A Comparative Study of Metal Content in Selected Polish and Romanian Honey Samples

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The aim of the study was to compare the heavy metal contamination and study the mineral content of Polish and Romanian honeys. We used Polish varietal honeys (n=18) produced in the year 2013-2014 in ecologically clean Podkarpackie region, and Romanian honeys (n=36) from Bihor district produced in 2013. The heavy metals concentration as well as mineral composition of honey was assayed by ICP-OES method with prior microwave mineralization. The cadmium and lead level in tested Polish honeys ranged from 0.007 to 0.021mg/kg and 0.02 to 0.098 mg/kg, respectively. Cadmium was not detected in Romanian honeys, and the lead contamination was lower than in Polish honey (0.018-0.05 mg/kg). Mercury was not detected in any tested honey samples. The aluminum content in Polish honeydew honeys was 34.6-times higher than in Romanian honeys. Honey samples from Poland were richest in potassium (2.5-fold for honeydew, lime and acacia, excluding heather), but they included less calcium. Generally, dark honeys include the highest mineral concentration and are more contaminated by heavy metals. However, all tested samples met the legal requirements for heavy metals residues. The geographical origin and soil composition strongly influenced honey chemical composition and the crucial factor for heavy metal transfer seems to be soil pH.

Key words: honey, heavy metal, nutritional minerals, environment contamination

Honey is a natural sweet substance produced by honeybees (*Apis mellifera* L.) from the nectar of blossoms or from secretions of living parts of plants or excretions of plant-sucking insects. It is known as a delicious food, which can be easily digestible and serves as rich source of nutritiously important complementary elements [1]. Indeed, medicinal importance of honey has been documented in the world's oldest medical literatures, and since ancient times, it has been known to possess antimicrobial properties as well as the ability to heal wounds [2]. As the composition of honey is variable and depends primarily on its floral source (type) and geographical origin [3], its health quality needs evaluation.

Honey contains a mixture of carbohydrates (mainly mono- and disaccharides up to 85% m/m), water (up to 20%) and other organic compounds (organic acid, amino acid, enzymes, vitamin) and inorganic ions being present to a minor extent [4]. Multiple minerals and trace elements were identified in honeys. The mean content of mineral substances in honey has been calculated to be 0.17% m/m, although it varies strongly depending on honey type [3]. However, the contribution of honey to the recommended daily intake (RDI) of the different trace elements is marginal [4].

While being a natural source of bio-elements, honey can also contain harmful elements to human health. Some of the heavy metals like iron, copper, zinc and manganese are essential in traces since they play an important role in biological systems, whereas lead and cadmium are non-essential metals which can be toxic even in trace amounts [5]. Lead and cadmium are considered the main toxic heavy metals and thus are most frequently studied [6]. It is well known that heavy metal contamination of nectar and honeydew honeys is increased in areas of industrial and intensified traffic [7].

Honey composition is influenced by the plant species, soil resources on growing, environmental conditions and by the beekeepers through the extraction and processing. Honey commercially available in Romanian markets, has a high variation in quality, largely assessed by color, flavor and density [8].

Bees and bee products have great potential for detecting and monitoring environmental pollution, as their way of life exposes them directly and indirectly to the impact of pollution [9, 10]. There is a good correlation between the level of heavy metals accumulation in soil and plants [11, 12]. The degree of contamination decreases in the following order: bees ≥ propolis > comb wax > honey. Low contamination of honey is probably due to *filtering* by the bees [6]. However, honey has been recognized as a biological indicator of pollution by heavy metals [3,13-16].

Honey is an important food for the human nutrition. As foodstuff used for healing purpose, honey must be free of unsafe contents [4]. Analysis of trace elements content in honey is necessary in food quality control and nutritional aspects because high levels can also be dangerous and cause toxicity [17]. Considering that honey is widely consumed by most people, especially in children, ill and elderly adults diet, and that heavy metals are systemic toxicants known to induce adverse health effects in humans, the monitoring of honey safety should be intensified.

The major minerals are mainly derived from the soil and nectar producing plants, but they may also come from anthropogenic sources, such as environmental pollution [18].

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Typically, honeydew honey has a higher chance of accumulating dust and dirt [19- 21] than nectar honey. Probably this availability is important for bees gathering honeydew in favorable conditions over a large surface of the leaves, stems, and even on the ground around the honeydew's plants.

Roman [15] examined honey samples originating from former airport of USSR troops, and the concentration of cadmium and lead determined in multifloral honey was on average of 0.03 mg/kg and 0.26 mg/kg, respectively. For comparison, honeys from the area of agri-farm contained 0.05 mg/kg Cd and 0.15 mg/kg Pb. In our study, lower contamination of Romanian honeys was observed.

Meanwhile, [14] found elevated amounts of heavy metals in honeys from ecologically clean areas in Romania (Sibiu region): multifloral (0.015 mg/kg Cd and 0.07 mg/kg Pb), lime (0.018 mg/kg Cd and 0.09 mg/kg Pb) and acacia (0.017 mg/kg Cd and 0.089 mg/kg Pb). Similarly, Solayman [18] detected a maximum of 0.24 mg/kg Cd and 0.15 mg/kg Pb in Turkish honey.

