

A comparison of the antioxidant activities and biomonitoring of heavy metals by pollen in the urban environments

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Abstract Pollen is one of the most valuable nutrients due to its content and antioxidant activity. In this study, its botanic origin, total phenol content (TPC), total flavonoid content (TFC), the hydrogen peroxide scavenging activity (HPSA) (in terms of SC_{50}), ferric reducing antioxidant power capacity (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (in terms of SC_{50}), metal-chelating activity (MCA) (%), and heavy metal content were examined to determine the quality of pollen that has been collected from seven different cities of Turkey. According to the melissopalynological analysis, the botanic origin of samples is six uni-flora and one multiflora. The TPC, TFC, HPSA, FRAP, DPPH, and MCA were found between 1360.70–2981.34 mg GAE/100 g, 74.23–111.74 mg CAE/100 g, 25.56–30.28 $\mu\text{g/mL}$, 71.92–73.86%, 52.26–53.27 $\mu\text{g/mL}$, and 43.97–65.21%, respectively. When obtained results compared to the standards (Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT) and α -Tocopherol (TOC)), pollen

samples showed the effective antioxidant properties with respect to HPSA, FRAP, and DPPH radical scavenging activity. In addition, it was observed that honey samples were being contaminated with most of the metals to some extent (Al, Cr, Mn, Fe, Ni, Cu, Zn, B, As, Te, U), while some heavy metals (Co, Cd, V, Ga, and Ag) were never determined in all samples. However, Pb was determined only in sample 2 and sample 4, Mo in sample 1 and sample 2. According to meteorological parameters, samples 3, 4, and 5 were distinguished from the other samples. Finally, the data indicate that pollen could be affected by environmental pollutions.

Keywords Antioxidant activity · Melissopalynological analysis · Pollen · Heavy metal · Biomonitoring

Introduction

Pollen is male gametophyte of the plants. Pollens are collected from different flowers by honey bee colonies (*Apis mellifera* L.) for the purpose of brood breeding and larval feeding. To improve beekeeping activities, it is also important to determine the main pollen sources of a region. Therefore, many studies were performed on honey bee (*Apis mellifera* L.) colonies such as (Gabriele et al. 2015) in Italia, (Ismail et al. 2013) in Egypt, (Feás et al. 2012) in Portugal, (Sabo et al. 2011) in Croatia, (Almeida-Muradian et al. 2005) in Brazil, and in Turkey (Altunatmaz et al. 2017; Kızılpınar et al. 2016).

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Pollen consists of proteins, carbohydrates, lipids, vitamins, minerals, and polyphenolic compounds. Polyphenolics mainly phenol and flavonoid can be responsible for human health and wellbeing. In addition, total phenol and flavonoid positively impact antioxidant power. Therefore, a great number of studies carried out antioxidant quality tests (Feás et al. 2012; Gabriele et al. 2015; Silva et al. 2009). Pollen is used as a drug to fight against some diseases such as cancer, heart diseases, and diabetes (Roman et al. 2016; Formicki et al. 2013). However, pollen is exposed to environmental pollutants such as heavy metals, organic pollutants, pesticides, a pathogenic microorganism, GMO (genetically modified organisms), and radioactivity (Bogdanov 2006). Among all these pollutants, heavy metals are industrial, agricultural, combustion events and traffic originated (Kocaer and Başkaya 2003; Bogdanov 2006; Dinkov and Stratev 2016). The high quantities of metals show the toxic effects while the limited quantities of heavy metals such as Fe, Cu, Zn, and Mn can be important for all living organisms. And other heavy metals are highly toxic (Ojuederie and Babalola 2017; Gergen et al., 2006). There are very few studies published on heavy metal contamination of pollen (Conti and Botre 2001; Madras-Majewska et al. 2002; Madras-Majewska and Jasinski 2003).

Pollen is considered valuable food for the human, but its content or the effect of environmental pollution on it is not evaluated by the consumer. However, there is the limited study about this situation in the literature. The main goal of this study is to draw attention to the heavy metal pollution in pollen samples. Additionally, it is also to produce useful information for beekeepers, the honey industry, and other industries that use pollen as a raw material in their product by characteristics of high-quality pollen.

Pollen is important bio-indicator; it reflects anthropogenic environment pollution (Dinkov and Stratev 2016). There are few studies that show botanic origin, antioxidant power, and heavy metal content of pollen in Turkey. Therefore, the objectives of this study were to examine (i) botanic origin, (ii) antioxidant activities, and (iii) the effectiveness of pollens as a bio-indicator of the presence of heavy metals in the environment by comparing results from different sampling areas. Pollen is considered valuable food for the human, but its content or the effect of environmental pollution on it is not evaluated by the consumer. However, there is the

limited study about this situation in the literature. The main goal of this study is to draw attention to the heavy metal pollution in pollen samples. Additionally, it is also to produce useful information for beekeepers, the honey industry, and other industries that use pollen as a raw material in their product by characteristics of high-quality pollen.

