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Botanical sources and heavy metals contents of honey produced by *Apis mellifera* in an ecotone region of the state of Bahia, Brazil

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Abstract: The present study investigated by palynological and chemical analysis (Flame Atomic Absorption Spectrometry) about the botanical origin and the heavy metals content (arsenic, cadmium, chromium, lead and mercury) of monthly honey samples of *Apis mellifera* L. over two years. The pollen types Apiaceae, *Mimosa caesalpiniifolia*, *M. tenuiflora* and *Myrcia* indicated the main floristic sources used by bees. *M. tenuiflora* was the most frequent of the pollen types, and because it predominates in different months in each year, which may indicate more than one species of the genus being foraged by the beehive. The climatic influence (rainfall and temperature) on the pollen diversity was investigated and was not statistically supported. The chemical analysis showed that the heavy metal content of the samples were below their respective limits of quantification, and, therefore, the samples can be considered safe for human consumption.

Key words: Caatinga, Cerrado, Melissopalinology, nectar sources, pollen spectra, honey contaminant.

INTRODUCTION

Honey is a natural sweet substance produced by bees through the combination of their own secretions with nectar collected from flowers (Codex Alimentarius 1981). Any pollen grains found in honey are accidentally inserted because of the foraging behavior of the bees. The pollen grains present in bee products can be typified by palynological studies and are thus important sources of information about the plant species used as food sources by the hive (Barth 1989, 2004). Chemically, honey is composed of approximately 25% water with a high concentration of carbohydrates (~95% of solids), and lesser amounts of organic acids, proteins, vitamins, and minerals (~1%) (White Jr. & Donner 1980). Some of the minerals found in honey include heavy metals and trace elements. These are metals and semimetals associated with contamination and toxicity to human health, depending on the element and its chemical form (Duffus 2002).

Heavy metals are emitted continuously in the environment by natural and anthropogenic agents, and since they are not degraded, they remain active in the physical and biological cycles (Porrini et al. 2003). Industry and traffic are considered the main sources of environmental contamination by humans (Bogdanov 2006). In honey, the entry of metals may be due to improper handling during the production and storage of honey, or by contact with contaminated water, air, soil, and plants in the foraging area (Caroli et al. 1999, Porrini et al. 2003, Bogdanov 2006, Pisani et al. 2008, Zhelyazkova 2012).

The floral origin of honey influences its total mineral content, as recorded by Fredes & Montenegro (2006) in Chile, Conti et al. (2007) in Italy, Bilandžić et al. (2012) in Croatia, and Formicki et al. (2013) in Poland. These studies have shown that melissopalynological and chemical studies can provide important information about the main botanical sources used by honeybees as food and their influence on the heavy metal content of honey, while also assessing the potential of bees and their products as bioindicators of environmental contamination (Porrini et al. 2003).

The floral origins of Brazilian honey have been studied since the 1960s (Barth 1990). Robust studies have previously been conducted in Northeast Brazil and serve as references for beekeeping in the region, especially in the states of Bahia (Oliveira & Santos 2014), Piauí (R. Borges, unpublished data) and Sergipe (Silva & Santos 2015). On the other hand, studies on heavy metals in honeys have been reported more recently, including reports by G. Sodré (unpublished data) in the northeastern region, in addition to the works of Mendes et al. (2006), M. Magalhães (unpublished data), R. Ribeiro (unpublished data), Andrade et al. (2014) and Souza et al. (2014) in other parts of the country. Considering the size, biodiversity, and beekeeping potential of the country, scientific investigations in this area remains insufficient.

The present study was formulated to evaluate the variation in the botanical constitution and heavy metal content of honey produced over two years in a commercial apiary in the interior of the state of Bahia, and thus to contribute to bee research in Northeast Brazil.

MATERIALS AND METHODS

Study area

Sampling was carried out in a commercial apiary in Caetité (13°58’41.45”S, 42°27’24.71”W) (Figure 1), a municipality located in the micro-region of the Serra Geral in the state of Bahia (IBGE-Instituto Brasileiro de Geografia e Estatística 2016). Its vegetation is characterized by an ecotone between the cerrado, caatinga, and seasonal forest, (SEI - Superintendência de Estudos Econômicos e Sociais da Bahia 2015), which is constantly threatened by environmental impacts from mining activities. The apiary is located 763 m to the west of highway BR 122, which connects Caetité to its district Maniaçu. The hives are installed in an anthropic rural area surrounded by farms in Caatinga.

