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RESEARCH ARTICLE

Bee pollens as biological indicators: An ecological assessment of pollution in Northern Turkey via ICP-MS and XPS analyses

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Abstract

In this study, pollens were collected from 25 different locations of Northern Turkey to investigate pollution monitoring. Surface chemistry of pollen samples was characterized by X-ray photoelectron spectroscopy (XPS). Then the concentrations of certain elements (Li, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, Ba, and Pb) in pollen samples were determined by inductively coupled plasma mass spectrometry (ICP-MS) for the evaluation of environmental pollution. The levels of elements were detected in the following ranges (minimum–maximum, mg/kg dry pollen): Li (0.18–0.39), Al (24.98–308.04), V (6.18–98.58), Cr (1.05–6.81), Mn (13.85–95.91), Fe (52.20–326.26), Co (0.15–0.34), Ni (1.66–10.79), Cu (8.61–19.01), Zn (20.47–70.02), As (1.22–2.65), Se (0.39–0.67), Cd (0.05–0.74), Ba (0.73–16.30), and Pb (0.00–0.26). It has been concluded that there is a correlation between the pollen samples with high heavy metal concentrations and traffic density as these regions are closer to the road in the northern region. It is exposed to pollution from various sources such as intensified urbanization and tourism activities carried out on land and sea; industrial activities are increasing rapidly due to the opportunities offered by the coastal areas, sea transportation, and agricultural, domestic, and industrial pollution coming from the inner regions through rivers and streams. In this sense, pollens can be used as potential bio-indicators for monitoring heavy metal pollution and gives an idea about how we can use them for future assessing purposes.

Keywords Bee pollen · Environmental pollution · Biological indicator · XPS · ICP-MS

Introduction

Bee pollen is a mixture of saliva and nectar (or honey) produced by young bees when they land on a flower. They have a unique chemical composition that includes carbohydrates, proteins, and bioactive compounds such as amino acids, lipids, vitamins, carotenoids, and polyphenols. Therefore, honeybee pollens are valuable natural herbal substances with a variety of therapeutic applications for humans, especially in medical and nutritional uses (Ares et al. 2018; Denisow and Denisow-Pietrzyk 2016; Komosinska-Vassev et al. 2015; Margaoan et al. 2019; URCAN et al. 2017). They have antioxidant, anti-inflammatory, antibacterial, anticarcinogenic, and antiallergic activities that affect different body functions appropriately (Denisow and Denisow-Pietrzyk 2016; Kieliszek et al. 2018; Komosinska-Vassev et al. 2015; Kostić et al. 2020; Li et al. 2018).

Besides organic compounds, honeybee pollens are rich sources of various minerals (Kieliszek et al. 2018; Kostić et al. 2015; Li et al. 2018). The role of nutritional and physiologically important elements in completing deficiencies is of great importance. However, the inherent elements of bee pollen material enable them to be used not only for nutrition purposes but also for other purposes. The presence of minor and trace elements selected is important in detecting and monitoring environmental pollution (Conti and Botre 2001).

In recent years, there have been studies suggesting that bee products and pollen are important indicators in

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monitoring environmental pollution by being affected by environmental pollution. Various technological (mining, air and water pollution, various wastes) and natural pollution (originating from mines and soil structure) pass to all these biological products through soil, air, and water. Despite the nutritional value of pollen and its ability to prevent many diseases, it is also known that heavy metals that may be present in them can lead to many types of cancer (Antwi et al. 2015; Matés et al. 2010; Turkdogan et al. 2003). Pollution in water, soil, and air can be measured directly, and these results can provide information about environmental pollution, in this case information about heavy metal to be taken from drinking water or breathing air. However, this information does not give clear information about how much of the pollution affects living things and how much of it is passed on to humans due to biological material. In this case, the composition of pollens is directly affected by the plants as they are exposed to chemicals through air, water, and soil (Altunatmaz et al. 2017). These pollens may include some heavy metals that may come from the air by adsorption. In addition, some of them might comprise these heavy metals in their structure via plants. Pollen, which is used as an important food source in this respect, is considered among the important biomarkers (Aldgini et al. 2019) that can also be considered as a measure of natural and anthropogenic environmental pollution (Dinkov and Stratev 2016). In Turkey, several researchers have investigated some heavy metal levels by ICP-MS analyses in pollen samples and used them as bio-indicators (Altunatmaz et al. 2017; Altunatmaz et al. 2018; Matin et al. 2016; Silici et al. 2016; Temizer et al. 2018). Several works have also shown that bee products can be used as a bio-indicator based on the results of various heavy metals analyzed (Al Naggar et al. 2013; Formicki et al. 2013; Morgano et al. 2012). Hence, it has been revealed that pollens and bee products are important bio-indicators that provide information about environmental pollution.

