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Native Trees as Biomonitors of Chemical Elements in the Biodiversity Conservation of the Atlantic Forest

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Abstract. The Atlantic Forest, a hotspot in the world with a great diversity of plants and animals, is located in the most industrialized area of Brazil, a potential source of chemical elements for the atmosphere. From its original area about 10% has been preserved mainly through the implementation of conservation units, among which the Parque Estadual Carlos Botelho (PECB) is one of the most representative. Here, leaves of the predominant species in the PECB were analyzed by instrumental neutron activation analysis (INAA) for the establishment of natural backgrounds of As, Ba, Br, Ca, Cd, Ce, Co, Cs, Fe, Hg, K, Na, Rb, Sc, Se, Sr and Zn. Biomonitoring of the Atlantic Forest was realized through the tree community study taking in account the interspecies and the intraspecies variability of chemical concentrations. Results pointed out the low status of pollution based on the concentrations of chemical elements of environmental concerning. However, Br concentrations were higher in the understory species, which could be related to the possible effects of atmospheric pollution or sea influence. In addition, some *Hyeronima alchorneoides* trees showed to be hyperaccumulators of Co.

Key words: Atlantic Forest, biomonitoring, chemical elements, INAA, bromine, cobalt.

1. Introduction

Tropical forests are characterized by the low availability of nutrients in the soil, for that reason the sustainability depends on the continuous supply of chemical elements from atmospheric sources (Waring and Schlesinger, 1985). In such forests, biochemical cycling and other conserving mechanisms have a fundamental function, considering the high potential for nutrient loss in the wet tropics (Jordan, 1985). Therefore, the assessment of plant nutritional status and atmospheric contributions, as well as the distribution of trace elements in the biological compartments, are priorities for understanding and maintaining the biodiversity in these environments.

Biological compartments of the ecosystem can be employed as monitors of atmospheric inputs, due to their capacity in accumulating chemical elements, in spite of the adaptability to the environmental chemical variations (Markert, 1991; Kabata-Pendias and Pendias, 1984; Koyama *et al.*, 1987). The use of vascular plants for biomonitoring has advantages related to easy taxonomic identification, well-known physiology and possibility of selecting different parts (leaf, fruit, branch and root) for analysis (Witting, 1993).

A database containing results for as many elements in biomonitors as possible should be generated in order to establish background levels and to provide information for the identification of the element sources in a given ecosystem. For this purpose, multielement analytical methods are normally used, and instrumental neutron activation analysis (INAA) has a special role, due to its ability to analyze untreated solid samples for many elements with a high degree of sensitivity and selectivity (Smodiš and Bleise, 2002). Moreover, it is possible to evaluate different groups of elements, including nutrients, pollutants and trace elements.

The increasing environmental problems in Brazil have pushed the development of biomonitoring studies as a relevant tool for establishing the natural background and for studying the contamination by chemical elements. There is little information on the distribution of chemical elements in Brazilian ecosystems, chiefly in the Atlantic Forest, a hotspot of the global diversity of plants and animals (Myers *et al.*, 2000). A previous work, based on the analysis of tree leaves from eight species of the Atlantic Forest (França *et al.*, 2004), revealed measurable concentrations of Br, Ca, Co, Cs, K, Rb, Sr and Zn.

Because of the high biodiversity, the study of Brazilian forests in intricate, requiring multidisciplinary approaches. Thereby, the Virtual Institute of Biodiversity - BIOTA Program was implemented in 1999 by the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP) for the characterization of biodiversity in the São Paulo State.

One of the BIOTA projects is “Diversity, dynamics and conservation in São Paulo State forests: 40 ha of permanent parcels,” which aims at understanding the dynamics, generation and maintenance of the biodiversity, as well as adapting strategies for conservation, management and restoration. Four conservation units were selected for implantation of 0.1 km^2 permanent parcels or long-term plots, as to represent the major vegetation types of the state—Slope Atlantic Forest, Restinga Forest, Semideciduous Seasonal Forest and Cerradão. Several surveys have been carried out in these plots, involving aspects of botany, chemistry, climatology, ecology and microbiology.

