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Honey from Stingless Bee as Indicator of Contamination with Metals

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Abstract

Melipona scutellaris Latreille (Apidae, Meliponini) is one of the main species of stingless bees used in beekeeping in the Northeast of Brazil. We examined the honey of *M. scutellaris* as an indicator to evaluate the levels of metals at sampling sites subject to a broad spectrum of environmental pollutants. The collections were carried out in the urban-industrial area of Salvador, Bahia and the metropolitan region. Samples (n = 58) were submitted to the nitroperchloric digestion procedure. We used the inductively coupled plasma optical emission spectrometry technique (ICP OES) to determine the concentration of metals (Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn) in the samples. The studied metals were detected among the samples, which presented tolerable levels according to current Brazilian legislation and recommendations from the World Health Organization (WHO), except for Cr, which presented mean values higher than the threshold for all sampling sites. The detection of the analyzed metals indicates that the honey of *M. scutellaris* is a useful tool to evaluate the presence of environmental contaminants; therefore, it can be considered a good indicator of environmental contamination for monitoring a particular region and preventing issues due to the release of metals into the environment.

Introduction

Bees can carry contaminants from the environment to the beehive and consequently to honey, due to their foraging activity. When foraging areas for bees are polluted, several undesirable chemicals may be introduced into honey through nectar, pollen or sugary exudates from plants growing on contaminated soil and/or absorbing contaminated water. Additionally, elements in the atmosphere also are important source of contaminants that may mix with the resources collected by bees (Porrini et al., 2003; Stecka et al., 2014; Di et al., 2016).

Honey can be contaminated with inorganic chemical elements during raw material collection by bees or in the honey extraction process. Moreover, different weather conditions, seasons and honey botanical origin are variables that affect of metal contents in bee products. As bee products are the final stage of a bioaccumulation process, chemical study on honey can provide useful information on the environmental quality of the area where bees feed (Pisani et al., 2008; Stecka et al., 2014; Silici et al., 2016).

Plants can accumulate metals in their tissues due to their ability to adapt to various chemical conditions in the environment. Therefore, some species are considered accumulators of metals found in the soil, water and air (Malavolta, 1994). The geographic and botanical origin, as well as anthropogenic factors near colonies are determinant for the presence of high concentrations of metals in bee products (Bogdanov et al., 2007; Silici et al., 2016). In this sense, the pollen analysis of honey in bee products is very important because it helps a better understanding of the levels of metals in samples from different sites.

The presence of toxic metals in honey can threaten human health (Ru et al., 2013). Additionally, it can serve as an indicator of environmental pollution. Thus, many studies

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have investigated metal concentration in the body of bees and in their products (Porrini et al., 2003; Batista et al., 2012; Pohl et al., 2012; Bastías et al., 2013; Aghamirlou et al., 2015; Nascimento et al., 2015; Martin et al., 2016; Steen et al., 2016; Zarić et al., 2016; Bonsucesso et al., 2018). However, our study is the first carried out in an industrial urban area using honey of *Melipona scutellaris* Latreille, a stingless bee, as an indicator of environmental quality.

Bees react to changes in the environment they inhabit, especially in relation to amounts of toxic metals in the soil, air and plants. This characterizes bees as reliable indicators of environmental quality, allowing their use in biomonitoring (Zhelyazkova, 2012).

Stingless bees (Meliponini) have potential for use as indicators of environmental contamination with toxic metals (Nascimento et al., 2015). These bees have an atrophied sting, which facilitates management (Camargo et al., 2017), and are commonly reared in an urban environment, where there are many anthropogenic activities near the beehives, thus the bees are more exposed to loads of pollutants, especially in large urban centers.

M. scutellaris (Apidae, Meliponini) is one of the main species used in the beekeeping activity in northeastern Brazil (Villas-Bôas, 2012). It was the most frequent species in the region evaluated in this study and presented a satisfactory honey production, allowing its use for this research. This study used honey from *M. scutellaris* as an indicator to evaluate the levels of metals at sampling sites subject to a broad spectrum of environmental pollutants.

