Taylor & Francis
Taylor & Francis Group

Journal of Apicultural Research

ISSN: 0021-8839 (Print) 2078-6913 (Online) Journal homepage: https://www.tandfonline.com/loi/tjar20

Determination of inorganic elements in geopropolis samples by inductively coupled plasma optical emission spectrometry

Jean Sanger Siqueira, Cleide Samara Tavares Mescouto, Michelle de Souza Lemos, João Batista Pereira Junior, Giorgio C. Venturieri, Heronides Adonias Dantas Filho & Kelly das Graças Fernandes Dantas

To cite this article: Jean Sanger Siqueira, Cleide Samara Tavares Mescouto, Michelle de Souza Lemos, João Batista Pereira Junior, Giorgio C. Venturieri, Heronides Adonias Dantas Filho & Kelly das Graças Fernandes Dantas (2020): Determination of inorganic elements in geopropolis samples by inductively coupled plasma optical emission spectrometry, Journal of Apicultural Research, DOI: 10.1080/00218839.2020.1745435

To link to this article: https://doi.org/10.1080/00218839.2020.1745435

Published online: 28 Apr 2020.

Journal of Apicultural Research, 2020
https://doi.org/10.1080/00218839.2020.1745435

ORIGINAL RESEARCH ARTICLE

Determination of inorganic elements in geopropolis samples by inductively coupled plasma optical emission spectrometry

Jean Sanger Siqueira[a], Cleide Samara Tavares Mescouto[a], Michelle de Souza Lemos[a], João Batista Pereira Junior[a], Giorgio C. Venturieri[b], Heronides Adonias Dantas Filho[a] and Kelly das Graças Fernandes Dantas[a]*

[a]*Grupo de Espectrometria Analítica Aplicada, Faculdade de Química, Universidade Federal do Pará, Belém, Pará, Brazil;* [b]*Empresa Brasileira de Pesquisa Agropecuária, Centro de Pesquisa Agroflorestal da Amazônia Oriental, Embrapa Amazônia Oriental, Pará, Brazil*

(Received 13 October 2017; accepted 13 August 2019)

Information on the mineral composition of geopropolis produced by bees native to the Amazon region is of great importance for the chemical characterization of this product. This study aimed to determine the Ca, Cu, Fe, K, Mg, Mn, P, and Zn contents in 22 samples of geopropolis from northern Brazil by inductively coupled plasma optical emission spectrometry (ICP OES). Geopropolis samples were digested in a microwave oven using concentrated nitric acid and hydrogen peroxide and subsequent addition of hydrofluoric acid. The accuracy of the analytical procedures was assessed by elemental determination using certified reference material Poplar Leaves (GBW 07604). Analyte recoveries ranged between 88% and 99%, and there was no statistical difference ($p < 0.05$) between the determined and certified values. The accuracy of the measurements was evaluated by the addition and recovery test, and the recoveries ranged between 93% and 118%. The results showed high levels of Fe, Ca, Mg, K, and P in the samples. Principal component analysis (PCA) was performed and the results showed that there was better separation and grouping of the samples in relation to the geographic region, indicating that the mineral composition of geopropolis can vary according to the characteristics of the region. Thus, this study reported for the first time information about the mineral composition of geopropolis produced in northern Brazil, which can serve as a database for the characterization of the product in this region.

Keywords: geopropolis, stingless bee, inorganic elements, chemical composition, food analysis, ICP OES

Introduction

Meliponines, known as stingless bees, are native species that occupy much of the tropical region on the planet, especially South America. These bees develop an important role in pollination, and they can produce honey, propolis, and geopropolis in small amounts (Dutra et al., 2008; Menezes, 2005). Geopropolis is a mixture of clay and propolis prepared by stingless bees (Meliponinae). The use of geopropolis in hives provides protection against insects and pathogens, and strengthens the honey combs (Cardozo et al., 2015; Nogueira & Neto, 1997; Souza et al., 2013). Geopropolis has been used for the treatment of inflammatory diseases, hemorrhoids, gastritis, and cough. Some authors have reported that geopropolis has antimicrobial, antitumor, antioxidant, anti-inflammatory, and analgesic properties (Cinegaglia et al., 2013; Franchin et al., 2012; Souza et al., 2013).

The chemical composition of geopropolis is complex and it varies according to the flora, bee species, geographic region, and the climate. Some studies have reported the presence of terpenes, fatty acids, saponins, and phenolic compounds (phenolic acids, flavonoids, and tannins), which highlight the antioxidant activity of geopropolis (Araújo et al. 2016; Cardozo et al., 2015; Dutra et al., 2008; 2014).

Reports in the literature about the inorganic elements in geopropolis are scarce. However, the study of inorganic elements is of great importance since they play a key role in the human body. Elements such as Mn and Zn are the active sites of some enzymes, hormones, vitamins, and nucleic acids, which play an important role in the maintenance of life metabolism (Siqueira et al., 2017). Bonsucesso et al. (2018) determined the concentration of toxic metals in geopropolis produced by *Melipona scutellaris* to evaluate environmental contamination in urban areas in the state of Bahia located in northeast Brazil. Some authors have investigated the inorganic constituents in propolis produced by *Apis mellifera* (Bonvehí & Bermejo, 2013; Finger et al., 2014; Formicki et al., 2013; Korn et al., 2013), or *Tetragonisca angustula* (Ataide de Oliveira et al., 2020). However, there are no studies of the inorganic composition of geopropolis produced by bees native to the Amazon region.

