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Inorganic Contaminants in Bee Pollen from Southeastern Brazil

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A set of experiments was carried out to validate a method for inorganic contaminants in honeybee-collected pollen, consisting of digestion of the samples in a closed microwave-assisted system and quantification of 10 inorganic contaminants by ICP OES. Forty-three samples of Brazilian bee pollen, collected in southeastern Brazil during one year, were analyzed. Determination of these analytes is important both as bioindicators of pollution and to verify the safety of consuming the pollen itself. The method had satisfactory performance, with good accuracy and precision. The ranges of the mean levels were 10.4−268.0 mg/kg for Al, <0.01−1.38 mg/kg for As, 2.78−17.63 mg/kg for Ba, 0.003−0.233 mg/kg for Cd, <0.01−1.11 mg/kg for Co, <0.01−2.32 mg/kg for Cr, <0.10−1.13 mg/kg for Ni, <0.01−0.44 mg/kg for Pb, <0.035−1.33 mg/kg for Sb, and <0.0004−0.0068 mg/kg for Hg. Contamination seemed to occur in the following decreasing order: São Paulo > Minas Gerais > Espírito Santo. Generally higher levels of all studied contaminants were observed in samples produced in an urban site, compared to those of a rural site. Al, Cd, Co, and Pb tended to have higher levels during the dry months (July−October). Ingestion estimates showed that Al and As would have the highest contributions to the adult diet, reaching 27 and 8%, respectively, of the provisional tolerable weekly intake (PTWI) values, considering a daily portion of 25 g.

KEYWORDS: Bee pollen; inorganic contaminants; ICP OES; aluminum; arsenic; cadmium; lead

INTRODUCTION

With its extensive territory, diversified flora, and favorable climate throughout the year, Brazil has great potential for apiculture (*1*). Among the apiary's products, bee-collected pollen has nutritive and therapeutic properties that make it attractive in terms of human health (*2*−*8*). It is the major source of protein, lipids, minerals, and vitamins for the bee colony itself (*9*). More studies are needed on the nutrient and bioactive compound composition of this product to promote its greater production and commercialization in domestic and international markets. It is also important, however, to verify the presence of contaminants that are harmful to health. Although a good source of nutrient minerals, the pollen can also contain toxic heavy metals (*10*−*16*).

On the other hand, increased pollution of the air in both urban and industrial areas has generated growing interest oin bioindicator-based techniques for the detection and evaluation of environmental contamination in recent years (*17*−*19*). Considering that bee foraging activity generally extends over a superficial area of about 7 km^2, many authors have proposed apiculture products (honey, pollen, propolis, and wax) and honeybees as bioindicators of chemical pollution brought about by different factors such as metal-containing agricultural chemicals, disposal of wastes from mines or mills, industrial emissions, and emissions from vehicles (*12*, *17*−*22*).

From the literature, possible contaminants and the means by which they can be introduced into the environment can be found. Aluminum in the environment can be a result of acidic precipitation. Acidification of catchments leads to increased Al concentrations in soil and freshwaters, affecting large parts of both aquatic and terrestrial ecosystems (*23*−*25*). It has been used in many industrial applications, which result in possibilities of human contact with the metal and consequent impact on human populations (*25*).

Arsenic is ubiquitious in the environment, being present in rock, soil, dust, water, and air (*26*). Air and water are the principal means of As contamination, which can also be due to the use of As-based compounds in agriculture (*27*).

Barium is widely used in the manufacture of glass, ceramics, television picture tubes, fireworks, insecticides, depilatories, etc., and also has many applications in the automotive industries (*28*). It is also part of the composition of urban street dust and is recognized as a valuable tracer for vehicle emissions (*29*, *30*).

Ambient air Cd concentrations increase from rural to urban and industrialized areas. Leita et al. (*18*) found Cd levels as high as 2.1 mg/kg in bee pollen collected in hives located about 50 m from

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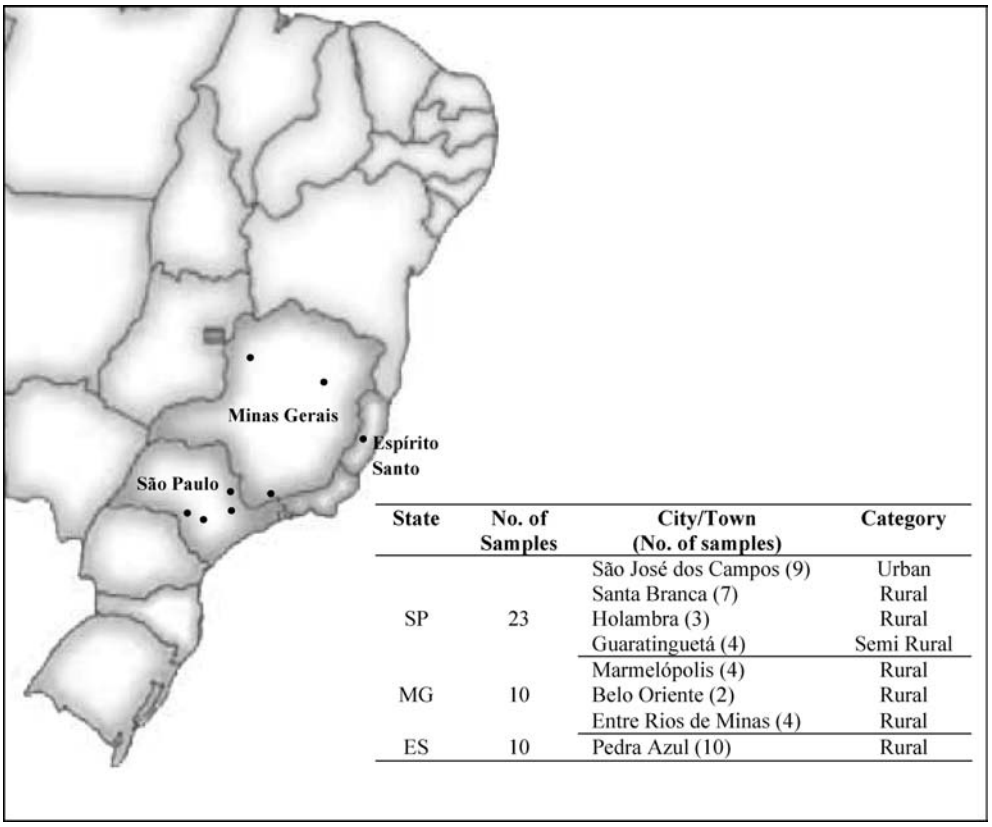

