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Perturbation vectors to evaluate air quality using lichens and bromeliads: a Brazilian case study

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Abstract Samples of one lichen species, *Parmotrema crinitum*, and one bromeliad species, *Tillandsia usneoides*, were collected in the state of Rio de Janeiro, Brazil, at four sites differently affected by anthropogenic pollution. The concentrations of aluminum, cadmium, copper, iron, lanthanum, lead, sulfur, titanium, zinc, and zirconium were determined by inductively coupled plasma–atomic emission spectroscopy. The environmental diagnosis was established by examining compositional changes via perturbation vectors, an underused family of methods designed to circumvent the problem of closure in any compositional dataset. The perturbation vectors between the reference site and the other three sites were similar for both species, although body concentration levels were different. At each site, perturbation vectors between lichens and bromeliads were approximately the same, whatever the local pollution level. It should thus be possible to combine these organisms, though physiologically different, for air quality surveys, after making all results comparable with appropriate correction. The use of perturbation vectors seems particularly suitable for assessing pollution level by biomonitoring, and for many frequently met situations in environmental geochemistry, where elemental ratios are more relevant than absolute concentrations.

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Introduction

Air monitoring studies based on the ability of living organisms to bio-accumulate metals are abundant in the literature (see reviews by Conti and Cecchetti 2001, Ares et al. 2012, and Tarricone et al. 2015). Several species of lichens and bromeliads have proved their efficiency and relevance for the study of air quality over time and/or space (Varrica et al. 2000; Szczepaniak and Biziuk 2003; Husk et al. 2004; Cardoso-Gustavson et al. 2016), because they are able to accumulate metals from the atmosphere through wet and dry deposition, in amounts far beyond their physiological needs (e.g., Conti and Cecchetti 2001; Brighigna et al. 1997; Nimis et al. 1990; Garty 2001; Vianna et al. 2011). The underlying idea is to compare the concentration levels of environmental xenobiotics in native bioaccumulators, collected from potentially polluted areas, with those sampled from clean reference areas (Bosch-Roig et al. 2013). Another alternative is to transplant unpolluted individuals to contaminated areas, and to observe any compositional changes after a given period of time (Rodriguez et al. 2011; Bermudez et al. 2009). For lichens, three main physiochemical processes and intracellular mechanisms leading to differential metal incorporation in tissues are involved (Richardson 1995; Szczepaniak and Biziuk 2003; Kularatne and de Freitas 2013): intercellular absorption by an exchange process, intercellular accumulation, and metal-rich particle entrapment, but their respective roles are not fully understood. It seems likely that both metal accumulation and release depend on several environmental factors. Like lichens, epiphytic plants from the *Tillandsia* genus absorb and accumulate in their tissues necessary nutrients and metals from the atmosphere (Bermudez et al. 2009). They are highly ramified in long, thin leaves, resulting in a high area/mass ratio, and many absorption and adsorption sites (Malm et al. 1998). Although numerous studies have used them for air quality assessment during the two last decades, the accumulation processes of metals in *Tillandsia* are probably less well identified than for lichens. In any case, several factors proper to each individual (morphology, exposure, age, species, etc.), with other factors of abiotic relevance (temperature, hygrometry, season, etc.), probably play a role in these accumulation processes, for both lichens and bromeliads (Szczepaniak and Biziuk 2003; Ayrault et al. 2007). The transplant approach, with better control of exposure, is therefore often preferred (Ayrault et al. 2007). In the case of direct collection in the field, a common practice consists in normalizing metal concentrations to some element of dominantly crustal origin, such as titanium, aluminum, or rare earth elements (Dongarrà et al. 2003; Monna et al. 1999), because it is believed that the above-mentioned factors of variability act with approximately the same intensity on both numerator and denominator (Monna et al. 2012). However, correlations made from variables using a common divisor run the risk of producing spurious results, particularly when the denominator is highly variable (van der Weijden 2002), so that the use of multivariate statistics based on correlations, such as principal component analysis, is not recommended (Pasquet et al. 2016). From a statistical point of view, treating raw concentration data (i.e., without normalization) is not a better option. Compositional data are subject to the constant-sum problem: when one component increases, one or several others simultaneously decrease, inducing negative correlations with the increasing variable (the so-called *negative bias* problem). Positive correlations between minor elements may also be noticed. This can be viewed as the simple effect of variable dilution or, in the case of bio-accumulators, as their capacity to incorporate dust, related to morphology, exposure, and age. Although these mathematical problems have been identified for several decades (Chayes 1960; Aitchison 1986), or even centuries (Pearson 1897), environmental geochemists continue widely and routinely to apply inappropriate statistical methods. Yet better statistical/mathematical solutions exist and can easily be implemented, as accessible and comprehensive tools are now available (van den Boogaart and Tolosana-Delgado 2013). The aim of the present study is to explore their potential for assessing air quality via bio-accumulators, more particularly by focusing on perturbation vectors, as a means of expressing compositional changes for comparing samples (Von Eynatten et al. 2002; Aitchison and Ng 2005). Four sites differently affected by anthropogenic pollution were therefore selected around Rio de Janeiro, Brazil. Samples belonging to one lichen species, *Parmotrema crinitum*, and to one bromeliad species, *Tillandsia usneoides*, were collected and analyzed for their aluminum (Al), cadmium (Cd), copper (Cu), iron (Fe), lanthanum (La), lead (Pb), sulfur (S), titanium (Ti), zinc (Zn), and zirconium (Zr) contents. The present study seeks to provide a promising new way to represent the response of these bioaccumulators to variable air pollution, using perturbation vectors.

