



RESEARCH ARTICLE - BEES

Pollen storage by stingless bees as an environmental marker for metal contamination: spatial and temporal distribution of metal elements

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Article History

Edited by

Celso Martins, UFPB, Brazil

Received 13 November 2017

Initial acceptance 08 December 2017

Final acceptance 21 January 2018

Publication date 09 July 2018

Keywords

Bee; Pollen; suspended particulate material; mining activities; Iron Quadrangle.

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Abstract

Since the middle of the 20th century, human activities have led to overall ecosystem contamination and to major modifications in landscape structure and composition. Mining activities represent a major source of environmental contamination by metal residues. The objective of our study was to evaluate the presence of heavy metals and other elements on stingless bee pollen, and compare them to samples of Suspended Particulate Material (SPM) in five points a Mineral Province, in Brazil. More than 50 elements were identified by ICP-OES and ICP-MS, after microwave digestion. Overall, we found a strong relation among elements present on pollen and SPM. Samples from the four areas exhibited higher levels of minerals compared to the reference site. Mineral levels varied widely within the two seasonal periods. Some elements, like Pb, Cd, As, Cu, Zn, and Fe were found at levels considered potentially toxic to human health. Pollen stored by stingless bees was a successful bioindicator, and demonstrated the value of quantitative ecological information for detecting air pollution.

Introduction

Since the middle of the 20th century, growing industrialization, urbanization, transportation and agriculture has led to overall ecosystem contamination and major modifications to landscape structure and composition. The presence of metals in the environment can be the result of external sources such as industrial smelter pollution, emissions from ferrous metallurgy, external mining activities and busy highway traffic (Bilandžić et al., 2011; Lambert et al., 2012).

Mining activities represent a major source of environmental contamination by metal residues (Freedman & Hutchinson, 1981; Perugini et al., 2011). In the Brazilian state of Minas Gerais, mine exploitation in the Iron Quadrangle reached its peak towards the middle of last century and the activity is still intense (Meneses et al., 2011). One of the consequences of this industry is the daily release of huge amounts of dust into the atmosphere (Meneses et al., 2011).

The rocks (or soils) of Iron Quadrangle naturally have high levels of metals that can be considered toxic for human health (Rapini et al., 2008; Azevedo et al., 2012; Messias et al., 2013; Carvalho-Filho et al., 2010). On the other hand, these regions shelter a relatively high variety of very poorly studied metallophilic plants, living in a harsh environment with high concentrations of metals in the soil and subject to atmospheric deposition of those toxic elements, due to the mining activities. Studies carried by Valim (2012) and Baêta (2012) for example, found that relevant quantities of elements such as Al, Ba, Ca, Fe, K, Na, Mg, Mn, S, Sr, and Zn are deposited annually on the soil surface and vegetation via wet and dry deposition. According to these authors, this deposition depends on the geochemical nature of the environments that may be affected by weathering and anthropic activities, being sources of input of both water and nutrients and even pollutants for plants and animals.



Due to their enormous diversity (species richness and abundance) and role in ecosystem functioning, the use of insects as bioindicators has been increasing (McGeoch, 2007; Lambert et al., 2012). The functions and use of bioindicators are better depicted if we take a look at the bees. The idea of employing bees in environmental monitoring is not a new one. Since 1970, honeybees have been increasingly employed to monitor heavy metals in territorial and urban areas, pesticides in rural areas and radio nuclides (see Pohl, 2009 for a revision; Oliveira et al., 2017).

Besides honeybees, their products have been employed in studies aiming to determine levels of metal pollution in different ecosystems (agricultural, urban and industrial; e.g. Bratu & Beorgescu, 2005; Bilandžić et al., 2011; Morgano et al., 2012; Formick et al., 2013; Lambert et al., 2012; Leita et al., 1996). In many countries, there are no reports regarding the use of stingless bees to measure pollen contamination by toxic elements or as environmental markers in mining areas.

Apparently, mines, steelworks, industrial and urban areas, and highways in or near bee foraging areas can result in an increase in the concentrations of certain metals (e.g., Al, Ba, Ca, Cd, Cu, Mg, Mn, Ni, Pb, Pd or Zn) in bee matrices (honey and pollen) due to pollution from chemical wastes and exhaust fumes (Wayne, 1983; Bilandžić et al., 2011; Formick et al., 2013). The fundamental difference between heavy metals and other pollutants, like pesticides, is their mode of introduction and their environmental fate. Pesticides are scattered both in time and space and, depending on the type of chemical compound, are degraded by various environmental processes over longer or shorter periods of time. Heavy metals, on the other hand, are emitted in a continuous manner by various natural and anthropic sources and, since they are not degraded, are continuously kept “in play”, thus entering into physical and biological cycles (Matina et al., 2016).

In this scenario, we propose the use of stingless bees as active environmental markers in mining areas; they have been mostly used in standard exploration procedures of mining companies as efficient mineral prospectors. Stingless bees, like other bee species, are exposed to atmospheric pollutants during their foraging activities. Their hairy bodies easily hold residues, and they may be exposed to contaminants via contaminated food resources such as nectar, pollen or water (Pohl, 2009). Heavy metals present in the atmosphere can be deposited on these hairy bodies and be brought back to the hive with pollen, or they may be absorbed together with nectar, water or honeydew (Caroli et al., 1999; Matei et al., 2004; Rodriguez Garcia et al., 2006; Bogdanov, 2006). Furthermore, almost all environments (soil, vegetation, water and air) are sampled by stingless bees, and thus provide numerous indicators (through foraging) for each season. Finally, a variety of potentially contaminated materials are brought into the hive (nectar, pollen, honeydew, propolis and water) and stored accordingly.

A number of variables need to be considered when using bees, or beehive products such as pollen, to monitor

heavy metals in the environment. These include flight distance, proximity to the contamination source, weather (rain and wind can clean the atmosphere or transfer heavy metals to other environmental sectors), season (nectar flow, which is usually greater in spring than in summer and autumn, could dilute pollutants) and botanical origin of the honey (nectar of flowers with an open morphology and honeydew are much more exposed to pollutants).

The present study reports the first effort to use stingless bees as ecological indicators. Our purpose was to investigate whether stingless bees are an effective environmental marker of contamination for monitoring environment pollution caused by mining activities through the analysis of pollen matrices over two climatic periods (dry and wet season) by comparing their contaminant content with suspended particulate matter (SPM). The study areas were located in mining sites that are susceptible to various levels of contamination due to different kinds of minerals present in the rocks.

Materials and methods

Sampling sites and biological matrices

Sampling of pollen matrices of *Tetragonisca angustula* (Latreille) beehives and suspended particulate matter (SPM) were performed from February to April (wet period) and August to September (dry period) of 2013 in five areas of the Iron Quadrangle, Minas Gerais, Brazil (Figure 1). We avoid sampling during periods of intense rainfall, in wet period. Suspended particulate matter (SPM) is here considered as finely divided solids that may be dispersed through the air from mining activities.

This area is known worldwide as one of the most important iron and gold producers of the world consequently undergo environmental contamination (CPRM 2014, Figueiredo et al., 2000). The substrates in this region are rich in iron, manganese and aluminum, and have given rise to the development of Rupestrian Fields (Campos Rupestres), also called Ferruginous Rupestrian Fields, ferruginous rocky outcrops, or “canga” vegetation (Schaefer et al., 2016; Silveira et al., 2016).

