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Detection of heavy metals in common vegetables at Varaždin City Market, Croatia

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The present study was aimed at the estimation of heavy metal content in vegetables sold at the city market of one of the densely populated Croatian cities, Varaždin, and to establish the relationship between their levels and possible sources of contamination. Twenty-eight samples of the most common diet vegetables (red and white potato, onion, carrot, common bean, lettuce, and cabbage) were randomly bought at the market in September and October 2013. Using the atomic absorption spectrometry method, concentrations of nine heavy metals (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn) were measured in the selected samples. The results showed that, in five out of 28 samples analysed, six concentrations exceeded the maximum levels provided for in the regulations: five for Pb and one for Cd. Maximum regulated levels for Pb were exceeded in two samples of red potato, two samples of common bean, and one sample of carrot (17.9 %), and for Cd in a sample of red potato (3.6 %). In conclusion, the cause of the overstepping of the maximum levels for Pb and Cd in the vegetables analysed was most likely the contaminated soil. The possible sources of soil contamination include traffic, nearby industry, floodwaters of rivers and streams, and the use of pesticides and fertilisers in agricultural production.

KEY WORDS: *cadmium; lead; maximum permitted level; food analysis; atomic absorption spectrometry*

Soil is a multifunctional good with many important roles: ecological regulation, filtration of water, universal buffering, regulation of climate, as a source of biodiversity, and, most importantly, in supplying plants with water, air and nutrients, therefore enabling the production of organic compounds in the process of photosynthesis (1, 2). All heavy metals are naturally present in the soil; however, elevated levels most frequently occur due to contamination, which can be of anthropogenic or natural origin. Anthropogenic activities making major contributions to soil contamination include industry, mining, waste management, traffic, agriculture, artificial fertilisers, metal-based pesticides, municipal sewage wastes, and irrigation (1, 3, 4). Some activities cause mobilisation of heavy metals, increasing their circulation through soil, water, and air and their transfer to the human food chain (5). The contamination of agricultural soil is of special concern (3, 5, 6). In Croatia, regulations define the maximum permitted levels of heavy metals in agricultural soil (7, 8).

The concentration of heavy metals in plants depends on the species, cultivar, growth stage, and organ of the plant, the concentration and bioavailability of heavy metals in the soil (strongly influenced by soil pH), and environmental conditions (9-12). Vegetables, an important dietary source of essential nutrients, may also contain elevated concentrations of heavy metals due to high transfer into the harvested organ (4).

Heavy metals may translocate to the shoots (e.g. Cd, Fe, Zn) or accumulate in roots (e.g. As, Cr, and Pb), and the concentrations of heavy metals are generally the highest in roots, followed by stem, leaves, and fruits (13-15).

For living organisms, heavy metals can be essential (e.g., Zn, Cu, Mn, Cr, Ni), utilised in small quantities in their metabolism and maintained at optimal levels through homeostasis, or non-essential and toxic (e.g., Cd, Pb, Hg, As) (11, 16, 17). In higher plants, essential micronutrients include Fe, Mn, B, Zn, Cu, Mo, Cl, and Ni, and non-essential As, Cd, Hg, and Pb (18). Essential heavy metals in plants are involved in many important processes, including redox processes, functions of metalloenzymes and metalloproteins, photosynthesis, respiration, expression, and regulation of genes, synthesis of proteins and plant defence mechanisms (19). The concentrations necessary for plant growth (critical deficiency concentrations) and toxic concentrations (critical toxicity concentrations) vary considerably depending on the plant species, plant age and concentration of other elements (18). For some, the line between micronutrient and phytotoxic substance is very thin (1, 11).

Regarding bioavailability for plant uptake, heavy metals can be bioavailable (e.g. As, Cd, Cu, Ni, Zn), moderately bioavailable (Mn), or least bioavailable (Cr, Pb) (20). Uptake of metals is influenced by solubility of the metal, complexation and chelation reactions, pH of soil solution, and concentration of metals (11). In alkaline soils, the

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availability of Fe, Cu, Mn, and Zn is very low, while in acid soils, concentrations of Al and Mn may rise to toxic levels and negatively influence plant growth (21).

The toxic effect of heavy metals, both essential and non-essential, depends on the type of target organism, the conditions of intake, the availability and concentration of the heavy metal and the kinetics of uptake, while the mechanisms of heavy-metal ion toxicity include the blocking of the functional group of biomolecules, displacing the essential metal ion in biomolecules, and modification of the active conformation of biomolecules (22). Heavy metals may affect cellular oxidation state, lipid peroxidation, breaking of DNA strands, protein expression and folding, degradation in proteasome, protein interactions, cell cycle and apoptosis; they are persistent and toxic, and they accumulate through the food chain (17, 23-25).

