Journal of Elementology ISSN 1644-2296

Dżugan M., Zaguła G., Wesołowska M., Sowa P., Puchalski Cz. 2017. *Levels of toxic and essential metals in varietal honeys from Podkarpacie*. J. Elem., 22(3): 1039-1048. DOI: 10.5601/jelem.2016.21.4.1298

ORIGINAL PAPER

LEVELS OF TOXIC AND ESSENTIAL METALS IN VARIETAL HONEYS FROM PODKARPACIE*

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Abstract

The mineral composition of honey largely depends on its botanical origin as well as on the climatic conditions and the geographical area where it was produced. The aim of the study was to determine the content of toxic (Cd, Pb, Hg, Al and Tl) and essential (Ca, Mg, Zn, Fe, Mn, K, Cu) elements in honey samples obtained in Podkarpacie, a region in the south-east of Poland. Varietal honeys (n = 106) including nectar (multifloral, dandelion, oilseed rape, goldenrod, linden, buckwheat) and honeydew honeys were collected directly from beekeepers. The mineral composition of honey was determined by the ICP-OES method preceded by wet mineralization. Mercury was undetected while the concentration of other heavy metals in all the studied honey samples (0.01-0.03 mg kg^{-1} for Cd and 0.02-0.09 mg kg^{-1} for Pb) were below the maximum allowable contaminant limits. The level of aluminium in honeydew honey was higher than in other varietal honeys (from 10-fold more than in dandelion to 95-fold more than in oilseed rape honey). The honeys were confirmed to be rich in potassium (310.6-2548.4 mg kg^{-1}), calcium (34.7-108.6 mg kg^{-1}) and magnesium (23.7-63.3 mg kg^{-1}). The content of the other microelements was determined in the following order: Mn>Fe≥Zn>>Ni≥Cu. The highest levels of microelements were found in the buckwheat honeys (Mn – 7.82; Fe – 0.21; Zn – 2.90; Cu – 0.86; Ni – 0.20 mg kg^{-1}). Significant differences ($p < 0.05$) in the mineral content between the honey types were found, and dark honeys (honeydew and buckwheat honeys) contained more essential elements than light honeys.

Keywords: honey, geographical origin, mineral content, heavy metals, ICP-OES.

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* The study was financed by University of Rzeszow (PB/KChTŻ/2015).

INTRODUCTION

Honey is the nature's original sweetener. It has been used as food for at least six thousand years and for much of that time was the sole source of sweet for much of the world's population (White, Doner 1980). At first approximation, honey is a supersaturated sugar solution. However, honey is much more than this. Its unique (though variable) combination of components makes honey a prized addition to a diet. The mineral content of honey has a 50-fold range of values, the largest of any dietary component. The US FDA standards note that most honey can be classified as a low-sodium or sodium-free food. Trace elements found in honey include chromium, lithium, nickel, lead, tin and zinc (White, Doner 1980, de Andrade et al. 2014, Soleyman et al. 2016).

The composition of minerals in honey depends largely on the raw material from which honey was produced (botanical origin) as well as the climatic conditions and geographical area (Bogdanov et al. 2003, 2006, 2007, de Andrade et al. 2014, Solayman et al. 2016). Among minerals present in honeys, presence of toxic metals, including heavy metals, must be scrutinized due to negative impact on human's health. This group contain toxic metals without any physiological function (non-essential), essential metals as well as semi-metallic elements (As and Se). The essential heavy metals are required for biological functioning, although an excess amount of such metals produces cellular and tissue damage leading to a variety of adverse effects and human diseases. For some elements, including chromium and copper, there is a very narrow range of concentrations between beneficial and toxic effects (Roman 2003, Bratu, Georgescu 2005, de Andrade et al. 2014). In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by these metals. On account of the fact that presence of heavy metals in food products can be dangerous to human health, allowable concentrations were determined in *Regulation of the Minister of Health* (Dz.U. 2003 no. 37 item 326).

