

TOXIC ELEMENTS IN SERBIAN SUNFLOWER HONEY ORIGINATING FROM VARIOUS REGIONS

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Abstract: The concentrations of As, Cd, Hg, Ni and Pb were determined in fifteen sunflower honey samples collected from 9 locations in the Republic of Serbia during 2019. The elements were analysed using inductively coupled plasma mass spectrometry (ICP-MS). Mean levels of elements (mg kg^{-1}) in all sunflower honey samples were as follows: 0.004 for As, 0.003 for Cd, 0.077 for Ni and 0.051 for Pb. The mercury content in all honey samples was below the detection limit of the applied method ($< 0.001 \text{ mg Hg kg}^{-1}$ honey). The lead level in all the examined honey samples was below maximum permissible value (0.10 mg kg^{-1}). The highest values of elements (mg kg^{-1}) were: 0.096 for Pb (in Kanjiža), 0.025 for As (Kikinda sample), 0.008 for Cd (Senta) and 0.125 for Ni in the honey originating from Svrlijig. None of the 5 toxic elements analysed exceeded the maximum permissible level.

Key words: sunflower honey, toxic elements

Introduction

Honey is a foodstuff with nutritional, sensorial and potentially therapeutic properties (*Przybyłowski and Wilczyńska, 2001; Bilandžić et al., 2011; Sergalio et al., 2019*). These properties are related to the chemical composition of honey. As food, honey must be free from contaminants. Honey contains about 200 substances, mainly sugars such as glucose, fructose and sucrose. It also contains, but in much smaller quantities, proteins, organic acids, vitamins, minerals, pigments, phenolic compounds, volatile compounds, and solid particles derived from honey harvesting (*Bogdanov et al., 2008; Da Silva et al., 2016*). Mineral concentrations in honey depend on the botanical origin, climate conditions, but also significantly on geographical origin and type of soil where plant grows (*Bilić-Šobot, 2020; Živkov Baloš et al., 2018; Lazarević et al., 2017; Uršulin-Trstenjak et al., 2015*). Mineral content of honey contributes to the colour of honey. Darker honey types are richer

in minerals. Black locust and sunflower honey are characterized by low concentrations of ash and minerals, compared to meadow, chestnut and honeydew honey (Lasić et al., 2018; Dhahir and Hemed, 2015; Uršulin-Trstenjak et al., 2015).

Honey can be a useful indicator of environmental pollution (Đogo Mračević et al., 2020; Sergalio et al., 2019; Lazarević et al., 2017; Moniruzzaman et al., 2014; Bilandžić et al., 2011). Honeybees may be exposed to toxic elements pollution (arsenic, mercury, lead, cadmium, nickel) in an area of around 7 km² surrounding the hives (Đogo Mračević et al., 2020). During foraging, bees are exposed to pollutants. Their hairy bodies can gather various particles from the atmosphere or they may be exposed to contaminated pollen or water (Porrini et al., 2003; Lambert et al., 2012; Costa et al., 2019). Contamination of honey by toxic elements may be a result of industrial development, urbanization and transport (Hamad et al., 2020; Tutun et al., 2019; Lambert et al., 2012; Bilandžić et al., 2011). In addition to the listed sources, contamination of honey may be caused by incorrect procedures applied during harvesting, fumigation, extraction and processing, storage and conservation phases (Bartha et al., 2020).

Sunflower honey is traditional honey with exceptional healing properties and nutritional value (Sari and Ayyildiz, 2012). Sunflower is cultivated in the southern regions, with abundant sunshine and where the climate is favourable for growing this plant. It is important to point out that the literature clearly shows that sunflower can accumulate high concentrations of toxic elements (As, Pb, Cu, Cd, Ni, Cr, Co), mainly in shoots and roots (Dhiman et al., 2017; Stoikou et al., 2017; Angelova et al., 2016; Garcia et al., 2006). Since growing sunflower plants has considerable potential to accumulate toxic elements contaminants, they are considered “hyperaccumulators” of toxic elements (Dhiman et al., 2017).

