CONTENT OF HEAVY METALS, MICRO AND MACRO ELEMENTS IN BULGARIAN HONEYDEW HONEY

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Abstract

The aim of this study was to determine the content of heavy metals, trace and macro elements in honeydew honey collected in Bulgaria. A total of 13 honey samples purchased from apiaries and hypermarkets were tested. The honey samples were prepared for analysis by digestion with nitric acid in a Mars 6 microwave system and analysed by ICP-OES. The Hg content of the samples was determined directly (without digestion) with a mercury analyser. Most of the honeydew honey on the market is genuine, with electrical conductivity values ranging from 0.8 to 1.4 mS/cm. Four of the samples tested were adulterated by mixing with nectar honey. Of the macroelements, the highest content was K (1074-1912 mg/kg), followed by Ca (93.7-192.5 mg/kg), Mg (74.5 -149.7 mg/kg), P (71.1-149.8 mg/kg), Na (41.8-130.8 mg/kg) and Al (0.12-5.8 mg/kg). Of the trace elements, Mn (7.3-28.1 mg/kg) was the highest, followed by Fe (3.1-11.7 mg/kg), B (3.8-9.0 mg/kg), Zn (0.64-2.9 mg/kg), and Cu (0.56-1.15 mg/kg). Toxic metal contents were very low and ranged from 0.01 to 0.63 mg/kg for Pb, 0.15 to 0.39 mg/kg for As, and 0.03 to 5.86 ng/g for Hg. Variability was found in the elemental composition of the honeydew honey, the differences being due to the geographical origin of the honey. The content of toxic metals Pb, Cd, As and Hg in honeydew honey does not exceed the maximum permissible values (except for 2 samples for Pb), indicating minimal environmental contamination in the areas where the honeydew honey is produced. Bulgarian honey meets the requirements for a safe product for human consumption.

Keywords: *honey, heavy metals, trace elements, mineral content*

1. INTRODUCTION

Honeydew honey is produced by honey bees from excreta of plant-sucking insects (Hemiptera) on various plants, mainly conifers such as fir (*Abies alba*) and spruce (*Picea abies*), but also deciduous trees such as oak (*Quercus spp*.) and lime (*Tilia spp*.) (Bogdanov, 2009; Persano Oddo et.al., 2004). When insects feed on plant sap, they absorb essential nutrients and excrete the sugar-rich residues in a sticky liquid known as honeydew. These exudates are collected by honey bees and converted by them into honey known as honeydew, forest, fir or spruce honey (Shaaban et al., 2021). The composition of honeydew honey is determined by the phloem sap of the plant, the insect producing the honeydew, the bees in the honey production process (Shaaban et al., 2021), and various environmental conditions (Fischer and Shingleton, 2001).

The chemical composition of honeydew honey differs from that of nectar honey (Bogdanov et al., 2004; Seraglio et al., 2019). Honeydew honey has a darker colour, and has higher values of electrical conductivity, pH, ash, disaccharides and trisaccharides and a lower level of monosaccharides. Due to the specific origin of honeydew honey, its market price is higher than that of nectar honey. Honeydew honey is often adulterated by mixing with nectar honey or by incorrect labelling of the origin of the honey.

The mineral content of honey is one of the properties to consider when assessing its nutritional value (Honeydew honey et al., 2017). The mineral content of honeydew honey is usually higher compared to honeydew honey due to the higher amount of K and P (Escuredo et al., 2012). The mineral composition of honey depends on its botanical and geographical origin (including soil and climatic conditions). The content of macro and trace elements is mostly related to the soil composition and the type of plants from which honey bees collect food. Elevated concentrations of trace elements and toxic elements (Pb, Cd, Cr and As) in honeydew honey may result from anthopogenic contamination, use of fertilizers and plant protection products (Kovacik, et al., 2016) or improper procedures during honey processing and storage (Atanassova et al., 2012). Due to its acidity, honey can cause container corrosion during honey collection, production and storage and metals such as Al, Cd, Cr, Fe and Pb can migrate into the honey. Sugar syrup, as food for bees, can also be a significant source of Mn, Fe, Cd and Pb

Heavy metals and other contaminants in honey have been investigated as honey is a good indicator of contamination levels (Staniskienė et al., 2006; Sereviciené et al., 2022). Honey can be contaminated from two sources: the environment in which the bees collect honey and the method of beekeeping. In the case of environmental contamination, the bees also become contaminated and carry the contaminants from the environment into the hive itself or into the raw materials collected. The foraging area of bees covers about 7 km^2. When foraging for nectar, honeydew and pollen in such a large area, bees come into contact with plants, air, water and soil (Pohl, 2009). The amount of heavy metals in plants varies depending on soil contamination, plant type and location (distance to the source of contamination) (Koptsik et al., 2022|. Metals move through the root system to the honey bee plants, enter the nectar, and then the honey produced by the bees (Pohl, 2009).

This study aims to determine the content of heavy metals (Pb, Cd, As, Hg), trace elements (Cu, Zn, Fe, Mn, B) and macronutrients (K, Ca, Mg, Al and Na) in honeydew samples (after preliminary authentication) collected from different geographical regions of Bulgaria.

