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TOXINS IN HONEY – A STUDY BY ICP-MS

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ABSTRACT

This paper represents a benchmark study that compares trace toxic elemental concentrations in honey products from various countries to establish a set of guidelines against which subsequent honey analyses could be referenced. Honey is a popular food item, widely consumed as part of a dietary regimen. Honey products originating from nine different countries were analyzed for toxic trace elements following microwave digestion in mild acid media (3% HNO_3). A comparative study of this nature has not been previously reported and could provide useful data particularly from an environmental and nutritional perspective. High-performance ICP-MS was employed to examine the levels of a range of noxious elements in aqueous samples. The performance of the instrument was validated using certified reference standards. Minor fluctuations in performance were adjusted by use of an internal standard. Sixteen elements were selected for investigation and the mean concentration ranges are summarized as follows: Li, Be, Ag, Cd, Sb, Hg, Tl, Bi, Th, U occurred in the range 1-25 μg/L; Pb, Se V, Ni, Cr, Al were observed in the interval 0.1- 4 mg/L. These toxins tend to originate from natural flora associated with the honey or from the chemical processes linked to the production of honey. In some samples "spikes" or elevated elemental levels were observed, which could be used to identify or "fingerprint" the country of origin of the corresponding honey sample. Our work could be considered a valuable source of reference data, and could contribute to environmental, food and forensic science.

Keywords: ICP-MS, honey, toxic trace elements.

INTRODUCTION

It is well known that honey originates from floral and plant discharges and is collected from honeycombs constructed by apocrital insects, such as bees. The origin and mechanistic pathway of toxic elemental impurities in honey is not clearly understood. These toxic elements could be characteristic of the soil and water that feed the flowers and plants (Williams and Pillay, 2005), or they could originate from the insects themselves or the honey-making process. The machinery, additives and preservatives that are associated with the production of honey represent a highly feasible source of these impurities, and could be considered a significant factor in contributing to the levels of these impurities. Honey products from nine different countries were studied for trace toxic elements by ICP-MS (inductively coupled plasma mass spectrometry). The investigation is original from the perspective that a comparative study of this nature has not been previously documented. In this respect our research could be considered a baseline study to provide a guideline for typical concentrations of selected trace toxic elements in honey.

Very few current analytical techniques possess the capability to detect ultra trace metal toxins. However, ICP-MS has demonstrated its superiority in this respect (Stephen *et al*., 2014). Other contemporary techniques such as XRF (X-ray fluorescence) (Punyadeera *et al*., 1997), PIXE (particle-induced X-ray emission) (Peisach *et al*., 1994) and PIGE (particle induced gamma emission) (Pillay and Peisach, 1992) are not sufficiently sensitive; whereas alternative trace techniques such as neutron activation (Pillay, 2002) and electrothermal analysis (Govender *et al*., 2001) have distinct drawbacks which make them inadequate. ICP-MS is multi-elemental, rapid, and has the capacity to reach lower limits of detection in the ppt or ng/L region (Greenfield, 1994). The honey products subjected to investigation were analysed for sixteen trace elements, all considered to be toxic at elevated levels. From the perspective of sustainable development, our work could be considered as particularly relevant as toxins in honey are of global significance. Food studies and forensic science could also benefit from this study.

MATERIALS AND METHODS

Instrumentation / Sample Treatment

Samples of honey of different brands and from a variety of countries were procured from retail venues located in

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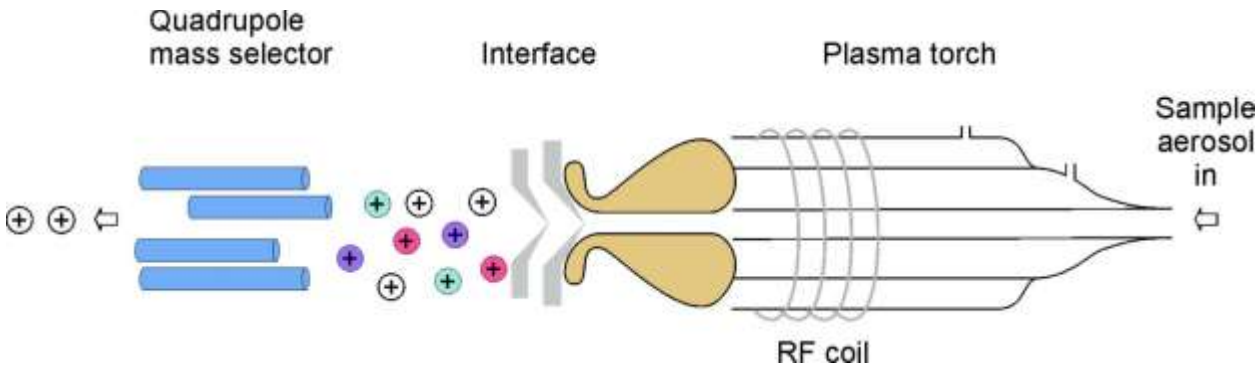

Fig. 1. The quadrupole mass filter responsible for highly discrete mass separation.

