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Elemental Composition of Honeys from Greece-Possible Use as Environmental Indicators

Sager M[1]* and Maleviti E[2]

[1] *Special investigations in element analysis, Austrian Agency for Health and Food Safety, Spargelfeld strasse 191, 1220 Vienna, Austria*
[2] *Centre for Environmental Strategy, School of Engineering, University of Surrey, GU2 7XH Guildford, Surrey, Great Britain*

Abstract

The evaluation of trace element concentration patterns of honey samples was tested either to indicate the authenticity of the sampling location, or to indicate environmental contaminations. Greece was selected because of its heterogenous structure, having plains (<100 m above sea level), hills (100-500 m) and mountains, as well as big industrial cities (Athens, Patras), small towns and rural areas at rather close distances to one another, as well as occasionally proximity to the seaside. Contrary to plant leaves, honey samples might be indicative for an area of 7 km². 4 grams of honey were gently digested with nitric acid in open Erlenmeyer flasks on a hot plate, made up to 25 ml with water, and submitted to multi-element determinations by ICP-OES (inductively coupled plasma optical emission spectroscopy), reading 25 elements, of which 16 could be finally used; others were below detection limits. In addition, Cd-Cr-Mo-Pb were determined by graphite furnace AAS (atomic absorption spectrometry).

The botanical origin largely dominated the trace element concentration patterns based on sample weights. Honeydew honeys were higher in ash and most element contents, except boron. Because honeydew honeys were largely found in the mountains and rural areas, this counteracted effects of enviromental pollution. Ash-based concentratin data, however, might reflect dust immission within the area of the bees´ activity. Within ash-based concentration data, some environmental trends could be noticed. Na-B-Cr-Mo-V-Zn decreased with the distance to the sea, B-Li-Na-V increased with increasing population, and Cr-Li-Ni increased with increasing height above sea level, the latter reflecting abrasion of the Greek basic rocks. With respect to their mean occurrence in the earth crust, K-P-S, and particularly B get enriched in ash-based concentration data of honey samples. Possible environmental contaminations were defined as outliers within the ash-based sample dataset.

Keywords: Honey; Trace elements; Environmental pollution indicator

Introduction

In the EU (European Union), honey is considered as food of animal origin, according to the directive 23/1996 [1]. Honey is one of the least mineralized foods. Its mineral contents are generally less than common plant samples, and even cereals. Honey from wood contains about 0,5% of ash, and below 0,2% of ash from blossoms. It contains 82% sugars on the average, its protein contents is usually lower than 0,5%. Annual consumption of honey is about 1,2 kg per person in Germany and 0,4 kg per person in Italy [1]. The majority of consumers are children [2]. Honeys reflect the mineral components of plants, soil and atmosphere, variabilities caused by floral density, season and rainfall, as well as the equipment [3]. The native bee species in Europe and Africa is *Apis mellifera*, whereas in Asia it is *Apis cerana, Apis dorsata, Apis florea* and *Apis laboriosa* [4]. Honeybees are continuously exposed to potential pollutants present in widespread foraging areas. Bees collect honey within an area of about 7 km², from spring till autumn, contrary to sampling of green plants from one spot. They distribute the honey homogenously between various honeycombs within the same hive. At the same sampling site, however, some hive to hive variations might appear [5]. Sampling in Greece offers the opportunity to evaluate influences of the distance to the sea, sampling height above sea level, population density and type of plants upon the composition of honey. These effects are intercorrelated, however. Modern multi-element methods permit the determination of a lot of elements simultaneously, provided, there is complete recovery in the digestion procedure. For some techniques, like graphite furnace AAS (atomic absorption spectrometry) or flame-AAS, simple dilution may be adequate also. Neutron activation analysis without any further digestion permits the simultaneous determination of Cl and Br in addition to many cations, but no Pb [6]. Pattern recognition techniques have been widely applied to food chemistry in recent years. The application of multivariate analyses proved to be extremely useful for grouping and detecting honey of various origins, geographical and botanical [7].

Material and Methods

Honeys were sampled in summers 1998 and 1999 by biologist Brigitte Schaufler, directly from the producers, and were analyzed in the lab of the author during 2001/2002. For the current data evaluation, the sampling points were identified from a google-map, from which the distance to the sea was estimated by the shortest aerial line to assign the categories <5 km, 5-20 km, and >20 km. Because working bees fly about 2 km, samples got more than 5 km from the seaside have not been presumably directly affected by the sea. The height above sea level was categorized in plains (<100 m), hills (100-500 m) and mountains (>500 m). The population density was categorized into cities (Athens, Patras and suburbs), smaller towns (>5000 inhabitants) and rural areas. Because these categorical variables are intercorrelated, a combined

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***Corresponding Author:** M Sager, Special investigations in element analysis, Austrian Agency for Health and Food Safety, Spargelfeld strasse 191, 1220 Vienna, Austria, Tel: 0043 50555 32801; E-mail: manfred.sager@ages.at

