Palynological, physical, and chemical data on honey from the Kazanlak region (Central Bulgaria)

Juliana R. Atanassova[1], Lilyana D. Yurukova[2] & Maria A. Lazarova[2]

[1] Department of Botany, Faculty of Biology, University of Sofia "St. Kl. Ohridski", 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria, e-mail: atanassova_juliana@abv.bg

[2] Institute of Botany, Bulgarian Academy of Sciences, Acad. Georgi Bonchev St., bl. 23, Sofia 1113, Bulgaria

Received: November 04, 2008 ▹ Accepted: February 02, 2009

Abstract. A study of 17 honey samples from five localities – the villages Enina, Buzovgrad and Sahrane and the towns Kazanlak and Shipka in Central Bulgaria – collected in 2006, 2007 and 2008, is presented. The aim was to identify the botanical nectar sources important for the bees. Nineteen elements in five honeys were analyzed by ICP-AES method in a certified laboratory. The results obtained for the elements, water content, pH, and electrical conductivity were compared with literature data. Fifty-eight pollen types of nectariferous plants were identified in the analyzed samples, and two monofloral honeys were recognized: *Robinia pseudoacacia* honey from Buzovgrad village and *Stachys*-type honey from Enina village.

Key words: electrical conductivity, honey, macroelements, melissopalynology, microelements, water content

Introduction

Diversity of vegetation near Kazanlak town makes possible diversification of apicultural production. In this context, melissopalynological studies are particularly relevant, because they indicate the resources utilized by honeybees as nectar sources. Melissopalynological investigations are few in Bulgaria (Bozilova & Anchev 1969; Bozilova & Chan 1976; Lazarova & Bozilova 2001, 2002; Atanassova & Kondova 2004; Atanassova & al. 2004). No palynological investigations and studies of the content of microelements of honey from this region have been made.

The aim of this work was to establish the botanical origin of honey in the region and to identify the nectar sources important for the bees. Of interest are also the intra-annual and inter-annual variation of the pollen spectra and the detection of different sources of nectar during the apicultural period. The physicochemical parameters – water content, pH, electrical conductivity, and content of microelements – are important indicators of the quality of honey. Electrical conductivity is best known and is one of the most important honey characteristics (European Commission 2002; Bogdanov & al. 2004). The different elements among the honey types may reflect the different geographical origin of honeys. The most pronounced differences, especially of trace elements, were between honeydew and blossom type of honey (Ivanov & Chervenakova 1984; Feller-Demalsy & al. 1989; Sevlimli & al. 1992). The heavy metals Pb and Cd and the toxic elements Cr and As could reflect the presence of contaminants due to environmental pollution, or pharmacological (anti-parasitical or acaricidal) treatment of honey, or incorrect procedures during the honey-processing and conservation phases (Pisani & al. 2008).

Area of investigation

The area of investigation had a radius of about 20 km around Kazanlak town in Central Bulgaria (Fig. 1), between the Central Balkan Range and Sredna Gora Mts. Shipka town and Enina village are located at the foot of the Central Balkan Range, at 700–800 m a.s.l. Kazanlak town and Sahrane village lie in the lowlands, and Buzovgrad village is 3 km southwards of the town, on a hill at about 500-600 m a.s.l. (Fig. 1).

The climate of the region is mildly continental (Velev 2002). The diversity of vegetation depends on topography and cultivation. The foothills of the Central Balkan Range are covered by deciduous forests, dominated by *Quercus dalechampii* and forests and shrubs of *Carpinus orientalis,* as well as planted *Pinus sylvestris* and *P. nigra* forests (Bondev 1991). During the last 20 years *Robinia pseudoacacia* has expanded in the deciduous forests in that area. The lowlands are covered by xerothermic grass communities, with prevalence of *Dichantium ischemum, Poa bulbosa* and *Chrysopogon gryllus*. Mesophylous grass communities with *Festuca pratensis*, *Poa sylvicola*, *Alopecurus pratensis*, and *Lolium perenne* are also widespread, replacing the forests of *Ulmus minor*, *Fraxinus oxycarpa* and *Quercus pedunculiflora* (Bondev 1991). *Alnus glutinosa*, *Fraxinus oxycarpa*, *Ulmus minor*, and *Salix alba* grow along the rivers. Near the apiaries we have collected and identified many herb species visited by the bees.

