Trace Element Content of Polyfloral Honey and Beeswax from the Vicinity of Non-Ferrous Metal Plant

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Abstract

Bees and their products occupy an important place in the trophic chain “toxicant-soil-plant-bee-bee product-man”, so it has been suggested that bees and bee products should be considered as not a costly method for monitoring of the environmental pollution. Aim of the present study was to analyse trace element content of polyfloral honey and beeswax, produced in three beehives, situated in the vicinity of non-ferrous metal plant and to assess the hazardous risk at consummation. For the purposes of the study, three beehives in this area have been chosen, as follows: BH1 – located at distance of 3.8 km from the smelter, SW; BH2 – at 4 km distance, NE; BH3 – at 4.6 km distance, SE. Content of Al, Cr, Co, Cu, Fe, Mn, Ni, Pb, Sr and Zn was determined by ICP-MS. Data revealed approximately low content of heavy metals and toxic elements. Beeswax samples were found to contain significantly more Cr, Cu, Fe, Pb and Zn, in comparison with the honey samples (p<0.05). On the basis of the trace elements content in bee products, the hazardous risk at consummation could be arranged in the following descending order: BH2 > BH1 > BH3.

Keywords: bee, beeswax, biomonitoring, honey, metal pollution, Plovdiv

Introduction

Honey is sweet substance produced by honeybees (Apis mellifera), mainly from the nectar of flowers of various plants. The wax is secreted by the wax glands of the worker bees and is used mainly for building the wax cakes. There are a number of varieties of honey available in the market or directly from the beekeepers today. According to the type of plant which is collected nectar, honey is polyfloral (from different plants) or unifloral (mostly from one species of plants) (Yurukova et al., 2010). Bees and their products occupy an important place in the trophic chain “toxicant-soil-plant-bee-bee product-man”. Although bee honey is mainly used for food (directly or in the composition of some foodstuffs), the literature cites its other uses, namely as an indicator of the environmental pollution (Tong et al., 1975; D’Ambrosio and Marchesini, 1982; González Paramás et al., 2000; Batista et al., 2012; Matin et al., 2016). In fact, it has been suggested that bees and bee products should be considered as not a costly method for trace element monitoring, since bees fly a huge amount of space, confined within
a certain radius of the hive's location (3-4 km), in search of food, which they come into contact with pollutants (in the air, water, soil, and plants) and import them into the bees’ products (Tong et al., 1975; Bogdanov et al., 1986; Dinkov and Stratev, 2016; Matin et al., 2016). There bees have a filtering ability against the toxins and send signals in two ways: by high mortality and/or by deposition of pollutants in bee organism or in bee products. Pollutants may come from the environment in bee colonies in various ways: i) through the air and water they get on the body hairs of the bees or in the bee organism and are thus transferred to the hive; ii) from air, water and soil they accumulate in plants and on their surfaces (leaves, flowers), pass into nectar, mana, pollen and plant resins. The bees collect and process these products, whereby the available contaminants are accumulated in honey, pollen and bee glue (propolis) (Przybylowski and Wilczynska, 2001; Ioannidou et al., 2004).

It is known that contaminants can reach the raw materials of bee products (nectar, honeydew, pollen, plant exudates) by air, water, plants and soil due to industry and motor traffic, and then be transported into the bee hive by the bees and after that in honey products (Bogdanov et al., 1986). There is a lack of data concerning the trace element content of bee products form the so-called “hot points” of environmental pollution, most of them associated with the metal industry.

Aim of the present study was to analyse trace element content of polyfloral honey and beeswax, produced in three beehives, situated in the vicinity of non-ferrous metal plant (KCM 2000 – Plovdiv, Bulgaria) and to assess the hazardous risk at consummation.

Materials and Methods

Study area and beehives location

Non-ferrous metal plant “KCM 2000”-Plovdiv is the biggest company for Pb and Zn production in SE Bulgaria. Its vicinity is one of the hot-spots in the country in terms of heavy metal pollution. For the purposes of the study, three beehives in this area have been chosen, as follows: BH1 – located at distance of 3.8 km from the smelter, SW; BH2 - located at distance of 4 km, NE; BH3 – located at distance of 4.6 km, SE (Fig. 1). Representative polyfloral honey and beeswax samples were harvest of 2017 and have been obtained by certified beekeepers from the abovementioned plots.