Materials and methods

Pollen samples

Pollen samples were collected from seven regions (numbered from 1 to 7) of Turkey in 2016 (Fig. 1). Pollen analysis was carried out according to the Louveaux method with minor modifications (Louveaux et al. 1978). According to the method, 2 g of pollen samples was mixed with 50 mL water for 15 min. Then, 10 mL of this mixture was attenuated with 30 mL water. After this stage, the routine pollen analysis procedures were applied. The microscope examination (Nikon Eclipse Ci) was performed in the bright field with a magnification of $\times 1000$. Pollen diagnosis was done according to reference slides obtained from local flora, and related palynology books and atlases (Sorkun 2000; Erik and Doğan 2002).

Sample preparation

All the reagents used were pro-analysis: 2,2-diphenyl-1-picrylhydrazyl, butylated hydroxyanisole, butylated hydroxytoluene, gallic acid, quercetin, α -tocopherol (Sigma); folin-ciocalteu's reagent, absolute ethyl alcohol (Merck). All other chemicals were analytical grade.

Two grams of pollen samples mixed with 100 mL absolute ethanol and this suspension was mixed for 24 h at the room temperature by using a magnetic stirrer. Then, this extraction solution was filtered via Whatman no: 4 filter paper and stored at the 4 °C.

Measurement of total phenol content

Amount of total phenol compound of pollen extract used in this research was determined according to the Folin-Ciocalteu reactive and the Slinkard and Singleton method (Slinkard and Singleton 1977). The sample was placed in ethyl alcohol solution (0.5 mL) and then deionized water (7.0 mL) was added. After 3 min,



Fig. 1 Map of collection locations of honey bee pollen samples in Turkey (Google earth)

Na_2CO_3 (2%, 2.0 mL) was added and incubated at room temperature shaking occasionally for 2 h. The absorbance of compounds was measured at 760 nm. Total phenol compound of the sample was calculated with the aid of gallic acid calibration curve ($R^2 = 0.9827$).

Measurement of total flavonoid content

Total flavonoid component of pollen extracts was measured according to the aluminum chloride colorimetric method (Chung et al. 2002). Ethyl alcohol solution (0.5 mL) and deionized water (1.5 mL) were added. Then, $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (10.0%, 0.1 mL) and 1 M CH_3COOK (0.1 mL) were added and were diluted by adding 2.8 mL deionized water. After it was incubated at room temperature for 30 min, its absorbance was immediately measured at 415 nm. Sample's total flavonoid compounds were calculated with the aid of the catechin's calibration curve which was used as a standard ($R^2 = 0.9720$).

Hydrogen peroxide scavenging activity designation

Hydrogen peroxide scavenging activity of the sample used in the study (prepared with 0.04 M, pH = 7.4 using phosphate buffer) was done according to Ruch method (Ruch et al. 1989). For this, 3.4 mL was taken from the sample, and 0.6 mL of H_2O_2 (40 mM) (0.04 M, pH = 7.4 prepared from phosphate buffer) was added. After

10 min, the absorbance of the mixture was measured at 230 nm versus blank sample. Phosphate buffer (0.04 M, pH = 7.4) which does not include hydrogen peroxide was used as a blank sample. The decrease in absorbance value shows that hydrogen peroxide scavenging activity designation of the sample is high. Hydrogen peroxide scavenging activity designation of the sample is expressed as SC_{50} values ($\mu\text{g/mL}$).

Determination of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of pollen extract solutions which were prepared by ethyl alcohol was implemented according to Blois method (Blois 1958). 3.0 mL was taken from sample solutions and 1.0 mL DPPH $^{\bullet}$ (0.1 mM) prepared by adding absolute ethanol was mixed with it and then blended properly. It was kept at room temperature in dark. After 30 min, an absorbance of mixtures was measured at 517 nm. Free radical scavenging activity was evaluated by drawing free calibration graphics. Free radical scavenging activities of reaction mixtures were calculated by using absorbance estimations 30 min later. A decrease in absorbance estimation is a demonstration of high rate of free radical scavenging activity. Free radical scavenging activity of the sample is expressed through the type of SC_{50} ($\mu\text{g/mL}$).

Designation metal-chelate activity

The metal-chelate activity (MCA) of pollen extract solution and standard antioxidant materials prepared in absolute ethanol was practiced according to the Dinis method (Dinis et al. 1994). 0.4 mL was taken from extract solution and standard antioxidant material, and it was incubated for 10 min by adding 0.05 mL FeCl₂ (2 mM) on it. Then, it was completed to 4 mL by adding 0.2 mL ferrozine (5 mM) and absolute ethanol. This mixture was incubated at room temperature after those were shaken potently and the absorbance of mixtures was calculated at 562 nm. A decrease of absorbance estimation is the demonstration for high level of metal-chelate activities of extracts and standard antioxidant materials. Metal-chelate activity of extract and standard antioxidant material were calculated according to the formula below:

Ferrous ions chelating activity (%)

$$= [1 - (A_s/A_c)] \times 100 \quad (1)$$

where A_c is control's absorbance values and A_s is extracts or standard materials absorbance values.

