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# Characteristics of Honey from Serpentine Area in the Eastern Rhodopes Mt., Bulgaria

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**Abstract** Honey samples collected during 2007–2010 from serpentine and non-serpentine localities in the Eastern Rhodopes Mt. (Bulgaria) were characterized on the basis of their pollen content by qualitative melissopalynological analysis and physicochemical composition. Water content, pH, electrical conductivity, macroelements—K, Ca, Mg, P, and microelements—As, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb, and Zn were determined after the Harmonised Methods of the International Honey Commission and ICP-AES method. The results from serpentine honey samples were compared with data from bee pollen collected from the same serpentine area. Different elements have different concentrations in honey from the same botanical type even collected from the same geographical region, same locality, and same beehive but in different vegetation season. The elements Mg, Mn, Ni, and P contribute mostly for separation of the serpentine honey samples based on measured elemental concentrations and performed principal component analysis. The element concentrations were higher in bee pollen and above the permissible limits for the toxic metals Cd and Pb. No specific indicator plant species was found for identification of the geographical origin of serpentine honey in relation to the forage of bees.

**Keywords** Honey · Chemical elements · Pollen · Serpentine

Lilyana Yurukova has passed away.

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## Introduction

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. Honey contains several other compounds in traces like proteins and enzymes, amino acids, pigments, substances responsible for its flavor and aroma [1]. 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 [2]. A lot of data demonstrated the localization of metals in pollen grains as well [3–6].

Being a local product, the quality of honey (physical and chemical characteristics) is strongly influenced by its botanical and geographical origin and many environmental factors [7]. Different criteria are accepted for the standardization of honey and bee products at international and national levels [8–10]. An important aspect of the quality of honey is the presence of metals which is directly related to the chemical composition of the soils in areas where bees forage [1]. Honey and bee pollen may be useful as an environmental indicator for assessing the presence of environmental pollution with toxic metals [7, 11]. Metal contamination of honey could be a result of different human activities such as agricultural practice, industries, waste dump, and traffic [1, 12]. Metal contamination of honey could be also a result of dust coming from naturally metalliferous soils like serpentine. Serpentines and their soils are characterized by toxic quantities of metals (particularly Ni and Cr), low Ca/Mg ratio, drought, and wide temperature fluctuations [13]. Such soils could be environmental pollutant for honey and bee products even when they are produced far from urbanized territories. Some plants restricted to the serpentines have a remarkable ability to accumulate or hyperaccumulate inorganic components from the environment and transport them to all plant parts. This phenomenon is known in approximately 400 species worldwide in a range of different plant families [14] and in the Balkan peninsula they are mainly representatives of Brassicaceae family [15]. Some elements such as Cu, Fe, Zn, and Ni are mineral nutrients for plants [13] but in concentrations above tolerance limits they become environmental pollutants and phytotoxic. Even at low concentrations the trace elements As, Pb, and Cd are considered as toxic [16, 17] and can damage the quality of human life [18].

Recently, the interest in the concentrations of metals in different honeys arose and their presence has been determined in several countries such as Turkey [19–21], Italy [1], Poland [22], Bosnia and Hercegovina [23], Serbia [24], Greece [25, 26], Romania [27], and Iran [28]. However, little attention was paid on serpentine soils as a source of contamination of honey [29]. In addition, still little is known about metals in bee pollen [30, 31] although the concentrations of metals there are always higher than in honey [32]. Apart from this fact, the monitoring on honey safety is very important. There are no global standards for permissible levels of chemical elements in honey and bee products. The maximal permissible levels only for Cd and Pb (0.1 and 1.0, mg $kg^{-1}$, respectively) are fixed but not for honey [8, 9, 33, 34]. Actually no specific legislation exists on maximal residual levels of metals in honey in Bulgaria [10]. The levels of metals accepted for other food products are recommended for honey as well [32]. The accepted standards for honey quality in Bulgaria are regulated by BDS 3050–80 "Bee honey. Rules for sample collection and methods of treatment", BDS 2673–89 "Bee honey" [35].

The aims of this study were the following: (1) evaluate the effect of soil on the quality of honey produced from serpentine area in the Eastern Rhodopes Mt.; (2) compare element concentrations in honey samples from serpentine and non-serpentine areas; (3) compare the differences between the elemental concentrations in honey and bee pollen samples from serpentines; and (4) discuss the possibilities for identification of the botanical origin of serpentine honey on the basis of pollen spectra.

## Material and Methods

### Area of Investigation

Ten honey samples were obtained directly from beekeepers from two sites in the Eastern Rhodope Mt.: serpentine area near Fetler village (41°21′53.19″ N, 25°18′50.42″ E) and non-serpentine area southeastward from Gruevo village, Kardzali region, Dambala locality (41°33′53.06″N, 25°27′23.88″E). The beehives were localized at altitudes 455 and 763 m, respectively. Two bee pollen samples collected from the same serpentine area were also analyzed and compared with the data from honey samples. The climate in the Eastern Rhodope Mt. is characterized by comparatively mild winters with a characteristic southern wind and relatively cool summers [36].

The natural vegetation at both sites is of southern-European and anatolian character represented mainly by *Quercus frainetto* Ten., *Q. pubescens* Willd., and *Q. cerris* L. These species form forests on slopes with south-southeastern exposure not far from the placement of the beehives. The most common accompanying plant species in these oak forests are *Prunus mahaleb* L., *Viburnum lantana* L., *Pistacia terebinthus* L., *Acer monspessulanum* L., and *Euonymus verrucosus* Scop. On some north-facing slopes and in the humid ravines are developed plant communities of *Carpinus betulus* L. and *Carpinus orientalis* Mill. Presently, the open serpentine areas are protected partly from erosion by artificial plantations of *Pinus nigra* Arn. and *Robinia pseudoacacia* L. For the restoration of the plant cover after deforestation in the past, the pioneer role of *Rubus*, *Crataegus*, *Rosa*, *Prunus*, *Pyrus*, *Cistus*, and *Pistacia* species is of great importance together with grasses from *Festuca*, *Dichantium*, *Koeleria*, *Chrysopogon*, and *Poa* genera that form a dense herb cover and favor the growth of other plants like *Trifolium*, *Lotus*, *Dorycnium*, *Coronilla*, etc.

All samples studied were examined for their botanical origin, physicochemical, and elemental properties. The origin, organoleptic characteristics, physicochemical parameters, number of honey and bee pollen samples, and date of collection are presented in Table 1.

### Melissopalynolgical Analysis

In order to obtain more detailed analysis about pollen grains used from bees as forage, honey samples were collected during the years 2007–2010. The method described by Louveaux et al. [37] was followed in the laboratory preparations and qualitative melissopalynological analysis of honey samples. The number of pollen grains from nectar-producing plants counted in each honey sample was 500. Pollen identification was carried out with a light microscope at ×400. For comparison, the reference collection and the book of Beug [38] were used. All honeydew elements (HDE) such as mold hyphae and spores, and unicellular algae were also counted in each honey sample.

The frequency of the pollen types in honey was expressed as percentage of the pollen sum which included pollen grains only from nectar-producing plants (Pn). The pollen grains of wind-pollinated and nectarless plants (Pa) were calculated separately. The honeydew index was calculated as a ratio of HDE to Pn [37].

Two mixed bee pollen samples each weighing 0.05 g were analyzed. All pollen grains were included in the pollen sum for calculation of the percentage values.

