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Pesticides Residues and Metals in Plant Products from Agricultural Area of Belgrade, Serbia

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Abstract The objective of study was to assess the levels of selected metals and pesticides in plant products from agricultural area of Belgrade, Serbia in order to indicate their possible sources and risks of contamination and to evaluate their sanitary probity and safety. The concentrations of cadmium, copper, iron, manganese, nickel, lead and zinc were below limits established by national and international regulations (maximum found concentrations were 0.028, 1.91, 11.16, 1.77, 0.605, 0.073 and 1.76 mg kg^{-1} respectively). Only residue of one of examined pesticides was found in amount below MRL (bifenthrin 2.46 μg kg^{-1}) in only one of analysed samples, while others were below detection limits. Obtained results indicate that crops from examined agricultural areas are unpolluted by contaminants used for plant protection and nutrition, indicating good agricultural practice regarding pesticides and fertilizer usage as well as moderate industrial production within examined areas.

Keywords Pesticides · Metals · Plants · Monitoring

The main concern regarding increasing pollution levels is about contamination of environment with pesticide and metals as one of the most hazardous groups of contaminants due to their persistence and toxicity (Adriano 2001; Ecobichon 2001). When environment is contaminated with pesticides and metals, they may easily enter into human food chain through the plants. Eventually, the intake of food contaminated with those chemicals can lead to intoxication episodes that can be described as acute or, when the disease appears after a latent period of time, long-term intoxications. Thus, as a consequence of an outstanding concern for human health, environmental monitoring projects including assessment of the content of pesticide residues and metal concentration in plant products have been performed throughout the European countries.

Monitoring of pesticide residues in agricultural products of market producers allows determination and control of a correct use of plant protection products conforming to the good agricultural practice applied in the conventional, integrated and ecological production, and determination of origin and cause of the residues found (Baša Česnik et al. 2006). Additionally, inclusion of metals concentration assessment, associated with not only pesticides and fertilizers use but also with atmospheric deposition from town wastes, industrial emissions, and metal production (McLaughlin and Singh 1999), in the monitoring of those plant products, provides insight of the level of food safety on market.

Regular and stringent food quality control, performed throughout numerous countries, is based on Codex Alimentarius and other Food Agriculture Organization/World Health Organization (FAO/WHO) food standards (FAO/WHO, CAC/MRL 2008). Maximum residue levels (MRLs) that limit the types and amounts of residues that can be legally present on foods are set by regulatory bodies worldwide, while in Europe, European Union Council Directive 91/414/EEC, together with two new recent pieces of regulation - Regulation EC 1107/2009 and Directive 2009/128/EC, sets the rules about the regulatory framework by which MRLs are set. In the process of European integration, our country has adapted its work to the EU, thus the Serbian Law on Health Suitability of Food

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Products and Products of General Use, adopted in 1992. was, to the present, supplemented several times with the regulations concerning the permitted amount of pesticides, metals and metalloids and other toxic substances, chemotherapeutics, anabolic and other substances in food (Official Gazette RS 28/11 2011).

Systematic monitoring of the city of Belgrade environment (air, soil, drinking and river water quality, communal noise, and radioactivity) has been regularly organized by the Secretariat for Environmental Protection of the City of Belgrade Assembly, and in 2006. this Secretariat approved Project named Toxic Elements and Pesticides in Agricultural Land and Plant Products in the Belgrade Area. The present study was carried out as part of final Project phase, thus this paper contributes to the determination of the pollution levels by pesticides and metals in plant products cultivated in the Belgrade area, Serbia.

