Polycyclic aromatic hydrocarbons and risk elements in honey from the South Moravian region (Czech Republic)

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Abstract

The aim of this study was to determine the actual content of some exogenous contaminants in the honey from the South Moravian region of the Czech Republic. The content of polycyclic aromatic hydrocarbons (PAHs) and risk elements (Mercury - Hg, Cadmium - Cd, Lead - Pb, and Arsenic - As) in multifloral blossom and honey dew samples of honey were determined by high-performance liquid chromatography and atomic absorption spectroscopy methods. The samples were collected form beekeepers and from retail stores located in South Moravia in the Czech Republic. Concentrations of individual PAHs in honey samples ranged between 0.02 μg·kg^{-1}–1.93 μg·kg^{-1}. The limit of benzo[*a*] pyrene for infant formula (1.0 μg·kg^{-1}) was not exceeded and fluoranthene was not quantified in any of the samples. Concentrations of Hg, Cd, and Pb were in the range of 3.24 μg·kg^{-1}–11.31 μg·kg^{-1}, 0.95 μg·kg^{-1}–32.35 μg·kg^{-1}, and 22.80 μg·kg^{-1}–177.85 μg·kg^{-1}, respectively. Concentration of As exceeded the detection limit only in three samples, ranging from 3.51 μg·kg^{-1} to 4.35 μg·kg^{-1}. Acceptable limits for trace elements in infant formula were met. Results of this study complete present knowledge of the contaminant content in Czech honey and confirmed high quality and safety of honey from the South Moravian region.

Persistent organic pollutants, toxic elements, HPLC, atomic absorption spectroscopy

Honey contains a number of nutritionally valuable compounds and is appreciated for its healing and prophylactic properties. These result from its composition and are reflected by physicochemical and chemical indicators (Lachman et al. 2010). According to some authors, honey is considered an environmental marker due to its ability to contain harmful substances coming from polluted environment and beekeeping practices, e.g. trace elements (Conti and Botre 2001), pesticides (Blasco et al. 2003), antibiotic residues (Hammel et al. 2008), and polycyclic aromatic hydrocarbons (PAHs) (Dobrinas et al. 2008). On the other hand, honeybees use their own detoxification mechanisms to decrease the content of harmful substances (Ferreira et al. 2010; Niu et al. 2011). The PAHs belong to the family of persistent organic pollutants with properties negatively impacting the human organism such as carcinogenicity, mutagenicity, etc. (Ramesh et al. 2004). Inhaled air and foodstuffs represent the main exposure source of PAHs for most of the population (Alexander et al. 2008). Data on the PAHs content in honey are very rare, nevertheless, some authors reported high concentrations of PAHs in honey (Dobrinas et al. 2008). Trace concentration of metals in honey and their high variability are dependent very strongly on the botanical and geographical origin, and less on the climatic and seasonal criteria (Čelechovská and Vorlová 2001). Remarkably elevated values of trace elements in honey were reported in samples from industrial and otherwise polluted areas (Bogdanov et al. 2007). The influence of environmental anthropogenic contaminations on the risk element content in honey is still discussed (Claudianos et al. 2006; Fredes and Montenegro 2006; Tuzen et al. 2006) because of evident low metal bioaccumulation (Conti and Botre 2001). On the other hand, according to previous studies the presence of toxic metals in honey is

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significantly influenced by the environmental pollution (Toporčák et al. 1992; Pisany et al. 2008).

The aim of our study was to determine the actual content of exogenous contaminants, i.e. PAHs and toxic elements (Hg, Cd, Pb, and As) in honey from the South Moravian region of the Czech Republic, to compare our results with known data obtained from previous studies, and to confirm the quality and safety of the honey tested.

Materials and Methods

Sample characterization

Samples of honey were obtained from beekeepers located around the city of Brno (honey no. 1–10) and from Brno retail stores (honey no. 11–20). The country of origin of all honey samples was declared as the Czech Republic. Samples no. 1 and 2 were honey dew honeys and the remaining samples were multifloral blossom honeys.

