CONCENTRATION OF SELECTED ELEMENTS IN HONEYBEE-COLLECTED POLLEN

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Summary

The objective of the study was to investigate the macro- and micro-nutrient composition of the multifloral pollen harvested in different countries (Poland, South Korea and China). A total of 27 pollen samples collected by bees as pollen loads were included in the study. The material was assayed for the contents of dry matter, total ash and selected elements. The contents were determined using Atomic Absorption Spectrometry (AAS) following wet digestion of the samples with concentrated sulfuric and nitric acids.

In the honeybee-collected pollen samples from different countries the following minerals were assayed: calcium (Ca), manganese (Mn), magnesium (Mg), zinc (Zn), copper (Cu), iron (Fe), sodium (Na) and potassium (K). Potassium followed by magnesium, sodium and calcium occur in the highest concentrations. Potassium accounted for as much as 59% of total assayed minerals, magnesium for 18%, sodium for 12%, calcium for 8% and the remaining elements jointly about 3%. The contents of elements in the samples can be put in the following decreasing order: K>Mg>Na>Ca>Fe>Mn>Zn>Cu. In addition, it was found that contents of ash and of the minerals varied substantially from sample-to-sample which could be related to different botanical origin. Pollen samples harvested in Poland, as compared to those from other countries, were characterized by a significantly higher content of calcium, samples from China had a significantly higher content of sodium, potassium and manganese and samples from South Korea were significantly higher in zinc and iron. Of all the minerals under investigation manganese showed the highest variation. Pollen harvested by bees as pollen loads, due to its high content of minerals, can be used by man as a natural source of minerals.

Keywords: pollen loads, ash, macro- and micro-nutrients, botanical origin, AAS.

INTRODUCTION

According to different authors ash content of pollen varies from 1.55% to 6.05% (Nation and Robinson 1971, McLellan 1977, Youssef et al. 1978, Zalewski and Szymaniuk 1985, Serra Bonvehi et al. 1986, Szczęsna et al. 1995, Serra Bonvehi and Escola Jorda 1997). Polish authors (Zalewski and Szymaniuk 1985, Szczęsna et al. 1995) found higher values for that constituent with an average of more than 3% whereas Spanish authors reported values less than 2% (Serra Bonvehi and Escola Jorda 1997).

A detailed analysis of pollen revealed the presence of magnesium, strontium, zinc, manganese, iron, aluminum, copper, boron, silicon and phosphorus. In some samples nickel and chromium were also found (Nation and Robinson 1971, Lopez and Nicotra 1975, McLellan 1977, Herbert and Shimanuki 1978, Youssef et al. 1978, Kauffeld 1980, Loper et al. 1980, Solberg and Remedios 1980, Echigo et al. 1986, Serra Bonvehi et al. 1986, Herbert and Miller-Ihli 1987, Serra Bonvehi and Escola Jorda 1997, Somerville and Nicol 2002). Potassium, magnesium, calcium and sodium were found at the highest concentrations. Iron, manganese, zinc and copper were also relatively high. All the above-mentioned investigators emphasize the dependence of the content of individual elements in pollen on its botanical origin. In addition, Herbert and Miller-Ihli (1987) found high seasonal variation for the content of individual elements in pollen loads which was not only related to species providing pollen at a given period but was also dependent on soil conditions. Some authors report calcium and magnesium contents of pollen loads to be relatively stable (McLellan 1977) whereas others report it to be subject to substantial variation (Herbert and Miller-Ihli 1987). Of other elements, iron and manganese are also highly variable whereas copper and zinc are relatively stable (Herbert and Miller-Ihli 1987).

While being a natural source of bio-elements pollen can also contain elements harmful to human health: cadmium, lead, mercury and arsenic (Free et al. 1983, Lipińska and Zalewski 1989, Migula 1990, Konopacka et al. 1993, Szczęsna et al. 1993). Pollen loads are assayed for heavy metal contents mainly because of the contamination of environment with those metals. The authors of those studies report on ever more frequent cases of admissible contents of those health-compromising metals being exceeded and point to the need for their level in honeybee-collected pollen to be constantly monitored. Pollen production-oriented apiaries should not be located in heavily industrialized areas, in a close proximity of heavy traffic roads or close to large urban agglomerations.

