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Comprehensive Characterization of a Bee Pollen Sample from Erzurum (Türkiye) Using Integrated Palyno-Morphological, Elemental, and Volatile Analyses

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Abstract

Bee pollen (BP) is a natural product widely recognized for its rich nutritional composition and diverse biological activities. In this study, a comprehensive characterization of bee-collected pollen was performed by integrating palyno-morphological, elemental, and volatile compound analyses. Pollen samples were collected from the Olur district (Erzurum, Türkiye) and analyzed using light microscopy (LM) and scanning electron microscopy (SEM) to determine their morphological features. The results revealed distinct pollen morphotypes belonging to different plant taxa, characterized by variations in shape, aperture type, and exine ornamentation, which are critical for botanical identification. Elemental composition was determined using inductively coupled plasma–optical emission spectrometry (ICP-OES), revealing potassium (K), calcium (Ca), and magnesium (Mg) as the most abundant elements, followed by iron (Fe), manganese (Mn), and zinc (Zn). Trace elements such as copper (Cu), boron (B), nickel (Ni), and cadmium (Cd) were detected at low concentrations. These findings indicate that BP represents a valuable source of essential minerals with potential nutritional significance. The volatile profile of BP was analyzed using headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME/GC–MS). A complex mixture of volatile compounds was identified, dominated by aldehydes and terpenes. The major compounds included pelargonaldehyde, α-pinene, capronaldehyde, and enanthaldehyde, highlighting the influence of botanical origin on aroma composition. The diversity and variability of volatile compounds further confirm the complex chemical nature of BP. Overall, the integration of microscopic, elemental, and volatile analyses provides a multidimensional understanding of BP composition.

Keywords: Bee pollen, Palynology, Light microscopy, Scanning electron microscopy, Mineral composition, Volatile compounds

1. INTRODUCTION

BP, defined as the male gametophyte of flowering plants collected and processed by honeybees, has gained increasing attention as a natural functional food due to its rich nutritional composition and wide range of biological activities (Kolayli et al., 2024). It is formed through the agglutination of pollen grains with nectar and bee secretions, resulting in a complex matrix containing macronutrients such as proteins, carbohydrates, lipids, and dietary fiber, as well as micronutrients including minerals, vitamins, phenolic compounds, and volatile constituents. Owing to this diverse composition, BP has been widely recognized as a valuable dietary supplement with antioxidant, antimicrobial, anti-inflammatory, and other health-promoting properties (El Ghouzi et al., 2023).

Melliferous flora constitutes the fundamental ecological basis of apiculture, directly influencing honey yield, quality, and botanical origin. The identification and characterization of bee forage plants are therefore essential for sustainable beekeeping and honey authentication studies. Palynology, particularly melissopalynology, has emerged as a reliable scientific approach for determining the botanical and geographical origin of honey through the microscopic analysis of pollen grains (Jamwal, 2021; Khan et al., 2024). Pollen morphology provides diagnostic taxonomic characters such as size, shape, aperture type, exine thickness, and surface

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ornamentation, which are valuable for distinguishing closely related taxa and identifying floral sources of honey (Türker et al., 2025).

Recent advances in microscopy have significantly enhanced palynological investigations. Light Microscopy (LM) allows quantitative measurement of pollen parameters including polar diameter, equatorial diameter, P/E ratio, colpi dimensions, and exine thickness, while Scanning Electron Microscopy (SEM) provides high-resolution visualization of exine sculpturing and surface ornamentation patterns (Zafar et al., 2022; Khan et al., 2024). SEM-based studies have demonstrated that pollen surface features such as psilate, scabrate, echinate, reticulate, and rugulate ornamentations possess strong taxonomic and diagnostic significance in melliferous plant identification. These micro-morphological characteristics contribute not only to systematic botany but also to honey authentication and quality control.

