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Research Article

EVALUATION OF THE CONTENT OF SELECTED HEAVY METALS IN SAMPLES OF POLISH HONEYS

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ABSTRACT

This paper presents the result of the determination of the total content of Cu, Pb and Zn by the method of atomic absorption spectrometry with atomization in an air-acetylene flame in Polish honeys samples. The research material was a honeydew, monofloral honey and buckwheat honey. For the mineralization of samples, the following solutions were applied: 1) $HNO_{3(conc)}$, 2) $HNO_{3(conc)}$ and $H_2O_{2(conc)}$ in the volume ratio equal to 4 : 1 and 3 : 1. On the basis of the results and recommended food standards the percentage of the recommended dietary allowances (RDA) in connection with the consumption of 100 g of product were estimated. To verify the results validation of analytical method used was carried out. It was included defining the following validation parameters values: the limit of detection and quantification; linearity and measurement range; repeatability and accuracy of the results. The contamination of the analyzed honeys by Pb is higher than the acceptable level of contamination of this element. The highest contents of Cu and Zn were characterized by monofloral honeys.

Keywords: bee products, heavy metals, FAAS.

INTRODUCTION

The western honey bee *(Apis mellifera)* is respected in the world as a strategic pollinator of crops. Bee products (honey, propolis, royal jelly) have been widely used in the food industry and medicine. The most popular is honey, which is a valuable nutrient. It has antibacterial and immunogenic properties. First references on them appeared in the early Paleolithic Era, within cave paintings [Kędzierska-Matysek et al. 2013]. It is produced from nectar of plants, secretions of living parts of plants, as well as from insects' secretions [Koszowska et al. 2013]. The basis for the universal use of honey in diet is in its complex chemical composition. Significant importance is attributed to energy, building and regulating nutrients, which are necessary for the proper development and functioning of the body. The quality of honey is determined by climate, environment, soil, origin of the honey, and storage conditions [Wantusiak et al. 2011].

Honey bees can be used to monitor the condition of the natural environment.While collecting flower pollen or honeydew, insects are exposed to frequent contacts with contaminants which are settling on plant shoot system and can be transferred into honey. Apiculture products can be contaminated with chemicals derived from agricultural and industrial operations. Their analysis allows determining the level of contamination with heavy metals, pesticides, xenobiotics, and radioactive substances. Honey can accumulate heavy metals such as lead, zinc, cadmium and copper. Due to the relatively low penetration range of these sediments (2–3 km), their concentration in bees' bodies and apiculture products determines the local contamination level of the nearest surrounding [Kisała and Dżugan 2009]. The level of environmental pollution can be reflected in the composition of honeys.

The presence and circulation of heavy metals in ecosystems is a common phenomenon. The exceeded natural content of elements caused by agriculture and industrial operation, as well as municipal services, can be a potential environmental threat [Piontek 2014]. Most of the heavy metal compounds can be characterized by greater stability, which means that once introduced into ecosystems, they remain there forever and can be transported over long distances. Lead, zinc and copper poisoning is particularly dangerous for both animals and humans. Therefore, it is important to determine places where the concentration of these elements significantly exceeds acceptable standards, and to identify potential access ways into living organisms.

Apiculture products may be contaminated directly (apiculture practice) or indirectly (environment, agriculture). Heavy metals, radioactive elements and permanent organic pollutants (pesticides, PCBs, PAHs) are the most common contaminants of these food products [Fernández et al. 2002]. In order to determine the content of the above-mentioned substances, a number of different analytical procedures are used. The correctness of the final result is determined by all the stages of the analytical procedure, i.e., sample collecting and preparation, isolation of analytes from the products, purification of the received extracts, and the final determination. Choosing the right technique for the final determination depends on the properties of the determined component. The chosen methodology should be characterized by high sensitivity, precision, selectivity, and the repeatability and linearity of results [Gąsior 2007]. Various analytical chromatographic methods and spectroscopic techniques, such as AAS, ICP-AES, ICP-MS are used for the determinations of trace elements [Synak et al. 2010].

