Levels of Trace Elements and Rare Earth Elements in Honey From Jordan

Yahya R Tahboub (✉ tahboub@just.edu.jo)

Jordan University of Science and Technology <https://orcid.org/0000-0003-4691-9541>

Abd Al-Majeed A. Al-Ghzawi

Jordan University of Science and Technology

Shaker S Al-Zayadneh

Jordan University of Science and Technology

Mohammad S AlGhotani

Jordan University of Science and Technology

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Abstract

Honey is a common sweetener in the Jordanian diet with annual consumption of about one thousand tons, two-third of them are imported. It is believed that the elemental profile of honey is an indicator of safety and origin. In the literature, there is a lack of studies concerning levels of trace elements in honey in Jordan. A total 49 elements including 18 rare earth elements (REEs) were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) in mono-floral, and multi-floral imported honey samples, and multi-floral local samples. Regarding mono-floral samples, Black forest samples has the highest total metal content, while Acasia has the lowest total metal content. Local multi-floral honey has the largest Sr, and total REEs levels, while it has the lowest Mn levels. Very low levels of toxic elements were found in all samples, indicating the safety of honey in Jordan for human consumption. Results of this study showed that advanced statistical models are required to discriminate between multi-floral imported and local honey.

Introduction

Genuine pure honey is a natural product produced entirely by bees. It is a natural sweetener containing sugars, and small quantities of minerals, vitamins, fatty acids, and antioxidants (Alvarez-Suarez et al. 2010; Solayman et al. 2016). Its nutritious components make honey a major component in food industries and its medicinal properties including antimicrobial activities give honey a role in pharmaceutical industries. Thus, pure honey has significant economic value. However, honey is subjected to a wide range of adulteration by corn syrup and sugar cane and mislabeling of the geographic origin (Alvarez-Suarez et al. 2010; Silvano et al. 2014; Sobrino-Gregorio et al. 2018)

It has been known, that some mislabeled honey raises health and safety concerns, and may contain antibodies, toxins and alkaloids (Norsuzila Ya'acob¹, Mardina Abdullah^{1, 2} and Mahamod Ismail¹ et al. 1989; Salvador et al. 2019)

Honey in Jordan is either locally produced or exported from neighboring countries (Yemen, Egypt) or from European countries (Germany, Spain, Turkey). In 2019 Jordan produced about 320 tons of honey and imported about 600 tons.

Identification and authenticity of pure honey is a multidimensional and complex task and it have been done based on honey properties (Berriel, Barreto, and Perdomo 2019; Bogdanov and Gallman 2008; Salvador et al. 2019; Zhou et al. 2018)

Classical methods include pH, sugar content, moisture content, ash content, and free acidity (Anklam 1998; Khalafi, Goli, and Behjatian 2016; Lazarević et al. 2012; Silvano et al. 2014). Modern methods include chromatographic methods for measurements for sugar profile, amino acid profile, and phenolic profile (Karabagias 2016; Khalafi, Goli, and Behjatian 2016; Lazarević et al. 2012; Ng and Reuter 2015; Silvano et al. 2014; Tosun 2013; Zhou et al. 2018). Atomic spectroscopic methods are employed for the determination of trace element profile that has been reflected the botanic origin, geographic origin, and the

possibility of pollution with toxic elements (Adugna et al. 2020; Gallmann 2007; Rashed and Soltan 2004). Inductively coupled plasma-mass spectrometry (ICP-MS), which will be employed for elemental monitoring in this study has the advantage of other atomic spectroscopic methods of being, multi-element, more sensitive, selective, and less amenable to matrix effects (Czipa, Andrási, and Kovács 2015; Spiric et al. 2019; Squadrone, Brizio, Stella, Mantia, et al. 2020; Squadrone, Brizio, Stella, Pederiva, et al. 2020)

In this work, we will assess the safety, botanic and geographic origins of honey in Jordanian market (local and imported) by monitoring minor elements (Na, Mg, P, K, and Ca), essential trace elements (Fe, Zn, Mn, Cu, Ni, Cr, Se, Mo, and Co), nonessential trace elements (Li, Be, Al, V, Ga, Sr, Ag, Sn, Cs, Ba, Bi, and Cu), potentially toxic elements (As, Cd, In, Tl, and Pb), and 18 REEs (Y, Nb, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, and Th).

Materials And Methods

Reagents

Chemicals and standards used were analytical grade. HNO_3 (69% w/w, extra pure) and H_2O_2 (35% w/w, extra pure) were purchased from Sigma (St. Luis MO USA). Ultrapure deionized water (Milli-Q water) was employed for preparing standard solutions and sample solutions. A stock solution of combined elements (10.0 mg/L each) was purchased from Merck (Darmstadt, Germany). All plastic containers, pipette tips, polypropylene flasks, Pyrex glass digestion tubes, and reagents that came into contact with samples or standards were checked for contamination.

