

## Urban honey - the aspects of its safety

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To contribute to the development of urban beekeeping, we designed this study to obtain more information about the contamination of urban bee products with toxic metals, polycyclic aromatic hydrocarbons (PAHs), and pesticides. The samples of honey (N=23), pollen (N=13), and floral nectar (N=6) were collected from the experimental stationary apiary of the Belgrade University Faculty of Agriculture located in centre of Zemun (a municipality of the Belgrade metropolitan area) in 2015 and 2016. Metals (Pb, Cd, As, Cu, Zn, Fe, Mn, Ni, Cr, and Hg) were determined with inductively coupled plasma quadrupole mass spectrometry (ICP-QMS). Polycyclic aromatic hydrocarbons (PAHs) were analysed with high-performance liquid chromatography with fluorescence detection (HPLC-FLD). Pesticides were analysed with gas chromatography - mass spectrometry (GC-MS). The honey samples were generally within the European and Serbian regulatory limits. The levels of all the 123 analysed pesticides were below the limit of quantification (LOQ). Regarding PAH levels in honey, the highest content was found for naphthalene. The elevated levels of Hg and Cr and of PAHs in the pollen samples indicated air pollution. Pesticide residues in pollen, however, were below the LOQ. In nectar, metal levels were relatively similar to those in honey. Our results suggest that the investigated urban honey meets the regulatory requirements for metals, PAHs, and pesticides and is therefore safe for consumption.

KEY WORDS: *beekeeping; contaminants; pollen; urban areas*

Back in 1901, in his book *The Life of the Bee*, Maurice Maeterlinck made an interesting point, relevant for all mankind: "If the bee disappeared off the face of the earth, man would only have four years left to live" (1). Bees are efficient and reliable pollinators; they are methodical collectors of nectar and pollen, do not destroy the plant, and maintain the biodiversity and productivity of both natural and agricultural ecosystems (2). They also contribute to human wealth through the production of honey and other products (pollen, wax, propolis, and royal jelly).

In terms of environmental monitoring, bees are excellent mobile samplers and bioindicators of chemical contamination, as they come into contact with a variety of pollutants during their foraging flights (2-4). Contaminants cause high mortality rates of bees and end up in honey and other bee products (5) either indirectly, from the environment or agricultural practice, or directly, from beekeeping practice. The most important environmental contaminants are toxic (heavy) metals, pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), bacteria, genetically modified organisms, and radioactivity. Contaminants that originate from inappropriate beekeeping practice include substances used to control bee pests and diseases (acaricides, antibiotics, pesticides, etc.)

and heavy metals from the honey storage equipment and containers (6-8).

Unfortunately, bees are in decline across the globe. Over the past 10 to 15 years, beekeepers have been reporting unusual drops in bee numbers and colony losses throughout Europe and the USA. Statistics show a devastating 25 % loss of honey bees in Europe since 1985 (45 % in the UK alone since 2010) and 40 % in the USA since 2006 (9, 10). This phenomenon has been termed *colony collapse disorder* (CCD) and cannot be attributed to any single cause. The scientific community, however, points to the following possible contributing factors that act in combination or separately: beekeeping practices, environmental factors, chemical factors, and biological agents, which together create stress, weaken the bee's immune system, and pave way for pests and pathogens to kill colonies (9, 11).

In view of the ecological and economic importance of bees, this has raised the issue of their monitoring and protection all over the world. In 2011, the European Environmental Agency (EEA) started The Project City Bees on the assumption that bees were less exposed to pesticides in the cities (12, 13). The consequent assumption is that honey produced in the cities would be free from impurities, especially as the bees filter the nectar. However, there are too few (or too narrow) reports on the quality and the safety of honey produced exclusively in the cities to either refute or support it.

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The main aim of this study was, therefore, to obtain, for the first time, a more comprehensive information about the contamination of the so-called *urban honey* with toxic metals, polycyclic aromatic hydrocarbons (PAHs), and pesticide residues. Beside the honey, we analysed pollen and floral nectar to better understand the impact of urban environment on their safety aspects.

Another aim was to compare our findings with the relevant regulations. The EU has set maximum residue levels (MRLs) for a wide spectra of pesticides with its EC Regulation No. 396/2005 (14) and for pharmacologically active substances in honey with the Regulation No. 37/2010 (15). Serbia has also set MRLs for pesticides in honey (16). As for heavy metals, the EU regulations define the maximum level (ML) of lead alone, while Serbian regulations have also included cadmium, arsenic, zinc, copper, and iron in honey (16, 17). However, no MLs for PAHs in honey and other bee products have yet been established. Even so, their presence could certainly impair the safety of honey and in the city area they mostly reflect contamination from traffic and combustion of fossil fuels, which are still used for heating in many households in Zemun.

The idea to investigate urban honey came from an earlier collaboration with the Serbian Federation of Beekeeping Associations (SPOS, in which we analysed 379 monofloral and polyfloral honeys from all over Serbia for their physicochemical parameters (including metal content). Having found that heavy metal content, cadmium, for example, did not exceed the Serbian limits (18), we wanted to broaden our research.

## MATERIALS AND METHODS

### *Sample collection*

Samples for this study were obtained from an experimental stationary apiary located in the centre of Zemun, a municipality belonging to the Belgrade metropolitan area. The samples were collected by trained personnel of the Belgrade University Faculty of Agriculture. The bee colonies were kept in standard Langstroth's type beehive with 10 frames. The apiary had been monitored by the experts of the same institution to exclude any contamination caused by beekeeping, such as inadequate beehive treatment or equipment, and limit contamination to environmental sources.

