Multi-elemental analysis by FRXDE on pollen produced by the native bee *Scaptotrigona aff. postiça*

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ABSTRACT
Brazil is one of the largest suppliers of bee products in the world. It is currently the 5th largest exporter of honey and derivatives and a significant part of its production originates from the south and northeast. Specifically, the species *Scaptotrigona aff. postica*, from the region of Barra do Corda in the State of Maranhão (MA), produces pollen which is one of the main activities of the local economy (food supplement). However, there are no data on its mineral composition, although there is a history of applications in the nutritional field. In this context, this research aims to generate integrated and interdisciplinary knowledge of the pollen produced by this pollinating species, through multi-element investigation (identification and quantification of chemical elements) using the Energy Dispersive X-ray Fluorescence (XRF) technique. The elemental composition of pollen emphasizes its nutritional relevance (Ca, Cl, Fe, K, Mn and P) and the results of the concentrations showed little seasonal variation, serving as a model for the safe expansion of meliponiculture in the region. In the technological sphere, this analysis is a tool for tracking (provenance), which allows the identification of fraud and/or adulteration, in addition to adding quality control to its commercialization.

Keywords: Bee pollen, XRF, Nutrition, Ion dosage, Dietary supplement.

1 INTRODUCTION

According to the Ministry of Agriculture and Food Supply [1], bee pollen is the result of the agglutination of flower pollen, carried out by bees, through nectar and their salivary substances. Pollen is relevant in the nutrition of bees and can also be used as a food supplement for humans, as it is a protein source, but its production and commercialization is still small compared to honey and propolis. However,

in recent years the demand for natural and healthy products has grown significantly and products from meliponiculture have started to be consumed on a larger scale in Brazil [2,3].

Meliponiculture is the breeding of stingless bees (Meliponineos) for the production and commercialization of hives, honey, propolis, pollen and resins, in addition to these bees being the main agents of pollination and conservation of local biodiversity. Among bee products, pollen has gained prominence in the commercial sphere for being a protein source (16 to 40%, containing several amino acids) and rich in carbohydrates, lipids, minerals, fibers, hormones and vitamins in its composition. Specifically, the pollen produced by the species *Scaptotrigona aff. postica*, (from the Municipality of Barra do Corda, Maranhão - MA) is widely used as a food supplement as well as in the prevention of colds and flu by the local population, in addition to being of interest in sports medicine, as it is considered a nutritional supplement that improves muscle endurance and the immune system of the athlete, in addition to providing elasticity to the tissues.

In recent years, the species *Scaptotrigona aff. postica (*MA) has been investigated at the Institute for Energy and Nuclear Research (IPEN/SP) in partnership with the Butantan Institute (IBu/SP) and the Pontifical Catholic University of Paraná (PUCPR), and the results of investigations with propolis have shown promise in the area of health [4-9]. In this study we propose to generate integrated and interdisciplinary knowledge of the pollen produced by the species *Scaptotrigona aff. postica*. The focus is to fill in the gaps on the multi-elemental characterization of the pollen produced by this bee, generating original knowledge of the mechanism of interdependence between essential and non-essential elements, in addition to adding security in the nutritional scope of this input. An important result of these analyses is the identification of toxic elements, such as Arsenic (As), Lead (Pb), Cadmium (Cd), Chromium (Cr), Mercury (Hg) and Nickel (Ni), which even in low concentrations, are very dangerous to health, restricting their nutritional use. For these qualitative-quantitative analyses of the chemical elements (ion dosage) that make up pollen, the analytical technique of Energy Dispersive X-ray Fluorescence (XRF) will be used.

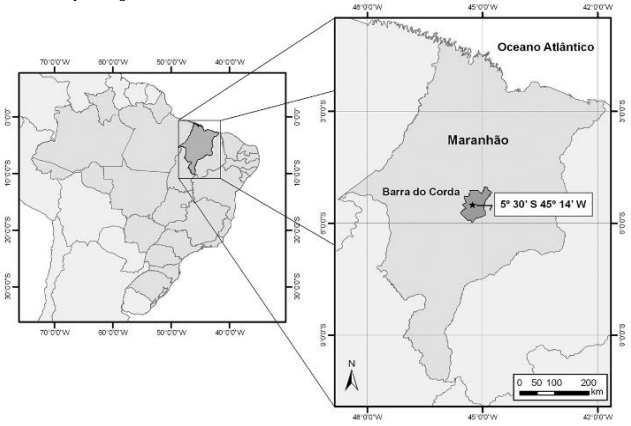
2 EXPERIMENTAL

In the analytical process of Energy Dispersive Ray Fluorescence (XRF) [10], the interaction of the X-ray beam (generated by a targeted X-ray tube) excites the chemical elements present in the sample in question (pollen). In the process of deexcitation, there is the emission of characteristic X-rays, related to the chemical elements that compose it, which are identified and quantified by spectral analyses obtained by dedicated *software* [11,12].