Since the Amazon region presents important characteristics, such as diverse flora, a high number of bee species, and a hot and humid climate, studies on the chemical composition of geopropolis, especially with

*Corresponding author. E-mail: kdgfernandes@ufpa.br

regards to the content of inorganic elements, are fundamental for the characterization of these products in this region. Thus, this study aimed to determine the levels of inorganic elements (Ca, Cu, Fe, K, Mg, Mn, P, and Zn) in samples of geopropolis and to evaluate the similarities and differences between the samples in relation to the collection region and bee species (*Melipona flavolineata, Melipona fasciculata, Melipona seminigra*) from the Amazon region.

Materials and methods

Reagents and materials

All reagents used were of analytical grade. All dilutions were made using ultrapure water (resistivity of 18.2 MΩ cm) obtained from a Synergy-UV water purification system (Millipore, Bedford, USA). All glassware and plastic bottles used were previously decontaminated by immersion in a 10% (v v^{-1}) HNO_3 solution for 24 h and washed with deionized water prior to use.

Nitric acid (Quimex, São Paulo, Brazil), H_2O_2 (30%, w w^{-1}) (Impex, Brazil), and HF (40%, w v^{-1}) (Dinâmica, São Paulo, Brazil) were used to digest the samples. H_3BO_4 (Vetec, Rio de Janeiro, Brazil) was used for complexation of the remaining fluoride.

Standard solutions for calibration were prepared by suitable dilution of the stock solutions containing 1000 mg L^{-1} of Ca, Cu, Fe, K, Mg, Mn, P, and Zn (Sigma, USA). The analytical curves were constructed with 2.0, 4.0, 6.0, 8.0, and 10.0 mg L^{-1} of Ca, Cu, Fe, K, Mg, Mn, P, and Zn in 5.0% (v v^{-1}) nitric acid.

Sample collection

Twenty-two geopropolis samples corresponding to three different species of bees (*M. fasciculata*, *M. flavolineata*, and *M. seminigra*) were collected in apiaries of different cities of the State of Pará, northern Brazil, as shown in Table 1. After collection, the samples were identified, packed, and transported to the laboratory in polyethylene bags.

Instrumentation and conditions

A cryogenic mill (SPEX SamplePrep, model 6770, Metuchen, NJ, EUA) was used for grinding the geopropolis samples. Digestion of the samples was performed in a microwave oven (Start E, Milestone, Sorisole, Italy). A Lab-Line shaker table (3520, EUA) and a Centribio centrifuge (model 80-2B, Brazil) were used to solubilize and centrifuge the samples after digestion, respectively.

An inductively coupled plasma optical emission spectrometer (ICP OES) with radial and axial view (iCAP 6500 Duo Thermo Scientific, Cambridge, UK) and operational software (iTEVA) was used for the determination of Ca, Cu, Fe, K, Mg, Mn, P, and Zn in geopropolis samples. Operating parameters used were as follows: 1.15 kW (RF power), 12 L min^{-1} (plasma flow rate), 0.5 L min^{-1} (auxiliary gas flow rate), and 0.5 L min^{-1} (nebulizer flow rate). A concentric nebulizer and a cyclonic type spray chamber were used to introduce the digested samples into the plasma. Analytical lines of Ca I 317.933 nm, Cu I 224.700 nm, Fe I 239.562 nm, K II 776.490 nm, Mg I 279.553 nm, Mn I 259.373 nm, P II 185.942 nm, and Zn II 213.856 nm were measured. Argon (99.999% pure, Linde Gases, Ananindeua, PA, Brazil) was used to purge the optics and to form the plasma.

Table 1. Species and location of geopropolis samples.

Samples	Species	Location
G1	*Melipona fasciculata*	Belém, PA
G2	*Melipona flavolineata*	Belém, PA
G3	*Melipona seminigra*	Belém, PA
G4	*Melipona fasciculata*	Belém, PA
G5	*Melipona fasciculata*	Bragança, PA
G6	*Melipona fasciculata*	Tracuateua, PA
G7	*Melipona flavolineata*	Barcarena, PA
G8	*Melipona flavolineata*	Igarapé-Miri,PA
G9	*Melipona fasciculata*	São João de Pirabas, PA
G10	*Melipona flavolineata*	Igarapé-Miri, PA
G11	*Melipona flavolineata*	Igarapé-Miri, PA
G12	*Melipona fasciculata*	Tracuateua, PA
G13	*Melipona flavolineata*	Colares, PA
G14	*Melipona flavolineata*	Vigia, PA
G15	*Melipona flavolineata*	Colares, PA
G16	*Melipona flavolineata*	St. Antônio do Tauá, PA
G17	*Melipona fasciculata*	São Caetano de Odivelas, PA
G18	*Melipona flavolineata*	São Caetano de Odivelas, PA
G19	*Melipona flavolineata*	Vigia, PA
G20	*Melipona flavolineata*	St. Antônio do Tauá, PA
G21	*Melipona seminigra*	Belterra, PA
G22	*Melipona fasciculata*	São João de Pirabas, PA

Sample preparation

Prior to analysis, the samples were ground in a cryogenic mill. A two-step program was applied: step I (prefreezing), 10 min; step II (milling), 2 min intercalated by cycles of freezing of 2 min. After grinding, the samples were stored in volumetric flasks in a desiccator.

A mass of 0.25 g was weighed for each sample in triplicate ($n = 3$) and digested with 4.0 mL of 14.0 mol L^{-1} HNO_3 and 4.0 mL of 30% (w w^{-1}) H_2O_2 in a microwave oven. The heating program consisted of the following 3 steps: 800 W, 180 °C for 10 min; 800 W, 180 °C for 20 min; and ventilation for 50 min. After digestion, the digested part and the residue (silicate compounds that were not digested) were quantitatively transferred to a 14 mL volumetric flask and 10.0 mL of ultrapure water were added. Separation of the supernatant and residue was carried out by centrifugation at 3000 rpm for 30 min. The residue was dissolved at room temperature by adding 1.0 mL of HF (40%, w v^{-1}), and after dissolution 1 g of H_3BO_4 was added for complexation of the remaining fluoride. The resulting mixture was added to the previously collected supernatant (liquid phase of the digested part) and the volume was adjusted to 14.0 mL with ultrapure water. Blank experiments were carried out in the same way.