In Serbia, honey production is well-developed thanks to the suitable climate and geographic location, so sunflower honey is one of the most commonly produced kind of honey (Živkov Baloš et al., 2021). There is very little data on toxic elements in sunflower honey available in the literature, so the purpose of this study was to determine concentrations of trace (toxic) elements (As, Cd, Hg, Ni and Pb) in sunflower honey in order to obtain information about honey safety.

Material and Methods

Samples: A total of 15 samples of sunflower honey harvested in 2019 were collected from beekeepers in various regions of the Republic of Serbia. The sampling included locations in the following municipalities: Kanjiža (4 samples), Kikinda (2 samples), Čelarevo (4 samples), Sremska Mitrovica (1 sample), Senta (1 sample), Žabalj (1 sample), Osečina (1 sample) and Svrlijig (1 sample). All the collected samples were in their original packaging (jars) and were transferred to the

laboratory of Scientific Veterinary Institute “Novi Sad” for examination. Manufacturers used field observations for botanical origin determination. Honey analyses were carried out immediately after sampling. The honey analysis procedures were performed in two replicates.

Sample preparation: The samples (1 g) were prepared applying the microwave (Ethos, Labstation Microwave, Milestone), digestion method (14) with the use of the mixture $\text{H}_2\text{O}_2/\text{HNO}_3$ (1:4, v/v). After this process, the samples were transferred to 50 mL volumetric flasks and diluted with deionized water.

Determination of elements: The contents of Pb (NoG-M, IT 0.1 s/P), Cd (NoG-M, IT 1 s/P), As (He-M, IT 1 s/P), Ni (He-M, IT 1s/P) and Hg (NoG-M, IT 1 s/P) were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) on ICP-MS 7700 mass spectrometer (Agilent Technologies). Solutions used for calibration were prepared from commercial stock standard solutions with 1000 mg/l of each element (Accustandard). To calculate the recovery percentage, 6 samples has been spiked with known amounts of Cd, As, Hg, Ni and Pb analytical standards. Obtained results are presented in Table 1.

Table 1. Isotopes, limit of detection (LOD) and recovery rates for monitored elements

Element	Isotope	LOD (mg kg^{-1})	Recovery (%)
Cd	^{111}Cd	0.001	96.1
As	^{75}As	0.001	100.4
Hg	^{201}Hg	0.001	83.7
Pb	^{208}Pb	0.001	88.1
Ni	^{60}Ni	0.001	92.6

Data analysis: All the calculations and statistical analyses were performed using the PAST software package, version 2.12, Oslo, Norway. The data were grouped according to the samples of sunflower honey and presented as mean, standard deviation, minimum, and maximum values.

Results and Discussion

Table 2 shows the values of toxic elements detected in sunflower honey samples. The obtained results were compared with the literature data presenting the highest and lowest mean values of investigated elements.

In the present study, lead concentrations ranged from 0.002 to 0.096 mg kg^{-1} with a mean value of 0.051 mg kg^{-1} in fifteen sunflower honey samples from all the examined locations. The highest Pb level was 0.096 mg kg^{-1} found in the sample from Kanjiža, and the lowest was 0.002 mg kg^{-1} in the sample from Čelarevo. The average lead level found in our investigation was similar to the levels found in honey samples from Croatia (65.2 $\mu\text{g kg}^{-1}$; reported by *Bilandžić et al.*, (2011) and 0.02-0.11 mg kg^{-1} ; reported by *Uršulin-Trstenjak et al.*, (2015)),

Romania (51.674 $\mu\text{g kg}^{-1}$; Oroian et al., 2016) and Poland (0.048 mg kg^{-1} ; Przybyłowski and Wilczyńska, 2001). However, the Pb concentrations were lower than those found in sunflower and other types of honey from Turkey (with maximum content of 0.48 mg g^{-1} ; Citak et al., 2012), (0.80 mg kg^{-1} ; Tutun et al., 2019), Iran (507.58 $\mu\text{g kg}^{-1}$; Aghamirlou et al., 2015), Iraq (0.108 – 0.820 mg kg^{-1} ; Dhahir and Hemed, 2015), Malaysia (0.36 mg kg^{-1} ; Moniruzzaman et al., 2014), and from polluted areas in Egypt (Hamad et al., 2020) and Romania (Bartha et al., 2020). In the current study, the average lead level was higher than those reported in other studies carried out in our country and Croatia, Malaysia, France, and Saudi Arabia (Đogo Mračević et al., 2020; Lasić et al., 2018; Bilandžić et al., 2017; Chua et al., 2012; Lambert et al., 2012; Aljedani, 2017).

