Bull Environ Contam Toxicol (2012) 88:985–989
DOI 10.1007/s00128-012-0585-z

Lead Content in Multifloral Honey from Central Croatia over a Three-Year Period

Nina Bilandžić · Marija Sedak · Maja Đokić · Božica Solomun Kolanović · Ivana Varenina · Đurđica Božić · Branimir Šimić · Ana Končurat · Mate Brstilo

Received: 17 November 2011 / Accepted: 28 February 2012 / Published online: 14 March 2012

Abstract Lead concentrations were analysed by atomic absorption spectrometry in multifloral honeys collected in central Croatia (Zagreb County) during a three-year period from 2009 to 2011. The mean levels of elements (µg/kg) in honey samples measured were: 90.8 in 2009, 189 in 2010 and 360 in 2011. Significant differences were observed, and Pb levels determined in 2009 were significantly lower than those in 2010 and 2011 ($p < 0.05$, both). In 2009 there was no concentration found above 300 µg/kg. However, in 2010 and 2011 levels exceeding 300 µg/kg were found in 28.6 % and 25 % of samples. Trace element levels of Pb determined in multifloral honey were generally higher than concentrations obtained from other geographical origins and neighbouring countries. These high concentrations of Pb may be related to the fact that the central region is becoming increasingly urban and the network of motorways is growing. Accordingly, the risk of positioning hives near zones of busy highways and railways is increasing. This underlines that particular attention should be paid to toxic Pb levels, due to their gradual increased during the study period.

Keywords Lead · Multifloral honey · Pollution · Croatia

N. Bilandžić (✉) · M. Sedak · M. Đokić · B. S. Kolanović · I. Varenina · Đ. Božić
Laboratory for Residue Control, Department of Veterinary Public Health, Croatian Veterinary Institute, Savska cesta 143, 10000 Zagreb, Croatia
e-mail: bilandzic@veinst.hr

B. Šimić
Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia

A. Končurat
Laboratory for Culture Media Preparation and Sterilisation, Križevci Veterinary Institute, 48260 Križevci, Croatia

M. Brstilo
General Department, Croatian Veterinary Institute, Savska cesta 143, 10000 Zagreb, Croatia

Rapid developments and increases in mining and industrial activities have gradually redistributed many of the toxic metals from the earth's crust to the soil, atmosphere and waters, foods and plants. Metals accumulate in the topsoil, thus endangering crops, vegetables and microflora and causing their incorporation into the food chain, which can entail a wide variety of adverse effects on animals and humans due to contaminant accumulation. Soils contaminated by metals, especially Pb, are an environmental issue and a priority.

Honey is a natural, sweet product and its chemical composition gives it many nourishing, prophylactic and healing properties. It is also used as an ingredient for sweetness, colour, flavour, caramelisation and viscosity in many type of food products (Rashed and Soltan 2004). The mineral content in honey ranges from about 0.04 % in pale honeys to 0.2 % in dark honeys (Anklam 1998). Investigations of environmental, geographical and botanical aspects of the mineral content in honey have shown that botanical factors have the greatest influence on trace element content with regard to soil and climate characteristics (Bogdanov et al. 2007). Elevated toxic element levels in honey, such as heavy metals (As, Cd, Hg, Pb), may be the result of elevated metal contents in plant nectar. High heavy element contents have been determined in honey from industrial areas with heavy industrial activities or near busy highways (Toporcák et al. 1992).

Widespread environmental contamination with Pb during the past 50 years has been caused by 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). It is a ubiquitous though biologically non-essential element, and its toxicity is caused by the ability of Pb^{2+} to interfere with several enzymes. Lead may cause a large variety of toxic effects, including gastrointestinal, muscular, reproductive, neurological or behavioural effects, including sleeplessness, fatigue, hearing and weight loss, and genetic malfunctions (Johnson 1998). Exposure to Pb may manifest as peripheral nerve degeneration with detrimental effects on intelligence in the case of prolonged ingestion of small amounts of metal. It may also cause poisoning in domestic animals, with elevated blood Pb levels detected in cattle from industrial areas (Swarup et al. 2005). Lead is one of the least soluble metals with a very long retention time, and its contamination is mainly restricted to surface soil in boreal forests that are rich in humic material with a podsolic stratification. Honeybees forage on plants growing over a relatively large area (more than 7 km^2) and move from flower to flower, and are thus in constant contact with air, water and soil, branches and leaves. Therefore, honey is the result of a bio-accumulative process that is useful for collecting information about the environment, and thus may be considered a bioindicator of environmental pollution (Conti and Botre 2001; Pisani et al. 2008).

