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

NO NASCIMENTO, HA NALINI, F ATAIDE, AT ABREU, Y ANTONINI
1 - Universidade Federal de Ouro Preto, Departamento de Biodiversidade Evolução e Meio Ambiente, Instituto de Ciências Exatas e Biológicas, Ouro Preto, Minas Gerais, Brazil
2 - Universidade Federal de Ouro Preto, Departamento de Geologia, Escola de Minas, Ouro Preto, Minas Gerais, Brazil

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.

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; Bilandzić 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; Bilandzić 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 *Tetragnostisca 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 RSDxBEC)/100 and LOQ = 5 x 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.

<p>| Table 1. Limits of Quantification (LOQ) for major, minor and trace elements determined by ICP-OES and ICP-MS. |
|-----------------|--------|-----------------|</p>
<table>
<thead>
<tr>
<th>Element</th>
<th>SPM (µg/L)</th>
<th>Pollen (*µg/kg; **mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>8.920</td>
<td>3.674**</td>
</tr>
<tr>
<td>As</td>
<td>104.000</td>
<td>0.0328*</td>
</tr>
<tr>
<td>Ba</td>
<td>0.480</td>
<td>0.121*</td>
</tr>
<tr>
<td>Cd</td>
<td>6.640</td>
<td>0.00733*</td>
</tr>
<tr>
<td>Co</td>
<td>26.300</td>
<td>0.00766*</td>
</tr>
<tr>
<td>Cr</td>
<td>17.100</td>
<td>0.142*</td>
</tr>
<tr>
<td>Cu</td>
<td>5.370</td>
<td>0.896**</td>
</tr>
<tr>
<td>Fe</td>
<td>7.040</td>
<td>3.068**</td>
</tr>
<tr>
<td>Mn</td>
<td>2.280</td>
<td>0.382**</td>
</tr>
<tr>
<td>Ni</td>
<td>37.000</td>
<td>0.214*</td>
</tr>
<tr>
<td>Pb</td>
<td>181.000</td>
<td>0.0639*</td>
</tr>
<tr>
<td>Sb</td>
<td>0.0296*</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>2.740</td>
<td>0.044**</td>
</tr>
<tr>
<td>Zn</td>
<td>6.210</td>
<td>0.634**</td>
</tr>
</tbody>
</table>
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 quantile 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>
<thead>
<tr>
<th>Element</th>
<th>CT</th>
<th>IM</th>
<th>VM</th>
<th>BM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average±SD</td>
<td>Min/Max</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>78.18±11.69</td>
<td>470±381</td>
<td>318.7±114.5</td>
<td>140.5±27.4</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Co</td>
<td>24.0±10.12</td>
<td>104.99±9.88</td>
<td>118.5±71.7</td>
<td>38.68±11.54</td>
</tr>
<tr>
<td>Cr</td>
<td>11.87±35.33</td>
<td>94.51±114.14</td>
<td>57.2±242.7</td>
<td>29.69±55.85</td>
</tr>
<tr>
<td>Cu</td>
<td>6552±206</td>
<td>11590±4287</td>
<td>2690±681</td>
<td>461.1±66.2</td>
</tr>
<tr>
<td>Fe</td>
<td>6315/6962</td>
<td>6095/15686</td>
<td>1821/3431</td>
<td>415/507</td>
</tr>
<tr>
<td>Mn</td>
<td>59.2±32.3</td>
<td>160.8±114</td>
<td>135.7±76.6</td>
<td>61.89±15.17</td>
</tr>
<tr>
<td>Ni</td>
<td>29.4/106.1</td>
<td>33.8/273.1</td>
<td>62.7/213.3</td>
<td>46.42/76.51</td>
</tr>
<tr>
<td>Pb</td>
<td>864±207.5</td>
<td>211.5±172.1</td>
<td>3925±2038</td>
<td>1591±649</td>
</tr>
<tr>
<td>Sb</td>
<td>717.9/1277</td>
<td>33.4/142.9</td>
<td>1976/5997</td>
<td>876/2560</td>
</tr>
<tr>
<td>Zn</td>
<td>4544±4950</td>
<td>3454±1773</td>
<td>2559±725</td>
<td>8070±1715</td>
</tr>
<tr>
<td>Average±SD</td>
<td>Min/Max</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>68.3/101</td>
<td>102/836</td>
<td>207/461</td>
<td>116/179</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Co</td>
<td>2.47/10.12</td>
<td>104.99±9.88</td>
<td>118.5±71.7</td>
<td>38.68±11.54</td>
</tr>
<tr>
<td>Cr</td>
<td>11.87±35.33</td>
<td>94.51±114.14</td>
<td>57.2±242.7</td>
<td>29.69±55.85</td>
</tr>
<tr>
<td>Cu</td>
<td>6552±206</td>
<td>11590±4287</td>
<td>2690±681</td>
<td>461.1±66.2</td>
</tr>
<tr>
<td>Fe</td>
<td>6315/6962</td>
<td>6095/15686</td>
<td>1821/3431</td>
<td>415/507</td>
</tr>
<tr>
<td>Mn</td>
<td>59.2±32.3</td>
<td>160.8±114</td>
<td>135.7±76.6</td>
<td>61.89±15.17</td>
</tr>
<tr>
<td>Ni</td>
<td>29.4/106.1</td>
<td>33.8/273.1</td>
<td>62.7/213.3</td>
<td>46.42/76.51</td>
</tr>
<tr>
<td>Pb</td>
<td>864±207.5</td>
<td>211.5±172.1</td>
<td>3925±2038</td>
<td>1591±649</td>
</tr>
<tr>
<td>Sb</td>
<td>717.9/1277</td>
<td>33.4/142.9</td>
<td>1976/5997</td>
<td>876/2560</td>
</tr>
<tr>
<td>Zn</td>
<td>4544±4950</td>
<td>3454±1773</td>
<td>2559±725</td>
<td>8070±1715</td>
</tr>
</tbody>
</table>