Sample collection

Monthly samples were collected from honeybees (*Apis mellifera* L.) between March 2015 and February 2017. A beehive was provided by the apiary owner, from which the samples were collected by removing all the honey content from the pre-identified frame, ensuring that the new production corresponded to the following sampling month. During the 24 month-study, 21 samples were collected, because of insufficient nectar flow between September–November, 2016. Samples were collected directly from the frame without any contact with the metallic structures of the beekeeping equipment. The honey was stored in polypropylene tubes, labeled, and conditioned at room temperature.

Botanical characterization

The samples were chemically treated as per the method described by Louveaux et al. (1978) with the addition of 50 mL absolute ethyl alcohol to the solution, an adaptation proposed by Jones & Bryant Jr. (2004), which aims to minimize the

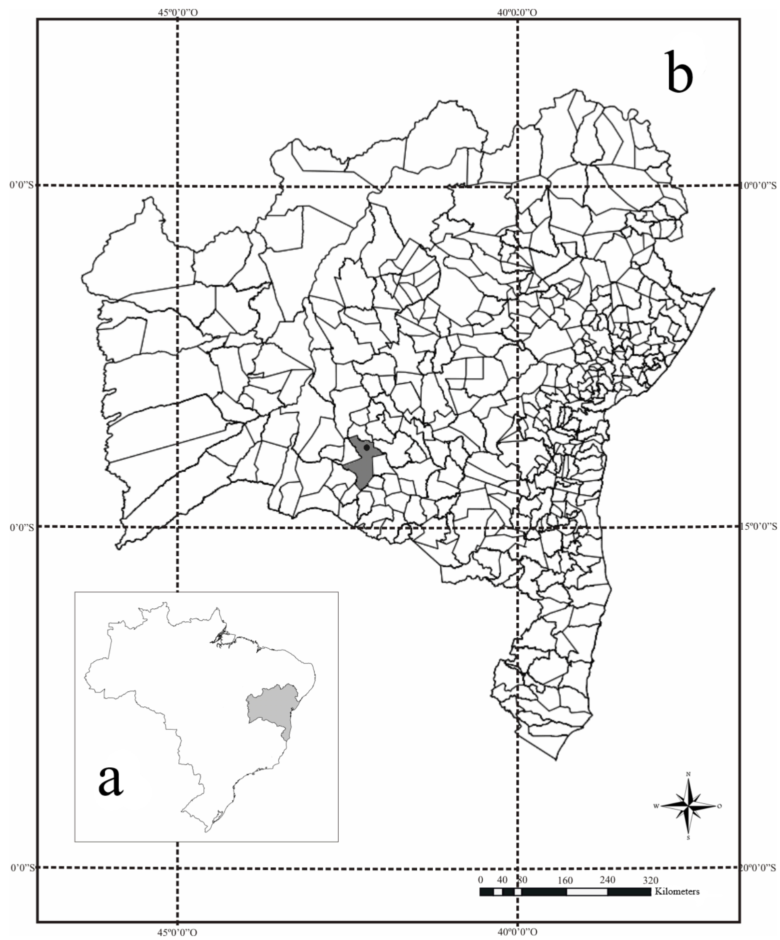

Figure 1. **Location of the study area. In gray: (a) state of Bahia; (b) the municipality of Caetité.**

loss of pollen grains. The sediment was then subjected to acetolysis (Erdtman 1960) and mounted on slides with glycerinated jelly.

A minimum of 500 pollen grains per sample were counted for the palynological census (Moar 1985), which were classified according to Louveaux et al. (1978): predominant (>45%), secondary (15–45%), important minor (3–15%), minor (1–3%), or trace (<1%) pollen.

The typification of the pollen grains was performed by comparison with the slide collection of the Laboratório de Micromorfologia Vegetal (LAMIV) of the Universidade Estadual de Feira de Santana (UEFS) and by consulting pollen catalogs (i.e., Chávez et al. 1991, Roubik & Moreno 1991, L. Silva, unpublished data) Brazilian online databases such as INCT-Herbário Virtual da Flora e dos Fungos (2017) and Flora do Brasil 2020 (2018) were also consulted.

Climatic data

Rainfall and temperature data were obtained from the Caetité Meteorological Station through the Instituto Nacional de Meteorologia (INMET 2017).