Honeybees are considered as a good biological indicator of the environmental condition (Conti, Botre 2001, Przybyłowski, Wilczyńska 2001, Fakhimzadeh et al. 2005). Furthermore, bees are able to detect chemical substances in the environment at a level that is difficult or impossible to detect by other methods (Zhelyakova et al. 2010). Limitation of the potential use of apiculture for biomonitoring of metal pollution is caused by the incorporation of metal components and metal-based wood preservatives in commercial beehives (van der Steen et al. 2012). Bees collect and process the plant products (nectar, pollen and mildew) and available pollutants accumulate in honey and other bee products, like pollen and propolis (Celli, Maccagnani 2003, Zhelyazkova et al. 2010, Roman et al. 2011, Sadeghi et al. 2012).

The aim of the study was to compare the mineral content of honeys from Podkarpacie, a region in the south-east of Poland, according their botanical origin. Another objective was to test the level of honey contamination with heavy metals.

MATERIAL AND METHODS

Samples

Honey samples including multifloral (n = 32), dandelion (n = 9), oilseed rape (n = 10), goldenrod (n = 19), linden (n = 6), buckwheat (n = 5) and honeydew (n = 25) were collected in 2013 from 30 apiaries localized in South-Eastern Poland in different part of the Province of Podkarpacie (*województwo podkarpackie*) – Figure 1. From each research site, multifloral honeys

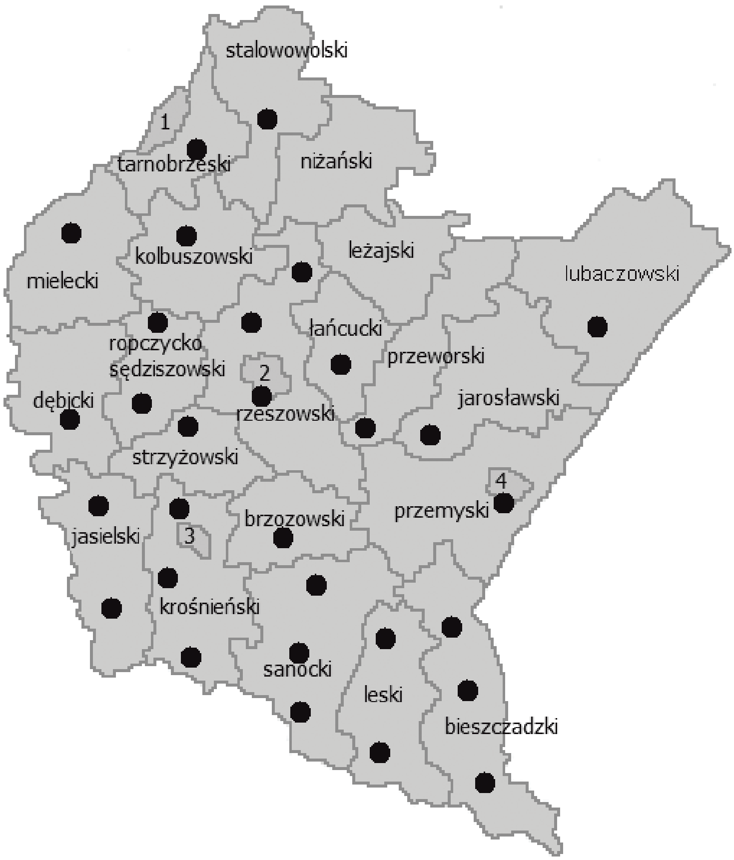

Fig. 1. The honey sampling sites in Podkarpacie

were obtained and monofloral honey samples were collected according to the availability. Honeys were stored at room temperature until analysis.

