Essential and toxic elements in honeys from a region of central Italy

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Abstract

Levels of iron (Fe), manganese (Mn), chromium (Cr), copper (Cu), zinc (Zn), mercury (Hg), cadmium (Cd) and lead (Pb) in several kinds of honey produced in a region of Central Italy were determined by atomic absorption spectroscopy (AAS). The degree of humidity, sugar content, pH, free acidity, combined acidity (lactones) and total acidity were also measured. These elements were found to be present in honey in various proportions depending upon (1) the area foraged by bees, (2) flower type visited for collection of nectar, and (3) quality of water in the vicinity of the hive. Strong positive correlations occurred between Pb vs Hg, Pb vs Cd, Pb vs Fe, Pb vs Cr, Hg vs Cd, and Hg vs Fe. The honey products produced in Central Italy were of good quality, but not completely free of heavy metal contamination. Compared with established recommended daily intakes, heavy metals or trace element intoxication following honey consumption in Italy is not a concern for human health.

Keywords: Italian honeys; Essential and toxic elements; Atomic Absorption Spectrometry

Introduction

Honey, a product of the elaboration of flower nectar or honeydew by bees, is one of the most complex foods produced naturally (Pisani et al., 2008; Tuzen et al., 2007).

Honey provides energy, with valuable nutritional, healing and prophylactic properties attributed to chemical composition and predominant simple sugars content (Belitz et al., 2004). The general features and elemental composition of honey depend upon its botanical and geographical origin. Honey contains mixture of different carbohydrates, including fructose, glucose, maltose, sucrose, high sugars, proteins, amino acids, vitamins and minerals (Buldini et al., 2001). The contribution of minerals is relatively low and normally accounts for 0.1–0.2% of nectar honeys (Pohl, 2009); this content depends upon the soil type.

Honey is also used as an ingredient or preservative in foodstuffs because of its flavor, color and sweetness. This foodstuffs has healing properties where the moisturizing action of honey around a wound facilitates healing process and high viscosity of honey inhibits infections to penetrate into the body. The antibacterial properties are due to its low acidity and low-level hydrogen peroxide release (Akbari et al., 2012).

Honey may be useful as biomonitor for collecting information regarding the environment within the bees' forage area (a surface of more than 7 km^2). Honey bees come into a contact with different parts of the surroundings and are exposed to potential pollutants when they forage for nectar, pollen, honeydew, or other exudates within such a territory (Bratu and Georgescu, 2005). In this manner, contaminants in air, water, and soil reach the honey and change its composition and quality. Usually, in a neighborhood of mines and steelworks, industrialized and urban areas, or highways in the forage area result in contamination of the apiary locality and this is reflected by higher levels of certain metals in honey, associated with chemical wastes, exhausts, or other pollutants emitted (Yarsan et al., 2007). Honey originating from rural areas, where there is a relatively low density of automotive traffic, typically presents with low levels of heavy metals such as cadmium (Cd) and lead (Pb) (Bratu and Georgescu, 2005). In addition the extent of the contamination may be dependent upon the floral origin of honey plants visited by bees. Accordingly, honeys elaborated from nectar of aromatic plants are characterized by high concentrations of heavy metals since they tend to concentrate pollutants more readily than herbaceous plants.

Honey might also be polluted with certain metals during harvesting and processing and this is primarily related to the apiculture technology used by beekeepers. Different conditions of honey processing result in significant differences in the content of aluminum (Al), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), nickel (Ni), and zinc (Zn). These metals may be released from materials (stainless steel, galvanized steel, and Al) in tools and equipment used for honey crop, extraction, centrifugation, or ripening. The materials of the containers applied for honey processing, shipping, and storage of the extracted and ripened honey or lids may also contribute to an increase in metals in honey (Pisani et al., 2008; Yarsan et al., 2007). The extent of this process is facilitated by honey acidity (pH 3.5–4.8) and its corrosive effect on the surface of tools and containers (Pohl, 2009).

