



Mineral composition of bee pollen and its relationship with botanical origin and harvesting period

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ABSTRACT

In the present study, the mineral composition of seventy-one bee pollen samples from four different apiaries was determined by means of inductively coupled plasma-optical emission spectrometry. The results showed that there were no significant differences in relation to the overall mineral content per sample in terms of the apiary of origin or the harvesting period; the most common elements were phosphorus and potassium with concentrations ranging from 2.3 to 5.1 g/kg (dry weight). Moreover, the mineral content of the samples analyzed was similar to or higher than the recommended as well-balanced food for bees. Regarding the nutritional value for humans, bee pollen samples could be considered as a food rich in copper, iron, magnesium, manganese, and phosphorus. Finally, a canonical discriminant analysis was performed, and it was found that the apiary of origin could be distinguished by using the first three canonical functions; furthermore, over 90% of the samples could be correctly assigned to their corresponding apiary. The results were even better as regards the harvesting period, as only the first two canonical functions were sufficient to make a distinction between the different harvesting periods, resulting in a perfect match (100% of success rate).

1. Introduction

Insect pollination is crucial for the maintenance of ecosystems and food production. Among the pollinators, honeybees are the most effective on an extensive range of flowering plants (Cruz et al., 2022). Currently, the honeybee population has declined in many parts of the world, due to a series of factors such as climatic conditions (Klein et al., 2017), agricultural practices (Bednarska et al., 2022), or diseases (Goulson et al., 2015). Nevertheless, bee nutrition is a key factor that will determine whether a larva will develop into a queen or a worker (M. Haydak, 1970). Consequently, poor nutrition could affect susceptibility to disease and thus decreased the longevity. Bee pollen is an essential product for honeybee growth, and it is collected by bees from flowers and then mixed with their own secretions, nectar and regurgitated

honey, to create a final product in the form of granules (Tutun et al., 2022). It is also an important nutritional supplement for the human diet due to its related compounds of high therapeutic value (Ares et al., 2018; Aylanc et al., 2021). Bee pollen is characterized for being a natural source of proteins, amino acids, lipids, carbohydrates, phenolic compounds vitamins, or minerals, among others (Ares et al., 2022; Erdoğan et al., 2022; Zhang et al., 2022; Thakur and Nanda, 2021; Taha et al., 2019; Gardana et al., 2018; Karabagias et al., 2018; Li et al., 2017; Lv et al., 2015). The mineral content is essential for ensuring the protection of the cell, metabolic activities, homeostasis, and overall health. A combination of calcium (Ca), phosphorus (P), and magnesium (Mg) has been deemed necessary to regulate the haemolymph osmotic pressure, as well as inter- and intracellular fluids; meanwhile minor elements such as iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn) are crucial for

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growth, development, and the reproductive function research (Matuszewska et al., 2021; De-Melo et al., 2018; Kalaycioglu et al., 2017). Therefore, an insufficiency of these elements can cause various metabolic disorders, acute growth malfunctions and even cause fatal diseases (Quintaes and Diez-Garcia, 2015). However, during foraging bees may acquire toxic elements as mercury (Hg), nickel (Ni), lead (Pb), cadmium (Cd), and chrome (Cr) from urban and industrial areas and these are finally transferred to the beehives. This may present a risk for the health of both bees and humans, who would consume these products (Conti et al., 2022; Kostić et al., 2022; Zafeiraki et al., 2022; Yang et al., 2013). As in the case of other bee pollen constituents, the mineral composition is greatly affected by several factors, mainly soil, climate, geographical origin and botanical species (Liolios et al., 2019; Taha, 2015; Yang et al., 2013), since the plants accumulate different amounts of mineral salts. The mineral profile can be used as a biomarker of botanical and geographical origins of bee pollen, which is a quite relevant issue these days in terms of combating fraud in the beekeeping industry, owing to adulteration with pollen from non-declared origins (Wang et al., 2022; Wang et al., 2021). Moreover, monitoring the presence of certain minor elements is important for evaluating environmental pollution (Huseyin and Ali, 2022; Álvarez-Ayuso and Abad-Valle, 2017), as well as the quality and safety of this bee product.

Thus, the main goal of the present study was to determine mineral content by means of inductively coupled plasma-optical emission spectrometry (ICP-OES), which has been successfully employed for this purpose in many publications (Costa et al., 2019; Altunatmaz et al., 2017; Sattler et al., 2016; Yang et al., 2013; Somerville and Nicol, 2002). Seventy-one bee pollen samples were collected from four different apiaries located in the same geographical area (Marchamalo, Guadalajara, Spain). These samples came from three consecutive foraging periods (April-May; June; July-August) in 2018. A further aim of this study was to assess the potential of mineral elements as bee pollen markers. To our knowledge, this is the first study to consider whether bee pollen samples can be classified, by means of canonical discriminant analysis (CDA) focusing on mineral content, on the basis of the corresponding apiary of origin and the harvesting periods.