This article evaluates the results of the presence of elements found in honeybee pollens in Northern Turkey which has specific geographic properties. Geographic properties are important for pollens in terms of both trace metals and minerals. Although trace elements are necessary for humans, toxic metals can also be transported through pollens due to environmental conditions. Therefore, it is important to examine the mineral content of pollens in terms of both human health and indirectly to see the environmental pollution effect (Pohl et al. 2020).

The purpose of this study is to determine the elemental composition of honeybee pollen samples by ICP-MS collected from Northern Turkey including heavy metals and to designate how pollens can be used as biological indicators of environmental pollution. It has also been shown by XPS that functional groups present on the pollen surfaces can be associated with heavy metal sequestration.

Materials and methods

Collection of samples and pre-treatment

Honeybee pollen samples from 25 different locations of Marmara and Black Sea regions of Turkey were obtained. In order to avoid the moisture absorption of honeybee pollen samples, they were kept in glass bottles and stored at − 20 °C. Finally, they were dried at 40 °C in the oven and grounded with the aid of a mortar prior to acid digestion. A minimum of 2 and a maximum of 3 samples were collected from each location. The name and the GPS coordinates of all locations were given in Table 1. The location of all sampling sites were also designated in Fig. 1 using Google Maps according to their GPS coordinates.

Table 1 Distribution of honeybee pollen samples in Northern Turkey and their surface elemental composition by mass (%) via XPS

Pollen No	Sampling locations	GPS coordinates *X* and *Y*
1	Balıkesir Çamlık	39.66241, 27.81656
2	Balıkesir Aygören	39.64758, 27.95522
3	Bursa Osmangazi	40.17944, 29.02272
4	Bursa İnegazi	40.1308, 28.8681
5	Kocaeli Tepeköy	40.80067, 29.97694
6	Kocaeli Umuttepe	40.82402, 29.92516
7	Yalova Karadere	40.64458, 29.47886
8	Yalova Hasan Baba	40.63114, 29.12452
9	Yalova Gelincik	40.66564, 29.30856
10	İstanbul Polonez	41.10857, 29.16601
11	İstanbul Beykoz	41.14338, 29.17801
12	İstanbul Bahçeköy	41.17941, 28.98587
13	Çorum İskilip	40.73093, 34.47108
14	Çorum Oğuzlar	40.75929, 34.69897
15	Çanakkale Hamidiye	40.10188, 26.41503
16	Çanakkale Ulupınar	40.09635, 26.47668
17	Ardahan Sabaholdu	41.30551, 43.11375
18	Ardahan Sevimli	41.16224, 42.98613
19	Ardahan Center	41.0691, 42.78529
20	Rize Taşlık	41.03807, 40.60577
21	Rize Pekmezli	41.01239, 40.58819
22	Ordu Perşembe	41.06662, 37.76797
23	Ordu Devecik	40.9647, 36.98829
24	Bayburt Erenli	40.26193, 40.24675
25	Bayburt Kırkpınar	40.28031, 39.96487

Fig. 1 Location of 25 sampling sites for bee pollen samples collected from Northern Turkey

Sample preparation for measurements

To decompose the organic matrix of the dried honeybee pollen samples and release the elements into their solutions, the closed-vessel high-pressure microwave-assisted wet digestion (MWD) was applied. After weighing the pollen samples in PFA Teflon vessels, 2.0 mL of HNO_3 (nitric acid) and HCl (hydrochloric acid) mixture (5:2 v/v) was added (Adaskeviciute et al.2019; Temizer et al.2018). The pollen samples were left to dissolve using microwave digestion oven (CEM Mars 5 digestion oven) under the conditions of 1600 W and 210 °C for 20 min. After, the vessels were allowed to cool. Finally, the solutions were vortexed, and deionized water was added, totalling its volume to 10 mL prior to ICP-MS analyses. The experiments were performed three times, and relative standard deviations were calculated for each pollen sample.

