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Palynological and physicochemical characterization of *Apis mellifera* L. bee pollen in the Southern region of Brazil

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Abstract

Bee pollen has been used for many years in both traditional medicine and supplementary nutrition, as well as in alternative diets, mainly due to its nutritional properties and health benefits. Bee pollen production is a recent activity in Brazil, having begun in the late 1980s. However, the country has the potential of being a large world producer of high quality pollen, particularly because of the great diversity of tropical flora and the resistance of the Brazilian *Apis mellifera* bee races. Thirty-six samples of bee pollen from the Southern region of Brazil were analyzed regarding pollen types and physicochemical and nutritional composition. Only one sample was considered monofloral, which was exclusively composed by pollen from the Asteraceae family). The State of Paraná showed a greater variety of pollen types, 18 in total, representing 82% of the total number identified in this study. The bee pollen in the States of Rio Grande do Sul and Paraná showed a higher number of samples with humidity content above the standard permitted by the Brazilian legislation, i.e. over 4%. The bee pollen was characterized by its high protein content with average values of 20.47%. The analysis regarding humidity, lipids and sugar showed no statistical differences among the samples ($p<0.05$). The pollen samples had a high concentration of reducible sugars (48%). The predominant minerals in the samples PR, SC and RS were phosphorus (7102.29, 6873.40, 6661.73 mg/kg of pollen), followed by potassium (5383.73, 4997.77, 4773.26 mg/kg of pollen), calcium (1179.05, 961.93, 848.36 mg/kg of pollen) and magnesium (818.02, 679.01, 725.89 mg/kg of pollen). Statistical analysis (Tukey test) demonstrated no significant difference between the contents of calcium, copper, iron, phosphorus and sodium in the pollen samples of the South of Brazil. However, the samples from the State of Paraná contained the highest contents of potassium and differed statistically from the samples of the State of Rio Grande do Sul.

Key words: Chemical composition, bee pollen, *Apis mellifera*, minerals, Asteraceae family.

Introduction

Bee pollen is an agglomerate of pollen grains from various botanical sources, which are collected by the bees and mixed with nectar and secretion from the hypopharyngeal glands, such as α and β-glycosidase enzymes. Pollen contains nutrients, such as carbohydrates, proteins, amino acids, lipids, vitamins and minerals, in addition to carotenoids, flavonoids and phytosterols, which is the reason why it is used by humans as an alternative food source and/or food supplement [1].

Each pollen type has its own specific characteristics related to the genetics of the floral species and plantations visited by the bees. During the pollen harvest (collection) the bees might show preferences regarding the floral sources [2, 3]. The nutritional quality of the pollen is one of the factors that makes it attractive to bees[4]. Honeybees collect a substantial quantity of pollen, approximately 30 mg, at each visit to the field and to obtain such a load they have to visit many flowers [5]. Bee pollen can be *monofloral*, characterized by single botanic taxonomy in the pollen load of the "corbiculae", that maintains the constant organoleptic and biochemical properties of the original plant. When the bees visit other flowers, or mix the "pollen loads" from various flowers, these are called *heterofloral* and present a variety of biochemical properties [6].

Diversity and frequency of pollen grains in bee pollen can be determined by means of pollen analysis of the morphological characteristics, which indicates the probable original botanical species. The quantity of each pollen type can also be an indication of the quality of the product. In Brazil, cooperatives and apiculture associations don't use palynological analyses to qualify the market's apicultural products [7].

Pollen, as well as other apiculture products, such as propolis and honey, have gained attention due to their antibacterial [8, 9], antifungal [10], anti-inflammatory [11], antioxidant and immunomodulatory therapeutic properties [12]. Pollen extract (Cernilton®) is often used in the treatment of some cases of benign prostatitis [12] and in reducing oral sensitivity in children with pollen allergy [13].

The objective of this study was to identify the pollen component

types and to verify the monofloral and heterofloral conditions of each sample and further to determine the physicochemical and nutritional composition of the dehydrated bee pollen from three states in the Southern region of Brazil.