- In the meadows and grasslands were: *Astragalus onobrychis*, *Dorycnium herbaceum*, *Lotus corniculatus*, *Medicago sativa*, *Melilotus alba*, *M. officinalis*, *Trifolium arvense*, *T. patense*, *T. repens*, *Vicia grandiflora*, *Arabis turrita*, *Berteroa incana*, *Erysimum diffusum*, *Lepidium perfoliatum*, *Sisymbrium orientale*, *Raphanus raphanistrum*, *Rorippa prolifera*, *Thlaspi* sp., *Ajuga chamaepytis*, *A. reptans*, *Ballota nigra*, *Marrubium peregrinum*, *Mentha longifolia*, *M. spicata*, *Salvia verticillata*, *S. pratensis*, *Sideritis montana*, *Thymus* sp., *Daucus carota*, *Orlaya grandiflora*, *Malva sylvestris*, *M. neglecta*, *Arctium lappa*, *Centaurea diffusa*, *C. rhenana*, *Eupatorium cannabinum*, *Taraxacum officinale*, *Anchusa officinalis*, *Linaria vulgaris*, etc.
- In the open forest near the apiaries in Enina village we found: *Clinopodium vulgare*, *Melissa officinalis*, *Satureja pilosa*, *Stachys recta*, *Teucrium chamaedrys*, and on rocky grounds *Rhodiola rosea*, *Sedum acre*, and *S. album.*
- In the inhabited lands and along roads were: *Carduus acanthoides*, *Cirsium arvense*, *Cichorium intybus*, *Centaurea cyanus*, *C. solstitialis*, *Echium vulgare*, *E. italicum*, *Dipsacus laciniatus*, *Datura stramonium*, *Verbascum* spp., etc.

All apiaries were close to gardens dominated by trees and shrubs of *Rosaceae* (*Prunus, Malus*, *Pyrus*, *Rubus* etc.)

Material and methods

The seventeen analysed honey samples had been collected once or, if possible, twice during consecutive apicultural periods, in most cases from the same locations. The locations of the apiaries were selected for their different topography and vegetation. Two apiaries were in Kazanlak town – one of them (**1K**) located at the foot of the Tyulbeto Hill had various ornamental trees and bushes, such as *Sophora japonica*, *R. pseudoacacia*, *Spirea* spp., and different species of *Cupressaceae* and *Pinaceae.* Another apiary was in an open area with meadows and uncultivated grounds (**2K**). The apiary in Sahrane village (**1Sh**) had the same surroundings: meadows and pastures. Two apiaries were located in Enina village, in the vicinity of a deciduous forest and a forest dominated

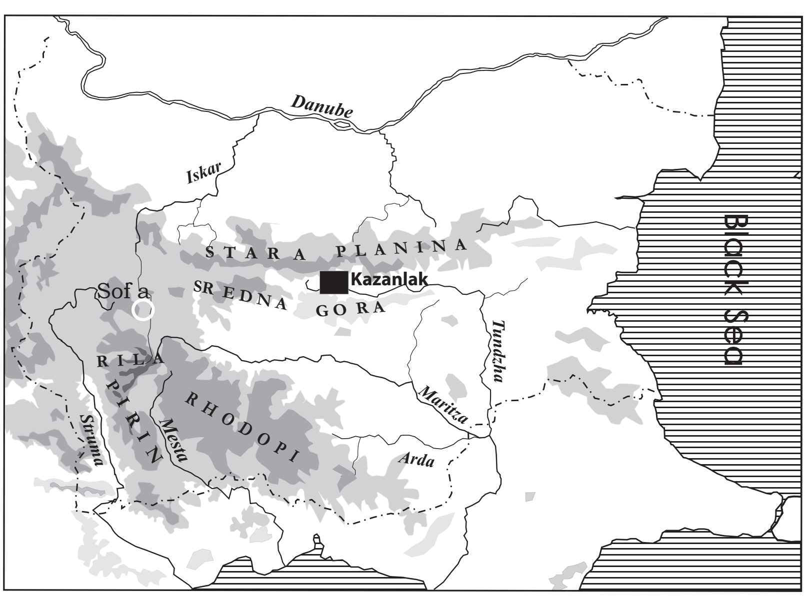

Fig. 1. Map of Bulgaria with the region of investigation.

by *P. nigra* (**3E** and **4E**). Another apiary was in Shipka town (**5S**), near a deciduous forest and pasturelands, and still another one in Buzovgrad (**6B**) was in a deciduous forest dominated by *Quercus dalechampii*, *Carpinus orientalis* and *Robinia pseudoacacia*.

The samples obtained in June and the first half of July, were considered as spring honeys. Those collected in the second half of July and in August and September were considered as summer honeys. Only from Enina (sample **3E1** and **3E2**) and from Buzovgrad village (sample **6B1** and **6B2**) it was possible to collect two honey samples in 2007: once in spring and once in summer, because of the more favourable climatic conditions. The precipitation in spring and the high temperatures and dry summer in 2008 resulted in low honey production in the region, and samples were collected only once: at the end of August and the first half of September.