State	No. of Samples	City/Town (No. of samples)	Category
SP	23	São José dos Campos (9)	Urban
		Santa Branca (7)	Rural
		Holambra (3)	Rural
		Guaratinguetá (4)	Semi Rural
MG	10	Marmelópolis (4)	Rural
		Belo Oriente (2)	Rural
		Entre Rios de Minas (4)	Rural
ES	10	Pedra Azul (10)	Rural

Figure 1. Localization of bee pollen sample collection areas in southeastern states: São Paulo (SP), Minas Gerais (MG), and Espírito Santo (ES).

an urban area with high traffic density. Cadmium also reaches water and soil through weathering and erosion of soils and bedrock, atmospheric deposition, direct discharge from industrial operations, leakage from landfalls and contaminated sites, and the dispersive use of sludge and fertilizers in agriculture (*31*). Moreover, antiwear protectants incorporated in lubricants often contain Cd among other metals, which are released to the environment (*18*).

Cobalt can either be naturally found in soil, dust, and seawater or released to the environment from burning coal and oil, from car, truck, and airplane exhausts, and from industrial processes that use the metal. Atmospheric concentrations of Co tend to be higher in heavily industrialized cities (*32*).

Chromium is found in the air, soil, and water. The pollution of air by this element and its compounds comes primarily from industrial use (e.g., combustion activities at utilities) and emissions from road dust (*33*).

Nickel is released into the atmosphere by power plants, trash incinerators, and industries that make or use Ni or Ni compounds (*34*,*35*). In the air it attaches to small particles of dust that settle to the ground or are taken out of the air in rain or snow. It usually takes a long time for nickel to be removed from air.

Lead levels in the environment are considered to be a reliable índex of environmental contamination (*19*). The element can be disseminated by fuel combustion, industrial activities, and use of fertilizers and insecticides (*36*). Its content in plants depends on the soil composition and can be high, especially in mining areas (*37*).

Antimony and its compounds are present in the environment as a result of natural processes and human activities. There are many industrial uses for Sb compounds, which are considered to be pollutants (*38*).

Mercury can be transported through long distances, aggregated to particles in suspension in the atmosphere, and is considered to be an indicator of urban and industrial pollution, indicated for global monitoring of pollution. Among the causes of environmental contamination by Hg are petroleum and other fossil combustibles; eletro-electronic, paint, and metal industries; producers of steel and iron; deforestation; and gold mines (*39*, *40*). This element can be captured by the leaves of plants, in which they cause damage to the roots, aside from interfering with water absorption, photosynthesis, and respiration. Its transfer to cultivated soil provokes elevation of its level in the vegetables produced (*41*).

The objectives of this study, therefore, were (1) to validate a method for inorganic contaminants, using digestion of the samples in a closed microwave-assisted system and quantification by inductively coupled plasma optical emission spectroscopy (ICP OES); (2) to determine aluminum (Al), arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), cobalt (Co), nickel (Ni), lead (Pb), antimony (Sb), and mercury (Hg) in samples from different producing sites in southeastern Brazil; and (3) to estimate potential exposure to Al, As, Cd, Pb, and total Hg, using the provisional tolerable weekly intake (PTWI).

MATERIALS AND METHODS

Sampling. A total of 43 samples of dehydrated, granulated bee pollen ready for commercialization (dry, clean, packed products) were acquired directly from apiculture producers of three Brazilian states of the southeastern region, São Paulo (SP), Espírito Santo (ES), and Minas Gerais (MG) (**Figure 1**), between 2007 and 2008 during the period of production. The samples, weighing 200−330 g, were taken to our laboratory mostly packed in plastic bags with a few in glass containers, simulating actual commercialization practices. Because the production process includes collection, cleaning, dehydration, packaging, and transportation (no more than 2 or 3 days), samples arrived at our laboratory within the month of production.

