8. A study of Hg and Pb content in pot-honey (Apidae: Meliponini)

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Abstract

Stingless bee or pot-honey has been previously studied to ascertain composition, bioactivity and sensory attributes. In this study, the honey produced by six meliponines in Argentina (*Tetragonisca fiebrigi*), Australia (*Tetragonula carbonaria*, *Austroplebeia simei*), Brazil (*Melipona manaosensis, Melipona seminigra pernigra*) and Venezuela (*Melipona favosa*), was evaluated with spectroscopic techniques for lead and mercury content. Lead values varied between 109.75 and 332.33 µg/kg, and mercury between 12.96 and 145.21 µg/kg. This is similar to honey of *Apis mellifera*.

Keywords:
Argentina, Australia, Brazil, ET-AAS, CV-AAS, pot-honey, lead, Meliponini, mercury, Venezuela

Introduction

Meliponine honey is produced and stored in pots, not in combs like *Apis*. This honey was widely relished before Columbus (Schwarz, 1948) and medicinal properties are attributed to it (Vit et al., 2004). Stingless bees (Hymenoptera, Apidae, Meliponini) show their greatest biodiversity in the Neotropics, with over 400 species (Camargo and Pedro, 2007). Besides the classical physicochemical standards of quality for stingless bee honey, reviewed by Souza et al. (2006), trace elements are also of interest in honey qualiy.

The sources of bee product contamination vary according to the residue. Lead content in honey has been previously studied an indicator of environmental contamination derived from urban traffic, and suggested as a bioindicator of environmental pollution (Conti and Botré, 2001). Mercury comes from industrially contaminated areas based on processing (Toporcák et al., 1992).

The objective of this work was to evaluate the content of mercury and lead in honey produced by six species of stingless bees, by cold vapor generation atomic absorption spectroscopy (CV-AAS) and Electrothermal Atomic Absorption Spectrometry (ET-AAS), respectively.

8.1 Mercury and lead in pot-honey

8.1.1 Stingless bee species

In Table 1 the entomological origin of the honey is given.

Table 1: Entomological and geographical origin of honey samples

Nr.	Stingless bee species (country of origin)
1	*Melipona favosa* (Fabricius, 1789) (Venezuela)
2	*Tetragonisca fiebrigi* (Schwarz, 1938) (Argentina)
3	*Melipona manaosensis* Schwarz, 1932 (Brazil)
4	*Melipona seminigra pernigra* Moure & Kerr, 1950 (Brazil)
5	*Tetragonula carbonaria* (Smith, 1854) (Australia)
6	*Austroplebeia symei* (Rayment, 1932) (Australia)

8.1.2 Honey samples

Honey collected by syringe extraction from colonies of stingless bees in Argentina, Australia, Brazil and Venezuela was frozen in plastic flasks until analysis.

8.1.3 Mineralization

Honey mineralization was performed in triplicate (0.5 g honey digested with 1 ml ultrapure nitric acid and 0.5 ml hydrogen peroxide during 30 sec) in a conventional microwave oven (NN-H264SF INVERTER, Panasonic, Spain). The mineralized honey samples were diluted to a final volume of 25 ml with deionized water. All reagents were analytic grade, purchased from Merck.

8.1.4 Aqueous calibration and standard addition for Hg

Mercury was measured with a flow injection cold vapor generation atomic absorption spectrometry FI-CV-FAAS system, Perkin-Elmer model 3100, coupled to a personal computer and Gem Desktop Gem/3 software (Germany). A stock solution of 1000 µg/L metallic mercury was used to prepare 50 mL of 0-5-10-20-30 µg/L Hg solutions for calibration, to establish limits of detection and quantification in the working interval. A standard addition curve was made up by adding 200 µL of the diluted mineralized honey to each concentration of the aqueous calibration standard (0-30 µg/L Hg). Aqueous calibration graphs and standard addition curves were obtained for mercury.

8.1.5 Aqueous calibration and standard addition curves for Pb

Lead was determined in the honey solution by ETA-AAS with a Perkin-Elmer, model 4100ZL, coupled to a personal computer and PE- 4100ZL, Gem Desktop Gem/3, version PC 3.7 software (Germany). A stock solution of 1000 ppb lead was used to prepare 50 mL of 0-5-10-20-30-40 ppb dilutions for the calibration curve, to establish limits of detection and quantification in the working interval. A standard addition curve was derived by adding 200 µL of the dilute mineralized honey to each concentration of the aqueous calibration standard (0-40 ppb Pb). An aqueous calibration graph and standard addition method for lead were thus obtained.

8.1.6 Accuracy

The recovery study of each solution was performed to determine accuracy of the method. The absorbance of the honey sample and the stock Hg and Pb were measured. Percentage recovery values were within the recommended international standards (100 ± 5 %).

8.1.7 Analytical characteristics to validate Hg determination

In Table 2 analytical characteristics are given for the FI-CV-FAAS system to measure Hg in honey

Table 2. Operating conditions of the FI-CV-FAAS system

Characteristics	Values
Linear regression equation	Abs = 0.0008 + 0.0056[Hg]
Coefficient of determination	0.9992
Sensitivity check	0.0060 µg/kg
Limit of detection (LOD)	1.06 µg/kg
Limit of quantification (LOQ)	1.98 µg/kg
Working interval (LOQ – LOL)	1.98 - 25 µg/kg
Relative standard deviation (RSD)	3 %

8.1.8 Analytical characteristics to validate Pb determination

In Table 3 analytical characteristics are given for the ET-AAS system to measure Pb in honey.

