



## A Comprehensive Investigation of the Mineral Composition of Brazilian Bee Pollen: Geographic and Seasonal Variations and Contribution to Human Diet

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A composição de minerais foi investigada em 154 amostras de pólen apícola, provenientes de diferentes regiões do Brasil. A técnica de ICP OES foi utilizada para determinar Ca, Cu, Fe, K, Mg, Mn, Na, P, Se e Zn, após mineralização em sistema de microondas. Utilizando parâmetros instrumentais otimizados conseguiu-se boa exatidão e precisão na determinação simultânea dos minerais. Em geral, as amostras dos estados do Nordeste apresentaram teores de minerais mais elevados e produção constante durante o ano. Os minerais Mn, Se, Cu, Zn, Fe são os que poderiam ter maior contribuição na dieta, podendo atingir 70, 37, 27, 17 e 17%, respectivamente, da ingestão dietética diária recomendada no Brasil. A análise de componentes principais mostrou que Ca, Cu, Fe, K, Mg, Mn, Na, P e Zn podem ser usados na classificação do pólen nacional em função da origem geográfica. Os teores dos minerais variaram amplamente ao longo do ano.

The mineral composition of bee pollen was investigated in 154 samples from different Brazilian regions. Ca, Cu, Fe, K, Mg, Mn, Na, P, Se and Zn were determined by ICP OES after microwave mineralization. Employing optimized instrumental parameters, the method was shown to have a good precision and accuracy for the simultaneous determination of minerals. Overall, samples from the Northeastern states presented significantly higher amounts of minerals and showed constant production throughout the year. Manganese, Se, Cu, Zn and Fe were the elements that showed the greatest possible contributions to the diet, contributing with 70, 37, 27, 17 and 17%, respectively, of the Brazilian recommended daily intake. Principal component analysis (PCA) was applied to study geographic effects. The elements Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn were important in explaining the classification of pollen according to their geographical origin. The mineral levels varied widely during the year.

**Keywords:** bee pollen, minerals, ICP OES

### Introduction

Bee pollen, often referred to as a natural supplement, is one of the emerging bee products valued by its high content of nutrients and other health-promoting constituents. Brazilian honey has become an important product for domestic consumption and export, the latter having increased by 12% of its value in 2010. The major importing country is the United States, followed by Germany, the

United Kingdom, Canada, Austria, France and Spain.<sup>1</sup> Official data for the pollen is not available, but it is known that the current production is not sufficient to meet the national demand. Beekeeping has gradually increased in Brazil favored by plant diversification, vast land area and favorable climatic conditions (tropical climate).<sup>2</sup>

Bee pollen has 13 to 55% carbohydrates, 10 to 40% proteins, 1 to 20% lipids, 3 to 8% water, minerals (500-3000 mg 100 g<sup>-1</sup>), resins, vitamins (20-100 mg 100 g<sup>-1</sup>) and other compounds such as flavonoids (40-3000 mg 100 g<sup>-1</sup>) and antibiotic substances.<sup>3-7</sup> Brazilian studies on the

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macronutrient and micronutrient composition of bee pollen, however, are still limited.<sup>8,9</sup>

The chemical composition of bee pollen is known to vary with the methods of extraction and storage, the floral origin (plant species), including age and nutritional status of the plant, and environmental conditions.<sup>10</sup> According to O'Rourke and Buchmann,<sup>11</sup> pollen composition may vary in different locations, between seasons and years. Funari *et al.*<sup>8</sup> found variations in mineral composition of Brazilian bee pollen collected in one apiary from Botucatu during a period of four months. Knowledge of the nutritional value and quality of bee pollen produced in different regions may contribute to the choice of the place to install apiaries.<sup>12,13</sup>

Some mineral elements have already been quantified in bee pollen samples from several countries: potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), sodium (Na), sulfur (S), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), chromium (Cr), nickel (Ni) and selenium (Se).<sup>6,8,14-19</sup> The number of samples analyzed, however, is limited, varying from one<sup>19</sup> to fifty<sup>16</sup> *per* study. Two of them are Brazilian studies, involving 24 samples collected in only one sampling site from the state of São Paulo<sup>8</sup> and 36 samples from Southern Brazil.<sup>9</sup>

The main analytical techniques used to determine mineral elements in bee pollen samples are atomic absorption spectrometry (AAS),<sup>7,17,18,20</sup> electrothermal atomization atomic absorption spectrometry (ET-AAS),<sup>21</sup> inductively coupled plasma optical emission spectrometry (ICP OES),<sup>6,16</sup> and X-ray fluorescence spectrometry (XRF).<sup>15</sup>

Baldi-Coronel *et al.*<sup>17</sup> determined K, Ca, Mg, Na, Fe, Zn, Mn and Cu by AAS in bee pollen ash obtained by calcination of samples at 550 °C. Szczesna found in decreasing order the minerals K > Mg > Na > Ca > Fe > Mn > Zn > Cu in bee pollen samples collected in Poland, South Korea and China, after sample mineralization in acid medium using an open microwave-assisted system and quantification by AAS.<sup>18</sup> Somerville and Nicol<sup>16</sup> used ICP OES to quantify K, P, S, Ca, Mg, Na, Fe, Zn, Mn and Cu in bee pollen from New South Wales after sample digestion in closed microwave-assisted system. Serra-Bonvehí and Escolà-Jordà<sup>6</sup> determined Fe, Ca, Zn, K, Na, Cu, Mg, P and Mn in Spanish bee pollen, also using ICP OES after calcination. Comparing dry and wet digestion, Kump *et al.*<sup>15</sup> obtained the best results by ashing the sample in the presence of diluted H<sub>2</sub>SO<sub>4</sub> (1:4). These authors used XRF to determine K, Ca, Mn, Fe, Ni, Cu, Zn, Rb and Pb in bee pollen samples from Slovenia.

