Determination of Macro and Trace Element Levels in Honey from the Lower Amazonian Region, Brazil

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Graphical Abstract

Possible sources of macro and micronutrients, as well as potentially toxic elements in honey

The aim of this study was to quantify the macro and trace element concentrations in *Apis mellifera* and *Melipona interrupta* honey samples from the Lower Amazonian region in order to evaluate if the samples of different geographical origin and/or species could be distinguished by their mineral content. In addition, it was evaluated the presence of potentially toxic metals in honey samples. The metal contents were determined by inductively coupled plasma optical emission spectrometry (ICP OES) and the most abundant metals found in the samples were K, Ca, Mg, and Na. The total metal (K, Ca, Mg, Na, Cd, Co, Ni, Fe, Mn, Cr, Al and Ba) concentration ranged from 127.7 ± 1.4 to $1844.4 \pm 45.2 \mu\text{g g}^{-1}$ and from 102.7 ± 2.0 to $639.0 \pm 15.3 \mu\text{g g}^{-1}$ in *Apis mellifera* and *Melipona interrupta* honey, respectively. All mineral content levels found in the honey samples were lower than the maximum established by Brazilian and international law (Cd and Cr $0.1 \mu\text{g g}^{-1}$, Pb $0.30 \mu\text{g g}^{-1}$, Ni $5 \mu\text{g g}^{-1}$, Cu $10 \mu\text{g g}^{-1}$, Zn $50 \mu\text{g g}^{-1}$). Furthermore, Cu, Pb, and Zn were not detected in any of the samples. However, potentially toxic elements, such as Cd, Co, and Ni, were detected in most of the commercial samples and in *Apis mellifera* honey from beehives that were close to livestock fields and/or soybean areas. Hierarchical cluster analysis (HCA) was used to study the mineral contents and it was possible to distinguish eight different groups of honey. However, the *Melipona interrupta* honey could not be separated into different groups.

Keywords: Trace elements, mineral content, toxic metals, hierarchical component analysis

INTRODUCTION

The mineral composition of honey consists of approximately 27 elements, and several studies have reported that it is possible to separate honey from different floral and geographical origins using the presence of specific mineral groups [1-3]. These metals are classified into macro elements, such as: K, P, and Ca, and trace elements, such as: Pb, Cd, Zn, Fe, Mg, Mn, Al, Ba, Ag, Cr, As, etc., which are not considered to be essential components of honey [4]. The trace element content in honey varies, but it is typically below $1.0 \mu\text{g g}^{-1}$ and is normally associated with its color. For example, dark honey has the highest metal contents [5-6]. Brazil is one of the largest producers of honey (FAO 2014) [7] and its production is widely distributed across all regions of the country, which has led to a large variety of honey types [8]. The Lower Amazonian region is a promising area for beekeeping due to its special flora and climate characteristics, although it is considered to be a secondary activity [8]. However, this region has been suffering from agricultural expansion since the 1970s, and has mainly occurred along the BR 163 highway [9].

Several potentially toxic elements bioaccumulate in soils and could affect plant and, consequently, honey quality. Therefore, it is important to evaluate the mineral composition of honey, especially because some minerals are essential for human health. It is also important because some trace elements, such as Cd, Hg, and Pb, are non-essential but toxic in very small concentrations and some, such as Cr, Fe, Mn, Ni, Se, and Zn are essential at certain content levels, but can bioaccumulate to toxic levels (plant-animal-human) under certain circumstances [5].

It has been suggested that honey could be used as bio-indicator of environmental pollution if it is produced from the nectar of different plants, particularly if the nectar bioaccumulates heavy metals [5]. However, honey could also be contaminated with different minerals during processing and storage (e.g., contaminated equipment and tools, and poor hygiene) [6,10-11].

Some authors have found large concentrations of heavy metals in honey produced in areas where industrial and/or agricultural activity is high. These types of activity are considered the most important sources of honey contamination [12-13]. The use of multivariate analysis methods such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) have been used for statistical data processing in order to classify honey samples according to their properties in different groups according to their botanical origin, species or geographical origin [4,6,14-18].

The first aim of this study was to determine the metal contents (macro and trace elements) in *Apis mellifera* and *Melipona interrupta* honeys from the Lower Amazonian region. Secondly, to evaluate whether it is possible to differentiate honey by its geographical origin or bee species through the determination of its mineral content. In addition, the degree of contamination of these samples by potentially toxic metals was evaluated.

MATERIALS AND METHODS

Honey samples

A total of 46 honey samples from the Lower Amazonian region were collected between November 2015 and July 2017. Sixteen samples were obtained from commercial sources and 30 samples were obtained through manual extraction where no metal instruments were used and without the use of smoke or chemicals. These samples were collected from two bee species. A total of 33 samples came from *Apis mellifera* (M01–M33) and 13 samples came from Jandaíra (*Melipona interrupta*) (J01–J13). Thirty five samples (M01–M20, M30–M33, and J1–J11), four samples (M26–M29), and seven samples (M21–M25 and J12–J13) were obtained from Santarém ($02^{\circ}26'34''\text{S}$ and $54^{\circ}42'28''\text{W}$), Belterra ($02^{\circ}38'09''\text{S}$ and $54^{\circ}56'13''\text{W}$) and Itaituba ($04^{\circ}16'33''\text{S}$ and $55^{\circ}59'02''\text{W}$), respectively, located in the state of Pará, Brazil (Figure 1 and Table I). All honey samples were stored in Falcon tubes, protected from light, and kept at room temperature until needed for further analysis.

Figure 1. Geographic location of the beehives where the honey samples were collected.