Method validation

As reported, an appropriate certified reference material (CRM) of the bee pollen matrix is not commercially available (Pohl et al. 2020). For this reason, certified reference material, CRM (BCR®279, sea lettuce *Ulva lactuca*), was used for the validation of our method due to its complexity as bee pollen matrix. According to the analyses performed ($n=6$), the results obtained are consistent with CRM values of the elements present in the sample.

ICP-MS analysis of elements

Once the method has been validated, a total of 25 elements were detected, including mineral and heavy metals (Li, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, Ba, and Pb) on 25 pollen samples using ICP-MS instrument (Bruker 820-MS ICP-MS spectrometer). In ICP-MS analyses, Sc (45), Y (89), and In (115) elements were used as internal standards to compensate the matrix effects. The measurements were repeated 10 times, and the mean values were determined. ICP-MS plasma conditions were given as follows: plasma flow, 16.50 L/min; auxiliary flow, 1.65 L/min; sheath gas flow, 0.20 L/min; nebulizer flow, 0.98L/min; sampling depth, 6.00 mm; and power, 1.40 kW.

XPS analyses of bee pollen samples and data acquisition

The pollen samples were also dried in the oven at 40 °C prior to XPS analyses. After, they were placed into Thermo Scientific K-Alpha XPS instrument sample holder using carbon tape. Subsequently, monochromatic Al Kα X-rays (1486.6 eV) with a diameter of 300 µm were sent to each sample surface. Survey spectra and high-resolution spectra for each sample were acquired with pass energies of 30 eV and 200 eV, respectively, by taking 10 scans to improve the resolution. The pressure in the analysis chamber was 1×10^{-8} mbar. After the analyses ($n=3$), data were evaluated using Avantage XPS software (5.9915) package. Peak fitting was performed using Gaussian/Lorentzian peak shapes and a Shirley/Smart type background.

Results

Evaluation of elemental analysis data in bee pollens

In the present study, the concentrations of 15 elements in bee pollen samples were selected and determined since they may reflect the environmental pollution in Northern Turkey. These elements were, namely, Li, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, Ba, and Pb. The results of the analyses were given in Table 2 as mg/kg pollen dry weight.

The minimum and maximum levels of elements were found as followed: Li (0.18 mg/kg) in Ordu Devecik and Ardahan Merkez (0.39 mg/kg); Al in Bayburt Kırkpınar (24.98 mg/kg) and Ardahan Sabaholdu (308.04 mg/kg); V in Yalova Karadere (6.18 mg/kg) and Ordu Perşembe (98.58 mg/kg); Cr in Rize Pekmezli (1.05 mg/kg) and Ordu Perşembe (6.81 mg/kg); Mn in Bayburt Kırkpınar (13.85 mg/kg) and Balıkesir Çamlık (95.91 mg/kg); Fe in Bayburt Kırkpınar (52.20 mg/kg) and Çanakkale Hamidiye (326.26 mg/kg); Co in Bursa İnegazi (0.15 mg/kg) and Balıkesir Çamlık (0.34 mg/kg); Ni in Bayburt Kırkpınar Village (1.66 mg/kg) and Balıkesir Çamlık (10.79 mg/kg); Cu in Bayburt Kırkpınar (8.61 mg/kg) and Çanakkale Hamidiye (19.01 mg/kg); Zn in Bayburt Kırkpınar (20.47 mg/kg) and Çanakkale Hamidiye (70.02 mg/kg); As in Yalova Karadere (1.22 mg/kg) and Ordu Perşembe (2.65 mg/kg); Se in Çorum Oğuzlar (0.39 mg/kg) and Ardahan Center (0.67 mg/kg); Cd in Yalova Gelincik (0.05 mg/kg) and Balıkesir Aygören (0.67 mg/kg); Ba in Ordu Perşembe (0.73 mg/kg) and Balıkesir Aygören (16.30 mg/kg); and Pb in many locations (< 0.005 mg/kg) and Ardahan Sevimli (0.26 mg/kg). According to these results, it might be deduced that bee pollen samples in Ordu Perşembe were highly contaminated with V, Cr, As, Se, and Ba. It had also high levels of Al, Mn, Ni, Cu, and Zn, whereas it was very contaminated with Cd and Pb. On the other hand, Bayburt Kırkpınar had the lowest pollution levels relatively when these elements were considered. Among the analyzed bee pollen samples, the content of