PAHs analysis

We monitored the content of 15 PAHs pollutants in honey according to the US EPA (EPA/630/R-98/002, 1986), namely acenaphtene (Ace), anthracene (Ant), benzo[*a*]anthracene (BaA), benzo[*a*]pyrene (BaP), benzo[*g,h,i*] perylen (BghiP), benzo[*b*]fluoranthen (BbF), benzo[*k*]-fluoranthene (BkF), chrysene (Chr), dibenzo[*a,h*] anthracene (DahA), fluorene (Fl), fluoranthene (Fu), indeno[*1,2,3-c,d*] pyrene (IPy), naphthalene (Na), phenanthrene (Phe), and pyrene (Py). The honey sample (10 g) was mixed with anhydrous sodium sulphate, and 40 ml of dichloromethane were added and mixture was extracted by means of ultrasonic bath and Ultra Turrax. After filtration the solvent was evaporated to dryness (Büchi, Flawil, Switzerland), redissolved in 1 ml of acetonitrile, filtered (0.45 μm nylon membrane filter) and analysed by HPLC – Waters 2695 Alliance chromatographic system equipped with Empower 2 software, and the Waters 2475 Fluorescence Detector (Waters, Milford, USA) using an excitation and emission wavelength program. The PAH C18 column (250 mm × 4.6 mm I.D. 5 μm – Waters, Milford, USA) was used. Column temperature was 30 °C, mobile phase A (water) and B (acetonitrile). The gradient elution was programmed as follows: 50% B (0–5 min), 50% B to 100% B linearly (5–20 min), 100% B (20–28 min), 100% B to 50% B linearly (28–32 min). The flow rate was set at 1.4 $ml \cdot min^{-1}$. Quantification was carried out by the external standard method.

Risk element analysis

Risk elements Cd and Pb were determined by electrothermal atomization HR-CS AAS (High Resolution Continuum Source Atomic Absorption Spectrometry) on the ContrAA 700 apparatus (Analytik, Jena, Germany) after mineralization of the sample with concentrated nitric acid and hydrogen peroxide in a laboratory autoclave with microwave heating (Ethos Sel, Milestone, Sorisole, Italy). For determination of As, the sample (after mineralization in autoclave) was ashed with the addition of magnesium nitrate in a muffle oven at 450 °C. The ash was dissolved in hydrochloric acid and As^{V} was reduced to As^{III}. Arsenic was determined by hydride technique in graphite atomizer, HS 60 (Analytik). The apparatus was set to the conditions recommended by the producer and the temperature program was optimized for the individual elements (see Table 1). The measurement of Hg was done by HR-CS AAS on an Advanced Mercury Analyzer AMA – 254 (ALTEC, Dvůr Králové nad Labem, Czech Republic).

Table 1. Additional information about the experimental conditions of risk elements analysis in honey.

Element	λ (nm)	Atomization (°C)	Modificator	Calibration ($\mu g \cdot l^{-1}$)
Cd	228.8018	1500	$Pd(NO_3)_2$	0.5–4.0
Pb	283.3060	1900	$Pd(NO_3)_2$	5.0–40.0
As	193.6960	2200	Ir	0.25–2.0

Statistics

Basic statistical characteristics (mean, standard deviation, minimum, maximum) for the exogenous elements were calculated using Microsoft Excel 2003. Statistical assessment of the data was carried out using the statistical and graphic software STAT Plus (Matoušková et al. 1992).

Results

Polycyclic aromatic hydrocarbons

Limit of detection (LOD) and limit of quantification (LOQ) ranged from 0.01 $\mu g \cdot kg^{-1}$ to 0.21 $\mu g \cdot kg^{-1}$ and from 0.02 $\mu g \cdot kg^{-1}$ to 0.68 $\mu g \cdot kg^{-1}$, respectively. Recoveries of PAHs ranged from 60.5% (Fl) to 103.2% (Ace). Repeatability expressed as relative standard deviation (RSD) of 12 parallel measurements was no higher than 3.0%. The average concentrations of individual

PAHs in samples from beekeepers and in samples from retail ranged from 0.02 μg·kg^{-1} to 1.60 μg·kg^{-1} and from 0.04 μg·kg^{-1} to 1.93 μg·kg^{-1}, respectively. Maximum value of BaP was 0.83 μg·kg^{-1} (samples from retail) and 0.81 μg·kg^{-1} (samples from beekeepers) (Table 2, 3).

Table 2. Concentrations of polycyclic aromatic hydrocarbons (μg·kg^{-1}) in honey samples collected from beekeepers.