Determination of heavy metals contents

Triplicate samples of honey (0.5 g), diluted with 5 mL of nitric acid (Merck, Darmstadt, Germany), were digested in a microwave (Mars Xpress, CEM, São Paulo, Brazil) as described in Table I. The digested samples were then diluted (1:1) with distilled water and used for reading.

The levels of As, Cd, Cr, Pb, and Hg were determined via flame atomic absorption

Table I. **Operating conditions for the digestion of honey samples by microwave.**

Steps	Power	Temperature °C	Heating time	Cooling time
1	800	room temp. - 120	5 min	-
2	800	120	10 min	-
3	800	120 - 160	5 min	-
4	800	160	5 min	-
5	0	room temp.	-	5

spectrometry (FAAS) (Varian AA 240, Agilent Technologies Inc., California, USA), equipped with individual hollow cathode lamps under appropriate wavelengths (Table II). For determining As and Hg levels, a steam generation accessory (VGA-77, Varian, Mulgrave, Australia) was used. Five milliliters of concentrated nitric acid with 5 mL of distilled water were used as the blank. Standard solutions of the metals at a concentration of 1000 ppm (Qhemis-Hexis, São Paulo, Brazil) were used to prepare working solutions after appropriate dilution (Table II). Distilled water was used in all dilution procedures. All reagents used were of analytical grade.

Data analysis

In order to relate the diversity of pollen types with climatic data (rainfall and temperature), we constructed General Linearized Mixed Models (GLMM's, glmmPQL function in the "MASS" package, Venables & Ripley 2002). We chose this method of analysis because there is an argument in the function that makes the correction for time-repeated data, in this way it controls temporal autocorrelation effects. Since we accessed monthly climate data, from the month prior to collection and from the current month to collection, as well as an average of two months of sampling, we constructed separate sets of models. In all models we use Poison as the distribution of errors. The probability of the GLMM models was tested by ANOVA (Type II sums of squares test hypotheses: Anova function, package "car", Fox & Weisberg 2011) All analyzes were done in the R environment (R Core Team 2018).

RESULTS

Froral origin, pollen diversity and climate influency

The bee floral diversity of the study area was represented by 95 pollen types, of which 79 were identified and associated with 30 botanical families (Table III and Figure 2). Table III presents the pollen spectrum of all samples in categories (classification by Louveaux et al. 1978), which are especially informative for local beekeepers as it highlights the main food sources exploited by bees. The spectra indicated that Fabaceae species were the main food source for *A. mellifera*. Pollen types in this family were recorded in the spectra of all sampled months. Other families were also frequent among the samples, although with different contributions in the spectra; for example, Rubiaceae, the family with the second highest number of pollen types, was represented mainly by important, minor, and trace pollen.

Table II. Individual conditions of the metals for reading under FAAS.

Element	Wavelength (nm)	Flame composition	LOQ (ppm)	LOD (ppm)	Standards solution (ppm)
As*	193,7	Air-Acetylene + nitrous oxide + hydride generator	2,00	0,660	2,0 - 4,0 - 6,0 - 8,0 - 10,0
Cd	228,8	Air-Acetylene + air	0,02	0,006	0,2 - 0,4 - 0,6 - 0,8 - 1,0
Cr	357,9	Air-Acetylene + air	0,06	0,020	0,2 - 0,4 - 0,6 - 0,8 - 1,0
Hg*	253.7	Air-Acetylene + nitrous oxide + hydride generator	2,00	0,660	2,0 - 4,0 - 6,0 - 8,0 - 10,0 - 12,0
Pb	217,0	Air-Acetylene + air	0,10	0,033	0,2 - 0,4 - 0,6 - 0,8 - 1,0

***Values in ppb.**

During the 24-month study, the main botanical sources that composed the pollen spectra varied among the four pollen types: Apiaceae, *Mimosa caesalpiniifolia*, *M. tenuiflora*, and *Myrcia* (Table III and Figures 3, 4). The pollen type *M. tenuiflora* predominated in a larger number of samples and was present in all months of the study. Because it predominated at different times in each year of study (Figure 3), this pollen type might indicate the presence of more than one species of the genus in the area. In addition to the above pollen types, *Begonia*, *Eucalyptus*, *Mimosa pudica*, *M. ursina, Mikania*, *Mitracarpus hirtus*, *Raphiodon* and Myrtaceae (Table III and Figure 4) were characterized as secondary pollen, and thus indicated important taxa for bee feeding.