Table 2 *Concentration (mg/kg) of elements determined in the honeybee pollen samples collected from Northern Turkey

Pollen No	Li	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Cd	Ba	Pb
1	0.24	108.21	12.99	1.15	95.91	257.37	0.34	10.79	14.28	62.54	1.41	0.50	0.09	4.17	0.00
2	0.23	48.91	19.45	1.29	17.34	71.80	0.19	2.99	11.68	38.62	1.30	0.59	0.74	16.30	0.01
3	0.22	63.02	15.64	1.45	25.66	117.09	0.17	4.26	12.38	44.55	1.52	0.45	0.07	4.56	0.00
4	0.24	89.90	17.96	1.53	21.10	157.08	0.15	4.40	13.01	48.15	1.54	0.56	0.08	2.32	0.03
5	0.27	87.16	19.10	2.16	58.47	206.64	0.29	10.19	16.44	56.64	1.45	0.43	0.27	3.78	0.09
6	0.26	80.45	16.53	1.43	33.10	134.07	0.20	5.12	13.28	40.16	1.45	0.48	0.14	3.83	0.00
7	0.22	77.85	6.18	1.15	36.23	105.57	0.18	3.90	10.39	30.79	1.22	0.40	0.24	8.42	0.09
8	0.23	97.21	18.55	2.20	69.44	236.07	0.26	9.60	13.59	54.00	1.64	0.43	0.10	7.21	0.07
9	0.21	62.12	15.15	1.29	22.55	132.74	0.20	3.74	12.39	43.63	1.49	0.51	0.05	4.51	0.06
10	0.23	76.64	12.31	1.52	49.10	167.14	0.27	8.13	12.66	41.66	1.31	0.42	0.20	3.55	0.00
11	0.26	155.03	24.54	1.96	24.09	177.69	0.22	5.23	12.83	40.24	1.59	0.50	0.20	2.65	0.04
12	0.23	63.22	74.55	5.21	29.81	121.85	0.24	5.22	12.50	37.45	2.09	0.48	0.18	2.50	0.03
13	0.28	183.71	17.57	2.11	95.59	326.26	0.32	10.00	19.01	70.02	1.55	0.52	0.19	14.15	0.24
14	0.32	145.69	11.51	1.08	28.85	160.44	0.21	4.31	12.80	32.17	1.43	0.54	0.07	1.96	0.00
15	0.26	157.35	17.13	1.90	21.25	171.33	0.24	5.44	10.76	41.56	1.49	0.52	0.07	9.32	0.03
16	0.20	55.21	24.34	2.20	21.37	85.44	0.24	7.05	9.86	38.54	1.33	0.39	0.16	4.28	0.00
17	0.23	308.04	29.31	2.52	42.08	136.26	0.22	4.90	14.03	34.61	1.59	0.55	0.10	3.22	0.00
18	0.28	227.53	21.67	1.73	34.97	222.16	0.33	6.23	16.42	48.80	1.43	0.49	0.53	3.65	0.26
19	0.39	214.39	22.54	1.76	30.20	223.77	0.24	6.68	17.88	34.76	1.53	0.67	0.06	2.65	0.00
20	0.21	43.41	18.42	1.37	23.19	79.66	0.26	5.52	9.39	28.59	1.34	0.46	0.14	2.34	0.00
21	0.23	77.10	12.92	1.05	18.93	141.81	0.25	5.96	9.44	27.79	1.45	0.48	0.13	2.55	0.00
22	0.25	51.57	98.58	6.81	22.24	116.22	0.27	5.79	16.78	44.96	2.65	0.44	0.13	0.73	0.00
23	0.18	79.73	22.17	1.47	37.23	105.79	0.19	3.51	14.26	35.34	1.48	0.53	0.07	2.81	0.00
24	0.20	51.66	17.64	1.19	31.51	81.51	0.20	2.71	18.93	34.70	1.42	0.45	0.09	3.05	0.00
25	0.19	24.98	21.01	1.37	13.85	52.20	0.19	1.66	8.61	20.47	1.45	0.46	0.07	1.19	0.00

*Relative standard deviation of the elemental composition of honeybee pollen samples were all below < 2%

(Results from 3 analytical replicates were calculated, and the data for each experiment were mean values)

Se, Cd, and Pb levels was found to be low in all of the locations where concentrations of As were also relatively high.

