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Metals in Honeys from Different Areas of Southern Italy

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Abstract The aim of the study was to quantify the cadmium (Cd), lead (Pb), chromium (Cr) and arsenic (As) contents in ninety honey samples from nine areas of southern Italy. Results showed that As content was below the detection limit, while Cd, Pb, and Cr contents were below the recommended maximum acceptable levels. Mean Cd, Pb, and Cr contents were 0.013, 0.289 and 0.707 mg kg^{-1}, respectively. The metal contents in honey varied greatly depending on considered area. Correlations between the metals were statistically significant ($p < 0.05$), suggesting that polluting sources involve the simultaneous presence of metals in honey.

Keywords As · Cd · Pb · Cr · Environmental pollution · Geographical area

Metals are natural trace components of the Earth, but in the last century, human activity has increased their concentration, reaching in some cases toxic thresholds for flora, fauna and humans (Adriano 1992). Among the most polluting activities are mining companies (Hutton and Symon 1986), foundries, combustion by-products, and engine discharges (UNEP/GPA 2004). Peplow (1999) reported that metals persist for long periods of time after their release into the environment. Some metals such as iron (Fe), zinc (Zn), chromium (Cr), cobalt (Co), copper (Cu), molybdenum (Mo) and manganese (Mn) are constituents of various bio-active compounds, but if they exceed safety levels, they can be toxic (Codex Alimentarius Commission 1993). Other metals, such as arsenic (As), lead (Pb), cadmium (Cd) and methyl forms of mercury (Hg) have no biological roles and their presence causes environmental pollution. Even at very low concentrations, all these elements can be toxic both for man and other living species because they bind with cellular structures and hinder the performance of certain vital functions (Jarup 2003).

Honey is produced by honeybees from the nectar of different plants and honeydew, and it is also considered the result of a bioaccumulation process of many metals that can play an important role in a number of biochemical processes (Garcia et al. 2005). The amount of micro- and macro-elements in honey depends on its botanical origin and soil composition on which the plants grow (Lachman et al. 2007; González-Miret et al. 2005). In recent years, many researchers have detected a high correlation between metal contents in bee products and their content into the environment, suggesting the use of honey as a indicator of environmental contamination (Staniškienė et al. 2006; Przybylowski and Wilczynska 2001). The aim of this study was to evaluate four metals (Cd, Pb, Cr, and As) content, which are considered among the most dangerous to humans, in honeys from nine areas of southern Italy.

Materials and Methods

During 2012, ninety honey samples were collected directly from beekeepers in nine geographical areas of southern Italy (ten honey samples for each area; Fig. 1). Sampling sites are large areas with different pedology characteristics, density, and productive activities (Table 1) and they are characterized by the high presence of wild flowers and botanical species in all altitudes. Honey samples (about

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Fig. 1 The map showing the sampling sites

Legend

	Area
1	Tarantino
2	Penisola Sorrentina
3	Camastra-Dolomiti Lucane
4	Leccese
5	Basso Pollino
6	Collina Materana
7	Potentino
8	Vulture Melfese
9	Cilento

Table 1 Pedological, altimetric and economic characteristics of the considered areas

Area	m above sea level	Pedological characteristics	Density[a] (inhabitants/km^2)	Presence of industries with high environmental impacts	Agricultural activity
Tarantino	130–480	Sandy-clayey soil	Medium	High	Intensive
Penisola Sorrentina	0–600	Marble-clayey soil	High	Low	Intensive
Camastra-Dolomiti Lucane	700–1.100	Clayey soil	Low	Low	Extensive
Leccese	57	Chalky soil	Medium	Low	Semi-intensive
Basso Pollino	200–1.000	Chalky soil	Low	Low	Semi-intensive
Collina Materana	20–770	Silty-clayey soil	Low	Low	Semi-intensive
Potentino	400–1.100	Clayey soil	Medium	High	Extensive
Vulture Melfese	350–730	Volcanic soil	Low	High	Intensive
Cilento	450–650	Chalky soil	Low	Low	Semi-intensive

[a] Density: high >600 inhabitants/km^2; medium: between 300 and 600 inhabitants/km^2; low <300 inhabitants/km^2

250 g) were placed into clean glass bottles and stored at room temperature in the dark until analysed.

