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Concentration of macro- and microelements in honey using inductively coupled plasma-optical emission spectroscopy (ICP-OES)

Antonio Francisco de Oliveira[1], Charles Kiefer[1]*, Elaine Silva de Pádua Melo[2], Brenda Farias da Costa Leite Lopes[1], Gabriela Puhl Rodrigues[1], Valter Aragão do Nascimento[2]

[1] Universidade Federal de Mato Grosso do Sul, Faculdade de Medicina Veterinária e Zootecnia, Campo Grande, MS, Brasil.
[2] Universidade Federal de Mato Grosso do Sul, Faculdade de Medicina, Campo Grande, MS, Brasil.

***Corresponding author:**
charles.kiefer@ufms.br

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ABSTRACT - The objective of this work was to evaluate the composition of macro- and microelements in samples of bee honey (*Apis mellifera*) produced in different floral resources: aroeira (*Myracrodruon urundeuva*), cissus (*Cissus rhombifolia*), eucalyptus (*Eucalyptus* spp.), orange (*Citrus sinensis*), and honey of Jataí (*Tetragonisca angustula*). The content of macro- and microminerals in the honey samples were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES). The results showed concentration levels of macro- (Ca, P, K, Mg, and Na) and microelements (Al, As, Cu, Fe, Mn, Ni, and Se) in the samples of honey analyzed. The present study revealed that there were Na concentrations in tested samples analyzed. Cadmium, Co, Cr, and Mo were not detected in any sample. The concentration of heavy metals such as As detected in the honey of *A. mellifera* and *T. angustula* were above the tabulated values. *T. angustula* honey had higher concentration of minerals when compared with *A. mellifera* honey, except for Fe, Mn, and Ni, in which aroeira honey had a higher concentration. Considering the determination of heavy metals, the study reveals that honey produced by bees can be excellent bioindicators of environmental pollution.

Keywords: *Apis mellifera*, bee, Jataí, minerals, *Tetragonisca angustula*

1. Introduction

Inductively coupled plasma-optical emission spectrometry (ICP-OES) is an analytical technique that quantifies elements (metals, semi-metals, and rare earths) in different types of samples and can be used for analyzing honey samples. Honey can be defined as a sugar solution formed by fructose, glucose, and other carbohydrates (Rodríguez et al., 2012), containing amino acids, organic acids, vitamins, minerals, enzymes, flavonoids, pigments, and pollen (Li et al., 2012). The quality of honey depends on its floral origin and chemical composition (Tafere, 2021). Due to the different possibilities of combinations of origin, honey can present great variations in its composition (Braghini et al., 2017) and even slight variations when produced by different species in the same region (Dakshayini et al., 2024).

Native bees play a significant role in pollination due to their foraging behavior with high visitation efficiency (Sajjad et al., 2023). However, bees have been threatened worldwide due to the degradation of natural habitats and the intensification of agriculture (Orford et al., 2015). In addition, global warming resulting from climate change could cause the disappearance of many bee species. The arid climate, for example, is not favorable for *A. mellifera* (Ali et al., 2020), while some native bee species are able to survive extreme conditions (Sajjad et al., 2023), a fact that highlights the importance of native bees in ecosystems.

The chemical composition of honey can vary according to the climate, soil, floral origin, and metallic composition, and the specific compositions of each honey can be used to characterize and distinguish the honey produced in different geographical regions (Uršulin-Trstenjak et al., 2015; Solayman et al., 2016). The constituents of honey can be used to establish criteria for quality control and markers of geographical (Valverde et al., 2022; Pavlin et al., 2023) and botanical (Pavlin et al., 2023) origin. In addition, the honey produced by bees can be used as a bioindicator of environmental pollution in the region (Bastías et al., 2013), considering that it may contain heavy metals, pesticides, and environmental contaminants (Nayik and Nanda, 2015) that can make honey a toxic food (Przybylowski and Wilczynska, 2001). Studies considering the evaluation of the quality of honey from bees based on the determination of metallic elements using atomic absorption spectrometry are limited and could guide the complementation of information from the Regulamento Técnico de Identidade e Qualidade do Mel ("Technical Regulation of Identity and Quality of Honey"; MAPA, 2000). Since this only shows the value for the maximum permitted concentration of minerals (60.0 g kg^{-1}). The concentration of minerals in honey can affect their quality and improve their commercial value (Valverde et al., 2022; Dakshayini et al., 2024). Honey with 0.04 percent mineral content have a lighter color, while those with a concentration of 0.20 percent have a darker color (Bogdanov et al., 2007), and color can influence consumer choice.