Heavy metal analysis

In the present study, bioaccumulation and biosorption of heavy metals in pollen samples were studied and 20 heavy metal levels of the samples were determined by using ICP-MS Spectrometer (Model Bruker 820-MS). Microwave digestion method was used to prepare the pollen samples. 0.5 g of each samples was digested in 5 mL of HNO₃ (ultrapure) and 2 mL of HCl (ultrapure) at 210 °C at 1600 W for 35 min (20 min for the solution preparation and 15 min for incubation) in CEM MARS-5 Closed Vessel Microwave Digestion System. These solutions were colorless and clear after the cooling samples were placed in a capped falcon tube and were completed to 50 mL with ultrapure water. And these samples were analyzed by Bruker 820-MS ICP-MS after filtered through a membrane filter (0.45 µm). A calibration curve was made by using the certified multi-element standard. An intermediate stock of 10 mg/L was prepared from the main stock solution and a calibration curve was drawn from standard stocks of 5, 10, 20, 50, 100, and 250 µg/L. Samples were prepared in triplicate and 10 readings were taken in each parallel ICP-MS

device. The blank sample prepared in 1% HNO₃ solution was read 20 times. The standard slope, LOD (detection limit), and LOQ (determination limit) were determined 3, 3, and 10 times, respectively.

Statistical analysis

Statistical analyses were carried out using R software. Spearman correlation analysis was performed to analyze the relationship between meteorological parameters and heavy metal contents of pollens. To identify structures and to emphasize variation and bring out strong patterns in a dataset cluster and principal component analysis (PCA) was performed. Among meteorological parameters (between years 1992 and 2016), mean temperature, mean sunshine hours, and mean of total monthly rainfall were all obtained from the Republic of Turkey Ministry of Environment and Forestry, State Meteorological Internet Service.

Results and discussions

Pollen spectrum

In this study, *A. mellifera* colonies prefer plants to get pollen (Table 1) and dominant pollens as *Rutaceae* in sample 1, *Sambucus* L. in sample 4, *Brassicaceae* in sample 2, *Astragalus* L. in sample 5, *Papaver* L. in sample 6, and *Ononis* L. in sample 7, whereas seconder pollens as *Gentiana* L. sample 1, *Asteraceae-Lakun* typ, *Geraniaceae*, *Sambucus* in sample 3, and *Lathyrus* L. and *Myrtaceae* in sample 7. Botanic origin of samples was agreement with their regional vegetation. Many studies emphasized that these plants are important for honey bees (Çelemlı and Sorkun 2012; Diaz-Losada et al. 1998; Sorkun 2008; Serra Bonvehí 1988).

Hydrogen peroxide and DPPH free radical are used to determine antioxidant and radical scavenging properties of natural and synthetic materials. Results from these tests can be used for evaluation of antioxidant activity. In DPPH scavenging activity assay, DPPH stable radical accepts given hydrogen or electron by donor molecule. Consequently, a non-radical form of DPPH occurs in the reaction. While a radical form of DPPH absorbs at 517 nm, a non-radical form does not absorb. Thereupon, the degree of discoloration indicates the scavenging potentials of the antioxidant compounds which can be detected at 517 nm (Kaştaş et al. 2017).

Table 1 Pollen grains belong to taxa and its percent amount

Taxa	Samples						
	S1	S2	S3	S4	S5	S6	S7
<i>Achillea</i>	—	8.6	—	—	—	—	—
<i>Anchusa</i>	—	—	9.7	—	—	—	—
<i>Anthemis</i>	—	—	—	3.95	—	—	—
Apiaceae	—	—	—	5.26	1.5	—	—
Asteraceae-echinate typ	—	—	—	—	3	4.83	1.09
Asteraceae-lakun typ	1	12.24	18.79	3.95	5	—	2.72
<i>Astragalus</i>	—	—	—	—	75	—	—
Brassicaceae	—	1.91	—	—	—	—	—
<i>Campanula</i>	—	0.38	1.82	—	—	—	—
<i>Castanea</i>	—	—	—	7.89	—	—	—
<i>Centaurea</i>	—	—	1.82	10.53	4	0.2	—
<i>Dipsacus</i>	—	—	—	—	0.5	—	—
<i>Epilobium</i>	—	—	1.21	—	0.5	—	—
Fabaceae	—	3.25	—	5.26	—	1.01	—
<i>Gentiana</i>	20	—	10.3	5.26	—	—	—
Geraniaceae	—	1.91	18.18	—	5	1.01	—
Lamiaceae	—	0.96	—	1.32	2.5	—	1.63
<i>Lathyrus</i>	—	—	—	—	2.5	—	19.57
<i>Myosotis</i>	—	—	—	—	—	3.42	—
Myrtaceae	—	—	—	—	—	—	17.93
<i>Onobrychis</i>	5	—	—	7.89	—	—	—
<i>Ononis</i>	—	—	—	—	—	—	52.17
<i>Papaver</i>	—	—	—	—	—	89.13	—
<i>Plantago</i>	—	—	—	—	0.5	—	—
Poaceae	—	—	—	—	—	0.4	—
Rhododendron	—	—	14.55	—	—	—	—
Rosaceae	—	3.82	—	1.32	—	—	4.89
Rutaceae	70	—	—	—	—	—	—
<i>Sambucus</i>	—	66.92	15.15	47.37	—	—	—
<i>Sanguisorba</i>	2.5	—	—	—	—	—	—
<i>Thalictrum</i>	—	—	8.48	—	—	—	—
<i>Trifolium</i>	1.5	—	—	—	—	—	—

