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Determining the Levels of Trace Elements Cd, Cu, Pb and Zn in Honey of Stingless Bee (Hymenoptera: Apidae) Using Voltammetry

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Abstract

Determining the levels of heavy metals in honey is a measure to control its quality. The objective of this study was to determine the contents of Cd, Cu, Pb and Zn in honey of stingless bee. The differential pulse anodic stripping voltammetry (DPASV) was used to determine the trace elements in honey samples. Pb was detected in 100% of the samples, Cu and Zn in 98.15%, and Cd only in 33.33%. The trace elements analyzed detected in the samples remained within tolerable levels in foods for human consumption.

Keywords

Heavy Metals, Stingless Bees, Voltammetry, Honey

1. Introduction

There is a growing concern with food-related risks to human health caused by toxic contaminants. The anthropogenic impact on the environment, mainly air pollution, is a major concern worldwide. The inhalation of particulate matter and the consumption of contaminated food and water are the most common routes of direct and in-

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direct contamination for humans [1].

Many heavy metals have cumulative properties [2] that are particularly worrisome for children, as children can ingest relatively higher amounts of metals than adults, considering the ratio amount/body weight [3].

Heavy metals such as Cd, Pb, Cu, Cr, Mn, Hg and Zn are the most studied due to their effects on human health [4]. Al, Cd, Co, Cu, Pb, Sr, and Zn are the main trace elements that contaminate bee honey [5].

These contaminants in bee honey can occur because of geographic and botanical origin, as well as anthropogenic factors around the colonies [6]. Trace elements in the atmosphere can deposit on the body hair of bees and they are transported to the colonies with pollen or they can still be absorbed with the flower nectar or transported by water or honeydew [7].

Honey products that contain trace elements at levels above limits established by the legislation pose a threat to human health due to their negative and cumulative effects in the body. Scientific studies have shown the negative effects of several heavy metals on human health [8].

In search of nectar, honeydew, pollen, and plant exudates within their radius of action, bees are exposed to plants, water, air and soil. At sites where environmental contamination exists, bees also contaminate themselves and transport pollutants from the surroundings to the colonies or collect contaminated raw material [7] [9]. Therefore, bees contaminate honey products affecting their composition and compromising their quality.

It is important to measure the levels of heavy metals in honey as a method to ensure product quality. For that propose, voltammetry, the electrochemical technique where the qualitative and quantitative data of a chemical substance are obtained from records of current-potential curves, can be used. This technique is less costly when compared with other methods available. Thus, the objective of this study was to determine the levels of Cd, Cu, Pb and Zn in honey of Meliponinae using voltammetry.

2. Material and Methods

The study site was the municipality of Guaraqueçaba, Paraná State, Brazil (25˚17'15"S; 48˚19'1"W, altitude 20 m) where beehives were already structured. Apiculture in Paraná State is an activity performed by small producers, and this region shows great potential for apiculture due to the local flora species and the adaptation of stingless bees.

Samples, consisting of 50 mL of honey, were obtained directly from local producers during the peak honey production in the region. We collected 30 samples of species of stingless bees: *Cephalotrigona capitata* (n = 2), *Melipona bicolor* (n = 4), *Melipona marginata* (n = 3), *Melipona mondury* (n = 3), *Melipona quadrifasciata* (n = 4), *Melipona scutellaris* (n = 4), *Melipona seminigra* (n = 3), *Scaptotrigona xanthotricha* (n = 3) and *Tetragonisca angustula* (n = 4).

The procedure of sample digestion was carried out at the Laboratory of Plant Tissues at the Soil Science Department of College of Agriculture "Luiz de Queiroz", University of Sao Paulo, Piracicaba, Sao Paulo State, Brazil, following the methodology of Krug [10].

