X-RAY SPECTROMETRY
X-Ray Spectrom. 2007; **36**: 215–220

(www.interscience.wiley.com) DOI: 10.1002/xrs.944

Honey characterization by total reflection x-ray fluorescence: evaluation of environmental quality and risk for the human health†

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Received 28 July 2006; Revised 30 November 2006; Accepted 1 December 2006

Honey is produced by *Apis Mellifera* bees, primarily from floral nectars. The bees are estimated to forage on plants growing in a relatively large area of more than 7 km^2. If it is assumed that any hive includes at least 1000 worker bees and that each of them forages on 1000 flowers per day, the honey produced daily can be considered the outcome of at least one million interactions. In this way, the foraged area is effectively sampled for trace elements. The mineral content of honey is recognized as an environmental indicator. In this work, it is used as an indicator of toxic metal contamination in an extensive area in the central-west of Argentina. Metal identification and quantification was done by total reflection x-ray fluorescence (TXRF). The level of contamination was evaluated taking into account the concentration values suggested by the Codex Alimentarium Commission. Chemometric tools were employed to relate the honey composition to the environmental contamination of the studied areas. Copyright © 2007 John Wiley & Sons, Ltd.

INTRODUCTION

The purpose of the present paper is to provide a starting point to assess the usefulness of total reflection x-ray fluorescence (TXRF) characterization of honey as an indicator of heavy-metal contamination.

Honey has been always considered a delicious food, but it is also the result of a bio-accumulative process that is useful to collect information about the environment within the bees' forage area. Bees are estimated to forage on plants growing in a relatively large area[1,2] of more than 7 km^2. If it is assumed that any hive includes at least 1000 worker bees and that each of them forage on 1000 flowers per day, the honey produced daily can be considered the outcome of at least one million interactions. In this way, the forage area is effectively sampled for trace elements and the concentration in honey of heavy and transition metals reflects their levels in the foraged area.[3–7]

The mineral content of honey, usually calculated to be about 0.17%, has been recognized as an environmental indicator at least since 1984.[8] In addition, the determination of heavy metals in honey is of interest for quality control when considering it as food. High levels of metals are undesirable because of their known or assumed toxicity.

Considered as an analytical sample, honey is one of the most complex mixtures of carbohydrates produced in nature. Glucose and fructose are the major components (65–75% of total soluble solids), and other oligosaccharides are present with small quantities of proteins, amino acids and vitamins. In addition, honey contains 15–20% water.[9] Metal determination in sugar-rich foodstuffs has been a challenging analytical task because of the interference caused by the organic matrix. As dilution may reduce concentrations below the limit of detection, pre-treatment of the sample is usually required to destroy the matrix. Currently, the most accepted approach to mineralization seems to be microwave oven digestion, which allows reduced reagent amounts, temperature and the time required.

As to the analytical technique, several have been proposed to determine metallic impurities in honey. The detection limit has proved to be the most important limiting factor of most of them.[10,11] Among the possible techniques, TXRF is very attractive for trace analysis owing to the easy operation of the equipment and satisfactory sensitivity coupled with the advantage of multielemental analysis.

In this work, honey is oxidized by microwave oven digestion and the contents of heavy metals are simultaneously determined by TXRF.

EXPERIMENTAL

Sample preparation

Honey samples coming from 14 different locations in Argentina were stored in dark in tightly closed plastic bottles so as to prevent metal contamination. In order to obtain homogeneous samples, the bottles were slightly heated in a hot water bath until the honey was liquefied.

Three subsamples were taken from each sampling site.

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†Paper presented as part of a special issue of papers from the 2006 European X-ray Spectrometry Conference, Paris, France, 19–23 June, Part 4.