Sampling and sample preparation

Local honey samples ($n = 12$) were obtained directly from honey producers, and imported honey samples ($n = 18$) were purchased from local markets. All samples were labeled with their flora type. Samples were stored in brown bottles at room temperature.

A 1.00 g honey sample in triplicate was mixed with 6.0 mL HNO_3 and 2.0 mL of H_2O_2 as a catalyst and was digested using ETHOS 1-advanced Microwave Digestion Lab Station (Milestone S.r.l, Italy). The thermal program included a gradual rise in temperature for 12 minutes to reach 200 °C and then constancy of temperature at 200 °C for 20 minutes, and finally a gradual decline for 12 minutes to get to room temperature. The digestion was completed, as indicated by the appearance of colorless solution, completely clear, and homogenous solutions. The clear mixture was left to cool down and the contents of the tubes were transferred to digestion tubes and evaporated to near dryness at 110 °C and then diluted to 20 mL with 1 % HNO_3 solution.

The obtained solutions were measured for the targeted elements using ICP-MS. (Agilent 7500a Series ICP-MS). ICP-MS configuration and operating parameters, including selected isotopes are presented in Table 1S.

Calibration and quality control

External calibration was employed by sequential dilution of the multi-elemental calibration standard to prepare six calibration standards ranged from 0.10 to 20 µg/L for REEs, 0.10 to 100 µg/L for trace and potentially toxic elements and from 10 to 1000 µg/L for minor elements. Absolute intensities, counts per second (Cps), versus concentration were employed in statistical analysis. Linear least squares method was applied for calculation of statistical parameters for calibration curves. Elemental concentrations were calculated from respective calibration curves.

Calibration curves were linear over the range of the three calibration ranges with correlation coefficients better than 0.999.

The limit of detection (LOD), defined as $3S_b / a$, where S_b is the standard deviation of intercept and “a” is the slope of the calibration curve was obtained for each element. Method limit of detection (MLOD) was calculated by multiplying LOD by the dilution factor (20). Method limit of quantitation (MLOQ) for each element was calculated the same way but employing 10 S_b . Precision (% RSD) defined as $(S_a / a) \times 100$, where S_a is the standard deviation of the slope of the calibration curve.

For minor elements, method limits of detection (MLODs) vary between 1.46 µg/L and 6.52 µg/L for Mg and P respectively. For trace elements, MLODs vary between 0.008 µg/L and 4.21 µg/L for Ga and Mn respectively. For REEs, MLODs vary between 0.07 µg/L and 0.54 µg/L for Sm and Eu respectively. Precisions (% RSD) were less than 5 % for majority of targeted elements except for K (8.50 %), Li (7.36 %), P (7.01 %), Mg (5.71 %), V (5.38 %), and As (5.%). Detailed calibration results are presented in Table 2S.

Quality control studies were performed on a composite sample prepared from the digest of the 27 olive oil samples. The composite sample was certified for targeted elements by the standard addition method (Tahboub et al. 2021) and was employed for further quality control measurements.

Accuracy and recovery results were above 94 % and precision results (% RSD) were less than 6 % for all determined elements. Accuracy, precision and recovery results on certified sample and post-spiked certified samples are presented in Table 3S.

2.5 Statistical analysis

Statistical analysis for analyzed samples including mean $\pm$ SD, median, range, one way ANOVA, and Pearson's correlations were performed by Microsoft Excel 2013.

Results And Discussion

The analytical results for the basic 31 elements and 18 rare earth elements (REEs) in collected honey samples (n = 30) are displayed in Table 1 and Table 2.

Levels for each targeted element (mg/kg or µg/kg) includes mean $\pm$ SD, and range (minimum-maximum). Also, the elements were categorized to local and imported, and the level of each element in its category is reported in Table 1 and Table 2, as mean $\pm$ SD. The basic elements were classified to minor elements, n = 5,

(Na, Mg, P, K, and Ca), essential trace elements, $n = 9$, (Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, and Mo), non-essential elements ($n = 12$), (Li, Be, Al, V, Ga, Sr, Ag, Sn, Cs, Ba, Bi, and U), and potentially toxic elements ($n = 5$), (As, Cd, In, Tl and Pb).

Minor elements

Minor elements, Na, Mg, P, K, and Ca represent more than 90 % of total elemental content in most foods including honey, and their levels are related to their presence in soil, fertilizer and irrigation water. Their decreasing order in honey was $K > > Ca > P > Na > Mg$. The same order holds for imported honey samples, however, in local samples Na is larger than P. Large variations in elemental levels between samples is attributed to botanic and geographic origins. Large levels for $\sum$ minor were observed for Black forest mono-floral honey samples, and small levels were observed for Acasia mono-floral honey samples. Both of them are imported from European countries. P-values ($p > 0.05$) indicate no significant difference between levels of minor elements in local and imported honey. However, a smaller p-value for Na levels between local and imported samples is due to significantly large salinity of irrigation water in Jordan (Na is around 1000 mg/L).