In a previous study, the occurrence of inorganic contaminants in bee pollen from Southeastern Brazil was reported.<sup>22</sup> The purpose of the present paper was to analyze

bee pollen samples from Brazilian producing regions for the mineral elements Ca, Cu, Fe, K, Mg, Mn, Na, P, Se and Zn. To reach this goal, a method using sample digestion in closed microwave-assisted system and quantification by ICP OES was optimized. While food safety was the concern of the previous paper, the current work deals with the nutritional value, geographic and seasonal variations of this increasingly important food product.

## Experimental

### Sampling

A total of 154 Brazilian dehydrated bee pollen samples ready for commercialization (dry, clean and packaged products) were directly purchased from apiculture producers of Southern, Southeastern, Central, Northeastern and Midwestern regions (Table 1) during a year. Samples (weighing 200 to 300 g) were transported to Campinas, SP, mostly in plastic bags, some in glass containers, simulating commercialization practices.

**Table 1.** Localization of bee pollen sample collection regions

Region	State	City (number of sampling sites)
Southern	RS	Cruz Alta (9)
	SC	Campos Novos (17), Porto União (13)
	PR	Ilha Grande (3), Lapa (7)
Southeastern	SP	São José dos Campos (9), Santa Branca (7), Holambra (3), Guaratinguetá (4)
	MG	Marmelópolis (4), Belo Oriente (2), Entre Rios de Minas (4)
	ES	Pedra Azul (10)
Central	DF	Federal Distict (3)
Northeastern	BA	Canavieiras (24), Ilhéus (10), Norte Bahia (3)
	SE	Neópolis (13), Aracaju (1), Salgado (4)
	PI	Teresina (1)
Midwestern	CE	Trairí (1)
	MT	Cáceres (2)

RS, Rio Grande do Sul; SC, Santa Catarina; PR, Paraná; SP, São Paulo; MG, Minas Gerais; ES, Espírito Santo; MT, Mato Grosso; BA, Bahia; CE, Ceará; PI, Piauí; SE, Sergipe; DF, Federal District.

### Sample preparation

In the laboratory, the samples were packed under vacuum in polyamide and polyethylene bags, to avoid absorption of moisture and oxygen, and stored in a freezer at -16 °C until analyses were carried out (maximum of 3 months). On the day of analysis, samples were quartered in a stainless steel quartering equipment and ground in a refrigerated mill with

tungsten helix (M20, IKA Labortechnik, Staufen, Baden, Ge). The samples were then sieved using a 30-mesh (600  $\mu\text{m}$ ) sieve to standardize pollen particles size.

#### Analytical determination

Digestion was performed in a closed microwave acid digestion unit with 41 digestion bottles (Microwave Digestion System Start D, Milestone, Sorisole, Italy), using nitric acid 65% (m/m) (Merck, Farmstadt, Germany) and hydrogen peroxide 30% (v/v) (Merck, Darmstadt, Germany) as oxidizing agents. A sample of 0.65-0.70 g was weighed in the digestion bottle and 10 mL of concentrated nitric acid were added, followed by 3 mL of 30% hydrogen peroxide. Digestion was performed in two phases, with 30 min of cooling after each phase. Power was maintained at 1000 W and external temperature of the reaction vessel was at 110 °C. For phase 1 three stages of 20, 20 and 30 min were undertaken, the internal temperature of the reaction vessel being kept at 140, 160 and 180 °C, respectively. Phase 2 had two stages of 20 and 30 min with the internal temperature of the reaction vessel at 180 and 200 °C, respectively. Once digestion was completed, the bottle's content was transferred to a 25 mL volumetric flask, the volume being completed with a 5% HCl solution (v/v).<sup>22</sup>

Quantification of mineral elements was performed by ICP OES, using a Varian Vista MPX model (Mulgrave Victoria, Australia), with axial vision, equipped with a radio frequency (RF) source of 40 MHz, a Charge Coupled Device (CCD-type) simultaneous multi-element solid state detector, a peristaltic pump, a spraying chamber and a sea spray nebulizer. The system was controlled by ICP Expert software and 99.996% liquid argon was used as plasma gas (Air Liquid, SP, Brazil). Operating conditions of the ICP OES equipment were: forward power, 1000 W; nebulizer Ar flow rate, 0.9 L min<sup>-1</sup>; auxiliary argon flow, 1.5 L min<sup>-1</sup>; cooling Ar flow rate, 15 L min<sup>-1</sup>; background correction, 2 points; integration and reading time, 10 s; replicate number, 3 and axial torch configuration. The analytical line wavelengths were: Ca, 317.933 nm; Cu, 324.754 nm; Fe, 259.940 nm; K, 766.491 nm; Mg, 280.270 nm; Mn, 257.610 nm; Na, 589.592 nm; P, 213.618 nm; Se, 196.026 nm; Zn, 206.200 nm.

Stock solutions at 10,000 mg L<sup>-1</sup> for Ca, K, Mg, Na (Titrisol - Merck) and P (Qhemis High Purity) and at 1,000 mg L<sup>-1</sup> for Cu, Fe, Mn, Zn (Merck) and Se (Qhemis High Purity) were used for preparing the standard solutions in 5% HCl v/v. The concentration ranges of the standard solutions were: 0.005 to 0.5 mg L<sup>-1</sup> of Cu and Se, 0.05 to 2.5 mg L<sup>-1</sup> of Fe, Mn and Zn, 0.5 to 15.0 mg L<sup>-1</sup> of Na, 5.0 to 75.0 mg L<sup>-1</sup> of Ca, P and Mg and 25.0 to 150.0 mg L<sup>-1</sup> of K.

#### Assurance of quality in analytical procedure

Method accuracy was evaluated by recovery tests conducted at two levels, covering the concentration ranges in the samples. The limit of detection (LOD) and limit of quantification (LOQ) values were calculated as suggested by Mermet and Poussel:<sup>23</sup>  $\text{LOD} = (3 \text{ RSD BEC})/100$  and  $\text{LOQ} = 5 \text{ LOD}$ , where RSD is the relative standard deviation and BEC is the background equivalent concentration, determined experimentally ( $n = 8$ ). Method precision was evaluated by the coefficient of variation of 8 repetitions.