On arrival, 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 the analyses were carried out (maximum of

3 months). On the day of analysis, the samples were quartered in a stainless steel quartering device and ground in a refrigerated mill with a tungsten helix (M20, IKA Labortechnik, Wasserburg, Germany). The resulting powder was sieved through a 30 mesh (600 μm) sieve to standardize the particle size of the pollen.

Analytical Method and Instrumentation. The digestion of the samples was carried out in a microwave acid digestion unit (Microwave Digestion System Start D, Milestone, Sorisole, Italy), using HNO_3 (Merck, Darmstadt, Germany) and H_2O_2 (Merck) as oxidizing agents. A sample of 0.65−0.70 g was weighed, and 10 mL of concentrated HNO_3 was added, followed by 3 mL of 30% H_2O_2. Digestion was done in two steps, using the following conditions: step 1, potency = 1000 W, temperature = 180 °C, time = 70 min; step 2, potency = 1000 W, temperature = 200 °C, time = 50 min. After digestion, the sample was transferred to a 25 mL volumetric flask, and the volume was completed with a 5% solution of HCl (v/v).

Quantification of the inorganic contaminants was carried out using an ICP OES unit (Vista MPX, Varian, Mulgrave, Australia), with axial vision, equipped with a radiofrequency source (RF) of 40 MHz, a simultaneous multielement detector of solid state type charge-coupled device (CCD), a peristaltic pump, a nebulizing chamber, and a nebulizer sea spray. The system was controlled by ICP Expert software, utilizing argon with a purity of 99.996% (Air Liquid, SP, Brazil) as both plasma liquid and auxiliary gas. The operating conditions were as follows: potency, 1000 W; nebulizing rate, 0.9 L/min; flow rates of argon and the auxiliary gas, 1.5 and 15 L/min; integrating and reading times, 10 and 3 s; number of replicates, 3. The wavelengths (nm) used were Al, 308.215; As, 193.696; Ba, 455.403; Cd, 214.439; Co, 228.615; Cr, 276.653; Ni, 221.648; Pb, 220.353; Sb, 206.834; and Hg, 194.164.

Hydride Generator. For the quantification of total mercury, a hydride generator (VGA77, Varian, Mulgrave, Australia) coupled with ICP OES was used. For the generation of mercuric hydride, $SnCl_2$ in 10 mol/L HCl was employed as reductant. The operating conditions were as follows: sample flow rate, 9 mL/min; acid flow rate, 1 mL/min; $SnCl_2$, 25% (m/v); flow rate, 1 mL/min; carrier gas pressure, 350 kPa; carrier gas flow rate, 100 mL/min.

Standard Curves. For the standard curves, a multielement standard solution was prepared in 5% HCl v/v from stock solutions of 1000 mg/L of Co, Ni, Ba, Cd, Cr, Pb, Al (Merck), As (High Purity Standards, Charleston, SC), Sb (Specsol, Jacareí, Brazil), and Hg (Qhemis High Purity, Jundiaí, Brazil). The concentration ranges for the standard curves were 0.005−0.5 mg/kg for Co, Ni, Ba, Cd, As, Cr, Pb, and Sb; 0.05−2.5 mg/kg for Al; and 0.0005−0.01 mg/kg for Hg.

Evaluation of Method Performance. Because a reference material was not available for bee pollen, recovery tests at two levels for each of the 10 inorganic contaminants were carried out to evaluate the accuracy of the method. The limits of detection (LOD) and quantification (LOQ) were calculated according to the method of Mermet and Poussel (*42*). Precision was evaluated through the coefficient of variation (CV) of eight repetitions.

Statistical Analysis. To verify if the means of inorganic contaminants obtained for the dehydrated bee pollen samples from different states were statistically different at $p < 0.05$, the Tukey multiple-comparison test (by the approximation method with different numbers of repetition per state) was applied using the software Statistica for Windows 5.5 (StatSoft Inc., Tulsa, OK).

Principal Component Analysis (PCA). Using mean values, a data matrix (43 × 10) was built, in which samples were put in lines and the concentrations of the pollen contaminants were put in columns (variables). Data were preprocessed and then submitted to multivariate analysis and PCA, using Statistica program version 5.0.

Calculation of Potential Exposure. For Al, As, Cd, Pb, and Hg, for which PTWI values have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the percentages of PTWIs were estimated (*43*−*45*). This was based on daily portions of 25 g (approximately 3 tablespoons) for adults (considering a body weight of 60 kg) and 10 g for children (body weight of 34.5 kg). From the estimated weekly intake, the contribution of each contaminant was calculated and expressed as percentage of PTWI.

RESULTS AND DISCUSSION

Method Performance. The method for sample preparation using digestion in a closed microwave-assisted system permitted quantification of Al, As, Ba, Cd, Cr, Co, Ni, Pb, Sb, and Hg in a single sample preparation. The residual carbon was 3.7 ± 0.6 g/100 g, indicating a low level of organic matter after digestion of the sample and therefore efficient mineralization. The digestion time was approximately 120 min.