Essential trace elements

Levels of essential trace elements in honey samples varied between 19.4 and 0.011 mg/kg for Fe and Co, and were found in the decreasing order: $Fe > > Mn > Zn > Cu > Cr > Ni > Se > Mo > Co$.

Iron, Fe, is an element required for essential functions in cells related to oxygen transport, oxidase activities and energy metabolism (Squadrone, Brizio, Stella, Mantia, et al. 2020). Levels of Fe, varied between 7.03 mg/kg for an imported multi-floral sample and 49.3 mg/kg for a local multi-floral sample. No significant difference was observed for Fe mean levels between local and imported samples.

Manganese, Mn, is a part of metallo-enzymes involved in various metabolisms (Squadrone, Brizio, Stella, Mantia, et al. 2020). Mn levels in honey varied between 0.13 mg/kg for an imported mono-floral sample (Acasia) and 13.3 mg/kg for an imported Black forest mono-floral sample. There is relatively significant difference in levels of Mn between local and imported samples.

Zinc, Zn, is elaborated in various physiological functions and is ubiquitous in the body (Jurowski et al. 2014). Zn levels vary between 0.48 mg/kg for an imported Acasia mono-floral sample and 3.72 mg/kg for an imported multifloral sample. There is no significant difference between levels of Zn in local and imported samples.

Copper, Cu, is a part of several physiological processes in human body. Cu levels varied between 0.145 mg/kg for a local multi-floral sample and 2.34 mg/kg for another local multi-floral sample. There is no significant difference between levels of Cu in local and imported samples.

Chromium, Cr, in trivalent form is involved in metabolism of carbohydrates. Levels of Cr varied between 0.06 mg/kg for a local multi-floral sample and 0.31 mg/kg for another local multi-floral sample. No significant difference was observed between levels of Cr in local and imported samples.

Other essential elements Ni, Se, Mo, and Co were detected with lower levels and had less impact in nutrition or safety assessment.

Nonessential trace elements

Among the 12 targeted nonessential elements, Al, Sr, Ba and Sn had significant levels, and was found in the decreasing order: Al > > Sr > Ba > Sn.

Aluminum, Al, was detectable in all samples ranging from 2.67 mg/kg for an imported Acasia mono-floral sample to 21.9 mg/kg for an imported multi-floral sample. Mean levels of Al in local and imported samples were significantly similar.

Strontium, Sr, was measured in significant levels in all samples ranging from 0.075 mg/kg for an imported Acasia mono-floral sample to 1.62 mg/kg for an imported multi-floral sample. Mean Sr levels between local and imported samples are significantly different. Sr could be employed as elemental marker to distinguish between local and imported honey.

Barium, Ba, levels vary between 0.059 mg/kg for an imported multi-floral sample and 1.06 mg/kg for a Black forest mono-floral sample. There is no significant difference in mean Ba levels between local and imported samples.

Tin, Sn, was found at low levels in honey, with lowest concentration, 0.033 mg/kg, for an imported Black forest mono-floral honey sample, and a highest concentration, 0.186 mg/kg, for a local multi-floral sample. Sn levels in local samples are slightly higher than imported samples.

Potentially toxic trace elements

Thallium, Tl, and indium, In, had lower levels to be considered for toxicity and assessment. Arsenic, As, is a highly toxic element. Its levels were not detected (ND) in 50 % of the samples and was up to 6.9 µg/kg in an imported multi-floral sample. Cadmium, Cd, and lead, Pb, are common pollutants that can enter the food chain after contamination of flowering plants. Cd levels were low in most samples and reached 6.7 µg/kg in an imported Black forest mono-floral sample. Pb levels was low in most samples and reached 31.5 µg/kg in the same imported Black forest mono-floral sample. Levels of Cd and Pb as well As are much lower than regulated levels in food (Paz 2017)

Rare earth elements

REEs are concentrated in different geological environments and their presence in honey is related to geographic origin and geochemical soil composition. Table 2 presents levels of 17 REE. Significant levels were detected for La, Ce, Pr, Nd, Sm, and Th with a decreasing order: Ce > Nd > La > Th > Pr > Sm. Total REE levels vary between 41 µg/kg for an imported mono-floral sample and 583 µg/kg for a local multi-floral sample with a mean of 145 µg/kg and SD of 157 µg/kg. Local samples had significantly larger mean than imported samples. Significantly larger deviations, mainly in imported samples, were due to the difference in geographic origins between imported countries.