Four sites were located in mining sites of iron (SM), limestone (BM), gravel (IM) and soapstone (VM) mines, and one control site (CT). Besides the past intensive mining activity in a radius of 3 km, the CT site is today a Natural Protected area, covered by forest.

Three samples in each period (dry and wet) were taken from the bee hives of *Tetragosnica angustula* (3 colonies in each sampling site, totaling 90 samples) installed one km distance to the main mining pit. The distance of 1km is twice a maximum distance that *T. angustula* workers can fly (Nogueira-Neto, 1997). The samples were collected from the same colonies throughout the survey, with the exception that dead colonies were replaced in order to maintain the number of hives sampled. Pollen was collected directly from uncapped pollen combs in order to assure fresh pollen was collected.

These field-collected pools were immediately placed on ice and then stored at 22°C until analysis. Colonies used for the experiment were obtained from natural areas, outside of Iron Quadrangle.

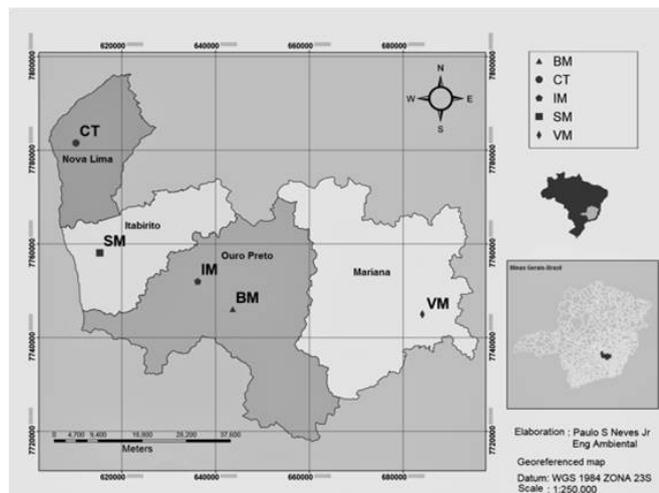


Fig 1. Sampling areas of the Iron Quadrangle, Minas Gerais, Brazil. BM (Bemil), CT (Control area), IM (Irmãos Machado), SM(SAFM), VM (Viamar).

Sample preparation

A simple system made from a high-density polyethylene (HDPE) bottle connected to a HDPE funnel was used for total SPM collection, following Azimi et al. (2003) (Supplementary Material Figure 1). These collectors were arranged in a north-south-east-west direction, relative to the bee's nest with a collector next to it in a central position. The five collectors per sampling area were arranged distant about 50 meters from the central. Collection bottles were filled in advance with 100 mL of a solution of 10% nitric acid (65% Suprapur nitric acid, Merck) and Mili-Q water in order to dissolve particulate matter and to avoid trace metal adsorption by bottle walls. The collection bottles were filled with concentrated nitric acid after the sampling period (7 days) to reach a final pH of 1, and samples were kept in a dark room at 5°C for one month in order to dissolve most of the particles. After storage for one-month, samples were filtered under a class 100 laminar hood with 0.45 µm porosity filters (Sartorius, cellulose nitrate). The sub-samples obtained were kept at 5 °C until analysis. Materials were washed following the procedures of Azimi et al. (2003).

Sample preparation was performed according to previously described methods (Miller & Miller, 2005; Vinas et al., 2000). Triplicates of 0.5 g of each samples of pollen were incinerated in a microwave oven (Step1: Ramp time - 10 min until 200°C; Step 2: Hold time - 15 min at 200 °C; Step 3: Cooling - 30 min) after acid digestion with 7 mL 65% m/m nitric acid (Suprapur, Merck, Darmstadt, Germany) and 1 mL 30% m/m hydrogen peroxide (Suprapur, Merck). All solutions were prepared with deionized water obtained by passing distilled water through a Millipore Milli-Q water purification system (Waters Corporation, Milford, MA, USA)

with a final volume of 50 mL. Chemical element content of the solutions was measured by ICP-OES (optical emission spectrometry with inductively coupled plasma) and ICP-MS (mass spectrometry with inductively coupled plasma).

Instruments and equipment

Analyses were carried out using a Microwave Milestone Ethos s1, ICP-MS (model Agilent 7700x) and ICP-OES (model Agilent 725). Analyses employed the following procedure: optimization of the instrument, calibration with standard solutions, analysis of the sample blank consisting of 2% ultra-pure nitric acid and analysis of the reference materials (after every 10 samples). The analyses were performed at the Laboratory of Geochemistry of the Geological Department of the Federal University of Ouro Preto (Brazil).

Assurance of analytical quality

Method precision was evaluated by recovery tests conducted at two concentration levels covering the concentration ranges in the samples. These solutions were analyzed after every ten samples and the coefficient of variation was determined for 3 repetitions due to the heterogeneity of the pollen samples.

Because of the unavailability of certified reference material for bee pollen, a certified reference material (Apple leaf, Nist SRM 1515) was evaluated to assess method accuracy for the recovery of elements.

Limit of detection (LOD) and limit of quantification (LOQ) values were calculated as suggested by Mermet and Poussel (1995): $LOD = (3 RSD \times BEC) / 100$ and $LOQ = 5 \times LOD$, where RSD is the relative standard deviation of blank samples and BEC is the background equivalent concentration, determined experimentally (n = 10). Limits of quantification (LOQ) are provided in Table 1.

Table 1. Limits of Quantification (LOQ) for major, minor and trace elements determined by ICP-OES and ICP-MS.

Element	SPM (µg/L)	Pollen (*µg/kg; **mg/Kg)
Al	8.920	3.674**
As	104.000	0.0328*
Ba	0.480	0.121*
Cd	6.640	0.00733*
Co	26.300	0.00766*
Cr	17.100	0.142*
Cu	5.370	0.896**
Fe	7.040	3.068**
Mn	2.280	0.382**
Ni	37.000	0.214*
Pb	181.000	0.0639*
Sb		0.0296*
Y	2.740	0.044**
Zn	6.210	0.634**

Statistical analysis

In total, 54 pollen and 60 SPM samples were analysed. For the statistical analyses, the site SM had to be excluded – the colonies perished due to high levels of dust deposited outside the nests. The statistical parameters for concentrations (mean, median and standard deviation) were calculated from all the analyzed samples of each matrix, and not only from samples for which residues were detected or quantified. When a compound was not quantified (< LOQ), the concentration used for statistical analysis was LOQ/2 (Hewett & Ganser, 2007).

Statistical analyses were performed only for pollen and SPM residues that were detected or quantified at least once. Comparisons of the means of element concentrations in pollen between climatic periods (dry and wet) were performed using the Friedman test (non-parametric test for paired samples).

The effects of climatic period and site location on metal concentrations were evaluated using a general linear mixed effect model (GLM). To be valid, a linear mixed effects model must exhibit independent and normally distributed residuals, which were assessed for each model through diagnostic plots using R-packages (plots of deviance residuals versus fitted values and normal quartile plot of Pearson residuals; plots not shown). Contrast analyses comparing metal concentrations among sites using metal as the explanatory variable were then been performed using R software (R Development Core Team 2010). AGLM analysis was used to test if the atmospheric deposition (SPM), as explanatory variable, was the primary factor for metal concentrations in pollen.