Material and Methods

Study site

The collections were carried out in urban-industrial area of Salvador and metropolitan region, Bahia, Brazil (Fig 1). The selected meliponaries were installed in urban (sites A-D), semi-rural (site E) and rural environments (site F), near the petrochemical Complex of Camaçari (Site E-F), sanitary landfill of Salvador and highways Cia-Aeroporto (site A-D), Base Naval Road and BA 093 (site A) (Table 1). The colonies were exposed to a very high range of pollutants that may be present in the atmosphere, soil and water. One month before sample collection, we prepared the colonies used in the experiment, leaving these colonies with empty honey pots, which allowed collection of honey stored by bees during the sampling period.

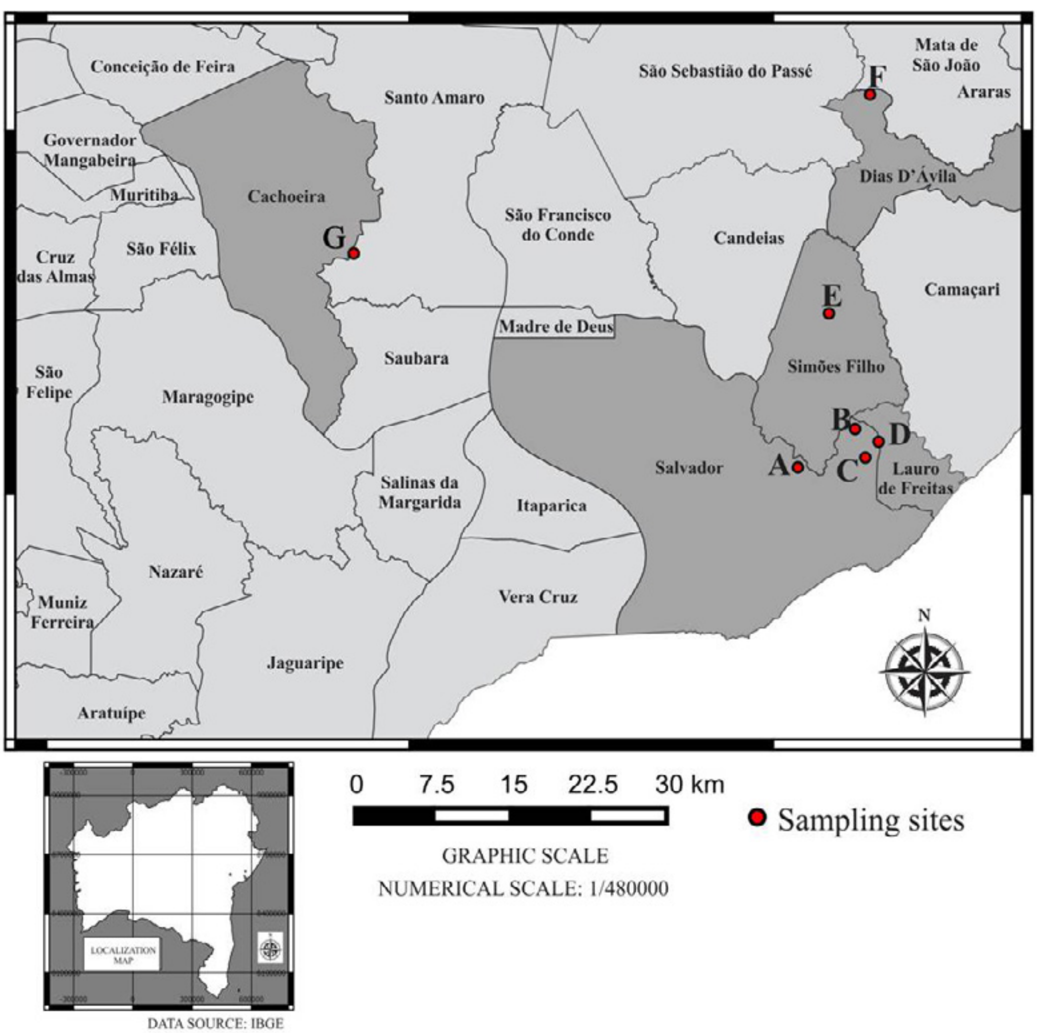

Fig 1. Geographical location of sampling sites in Salvador and the metropolitan region, state of Bahia, Brazil.

A meliponary installed in a non-urban area was used to collect control samples, located in Baía do Iguape, Cachoeira, Bahia. The Marine Extractive Reserve of the Baía do Iguape (currently known as Iguape) is a federal conservation unit in Brazil categorized as an extractive reserve (RESEX) in the state of Bahia.