We collected 23 samples of unprocessed polyfloral honey (16 in 2015 and seven in 2016) and 13 samples of bee pollen (10 in 2015 and three in 2016 year) taking care that honey and pollen samples corresponded to each other by collecting them at the same time. Honey samples were taken with 20 mL sterile syringes from parts of a young honeycomb that did not come into contact with the supporting wire. During collection, we did not use smoke.

Pollen samples were collected by placing plastic pollen collectors (pollen catching traps) at the entrance to the beehive. We also took six samples of nectar in 2016 by extracting it from the foragers' honey stomachs. Table 1 gives an overview of the collected samples.

Honey and pollen samples were stored in polyethylene containers (Lab Logistics Group GmbH, Meckenheim, Germany) suitable for foodstuff according to the EC

**Table 1** *The overview of the analysed samples of honey, bee pollen and nectar*

Sample	Description
h1	honey, April 2015
h2	honey, April 2015
h3	honey, April 2015
h4	honey, April 2015
h5	honey, May 2015
h6	honey, May 2015
h7	honey, May 2015
h8	honey, May 2015
h9	honey, June 2015
h10	honey, June 2015
h11	honey, July 2015
h12	honey, July 2015
h13	honey, July 2015
h14	honey, July 2015
h15	honey, August 2015
h16	honey, August 2015
h17	honey, April 2016
h18	honey, April 2016
h19	honey, June 2016
h20	honey, June 2016
h21	honey, June 2016
h22	honey, June 2016
h23	honey, October 2016
p1	pollen, April 2015
p2	pollen, April 2015
p3	pollen, May 2015
p4	pollen, May 2015
p5	pollen, May 2015
p6	pollen, May 2015
p7	pollen, June 2015
p8	pollen, June 2015
p9	pollen, July 2015
p10	pollen, July 2015
p11	pollen, April 2016
p12	pollen, June 2016
p13	pollen, October 2016
n1	nectar, April 2016
n2	nectar, April 2016
n3	nectar, June 2016
n4	nectar, June 2016
n5	nectar, June 2016
n6	nectar, June 2016

Regulations No. 1935/2004 and 10/2011 (19, 20). Honey samples were kept at room temperature, in the dark. Pollen samples were stored in a refrigerator at 4-8 °C. Nectar samples were stored in safe-lock tubes (Eppendorf AG, Hamburg, Germany) and deep-frozen at -18 °C until analysis. The pollen grains were powdered with a pestle in a mortar for homogeneity just before analysis.

#### *Chemicals and materials*

All chemicals were of analytical grade and supplied by Merck (Darmstadt, Germany): nitric acid 65 % (for analysis EMSURE® ISO), hydrogen peroxide 30 % (for analysis EMSURE® ISO) and acetonitrile ≥99.9 % (LiChrosolv®). The QuEChERS kits for PAHs with salt packets containing 4 g of anhydrous magnesium sulphate (MgSO<sub>4</sub>) and 0.5 g of sodium chloride (NaCl), the QuEChERS kits for pesticides with salt packets containing 4 g MgSO<sub>4</sub>, 1 g NaCl, 1 g trisodium citrate dihydrate, and 0.5 g disodium hydrogen citrate sesquihydrate, and 6 mL centrifuge tubes with 900 mg anhydrous MgSO<sub>4</sub>, 150 mg of primary secondary amine (PSA), and 150 mg octadecylsilane (ODS, also known as C18) for dispersive solid-phase extraction (dSPE) were purchased from Restek (Bellefonte PA, USA).

Deionised water (electrical resistivity 18.2 MΩ cm<sup>-1</sup>) was obtained using the Simplicity® water purification system (Merck Millipore, Burlington MA, USA).

For the quantification of metals, we used multi-element stock solution (1,000 g L<sup>-1</sup> of trace elements, Merck, Germany) to prepare intermediate multi-element standard solutions. Instead of certified reference materials, we used the leftover of the test material from the Fapas proficiency testing “Metallic contaminants in honey” (07286, Fapas, London, UK) for quality control of the analytical procedure.

For PAH analysis standard we used the PAH mix 16 (100 mg L<sup>-1</sup>) from Neochema (Bodenheim, Germany).

For pesticide residue analysis we used the following multiresidue pesticide standards: Organochlorine Pesticide Mix AB#3, 531.2 Carbamate Pesticide Cal Mix, Minnesota Ag List 1 Pesticides Mix A, 527 Pesticide Calibration Standard #1, 8140/8141 OP Pesticide Calibration Mix A (all by Restek), Pesticide Mix E27, Pesticide Mix F30, and Pesticide Mix E30 (all by Lab Standards, Castellana Grotte, Italy) as well as single residue pesticide standards: biphenyl and prothioconazole-dethio by Sigma-Aldrich, (Taufkirchen, Germany), fipronil-sulphone by Lab Standards, and burpofezin, dimethomorph, diphenylamine, fenamidone, fenhexamid, ipodione, terbuthylazine, and tetramethrin by Dr. Ehrenstorfer GmbH (Augsburg, Germany). The analytical-grade solution of triphenyl phosphate (TPP), used as internal standard in pesticide analysis, was purchased from Restek.

