The effect of collecting area on the element content of Hungarian acacia honeys

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Summary

Six macroelements and twelve microelements were identified in thirty-six Hungarian acacia honeys collected from ten counties by inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). One-Way ANOVA (LSD and Dunnett T3 test) and linear discriminant analysis (LDA) were used to determine the statistically verified differences among the honey samples with different geographical origin.

   Significant differences were established among the samples from different counties in Na, P, S, Fe, Ni, Cu and Sr concentrations. Based on the macroelement content of honeys, the separation of samples with different geographical origin was not successful because the percent of correctly categorised cases was only 64.9%. However, examining the As, B, Ba, Cu, Fe Mn, Ni and Sr concentration, the separation of different groups was convincing since the percent of correctly classified cases was 97.2%. Thus, the examination of microelement concentration may be able to determine the geographical origin of acacia honeys.

Keywords: acacia honey, collecting area, element content

Introduction

Honey is a natural substance produced by *Apis mellifera* bees from nectar and/or honeydew. In Hungary the annual honey production was 26,360 tonnes in 2016 (FAOSTAT) due to the environmental conditions of our countries that are favourable for honey production. Honey is a very complex food containing many essential nutrients, in particular micro and trace elements, proteins, amino acids, vitamins and enzymes. The botanical origin, harvesting treatment and storage has important effect to the composition and properties of honeys. Honey has a low mineral content (0.1–0.2% in nectar honeys) which varies depending on the soil conditions (Pohl et al., 2012) and extraction techniques (Hernández et al., 2005), rendering it suitable as an environmental indicator (Almeida-Silva et al., 2011). Anthropogenic activities (e.g. smelting, burning of fossil fuel, use of fertilizers and pesticides, transport) may also affect soil and air properties that influence the trace element content of plants (Kabata-Pendias and Mukherjee, 2007).

The aims of this study were to: (i) determine the macro, micro and trace element content in 36 Hungarian acacia honeys; (ii) test the influence of the collecting area on the element content of examined acacia honey samples.

Materials and methods

Honey samples

In this study 36 acacia *(Robinia pseudoacacia)* honey samples were analysed. All samples were collected from Hungarian beekeepers from 10 counties of Hungary (*Table 1*). The collecting year of examined samples was 2016; all samples were collected and stored in new, sterile glass jars at room temperature in the dark until analysis. Melissopalynological analysis was carried out to determine the botanical origin of examined honey samples using the method described by MSZ 6950-3:1977 (microscopic analysis of honey). Predominant pollen (>50% of pollen grains counted) was determined.

Table 1. *Geographical origin of examined samples*

Sample	County	Abbrevation
1–5	Hajdú-Bihar	HB
6–8	Békés	BE
9–14	Szabolcs-Szatmár-Bereg	SSB
15–17	Bács-Kiskun	BK
18–20	Csongrád	CS
21–24	Borsod-Abaúj-Zemplén	BAZ
25–27	Heves	HE
28–30	Zala	ZA
31–33	Baranya	BA
34–36	Veszprém	VE

Determination of element content

The digestions of honey samples were carried out according to the method of Kovács et al. (1996). Nitric acid (69%v/v) and hydrogen-peroxide (30%v/v) from VWR International Ltd. (Radnor, USA) were used to the digestion of samples and ultrapure water produced by a Milli-Q water purification system (Millipore SAS, Molsheim, France) was used to the preparation of solutions and dilutions. Depending on the anticipated concentration, determination of B, Ca, K, Mg, Na, P and S was carried out using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry; Thermo Scientific iCAP 6300, Cambridge, UK) and determination of Al, Mn, Fe, Ni, Cu, Zn, As, Sr, Cd, Ba and Pb was carried out using ICP-MS (Inductively Couples Plasma Mass Spectrometry; Thermo Scientific XSeries 2, Bremen, Germany). The

applied wavelengths, isotopes and LDs (detection of limits) are shown in *Table 2*.

