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Araştırma Makalesi

Farklı Botanik Kaynaklı Arı Polenlerinin Element Analizi ile Değerlendirilmesi

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Öz

Arı poleni, mineraller de dahil olmak üzere pek çok besin maddesi içermektedir. Elementler arı poleninde yer alan minör bileşenlerdir ve polenin kalitesinin belirlenmesinde kullanılan önemli parametrelerden biridir. Bir gıda takviyesi olarak, arı poleninin kalitesini ve güvenilirliğini belirlemek için, temel makro ve mikro elementlerin ve zararlı ağır metallerin konsantrasyonlarının saptanması gerekmektedir. Bu çalışmanın amacı, farklı botanik kaynaklardan elde edilen arı polenlerinin element içeriklerinin belirlenmesidir. Bu çalışmada, ilk olarak arı poleni örneklerinin botanik kökenlerini saptamak için melissopalinolojik analiz yapılmıştır. Daha sonra örneklerde yer alan 13 elementin konsantrasyonu, İndüktif Eşleşmiş Plazma-Kütle Spektrometrisi (ICP-MS) ile ölçülmüştür. Örneklerde en yüksek konsantrasyonlu element potasyum (K) 4840-9623, ardından sırasıyla magnezyum (Mg) 128.1-808.1, kalsiyum (Ca) 261.3-424.7, sodyum (Na) 176.3-356.7, demir (Fe) 67.7-120.3, Çinko (Zn) 6-57.6, Manganez (Mn) 15.1-33.9, Bakır (Cu) 0.8-9.7, Nikel (Ni) 1.1-5.1, Krom (Cr) 2.0-3.3, Selenyum (Se) 0.16-1.0 ve Kobalt (Co) 0-0.24 mg kg^{-1} olarak bulunmuştur. Hedef tehlike katsayısı (THQ) ve tehlike indeksi (HI) metodolojilerinin kullanılmasıyla, arı poleninde bulunan belirli elementlerin tüketiminin insan sağlığına etkisi değerlendirilmiştir. Araştırmamıza göre, arı poleninin düzenli tüketiminin (THQ <1; HI< 1) yetişkinler için güvenli olduğu belirlenmiştir. Ancak çalışılan arı polenlerinin, çocukların düzenli tüketimine uygun olmadığı saptanmıştır.

Anahtar kelimeler: *Melissopalinoloji, arı poleni, ICP-MS, element, ağır metal, sağlık risk değerlendirmesi*

Evaluation of Bee Pollen from Different Botanical Origins by Elemental Analysis

Abstract

Bee pollen contains many nutrients, including minerals. Elements are minor substances of bee pollen, they play a crucial role in identifying its quality. As a food supplement, concentrations of essential macro and microelements, and harmful trace elements have to be verified to determine its quality. This study aimed to identify the element contents of edible bee pollen from different botanic sources to determine its safety. Firstly, we applied melissopalynological analysis to find the botanical origins of bee pollen samples. Then, it was determined the concentrations of 13 elements in the samples. Element concentration was measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Potassium (K) occurred at the highest concentrations in samples 4840-9623, followed by magnesium (Mg) 128.1-808.1, calcium (Ca) 261.3-424.7, sodium (Na) 176.3-356.7, iron (Fe) 67.7-120.3, Zinc (Zn) 6-57.6, Manganese (Mn) 15.1-33.9, Copper (Cu) 0.8-9.7, Nickel (Ni) 1.1-5.1, Chromium (Cr) 2.0-3.3, Selenium (Se) 0.16-1.0 and Cobalt (Co) 0-0.24 as mg kg^{-1} respectively. Through the use of the target hazard quotient (THQ) and hazard index (HI) methodologies, the human health risk posed by the consumption of specific elements included in bee pollen was assessed. According to our research, bee pollen was safe for adults to consume (THQ <1; HI< 1), even if they consume it regularly, but studied bee pollen was not suitable for consumption by children regularly.