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category termed "location" was created thereof. 4 g of honey sample was weighed into 100 ml Erlenmeyer flasks, 30 ml of suprapure nitric acid was added and gently heated on a hot plate to about 70°C in a fume cupboard. After start of the exothermic reaction, heating could be minimized, and then slowly scaled up to about 140°C. Contrary to wet digestion of green plants, addition of perchloric acid was not necessary. After the samples had gone to almost dryness, the residues were dissolved in 25 ml ultra-pure water (de-ionized water, purified by reverse osmosis). The resulting solutions were run undiluted on an ICP-OES (inductively coupled plasma optical emission spectrometer; Perkin-Elmer 3000 XL) for multi-element analysis. Cd, Pb, Cr and Mo were determined by graphite furnace AAS and standard addition (Perkin Elmer 3030 Z), because of insufficient detection limits in the ICP-OES. K as the main cation was determined by flame-AAS after dilution 1+49 or 1+99 versus pure K- solutions. Surprisingly, there was quantitative recovery of B, As and others from this procedure. The recovery of sulfur, added as methionine, however, was just 60-85%; these data should be handled as informative only. As, Be and Tl were read, but were below detection limit throughout. Ba traces showed some interactions with the glass, therefore no Ba-data are given. The ash contents were calculated as the sum of the oxides of all elements (except C and N), like it has been widely usual in earth sciences. For statistical evaluations, the program IBM-SPSS Statistics version 20 was used. Within results and discussion, for reasons of simplicity, elements within groups will be given in alphabetical order (of the element symbol).

Results

With respect to the low levels of "mineral substances" present in honey, reasonable data from the optical ICP could only be obtained, because 4 g sample finally got enriched in 25 ml digestion solution, and suprapure acid was available. Currently, an ICP-MS (inductively coupled plasma mass spectrometer) is used in addition.

Some samples have been labelled as honeydew, floral, or mixed honeys. Honeydew honeys were higher in Al-Cd-Ca-Cr-Cu-K-Mg-Mn-Ni-P-S-Zn contents, and thus also in ash. Based on ash contents, floral honey contained less Al-K and more B-Ca-Fe-Na-Sr-Zn.

With the distance to the sea, K-Mg-Mn concentrations in the honeys increased on sample weight basis. This causes an increase of ash contents with the distance to the sea as well. Within the ash-based data, Na-B-Cr-Mo-V-Zn decreased with the distance to the sea. Cr-Li-Ni concentrations showed increasing trends with increasing height above sea level.

At the first look, there were hardly linear trends with population density, because most weight based element contents decreased from large towns to rural areas to small towns. The presumable reason for this effect derives from decline of aerial dust concentration versus an increase in honeys from trees, which carry larger loads of "mineral substances". Based on ash contents, B-Li-Na-V increased with increasing population.

In order to recognize multi-element correlations, a lot of factor analyses were tried, and the factor scores plotted against each other, marked by the categories sea level, distance to the sea, population density, and plant origin. Separated ranges in the factor plots could only be achieved versus plant origin, irrespectively of the set of variables used. From the weight-based dataset, high factor weights for Na-K, Al-Fe, Zn-Cd, Pb-Cd, and Mo-Cu hardly appeared in the same component, as it might be expected from geochemical relationships. However, factor analyses of the dataset based on ash, roughly reflected geochemical relationships, within the first 2 components, like Ca-S-Sr, and Al-Li-Mg-Mn, also, if some variables were omitted. Significant relations between factor plots and sampling categories could not be established.

Plots of the factor scores versus single element contents might reveal possible contaminations as outliers. But as correlations were overall poor, looking for outliers is meaningless. From cluster analyses, no proper combination of elements could be found to assign clusters to the categories sea level, distance to the sea, population density or plant origin. Partial correlation analysis assuming the categories as variables and the measured data as independents revealed that the categories sea level and distance to the sea, as well as sea level and population density were highly intercorrelated. This led to the creation of a newly mixed category to mark the locations as coastal cities (=1), small coastal towns (=2), coastal rural plains (=3), coastal hills (=4), inner plains (=5), hills (=6), and mountains (=7). Highest median concentrations of weight based data occurred in the coastal cities for B-Ca-Cr-Fe-Li.-Na-P-Sr-V and in the mountains for Al-Cd-K-Mg-Mn-Ni-S. Referring to ash-based data, medians of C-Cr-Fe-Li-Na-S-Sr-V- concentrations were highest in coastal cities, B-Cd-Mo-Ni-Zn at coastal hills, Co-Cu-P in the inner plains, and just K in the mountains. The medians were hardly changed by the rejection of outliers. If each variable is treated separately, the set of 50 samples seems sufficient to evaluate outliers. These outliers are given in Tables 1 and 2, together with the sampling points, and the