Table 2. *Applied wavelengths and isotopes and LDs*

ICP-OES		
Elements	Wavelengths (nm)	LDs (mg kg^{-1})
B	249.6	0.12
Ca	317.9	2.38
K	766.4	0.53
Mg	279.5	0.10
Na	589.5	0.49
P	185.9	0.49
S	182.0	0.11
ICP-MS		
Elements	Isotopes (amu)	LODs (µg kg^{-1})
Al	27	39.90
Mn	55	3.44
Fe	56	77.40
Ni	60	2.92
Cu	65	27.40
Zn	66	45.30
As	75	1.19
Sr	88	1.09
Cd	111	0.19
Ba	137	0.65
Pb	206	0.93

Statistical analysis

All analytical analysis was carried out in triplicate. Data are described using general terms (mean, standard deviation, minimum and maximum values), One-Way ANOVA with LSD or Dunnett T3 tests and LDA (Linear Discriminant Analysis). SPSS for Windows (version 13; SPSS Inc. Chicago, Illinois, USA) was used for all calculations.

Results and discussion

The element concentration of examined Hungarian acacia honey samples are shown in *Table 3*. According to total element concentrations K (164±43 mg kg^{-1}) was the most abundant of the minerals analysed followed by P (41.8±6.6 mg kg^{-1}), S (15.6±3.1 mg kg^{-1}), Ca (13.1±3.6 mg kg^{-1}), Mg (4.91±0.81 mg kg^{-1}) and Na (3.54±0.75 mg kg^{-1}). The highest K content was measured in a sample from HB (287±4 mg kg^{-1}) and the lowest concentration was determined in a sample from BK (111±4 mg kg^{-1}).

Samples from HB showed the highest mean K concentration (229±47 mg kg^{-1}) and the lowest mean K content were measured in samples from

BE (125±6 mg kg^{-1}) and BK (130±27 mg kg^{-1}). The P concentrations were ranged between 32.0±1.2 mg kg^{-1} (BK) and 56.1±0.6 mg kg^{-1} (VE). Examining the counties, samples from VE showed the highest (52.4±3.6 mg kg^{-1}) and BE and BAZ showed the lowest mean P concentrations (33.6±1.5 and 33.9±1.4 mg kg^{-1}).The highest and lowest S contents were measured in a sample from HB (22.9±0.3 mg kg^{-1}) and BE (7.21±0.03 mg kg^{-1}). The mean S concentrations of samples from different counties showed similar results.

The samples from HB showed the highest S content (20.1±2.3 mg kg^{-1}) and the samples from BE showed the lowest S concentrations (10.0±0.4 mg kg^{-1}).The lowest Ca content was determined in a sample from BE (7.21±0.6 mg kg^{-1}) and the samples from this county showed the lowest mean Ca concentrations (7.94±0.65 mg kg^{-1}). Similar mean Ca concentrations were measured in samples from CS and BA (8.54±0.21 and 8.55±0.53 mg kg^{-1}). Samples from SSB showed the highest mean Ca contents (17.2±1.6 mg kg^{-1}); however the highest Ca concentration was measured in a sample from VE (19.5±0.3 mg kg^{-1}).

Analysed samples showed similar mean Mg, Na and B contents. Mg concentrations ranged from 3.64±0.05 mg kg^{-1} (CS) to 6.92±0.06 mg kg^{-1} (VE); however the mean Mg content of samples from different counties did not show big variance. The lowest mean values were determined in samples from CS and ZA (4.14±0.59 and 4.20±0.10 mg kg^{-1}) and the highest mean contents were measured in samples from HB, HE and VE (5.78±0.57, 5.77±0.67 and 5.64±1.11 mg kg^{-1}). The highest Na content was measured in a sample from BA (1.89±0.06 mg kg^{-1}) and the mean Na concentration of samples from different counties was the lowest in samples from VE and BA (2.42±0.61 and 2.50±0.54 mg kg^{-1}). The highest Na concentration was determined in a sample from HB (5.20±0.11 mg kg^{-1}) and the samples from this country showed the highest mean Na content (4.53±0.55 mg kg^{-1}).

Samples from HE showed the highest mean B content (3.85±0.37 mg kg^{-1}) and a sample from this county showed the highest B concentration (4.28±0.12 mg kg^{-1}). The lowest B content was measured in a sample from ZA (2.03±0.02 mg kg^{-1}) and samples from this county showed the lowest mean B concentration (2.57±0.27 mg kg^{-1}).

Examining the element content of samples from 10 counties statistically verified differences ($p < 0.05$) were determined in 14 cases for K, 22 cases for Na, 13 cases for Ca, 28 cases for P, 22 cases for S, 15 cases for Mg and 11 cases for B. Therefore the highest differences in element content of samples from different counties were found in case of P, S and Na content.