Table 1. Repeatability data obtained with certified reference standards (Fluka 70007).

Measurement	Be	Mg	Co	Ni	In	Ce	Bi
1	10.8	10.5	9.8	9.9	9.6	9.8	9.2
2	10.7	10.5	10.6	10.2	9.5	9.4	9.6
3	10.7	10.6	9.8	10.1	9.5	9.3	9.3
Mean ± RSD	10.7±0.54%	10.5±0.6%	10.1±4.6%	10.1±1.5%	9.5±0.6%	9.5±2.8%	9.4±2.2%

the vicinity. The samples were treated in aqueous acid media (3% HNO_3) and subsequently solubilized in a microwave digester. The digested samples underwent further dilution in ultra-pure aqueous media and were transferred to special vials for ICP-MS analysis. Each liquid solution thus prepared was converted to an aerosol via an aspirator/nebulizer unit in the instrument itself and transported to a high-temperature argon plasma (6000-8000 K) where it was ionized, and the ions, characteristic of the elements of interest, were carried to a mass spectrometer for detection. The instrument is equipped with a quadrupole mass filter that acts as a multi-elemental selector system (Fig. 1). An oscillating electrostatic field permitted ions of a single mass-to-charge ratio (m/z) to pass through the filter so that at any given instant discrete mass separation up to 2400 amu (atomic mass units) per second was attained. The technique is rapid, multi-elemental, high-performance and facile. It is highly sensitive, capable of ultra-trace analysis, and ideally suited for environmental studies.

Spectra arising from the toxins of interest were recorded, and numerical analysis was undertaken by comparing sample measurements with suitable reference standards. Advanced software was deployed to correct for interferences and matrix effects. Instrumental drift and minor aberrations in performance were adjusted by the use of internal standards. Linear calibration and background correction was achieved by the use of certified standards. The nebulizer gas flow in the system was 0.80 L/min. Prior to application the performance of the instrument was validated for repeatability (Stephen, 2015).

RESULTS AND DISCUSSION

Repeatability

Appropriate multiple reference standards (Fluka 70007) were available to establish the validity of the study and to evaluate the analytical performance of the technique. Of significance, is that the instrumental performance of the method was duly investigated by recording replicate measurements (n = 3) for each sample and standard. Three individual sample aliquots are aspirated into the instrument in quick succession and the system calculates the standard deviation. If the relative standard deviation (RSD) surpasses 10% the sample is re-analyzed to determine whether certain interfering factors are influencing the results. In each case the RSD was carefully examined and for our study RSDs <5% were attained for certified material (Table 1) indicating that the instrumental performance of the system was satisfactory.

Toxic elemental concentrations

As mentioned earlier, trace metal toxins could arise from floral discharges or from the honey-making process. It is important to bear in mind that as the honey samples of interest originated from different countries (north and south of the equator) the distribution of the experimentally determined trace concentrations is unique in each case. This unique elemental profile facilitates linking each sample with its corresponding country ("fingerprinting"). These distributions are presented in Figures 2-6, and are discussed in detail below. An important point to note is that the data presented in Figures 2-6 are log plots that tend to amplify small differences.

