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Toxic Metals in the Crude Propolis and Its Transfer Rate to the Ethanolic Extract

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Abstract

Concentrations of six toxic metals (Ni, Cr, Hg, Cd, Pb and Sn) in 106 samples of Brazilian crude propolis and the transfer rate of these contaminants to ethanolic extract of propolis were evaluated by atomic absorption spectrophotometry. The results show the presence of all the analyzed metals in the samples of crude propolis of São Paulo and Minas Gerais States. Regarding the transfer of these metals to ethanolic extract of propolis, a significant reduction was observed for all metals analyzed. The crude propolis can be considered as an indicator of toxic metals in the environment and the reduction observed in the ethanolic extract of propolis makes the product safe for consumption.

Introduction

Crude propolis (CP) is a resinous material harvesting by the bees surrounding the hives, with important protecting role for a bee colony. It has a strong and characteristic aroma, representing a complex set of substances (55% resins and balsams, 30% waxes, 10% volatile oils and about 5% pollen) and mechanical impurities (Banskota et al., 2001; Sonmez et al., 2005), that depends on the geographic region, botanical source and bee specie (Finger et al., 2014). This bee product has used since ancient time, due the several biological and therapeutic properties, being widely used as antibacterial, antiviral, antitumor, antifungal, antioxidant, immunomodulatory, presenting many other biological activities (Orsi et al., 2006; Sforcin, 2007; Kunimasa et al., 2011; Canale et al., 2017).

Among the various substances present in its composition, the largest groups of isolated compounds are that of flavonoids, being also aromatic aldehydes, phenolic acids, organic acids, vitamins, amino acids, minerals, among others (Banskota et al., 2001; Falcão et al., 2010; Huang et al., 2014). In addition, the CP has a high content of minerals, such as aluminum, vanadium, iron, calcium, silicon, manganese, among others (Marcucci et al., 2001).

As a natural product, the CP can be subject to the presence of toxic elements in its composition, being directly associated with the environmental pollution of the anthropic origin around the apiaries (Gong et al., 2012; Bonvehí & Bermejo, 2013). The contamination of the environment by toxic metals is a constant concern of the society, arising from industrialization and urbanization (Arslan & Arikan, 2013). CP collected from hives installed in apiaries near contaminated areas may present potentially toxic metals in their composition. Finger et al. (2014) found high levels of cadmium, chromium and lead in crude propolis from different regions of Paraná State - Brazil, allowing the identification of specific areas with environmental contamination. CP can be considered an indicator of environmental contamination,

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as observed by several authors that investigated the Serbian, Spanish, Macedonia, Italian, among others propolis (Bonvehí & Bermejo, 2013; Popov et al., 2017; Tosic et al., 2017).

However, data about the transfer rate of the metals toxics presents in the CP to ethanolic extract of propolis (EEP) is scarce in scientific literature. Depending on the toxic metals concentration on the EEP, the biological activity of the extract can be affected (Bonvehí & Bermejo, 2013). The principal form of consumption of the propolis occurs in the form of extract and derived products (Canale et al., 2017), and knowing the safety of this product for consumption is important.

Thus, the goal of this study was to evaluate the presence of six toxic metals (Ni, Cr, Hg, Cd, Pb and Sn) in 106 samples of Brazilian crude propolis (CP) and the transfer rate of these contaminants to the ethanolic extract of propolis (EEP).

Materials and Methods

Sample Collection

Samples of CP were collected in apiaries located in Rio Grande do Norte (RN) (N = 3), Pernambuco (PE) (N = 1), Ceará (CE) (N = 1), Goiás (GO) (N = 4), Tocantins (TO) (N = 1), Rio Grande do Sul (RS) (N = 9), Mato Grosso (MT) (N = 3), Minas Gerais (MG) (N = 7), São Paulo (SP) (N = 50), Paraná (PR) (N = 15), and Santa Catarina (SC) (N = 12) States, totaling 106 samples from 79 cities (Fig 1). The harvested propolis kept in a freezer until analysis. Samples were provided by beekeepers.

Preparation and analysis of potentially toxic metals in crude propolis and alcoholic extract

Each CP samples was crushed and homogenized with amortar and pistil. Then, 1 gram was separated and homogenized again. Subsequently, 100 mg of each sample was placed in “Pyrex 50 mL” test tubes and then nitric acid and perchloric acid were added in a ratio of 9: 1.