Atomic absorption spectrometry (AAS) is the most commonly used method for the determination of heavy metals. The method is characterized by greater selectivity, ppb detection limit and the possibility of determination of 70 elements [Szczepaniak 2011]. Sergiel and Pohl [Sergiel and Pohl 2013] determine the concentration of Ca, Cu, Fe, Mg, Mn and Zn in honey using ASA technique and stated that dark honeys are characterized by a substantial amount of Mn. Also, Kędzierska-Matysek et al. [Kędzierska-Matysek et al. 2013], Naggar et al. [Naggar et al. 2013] and Przybyłowski et al. [Przybyłowski et al. 2003]] carried out a quantitative analysis of honey samples using ASA technique. Another, equally common method for determination of heavy metals in environmental samples is inductively coupled plasma atomic emission spectrometry (ICP-AES). ICP method is characterized by greater reproducibility and accuracy of results, makes it possible to determine elements that are difficult to determine within other methods. Moreover, ICP method allows for simultaneous determination of almost all elements in a single excited state [Stefánsson et al. 2007]. Roman [Roman 2007], within this method, described the content of selected trace metals in fresh flower pollen and noted a high content of cadmium in most of the analyzed samples. ICP-AES method was also used for the determination of trace elements in honey samples [Nowak and Piotrowski 2011, Roman 2003, Madejczyk and Baralkiewicz, 2008]. Inductively coupled plasma mass spectrometry (ICP-MS) is also used for the determination of heavy metals in apiculture products. This method is characterized by greater sensitivity and selectivity and allows for the analysis of single and multi-elements. The quantitative analysis of apiculture products using this procedure was carried out, among others, by Chudzińska [Chudzinska and Baralkiewicz 2010, 2011], Yücel [Yücel and Sultanoğlu 2013] and Chua [Chua et al. 2012]. Chromatographic methods, i.e., ion exchange chromatography, are also used for the determination of heavy metals in apiculture products [Buldini et al. 2001]. This technique was used in 1989 to determine the content of alkali and alkaline metals in honey samples [Perez Cerrada et al. 1989]. The content of heavy metals in apiculture products can also be determined by voltammetric measuring method (e.g., DPASV) [Buldini et al. 2001, Sanna et al. 2002].

The aim of this thesis was to determine the content of selected heavy metals (Pb, Zn, Cu) in the Polish honey samples and to define the impact of the composition of mineralizing solution on determined results, as well as to estimate the percentage of the recommended dietary allowances (RDA) in connection with consumption of 100g of a product based on the results of the total content of determined metals in the analyzed honeys and recommended food standards.

MATERIALS AND METHODS

Reagent and instrumentation

All chemicals and reagents were of analytical grade or higher purity and were obtained from POCH, Poland. The solutions were prepared by dissolving appropriate compounds in doubledistilled water from the Water Purification System (SolPure 7 POLL LAB, Poland). Standard solutions were prepared from standard solutions for atomic absorption (Sigma-Aldrich Chemie GmbH, Switzerland) – the concentrations of all metals in standard solutions were the same and equals 1000 $\mu g \cdot cm^{-3}$ in 1% of HNO_3. Working standard solutions containing Cu, Pb and Zn were prepared by serial dilution of the appropriately of standard solution for atomic absorption.

The flame atomic absorption spectrometer Perkin-Elmer 3100 Model (Shelton Instruments, CT USA) was used for Cu, Pb and Zn determination in the all solutions. Measurements were performed at wavelength 324.8 nm (Cu), 213.9 nm (Zn) and 217.0 nm (Pb) using a yellow fuel-rich air-acetylene flame (acetylene flow velocity 2.0 $dm^3 \cdot min^{-1}$; air flow velocity 8.0 $dm^3 \cdot min^{-1}$) and burner height of 3 mm. The analytical lines were selected using a slit width 0.7 mm (Cu, Pb and Zn). Hollow cathode lamp at 10 mA (Cu, Pb and Zn) was used [The Perkin – Elmer Corporation 1982].