Palyno-morphological characterization of bee forage plants has been extensively applied across different regions. For instance, Jamwal (2021) investigated selected Fabaceae members and highlighted the importance of pollen shape class, aperture type, and exine ornamentation in species discrimination. Similarly, Zafar et al. (2022) analyzed pollen ultrastructure of honeybee floral species using LM and SEM and emphasized the taxonomic value of exine sculpturing and aperture configuration in identifying frequently visited bee flora. More recently, Khan et al. (2024) demonstrated that combining melissopalynology with advanced microscopic authentication techniques enables accurate determination of honey botanical origin, supporting honey purity assessment and market credibility.

Recent research has emphasized the strong relationship between BP composition and antioxidant activity. Anık & Vardar (2024) reported that different color fractions of BP exhibited variations in total phenolic, flavonoid, and carotenoid contents depending on their botanical origins. Similarly, Boulfous et al. (2025) demonstrated that monofloral and polyfloral bee pollen samples from Algeria contained significant amounts of phenolic compounds and showed considerable antioxidant potential evaluated through DPPH, FRAP, and TAC assays. Their study also identified several bioactive phenolic compounds, including rutin, myricetin, naringenin, resveratrol, and kaempferol, using HPLC analysis.

Comprehensive characterization of BP therefore requires the integration of multiple analytical approaches. Element analysis provides insight into nutritional and safety aspects, while volatile compound analysis contributes to the identification of aroma-active and potentially bioactive molecules. Together with other chemical profiling techniques, these analyses enable a more complete understanding of the composition-function relationship in BP.

This study aims to investigate the palyno-morphological characteristics of selected BP samples using light microscopy and scanning electron microscopy techniques. By integrating microscopic characterization with palynological analysis, the study seeks to contribute to sustainable apiculture, honey quality assurance, and the understanding of bee-floral diversity. In addition, comprehensive chemical characterization of BP was conducted through elemental and volatile compound analyses to evaluate its composition. Overall, the study aims to provide an integrated assessment of the botanical origin, morphological features, and chemical composition of bee pollen samples.

2. MATERIALS AND METHODS

2.1. Material

2.1.1. Study area and sample collection

A Bee-collected pollen samples were obtained from a local beekeeper operating in Olur district, Erzurum Province, Türkiye (altitude ≈ 1,350 m). The region is characterized by a continental climate with distinct seasonal variation and diverse steppe and montane vegetation, providing a heterogeneous floral source for honeybees.

Pollen was collected using front-mounted pollen traps installed at the hive entrance during the peak flowering season (May–July 2025). Freshly collected pellets were manually cleaned to remove debris and stored in sterile glass containers. Samples were air-dried at room temperature (22–24 °C) for 48 h and subsequently preserved at 4 °C until analysis.

2.2. Analysis

2.2.1. Sample processing and preparation for light microscopy (LM)

Collected pollen pellets were first manually sorted according to their color in order to preliminarily distinguish potential botanical origins. Color-based separation was performed under a stereomicroscope to minimize cross-contamination among pellet groups.

For light microscopy analysis, permanent slides were prepared using basic fuchsin-stained glycerin jelly. A small amount of homogenized pollen powder was transferred onto a clean glass slide and mounted in warm glycerin jelly containing basic fuchsin stain. The preparation was gently heated to allow proper spreading and staining of pollen grains. Coverslips were carefully placed to avoid air bubbles, and the edges were sealed with paraffin wax to produce permanent preparations (Louveaux et al., 1978).

Prepared slides were examined under a research light microscope (Nikon Eclipse E100, Japan) at 40× and 100× magnifications. For each sample type (7 color-separated), a minimum of 30 pollen grains per morphotype were analyzed.

2.2.2. Preparation for scanning electron microscopy (SEM)

For SEM analysis, intact pollen pellets were processed without acetolysis in order to preserve natural surface morphology. Approximately one pollen pellet was dissolved in 0.5 mL of methanol and vortexed briefly to obtain a homogeneous suspension. A small aliquot of the suspension was pipetted onto aluminum SEM stubs previously covered with double-sided conductive carbon tape. The suspension was allowed to air-dry at room temperature to ensure uniform distribution of pollen grains across the stub surface. After complete solvent evaporation, the mounted samples were sputter-coated with gold for 6 minutes to enhance electrical conductivity and prevent charging during imaging. SEM observations were carried out under high-vacuum conditions at an accelerating voltage of 15 kV. Micrographs were obtained at magnifications ranging from 200× to 2,000× for detailed examination of exine's surface morphology. All micrographs were recorded digitally for subsequent morphometric evaluation.