**Table 1** Honey and bee pollen samples studied: origin, date of collection, organoleptic characteristics, and physicochemical parameters

| Sample no. | Site locality | Color | Physical state | Water content (%) | pH | Electrical conductivity (mS/cm) | Date of collection |
|---|---|---|---|---|---|---|---|
| Honey from serpentine site (S) | | | | | | | |
| 1 | Fetler village | Light yellow | Liquid | 19.0 | 3.29 | 0.237 | 10 May 2008 |
| 2 | | Light yellow | Liquid | 19.0 | 3.29 | 0.257 | 26 May 2008 |
| 3 | | Amber | Liquid | 16.0 | 3.90 | 0.760 | 30 June 2008 |
| 4 | | Creamy white | Fine crystallized | 18.5 | 3.33 | 0.199 | 26 May 2009 |
| 5 | | Yellow | Liquid | 17.5 | 3.71 | 0.359 | 05 June 2009 |
| 6 | | Light yellow | Liquid | 17.2 | 3.47 | 0.186 | 26 May 2010 |
| 7 | | Light yellow | Liquid | 17.5 | 3.41 | 0.179 | 05 June 2010 |
| Honey from non-serpentine site (NS) | | | | | | | |
| 8 | Gruevo village Kardzali–Dambala | Amber | Liquid | 18 | 4.19 | 0.933 | 20 May 2007 |
| 9 | | Light amber | Liquid | 15 | 4.12 | 0.822 | 05 June 2007 |
| 10 | | Light yellow | Liquid | 16.1 | 3.61 | 0.606 | 30 June 2007 |
| Bee pollen from serpentine site (S) | | | | | | | |
| 1p | Fetler village | Golden rod | | | | | 26 May 2009 |
| 2p | | Yellow | | | | | 05 June 2010 |

### Physicochemical Analysis

The routine physicochemical analysis included water content (honey refractometer Atago HHR-2 N 12–30 %; Atago Co., Ltd., Tokyo, Japan), EC (mS/cm, ±1 %) in 20 % solution at 20 °C (MultiLine P3; WTW, Weilheim, Germany), and pH (20 % solution, ±0.01, Jenway pH-meter; Bibby Scientific Ltd., Staffordshire, UK). The Harmonised Methods of the International Honey Commission [39] do not require any method for identification of chemical elements in honey. About 10 g of material was treated with 15 ml nitric acid (9.67 M) overnight. The wet-ashing was continued with heating in a water bath followed by the addition of 2 ml hydrogen peroxide. This treatment was repeated until reaching full digestion. The filtrate (through filter paper Filpap KA 2; Filpap, Štětí, Czech Republic) was diluted with double-distilled water (0.06 μS/cm) up to 25 ml. All solutions were stored in plastic flasks. The macroelements K, Ca, Mg, and P and the microelements As, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb, and Zn were determined by atomic emission spectrometry with the inductively coupled plasma system (ICP-AES) of VARIAN VISTA-PRO. The analytical precision was checked by three replications and blanks and by stock standard solutions (1000 μg/l Merck) for the preparation of working aqueous solutions.

### Data Analysis

Basic descriptive statistics, Tukey's test for independence of groups, and one-way ANOVA, were used to determine the differences between the serpentine and non-serpentine honey samples on the basis of element concentrations measured. The results were considered significant at $P<0.05$. Principal component analysis (PCA) was used to show the loadings for each chemical element and which of them contributed most for differences between two groups of samples (serpentine and non-serpentine). Eigenvalues were extracted from the correlation matrix. All statistics were performed using software program StatSoft (StatSoft. Inc. Tulsa, OK, USA)—Statistica 7.

## Results

### Melissopalynological Data

According to the palynological results, all honey samples were classified as multifloral blossom honeys [35, 37]. The honeydew index was very low in all samples studied (0.1–0.6) wide below the limit of 3 for honeydew honey [37]. The highest percentage values for *Onosma* (28.9 %), *Potentilla/Fragaria*-type (27.7 %), and *Cynoglossum* (26.5 %) (samples 6, 7, and 5, respectively) for the serpentine honey samples were found. A total of 64 pollen taxa were identified in the analyzed honey samples, 50 of them from nectar-producing plants. The results of the qualitative pollen analysis indicated the diversity of plant resources utilized by honeybees in the region of investigation. The basic botanical sources for honey production were species from the families Boraginaceae (*Onosma, Cynoglossum, Lithospermum*), Fabaceae (*Trifolium, Dorycnium, Medicago, Robinia, Genista,* etc.), Rosaceae (*Prunus*-type, *Rubus*-type, *Potentilla/Fragaria* type), Brassicaceae, etc. (Table 2).

For the honey from the non-serpentine area, the highest percentage values for *Potentilla/Fragaria*-type (11.2 %), *Trifolium* (27.7 %), and *Vicia* (16.5 %) were found. Important for honey production were also species from *Lotus*, *Genista*, *Tilia*, *Prunus*, *Paliurus*, Brassicaceae, etc. (Table 2).

The botanical origin of bee pollen from the serpentine area was also identified. The basic pollen sources for the bees were nectar and pollen producers such as *Rubus*, *Potentilla*, *Fragaria*, *Prunus*, *Paliurus*, Brassicaceae and nectarless plants as *Plantago*, *Sambucus*, and *Cistus*.

## Organoleptic and Physicochemical Characteristics of the Samples

The main organoleptic and physicochemical characteristics of the honey samples studied are presented in Table 1. The water content in all honey samples was within the range of 16.0 to 19.0 % which is below 20 %, a value considered as limit for good quality of honey according to the Bulgarian State Standards [10]. The values for pH and the electrical conductivity were also in range considered as normal according to the Bulgarian legislation [10]. The pH values measured in the samples were within the range of 3.29–4.19. The electrical conductivity varied in the range from 0.179 to 0.933 mS/cm.

## Element Concentrations of the Samples

The elements analyzed in honeys and bee pollen are listed in Table 3 and the comparison between serpentine and non-serpentine honeys is presented in Table 4. The relative concentrations of the studied elements tested in the serpentine and non-serpentine honey samples varied considerably. Variation of the element concentrations was found even in honey samples collected from the same locality but in different periods of the year. The mean concentrations of evaluated elements decreased in the following order:

$$K > P > Ca > Mg > Na > Fe > Mn > Zn > Ni > Pb > Cu > As > Cr = Co = Cd \text{ for serpentine and}$$

$$K > Ca > P > Na > Mg > Fe > Zn > Mn > Cu > Pb > Ni = Cr = Co = Cd > As \text{ for non-serpentine samples.}$$

Normally the elements K, Ca, Mg, Na, and P in honeys have higher concentrations. The highest values were measured for K in all analyzed samples while most concentrations of P were higher in serpentine honeys. Differences were found between both groups of samples in relation to Ca and Mg. The mean Ca concentrations in serpentine and non-serpentine honey samples were 41.71 mg $kg^{-1}$ and 76.33 mg $kg^{-1}$ respectively, while those for Mg were 20.71 mg $kg^{-1}$ and 15.31 mg $kg^{-1}$. The highest concentration of Mg (48 mg $kg^{-1}$) was measured in serpentine honey sample No. 3 in which the highest concentrations for K, P, and Mn were also found.

The concentrations of Fe in the analyzed honeys vary considerably. The mean Fe concentrations for non-serpentine samples were higher compared to serpentine honeys. The highest measured concentration was 19.25 mg $kg^{-1}$. The concentrations for Ni and Mn, elements with higher values in serpentine soils [13], were higher in most samples collected from the serpentine area—1.2 mg $kg^{-1}$ and 4.7 mg $kg^{-1}$, respectively.

Very low concentrations were measured for Cr, Co, Cd, and As in both types of honey samples. The concentrations of Cu, Cr, Co, and Cd were more or less equal in the serpentine and non-serpentine samples while those for As in the serpentine samples were more variable reaching a maximal value of 0.63 mg $kg^{-1}$.