Within the scope of such BIOTA’s project, this paper provides estimates of the background concentrations of As, Ba, Br, Ca, Cd, Ce, Co, Cs, Fe, Hg, K, Na, Rb, Sc, Se, Sr and Zn in the Atlantic Forest by studying the long-term plot implanted in the “Parque Estadual Carlos Botelho” (PECB). As the most abundant tree species are responsible for the major part of chemical elements cycling (Golley *et al.*, 1978), the composition of leaves from the predominant native trees was determined by INAA.

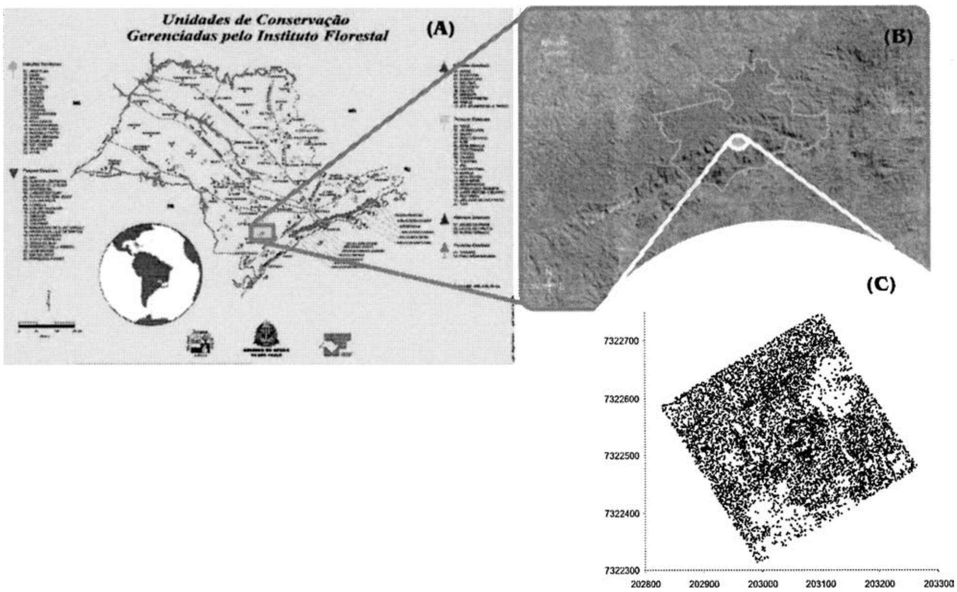

Figure 1. (A) Conservation units of São Paulo State; (B) Parque Estadual Carlos Botelho; (C) Trees identified in the long-term plot.

2. Experimental

The Parque Estadual Carlos Botelho (PECB) with 380 km^2 of well-preserved vegetation is located at the southwest portion of São Paulo State, Brazil, among the municipalities of São Miguel Arcanjo, Capão Bonito and Sete Barras. The long-term plot (0.1 km^2) was installed in the PECB at 800 m altitude facing the Atlantic Ocean (Figure 1). According to the databank of the BIOTA's project, about 10,600 trees with diameters higher than 5 cm were mapped and individually marked on the field. A phytosociological inventory has identified all marked trees to the species level. The palm *Euterpe edulis* is the dominant understory species, totalizing 31% of the identified trees.

Ten trees from each of the predominant species in the area were selected for sampling (Table I). Leaves (approximately 500 g) were collected from the middle-crown and lower-crown at February 2003. Information about health (injury and herbivory) was also compiled. The distribution of the sampled trees (Figure 2) was established according to the natural occurrence and local accessibility. Due to the misidentification of two trees, only eight samples of *Chrysophyllum inornatum* were analyzed.

The leaves were washed with water and oven-dried at 60°C until constant weight. After particle size reduction (0.5 mm), test portions of 250 mg were transferred to polyethylene vials (Vrije Universiteit, Amsterdam) for irradiation with neutrons. The certified reference materials INCT-TL-1 Tea Leaves and IAEA-336 Lichen