Table I. Main characteristics of the different beehive areas

ID sample	Description of the beehive areas
M01	Soybean field $\pm$ 300 m, highway $\pm$ 500 m, obtained from the beestock
M02	Located in a secondary forest, Livestock area $\pm$ 500, highway $\pm$ 300 m, obtained from the beestock
M03	Soybean field and livestock area $\pm$ 500 m, highway $\pm$ 2000 m, obtained from the beestock
M04	Located in secondary forest, passion fruit plantation $\pm$ 300 m, highway $\pm$ 1500 m, obtained from the beestock
M05	Located in secondary forest, highway $\pm$ 2000 m, obtained from the beestock
M06	Soybean field $\pm$ 500 m, highway $\pm$ 1000 m, obtained from the beestock
M07	Livestock area $\pm$ 500 m, highway $\pm$ 3000 m, obtained from the beestock
M08	Vegetable garden $\pm$ 1000 m, livestock area $\pm$ 1500 m, highway $\pm$ 3000 m, obtained from the beestock
M09	Soybean field $\pm$ 500 m, highway $\pm$ 1000 m, obtained from the beestock
M10	Located in secondary forest, livestock area $\pm$ 500 m, highway $\pm$ 300 m, obtained from the beestock
M11	Soybean field and livestock area $\pm$ 500 m, highway $\pm$ 2000 m, obtained from the beestock
M12	Located in secondary forest, passion fruit plantation $\pm$ 500 m, highway $\pm$ 1500 m, obtained from the beestock
M13	Located in secondary forest, highway $\pm$ 2000 m, obtained from the beestock
M14	Soybean field $\pm$ 1000 m, highway $\pm$ 1500 m, obtained from the beestock
M15	Livestock area $\pm$ 500 m, highway $\pm$ 300 m, obtained from the beestock
M16	Vegetable garden $\pm$ 1000 m, livestock area $\pm$ 1500 m, highway $\pm$ 3000 m, obtained from the beestock
M17	Commercial sample
M18	Commercial sample
M19	Commercial sample
M20	Commercial sample
M21	Located in secondary forest, freshly harvested honey
M22	Located in secondary forest, freshly harvested honey
M23	Acerola (<i>Malpighia emarginata</i>), cupuaçu (<i>Theobroma grandiflorum</i>), papay (<i>Carica papaya</i>), orange (<i>Citrus aurantium</i>), and lemon (<i>Citrus limonum</i>) trees.
M24	Acerola (<i>Malpighia emarginata</i>), cupuaçu (<i>Theobroma grandiflorum</i>), papay (<i>Carica papaya</i>), orange (<i>Citrus aurantium</i>), and lemon (<i>Citrus limonum</i>) trees.
M25	Acerola (<i>Malpighia emarginata</i>), cupuaçu (<i>Theobroma grandiflorum</i>), papay (<i>Carica papaya</i>), orange (<i>Citrus aurantium</i>), and lemon (<i>Citrus limonum</i>) trees.
M26	Mango (<i>Mangifera indica</i> L.), orange (<i>Citrus aurantium</i>), Brazil nut (<i>Bertholletia excelsa</i>), cashew (<i>Anacardium occidentale</i>) and açai (<i>Euterpe oleracea</i>) trees.
M27	Mango (<i>Mangifera indica</i> L.), orange (<i>Citrus aurantium</i>), Brazil nut (<i>Bertholletia excelsa</i>), cashew (<i>Anacardium occidentale</i>), and açai (<i>Euterpe oleracea</i>) trees.
M28	Mango (<i>Mangifera indica</i> L.), orange (<i>Citrus aurantium</i>), Brazil nut (<i>Bertholletia excelsa</i>), cashew (<i>Anacardium occidentale</i>), and açai (<i>Euterpe oleracea</i>) trees.
M29	Mango (<i>Mangifera indica</i> L.), orange (<i>Citrus aurantium</i>), Brazil nut (<i>Bertholletia excelsa</i>), cashew (<i>Anacardium occidentale</i>), and açai (<i>Euterpe oleracea</i>) trees.
M30	Commercial sample
M31	Commercial sample
M32	Commercial sample
M33	Commercial sample

Table I. Main characteristics of the different beehive areas (Cont)

ID sample	Description of the beehive areas
J01	Located in secondary forest, soybean field $\pm$ 1000 m, highway $\pm$ 1000 m
J02	Located in secondary forest, soybean field $\pm$ 1000 m, highway $\pm$ 1000 m
J03	Located in secondary forest, soybean field $\pm$ 1000 m, passion fruit plantation $\pm$ 300 m, highway $\pm$ 1500 m
J04	Soybean field $\pm$ 1000 m, highway $\pm$ 1000 m
J05	Located in a secondary forest, soybean field $\pm$ 1000 m, highway $\pm$ 1000 m
J06	Commercial sample
J07	Commercial sample
J08	Commercial sample
J09	Commercial sample
J10	Commercial sample
J11	Commercial sample
J12	Commercial sample
J13	Commercial sample

M: *Apis mellifera* honey; J: Jandaíra (*Melipona interrupta*) honey.

Sample preparation

Microwave assisted digestion

Approximately 1.0 g of the sample was weighed in polytetrafluoroethylene (PTFE) tubes and then mixed with 2.0 mL of HNO_3 , 14.0 mol L^{-1} (Vetec, Rio de Janeiro, RJ, Brazil) that had been previously distilled in a quartz stillatory (Kurner, Analysentechnik, Rosenheim, Germany); 2.0 mL of H_2O_2 , 30% (v/v) (Vetec, Rio de Janeiro, RJ, Brazil); and 0.5 mL of yttrium solution (100.0 mg L^{-1}) (Sigma Aldrich, St. Louis, MO, USA), which was the internal standard. The mixture was divided into three fractions and then was subjected to a heating program in a closed microwave oven (CEM/Mars Xpress, Matthews, NC, USA), which consisted of the following steps: 1 min at 320 W, 2 min at 120 W, 5 min at 320 W, 5 min at 520 W, and 5 min at 720 W. Then, the resulting mixture was left to cool and was quantitatively transferred to polypropylene vials. The volume of the final solution was made up to 25.0 mL with ultrapure water with a resistivity of 18 M Ω cm. The ultrapure water had been obtained from a Milli-Q system (Millipore, Bedford, MA, USA) before analysis. Reagent blank containing ultrapure water, HNO_3 , and H_2O_2 was subjected to the same treatment as the honey samples.