Materials and methods

Study site

The study area is located in the State of Rio de Janeiro (southeastern Brazil). It encompasses the Rio de Janeiro metropolitan area (RJMA), rural areas, and protected forests (Fig. 1). The RJMA is the second largest urban agglomeration in Brazil, with 12.2 million inhabitants, and it possesses the second largest industrial park, including metallurgy, shipping yards, oil refineries and petrochemical, gas-chemical, textile, printing, and pharmaceutical industries. The relief of the study area is contrasted, with coastal plains, intermittent deep valleys, and mountains (2000 m asl), 60 km north of the seashore. These mountains are part of the Brazilian crystalline complex, with granitic and metamorphic rocks, producing sediments deposited in plains and valleys. The annual average temperature in this region ranges from 13 to 23 °C, while total annual precipitation ranges from 1000 to 3600 mm, typical of a humid tropical climate (Castro 2008). The dry season extends from May to July, and the rainy season from December to February.

Main features of sampled lichens and bromeliads

Tillandsia usneoides, also known as Spanish moss, is an epiphytic bromeliad found throughout Central and South America (Malm et al. 1998; Figueiredo et al. 2004). It presents morphological and physiological characteristics that make it a very suitable bioindicator of metal pollution (Schrimpff 1984; Benzing and Bermudes 1991; Padaki et al. 1992; Pyatt et al. 1999). Water, nutrients, minerals and also metals, metalloids, and pollutants are directly derived from the atmosphere, and not from the substrate (Bermudez et al. 2009). *Tillandsia usneoides* lacks an extensive vascular system, and its stem and leaves are covered by peltate trichomes (scales) that protect it from UV radiation and desiccation. *Parmotrema crinitum* is a lichen widespread in humid habitats, in both temperate and tropical regions. This lichen, loosely attached to the substratum, whether bark or rock, is characterized by a foliose thallus, leafy gray lobes, large ciliate isidia, and is able to withstand prolonged drying. Because they have no roots, the substrate on which they live is not thought to contribute significantly to metal content (Loppi and Pirintsos 2003). Like plants from the *Tillandsia* genus, they obtain nutrients directly from the air.

Sample collection and analysis

Samples were collected at four sites within the state of Rio de Janeiro (Fig. 1). The RJ site is in the Botanical Garden of RJMA, an urban location, almost at sea level, 3 km from the seashore. Two other sites (PB and PH) are located in the "Parque Nacional da Serra dos Órgãos" (Fig. 1), a biological reserve of 20,024 ha, housing endemic species of the Atlantic Forest, and high-altitude ecosystems, 60 km north of RJMA. This park is a hotspot for biodiversity conservation (Castro 2008), but is nevertheless potentially subjected to atmospheric deposition from RJMA, due to a cold front coming in from the south. Although PB and PH are geographically close to each other (4.5 km), PB is located in the foothills at 410 m asl, while PH is at an altitude of 1290 m. The LUM site is close to the small town of Lumiar (750 m asl), about 110 km NE of RJMA (Fig. 1). Except for some small-scale agricultural activity, there is no clear anthropogenic source nearby.