Although not very reactive oxygen species, hydrogen peroxide is generated in living organisms, this active compound can be toxic to cells due to hydroxyl radical formation. For this reason, hydrogen peroxide scavenging activity is seen as very important property by the researchers (Temel et al. 2015). Hydrogen peroxide and DPPH radical scavenging activities of pollen samples range from 25.56 to 30.28 and 52.26 to 53.27 $\mu\text{g/mL}$, respectively. Graikou et al. (2011) studied Greek pollen

and its DPPH radical scavenging activity was found as 181.40 $\mu\text{g/mL}$. Freire et al. (2012) investigated Bahia (Brazil) pollen samples. Their DPPH radical scavenging activities range from 10.70 to 209.10 $\mu\text{g/mL}$. Stojko et al. (2015) carried out the antioxidant activity of Polland pollen by using the different extraction mixtures and their DPPH radical scavenging activities were indicated in the range of 20.91–178.09 $\mu\text{g/mL}$. Fadzilah et al. (2017) performed the Trigona pollens obtained

from Malaysia and their activities range from 860 to 3240 $\mu\text{g/mL}$. Feás et al. (2012) practiced Portugal pollen samples and they found the DPPH radical scavenging activities in the range of 2000–4300 $\mu\text{g/mL}$. Ulusoy and Kolaylı (2014) studied Anzer pollen samples and their activities range from 650 to 8200 $\mu\text{g/mL}$. DPPH radical scavenging activities of investigated pollen samples are generally higher than compared to literature data.

Ferric reducing power (FRAP) assay can be used as a significant evidence of potential antioxidant activity. In this assay, antioxidant compounds bring about a reduction from $[\text{Fe}(\text{CN})_6]^{3-}$ complex to $[\text{Fe}(\text{CN})_6]^{4-}$. When Fe^{2+} interacts with the composed complex, Perl's Prussian Blue ($\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$) complex occur and this complex can be monitored at 700 nm easily (Güder et al. 2014). Ferric reducing the power of pollen samples were changed in the range of 71.92–73.86%. According to obtained results, samples show very effective FRAP activity than the standards except for BHA. Ulusoy and Kolaylı (2014) found the FRAP activity of Anzer pollens between the range of 11.77–105.06 mmol Trolox/g pollen.

Phenolic components show antioxidant properties via free radicals scavenging activity. These radicals are the active oxygen species such as singlet oxygen, hydroxyl radicals, and nitric oxide radicals. The other important group is flavonoids and their products in plants. They function as antioxidant components as well as phenolic compounds (Güder and Korkmaz 2012). Total phenolic and flavonoid contents of pollen samples changed from 1360.70 to 2981.34 mg GAE/100 g and 74.23 to 111.74 mg CAE/100 g, respectively. Whereas hydrogen peroxide scavenging activities of samples are higher than standards, DPPH radical scavenging activities are lower. Carpes et al. (2007) studied phenolic and flavonoid contents obtained from Brazil. Total phenolic contents range from 360 to 1090 mg GAE/100 g. Freire et al. (2012) carried out total phenol content (TPC) of Bahia (Brazil) pollen samples, their TPC change from between 4150 and 21,320 mg GAE/100 g. Rebiai and Lanez (2012) studied TPC and total flavonoid content (TFC) of Northern Algeria pollen samples and their TPC and TFC range from 26.69 to 36.02 mg GAE/100 g and 5.71 to 14.57 mg RE (Rutin)/100 g, respectively. Stojko et al. (2015) determined the TPC and TFC of Polland pollen as 2730 mg GAE/100 g and 2022 mg QE/100 g, respectively. Cosmulescu et al. (2015) studied Romania pollen samples and their TPC

and TFC contents were found in the range of 1080–1764 mgGAE/100 g and 732–795 mg QE/100 g, respectively. Fadzilah et al. (2017) investigated the Trigona pollens obtained from Malaysia, and TPC and TFC of pollen samples range from 3346 to 13,593 mg GAE/100 g and 1528 to 3180 mg QE/100 g, respectively. Ulusoy and Kolaylı (2014) determined TPC of Anzer pollen samples between the range of 4407–12,410 mg GAE/100 g.