Sampling

The samples (n = 58), composed each of approximately 250 g of honey, were collected directly from the colonies of *M. scutellaris* with disposable syringes, placed in properly identified sterile plastic containers. The sampling period was one year, between August 2014 and July 2015.

Table 1. Description of sampling sites in Salvador and the metropolitan region, Bahia, Brazil.

Sites	Municipality	Environment	Anthropogenic influence
Meliponary A	Salvador 12º51'32.4" S 038º27'9.90" W	Urban area	Near (~ 600 m distance) highway Base Naval, with intense traffic of vehicles
Meliponary B	Salvador 12º49'58.7" S 038º22'27.4" W	Urban area	Region of the Industrial Center of Aratu (CIA), highway CIA-Aeroporto, with intense traffic of vehicles
Meliponary C	Salvador 12º51'28.3" S 038º21'54.3" W	Urban area	Region of the Industrial Center of Aratu (CIA), highway CIA-Aeroporto, with intense traffic of vehicles, near a landfill and unpaved road
Meliponary D	Lauro de Freitas 12º50'38.1" S 038º21'12.1" W	Urban area	Metropolitan Region of Salvador, Bahia. unpaved road, near (~ 800 m distance) an indian reserve
Meliponary E	Simões Filho 12º43'55.5" S 038º23'51.6" W	Semi-rural	Metropolitan Region of Salvador, Bahia. Farm located approximately 300 m from highway BA 093, with intense traffic of vehicles, near the petrochemical complex of Camaçari, Bahia
Meliponary F	Dias D'Ávila 12º32'28.0" S 038º21'42.3" W	Rural	Metropolitan Region of Salvador, Bahia. Farm located approximately 8 km from highway BA 093 with intense traffic of vehicles
Meliponary G	Cachoeira 12º38'25.3" S 38º51'42.0" W	Non-urban area	Federal conservation unit of Brazil. Marine Extractive Reserve of Baía do Iguape (Control)

Preparation of samples

We used the method of nitro-perchloric digestion proposed by Malavolta et al. (1989) in the preparation of samples to identify the metals. We used 2 g of each honey sample, the evaluations of each sample being performed in triplicate. All the glassware used was placed in a 10% nitric acid solution (HNO_3) for 24 h for decontamination, after which all the material was washed with ultrapure water (18.2 MΩ.cm). We used a standard solution (blank solution) containing only acids, which was submitted to the same procedures for the digestion of honey samples. For the analyses, reagents of certified analytical grade were used.

Determination of metal concentration

Metals cadmium (Cd), copper (Cu), lead (Pb), cobalt (Co), chromium (Cr), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn) were selected for this study because of their importance as environmental contaminants. To determine the concentration of metals in the samples, the Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) technique was used in the ICP (Spectrometer) Thermo Scientific iCAP 6000 Series, Model 6300 Duo. Table 2 presents the analysis conditions of ICP OES.

Accuracy of the analytical method was evaluated in terms of repeatability of experimental results of real samples (triplicate) and expressed as standard deviation of the mean. Accuracy was verified by calibration (using standard solutions).

Pollen analysis

To determine the botanical origin of honey, samples were prepared according to the methodology described by Jones and Bryant Jr. (2004) and later submitted to the acetolysis process of Erdtman (1960). The resulting pellet was mounted on slides for microscopy, followed by the identification and counting of the pollen grains that compose the pollen spectrum of the sample. The pollen types were identified using specialized literature, such as Barth (1989), Roubik and Moreno (1991), Punt et al. (2007) and consulting database and images of the Palinoteca of the Universidade Federal do Recôncavo da Bahia, Brazil. Frequency class of each pollen type was determined according to Louveaux et al. (1978), classified as: Predominant Pollen (PP- > 45% of total grains), Secondary Pollen (SP-16 to 45%), Important Minor Pollen (IMP – 3 to 15%) and Minor Pollen (MP - < 3%).

Data analysis

First, the mean and standard deviation for all metals investigated and detected at each site were calculated. Subsequently, the data were submitted to the Shapiro-Wilk and Levene tests to verify the residue normality and homogeneity of the variances, respectively. The data did not present the assumptions for one-way ANOVA. Thus, we chose to analyze the data by the Kruskal-Wallis nonpanametnic rank analysis of variance (H statistic) to establish an overall difference between the seven sites, and then by *post hoc* Bonferroni-Dunn test with adjustment of *p*-values for the pairwise multiple comparison of mean ranks. Statistical significance was set at $p < 0.05$. All analyses were carried out in triplicate. The “R” statistical and programming environment version 3.0.2 (R Core Team, 2015) was used.