Microwave digestion procedure for CRM was performed to validate the method based on accuracy and precision. To improve the validation study, an in-house secondary reference standard solution at the concentration of 10 mg/L was produced by using the multi-element calibration solution (Merck 1.11355-ICP multi-element standard solution IV for ICP-MS). As described by previous studies (Arica et al. 2018; Horwitz 1982), precision was calculated in terms of coefficient of variation, whereas accuracy was expressed by relative error. The results of the validation study were given in Table 3, and it demonstrates that the method is accurate and precise.

Elemental composition bee pollen surface by XPS

Survey and partial screening of the elements on the pollen surface were performed by XPS, and information about the functional groups on the pollen surface was acquired. The survey spectra for the pollen samples were given in Fig. 2. Elemental characterization of pollens with % values by mass was also obtained quantitatively. In order to get information about the surface chemistry of the pollens and the functional groups present on the surface, C 1 s spectra of bee pollens were provided in supplementary part (Fig. S1).

As seen in Table 4, most pollen samples consist of C and O elements. In some examples, N element was also found, meaning that each pollen sample surface has different compositions.

Discussion

Bee pollens as bio-indication of environmental pollution

When the results obtained from many studies conducted in different countries are examined, the max and min values related to the elements are as follows, respectively: Al, 0.10–836 (mg/kg); Co, 0.01–1.2 (mg/kg); Fe, 6.6–1180 (mg/kg); Zn, 5.1–162 (mg/kg); Ni, 0.001–6.85 (mg/kg); Mn, 5.1–429.8 (mg/kg); Cu, 0.11–27.7 (mg/kg); Cr, 0.002–42 (mg/kg); Pb, 0.001–14.20 (mg/kg); As, 0.007–14.710 (mg/kg); Ba, 0.032–17.63 (mg/kg); V, 0.04–3.94 (mg/kg); Li, 0.02–2.35 (mg/kg); Cd, 0.001–15.40 (mg/kg); and Se, 0.01–5.48 (mg/kg) (Pohl et al. 2020; Sattler et al. 2016).

When reviewing the results obtained from studies conducted in Turkey, some elements belonging to the max and min values are as follows: Al, 1.20–108.5 (mg/kg); Co, 0.00 (mg/kg); Fe, 28.6–725 (mg/kg); Zn, 10.3–162 (mg/kg); Ni, 0.002–2.56 (mg/kg); Mn, 8.2–201.0 (mg/kg); Cu, 2.7–15.00 (mg/kg); Cr, 0.13–7.94 (mg/kg); Pb, 0.25–0.67 (mg/kg); As, 0.006–8.51 (mg/kg); Ba, not analyzed; V, 0.84–3.94 (mg/kg); Li, not analyzed; Cd, 0.007–0.297 (mg/kg); and Se, 0.42–19.88 (mg/kg) (Altunatmaz et al. 2017; ALTUNATMAZ et al. 2018; Kalaycıoğlu et al. 2017; Matin et al. 2016; Silici et al. 2016; Temizer et al. 2018).

When the results are examined, Ni and V levels are particularly striking. It has been observed that these two elements are higher than their maximum values found in studies in the literature. Another point to be emphasized is that there are few studies on vanadium. According to

Table 3 Method validation results using certified reference material (BCR®279, sea lettuce *Ulva lactuca*)

