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Significance of the apiary location for the content of copper and zinc in the bee pollen

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Abstract

Bee pollen is one of the most valuable direct products of honey bees. Bees as well as humans use pollen in their nutrition as a high-quality supplement. Therefore, information about the mineral content of pollen is valuable. The aim of this study was to determine the amounts of copper (Cu) and zinc (Zn) in the bee pollen collected from the three locations (Kozarska Dubica, Petrovo, and Srbac). Quantification of copper and zinc was done by atomic absorption spectrophotometry after acid digestion (HNO_3+$HClO_4$) of pollen. The highest contents of both elements were found in the bee pollen from Kozarska Dubica (8.41 mg Cu/kg, 29.52 mg Zn/kg). The lowest Cu content was found in the samples from Petrovo (6.19 mg/kg), while the lowest Zn content was determined in the pollen from Srbac (22.06 mg/kg). Additionally, there were statistically significant differences in the content of both elements between pollen originating from different locations. As the location of the apiary was mainly determined by its proximity to potential sources of metal contamination, botanical origin of pollen and technology of pollen collection, it is important to investigate their impact in more detail on the elemental content of pollen in the future.

Key words: bee products, pollen, copper, zinc, honey bees

Introduction

Bee pollen is one of the apicultural products collected from flowering plants by honey bees (*Apis mellifera*). It is made of natural flower pollen mixed with small quantities of nectar and bee secretions, which are finally created in the form of granules (Campos et al. 2008). Beekeepers place pollen traps at the hives entrance to collect pollen for commercial use (Aylanc et al., 2021).

Pollen present in the beehives is the source of nutrients necessary for the growth of honey bees during all developmental stages, especially in the stage of larvae and their youth (Avni et al., 2014). Moreover, it has important nutritional and therapeutic characteristics for humans. Pollen has been consumed daily all around the world since ancient times and has gained increased attention in the 21st century owing to a tendency for natural diet supplementation (Yang et al. 2012). According to Pascoal et al. (2014), pollen is the "only perfectly complete food" due to the presence of all essential amino acids needed by the human organism.

Pollen contains a variety of nutrients and compounds, including proteins (10-40%), carbohydrates (13-55%), lipids (1-13%), dietary fibre (14-20%), minerals, vitamins, and several other bioactive compounds (Salzar-Gonzales & Diaz-Moreno, 2016, Aylanc et al., 2021). Mineral substances present in the pollen include macroelements: potassium (K), phosphorus (P), calcium (Ca), sodium (Na), magnesium (Mg), and microelements such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and others (Salzar-Gonzales & Diaz-Moreno, 2016). The composition of bee pollen depends on its botanical origin, geographic, and climatic characteristics (Pascoal et al., 2014, Aylanc et al., 2021). Additionally, beekeeper activities, processes, or storage treatments in commercial production are important factors for the final content and quality of the bee pollen (Yang et al. 2012).

The quantification of microelements such as Zn and Cu in pollen is valuable for the assessment of pollen quality and safety. It is well known that zinc and copper are essential elements, necessary in small doses, while their presence in elevated concentrations in food and diet supplements is not desirable. According to Aldgini et al. (2019), the maximum accepted limits for the contents of these elements in the bee pollen used in human nutrition are 60 mg Zn/kg and 20 mg Cu/kg.

Considering all of the above and the lack of data about the mineral content of pollen originating from our country, the aim of our study was to determine the content of copper (Cu) and zinc (Zn) in the bee pollen collected in the northern part of Bosnia and Herzegovina at three locations: Kozarska Dubica, Petrovo, and Srbac.

Material and Methods

For the purpose of this research, pollen samples from three locations in the north of the Republic of Srpska were obtained. In this area plains dominate, mainly formed in the river valleys of Sava, Vrbas, Bosna, Ukrina, and other smaller water streams, with hills and low to middle-high mountains (Kozara, Motajica, Ozren). The moderate continental climate prevails here, and industrial and agricultural production are well developed. In addition, the north of the Republic of Srpska is characterized by intensive agricultural production, including the use of pesticides, which makes it difficult to produce healthy and safe bee products. On the other hand, the same area abounds in generous and long-lasting bee grazing during the whole year (particularly early spring, fruit, main, and meadow grazing). Samples of pollen were collected at the three apiaries located in the municipalties of Kozarska Dubica, Petrovo, and Srbac during the spring (from April to June) of 2022.