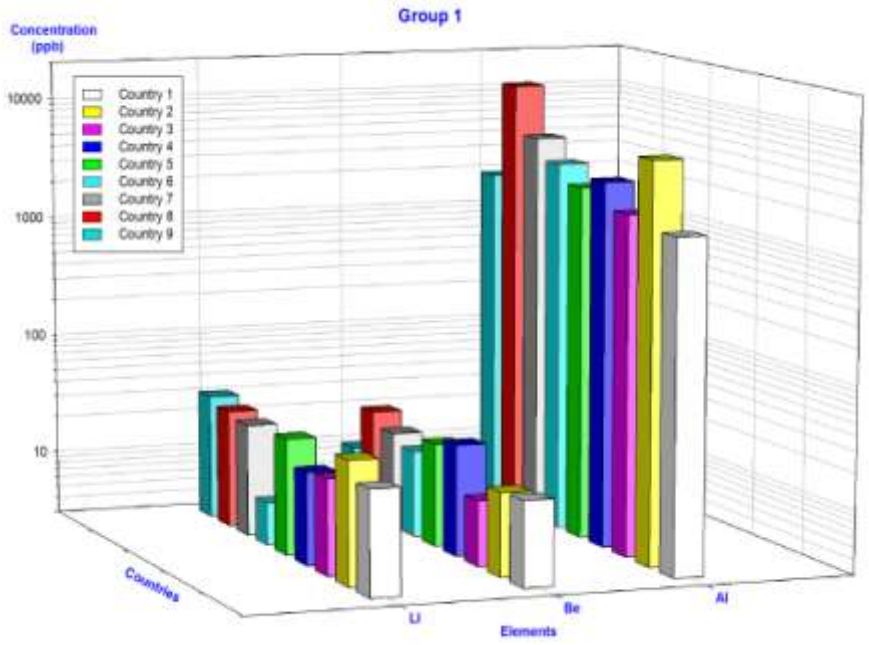

Fig. 2. Concentrations of Li, Be and Al in honey samples.

Lithium / beryllium: the health effects of lithium (Li) and beryllium (Be) at elevated doses are not widely documented and the impact of their toxicology is the subject of constant clinical research. It is known that Li in small doses can be beneficial, but elevated levels in the human body could lead to hypothyroidism and kidney disorders (Marcus, 1994). Lithium could replace sodium in body fluids but the exact biochemical mechanism of this displacement is not clearly understood. Beryllium on the other hand could replace magnesium in specific enzymes. It is a known carcinogen and its toxicity has become an occupational hazard (Stange *et al*., 1996). Our results showed that Li occurred at an average level of 23 µg/L; while Be was recorded at an average of 17 µg/L (Fig. 2). The trend in the Li levels were relatively consistent, with the exception of sample#6, which dropped to 7 µg/L, about three times lower than the average. This deficiency in Li in honey-sample#6 could reflect the nature of the environment from which it was sourced. The Be trend was equally consistent with no dramatic highs or lows. The above average values for Li and Be are well within the acceptable adult oral dose, however, honey is commonly used as food for children and could be administered in diminished quantities or at suitable intervals to minimize any negative health effects.

Aluminium: it is widely acknowledged that abnormal intake of aluminium could result in Alzheimer's disorder (Cooke and Gould, 1991). Aluminium (Al) occurs at fluctuating levels in foodstuff, but in bread and cheese typical mean values are about 5 µg/g. The mean Al concentration in this study was 4 mg/L (Fig. 2). However, the trend in Figure 2 shows that a "spike" in Al levels was observed for sample#8 at about 12 mg/L. This elevated level for sample#8 suggests that the equipment employed to process the honey could be contaminated with Al, and signifies that the general technological production of honey could be revisited to institute some form of remediation.

Toxic transition metals (vanadium/chromium/nickel): vanadium (V), chromium (Cr) and nickel (Ni) were selected for study as each of them could be considered toxic in high doses. All three transition metals are linked to chronic disorders. Vanadium at elevated levels is associated with pneumonia (Rodriguez *et al*., 2003); nickel is linked to respiratory disorders (Das *et al*., 2008); and hexavalent chromium is a designated carcinogen (Baruthio, 1992). The experimentally determined mean levels for this trio were: V: 0.7 mg/L; Cr: 1 mg/L; and Ni: 4 mg/L. The recorded data are within acceptable limits; however, it is clear from Fig 3 that two of the honey products displayed unusually pronounced levels. For example sample#6 showed a higher than normal level of Cr (4 mg/L); and sample#8 displayed a higher than average concentration of Ni (8 mg/L). Here again, it is suggestive that these elevated levels could have originated from the metal equipment associated with honey production and it is recommended that in the interest of sustainable living such processes should be overhauled to eradicate toxic elements.