2.2.3. Macro and micro element analysis

Elemental analysis of the BP samples was performed according to the method described by (Demir Kanbur et al., 2021) using inductively coupled plasma–optical emission spectrometry (ICP-OES). For sample preparation, 300 mg of each BP sample was accurately weighed into Teflon digestion vessels. A mixture of 5 mL concentrated nitric acid (HNO_3) and 3 mL hydrogen peroxide (H_2O_2) was added to each vessel. The samples were digested using a microwave-assisted wet digestion system under controlled conditions. After digestion, the solutions were allowed to cool and were diluted to an appropriate volume with ultrapure water. The concentrations of selected elements, including Cd, Cu, Fe, Mn, Na, Ni, Al, Zn, B, Mg and K were determined using an ICP-OES instrument (Optima 7000 DV). The operating conditions of the ICP-OES were set as follows: RF power 1.0 kW, plasma gas flow (Ar) 15 L/min, auxiliary gas flow (Ar) 1.5 L/min, nebulizer gas flow (Ar) 0.75 L/min, RF generator frequency 40 MHz, and pump speed 15 rpm. Calibration was performed using five multi-element standard solutions within a concentration range of 0.02–1.0 mg L^{-1}, and calibration curves with correlation coefficients ($R^2 > 0.99$) were accepted. Element concentrations were expressed as mg kg^{-1} of BP.

2.2.4. Aroma analysis

The volatile aroma compounds of the BP samples were analysed using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS), following the method described by Koyuncu (2025) with minor modifications. For HS-SPME extraction the samples were placed in 15 mL amber vials, sealed with PTFE-silicone septa, and equilibrated at 60 °C for 15 min under continuous stirring. Volatile compounds were then extracted using an 85 µm carboxen/polydimethylsiloxane (CAR/PDMS) fibre for 30 min.

GC–MS analysis was performed using a GC–MS (Shimadzu QP2010 Ultra) device with a 30 m 5-Ms column (30 m × 0.25 mm × 0.25 µm). The injector temperature was set to 250 °C, and desorption was carried out for

5 min. The oven temperature program started at 40 °C (held for 4 min), increased to 90 °C at 3 °C/min, then to 130 °C at 4 °C/min (held for 4 min), and finally to 240 °C at 5 °C/min, where it was held for 8 min. Helium was used as the carrier gas at a constant flow rate of 1.2 mL min^{-1}. The mass spectrometer was operated in electron impact (EI) ionization mode at 70 eV with a scan range of *m/z* 30-600 and a split ratio of 1:10. Volatile compounds were identified by comparing their mass spectra with those available in the NIST and Wiley mass spectral libraries. Retention indices were calculated using a homologous series of n-alkanes (C8–C32) analysed under identical chromatographic conditions and were used to support compound identification. Semi-quantitative determination of volatile compounds was performed based on relative peak area percentages obtained from the total ion chromatograms.

3. RESULTS AND DISCUSSION

The morphological characteristics of the pollen samples were investigated using scanning electron microscopy (SEM) and light microscopy. The images are presented in Figure 1 (1a–7b), where "a" denotes SEM micrographs and "b" represents light microscopy images. The analyzed taxa include *Ranunculus* spp. (Figure 1-1a-1b), *Helianthemum* spp. (Figure1- 2a-2b), *Centaurea* spp. (Figure1- 3a-3b), Fabaceae (possibly *Trifolium* spp.) (Figure 1- 4a-4b), Caryophyllaceae (Figure 1-5a-5b), Liliaceae (Figure 1-6a-6b), and an unidentified taxon (Figure 1-7a-7b). The BP sample collected from the Olur district of Erzurum (Türkiye) was characterized by the dominance of these pollen types within the pollen pellets.