Table I. Characteristics of trees and leaves sampled in the PECB long-term plot

		Injury (%)		Infestation (%)	
Species	Family	min	max	min	max
BM *Bathysa meridionalis*	Rubiaceae	0	40	5	40
CI *Chrysophyllum inornatum*	Sapotaceae	10	25	5	15
EC *Eugenia cuprea*	Myrtaceae	5	60	1	10
EE *Euterpe edulis*	Arecaceae	5	20	1	10
GG *Garcinia gardneriana*	Clusiaceae	5	50	1	10
GF *Gomidesia flagellaris*	Myrtaceae	10	20	1	20
GO *Guapira opposita*	Nyctaginaceae	5	20	5	30
HA *Hyeronima alchorneoides*	Euphorbiaceae	0	15	1	10
TG *Tetrastylidium grandifolium*	Olacaceae	5	30	1	10
VB *Virola bicuhyba*	Myristicaceae	0	20	1	20

were included in the analytical series for quality control. Ni-Cr alloy wires of 10 mg were employed for thermal neutron flux monitoring (França *et al.*, 2003a). The irradiation was carried out in the nuclear research reactor IEA-R1m, of the Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN), São Paulo, at a thermal neutron flux of 10^{13} cm^{-2} s^{-1} during 8 h. Two different germanium detectors (45% and 50% relative efficiencies at ^{60}Co 1332 keV, from Ortec) were used for measuring the induced radioactivity. Chemical elements were determined by k_0-method instrumental neutron activation analysis (k_0-INAA) using the Quantu software package (Bacchi and De Nadai Fernandes, 2003).

Exploratory statistical analysis was performed by factorial model based on the principal components of standardized concentrations as described elsewhere (Johnson and Wichern, 1998). Data were transformed attending the requirements related to the normality and outliers detection. Factor loadings were useful for variable separation, while factor scores were applied to the evaluation of variability of chemical composition.

3. Results and Discussion

A good agreement was verified between the obtained and assigned concentrations for the certified reference materials (Table II) evidencing the analytical quality for the determination of 15 chemical elements in leaves.

Compared to species growing in other natural ecosystem of the São Paulo State (França *et al.*, 2003b), the tree leaves of the Atlantic Forest have similar concentrations of Ca, K, Na, Rb and Sr (Table III). In fact, high efficiency in the absorption and maintenance of nutrients in biological compartments are normally observed

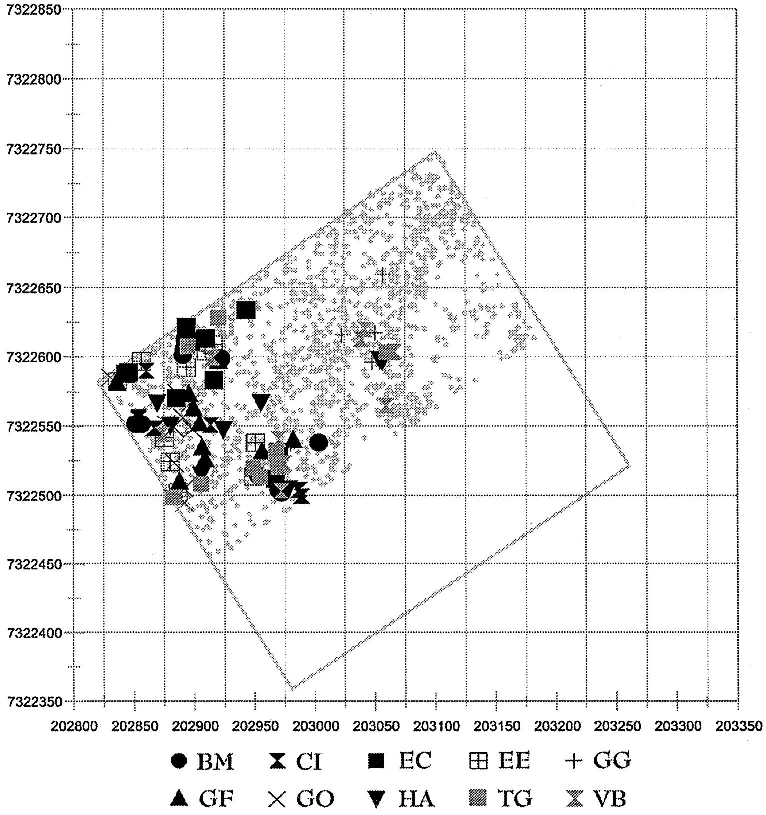

Figure 2. Spatial distribution of sampled trees in the PECB long-term plot. Dots refer to all trees available for selection. BM = *Bathysa meridionalis*, CI = *Chrysophyllum inornatum,* EC = *Eugenia cuprea*, EE = *Euterpe edulis*, GG = *Garcinia gardneriana*, GF = *Gomidesia flagellaris*, GO = *Guapira opposita*, HA = *Hyeronima alchorneoides*, TG = *Tetrastylidium grandifolium*, VB = *Virolo bicuhyba.*