#### *Metal analysis*

Test portions of about 0.5 g of honey, 0.3 g of pollen and 0.4-0.5 g of nectar were treated with 7 mL of nitric acid

and 2 mL of hydrogen peroxide in polytetrafluoroethylene (PTFE) vessels and then mineralised in a microwave closed digestion system (Ethos Touch, Milestone, Bergamo, Italy) by heating up to 180 °C for 15 min, followed by heating up to 220 °C for 15 min, and then heating up to 240 °C for 10 min. After digestion, honey and pollen samples were quantitatively transferred into 50 mL volumetric flasks and diluted with deionised water. The digested samples of nectar were transferred into 25 mL volumetric flasks and also diluted with deionised water.

Trace element (Pb, Cd, As, Cu, Zn, Fe, Mn, Ni, Cr, and Hg) levels were determined with inductively coupled plasma quadrupole mass spectrometry (ICP-QMS) (iCAP Q, Thermo Scientific X series 2, Waltham MA, USA). The entire system was controlled with the Qtegra Instrument Control Software (Thermo Scientific, Waltham, MA, USA). Instrumental conditions were as follows: Rf power 1548 W; gas flows: 13.9, 1.09, 0.8 L min<sup>-1</sup>; acquisition time: 3×50 s; points per peak: 3; dwell time: 10 ns; detector mode: pulse. The measured isotopes were: <sup>50</sup>Cr, <sup>55</sup>Mn, <sup>57</sup>Fe, <sup>60</sup>Ni, <sup>65</sup>Cu, <sup>68</sup>Zn, <sup>75</sup>As, <sup>111</sup>Cd, <sup>202</sup>Hg, and <sup>208</sup>Pb.

#### *PAH analysis*

As we mentioned earlier, there are no MLs for PAHs in honey, but we decided to analyse 15 of the 16 PAHs frequently found in environmental monitoring samples (according to the United States Environmental Protection Agency): acenaphthene, anthracene, benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene, fluoranthene, fluorine, indeno[1.2.3-cd]pyrene, naphthalene, phenanthrene, pyrene, and their sum.

Samples of 5±0.1 g honey and 2±0.1 g pollen were weighed into a 50 mL polypropylene centrifuge tube and added 10 mL of deionised water. After 30 min, 10 mL of acetonitrile was added and the tube hand-shaken for 1 min. As soon as the QuEChERS salt kit for PAHs was added the sample was hand-shaken again for 1 min and then centrifuged at 1507 g for 5 min. Then 6 mL of the acetonitrile fraction was transferred into a 6 mL dSPE polypropylene tube. The tube was hand-shaken for 1 min and centrifuged at 1507 g for 5 min. Five mL of the clear solution was transferred into a 15 mL glass tube, and the eluate was evaporated to dryness at 60 °C using a gentle stream of nitrogen. The residues were dissolved in 1 mL of acetonitrile. Extracts of the samples in acetonitrile were passed through a 0.45 µm nylon membrane filter and analysed with a Thermo Spectra System (Thermo Scientific) high-performance liquid chromatographer with fluorescence detection (HPLC-FLD) equipped with the FL2000/FL3000 ULTRAFLUOR fluorescence detector and ChromQuest 4.2.35 software (Thermo Scientific) by injecting 50 µL of the sample into the Agilent Zorbax Eclipse PAH C18 column (4.6×250 mm; i.d. 5 mm, Agilent, Santa Clara CA, USA). The column temperature was ambient (25 °C);

mobile phase A (water) and B (acetonitrile). The gradient elution was programmed as follows: 50 % B (0-0.7 min) with 1.5 mL min<sup>-1</sup> flow rate; 50 % B to 75 % B linearly (0.7-12 min) with 2.0 mL min<sup>-1</sup> flow rate; 100 % B (12-25 min) with 2.0 mL min<sup>-1</sup> flow rate; and 100 % B to 50 % B linearly (25-30 min) with 1.5 mL min<sup>-1</sup> flow rate. For quantification we used the external standard method.

#### *Pesticide analysis*

Samples of 5±0.1 g honey and 2±0.1 g of pollen were weighed into a 50 mL polypropylene centrifuge tube and added 10 mL of deionised water and 50 µL of internal standard solution (TPP at 20 µg mL<sup>-1</sup>). After 30 min, 10 mL of acetonitrile was added, the tube was hand-shaken for 1 min. As soon as the QuEChERS salt kit for pesticides was added the sample hand-shaken again for 1 min and then centrifuged at 1507 g for 5 min. Then 6 mL of the acetonitrile fraction was transferred into a 6 mL dSPE polypropylene tube. The tube was hand-shaken for 1 min and centrifuged at 1507 g for 5 min. Five mL of the clear solution was transferred into a 15 mL glass tube, and the eluate was evaporated to dryness at 60 °C using a gentle stream of nitrogen. The residues were dissolved in 1 mL acetonitrile, and 1 µL of the sample was injected into a HP-5MS UI 0.25 µm, 0.25 mm×30 m column for gas chromatography mass spectrometry (GC-MS) a 7890A GC system, a 5975C MS system, and a 7683B autosampler (Agilent Technologies). The column was set to constant pressure. The injector temperature was 250 °C, and samples were injected in the splitless mode (the split vent opened after 30 s). The column temperature was programmed as follows: the initial temperature started at 70 °C (for 2 min), increased to 150 °C at the rate of 25 °C min<sup>-1</sup>, then immediately increased to 200 °C at 3 °C min<sup>-1</sup>, and ramped to 280 °C at 8 °C min<sup>-1</sup> (held for 10 min). The total run time was 41.87 min. The MS ionisation potential was 70 eV, and the temperatures were as follows: ion source 230 °C, transfer line 280 °C, and analyser 150 °C. The mass spectrometer was operated in the scan and selected-ion monitoring (SIM) mode.

