ASSESSMENT OF HEAVY METALS CONTENT IN POLLEN FROM THE COMBS (BEE BREAD) IN REGIONS WITH DIFFERENT ANTHROPOGENIC IMPACT

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Abstract: The object of this study was the pollen carried by bees and stored in the combs. The aim of the study was to assess the heavy metals (Cu, Zn, Pb, Cd, Ni, Mn and Fe) quantity in the pollen from different villages of South-Central Bulgaria with different anthropogenic impact. Samples are obtained by extracting the pollen from cells of honeycombs, originating from 8 villages, situated in 5 municipalities in the Stara Zagora region: municipality of Gurkovo – Gurkovo town; municipality of Maglizh – Yagoda village; municipality of Stara Zagora – Stara Zagora town, Equestrian grounds, Preslaven village and Arnautito village; municipality of Radnevo – Polski Gradets village; municipality of Galabovo – Mednikarovo village and Pomoshtnik village. A total of 68 samples of pollen were analyzed by atomic absorption spectrometry. It has been found that the studied elements are arranged in the following descending order: Fe>Mn>Zn>Cu>Pb>Ni>Cd. In some of the settlements there is a difference in the Cu, Zn and Mn position in the established range. These results may be explained by the origin of pollen, soil type, the presence of anthropogenic influence in the region, etc.

Key words: heavy metals; pollen; combs, bees

INTRODUCTION

Information from specialized beekeepers’ literature points out that bees and bee products can be used for environmental monitoring – Crane (1984); Ivanov and Chervenakova (1984); Zalevski et al. (1987); Tureček et al. (1991); Bilalov et al. (1992), Conti and Botre (2001); Porrini et al. (2003); Wilczynska and Przybylowski (2003); Zhelyazkova (2011a, b); Zhelyazkova et al. (2011). Air, soil and water polluters (heavy metals, radioactive substances, pesticides, etc.) are deposited in nectar, blight, pollen and other parts of plants. Honey bees collect these products and take them to beehive nests, store them in the honeycombs and subject them to specific processing. In this way environmental pollutants enter the end honey
products used by humans – honey (nectar, dew), pollen, wax, propolis, royal jelly – Ivanov and Chervenakova (1984); Bogdanov et al. (2002); Piro et al. (2003).

Based on data by Rusakova and Martynova (1994), not all bee products reflect the state of the environment equally well. It is believed that pollen provides more accurate information on environmental pollution – Ravetto et al. (1988); Tureček et al. (1991); Jablonski et al. (1995); Fakhimzadeh and Lodenius (2000); Conti and Botre (2001); Bogdanov (2004); Ossintseva (2004); Zhelyazkova (2011a); Zhelyazkova et al. (2011). In our country as early as 1980, Toshkov et al. (quoted by Ivanov and Chervenakova, 1984) reported higher levels of the elements copper and zinc in pollen obtained from areas with industrial plants.

According to a number of authors, bee bread (pollen stored in honeycombs) is a product suitable for biomonitoring of the environment – Cermakova (1997); Fakhimzadeh and Lodenius (2000); Porrini et al. (2003); Majewska and Jasinski (2005); Chauzat et al. (2006); Spodniewska and Romaniuk (2006); Bernal et al. (2010); Kozmus et al. (2010); Zhelyazkova et al. (2008); Zhelyazkova (2009); Zhelyazkova (2011a); Zhelyazkova et al. (2011). It is assumed that this pollen is the best indicator of environmental pollution with radioactive substances, pesticides and heavy metals.

Taking into account that pollen is used by humans as food additive and that there are possibilities for its contamination with toxic substances, it is appropriate to study its composition, including identifying some heavy metals content.

The aim of the study was to assess the heavy metals (Cu, Zn, Pb, Cd, Ni, Mn and Fe) quantity in the pollen from the combs (bee bread) from some villages of South Central Bulgaria with different anthropogenic impact.

MATERIAL AND METHODS

Sampling was carried out during the 2007–2012 period twice during the active apicultural season. The apiaries are selected at random. The study includes normally developed healthy bee colonies housed in Dadan-Blatt and Langstroth-Ruth hives.