Honey samples

Research material consisted of four Polish honeys:

1) honeydew honey (honey from Polish apiaries), apiary T. Kozak Family Apiary in Roztocze region, Kozaki 8, 23-412 Łuków.
2) monofloral honey I, APIS Apiculture Cooperative in Lublin, Diamentowa 23, 20-472 Lublin.
3) monofloral honey II, produced in Poland for Tesco/Poland by Huzar, Kapelanka 56, 30-347 Kraków.
4) buckwheat honey, APIS Apiculture Cooperative in Lublin, ul. Diamentowa 23, 20-472 Lublin.

Analytical methods

For the determination of the total content of Cu, Zn and Pb in the analyzed honeys, three different mineralizing solutions are used [Santelli et al. 2006]. Mineralization was carried out in an open system, at elevated temperature. Particulars of the various procedures are summarized in Figure 1.

Determination of the concentrations of Cu, Zn, Pb in mineralizing solutions were performed by atomic absorption spectrophotometry with excitation flame: acetylene-air (FAAS). The assay conditions of the tested metals are summarized in Table 1.

Validation

Considering the fact that honey is a complex sample, the validation of analytical methodologies is crucial. Its primary objective is to obtain reliable analytic results.

Implementation of the solubilization method of honey samples with the usage of a variety of mineralizing solutions was under validation process, which included defining the following validation parameters values: the limit of detection and quantification; linearity and measurement range; repeatability and accuracy of the results.

Blind, master and actual samples were used in the validation process. Measurements were performed in triplicate, and the individual parameters were determined by the generally accepted principles of statistical analysis of the results (Student's t-test, p = 95%).

The measurement range and linearity is determined on the basis of the standard solutions used for calibration of the spectrometer. The concen-

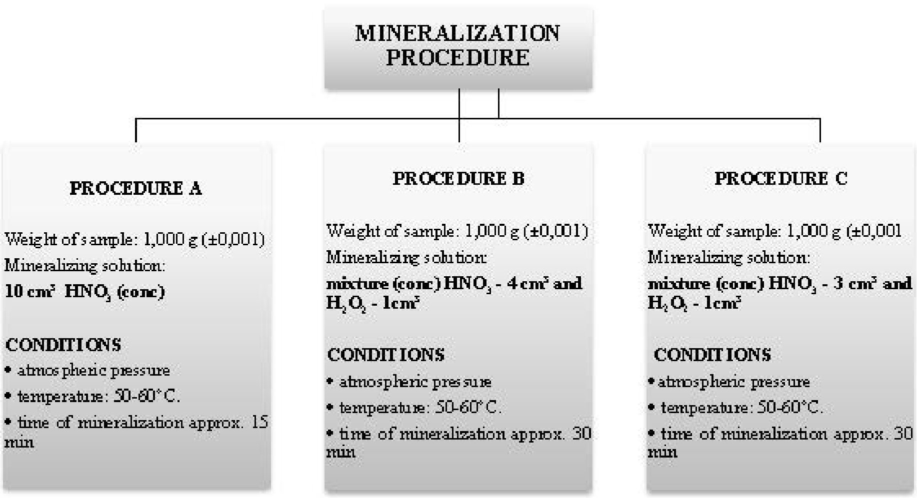

Figure 1. The detailed conditions of mineralization procedures

Table 1. Optimal instrumental parameters for FAAS determination of metals

Parameter	Metal		
	Cu	Pb	Zn
Wavelength [nm]	324.8	213.9	217.0
Lamp current [%]	100	75	75
Spectral width slit [nm]	0.7	0.7	0.7
Acetylene flow rate [dm^3/min]	0.8–1.0	0.8–1.0	0.8–1.0
Time of measurement [sec.]	3	3	3
Sensivity [mg/dm^3]	0.045	0.190	0.084
Background correction	off	off	off
Concentration of standard solutions [mg/cm^3]	2, 4, 5	9, 20	1, 3, 5, 6

tration range of the standard solutions used in the calibration process for each metal resulted from recommendations of the spectrometer producer. The linear regression equations for each metal were determined on the basis of standard solutions analysis with concentration presented in Table 1. The level of compatibility of measurement points with a predetermined linear function was determined by the linear coefficients of determination (R2). Its value was higher than 0.9700 for mineralization methods used for all determined metals. Table 2 presents the linear values of the coefficients of determination and linear ranges of determined metals by FAAS method.

