA-3200RL Inductively Coupled Plasma Optical Spectrometer (ICP-OES). The remaining elements (lead (Pb), mercury (Hg), cadmium (Cd), copper (Cu), zinc (Zn), aluminium (Al), boron (B), sodium (Na), barium (Ba), chromium (Cr), strontium (Sr), rubidium (Rb), manganese (Mn), and molibdenum (Mo)) were determined by an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) Perkin Elmer ELAN-6000. Two replicate subsamples were analysed and a standard reference sample (Bovine Liver SRM-1577a) certificated by the National Bureau of Standards (NBS) was included in the analysis. Metal analyses were carried out at the Spectroscopy Service of the University of Barcelona.

Statistical Methods

The main goal was to compare two populations described by compositional samples. The origen of the samples suggested that some differences should exist. A first step was to represent the compositional samples by some coordinate system. The selection of such a coordinate system was done in order to improve the interpretability of each coordinate. Therefore, we selected orthonormal balances as coordinates.

Once the reference system was fixed, the second step was to proceed to mean comparisons between the two populations in an standard way: check equal variances, check normality and, finally, compare mean coordinates (e.g. ANOVA or t-student) or, alternatively, mean range coordinate (e.g. Mann-Whitney test). Interpretation of results completed the statistical analysis.

We fucussed our attention in the selection of the balances that represent the compositional vectors. As stated in (Egozcue and Pawlowsky-Glahn, 2005b), an orthonormal coordinate system of balances can be obtained designing a sequential binary partition (SBP) of the compositional vector. We started with the complete composition ($D = 19$ parts) and we divided it into two groups of parts. The idea was to group parts that are assumed affine in some interpretable sense. The process was repeated within each group thus partitioning it into two new sub-groups. The procedure stopped when all groups had a single part, i.e. at the $D - 1 = 18$ order partition.

In our case, some partitions were meaningful but other ones were selected to complete the orthonormal system of coordinates. The selected SBP was encoded in Table 1. Labels of parts were presented horizontally and order of partition vertically. The first row indicates the order 1 partition into two groups. First and second groups are labelled $+1$ and -1 respectively. Therefore, group $+1$ was made of a single part (Al) and the second group contained all other parts. Second row in Table 1 divides the -1 group of the first order partition into two new groups ($+1$, -1, in the second row). Parts not involved in a partition are marked with 0. For instance, at order 8 partition we grouped {Zn,Cu} versus {Rb,Mn,Mo}.

The explanation of the selected SBP is an important issue because the interpretation of statistical results depend on it. A brief description follows.

order 1 {Al} vs. {Ca, K, Fe, B, P, S, Na, Zn, Ba, Rb, Sr, Cu, Mn, Hg, Cd, Mo, Cr, Pb}. Relative presence of Al may be due to impurities and probably not directly related to environmental pollution. Generally, levels of Al vary largely between items and, when appearing in high concentrations, they are considered as a product of external contamination of the samples;

order 2 {B} vs. {Ca, K, Fe, P, S, Na, Zn, Ba, Rb, Sr, Cu, Mn, Hg, Cd, Mo, Cr, Pb}. Relative presence of B may be due to impurities and probably not directly related to environmental pollution. This case is similar to Al;

order 3 {Hg, Cd, Pb} vs. {Ca, K, Fe, P, S, Na, Zn, Ba, Rb, Sr, Cu, Mn, Mo, Cr}. Elements in the first group are typical pollutants in deltaic areas, particularly, in the Ebro delta; they are *non-essential* elements. Elements in the second group are *essential*;

order 4 {Hg, Cd} vs. {Pb}. Spill and assimilation route of Pb may be different of those of Hg and Cd. However, this is a routine partition to analyse the three part subcomposition Hg, Cd, Pb;

order 5 {Hg} vs. {Cd}. Routine partition to analyse the three part subcomposition Hg, Cd, Pb;

order 6 {Ca, K, Fe, P, S, Na, Ba, Sr} vs. {Zn, Rb, Cu, Mn, Mo, Cr}. First group is made of major elements and the second one are trace elements;

order 7 {Zn, Rb, Cu, Mn, Mo} vs. {Cr}. Cromium is separated because its concentration may be affected by artifacts of the analytic process;

order 8 {Zn, Cu} vs. {Rb, Mn, Mo}. Zn and Cu are elements involved in de-toxificant processes and well-regulated by metabolism and they are separated from other trace elements;

order 9 {Zn} vs. {Cu}. These elements interact largely, e.g. they compete for absortion in the digestive tract.