The survey comprises 8 settlements from 5 municipalities in Stara Zagora region – (Figure 1), namely: town of Gurkovo (municipality of Gurkovo), settlements in the area of Maritsa East Energy Complex – village of Polski Gradets (municipality of Radnevo): village of Pomoshtnik and village of Mednikarovo (municipality of Galabovo); settlements in the Sarnena Sredna Gora region – village of Yagoda (municipality of Maglizh); settlements in the municipality of Stara Zagora – village of Arnauto, Equestrian base (in close proximity to the industrial area of the city and a road with heavy traffic), village of Preslaven (close to the former enterprise "Agrobiochim" EAD). The selected settlements have different degree of anthropogenic impact.

Fig. 1. Villages included in the study (region of Stara Zagora)
Pollen samples were obtained by taking out the pollen stored in the cells by means of a spatula.

Subject of the study are the levels of some heavy metals and metalloids in bee pollen: copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), nickel (Ni), manganese (Mn) and iron (Fe). A total of 68 bee bread samples have been analyzed. Analyses have been carried out at the Scientific Laboratory of the Faculty of Agriculture at Trakia University – Stara Zagora, using modern reference methods, legalized by ISO standards (ISO 11047) and atomic absorption spectrometry by methods for feed, milk, water and other biological materials.

The results were statistically processed on a computer (Excel software).

RESULTS AND DISCUSSION

The summarized results from the two sampling periods on the contents of the analyzed heavy metals in the bee bread samples are given in Table 1 and Figures 2 – 8. The following results have been established:

**Copper (Cu).** Copper content in the pollen samples analyzed ranges from 6.72±0.67 mg/kg (village of Arnautito – low level of anthropogenic impact) to 13.44±1.56 mg/kg (village of Polski Gradets – Maritsa East Energy Complex with high level of anthropogenic impact). In 5 of the settlements average values of Cu in pollen above 10 mg/kg have been determined (Figure 2).

**Zinc (Zn).** The maximum value for zinc content has been determined for pollen samples from the village of Pomoshtnik (Maritsa East Energy Complex – high level of anthropogenic impact) and the minimum is in the samples from the village of Mednikarovo. Values close to the maximum ones have also been found for pollen from the village of Polski Gradets located close to TPP 2 and TPP 3 of Maritsa East Energy Complex. In the pollen samples from the Equestrian ground, the town of Gurkovo and the village of Mednikarovo amount of Zn below 10 mg/kg has been determined (Figure 3).

<table>
<thead>
<tr>
<th>Villages</th>
<th>n</th>
<th>Cu</th>
<th>Zn</th>
<th>Pb</th>
<th>Cd</th>
<th>Ni</th>
<th>Mn</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gurkovo</td>
<td>9</td>
<td>11.54±0.76</td>
<td>10.00±0.21</td>
<td>0.92±0.04</td>
<td>0.16±0.031</td>
<td>0.69±0.05</td>
<td>8.52±0.36</td>
<td>51.83±0.85</td>
</tr>
<tr>
<td>Arnautito</td>
<td>5</td>
<td>6.72±0.67</td>
<td>12.36±0.18</td>
<td>0.65±0.02</td>
<td>–</td>
<td>0.75±0.05</td>
<td>9.85±0.19</td>
<td>28.49±1.45</td>
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<tr>
<td>Polski Gradets</td>
<td>10</td>
<td>13.44±1.56</td>
<td>18.17±2.25</td>
<td>1.20±0.12</td>
<td>0.19±0.03</td>
<td>0.40±0.07</td>
<td>27.89±5.25</td>
<td>116.35±10.21</td>
</tr>
<tr>
<td>Pomoshtnik</td>
<td>10</td>
<td>10.29±0.31</td>
<td>19.43±2.07</td>
<td>1.47±0.10</td>
<td>0.27±0.04</td>
<td>0.38±0.02</td>
<td>35.43±0.79</td>
<td>142.9±6.92</td>
</tr>
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<td>Mednikarovo</td>
<td>8</td>
<td>10.97±0.35</td>
<td>8.54±0.15</td>
<td>0.89±0.02</td>
<td>0.07±0.002</td>
<td>0.71±0.08</td>
<td>8.54±0.13</td>
<td>53.41±0.65</td>
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<td>Yagoda</td>
<td>8</td>
<td>10.12±0.11</td>
<td>10.40±0.18</td>
<td>0.85±0.02</td>
<td>0.20±0.002</td>
<td>0.77±0.06</td>
<td>10.33±0.30</td>
<td>54.67±1.06</td>
</tr>
<tr>
<td>Equestrian ground</td>
<td>8</td>
<td>9.38±0.64</td>
<td>9.54±0.20</td>
<td>0.97±0.05</td>
<td>0.13±0.028</td>
<td>0.54±0.04</td>
<td>6.95±0.45</td>
<td>51.15±0.50</td>
</tr>
<tr>
<td>Preslavens</td>
<td>10</td>
<td>7.82±0.60</td>
<td>13.43±1.45</td>
<td>0.73±0.04</td>
<td>–</td>
<td>1.06±0.06</td>
<td>18.22±3.24</td>
<td>32.08±3.23</td>
</tr>
</tbody>
</table>