Between 12 and 15 specimens of lichen (*P. crinitum*) were sampled at each of these four sites, always within a ~ 10-m-radius circle. In addition, around 15 specimens of *T. usneoides* were sampled at the LUM and RJ sites, but *T. usneoides* could not be found at the PB and PH sites. All samples were collected at an elevation of at least 1.5 m from the ground, always on living trees. Although the soils present different types, yellow-red podzol for RJ, red-yellow latosol for PB and PH, and cambisol for LUM (Ker, 1997), the sampling elevation in relation to the ground, together with the epiphyte nature of the organisms sampled prevent direct metal uptake from local soils. In the laboratory, lichens (consisting of a single thallus) and bromeliads were dried at 45 °C up to constant weight. The *T. usneoides* samples were composed of the most exterior part of the stem (ca 3 cm). All samples were manually powdered in a pre-cleaned agate mortar. About 60–100 mg (precisely weighed) was totally digested in a mixture of 2 mL each

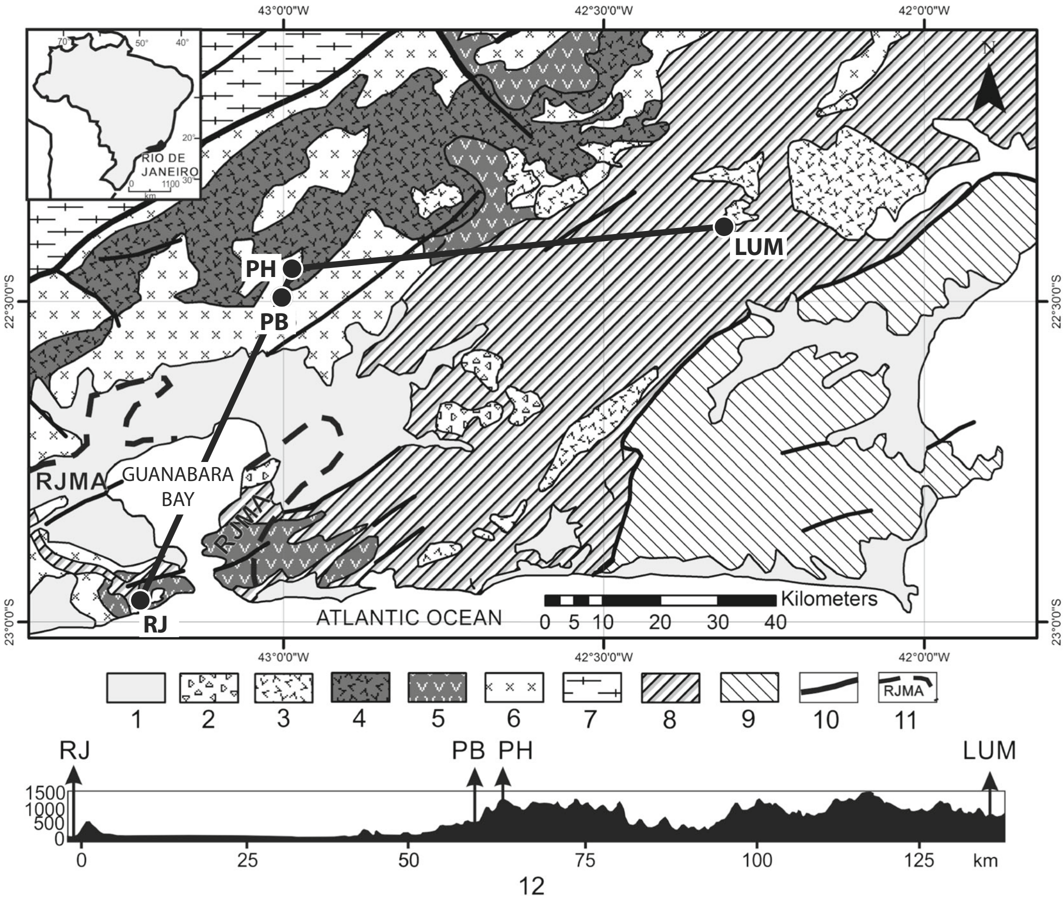

Fig. 1 Simplified geological map of the study area in the state of Rio de Janeiro, showing sampling sites: RJ (22° 58.07 S, 43° 13.43 W), PB (22° 29.55 S, 43° 00.12 W), PH (22° 27.12 S, 43° 00.07 W) and LUM (22° 21.02 S, 42° 19.72 W) and geological information: 1. Cenozoic deposits, 2. KT igneous alkaline rocks, 3. biotite granites (510–480 Ma), 4. Granites (560 Ma), 5. leucogranites and charnockites (590–560 Ma), 6. Rio Negro magmatic arc and related suites (790–620 Ma), 7. Paraíba do Sul complex, 8. paragneisses, 9. orthogneisses, amphibolites and paragneisses, 10. Faults, 11. Rio de Janeiro Metropolitan Area (RJMA), 12. Topographic profile throughout the sampling sites

of concentrated HNO_3, HCl, and HF acids of Suprapur grade (Merck, Germany). The solutions were evaporated, retaken with 2 mL of nitric acid, properly diluted with Milli-Q demineralized water, and analyzed with a Spectro ARCOS inductively coupled plasma–atomic emission spectroscope assisted by a Setac ultrasonic nebulizer, for elements presenting a potentially anthropogenic origin (Cd, Cu, Pb, S, and Zn), and for those predominantly of crustal origin (Al, Fe, La, Ti, and Zr). Concentrations were expressed on a dry mass basis. Eight blanks and certified reference materials (CRMs), namely