In MCA, the complex is generated between ferrozine and Fe^{2+} . When chelating another agent added to the reaction tube, the intensity of the red color of ferrozine- Fe^{2+} complex decreases because of the disruption of a complex. For this reason, lower absorbance indicates higher metal-chelating activity of chelating agent (Alaşalvar et al. 2017). Antioxidant activity, total phenol, total flavonoid content, and metal-chelating activities of the pollen samples are represented in Table 2. Metal-chelating activities of the pollen samples range from 43.97 to 65.21% in this study; however, samples are not forceful as well as standards.

Heavy metal contents of pollen samples

The findings of heavy metal contents in tested pollen samples are shown in Table 3. According to the results, all pollen samples were determined to be contaminated with most of the tested heavy metals to some extent. Some of the heavy metals (Co, Cd, V, Ga, and Ag) were never encountered in all pollen samples.

Table 2 Antioxidant activity, total phenol, and total flavonoid content of samples

Sample	HPS ^a	FRAP ^b	DPPH ^a	TPC ^c	TFC ^d	MCA ^b
Sample 1	27.10	73.16	53.24	1605.25	78.09	43.97
Sample 2	27.13	72.95	53.27	2981.34	111.74	50.76
Sample 3	29.09	71.92	53.04	1660.00	92.13	65.21
Sample 4	26.71	73.86	52.47	1360.70	74.23	60.82
Sample 5	26.27	72.51	52.51	1824.26	92.04	49.14
Sample 6	25.56	72.29	52.26	1692.86	111.69	54.65
Sample 7	30.28	72.58	52.39	1583.35	110.49	55.93
BHA	184.13	92.02	8.53	—	—	89.95
BHT	147.49	54.16	9.01	—	—	86.26
TOC	216.26	32.98	11.97	—	—	93.41

^aSC₅₀ value ($\mu\text{g/mL}$)

^bActivity % (at the 100 $\mu\text{g/mL}$ concentration)

^cmg GAE/100 g

^dmg CAE/100 g

Table 3 Heavy metal contents of tested pollen samples

Metals (mg/kg)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Al	83.301	59.089	35.709	92.359	108.472	11.991	13.605
Cr	0.201	0.322	0.491	0.368	0.437	0.537	0.240
Mn	14.943	14.593	12.357	21.594	21.278	12.471	39.918
Fe	89.062	74.812	51.916	91.034	125.321	37.184	33.556
Co	—	—	—	—	—	—	—
Ni	0.651	—	1.285	0.354	0.339	0.637	0.629
Cu	9.072	2.679	4.755	4.712	5.192	2.984	5.240
Zn	22.719	13.276	10.298	14.217	23.504	19.048	21.424
Cd	—	—	—	—	—	—	—
Pb	—	0.572	—	0.103	—	—	—
B	11.660	19.320	1.172	16.400	31.538	6.326	16.045
V	—	—	—	—	—	—	—
Ga	—	—	—	—	—	—	—
As	6.100	8.182	7.106	6.251	7.354	8.507	5.710
Mo	0.392	0.038	—	—	—	—	—
Ag	—	—	—	—	—	—	—
Te	0.112	0.134	0.139	0.129	0.124	0.153	0.099
Tl	0.025	0.009	—	0.002	0.007	—	—
Bi	0.057	—	0.108	0.031	0.055	0.167	—
U	0.126	0.138	0.138	0.129	0.125	0.129	0.094

Among all heavy metals, Fe had the highest content with 33.556–125.321 mg/kg in all pollen samples. Following Fe, Al, Mn, and Zn had the other highest concentration levels. These findings are quite high compared to other studies (Kalbande et al. 2008). The other metals in all pollen samples were determined in increasing order of U (0.094–0.138 mg/kg), Te (0.099–0.139 mg/kg), Cr (0.201–0.537 mg/kg), B (1.172–31.538 mg/kg), Cu (2.984–9.092 mg/kg), and As (6.100–8.507 mg/kg). Ni was determined ranged from 0.339 and 0.651 mg/kg in all pollen samples except for sample 2.