Principal component analysis (PCA) was used to investigate the multivariate structure of the dataset and to highlight possible trends among the data. Principal component analysis reduces the number of dimensions of a dataset by determining a linear combination of initial variables that maximizes the information content of a group of data. This is achieved by decomposition of the correlation matrix into eigenvalues and eigenvectors, with the eigenvectors representing the linear combination of coefficients and the corresponding eigenvalues representing the variance described by each linear combination. Since the eigenvalues are in decreasing order, the first linear components account for the largest amount of variance. Once derived, the principal components (PCs) can be used for further analyses to visualize groupings of the data, to verify the presence of outlier values and to determine the variables that discriminate among groups. For all these statistical tests, a significant effect was considered to have a type-I error risk of $p < 0.05$.

Results

Forty-seven chemical elements were detected and quantified from the pollen samples and 34 elements from the SPM samples. Mean contents, standard deviations and concentration ranges obtained for the elements of the 54 dehydrated bee pollen samples and 60 SPM samples from the

four sites in the Iron Quadrangle are shown in Tables 2 and 3, respectively.

Table 2. Mineral contents in $\mu\text{g}/\text{Kg}$ and mg/Kg for 54 dehydrated bee pollen samples collected from four sites in the Iron Quadrangle, MG, Brazil. (*) average of concentration no samples with <LOQ. Samples read in ICP-OES: mg/Kg (Al, Fe, Mn and Zn). Samples read in ICP-MS: $\mu\text{g}/\text{Kg}$ (As, Ba, Cd, Co, Cr, Cs, Cu, Ni, Pb, Sb and Y). Codes for areas are as in Figure 1.

Element	Sampling sites			
	CT	IM	VM	BM
	Average \pm SD Min/Max			
Al	78.18 \pm 11.69	470 \pm 381	318.7 \pm 114.5	140.5 \pm 27.4
	68.3/101	102/836	207/461	116/179
As	24.01 \pm 10.12	104.99 \pm 9.88	118.5 \pm 71.7	38.68 \pm 11.54
	11.87/35.33	94.51/114.14	57.2/242.7	29.69/55.85
Ba	6552* \pm 206	11590 \pm 4287	2690 \pm 681	461.1* \pm 65.2
	6315/6692	6095/15686	1821/3431	415/507.2
Cd	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ	<LOQ
Co	59.2 \pm 32.3	160.8 \pm 114	135.7 \pm 76.6	61.89 \pm 15.17
	29.4/106.1	33.8/273.1	62.7/213.3	46.42/76.51
Cr	864 \pm 207.5	211.5 \pm 172.1	3925 \pm 2038	1591 \pm 649
	717.9/1277	33.4/412.9	1976/5997	876/2560
Cu	4544 \pm 4950	3454 \pm 1773	2559* \pm 725	8070 \pm 1715
	109/10145	1399/5734	1878/3321	6369/10934
Fe	351.3 \pm 122.3	603 \pm 392	318.5 \pm 123.7	534.3 \pm 54
	213/482	218/973	205/454	463/607
Mn	73.5 \pm 27.2	31.63 \pm 6.96	34.41 \pm 5.13	63.87 \pm 12.98
	47.247.2	24.55/39.58	28.32/39.31	49.99/79.58
Ni	562.3 \pm 96.6	158.5 \pm 169.6	2655 \pm 494	775.1 \pm 136.9
	438.5/695.6	18.1/114.1	2094/3222	590.7/916.9
Pb	<LOQ	363.2 \pm 157.1	<LOQ	<LOQ
	<LOQ	129.9/472.1	<LOQ	<LOQ
Sb	210.3 \pm 28.6	286.8 \pm 117.8	216.1 \pm 27	277.4 \pm 92.9
	182.2/255.2	186.8/503.7	187.4/262.3	199.3/426.9
Y	9.027 \pm 2.35	285 \pm 251	28.16 \pm 11.21	32.9 \pm 27.2
	6.907/13.091	43/535	13.05/42	6.2/61.8
Zn	33.16 \pm 5.51	29.98 \pm 2.93	36.2 \pm 2.5	40.71 \pm 6.83
	26.78/38.66	27.02/33.25	32.82/40.24	33.67/50.91

Spatial and temporal distribution of metal elements

For comparisons among sites and seasons we chose to analyze the elements here classified as heavy metals due their toxicity and potential to cause damage to the environment: Cr, Co, Ni, Cu, As, Y, Sb, Cs, Ba, Cd, Pb, Al, Fe, Mn and Zn. Mean concentration of each of these 15 elements in pollen samples are shown in Table 2 and in SPM samples in Table 3. In general, wide within-site and among-sites concentration ranges were obtained for almost all elements both in pollen and SPM samples (Table 4). Three element groups can be

Table 3. Mineral contents in $\mu\text{g/L}$ for 60 Suspended Particulate Material (SPM) samples collected from four sites in the Iron Quadrangle, MG, Brazil (*) average of concentration no samples with <LOQ. Codes for the areas are as in Figure 1.

Elements	Sampling sites			
	CT	IM	VM	BM
	Average \pm SD Min/Max			
Al	138.7 \pm 94.9	744 \pm 559	322.1 \pm 235.3	346 \pm 429
	39.4/286	90/1838	70.5/754	17/1454
As	0.1267 \pm 0.0635	0.1042 \pm 0.0458	0.2359 \pm 0.1666	0.927 \pm 1.997
	0.051/0.282	0.045/0.185	0.085/0.619	0.072/5.837
Ba	4.73 \pm 3.57	135.4 \pm 297.9	8.32 \pm 4.9	12.22 \pm 11.56
	1.99/13.23	6.2/976	3.57/16.11	2.28/41.71
Cd	0.02028 \pm 0.01607	0.0958 \pm 0.1925	0.0618 \pm 0.1173	0.0138 \pm 0.00898
	0.00078/0.0623	0.0075/0.6371	0.0117/0.3924	0.00078/0.0292
Co	0.593 \pm 1.345	0.516 \pm 0.2887	0.4308 \pm 0.2581	0.4319 \pm 0.2129
	0.059/4.409	0.173/0.871	0.185/0.89	0.396/0.707
Cr	8.61 \pm 7.71	4.9 \pm 5.13	12.11 \pm 11.29	5.36 \pm 7.14
	1.21/21.77	0.95/13.45	1.53/29.38	0.51/20.76
Cu	3.98 \pm 3.77	6.81 \pm 6.52	6.85 \pm 5.87	2.776 \pm 3.009
	0.73/12.86	0.51/16.66	0.37/19.41	0.15/10.51
Fe	108.6 \pm 60.6	1328 \pm 1112	315.2 \pm 255.2	2751 \pm 3518
	50.9/241	144/3147	101/818	169/12030
Mn	101.1 \pm 101.9	330 \pm 677	65.2 \pm 80.7	779 \pm 848
	11.2/344	21/2244	12.7/275	39/2918
Ni	2.169 \pm 2.656	0.6039 \pm 0.204	5.61 \pm 4.43	1.083 \pm 0.471
	0.291/7.11	0.323/0.886	1.15/13.42	0.46/1.76
Pb	0.643 \pm 0.523	1.125 \pm 0.658	1.718 \pm 2.193	0.5332 \pm 0.2472
	0.259/2.03	0.697/2.93	0.344/7.5	0.188/0.864
Sb	2.562 \pm 0.6	1.827 \pm 0.376	2.738 \pm 1.341	1.38 \pm 0.601
	1.92/4.07	1.21/2.26	1.95/6.5	0.287/2.29
Y	<LOQ	3.125* \pm 0.233	<LOQ	8.38 \pm 10.59
		2.96/3.29		2.97/31.82
Zn	106.7 \pm 170.5	151.3 \pm 66.1	121.8 \pm 97	100.4 \pm 55.7
	18.8/586	96.6/314	55.8/373	58.4/247

differentiated: elements that are very abundant – present in the four sites in high concentrations (Cr,Cu, Ni,Fe, Ba); elements in moderate concentration; and trace elements (Pb, Cd, Cs, Y and Zn). Metal concentration in pollen was expressed in $\mu\text{g/Kg}$ (Cr, Co, Ni, As, Y, Sb, Cs e Ba, Cd, Pb) or mg/Kg (Cu, Al, Fe, Mn, Zn) and for SPM in $\mu\text{g/L}$ (Cr, Co, Ni, Cu, As, Y, Sb, Cs, Ba, Cd, Pb, Al, Fe, Mn and Z).