	Ash, mg/kg		K, mg/kg		P. mg/kg		Ca, mg/kg		Mg, mg/kg	
coastal cities	**2236**	1617 - 3600	**1322**	882 - 1718	**107,0**	101,6 - 124,1	**78,0**	46,3 - 96,8	**31,8**	21,5 - 38,0
small coastal towns	**2403**	442 – 3987	**1651**	752 – 2149	**101,0**	74,5 - 134,9	**19,1**	13,0- 57,7	**29,3**	9,7 - 50,3
coastal rural plains	**1143**	771 – 2729	**791**	453 – 1809	**40,6**	34,5 - 129,9	**23,4**	14,2 - 34,4	**13,4**	5,6 - 38,6
coastal hills	**635**	548 – 2729	**391**	311 – 732	**40,1**	31,7 - 48,4	**18,0**	11,3- 51,2	**6,8**	5,9 - 13,7
inner plains	**701**	513 - 913	**418**	308 – 523	**48,9**	32,0 - 56,1	**24,8**	10,6- 43,8	**9,8**	4,9 - 14,4
hills	**1722**	486 – 4061	**1011**	274 – 2696	**63,7**	18,6 - 178,4	**47,6**	16,7 - 88,7	**35,5**	5,5 – 84,4
mountains	**3700**	1256 – 5364	**2494**	712 – 3693	**93,1**	74,7 - 228,0	**35,8**	24,6 - 76,0	**63,6**	27,7 - 81,9
	Na, mg/kg		S, mg/kg		B, mg/kg		Al, mg/kg			
coastal cities	**42,0**	31,2 – 58,2	**30,8**	26,3 - 35,9	**4,54**	3,10 – 7,90	**2,21**	1,60 - 3,46		
small coastal towns	**11,4**	9,6 - 23,1	**24,7**	17,0 - 35,4	**2,89**	2,32 – 3,52	**2,89**	0,62 – 11,9		
coastal rural plains	**19,9**	6,7 - 31,0	**16,4**	4,8 - 34,4	**4,31**	1,91 – 7,02	**2,40**	0,18 - 9,65		
coastal hills	**12,0**	7,2 - 20,0	**10,3**	5,4 - 13,6	**3,10**	0,47 – 3,79	**0,84**	0,46 - 1,92		
inner plains	**10,3**	8,7 - 11,0	**8,8**	7,2 - 16,2	**2,64**	1,60 - 3,25	**0,90**	0,56 - 1,20		

Hills	**15,0**	4,7 - 24,2	**25,2**	11,9 - 43,8	**4,82**	3,23 - 6,50	**1,11**	0,46 - 2,11		
mountains	**17,5**	8,9 - 79,9	**29,9**	14,2 – 60,6	**4,10**	0,81 - 5,80	**3,19**	0,54 – 20,3		
	Fe, mg/kg		Mn, mg/kg		Cu, mg/kg		Ni, mg/kg		Zn, mg/kg	
coastal cities	**6,30**	3,71 - 7,09	**0,43**	0,31 - 0,74	**0,39**	0,25 - 0,46	**0,207**	0,095 - 0,243	**1,19**	0,65 - 1,66
small coastal towns	**2,35**	1,87 - 3,08	**1,30**	0,73 - 1,75	**0,24**	0,18 - 0,52	**0,115**	0,048 - 0,225	**1,65**	1,39 - 1,73
coastal rural plains	**1,92**	1,55 - 6,25	**0,24**	0,13 - 1,33	**0,38**	0,10 - 0,48	**0,048**	0 - 0,182	**1,81**	1,75- 4,87
coastal hills	**1,08**	0,65 - 9,27	**0,15**	0,06 - 0,26	**0,14**	0,09 - 0,20	**0,057**	0,030 - 0,199	**0,89**	0,29 - 2,71
inner plains	**1,03**	0,88 - 2,12	**0,19**	0,07 – 0,75	**0,14**	0,11 - 0,17	**0,065**	0,053 - 0,098	**1,13**	0,28 - 1,93
hills	**3,70**	0,35 - 5,43	**0,91**	0,04 – 12,94	**0,52**	0,05 - 0,75	**0,161**	0,044 - 0,234	**1,73**	0,75 - 4,48
mountains	**3,31**	1,05 - 5,41	**1,46**	0,35 - 4,04	**0,49**	0,29 - 0,72	**0,165**	0,088 - 1,822	**1,27**	0,64 - 4,33
	Cd, µg/kg		Pb, µg/kg		Cr, µg/kg		Co, µg/kg			
coastal cities	**1,55**	1,13 - 1,66	**43,4**	4,7 – 154,1	**41,00**	6,20 - 47,8	**10,25**	6,18 - 11,15		
small coastal towns	**1,59**	0,31 - 2,18	**6,1**	2,6 – 42,3	**9,16**	7,83 - 13,0	**8,30**	1,73 - 14,9		
coastal rural plains	**0,80**	0,61 – 12,45	**68,8**	67,2- 311,5	**8,55**	5,20 - 13,9	**1,15**	0 - 13,2		
coastal hills	**0,81**	0,18 - 1,31	**16,4**	4,8 – 60,4	**4,78**	3,60 – 10,7	**1,18**	0 - 12,1		
inner plains	**0,68**	0,65 - 0,72	**15,4**	5,5 - 38,5	**3,10**	1,50 – 15,5	**5,25**	0 - 10,2		
Hills	**0,85**	0,10 - 3,56	**20,5**	0 - 50,9	**11,80**	4,20- 19,6	**11,54**	3,58- 13,9		
Mountains	**1,63**	0,80 - 5,33	**31,1**	6,9 - 51,6	**14,10**	9,00 – 20,7	**4,52**	0 - 24,1		
	Sr, µg/kg		Li, µg/kg		Mo, µg/kg		V, µg/kg			
coastal cities	**142**	104 – 197	**10,0**	1,4 - 11,1	**5,30**	4,77 – 9,50	**24,0**	3,0 - 30,0		
small coastal towns	**26**	23 – 50	**1,5**	0,9 - 2,4	**4,88**	2,98 - 5,80	**0,7**	0 - 5,2		
coastal rural plains	**36**	34- 170	**1,6**	0,4 - 2,3	**8,80**	4,95 – 9.30	**2,3**	2,0 - 7,0		
coastal hills	**25**	10 - 46	**1,2**	0,9 - 3,6	**5,30**	1,80 - 9,60	**0**	0 - 7,3		
inner plains	**28**	7 - 88	**3,0**	0,8 - 4,3	**5,80**	3,90 - 8,10	**0,1**	0 - 4,1		
Hills	**72**	44 - 124	**4,2**	1,7 – 72,3	**6,00**	4,10 - 8,80	**2,5**	0 - 3,0		
Mountains	**84**	43 - 128	**4,3**	1,7 – 8,3	**6,10**	2,50 - 16,9	**1,6**	0 – 6,3		