for plants growing in oligotrophic ecosystems (Arnason *et al.*, 1984). Furthermore, the Atlantic Forest has a low status of As, Cd, Hg and Se, considering the average content ranges in plants provided by Market (1998)—As: 0.01–1.5 mg kg^{-1} Cd: 0.03–0.5 mg kg^{-1}; Hg: 0.005–0.2 mg kg^{-1}; Se: 0.01–2 mg kg^{-1}.

Among the investigated species, the palm *Euterpe edulis* showed the lowest concentrations, with exception of Cs and Zn. Such behavior, associated to the ecological relevance of *E. edulis* and its wide distribution within the PECB plot, corroborates the application of this species to establish the natural background of chemical elements in the Atlantic Forest.

A comparison between the inter- and intraspecific variability of chemical concentrations can be found in Figure 3, where the error bars represent the dispersion of results, expressed as standard deviation. In general, *Guapira opposita* (GO) showed the most significant variability of chemical composition, in spite of the

Table II. Results (mg kg^{-1}) for chemical elements in the certified reference material compared to the reference values

	INCT-TL-1 Tea Leaves ($n = 11$)				IAEA-336 Lichen ($n = 11$)			
	Obtained		Reference		Obtained		Reference	
	Mean	u_c	Mean	U	Mean	u_c	Mean	95% interval
As	< 0.14		0.11	0.02	0.69	0.06	0.63	0.55–0.71
Br	12.4	0.4	12.3	1.0	13.6	0.4	12.9	11.2–14.6
Ca*	0.55	0.02	0.582	0.052	0.257	0.013	not available	
Cd	< 0.6		0.030	0.004	< 0.6		0.117	0.100–0.134
Co	0.392	0.012	0.387	0.042	0.317	0.011	0.29	0.24–0.34
Cs	3.46	0.15	3.61	0.37	0.120	0.007	0.110	0.097–0.123
Fe	506	12	432		457	12	430	380–480
Hg	< 0.03		0.0049	0.0007	0.17	0.02	0.17	0.15–0.19
K*	1.61	0.03	1.70	0.12	0.194	0.006	0.184	0.164–0.204
Na	20.9	0.6	24.7	3.2	348	6	320	280–360
Rb	83	3	81.5	6.5	1.95	0.15	1.76	1.54–1.98
Sc	0.251	0.006	0.266	0.024	0.192	0.005	0.17	0.15–0.19
Se	< 0.10		0.076		0.24	0.03	0.22	0.18–0.26
Sr	23	2	20.8	1.7	11.4	1.8	9.3	8.2–10.4
Zn	32.5	1.3	34.7	2.7	30.7	1.2	30.4	27–33.8

*Values in % (g g^{-1}).
U_c = mean combined uncertainty.

similar environmental conditions among the trees (110 m of maximum distance). Some accumulation of Br, Na, Ca and Sr could be verified in leaves of this species. The highest concentrations of Se are obtained in leaves of *Tetrastylidium grandifolium* and *Eugenia cuprea*, which also shows the highest Hg levels. There is some indication of other sources, beside soil, contributing for the concentration of both Hg and Se in these plants. The possibility of atmospheric contribution for Hg concentration in leaves was already discussed elsewhere (Ferrari *et al.,* 2003).

The factor analysis, followed by plotting of factor values, provided a better understanding of Br, Ca, Co, Cs, Fe, K, Na, Rb, Sc, Sr and Zn distribution (Table IV). The grouping of variables in factors revealed sources of chemical elements or similar chemical behavior, which was noticed for Rb and K, as well as for Ca and Sr. Factor 3 is connected with soil particles adhered to the surface of leaves, since the Fe and Sc concentrations in plants are usually related to soil contamination (Fernandes, 1993). There is also an evidence of marine influence due to the correlation of Br and Na with factor 4 (Djingova and Kuleff, 1993).