The aim of the study was to compare the heavy metal contamination and study the mineral content of Polish and Romanian honeys depending on its geographical and botanical origin.

Experimental part

Materials and methods

The material consists in fifty four honey samples purchased from apiaries localized in Bihor district, North-West Romania and in Podkarpackie region, South -Eastern Poland, presented in table 1.

Metal content determination

The content of 13 elements including toxic heavy metals (Al, Cd, Cr, Ni, Pb and Hg) as well as nutritional minerals (Ca, Cu, Fe, K, Mg, Mn and Zn) was determined by optical emission spectrometry with inductively-induced plasma (ICP-OES) using a Thermo iCAP 6500 spectrophotometer (Thermo Fisher Scientific Inc., USA). Before the determination of elements, wet mineralization was carried out. The samples of honey (1 g) were weighed into Teflon vessels, and added with 8 mL of concentrated Nitric acid (65% POCH, Poland). The mineralization of the honey samples was performed by using microwave mineralizer Milestone Ethos Ultrawave-One (Milestone SRL, Italy) for about 45 min in Teflon containers. After cooling, the samples were transferred quantitatively to a 50 cm^3 flask and supplemented with redistilled water to the mark. Next they were examined by ICP-OES spectrometry. The precision of the analytical method was evaluated in terms of repeatability of the experimental results of real samples and expressed as standard deviation (S.D). The accuracy was verified by calibration (using standard solutions). Additionally the internal standard (^{89}Y, ^{173}Yb) was applied for ICP-OES technique to correct the matrix effects.

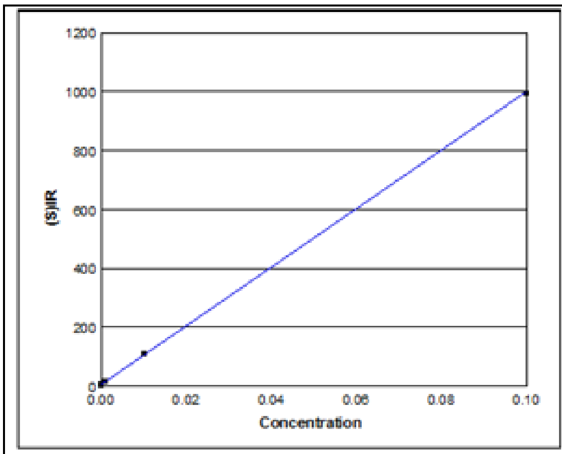
The standard curve linearity range for Cd was from 1μg/L to 100 μg/L with correlation on level 0.9998 and for Pb was from 5μg/L to 500μg/L with correlation on level 0.9996 (fig. 1).

Statistical analysis

The honey samples were classified in eight groups respecting the country and honey type factors. All the data was subjected to non-parametric analysis of variance. Mann-Whitney test (P =0.05) was done in order to establish

Table 1
ANALYSED HONEY SAMPLES CODIFICATION

Sample code	Nr of sample	Type of honey	Botanical origin
Romanian honey			
RO_ACC	6	Acacia	*Robinia pseudoacacia*
RO_HDW	18	Honeydew	*Unknown*
RO_HTH	6	Heather	*Calluna vulgaris*
RO_LM	6	Lime	*Tillia*
Polish honey			
PL_ACC	4	Acacia	*Robinia pseudoacacia*
PL_HDW	5	Honeydew	*Unknown*
PL_HTH	4	Heather	*Calluna vulgaris*
PL_LM	5	Lime	*Tillia*



Element Name:	Cd
Element Wavelength :	Cd 228.802 nm
Concentration Units :	ppb
Date of Calibration :	22/05/2015 10:37:12
Date of Fit:	22/05/2015 10:37:12
Type of Fit:	Linear
Correlation:	0.9998
A0 (Offset):	4.410
A1 (Gain):	9.960
A2 (Curvature):	0.0000
n (Exponent):	1.000

Reslope		QC Normalize	
Slope:	1.000	Slope factor:	1.000
Y Int:	0.0000	Offset:	0.0000

Standard Name	Stated	Found	Diff	% Diff	(S)IR	Stddev	Emphasis
Blank	0.0000	0000001879	-0.0000001879	0.0000	4.408	1.236	1
#1	0.001000	0.001152	0.0001518	15.18	15.88	1.214	1
#2	0.01000	0.01042	0.0004183	4.183	108.2	0.4115	1
#3	0.1000	0.09943	-0.0005701	-0.5701	994.7	4.931	1