Multifloral honey is one of the most common honey types in Croatia. The aim of the present study is to examine the concentrations of Pb in multifloral honey collected over 3 years in the same geographical region, i.e. in Zagreb County in central Croatia.

Materials and Methods

A total of 41 multifloral honey samples were collected over a three-year period, from 2009 to 2011. Samples were produced and collected from individual beekeepers near towns in the central region, i.e. in Zagreb County located in northwestern Croatia (Fig. 1).

Zagreb County covers an area of about 3,078 km^2 with 9 urban centres having a total population of 96,928 and 25 municipalities, together with more than 300,000 inhabitants. This is the most populated, urbanized and industrialized region in Croatia. The capital city of Zagreb has more than 800,000 inhabitants and pronounced industrial activities: production of electronic devices, chemical and pharmaceutical industries, textile industry and food production. Also, small scale industry is situated near the small settlements. The capital and neighbouring cities are crossroads of European transport routes, with a network of busy highways and railways. Airport facilities are also located in the southerneastern part of the capital.

Upon collection, all honey samples (500 g) were placed into clean glass bottles, labelled and transferred to the laboratory and kept at 4–8°C until analysis.

The acids used, HNO_3 and HCl, were of analytical reagent grade (Kemika, Croatia). Ultra high purity water processed through a purification system NIRO VV UV UF 20 (Nirosta d.o.o. Water Technologies, Osijek, Croatia) was used for all dilutions. Calibrations were prepared with element standard solutions of 1 g L^{-1} of Pb (Perkin Elmer, USA). Stock solution was diluted in HNO_3 (0.2 %). As matrix modifiers in each atomization for Pb, 0.005 mg $NH_4H_2PO_4$ (Perkin Elmer, USA) and 0.003 mg $Mg(NO_3)_2$ (Perkin Elmer, USA) were used.

Plastic and glassware were cleaned by soaking in diluted HNO_3 (1/9; v/v) and by subsequent rinsing with high purity water and drying prior to use.

Honey samples (0.5 g) were digested with 4 mL HNO_3 (65 % v/v) and 2 mL H_2O_2 (30 % v/v) with a Multiwave 3000 microwave closed system (Anton Paar, Germany). A blank digest was carried out in the same way. The digestion programme began at a power of 500 W, ramped for 1 min and hold for 4 min. The second step began at a power of 1,000 W ramped for 5 min and hold for 5 min. The third step began at a power of 1,400 W, ramped for 5 min and hold for 10 min. Digested samples were diluted to a final volume of 50 mL with double deionised water.

Detection limits for Pb were determined as the concentration corresponding to three times the standard deviation of twenty blanks. All samples were run in batches that included blanks, a standard calibration curve, two spiked honey samples, and one duplicate. To calculate the recovery percentage, ten honey samples were spiked with known amounts of Pb.

The analyses of Pb were performed by graphite furnace-atomic absorption spectroscopy with an AAnalyst 800 atomic absorption spectrometer (Perkin Elmer, USA), equipped with an AS 800 autosampler (Perkin Elmer, USA) set at 283.3 nm. Argon was used for graphite furnace measurements. Pyrolytic-coated graphite tubes with a platform were used.

All calculations and statistical analysis were performed using the software package Statistica 6.1 (StatSoft® Inc., USA). Data were log-transformed to improve normality prior to analysis to meet the underlying assumptions of the analysis of variance. One-way analysis of variance was used to test for differences in honey metal concentrations. The differences between the metal concentrations within different years were analyzed using the *t* test. A probability level of $p \leq 0.05$ was considered statistically significant.