Table 3. *Macro- and microelement contents of examined acacia samples with different geographical origin*

	HB (n=5)	BÉ (n=3)	SSB (n=6)	BK (n=3)	CS (n=3)
Ca	15.8±2.2	7.94±0.64	17.2±1.6	15.1±0.9	8.54±0.21
K	229±45	125±6	154±30	130±27	143±34
Mg	5.78±0.59	4.28±0.29	4.81±0.71	4.74±0.34	4.14±0.59
Na	4.53±5.54	3.11±0.23	3.76±0.49	3.39±0.12	3.74±0.29
P	44.4±5.1	33.6±1.5	46.3±4.7	36.6±4.0	36.6±1.3
S	20.1±2.3	10.0±0.4	15.5±1.5	12.8±0.8	15.6±2.3
Al	133±46	293±19	129±44	<LD	149±22
As	12.3±2.4	6.92±0.24	41.1±2.1	29.7±2.1	35.0±3.5
B	2.84±0.67	2.95±0.7	3.53±0.32	3.19±0.61	3.42±0.29
Ba	15.3±5.3	79.6±3.1	20.5±5.8	8.14±0.26	5.54±1.02
Cd	0.45±0.12	<LD	<LD	<LD	<LD
Cu	141±24	69.8±2.0	118±14	155±11	170±4
Fe	597±51	315±9	583±66	310±14	1175±44
Mn	186±62	68.5±2.8	114±9	63.5±11.5	50.6±1.0
Ni	25.0±3.4	29.8±1.0	24.9±3.3	14.0±1.8	12.3±1.5
Pb	<LD	<LD	6.35*	<LD	8.65±0.82
Sr	26.1±3.1	16.4±0.5	22.3±3.3	18.2±2.6	22.3±2.4
Zn	238±33	151±10	104±12	351±26	248±3
	BAZ (n=4)	HE (n=3)	ZA (n=3)	BA (n=3)	VE (n=3)
Ca	11.4±0.8	12.7±1.1	11.8±1.6	8.55±0.53	16.0±3.0
K	163±32	146±30	201±25	144±31	169±33
Mg	4.67±0.80	5.77±0.67	4.20±0.10	4.71±0.39	5.64±1.11
Na	4.19±0.51	3.51±0.27	3.18±0.07	2.50±0.54	2.42±0.61
P	33.9±1.4	43.3±1.3	47.0±1.3	40.2±4.2	52.4±3.6
S	18.1±0.7	15.8±2.4	15.7±0.9	14.5±2.1	14.3±2.0
Al	151±36	104±9	113±7	99.3±11.6	149±32
As	7.43±1.4	29.1±2.4	18.3±1.5	34.5±1.3	14.6±1.0
B	3.05±0.17	3.85±0.40	2.57±0.27	2.62±0.25	3.09±0.30
Ba	12.7±1.8	14.4±0.7	21.0±1.6	10.0±2.4	19.9±2.2
Cd	0.09±0.04	0.33±0.04	<LD	<LD	<LD
Cu	135±11	155±29	232±25	172±7	76.4±3.4
Fe	567±54	442±23	723±11	137±5	614±24
Mn	88.7±6.1	90.3±2.6	43.6±3.4	59.0±4.7	51.6±1.7
Ni	17.4±2.2	105±5	24.8±1.6	21.5±2.3	19.8±1.1
Pb	31.1*	<LD	8.64±0.80	4.20±0.24	<LD
Sr	16.4±3.2	22.9±2.4	10.5±0.3	13.0±1.9	17.3±1.6
Zn	1583±299	240±21	185±10	<LD	131±23

Note: Ca, K, Mg, Na, P, S and B expressed in mg kg^{-1}; Al, As, Ba, Cd, Cu, Fe, Mn, Ni, Pb, Sr and Zn expressed in µg kg^{-1}, *Pb content were measured only one sample.

Al concentrations were under LD in samples from BK and the samples from BA also showed lower Zn content than the LD. Pb concentrations could be measured only in 11 samples (1 sample from SSB, every sample from CS, ZA and BA and 1 sample from BAZ. Cd content was above LD in 12 samples (every sample from HB, BAZ and HE counties).