Fig. 1. Standard curve linearity for Cadmium

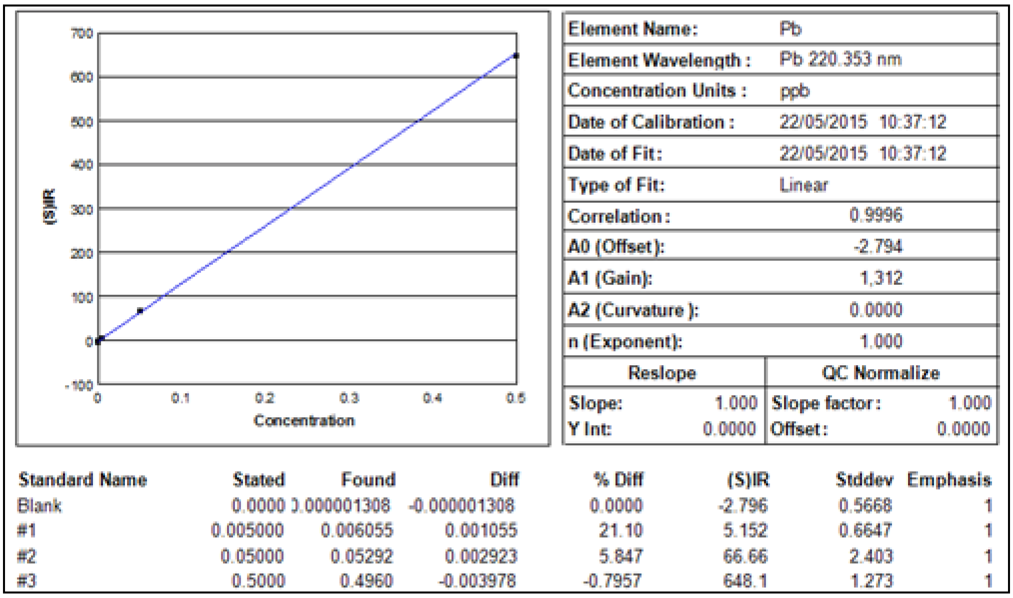


Fig. 1. Standard curve linearity for Lead

the significant differences between two independent sample means (PAST 3.05, [22]).

Metals content of each honey sample can be called metal sample profile. In order to describe the differences between the metal profiles from the honey samples, a multivariate sequence was conducted. This sequence consists of four multivariate statistical methods: principal component analysis (PCA), linear discriminant analysis (LDA), multivariate analysis of variance (MANOVA) (P = 0.05) and hierarchical cluster analysis (HCA) (PAST 3.05, [23]).

Results and discussion

Metal content determination

Analysing the results of toxic metals in tested honey samples, the mean values are summarized in table 2, while the interval plot of toxic metals content with 95% confidence intervals for factors: Country and Honey Type are represented in figure 2. The results showed that in the selected honey samples, heavy metals accumulated differently depending on the species and origin.

Mercury was not detected in any samples and Cadmium was not detected in Romanian honeys. Among the studied honeys, in the case of cadmium, the highest content has been demonstrated for polish honeydew honeys (0.021

Table 2

NON-PARAMETRIC MANN-WHITNEY TEST OF MEANS (P =0.05) RESULTS PRESENTED AS OBSERVATIONS NUMBER (N), MEAN, STANDARD DEVIATIONS (SD) AND STATISTICAL SIGNIFICANCE (*P*-VALUE)FOR TOXIC METAL CONTENT OF SELECTED HONEY SAMPLES. BOLD *P*-VALES PRESCRIBE STATISTICAL SIGNIFICANT DIFFERENCES OF MEANS

Type of honey	Country	Al[mg/kg]	Cd[mg/kg]	Cr[mg/kg]	Ni[mg/kg]	Pb[mg/kg]
		mean	mean	mean	mean	mean
		SD	SD	SD	SD	SD
ACC	PL (N=4)	0.596	0.019	0.037	0.184	0.050
		0.241	0.006	0.006	0.368	0.063
	RO (N=6)	0.046	0.000	0.030	0.046	0.027
		0.051	0.000	0.046	0.005	0.030
	p-value *	**0.014**	**0.014**	0.441	0.232	0.825
HDW	PL (N=5)	41.446	0.021	0.059	1.241	0.098
		17.951	0.005	0.020	0.426	0.103
	RO (N=18)	1.197	0.000	0.022	0.125	0.018
		0.321	0.000	0.029	0.045	0.021
	p-value *	**0.001**	**< 0.0001**	**0.016**	**0.001**	0.071
HTH	PL (N=4)	0.596	0.019	0.037	0.722	0.050
		0.241	0.006	0.006	0.368	0.063
	RO (N=6)	2.696	0.000	0.013	0.398	0.031
		0.299	0.000	0.022	0.028	0.028
	p-value *	**0.010**	**0.010**	**0.043**	**0.010**	0.070
LM	PL (N=5)	0.505	0.007	0.055	0.224	0.020
		0.706	0.007	0.019	0.178	0.033
	RO (N=6)	0.366	0.000	0.007	0.000	0.050
		0.459	0.000	0.009	0.000	0.025
	p-value *	**0.784**	**0.315**	**0.008**	**0.008**	0.167
LIMIT	RO/EU **	-	0.02	-	-	0.2
	PL***	-	0.03	-	-	0.3

* Mann-Whitney test *p*-value; ** Regulation of the Polish Minister of Health of 13 January 2003 ;