2. MATERIALS AND METHODS

2.1. Materials

Thirteen honeydew honey samples were collected from different regions of Bulgaria (Strandzha Mountain, Eastern Rhodopes, Sredna Gora, Ludogorie, Plovdiv, Sofia, Kalofer, Ludogorie, Zlatograd, and Varna) (Fig..1). Most of them are taken directly from the producers and some are bought from the commercial network. All samples in their original glass packaging were stored at room temperature in the dark until analysis.

Fig. 1. Location of the analysed honeydew honey samples from different regions of Bulgaria

2.2. Methods

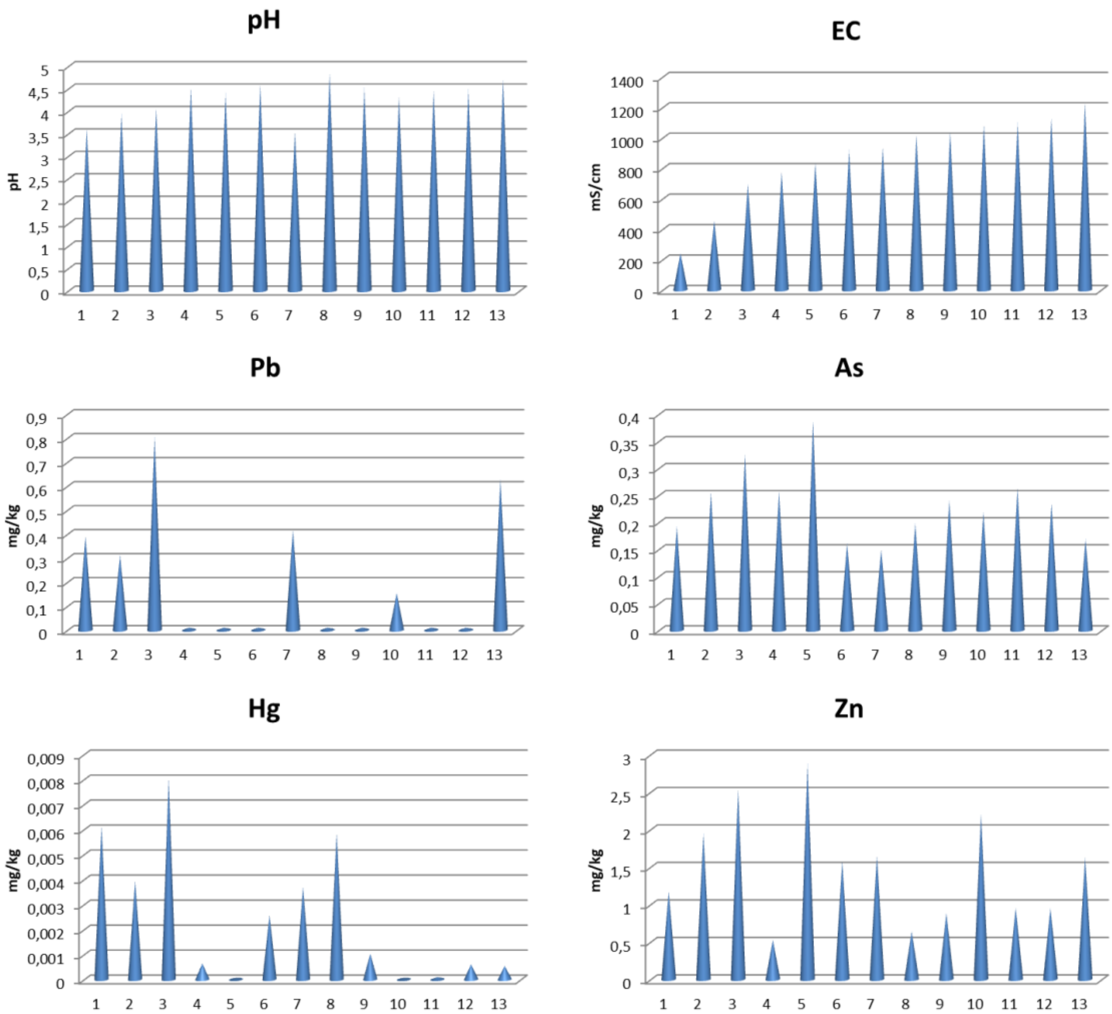
Two main physicochemical parameters of the honeydew honey - electrical conductivity and pH, as well as the content of heavy metals, macro- and trace elements were determined. pH and electrical conductivity were measured on 20% aqueous solutions. Homey samples were prepared for analysis by the microwave mineralization method. A 1 g sample was weighed to the nearest 0.001 g in a Teflon vessel and 10 mL HNO_3 (65%) was added. After gently shaking the containers with the samples and the acid, they are placed in a water bath at 45-50°C for 15 minutes, then removed and placed in a cold water bath for 15 minutes. After cooling, the containers are closed and placed in the microwave system (MARS 6, CEM Corporation, USA). The mineralization program includes 3 steps: (1) 20 minutes at 200 °C, pressure rise to 800 PSI, (2) 15 minutes holding time at 200 °C with 800 PSI pressure, and (3) 30 minutes for cooling. After cooling, the samples are quantitatively transferred to a 25 cm^3 volumetric flask. A blank is also included in each series of mineralized honey samples to assess the purity of the reagents and glassware used.