2. Materials and methods

2.1. Reagents and standards

A multi-element standard solution mixture prepared in diluted nitric acid (Aluminium, Al; Barium, Ba; Bismuth, Bi; Calcium, Ca; Cadmium, Cd; Cobalt, Co; Chrome, Cr; Copper, Cu; Iron, Fe; Gallium, Ga; Indium, In; Potassium, K; Lithium, Li; Magnesium, Mg; Manganese, Mn; Sodium, Na; Nickel, Ni; Lead, Pb; Strontium, Sr; Zinc, Zn), and standards solutions of some individual elements (Phosphorus, P (ultrapure water); Selenium (ultrapure water), Se; Arsenic (ultrapure water), As; ⁸⁹Yttrium (diluted nitric acid), ⁸⁹Y-internal standard, IS) were all purchased by Sigma Aldrich (Merck KGaA, Darmstadt, Germany). Two certified reference materials (CRM) were used for assessing the accuracy of the method, NCS DC73349 (branches and leaves of bush) and INCT-MPH-2 (mixed polish herbs), and they were obtained from LGC Standards (Teddington, Middlesex, UK). It should be noted that these CRMs were selected as there is not an available CRM for bee pollen, and because all the elements studied in the present work were not included in each of the CRM separately. A microwave extraction system (MARS, CEM, NC, USA), nitric acid and peroxide hydrogen (Sigma Aldrich, Merck KGaA, Darmstadt, Germany), ultrapure water (Millipore Milli-RO plus and Milli-Q systems, Bedford, MA, USA), and filter paper (ref. 1242, 90 mm; Letslab, Barcelona, Spain) were used for sample treatment.

2.2. Sample obtention and treatment

2.2.1. Samples

Bee pollen samples were obtained from four experimental apiaries

with homogeneous colonies of *Apis mellifera iberiensis* (n = 71). Three of the apiaries, Pistacho (PI), Tio Natalio (TN) and Monte (MO), were located on the Centro de Investigación Apícola y Agroambiental (CIAPA) estate in Marchamalo, whilst the fourth, Fuentelahiguera (FU), was in the municipality of Fuentelahiguera de Albatages; all of these were in the province of Guadalajara (Spain). Location and information concerning the apiaries and vegetation are included in the [Supplementary Material](#) (see [Table 1S](#) and [Fig. 1S](#)). During the study period, which comprised three consecutive foraging periods in 2018 (April and May; June; July and August), bee pollen samples were collected using pollen traps placed at the entrance to the hive. Every fortnight the pollen trap was closed for a period of 24 h. After the collection period, the bee pollen was immediately transferred to the laboratory where it was frozen until a palynological analysis was carried out (see [Supplementary Material, Table 1S](#)). The results of the contents of corbicular pollens mostly collected in the samples corresponding to each period and colony are summarized in [Table 2S](#) (see [Supplementary Material](#)). Whenever the majority taxon in the composition of the collected sample is well defined (greater than 80%; [Ares et al., 2022; Campos et al., 2008](#)), in contrast to those where this requirement is not met, the denomination multifloral (MF) has been applied ([Escuredo et al., 2012](#)). The results of the palynological analysis provided information about the predominant pollen type: thirty of the analyzed samples were monofloral (42%), while the others were classified as polyfloral. Distribution of the monofloral bee pollen depending on the beehives was as follows: FU (72%) > PI (47%) > MO (23%) > TN (13%). Of the different types of pollen *Brassica*, *Rosa*, *Rubus* and *Cytisus* predominated. These results were consistent with the Database of Pollinator Interactions (Database of pollinator interactions ([DoPI](#)), 2022) where it was stated that *A. mellifera* demonstrated great interest in these species. The number of interactions registered for *Brassica* totalled more than 700, *Rosa* (284), *Rubus* (7588) and *Cytisus* (17).

2.2.2. Sample preparation

Bee pollen samples were dried until the mass stabilized; humidity was around 12% in all cases. Next, samples were individually mixed, ground, and pooled for optimum sample homogeneity (see [Supplementary Material, Fig. 2S](#)). Then, 0.2 g of each sample were weighted and placed into a vessel (100 mL) of a microwave extraction system to be digested. The experimental conditions were adapted from a previous work ([Pohl et al., 2020](#)). Briefly, 3 mL of nitric acid (69% in ultrapure water), 1.5 mL of peroxide hydrogen (33% in ultrapure water) and 5.5 mL of ultrapure water were added in each vessel and the samples were further digested by using the following temperature program: heated during 10 min up to 200 °C, 15 min hold time, and cooled below 40°C during 30 min. The digestion solution was filtered, and quantitatively transferred to a volumetric flask, the final volume was made up to 25 mL with ultrapure water. To avoid any type of contamination, the laboratory material used was previously immersed in a solution of *aqua regia* (overnight), and it was washed several times with ultrapure water.

2.3. Instrumental analysis

In the present study, the mineral elements were determined by inductively coupled plasma - optical emission spectrometry (ICP-OES; iCAP-PRO X Duo Spectrometer, Thermo Scientific, Waltham, Massachusetts, USA). The optimal ICP-OES conditions, along with the selected wavelengths and IS are presented in [Table 1](#). The ICP-OES operated in Intelligent Full Range (iFR) analysis mode that can measure wavelengths between 167.021 and 852.145 nm in one simultaneous measurement, significantly reducing analysis times and the consumption of Ar. In addition, the acquisition mode was also selected depending on the concentration of the mineral elements (radial, majoritarian elements; axial, minoritarian elements). To avoid ion signal fluctuations caused by the matrix, a diluted internal standard (IS) solution (10 mg/L of ⁸⁹Y) was used, which was distributed in all solutions and samples by using a

Table 1
Selected conditions for ICP-OES analysis.