Materials and Methods

Materials: All solvents were of analytical grade and obtained from Merck (Merck KGaA Frankfurter, Darmstadt, Germany). Thirty-six dehydrated apicultural pollen samples were collected from different apiaries in locations in the Southern region of Brazil, sixteen being from the State of Paraná (PR samples), ten from the State of Santa Catarina (SC samples) and ten from the State of Rio Grande do Sul (RS samples), during the period from August 2005 to April 2006 (Fig. 1). After collection, each sample was separately crushed, homogenized and stored at 5ºC in a freezer for later analysis. All analyses were performed in triplicate.

Palynology: Scanning electronic microscopy (SEM) analysis of the pollen grains was performed in the laboratory facilities of the Nucleus of Support for Research in electronic microscopy applied to cattle raising research (NAP/MEPA) at ESALQ-USP, Piracicaba-São Paulo. Approximately 2 g of each sample was considered representative for pollen analysis [14]. The pollen loads were grouped up into subsamples according to their coloring and each subsample was weighed and metallized with gold and then analyzed by scanning electronic microscopy (SEM) in Digital Scanning Microscope DSM 940 A (Zeiss Co.). In this experiment approximately 350 pollen grains of each sample were identified and counted and from this total the percentage of each pollen type was established as: "Predominant pollen" or "dominant" (> 45% do total) (PD); "Accessory or secondary pollen" (16-45%) (PA); "Important isolated pollen" (3-15%) (PII) and "Occasional isolated pollen" (<3%) (PIO) [15].

Determination of physicochemical characteristics: The humidity content was determined in an oven at 60ºC, until a constant weight of a previously crushed 2 g pollen dry basis sample was obtained[16].

Figure 1. Collection sites of bee pollen in the Southern region of Brazil. PR: State of Paraná; SC: State of Santa Catarina; RS: State of Rio Grande do Sul.

Total nitrogen was determined by the Kjeldahl method from a 0.7 g crushed pollen sample, using a factor of 6.25 for conversion into protein [16].

The total lipids were determined by gravimetry from 2 g of crushed pollen extracted with petroleum ether in a Soxhlet apparatus for approximately 4 hours [17].

The total sugar and reducible sugar contents were determined spectrophotometrically at 510 nm, in accordance with the Somogyi-Nelson method with a few modifications [18].

The minerals Fe, Ca, Zn, K, Na, Cu, Mg and Mn were determined after incineration of 1.5 g of pollen at 550 ºC, until a constant weight was obtained. The ash was solubilized with 25 ml of HNO_3 50%, heated in a water bath for 30 min, filtered and the minerals were determined by atomic absorption spectrophotometry (Varian Model Spectra AA 100 & 200). The phosphorus content was determined by an ultraviolet spectrophotometer (Shimadzu Model UV-1601 PC) at 650 nm [16].

Statistical analysis: Data was submitted to analysis of variance (ANOVA). The Student's *t*- test was used to assess the significant differences between means, sixteen of which from the State of Paraná (PR samples), ten from the State of Santa Catarina (SC samples) and ten from the State of Rio Grande do Sul (RS samples), (comparison of means) at the level of $p<0.05$, using the software SAS V9.

Results and Discussion

According to Barth [19], studies regarding pollen analysis of apicultural products in Southern Brazil are not frequent. Nevertheless, it is known that honey from this region is predominantly of Asteraceae (Compositae), especially *Senecio brasiliensis,* "maria-mole" and *Lithrea* sp. "aroeira" [15]. In other studies, Barth and Dutra [7, 19] confirmed that the pollen types of various species of Asteraceae, *Eucalyptus, Myrcia* and *Mimosa scabrella,* frequently occur in the honey from the Southern region of Brazil.