The apiary of Kozarska Dubica is located in the village of Milića Gaj, at the meadow, at an altitude of 300 m, about 20 km from the town of Kozarska Dubica, beside the main road Kozarska Dubica - Banja Luka. This apiary has a capacity of 100 bee colonies and is of the migratory type. Honey, pollen, propolis, queens, and swarms of bees are produced here. The dominant bee grazings are fruit grazing, acacia, linden, and meadow plants. The second studied apiary belongs to the municipality of Srbac and is located in the village of Crveni Jazak, 10 km from Srbac. The apiary is located at an altitude of 130 m and its capacity is 60 production bee colonies in LR hives. The most abundant grazing for the bees in this apiary are raspberry, acacia, and *Amorpha fruticosa*. The third appiary is located on the territory of the municipality Petrovo between villages Boljanić and Karanovac, at a pasture at an the altitude of 400 m, 18 km from Petrovo Selo, where the administrative center of this municipality is located, and 26 km from the town of Doboj, which is the biggest town, as well as industial and road centre of this region. The apiary has a capacity of 100 bee colonies. It is far from the main roads. The nearest frequent road is the local road from Doboj to Petrovo, situated about 10 km from the apiary. This location is dominated by meadows and generous pine grazing.

Sampling of the pollen was done from three different beehives randomly selected in the apiary at each of the three sampling locations. The samples were collected using external pollen traps placed at the entrance of LR hives. After collection, samples of pollen were transferred to a sterile plastic container and properly labelled. An average of 100 g of bee pollen samples were taken from each of selected beehives. The samples were kept in plastic containers at -40C prior to analysis.

Pollen samples were digested according to the procedure described by Altunatmaz et al. (2017), with some minor modifications. 1.0 g of the dried pollen samples was weighed on an analytical balance and transferred into the clean digestive tubes. After adding 10 mL of concentrated nitric acid (HNO_3), the tubes were covered with glass funnels and then left to stand overnight. In the morning, the contents of the tubes were slowly heated on the digestive block at a temperature of around 800C for 2 hours. After that, the contents of the tubes were cooled to room temperature, 5 mL of concentrated perchloric acid was added, and the boiling continued until the content in them became clear. The digestion solution was quantitatively transferred to a volumetric flask of 50 mL and filled up with deionized water. Finally, the contents of copper (Cu) and zinc (Zn) were quantified by the method of flame atomic absorption spectrophotometry. The measurements of both elements were done in the air-acetylene flame at wavelengths of 324.75 nm for Cu and 213.70 nm for Zn. Additional deuterium background correction of the signal was included during the Zn measurements. All analytical procedures were triplicated, and all chemicals used were analytically pure (p.a. grade). To avoid any type of contamination, all laboratory glassware was previously immersed in 10% HNO3 overnight and washed several times with deionized water. Statistical and graphical analysis of the results obtained after chemical analyses was conducted with the assistance of the statistical software package SPSS 22 (IBM 2013).

Results and Discussion

The measured contents of copper and zinc in the analyzed samples of bee pollen are shown in Table 1. The highest content of both elements was found in the pollen from Kozarska Dubica: 8.41 mg Cu/kg and 29.52 mg Zn/kg. The lowest Cu content was determined in the samples from Petrovo (6.19 mg/kg), while the lowest Zn content was determined in the pollen from Srbac (22.06 mg/kg). Furthermore, the determined contents of both elements in all analyzed samples were lower than their maximum accepted limits (60 mg Zn/kg and 20 mg Cu/kg) in bee pollen (Aldgini et al. 2019). Therefore, we can conclude that the analyzed pollen samples are safe for human consumption considering the content of copper and zinc.

