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ORIGINAL PAPER

AN MIP-AES STUDY OF HEAVY METALS IN EGYPTIAN HONEY: TOXICITY ASSESSMENT AND POTENTIAL HEALTH HAZARDS TO CONSUMERS

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Abstract

Heavy metals and trace elements in honey are beneficial for nutrition, but at certain levels they can aggravate health problems. The current study aimed to assess the content of selected heavy metals in honey samples collected from Egypt using the recently introduced Microwave Plasma – Atomic Emission Spectroscopy (MIP-AES), and to estimate their associated risk according to the Egyptian Standards. The analytical method was validated embracing well-established guidelines, exhibiting sufficient LOQs, in the range of 0.96-14.85 μg kg^{-1}. Recoveries at two concentration levels varied from 90-99%, with RSD% values not surpassing 13%. The matrix effect was alleviated by using an appropriate dilution (a dilution factor of approximately 50 was implemented) after the digestion step, reaching a good compromise for minimizing ME for all elements. Samples were analyzed for cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn). Analyses assisted by a robust statistical validity test revealed that mean concentrations of Cd, Cu, Fe, Pb, and Zn were 5, 128, 462, 123, and 244 μg kg^{-1}, respectively. Out of the metals analyzed, Fe was the most abundant, followed by Zn, Cu, and Pb, while Cd was present in low concentrations. The amounts of studied heavy metals in honey were less than the recommended threshold levels according to the standard set by the Egyptian Organization of Standardization for honey. The average daily intakes (ADI) of the detected heavy metals were much below

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the oral daily intake reference dose (RfD) suggested by the European regulations. The low calculated hazard index (HI) of the five heavy metals indicated that the intake of a single metal by consuming specific honeys did not pose a significant chronic-toxic risk for consumers.

Keywords: chemical analysis, heavy metals, honey, MIP-AES, risk assessment.

INTRODUCTION

Honey is a beneficial and healthy sugary food produced naturally by honeybees (EC 2002). It has a rich and diverse chemical composition including sugars such as fructose and glucose, proteins, amino acids (Pereira et al. 1998, Iglesias et al. 2004, Mahmoudi et al. 2014), organic acids (Codex… 2001), antioxidant components (Moniruzzaman et al. 2014), and minerals (~ 0.17%) both macro- (calcium (Ca), magnesium (Mg), and potassium (K)) and micro-elements (chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), sodium (Na), and zinc (Zn). The latter renders honey worth investigating both from the nutritional aspect but also in relation to consumers' safety.

Although honey and the beekeeping sector in general have a long tradition in Egypt, the levels of heavy metals in Egyptian honey are scarcely reported (Rashed, Soltan 2004, Rashed et al. 2009). Hence, this gap in knowledge needs to be continuously investigated and supplied with data from robust analytical monitoring studies. In the same context, the fingerprinting of heavy metals and essential elements in honey relies mainly on its botanical origin (Oroian et al. 2016, Bogdanov et al. 2007) as well as the climatic conditions and geographical location (Bogdanov et al. 2007, Dzugan et al. 2017). A principal route of the import of heavy metals into honey is during its production because of their presence in the pollen and nectar that bees bring to the hive after foraging. In addition, anthropogenic sources affect heavy metal concentrations in the environment, exemplified by contaminated water and agrochemicals such as fertilizers. It has been reported that heavy metals could also be relocated from the soil to nectar and pollen through the root system of a plant and distributed throughout the entire plant including the nectar and pollen (Xun et al. 2017). The increase in heavy metal concentrations in the plants follow a similar pattern as the content of heavy metals in soil (Singh et al. 2010) and the translocation is extensively studied (cf. Pachura, et al. 2016). Thus, the concentration of heavy metals in honey might disclose their amounts in the environment around the hive (Kujawski, Namiesnik 2008, Mahmoudi et al. 2015, Mahmoudi, Pakbin 2015, Mahmoudi et al. 2017), wich has stimulated several research teams to use honey as a biological marker of the environmental pollution (Przybylowski, Wilczynska 2001, Celli, Maccagnani 2003, Atrouse et al. 2004, Rashed, Soltan 2004, Munoz, Palmero 2006, Naccari et al. 2014, Sitarz-Palczak et al. 2015).