Table 2. Conditions of analysis in ICP OES for quantification of metals in honey.

Parameters - ICP OES	Conditions of analysis
Power RF	1150 W
Nebulization flow	0.70 L/min
Auxiliary gas flow	0.50 L/min
Internal Standard	Ítrio (Y)
Integration time and reading	15 s
Purity of gas (Argon)	99.999%
Metal	**Wavelength (nm)**
Cd	226.5 axial
Co	228.6 axial
Cr	267.7 radial
Cu	324.7 radial
Fe	259.9 radial
Mn	257.6 radial
Mo	202.0 axial
Ni	231.6 axial
Pb	220.3 axial
Zn	213.8 axial

Results

The metals studied were detected in the samples and presented tolerable levels according to current Brazilian legislation and recommendations of the World Health Organization (WHO) (Brasil, 1965, 2009; WHO, 1982; 1983; Mercosur, 2011), except for Cr that presented mean values higher than that established for all sampling sites (Table 3). Honey samples analyzed in this study presented Cd and Pb values below the detection threshold in 98% of the samples (Table 3), both detected only in one sample, Cd in meliponary A and Pb in meliponary B.

Table 3. Means (mg/kg) and standard deviations of metal concentrations detected in honey samples of *Melipona scutellaris* from different sites.

Metal	Sampling sites							Brazilian Legislation*	WHO* (mg/kg/day)
	A (n=9)	B (n=8)	C (n=10)	D (n=12)	E (n=9)	F (n=10)	G (n=10) Control		
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
Cd[a]	0.0010±0.0030	<LD	<LD	<LD	<LD	<LD	<LD	0.5000	0.0070
Co[a]	0.0020±0.0030	0.0069±0.0078	0.0060±0.0014	0.0038±0.0035	0.0010±0.0015	0.0038±0.0036	<LD	-	-
Cr[a]	0.2806±0.3621	0.3426±0.2997	0.4849±03477	0.5168±0.4750	0.5513±0.5033	0.3097±0.1152	<LD	0.1000	0.00003 - 0.00013
Cu[b]	0.2936±0.1580	0.3335±0.1659	0.4563±0.1281	0.3667±0.1203	0.3196±0.1447	0.6688±0.3869	<LD	10.000	0.050 - 0.5000
Fe[a]	1.2108±0.8488	2.4829±1.0377	1.8802±0.4813	1.5311±0.5983	2.9585±1.9328	1.4801±1.0839	3.6000±0.8000	-	-
Mn[b]	0.2629±0.0916	0.4274±0.0869	0.6060±0.4755	0.2405±0.0785	0.7782±0.3323	1.2035±0.4944	<LD	-	-
Mo[b]	0.0226±0.0371	0.0063±0.0117	0.2071±0.1180	0.1698±0.1446	0.2818±0.1756	0.1395±0.0553	<LD	-	-
Ni[a]	0.0152±0.0087	0.0137±0.0081	0.0145±0.0104	0.0241±0.0198	0.0176±0.0060	0.0179±0.0061	<LD	5.0000	0.5000
Pb[a]	0.0007±0.0020	<LD	<LD	<LD	<LD	<LD	<LD	0.5000	0.0500
Zn[a]	0.4000±0.1994	0.9166±0.5042	0.7044±0.7663	0.8428±0.5872	0.9049±1.1143	0.9278±0.5063	<LD	50.000	0.300 - 1.0000

[a]No significant differences among the seven sites by Kruskal-Wallis test. [b]Significant overall difference among sites by Kruskal-Wallis test.
LD = limit of detection, where: Cd, Cu and Mn (LD = <0.005 mg/kg); Co, Cr, Mo, Ni and Zn (LD = <0.002 mg/kg); Fe and Pb (LD = <0.001 mg/kg)
*Brasil (1965; 1998; 2009) and Recommended Ingestion by WHO (World Health Organization) (WHO, 1982; 1991;1993; 1996).

The highest mean concentrations among the samples of the studied areas were recorded for Fe, Zn, Mn, Cr and Cu. As expected among control samples, concentrations of the metals evaluated were below the detection limit, except for Fe that presented a mean of M = 3.6000, SD = 0.8000 mg/kg.