Table 2. Toxic elements content in sunflower honey from different location in Serbia

Location	Pb	As	Hg	Cd	Ni
	mg kg^{-1}				
Kanjiža	0.033	0.005	<0.001	0.002	0.053
Kanjiža	0.028	0.004	<0.001	0.002	0.058
Kanjiža	0.096	0.005	<0.001	0.001	0.045
Kanjiža	0.008	0.003	<0.001	0.002	0.048
Sr. Mitrovica	0.034	0.004	<0.001	0.002	0.099
Kikinda	0.063	0.025	<0.001	0.001	0.057
Kikinda	0.016	0.004	<0.001	0.003	0.078
Senta	0.089	0.005	<0.001	0.008	0.095
Osečina	0.040	0.005	<0.001	0.005	0.071
Čelarevo	0.046	0.004	<0.001	0.005	0.069
Čelarevo	0.043	0.005	<0.001	0.004	0.094
Čelarevo	0.002	0.003	<0.001	0.002	0.053
Čelarevo	0.081	0.005	<0.001	0.005	0.123
Žabalj	0.085	0.004	<0.001	0.004	0.093
Svrljig	0.094	0.004	<0.001	0.003	0.125
Mean value	0.051	0.006	< LOD	0.003	0.077
Standard deviation	0.032	0.005		0.002	0.026
Minimum	0.002	0.003		0.001	0.045
Maximum	0.096	0.025		0.008	0.125

LOD- limit of detection

Lead is a natural component of the biogeosphere. It enters the environment from metal smelters, coal-fired power plants, from sewage sludge, waste oil, or is a result of solid waste combustion. However, the dominant anthropogenic emission of Pb in the environment is the result of the use of organo-lead compounds - additives in the oil industry. The lead used in automobile fuel was forbidden a few years ago. However, air and water contamination is still high (Lambert et al., 2012). Lead is one of most widespread contaminants in the environment, and its

content is examined in all environmental studies (*Bilandžić et al., 2011*). Lead is the only metal whose maximum content in honey is limited by regulations. The maximum permissible value of lead is prescribed by national regulation on maximum concentrations of certain contaminants in food (Official Gazette, 81/2019). This regulation is harmonized with the European regulation (*Commission Regulation, 1005/2015*). Maximum permissible value is set at 0.10 mg of Pb/kg for honey. The lead content in all the examined honey samples was below 0.10 mg kg⁻¹. However, lead content in some samples (Kanjiža, Senta, Svrlijig) was very close to the maximum permissible value (0.096, 0.089 and 0.094 mg kg⁻¹). Higher concentrations of Pb in sunflower honey samples from these three locations may be the result of the location of hives in the areas, as they were near roads, industrial or building sites.

Arsenic levels ranged from 0.003 to 0.025 mg kg⁻¹ and mean content in all honey samples was 0.006 mg kg⁻¹. The highest As level was 0.025 mg kg⁻¹ in the sample from Kikinda, while the lowest was 0.003 mg kg⁻¹ in the samples from Kanjiža and Čelarevo. In comparison with the levels found in the literature, mean As level was higher than the values found in Malaysia (< LOD; Chua et al., 2012) and Iran (< LOD; Aghamirlou et al., 2015). Similar results for As in honey were reported in Croatia (1.97 μg kg⁻¹ reported by *Bilandžić et al., (2011)* and 0.62 – 6.95 μg kg⁻¹ reported by *Bilandžić et al., (2017)*) and Romania (3.49 μg kg⁻¹; *Oroian et al., 2016*). Arsenic (As) is a common contaminant, found both naturally and as a result of human activity. Industrially produced arsenic mostly originates from agricultural products such as insecticides, herbicides, fungicides, algicides, wood preservatives, and growth stimulators for plants and animals. The use of pesticides containing arsenic and other chemical products in agriculture results in arsenic accumulation in soil and plants. Consequently, arsenic is usually found as a trace element in both food and feed (*Roy and Saha, 2002; Mandal, 2017*). Natural distribution of As is associated with igneous and sedimentary rocks. High As concentrations in groundwater and drinking water are registered throughout Pannonian Basin (*Kristoforović-Ilić et al., 2009; Kostić et al., 2016; Senila et al., 2017*). Most of As-contaminated areas in Vojvodina are in the region of alluvial formation along the banks of the rivers Danube and Tisa, and confluent rivers Zlatica, Begej, Tamiš and Nera (*Kristoforović-Ilić et al., 2009*). Taking into account these facts, it can be assumed that the high concentration of As in the sample from the area of Kikinda (0.025 mg kg⁻¹) is related to naturally contaminated groundwaters.