Parameter	Value
Forward power (W)	1150
Ar gas flow rated (L/min)	
Nebulizer (L/min)	0.5
Coolant (L/min)	12.5
Auxiliary (L/min)	0.5
Nebulizer gas pressure (bar)	1.92
Replicates	3
Uptake time (s)	50
Pump speed (rpm)	45
Element	Wavelengths (nm)
	589.592 Na 257.610 Mn 196.090 Se
	766.490 K 213.856 Zn 455.403 Ba
	315.887 Ca 267.716 Cr 670.791 Li
	279.553 Mg 221.647 Ni 407.771 Sr
	167.079 Al 288.616 Co 294.364 Ga
	177.495 P 214.438 Cd 325.609 In
	259.940 Fe 220.353 Pb 223.061 Bi
	324.754 Cu 189.042 As
	371.030 ⁸⁹ Y

second channel of the peristaltic pump.

2.4. Statistical analysis

The calculations for CDA required in this paper were performed using SAS PROC CANDISC (version 9.4; SAS Institute Inc., Cary, NC, USA). CDA obtains linear combinations of the quantitative variables that emphasize the differences among the groups (Ares et al., 2022; Jobson, 1991; Oda et al., 2020). To determine how many canonical functions must be used in the CDA, one should consider the possible proportion of accumulated variability explained by the canonical functions, at least 90%. The data base used in the present study comprised the response of each sample to the qualitative variable (apiary of origin or harvesting period) and the three analyses of each individual sample for each mineral element (quantitative variables).

3. Results and discussion

3.1. Method performance

The limits of detection (LODs) and quantification (LOQs) were experimentally determined, and were estimated to be three and ten times the signal-to-noise (S/N) ratio, respectively (see Table 2). Calibration curves were constructed by plotting the signal on the y-axis (analyte peak area) against the analyte concentration on x-axis. Quantification of major elements (Al, Ca, K, Mg, Na, P) was carried out by means of a calibration curve ($n = 6$) that covered a range of concentrations from 10 to 150 mg/L (10, 25, 50, 75, and 150 mg/L), corresponding to concentrations between 1250 and 18,750 mg/kg (1250, 3125, 6250, 9375, and 18,750 mg/kg) depending on the proposed sample treatment; meanwhile, calibrations curves ($n = 6$) covering concentrations between LOQ and 10 mg/L (LOQ, 0.05, 0.10, 1, 5, 10 mg/L) or (LOQ, 6, 13, 125, 625, and 1250 mg/kg) were used to measure minority elements (As, Ba, Bi, Cd, Co, Cr, Cu, Fe, Ga, In, Li, Mn, Ni, Pb, Se, Sr, Zn). All the calibration standards were prepared by diluting the commercial standards with a 2% solution of nitric acid in ultrapure water. The graphs obtained in all the calibration curves were straight lines, with the coefficient of the determination values (R^2) higher than 0.99; residual analysis revealed a random scatter with no systematic tendency (data not shown). Experiments for precision, which was expressed as relative standard deviation (%RSD), were performed concurrently by repeated analysis with a standard solution of mixed mineral elements (majority, 6250 mg/kg; minority, 125 mg/kg), and bee pollen samples ($n = 6$; intra-day precision), or over three consecutive days ($n = 6$; inter-day precision). The %RSD values obtained for the

Table 2

Acquisition mode (R, radial for majoritarian elements; A, axial for minoritarian elements), determination coefficients (R^2), limits of detection (LOD), limits of quantification (LOQ) of the analyzed minerals by ICP-OES (see experimental conditions in Subsection *Instrumental Analysis* and Table 1).

Mineral	Acquisition Mode	R^2	LOD (mg/kg)	LOQ (mg/kg)
Al	R	0.9989	0.5	1.8
As	A	0.9991	0.5	1.5
Ba	A	0.9990	0.3	1.0
Bi	A	0.9995	0.7	2.5
Ca	R	0.9988	0.6	2.0
Cd	A	0.9992	0.2	0.6
Co	A	0.9999	0.4	1.2
Cr	A	0.9984	0.4	1.4
Cu	A	0.9979	0.2	0.7
Fe	A	0.9995	0.1	0.5
Ga	A	0.9971	0.8	2.8
In	A	0.9976	0.7	2.2
K	R	0.9983	2.4	7.9
Li	A	0.9994	0.3	1.0
Mg	R	0.9991	0.8	2.7
Mn	A	0.9997	0.5	1.7
Na	R	0.9988	1.0	3.3
Ni	A	0.9991	0.6	1.8
P	R	0.9994	0.7	2.4
Pb	A	0.9984	0.2	0.7
Se	A	0.9989	0.6	2.2
Sr	A	0.9991	0.2	0.8
Zn	A	0.9993	0.3	1.2

areas and retention times were below or equal to 15% in all cases (data not shown). Finally, accuracy was examined by analyzing two different CRMs (NCS DC73349 and INCT-MPH-2), which were prepared following the same procedure as that applied to the bee pollen samples. The results were in good agreement with the CRMs shown in Table S3 (see Supplementary Material). Recoveries for the elements contained in the CRMs were in the range of 87–116%.