Chemical analysis

About 10 g honey or beeswax was treated with 15 ml nitric acid (9.67 M) overnight. The wet ashed procedure was continued with heating on a water bath, following by addition of 2 ml hydrogen peroxide. This treatment was repeated till full digestion. The filtrate was diluted with double distilled water (0.06 μS cm⁻¹) up to 25
ml. The content of ten trace elements (Al, Cr, Co, Cu, Fe, Mn, Ni, Pb, Sr and Zn) was determined by inductively coupled plasma–mass spectrometry (Agilent 7700 ICP–MS) and presented in mg kg$^{-1}$ fresh weight. The detection limits were 0.002 mg l$^{-1}$ for Mn and Sr, 0.004 mg l$^{-1}$ for Co, Cr, Cu, Ni and Zn, 0.03 mg l$^{-1}$ for Pb, 0.04 mg ml$^{-1}$ for Al and Fe. Analytical precision was checked with replicating (deviation between the duplicates was below 5% in all cases), blanks and stock standard solutions (1000 μg l$^{-1}$ Merck) for the preparation of working aqueous solutions. The quality control was assured with plant reference material (NCS DC73348). The measured concentrations were in agreement with the recommended values. The value for each sample was the average of 3 subsamples and for each subsample was the mean of 3 analytical determinations.

**Statistical analysis**

In order to determine the significant differences between two studied bee products, obtained from three different bee hives, analysis of variance (ANOVA) followed by Student’s t-test were performed (p< 0.05). SPSS (version 19.0, SPSS Inc., Chicago, IL, USA) for Windows was used.

**Results and Discussion**

Data revealed an approximately low content of heavy metals and toxic elements in both honey and beeswax samples (Fig. 2) according to the Maximum Admitted Levels or Maximum Residue Limits, proposed by Bogdanov et al. (1986), Matei et al. (2004), Bratu and Georgescu (2005). Most abundant trace element in all studied samples was found to be Al (3.9 mg kg$^{-1}$ in honey and 4.7 mg kg$^{-1}$ in beeswax from BH 2), followed by Fe (1.6 mg kg$^{-1}$ in honey and 3.2 mg kg$^{-1}$ in beeswax from BH 2), and Zn (1.1 mg kg$^{-1}$ in honey and 3.8 mg kg$^{-1}$ in beeswax from BH 1). Least content in analysed samples showed Co (0.004 mg kg$^{-1}$ in honey and 0.006 mg kg$^{-1}$ in beeswax from BH 1), Cr (0.02 mg kg$^{-1}$ in honey and 0.05 mg kg$^{-1}$ in beeswax from BH 3) and Ni (0.06 mg kg$^{-1}$ in honey and 0.03 mg kg$^{-1}$ in beeswax from BH 3) (Fig. 2).

Data revealed that the samples from the three studied bee hives reflected to the different proportion of trace elements content of honey and beeswax. In BH 1 samples the descending order of elements was Zn > Fe > Cu > Mn > Pb = Al > Sr > Ni = Cr > Co (in honey) and Zn > Fe > Al > Cu > Mn > Sr > Pb > Ni > Cr > Co (in beeswax). In BH 2 samples the descending order was found to be Al > Fe > Zn > Pb > Mn > Cu > Sr > Ni > Cr > Co (both in honey and beeswax). In BH 3 samples the descending order of trace elements was Al > Fe > Zn > Mn > Cu > Sr > Ni > Pb > Cr > Co (in honey) and Fe > Zn > Al > Cu > Mn > Sr > Cr > Pb = Ni > Co (in beeswax).

Our previous studies on trace elements content of polyfloral honey, obtained from bee hives situated on the area of Plovdiv city in 2009, have showed that the descending order was Fe > Zn > Ni > Al > Pb > Cu > Sr > Mn > Cr > Co (Yurukova et al., 2010). Significant higher content of Al, Cu, Pb and Zn were found in the honey samples from vicinity of non-ferrous metal plant ("KCM 2000"-Plovdiv, Bulgaria) in comparison with the honey samples from city of Plovdiv (p<0.05), while a significant lower content was proven for Fe and Sr (p<0.05). Elevated concentrations of the above mentioned four elements, found in bee products from the vicinity of the smelter, were related with its activity. They could be connected with the hazardous emission from the plant (mainly into air) and reflected the environmental state of the surrounding area.