Key words: *Melissopalynology, bee pollen, ICP-MS, element, heavy metal, health risk assessment*

Introduction

Angiosperm plant pollen is agglutinated by nectar and oral enzymes (e.g. amylase, catalase) secreted by honey bees (*Apis mellifera*), stored in pollen basket on their hind legs and pollen loads, which is called bee pollen in the pellet structure, is formed (Stanciu et al., 2012). Various vitamins, enzymes, coenzymes, steroids, mineral substances, and flavonoids form the structure of bee pollen (Pascoal et al., 2014). Due to its structure, it is the most important protein, lipid, vitamin, and mineral source for young bees and humans. Its nutritional and chemical composition varies according to the geographical and botanic origin and other factors such as soil type, processing, climatic conditions, and beekeeping activities (De-Melo et al., 2016).

Bee pollen is a significant bee product gaining popularity as a functional food. This bee product is known for its high content of compounds and health-promoting effects on human physical and mental health, which make it the latest trend in dietary supplements (Asmae et al., 2021). It is used in apitherapy with antibacterial, antioxidant, anti fungicidal, anti-inflammatory, and hepatoprotective properties. Because of its nutritional value, bee pollen is being evaluated as a nutraceutical and an inartificial product (Denisow and Denisow-Pietrzyk, 2016).

Minerals are essential for human metabolism. Since the body cannot produce minerals, the diet should be included to ensure an adequate daily intake (Stanciu et al., 2012). In addition to macronutrients like K, Na, Ca, and Mg, bee pollen also contains micronutrients like Cu, Zn, Mn, and Se (Aldgini et al., 2019). Ca and Mg help the development and protection of bone tissue and regulate the osmotic pressure of intercellular and cellular fluids as well as blood. Fe, Mn, Co, Cu, and Zn elements play a crucial role in the body's growth, development, blood formation, and reproduction (Thakur and Nanda, 2020). Cr and Co are also fundamental for the metabolism of lipids, carbohydrates, and the production of proteins. Moreover, Co constitutes an integral part of vitamin B12 while Mn is a cofactor of such classes of enzymes as lyases, transferases, isomerases, etc. (Foulquier and Legrand, 2020). Ni takes part in hormonal and lipid metabolism, some enzyme activations, and stabilization of DNA and RNA. Se is necessary for the suitable course of vital mechanisms in the human body. Therefore, deficiency or excess of these essential elements can cause various metabolic disorders, acute growth defects, and even fatal diseases (Thakur and Nanda, 2020).

Due to urbanization, mining, industrialization, and agricultural activities, the concentration of chemicals increases and pollutes the environment (Tutun et al., 2022). Toxic elements are highly harmful to living organisms and are important environmental pollutants (Jaishankar et al., 2014). Bee products can provide data on elemental emissions from various sources and information about potential risks to human health (Conti et al., 2022). Therefore, it means that all bee products must be strictly controlled in terms of element contents.

The quality of bee pollen has been investigated over the last few years. There are studies about the identification of macro, micro, and hazardous elements in honey bee products, generally using these elements as bioindicators and evaluating the quality of edible bee pollen (Temizer et al., 2018; Aldgini et al., 2019; Mayda et al., 2020).

Even though there has been numerous research on the elements found in bee pollen, studies involving risk assessment are limited. This study aimed to identify the element contents of edible bee pollens from different botanic sources to determine its safety. Firstly, we applied melissopalynological analysis to find the botanical sources of samples. Then, it was determined the concentrations of 13 elements. To determine the potential risk of bee pollen to consumers in terms of public health, this study also sought to examine health risk assessment.

Material and Methods

Samples

Bee pollen samples were taken from different markets where local products are sold in (P1) Adana, (P2, P3, P4) Muğla, and (P5) Antalya.