The metal contents in the honey were determined by inductively coupled plasma optical emission spectroscopy (ICP OES) model Vista-MPX CCD (Varian, Mulgrave, VIC, Australia) with an automatic sampling system (ASX 520, Cetac Technologies, Omaha, NE, USA). Each sample was analyzed in triplicate. A cyclonic spray chamber and concentric nebulizer were used. The metal determinations were carried out using the manufacturer recommended conditions for power (1.4 kW), generator frequency (40 MHz), plasma gas flow (15 L min^{-1}), auxiliary gas flow (1.5 L min^{-1}), nebulizer gas flow (0.75 L min^{-1}) and sample uptake rate (1.0 mL min^{-1}). The emission intensity scan duration was 60 s. The following analytical wavelengths (nm) were selected: Al (396.152), Ba (455.403), Ca (318.127), Cd (226.502), Co (231.160), Cr (276.716), Cu (327.395), Fe (238.204), K (766.491), Mg (280.270), Mn (257.610), Na (589.592), Ni (231.604), Pb (220.353) and Zn (213.857).

The absence of certified honey samples meant that recovery tests were performed to validate the method. The recovery percentage was calculated by processing five honey samples spiked with known amounts of element analytical standards.

Calibration of the samples against matrix matches was undertaken so that the macro and trace element concentrations could be calculated and to correct for matrix interferences. One sample of base

honey from *Apis mellifera* (M10) honey was selected. Then, standard stock solutions containing 1000 mg L⁻¹ of Zn, Pb, Ni, Cd, Co, Fe, Mn, Cr, Mg, Cu, Al, Ca, Ba, Na, or K were purchased from Merck (Darmstadt, Germany). Analytical calibration standards were prepared daily and the concentrations ranged from 40 to 400 µg L⁻¹ for all analyzed elements. The base honey was prepared in the same manner as the samples. Yttrium was added as the internal standard to a final concentration 10 µg L⁻¹. This sample was digested in a microwave oven and after dilution in the same way as the other samples.

The limits of detection (LOD) were calculated using the equation $LOD = (3 \times RSDB_{blank} \times BEC)/100$ and $LOQ = (10 \times RSDB_{blank} \times BEC)/100$. The limits of quantification (LOQ) were determined as three times the LOD value ($3 \times LOD$). Where BEC (background equivalent concentration) is the blank intensity divided by the angular coefficient of the analytical curve and RSDB_{blank} is the relative standard deviation of the blank ($n = 10$). The equation for the BEC can also be written as $BEC = C_{Element} / SBR$, where $C_{Element}$ is the element concentration and SBR (signal-bottom ratio) is the ratio between the intensity of the pure analytical signal and the background signal, which can be calculated $SBR = (I_{Analytical} - I_{Blank}) / I_{Blank}$. The LOD and LOQ values are given in Table II.

All analyses were run in triplicate and the results were expressed as means $\pm$ the standard deviation (SD). A Pearson's correlation analysis was performed on the mean values for each metal content using Minitab Statistical Software 14 (Minitab, State College, PA, USA).

Statistical multivariate analysis by Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were undertaken using the complete linkage with the square Euclidian distance to evaluate if *Apis mellifera* and *Melipona interrupta* honey could be distinguished by their mineral contents. The results of the analysis are presented in a dendrogram plot that shows the similarity level between sample results provided by the Minitab Statistical Software 14 (Minitab Inc., 2010) and free software R version 3.1.1 (R Core Team, 2017).

RESULTS AND DISCUSSION

Macro and trace element concentrations in honey from different areas of Brazil

Table II shows the limits of detection and quantification as well as the percentage of recovery of the analyzed elements. The recovery obtained for all elements ranged from 67.0% (Al) to 113.1% (K).

A total of 15 metals were simultaneously determined using ICP OES and the average macro and trace element concentrations in the honey samples are given along with their standard deviations in Tables IV and V, respectively.

Total metal concentration was obtained from the sum of the mean concentrations of each element, which ranged from 127.7 (M26) to 1844.4 µg g⁻¹ (M23) and from 102.7 (J05) to 639.0 µg g⁻¹ (J07) for *Apis mellifera* and *Melipona interrupta*, respectively. The most abundant elements found in the honey samples were K, Na, Ca, and Mg with maximum concentrations of 1618.0 µg g⁻¹ (M23), 200.3 µg g⁻¹ (M32), 167.5 µg g⁻¹ (M32), and 134.2 µg g⁻¹ (M32), respectively (Table III).

Table II. Major (Mg, Ca, Na, K) and trace element (Zn, Pb, Cd, Co, Ni, Fe, Mn, Cr, Cu, Al, Ba) levels, and recoveries from spiked honey samples

Element	LOD (ng g ⁻¹)	LOQ (ng g ⁻¹)	Spike values (µg g ⁻¹)	Measured value (µg g ⁻¹)	Recovery (%)
Mg	0.19	0.66	500	391.67 $\pm$ 12.89	76.6 $\pm$ 2.2
Ca	0.36	1.17	500	425.35 $\pm$ 15.39	81.6 $\pm$ 3.3
Na	0.29	0.96	500	486.54 $\pm$ 39.92	95.5 $\pm$ 6.6
K	1.59	5.30	500	565.50 $\pm$ 34.90	113.1 $\pm$ 7.0
Zn	0.23	0.77	500	410.66 $\pm$ 16.32	82.1 $\pm$ 3.3
Pb	0.21	0.69	500	448.44 $\pm$ 17.20	89.7 $\pm$ 3.4