Table 1: Median concentration and ranges (corrected for outliers) at different kinds of location, based on sample weight.

location category. In order to find presumable contaminations, the dataset has been evaluated based both upon sample weight as such, as well as upon the calculated ash contents. Versus mean crust values, the ash of honey is strongly enriched with B, P, Zn and K. Enrichment of boron turned out to be 1000 to 6000 fold. To the contrary, it is depleted in Al, Fe, Na and Cr (among the elements investigated). A lot of other elements, however, occur in the ash of honey in about the same order of magnitude like the mean of the earth crust, and are just diluted by the sugar matrix (alphabetically): Ca, Cu, Li, Mg, Mn, Mo, Ni, Pb, Sr [5] (Table 3).

After factor analysis, no proper fields could be assigned to the mixed location categories from factor plots against one another. Factor analysis [5] of the entire data set (including outliers) does not yield very clear relations; the first component contains high factor loads of Cd/Cu/K/Li/Mg/Mo/Na/P/S/Sr. After rotation, the rather unusual relation Fe-Pb remains. Cd, Pb and Zn appear mainly in different factors, and Na is quite independent from K. (Table 4 and 5).

Discussion

The original intention of sampling bee honey was just to establish a method to monitor atmospheric pollution over the entire area, where the bees collect their honey. The real situation, however, is much more complex. Bees avoid streets used by traffic; at least they never come back from a highway. The type of plant is of great influence, and in gardens and urban parks, a lot of nonnative species can be found, thus changing the expected composition. Honey contains some amount of aromatic carbonic acids, e.g. benzoic acid, phenylacetic acid, mandelic acid, phenylpropanoic acid and others [8]. Therefore its pH is slightly acid. Within the above sample set, median pH was 4,0 (range pH 3,5-4,8). This is sufficient to dissolve some of adherent dust. Usually, more than one hive is acting at the same site. Therefore, samples were received from beekeepers, who usually mix the honey of their various hives on site. Within a previous study, in order to investigate the hive to hive variations, 5 hives were sampled from a rape field near Wiener Neustadt, 5 near Holzing (Amstetten region), and 4 near Hollabrunn (all in Lower Austria). From each hive, 4 different honeycombs were taken as separate samples, to obtain the precision of the sampling+analysis within one single hive. Though concentrations for this rape honey were generally lower for the majority of elements, than for the reference limetree samples, the (absolute) precision within a single hive was the same, just Mn gave an additional variation due to sampling from different honeycombs. In all 3 data sets, however, significant differences among hives frequently emerged, like one hive contrary to the 4 others, or 2 hives contrary to the 3 others. Theses deviations were irregulary, and no general trend could be observed. The most plausible explanation might be the collection of honey from adjacent other plants accidentially found [5]. Honeydew honey has in general a higher total content of "mineral substances" than nectar or floral honeys, in particular K, Na, Mg, Fe, Cu, and Mn. In Austria, most significant differences between floral and honeydew honeys appeared in B-Cu-Mn [4]. Environmental contamination means a deviation from the level supposed to occur without human activities.