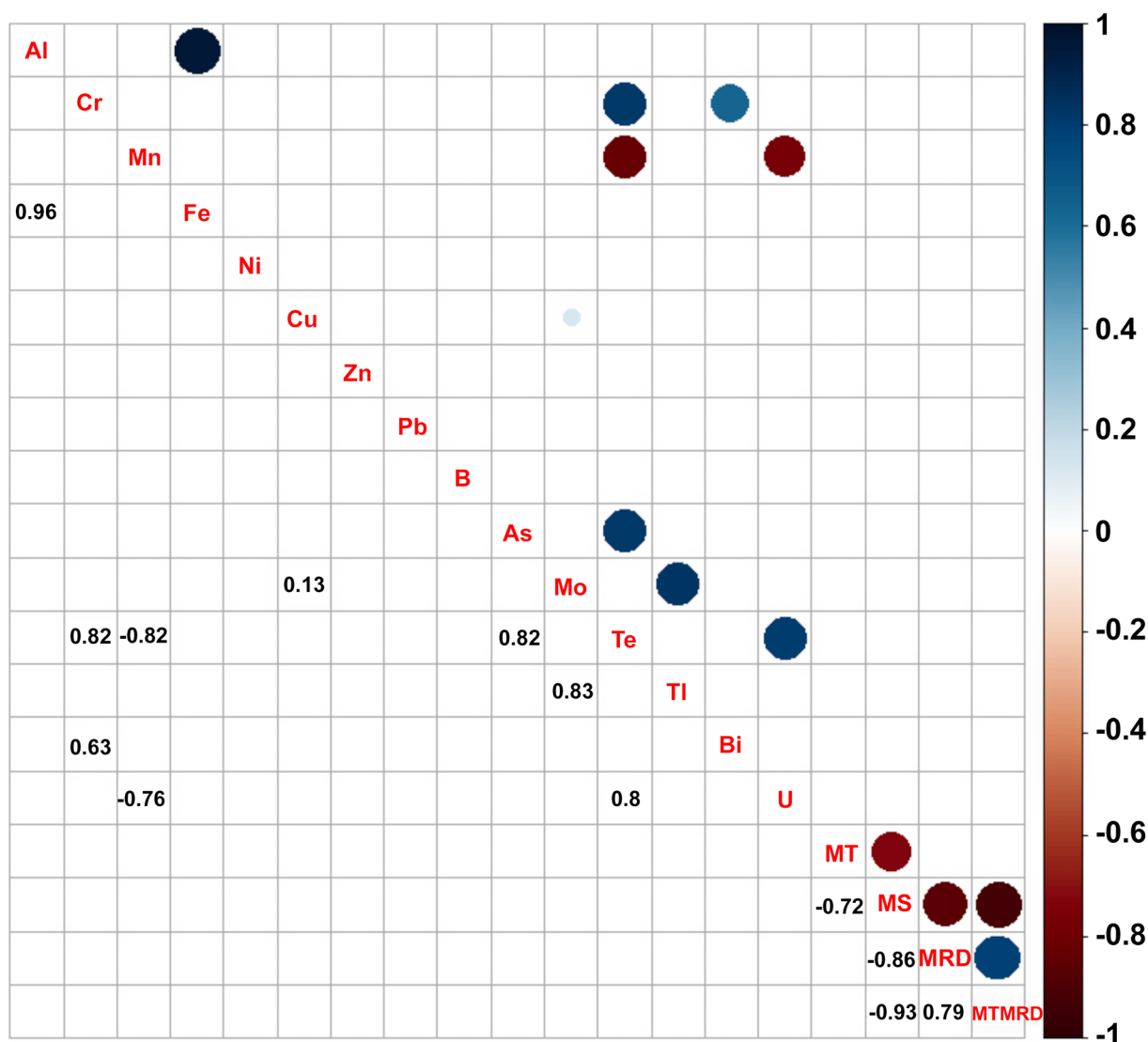
Bi was found as 0.031–0.167 mg/kg in all pollen samples except for samples 2 and 7. Pb was found as 0.572 and 0.103 mg/kg in sample 2 and sample 4

collected in Gümüşhane and Giresun (cities in Turkey), respectively. Tl observed in samples 1, 2, 4, and 5 had found a lowest concentration with 0.002–0.025 mg/kg while the other heavy metals had important amounts. And Mo was also found with low quantities only in samples 1 and 2 collected in Erzincan and Gümüşhane cities. The pollen samples taken in Afyon and Kırklareli cities had the lowest heavy metal contents and amounts. The trend of the presence of heavy metals in all pollen samples is shown as sample 5 > sample 4 > sample 1 > sample 2 > sample 3 > sample 6 > sample 7.

Fe, Al, Mn, and Zn were determined to be bio-sorbed and bio-concentrated in remarkable quantities in all pollen samples compared to the literature (Harmanescu et al.

Table 4 Meteorological parameters of tested pollen samples

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Mean temperature	10.9	9.7	14.3	14.4	14.4	11.3	13.3
Mean sunshine (h)	81	67.1	49.4	43.9	52.6	80.2	68.1
Mean rainfall days	100.3	117.2	172.5	159.9	155.8	107.3	98.4
Mean of total monthly rainfall	373.5	464.2	2304.1	1288.4	1042.2	439	570.1



MT: Mean temperature; MS: Mean sunshine (hours); MRD: Mean rainfall days; MTMRD: Mean of total monthly rainfall days