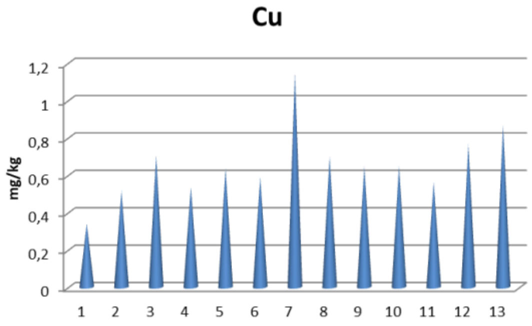
The elements were determined using an ICP-OES spectrometer (Prodigy model 7, Teledyne Leeman Labs, USA). The mercury content was determined directly (without digestion) by mercury analyzer MA 3000.

Analyses were performed in triplicate, and all data were expressed as mean values. Data processing was performed with the statistical program SPSS 26.

3. RESULTS

The results obtained for pH, electrical conductivity, heavy metal content, macro and trace elements are presented in Fig. 2. Tables 1 and 2 show the minimum, maximum and average values of the studied elements and the results for honeydew honey from other authors. The results of the study were also compared with the maximum levels of heavy metals specified in the recommendations of the World Health Organization (WHO), the Joint Codex Alimentarius Commission (FAO), and Commission Directive 2001/110/EC relating to honey defines honeydew honey of plant and animal origin, so that the limit value can only be set as a range: for Pb 0.1-0.5 mg/kg, while for Cd it is 0.05-0.3 mg/kg, according to Commission Regulation (EC) No 1881/2006 of 19 December 2006.

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mg/kg
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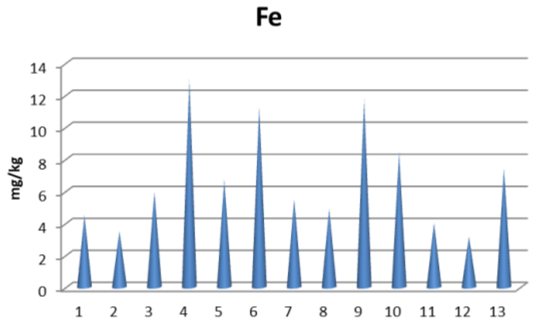
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mg/kg
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14
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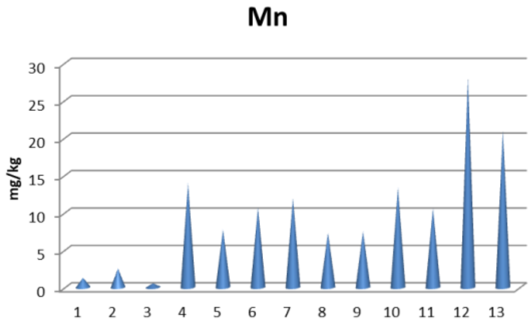
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mg/kg
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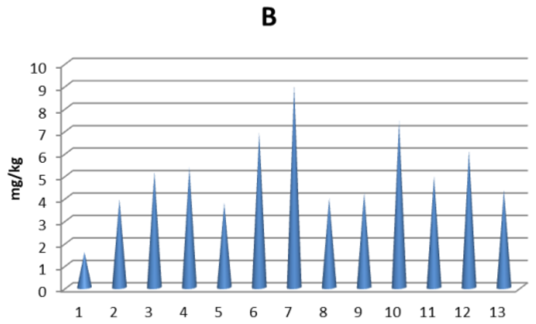
5
10
15
20
25
30
1 2 3 4 5 6 7 8 9 10 11 12 13


