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Mineral analysis of pollen by Total Reflection X-Ray Fluorescence

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HIGHLIGHTS

- This is the first report of an analysis of Argentine bee pollen by TXRF.
- The grand means of mineral composition of bee pollen exhibited that K, P, S and Ca were the predominant elements.
- The ranges of elemental concentrations found in bee pollen using TXRF is comparable to those found from other techniques.

The preservation of the health of bees, whether farmed or wild, is part of the good management of the environment, food security and the enhancement of world agriculture (OIE, 2014). Pollination of crops by animals is an essential part of global food production, but evidence suggests that wild pollinator populations may be declining while a number of problems are besetting managed honey bee colonies (Bailes et al., 2015). Around 75% of the 115 highest producing crops worldwide benefit from greater yields when pollinated by animals, mainly bees (Klein et al., 2007). Adequate nutrition is the support for the growth and development of a healthy bee colony. The factors responsible for the amount of produced progeny, the longevity, the health of adults, survival and productivity of a colony of bees are carbohydrates (honey), proteins (pollen), lipids, vitamins and minerals available to bees, but despite its importance, these last three nutrients (lipids, vitamins and minerals) are little studied (Brodschneider and Crailsheim, 2010). Although the bees, other insects and animals, have mineral requirements that are essential for many biochemical reactions within the body the real mineral dietary needs for bees have not been well established (Herbert, 1997). The dominant source of minerals for bees is pollen, with secondary quantities that normally come from the nectar (Somerville and Nicol, 2002).

For plants the pollen is the male gamete to fertilize the female gamete which will generate the seeds for the perpetuation of the species. Bee pollen is the result of the agglutination of flower pollens, made by worker honey bees, with nectar (and/or honey) and salivary substances, and collected at the hive entrance (Campos et al., 2008) with bee pollen traps.

There is strong evidence that the minerals in some sources of pollen concentrations are so high that there is a negative effect on breeding and productivity (Black, 2006), or that the forage can be repelled by high concentrations of potassium and phosphorus (Afik et al., 2006; Hagler, 1990; Wiest et al., 2011).

Afik et al. (2006) has suggested that the syndrome of depopulation of the colonies (Colony Collapse Disorder, CCD) that is observed in the beehives for years in Queensland is very likely due to high concentration of trace elements.

In addition to essential minerals, bee pollen may also contain pollutants since it may be exposed to various sources of pollution according to its environment (Black, 2006; Wiest et al., 2011). This could also create a problem of food safety, since the pollen is known for its nutritional and therapeutic effects and has had a growing interest in recent years due to the trend towards a gradual dietary supplementation with natural products for humans (Funari et al., 2003; De-Melo et al., 2015).

Argentine beekeeping has a leading role at the global level; it became the world's second-largest producer of honey in the year 2006 and for several years was the first or second world exporter of honey (Somerville and Nicol, 2002). The preservation of the health of bees is essential to sustain and increase domestic production and ensuring biodiversity.

Few studies have been conducted in our country about the mineral content of bee products; Baldi Coronel et al. (2004) studied the mineral content of sodium, potassium, calcium, magnesium, iron, copper, manganese and zinc in pollen. Bailes et al. (2015) and Patrignani et al. (2015) evaluated the geographical discrimination of honey through differences in elemental concentrations. Studies approaches mineral contributions to the hive and its seasonal variation around the world are scarce; therefore a multielemental characterization could contribute with knowledge about mineral nutrition of bees.

Total-reflection X-ray fluorescence (TXRF) is a nuclear analytical technique of simultaneous multielemental analysis which is quite economical with respect to costs of instrumentation and maintenance, as well as low in time consumption. Quantification is performed by internal standardization, either by the addition of the analyte element