The presence of any form of mercury is considered undesirable and dangerous in the natural environment (*Dobrowolska and Melosik, 2002*). The source of mercury soil contamination are mineral fertilizers, fungicides and disinfectants in agriculture, as well as the use of waste sludge to fertilize arable land. Emitters of mercury in the atmosphere are metal smelters, burning fossil fuels and burning waste material. There is almost no literature data on mercury

content in honey. In the present study, the content of Hg was below the limit of detection by ICP MS method ($< 0.001 \text{ mg kg}^{-1}$). *Bilandžić et al. (2011)* reported that mean Hg content in honey from Croatia was $2.72 \text{ } \mu\text{g kg}^{-1}$ honey, while *Oroian et al. (2016)* found $0.73 \text{ } \mu\text{g Hg/kg}$ in honey samples from Romania.

The concentration of cadmium in the environment increases significantly due to the industrial production of plastics, dry batteries, paints and other products that contain this element, and also through phosphate fertilizers that contain significant amounts of Cd (*Satarug et al., 2003*). The mean cadmium content in the tested sunflower samples was 0.003 mg kg^{-1} (0.001 to 0.008 mg kg^{-1}). These results were similar to those from Croatia (with mean Cd amounting 0.005 mg kg^{-1} reported by *Lasić et al. (2018)* and ranging from 0.003 to 0.011 mg kg^{-1} reported by *Uršulin-Trstenjak et al., (2015)*) and Romania ($1.19 \text{ } \mu\text{g kg}^{-1}$; *Oroian et al., 2016*). Mean Cd level in sunflower samples was higher than the levels found in Malaysia ($< \text{LOD}$; *Chua et al., 2012*) and Saudi Arabia ($< \text{LOD}$; *Aljedani, 2017*). Cadmium concentrations in this study were lower than those found in honey samples from Egypt ($0.01 - 0.03 \text{ mg kg}^{-1}$; *Hamad et al., 2020*), Iran ($27.62 \text{ } \mu\text{g kg}^{-1}$; *Aghamirlou et al., 2015*), Iraq ($0.210 - 0.894 \text{ mg kg}^{-1}$; *Dhahir and Hemed, 2015*), and Malaysia (0.35 mg kg^{-1} ; *Moniruzzaman et al., 2014*). *Bartha et al. (2020)* have found very high concentrations of Cd ($0.05 - 3.81 \text{ mg kg}^{-1}$) in polyfloral honey from polluted areas in Romania.