PAHs	Honey samples										Range
	1	2	3	4	5	6	7	8	9	10	
Na	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ace	< LOQ	< LOQ	< LOQ	1.12	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ – 1.12
Fl	0.58	1.30	0.81	1.60	< LOQ	1.22	0.82	< LOQ	0.65	1.35	< LOQ – 1.60
Phe	0.71	0.83	0.67	0.71	1.26	0.72	0.84	0.08	1.58	0.87	0.08 – 1.58
Ant	0.71	0.73	0.72	0.73	0.74	0.72	0.72	0.04	0.74	0.75	0.04 – 0.75
Fu	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Py	0.44	0.49	0.47	0.45	0.56	0.51	0.47	< LOQ	0.58	0.53	< LOQ – 0.58
BaA	1.39	1.41	1.39	1.40	1.40	1.40	1.39	0.04	1.41	1.41	0.04 – 1.41
Chr	1.22	1.28	1.24	1.25	1.25	1.22	1.23	0.05	1.31	1.26	0.05 – 1.31
BbF	0.45	0.46	0.42	0.02	0.45	0.41	0.44	< LOQ	0.03	0.92	< LOQ – 0.92
BkF	0.82	0.82	0.82	0.82	0.82	0.83	0.82	< LOQ	0.83	0.84	< LOQ – 0.84
BaP	< LOQ	< LOQ	< LOQ	< LOQ	0.80	< LOQ	0.79	< LOQ	0.81	< LOQ	< LOQ – 0.81
DahA	0.71	0.69	0.86	0.71	0.71	0.71	0.35	< LOQ	0.71	0.73	< LOQ – 0.86
BghiP	0.99	0.99	0.98	0.99	0.99	0.99	0.99	< LOQ	0.99	1.10	< LOQ – 1.10
Ipy	0.79	0.39	0.78	0.78	0.78	0.79	0.78	< LOQ	0.79	0.81	< LOQ – 0.81

PAHs – polycyclic aromatic hydrocarbons, Na – naphthalene, LOQ – limit of quantification, Ace – acenaphtene, Fl – fluorene, Phe – phenanthrene, Ant – anthracene, Fu – fluoranthene, Py – pyrene, BaA – benzo[*a*]anthracene, Chr – chrysene, BbF – benzo[*b*]fluoranthene, BkF – benzo[*k*]-fluoranthene, BaP – benzo[*a*]pyrene, DahA – dibenzo [*a,h*] anthracene, BghiP – benzo[*g,h,i*]perylene, Ipy – indeno[*1,2,3-c,d*] pyrene

Table 3. Concentrations of polycyclic aromatic hydrocarbons (μg·kg^{-1}) in honey samples collected from retail.

PAHs	Honey samples										Range
	11	12	13	14	15	16	17	18	19	20	
Na	< LOQ	< LOQ	1.30	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ – 1.30
Ace	< LOQ	< LOQ	1.10	0.53	1.92	< LOQ	1.47	< LOQ	< LOQ	1.14	< LOQ – 1.47
Fl	0.55	0.62	0.88	< LOQ	< LOQ	1.29	0.24	0.80	0.63	1.93	< LOQ – 1.93
Phe	0.68	1.30	1.50	0.06	0.31	1.80	0.85	0.77	0.92	0.13	0.06 – 1.50
Ant	0.73	0.74	0.05	< LOQ	< LOQ	0.74	0.04	0.73	0.73	0.43	< LOQ – 0.74
Fu	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Py	0.54	< LOQ	0.97	< LOQ	< LOQ	0.58	< LOQ	0.53	0.54	< LOQ	< LOQ – 0.97
BaA	1.41	1.40	0.20	0.05	0.13	1.40	0.11	1.41	1.40	0.52	0.05 – 1.41
Chr	1.28	1.28	0.24	0.10	0.13	1.26	0.19	1.30	1.26	0.42	0.10 – 1.30
BbF	0.44	0.44	< LOQ	< LOQ	< LOQ	0.45	< LOQ	0.91	0.44	< LOQ	< LOQ – 0.91
BkF	0.83	0.41	0.04	< LOQ	< LOQ	0.83	0.04	0.83	0.83	0.04	< LOQ – 0.83
BaP	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.83	0.81	< LOQ	< LOQ – 0.83
DahA	0.71	0.36	0.09	< LOQ	< LOQ	0.71	0.04	0.71	0.72	0.10	< LOQ – 0.72
BghiP	0.99	0.50	0.10	< LOQ	< LOQ	0.99	0.11	0.99	1.00	0.17	< LOQ – 1.00
Ipy	0.79	0.39	< LOQ	< LOQ	< LOQ	0.79	< LOQ	0.80	0.79	0.19	< LOQ – 0.80

