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Antioxidant properties, element contents and antimicrobial activities of bee pollen collected by *Apis mellifera* L. in Türkiye

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Abstract

Aim of study: Recently, pollen has become a preferred nutritional supplement because of its complex composition. We examined the botanical origin, total phenolic/flavonoid content (TPC/TFC), antioxidant/antimicrobial activity, and element content of pollen samples collected from honeybees. This study also examined whether the elements contained in pollen, when consumed as food, posed a risk to human health.

Area of study: Ten mixed pollen samples were randomly collected from honeybees in the apiaries of four different Turkish regions, which fall among the three phytogeographic regions of Türkiye.

Material and methods: We evaluated total flavonoid (TFC) and phenolic (TPC) contents; antioxidant activities (radical scavenging activity, hydrogen peroxide scavenging activity - HPSA, ferric reducing antioxidant power - FRAP, and ferrous ion chelating activity - FICA), element concentrations and antimicrobial activity.

Main results: According to the melissopalynological analysis, one sample was determined to be unifloral and nine samples were found to be multifloral. The values found ranged 271.42-601.85 mg GAE/100 g TPC, 23.53-34.50 mg CAE/100 g TFC, 22.19-23.78 µg/mL DPPH, 6.50-78.40 µg/mL ABTS, 20.43-150.94 µg/mL HPSA, 97.26-99.83% FRAP and 74.84-91.79% FICA. Rosmanic acid, p-coumaric acid, quercetin, apigenin, and naringin were identified in all samples, while catechin was detected only in S6 and S7. Element contents were found Mg > Fe > Mn > Zn > Cu > Se > Cr > Ni > Cd > Co. All the samples had high antibacterial activity against *Bacillus cereus* (MIC= 4.17-8.33 g/mL), and against *Staphylococcus aureus* (MIC= 8.33 g/mL), except S3 and S4.

Research highlights: Different levels and combinations of these components are efficient in the antioxidant and antibacterial activity of pollen.

Additional key words: antimicrobial activity; antioxidant activity; bee products; elements; melissopalynological analysis; pollen.

Abbreviations used: ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)); CAE (catechin equivalent); DPPH (2,2-diphenyl-1-picrylhydrazyl); EDI (estimated daily intake); FICA (ferrous ion chelating activity); FRAP (ferric reducing antioxidant power); GAE (gallic acid equivalent); HPLC (high performance liquid chromatography); HPSA (hydrogen peroxide scavenging activity); ICP-MS (inductively coupled plasma mass spectrometry); LOD (limit of detection); LOQ (limit of quantification); MHA (Muller-Hinton Agar); MHB (Mueller-Hinton Broth); MIC (minimum inhibitory concentration); PCA (principal component analysis); RfD (oral reference dose), SHI (sum of hazard index); SRC (Spearman's rank correlation coefficient; TFC (total flavonoid content); THI (target hazard index); TPC (total phenolic content).

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Introduction

Türkiye has a particularly rich biodiversity, with a characteristic climate, topography, and geomorphology, and is divided into three phytogeographic regions: Euro-Siberian, Irano-Turanian, and Mediterranean. This biodiversity enables the beekeepers to perform their beekeeping activities with both traditional and modern methods. Many melissopalynological studies have stated that plant species belonging to the families Fabaceae, Asteraceae, Lamiaceae, Rosaceae and Brassicaceae are important for beekeeping (De Souza *et al.*, 2019; Lau *et al.*, 2019). In Türkiye, there are 71 genera and 1013 species of which 400 are endemic in the Fabaceae family; 140 genera and 1186 species (446 endemic) in the Asteraceae family; 45 genera and 574 species (256 endemic) in the Lamiaceae family; 37 genera and 297 species (58 endemic) in the Rosaceae family; and 606 species (226 endemic) in the Brassicaceae family (Davis, 1965-1985; Erik & Tarıkahya, 2004; Mutlu & Karakuş, 2015).

Botanical richness enables beekeepers to produce bee products with various chemical and physical properties. Since ancient times, bee products have been among the most valuable products, which are indispensable for people because of their nutritional and therapeutic properties. Pollen, as one of these bee products, contains many bioactive components (phenolic and flavonoid compounds), vitamins, minerals, and pigments, as well as primary metabolites such as carbohydrates, proteins, and lipids (Denisow & Denisow-Pietrzyk, 2016). These phenolic and flavonoid components vary directly with the botanical origin, and indirectly depend on factors such as climate and soil (Komosinska-Vassev *et al.*, 2015; Temizer *et al.*, 2018). Additionally, the differences in the antioxidant activity properties of pollen are closely related to the chemical structure of these components (Rzepecka-Stojko *et al.*, 2015). Furthermore, many researchers claimed that pollen has high antibacterial (Basim *et al.*, 2006; Özkalp & Özcan, 2010), antifungal (Özcan, 2004), anti-inflammatory (Di Paola-Naranjo *et al.*, 2004) and antimutagenic (Pascoal *et al.*, 2014) effects.

The food supply needed due to the rapid increase in the world population, the use of synthetic chemical pesticides, mineral fertilizers, growth regulators and hormones in agricultural activities is increasing and as a result, pollen is also affected by the increasing environmental pollution. Many studies have emphasized that pollens are used as a bioindicator in environmental pollution (Temizer *et al.*, 2018; Aldgini *et al.*, 2019). The substances that are required at a minimum level to sustain the survival of living things, especially plants, are called trace elements. When the amounts of these elements exceed the tolerable limit values, they are defined as toxic contaminants. Essential and toxic elements that cause adverse effects for human health in pollen have been investigated in many countries, such as Türkiye, Bulgaria, Poland, Brazil, Romania, and Jordan (Dinkov & Stratev, 2016; Roman *et al.*, 2016; Temizer *et al.*, 2018; Aldgini *et al.*, 2019).

The production and nutritional value of bee products other than honey such as pollen are ignored, although beekeeping is an important and common activity around the world. This study investigates the botanical origin, total phenolic/flavonoid components, antioxidant, and antimicrobial activities of the pollen samples. The levels of elements in the pollen were also determined to evaluate the health risks and environmental pollution.

Material and methods

Pollen sample analysis

Pollen samples were obtained at the end of the pollen flow season in 2018. In this study, each mixed pollen sample was randomly collected from different beekeepers. They were collected from four different locations: sample S1 from İspir-Erzurum, which has a semi-humid climate and is located in the Irano-Turanian phytogeographical region (40° 29’ 3.2424’’ N - 41° 0’ 11.0268’’ E); samples S2, S3, and S4 from Bingöl, which has a semi-humid climate and is located in the Irano Turanian phytogeographical region (38° 53’ 7.2564” N - 40° 29’ 53.8476” E); sample S5 from Bulancak-Giresun, which has a humid climate and is located in the Euro-Siberian phytogeographical region (40° 56’ 15.0396” N - 38° 13’ 55.3584” E); and samples S6, S7, S8, S9 and S10 from Fethiye-Muğla, which has semi-drought and humid climate and is located in the Mediterranean phytogeographical region (36° 37’ 34.04” N - 29° 6’ 33.23” E) (Davis, 1965-1985; TSMS, 2022).

All pollen samples (2 g) were dissolved in 50 mL of absolute ethanol and pollen slides were elaborated according to Wodehouse (1935). At least 500 pollen grains were counted on each slide to determine its composition (Freire *et al.*, 2012).The description of pollens as form or type was conducted according to the method of Mateo & Bosch-Reig (1998). A Nikon Eclipse Ci model microscope was used for examinations, including taxon identification at x1000 magnification and counting at x400 magnification. Monofloral and heterofloral properties of pollen were classified according to Freitas *et al.* (2013).

