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**Evaluation of matrix effect on the determination of rare earth elements and As, Bi, Cd, Pb, Se and In in honey and pollen of native Brazilian bees (*Tetragonisca angustula* – Jataí) by Q-ICP-MS**

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**Abstract**

Bees are considered the main pollinators in natural and agricultural environments. Chemical elements from honey and pollen have been be used for monitoring the environment, the health of bees and the quality of their products. Nevertheless, there are not many studies on honey and pollen of native Brazilian bees. The goal of this work was to determine important chemical

elements (Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Ho, Er, Tm, Lu and Yb) along with As, Bi, Cd, Pb, Se and In, in honey and pollen of native Brazilian bees, assessing analytical interferences from the matrix. A proposed analytical method was developed for these elements by quadrupole ICP-MS. Matrix effect was verified in honey matrix in the quantification of As, Bi and Dy; and in pollen matrix for Bi, Cd, Ce, Gd, La, Pb and Sc. The quality of the method was considered satisfactory taking into consideration the recovery rate of each element in the spiked solutions: honey matrix (91.6%-103.9%) and pollen matrix (94.1%-115.6%). The quantification limits of the method ranged between 0.00041-10.3 μg L$^{-1}$ for honey and 0.00041-0.095 μg L$^{-1}$ for pollen. The results demonstrate that the method is accurate, precise and suitable.

**Keywords**: Honey; Pollen; Native bees; Matrix effect; Rare earth elements; ICP-MS

## 1. Introduction

Honey and pollen are natural products commonly found in hives [1]. Honey produced by *Apis mellifera* bees is defined as a natural sweet substance produced by bees from the nectar of plants, either from secretions of living parts of plants, or excretions of plant-sucking insects. Honey bees collect and transform these raw materials and combine them with specific substances produced by them, depositing, dehydrating and storing them in honeycombs to ripen and mature it [1]. The honey produced by native wild stingless bees is stored in pots rather than in combs. This honey is also nectar or honeydew that has been concentrated and transformed [2]. Bees collect pollen as source of protein, and nectar as source of sugars, thereby promoting fertilization of plants. During their activities, bees cover a large area around the hive [3]. It is estimated that the honey produced in one day on a comb is the result of at least 1 million interactions between bees and flowers [4]. Bees are considered the main pollinators in natural and agricultural environments [5], and represent an important element for the preservation of plant biodiversity [3]. This process is considered an environmental service or ecosystem service [5], and has large importance even in cultures that are not dependent on pollinators such as bees [6,7]. Some plants have their production success more affected by the pollination than the

fertility of the soil or climate conditions [8]. Pollination is not the only ecosystem service of bees. They yield products which are used as food [5], cosmetics and pharmaceuticals [9,10].

In recent decades the concern with the preservation of bees has been growing due to the drastic losses of colonies. The decline of these colonies in winter 2009-2010 ranged from 7-30% in Europe; 16-25% in six Canadian provinces; and 40% in Nova Scotia [11]. The United States lost 44% of their honey bee colonies from April 2015 to April 2016 [12]. Among the many causes [13] for this decline is the Colony Collapse Disorder (CCD), a mysterious syndrome affecting bee hives, especially in Europe and in the US [12,14]. Other factors that have contributed to the reduction of the number and variety of species of bees are mite infestation, growth of cities, use of pesticides, and the reduction of forests [14]. Moreover, native wild stingless bees also are vulnerable to competition from the invasive Africanized honey bees [15]. Alterations on pollination activity impair important ecosystem functions upon which our food supply depends, also interfering with the chemical composition and quality of products from bees [16].

The products of European and Africanized honey bees (*Apis mellifera*) are the most used ones in surveys on honey bee decline. Studies have shown that wild and native bees, *e.g.* stingless bees, are responsible for a considerable proportion of pollination [17] of different species of plants [18]. Hence, preserving the population of native bees and their colonies is of crucial importance for the maintenance of food resources [14].

During the pollination process, the honey bees come into contact with the environment [19]. Thereby the mineral composition of honey and pollen represents a fingerprint of their geographical and botanical origins [20-22], as well as environmental contamination [23-31], which enables their use for environment monitoring. Currently, there are not many studies using the chemical elements in honey and pollen involving Brazilian native bees [31-34]. Because of the lack of knowledge regarding these products, their possible use for monitoring the bees and their products is difficult [15,35].

Among the chemical elements, studied in the present work, the Rare Earth Elements (REE) are a chemically uniform group, and they are known for their great importance as indicators of soil

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processes, which contribute to increased interest in these elements in environmental sciences [36]. The remaining studied chemical elements, *i. e.,* As, Bi, Cd, Pb, Se and In constitute persistent toxins, given their natural affinity for sulfhydryl groups of enzymes [33,34]. All of these elements occur in rocks and are mobilized by natural mechanisms such as weathering and biological activity, and due to anthropogenic activities such as mining for example.

The determination of chemical elements in honey and pollen matrices is a difficult analytical task [16,37]. Several techniques have been used in their determination in bee products. Presently, the most widely acceptable method for digestion of this type of matrix is the microwave oven, which has allowed the reduction of reagents quantities, temperature and time [31,38]. Tuzen et al. [39] compared three different methods of digestion in honey samples collected in the region of Turkey, and concluded that the analysis carried out in samples solubilized with the microwave oven had better rate recovery of the chemical elements. According to Linge [40], the most recommended technique for determination of chemical elements in honey and pollen is the ICP-MS (Mass Spectrometry - Inductively Coupled Plasma). This technique is considered a reliable and fast analytical method for trace and ultra trace elements [40,41].