The metals Zn and Pb demonstrated different levels in both groups of honey samples. The mean concentration of Zn in the non-serpentine honeys was twice higher compared to the serpentine honeys. Although serpentine soils are not rich in Pb, the concentrations found in the serpentine honey samples are higher compared to the non-serpentine samples with mean values of 0.21 and 0.08 mg $kg^{-1}$, respectively.

In addition, the elemental concentrations for two bee pollen samples collected from the serpentine area (Table 3) were analyzed and compared with the serpentine honey samples. The results showed higher concentration for all elements in bee pollen. The order of mean element concentrations of bee pollen is:

$$K > P > Ca > Mg > Na > Fe > Zn > Mn > Cu > Pb > Ni > As > Cr > Co > Cd.$$

## Principal Component Analysis

The relations between the two groups of honey samples (serpentine and non-serpentine) based on measured elemental concentrations were performed using the principal component analysis (PCA). The ordination of the dataset accounts for 78.5 % of the total variance with the first three principal component (PC) factors; F1, F2, and F3 account for 35.33, 25.17, and 18 %, respectively. Eigenvalues were extracted from the correlation matrix. Percentage eigenvalues and factor loadings on the axes are reported in Table 5. The elements Mg, Mn, Ni, and P are separated from other elements because of their positive correlations with both principal components and mostly contribute for separation of the serpentine honey samples (Fig. 1). These honeys are generally characterized by higher concentrations of Mg, Mn, Ni, and P. The same elements have clear

**Table 2** Percentage of each pollen taxa in honey and bee pollen samples

| Pollen taxa | Sample no. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1p | 2p |
| Nectar producing (Pn) | | | | | | | | | | | | |
| *Prunus* type | 12.4 | 7.4 | | 7.7 | 1.6 | 12.2 | 1.8 | 5.1 | 1.1 | 5.5 | 11.5 | |
| *Rubus* type | 0.5 | 1.3 | | 0.5 | | 20.8 | 3.4 | | | | 31.7 | |
| *Potentilla/Fragaria* type | 15.5 | 13.1 | | 6.4 | 1.6 | 2.0 | 27.7 | 11.2 | 1.1 | 5.4 | 20.7 | 1.4 |
| Rosaceae | 7.6 | 12.0 | 5.7 | | 4.5 | 4.2 | 10.7 | | | | | |
| Brassicaceae | 5.5 | 2.0 | 3.7 | 10.6 | 6.2 | 4.3 | 5.9 | 7.5 | 4.2 | 11.3 | 6.8 | 12.1 |
| *Trifolium repens* type | 16.0 | 23.0 | 13.8 | 25.5 | 5.1 | 10.5 | 1.6 | | | | | |
| *Trifolium type* | 11.7 | 13.7 | 6.7 | 5.0 | 8.4 | | 5.3 | 10.9 | 27.7 | 10.9 | | |
| *Dorycnium* | | 5.3 | 19.6 | 9.9 | 12.3 | | 13.4 | | 1.0 | | | |
| *Medicago* | 4.1 | 8.9 | 4.0 | 6.1 | | | | | | | | |
| *Robinia* | | 1.2 | 5.0 | | 4.2 | | | | | | | |
| *Melilotus* | | | | 3.0 | 1.9 | | | | | | | |
| *Vicia* | | | | | | | | 9.2 | 4.5 | 16.5 | | |
| *Lotus* | | 1.5 | | | 1.7 | | 1.3 | 7.8 | 6.3 | 9.1 | | |
| *Genista* | | | 5.8 | 3.5 | 2.2 | | 12.3 | 7.1 | 4.8 | | | |
| *Onobrychis* | | | | | 2.0 | | | | | | | |
| *Crepis/Taraxacum type* | 2.4 | 1.6 | | 1.2 | | 3.5 | | | | | | |
| *Matricaria/Achillea* type | | | 1.1 | | | | | 3.7 | 1.0 | | | |
| *Centaurea jacea* type | | | | | | | | 1.5 | 0.5 | | | |
| *Cirsium* type | | | | | | | | 1.3 | | | | |
| *Cynoglossum* | | | | 3.9 | 26.5 | | | | | | | |
| *Onosma* | | | | | | 28.9 | | | | | | |
| *Echium* | | | 6.3 | | 1.9 | 2.2 | 4.5 | | 1.9 | | | |
| *Myosotis* | | 0.4 | | | 2.0 | | | | | | | |
| *Lithospermum* | 10.2 | | | 1.1 | | | | | | | | |
| *Symphytum* | | | | | 0.5 | | 2.4 | | | | | |
| Apiaceae | 3.6 | 1.0 | | 4.0 | 2.7 | | 1.6 | 3.0 | | 7.4 | | |
| *Heracleum* | | | 2.0 | | | | | | | | | |
| *Stachys* type | 4.5 | 1.9 | 1.1 | 3.7 | 7.6 | | | 4.0 | | | | |
| *Mentha* type | 3.8 | | 1.0 | 0.5 | 1.2 | | | 2.3 | 8.4 | 3.1 | | |
| *Salvia* | | 0.7 | 2.0 | 1.3 | 0.6 | | 3.5 | | | | | 3.1 |
| *Viburnum* | | | | 0.4 | 0.3 | | | | | | | |
| *Tilia* | | | 2.6 | | 0.6 | | | 8.9 | 9.5 | 6.7 | | |
| *Acer* | 0.5 | | 0.3 | | 0.7 | | 0.5 | 0.9 | | 3.6 | | |
| *Syringa/Ligustrum* | | | | | 1.0 | 1.7 | | | | | | |
| *Paliurus type* | | | 5.1 | 1.3 | | | | | 15.3 | 7.7 | 7.0 | 38.5 |
| *Campanula* | | | 4.9 | | | | 1.0 | | | | | |
| Caryophyllaceae | 0.2 | | | 0.5 | | | | | | 2.7 | | 3.0 |
| *Silene* | | | 0.9 | | | | | | | | | |
| *Cerastium* | | | 2.8 | | | | | | | | | |
| *Geranium* | | | 0.8 | 0.4 | | | | | | | | |
| *Hyosciamus* | | | | | | | | | | 1.0 | | |
| Ranunculaceae | 0.5 | 2.0 | 2.1 | 1.6 | 0.5 | 0.5 | 2.3 | 0.5 | | | | |
| *Helleborus* | 0.2 | | | | | | | | | | | |
| *Ranunculus* type | | | | | | 2.3 | | | | | | |
| Scrophulariaceae | | 1.5 | 2.2 | 1.5 | | 2.1 | | | | | | |
| *Veronica* | 0.5 | 1.2 | | | 1.2 | 3.4 | | | | | | |

