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IMPACT OF ENVIRONMENTAL CONDITIONS ON HEAVY METAL CONCENTRATION IN HONEY SAMPLES

H. ALIU[a], S. MAKOLLI[b]*, S. DIZMAN[c], S. KADIRI[d], G. HODOLLI[b]

[a]*Faculty of Agriculture and Veterinary, University of Pristina, 10 000 Pristina, Kosovo*
[b]*University for Business and Technology UBT, 10 000 Pristina, Kosovo*
E-mail: *samimakolli@hotmail.com*
[c]*Department of Physics, Faculty of Arts and Sciences, Recep Tayyip Erdogan University, 53 100 Rize, Turkey*
[d]*AAB College, 10 000 Pristina, Kosovo*

Abstract. The aim of the present study is determination of heavy metals in honey samples with different botanic origin produced by Kosovo farmers and as well as the comparison of gathered results with the specific environmental conditions, with particular emphasis on industrial and agricultural areas where the concentration of heavy metals is expected to be higher than at forester areas. Concentrations of thirty-four heavy metals were investigated in 30 different honey samples but are reported only the most important elements. For this reason, Inductively coupled plasma mass spectrometry (ICP-MS) techniques was used. All metals were detected in 78.49% of the honey samples. Values of pH for tested honey samples show that they are all acidic, with range of pH values from 3.41 to 4.97, with mean value of pH 4.07 ± 0.33. The most abundant element in investigated honey was Mg (44.831 mg kg^{-1}), followed by Al (12.013 mg kg^{-1}), Fe (8.859 mg kg^{-1}), Zn (4.814 mg kg^{-1}), Mn (3.378 mg kg^{-1}), Cr (2.472 mg kg^{-1}), Se (1.599 mg kg^{-1}) and Cu (1.105 mg kg^{-1}), the mean of others elements was less than 1 mg kg^{-1} (Co, Ni, As, U and Cd). In neither of the analysed samples has not been found the presence of any of the lead isotopes: 206, 207 or 208. In general, the heavy metal concentrations in investigated honey samples collected in regions of Kosovo generally fit international standards but the concentration of heavy metals in honey samples is strongly dependent on the environmental conditions.

Keywords: heavy metals, honey, environment, bee.

AIMS AND BACKGROUND

There is a growing public concern over the potential accumulation of heavy metals in environment, especially at industrialised and urbanised areas all around the world. There is acommunication of mass between air, water and soil. So, the food is affected directly by environment where it was produced. Therefore, honey has been analysed in many aspects. It is well known that honey contains chemical components of the plants from which the honeybees collect their pollen and nectar, so the content of elements and their quantity present in honey depend on the type

* For correspondence.

of soil in which the plant and nectar were[1]. According to this paragraph, many researcher proposed to use honey like as environment bio-indicator[2]. Honeybees interact strongly with vegetables, air, soil, and water in the vicinity of the hive and as a consequence, pollutants from these sources are translated to the honeybees and to the hive products[3]. So, heavy metals in honey are strong linked to metal content of soil, water and air where bees collect nectar. Honey is useful for collecting information about the environment within the bees 'collecting area', which is about 7 km^2 (Ref. 4).

Honey is a natural food that is widely used in human daily life. Honey is produced by *Apis mellifera* (Genus name) from flower nectar or honeydew. It is proved that honey has many health benefits for humans[5]. Also, honey is classified like as antioxidant and anti-bacterial[6,7].

The colour of honey varies from nearly colourless to dark brown. The consistency can be fluid, viscous or partly to entirely crystallised. Flavour and aroma vary, and are due to the plant origin. Honey is a characteristically quite acidic item, its pH being between 3.2 and 4.5, which is low enough to be inhibitory to many animal pathogens. Gluconic acid was found to be the principal acid in honey[8].

Nowadays is estimated that there are 56 million bees worldwide and average of 1.2 million tons of honey is produced annually worldwide in one year[9].

