ORIGINAL RESEARCH ARTICLE

Minerals in honey: environmental, geographical and botanical aspects.

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Summary

Honey usually contains a variety of mineral substances. Ninety five samples of known geographic and botanic origin were analyzed in order to detect possible contamination by common air pollution or other pathways. It was found that most elements correlate significantly with electrical conductivity. The contamination level of the toxic elements Pb and Cd measured in our study was low. There were differences between the honeys produced in the different areas only with regard to Fe and Cr content. The concentrations of the other trace elements measured in the present study, Cr, Ni, Cu, Zn, Mn and Fe, were similar to the values found in other recent studies. All of the trace elements determined in this work can be both from natural sources (soil, plants) and anthropogenic sources. Pb, Cd, and Zn especially, but also Cu, Cr, and Ni, are well known as potential air or soil contaminants of anthropogenic origin, but are of course also found as natural ingredients of soil minerals, as are also Fe and Mn. Variation in trace element content in different honey types is primarily due to botanical origin rather than geographical and environmental exposition of nectar sources.

Minerales en la miel: aspectos ambientales, geográficos y botánicos.

La miel normalmente contiene una variedad de sustancias minerales. Noventa y cinco muestras con origen geográfico y botánico conocidos fueron analizadas con el objetivo de detectar la posible contaminación por la polución del aire o de otras vías. Se encontró que la mayoría de los elementos se correlacionan significativamente con la conductividad eléctrica. El nivel de contaminación medido en nuestro estudio de los elementos tóxicos de Cd y Pb fue bajo. Sólo hay diferencias entre las mieles producidas en diferentes áreas en relación con el contenido de Fe y Cr. Las concentraciones medidas en el presente trabajo de otros oligoelementos como Cr, Ni, Cu, Zn, Mn y Fe, fueron similares a los valores encontrados en otros estudios recientes. Todos los oligoelementos determinados en este estudio pueden ser tanto de fuentes naturales (suelo, plantas) como de fuentes antropogénicas. Especialmente el Pb, Cd, Zn, pero también el Cu, Cr y Ni, son bien conocidos como contaminantes potenciales del aire o el suelo de origen antropogénico, pero además, son componentes naturales de los minerales del suelo, como lo son también el Fe y Mn. La variación del contenido de oligoelementos en los distintos tipos de miel se debe principalmente al origen botánico de las fuentes de néctar más que al geográfico y ambiental.

Keywords: honey, minerals, trace elements, heavy metal contamination.

Introduction

Honey contains varying amounts of mineral substances, ranging from 0.02 to 1.03g/100g (White, 1975). Potassium, with an average of about one third of the total, is the main mineral element, but there is a wide variety of trace elements. Several investigations have shown that the trace element content of honey depends on the botanical origin of honey, light blossom honeys having a lower content than dark honeys such as honeydew, chestnut and heather (Feller-Demalsy et al., 1989; Gonzalez-Miret et al., 2005; Sevlimli et al., 1992). It has been shown to be possible to differentiate between ling (Calluna vulgaris), heather (Erica sp.), rosemary (Rosmarinus officinalis), thyme (Thymus vulgaris), lavender (Lavandula sp.) and oak (Quercus sp.) honeydew honeys by measuring Mg, Ca, Al, Fe, Mn, Zn, B, Cu, Co, Cr, Ni, Cd and P (Nozal Nalda et al.,2005) and subsequent discriminant analysis. On the other hand, the mineral content of honey can also depend on the geographical origin, and it is possible to differentiate between honeys of different geographical origins (Hernandez et al., 2005; Lasceve and Gonnet, 1974; Latorre et al., 1999).

When interpreting the data, it should be taken into consideration that some heavy metals can be of anthropogenic origin. Indeed honey can be used as an indicator of pollution by heavy metals, mostly Cd, Pb, Cr and Ni (Porrini et al., 2003). Reviewing various studies on honey heavy metal content has shown, however, that lead contamination of honey in polluted and non polluted areas is not significantly different, due to the high variability of the data, but the highest values were often found in polluted areas (Bogdanov, 2006). The data variability is probably due to the different botanical origin of the honey samples examined.