**Table 2** (continued)

| Pollen taxa | Sample no. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1p | 2p |
| *Linaria* | | | | | | | | | 6.4 | 9.1 | | |
| *Linum* | | 0.1 | | | | | | | | | | |
| *Primulaceae* | 0.1 | | | | | 1.2 | | | | | | |
| Liliaceae | 0.1 | | | 0.1 | | | 0.2 | | | | | |
| *Convolvulus* | | | 0.1 | | | | | 2.9 | 1.1 | | | 5.1 |
| *Evonymus* | | | | | 0.5 | | | | | | | |
| *Cornus* | | | | | | | | 8.5 | 5.1 | | | |
| *Loranthus* | | | 0.5 | | 0.2 | 0.2 | | | | | | |
| *Saxifraga* | | 0.4 | | | | | | | | | | |
| *Sedum* | | 0.1 | | | | | | | | | | |
| *Cistus* | | | | | | | | | | | | 7.5 |
| *Salix* | | | | | | | | 4.4 | | 1.7 | | |
| Nectarless (Pa) | | | | | | | | | | | | |
| *Quercus* | | | | 0.1 | | | | | | 0.7 | | 0.6 |
| *Carpinus betulus* | 0.6 | 0.5 | | | | 1.3 | | | | | | |
| *Betula* | 0.2 | | | 0.2 | | | | | | | | |
| *Fagus* | | 0.2 | | 0.5 | | | | | | | | 0.1 |
| *Picea* | | | | 0.2 | | 0.7 | | | | | | |
| *Pinus* | | | | | | | | | | | 1 | |
| *Vitis* | 0.2 | | | | | | | | | | | |
| *Sambucus* | 0.2 | | | | | | | | | | 19.1 | |
| Papaveraceae | 0.4 | | | | | | | | | | | |
| *Hypericum* | | 0.2 | | | | | | | | | | |
| Poaceae | 1.4 | | 2.9 | 2.7 | 1.8 | 1.7 | 3 | 4.0 | 1.6 | | | |
| *Plantago* | 0.5 | | 8.8 | 1.4 | 6.6 | | | 0.8 | 5.2 | 4.3 | | 28.6 |
| *Rumex* | | 0.4 | 4.0 | | 2.4 | | 2.7 | | | | 1.9 | |
| Chenopodiaceae | | | 2.1 | 0.4 | | | | | | | | |
| *Sanguisorba minor* | | | 1.2 | | | | 4.9 | | | | | |
| *Artemisia* | | | | | | | | | 1.8 | | | |

Sample number as in Table 1

negative correlations with Fe, Co, Ca, Cr, and Zn. The result demonstrates that K, Na, and Cr have positive coordinates and correlations with factor 2 and negative correlations with Cd, As, Cu, and Pb. Higher concentrations of K and Na characterize the non-serpentine honey samples.

The relationships between samples on the basis of their elemental concentrations were presented when cases (samples) were projected on the factor-plane (Fig. 2). Most of the serpentine samples show positive coordinates and correlations with factor 1 which contribute to their separation. The group of serpentine samples is more dispersed in the factor-plane because of different correlations to both factors. The non-serpentine samples have negative coordinates with F1 and F2 and form a group on the plot area clearly separated from the serpentine one.

## Discussion

### Melissopalynological Data and Physicochemical Characteristics

The palynological data showed quite similar botanical origin of the honey from serpentine and non-serpentine areas. Some differences in the pollen spectra of the analyzed samples from the same locality were found (Table 2) reflecting the climatic conditions of the study territory during the years. The representatives of Fabaceae, Rosaceae, and Brassicaceae families were important for honey production at both sites. The higher percentage values of Boraginaceae pollen characterized the composition of honey from the serpentine area (Table 2). The highest percentage value (28.9 %) was calculated for

**Table 3** Elemental concentrations (mg $kg^{-1}$) in honey samples and bee pollen

| Sample no. | Ca | Mg | K | Na | P | Fe | Ni | Mn | Cr | Co | Cd | As | Cu | Zn | Pb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Honey | | | | | | | | | | | | | | | |
| 1 | 38 ± 2 | 13 ± 1 | 272 ± 18 | 15 ± 2 | 61 ± 7 | 1.1 ± 0.1 | 0.21 ± 0.02 | 0.43 ± 0.02 | 0.02 ± 0.01 | 0.01 ± 0.001 | 0.01 ± 0.0 | 0.01 ± 0.0 | 0.1 ± 0.0 | 0.66 ± 0.02 | 0.08 ± 0.01 |
| 2 | 50 ± 4 | 19 ± 1 | 236 ± 11 | 13 ± 2 | 75 ± 5 | 4.5 ± 0.3 | 1.2 ± 0.1 | 0.63 ± 0.02 | 0.02 ± 0.01 | 0.01 ± 0.001 | 0.01 ± 0.0 | 0.01 ± 0.0 | 0.19 ± 0.01 | 0.72 ± 0.04 | 0.14 ± 0.1 |
| 3 | 50 ± 3 | 48 ± 9 | 770 ± 43 | 9.3 ± 1 | 131 ± 10 | 1.7 ± 0.1 | 0.62 ± 0.03 | 4.7 ± 1 | 0.01 ± 0.0 | 0.01 ± 0.003 | 0.01 ± 0.0 | 0.01 ± 0.0 | 0.23 ± 0.01 | 0.68 ± 0.02 | 0.08 ± 0.01 |
| 4 | 24 ± 2 | 9 ± 1 | 184 ± 10 | 8.6 ± 1 | 45 ± 14 | 0.71 ± 0.03 | 0.15 ± 0.02 | 0.31 ± 0.01 | 0.01 ± 0.0 | 0.01 ± 0.0 | 0.01 ± 0.002 | 0.01 ± 0.0 | 0.05 ± 0.0 | 0.38 ± 0.02 | 0.08 ± 0.01 |
| 5 | 33 ± 2 | 18 ± 2 | 592 ± 16 | 13 ± 2 | 103 ± 9 | 2.9 ± 0.2 | 0.44 ± 0.02 | 0.99 ± 0.1 | 0.01 ± 0.001 | 0.01 ± 0.0 | 0.01 ± 0.0 | 0.01 ± 0.0 | 0.1 ± 0.01 | 0.39 ± 0.02 | 0.35 ± 0.04 |
| 6 | 54 ± 3 | 28 ± 8 | 136 ± 9 | 6.3 ± 1 | 114 ± 9 | 3.4 ± 0.02 | 0.06 ± 0.01 | 0.55 ± 0.04 | 0.01 ± 0.0 | 0.01 ± 0.0 | 0.02 ± 0.001 | 0.19 ± 0.02 | 0.24 ± 0.03 | 0.77 ± 0.05 | 0.29 ± 0.02 |
| 7 | 43 ± 3 | 10 ± 1 | 166 ± 14 | 9.7 ± 1 | 36 ± 3 | 7.4 ± 0.04 | 0.34 ± 0.03 | 0.35 ± 0.02 | 0.01 ± 0.003 | 0.01 ± 0.003 | 0.04 ± 0.01 | 0.63 ± 0.3 | 0.49 ± 0.05 | 0.53 ± 0.02 | 0.44 ± 0.04 |
| 8 | 94 ± 7 | 17.2 ± 3 | 1900 ± 98 | 20.2 ± 3 | 62 ± 4 | 1.97 ± 0.05 | 0.02 ± 0.001 | 0.54 ± 0.04 | 0.02 ± 0.00 | 0.02 ± 0.001 | 0.02 ± 0.003 | 0.01 ± 0.002 | 0.18 ± 0.02 | 1.71 ± 0.1 | 0.08 ± 0.01 |
| 9 | 44 ± 3 | 8.3 ± 1 | 1176 ± 112 | 9.3 ± 1 | 29.2 ± 2 | 4.29 ± 0.04 | 0.02 ± 0.003 | 0.43 ± 0.03 | 0.02 ± 0.003 | 0.02 ± 0.004 | 0.02 ± 0.003 | 0.01 ± 0.002 | 0.16 ± 0.06 | 0.71 ± 0.04 | 0.08 ± 0.01 |
| 10 | 91 ± 6 | 20.2 ± 4 | 929 ± 25 | 16.8 ± 3 | 85.2 ± 5 | 19.25 ± 0.04 | 0.02 ± 0.002 | 0.71 ± 0.04 | 0.02 ± 0.001 | 0.02 ± 0.002 | 0.02 ± 0.001 | 0.01 ± 0.002 | 0.19 ± 0.02 | 1.68 ± 0.12 | 0.08 ± 0.01 |
| Bee pollen | | | | | | | | | | | | | | | |
| 1p | 1500 ± 98 | 787 ± 29 | 3746 ± 202 | 161 ± 20 | 3241 ± 181 | 79 ± 12 | 2 ± 0.2 | 15 ± 1 | 0.61 ± 0.1 | 0.3 ± 0.04 | 0.27 ± 0.01 | 1.3 ± 0.3 | 3.1 ± 0.3 | 22 ± 2 | 8.1 ± 0.2 |
| 2p | 2211 ± 112 | 864 ± 42 | 3533 ± 168 | 68 ± 7 | 3024 ± 112 | 53 ± 5 | 1.4 ± 0.2 | 17 ± 2 | 0.1 ± 0.01 | 0.16 ± 0.01 | 0.1 ± 0.03 | 0.97 ± 0.1 | 17 ± 2 | 19 ± 2 | 3.5 ± 0.1 |