Microscopic Analysis

For microscopic examination 5 gr bee pollen samples were dissolved in 50 mL 96% ethanol. Then, it was vortexed for one minute. 20 µL solution was taken from this mixture and placed on the microscopic slide. Nikon Eclipse Ci brand microscope was used for identification. Pollen grains were determined at 400X and 1000X magnification. A minimum of 500 pollen grains were counted on each slide to the evaluation of the relative abundance of pollen types. The sample was categorized in terms of pollen frequency using the following classes: predominant pollen (PP) (≥ 45%), secondary pollen (SP) (15–45%), important pollen (IP) (3–15%), and minor pollen (MP) (< 3%) (Barth et al. 2010).

Element analysis

Element concentrations of bee pollen samples were determined with inductively coupled plasma mass spectrometry ICP-MS (Bruker 820-MS) according to the method by Temizer et al., (2018) with some modification. For microwave digestion, 1 g of bee pollen was added to a teflon container along with 10 mL of concentrated nitric acid (65%, Merck, Germany). This solution was microwaved at 200 °C for 15 minutes to digest it, with the maximum temperature increased to within 15 minutes. The digested sample solutions were quantitatively diluted with deionized water to 50 mL after cooling. This solution was diluted at 1:99 with suprapur nitric acid/deionized water in 10 mL of the original volume.

Table 1. The botanical origin of bee pollen samples *

	Samples				
Family/Taxa	P 1[a] %	P 2 %	P 3%	P4%	P5%
Anacardiaceae					
Pistacia			12.7(IP)	13.3(IP)	
Apiaceae					
Ferula	17.69(SP)				
Pimpinella			5.2(IP)	3.4(IP)	4.3(IP)
Asteraceae					
Artemisia			2.6(MP)		
Matricaria				5.4(IP)	
Taraxacum			3.1(IP)	1.2(MP)	
Centaurea	3.08 (IP)	3.92(IP)			4.2(IP)
Xeranthemum		5.88(IP)			3.4(IP)
Brassicaceae					
Brassica					3.6 (IP)
Sisymbrium	9.23(MP)				
Cistaceae					
Cistus			35(SP)	27.8(SP)	25.4(SP)
Cornaceae					
Cornus			19.5(SP)	0.9(MP)	14.2(IP)
Fabaceae					
Astragalus	30.77(SP)			3.8(IP)	
Lotus		4.9(IP)			
Medicago	7.69(IP)			4.8(IP)	
Melilotus		74.51(DP)			
Trifolium	31.54(SP)		1.98(MP)	20.3(SP)	28.6(SP)
Moraceae					
Morus				1.2(MP)	
Oleaceae					
Olea		10.78(IP)	4.6(IP)	5.8(IP)	
Papaveraceae					
Papaver					10.4(IP)
Polygonaceae					
Rumex					1.2(MP)
Ranunculaceae					
Ranunculus			3.8(IP)	3.4(IP)	2.6(MP)
Rosaceae					
Rubus				3.6(IP)	2.3(MP)
Scrophulariaceae					
Verbascum			11.7(IP)	5.1(IP)	

a : Pollen samples

Health risk assessment

Health risk assessment is required to understand the potential hazard of foods. Among the risk assessment methods, the target hazard quotients (THQ) and the hazard index (HI) are the most widely used methods for human health risk assessment (Erdoğan et al., 2022).

In the current study, the rate of potentially toxic elements (Cr, Mn, Fe, Co, Ni, Cu, Zn, Se and Cd) accumulation and potential risk levels were calculated based on the amount of pollen consumed daily. The risk assessment of pollen samples was calculated given the equation below (Eq1.):

THQ=(CxDPC/BW)/RfD

Where C represents the potentially toxic element content in pollens (mg kg^{-1} dry weight); DPC (daily pollen consumption) was calculated at 40 g for adults and 20 g for children; BW (average body weight): 15 kg for children and 70 kg for adults (Zafeiraki et al., 2022). The RfD limit values of Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, and Cd were as follows: 1.5, 0.14, 0.7, 0.0003, 0.02, 0.04, 0.3, 0.005 and 0.001 mg kg^{-1} BW (Shomar and Rashkeev, 2021).