The limit of detection and determination for each metal was estimated by the following relations: 3 × SD / m 10 × SD / m respectively, where m is the slope of the calibration curve, and SD is the standard deviation for the determination of the metal in 9 blind sample solutions [Wood 1999, Araujo 2009]. The values for the determination of Cu, Pb and Zn by FAAS method are summarized in Table 2.

The accuracy and repeatability of the results was estimated on the basis of the recovery tests and the value of the coefficient of variation (CV) respectively. 9 simultaneous analyses of master samples containing the standard element, i.e., specified metal, were carried out to determine the recovery value of metals with the usage of the

Table 2. Calibration and analytical performance data

Parameter	Cu	Pb	Zn
Correlation coefficient	0.999	0.997	0.999
LOD [μg/dm^3]	0.3	0.6	0.9
LOQ [μg/dm^3]	1.2	1.8	3.1
Linear range	5	20	1

mineralizing solution. Determination of metals (Cu, Pb and Zn) in the obtained solution was performed in triplicate.

DISCUSSION

The total content of metals in the studied samples of honey

The results of the total content of metals in studied honey samples are summarized in Table 3.

Honeydew honey

The concentration of Cu in the analyzed honeys samples amounted to 0.96–30.10 mg/kg, the content of Zn was estimated between 1.93–8.99 mg/kg, and Pb ranged from 1.95 to 39.0 mg/kg. Significant differences were observed in the individual elements of the same types of honey samples. These changes may be due to different soil and weather conditions, as well as by the final product receiving technology (technical contamination). Comparable Zn levels were noted by Rashed et al. [Rashed and Soltan 2004].

Monofloral honey I

The concentration of Cu in the analyzed honey samples ranged from 1.98 to 7.96 mg / kg, content of zinc from 3,92–8,77 mg/kg and lead from 0.97 to 4.97 mg/kg. The average content of Cu, Zn and Pb is similar for most samples. Comparable levels of Cu, Zn and Pb were noted by Pohl [Pohl 2009].

Monofloral honey II

The content of Cu and Pb in the analyzed honey samples amounts from 0.98–1.99 mg/kg and 1.97–4.95 mg/kg respectively. Average content level of these elements is similar in all honey samples. Comparable Cu content noted Munoz et al. [Munoz and Palmero 2006] and Pb noted Rashed et al. [Rashed and Soltan 2004]. The concentration of Zn amounts from 0.99–10.92 mg/kg. Significant differences in the content of the element in individual samples may be due to the different soil and weather conditions, and may be caused by technological containments. The same content of Zn in honey samples was noted by Stankovska et al. [Stankovska et al. 2008].

Buckwheat honey

Concentration of Cu and Pb in the analyzed honey samples amounts from 0.97–2.97 mg/kg and 0.97–0.99 mg/kg respectively. The average content level of these metals is similar in all

Table 3. The total content of metals in honey determined by FAAS method (n = 9, p = 99.5%)

Metal	The metal content [mg/kg]			
	Minimum	Maximum	Average	Acceptable content*
	Honeydew honey			
Cu	0.96	30.10	5.10	< 1.08
Zn	1.93	8.99	2.93	3.4 – 47.7
Pb	1.95	39.0	8.30	< 0.259
	Monofloral honey I			
Cu	1.98	7.96	3.18	
Zn	3.93	8.77	2.93	
Pb	0.97	4.97	2.64	
	Monofloral honey II			
Cu	0.98	1.99	0.99	
Zn	0.99	10.92	2.20	
Pb	1.97	4.95	3.30	
	Buckwheat honey			
Cu	0.97	2.97	1.64	
Zn	2.96	42.18	8.97	
Pb	0.97	0.99	0.66	

* Maximum permissible concentration according to Polish Standards PN-88/A-77626.

honey samples. Comparable copper content in the honey samples were noted by Nanda et al. [Nanda et al. 2003]. The Zn content amounts from 2.96 to 42.18 mg/kg. Significant difference in the value of this element may be due to different soil and weather conditions, as well as different level of environmental pollution.

The influence of different type of mineralizing solutions on the total content of Cu, Zn, Pb in honey samples

The results of the kind of mineralization solutions on the total content of metals in the studied honey samples are presented in Figure 2.