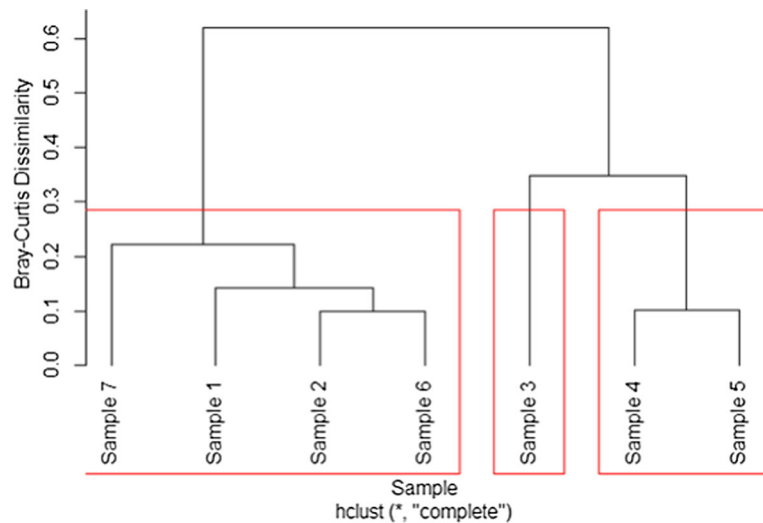
Fig. 2 Significant correlation matrix of meteorological parameters and heavy metal contents of pollen samples

2007; Kalbande et al. 2008; Arslan and Arkan 2013). Especially, due to traffic and human consumption, low Pb concentrations of only two samples among tested pollen samples were a positive finding. But, these findings were quite high compared to other studies (Dinkov and Stratev 2016; Conti and Botre 2001; Harmanescu et al. 2007; Kalbande et al. 2008). As a result of air pollution and agricultural activities, As was determined at considerable quantities in all pollen samples. Data related to the Cr and Cu compared to the other studies were found quite high concentration in all pollen samples (Conti and Botre 2001; Harmanescu et al. 2007; Kalbande et al. 2008;

Arslan and Arkan 2013). The most important factors of nickel contamination are mine spoilage, liquidation furnaces, and refinery arresters. The lower Ni concentrations compared to the literature were found in pollen samples (Formicki et al. 2013; Kalbande et al. 2008).

Meteorological parameters

Meteorological parameters (between years 1992 and 2016), mean temperature, mean sunshine hours, and mean of total monthly rainfall were all obtained from the Republic of Turkey Ministry of Environment and

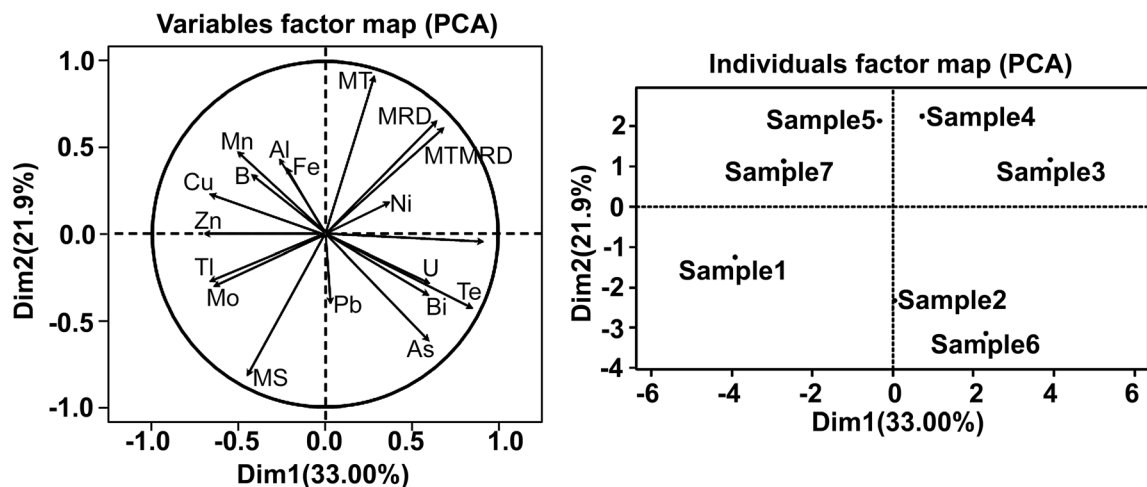
Fig. 3 Bray-Curtis dissimilarity dendrogram

Forestry, State Meteorological Internet Service (Table 4).