The content of certain metals in honey is of interest in terms of quality and potential adverse human health risks. Excessive levels of some heavy metals are undesirable due to toxicity and adverse effect exerted on humans (Buchanan et al, 2011; Carneiro et al., 2014 Ginsberg, 2012, Nunes et al, 2014; Losfeld et al., 2014). However, the admissible levels for contaminant metals in honey including Cd, Pb, Hg, Cu, and Zn are not established. The directive of the European Union Commission related to honey includes some general and specific properties of honey composition but no guidelines regarding heavy metals content (EC 2001/110). No specific regulation regarding the presence of heavy metals, except for a statement that honey shall be free from them in amounts which may result in a hazard to the human health, is included in the Codex Alimentarius (Codex Alimentarius, 2001). Consequently, the tolerable levels of individual metals that exhibit a potential threat or latent toxicity are regulated by national legislation or standards for other food products (EC 2006/1881).

Taking into account that apiculture is popular in Italy, in fact the number of hives in Italy exceeds 1,000,000 and yearly the country produces approximately 20,000 tons of honey (Benvenuti et al., 2009), it was decided to determine the levels of 8 elements in honeys, subdivided into micro-essential (Mn, Fe, Cu, Zn and Cr) and nonessential or toxic (Cd, Pb and Hg).

Manganese, Fe, Cu and Zn are essential elements required for enzyme metabolism. These elements exert immunomodulatory functions and thus influence susceptibility to the course and the outcome of a variety of viral infections (Desideri et al., 2011). Chromium is an essential element that acts as co-factor in insulin synthesis and in regulation of cholesterol and blood triglyceride; however reduction of Cr VI to lower oxidation states results in carcinogenicity (Demirezen and Uric, 2006; Shi et al, 1999).

Cd, Pb, Hg have no known physiological functions. These metals were reported to be toxic and should be considered as a high risk factor to public health in general (Counter et al, 2009; Ginsberg, 2012; Ratcliffe et al., 1996). Lead toxicity is produced by the ability of Pb to interfere with several enzymes. Lead intoxication damages the nervous, immune and hematopoietic system and may also result in dysfunction of renal tubules, liver and cardiovascular system (Desideri et al., 2012; Luebke et al, 2006). Contamination from Pb is attributed to the use of Pb in batteries, bearing metals, cable coverings, gasoline additives, explosives and ammunition and in the manufacture of pesticides, antifouling paints and analytical reagents (Johnson, 1998). Cadmium is highly toxic to humans adversely affecting brain metabolism and other severe effects on prostate, kidney, liver, lungs and bones (Citak et al., 2012; Ginsberg, 2012). Cadmium is employed in several industrial processes such as: (a) protective coatings (electroplating) for metals like Fe; (b) preparation of Cd-Ni batteries, control rods and shields within nuclear reactors and television phosphors. For non-smoking population the major exposure pathway is through food. Cadmium is readily taken up by plants. Potential source of Cd toxicity is the use of commercial sludge for fertilizing agricultural fields. Mercury is a neurotoxic poison that produces neurobehavioral neuroendocrine effects, immunotoxicity, renal damage and gastrointestinal toxicity (Chen et al., 2011; Sweet and Zelikoff, 2001). The most important anthropogenic sources of Hg pollution in the environment are industrial and urban discharges, mining and combustion, as well as agricultural materials (Ru et al., 2013).

Taking into account that safety of the honey is of great importance, the aim of this study was to determine the levels of Fe, Cr, Mn, Zn, Cu, Pb, Cd and Hg by atomic absorption spectroscopy (AAS) in 21 samples of multifloral honey for (1) quality assessment and control and (2) investigate possible environmental contamination.

MATERIALS AND METHODS

Samples and sampling

Twenty-one samples of honey (19 wildflower, 1 honeydew and 1 acacia) were analyzed. The samples were produced and collected in 2013 from individual beekeepers in Central-Eastern

Italy (Pesaro-Urbino Province) near S. Marino, an area with small-scale mixed farming and with scarce big industries. Further, the urban centers are small and the main town, Urbino, is an historical city.