Moreover, honey production has been shown to have a positive economic impact. According to Acibuca (2024), honey, along with other animal products, significantly contributes to the agricultural gross domestic product (AGDP), highlighting the economic benefits of enhancing honey production as part of sustainable agricultural strategies.

We also found that there are no studies comparing the composition of macro- and microelements of honey from different flowering areas, as well as a comparison between honey produced by the Africanized bees *Apis mellifera* and native bees *Tetragonisca angustula*, also known as the Jataí or Jataí-Amarela bees, using the ICP-OES technique. Therefore, this study was carried out to evaluate the concentrations of macro- and microelements in honey associated with different blooms produced by *A. mellifera* and *T. angustula*.

2. Material and methods

The experiment was conducted in Campo Grande, MS, Brazil (20°26'37" S latitude and 54°38'52" W longitude). The predominant climate in the region is tropical semi-humid. This type of climate is characterized by hot, rainy summers and dry, cold winters.

2.1. Sample collection

Samples of honey produced by *A. mellifera* from different flowerings: aroeira (*Myracrodruon urundeuva*), cissus (*Cissus rhombifolia*), eucalyptus (*Eucalyptus* spp.), orange (*Citrus sinensis*), and also honey samples produced by Jataí bees (*T. angustula*) were collected in the state of Mato Grosso do Sul, Brazil.

The honey samples produced by *A. mellifera* were collected between September 2017 and February 2018, according to the flowering predominance. The honey samples from the Jataí bees were collected regardless of the bloom. Ten samples of around 50 g of each type of honey were taken after centrifugation and decanting. The honey samples were placed in sterilized glass jars and stored in the dark at room temperature until analysis.

2.2. Acid digestion procedure and quantification of macro- and microelements using ICP OES

A quantity of 1.0 g of each honey sample was weighed separately using an analytical balance with 0.01-g resolution (Marte, model AL 500C, São Paulo) and placed in tubes, and then 2 mL of HNO_3 (65% Merck, Darmstadt, Germany), 1 mL of H_2O_2 peroxide (35%, Merck, Darmstadt, Germany), and 1 mL of ultrapure water (18 MΩcm, Milli-Q Millipore, Bedford, MA, USA) were added. The mixture in each tube underwent a stirring process using a vortex tube shaker (Nova). After stirring, 30 mL of ultrapure water (18 MΩcm, Milli-Q Millipore, Bedford, MA, USA) were added to the tubes.

After preparing the honey samples, the analysis was performed using the ICP-OES technique, model 6000 Duo, with an axial view (Thermo Scientific), considering the instrumental analytical conditions (Table 1).

For the ICP-OES method, multielement stock solutions containing 1,000 mg L^{-1} of Al, As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Se, and Zn were obtained from SpecSol (SpecSol, Quimlab, Brazil), and analytical calibration standards were prepared. For each element detected (Table 2), the quantification limit (QL), detection limit (DL), and correlation coefficient (R^2) were established (Long and Winefordner, 1983). The entire experimental procedure was performed in triplicate.

Table 1 - Instrumental analytical conditions for ICP OES of elemental analyzes for honey samples

Parameter	Setting
Rf power (W)	1,250
Sample flow rate (L min^{-1})	0.45
Plasma gas flow rate (L min^{-1})	12
Integration time (s)	5
Stabilization time (s)	20
Nebulizer pressure (psi)	20
View mode	Axial
Argon gas, 99,999%	Ar
Number of replicates	3

Table 2 - External calibration parameters: elements and element wavelengths, detection (DL) and quantification (QL) limits, adjustment equation, and correlation coefficient (R^2) used in ICP OES

Element	Wavelength (nm)	DL	QL	Value of y	R^2
Al	396,152	0.0006	0.002	33997x + 314	0.999
As	189,042	0.002	0.006	344x – 2.033	0.999
Ca	422,673	0.002	0.008	123742x + 28251	0.999
Cd	228,802	0.0002	0.0008	9325x + 11.425	0.999
Co	238,892	0.003	0.01	3931x + 11.484	0.999
Cr	425,435	0.0008	0.003	24644x + 225.558	0.999
Cu	324,754	0.0003	0.0009	28752x + 177.475	0.999
Fe	259,940	0.002	0.006	5036x + 37.720	0.999
K	769,896	0.2	0.6	173636x + 25276	0.999
Mg	285,213	0.0002	0.0007	8544x + 524.487	0.999
Mn	257,610	0.0002	0.0008	29928x + 18.838	0.997
Mo	202,030	0.001	0.004	1717x + 9.518	0.997
Na	589,592	0.0006	0.002	404769x + 25262	0.999
Ni	221,647	0.005	0.02	2624x + 23.360	0.999
P	185,942	0.02	0.05	108x + 3.348	0.999
Se	196,090	0.003	0.009	265x + 3.631	0.999
Zn	213,856	0.0007	0.002	4990x + 41.654	0.999