*** Council Directive 2001/110/EC relating to honey

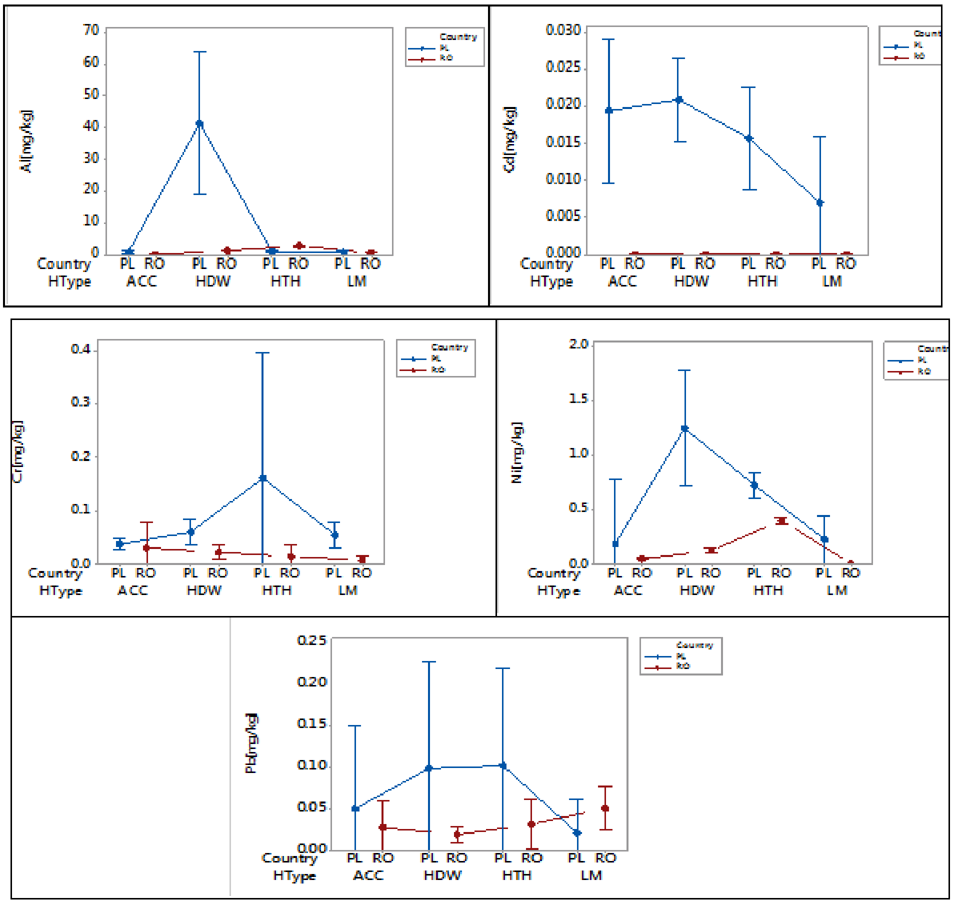


Fig. 2. Interval plot of toxic metals (mg/kg) content with 95% confidence intervals for factors: Country and Honey Type (HType).

mg/kg), while for the polish lime honeys the lowest content (up to 0.007 mg/kg) (table 2). The highest levels of lead were found in Polish heather and honeydew honeys (on the average value 0.05 mg/kg, respectively 0.098mg/kg).

The chromium mean values in Polish samples ranged from 0.037 to 0.059 mg/kg and were significantly higher in comparison with Romanian honeydew, heather and lime honey (7.7 times higher than in case of Romanian lime honey) samples and nonsignificant difference in case of acacia honey.

The effect of the botanical origin of honey was most evident for honeydew honey, which was more contaminated by aluminum and nickel than other nectar honeys. Moreover, there was a significant difference in the concentration of aluminum between Polish and Romanian samples. The aluminum content in Polish honeydew honeys was 34.6 higher than in Romanian honeydew honeys.

The nickel content in Polish honeydew honey was 10 times higher than in Romanian samples, and heather honey was 2 times higher than in Romanian samples.

The analysis of the data shows that all of the tested honey does not exceed the limit values contained in the European Honey Directive of the European Honey Commission [24] and in the Regulation of the Polish Minister of Health of 13 January 2003 [25] on the list of acceptable amounts of additives and other foreign substances added to foodstuffs (table 2).

In terms of the content of the limited heavy metals, studied honeys were characterized by high quality and well meet the requirements of the standard.

Comparing the contents of honey's macro and microelements in terms of botanical origin, results are very different (table 3, fig.3). Polish honeys are a more abundant source of potassium than the analogical type of Romanian honeys, excluding heather honeys. On the opposite, the level of calcium in Romanian samples (besides acacia honey), was 7.5 times higher in case of honeydew honey in comparison with Polish honeys, and 3.2 and 2.5 times higher in case of heather respectively lime honey. This tendency was not observed with magnesium.

The results obtained for Polish honeys are strongly supported by other authors' findings [19,26,27]. Madejczyk and Baralkiewicz [26] studied the mineral content of rape and honeydew honey originated from various locations within Poland, including honeydew honey from Podkarpackie region. It was shown, that potassium is the most abundant mineral, and showed the concentration from 7.7 to 2612.2mg/kg. The other major elements, Mg and Ca were present in concentrations ranged between 0.07 -19.38 and 3.3-159.2 mg/kg, respectively. The similar levels for other studied elements were obtained: 1.0-16.1 mg/kg for Fe, 0.13-9.93 mg/kg for Zn, 0.02-7.37 mg/kg for Mn and 0.26-1.82 mg/kg for Cu. Obtained results are also similar to those described for foreign honeys [19, 28, 29]. Moreover, the concentration of elements in dark honeydew honey was several folds higher than in rape honeys [19, 26].