B
mg/kg
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1
2
3
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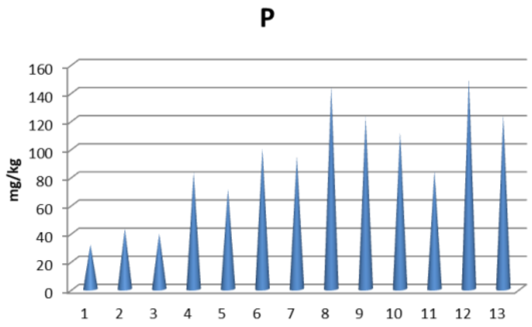
6
7
8
9
10
1 2 3 4 5 6 7 8 9 10 11 12 13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mg/kg
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60
80
100
120
140
160
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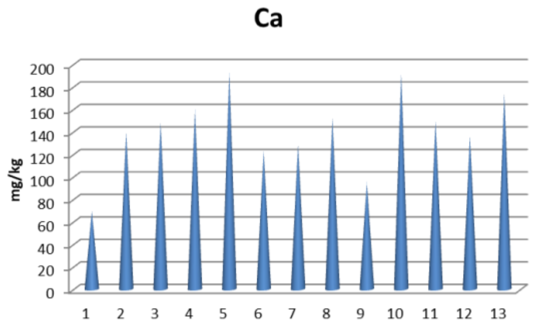
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160
180
200
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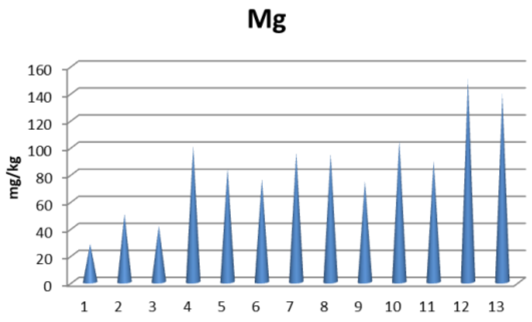
Mg
mg/kg
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40
60
80
100
120
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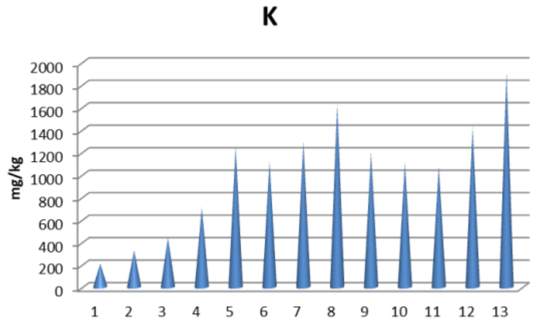
160
1 2 3 4 5 6 7 8 9 10 11 12 13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mg/kg
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200
400
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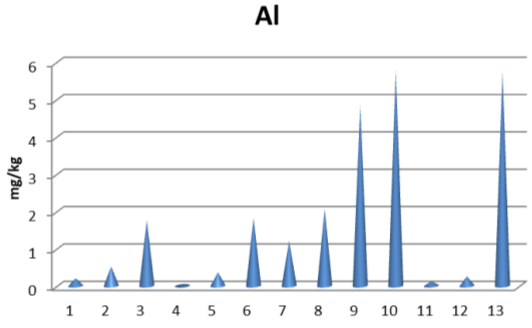
800
1000
1200
1400
1600
1800
2000
1 2 3 4 5 6 7 8 9 10 11 12 13


Al
mg/kg
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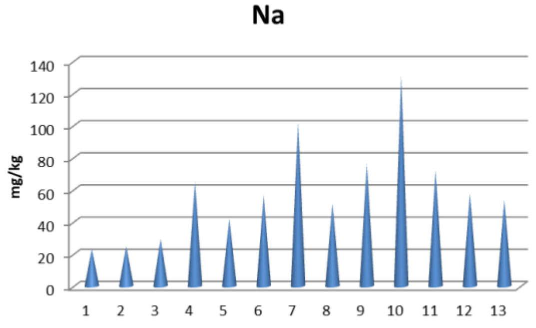
1
2
3
4
5
6
1 2 3 4 5 6 7 8 9 10 11 12 13


Na
mg/kg
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20
40
60
80
100
120
140
1 2 3 4 5 6 7 8 9 10 11 12 13

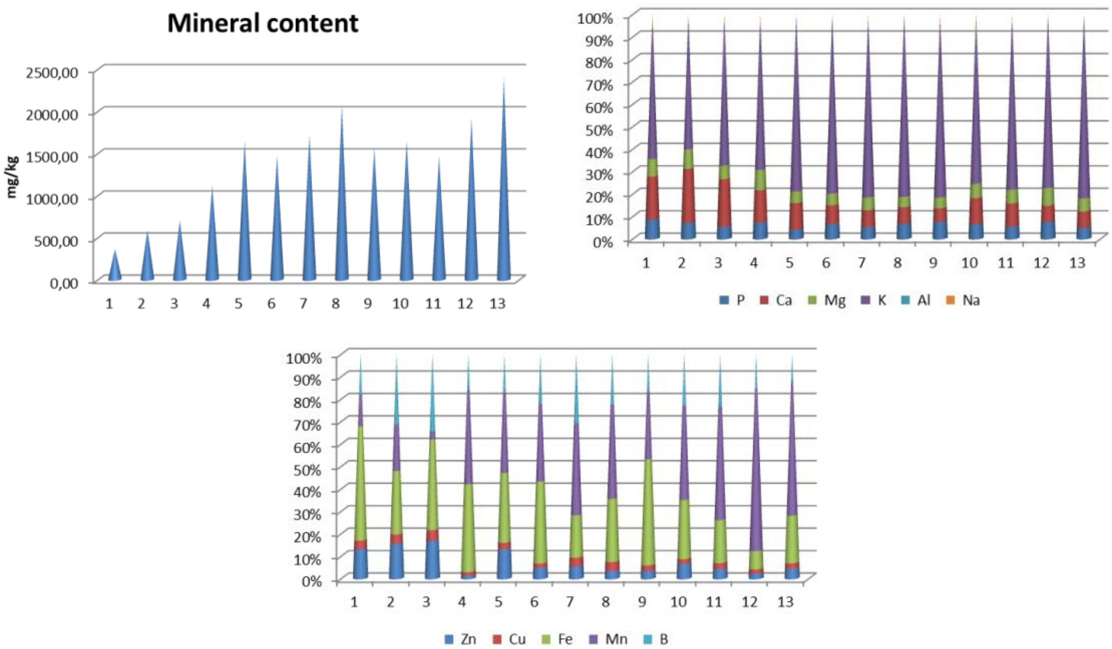

Fig. 2. pH, electrical conductivity, content of heavy metals, micro and macro elements in honeydew honey from different regions of Bulgaria