Samples coming from the sample site CT differed significantly ($p<0.01$) from those found in samples coming from mining areas. Pollen from site CT had, in general, lower concentrations of elements than the other sites with the exception of Ba, Cu, Mn and Ni elements. For SPM the concentrations of most of the elements in CT were highly variable in relation to the mining areas. The Fe concentration was very similar in the pollen samples of the four sampling sites, however, for SPM samples, values of Fe from CT

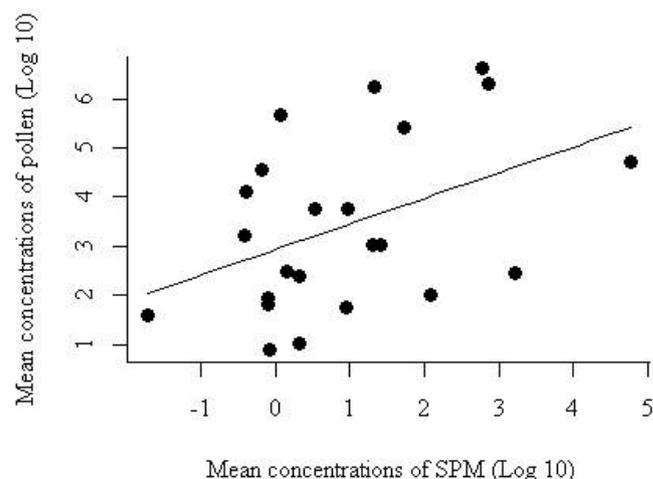
**Fig 2.** Relationship between mean concentration of metals on pollen and in Suspended Particulate Material (SPM).

Table 4. T-test values for the comparison of element concentrations in the dry and wet seasons for both for pollen and SPM samples. (*) sample < LOQ; bold numbers P < 0.05; P = P value; T = t-test value. Codes for areas are as in Figure 1.

QE	Sampling sites															
	CT				IM				VM				BM			
	Pollen		SPM		Pollen		SPM		Pollen		SPM		Pollen		SPM	
	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T
Al	0.099	-1.95	0.405	1.05	0.003	-4.98	0	-31.4	0.032	2.93	0.024	-6.32	0.137	-1.85	0.892	-0.15
As	0.695	-0.42	0.006	-6.91	0.003	-4.58	*	*	0.745	0.34	0.087	-3.16	0.451	0.83	0.978	0.03
Ba	0.125	-1.94	*	*	0.367	1.02	0.179	-2.04	0.173	1.55	0.005	-7.55	0.084	-2.29	*	*
Cd	0.625	-0.53	*	*	0.380	0.99	*	*	0.298	-1.19	*	*	0.221	1.34	*	*
Co	0.361	-1.03	0.029	-5.73	0	-7.74	0.018	-7.43	0.082	2.17	0.001	-26.34	-1.960	-1.96	0	-24.79
Cr	0.006	5.42	0.453	0.92	0.026	3.43	0.01	-9.93	0.004	6.16	0.001	-28.61	0.107	2.08	0.399	0.98
Cu	0.299	-1.16	*	*	0.345	1.01	0.011	5.66	0.075	-2.39	*	*	0.514	0.72	0.379	-1.12
Fe	0.050	-2.57	0.001	-11.68	0	-9.22	0.001	-28.84	0.053	2.72	0.010	-10.18	0.174	-1.65	0.035	-3.69
Mn	0.327	-1.09	0.004	-16.00	0.511	0.72	0.002	-11.05	0.300	-1.19	0.007	-12.23	0.089	-2.11	0.005	-7.60
Ni	0.252	-1.34	0.019	4.64	0.085	2.00	0.967	-0.05	0.445	0.81	0.008	-6.43	0.286	-1.17	0.010	-5.86
Pb	0.527	-0.69	*	*	0.402	0.94	*	*	0.714	-0.39	*	*	0.783	0.29	*	*
Sb	0.587	-0.59	0.164	-2.16	0.520	0.68	0.132	-2.47	0.367	-1.02	0.437	-0.96	0.560	0.62	0.103	-2.87
Y	*	*	0.792	-0.30	*	*	0	-29.2	*	*	0.028	-3.98	0.710	0.40	0.001	-12.34
Zn	0.243	-1.37	0.006	12.36	0.768	0.31	0.654	-0.5	0.531	-0.67	0.140	1.99	0.514	0.72	0.068	-3.63

and VM were much lower. Samples of SPM did not differ significantly in Sb and Cd concentration among the sites. We find a relation among elements presence on pollen and SPM samples in our sites ($F=4.92$, $p=0.03$) (Figure 2).

Pronounced variation within-sites and among-sites was observed in the levels of all elements during the two climatic periods both in pollen and in SPM samples (Tables 5 and 6). For pollen, the highest mean concentration was found in samples collected in the dry season, and all 15 elements differed significantly between the dry and wet seasons (Table 4 and 5). The concentration of Sb did not differ significantly between the two seasonal sampling periods for the four sampling sites, whereas site VM had the greatest differences in concentrations between dry and wet season (Table 4 and 5).

For the SPM samples, the concentrations of most of elements did not differ significantly between wet and dry period; only Cr and Al at sites CT, IM and VM, and Co, As and Fe at site IM differed significantly between climatic periods (Table 4 and 6).

The grouping of samples based on their similarities through PCA (Figure 3 and 4) indicates which chemical elements present in the pollen and SPM samples are responsible for clustering in both climatic periods. For pollen samples, the CCA explained 64.7% of the metal concentration variation between wet and dry climatic period. Samples from IM had higher concentrations of Cd, Sb, Ba, Cs, Fe, Pb, Y, Fe, Al, and Mn in the dry season (Figure 3A and B), while those from VM had higher concentrations of As, Co and Al in the dry climatic period.

For SPM samples, only 45.6% of the variation was explained by metal concentration in wet and dry climatic periods. But we found differences only for site SM, that had higher concentrations of Co, Mn, Al and Fe (Figure 4A and B).

Discussion

Our results showed that various quantities of toxic elements had accumulated in the stingless bee pollen samples, as expected. The level of accumulation depended on the type of rock being mined near where the pollen samples originated, as well as the sampling season. In the present study, pollen load was found to be the more contaminated matrix since we were able to detect and quantify more elements there than in SPM, maybe because bees fly in many directions searching for food, gathering “contaminated” pollen from a broader area. It must be noted that this pollen matrix has not been widely studied in other monitoring programs (Borgdanov, 2006; Chauzat et al., 2011; Oliveira et al., 2017). The samples from pollen loads from *T. angustula* proved to be a good indicator of the presence of metal released in the atmosphere and subsequently throughout the environment, exhibiting differences in the composition of metals among the sampling sites.

