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A comparison between the mineral content of flower and honeybee collected pollen of selected plant origin (*Helianthus annuus* L. and *Salix* sp.)

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Abstract

The macro and micronutrient content of Helianthus annuus L. and Salix sp. flower pollen in comparison with honeybee collected pollen of the same plant origin were determined. Potassium, magnesium, calcium, iron and zinc were analyzed by atomic absorption spectroscopy after dry ashing with air-acetylene flame. The pollen composition presented valuable nutrients, among which may be mentioned minerals and oligoelements, which was the objective of this study. Both analyzed plant species have their own specificity in terms of the mineral content. Potassium occurred at the highest concentration in all tested pollen samples, with average concentration of 7294.70 mg kg-^{1} in flower pollen and 4334.17 mg kg^{-1} in honeybee-collected pollen, followed by calcium (5492.78 and 2020.23 mg kg^{-1} respectively) and magnesium (1764.86 and 692.61 mg kg^{-1} respectively). In addition, the determined oligoelements presented average values of 1599.09 (Fe) and 75.14 mg kg^{-1} (Zn) in flower and honeybee collected pollen, respectively. Similarly, the determined average zinc levels were 75.01 mg kg^{-1} in flower pollen and 35.83 mg kg^{-1} in honeybee-collected pollen. Our results indicate that flower pollen in comparison with bee pollen contains higher levels of valuable minerals.

Keywords: flower pollen, honeybee collected pollen, major components, oligoelements, flame atomic absorption spectrometry

1. Introduction

Modern nutritional research confirmed that minerals and trace elements are of vital importance to the human body. Calcium constitutes a major component of bones, it is essential for the normal functioning of cardiac muscles, blood coagulation, milk clotting and the regulation of cell permeability. Magnesium regulates nerve simulation, muscle contraction and maintain osmotic equilibrium. Potassium constitutes an essential part in ionic balance of the human body and maintains tissue excitability (M.M. Eschleman [2]). Zn and Fe are components of many metal enzymes, playing an important role in promoting the metabolism of organisms, strengthening the immune system and preventing diseases (G. DONER & al. [1]).

Pollen is a natural product, containing valuable nutrients, among which high levels of minerals, which was the objective of this study. Considering its nutritional composition and according to the traditional usage, honeybee collected pollen (bee pollen) may be consumed everyday by humans as dietary supplement, contributing to functional and harmonious balance of the body (CRANE, 1983 cited by L.B ALMEIDA-MURADIAN & al. [3]).

Bee pollen is actually pollen from flowers collected by bees for their own nutritional purposes, because flower pollen is incredibly nutrient dense, providing the honeybee all of the nutrients that it needs for growth and development. Bee pollen is defined as the result of the

agglutination of flower pollen, nectar and honeybee salivary substances, carried out by worker bees and collected at the hive entrance.

In this paper, a comparison between the concentration of selected macro (K, Ca, Mg) and microelements (Fe, Zn) in flower and bee pollen of the same plant origin was performed, respectively *Helianthuus annuus* L. and *Salix* sp., by flame atomic absorption spectrometry after dry ashing.

2. Materials and Methods

2.1. Chemicals

Standard stock solutions of each evaluated elements, containing 1001 ± 2 mg l^{-1} for K, Ca, Mg, Zn and 1000 mg l^{-1} for Fe ipn 0.5 mol l^{-1} HNO_3 were purchased from Merck-Germany. Hydrochloric acid (37%) was from Merck, nitric acid (65%) from Fluka-Germany and de-ionized water (18.2 MΩ cm^{-1}) was produced in our lab. All reagents used in the experiments were of analytical grade. Plastic and glassware were cleaned by soaking in 10% HNO_3 and rinsed with distilled water prior to use.

2.2. Botanical origin identification of bee pollen pellets

Samples of bee pollen, obtained from pollen traps, were purchased on the market or from local beekeepers from Transylvanian area of Romania in 2009. The flower pollen for the study was collected by hand, directly from anthers of flowers and the plant taxon was identified with the botanic atlas (L. POPOVICI & al. [4]). All samples were stored at -4ºC until analyzed. The flower origin of bee pollen pellets was identified by color and light microscope examination by palynological analysis, using the acetolysis method (G. ERDTMAN [5]). Pollen types were identified by comparison with pollen reference slides made by the authors of the present work, and then compared with pollen descriptive images (G. ERDTMAN [5]). In addition, the pollen reference slides were prepared from anthers of flower for an accurate botanic identification of the bee pollen samples.

