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Trace elements and heavy metals in multifloral honeys from Serbia

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Abstract. This study was to determine the contents and any correlations of As, Cu, Zn, Fe, Cd and Pb in multifloral honeys. Honey, among other bee products, is a good bioindicator since it can reveal the connections between soil, plants and honeybees. Ninety-two samples of multifloral honey were collected from the retail market during the 2018 vegetation season and analyzed to determine mineral content. Analysis of the elements was performed by inductively coupled plasma mass spectrometry (ICP-MS). The most abundant element was Fe, with average concentration of 2.21 ± 1.00 mg/kg, followed by Zn, Cu, Pb, As and Cd. The results obtained show positive correlations: Zn-As, Fe-As, Fe-Cu, Fe-Cd, Cd-Cu and Cd-Pb. Negative correlations are noticeable between Pb and all other minerals except Cd.

1. Introduction

Honey is the natural sweet substance produced by honey bees (*Apis mellifera*) from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature [1]. Honey as food is an important source of energy and also has multiple healing properties, whether it is floral honey, or honeydew honey.

About 90-95% of the dry matter of honey is sugar, followed by water, in which organic acids and mineral compounds are dissolved. The numerous constituents of honey include enzymes, amino acids, organic acids, carotenoids, vitamins, minerals, and aromatic substances. Honey is rich in flavonoids and phenolic acids that exhibit a wide range of biological effects and act as natural antioxidants [2]. The composition, color, aroma and flavor of honey depend mainly on the flowers, geographical region, climate and honeybee species involved in its production, and are also affected by weather conditions, processing, manipulation, packaging, and storage time [3]. Honey contains components that have antioxidant activity, and also, honey is useful because of its antimicrobial components [4,5]. Mineral content determination in honey is important because honey is a valuable dietary supplement. Trace elements in honey are valuable as indicators of geographical origin. Some heavy metals are very toxic, so it is important to determine their content from the food safety point of view. There are no defined criteria for most of the minerals in honey. As an important ingredient of many dietary supplements, honey should meet requirements regarding Pb content (6).

Nevertheless, honey is good bioindicator, so it is possible to use analysis of honey to determine the relationships of minerals in soil, plants and bee products. Some of the minerals co-exist in positive relationships with plants, nectar and bee products, respectively. It is possible to determine relationships between the occurrences of different elements in certain bee products [7]. Multifloral honey, the most common honey type in Serbia, was chosen for this study [8,9]. The aim of this study was to determine the contents of As, Cu, Zn, Fe, Cd and Pb in multifloral honey as well as their correlations.

2. Materials and Methods

Ninety-two samples of multifloral honey were collected from the market during one vegetation season (from March until November 2018) and analyzed to determine mineral content, particularly some trace elements and heavy metals commonly found in honey. Honey samples were kept at 4ºC until analysis, thawed at room temperature (RT) and then homogenized. An amount, approximately 0.5 g, of each honey sample was transferred into a Teflon vessel with 5 ml nitric acid (67% TraceMetal Grade, Fisher Scientific, Loughborough, UK) and 1.5 ml hydrogen peroxide (30% analytical grade, Sigma-Aldrich, St. Louis, MO, USA) for microwave digestion. The microwave (Start D, Milestone, Sorisole, Italy) programme consisted of three steps: 5 min from RT to 180°C, 10 min hold at 180°C, and 20 min vent. After cooling, the digested sample solutions were quantitatively transferred into disposable flasks and diluted to 100 ml with deionized water produced by a water purification system (Purelab DV35, ELGA, High Wycombe, Buckinghamshire, UK). Analysis of the following elements: Fe, Zn, Cu, Cd, Pb, and As, was performed by inductively coupled plasma mass spectrometry (ICP-MS) (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany). The most abundant isotopes were used for quantification. The statistical analysis was performed using the GraphPad Prism version 7.00 software. The concentrations of heavy metals in multifloral honey types were expressed as the minimum, maximum, and mean ± standard deviation (SD) and were subjected to analysis of variance (One-way ANOVA). The parameters were analyzed using the Student's t-test at the probability of 0.05. Pearson's correlation analysis was applied to examine the relationship between heavy metal concentrations.