2.3. Statistical analyses

The concentrations of macro- and microelements in honey from different blooms produced by *A. mellifera* and *T. angustula* were analyzed using descriptive statistics (mean and standard deviation) using the statistical analysis in Excel, de according to Ribeiro Júnior (2013). The statistical model used to calculate the mean was:

$$\bar{y} = \frac{y_1 + y_2 + ... + y_n}{n} = \frac{\sum_{i=1}^{n} y_i}{n}$$

in which $\bar{y}$ = arithmetic mean of the responses variable, y_i = value of variable i, and n = number of observations.

The statistical model used to calculate the standard deviation was:

$$S_y = \sqrt{S_y^2}$$

in which S_y = average of the absolute deviations of each value y_1, y_2, ..., y_n from $\bar{y}$.

3. Results

The results obtained were limited to macro- (Ca, P, K, Mg, and Na) and microelements (Al, As, Cu, Fe, Mn, Ni, Se, and Zn) in honey (Table 3). From the results obtained, we established a decreasing order for the concentration of the quantified elements in honey. For eucalyptus blossom, it was: Ca> P> Mg> Na> Al> Fe> As> Se> Mn> Zn> Cu; for orange blossom, it was: Ca> P> Na> Mg> Al> Fe> As> Mn> Cu; for cissus blossom, it was: P> Ca> Mg> Na> Fe> Al> Se> As> Mn> Zn> Cu; for aroeira blossom, it was: P> Ca> Mg> Na> Fe> Al> Mn> Se> As> Zn> Ni> Cu; and for *T. angustula* honey, it was Ca> K> P> Mg> Na> Zn> Fe> Al> Se> As> Mn> Ni> Cu.

Table 3 - Concentration of macro- and microelements (mg kg^{-1}) detected in honey from different flowering periods produced by *A. mellifera* and *T. angustula* (Jataí)

Element	Flowering				Jataí
	Eucalyptus	Orange	Cissus	Aroeira	
Macroelements					
Ca	77.75±2.35	59.38±3.41	22.55±1.61	38.00±0.46	125.95±2.33
P	69.40±0.59	55.82±5.23	50.49±0.60	63.34±0.10	99.94±0.12
K	<QL	<QL	<QL	<QL	113.73±17.46
Mg	50.3±0.58	21.49±0.49	14.34±0.61	27.29±0.06	54.80±1.74
Na	41.17±0.21	27.05±2.50	11.67±0.73	17.44±0.13	50.42±1.24
Microelements					
Al	2.34±0.05	2.62±0.15	2.87±0.12	2.65±0.04	2.46±0.09
As	2.05±0.06	1.61±0.12	1.17±0.01	1.37±0.07	1.40±0.10
Fe	2.05±0.06	2.21±0.20	3.22±0.06	3.44±0.05	2.59±0.07
Se	1.66±0.05	<QL	1.29±0.05	1.67±0.11	1.81±0.16
Mn	0.78±0.06	0.58±0.08	0.98±0.01	2.44±0.03	1.17±0.02
Zn	0.73±0.01	<QL	0.32±0.00	0.37±0.01	6.97±0.09
Cu	0.08±0.01	0.14±0.01	0.04±0.00	0.08±0.00	0.15±0.02
Ni	<QL	<QL	<QL	0.31±0.01	0.25±0.02
Cd	<QL	<QL	<QL	<QL	<QL
Co	<QL	<QL	<QL	<QL	<QL
Cr	<QL	<QL	<QL	<QL	<QL
Mo	<QL	<QL	<QL	<QL	<QL

<QL - below quantification limit.

We found that the elements Cd, Co, Cr, and Mo were below the limit of quantification in all evaluated honey samples. Potassium was below the limit of quantification for eucalyptus, orange, cissus, and aroeira flowering, being quantified only in *T. angustula* honey. Nickel was not detected in eucalyptus, orange, and cissus flowerings; on the other hand, it was detected in aroeira flowering and *T. angustula* honey. Se and Zn were not detected in orange flowering, but were detected in eucalyptus, cissus, aroeira, and *T. angustula* honey samples.