Kosovo has an estimated population on the 2011 census population of 1.734 million. Kosovo has a population density of 159 people per km^2. The capital and largest city is Pristina, which has a population of 210 000 (250 people per km^2). People of Kosovo are the Europe fastest growing group with a growth rate of 1.3% per year[10]. Heavy industries were active for decades and some of them are still in use, like mining extracting ore enriched with: Pb, Zn, Ag, Ni, Fe, Bi, Cd, Se, Te, In and Ge (Ref. 11) also coal-fired power plants are in use[12].

The present study aimed evaluating the quality of Kosovo honey in terms of heavy metals as well as the comparison of gathered results with the specific environmental conditions, with particular emphasis on industrial and agricultural areas where the concentration of heavy metals is expected to be higher than at forester areas. It is the first intention on state level to do such analysis. Also pH of samples is analysed, because it has an important link to the growth of bacteria. It is well known that bacteria are generally neutrophils.

EXPERIMENTAL

Samples of honey from 30 apiaries were selected to participate to this survey. The gird sampling pattern method was applied for homogeneous geographically sampling process in the country. So, the territory of Kosovo was divided to 30 equal surface units with rectangular shape, as shown in Fig. 1.



Fig. 1. Kosovo map with sampling sites

One cell represents around 363 km^2 and in each of cell was taken one sample for further analysis. Geographical sample locations were recorded by portable GPS during the sampling time, digital map with sampling location was prepared by MapInfo Professional software version 11.5. Honey samples were collected on 900 g glass jars.

The analysis of heavy metal in honey samples was done using Inductively coupled plasma mass spectrometry (ICP-MS), type Bruker 820 (Karlsruhe, Germany) in high sensitivity mode. ICP-MS was successfully used for multi-elements determinations in a single measurement[3]. The ICP-MS plasma parameters were: plasma flow: 18.00 l/min, auxiliary flow: 1.80 l/min, sheath gas flow: 0.20 l/min, nebulizer flow: 1.01 l/min, sampling depth: 6.50 mm, power: 1.40 kW, pump rate: 4 rpm and stabilisation delay 10 s. Method validation and calibrations for measuring heavy elements in honey samples are described elsewhere[13].

For pH measurements was used Hanna Checker Digital pH detector. Measurements were done at same conditions.

RESULTS AND DISCUSSION

The pH values of all samples are acidic, with range of pH values from 3.41 to 4.97, with mean value of pH 4.07 ± 0.33. The mean value of pH samples by regions are: 4.39, 4.18, 4.08, 4.03, 3.98, 3.93 and 3.91 for Peja, Gjilani, Mitrovica, Gjakova, Prishtina, Prizren and Ferizaj, respectively. All results of pH values of tested honey are within limits (3.0–5.6) established by internationals organisations[14,15]. pH values detected in honey of Kosovo are presented by regions. Similar pH values of honey samples are reported by national[16] and international researchers[17,18]. pH is an useful index of possible microbial growth. Acidophiles bacteria grow optimally at a pH

near 3.0. Most bacteria grow in a neutral and mildly alkaline environment, while yeasts and moulds are capable of growth in acidic medium (pH = 4.0–4.5) and do not grow well in alkaline media[19]. So, only acidophiles bacteria can be found on tested honey samples and most of them can be thrive usually below pH 2.0. It can be topic of another research.

Results of 14 elements from honey samples are presented in Table 1. It shows that the concentrations of heavy metals have a wide range of results for the different honey samples.

Five minerals groups can be differentiated based on concentration of minerals on honey samples: very abundant elements, abundant elements, medium concentration elements, trace elements and elements not detected.