Each sample, furnished by the local Health Agency (ASUR), was accompanied by a sheet in which the type of honey and provenance were indicated. Table 1 shows the locations of production, kind and botanical origin of the honeys sampled.

Determination of sugars and degree of humidity.

The degree of humidity (%) and the sugar content (expressed in degrees Brix) of honey were determined by using a honey-meter (Bertuzzi of Brugherio, Italy) that uses the principle of refraction of the liquid (at a temperature of 20 °C) which varies with their concentration.

Determination of pH, free acidity, combined acidity (lactones) and total acidity

The acidity of honey is due to the presence of organic acids in varying quantities depending on the botanical origin of honey. These acids are in a state of equilibrium between the free form and variable combined form (lactones). The pH was determined by a direct potentiometric measure method (pH meter 507, Crison Instruments, Spain). The determination of free acidity, combined acidity (lactones) and total acidity is based on measuring potentiometric of the pH and on the acid-base reactions, carried out with two successive titrations to end point. The free acidity was measured by direct titration, lactones hydrolyzed after the addition of alkali to the solution of honey were then determined by back titration.

Atomic absorption spectroscopy (AAS)

For the measure by AAS, it is necessary to completely solubilize the samples; for this purpose, one g of each sample was digested and mineralized (MDS 2000 CEM, Italy) with a mixture of concentrated HNO_3/H_2O_2 (12+3 ml). All chemicals used in sample treatment were ultrapure grade (HNO_3 65%, H_2O_2 30%, Merck Suprapur, Darmstadt, Germany); ultrapure water (Milli-Q system, Millipore Corporation, USA) was used for all solutions. All glassware was cleaned prior to use by soaking in 10% v/v HNO_3 for 24 hr and rinsed with Milli-Q water. The metal concentration was measured using a Perkin Elmer Analyst 300 atomic absorption spectrophotometer with flame atomization (FAAS), graphite furnace system (GFAAS) with HGA 800 auto sampler and with flow injection analysis system (FIAS 100). For Zn the FAAS was employed, while for Cd, Cr, Cu, Fe, Mn and Pb, the GFAAS with HGA 800 system was used. The chemical modifiers are useful to avoid loss of analyte in any of the steps of heating the graphite furnace or decrease the matrix effects. $NH_4H_2PO_4$ was used as matrix modifier for Cd and Pb, and Mg $(NO_3)_2$ for Cr, Fe and Mn. Hg content was determined using FIAS 100; all samples and standard solutions were stabilized by the addition of 1-2 drops of a 5% (w/v) $KMnO_4$ solution. The standard solutions of metals were prepared from stock standard solutions of ultrapure grade, AA Certipur® 1000 mg/L (Merck Millipore, Darmstadt, Germany). The precision metal determinations, based on variation in replicate analyses (n = 2) on the same sample, was 10% lower. The accuracy of the method was evaluated using recovery tests with BCR certified reference materials CRM 482. Three sample of reference material and blanks were included in each analytical batch. Results were in good agreement with certified values, recovery ranged between 91% and 113% attesting to the reliable quality of the analytical methods.

Statistical analyses

Data were grouped according to the origin area and, for every group, the concentrations, expressed as median and arithmetical mean with relevant standard deviation, minimum and maximum values are reported. Pearson coefficients (r) were also calculated in order to highlight correlations between metals content.

RESULTS AND DISCUSSION

Chemical characteristic and essential and toxic elements content

Table 2 shows the content of sugars and degree of humidity, pH, free acidity, combined and total acidity acidity. The values obtained are in good agreement with those reported by the Council Directive 2001/110/EC related to honey (EC, 2001).