order 10 {Mn} vs. {Rb, Mo}. Routine partition;

order 11 {Rb} vs. {Mo}. Routine partition;

order 12 {K, Fe, S, Na} vs. {Ca, P, Ba, Sr}. First group: abundant essential elements in organisms; second group: essential elements characteristic of bones and their associates Ba and Sr;

order 13 {P} vs. {Ca, Ba, Sr}. P and Ca are major element in bones; Ca, is associated with trace elements Ba and Sr;

order 14 {Ca} vs. {Ba, Sr}. Major versus trace elements in bones.

order 15 {Ba} vs. {Sr}. Routine partition;

order 16 {Fe, S} vs. {K, Na}. Comparison between elements involved in homeostatic processes (K, Na) and the other abundant essential elements;

order 17 {K} vs. {Na}. Routine partition;

order 18 {Fe} vs. {S}. Routine partition.

The SBP process can be visualized in the CoDa-dendrogram in Figure 1. Figures 2 and 3 are magnifications of two sub-branches of the complete tree.

A binary partition of order i has an associated balance-coordinate which is computed as

$$b_i = \ln \frac{(\prod x_+)^{a_+}}{(\prod x_-)^{a_-}} \ ,$$

where x_+ and x_- denote the parts labelled with $+$ and $-$ respectively and the powers are

$$a_+ = \sqrt{\frac{n_-}{n_+(n_+ + n_-)}} \quad ; \quad a_- = \sqrt{\frac{n_+}{n_-(n_+ + n_-)}} \ ,$$

being n_+ and n_- the number of parts labelled with + and − respectively.

The meaning of a balance between two groups of parts is explained in Egozcue and Pawlowsky-Glahn (2005a), (2005b), the former one in this volume. Briefly, the balance represents what remains of a composition after projecting on the subcomposition associated with the two groups together and, then, filtering out what is related to the subcompositions associated with each group separately. Balances, as defined by the SBP in Table 1 are orthonormal coordinates of the whole composition and, therefore, are well suited to represent the studied samples.

The tree structure of the dendrogram describes the process of SBP presented in the previous section and explains the meaning of each balance. Horizontal bars to the right of a vertical segment represent the fraction of the total variance of the sample explained by the balance corresponding to the vertical bar. Vertical lines are assumed to be balance axes, ranging from -8 to +8 in Figures 1 and 2 and from -6 to 6 in Figure 3. Horizontal segments to the right touch the vertical bar at the mean balance value. Moreover, quantile (0.05, 0.25, 0.50, 0.75, 0.95) box-plots are shown attached to the vertical balance axes.

After computing the sample balances for both populations, normality of the balance was checked using Kolmogorov-Smirnov goodness-of-fit test. We also proceeded to test equal variance of balance for the two populations. If normality of a particular balance for both samples had not been rejected, e.g. the p-value is greater than 0.05, the null hypothesis for equal balance mean was considered and then tested with standard ANOVA techniques, taking into account rejections of equal variance. If normality of balance was rejected for one population, p-value less than 0.05, mean ranges were compared using the non-parametric Mann-Whitney test.

3 Results and discussion

The CoDa-dendrogram in Figure 1, complemented with Figures 2 and 3, shows the main features of the two samples (Ebro Delta, blue; Medas Islands, red). Basic descriptive statistics of the balances for each site are shown in Table 2; this table also shows Kolmogorov-Smirnov p-values testing normality for each balance and for the two samples. Comparison of mean balances of the two populations is also presented in Table 2.

Results revealed that, for most balances, variance was larger in Ebro sample than in Medas sample. (see Figures 1, 2, 3. In general, this can be understood as a differential individual response to pollutants in the sense that some individuals might be more influenced than other ones depending on particular circumstances such as sex, sexual maturation, age, habitat or exposure to contamination.

Differences observed in the balances concerning relationships between Al and B (orders 1 and 2) with respect to the remaining metals may be partially due to the external contamination inherent to the analytical methodology employed. Additionally, boron is an element that naturally occurs in soil and water and its essentiality is still under discussion. In fact, the abundance of this element in the Ebro Delta might be associated with human activities, as has been described in other wetlands (Powell et al., 1997). Since information on the anthropogenic sources of this element in the environment is, in general, scarce and particularly null in the Ebro Delta, further information is needed to interpret this result.