The "pollen loads" had a mixture of pollen types from many different floral species, whose coloring varied from light yellow to darker colors, such as purple and brown. Twenty-two pollen types were identified in the 36 bee pollen samples from the Southern region of Brazil. With exception of sample PR 12, which was classified as monofloral, all the other samples of bee pollen presented at least two pollen types. The bee pollen PR 12 from one of the apiaries in União da Vitória, State of Paraná, had pollen exclusively from the *Baccharis* type (Asteraceae) (Table 1).

Pollen from the Euphorbiaceae family (Fig. 2, Image 1) and Asteraceae (*Elephantopus* type) (Fig. 2, Image 2) were dominant (>45%) only in samples PR 01 and PR 09, respectively (Table 1). Thus, with exception of samples RS 06 and RS 08, all the samples from the State of Rio Grande do Sul contained the *Elephantopus* type as accessory pollen (15 - 45%) and important isolated pollen (3 – 15%) (Table 3, Fig. 2, Image 2).

In the States of Paraná and Santa Catarina the *Eupatorium* type was present in 50% of the samples, while in Rio Grande do Sul the *Elephantopus* type was present in 80% of the samples (Tables 1, 2 and 3). The

Table 1. Pollen types of bee pollen samples observed in the State of Paraná.

*Image	Pollen types	Bee pollen samples from the State of Parana															
		01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16
1	Euphorbiaceae	PD															
2	Asteraceae *Elephantopus*	PII		PIO						PD					PIO		
3	Asteraceae *Eupatorium*		PD		PD		PA	PIO	PA		PD				PII		PA
4	Asteraceae *Gochnatia*				PII				PIO			PA					
5	Asteraceae *Baccharis*					PD							PD			PII	
6	Sapindaceae *Matayba*	PII															
7	Myrtaceae *Eucalyptus*		PII		PA							PA					PA
8	Mimosaceae *Mimosa scabrella*		PA				PA								PA		
9	Verbenaceae *Aegiphila*		PII														
10	Brassicaceae			PD			PII							PII	PD	PA	
11	Arecaceae Type I					PII		PD						PA			
12	Arecaceae Type 2													PA			
13	Anacardiaceae *Schinus*									PA							
14	Anacardiaceae Type I					PII											
15	Anacardiaceae *Astronium*					PIO					PII						
16	Leguminosae					PIO											
17	Boraginaceae *Cordia*							PA									
18	Arecaceae															PD	

* Images from the Scanning Electronic Microscopy (SEM); DP = dominant pollen (> 45%); PA = accessory pollen (15 - 45 %); PII = important isolated pollen (3 – 15%); PIO = occasional isolated pollen (< 3%).

pollen type *Mimosa scabrella*, characteristic from the Curitiba Paraná region, appeared as accessory pollen (15-45%) only in samples PR 02 (Lapa), PR 06 (União da Vitória) and PR 14 (Curitiba). However, this pollen type was not found in the other states of the Southern region (Table 1, Fig. 2, Image 8).

In the sample PR 11 was detected the *Gochnatia* (Asteraceae) type (Table 1, Fig. 2, Image 4) in the accessory pollen category and the *Eucalyptus* (Myrtaceae) type as isolated pollen (Fig. 2, Image 7).

The State of Paraná (PR samples) showed the biggest variety (18 pollinic types) representing 82% of the total identified in this study (Table 1). These results corroborate other studies with *Apis mellifera*, that confirmed the presence of *Allophylus*, *Baccharis*, *Campomanesia*, *Cecropia*, *Citrus*, *Eucalyptus*, *Matayba*, *Mimosa scabrella*, *Paspalum* and *Vernonia* in the honey from the State of Paraná, and found it to be a basically heterofloral product, but according to the results of this author, the highest occurrence was of *Eucalyptus* [2].