Analytical methods

Before the determination of elements, wet mineralization was carried out. The samples of honey were weighed (about 1 g) into Teflon vessels and added 8 cm^3 of concentrated HNO_3 (65% POCH). The mineralization of honey samples was performed in a microwave mineralizer Milestone Ethos Ultrawave-One (Milestone SRL, Italy) and lasted about 45 minutes. After cooling, the samples were transferred quantitatively to 50 cm^3 flasks and replenished with redistilled water to the mark. The concentrations of 13 metals (Cd, Pb, Hg, Al, Tl, Ca, Mg, Zn, Fe, Ni, Mn, K, Cu) were determined by optical emission spectrometry with inductively-induced plasma (ICP-OES) using a Thermo iCAP 6500 spectrophotometer (Thermo Fisher Scientific Inc., USA). The detection threshold obtained for each element was no less than 0.01 mg kg^{-1} (with the assumed detection capacity of the measuring apparatus at a level exceeding 1ppb). A curve fit factor for the elements studied was above 0.99. All the analyses were made in three independent replications for each sample. The targeted repeatability expressed as the relative standard deviation (RSD) and targeted recovery were 20% and 97% to 102%, respectively. The method was validated using certified reference material (NIST – 1515). In order to identify the relevant measurement lines and avoid possible interferences, the method of adding an internal standard was applied. Yttrium and ytterbium ions (at concentrations of 2 mg dm^{-3} and 5 mg dm^{-3}, respectively) were used as internal standards.

Statistical analysis

For statistical analysis, Statistica 10.0 (Statsoft, Poland) was used. The results were expressed as mg kg^{-1} fresh weight. Mean, standard deviations and max-min values were shown. Statistical differences using one-way analysis of variance (ANOVA) followed by the Tukey's multiple range test and Pearson's correlation were calculated. Cluster analysis was performed to find similarities between analyzed groups based on the average value of each metal and using the complete linkage as a connection method and the Euclidean distance.

RESULTS AND DISCUSSION

The content of the toxic and essential metals analyzed with the ICP-OES method is presented in Table 1. The amounts of cadmium, lead and mercury should not exceed the limits (in mg kg^{-1}) such as 0.03 for Cd, 0.30 for Pb 0.01 for Hg. Only one sample of honeydew and one of dandelion honey did not

Table 1

Content of toxic and essential metals in honey from Podkarpacie (mg kg^{-1})