The Kruskal-Wallis test statistics was significant for Cu ($\chi^2(5)$ = 13.84; p = 0.02), Mn ($\chi^2(5)$ = 43.13; p = 0.0001), Mo ($\chi^2(5)$ = 34.59; p = 0.0001). There were no differences in the Cd ($\chi^2(5)$ = 2.33; p = 0.80), Pb ($\chi^2(5)$ = 6.25; p = 0.28), Zn ($\chi^2(5)$ = 9.65; p = 0.09), Ni ($\chi^2(5)$ = 4.85; p = 0.44), Cr ($\chi^2(5)$ = 5.28; p = 0.38) between sites (Table 3). Although the Kruskal-Wallis test showed some differences for Fe ($\chi^2(5)$ = 14.37; p = 0.01) and Co ($\chi^2(5)$ = 12.30; p = 0.03) between sites, these possible differences were not found when adjusting the probability values for pairs of multiple comparisons.

The mean rank of Cu (mg/kg) was significantly greater in site F compared with site A (p = 0.03). The mean rank of Mn (mg/kg) was significantly different between sites A and E (p = 0.003) and A and F (p = 0.0001). The mean rank of Mn (mg/kg) was also significantly greater in site C compared with site D (p = 0.02) and different from sites E (p = 0.0001) and F (p = 0.0001). There was no difference in Mo between sites A and B (p = 1.00) (Table 4).

Additionally, in the determination of the botanical origin of samples, we identified 85 pollen types, distributed in 27 families. In all sampling sites, the Fabaceae family showed the highest number of pollen types, representing 33% of the total, followed by the families Anacardiaceae (9%), Arecaceae (9%) and Euphorbiaceae (6%). The honeys analyzed were classified as multifloral with expressive representation of pollen types of the family Fabaceae, and the *Mimosa caesalpiniifolia* type occurred among samples from all the studied sites.

Table 4. Mean ranks ($\overline{R}_j$) of metals in honey samples from seven different sites.

Sites	Mean ranks ()		
	Cu	Mn	Mo
A	19.7a	13.9ab	11.5a
B	24.6ab	29.6abc	8.4a
C	36.1ab	33.9ac	39.6b
D	28.3ab	10.6b	33.5b
E	22.3ab	43.6c	43.5b
F	43.5b	49.1c	35.1b

* Different letters in the same column indicate significant differences (p <0.05) according to the Bonferroni-Dunn test.

Discussion

Although many metals can be sequestered and transferred to the body of bees and their products, those usually detected are Cd and Pb (Satta et al., 2012; Hladun et al., 2015). In our study the Cd was only detected in a sample of the meliponary A collected in August 2014. In this sample, the concentration determined for Cd was 0.0050 mg/kg. Current standards for honey (Brasil, 2009) establish a threshold of 0.5000 mg/kg for this metal. The World Health Organization (WHO) suggests as tolerable limit of daily intake of Cd 0.0070 mg/kg for adults with 70 kg of body weight (WHO, 1993).

In the sample where this metal (Cd) was detected, the pollen analysis showed that it is multifloral honey. However, the pollen type *Mimosa caesalpiniifolia* (Fabaceae) occurred as secondary pollen (SP -16 to 45%) and *Mimosa quadrivalvis* and *Mimosa tenuiflora* (Fabaceae) as minor pollen (MP - <3%). Note that types *M. quadrivalvis* and *M. tenuiflora* were identified only in the August sample of meliponary B. This may have contributed to Cd detection in this sample, since species of Fabaceae may be Cd accumulators, according to Barbosa et al. (2017). In addition, climatic conditions may have contributed to the detection of Cd levels in the sample evaluated.

Cd is released into the environment through various industrial processes and enters the food chain from absorption by contaminated soil plant or water. Thus, Cd concentration in distinct areas depends on many variables, leading to a variation in the concentration in samples of different honey origin (Naccari et al., 2014; Aghamirlou et al., 2015; Silici et al., 2016). This variation in concentration also occurred in our study for the evaluated metals and can be observed in the different sampling sites (Table 3).

Perugini et al. (2011) found that Cd levels were significantly higher in urban areas than in non-residential areas. Aghamirlou et al. (2015) and Silici et al. (2016) also reported a similar result. The samples evaluated in our study were obtained in industrial urban area and rural did not present high concentrations of the Cd in the sample in which they was detected, indicating that the concentration of metals is variable and depends on factors such as botanical and geographical origin of the sample and climatic conditions.