The frequency of pollen types among the samples is considered an important factor, since it indicates the period during which the species were flowering in the area contributing to the production and maintenance of the colony. In descending order, the most frequent types (> 50%) were: *M. tenuiflora* > *M. caesalpiniifolia* > Apiaceae > *Raphiodon* > *Evolvulus glomeratus* > *M. ursina* > *Hyptis* > *Richardia grandiflora* > *Myrcia* (Table III and Figure 3).

The influence of monthly climatic factors (temperature and rainfall) on the composition of the pollen spectra was investigated through Pearson analysis: climatic factors × pollen diversity and climatic factors × pollen frequency. The average temperature in the municipality ranged from ca. 20–26 °C, while total precipitation ranged from 0– 0.6 mm³ in the driest months, to 63.4–400 mm³ in the rainy season (Figure 5). The results of this analysis showed that there was no statistically significant correlation ($p > 0.05$) among the variables considered.

Heavy metals contents

The chemical analyses using FAAS showed that the samples presented lower readings than those obtained for the lowest point of the standard curve of each metal. In other words, the concentrations of As, Cd, Cr, Hg, and Pb in the honey samples were below their respective limits of quantification (Table II), therefore, the samples were considered safe for human consumption.

Table III. Pollen types from honey of *Apis mellifera* L. produced over two years in the study area. Frequency Classes: P = predominant pollen, S = secondary pollen, I = important minor pollen, m = minor pollen, t = trace pollen, % = frequency between spectra.

Floral sources		2015										2016									2017		
Family	**Pollen types**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Dec**	**Jan**	**Feb**	**%**
Amaranthaceae	*Alternanthera* 1		t	t	t								t	m									24
	Alternanthera 2																	t					5
	Gomphrena	t	I	m	m		t		t				t								m		38
Anacardiaceae	*Myracrodruon urundeuva*				t	t			t	t					t	t	I		I	I			43
Apiaceae	Apiaceae	S	t			S		t		t	P	P	I	S	I	m	m	I	I		t	t	71
Apocynaceae	Apocynaceae																			t			5
Arecaceae	*Cocos nucifera*											I											5
	Cocos 1												m										5
	Syagrus coronata		t																				5
Asteraceae	*Ageratum*													t									5
	Aspilia				t																		5
	Mikania		t			t	t						S	t	m	t			t	t		t	48
	Elephantopus			t																			5
	Vernonanthura													t									5
Begoniaceae	*Begonia*																				S		5
Bignoniaceae	*Anemopaegma*										t												5
	Bignoniaceae																					m	5
	Fridericia						t											t					10
Boraginaceae	*Cordia*													t									5
	Helitropium					t					t			t									14
Cannabaceae	*Celtis*									t													5
Concolvulaceae	Evolvulus glomeratus	m	I	t	m	I	m		t		I	m	m	I	m		t	t					67
	Jacquemontia	t					m							t									14
Cucurbitaceae	*Momordica charantia*										t												5
Cyperaceae	Cyperaceae												t					t					10
Dilleniaceae	*Davilla*			t																			5
Euphorbiaceae	*Croton* 1					m					m	t		t						t	t	t	33
	Croton 2										t							t					10
	Croton 3												t										5
	Croton 4														t			t					10
	Croton 5																	t					5
	Euphorbiaceae		t																		t		10

Table III. Continuation.