	K	Ca	Mg	Al	Na	P
This research	1074-1912 (1335)	94.7-192.5 (149.1)	74.5-150 (101)	0.12-5.77 (2.47)	41.8-130.8 (71.2)	71.1-149.8 (112.3)
Bulgaia (Ref.3)	1331	103	83	1.3	17	123
Bulgaria (Ref.57)	1080-2114 (1522)	98-178 (128)	59.3-182 (88.1)		12.7-28.8 (19.6)	88.4-210 (135)
Italy (Ref.17)	2569	397.9	64.2		62.4	
Italy (Ref.38)	3440	356	139		148	
Czech (Ref.28)		34.9	70.1	14.3		
Poland (Ref.30)	1744-3659	3.3-48.2	1.4-6.6	5.1-36.3	10.3-88.4	
Poland (Ref.13)	1744 -4222 (2641.9)	Nd-62.9 (21.4)	1.48-64.7 (26.1)	5.14-46.7 (25.7)	10.3-88.4 (28.4)	
Poland (Ref.52)	2088-2952 (2572)	11.2-115.8 (39.6)	33.5-65.1 (41.9)	13.0-33.6 (22.8)	8.3-16.4 (12.0)	444.7-899 (555.6)
Poland (Ref.43)	2957.4	69.9	65.74	41.47		
Romania (Ref.43)	1201.3	523.4	46.4	1.12		
Spain (Ref.20)	239.7	14.7	21.3		3.9	16.3

Table 1. Mineral composition of honeydew honey (mg/kg)

	Fe	Mn	Cu	Zn	B	Pb	As
This research	3.2-11.7 (7.00)	7.3-28.1 (13.2)	0.6-1.2 (0.7)	0.6-2.9 (1.5)	3.8-9.0 (5.6)	0.01-0.63 (0.14)	0.15-0.39 (0.22)
Bulgaria (Ref.3)	3	12	0.5	1.2		< 0.3	< 0.3
Bulgaria (Ref.57)	1.8-13.1 (4.0)	3.7-27.2 (12.3)	0.4-1.2 (0.7)	0.4-4.6 (1.4)		< 0.2	< 0.1
Italy (Ref.17)	8.6	0.9	1.9				
Italy (Ref.38)	10.1	1.7	4.4	1.87			0.01
Czech (Ref.28)		7	0.4	2.5	30.4	0.09	
Poland (Ref.30)	3.8-16.1		nd-1.8	nd-9.9	1.0-6.8		
Poland (Ref.13)		2.7-7.4 (4.2)	0-2.5 (1.2)	1.4-39.7 (5.6)	1.01-12.9 (4.1)	nd-0.89 (0.21)	
Poland (Ref.52)	1.8-7.1 (3.1)	5.2-9.9 (7.0)	1.2-1.9 (1.6)	0.66-9.2 (1.6)		0.02-0.12 (0.05)	0.74-0.79 (0.76)
Poland (Ref.43)	3.97	4.22	1.31	1.86		0.098	
Romania (Ref.43)	1.92	4.27	0.66	2.16		0.018	
Spain (Ref.20)	0.5		0.1	4.9			0.07

Table 2. Content of microelements (mg/kg) in honeydew honey

4. DISCUSSION

4.1. Physicochemical characteristics of honeydew honey

According to the Directive 2014/63/EU, in order for honey to be classified as mana, its electrical conductivity must be greater than 0.8 mS/cm, and for other types of honey it must be in the range of 0.2 to 0.8 mS/cm. In the honey analyzed in this study, the electrical conductivity ranges from 836 to 1229 mS/cm, which clearly indicates that the honey can be classified as a mana honey. In Samples 1, 2 and 3, the electrical conductivity was significantly lower (240-694 mS/cm), and in Sample 4 it approached the 0.8 mS/cm limit (773 mS/cm). The results obtained strongly suggest that samples 1-4 are not true honeydew honey but mixed honey (honeydew and nectar honey), despite being marketed as honeydew honey.

Similar results were obtained for Croatian (0.80-1.16 mS/cm) and Macedonian honeydew honey (0.80-1.30 mS/cm) (Primoras et al.. 2009) and Bulgarian honey (0.8-1.4 mS/cm) (Atanassova et al.,2016; Marinova et al., 2015, Yurukova et al., 2008), Polish honey (0.9 mS/cm (Madejczyk and Baralkiewicz, 2008), 1.028-1.414 mS/cm (Tomczyk et al., 2022) and 1.140 (Rybak-Chmielewska et al., 2013), Italian honey (1.10 mS/cm) (Seraglio et al., 2019). The present study confirms that conductivity can be used as a parameter to distinguish honeydew honey from mixed and nectar honey.