Environmental, geographical and botanical factors thus all have an influence on the trace element content of honey, and it is necessary to consider all of these factors when studying honey trace element composition. Many of the studies cited above have not considered these different factors. The present study was therefore performed in order to study honey trace element contents, considering environmental, geographical and botanical factors. We also included the electrical conductivity (EC), as it correlates significantly to honey mineral content and is frequently used for the characterisation of the botanical origin of honey (Bogdanov et al.,2004). The trace elements were measured by inductively coupled sector-field plasma mass spectrometry (ICP-SFMS). In contrast to quadrupole-based ICP-MS, the capabilities of ICP-SFMS are significantly improved by virtue of enhanced sensitivity and separation of polyatomic interferences in a high resolution mode.

Materials and Methods

Samples

Ninety five honey samples harvested between 1998 and 2001 were from known Swiss production areas. With respect to possible influences of climatic and anthropogenic factors, we divided the samples into 4 groups: 13 samples from the cities of Berne and Basel, both with more than 100,000 inhabitants; 18 samples were harvested in villages with less than 5,000 inhabitants; 50 samples were from rural areas lying lower than 700 m above sea level, and 14 were from mountainous areas lying higher than 1,000 m above sea level. The honey production areas of the last two groups were more than 3km away from the nearest village. The data for the different samples are summarised in Table 1.

Table 1. Botanical origin in the different production areas

Area		Honey Types
City,	n = 13	10 mixed blossom, 2 mixed honeydew, 1 blend blossom-honeydew
Village,	n = 18	10 mixed blossom, 1 linden, 1 dandelion, 3 mixed honeydew, 3 blends blossom-honeydew
Rural,	n = 51	7 acacia, 7 chestnut; 7 dandelion; 7 linden; 6 rape, 7 fir, 5 mixed blossom, 5 mixed honeydew
Mountain,	n = 13	7 rhododendron, 6 mixed mountain blossom

The botanical origin of honeys is often classified into just two classes: blossom and honeydew honey. The correspondence of the samples to blossom or honeydew origin was based on the measurement of the EC according to the honey standards of the European commission and the Codex Alimentarius (Codex Alimentarius Committee on Sugars, 2001; European Commission, 2002) and on the sensory properties of the samples. According to these standards honeys with EC values higher than 0.8mS/cm are considered as honeydew honeys, while honeys with lower values are blossom honeys or blends of blossom with honeydew honey. Generally, honeys with EC values smaller than 0.5mS/cm are considered as blossom honeys (Talpay, 1985). According to these definitions 54 of our samples were classified as blossom honeys, 19 as honeydew honeys, whilst 22 were blends of honeydew and blossom honeys.

The specific botanical origin of the honeys was determined according to the combined appreciation of the sensory, microscopical and chemical properties of honey, determined according to the methods of the European Honey Commission (Persano Oddo and Bogdanov, 2004; Persano Oddo and Piro,2004; Piana et al.,2004; von der Ohe et al.,2004): 7 acacia (Robinia pseudoacacia), 7 rhododendron (Rhododendron ferrugineum and Rhododendron hirsutum), 7 chestnut (Castanea sativa), 8 dandelion (Taraxacum officinalis), 8 lime (Tilia sp.), 6 rape (Brassica napus) and 7 fir which in this area is usually a mixture of white fir (Abies alba) and red fir (Picea abies). 25 honeys were of mixed blossom non-mountain, and 6 honeys were of mixed blossom mountain origin. The rest of the samples (n = 14) were unspecified blends (honeydew blends, blends of honeydew with blossom honey).

