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NATIVE TREES OF THE ATLANTIC FOREST: BIOMONITORING OF CHEMICAL ELEMENTS

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ABSTRACT

The increasing environmental problems in Brazil have pushed the development of biomonitoring processes as a relevant tool either to establish the natural background or to study the contamination by chemical elements. 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. 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. A multidisciplinary study was launched by BIOTA/FAPESP (The Virtual Institute of Biodiversity, Fundação de Amparo à Pesquisa do Estado de São Paulo) aiming at a comprehensive environmental characterization of biodiversity. For biomonitoring purposes, leaves of the predominant species were analyzed by instrumental neutron activation analysis. Results showed the prevalence of inter-species over the intra-species variability of chemical concentrations. Bromine concentrations were higher in the understory species and it could be related to the possible effects of atmospheric pollution or the influence of sea. It is suggested that some *Hyeronima alchorneoides* trees as hyperaccumulators of Co.

1 INTRODUCTION

The atmosphere is a considerable source of chemical elements for ecosystems, affecting the sustainability of biodiversity [1]. Biologic components of the ecosystem can be employed as monitors of atmospheric inputs, due to their capacity in accumulating chemical elements, especially heavy metals, in spite of the adaptability to the environmental chemical variations

[2, 3, 4]. The use of superior plants for biomonitoring has advantages related to easier taxonomic identification, well-known physiology and possibility of selecting different parts for analysis (leaf, fruit, branch and root) [5].

In Brazil, the biomonitoring concepts have been applied [6, 7] for the investigation of anthropic influence on the biodiversity hotspots and for the establishment of reference protocols for environmental impact assessment. Besides, some authors revealed that the real point of interest is often not the actual concentration of heavy metals in the air or the amount of deposition but their effective concentrations in higher plants, especially in plants used to feed animals [8], which is the case for the tree species found in the Atlantic Forest.

Considering the high biodiversity, the study of Brazilian forests is intricate, requiring multidisciplinary approaches, particularly for the Atlantic Forest, a hotspot of the global diversity of plants and animals [9]. Thereby, the Virtual Institute of Biodiversity - BIOTA Program was implemented at 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" that aims at understanding the dynamics, generation and maintenance of the biodiversity as well as to adequate strategies for conservation, management and restoration. For implantation of 0.1 km^2 permanent parcels or long-term plots, the four major vegetation types of the state were selected - Slope Atlantic Forest, Restinga Forest, Semideciduous Seasonal Forest and Cerradão, which are respectively represented by the Parque Estadual Carlos Botelho (PECB), the Parque Estadual Ilha do Cardoso, the Estação Ecológica de Assis and the Estação Ecológica de Caetetus conservation units. Several surveys have been carried out in these plots, involving aspects of botany, chemistry, climatology, ecology and microbiology.

The objective of this paper is to provide estimates of the background concentrations of chemical elements in the Atlantic Forest by studying the PECB plot. As the most abundant tree species are responsible for the major part of chemical elements cycling [10], the composition of leaves from the predominant native trees was determined by instrumental neutron activation analysis (INAA).

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). Maximum and minimum temperature values as well as precipitation data along the six-month period are shown in Figure 2. According to the data bank 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 the marked trees to the species level. The palm *Euterpe edulis* is the dominant understory species, totalizing 31% of the identified trees.

Ten trees of each of the ten most predominant species in the area were selected for sampling (Table 1). Leaves (approximately 500 g) were collected from the middle- and lower-crown at February 2003. Information about health (injury and herbivory) was also compiled. The distribution of the sampled trees (Figure 3) was established according to the natural occurrence and accessibility. Since one tree was erroneously identified, only nine samples of *Chrysophyllum inornatum* were analyzed.