#### *Quality parameters of honey*

Beside the metal content, PAHs, and pesticide residues, the honey samples were analysed for quality according to Harmonised Methods of the International Honey Commission (IHC) (21).

## RESULTS AND DISCUSSION

Our samples of urban honey met the basic physicochemical requirements for good quality, which supports the recent report on honey from stationary apiaries located in Serbian cities (22).

#### *Heavy metal content*

Table 2 shows the content of Pb, Cd, As, Cu, Zn, and Fe and the corresponding Serbian MLs (16) in honey samples. Except for Cu, all the urban honey samples met the requirements of the Serbian regulations, even though several studies warned about higher Pb in urban honey from polluted areas near busy roads and railways (5, 23). They ranged from <0.003 to 0.085 mg kg<sup>-1</sup> in 2015 and from <0.003 to 0.107 mg kg<sup>-1</sup> in 2016. Similarly, Cd and As levels kept very low in both years, whereas reports for Croatian honey were higher and ranged from 0.001 to 0.024 mg kg<sup>-1</sup> for Cd and 0.004-0.105 mg kg<sup>-1</sup> for As (24).

The levels of Cu, Zn, Fe, Mn, Ni and Cr were scattered even in samples from the same month. This variation, especially of Cu, which exceeded the MLs in four samples, was most likely related to the diversity of the foraged plant species, but some of it may be owed to the presence of pollen particles, because the sampled honey was unprocessed. The content of Mn and Ni was similar to the one reported in Romanian honey (0.868-2.529 mg kg<sup>-1</sup> for Mn and 0.122-0.325 mg kg<sup>-1</sup> for Ni), while Cr content was significantly higher (0.029-0.051 mg kg<sup>-1</sup>) (25).

Data about mercury content in urban honey are scarce. Our results correspond to those reported in areas affected with industrial pollution in Slovakia, where it ranged from 0.050 to 0.212 mg kg<sup>-1</sup> (26). Small amounts of mercury (0.083±0.011 µg kg<sup>-1</sup> of dry matter) were also found in honey samples from apiaries located in the area of the University of Veterinary Medicine and Pharmacy in Košice, also in Slovakia (27). In Greek honey from both rural and industrialised areas mercury content was lower than 0.05 mg kg<sup>-1</sup> (28).

As there are no regulations for metal content in bee pollen, we can only compare our findings (Table 2, p1-p13) with similar recent studies, particularly those investigating pollen from urban areas. Average Pb and Cd contents (0.17 mg kg<sup>-1</sup> and 0.05 mg kg<sup>-1</sup>, respectively) were similar to those reported for Polish pollen from stationary apiaries located in an industrial area (29). On the other hand, Cd content in bee pollen obtained in this study corresponds well to those reported earlier in samples not only from the Belgrade surroundings but also from non-urban parts of Serbia (30). The same is true for the content of Cu, Zn, Fe, Mn and Ni.

In 2015, Hg levels ranged from 0.073 to 0.198 mg kg<sup>-1</sup> in 2015, while in 2016 they were higher and ranged from 0.128 to 0.519 mg kg<sup>-1</sup>. The Slovakian study of bee pollen from the apiaries in Košice (27) reported lower Hg levels (0.05134±0.000038 mg kg<sup>-1</sup> of dry matter), and so did the Greek study (<0.06 mg kg<sup>-1</sup>) (28). The elevated Hg levels in our study may be attributed to different anthropogenic activities, especially combustion of fossil fuels containing toxic metals at trace levels (26), which are still used for heating in many households of Zemun and its surroundings.

Cr levels ranged from 2.474 to 5.998 mg kg<sup>-1</sup> and were significantly higher than those reported for Polish (29) and