Table II. Major (Mg, Ca, Na, K) and trace element (Zn, Pb, Cd, Co, Ni, Fe, Mn, Cr, Cu, Al, Ba) levels, and recoveries from spiked honey samples (Cont)

Element	LOD (ng g ⁻¹)	LOQ (ng g ⁻¹)	Spike values (µg g ⁻¹)	Measured value (µg g ⁻¹)	Recovery (%)
Cd	1.30	4.34	500	374.67 ± 16.39	74.9 ± 3.3
Co	0.87	2.90	500	372.05 ± 17.50	74.4 ± 3.5
Ni	2.71	9.04	500	372.79 ± 13.98	74.6 ± 2.8
Fe	0.28	0.93	500	378.70 ± 17.24	75.7 ± 3.4
Mn	0.40	1.34	500	374.67 ± 16.39	74.9 ± 3.3
Cr	1.66	5.53	500	391.40 ± 16.34	78.3 ± 3.3
Cu	1.19	3.99	500	385.11 ± 15.70	77.0 ± 3.1
Al	0.28	0.94	500	335.23 ± 13.84	67.0 ± 2.8
Ba	1.13	3.79	500	337.29 ± 12.11	67.5 ± 2.4

LOD: Limits of detection LOQ: Limits of quantification

Table III. Average Mg, Ca, Na, and K concentrations in the honey samples (µg g⁻¹)^a

ID sample	Mg	Ca	Na	K
M01	21.98 ± 0.44	43.39 ± 0.35	42.18 ± 0.49	364.87 ± 7.13
M02	8.82 ± 0.20	22.32 ± 0.17	28.04 ± 0.17	201.71 ± 5.25
M03	19.55 ± 0.23	41.91 ± 0.56	51.50 ± 0.50	448.17 ± 10.79
M04	27.18 ± 0.30	47.67 ± 0.72	73.40 ± 0.58	592.40 ± 15.36
M05	60.06 ± 0.19	117.63 ± 0.52	82.91 ± 0.86	1418.06 ± 30.12
M06	51.39 ± 0.20	89.22 ± 0.91	90.12 ± 1.00	802.92 ± 6.69
M07	71.14 ± 0.20	131.02 ± 0.49	103.91 ± 1.10	1404.06 ± 42.84
M08	88.68 ± 0.43	113.76 ± 0.52	98.40 ± 0.44	1465.84 ± 27.13
M09	27.85 ± 0.46	51.72 ± 0.41	69.31 ± 0.57	483.35 ± 4.86
M10	12.05 ± 0.27	26.78 ± 0.32	68.21 ± 1.82	234.47 ± 6.41
M11	20.24 ± 0.36	42.91 ± 0.49	81.62 ± 1.34	441.82 ± 8.76
M12	13.74 ± 0.28	23.07 ± 1.07	55.59 ± 0.78	233.42 ± 13.32
M13	36.37 ± 0.19	70.25 ± 0.66	83.02 ± 1.07	761.18 ± 3.04
M14	36.73 ± 0.26	59.46 ± 1.24	89.99 ± 0.96	568.89 ± 5.92
M15	43.66 ± 0.50	81.96 ± 0.35	87.80 ± 0.64	695.98 ± 20.12
M16	45.46 ± 0.41	63.25 ± 0.89	82.94 ± 0.28	737.39 ± 12.80
M17	13.86 ± 0.55	98.41 ± 1.90	137.33 ± 3.43	667.00 ± 2.17
M18	7.99 ± 0.36	17.11 ± 0.44	67.19 ± 2.57	161.83 ± 2.13
M19	15.28 ± 0.75	29.92 ± 1.23	68.26 ± 1.64	333.25 ± 6.18
M20	23.83 ± 0.38	29.21 ± 2.79	62.34 ± 1.12	198.45 ± 2.28
M21	9.26 ± 0.21	36.74 ± 0.46	59.98 ± 0.66	118.86 ± 6.75

Table III. Average Mg, Ca, Na, and K concentrations in the honey samples ($\mu\text{g g}^{-1}$)^a (Cont)