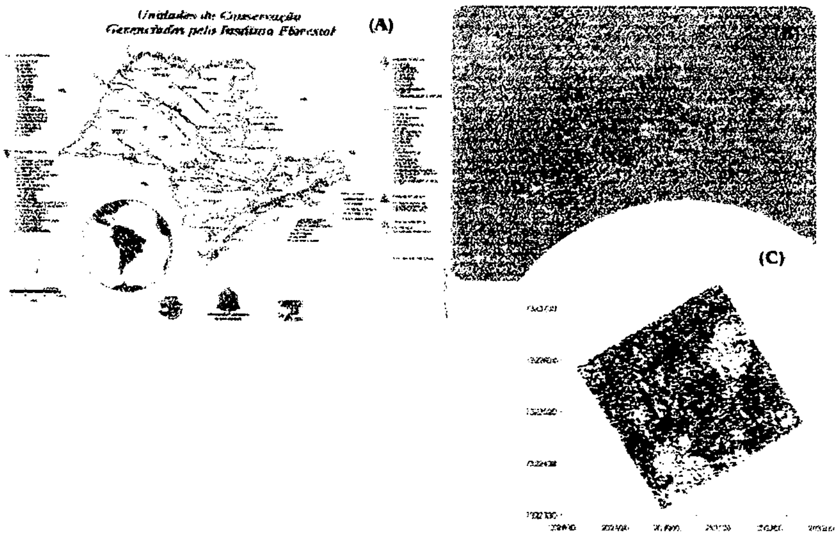

Figure 1: (A) Conservation units of São Paulo State. (B) Parque Estadual Carlos Botelho. (C) Trees identified in the long-term plot

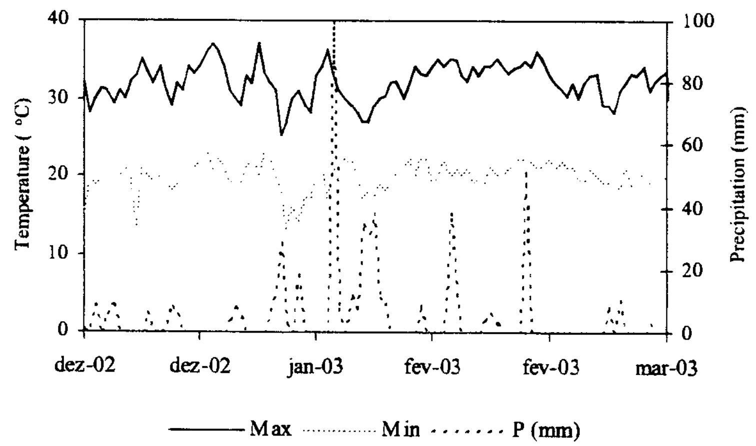

Figure 2: Six-month records of maximum (Max) and minimum (Min) temperatures, and precipitation (P) in Sete Barras. Source: Instituto Agronômico de Campinas, 2003.

Table 1. Characteristics of trees and leaves sampled in the PECB long-term plot

Species		Family	Injury (%)		Infestation (%)	
			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

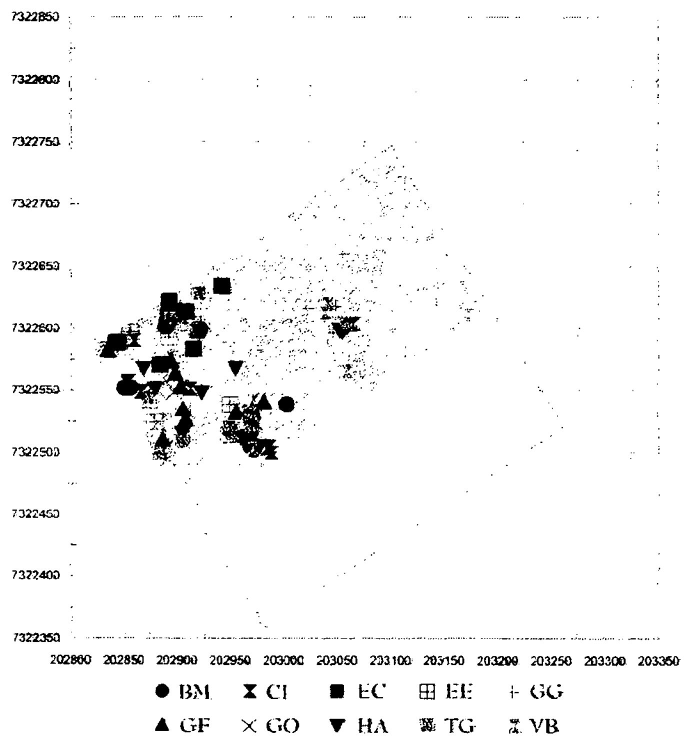

Figure 3: Spatial distribution of sampled trees in the PECB long-term plot. Dots refer to all trees available for selection

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 were included in the analytical series for quality control. Ni-Cr alloy wires of 10 mg were employed for thermal neutron flux monitoring [11]. 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 hours. Two different germanium detectors

(45% and 50% relative efficiency at ^{60}Co 1332 keV) from Ortec were used for the measurements of induced radioactivity. Chemical elements were determined by k_0-method instrumental neutron activation analysis (k_0-INAA) using the Quantu software package [12].