PAHs – polycyclic aromatic hydrocarbons, Na – naphtalene, LOQ – limit of quantification, Ace – acenaphtene, Fl – fluorene, Phe – phenanthrene, Ant – anthracene, Fu – fluoranthene, Py – pyrene, BaA – benzo[*a*]anthracene, Chr – chrysene, BbF – benzo[*b*]fluoranthene, BkF – benzo[*k*]-fluoranthene, BaP – benzo[*a*]pyrene, DahA – dibenzo [*a,h*] anthracene, BghiP – benzo[*g,h,i*]perylene, Ipy – indeno[*1,2,3-c,d*] pyrene

Risk elements

The method AAS showed the following detection limits (defined as the concentration equivalent to three times the standard deviation of the analytical blank signal, n = 10) 0.25 $\mu g \cdot kg^{-1}$, 4.5 $\mu g \cdot kg^{-1}$ and 0.9 $\mu g \cdot kg^{-1}$ for Cd, Pb and As, respectively. The recovery test ranged from 93.5% to 104.1% for individual elements. Detection limit for mercury was 0.01 $ng \cdot kg^{-1}$. Concentrations of Hg, Cd, and Pb were found in the range of 3.24 $\mu g \cdot kg^{-1}$ to 11.31 $\mu g \cdot kg^{-1}$, 0.95 $\mu g \cdot kg^{-1}$ to 32.35 $\mu g \cdot kg^{-1}$, and 22.8 $\mu g \cdot kg^{-1}$ to 177.85 $\mu g \cdot kg^{-1}$, respectively. Concentration of arsenic exceeded the detection limit only in three samples, ranging from 3.51 $\mu g \cdot kg^{-1}$ to 4.35 $\mu g \cdot kg^{-1}$ (Table 4 and 5). Table 6 presents comparisons of toxicology limits of provisional tolerable weekly intake (PTWI), and the calculated amount of honey that would meet the toxicology limit (NTL – weight of honey sample

Table 4. Concentration of trace elements ($\mu g \cdot kg^{-1}$) in honey collected from beekeepers.

Trace elements	Honey samples										Range	Acceptable limits ($\mu g \cdot kg^{-1}$)[a]
	1	2	3	4	5	6	7	8	9	10		
Hg	5.06	8.73	11.31	9.49	8.69	6.22	6.53	6.84	6.97	5.78	5.06-11.31	20
Cd	1.25	4.05	12.4	32.35	3.05	0.95	1.55	19.6	4.15	1.85	0.95-32.35	100
Pb	39.25	177.85	24.1	45.5	41.9	37.15	22.8	76	97.4	51.6	22.8-117.85	200
As	< 1.00	4.35	< 1.00	3.51	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00-4.35	100

[a] Acceptable limits for infant formula (Decree no. 305/2004 Coll. and Commission Regulation (EC) No. 1881/2006)

Table 5. Concentration of trace elements ($\mu g \cdot kg^{-1}$) in honey collected from beekeepers and from retail.

Trace elements	Honey samples										Range	Acceptable limits ($\mu g \cdot kg^{-1}$)[a]
	11	12	13	14	15	16	17	18	19	20		
Hg	7.23	5.97	5.95	3.24	4.44	4.99	4.49	5.18	4.29	3.78	3.24-7.23	20
Cd	5.8	1.65	1.5	21.65	21.65	3.9	2.25	1.35	4.15	7.55	1.35-21.65	100
Pb	69.75	31.55	31.05	37.05	78.4	78.9	53.8	46.55	94	34.75	31.05-94.00	200
As	3.65	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00-3.65	100

[a] Acceptable limits for infant formula (Decree no. 305/2004 Coll. and Commission Regulation (EC) No. 1881/2006)

Table 6. Comparison of maximum concentration of pollutants in honey with toxicology limits of the World Health Organization.