Balance between non-essential and essential elements (order 3) shows a relative increase of non-essential elements in the wetland area (Ebro). Inspection of Figure 1 and Table 2 reveals that for balance {Hg, Cd} vs. { Pb} the variance appears to be larger in the Ebro Delta than in Medas Island, and means differ substantially. This result is coherent with the environmental contamination observed in that wetland, where the lead from shot pellets is the main source of heavy metal pollution in this area, one with the highest concentration of pellets in soil in all over the world (Mañosa et al., 2001; Mateo et al., 1997). By natural corrosion, lead is transferred to soil, where it becomes available to animals, basically trough the diet.

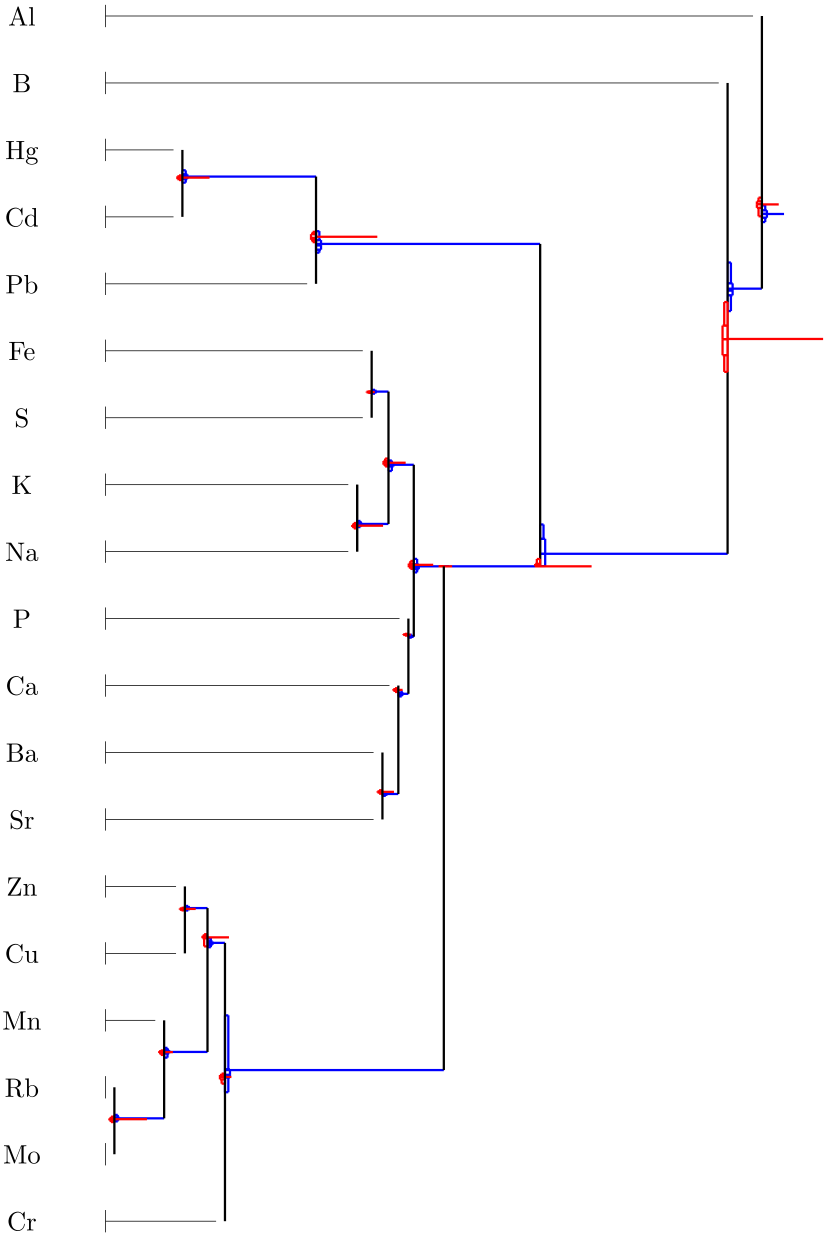

Figure 1: CoDa-dendrogram of Ebro and Medas samples following the partition in Table 1: Ebro in blue, Medas in red. Vertical bars scaled from −8 to +8.

Table 1: Code of the sequential binary partition. For each order, +1, −1, 0 respectively indicate first group, second group, and element does not participate in the partition.