For laboratory preparation and qualitative analysis, we have followed the method of Louveaux & al. (1978): 400–500 pollen grains were counted in each sample. The frequency of pollen types is expressed as percentage of the pollen sum (P), which includes pollen grains only from nectar-producing plants. The pollen types in each sample were classified into four groups: dominant pollen (d) (over 45 % of P); secondary pollen (s) (16–45 %); important minor pollen (i) (3–15 %), and minor pollen (m) (under 3 %). Sporadically presented minor pollen taxa (*Campanula*, *Linum*, *Aesculus*, *Pedicularis*, *Solanum*, *Malva*, *Hedera*, *Cerastium*, *Fraxinus ornus*, and *Reseda*) are not included in the tables. If one pollen type constituted <45 % of the total number of pollen grains, the sample was classified as monofloral. Pollen taxa were identified by comparison, with a reference collection obtained from the melliferous plants in the vicinity of the investigated apiaries and with *Leitfaden der Pollenbestimmung* (Beug 2004).

For quantitative analysis, the method described by Moar (1985) was followed. We added tablets containing a known number of spores of *Lycopodium clavatum* (Stockmarr 1971). Pollen concentration was calculated and compared according to the classification proposed by Maurizio (1939). The five classes according to the pollen-grain content in 1 g of honey were: I – less than 2000 pollen grains; II – 2000 to 10 000 pollen grains; III – 10 000 to 50 000 pollen grains; IV – 50 000 to 100 000 pollen grains; V – over 100 000 pollen grains.

The ratio of honeydew elements to pollen grains of nectariferous plants was also calculated (HDE/P) (Louveaux & al. 1978). Identification of the plant species collected in the investigated region followed Jordanov (1963–1979), Velchev (1982–1989), and Kozhuharov (1992).

Only 5–6 honey samples were used to measure the additional parameters because of the cost. Routine physicochemical analysis included water content (honey refractometer Atago HHR-2N 12–30 %, Japan), electrical conductivity (mS/cm in 20 % solution at 20 °C using MultiLine P3, WTW, Germany), and pH (20 % solution, Jenway pH-meter 3310, England). The values of conductivity and pH were calculated on a dry-weight basis. About 5-6 g of honey was treated with 15 ml of nitric acid (9.67 M) overnight. The wet-ash procedure was continued with heating in a water bath, followed by addition of 2 ml hydrogen peroxide until full digestion. The filtrate was diluted with doubly distilled water (0.06 μS/cm) to 25 ml. All solutions were stored in plastic flasks. Duplicates of each sample were prepared independently. The elements Al, As, Ca, Co, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Sr, V, and Zn were determined in a certified laboratory by atomic emission spectrometry with inductively coupled plasma (ICP–AES) on a VARIAN VISTA-PRO instrument. The detection limits were 0.002 mg/l for Mn and Sr, 0.004 mg/l for Cd, Co, Cr, Cu, and Ni, 0.005 mg/l for Zn, 0.02 mg/l for As and V, 0.03 mg/l for Pb, 0.04 mg/l for Al and Fe, 0.5 mg/l for Ca, K, Na, P, and S, and 1 mg/l for Mg. The analytical precision was verified by triplicating (deviation was below 5 % in all cases) and by use of blanks and standard stock solutions. Quality control was ensured by reference-plant material (CRM 281, ryegrass). The measured concentrations were in good agreement with the recommended values. All concentrations are presented in mg/kg.

Results

A total of 73 pollen taxa were identified in the analyzed honey samples, 59 of them from nectar-producing plants. Two pollen taxa were dominant: *R. pseudoacacia* exceeded 45 % in sample **6B1**-2007 (spring honey) from Buzovgrad village (Table 1) and *Stachys* type in sample **3E1**-2008 (summer honey) from Enina village (Table 2). The honeys were classified as monofloral (Louveaux & al. 1978). Secondary pollen (s) came from two pollen taxa in spring honey: *Brassicaceae* and *Prunus* type. *Brassicaceae* had high percentages in the pollen spectra of honey samples **1K1**-2006, **1K1**-2007 from Kazanlak town (21.4 % – 39.7 %) and in **3E1**-2006, **3E1**-2007 (35.5 % – 39.8 %) from Enina village (Table 3 and 2). *Prunus* type predominated in the pollen spectra of sample 4**E1**-2007 (31.7 %) from Enina village. Secondary pollen taxa in the summer-honey pollen spectra were *Brassicaceae*, *Stachys* type, *Trifolium*, *Lotus*, *C. cyanus*, and *Echium*. The pollen analyses established dominance of *Trifolium* in summer honey from Kazanlak town (**2K1**-2007 with 41.5 % and **2K1**-2008 with 30.8 %) and in summer honey from Buzovgrad village (**6B2**-2007 with 23.5 %). *Lotus* was the dominant pollen

Table 1. Pollen content in the honey samples collected in 2007.