The lead metal (Pb) in our study was also detected only in a sample of the Meliponary B also collected in August 2014, in this sample concentration determined for Pb was 0.0075 mg/kg. Current standards for honey (Brasil, 2009) establish a threshold of 0.5000 mg/kg for this metal. The WHO suggests as tolerable limit of daily intake of Pb 0.0500 mg/kg for adults with 70 kg of body weight (WHO, 1993).

Lead was the metal that presented the lowest concentration in a study by Di et al. (2016). Morgano et al. (2010) reported different results by comparing pollen stored by bees, another hive product, from semi-rural environments and high-emission pollutants sites of automotive vehicles in Brazil, where they observed very high Pb concentrations (0.44 mg/kg). In our study, Pb was detected in only one sample from site A, this meliponary is located near a highway where there is intense traffic of motor vehicles.

Considering that Pb has limited accumulation in plants and that even in contaminated soils this metal is not readily bioavailable to plants (Hladun et al., 2015, Davies et al., 2003), its low concentration is justified in floral nectar, raw material for honey production. In addition, nectar is less exposed to environmental contamination from other sources such as air (Silveira et al., 2013). This may have a correlation with Pb concentration below the detection limit in 98% of the samples analyzed in our study.

Chromium (Cr) presented a mean concentration ranging from 0.2806 (*SD* = 0.3621) to 0.5513 (*SD* = 0.5013) mg/kg. The presence of this element in the samples at concentrations above the limit stipulated by the Brazilian legislation (Brasil,1965) can be related to anthropogenic factors in the sites of origin of the samples, which are mostly located in an urban-industrial environment (Table 1 and Fig 1). Cr is a natural element in the earth's crust and is released into the environment by natural and anthropogenic sources. Companies most contribute to Cr release from metal processing, tanning facilities, chromate production, stainless steel welding and electroplate iron production. The population is exposed to Cr by inhalation, food intake and drinking water contaminated with this metal (Atsdr, 2012a).

Contamination by Cr in honey probably comes from the load of pollutants in the atmosphere that can contaminate honey directly through, nectar or honeydew or the bees add them involuntarily. Metals in the air can be deposited on bee body when during foraging activities; thus, they can be carried to the hive (Porrini et al., 2003; Bogdanov et al., 2007; Orioan et al., 2016).

The mean values of Cr found by Bogdanov et al. (2007) were higher in samples collected in the city (0.010 mg/kg) than in rural areas (0.004 mg/kg). According to the authors, the Cr content in honey depends on climatic conditions and differences in the Cr content in the different sample areas are attributed to environmental or geographic factors.

Zn concentration in the honey samples analyzed presented mean values ranging from 0.4000, (*SD* = 0.1994) to 0.9278 (*SD* = 0.5063) mg/kg (Table 3). The maximum permissible concentration is 50.00 mg/kg for Zn according to Decree No. 55871/65 (Brasil, 1965). However, in Brazil, there are no updated guidelines on acceptable Zn levels, specific for honey. The WHO establishes 0.30 to 1.00 mg/kg as the tolerable limit for daily intake (WHO, 1982). Aghamirlou et al. (2015) reported that Zn was the most abundant metal in all honey samples, with a mean of 148 µg/kg (ranging from 122 to 6638 µg/kg) and Cu, Cr, Cd, Pb and Ni were also detected and presented lower means in comparison to Zn.

Iron (Fe) was the chemical species with the highest concentrations; however, in compliance with the limits required by the WHO (Table 3). Fe is considered a contaminant mainly in the oxide and hydroxide forms. Fe is an essential metal and required by all life forms. In the human body, Fe has important functions in metabolism, such as protein synthesis and oxidative metabolism. The mean daily Fe intake for men is estimated at 17.00 mg and for women at 9-12.00 mg (WHO, 1983). Acute toxicity of Fe ingested from distinct dietary sources is not reported, and idiopathic hemochromatosis is a disease characterized by long and slow accumulation of Fe in tissues without evidence of excessive dietary intake (WHO, 1983).