Selenium/silver/antimony: negative health effects linked to selenium (Se), silver (Ag) and antimony (Sb) tend to vary and in some cases are not widely known. Abnormal intake of Sb and Se are related to adverse endocrine

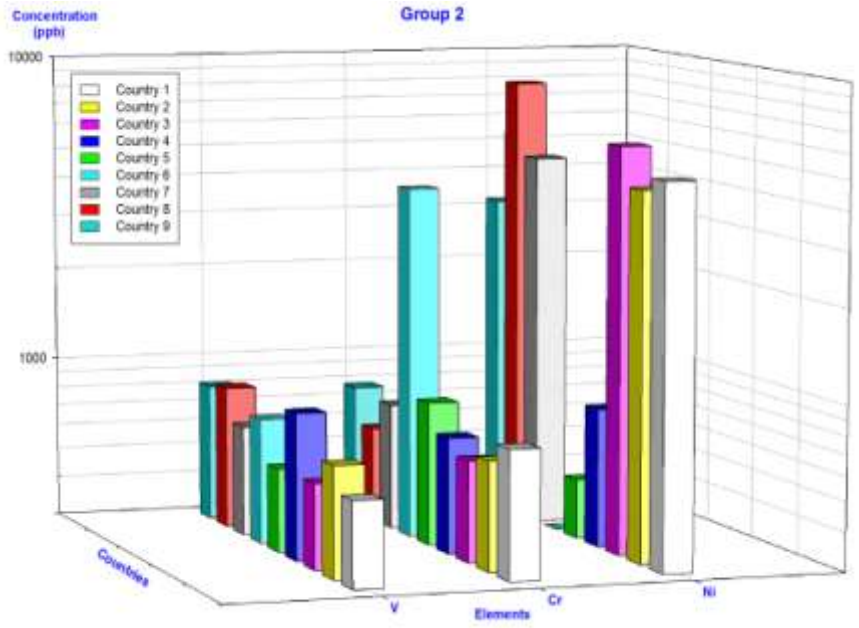

Fig. 3. Concentrations of V, Cr and Ni in honey samples.

disorders (Cooper and Harrison, 2009; Vinceti *et al*., 2001) while elevated levels of Ag in the human body could be associated with renal and lung afflictions (Drake and Hazelwood, 2005). The experimentally recorded mean levels of Ag and Sb in Figure 4 are in the region of 12 µg/L; while that of Se is close to 300 µg/L. These concentrations are within acceptable limits. The general trend reveals that sample#9 reflects elevated concentrations for Ag and Sb at 30 µg/L. The origin of these elements in honey could be natural resources.

Heavy metals (cadmium / lead / mercury): the hazardous nature of cadmium (Cd), mercury (Hg) and lead (Pb) cannot be overstated. All three heavy metals are linked to bodily disorders. Cadmium at abnormal levels causes renal problems (Jeffrey *et al*., 1980); Pb is associated with neuro disorders (Rosen, 1995) and mercury in the organic form is carcinogenic (Ratcliffe *et al*., 1996). The permissible dietary intake of these metals is <1 µg/g. The experimentally determined mean levels for these metals (Fig. 5) are within acceptable limits: Cd: 7 µg/L; Hg: 5 µg/L; Pb: 133 µg/L. The general trends for Cd and Hg varied at levels <10 ug/L with no dramatic fluctuations. On the other hand, the profile of Pb was not as smooth and displayed a "spike" at ~250 µg/L for sample#7. Honey is usually consumed in diets on a regular basis and although these heavy metals are within allowable limits, the data suggest that the intervals of consumption could be increased for reduced oral dose, especially for children.

Thallium / Bismuth / Actinides (*thorium, uranium*): thallium (Tl), bismuth (Bi), thorium (Th), and uranium (U) are considered "exotic" elements whose toxicology is undergoing continued clinical research (Najem and Voyce, 1990; Slikkerveer and de Wolff, 1989). It is unusual to find these trace elements in foodstuff and the experimental data tend to signify that these toxins could possibly originate from the soil and water associated with the honey products. The health effects of these trace metals are not widely understood. Thallium is a known pesticide, and can be lethal even in small doses (~15mg/L) (Kazantzis, 2000). Bismuth, Th and U are linked to various non-specific disorders but renal failure at elevated doses is characteristic of these particular toxins (Brugge and Buchner, 2011). Figure 6 portrays a trend for Tl that fluctuates at levels <10 µg/L, with a mean value of 5 µg/L. However, sample#6 displays an elevated level for this metal at roughly 25 µg/L. It is unclear why Tl is present in sample#6 at a level five times above the mean and information of this nature could prompt routine analysis of honey products combined with some form of de-metallization of this element at the production stage. The mean values of Bi, Th and U were 2, 8 and 1 µg/L, respectively. Sample#9 revealed a higher than average level of Bi and Th at 12 and 20 µg/L, respectively. Sample#3 also displayed an elevated level of Th at about 24 ug/L. Here again, these anomalies could be due to environmental contaminants.