3.2. Mineral content

Mineral content was determined in seventy-one samples of bee pollen from four apiaries located in Marchamalo (PI, $n = 44$; MO, $n = 12$; TN, $n = 8$; FU, $n = 7$). All the samples were analyzed in triplicate. It is worth noting that eight minerals (As, Bi, Cd, Co, Ga, In, Pb and Se) were not detected in any of the samples analyzed. The results are listed in Tables 3–5 and Table 4S (see Supplementary Material), where frequency (the number of samples in which a mineral was detected/the total number of samples) and concentration intervals (majority elements, g/kg; minority elements, mg/kg) are shown. Firstly, no significant differences were detected in relation to the overall mineral content per sample depending on the apiary of origin or the harvesting period; however, individual mineral content varied according to the origin of bee pollen. For example, the following general trend may be observed regarding the minerals found in higher concentration in bee pollen: P (2.3–5.1 g/kg) > K (2.3–4.9 g/kg) > Ca (0.58–2.8 g/kg) > Mg (0.36–1.3 g/kg) > Na (82–612 mg/kg). In the case of microelements, the general pattern was as follows: Fe (52.3–133 mg/kg) > Mn (12–119 mg/kg) > Zn (3.0–67.5 mg/kg) > Cu (14.7–45.7 mg/kg). These tendencies were observed for each hive, and maximum values were in most cases related to monofloral bee pollen. A similar scenario was observed in previous studies conducted in other countries (see Supplementary Material, Table 5S; Lilek et al., 2021; Taha and Al Kahtani, 2020; Costa et al., 2019; Altunatmaz et al., 2017; Sattler et al., 2016; Taha, 2015; Yang et al., 2013; Somerville and Nicol, 2002), as P and K were the predominant elements, followed by Ca, Mg and Na. However, the concentrations detected in the present study were slightly lower than in the previous ones. This could be attributed to the particularity of the geographical area, and the mineralogy of the soil (see lithological maps for the area under study in

Table 3

Frequency and concentration range of each mineral in the four apiaries.

Mineral	FU		PI		MO		TN	
	FR	CR	FR	CR	FR	CR	FR	CR
Al ^A	100	4.4–27	100	13–20	100	5.8–124	100	26–94
Ba ^A	100	< LOQ-3.9	100	< LOQ	100	< LOQ	100	< LOQ
Ca ^B	100	1.5–2.7	100	0.58–2.11	100	1.4–1.9	100	0.75–2.8
Cr ^A	0	<LOD	9	< LOQ	0	<LOD	0	<LOD
Cu ^A	100	15–36	100	17–21	100	21–46	100	22–41
Fe ^A	100	61–125	100	53–61	100	58–134	100	77–110
K ^B	100	3.7–4.2	100	3.0–4.2	100	2.3–4.7	100	3.0–4.8
Li ^A	57	<LOQ-2.8	71	<LOQ-2.8	75	<LOD-3.0	50	<LOQ
Mg ^B	100	0.9–1.0	100	0.61–1.1	100	0.36–1.1	100	0.55–1.3
Mn ^A	100	15–119	100	12–22	100	17–54	100	14–38
Na ^B	100	100–315	100	90–390	100	88–241	100	94–106
Ni ^A	29	<LOQ-1.9	5	<LOD-1.9	0	<LOD	0	<LOD
P ^B	100	3.7–5.0	100	3.2–4.8	100	2.3–4.7	100	3.1–4.9
Sr ^A	71	<LOQ-6.5	91	<LOQ-2.4	92	<LOD-4.8	88	<LOQ-6.9
Zn ^A	100	31–63	100	24–37	100	36–67	100	41–63

FR, frequency (%). Number of samples in which a mineral was detected (> LOD)/total number of samples (Fuentelahiguera (FU), n = 7; Pistacho (PI), n = 45; Monte (MO), n = 12; T í o Natalio (TN), n = 8) * 100; CR, concentration range (Amg/kg or Bg/kg; dry weight).

Table 4

Frequency and concentration range of each mineral depending on the harvesting period.

Mineral	AM		JN		JA	
	FR	CR	FR	CR	FR	CR
Al ^A	100	5.8–51	100	4.4–124	100	14–203
Ba ^A	100	<LOQ-3.9	100	<LOQ	100	<LOQ
Ca ^B	100	1.4–2.1	100	0.6–2.7	100	1.5–2.8
Cr ^A	0	<LOD	13	<LOQ	0	<LOD
Cu ^A	100	15–28	100	21–42	100	21–46
Fe ^A	100	53–87	100	72–132	100	84–133
K ^B	100	3.7–4.9	100	3.1–4.7	100	2.3–4.8
Li ^A	95	<LOD-3.0	100	<LOQ-2.9	0	<LOD
Mg ^B	100	0.7–1.1	100	0.5–1.1	100	0.3–1.3
Mn ^A	100	12–34	100	13–118	100	14–120
Na ^B	100	88–219	100	94–390	100	102–155
Ni ^A	0	<LOD	10	<LOD-1.9	0	<LOD
P ^B	100	3.6–5.0	100	3.1–5.1	100	2.3–4.8
Sr ^A	90	<LOD-5.5	90	<LOD-6.5	95	<LOQ-4.8
Zn ^A	100	32–49	100	24–67	100	39–58

FR, frequency (%). Number of samples in which a mineral was detected (> LOD)/total number of samples (Fuentelahiguera (FU), n = 7; Pistacho (PI), n = 45; Monte (MO), n = 12; T í o Natalio (TN), n = 8) * 100; CR, concentration range (Amg/kg or Bg/kg; dry weight).

Supplementary Material, Fig. 3S). The high levels of P, K and Ca could be tentatively explained by bee pollen taxa, the nature of the matrix and their specific role in plant nutrition. For example, high P content is directly related to the major taxon and the nature of the matrix studied, highlighting a high protein content (up to 50%) and that of phospholipids (up to 10%). Meanwhile, K is an essential nutrient in plant nutrition that is involved in several processes, such as water uptake, photosynthesis and enzymatic activity, among others; in addition, Ca participates in the physiological processes of the plant as well as in root

Table 5

Total and mean content per sample (mg/kg; dry weight) of minerals in the bee pollen samples grouped by apiary (Fuentelahiguera, FU; Pistacho, PI; Monte, MO; T í o Natalio, TN) and harvesting period (April–May, AM; June, JN; July–August, JA).