The pH in the honeydew honey ranged from 3.54 to 4.85 (average pH 4.43), In the samples of mixed nectar and honeydew honey the pH content was lower (pH 3.61-4.06), except for sample 4 (pH 4.53). Similar results were obtained by other authors (pH 4.3 (Atanassova et al., 2016), pH:4.66 (Tomczyk et al., 2022), pH 4.63 (Rybak-Chmielewska et al., 2013), pH 4.60 (Seraglio et al., 2019) and pH 4.48 (Tarapatsky et al., 2021).

4.2. Macroelements

Of the macronutrients, the K content was the highest in the samples tested. This is in agreement with other studies showing that K is the most abundant element in all types of honey (Escuredo et al., 2012). In honeydew honey the K content ranged from 1074 to 1912 mg/kg, while in mixed nectar and honeydew honey the content was significantly higher (209.9 - 706.8 mg/kg). Similar results were obtained for Bulgarian honey (1121 mg/kg, Atanassova et al, 2016), 1522 mg/kg, Yurukova et al., 2008),1600 mg/kg, Nikolova et al., 2013). Significantly higher values for K were found in Polish honey (2572, Tomczyk et al., 2022), 2642 mg/kg, Chudzinska and. Baralkiewicz, 2010 and 2957 mg/kg, Purcarea et al.,2017) and Italian honey (2569 mg/kg, Conti et al., 2007 and 3440 mg/kg, Pisani et al., 2008) (Table 1).

The Ca content ranged from 94.7 to 199.6 mg/kg, and no significant differences in Ca content were found between the honeydew honey and the mixed honey; only sample 1 had a lower Ca content (69.1 mg/kg). Similar results were obtained for Bulgarian honeydew honey (103 mg/kg, Atanassova et al. 2016, 123 mg/kg, Yurukova et al. 2008 and 127.7 mg/kg, Nikolova et al.,2013). Lower results were found in honeydew honey from Poland, Italy, Spain and the Czech Republic (Table 1). High Ca content was found in Italian honeydew honey (Pisani et al. 2008) which is probably due to the presence of carbonate rocks in the sampling area. Environmental and soil conditions are therefore factors that directly influence the mineral composition of honeydew honey.

The P content of the honeydew honey ranged from 71.1 to 149.8 mg/kg (mean 111.3 mg/kg), while the mixed honey had a lower P content (31.55 - 4 2.89 mg/kg), except for sample 4, which was closer in value to the honeydew honey (100.5 mg/kg). Similar results were found by Atanassova et al. (2012) (123 mg/kg) and Yurukova et al. (2008) (135 mg/kg) for Bulgarian honeydew honey. Significantly higher values for P were found in Polish honey (556.5 mg/kg)(Tomczyk et al,. 2022).

The Mg content ranged from 74.5 to 149.7 mg/kg (mean 101.0), while the mixed honey had a lower content (28.13-50.40 mg/kg), with the exception of sample 4, which was closer in value to honeydew honey (100.5 mg/kg). Similar results were found by Atanassova et al. (2012) (83 mg/kg),Yurukova et al. (2008) (88.1 mg/kg) and Nikolova et al., (2013) (109.1 mg/kg) for Bulgarian honeydew honey.

The Na content ranges from 41.8 to 130.8 mg/kg (average 71.2 mg/kg). Lower results were obtained for Bulgarian (Yurukova et al.,2008; Atanassova et al.,2012), and Polish and Spanish honeydew honey.

The Al content ranged from 0.12 to 5.77 mg/kg (mean 2.46 mg/kg). The Al content ranged from 5.19 to 20.2 mg/kg in Czech honeydew honey (Lachman et al., 2007), which is higher than in Bulgarian honeydew honey. High Al content in honeydew honey was found in Polish (22.8 -25.7 mg/kg) (Tomczyk et al,. 2022) and Romanian honey (41.57 mg/kg) and is probably due to contamination with this element.

The total mineral content of honeydew honey varies from 1471.6 to 2407.3 mg/kg. The content of mixed honey (samples 1-3) is lower (361.8 - 700 mg/kg), except for sample 4 -1114.6 mg/kg. According to literature data, the total mineral content of honey usually amounts to 0.1% to 0.2% of the composition of nectar honey, but may exceed 1% in honeydew honey (Tomczyk et al,. 2022).

4.3. Microelements

Of the trace elements, the highest Mn content is found in honeydew honey. The Mn content ranges from 7.3 to 28.1 mg/kg (average 13.2 mg/kg). Lower average values were obtained for Bulgarian honeydew honey by Atanassova et al. (2012) and Yurukova et al. (2008) (12-12.3 mg/kg) and Nikolova et al. (2013) (15.6 mg/kg). Lower values were found for Italian honey (0.9-1.70 mg/kg), Czech and Slovenian honey (7.0 mg/kg) (Golob et al.,2005).

The iron content ranged from 3.15 to 11.73 mg/kg (mean 7.00 mg/kg) Similar results were obtained for Italian honey, while the iron values in the studies for Bulgarian honeydew honey by Atanassova et al. (2012) and Yurukova et al. (2008) (3-4 mg/kg) and Nikolova et al. (2013) (5.0 mg/kg), Polish, Romanian and Spanish honey were lower (Table 2).