Sample N / Pollen taxa	1K1 Spring %	2K1 Summer %	3E1 Spring %	4E1 Spring %	4E2 Summer %	5S1 Summer %	6B1 Spring %	6B2 Summer %
Prunus type			15.9	31.7		1.3	10.8	
Rubus type			11.0					
Potentilla type	7.7		2.9	4.3			8.1	
Rosaceae		2.7	5.5		6.7	9.8		10.8
Brassicaceae	39.7	0.4	39.8	18.7	196	11.2	7.5	11.1
Trifolium	0.8	41.5		1.7	5.6	2.2	4.6	23.5
Robinia	14.8		1.2				**47.1**	
Melilotus		13.7			5.9	0.9		7.4
Lotus		2.7			6.7			
Vicia type		6.9	2.8	14.5			4.5	3.1
Helianthus type		9.6				2.4		3.1
Cirsium type		7.8			0.6	7.6		
Centaurea jacea type		1.8						
C. cyanus	2.6					16.1		
Crepis/Taraxacum type	1.1					7.6		
Matricaria/Achillea type			3.1	4.5	8.1		3.4	2.5
Echium					4.2	4.8		8.7
Pulmonaria type	0.3	0.4						
Apiaceae			2.4			1.3		
Daucus type					2.1			2.7
Heracleum		0.4			1.2			
Stahys type	3.7	2.6	2.2	2.2	7.0	3.9	1.8	2.5
Mentha type					2.0	1.8		
Salvia					2.5	1.8		
Euphorbia	12.8		1.0			1.3	0.9	10.2
Knautia					1.9			
Gentiana				10.0				
Ranunculus acris type		4.8						
Ranunculaceae				0.6			4.3	
Verbascum	2,5		1.2		7.0	10.7		2.7
Linaria		0.9	0.6			2.4		3.3
Sedum			0.6		6.4			
Convolvulus	1.1		0.6		0.8		1.2	
Liliaceae			2.6	0.6				
Allium				4.5				
Tilia	1.9					1.3		6.2
Acer	1.7			2.2		0.2	1.8	
Syringa/Ligustrum	4.0				0.8	2.8		
Viburnnum	3.4					0.6		
Salix	1.1		4.1					
Rhamnus/Paliurus								6.8
Ailanthus						5.2		

(30.8 %) in summer honey from Sahrane village (**1Sh1**-2006), and *Centaurea cyanus* had high percentages in the pollen spectra of sample **5S1**-2007 from Shipka town (16.1 %). Pollen grains of *Echium* were found in most investigated samples, but the highest level (29.6 %) was established in sample **3E**-2008 from Enina village. The high variety of pollen types (i) in spring honey came from *Rosaceae*, (*Rubus* type, *Potentilla* type), from family *Fabaceae* (*Robinia pseudoacacia*, *Lotus*, *Melilotus*, *Vicia* type), and from *Stachys* type, *Brassicaceae*, *Euphorbia*, *Syringa/Ligustrum*, *Salix*, *Tilia*, etc. In summer honeys, some important minor pollen was that of *Cirsium* type, *Helianthus* type, *Crepis/Taraxacum* type, *Matricaria/Achillea* type, *Brassicaceae*, *Echium*, *Mentha* type, *Salvia*, *Verbascum*, *Linaria*, *Sedum*, *Euphorbia*, etc. (Tables 1, 2, 3).

All honey samples contained a low content but high variety of pollen grains from nectarless plants, such as *Pinus*, *Quercus*, *Betula*, *Corylus*, *Fagus*, *Rumex*, *Plantago*, *Artemisia*, *Helianthemum*, *Papaver*, *Hypericum*, *Chenopodiaceae*, *Cyperaceae*, and *Poaceae*.

Table 2. Pollen content in the honey samples collected in 2008.