**Table 2** Metal concentrations in honey (h), bee pollen (p), and nectar (n) samples collected in 2015-2016

Samples	Metals (mg kg <sup>-1</sup> )									
	Pb	Cd	As	Cu	Zn	Fe	Hg	Mn	Ni	Cr
h1	0.004	<0.002	<0.001	0.532	1.804	3.721	0.082	0.847	0.406	0.149
h2	0.015	0.002	<0.001	<0.015	1.319	3.534	0.078	1.241	0.071	<0.005
h3	<0.003	<0.002	0.004	0.398	1.203	3.762	0.062	0.837	0.203	0.137
h4	0.033	0.002	0.001	0.531	1.635	5.581	0.174	1.113	0.203	0.094
h5	0.016	0.003	0.001	0.497	1.301	3.730	0.102	0.792	0.157	<0.005
h6	0.042	<0.002	<0.001	<0.015	2.311	2.753	0.085	0.839	0.093	0.322
h7	0.085	<0.002	<0.001	<0.015	0.008	1.981	0.257	0.079	<0.010	0.018
h8	<0.003	<0.002	<0.001	<0.015	0.290	7.339	0.036	0.186	0.017	0.367
h9	0.011	<0.002	<0.001	<0.015	1.022	3.721	0.048	0.380	0.029	0.090
h10	0.020	<0.002	<0.001	<0.015	<0.002	1.420	0.096	0.136	0.050	0.535
h11	0.048	0.004	0.005	0.790	3.456	7.843	0.034	0.798	0.079	0.118
h12	0.033	0.005	0.009	<b>1.362</b>	1.121	5.142	0.072	1.590	0.261	0.173
h13	0.028	<0.002	<0.001	0.478	<0.002	6.225	0.019	0.904	0.182	0.379
h14	0.038	0.004	0.006	<b>1.158</b>	1.418	6.786	<0.002	0.999	0.134	0.120
h15	0.015	0.008	0.002	<b>1.425</b>	1.367	6.042	0.013	0.904	0.344	0.065
h16	0.017	0.002	0.001	0.937	1.042	4.423	0.012	0.872	0.169	0.008
h17	0.007	0.002	<0.001	<0.015	0.427	<0.012	<0.002	0.787	0.162	0.755
h18	<0.003	0.005	<0.001	0.167	1.257	<0.012	<0.002	0.838	0.404	0.319
h19	<0.003	<0.002	<0.001	<0.015	<0.002	1.119	<0.002	0.405	<0.010	0.833
h20	0.107	<0.002	<0.001	<0.015	0.723	<0.012	<0.002	0.817	0.091	0.439
h21	0.045	<0.002	<0.001	0.644	4.346	5.247	<0.002	1.475	0.263	0.275
h22	0.032	<0.002	<0.001	<0.015	0.574	2.810	<0.002	0.645	0.022	0.005
h23	0.083	0.009	<0.001	<b>1.781</b>	1.381	10.054	<0.002	2.428	0.538	0.342
<b>MLs</b>	0.50	0.03	0.50	1.0	10.0	20.0	-	-	-	-
p1	0.164	0.162	<0.003	2.587	24.95	113.83	0.198	17.14	0.613	5.998
p2	0.132	0.097	<0.003	3.106	29.52	107.98	0.155	19.34	0.326	4.898
p3	0.167	0.006	<0.003	16.89	33.54	89.03	0.151	27.24	2.258	5.787
p4	0.051	0.040	<0.003	5.348	27.22	68.32	0.123	20.69	0.634	3.917
p5	0.073	0.041	<0.003	4.527	30.74	71.43	0.118	24.12	0.217	2.474
p6	0.370	0.032	<0.003	4.847	26.76	64.90	0.128	22.07	0.154	3.757
p7	0.058	0.008	<0.003	4.849	26.18	65.01	0.073	23.00	0.554	2.749
p8	0.057	0.025	<0.003	4.171	29.56	63.46	0.093	18.50	0.565	3.395
p9	0.138	0.015	<0.003	23.08	30.76	94.37	0.083	22.30	1.867	5.767
p10	0.151	0.024	<0.003	30.67	33.64	102.39	0.051	26.05	2.773	4.103
p11	0.686	0.118	<0.003	13.482	32.53	106.86	0.519	23.67	1.770	3.335
p12	0.091	0.010	<0.003	8.101	39.24	97.33	0.206	21.37	1.870	3.370
p13	0.079	0.127	<0.003	4.023	23.14	91.80	0.128	15.43	0.240	3.083
n1	0.059	0.002	0.134	<0.150	<0.012	1.010	0.061	1.242	0.296	0.380
n2	0.062	0.003	0.029	0.296	<0.012	0.189	0.098	0.844	0.094	0.585
n3	0.052	0.003	0.004	1.318	<0.012	5.769	0.022	1.489	0.299	1.138
n4	0.117	0.008	<0.003	2.227	<0.012	6.117	0.023	1.942	1.075	0.351
n5	<0.021	0.004	<0.003	<0.150	<0.012	2.876	0.025	0.362	<0.009	0.450
n6	0.039	<0.002	<0.003	<0.150	<0.012	0.569	<0.018	0.540	<0.009	0.408

Note: bolded figures exceed the Serbian maximum levels (MLs) for honey (16)