Among the 15 chosen elements, the highest concentrations were found for Barium, Chrome, Copper, Iron and Nickel, both in pollen and SPM, independent of the region from which the samples originated. These elements are widespread throughout the Iron Quadrangle since they are abundant components of its rock and soil (Costa et al., 2015; Oliveira et al., 2017).

Even though it is not possible to quantitatively compare our results with those of other monitoring programs, since only a few have been published and they considered different chemical elements, some qualitative comparisons can be made. Our findings were in line with other published studies in finding many different elements present in bee matrices in industrial areas. High heavy element concentrations, as we found, has been reported previously for pollen and honey coming from areas with heavy industrial activities or busy

Table 5. Mineral contents in $\mu\text{g}/\text{Kg}$ and mg/Kg for 54 dehydrated bee pollen samples collected during wet (W) and dry (D) season, from four sites in the Iron Quadrangle, MG, Brazil. (*) average of concentration no samples with <LOQ. Samples read in ICP-OES: mg/Kg (Al, Fe, Mn and Zn). Samples read in ICP-MS: $\mu\text{g}/\text{Kg}$ (As, Ba, Cd, Co, Cr, Cs, Cu, Ni, Pb, Sb and Y). Codes for areas are as in Figure 1.

	BM-W	BM-D	CT-W	CT-D	IM-W	IM-D	VM-W	VM-D
Al	138.6667	142.3333	83.1333	73.2333	122.6667	816.6667	219.0000	418.3333
As	38.8373	38.5166	15.1382	32.8802	1.2119	104.9892	63.2459	173.8340
Ba	36.9478	319.8446	35.6247	6551.5150	8797.5120	14383.4800	2089.0270	3290.3260
Cd	3.8774	3.9120	3.7385	3.8832	3.8453	3.8776	3.7891	3.803702
Co	48.0900	75.6960	31.3442	87.1070	60.267	261.3072	66.0247	205.4273
Cr	1851.7450	1330.2330	943.3929	784.6034	57.4582	365.4744	2068.8300	5780.761
Cu	7305.7980	8834.9580	8978.2190	110.0410	4979.3780	1927.9390	107.3742	2558.859
Fe	491.0000	577.6667	241.3333	461.3333	246.0000	960.6667	207.6667	429.3333
Mn	52.4100	75.3367	48.9000	98.1600	25.3833	37.8833	29.7900	39.02667
Ni	656.8879	893.3705	643.2998	481.3357	110.4292	206.6642	2224.7640	3085.544
Pb	9.8713	9.9571	9.5179	9.8863	49.8360	440.9491	9.6466	9.683775
Sb	207.7850	347.0570	191.1601	229.4160	203.2694	370.2805	205.3813	226.7653
Y	8.3697	57.4485	8.7082	9.3452	56.1115	513.9255	19.0124	37.30830
Zn	35.2533	46.1733	38.1233	28.1868	29.3366	30.6233	37.8100	34.59333

Table 6. Mineral contents in $\mu\text{g/L}$ for 160 Suspended Particulate Material (SPM) samples collected during wet (W) and dry (D) season, from four sites in the Iron Quadrangle, MG, Brazil (*) average of concentration no samples with <LOQ. Codes for the areas are as in Figure 1.

	BM-W	BM-D	CT-W	CT-D	IM-W	IM-D	VM-W	VM-D
Al	150.5860	541.4000	145.2520	132.0880	651.4000	835.9880	451.0060	193.1060
As	13.5000	0.1344	0.1080	0.1454	0.0980	0.1104	0.2356	0.2362
Ba	68.1200	176.3000	4.8480	4.6160	239.5120	31.2060	10.6280	6.0120
Cd	0.0132	0.0092	0.0132	0.0274	0.1634	0.0290	0.0946	0.0290
Co	0.2781	0.4994	10.0120	0.1850	0.5070	0.5250	0.5484	0.3132
Cr	649.9200	42.9920	94.8280	7.7300	60.3760	37.65400	19.2420	4.9740
Cu	347.2000	20.8080	2.3160	5.6340	10.7560	2.8700	3.9920	9.7120
Fe	1109.8000	4391.6000	100.9400	116.1680	1106.2000	1.5490	453.0000	177.4000
Mn	346.9760	1210.6000	109.7600	92.4160	530.5260	129.486	37.7220	92.7480
Ni	0.6324	1.3220	21.6260	2.1760	0.7606	0.4472	86.6380	2.5580
Pb	0.5090	0.4522	0.3956	0.8900	13.0960	0.9410	27.9580	0.6408
Sb	14.6140	1.2980	2.6820	2.4420	1.8440	1.8100	2.3020	3.1740
Y	77.9760	418.9580	0.4315	0.4315	0.4315	151.0407	0.4315	0.4315
Zn	110.5300	90.364	57.206	156.122	167.2	135.314	148.244	95.272

highways (D'Ambrosio & Marchesini, 1982; Gajek et al., 1987; Oliveira et al., 2017).

The spatial distribution of metal elements was directly related to the region/mining type, since almost all five sampling areas had a high degree of similarity in the presence and

concentration of metals, including, to our surprise, the control area. Thus, the elements detected in the pollen samples of this study are a “mirror” of the rock type as well as the SPM composition. It is important to notice that the most similar sampling sites were those with the most similar mining rock,

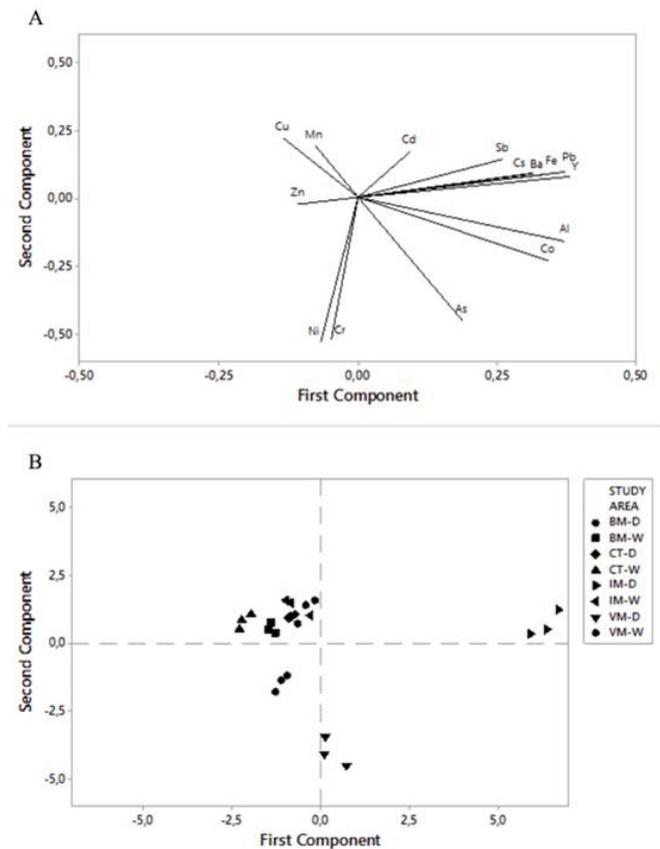


Fig 3. Loading Plot (A) and Score Plot (B) - Principal components 1 and 2 - obtained from mean composition of mineral elements in 40 dehydrated bee pollen samples from four areas in the Iron Quadrangle in dry (D) and wet (W) seasons.