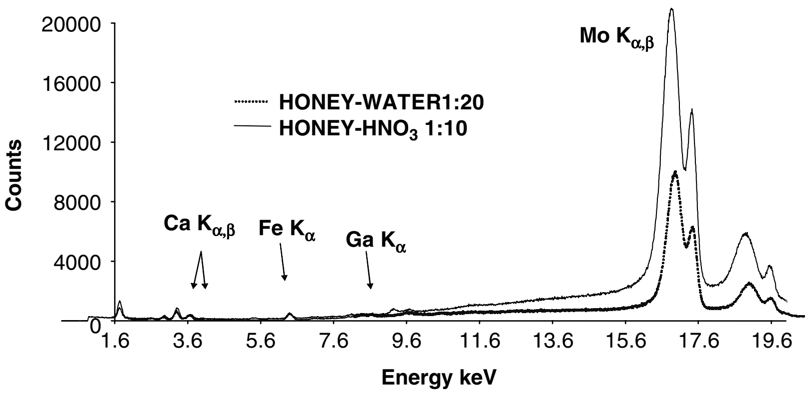

Figure 1. TXRF spectra from samples of honey in different media.

Different procedures were experimented with in order to minimize sample manipulation, which is an unavoidable condition when dealing with trace elements.

Different solvents were tested in various proportions and conditions in order to achieve the thin-film condition and obtain reproducible results. Since honey diluted in water does not reach thin-film conditions, the analysis shows high background spectra and it does not allow the detection of trace elements (Fig. 1). The other solvents tried (ethanol, hexane, acetone, butanol, methylethylcetone, ethylacetate) did not dissolve honey. This problem was solved by eliminating the organic material through previous digestion of the sample. Closed microwave digestion is a fast way to achieve this. Different digestion programmes were tested, and finally the procedure described in Table 1 was adopted.

Table 1. Microwave digestion programme for mineralization

Digestion time (min)	Power (W)
1	250
1	0
	250
5	400
5	650
1	0

Five portions of 1.0 g from each honey sample were weighed into a Teflon vessel with 3 ml of distilled nitric acid (HNO_3) and 1 ml of hydrogen peroxide (H_2O_2).

The solutions were mineralized using the microwave digestion programme shown in Table 1.

The digestion vessel was then cooled to room temperature and the resulting solution was diluted to 5 ml with Milli-Q water. An internal standard was added (Ga = 2 mg l^{-1}). An aliquot of the solution was deposited on a reflecting surface and dried using an infrared lamp to a thin solid film. The quartz surface with the sample was finally placed in the x-ray spectrometer for analysis.

The results obtained after digestion show how this simple procedure improves the TXRF analysis (Fig. 2).

Instruments

Sample digestion was performed in a Microwave Lab Station MILESTONE 1200 (Milestone, Sorisole, Italy).[12]

Multielemental analysis was carried out using an x-ray fluorescence system in the total reflection geometry.[13]

The spectrometer consisted of a Seifert x-ray generator and a fine-focus x-ray tube with a Mo anode. The detection and data acquisition system consisted of a[22] 80 mm^2 Si(Li) detector with 166 eV FWHM for 5.9 keV, a 0.008-mm thick Be window, an Ortec 672 fast spectroscopy amplifier and an analogue to digital converter (ADC) Nucleus PCA2.

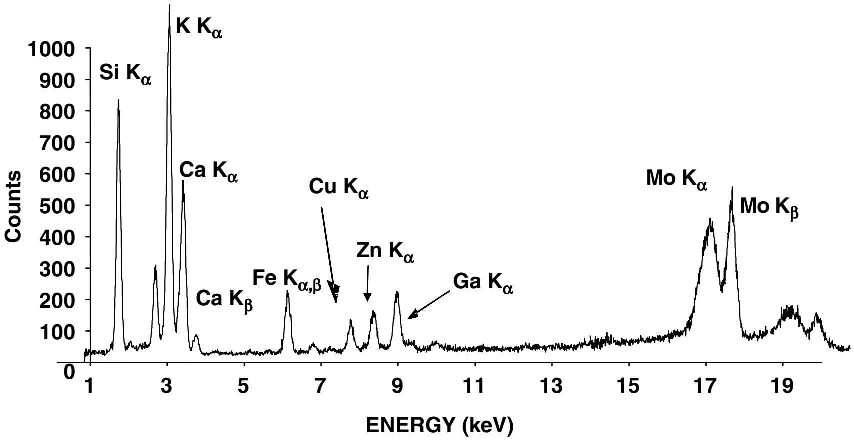

Figure 2. TXRF spectra of a sample of honey after microwave digestion.