Fabaceae	Aeschynomene																t						5
	Anadenanthera colubrina																t						5
	Chamaecrista ramosa		t		m							t											14
	Chamaecrista nictitans						t																5
	Copaifera														m	t	t						14
	Dioclea												m	t									10
	Diptychandra					t																	5
	Fabaceae 1				t	t							t							t		t	24
	Fabaceae 2						t																5
	Galactia jussiaeana									t													5
	Inga vera										m	m		t	t			t	t				29
	Machaerium								t			m	l										14
	Mimosa caesalpiniifolia			t	t	t			t	t	t	l	l	m	P	P	l	P	t	l	t	t	81
	Mimosa pudica		l					t				t	S	t				t					29
	Mimosa tenuiflora	P	P	P	P	S	P	m	S	S	m	l	l	S	l	l	P	l	S	P	P	P	100
	Mimosa ursina	l	m			S	t			t	m	m	m	m	t	t		m					57
	Neptunia plena											t											5
	Pityrocarpa moniliformis						m																5
Lamiaceae	*Hyptis*	t		t	t	t	m					t	m	m	t			t			t	t	57
	Raphiodon	m	l	l	l	l	S		t	t			t	m				t		l	l	S	67
Mapighiaceae	*Byrsonima*													l	t	t	t	t					24
Malvaceae	*Herissantia*	t	t		t		t														t		24
	Malvaceae																				t		5
	Melochia tomentosa					t					t	t											14
	Waltheria arckemanianna																				t		5
Myrtaceae	*Eucalyptus*					t		m	m	m						t	m		S	m	t	m	48
	Myrcia				t	l		P	P	P		t			t		t	t		t	t		52
	Myrtaceae							S	S	l		t							l	t			29
Nyctaginaceae	Guapira							t	t	t							t						19
	Nyctaginaceae					t																	5
Planttaginaceae	*Angelonia*							t															5
Phytolacaceae	*Microteia*											t											5
Poaceae	Poaceae										t	t											10
Polygalaceae	*Polygala*				t							t	t										14
Polygonaceae	*Polygonum*													t									5
Rubiaceae	*Borreria verticillata*										t			t									10
	Hexassepalum apicullata						t						t		m						t	m	24
	Guettarda														t								5
	Mitracarpus frigidus									t													5
	Mitracarpus hirtus			l	l					t				S				t		t			29
	Richardia grandiflora	t	t	t	t		t				t		t	l	t						t	l	52
	Rubiaceae																					t	5
Sapindaceae	Cardiospermum												t										5
	Cupania					t																	5
	Serjania														t								5
Urticaceae	*Cecropia*									t		t											10
Verbenaceae	*Lippia*									t													5
Pollen types not identified		0	0	0	0	0	1	0	0	1	1	1	0	3	0	0	5	4	0	0	0	0	
Total of pollen types		10	14	11	15	18	15	8	11	16	15	20	21	27	17	9	13	18	8	12	16	13	

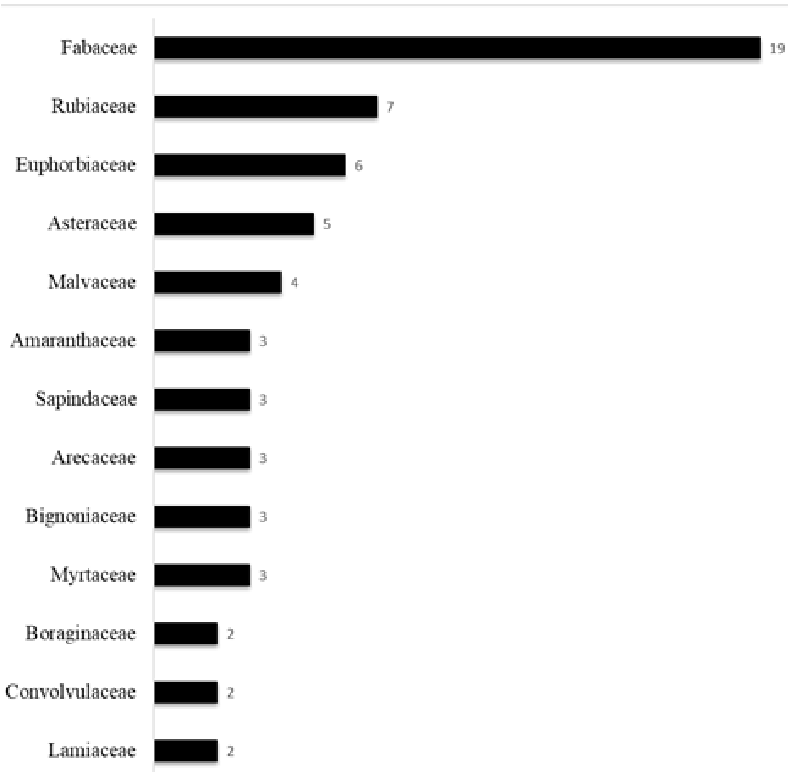

Figure 2. Most representative botanical families regard to the number of pollen types.