Safety assessment

Honey is a popular sweetener in Jordanian diets. An adult usually consumes 10–50 g daily. Thus, honey may contribute significantly for total daily intakes of elements. Table 3 presents levels of estimated daily intake (EDI), recommended daily intake (RDI) (Paz 2017), and percentage contribution of each metal. The percentage contributions of Na, Mg, P, K, Ca, Mn and Zn are negligible relative to respective EDI (< 1.0 %). Little percentage contributions were observed for the rest of essential elements. Levels of potentially toxic elements (As, Cd, and Pb) were negligible to their RDI values, indicating the safety of Jordanian honey for human consumption.

Assessment and Authentication

Botanic and geographic origins are usually considered in assessment and authentication of honey (Gallmann 2007; Salvador et al. 2019; Squadrone, Brizio, Stella, Mantia, et al. 2020; Squadrone, Brizio, Stella, Pederiva, et al. 2020; Zhou et al. 2018). Results of Tables 1 and 2 indicated that classification of samples by local and imported did not give a clear picture of authentication. Calculated ANOVA p-values were, $p > 0.05$, indicating no significant difference between local and imported honey, even, levels of Na, Mn, and Sr were significantly different. A closer look to imported samples, they were consisted of Black forest mono-floral samples ($n = 5$), Acasia mono-floral samples ($n = 5$), and multi-floral samples ($n = 8$). All local samples were multi-floral ($n = 12$). Thus, botanic origin was considered in assessment and authentication. Levels of selected elements (mean $\pm$ SD) are presented in Table 4 and graphically in Fig. 1(A-E).

From the first look, precisions (%RSD) of results were much smaller than those in Tables 1 and 2, especially for mono-floral samples. Black forest samples have the highest $\sum$ minor elements, while Acasia samples were the lowest. While it was easier to discriminate between mono-floral and multi-floral, it needs more attention to discriminate between multi-floral-local and multi-floral-imported samples. Sodium, Na, strontium, Sr, and $\sum$ REE were significantly larger in multi-floral-local samples. These elements may be considered markers for multi-floral-local samples.

Pearson's correlation coefficients between targeted elements themselves in honey samples were computed and are presented in Table 5.

In this study we combined Black forest and Acasia samples in one category under the title "mono-floral". The other two categories were multi-floral-imported and multi-floral-local. Rare earth elements in the three categories were positively correlated with each other ($1.00 > r > 0.80$) and negatively correlated with other elements, thus, they were not considered in this study.

Selected correlations were based on categories with positive correlation coefficients ($r > 0.50$). Among the 60 selected correlations, largest positive correlation coefficients were distributed as 22 for multi-floral-local, 11 for multi-floral-imported, and 27 for mono-floral. Also, negative correlations were observed as 13 for multi-floral-local, 4 for multi-floral-imported, and 6 for mono-floral. The correlations Na/Mg, Na/Se, Mg/Se, Cr/Fe, Cr/Co, Cr/Ni, Fe/Ni, and Al/Sr were distinctive for multi-floral-local. The correlations K/Fe, K/Cu, $\sum$ minor/Cu, Fe/Cu, Co/Mo and Se/Sn were distinctive for multi-floral-imported.

Comparison with previous studies

Various studies were reported for multielemental measurements (minor and trace) in honey from popular honey producing countries. A summary of these results were reported by P. Phohl et al (Marcovecchio et al. 2015). Our results were within the lower half of reported ranges. RREs in honey from Italy, Balkans, Kazakhstan, South America and Tanzania were reported by S. Squadrone et al. They reported $\sum$ REE in the range of 9.0–65 $\mu\text{g/kg}$. Our results were 67 $\mu\text{g/kg}$ for Acasia, 79 $\mu\text{g/kg}$ for Black forest, 128 $\mu\text{g/kg}$ for multifloral-imported and 384 $\mu\text{g/g}$ for multifloral-local. A mean of 55 mg/kg $\sum$ REE were reported in soil from Jordan.

Conclusions

Forty eight elements including 17 rare earth elements were analyzed for honey in Jordan including multi-floral-local samples ($n = 12$), multi-floral-imported samples ($n = 8$) and mono-floral-imported samples ($n = 10$). Our results indicated a wide range for each element, with an order larger than 10 between maximum/minimum concentration. Precision (% RSD) was larger than 50 % for most elements, supporting the domination of the geographic origin. Potassium, K, dominated minor elements with a mean 718 mg/kg. Four essential elements, Fe, Mn, Zn, and Cu had significant concentrations, and Al, Sr, and Ba were the only nonessential elements with significant concentrations. Levels of potentially toxic elements, As, Cd, and Pb in all samples were much lower than their guideline limits, indicating the safety of honey in Jordan for human consumption.