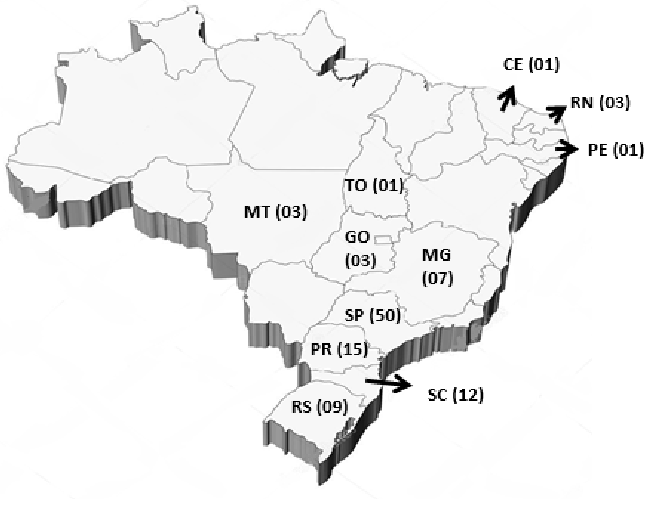

Fig 1. Samples number of CP collected in the Brazilian States. Rio Grande do Norte (RN), Pernambuco (PE), Ceará (CE), Goiás (GO), Tocantins (TO), Rio Grande do Sul (RS), Mato Grosso (MT), Minas Gerais (MG), São Paulo (SP), Paraná (PR) and Santa Catarina (SC) State (From https://pt.depositphotos.com/8347343/stock-photo-map-of- brazil-with-states.html.).

For the EEP preparation, 30 g of the CP homogenized was diluted in 100 mL of the ethanol 70% (Orsi et al., 2000). The solutions remained under the light, under frequent agitation, for seven days. After this period, the solutions were gravity filtered with a commercial 40 mesh paper filter. Then, 1 mL of each EEP sample was homogenized and placed in “Pyrex 50 mL” test tube, and nitric acid and perchloric acid were added in a ratio of 9: 1.

The CP and EEP samples (residue was not analyzed) were then placed in “Tecnal model TE-040/25” thermostatic digestion block exhaust hood and heated at 250 ºC for two and a half hours for digestion and elimination of organic matter. After this period, the samples were resuspended in distilled water, the volume was completed to 25 mL.

The qualitative and quantitative analyses were carried out with an atomic absorption spectrophotometry, according to the methodology described by Sarruge and Haag (1974), with equipment of the brand “Varian”, model “Spectro 12/1475”. The calibration, for the spectrophotometric analyses, was performed with standard solutions, according to each metal analyzed. The detection limits, in mgKg^{-1} of Pb, Ni, Hg, Cr, Cd and Sn were 0.025; 0.005; 0.01; 0.004; 0.002; 0.03 respectively. The samples analyses were performed in duplicate.

For the calculation of the concentrationof metals found in the CP and EEP, the following formula was used:

Metal concentration = [(reading metal - blank reading) × Vol]/ Sample (100 mg CP or 1 mL EEP)

Statistical analysis

For the quantitative variables observed in the samples, the results were compared by analysis of variance (ANOVA) with Kruskal-Wallis test for comparing means at the 5% level of significance (Zar, 1996).

Results

How the honeybees collect the vegetal resin to produce the propolis (CP), this product may present contamination coming from the environmental, as toxic metals. In this work, we observed that the CP produced in different regions of Brazil shows toxic metals depending on the collect place (Table 1).

Lead content in crude propolis ranged from 0.70 ± 0.8 (Minas Gerais State) to 6.88 ± 8.8 mgkg^{-1} (São Paulo State), and after processing ranged from 0.10 ± 0.0 to 1.93 ± 1.1 mgL^{-1}, with reduction average of 91.90%.

The nickel content in crude propolis ranged from 0.10 ± 0.0 (Paraná and Rio Grande do Sul States) to 42.50 ± 0.0 mgkg^{-1} (Tocantins State). After the processing, the EEP nickel presence do not observed.

The mercury content in crude propolis ranged from 0.10 ± 0.0 (Tocantins State) to 1.20 ± 0.3 mgkg^{-1} (Rio Grande do Norte State); after processing ranged from 0.30 ± 0.0 to 0.50 ± 0.1 mgL^{-1}, with reduction this element from 48.28% to 100.00%.