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 and SPM 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 µg/L for 60 Suspended Particulate Material (SMP) 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.

<table>
<thead>
<tr>
<th>Elements</th>
<th>CT</th>
<th>IM</th>
<th>VM</th>
<th>BM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average±SD</td>
<td>Min/Max</td>
<td>Average±SD</td>
<td>Min/Max</td>
</tr>
<tr>
<td>Al</td>
<td>138.7±94.9</td>
<td>744±559</td>
<td>322.1±235.3</td>
<td>346±429</td>
</tr>
<tr>
<td>As</td>
<td>0.1267±0.0635</td>
<td>0.1042±0.0458</td>
<td>0.2359±0.1666</td>
<td>0.927±1.997</td>
</tr>
<tr>
<td>Ba</td>
<td>4.73±3.57</td>
<td>135.4±297.9</td>
<td>322.1±235.3</td>
<td>4.73±3.57</td>
</tr>
<tr>
<td>Cd</td>
<td>0.02028±0.01607</td>
<td>0.0958±0.1925</td>
<td>0.0618±0.1173</td>
<td>0.0138±0.00898</td>
</tr>
<tr>
<td>Co</td>
<td>0.593±1.345</td>
<td>0.516±0.2887</td>
<td>0.4308±0.2581</td>
<td>0.4319±0.2129</td>
</tr>
<tr>
<td>Cr</td>
<td>0.059/4.009</td>
<td>0.173/0.871</td>
<td>0.185/0.89</td>
<td>0.396/0.707</td>
</tr>
<tr>
<td>Cu</td>
<td>8.61±7.71</td>
<td>4.9±5.13</td>
<td>12.11±11.29</td>
<td>5.36±7.14</td>
</tr>
<tr>
<td>Fe</td>
<td>121.8±97</td>
<td>121.8±97</td>
<td>121.8±97</td>
<td>121.8±97</td>
</tr>
<tr>
<td>Mn</td>
<td>101.1±101.9</td>
<td>330±677</td>
<td>65.2±80.7</td>
<td>779±848</td>
</tr>
<tr>
<td>Pb</td>
<td>2.169±2.656</td>
<td>0.6039±0.204</td>
<td>5.61±4.43</td>
<td>1.083±0.471</td>
</tr>
<tr>
<td>Sb</td>
<td>2.562±0.6</td>
<td>1.827±0.376</td>
<td>2.738±1.341</td>
<td>1.38±0.601</td>
</tr>
<tr>
<td>Y</td>
<td>&lt;LOQ</td>
<td>0.51/20.76</td>
<td>0.396/0.707</td>
<td>0.15/10.51</td>
</tr>
<tr>
<td>Zn</td>
<td>106.7±170.5</td>
<td>151.3±66.1</td>
<td>121.8±97</td>
<td>100.4±55.7</td>
</tr>
</tbody>
</table>

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 µg/K (Cr, Co, Ni, As, Y, Sb, Cs e Ba, Cd, Pb) or mg/K (Cu, Al, Fe, Mn, Zn) and for SPM in µg/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
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 valu; T = t-test value. Codes for areas are as in Figure 1.