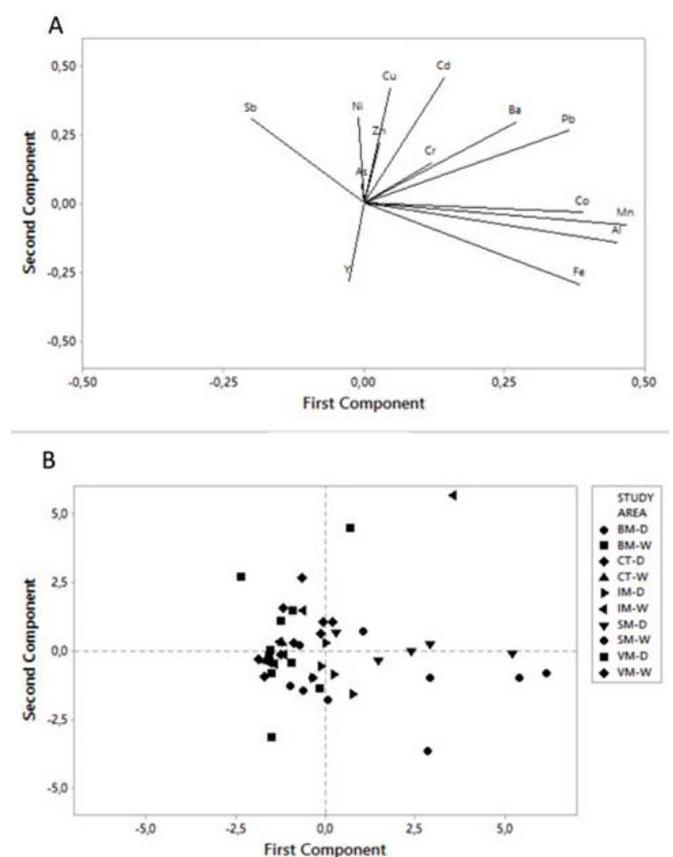


Fig 4. Loading Plot (A) and Score Plot (B) - Principal components 1 and 2 - obtained from mean composition of mineral elements in 60 SPM samples from four areas in the Iron Quadrangle in dry (D) and wet (W) seasons.

like BM and IM (ore for crushed stone), which had a high degree of similarity, while SF (iron ore) was more similar to CT. Besides CT is a Natural Protected Area, it is in a region with many abandoned or reclaimed iron ore mining. According to Navarro et al. (2008) residues from mining and metallurgical operations are often dispersed by wind and/or water after their disposal. According to Costa et al. (2015), the Iron Quadrangle possesses some signature minerals that represent the main rock or soil. This also could explain the similarities in metal presence among CT site and SF (iron ore), for example.

In our sampling sites, As was quantified in both pollen and SPM, although in low concentrations. The significant presence of As in this lithotype is corroborated by its occurrence in arsenopyrite, loellingite, realgar and arseniferous pyrite and the local gold mineralization. Furthermore, the Geochemical map of the Iron Quadrangle showed the highest density of positive As, Cd, Cu, Ni, Cr and Zn anomalies (Costa et al., 2015) According to these authors, these elevated concentrations were due to the interaction between geology and human interference.

For Cd, however, we found concentrations above that recommended by Brazilian regulation only in SPM. Also, according to Costa et al. (2015), the Iron Quadrangle possesses a natural liberation of trace elements, such as Cd, from rocks and soils, which may explain the higher level of this element in SPM samples.

Lead was detected in SPM samples of the four studied sites, however, for pollen samples it was quantified only in samples from IM. Despite occurring in low concentrations, the amount of Pb in SPM samples was detected and quantified for all sampling sites. Again, according to Costa et al. (2015), Pb distribution in the Iron Quadrangle is characterized by low concentrations. It is important to note, however, that the highest Pb concentrations were found in areas heavily impacted by mining activities, and related to rock types such as granites and gneisses, as are found at the IM mining site. Perugini et al. (2011) reported similar results, with a significant difference in Pb concentration in bees among stations located in urban areas and stations located in natural reserves; the highest mean concentrations were detected in honeybees collected from hives with surroundings characterized by intense air traffic and motor vehicle circulation.

Zinc distribution and concentration was found to be very similar among sampling sites, both for pollen and SPM samples, however, the concentrations were low. This finding is corroborated by the fact that a large part of the Iron Quadrangle possesses Zn values below the limits of intervention established by environmental legislation (Pereira et al. 2007).

Climatic seasons

The low level of variation observed in the concentration of almost all chemical elements within each of the two seasonal periods, even though the highest mean concentration of some elements was found in samples collected in dry

climatic period, only a few elements had significantly different concentrations than in the wet period. Although this variation presents a challenge to the appraisal of geographic effects, it also can reflect the influence of climate on the concentration of metal in the environment. It should be also considered that in Iron Quadrangle the wet period (October to March) are marked for days of intense rainfall, followed by weeks with no rain, what could explain the similarities in metal concentration among the climatic periods.

For pollen samples, greater differences between climatic periods were found for Al, Co, Cr and Fe. Pollen seemed to be more sensitive to climatic influences than SPM in the present study, which was expected since mining activity does not stop, even during the rainy season.

Foraging bees must search for food on flowering plants that, in the case of mining areas, only exist inside the remaining small patches of forest. Nonetheless, these plants still continuously receive high volumes of SPM deposition on their flowers.

However, for SPM pronounced differences were found between seasons, with higher concentrations of more elements during the dry season. In the Iron Quadrangle, there is very little rainfall in the dry season and the amount of dust in the atmosphere is very high. Therefore, it seems likely that this temporal distribution of contamination levels is linked to meteorological conditions and/or to the different types of plants foraged by bees (Perugini et al., 2011). Azimi et al. (2003), also found high levels of variation in the concentration of elements (Pb, Cd and Cu) among studies conducted in different years and seasons. Lambert et al. (2012) also compared the concentration of Pb among dry and wet seasons and found higher values for the wet season.

Past studies (Magalhaes, 2007; Oliveira et al., 2017) have shown that differences in contamination levels might be related to the landscape, but they have also revealed a relationship between urbanization, industrialization or anthropogenic activities and high contaminations in matrices. In our study, the general trends were influenced by certain contaminants. Some chemical elements have already been quantified in bee pollen samples from several countries: potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), sodium (Na), sulfur (S), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), chromium (Cr), nickel (Ni) and selenium (Se) (e.g. Azimi et al., 2013; Bilandžić et al., 2011, Bratu & Beorgescu, 2005; Chauzat et al., 2011). The number of samples analyzed, however, is limited, varying from one to fifty per study. Two of these were studies in Brazil (Magalhaes, 2007; Oliveira et al., 2017) and involved 24 samples collected from only one sampling site in the state of São Paulo, and 36 samples from southern Brazil.