The challenge of these chemical analyzes is the organic complex matrix. It can affect the analysis of individual components [32], due to colloidal particles and the large variety of organic compounds present, which can adsorb ions from solution, thereby causing bias in the actual analytical result [52]. According to Taylor [43] the analytes are all depressed depending on the level of the interference being more serious as the analyte mass decreases and the mass matrix increases, this effect is not well understood yet.

Analytical results must be unambiguous and reflect the actual composition of the sample. Hence a reliable instrumental method must be chosen, leading to results with proper traceability to ensure quality results and reliable values [42]. However, achieving traceability of measurement results in these materials is a complex task due to the lack of corresponding certified reference materials (CRM). Due to the high carbohydrate content, it is not easy to obtain reference material equivalent to honey in the market [16]. Caroli et al. [44] initiated a major effort to

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generate a specific CRM for chemicals based on honey matrix. Usable results are not yet available. For this reason, some authors use CRMs with other matrix nature, *e. g.*, SRM 1515 - Apple leave [16,27,39] and BCR 191 - Brown Bread [44].

In the case of lack of corresponding CRM, the method of additions is the most suitable to ensure the quality of the analysis CRM [45]. It has been applied mainly to overcome the problems caused by high content of carbohydrates found in the analyses with honey and pollen matrices [16,46-48]. However, it is laborious, expensive and cannot be used routinely. An alternative solution is the spike method, in which a known amount of analyte is added to a solution of the sample matrix and to a solution made with the standard solvent (water). The two sets of responses are compared based on values calculated from an analytical curve. If the value observed for the spike shows no significant difference with the value obtained for the analyte prepared in standard diluent (recovery), the sample matrix is considered valid and free of interference. If the recovery rate differs significantly, *i.e.*, higher than 120% or lower than 80%, then components in the sample matrix may be causing interference, and adjustments must be made to the methodology. One strategy is to use a standard diluent whose composition matches the final sample matrix. A second strategy is to alter the sample matrix. In this work we adopted the spike method to check for the occurrence of matrix interference [49]. Finally internal standards are frequently used in ICP-MS analyses to correct for variations in the instrument response (drift) in the course of analysis and to correct the analyte concentrations in the samples [50].

The aim of this work was to assess the matrix effect and to propose an analytical method to quantify REE (Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Ho, Er, Tm, Lu and Yb) and As, Bi, Cd, Pb, Se and In in honey and pollen of native Brazilian bees (*Tetragonisca angustula* – Jataí). Material digestion was carried out in microwave oven, a procedure that has allowed reductions in the amounts of reactants and digestion time [31], and analytical measurements were obtained by the Q-ICP-MS technique (Inductively Coupled Plasma Mass Spectrometry).

## 2. Experimental

To verify the matrix effect (honey and pollen) in determining REE (Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Ho, Er, Tm, Lu and Yb), e As, Bi, Cd, Pb, Se and In, calibration curves of aqueous solutions, solutions with honey matrix and solutions with the pollen matrix were prepared. Evidence of the occurrence of interference was sought by comparing the slopes of the calibration curves of each matrix (honey and pollen) with the slope of the curve made with aqueous solution. Any differences in these values may indicate matrix interference in the determination of each element, since in aqueous solution the components of the original matrix are absent. Other figures of merit were also calculated to evaluate the quality of results [51]. Material digestion was carried out in microwave oven. This procedure has allowed reductions in the amounts of reactants and digestion time [31]. Analytical measurements were obtained by the quadrupole ICP-MS (Q-ICP-MS) technique (Inductively Coupled Plasma Mass Spectrometry). The ICP-MS is considered a reliable and fast analytical method for trace and ultra trace elements [40,41].

## 2.1. Reagents and solutions

All solutions were prepared using water (18.2 MΩcm resistivity) purified with Millipore Mill-Q® purification system (Direct-Q 3). The reagents used in the digestion were: concentrated nitric acid ($HNO_3$ 65% m/m, Suprapur®, Merck, Germany) and hydrogen peroxide ($H_2O_2$ 30% m/m, Suprapur®, Merck). A solution $HNO_3$ 1% v/v (Suprapur®, Merck, Ultrapure) solution was used to clean the Q-ICP-MS apparatus between quantifications. Analytical curves were prepared with multi-element standard solution CertiPUR® (Merck, Darmstadt, Germany) at concentration of 10 mg $L^{-1}$ (As, Bi, Cd, In, Pb, Se), and High-Purity mono-element standard solutions at a concentration of 10 mg $L^{-1}$ each (REE) including Tb (internal standard).

## 2.2. Instruments and apparatus

Digestions were performed in a microwave oven (Milestone Ethos 1). Configurations and instrumental parameters used are reported in Table 1. The element concentrations were measured with a Q-ICP-MS (Inductively Coupled Plasma Mass Spectrometry - Agilent 7700x). The operational condition of the Q-ICP-MS equipment were optimized using a tuning solution (Ce, Co, Li, Tl and Y 1 μg $L^{-1}$ Agilent Technologies standard solution) aiming to obtain

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maximum intensity for mass/charge ratio of the following isotopes ($^7$Li, $^{59}$Co, $^{89}$Y, $^{205}$Tl) keeping the oxide formation ($^{140}$Ce$^{16}$O$^+$/$^{140}$Ce) below 1.5% and the double charge formation ($^{140}$Ce$^{2+}$/$^{140}$Ce$^+$) below to 3.0%. Counts for $^7$Li, $^{89}$Y e $^{205}$Tl were checked, and kept to a minimum, *i.e.,* 8000 cps, 19000 cps and 18000 cps respectively, for no gas mode; and for $^{59}$Co, $^{89}$Y and $^{205}$Tl in collision cell mode (He), *i.e.,* 5000 cps, 6000 cps and 11000 cps respectively. All operating conditions are shown in Table 2. Q-ICP-MS was coupled to a collision cell to reduce spectral interferences from polyatomic ions.