Sample number as in Table 1

Values are means of three measurements per chemical element per sample

**Table 4** Comparison between serpentine ($n = 7$) and non-serpentine honeys ($n = 3$)

| | Serpentine honeys | Non-serpentine honeys |
|---|---|---|
| Ca | *41.71 ± 10.75a* | *76.33 ± 28.04b* |
| Mg | 20.71 ± 13.66a | 15.23 ± 6.19a |
| K | *336.57 ± 244.95a* | *1335.0 ± 504.65b* |
| Na | 10.70 ± 3.05a | 15.43 ± 5.58a |
| P | 80.71 ± 36.12a | 58.80 ± 28.14a |
| Fe | 3.10 ± 2.32a | 8.50 ± 9.38a |
| Ni | 0.43 ± 0.39a | 0.02 ± 0.00a |
| Mn | 1.14 ± 1.59a | 0.56 ± 0.14a |
| Cr | *0.01 ± 0.05a* | *0.02 ± 0.00b* |
| Co | 0.01 ± 0.00a | 0.02 ± 0.00a |
| Cd | 0.02 ± 0.01a | 0.02 ± 0.00a |
| As | 0.12 ± 0.23a | 0.01 ± 0.00a |
| Cu | 0.20 ± 0.15a | 0.18 ± 0.1a |
| Zn | *0.59 ± 0.16a* | *1.37 ± 0.57b* |
| Pb | 0.21 ± 0.15a | 0.08 ± 0.00a |

Data are means ± SDs. Significant value based on one-way ANOVA are in italics. Values within rows followed by the different letters are significantly different according to Tukey's test ($P < 0.05$)

*Onosma* pollen in sample No. 6. Bearing in mind that *Onosma pavlovae* Petrova & Kit Tan is the only representative of the genus in the studied area, we can conclude that it is a good meliferous plant. Surprisingly low are the pollen percentages of Caryophyllaceae, *Silene*, and *Cerastium* in the serpentine

**Table 5** Percentage eigenvalues, variance explained by first three components, and factor loadings of the variables

| | Factor 1 | Factor 2 | Factor 3 |
|---|---|---|---|
| Eigenvalues % | 35.33 | 25.17 | 18.00 |
| Cumulative % | 35.33 | 60.50 | 78.50 |
| Variables | | | |
| Ca | −0.819 | −0.132 | 0.507 |
| Mg | 0.139 | 0.603 | 0.775 |
| K | −0.817 | 0.045 | 0.147 |
| Na | −0.789 | 0.005 | 0.147 |
| P | 0.139 | 0.608 | 0.690 |
| Fe | −0.427 | −0.396 | 0.366 |
| Ni | 0.394 | 0.351 | 0.116 |
| Mn | 0.185 | 0.612 | 0.629 |
| Cr | −0.794 | −0.001 | −0.268 |
| Co | −0.902 | −0.218 | 0.021 |
| Cd | 0.031 | −0.929 | 0.318 |
| As | 0.474 | −0.808 | 0.923 |
| Cu | 0.274 | −0.680 | 0.293 |
| Zn | −0.897 | −0.109 | 0.362 |
| Pb | 0.614 | −0.571 | 0.218 |

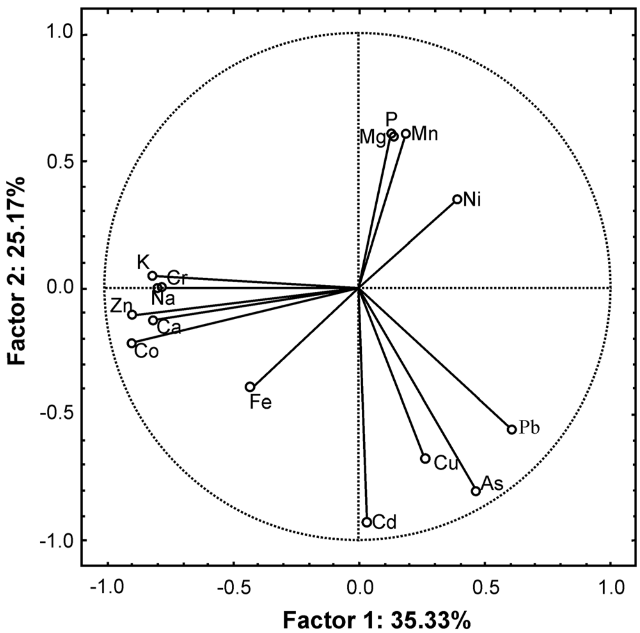



**Fig. 1** Principal-component-analyses plot variables (vectors). Length of the vectors is proportional to the strength of the correlations between variables and one of the PCs (factors)

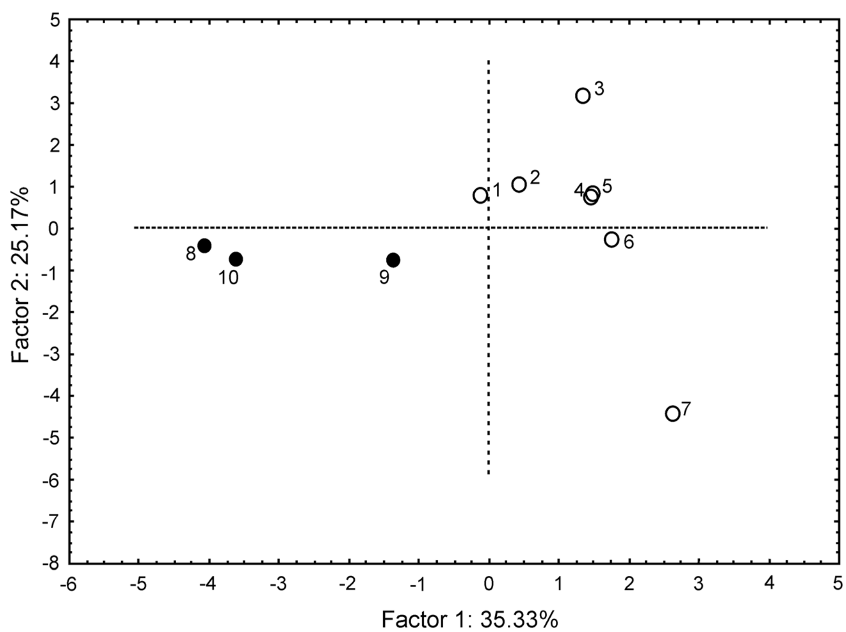
honey samples, although representatives of this family are very well presented on serpentines in the Balkans [40]. Pollen grains from rare and endemic plants growing at the serpentine site like *Silene fetlerii* D. Pavlova, *Convolvulus boissieri* Stend. subsp. *parnassicus* (Boiss. et Orph.) Kuzm., *Anthemis rumelica* (DC) Ferdinand, *Micromeria dalmatica* Benth. subsp. *bulgarica* (Velen.) Guinea, *Genista rumelica* Velen., and *Potentilla regis-borisii* Stoj. were difficult for identification at species and subspecies level and were included in an appropriate pollen type.