peach leaves NIST-1547, harbor sediment PACS-1, marine sediment BCSS-1, basalt BCR-2, freshwaters (SLRS-4 and SLRS-5), were also processed, together with sample batches. Blanks appeared to be low with respect to the elemental compositions of biological samples (see Supplementary materials, SM1). Concentrations measured for CRMs did not differ from certified values by more than 10–15%, at least when the concentration levels sufficiently exceeded the LODs (Supplementary materials, SM1). The results of five duplicates indicated a suitable replicability with respect to the range of variation observed throughout the study (Supplementary materials, SM1).

Mathematical treatment

Fundamentals of compositional data analysis (often abbreviated as CoDA) are briefly presented here for readers who are not familiar with the approach (more details can be found in Aitchison 1986; Aitchison 1992; Barceló-Vidal et al. 2001). The underlying concept is that since compositional data carry relative information, absolute values are therefore of limited interest, and only the ratios between variables are relevant. In this sense, the approach is somewhat similar to ratio-based isotopic geochemistry, or to the principles underlying the enrichment factor. Let **x** denote a positive vector whose D components are a subset of measured concentrations $\mathbf{x} = [x_1,\ldots,x_D]$, summing up to a constant κ, with $\kappa \leq 1$. The vector **x** can be reclosed to $\kappa = 1$ as follows:

$$\mathcal{C}(\mathbf{x}) = \left[\frac{x_1}{\sum_{i=1}^{D} x_i}, \ldots, \frac{x_D}{\sum_{i=1}^{D} x_i}\right] \tag{1}$$

Once reclosed, two samples, say **a** and **b**, for which Zn, Pb, and Cu concentrations have been measured: **a** = [24, 12, 4] and **b** = [12, 6, 2], become compositionally equivalent and equal to [0.6, 0.3, 0.1], because their internal ratios are the same (Barceló-Vidal 2001). After closure, the sample space is not the real space R^D, but a simplex S^D, in which the perturbation plays the role of sum or translation (Aitchison and Ng 2005). Let $\mathbf{x} = [x_1,\ldots,x_D]$ and $\mathbf{y} = [y_1,\ldots,y_D]$ denote two compositional vectors in S^D. Then **z**, corresponding to the perturbation of **x** by **y** in S^D, is given by:

$$\mathbf{z} = \mathbf{x} \oplus \mathbf{y} = \mathcal{C}[x_1 y_1, \ldots, x_D y_D] \tag{2}$$