Table 3 shows the Cr, Mn, Fe, Cu, Zn, Cd, Hg, Pb concentration (ppm), median, arithmetical mean and standard deviation. For element concentrations below the detection limit, for the mean, LOQ was considered. Fe, Zn and Pb were determined in 16 of the 21 samples examined. The variation of elemental content from honey to honey is mainly attributed to differences in botanical structure, as well as in the mineral composition of soil in which plants were cultivated. Other factors responsible for a variation in elemental content may be preferential absorbability of the plant, use of fertilizers, irrigation water and climatic conditions.

The essential elements analyzed, Fe, Cr, Mn, Cu and Zn, ranged from 0.16 to 4 ppm (mean 0.95 ± 1.25), from <0.02 to 0.67 ppm (mean 0.27 ± 0.23), from 0.37 to 2.93 ppm (mean 0.87 ± 0.56), from 0.13 to 0.79 ppm (mean 0.39 ± 0.18) and <0.1 to 0.98 ppm (mean 0.3 ± 0.32) respectively. Fe, Mn and Cu were detectable in 100% of samples, Cr in 71 % and Zn in 37%.

Among the toxic elements, Hg ranged from <0.001 to 0.034 ppm (mean 0.007 ± 0.009), Pb ranged from 0.01 to 0.45 ppm (mean 0.18 ± 0.16) and Cd ranged from <0.001 to 0.053 ppm (mean 0.02± 0.018). Pb was detectable in 100% of samples, Cd in 86 % and Hg in 69%.

Figure 1 shows, for all tested metals, comparison of mean content calculated for every area. Small differences were found for different areas. Urbino area showed the highest content of Pb, Cd, Cr, Fe, Cu. The Pb and Cd concentrations may be attributed to the fact that this is an area with a relatively higher density of inhabitants and greater vehicular traffic.

Heavy metal contamination and trace element composition might be caused not only by external sources but also by inappropriate actions during the processing and conservation steps. As a matter of act, the acidic property of honey may lead to release of elements (such as Cr, Pb, Cd and Zn) from metallic tools and containers (Pisani et al., 2008). However, contamination during the manufacturing process might considered unlikely, as the food operators, including the primary producers, need to comply with its regulations related to legislation on materials and articles intended for contact with foodstuffs (EC 1935/2004 and amendments; MD 21/03/1973 and amendments).

The determined contents were generally comparable to those reported by other investigators (Table 4) for others Italian or European regions. However, it is difficult to draw conclusions by extrapolating data from different botanical types of honey, as this characteristic may substantially affect chemical composition. Further, different methods of sample solubilization and different analytical techniques may also affect the results.

Pearson coefficients (r) are presented in Table 5. Strong positive correlations were noted between Pb vs Hg, Pb vs Cd, Pb vs Fe, Pb vs Cr, Hg vs Cd, and Hg vs Fe.

Potential health hazards resulting from honey consumption

In addition to the environmental concern regarding honey element composition, quality control of honey is also important given the increasing global trends in total honey production and the fact that European Union is the world's largest consumer of honey (Bilandzic et al., 2012). There are no specific regulation regarding the presence of heavy metals, whose origin in honey is usually anthropogenic. A statement that honey shall be free from metals in amounts which may result in a hazard to human health, is included either in the Codex Alimentarius (Codex Alimentarius, 2001). For heavy metals, although they were considered by the Directive 96/23/CE, no limits have been established (European Commission, 1996). The Directive 2001/110/EC of the European Union Commission relating to honey includes some general and specific properties of honey composition but no guidelines about the content of heavy metals (European Commission, 2001). The regulation EC 1881/2006, which set the maximum levels for certain contaminants in foodstuffs, does not address apiary products (EC, 2006). Consequently there are currently no legal criteria with which to compare the results obtained in our studies. However, to have a basis for comparison, the maximum limits of Pb and Cd permitted by EC Regulation 1881/06 in bivalve molluscs (1.5 ppm for Pb and 1 ppm for Cd) were taken as reference taking into account the characteristics of bioaccumulation in the bees and the annual per capita consumption of honey. The content of Cd and Pb in honey samples tested were all below the maximum levels indicated in Community legislation.