Special attention was paid on the quantity of pollen grains from plants known as Ni-hyperaccumularors [15] such as *Alyssum murale* Waldst. & Kit. subsp. *murale*, *Thlaspi ochroleucum* Boiss. & Heldr., and *Th. praecox* Wulfen in Jacq. It is known that the botanical origin of nectar affects to a certain extent the chemical composition of honey [41]. The pollen morphological differences between these species are very weak and the pollen grains calculated are placed in one pollen group (Brassicaceae). The highest percentage of Brassicaceae pollen was found in honey sample No. 4 (10.6 %) and in bee pollen sample No. 2 (12.1 %). Brassicaceae species are important but not essential sources for nectar and pollen. It is quite possible that these plants are avoided by bees because of the elevated Ni concentrations in their organs. Although the chemical composition of bee pollen can be correlated with the plant species from which pollen was collected [30], it is difficult to confirm this with our data for Brassicaceae pollen because of the insufficient material analyzed.

The nectarless plants in this region resemble 28.06 % of all plants found in the studied area but their pollen is also included in honey. All honey samples contained a low percentage of pollen grains from nectarless plants such as Poaceae, *Plantago*, *Rumex*, *Sanguisorba minor* Scop., and Chenopodiaceae (Table 2). The quantity of pollen grains from *Plantago* and *Rumex* in honey samples is greater compared to the one from Poaceae species which are the best presented in the serpentine flora. *Plantago carinata* Scop. and *Rumex acetosella* L. are the characteristic species of the serpentine terrains not only in Bulgaria but in other Balkan countries [40].

Despite that the identification of pollen in honey was performed to the lowest possible taxonomic level (mainly genus level), it does not allow the use of specific indicator species for

**Fig. 2** Principal-component-analyses plot cases (honey samples). The origin of honey samples and their numbers are given in Table 1. Serpentine samples—*open circle*; non-serpentine samples—*closed circle*



identification of the geographical origin in relation to the forage of bees. Pollen grains of *Vicia*, *Artemisia*, *Centaurea jacea*-type, *Cirsium*-type, *Matricaria/Achillea*-type, *Linaria*, *Hyosciamus*, etc., were related to non-serpentine pollen samples and obviously reflected the meadow type of vegetation around the beehives at this site. The variation in the pollen spectra of honey collected from the same locality and even the same beehive were also a result of the differences in the seasons during the years of collection which influenced not only the pollen composition but also the nectar production [42].

The physicochemical characteristics of our honeys (Table 1) are close to the results reported for different honeys in Europe [43] and fall in the ranges accepted for honey quality and international regulatory standards [44]. Although one of the honey samples has a value higher than 0.8 mS/cm which is typical for honeydew honeys, we consider it as a blossom honey because many exceptions to this rule were also established [45, 46].

**Elemental Concentrations**

Metalliferous soils with abnormally high concentrations of some of the elements that are normally present as minor constituents (200–2000 mg $kg^{-1}$, e.g., Mn) or trace constituents (0.01–200 mg $kg^{-1}$, e.g., Zn, Cu, Ni, Cr, Pb, As, Co, Se, Cd), vary widely in their effects on plants [47] and pollinators [5].

The element concentrations vary between honey samples and clear separation of serpentine from non-serpentine honeys were found on the basis of the higher concentrations of Ni, Mg, and Mn. Higher concentrations of Ni, Mg, Mn, and P in serpentine honey samples in comparison to non-serpentine (Tables 3 and 4) could be accepted as a result of the effect of the soil and confirmed by performed PCA (Fig. 1). The detected concentrations of Ni in honeys from Bulgarian serpentine area are similar to the data of Ramalhosa et al. [29] measured for multifloral honey originating from Morais serpentine site in Portugal. Higher Ni concentrations in Czech honeys reported by Lachman et al. [48] and in Slovakia honeys reported by Kováčik et al. [49] are related to honeydew honeys while nectar honeys have Ni in concentrations similar to our non-serpentine samples. Although the concentration range of Ni in Bulgarian serpentine honey vary between 0.06 and 1.2 mg $kg^{-1}$, in most samples they are higher compared to data provided for non-serpentine multifloral honeys from Bulgaria [50], Morocco [51], Turkey [19, 21], and Poland [22]. High variation of Ni values was established also for unifloral Bulgarian honeys [46]. The most pronounced differences of accumulated elements in honey types, especially for trace elements, were found between honeydew and blossom type of honey [7, 48, 50]. The following Ni levels were reported for multifloral honeys from different countries: 0.0–0.3 [19]; 0.078–0.42 [51]; 0.1–0.8 [22]; 0.28–0.88 mg $kg^{-1}$ [21] from non-serpentine areas and 0.06–1.1 mg $kg^{-1}$ [29] from serpentine areas.

The variation in trace element content in different honey types is considered primarily due to botanical origin rather than geographical and environmental exposition of nectar sources [7]. Our data demonstrate variation of metal concentrations in honeys even collected from the same locality and the same beehive but from different vegetation seasons and could be considered primarily a result of environmental influence or contamination. Even low, there is a possibility Ni to fall in honey by pollen from Ni hyperaccumulator plant species. Such assumption needs more precise analyses and future experiments. Also Kováčik et al. [49] suspect the occurrence of Ni-accumulating plant species in the locality where elevated Ni concentration in forest honey were found.

Worldwide there are no accepted maximum limits for Ni in honey. The allowable amount in Bulgaria for Ni varies in different foods from 0.1 mg $kg^{-1}$ (milk) to 8.0 mg $kg^{-1}$ (tea, cacao powder) [10]. Although bee pollen has higher metal concentrations than honey [7], the measured Ni both in bee pollen and honey is in range considered as normal.

The mean Mg concentrations from serpentine honey samples were higher not only from the mean concentrations of non-serpentine samples but also from previous data for multifloral honeys from Bulgaria [50]. Magnesium concentrations from serpentine honey samples were lower than the data for serpentine honeys from Portugal [29]. The relations between Mg and Ca concentrations in serpentine soils and plants are quite important characteristics and part of the serpentine syndrome [13]. Also, lower Ca and higher Mg concentrations found in serpentine honey samples in comparison to the non-serpentine could be related to the available concentrations of Ca and Mg in the serpentine soil ($Ca:Mg < 1$). We suggest that elevated Mg concentrations in serpentine honeys are characteristic for honeys originating from such areas. Similar results for Ca and Mg in serpentine honey samples were reported for Portugal [29]. Our findings for Ca concentrations are in the range suggested for honey by Szefer and Grembecka [17]. Calcium, potassium, and phosphorus were the most abundant elements in both studied groups and synchronized with previous reported data [49, 52].

The results of PCA demonstrate that Mn is the third element important for the separation of both groups of honey samples. This element has elevated concentrations in serpentine soils [13, 53]. Similar results were also reported for Slovakian honeys [49]. Manganese is important for biological processes and toxic only at much higher levels than those encountered in honey [7]. Kováčik et al. [49] consider Ni and Mn among the most discriminating variables which can be used to distinguish between different honey types. Their results are in accordance with our data.

Although the concentrations of Fe, Co, and Cr from serpentine soils are normally elevated, they have low concentrations in serpentine honeys (Table 3). These metals show similar values to the ones found by Bogdanov et al. [7], Chakir et al. [51], Deribaşı et al. [20], Grembecka and Szefer [22], Tuzen et al. [19], and Yurukova et al. [50] and could not be considered important for separation of serpentine honey samples. The maximal level of 19.25 mg kg$^{-1}$ Fe determined in this study was higher compared to previous reports from Bulgaria [50], Turkey [19, 20], Poland [22], Bosnia and Hercegovina [23] and summarized data presented by Bogdanov et al. [7]. The detected concentrations of Cr and Co were very low (<0.01–0.02 mg kg$^{-1}$) in both group of samples. The concentration of Cr depends on the climatic conditions [7]. Small quantities of the element in serpentine samples and the lack of significant variation between concentrations found in our study do not confirm this.