Table III. Results (mg kg^{-1}) for chemical elements in the leaves. Standard deviations calculated for results from ten independent samples

	Bathysa meridionalis BM ($n = 10$)		Chrysophyllum inornatum CI ($n = 8$)		Eugenia cuprea EC ($n = 10$)		Euterpe edulis EE ($n = 10$)		Gomidesia flagellaris GF ($n = 10$)	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
As	< 0.11		< 0.11		< 0.11		< 0.11		< 0.11	
Br	3.3	2.6	18.9	10.2	21.3	6.4	5.7	1.9	14	2
Ca*	0.8	0.2	1.2	0.4	1.1	0.2	0.39	0.09	0.76	0.08
Cd	< 0.6		< 0.6		< 0.6		< 0.6		< 0.6	
Co	0.19	0.14	0.10	0.06	0.17	0.12	0.14	0.34	0.19	0.06
Cs	0.17	0.06	0.14	0.08	0.09	0.03	0.23	0.09	0.18	0.06
Fe	186	151	110	58	137	27	132	54	93	16
Hg	0.038	0.017	0.12	0.03	0.18	0.04	0.05	0.02	0.097	0.017
K*	2.0	0.6	1.3	0.4	0.7	0.2	0.9	0.2	0.8	0.2
Na*	0.11	0.04	0.065	0.012	0.06	0.05	0.13	0.11	0.11	0.05
Rb	66	29	31	12	18	5	28	7	22	5
Sc	0.046	0.046	0.016	0.008	0.035	0.008	0.024	0.012	0.020	0.005
Se	0.21	0.09	0.20	0.05	0.55	0.18	0.19	0.07	0.23	0.05
Sr	130	34	290	116	200	66	28	10	126	40
Zn	37	8	14	3	19	5	44	14	11	2

(*Continued on next page.*)

Table III. (*Continued*)

	Garcinia gardneriana GG ($n = 10$)		Guapira opposita GO ($n = 10$)		Hyeronima alchorneoides HA ($n = 10$)		Tetrastylidium grandifolium TG ($n = 10$)		Virola bicuhyba VB ($n = 10$)	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
As	< 0.11		< 0.11		< 0.11		< 0.11		< 0.11	
Br	5.8	1.7	33	18	7	2	20	5	2.6	1.6
Ca*	0.8	0.2	1.3	0.5	0.7	0.2	0.4	0.2	0.7	0.2
Cd	< 0.6		< 0.6		< 0.6		< 0.6		< 0.6	
Co	2.7	2.3	0.14	0.05	15	36	0.042	0.016	2.2	6.5
Cs	0.13	0.04	0.22	0.07	0.11	0.05	0.18	0.08	0.12	0.06
Fe	62	22	93	23	64	13	114	38	78	28
K*	0.7	0.2	2.5	0.7	1.4	0.3	1.12	0.13	1.18	0.26
Hg	0.06	0.02	0.032	0.011	0.029	0.012	0.044	0.014	0.08	0.04
Na*	0.11	0.03	0.74	0.12	0.054	0.01	0.22	0.04	0.05	0.04
Rb	19	5	73	21	40	13	27	7	39	10
Sc	0.011	0.004	0.019	0.007	0.007	0.002	0.024	0.012	0.011	0.008
Se	0.33	0.17	0.15	0.03	0.13	0.04	0.54	0.12	0.10	0.02
Sr	183	73	291	87	105	25	93	35	108	39
Zn	51	20	27	13	25	5	17	3	25	6

*Values in % ($g\,g^{-1}$).