Elemental profiles have strong influence in discrimination between different types and origins of honey. Selected, elements may be adequate for mono-floral honey due to domination of botanic origins. However, for multi-floral honey more elements are needed combined with correlations and statistical models such as principal component analysis (PCA). Also, elemental profiles for water and soil from various geographic regions are essential for accurate assessment and authentication.

Declarations

Ethical Approval Not applicable

Consent to Participate Not applicable

Consent of publication Not applicable

Data availability All data generated or analyzed during this study are included in the published article and its supplementary information files.

Competing interests The authors declare that they have no competing interests.

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Authors' Contributions The authors contributed to this research in the following manner. Conceptualization: Y. Tahboub and A. Al-Ghzawi; Supervised the whole stages, from collection of samples to writing of manuscript. S. Al-zayadneh and M. AlGhotani; Validated the method, and performed analysis of elements.

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Tables

Table 1 Statistical results for minor and trace elements in honey samples ^a: Total (mean±SD, range) ^b, local (mean±SD)^c and imported (mean±SD)^d and p-values between local and imported.

Minor elements

Element	Total (mg/kg) (Mean ±SD)	Range (mg/kg) (Mean ±SD)	Local (mg/kg) (Mean ±SD)	Imported (mg/kg) (Mean ±SD)	p-value
Na	66.9 ± 49.3	19.2 - 258	88.6± 65.7	54.8 ± 35.6	0.07
Mg	43.4 ± 27.4	9.1 – 107.8	37.6 ± 23.5	46.3 ± 29.2	0.42
P	74.3 ± 48.4	12.3 – 187.3	59.5 ± 21.0	81.7 ± 56.5	0.24
K	718 ± 746	27.7 - 2404	624 ± 710	840 ± 771	0.46
Ca	100.0 ± 44.9	26.9 - 205	88.4 ± 50.3	106 ± 41.9	0.30
Σ _{minor}	1052 ± 844	132 - 2872	898 ± 673	1129 ± 738	0.32

Essential trace elements

Cr	0.180 ± 0.053	0.06 – 0.306	0.175± 0.073	0.182 ± 0.043	0.77
Mn	1.96 ± 3.48	0.13 – 13.40	0.435 ± 0.160	2.77 ± 13.3	0.09
Fe	19.4 ± 11.0	7.03 - 49.3	21.3 ± 13.8	18.5 ± 9.66	0.24
Co	0.011 ± 0.010	0.003 – 0.049	0.008 ± 0.005	0.012 ± 0.012	—
Ni	0.123 ± 0.181	0.030 – 0.688	0.050 ± 0.015	0.160± 0.214	0.11
Cu	0.606 ± 0.478	0.145 – 2.34	0.615 ± 0.650	0.601 ± 0.382	0.95
Zn	1.67 ± 0.785	0.48 – 3.72	1.92 ± 0.681	1.51 ± 0.821	0.23
Se	0.041 ± 0.019	0.005 – 0.105	0.041 ± 0.025	0.041 ± 0.016	—
Mo	0.030 ± 0.013	0.020 – 0.096	0.029 ± 0.003	0.031 ± 0.016	—

Non-essential trace elements.

Li	18.3 ± 18.0	0.29 – 72.9	15.6± 9.30	19.6 ± 21.2	—
Be	1.30 ± 0.43	0.42 – 2.31	1.30± 0.43	1.30 ± 0.45	—
Al	4670 ± 3510	2660 - 21900	4442 ± 1529	4985 ± 1950	0.80
V	11.6 ± 6.80	5.14 – 37.7	15.7 ± 9.4	9.5 ± 3.9	—
Ga	13.6 ± 18.8	ND – 78.6	11.0 ± 7.3	14.9± 22.6	—
Sr	580 ± 440	75.0 – 1620	781 ± 380	480 ± 437	0.07
Ag	35.2 ± 69.9	5.70 – 386	27.7 ± 23.2	39.1 ± 84.7	—
Sn	56.9 ± 29.9	33.6 – 186	70.7 ± 46.7	50.1 ± 13.3	—
Cs	4.80 ± 8.60	0.17 – 37.9	2.00 ± 3.61	6.2 ± 10.1	—
Ba	251 ± 233	59 – 1060	204 ± 87	278 ± 278	0.44
Bi	0.77 ± 0.48	0.28 – 2.56	0.78 ± 0.39	0.77 ± 0.53	—
U	1.23 ± 0.65	0.49 – 3.23	1.31 ± 0.51	1.23 ± 0.65	—