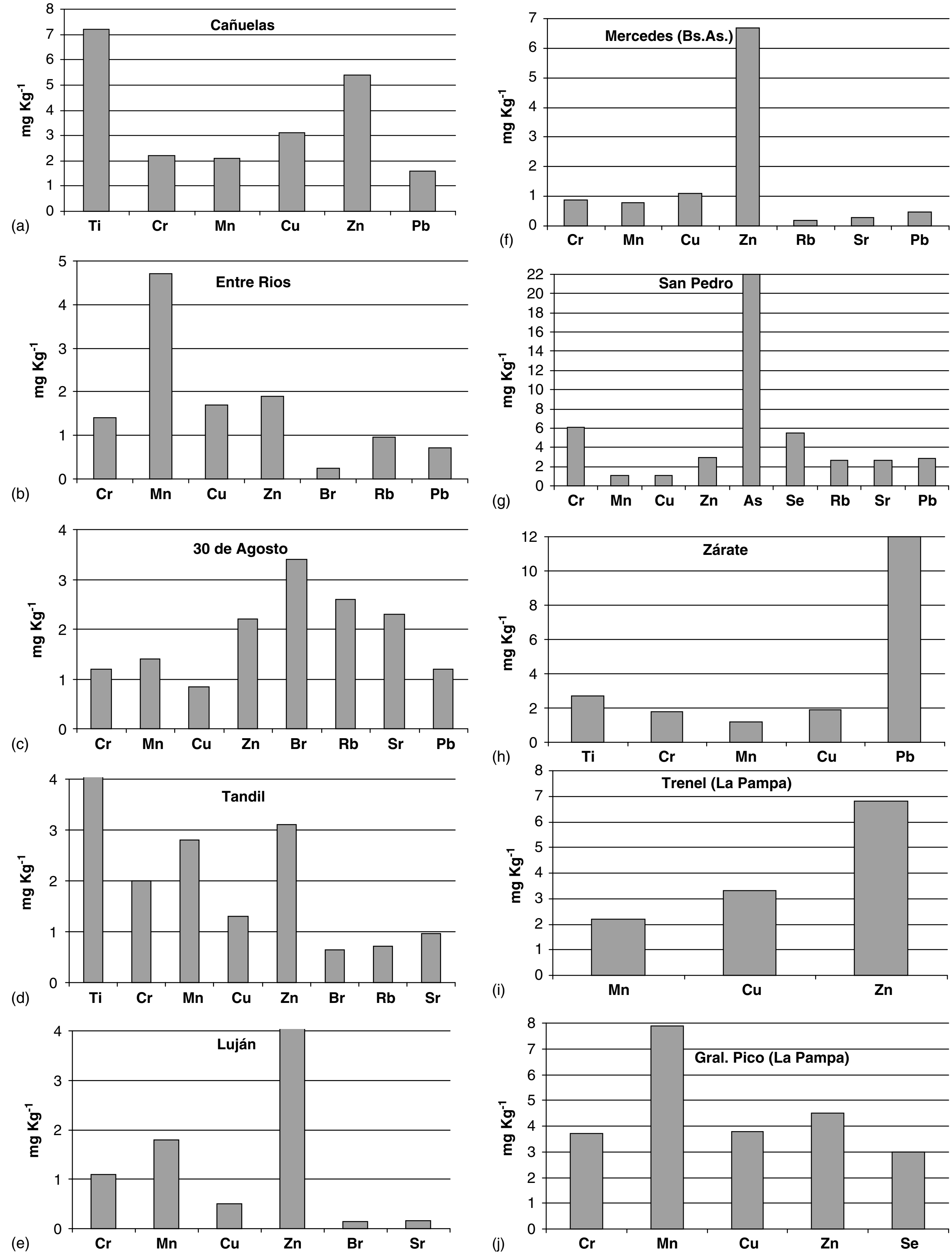

Figure 3. Experimentally obtained mean values for metal contents (milligram of metal per