Apiary of origin			Harvesting period		
Apiary of origin	Total content	Mean content	Harvesting period	Total content	Mean content
FU	82,582	11,797	AM	236,083	11,804
PI	48,7161	11,071	JN	245,995	10,695
MO	12,8460	10,705	JA	221,206	10,533
TN	84,868	10,608			

growth (Amadou et al., 2022, Bereta Lanza and Dos Reis, 2021). Furthermore, a seasonal variation in the content of some minerals was observed. A decrease in P, K and Mg content was found during the summer months, a phenomenon which had been reported in previous studies and which was related to the highest concentration of trapped pollen during spring (Taha and Al-Kahtani, 2020), while an increase was detected in the concentration of all micronutrients during the summer months. This might be explained by the rainy season, especially the months of April and May, leading to waterlogged soils, and thereby reducing plants' micronutrient uptake (Bondy, 2014).

Moreover, it was also observed that mineral content for the majority minerals of the samples analyzed was higher than that recommend for constituting a well-balanced food for bees (see values in Supplementary Material, Table 6S), despite concentrations of Cu and Zn being within the established ranges. High values were detected for Fe, but these were comparable to those of studies from other countries. It is noteworthy that high concentrations of Mn (> 100 mg/kg) were reported in samples from the FU apiary. These unexpected results could be explained by its geographical proximity to a chicken farm, and the corresponding relationship with poultry manure production. This product contains essential plant nutrients in high concentrations, which include P, K, Ca, Mg, and Mn (>2000 mg/kg), and therefore it is usually employed as a fertilizer (Agbede and Oyewumi, 2022). Thus, it is necessary to continue with these studies, and to prevent bees from being fed with this pollen, since high concentrations of Mn could be lethal for them.

As for assessing the nutritional value in humans, the dietary reference intake (DRI) values recommended by the European Food Safety Authority (EFSA, 2023) were selected. We also used the criteria classification as a "source food" of a certain mineral per serving (20 g, recommended bee pollen intake/day; Végh et al., 2021) if providing at least 15% of DRI. On that basis, the bee pollen samples assayed could be

considered as a food substance rich in Cu, Fe, Mg, Mn, and P (see values in [Supplementary Material, Table 6S](#)).

Previously, Al was considered an innocuous element due to its abundance in the Earth's crust. Nowadays it is known that this could be toxic for plants and animals, and, moreover, humans' exposure to it should be controlled ([Bondy, 2014](#)). In addition, the growing prevalence of acid rain can affect to the discharge of larger amounts of Al salts from insoluble minerals, leading to greater bioavailability. In fact, Al content was highly variable (4.4–179 mg/kg) among apiaries and the harvesting period. In general terms, Al concentration increased during the summer months, with PI apiary displaying the highest content. Similarly, a large amount of Al was reported in other bee products, and was related to the type of soil from a rural areas, as in the case under study ([Ferreira et al., 2021](#)). The scientific committee of the Spanish Agency for Food Safety and Nutrition (AESAN) considers suitable the intake safety threshold set by EFSA at 1 mg Al/kg per week ([AESAN, 2009](#)). All the bee pollen samples analyzed exhibited concentrations which were above those recommended, being therefore inadvisable for human consumption.

However, it would be appropriate to investigate the origin of these concentrations and determine whether they could affect the health of bees. Also, it is important to note that some inorganic contaminants were detected in the samples. Li was found in 69% of the samples (maximum value: 3.0 mg/kg), Sr in 89% of the samples (maximum value: 6.9 mg/kg), while Ba was detected in all of them (<LOQ in all cases). Ba is used as an insecticide in barium fluorosilicate or carbonate form, and the presence of Sr may be due to the use of fertilizers or pesticides ([Zarić et al., 2022](#)). Finally, residues of heavy metals were not observed in any of the samples, while other toxic elements like Ni and Cr were only detected in several samples at lower concentrations, which are below the tolerated limits ([EFSA, 2018](#)).

3.3. Statistical analysis

As it was mentioned in the Introduction, one of the objectives of this work was to evaluate the potential of mineral elements as bee pollen markers. Therefore, two different statistical examinations (CDA) were