The Joint FAO/WHO Expert Committee on Food Additives has designed for Hg a Provisional Tolerable Weekly Intake (P.T.W.I) of 0.3 mg (0.042 mg/day) for a 70 kg person (0.004 mg/kg body weigh/week) (FAO/WHO, 2010a). A Provisional Tolerable Monthly Intake (P.T.M.I) of 1.75 mg (0.058 mg/day) for a 70 kg person (0.025 mg/kg body weigh/month) was designed for Cd (FAO/WHO, 2010b) and for Pb a P.T.W.I of 1.75 mg (0.25 mg/day) for a 70 kg person (0.025 mg/kg body weigh/week) was indicated. For tested sample (Code N.1) with the highest Pb (0.45 ppm), a 20 g daily honey consumption turns into a weekly intake of 0.063 mg. This intake only represents about 3.6% of PTWI for Pb. For tested sample (Code N.2) with the highest Cd (0.053 ppm), a 20 g daily honey consumption represents a monthly intake of 0.0318 mg. This intake only represents approximately 1.8% of PTWI for Cd. For tested sample (Code N.14) with the highest Hg (0.034 ppm), a 20 g daily honey consumption represents a weekly intake of 0.0042 mg. This intake only represents approximately 1.4% of PTWI for Hg. Therefore, although the honeys samples are not completely pollution free, heavy metal intake from honey is well below the recommended dose and, from this point of view, the consumption of these honey products is not considered dangerous for human health.

CONCLUSIONS

A total of 8 elements (essential, non-essential and toxic) were determined by AAS in 21 types of honey produced in central Italy. Quality control is important given the increasing global trends in total honey production and the fact the European Union is the world's largest consumer of honey.

The elements were found to be present in honey in various proportions depending on the area foraged by bees from flower type visited for the collection of nectar, the quality of water in the vicinity of the hive. The honeys produced in central Italy were of good quality, but not completely free of heavy metals. Comparison with the recommended daily intakes, it is possible to conclude that heavy metal intoxication resulting in human adverse effects is not a concern.

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Table 1

List of honey samples, location of production, kind and botanical origin

Sample code	Kind of honey	Botanical origin	Sampling area
1	wildflower	Alfalfa, Clover, Sulla	Urbino
2	wildflower	Alfalfa	Urbino
3	wildflower	Sulla, Grasses	Urbino
4	wildflower	Alfalfa, Woods, Sunflower	Urbino
5	wildflower	Alfalfa, Woods	Urbino
6	wildflower	Alfalfa, Woods, Sunflower, Coriander	Urbino
7	wildflower	Woods	Urbino
8	wildflower	Alfalfa, Sunflower, Bramble	Urbino
9	wildflower	Lime, Pastures	Urbino
10	wildflower	Alfalfa, Sulla, Woods	Foglia Valley
11	wildflower	Alfalfa, Woods	Foglia Valley
12	wildflower	Alfalfa, Chestnut, Woods	Foglia Valley
13	wildflower	Alfalfa, Woods	Foglia Valley
14	wildflower	Alfalfa, Woods, Clover, Orchard	Foglia Valley
15	wildflower	-	Metauro Valley
16	wildflower	Alfalfa, Robinia, Sulla	Metauro Valley
17	wildflower	Alfalfa, Cherry	Metauro Valley
18	wildflower	Meadows, Pastures, Woods	Metauro Valley
19	acacia honey	Robinia pseudoacacia	Candigliano Valley
20	honeydew	Woods, Orchard, Lime	Candigliano Valley
21	wildflower	Alfalfa, Sunflower, Robinia, Lime	Candigliano Valley