Potentially toxic elements

As	3.10 ± 1.50	ND – 6.90	3.10± 1.60	3.10 ± 1.51	0.87
Cd	3.02 ± 1.18	0.38 – 6.47	2.90 ± 0.60	3.10 ± 1.41	0.42
In	0.68 ± 0.21	0.40 – 1.52	0.72 ± 0.20	0.68 ± 0.23	0.24
Tl	1.33 ± 2.56	0.16 – 13.4	10.7 ± 2.82	1.73 ± 3.07	0.46
Pb	13.6 ± 6.60	3.61 – 31.5	11.1 ± 2.5	15.0 ± 7.52	0.30

^a Four replicates, ^b n=30, ^c n=12, ^d n=18

Table 2 Statistical results for rare earth elements in honey samples: Total (mean±SD, range)^a, local (mean±SD)^b and imported (mean±SD)^c and p-values between local and imported.

Element	Total (mg/kg) (Mean $\pm$ SD)	Range (μ g/kg) (Mean $\pm$ SD)	Local (μ g/kg) (Mean $\pm$ SD)	Imported (μ g/kg) (Mean $\pm$ SD)	p-value
Y	2.42 $\pm$ 0.92	1.32 – 4.43	3.20 $\pm$ 0.98	2.15 $\pm$ 0.71	—
Nb	5.30 $\pm$ 12.9	0.33 – 66	2.60 $\pm$ 3.80	6.8 $\pm$ 15	0.27
La	19.4 $\pm$ 21.9	3.9 - 78	35.0 $\pm$ 27.0	12.5 $\pm$ 15.5	0.08
Ce	66 $\pm$ 82	15.1 – 314	111 $\pm$ 100	48 $\pm$ 69	0.28
Pr	5.5 $\pm$ 7.7	1.10 – 28.3	9.4 $\pm$ 9.1	3.9 $\pm$ 6.7	0.13
Nd	20.8 $\pm$ 31.4	3.40 –116	36.0 $\pm$ 37.0	14.3 $\pm$ 27	0.08
Sm	3.60 $\pm$ 4.20	0.90 – 15.9	5.8 $\pm$ 4.8	2.70 $\pm$ 3.60	0.38
Eu	0.47 $\pm$ 0.38	0.22 – 1.28	0.48 $\pm$ 0.33	0.47 $\pm$ 0.33	—
Gd	1.40 $\pm$ 1.80	ND-6.3	2.33 $\pm$ 2.02	1.29 $\pm$ 0.30	0.45
Tb	0.23 $\pm$ 0.12	0.10 – 0.58	0.30 $\pm$ 0.14	0.28 $\pm$ 0.26	—
Dy	1.01 $\pm$ 0.38	0.41– 1.70	1.44 $\pm$ 0.52	1.34 $\pm$ 1.48	—
Ho	0.37 $\pm$ 0.21	0.11 – 1.07	0.37 $\pm$ 0.15	0.39 $\pm$ 0.22	—
Er	0.41 $\pm$ 0.24	ND-0.93	0.54 $\pm$ 0.18	0.40 $\pm$ 0.28	—
Tm	0.18 $\pm$ 0.18	ND – 0.80	0.17 $\pm$ 0.14	0.16 $\pm$ 0.20	—
Yb	0.55 $\pm$ 0.28	0.16 – 1.36	0.58 $\pm$ 0.26	0.54 $\pm$ 0.29	—
Lu	0.28 $\pm$ 0.18	0.02 – 0.86	0.23 $\pm$ 0.16	0.29 $\pm$ 0.18	—
Hf	0.46 $\pm$ 0.45	0.040 – 1.80	0.58 $\pm$ 0.51	0.39 $\pm$ 0.36	—
Th	15.2 $\pm$ 14.3	3.9 – 58	23.0 $\pm$ 17.0	23.0 $\pm$ 17.2	—
Σ REE	145 $\pm$ 157	41-583	233 $\pm$ 185	107 $\pm$ 133	0.14

^a Four replicates, ^b n=30, ^c n=12, ^d n=18

Table 3 Estimated daily intake (EDI), recommended daily intake (RDI) and percentage contribution for selected elements..

Element	Mean mg/kg	EDI ^a mg/day	RDI [29,30] mg/day	% Contribution
Na	66.9	0.67-3.35	2300	0.03-0.15
Mg	43.4	0.434 – 2.17	420	0.10-0.15
P	74.3	0.743-10.9	700	0.11-0.55
Al	4.67	0.047 – 0.235	20	0.24 – 1.18
K	718	7.18 – 35.9	4700	0.15-0.76
Ca	100	1.0 – 5.0	1000	0.10-0.50
Cr	0.18	0.0018 – 0.009	0.120	1.5– 7.5
Fe	19.4	0.19 – 0.95	18	1.05-5.30
Mn	1.96	0.02-0.10	3.0	0.67-3.0
Zn	1.7	0.017 – 0.085	15	0.11 – 0.55
Co	11 µg/kg	0.11 – 0.55	6.0 µg	1.83 – 9.16
Ni	0.13	0.0013 – 0.0065	0.15	0.87 – 4.33
Cu	0.61	0.0061 – 0.035	2.0	0.30-1.50
As	3.1 µg/kg	0.031 – 0.155	150 µg	0.02 – 0.10