Because of the unavailability of a certified reference material for bee pollen, a certified reference material, NIST 1566a (oyster tissue), was used only to validate analytical control of the laboratory under existing working conditions. This material has certified values for Ca, Cu, Fe, K, Mg, Mn, Na, Se and Zn. Results were assessed by the relative error.

#### Statistical analysis

In order to verify if the means obtained for mineral elements of dehydrated bee pollen samples from different states could be considered statistically different at a significance level of  $p < 0.05$ , Tukey's multiple comparison test was applied (employing the approach method with different repetition numbers *per* state) using the software Statistica for Windows 5.5 (StatSoft Inc., Tulsa, USA).

#### Hierarchical cluster analysis (HCA) and principal component analysis (PCA)

Using mean values, a data matrix (154  $\times$  10) was built, in which samples were put in lines and the concentration of minerals were put in columns (variables). Data were preprocessed and then submitted to HCA and PCA, using Statistica program version 5.0. The HCA statistical analysis was performed with the purpose of verifying similarities between samples from different states, clustering samples by similarity from the Euclidean distance calculated. HCA results were presented as a bi-dimensional graph called dendrogram. PCA analysis was used as a chemometric tool to verify which mineral elements were important for the separation of the groups formed.

#### Estimation of microelement daily intake

Contribution of mineral elements in bee pollen to dietary intake was estimated according to the recommended daily intake (RDI) values established by Brazilian regulation for adults,<sup>24</sup> based on the recommended nutrient intakes

(RNI) for Ca, Fe, Mg, Zn and Se<sup>25</sup> and the dietary reference intakes (DRI) values for Cu, Mn<sup>26</sup> and P.<sup>27</sup> Potassium DRI level was adopted from the Institute of Medicine,<sup>28</sup> since the Brazilian regulation does not establish a recommendation for this mineral. To estimate the microelement daily intake, the portion size for bee pollen recommended by Lengler was used, which is 25 g (approximately three tablespoons) for adults.<sup>29</sup>

## Results and Discussion

### Method performance

Sample digestion using a microwave-assisted closed system allowed quantification of the mineral elements Ca, Cu, Fe, K, Mg, Mn, Na, P, Se and Zn in a single sample preparation. The residual carbon content was 0.15 g kg<sup>-1</sup>, indicating low level of organic matter after sample digestion. The time spent to digest the samples was approximately 180 min.

The method exhibited good performance, confirmed by *r* values close to 1, LOD and LOQ with appropriate sensitivity to determine the minerals and satisfactory CV at the detection levels achieved (Table 2). The working standards solutions for the calibration and reagent blanks used for the estimation of the linearity ranges and limits of detection and quantification were subjected to the digestion

procedure. Recovery results in pollen matrix were within the range of 70 to 106%, mostly close to 100%, indicating adequacy of the analytical method.

The results obtained in the laboratory analytical control evaluation, using the oyster tissue NIST 1566a certified reference material, under the same preparation conditions for bee pollen samples, showed adequacy of the method. For all elements, relative error was below 8% (Table 2).

### Composition of mineral elements in Brazilian bee pollen sample

Mean contents, standard deviations and concentration ranges obtained for the minerals in the 154 dehydrated bee pollen samples from 11 states and the Federal District are shown in Table 3. In general, wide within-state and between-state concentration ranges were obtained, especially for Se, Na and Cu, which had standard deviations higher than the means.

A perusal of the between-state variation shows higher Ca levels in PI, CE, SE, BA and MT samples, while those of SE, CE and PI had higher amounts of P. There was no significant variation of Cu and Mn among the states. Samples from MG and DF had higher levels of Fe, than those of the other states, but the differences did not reach significant levels. Samples from SE, DF, PI, CE, BA and MT had greater amounts of K and Mg, but for the latter, PR also had higher

**Table 2.** Performance characteristics of the method for determining bee pollen mineral elements

Element	<i>r</i>	LOD / (mg L <sup>-1</sup> )	LOQ / (mg L <sup>-1</sup> )	CV / %	Oyster tissue / (mg kg <sup>-1</sup> )		Fortification level / (mg L <sup>-1</sup> )	Recovery / %
					Certified Value	Measured Value*		
Ca	0.9999	0.001	0.003	5.6	793 ± 15	838 ± 20	20.05 31.00	106 96
Cu	1.0000	0.003	0.01	1.7	67.7 ± 1.2	71.6 ± 1.6	0.10 0.15	85 87
Fe	0.9998	0.001	0.03	1.7	212.9 ± 1.3	205.8 ± 6.8	0.10 1.00	94 98
K	0.9952	0.006	0.03	2.7	6531 ± 156	6520 ± 90	20.05 36.00	86 97
Mg	0.9954	0.0001	0.001	1.5	1100 ± 30	1085 ± 23	30.00 50.00	71 80
Mn	0.9998	0.0001	0.0004	7.0	18.7 ± 0.1	18.5 ± 0.2	0.80 1.00	80 73
Na	0.9992	0.004	0.02	3.5	3482 ± 140	3297 ± 530	2.00 4.00	90 85
P	0.9998	0.009	0.04	2.5	—	—	25.00 40.00	94 94
Se	0.9995	0.01	0.07	13.5	1.88 ± 0.32	2.06 ± 0.15	0.01 0.10	70 102
Zn	0.9999	0.004	0.02	2.3	1420 ± 17	1424 ± 46	0.80 1.00	103 92

*r*, coefficient of correlation; LOD, limit of detection; LOQ, limit of quantification; CV, coefficient of variation; \**n* = 3.