The neutral element of the perturbation is **e** $= \mathcal{C}[1, \ldots, 1] = [1/D,\ldots,1/D]$, and $\mathbf{x} = \mathbf{x} \oplus \mathbf{e}$. The inverse of a perturbation **y** is defined as the closure of the inverse of **y**:

$$\mathbf{y}^{-1} = \mathcal{C}\left[y_1^{-1}, \ldots, y_D^{-1}\right] \tag{3}$$

The operation of this inverse on **x,** noted $\mathbf{x} \oplus \mathbf{y}^{-1}$ or $\mathbf{x} \ominus \mathbf{y}$, corresponds to the perturbation vector, which expresses the change from **y** to **x**. For our previous example, $\mathbf{b} \ominus \mathbf{a}$, which corresponds to compositional changes from **a** to **b**, equals [1/3, 1/3, 1/3], i.e., the neutral element, as **a** and **b** are compositionally equivalent. Now, let us consider a new composition **c** = [50, 20, 10], $\mathbf{c} \ominus \mathbf{a} = \mathbf{c} \ominus \mathbf{b} = [0.324, 0.406, 0.270]$, note that the sum of the elements equals 1. This subtraction operator will be of particular interest in the following.

To circumvent the problem of closed data, Aitchison (1986) introduced the centered log-ratio transformation:z

$$clr(\boldsymbol{x}) = \left[\ln\frac{x_1}{gm(\boldsymbol{x})}, \ln\frac{x_2}{gm(\boldsymbol{x})}, \ldots, \ln\frac{x_D}{gm(\boldsymbol{x})}\right] \tag{4}$$

where $gm(\mathbf{x})$ denotes the geometric mean of the D parts: $gm(\boldsymbol{x}) = \left(\prod_{i=1}^{D} x_i\right)^{1/D}$. Then, the structuration inside the compositional dataset can be explored using a compositional biplot, which displays the relative variation of a multivariate dataset by projection on to a plane, most of the time defined by the first two or three principal components (Aitchison and Greenacre 2002). This representation allows samples and variables to be depicted together, but differs from the classical biplot of Gabriel (1971) in the sense that *clr*-transformed data are used as inputs. The links between two arrow heads (i.e., the projection of variables) are of interest. They correspond to the log ratios between the two components involved (for more details see Aitchison and Greenacre 2002; van den Boogaart and Tolosana-Delgado 2013). The application of some other multivariate analyses, such as multiple analysis of variance (MANOVA), undertaken to compare multivariate population means from several groups, nevertheless raises particular problems, as the covariance matrix of *clr*-transformed data is always singular. As an alternative, Egozcue et al. (2003) developed the *ilr*-transformation (*ilr* for isometric log-ratio), which allows a composition in the D-part Aitchison-simplex to be isometrically transformed into a (D-1) dimensional Euclidian vector. The *ilr*-transformation is performed following:

$$ilr(\mathbf{x}) = \mathbf{z} = [z_1, \ldots, z_{D-1}] \in \mathbb{R}^{D-1}, \quad z_i = \sqrt{\frac{i}{i+1}} \ln \frac{\sqrt[i]{\prod_{j=1}^{i} x_j}}{x_{i+1}}, for\ i = 1, \ldots, D-1 \tag{5}$$

Interestingly, the covariance matrix of the *ilr*-transformed dataset is invertible, so that MANOVA, and possibly post-hoc tests, can be applied straightforwardly on *ilr*-coordinates (Kovacs et al. 2006). In the following, all data transformations, operations, and statistical procedures used the packages compositions (van den Boogaart and Tolosana-Delgado 2008), FactoMineR (Lê et al. 2008), and Hotelling, developed for the free R software, http://www.r-project.org/ (R Development Core Team 2008).

Table 1 Raw geometric mean of elemental concentrations measured in lichens and bromeliads by site: RJ for Rio de Janeiro's botanical garden, PB and PH for the Parque Nacional da Serra dos Órgãos, and LUM for Lumiar. Concentrations are expressed in μg g^{-1} on a dry mass basis

	Al	Ti	Fe	Zr	La	Cd	Cu	Pb	S	Zn
					Geometric means					
Lichens (μg g^{-1})										
RJ (n = 15)	1960	170	1050	3.5	3.3	0.43	8.1	21.5	1100	32.8
PB (n = 15)	670	40	310	1.1	1.4	0.34	6.9	4.0	810	23.6
PH (n = 15)	790	51	450	1.1	1.4	0.34	7.4	3.4	850	30.0
LUM (n = 11)	3690	250	1600	9.5	6.0	0.27	6.3	5.6	580	25.8
Bromeliads (μg g^{-1})										
RJ (n = 15)	1040	79	570	1.3	2.0	0.45	7.7	7.2	960	33.0
LUM (n = 15)	1870	110	900	1.5	2.0	0.30	4.2	3.7	610	14.3