ID sample	Mg	Ca	Na	K
M22	9.32 $\pm$ 0.11	42.63 $\pm$ 0.44	72.02 $\pm$ 0.53	68.69 $\pm$ 4.59
M23	34.91 $\pm$ 0.13	103.17 $\pm$ 0.62	81.28 $\pm$ 0.52	1618.01 $\pm$ 43.76
M24	35.62 $\pm$ 0.10	74.44 $\pm$ 0.54	60.97 $\pm$ 0.41	1111.71 $\pm$ 28.37
M25	47.44 $\pm$ 0.62	84.99 $\pm$ 0.22	81.83 $\pm$ 0.34	1159.15 $\pm$ 23.20
M26	6.36 $\pm$ 0.35	32.77 $\pm$ 0.10	48.53 $\pm$ 0.21	36.41 $\pm$ 0.70
M27	15.38 $\pm$ 0.79	78.45 $\pm$ 0.97	66.59 $\pm$ 0.54	113.49 $\pm$ 1.12
M28	8.98 $\pm$ 0.50	24.10 $\pm$ 0.34	60.03 $\pm$ 0.20	196.00 $\pm$ 0.98
M29	17.62 $\pm$ 0.36	29.73 $\pm$ 0.76	77.35 $\pm$ 0.45	265.23 $\pm$ 1.33
M30	20.14 $\pm$ 0.91	78.48 $\pm$ 1.38	81.25 $\pm$ 1.03	145.68 $\pm$ 4.49
M31	47.95 $\pm$ 0.62	100.76 $\pm$ 0.94	110.55 $\pm$ 2.21	1264.94 $\pm$ 16.74
M32	134.17 $\pm$ 0.44	167.50 $\pm$ 1.94	200.29 $\pm$ 4.34	867.12 $\pm$ 20.05
M33	11.89 $\pm$ 0.28	19.40 $\pm$ 0.23	59.92 $\pm$ 0.81	172.56 $\pm$ 3.87
J01	2.80 $\pm$ 0.11	14.82 $\pm$ 0.30	75.60 $\pm$ 1.67	40.82 $\pm$ 3.56
J02	10.70 $\pm$ 0.27	34.44 $\pm$ 0.32	103.39 $\pm$ 1.90	130.67 $\pm$ 7.22
J03	4.72 $\pm$ 0.11	15.42 $\pm$ 0.58	67.33 $\pm$ 0.89	90.02 $\pm$ 2.58
J04	3.30 $\pm$ 0.07	13.43 $\pm$ 0.20	52.40 $\pm$ 2.47	52.23 $\pm$ 1.35
J05	2.79 $\pm$ 0.10	13.78 $\pm$ 0.21	53.72 $\pm$ 0.96	30.75 $\pm$ 0.72
J06	18.41 $\pm$ 0.32	79.75 $\pm$ 1.62	79.59 $\pm$ 5.22	233.35 $\pm$ 8.57
J07	34.50 $\pm$ 0.28	84.60 $\pm$ 4.51	104.13 $\pm$ 2.22	412.22 $\pm$ 8.22
J08	30.34 $\pm$ 0.54	81.59 $\pm$ 5.03	78.20 $\pm$ 2.69	366.76 $\pm$ 6.00
J09	11.93 $\pm$ 0.15	64.74 $\pm$ 2.11	67.56 $\pm$ 2.84	121.43 $\pm$ 6.42
J10	16.55 $\pm$ 0.79	20.17 $\pm$ 0.72	65.93 $\pm$ 4.12	109.34 $\pm$ 6.00
J11	19.94 $\pm$ 0.57	30.32 $\pm$ 0.46	60.58 $\pm$ 3.50	136.72 $\pm$ 9.02
J12	24.10 $\pm$ 0.46	56.29 $\pm$ 1.26	64.93 $\pm$ 1.93	202.14 $\pm$ 4.04
J13	12.28 $\pm$ 0.28	49.24 $\pm$ 0.13	67.56 $\pm$ 1.32	266.34 $\pm$ 3.39

^aAverage of three determinations $\pm$ the standard deviation. M: *Apis mellifera* honey; J: Jandaíra (*Melipona interrupta*) honey.

Table IV. Average Cd, Co, Ni, Fe, Mn, Cr, Al, and Ba concentrations in the honey samples ($\mu\text{g g}^{-1}$) and their standard deviations^a

ID	Cd	Co	Ni	Fe	Mn	Cr	Al	Ba
M01	< LOD	0.13 ± 0.04	0.07 ± 0.01	< LOD	1.65 ± 0.07	< LOD	< LOD	< LOD
M02	0.016 ± 0.003	0.26 ± 0.03	< LOD	< LOD	1.52 ± 0.02	< LOD	< LOD	< LOD
M03	< LOD	0.20 ± 0.02	0.20 ± 0.01	< LOD	0.65 ± 0.01	< LOD	< LOD	< LOD
M04	0.006 ± 0.001	0.28 ± 0.02	< LOD	< LOD	1.50 ± 0.04	< LOD	< LOD	< LOD
M05	0.031 ± 0.002	0.17 ± 0.02	0.02 ± 0.00	< LOD	1.87 ± 0.04	< LOD	< LOD	< LOD
M06	< LOD	0.06 ± 0.02	0.03 ± 0.01	0.42 ± 0.02	0.99 ± 0.05	< LOD	0.61 ± 0.01	< LOD
M07	0.018 ± 0.004	0.37 ± 0.02	0.26 ± 0.02	1.18 ± 0.09	2.35 ± 0.09	< LOD	1.84 ± 0.02	0.14 ± 0.01
M08	0.011 ± 0.002	0.20 ± 0.03	0.05 ± 0.01	0.40 ± 0.03	1.33 ± 0.05	< LOD	0.43 ± 0.02	< LOD
M09	< LOD	0.25 ± 0.02	0.02 ± 0.00	< LOD	1.71 ± 0.07	0.03 ± 0.00	< LOD	< LOD
M10	0.031 ± 0.005	0.37 ± 0.02	0.10 ± 0.01	< LOD	1.36 ± 0.03	0.01 ± 0.00	< LOD	< LOD
M11	0.063 ± 0.002	0.21 ± 0.03	0.20 ± 0.01	0.13 ± 0.09	0.50 ± 0.02	< LOD	0.37 ± 0.01	< LOD
M12	0.008 ± 0.002	0.14 ± 0.02	< LOD	< LOD	0.61 ± 0.04	< LOD	< LOD	< LOD
M13	< LOD	0.34 ± 0.08	0.23 ± 0.03	0.12 ± 0.02	1.08 ± 0.04	< LOD	< LOD	< LOD
M14	0.012 ± 0.003	< LOD	< LOD	0.20 ± 0.02	0.66 ± 0.05	< LOD	0.44 ± 0.01	< LOD
M15	0.045 ± 0.005	0.06 ± 0.01	< LOD	0.74 ± 0.07	1.39 ± 0.07	< LOD	0.54 ± 0.01	< LOD
M16	0.057 ± 0.008	0.07 ± 0.02	0.05 ± 0.01	< LOD	0.75 ± 0.02	0.05 ± 0.01	0.13 ± 0.01	< LOD
M17	0.005 ± 0.001	0.13 ± 0.02	< LOD	< LOD	3.48 ± 0.26	< LOD	0.14 ± 0.01	0.41 ± 0.03
M18	0.020 ± 0.003	0.4 ± 0.04	0.11 ± 0.01	< LOD	0.43 ± 0.03	< LOD	1.49 ± 0.05	< LOD
M19	< LOD	0.31 ± 0.03	0.20 ± 0.01	< LOD	0.47 ± 0.01	0.01 ± 0.00	0.14 ± 0.01	0.15 ± 0.008
M20	< LOD	0.10 ± 0.02	< LOD	< LOD	0.21 ± 0.02	< LOD	0.14 ± 0.02	< LOD
M21	< LOD	0.03 ± 0.01	0.22 ± 0.02	3.48 ± 0.10	0.38 ± 0.02	< LOD	1.06 ± 0.05	< LOD
M22	0.002 ± 0.001	0.36 ± 0.02	< LOD	3.28 ± 0.09	0.25 ± 0.01	< LOD	0.97 ± 0.01	< LOD
M23	0.003 ± 0.001	0.02 ± 0.01	0.16 ± 0.02	2.66 ± 0.06	0.61 ± 0.01	< LOD	3.57 ± 0.03	< LOD
M24	< LOD	0.05 ± 0.01	< LOD	2.52 ± 0.05	0.92 ± 0.02	< LOD	4.14 ± 0.02	< LOD
M25	< LOD	< LOD	< LOD	2.04 ± 0.17	1.73 ± 0.04	< LOD	4.52 ± 0.02	0.02 ± 0.01
M26	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	3.60 ± 0.04	< LOD
M27	< LOD	< LOD	< LOD	< LOD	0.14 ± 0.01	< LOD	2.52 ± 0.02	< LOD
M28	< LOD	< LOD	< LOD	0.46 ± 0.04	0.17 ± 0.01	< LOD	2.10 ± 0.03	< LOD
M29	< LOD	< LOD	< LOD	< LOD	0.32 ± 0.01	< LOD	1.64 ± 0.03	< LOD
M30	0.027 ± 0.003	0.07 ± 0.01	0.10 ± 0.01	56.35 ± 0.13	0.38 ± 0.03	0.01 ± 0.00	< LOD	0.003 ± 0.001
M31	0.021 ± 0.004	0.03 ± 0.01	0.32 ± 0.03	1.00 ± 0.07	3.43 ± 0.14	< LOD	1.26 ± 0.01	< LOD
M32	0.010 ± 0.001	0.08 ± 0.01	0.05 ± 0.00	1.53 ± 0.09	1.77 ± 0.03	< LOD	4.49 ± 0.05	1.26 ± 0.11
M33	0.007 ± 0.002	0.24 ± 0.01	0.04 ± 0.01	< LOD	0.37 ± 0.04	< LOD	0.01 ± 0.00	0.15 ± 0.02
J01	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	2.19 ± 0.04	< LOD
J02	< LOD	< LOD	< LOD	< LOD	0.23 ± 0.02	< LOD	3.11 ± 0.05	0.09 ± 0.02
J03	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	1.66 ± 0.03	< LOD