**Table 3.** Mineral contents of 154 dehydrated bee pollen samples collected from 11 Brazilian states and the Federal District

State/Federal District	N	mineral elements / (mg kg <sup>-1</sup> )									
		calcium	copper	iron	potassium	magnesium	manganese	sodium	phosphorus	selenium	zinc
Paraná	10	mean ± SD	7.75 ± 9.95 <sup>a</sup>	76.6 ± 33.8 <sup>b</sup>	4205 ± 2272 <sup>bc</sup>	1315 ± 827 <sup>a</sup>	46.5 ± 16.8 <sup>a</sup>	17.7 ± 12.0 <sup>b</sup>	4760 ± 909 <sup>b</sup>	0.61 ± 0.92 <sup>b</sup>	38.7 ± 8.8 <sup>b</sup>
		Median	7.13	67.3	3315	1113	45.7	15.8	4456	0.158	39.7
		Interval	3.19-12.12	38.3-131.4	2237-9910	780-3621	22.0-81.5	1.7-33.2	3826-6903	<0.01-2.73	24.0-51.4
Santa Catarina	30	mean ± SD	10.04 ± 3.34 <sup>a</sup>	72.8 ± 34.5 <sup>b</sup>	4683 ± 1818 <sup>b</sup>	966 ± 337 <sup>b</sup>	74.2 ± 51.8 <sup>a</sup>	15.7 ± 12.6 <sup>b</sup>	4458 ± 1144 <sup>bc</sup>	0.52 ± 0.56 <sup>b</sup>	37.9 ± 7.5 <sup>b</sup>
		Median	9.98	64.8	4235	1011	55.4	12.7	4669	0.28	37.3
		Interval	4.76-18.33	28.7-181.7	1431-8297	348-1633	15.8-210.7	3.1-62.0	2177-6650	<0.01-1.75	23.6-57.6
Rio Grande do Sul	9	mean ± SD	10.87 ± 2.33 <sup>a</sup>	164 ± 117 <sup>b</sup>	3869 ± 1167 <sup>b</sup>	994 ± 245 <sup>b</sup>	47.0 ± 15.7 <sup>a</sup>	43.7 ± 19.6 <sup>a</sup>	4658 ± 1078 <sup>b</sup>	0.56 ± 0.79 <sup>b</sup>	47.0 ± 6.0 <sup>a</sup>
		Median	10.67	179.0	3831	1037	48.0	46.0	4303	0.33	46.5
		Interval	8.86-16.22	11.1-373.3	1627-6047	614-1299	23.0-75.5	9.8-77.2	3303-6266	<0.01-2.35	39.4-56.3
São Paulo	23	mean ± SD	9.67 ± 3.85 <sup>a</sup>	94.4 ± 59.4 <sup>b</sup>	4434 ± 1840 <sup>b</sup>	884 ± 215 <sup>b</sup>	71.2 ± 33.9 <sup>a</sup>	29.3 ± 16.5 <sup>b</sup>	4397 ± 909 <sup>bc</sup>	0.53 ± 0.94 <sup>b</sup>	53.6 ± 11.2 <sup>a</sup>
		Median	8.57	75.1	4080	865	55.1	24.3	4444	0.17	52.8
		Interval	4.71-20.15	23.8-261.4	2385-7972	466-1456	39.5-155.2	8.7-63.8	2820-5934	<0.01-4.28	36.7-75.8
Minas Gerais	10	mean ± SD	11.31 ± 6.00 <sup>a</sup>	202 ± 135 <sup>a</sup>	3182 ± 811 <sup>b</sup>	789 ± 303 <sup>c</sup>	40.9 ± 14.6 <sup>a</sup>	15.8 ± 13.9 <sup>b</sup>	3399 ± 747 <sup>bc</sup>	0.31 ± 0.52 <sup>b</sup>	41.1 ± 15.3 <sup>b</sup>
		Median	9.45	182.8	3223	711	40.3	14.2	3319	0.01	37.1
		Interval	4.20-25.40	81.7-551.6	2153-4308	413-1260	20.1-72.0	<0.004-40	2323-4736	<0.01-1.61	26.7-72.9
Espírito Santo	10	mean ± SD	10.09 ± 1.74 <sup>a</sup>	71.5 ± 16.8 <sup>b</sup>	3509 ± 1235 <sup>b</sup>	915 ± 446 <sup>b</sup>	59.9 ± 34.1 <sup>a</sup>	76.5 ± 84.6 <sup>a</sup>	3257 ± 707 <sup>bc</sup>	0.19 ± 0.40 <sup>b</sup>	40.1 ± 8.8 <sup>b</sup>
		Median	10.37	72.9	3421	932	55.5	50.2	3315	0.01	39.6
		Interval	6.88-12.30	39.3-96.0	1980-6014	432-1772	11.9-126.0	10.8-288.0	2312-4083	<0.01-1.31	28.5-51.4
Federal District	3	mean ± SD	10.19 ± 2.72 <sup>a</sup>	151.9 ± 57.4 <sup>b</sup>	7044 ± 1693 <sup>a</sup>	1384 ± 77 <sup>a</sup>	39.2 ± 16.4 <sup>a</sup>	1.06 ± 1.05 <sup>c</sup>	5078 ± 425 <sup>b</sup>	1.65 ± 1.38 <sup>b</sup>	42.7 ± 0.8 <sup>a</sup>
		Median	10.83	156.4	6428	1388	36.3	1.07	5170	1.22	42.4
		Interval	7.21-12.53	92.4-207.0	5745-8958	1305-1458	24.4-56.9	<0.004-2.11	4615-5459	0.54-3.19	42.1-43.6
Bahia	37	mean ± SD	9.20 ± 1.80 <sup>a</sup>	47.2 ± 18.9 <sup>b</sup>	5960 ± 1588 <sup>a</sup>	1444 ± 341 <sup>a</sup>	76.4 ± 30.3 <sup>a</sup>	233 ± 301 <sup>a</sup>	5239 ± 744 <sup>b</sup>	0.44 ± 0.70 <sup>b</sup>	51.5 ± 7.8 <sup>a</sup>
		Median	9.01	44.0	5767	1510	72.0	126	5303	0.01	53.2
		Interval	6.31-13.50	20.4-177.0	2908-9602	792-2129	29.1-147.0	29-1466	3808-6353	<0.01-2.56	32.6-73.4
Sergipe	18	mean ± SD	8.62 ± 2.82 <sup>a</sup>	43.0 ± 11.9 <sup>b</sup>	7376 ± 1123 <sup>a</sup>	1597 ± 222 <sup>a</sup>	48.4 ± 7.8 <sup>a</sup>	89.1 ± 131.2 <sup>a</sup>	6886 ± 980 <sup>a</sup>	0.50 ± 0.50 <sup>b</sup>	52.6 ± 13.2 <sup>a</sup>
		Median	7.75	38.6	7554	1606	48.5	58.3	7229	0.038	54.1
		Interval	6.54-18.24	31.2-80.0	5154-8929	951-1992	28.4-61.2	6.7-597.0	4269-8165	<0.01-1.4	5.1-65.3
Piauí	1	mean	10.13 <sup>a</sup>	66.9 <sup>b</sup>	6542 <sup>a</sup>	1627 <sup>a</sup>	140 <sup>a</sup>	3.21 <sup>c</sup>	5778 <sup>a</sup>	0.87 <sup>b</sup>	76.1 <sup>a</sup>
Ceará	1	mean	8.85 <sup>a</sup>	38.3 <sup>b</sup>	6413 <sup>a</sup>	1668 <sup>a</sup>	61.8 <sup>a</sup>	84.1 <sup>a</sup>	6439 <sup>a</sup>	4.45 <sup>a</sup>	56.3 <sup>a</sup>
Mato Grosso	2	mean ± SD	13.0 ± 0.9 <sup>a</sup>	87.5 ± 4.9 <sup>b</sup>	4993 ± 123 <sup>a</sup>	1744 ± 124 <sup>a</sup>	49 ± 3 <sup>a</sup>	42 ± 0 <sup>a</sup>	3691 ± 687 <sup>b</sup>	<0.01	42.6 ± 4.6 <sup>a</sup>
		Interval	12.4-13.6	84.0-91.0	4906-5080	1656-1832	47-51	42-42	3205-4177	<0.01	39.3-45.9
Total	154	general mean ± SD	9.6 ± 3.2	82 ± 69	5089 ± 1981	1179 ± 455	64 ± 35	85 ± 177	4828 ± 1309	0.51 ± 0.78	46.7 ± 11.6
		median	9.0	60.0	4919	1114	53	34	4773	0.13	46.4
		Interval	3.2-25.4	11.1-551.6	1431-9910	348-3621	12-211	<0.004-1466	2177-8165	<0.01-4.45	5.1-76.1