However, it should be noted that in comparison with the results of other authors, honey from both tested areas fall very favorably and are very clean.

The specific for Polish region seems to be high concentration of aluminum in honeydew honey (41.4 mg/kg on average), chromium (0.060 mg/kg) and nickel (1.24 and 0.72 mg/kg for honeydew and heather honeys, respectively). Madejczyk and Baralkiewicz [26] found similar concentration of these elements in Polish honeys which ranged between 0.3-35.1 for Al, 0.005-0.093 for Cr and 0.02-1.33 mg/kg for Ni. Moreover, level of these metals was much higher in honeydew honey (dark) than in rape (light) honeys which support our observations.

The content of heavy metals in honey is dependent on various factors such as: the region of the country, the surroundings of the apiaries, the presence of heavy industry

Table 3

NON-PARAMETRIC MANN-WHITNEY TEST OF MEANS (P =0.05) RESULTS PRESENTED AS OBSERVATIONS NUMBER (N), MEAN, STANDARD DEVIATIONS (SD) AND STATISTICAL SIGNIFICANCE (*P*-VALUE)FOR NUTRITIONAL MINERAL CONTENT OF SELECTED HONEY SAMPLES. BOLD *P*-VALES PRESCRIBE STATISTICAL SIGNIFICANT DIFFERENCES OF MEANS

Type of honey	Country	Ca[mg/kg]	Cu[mg/kg]	Fe[mg/kg]	K[mg/kg]	Mg[mg/kg]	Mn[mg/kg]	Zn[mg/kg]
		mean SD	mean SD	mean SD	mean SD	mean SD	mean SD	mean SD
ACC	PL (N=4)	89.013	0.186	2.979	755.510	39.592	2.149	2.471
		26.081	0.248	2.146	32.793	8.867	1.030	2.197
	RO (N=6)	45.160	0.347	0.396	213.552	3.128	1.103	0.371
		1.222	0.027	0.054	8.565	0.107	0.092	0.010
	***p*-value** *	**0.014**	0.238	0.109	**0.014**	**0.014**	**0.014**	**0.010**
HDW	PL (N=5)	69.859	1.306	3.974	2957.471	67.537	4.216	1.817
		32.588	0.265	2.399	387.417	7.833	1.307	1.549
	RO (N=18)	523.940	0.655	1.923	1201.296	46.392	4.274	2.158
		186.265	0.111	0.517	157.716	10.749	1.820	1.484
	***p*-value** *	**< 0.0001**	**0.001**	0.192	**< 0.0001**	**0.005**	**0.479**	**0.526**
HTH	PL (N=4)	89.013	0.186	2.979	755.510	39.592	2.149	2.471
		26.081	0.248	2.146	32.793	8.867	1.030	2.197
	RO (N=6)	284.473	0.866	1.594	1680.685	47.449	5.724	3.141
		40.939	0.047	0.061	107.904	4.129	0.409	0.405
	***p*-value** *	**0.010**	**0.010**	**0.010**	**0.110**	**0.110**	**0.070**	**0.010**
LM	PL (N=5)	76.156	0.144	0.202	1277.162	28.628	4.587	1.085
		22.071	0.018	0.451	301.037	8.181	1.829	0.374
	RO (N=6)	501.040	0.493	0.598	507.152	23.619	1.391	1.952
		11.645	0.060	0.077	67.812	3.375	0.116	0.069
	***p*-value** *	**0.004**	**0.004**	0.112	**0.004**	**0.315**	**0.004**	**0.008**