Table IV. Average Cd, Co, Ni, Fe, Mn, Cr, Al, and Ba concentrations in the honey samples ($\mu\text{g g}^{-1}$) and their standard deviations^a (Cont)

ID	Cd	Co	Ni	Fe	Mn	Cr	Al	Ba
J04	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	1.40 ± 0.04	< LOD
J05	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	1.62 ± 0.02	< LOD
J06	< LOD	< LOD	< LOD	< LOD	0.68 ± 0.05	< LOD	9.13 ± 0.07	< LOD
J07	< LOD	0.10 ± 0.01	< LOD	< LOD	0.59 ± 0.03	< LOD	2.88 ± 0.04	< LOD
J08	< LOD	< LOD	< LOD	< LOD	0.49 ± 0.03	< LOD	2.98 ± 0.03	0.05 ± 0.01
J09	< LOD	< LOD	< LOD	< LOD	0.14 ± 0.03	< LOD	2.63 ± 0.02	< LOD
J10	0.044 ± 0.005	0.02 ± 0.01	0.31 ± 0.02	< LOD	0.13 ± 0.01	0.02 ± 0.00	0.33 ± 0.01	< LOD
J11	0.017 ± 0.002	0.27 ± 0.02	0.21 ± 0.01	0.52 ± 0.06	0.34 ± 0.03	< LOD	0.36 ± 0.01	< LOD
J12	0.015 ± 0.004	0.01 ± 0.00	< LOD	7.59 ± 0.12	0.78 ± 0.02	< LOD	3.00 ± 0.03	< LOD
J13	< LOD	0.27 ± 0.02	< LOD	0.72 ± 0.03	0.57 ± 0.02	< LOD	0.76 ± 0.01	1.38 ± 0.23

^aAverage of three determinations $\pm$ standard deviation. ID Sample; M: *Apis mellifera* honey; J: Jandaíra honey. LOD: limit of detection.

There were also a few potentially toxic trace elements, such as Cd, Co, Ni, Fe, Mn, and Al, detected in most of the *Apis* honey samples and some of the *Melipona interrupta* samples (Table IV). However, there was generally less than $1.00 \mu\text{g g}^{-1}$ of these elements present, except for Fe, Mn, and Al in some samples (Table IV).

The correlations between these metals ranged from -0.114 to 0.848 for Ca, which had a negative correlation with Cr content ($r^2 = 1.3\%$) and a strong positive correlation with Mg content ($r^2 = 72.0\%$) (Table V). The correlations between Cr and Na, and K contents whereas 0.749 ($r^2 = 56.1\%$) and with K was 0.772 ($r^2 = 59.6\%$), this perhaps shows that the composition of these elements at the sampling honeys, increase when the each one increases, therefore in all samples the calcium content was directly influenced by Na, Mg and K contents representing the major total variation of data. The Mn contents show the major value of correlation between a trace metal (0.605) ($r^2 = 36.6\%$) (Table V).