The SEM images of *Ranunculus* spp. (Figure 1-1a) pollen grains are typically tricolpate, isopolar, and radially symmetrical. They are generally subprolate to spheroidal in shape. The exine is well developed and exhibits microechinat-perforate ornamentation, resulting in an irregular surface pattern. The colpi are elongated and clearly defined. SEM observations reveal a moderately thick exine with a distinct surface texture.

In *Helianthemum* spp. (Figure 1-2a), pollen grains are tricolporate, isopolar, and mostly prolate-spheroidal. The exine ornamentation is finely perforate-striate, giving the surface a relatively smooth and uniform appearance. The colpi are narrow and extend along the polar axis. Compared to Ranunculus, the surface structure appears less pronounced under SEM. *Centaurea* spp. (Figure 1-3a) pollen grains were generally tricolporate and exhibited a microechinate exine ornamentation with a comparatively rougher surface texture than those observed in Fabaceae pollen. The corresponding LM image (Figure 1-3b) confirmed their characteristic shape and aperture configuration. Members of the Fabaceae family (Figure1- 4a), possibly *Trifolium* spp., exhibit relatively regular pollen morphology. SEM images suggest a psilate to finely reticulate exine pattern with a more uniform surface compared to *Centaurea* spp. The light microscopy image (Figure 1-4b) shows homogeneous pollen grains in terms of shape and size distribution.

In the case of Caryophyllaceae (Figure 1-5a), pollen grains are typically periporate, radially symmetrical, and spheroidal. The exine ornamentation is granulate to microperforate, producing a finely textured surface. Numerous pores are distributed across the exine, which is a characteristic feature of this family. SEM observations reveal a moderately rough but evenly structured surface. The pollen grains appear moderately textured, differing from the smoother surfaces observed in Fabaceae. The light microscopy image (Figure 1-5b) provides an overview of pollen size and general morphology.

The pollen grains belonging to Liliaceae (Figure 1-6a) show characteristic morphological features, often associated with monosulcate or elongated structures typical of monocotyledons. Pollen grains are typically monosulcate, reflecting their monocotyledonous origin. They are generally ellipsoidal to elongated in shape. The exine ornamentation is reticulate. A single elongated sulcus extends along the longitudinal axis of the grain. SEM observations confirm a simple and less ornamented structure compared to eudicot taxa. SEM images indicate a relatively smooth to slightly ornamented exine. The corresponding light microscopy image (Figure 1-6b) confirms the elongated shape and overall structure of the grains.

Finally, the pollen grains identified as *Dianthus* spp. (Figure 1-7a and Figure 1-7b) exhibited spheroidal morphology with pantoporate apertures, which are characteristic features of many members of the Caryophyllaceae family. SEM observations revealed a microechinate exine ornamentation pattern, while LM images clearly showed the distribution of pores across the pollen surface. The combined microscopic observations supported the identification of this pollen type as *Dianthus* spp.

Overall, the comparative analysis of SEM and light microscopy images demonstrates that each taxon possesses distinct pollen morphology, particularly in terms of exine ornamentation, surface texture, and structural complexity. SEM provides detailed insights into microstructural features, while light microscopy offers a broader view of pollen shape and distribution. The combined use of these techniques enables a comprehensive evaluation of pollen morphology and supports taxonomic differentiation among the studied groups.