In the presented work, five heavy metals were selected for analysis: copper (Cu), zinc (Zn), and iron (Fe) classified as essential metals, and lead (Pb) and cadmium (Cd), which are considered invariably harmful (Akoto et al. 2014). Cadmium is a heavy metal with a plethora of industrial applications but also of high environmental concern. Pb, which naturally occurs in the environment, has several anthropogenic sources as well, such as fossil fuels etc. Both Cd and Pb exhibit a high degree of toxicity and are classified as priority metals with human health importance (Tchounwou et al. 2012). Notwithstanding, Codex reports that "honey shall be free from heavy metals in amounts which may represent a hazard to human health" (Codex-Alimentarius 1993). Therefore, the analysis of trace elements content in honey is indispensable for providing safe and quality honey to consumers, considering that all heavy metals are toxic to humans if they are present in surplus quantities.

Principal analytical methods for the detection and quantitation of heavy metals in several commodities are atomic absorption spectrometry (AAS) and atomic emission spectrometry (AES). Both techniques are highly applicable when a sample is in solution or readily solubilized. AAS is cost-effective and rather simple; AES has the advantage of increased input due to its multielement monitoring capability. Although several improved and modern techniques have emerged, such as the inductively coupled plasma (ICP) with mass spectrometry (ICP-MS), AAS and AES still constitute a fundamental pillar in toxic and essential elemental analysis. Inductively coupled plasma optical emission spectroscopy (ICP-OES) is also a useful technique widely applied for the same purpose in several matrices (Hou, Jones 2000, Li et al. 2013, Dzugan et al. 2018) using argon to create the plasma. Despite undisputable benefits of ICP-OES, one main disadvantage is the high-temperature plasma that requires a continuous flow of argon, increasing the operating cost substantially. Last but not least, ICP-OES interferences are abundant and difficult to cope with in the spectra analysis.

In this context, Microwave Induced Plasma – Atomic Emission Spectroscopy (MIP-AES) has emerged as a cutting edge, viable alternative to ICP--OES. After its commercial inauguration in 2011, several applications have demonstrated its significance (cf. Balaram et al. 2013, Li et al. 2013, Kamala et al. 2014, Tanabe et al. 2016). More specifically, another approach is used in MIP-AES to generate and maintain the plasma (Hammer 2008, Chalyavi et al. 2017). Owing to the lower plasma temperature, it assists easier ionization of the matrix, providing elements usually in their atomic state, and spectra devoid of substantial interferences. In addition, the advent of nitrogen-based MIP-AES (supported by a nitrogen generator) has drastically decreased the operation cost for such type of analyses that typically requires flammable or other expensive gases (MIP-AES).

With this in view, the current study aimed to measure the content of the aforementioned heavy metals in a substantial number of honey samples col-

lected from different places in El-Menofiyia Governorate, Egypt. In this context, an MIP-AES method was developed, validated and used in this direction. Last but not least, an assessment of risk associated with heavy metals was made, compared to the Egyptian Standards for honey.

MATERIALS AND METHODS

Chemicals and standards

Honey samples were digested with concentrated nitric acid (65% HNO_3, Sigma Aldrich, Germany) and hydrogen peroxide (30% H_2O_2 pure p.a, Sigma Aldrich, Germany). The element standard concentrated stock solutions (1,000 mg L^{-1}) of Cd, Cu, Fe, Pb, and Zn were obtained from Merck (Merck, Darmstadt, Germany) and used to prepare working solutions after appropriate dilution. Ultrapure water was used in all dilutions. The glassware, porcelain crucibles and tools used in the present study were soaked in detergent, rinsed with tap water, soaked in 15% nitric acid, rinsed with distilled water, and kept in an oven at 110°C until use (Fredes, Montenegro 2006).

