LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XL, 2007, TIMIŞOARA

HEAVY METALS CONTENTS OF BEE'S POLLEN FROM DIFFERENT LOCATIONS OF ROMANIA

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Summary

This study reports the heavy metals contents of eight bee pollen purchased from eight different locations of Romania. The quantified metals for each sample were: Fe, Mn, Zn, Cu, Ni, Pb and Cr. Heavy metals contents were determinated by flame atomic absorption spectrometry (F-AAS) with high-resolution continuum source ContrAA 300 spectrometer. Multivariate analyses were using to interpret statistically the obtained results.

Key words: heavy metals, bee's pollen, flame atomic absorption spectrometry, multivariate analyses

Pollen collected by bees (*Apis mellifera*) is an apicultural product, used in human diet for its nutritional value. It is made of natural flower pollen mixed with small quantities of nectar and bee secretions [Silva et al., 2006]. Pollen is the male genetic material of flowers and represents the main source of normal non-liquid food for bees [Mondal et al., 1998]. It is present as a fine powder in the plants. The morphology, size and color of pollen vary in relation to the species of plants.

Bee pollen is used for its therapeutic properties. Daily ingestion of bee pollen is recommended because it is capable to regulate the function of intestines and has benefits on cardiovascular system, skin and vision. It is used also in chronic prostates treatment for its presumed anti-inflammatory and anti-androgenic effects [Narajo et al., 2004].

The heavy metals contents of pollen are variable, due to the factors like differences between the plants species, geographical area, conditions of drying process. The accessibility of heavy metals for plants depends on soil reaction, mineral colloids, soil humidity, microbiological activity and organic matter content. Organic matter, especially humus compounds, can form organic-metallic compounds with high mobility in soil solutions and with high availability for plants [Kabata & Pendias, 2001] and pollen. The heavy metals like Fe, Mn, Cu and Zn can be essential in small quantities for plants, animals and humans, but becomes toxic in high quantities. Other heavy metals such Cr, Ni, Pb are very toxic [Gergen et al., 2006].

Materials and methods

Pollen Samples

Eight bee pollen samples were collected from eight markets, situated in different location of Romania: **Sample1**-Pollen from Brad, Hunedoara; **Sample2**-Pollen A from Cluj-Napoca, Cluj; **Sample3**-Pollen from Baia Mare, Maramures; **Sample4**-Pollen from Comanesti, Bacau; **Sample5**-Pollen B from Cluj-Napoca, Cluj; **Sample6**-Pollen from Targu Jiu, Gorj; **Sample7**-Pollen from Zalau, Salaj; **Sample8**-Pollen from Bucuresti.

Pollen samples preparation

The heavy metals from bee pollen samples were analyzed after dry burning of 10 g in the quartz capsules at 650^{o}C for 4 hours. After complete burning a nitric acid 0.5 N solution was added up to 50 mL. The solutions obtained were used for total heavy metals contents determination by flame atomic absorption spectrometry (F-AAS) with high-resolution continuum source.

Reagents

The standard solutions (1000 mg/L) were analytical grade from Riedel de Haen (Germany). The nitric acid 65% solution used was of ultra pure grade (Merck, Germany). All solutions were prepared using deionized water.

Heavy metals determination

Analysis of heavy metals was made with ContrAA-300, Analytik-Jena device, by flame atomic absorption spectrometry (FASS) in air/acetylene flame. The device working parameters (air, acetylene, optics and electronics) were adjusted for maximum absorption for each element. Acetylene was of 99.99 % purity. Under the optimum established parameters, standard calibration curves for metals were constructed by plotting absorbency against concentration [Gergen et al., 2006]. In a definite range for each metal a good linearity was observed. The correlation coefficient for the calibration curves (r^2) ranged between 0.9745 - 0.9891. All analyses were made in triplicate and the mean values were reported. All the values obtained for metals contents in pollen samples were calculated in mg/kg pollen.

Statistical interpretation of data obtained using multivariate analyses was performed with Statistica-6 software.