Analytical methods

The electrical conductivity (EC) was measured according to the harmonised methods of the European Honey Commission and is expressed in mS/cm (Bogdanov *et al.*, 1997). Trace element measurements were performed using an Element Inductively Coupled Plasma Sector Field Mass Spectrometer (Finnigan MAT, Bremen, Germany). The mass analyzer of this instrument consists of magnetic and electric sector fields in a reversed Nier–Johnson geometry. This geometry, combined with different slit-widths, allows signal acquisition at high resolution. ^{111}Cd and ^{208}Pb were measured at a resolution of m/$\triangle$m = 300 (10% valley definition). To separate the elements from interfering molecular ions, ^{52}Cr, ^{55}Mn, ^{60}Ni, ^{63}Cu and ^{66}Zn were measured at a nominal resolution of m/$\triangle$m = 4000. ^{103}Rh (internal standard) was measured in both resolution settings. The electric scan acquisition mode was used with nine scans performed at each resolution. Optimization of the system has been performed on the ^{115}In signal (1.0 ng/ml) and was restricted to adjustments of sample (1.03 l/min) and auxiliary (0.85 l/min) argon flow rates to obtain a stable response and a maximum signal. 1.17 kW Rf-power was applied at a cool gas flow rate of 15.2 l/min.

The experiments were performed using a micro-flow nebulizer (AR30-1-FM02, Glass Expansion, Melbourne, Australia) and a low volume (20 cm^3) cyclonic spray chamber (Twinnabar, Glass Expansion). The nebulizer was run by using a low pulsation tubing pump that is designed for low-flow fluid transfer (IPC, Ismatec, Zürich, Switzerland), which was operated at a sample uptake rate of 0.3ml/min. The take-up and wash times were 2 and 4 min, respectively.

Containers used for the handling and storage of sample and standard solutions were polypropylene (PP) vials. Deionized water (18 MΩ, Barnstead system) and suprapur® nitric acid (Merck, Darmstadt, Germany) were used for preparation of the solutions. The samples were dissolved in 0.6 % nitric acid to a final honey concentration of 1.0 %.

The calibration standard was prepared by diluting a commercially available multi-element standard (ICP VI, Merck) with a solution containing 0.6 % nitric acid and 0.85% fructose (MicroSelect, Fluka, Buchs, Switzerland). Fructose was added to match the monosaccharide matrix of the honey. All elemental concentrations were 1.0ng/ml, except iron and zinc were at a level of 10.0ng/ml. The samples and reagents were prepared daily in order to avoid losses and contamination. To correct for instrumental drift and plasma fluctuations, all solutions were spiked with a rhodium solution as internal standard to a final concentration of 1.0ng/ml.

A quality control sample (SRM) was prepared from the standard reference material NIST1640 (National Institute of Standards, Gaithersburg, MD, USA) by 20-fold dilution with 0.85% fructose in 0.6% nitric acid.

Statistical methods

Univariate statistical analysis of group means and medians was based on parametric analysis of variance (ANOVA) and non-parametric Kruskal-Wallis ANOVA followed by multiple testing of pair wise differences (Tukey-Kramer and Mann-Whitney U tests, respectively). The tentative classification according to the botanical origin was performed by multivariate linear discriminant analysis (LDA) of the sample scores from principal component analysis (PCA). Descriptive and inferential statistics was done using SYSTAT Vs. 11 (Systat Software Inc. Richmond, California, USA). Outliers (4 values of Ni, 1 value of Fe) were eliminated based on Grubbs test and plausibility verification based on expert knowledge, before statistical evaluation.

Results

Method

Five series were run on different days to measure the honey samples. To emulate the measurement solution of the honey (natural water) the SRM NIST 1640 was dissolved in the fructose/ nitric acid matrix as well (*Table 2*). The certified values agree closely with the obtained concentrations at the 95 % confidence interval (CI).