When the THQ value is less than 1, it is considered safe for consumers exposed to this product. If THQ is equal to or higher than 1, the substance is considered unsafe for human health. Hazardous Index (HI) is calculated as the sum of the target hazard quotients for all elements. Also If HI is equal to or higher than 1, the substance is considered unsafe for human health (Conti et al., 2022).

Results and Discussion

Floral sources of pollen samples

Melissopalynology is a trustworthy method for examining pollen grains to detect the geographic and botanical sources of bee pollen. Table 1. lists the identified pollen grains and their frequency based on pollen analysis of sample data. There were 26 distinct pollen types in the samples' pollen spectrum, including 25 nectariferous taxa and 1 non-nectariferous taxon. Nearly all samples included the pollen types *Trifolium* spp., *Olea* spp., *Cistus* spp., and *Ranunculus* spp. A significant amount of *Cistus* spp. pollen grains were observed (17.6% of the samples).

Additionally, it was classified as secondary pollen in three samples (P3, P4, P5). *Trifolium* spp., which was discovered in 16.5% of the samples, *Melilotus* spp., *Cornus* spp., and *Astragalus* spp., which were each present in 14.9%, 6.9%, and 6.9% of the samples, respectively, were other prevalent pollen types. The most widely represented family was Asteraceae, Fabaceae, and Cistaceae.

According to the melissopalynological analysis, if a taxon accounted for more than 90% of the pollen source, it was categorized as monofloral (da Silva de Freitas et al. 2015). In the present study, bee pollen samples were classified as heterofloral. Plant taxa determined in the samples can change concerning flora reached by honey bees at harvest time. However, the melissopalynological study revealed plant species in bee pollen samples that were identical to those found in previous studies (Temizer et al., 2018; Bakchiche et al., 2020; Mayda et al., 2020) . The majority of pollen taxa, as determined, were members of the Fabaceae and Asteraceae families. Many studies declared that Asteraceae and Fabaceae family is dominant and important for honey bees (da Silva de Freitas et al. 2015).

Element Content

In this study, 13 different macro, and microelements and heavy metals were determined in bee pollen samples by ICP-MS. The average values of the analyzed elements were given in Table 2.

Although found in trace amounts in foods, the presence of macroelements is essential for the normal growth and function of the human body (Zafeiraki et al. 2022). K is one of the macroelements essential for life. The results showed that the highest element concentration was found for K. Concentrations of K in this study does not differ significantly from other studies (Taha and Al-Kahtani, 2020; Asmae et al., 2021). Na and Ca compounds of many foodstuffs, are necessary for humans to obtain regulation of human metabolism. Most studies reported that Na content was below 1000 mg/kg but this value reached 6223, 8350, and 1466 in Turkish, Saudi Arabian, and Brazilian bee pollen respectively (Morgano et al., 2010; Kalaycıoğlu et al., 2017; Taha and Al-Kahtani, 2020). Similar to this work, Na level was less than 400 mg/kg in more than 75% of Brazilian pollen samples (Morgano et al. 2012). Due to its higher K and lower Na levels, bee pollen contains higher K: Na ratio to provide the optimum electrolytic balance in the body (Carpes et al. 2009). Brazilian bee pollen's Ca content varied from 643 to 4670 mg/kg, these values were higher than the values determined in current study (Morgano et al., 2012; De-Melo et al., 2016).

Mg is one of the most abundant essential macroelements for humans (Pohl et al. 2020). The second most abundant element was Mg according to this study. Kostić et al. (2017), found Mg concentration 730–1030 mg kg^{-1}, Aldgini et al. (2019) showed that the range of Mg 1575.19–

641.388 mg/kg in bee pollen from Jordan and China. Altunatmaz et al. (2017) showed that Mg elements in bee pollen vary from 271.107 to 1278.340 µg/g. Mayda et al. (2020), declared that Mg concentration varies from 784.23–1266.12 mg/kg in bee pollen. Kastrati et al. (2021), exhibited Mg concentrations in bee pollen ranging from 393 to 762 mg/kg. In present study, Mg levels found 128 - 808 mg/kg in the samples. Similar Mg concentrations were found compared to the literature.