The Na correlation coefficients ranged from -0.057 to 0.749 , being -0.057 with Co contents and 0.749 with Ca contents. The Na had a moderate correlation with Mn (0.488) among the trace metals, as the Ca contents. The others macro elements had the ranges of their correlation coefficients: -0.083 with Fe and 0.772 with Ca (for K contents); and -0.019 with Fe and 0.848 with Ca (for Mg contents).

The Co contents showed a weak positive correlation with Ni contents (0.300) ($r^2 = 9.0\%$), however, it presented moderate negative correlation with Al content (-0.507) ($r^2 = 25.0\%$) (Table V). All others elements showed low correlations with others elements with the remark for the coefficients between Cr and Cd (0.634) that could represent a risk for the human health. If the continuous monitoring under the same sampling and analysis conditions shows that Cr or Cd continue to be present at high levels, then it may indicate that an increase in Cr leads to an increase in Cd and vice versa.

The levels of Cu, Pb and Zn in the honey samples were below the limit of detection. Cadmium was detected in 57.60% and 23.07% of *Apis* and *Melipona* honey samples, respectively, with concentrations ranging from 0.02 (M22) to 0.063 (M11) $\mu\text{g g}^{-1}$. In addition, Ni and Co were detected in more than half of the *Apis mellifera* honey, with concentrations ranging from $0.025 \mu\text{g g}^{-1}$ (M09) to $0.325 \mu\text{g g}^{-1}$ (M31) $\mu\text{g g}^{-1}$, and from $0.016 \mu\text{g g}^{-1}$ (M23) to $0.373 \mu\text{g g}^{-1}$ (M10), respectively (Table IV).

Table V. Pearson's coefficients for macro and trace elements

	Al	Ba	Ca	Cd	Co	Cr	Fe	K	Mg	Mn	Na
Ba	0.098										
Ca	0.288	0.327									
Cd	-0.322	-0.095	0.089								
Co	-0.507	0.147	-0.007	0.146							
Cr	-0.229	-0.073	-0.114	0.634	0.120						
Fe	-0.081	-0.034	0.120	0.149	-0.067	0.010					
K	0.056	0.064	0.772	0.162	0.119	-0.020	-0.083				
Mg	0.128	0.363	0.848	0.187	0.080	0.007	-0.019	0.738			
Mn	-0.124	0.184	0.605	0.164	0.281	-0.041	-0.076	0.645	0.504		
Na	0.227	0.513	0.749	0.112	-0.057	-0.014	0.034	0.458	0.734	0.488	
Ni	-0.253	-0.081	0.092	0.357	0.300	0.212	0.070	0.244	0.132	0.209	0.072

Multivariate analysis

Figure 2 shows the scores and loadings plots of macro and trace elements determined in honey samples. The explained variance of PC1 and PC2 was 51.8%. The contents of Fe, Co and K were similar and had the same weight to separation as well as Na, Cd and Mg and Ca, Ba, Al and Ni. The contents of Mn and Cr were isolated. The content of Cr has a diametrical opposition in relationship to Al, Ba and Ca contents, this may explain the low and negative correlation between these variables (-0.229 (Cr-Al); -0.073 (Cr-Ba); -0.114 (Cr-Ca)).

The *Apis mellifera* samples had the major of metal contents responsible to distinguish different geographical location while the *Melipona interrupta* had the majority of samples with contents of Fe, Co, K, Cd, Mg and Na very different in relationship to *Apis mellifera* samples. Only two samples from *Melipona interrupta* had similar contents of Mn in comparison to samples from other bee species.

Some samples of *Apis mellifera* had similar characteristics to the others samples (M26, M27, M28 and M29). The majority of *Apis* samples had contents of Ni, Al, Ba and Ca similar. Two samples had the same contents of Cd and Mg. Some samples of *Apis* and *Melipona* had similar Cr contents (M23, M25, M06 and M14; M24, J07 and J13, respectively). One sample of *Apis* (M32) was an outlier.

Figure 2. Principal component analysis of the averages of metal concentration in *Apis mellifera* (■) and *Melipona interrupta* (●) honey. Left: Scores. Right: Loadings.

The final partition of dendrogram was at 98.1% similarity (Figure 1). This meant that eight clusters were formed based on the similarity between the samples. The M01, M19, J07, and J08 grouping contained relatively high metal contents, which were similar to the contents in samples M03, M11, and M09 (Figure 3). The major grouping was at 91.8% similarity (groups II and III, b and c) and this grouping contained 12 sample types. A large number of the “M” samples were around 99.5% similar; and 14 samples among “J” and “M” sample types had 99.1% similarity. These sample types were differentiated from the first group where the similarity between the sample types was only 69.9%. The groups included many samples from the different types of bees and therefore formed a heterogeneous cluster.

Figure 3. Dendrogram of the macro and trace element concentrations in the honey samples. M: *Apis mellifera* honey and J: *Melipona interrupta* honey.

Others sample types grouped into three clusters with 95.2% similarity, Cluster IV (d) included M04 and M14, which had similar K contents, and M15 and M17, which had high K content similarities with M04 and M14. Cluster V (e) contained sample types M13, M06, and M16 because they had similar Na, K, and Mn contents (Table III). Cluster VI (f) contained just one sample type (M32). This sample was separated from the others because it had high macro-element contents (Table III). Clusters V and VI had 98.1% similarity. The similarity between these two clusters was lower than between the previous groups and there was no similarity between Clusters V and VI and the other groups.

Group VII (g) contained M05, M07, M08, and M23, which all had similar K contents (Table III). The Fe content was higher than in the other clusters, which may explain the reduced similarity between this cluster and the others (Table III). This cluster had 89.8% similarity with group VIII (h), which contained M31, M24, and M25. The sample types had similar trace element compositions, but M31 had different Ca, Na, and K contents. Therefore, these elements were the factor that distinguished M31 from the others samples (Table III). In addition, the “J” samples were not grouped into their own clusters by the analysis. Instead, they were distributed among the “M” samples (Figure 3).