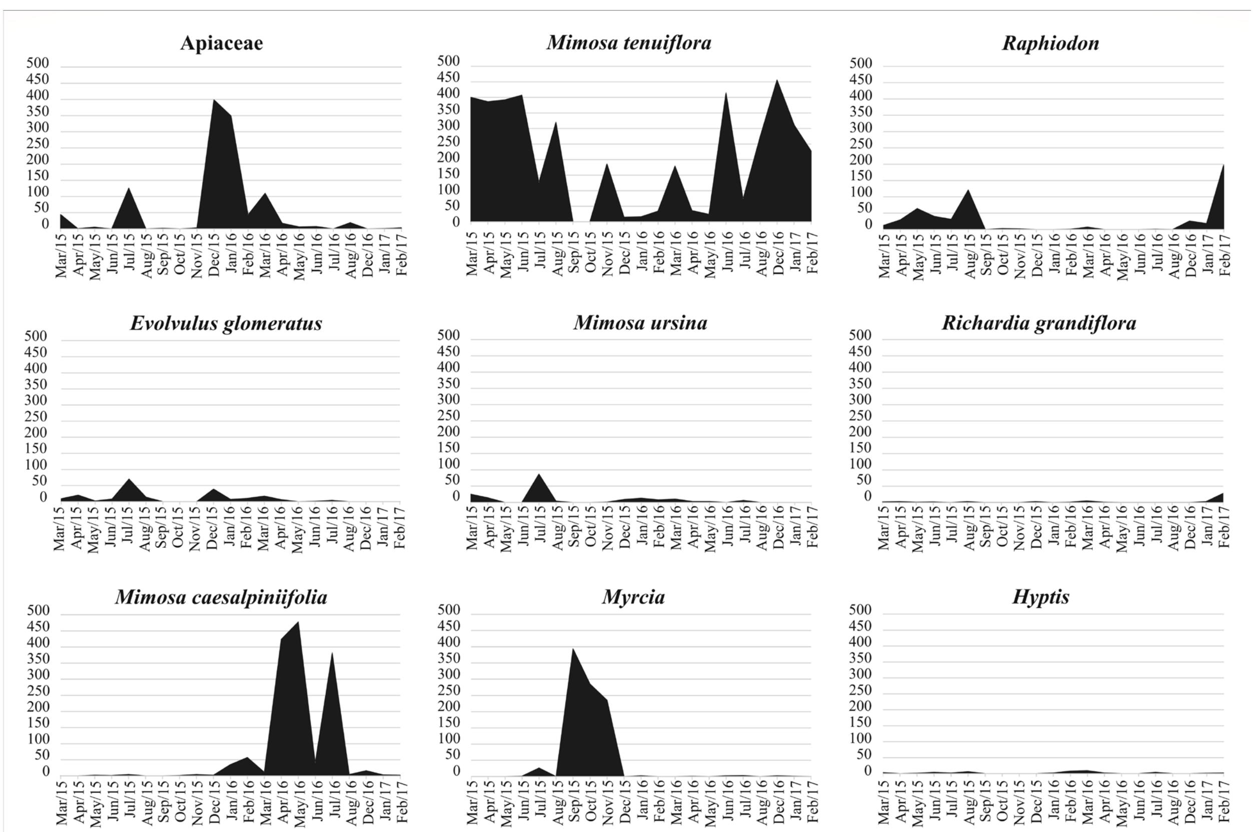

Figure 3. The frequency of pollen types with higher occurrence in the honey samples (> 50%).