Sample N / Pollen taxa	2K1 Summer %	3E1 Summer %	4E1 Summer %	5S1 Summer %	6B1 Summer %
Potentilla type		1.2	0.6	1.1	1.3
Agrimonia type		1.2			
Rosaceae	0.7	0.6		1.1	
Brassicaceae	16.9	3.9	35.1	6.3	10.8
Trifolium	30.8	2.3		2.2	3.4
Melilotus	10.3			4.2	
Lotus	3.1	1.2			4.8
Vicia type		1.2	1.6	1.3	1.5
Onobrychis					4.6
Helianthus type	3.2				3.7
Cirsium type	10.9		1.2	0.9	1.3
Centaurea jacea type	2.0		0.6		1.8
C. cyanus				0.7	5.5
Crepis/Taraxacum type			0.6		
Matricaria/Achillea type	2.0		1.2	4.0	5.5
Echium	2.0	29.6	10.0	16.3	7.4
Pulmonaria type			0.3		3.7
Apiaceae		1.0			
Daucus type	0.9			2.4	
Eryngium			1.6	3.1	1.8
Heracleum			1.6	0.7	
Tilia	1.8			0.7	3.7
Stachys type	7.8	**49.8**	26.9	12.6	6.8
Mentha type		1.2		4.6	3.7
Salvia		2.3	1.0	5.1	
Euphorbia	0.9		1.2	1.8	1.1
Verbascum		0.6	2.7	12.1	2.8
Linaria			9.3	0.7	1.8
Centaurium					
Gentiana			1.8		0.9
Ranunculus acris type	3.3	1.2		1.3	15.2
Convolvulus					1.5
Acer		1.2	1.2	1.3	3.7
Syringa/Ligustrum					
Viburnum	0.4				
Salix					
Rhamnus/Paliurus					
Ailanthus				2.9	
Liliaceae	4.2		1.0		
Galium				1.5	

Table 3. Pollen content in the honey samples collected in 2006.

Sample N / Pollen taxa	1K1 Spring %	3E1 Spring %	5S1 Summer %	7Sh1 Summer %
Prunus type		11.2		
Rubus type	8.9	14.5		
Potentilla type	2,6	1.8	2.4	1.5
Rosaceae			2.9	3.4
Brassicaceae	21.4	35.5	16.7	13.6
Trifolium	19.6	4.3	7.0	3.0
Robinia	8.5	2.2	7.0	
Melilotus	5,6		2.9	1.5
Lotus	3.0		0.6	30.8
Vicia type	6.3	1.2	3.5	3.0
Onobrychis		0.4	2.1	
Crepis/Taraxacum type			0.9	0.7
Cirsium type			5,6	0.7
Centaurea cyanus	4,6		7.2	5.9
Matricaria/Achillea type	3.0	0.6	2.0	0.7
Echium	2.3	0.6	1.6	10.6
Pulmonaria type		0.4	0.3	
Lithospermum				5.7
Apiaceae		2.3		1.2
Daucus type		2,0		1.5
Heracleum				0.7
Ranunculus acris type				1.0
Stachys type	3,2	6.8	13.7	6.1
Mentha type		1.2	1.2	5.4
Euphorbia	9.3	2.2		
Gentiana		1.8		
Polygonum aviculare		0.6	1.6	
Convolvulus	0.6		0.6	
Tilia		0.6		
Acer		3.3		
Syringa/Ligustrum		2.4		
Salix		3.3		
Verbascum			14.3	0.2
Linaria				1.7
Galium			2.4	

Quantitative analysis has shown low pollen concentrations in the studied honey samples: 2 honeys were assigned to class III, and 15 honey samples were included in class II (Maurizio 1939) (Table 4). Honeydew indicators were scarce (Table 4).

The water content in honeys was within the range of 12.2 % to 17.8 %, the average pH value was 3.48±0.23, and the electrical conductivity varied in a wide range from 0.117 to 0.428 mS/cm (Table 5). Heavy metals, such as Cd, Co, Pb, and Ni and toxic elements such as As, Cr, and V in most cases were under the detection limits (Table 6). Zinc varied for more than one order of magnitude among the different honey samples, followed by K and Mn (4 times), Al, Cu, Fe, P, and S (3 times). The maximum values of six elements (Cu, K, Mg, P, S, and Zn) were found in the honey from Shipka town (5S-1). The highest concentrations of Fe, Ni, and Na were in the honey produced in the area of Tyulbeto Hill (1K-1), whereas the highest content of Mn and Sr was in the honey sample from Enina village (3E-1). Calcium had maximum value in the sample from Kazanlak district (2K-1), and aluminium in the Buzovgrad region (6B-1). Only the changes in zinc concentrations were significant.

Table 4. HDE/P ratio and pollen concentration grains/g .