Table 2. Repeated measurement (n=5, between-run) of the SRM NIST 1640 in a fructose matrix. (CI – confidence interval)

Element	Concentration (µg/l)	95 % CI (µg/l)	Certified (µg/l)
Cd	23.7	± 0.87	22.8
Pb	28.5	± 0.92	27.9
Cr	36.2	± 2.69	38.6
Mn	123.0	± 12.0	121.0
Fe	35.6	± 5.16	34.3
Ni	27.3	± 7.28	27.4
Cu	88.8	± 14.2	85.2
Zn	54.5	± 29.2	53.2

Data analysis

Table 3 shows some basic descriptive statistics for all samples (after elimination of the outliers). Fe, Mn, and Zn are the main trace elements while Cd and Cr had the lowest concentration. The data are not normally distributed as can be seen by the differences between mean and median values in *Table 3*. Also, skewness and curtosis, as well as the diagrams of the data indicated positively skewed distributions (data and graphs not shown). Furthermore, in many cases the groups tested with ANOVA were not homoscedastic, nor were the residuals normally distributed. These observations indicate that only the non-parametric statistical comparisons among groups are adequate. Therefore, tests results given later on are all based on Kruskal-Wallis and Mann-Whitney tests.

In *Table 4* the Pearson correlation coefficients between all measurands are given. There were significant correlations (individual significance level is 0.05) between the electrical conductivity and most of the elements, except Pb and Cr. In

Table 3. Descriptive statistics of all honey samples (n = 95, n_{Fe} = 94, n_{Ni} = 91). Concentrations are in mg/kg, EC in mS/cm.

	Cd	Pb	Cr	Mn	Fe	Ni	Cu	Zn	EC
Minimum	< 0.001	0.003	< 0.001	0.125	0.136	< 0.001	0.051	0.016	0.10
Maximum	0.026	0.329	0.037	12.354	9.852	1.966	3.317	4.133	2.41
Median	0.001	0.023	0.003	0.910	0.916	0.062	0.417	0.873	0.46
Mean	0.003	0.041	0.005	2.063	1.390	0.235	0.656	1.041	0.57

Table 4. Pearson correlation coefficients of the measurands. Correlations in italics are significant (individual significance level: 0.05)

	Cd	Pb	Cr	Mn	Fe	Ni	Cu	Zn
Cd	1							
Pb	0.0797	1						
Cr	-0.0051	*0.2217*	1					
Mn	*0.5324*	0.0440	0.0045	1				
Fe	*0.5743*	*0.3621*	*0.2615*	*0.4744*	1			
Ni	*0.8355*	0.1759	-0.0371	*0.4248*	*0.6645*	1		
Cu	*0.8476*	0.0823	0.0903	*0.4862*	*0.5644*	*0.7857*	1	
Zn	*0.6116*	*0.2481*	0.1745	*0.4080*	*0.7545*	*0.6086*	*0.5424*	1
EC	*0.3323*	0.1246	0.1618	*0.6652*	*0.4163*	*0.2904*	*0.4000*	*0.3255*

agreement with this, Pb and Cr did not correlate significantly with the majority of elements. Exceptions were the significant correlations between Pb on one hand and Cr, Fe and Zn on the other and between Cr on one hand and Fe on the other. As Pearson's correlation measures only the strength of linear associations between the variables we also computed the matrix of Spearman correlations (data not shown) which measures monotonic relations as well. Four Spearman correlation coefficients were higher than 0.6 (Cd/Cu, Fe/Zn, Cu/Zn, and EC/Mn) and only four were statistically not significant (Pb/Mn, Pb/Ni, Cr/Ni, and EC/Ni).