Table 1. Performance Characteristics of the Method for Determining Bee Pollen Inorganic Contaminants[a]

element	λ (nm)	r	sensitivity (L mg^{-1})	LOD (mg/L)	LOQ (mg/L)	CV (%)
Al	308.215	0.9998	898	0.03	0.07	5.7
As	193.696	1.0000	52	0.01	0.07	5.1
Ba	455.403	1.0000	540454	0.0004	0.002	2.3
Cd	214.439	1.0000	3215	0.001	0.007	6.7
Co	228.615	1.0000	2310	0.01	0.05	9.1
Cr	276.653	1.0000	3842	0.01	0.06	6.7
Hg	194.164	0.9999	9816	0.0004	0.002	6.4
Ni	221.648	1.0000	375	0.01	0.05	5.9
Pb	220.353	0.9999	328	0.01	0.06	5.5
Sb	206.834	0.9999	127	0.04	0.2	5.0

[a] λ, wavelength; r, coefficient of correlation; LOD, limit of detection; LOQ, limit of quantification, n = 10; CV, coefficient of variation, n = 8.

The performance of the method was good, as shown by the coefficient of correlation (r) equal or close to 1, LOD and LOQ indicating adequate sensitivity for the contaminants analyzed, and statisfactory CVs at the LOQs obtained (**Table 1**).

Mean recovery percentages varied from 79 to 123%, mostly close to 100%, indicating adequacy of the analytical method.

Levels of Inorganic Contaminants. The means, standard deviations, and ranges of the inorganic contaminant concentrations found in the 43 samples of bee pollen analyzed are presented in **Table 2**. There was very wide within-state variation in the levels of all analytes. Mainly because the levels varied considerably within the same state, the between-state variation was not significant for Al, As, Ba, Co, Cr, and Hg. MG and SP presented significantly higher levels of Ni and Sb, respectively. Levels of Cd and Pb were also significantly lower in ES.

Al, Ba, and Ni were the most frequent inorganic contaminants found in all bee pollen samples analyzed, followed by Cr, Co, Cd, Pb, As, Sb, and Hg.

Aluminum was found in all of the bee pollen samples analyzed (10.4−314.9 mg/kg). The southeastern Brazil state that presented the highest concentrations was Minas Gerais (14.4−314.9 mg/kg). Aluminum was determined in bee pollen only in this and another Brazilian work (*15*). Al ranges obtained in the present study are higher than those obtained by Santos (*15*) for samples from the southeastern region (< 0.05−93.6 mg/kg, SP, n = 12 and MG, n = 5) in years prior to the period of our work, indicating a possible increase of environmental contamination of Al in this region.

Sixty percent of the samples had As, 30% of which (n = 8) had levels above the Brazilian maximum tolerable level (MTL) established for foods, other than juices, syrups, soft drinks, and beverages (1.00 mg/kg) (*46*). Barium was present in all samples. There is no Ba MTL established by Brazilian regulation.

Cadmium, present in 79% of the samples, showed a wider range in the present work (< 0.001−0.233 mg/kg) compared with those encountered in samples from Italy (< 0.015−0.090 mg/kg, wet weight) (*19*), the United Kingdom (< 0.07−0.13 mg/kg), and Spain (0.07−0.10 mg/kg) (*13*).

Although all samples presented Cd levels below the Brazilian MTL (1.00 mg/kg) (*46*), it is noteworthy that the five samples with the highest levels were from urban or semirural areas. Three had Cd levels from 0.102 to 0.126 mg/kg and were collected in an area of preservation and environmental recovery near the urban center of São José dos Campos (SP), an important metal and weapon

Table 2. Inorganic Contaminant Levels in Dehydrated Bee Pollen Samples Collected in 2007−2008 in Southeastern Brazil States[a]

			inorganic contaminants (mg/kg)								
state	*N*	*n*	aluminum	arsenic	barium	cadmium	cobalt	chromium	nickel	lead	antimony	mercury
SP	23	4	av ± SD	80.3 ± 89.0a	0.46 ± 0.50a	6.58 ± 3.36a	0.051 ± 0.054a	0.15 ± 0.22a	0.38 ± 0.49a	0.71 ± 0.27b	0.17 ± 0.13a	0.45 ± 0.38a	0.0013 ± 0.0017a
			median	34.4	0.24	5.71	0.033	0.08	0.21	0.71	0.14	0.45	0.0006
			interval	10.4−268.0	<0.01−1.38	2.78−17.63	0.003−0.233	<0.01−1.11	<0.01−2.32	0.10−1.13	<0.01−0.44	<0.035−1.33	<0.0004−0.0068
MG	10	3	av ± SD	143.1 ± 122.5a	0.36 ± 0.47a	5.39 ± 2.80a	0.023 ± 0.018a	0.06 ± 0.04a	0.39 ± 0.27a	1.75 ± 1.97a	0.11 ± 0.12a	0.14 ± 0.19b	0.0006 ± 0.0005a
			median	135.8	0.12	5.38	0.019	0.06	0.37	1.07	0.07	0.04	0.0004
			interval	14.4−314.9	<0.01−1.26	0.32−9.83	<0.001−0.062	<0.01−0.12	0.62−0.97	0.12−6.85	<0.01−0.32	<0.035−0.57	<0.0004−0.0016
ES	10	1	av ± SD	74.9 ± 56.9a	0.29 ± 0.58a	2.74 ± 1.21a	0.007 ± 0.010b	0.24 ± 0.42a	0.19 ± 0.11a	0.63 ± 0.25b	0.02 ± 0.03b	0.13 ± 0.11b	0.0006 ± 0.0005a
			median	56.3	0.01	2.31	0.001	0.02	0.16	0.65	0.01	0.083	0.0004
			interval	18.9−156.8	<0.01−1.83	1.41−5.10	<0.001−0.025	<0.01−1.22	0.05−0.38	0.12−0.92	<0.01−0.10	<0.035−0.32	<0.0004−0.0020
total	43	8	general av ± SD	93.6 ± 93.9	0.40 ± 0.51	5.41 ± 3.22	0.034 ± 0.044	0.15 ± 0.26	0.34 ± 0.38	0.93 ± 1.04	0.12 ± 0.13	0.30 ± 0.33	0.0010 ± 0.0013
			median	45.0	0.13	4.91	0.061	0.06	0.21	0.75	0.09	0.15	0.0004
			interval	10.4−314.9	<0.01−1.83	0.32−17.63	<0.001−0.233	<0.01−1.22	<0.01−2.32	0.10−6.85	<0.01−0.44	<0.035−1.33	<0.0004−0.0068