Discussion

Since there is no certified and appropriate commercial reference material for honey, the recovery rate was estimated by the use of spiked concentrations of all elements investigated. The percentage of recovery of the analyzed elements was high (ranging from 67% to 113% at a spiking concentration of $500 \mu\text{g g}^{-1}$), indicating that the method is suitable.

It is clear that all the honey samples analyzed in this study had total mineral contents that were similar to those found by Silva *et al.* (2013) in northwestern Pará, Brazil. Silva *et al.* (2013) also found lower amounts of these minerals in *Melipona fasciculata* samples compared to *Apis mellifera* and other species of meliponae in the state of Pará [4].

In general, the macro element levels in the analyzed samples were similar to those found in Brazilian honey [4,19-20], as well as in Spanish multifloral/monofloral honey [1], Portuguese multifloral and monofloral honey [21] and Malaysian honey [22]. The trace elements levels were in agreement with those found in other studies [4,10]. The strong correlation between Ca and Mg content could be related to plant growth and the close relationship between Ca and Mg uptake. Interesting, the relationship between Ca and Mn content is reported to be a potential fingerprint for honeys from different regions [23].

The presence of heavy metal in honeys is related to industrial pollution levels near to the apiaries, which means that honey could potentially be used as a biological pollution indicator [24]. The negative correlation between Co and Al could be a good potential indicator because when Co increases in the honey Al decreases and vice versa. The presence of these heavy metals may be due to intense agricultural activity close to the nectar collecting regions or to contamination of the groundwater absorbed by trees in the nectar collection area [3]. Cadmium levels were not thought to be high in honey [25]. However, in this study, the positive correlation between these elements was significant and represented 40% of the determination coefficient. Furthermore, Cd levels were similar to those found in Polish honey from an industrialized region [26] and were higher than those found in French [27] and Brazilian honey [2]. Nevertheless, no honey sample analyzed in this study had Cd concentrations that were higher than the maximum recommended ($0.1 \mu\text{g g}^{-1}$) by international law [28]. Presence of this trace element may indicate anthropogenic activities or the impact of environmental pollution [29-30].

The presence of higher Fe and Al contents in the samples analyzed in this study could be associated with the predominance of latosols and argisols in the study region, which are rich in these metals [31]. Although the Fe, Mn, and Al concentrations were higher than the other trace metals [32].

The absence of Pb, Cu, and Zn in the analyzed samples corroborated the results obtained by Silva *et al.* (2013) when they analyzed 27 samples from the North region of Brazil [4]. The presence of Cd, Co, Fe, K, Mg, Mn, Pb and Na in higher concentrations may also be associated with substances provided for the nutrition of bees (e.g. industrial syrups) [6,33]. Most of the metals present in honey come from plants that produce soil and nectar. However, the presence of some metals, mainly Cd, Cr, Cu, Fe, Ni, Pb and Zn, may indicate environmental pollution or anthropogenic alteration [6].

All mineral content levels found in the honey samples were lower than the maximum established by Brazilian and international law (Cd and Cr $0.1 \mu\text{g g}^{-1}$, Pb $0.30 \mu\text{g g}^{-1}$, Ni $5 \mu\text{g g}^{-1}$, Cu $10 \mu\text{g g}^{-1}$, Zn $50 \mu\text{g g}^{-1}$) [28].

The M01, M19, J07, and e J08 grouping may be explained by their similar macro element contents (Na, Ca, Ba, Mg, and K) (Table III). The differences between M03, M11, and M09 may be due their similar macro and trace element contents (Tables III and IV). These sample types may lead to similar contamination results because their trace element contents were almost the same. The high similarity between the 32 samples in Groups I and II may be due to the high homogeneity between the macro and trace metal contents in each sample type.

The absence of clusters among the *Melipona interrupta* samples (Figure 2) may indicate that the individual *Melipona interrupta* sample types were highly heterogeneous, which suggests that floral nectar composition was highly variable and that there was a relationship between the “J” samples and

the composition of the *Apis mellifera* samples. The trace-elements in a soil affect honey composition and the macro elements are influenced by the floral origins of the nectar [18]. The high similarity between some “J” and “M” samples may indicate that the soil in the area and the floral nectar collected by the different bee species were similar (Figure 2).

CONCLUSIONS

The results produced by this study showed that the total mineral contents in the analysed honey were similar to those reported by previous studies in the same region and from other Brazilian regions. The most abundant elements in the honey samples were K, Ca, Mg, and Na. The K contents in the *Melipona interrupta* honey samples were generally lower than in the *Apis mellifera* honey. In addition, Cu, Pb, and Zn were not detected in any honey sample analysed.

Potentially toxic trace elements, such as, Cd, Co and Ni were detected in most of the *Apis mellifera* honey samples, and they could be associated with the use of agrochemicals in areas near to the beehives and in the *Apis mellifera* flightpaths.

The Pearson's coefficients showed that Mg, Na, and Mn were highly positively correlated with Ca. In addition, there was a moderate positive correlation between Ni and Cd and a moderate negative correlation between Co and Al.

The PCA of the macro and trace elements showed that the mineral content of the samples changes with respect to geographical origin. However, there is no specific separation between honeys of different species.

The HCA of the macro and trace elements showed that the honey sample types could be aligned into eight groups. However, the *Melipona interrupta* honey samples did not form an isolated group, which may indicate a potentially high variation in floral nectar composition or that the honeys produced by these species may have been altered by the addition of *Apis mellifera* honey.

Agricultural activities are increasing in the Lower Amazonian region, which means that more research should be conducted periodically to determine the concentrations of potentially toxic heavy metals and to monitor environmental contamination in this region.

Conflict of Interest Statement

The authors declare no conflict of interest.

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