Elemental analysis

Ca, Cu, Fe, K, Mg, Mn, P, and Zn were determined in digests by ICP OES. The final acidity was 5% v v^{-1}.

The accuracy of the analytical procedures was assessed by Ca, Cu, Fe, K, Mg, Mn, P, and Zn determination using certified reference material Poplar Leaves (GBW 07604) that was digested using the same procedure for geopropolis samples.

Figures of merit

Validation of the analytical procedures is an essential condition for evaluating the appropriateness of the procedure for the method of analysis. In these processes, parameters known as figures of merit are used to confirm the validation of the proposed procedure. In this study, the figures of merit used for this purpose were analytical curve linearity (R2), limit of detection (LOD), and limit of quantification (LOQ).

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following equations: $LOD = (3 \times RSD_{blank} \times BEC)/100$ and $LOQ = (10 \times RSD_{blank} \times BEC)/100$, where BEC is the C_{SR} (concentration of the multi-element reference solution) divided by SBR (analytical signal/background signal) and RSD_{blank} is the relative standard deviation for 10 consecutive blank measurements (Thomsen et al., 2000). The figures of merit are presented in Table 2.

Statistical analysis

For a better interpretation of the obtained data and extraction of information, statistical analysis was performed in Minitab Statistical Software version 18.1 (Minitab Inc., State College, Pennsylvania, USA) for Windows. Principal component analysis (PCA) was applied to verify the correlation of the inorganic elements with the sample collection regions and the species of bees producing geopropolis. The correlation between the concentrations of the elements was established by the Pearson correlation coefficient (r) in the bivariate linear correlations ($p < 0.05$).

Results

Concentrations of inorganic elements in geopropolis

Table 3 shows the levels of Ca, Cu, Fe, K, Mg, Mn, P, and Zn obtained in digestates of geopropolis samples by ICP OES. The results showed good precision under the analytical conditions used, and the RSD values were less than 5%.

Calcium is a necessary element for growth and normal development of the skeleton and teeth. It is involved in various metabolic processes, such as blood clotting and muscle contraction, and a high dietary intake of Ca may cause excessive calcification of bones and soft tissue such as the kidneys (Underwood, 1981). Levels of Ca were obtained in samples ranging from 146.2 mg kg^{-1} to 3888.0 mg kg^{-1}. The highest concentration of Ca was found in G16 of the Santo Antônio do Tauá City. Bonvehí and Bermejo, (2013) found higher values of Ca when compared to levels obtained in this study.

Table 2. Figures of merit in the determination of Ca, Cu, Fe, K, Mg, Mn, P and Zn in geopropolis samples by ICP OES.

Elements	R^2	LOD (mg kg^{-1})	LOQ (mg kg^{-1})
Ca	0.9991	0.7	2.5
Cu	0.9996	0.1	0.3
Fe	0.9995	0.5	1.8
K	0.9992	9.1	30.3
Mg	0.9986	0.01	0.2
Mn	0.9641	9.7	32.3
P	0.9988	0.5	1.7
Zn	0.9989	0.4	1.3

R^2: correlation coefficient; LOD: limit of detection; LOQ: limit of quantification.

Copper is an element that participates in the synthesis of hemoglobin. Intoxication by this element is characterized by the accumulation in the liver, causing nausea and vomiting (Yeung & Laquatra, 2003). The contents of Cu obtained in geopropolis ranged from 1.6 mg kg^{-1} to 21.9 mg kg^{-1}. These values are above the levels of Cu found in propolis by Korn et al. (2013) (0.3–2.6 μg g^{-1}). Cvek et al. (2008) found Cu (15.8 mg kg^{-1}) in propolis samples close to the levels obtained for geopropolis samples studied here. Except for G21, the other samples showed Cu concentrations similar to the levels found by Gong et al. (2012) in propolis samples from China.

Iron acts especially on hemoglobin preventing anemia (Schümann et al., 2007). Fe was the most abundant element found in geopropolis samples, and it showed minimum and maximum concentrations in G19 (2358.1 mg kg^{-1}) and G21 (23128.8 mg kg^{-1}), respectively. In this study, Fe levels obtained were higher than the values found in propolis samples from Poland (Gong et al., 2012), Argentina (Cantarelli et al., 2011), and Spain (Bonvehí & Bermejo, 2013). These high iron levels in geopropolis samples can be related to the soil.

Potassium acts on the body regulating osmotic pressure (Yeung & Laquatra, 2003). The concentrations of K in samples ranged from 169.3 mg kg^{-1} to 2046.3 mg kg^{-1}. G20 presented the highest contents of K (Santo Antonio do Taua City). Bonvehí and Bermejo, (2013) obtained high concentrations of K (735–4790 mg kg^{-1}) in propolis samples from different areas of southern Spain.

Magnesium is a mineral that participates in many biochemical and physiological processes, such as glucose metabolism, synthesis of proteins, and neuromuscular transmission (Magnoni & Cukier, 2004). Mg showed high concentrations in geopropolis, ranging from 126.2 mg kg^{-1}

Table 3. Concentrations of Ca, Cu, Fe, K, Mg, Mn, P and Zn (in mg kg^{-1}) in 22 geopropolis samples determined by ICP OES (mean ± SD, $n = 3$).