	K, %		P, %		Ca, %		Mg, %		Na, %	
Mean crust	**2,14**		**0,076**		**3,85**		**2,20**		**2,36**	
coastal cities	**59,78**	56,5 – 65,5	**4,64**	4,44 – 5,14	**4,20**	1,77 – 5,31	**1,37**	1,03 -. 1,70	**2,52**	1,18 – 4,86
small coastal towns	**67,24**	50,9 – 69,1	**4,51**	3,92 – 8,07	**1,28**	0,42 – 3,88	**1,27**	0,79 – 1,58	**0,95**	0,24 – 2,90
coastal rural plains	**66,29**	58,8 - 69,2	**4,47**	3,55 – 4,76	**1,25**	0,86 - 4,46	**1,42**	0,49 – 1,74	**0,86**	0,73 – 2,71
coastal hills	**62,21**	57,4 – 68,9	**5,55**	3,74 – 5,78	**2,44**	1,07 – 4,74	**1,04**	0,93 – 1,29	**1,51**	1,14 – 1,87
inner plains	**60,02**	56,3 – 63,8	**5,84**	5,27 – 6,42	**3,59**	2,07 – 4,80	**1,58**	0,81 – 1,75	**1,38**	1,11 – 1,43
hills	**63,19**	56,4 – 67,3	**4,39**	3,33 – 5,84	**3,46**	1,09 – 4,60	**1,61**	1,28 – 2,27	**0,64**	0,32 – 1,76
mountains	**68,22**	62,1 – 72,4	**3,86**	2,49 – 4,86	**1,23**	0,42 – 4,87	**1,51**	1,35 – 2,22	**0,73**	0,14 – 3,83
	Al, %		S, mg/kg				B, mg/kg			
Mean crust	**7,96**		**0,070**				**11**			
coastal cities	**0,137**	0,070 – 0,257	**1,48**	1,15 – 1,73			**4113**	1385 - 5205		
small coastal towns	**0,231**	0,082 – 0,406	**1,16**	0,89 – 2,22			**1746**	883 - 6547		
coastal rural plains	**0,210**	0,024 – 0,354	**1,26**	0,42 – 2,12			**1667**	1578 - 9111		
coastal hills	**0,119**	0,046 – 0,283	**1,19**	0,78 – 1,66			**4440**	2153 - 6987		
inner plains	**0,079**	0,053 – 0,131	**1,27**	1,26 – 1,41			**3559**	3114 - 7914		
hills	**0,105**	0,032 – 0,122	**1,29**	0,80 – 2,64			**1879**	276 - 9254		
mountains	**0,200**	0,047 – 0,347	**1,04**	0,74 – 1,62			**707**	89 - 3513		
	Fe, mg/kg		Mn, mg/kg		Cu, mg/kg		Ni, mg/kg		Zn, mg/kg	
	43200		**716**		**25**		**56**		**65**	
coastal cities	**2456**	1386 - 8222	**329**	203 - 438	**160**	125 - 206	**77,8**	60,0 - 104,4	**485**	426 - 2370
small coastal towns	**1649**	647 - 2037	**541**	227 - 1396	**160**	102 - 534	**61,4**	38,8 - 138,1	**882**	415 - 3150
coastal rural plains	**2005**	1679 - 2289	**307**	117 - 487	**175**	90 - 497	**62,3**	0,0 - 66,7	**1535**	663 - 6311
coastal hills	**1737**	1053 - 5270	**243**	96 - 341	**162**	112 - 181	**112,7**	35,5 - 251,8	**1541**	341 - 3040
inner plains	**1410**	821 - 1448	**331**	122 - 939	**215**	120 - 219	**93,7**	83,2 - 103,3	**678**	455 - 2116
hills	**1167**	695 - 2149	**652**	83 - 3688	**125**	106 - 207	**87,8**	35,3 - 199,2	**1037**	330 - 1710
mountains	**924**	473 - 1575	**591**	373 - 794	**176**	75 - 269	**97,0**	25,6 - 486,8	**517**	147 - 807
	Cd, mg/kg		Pb, mg/kg		Cr, mg/kg		Co, mg/kg			
	0,10		**14,8**		**126**		**24**			
coastal cities	**0,74**	0,31 - 1,37	**37,7**	5,5 - 215,6	**14,85**	2,36 - 31,49	**4,45**	0,15 - 7,17		
small coastal towns	**0,71**	0,68 - 0,92	**7,7**	1,2 - 20,5	**5,19**	3,26 - 6,41	**4,36**	1,43 - 8,65		
coastal rural plains	**0,79**	0,70 - 4,56	**89,3**	24,6 - 272,6	**6,75**	5,09 - 7,48	**1,49**	0,00 - 4,82		
coastal hills	**0,83**	0,33 - 1,10	**20,5**	7,6 - 63,6	**6,87**	0,00 - 10,07	**2,29**	0,00 - 14,87		
inner plains	**0,82**	0,71 - 0,91	**14,9**	9,0 - 25,1	**3,39**	1,26 - 16,30	**6,30**	4,14 - 9,05		
hills	**0,38**	0,21 - 0,76	**6,5**	-0,4 - 110,9	**4,21**	0,60 - 13,12	**3,96**	2,29 - 7,29		
mountains	**0,71**	0,34 - 1,86	**8,8**	1,3 - 19,4	**5,04**	0,39 - 7,17	**2,65**	0,00 - 7,86		
	Sr, mg/kg		Li, mg/kg		Mo, mg/kg		V, mg/kg			
Mean crust	**333**		**18**		**1,1**		**98**			
coastal cities	**68,6**	19,3 - 111,4	**5,0**	1,6 - 14,1	**5,53**	2,17 - 7,50	**12,21**	1,14 - 13,42		
small coastal towns	**27,8**	8,6 - 51,8	**1,4**	0,4 - 3,7	**2,09**	1,21 - 6,73	**1,07**	0,00 - 11,69		
coastal rural plains	**29,6**	13,3 - 221,0	**0,8**	0,5 - 1,4	**4,33**	3,41 - 11,42	**2,57**	2,03 - 2,59		
coastal hills	**34,0**	9,4 - 55,5	**2,0**	0,8 - 2,7	**6,35**	2,13 - 9,13	**0,00**	0,00 - 4,26		
inner plains	**34,0**	12,9 - 60,1	**3,3**	2,5 - 4,7	**4,27**	2,30 - 7,30	**0,13**	0,00 - 1,60		
hills	**35,4**	13,2 - 95,8	**3,7**	0,8 - 16,6	**3,02**	1,53 - 8,44	**0,77**	0,00 - 6,94		
mountains	**27,9**	10,5 - 73,5	**1,6**	0,3 - 4,7	**2,10**	1,14 - 4,43	**0,74**	0,00 - 2,92		

Table 2 Median concentration and ranges (corrected for outliers) at different kinds of location, based on ash.