Results and discussion

Lichens

Geometric mean compositions of lichens from each of the study sites are given in Table 1 (the entire raw dataset can be found in Supplementary Material SM2). The same decreasing pattern (LUM > RJ > PB~PH) is observed for all lithophilic elements (Al, Ti, Fe, Zr, and La), but the geometric means of Cd, Cu, S, Pb, and Zn are higher at RJ. The data were further explored using the covariance biplot (Fig. 2a). The first two principal components explain over 83% of the total variance (66.8 and 16.6% respectively). Two groups of variables were clearly identified by the sign of loadings on component 1, and the proximity of their arrow heads: (i) on the left side of the biplot, the lithophilic elements: Al, Fe, La, Ti, and Zr; and (ii) on the right side: S, Cd, Cu, and Zn. The proximity of these elements (i.e., their links are short) within each group indicates relatively constant log-ratios, while the largest links observed between [Zn, Cu, Cd, and S], on the one hand, and [Al, Fe, La, Ti, Zr], on the other hand, indicate the most relative variations across the lichens. Lead lies apart, in the upper part of the biplot. The sample distribution in this ordination space points to three distinct groups, without any overlapping: LUM, RJ, and PH-PB, with the LUM samples least affected by anthropogenic inputs. The predominance of inter-site variability over intra-site variability was more formally tested using a MANOVA, performed

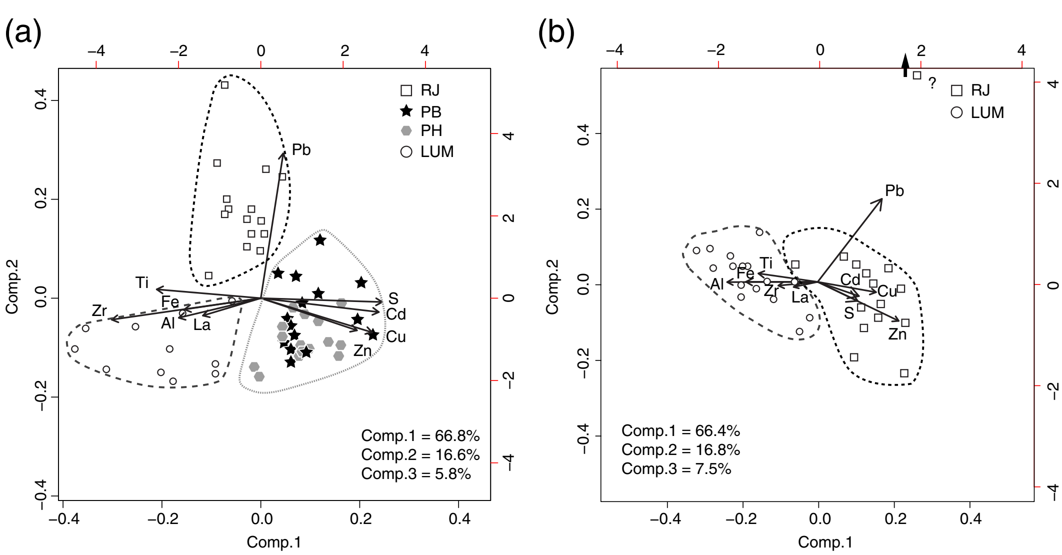

Fig. 2 Covariance biplots for (**a**) lichens at RJ, PB, PH, LUM, and for (**b**) bromeliads at RJ and LUM

on *ilr*-coordinates. As expected, the null hypothesis, where vectors corresponding to mean compositions would be the same at all sites, was significantly rejected ($p < 10^{-6}$). Pairwise tests using Hotelling's T-squared statistics suggest that all four study sites were significantly different ($p < 0.05$). Nevertheless, when a Bonferroni correction was introduced to counteract the problem raised by multiple comparisons (Hochberg 1988), the samples from PH and PB were no longer significantly different (see Supplementary Material SM4 for more details).