N, number of samples. Values with different letters (a, b, c) in the same column are significantly different at P ≤ 0.05.



levels. Samples from BA, SE, CE, RS and MT had higher amounts of Na. The CE samples presented the highest level of Se ( $4.45 \text{ mg kg}^{-1}$ ), comparable to a single sample from Santa Branca, SP ( $4.28 \text{ mg kg}^{-1}$ ). Higher levels of Zn were found in samples from PI, CE, SP, SE, BA, RS, DF and MT.

Overall, greater amounts of minerals were found in bee pollen samples from Northeastern (CE, SE, PI, BA), Midwestern (DF) and Central Brazil (MT). One state from Southeastern Brazil (MG) presented the highest average level of Fe. Nevertheless, it should be emphasized that the interpretation of these data is limited by the small number of samples collected in PI, CE, MT and DF, where bee pollen production was low during the period of the present study.

The predominant minerals of Brazilian bee pollen were K, P, Ca and Mg comprising 37, 36, 16 and 9%, respectively of the mean composition. Present in lower proportions were Na (0.63%), Fe (0.60%), Mn (0.47%), Zn (0.34%), Cu (0.07%) and Se (0.004%).

The wide range of K levels found in the present study ( $1431\text{--}9910 \text{ mg kg}^{-1}$ ), covered the ranges obtained in Brazilian studies and those of other countries. Funari *et al.*<sup>8</sup> and Carpes *et al.*<sup>9</sup> found  $5900\text{--}7200 \text{ mg kg}^{-1}$  and  $3722\text{--}6364 \text{ mg kg}^{-1}$  K in samples from SP and from PR, SC and RS, respectively. Samples from Spain,<sup>6</sup> Australia,<sup>16</sup> Poland, South Korea, China<sup>18</sup> and Argentina<sup>17</sup> presented the following K ranges:  $2485\text{--}6411 \text{ mg kg}^{-1}$ ,  $2200\text{--}3800 \text{ mg kg}^{-1}$ ,  $2843\text{--}4854 \text{ mg kg}^{-1}$ ,  $3455\text{--}5489 \text{ mg kg}^{-1}$ ,  $4247\text{--}5976 \text{ mg kg}^{-1}$ ,  $2263\text{--}9784 \text{ mg kg}^{-1}$ , respectively.