Cd	3.02 µg/kg	0.032 – 0.16	75 µg	0.042– 0.213
Pb	13.6 µg/kg	0.136 – 0.68	250 µg	0.054 – 0.272

^a Based on an adult consuming 10-50 g honey

Table 4 Statistical results for targeted elements in honey samples based on flora: Black forest, Acasia , multi-floral (imported), and multi-floral (local).

Element	Black forest (Mean $\pm$ SD)	Acasia (Mean $\pm$ SD)	Multi-floral (imported) (Mean $\pm$ SD)	Multi-floral(local) (Mean $\pm$ SD)
Minor elements				
Na (mg/kg)	50 $\pm$ 23	20 $\pm$ 1.5	66 $\pm$ 39	89 $\pm$ 70
Mg (mg/kg)	67 $\pm$ 28	14.5 $\pm$ 1.6	45 $\pm$ 26	39 $\pm$ 24
P (mg/kg)	136 $\pm$ 54	42 $\pm$ 3.5	68 $\pm$ 49	60 $\pm$ 22
K (mg/kg)	1750 $\pm$ 677	222 $\pm$ 38	615 $\pm$ 569	668 $\pm$ 738
Ca (mg/kg)	128 $\pm$ 27	54 $\pm$ 16	110 $\pm$ 41	91 $\pm$ 52
Σ Minor	2131 $\pm$ 825	420 $\pm$ 72	904 $\pm$ 574	947 $\pm$ 743
Essential elements				
Cr (μ g/kg)	178 $\pm$ 64	164 $\pm$ 47	187 $\pm$ 33	180 $\pm$ 76
Mn (mg/kg)	7.64 $\pm$ 4.52	0.201 $\pm$ 0.072	1.25 $\pm$ 2.42	0.432 $\pm$ 0.169
Fe (mg/kg)	16.1 $\pm$ 9.80	12.6 $\pm$ 6.04	20.9 $\pm$ 10.0	22.8 $\pm$ 13.6
Co (μ g/kg)	29 $\pm$ 15	4.0 $\pm$ 1.70	7.5 $\pm$ 3.1	8.7 $\pm$ 4.7
Ni (μ g/kg)	482 $\pm$ 209	53 $\pm$ 13	52 $\pm$ 20	48 $\pm$ 14
Cu (μ g/kg)	1066 $\pm$ 329	196 $\pm$ 34	508 $\pm$ 249	661 $\pm$ 672
Zn (mg/kg)	2.04 $\pm$ 0.653	0.856 $\pm$ 0.414	1.51 $\pm$ 0.857	1.93 $\pm$ 0.73
Se (μ g/kg)	35 $\pm$ 12	31 $\pm$ 12	46 $\pm$ 18	40 $\pm$ 27
Mo (μ g/kg)	45 $\pm$ 28	23 $\pm$ 2.5	26 $\pm$ 3.0	29 $\pm$ 3.3
Selected non-essential elements				
Al (mg/kg)	5.11 $\pm$ 1.13	2.71 $\pm$ 0.19	5.62 $\pm$ 5.84	4.54 $\pm$ 1.59
Sr (μ g/kg)	535 $\pm$ 432	80 $\pm$ 3.9	557 $\pm$ 449	838 $\pm$ 354
Ba (μ g/kg)	591 $\pm$ 418	80 $\pm$ 35	191 $\pm$ 83	202 $\pm$ 92
Sn (μ g/kg)	82 $\pm$ 15	82 $\pm$ 1.4	100 $\pm$ 21	117 $\pm$ 78
Ag (μ g/kg)	9.3 $\pm$ 2.4	8.6 $\pm$ 1.5	59 $\pm$ 106	28 $\pm$ 24
Potentially toxic elements				
As (μ g/kg)	3.5 $\pm$ 1.5	1.5 $\pm$ 1.6	3.3 $\pm$ 1.37	3.1 $\pm$ 1.7
Cd (μ g/kg)	4.5 $\pm$ 1.5	2.3 $\pm$ 1.7	2.7 $\pm$ 0.90	2.9 $\pm$ 0.60
Pb (μ g/kg)	16 $\pm$ 11	20 $\pm$ 5.8	13 $\pm$ 6.0	11 $\pm$ 2.5