Pollen extract preparation

Five grams of pollen sample were added to 60 mL of absolute ethanol and this suspension was stirred at room temperature for 24 h by using a magnetic stirrer. This extraction solution was then filtered through Whatman no: 4 filter paper and stored at 4 °C.

Total phenolics content (TPC) and total flavonoids content (TFC)

It has been reported that ethanol is the best solvent for pollen extraction for measuring TPC/TFC and antioxidant activities (Karkar *et al.*, 2018). We used ethanol as a solvent in determining these parameters in the samples. TPC of pollen extracts was determined according to the Folin-Ciocalteu reagent and Slinkard & Singleton´s (1977) method. Absorbances of the compounds were measured at 760 nm. TPC of the samples was calculated using the gallic acid calibration curve, which was used as a standard ($R^2 = 0.9995$).

The TFC of pollen extracts was determined according to the aluminium chloride colorimetric method (Chung *et al.*, 2002). The absorbances were measured using a spectrophotometer (Optizen Pop UV/Vis Single Beam) at 415 nm. TFC of the samples were calculated using catechin's calibration curve, which was used as a standard ($R^2 = 0.9979$).

Determination of phenolic and flavonoid components

Fragmentation profiles for the identified phenolic compounds were performed in the Agilent 1260 High Performance Liquid Chromatography (HPLC) system, and these compounds were determined by matching standard peak retention times run under equal HPLC conditions. A reverse phase column, Inertsil ODS-2 GL Sciences Inc. 5 µm (4.6×250 mm) C18 and Shimadzu SPD-M10 Avp PDA detector (270 nm) was used during fragmentation in a Shimadzu Prominence HPLC (Liu *et al.*, 1997). System flow was 1.5 mL/min and system temperature was 25ºC. The following segmented gradient elution was used: 0-6.5 min, 90% A; 6.5-7.5 min, 89% A; 7.5-9 min, 87% A; 9-15 min, 20% A; 15-17 min, 95% A; 17-35 min, B (Miura *et al.,* 2002). The mobile phase includes A, which is 97.5 water: 2.5 phosphate (v:v), B is acetonitrile. The measurements were repeated at least twice.

Antioxidant capacity assays

— DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. The DPPH radical scavenging activity of pollen extracts was determined according to Blois (1958) with minor modifications. Sample solutions (3.0 mL) were mixed with 0.1 mM DPPH (1.0 mL) prepared by adding absolute ethanol. The mixture was incubated for 30 min at room temperature in a dark environment and the absorbance was measured at 517 nm using a spectrophotometer. The free radical scavenging activities of the reaction mixtures were calculated using the absorbance values after 30 min. The decline in absorbance is an indicator of the high rate of free radical scavenging activity in the samples, which was expressed in SC_{50} (µg/mL).

— ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity. The ABTS radical was dissolved in 7 mM potassium persulfate solution (2:1) and incubated for 16 h at room temperature in a dark environment. The prepared solution was diluted using 5 mM phosphate buffer (pH 7.4) so that the absorbance value reached 0.700 ± 0.020. Pollen extract samples (20 mL) were homogenized with 2 mL of the solution, kept for 5 min and measured in the spectrophotometer (Gökce *et al.*, 2019).

— Hydrogen peroxide scavenging activity (HPSA). The HPSA of pollen samples (using 0.04 M phosphate buffer, pH 7.4) was performed according to Ruch *et al.* (1989). The HPSA of the samples was expressed in SC_{50} (µg/mL).

— Ferric reducing antioxidant power (FRAP). The FRAP of pollen extracts and standard antioxidant solutions prepared with absolute ethanol was determined according to Oyaizu (1986). The absorbance values of the samples were measured using a spectrophotometer at 700 nm. The increase in absorbance values in the reaction mixture is an indicator of the high level of reducing power in extracts and standard antioxidant substances (Eq. 1):

$$\text{FRAP (\%)} = (\text{As/Ac}) \times 100 \quad (1)$$

where As: extracts or standard materials absorbance values, Ac: control's absorbance values.

— Ferrous ion chelating activity (FICA). The FICA of the solutions prepared with pollen extracts and standard antioxidant substances in absolute ethanol was performed according to Dinis *et al.* (1994). The absorbance values of the mixtures were measured at 562 nm. FICA of the extracts and standard antioxidant substances was calculated according to Eq. (2):

$$\text{FICA (\%)} = [1-(\text{As/Ac})] \times 100 \quad (2)$$

Analysis of elements

In this study, Mg (microelement), Fe, Zn, Mn, Ni and Cu (trace elements), Se, Cr and Co (ultra-trace elements), and Cd (toxic element) were examined by inductively coupled plasma mass spectrometry (ICP-MS) (Model Bruker 820-MS). The microwave digestion method was used for the preparation of pollen samples: 0.5 g of each sample was digested in CEM MARS for 5 min with $HN0_3$ (ultrapure) and in 2 mL of HCl (ultra-purification) for 15 min at 1600 Watt in 210 mL. These solutions, which were placed in a cooling sample lid falcon tube and completed to 50 mL with distilled water, were analysed by Bruker 820-MS ICP-MS by filtering through a membrane filter (0.45 µm). The calibration curve was obtained using a certified multi-element standard. An intermediate stock of 10 mg/L was prepared from the main stock solution and a calibration curve was plotted from standard stocks of 5, 10, 20,

50, 100, and 250 µg/L. Samples were prepared in triplicate and ten readings were taken on each parallel ICP-MS. The blank sample prepared in 1% HNO_3 solution was measured 20 times. The standard slope, LOD (limit of detection) and LOQ (limit of quantification) were determined three, three and ten times, respectively (Temizer *et al.*, 2018).

Antimicrobial activity

Antimicrobial activities of pollen extracts were tested against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 10876) and *Candida albicans* (ATCC 10231) strains.

The strains were stored in a medium containing 15% glycerol and kept at -80 °C. Microbial cultures were incubated in sterile Mueller-Hinton Broth (MHB) for 6 h at 37 °C. Each culture was then transferred to a fresh MHB and incubated at 37 °C for 24 h. A medium containing 0.5X McFarland solution (approx. a culture density of 1.5×10^8 cells/mL) was prepared.

Antimicrobial activities of pollen extracts were studied using the agar diffusion test method (Rios *et al.*, 1988). Pollen extracts were prepared at a concentration of 8.33 g/mL in ethanol. Ethanol was used as a negative control. Each strain was inoculated onto Muller-Hinton Agar (MHA) medium with sterile swabs. Each pollen extract solution (100 µL) and controls were dropped into 8 mm diameter wells opened in the medium. Petri plates were incubated at 37 °C for 24 h. After incubation, the diameter of the inhibition zone around each well was measured and recorded. Antimicrobial activity was expressed as the diameter of the regions of inhibition generated by the tested extract. Each test was run three times.

The minimum inhibitory concentration (MIC) was determined using the microplate method (Wiegand *et al.*, 2008). Pollen extract (8.33 g/mL) was serially diluted to 50% with MHB medium and 50 µL of a microorganism culture solution was added to 12 wells of a 96-well microplate. The microplates were incubated at 37 °C for 24 h. The absorbance values of the samples after incubation were measured at 450 nm using an automated microplate reader (Multiskan FC, Thermo).