Sample collection

One hundred samples of honey of different botanical origin, not thermally treated nor pasteurized, were obtained from beekeepers of El-Menofiyia Governorate (Egypt) in the summer of 2017. The number of samples was considered sufficient to obtain a representative overview of the honey produced (concerning heavy metals' presence) in the region, accompanied by statistical robustness. All samples were placed and stored in glass bottles and kept at 4-5°C in the dark before analysis. As a blank sample, a market organic honey sample tested free from heavy metals was used.

Sample preparation

A portion of 2 g of honey was placed in porcelain crucibles and heated from 60°C to 80°C for at least 12 h on a hot plate. The crucibles were then burned in muffle oven to ash at 450°C for 4 h. The ash was treated with 2 mL of 65% HNO_3 solution, and 2 mL hydrogen peroxide (30% H_2O_2 pure) for destroying any organic residue left after the burning. Each sample was placed in a 50-mL flask bottle, replenished with ultrapure water and filtered for instrumental analysis (for sample preparation procedures see Akbari et al. 2012, Mejías, Garrido 2017). A similar procedure was followed for the preparation of the blank honey extract used for matrix-matched calibration standards.

Quantitative analysis

Quantitative determination of Cd, Cu, Fe, Pb, and Zn was conducted by Microwave Plasma-Atomic Emission Spectroscopy using an Agilent MP-AES 4100 instrument (Agilent, Santa Clara, CA, USA) accompanied by an Agilent 4107-nitrogen generator (for separate compartments of the instrument) – Table 1. The Agilent MP Expert software was used to subtract the background signal from the analytical signal automatically. The background spectrum from the blank solution was recorded and automatically subtracted from each standard and sample solution analyzed. Prior to analytical runs, a standard mixture of the five elements at 200 μg L^{-1} was injected to optimize parameters such as nebulizer pressure. The software was also used to optimize the same pressure and viewing position for each wavelength selected to maximize sensitivity. The MIP-AES operating parameters and conditions are depicted in Table 1. Also, a matrix-matched standard curve of each studied element was established and used to calculate its concentration in the unknown samples, while two measurements were performed for each honey sample.

Table 1

MP-AES operating parameters and conditions

Operating parameters-conditions	
Nebulizer	One Neb
Nebulizer pressure	140-240 kPa (optimized per element)
Gas flows	Air 25 L^{-1} min, Optics purge 10 L^{-1} min, Ar 1.5 L^{-1} min, N_2 20 L^{-1} min
Spray chamber	Double pass glass cyclonic
Power of magnetron output	6480 K joules h^{-1}
Torch	Quartz torch
Plasma viewing	Axial
Read time	15-30 s
Stabilization time	15 s
Sample uptake delay	5-10 s
Number of replicates	3
Optical system	Czerny-Turner design monochromator with 600 mm focal length and fixed entrance slit
Detector	Back-thinned solid-state CCD detector (532×128 pixels)
Analytes (wavelengths)[a]	Cu 324.75 nm; Fe 259.94 nm; Pb 405.78 nm; Zn 213.86 nm; Cd 228.80 nm

[a] correspond to optimized spectral line for each element, considering maximum intensity

Analytical method validation

The analytical method was validated following several AOAC guidelines for the determination of metals and trace elements in foods (AOAC 1990), taking into account also bibliographic recommendations based on IUPAC definitions (Thomsen et al. 2003). Calibration standards (quality control samples) were regularly performed to evaluate the accuracy of the analytical method. Working calibration standards of the mixture of Pb, Cd, Zn, Cu, and Fe were prepared from a mixed standard stock solution of 100 mg L^{-1} prepared from the individual stock solutions. Matrix-matched calibration standards were prepared from blank organic honey sample extract. Standard and blank solutions were also analyzed in the same way as the digested unknown samples. Linearity was assessed from standard mixtures at six concentration levels. Linear calibration curves were established over the range of 1 to 1000 µg kg^{-1}, with the exception of Fe in which the range varied from 5 to 1000 µg kg^{-1}. The limits of detection (LOD) and limits of quantification (LOQ) were determined for each element. Specifically, LOD was calculated as 3 x standard deviation (SD) derived from 10 measurements in the blank matrix (independently prepared), divided by the slope of the analytical curve. LOQ was calculated as 10 x SD divided by the slope of the analytical curve.