Geographical origin and environmental influences
The Kruskal-Wallis tests between the honeys produced in the different production areas revealed significant differences regarding only the Cr and Fe content and the EC. The significant differences of EC are probably due to differences of the botanical origin of the samples (see also discussion). The highest average Cr values were found in the city samples, 0.010mg/kg, followed by the village samples with 0.006mg/kg, and the rural and the mountain samples with an average of 0.004 mg/kg. There was a significant difference between the samples originating from the countryside on one hand and the city and the village samples on the other hand (Mann-Whitney tests, Bonferroni significance level: 0.0083). The average Fe values in the sample from mountain, rural, city and village area were 0.73, 1.34, 1.70 and 1.80 mg/kg,

respectively. There were significant differences between a) the mountain honeys compared to the city and the village honeys and b) the country samples versus the village samples (Mann-Whitney tests, Bonferroni significance level 0.0083).

The levels of Pb and Cd did not significantly depend on the geographical origin of honey. The highest Pb values were found in samples from village and country areas, while all values in the city honeys were relatively low.

Differences due botanical origin of honey
With exception of Ni the concentration of the other elements in the honeydew samples was significantly higher in the blossom honeys (*Table 5*).

Comparisons of the elements in the different unifloral honeys by Kruskal-Wallis tests revealed significant differences in these groups (*Table 6*). The order of total trace element content was as follows: fir > chestnut > mountain blossom > rhododendron > mixed blossom > lime > rape > dandelion > acacia. Regarding conductivity chestnut and fir honeys have the highest EC values as expected while acacia honeys have lowest values. But for the other types the order does not follow that of total trace elements.

In multivariate analysis a tentative classification of the honeys by PCA and subsequent LDA of the PC scores was carried out (*Table 7*). On average, 76 % of the honeys were classified correctly. Acacia, chestnut and fir honeys were classified best,

while other honeys like the mountain, rhododendron and mixed blossom were less successfully classified.

In order to estimate the importance of individual measurands for classification we also performed LDA directly with standardized measurands applying backward elimination of variables. Table 8 shows that the electrical conductivity with a partial F-to-remove value of 32 is the most useful measurand for the classification. Ni was the most important element for classification, Fe, Mn and Cd having also a small importance. The F values for the other elements being smaller than 4, these elements were not used for the classification functions. It is clear that all these results from multivariate evaluations must be interpreted with care because the number of samples per group is very small in most cases.

Table 5. Trace elements and electrical conductivity of blossom (n = 54) and honeydew (n = 19). Concentrations are in mg/kg, EC in mS/cm. p-values refer to the Mann-Whitney U test.

		Cd	**Pb**	**Cr**	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**EC**
B	Minimum	0.000	0.003	0.000	0.125	0.136	0.002	0.051	0.016	0.10
	Maximum	0.008	0.329	0.014	4.440	4.187	1.190	2.023	2.477	0.50
	Median	0.001	0.020	0.003	0.541	0.819	0.063	0.383	0.708	0.38
	Mean	0.002	0.037	0.004	0.764	0.941	0.152	0.429	0.822	0.33
H	Minimum	0.000	0.012	0.001	0.569	0.386	0.028	0.228	0.408	0.85
	Maximum	0.026	0.179	0.026	10.197	9.852	1.966	3.317	4.133	2.41
	Median	0.005	0.028	0.004	5.422	2.833	0.057	1.320	1.567	0.98
	Mean	0.008	0.048	0.007	5.720	2.769	0.665	1.450	1.528	1.18
	p-value	0.001	0.032	0.031	< 0.001	0.003	0.34	< 0.001	0.001	< 0.001

Table 6. Trace elements and electrical conductivity in unifloral and mixed blossom honeys Concentrations are in mg/kg, EC in mS/cm, p- values refer to Kruskal-Wallis tests.