Exploratory statistical analysis was performed by factorial model based on the principal components of standardized concentrations as described elsewhere [13]. Data were transformed attending the requirements related to the normality and outliers detection. Factor loadings were useful for the 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 2) evidencing the analytical quality for the determination of 12 chemical elements in leaves.

Table 2. Results (mg kg^{-1}) of chemical elements in the certified reference materials 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
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	
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*
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*
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

Compared to species growing in the Semideciduous Forest ecosystem of the São Paulo State [8], the tree leaves of the Atlantic Forest have similar concentrations of Ca, K, Na, Rb and Sr (Table 3), in spite of the low nutrient status in the soil soluble fraction from both areas. High efficiency in the absorption and maintenance of nutrients in biological compartments are normally observed for plants growing in oligotrophic ecosystems [14].

Among the investigated species, the palm *Euterpe edulis* showed the lowest concentrations, with exception of Cs and Zn. Such behavior corroborated the application of this palm to establishing the natural concentration background of the Atlantic Forest associated to its wide distribution within the PECB plot, ecological relevance and extinction risk.

Table 3. Concentrations (mg kg^{-1}) of chemical elements in the leaves. Standard deviations calculated for the results from ten independent samples

	Bathysa meridionalis BM (n=10)		*Chrysophyllum inornatum* CI (n=9)		*Eugenia cuprea* EC (n=10)		*Euterpe edulis* EE (n=10)		*Gomidesia flagellaris* GF (n=10)	
	Mean	*STD*	Mean	*STD*	Mean	*STD*	Mean	*STD*	Mean	*STD*
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*
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*
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*
Sr	130	*34*	290	*116*	200	*66*	28	*10*	126	*40*
Zn	37	*8*	14	*3*	19	*5*	44	*14*	11	*2*

	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	*STD*	Mean	*STD*	Mean	*STD*	Mean	*STD*	Mean	*STD*
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*
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*
Na*	0.11	*0.03*	0.74	*0.12*	0.054	*0.018*	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*
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})

A comparison between the inter- and intra-specific variability of chemical concentrations can be found in Figure 4, 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 similar environmental conditions (110 m of maximum distance among trees). Some accumulation of Br, Na, Ca and Sr could be verified in leaves of this species.

The visualization of prevalence of the inter-specific over intra-specific variability of chemical elements was improved through factor analysis (Table 4) followed by dendrogram of factor scores. The grouping of variables in factors revealed sources of chemical elements. Factor 3 is connected to soil particles adhered to the surface of leaves, since Fe and Sc concentrations in plants are usually related to soil contamination [15]. There is also an evidence of marine influence due to the correlation of Br and Na concentrations with factor 4 [16].