Samples	Ca	Cu	Fe	K	Mg	Mn	P	Zn
G1	1336.7 ± 43.3	10.4 ± 0.7	4180.9 ± 6.2	654.8 ± 7.9	1167.3 ± 7.4	50.5 ± 1.8	475.8 ± 9.2	7.2 ± 0.8
G2	609.5 ± 13.4	8.9 ± 0.5	6670.5 ± 20.9	169.3 ± 1.8	409.7 ± 3.9	48.9 ± 1.0	356.6 ± 5.7	11.0 ± 0.7
G3	146.2 ± 8.1	6.5 ± 1.3	7511.6 ± 74.7	182.2 ± 4.9	126.2 ± 5.1	45.7 ± 3.0	299.5 ± 40.2	3.9 ± 0.3
G4	448.3 ± 26.6	7.3 ± 0.7	3755.0 ± 68.3	540.2 ± 0.2	424.5 ± 0.6	38.9 ± 3.9	455.4 ± 47.6	8.9 ± 2.6
G5	590.3 ± 31.6	9.3 ± 0.4	6121.4 ± 147.7	1037.1 ± 18.9	1377.5 ± 54.7	63.9 ± 0.5	304.6 ± 13.6	7.7 ± 0.5
G6	1412.5 ± 3.0	12.5 ± 1.1	6554.8 ± 75.2	959.6 ± 2.4	1494.0 ± 1.5	77.6 ± 0.9	192.4 ± 5.1	11.2 ± 0.6
G7	1407.6 ± 31.8	9.6 ± 0.3	6349.2 ± 159.3	709.7 ± 13.4	980.6 ± 17.7	64.3 ± 2.7	413.2 ± 0.5	12.6 ± 0.6
G8	979.4 ± 49.2	13.9 ± 0.3	4915.3 ± 226.4	984.8 ± 40.8	702.4 ± 30.3	80.0 ± 3.4	232.9 ± 60.4	6.5 ± 0.6
G9	1032.5 ± 26.5	5.9 ± 0.3	6465.4 ± 55.9	920.9 ± 14.4	2159.4 ± 36.0	74.0 ± 1.2	268.2 ± 2.0	8.2 ± 1.0
G10	1584.0 ± 46.7	7.9 ± 0.4	6511.6 ± 59.7	1455.1 ± 14.6	1382.5 ± 20.5	156.7 ± 4.9	345.0 ± 9.6	6.0 ± 0.2
G11	1737.8 ± 15.3	7.6 ± 0.1	6285.3 ± 74.1	1314.1 ± 3.8	1178.2 ± 13.4	121.9 ± 0.2	323.6 ± 3.3	68.2 ± 3.0
G12	723.7 ± 1.9	8.0 ± 0.1	9179.0 ± 83.5	898.2 ± 10.1	1743.2 ± 22.0	94.4 ± 1.3	143.0 ± 12.2	7.0 ± 0.4
G13	1552.2 ± 66.4	10.1 ± 0.1	6267.4 ± 49.5	526.9 ± 4.6	612.2 ± 11.0	56.0 ± 0.7	564.1 ± 17.9	17.5 ± 2.6
G14	1256.9 ± 13.0	10.9 ± 0.7	6400.0 ± 58.6	771.8 ± 36.1	632.0 ± 24.4	55.6 ± 3.2	475.1 ± 44.0	18.3 ± 2.0
G15	1352.8 ± 43.6	7.3 ± 0.5	3168.8 ± 89.1	917.6 ± 30.9	599.7 ± 27.5	37.1 ± 1.2	622.9 ± 12.8	9.3 ± 4.0
G16	3888.0 ± 92.3	10.5 ± 0.1	4982.2 ± 47.3	1548.2 ± 44.2	1182.3 ± 9.5	64.6 ± 1.0	799.8 ± 21.2	12.1 ± 1.4
G17	2451.1 ± 57.2	4.5 ± 0.1	4813.5 ± 13.6	872.2 ± 1.0	1333.1 ± 6.3	53.6 ± 0.5	802.6 ± 0.5	11.0 ± 1.3
G18	1215.9 ± 23.4	5.5 ± 0.1	7957.7 ± 8.1	1164.2 ± 4.9	592.5 ± 7.7	57.4 ± 1.7	594.5 ± 3.9	9.9 ± 1.5
G19	3357.6 ± 230.4	1.6 ± 0.5	2358.1 ± 169.1	615.8 ± 52.6	3905.0 ± 212.7	23.3 ± 2.4	257.0 ± 60.0	11.5 ± 2.3
G20 G21	3871.3 ± 105.9	5.4 ± 0.1	3019.7 ± 29.6	2046.3 ± 23.5	1762.6 ± 28.2	51.2 ± 0.5	1772.2 ± 4.0	20.4 ± 0.7
	1104.5 ± 11.4	21.9 ± 0.1	23128.8 ± 63.8	245.9 ± 5.7	232.0 ± 1.2	150.8 ± 1.1	602.3 ± 2.0	21.2 ± 0.3
G22	818.9 ± 35.4	3.0 ± 0.2	2625.3 ± 9.8	326.5 ± 4.3	636.7 ± 10.6	42.9 ± 1.3	352.0 ± 3.6	3.6 ± 0.8

Table 4. Correlation between the concentrations of different mineral elements.

	Ca	Cu	Fe	K	Mg	Mn	P
Cu	−0.216						
Fe	−0.270	0.747					
K	0.628	−0.159	−0.288				
Mg	0.550	−0.445	−0.322	0.330			
Mn	−0.101	0.551	0.656	0.217	−0.107		
P	0.643	−0.087	−0.099	0.536	−0.026	−0.158	
Zn	0.216	0.106	0.143	0.263	0.011	0.362	0.117

*Correlation with significance at $p < 0.05$.

to 3905.0 mg kg^{-1}. High Mg concentrations were found in G3 (Belém city) and G19 (Vigia city). Mg levels obtained were higher than those found by Korn et al. (2013) in propolis samples (157–387 mg kg^{-1}) from northeast Brazil. On the other hand, Finger et al. (2014) found Mg contents higher (530–4660 mg kg^{-1}) than those obtained in this study.