Five grams of each honey sample were placed in porcelain crucibles and heated in an oven to 80°C for about 12 h. Crucibles with the samples were then placed in muffle ovens and burned to ash at 450°C. Five mL of HNO_3 (0.1 M) plus 1 mL of H_2O_2 (30 %, v/v) was added to a cup containing the white ash, the mixture was stirred and then heated on a hotplate to almost complete dryness. Two mL of the HNO_3 (0.1 M) was added, and the mixture was made up to a 10 mL with distilled water. The metal concentration was determined by means of quadrupole inductively coupled plasma mass spectrometry (ICP-QMS; Elan DRC II, Perkin-Elmer SCIEX, CT, USA). Blanks (only HNO_3 and H_2O_2) and a standard stock solution of 50 mg kg^{-1} for each element were analyzed for reference purposes. Typical daily instrumental parameters are given in Table 2. High purity He (99.9999 %) and H_2 (99.9995 %) were used, in order to minimize potential problems caused by unidentified reactive contaminant species in the cell. Reference wavelengths for each metal were chosen to avoid interferences with other analyzed elements. Before use, all glassware and plastic containers were cleaned by washing with 10 % ultra-pure grade HNO_3 for at least 24 h and then copiously rinsed with ultra-pure water before use.

In order to analyze samples for all elements and to correct for matrix interferences, calibration of samples against matrix matching was used. For this purpose, a synthetic honey sample (0.85 % fructose) with low concentrations of the elements was selected to be studied.

Calibration solutions were prepared from multi-elemental standard stock solutions of 1.000 mg L^{-1} and the calibration curves were obtained by using at least 6 diluted solutions. Reagent blanks containing ultra-pure water were additionally analyzed in order to control the purity of the reagents and the laboratory equipment. Standards and blanks were subjected to the same treatment as honey samples. Rhodium was added as the internal standard to a final concentration of 10 μg L^{-1}. Results were expressed as mg of metal per kg of fresh honey (mg kg^{-1}). Each sample was analyzed in triplicate. Quality assurance was evaluated for the validation of the analytical methods for determination of elements in honey samples, namely: linearity, limits of detection (LOD) and limits of quantification (LOQ) and precision. The analytical quality control was verified by the recovery tests with synthetic honey sample spiked with Pb, Cd, As and Cr (10 ng mL^{-1}).

Statistical analysis was performed using the general linear model (GLM) procedure of statistical analysis system (SAS 1996) using a monofactorial model. Differences between metal concentrations in different geographical areas were analysed using Student's *t* test. A Pearson's correlation test was conducted to determine the linear correlation among the variables. Differences between means at the 95 % ($p < 0.05$) confidence level were considered statistically significant. Data were expressed as mean ± standard deviation.

Table 2 ICP-MS operating conditions and measurement parameters

Spectrometer	Elan DRC II (Perkin-Elmer SCIEX, CT, USA)
Nebulizer	Mehinard
Spray chamber	Cyclonic
Interface	Pt cones
Mass analizzator	Quadrupole
RF power (W)	1.200
Ar gas flow rate (L min^{-1})	
Plasma	15
Auxiliary	1.0
Nebulizer	0.85
Sample uptake rate (mL min^{-1})	1
Lens voltage (V)	7.75
Dwell time (msec)	50
Optimized	On mass Fe^{56}
Number of replicates	3
Isotopes	As^{75}, Pb^{208}, Cr^{53}; Cd^{111}
Internal standard	Rh^{103}
Spectral lines (nm)	As, 193.696; Pb, 217.005; Cr, 283.563; Cd 228.802

Results and Discussion

Performance characteristics of the method for the elements analysed are showed in Table 3.

Comparison of metal contents in honey samples from different geographical areas, and mean, minimum and maximum value of each heavy metal are reported in Table 4. The As content in considered honeys was below the detection limit. The high variability detected in honey samples around the mean value strengthen the widely accepted theory that metal contents in honey varies greatly depending on considered area (Staniškienė et al. 2006; Przybylowski and Wilczynska 2001).