Materials and Methods

On the territory of Belgrade, agricultural land covers an area of about 322,292 ha, i.e. 70% of the total city area. This study was conduced on private farms and on Agricultural Corporation PKB—Belgrade, on ten localities: Ušće (Obrenovac), Zeoke (Lazarevac), Šiljakovac (Barajevo), Nemenikuće (Sopot), Jagnjilo (Mladenovac), Padinska skela, Veliko selo, Borča and Slanci (Palilula) and Batajnica (Zemun). Sampling sites for this phase of Project were determined in order to get representative plant samples of the main agricultural land from Belgrade territory. Plant samples from plastic sheds in this area were included in the study as well. Plants collected from June to October 2009. at 50 random agricultural plots from described agricultural areas were: cabbage (*Brassica oleracea* L. var. *capitata*), cucumber (*Cucumis sativus*), pepper (*Capsicum annum*), tomato (*Solanum lycopersicum*), zucchini (*Cucurbita pepo*) and apricot (*Prunus armeniaca*). Plant sampling was done in accordance with relevant European Union protocol.

As most of the vegetable and fruit samples analyzed were raised on private farms and, therefore, no records of protection product applications, despite distributed questionnaires, were available, selection of 17 pesticides studied in this research was based on active ingredients previously or recently registered for use in plant protection in our country (Janjic and Elezovic 2010), as well as their purpose and aplication time in production year when sampling was performed. Prior to analysis, 1 kg of plant samples was sliced and homogenized. Extraction of pesticides from plant samples was performed by QuEChERS procedure folowed by dSPE purification (Anastassiades et al. 2003). Quantification was performed using fortified untreated plant samples. Each plant sample was analyzed in triplicate. A gas chromatograph/mass spectrometer (CP-3800/Saturn 2200, Varian, Australia) with a 30 m × 0.25 mm × 0.25 µm, VF-5 ms column was used as detection device. Injector temperature was set to 270°C. The GC was programmed as follows: initial temperature was 120°C, then increased to 170°C at 8°C min^{-1} and held for 4.5 min, increased to 280°C at 9°C min^{-1} and held for 5.5 min. Helium was used as the carrier gas and its flow rate was 1.1 mL min^{-1}. The ion trap mass spectrometer was operated in the electron impact/selected ion monitoring (EI/SIM) mode. The ion trap and transfer line temperatures were set to 220 and 250°C, respectively. One specific pesticide ion was selected for detection and quantification, while a second one was used for confirmation. The ions inspected (m/z) were as follows: 160 (188) for alachlor, 181 (166) for bifenthrin, 314 (258) for chlorpyrifos, 204 (125) for clomazone, 179 (199) for diazinon, 267 (241) for endosulfan, 260 (277) for fenitrothion, 283 (383) for fluazifop-P-butyl, 127 (173) for malathion, 198 (215) for metribuzin, 72 (100) for napropamide, 252 (317) for oxyfluorfen, 252 (191) for pendimethalin, 201 (186) for simazine, 306 (264) for trifluralin and 214 (198) for vinclozolin.

For determination of the pollution levels by metals in invesigated plant samples, following elements were chosen: cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn). This choice was based on knowledge about use of mineral fertilizers with microelements and some Cu- or Zn-based fungicides in horticultural production on chosen agricultural areas. Besides, as the agricultural area of the Obrenovac and nearby Barajevo and Lazarevac territory are situated in the vicinity of coal-fired thermal power plants (TENT A and B), and because fly and bottom ash particles emitted as coal combustion products are significantly rich in toxic elements such as Cd, Ni and Pb (Baba et al. 2003), causing severe environmental problems, the determination of those metal contents in the plant products were also included. Prior to analysis, one kilogram of plant samples was sliced dried and ground according to AOAC official method 922.02., and determination of Cd, Cu, Fe, Mn, Ni, Pb and Zn concentrations in plant extracts was done by flame atomic absorption spectrometry (FAAS) according AOAC official method 975.03 (Horwitz 2002). A Varian SpectrAA 220 atomic absorption spectrometer with air/acetylene oxidizing flame and deuterium background correction was used.