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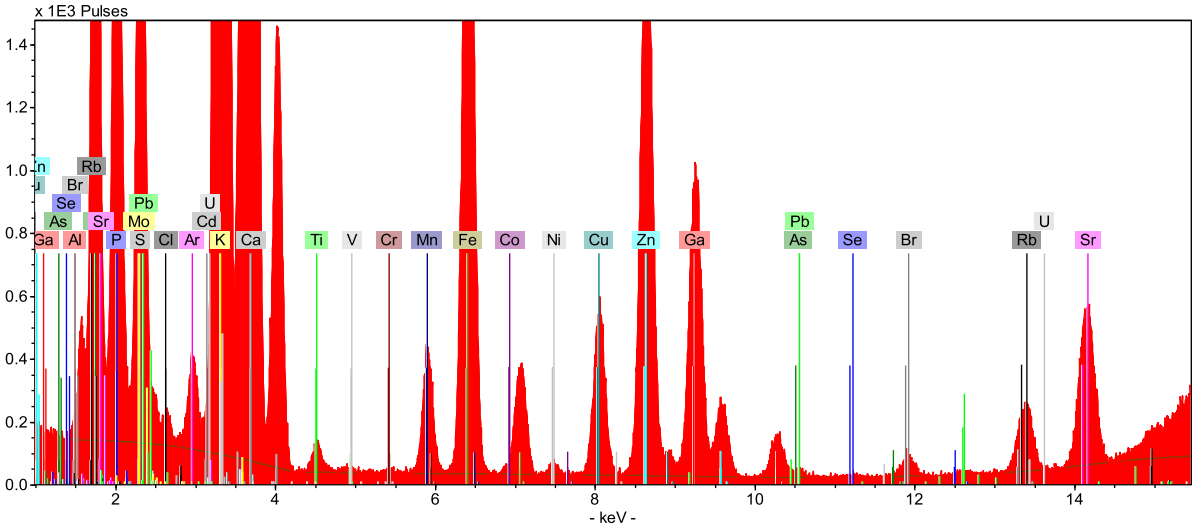

Fig. 1. Spectrum of a analysed pollen sample.

Table 1
Elemental concentration in mg kg^{-1} present in dehydrated bee pollen samples from Argentina. ND: Not detected.

	Al	P	S	Cl	K	Ca	Ti	V	Cr	Mn
September	ND	5679	1792	32,3	7122	818	3,2	0,1	0,1	31,0
October	43,4	5440	2071	43,5	6101	1042	9,1	0,7	0,4	37,3
November	37,2	5838	1888	36,6	6059	1159	9,0	0,8	ND	34,8
December	17,5	5354	1755	30,5	5446	1067	3,5	ND	ND	32,6
January	7,3	4393	1293	27,8	4178	1297	5,1	ND	ND	18,3
February	61,2	3795	1397	35,2	4328	1449	6,7	0,4	0,1	19,3
March	47,9	1937	1022	44,2	4572	1658	9,7	0,5	0,1	13,5
Grand mean	35,8	4634	1602	35,7	5401	1213	6,6	0,5	0,2	26,7
Standard deviation	20,0	1399	373	6,2	1097	280	2,7	0,3	0,1	9,4
Max value	61,2	5838	2071	44,2	7122	1658	9,7	0,8	0,4	37,3
Min value	7,3	1937	1022	27,8	4178	818	3,2	0,1	0,1	13,5

	Fe	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Br**	**Rb**	**Sr**	**Pb**
September	63,6	0,1	11,9	44,9	0,1	0,3	0,1	2,5	4,4	ND
October	109,1	7,0	15,0	71,7	0,1	0,2	0,2	3,4	4,6	ND
November	104,9	ND	26,0	59,8	0,1	0,2	0,6	4,1	5,1	ND
December	77,6	0,3	25,0	59,6	0,1	0,2	0,3	4,1	5,1	ND
January	59,4	1,3	14,1	41,1	0,1	ND	0,6	3,9	4,0	ND
February	114,1	0,7	15,4	44,8	0,2	0,1	1,0	3,4	9,2	ND
March	121,5	1,0	11,8	26,9	0,2	ND	0,7	4,1	6,1	ND
Grand mean	92,9	1,7	17,0	49,8	0,1	0,2	0,5	3,7	5,5	ND
Standard deviation	25,5	2,6	6,0	14,9	0,0	0,1	0,3	0,6	1,8	ND
Max value	121,5	7,0	26,0	71,7	0,2	0,3	1,0	4,1	9,2	ND
Min value	59,4	0,1	11,8	26,9	0,1	0,1	0,1	2,5	4,0	ND

All results are expressed as mean (n = 5).

itself or of another element (i.e., previously not present in the sample). No labor intensive calibration is required. Because of the small sample volume needed for analysis, in general no matrix effects occur (Klockenkämper and von Bohlen, 2014), even though there can be matrix effects if organic samples are analysed directly (i.e. no acid digestion).