The pollen type Brassicaceae (Fig. 3, Image 10) was found in the bee pollen of three states in the Southern region of Brazil but in the State of Rio Grande do Sul it only appeared in 30% of the samples as dominant pollen (>45%). However, the *Eucalyptus* type was the dominant one, being present in 20% of the samples from the State of Santa Catarina (Table 2). The State of Santa Catarina is also an important producer of honey, bee pollen and propolis, but knowledge about the pollen grains found in these products is poor [19]. The bracatinga (*Mimosa scabrella*) is a very important apicultural plant in the State of Santa Catarina [15] and its honey was analyzed and classified as melato honey [20]. Melato contains enzymes derived from salivary gland secretions and from the intestine of the plant sucker insects and it has always been classified as inferior honey compared with floral honey on the internal market, by being less attractive due to its dark color and strong flavor, however, since the 80's the German market has shown great interest in this product [20].

Pollen from the Leguminosae (Table 2, Fig. 3, Image 16) and Arecaceae (Table 2, Fig. 4, Image 18) families appeared individually in only one of the 36 analyzed samples. These pollen types were found in the PR 05 sample, collected in the Pato Branco region, and the in PR 15 sample, collected in the Balsa Nova region, both in the State of Paraná.

In sample RS 06 *Eucalyptus* was the dominant pollen, however, the pollen type from the Rosaceae family was observed as accessory pollen, and was unprecedented among the 36 analyzed samples of bee pollen (Table 3, Fig. 4, Image 21). The pollen type *Struthanthus* (Loranthaceae) was present only as isolated pollen (3-15%) in the sample RS 09 (Table 3, Fig. 4, Image 22).

The humidity content in the samples ranged from 1.69% to 7.84%, with a mean of 4.19% (between 36 samples). Results showed that 47% of the samples were outside the Brazilian Legislation standards, which only allows the sale of dehydrated pollen with a maximum humidity content of 4% [21]. The bee pollen in the States of Rio Grande do Sul (RS) and Paraná (PR) presented the largest number of samples with humidity levels outside the Brazilian Legislation standard, which corresponds to 60 and 50% of the samples, respectively. However, there were no statistical differences among the bee pollen samples in the three analyzed states (Table 4).

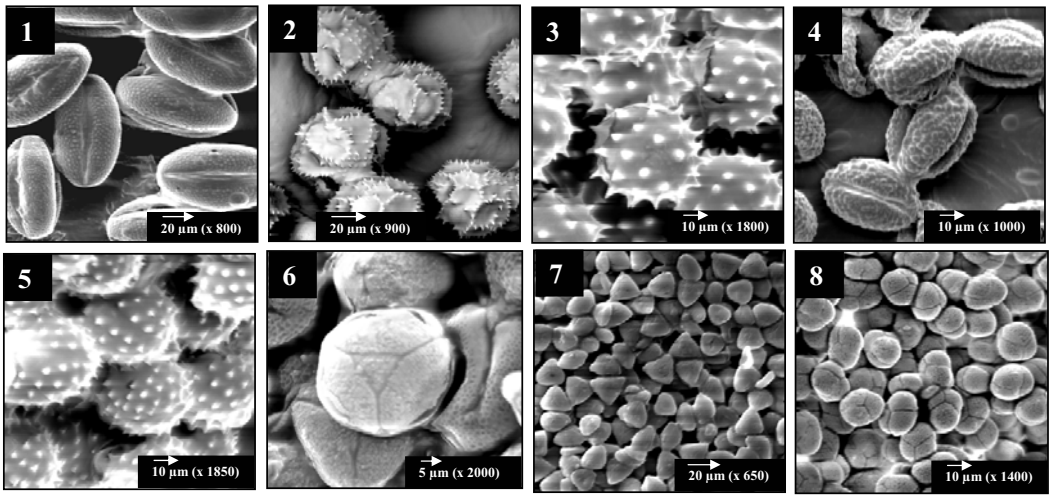

Figure 2. Pollen types observed in pollen loads in the Southern region of Brazil. Images: 1. Euphorbiaceae; 2. Asteraceae *Elephantopus*; 3. Asteraceae *Eupatorium*; 4. Asteraceae *Gochnatia*; 5. Asteraceae *Baccharis*; 6. Sapindaceae *Matayba*; 7. Myrtaceae *Eucalyptus*; 8. Mimosaceae *Mimosa scabrella*.