**Table 3** PAH concentrations in honey (h) and bee pollen (p) samples collected in 2015-2016

Samples	PAHs ( $\mu\text{g kg}^{-1}$ )															
	Benzo[a]anthracene	Chrysene	Benzo[b]fluoranthene	Benzo[a]pyrene	Naphthalene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benzo[k]fluoranthene	Indeno[1.2.3-cd]pyrene	Dibenzo[a,h]anthracene	Benzo[g,h,i]perylene	Sum of PAHs
h1	0.2	<0.9	<0.3	0.3	4.0	<0.3	0.3	0.9	0.28	<0.2	<0.8	0.16	<0.2	1.0	<2.0	7.9
h2	0.2	<0.9	<0.3	0.2	4.0	<0.3	0.3	1.2	<0.04	<0.2	<0.8	0.24	<0.2	1.0	<2.0	7.1
h3	0.2	1.2	<0.3	0.4	3.6	<0.3	0.2	0.9	0.32	<0.2	<0.8	0.28	<0.2	1.0	<2.0	8.1
h4	0.4	<0.9	<0.3	0.5	3.6	0.2	0.5	1.5	0.36	<0.2	<0.8	0.32	<0.2	1.4	<2.0	9.6
h5	0.3	<0.9	<0.3	0.2	7.6	0.4	0.3	1.0	0.20	<0.2	<0.8	0.2	<0.2	1.4	<2.0	11.6
h6	0.3	<0.9	<0.3	0.2	11.6	0.4	0.8	2.2	<0.04	<0.2	<0.8	0.16	<0.2	1.8	<2.0	17.5
h7	0.4	<0.9	<0.3	0.2	3.6	<0.3	<0.2	0.9	0.18	<0.2	<0.8	0.13	<0.2	1.5	<2.0	6.9
h8	0.2	<0.9	<0.3	<0.2	7.6	<0.3	0.4	1.3	<0.04	<0.2	<0.8	0.20	<0.2	1.2	<2.0	10.9
h9	0.3	1.0	<0.3	<0.2	11.6	2.2	0.4	1.1	0.12	<0.2	<0.8	0.16	<0.2	1.2	<2.0	18.1
h10	0.3	1.0	<0.3	<0.2	7.6	2.1	0.2	1.2	0.04	0.3	<0.8	0.08	<0.2	0.8	<2.0	13.6
h11	0.2	0.9	<0.3	<0.2	7.6	0.4	0.4	1.2	<0.04	<0.2	<0.8	<0.06	<0.2	1.0	<2.0	11.7
h12	0.3	1.1	<0.3	<0.2	4.1	<0.3	0.3	0.9	<0.04	<0.2	<0.8	<0.06	<0.2	1.1	<2.0	7.8
h13	0.2	<0.9	<0.3	<0.2	5.3	<0.3	0.5	1.5	0.05	<0.2	<0.8	0.06	<0.2	1.3	<2.0	9.7
h14	0.2	<0.9	<0.3	0.2	6.4	<0.3	0.5	1.4	0.11	<0.2	<0.8	0.19	<0.2	1.6	<2.0	11.4
h15	0.3	1.1	<0.3	<0.2	3.5	<0.3	<0.2	0.3	0.12	<0.2	<0.8	0.16	<0.2	1.0	<2.0	6.5
h16	0.2	1.2	<0.3	<0.2	3.8	<0.3	0.2	0.8	0.05	<0.2	<0.8	0.06	<0.2	0.9	<2.0	7.2
h17	<0.1	<0.9	<0.3	<0.2	5.6	0.8	0.6	1.6	0.10	<0.2	<0.8	0.08	<0.2	1.1	<2.0	9.9
h18	0.1	<0.9	<0.3	<0.2	3.7	0.2	0.2	0.6	<0.04	<0.2	1.5	<0.06	<0.2	0.7	<2.0	7.0
h19	0.2	<0.9	<0.3	<0.2	<0.8	0.4	<0.2	0.7	<0.04	<0.2	<0.8	0.07	<0.2	1.4	<2.0	2.8
h20	0.1	<0.9	<0.3	<0.2	4.0	0.4	0.2	0.6	<0.04	<0.2	<0.8	<0.06	<0.2	0.8	<2.0	6.1
h21	<0.1	<0.9	<0.3	<0.2	2.4	<0.3	<0.2	0.2	0.12	0.3	<0.8	0.10	0.4	1.5	<2.0	5.0
h22	<0.1	<0.9	<0.3	<0.2	8.7	<0.3	0.3	0.6	0.11	<0.2	<0.8	<0.06	<0.2	1.1	<2.0	10.8
h23	<0.1	<0.9	<0.3	<0.2	7.0	<0.3	<0.2	0.6	0.10	<0.2	<0.8	<0.06	<0.2	0.8	<2.0	8.5

Table 3 continued

Samples	PAHs ( $\mu\text{g kg}^{-1}$ )															
	Benzo[a]anthracene	Chrysene	Benzo[b]fluoranthene	Benzo[a]pyrene	Naphtalene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benzo[k]fluoranthene	Indeno[1.2.3-cd]pyrene	Dibenzo[a,h]anthracene	Benzo[g,h,i]perylene	Sum of PAHs
<b>Range</b>	<0.1-0.4	<0.9-1.2	<0.3	<0.2-0.5	<0.8-11.6	<0.3-2.2	<0.2-0.8	0.6-2.2	<0.04-0.36	<0.2-0.3	<0.8-1.5	<0.06-0.32	<0.2-0.4	0.7-1.8	<2.0	2.8-18.1
p1	<0.3	<2.0	<0.6	<0.4	<2.0	5.5	7.2	33.0	3.2	5.6	<2.0	<0.2	<0.4	<0.8	<5.0	54.5
p2	2.0	<2.0	12.9	2.2	<2.0	11.4	2.6	<0.3	0.6	<0.5	<2.0	1.8	<0.4	<0.8	<5.0	33.5
p3	1.2	<2.0	7.3	1.1	<2.0	10.3	2.9	<0.3	<0.1	<0.5	<2.0	0.6	<0.4	<0.8	<5.0	23.4
p4	<0.3	<2.0	<0.6	2.8	<2.0	1.1	2.5	<0.3	<0.1	<0.5	<2.0	<0.2	<0.4	<0.8	<5.0	6.4
p5	<0.3	<2.0	<0.6	1.5	<2.0	13.8	3.4	0.6	<0.1	<0.5	<2.0	0.3	<0.4	<0.8	<5.0	19.6
p6	0.6	<2.0	<0.6	1.4	<2.0	9.1	3.0	0.3	0.2	0.7	<2.0	3.9	31.0	<0.8	<5.0	50.2
p7	0.3	<2.0	<0.6	6.8	<2.0	9.7	3.7	<0.3	0.7	0.7	<2.0	5.0	41.4	<0.8	<5.0	68.3
p8	<0.3	<2.0	1.4	<0.4	<2.0	7.3	2.2	<0.3	<0.1	1.4	<2.0	0.3	<0.4	<0.8	<5.0	12.6
p9	0.4	5.0	<0.6	<0.4	<2.0	7.7	5.2	6.3	<0.1	<0.5	6.0	0.5	5.0	<0.8	<5.0	36.1
p10	<0.3	3.0	<0.6	1.7	29.0	3.8	2.7	14.8	1.9	<0.5	27.0	<0.2	3.6	<0.8	<5.0	87.5
p11	<0.3	<2.0	<0.6	<0.4	51.0	4.2	8.6	0.3	6.0	<0.5	22.0	<0.2	<0.4	<0.8	<5.0	92.1
p12	1.9	8.0	0.9	2.4	49.0	5.0	<0.4	45.7	11.6	7.0	<2.0	2.1	5.1	2.3	<5.0	141.0
p13	0.8	13.0	<0.6	<0.4	39.0	12.0	7.5	74.2	5.4	4.9	<2.0	0.4	5.1	1.0	<5.0	163.3
<b>Range</b>	<0.3-2.0	<2.0-13.0	<0.6-12.9	<0.4-6.8	<2.0-51.0	1.1-13.8	<0.4-8.6	<0.3-74.2	<0.1-11.6	<0.5-7.0	<2.0-27.0	<0.2-5.0	<0.4-41.4	<0.8-2.3	<5.0	6.4-163.3