In recent years, the arrival on the market of compact TXRF equipment using new technologies with competitive costs against conventional equipment AAS and ICPS has promoted the TXRF approach in analytical chemistry in laboratories around the world (Marguí et al., 2014). In different interlaboratory comparisons, TXRF showed good performance in comparison with methods such as electrothermal atomic absorption method (ET-AAS), inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma-mass spectrometry (ICP-MS), Rutherford Backscattering Spectrometry (RBS) and Instrumental Neutron Activation Analysis (INAA) Klockenkämper and von Bohlen, 2015). The sensitivity of the TXRF technique and the small amounts of sample required for analysis of pollen make TXRF a suitable technique for the analysis of such samples.

The aim of this work was to use the technique TXRF to analyse mineral composition of Argentinian bee pollens. TXRF performed by S2 Picofox Bruker equipment never has been used for analyse mineral content in this type of samples.

1. Experimental

The multiflower bee pollen samples (n = 35) were collected from 5 hives located next to each other and equipped with plastic pollen traps at the Ezeiza Atomic Center, Ezeiza, Buenos Aires province, Argentina. The sampling was made in the morning between 10:00 a.m. and 12:00 a.m. once a month at the same time during all the foraging stations from September to March. The samples were dried in oven at 50 °C (ANMAT, 2018) for 24 h s, homogenized and stored at −20 °C until analysis. The acid decomposition by microwave digestion was selected because this preparation procedure is preferable to the pollen suspension in terms of reproducibility and representativeness of the whole sample and the higher background of the suspension spectrum leads to higher detection limits in the whole spectral range (Pepponia et al., 2004). Samples of 0.5 gm of pollen was treated by acid digestion in closed microwave system Milestone brand with 3 ml of sub boiling nitric acid and 1 ml hydrogen peroxide 30% (v/v) (Merck Suprapur) as oxidizing agents. The liquid of digestion was taken to 10 ml flask with Milli-Q® ultrapure quality water. Gallium 1 mg L^{-1} was added to sample as internal standard.

Quantification of mineral elements was performed in 5 µl of sample with a Benchtop TXRF Spectrometer S2 Picofox (Bruker AXS Microanalysis GmbH, Berlin, Germany), equipped with an X-ray tube microfoco (Mo anode, 50 kV and 750 µA), a multi-layer monochromator (Ni/C), X flash® silicon drift detector with 30 mm2 area with a resolution of around 150 EV in MnKα-line. The treatment of x-ray spectra and calculations were performed using SPECTRA 5, 3e FRXRT S2 Picofox of BRUKER software. Instrument parameters for the quality control were monitored, including sensitivity and accuracy of the quantification. The accuracy of the measurements was calculated by determining the concentration of the elements in a standard solution (ICP Multi-Element Standards Certipur® IV, Traceable to NIST). The lower limit detection (LLD) was calculated as recommended for the instrument (Bruker Nano GmbH, 2011; Patrignani et al., 2015).

2. Results and discussion

In the pollen samples, 20 minerals (Al, P, S, Cl, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Br, Rb, Sr and Pb) were examined by TXRF in the level of mg kg^{-1}. The spectrum of one sample of analyzed pollen is shown in Fig. 1. Mean contents (n = 5), standard deviations and maximum and minimum values for the minerals in the 35 dehydrated bee pollen samples from 7 months are shown in Table 1. The grand means of mineral composition of bee pollen exhibited that K (5401 mg kg^{-1}), P (4634 mg kg^{-1}), S (1602 mg kg^{-1}) and Ca (1213 mg kg^{-1}) were the predominant elements.

Present in lower concentrations were the remaining elements, except Pb that was not found in any sample (LLD = 0.005 mg L^{-1}); Al (LLD = 2 mg L^{-1}), V (LLD = 0.010 mg L^{-1}), Cr (LLD = 0.010 mg L^{-1}), Ni (LLD = 0.005 mg L^{-1}) and Se (LLD = 0.003 mg L^{-1}) were not found in all the samples.