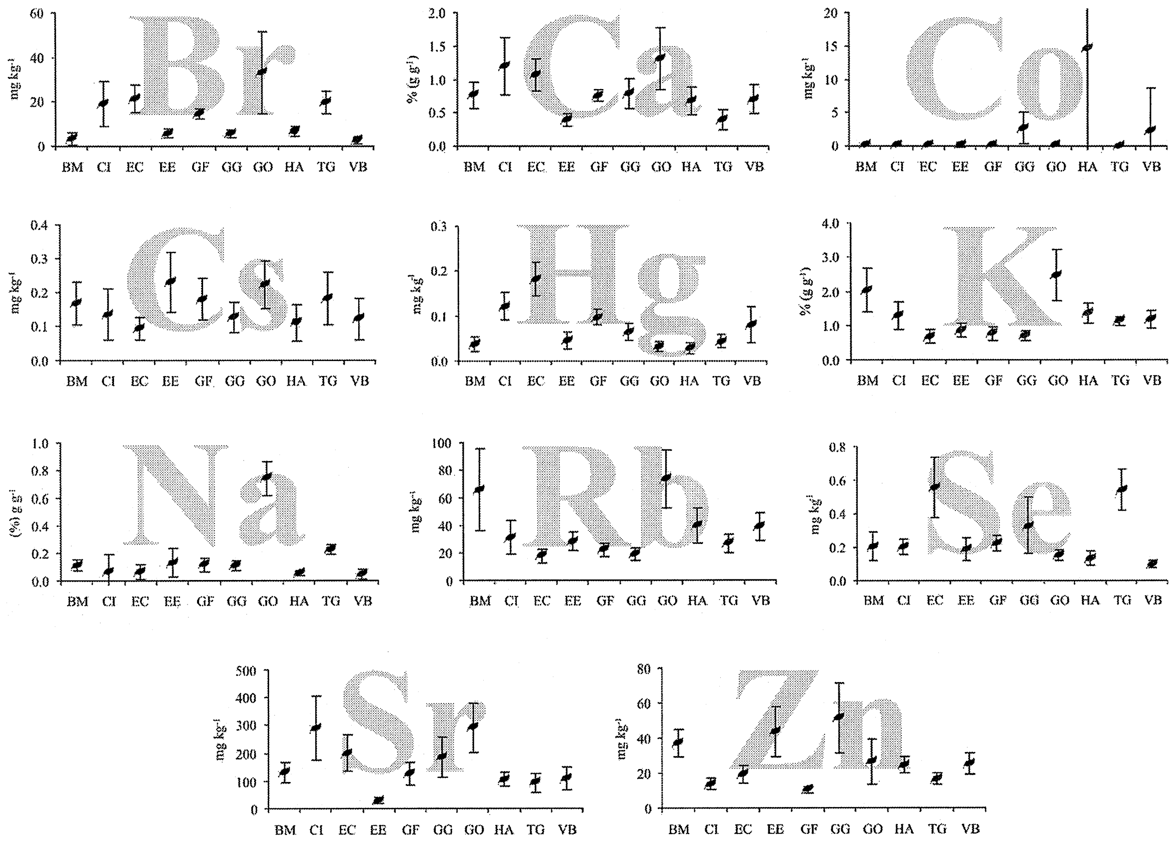

Figure 3. Average element concentrations in the leaf samples. Error bars indicate the standard deviation ($n = 10$; $n = 8$ for CI). BM = *Bathysa meridionalis*, CI = *Chrysophyllum inornatum*, EC = *Eugenia cuprea*, EE = *Euterpe edulis*, GG = *Garcinia gardneriana*, GF = *Gomidesia flagellaris*, GO = *Guapira opoosita*, HA = *Hyeronima alchorneoides*, TG = *Tetrastylidium grandifolium*, VB = *Virola bicuhyba*.

Table IV. Loading factors provided by principal component factor analysis

	Factor					
	1	2	3	4	5	Communality*
Sr	0.48	−0.11	0.16	0.53	−0.58	0.893
Ca	0.92	0.10	0.14	−0.07	0.00	0.888
Br	0.26	−0.25	−0.40	−0.02	0.60	0.879
K	−0.32	0.20	0.13	0.79	0.08	0.940
Rb	0.08	0.00	0.96	0.02	0.02	0.947
Sc	0.13	0.95	−0.02	0.16	−0.03	0.914
Fe	0.33	0.39	0.00	0.76	−0.08	0.919
Cs	0.02	0.95	−0.05	0.21	0.06	0.794
Na	0.16	−0.09	0.93	0.12	−0.07	0.853
Zn	0.94	0.08	0.06	0.06	−0.07	0.831
Co	−0.12	0.10	0.13	0.04	0.89	0.644

*Portion of the variance of the results that contributed to the common factors.