kilogram of honey) in all sampling sites.

DOI: 10.1002/xrs

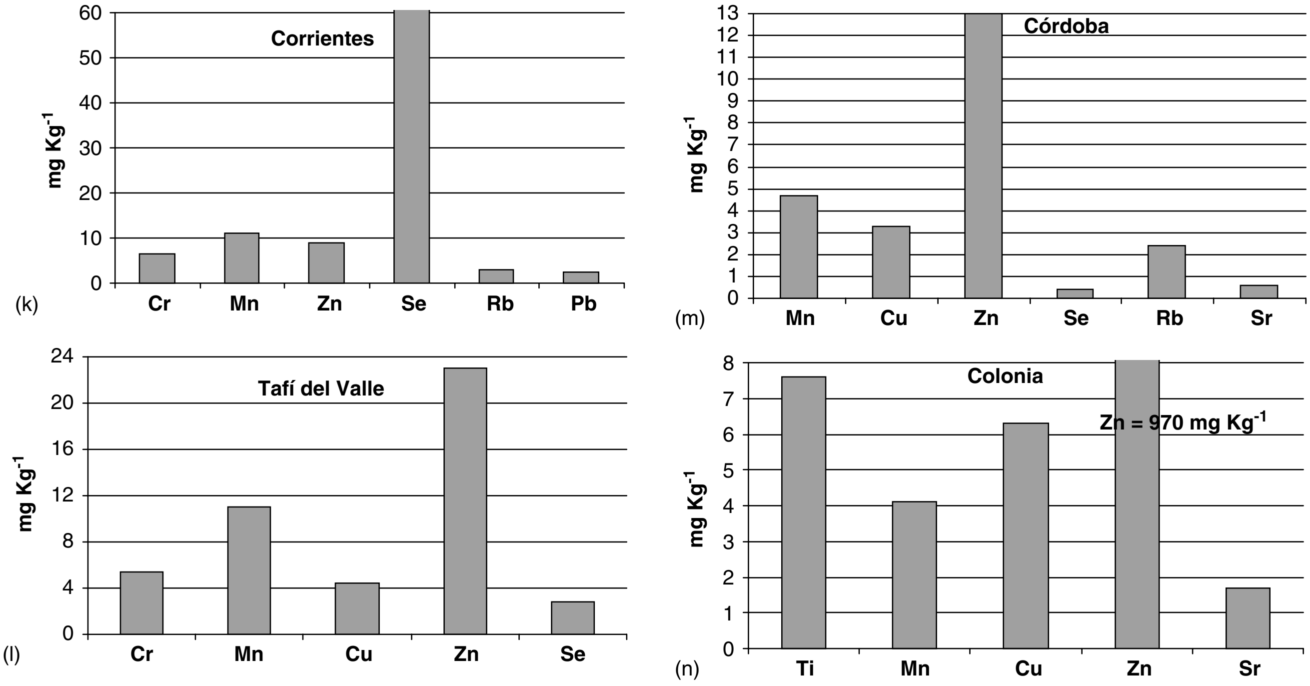

Figure 3. (*Continued*).