"Fingerprinting"/Provenancing

The feasibility of employing trace toxic elements in honey for use in "fingerprinting" or provenancing studies was examined. The rationale behind fingerprinting is to identify honey produced from a specific region, based on trace elemental levels (Punyadeera *et al*., 1997). Uniquely elevated levels or 'spikes' in the recorded concentrations

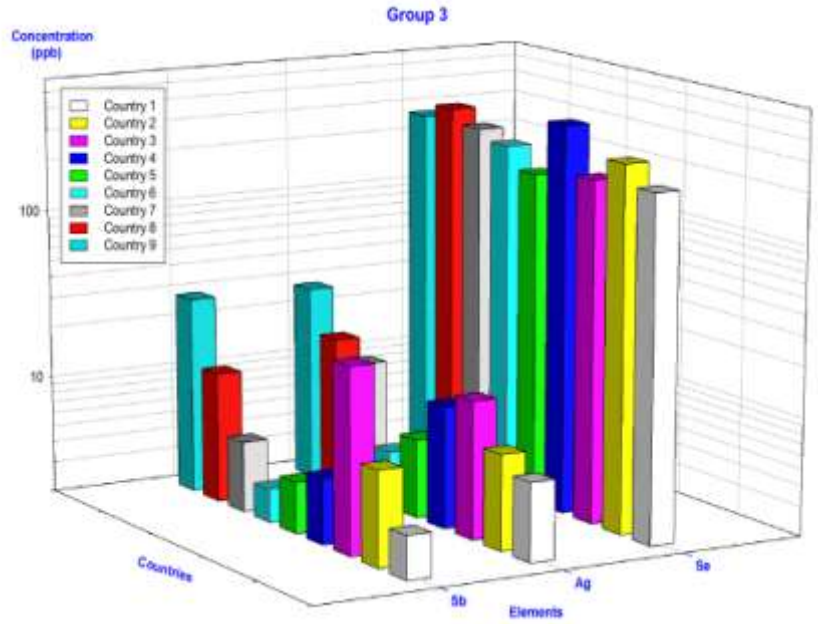

Fig. 4. Concentrations of Sb, Ag and Se in honey samples.

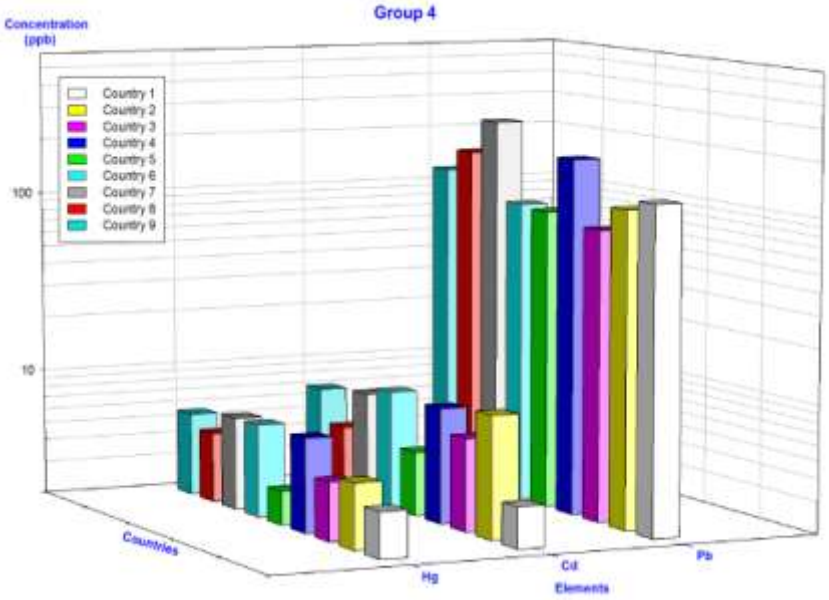

Fig. 5. Concentrations of Hg, Cd and Pb in honey samples.

tend to facilitate the task of "fingerprinting". Modern fingerprinting exercises could include pattern recognition methods that deploy elaborate software. Therefore, this particular area could be complex and the subject of a more detailed future study. However, the fact that the nine samples investigated in this work originated from nine individual countries lends itself to the notion of simple provenancing based on trace elemental content or ratios. For example honey sample#6 displays the highest chromium and thallium concentrations and the parameter Cr/Tl (~150) could be used to "fingerprint" this specific honey product and the country of its origin. Likewise, honey sample#8 reflects pronounced aluminium and nickel levels and the Al/Ni ratio (~1.5) could be employed in the same way. The Bi/Ag ratio (~2) for honey sample#9 is another useful example of applying such parameters in identification studies. It is interesting to note that such parameters could be helpful in forensic science, in tracing honey products to certain outlets that retail honey from specific countries. Another possibility is that such ratios and similar parameters could be "fine-tuned" or refined and used for "mapping" local honey farms in a district or locality.