Manganese is an essential micronutrient that acts as a cofactor of various enzymes, such as carboxylase. Mn deficiency in humans includes weight loss, dermatitis, nausea, vomiting, hypocholesterolemia, and prolonged clotting time (Yeung & Laquatra, 2003). The Mn content in the studied geopropolis ranged from 23.3 mg kg^{-1} (sample G19) to 156.7 mg kg^{-1} (sample G10). These values were higher than levels (3.6–88.2 mg kg^{-1}) obtained by Gong et al. (2012) in propolis. Finger et al. (2014) found values (20–140 mg kg^{-1}) close to the levels obtained in this study.

Phosphorus is an element that participates as a cofactor in certain enzymes. P deficiency in humans may cause many events, such as bone pain, myopathy, hypoglycemia, insulin resistance, delirium, memory loss, anorexia, and tachycardia (Epstein & Bloom, 2006; Franco, 1999; Yeung & Laquatra, 2003). High content of P was found in G20 (1772.2 mg kg^{-1}) of the Santo Antônio do Tauá city. P levels found in the other samples were similar to those obtained by Bonvehí and Bermejo, (2013).

Zinc is essential for normal growth and development of the skeleton and plays many roles in carbohydrate, lipid, protein, and nucleic acid metabolism and cell growth (Siqueira et al., 2017; Scherz & Kirchhoff, 2006). The geopropolis samples showed lower Zn levels, ranging from 3.60 mg kg^{-1} to 68.22 mg kg^{-1}. Formicki et al. (2013) and Cantarelli et al. (2011) reported Zn contents (25–72 µg g^{-1} and 11–105 µg g^{-1}) in propolis samples above those obtained in this study.

Correlation analysis

Correlation analysis of Ca, Cu, Fe, K, Mg, Mn, P, and Zn concentrations (variables) was performed, as shown in Table 4. The coefficient of values ranged from 0 to 1 (or −1), indicating weak to strong correlations between variables. According to Dancey and Reidy (2006), the correlations between the variables are considered

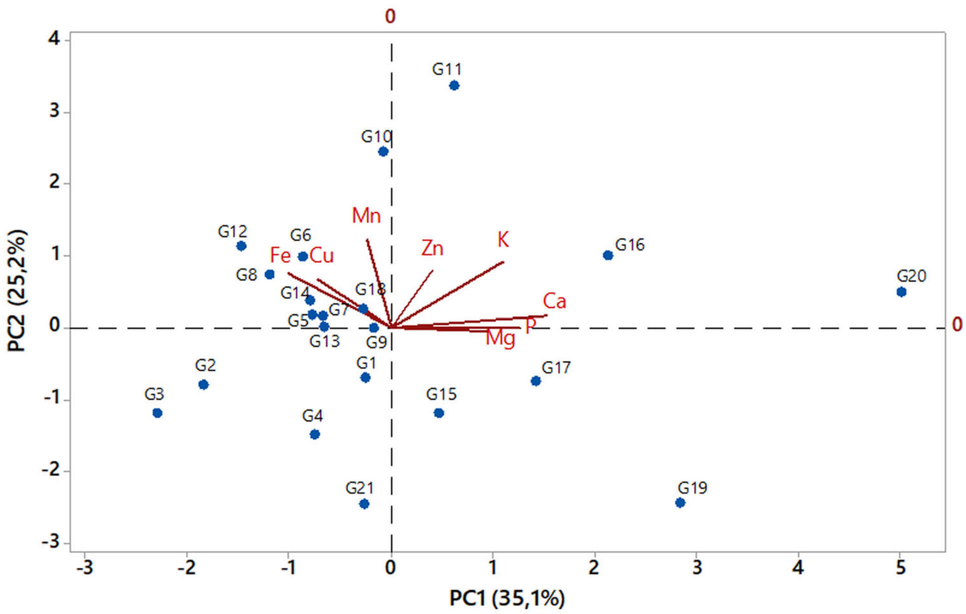

Figure 1. Scores and loadings biplot obtained from PCA data of mineral concentrations in geopropolis samples.