[a] *N*, number of samples; *n*, number of sampling sites. Values with different letters (a, b) in the same column are significantly different at $P < 0.05$.

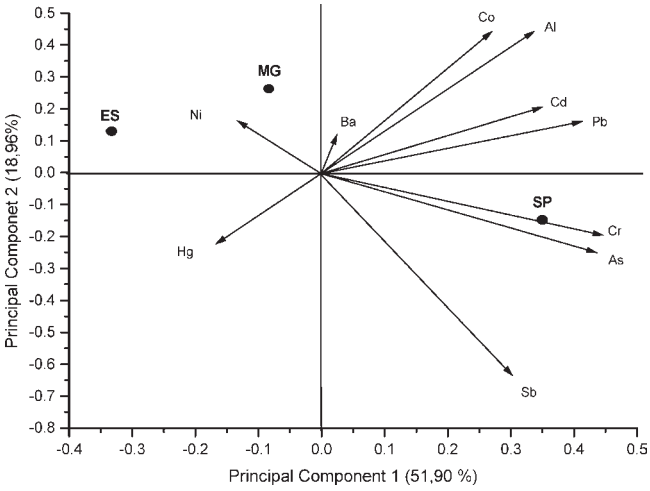

Figure 2. Principal component analysis obtained from mean composition of elements present in 43 dehydrated bee pollen samples from southeastern Brazil states: ES (Espírito Santo), MG (Minas Gerais), and SP (São Paulo) and rural, semirural, and urban areas of SP.

industrial region. Two samples with Cd levels of 0.104 and 0.233 came from Guaratinguetá (SP), where the hives were located in a neighborhood described by the producer as a semirural region. A previous work reported environmental contamination with Pb and Cd in this region caused by a factory producing lead lingots (*47*). It is possible that the presence of Cd in one-third of the samples collected in this region is due to remnants of previous contamination, aside from vehicle emissions. Lead was also present in four of these five samples at levels of 0.19−0.44 mg/kg.

Cobalt was found in 81% of the samples analyzed. Three samples (one from SP and two from ES) had Co levels above 0.80 mg/kg, the other samples having levels below 0.30 mg/kg.

Ninety-three percent of the samples had Cr. The levels (< 0.01−2.32 mg/kg) varied more than those of Italian samples (< 0.030−0.112 mg/kg, wet weight) (*19*), which the authors considered to be very low. A total of 74% ($n = 32$) of the samples analyzed had Cr levels higher than the Brazilian MTL for Cr (0.10 mg/kg) for food (*46*).

Although Cr is toxic in the hexavalent form, the trivalent form (more stable, found in plants, generally associated with organic complexes) is considered to be an essential nutrient for humans, often a limiting mineral in the diet (*48*). Thus, future studies should determine the form in which this element is present in bee pollen.

Encountered in 100% of the samples analyzed, Ni presented the highest level in MG (0.12−6.85 mg/kg), but only one sample from this state had Ni level superior to the Brazilian MTL (5.00 mg/kg) (*46*). Kump et al. (*11*) found Ni levels of 2.5 ± 0.5 and 3.1 ± 2.3 mg/kg in samples from Slovenia.

Lead was present in 63% of the samples analyzed. As with Cd, the state of SP presented the highest levels of Pb (< 0.01 − 0.44 mg/kg), but all samples had lower values than the Brazilian MTL established for majority of foods (0.8 mg/kg) (*49*).

Kump et al. (*11*) found Pb concentrations of 3.5 ± 2.3 by X-ray fluorescence spectrometry and 4.7 ± 0.9 mg/kg by total reflection X-ray fluorescence spectrometry in bee pollen from Slovenia, much greater than those obtained in the present work (< 0.01−0.44 mg/kg). It was also reported in other European studies (< 0.02−0.329 mg/kg in Italian pollen, 0.3−1.5 mg/kg in British pollen, 0.1−0.14 mg/kg in Spanish pollen) (*13*, *19*). Saavedra et al. (*14*) found a Pb level of 0.268 mg/kg in a fresh bee