To assess recovery, a known amount of the specific elements was added to blank matrix honey at two concentration levels (at 10 and 100 µg kg^{-1}, Table 1), and the whole procedure were repeated (identically as above) to calculate the recoveries using six replicates. Precision (repeatability and reproducibility) was investigated at 100 µg kg^{-1} using repeated measurements (n=10) of the same sample on the same day, or different preparations (n=10) at different days (n=10) and consequent measurements. Its criterion was the RSD% value obtained. The matrix effect (ME) was also assessed using matrix-matched calibration standards, applying an appropriate dilution after the digestion step. All data were corrected according to the values of a recovery percentage. Concentrations were expressed as mean ± SD, minimum and maximum values, and analysis of variance technique (ANOVA) was utilized. The probability of 0.05 or less ($p \leq 0.05$) was considered significant. Statistical analyses were carried out using Microsoft Excel 2016.

RESULTS AND DISCUSSION

Method validation

The five elements, namely Cd, Cu, Fe, Pb and Zn, were simultaneously determined using MIP-AES after the acid-digestion sample preparation step. Regression coefficient (r^2) values were > 0.999 in the studied range (Table 1), thus being acceptable. The capability of the method as a routine analysis

method was estimated initially through the determination of the LOD and LOQ of each element. LOQs were in the range of 0.96-14.85 μg kg^{-1} (presented along with LODs in Table 1). The latter are considered competitive to the ones reported using same instrumentation for other matrices (for comparison with Agilent MP-AES handbook see MP-AES-Agilent--Technologies). Li and coworkers reported also instrumental LODs and LOQs that were in the same order of magnitude (Li et al. 2013) with the presented in this work.

The analytical quality control was evaluated by the recovery experiments for the five selected elements, spiking at the two concentration **levels.** The recoveries (Table 1) were in the range of 90-99% with acceptable RSD% values, not exceeding 13%. Precision of the analytical method was corroborated by the acceptable RSD% values (Chudzinska et al. 2012) – Table 2.

Table 2

Analytical figures of merit for the MIP-AES method

Element	Linearity range (μg L^{-1})	Regression coefficient r^2	LOD[a] (μg kg^{-1})	LOQ (μg kg^{-1})	Recovery (%) ± RSD%[a].*		Precision*	
					10 μg kg^{-1}	100 μg kg^{-1}	repeatability (RSD%)	reproducibility (RSD%)
Cd	1-1000	0.999	0.29	0.96	99±6.5	91±5.3	2.1	3.9
Cu		0.999	2.8	9.24	90±7.8	91±4.4	3.7	7.3
Pb		0.999	0.5	1.65	91±12.0	95±9.1	1.3	6.5
Zn		0.999	3.1	10.23	90±10	93±12.7	4.2	11.8
Fe	5-1000	1.000	4.5	14.85	96±9.0	90±8.1	1.1	7.5

[a] 99% confidence intervals and degrees of freedom (df) = 9, *no significant difference was observed between replicate values, p>0.05

With regard to ME, a low effect was observed for the five elements under the established experimental conditions. The latter was assessed considering the dilution impact after the digestion step, which - based on bibliography – is a key determinant of ME (Li et al. 2013) in such type of analysis. Hence, a dilution factor of approximately 50 proved to be a good compromise for minimizing ME for all elements. Albeit, the use of matrix-matched calibration standards addressed any possible matrix effect.

Heavy metals in honey

Results in Table 3 show the minimum and maximum levels of mineral elements and the mean values obtained for the 100 honey samples. The values of the heavy metals concentrations have been compared with those established by the EOS (1993).