Serra-Bonvehí, and Escolà-Jordà<sup>6</sup> found in Spanish samples phosphorus values ten times lower ( $295\text{--}854 \text{ mg kg}^{-1}$ ) than those of the present study ( $2177\text{--}8165 \text{ mg kg}^{-1}$ ). Our results corroborated the ranges found by Somerville and Nicol<sup>16</sup> for Australian ( $1400\text{--}8000 \text{ mg kg}^{-1}$ ) and Brazilian samples ( $3900\text{--}5000 \text{ mg kg}^{-1}$  in samples from SP and  $5648\text{--}8865 \text{ mg kg}^{-1}$  in samples from PR, SC and RS).<sup>8,9</sup>

Our pollen samples presented higher Ca levels ( $828\text{--}4670 \text{ mg kg}^{-1}$ ) than those found in Spanish ( $253\text{--}850 \text{ mg kg}^{-1}$ ),<sup>4</sup> South Korean ( $105\text{--}694 \text{ mg kg}^{-1}$ ) and in some Chinese ( $181\text{--}974 \text{ mg kg}^{-1}$ ) and Polish ( $542\text{--}1080 \text{ mg kg}^{-1}$ ) samples.<sup>18</sup> Our levels were closer to the ones found in Australian ( $360\text{--}3100 \text{ mg kg}^{-1}$ ),<sup>16</sup> Argentinian ( $160\text{--}2490 \text{ mg kg}^{-1}$ )<sup>17</sup> and Brazilian ( $1500\text{--}3000 \text{ mg kg}^{-1}$ ,  $292\text{--}2149 \text{ mg kg}^{-1}$ ) samples.<sup>8,9</sup>

Magnesium levels found in present study ( $348\text{--}3621 \text{ mg kg}^{-1}$ ) covered the levels found in most of the other studies with ranges of  $742\text{--}1723$  for Polish,  $987\text{--}1762$  for South Korean,  $1126\text{--}1893$  for Chinese,<sup>18</sup>  $401\text{--}2210$  for Argentinian,<sup>17</sup>  $600\text{--}1000$  and  $463\text{--}1235 \text{ mg kg}^{-1}$  for Brazilian samples.<sup>8,9</sup> Spanish and Australian samples showed some lower Mg levels, with ranges between:  $273\text{--}816$  and  $220\text{--}2700 \text{ mg kg}^{-1}$ .<sup>6,16</sup>

Sodium levels in the present study ( $<0.004\text{--}1466 \text{ mg kg}^{-1}$ ) tend to be lower than those of Spanish ( $614\text{--}2375 \text{ mg kg}^{-1}$ ),<sup>7</sup> Polish ( $293\text{--}2191 \text{ mg kg}^{-1}$ ) and Chinese pollen ( $1072\text{--}2447 \text{ mg kg}^{-1}$ ),<sup>18</sup> but in agreement with those found in Brazilian ( $12\text{--}636 \text{ mg kg}^{-1}$ ),<sup>9</sup> Argentinian ( $46\text{--}554 \text{ mg kg}^{-1}$ ),<sup>17</sup> Australian ( $16\text{--}480 \text{ mg kg}^{-1}$ )<sup>16</sup> and South Korean ( $454\text{--}1113 \text{ mg kg}^{-1}$ ) samples.<sup>18</sup>

Our Fe and Cu levels have wider ranges ( $11\text{--}552$  and  $3.2\text{--}25.4 \text{ mg kg}^{-1}$ ), that cover the results obtained in other studies. Fe ranged from  $11.1$  to  $80.3$ ,<sup>6</sup>  $14$  to  $520$ ,<sup>16</sup>  $40.4$  to  $136.1$ ,  $74.3$  to  $365.9$ ,  $59.0$  to  $182.3$  and  $23$  to  $229 \text{ mg kg}^{-1}$  in samples from Spain, Australia, Poland, South Korea, China and Argentina, respectively.<sup>17,18</sup> In Brazilian bee pollen samples from SP and PR, SC and RS, Fe levels ranged from  $90.7$  to  $145.0$  and  $27.9$  to  $203.3 \text{ mg kg}^{-1}$ , respectively.<sup>8,9</sup>

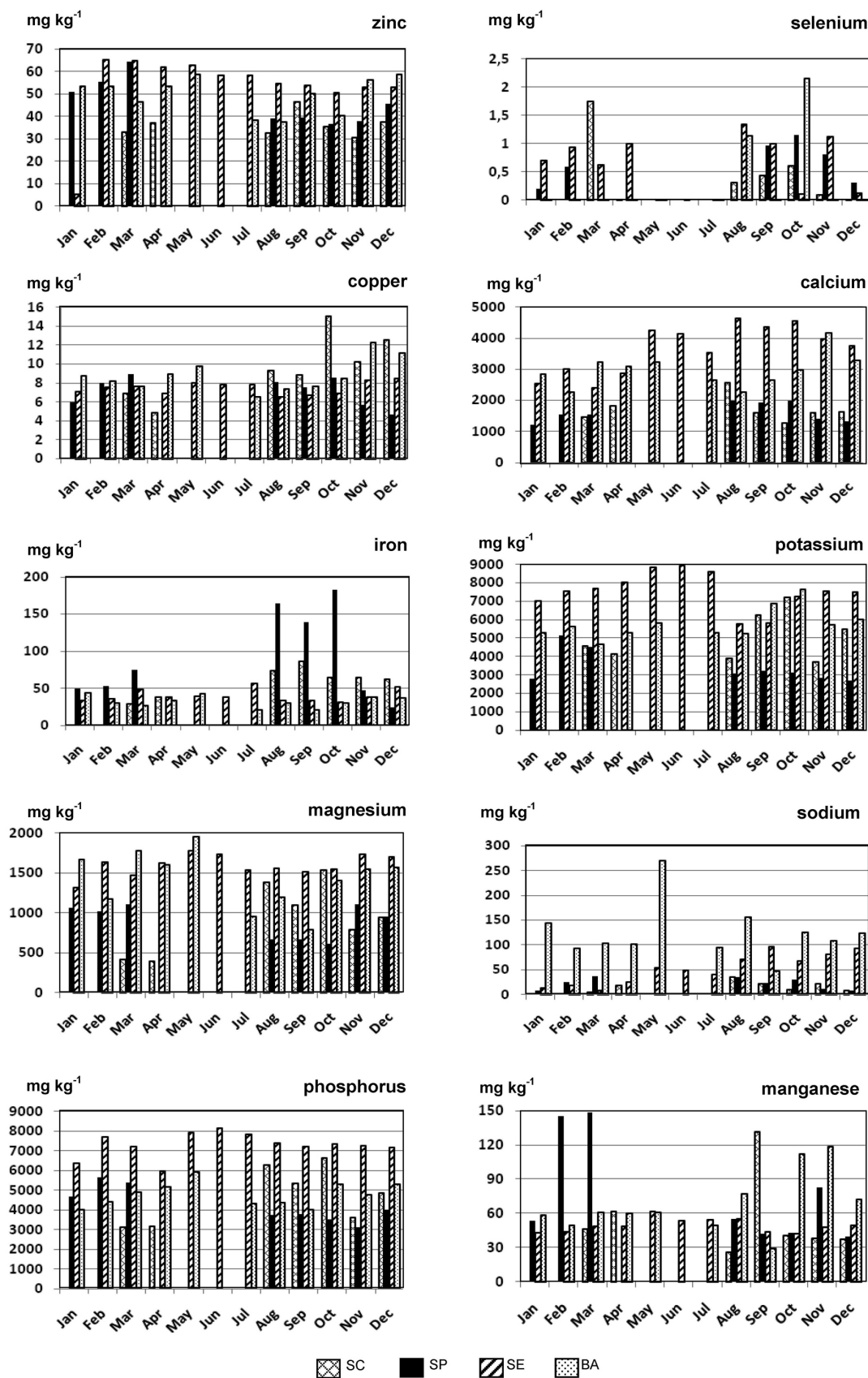
Copper ranges of  $4\text{--}16$ ,  $3\text{--}42$ ,  $5.6\text{--}23.9$ ,  $5.3\text{--}14.7$ ,  $3.2\text{--}10.0$  and  $6.0\text{--}20.0 \text{ mg kg}^{-1}$  were obtained in samples from Spain, Australia, Poland, South Korea, China and Argentina, respectively.<sup>6,16-18</sup> In Brazilian bee pollen samples from SP and PR, SC and RS, Cu ranges were  $10.0\text{--}18.0$  and  $4.5\text{--}17.8 \text{ mg kg}^{-1}$ , respectively.<sup>8,9</sup>