2.3. Atomic Absorption Spectroscopy measurements

The spectroscopy measurements was performed using an Atomic Absorption Spectrophotometer AA-6300 produced by Shimadzu-Japonia, equipped with deuterium lamp for background correction and hallow-cathode lamps for each of the element studied (single element lamps), burner system for flame analysis with a suitable acetylene cylinder and air compressor, auto sampler ASC-6100F. Analytical calibration curves (r^2=0.9990-0.9999) were prepared, with five concentration points, for each evaluated elements. The following wavelengths were used for the studied metals: potassium 766.5 nm, calcium 422.7 nm, magnesium 285.2 nm, iron 248.3 nm and zinc 213.9 nm.

2.4. Samples Preparation

About 2 g of pollen were placed in a quartz crucible and ashed in a muffle furnace at 450°C over the night. Ash was digested by treating with hydrochloric acid 6M (5 ml) and then evaporated. The resulting white ash was dissolved with 25 ml nitric acid 0.1 M (SR EN 14082 [6]). A blank digest was also carried out in the same way. Lanthanum nitrate [La $(NO_3)_3$x6H2O] were used as matrix modifier.

2.5. Statistical analysis

All determinations were performed in triplicate and results are expressed as mean ± standard deviation. The relationship between different mineral content of the samples was analyzed by Pearson correlation coefficient. The data were analyzed by an analysis of variance (one way ANOVA) ($p<0.05$) and means compared using Tukey Test. Results were processed by Microsoft Excel and Origin 7 software.

3. Results and Discussion

3.1. Botanical identification of bee pollen loads

The bee pollen loads were first separated by color from the complex mixtures of pollen pellets coming from different species of plants, resulting monochromatic pollen loads with uniform color. The microscopic examination was the principal tool for the selection of the bee pollen pellets coming from one species. In each microscopic preparation, pollen was determined, when possible, into genus, species or family. The selected pollen load samples studied here presented the following colors: red orange for *Helianthus annuus* L. and light maroon for *Salix* sp.

3.2. Mineral content of pollen samples

Potassium was the most abundant mineral in the analyzed pollen samples. The highest significant potassium level ($p< 0.05$) was found in *Salix* sp. pollen (8686.64 mg kg^{-1}), followed by *Helianthus annuus* L. pollen (5421.85 mg kg^{-1}). Significant low values ($p< 0.05$) were determined for *Salix* sp. bee pollen (5421.85 mg/kg) and *Helianthus annuus* L. bee pollen (3246.50 mg kg^{-1}) (Table 1). These values were in the range with those previously reported for multifloral bee pollen, namely 4000 mg kg^{-1} (M.T.O. VILLANUEVA & al. [7]), 5530 mg kg^{-1} (D.C. SOMERVILLE & al. [8]), 2843-5976 mg kg^{-1} (TERESA SZCZESNA [9]) and 4950-5131 mg kg^{-1} (G.G. SALAMANCA & al. [10]).

Table 1. The macro and micromineral content of flower and honeybee collected pollen of selected plant origin*

Botanical name of floral species	Mineral content (mg kg^{-1})				
	K	Ca	Mg	Fe	Zn
Honeybee collected pollen					
Helianthus annuus L.	3246.50 ±38.96	1409.79 ±28.20	376.94 ±4.83	27.42 ±1.37	31.61 ±1.58
Salix sp.	5421.85 ±65.06	2630.67 ±52.61	1008.28 ±20.17	122.87 ±6.14	40.06 ±2.00
Flower pollen					
Helianthus annuus L.	5902.76 ±70.83	8090.28 ±91.81	1336.60 ±26.73	2872.89 ±93.64	70.28 ±3.51
Salix sp.	8686.64 ±94.24	2895.28 ±57.91	2193.13 ±43.86	325.29 ±16.26	79.74 ±3.99

*Samples analyzed in triplicate.