Cr is one of the important essential elements. Cr was found 1.95-3.3 mg/kg in samples. Altunatmaz et al. (2017), noted 0.124-1.595 µg/g Cr in bee pollen samples. Kostić et al. (2017) determined that 0.094-0.239 mg kg^{-1} Cr in different Serbian maize hybrids bee pollen. Temizer et al. (2018) found 0.201-0.537 mg/kg Cr in bee pollen samples from Turkey.

Mn is recognized as an essential trace element and required for the development, growth, and maintenance of health (Avila et al., 2013). Kostić et al. (2017) were found 11-21 mg kg^{-1} from Serbia, Altunatmaz et al. (2017) were detected 8.151-201.036 µg/g/pollen Mn in bee pollen samples from Turkey. According to current study Mn concentration was found 15-34 mg/kg as shown in Table 2.

Fe is an abundant element on earth and is a biologically essential element for living organisms (Abbaspour et al. 2014). Our result showed 68-120 mg/kg Fe elements in bee pollen samples. Similar to our work, Altunatmaz et al. (2017), and Mayda et al., (2020) detected that 28.603-725.360 µg/g and 140.17-239.55 mg/kg Fe element respectively.

Co is very common in the natural environment and may occur as an effect of anthropogenic activity (Czarnek et al. 2015). In the current study, the range of concentrations found for Co 0-0.024 mg kg^{-1} in bee pollen samples usually matches those reported in the literature (Altunatmaz et al. 2017; Mayda et al. 2020).

Ni is an essential element for human nutrition but excessive soluble Ni element is hepatotoxic and nephrotoxic(Harmanescu et al. 2007). Ni was present in the range of 1.13–5.06 mg kg^{-1}in bee pollen samples These findings were higher than the earlier studies which have been done in Turkey, and Romania (Altunatmaz et al., 2017; Aldgini et al., 2019).

Table 2. Element content of pollen samples

	Samples							
Elements mg/kg	P1	P2	P3	P4	P5	Mean[a]	Min[b]	Max[c]
Ca	261	294	359	425	327	333.204	261.33	424.68
Na	350	263	357	250	176	279.2	176.33	356.7
K	9623	8667	6753	4840	7710	7518.678	4840.03	9623.33
Mg	808	672	400	128	536	508.88	128.08	808.08
Cr	1.95	2.22	2.76	3.30	2.49	2.544	1.95	3.3
Mn	34	20	15	17	25	22.2	15.1	33.85
Fe	110	96	68	120	82	95.276	67.72	120.3
Co	0	0.008	0.024	0.005	0.016	0.0106	0	0.024
Ni	1.13	2.44	5.06	1.2	3.75	2.716	1.13	5.06
Cu	9.68	5.26	3.58	1.9	0.84	4.252	0.84	9.68
Zn	57.6	33.8	13.9	6.0	10.0	24.3	6.0	57.6
Se	1	0.72	0.16	0.4	0.44	0.544	0.16	1
Cd	0.19	0.15	0.07	0.01	0.11	0.106	0.01	0.19
TE[d]	11258.17	10056.62	7977.824	5792.945	8873.816			

a : Mean value; b : Minimum; c : Maximum; d: Total Element

Zn is one of the essential elements for all life forms and the accepted limit for Zn for human nutrition is 40 mg/day (Harmanescu et al., 2007). The results showed that the concentration of Zn in all samples was below the accepted limit (Table 2). The Zn concentration in this study was between 6 mg/kg and 57.6 mg/kg. These results were similar to some previous studies (Altunatmaz et al., 2017; Aldgini et al., 2019; Çobanoğlu et al. 2022).

Se is an essential element, considered supplemental food and antioxidant. The acceptable limit is 0.04 mg/day and the results showed that the concentration of Se for all samples was below the acceptable limit. Altunatmaz et al. (2017), declared that 0.593-5.085 mg/kg Se in bee pollen samples. Mayda et al. (2020) found 0.01-0.05 mg/kg Se in bee pollen.