Procedure A

The honeydew honey is characterized by the highest content of lead and copper, while buckwheat honey has the highest content of zinc. The results were compared with Polish Standard PN-88/A-77626 values. It was noted that in the case of honey dew, the content of copper and lead is higher than acceptable level, while the zinc concentration is below the limit. In the monofloral honey I the acceptable concentration of copper and lead was higher and the content of lead is acceptable. The monofloral honey II has an increased content of lead. The concentration of copper and zinc is normal. In buckwheat honey the content of lead is higher. The highest content level based on the weight of the sample was noted for lead in honeydew honey sample, i.e., 14.95 mg/kg.

Procedure B

In the case of the concentrated HNO_3 + H_2O_2 solutions used as mineralizing solution in 4:1 proportion, the highest lead content has monofloral honey I. The highest content of zinc and copper was noted in honeydew honey. The obtained results were compared with the Polish Standard PN-88/A-77626 data. It was noted that in all honey samples levels of copper and lead were higher than acceptable. In the case of zinc, honeydew honey, monofloral honey I and monofloral honey II, the concentrations are below the standard level, while in buckwheat honey the content of Zn fits acceptable standards. Among the determined elements the highest content of metal based on the weight of the sample was noted for Zn in buckwheat honey, i.e., 16,70 mg/kg.

Procedure C

Monofloral honey I is characterized by the highest content of lead. The highest content of zinc and copper was found in the honeydew honey. The results were compared with the Polish Standard PN-88 / A-77626 data. In the case of this mineralization/digestion procedure honeydew honey appears to have a high content of copper and lead and content of zinc is normal. The monofloral honey I is characterized by higher levels of copper and lead, while the zinc content is below the standard level. The monofloral honey II has higher level of lead. Concentration of copper and zinc is too low in relation to the standard level. The buckwheat honey has a high content of lead, while the concentrations of copper and zinc fit the standard levels. Among the determined maximum content of metals based on the weight of the sample was observed for Cu honeydew (10.40 mg/kg). From the determined metals the highest content of Cu was noted in buckwheat honey.

The obtained results indicate that the most effective mineralizing solution is the $HNO_{3(conc)}$ solution for all determined metals.

Estimation of derived analyzed metals with food ration

In accordance with the recommendations of the FAO Nutrition Committee/WHO Nutrition Committee the higher content of heavy metals in animal raw materials and products does not dis-

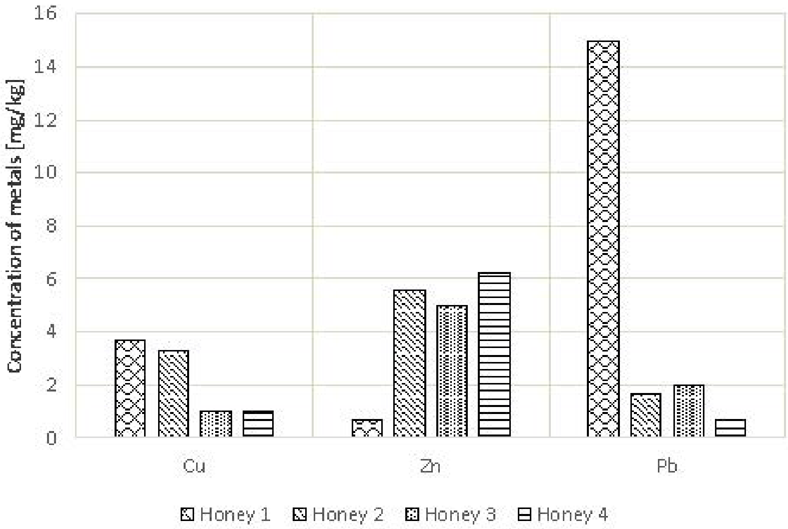

Figure 2. The content of Cu, Pb and Zn in the samples of honey [mg/kg] using as mineralization solution $HNO_{3(conc)}$