The wide range of K levels found in the present study, 4178–7122 mg kg^{-1}, covered the range obtained 2263–9784 mg kg^{-1} in an Argentine pollen study (Baldi Coronel et al., 2004) and those of other studies, for example 1431–9910 mg kg^{-1} (Morgano et al., 2012), 5900–7200 mg kg^{-1} (Funari et al., 2003 and Carpes et al., 2009). Samples from Spain, Australia, Poland, South Korea and China presented the following K ranges: 2485–6411 mg kg^{-1}, 2200–3800 mg kg^{-1}, 2843–4854 mg kg^{-1}, 3455–5489 mg kg^{-1}, 4247–5976 mg kg^{-1}, respectively.

Analyzing the concentration ranges obtained in this study for Ca (818–1658 mg kg^{-1}), Fe (59,4–121,5 mg kg^{-1}), Cu (11,8–26,0 mg kg^{-1}), Mn (13,5–37,3 mg kg^{-1}) and Zn (26,9–71,7 mg kg^{-1}), matching or close to the published range values by Baldi Coronel et al. (2004), were found: Ca (160–2496 mg kg^{-1}), Fe (23–229 mg kg^{-1}), Cu (6–20 mg kg^{-1}), Mn (14–57 mg kg^{-1}) and Zn (23–106 mg kg^{-1}).

In general, wide ranges of concentrations between months were obtained, especially for Ni, which had standard deviation higher than the mean; according to O'Rourke and Buchmann (1991) pollen composition may vary between seasons.

The monthly average concentrations for minor elements are in Fig. 2; K and P had a decrease in concentration toward the end of season, not so marked for S. On the other hand, Ca exhibits behaviour slightly upward, different from the rest of the elements.

In Fig. 3, the concentration of trace elements had a decrease in the month of January. This is coincident with the maximum value of mass of produced pollen per hive (Table 2).

As the number of samples is limited any observed trend is simply suggestive; a future work will seek to analyze more samples to arrive at more statistically sound observations.

3. Conclusions

The ranges of elemental concentrations found in bee pollen using Total-reflection X-ray fluorescence is comparable to those found from other techniques.

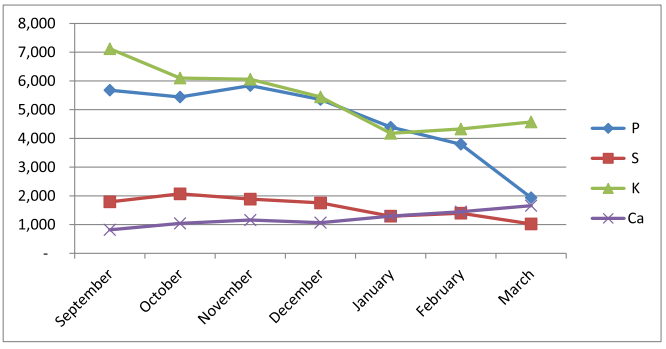

Fig. 2. Mean values in mg kg^{-1} for minor elements in all foraging season.

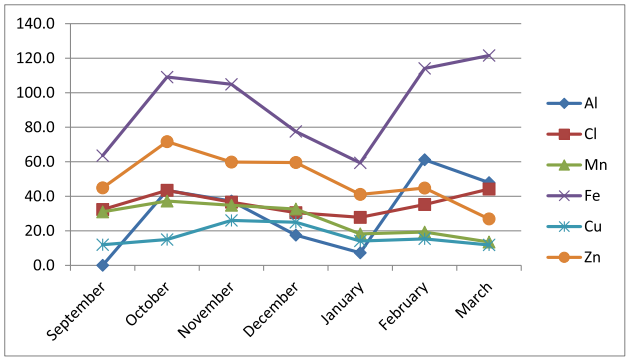

Fig. 3. Mean values in mg kg^{-1} for 6 trace elements for all foraging season.

Table 2
Mean dried mass per hive of bee pollen for each month in grs.

Month	Mean per hive (n = 5)
September	0,806
October	0,752
November	0,881
December	0,861
January	0,944
February	0,835
March	0,920

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