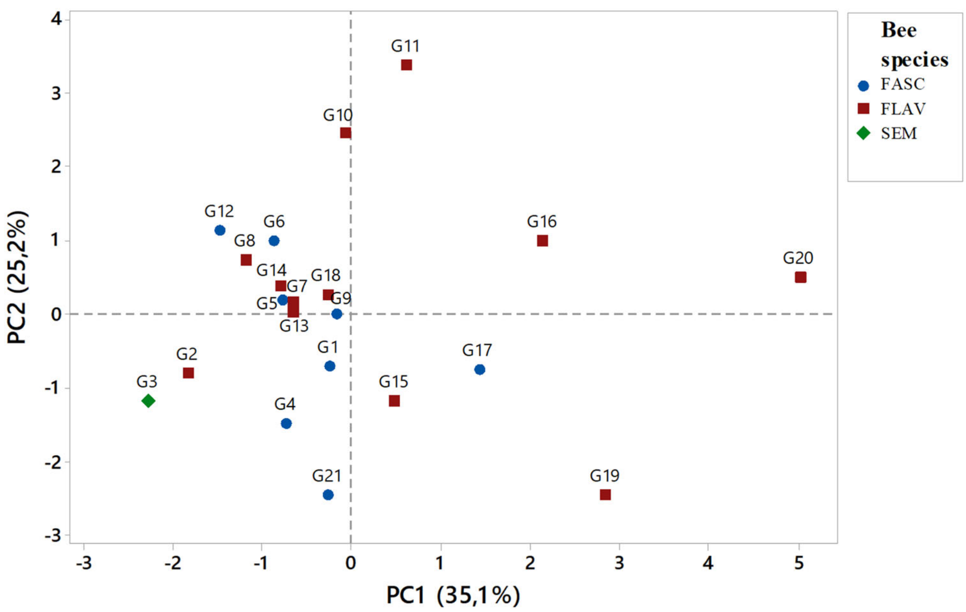

Figure 2. Scores plot for bees species producing geopropolis: *Melipona fasciculate* (FASC), *Melipona flavolineata* (FLAV), *Melipona semi-nigra* (SEM).

weak, moderate, and strong when the values obtained for "r" are between 0.10 and 0.30, 0.40 and 0.60, and 0.70 and 1.00, respectively. According to this classification, a strong positive correlation was observed between Fe and Cu contents in the geopropolis samples ($r = 0.747$). Moderate and positive correlations were found between K and Ca contents ($r = 0.628$), Mn and Fe ($r = 0.656$), and P and Ca ($r = 0.643$). The Mg and Ca, Mn and Cu, and P and K contents presented a moderate correlation at a lower level of significance. Similar to this study, Korn et al. (2013) found a positive and strong correlation between Fe and Cu contents ($r = 0.9382$) and a moderate correlation between Mn and Fe contents ($r = 0.6434$) in samples of propolis collected in northeastern Brazil.

Principal component analysis

The relationship between the inorganic constituents in geopropolis was verified by PCA. The contents of the elements (Ca, Cu, Fe, K, Mg, Mn, P, and Zn) in 22 geopropolis samples were used as variables (loadings) for the formation of the original matrix (22×8). However, the presence of the G21 (Belterra) sample showed low variance in the data for the first and second principal components, indicating an outlier in the formation of principal component groups.

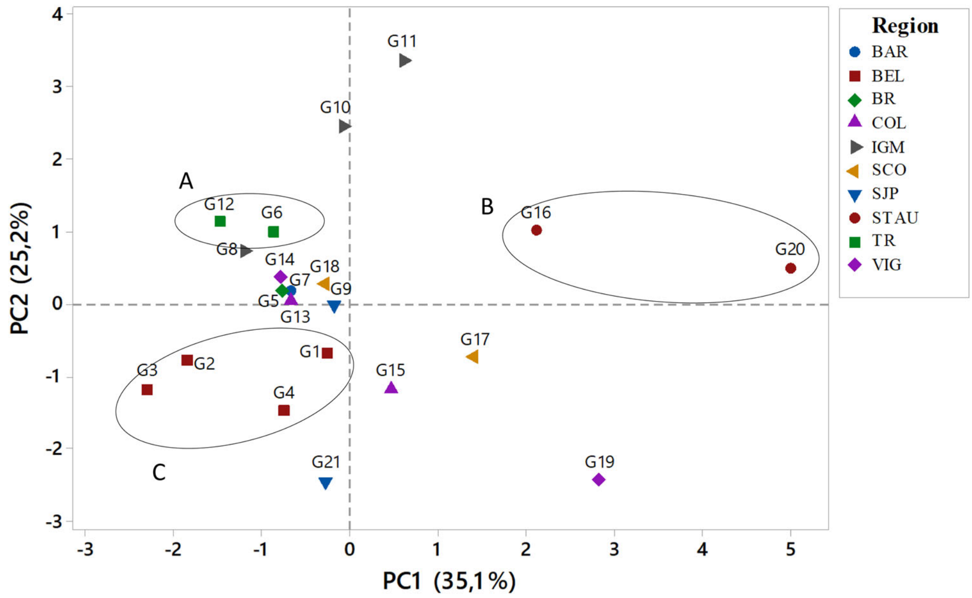

Figure 3. Scores plot for geographic region of geopropolis sample collection. Regions: Barcarena (BAR), Belém (BEL), Bragança (BR), Colares (COL), Igarapé-Miri (IGM), São Caetano de Odivelas (SCO), São João de Pirabas (SJP), Santo Antônio do Tauá (STAU), Tracuateua (TR), Vigia (VIG).

Table 5. Accuracy of the method by certified reference material, GBW 07604. Data are mean ± SD ($n = 3$).

Elements	Certified value (mg kg^{-1})	Determined value (mg kg^{-1})	Recovery (%)
Ca	18100	18048 ± 147	99.7
Cu	9.3	8.5 ± 0.7	91.6
Fe	274	227.4 ± 3.4	83.1
K	–	–	–
Mg	6500	6280.4 ± 59.3	96.6
Mn	45	43.4 ± 0.4	96.5
P	–	–	–
Zn	37	38.8 ± 2.1	104.8

Consequently, the data for this sample (scores) were taken from the original matrix, and a new matrix (21 × 8) was obtained to evaluate the groups formed by the PCA.

Through the results (Figure 1), it was observed that Ca, P, and K presented the highest coefficients for the first component of the correlation matrix. The samples of Santo Antônio do Tauá presented the highest concentrations of Ca (G16), P, and K (G20). The proximity of the coefficients obtained for these elements agrees with the values of the Pearson correlation analysis. Mn presented the highest correlation coefficient, associated with the second principal component. As expected, Fe and Cu presented similar behavior in the two components, which corroborate the correlation analysis shown in Table 4.

In relation to the analysis of the scores, the samples were grouped by bee species producing geoprópolis (Figure 2) and by geographic region (municipalities) of sample collection (Figure 3).