Elements presenting the possibility of polyatomic or molecular spectral interference (As, Cd, Sc and Y) were analyzed in collision cell mode (3.5 mL$^{-1}$ min flow rate for He) and the results for NIST 1515 SRM were not good in agreement to certified values (Table 2).

*2.3. Honey and pollen samples*

The procedure was applied on honey and pollen samples which came from five different areas within Quadrilátero Ferrífero, south-central state of Minas Gerais, Brazil. All samples were collected from native Brazilian bees (jataí). The samples were stored in glass bottles and kept at 4–5 $^o$C in dark place until analysis.

*2.4. Matrix extracts*

Matrix extracts are solutions prepared with honey and pollen separately in order to set up analytical curves set up with the actual material to be analysed (matrix matching). This matrix was prepared with a blend of samples (pool) of honey collected from native bee (jataí) hives from 10 different sources. A blend of these 10 honey samples were homogenized in a water bath and digested using a microwave oven decomposition system (Milestone Ethos 1). Aliquots of 1.0 g (± 0.1 mg) of this honey pool were accurately weighed in a PTFE digestion vessel. A volume of 7 mL of HNO$_3$ (65% m/m) and 1 mL H$_2$O$_2$ (30% m/m) were added for digestion. This mixture was pre-digested for 12 hours followed digestion in microwave oven (Table 1). After digestion the vessels were closed and kept at room temperature for 12 hours until complete cooling was achieved. This procedure aimed to reduce possible losses of elements by volatilisation and to optimise digestion. The resultant solutions were quantitatively transferred to 50 mL volumetric flasks and the volume was completed with ultrapure water.

The pollen matrix extract was prepared the same way with the difference that a mass of 0.5 g (± 0.1 mg). Both pools were stored in the dark and kept at a temperature of 4-5 °C. Previously to the preparation of analytical curves, matrices were homogenised and allowed to reach room temperature. Honey and pollen samples, blank solutions and SRM 1515 samples were prepared in the same way.

*2.5. Analytical procedures*

Calibration curves (10 mL each) were prepared from the stock solution with 10 mg L$^{-1}$ for the following elements: As, Bi, Cd, Ce, Dy, Eu, Er, Gd, Ho, In, La, Lu, Nd, Pb, Pr, Sc, Se, Sm, Tm, Y and Yb at concentrations: 0.125, 0.250, 1.0, 5.0, 10.0, 50.0 e 100.0 µg L$^{-1}$. To test possible matrix effect three groups of calibration curves were prepared: one aqueous curve and two matrix matching calibration curve (n=3 for both). The matrix matching calibration curves were prepared using 4 mL of matrix extract and in the case of curves of aqueous solutions it was added HNO$_3$ 1% v/v. The internal standard was added in the solutions used in the curve at a concentration of 2 µg L$^{-1}$. All analytical solutions used to set up the analytical curve were weighted in an analytical balance with 0.1 mg accuracy. Concentrations were adjusted according to the solution masses by the spectrometer manufacturer software

*2.6. Quality assurance*

Several approaches for determining the limit of detection (LOD) and limit of quantification (LOQ) are possible [41,52,53]. For this work we used the approach based on the measurement of the standard deviation of 10 blank solutions. This value was then multiplied by the BEC (background equivalent concentration) and divided by 100 (Equation 2 and 3) [53].

The certified Reference Materials (CRM) NIST (National Institute of Standards and Technology, Gaithersburg, USA) SRM 1515 (Apple Leaves) were used as external standards to monitor the quality of the analysis. In addition to that, spiked solutions were used. They were prepared using stock solutions with 4 mL matrix matching. Spiked solutions had final concentrations of 3 µg L$^{-1}$ for all elements evaluated in the curves and 2 µg L$^{-1}$ for the internal standard with a final volume of 10 mL. Blank solutions were used in the determination of LOD

and LOQ. They were prepared under the same conditions as the matrix and analysed by Q-ICP-MS.

LOD and LOQ were determined for each element in honey and pollen matrices. The calculation of their values was based on the BEC (background equivalent concentration) combined with the standard deviation of blank samples. The BEC is defined as the analyte concentration that produces a signal equal to that of the background and can be calculated according to equation (1), as proposed in the literature [53,54].

$$BEC = \frac{I_{\text{Blank}}}{m} \qquad (1)$$

Where:

$I_{\text{Blank}}$: Blank signal intensity;

m: Slope of the analytical curve;

The minimum concentration that can be determined with greater accuracy will depend on the ratio of statistical fluctuations between background signal and analytical signal, and can be calculated by equations (2) and (3):

$$LOD = \left( \frac{3\ RSD_{\text{Blank}}\ x\ BEC}{100} \right) \qquad (2)$$

$$LOQ = \left( \frac{10\ RSD_{\text{Blank}}\ x\ BEC}{100} \right) \qquad (3)$$

Where:

$RSD_{\text{Blank}}$: relative standard deviation to the blank (n = 10).

*2.7. Matrix effect measures*

The incidence of matrix effect was determined comparing the slope of the aqueous calibration curves with correspondent slopes of each of the matrix extract curves. Regression parameters such as slope (*a*), interception (*b*), determination coefficient (*r²*) and standardized residues were calculated with Minitab, statistical package software, version 14.0 (Minitab Inc., USA) (Table 3). To evaluate eventual significant differences between parameters of different curves, t-Student and F-Fischer tests were used [55].