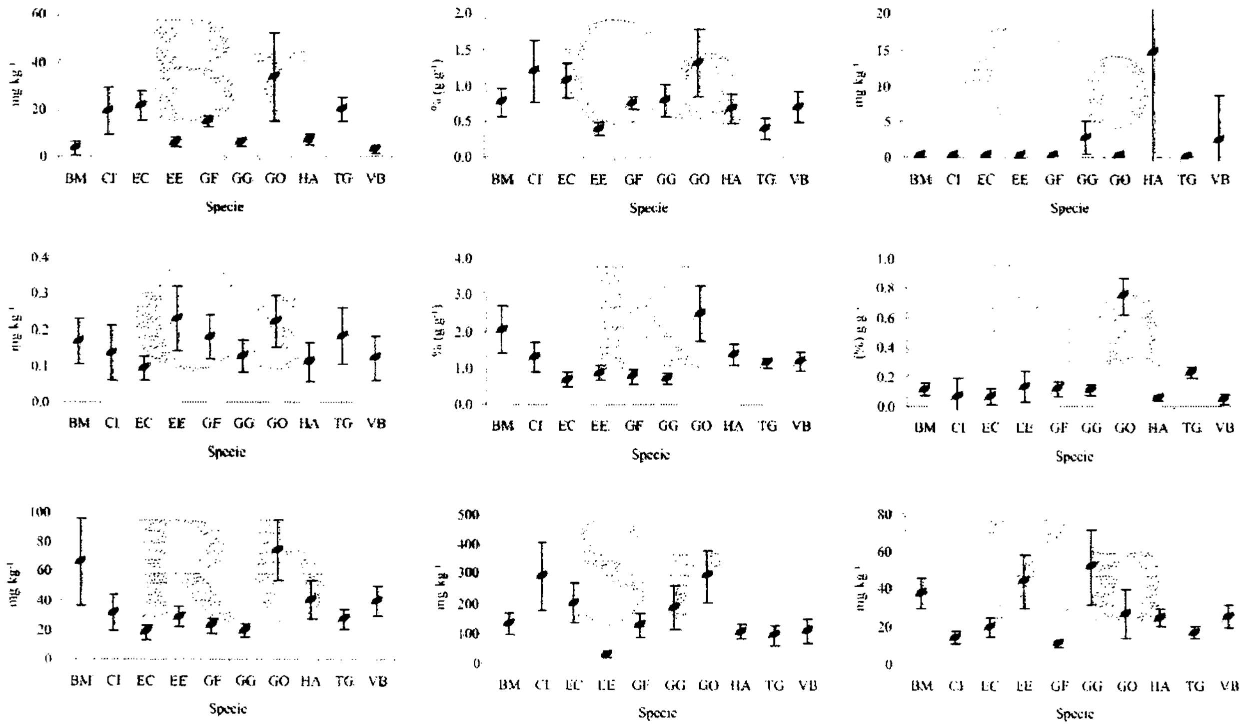

Figure 4: Comparison of the concentrations of chemical elements. Error bars indicate the standard deviation (n = 10; n = 9 for CI). BM = *Bathysa meridionalis*, CI = *Chrysophyllum inornatum*, EC = *Eugenia cuprea*, EE = *Euterpe edulis*, GG = *Garcinia gardneriana*, GF = *Gomidesia flagellaris*, GO = *Guapira opposita*, HA = *Hyeronima alchorneoides*, TG = *Tetrastylidium grandifolium*, VB = *Virola bicuhyba*

Table 4. Results of factor analysis

	Factor					
	1	2	3	4	5	Communality*
Sr	0.935					0.893
Ca	0.924					0.888
Br	0.476			0.533		0.879
K		0.946				0.940
Rb		0.948				0.947
Sc			0.927			0.914
Fe			0.955			0.919
Cs				0.793		0.794
Na				0.764		0.853
Zn					0.888	0.831
Co					0.599	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 [13]. In fact, the unweighted pair-group combined with the Chebychev distance resulted in a consistent dendrogram for species grouping (Figure 5). *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.

The clustering results suggest the occurrence of an intrinsic composition of plants. As an example, all *Garcinia gardneriana* trees were kept together in the dendrogram, in spite of their distinct localization in the long-term plot. For *Virola bicuhyba* the clustering was not well-defined, probably due to the ecological behavior of the species or the clustering method deficiency. Hierarchical clustering methods are considered to be quite sensitive to outliers because sources of error and variation could not be formally included in the calculations [13].