Copper (Cu) was detected in samples from all sites (except in meliponary G - control) with a mean concentration lower than 10.00 mg/kg, which is the maximum concentration established by Ordinance No. 685/98 (Brasil, 1998), presenting mean values that ranged from 0.2936 (*SD* = 0.1580) to 0.6688 (*SD* = 0.3869) mg/kg between samples (Table 3). The mean rate of Cu was significantly higher in site F compared to site A (Table 4). Meliponary A is located in an urban setting while site F is in a rural area. Lower concentrations of Cu in meliponary F was expected because it is far from the urban perimeter. However, this can be explained by the probable high pollutant load emitted by motor vehicle traffic from highway BA 093 that is approximately 8 km from this sampling site (Table 1 and Fig 1).

In addition, as it is a rural area with cultivation of *Cocus nucifera* L. (Arecaceae), *Manihot esculenta* Crantz. (Euphorbiaceae), *Musa* spp. (Musaceae), *Passiflora edulis* Sims. (Passifloraceae) and *Zea mays* L. (Poaceae), farmers can adopt measures of pest control with agrochemicals. Cu may be present in honey due to the use of agrochemicals (fungicides) in agriculture and concentration this metal varies according to the botanical origin, geographical and anthropogenic activities (Orioan et al., 2016).

In the honey samples of meliponary F, the pollen type *Cocus nucifera* had a relative frequency of 50.00% and the Euphorbiaceae type occurred as predominant pollen (PP = 45.20%), indicating that bees may have collected material contaminated with Cu, which reveals the importance of considering the botanical origin of samples. Families Arecaceae and Euphorbiaceae are the most representative of 27 botanical families identified in the pollen spectrum of the samples evaluated.

Detection of Cu in the samples of all meliponaries studied is possibly because they are located in an environment with a certain anthropization degree, such as proximity to roads, a petrochemical center and industrial areas. Urban areas are overloaded with pollution from various sources. Vehicle traffic is one of the main pollution sources in urban centers (Silva et al., 2014). The presence of Cu in urban areas is generally explained by anthropogenic and industrial activities, and brake pads from motor vehicles are considered a significant Cu source (Zarić et al., 2016).

Cu is a vital element for the health of living beings. However, excessive intake of Cu can cause adverse effects to human health. Therefore, it is necessary to consider the daily intake of Cu from different food sources, and daily intake for Cu of 0.05 to 0.5 mg/kg of body weight is tolerable (WHO, 1982; Aghamirlou et al., 2015).

Cobalt (Co) was detected in honey samples with concentrations ranging from 0.0006 (*SD* = 0.0014) to 0.0069 (*SD* = 0.0078) mg/kg. Co is a natural element found in rocks, soil, water and plants. This metal is used to produce alloys used in manufacture of aircraft engines, cutting and roughing tools. Co compounds are also used in the industry to color glass, ceramics and paints (Atsdr, 2004). Therefore, Co in the samples evaluated may come from natural sources of the environment and from anthropogenic activities. The tolerable limit for Co in water is 0.050 mg/L established by resolution No. 357 of the National Council of the Environment – CONAMA, published in March 2005. Brasil (2009) does not establish a limit for this metal in honey.

Among the metals evaluated and detected in the samples, trace elements such as Cu, Fe, Mn and Zn are important for human metabolism and are required in small quantities (WHO, 1996; Atsdr, 2012a; Reynaud, 2014). Thus, detection of these metals at a tolerable level (except Cu) indicates that these samples do not present toxic levels that make their consumption impossible, considering only these metals. To ensure honey quality for human consumption, other complementary analyses are necessary, such as determination of physicochemical and microbiological parameters.

Manganese (Mn) was detected in the samples (except meliponary G - control) with mean concentrations ranging from 0.2405 (*SD* = 0.0785) to 1.2035 (*SD* = 0.4944) mg/kg. Mn occurs naturally in many types of rocks and soils. Mn is mainly used in steel production and processing, along with cast iron and superalloys. Mn can also be used as a fuel additive for automotive vehicles, such as gasoline. Mn in additives rapidly degrades the environment when exposed to sunlight, releasing this metal. In this way, its presence in the environment and in the products of the hive can come from natural sources or anthropogenic activities. Emphasizing that Manganese is a trace element and is necessary for good health (Atsdr, 2012b).