*** Mann-Whitney test *p*-value**

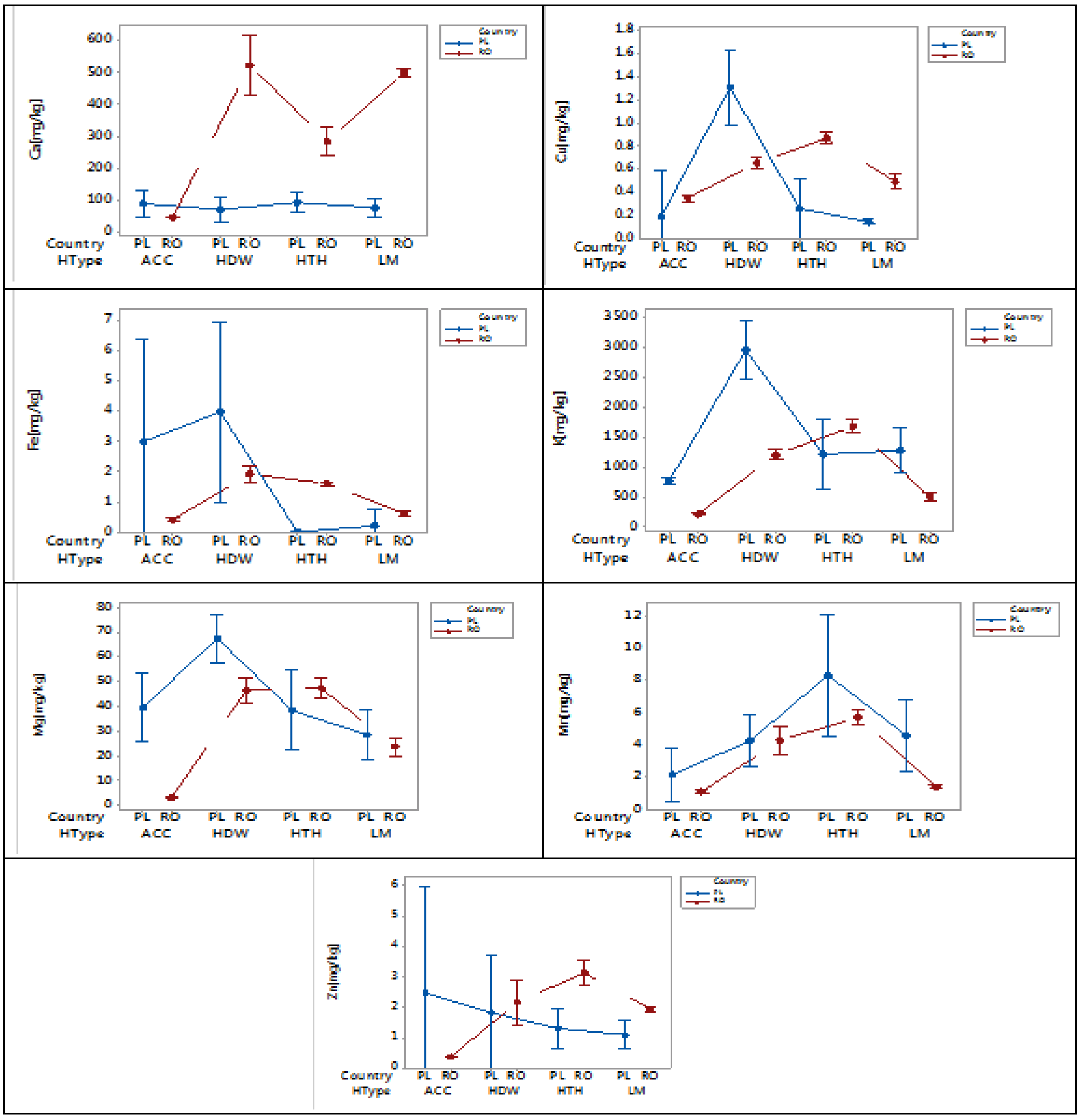


Fig. 3. Interval plot of nutritional minerals (mg/kg) content with 95% confidence intervals for factors: Country and Honey Type (HType)

in the area, the various communication routes present and the variations of the honey. The environmental pollution in urban areas increased the Cd content in nectar and honeydew honeys as compared to non-urbanized areas [30]. However, Podkarpackie is an ecologically clean region of Poland. Thus, elevated concentration of toxic metals is not the consequence of industrial activity. There are two probable explanations for this phenomenon: (1) elevated geochemical background resulting from specific composition of the bedrock (Carpathian Flysch) and (2) soil acidity (*p*H 3.5-5.5) which favors some metal (Cd, Al, Ni) migration and increases their bioavailability for plants.

Statistical analysis

Principal component analysis (PCA) used the variables (i.e. metals content) correlation matrix and the *between groups* calculation algorithms. In this way, there are correlations between the principal axes, variable vectors (loadings) and honey samples (scores). All these correlations are represented graphically in the PCA biplot (fig. 4). The first three principal components (PC1, PC2 and PC3) describe 85.99% of total variance and have eigenvalues greater than the unit.

Small angles between the variable vectors prescribe strong correlations between the variables. As a consequence the variables can be classified in groups. The PCA based variables groups are: VarG1: Al, K and Mg; VarG2: Cu and Fe; VarG3: Ni; VarG4: Cd, Mn and Pb; VarG5: Zn; VarG6: Ca and VarG7: Cr.

In the PCA biplot, honey sample groups are represented by convex hulls. Their reciprocal positions with other honey sample groups will define the relative differences of the metals content (i.e. metals abundance) that are provided by the variable vectors.

The Polish honeydew honey samples (PL_HDW) have the highest abundance of the first three variable groups (i.e. VarG1: Al, K and Mg; VarG2: Cu and Fe; VarG3: Ni). This is due to the fact that the respective variable vector endpoints are oriented towards this honey sample (PL_HDW). The Romanian honey samples RO_LM and RO_ACC have the lowest abundance of the first five variable groups, but high abundance of Ca and Cr, respectively. Based on the PCA of metal profiles all the metals abundance differences can be establish.

However, according the PC1 and PC2 biplot (i.e. 2D), there are some overlapping variable groups: RO_HDW, PL_ACC and PL_LM. This situation is alleviated considering the first three PC's (i.e. 3D) biplot representation (graph not shown), but still closed range honey sample groups are present.