**3. Results and discussion**

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*3.1. Matrix effect*

The matrix effect was evaluated by comparing the slope of the curves obtained by the calibration with and without matrix matching. The results are shown in Table 3. The equations of the analytical curves describe the relationship of ratio (counts per second - CPS) on the y axis and the concentration of elements ($\mu g\ g^{-1}$) on the x axis. The t-Student test show significant differences between the slopes of the curves with aqueous matrix and honey matrix for the following elements: As, Bi and Dy. The other ones showed no significant difference at a significance level of 0.05.

Matrix effect was also observed for pollen curves. The t-Student test show significant differences between the slopes of the curves with aqueous matrix and pollen matrix for the following elements: Bi, Ce, Gd, La, Pb and Sc were revealed significant differences for the angular coefficients of their curves at a significance level of 0.05. The other elements exhibited no significant differences, which suggest the occurrence of matrix effect interference for this kind of material. The results shown in Table 3 for honey followed the same trend observed in the analytical curves of pollen.

*3.2. Linearity of calibration curves*

Linearity was assessed with the determination coefficient ($r^2$) who was used to check for the adjustment of the data to a linear model – values close to 1 indicate good adjustment. For this work values ranging from 0.125 to 100 $\mu g\ L^{-1}$ were studied. The calibration curves for As, Bi, Cd, Ce, Dy, Eu, Er, Gd, Ho, In, La, Lu, Nd, Pb, Pr, Sc, Se, Sm, Tm, Y and Yb in honey and pollen of native Brazilian bees – Jataí (Table 3) were linear in this range.

*3.3. Limits of detection (LOD) and limits of quantification (LOQ)*

After choosing the appropriate curve, a new one was constructed and 10 blank sample solutions were measured. The standard deviations of the responses were calculated and these values were used to obtain the LOD and LOQ [56].

Limits of detection and limits of quantification of 21 chemical elements determined in the honey matrix and pollen matrix are displayed in Table 4. The quantification limits of the

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method ranged from 0.00041 (Tm) to 10.3 (Pb) µg L$^{-1}$ for honey and from 0.00041 (Tm) to 0.095 (Pb) µg L$^{-1}$ for pollen.

*3.4. Accuracy and precision*

Due to the lack of a certified reference material for the analysis of honey and pollen, accuracy was determined by the recovery rate of elements both in SRM 1515 samples and in spiked solutions.

The SRM 1515 reference material, despite being the most used one in the literature, is not similar to the organic material used in this study, but only As, Cd, Pb and Se have certified tabulated reference values [57]. The analysis of the methodology development of honey showed a recovery rate of SRM 1515 for Pb (63.7%) and Cd (100.5%), when compared with certified and tabulated values. The recovery rate of SRM 1515 for As and Se were not assessed because the contents of these elements are below their limit of quantification (LOQ) (Table 5).

Other elements of SRM 1515 presented recovery rate outside the range considered satisfactory when compared with non-certified tabulated reference value or compared with other research values determined and provided by Georem [58]. The recovery rates these elements were: Gd 78.4%, Ho 70.9%, Lu 73.9%, Nd 77.2%, Tm 77.0% and Yb 49.9% (Table 5).

The quality of procedures for extraction and quantification of elements in the studied methodology was considered satisfactory when evaluating the recovery elements in the spiked samples with honey matrix (91.6% to 103.9%) (Table 5).

The recovery rate was satisfactory when compared with non-certified tabulated reference values remained between 83.6% (Tm) and 109.7% (Eu) (Table 6). However the recovery rate of SRM 1515 for the analysis of pollen exhibited values outside the range considered satisfactory (Ho 76.5%, Sc 72.2%, Sm 78.7% and Yb 54.6%). The recovery rate of SRM 1515 for Pb was 63.9%, compared with certified tabulated reference values (Table 6).

The elements As and Se were not determined in SRM 1515 in the analysis of honey (Table 5), because their concentrations in this material were below the LOQ. Similar result was observed in the analysis of pollen when the elements As, Cd and Se were not determined in SRM 1515 (Table 6). The elements Bi, Dy, Er, In and Y were determined in SRM 1515 in the honey and

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pollen analysis. The recovery rates of these elements determined in SRM 1515 were not calculated, because there are no certified tabulated reference values for comparison.

A possible explanation is that the digestion method used was sufficient to digest pollen but not the reference material SRM 1515, a result that can be confirmed with the satisfactory recovery rate of the spiked samples, which ranges from 94.1% (Bi) to 115.6% (Ho) (Table 6).

*3.5. Application of the procedure to honey and pollen samples*

In an attempt to characterize native Brazilian honey and pollen from the point of view of their mineral content, 21 elements were quantified from five different areas. The results for three independent samples for each area are listed in Table 7.

## 4. Conclusions

These results demonstrated the interference of the honey organic matrix in the quantification of elements As, Bi and Dy, as well as the interference of the pollen organic matrix in the quantification of elements Bi, Cd, Ce, Gd, La, Pb and Sc by Q-ICP-MS. The methodology herein proposed led to low detection limits for the quantification of trace and ultra-trace elements for honey (0.00041 to 10.3 $\mu$g L$^{-1}$) and pollen (0.00041 to 0.095 $\mu$g L$^{-1}$). The recovery rates for NIST 1515 SRM were not satisfactory in agreement to certified values. However, the spiked solution method shows also satisfactory recovery rates for honey (91.63% to 104.60%) and pollen (92.77% to 115.59%). This study indicated that method is accurate, precise and suitable for elements (As, Bi, Cd, Ce, Dy, Eu, Er, Gd, Ho, In, La, Lu, Nd, Pb, Pr, Sc, Se, Sm, Tm, Y and Yb) for honey of native Brazilian native bees – Jataí; and the same elements for pollen of the same origin.