For Mn, our results ( $12\text{--}211 \text{ mg kg}^{-1}$ ) tend to be lower than some levels found in some Chinese pollen samples ( $13.2\text{--}429.9 \text{ mg kg}^{-1}$ ),<sup>18</sup> but are mostly in accordance with other work that obtained the following results:  $7.4\text{--}18.3 \text{ mg kg}^{-1}$  for Spanish samples,<sup>6</sup>  $5\text{--}110 \text{ mg kg}^{-1}$  for Australian samples,<sup>16</sup>  $13.3\text{--}59.7$  and  $25.6\text{--}99.4 \text{ mg kg}^{-1}$  for Polish and South Korean samples,<sup>18</sup> respectively and  $14\text{--}57 \text{ mg kg}^{-1}$  for Argentinian samples.<sup>17</sup> Brazilian samples from SP and PR, SC and RS presented ranges of  $19.0\text{--}54.8 \text{ mg kg}^{-1}$  and  $18.7\text{--}129.4 \text{ mg kg}^{-1}$ , respectively.<sup>8,9</sup>

In the present work Zn levels ranged from  $41.4\text{--}65.3 \text{ mg kg}^{-1}$ , with only one sample with a lower level ( $5.1 \text{ mg kg}^{-1}$ ). With the exception of this last sample, our results are within the ranges of most studies:  $19\text{--}81 \text{ mg kg}^{-1}$  for Spanish samples,<sup>6</sup>  $16\text{--}340 \text{ mg kg}^{-1}$  for Australian samples,<sup>16</sup>  $25.6\text{--}53.6$ ,  $23.7\text{--}60.7$  and  $23.9\text{--}38.3 \text{ mg kg}^{-1}$  for Polish, South Korean and Chinese samples,<sup>18</sup> respectively, and  $23\text{--}106 \text{ mg kg}^{-1}$  for Argentinian samples.<sup>17</sup> Brazilian samples from SP and PR, SC and RS had ranges of  $48.0\text{--}118.0$  and  $36.1\text{--}82.1 \text{ mg kg}^{-1}$ , respectively.<sup>8,9</sup>

#### Seasonal and geographical effects

Variations of mineral levels of samples from the same sites in SP (São José dos Campos), SC (Campos Novos), BA (Canavieiras) and SE (Neópolis) during the year are shown in Figure 1. SP and SC are located in Southeastern and Southern areas of the country, respectively, while BA and SE are located in Northeastern region.



**Figure 1.** Variation of minerals levels of bee pollen during one year of production from the same sites of the following states: Santa Catarina (SC), São Paulo (SP), Sergipe (SE) and Bahia (BA).

Pronounced variations are observed in the levels of all minerals during one year of bee pollen production. Although this variation presents a challenge to the appraisal of geographic effects, one can still observe that Brazilian pollen production is constant in Northeastern states (BA and SE), and the mineral levels tend to be higher, especially for Zn, Ca, K, Mg and P. In fact, apiculture has grown in this area of the country, which is considered to be prominent for beekeeping activity. The Northeastern region is one of the few in the world with the potential to produce organic bee products in large quantity, due to the great diversity of plant species and microclimates, along with vast unexplored land areas, free of conventional farming activity.