The Pb content in honey samples ranged from 0.010 to 1.390 mg kg^{-1}, with an average value of 0.289 mg kg^{-1} (Table 4). These values were lower than those found in Polish honey (0.396–1.730 mg kg^{-1}) (Dobrzancki et al. 1994), in Egyptian honey (4.200 mg kg^{-1}) (Rashed and Soltan 2004), and in Saudi Arabia honey (1.81 mg kg^{-1}) (Bibi et al. 2008). Current results were higher than those found in German honey (0.040–0.273 mg kg^{-1}) (Ruhnke 1993). Generally, our results were similar to or higher than those found in Italian honeys by other authors (Table 5), while they were substantially lower than those reported by D'Ambrosio and Marchesini (1982).

Basso Pollino, Collina Materana, Vulture Melfese, Leccese, and Cilento honeys do not show any significant differences in Pb concentrations. These areas, except for the Vulture Melfese area, are characterized by the presence of small and medium-sized industries with a low environmental impact. The highest Pb content, in Penisola Sorrentina honeys, a well known tourist area, could be due to

Table 3 Performance characteristics of the method for the elements analysed

Metals	R^2	Linearity range (ng mL^{-1})	LOD[a] (ng mL^{-1})	LOQ[b] (ng mL^{-1})	Precision intra-day (% RSD)[c] n = 6	Precision inter-day (% RSD)[c] n = 6	Recovery[d] % n = 10
As	0.9998	0.5–50	0.030	0.12	3.45	4.36	99.6
Cd	0.9993	0.5–50	0.012	0.04	3.40	5.30	99.8
Pb	0.9996	10–1.000	0.014	0.05	2.88	3.27	98.5
Cr	0.9997	0.2–100	0.030	0.10	1.05	2.00	99.7

[a] *LOD* Limit of detection (3.3 SD/slope of the calibration curve)

[b] *LOQ* Limit of quantification (10 SD/slope of the calibration curve)

[c] *RSD* relative standard deviation

[d] Recovery percent from synthetic honey sample spiked with standard solution

high movement of vehicles, especially in the spring-summer period; while the high Pb content, in Potentino honeys, could be due to the presence of chemical industries and steel mills that have a high environmental impact.

Potentino area is also characterized by both local and transit traffic, determined by a high concentrations of cars (71cars/100 inhabitants) and by the presence of an important road knot for the region (Euromobility 2012). Tarantino is characterized by a high presence of industries (iron and steel), power stations and refineries. Camastra-Dolomiti Lucane is mainly agricultural-pastoral, with a low population density, and it is included in the Natural Park of Dolomiti Lucane, representing one of the main green lungs of southern Italy. The high Pb content can be linked to the petroleum extraction-related activities. Indeed, Viggiano oil center (Basilicata region), that is considered the greatest of continental Europe, is only 25 km far.

Cd levels found in honey samples showed a low variability around the mean value ranging from 0.001 to 0.040 mg kg^{-1}, with an average value of 0.013 mg kg^{-1} (Table 4). These values were lower than those found in Turkish (Tuzen et al. 2007), Macedonian (Stankovska et al. 2006), and French (Devillers et al. 2002) honeys, while they were higher than the values reported by Rodríguez García et al. (2006) in Spanish honey. In general, Cd pollution is caused by high industrialization, mainly from smelting and refining of non-ferrous metals, and fossil fuel combustion and municipal waste incineration. In areas with intensive agriculture, it is caused by high consumption of mineral fertilisers and pesticides.

The Cr content in honey samples showed a high variability around the mean value, ranging from 0.020 to 2.040 mg kg^{-1} (Table 4). The average Cr value (0.707 mg kg^{-1}) was higher than that found by Caroli et al. (1999) in Italian honeys. Cr pollutants may contaminate honey in several ways which are likely to be encountered either in gaseous form when petroleum products are used as fuel or packed (Shukla et al. 2007). The high and significant variability among the honey samples from considered areas might be due to the different geographical origin of the studied honeys (Pohl 2009). In addition, it should not be underestimated that the Cr content in honey

Table 4 Comparison of heavy metal contents in honey samples from different geographical areas, and mean, minimum and maximum value of each heavy metal