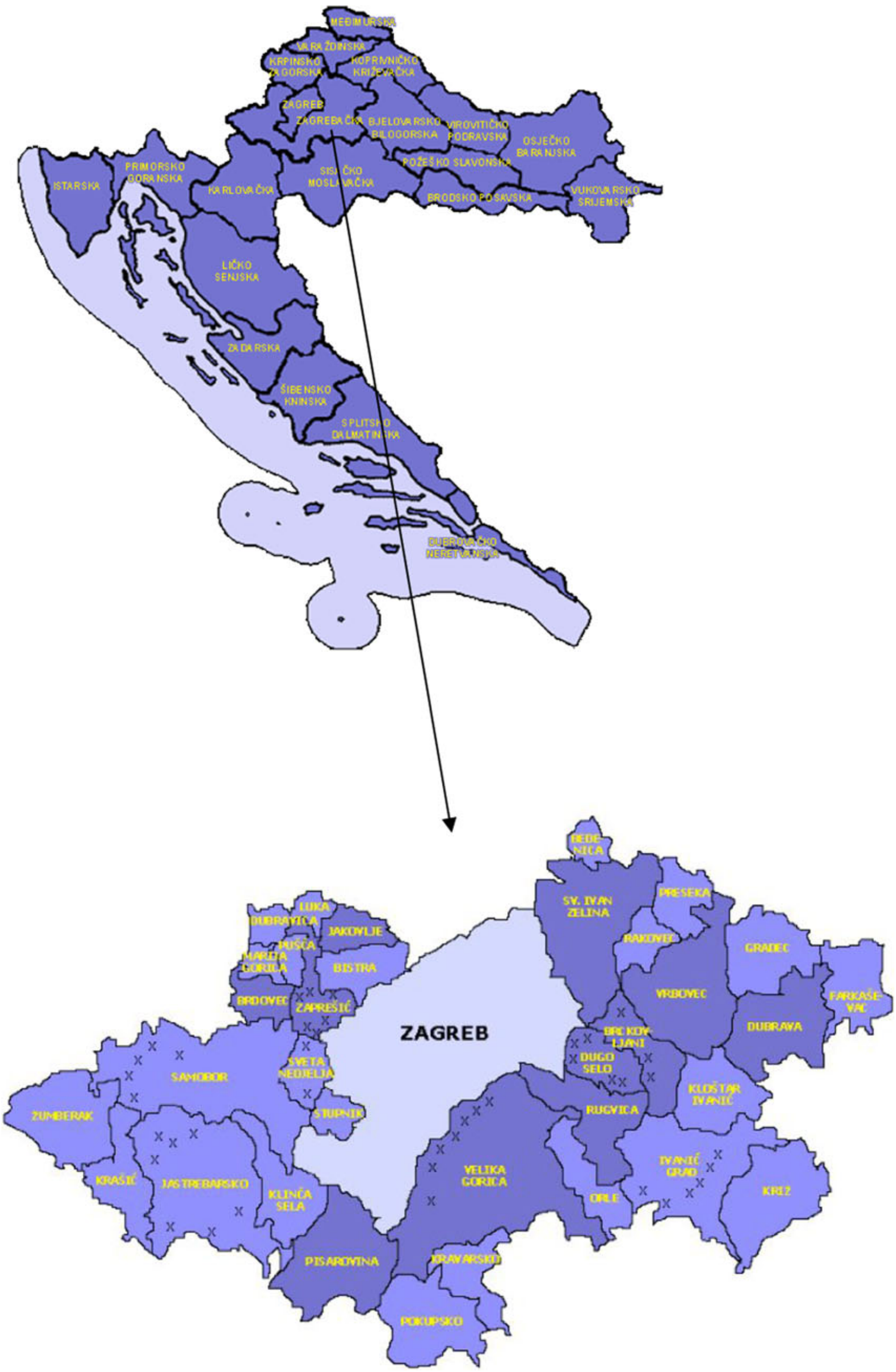

Fig. 1 Map of Zagreb County showing sampling location of multifloral honey samples

Results and Discussion

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 the European Union is the world's largest consumer of honey (Vanhanen et al. 2011). Usually, the heavy metal content of honey is sufficiently small that even 100 g daily intake of honey would not contribute appreciably to dietary requirements. A provisional tolerable weekly intake of 25 µg/kg b.w. has been established as an acceptable level of Pb that may accumulate in the body and can be ingested on a weekly basis (WHO 2000).

On the other hand, when the environment consists of one or more metals, the content in honey may be enhanced and thereby influence health. Toxic trace metal contents have been presented in different honey types, mainly multifloral, from European countries during the past 10 years (Przybyłowski and Wilczyńska 2001; Bratu and Georgescu 2005; Golob et al. 2005; Tuzen et al. 2007; Pisani et al. 2008; Silici et al. 2008).

In the present study, Pb concentrations were determined in multifloral honey collected over 3 years from the same geographical origin. There are no mining activities in Zagreb County or in the territory of Croatia. However, industrial production is centred around large cities, primarily in the central Zagreb County region.