Table 2. Pollen types observed in the samples of bee pollen in the State of Santa Catarina.

*Image	Pollen types	Bee pollen samples from the State of Santa Catarina									
		01	02	03	04	05	06	07	08	09	10
1	Euphorbiaceae							PA	PII		PII
2	Asteraceae *Elephantopus*		PII								PA
3	Asteraceae *Eupatorium*	PD		PII				PA	PA	PA	
4	Asteraceae *Gochnatia*			PII	PII					PD	PII
5	Asteraceae *Baccharis*	PII			PA		PII	PII			
7	Myrtaceae *Eucalyptus*			PD		PD					
10	Brassicaceae		PD				PII				
11	Arecaceae Type I					PII					PD
14	Anacardiaceae Type I				PA					PII	
16	Leguminosae								PA		
19	Anacardiaceae Type 2				PA	PA		PII			
20	Rosaceae *Prunus*						PD				

* Images from the Scanning Electronic Microscopy (SEM); DP = dominant pollen (> 45%); PA = accessory pollen (15 - 45 %); PII = important isolated pollen (3 – 15%); PIO = occasional isolated pollen (< 3%).

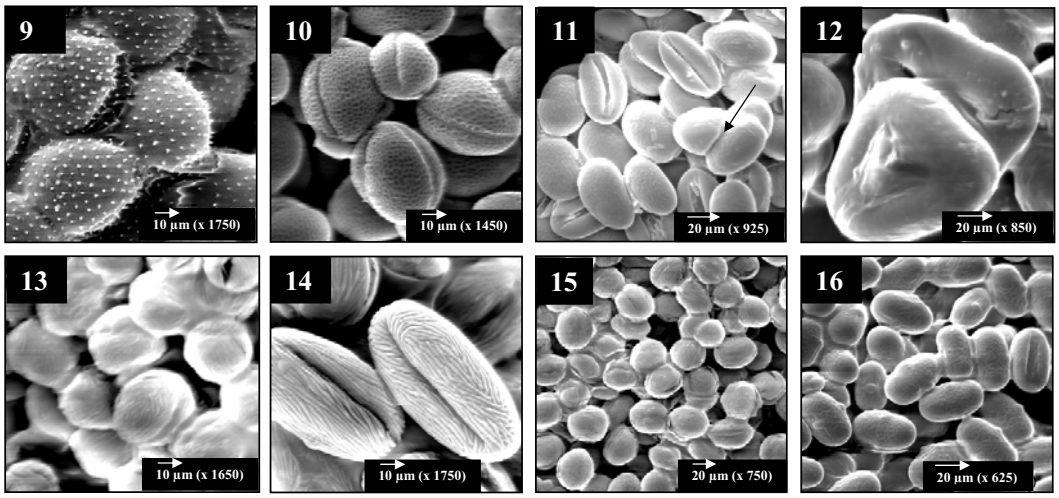

Figure 3. Pollen types observed in pollen loads in the Southern region of Brazil. Images: 9 Verbenaceae *Aegiphila*; 10. Brassicaceae; 11. Arecaceae Type I, on black arrow *Pinus*; 12. Arecaceae Type 2; 13. Anacardiaceae *Schinus*; 14. Anacardiaceae Type I; 15. Anacardiaceae *Astronium*; 16. Leguminosae.

Table 3. Pollen types of bee pollen samples observed in the State of Rio Grande do Sul.