In order to increase the 3D honey sample group distances, the linear discriminant analysis (LDA) was conducted. LDA is a PCA based multivariate analysis but the resulting canonical axes (Axis 1 and Axis 2) provide maximal and second to maximal separation between all groups (fig. 5). The LDA result in 3D representation has full non-overlapping honey sample groups, but with close distances between RO_HDW, PL_ACC and PL_LM sample groups. Hierarchical cluster analysis (HCA), with Ward's algorithm, was done to calculate the clustering scheme presented as dendrogram in figure 6. Bottom-right frame represents the percentage of within-class variance that

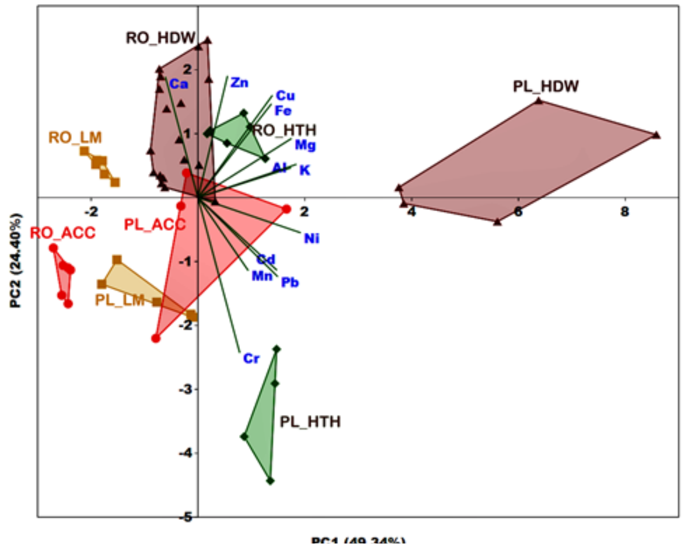


Fig. 4. Principal component analysis biplot

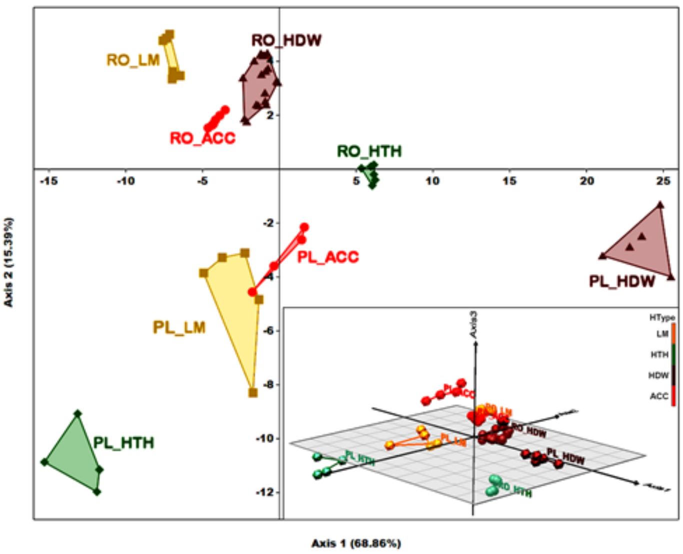


Fig. 5. Linear discriminant analysis biplot, 2D and 3D (bottom-right frame) representations

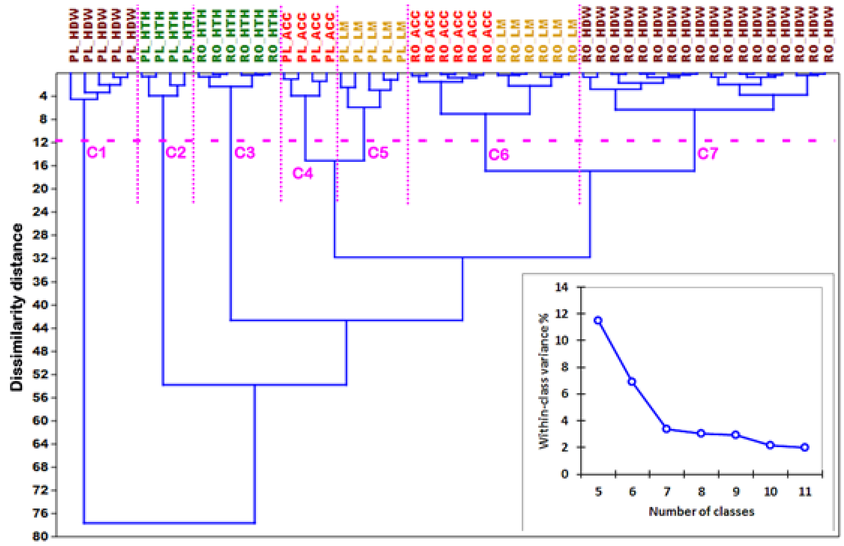


Fig. 6. Hierarchical cluster analysis dendrogram of first three canonical coordinates of the honey samples.

should be below 5% (0.05) level to prescribe the proper clusters number. In this case the within-class variance of 3.378% prescribes seven clusters. The same cluster number is obtained by using the multivariate MANOVA (P = 0.05) of metal profiles of the honey samples. In this case all the pairwise multicomparisons between the honey samples have the statistical significances $p < 0.0001$, except the RO_ACC and RO_LM that have $p = 0.083$. These two honey sample groups gather in one cluster, all the other six sample groups consist of six clusters (fig. 6).

Conclusions

Our results indicate that honey produced in apiaries in Bihor district (Romania) were less contaminated with toxic metals (Cd, Pb, Al) than samples originated from Podkarpackie region (Poland). All studied honeys contain heavy metals in concentrations well below permitted levels by European legislation. Therefore, these can be regarded as safe for human consumption.

