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The nickel content in honey derived from serpentine and non-serpentine areas of Kosovo

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Abstract-

The purpose of this paper is to find out the nickel level in the serpentine multi-floral-honey obtained from the honeybeeswith higher nickel concentration than the non-serpentine flora. The average value of Ni concentration in the aerial part of examined flora samples comprised of 10 different plant species resulted to be 336mgkg^{-1}. The honey was collected from 1 apiary three years in the row,which is situated in serpentine area of south-eastern part of Kosovo-Rezhance and from 3 other apiaries from non-serpentinearea. Obtained results have revealed higher concentration of nickel in the serpentine multi-floralhoneythan those in the non-serpentine honey.

The average Ni level in the serpentine multi-floral honey was 3.71mg.kg^{-1}, with the highest concentration of 3.96mgkg^{-1}. Whereas the average levels of Ni in the non-serpentine flora was1.66 mg.kg^{-1}.with the highest reading of1.92 mgkg^{-1}.

In conclusion, the entire amount of nickel in the honey comes either from nectar collected by honey bees in the Ni accumulative and hyper-accumulative serpentine flora or by dust coming from serpentine soils, which is abundant on Ni content.

Keywords: Honey, Ni, Serpentine flora, non-serpentine flora, beehive

Introduction

Honey is the natural sweet substance produced by honey bees 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.

Honey consists essentially of different sugars, predominantly fructose and glucose as well as other substances such as organic acids, enzymes and solid particles derived from honey collection

Honeybees visit flowers of diverse plant species collecting nectar and pollen grains producing honey. Pollen is the bee's major source of protein, minerals, and vitamins, while nectar is the major source of carbohydrates from which honeybees obtain their energy [3].

Honey shall be free from heavy metals in amounts which may represent a hazard to human health. The products covered by this standard shall comply with those maximum levels for heavy metals established by the Codex Alimentarius Commission.

Also, some essential elements (P, Fe, Al, Mg, Cu, Mn, Si, Ca, K, and Na) naturally distributed in the soil are included in the nectar transported via plant's root system [22]. A lot of data demonstrated the localization of metals in pollen grains as well [6–28].

Honey may contain metals of which some may be beneficial or injurious if consumed. Some essential metals are involved in numerous biochemical processes and adequate intake of certain essential metals relates to the prevention of deficiency diseases. The essential metals may become toxic when the metal intake is excessively elevated [18]. Heavy

metals such as lead (Pb) are toxic even at trace levels [12].

Bee honey can be a good source of major and trace elements needed by humans, where it contains metals up to 0.17%. Metals such as Cr, Co, Cu, Fe, Mn and Zn are essential for humans, and they may play an important role in a number of biochemical processes [13, 17]. Some of them are present at the trace level and may become toxic if they exceed safe limits [8].

Nickel produces more cases of allergic contact dermatitis than all other metals together.Therefore, a good knowledge of the presence of nickel in food is helpful for the management of nickel allergy.

The presence of sufficient amounts of nickel in the diet of a nickel-sensitive person can provoke dermatitis. It has been observed that nickel sulphate when orally administered in the range of 600-5,600 mg as a single dose may provoke hand eczema. [14] The hands are the most commonly affected sites for systemic nickel dermatitis. However, other body areas may be affected as well.

Materials and Methods

The serpentine region where the honey was collected lies in the south-eastern part of Kosovo with geographical latitude and longitude of $42^0$8.7426'N; 21015.54696'E and altitude of 650 m above the sea level with Rezhance being the closest settlement to this location. This serpentine region covers an area of 215 hectares containing several kinds of low herbal plants known to accumulate or hyper-accumulate nickel, serving also as nectar–plants for the honeybees.

Initially sampling was conducted in the end of May 2014 by digging out a soil profile and taking the soil samples and collecting the entire flora present therein at the time of sampling. These samples were then dried at the room temperature, ground to 2 mm size and sent to the lab for determination of heavy metals. Since the results showed the high presence of nickel in both soil and plants, in September 2014 we carried out with the sampling of honey from the hives which were in the vicinity of this area.

For three consecutive years, in 2014, 2015 and 2016 the honey samples were taken from the same apiary consisted of 10 hives to determine the level of Ni in honey produced by bees from nectar collectioninserpentine multi-floral location. According to the beekeeper he has a total of 10 hives and honey is harvested only once a year, in August, so the taken sample was very representative and was considered as a composite sample. The hill, which is located just 200 m (Fig.1) from the apiary, is covered with Alyssum murale which is known as a hyper-accumulator plant of Ni and can be attractive to honeybees to collect the nectar from it as well. In addition, three samples were collected in other non-serpentine regions to compare the results ofNi found in honey coming from both serpentine and non-serpentine flora.

The samples were taken in the plastic containerand sent to the lab for determination of their Ni quantities. Objective of this paper was to determine: 1) The Ni quantity in these serpentine plants and 2) the possibility of the Ni entry into the food chain through the honey.