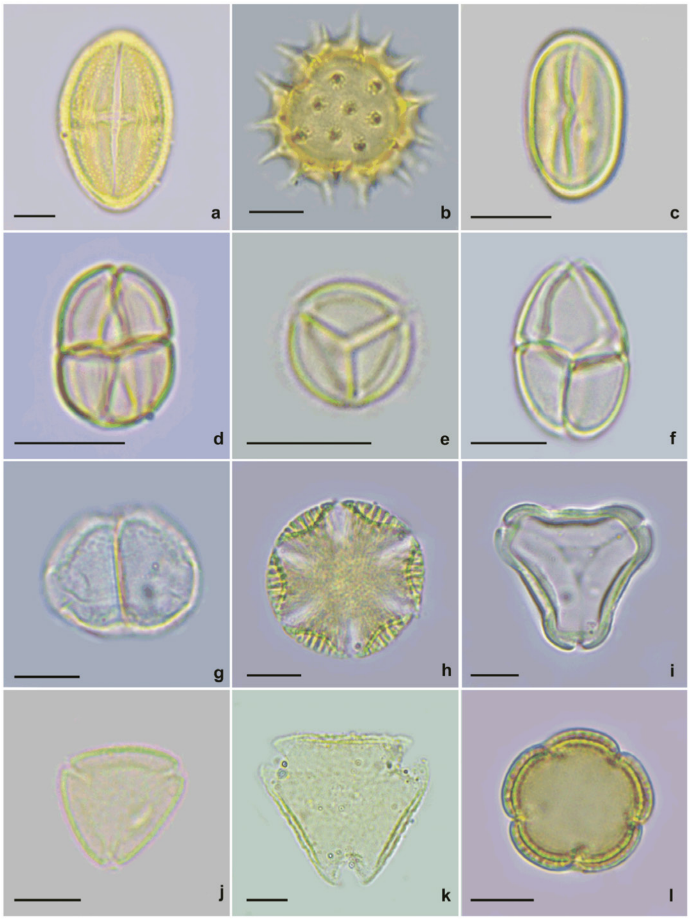

Figure 4. **Predominant and secondary pollen types identified in the honey samples: (a) Apiaceae; (b) *Mikania* (Asteraceae); (c) *Begonia* (Begoniaceae); (d) *Mimosa caesalpiniifolia* (Fabaceae); (e) *M. pudica* (Fabaceae); (f) *M. tenuiflora* (Fabaceae); (g) *M. ursina* (Fabaceae); (h) *Raphiodon* (Lamiaceae); (i) *Eucalyptus* (Myrtaceae); (j) *Myrcia* (Myrtaceae); (k) Myrtaceae; (l) *Mitracarpus hirtus* (Rubiaceae). Scale bar: 10 µm.**

DISCUSSION

The pollen spectra of honey produced in Caetité generally resemble those of honey from other regions of Bahia (See Table III). For example, the regions of Recôncavo; northeast, west, and middle São Francisco; and Serra Geral (to which the municipality belongs), had pollen spectra counts exhibiting predominant *Mimosa* L. content. (Oliveira & Santos 2014). In the remaining regions of the state, the honey samples exhibited pollen counts with other predominant pollen types from native and exotic genera such as *Coffea*, *Copaifera*, *Elaeis*, *Eucalyptus*, *Euterpe*, *Schinus*, *Syagrus*, *Tapirira*, and more. (Moreti et al. 2000, Oliveira et al. 2010, Oliveira & Santos 2014, Nascimento et al. 2015).

Predominant pollen types in honey can generate a false impression of the major nectariferous source used by bees and mask the importance of other honey plants. This holds true for the overrepresented species in the spectra, as they produce a much larger quantity of pollen grains than nectar (Barth 1989). Among the predominant pollen types identified, three indicate species that produce large quantities of pollen grains: *M. caesalpiniifolia*, *M. tenuiflora*, and *Myrcia* (Barth 1989, Proença & Gibbs 1994).

The Apiaceae type, predominant in two samples, is related to exotic vegetables such as *Daucus carota* L., *Eryngium foetidum* L., and *Foeniculum vulgare* Mill. that can offer both nectar and pollen to honeybees (Pérez-Bañón et al. 2007). The most frequent pollen types of the genus *Mimosa* indicated species known by the people of the study area because of their beekeeping importance. The “Sabiás”

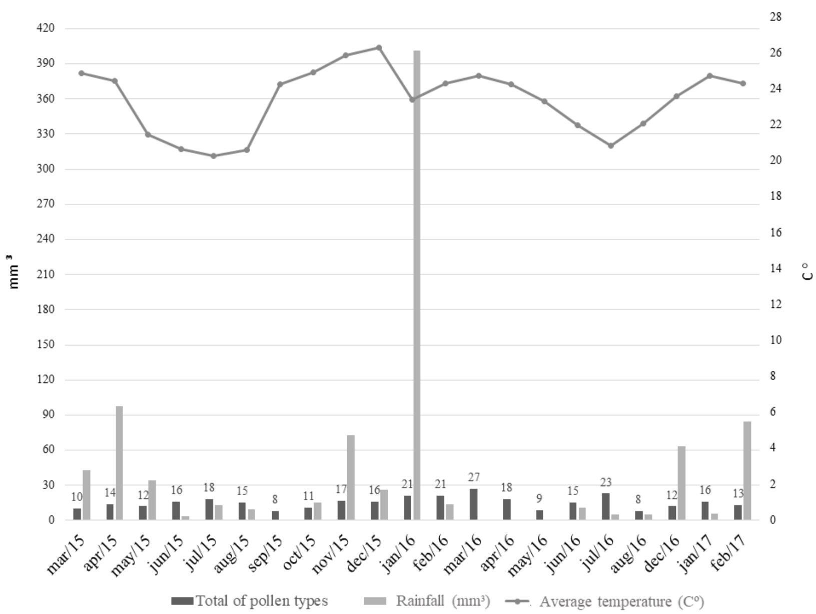

Figure 5. **Monthly pollen diversity and climatic data of the study area (rainfall and temperature).**

(*M. caesalpiniifolia*) and "Juremas-pretas" (*M. tenuiflora*), as they are popularly called, produce large amounts of pollen and also supply nectar to bees (Freitas & Silva 2006).