Label	Sampling site		Plants	Outliers	Outliers ash-based
A1	Athens, Agricultural Univ.	1	Eukalyptus	B,Cr,Mo,V	Li, V
A2	Athens, Agricultural Univ.	1	Eukalyptus	Cr, V	Cr, V
G15	Laconia, Areopolis-Lagra	1	blossoms		
G25	Messinia, Kalamata	1	Orange, blossoms		
P1	Patras, city	1		Cr, Na, Pb, Sr, V	Na, V
G10	Messinia, Kalamata-Avia	2	Thymian		
G6	Messinia, Kalamata-Avia	2	trees	Al	Al
G7	Messinia, Kalamata-Avia	2	trees/Salvia		Cu, K, P, V
G8	Messinia, Kalamata-Avia	2	Salvia		
G9	Messinia, Kalamata-Avia	2	trees		
P15	Achaia, Rogitika, Paralia	2	Orange, flowers		
G16	Laconia, Skala	3	Orange,blossoms		B, Cu, Sr
G28	Zizanio-Koroni	3	blossoms		Cd
P13	Achaia, Gomosto, Movri	3		Pb	Mg
G11	Messinia, Katafygio	4	Thymian/Salvia/Trifolium		
G19	Laconia, Mani	4	Salvia, blossoms		
G21	Laconia, Mani +Sparti	4	Orange, blossoms		Co, Mo, S
G4	Messinia, Sotirianika	4	blossoms	Fe, Pb	Ca, Co, Cr, Fe, Pb
G5	Messinia, Stouropigion	4	Thymian		
P10	Elia, Tragana, Gargalionoi	4		Fe	Mg
P4	Anatoliki-Mani	4	Phlomis		
G12	Messinia, Agios Nicon	5	Thymian/Origanum/Vicia		
G13	Messinia, Agios Nicon	5	Vicia,Thymian,Origanum	Zn	Cr, Fe, Mo, Zn
G26	Elia, Tropaia	5	blossoms		Sr
P6	Thermon, Agrinio	5	blossoms		
P9	Achaia, Arla, Olenia	5	Erica		Mg, P
G1	Arcadia, Leontari	6	blossoms/Orange		
G2	Arcadia, Leontari	6	blossoms		
G22	Laconia, Sparti	6	Blossoms, Eukalyptus	Al, P, S	
G23	Laconia, Spartis-Skalas	6	Orange, blossoms		B, Ca, K, Mo, S, Sr
G24	Laconia, Spartis-Amyklon	6	Orange, Salvia, blossoms		B, Co, S
P18	Euboea	6	Coniferes		
P5	Lefkas island	6	Thymian/ blossoms		
P7	Kalavryta	6	blossoms	Li, Mg, Mn	Li, Mn
P8	Kalavryta	6	trees	Li, Mn	Li, Mn
G17	Laconia, Parnon	7	trees, Thymian	Al	
G18	Laconia, Taygetus	7	trees, Thymian	Mo	
G20	Laconia, Parnon	7	trees, Thymian	Al, K, Mg, Mo, S, Sr	
G27	Messinia, Vlaseika Aipeion	7	mixed		Ca, K, Na
G3	Arcadia, Vytina	7	trees	Ni, S	Cd
P11	Achaia, Leontio	7			
P14	Helia, Tsipiana, Lasion	7			
P19	Achaia, Kalentzi	7		Ni	Ni
P2	Karpenissi 80%, Arta 20%	7		Al, S	Al
P3	Arta	7	Orange		

Table 3: Sampling sites and outliers, detected by treating each variable separately.

Contamination sources are dust input by the bees themselves, as well as dirt from processing and storing the honey. In urban areas, Cr, V, and Pb might be higher than expected. Top Pb concentrations were e.g. found in Kathmandu/Nepal (Sager et al., unpublished). If these effects come from unexpected plant origins, this is not seen in ash-based data. Similarly, in the samples from Athens, elevated B and Mo may be due to exotic plants. Because Patras gets mainly winds from the seaside from its west, high Na appeared, but not so much in Athens.

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Component Matrix[a]

	Component					
	1	2	3	4	5	6
	35,9 %	16,0 %	8,9 %	7,6 %	6,3%	5,4 %
Al	,501	-,657	,086	,378	,246	,060
B	,281	,323	-,626	,086	,352	-,025
Ca	,470	,558	,260	-,316	,320	-,242
Cd	,615	-,290	-,017	,433	-,182	-,018
Co	,746	,049	-,057	,051	-,417	-,190
Cr	,448	,536	,180	,205	,482	,018
Cu	,861	,046	-,218	-,103	-,363	,064
Fe	,289	,152	,735	,206	-,225	-,066
K	,817	-,479	,083	,006	,114	-,007
Li	,650	,255	-,171	-,556	-,227	,035
Mg	,825	-,359	,217	-,190	,141	-,121
Mn	,446	-,278	,329	-,681	,145	-,153
Mo	,567	-,068	-,111	,049	,241	,507
Na	,580	,581	-,285	,045	-,291	,041
Ni	,244	-,025	-,134	,222	-,281	-,267
P	,882	-,354	-,090	,054	-,022	,006
Pb	,067	,561	,583	,330	-,084	-,111
S	,899	-,317	,002	,133	,144	-,045
Sr	,667	,495	-,035	-,026	,048	,017
V	,530	,680	-,090	,233	,143	,054
Zn	,136	,119	,347	-,137	-,202	,794

a. 6 components extracted.