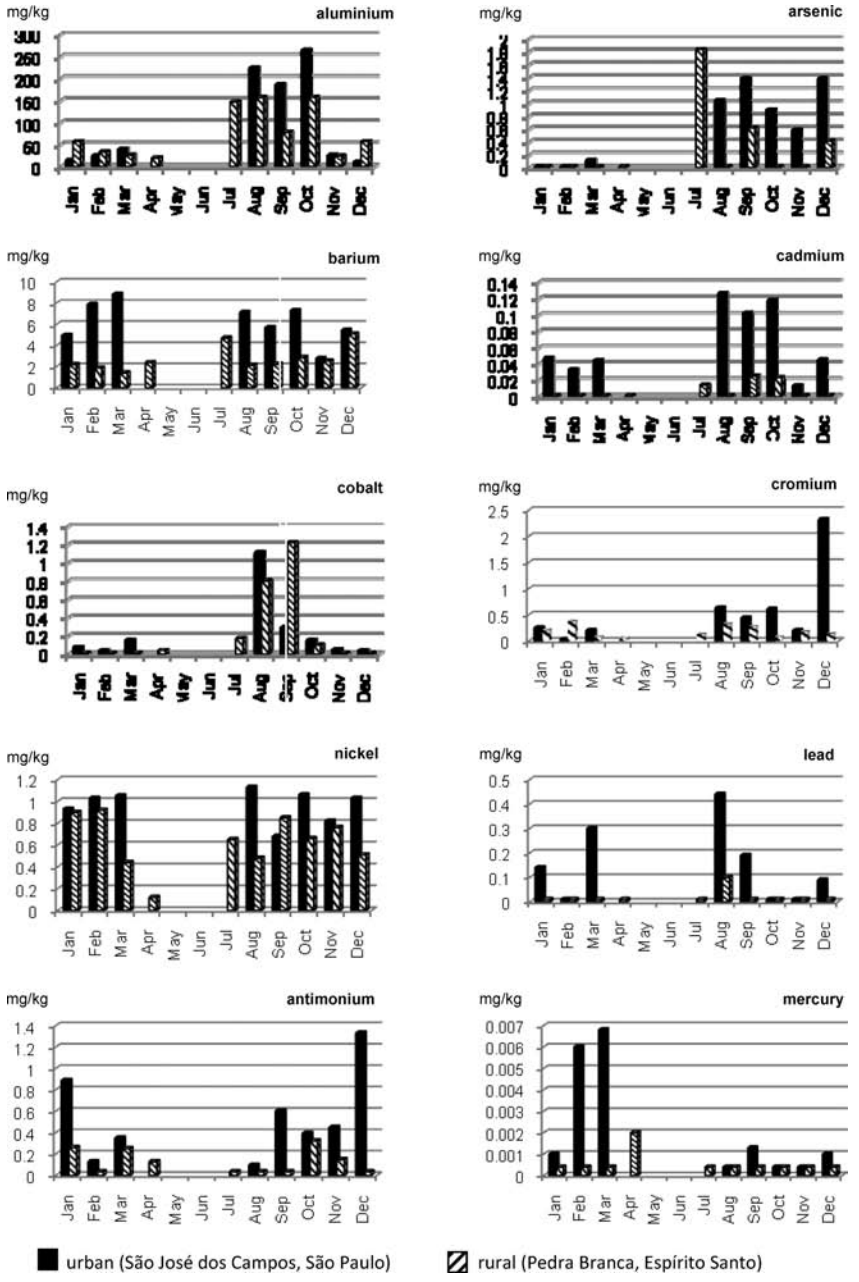

Figure 3. Annual variation of inorganic contaminant levels in bee pollen samples collected in urban and rural sites in two southeastern Brazil states.

pollen sample from Venezuela. Santos (*15*) obtained a range of Pb for Brazilian pollen (< 0.05−0.41 mg/kg) that is similar to the present work, except one sample from SP that presented a high value (5.69 mg/kg), agreeing with those of Kump et al. (*11*). This sample was collected from a site near a manufacturer of automobile batteries that had been fined for contaminating the soil and air with Pb residues.

Fifty-eight percent of the samples had Sb with values ranging from < 0.035 to 1.33 mg/kg. To date there is no reference of Sb levels in bee pollen in the scientific literature. The Sb levels encountered in this work are lower than the Brazilian MTL (2.00 mg/kg) (*46*).

Mercury was the least frequent inorganic contaminant, present in 35% of the samples. No sample surpassed the Brazilian MTL for Hg (0.01 mg/kg) (*46*). Mercury was determined in bee pollen only in this and another Brazilian work (*15*) in which higher levels of Hg were found for the national range (< 0.00012−0.01932 mg/kg) and for the southeastern region (< 0.00012−0.01652 mg/kg) than the present study (< 0.0004−0.0068 mg/kg).

The lower levels of Hg encountered in the present study in relation to those found previously by Santos (*15*) may indicate a decreasing tendency of urban and industrial pollution with this metal in the southeastern region of Brazil.

Although presenting the two samples with the highest As (1.83 mg/kg) and Co (1.22 mg/kg) levels, the city of Pedra Azul (ES) showed generally lower levels of inorganic contaminants, with 42% of results below the method's LOD. The corresponding percentages were 28 and 12% for MG and SP samples, respectively, thus indicating higher environmental pollution indices in these states.

Principal Component Analysis. A multivariate data analysis was applied to the trace element data. In **Figure 2**, the score plot resulting from PCA, applied to mean values, shows that pollen samples from São Paulo state were discriminated by the elements Cr and As. Nickel levels were important to discriminate mainly samples from MG state and, to a lesser extent, samples from ES.