Table 1. Heavy metal concentration in honey samples of Kosovo

	Element	Mean (mg kg^{-1})	(Min – Max) (mg kg^{-1})	Standard deviation	Element presented on samples (%)
Very abundant elements	Mg-24	44.831	(15.703–137.955)	26.494	100
	Al-27	12.013	(1.289–103.032)	18.438	100
	Fe-57	8.859	(0.630–23.427)	4.932	100
Abundant element	Zn-66	4.814	(1.021–18.047)	3.064	100
	Mn-55	3.378	(0.401–33.298)	6.339	100
	Cr-52	2.472	(0.374–6.087)	1.236	100
	Se-78	1.599	(0.325–2.147)	0.509	100
	Cu-65	1.105	(0.337–1.977)	0.408	100
Medium elements	Co-59	0.811	(0.007–1.151)	0.341	100
	Ni-60	0.651	(0.305–1.442)	0.271	100
	As-75	0.477	(< DL–5.146)	1.212	20
Trace element	U-238	0.062	(0.009–0.172)	0.032	100
	Cd-114	0.049	(< DL–0.237)	0.059	97.7
Not detected	Pb-206	< DL	(< DL – 0 < DL)	< DL	0

< DL – less than Detection limit.

The first group consists of Mg, Al and Fe. Quantitatively the most important is Mg and highest concentration of Mg is detected in honey sample from Pristina region, with average 61.591 mg kg^{-1}, followed by other regions and the cleanest honey samples from Mg belongs to Mitrovica region with 26.643 mg kg^{-1}. The most polluted region with Al in honey samples again was the Pristina region, with an average value of 24.010 mg kg^{-1} and the cleanest resulted Ferizaj with 3.903 mg kg^{-1}. In case of Fe, the highest content was detected in honeys from Peja region with 14.0.13 mg kg^{-1}, followed by Pristina, Mitrovica, Gjilan, Gjakova, Prizren

and the cleanest honey samples from Fe resulted Ferizaj region with mean value 4.541 mg kg^{-1}.

The second mineral group, named abundant elements is composed by Zn, Mn, Cr, Se, and Cu with mean of 4.814, 3.378, 2.472, 1.599 and 1.105 3.195 mg kg^{-1}, respectively. The highest concentration of elements is detected in regions: Mitrovica (Zn – 7.4477 g kg^{-1}), Peja (Mn – 9.534 mg kg^{-1}), Gjilan (Cr – 3.641 mg kg^{-1}), Prishtina (Se – 1.885 mg kg^{-1} and with Cu – 1.358 mg kg^{-1}).

In Fig. 2 are presented all results collected for very abundant and abundant groups. There are presented also standard deviations and mean of each group. Ordinate is presented in logarithmic scale.