Serbian (30) bee pollen (0.078-0.965 mg kg<sup>-1</sup> and 0.169-0.465 mg kg<sup>-1</sup>, respectively).

As bee pollen is considered a valuable apitherapeutic product (31), its safety is very important and requires further investigation of metal contaminants in pollen from urban sites.

Table 2 also shows metal content in the samples of nectar collected in April and June 2016. Levels of metals in nectar samples were quite scattered, more or less similar to honey samples taken in the same period. This could be explained by the fact that bees visit different plants and use different plant exudates during the production of honey.

**Table 4** The overview of pesticides analysed in the honey and bee pollen samples

Chemical class of pesticides analysed (chemical name)	No. of pesticides
2,6-Dinitroaniline (pendimethalin, trifluralin)	2
Amine (diphenylamine)	1
Anilide (fenhexamid)	1
Benzimidazole (thiabendazole)	1
Carbamate, N-methyl carbamate and thiocarbamate (carbaryl, carbofuran, chlorpropham, iprovalicarb, propamocarb, methiocarb, methomyl, oxamyl, pirimicarb, propoxur, thiobencarb)	11
Carboxamide (boscalid)	1
Chitin synthesis inhibitors (buprofezin)	1
Chloroacetanilide (alachlor, metolachlor)	2
Conazole (difenoconazole, epoxiconazole, fluquinconazole, flutriafol, imazalil, myclobutanil, penconazole, prochloraz, propiconazole, prothioconazole-desthio, triadimefon, triadimenol)	12
Cyclodiene ( <i>cis</i> -chlordane, <i>trans</i> -chlordane, chordecone)	3
Dicarboximid (procymidone)	1
Dicarboximide (iprodione, vinclozolin)	2
Imidazole (fenamidone)	1
Juvenile hormone mimic (pyriproxyfen) and other carbamate/juvenile hormone mimic (fenoxycarb)	2
Keto-enol (spiromesifen)	1
Methoxyacrylate (azoxystrobin)	1
Morpholine (dimethomorph)	1
Organochlorine (aldrin, p,p'-DDD, p,p'-DDE, p,p'-DDD, dieldrin, endosulfansulfate, alpha-endosulfan, beta-endosulfan, endrin, keto-endrin, alpha-HCH, beta-HCH, delta-HCH, gamma-HCH, heptachlor, <i>cis</i> -heptachlorepoxyd, <i>trans</i> -heptachlorepoxyd, hexachlorobenzene, methoxychlor, oxychlordane)	20
Organophosphorus (chlorpyrifos, chlorpyrifos-methyl, coumaphos, dichlorvos, dimethoate / omethoate, ethoprophos, etrimfos, famphur, fenitrothion, fensulfothion, fenthion, isocarbophos, malathion, methacrifos, mevinphos, parathion, parathion-methyl, phosphamidon, pirimiphos-methyl)	20
Organothiophosphate (diazinon)	1
Phenol (orthophenylphenol)	1
Phenylsulfamide (dichlofluanid)	1
Pyrazole (fipronil, tebufenpyrad)	2
Pyrethroid (bifenthrin, bioallethrin, cyfluthrin, lambda-cyhalothrin, cypermethrin, deltamethrin, fenvalerate / esfenvalerate, fenpropathrin, permethrin, tefluthrin, tetramethrin)	12
Pyrimidine (bupirimate, cyprodinil, mepanipyrim, pyrimethanil)	4
Quinoline (ethoxyquin, quinoxifen)	2
Strobin (kresoxim-methyl, trifloxystrobin)	2
Triazine (cyanazine, prometon, prometryn, propazine, simazine, terbuthylazine)	6
Triazinone (hexazinone, metribuzin)	2
Unclassified (biphenyl, fenazaquin, fenpropidin, propargite)	4
Uracil (bromacil)	1
Xylylalanine (metalaxyl)	1



Besides, even the bees from same apiary may visit sites with different levels of contamination (32).

#### *Content of polycyclic aromatic hydrocarbons*

Table 3 shows PAH content in the honey and pollen samples. Sums of all 15 PAHs in honey ranged from 2.8-18.1  $\mu\text{g kg}^{-1}$ . Our benzo[a]pyrene levels in honey correspond to those reported in the studies of Czech (33) and Polish honey (34). It is interesting that the sum of benzo[a]anthracene, chrysene, benzo[b]fluoranthene, and benzo[a]pyrene in French honey taken from non-urban sites (0.03-5.80  $\mu\text{g kg}^{-1}$ ) was higher than the sum of these PAHs in our samples (35).