Order	Ca	K	Fe	B	P	S	Na	Al	Zn	Ba	Rb	Sr	Cu	Mn	Hg	Cd	Mo	Cr	Pb
1	−1	−1	−1	−1	−1	−1	−1	+1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1
2	−1	−1	−1	+1	−1	−1	−1	0	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1
3	−1	−1	−1	0	−1	−1	−1	0	−1	−1	−1	−1	−1	−1	+1	+1	−1	−1	+1
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+1	+1	0	0	−1
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+1	−1	0	0	0
6	+1	+1	+1	0	+1	+1	+1	0	−1	+1	−1	+1	−1	−1	0	0	−1	−1	0
7	0	0	0	0	0	0	0	0	+1	0	+1	0	+1	+1	0	0	+1	−1	0
8	0	0	0	0	0	0	0	0	+1	0	−1	0	+1	−1	0	0	−1	0	0
9	0	0	0	0	0	0	0	0	+1	0	0	0	−1	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	−1	0	0	+1	0	0	−1	0	0
11	0	0	0	0	0	0	0	0	0	0	+1	0	0	0	0	0	−1	0	0
12	−1	+1	+1	0	−1	+1	+1	0	0	−1	0	−1	0	0	0	0	0	0	0
13	−1	0	0	0	+1	0	0	0	0	−1	0	−1	0	0	0	0	0	0	0
14	+1	0	0	0	0	0	0	0	0	−1	0	−1	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	+1	0	−1	0	0	0	0	0	0	0
16	0	−1	+1	0	0	+1	−1	0	0	0	0	0	0	0	0	0	0	0	0
17	0	+1	0	0	0	0	−1	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	+1	0	0	−1	0	0	0	0	0	0	0	0	0	0	0	0	0

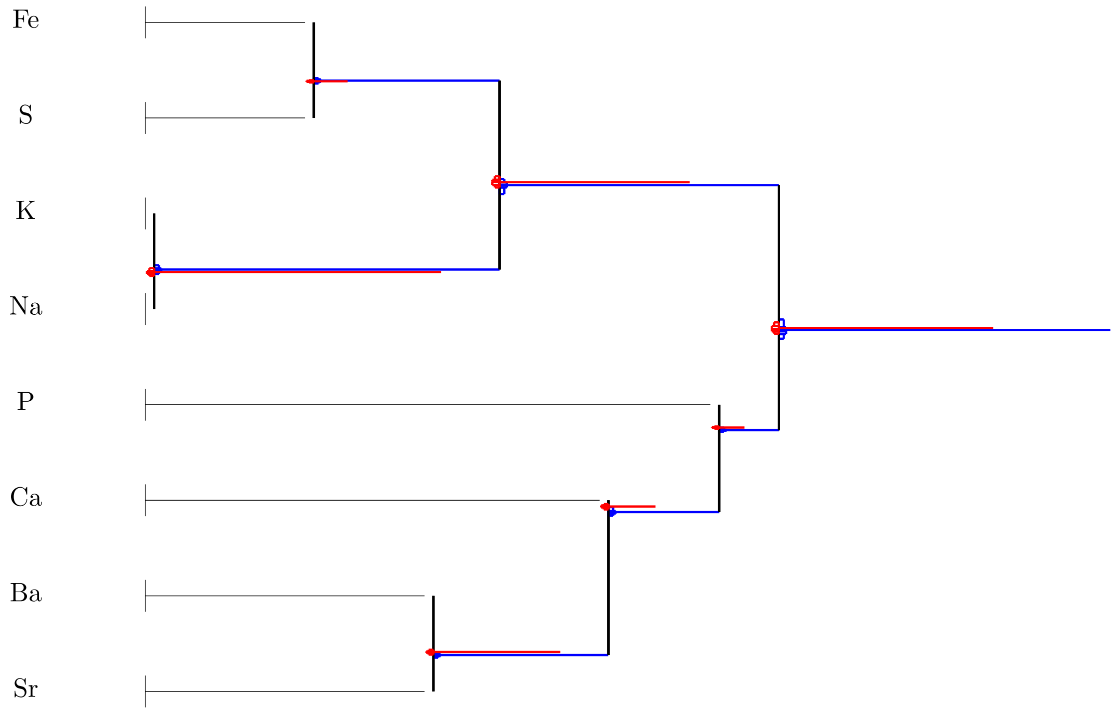

Figure 2: CoDa-dendrogram of Ebro and Medas samples for the subcomposition {Ca, K, Fe, P, S, Na, Ba, Sr}, following the partition in Table 1: Ebro in blue, Medas in red. Vertical bars scaled from −8 to +8.