*Image	Pollen types	Bee pollen samples from the State of Rio Grande do Sul									
		01	02	03	04	05	06	07	08	09	10
2	Asteraceae *Elephantopus*	PA	PII	PII	PII	PA		PII		PA	PII
3	Asteraceae *Eupatorium*				PA	PA	PII		PII		
4	Asteraceae *Gochnatia*							PA			
5	Asteraceae *Baccharis*									PA	
6	Sapindaceae *Matahyba*	PA	PD						PD		
7	Myrtaceae *Eucalyptus*						PD	PA		PD	
10	Brassicaceae	PA	PA	PD	PD						PD
11	Arecaceae Type I		PA	PA		PA					PA
20	Rosaceae *Prunus*		PIO								
21	Rosaceae						PA				
22	Loranthaceae *Struthanthus*									PII	

* Images from the Scanning Electronic Microscopy (SEM); DP = dominant pollen (> 45%); PA = accessory pollen (15 - 45 %); PII = important isolated pollen (3 – 15%); PIO = occasional isolated pollen (< 3%).

The total protein content ranged from 15.04 to 27.69 % with a mean of 20% (between 36 samples), and the protein contents of the samples from the State of Santa Catarina (SC) differed statistically from those from the States of Rio Grande do Sul (RS) and Paraná (PR) (Table 4). All the analyzed samples presented a protein content of above 8%, thus being in accordance with the Brazilian technical regulation [21]. Protein contents higher than 25.9% content have also been found in bee pollen produced in Minas Gerais [22] and in Southeast Australia [23]. The greatest part of the nitrogen present in pollen is found in the protein fraction, which is the second most abundant group of nutrients, after the carbohydrates.

The high protein content (20.34, 22.60 and 18.55%), reducible sugars (50.01, 47.43 and 48.18 g/100 g) and low lipid content (5.07, 4.59 and 4.80%) found in the PR, SC and RS samples, make pollen an excellent food supplement (Table 4). These results corroborate those of Almeida-Muradian *et al.* [24], who assessed ten samples from the Southern region of Brazil and found contents of humidity, proteins, lipids and ash of 7.4, 20, 6 and 2.2%, respectively. The high reducible sugar content in pollen can be explained by the presence of honey or nectar in the fluid that cements the pollen grains [25]. The lipid content from the 36 samples of bee pollen (4.86± 0.65 g/100 g) was similar to that described by Somerville [26], who found a variation of 0% of lipids for *Eucalyptus macrorhyncha* pollen and 11.2% for *Hypochoeris radicata* pollen in the southeast of Australia.

The predominant minerals in the samples from PR, SC and RS were phosphorus (7102, 6873 and 6662 mg/kg of pollen), followed by potassium (5384, 4998 and 4773 mg/kg of pollen), calcium (1179, 962 and 848 mg/kg of pollen) and magnesium (818, 679 and 726 mg/kg of pollen). Statistical analysis through the Tukey test showed no significant difference between the contents of calcium, copper, iron, phosphorus, magnesium and sodium in the pollen samples from the South of Brazil. However, regarding potassium, the samples from State of Paraná (PR) presented the highest contents and differed statistically from the samples from State of Rio Grande do Sul (RS) (Table 4). The mean zinc content was 55.22±2.88 mg/kg of pollen from the State of Rio Grande do Sul (RS) and differed statistically from the samples from the State of Santa Catarina (SC) ($p<0.05$). The iron and zinc contents corresponded to 15% of the recommended daily allowance (RDA) (Table 4). According to Wesh and Marston[27] the presence of zinc, copper, iron and a high rate of potassium/sodium make bee pollen an interesting food for diets with a defined electrolytic balance. With the exception of the zinc content, the other minerals from bee pollen in the State of Paraná were shown to have higher mineral contents than that in the other states of the Southern region.