We weighed approximately 2 g of each sample in digestion tubes (50 mL) and added 5 mL of concentrated nitric acid 65% (HNO_3, Sigma-Aldrich®). The mixture was kept at rest for 24 h to facilitate the decomposition of organic compounds. Subsequently, the tubes were placed in the digestion block and heated up to 160˚C for 30 min. Next, the samples were left to cool and 2 mL of nitric acid and 1.5 mL of concentrated perchloric acid ($HClO_4$, Sigma-Aldrich®) were added following this sequence, because otherwise there is a risk of explosion. Afterwards, the balloons were returned to the digestion block and remained there for approximately 1:30 min, observing the whitening of the liquid. Next, the balloons were kept on the plate until the total evaporation of gases. At the end of the procedure, a small blade of the balloon sample was transferred to the 25 mL flask, and ultra-pure water was added (18.2 Mohm.cm) at 25˚C to complete the flask volume. Subsequently, the samples were transferred to sterilized 50 mL Falcon Tubes and stored in a refrigerated chamber.

All glassware used was placed in HNO_3 at 10% for 24 h for decontamination. Prior to use, all material was rinsed in ultra-pure water. We used a standard solution (white solution) containing only the acids, which were subjected to the same digestion procedures used in the honey samples.

To determine trace elements in the honey samples, the Differential Pulse Anodic Stripping Voltammetry (DPASV) technique was used.

A voltammetric analyzer 767 VA Computrace Metrohm© was used to conduct electrochemical measures. The analyzer was connected to a computer with the software program 767 VA Computrace 1.3.1 version Metrohm®

installed to register the measurements. The calibration was made by adding a pattern to every sample analyzed, following the parameters and conditions of the analyses (**Table 1**).

The assumptions were tested in the analysis of variance by means of the optimal transformation family of Box-Cox [11] and the Hartley test [12] was used to verify the homogeneity of variances. The experimental design was completely randomized and the treatments consisted of honey samples of species *C. capitata*, *M. bicolor*, *M. marginata*, *M. mondury*, *M. quadrifasciata*, *M. scutellaris*, *M. seminigra*, *S. xanthotricha* and *T. angustula* that were analyzed separately, that is, only one factor.

After verifying the assumptions of ANOVA, the F-test ($p < 0.05$) was used to verify possible differences between treatments. The Tukey test was used ($p < 0.05$) for the variables that showed difference. The data were analyzed using the computer program SAS/STAT [13].

3. Results and Discussion

The trace elements identified in the samples remained within the acceptable levels in foods for human consumption according to the Brazilian legislation (**Table 2**). Pb was detected in 100% of the samples, Cu and Zn in 98.15% and Cd only in 33.33%.

Table 1. Parameters and conditions used in the voltammetric cell to determine the levels of Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+}.

Voltammetric parameters	Unit	Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+}
Volume of diluted sample	mL	1
Volume of ultra-pure water	mL	10
Electrolytes		
KCl (1.5 mol·L^{-1}) and $C_2H_3NaO_2$ (0.5 mol·L^{-1})	mL	2
Electrode of work		HMDE
Number of drops 4		4
Number of additions 2		2
Number of replications		3
Electrode of reference		Ag/AgCl (KCl 3 mol·L^{-1})
Auxiliary electrode		Platinum
Speed of shaking	rpm	2000
Purge time (ultra-pure N_2)	s	300
Additional purge time	s	10
Deposition potential	V	1.15
Deposition time	s	90
Equilibrium time	s	10
Pulse range	mV	0.05
Initial potential	V	−1.15
Final potential	mV	50
Tension degree	mV	6
Time of tension degree	s	0.1
Scanning rate	V/s	0.06
Zn^{2+}	V	0.98
Cd^{2+}	V	0.61
Pb^{2+}	V	0.38
Cu^{2+}	V	0.16

Table 2. Comparison between the average values of Cd (μg·kg^{-1}) and Cu, Pb and Zn (mg·kg^{-1}) in honey samples of stingless bees.