	PTWI (WHO)	Honey	c ($\mu g \cdot kg^{-1}$)	D (μg)	NTL (kg)
Hg	4 $\mu g \cdot kg^{-1}$ bw per week [a]	beekeepers	11.31	280	24.76
		retail	7.23		38.72
As	15 $\mu g \cdot kg^{-1}$ bw per week [b]	beekeepers	4.35	1050	241.4
		retail	3.65		287.7
Pb	25 $\mu g \cdot kg^{-1}$ bw per week	beekeepers	177.85	1750	9.84
		retail	94.00		18.61
Cd	7 $\mu g \cdot kg^{-1}$ bw per week	beekeepers	32.35	490	15.15
		retail	21.65		22.63

PTWI – provisional tolerable weekly intake (JECFA/72/SC 2010), WHO – World Health Organization, bw – body weight, c – maximum concentration, D – tolerable weekly amount of pollutant expressed in micrograms per person; D = PTWI × W, W – average personal weight (70 kg), NTL - weight of honey sample for filling toxicology limits expressed in kilograms per week; NTL = D/c, [a] – value for inorganic Hg (other than fish and shellfish), [b] – value for inorganic As

for meeting toxicology limits). Calculation was carried out for maximal concentration of target analytes in honey.

Discussion

There were no significant differences of PAH and trace element contents between honey samples collected form beekeepers and from Brno retail stores, and between blossom and honey dew honey.

The results of polycyclic aromatic hydrocarbon content in honey obtained in our study correspond with data published by Perugini et al. (2009) from Italy and are lower than PAH values in honey from Romania (Dobrinas et al. 2008). Maximum residue levels (MRLs) for PAHs in honey are not set. The Commission Regulation only sets limits of BaP for oils, fats, smoked meats, smoked fish and sea food, processed cereal-based food, baby food, infant's formula and milk and dietary foods for special medical purposes intended specifically for infants. Our results may be compared with limits for BaP in uncontaminated food matrix, e.g. infant formula (1.0 $\mu g \cdot kg^{-1}$) (Commission Regulation (EC) No 1881/2006). This limit was not exceeded in any of the samples.

The values of risk elements could be compared with maximum levels of heavy metals in children's food. The maximum level limits for mercury, cadmium, and arsenic in children's and infant's food is 20 $\mu g \cdot kg^{-1}$, 100 $\mu g \cdot kg^{-1}$, and 100 $\mu g \cdot kg^{-1}$, respectively (Decree no. 305/2004 Coll.), for lead 20 $\mu g \cdot kg^{-1}$ (Commission Regulation (EC) No 1881/2006). Lead fills toxicology limits. The value of lead in honey in our study are similar to results from Lithuania (Juodisius and Simoneliene 2009). Higher concentration of lead and cadmium were detected in Turkey (Leblebici and Aksoy 2008). Concentration of cadmium in our samples was higher than in the samples from Lithuania (Juodisius and Simoneliene 2009), Italy (Pisany et al. 2008), and Canary Islands (Trias et al. 2008). The samples analyzed in our study were collected from an urban area, so possible sources of contamination include anthropogenic activity, industry or traffic. The concentrations of mercury and arsenic in our samples were very low compared to the concentrations in honey from other European countries (Pisany et al. 2008) and correspond to the amount of these elements in honey from an area with no industrial load (Toporčák et al. 1992). The concentration of trace elements did not exceed acceptable limits for infant's food. In reference to these results of trace elements analysis and the average consumption portion of honey (20 g) we can conclude that the daily intake of honey poses a very low hygienic risk for consumers.

In conclusion, our results confirm that Czech honey from the South Moravian region of the Czech Republic may be considered safe food of high quality indicating good hygiene of manufacturing and bee keeping practices.

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References

Alexander J, Benford D, Cockburn A, Cravedi J, Dogliotti E, Di Domenico A, Fernández-Cruz M, L, Fink-Gremmels J, Fürst P, Galli C, Grandjean P, Azyl J, Heinemeyer G, Johansson N, Mutti A, Schlatter J, Van Leeuwen R, Van Peteghem C, Verger P 2008: Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on polycyclic aromatic hydrocarbons in food 2008. EFSA J **724**: 1-114

Blasco C, Fernández M, Pena A, Lino C, Silveira MI, Font G, Picó Y 2003: Assessment of pesticide residues in honey samples from Portugal and Spain. J Agr Food Chem **51**: 8132-8138

Bogdanov S, Haldimann M, Luginbuhl W, Gallmann P 2007: Minerals in honey: Environmental, geographical and botanical aspects. J Apicult Res **46**: 269-275