Factor 3, which is not representative of the composition of leaves, was not included in the clustering analysis. The exploratory discrimination was performed according to the recommendations for hierarchical clustering procedures. Several methods were tried and, within a given method, different ways of assigning distances were used (Johnson and Wichern, 1998). The unweighted pair-group combined with the Chebychev distance resulted in a consistent dendrogram for species grouping (Figure 4). *Gomidesia flagellaris* and *Eugenia cuprea* belonging to the Myrtaceae family were grouped together, as well as other species. However, more chemical elements would be necessary to enhance the species discrimination within the same family. It is interesting to point out that *Guapira opposita* (GO) was the most distinct group of plants, probably due to the accumulation of some elements. As an example, all *Garcinia gardneriana* trees were kept together in the dendrogram, in spite of their distinct localization in the long-term plot and, consequently, growing in different soil types.

The clustering results suggest the occurrence of an intrinsic composition of plants, which is valuable information for selecting the species for each biomonitoring necessity, since the ecological adaptation of plant species is related to the capacity of accumulating chemical elements. In fact, the most relevant information in biomonitoring is commonly deduced from changes in the behavior of the monitor organism or in the concentration of specific substances in the monitor tissues (Wolterbeek, 2002).

Bromine concentrations were higher for *Chrysophyllum inornatum, Eugenia cuprea, Gomidesia flagellaris, Guapira opposita*, and *Tetrastylidium grandifolium.*

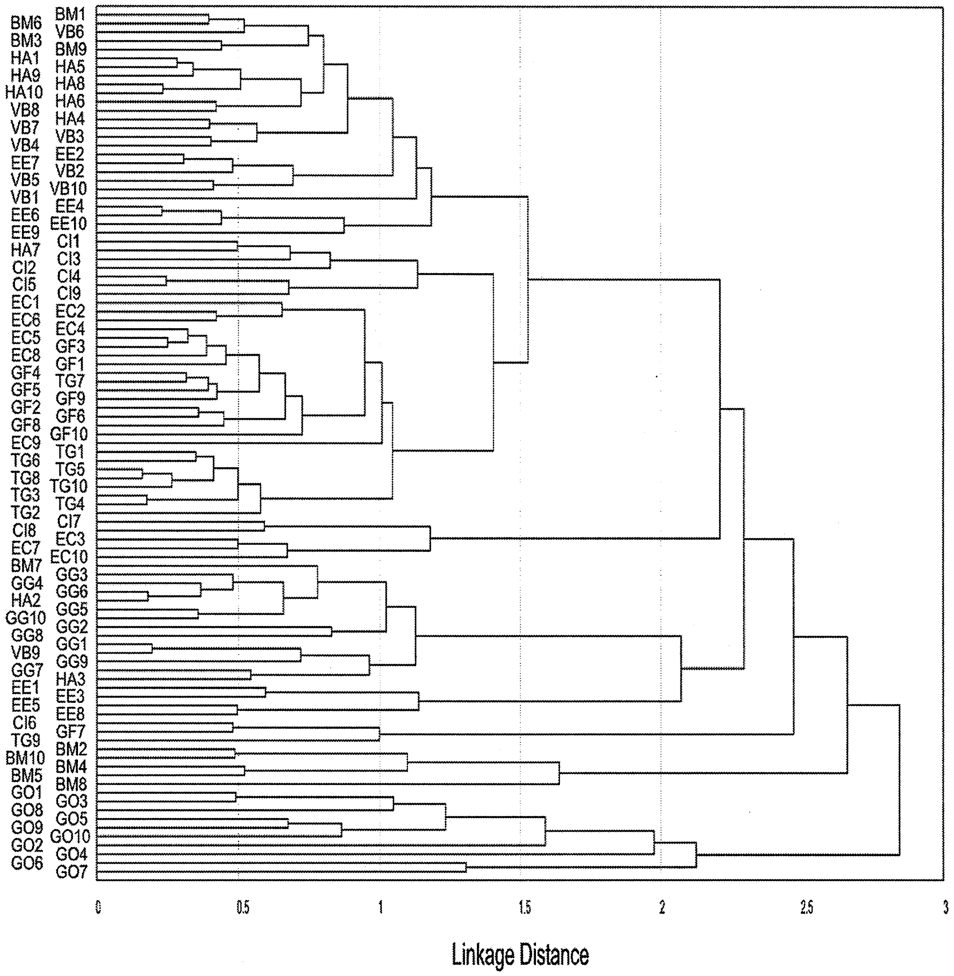

Figure 4. Dendrogram built from the concentrations of 12 chemical elements using the Chebychev distance and unweighted pair-group average. Numbers indicate the different sampled trees for each specie. BM = *Bathysa meridionalis*, CI = *Chrysophyllum inornatum*, EC = *Eugenia cuprea*, EE = *Euterpe edulis*, GG = *Garcinia gardneriana*, GF = *Gomidesia flagellaris*, GO = *Guapira opposita*, HA = *Hyeronima alchorneoides*, TG = *Tetrastylidium grandifolium*, VB = *Virola bicuhyba*.