Bromeliads

The total variance carried by the first two principal components, and the variable grouping are the same as for lichens (Fig. 2b). Lithophilic variables are clustered together, while Cu, Zn, S, and Cd form a distinct group on the positive side of loadings of component 1. Lead was once again apart from the other vectors. The MANOVA indicated that the vector means at RJ and LUM are significantly different ($p < 10^{-6}$), due to Cd, Cu, Zn, S, and Pb enrichments at RJ compared to LUM.

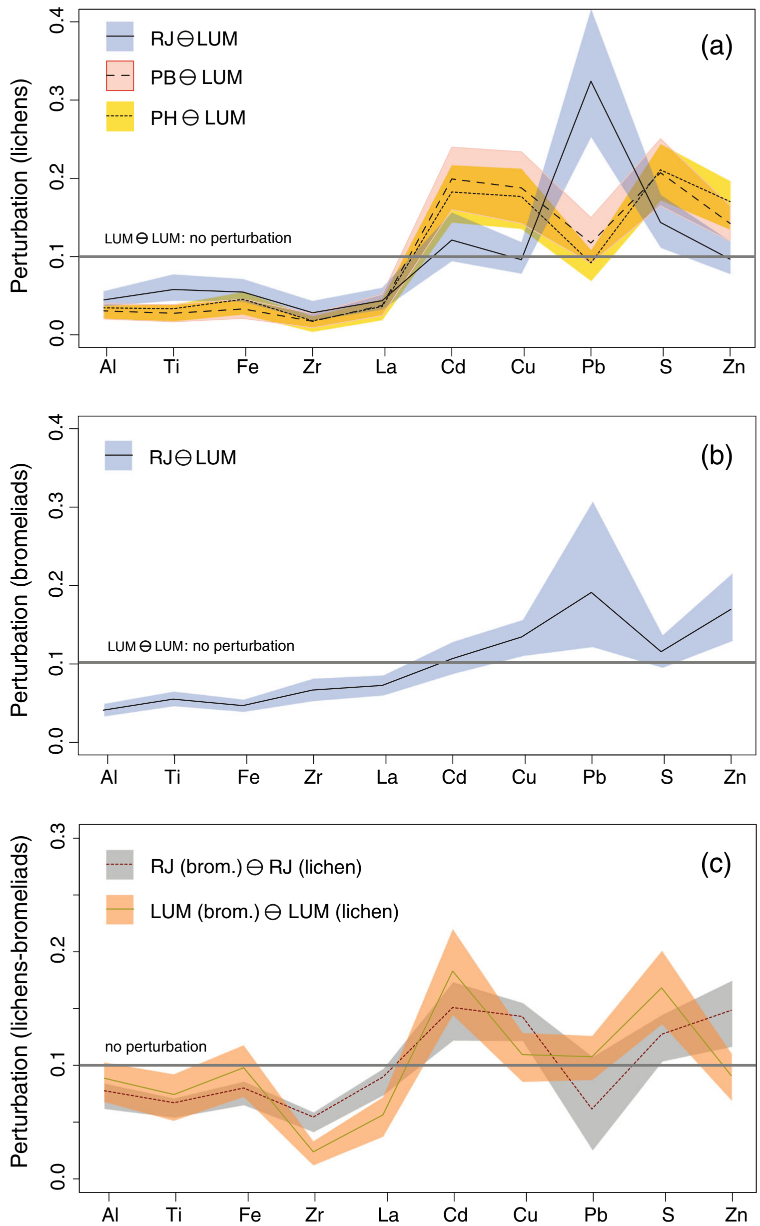

Fig. 3 Graphical representation of perturbation vectors: (**a**) between LUM, chosen as reference, and PB, PH, and RJ for lichens; (**b**) between LUM, chosen as reference, and RJ for bromeliads; (**c**) between lichens and bromeliads at LUM and RJ. The 95% confidence intervals were evaluated by bootstrapping