The multivariate analysis sequence (PCA, LDA, MANOVA and HCA) prescribes seven clusters of honeys from eight singleton honey sample groups. The clustering offers the information about the multivariate differences of the honeys' metal sample profiles.

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References

1.AL-QUASSEMI R., ROBINSON R.K., Nutr. Food Sci., **33**, nr.6, 2003, p. 254.
2.MANDAL M.D, MANDAL S., Asian Pac J Trop Biomed., **1**, nr 2, 2011, p. 154.
3.BOGDANOV S., HALDIMANN M., LUGINBUHL W., GALLMANN P. J., Apic. Res. Bee World, **46**, nr.4, 2007. p. 269.
4.BOGDANOV S., JURENDIC P., GALLMANN P., J Am Coll Nutr.**27,** nr.6, 2008, p. 677.
5.TEMAMOGULLARI F., YAZAR S., BASKAYA R., Eurasian J. Vet. Sci., **28**, nr.1, 2012, p. 38.
6.BERINDE Z.M., MICHNEA A. M., Am. J. Sci. Arts, **23**, nr.2, 2013, p. 173.
7.BOGDANOV S. Apidologie, **37,** 2006, p.1.
8.STIHI, C., RADULESCU, C., CHELARESCU, E.D., CHILIAN, A., TOMA, L.G., Rev.Chim. (Bucharest), **64,** no.9, 2013, p.1000.
9.MADRAS-MAJEWSKA B., JASINSKI Z,. J. Apic. Sci. **47,** nr.2, 2003, p. 47.
10.VAN DER STEEN J.J., DE KRAKER J., GROTENHUIS T., Environ. Monit. Assess., **184**, nr.7, 2012, p. 4119.
11.ROMAN A., MADRAS-MAJEWSKA B., POPIELA-PLEBAN E., J. Apic. Sci., **55,** nr. 2, 2012, p. 97.
12.ZHELYAZKOVA I., Bulgarian Journal of Agricultural Science., **18**, nr.3, 2012, p.435.
13.PRZYBY£OWSKI P., WILCZYNSKA A., Food Chem., **74**, nr.3, 2001,p. 289.
14.BRATU, L., GEORGESCU, C. Rev. Chim.(Bucharest), **56,** no.2, 2005, p.118
15.ROMAN A., Acta Agrophys., **1**, nr.2, 2003, p. 295.
16.FORMICKI G., GREÑ A., STAWARZ R., ZYŒK B., GA£ A., Pol. J. Environ. Stud., **22**, nr.1, 2013, p.99.
17.NACCARI C., MACALUSO A., GIANGROSSO G., NACCARI F., FERRANTELLI V. (2014). J.Food. Res., **3**, nr.2, 2014,p. 107.
18.MD. SOLAYMAN, MD. ASIFUL ISLAM, SUDIP PAUL, YOUSUF ALI, MD. IBRAHIM KHALIL, NADIA ALAM, AND SIEW HUA GAN, Compr. Rev. Food Sci. Food Saf., 15, 2016, p.219.
19.KEDZIERSKA-MATYSEK M., LITWINCZUK Z., KOPERSKA N., BAR£OWSKA J., Nauka Przyr. Technol., 7, nr.3, 2013, p.1.
20.KACANIOVA M., KNAZOVICKA V., MELICH M., FIKSELOVA M., MASSANYI P., STAWARZ R., HASCIK P., PECHOCIAK T., KUCZKOWSKA A., PUTALA A. J., Environ. Sci. Health, Part A: Environ. Sci. Eng., 44, nr.4, 2009, p.414.
21.CELECHOVSKA O., VORLOVA L., Acta Vet. Brno, 70, nr.1, 2001, p.91.
22.HAMMER, O., HARPER, D.A.T., Paleontological Data Analysis, Wiley-Blackwell Publishing, Malden, MA, USA, May 2008, p.35-39.
23.HAMMER, O., HARPER, D.A.T., Paleontological Data Analysis, Wiley-Blackwell Publishing, Malden, MA, USA, May 2008, p.78-105.
24.***Council Directive 2001/110/EC relating to honey.
25.***Regulation of the Minister of Health from 13.01.2003 (Dz.U. 2003, no. 37, pos. 326) concerning the levels of chemical and biological pollutants present in food components, permissible additional substances, substances aiding the processing, or on food surface. No. 37, annex 1 (in Polish).
26.MADEJCZYK M., BARALKIEWICZ D., Anal. Chim. Acta, **617**, nr.1-2, 2008, p.11.
27.CHUDZINSKA M., BARALKIEWICZ D., Food Chem. Toxicol. **49**, nr.11, 2011, p. 2741.
28.FREDES C., MONTENEGRO G., Cienc. Invest. Agrar., **33**, nr.1, 2006, p.50.
29.GONZALEZ PARAMAS A.M., GOMEZ BAREZ J.A., GARCIA-VILLANOVA R.J., RIVAS PALA T., ARDANUY A.R., SANCHEZ SANCHEZ J., J. Sci. Food Agric., **80**, nr.1, 2000, p.157.
30.PORRINI C., SABATINI A.G., GIROTTI S., GHINI S., MEDRZYCKI P., GRILLENZONI F., BORTOLOTTI L., GATTAVECCHIA E., CELLI G. Apiacta, **38**, 2003, p.63.

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