Accuracy

The accuracy of the method for determining Ca, Cu, Fe, K, Mg, Mn, P, and Zn in geopropolis was verified using the certified reference material Poplar Leaves (GBW 07604) (National Research Centre for CRM, Langfang, China). The comparison between the determined and certified values for analytes is presented in Table 5. Analyte recoveries were in the range of 88–99%. The *t* test, at a 95% confidence level, showed that there was no significant difference between determined and certified values. However, the accuracy of the method was also evaluated by adding 3, 5, 7, and 9 mg L^{-1} of all analytes in the samples prior to digestion. Thereafter, the samples were subjected to the digestion process, assisted by microwave radiation. Afterwards, the elements were determined by ICP OES. Recoveries ranged from 82% to 118%.

The accuracy of the measurements by ICP OES was verified by the recovery test. Aliquots of Ca, Cu, Fe, K, Mg, Mn, P, and Zn (3.0, 5.0, and 7.0 mg L^{-1}) were added to the digested samples of geopropolis. All recovery values were satisfactory and ranged from 96% to 103% for Ca, 93% to 97% for Cu, 96% to 98% for Fe, 98% to 101% for K, 98% to 102% for Mg, 94% to 99% for Mn, 97% to 98% for P, and 94% to 99% for Zn.

Discussion

Microwave-assisted digestion and quantification by ICP OES was shown to be effective in the determination of inorganic constituents in geopropolis samples. Low concentrations of Cu, Mn, and Zn were found in samples. On the other hand, Ca, Fe, K, Mg, and P were measedured at high levels. The results of the Pearson correlation analysis showed that there was a strong and positive correlation between Fe and Cu contents and moderate and positive correlation between Mn and Fe contents in the geopropolis samples.

According to the results of PCA, there was better separation and grouping of the samples when they were related to the municipalities, indicating a strong influence of the geographic region on the mineral composition of geopropolis, which can be related to the flora, type of soil, and climate characteristic of each region. The formation of three main groups was observed. Group A was composed exclusively of samples from the municipality of Tracuateua and group B was composed of samples from Santo Antônio do Tauá. Group C was composed only of geopropolis samples collected in Belém. Other studies also verified the correlation of propolis and geopropolis samples with the respective collection sites in different geographical regions (Bonsucesso et al., 2018; Bonvehí & Bermejo, 2013; Cantarelli et al., 2011; Cvek et al., 2008; Gong et al., 2012; Korn et al., 2013).

This study is of great importance to the knowledge of the chemical composition of geopropolis produced in northern Brazil and can serve as a database for mineral characterization of the products in this region.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The authors are grateful to Fundação Amazônia de Amparo a Estudos e Pesquisas do Pará (FAPESPA) (ICAAF No. 012/2012) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Processo no. 562994/2010-6) for research funding and fellowships.

ORCID

Jean Sanger Siqueira http://orcid.org/0000-0002-1880-7254
Cleide Samara Tavares Mescouto http://orcid.org/0000-0003-0691-7998
Michelle de Souza Lemos http://orcid.org/0000-0003-4611-5148
João Batista Pereira Junior http://orcid.org/0000-0002-7851-8235
Giorgio C. Venturieri http://orcid.org/0000-0001-9644-4439
Heronides Adonias Dantas Filho http://orcid.org/0000-0001-5698-5441
Kelly das Graças Fernandes Dantas http://orcid.org/0000-0002-5424-2908

References

Araüjo, K. S. S., Santos Júnior, J. F., Sato, M. O., Finco, F. D. B. A., Soares, I. M., Barbosa, R. S., Alvim, T. C., Ascêncio, S. D., & Mariano, S. M. B. (2016). Physicochemical properties and antioxidant capacity of propolis of stingless bees (Meliponinae) and Apis from two regions of Tocantins, Brazil. *Acta Amazonica*, *46*(1), 61–68. https://doi.org/10.1590/1809-4392201501045

Ataide de Oliveira, F., Abreu, A. T. D., Nascimento, N. D. O., Froes, R. E. S., Nalini, Jr, H. A., & Antonine, Y. (2020). Mineral content in honey and pollen from native stingless bees *Tetragonisca angustula* (Latreille, 1811) in the Iron Quadrangle, Brazil. *Journal of Apicultural Research*, 1–12. https://doi:10.1080/00218839.2020.1730578

Bonsucesso, J. S., Gloaguen, T. V., Nascimento, A. S., Carvalho, C. A. L., & Dias, F. S. (2018). Metals in geopropolis from beehive of Melipona scutellaris in urban environments. *Science of the Total Environment*, *634*, 687–694. https://doi.org/10.1016/j.scitotenv.2018.04.022

Bonvehí, J. S., & Bermejo, F. J. (2013). Element content of propolis collected from different areas of South Spain. *Environmental Monitoring and Assessment*, *185*(7), 6035–6047. https://doi.org/10.1007/s10661-012-3004-3

Cantarelli, M. A., Camiña, J. M., Pettenati, E. M., Marchevsky, E. J., & Pellerano, R. G. (2011). Trace mineral contente of Argentinean raw propolis by neutron activation analysis (NAA): Assessment of geographical provenance by chemometrics. *LWT: Food Science and Technology*, *44*(1), 256–260. https://doi.org/10.1016/j.lwt.2010.06.031

Cardozo, D. V., Mokochinski, J. B., Machado, C. S., Sawaya, A. C. H. F., Caetano, I. K., Felsner, M. L., & Torres, Y. R. (2015). Variabilidade Química da geoprópolis produzida pelas abelhas sem ferrão Jataí, Mandaçaia e Mandurí. *Revista Virtual de Química*, *7*(6), 2457–2474. https://doi.org/10.5935/1984-6835.20150146

Cinegaglia, N. C., Bersano, P. R. O., Araújo, M. J. A. M., Búfalo, M. C., & Sforcin, J. M. (2013). Anticancer effects of geopropolis produced by stingless bees on canine osteosarcoma cells *in vitro*. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 1–6. https://doi.org/10.1155/2013/737386

Cvek, J., Medić- Šarić, M., Vitali, D., Vedrina-Dragojević, I., Smit, Z., & Tomić, S. (2008). The content of essential and toxic elements in Croatian propolis samples and their tinctures. *Journal of Apicultural Research and Research*, *47*(1), 35–45. https://doi.org/10.1080/00218839.2008.11101421

Dancey C., & Reidy, J. (2006). *Estatística sem matemática para psicologia: Usando SPSS para Windows*. Artmed, Porto Alegre.