Examining the micro element concentrations of samples, considered separately, the lowest Al, Ba, Cu, Fe, Mn, Ni, Sr and Zn contents were measured in the following sample from BA (87.0±2.3 µg kg⁻¹), CS (4.36±0.12 µg kg⁻¹), BE (67.5±1.1 µg kg⁻¹), BA (134±2 µg kg⁻¹), ZA (39.9±0.5 µg kg⁻¹), CS (10.9±0.2 µg kg⁻¹), ZA (10.2±0.3 µg kg⁻¹) and SSB (91.0±1.5 µg kg⁻¹) county. The highest Al, Ba, Cu, Fe, Mn, Ni, Sr and Zn concentrations were measured in the following sample from BE (310±6 µg kg⁻¹), BE (82.1±1.0 µg kg⁻¹), ZA (256±3 µg kg⁻¹), CS (1212±13 µg kg⁻¹), HB (230±6 µg kg⁻¹), HE (110±3 µg kg⁻¹), HB (29.5±1.1 µg kg⁻¹) and BAZ (1940±12 µg kg⁻¹) county.

Examining the micro element concentrations by county, the lowest mean Al, Ba, Cu, Fe, Mn, Ni, Sr and Zn contents of different counties was measured in samples from BA (99.3±11.6 µg kg⁻¹), CS (5.54±1.02 µg kg⁻¹), BE (69.8±2.0 µg kg⁻¹), BA (137±5 µg kg⁻¹), ZA (43.6±3.4 µg kg⁻¹), CS (12.3±1.5 µg kg⁻¹), ZA (10.5±0.3 µg kg⁻¹) and SSB (104±12 µg kg⁻¹) county.

The highest Al, Ba, Cu, Fe, Mn, Ni, Sr and Zn concentrations of different counties was determined in samples from BE (292±19 µg kg⁻¹), BE (79.6±3.1 µg kg⁻¹), ZA (232±25 µg kg⁻¹), CS (1175±44 µg kg⁻¹), HB (186±62 µg kg⁻¹), HE (105±5 µg kg⁻¹), HB (26.1±3.1 µg kg⁻¹) and BAZ (1583±299 µg kg⁻¹) county.

Statistically verified differences were found among the micro element contents of the most examined samples from different counties mainly in case of Fe (32 cases), Ni (34 cases), Cu (32 cases) and Sr (29 cases).

In our study the samples collected in different counties showed statistically verified differences in case of element content. However, in our previous studies we verified the macro element content of honeys depends on mainly the botanical origin (Czipa et al., 2018) and the effect of collecting area on the macro element concentration of different honey types is not so significant (Czipa et al., 2017). Conversely the micro element content of honeys is influenced by collecting area; therefore we suppose that when we would like to determine statistically verified differences among the honeys with same botanical origin; we have to examine the micro element contents. For this reason more LDAs were carried out to confirmation this hypothesis.

Figure 1 showed the results of LDA when only macro elements were analysed. Examining the equality of group means, based on the Wilks' Lambda Ca had the highest effect to the discriminant function (0.189) followed by P (0.214), S (0.227), Na (0.249), K (0.415) and Mg (0.457). Every variable were significant (P value<0.005). Six canonical discriminant functions were used in the analysis. The correlation was very high in case of first and second function (0.962 and 0.951). Third and fourth function showed middle correlation (0.784 and 0.709) and fifth and sixth function showed the lowest correlation (0.653 and 0.468). The first

function explained 92.5%, the second 90.4%, the third 61.5%, the fourth 50.3%, the fifth 42.6% and the sixth 21.9%. In the first dimension honeys from VE showed the highest group centroid (6.29) followed by honeys from SSB (2.63), ZA (1.40), BK (0.87) and HE (0.35). Centroid values was lower than zero in case of honeys from BA (-0.22), BE (-0.71), HB (-1.86), CS (-3.39) and BAZ (-5.07). In the second dimension honeys from SSB showed the highest group centroid (3.10) followed by honeys from HB (3.00), BAZ (1.15) and BK (0.39). Centroid value was lower than zero in case of honeys from ZA (-0.59) followed by honeys from HE (-1.08), VE (-1.12), CS (-1.22), BA (-4.09) and BE (-5.02).