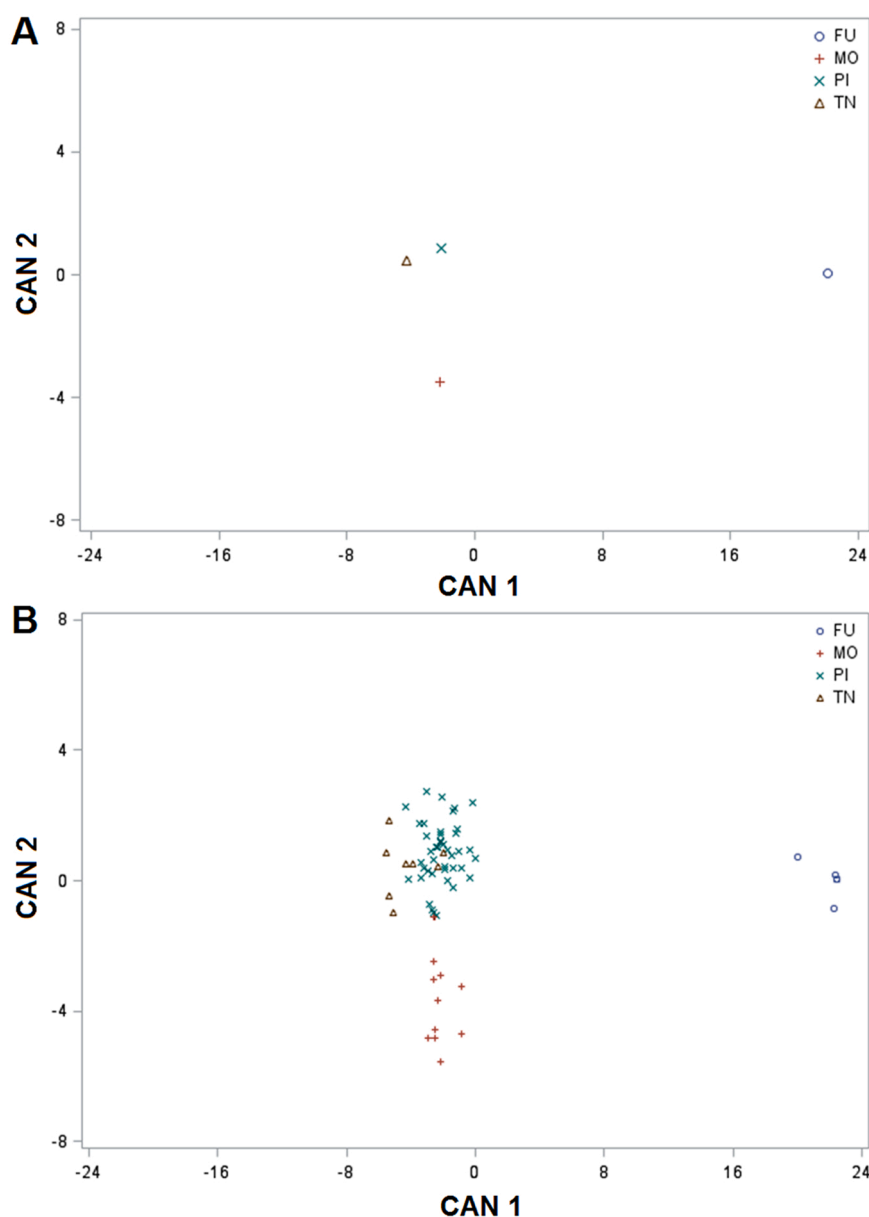


Fig. 1. A) Representation of the apiaries (FU, 1; MO, 2; PI, 3; TN, 4) as averages of canonical functions 1 and 2; B) Representation of individual bee pollen samples (from the apiaries (FU, 1; MO, 2; PI, 3; TN, 4) as representations of canonical functions 1 and 2.

performed in line with the apiary of origin (PI, $n = 44$; MO, $n = 12$; TN, $n = 8$; FU, $n = 7$) or the harvesting period (April-May, $n = 20$; June, $n = 30$; July-August; $n = 21$). Eight of the elements (As, Bi, Cd, Co, Ga, In, Pb, and Se) were not included in the CDA, as they were not identified in any of the samples.

3.3.1. Apiary of origin

In this study, seventy-one samples were analyzed, distributed among four classes (apiaries: FU, MO, PI, and TN), with fifteen variables (each variable was analyzed in triplicate, representing forty-five measurements per apiary). The results showed that the first three canonical functions were sufficient to explain 100% of original data variability (data not shown). Next, the weighting of the first three canonical functions was obtained (see [Supplementary Material, Table 7S](#)). The signs (positive or negative) indicate relevance in each of the canonical variables selected. Subsequently, the averages of the first three canonical functions for the four apiaries were obtained (see [Supplementary Material, Table 8S](#)) and represented graphically ([Figs. 1A and 2A](#)). The location of the points in the graphic representation is the result of the

positive or negative weight of the canonical functions. As can be seen in [Fig. 1A](#), two of the apiaries (MO and FU) could be differentiated from the other two apiaries by means of the first two canonical functions. The FU apiary was the only one with positive values for both canonical functions, and consequently it is located in the right section of the graph; meanwhile, MO apiary exhibited negative values for both functions, and is therefore situated on the lower left side of the graph. The other two apiaries (TN and PI) had the same signs for both functions, albeit with differing absolute values. However, a more distinct separation between these was observed by comparing canonical functions 1 and 3 (see [Fig. 2A](#)). In this regard, the TN apiary had a positive value for the third canonical function, while the value was negative for the PI apiary. As may be expected, the same phenomenon was observed when comparing the individual values for each sample (see [Figs. 1B and 2B](#)), although in these graphs the separation was not as clearly visible as with the average values due to the large number of points. According to the data included in [Table 7S](#) (see [Supplementary Material](#)), it can be concluded that the elements which influenced this separation between apiaries the most were Ni and Cr, due to their corresponding weights having the highest