Results and Discussion

The results of pesticide residues determined in plant samples are summarized in Table 1. Because different

Table 1 Pesticide residues in plant samples

Plant	Alachlor	Bifenthrin	Fenitrothion	Fluazifop-*P*-butyl	Chlorpyrifos	Clomazone	Diazinon	Endo-sulfan I	Endo-sulfan II	Malathion	Metribuzin	Napropamide	Oxy-fluorfen	Pendi-methalin	Simazine	Tri-fluralin	Vinclozolin
Pepper																	
(16 samples)[a]																	
LOD	–	–	–	4	–	3	1	–	–	1	–	16	–	5	–	1	–
f.c.				nd		nd	nd			nd		nd		nd		nd	
Cucumber (6 samples)[b]																	
LOD	–	1.9	1	–	–	–	3	–	–	3	–	10	–	–	–	–	1
f.c.		2.46 (one sample)	nd				nd			nd		nd					nd
Tomato																	
(18 samples)[c]																	
LOD	–	–	–	–	–	–	–	–	–	17	17	19	–	12	–	5	–
f.c.										nd	nd	nd		nd		nd	
Appricot																	
(1 sample)[d]																	
LOD	12	–	9	6	7	–	–	14	13	7	–	–	3	–	2	–	–
f.c.	nd		nd	nd	nd			nd	nd	nd			nd		nd		
Zucchini																	
(1 sample)[e]																	
LOD	–	10	6	–	–	–	4	–	–	3	–	12	–	–	–	–	3
f.c.		nd	nd				nd			nd		nd					nd
Cabbage																	
(8 samples)[f]																	
LOD	–	111	14	10	–	–	11	–	–	–	–	–	–	–	–	–	–
f.c.		nd	nd	nd			nd										

LOD limit of detection μg kg^{-1}, *f.c.* found content μg kg^{-1}, – not analyzed, *nd* not detected

[a] One from Batajnica, four from Padinska Skela, one from plastic shed in Jagnjilo, one from plastic shed in Nemenikuće, two from plastic sheds in Zeoke, five from plastic sheds in Šiljakovac and two from plastic sheds in Ušće; [b] one from Batajnica, two from Padinska Skela, one from plastic shed in Nemenikuće, one from open field in Nemenikuće and one from plastic shed in Zeoke; [c] four from Padinska Skela, one from plastic shed in Batajnica, one from plastic shed in Jagnjilo, two from plastic sheds in Nemenikuće, two from plastic sheds in Zeoke, two from plastic sheds in Šiljakovac and six from plastic sheds in Ušće; [d] from Nemenikuće; [e] from Nemenikuće; [f] one from Borča, four from Slanci and three from Veliko Selo

pesticides are registered for each plant product protection, different compounds were studied in every commodity type. The RSD values of three consecutive QuEChERS-GC/MS analysis of each plant sample were lower than 12.3%. The recovery values at the 0.03 mg kg^{-1} fortification level varied from 72.4% to 103.2% for all pesticides studied. Limit of detection (LOD) values, presented in Table 1, were computed as three times the baseline noise (S/N = 3) at the lowest detectable concentration.

Concentrations of all active ingredients studied in all plant samples were below the relevant LOD values (Table 1). According to results, only residues of bifenthrin, was found in amount of 2.46 μg kg^{-1} in cucumber sample from open field on Batajnica locality. The obtained result is significantly below the MRL of bifenthrin established for cucumber by national and international regulations (FAO/WHO, CAC/MRL 2008; Official Gazette RS 28/11 2011). Due to obtained results, it can be concluded that this insecticide had been applied according to the instructions. Levels of pesticide residues in cabbage, cucumber, pepper, tomato, zucchini and apricot cultivated on Belgrade agricultural area in 2009 do not give any cause for alarm as 98% examined samples did not contain any residues and exceeding maximum residue levels were not found. Comparing with the results of pesticide residues analysis in first phase of monitoring Project (Marković et al. 2010), it can be concluded that within examined areas good agricultural practice regarding pesticides usage in plant protection was achieved in recent years. Similar conclusion was earlier given by Škrbić and Predojević (2008) regarding the residue levels of pesticides in crops from Vojvodina, Serbia, and Lazić et al. (2009) regarding pesticide residues in vegetable samples from the market of the Republic of Serbia during 2007. As far as health problems caused by the presence of pesticide residues in foodstuffs are concerned, several countries have carried out food-contamination monitoring programs (Baša Česnik et al. 2010; Kmellár et al. 2010). There were several MRL exceeding for vegetables in various countries, indicating strong need for further regular monitoring of pesticide residues in plant products in order to have a good quality control of food with aim to avoid over consumption and their cumulative toxicities in long-term use.