Zinc concentrations ranged from 0.64 to 2.9 mg/kg (mean 1.5 mg/kg). Similar results were obtained by Atanassova et al. (2012) (1.2 mg/kg) and Yurukova et al. (2008) (1.4 mg/kg). It has been found that the zinc content of honeydew honey from Siena County in Italy reaches up to 1.820 mg/kg (Pisani et al.,2008), in Italy - up to 3.205 mg/kg (Buldini et al., 2001), in Romania - 3.3 mg/kg Zn (Bratu & Beorgescu, 2005), and mm Slovenia - 3.61 mg/kg (Golob et al., 2005,). Madejczyk and Baralkiewicz (2008) associated the higher zinc value in honeydew honey with the presence of this element in plants and soil in Poland, where the samples were collected. On the other hand, storage of honey in galvanized containers may be a source of Zn contamination in honey (Gonzalez et al., 2000).

The Cu content ranged from 0.56 to 1.15 mg/kg (mean 0.73 mg/kg), with no significant differences between the mannoia and the mixed honey, except for sample 1 (0.32 mg/kg). Similar results were obtained for Bulgarian honeydew honey by Atanassova et al. (2012) (0.5 mg/kg) and Yurukova et al. (2008) (0.73 mg/kg) and Nikolova et al. (2013) (0.99 mg/kg). Higher values were obtained for Italian honeydew honey (1.9-4.4 mg/kg) (Conti et al., 2007; Pisani et al., 2008).

The B content ranged from 3.8 to 9.0 mg/kg (mean 5.6 mg/kg), with no significant differences between honeydew and mixed honey, except for sample 1 (1.58 mg/kg). Significantly higher results were obtained for Czech honeydew honey (30.4 mg/kg).

4.4. Heavy metals

The oobtained results show that the heavy metals Pb, As and Hg are detected in honeydew honey, while Cd is below the quantification limits of the method used. The Pb content ranged from 0.01 to 0.63 mg/kg (mean 0.14 mg/kg). According to Regulation (EC) No 1881/2006 of 19 December 2006, the maximum limit for lead should not exceed the range 0,1-0,5 mg/kg. Higher values than the maximum limit for lead were found only in 1 sample of honeydew honey and 1 sample of mixed honey, probably due to contamination of soils and plants. Due to the stickiness of the honeydew released on the leaves and other plant parts, airborne toxic metals adhere to the surface of the plant leaves and accumulate in the honeydew and pass into the resulting honeydew honey.

Studies by Atanassova et al. (2012) and Marinova et al. (2015) show that the Pb content of Bulgarian honeydew honey can reach up to 0.25 mg/kg and 0.31 mg/kg, respectively. Antonescu and Mateescu (2001) reported that Romanian honey contains Pb in the range of 0.1-200 μg/kg. The measured Pb concentration in this study was higher than that found in honey samples from Croatia (65.2 μg/kg, Bilandzis et al., 2011), Turkey (17.6-32.1 μg/kg, Tuzen & Soylak, 2005), Poland (0.048 mg/kg, (Przybylowski and Wilczynska, 2001), Romania (0.07 μg/kg, Bratu & Beorgescu, 2005). Higher levels of lead were found in honey from Luthuana (1,65 mgkg, Sereviciene et al., 2022), Italy (1,39 mg/kg, Perna et al., 2004; 2370 μg/kg, D'Ambrosio & Marchesini,1982), Romania (0.76-3.41 mg/kg, Bartha et al., 2020) and Slovenia (5.94 mg/kg, Golob et al., 2005).

The As content ranges from 0.15 mg/kg to 0.39 mg/kg, which is within the maximum level (10-500 μg/kg) according to the provisions of the Codex Alimentarius (FAO/WHO, 1993) and the Commission Regulation (No 1881/2006). Studies by Atanassova et al. (2016) show that the As content of Bulgarian honeydew honey can reach up to 0,32 mg/kg, respectively. Lower values for As have been found in honey from Siena County, Italy (6.59 and <0.5 μg/kg) (Pisani et al., 2008; Caroli et al.,1999), Croatia (19.7 μg/kg, Bilandzic et al., 2011), while in Slovenian honey the values range from 1.24 to 1.49 mg/kg (Golob et al., 2005). A probable cause of As contamination of honey is water, soil and air pollution, as well as the use of As-based pesticides. As is absorbed by plants and, therefore, may be present in honey.

Hg is one of the most dangerous heavy metals that can threaten human health at very low concentrations (Nabi, 2014). The maximum concentration of Hg in honey specified in European Commission Regulation 2018/73 of 16 January 2018 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council is 0.01 mg/kg.