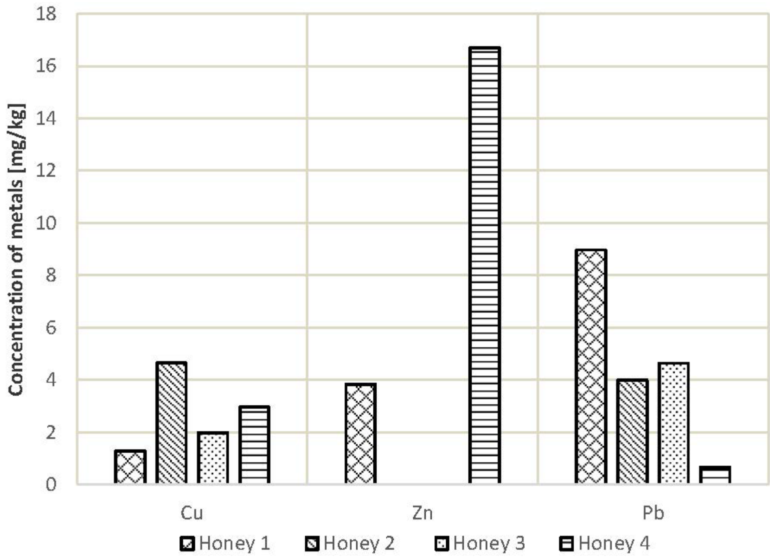

Figure 3. The content of Cu, Pb and Zn in the samples of honey [mg/kg] using mixture of concentrated solutions $HNO_3 + H_2O_2$ in ratio 4:1

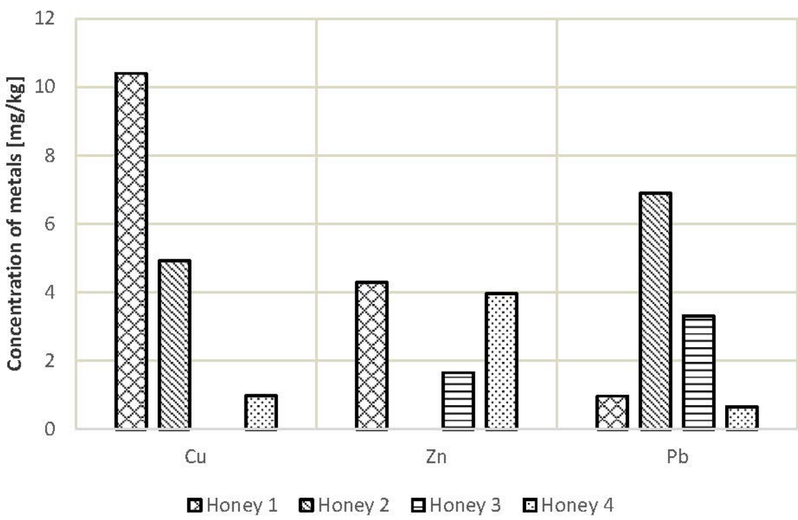

Figure 4. The content of Cu, Pb and Zn in the samples of honey [mg/kg] using mixture of concentrated solutions $HNO_3 + H_2O_2$ in ratio 3:1

Table 4. RDA [%] for Cu and Zn for adults connection with consumption of 100g of a product

Parameter	RDA [%]			
	Cu		Zn	
	Female	Male	Female	Male
	Honeydew honey			
Minimum	10.60		2.41	1.75
Maximum	334.40		11.24	8.17
Average	56.62		3.66	2.66
Monofloral honey I				
Minimum	22.20		4.90	3.50
Maximum	88.40		10.90	7.90
Average	35.41		6.90	4.96
Monofloral honey II				
Minimum	10.80		1.24	0.99
Maximum	22.00		13.65	9.93
Average	10.96		2.75	2.01
Buckwheat honey				
Minimum	1.10		3.70	2.70
Maximum	32.60		52.73	38.35
Average	17.12		11.21	7.82

qualify them for human consumption if the level of these metals corresponds with daily food intake. The coverage level of the recommended dietary allowance (RDA) for Cu and Zn per 100 g of honey for adults was calculated. The test results presented in Table 4 are in accordance with the coverage level of the recommended dietary allowance for Cu and Zn for adults.