<table>
<thead>
<tr>
<th>QE</th>
<th>CT</th>
<th></th>
<th></th>
<th></th>
<th>IM</th>
<th></th>
<th></th>
<th></th>
<th>VM</th>
<th></th>
<th></th>
<th>BM</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pollen</td>
<td>SPM</td>
<td>Pollen</td>
<td>SPM</td>
<td>Pollen</td>
<td>SPM</td>
<td>Pollen</td>
<td>SPM</td>
<td>Pollen</td>
<td>SPM</td>
<td>Pollen</td>
<td>SPM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>T</td>
<td>P</td>
<td>T</td>
<td>P</td>
<td>T</td>
<td>P</td>
<td>T</td>
<td>P</td>
<td>T</td>
<td>P</td>
<td>T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>0.099</td>
<td>-1.95</td>
<td>0.405</td>
<td>1.05</td>
<td>0.003</td>
<td>-4.98</td>
<td>0</td>
<td>-31.4</td>
<td>0.032</td>
<td>2.93</td>
<td>0.024</td>
<td>-6.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As</td>
<td>0.695</td>
<td>-0.42</td>
<td>0.006</td>
<td>-6.91</td>
<td>0.003</td>
<td>-4.58</td>
<td>*</td>
<td>*</td>
<td>0.745</td>
<td>0.34</td>
<td>0.087</td>
<td>-3.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ba</td>
<td>0.125</td>
<td>-1.94</td>
<td>*</td>
<td>*</td>
<td>0.367</td>
<td>1.02</td>
<td>0.179</td>
<td>-2.04</td>
<td>0.173</td>
<td>1.55</td>
<td>0.005</td>
<td>-7.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.625</td>
<td>-0.53</td>
<td>*</td>
<td>*</td>
<td>0.380</td>
<td>0.99</td>
<td>*</td>
<td>*</td>
<td>0.298</td>
<td>-1.19</td>
<td>*</td>
<td>*</td>
<td>0.221</td>
<td>1.34</td>
<td>*</td>
</tr>
<tr>
<td>Co</td>
<td>0.361</td>
<td>-1.03</td>
<td>0.029</td>
<td>-5.73</td>
<td>0</td>
<td>-7.74</td>
<td>0.018</td>
<td>-7.43</td>
<td>0.082</td>
<td>2.17</td>
<td>0.001</td>
<td>-26.34</td>
<td>-1.960</td>
<td>-1.96</td>
<td>0</td>
</tr>
<tr>
<td>Cr</td>
<td>0.006</td>
<td>5.42</td>
<td>0.453</td>
<td>0.92</td>
<td>0.026</td>
<td>3.43</td>
<td>0.01</td>
<td>-9.93</td>
<td>0.004</td>
<td>6.16</td>
<td>0.001</td>
<td>-28.61</td>
<td>0.107</td>
<td>2.08</td>
<td>0.399</td>
</tr>
<tr>
<td>Cu</td>
<td>0.299</td>
<td>-1.16</td>
<td>*</td>
<td>*</td>
<td>0.345</td>
<td>1.01</td>
<td>0.011</td>
<td>5.66</td>
<td>0.075</td>
<td>-2.39</td>
<td>*</td>
<td>*</td>
<td>0.514</td>
<td>0.72</td>
<td>0.379</td>
</tr>
<tr>
<td>Fe</td>
<td>0.050</td>
<td>-2.57</td>
<td>0.001</td>
<td>-11.68</td>
<td>0</td>
<td>-9.22</td>
<td>0.001</td>
<td>-28.84</td>
<td>0.053</td>
<td>2.72</td>
<td>0.010</td>
<td>-10.18</td>
<td>0.174</td>
<td>-1.65</td>
<td>0.035</td>
</tr>
<tr>
<td>Mn</td>
<td>0.327</td>
<td>-1.09</td>
<td>0.004</td>
<td>-16.00</td>
<td>0.511</td>
<td>0.72</td>
<td>0.002</td>
<td>-11.05</td>
<td>0.300</td>
<td>-1.19</td>
<td>0.007</td>
<td>-12.23</td>
<td>0.089</td>
<td>-2.11</td>
<td>0.005</td>
</tr>
<tr>
<td>Ni</td>
<td>0.252</td>
<td>-1.34</td>
<td>0.019</td>
<td>4.64</td>
<td>0.085</td>
<td>2.00</td>
<td>0.967</td>
<td>-0.05</td>
<td>0.445</td>
<td>0.81</td>
<td>0.008</td>
<td>-6.43</td>
<td>0.286</td>
<td>-1.17</td>
<td>0.010</td>
</tr>
<tr>
<td>Pb</td>
<td>0.527</td>
<td>-0.69</td>
<td>*</td>
<td>*</td>
<td>0.402</td>
<td>0.94</td>
<td>*</td>
<td>*</td>
<td>0.714</td>
<td>-0.39</td>
<td>*</td>
<td>*</td>
<td>0.783</td>
<td>0.29</td>
<td>*</td>
</tr>
<tr>
<td>Sb</td>
<td>0.587</td>
<td>-0.59</td>
<td>0.164</td>
<td>-2.16</td>
<td>0.520</td>
<td>0.68</td>
<td>0.132</td>
<td>-2.47</td>
<td>0.367</td>
<td>-1.02</td>
<td>0.437</td>
<td>-0.96</td>
<td>0.560</td>
<td>0.62</td>
<td>0.103</td>
</tr>
<tr>
<td>Y</td>
<td>*</td>
<td>*</td>
<td>0.792</td>
<td>-0.30</td>
<td>*</td>
<td>*</td>
<td>0</td>
<td>-29.2</td>
<td>*</td>
<td>*</td>
<td>0.028</td>
<td>-3.98</td>
<td>0.710</td>
<td>0.40</td>
<td>0.001</td>
</tr>
<tr>
<td>Zn</td>
<td>0.243</td>
<td>-1.37</td>
<td>0.006</td>
<td>12.36</td>
<td>0.768</td>
<td>0.31</td>
<td>0.654</td>
<td>-0.5</td>
<td>0.531</td>
<td>-0.67</td>
<td>0.140</td>
<td>1.99</td>
<td>0.514</td>
<td>0.72</td>
<td>0.068</td>
</tr>
</tbody>
</table>
Table 5. Mineral contents in µg/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: µg/Kg (As, Ba, Cd, Co, Cr, Cs, Cu, Ni, Pb, Sb and Y). Codes for areas are as in Figure 1.