		Cd	**Pb**	**Cr**	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**EC**
Acacia	mean	0.000	0.013	0.003	0.453	0.278	0.056	0.180	0.217	0.120
	sd	0.000	0.006	0.001	0.277	0.174	0.043	0.169	0.123	0.018
Chestnut	mean	0.001	0.030	0.003	6.167	0.602	0.041	0.399	0.662	1.350
	sd	0.000	0.033	0.002	2.006	0.170	0.015	0.171	0.188	0.197
Dandelion	mean	0.002	0.028	0.001	0.258	0.470	0.064	0.471	0.645	0.470
	sd	0.001	0.025	0.001	0.124	0.115	0.043	0.211	0.429	0.049
Lime	mean	0.002	0.038	0.003	1.292	0.654	0.040	0.382	0.999	0.660
	sd	0.001	0.036	0.004	0.873	0.426	0.025	0.112	0.825	0.112
Rape	mean	0.001	0.020	0.002	0.416	0.699	0.031	0.265	0.692	0.220
	sd	0.000	0.008	0.001	0.071	0.114	0.008	0.039	0.094	0.024
Rhododendron	mean	0.005	0.053	0.006	1.400	0.570	0.150	0.959	0.973	0.310
	sd	0.003	0.030	0.004	1.417	0.171	0.105	0.589	0.347	0.113
Fir	mean	0.016	0.037	0.005	6.077	3.436	1.569	2.605	1.963	0.970
	sd	0.005	0.018	0.003	1.948	0.863	0.289	0.670	0.389	0.109
Mountain blossom	mean	0.001	0.017	0.003	1.991	1.010	0.196	0.310	1.016	0.440
	sd	0.001	0.012	0.002	0.811	0.294	0.173	0.169	0.334	0.089
Mixed blossom	mean	0.001	0.034	0.005	0.900	1.545	0.101	0.449	1.017	0.390
	sd	0.001	0.045	0.003	0.827	0.557	0.127	0.179	0.510	0.084
	p	0.000	0.020	0.003	0.000	0.000	0.000	0.000	0.000	0.000

Table 7. Linear Discriminant Analysis: Jackknife classification matrix. Number of samples in brackets

	Ac	Ro	Mo	Mb	Ch	Da	Li	Ra	Fir	% correct
Acacia (7)	7	0	0	0	0	0	0	0	0	**100**
Rhododendron (7)	0	4	1	0	0	2	0	0	0	**57**
Mountain (6)	0	0	1	2	0	2	0	1	0	**17**
Mixed blossom (16)	1	0	1	11	0	2	0	1	0	**69**
Chestnut (7)	0	0	0	0	7	0	0	0	0	**100**
Dandelion (8)	0	0	0	0	0	7	1	0	0	**88**
Lime (8)	0	1	0	0	0	1	6	0	0	**75**
Rape (6)	1	0	0	0	0	0	0	5	0	**83**
Fir (5)	0	0	0	0	0	0	0	0	5	**100**
Total	9	5	3	13	7	14	7	7	5	**76**

Table 8. Partial F values in Linear Discriminant Analysis

Measurand	F-to-remove	Measurand	F-to-enter
Cd	4.06	Pb	0.55
Mn	5.54	Cr	3.13
Fe	12.5	Cu	2.68
Ni	13.68	Zn	1.81
EC	32.06		

Discussion

Botanical factors

The results show that most elements correlate significantly with electrical conductivity, the most widely used parameter for the determination of the botanical origin of honey. The trace elements Ni, Fe, Mn and Cd, combined with the electrical conductivity could be used for the classification of unifloral honeys. Due to the small number of samples used for each type of honey, these results are of preliminary nature and more extensive studies with a higher number of samples per honey type should be carried out. Most studies published on the discrimination of unifloral honeys do not include polyfloral honeys (Bogdanov *et al.*, 2004). Indeed, it is much easier to discriminate unifloral honeys. However, the main problem in routine honey control is to decide whether a honey is unifloral or polyfloral. The fact that trace elements can be used for this purpose is promising. Further studies with more unifloral and polyfloral honeys, including also more trace elements, should be carried out in order to verify the potential of trace element analysis for the classification of unifloral honeys.