Cd is very toxic to many organisms but the acceptable limit is ranged between 0.05 and 1.00 mg/kg (Harmanescu et al., 2007). Except Sample P4, the values of Cd were higher than the acceptable limits (<0.05 mg/kg). Our results were similar to the previous studies (Altunatmaz et al., 2017; Aldgini et al., 2019). However Dinkov and Stratev (2016), declared that Cd concentrations in bee pollens varied from 0.019 to 0.030 mg/kg and were lower than acceptable norms. Morgano et al. (2010) found between 0.003 and 0.233 mg/kg of Cd in bee pollen.

Risk Assessment

THQ and HI values in nutrients are considered meaningful parameters for assessing health risks (Erdoğan et al., 2022; Zafeiraki et al., 2022). We may conclude that bee pollen is safe for consumption by both adults and children if TH<Q1 and HI<1, even when taking into account the probability of consuming them regularly. Risk assessment for human health related to pollen consumption was evaluated. Table 3 lists the THQ values of potentially toxic elements (Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Cd) in bee pollen.

Table 3. THQ and HI values of samples for adults and children

	Samples/THQ									
Elements	P1		P2		P3		P4		P5	
Cr	0.000743	0.001733	0.000846	0.001973	0.001051	0.002453	0.001257	0.002933	0.000949	0.002213
Mn	0.138163	0.322381	0.082245	0.191905	0.061633	0.14381	0.068163	0.159048	0.102857	0.24
Fe	0.090057	0.210133	0.078465	0.183086	0.055282	0.12899	0.098204	0.229143	0.066873	0.156038
Co	0	0	0.015238	0.035556	0.045714	0.106667	0.009524	0.022222	0.030476	0.071111
Ni	0.032286	0.075333	0.069714	0.162667	0.144571	0.337333	0.034286	0.08	0.107143	0.25
Cu	0.138286	0.322667	0.075143	0.175333	0.051143	0.119333	0.027143	0.063333	0.012	0.028
Zn	0.10979	0.256178	0.064381	0.150222	0.026438	0.061689	0.011505	0.026844	0.018971	0.044267
Se	0.114286	0.266667	0.082286	0.192	0.018286	0.042667	0.045714	0.106667	0.050286	0.117333
Cd	0.108571	0.253333	0.085714	0.2	0.04	0.093333	0.005714	0.013333	0.062857	0.146667
HI	0.732182	1.708425[a]	0.554032	1.292742[a]	0.444118	1.036276[a]	0.30151	0.703524	0.452413	1.055629[a]

In this study, THQ values were below 1, indicating that the exposed human population should be safe for all of the examined elements (Zafeiraki et al., 2022).

According to the relative contributions of the various elements to total HI in P1, P2, P3, and P5 pollen samples accumulated trace element quantities in greater proportions. Since the HI values of these samples were greater than 1, it was determined that the regular consumption of 20 g of these pollen samples by children was not suitable because of the element accumulation in the body.

Conclusion

Exposure of heavy metals to nature and humans has risen dramatically since the industrial revolution. Although, the presence of heavy metals in bee pollen can threaten the health of living organisms. Environmental sources pollutants can contaminate the raw materials of bee products (pollen, honeydew, nectar, plant exudates) through air, water and soil, and then they are transported to the hive by bees. Air and soil contain heavy metals, especially from traffic and industry, which can also contaminate the bee colony and its products. Several studies have shown heavy metals as important contaminants of bee pollen.

This means that all bee products should be strictly controlled in terms of quality and safety. Therefore, these products should be evaluated in terms of health risk assessment before consumption.

Declaration of competing interest

The authors of this article declare that they have no known competing financial interests or personal relationships that could have appeared to affect this work.

Author's contributions

İKT supplied the bee pollen samples. DNÇ and İKT conducted the palynological analyses, elemental analysis, calculated hazard risk assessment and wrote the manuscript and approved its final version.

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