Honeydew honey

The coverage level of the recommended dietary allowance (RDA) for Cu in consumption of 100 g is the same for women and men. The average content of Cu in honeydew honey amounts to 55.56% of the recommended dietary allowance for Cu in 100 g of a product. In the case of honeydew honey, the highest RDA value for copper has sample 9, which amounts to 334.4%. The highest coverage level of recommended dietary allowance for zinc for women and men can be observed in the case of sample 8. It amounts to 11.24% for women and 8.17% for men. The average content of Zn for women in honeydew honey is 3.33% and for men it amounts to 2.66% of the recommended dietary allowance for zinc per 100 g of a product.

Monofloral honey I

The average content of Cu in monofloral honey I is 32.1% of the recommended dietary allowance for Cu per 100 g of a product. The highest RDA value for copper has sample 5 (88.4%). The RDA for Zn for men and women differ considerably. The recommended dietary allowance for women is higher. The highest RDA value for women is in sample 1 (10.9%). Sample 1 also has the highest RDA for men (7.97%). The average content of Zn in monofloral honey I for women amounts to 2.31%, while for men 0.56% of the recommended dietary allowance for zinc per 100 g of a product.

Monofloral honey II

The average content of Cu in the analyzed sample for women and men is 10.96%. The highest coverage value of the recommended dietary allowance is in sample 5 and amounts to 22.1%. On the other hand, the RDA for zinc for men and women is the highest in the sample 2 and amounts to 13.65% and 9.93% respectively. The average content of Zn per 100 g of a product for women in monofloral honey is 2.75% and for men is 2.01%.

Buckwheat honey

The average content of copper in buckwheat honey is 17.12% of the recommended dietary allowance for Cu per 100 g of a product. The highest coverage value of recommended dietary allowance for copper is in the sample 4 and amounts to 32.8%. In the case of zinc, the highest RDA was recorded for both women and men in the sample 5 and amounts to 52.73% and 38.35% respectively.

Table 5. Recoveries [%] and coefficients of variation [%] for the studied elements of honey samples prepared with mineralization methods (procedure A)

Metal	Recoveries [%]	Coefficients of variation [%]
Honeydew honey		
Cu	99.0	5.3
Pb	95.5	11.8
Zn	99.8	8.6
Nectar honey I		
Cu	98.0	13.7
Pb	95.0	14.2
Zn	98.5	2.6
Nectar honey II		
Cu	99.5	3.2
Pb	99.5	8.6
Zn	99.0	1.6
Buckwheat honey		
Cu	95.5	3.6
Pb	98.0	3.9
Zn	99.7	1.2

The average content of Zn per 100 g of a product in monofloral honey for women is 11.21% and for men 7.82%.

Validation

Collected recovery values and calculated values of coefficient of variation in accordance with the applied mineralizing solutions are presented in Table 5. For all determined metals, the recovery was in the optimum level between 70 and 120%. The highest levels of recovery of analyzed metals were obtained for mineralizing methods with the concentrated HNO_3 solution. Due to the fact that the coefficient of variation for all analyzed samples of all metals does not exceed 20%, it can be assumed that the obtained results with the usage of all three mineralizing solutions are repeatable.

CONCLUSIONS

1. Honey bees can be used as environmental pollution bioindicators. Analysis of heavy metals in apiculture products provides information on local contamination of ecosystems.
2. Maximum level of Pb was found in honeydew honey (39.00 mg/kg). The highest content of Zn metal (8.77 mg/kg) was found in monofloral honey I. Zn has the highest concentration level in monofloral honey II and buckwheat honey, which amounts to 10.92 mg/kg and 42.18 mg/kg. respectively.
3. The evaluation of the total content of Cu and Zn showed that the analyzed honeys contain low, without any health restrictions, concentrations of analyzed metals. The contamination of analyzed honeys by Pb is higher than acceptable level of contamination by this element and therefore, may pose a health threat.
4. Type of mineralizing solution affects the result of the determination of the total content of Cu, Zn and Pb in honey samples. The highest concentration of the analyzed metals was obtained with the use of concentrated HNO_3 solution.
5. Due to the fact that honey bees are very good bioindicators and due to the content of heavy metals, the environment needs a constant monitoring of heavy metals level in honeys and apiculture products.

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