A classic example of a nectariferous group underrepresented in pollen spectra is the Lamiaceae (Barth 1989). In this study, the family was represented in greater frequency (secondary and important pollen) by *Raphiodon*. Thus, considering their importance in the spectra and their floral characteristics (Dias & Kiill 2007), species indicated by this pollen type can be considered as important sources of nectar for honeybees. This example can also be used for other nectariferous species which have been indicated in the spectra, such as *Eucalyptus* sp. (Myrtaceae), *Mitracarpus hirtus* (Rubiaceae) and *Myracrodruon urundeuva* (Anacardiaceae) (Barth 1989, 1990, Davis 1997).

Some pollen types were important, not necessarily because of their abundance in the spectra, but because of their frequency during the sampling months (i.e., *M. tenuiflora*, M. *caesalpiniifolia*, Apiaceae, *Raphiodon*, *Evolvulus glomeratus*, *M. ursina*, *Hyptis*, *Richardia grandiflora* and *Myrcia*), which indicated beekeeping species useful for maintenance. According to Freitas & Silva (2006), the species of maintenance, unlike species of production, are those that do not produce excess nectar for commercial purposes, but ensure that the colonies remain strong and do not abandon the beehive, especially in the driest months.

Climatic factors can influence the phenological patterns of plant species (Machado et al. 1997), the behavior of honeybees (Szabo 1980) and, consequently, the pollen diversity of bee products (Andrada & Tellería 2005, Simeão et al. 2015). Nevertheless, this correlation has not been clearly observed in our experiment as well as in other works with Brazilian bee products (R. Borges, unpublished data, Alves & Santos 2018, Matos & Santos 2019). Considering only species blooms, the authors generally expect a positive relationship between water availability in the environment and diversity in pollen spectra.

Regarding the presence of heavy metals in the studied samples, the results indicated that although the municipality of Caetité is a focus of mining industries, the probable sources of pollution have low influence on the study area. In more developed cities, the heavy metal content may be higher than the maximum values allowed by Brazilian legislation, which is, in mg/kg (or ppm), 0.1 for Cd and Cr, 0.3 for As and Pb, and 0.5 for Hg (BRASIL 1987, 2008, 2013).

Cr contamination is very common in Brazilian honey, according to the literature. The highest Cr contents were observed in honey from cities such as Teresópolis-RJ (0.42 mg/kg), Betim-MG (0.53 mg/kg) and Moju-PA (0.83 mg/kg) (M. Magalhães 2010, unpublished data, R. Ribeiro 2010, unpublished data, Souza et al. 2014, respectively), while Pb levels ranged from 0.14 mg/kg in Guarapuava-PR to 1.30 mg/kg in Moju-PA (Souza et al. 2014). On the other hand, the levels of As, Cd, and Hg are generally low or undetectable by analytical methods and are thus considered to be absent (G. Sodré, unpublished data, Mendes et al. 2006, Souza et al. 2014).

Although our study detected metallic contaminants at very low values in the samples., honeybees (and their products) are considered excellent ecological (Porrini et al. 2003). Studies have shown that environmental factors such as pollution, climate, and botanical origin influence the mineral composition of honey (Bogdanov 2006, Bilandžić et al. 2012).

This study presented important information about the environmental conditions of a city with increasing urban development, and contributed to the beekeeping knowledge of the local community. However, additional studies are needed, both to cover a larger sampling area and to assess the potential of other bee products as bioindicators, since the literature shows that products such as bee pollen, propolis, and wax generally accumulate more minerals than honey (Birge & Price 2001, M. Magalhães, unpublished data, Formicki et al. 2013).

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CSJ, FS, and TS conceived the study and designed experiment; CSJ, AG and GRS performed the chemical analyzes; CSJ, BAL and EAM conducted palynological studies; RSA performed data analysis; All authors read and approved the final manuscript.