The content of other PAHs, such as chrysene, phenanthrene, and anthracene in our honey samples were similar to those in Italian honey samples originating from polluted areas (36).

Naphthalene had the highest concentrations in the honey samples (<0.8-11.6  $\mu\text{g kg}^{-1}$ ), but they were much lower than, for example, those reported by Dobrinas et al. (37) (up to 665.0  $\mu\text{g kg}^{-1}$ ) in honey from Romanian urban areas (37).

The sums of the 15 analysed PAHs in pollen ranged from 6.4 to 163.3  $\mu\text{g kg}^{-1}$ . The sum of benzo[a]anthracene, chrysene, benzo[b]fluoranthene, and benzo[a]pyrene was lower than reported for French pollen (0.22-129.41  $\mu\text{g kg}^{-1}$ ) from non-urban sites (35).

The highest levels in the pollen samples were those of phenanthrene, naphthalene, and indeno[1,2,3-cd]pyrene; their ranges were <0.3-74.2  $\mu\text{g kg}^{-1}$ , <2.0-51.0  $\mu\text{g kg}^{-1}$ , and <0.4-41.4  $\mu\text{g kg}^{-1}$ , respectively. Naphthalene was higher in all three pollen samples from 2016 (39.0-51.0  $\mu\text{g kg}^{-1}$ ) than in those from 2015 (<0.2  $\mu\text{g kg}^{-1}$ ). Similar was for phenanthrene (74.2  $\mu\text{g kg}^{-1}$  vs. 45.7  $\mu\text{g kg}^{-1}$ , respectively). This could be attributed to air pollution by traffic and fossil fuel heating, as well as weather conditions favouring the distribution of PAHs (36).

As expected, the PAH levels in pollen were higher than in honey, because they are highly lipophilic, and pollen contains 4-8 % of lipids, in some cases even 22.4 % (30, 38, 39), while honey contains none. It is mainly composed of sugars, amino acids, organic acids, minerals, and other relatively hydrophilic constituents (40).

#### *Pesticide content*

Table 4 shows how many honey and bee pollen samples showed the presence of one or more of the 123 pesticides analysed by GC-MS. However, none of the detected pesticide residues went above the LOQ of the method (0.01  $\text{mg kg}^{-1}$ ).

There are not many data about the presence of pesticides in honey and other bee products from exclusively urban sites (41, 42). Lambert et al. (43) reported higher, but not significantly higher contamination of rural honey than honey from other landscapes, including urban (43).

## CONCLUSION

To the best of our knowledge, this is the first more comprehensive report on the safety aspects of urban honey, pollen, and nectar in terms of toxic metals, PAHs, and pesticide residues.

Our honey samples met the European and Serbian regulations for pesticides and metals (Pb, Cd, As, Cu, Zn, and Fe). Our results suggest that the city environment does not pose greater risk of honey contamination if good beekeeping practices are followed. Pollen contamination needs further investigation, especially of air pollutants, as is indicated by elevated levels of Hg, Cr, and PAHs.

We believe that our findings will encourage the development of urban beekeeping with its undeniable benefits for urban residents and the environment.

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### Zagađivala u pčelinjim proizvodima urbanog podrijetla

Glavni cilj ove studije, kao doprinosa razvoju koncepta urbanog pčelarstva, bio je dati informacije o određenim zagađivalima - toksičnim metalima, policikličkim aromatskim ugljikovodicima (PAHs) i pesticidima koji bi mogli biti prisutni u pčelinjim proizvodima (med i pelud) isključivo urbanog podrijetla. Uzorci meda (23), peludi (13) i cvjetnog nektara (6) iz 2015. i 2016. godine dobiveni su iz eksperimentalnog stacionarnog pčelinjaka Poljoprivrednog fakulteta u središtu Zemuna (Beograd). Sadržaj metala (Pb, Cd, As, Cu, Zn, Fe, Mn, Ni, Cr i Hg) određen je pomoću induktivno spregnute plazme kvadrupolske masene spektrometrije (ICP-QMS). Policiklički aromatski ugljikovodici analizirani su tekućinskom kromatografijom visokog učinka uz fluorescentnu detekciju (HPLC-FLD). Pesticidi su analizirani plinskom kromatografijom s masenom spektrometrijom (GC-MS). Uzorci meda ispunjavali su europske i srbijanske službene propise vezane za najveće dopuštene količine određenih metala. Koncentracija 123 analizirana pesticida bila je ispod granice kvantifikacije (LOQ). Što se tiče sadržaja PAH u medu, najveća koncentracija pronađena je za naftalen. Povišene vrijednosti za neke metale (Hg, Cr) i PAH u uzorcima peludi upozoravaju na onečišćenje zraka kojem je pelud izložen. Što se tiče ostataka pesticida u peludi, oni su bili ispod LOQ-a. Sadržaj metala u nektaru bio je do određene mjere sličan onomu u medu. Općenito se može zaključiti da je ispitani med s urbanoga područja u pogledu sadržaja metala, PAH i pesticida u skladu s europskim i srbijanskim propisima.

KLJUČNE RIJEČI: kontaminanti; med; pelud; urbana područja; pčelarstvo