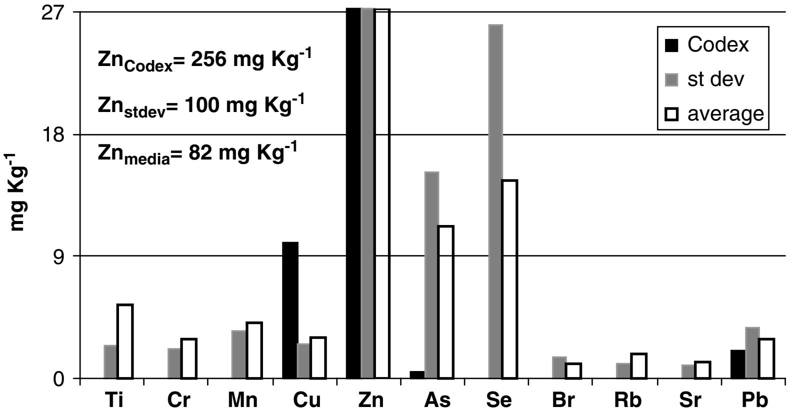

Figure 4. Standard deviation and median of all values for metal contents compared with the admissible value for the four controlled metals in honey.

A total reflection module, designed at the Atominstitut der Östereichischen Universitäten, fitted with a cut-off-filtered radiation from a fine-focus diffraction molybdenum anode x-ray tube was employed.[14]

The excitation condition was 50 kV and 30 mA in all cases. The acquisition time for each spectrum was 500 s.

The simple quantitative analysis and regression of count rates *versus* concentration in the QXAS software package from the International Atomic Energy Agency was employed for quantification of the data.[15] The analysis was fast: the time required for the digestion of 8 samples was about 15 min.

For each subsample five measurements were made.

RESULTS AND DISCUSSION

Analytical results

In order to evaluate the contamination, the major elements detected in honey samples, such as K, Ca and Fe, were

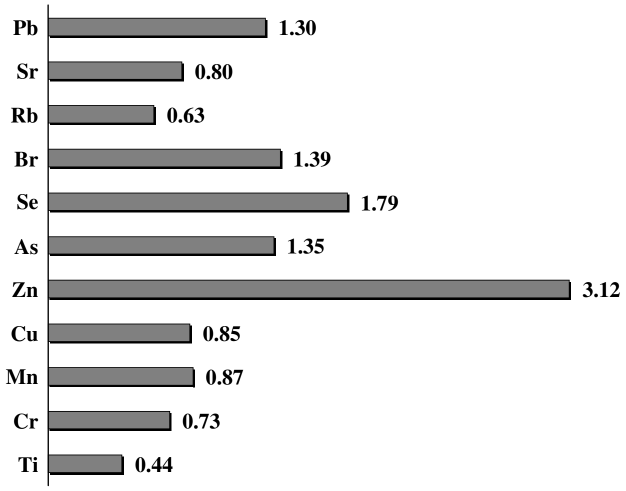

Figure 5. Coefficients of variation for all detected metals in honey.