Species	Trace elements			
	Cd	**Cu**	**Pb**	**Zn**
Cephalotrigona capitata	3.490 a	0.125 b	1.195 ab	3.705 a
Melipona bicolor	0.000 a	0.305 b	1.210 ab	7.185 a
Melipona marginata	3.223 a	0.176 b	1.186 ab	4.833 a
Melipona mondury	0.000 a	0.343 b	1.333 a	6.476 a
Melipona quadrifasciata	0.000 a	0.392 b	1.215 ab	4.885 a
Melipona scutellaris	6.533 a	0.377 b	1.100 b	6.030 a
Melipona seminigra	2.043 a	0.243 b	1.220 ab	7.430 a
Scaptotrigona xanthotricha	3.307 a	0.395 b	1.076 b	3.677 a
Tetragonisca angustula	6.165 a	1.065 a	1.080 b	6.340 a

Means followed by the same letter in the rows do not differ statistically ($p > 0.05$).

The levels of Cu and Pb showed a statistical difference in the honey samples. Cd and Zn did not differ statistically (**Table 2**).

Cd had low occurrence in the honey samples and when detected, it showed levels within the established limits by the World Health Organization (WHO). Cd is not found in its pure state in nature. It is mainly associated with sulfides in ores of Cu, Pb and Zn, and it is a relatively rare element. Because Cd has several physical and chemical properties similar to those of Zn, the two metals usually occur together in nature, found in traces, 2 - 3 mg·kg^{-1}, of extracted Zn ore [14].

For Cd, the WHO established an intake of 7 μg·kg^{-1} of body weight/week as acceptable limit, for adults as well as babies and children [15]. The Brazilian legislation establishes a maximum limit of 1.0 mg·kg^{-1} of Cd consumption [16]. Cu levels in the honey samples remained within the acceptable limits established ANVISA (Brazilian Agency for Health Surveillance), Ordinance No. 685, August 27, 1998, which prescribes the maximum residue limits for inorganic contaminants in foods and establishes up to 1 mg·kg^{-1} for Cu in honey.

The honey samples showed Zn levels within the acceptable limits, since the recommended daily consumption is 7.5 mg·kg^{-1} for children and 13.5 mg·kg^{-1} for adults [17].

For the WHO, the acceptable limits of Pb consumption is 50 μg·kg^{-1} body weight/week for adults and 25 μg·kg^{-1} body weight/week for children [18].

The average levels of Pb in the samples ranged from 1.040 to 1.333 mg·kg^{-1}. The Brazilian Ministry of Health reassessed the acceptable levels for Pb in food and decreased the acceptable levels from 8.0 mg·kg^{-1} to 0.8 mg·kg^{-1} for most foods, however, there is no specific reference for honey [19].

Araújo [20] studied honey of *Apis mellifera* and found that Pb was the element that showed, on average, the highest levels, about 2.11 mg·kg^{-1}. Followed by the Zn, with an average of 1.56 mg·kg^{-1} and Cu with average values of 0.35 mg·kg^{-1}. Cd was not found in any sample analyzed. Silveira *et al*. [21] studied honey pollen in the same area studied by Araújo [20] and found higher levels of these elements, compared to levels found for honey, with the following average values of 540.7 mg·kg^{-1}, Pb (28.7 mg·kg^{-1}), Cu (56.55 mg·kg^{-1}) and Cd (280.75 mg·kg^{-1}).

The levels of heavy metals in honey are typically very low and even an intake of 100 g daily would not significantly contribute to dietary requirements. However, if there is an increase of one or more trace elements in the environment and if the levels of these elements increase in honey, the product quality can be significantly compromised [22].

Bilandžić *et al*. [22] verified that in multifloral honeys, Pb and Cu levels were smaller than in monofloral honeys obtained in the same geographic region. Consequently, these results indicate a difference in the levels of trace elements in honeys of different botanical origin, even if it is produced in the same geographic region. The results indicate the need to monitor the levels of these trace elements in honey samples and to study the possible sources of contamination to ensure product quality.

In addition to the environmental importance, determining trace elements is essential for the quality control of honey, given that total honey production worldwide is on the rise. In the last decade, new levels of trace elements were established for different types of honey in European countries as reported by [23]-[31].

4. Conclusion

The levels of trace elements (Cd, Cu, Pb and Zn) analyzed in the honey samples of stingless bees in this study remained within the acceptable levels for human health.

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