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**Table 1** Configurations and instrumental parameters for the microwave oven digestion.

| Step | Temperature ($^o$C) | Ramp time (min) | Hold time (min) |
|------|------------|-----------|-----------|
| 1 | 200 | 10 | |
| 2 | 200 | | 15 |
| 3 | Cooling, 30 min | | |

**Table 2** Parameters and operating conditions used for determination of chemical elements by ICP-MS Agilent equipment, model 7700x.

| Spectrometer | Agilent 7700 x |
|---|---|
| Spray chamber | Cyclonic |
| Nebuliser | Peek Mira Mist Nebuliser (0.1 rps) |
| Interface | Ni cones |
| Mass analyser | Quadrupole |
| RF Power (W) | 1550 |
| Ar-gas flow rates (L min$^{-1}$) | |
|   Plasma | 15 |
|   Auxiliary | 1.2 |
|   Nebuliser | 1.2 |
| Spray chamber temperature | 2 $^{o}$C |
| Dwell time (ms) | 100-300 |
| Number of replicates | 3 |
| Internal standard | Tb$^{159}$ |
| Maximum oxide and double charge production | $^{140}Ce^{16}O^{+}/^{140}Ce < 1.5\%$ and $^{140}Ce^{2+}/^{140}Ce^{+} < 3.0\%$ |
| Standard mode | |
| Delay | 5 s |
| Isotopes | Bi$^{209}$, Ce$^{140}$, Dy$^{162}$, Eu$^{152}$, Er$^{167}$, Gd$^{157}$, Ho$^{165}$, In$^{115}$, La$^{139}$, Lu$^{175}$, Nd$^{144}$, Pb$^{208}$, Pr$^{141}$, Se$^{79}$, Sm$^{150}$, Tm$^{169}$, Yb$^{173}$ |
| Sensibility | $^{7}$Li > 8000 cps, $^{89}$Y > 19000 cps, $^{205}$Tl > 18000 cps |
| Collision cell mode | |
| Delay | 30 s |
| Isotopes | As$^{75}$, Cd$^{111}$, Sc$^{45}$, Y$^{89}$ |
| Sensibility | $^{59}$Co > 5000cps, $^{89}$Y > 6000 cps, $^{205}$Tl >11000 cps and $^{78}$Ar$_2$ < 30 cps |
| He gas flow | 3.5 mL min$^{-1}$ |

**Table 3** Average slopes and correlation coefficients (r$^2$) for analytes determined in aqueous matrix solution calibration (n = 6), honey matrix solution matching calibration (n = 3) and pollen matrix solution matching calibration (n = 3) by ICP-MS. ($^{a/}$) slopes values of the second and fifth column and ($^{b/}$) slopes values of the second and eight column was different according to Student t-test at a level of 0.05 of significance.

| Elements | Aqueous matrix solution | | | Curve with honey matrix | | | Curve with pollen matrix | | |
|---|---|---|---|---|---|---|---|---|---|
| | Slope | RSD | Correlation coefficient (r$^2$) | Slope | RSD | Correlation coefficient (r$^2$) | Slope | RSD | Correlation coefficient (r$^2$) |
| As $^{a/}$ | 0.037 | 0.016 | 0.9999 | 0.018 | 0.00036 | 0.9999 | 0.0265 | 0.00027 | 0.9998 |
| Bi $^{a/\ b/}$ | 0.66 | 0.018 | 0.9997 | 0.62 | 0.0035 | 0.9997 | 0.573 | 0.012 | 0.9989 |
| Cd $^{b/}$ | 0.15 | 0.0025 | 0.9999 | 0.077 | 0.00052 | 0.9999 | 0.120 | 0.0034 | 0.9999 |
| Ce $^{b/}$ | 0.72 | 0.076 | 0.9999 | 0.74 | 0.018 | 0.9994 | 0.604 | 0.010 | 0.9998 |
| Dy $^{a/}$ | 0.25 | 0.004 | 0.9998 | 0.24 | 0.0039 | 0.9996 | 0.254 | 0.019 | 0.9998 |
| Er | 0.33 | 0.0061 | 0.9998 | 0.32 | 0.0063 | 0.9997 | 0.341 | 0.020 | 0.9993 |
| Eu | 0.45 | 0.011 | 0.9998 | 0.44 | 0.0090 | 0.9997 | 0.455 | 0.028 | 0.9999 |
| Gd $^{b/}$ | 0.16 | 0.0029 | 0.9999 | 0.15 | 0.0030 | 0.9995 | 0.168 | 0.0039 | 0.9998 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Ho | 0.99 | 0.016 | 1.0000 | 0.96 | 0.019 | 0.9995 | 1.009 | 0.0049 | 0.9999 |
| In | 0.69 | 0.028 | 0.9999 | 0.70 | 0.0048 | 0.9999 | 0.670 | 0.015 | 0.9999 |
| La [b/] | 0.72 | 0.088 | 0.9999 | 0.74 | 0.017 | 0.9996 | 0.576 | 0.0057 | 0.9996 |
| Lu | 1.03 | 0.027 | 1.0000 | 0.99 | 0.017 | 0.9999 | 1.043 | 0.0087 | 0.9999 |
| Nd | 0.11 | 0.0054 | 0.9999 | 0.11 | 0.0024 | 0.9995 | 0.106 | 0.012 | 0.9994 |
| Pb [b/] | 0.19 | 0.0052 | 0.9999 | 0.18 | 0.00052 | 0.9999 | 0.175 | 0.037 | 0.9999 |
| Pr | 0.88 | 0.058 | 0.9999 | 0.89 | 0.017 | 0.9997 | 0.829 | 0.0089 | 0.9998 |
| Sc [b/] | 0.074 | 0.0040 | 0.9997 | 0.074 | 0.0011 | 0.9999 | 0.362 | 0.038 | 0.9999 |
| Se | 0.004 | 0.0039 | 0.9999 | 0.0048 | 0.0041 | 0.9999 | 0.0042 | 0.0034 | 0.9999 |
| Sm | 0.14 | 0.0047 | 0.9999 | 0.14 | 0.0027 | 0.9996 | 0.140 | 0.0048 | 0.9997 |
| Tm | 1.03 | 0.022 | 0.9998 | 0.99 | 0.022 | 0.9996 | 1.051 | 0.035 | 0.9999 |
| Y | 0.30 | 0.045 | 0.9998 | 0.44 | 0.0069 | 0.9996 | 0.287 | 0.00030 | 0.9998 |
| Yb | 0.24 | 0.0078 | 0.9999 | 0.23 | 0.0043 | 0.9996 | 0.243 | 0.00063 | 0.9998 |