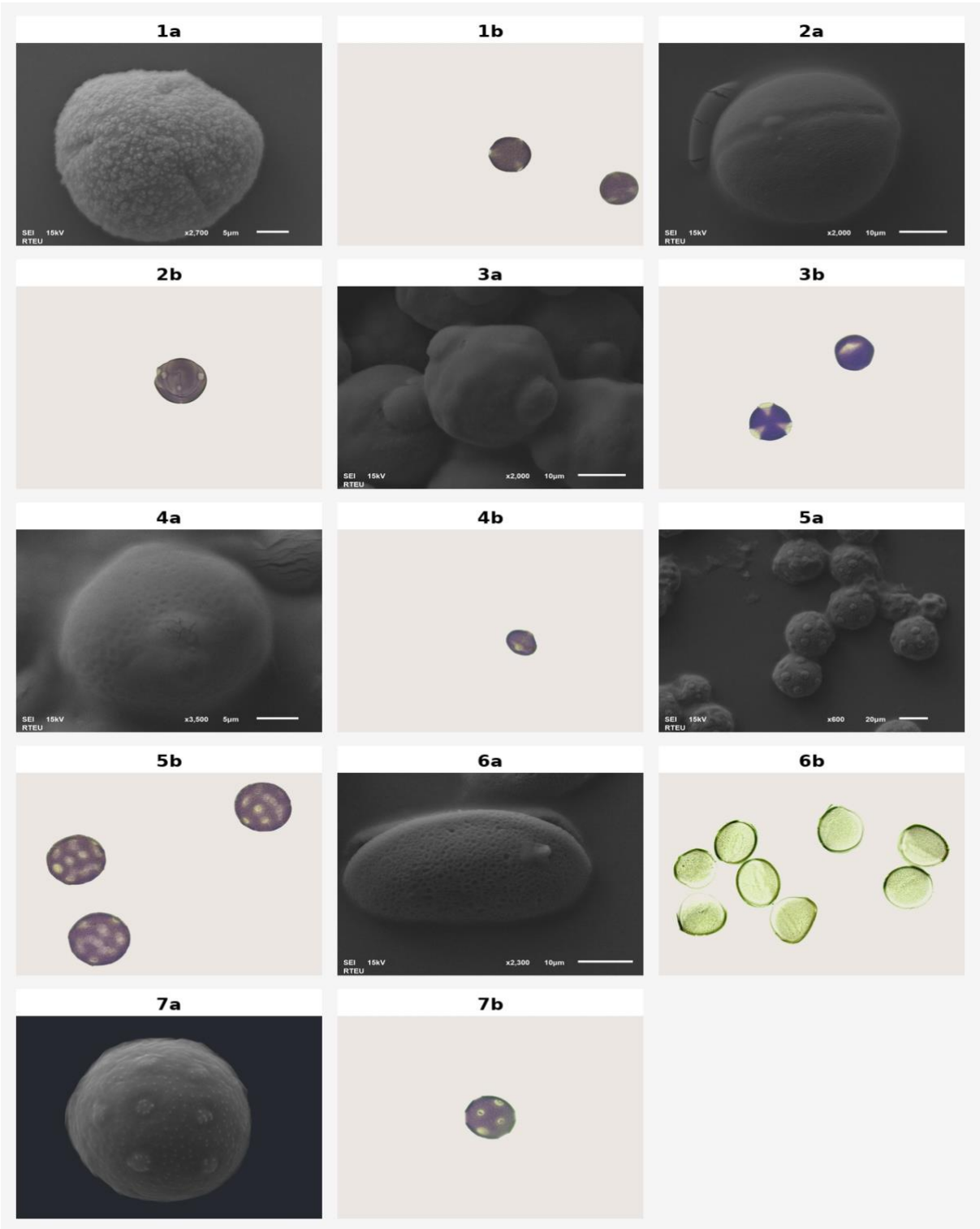

Figure 1. (a) Scanning electron microscopy (SEM) images; (b) light microscopy images (40x). (1a–7a) SEM micrographs showing detailed exine ornamentation and surface morphology; (1b–7b) corresponding light microscopy images showing general shape and size of the pollen grains. The analyzed taxa include: (1) *Ranunculus* spp., (2) *Helianthemum* spp., (3) *Centaurea* spp., (4) *Fabaceae* (possibly *Trifolium* spp.), (5) *Caryophyllaceae*, (6) *Liliaceae*, and (7) *Dianthus* spp.

The highest concentration of elements in BP was found for potassium (K) with a value of 2521.88 mg/kg, followed by calcium (Ca) at 724.61 mg/kg and magnesium (Mg) at 624.69 mg/kg. These elements are known to play significant roles in various physiological processes. The concentration of iron (Fe) was also notable, measuring 268.36 mg/kg, which may contribute to the nutritional value of BP (Table 1).