Claudianos C, Ranson H, Johnson, RM, Biswas S, Schuler MA, Berenbaum MR, Feyereisen R, Oakeshott JG 2006: A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. Insect Mol Biol **15**: 615-636

Conti ME, Botre F 2001: Honeybees and their product as potential bioindicators of heavy metals contamination. Environ Monit Assess **69**: 267-282

Čelechovská O, Vorlová L 2001: Groups of honey – physicochemical properties and heavy metals. Acta Vet Brno **70**: 91-95

Decree No. 305/2004 Coll., determining types of contaminating and toxic substances and their admissible levels in foodstuffs. Collection of Laws, Czech Republic, 2004, no. 100, pp. 6398-6406

Dobrinas S, Birghila S, Coatu V 2008: Assessment of polycyclic aromatic hydrocarbons in honey and propolis produced from various flowering trees and plants in Romania. J Food Compos Anal **27**: 71-77

EPA/630/R-98/002 1986: Guidance for health risk from exposure to chemical mixtures. Federal Register, No. 185, **51**: 34014-34025

EU. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, 2006, pp. 364/5-364/24

Ferreira RS, Anderlini RP, Anna OABES, Pimenta DC, Orsi RD, Barraviera B 2010: New nanostructured silica adjuvant (SBA-15) employed to produce antivenom in young sheep using crotalus durissus terrificus and apis mellifera venoms detoxified by cobalt-60 2010. J Toxicol Environ Health **73**: 926-933

Fredes C, Montenegro G 2006: Heavy metals and other trace elements contents in Chilean honey. Cienc Investig Agrar **33**: 50-58

Hammel YA, Mohamed R, Gremaud E, LeBreton, MH**,** Guy PA 2008: Multi-screening approach to monitor and quantify 42 antibiotic residues in honey by liquid chromatography–tandem mass spectrometry. J Chromatogr A **1177**: 58-76

JECFA/72/SC 2010: Joint FAO/WHO Expert Committee on Food Additives. Seventy-second meeting, Rome, 16 – 25 February 2010. Summary and Conclusions. Food and Agriculture Organization of the United Nations, Rome, 16 p.

Juodisius E, Simoneliene A 2009: Trace metals and pesticide residues in honey on the Lithuanian market. Vet Med Zoo **45**: 27-32

Lachman J, Orsák M, Hejtmánková A, Kovářová E 2010: Evaluation of antioxidant activity and total phenolics of selected Czech honeys. Food Sci Technol-Leb **43**: 52-58

Leblebici Z, Aksoy A 2008: Determination of heavy metals in honey samples from Central Anatolia using plasma optical emission spectrophotometry (ICP-OES). Pol J Environ Stud **17**: 549-555

Matoušková J, Chalupa J, Cígler M, Hruška K 1992: STAT Plus – Manual, version 1.01. (in Czech). Veterinary Research Institute, Brno, 168 p

Niu GD, Johnson RM, Berenbaum MR, May R 2011: Toxicity of mycotoxins to honeybees and its amelioration by propolis. Apido **42**: 79-87

Perugini M, Serafino G, Giacomelli A Medrzycki P, Sabatini AG, Persano OL, Marinelli E, Amorena M 2009: Monitoring of polycyclic aromatic hydrocarbons in bees (*Apis mellifera*) and honey in urban areas and wildlife reserves. J Agr Food Chem **57**: 7440-7444

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

Ramesh A, Walker SA, Hood DB, Guillen MD, Schneider K, Weyand EH 2004: Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. Int J Toxicol **23**: 301-333

Toporčák J, Legáth J, Kulková J 1992: Koncentrácia ortute vo vzorkách včiel a medu, pochádzajúcich z priemyselne exponovanej a neexponovanej oblasti (Levels of mercury in samples of bees and honey from areas with and without industrial contamination). Vet Med-Czech **37**: 405-412

Trias I, Rubio C, Gonzalez-Iglesias T, Gutierrez AJ, Gonzalez-Weller D, Hardisson A 2008: Metals in fresh honeys from Tenerife Island, Spain. Bull Environ Contam Toxicol **80**: 30-33

Tuzen M, Silici S, Mendil D, Soylak M 2006: Trace element levels in honeys from different regions of Turkey. Food Chem **103**: 325-330