**Table 4** Results of analytical parameters of the studied chemical elements for honey matrix and pollen matrix. LOD – limit of detection, LOQ - limit of quantification, BEC - background equivalent concentration).

| Elements | Curve with honey matrix | | | Curve with pollen matrix | | |
|---|---|---|---|---|---|---|
| | LOD ($\mu g\ L^{-1}$) | LOQ ($\mu g\ L^{-1}$) | BEC | LOD ($\mu g\ L^{-1}$) | LOQ ($\mu g\ L^{-1}$) | BEC |
| As | 0.54 | 1.8 | 0.35 | 0.0036 | 0.012 | 0.13 |
| Bi | 0.018 | 0.059 | 0.011 | 0.0025 | 0.0083 | 0.016 |
| Cd | 0.033 | 0.11 | 0.021 | 0.0059 | 0.020 | 0.084 |
| Ce | 0.040 | 0.13 | 0.085 | 0.022 | 0.072 | 0.17 |
| Dy | 0.0014 | 0.0046 | 0.0036 | 0.0012 | 0.0040 | 0.0076 |
| Er | 0.00070 | 0.0023 | 0.0019 | 0.00087 | 0.0029 | 0.0043 |
| Eu | 0.0014 | 0.0047 | 0.0012 | 0.00018 | 0.00061 | 0.0037 |
| Gd | 0.0019 | 0.0064 | 0.0048 | 0.0011 | 0.0038 | 0.010 |
| Ho | 0.00028 | 0.00094 | 0.00054 | 0.00026 | 0.00087 | 0.0016 |
| In | 0.0012 | 0.0039 | 0.00076 | 0.00018 | 0.00061 | 0.0018 |
| La | 0.017 | 0.056 | 0.042 | 0.0028 | 0.0095 | 0.078 |
| Lu | 0.0017 | 0.0056 | 0.083 | 0.00085 | 0.0028 | 0.092 |
| Nd | 0.0087 | 0.029 | 0.028 | 0.0023 | 0.0078 | 0.059 |
| Pb | 3.1 | 10 | 2.2 | 0.029 | 0.095 | 2.2 |
| Pr | 0.0026 | 0.0087 | 0.0077 | 0.00054 | 0.0018 | 0.015 |
| Sc | 0.012 | 0.039 | 0.031 | 0.0057 | 0.019 | 0.04 |
| Se | 0.81 | 2.7 | 0.54 | 0.022 | 0.77 | 0.55 |
| Sm | 0.0022 | 0.0072 | 0.0042 | 0.00066 | 0.0022 | 0.0093 |
| Tm | 0.00012 | 0.00041 | 0.00027 | 0.00012 | 0.00041 | 0.00051 |
| Y | 0.016 | 0.054 | 0.025 | 0.0079 | 0.026 | 0.050 |

| Yb | 0.00077 | 0.0026 | 0.0016 | 0.00056 | 0.0019 | 0.0034 |

**Table 5** Recovery rate of elements for the SRM-1515 Apple Leaves certified material (n = 2) and the spiked solution with honey matrix.

| Elements | SRM 1515 Reference | SRM 1515 Measured | Recovery rate | Added | Measured mass fraction | Recovery rate (%) |
|---|---|---|---|---|---|---|
| | conc (ug g$^{-1}$) | conc. (ug g$^{-1}$) | (%) | (ug g$^{-1}$) | Spiked samples (ug g$^{-1}$) | |
| As | 0.04+/-0.007 | <LOQ | - | 3.110 | 3.232 | 103.9 |
| Bi | | 0.0020 | | 3.110 | 2.891 | 93.0 |
| Cd | 0.013+/-0.002 | 0.011 | 100.5 | 3.110 | 3.014 | 96.9 |
| Ce | 3.0 [a] | 2.5 | 83.1 | 3.054 | 2.878 | 94.2 |
| Dy | | 1.37 | | 3.040 | 2.860 | 94.1 |
| Er | | 0.46 | | 3.084 | 2.826 | 91.6 |
| Eu | 0.2 [a] | 0.20 | 102.2 | 3.065 | 2.923 | 95.4 |
| Gd | 3.0 [a] | 2.4 | 78.4 | 3.060 | 2.859 | 93.4 |
| Ho | 0.32 [b c] | 0.23 | 70.9 | 3.060 | 2.873 | 93.9 |
| In | | 0.0020 | | 3.110 | 3.025 | 97.3 |
| La | 20 [a] | 17 | 82.7 | 3.063 | 2.907 | 94.9 |
| Lu | 0.02 [b c] | 0.015 | 73.9 | 3.050 | 2.863 | 93.9 |
| Nd | 17 [a] | 13 | 77.2 | 3.063 | 2.835 | 92.6 |
| Pb | 0.47+/-0.024 | 0.28 | 63.7 | 3.110 | 2.969 | 95.5 |
| Pr | 4.17 [b c] | 3.4 | 81.2 | 3.062 | 2.884 | 94.2 |
| Sc | 0.03 [a] | 0.029 | 97.9 | 3.056 | 2.994 | 98.0 |
| Se | 0.05+/-0.009 | <LOQ | - | 3.110 | 3.253 | 104.6 |
| Sm | 3.00 [a] | 2.2 | 72.6 | 3.053 | 2.848 | 93.3 |
| Tm | 0.051 [b c] | 0.039 | 77.0 | 3.063 | 2.842 | 92.8 |
| Y | | 8.1 | | 3.054 | 3.027 | 99.1 |
| Yb | 0.3 [a] | 0.15 | 49.9 | 3.057 | 2.852 | 93.3 |