**Table 1**

*Content of heavy metals in bee pollen (bee bread) from the region of Stara Zagora*

**Fig. 2.** Content of copper (Cu) in bee pollen (bee bread) (mg/kg)

**Fig. 3.** Content of zinc (Zn) in bee pollen (bee bread) (mg/kg)
Lead (Pb). The lead content in the analyzed pollen samples ranges from 0.65 ± 0.02 mg/kg (village of Arnautito – low level of anthropogenic impact) to 1.47 ± 0.10 mg/kg (village of Pomoshtnik close to TPP 3 of Maritsa East Energy Complex). A value close to the maximum one has been determined for pollen from the village of Polski Gradets (an area with high level of anthropogenic impact). Based on Pb content, pollen samples can be divided into 3 groups: below 0.8 mg/kg (the villages of Arnautito and Preslaven); from 0.8 to 1.00 mg/kg (the town of Gurkovo, Equestrial grounds Stara Zagora and the villages of Yagoda and Mednikarovo); over 1.00 mg/kg (the villages of Pomoshtnik and Polski Gradets) (Figure 4). According to data by Bogdanov (2006), the element lead is very poorly transformed from the soil through the plants to the flower (pollen, respectively), therefore, as a pollutant it is mainly in the atmospheric air. In this respect, it can be assumed that the determined higher values for the quantity of Pb in the bee bread are probably due to: the intensive road traffic (town of Gurkovo); the proximity of the Equestrial base to the industrial area of the town of Stara Zagora and a busy road; the small distance of the village of Yagoda to the central area of the Zmeyovo military ground, Sarnena Sredna Gora mountain; the production activity of TPP of Maritza East Energy Complex for the villages of Pomoshtnik and Polski Gradets.

The results for the Pb and Cd contents in the pollen samples from the surveyed villages are higher than those reported by Campos et al. (2008) – not more than 0.1 mg/kg for Cd and 0.5 mg/kg for Pb. This requirements refer to pollen loads carried by bees and not to pollen stored in the combs (bee bread).

Nickel (Ni). In 7 from the 8 settlements included in the study nickel content in bee bread samples is within the limits of 0.38 ± 0.02 mg/kg to 0.77 ± 0.06 mg/kg. The maximum value has been established in the bee bread from the village of Preslaven (1.06 ± 0.06 mg/kg), a village located in close proximity to the former „Agrobiochim“ EAD company – Stara Zagora (Figure 6). The company produced nitrogen fertilizers, phosphorus containing compounds, products of organic synthesis etc. for 40 years. The study by Eneva and Todorova (2004) proved presence of technogenic impact of its production activity from the near past in relation to heavy metal content in the soil.
Manganese (Mn). Depending on manganese content bee bread samples can be divided into 3 groups: under 10 mg/kg (town of Gurkovo, Equestrian base in the town of Stara Zagora, villages of Arnautito and Mednikarovo); between 10 and 20 mg/kg (villages of Yagoda and Preslaven); over 20 mg/kg (villages of Pomoshtnik and Polski Gradets) (Figure 7).