Varimax-Rotated Component Matrix[a]

	Component					
	1	2	3	4	5	6
Al	,927	-,080	-,158	-,076	,003	-,019
B	,059	,578	,174	-,143	-,546	-,144
Ca	-,052	,700	,123	,552	,206	-,067
Cd	,704	,012	,345	-,209	,149	-,043
Co	,404	,121	,739	,114	,173	-,084
Cr	,159	,852	-,062	,045	,191	,048
Cu	,448	,177	,801	,180	-,066	,161
Fe	,169	,104	,098	,093	,822	,101
K	,883	,044	,192	,309	-,007	,043
Li	,055	,240	,677	,538	-,165	,208
Mg	,759	,109	,193	,543	,086	,004
Mn	,292	-,067	,027	,893	,039	,055
Mo	,514	,324	,084	,012	-,224	,479
Na	-,012	,515	,750	-,069	-,012	,103
Ni	,180	-,030	,376	-,148	,088	-,272
P	,831	,102	,409	,196	-,077	,041
Pb	-,173	,412	,027	-,118	,757	-,001
S	,883	,233	,273	,200	-,009	-,016
Sr	,158	,648	,452	,165	,074	,110
V	,054	,822	,349	-,118	,094	,076
Zn	-,006	-,005	,068	,030	,219	,888

Extraction Method: Principal Component Analysis.

Table 4: Results of factor analysis of weight-based data.

Apart from dirt, some elements might be elevated from local geology, but the influence of the soil composition on honey is usually marginal. The same set of elements like given in this work was determined in pure rape honey grown in Austria at 3 different sites. In rape honey, the level of mineral substances is generally low. Significant correlations between concentrations found in honey and in the aqua regia digest of soils were just found for Fe, Mn, Mo, and Ni, but not for all the others [9].

Because dust adhesion to blossoms and bees is one of the sources of the "mineral substances" composition of honeys, this is the reason

Component Matrix[a]

	Component						
	1	2	3	4	5	6	7
Al	-,388	-,603	-,201	-,052	,020	,078	,319
B	,687	-,085	-,381	-,141	-,357	-,300	-,019
Ca	,829	,267	,226	-,305	-,086	-,138	,104
Cd	-,057	-,228	-,188	,017	,490	,285	,359
Co	,456	-,093	,011	-,590	-,074	,441	-,160
Cr	,564	-,057	,536	,304	,060	-,281	,237
Cu	,435	,239	-,217	,417	,328	,251	-,041
Fe	,383	-,358	,729	-,024	,025	,318	-,064
K	-,936	-,062	,210	,067	-,019	-,100	-,034
Li	,203	,538	,036	,308	-,140	,442	-,153
Mg	-,048	,731	,135	,039	-,108	-,052	,297
Mn	-,264	,639	,106	,045	-,369	,404	,082
Mo	,591	-,376	-,015	,359	-,498	-,097	-,029
Na	,553	,195	-,089	,249	,511	-,012	-,224
Ni	-,005	-,008	-,054	,070	,179	-,262	-,751
P	,512	-,277	-,557	,129	,064	,440	-,045
Pb	,377	-,214	,719	-,305	,255	,191	-,070
S	,787	-,066	-,387	-,188	-,190	,040	,139
Sr	,715	,200	,050	-,226	-,003	-,223	,033
V	,457	,204	-,062	,115	,510	-,268	,279
Zn	,318	-,360	,270	,709	-,287	,091	,039

Extraction Method: Principal Component Analysis.

7 components extracted.

Varimax-Rotated Component Matrix[a]

	Component						
	1	2	3	4	5	6	7
Al	-,305	-,131	-,262	,034	-,516	,234	,407
B	,855	-,203	-,017	,213	-,146	,103	-,112
Ca	,822	,380	,197	,001	,173	-,223	,005
Cd	-,216	,039	,347	-,159	-,250	,284	,451
Co	,486	,489	-,158	-,268	,078	,461	,005
Cr	,270	,406	,350	,511	-,057	-,457	,083
Cu	,101	-,052	,675	,159	,265	,274	-,023
Fe	,024	,891	-,018	,339	-,022	,046	,032
K	-,801	-,160	-,412	-,146	-,081	-,274	-,024
Li	,024	,023	,205	,133	,754	,168	-,085
Mg	,069	-,153	,095	-,169	,590	-,456	,195
Mn	-,152	-,096	-,236	-,116	,819	-,056	,152
Mo	,468	,017	-,077	,788	-,093	,135	-,066
Na	,212	,107	,760	,034	,052	,108	-,274
Ni	-,084	-,034	,116	-,050	-,135	,053	-,791
P	,332	-,088	,300	,193	-,064	,778	,103
Pb	,102	,934	,078	-,021	-,095	-,078	-,011
S	,842	-,035	,141	,120	-,041	,317	,137
Sr	,721	,196	,248	-,016	,043	-,166	-,077
V	,285	-,006	,709	-,061	-,117	-,242	,133
Zn	-,049	,152	,086	,928	,030	,058	,037

Extraction Method: Principal Component Analysis.