The Hg content in honey was very low (0.03 - 8.06 μg/kg, average 1.61 μg/kg) and did not exceed the permissible limits in any of the samples analysed. Similar results were obtained for honey from Poland (0.01- 1.71 μg/kg, average 0.43 μg/kg, Fischer et al., 2022), Italy (0.04-1.46 μg/kg, average 0.19

µg/kg, Quinto et al., 2016), Czech Republic (0.67-2.93 µg/kg, Celechovska and Vorlová, 2001), Slovakia (0.079 - 0.081 µg/kg, Toth et al., 2016). Toporcák et al. (1992) in a study of honey samples from Slovakia found that. the Hg content for honey obtained from contaminated areas was in the range of 50-212 µg/kg, while from non-contaminated areas it was several times lower (1-3 µg/kg).

Comparison of the results obtained with the studies of other authors shows differences in Hg content in honey due to the geographical origin of the honey. Hg content in honey from Croatia is 2.72 µg/kg (Bilandzic et al., 2011), in Greece - 50 µg/kg (Maragou et al., 2016), in Serbia - 73 and 519 µg/kg, (Jovetic et al., 2018), and in Iran - 3.03 mg/kg, (Akbari et al., 2012). The elevated level of Hg is attributed to anthropogenic sources of air pollution, especially fossil fuel combustion.

The botanical and geographical origin of honeydew honey has a significant influence on the chemical composition, which is confirmed by the data presented in Tables 1 and 2. Despite the observed differences in the composition of honeydew honey, there are common trends - K is the most abundant element, in an amount > 70% of the elements contained in honeydew honey, Ca, Mg, Na and P are in concentrations higher than 1%, the content of Cu, Mn, Fe, Zn and B c is lower than 1%, and elements such as As, Cd, Pb are less than 0.1% (Fig. 2).

Attempts have been made to differentiate honeydew from nectar honey of different botanical and geographical origins on the basis of elemental composition. Chudzinska and Baralkiewicz (2010) suggested K and Mn as the most appropriate descriptors to distinguish between honey types, Quirantes-Pine et al. (2024) - Mg, Al and Mn, Oroian et al. (2015) - K, Mg and Ca. Vasic et al. - Mg, Mn and Ba, Bilandzic et al. (2017) - Mg, Mn and Ba as descriptors. Obtained results from this research show that K, phosphorus, and manganese can be used to distinguish mannous from mixed honey.

Further studies on honeydew honey and its physicochemical properties and composition are needed to ensure the quality, safety and authenticity of this product.

4.5. Cluster analysis

Hierarchical cluster analysis was performed. A dendrogram was constructed to graphically represent the formed clusters. The dotted horizontal line on the dendrogram shows the rescaled distance at which the clusters were formed.

The results of the cluster analysis are presented in Figure 3 by means of a dendrogram. The first cluster consists of two subclusters that are more homogeneous. The first subcluster brings together samples 6, 9, 4, 11, 8 and 10, similar in pH, Pb, Hg, Cu and Na and with the least Euclidean distance between them. The second subcluster combines variants 12 and 13, which are similar in EC, As, Hg, Cu, P and Mg.

The second main cluster includes samples 2, 3 and 1, which are of mixed (mannic and nectaric) Cu. They are most similar in pH, Hg, P and Na.

The outliers are samples 1 and 4, which have a large difference in EC, Pb, Fe, Mn, Ca, Mg, K and Na.

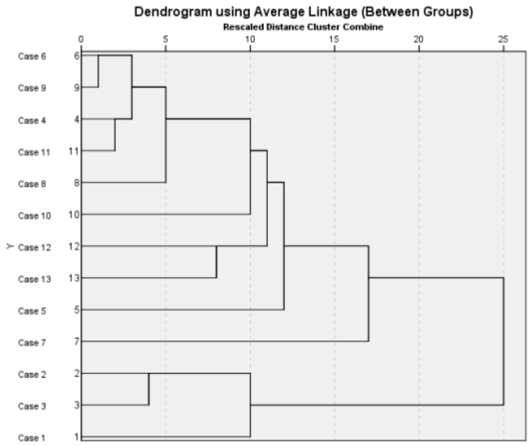

Fig. 3. Dendrogram of the hierarchical cluster analysis from the honeydew honey

5. CONCLUSIONS

1. The authenticity of the investigated honeydew honey samples from different regions of Bulgaria was proved by determining their electrical conductivity. Most of the honeydew honey on the market is genuine, with electrical conductivity values ranging from 0.8 to 1.4 mS/cm. Four of the samples tested were adulterated by mixing with nectar honey.
2. The honeydew honey could be distinguished from the mixed honey by its K, phosphorus and manganese content.
3. Variability was found in the elemental composition of the honeydew honeys studied, the differences being due to the geographical origin of the honey. The probable reason is also the different vegetation in the forests where the honeydew honey was extracted, as well as the different amount of these elements in the soil.
4. The order of accumulation of macroelements is as follows: K>Ca>Mg>P>Na>Al, of trace elements: Mn> Fe>B>Zn>Cu.
5. The content of toxic metals Pb, Cd, As and Hg in honeydew honey is low and does not exceed the maximum permissible values (except for two samples for Pb), indicating minimal environmental pollution in the areas where honeydew honey was produced.
6. Bulgarian honeydew honey is low in toxic metals and poses no risk to consumers. Bulgarian honeydew honey meets the requirements for a safe product for human consumption.

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