The pollen samples were obtained from the municipality of Barra do Corda (MA). The city is located in the geographic center of the State of Maranhão (**Figure 1**) at the confluence of the Corda and Mearim Rivers. Pollen was collected every 3 months for one year, depending on the seasons (summer, autumn and

winter and spring). The samples, prepared in duplicate, were selected from two hives (named C1: hive 1 and C2: hive 2). The hives were renewed, i.e., after each collection they were emptied and renewed for reliable sampling. It is important to note that the soil did not receive any type of fertilizer.

Figure 1. Municipality of Barra do Corda, Maranhão, Brazil *Source: Scielo.br.*



The samples were partitioned, crushed in mortar and compacted into pellets (~200mg). The XRF measurements were performed using an XRF spectrometer (model X-123 SDD Complete X Ray Spectrometer - Amptek®) consisting of an X-ray tube with silver (Ag) target, 25 mm2 x 500 μm Si Drift (SDD) silicon detector and 12.5 μm beryllium window. The measurements were performed using a current of 5 μA, an operating voltage of 30 KV and an excitation time of 300 s.

3 RESULTS

Table **1** shows the pollen results (Mean Value and Standard Deviation) obtained by the XRF technique. Figure 1 was prepared to evaluate the behavior of the concentration of each element as a function of the collections by season of the year, in the period of 1 year. In the elaboration of these curves, the concentrations were normalized according to the first collection.

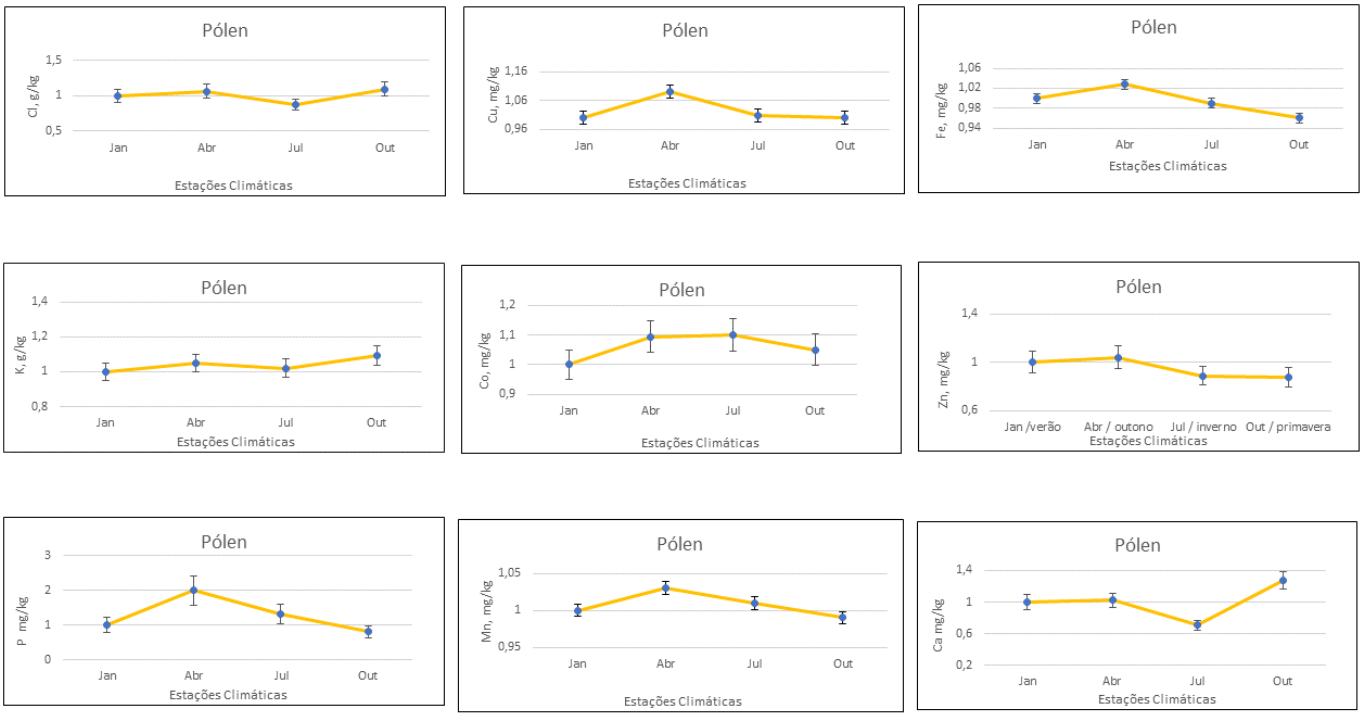
Table 1. Concentration of the elements in the samples of pollen produced by the species *Scaptotrigona aff. postica* (MA)

mg/kg	Average Value	±1 Standard Deviation	CV, %
Cl	715	67	0,09
K	287	28	0,10
P	13,45	0,13	0,01
Cu	2,65	0,31	0,12
Co	2,01	0,19	0,09
Mn	1,41	0,14	0,04
Zn	1,09	0,03	0,03
Fe	1,08	0,09	0,08
Ca	0,232	0,021	0,09

The behavior of the concentrations of the investigated elements in pollen, as a function of the collections by climatic seasons, expressed by the Coefficient of Variation (CV), emphasizes that throughout the year the contents of the elements vary little (1% for P to 10% for K). Considering that the climate in this region is tropical with an average temperature of 26.9 °C and with little variation throughout the year (less than 6 °C),[1] together with the conservation of the vegetation, they guarantee a balanced system, that is, subject to few changes. In addition, it is important to note that no type of pesticide is used at the collection site.