There is no benchmark for the maximum concentration of heavy metal allowed in pollen, as there is for honey, although, for pollen, there is for few individual elements like As (0.30 µg/Kg), Pb (0.30 µg/Kg) and Cd (0.10 µg/Kg)

(ANVISA 1998). For As in pollen samples, for example, we found concentrations many times higher than those established by regulations. For samples of Pb, from SPM, at the site IM, the concentrations were also much higher than those established by regulations for honey. It is important to note that the concentrations of other elements, without benchmark values for pollen and honey, were also very high. Other chemical elements that have a limit established for food in general, or for food other than honey and pollen, are: Cr (0.10 mg/kg), Cu (10.0 mg/kg), Ni (5.0 mg/kg), Sb (2.0 mg/kg), Zn (50.0 mg/Kg) (ANVISA1998), Al (7.0 mg/Kg), Fe (found no tolerance limit established for human consumption, but found a stipulated maximum value of 0.8 mg/Kg body weight), Mn (between 780 – 930 mg/kg) (ANVISA1998). Due to the deficiency of regulations, it can be inferred that many of the samples had anomalous values exceeding stipulated limits when considering the few regulations that do exist for human consumption as mentioned above.

We assumed that the contribution of chemicals elements on pollen, caused by translocation via soil, may be much lower than that of atmospheric deposition. More than 600 plant species were identified in the Iron Quadrangle, but information about metal concentrations in leaves and other plant parts is available for only 14 species (Porto & Silva, 1989; Ribeiro et al., 2016). In a more recent effort, foliar metal concentrations, specifically AL, Fe and Mn, were determined for 55 plant species (31 botanical families) (Antonella Schettini pers. comm.), with the ability of accumulating at least one of these metals in the leaves seemingly present in most of them, however, varying from minimum to maximum concentration by a factor of 47 for Mn, 50 for Fe and 314 for Al. According to Ribeiro et al. (2016) three plant species visited by *T. angustula* (*Eremanthus incanus*, *E. erytropappus* and *Baccharis* sp.) have the ability to translocate metals such as Pb, Cd, Cu, Cr, Co, Y, Ni, Ba, Fe, Al, Zn, and Mn to leaves, but not to flowers.

Wide within-area and among-areas variation was found in the mineral composition of dehydrated bee pollen, and Fe, Ba, Al and Mg were the major chemicals elements present. The five areas exhibited differences in chemical composition. In terms of mean values, bee pollen may be considered a rich source of information as a bioindicator for biomonitoring.

It should be mentioned that pollen matrices from stingless bees proved to be an efficient environmental marker for metal contamination of the environment since we found significant differences in the quality and quantity of metals among the four sampling sites. Since the sampling sites were associated with different types of mining, the metal composition present likely reflects the mineral composition of the dust generated by mining activities, which accumulates on flowers and consequently is carried to nests by stingless bee workers.

Acknowledgments

The authors gratefully acknowledge CNPq for scholarships to YA and HANJ, CAPES for the scholarship to FA, and CAPES for the scholarship to NON. Many thanks also to the following mining companies for the permission: SAFM, Viamar, Pedreira Irmãos Machado and Bemil. We also like to thank Adam Roman and John Trumble for the suggestions made in an earlier version of the manuscript.

References

- ANVISA - Agência Nacional de Vigilância Sanitária; Brazilian Ministry of Health. (1998). Portaria N. 27, 13 de Janeiro de 1998: Informação Nutricional Complementar, Brasília, DF, Brasil.
- Azevedo, U.R., Machado, M.M.M., Castro, P.T.A., Renger, F.E., Trevisol, A. & Beato, D.A.C. (2012). Geoparque Quadrilátero Ferrífero (MG) – proposta. In: Schobbenhaus, C. & Silva, C.R. (org). Geoparques do Brasil. CPRM – Serviço Geológico do Brasil.
- Azimi, S., Ludwig, A., Théev Venot, D.R. & Colin, J.L. (2003). Trace metal determination in total atmospheric deposition in rural and urban areas. *The Science of the Total Environment*, 308: 247-256.
- Baêta, H.E. (2012). Contribuição da deposição úmida (chuva e neblina) nas relações hídricas e nutricionais de fisionomias de campos ferruginosos na Serra da Brígida, ouro preto, MG. Dissertação (Mestrado) – Instituto de Ciências Exatas e Biológicas, Universidade Federal de Ouro Preto, Minas Gerais
- Bilandžić, N., Okić, M., Sedak, M., Kolanović, B.S., Varenina, I., Končurat, A. & Rudan, N. (2011). Determination of trace elements in Croatian floral honey originating from different regions. *Food Chemistry*, 128: 1160-1164. doi: 10.1016/j.foodchem.2011.04.023
- Bogdanov, S. (2006). Contaminants of bee products. *Apidologie*, 37: 1-18. doi: 10.1051/apido:2005043
- Bratu, I. & Beorgescu, C. (2005). Chemical contamination of bee honey – identifying sensor of the environment pollution. *Journal of Central European Agriculture*. 6: 95-98.
- Caroli, S., Forte, G., Iamiceli, A.L. & Galoppi, B. (1999). Determination of essential and potentially toxic trace elements in honey by inductively coupled plasma-based techniques *Talanta*, 50: 327-336.
- Carvalho-Filho, A., Curi, N. & Shinzato, E. (2010). Relações solo-paisagem no Quadrilátero Ferrífero em Minas Gerais. *Pesquisa Agropecuária Brasileira*, 45: 903-916.
- Chauzat, M.P., Martel, A.C., Cougoule, N., Porta, P., Lachaize, J., Zeggane, S., et al. (2011). An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera: Apidae) to