Figure 1. *LDA of acacia honeys examining K, Ca, Mg, Na, P and S*
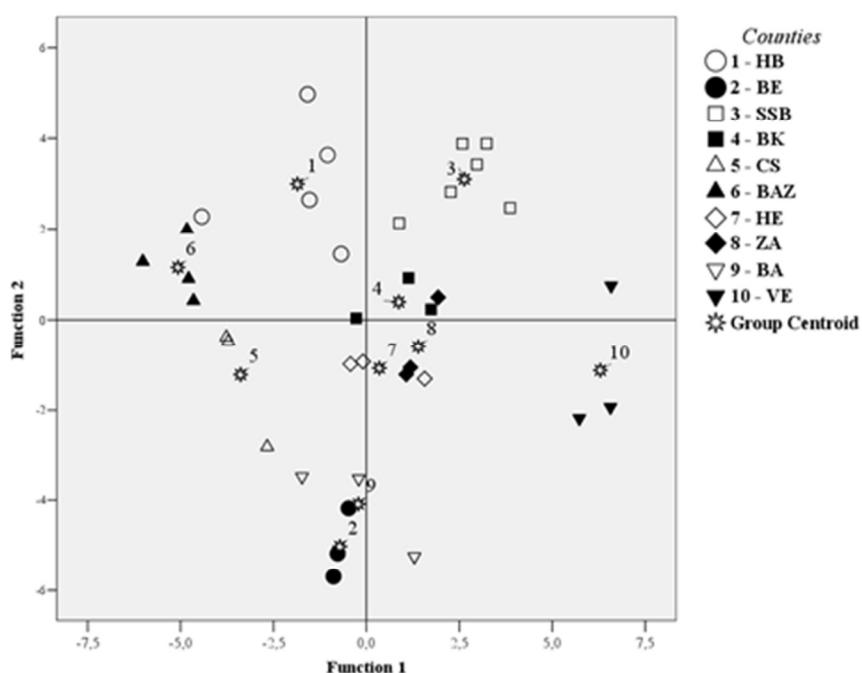
According to the cross validation in case of honeys from HB the number of correctly categorized cases was only 1 (20%), with 1–1 sample in the group of SSB, BK, BAZ and HE. In case of honeys from SSB this number was 5 (83.3%), because 1 sample was assigned to the group of BK. In case of honeys from CS this number was only 1 (33.3%), with 1–1 sample in the group of BE and BAZ. This number was only 1 in case of honeys from HE, because 1 sample was assigned to the group of ZA and BA. In case of honeys from BA this number was also only 1, with 1–1 sample in the group of BE and HE. The percent of correctly categorized

135

cases was 100% in case of honeys from BE, BK, BAZ, ZA and VE. Overall 69.4% of cross-validated grouped cases were correctly classified.

From the examined micro elements, As, B, Ba, Cu, Fe, Mn, Ni and Sr concentration were involved in LDA. Al, Zn, Cd and Pb content were missed out from this analysis because these elements were not measured in every sample. The result of LDA showed in *Figure 2*. According to the Wilks'Lambda (based on the test of equality of group means) every variables has significant effect and Ni (0.009) has the highest effect to the discriminant function followed by As (0.018), Fe (0.021), Ba (0.026), Cu (0.099), Sr (0.184), Mn (0.186) and B (0.445).