Trace elements such as zinc (Zn) and manganese (Mn) were present at moderate concentrations of 24.30 mg/kg and 185.16 mg/kg, respectively. These elements are essential for various metabolic functions. Copper (Cu) and boron (B) were detected at lower concentrations, 6.48 mg/kg and 8.36 mg/kg, respectively. Nickel (Ni) and cadmium (Cd) were found in very small amounts, with concentrations of 0.78 mg/kg and 0.16 mg/kg, respectively (Table 1).

The elemental composition of BP suggests that it may be a valuable source of essential minerals, particularly for individuals seeking to supplement their diets with bioavailable nutrients. Further studies on the bioavailability and potential health benefits of these elements in BP are warranted.

Table 1. Elemental Concentrations (mg kg^{-1}) in BP

Sample	Fe	Zn	Cu	Mn	Ni	B	Cd	Ca	Na	Mg	K	Al
BP	268.36	24.30	6.48	185.16	0.78	8.36	0.16	724.61	383.83	624.69	2521.88	150.55

The mineral composition of BP also showed significant variation based on botanical origin. Kolayli et al. (2024) reported that potassium was the most abundant element in both BP and BB, followed by calcium and magnesium. These findings are consistent with our results, where potassium was the most prevalent element in BP. Additionally, both studies found that the mineral content varied significantly depending on the plant species from which the pollen was collected, reinforcing the importance of botanical and environmental factors in the mineral profile of bee products (Figure 2).

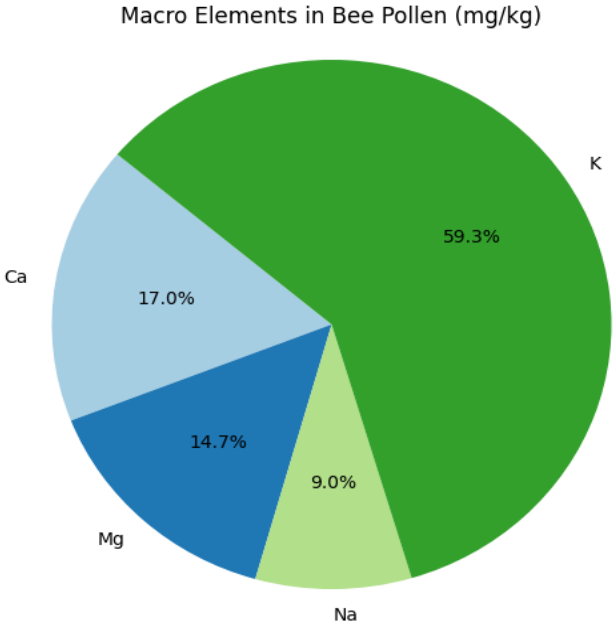

Figure 2. Macro elements in BP (mg/kg)

The mineral content in BP, particularly trace elements such as iron, manganese, copper, and zinc, plays a crucial role in human health, offering significant benefits despite their relatively low concentrations (Altunatmaz et al., 2017). Several studies, including those by El Ghouzi et al. (2023), Aylanc et al. (2023a), and Mayda et al. (2020), provide valuable insights into the bioavailability and functionality of these elements in BP.

Iron is essential for oxygen transport in the body and plays a critical role in cellular respiration and metabolism. El Ghouzi et al. (2023) and Aylanc et al. (2023a) noted the presence of iron in BP, albeit at trace levels. Iron

concentrations in various BP samples range from 30 mg/kg to 200 mg/kg. Mutlu et al. (2025) further emphasized the bioaccessibility of iron during simulated digestion, highlighting that iron is less bioavailable in BP compared to other elements like potassium. Despite this, BP still contributes a notable fraction of the recommended dietary intake of iron (Figure 3).

Manganese is a crucial cofactor for enzymes involved in bone formation, blood clotting, and the metabolism of amino acids, cholesterol, and carbohydrates. Aylanc et al. (2023b) found manganese levels in BP to vary significantly, depending on its botanical origin. Manganese bioaccessibility, as noted in the study, is relatively higher during the gastric phase of digestion, which aligns with findings from (El Ghouzi et al., 2023), where manganese bioaccessibility was generally higher in the gastric stage compared to the intestinal phase (Figure 3).