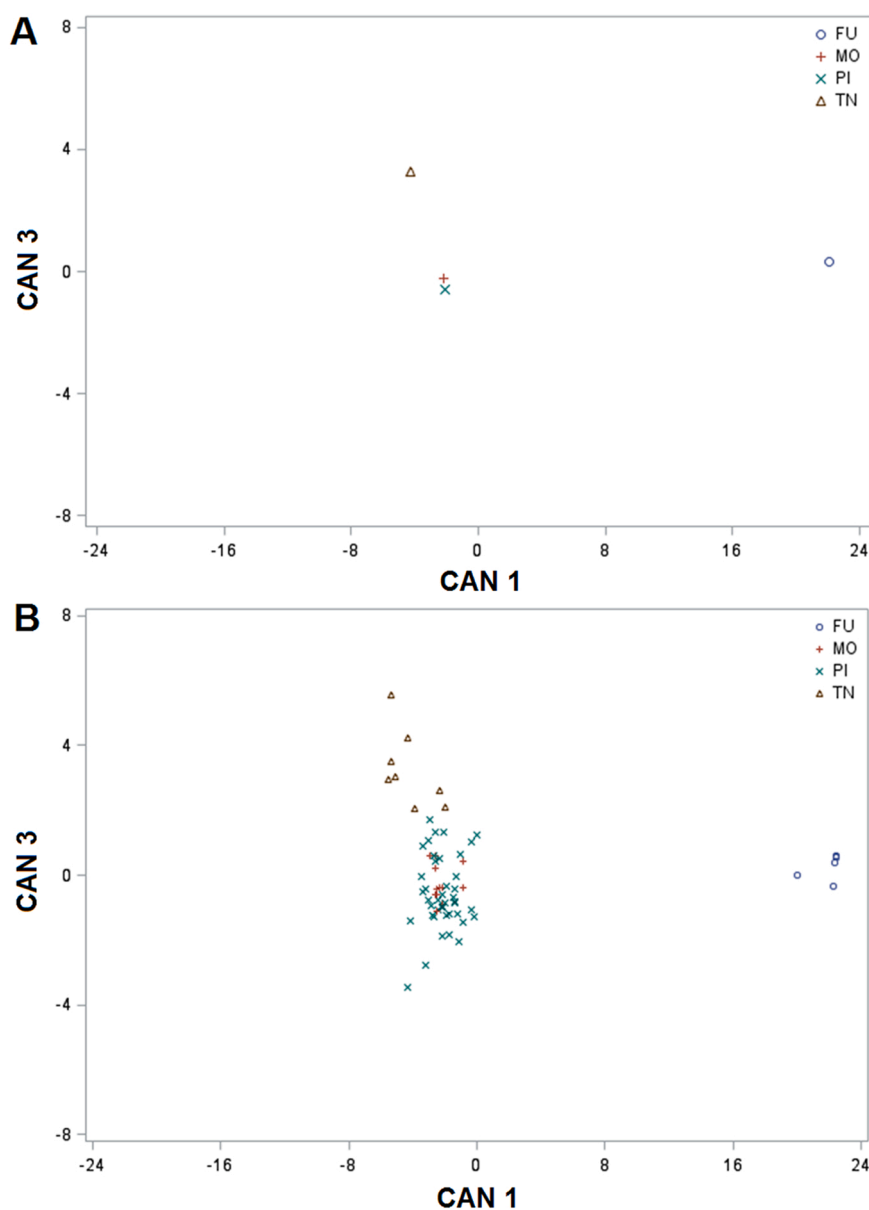


Fig. 2. A) Representation of the apiaries (FU, 1; MO, 2; PI, 3; TN, 4) as averages of canonical functions 1 and 3; B) Representation of individual bee pollen samples from the apiaries (FU, 1; MO, 2; PI, 3; TN, 4) as representations of canonical functions 1 and 3.

values for all the canonical functions; moreover, Ba and Li also had a significant influence in this separation, yet to a lesser extent than the above-mentioned elements. These results are very interesting, since the compounds that influenced separation the most were not in fact those found at the highest concentration (see Tables 3 and 4). Therefore, the importance of evaluating the content of both majority and minority elements in bee pollen is demonstrated, not only in order to evaluate its nutritional power, but also to distinguish its origin. Subsequently, a CDA was performed by quadratic discriminant function and the first three canonical functions, with the results shown in Table 9S (Supplementary Material). Cross-validation concluded that quadratic discriminant analysis made possible an excellent classification of the bee pollen samples in terms of their apiary of origin, with over 90% being correctly assigned, and in the case of three of the apiaries (FU, MO, and TN), the success rate was 100%. To our knowledge, this is the first time that the apiary of origin has been distinguished by means of the mineral content of bee pollen samples, and, more importantly, that most of the samples (> 90%) could be correctly assigned to their apiary of origin. Thus, it can be concluded that these results have confirmed the potential of mineral

elements as markers of the geographical origin, and more specifically of the apiary of origin.

3.3.2. Harvesting period

The sample size (seventy-one) and number of variables (fifteen) were the same as for the study based on the apiary of origin-based study, but in this case, they were distributed among three classes (harvesting periods: April-May, June, and July-August). Here, it is worth mentioning that the first two canonical functions were sufficient to account for 100% of data variability (data not shown), which is a better result than the one obtained for the previous study. Subsequently, the weights of both canonical functions were obtained, and the average values calculated (see Supplementary Material, Tables 10S, and 11S). Graphical representation of the average values (see Fig. 3A) or the individual values (see Fig. 3B) showed a clear differentiation of the three harvesting periods based on the mineral content, which is a considerable improvement compared with that of the apiary of origin. As can be appreciated, the June period displays positive values for both canonical functions, while the other two harvesting periods have opposite values for both canonical