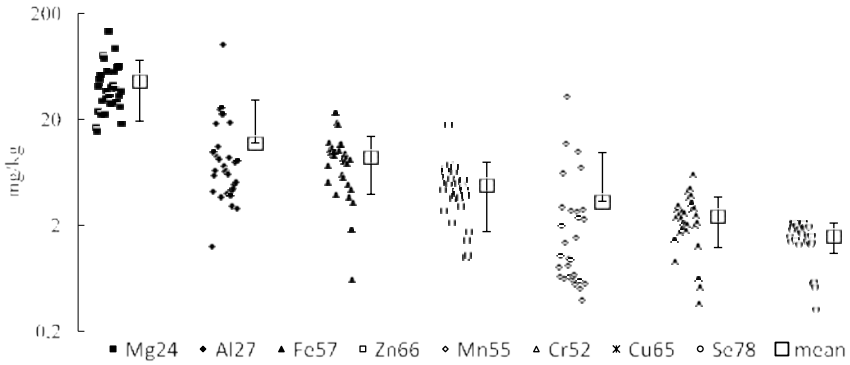

Fig. 2. Results of heavy elements grouped as abundant and very abundant elements

The third mineral group, named medium elements, is composed by: Co, Ni, and As, where they ranged from 0.007–1.151, 0.305–1.442 and 0.0–5.146 mg kg^{-1}, respectively. From tested honey samples, only 20% resulted with As element. Fourth element group, named tracer elements, is composed by U and Cd, where they ranged within 0.009–0.172 and 0.000–0.237 mg kg^{-1}, respectively. Uranium 238 was present in 100% of samples and it is interesting due to the past of Kosovo when Depleted uranium (DU) where used for military reasons[20]. There are no data available for U238 concentration in honey samples from different countries but in another study should be determined the concentrations of DU in honey. Cd was detected in 97.7 % of all samples. The fifth group is composed only by Pb element. Pb is not detected at any honey samples, there were measured Pb isotopes: Pb-206, Pb-207 and Pb-208. Typical detection limits for our ICP-MS (Bruker–MS) is 0.3 ng/l. In Fig. 3 are presented all results collected for medium and trace element groups. Also, there are presented standard deviations and the means of each heavy metal of this group. The ordinate is presented in logarithmic scale.

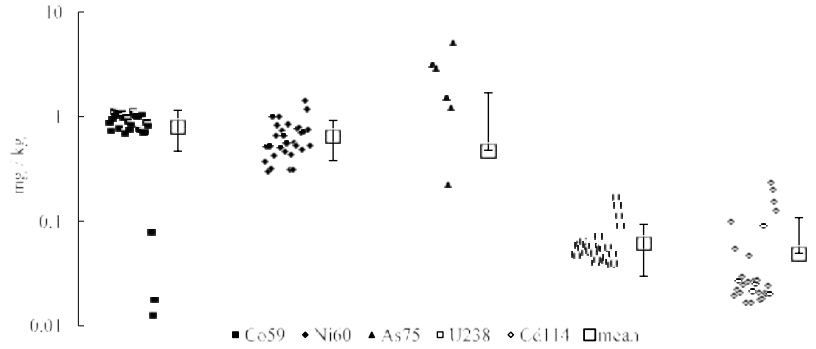

Fig. 3. Results of heavy elements grouped as medium and trace elements

Similar results are presented in other papers. At country level there are available results only for Cu, Pb and Zn (Ref. 16). Similar results were reported by Atiken et al.[21] and another publication for heavy metals in honey samples from south and east of Turkey shows Fe as the most abundant element followed by Al, Cu, Zn and the others[22]. It is proven by Crane[23] that dark honey exhibited twice as much as elements concentrations than light honey.

There is no possibility to conclude which region of Kosovo has more polluted honey with heavy metals than others, because some elements are more abundant in one region and other elements are more abundant in another region. It is due to the botanic origin of honey, as Fernández-Torres[24] stated that Zn, Mn and Mg concentrations are strongly dependent on the kind of botanical origin.

CONCLUSIONS

There are not official data available yet for the average consumption of honey by people at country level. So, mean consumption of 400 g for person for year of honey can be calculated. A rough estimation of the average intake of minerals from food item for the Kosovo population can be drawn. The values of minerals intake from honeys are somehow quite low. We can speculate that honey does not contribute for a significant proportion of minerals recommended dietary allowances, honey can contribute from 0.01 to 0.05% of the recommended daily requirements of K, Mg, Cu, Fe, Mn and Zn (Ref. 25).

The most abundant element in Kosovo honey was Mg (44.831 mg kg^{-1}), followed by Al (12.013 mg kg^{-1}), Fe (8.859 mg kg^{-1}), Zn (4.814 mg kg^{-1}), Mn (3.378 mg kg^{-1}), Cr (2.472 mg kg^{-1}), Se (1.599 mg kg^{-1}) and Cu (1.105 mg kg^{-1}), the mean of others elements were less than 1 mg kg^{-1} (Co, Ni, As, U and Cd). We could not detected Pb in any honey sample.

In general, the heavy metal concentrations in investigated honey samples collected in regions of Kosovo generally fit international standards. The levels of

analysed metals in honeys samples are in safety baseline levels for human consumption. In further research for heavy metals in Kosovo honey should be paid attention also to DU concentration.

About pH values of tested honey samples, can be concluded that they are all acidic, with range of pH values from 3.41 to 4.97, with mean value of pH 4.07 ± 0.33. So, only acidophiles bacteria can be found on tested honey samples.

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