As for metals, the mean value concentrations in sampled plants are presented in Table 2. The RSD values for three replicate analyses were not higher than 10%. Linearity of calibration curves for metal content determination was attained for the given concentration ranges and confirmed by regression coefficients (R) better than 0.997. The concentrations obtained in this study for the different plant samples analyzed present Fe as the element with the highest concentration levels, followed by Zn, Mn, Cu, Ni, Pb and Cd. There were no significant differences in this sequence among different vegetable species. As can be seen from the results, metal contents in analyzed plant samples do not exceed the maximum permitted values established by the Serbian national regulations (Official Gazette RS 28/11 2011) and mostly do not exceed the maximum permitted metal concentrations laid down in codex general standard for contaminants and toxins in food and feed (FAO/WHO 193-1995). Slightly higher concentrations of Ni were evidenced in Sopot and Lazarevac situated in the vicinity of coal-fired thermal power plants, and Palilula which is closer to urban regions in Belgrade and where the sources of Ni could largely be from the burning of fossil fuels, ashes from electricity and heating plant chimneys. But given that the average Ni content within all plants reported here is low, it is most likely that the sources of Ni found in some plants from studied areas are from natural—geochemical origin, with addition of smaller Ni quantities probably from phosphorous fertilizers. Besides Ni, higher concentration of Pb (slightly above maximum level defined by Codex Alimentarius Standard) was detected in only one tomato sample from near urban area (Padinska Skela), and slightly higher concentration of Cd were detected in plants cultivated on territories in the vicinity of coal-fired thermal power plants (Ušće), and near urban areas of Belgrade (Padinska Skela).

In general, compared with the metal concentration obtained in first phase of monitoring Project (Marković et al. 2010), concentration of investigated elements were significantly lower, indicating improvement in agricultural practice as well as moderate industrial production within examined areas. Besides, the obtained Cd, Zn and Pb concentrations are lower than the values reported for vegetables grown in Spain (Bordajandi et al. 2004) and UK (Alexander et al. 2006), while Cu concentrations fit well into the Cu content ranges published by those authors.

Considering all, the examined plants can be considered unpolluted and of satisfactory sanitary probity and safety, indicating good agricultural practice regarding pesticides and fertilizer usage as well as moderate industrial production within examined areas. Further regular monitoring of pesticide residues and metals levels in plant products is absolutely essential in order to have a good quality control of plant raw materials and to determine the presence of some contaminants, especially toxic elements, to avoid over consumption and their cumulative toxicities in long-term use.