a / Non-certified tabulated reference values, other certified values SRM [58]

b / Values provided by GEOREM [57]

c / Kucera et al. [59]

d / Mizera et al. [60]

**Table 6** Recovery rate of elements for the SRM 1515-apple leaves certified material (n = 2), and the spiked solution with pollen matrix.

| Elements | SRM 1515 Reference | SRM 1515 Measured | Recovery rate (%) | Spiked Solution | Measured mass fraction | Recovery rate (%) |
|---|---|---|---|---|---|---|
| | conc. (μg g$^{-1}$) | conc. (μg g$^{-1}$) | | (μg g$^{-1}$) | Spiked samples (μg g$^{-1}$) | |
| As | 0.04+/-0.007 | - | - | 3.042 | 3.163 | 104.0 |
| Bi | - | 0.0010 | - | 3.042 | 2.863 | 94.1 |
| Ce | 3.0 [a] | 2.7 | 91.7 | 3.054 | 2.909 | 95.3 |
| Dy | - | 1.5 | - | 3.040 | 2.947 | 96.9 |
| Er | - | 0.51 | - | 3.084 | 2.945 | 95.5 |
| Eu | 0.2 [a] | 0.22 | 109.7 | 3.065 | 2.909 | 94.9 |
| Gd | 3.0 [a] | 2.5 | 85.0 | 3.060 | 2.994 | 97.8 |
| Ho | 0.32 [b c] | 0.24 | 76.5 | 3.060 | 3.537 | 115.6 |
| In | - | 0.0010 | - | 3.042 | 2.916 | 95.9 |
| La | 20 [a] | 18 | 92.1 | 3.063 | 2.973 | 97.0 |
| Lu | 0.02 [b c] | 0.017 | 83.9 | 3.035 | 2.96 | 97.5 |
| Nd | 17 [a] | 14.6 | 86.0 | 3.063 | 2.936 | 95.8 |
| Pb | 0.47+/-0.024 | 0.29 | 63.9 | 3.042 | 2.951 | 97.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Pr | 4.17 [b c] | 3.7 | 89.6 | 3.062 | 3.3 | 107.8 |
| Sc | 0.03 [a] | 0.022 | 72.2 | 3.041 | 2.973 | 97.8 |
| Se | 0.05+/-0.009 | - | - | 3.042 | 3.148 | 103.5 |
| Sm | 3.00 [a] | 2.36 | 78.7 | 3.053 | 2.897 | 94.9 |
| Tm | 0.05 [b c] | 0.043 | 83.6 | 3.063 | 2.942 | 96.04 |
| Y | - | 8.8 | - | 3.039 | 2.982 | 98.1 |
| Yb | 0.3 [a] | 0.16 | 54.6 | 3.042 | 2.92 | 96.0 |

a / Non-certified tabulated reference values, other certified values SRM [58]

b / Values provided by GEOREM [57]

c / Kucera et al. [59]

d / Mizera et al. [60]