Usually serpentine soils are not rich in Cu, Zn, and Pb and their effect on serpentine honeys is expected to be low. These metals do not contribute to separation of the groups of samples which is confirmed by the PCA analysis. The concentrations of Zn in non-serpentine honeys were twice higher compared to the serpentine honeys. This fact could be a result of environmental contamination related to the Zn mineralization and Zn smelting operation in the region of the town of Kardzali, 10 km from the place where beehives are localized. Despite that Zn concentrations in non-serpentine honeys were higher than serpentine honeys, our values were lower than the maximal concentrations provided for Turkey [19], Poland, [22] and Iran [28]. Honey contamination with Zn up to 41.25 mg kg$^{-1}$ was reported by Vincevica-Gaile et al. [54] when honey storage is in galvanized containers.

The metals Pb, Cd, and As do not contribute for separation of serpentine and non-serpentine honeys. They are considered as indicators for honey contamination [32]. These elements could reflect the presence of contaminants due to environmental contamination or pharmacological (antiparasitical or acaricidal) treatment of honey or by incorrect procedures during honey processing and conservation phases [50]. Lead is present in most soils and rocks at concentrations below 50 mg kg$^{-1}$ and generally shows relatively little mobility in the soils and into vegetation. Significant translocation of this metal to the upper parts of the plant is uncommon [47]. Lead contamination of honey is usually related to air pollution by motor traffic or industry and for good quality of honeys is recommended beehives to be placed far from the roads [55]. No significant differences were found between Pb contamination of honey in polluted and non-polluted areas due to the considerable natural variation of the data but the highest values were often found in polluted areas [32]. Cadmium is usually transported from soil to plant and contaminates nectar and honeydew. Elevated soil Cd concentrations can be found in soils containing waste from mining Zn sulfides and other Zn ores, but may also occur in soils treated with industrial wastes or with certain Cd-rich phosphatic fertilizers, or soils subjected to irrigation with Cd-enriched waters [47]. This metal originates from metal industry and might reach honey also by air. The limits of 0.1 mg kg$^{-1}$ for Cd and 1 mg kg$^{-1}$ for Pb [8, 9, 33] are not exceeded in all studied honey samples while in bee pollen they are over the proposed limits. The mean As concentration for all serpentine honey samples was 0.12 mg kg$^{-1}$ which was below the maximal allowable level (0.01–0.5 mg kg$^{-1}$) [33, 34]. The mean concentrations for this metal from non-serpentine honeys were similar to the results reported for Iran [28], Bulgaria [50], and Bosnia and Hercegovina [23]. Contamination of honey with As can be caused by non-ferrous metallurgy, factories, and agrochemicals such as fertilizers and arsenic-based pesticides [31].

Therefore, it was concluded that there was no health hazard associated with consumption of honeys collected from serpentines. We recommend a strict control on the origin of bee pollen before its direct use as food additive or medication.

## Conclusions

The results suggest possible negative effect of the elevated metal concentrations of serpentine soils on the quality of bee pollen and honey. Different elements have different concentrations in honey from the same botanical type even collected from the same geographical region, same locality, same beehive, but in different vegetation season which means that environmental factors and contamination are of great importance for the elemental concentrations. For more precise analysis of trace element content in honey, special attention should be paid on the environmental characteristics of the locality, altogether with the geographical region and the botanical spectrum. The slight differences in pollen composition found between serpentine and non-serpentine honey samples do not allow the use of specific indicator plant species for identification of the geographical origin in relation to the forage of bees.

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.

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#### Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no competing interests.

## References


1. Naccari C, Macaluso A, Giangrosso G, Naccari F, Ferrantelli V (2014) Risk assessment of heavy metals and pesticides in honey from Sicily (Italy). J Food Res 3(2):107–117. doi:10.5539/jfr.v3n2p1
2. Matei N, Birghila S, Dobrinas S, Capota P (2004) Determination of C vitamin and some essential trace elements (Ni, Mn, Fe, Cr) in bee products. Acta Chim Slov 51:169–175
3. Breygina M, Matveyeva N, Polevova S, Meychik N, Nikolaeva Y, Mamaeva A, Yermakov I (2012) Ni2+ effects on *Nicotiana tabacum* L. pollen germination and pollen tube growth. BioMetals 25:1221–1233. doi:10.1007/s10534-012-9584-0
4. Meindl GA, Ashman T-L (2013) The effects of aluminum and nickel in nectar on the foraging behavior of bumblebees. Environ Pollut 177:78–81. doi:10.1016/j.envpol.2013.02.017
5. Meindl GA, Bain DJ, Ashman T-L (2013) Edaphic factors and plant-insect interactions: effects of soil chemistry on pollination and florivory. Oecologia 173:1355–1366. doi:10.1007/s00442-013-2711-y
6. Pavlova D, De la Fuente V, Sanchez-Mata D, Rufo L (2014) Pollen morphology and localization of Ni in some Ni-hyperaccumulator taxa of *Alyssum* L. (Brassicaceae). Plant Biosyst Int J Asp Plant Biol. doi:10.1080/11263504.2014.989284
7. Bogdanov S, Haldimann M, Luginbuhl W, Gallmann P (2007) Minerals in honey: environmental, geographical and botanical aspects. J Apic Res Bee World 46(4):269–275
8. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February (2005) on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. Official Journal European Union 16.3.2005 L 70/1
9. Food Standards Agency (2003) The Honey Regulations 2003, Guidance Notes. https://www.food.gov.uk/northern-ireland/niregulation/niguidancenotes/honeyregsguide. Accessed 30 September 2015
10. Bulgarian Ministry of Health (2006) Ordinance № 31/29.07.2004 on the maximum allowable food contaminants. State Newspaper no. 51/23.06.2006. http://lex.bg/bg/laws/ldoc/2135493121. Accessed 30 September 2015 (in Bulgarian)
11. Pisani A, Protano G, Riccobono F (2008) Minor and trace elements in different honey types produced in Siena County (Italy). Food Chem 107:1553–1560. doi:10.1016/j.foodchem.2007.09.029
12. Özcan M, Al Juhaimi F (2012) Determination of heavy metals in bee honey with connected and not connected metal wires using inductively coupled plasma atomic emission spectrometry (ICP–AES). Environ Monit Assess 184(4):2373–2375. doi:10.1007/s10661-011-2123-6
13. Brooks R (1987) Serpentine and its vegetation. A multidisciplinary approach. Dioscorides Press, Portland, Oregon
14. Van der Ent A, Baker AJM, Reeves R, Pollard J, Schat H (2013) Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362:319–334. doi:10.1007/s11104-012-1287-3
15. Bani A, Pavlova D, Echevarria G, Mullaj A, Reeves R, Morel J, Sulçe S (2010) Nickel hyperaccumulation by species of *Alyssum* and *Thlaspi* (Brassicaceae) from the ultramafics of Balkans. Bot Serbica 34:3–14
16. Samiullah N (2006) Cadmium toxicity and tolerance in plants. Alfa Science International, Oxford
17. Szefer P, Grembecka M (2007) Mineral components in foods of animal origin and in honey. In: Szefer P, Nriagu J (eds) Mineral components in foods. CRC, Taylor Francis Group, London, pp 163–230
18. Altundag H, Albayrak S, Dundar MS, Tuzen M, Soyak M (2015) Investigation of the influence of selected soil and plant properties from Sakarya, Turkey on the bioavailability of trace elements by applying in vitro digestion model. Biol Trace Elem Res 168(1):276–285. doi:10.1007/s12011-015-0330-7
19. Tuzen M, Silici S, Mendil D, Soylak M (2007) Trace element levels in honeys from different regions of Turkey. Food Chem 103:325–330. doi:10.1016/j.foodchem.2006.07.053
20. Derebaşı E, Bulut G, Col M, Güney F, Yaşar N, Ertürk Ö (2014) Physicochemical and residue analysis of honey from Black Sea region of Turkey. Fresenius Environ Bull 23(1):10–17
21. Altundag H, Bina E, Altintig E (2015) The levels of trace elements in honey and molasses samples that were determined by ICP-OES after microwave digestion method. Biol Trace Elem Res doi:10.1007.s1 12011-015-0468-3
22. Grembecka M, Szefer P (2013) Evaluation of honeys and bee products quality based on their mineral composition using multivariate techniques. Environ Monit Assess 185:4033–4047. doi:10.1007/s10661-012-2847-y
23. Mujić I, Alibabić V, Jokić S, Galijašević E, Jukić D, Sekulja D, Bajramović M (2011) Determination of pesticides, heavy metals, radioactive substances, and antibiotic residues in honey. Pol J Environ Stud 20(3):719–724
24. Lacarević K, Trifković J, Andrić F, Tesic Z, Anđelković I, Radović N, Milojković-Opsenica D (2013) Quality parameters and pattern recognition methods as a tool in tracing the regional origin of multifloral honey. J Serbian Chem Soc 78:1875–1892
25. Dimou M, Tananaki C, Liolios V, Thrasyvoulos A (2014) Pollen foraging by honey bees (*Apis mellifera* L.) in Greece: botanical and geographical origin. J Apic Sci 58(2):11–23. doi:10.2478/jas-2014-0018
26. Sager M, Maleviti E (2014) Elemental composition of honeys from Greece—possible use as environmental indicators. J Nutr Food Sci S8:002. doi:10.4172/2155-9600.S8-002
27. Bratu I, Georgescu C (2005) Chemical contamination of bee honey—identifying sensor of the environment pollution. J Cent Eur Agric 6(1):467–470
28. Aghamirlou H, Khadem M, Rahmani A, Sadeghian M, Mahvi A, Akbarzadeh A, Nazmara S (2015) Heavy metals determination in honey samples using inductively coupled plasma-optical emission spectrometry. J Environ Health Sci Eng 13:39–46
29. Ramalhosa E, Magalhaes M, Afonso MJ, Plasencia P, Saraiva D, Azevedo J, Castro M (2013) Metal contents in honey and mushrooms from serpentine soils in the Morais site, Portugal. In: Petrotos D, Filintas A (eds.) Proceedings of FaBE (2013) International Conferences on Food and Biosystems Engineering, 30 May - 02 June 2013, Skiathos Island Greece 1:167–173
30. Taha E-K (2015) Chemical composition and amounts of mineral elements in honeybee-collected pollen in relation to botanical origin. J Apic Sci 59(1):75–81. doi:10.1515/jas-2015-0008
31. Wróblewska A, Stawiarz E (2015) Botanical characteristics of Polish honeys from Natura 2000 habitats. J Apic Sci 59(1):39–49. doi:10.1515/jas-2015-0003
32. Bogdanov S (2006) Contaminants of bee products. Apidologie 37:1–18. doi:10.1051/apido:2005043
33. The Commission of the European Community (2006) Setting maximum levels for certain contaminations in foodstuffs, Commission Regulation (EC) No 1881/2006. Off J Eur Union 2006 L364/9
34. Codex Alimentarius (1993) Standard for honey. Ref Nr. CL 1993/14, SH FAO/WHO, Rome
35. Bulgarian Ministry of Agriculture and Forests (2005) Ordinance № 48/11.11.2003 on the procedure and methods of sampling and analysis of honey. State Newspaper no. 103/01.09.2005. http://www.lex.bg/bg/laws/ldoc/2135474656. Accessed 30 September 2015 (in Bulgarian)
36. Moraliysky E, Goceva A (1989) Temperature of the air. In: Michev K, Velchev V, Zjapkov L, Velev S, Tischkov Ch. (eds.) Natural and economic potential of the mountains in Bulgaria. I. Nature and