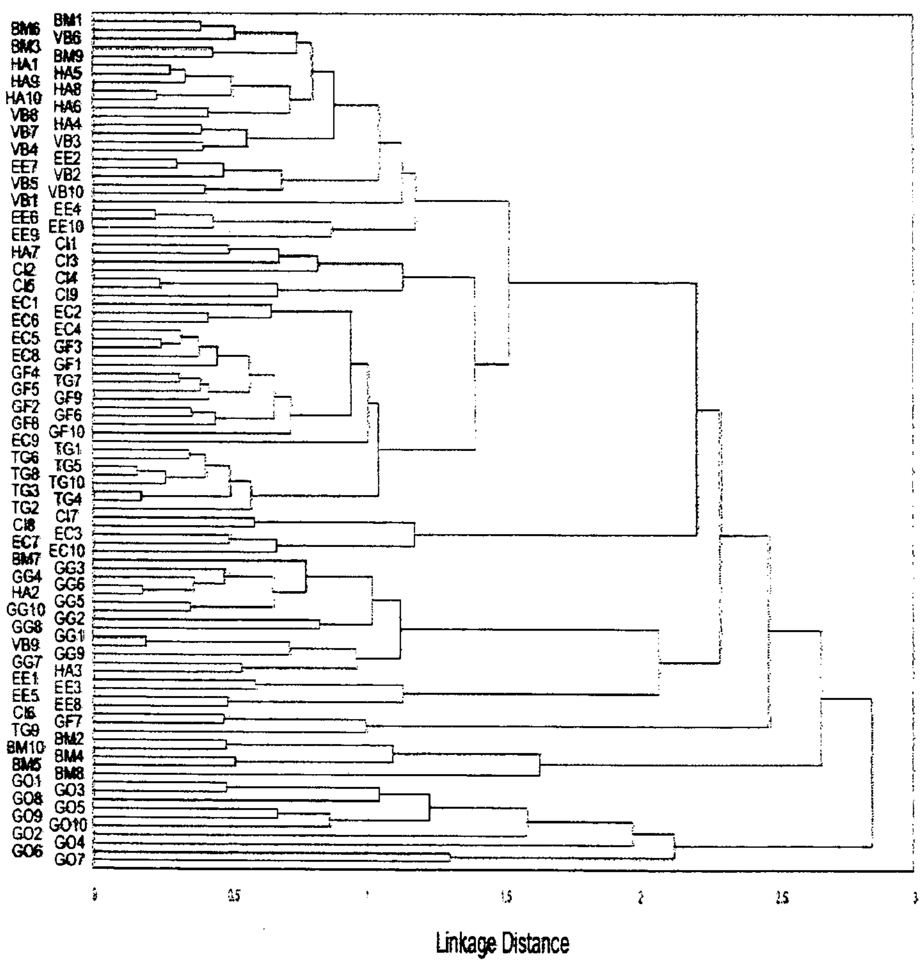

Figure 5: Dendrogram built from the concentrations of 12 chemical elements using the Chebychev distance and unweighted pair-group average. Numbers indicate the different sampled trees. BM = *Bathysa meridionalis*, CI = *Chrysophyllum inornatum*, EC = *Eugenia cuprea*, EE = *Euterpe edulis*, GG = *Garcinia gardneriana*, GF = *Gomidesia flagellaris*, GO = *Guapira opposita*, HA = *Hyeronima alchorneoides*, TG = *Tetrastylidium grandifolium*, VB = *Virola bicuhyba*

Bromine concentrations were higher for *Chrysophyllum inornatum*, *Eugenia cuprea*, *Gomidesia flagellaris*, *Guapira opposita*, and *Tetrastylidium grandifolium*. In the leaves from one tree of *Guapira opposita* the concentration of Br reached 80 mg kg^{-1}, while the concentration in the soils of the region did not exceed 40 mg kg^{-1} [17]. Br concentrations higher than 40 mg kg^{-1} in plants can be associated to atmosphere pollution [3] or, as well as for Na, to the influence of the sea [16]. Considering that no correlation was noticed between Br and Na for this species (Figure 6), the high Br concentration seems to be an evidence of the impact of atmospheric pollution on the plants growing in the PECB.

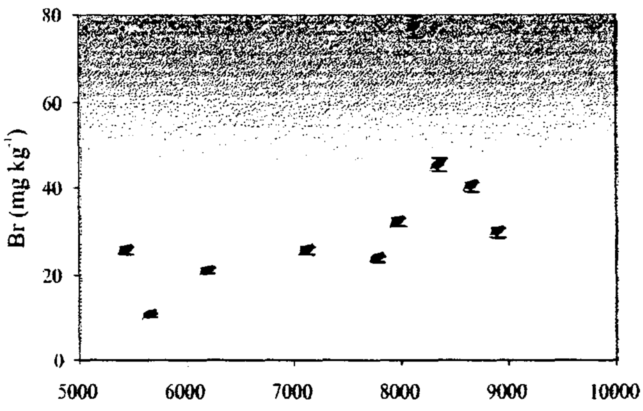

Figure 6: Scatter plot of the Br and Na concentrations of the leaves of *Guapira opposita* trees. Error bars represent the combined uncertainty

Plants can accumulate cobalt in trees leaves [4], particularly species of Euphorbiaceae family like *Pachystroma longifolium*, which attained up to 30 mg kg^{-1} in mature leaves [8]. Several plant races (ecotypes) have been reported to be "hyperaccumulators" leading to bioaccumulation figures considerably higher than found for other plant species in the same locality, often by a factor between 10 and 1000 [18]. Cobalt concentration in leaves of *Hyeronima alchorneoides* (Euphorbiaceae family) ranged from 0.12 to 0.61, however two leaf trees showed concentrations of 114 ± 6 and 26.8 ± 1.8 mg kg^{-1}, respectively. Therefore, a factor of 80 to 300 was found for Co concentrations of these trees in comparison to the other plants growing in the same locality, suggesting these plants as hyperaccumulators. There is no evidence of the influence of geochemical variability (concentrations in soils varied from 3 to 6 mg kg^{-1} [17]). Besides, the trees showing high Co concentration are growing in different microhabitats.

4 CONCLUSIONS

The analysis of the ten species provided the background concentrations of Br, Ca, Co, Cs, K, Na, Rb, Sr and Zn in the Atlantic Forest. The results showed the prevalence of inter-species over the intra-species variability of chemical composition. A satisfactory exploratory discrimination of tree species by chemical composition was realized through clustering of factor scores. 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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