Estimated daily mineral intake

The intake of elements in the diet is considered 0.04 mg/day for adults and 0.02 mg/day for children (Zafeiraki *et al.,* 2022). The equation below was used to get estimated daily intake (EDI):

$$EDI = \frac{c}{BW} \times AFC \qquad \text{(Eq. 3)}$$

where c refers to the element level in bee pollen; AFC is the amount of food consumption (mg/day), calculated as 0.04 mg for adult consumption and 0.02 mg for child consumption (Zafeiraki *et al.,* 2022); and BW is the consumer´s mean body weight (70 kg for male adults, 60 kg for female adults, and 15 kg for children) (Azanu *et al.,* 2018; Tutun *et al.,* 2022; Zafeiraki *et al.,* 2022).

Health risk assessment

This analysis investigated the potential health risks associated with consuming pollen as a food. As a result, only oral consumption was used to determine the THI (target hazard index) and SHI (sum of hazard index) evaluations; THI (Eq. 4) being used to describe the health hazards associated with just one chemical, and SHI (Eq. 5) the risk of exposure to two or more chemicals (Zafeiraki *et al.,* 2022):

$$THI = \frac{CFxCDxEDI}{RfDxTA} \qquad \text{(Eq. 4)}$$

where CF is contact frequency (365 days/year); CD is contact duration (70 years for adults and 4 years for children); RfD (mg/kg·day) is the oral reference dose for which the health-based recommendation value; and TA is the mean lifetime (365 days/year 70 years). The RfD levels for Mn, Fe, Co, Ni, Cu, Zn, Cr, Cd are defined in Demir *et al*. (2020) and for Se in Singh *et al*. (2019). There is no noticeable health risk when the computed THI and SHI values (Eq. 5) are both lower than 1.

$$SHI = \sum_{i=1}^{N} THIi \qquad \text{(Eq. 5)}$$

where N is the total number of distinct elements found in each sample of bee pollen.

Statistical analysis

Statistical evaluations were performed from several methodological perspectives to analyse the pollen samples in terms of their antioxidant activity, contents of the elements and phenolics they contain.

The linear relationships between element, antioxidant, and phenolic contents in the pollen samples were examined. The Pearson correlation coefficient was used for variables complying with the normality condition as tested by the Shapiro & Wilk (1965) test. For those not complying, the non-parametric Spearman's Rank Correlation Coefficient (SRC) (Spearman, 1904) was used.

Pollen samples were submitted to complete-linkage clustering analysis to be classified in terms of element, phenolic, and antioxidant contents (Defays, 1977). The findings of the clustering analysis have been visualized in the form of a dendrogram.

PCA has been widely used as an exploratory tool for data analysis (Pearson, 1895; Hotelling, 1933). Principal component analysis (PCA) was also performed based on

Table 1. Taxa determined in pollen samples and their incidence percentages (%).

Taxa	Pollen samples									
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
Acacia sp.						2.1				
Cistaceae						10.3		14		
Rosaceae		98.4	80.3			2.7	15.9	2.3		
Pistacia sp.						57.5	3.7			50.5
Brassicaceae	62.1				2.4	11.5		64.3	3.1	
Thalictrum sp.					0.8	0.7				
Moraceae						0.7				
Taraxacum sp.						1.4				
Lamiaceae (3-colpate type)						8.2				
Geraniaceae	4.5					1.4	1.2		26.2	37.4
Lamiaceae (6-colpate type)				26.3		0.7	26.8	0.8		
Asteraceae (echinatetype)		1.6	17.9	12.3		0.7	1.2		1.5	
Apiaceae									53.8	
Epilobium sp.									9.2	
Boraginaceae	2.3						14.6		3.1	4.7
Centaurea sp.			1.4		2.4				3.1	
Onobrychis sp.					86.4		34.1			
Chenopodiaceae							2.4			
Trifolium sp.				48.2						
Daucus sp.				2.6						
Sanguisorba sp.				9.6						
Myosotis sp.				0.9	8					
Lotus sp.										
Fabaceae	26.3									4.7
Morus sp.										2.8
Ericaceaae										
Asteraceae (lacunate type)	2.3					2.1				
Betula sp.			0.5							
Campanula sp.	0.8									

element, antioxidant, and phenolic contents to determine the patterns in the data structure. To determine the number of principal components, two different statistical perspectives such as eigenvalues and the ratio of explaining the total variance can be considered. In this study, both perspectives were evaluated to select components.

Results and discussion

Pollen spectrum

The floral origin of bee pollen samples (n = 10) was determined, and the taxa found are given in Table 1. Sample S2 was classified as monofloral, and the other samples as heterofloral because each taxon was represented by less than 90% (Table 1). In this study, although some pollen samples (S2-S3-S4 and S6-S7-S8-S9-S10) were collected from different beekeepers in the same locality, their profiles differed from each other. This can be explained by the rich plant diversity in Türkiye (over 11,500 taxa, near to all Europe) (Alimoğlu *et al*., 2021).

The Apiaceae family was the predominant type of pollen in S1; Rosaceae family was the predominant type of pollen in S2 and S3; Fabaceae family was predominant type of pollen in S4 (*Trifolium* genus) and S5 (*Onobrychis* genus). The *Pistacia* genus was dominant in S6, S7, and S10 samples collected in Muğla. Many studies have reported that these taxa are crucial for the survival of bees

Table 2. TPC, TFC and antioxidant activities of pollen samples.

Samples	TPC[a]	TFC[b]	FICA[c]	HPSA[d]	ABTS[e]	DPPH[f]	FRAP[g]
1	316.57	32.27	90.19	20.43	64.98	22.99	98.65
2	271.42	25.86	91.61	150.94	60.48	22.71	97.26
3	545.41	23.53	74.84	20.35	74.99	22.57	98.36
4	601.85	24.66	83.34	21.77	72.65	22.74	98.74
5	276.55	26.44	88.28	24.17	78.4	23.78	99.45
6	412.01	29.42	91.79	21.37	75.1	22.47	99.75
7	368.91	30.65	91.44	22.04	62.17	22.83	99.83
8	507.44	34.5	84.64	23.57	73.8	22.19	99.83
9	302.21	32.36	79.03	23.14	66.78	23.34	99.54
10	392.51	28.87	91.23	22.23	64.56	22.94	99.75

[a] TPC: total phenolic content, mg GAE/100 g. [b] TFC: total flavonoid content, mg CAE/100 g. [c] FICA: ferrous ion chelating activity, % (at the 100 µg/mL concentration). [d] HPSA: hydrogen peroxide scavenging activity, SC50 (µg/mL). [e] ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid. [f] DPPH: 2,2-diphenyl-1-picryl-hydrazyl. [g] FRAP: ferric reducing antioxidant power.

(Freire *et al.*, 2012; Freitas *et al.*, 2013; Temizer *et al.*, 2019). This vegetation also affects the phenolic compound, antioxidant, and antimicrobial activities of bee pollen.

TPC and TFC

TPC, TFC and antioxidant activities of the pollens are given in Table 2 and the concentration of phenolic substances in Table 3. Rosmanic acid, p-coumaric acid, quercetin, apigenin and naringin were found in all pollen samples.

TPC and TFC of the pollen samples ranged 271.42-601.85 mg GAE/100 g and 23.53-34.50 mg CAE/100 g, respectively (Table 2). Özcan *et al.* (2019) collected pollen samples from different regions of Türkiye and determined the total content of phenolic substances in the pollen in 434.17-719.58 mg GAE/100 g, values quite compatible with the results of our study. De-Melo *et al.* (2018) analysed the pollen of different botanical origins in Brazil and reported total phenolic and flavonoid contents of 560-2970 mg GAE/100 g and 25.74-1630.39 mg CAE/100 g, respectively. These values were quite higher than our results. The total flavonoid contents of the pollen samples in Malaysia (15.28-31.80 mg CAE/100 g) were very similar to our results (Harif Fadzilah *et al.,* 2017).