Statistical evaluation

A statistically significant correlation analysis of meteorological parameters and heavy metal contents of pollens are shown in Fig. 2. Positive correlations are displayed in blue and negative correlations in red color. Color intensity and the size of the circle are proportional to the correlation coefficients. Figure 2 shows that non-significant correlation was observed between meteorological parameters and heavy metal contents. Higher

significant positive correlations were observed between Al-Fe ($r = 0.96$, $p < 0.05$), Cr-Te ($r = 0.82$, $p < 0.05$), As-Te ($r = 0.82$, $p < 0.05$), Mo-Ti ($r = 0.83$, $p < 0.05$), Te-U ($r = 0.80$, $p < 0.05$), mean rainfall days-mean of total monthly rainfall ($r = 0.79$, $p < 0.05$), whereas higher significant negative correlations were observed between mean temperature-mean sunshine (hours) ($r = -0.72$, $p < 0.05$), mean sunshine (hours)-mean rainfall days ($r = -0.86$, $p < 0.05$), mean sunshine (hours)-mean of total monthly rainfall ($r = -0.93$, $p < 0.05$). Lower significant positive correlations were observed for Cr-Bi ($r = 0.63$, $p < 0.05$) and Cu-Mo ($r = 0.13$, $p < 0.05$), respectively.

**Fig. 4** PC1-PC2 generated from a correlation matrix of PCA

To quantify the difference between collected ecological data at different sampling locations, Bray-Curtis is one of the most well-known and reasonable way to achieving this goal. Ward's method joins clusters based on minimizing the within-group sum of squares, and it tends to produce compact well-defined clusters.

The results of the cluster analysis are shown in a dendrogram in Fig. 3. A dendrogram illustrates the relationships between all the samples based on a defined measure of similarity. In this study, the dendrogram reflects the relationship between samples (city) based on heavy metal and meteorological parameters. The samples within a cluster are much more similar to each other than samples in other clusters. Clusters grow by the joining of smaller clusters but the similarity decreasing.

The y-axis is the measure of the dissimilarity (1-similarity) at which clusters join. The most similar (90% similarity) samples were sample 2–sample 6 and sample 4–sample 5. Based on 30% dissimilarity (70% similarity), seven samples joined at similar levels, suggesting the presence of three main clusters that displayed by red boxes. The first main cluster contained four samples: sample 1, sample 2, sample 6, and sample 7. Sample 2 and sample 6 were the most similar and join to form the first cluster, followed by sample 1 and sample 7. The second main cluster contained only sample 3 that is the most different sample. The third main cluster contained sample 4 and sample 5.

Principal component analysis (PCA) was performed to visualize the patterns in the dataset (Fig. 4). The first three principal components (PCs) represented 74.46% of total variance. With Eigen values of 6.271, 4.163, and 3.713, the first three PCs that were estimated from the eigenvectors of the correlation matrix represented 33.003, 21.913, and 19.545% of the total variance. The pollen samples were well separated and classified into subgroups in the PC1-PC3 plot. Although PC1-PC3 also showed a classification, its plot was not included here since the PC1-PC2 plot gave convenient results.

From Fig. 4a, Zn was correlated with PCs directly. The negative part of PC1 was related with Cr, Te, Bi, U, As, Pb, mean sunshine hours, Mo, and Ti; the rest of the variables were related with the positive part of PC1. From Fig. 4b, the score plot indicated outliers (sample 3, sample 6, sample 1, and sample 7) that distinguished from the other samples especially by significantly higher contents of heavy metal or meteorological parameters. Due to the highest mean rainfall days and mean of total monthly rainfall, sample 3 differentiated

from the other samples. Samples 4 and 5 have the similar mean temperature. Especially high contents of Al-Fe-Mn-B in sample 7, Ti-Mo in sample 1, and Pb in sample 2 were observed. Both cluster and PCA gave similar results. Therefore, the given variables can be used to classify these pollen samples.

Conclusion

The biochemical properties of pollen samples vary according to their botanical origin and samples have a potential for pharmaceutical, medical, and food industry. All the samples have pollen grains belong to *Asteraceae* and *Fabaceae* family. These families are important for beekeeping activities. A part of the presented study aimed to evaluate whether pollens can be taken into consideration as reliable bio-indicator for heavy metal pollution. The plants are exposed to air pollutants such as traffic, dust, and burning and sorbed them. This situation cause unwanted impact on viability and biochemical composition of pollens. The quality of pollens is a measure of the quality of regional and local environment. Therefore, pollens exposed to pollution had to be considered as an allergen; moreover, pollen allergens increase allergy symptoms in polluted cities. From the statistical analysis, it was obtained that seven pollen samples were clustered in three groups according to the 70% similarity. PCA indicated that the difference between samples according to meteorological parameters was observed especially in sample-3 by mean rainfall days and mean of total monthly rainfall, in samples 4 and 5 by similar mean temperature. Sample 1, sample 2, and sample 7 differentiated due to heavy metal contents. Finally, our results indicate that pollens were effective in assessing the level of heavy metal pollution in the environment. Additionally, different plant species and organs of plant may have different contents and type of heavy metals. Therefore, complex studies were performed on all bee products can be useful for monitoring of environmental pollution by heavy metals. Also, antioxidant activity can be seen very important properties because of the protective effect against the exogenous and endogenous oxidant molecules in living organisms. Therefore, the formation and elimination of the radical form must be balanced. These antioxidant components in these samples will be isolated and characterized by using chromatographic and spectroscopic techniques in the future.

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