Cadmium content determined in the present study was within the range reported for Turkish bee pollens by (Altunatmaz et al., 2017), indicating that the analyzed samples were not exposed to unusually high cadmium contamination. However, the study also emphasized that some bee pollen samples may exceed recommended international limits for Cd depending on environmental conditions and geographical origin (Figure 3).

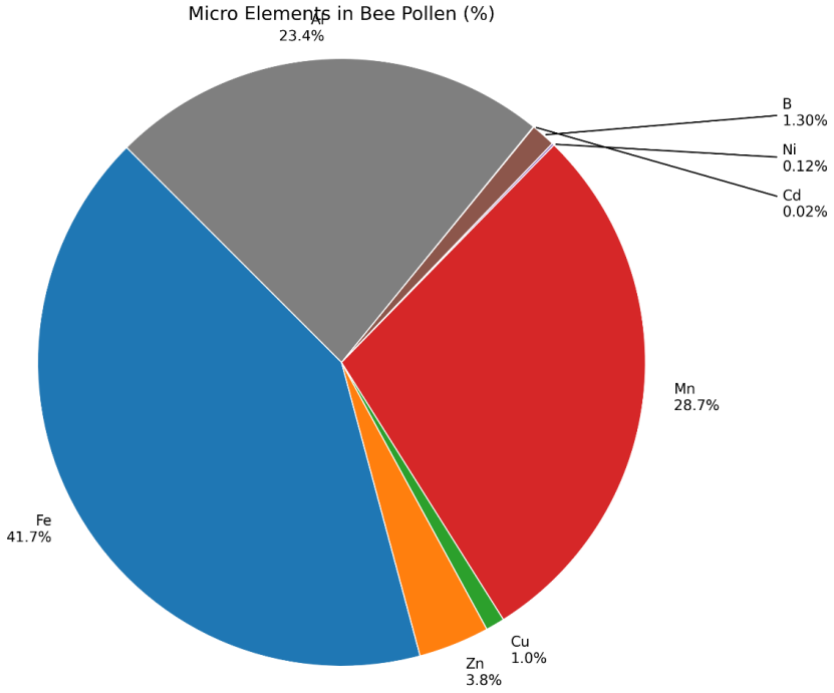

Figure 3. Micro elements in BP (mg/kg)

Results are expressed as mean ± standard error (SE) of two independent analyses. Considering that SPME is an equilibrium-based and semi-quantitative extraction technique, the relatively high standard deviations observed for certain volatile compounds are within acceptable limits and consistent with previous studies (Table 2).

The volatile composition of the sample was analyzed by solid-phase microextraction coupled with gas chromatography (SPME-GC), and the identified compounds are presented as mean ± standard error (SE) of two independent analyses. A complex profile of volatile compounds was observed, accounting for 100% of the total composition.

The major constituents were pelargonaldehyde (14.80 ± 2.78%), α-pinene (13.80 ± 4.12%), capronaldehyde (12.45 ± 1.56%), and enanthaldehyde (10.13 ± 0.41%). These findings indicate that aldehydes and terpenes are dominant in the volatile fraction. Similar volatile profiles have been reported in bee products, where aldehydes, hydrocarbons, and terpenes constitute the main chemical classes. In particular, compounds such as

dodecane, tridecane, and nonanal derivatives have been previously identified as key volatiles in BP and related matrices using SPME-GC-MS (Baky et al., 2023).

The presence of α-pinene as a major component is consistent with previous studies reporting terpene-derived compounds as important contributors to the volatile profile of BP and bee bread. These compounds are strongly associated with plant origin and contribute significantly to aroma characteristics. Moreover, volatile hydrocarbons and oxygenated compounds, including aldehydes and alcohols, have been widely reported in BP samples, confirming the complex chemical nature of such matrices (Aylanc et al., 2023).

A relatively high variability was observed for certain compounds, particularly α-pinene and tridecane. This can be attributed to the equilibrium-based and semi-quantitative nature of the SPME technique. Variations in extraction conditions, such as temperature, exposure time, and matrix interactions, can significantly affect the adsorption efficiency of volatile compounds. Such variability is commonly reported in SPME-based studies and is considered acceptable.