<table>
<thead>
<tr>
<th></th>
<th>BM-W</th>
<th>BM-D</th>
<th>CT-W</th>
<th>CT-D</th>
<th>IM-W</th>
<th>IM-D</th>
<th>VM-W</th>
<th>VM-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>138.6667</td>
<td>142.3333</td>
<td>83.1333</td>
<td>73.2333</td>
<td>122.6667</td>
<td>816.6667</td>
<td>219.0000</td>
<td>418.3333</td>
</tr>
<tr>
<td>As</td>
<td>38.8373</td>
<td>38.5166</td>
<td>15.1382</td>
<td>32.8802</td>
<td>1.2119</td>
<td>104.9892</td>
<td>63.2459</td>
<td>173.8340</td>
</tr>
<tr>
<td>Ba</td>
<td>36.9478</td>
<td>319.8446</td>
<td>35.6247</td>
<td>6551.5150</td>
<td>8797.5120</td>
<td>14383.4800</td>
<td>2089.0270</td>
<td>5780.7610</td>
</tr>
<tr>
<td>Co</td>
<td>48.0900</td>
<td>75.6960</td>
<td>31.3442</td>
<td>87.1070</td>
<td>60.267</td>
<td>261.3072</td>
<td>66.0247</td>
<td>205.4273</td>
</tr>
<tr>
<td>Cr</td>
<td>1851.7450</td>
<td>1330.2330</td>
<td>943.3929</td>
<td>784.6034</td>
<td>57.4582</td>
<td>365.4744</td>
<td>2068.8300</td>
<td>5780.7610</td>
</tr>
<tr>
<td>Cu</td>
<td>7305.7980</td>
<td>8834.9580</td>
<td>8978.2190</td>
<td>110.0410</td>
<td>4979.3780</td>
<td>1927.9390</td>
<td>107.3742</td>
<td>2558.859</td>
</tr>
<tr>
<td>Fe</td>
<td>491.0000</td>
<td>577.6667</td>
<td>241.3333</td>
<td>461.3333</td>
<td>246.0000</td>
<td>960.6667</td>
<td>207.6667</td>
<td>429.3333</td>
</tr>
<tr>
<td>Mn</td>
<td>52.4100</td>
<td>75.3367</td>
<td>48.9000</td>
<td>98.1600</td>
<td>25.3833</td>
<td>37.8833</td>
<td>29.7900</td>
<td>39.0124</td>
</tr>
<tr>
<td>Ni</td>
<td>656.8879</td>
<td>893.3705</td>
<td>643.2998</td>
<td>481.3357</td>
<td>110.4292</td>
<td>206.6642</td>
<td>2224.7640</td>
<td>3085.544</td>
</tr>
<tr>
<td>Sb</td>
<td>207.7850</td>
<td>347.0570</td>
<td>191.1601</td>
<td>229.4160</td>
<td>203.2694</td>
<td>370.2805</td>
<td>205.3813</td>
<td>226.7645</td>
</tr>
<tr>
<td>Y</td>
<td>8.3697</td>
<td>57.4485</td>
<td>8.7082</td>
<td>9.3452</td>
<td>56.1115</td>
<td>513.9255</td>
<td>19.0124</td>
<td>37.3083</td>
</tr>
<tr>
<td>Zn</td>
<td>35.2533</td>
<td>46.1733</td>
<td>38.1233</td>
<td>28.1868</td>
<td>29.3366</td>
<td>30.6233</td>
<td>37.8100</td>
<td>34.59333</td>
</tr>
</tbody>
</table>