[1] According to data from the National Institute of Meteorology (INMET), in the period from 1961 to 2019 the lowest temperature recorded was 10.2 C (on August 4, 1966) and the highest reached 40.9 C (September 23, 1962).

Figure 2. Behaviour of the concentration of the elements Cl [A], K [B], P [C], Cu [D], Co [E], Mn [F], Fe [G], Zn [H] and Ca [I] in pollen samples. Normalized data based on the first collection.



Regarding mineral content, the most abundant element found was Cl (715 ± 67 mg/kg), followed by K (287 ± 27 mg/kg) and P (13.45 ± 0.13 mg/kg) and, in lower concentrations, Cu (2.65 ± 0.31 mg/kg), Co (2.01 ± 0.19 mg/kg), Mn (1.41 ± 0.14 mg/kg), Zn (1.09 ± 0.03 mg/kg), Fe (1.08 ± 0.09 mg/kg) and Ca (0.232 ± 0.021 mg/kg). Figures **3 and 4 show the quantification of pollen** as a function of the major elements (**Figure 3**) and traces (**Figure 4**) in percentage terms . The pollen produced by the species *Scaptotrigona aff. postica* (MA) is mainly rich in potassium and has a low calcium content. The high concentration of Cl is due to the compounds formed with the major elements (K and P) and the other trace elements (Ca, Co, Cu, Fe, Mn and Zn).

Figure 3. Illustrative scheme of the multi-elemental composition of pollen as a function of the majority elements

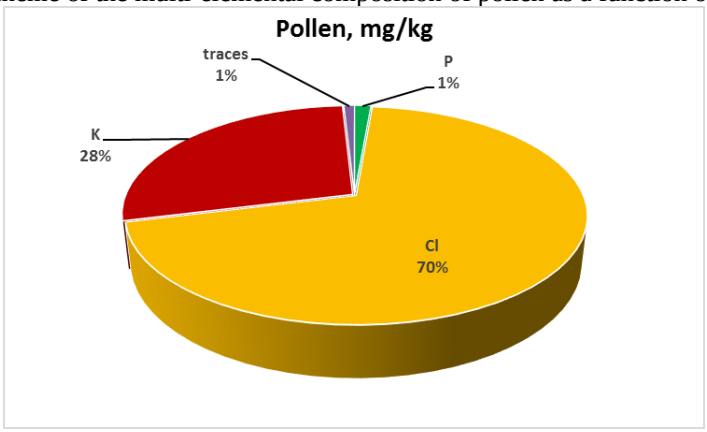
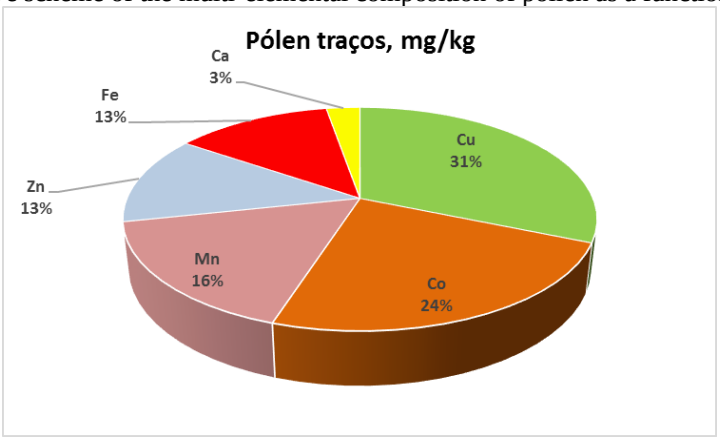


Figure 4. Illustrative scheme of the multi-elemental composition of pollen as a function of trace elements



These data constitute the first quantitative estimate of pollen produced by colonies of *Scaptotrigona aff bees. and* generate knowledge that ensures its nutritional use. In addition, considering that the demand for natural products has increased (Brazil stands out as a world exporter of honey and derivatives [3]), these data also contribute to the verification of food quality and safety, with regard to adulteration and storage conditions.

4 CONCLUSION

The knowledge acquired in the investigation of pollen produced by colonies of *Scaptotrigona aff. postica* (MA) adds improvements in the local market, which will allow to expand the commercial interest of this nutritional input, in addition to serving as a model for the safe expansion of meliponiculture in the region.

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