Since plants take up heavy metals, due to absorption from both polluted soil and environment, monitoring of their levels in vegetables is essential for preventing excessive build-up of metals in the food chain.

The aim of this study was to investigate the heavy-metal content of common diet vegetables sold at the market in Varaždin, Croatia. Vegetable samples were collected based on dietary preferences common in continental Croatia (lettuce, cabbage, red and white potato, onion, carrot and common bean). The presence of nine heavy metals – arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) was estimated.

MATERIALS AND METHODS

Study location

Varaždin was selected as one of the densely populated cities of Croatia, characterised by different industrial and commercial enterprises as potential sources of pollution, and relatively heavy traffic around and within the city. At the same time, large-scale vegetable production is conducted in this area, largely to supply the main city market. The climate in Varaždin region is continental. The mean annual air temperature is 10.2 °C and mean monthly air temperature ranges from -3.7 °C in January to 26.0 °C in July (26). Also, the mean annual precipitation is 843.1 mm and mean monthly precipitation ranges from 38.9 mm in January to 96.5 mm in June (26).

Vegetable samples

In September and October 2013, 28 samples of seven vegetables presumed to be most common in the diet of the continental region of Croatia were bought at 11 different sales locations at the city market in Varaždin: lettuce (*Lactuca sativa* L., fam. *Asteraceae*) cultivar "crystal", cabbage (*Brassica oleracea* L. var. *capitata* L., fam. *Brassicaceae*) white or green cultivar, carrot (*Daucus carota* L. subsp. *sativus* (Hoffm.) Schübl. & G. Martens, fam. *Apiaceae*), onion (*Allium cepa* L., fam. *Amaryllidaceae*), common bean (*Phaseolus vulgaris* L., fam. *Fabaceae*), and potato (*Solanum tuberosum* L., fam. *Solanaceae*).

Sample preparation for laboratory analysis

Samples were treated identically as during their preparation for human consumption. After washing with running water, fresh vegetable samples were weighed on a technical scale, chopped into smaller pieces, and air-dried at room temperature. Once dry, the samples were weighed again on a technical scale and ground.

Extraction of heavy metals soluble in a mixture of nitric and perchloric acids

Heavy metals were extracted from plant tissues into a solution. To 1 g of each sample (measured with the Kern ABJ 220-4M analytical scale) placed in a 50 mL laboratory glass, 10 mL of 65.0 % HNO_3 (p.a., Kemika, Zagreb, Croatia) was added. The samples were digested in a water bath at 50 °C until the release of gaseous NO_2 stopped. Afterwards, 3 mL of 70.0 % $HClO_4$ (p.a., Kemika, Zagreb, Croatia) was added to the samples, which were then once again digested. The cooled samples were filtered through a filter paper (70 mm diameter, blue ribbon, Munktell – Grade 391) into volumetric flasks and diluted with deionised water (deionised with Millipore DirectQ 3 Water Purification System, Molsheim, France) up to a total volume of 50 mL. For the purpose of heavy metal detection, the samples were further diluted with deionised water in the proportions 1:5, 1:10, and 1:100.

Atomic absorption spectrometry (AAS)

The concentrations of heavy metals in the vegetable samples were detected with atomic absorption spectrometry (AAS). The presence of As, Cd, Cr, Ni, and Pb was measured by graphite-furnace AAS (detection limits of 0.05 μg L^{-1}, 0.002 μg L^{-1}, 0.004 μg L^{-1}, 0.07 μg L^{-1}, and 0.05 μg L^{-1}, respectively) and the presence of Cu, Mn, and Zn by air-acetylene flame AAS (detection limit of 0.0015 μg L^{-1} for all three heavy metals), both using a Perkin Elmer AAnalyst 800 atomic absorption spectrometer (PerkinElmer, Inc., Shelton, Connecticut, USA). The presence of Hg was measured by hydride-generation technique (detection limit of 0.009 μg L^{-1}) using a Perkin Elmer AAnalyst 800 atomic absorption spectrometer coupled with a Flow Injection for Atomic Spectroscopy (FIAS) 100 system (PerkinElmer, Inc., Shelton, Connecticut, USA). Hollow cathode lamps (HCL) were used as sources of radiation for detection of Cd, Cr, Cu, Mn, Ni, Pb, and Zn, while electrodeless discharge lamps (EDL) were used for detection of As and Hg. For each heavy metal determined, the respective atomic spectroscopy standard, grade pure, was used as standard reference material (PerkinElmer, Inc., Shelton, Connecticut, USA). In all the vegetable samples, measurement of each heavy metal was performed once.