Hierarchical clustering analysis from phenolic contents of pollen samples

Pollen samples were classified by a hierarchical clustering method in terms of phenolic contents. The dendrogram indicated that the pollen samples can be split into three main clusters (Fig. S1 [suppl]). PCA was also performed (Fig. S2a [suppl]) to determine the patterns in the data structure based on phenolic contents. Fig. S2b [suppl] represents the score plot of samples for PC1 and PC2, where S2 significantly dissociates from all other samples in terms of phenolic contents, parallel to the clustering analysis results.

Antioxidant capacity assays

HPSA and DPPH radical scavenging activity

HPSA and DPPH radical scavenging activities of pollen samples are ranged 20.43-150.94 and 22.19-23.78 µg/mL, respectively (Table 2). HPSA values of pollen samples collected from different regions of Türkiye by Temizer *et al.* (2018) were in the range 25.56-30.28 mg/mL. The HPSA values were similar to this study except for S2. The level of p-coumaric acid determined in S2 was also lower than in the rest of samples. Therefore, HPSA is also considered to be caused by p-coumaric acid. S5 and S9 had a high HPSA activity, but these samples were found to have low p-coumaric acid levels. A high HPSA activity value may be related to the amount of gallic acid and apigenin. The DPPH radical scavenging activity of the pollen samples was higher than that reported by Harif Fadzilah *et al.* (2017) and Sun *et al.* (2017), but similar to that of the pollen samples collected from Brazil (Freire *et al.,* 2012).

FRAP and FICA

The FICA and FRAP of the pollen samples varied between 74.84-91.79% and 97.26-99.83%, respectively (Table 3). Kao *et al.* (2011) and Sardar *et al.* (2014) found metal-chelating activities in pollen samples in the range 11.27-73.50% and 41.40-58.80%, respectively. Almost all pollen

Table 3. Contents of phenolic substances in pollen samples (mg/g). ND: not detected.

Samples	Gallic acid	Catechin	Chlorogenic acid	p-coumaric acid	Hesperidin
S1	ND	ND	0.0051±0.00	0.0036±0.00	0.0383±0.01
S2	0.0076±0.01	ND	ND	0.0019±0.00	0.0077±0.00
S3	0.0021±0.00	ND	0.0010±0.00	0.0702±0.00	0.0087±0.00
S4	0.0102±0.00	ND	ND	0.0884±0.00	0.0061±0.00
S5	0.0226±0.00	ND	ND	0.0019±0.00	ND
S6	0.0034±0.00	0.0113±0.00	0.0027±0.00	0.0060±0.00	0.0127±0.00
S7	0.0030±0.00	0.0039±0.00	0.0023±0.00	0.0059±0.00	0.0098±0.01
S8	ND	ND	0.0026±0.00	0.0039±0.00	0.0283±0.00
S9	0.0010±0.00	ND	0.0021±0.00	0.0023±0.00	0.0128±0.00
S10	0.0022±0.00	ND	0.0688±0.00	0.0043±0.00	0.0305±0.00
	Rosmanic acid	**Quercetin**	**Apigenin**	**Naringin**	**Quinic acid**
S1	0.0278±0.00	0.451±0.01	0.0047±0.00	0.0688±0.00	ND
S2	0.187±0.00	0.064±0.00	0.0005±0.00	0.5643±0.00	ND
S3	0.0239±0.00	0.177±0.00	0.0002±0.00	0.0009±0.00	ND
S4	0.0259±0.00	0.2100±0.00	0.0007±0.00	0.0182±0.00	ND
S5	0.0101±0.00	0.0688±0.00	0.0018±0.00	0.0006±0.00	ND
S6	0.0593±0.00	0.0367±0.00	0.0002±0.00	0.0044±0.00	ND
S7	0.0529±0.03	0.4137±0.00	0.0005±0.00	0.0478±0.00	ND
S8	0.0357±0.00	0.1727±0.00	0.0006±0.00	0.0075±0.00	ND
S9	0.1407±0.00	0.0712±0.00	0.0304±0.00	0.0060±0.00	ND
S10	0.2045±0.00	0.0594±0.00	0.0022±0.00	0.0285±0.00	ND

samples showed an effective reduction in power activity. Temizer *et al.* (2017, 2018) reported that the iron reduction power in pollen samples was 71.92-73.86% and 72.29-72.58%, respectively. When compared with our study, it was determined that our pollen samples showed effective chelating activity. Analysing the components of all samples, p-coumaric acid, rosmanic acid, quercetin, apigenin, and naringin were the substances with a more prominent role in metal-chelating activity. The ability of these components to exhibit ferrous reducing power activity usually depends on substituted groups and phenolic structures.

ABTS radical scavenging activity

ABTS radical scavenging activities ranged 6.50-78.40 µg/mL. Freire *et al.* (2012) reported ABTS radical scavenging activities of pollen samples in Brazil as 6.0-97.20 µg/mL, quite similar to our study. Sun *et al.* (2017) determined ABTS radical scavenging activities as 3.19-3.85 µg/mL, lower than the values in our study. In our study, ABTS radical scavenging activity of S1 was higher than that of the other pollen samples (Table 2). The fact that S1 contains more hesperidin and quercetin than the other phenolic substances indicate an important role for these substances in ABTS radical scavenging activity. The phenolic groups in both compounds may be closely related to the substituted groups at the o-, m- and p- positions in the aromatic ring.

Hierarchical clustering analysis from antioxidant contents of pollen samples

A hierarchical clustering method was used to classify different locations according to the antioxidant contents of pollen samples. Fig. S3 [suppl] shows a dendrogram which illustrates the relationships among all pollen samples from the perspective of antioxidant content based on the pre-determined measure of similarity. Fig. S4a [suppl] shows a map for the antioxidant contents (PCA, PC1 and PC2). Fig. S4a [suppl] indicates that the positive part of PC1 was related to TPC, ABTS, FRAP and TFC, while the positive part of PC2 was related to HPSA, TPC and ABTS. For both PCs, the negative parts were related with these elements. Fig. S4b [suppl] shows that S2 significantly dissociates from the rest of samples in terms of antioxidant contents, parallel to the clustering analysis results. These findings demonstrate that the antioxidant contents can be used to classify these pollen samples.

Table 4. Elements detected in pollen samples (mg/kg). ND: not detected.

Samples	Mg	Cr	Mn	Fe	Co	Ni	Cu	Zn	Se	Cd
S1	613.76	1.534	21.628	123.1	ND	0.451	6.234	26.6	6.386	0.157
S2	633.28	4.423	32.752	156.25	ND	1.954	8.724	38.044	6.216	0.24
S3	899.16	3.396	29.029	183.92	ND	0.855	3.907	37.181	7.898	0.155
S4	128.47	1.722	38.832	192.21	ND	2.551	8.545	52.497	6.592	0.137
S5	807.83	2.349	36.707	87.104	ND	1.858	13.851	32.976	7.497	0.122
S6	700.63	3.633	20.707	63.591	0.006	3.269	6.164	27.82	5.942	0.163
S7	714.6	3.771	32.935	12.181	ND	3.392	6.936	33.086	6.173	0.138
S8	885.36	2.197	27.794	12.721	ND	2.463	6.734	29.406	6.317	0.153
S9	525.32	1.338	18.118	16.753	ND	2.475	5.968	32.658	6.416	0.193
S10	820.15	3.206	22.5	154.085	ND	1.486	6.467	30.781	6.04	0.112

Relationships between antioxidant and phenolic contents of pollen samples

The linear relationships between the antioxidant and phenolic contents of the pollen samples are shown in Table S1 [suppl]. Chlorogenic acid had a significant positive linear correlation with hesperidin (r=0.91; $p<0.001$) and TFC (r=0.57; $p=0.085$), at 0.05 and 0.10 significance levels, respectively; p-coumaric acid, at 0.05 significance level, had a significant positive linear correlation with TPC (r=0.91; $p<0.001$) and a significant negative linear correlation with HPSA (r=-0.74; $p=0.013$); hesperidin and TFC had a significant positive linear correlation (r=0.65; $p=0.042$), at 0.05 significance level.