The Cd concentrations achieved a mean value of 5.42 μg kg^{-1}. The bibliography (Table 4) provides higher mean levels of Cd in Iran, New Zealand,

Table 3

Concentration (μg kg^{-1}) of heavy metals in honey samples

Metal	Min.	Max.	Mean	SD*	Recommended level[a]
Cd	1.0	14	5.4	1.0	50
Cu	12.4	940	128	16	3500
Fe	60.0	5870	462	147	70000
Pb	1.7	1590	123	95	4500
Zn	19.2	2630	244	121	1500

* no significant difference was observed between replicate values, $p > 0.05$,
[a] recommended level (μg kg^{-1}) according to Egyptian Organization of Standardization (EOS 1993)

in Italy and in another Egyptian study published in 2009. The mean value reported herein for Cd was in the same range as in the other work from Egypt (reported in 2004), and in Turkey, Spain, Russia and two Polish studies (Table 4). With regard to the significant causes of Cd contamination that potentially affect honeybees foraging environments and honey are the use of agricultural chemicals and inorganic fertilizers that contain Cd and other heavy metals as well (Giuffre de Lopez Camelo et al. 1997), the presence of the metal in sewage sludge that can be used as agricultural fertilizer, smelting or mining, pigments and plastics.

The Cu content showed an average concentration of 128 μg kg^{-1}, which was practically the same as those found in honey samples from Iran 130 μg kg^{-1} (Akbari et al. 2012) and in the same order of magnitude as the levels detected in Romania 228.3 μg kg^{-1} (Oroian et al. 2015) and Poland 250 μg kg^{-1} (Dzugan et al. 2017). Elevated levels were reported in Spain 1280

Table 4

Comparison of mean Cd concentration in honey samples in literature

Mean Cd concentration or range (μg kg^{-1})	Country	Reference
5.42	Egypt	this work
10	Egypt	Rashed, Soltan (2004)
180	Egypt	Rashed et al. (2009)
0.9-17.9	Turkey	Tuzen et al. (2007)
4.4	Spain	Frias et al. (2008)
10	Poland	Dzugan et al. (2017)
15	Poland	Przybylowski, Wilczynska (2001)
18	Russia	Eskov et al. (2015)
149	New Zealand	Vanhanen et al. (2011)
305	Italy	Buldini et al. (2001)
390	Iran	Akbari et al. (2012)

(Frias et al. 2008) and much higher levels in Russia – 15 740 μg kg^{-1} (Eskov et al. 2015), although in the latter case, bee communities were in proximity to Cu anthropogenic sources. In Egypt, previous works demonstrated greater levels – 1750 μg kg^{-1} (Rashed, Soltan 2004). The primary sources of Cu pollution in the environment are industrial and urban discharges and the application of agricultural chemicals. Smaller amounts are also released naturally from the earth's crust (Oroian et al. 2015).

The Fe average concentration (462 μg kg^{-1}) was lower than those found in honey samples in a previous investigation conducted in Egypt, which equalled 34 000 μg kg^{-1} (Rashed et al. 2009) and 58 000 μg kg^{-1} (Rashed, Soltan 2004), and one order of magnitude lower than the results achieved in Spain (Frias et al. 2008). In the case of honey from Romania, a range of 19 156 to 28 285 μg kg^{-1} was determined (Oroian et al. 2015, 2016); a similar mean value (29 380 μg kg^{-1}) was reported in Russia (Eskov et al. 2015) and the respective range in Indian honey fluctuated from 8 860 to 13 250 μg kg^{-1} (Nanda et al. 2003).

Lead is a non-essential element, and it is well documented that it can induce neurotoxicity, nephrotoxicity, and many other adverse health effects (Garcia-Leston et al. 2010, Rahman et al. 2012). The measured mean Pb concentration (123 μg kg^{-1}) was much lower than those reported previously in Egypt, i.e. 1050 μg kg^{-1} (Rashed et al. 2009) and 4200 μg kg^{-1} (Rashed, Soltan 2004), but higher than in China, where it was determined at 33.9 μg kg^{-1} (Ru et al. 2013), in the Pomeranian region of Poland, 48 μg kg^{-1} (Przybylowski, Wilczynska 2001) in Romania (Oroian et al. 2015), in Spain, 37 μg kg^{-1} (Frias et al. 2008), in Saudi Arabia, from 40 to 80 μg kg^{-1} (Osman et al. 2007) and in France, 47 μg kg^{-1} (Lambert et al. 2012). The measured mean Pb concentration was in the same order of magnitude as found in honey samples from Iran, 390 μg kg^{-1} (Akbari et al. 2012), and practically the same as measured in Russia (Eskov et al. 2015).