Iron (Fe). The minimum value for iron content has been determined for the bee bread samples from the village of Arnautito and the maximum one for the samples from the village of Pomoshtnik. Value close to the minimum one has been established for the bee bread from the village of Preslaven. In 4 of the settlements (town of Gurkovo, Equestrian base in the town of Stara Zagora, villages of Yagoda and Mednikarovo) the reported Fe quantity in bee bread samples is between 50 and 55 mg/kg, and in 2 of the settlements with proven high level of anthropogenic impact (the villages of Polski Gradets and Pomoshtnik) – over 100 mg/kg (116.35 ± 10.21 mg/kg and 142.90 ± 6.92 mg/kg, respectively) (Figure 8).

Comparative analysis of the data from Table 1 and Figures 2 – 8 shows that the minimum values for the contents of the elements Cu, Zn, Pb, Cd, Mn and Fe were found in the bee bread samples from the villages of Arnautito and Mednikarovo. These results give reason to believe that in these settlements the level of anthropogenic impact concerning heavy metal and metalloid pollution is low.

From the obtained results it is evident that the maximum or close to these values for the contents of the studied elements are found in the bee bread samples from the villages of Pomoshtnik and Polski Gradets – settlements close to TPP-2 and TPP-3 of Maritsa East Energy Complex. In relation to that a conclusion can be made about technogenic impact of the production activity of TPPs in the region of the above settlements.

The data in Table 1 and Figures 2 – 8 show that, irrespective of the time and place of sample taking, the largest in bee bread samples is the quantity of iron and the lowest is the cadmium content. Based on the results, the studied heavy metals and metalloids are arranged in the following descending order: Fe>Mn>Zn>Cu>Pb>Ni>Cd for bee bread from the villages of Polski Gradets, Pomoshtnik, Mednikarovo; Fe>Mn>Zn>Cu>Ni>Pb for samples from the village of Preslav; Fe>Zn>Mn>Cu>Ni>Pb for the bee bread from the village of Arnautito; Fe>Zn>Mn>Cu>Pb>Ni>Cd for the bee pollen samples from the village of Yagoda; Fe>Zn>Cu>Mn>Pb>Ni>Cd for the bee pollen from the Equestrian base of the town of Stara Zagora; Fe>Cu>Zn>Mn>Pb>Ni>Cd for the samples from the town of Gurkovo. It is obvious that the above order for the individual controlled settlements differs by positions of the elements Mn, Zn and Cu, as well as that of Pb, Ni. Higher content of manganese in bee bread samples from settlements with proven anthropogenic impact – village of Polski Gradets, villages of Pomoshtnik and Preslaven can be commented. In our studies, Zhelyazkova et al. (2011), Zhelyazkova (2013) established a connection between extra loading of the soil ecosystem at the point in the villages of Polski Gradets and Preslaven with manganese and its salts and the higher content of the element in flowers of nectariferous plants and in the bee body.

The results found in the study are indicative of the bioindicator characteristic of bee bread.

CONCLUSIONS

The minimum values for contents of the elements Cu, Zn, Pb, Cd, Mn and Fe have been found in bee bread samples from the villages of Arnautito and Mednikarovo, which justifies the assumption...
that in these settlements the level of anthropogenic impact concerning pollution with heavy metals and metalloids is low.

The maximum or close to these values for contents of the studied elements have been found in bee bread samples from the villages of Pomoshnik and Polski Gradets – settlements close to TPP of Maritsa East Energy Complex. A reason for the obtained results could be the technogenic impact of the production activity of TPP in the region of these settlements.

The largest in the bee bread samples is the quantity of the element iron, and the lowest is the cadmium content, regardless of the time and place of sample taking. A descending order of the studied heavy metals and metalloids has been determined and it differs for the individual settlements. The specified order for the controlled settlements differs by the positions of the elements Mn, Zn and Cu, as well as that of Pb, Ni.

The results show that bee bread is a good bioindicator for assessing environmental quality for contents of heavy metals.

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Assessment of heavy metals content in pollen from the combs (bee bread) in regions with different anthropogenic impact


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