Analysis of elements

Table 4 shows the results of the heavy metals detected in the pollen samples: Mg > Fe > Zn > Mn > Cu > Se > Cr > Ni > Cd > Co (Table 4). Adaškevičiūtė *et al*. (2019) found in all bee pollen samples Mg (644-1009 mg/kg), Fe (45.04-76.40 mg/kg), Mn (15.34-66.49 mg/kg), Zn (20.04-31.99 mg/kg), Co (0.000-0.105 mg/kg) and Cu (0.00-6.05 mg/kg), but Cr was found in none of the samples. Tutun *et al.* (2022) detected in bee pollen samples from Türkiye were Fe (12.6-130 mg/kg), Ni (0.35-3.70 mg/kg) Zn (12.7-53.5 mg/kg), Mn (4.74-19.0 mg/kg), Cu (3.19-18.5 mg/kg), Co (<LOD-0.006 mg/kg) and Cr (0.05-0.91 mg/kg). Zafeiraki *et al*. (2022) detected in bee pollen samples from Greece Mg (892-6098 µg/g), Fe (78-1496 µg/g), Mn (13-324 µg/g), Ni (0.012-1.4 µg/g), Zn (40-451 µg/g), Cu (11-57 µg/g), Se (LOQ<-0.76 µg/g), Cd (0.03-1.1 µg/g). The Mg level obtained from this study was higher than in previous studies (Adaškevičiūtė *et al*., 2019; Zafeiraki *et al*., 2022). In our analysis, Fe values were found in a wide range (Table 4). These values were greater than in Adaškevičiūtė *et al.* (2019) and similar to those found by Zaferaki *et al.* (2022) and Tutun *et al.* (2022). The Zn levels detected were found to be much lower than those reported by Zafeiraki *et al*. (2022) and closely similar to those reported by Tutun *et al*. (2022). In this study, Mn values are similar to Tutun *et al*. (2022), and lower than those values reported by Zafeiraki *et al.* (2022) and Adaškevičiūtė *et al*. (2019). Cu levels in our samples were similar to the values reported by Adaškevičiūtė *et al*. (2019), but lower than those found by Tutun *et al*. (2022) and Zafeiraki *et al*. (2022). In this study, Co (0.006 mg/kg) was detected only in S6 and it is within the range reported by Adaškevičiūtė *et al*. (2019) and Tutun *et al*. (2022). Adaškevičiūtė *et al*. (2019) never detected Cr in samples, and the Cr level reported by Tutun *et al*. (2022) was low compared to our study. While the Cd level in our study was similar to that found by Zaferaki *et al*. (2022), the Se level was higher than the one reported by these authors. Tutun *et al.* (2022) detected Se and Cd below the detection limit in the pollen samples.

Correlations between element contents of pollen samples

The findings related to the relationships between elements are shown in Table S2 [suppl]. The linear correlation between Cr and Co (r=0.588; $p=0.074$), and between Fe and Co (r=-0.583; $p=0.077$) showed a significant value at 0.10 significance level, in a positive and negative way respectively. Moreover, both Cu (r=0.758; $p=0.011$) and Zn (r=0.745; $p=0.013$) had a significant positive linear correlation with Mn at 0.05 significance level.

Hierarchical clustering analysis of element contents in the pollen samples

Fig. S5 [suppl.] shows the results of the clustering analysis with a dendrogram, which illustrates the relationships among all pollen sample locations based on element contents according to Euclidean distance. The dendrogram explained that the pollen samples can be split into three main clusters. S4 dissociated from all other samples and forms the third cluster alone. Fig. S6a [suppl] illustrates the element map.

Table 5. Antimicrobial activity of pollen samples determined by agar diffusion and minimum inhibitory concentration (MIC) methods (SD ± 1.0, n=3).

Samples	Microorganisms	Zone of inhibition (mm)	MIC value (g/mL)
S1	*B.cereus*	13	8.33
	S.aureus	12	8.33
S2	*B.cereus*	13	8.33
	S.aureus	13	8.33
S3	*B.cereus*	12	8.33
S4	*B.cereus*	12	8.33
S5	*B.cereus*	13	8.33
	S.aureus	12	8.33
S6	*B.cereus*	13	8.33
	S.aureus	12	8.33
S7	*B.cereus*	13	8.33
	S.aureus	12	8.33
S8	*B.cereus*	12	8.33
	S.aureus	12	8.33
S9	*B.cereus*	14	4.17
	S.aureus	12	8.33
S10	*B.cereus*	12	8.33
	S.aureus	11	8.33

The positive part of PC1 was related to Cu, Zn, Mn, Fe, and Se, while the positive part of PC2 was related to Co, Cr, Ni, Cd, Cu, Zn, Mn. For each PC, the negative parts were related with these elements. For each PC, the negative parts were related with these elements. Fig. 6Sb [suppl] represents the score plot of samples for PC1 and PC2 and shows that S4 significantly dissociated from all other samples for element contains, parallel to the clustering analysis results. Besides, for PC1 and PC2, the proximity of the positions of S6, S7, and S9 on the plot indicates that these samples had a similar structure in terms of their element content.

Antimicrobial activity

The antimicrobial activity of ten pollen samples was investigated for three bacteria and one yeast. Different levels of antimicrobial activity in pollen samples were determined against *B. cereus* ATCC 10876 and *S. aureus* ATCC 29213 (Table 5). Sample S9 showed the highest antibacterial activity against *B. cereus* (14.00 mm, MIC= 4.17 mg/mL), and S2 the highest against *S. aureus* (13.00 mm, MIC=8.33 g/mL). No antimicrobial activity was observed against *E. coli* and *C. albicans* in any of the pollen samples. *S. aureus* and *B. cereus* are common human pathogens that cause serious infections in humans (Bottone, 2010; Campanile *et al.*, 2015). All pollen samples, except S3 and S4, showed antibacterial activity against *S. aureus*. When compared with Nikolaieva *et al.* (2019) and Kaškonienė *et al.* (2020), who investigated the antimicrobial activities of natural and fermented pollens, our values were very close to values of the natural pollens, whereas they were at a very low level compared to fermented pollens.

All pollen samples had the same characteristics and properties regarding MIC and inhibition zones of *C. albicans*, *B. cereus* and *E. coli.* When *S. aureus* is considered, all samples exhibited similar behaviour except for S3 and S4, which could not eliminate the bacteria. These findings can be statistically supported by a cluster analysis (Fig. S5 [suppl]), where S3 and S4 formed a cluster (70.97% similarity), and the other samples formed separate clusters.

EDI and health risk assessment

The risk to human health was assessed based on bee pollen intake, considering the RfD values together with EDI (Tables 6 and 7). In this study, the calculation of risk was made according to the worst-case scenarios, and it was determined that the THI values of the essential and toxic elements examined were not greater than 1, except for Se for men, women and children (Table 7). However, due to the high Se content, SHI values were found to be greater than 1 for men, women, and children. SHI values were greater than 1 due to Se, which is naturally found in the soil and is an essential element for humans. In addition, THI values of the toxic Cd element were found to be less than 1 for men, women, and children.