Maximum levels for heavy metals in vegetables

The results of the AAS analysis were compared with current regulations – i.e. Commission Regulation (EC) No 1881/2006 with amendments – which define the maximum levels for Cd and Pb in vegetables, while the maximum levels for As, Cr, Cu, Hg, Mn, Ni, and Zn in vegetables are not defined (27, 28). For Cd, the maximum levels in vegetables (mg kg^{-1} of wet weight) are defined as follows: in leaf vegetables 0.20; in stem vegetables, root vegetables, and peeled potatoes 0.10; in vegetables and fruit excluding leaf vegetables, stem vegetables, root vegetables and potatoes 0.050 (27, 28). For Pb, the maximum levels in vegetables (mg kg^{-1} of wet weight) are defined as follows: in *Brassica* vegetables and leaf vegetables 0.30; in vegetables including peeled potatoes and excluding *Brassica* vegetables and leaf vegetables 0.10; in legumes 0.20 (27, 28). Since the current regulations do not define the maximum levels for As and Hg in vegetables, the results of analysis were compared with the maximum permitted levels for As and Hg defined in a previous regulation (29). For As, the maximum permitted level in fruit and vegetables (mg kg^{-1}) was defined as 0.3 (29). For Hg, the maximum permitted levels in vegetables (mg kg^{-1}) were defined as follows: in leaf vegetables and peeled potato 0.05; in other fruit and vegetables 0.2 (29).

Critical deficiency and toxicity concentrations in vegetables

The concentrations of essential heavy metals (Cu, Mn, Ni, Zn) detected in the vegetables were compared with critical deficiency and toxicity concentrations (19), and the concentrations of non-essential heavy metals were compared with toxicity thresholds (As, Cd, Cr, Pb) (15) and limit for phytotoxic effects (Hg) (30). However, these concentrations were not available for all the metals analysed. In higher plants, the critical deficiency concentrations in dry weight are as follows: for Cu 1-5 mg kg^{-1} (in vegetative organs), for Mn 10-20 mg kg^{-1} (in leaves) and for Zn below 15-20 mg kg^{-1} (in leaves) (19). For Ni, the adequate range is from 0.01 to above 10 mg kg^{-1} (19). The critical toxicity concentrations in dry weight are as follows: for Cu above 20-30 mg kg^{-1} (in leaves of crops), for Mn 200-5300 mg kg^{-1} (in shoots), for Ni above 10-50 mg kg^{-1} (in crops), and for Zn from 100 to above 300 mg kg^{-1} (in leaves of crops) (19). The concentrations of non-essential As, Cd, Cr, and Pb were compared with plant toxicity thresholds of 20, 5-10, 1-2, and 10-20 mg kg^{-1}, respectively (15). The concentrations of Hg were compared with a limit for phytotoxic effects in food crops of 1 mg kg^{-1} (30).

RESULTS

In order to allow comparison with both regulations and deficiency and toxicity concentrations, thresholds and limits in plants, the results are presented as concentrations of fresh (Figure 1, Figure 2) and dry weight (Table 1).

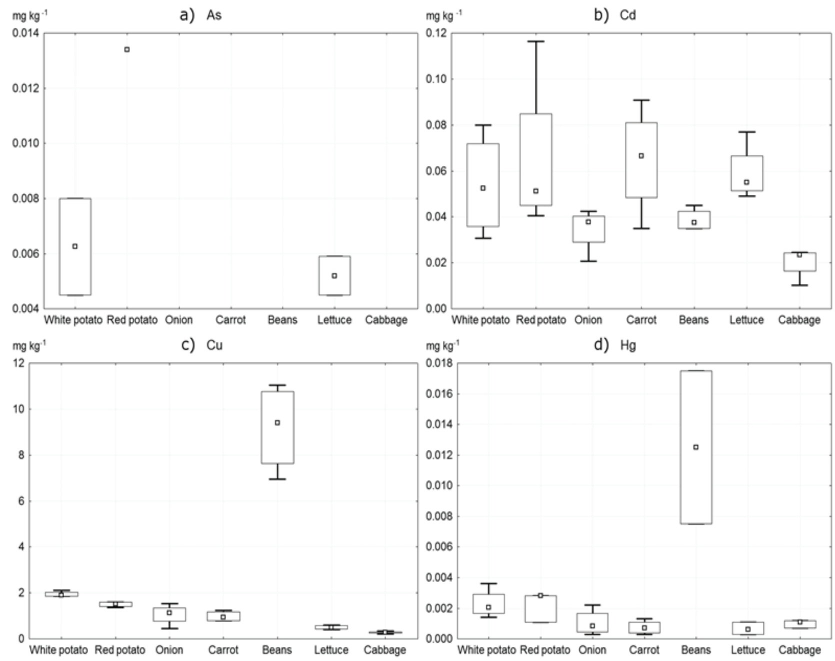

Figure 1 *Concentrations of a) arsenic (As), b) cadmium (Cd), c) copper (Cu) and d) mercury (Hg) in vegetable samples (fresh weight) collected at Varaždin market*