Table 2 Metal content of dry and fresh plant samples

Plant	Lo	Water cont. (%)	Concentration (mg kg^{-1} dry weight/mg kg^{-1} fresh weight)						
			Cd	Cu	Fe	Mn	Ni	Pb	Zn
Cucumber	X	95.4	nd/nd	6.57/0.30	30.51/1.40	11.47/0.53	nd/nd	0.30/0.014	27.19/1.25
	VI	96.0	0.10/0.004	9.64/0.39	102.08/4.08	6.62/0.26	nd/nd	1.92/0.077	65.28/2.61
	VI	95.0	0.10/0.005	9.88/0.49	66.62/3.33	2.73/0.14	nd/nd	0.10/0.005	17.98/0.90
	IV[a]	95.5	nd/nd	6.85/0.31	94.32/4.24	8.78/0.39	nd/nd	0.68/0.031	22.13/0.99
	IV	96.3	0.050/0.002	8.75/0.32	160.26/5.93	9.37/0.28	7.40/0.274	0.40/0.015	28.01/1.04
	II[a]	95.8	0.10/0.004	11.36/0.48	175.04/7.35	9.03/0.38	nd/nd	1.37/0.057	24.13/1.01
Pepper	X	91.5	0.05/0.004	5.19/0.44	80.95/6.88	6.06/0.51	nd/nd	0.17/0.014	11.33/0.96
	VI	91.7	0.30/0.025	10.39/0.86	16.60/1.38	5.95/0.49	nd/nd	0.26/0.021	22.50/1.87
	VI	91.2	0.28/0.025	10.63/0.93	61.54/5.41	6.19/0.54	nd/nd	nd/nd	25.36/2.23
	V[a]	92.5	0.44/0.033	6.47/0.48	72.25/5.42	8.32/0.62	0.68/0.051	0.15/0.011	11.97/0.90
	IV[a]	91.9	0.05/0.004	11.65/0.94	155.42/12.59	8.36/0.68	7.80/0.632	1.06/0.086	24.26/1.96
	II[a]	93.0	0.19/0.013	9.17/0.64	112.70/7.89	10.29/0.72	6.29/0.440	0.65/0.045	20.14/1.41
	II[a]	91.3	0.14/0.012	7.60/0.66	81.86/7.12	7.71/0.67	0.92/0.080	0.85/0.074	20.59/1.79
	III[a]	91.8	nd/nd	4.27/0.35	44.06/3.61	6.37/0.52	nd/nd	0.80/0.066	14.65/1.20
	III[a]	92.0	0.05/0.004	5.21/0.42	38.68/3.09	8.04/0.62	nd/nd	0.81/0.065	14.77/1.18
	III[a]	92.0	0.20/0.016	8.11/0.65	54.88/4.39	10.21/0.82	nd/nd	0.69/0.055	19.62/1.57
	III[a]	91.9	0.19/0.015	7.35/0.59	52.59/4.26	6.45/0.52	nd/nd	0.18/0.015	13.64/1.10
	III[a]	91.5	0.23/0.019	7.37/0.63	43.70/3.71	6.97/0.59	nd/nd	1.16/0.099	15.97/1.36
	I[a]	92.8	0.05/0.004	8.44/0.61	85.88/6.20	9.65/0.69	1.17/0.084	0.90/0.065	22.11/1.59
	I[a]	92.1	0.19/0.015	4.55/0.36	83.19/6.57	11.57/0.91	0.86/0.068	0.40/0.032	15.05/1.19
	VI	91.2	0.14/0.012	7.69/0.68	27.96/2.46	7.85/0.69	nd/nd	0.48/0.042	22.44/1.97
	VI	91.0	0.25/0.022	8.02/0.75	103.62/9.32	3.63/0.33	nd/nd	nd/nd	13.25/1.19
Tomato	VI	94.5	0.14/0.008	12.87/0.71	97.65/5.37	2.89/0.16	nd/nd	**2.0/0.110**	7.73/0.42
	VI	95.0	0.19/0.009	7.48/0.37	64.50/3.22	5.68/0.28	2.82/0.141	nd/nd	13.94/0.70
	VI	93.2	0.10/0.007	9.58/0.65	23.97/1.63	1.43/0.10	nd/nd	nd/nd	7.27/0.49
	VI	93.6	0.10/0.006	12.47/0.80	7.40/0.47	1.61/0.10	9.45/0.605	0.90/0.058	11.37/0.73
	X[a]	93.5	0.23/0.015	7.91/0.51	58.68/3.81	10.89/0.71	nd/nd	0.33/0.021	11.84/0.77