Table 6. Estimated daily intake (EDI) values of bee pollens for men (M), women (W) and children (C). ND: not detected.

Samples		Cr (III)	Mn	Fe	Co	Ni	Cu	Zn	Se	Cd
S1	M	0.00088	0.01200	0.07000	ND	0.00026	0.00300	0.01500	0.00400	0.00009
	W	0.00100	0.01400	0.08200	ND	0.00030	0.00400	0.01800	0.00400	0.00011
	C	0.00200	0.02800	0.16400	ND	0.00060	0.00800	0.03500	0.00800	<0.00001
S2	M	0.00300	0.01800	0.08900	ND	0.00100	0.00500	0.02200	0.00300	<0.00001
	W	0.00200	0.01900	0.12200	ND	0.00057	0.00300	0.02500	0.00500	0.00010
	C	0.00600	0.04300	0.20800	ND	0.00300	0.01200	0.05000	0.00800	<0.00001
S3	M	0.00200	0.01600	0.10500	ND	0.00049	0.00200	0.02100	0.00500	0.00009
	W	0.00200	0.01900	0.12200	ND	0.00057	0.00300	0.02500	0.01000	0.00010
	C	0.00500	0.03900	0.24500	ND	0.00100	0.00500	0.05000	0.01000	0.00021
S4	M	0.00100	0.02200	0.11000	ND	0.00100	0.00500	0.03000	0.00400	0.00008
	W	0.00100	0.02600	0.12800	ND	0.00200	0.00600	0.03500	0.00400	0.00009
	C	0.00200	0.05200	0.25600	ND	0.00300	0.01100	0.07000	0.00900	0.00018
S5	M	0.00100	0.02100	0.05000	ND	0.00100	0.00800	0.01900	0.00400	0.00007
	W	0.00200	0.02400	0.05800	ND	0.00100	0.00900	0.02200	0.00500	0.00008
	C	0.00300	0.04900	0.11600	ND	0.00200	0.01800	0.04400	0.01000	0.00016
S6	M	0.00200	0.01200	0.03600	<0.00001	0.00200	0.00300	0.01600	0.00300	0.00009
	W	0.00200	0.01400	0.04200	<0.00001	0.00200	0.00400	0.01900	0.00400	0.00001
	C	0.00500	0.02800	0.08500	0.00001	0.00400	0.00800	0.03700	0.00800	0.00022
S7	M	0.00200	0.01900	0.00700	ND	0.00200	0.00400	0.01900	0.00300	0.00008
	W	0.00300	0.02200	0.00800	ND	0.00200	0.00500	0.02200	0.00400	<0.00001
	C	0.00500	0.04400	0.01600	ND	0.00400	0.00900	0.04400	0.00800	0.00018
S8	M	0.00100	0.01600	0.00700	ND	0.00100	0.00400	0.01700	0.00400	0.00009
	W	0.00100	0.01900	0.00850	ND	0.00100	0.00400	0.02000	0.00400	<0.00001
	C	0.00200	0.03700	0.01700	ND	0.00300	0.00900	0.04000	0.00800	0.00020
S9	M	0.00100	0.01000	0.00900	ND	0.00100	0.00300	0.01900	0.00400	0.00011
	W	0.00100	0.01200	0.01100	ND	0.00100	0.00400	0.02200	0.00400	0.00013
	C	0.00200	0.02400	0.02200	ND	0.00300	0.00800	0.04300	0.00800	0.00026
S10	M	0.00200	0.01300	0.08800	ND	0.00085	0.00400	0.01700	0.00300	0.00006
	W	0.00200	0.01500	0.10300	ND	0.00099	0.00400	0.02100	0.00400	0.00007
	C	0.00400	0.03000	0.20500	ND	0.00200	0.00900	0.04100	0.00800	0.00015

Conclusions

Türkiye has suitable ecological conditions for beekeeping activities because it is at the intersection of three phytogeographical regions and has its own topographic and climatic conditions, which cause the blooms of plants located in different geographic locations to occur at different periods of the year. The taxa detected in pollen samples were found to belong to many plants regardless of whether the flowering period was long or short in this study. The antioxidant activities of pollen samples were evaluated using methods with different principles. The chemical structure of phenolic compounds was effective on antioxidant activity, although the total phenol and flavonoid levels in the pollen did not directly affect the antioxidant activity in our study. Our results showed as well that the amount and combinations of these compounds were effective on the antioxidant and antimicrobial activities of pollen.

Pollen samples were found to be rich in the essential element (Se); however, all pollen samples had toxic Cd contamination. According to the health risk assessment, if adults consume daily 0.04 mg of pollen and children 0.02 mg, there may be a possibility of toxic effects due to Se. Pollens have potential bioactive properties, its content needs to be studied extensively to be used for cosmetic, pharmacy, and food industry.

Table 7. Target hazard index (THI) and sum of hazard index (SHI) levels of bee pollens for men (M), women (W) and children (C).

		Cr (III)	Mn	Fe	Co	Cu	Zn	Se	Cd	Ni	SHI
S1	M	0.00058	0.08800	0.10049	ND	0.09000	0.05000	0.73000	0.09000	0.01300	1.16100
	W	0.00068	0.10300	0.11724	ND	0.10400	0.06000	0.85000	0.10400	0.01500	1.34000
	C	0.00100	0.20600	0.23448	ND	0.20800	0.12000	1.70300	0.20900	0.03000	2.68000
S2	M	0.00200	0.13400	0.12755	ND	0.12500	0.07200	0.71000	0.13700	0.05600	1.30700
	W	0.00100	0.13800	0.17516	ND	0.06500	0.08200	1.05300	0.10300	0.02800	1.62000
	C	0.00400	0.31200	0.29762	ND	0.29100	0.17000	1.65700	0.32000	0.13000	3.05000
S3	M	0.00100	0.11800	0.15014	ND	0.05600	0.07000	0.90300	0.08800	0.02400	1.38800
	W	0.00100	0.13800	0.17516	ND	0.06500	0.08200	1.05300	0.10300	0.02800	1.62000
	C	0.00300	0.27600	0.35032	ND	0.13000	0.16500	2.10600	0.20700	0.05700	3.23800
S4	M	0.00066	0.15800	0.15691	ND	0.12200	0.10000	0.75300	0.07800	0.07300	1.36900
	W	0.00077	0.18500	0.18306	ND	0.14200	0.10000	0.87900	0.09100	0.08500	1.59800
	C	0.00100	0.37000	0.36611	ND	0.28500	0.20000	1.75800	0.18200	0.17000	3.19600
S5	M	0.00090	0.15000	0.07111	ND	0.19800	0.06200	0.85700	0.07000	0.05300	1.40900
	W	0.00100	0.17500	0.08296	ND	0.23100	0.07300	0.10000	0.08100	0.06200	1.64300
	C	0.00200	0.35000	0.16591	ND	0.46200	0.14600	2.00000	0.16300	0.12400	3.28700
S6	M	0.00100	0.08500	0.05191	0.01100	0.08800	0.05300	0.68000	0.18600	0.09300	1.15500
	W	0.00100	0.09900	0.06056	0.01300	0.10300	0.06200	0.79200	0.10900	0.10900	1.23900
	C	0.00300	0.19700	0.12113	0.02700	0.20500	0.12400	1.58400	0.21700	0.21800	2.47900
S7	M	0.00100	0.13400	0.00994	ND	0.10000	0.06300	0.70500	0.07900	0.09700	1.09200
	W	0.00100	0.15700	0.01160	ND	0.11500	0.07400	0.82300	0.09200	0.11300	1.27400
	C	0.00300	0.31300	0.02320	ND	0.23100	0.14700	1.64600	0.18400	0.22600	2.54800
S8	M	0.00100	0.11300	0.01038	ND	0.09600	0.05600	0.72200	0.08700	0.07000	1.08600
	W	0.00100	0.13200	0.01212	ND	0.11200	0.06500	0.84200	0.10200	0.08200	1.26700
	C	0.00200	0.26500	0.02423	ND	0.22400	0.13100	1.68500	0.20400	0.16400	2.53400
S9	M	0.00051	0.07400	0.01368	ND	0.08500	0.06200	0.73300	0.11000	0.07000	1.07900
	W	0.00060	0.08600	0.01596	ND	0.10000	0.07300	0.85500	0.12900	0.08200	1.25900
	C	0.00100	0.17200	0.03191	ND	0.20000	0.14500	1.71100	0.25700	0.16500	2.51800
S10	M	0.00100	0.09200	0.12578	ND	0.10000	0.05900	0.69000	0.06400	0.04200	1.12400
	W	0.00100	0.10700	0.14675	ND	0.10000	0.06800	0.80500	0.07400	0.05000	1.31100
	C	0.00200	0.21000	0.29350	ND	0.20000	0.13700	1.61000	0.14900	0.01000	2.62300