The chromium content in crude propolis ranged from 0.10 ± 0.0 (Paraná State) to 20.25 ± 3.6 mgkg^{-1} (São Paulo State), however, after the processing this element was found in the EEP only in the samples of São Paulo State at 0.15 ± 0.1 mgL^{-1}.

The cadmium content in crude propolis ranged from 0.24 ± 0.1 (Paraná State) to 0.92 ± 0.3 mgkg^{-1} (Rio Grande do Sul State), and after processing ranged from 0.13 ± 0.1 to 0.20 ± 0.0 mgL^{1}.

Tin content in crude propolis ranged from 1.80 ± 0.0 (Ceará State) to 52.60 ± 10.7 mgkg^{-1} (São Paulo State), and after processing ranged from 0.10 ± 0.0 to 3.68 ± 4.3 mgL^{-1}, the percentage of reduction ranged from 71.81 to 100%.

Discussion

Evaluating the Brazilian states, we can observe that the all toxic metals analyzed were found in São Paulo and Minas Gerais, showing strong correlation with the industrialization of the southeastern region of the country. According to Finger et al. (2014), some potentially toxic metals are found in waste from chemical and electronic industries, especially the burning of fossilfuels such as petroleum. The increased presence of metals in CP may be indicative of contaminated environment, how verified by other authors (Bonvehí & Bermejo, 2013; Popov et al., 2017; Tosic et al., 2017). The incorporation of toxic metals into propolis may also be associated with several factors, such as the industrial activity, with the liberation of particles that can remain suspended in the air; the indiscriminate use of fertilizers and the practice of irrigation with contaminated water (Alloway, 1990; Liu et al., 2009; Sawidis et al., 2011). However, even areas of less anthropogenic contact and industrial activies are also subject to contamination, due to the transportation of metals, mainly through atmospheric (Steinnes et al., 1997), as observed for other states evaluated, at where the toxic metal contents varied (Table 1).

The deposition of these potentially toxic metals in propolis can occur either by gravity or by precipitation. Thus, water droplets would transport the particles to the impact areas, which would be areas where *Apis mellifera* L. bees collect plant resins for propolis. However, there is a possibility of transport of these molecules to waterways, after primary transport by rainfall (Gibbs, 1973). The movement of these pollutants can occur due to atmospheric circulation of particles, and soil deposition may occur in areas far from the pollutant source sites (Onder & Dursun, 2006). Thus, the deposition forms in the plants or soil can be natural, by atmospheric, precipitation or anthropogenic, through irrigation or fertilization; therefore, when they are fixed in plant resins, the material used to make propolis, will be collected by *A. mellifera* bees.

Toxic metals, because they are not biodegradable, can accumulate in living tissues along the food chain reaching humans mainly through food. Many organisms that feed on organic matter or natural resources from existing plants such as pollen and nectar can to absorb these toxic elements and thus be a potential risk to human health (Aguiar et al., 2002), being able to alter the cellular structures, enzymes and replace metal cofactors of enzymatic activities, essentials to the metabolism of living organisms. Thus, the excess or lack of these elements can lead to disorders in the body, and in extreme cases, to death (Cain et al., 2004).

These elements can be introduced into living tissue through water, food, respiration and even the skin itself. However, 90% of the intake of toxic metals and other contaminants occur through food consumption.Thus, the consumption of propolis as a beneficial natural product for humans can become a source of contamination of toxic metals to those who use it (Cain et al., 2004). Glinski (2000) suggests that the contamination of bee products with toxic metals poses a serious health risk to the population since it can promote a suppression of the immune system, toxic to the organism and, consequently, diseases.

However, the mainly way of human consumption is in the form of extracts or manufactured products (Finger et al., 2014; Canale et al., 2017), making it important the knowledge of the dynamics of transfer of this metals to products consumed by humans or animals. Then, we produced the ethanolic extract of propolis (EEP) of each CP sample and evaluated what would be the concentration of these after a simple extraction and filtration process.

We verified that there was a significant reduction in the concentration of all the metals analyzed in the EEP, ranging from 24.24 to 100.00%. This fact shows that the process of filtration and separation of the filtrate from the sludge eliminates some of the metals found in CP, reducing the risk of contamination by metals considered as toxics, and making the product safe for consumption.

During the preparation of CP by the honeybees, the natural occurrence of pollen from flowers occurs, as well as bees wax. Thus, the presence of hydrocarbons and fatty acids that presents in the CP shows low solubility in the ethanol and could be a source of retention to metals after the processing (Banskota et al., 2001; Vishchur et al., 2016). Also, according to Bezerra et al. (2009) there is also an affinity to toxic metals for the cellulose, that is presente in the filter paper; however, it is not possible to infer that the amount of metals absorbed and concentrated in the propolis ground was due to the extraction process by the filter.