The quality of data was checked by analysis of the recovery rate with spiked honey samples for Pb and showed good accuracy, with a recovery rate of 97.8. The limit of detection (LOD) for Pb was 4.7 µg/kg. The concentrations of Pb in multifloral honey are reported in Table 1. Statistical analyses with the one-way ANOVA showed a significant difference in Pb ($p < 0.01$) levels within studied years. Lead concentrations gradually increased over each year of measurement: 90.8, 189 and 360 µg/kg. Therefore, mean Pb levels found in the 2009 were significantly lower than those in 2010 and 2011 ($p < 0.05$, both).

The mean lead content in all honey samples investigated was 229 µg/kg. The highest mean Pb concentration was 1,521 µg/kg, measured in 2011. The results obtained in 2010 and 2011 were higher than previously determined values in the same region of Croatia (Bilandžic et al. 2011).

The frequency distribution of Pb concentration in different ranges in honey samples are presented in Table 2. In total 41.5 % honey samples measured Pb levels were below 100 µg/kg. However, Pb levels in range from 100 to 200 µg/kg were determined in 19.5 % samples and exceeding the 200 µg/kg in 39 % samples. Lead concentrations in range from 100 to 200 µg/kg were measured in 18 % of multifloral honey samples collected in 2009. However, there was no concentration found above 300 µg/kg. In 2010 and 2011 Pb concentrations exceeding 300 µg/kg were found in 28.6 % and 25 % of samples. Also, concentrations exceeding 200 µg/kg were found in 57.1 % and 37.5 % of samples.

Measured Pb concentrations in the present study were similar to Pb levels measured in honeys in Hungary, i.e. ranging from 18 to 163 µg/kg in floral honey, and from 17 to 144 µg/kg in accacia honey (Ajtony et al. 2007). However, the Pb contents in the present study were higher than levels determined in Turkey, a country known for its large honey production: 17.6–32.1 µg/kg (Tuzen and Soylak 2005), 8.4–106 µg/kg (Tuzen et al. 2007), 3.2–36.7 µg/kg (Silici et al. 2008). The observed concentrations were also higher than those found in honey (25–70 µg/kg) from Poland (Przybyłowski and Wilczyńska 2001) and in blossom multifloral honey (0.042 mg/kg) and rape blossom honey (0.096 mg/kg) from Czech Republic (Vorlová and Čelechovská 2002). Lead concentrations ranging from 0.0001 to 0.200 mg/kg were determined in Romanian honey (Antonescu and Mateescu 2001). Very low Pb contents were measured in different honey types from Romania (mg/kg): mixed flowers 0.07, Linden tree 0.09, rape 0.03, acacia tree 0.089 and dark honey 0.18 (Bratu and Georgescu 2005).

Lead content in multifloral honey measured in Italy in the past decade were (µg/kg): <50 in Lizzano and 620 in Bologna (Buldini et al. 2001), <2.0–63.0 in the City of Rome and Rome County (Conti and Botre 2001), 28.2–304 in Siena County (Pisani et al. 2008).

High Pb concentrations of 1.86–4.3 mg/kg of Pb were determined in acacia honey from Croatia's neighbouring country, Slovenia (Golob et al. 2005). Also, high Pb levels

Table 1 Lead concentrations (mean and range; (µg/kg, w.w.) in multifloral honey over a three-year period from central region of Croatia

Year	No. of samples	Mean (µg/kg)	Minimum (µg/kg)	Maximum (µg/kg)	SD (µg/kg)
2009	11	90.8[a]	19.7	226	71.7
2010	14	189[a]	22.6	441	139
2011	16	360[a]	27.5	1,521	493
All	41	229	19.7	1,521	334

[a] Vertically, letters show statistically significant differences between years at $p < 0.05$

Table 2 Frequency distribution of Pb concentrations in multifloral honey over a 3-year period in central region of Croatia

Year	No. of samples	Number of samples in range (μg/kg)				
		<50	50–100	100–200	200–300	>300
2009	11	5	1	3	2	–
2010	14	3	2	1	4	4
2011	16	2	4	4	2	4
All	41	10	7	8	8	8

of 6.3, 5.7 and 4.2 mg/kg were determined in Egyptian sesame, orange and clover honeys, respectively (Rashed and Soltan 2004). Furthermore, high Pb levels were measured in mixed flower honey of 920 μg/kg from Kashmir and 695 μg/kg from Ashok originating from India (Buldini et al. 2001).