3. Results and Discussion

Findings of the six examined trace elements are given in Table 1.

Table 1. Mean concentrations of selected metals in 92 Serbian multifloral honeys (mg/kg)

Element	Statistics	Multifloral honey
As	Mean ± SD	0.003±0.001
	Range $_{(min-max)}$	0.001-0.004
Cu	Mean ± SD	0.31±0.21
	Range $_{(min-max)}$	0.09-0.92
Zn	Mean ± SD	1.95±1.70
	Range $_{(min-max)}$	0.37-8.02
Fe	Mean ± SD	2.21±1.00
	Range $_{(min-max)}$	0.77-3.94
Cd	Mean ± SD	0.003±0.001
	Range $_{(min-max)}$	0.001-0.01
Pb	Mean ± SD	0.005±0.001
	Range $_{(min-max)}$	0.004-0.026

As, Cu, Zn, concentrations were greater compared to previous studies of multifloral honey samples, while Pb, Cd and Fe were lower [10]. The range of element concentrations for different honeys was greater for

Cu and Pb, lower for Zn, Cd and Fe, and about the same for As. Comparable results were obtained in Turkey for multifloral honeys: Zn (0.65-3.2 mg/kg), Fe (1.55-12.9 mg/kg), Cu (9.97-29.5 mg/kg), Cd (0.29-2.03 mg/kg), Pb (1.54-36.7 mg/kg) (11). Multifloral honey from Croatia had similar content of Cd, but greater concentrations of As, Cu and Pb (12).

Some pairs or even multiple elements can be found in natural matrices together, meaning that it is possible to determine correlations between them, in the same sample matrix, or even in different, sequential matrices, e.g. soil, plant nectar and honey. Correlations between the tested elements are presented in Table 2.

Table 2. Matrix of correlation coefficients (r)

Element	As	Cu	Zn	Fe	Cd	Pb
As	1	-0.099	0.252	0.383	-0.133	-1.00
Cu		1	-0.117	0.418	0.426	-0.107
Zn			1	-0.079	-0.100	-0.216
Fe				1	0.064	-0.391
Cd					1	0.268
Pb						1

Regarding the honey samples, there were positive correlations between all metals except Fe and Zn, since these two elements were negatively correlated. There were positive correlations between Pb and Ni, Pb and Cd, and Pb and Fe content in wax. There were strong positive correlations between Ni and Cd, Ni and Fe, and Ni and Mg. Cd content was positively correlated with contents of tested metals in wax and propolis [7].

Serbian national regulation [13] defines criteria for trace elements in honey, Table 3. The table also contains values of method performance for elements, showed as limit of detection (LOD).

Table 3. Maximum residue limits (MRLs) for heavy metal content [13] and limits of detection of the ICP-MS method for the tested elements

Element	MRL (mg/kg)	LOD (mg/kg)
As	0.5	0.001
Cu	1	0.02
Zn	10	0.12
Fe	20	0.08
Cd	0.03	0.001
Pb	0.1	0.002

The results obtained during this study show that the honeys' element concentrations were less than the maximum residue limits (MRL).

4. Conclusion
The most abundant element, on average, was Fe, with a mean concentration of 2.21mg/kg, followed by (mg/kg) Zn (1.95), Cu (0.31), Pb (0.005), As (0.003) and Cd (0.003). The widest range of concentrations (mg/kg) of the examined elements in the honeys was for Zn (0.37-8.02), then Fe (0.77-3.94), Cu (0.09-0.92), Pb (0.004-0.026), Cd (0.001-0.01) and As (0.001-0.004). The results obtained showed some positive correlations: Zn-As, Fe-As, Fe-Cu, Fe-Cd, Cd-Cu and Cd-Pb. The strongest negative correlation was noticeable between Pb and As (r= -1), followed by correlations of Pb and Fe (r=-0,391), Pb and Zn (r= -0,216), Cd and As (r= -0,133), Zn and Cu (r= -0,117), Pb and Cu (r= -0,107), Cd and Zn (r= -0,100) and finally Cu and As (r= -0,099).

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