The perturbation operator as a new environmental proxy

Let the LUM site, the most distant from Rio de Janeiro and its anthropogenic activity, be the reference site. To assess pollution levels in the environment via biomonitors, study sites are generally compared to reference sites (preferably unpolluted), either using raw concentrations or EFs. Our strategy rather consists in calculating perturbation vectors that delineate compositional changes between LUM (the least polluted) and the other sites (i.e., RJ⊖LUM, PB⊖LUM, and PH⊖LUM for lichens, and RJ⊖LUM for bromeliads). These calculations were performed after closure of the compositional vectors, so that the primitive relationships with absolute concentration values were lost. The 95% confidence intervals of the perturbation vectors were estimated by bootstrapping the original dataset with $n = 1000$ (Efron 1979), as shown in Fig. 3a, b. The key reading of such diagrams is the position of each variable with respect to the neutral change value: here 0.1 (1/10, as 10 variables were processed). Values above (below) 0.1 imply a relative increase (decrease), while the ratios of two elements presenting the same perturbation value (i.e., plotting at the same height on the diagram, whatever their position with respect to the $y = 0.1$ line) will be unchanged. Lichens from RJ, PH, and PB exhibited a relative depletion in lithophilic elements compared to lichens from LUM (Fig. 3a). At RJ, a strong increase was noticed in Pb, and to a lesser extent in S. Unsurprisingly, the perturbation vectors from LUM to PH, and from LUM to PB, both indicating a significant increase in Cd, Cu, S, and Zn, could be not distinguished from one another. With regard to *T. usneoides*, the perturbation vector between LUM and RJ (Fig. 3b) exhibits a pattern similar to that observed for lichens (Fig. 3a), but with a slight enrichment in Zn, and less marked relative enrichment in Pb. These findings are consistent with the environmental context in which the lichens and bromeliads lived. The strong Pb enrichment at RJ is not unexpected. Although leaded gasoline was finally phased out in Brazil by 1993 (Lovei 1998), lead concentrations in soils remain high, and lead-enriched particles, remobilized by wind and human activity, can still reach organisms living nearby. A similar process has been observed in an urban/periurban area of northeastern France (Cloquet et al. 2006). Automotive traffic also emits sulfur. It would be misleading to consider that Zn, Cd and Cu are unchanged between LUM and RJ. In fact, lithophilic elements are concomitantly depleted in the RJ samples, so that the ratios of [Zn, Cd, or Cu] over, e.g., Al, are much higher at RJ than at LUM, demonstrating an enrichment in elements of anthropogenic origin. The similarity between the perturbation vectors of PB and PH, sites only 4 km apart but at different altitudes, tends to indicate that the altitude factor has no influence, in our case, on the elemental ratios. These sites are more affected by anthropogenic inputs of Cd, Cu, S, and Zn than RJ, probably because these pollutants, emitted into the atmosphere from industries around Guanabara Bay, are partly transported by the prevailing southeast trade winds through the mountains.

Although raw concentrations were different in lichens and bromeliads, probably because these species possess their own physiological response, in terms of needs, or protection against certain elements, the perturbation vectors between LUM and RJ were quite similar for both species (Fig. 3a, b). Figure 3c presents this interesting feature in a different way, using the perturbation vectors between lichens and bromeliads, at LUM and RJ (Fig. 3c). Both perturbation vectors matched reasonably well, whatever the local degree of pollution. It would therefore have been possible to estimate the bromeliad values from the lichen measurements (or vice versa). Provided that the perturbation vector between species is known and well constrained, biomonitoring surveys could thus be based on a multispecies approach, once measurements have been corrected to make results comparable. After further validation, the procedure presented in this paper will offer new perspectives for biomonitoring studies, especially in cases where it is difficult or impossible to find enough individuals from the same species.

Conclusion

The perturbation vector allows the compositional differences between two sites to be summarized in an original, simple, and informative graphical way. Rapid comparisons can be made in terms of internal ratios, similarly to what is routinely done in other geochemical contexts (for instance, with rare earth elements, where the shapes of the REE spectra are of interest). Perturbation vectors not only appear to be particularly well adapted to biomonitoring studies but also to a huge number of situations frequently met in environmental geochemistry, where elemental ratios are more relevant than absolute concentrations. It should however be kept in mind that, as compositional data analysis only conveys ratio information, absolute values of the original concentrations are lost. This may be a drawback for ecotoxicological studies, where body concentration levels are of primary importance. Absolute concentrations should be therefore presented and discussed in parallel with perturbation vectors.

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