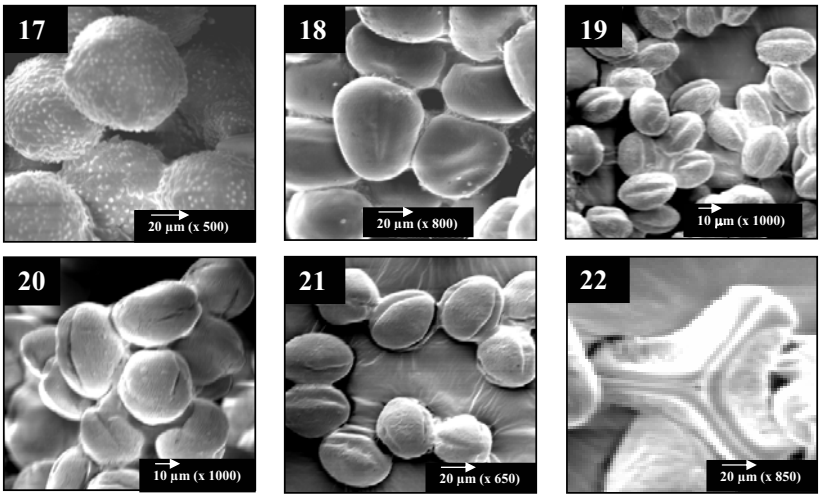

Figure 4. Pollen types observed in pollen loads in the Southern region of Brazil. Images: 17. Boraginaceae *Cordia*; 18. Arecaceae; 19. Anacardiaceae Type 2; 20. Rosaceae *Prunus*; 21. Rosaceae; 22. Loranthaceae *Struthanthus.*

Table 4. Chemical composition of bee pollen samples of the Southern region of Brazil.

Analysis	Bee pollen		
	PR[1]	SC[2]	RS[2]
aw*	0.38±0.01 [a]	0.38±0.01[a]	0.37±0.01[a]
Humidity (%)	4.39±0.42 [a]	3.45±0.53 [a]	4.85±0.43 [a]
Protein (%)	20.34±0.55 [b]	22.60±0.01 [a]	18.55±0.01 [b]
Lipids (%)	5.07±0.16 [a]	4.59±0.20 [a]	4.80±0.20 [a]
Total sugar**	53.09±1.05 [a]	50.44±1.32 [a]	50.16±1.32 [a]
Reducing sugar**	50.01±1.03 [a]	47.43±1.30 [a]	48.18±1.30 [a]
Ca[3]	1179.05±89.99 [a]	961.93±113.84[a]	848.36±120.00 [a]
Cu	12.05±0.74 [a]	11.29±0.94 [a]	10.41±0.99 [a]
Fe	86.66±8.40 [a]	59.48±10.63 [a]	75.64±11.88 [a]
P	7102.29±219.78[a]	6873.40±278.00 [a]	6661.73±293.03 [a]
Mg	818.02±44.72 [a]	679.01±56.57 [b]	725.89±59.63 [b]
Mn	73.51±5.64 [a]	68.68±7.14 [a]	42.60±7.53 [b]
K	5383.73±118.38 [a]	4997.77±149.74 [a.b]	4773.26±157.84 [b]
Na	215.35±35.13 [a]	191.02±44.43 [a]	192.39±46.84 [a]
Zn	51.52±2.16 [a.b]	45.07±2.73 [b]	55.22±2.88 [a]

* water activity (22ºC) **pollen g/100 g [1] mean of sixteen sample from the State of Paraná (PR)±SD; [2] mean of ten samples from the State of Santa Catarina (SC) and Rio Grande do Sul (RS) ±SD; [3] bee pollen mg/kg; Different letters between columns mean there was statistical difference at the level of 5% by the Tukey test.

The mineral composition of pollen does not depend only on the floral origin of pollen, but also on plant growth conditions, such as soil and geographic origin. There are differences in the mineral content of pollen collected by bees and pollen collected directly from the flower [25].

Conclusions

With exception of the PR 12 sample, all the others were classified as heterofloral. The pollen types found in greater number in the samples were from the Asteraceae family. The State of Paraná presented a richer variety (18 pollen types), representing 82% of the total identified in this study. With exception to humidity content, all the physicochemical parameters of the analyzed dehydrated bee pollen samples were in accordance with the Brazilian legislation. The predominant minerals in the samples were phosphorus, followed by potassium, calcium and magnesium. With the exception of the zinc content, the other minerals in the samples from the State of Paraná were shown to have higher mineral contents those in the other states of the Southern region. This can be explained by the great diversity of pollen types found in the bee pollen in this state.

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