Sample N/ Year of collection	HDE/P	Pollen concentration grains/1g	Class (Maurizio 1939)
1K-1, Spring 2006	0.02 Practically none	4 220	II
3E-1, Spring 2006	0.13 Few	5 296	II
5S-1, Summer 2006	0.10 Few	5 890	II
7Sh-1, Summer 2006	0.18 Few	4 487	II
1K-1, Spring 2007	0.02 Practically none	6 539	II
2K-1, Summer 2007	0.13 Few	6 380	II
3E-1, Spring 2007	0.05 Practically none	6 671	II
4E-1, Spring 2007	0.07 Practically none	3 884	II
4E-2, Summer 2007	0.10 Few	2 845	II
5S-1, Summer 2007	0.10 Few	5 890	II
6B-1, Spring 2007	0.01 Practically none	4 026	II
6B-2, Summer 2007	0.55 Few	3 337	II
2K-1, Summer 2008	0.36 Few	3 800	II
3E-1, Summer 2008	0.14 Few	11 444	III
4E-1, Summer 2008	0.07 Practically none	16 970	III
5S-1, Summer 2008	0.04 Practically none	6 338	II
6B-1, Summer 2008	0.07 Practically none	3 474	II

Table 5. Water content, pH and electrical conductivity in honey samples from the region of Kazanlak.

Index	Water content, %	pH	Conductivity, mS/cm
2K1-2007	12.2	3.10	0.223
1K1-2007	16.1	3.39	0.217
3E1-2007	15.9	3.77	0.289
5S1-2007	17.8	3.45	0.428
6B1-2007	16.3	3.58	0.117
6B2-2007	16.2	3.58	0.337

Table 6. Concentrations of 19 macro- microelements (mg/kg) in studied honey samples from the region of Kazanlak.

Index	Al	As	Ca	Cd	Co	Cr	Cu	Fe	K
2K1	0.88	<0.1	86.2	<0.02	<0.02	<0.02	0.25	1.47	178
1K1	2.63	<0.1	46.1	<0.02	<0.02	<0.02	0.09	4.31	190
3E1	2.89	<0.1	73.7	<0.02	<0.02	<0.02	0.21	1.24	462
5S1	2.62	<0.1	75.2	<0.02	<0.02	<0.02	0.25	1.75	586
6B1	3.04	<0.1	52.8	<0.02	<0.02	<0.02	0.15	1.45	152

Index	Mg	Mn	Na	Ni	P	Pb	S	Sr	V	Zn
2K1	13.2	0.20	14.6	<0.02	44.1	<0.2	28.9	0.28	<0.1	1.26
1K1	8.9	0.11	32.7	1.02	34.2	<0.2	18.8	0.19	<0.1	0.23
3E1	19.4	0.46	16.9	<0.02	56.8	<0.2	37.3	0.30	<0.1	0.93
5S1	19.9	0.37	24.7	<0.02	72.8	<0.2	40.3	0.25	<0.1	3.91
6B1	8.5	0.14	15.0	0.08	28.4	<0.2	13.5	0.22	<0.1	0.30

Discussion

The results of qualitative pollen analysis indicate the diversity of resources utilized by honeybees in the region of investigation. The main botanical species for honey production were of the families *Fabaceae*, *Lamiaceae*, *Brassicaceae*, and *Rosaceae*. *Robinia pseudoacacia* was an important nectar source in spring, together with *Trifolium*, *Vicia*, and different species of *Rosaceae* and *Brassicaceae*. *Robinia* unifloral honey is common in Bulgaria, but its production varies greatly, depending on the climatic conditions in spring (precipitation, temperature, winds, etc. (Petkov 2006). Besides, Black Locust is a fine nectariferous plant but has a short flowering period (10–15 days). In interpreting the results of pollen analysis, mention deserves the fact that *Prunus* pollen grains have morphological characteristics similar to those of *Pyrus*, *Malus*, *Mespilus* (Beug 2004). The same applies to the pollen grains of *Potentilla* and *Fragaria*, and they were included in the general pollen types (*Prunus* type and *Potentilla* type). Gardens with cultivated trees and shrubs, predominantly of *Rosaceae,* are important for supplying the bees with nectar in spring.

In summer, numerous species of *Lamiaceae* (*Ajuga chamaepytis*, *Clinopodium vulgare*, *Ballota nigra*, *Marrubium peregrinum*, *Mentha spicata*, *Salvia verticillata*, *Stachys recta*, *Thymus* sp.), *Fabaceae* (*Lotus corniculatus*, *Medicago sativa*, *Melilotus alba*, *M. officinale*, *Trifolium pratense*, *T. repens*, *Vicia grandiflora*), *Brassicaceae* (*Brassica incana*, *Erysimum diffusum*, *Sisymbrium orientale*, *Raphanus raphanistrum*), *Boraginaceae* (*Echium vulgare*, *E. italicum*) etc., which have long periods of flowering (from May to August-September), supply the bees with nectar. Because of similar morphological characteristics, the pollen grains of *Lamium* and *Ballota* were included in the *Stahys* type. Pollen analysis shows an increase in the summer pollen spectra of the *Asteraceae* taxa, especially *Centaurea cyanus* and *Cirsium*. *Centaurea cyanus* is a weed infesting the cereal fields that produces high amounts of nectar in the dry summer periods (Bizev & al. 2003). The same applies to *E. vulgare* and *E. italicum*, which are widespread on uncultivated lands and in arid pastures (Ricciardelli d'Albore 1998).