Taking into account an extreme importance of mineral nutrients for human health new sources of those bio-elements should be constantly looked for. It was the motive behind the decision by the author of this study to carry out investigations aimed at studying pollen loads harvested in different countries (Poland, South Korea, China) for the contents of selected macro- and micro-nutrients. Such studies, especially of domestic pollen, were conducted but to a very limited extent and pollen is harvested by beekeepers on a regular basis and is recommended as a product possessing valuable nutritive and therapeutic properties.

MATERIAL AND METHODS

The study was conducted at Kon-Kuk University, Animal Resources Research Center, Seoul, Korea. The material for the study comprised samples of multifloral pollen that came from Poland (13 samples), South Korea (9 samples) and China (5 samples). Samples of domestic pollen were collected in June and July of 1997 at the apiary of the Institute of Pomology and Floriculture, Apiculture Division in Puławy, located on the premises of the Division's building and at a private apiary located a short distance (1-2 km) from the Division's building. The park of en extensive area of garden plots and home gardens were within the reach of bees' flights. The samples of the Korean pollen were collected in the months of April to August of 1997 in the apiary of the Insect Biotechnology Laboratory, Department of Industrial Entomology, National Institute of Sericulture and Entomology, Suwon and samples of Chinese pollen were harvested in April of 1997 in Anhui province of China. The study comprised a total of 27 samples of pollen collected by bees as pollen loads. Once collected, the samples were immediately dried at 40°C and stored at -21°C until analyzed.

The experiment material was assayed for the contents of dry matter, total ash and selected macro- and micro-nutrients (Ca, Mn, Mg, Zn, Cu, Fe, K and Na).

Dry matter content was determined by drying at 105°C to constant weight (Serra Bonvehi and Casanova 1987).

Ash content was assayed by mineralization at 500-550°C and weighting the residues following mineralization to constant weight (Serra Bonvehi and Escola Jorda 1997).

The contents of individual elements were determined using Atomic Absorption Spectrometry (AAS) following wet digestion with concentrated sulfuric and nitric acids. Pollen was digested using a microwave device MICRODIGEST 401 PROLABO. To this end a portion of about 1 g of pollen was digested according to the conditions listed in Table 1. Once digested, the sample was dissolved in 2 ml of 2 N HCl and then transferred volumetrically to a 25 ml volumetric flask. The assays of individual metals were done by the AAS method using PERKIN ELMER 3110 atomic absorption spectrophotometer equipped with appropriate cathode lamps.

To assay pollen samples for: Zn, Fe, Cu and Mn non-diluted after digestion solutions were used. The solutions were diluted with deionized water 10 times for Ca assays, 100 times for Na assays and 1000 times for Mg and K assays. Alongside, the so called reagent blind was done. For individual metals detectability and determinability limits, linearity range, recovery as well as repeatability and reproducibility of the method were determined.

The data concerning the contents of ash and analysed elements were converted to dry matter.

Pollen samples from Poland were additionally subjected to melissopalynological analysis to determine their botanical origin (Szczęsna 2006). Pollen analysis was done according to recognized methods used in melissopalynological studies as recommended by the International Commission of Bee Botany of International Union of Biological Sciences (Louveaux at al. 1978).

The data on the contents of total ash and macro- and micro-elements in the tested samples of multifloral pollen from three different countries (Poland, South Korea and China) were analyzed using one-way ANOVA with an unequal number of replications. Differences between means were evaluated using Duncan's test at a significance level of α = 0.05. The variability among pollen loads samples from Poland, South Korea and China for the constituents tested were evaluated by determining standard deviation for each country.