Area	Concentrations (mg kg^{-1})		
	Pb	Cr	Cd
Tarantino	0.340 ± 0.165^{a}	0.686 ± 0.435^{a}	0.010 ± 0.007^{a}
Penisola Sorrentina	0.900 ± 0.302^{b}	0.977 ± 0.574^{b}	0.018 ± 0.013^{b,c}
Camastra-Dolomiti Lucane	0.238 ± 0.198^{c}	1.016 ± 0.467^{b}	0.018 ± 0.008^{b,c}
Leccese	0.140 ± 0.107^{d}	0.329 ± 0.213^{c}	0.008 ± 0.005^{a}
Basso Pollino	0.165 ± 0.119^{d}	0.720 ± 0.318^{a,d}	0.011 ± 0.005^{a}
Collina Materana	0.156 ± 0.126^{d}	0.724 ± 0.320^{a,d}	0.010 ± 0.006^{a}
Potentino	0.332 ± 0.327^{a}	0.874 ± 0.770^{b,d}	0.020 ± 0.015^{c}
Vulture Melfese	0.168 ± 0.173^{d}	0.715 ± 0.587^{a,d}	0.016 ± 0.010^{b}
Cilento	0.166 ± 0.118^{d}	0.321 ± 0.540^{c}	0.009 ± 0.004^{a}
Mean	0.289 ± 0.296	0.707 ± 0.528	0.013 ± 0.010
Minimum	0.010	0.020	0.001
Maximum	1.390	2.040	0.040

Mean values from three repetition ± standard deviations

means in the same column with different letters are significantly different according to the Student's t-test ($p < 0.05$)

Table 5 Comparison of the Pb content of honeys produced in different Italian regions

	Geographical origin	Year of production	Pb (mg kg^{-1})		
			Mean	Max.^{a}	Min.^{b}
Present study	Southern Italy	2012	0.290	1.390	0.010
Pisani et al. (2008)	Siena County (Tuscany)	2004	0.076	0.304	0.028
Galeno et al. (1992)	Liguria	1992	0.075	0.237	<0.008
Sangiorgi and Ferretti (1996)	Emilia-Romagna and Lombardy	1993	0.037	0.220	
Abete and Voghera (1999)	Turin County (Piedmont)	1996–1997	0.065	0.162	0.028
Delbono et al. (1999)	Emilia-Romagna	1996–1998	0.150	0.424	0.018
D'Ambrosio and Marchesini (1982)	Italy	1982	2.370		

[a] *Max.* maximum, [b] *Min.* minimum

depends on the weather condition (Petrovic et al. 1994). The highest Cr concentration was found in Camastra-Dolomiti Lucane honeys (1.016 mg kg^{-1}, $p < 0.05$), followed by Penisola Sorrentina (0.977 mg kg^{-1}) and Potentino (0.874 mg kg^{-1}) honeys; while the lowest values were detected in Cilento and Leccese honeys (0.321 and 0.329 mg kg^{-1}, respectively). As evidenced for Pb content, Camastra-Dolomiti Lucane honeys are characterized by a high Cr and Cd content. Since there are no metallurgical and chemical manufacturing industries in this area, it is possible to confirm the hypothesis of a connection with the petroleum extraction-related activities.

Pearson correlation coefficients were calculated between the three metals. Statistically significant positive correlations were detected between Pb and Cd, Pb and Cr, and Cd and Cr contents ($p < 0.001$). These correlations allow the speculation that the polluting sources involve the simultaneous presence of metals. These results disagree with that found by Roman and Popiela (2011), while Frías et al. (2008) found a direct statistical correlation between Cd and Pb contents in Tenerife honey. Pratt and Sikorski (1982) demonstrated the relationship between metals content in organisms of worker bees and honey. The Pb, Cr and Cd contents in honey samples from 9 considered areas, although relatively high, resulted below the levels of risk defined by WHO (1982). At present, there are no Communitarian Regulations regarding Cd and Pb contents in honeys, even if the recommended maximum acceptable levels suggested by European Union is 0.1 mg $kg^{-1}$for Cd and 1 mg kg^{-1} for Pb (Byrne 2000).

In conclusion, the Pb, Cd, and Cr levels in honeys from studied areas were influenced by local environmental conditions and results were known to be within the acceptable limits. No specific legislation on honey's heavy metal contents exists; however, the content detected in our samples was below the levels defined for other types of food. Therefore, the present study confirms that honey can indicate the level of metals present in all environmental sectors (air, water, vegetation and soil) relevant to the bees pasture area.

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