Table 2

Chemical composition of the honey samples

Sample code	Humidity %	Sugars (Brix)	pH	Free acidity (meq/kg)	Combined acidity (meq/kg)	Total acidity (meq/kg)
1	17.7	80.6	4.00	28.1	4.3	32.4
2	18.3	80.0	3.74	34.4	2.0	36.4
3	18.9	79.4	4.08	32.1	2.2	34.3
4	18.4	80.2	3.98	29.9	3.5	33.4
5	18.2	80.4	4.18	31.1	5.7	36.8
6	18.4	80.2	3.94	24.7	5.3	30.0
7	18.4	80.2	4.05	28.8	4.8	33.6
8	20.0	78.6	3.98	29.8	3.2	33.0
9	18.8	79.8	4.30	31.3	5.1	36.4
10	16.8	81.5	4.08	26.7	1.1	27.8
11	17.9	80.4	3.85	23.4	2.5	25.9
12	16.9	81.2	3.92	24.0	1.3	25.3
13	16.9	81.2	3.87	21.7	1.7	23.4
14	16.1	82.0	3.78	22.0	1.9	23.9
15	18.9	79.4	3.90	23.9	2.0	25.9
16	18.9	79.4	3.90	23.4	3.3	26.7
17	18.3	80.0	3.99	27.5	3.3	30.8
18	19.0	79.6	3.85	31.1	3.1	34.2
19	18.0	80.6	3.87	16.2	2.3	18.5
20	18.2	80.4	4.38	37.9	4.6	42.5
21	18.2	80.4	3.81	35.7	4.3	40.0
Median	18.3	80.2	3.9	27.5	3.1	30.8
Mean	18.1	80.3	4.0	27.4	3.1	30.4
Stand. Dev.	0.9	0.8	0.2	5.6	1.4	6.4
Min.	16.1	78.6	3.7	16.2	1.1	18.5
Max.	20.0	83.0	5.4	37.9	5.7	42.6

Table 3

Content (ppm) of essential and toxic elements (N.D. = not determined)

Sample code	Cr	Mn	Fe	Cu	Zn	Cd	Hg	Pb
1	0.53	0.84	3.76	0.55	<0.10	0.027	0.012	0.45
2	0.25	0.44	N.D.	0.33	N.D.	0.053	N.D.	0.39
3	0.61	0.80	1.77	0.46	0.54	0.021	0.014	0.31
4	<0.02	1.10	0.30	0.44	<0.10	0.004	0.002	0.07
5	0.30	1.66	0.41	0.56	<0.10	<0.001	<0.001	0.03
6	0.55	0.37	N.D.	0.52	N.D.	0.050	N.D.	0.35
7	0.21	0.40	N.D.	0.47	N.D.	0.052	N.D.	0.34
8	0.31	0.76	N.D.	0.37	N.D.	0.038	N.D.	0.22
9	0.62	0.7	N.D.	0.79	N.D.	0.043	N.D.	0.33
10	<0.02	1.25	0.98	0.29	0.98	<0.001	<0.001	0.04
11	0.16	0.78	0.26	0.21	<0.10	0.031	0.014	0.26
12	0.11	0.74	0.27	0.23	<0.10	0.008	0.003	0.01
13	<0.02	0.79	0.21	0.20	<0.10	0.002	<0.001	0.01
14	0.50	0.81	4.00	0.23	<0.10	0.022	0.034	0.44
15	<0.02	0.47	0.31	0.30	<0.10	0.003	<0.001	0.08
16	0.25	0.79	0.19	0.22	0.38	0.004	0.002	0.04
17	0.67	0.74	1.68	0.38	<0.10	0.016	0.001	0.18
18	0.42	0.56	0.28	0.35	<0.10	0.020	0.009	0.19
19	0.04	0.53	0.16	0.13	0.75	0.002	0.001	0.02
20	<0.02	2.93	0.36	0.78	0.55	0.023	0.010	0.03
21	<0.02	0.74	0.20	0.33	0.61	<0.001	<0.001	0.01
Median	0.25	0.76	0.31	0.35	0.10	0.020	0.002	0.18
Mean	0.27	0.87	0.95	0.39	0.30	0.020	0.007	0.18
Stand. Dev.	0.23	0.56	1.25	0.18	0.32	0.018	0.009	0.16
Min	<0.02	0.37	0.16	0.13	<0.10	<0.001	<0.001	0.01
Max	0.67	2.93	4.00	0.79	0.98	0.053	0.034	0.45