Dutra, R. P., Abreu, B. V. B., Cunha, M. S., Batista, M. C. A., Torres, L. M. B., Nascimento, F. R. F., Ribeiro, M. N. S., & Guerra, R. N. M. (2014). Phenolic acids, hydrolyzable tannins, and antioxidant activity of geopropolis from the stingless bee melipona fasciculata Smith. *Journal of Agricultural and Food Chemistry*, *62*, 2549–2557. https://doi.org/10.1021/jf404875v

Dutra, R. P., Nogueira, A. M. C., Marques, R. R. O., Costa, M. C. P., & Ribeiro, M. N. S. (2008). Avaliação farmacológica de geoprópolis de Melipona fasciculata Smith da Baixada maranhense, Brasil. *Revista Brasileira de Farmacognosia*, *18*(4), 557–562. https://doi.org/10.1590/S0102-695X2008000400010

Epstein, E., & Bloom, A. (2006). *Nutrição mineral de plantas*. Editora Planta.

Finger, D., Filho, I. K., Torres, Y. R., & Quináia, S. P. (2014). Propolis as an indicator of environmental contamination by metals. *Bulletin of Environmental Contamination and Toxicology*, *92*(3), 259–264. https://doi.org/10.1007/s00128-014-1199-4

Formicki, G., Grén, A., Stawarz, R., Zyśk, B., & Gal, A. (2013). Metal contente in honey, própolis, wax, and bee pollen and implications for metal pollution monitoring. *Polish Journal of Environmental Studies*, *22*(1), 99–106.

Franchin, M., da Cunha, M. G., Denny, C., Napimoga, M. H., Cunha, T. M., Koo, H., de Alencar, S. M., Ikegaki, M., & Rosalen, P. L. (2012). Geopropolis from *Melipona scutellaris* decreases the mechanical inflammatory hypernociception by inhibiting the production of IL-1β and TNF-α. *Journal of Ethnopharmacology*, *143*(2), 709–715. https://doi.org/10.1016/j.jep.2012.07.040

Franco, G. (1999). *Tabela de Composição de Alimentos*. Ed. Livraria Atheneu.

Gong, S., Luo, L., Gong, W., Gao, Y., & Xie, M. (2012). Multivariate analysis of element concentrations revealed the groupings of propolis from different regions in China. *Food Chemistry*, *134*(1), 583–588.

Korn, M. G. A., Guida, M. A. B., Barbosa, J. T. P., Torres, E. A., Fernandes, A. P., Santos, J. C. C., Dantas, K. d G. F.,

& Nóbrega, J. A. (2013). Evaluation of sample preparation procedures for trace element determination in Brazilian propolis by inductively coupled plasma optical emission spectrometry and their discrimination according to geographic region. *Food Analytical Methods*, *6*(3), 872–880. https://doi.org/10.1007/s12161-012-9497-0

Magnoni, D., & Cukier, C. (2004). *Perguntas e Respostas em Nutrição Clínica*. Roca.

Menezes, H. (2005). Própolis: uma revisão dos recentes estudos de suas propriedades farmacológicas. *Arquivos do Instituto Biológico*, *72*(3), 405–411.

Nogueira, P. & Neto, (1997). *Vida e Criação de abelhas indígenas sem ferrão*. Nogueirapis.

Scherz, H., & Kirchhoff, E. (2006). Trace elements in foods: Zinc contents of raw foods-a comparison of data originating from different geographical regions of the world. *Journal of Food Composition and Analysis*, *19*(5), 420–433. https://doi.org/10.1016/j.jfca.2005.10.004

Schümann, K., Ettle, T., Szegner, B., Elsenhans, B., & Solomons, N. W. (2007). On risks and benefits of iron supplementation recommendations for iron intake revisited. *Journal of Trace Elements in Medicine and Biology*, *21*(3), 147–168. https://doi.org/10.1016/j.jtemb.2007.06.002

Siqueira, J. S., Pereira Júnior, J. B., Lemos, M. S., Dantas Filho, H. A., & Dantas, K. G. F. (2017). Optimization of a digestion method using diluted acid in bee pollen samples for determination of Fe, Mn, and Zn by flame atomic absorption spectrometry. *Food Analytical Methods*, *10*, 759–763. https://doi.org/10.1007/s12161-016-0625-0

Souza, S. A., Camara, C. A., Silva, E. M. S., & Silva, T. M. S. (2013). Composition and antioxidant activity of geopropolis collected by Melipona subnitida (Jandaíra) bees. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 801383.

Thomsen, V., Roberts, G., & Burguess, K. (2000). The concepts of background equivalent concentration in spectroscopy. *Spectroscopy*, *15*, 33.

Underwood, E. J. (1981). *The mineral nutrition of livestock*. Academic press.

Yeung, D. L., & Laquatra, I. (2003). *Heinz handbook of nutrition*. H.J. Heinz Company.