4. Discussion

The presence of minerals in honey can originate from both natural sources such as plants and soil, as well as anthropogenic sources like pollutants (Dakshayini et al., 2024), and the concentration can vary depending on the botanical origin, climatic conditions, extraction, and storage techniques (Thakur et al., 2022).

In general, it has been observed that K is the mineral with the highest concentration in most honey samples (Alqarni et al., 2014; Hungerford et al., 2020; Thakur et al., 2022). However, according to the results obtained, K concentrations for honey from the eucalyptus, aroeira, orange, and cissus flowering plants are below the QL. On the other hand, K (113.73±17.46 mg kg^{-1}) was detected in *T. angustula* honey. According to tabulated information, the average K concentration in the honey of *A. mellifera* is 990.0 mg kg^{-1}. However, based on the study carried out by Czipa et al. (2024), it is possible to infer that there may be a large variation in K concentration among the honey samples (87.7 to 1,560.0 mg kg^{-1}) of *A. mellifera*. A similar result was found by Grembecka and Szefer (2013) (166.0 to 736.0 mg kg^{-1}). For example, honey from Spain contains between 639.0 and 1,845.0 mg kg^{-1} of K (Fernández-Torres et al., 2005).

We must consider that the variations in the results are due to the variation between the flora in the different geographic regions (Uršulin-Trstenjak et al., 2015). Considering the effects of the regional flora on the mineral concentration in honey, some researchers infer that minerals can serve as indicators of the geographical origin of honey (Edo et al., 2022).

In our study, the highest concentrations of macrominerals obtained in *A. mellifera* honey were of Ca and P. The Ca values of cissus and aroeira hives are within the range of 27.6 to 72.8 mg kg^{-1} observed by Grembecka and Szefer (2013) in *A. mellifera* honey harvested in Europe. On the other hand, as recommended for this element for *T. angustula* honey, it was superior to that chosen by the referred author. Higher variations in the Ca concentration (111.0 to 257.0 mg kg^{-1}) were observed by Fernández-Torres et al. (2005) in *A. mellifera* honey in Spain.

In our study, all the honeys had P concentrations higher than the 40.0 mg kg^{-1} of P estimated by TACO (2006). Higher variations (63.8 to 143.0 mg kg^{-1}) than those observed in the present study were also found by Fernández-Torres et al. (2005) in *A. mellifera* honey. Even greater variations (35.7 to 696.0 mg kg^{-1}) were found by Grembecka and Szefer (2013) in *A. mellifera* honey.

The Mg concentrations determined in the honey were lower than the value established by TACO (2006), which is 60.0 mg kg^{-1}. In the literature, it is possible to verify that there can be a great variation in the Mg concentration in *A. mellifera* honey, as verified by Grembecka and Szefer (2013), who observed a variation of 5.50 to 49.3 mg kg^{-1}.

According to the results obtained, we can infer that *T. angustula* honey, in addition to being the only one with K concentration, has higher concentrations of minerals when compared with *A. mellifera* honey, except for Fe, Mn, and Ni in that Aroeira honey had a higher concentration. We observed that *T. angustula* honey had a Zn concentration approximately 20 times higher than the average concentration in *A. mellifera* honey. Honey with a higher mineral concentration generally have a darker color and greater medicinal value (Thakur et al., 2022). This shows its medicinal value, since Zn possesses therapeutic benefits in several chronic diseases in humans, such as atherosclerosis, several malignancies, autoimmune diseases, Alzheimer's disease and other neurodegenerative disorders, cancer, diabetes, depression, aging, and Wilson's disease (Chasapis et al., 2020).

There are no maximum limits stipulated by Brazilian legislation for the concentration of most minerals in honey (TACO, 2006). The concentrations of Ni, Cu, and Zn detected for all honey samples are below the limits stipulated by decree 55.791/65 (Brasil, 1965), which determines maximum concentrations of 5.0, 30.0, and 50.0 mg kg^{-1}, respectively. In turn, the quantified Se concentrations are above the maximum concentration of 0.05 mg kg^{-1} stipulated by Decree 55.791/65 (Brasil, 1965).

Higher Fe concentrations were found in the honey from the aroeira and cissus blossoms compared with the other honey samples. In addition, the Fe concentration observed in aroeira and cissus honey samples is higher than the average Fe value established by the food tables (TACO, 2006). Other studies in the literature obtained Fe concentrations of 3.76 mg kg^{-1} (Souza et al., 2014) and high variations (0.8 to 6.7 mg kg^{-1}) were recorded (Grembecka and Szefer, 2013) for *A. mellifera* honey.