RESULTS

Ash content of the tested samples ranged from 2.08 to 3.19% of dry matter (DM) for the samples from Poland, from 2.17 to 3.66% DM for the samples from Korea and from 2.78 to 3.33% DM for the samples from China (Table 2). The average content of that constituent was at a similar level in the three countries under comparison. It was found that pollen samples from

Table 1

Pollen digestion conditions using MICRODIGEST 401, PROLABO (200 W) device.

Action	Acid	Acid dosage speed (ml/min)	Acid volume (ml)	Energy (%)	Time (min)
1	H_2SO_4	10	10	25	15
2	HNO_3	5	5	40	10
3	HNO_3	5	2	50	10
4	-	-	-	60	10

Korea were characterized by greater variability for ash content when compared to pollen samples from the remaining countries. The standard deviation was 0.47 for the pollen samples from Korea, 0.40 for those from China and 0.32 for those from Poland.

The following elements were assayed in the tested pollen samples: sodium, potassium, calcium, magnesium, manganese, zinc, iron and copper. Regardless of origin, potassium occurred at the highest concentrations in all tested pollen samples. It accounted for as much as 59% of the total content of minerals determined in the samples (Fig. 1). The second largest with respect to content level was magnesium (18%) followed by sodium (12%) and calcium (8%). The remaining elements: iron, manganese, zinc and copper accounted jointly for about 3% of the total content of minerals in the tested pollen samples. The elements followed the following decreasing order with respect to their content of pollen: K>Mg>Na>Ca>Fe>Mn>Zn>Cu.

Samples from China were the highest in potassium averaging 5434 mg/kg, samples from Poland were the lowest averaging 3903 mg/kg DM (Table 2). In the samples from Korea, potassium content averaged 4350 mg/kg DM. The average magnesium content was the highest for the samples from China (1434 mg/kg DM), the averages for the samples from Korea and Poland being at a similar level, 1274 and 1305 mg/kg DM, respectively. Sodium ranged from 293 to 2191 mg/kg DM for the samples from Poland, from 454 to 1113 mg/kg DM for the samples from Korea and from 1072 to 2447 mg/kg DM for the samples from China. The highest sodium content was determined in the samples from China averaging 1549 mg/kg DM. The samples from Korea and Poland were much lower in that constituent averaging 678 and 739 mg/kg DM, respectively. The pollen samples from Poland were the highest in calcium, an average of 762 mg/kg DM. The average content of that constituent in the samples from Korea and China was much lower – 430 and 441 mg/kg DM respectively. Iron content of the tested samples ranged from 40.4 to 136.1 mg/kg DM for the samples from Poland, from 74.3 to 365.9 mg/kg for the samples from Korea and from 59.0 to 182.3 mg/kg DM for the samples from China. The average for the samples from Korea (176.7 mg/kg DM) was nearly three times as high as that for the samples from Poland (65.4 mg/kg DM) and over 1.5 times higher than that for samples from

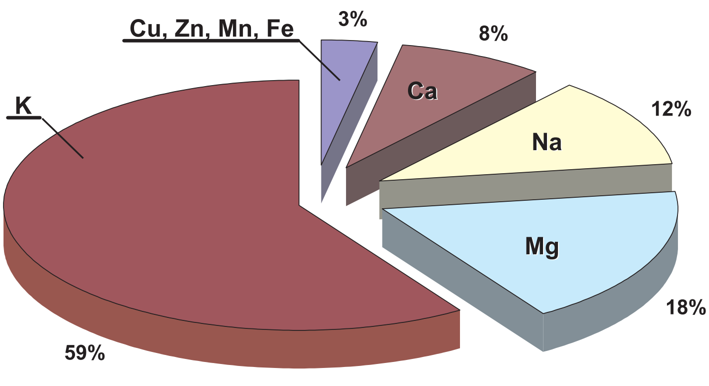

Fig. 1. Percentage of macro- and micro-nutrients content expressed as total minerals content in honeybee-collected pollen.

Table 2

Ash content (% DM) and mineral composition (mg/kg DM) of multifloral pollen samples originating from different countries.