There was no risk at consummation of bee products from this area due to the “filtering” ability of bees against heavy metals, reported by Bogdanov et al. (2003). Their studies in Switzerland have shown that the content of heavy metals in bees and their products was higher in industrialized areas and in those with heavy traffic, reducing the degree of pollution in the following order: bees > propolis> beeswax> honey. Data from Switzerland about the content of Pb (0.02-0.37 mg kg$^{-1}$) and Zn (0.5–2.8 mg kg$^{-1}$) in honey from industrialized areas were in good agreement with our results.

There are some other studies in Europe concerning the mineral content of honey with different geographic origin, published by Golob et al. (2005), Bogdanov et al. (2007), Chudzinska and Baralkiewicz (2010), Bilandžić et al. (2014), Ördög et al. (2017).

Data from Slovenia revealed that the average content of main mineral elements (measured in 79 samples from nectar honey) was 1100 mg kg$^{-1}$ K, 243 mg kg$^{-1}$ Cl, 56 mg kg$^{-1}$ S, 33 mg kg$^{-1}$ Ca, and 2.5 mg kg$^{-1}$ Mn (Golob et al., 2010). Average element contents of unifloral honey samples from the Hungarian Great Plain, reported by Ördög et al. (2017), showed significant differences according to the studied honey types. The Mn concentration...
in their study was significantly lower in the black locust honey (0.13 mg kg\(^{-1}\)) compared to the sunflower (0.19 mg kg\(^{-1}\)) and oilseed rape honeys (0.23 mg kg\(^{-1}\)). However, the Mn content in our honey samples varied in the range 0.24-0.33 mg kg\(^{-1}\), and was in good agreement with the Hungarian values. The black locust honeys from Slovenia (Golob et al., 2005) and oilseed rape honeys from Switzerland (Bogdanov et al., 2007) contained more Mn than those from the surroundings of the non-ferrous metal plant “KCM 2000”.

**Figure 2.** Trace element content of polyfloral honey and beeswax from the vicinity of non-ferrous metal plant (“KCM 2000”-Plovdiv, Bulgaria). In each column, the values with different letters indicate significant differences (p < 0.05)
The average Pb content (0.050 mg kg\(^{-1}\)) was the highest in the black locust honey from Hungary (Ördög et al., 2017). Chudzinska and Baralkiewicz (2010) found similar levels of Pb (0.44 mg kg\(^{-1}\)) and in the Polish oilseed rape honey and Bogdanov et al. (2007) measured similar levels of Pb in Swiss black locust and oilseed rape honey. However, Bilandžić et al. (2014) found higher Pb content (0.76 mg kg\(^{-1}\)) in the black locust and oilseed rape honey from Croatia. When compare the Pb content with our findings, it is obvious that although the activity of non-ferrous metal plant nearby, there is no contamination of bee products from this area.

It is known that these metals in honey content are related to soil characteristics and botanical origin (Pohl, 2009). However, the mineral properties do not show the quality of honey according to the EU Directive (EU, 2001). So, the determination of these metals is beneficial to evaluate the nutritional value of honey and to designate the differentiation of a botanical origin (Alves et al., 2013). Some comparisons of honey content found in the present study with the hygiene norms of WHO (Toxicological Evaluation of Certain Food Additives and Contaminants, 1982 and Evaluation of Certain Food Additives and Contaminants, 2006) and Agency for Toxic Substances and Disease Registry (ATSDR, 2005) have been also made in terms of better assessment of hazardous risk from heavy metals uptake.