The analysis of Cu contents in the pollen originating from different studied locations (Tab. 1, Fig. 1) indicated statistically highly significant differences ($p<0.001$). Namely, the Kozarska Dubica pollen had a significantly ($p<0.001$) higher content of Cu in comparison to Petrovo and Srbac, between which there was no significant difference (p= 0.584). Besides, the determined contents of pollen in our study (6.19-8.41 mg Cu/kg) were on average 3-4 times higher than those found in the pollen from Macedonia (1.5-2 mg Cu/kg, Bogdanova-Popov et al., 2022). However, the range of Cu content in the pollen from Serbia (4.4-10.7 mg/kg, Kostić et al., 2015), Greece (4-22 mg/kg, Liolios et al., 2019), Romania (5.5-12.1 mg/kg, Harmanesku, 2007), Turkey (3.73-14.99 mg/kg, Altunatmaz et al., 2017) Jordan (0.03-11.4 mg/kg, Aldgini et al., 2019), and Saudi Arabia (5.8-6.8 mg/kg, Taha & Al-Kahtani, 2020) are not significantly different from those found in our work. On the other hand, the pollen in Brazil (7.7-19.7 mg/kg, De Melo et al., 2018) and China (average value: 17.35 mg/kg, Yang, 2013) contained up to 2 times more Cu.

Tab. 1. Contents (mg/kg) of copper (Cu) and zinc (Zn) measured in the pollen samples at the studied locations

Location	Cu (mg/kg)			Zn (mg/kg)		
	$\overline{X}$	±	SD	$\overline{X}$	±	SD
K. Dubica	8,41	±	0,09	29,52	±	1,45
Petrovo	6,19	±	0,20	24,43	±	0,73
Srbac	6,28	±	0,55	22,06	±	3,29
$F_{location}$, $p_{location}$	119.58**, p<0.001			29.21**, p<0.001		
$HSD_{location}$	0.41			2.49		

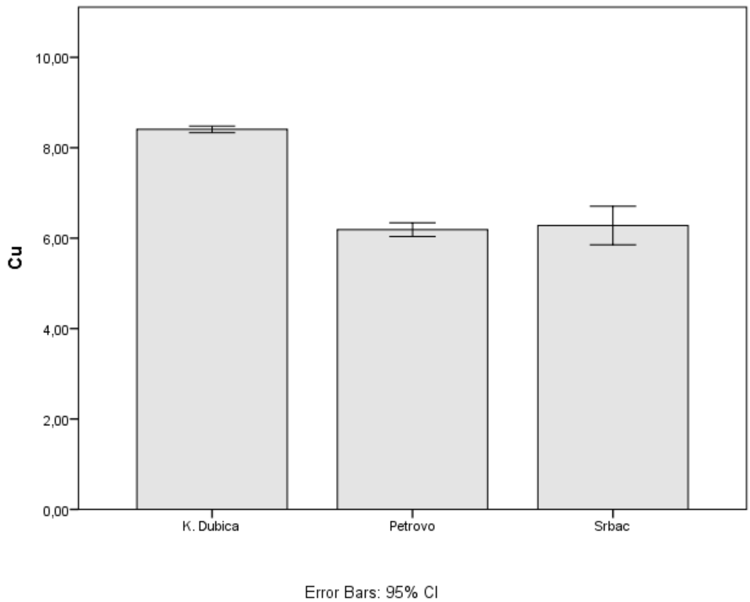

Fig. 1. Contents of Cu (/mg/kg) in the pollen samples according to the studied locations

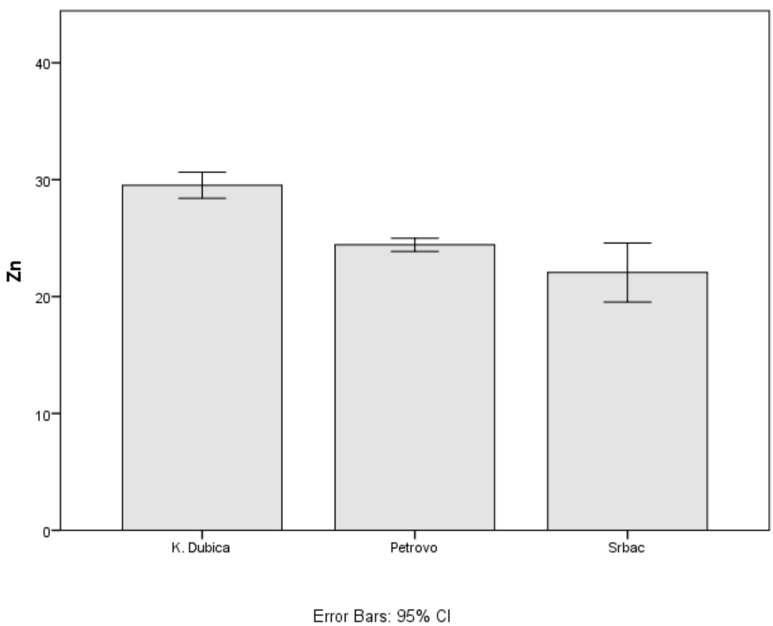

Fig. 2. Contents of Zn (mg/kg) in the pollen samples according to the studied locations