Rare earth elements				
Y (µg/kg)	2.40± 0.73	1.8 ± 0.33	2.11± 0.79	3.2± 1.0
Nb (µg/kg)	15.6 ± 28	3.5 ± 3.5	2.7 ± 4.3	2.62 ± 3.85
La (µg/kg)	8.2 ± 3.5	10.0 ± 6.1	16 ± 21	35.1 ± 27.4
Ce (µg/kg)	29.6 ± 18.5	31.2 ± 6.9	63 ± 92	111 ± 100
Pr (µg/kg)	2.01 ± 0.84	2.20 ± 1.37	5.5 ± 9.1	9.4 ± 9.2
Nd (µg/kg)	6.7 ± 3.6	8.1 ± 5.3	20 ± 37	36 ± 37
Sm (µg/kg)	1.6 ± 0.5	1.70 ± 0.50	3.6 ± 4.9	5.8 ± 4.8
Th (µg/kg)	13.2 ± 10.9	8.0 ± 5.9	13 ± 14	23 ± 17
ΣREE (µg/kg)	79.2 ± 35.7	66.5 ± 12.7	132 ± 121	233 ± 135

Table 5 Pearson's correlation coefficients between selected elements in honey

Elements	Unifloral	Multifloral (imported)	Multifloral (local)	Elements	Unifloral	Multifloral (imported)	Multifloral (local)
Na/Mg	0.49	0.30	0.70	Cr/Fe	0.33	0.45	0.79
Na/K	0.49	-0.33	0.53	Cr/Co	0.01	0.27	0.83
Na/Ca	0.70	0.22	0.85	Cr/Ni	0.00	0.17	0.73
Na/ $\sum$ minor	0.51	-0.23	0.61	Mn/Fe	0.21	0.43	0.61
Na/Se	0.67	0.18	0.91	Mn/Co	0.96	0.53	0.64
Na/Mo	-0.15	0.41	0.43	Mn/Ni	0.77	-0.01	0.51
Mg/K	0.97	0.46	0.50	Mn/Cu	0.86	0.53	0.17
Mg/Ca	0.89	0.48	0.72	Mn/Al	0.99	0.97	0.64
Mg/ $\sum$ minor	0.97	0.56	0.58	Mn/Sr	0.59	0.06	0.54
Mg/Se	0.03	0.19	0.71	Mn/Sn	0.29	0.03	0.74
Mg/P	0.93	0.72	0.37	Mn/Ba	0.61	0.32	0.63
Mg/Fe	-0.21	0.55	-0.68	Fe/Co	0.00	0.72	0.96
Mg/Co	0.72	0.57	-0.60	Fe/Cu	0.44	0.88	0.29
Mg/Ni	0.79	0.86	-0.63	Fe/Ni	-0.22	0.49	0.93
Mg/Cu	0.51	0.54	-0.42	Fe/Sn	0.44	0.35	0.77
P/K	0.95	0.89	0.60	Co/Ni	0.90	0.60	0.86
P/Ca	0.85	0.42	0.35	Co/Cu	0.80	0.79	0.33
P/ $\sum$ minor	0.96	0.91	0.57	Co/Mo	0.05	0.56	-0.12
P/Fe	-0.36	0.72	-0.25	Ni/Sn	0.05	0.10	0.66
P/Ni	0.84	0.78	-0.30	Ni/Mo	0.36	0.52	-0.33
P/Cu	0.44	0.80	-0.46	Ni/Cu	0.72	0.52	0.24
K/Ca	0.94	0.42	0.87	Cu/Al	0.89	0.44	0.71
K/ $\sum$ minor	1.00	0.99	0.99	Mo/Ba	-0.03	0.51	0.11
K/Fe	-0.15	0.55	-0.40	Al/Sr	0.61	0.20	0.77
K/Ni	0.75	0.61	-0.26	Al/Sn	0.22	0.06	0.55
K/Cu	0.52	0.64	-0.45	Al/Ba	0.67	0.54	0.45

Ca/ Σ minor	0.95	0.51	0.91	Se/Sn	0.31	0.69	0.07
Ca/Se	0.30	0.30	0.80	Se/Ba	0.55	0.51	0.77
Σ minor/Fe	-0.15	0.59	-0.56	Sr/Ba	0.84	0.48	0.74
Σ minor/Cu	0.53	0.65	-0.42	Sr/Sn	0.65	0.71	0.77

Figures

Figure 1

1A Minor elements in honey (mean $\pm$ SD, mg/kg) 1B Trace essential elements in honey (mean $\pm$ SD, mg/kg or µg/kg). 1C Nonessential elements in honey (mean $\pm$ SD, mg/kg or µg/kg) 1D Potentially toxic elements in honey (mean $\pm$ SD, µg/kg) 1E Rare earth toxic elements in honey (mean $\pm$ SD, µg/kg)

Supplementary Files

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