In respect with the calcium content, there is a significant difference, within a confidence level of 95%, between the obtained results (Table 1). The calcium content was significantly ($p\leq0.05$) higher (5.7 times) for *Helianthus annuus* L. pollen (8090.28 mg kg^{-1}) in comparison with bee pollen from the same plant species (1409.79 mg kg^{-1}). Similarly, for the *Salix* sp., the calcium concentrations differ significantly ($p< 0.05$), with low levels in bee pollen samples (2630.67 mg kg^{-1}) and higher values in flower pollen ones (2895.28 mg kg^{-1}). D.C. SOMERVILLE & al. [8] reported similar calcium levels for multifloral bee pollen (1146 mg kg^{-1}). Calcium levels registered by TERESA SZCZESNA [9] presented high variability, ranged between 105-1080 mg kg^{-1}. The average calcium concentrations determined in this work are higher than those obtained by G.G. SALAMANCA & al. [10] (422-448 mg kg^{-1}) and similar with those reported by T. ECHIGO & al. [11] (1600 mg kg^{-1}).

Very significant difference ($p< 0.05$) was found between flower and bee pollen samples in respect with the magnesium content (Table 1). *Salix* sp. pollen contained the highest magnesium content (2193.13 mg kg^{-1}) followed by *Helianthus annuus* L. bee pollen (1336.60 mg kg^{-1}). Low levels of magnesium were determined in bee pollen of the corresponding plant species, namely 1008.28 mg kg^{-1} for *Salix* sp. and 376.94 mg kg^{-1} for *Helianthus annuus* L. High variability presented the magnesium levels of multifloral bee pollen harvested from Poland, China and Korea, with values ranged between 53.2-429.8 mg kg^{-1} (TERESA SZCZESNA [9]). Our results are higher than those reported by G.G. SALAMANCA & al. [10] for multifloral bee pollen harvested from Columbia (81.6-98.5 mg

kg^{-1}). In comparison with our results, with exception of *Helianthus annuus* L. flower pollen, D.C. SOMERVILLE & al. [8] and T. ECHIGO & al. [11], determined lower iron levels in multifloral bee pollen produced in Australia and Japan, with average values of 716 mg kg^{-1} and 950 mg kg^{-1} respectively.

The significantly richest ($p< 0.05$) samples, in respect with the iron content, belonged to the flower pollen of *Helianthus annuus* L., with values of 2872.89 mg kg^{-1} (Table 1). In comparison, the unifloral bee pollen originated from the same plant species contained only 27.42 mg kg^{-1} iron. The iron content of *Salix* sp. pollen samples registered values of 325.29 mg kg^{-1} (flower pollen) and 122.87 mg kg^{-1} (bee pollen). In the case of the iron content, the bee pollen samples from the plant species analyzed in this work did not differ significantly ($p< 0.05$). Iron values in multifloral bee pollen have been previously reported in the range of 40.4-136.1 mg kg^{-1} in Poland samples, 74.3-365.9 mg kg^{-1} in South Korea samples and 59.0-182.3 in China samples (TERESA SZCZESNA [9]). Similar results were obtained by M.T.O. VILLANUEVA & al. [7] for the commercial bee pollen from Spain (40 mg kg^{-1}). The iron content of bee pollen samples analyzed in this work are not in concordance with those obtained by G.G. SALAMANCA & al. [10] and T. ECHIGO & al. [11], which were much higher (2221-2576 mg kg^{-1} and 300 mg kg^{-1} respectively).

The zinc levels determined in bee pollen samples did not differ significantly ($p< 0.05$), with values of 31.61 mg kg^{-1} and 40.06 mg kg^{-1} for bee pollen coming from *Helianthus annuus* L. and *Salix* sp., respectively. Significant higher levels were determined in flower pollen of the same plant species, namely 70.28 mg kg^{-1} and 79.74 mg kg^{-1}, respectively (Table 1). Zinc concentration values obtained are in agreement with those previously reported for multifloral bee pollen, by TERESA SZCZESNA [9], M.T.O. VILLANUEVA & al. [7] and D.C. SOMERVILLE & al. [8], which determined 23.7-60.7 mg Zn/kg, 58 mg Zn/kg and 36.6 mg Zn/kg respectively. In addition, the zinc levels are much lower than those previously reported by G.G. SALAMANCA & al. [10] for Columbian bee pollen, which reported values ranging between 162-311 mg kg^{-1}.