Figure 2. *LDA of acacia honeys examining As, B, Ba, Cu, Fe, Mn, Ni and Sr*
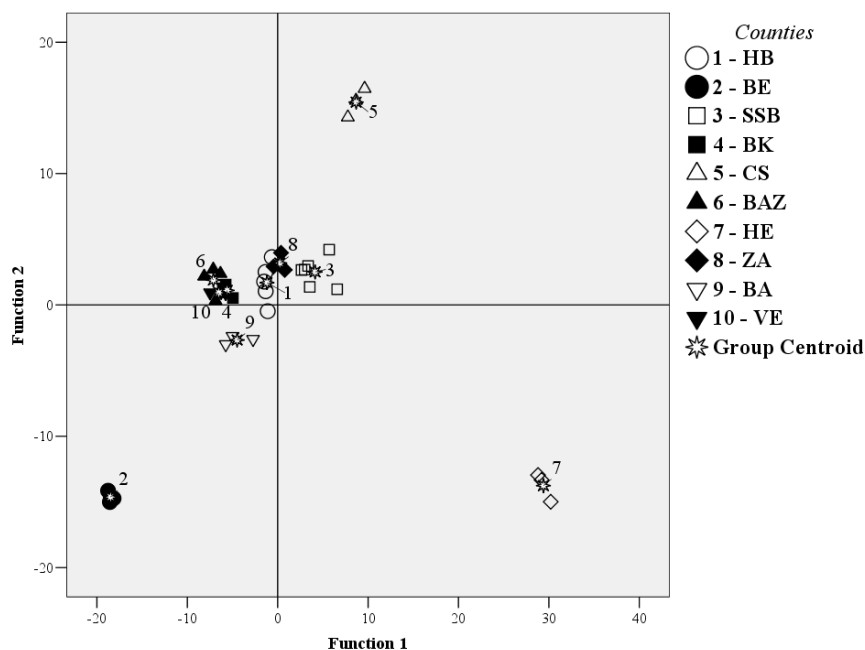
Eight canonical discriminant functions were used in this analysis. The correlations was very high in case of first, second, third, fourth and fifth function with 0.997, 0.994, 0.992, 0.985 and 0.966 value of canonical correlation. In case of sixth function this value was lower (0.860) and the seventh and eighth function showed the lowest values (0.543 and 0.348).

The first function explained 99.4%, the second 98.8%, the third 98.4%, the fourth 97.0%, the fifth 93.3%, the sixth 74.0%, the seventh 29.5% and the eighth only 12.1%.

In the first dimension the honeys from HE (29.4) showed the highest group centroid followed by honeys from CS (8.68), SSB (4.13), ZA (0.23),

HB (-1.19), BA (-4.49), BK (-5.60), VE (-6.51), BAZ (-7.12) and BE (-18.5). In the second dimension the honeys from CS (15.4) showed the highest group centroid followed by samples from ZA (3.19), SSB (2.51), BAZ (1.90), HB (1.70), BK (1.11), VE (0.93), BA (-13.8) and BE (-14.6).

According to the cross-validation in case of honeys from HB the number of correctly categorized cases was 4 (80%), with 1 sample in the group of BAZ. In case of other samples from different county the percent of correctly categorized cases was 100%. Overall 97.2% of cross-validated grouped cases were correctly classified.

Conclusion

In this study 6 macro and 13 micro elements in 36 Hungarian acacia honey samples collected in 10 counties were analysed. The concentration of macro elements decreased in the following order: K>P>S>Ca>Mg>Na.

Honeys from HB showed the highest mean K, Mg, Na and S concentrations, the highest mean Ca content was determined in samples from SSB and the highest mean P concentration was measured in samples from VE. Honeys from BE showed the lowest mean K, Ca, P and S concentrations, the lowest mean Mg content was measured in samples from CS and the lowest mean Na content was determined in samples from VE. Statistically verified differences were established mainly in case of mean Na, P and S concentrations of honeys from different counties.

Based on LDA, the analysis of macro element contents did not show obvious results because only 69.4% of cross-validated grouped cases were correctly classified. In 5 cases (HB, SSB, CS, HE and BA) the classification was not successful.

The concentration of micro elements decreased in the following order: B>Fe>Zn>Al>Cu>Mn>Ni>As>Ba>Sr>Pb>Cd. The highest mean Al and Ba contents were determined in samples from BE, the highest mean B and Ni content were measured is samples from HE and the highest mean Mn and Sr contents were determined in samples from HB. Honeys from CS showed the highest mean FE content, samples from ZA showed the highest mean Cu content and samples from BAZ showed the highest mean Zn concentration. Examining the toxic elements, every examined honey contained As; however the highest mean As content was determined in samples from SSB. The highest Pb content was measured a honey from BAZ and the highest Cd concentration was determined in samples from HB, every samples from this county contained Cd.

In samples from BK the Al content was under DL and the Zn concentration was determined also under DL in samples from BA. The Pb and Cd contents were under DL in many samples. In case of micro elements statistically verified differences were determined mainly in case

of Fe, Ni, Cu and Sr. Based on LDA the analysis of As, B, Ba, Cu, Fe, Mn, Ni and Sr the results of cross validation was obvious because the percent correctly classified cases was 97.2%. Therefore 9 groups were correctly classified and in case of only one group (samples from HB) was 80% this value.

Even though the same botanical origin, the micro element content was suitable for separation of samples from different geographical origin.

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