Environmental and geographical factors

As pointed out in the introduction, honey has often been used as an indicator of heavy metal contamination. Pb, Cd are the most toxic trace elements, which have predominantly an anthropogenic source. Therefore, these elements are frequently studied in honey. The contamination level of the toxic elements Pb and Cd measured in our study was low and the honey safe. There are no specific MRL values for honeys, but values of 0.1 mg/kg for Cd and 1 mg/kg for Pb has been suggested for the EU (Byrne, 2000). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended provisional tolerable weekly intakes (PTWI) of cadmium and lead of 7 µg/kg body weight (bw) and 25 µg/kg bw, respectively. Based on the annual per capita honey consumption in Switzerland (1.4 kg/person) and the mean concentrations listed in *Table 2*, the weekly contributions of cadmium and lead to the overall dietary intake of these contaminants would be very small and well below the recommended values. The Pb levels found were comparable to those reported in other recent studies, while the Cd levels are lower, by approximately a factor of 2 (Conti and Botre, 2001; Tuzen, 2002; Tuzen and Soylak, 2005). The level of these two elements was significantly lower in the blossom honeys than in the honeydew honeys, a finding which was also reported in other studies (Altmann, 1983; Bogdanov *et al.*, 1986). The Pb and the Cd levels, with averages of 0.041 and 0.003 mg/kg found in this study, were considerably lower than the means of 0.17 and 0.007 mg/kg found in an earlier study carried out in honeys harvested in Switzerland some 20 years ago (Bogdanov *et al.*, 1986). The Pb concentration decrease can be explained by the use of car engine catalysts during the past 10 years. Also, the utilisation of Cd has been restricted in Switzerland in recent years.

There were differences between the honeys produced in the different areas only regarding Fe and Cr content and EC. The difference of the EC values is probably due to differences in the botanical origin of honey. On the other hand, Pb and Cr were the only elements which did not significantly correlate to the electrical conductivity of honey. One explanation for this behaviour is that these elements are present in honey as metals or non-dissociating compounds, and not as complex cations.

Another explanation is that they do not reach honey via the nectar or honeydew, but reach it probably directly via air or are added by the bees. Pb, and to a smaller degree also Cr, are air contaminants and can directly contaminate nectar and honeydew. As the results of this study show Cr is not an important element for the differentiation of the unifloral honeys. It seems that the differences of the Cr content in the different sample areas are due to environmental or geographical factors. It has been postulated that the Cr content of honey depends on the climatic conditions (Petrovic *et al*., 1994). The interpretation of the differences in Fe content between the samples of the various production areas is more difficult. Fe is an important element for the classification of unifloral honeys. The statistically significant differences between samples from different origins might be due to differences of the botanical origin.

The concentrations of the other trace elements measured in the present study, Cr, Ni, Cu, Zn, Mn and Fe are similar to the values found in other recent studies (Golob *et al*., 2005; Nozal Nalda *et al*., 2005; Terrab *et al*., 2004). These elements are important for biological processes and are toxic only at much higher levels than those encountered in honey. Indeed, the toxic levels for these contaminants lie in the higher ppm range.

All of the trace elements determined in this work can be both from natural sources (soil, plants) and anthropogenic sources. Pb, Cd, and Zn particularly, but also Cu, Cr, and Ni, are well known as potential air or soil contaminants of anthropogenic origin, and are of course also found as natural ingredients of soil minerals, as are Fe and Mn. Apart from direct excretion via nectar, such elements might find other ways to honey: by deposition as dusts or aerosols onto flower and nectars, onto leaf surfaces and honeydew or on the bees themselves. Our results show that the botanical factors have the greatest influence on the trace element content of honey. Thus, in future studies using honey as an indicator of heavy metal contamination, the botanical origin of honey should be taken into consideration. Ideally, the same type of honey from different areas should be studied. The use of trace elements for the classification of unifloral studies should also be proven in further studies by increasing the number of samples in order to reach statistically more reliable conclusions.

Acknowledgements

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