With regard to Zn, mean concentration (244 μg kg^{-1}) it was much lower than previous works in Egypt (Rashed, Soltan 2004, Rashed et al. 2009), lower than that reported by Oroian et al. (2015), by Buldini et al. (2001) in the case of Italian honeys (3205 μg kg^{-1}), by Golob et al. (2005) (3610 μg kg^{-1}, Slovenian honeys) and by Osman et al. (2007), in Saudi Arabia honeys (range between 200-750 μg kg^{-1}). Oroian et al., (2015) reported that honey contamination with Zn could be produced during the harvesting and storage when the honey is transferred inside galvanized recipients. An alternative insight was provided by Rashed and Soltan (2004) who contemplated that the Zn content in honey is dependent on the type of flowers bees are foraging.

Zn and Cu have been reported to provoke carcinogenicity to animals (Waalkes 2002) whereas Fe (the second most abundant metal on the earth crust) has been included in the list of metals with potential carcinogenicity that targets lung tissue (Waalkes 2002). However, from a nutritional aspect, Fe is a fundamental element for the majority of living organisms (Valko

et al. 2005) and its deficiency can lead to severe problems. Consequently, a proper equilibrium of essential elements is a prerequisite for a functional health status. Otherwise, heavy metals in honey by exceeding the tolerable levels might elicit health problems (Codex-Alimentarius 1993).

In the presented study, concentrations of Cu, Cd, Fe, Pb, and Zn in honey from different places in this governorate of Egypt was found less than the recommended level according to EOS (EOS 1993), except one sample containing Zn above the threshold concentration value (Table 3). Therefore, mean reported levels of Cd, Cu, Fe, Pb, and Zn demonstrate that honey was suitable for consumption in the vast majority of cases.

Human risk assessment and hazard quotients

The risk of heavy metals on human health was estimated to assess their chronic effects. The hazard quotient (*HQ*) was calculated for each tested heavy metal in honey *via* its consumption. The *HQs* have been computed following the equations 1,2 depicted below:

$$HQ = \frac{ADD}{RfD} \tag{1}$$

$$ADD = C \cdot \frac{IR}{BW} \tag{2}$$

Where: *ADD* – average daily metal intake (μg kg^{-1} d^{-1});

RfD – daily intake reference dose (μg kg^{-1} d^{-1}) suggested by the European regulations;

C – mean of heavy metal concentration in honey (μg kg^{-1});

IR – honey consumption rate (kg $person^{-1}$ d^{-1});

BW – mean body weight (60 kg).

The hazard index (*HI*) was used to approximate the total chronic-toxic risks of multiple heavy metals on the hypothesis of dose additivity and calculated as the summation of individual *HQ* values (equation 3):

$$HI = \sum HQs = HQ1 + HQ2 + HQ3 + \cdots HQn \tag{3}$$

The chronic-effects of heavy metals may occur if the hazard quotient is greater than one. The higher is the *HQ*; the higher is the chronic-toxic effect. The daily intake of each heavy metal, daily intake reference dose, hazard quotient, and hazard index results are presented in Table 5. The hazard index is the expression of the combined chronic-toxic effects of the heavy metals. It was assumed that an Egyptian inhabitant consumes 5.4 g of honey per day (EOS 1993). The body weight was presumed to equal 60 kg. The oral *RfD* used for computing the hazard quotient and index quotients were retrieved from EFSA reports and equalled 0.35, 15.7, 800, 3.57 and 142.86 μg kg^{-1} day^{-1} for Cd, Cu, Fe, Pb and Zn, respectively (EFSA 2006, 2012*a*,*b*).

Table 5

The daily intake of each heavy metal, daily intake reference dose, hazard quotient and hazard index

Metal	*ADD* (μg kg^{-1} $^{-1}d^{-1}$)	*RfD* (μg $kg^{-1}d^{-1}$)	*HQ*	*HI*
Cd	0.0004	0.35[a]	0.0011	0.005
Cu	0.0116	15.7	0.0007	
Fe	0.0421	800	0.00005	
Pb	0.0112	3.57	0.0031	
Zn	0.0222	142.8	0.0001	

[a] EFSA has also set a tolerable weekly intake (TWI) for Cd at 2.5 μg kg^{-1} bw. TWI is the amount of a given substance which can be consumed every week over the course of a lifetime without triggering any significant health effects for consumers.