Inorganic Contaminant Levels versus Location of Apiary. Figure 3 shows the variation during a year of inorganic contaminant levels

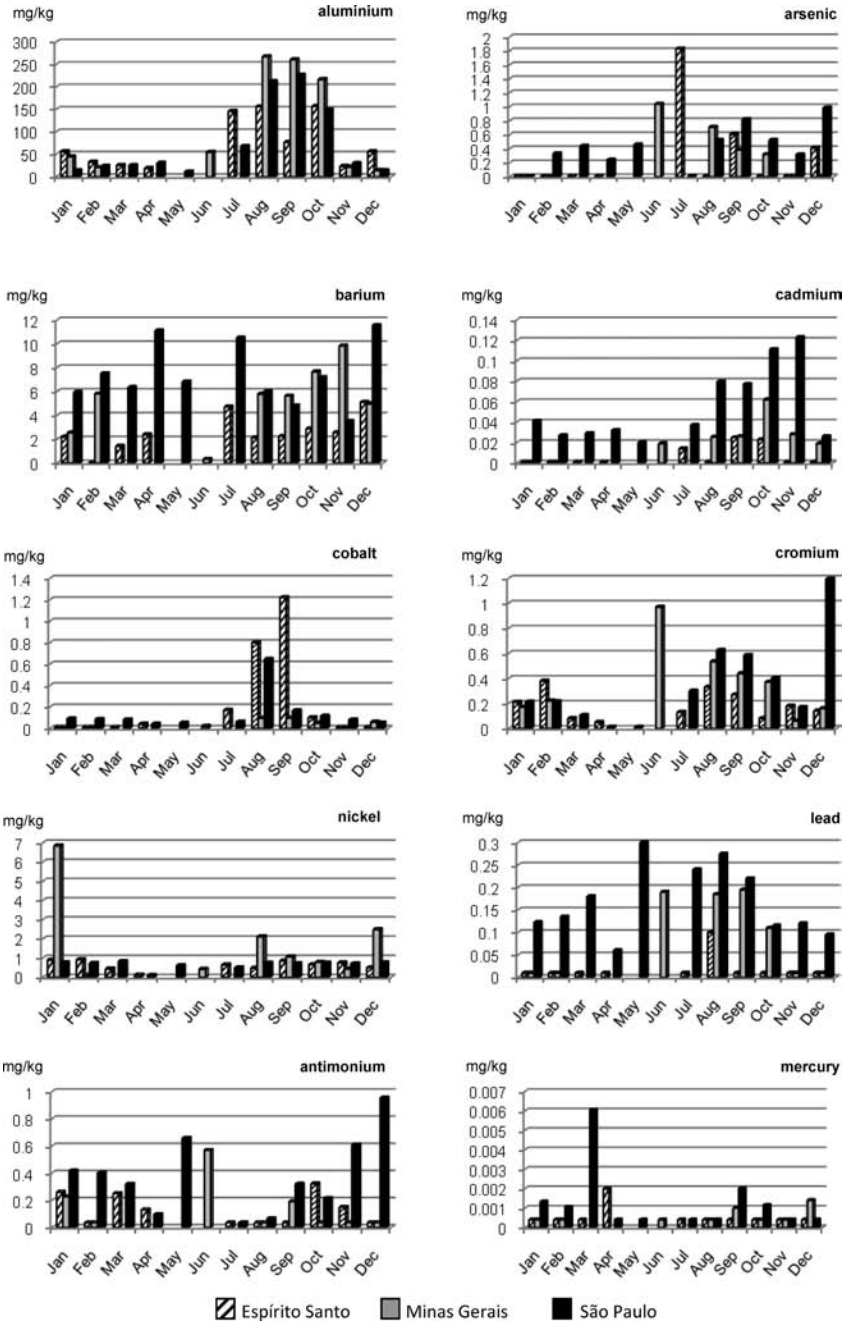

Figure 4. Inorganic contaminant average levels in bee pollen samples from southeastern Brazil states.

of bee pollen samples collected from urban and rural sites in the states of São Paulo and Espírito Santo, respectively. Generally higher levels of all studied contaminants can be seen in samples produced in the urban site. Exceptions were Al in bee pollens produced in January, February, and December, Co and Ni in samples collected in September, and Cr in samples produced in February. No comparisons can be made for samples collected in April and July, because there was no pollen production in the urban site during these months. These results confirm the greater pollution in urban areas and shows that bee pollen can indeed be a bioindicator of environmental pollution.

It may also be observed that Al levels of samples from both sites and As levels from the urban site tended to be higher from July to October. Cd had noticeably higher levels in August−October and Hg in February and March in the samples collected from the urban site. Plausible explanations for these variations during the year can be the subject of future research.

Inorganic Contaminant Levels versus Rainfall. Figure 4 shows inorganic contaminant mean levels in bee pollen samples collected in the three studied states (ES, SP, and MG) during the year. Aluminum levels tended to be higher from July to October, Cd from August to November, Co in August and September, and Pb from May to September.