Sample Analyses

Ash contents were determined by heating 10 g of honey precedence 100 °C to moisture amount decrease, after 500 °C to constant weight dry up with an infra-red lamp to prevent foaming (AOAC, 1984). Nickel (Ni) was measured using Perkin Elmer 3110 Atomic Absorption Spectrophotometer (AAS). Solution containing Ni ions was obtained by dissolution of ash in 10 ml percholic acid (60%) and nitric acid (65%) (Merck Darmstadt, Germany). The weight of the ash of the honey samples were measured and filtered by Whatman No 41 filter paper. Cd, Cu, Mn, Fe, Mg and Ni were determined directly in the ash solution by atomic absorption spectrometer (Perkin Elmer 3110 AAS). The instrument response was periodically checked with known standards. An airacetylene flame and hollow cathode lamp were

used for all samples. Calibration curves were prepared using dilutions of stock solutions. The wavelengths of 232.0 nm were used for the nickel reading.

Figure 1. Orthophoto of apiary and serpentine hill of Rezhance

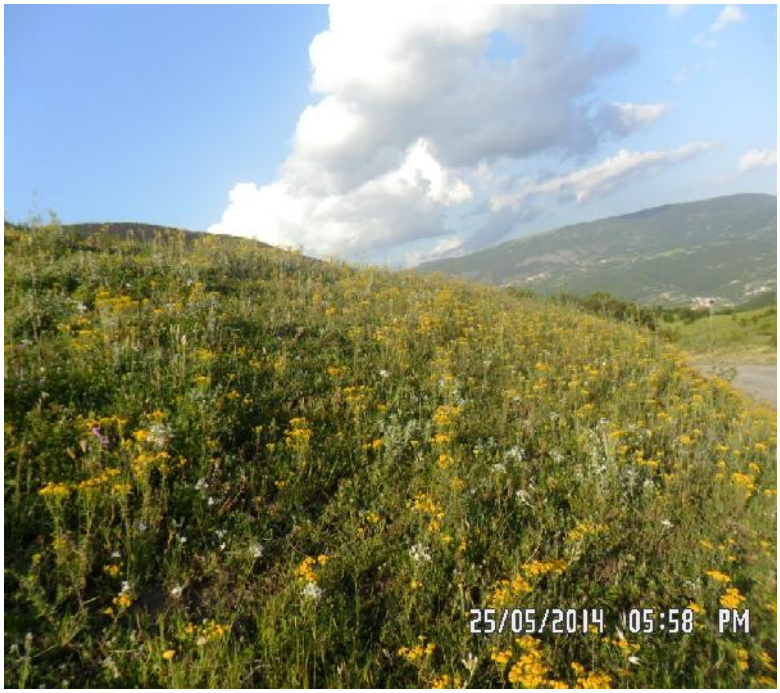

Figure 2. *Alyssum murale* in flowering stage in serpentine hill of Rezhance

Results and Discussions

From the table 1 is obviously seen that the level of Nickel in Rezhance is typically of serpentine environment, which means that this area is naturally occurring contaminated withNi and other heavy metals. The average amount of Ni in all three horizons was 1744 mgkg^{-1}, and might have adverse effect on entire environmental pollution.

As shown by the table 2, the plant *Alyssum murale,* which is known as hyper-accumulative plant of Ni, had the highest content of nickel reaching an amount of 3066 mgkg^{-1}, while the *Allium Cupani* had the lowest Ni content of 6.50 mg kg^{-1}. The average Ni content found in aerial part of 10 species of plants collected from this area amounts to336mgkg^{-1}.

The table 3 shows the Ni concentration in the honey taken in 6 samples in four sampling locations. As it can be seen from the table, the Ni content found in the honey exceeding for twice at least the amount of Ni found in the honey of non-serpentine flora. This Ni content varies in its amount from 3.36 to 3.92 mg kg^{-1}, whereas the average amount of Ni in those three samples from serpentine flora reaches a value of 3.71 mg kg^{-1}.

The table 3 also gives the Ni values in the three honey samples collected from the non-serpentine flora, whose findings indicate that the Ni level in these samples is considerably lower that the level found in the samples from the serpentine flora. These values vary from1.36 to1.92 mgkg^{-1}, with an average of 1.66 mg kg^{-1}, which is generally similar with findings from Poland [16]. But those are higher than outcomes found in Eastern Rhodopes mountain of Bulgaria [3].

The data were tested by t-paired test and the results were; t=12.2 in p<0.01, showing that the difference between Ni content in bee's honey located in serpentine and non serpentine areas is statistically significant.

The high Ni content is worrying because large quantity of Ni in the body poses a health threat. The Nickel level in the honey is so far higher compared to other foods documented. Nickel levels in food are generally in the range 0.01-0.1 mg/kg, but there are large variations [4, 9, 15, and 20]. Markedly higher levels had been reported in beans, seeds, nuts and wheatbran [31, 20] and in cacao [31]. The high content of nickel in the honey coming from serpentine regions could be from the hyperaccumulating plant sources which the pollen was taken from, or could be also as a result of dust coming from naturally metalliferous soils such as serpentine.