In the leaves from one tree of *Guapira opposita* the concentration of Br reached 80 mg kg^{-1}, whereas the concentration in the soils of the region did not exceed 40 mg kg^{-1} (França *et al.*, 2004). Br concentrations higher than 40 mg kg^{-1} in plants can be associated to atmosphere pollution (Kabata-Pendias and Pendias, 1984) or, as well as for Na, to the influence of the sea (Djingova and Kuleff, 1993). Considering that no correlation was noticed between Br and Na for this species (Figure 5), the high Br concentration seems to be an evidence of the impact of atmospheric pollution on the plants growing in the PECB.

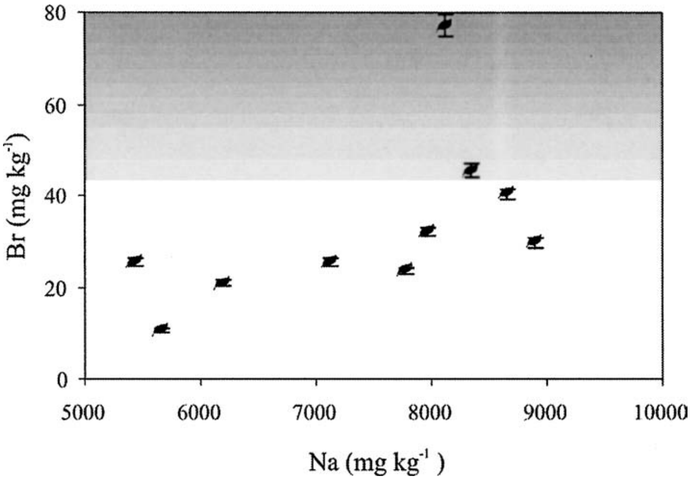

Figure 5. Scatter plot of Br and Na concentrations in the leaves of *Guapira opposita* trees. Error bars represent the individual uncertainty.

Cobalt concentration in leaves of *Hyeronima alchorneoides* (Euphorbiaceae family) normally ranged from 0.12 to 0.61, however for two samples taken from trees growing in different microhabitats the concentrations were 114 ± 6 and 26.8 ± 1.8 mg kg^{-1}. Therefore, an increase factor of 80 to 300 was found for Co in these trees comparing to the other plants, suggesting these plants as hyperaccumulators. Moreover, there was no evidence of the influence of geochemical variability, since concentrations in soils varied from 3 to 6 mg kg^{-1} (França *et al.*, 2004).

Plants can accumulate Co in trees leaves (Koyama *et al.*, 1987), which was particularly noticed for species of Euphorbiaceae family. *Pachystroma longifolium*, for instance, showed up to 30 mg kg^{-1} of Co in mature leaves (França *et al.*, 2003b). Several plant races (ecotypes) have been reported to be hyperaccumulators, leading to bioaccumulation figures considerably higher than found for other plants in the same locality, often by a factor between 10 and 1000 (Streit and Stumm, 1993).

4. Conclusions

The analysis of the 10 species provided the background concentrations of As, Br, Ca, Cd, Co, Cs, Hg, K, Na, Rb, Se, Sr and Zn in the Atlantic Forest. No contribution from atmospheric pollution was observed for As, Cd, Hg, and Se. Clustering of factor scores allowed a satisfactory exploratory discrimination of tree species by chemical composition. The clustering results suggest the occurrence of an intrinsic composition of plants, which is valuable for monitoring purposes. The obtained data indicate that some *Hyeronima alchorneoides* trees perform as hyperaccumulators of Co. Some high Br concentrations seem to be caused by atmospheric pollution, besides the marine contribution.

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