Elements	Reference material	*Certified value	*Measured value	RE%	CV%	R%	*LOD
Cu	BCR®279	13.1 ± 0.4	12.9 ± 0.3	1.5	2.32	98.5	0.003
Zn	BCR®279	51.3 ± 1.2	50.1 ± 1.10	2.3	2.19	97.7	0.09
As	BCR®279	3.09 ± 0.21	3.04 ± 0.16	1.6	5.26	98.4	0.001
Se	BCR®279	0.59 ± 0.04	0.58 ± 0.03	1.7	5.17	98.3	0.01
Cd	BCR®279	0.274 ± 0.022	0.271 ± 0.019	1.1	7.4	98.9	0.00001
Pb	BCR®279	13.5 ± 0.4	13.3 ± 0.40	1.5	3.0	98.5	0.001
Li	In-house reference	10.00 ± 0.50	9.75 ± 0.16	2.5	1.6	97.5	0.0008
Al	In-house reference	10.00 ± 0.50	9.99 ± 0.23	0.1	2.3	99.9	0.03
V	In-house reference	10.00 ± 0.50	10.16 ± 0.43	1.6	4.2	101.6	0.003
Cr	In-house reference	10.00 ± 0.50	10.13 ± 0.41	1.3	4.0	101.3	0.0009
Mn	In-house reference	10.00 ± 0.50	9.85 ± 0.29	1.5	2.9	98.5	0.002
Fe	In-house reference	100.00 ± 5.00	98.62 ± 2.50	1.4	2.5	98.6	0.02
Co	In-house reference	10.00 ± 0.50	9.98 ± 0.29	0.2	2.9	99.8	0.0005
Ni	In-house reference	10.00 ± 0.50	9.81 ± 0.27	1.9	2.8	98.1	0.003
Ba	In-house reference	10.00 ± 0.50	9.79 ± 0.19	2.1	1.9	97.9	0.2

*Concentration of elements determined is expressed in mg/kg

(Results from 6 analytical replicates were calculated, and the data for each experiment were mean values)