Results and discussions

The results obtained for the eight bee pollen analysed samples are presented in Table 1:

Table 1

The total heavy metals contents for analyzed bee pollen samples (ppm in pollen)

Samples/Metals	Cu ppm	Zn ppm	Ni ppm	Mn ppm	Fe ppm	Pb ppm	Cr ppm
1. Pollen from Brad, (Hunedoara)	5.51	33.93	0	28.58	**88.7**	2.08	0
2. Pollen A from Cluj-Napoca (Cluj)	11.36	45.96	0.72	84.95	51.9	0.22	0
3. Pollen from BaiaMare (Maramures)	**12.07**	46.15	0	42.13	68.3	1.12	0
4. Pollen from Comanesti (Bacau)	5.64	38.28	0	29.71	75.2	1.89	0
5. Pollen B from Cluj-Napoca (Cluj)	9.06	45.93	0.25	**98.25**	57.7	0.42	0
6. Pollen from Tg.Jiu (Gorj)	9.88	**54.35**	0	12.78	44.43	0.08	0
7. Pollen from Zalau (Salaj)	8.54	39.85	0	36.77	49.41	0.66	0.66
8. Pollen from Bucuresti	6.48	36.33	0.63	25.87	40.14	0.24	0
National limit in similar products, ppm	**20.0**	**60.0**	**-**	**-**	**-**	**1.0**	**-**

One of several trace heavy metals that are essential to life is *Cooper*. *Cooper* deficiency in humans is an exception, and would not occur if the content is more than 2 mg in the daily diet. The national accepted limit for *Cooper* in similar products is 20.0 mg/Kg [Ordinance 975/1998]. The two highest contents in *Cooper* were obtained for pollen from Baia Mare (12.07 ppm), respectively for pollen A from Cluj (11.36 ppm). In pollen from Brad was determinated the smaller quantity in *Cooper* (5.51 ppm).

Zinc is a constituent of about 300 enzymes and proteins that participate in all major metabolic processes. As an essential trace element *Zinc* can impair vital function either by deficiency or excess. Over exposure to *Zinc* by food, water and air commonly poses no risk to the general population. Dietary values for *Zinc* are between 9.4 to 11.0 mg/day for male adults and from 6.5 to 8.0 mg/day for female adults [Peganova & Eder, 2004]. The national accepted limit for *Zinc* in similar products is 60.0 mg/Kg [Ordinance 975/1998]. In pollen the smaller content in *Zinc* was obtained for pollen from Brad (33.93 ppm) and the highest for pollen from Tg. Jiu (54.35 ppm).

Manganese is one of the essential microelements for plants, animals and human beings. It is both a constituent and an activator of several enzymes and proteins in plant, animal and humans, and has around 20 identified functions. Crowley et al. [2000] reviewed Mn-containing and Mn-dependent enzymes and proteins, including their structures, functions and distributions. The recommended values for adults range from 2 to 5 mg Mn/day [Schäfer, 2004]. The highest content in Manganese (98.25 ppm) was determinated in pollen B from Cluj and the smaller (12.78ppm) in pollen from Tg.Jiu.

Other essential element for humans is *Iron*, ranging to approx 4200 mg/body. Approximately 60% of it is bound in haemoglobin and 10% in Fe-dependent tissue enzymes. The remaining 20% and 10% are stored as ferritin and respectively hemosiderin. The *Iron* turnover is approximately 30 mg/day [Schümann and Elsenhaus, 2004]. The contents in *Iron* for analysed bee pollen samples are between 40.14 and 88.7 ppm. The highest values for *Iron* content were obtained for pollen from Brad (88.7 ppm), pollen from Comanesti (75.2 ppm) and pollen from Baia Mare (68.3 ppm). The smaller content in *Iron* was obtained for pollen from Bucuresti (40.14 ppm).

Nickel is an essential element for human nutrition. The *Nickel* requirement of humans has been estimated to be 25-35 µg/day [Anke et al., 1995]. Excessive soluble *Nickel* compounds are hepatotoxic and nephrotoxic but as aerosols or dusts, insoluble *Nickel* compounds or elemental *Nickel* are very toxic (carcinogenic), justifying a lot of country imposed restricted limits, 0.05-1 mg/m^3 [Sunderman, 2004]. *Nickel* contents were detected in pollen A from Cluj-Napoca (0.72 ppm), in pollen from Bucuresti (0.63 ppm) and in pollen B from Cluj-Napoca (0.25 ppm).