**Table 7** Mean concentration ($\mu g\ kg^{-1}$) of elements in honey and pollen samples from Iron Quadrangle-MG, Brazil (n = 3), distributed by region, (mean $\pm$ SD[1/]).
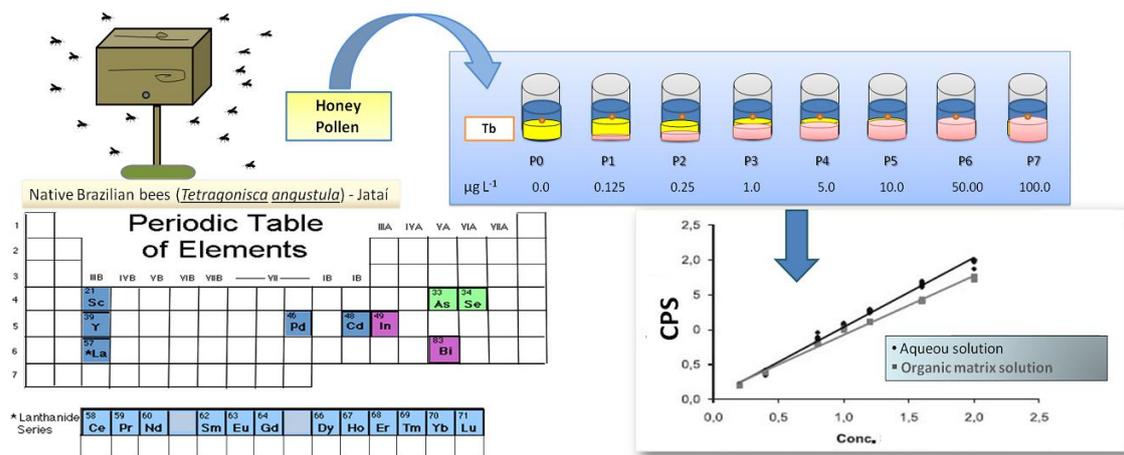
| Elements | Analysis of honey samples ($\mu g\ kg^{-1}$) | | | | | Analysis of pollen samples ($\mu g\ kg^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| As | 13.83± 1.90 | 4.23± 0.06 | 1.70± 0.01 | 1.70± 0.01 | 1.70± 0.01 | 361.30± 18.88 | 330.16± 4.49 | 93.21± 25.16 | 51.10± 22.19 | 7.00± 0.45 |
| Bi | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Cd | 0.11± 0.01 | 0.13± 0.06 | 0.11± 0.01 | 0.13± 0.06 | 0.30± 0.35 | 1.60± 0.01 | 1.64± 0.01 | 1.64± 0.01 | 1.60± 0.01 | 1.60± 0.01 |
| Ce | 0.23± 0.01 | 0.20± 0.01 | 7.32± 0.24 | 9.77± 0.81 | 0.20± 0.01 | 71.19± 6.40 | 129.72± 38.05 | 357.34± 20.69 | 116.60± 7.10 | 1.90± 0.01 |
| Dy | 0.31± 0.03 | 0.13± 0.06 | 0.34± 0.10 | 0.90± 0.17 | 0.17± 0.12 | 2.54± 0.17 | 5.69± 0.59 | 12.58± 3.16 | 9.60± 0.52 | 0.58± 0.10 |
| Er | 0.21±0. 03 | 0.60± 0.01 | 0.58± 0.01 | 0.67± 0.06 | 0.59± 0.02 | 1.41± 0.08 | 2.74± 0.21 | 6.68± 1.44 | 6.10± 0.52 | 0.43± 0.06 |
| Eu | 0.14± 0.02 | 0.10± 0.01 | 0.06± 0.01 | 0.28± 0.07 | 0.10± 0.01 | 2.07± 0.23 | 2.01± 0.47 | 3.89± 1.07 | 2.50± 0.17 | 0.27± 0.12 |
| Gd | 0.65± 0.02 | 0.23± 0.06 | 0.44± 0.06 | 1.20± 0.11 | 0.40± 0.20 | 5.70± 0.17 | 8.45± 0.37 | 20.33± 0.46 | 10.47± 0.64 | 0.20± 0.01 |
| Ho | 0.08± 0.01 | 0.10± 0.01 | 0.08± 0.01 | 0.21± 0.02 | 0.10± 0.01 | 0.60± 0.10 | 1.06± 0.14 | 2.39± 0.59 | 2.07± 0.12 | 0.10± 0.10 |
| In | <LOD | <LOD | <LOD | <LOD | <LOD | 0.10± 0.01 | 0.08± 0.01 | 0.39± 0.09 | 0.53± 0.29 | 0.10± 0.01 |
| La | 0.85± 0.27 | 0.27± 0.12 | 2.33± 0.12 | 3.55± 0.05 | 0.20± 0.01 | 47.20± 9.49 | 61.49± 4.78 | 163.31± 1.15 | 58.53± 4.39 | 7.85± 0.21 |
| Lu | <LOD | <LOD | <LOD | <LOD | <LOD | 0.20± 0.01 | 0.21± 0.01 | 0.61± 0.01 | 0.20± 0.01 | 0.20± 0.01 |
| Nd | 1.70± 0.14 | 0.68± 0.13 | 2.30± 0.23 | 2.96± 0.75 | 0.60± 0.01 | 32.65± 2.04 | 54.01± 13.58 | 125.43± 12.39 | 49.83± 3.52 | 3.90± 0.10 |
| Pb | <LOD | <LOD | <LOD | <LOD | <LOD | 36.33± 2.31 | 99.79± 26.23 | 463.31± 35.16 | 137.13± 79.27 | 1.20± 0.01 |
| Pr | 0.46± 0.08 | 0.20± 0.10 | 0.64± 0.08 | 1.08± 0.02 | 0.10± 0.01 | 9.33± 0.58 | 14.99± 3.37 | 34.79± 2.98 | 12.80± 1.04 | 1.13± 0.42 |
| Sc | <LOD | <LOD | <LOD | <LOD | <LOD | 8.53± 0.45 | 21.67± 0.58 | 46.14± 2.42 | 14.67± 1.85 | 12.43± 0.50 |
| Se | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Sm | 0.46± 0.05 | 0.20± 0.01 | 0.36± 0.14 | 0.99± 0.08 | 0.20± 0.01 | 6.80± 0.44 | 9.25± 0.68 | 21.96± 1.00 | 9.80± 0.52 | 0.50± 0.01 |
| Tm | <LOD | <LOD | <LOD | <LOD | <LOD | 0.20± 0.01 | 0.36± 0.03 | 1.06± 0.02 | 0.87± 0.12 | 0.10± 0.01 |
| Y | 2.02± 0.01 | 0.16± 0.10 | 0.55± 0.06 | 7.25± 0.04 | 0.11± 0.02 | 14.03± 1.00 | 28.13± 4.38 | 79.03± 4.27 | 65.90± 5.20 | 2.70± 0.14 |

| Yb | 0.04± 0.01 | <LOD | 0.04± 0.01 | 0.10± 0.01 | <LOD | 1.03± 0.40 | 2.24± 0.06 | 6.33± 0.39 | 5.57± 0.12 | 0.30± 0.01 |

[1/] SD -Standard deviation. LOD - Limit of detection.

Highlights

- Matrix effects were verified by Q-ICP-MS leading to a methodology for the correction.
- First results for REE contents for honey and pollen of Brazilian bees are presented.
- Toxic elements (As, Bi, Cd, Pb, Se and In) determination in this kind of matrix.