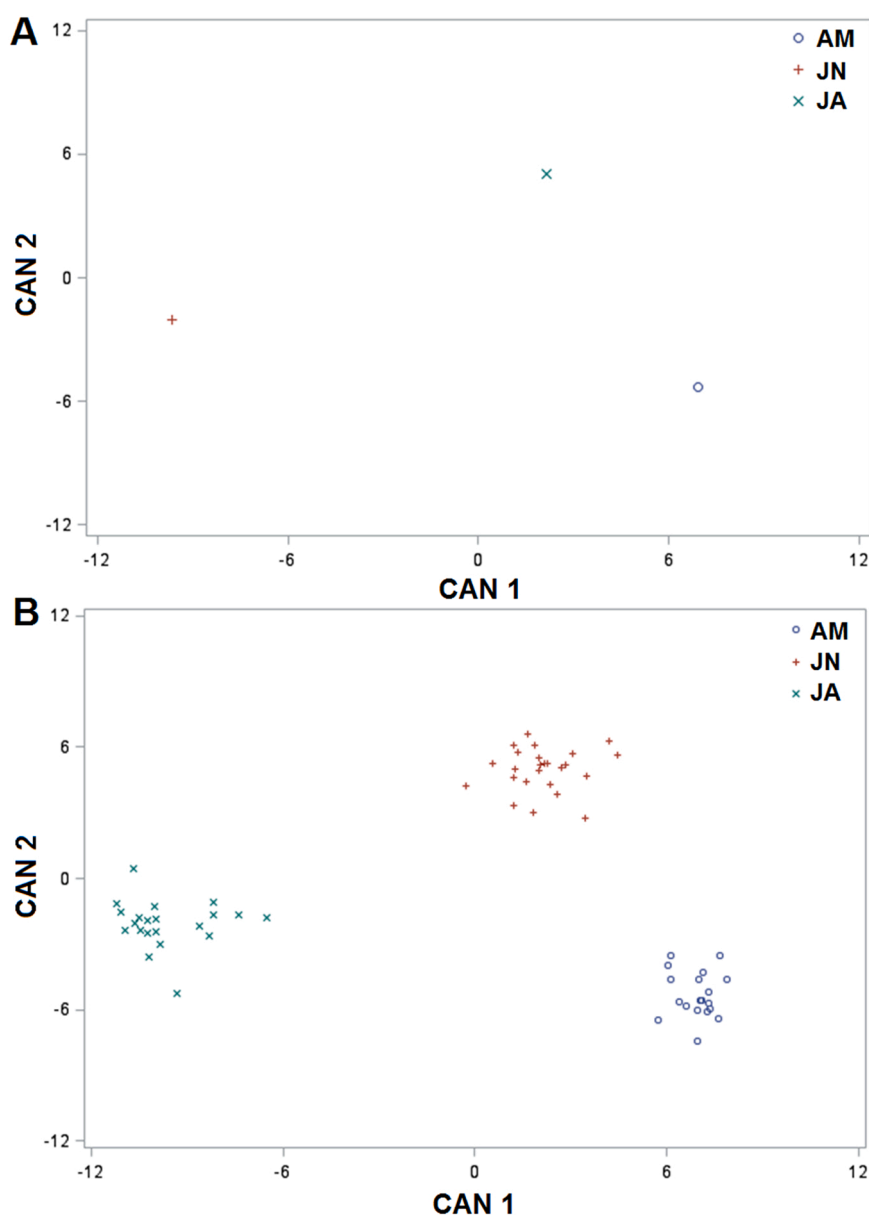


Fig. 3. A) Representation of the harvesting periods (April-May, AM; June, JN; July-August, JA) as averages of the first two canonical functions; B) Representation of individual bee pollen samples from the different harvesting periods (April-May, AM; June, JN; July-August, JA) as representation of the first two canonical functions.

functions, which facilitates their differentiation. In this case, and as was the case with the apiaries of origin, the elements with a greater weight of both canonical functions, and which, therefore, exerted a greater influence on the separation of the samples; these were followed by Ni and Cr, followed in this case by Ba, Sr and Li, which, as was previously mentioned, were not majority elements. Moreover, a cross-validation concluded that quadratic discriminant analysis made possible a perfect classification of the bee pollen samples in terms of their harvesting period, as the success rate was 100% in all cases (see [Supplementary Material, Table 12S](#)). There are not only excellent results, but also relevant findings, as it is the first time that bee pollen samples have been correctly classified according to the harvesting period by determining individual mineral content.

4. Conclusions

An analytical study of mineral content by ICP-OES was carried out on seventy-one samples of bee pollen from four different apiaries, located in Marchamalo (Guadalajara, Spain). P and K were the predominant elements, followed by Ca, Mg and Na, residues of heavy metals not being observed in any of the samples. Moreover, it can be concluded that not significant differences were detected in overall mineral content per sample according to the apiary of origin or the harvesting period; on the other hand, individual mineral content exhibited certain variations depending on the origin of bee pollen. This finding was also observed in each beehive, and maximum values could in most cases be related in most cases to monofloral bee pollen. Canonical discriminant analyses were conducted on the basis of the mineral content in the 71 bee pollen samples, and it was possible to assign over 90% of the samples to the corresponding apiary; meanwhile, all the samples were correctly classified according to the harvesting period. Finally, it can be concluded that a new perspective for classifying bee pollen samples has been provided, by demonstrating for the first time that mineral content can be used to discriminate bee pollen samples in relation to their harvesting period and apiary of origin.

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Silvia Valverde: Conceptualization, Methodology, Investigation, Supervision, Validation, Visualization, Writing – original draft. **Jesús Tapia:** Conceptualization, Formal analysis, Software, Visualization. **Araceli Pérez-Sanz:** Methodology, Investigation, Validation, Visualization. **Amelia V. González-Porto:** Conceptualization, Methodology, Investigation, Resources, Visualization. **Mariano Higes:** Conceptualization, Resources, Visualization. **Raquel Martín-Hernández:** Conceptualization, Funding acquisition, Project administration, Resources, Visualization. **Juan J. Lucena:** Funding acquisition, Project administration, Resources, Visualization. **José Bernal:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request. The datasets generated during the current study are included in this published article and the [Supplementary Material](#), or they are available from the corresponding author on reasonable request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jfca.2023.105235](https://doi.org/10.1016/j.jfca.2023.105235).

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