- monitor pesticide presence in continental France. *Environmental Toxicology and Chemistry*, 30: 103-111. doi: 10.1002/etc.361
- Costa, R.V.F., Matschullat, J., Garcia, M.L., Nalini-Junior, H.A. & Mendonça, F.P.C. (2015). Iron Quadrangle stream sediments, Brazil: geochemical maps and reference values. *Environ Earth Science*, 74: 4407-4417.
- CPRM. Serviço geológico do Brasil. (2014). Secretaria de geologia, mineração e transformação mineral – Ministério de Minas e Energia. Brasil.
- D'Ambrosio, M. & Marchesini, A. (1982). Heavy metal contamination of honey. *Atti Società Italiana Scienze Naturali*, 123: 342-348.
- FAO (1998). World reference base for soil resources. World Soil Resources Report 84, FAO/ISRIC/ISSS, Rome.
- Figueiredo, B.R., Gabriod, T. & Schwenkd, M. (2000). Human and environmental contamination in the Iron Quadrangle, Brazil. *Applied Geochemistry*, 15: 181-190.
- Formick, G., Gren, A., Stawarz, R., Zysk, B. & Gal, A. (2013). Metal Content in Honey, Propolis, Wax, and Bee Pollen and Implications for Metal Pollution Monitoring. *Polish Journal Environmental Study*, 22: 99-106.
- Freedman, B. & Hutchinson, T. (1981). Source of metal and contamination of terrestrial environments. Pp35-94. In: Leep, N. W. Leep (ed). *Effect of heavy metal pollution on plants*. Applied Science Publishers, London, 35-94.
- Gajek, O., Gdanski, M. & Gajewska, R. (1987). Metallic impurities in imported canned fruit and vegetables and bee honey. *Roczniki Panstwowego Zakladu Higieny*, 38:14-20.
- Hewett, P. & Ganser, G.H. (2007). A Comparison of Several Methods for Analyzing Censored Data. *The Annals of Occupational Hygiene*, 51: 611-632.
- Lambert, O., Puyo, M.P.S., Thorin, C., Larhantec, M. & Pouliquen, F.D.H. (2012). Bees, honey and pollen as sentinels for lead environmental contamination. *Environmental Pollution*, 170: 254-259. doi: 10.1016/j.envpol.2012.07.012
- Leita, L., Muhlbachova, G., Cesco, S., Barbattini, R. & Mondini, C. (1996). Investigation of the use of honey bees and honey bee products to assess heavy metals contamination. *Environmental Monitoring Assessment*, 43: 1-9.
- Magalhães, M. (2007). Mel e pólen de *Apis mellifera* como bioindicador de poluição ambiental por metais pesados. Dissertação de Mestrado. Universidade Federal de Ouro Preto, MG, Brazil, pp.66.
- Matei, N., Birghila, S., Dobrinas, S. & Capota, P. (2004). Determination of C Vitamin and some Essential Trace Elements (Ni, Mn, Fe, Cr) in Bee Products. *Acta Chimica Slovenica*, 51: 169-175.
- Matina, G., Kargarb, N. & Buyukisika, H.B. (2016). Bio-monitoring of cadmium, lead, arsenic and mercury in industrial districts of Izmir, Turkey by using honey bees, propolis and pine tree leaves. *Ecological Engineering*, 90: 331-335. doi: 10.1016/j.ecoleng.2016.01.035
- McGeoch, M.A. (2007). Insects and bioindication: Theory and progress. In: Stewart, A.J.A., New, T.R., & Lewis, O.T. (Eds), *Insect conservation biology: Proceedings of the Royal Entomological Society*, CABI, Wallingford, Oxfordshire, pp. 144-174.
- Meneses, I., Renger, F.E. & Deschamps, E. (2011). History and socioeconomy of Iron Quadrangle. In: Deschamps, E., Matschullat, J. (eds). *Arsenic: natural and anthropogenic. Arsenic in the environment*, pp 73–80, CRC Press London.
- Mermet, J.M. & Poussel, E. (1995). ICP Emission Spectrometers: 1995 Analytical Figures of Merit. *Applied Spectroscopy*, 49: 12A-18A.
- Messias, M.C.T.B., Leite, M.G.P., Meira Neto, J.A.A., Kozovits, A.R. & Tavares, R. (2013). Soil-Vegetation Relationship in Quartzitic and Ferruginous Brazilian Rocky Outcrops. *Folia Geobotanica*, 48: 509-521. doi: 10.1007/s12224-013-9154-4
- Miller, J.N. & Miller, J.C. (2005). *Statistics and Chemometrics for Analytical Chemistry*, Pearson Education, Harlow.
- Minussi, L.C. & Alves-Dos-Santos, I. (2007). Abelhas nativas versus *Apis mellifera*, Linnaeus, espécie exótica (Hymenoptera: Apidae). *Bioscience Journal*, 23: 58-62.
- Morgano, M.A., Martins, M.C.T., Rabonato, L.C., Milani, R.F., Yotsuyanagi, K. & Rodriguez-Amaya, D.B. (2012). A Comprehensive Investigation of the Mineral Composition of Brazilian Bee Pollen: Geographic and Seasonal Variations and Contribution to Human Diet. *Journal of Brazilian Chemical Society*, 23: 727-736.
- Nogueira-Neto, P. (1997). *Vida e criação de abelhas indígenas sem ferrão*. São Paulo: Nogueirapis, pp.445
- Oliveira, F.A., Abreu, A.T., Oliveira Nascimento, N.O., Froes-Silva, R.E., Antonini, Y., Nalini-Júnior, H.A. Jr. & Lena, J.C. (2017). Evaluation of matrix effect on the determination of rare earth elements and As, Bi, Cd, Pb, Se and In in honey and pollen of native Brazilian bees (*Tetragonisca angustula* - Jataí) by Q-ICP-MS. *Talanta*, 162C: 488-494. doi: 10.1016/j.talanta.2016.10.058
- Pereira, J.C., Guimarães-Silva, A. K., Nalini-Júnior, H.A., Pacheco-Silva E. & Lena, J.C. (2007). Distribuição, fracionamento e mobilidade de elementos traço em sedimentos superficiais. *Química Nova*, 30: 1249-1255.
- Perugini, M., Manera, M., Grotta, L., Abete, M.C., Tarasco, R. & Amorena, M. (2011). Heavy metals (Hg, Cr, Cd and Pb) contamination in urban areas and wildlife reserves: honeybees as bioindicators. *Biological Trace Element Research*, 140: 170-176. doi: 10.1007/s12011-010-8688-z

- Pohl, P. (2009). Determination of metal content in honey by atomic absorption and emission spectrometries. *Trends in Analytical Chemistry*, 28: 117-128.
- R language and environment for statistical computing (2010). R Foundation for statistical computing. Vienna, Austria.
- Rapini, A., Ribeiro, P.L., Lambert, S. & Pirani, J.R. (2008). A flora dos campos rupestres da Cadeia do Espinhaço. *Mega-diversidade*, 4: 15-23.
- Ribeiro, S.P., Londe, V., Bueno, A.P., Barbosa, J.S., Corrêa, T.L., Soeltl, T., Maia, M., Pinto V.D., Dueli, G.F. Sousa, H.C.D., Kozovits, A. R. & Nalini Jr, H.A. (2016). Plant defense against leaf herbivory based on metal accumulation: examples from a tropical high altitude ecosystem. *Plant Species Biology*, 32: 147-155. doi: 10.1111/1442-1984.12136
- Schaefer, C.E., Cândido, H.G., Corrêa, G.R., Nunes, J.A & Arruda, D.M. (2016). Soils Associated with Rupestrian Grasslands. In: Fernandes, G. W. (Ed.), *Ecology and Conservation of Mountaintop grasslands in Brazil* (pp. 55-69). Springer International Publishing. doi: 10.1007/978-3-319-29808-5.
- Silveira, F.A.O., Negreiros, D., Barbosa, N.P.U., Buisson, E., Carmo, F.F., Carstensen, D.W., Conceição, A.A., Cornelissen, T.G., Echternacht, L., Fernandes, G.W., Garcia, Q.S., Guerra, T.J., Jacobi, C.M., Lemos-Filho, J.P., Le Stradic, S., Morellato, L.P.C., Neves, F.S., Oliveira, R.S., Schaefer, C.E., Viana, P.L. & Lambers, L. (2016). Ecology and evolution of plant diversity in the endangered campo rupestre: a neglected conservation priority. *Plant and Soil*, 403: 129-152. doi: 10.1007/s11104-015-2637-8
- USDA. (1998). Keys to soil taxonomy. United States Department of Agriculture, New York.
- Valim, E.A.R. (2012). Ciclagem de nutrientes no sistema atmosfera-solo-planta em formação campestre e florestal sobre canga em Minas Gerais. Dissertação (Mestrado) - Instituto de Ciências Exatas e Biológicas, Universidade Federal de Ouro Preto, Minas Gerais
- Vinas, P., Pardo-Martinez, M. & Hernandez-Cordoba, M. (2000). Rapid determination of selenium, lead, and cadmium in baby food samples using electrothermal atomic absorption spectrometry and slurry atomization. *Analytica Chimica Acta*, 412: 121-130.
- Wayne, L. (1983). Bee miners join British Columbia God Hunt. *American Bee Journal*, 3: 635-637.