Minerals	Origin								
	Poland (n=13)			South Korea (n=9)			China (n=5)		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Total ash (% DM)	2.08 - 3.19	2.70 a	0.32	2.17 - 3.66	2.82 a	0.47	2.78 - 3.33	2.84 a	0.40
Calcium	542 - 1080	762 b	126	105 - 694	430 a	226	181 - 974	441 a	294
Manganese	13.3 - 59.7	26.3 a	16.4	25.6 - 99.4	55.1 a	27.4	13.2 - 429.8	142.7 b	187.3
Magnesium	742 - 1723	1305 a	293	987 - 1762	1274 a	285	1126 - 1893	1434 a	309
Zinc	25.6 - 53.6	36.8 a	6.9	23.7 - 60.7	47.4 b	10.5	23.9 - 38.3	28.9 a	5.8
Copper	5.6 - 23.9	9.3 a	5.3	5.3 - 14.7	10.1 a	3.4	3.2 - 10.0	7.5 a	2.6
Iron	40.4 - 136.1	65.4 a	30.9	74.3 - 365.9	176.7 b	100.2	59.0 - 182.3	108.2 a	59.0
Potassium	2843 - 4854	3903 a	603	3455 - 5489	4350 a	664	4247 - 5976	5434 b	736
Sodium	293 - 2191	739 a	551	454 - 1113	678 a	210	1072 - 2447	1549 b	557

Explanation:
a,b – significant statistical differences between the mean values compared in the rows at the significance level of $\alpha = 0.05$.

Table 3

Ash content (% DM) and mineral composition (mg/kg DM) of pollen samples from Poland depending on the botanical origin.

Minerals	Botanical origin								
	Brassicaceae (n=6)			*Artemisia* (n=2)			Multifloral (n=5)		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Total ash (% DM)	2.33 - 2.87	2.71	0.19	2.17 - 3.66	2.14	0.08	2.28 - 3.33	2.91	0.21
Calcium	542 - 1080	782	23	798 - 827	812	21	648 - 796	718	63
Manganese	14.2 - 59.7	23.7	18	13.3 - 13.7	13.5	0.3	16.60 - 51.2	34.7	15.5
Magnesium	1088 - 1485	1352	148	742 - 853	798	78	1024 - 1723	1450	265
Zinc	31.9 - 39.9	35.7	3.6	25.6 - 31.2	28.4	4.0	34.1 - 53.6	41.5	7.6
Copper	6.6 - 7.9	7.5	0.5	5.6 - 5.8	5.7	0.1	6.5 - 23.9	13.0	7.5
Iron	42.4 - 106.5	59.3	24.6	36.8 - 40.4	38.6	2.6	60.4 - 136.1	83.5	35.8
Potassium	3586 - 4274	3868	295	2843 - 3082	2963	169	3446 - 4854	4321	580
Sodium	293 - 1546	650	460	349 - 397	373	34	475 - 2191	992	698

China (108.2 mg/kg DM). Manganese content ranged from 13.3 to 59.7 mg/kg DM for the samples from Poland, from 25.6 to 99.4 mg/kg DM for the samples from Korea and from 13.2 to 429.8 mg/kg DM for the samples from China. The highest content of that element was determined in the samples from China – an average of 142.7 mg/kg DM and the lowest in those from Poland – an average of only

26.3 mg/kg DM. Zinc was the highest in the samples from Korea – an average of 47.4 mg/kg DM, slightly lower in those from Poland (36.8 mg/kg DM) and the lowest in the samples from China – an average of 28.9 mg/kg DM. Copper content of the samples ranged from 5.6 to 23.9 mg/kg DM for the samples from Poland, from 5.3 to 14.7 mg/kg DM for the samples from Korea and from 3.2 to 10.0 mg/kg DM for the samples from China with respective averages of 9.3, 10.1 and 7.5 mg/kg DM. The pollen samples harvested in Poland when compared to those from other countries were significantly higher in calcium, samples from China were significantly higher in sodium, potassium and manganese and those from South Korea were significantly higher in zinc and iron.