	V[a]	93.9	0.34/0.021	6.50/0.40	34.52/2.11	8.77/0.53	nd/nd	nd/nd	7.52/0.46
	IV[a]	95.7	0.20/0.009	7.12/0.31	63.17/2.72	4.21/0.18	nd/nd	1.08/0.046	15.64/0.67
	IV[a]	93.2	0.05/0.003	7.28/0.49	58.51/3.98	5.39/0.37	nd/nd	0.95/0.065	15.01/1.02
	II[a]	93.0	0.14/0.010	4.65/0.32	56.17/3.93	3.19/0.22	0.19/0.013	0.71/0.050	12.39/0.87
	II[a]	93.5	nd/nd	4.63/0.30	38.55/2.50	4.13/0.27	nd/nd	0.46/0.030	13.25/0.86
	III[a]	95.7	0.05/0.002	9.25/0.40	32.68/1.40	4.70/0.20	nd/nd	1.38/0.059	12.48/0.54
	III[a]	95.9	0.18/0.007	2.70/0.08	20.52/0.84	3.01/0.12	nd/nd	1.37/0.056	9.30/0.38
	I[a]	95.5	0.44/0.018	8.98/0.40	66.12/2.97	17.33/0.78	nd/nd	0.97/0.044	26.09/1.17
	I[a]	94.5	0.24/0.013	9.20/0.51	67.08/0.37	12.28/0.67	nd/nd	0.66/0.036	17.68/0.97
	I[a]	94.2	0.19/0.011	7.73/0.45	56.77/3.29	9.93/0.57	nd/nd	1.14/0.066	17.60/1.02
	I[a]	94.8	0.38/0.020	11.08/0.58	93.48/4.86	21.45/1.11	0.80/0.042	0.12/0.006	26.02/1.35
	I[a]	93.8	0.46/0.028	12.07/0.75	85.64/5.31	22.31/1.38	0.65/0.040	0.93/0.058	28.88/1.76
	I[a]	94.0	0.19/0.011	10.50/0.63	97.94/5.88	20.56/1.23	nd/nd	1.08/0.065	25.93/1.55
Cabbage	VIII	92.6	nd/nd	1.99/0.15	86.59/6.41	8.44/0.62	nd/nd	0.71/0.052	22.15/1.64
	IX	92.6	nd/nd	2.24/0.17	53.55/3.96	20.37/1.51	nd/nd	0.99/0.073	21.34/1.58
	IX	93.1	0.05/0.003	14.44/1.00	110.56/7.63	17.02/1.17	4.99/0.344	1.05/0.072	24.03/1.66
	IX	92.9	nd/nd	3.29/0.23	78.81/5.59	24.89/1.77	nd/nd	0.11/0.008	23.75/1.69
	IX	93.4	nd/nd	3.08/0.20	51.86/3.42	19.89/1.31	nd/nd	1.01/0.067	19.87/1.31
	VII	92.9	nd/nd	26.92/1.91	139.10/9.88	22.28/1.58	3.87/0.274	1.02/0.072	19.51/1.38
	VII	92.3	0.05/0.004	2.34/0.18	128.34/9.88	19.93/1.53	3.99/0.307	0.11/0.008	21.12/1.63

Table 2 continued

Plant	Lo	Water cont. (%)	Concentration (mg kg^{-1} dry weight/mg kg^{-1} fresh weight)						
			Cd	Cu	Fe	Mn	Ni	Pb	Zn
	VII	93.5	nd/nd	2.54/0.16	100.96/6.56	19.17/1.25	nd/nd	0.69/0.045	24.65/1.60
Apricot	IV	85.8	nd/nd	7.67/1.09	59.51/8.45	1.36/0.19	nd/nd	nd/nd	8.87/1.26
Zucchini	IV	94.4	nd/nd	7.30/0.41	202.92/11.16	7.54/0.42	9.99/0.559	0.51/0.028	22.74/1.27

Lo location: I Ušće, II Zeoke, III Šiljakovac, IV Nemenikuće, V Jagnjilo, VI Padinska skela, VII Veliko selo, VIIIBorča, IX Slanci, X Batajnica; *nd* not detected

Concentrations above maximum levels defined by Codex Alimentarius Standard are marked with bold letters

[a] Plastic shed

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