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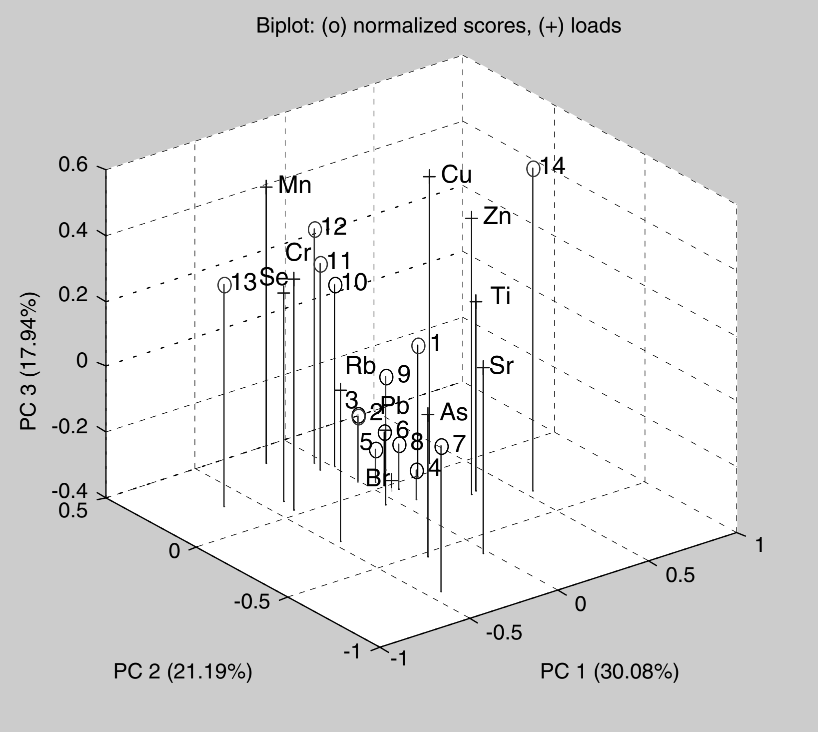

Figure 6. Biplot of normalized scores *versus* loadings for the three first principal components from the analysis of metals in honey samples: 1 — Cañuelas, 2 — Entre Ríos, 3–30 de Agosto, 4 — Tandil, 5 — Luján, 6 — Mercedes, 7 — San Pedro, 8 — Zárate, 9 — Trenel, 10 — Gral. Pico, 11 — Corrientes, 12 — Tafí del Valle, 13 — Códoba, 14 — Colonia.

avoided. Concentrations of these elements were similar to those reported by Golob *et al.*[16] and Adebiyi *et al.*[17]

The experimentally obtained mean values for metal contents (milligram of metal per kilogram of honey) in all the sampled sites are shown in Fig. 3. The standard deviation and the average of all these values compared with the allowed values for the four controlled metals in honey according to the International Codex[18] are shown in Fig. 4.

A high value of Zn, higher than that accepted, was observed in one of the samples (Colonia, Santiago del Estero). According to Braziewicz *et al.*[19] this high value could be attributed to the contact with the metallic honey extractor.

It is interesting to notice that Pb and As concentrations in some of these samples exceeded standards or guidelines for the protection of human health.[18] The high value of As detected in the San Pedro site agrees with previous reports of natural As contamination in this Pampa zone of Argentina.[20–22]

Coefficients of variation for all the detected metals are shown in Fig. 5. The elements with the highest observed variation were Zn, Br and As. It suggests a particular contamination with these elements in the studied samples.

In order to simplify the number of variables, the principal component analysis (PCA) was used. PCA is a technique for simplifying a dataset by applying a linear transformation that transforms the data to a new coordinate system such that the greatest variance by any projection of the data comes to lie on the first coordinate (called the first principal component), the second greatest variance on the second coordinate, etc.[23]

PCA as a biplot of normalized scores is shown in Fig. 6. Some of the sampling sites were clustered around Pb (Gral. Pico, Corrientes, Tafí del Valle and Córdoba), only one of them near the Zn (Colonia) and the others around Cr. This fact could be interpreted as a cluster around a common contaminant. This aggregation does not agree with the geographical location, but in the areas surrounding these cities are located iron and steel industries or tanneries that could be the sources of these contaminations.

CONCLUSIONS

We have been able to develop a method to carry out a multielemental analysis of trace metals in honey. It involves minimum sample manipulation, which is a great advantage when dealing with trace elements. Microwave oven digestion coupled with TXRF proved to be a simple and effective method, which has minimal reagent requirements, and it may be used to detect possible pollutants.

Few investigated samples were free of heavy metals. Some detected contaminants exceeded permissible limits. Determining the origin of the contaminants found in these samples would involve further analysis of soil, ground water and air. Consequently, the concentration of heavy metals in honey may be useful for assessing the presence of environmental contaminants.

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DOI: 10.1002/xrs