Table 2: Basic descriptive statistics, *p*-value of Kolmogorov-Smirnov normality test and *p*-value of equal mean ANOVA test or Mann-Whitney* equal mean rank test, for each balance

ord	groups	Site	mean	std.	min	max	KS-pv	Mean-pv
1	{Al}	Ebro	-3.62	0.33	-4.93	-2.88	0.922	
	{Ca, K, Fe . . . , Mo, Cr, Pb}	Medas	-3.06	0.29	-3.81	-2.59	0.555	0.000
2	{B}	Ebro	1.01	0.42	-0.01	2.08	0.468	
	{Ca, K, Fe . . . , Mo, Cr, Pb}	Medas	-0.70	0.70	-1.84	0.81	0.822	0.000
3	{Hg, Cd, Pb}	Ebro	-7.37	0.98	-9.82	-4.89	0.889	
	{Ca, K, Fe, . . . , Mn, Mo, Cr}	Medas	-8.20	0.52	-9.86	-7.17	0.734	0.000
4	{Hg, Cd}	Ebro	-2.04	1.07	-3.52	1.31	0.336	
	{Pb}	Medas	-0.97	0.56	-1.84	0.79	0.677	0.000
5	{Hg}	Ebro	1.61	0.83	-0.46	3.48	0.898	
	{Cd}	Medas	1.38	0.37	0.78	1.92	0.845	0.132
6	{Ca, K, Fe, P, S, Na, Ba, Sr}	Ebro	12.13	0.70	10.35	15.18	0.000	
	{Zn, Rb, Cu, Mn, Mo, Cr}	Medas	11.62	0.20	11.17	11.91	0.665	0.000*
7	{Zn, Rb, Cu, Mn, Mo}	Ebro	0.68	1.06	-1.25	4.41	0.000	
	{Cr}	Medas	0.29	0.18	-0.22	0.83	0.813	0.005*
8	{Zn, Cu}	Ebro	4.13	0.30	3.35	4.77	0.951	
	{Rb, Mn, Mo}	Medas	4.73	0.33	3.43	5.17	0.202	0.000
9	{Zn}	Ebro	2.81	0.34	1.94	3.52	0.986	
	{Cu}	Medas	2.62	0.23	2.20	3.52	0.837	0.006
10	{Mn}	Ebro	2.83	0.47	1.62	4.02	0.928	
	{Rb, Mo}	Medas	2.80	0.21	2.25	3.15	0.408	0.719
11	{Rb}	Ebro	0.57	0.50	-1.22	2.43	0.626	
	{Mo}	Medas	0.38	0.41	-0.49	1.75	0.945	0.058
12	{K, Fe, S, Na}	Ebro	-1.46	0.39	-2.14	-0.33	0.285	
	{Ca, P, Ba, Sr}	Medas	-1.32	0.31	-1.70	0.00	0.611	0.094
13	{P}	Ebro	4.19	0.16	3.90	4.63	0.309	
	{Ca, Ba, Sr}	Medas	4.51	0.10	4.33	4.81	0.989	0.000
14	{Ca}	Ebro	6.78	0.22	6.30	7.39	0.600	
	{Ba, Sr}	Medas	7.36	0.14	7.00	7.62	0.937	0.000
15	{Ba}	Ebro	-1.91	0.28	-3.03	-1.31	0.988	
	{Sr}	Medas	-1.39	0.24	-1.86	-0.62	0.595	0.000
16	{Fe, S}	Ebro	-0.84	0.36	-1.85	-0.18	0.564	
	{K, Na}	Medas	-0.59	0.30	-1.09	0.07	0.816	0.001
17	{K}	Ebro	-1.37	0.40	-2.28	-0.23	0.626	
	{Na}	Medas	-1.80	0.37	-2.46	-0.87	0.973	0.000
18	{Fe}	Ebro	-1.74	0.29	-2.43	-0.88	0.724	
	{S}	Medas	-1.85	0.12	-2.08	-1.52	0.973	0.042