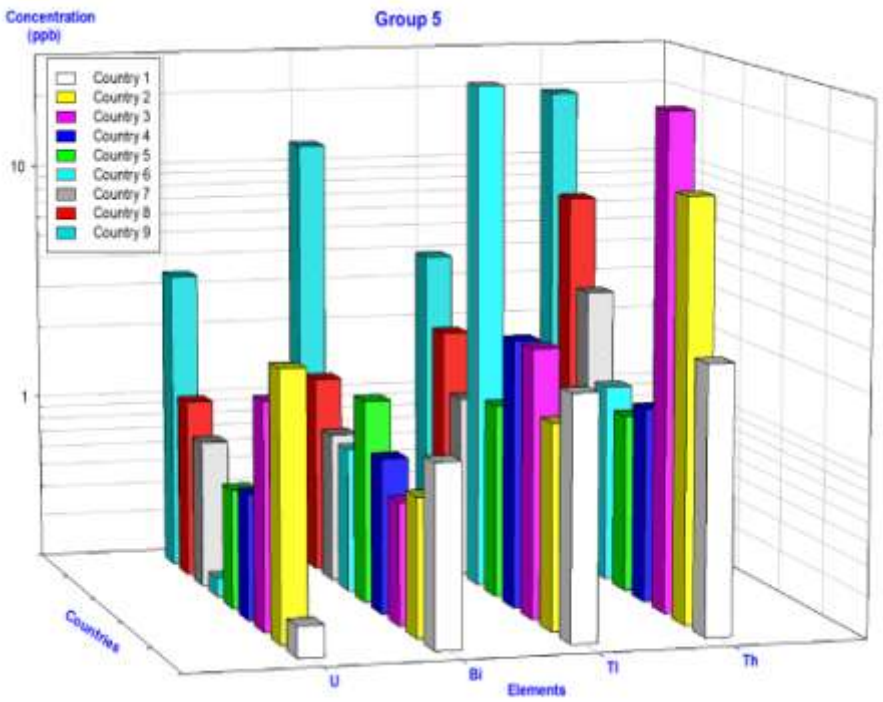

Fig. 6. Concentrations of U, Bi, Tl and Pb in honey samples.

Impact of the study

A comparative study of this nature, where honey products originating from various countries are investigated for metal toxins, has not been previously reported. Documentation of our experimental data is significant from two points of view: (i) human consumption of honey makes such studies essential; and (ii) it contributes to sustainable development. One notable feature observed in Figures 2-6 is that the elemental distribution for each honey sample (each country) is unique. This particular feature alone could be developed as a topic for further study and used for fingerprinting purposes. This unique regional distribution could stem from the trace elemental distributions in flora, soil and irrigation water associated with the region (country) and gives some indication of the "exotic" contaminants in the relevant environment. For example: sample#6 has an elevated Tl level; and sample#9 has above average levels of Ag, Bi and Th. These trace metals are unusual, not normally found at elevated levels, and reflects the nature of the environment from which the honey was sourced. A significant point is that some of these toxins could have originated from the honey-making process. Chemicals and reagents used in this process could have embedded impurities in them, which, in turn, could enter the honey product and contaminate it. In addition, the machinery and technical equipment used to produce the honey could be contaminated and could be responsible for the said impurities. The process of making honey should, therefore, be revisited and de-contamination operations could be considered. Thus the primary impact of this study is to make both honey manufacturers and consumers aware that certain impurities or toxins could be introduced into these products and remedial measures could be adopted to limit the presence of toxic elements in honey.

CONCLUSION

Our work illustrates that obtaining essential information on toxins in honey is in the interest of sustainability. Sustainable development is linked to maintaining a balanced environment, and our research presents data that could achieve this. The experimental results clearly demonstrate that certain toxins such as Al, Cr, Ni and Pb are present at above average levels in some samples and in such cases remedial measures could be taken to restrict the levels of these metals. The food industry would be interested in research of this nature, especially as honey is widely consumed by children. Our work contributes to both environmental studies and food science and a further extension to this study could include the application of trace elements in honey in pattern recognition and mapping studies.

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