By analyzing Zn contents in the pollen from the studied locations (Tab. 1, Fig. 2), a similar situation was observed as for Cu, i.e., there were statistically highly significant differences ($p<0.001$) between the locations. The Kozarska Dubica pollen had significantly ($p<0.001$) higher contents of Zn in comparison to Petrovo and Srbac between which there was no significant difference ($p=0.065$). Besides, the determined range of zinc content was significantly higher in Serbia (28.8-75.9 mg Zn/kg, Kostić et al., 2015), Greece (24-90 mg Zn/kg, Liolios et al., 2019), Romania (33.9-54.3 mg/kg, Harmanesku, 2007), Jordan (25.24-77.0 mg/kg, Aldgini et al., 2019), Brazil (30-101 mg/kg, De Melo et al., 2018), Saudi Arabia (32.8-44.1 mg/kg, Taha & Al-Kahtani, 2020), and China (average value 45.10 mg/g, Yang, 2013) compared with our results. The average Zn content in the pollen originating from these countries was 2-3 times higher than in our study. However, a similar concentration range of Zn was observed in the Macedonian pollen (22-37 mg/kg, Bogdanova-Popov et al., 2022).

Conclusion

All analyzed pollen samples are safe for human consumption considering the determined content of copper and zinc, which were lower than the allowed maximum values (60 mg Zn/kg and 20 mg Cu/kg) in bee pollen. Moreover, there were statistically significant differences in the content of both elements between the pollen originating from different locations. The highest contents of both elements were found in the Kozarska Dubica pollen (8.41 mg Cu/kg and 29.52 mg Zn/kg).

Given that all three studied apiaries are not relatively far from each other and that they are located in the same agroecological area with similar or identical climatic conditions, it is obvious that the difference in the contents of Cu and Zn in the examined pollen samples is not mainly caused by climatic influences. Additionally, all three locations are in rural areas, far enough from potential industrial pollutants and very frequent traffic. The difference in the content of both elements in the pollen from the studied apiaries is therefore most likely not caused by any of the metal sources in that group.

On the other hand, as we have already mentioned, we have different bee grazing in the examined locations. Hence, it is necessary to expand the research in terms of specifying the bee grazing in each locality and its influence on the content of Cu and Zn in the pollen. Further research about the dominant bee grazing during the different seasons as well as specific technological operations, equipment, and tools used in all three apiaries during the beekeeping process will provide more precise data. Owing to these data, it will be possible to obtain clearer conclusions about the reasons for the difference in the content of Cu and

Zn in the pollen. It will be interesting to expand the research to include more apiaries from the same region as well as other bee products.

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Значај локације пчелињака за садржај бакра и цинка у полену

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Сажетак

Полен је један од најзначајнијих пчелињих производа. Заступљен је у исхрани пчела и људи као високо квалитетни суплемент. Стога су подаци о минералном саставу полена драгоцјени. Циљ овог истраживања је да се установи садржај бакра (Cu) и цинка (Zn) у узорцима полена на три локације (Козарска Дубица, Петрово и Србац). Садржај бакра и цинка у полену одређен је методом атомске апсорпционе спектрофотометрије након киселинске дигестије (HNO_3+$HClO_4$). Највиши садржај оба елемента установљен је у узорцима полена из Козарске Дубице (8,41 mg Cu/kg, 29,52 mg Zn/kg). Најнижи садржаја бакра утврђен је у полену узоркованом у Петрову (6,19 mg Cu/kg), док је најмање цинка садржио полен из Србца (22,06 mg Zn /kg). Поред тога, установљен је статистички значајна разлика у садржају оба елемента у полену са различитих локација. Како је локација пчелињака углавном одређена са удаљеношћу од потенцијалних извора контаминације, ботаничким поријеклом полена и технолошким процесом скупљања и обраде истог, важно је наставити са истраживањем и утврдити детаљније њихов утицај на елементарни састав полена.

Кључне ријечи: пчелињи производи, полен, цинк, бакар, медоносне пчеле

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