Table 1.Nickel content in soil of serpentine site of Rezhance

Site # RezhanceNi mgkg^{-1}	
Rezhance- Horizon A	*3298*
Rezhance- HorizonB	1007
Rezhance–HorizonC	926

Metal contamination of honey could be a result of different human activities such as agricultural practice, industries, waste dump, and traffic [26, 27]. Serpentines and their soils are characterized by toxic quantities of metals (particularly Ni and Cr), low Ca/Mg ratio, drought, and wide temperature fluctuations [7].Recently, the interest in the concentrations of metals in different honeys and their presence has been determined in several countries such as Turkey [2, 33], Italy [26], Poland [19], Bosnia and Hercegovina [25], Serbia [21], Greece [11, 30], Romania [5], and Iran [1]. However, little attention was paid on serpentines oils as source of contamination of honey [29]. In addition, still little is known about metals in bee pollen [32, 34] although the concentrations of metals there are always higher than in honey [31].

Table 2. Heavy metals content in flora of serpentine site of Rezhance

	Site # Rezhance								
No.	Species	**Zn**	**Cd**	**Mn**	**Ni**	**Cu**	**Pb**	**Mo**	**Cr**	**Co**
		$mgkg^{-1}$	$mgkg^{-1}$	$mgkg^{-1}$	$mgkg^{-1}$	$mgkg^{-1}$	$mgkg^{-1}$	$mgkg^{-1}$	$mgkg^{-1}$	$mgkg^{-1}$
1	*Alyssum murale*	35.1	< 1	42.8	**3066**	3.3	3.8	< 1	2.9	5.5
2	*Onopordonacanthium*	32.6	< 1	46.8	**41.5**	8.9	5.8	< 1	67.7	2.2
3	*Achilleamillefolium*	26.4	< 1	25.2	**10.7**	11	4.3	< 1	6.1	< 1
4	*Allium Cupani*	14.6	< 1	9.8	**6.5**	2.5	2.2	< 1	4.7	< 1
5	*Thymus pulegioides*	16.9	< 1	35.6	**28.2**	5.4	3.5	< 1	29.3	1.2
6	*Chamomillarecutita*	30.3	< 1	36.4	**19.0**	9.9	4.5	8.5	17.0	1.3
7	*MinuartiaHybrida*	18.6	< 1	61.9	**63.1**	7.9	4.7	< 1	49.0	3.1
8	*Dentariapentaphyllos*	54.1	< 1	39.4	**29.0**	8.4	4.8	16.7	22.3	< 1
9	*Anchusaofficinalis*	12.3	< 1	66.4	**33.5**	6.4	5.8	< 1	42.0	< 1
10	*Cicerbitamuralis*	14.7	< 1	76.5	**60.6**	8.7	5.0	1.3	58.3	4.1

Table 3.Ni content in honey in serpentine and non-serpentine flora

Sample	Ni in honey from serpentine flora ($mg.kg^{-1}$)	Ni in honey from non-serpentine flora ($mg.kg^{-1}$)
Sample # 1	3.84	1.92
Sample # 2	3.37	1.71
Sample # 3	3.92	1.36
Average	**3.71**	**1.66**

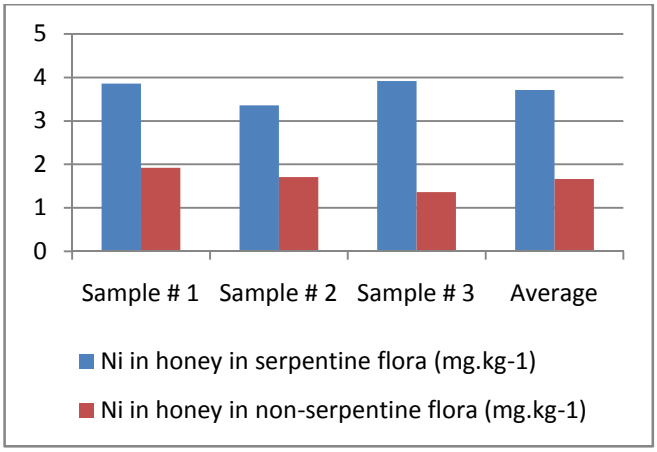

Figure 3. Difference of Ni concentration in honey in serpentine and non-serpentine flora

Conclusions

Apparently there is a difference on Nickel content between serpentine and non-serpentine honey. In conclusion, the entire amount of nickel in the honey comes either from nectar collected by honeybees in the Ni accumulative and hyper-accumulative serpentine flora or by elevated Ni content in serpentine soils of Rezhance as a result of dust generated by wind blowing.

There is no other source of Nickel that could have a direct impact on the Ni amount found in the honey samples since there is no other source of Ni in the area or its vicinity coming from a potential smelter or mine.

The beekeepers should be informed about possible negative effect of naturally metalliferous soils on the quality of honey and bee pollen and should pay attention to the environmental characteristics of the locality where they place beehives. A strict control on bee pollen used for medication is recommended in spite of further investigations in this aspect [3].

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