Honey may contain toxic elements (e.g., As and Cd), which have effects on human health (Czipa et al., 2024). In our study, Cd was not detected, since it showed values below the QL in all the honey samples evaluated. However, the As concentration obtained in all flowering honey samples and for *T. angustula* honey is above the 1.0 mg kg^{-1} stipulated by Decree 55.871/65 (Brasil, 1965) and normative instruction 14/2009 (MAPA, 2009). The values are also above the maximum permitted limits for inorganic contaminants for honey (0.3 mg kg^{-1}) stipulated by the Ministry of Health, according to resolution nº 42, of August 29, 2013 (ANVISA, 2013).

Arsenic has high absorption levels, depending on the solubility of the compound and the amount ingested, dose, frequency, and time of absorption. It is excreted by the liver and kidneys, acting in the body as an inhibitor of cellular respiration (Barra et al., 2000). Arsenic can cause environmental damage and comes from sources such as industrial (smelting, mining, electricity generation from coal, etc.) and agricultural activities (herbicides, insecticides, algicides, desiccants, wood preservatives, growth stimulants for plants and animals, etc.). In this way, all organisms are subject to direct contamination, or contamination of food.

Other great sources of As are coal furnaces, since the As present in coal becomes a pollutant of the air and a contaminant for food and water stored close to these environments (Baird and Cann, 2011). The content of heavy metals in *A. mellifera* honey can be considered as an indicator of natural or anthropogenic pollution since the concentration of heavy metals in bee samples reproduces the metal profile of the entire region visited by bees. Therefore, honey has been proposed as a viable bioindicator for environmental contamination by heavy metals in different regions. In a study carried out with honey samples collected in volcanic eruption regions in Chile demonstrated that pollution in honey can originate from natural and anthropogenic sources (Bastías et al., 2013).

The Al concentration obtained in all flowering honeys and in *T. angustula* honey are below the value of 3.43 mg kg^{-1} observed by Souza et al. (2014). Aluminum in plants reflects content in soil and water, and certain plants absorb more Al than others (Pennington, 1988). In particular, plants that grow in acidic soil can accumulate higher amounts of Al. It can be found in processed foods through food additives, as well as through the migration of contact materials with foods that contain Al (BMG, 2014). An interesting fact is that Al is listed as a heavy metal according to the definition of WHO (2011). However, we have not found an established limit for the concentration of this metal in our honey samples.

5. Conclusions

Some elements such as Mg (in *T. angustula*), Mn (in eucalyptus flowering), P (in all samples), and As (in all samples) are above the tabulated values. There were Na concentrations in all samples analyzed. The elements Cd, Co, Cr, and Mo were not detected in the samples. Nickel was not detected in the flowering of eucalyptus, orange, and cissus, but it was detected in the honey of the flowering of aroeira and *T. angustula*. The honey of *A. mellifera* and *T. angustula* bees can be used as an excellent bioindicator of environmental pollution in the evaluation of the degree of contamination of that environment or contamination of the hive. The results presented can be used to complement the Brazilian food table in relation to the missing elements in it.

Data availability

All data generated or analyzed during this study are included in this published article.

Author contributions

Conceptualization: Oliveira, A. F.; Kiefer, C. and Nascimento, V. A. **Formal analysis:** Oliveira, A. F.; Kiefer, C.; Melo, E. S. P. and Nascimento, V. A. **Investigation:** Oliveira, A. F.; Kiefer, C. and Nascimento, V. A. **Methodology:** Oliveira, A. F.; Kiefer, C.; Melo, E. S. P. and Nascimento, V. A. **Resources:** Oliveira, A. F.; Kiefer, C.; Melo, E. S. P. and Rodrigues, G. P. **Supervision:** Kiefer, C. and Nascimento, V. A. **Validation:** Kiefer, C.; Melo, E. S. P.; Rodrigues, G. P. and Nascimento, V. A. **Visualization:** Kiefer, C.; Lopes, B. F. C. L. and Rodrigues, G. P. **Writing – original draft:** Oliveira, A. F.; Kiefer, C.; Lopes, B. F. C. L.; Rodrigues, G. P. and Nascimento, V. A. **Writing – review & editing:** Kiefer, C. and Lopes, B. F. C. L.

Conflict of interest

The authors declare no conflict of interest.

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