Among the many factors that may contribute to increasing soil Al levels is a high pluviometric precipitation rate (*50*, *51*). **Figure 5** shows pluviometric precipitation levels in the three states during the studied period. It is noticeable that the monthly accumulated precipitation was higher from November to March, inversely related to the Al level in bee pollen, which was higher during the dry period. During rainy periods, soil leaching could favor Al accumulation in the plants, increasing its levels in bee pollen samples collected in the next blossoms that will occur during the dry period.

In southeastern Brazil, the dry season coincides with winter and is more prone to air pollution. Because Al, Cd, Co, and Pb

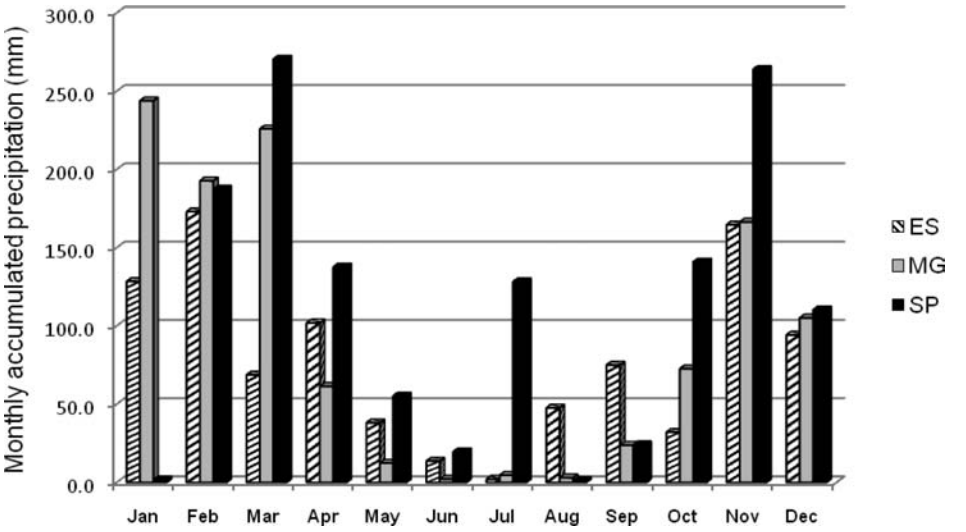

Figure 5. Monthly accumulated precipitation in southeastern Brazil states: Espírito Santo (ES), Minas Gerais (MG), and São Paulo (SP) states during a year (data conceded by National Institute of Meteorology (INMET)).

Table 3. Occurence of Inorganic Contaminants, Weekly Ingestion, and Percentage of PTWI Estimated for the Consumption of One Daily Portion of Dehydrated Bee Pollen of 25 g for Adults and 10 g for Children, Based on Average Values Found in 43 Samples Collected in Three Southeastern Brazil States during One Year

		weekly ingestion[a] (μg/kg of body wt)		mean percentage of PTWI[b]	
element	mean level (mg/kg)	adult, av	child, av	adult, av	child, av
aluminum	93.6	273.1	190.0	27	19
arsenic	0.40	1.16	0.81	8	5
cadmium	0.034	0.099	0.069	1	1
lead	0.12	0.360	0.250	1	1
mercury	0.0010	0.0028	0.0020	0.06	0.04

[a] Adult weight (> 17 years), 60 kg; child weight (7−12 years), 34.5 kg. [b] Al PTWI level = 1000 μg/kg of body wt/week; As PTWI = 15 μg/kg of body wt/week; Cd PTWI = 7 μg/kg of body wt/week; Pb PTWI = 25 μg/kg of body wt/week; Hg PTWI = 5 μg/kg of body wt/week (*52*).

can be dispersed by air (*10*, *18*, *25*, *32*), it is also reasonable to suspect that they accumulate in floral sources of bee pollen during the dry months.

Although the other contaminants may also be disseminated through water and/or by air, their levels did not show a definite pattern during the studied period.

Risk Evaluation. Table 3 presents the mean levels of some inorganic contaminants, weekly ingestion, and percentage of PTWI estimated for the consumption of one daily portion of dehydrated bee pollen. PTWI is expressed on a weekly basis to emphasize the long-term exposure risk to contaminants that may be accumulated in the human body. Al and As presented the highest possible contribution to the diets, reaching average PTWI values of 27 and 8% for adults and 19 and 5% for children, respectively.

In humans, the toxicity of Al has been associated with clinical complications and neurological dysfunctions, such as Alzheimer's disease (*53*). However, the principal consideration with respect to Al and health is the potential toxicity when exposure is excessive (*25*).

Arsenic exists in nature in a variety of chemical forms that differ in toxicity. The inorganic compounds are toxic, especially in the trivalent (As^{3+}) form, whereas the organic form has lower toxicity (*54*). Future work should investigate the chemical forms of As in the bee pollen samples, as well as the possible sources of contamination in the regions where the element is found.

On the basis of the levels of Ni encountered in the present work, its contribution to a daily portion (25 g) of bee pollen may reach up to 171 μg/day. This intake is not negligible if one considers a possible human sensitivity to this element coupled with the fact that Ni is also present in many other food sources (e.g., roots, vegetables, grains, bread, cocoa, chocolate, soy beans, oatmeal, nuts, almonds, and fresh and dried legumes) (*55*).

In summary, it can be concluded that bee pollen is (1) subject to contamination with inorganic elements and should be monitored to ensure safety and (2) sensitive to environmental pollution and can indeed serve as bioindicator.

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