Metal	Type of honey							Significant statistical differences*
	multifloral (M) n = 32	dandelion (D) n = 9	rape (R) n = 10	goldenrod (G) n = 19	buckwheat (B) n = 5	linden (L) n = 6	honeydew (H) n=25	
Cd	0.01±0.01 (0.00-0.03)	0.01±0.02 (0.00-0.04)	0.01±0.01 (0.00-0.02)	0.01±0.01 (0.00-0.03)	0.01±0.01 (0.00-0.02)	0.01±0.01 (0.00-0.02)	0.03±0.01 (0.01-0.05)	H M,D,R,G,B,L
Pb	0.03±0.04 (0.00-0.18)	0.04±0.06 (0.00-0.17)	0.04±0.05 (0.00-0.11)	0.04±0.04 (0.00-0.14)	0.02±0.02 (0.00-0.04)	0.04±0.05 (0.00-0.13)	0.09±0.16 (0.00-0.73)	-
Tl	0.23±0.45 (0.00-1.53)	0.06±0.12 (0.00-0.27)	0.16±0.25 (0.00-0.65)	0.23±0.39 (0.00-1.14)	0.38±0.62 (0.00-1.42)	0.23±0.34 (0.00-0.73)	0.19±0.33 (0.00-1.22)	-
Al	1.20±1.54 (0.00-5.81)	2.84±5.45 (0.15-17.13)	0.31±0.29 (0.00-0.75)	0.78±0.69 (0.05-2.52)	0.36±0.47 (0.00-1.11)	0.68±0.77 (0.00-1.66)	29.40±14.54 (10.70-63.47)	HM,D,R,G,B,L
Hg	n.d	n.d	n.d	n.d	n.d	n.d	n.d	-
Ca	88.23±47.89 (42.45-214.62)	82.12±28.80 (41.95-123.06)	55.44±8.3 (41.73-68.56)	108.57±24.94 (60.93-163.45)	34.71±14.70 (17.51-56.35)	75.36±19.84 (47.79-102.46)	62.40±41.83 (24.51-233.59)	MB; RG GR,B,H
Mg	33.55±12.12 (10.59-59.74)	29.73±15.03 (12.57-66.06)	23.39±3.97 (16.67-28.59)	39.19±8.83 (23.91-61.85)	23.73±8.26 (15.80-35.71)	29.21±7.45 (18.89-41.19)	63.16±10.77 (42.40-80.90)	HM,D,R,G,B,L; RG
K	1047.35±629.84 (192.84-2777.98)	1107.57±532.02 (431.21-2240.18)	310.59±75.43 (221.61-431.10)	838.06±340.94 (438.63-1610.00)	616.61±351.59 (381.32-1229.97)	1449.56±500.82 (1071.15-2311.54)	2548.43±500.41 (1755.96-3547.91)	H M,D,R,G,B,L; RM,D,L
Zn	1.61±2.40 (0.00-12.57)	2.97±7.32 (0.21-22.48)	0.53±0.57 (0.00-1.99)	1.38±1.59 (0.00-7.53)	2.90±5.18 (0.04-12.15)	0.65±0.48 (0.13-1.51)	2.33±4.54 (0.35-23.75)	-
Fe	1.78±3.39 (0.00-13.07)	5.89±9.42 (0.00-26.05)	1.37±2.37 (0.00-5.77)	1.52±2.20 (0.00-6.90)	0.21±0.46 (0.00-1.03)	2.74-6.23 (0.00-15.44)	4.81±7.20 (0.00-62.63)	-
Ni	0.23±0.31 (0.00-1.45)	0.27±0.42 (0.00-1.34)	0.15±0.18 (0.00-0.48)	0.47±0.81 (0.00-2.43)	0.20±0.34 (0.00-0.78)	0.26±0.23 (0.00-0.55)	1.11±0.47 (0.14-2.30)	HM,D,R,G,B,L
Mn	3.26±3.47 (0.26-16.72)	2.07±3.18 (0.31-9.53)	0.49±0.28 (0.25-1.24)	1.53±1.11 (0.25-4.82)	7.82±2.07 (5.56-9.88)	3.86±2.89 (0.67-6.48)	4.63±2.73 (2.11-13.89)	BM,D,R,G; HR,G
Cu	0.21±0.21 (0.00-0.77)	0.25±0.31 (0.05-1.01)	0.05±0.042 (0.00-0.12)	0.16±0.12 (0.00-0.41)	0.86±0.30 (0.56-1.29)	0.20±0.13 (0.12-0.47)	0.03±0.01 (0.01-0.05)	BM,D,R,G,L; HM,D,R,G,L

Results are expressed as mean value ± standard deviations and min-max, * significant differences between means in row ($p < 0.05$), n.d. – not detected meet the required standards for the cadmium content. An exceeded limit for lead in one sample of honeydew honey was observed. Meanwhile, mercury was not detected in any of the tested samples. Higher concentrations of heavy metals in honeydew honeys compared to other honeys were found ($p < 0.05$). Other authors reported that honeys from urban areas may contain elevated levels of heavy metals. Moreover, honeydew honey can accumulate more contaminants and dust (Formicki et al. 2013, Naccari et al. 2014, Marinova et al. 2015). Thus, the mineral composition of honey may be an indicator of environmental pollution (Przybyłowski, Wilczyńska 2001, Kanoniuk 2004). In this context, the results confirmed that Podkarpacie is an ecologically clean region in Poland and therefore ensures favourable conditions for honey production and beekeeping development.

The samples varied in the aluminium content, which was from 0.31 mg kg^{-1} (oilseed rape honeys) to 29.40 mg kg-1 (honeydew honey); moreover, significant differences between honeydew honeys and other samples were determined. Although it is a metal with a low acute activity (LD_{50} for mammals is 770-980 mg kg^{-1} m.c.), persistent exposure could lead to disorders in the circulatory, digestive and nervous systems or in bones (daily exposure reaching about 4-9 mg). Altundag et al. (2016) determined a lower concentration of this metal, i.e. 2.70-8.04 mg kg$^{-1,}$ while the results of Madejczyk, Barałkiewicz (2008) were similar to ours, i.e. 0.29-35.09 mg kg^{-1}. Scantiness of references on aluminium as well as thallium in honey should be highlighted. An elevated amount of this metal in honey can be caused by improper handling of honey, because acidic pH of honey (about 3.7) contributes to the release of this metal from containers to honey.