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...
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,

Table 6. Mineral contents in µg/L for 160 Suspended Particulate Matter (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.

<table>
<thead>
<tr>
<th></th>
<th>BM-W</th>
<th>BM-D</th>
<th>CT-W</th>
<th>CT-D</th>
<th>IM-W</th>
<th>IM-D</th>
<th>VM-W</th>
<th>VM-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>150.5860</td>
<td>541.4000</td>
<td>145.2520</td>
<td>132.0880</td>
<td>651.4000</td>
<td>835.9880</td>
<td>451.0060</td>
<td>193.1060</td>
</tr>
<tr>
<td>As</td>
<td>13.5000</td>
<td>0.1344</td>
<td>0.1080</td>
<td>0.1454</td>
<td>0.0980</td>
<td>0.1104</td>
<td>0.2356</td>
<td>0.2362</td>
</tr>
<tr>
<td>Cd</td>
<td>0.0132</td>
<td>0.0092</td>
<td>0.0132</td>
<td>0.0274</td>
<td>0.1634</td>
<td>0.0290</td>
<td>0.0946</td>
<td>0.0290</td>
</tr>
<tr>
<td>Co</td>
<td>0.2781</td>
<td>0.4994</td>
<td>10.0120</td>
<td>0.1850</td>
<td>0.5070</td>
<td>0.5250</td>
<td>0.5484</td>
<td>0.3132</td>
</tr>
<tr>
<td>Cr</td>
<td>649.9200</td>
<td>42.9920</td>
<td>94.8280</td>
<td>7.7300</td>
<td>60.3760</td>
<td>37.6540</td>
<td>19.2420</td>
<td>4.9740</td>
</tr>
<tr>
<td>Fe</td>
<td>1109.8000</td>
<td>4391.6000</td>
<td>100.9400</td>
<td>116.1680</td>
<td>1106.2000</td>
<td>1.5490</td>
<td>453.0000</td>
<td>177.4000</td>
</tr>
<tr>
<td>Mn</td>
<td>346.9760</td>
<td>1210.6000</td>
<td>109.7600</td>
<td>92.4160</td>
<td>530.5260</td>
<td>129.486</td>
<td>37.7220</td>
<td>92.7480</td>
</tr>
<tr>
<td>Ni</td>
<td>0.6324</td>
<td>1.3220</td>
<td>21.6260</td>
<td>2.1760</td>
<td>0.7606</td>
<td>0.4472</td>
<td>86.6380</td>
<td>2.5580</td>
</tr>
<tr>
<td>Pb</td>
<td>0.5090</td>
<td>0.4522</td>
<td>0.3956</td>
<td>0.8900</td>
<td>13.0960</td>
<td>0.9410</td>
<td>27.9580</td>
<td>0.6408</td>
</tr>
<tr>
<td>Sb</td>
<td>14.6140</td>
<td>1.2980</td>
<td>2.6820</td>
<td>2.4420</td>
<td>1.8440</td>
<td>1.8100</td>
<td>2.3020</td>
<td>3.1740</td>
</tr>
<tr>
<td>Y</td>
<td>77.9760</td>
<td>418.9580</td>
<td>0.4315</td>
<td>0.4315</td>
<td>0.4315</td>
<td>151.0407</td>
<td>0.4315</td>
<td>0.4315</td>
</tr>
<tr>
<td>Zn</td>
<td>110.5300</td>
<td>90.364</td>
<td>57.206</td>
<td>156.122</td>
<td>167.2</td>
<td>135.314</td>
<td>148.244</td>
<td>95.272</td>
</tr>
</tbody>
</table>

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)
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 was 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


monitor pesticide presence in continental France. Environmental Toxicology and Chemistry, 30: 103-111. doi: 10.1002/etc.361


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