Additionally, the diversity of volatile compounds observed in the present study is in agreement with previous findings, where a large number of volatile constituents (up to 119 compounds) were identified in bee bread samples using SPME-GC-MS. This high diversity is mainly attributed to differences in botanical origin, fermentation processes, and environmental factors influencing bee products (Kolayli et al., 2025).

It is well established that the chemical composition of BP and bee bread varies significantly depending on plant origin, geographical conditions, and seasonal factors. These variations directly influence both the qualitative and quantitative profiles of volatile compounds. Therefore, the differences observed between replicate analyses in the present study can be considered within the expected range for such natural products.

Overall, the results demonstrate that the analyzed sample possesses a complex volatile profile dominated by aldehydes and monoterpenes, with acceptable variability between replicates. The findings are consistent with previously reported studies on bee products and further confirm that SPME-GC is a suitable and reliable technique for profiling volatile constituents despite its inherent semi-quantitative limitations.

Table 2. Volatile profile of BP

Compound	**Mean±Standart Error**
Pelargonaldehyde	14.795±2.78
Pinene <alpha->	13.795±4.12
Capronaldehyde	12.45±1.56
Enanthaldehyde	10.125±0.41
Tridecane	7.575±3.28
Dodecane	6.93±1.44
Nonane	6.595±3.20
Hendecane	3.94±0.84
Hept-5-en-2-one <6-methyl->	2.355±0.14
Decane	2.16±0.40
Acetophenone <2,4-dimethyl->	1.985±1.40
Caprylaldehyde	1.8±0.43
Pinene <beta->	1.77±0.52
Heptadecane	1,41±0.35
Tetradecane	1.335±0.22
Verbenol	1.175±0.23
Myristate <isopropyl->	0.81±0.57
Cedrol	0.805±0.28
Hex-2(E)-enal	0.745±0.12
Pseudolimonene	0.745±0.14
Linalool <tetrahydro->	0.73±0.52
Ethylene brassylate	0.695±0.49
Benzaldehyde	0.605±0.14

Undecanal <2-methyl->	0.40
Angelate <isobutyl->	0.555±0.39
Hexadecane	0.55±0.39
Pentadecane	0.425±0.30
Myristic alcohol	0.39±0.28
Non-2(E)-enal	0.36±0.25
Hexanol <n->	0.325±0.23
Limonene	0.3±0.21
Formate <octyl->	0.28±0.20
Capryl alcohol	0.265±0.19
Benzene <amyl->	0.255±0.18
Tiglic aldehyde	0.2±0.14
Phenethyl alcohol	0.19±0.13

4. CONCLUSIONS

This study provides a comprehensive characterization of BP through the integration of palyno-morphological, elemental, and volatile compound analyses. The combined use of light microscopy (LM) and scanning electron microscopy (SEM) enabled detailed identification of pollen morphotypes and revealed significant structural differences among taxa, highlighting the importance of microscopic techniques in determining botanical origin.

Elemental analysis demonstrated that BP is a rich source of essential macro- and micro-elements, particularly potassium, calcium, and magnesium, along with notable amounts of iron, manganese, and zinc. The presence of these elements confirms the nutritional value of BP, while the low levels of potentially toxic elements indicate its relative safety for consumption.

The volatile compound analysis revealed a complex chemical profile dominated by aldehydes and terpenes, with major constituents such as pelargonaldehyde and α-pinene. These compounds reflect the botanical origin and contribute to the characteristic aroma profile of BP. The variability observed in volatile composition further emphasizes the influence of environmental and floral diversity.

Overall, the integration of morphological and chemical analyses offers a multidimensional approach to understanding BP composition. This study contributes to the evaluation of its botanical authenticity, nutritional significance, and functional potential. The findings support the use of BP as a valuable natural product and highlight the importance of combining complementary analytical techniques for its comprehensive assessment.

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AUTHOR CONTRIBUTION

EDK: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review and editing.

DECLARATIONS

Ethics Approval and Consent to Participate

This study did not involve any human participants or animals. Therefore, ethics committee approval was not required.

Conflict of Interest

The authors declare that there is no conflict of interest.

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