The most abundant macroelements were potassium, calcium and magnesium, especially in honeydew honey. Potassium was the dominant macroelement, but its concentration was very diverse and ranged from 310.59 mg kg^{-1} in oilseed rape honeys to 2548.43 mg kg^{-1} in honeydew honeys. The content of calcium varied between 34.71 (buckwheat) and 108.57 mg kg^{-1} (goldenrod honeys). The content of magnesium was quite similar in all nectar honeys (between 23.39-39.19 mg kg^{-1}), but twice as high in honeydew honeys (63.16 mg kg^{-1}). Other researches confirm that these metals (mainly potassium) are abundant in honey and that generally dark honey like honeydew honey contains much more elements than light honeys (Przybyłowski, Wilczyńska 2001, Madejczyk, Barałkiewicz 2008, Chudzińska, Barałkiewicz 2010). Additionally, the honey samples in study had more magnesium than samples analyzed by other authors: 14.4-29.6 mg kg^{-1} (Nowak et al. 2011) and 1.06-21.3 mg kg^{-1} (Chudzińska, Barałkiewicz 2010), except Formicki et al. (2013) who determined more magnesium in honey (between 43 - 86 mg kg^{-1}). Significant differences ($p < 0.05$) between honeydew honeys and other honeys in the content of macroelements (magnesium and potassium) were found.

Among the microelements, the highest content of zinc was found in dandelion and in buckwheat honeys (2.97 and 2.90 mg kg^{-1}, respectively), while the lowest one was in oilseed rape honeys (0.53 mg kg^{-1}). Our results are similar to other findings (Madejczyk, Barałkiewicz 2008, Chudzińska, Barałkiewicz 2010). Meanwhile, Nowak et al. (2011) determined a significantly higher zinc content in linden honey (about 13.41 mg kg^{-1}). Roman et al. (2011) reported an average value of Zn for multifloral honeys (3.58 mg kg^{-1}) that was higher than ours (1.61 mg kg^{-1}). The values of iron ranged from 0.21 mg kg^{-1} in buckwheat to 5.89 mg kg^{-1} in dandelion honeys. Further, individual samples showed a wide variation in the content of this metal. Other researches generally report low amounts of iron, especially in acacia honey (Bogdanov et al. 2007, Nowak et al. 2011, Formicki et al. 2013).

Significant differences ($p < 0.05$) in the amount of manganese were also found between buckwheat honeys and oilseed rape as well as goldenrod honeys and between honeydew honeys and the other samples. The amounts ranged between 0.49 (oilseed rape honeys) and 7.82 mg kg^{-1} (buckwheat honeys). This is in agreement with other authors' findings (Madejczyk, Barałkiewicz 2008, Chudzińska, Barałkiewicz 2010, Nowak et al. 2011) who showed that buckwheat honeys and honeydew honeys contained significantly more Mn than oilseed rape honeys. Big differences were also observed in the content of copper, whose lowest amount was found in honeydew honeys (0.03 mg kg^{-1}) and the highest one occurred in buckwheat honeys (0.86 mg kg^{-1}). The significantly highest Cu in buckwheat honeys, with and average value of 1.40 mg kg^{-1}, was also observed by Nowak et al. (2011), which was probably the ability of buckwheat to absorb some elements such as Cu from soil and to accumulate them. Generally, the honeys tested in this study contained less Cu than samples examined by others, especially in honeydew honeys: about 0.84 mg kg^{-1} (Marinova et al. 2015), 1.19 mg kg^{-1} (Chudzińska, Barałkiewicz, 2010) and from 0.26 to 1.82 mg kg^{-1} (Madejczyk, Barałkiewicz 2008).