Table 4

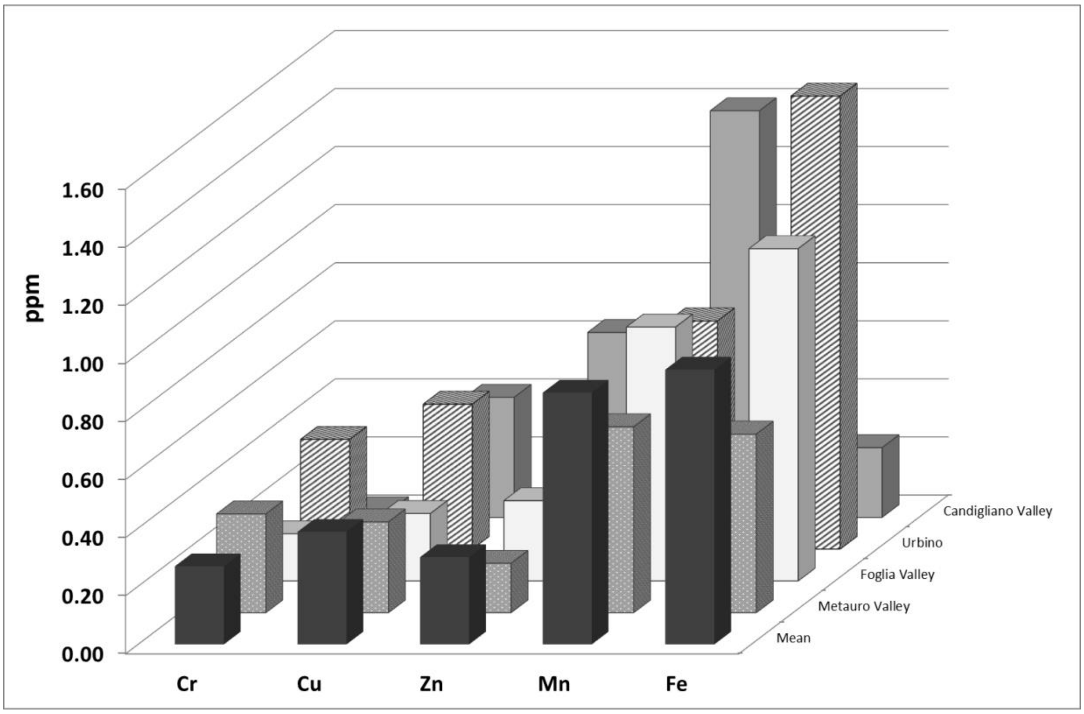
Comparison of the some metals contents (ppm) of honey produced in different Italia regions and in different European regions

	Cd	Hg	Pb	Cr	Mn	Fe	Cu	Zn
Marche, Italy (this paper)	<0.001-0.053	0.007	0.010-0.450	<0.02-0.67	0.37-2.93	0.16-4.00	0.13-0.79	<0.10-0.98
Bologna, Italy (Buldini et al., 2001)			<0.05-0.620					
Emilia Romagna, Italy (Delbono et al.,1999)	<0.003-0.013	<0.010	0.018-0.424		0.34-2.79		0.20-4.01	0.47-11.1
Rome,Italy (Conti and Botrè,2001)	<0.002-0.063		0.004-0.045	0.008-0.102				
Siena, Italy (Pisani et al., 2008)	0.001-0.015		0.028-0.304		0.13-16.9	0.97-13.7	0.172-5.90	0.72-3.66
Ireland (Downey et al., 2005)					0.9-10.2	4.7-31.3	1-2.3	1.6-22.5
Pomerania, Polland (Przybylowski,2001)	0.008-0.027		0.025-0.071					4.17-22.3
Central Croatia (Bilandzic et al.,2012)			0.020-1.521					
Thrace, Turkey (Citak et al., 2012)	<0.10-9.86		<0.11-0.48		<0.010-0.82	<0.025-14.0	<0.016-0.46	<0.064-1.98