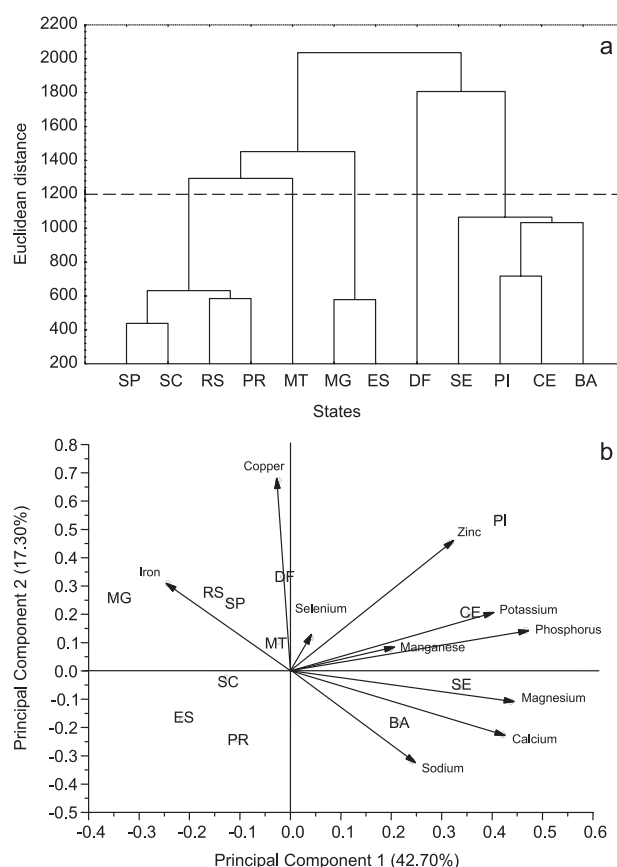
In SP and SC states bee pollen production is higher during the Spring season, which occurred from September 23 to December 12, decreasing during Summer (December 22 to March 19), Fall (March 20 to June 20), with low, if any production during the Winter season (June 21 to September 22). In addition, SP samples showed a tendency for higher Fe levels during August, September, and October and higher Mn levels during February and March.

The dendrogram obtained by HCA is shown in Figure 2a. Cluster formed among bee pollen samples from the Southern and Southeastern states of SC, RS, PR and SP indicates that these samples are similar in mineral composition. Samples from DF and MT exhibit low similarity. Samples from MG and ES show similarity and samples from the Northeastern region (SE, PI, CE and BA) make up another group with similar composition.

The grouping of the samples through their similarities can be better explained by the PCA (Figure 2b) that indicates which mineral elements are responsible for the clusters. The samples of BA and SE can be characterized by the Na, Ca and Mg contents. Those from PI and CE can be mainly defined by Zn, K, Mn and P. Cu influenced the samples from the Federal District while those of MG, RS, SP and MT are characterized by the Fe level.

#### Estimated micronutrient contribution of pollen to the diet

Contribution of the mineral elements in bee pollen to recommended daily intakes (RDI) established by Brazilian regulation *per* portion is shown in Table 4.<sup>30</sup> In terms of the mean values, high contribution could come from Mn (69.6% of RDI) and Se (37.5% of RDI). According to Brazilian regulation,<sup>31</sup> a contribution of more than 30% classifies the food as a rich source and above 15% as a source. In addition, Brazilian bee pollen may be a source of Cu (26.7% of RDI), P (17.2% of RDI) and Zn (16.7% of RDI). It is notice worthy that Cu content did not vary



**Figure 2.** Dendrogram (a) and principal components 1 and 2 (b) obtained from mean composition of mineral elements in 154 dehydrated bee pollen samples from 11 Brazilian states and the Federal District. Southern states: Rio Grande do Sul (RS), Santa Catarina (SC) and Paraná (PR). Southeastern states: São Paulo (SP), Minas Gerais (MG), Espírito Santo (ES). Midwestern state: Mato Grosso (MT). Northeastern states: Bahia (BA), Ceará (CE), Piauí (PI), Sergipe (SE). Central region: Federal District (DF).

**Table 4.** Percentage contribution of the mineral elements to Brazilian recommended daily intakes (RDI) for adults in relation to the mean levels found in the 154 Brazilian bee pollen samples

Element	Mean content / (mg kg <sup>-1</sup> )	RDI <sup>a</sup> / mg	RDI <sup>b</sup> / %
Sodium	85	2400	0.1
Calcium	2215	1000	5.5
Iron	82	14	14.6
Magnesium	1179	260	11.3
Zinc	46.7	7	16.7
Phosphorus	4828	700	17.2
Copper	9.6	0.9	26.7
Selenium	0.51	0.034	37.5
Potassium	5089	4700*	2.7
Manganese	64	2.3	69.6

<sup>a</sup>Brazilian Ministry of Health.<sup>24</sup> <sup>b</sup>Considering a 25 g portion.<sup>29</sup> \*Dietary Reference Intakes (DRI), according to Institute of Medicine,<sup>28</sup> not established by Brazilian regulation.



among the studied localities. The high K/Na ratio makes bee pollen potentially valuable for diets with defined electrolytic balance.

Selenium, Cu and Zn are considered antioxidant nutrients, since they are structural components of the antioxidant enzymes: Cu and Zn in superoxide dismutase (SOD) and Se in plasmatic glutathione peroxidase (GSHPx), respectively. Zn can also inactivate superoxide radicals in cytosol and mitochondria.<sup>32,33</sup> The presence of these three elements in Brazilian bee pollen, together with expressive amounts of flavonoids and others components points to this product as a potential source of antioxidants in the human diet.<sup>34-36</sup>

## Conclusions

The method used to determine the mineral elements, which consisted of microwave-assisted digestion in closed system and quantification by inductively coupled plasma optical emission spectrometry (ICP OES) showed good precision and accuracy, additionally allowing simultaneous determination of micro and macroelements in bee pollen samples. Wide within-state and between-state variations exist in the mineral composition of dehydrated bee pollen. K, P, Ca and Mg are the major mineral elements present in the pollen composition. Northeastern Brazilian states seem to be promising for bee pollen production, since samples from this region presented significantly higher amounts of minerals and showed constant production throughout the year. In terms of mean values, Brazilian bee pollen may be considered a rich source of Mn and Se and a source of Cu, P and Zn. The high K/Na ratio can make it a valuable product for diets with defined electrolytic balance; and the presence of Se, Cu and Zn makes it a potential source of antioxidant minerals to the human diet.

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