Palynological results show that the honey from every apiary has a different composition of pollen spectra in the successive years. The botanical composition of regional honey depends on the climatic conditions during the apicultural period. Multifloral honeys were frequent due to the fact that the common practice in the study area was to harvest honey only once during the apicultural period, storing it in the hive and extracting a mixture of honey at the end of the period. This made the interpretation of seasonal variations difficult.

According to the European honey standard (European Commission 2002), the conductivity of honeydew type should exceed 0.8 mS/cm. The honey samples studied by us had low conductivity, on the average of 0.269 ±0.108 mS/cm. Values of pH (3.45 ±0.23) in these honey samples were generally lower, as compared to the higher values of the honeydew type of honey (Soria & al. 2004). Water content in the honey samples from Kazanlak region was approximately similar to the ranges given for Slovenian honeydew honeys: 13.4 % and 18.0 % (Abramovič & al. 2008), and close to the average values for the main types of honeys – *Robinia*, multifloral, and honeydew (Dinkov 2003). The descending order of all analyzed macroelements and microlelements on the average was as follows: K>Ca>P>S>Na>Mg>Al>Fe>Zn>Mn>Sr>Cu> Ni, V, As, Pb, Cr, Co, Cd. Potassium was the most abundant element in all honey samples, with an average content of 313±198 mg/kg. The Czech, Slovak, and Polish honeys had higher nickel levels (e.g. 0.06–1.53 mg/kg in Czech honeys) than the honeys originating from other parts of the world (Lachman & al. 2007). The concentrations of Ni (<0.02 up to1.02 mg/kg) in the studied Bulgarian honey samples were insufficient to confirm this fact. Data observed in this study have shown a low content of heavy metals and toxic elements. A comparison with literature data of elements in honeys produced in Italy, Spain, Turkey, and Ireland (Pisani & al. 2008) has shown lower content of all analyzed elements, except for calcium in the Bulgarian samples from the region of Kazanlak. Besides the botanical origin of honey, the reason for these differences could be some geological and/or geochemical features.

Conclusion

The most important nectar-producing plants in the region of investigation were from the native flora, except for the representatives of family *Rosaceae* (*Prunus*, *Malus*, *Pyrus*, *Rubus*, *Fragaria*, etc.). Production of unifloral *Robinia* honey is possible under favourable climatic conditions in spring. The main nectar-producing plants belong to *Lamiaceae*, *Brassicaceae* and *Fabaceae*, distributed in the meadows, pastures, and uncultivated lands. Of particular importance for the bees in the region were the numerous species producing high quantity of nectar during the dry periods: *Centaurea cyanus*, *Cirsium*, *Echium vulgare*, *E. italicum*, etc.

The honeys under study had levels of water content, pH, and electrical conductivity within the limits set for European honey standard (European Commission 2002). Data observed in this study showed a low content of heavy metals and toxic elements in all honey samples.

Acknowledgements. This study is part of the Project N TK-B-1611 supported by the National Science Fund, Ministry of Education and Science, Bulgaria. We are grateful to Professor H.E. Wright and Dr. I. Stefanova from the University of Minneapolis for checking the English text of the manuscript. The authors are indebted to the anonymous reviewer for the valuable comments.

References

Abramovič, H., Jamnik, M., Burkan, L. & Kač, M. 2008. Water activity and water content in Slovenian honeys. – Food Control, **19**(11): 1086–1090.

Atanassova, J. & Kondova, V. 2004. Pollen and chemical-physical analysis of unifloral honey from different regions of Bulgaria. – Phytol. Balcan., **10** (2-3): 247-252.

Atanassova, J., Bozilova, E. & Todorova S. 2004. Pollen analysis of honey from the region of three villages in Western Bulgaria. – Phytol. Balcan., **10**(1): 45-50.

Beug, H-J. 2004. Leitfaden der Pollenbestimmung für Mitteleuropa und angrenzende Gebiete. Verlag Dr. Friedrich Pfeil, Munchen.