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References

Adaškevičiūtė V, Kaškonienė V, Kaškonas P, Barčauskaitė K, Maruška A, 2019. Comparison of physicochemical properties of bee pollen with other bee products.

Biomolecules 9(12): 819. https://doi.org/10.3390/biom9120819

Aldgini HMM, Abdullah Al-Abbadi A, Abu-Nameh ESM, Alghazeer RO, 2019. Determination of metals as bio indicators in some selected bee pollen samples from Jordan. Saudi J Biol Sci 26: 1418-1422. https://doi.org/10.1016/j.sjbs.2019.03.005

Alimoglu G, Guzelmeric E, Yuksel PI, Celik C, Deniz I, Yesilada E, 2021. Monofloral and polyfloral bee pollens: Comparative evaluation of their phenolics and bioactivity profiles. LWT 142: 110973. https://doi.org/10.1016/j.lwt.2021.110973

Azanu D, Styrishave B, Darko G, Weisser JJ, Abaidoo RC, 2018. Occurrence and risk assessment of antibiotics in water and lettuce in Ghana. Sci Total Environ 622: 293-305. https://doi.org/10.1016/j.scitotenv.2017.11.287

Basim E, Basim H, Özcan M, 2006. Antibacterial activities of Turkish pollen and propolis extracts against plant bacterial pathogens. J Food Eng 77: 992-996. https://doi.org/10.1016/j.jfoodeng.2005.08.027

Blois MS, 1958. Antioxidant determinations by the use of a stable free radical. Nature 181: 1199-1200. https://doi.org/10.1038/1811199a0

Bottone EJ, 2010. Bacillus cereus, a volatile human pathogen. Clin Microbiol Rev 23: 382-398. https://doi.org/10.1128/CMR.00073-09

Campanile F, Bongiorno D, Perez M, Mongelli G, Sessa L, Benvenuto S, *et al.,* 2015. Epidemiology of *Staphylococcus aureus* in Italy: first nationwide survey, 2012. J Glob Antimicrob Resist 3: 247-254. https://doi.org/10.1016/j.jgar.2015.06.006

Chung YC, Chang CT, Chao WW, Lin CF, Chou ST, 2002. Antioxidative activity and safety of the 50 ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. J Agric Food Chem 50: 2454-2458. https://doi.org/10.1021/jf011369q

Davis PH, 1965-1985. Flora of Turkey and the East Aegean Islands. Edinburgh University Press.

De-Melo AAM, Estevinho LM, Moreira MM, Delerue-Matos C, de Freitas ADS, Barth OM, de Almeida-Muradian LB, 2018. A multivariate approach based on physicochemical parameters and biological potential for the botanical and geographical discrimination of Brazilian bee pollen. Food Biosci 25: 91-110. https://doi.org/10.1016/j.fbio.2018.08.001

De Souza RR, de Abreu VHR, de Novais JS, 2019. Melissopalynology in Brazil: a map of pollen types and published productions between 2005 and 2017. Palynology 43: 690-700. https://doi.org/10.1080/01916122.2018.1542355

Defays D, 1977. An efficient algorithm for a complete link method. Comput J 20: 364-366. https://doi.org/10.1093/comjnl/20.4.364

Demir F, Kipcak AS, Dere Ozdemir O, Moroydor Derun E, 2020. Determination of essential and non-essential element concentrations and health risk assessment of some commercial fruit juices in Turkey. J Food Sci Technol 57(12): 4432-4442. https://doi.org/10.1007/s13197-020-04480-9

Denisow B, Denisow-Pietrzyk M, 2016. Biological and therapeutic properties of bee pollen: a review. J Sci Food Agr 96: 4303-4309. https://doi.org/10.1002/jsfa.7729

Di Paola-Naranjo RD, Sánchez-Sánchez J, González-Paramás AMA, Rivas-Gonzalo JC, 2004. Liquid chromatographic-mass spectrometric analysis of anthocyanin composition of dark blue bee pollen from *Echium plantagineum*. J Chromatogr A 1054: 205-210. https://doi.org/10.1016/j.chroma.2004.05.023

Dinis TC, Madeira VM, Almeida LM, 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 315: 161-169. https://doi.org/10.1006/abbi.1994.1485

Dinkov D, Stratev D, 2016. The content of two toxic heavy metals in Bulgarian bee pollen. Int Food Res J 23: 1343.

Erik S, Tarıkahya B, 2004. Türkiye Florası Üzerine. Kebikeç 17.

Freire KR, Lins A, Dórea MC, Santos FA, Camara CA, Silva T, 2012. Palynological origin, phenolic content, and antioxidant properties of honeybee-collected pollen from Bahia, Brazil. Molecules 17: 1652-1664. https://doi.org/10.3390/molecules17021652

Freitas A, de Arruda VAS, de Almeida-Muradian LB, Barth OM, 2013. The botanical profiles of dried bee pollen loads collected by *Apis mellifera* (Linnaeus) in Brazil. Sociobiology 60: 56-64. https://doi.org/10.13102/sociobiology.v60i1.56-64

Gökce H, Alpaslan YB, Zeyrek CT, Ağar E, Güder A, Özdemir N, Alpaslan G, 2019. Structural, spectroscopic, radical scavenging activity, molecular docking and DFT studies of a synthesized Schiff base compound. J Mol Struct 1179: 205-215. https://doi.org/10.1016/j.molstruc.2018.11.005

Harif Fadzilah N, Jaapar MF, Jajuli R, Wan Omar WA, 2017. Total phenolic content, total flavonoid and antioxidant activity of ethanolic bee pollen extracts from three species of Malaysian stingless bee. J Apic Res 56: 130-135. https://doi.org/10.1080/00218839.2017.1287996

Hotelling H, 1933. Analysis of a complex of statistical variables into principal components. J Educ Psychol 24: 417. https://doi.org/10.1037/h0071325

Kao YT, Lu MJ, Chen C, 2011. Preliminary analyses of phenolic compounds and antioxidant activities in tea pollen extracts. J Food Drug Anal 19(4): 3. https://doi.org/10.38212/2224-6614.2177