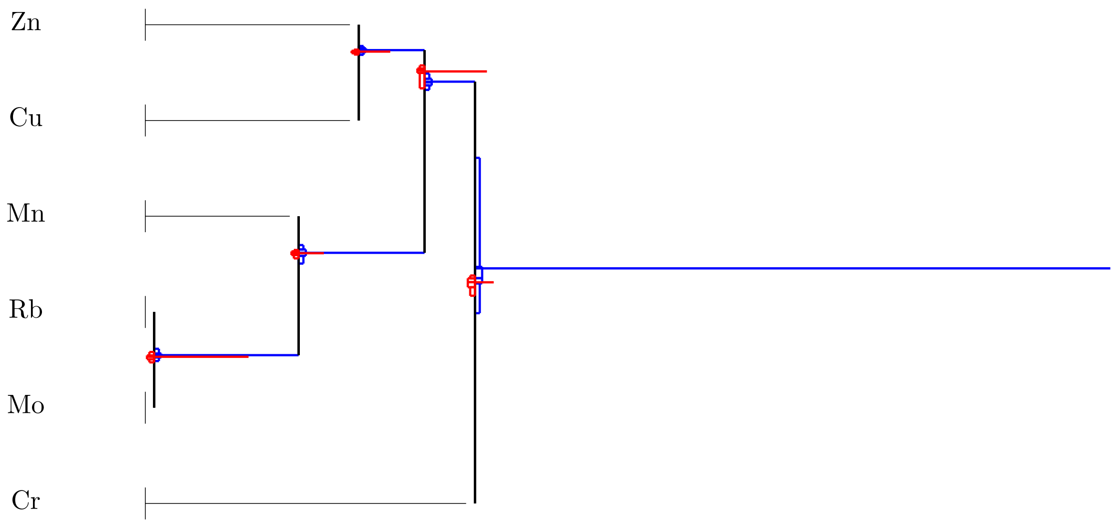

Figure 3: CoDa-dendrogram of Ebro and Medas samples for the subcomposition {Zn, Rb, Cu, Mn, Mo, Cr}, following the partition in Table 1: Ebro in blue, Medas in red. Vertical bars scaled from −6 to +6.

Results concerning order 6 showed that relationship between the major elements plus their associates and the trace elements diverged in both sites, being displaced towards the former group in the Ebro Delta. Examination of balances 7-9 revealed a significant increase of Zn and Cu at the Medas Islands. Both are essential elements well-regulated physiologically with important metabolic functions. Since in some cases, both metals are involved in detoxificant processes (Mas and Azcue, 1993), it follows that a larger amount of these metals should be expected in the Ebro Delta. However, it is worth mentioning that Cu may have a natural origen, particularly, from soil or from marine aerosol. This facts may explain the largest proportion of Cu in the Medas Islands. Moreover, both elements compete in the digestive tract and an increase of Cu will result in a deficiency in Zn as can be seen in balance 9.

The balance between the elements that conforms order 12 did not differ significantly between the polluted and the control area. Metals included in this balance were iron (involved in oxygen transport), sodium and potassium (responsible for intra e intercellular homeostasis) and calcium. The calcium is of crucial importance in the metabolism of vertebrates, especially in relation to hard tissues. Moreover, it is an element presents and available in large amounts in the environment (e.g. Scheuhammer, 1991) and associated with Sr and Ba in soil deposits. Transferred to bones, calcium is fixed with their associates in a similar ratio. Subsequent inspections of orders 13-18 revealed a noticeable increment of Sr, Fe and K in the Ebro Delta, suggesting that shrews have any homeostatic mechanism that allows regulating the balance between the elements considered in order 12. Additionally, this result suggests that the chemical contents of this mammal species are related to those presented in the environment.

4 Conclusions

Environmental pollution is rarely limited to a single chemical compound and the organisms are exposed to a wide range of stressors that interact among them producing antagonism or synergic effects (Bellés et al., 2002); obtained results confirm this result. The environmental metal pollution does not only affect the intake of a specific metal but also may disturb balances between several essential elements. Compositional data analysis provides a useful tool to interpret and understand, from an integrated viewpoint, relationships between chemical elements in eco-toxicological analyses. Additionally, this method allows us to identify meaningful global features that differ between polluted and reference populations.

The main difference, both in mean and variance, was found in the balance between essential and non-essential elements when comparing the polluted and the control population (order 3).

Results also show that the greater white-toothed shrew may be regarded as an effective bio-indicator of metal pollution in the Mediterranean climate.

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