Table 5: Factor analysis of the ash based dataset.

why honey can be selected as an environmental indicator substance. In order to reject the dilution done by various sugars, it is reasonable to compare data based on ash weight with mean crust values, or at least with local soil or geological data. In general, honeys contain K-P-S and in particular B enriched versus other element concentrations, with respect to their occurrence in the earth crust. Enrichment of boron is about 500 fold in floral honeys and 50 fold in honeydew honeys, which makes the main difference to commercial sugars, and is probably one of the reasons for health promoting actions. Lower amounts of Al, Fe, Cr, V, Li and even partially Mg may be due to the fact that digestions were done without hydrofluoric acid; these figures should rather be compared with local soil or geological data obtained from aqua regia extracts. Fluctuation of Mn and Sr, which usually dissolve quite well, could be also due to local geology. To the contrary, enrichments of Cd, Pb and sometimes Ni might be interpreted as contaminations, whereas enrichments of Cu-Mo-Zn might have physiological reasons also.

Relations to data found in the literature

Within the last 2 decades, a lot of data about element contents of honey have been published. This short review is limited to papers treating large sample numbers by sufficient sensitive methods. In Turkey, situations at the Western and Southern coast are like in Greece, whereas Anatolia is different. In honeys sampled all over Turkey, Cr-Cu-Mn-Pb were et the same level, whereas Al-Ni were lower, and Cd-Fe-Zn were higher than in Greece [10]. Honeys from Central Anatolia contained Cu-Fe-Mn-Zn within the same range than the samples collected in Greece in this work, apart from 2 outliers of Zn. Cd was generally higher in Anatolia (no traceable reason), and Pb was higher in some Greek urban samples [11]. In honeys from the Republic of Macedonia, where about the same geological formations are met like in Greece, but without influence of the sea, more Cd-Cu-Na, and less Fe-Mg-Mn, but about equal Ca-K-Zn levels were found [12]. Thus it seems that the source of Na is not just the marine aerosol, but there are antropogenic sources also.

In the Mediterranean area, thyme honeys are mainly produced in Greece, Italy Morocco and Spain. The thyme pollen is under-represented in thyme honeys. Element concentration levels in thyme honeys from Spain were reported about 10 times higher in Al-Cr-Co-Cr-Na-Sr-V, 5 times higher in Ca-Mg, about double in Fe-Li-Mo-P-S, and at the same level for Mn-Ni-Zn [13]. As the mean ash contents from mountain honeys were about 1,3 g/kg and from the coast were about 1,9 g/kg, cluster analysis could discriminate between thyme honeys from mountain and coastal regions, above all by differences in Li and P [13]. In Spanish honeys, obtained from stores and cooperatives, cluster analysis of element contents could discriminate between their origins as eucalyptus – heather – orange blossom – rosemary.

Heather and eucalyptus contained more „mineral substances“than the other groups. Alltogether, the Spanish honeys contained more B-Ca-Cu-Na-Sr-Zn, but about equal K-Mg-P than the Greece honeys presented within this work [3].

In honey samples from the Azores and mainland Portugal, correlations between element concentrations in honey, soil, tree bark and lichens was poor. Correlations of honey contents increased in the order soil < lichens < bark. The exact composition of honey depends largely on the flowers used by the bees, which in turn depends on the surrounding flora and ecosystem. Some metal traces, however, originate from production and storage processes such as centrifugation or maturation of the honey, like Sn [6]. Compared with the dataset from Greece presented in this work, Fe-Mg-Mn-Zn were at about the same level and Al lower. Floral honey from the Azores had less K, whereas Na contents tended to be higher, whereas in mainland Portugal, Na levels were about the same as in Greece [6].

In honeys from Brazilian cities, sampled in 4 regions of moderate to tropical climate, levels of Al-Co-Fe-P-Zn were equal to the levels found in Greece, whereas Mg-Mn-V were higher, and Cd-Cr-Cu-Mo-Ni-Pb were lower [2]. Some of these effects may be due to increase washout of air and soil in tropical regions, besides differences in the plant cover.

In honeys from the Czech Republic (24 samples from 24 regions), honeydew honeys contained higher levels of Al, B, Mg, Mn, Ni, and Zn, whereas Cu was equal and Ca was higher in floral honeys [14]. If all samples are taken in one group, the Greek samples contained Al-Cu-Mn-Zn at about the same level, but less B, Ca, Mg, and Ni than the samples from the Czech Republic [14]. Cluster analysis could discriminate between floral and honeydew honeys in combination of element content and electrolytic conductivity [14]. In samples from Chile, pH was within 3,5-5,5 which is the accepted range for honey. More Cd-Co-Cr-Cu-Sr, about equal levels of Al-Fe-Mn-Pb, and less Zn was found in Chilean honeys compared to Greece, but sampling height above sea level and plant origin had not been given [15]. Highest Pb and Cd came from bee hives that were close to roads and highways. High Al was related to storage in Al- containers [15,16].

Conclusions

Most of the data found in the literature, do not contain exact assignments to the floral or tree composition, nor did the samplers who sampled for this dataset.

The composition of honey is variable, but not as much as local geology. The effects of atmospheric immission and soil composition would be more clearly visible, if honey from the same plant origin could be compared, like it had been with rape honey [9]. In the ash-based dataset, differences between floral and honeydew honeys are largely minimized, and geological effects appear more distinctly. For comparisons with data from other parts of the world, however, only weight-based data have to be considered, because ash as such or several ash-forming elements has not been given.

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