Bizev, B., Gantshev, A & Tsonev, D. 2003. Melliferous Plants. Enovtshe Publ., Sofia (in Bulgarian).

Bogdanov, S., Ruoff, K. & Persano Oddo, L. 2004. Physicochemical methods for the characterization of unifloral honeys: a review. – Apidologie, **35**: 4–17.

Bondev, I. 1991. The Vegetation of Bulgaria. Map 1:60 000 with explanatory text. St. Kliment Ohridski Univ. Press, Sofia (in Bulgarian).

Bozilova, E. & Anchev, M. 1969. Pollen analysis of bee honey from Kyustendil, Znepole region. – God. Sofiisk. Univ. Biol. Fak., 2 Bot., **67**: 15-29 (in Bulgarian).

Bozilova, E. & Chan, N. 1976. Pollen and chemical analysis of honey samples from different districts of Bulgaria. – God. Sofiisk. Univ. Biol. Fak., 2 Bot., **67**: 15-29.

Dinkov, D. 2003. A scientific note on the specific optical rotation of three honeytypes from Bulgaria. – Apidologie, **34**: 319–320.

European Commission. 2002. Council Directive 2001/110/EC. – Off. J. Eur. Commun., **10:** 47-52.

Feller-Demalsy, M.J., Vincent, B. & Beaulieu, F. 1989. Mineral content and geographical origin of Canadian honeys. – Apidologie, **20**: 77–91.

Ivanov, T. & Chervenakova, Y. 1984. Content of some macro-, oligo- and microelements in bee honey, royal jelly and pollen. – Anim. Sci., **21**: 65–69.

Jordanov, D. (ed.). 1963-1979. Flora Reipubl. Popularis Bulgaricae. Vols **1**–**7**. In Aedibus Acad. Sci. Bulgaricae, Serdicae (in Bulgarian).

Kozhuharov, S. (ed.). 1992. Field Guide to the Vascular Plants in Bulgaria. Nauka & Izkustvo, Sofia (in Bulgarian).

Lachman, J., Kolihová, D., Miholová, D., Košata, J., Titěra, D. & Kult, K. 2007. Analysis of minority honey components: possible use for the evaluation of honey quality. – Food Chem., **101**(3): 973-979.

Lazarova, M. & Bozilova, E. 2001. Pollen and chemical analysis of honey from different floral regions of Bulgaria. – Phytol. Balcan., **7**(1): 101-112.

Lazarova, M. & Bozilova, E. 2002. Pollen and chemical analysis of honey from different floral regions of South Bulgaria. – Phytol. Balcan., **8**(2): 145-164.

Louveaux, J., Maurizio, A. & Vorwhol, G. 1978. Methods of Melissopalynology. International Bee Research Association. – Bee World, **59**(4): 139-157.

Maurizio, A. 1939. Untersuchungen zur quantitativen Pollenanalyse des Honing. – Mitt. Geb. Lebensm., **30**(1-2): 27-69.

Moar, N.T. 1985. Pollen analysis of New Zealand honey. – New Zealand J. Apic. Res., **28**: 39 – 70

Petkov, V. 2006. The Nectariferous Plants and Bee Forage in Bulgaria. Zahari Stoyanov Press and St. Kliment Ohridski Univ. Press, Sofia (in Bulgarian).

Pisani, A., Protano, G. & Riccobono, F. 2008. Minor and trace elements in different honey types produced in Siena County (Italy). – Food Chem., **107**(4): 1553-156.

Ricciardelli D'Albore, G. 1998. Mediterranean Melissopalynology. Istit. Entomol. Agraria Publ., Univ. degli Studi, Perugia.

Sevlimli, H., Bayulgen, N. & Varinioglu, A. 1992. Determination of trace elements in honey by INAA in Turkey. – J. Radioanal. Nucl. Chem. Letters, **165**: 319-325.

Soria, A.C., González, M., de Lorenzo, C., Martínez-Castro, I. & Sanz, J. 2004. Characterization of artisanal honeys from Madrid (Central Spain) on the basis of their melissopalynological, physicochemical and volatile composition data. – Food Chem., **85**: 121–130.

Stockmarr, J. 1971**.** Tablets with spores used in absolute pollen analysis. **–** Pollen & Spores, **13**: 615-621.

Velev, S. 2002. Climatic regioning. – In: **Kopralev, I**. (ed.), Geography of Bulgaria. Physical Geography. Socioeconomic Geography. Pp. 152-156. FarCom, Sofia.

Velchev, V. (ed.). 1982-1989. Flora Republ. Popularis Bulgarice. Vols **8**-**9**. In Aedibus Acad. Sci. Bulgaricae, Serdicae (in Bulgarian).