Table 3. Operating conditions of the ETA-AAS system

Characteristics	Values
Linear regression equation	Abs = 6.70E-04+ 4.54E-04 [Pb]
Coefficient of determination	0.9994
Sensitivity check	4.54E-04 µg/kg
Limit of detection (LOD)	0.0017 µg/kg
Limit of quantification (LOQ)	0.0058 µg/kg
Working interval (LOQ – LOL)	0.0058-100 µg/kg
Relative standard deviation (RSD)	4.68 %

8.1.9 Analytical results for mercury and lead content

In Table 4 we show that mercury content in honey varied between 12.96 and 145.21 μg/kg. Lead content was higher and varied between 109.75 and 332.33 μg/kg. There is no specific legislation on honey's mineral content. If we compare our results to the maximum fixed in the legislation for other types of food (WHO, 1993), the values for honey are low.

Table 4. Hg and Pb content of honey

Nr.	Hg (μg/kg)	Pb (μg/kg)
1	104.42 ± 0.001	212.84 ± 0.001
2	84.12 ± 0.001	115.17 ± 0.001
3	145.21 ± 0.001	171.59 ± 0.001
4	131.46 ± 0.002	109.75 ± 0.000
5	142.39 ± 0.001	332.33 ± 0.000
6	12.96 ± 0.001	167.04 ± 0.001

[1] See stingless bee species in Table 1.

8.2 Interpretation of mercury and lead values

A lower heavy metal content of honey, compared to the bee itself (Leita et al., 2006), possibly indicates that Pb is trapped in bee tissues during the physiological process of making honey. In a review on contaminants of bee products 0.001 to 1.8 mg Pb/kg honey is reported, but current use of lead-free fuels may diminish such levels (Bogdanov, 2006). Since 1977, honey with a lead concentration above 215 μg/kg cannot be sold in Germany (Otto and Jekat, 1977).

Lead content (μg Pb/kg honey) was studied in several regions of Italy: Emilia-Romagna (150 μg) (Delbono et al., 1999), Emilia-Romagna and Lombardy (37 μg) (Sangiorgi and Ferretti, 1996), Liguria (75 μg), Siena County, Tuscany (76 μg) (Pisani et al., 2008), Turin County, Piedmont (65 μg) (Abete and Voghera, 1999), Tuscany (172 μg) (Pinzauti et al., 1989), Veneto (230 μg) (Oddi and Bertani, 1987). However, in some contaminated areas, honey reaches 2,370 μg (D'Ambrosio and Marchesini, 1982).

There are few specific residue limits for honey (Piro and Mutinelli, 2003), making it difficult to discuss the toxicological importance of residues. A daily intake of 20 g honey is unimportant, according to the Provisional Tolerable Weekly Intakes (PTWIs) fixed for Hg and Pb (WHO, 1993). However, other sources of these metals could be present in the diet.

Frias et al. (2008) found 31.50 mg Pb/kg honey, and 46.32 mg Cd/kg honey from Tenerife. Roman et al. (2011) found lower levels of Pb in honey (0.98 mg/kg) than in propolis (5.74 mg/kg) from Poland, in agreement with Chudzinska and Baralkiewicz (2010) who also found Pb values lower than 1 mg/kg, with higher contents in rape honey. Similar values for these elements occur in honeydew honey from Spain, Soria Province (Nozal Nalda et al., 2005).

In Turkish honey the highest Pb level exists in cotton honey, 1.29 mg/kg (Sahinler Nuray et al., 2009). In honey from Slovakia, Pb is lower than 0.001 mg/kg, below their detectable limit (Kacainova et al., 2009). The level of metals were below the tolerable amount prescribed by the Czech Bylaw (298/1997) Pb - 8000 μg/kg and Hg - 500 μg/kg. The concentrations of Pb and Hg varied in individual groups of honey. Nevertheless, it is important to know that, the concentration of lead, is related to the pollution of the environment (Bohacenko et al., 1994), as well as the concentration of mercury in honey also depends on the contamination of the environment by this element (Toporcák et al., 1992).

The values obtained showed that the concentration levels of Pb and Hg were below of those reported in the literature for contaminated honey, according to the maximum concentration of pollutants in honey with toxicology limits of the World Health Organization (FAO/WHO, 2010) with a maximum of 280 μg Hg and 1,750 μg Pb. The Australian species present higher values of Hg, This may be related to the area where this species produces its honey.

Mercury and lead are non biological metals on humans and animals. Their ions are toxic and bind vital places: Pb^{2+} in much the same places as Ca^{2+} and Zn^{2+}, mercury is held strongly in S–Hg–CH_3 and CH_3Hg^+ compounds are lipid-soluble (Fraústo da Silva and Williams,1991). Excess of Pb causes injuries to the peripheral nervous system and Hg damages the central nervous system. N

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