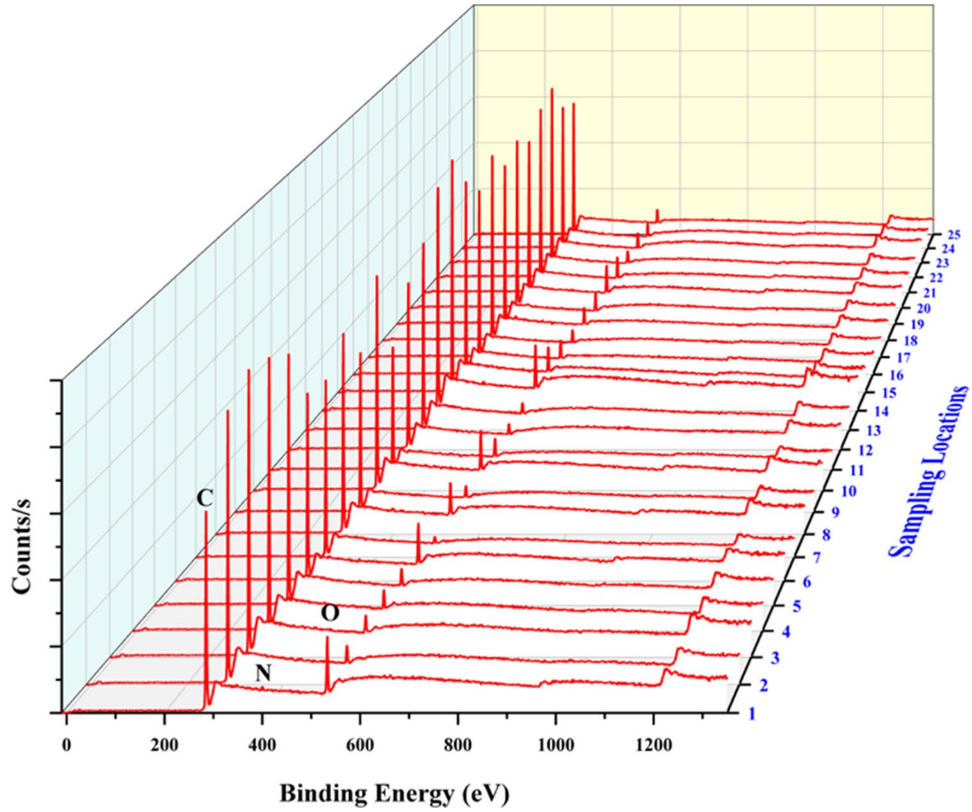

Fig. 2 XPS Survey spectra for bee pollen samples collected from 25 locations

Sattler et al. (2016), vanadium was detected in bee pollen for the first time in their own studies. In another study conducted in Turkey, maximum V concentration was reported to be 3.94 mg/kg. In this study, vanadium levels were found to be quite high. While the maximum value was 98.58 mg/kg, the average value was determined as 23.51 mg/kg. According to the reports of the State Planning Organization (DPT), there are no primary reserves or production of vanadium in Turkey. This does not mean that there is no source of vanadium in Turkey. Although there are no primary vanadium reserves established in Turkey, there may be a high level of secondary sources. There are various studies on Turkish coals and coal ashes for the determination of trace element contents. Among these, high levels of V, Co, and Ni were found when coal was burned. The ashes of these coal beds can be considered as a potential resource. As reported by DPT, asphaltite is one of many well-known vanadium resources in Turkey. Asphaltite coals are the most important resources, but not only for vanadium, but also for molybdenum and nickel. When coal is burned and turned into ash, the V_2O_5 content reaches to above 1%. It is clear that coal ash obtained from asphaltites is a serious source of vanadium and that sustainable vanadium production can be achieved from this source. In summary, the thermal power plant ash, bauxite processing wastes, and residues of petroleum or petroleum products may be potential vanadium resources in Turkey (UYSAL 2020). This indicates that the V source is of anthropogenic origin.

Cobalt analysis conducted by researchers in Turkey and Co was not detected in any samples of bee pollen (Temizer et al. 2018). In this study, Co values in bee pollens were found to be higher compared to other studies (Adaskeviciute et al. 2019; Kostić et al. 2015). It is known that most of the cobalt element is formed as a by-product of nickel refining (Pazik et al. 2016).

To the best of our knowledge, barium and lithium in bee pollen were not analyzed before in Turkey. The maximum levels of barium determined were close to the highest values in the world where the lithium values are relatively low (Pohl et al. 2020).

In this study, the results obtained for Cr, Cu, Mn, Zn Ni, Cd, and As are higher than the results found by Altunatmaz et al. (2017) on average. In another study conducted by Altunatmaz et al. (2018), 9 common elements were studied, and the average results were higher for the elements Cr, Cu, Fe, Mn, Zn, Cd, and Al except Se and Pb. When compared with a study performed by Kalaycıoğlu et al. (2017), the average Fe, Zn, Mn, Cu, Al, Ni, and V values found in the presents study are quite high. In addition, Temizer et al. (2018) collected bee pollen from similar regions of the Black Sea and investigated the concentrations of Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, As, and Pb elements. The average values we found for all elements are higher except Pb and As. Most

Table 4 Surface elemental composition of bee pollen samples by mass (%) via XPS analysis

Pollen No	C %	O %	N %
1	85.49	12.82	1.69
2	96.40	3.60	-
3	96.60	3.40	-
4	91.80	8.20	-
5	95.92	4.08	-
6	89.62	9.37	1.00
7	97.76	2.24	-
8	90.98	7.40	1.62
9	93.54	6.46	-
10	96,47	3,53	-
11	94.62	5.38	-
12	95.74	4.26	-
13	97.21	2.79	-
14	97.15	2.85	-
15	90.73	8.09	1.19
16	94.62	5.38	-
17	95.05	4.95	-
18	92.33	7.67	-
19	93.63	6.37	-
20	95.34	4.66	-
21	94.56	5.44	-
22	96.79	3.21	-
23	91.30	8.70	-
24	94.67	5.33	-
25	91.30	8.70	-

*Relative standard deviation of the elemental composition of honeybee pollen samples were all below < 1%

(3-point analyses were performed with XPS to calculate mean values with standard deviations)