Chromium is involved in insulin function. The actual TWA (Time Weighted Average) values for *Chromium* and *chromium compounds* are: for metal or Cr(III) compounds 0.5 mg/m^3 (irritation, dermatitis), for water soluble Cr(VI) compounds 0.05 mg/m^3 (liver, kidney, respiratory) and for insoluble Cr(VI) compounds 0.01 mg/m^3 (cancer, irritation), [Stoecker, 2004]. The *Chromium* content was found only for pollen from Zalau (0.66 ppm).

Lead is not an essential element for life and it is very toxic for the nervous system and the kidneys. A TLV-TWA (Threshold Limit Value - Time Weighted Average) of 0.05 mg Pb/m^3 is recommended in the US for occupational exposure to Pb and its inorganic compounds [Gerhardsson, 2004]. The national accepted limit for *Lead* in similar products is 1.0 mg/Kg [Ordinance 975/1998]. In analysed samples the highest content in *Lead* was determinated for pollen from Brad (2.08ppm), followed by pollen from Comanesti (1.89 ppm) and Baia Mare (1.12 ppm). All these values are up to the national limit (1.0 ppm).

For cluster analysis we used Statistica-6 software. The coefficients of matrix correlation of variables are presented in Table 2:

Table 2

The coefficients of matrix correlation of variables

	Cu	**Zn**	**Ni**	**Mn**	**Fe**	**Pb**	**Cr**
Cu	1.00	0.77	-0.13	0.41	-0.36	-0.55	0.00
Zn	0.77	1.00	-0.02	0.19	-0.45	-0.61	0.17
Ni	-0.13	-0.02	1.00	-0.68	0.47	0.58	-0.29
Mn	0.41	0.19	-0.68	1.00	-0.06	-0.28	0.11
Fe	-0.36	-0.45	0.47	-0.06	1.00	**0.94**	0.24
Pb	-0.55	-0.61	0.58	-0.28	**0.94**	1.00	0.09
Cr	0.00	0.17	-0.29	0.11	0.24	0.09	1.00

The very good correlation coefficient (0.94) for *Iron* and *Lead* permits us to exclude one of them. Also *Nickel* and *Chromium* are excluded because many values are closed to 0.0. Using *Cooper*, *Zinc*, *Iron* and *Manganese* like variables it was performed the calculation of variables - dendrogram, presents in Figure 1:

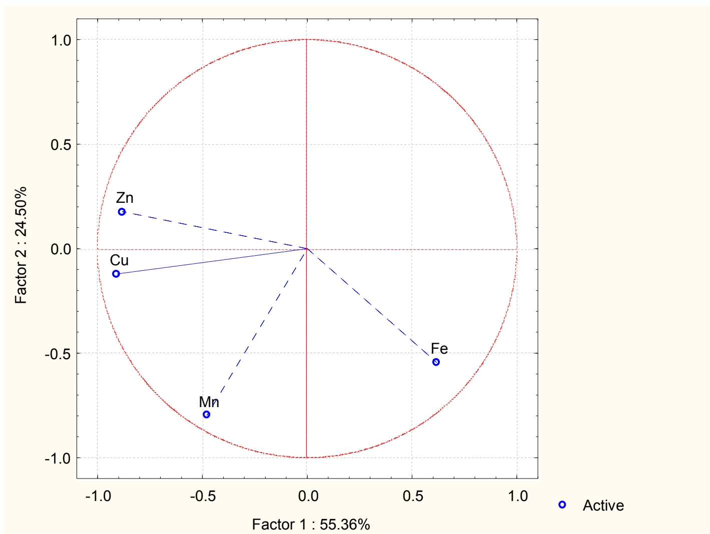

Figure 1. The graphical representation of variables - dendrogram

The correlations between factors and variables (factor loadings) are presented in Table 3:

Table 3

Factor-variable correlations (factor loadings)

	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**
Cu	*-0.908340*	-0.120509	-0.254008	0.309639
Zn	*-0.882248*	0.176549	-0.326701	-0.289372
Mn	-0.479254	*-0.798480*	0.352760	-0.091135
Fe	0.617427	-0.544806	*-0.566699*	-0.028695

The cases are represented by the all analysed pollen samples:

Sample 1 - Pollen from Brad, Hunedoara
Sample 2 - Pollen A from Cluj-Napoca, Cluj
Sample 3 - Pollen from Baia Mare, Maramures
Sample 4 - Pollen from Comanesti, Bacau
Sample 5 - Pollen B from Cluj-Napoca, Cluj

Sample 6 - Pollen from Targu Jiu, Gorj
Sample 7 - Pollen from Zalau, Salaj
Sample 8 - Pollen from Bucuresti

Using al the pollen samples it was performed the calculation of cases - dendrogram, presents in Figure 2:

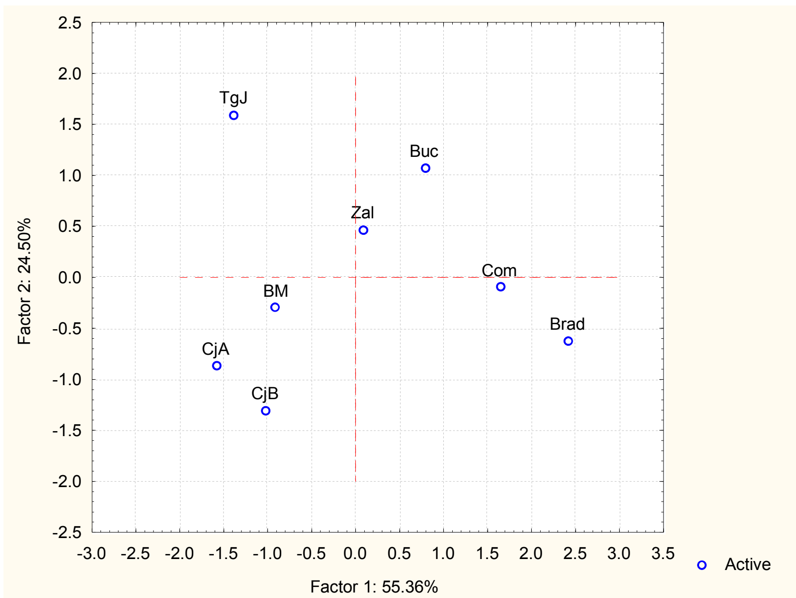

Figure 2. The graphical representation of cases - dendrogram

The graphical representation of variables - dendrogram (Figure 1) and cases – dendrogram (Figure 2) show that the pollen samples can be differentiate in four groups. The fist group is formed by three samples: pollen from Cluj A, pollen from Cluj B and pollen from Baia Mare. The high contents in *Manganese* are the main cause of the discrimination for first group from the others. The second group is formed by pollen from Brad and pollen from Comanesti. This group can be discriminated from the other analysed samples because has a high content in *Iron*. Pollen from Targu Jiu represents the third group. It has the highest content in Zinc and that's way it can be discriminated from the other three groups. The last group is formed by pollen from Bucuresti and pollen from Zalau witch contains all the four metals (*Cooper*, *Zinc, Manganese* and *Iron*) in medium values.

Conclusions

Multivariate PCA analyses using heavy metals contents can be useful tools to discriminate the pollen samples from different location.

Between al the analysed heavy metals the highest content was identified for non toxic metals *Iron* (40-89 ppm) and *Manganese* (26-98 ppm), followed by potential toxic metals *Cooper* (5-20 ppm) and *Zinc* (34-60 ppm). The values determinated for toxic metal *Lead* were between 0.08-2.08 ppm. The *Lead* contents in pollen from Brad (Hunedoara), Baia Mare (Maramures) and Comanesti (Bacau) are greater than accepted limit in Romania from similar food products (1.0 ppm).

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