The provisional maximum tolerable daily intake (PMTDI) of Pb is 0.5 mg kg\(^{-1}\) BW (body weight) for Cu (WHO, 1982), but too much Cu can lead to damaging effects in the body. In the present study, Cu concentrations were in the range of 0.21-0.4 mg kg\(^{-1}\) and were higher than values, reported from New Zealand (163-182 μg kg\(^{-1}\)) (Vanhanen et al., 2011) and Italy (310 μg kg\(^{-1}\)) (Pisani et al., 2008), and lower than values from Turkey (0.74-1.1 mg kg\(^{-1}\)) (Temizer et al., 2018) and Ireland (2 mg kg\(^{-1}\)) (Downey et al., 2005). Pb has not a beneficial role in human metabolism and can cause health problems (Aghamirlou et al., 2015). FAO and WHO (2006) reported that the acceptable limit for Pb was 2 mg kg\(^{-1}\). Pb can reach into plants through the air, water, and soil. So, some foods such as honey can contain this heavy metal. All studied honey samples from the vicinity of non-ferrous metal plant (“KCM 2000”-Plovdiv, Bulgaria) showed lower Pb content than the acceptable limits and the following findings in Italy (2.37 mg kg\(^{-1}\)) (D’Ambrosio and Marchesini, 1982), Turkey (0.51 mg kg\(^{-1}\)) (Temizer et al., 2018), and higher content than Poland (0.048 mg kg\(^{-1}\)) (Przybylowski and Wilczynska, 2001).

Zn is one of the main elements required for the body to keep the building proteins and immune system. High level of Zn can cause anemia and decreasing absorption of Cu and Fe (WHO, 1982). The mean suggested Zn as TDI in foods is predicted to be 12-15 mg/day or the PMTDI is 0.3-1.0 mg kg\(^{-1}\) BW (WHO, 1982; ATSDR, 2005). Our findings of Zn concentration in honey were between 0.47 mg kg\(^{-1}\) (BH 3) and 1.1 mg kg\(^{-1}\) (BH 1). They were lower than data from Siena county of Italy (1.820 mg kg\(^{-1}\)) (Pisani et al., 2008), Turkey (2.59-5.1 mg kg\(^{-1}\)) (Temizer et al., 2018), and New Zealand (1.18 mg kg\(^{-1}\)) (Vanhanen et al., 2011). Therefore, it could be think the intake of Zn from varied sources like honey with caution to its mineral content.

TDI value of Al is 8.57 mg/day for a 60 kg adult and PTWI value is 1 mg kg\(^{-1}\) BW (WHO, 2006). Our honey sample from BH 1 showed significantly lower content of Al (0.1 mg kg\(^{-1}\)) in comparison with the other two honey samples from BH 2 (3.9 mg kg\(^{-1}\)) and BH 3 (1.6 mg kg\(^{-1}\)). These findings were in agreement with data from different countries – Hungary (0.103-4.39 mg kg\(^{-1}\)) (Czipa et al., 2015), Brazil (0.23-7.40 mg kg\(^{-1}\)) (Batista et al., 2012), New Zealand (0.21-21.3 mg kg\(^{-1}\)) (Vanhanen et al., 2011), and quite lower from Turkey (6.51-18.81 mg kg\(^{-1}\)) (Temizer et al., 2018). Some researchers have considered that the source of Al and Zn can be the galvanized containers keeping honey (González Paramás et al., 2000).

Conclusion

Honey is considered valuable food for the human, but its content or the effect of environmental pollution on it is not evaluated by the consumer. The main goal of this study was to assess the hazardous risk at consummation of bee honey and beeswax, obtained from the vicinity of non-ferrous metal plant and to draw attention to the heavy metal pollution in honey. Additionally, it was also to produce useful information for beekeepers and the honey industry, by characteristics of high-quality honey.

Concentrations of trace elements in the studied honey and beeswax samples were approximately low according to the Maximum
Admitted Levels or Maximum Residue Limits, proposed in literature, and also according to the hygiene norms of Agency for Toxic Substances and Disease Registry (ATSDR, 2005) and WHO for Food Additives and Contaminants. Our study confirmed the filter ability of honey bees to different pollutants as heavy metals. Although the exceeded values in the environment (both in the air, water and soil) around the studied non-ferrous metal plant, especially of Mn, Ni, Pb and Zn, their content in honey and beeswax was quite low and there is no risk for human health. Spatial and seasonal fluctuations of heavy metals and toxic elements should be a subject of our future studies for confirmation of their high quality as food.

References


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