The mean nickel content in the tested sunflower honey samples was 0.077 mg kg^{-1} and the range of concentration was from 0.045 to 0.125 mg kg^{-1} . The highest Ni level was 0.125 mg kg^{-1} in the sample from Svrlijig, while the lowest amounted 0.045 mg kg^{-1} in the sample from Kanjiža. Similar to cadmium, Ni concentration in our study was higher than the levels found in Malaysia ($< \text{LOD}$; *Chua et al., 2012*), Turkey (mean 0.05 mg kg^{-1}) and Saudi Arabia ($< \text{LOD}$; *Aljedani, 2017*). The authors from Croatia (*Uršulin-Trstenjak et al., 2015*), Iraq (*Dhahir and Hemed, 2015*), Egypt (*Hamad et al., 2020*), Iran (*Aghamirlou et al., 2015*) and Romania (*Oroian et al., 2016*) have found higher Ni concentrations in honey samples (ranging between 0.09 and 1.86 mg kg^{-1} ; ranging between 0.117 and 0.440 mg kg^{-1} ; ranging between 0.24 and 1.29 mg kg^{-1} ; with mean value of $651.78 \text{ } \mu\text{g kg}^{-1}$; mean $122 \text{ } \mu\text{g kg}^{-1}$, respectively), in comparison to the results of our study. Similar results for Ni in honey were reported from Turkey ($< \text{LOD} - 9.86 \text{ } \mu\text{g kg}^{-1}$; *Citak et al., 2012*). Nickel is an essential and toxic element for humans, animals, plants and microorganisms. In the nature, this element is found in various forms, and is widely used in metallurgy, chemical and food industries, especially as a catalyst and pigment. Nickel has been studied more as a toxic element. High concentrations of Ni can cause allergies, cancer and non-malignant diseases of the respiratory tract. It has a toxic effect on the immune system. Also, nickel can interfere with DNA repair and lead to the production of free radicals, which causes an increase in the degree of lipid peroxidation and protein degradation (*Bangyuan et al., 2013*).

The data obtained in this research were also compared with the data from our previous research. In this study, a total of 40 samples of multi and polyfloral honey were collected from various localities in Serbia. The concentrations of lead and cadmium were in the range between 0.009 and 3.26 mg Pb kg⁻¹, and < LOD to 0.235 mg Cd kg⁻¹ (Mihaljev *et al.*, 2001). Since the research did not include certain locations, it can be concluded that the concentrations of lead and cadmium examined in this study are generally lower.

Conclusion

In this study, the mean concentrations of elements were measured in 15 sunflower honey samples from various locations in Serbia and they were decreasing in following order: Pb > Ni > As > Cd > Hg. The lead content in all the examined sunflower honey samples was below maximum permissible value. Regarding the fact that sunflower accumulates large amounts of metals in its tissues, the concentrations of toxic elements obtained in sunflower honey in our study are in line with the literature data on the concentrations of these elements in the honey originating from different botanical and geographical areas. In comparison to the data on sunflower honey from other countries, the concentrations of the examined elements in sunflower honey from Serbia are generally lower.

The obtained results are useful for improving the quality of honey production chain. Beekeepers should choose the location of their hives with caution. The procedures applied during the production and processing of honey should be in accordance with hygiene standards. It is very important to monitor the levels of elements in terms of their toxicity and because they can enter through root system or leaf surface of plants and thus access nectar.

Toksični elementi u suncokretovom medu sa različitih lokacija u Republici Srbiji

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Rezime

Cilj ovog istraživanja bio je dobijanje podataka o sadržaju toksičnih elemenata - As, Cd, Hg, Ni i Pb - u petnaest uzoraka suncokretovog meda koji su prikupljeni sa 9 lokacija u Republici Srbiji tokom 2019. godine. Koncentracije ispitivanih

elemenata su su dobijene primenom induktivno kuplovane plazme sa masenom detekcijom (ICP-MS). Srednje vrednosti koncentracija elemenata (mg kg^{-1}) u svim uzorcima suncokretovog meda bile su: 0,004 za As, 0,003 za Cd, 0,077 za Ni i 0,051 za Pb. Sadržaj žive u svim uzorcima meda bio je ispod granice detekcije primenjene metode ($<0,001 \text{ mg Hg kg}^{-1}$ meda). Sadržaj olova u svim ispitivanim uzorcima meda bio je ispod maksimalno dozvoljene vrednosti ($0,10 \text{ mg kg}^{-1}$). Najviši nivoi elemenata bili su (mg kg^{-1}): za Pb 0,096 (Kanjiža), za As 0,025 (Kikinda), za Cd 0,008 (Senta) i za Ni 0,125 (Svrljig). Nijedan od analiziranih toksičnih elemenata nije premašio maksimalno dozvoljeni nivo.

Ključne reči: suncokretov med, toksični elementi

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