In a recent study in New Zealand, Pb was not detected in five of ten honey types analysed, and the mean Pb content was 0.017 mg/kg (Vanhanen et al. 2011). It is suggested that New Zealand soils are classified as the lowest international Pb decile with a declining national median Pb level.

Lead is a widespread metal pollutant that can reach humans and animals through air, water and food. The fact that Pb is detected in honey is already evidence of pollution of the environment with this metal. The results of the present study show that the trace element levels of Pb determined in multifloral honey were generally higher than concentrations obtained from other geographical origins in Europe. These high concentrations of Pb in multifloral honey samples in the central region may be related to the fact that this region has become more urban and that the network of motorways is growing every year. Accordingly, the risk of positioning hives near to zones of busy highways and railways is increased. This underline that particular attention should be paid to toxic Pb levels regarding the fact that the Pb levels gradually increased during the study period.

Acknowledgments We thank Marijana Fluka and Mirjana Hren for assistance in sample preparation. This study was supported with a grant from the Croatian Veterinary Institute, Zagreb, Croatia.

References

Ajtony Z, Bencs L, Haraszi R, Szigeti J, Szoboszlai N (2007) Study on the simultaneous determination of some essential and toxic trace elements in honey by multi-element graphite furnace atomic absorption spectrometry. Talanta 71:683–690

Anklam E (1998) A review of the analytical methods to determine the geographical and botanical origin of honey. Food Chem 63: 549–562

Antonescu C, Mateescu C (2001) Environmental pollution and its effects on honey quality. Roman Biotech Lett 6:371–379

Bilandžić N, Đokić M, Sedak M, Kolanović BS, Varenina I, Končurat A, Rudan N (2011) Determination of trace elements in Croatian floral honey originating from different regions. Food Chem 128: 1160–1164

Bogdanov S, Haldimann M, Luginbühl W, Gallmann P (2007) Minerals in honey: environmental, geographical and botanical aspects. J Apicul Res 46:269–275

Bratu I, Georgescu C (2005) Chemical contamination of bee honey—identifying sensor of the environment pollution. J Cent Eur Agric 6(1):467–470

Buldini PL, Cavalli S, Mevoli A, Sharma JL (2001) Ion chromatographic and voltametric determination of heavy and transition metals in honey. Food Chem 73:487–495

Conti ME, Botre F (2001) Honeybees and their products as potential bioindicators of heavy metals contamination. Environ Monit Assess 69:267–282

Golob T, Doberšek U, Kump P, Nečemer M (2005) Determination of trace and minor elements in Slovenian honey by total reflection X-ray fluorescence spectroscopy. Food Chem 91:593–600

Johnson FM (1998) The genetic effects of environmental lead. Mutat Res 410:123–140

Pisani A, Protano G, Riccobono F (2008) Minor and trace elements in different honey types produced in Siena County (Italy). Food Chem 107:1553–1560

Przybyłowski P, Wilczyńska A (2001) Honey as an environmental marker. Food Chem 74:289–291

Rashed MN, Soltan ME (2004) Major and trace elements in different types of Egyptian mono-floral and non-floral bee honeys. J Food Comp Anal 17:725–735

Silici S, Uluozlu OD, Tuzen M, Soylak M (2008) Assessment of trace element levels in rhododendron honeys of Black sea Region, Turkey. J Hazard Mater 156:612–618

Swarup D, Patra RC, Naresh R, Kumar P, Shekhar P (2005) Blood lead levels in lactating cows reared around polluted localities; transfer of lead into milk. Sci Total Environ 347:106–110

Toporcák J, Legáth J, Kul'ková J (1992) Levels of mercury in samples of bees and honey from areas with and without industrial contamination. Vet Med 37:405–412

Tuzen M, Soylak M (2005) Heavy metal levels in microwave digested honey samples from middle Anatolia, Turkey. J Food Drug Anal 3:343–347

Tuzen M, Silici S, Mendil D, Soylak M (2007) Trace element levels in honeys from different regions of Turkey. Food Chem 103: 325–330

Vanhanen LP, Emmertz A, Savage GP (2011) Mineral analysis of mono-floral New Zealand honey. Food Chem 128:236–240

Vorlová L, Čelechovská O (2002) Activity of enzymes and trace element content in bee honey. Acta Vet Brno 71:375–378

WHO (2000) Evaluation of certain food additives and contaminants. Fifty-third Report of the Joint FAO/WHO Expert Committee on Food Additives. Technical Report Series 896. Geneva, Switzerland