The presence of nickel, one of non-essential elements was identified in honey. There was a significant difference in the content of Ni between honeydew honeys (1.11 mg kg^{-1}) and nectar honeys (0.5 mg kg^{-1}). Nowak et al. (2011) reported slightly higher concentrations of this metal in nectar honeys (from 0.54 to 1.25 mg kg^{-1}), while others found much lower Ni levels (Bodanov et al. 2007, Madejczyk, Barłkiewicz 2008, Chudzinska, Barałkiewicz 2010). However, the level of Ni in honeydew honey reported by the cited authors was in agreement with our results.

However, significant differences in mineral composition between honeydew honey and other monofloral honeys were observed, while significant correlations between metals occurring in honey were rare. A strong positive correlation was observed between: Cu and Al ($r = 0.82$), Al and K ($r = 0.80$), Al and Mg ($r = 0.80$), K and Cu ($r = 0.80$), while a less strong correlation appeared between: Al and Mg ($r = 0.77$), Mg and Cu ($r = 0.71$) and Cu with Ni ($r = 0.69$). These observations seem to indicate the specific chemical composition and properties of soil in Podkarpacie, which influenced the honey mineral content more than the botanical origin.

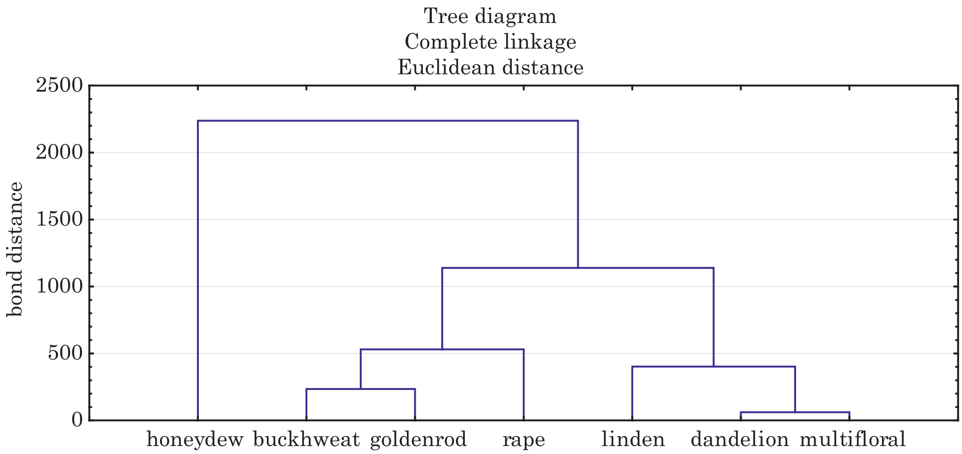

Fig. 2. Tree diagram based on average values of metals (complete linkage, Euclidean distance)

Cluster analysis was performed to find similarities between the analyzed types of honey based on average value of toxic and essential metals (Figure 2). Close correlation was found between dandelion and multifloral honeys (bond distance 61) and between buckwheat and goldenrod honeys (bond distance 234). The largest distance (bond distance 2238) was determined between honeydew honeys and oilseed rape honeys. However, in each case the distance between honeys was very large, which might be due to considerable differences in the composition of each sample in terms of individual elements.

CONCLUSIONS

Our results indicate that honeys produced in Podkarpacie are characterized by high quality in terms of heavy metal concentrations because the detected levels of all toxic metals were much below allowable limits. The lack of statistically significant differences in the content of heavy metals in honeys originating from various parts of the region probably reflects accidental exposure of bees to environmental pollution. Moreover, our results show that botanical factors have a strong influence on the macro- and trace element content of honey. Honeydew and buckwheat honeys were found to be the richest sources of macro- and microelements, whereas oilseed rape honey proved to be the poorest in the minerals. Concluding, honeys from Podkarpacie can be recommended as safe and beneficial food for human consumption.

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