Resources. Publishing House of Bulgarian Academy of Sciences, Sofia, pp 79–85 (in Bulgarian)
37. Louveaux J, Maurizio A, Vorwohl G (1978) Methods of melissopalynology. Bee World 59:139–157
38. Beug H-J (2004) Leitfaden der Pollenbestimmung fur Mitteleuropa und angrenzende Gebiete. Verlag Dr. Friedrich Pfeil, München
39. The Harmonised Methods of the International Honey Commission (2009) http://www.bee-hexagon.net/en/network.htm. Accessed 30 September 2015
40. Babalonas D (1989) Beitrag zur Flora des serpentinischen Vourinos-Gebirges (Nord-griecheland). Willdenowia 18:387–399
41. Terrab A, Dìez MJ, Heredia JF (2003) Palynological, physico-chemical and colour characterization of Moroccan honeys: I. River red gum (*Eucalyptus camaldulensis* Dehnh) honey. Int J Food Sci Technol 38(4):379–386. doi:10.1046/j.1365-2621.2003.00715.x
42. Negrão A, Barreto L, Orsi R (2014) Influence of the collection season on production, size, and chemical composition of bee pollen produced by *Apis mellifera* L. J Apic Sci 58(2):5–10. doi:10.2478/jas−2014-0017
43. Persano Oddo L, Piro R (2004) Main European unifloral honeys: descriptive sheets. Apidologie 35:38–81
44. Bogdanov S, Lüllmann C, Martin P, von der Ohe W, Russmann H et al (2015) Honey quality and international regulatory standards: review by the international honey Commission. Bee World 80(2):61–69
45. Bogdanov S, Gfeller M (2006) Classification of honeydew and blossom honeys by discriminant analysis. Alps Sci 500:1–6
46. Atanassova J, Yurukova L, Lazarova M (2012) Pollen and inorganic characteristics of Bulgarian unifloral honeys. Czech J Food Sci 30(6):520–526
47. Reeves R (2006) Hyperaccumulation of trace elements by plants. In: Morel JL, Echevarria G, Goncharova N (eds.) Phytoremediation of Metal-Contaminated Soils. NATO Science Series (IV) Earth and Environmental sciences 68:25–52
48. Lachman J, Kolihová D, Miholová D, Košata J, Titěra D, Kult K (2007) Analysis of minority honey components: possible use for the evaluation of honey quality. Food Chem 101(3):973–979. doi:10.1016/j.foodchem.2006.02.049
49. Kováčik J, Grúz J, Biba O, Hedbavny J (2015) Content of metals and metabolites in honey originated from the vicinity of industrial town Košice (eastern Slovakia). Environ Sci Pollut Res. doi:10.1007/s11356-015-5627-8
50. Yurukova L, Petrova S, Shopova N (2010) Inorganic characteristics of polyfloral honey in Plovdiv (Bulgaria). Biotechnol Biotechnol Equip Spec Issue 24:284–288. doi:10.1080/13102818.2010.10817849
51. Chakir A, Romane A, Barbagianni N, Bartoli D, Ferrazzi P (2011) Major and trace elements in different types of Moroccan honeys. Aust J Basic Appl Sci 5(4):223–231
52. Czipa N, Andrási D, Kovács B (2015) Determination of essential and toxic elements in Hungarian honeys. Food Chem 175:536–542
53. Kabata-Pendias A, Pendias H (1984) Trace elements in soils and plants. CRC Press, Boca Raton, FL, USA
54. Vincevica-Gaile Z, Klavins M, Rudovica V, Viksna A (2012) Potentially toxic metals in honey from Latvia: is there connection with botanical origin? In: Ramos R, Straupe I, Panagopoulus T (eds) Recent researches in environment, energy systems & sustainability. WSEAS Press, Faro, pp 158–163
55. Bogdanov S (2004) Quality and standards of pollen and beeswax. Apiacta 38:334–341