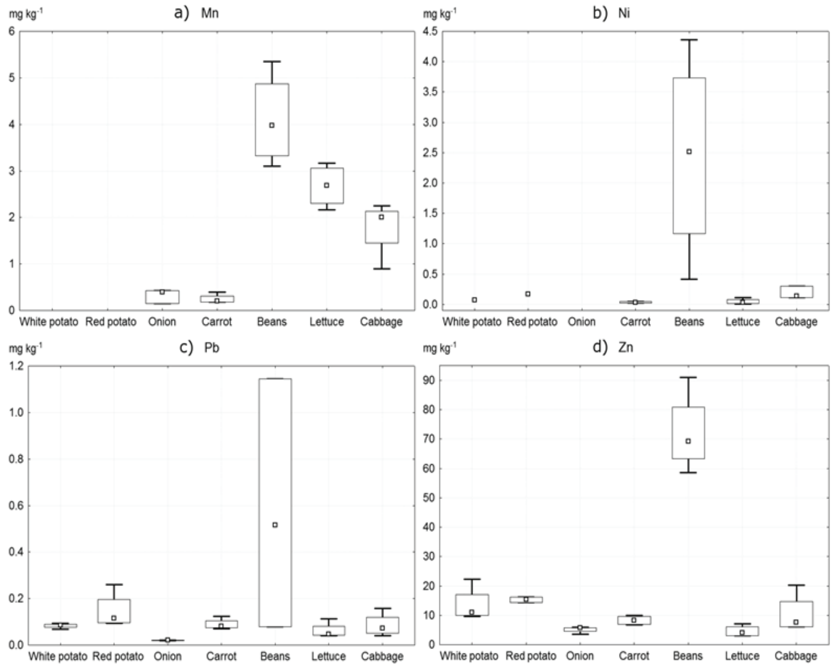

Figure 2 *Concentrations of a) manganese (Mn), b) nickel (Ni), c) lead (Pb) and d) zinc (Zn) in vegetable samples (fresh weight) collected at Varaždin market*

The concentrations of As in fresh weight for five samples ranged between 0.005 and 0.013 mg kg^{-1}, with the highest concentration detected in a sample of red potato (Figure 1a) at sales location OPG 6. The concentrations of Cd ranged between 0.01 and 0.12 mg kg^{-1}, with the highest concentration detected in a sample of red potato (Figure 1b) at sales location OPG 4. The concentrations of Cu ranged between 0.2 and 11.1 mg kg^{-1}, with the highest concentration detected in a sample of beans (Figure 1c) at sales location OPG 4. The concentrations of Hg in 24 samples ranged between 0.0003 and 0.0175 mg kg^{-1}, with the highest concentration detected in a sample of beans (Figure 1d) at sales location OPG 3. The concentrations of Mn in 19 samples ranged between 0.2 and 5.4 mg kg^{-1}, with the highest concentration detected in a sample of beans (Figure 2a) at sales location OPG 7. The concentrations of Ni in 15 samples ranged between 0.01 and 4.36 mg kg^{-1}, with the highest concentration detected in a sample of beans (Figure 2b) at sales location OPG 7. The concentrations of Pb in 26 samples ranged between 0.02 and 1.15 mg kg^{-1}, with the highest concentration detected in a sample of beans (Figure 2c) at sales location OPG 2. The concentrations of Zn ranged between 3.0 and 91.0 mg kg^{-1}, with the highest concentration detected in a sample of beans (Figure 2d) at sales location OPG 3. The heavy-metal content was below the detection limit of the instrument as follows: for As in 23 samples (82.1 %), for Cr in all samples analysed (100 %), for Hg in four samples (14.3 %), for Mn in nine samples (32.1 %), for Ni in 13 samples (46.4 %) and for Pb in two samples (7.1 %). The presence of Cd, Cu, and Zn was detectable in all 28 samples analysed.