The daily intake of each heavy metal analyzed, *RfD*, *HQs*, and *HI* are included in Table 3. All *ADD* values of individual metals were lower than one in the following order: Fe > Zn > Cu> Pb > Cd, while the HQ order was: Pb > Cd > Cu > Zn > Fe. The findings suggested that the intake of a single metal through honey consumption did not pose a significant potential chronic--toxic risk. The *HI* value of the five heavy metals from honey was 0.005. This result indicated that no significant chronic-toxic risk could be triggered by heavy metals due to honey consumption, and the measured mineral elements, considered as heavy metals, had no hazard effects. Similarly, Naccari et al. (2014) evaluated the risk using the target hazard quotient and reported that daily intake of metals with Sicilian honey was not to cause deleterious effects for consumers (Naccari et al. 2014). Overall, concentrations of heavy metals in honey are normally lower than the actual concentrations that bees carry on their bodies since heavy metal salts are selectively absorbed by stomach walls of bees and further dissolved inside nectar during the processing to honey (Eskov et al. 2015).

Based on results reported herein, honey serves as a mediocre bio-indicator of heavy metal contamination, especially when environmental or food samples are devoid of substantial contamination, as in this case. In the same context, the use of honey as an environmental bio-indicator is directly linked to plants origin-source (Mejias, Garrido 2017), which on the other hand does not always reflect the environmental stress provoked by diverse pollutants. The present results were supported and agree with several studies conducted in Finland (Fakhinzadeh, Lodenius 2000), India (Chandrama et al. 2014), Iran (Akbari et al. 2012, Mahmoudi et al. 2015) and Pakistan (Khan et al. 2014). On the other hand, several authors have reported that honey was a useful and sensitive biomarker for environmental contamination (Erbilir, Erdogrul 2005, Herrero-Lattore et al. 2017). To explore and standardize whether honey can be applied systematically a biomarker of environmental contamination, a more integrated study should be established taking into account an analy-

sis of environmental pollution sources including minerals in the soil, air, water, honey samples and additional elements in the analytical portfolio.

On the whole, assessing the progress on research on heavy metals in food commodities, from both an analytical point of view and health risk assessment, and as expressed on viewpoints and threshold values shaped through decades of substantial research efforts, any study that contributes to this domain is essential and can potentially be used in the refinement of such values. It is noteworthy that several agencies (such as the food and drug administration, FDA) have established toxic elements working groups, whose aim is to prioritize toxic metals and find ways and means to reduce exposure to them. This dynamic process is ongoing and eventually needs robust and powerful analytical methods, instruments and subsequent data. However, this process should be disconnected from the use of food commodities as sentinels of contamination. Some food commodities, such as honey, in respect to other apiculture matrices, are the least contaminated in the majority of cases (cf. LAMBERT et al. 2012) and a possible selection as an environmental sentinel should be investigated and evaluated on an individual basis.

CONCLUSIONS

A monitoring study of selected heavy metals in honey samples collected in Egypt was established employing the recently launched MIP-AES methodology and instrumentation. An analytical method was developed, validated and applied to analyze Cd, Cu, Fe, Zn and Pb in 100 honey samples from an Egyptian governorate characterized by profound beekeeping activity. The proper standards of Egyptian honey regarding levels of the chosen heavy metals were provided, together with a suitable risk assessment. The latter verified, through HQ and HI determinations, the safety of Egyptian honey in terms of a potential health hazard to consumers. The analytical method presented herein is currently expanded to other heavy metals of concern to provide further insight on their prevalence and thorough fingerprinting of heavy metals in Egyptian honey. Finally, although to our knowledge standard reference materials (SRMs) with certified concentrations of metals are not available for honey, we are currently planning to apply our method to other available SRMs to further characterize the accuracy of the MIP-AES analytical method.

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CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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