The CP can be considered as an indicator of toxic metals in the environment and the reduction observed in the EPP makes the product safe for consumption.

Table 1.Presence of toxic metals in the crude propolis (mgkg^{-1}) and ethanolic extract (mgL^{-1}) samples of propolis and reduction percentage of these metals in the extract.

METAL		SP	MG	RN	CE	PE	TO	GO	MT	PR	SC	RS
Pb	**CP**	6.88±8.8	0.70±0.8	2.47±1.4	0.90±0.0	LOD	LOD	3.3±2.5	3.50±4.0	1.02±1.7	1.80±1.0	1.15±1.1
	EEP	1.93±1.1	0.13±0.1	0.20±0.0	0.10±0.0	LOD	LOD	1.20±1.3	0.85±1.1	0.30±0.3	0.35±0.2	0.60±0.4
	RED	71.95%	81.43%	91.90%	88.89%	LOD	LOD	63.64%	75.71%	70.59%	80.56%	47.83%
Ni	**CP**	4.97±1.9	0.40±0.0	LOD	LOD	LOD	42.50±0.0	LOD	LOD	0.10±0.0	1.85±1.1	0.10±0.0
	EEP	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
	RED	100.00%	100.00%	LOD	LOD	LOD	100.00%	LOD	LOD	100.00%	100.00%	100.00%
Hg	**CP**	0.92±0.7	0.66±0.3	1.20±0.3	LOD	LOD	0.10±0.0	LOD	LOD	LOD	1.04+0.3	0.58±0.5
	EEP	LOD	0.50+0.1	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	0.30+0.0
	RED	100.00%	24.24%	100.00%	LOD	LOD	100.00%	LOD	LOD	LOD	100.00%	48.28%
Cr	**CP**	20.25±3.6	0.40±0.0	LOD	LOD	LOD	18.00±0.0	LOD	LOD	0.10±0.0	LOD	LOD
	EEP	0.15+0.1	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
	RED	99.26%	100.00%	LOD	LOD	LOD	100.00%	LOD	LOD	100.00%	LOD	LOD
Cd	**CP**	0.53±0.5	0.40±0.3	0.80±0.0	LOD	LOD	LOD	0.50±0.0	0.50±0.6	0.24±0.1	0.66±0.4	0.92±0.3
	EEP	LOD	0.17±0.1	LOD	LOD	LOD	LOD	LOD	LOD	LOD	0.20±0.0	0.13±0.1
	RED	100.00%	57.50%	100.00%	LOD	LOD	LOD	100.00%	100.00%	100.00%	69.70%	85.87%
Sn	**CP**	52.6±10.7	8.78±4.2	7.20±5.7	1.80±0.0	6.40±0.0	9.30±0.0	3.13±1.4	2.30±0.0	8.22±5.5	12.9±9.9	10.5±6.7
	EEP	3.68±4.3	1.00±0.1	0.10±0.0	LOD	LOD	LOD	0.20±0.0	0.6±0.0	1.59±0.9	1.95±1.2	2.96±0.6
	RED	93.00%	88.61%	98.61%	100.00%	100.00%	100.00%	93.61%	73.91%	80.66%	84.88%	71.81%

CP: crude propolis, EEP: ethanolic extract of propolis, RED: Percentage of reduction of metals, calculated by difference of the metals contents in the CP and the respectively EEP. *Comparing the averages which showed contamination levels. LOD: limits of detection. Abbreviatures of Brazilian States: SP - São Paulo, MG - Minas Gerais, RN - Rio Grande do Norte, CE - Ceará, PE - Pernambuco, TO - Tocantins, GO - Goiás, MT - Mato Grosso, PR - Paraná, SC - Santa Catarina, RS - Rio Grande do Sul.

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Authors' Contribution

RO ORSI, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing; DCB BARROS, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – review & editing; RCM SILVA, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – review & editing; JV QUEIROZ, Conceptualization, Formal analysis, Software, Visualization, Writing – review & editing; WLP ARAÚJO, Conceptualization, Formal analysis, Software, Visualization, Writing – review & editing; AJ SHINOHARA, Conceptualization, Data curation, Formal analysis, Methodology, Resources, Software, Validation, Visualization, Writing – review & editing.

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