Meliponaries E and F are respectively of semi-rural and rural environments and presented the highest mean concentrations of Mn. As the soil is a natural Mn source and many urban soils are no longer similar to original natural soils of a given site and may present lower availability of macro and micronutrients (Burghardt et al., 2015), higher Mn concentrations from meliponaries E and F are related to the greater Mn availability in soils of rural and semi-rural areas. The tolerable limit for Mn in water is 0.100 mg/L established by resolution No. 396 of the National Council of the Environment – CONAMA, published in April 2008. Brasil (2009) does not establish a limit for this metal in honey.

Molybdenum (Mo) was also detected in samples of all meliponaries (except in meliponary G - control), with the mean concentrations ranging from 0.0063 (*SD* = 0.0117) to 0.2818 (*SD* = 0.1756) mg/kg. Bonsucesso et al. (2018) conducted a study in the same sampling site of our research and reported that Mo was also detected in samples of *M. scutellaris* geopropolis of all meliponaries. The tolerable limit for Mo in water is 0.700 mg/L established by resolution No. 396 of the National Council of the Environment – CONAMA, published in April 2008. Atsdr (2017) recommends ingestion of 0.045 mg/kg. Data on toxicity of Mo in humans are limited. The association between Mo effects and thyroid diseases is indicated (Atsdr, 2017; Yorita & Christensen, 2013).

The variation (0.0137 (*SD* = 0.0081) to 0.0179 (*SD*= 0.0061) mg/kg) in the mean concentration of nickel (Ni) in honey samples (Table 3) may be related to several factors, such as nickel source and distance from the contamination source. Ni is present in air, water and soil and is usually evenly distributed throughout the soil profile (Aghamirlou et al., 2015). All samples presented Ni levels within the threshold (5.00 mg/kg) established by the Brazilian legislation (1965).

Batista et al. (2012) compared the concentration of metals in Brazilian honeys to that in other countries and observed that honeys in Brazil present higher average concentrations for Al, Mg and Ni and lower average concentrations of Cd, Cu and Pb. Additionally, the authors found that the mean values for P, Zn, Mn and Fe were very similar to those found for honey samples from other countries. Our results were similar to those found by Batista et al. (2012). Metal concentration in honey and other hive products is associated to floral origin, agricultural practices and soil characteristics, as well as environmental factors, such as pollution, which vary depending on the geographical location (Porrini et al., 2003; Bogdanov et al., 2007; Silici et al. 2016).

In a study on soil samples and geopropolis (bee stingless propolis) of *M. scutellaris*, Bonsucesso et al. (2018) found that most contents of the metals investigated in geopropolis had the soil as source. In addition, other external sources can contribute to the increase of metal concentration in geopropolis. Similar to our study, the authors verified that both soil and geopropolis had low contamination with metals, although the samples were obtained mainly from an urban-industrial region.

As metal concentration in honey can be related to its floral origin and the analyzed honey samples presented in its pollen spectrum a greater representation of the family Fabaceae (33% of total pollen types), this botanical family has some species that are considered hyperaccumulators of toxic metals. Possibly, there is some metal accumulator species among the identified pollen types of Fabaceae, as these metals can be absorbed by the plants and accumulated mainly in the roots and shoots (Vasconcelos et al., 2012). Barbosa et al. (2017) and Souza et al. (2013) cited some examples.

Therefore, the appropriate choice for the site to install the meliponary with the bee colonies needs to be considered to avoid areas very close to contamination sources due to anthropogenic activities. Malavolta (1994) highlighted that the useful life of toxic metals in the soil varies from 70-510 years for Zn, 13-1100 years for Cd, 300-1500 years for Cu and 740-5900 years for Pb. Therefore, if present in the soil, these metals can be absorbed by plants that constitute the bee flora.

The detection of the metals analyzed indicates that honey of *M. scutellaris* is a useful tool to evaluate the presence of environmental contaminants and can therefore be considered a good indicator of environmental contamination and be used to monitor a certain site to prevent problems caused by the release of metals into the environment. The samples in general presented tolerable levels of metals according to the current Brazilian legislation and recommendations of the World Health Organization.

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Authors' Contribution

AS Nascimento, DJ Oliveira and JS Bonsucesso designed the study and interpreted the data. Authors ED Chambó performed statistical analysis. BR Andrade elaboration of Figure 1 (map). Authors AS Nascimento, CAL Carvalho, ED Chambó, DJ Oliveira BR Andrade, and JS Bonsucesso participated in study conduction and data interpretation. Author AS Nascimento drafted the manuscript. All authors read and approved the final manuscript.

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