Detection limit values of elements were 0.002 mg kg^{-1} for K, 0.0025 mg kg^{-1} for Ca, 0.0007 mg kg^{-1} for Mg, 0.0057 mg kg^{-1} for Fe and 0.0024 for Zn. Detection limits corresponded to the concentration associated with three times higher the standard deviation of the background noise recorded in 10 assays of a sample with 3 times higher than the concentration of the expected detection limit. The accuracy of the measurements was evaluated with respect to the certified values by standard addition method with tree different concentration levels (0.1, 0.15 and 0.2 mg kg^{-1}). Mean recoveries of 96.77%, 97.42%, 97.99%, 93.29% and 93.34% respectively, were obtained for K, Ca, Mg, Fe and Zn. The relative standard deviations were less than 10% for all investigated elements.

Strong positive correlation (Table 2) was found only between calcium and iron content (r^2=1.000), potassium and magnesium levels (r^2=0.991), magnesium and zinc content (r^2=0.855) and potassium and zinc content (r^2=0.778). In addition, the iron and calcium levels determined in pollen samples analyzed here were not correlated with their zinc content (r^2=0.352, r^2=0.330).

Table 2. Pearson correlation matrix

	K	Ca	Mg	Fe	Zn
K	1				
Ca	-0.337**	1			
Mg	0.991**	-0.207**	1		
Fe	-0.315**	1.000	-0.185**	1	
Zn	0.778**	0.330**	0.855**	0.352**	1

** Significant at p<0.05.

The differences between the mineral concentrations of the flower pollen investigated in the present work may be determined by an association of factors mainly the plant specificity. The nutrient concentration of flower pollen is highly conserved within genera, families, and divisions (R.S. FARAG & al., 1980, T.H. ROULSTON & al., 2000, N. ALMARAZ-ABARCA & al., 2008 [12, 13, 14]). The concentration of minerals in pollen is related to their different botanical origin. Highest variation presents potassium, magnesium, calcium, manganese and iron levels, while the zinc content of pollen appeared to be more constant (E.W.J. HERBERT & al., 1987 cited by IRENE KELLER & al. [15]).

In regard to the plant specificity, the authors of the present study have previously determined significant differences between the polyphenolic content of unifloral bee pollen from different plant sources in Romania. Particularly, bee pollen of *Salix alba* L. (Salicaceae family, Genus *Salix*) contains the highest quantities of polyphenols than other unifloral bee pollen samples, including *Helianthus annuus* L. bee pollen (Compositae family, Genus *Helianthus*). (L.A. MARGHITAS & al., 2009 [16]).

T.H. ROULSTON & al. (2000) [13] suggested that pollen volume may correlate with the storage capacity of particular nutrients that influence pollen tube growth. Particularly, in respect with the flower pollen protein concentration of 377 plant species from 93 plant families, they determined that small pollen grains were generally protein-rich and the functional significance of protein-rich pollen in "buzz"-pollinated taxa could reflect selection to favor small grain size rather than to reward pollinators. This previous findings may also contribute to explain the mineral concentration differences determined in the present work, between the pollen of *Salix* sp. (polar axis: 16-19 μm, equatorial axis: 15-20 μm) and *Helianthus annuus* L. (polar axis: 34-40 μm, equatorial axis: 37-45 μm).

The decrease in mineral concentration in bee pollen samples in comparison with flower pollen probably reflects dilution due to the addition of regurgitated honey, which contains nectar and honeybee salivary substances, added by the bees, in order to prepare the pollen pellets and to transport them in the hive.

4. Conclusions

The comparison of our results, for the flower pollen of selected plant origin, with more literature data is rather difficult, since they have not been studied before. In general, the results obtained in the present work are in good agreement with those reported for bee pollen from other countries.

Future analysis is required, not only in determining other mineral element concentrations, but also in analyzing samples collected from different areas, in order to elucidate the potential differences due to substrate, such as soil and water mineral particularities, geographic conditions and environmental factors.

Our preliminary results may give an important contribution, considering that little pertinent information is available for this region of Romania, particularly for flower pollen of selected plant origin.

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