Karkar B, Şahin S, Güneş ME, 2018. Antioxidative effect of Turkish chestnut bee pollen on DNA oxidation system and its phenolic compounds. The Journal of Food 43: 34-42. https://doi.org/10.15237/gida.GD17055

Kaškonienė V, Adaškevičiūtė V, Kaškonas P, Mickienė R, Maruška A, 2020. Antimicrobial and antioxidant activities of natural and fermented bee pollen. Food Biosci 34: 100532. https://doi.org/10.1016/j.fbio.2020.100532

Komosinska-Vassev K, Olczyk P, Kaźmierczak J, Mencner L, Olczyk K, 2015. Bee pollen: Chemical composition and therapeutic application. Evid-based Compl Altern Med 2015: 297425. https://doi.org/10.1155/2015/297425

Lau P, Bryant V, Ellis JD, Huang ZY, Sullivan J, Schmehl DR, *et al.,* 2019. Seasonal variation of pollen collected by honey bees (*Apis mellifera*) in developed areas across four regions in the United States. PLoS One 14(6): e0217294. https://doi.org/10.1371/journal.pone.0217294

Liu F, Ooi V, Chang S, 1997. Free radicals scavenging activity of mushroom polysaccharide extract. J Life Sci 60: 763-771. https://doi.org/10.1016/S0024-3205(97)00004-0

Mateo R, Bosch-Reig F, 1998. Classification of Spanish unifloral honeys by discriminant analysis of electrical conductivity, color, water content, sugars, and pH. J Agric Food Chem 46: 393-400. https://doi.org/10.1021/jf970574w

Miura K, Kikuzaki H, Nakatani N, 2002. Antioxidant activity of chemical components from sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) measured by the oil stability index method. J Agric Food Chem 50: 1845-1851. https://doi.org/10.1021/jf011314o

Mutlu B, Karakuş Ş, 2015. A new species of *Sisymbrium* (Brassicaceae) from Turkey: Morphological and molecular evidence. Turk J Bot 39: 325-333. https://doi.org/10.3906/bot-1404-28

Nikolaieva N, Kačániová M, González JC, Grygorieva O, Nôžková J, 2019. Determination of microbiological contamination, antibacterial and antioxidant activities of natural plant hazelnut (*Corylus avellana* L.) pollen. J Environ Sci Health B 54: 525-532. https://doi.org/10.1080/03601234.2019.1603756

Oyaizu M, 1986. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. Japan J Nutr Diet 44: 307-315. https://doi.org/10.5264/eiyogakuzashi.44.307

Özcan M, 2004. Inhibition of *Aspergillus parasiticus* NRRL 2999 by pollen and propolis extracts. J Med Food 7: 114-116. https://doi.org/10.1089/109662004322984806

Özcan MM, Aljuhaimi F, Babiker EE, Uslu N, Ceylan DA, Ghafoor K, *et al.,* 2019. Determination of antioxidant activity, phenolic compound, mineral contents and fatty acid compositions of bee pollen grains collected from different locations. J Apic Sci 63: 69-79. https://doi.org/10.2478/jas-2019-0004

Özkalp B, Özcan MM, 2010. Antibacterial activity of pollen and propolis extracts. J Food Agric Environ 8: 17-19.

Pascoal A, Rodrigues S, Teixeira A, Feás X, Estevinho LM, 2014. Biological activities of commercial bee pollens: Antimicrobial, antimutagenic, antioxidant and anti-inflammatory. Food Chem Toxicol 63: 233-239. https://doi.org/10.1016/j.fct.2013.11.010

Pearson K, 1895. Note on Regression and inheritance in the case of two parents. Proc R Soc London, Ser A 58: 240-242. https://doi.org/10.1098/rspl.1895.0041

Rios J, Recio M, Villar A, 1988. Screening methods for natural products with antimicrobial activity: A review of the literature. J Ethnopharmacol 23: 127-149. https://doi.org/10.1016/0378-8741(88)90001-3

Roman A, Popiela-Pleban E, Migdał P, Kruszyński W, 2016. As, Cr, Cd, and Pb in bee products from a Polish industrialized region. Open Chem 14: 33-36. https://doi.org/10.1515/chem-2016-0007

Ruch RJ, Cheng SJ, Klaunig JE, 1989. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 10: 1003-1008. https://doi.org/10.1093/carcin/10.6.1003

Rzepecka-Stojko A, Stojko J, Kurek-Górecka A, Górecki M, Kabała-Dzik A, Kubina R, *et al.,* 2015. Polyphenols from bee pollen: Structure, absorption, metabolism and biological activity. Molecules 20: 21732-21749. https://doi.org/10.3390/molecules201219800

Sardar AA, Khan ZD, Perveen A, Farid S, Khan IU, 2014. In vitro antioxidant potential and free radical scavenging activity of various extracts of pollen of *Typha domigensis* Pers. Pak J Pharm Sci 27(2): 279-284.

Shapiro SS, Wilk MB, 1965. An analysis of variance test for normality (complete samples). Biometrika 52: 591-611. https://doi.org/10.1093/biomet/52.3-4.591

Singh DD, Thind PS, Sharma M, Sahoo S, John S, 2019. Environmentally sensitive elements in groundwater of an industrial town in India: Spatial distribution and human health risk. Water 11(11): 2350. https://doi.org/10.3390/w11112350

Slinkard K, Singleton VL, 1977. Total phenol analysis: automation and comparison with manual methods. Am J Enol Vitic 28: 49-55.

Spearman C, 1904. 'General intelligence,' objectively determined and measured. Am J Commun Psychol 15: 201-293. https://doi.org/10.2307/1412107

Sun L, Guo Y, Zhang Y, Zhuang Y, 2017. Antioxidant and anti-tyrosinase activities of phenolic extracts from rape bee pollen and inhibitory melanogenesis by cAMP/MITF/TYR pathway in B16 mouse melanoma cells. Front Pharmacol 8: 104. https://doi.org/10.3389/fphar.2017.00104

Temizer IK, Guder A, Turkmen Z, Celemli OG, 2017. Gas chromatography and mass spectrometry analysis, chemical contents and antioxidant properties of *Onobrychis* spp.(Fabaceae) pollen collected by honeybees. Fresenius Environ Bull 26: 962-968.

Temizer İK, Güder A, Temel FA, Esin A, 2018. A comparison of the antioxidant activities and biomonitoring of

heavy metals by pollen in the urban environments. Environ Monit Assess 190: 462. https://doi.org/10.1007/s10661-018-6829-6

Temizer İK, Güder A, Türkmen Z, 2019. Assessment of palynological characterization and total phenol-flavonoid content of some honeys from Ordu in Turkey. Erzincan Üniversitesi Fen Bilimleri Enstitüsü Dergisi 12: 1275-1282.

TSMS, 2022. Turkish State of Meteorological Service, TSMS (2021). https://www.mgm.gov.tr [02/07/2022].

Tutun H, Aluç Y, Kahraman HA, Sevin S, Yipel M, Ekici H, 2022. The content and health risk assessment of selected elements in bee pollen and propolis from Turkey. J Food Compos Anal 105: 104234. https://doi.org/10.1016/j.jfca.2021.104234

Wiegand I, Hilpert K, Hancock RE, 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols 3: 163. https://doi.org/10.1038/nprot.2007.521

Wodehouse RP, 1935. Pollen grains. McGraw-Hill Book Co., NY.

Zafeiraki E, Kasiotis KM, Nisianakis P, Manea-Karga E, Machera K, 2022. Occurrence and human health risk assessment of mineral elements and pesticides residues in bee pollen. Food Chem Toxicol 161: 112826. https://doi.org/10.1016/j.fct.2022.112826