The results from the study demonstrated that among the tested elements manganese showed the highest sample-to-sample variation within the particular countries. The standard deviation for that element was from 16.4 to 187.3. Highly variable were also the contents of iron, sodium and copper with standard deviation coming within the respective ranges of 30.9-100.2, 210-557 and 2.6-5.3.

Melissopalynological analysis of the tested bee pollen samples allowed the placement of the samples within three groups: group I – samples with the predominance of pollen from Brassicaceae family (over 65%), group II – samples with the predominance of pollen from the genus *Artemisia* (over 64%), group III – samples of multifloral pollen originated from other ruderal plants (*Rumex*, *Coryphyllaceae*, *Ranunculus*, *Centaurea cyanus*, *Majoran*a type) and from agricultural and horticultural plants (*Rubus* type, *Fragaria*, *Trifolium* type), trees and shrubs (*Syringa*, *Cornus*, *Robinia*, *Salix*) (Szczęsna 2006).

The pollen samples containing substantial percentage of pollen of *Artemisia* when compared to samples with a large percentage of Brassicaceae pollen and with samples of multifloral pollen were found to contain less ash and to be lower in all tested elements except calcium (Table 3). The ash content of samples with substantial percentage of *Artemisia* pollen was 2.14% DM whereas it was 2.71% and 2.91% in the two remaining groups, respectively. The calcium content of that pollen group (812 mg/kg DM) was similar to that of the remaining two groups. Instead, magnesium and sodium contents – 798 and 373 mg/kg DM, respectively – were lower ca. by half. Large group-to-group differences were also recorded for the contents of the remaining tested elements.

DISCUSSION

The ash content of 3% as determined in this study for multifloral pollen samples is in agreement with earlier studies both by the author (Szczęsna et al. 1995) and by other domestic investigators (Zalewski and Szymaniuk 1985). Spanish investigators obtained ash contents of pollen that were much lower being as low as ca. 2% (Serra Bonvehi et al. 1986, Serra Bonvehi and Escola Jorda 1997).

The results presented in this study showed potassium, magnesium, sodium and calcium to occur in the highest concentration of all the elements for which pollen loads were examined. The results do not always agree with those obtained in earlier studies by other investigators, especially as concerns the contents in a product tested. When compared to the results reported by Spanish researchers (Serra Bonvehi at al.1986, Serra Bonvehi and Escola Jorda 1997) comparable content values were obtained for potassium, calcium, zinc and copper. The results for magnesium were much higher in this study, the average magnesium content for the pollen from the three countries was 1338 mg/kg DM whereas the Spanish investigators obtained magnesium content values of ca. 450 mg/kg DM. Sodium was assayed at a low level (an average of 989 mg/kg DM) when compared to the results from Spain (an average of 1200 mg/kg). In addition higher contents of iron and manganese were found in this study. The differences in the contents of individual elements as reported by different authors can be explained by differences in geographic and botanical origin of the pollen samples tested. Of note are the extremely low contents of potassium, calcium, magnesium, manganese and zinc as determined in this study when compared to the values obtained by Nation and Robinson (1971), Echigo at al. (1986) and Herbert and Miller-Ihli (1987).

The manganese content in two samples from China was 238.2 and 429.8 mg/kg DM, respectively, whereas in the remaining pollen samples from that country it varied from 13.2 to 17.6 mg/kg DM. A high concentration for that element was also reported by McLellan (1977) for the pollen of Ericaceae (209 mg/kg DM) and Youssef et al. (1978) for the pollen of *Trifolium alexandrium*, *Zea mays*, *Vicia faba* and *Brassica kaber* (an average of ca. 200 mg/kg DM). Youssef et al. (1978) also found pollen to be relatively high in iron. According to those researchers it ranged from 843 mg/kg DM (*Brassica kaber*) to 1303 mg/kg DM (*Vicia faba*). In the studies by other authors, though, including this study values from 40 mg/kg DM (Serra Bonvehi and Escola Jorda 1997) to 190 mg/kg DM (Solberg and Remedios 1980) were reported for iron. Both in this study and in the studies by other investigators of all the tested elements it is manganese that showed the highest variation in content. Substantial variation in content was also showed by other elements.