of the results were higher when compared with the studies conducted in Turkey. This may also be related to the collection time. Al Naggar et al. (2013) reported that the collection time of bee pollen results in differences of up to 10 times in terms of metal content. Bee pollen collected in this study was typically exposed to air, water, and soil contamination from vehicle emissions, industrial emissions, dust spills, atmospheric deposits, wastes, or volcano activities. For rural areas and areas with high agricultural production, bee pollen contamination with selected elements might be often associated with natural fertilization and pests (Al Naggar et al. 2013; Aldgini et al. 2019; Altunatmaz et al. 2017; Formicki et al. 2013; Roman 2009; Sattler et al. 2016; Siqueira et al. 2017; Temizer et al. 2018). Especially, the northwestern regions have more industrial activity, and the higher population in these cities increased the pollution levels. On the other hand, the northeastern part of Turkey had a higher rural population which led to agricultural activities. As shown by several researchers, bee pollen has been highly susceptible to contamination from anthropogenic activities and can therefore serve as a highly sensitive biomarker of environmental pollution (Aldgini et al. 2019; Álvarez-Ayuso and Abad-Valle 2017; Conti and Botre 2001; Dima et al. 2012; Dinkov and Stratev 2016; Golubkina et al. 2016; Mejías and Montenegro 2012; Mejías et al. 2018; Morgano et al. 2010; Nascimento et al. 2018; Popescu et al. 2010; Roman 2009; Roman et al. 2016). Therefore, differences in concentration levels of elements found in bee pollen samples from different areas are related to their geochemistry, mineralogy, and population. They also reveal the complex relationships between urbanization, industrialization, and other anthropogenic activities that occur in these areas (Nascimento et al. 2018).

Surface chemistry of bee pollens and binding profiles

In the present study, surface chemistry of bee pollen samples was investigated by XPS. The XPS survey spectra of each pollen sample were demonstrated in Fig. 2 which indicates that pollen samples mainly consist of C, O, and sometimes N. High-resolution XPS spectra for the carbon element of pollen samples are given in Fig. S1. Accordingly, photoelectron peaks of carbon (C 1 s) at ~ 285 eV, nitrogen (N ls) at ~ 399 eV, and oxygen (O 1 s) at ~ 532 eV were found. While most pollen samples have C and O peaks, some pollen samples have N peaks (Rumble Jr et al. 1992). Based on the high-resolution C 1 s, N 1 s, and O 1 s spectra, the presence of functional groups in the pollen can be determined. When the C 1 s peaks of the pollen samples are examined, four different carbon bonds usually attract attention.

According to the partial spectra results of XPS from pollen samples, C peaks for all samples present four types of carbon bonding, each indicating a different functional group. The major carbon peak at ~ 285.0 eV represents C–C and/or C-H bond; ~ 286.5 eV indicates the presence of C–O–C and /or C–OH bond; ~ 287.9 eV shows O-C-O and/or C = O (carbonyl group) bond; and ~ 289.0 eV gives idea about O = C-O (carboxyl group) bonds. When oxygen peaks are examined, photoelectron peaks of 533.6 eV support the presence of carboxyl (C = O) groups, and peaks at 532.9 eV also show aliphatic C-O bonds from esters and alcohols. The presence of nitrogen peaks indicates that primary and secondary amine bonds are present in those pollens (C-N) (Bubert et al. 2002; Tan et al. 2018; Tang et al. 2016). When all the partial spectra of pollen samples were examined, it was seen that the related elements gave peaks close to their binding energy.

In all of the samples, based on the peak intensities, it can be said that the amount of each functional group explained here might change due to the structure of each pollen sample.

The XPS technique gives only information about surface chemistry of pollens as in all other samples. It does not require full composition of pollens. In the present study, XPS technique was used to give an idea about the functional groups present on the pollen surfaces on which the heavy metals were adsorbed. The functional groups on the surface of bee pollens are considered to be responsible for the adsorption of heavy metal pollution from air and/or pesticides. The metals in the environment are easily attached primarily to the functional groups on these pollen surfaces. This might be the major reasons that pollen samples have high metal uptake capacity.

Conclusion

This work emphasizes that the bee pollen is a candidate environmental specimen that can effectively be employed as a novel bio-indicator for monitoring ecological pollution. Involving XPS analyses, the study revealed the chemistry of surface functional groups having a high capacity of absorbing heavy metals. Herewith, a multi-element detection with ICP-MS has been performed to screen bee pollen samples since excess essential metals and even low levels of heavy metals are toxic to children, pregnant women, and older people. The use of this promising method in related samples will help future environmental studies.

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Author contribution Conceived and designed the experiment: MEŞ, AE, SDK, and ÖK.

Performed the experiment: MEŞ and AE.

Analyzed the data: MEŞ and AE.

Contributed materials/analysis: MEŞ, AE, SDK, and ÖK.

Wrote the article: MEŞ and AE.

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Data availability Not applicable.

Declarations

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate All authors declare that they participated in the study and in the development of this manuscript.

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