Table 5

Correlation between metal contents in tested products

	Cr	Mn	Fe	Cu	Zn	Cd	Hg	Pb
Cr		-0.2655	0.6877	0.3637	-0.3257	0.4861	0.4781	0.7097
Mn	-0.2655		-0.0975	0.4911	0.2276	-0.2494	0.0300	-0.3759
Fe	0.6877	-0.0975		0.0055	-0.1769	0.1662	0.7199	0.8678
Cu	0.3637	0.4911	0.0055		-0.0173	0.4063	0.0490	0.2567
Zn	-0.3257	0.2276	-0.1769	-0.0173		0.2929	-0.2105	-0.3286
Cd	0.4861	-0.2494	0.5256	0.4063	0.2929		0.7170	0.8004
Hg	0.4794	0.0300	0.7185	0.0490	-0.2105	0.7170		0.8082
Pb	0.7097	-0.3759	0.8678	0.2567	-0.3286	0.8004	0.8082	



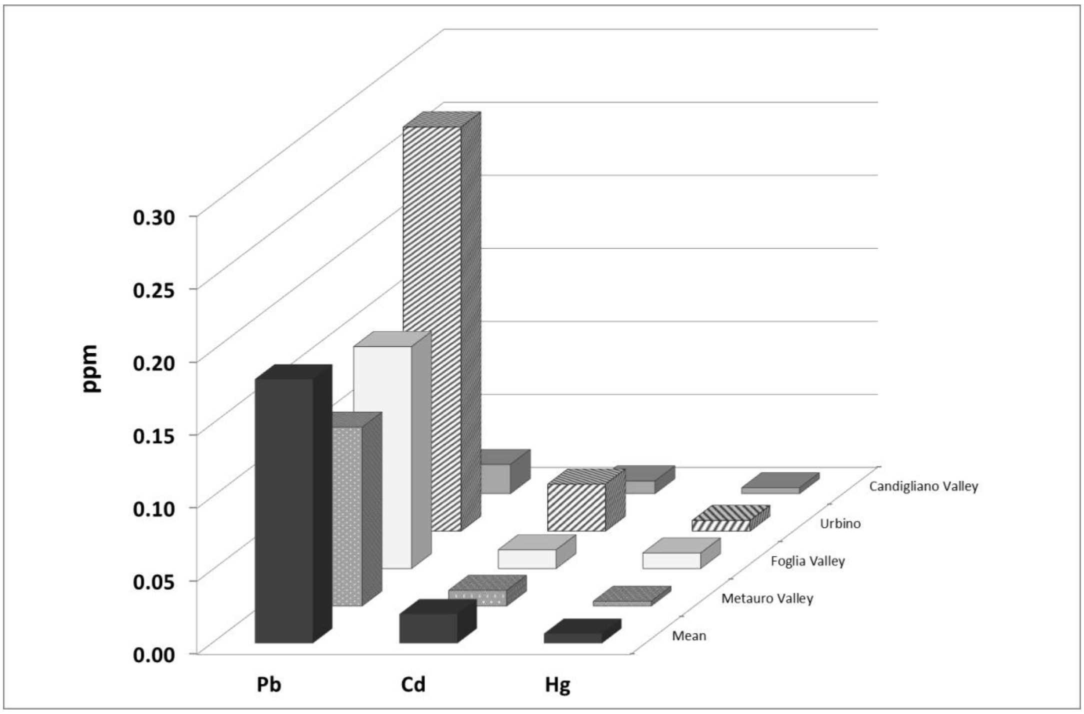


Fig. 1. Mean content (ppm) of tested metals in the four different areas of sampling.