Investigations of the pollen of ruderal species of the genus *Artemisia* and those of Brassicaceae family for their mineral composition are novel. In available literature there are no data concerning the pollen of those plants. The high concentration of the tested elements, especially in the pollen of Brassicaceae family, makes that variety of pollen an important potential source of macro- and micro-nutrients.

Compared with recommended dietary intakes (EC RDI) of elements the obtained contents of bee pollen point to a high nutritive value of that product which can be recommended as a natural source of macro- and micro-nutrients (Flynn et al. 2003). The product can be successfully used as different dietary formulas and supplements in order to enrich our food rations with valuable nutrients performing important functions in the human body.

CONCLUSIONS

1. Of element constituents of honey-bee-collected pollen potassium followed by magnesium, sodium and calcium occur in the highest concentrations. They account for 59, 18, 12 and 8% of the total elements content of pollen. Such elements as iron, manganese, zinc and copper account jointly for about 3%.
2. The concentrations of ash and of the minerals tested show substantial variation among samples from different countries. It is related to their different botanical origin. Manganese shows the highest variation.
3. Pollen collected by bees as pollen loads due to its high concentrations of minerals can be utilized by man as their natural source.

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ZAWARTOŚĆ WYBRANYCH SKŁADNIKÓW MINERALNYCH W PYŁKU ZBIERANYM PRZEZ PSZCZOŁY

Szczęsna T.

Streszczenie

Celem badań było poznanie zawartości wybranych makro- i mikroelementów w pyłku pszczelim mieszanym pozyskanym w różnych krajach (Polsce, Korei Południowej i Chinach). Łącznie badaniami objęto 27 próbek pyłku kwiatowego zebranego przez pszczoły w postaci obnóży. W zebranym materiale badawczym wykonano oznaczenia zawartości suchej masy, popiołu całkowitego oraz wybranych składników mineralnych. Zawartość poszczególnych pierwiastków oznaczono metodą Atomowej Spektrometrii Absorpcyjnej (ASA), po uprzedniej mineralizacji próbki na mokro za pomocą stężonych kwasów mineralnych: kwasu siarkowego (H_2SO_4) i kwasu azotowego (HNO_3).

W badanych próbkach pyłku pszczelego pochodzących z różnych krajów oznaczono następujące składniki mineralne: wapń, mangan, magnez, cynk, miedź, żelazo, potas i sód. W największych stężeniach wystąpiły: potas, magnez, sód i wapń. Stężenie potasu stanowiło aż 59% ogólnej zawartości oznaczonych składników mineralnych, magnezu – 18%, sodu – 12%, wapnia – 8%, a pozostałe badane składniki (żelazo, mangan, cynk i miedź) łącznie stanowiły 3%. Zawartość poszczególnych pierwiastków w badanych próbkach pyłku pszczelego można uszeregować w następującej malejącej kolejności: K>Mg>Na>Ca>Fe>Mn>Zn>Cu. Stwierdzono ponadto, że zawartość popiołu oraz oznaczonych składników mineralnych w pyłku pszczelim wykazywała duże wahania między próbkami, co mogło być związane z ich różnym pochodzeniem botanicznym. Próbki pyłku pozyskane w Polsce, w porównaniu z próbkami z pozostałych krajów, charakteryzowały się istotnie wyższą zawartością wapnia, próbki z Chin posiadały istotnie wyższą zawartość sodu, potasu i manganu, a próbki pyłku z Korei Południowej - istotnie wyższą zawartość cynku i żelaza. Spośród wszystkich badanych składników mineralnych mangan charakteryzował się największą zmiennością. Pyłek kwiatowy zbierany przez pszczoły w postaci obnóży ze względu na wysoki udział składników mineralnych może być wykorzystywany przez człowieka jako naturalne ich źródło.

Słowa kluczowe: obnóża pyłkowe, popiół, makro- i mikroskładniki, pochodzenie, AAS.