Overstepping the maximum level defined in the current regulations (27, 28) was detected for Cd in one sample (3.6 %), and for Pb in five samples (17.9 %) – more precisely: for both Cd (0.12 mg kg^{-1}) and Pb (0.26 mg kg^{-1}) in a sample of red potato; and for Pb alone in a sample of red potato (0.13 mg kg^{-1}), in a sample of carrot (0.12 mg kg^{-1}), and in two samples of beans (1.15 mg kg^{-1} and 0.52 mg kg^{-1}) (Fig. 1b, Fig. 2c). Since the maximum levels for As, Hg, Cu, Mn, Ni, and Zn are not defined in current regulations, no possible transgression could be determined. When the detected concentrations of As and Hg were compared with the maximum permitted levels defined in the previous regulation (29), no transgression was observed.

In comparison with critical deficiency and critical toxicity concentrations (19) of essential heavy metals in higher plants (in dry weight), the concentrations of Cu (2.1-12.3 mg kg^{-1}) were either within the critical deficiency concentration range (in all cabbage samples and one onion sample) or above it, and below the critical toxicity concentrations in all samples (Table 1). The concentrations of Mn (1.2-62.3 mg kg^{-1}) were above the critical deficiency concentration range in lettuce and one cabbage sample, within the range in one cabbage sample, and below it in potatoes, onion, carrot, beans, and two samples of cabbage, and below the critical toxicity concentrations in all samples. The concentrations of Ni (0.15-4.36 mg kg^{-1}) were within the adequate range and below the critical toxicity concentrations in all samples. The concentrations of Zn (28.0-198.0 mg kg^{-1}) were above the critical deficiency concentration range in all samples and within the critical toxicity concentration range in two samples of lettuce and one sample of cabbage, and below the critical toxicity range

Table 1 *Concentrations of As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn detected in vegetable samples (dry weight) collected at Varaždin market. dl=detection limit of instrument*

	Arithmetic mean (mg kg^{-1} of dry weight)±standard deviation (mg kg^{-1}) Range (minimum - maximum) (mg kg^{-1})								
	As	**Cd**	**Cr**	**Cu**	**Hg**	**Mn**	**Ni**	**Pb**	**Zn**
White potato	0.027±0.011	0.23±0.09	<dl	8.2±0.5	0.0098±0.0041	<dl	0.31*	0.36±0.04	57.4±25.1
	0.019-0.034	0.13-0.34		7.9-9.0	0.0060-0.0155			0.30-0.40	41.0-94.5
Red potato	0.053*	0.26±0.14	<dl	5.9±0.4	0.0088±0.0038	<dl	0.67*	0.58±0.31	60.4±4.2
		0.16-0.46		5.4-6.4	0.0045-0.0110			0.37-1.03	56.5-64.0
Onion	<dl	0.28±0.08	<dl	8.5±3.6	0.0084±0.0069	2.6±1.2	<dl	0.17±0.01	41.9±9.3
		0.17-0.34		3.6-12.3	0.0025-0.0180	1.2-3.5		0.16-0.18	28.0-47.0
Carrot	<dl	0.49±0.18	<dl	7.4±1.7	0.0054±0.0033	1.9±0.8	0.27±0.17	0.68±0.18	63.3±12.1
		0.27-0.69		6.0-9.4	0.0020-0.0095	1.4-3.0	0.15-0.39	0.54-0.94	51.0-75.5
Beans	<dl	0.04±0.00	<dl	9.2±1.9	0.0125±0.0050	4.1±1.0	2.45±1.68	0.25±0.23	72.0±13.7
		0.04-0.05		7.0-11.1	0.0075-0.0175	3.1-5.4	0.42-4.36	0.08-0.52	58.5-91.0
Lettuce	0.102±0.020	1.16±0.24	<dl	9.8±1.7	0.0127±0.0081	52.7±9.0	0.87±0.90	1.22±0.67	90.3±38.4
	0.088-0.116	0.97-1.52		8.1-11.8	0.0055-0.0215	42.7-62.3	0.15-2.15	0.82-2.22	59.5-139.5
Cabbage	<dl	0.20±0.07	<dl	2.7±0.5	0.0097±0.0028	17.4±5.9	1.81±1.0	0.83±0.49	101.3±65.9
		0.10-0.24		2.1-3.4	0.0065-0.0115	8.7-21.9	1.11-2.95	0.40-1.53	59.5-198.0
Total	0.062±0.040	0.38±0.37	<dl	7.4±2.8	0.0094±0.0050	16.4±20.6	1.35±1.29	0.61±0.47	69.5±33.6
	0.019-0.116	0.04-1.52		2.1-12.3	0.0020-0.0215	1.2-62.3	0.15-4.36	0.08-2.22	28.0-198.0

**Only one sample above detection limit* in all other samples. In comparison with plant toxicity thresholds (15) and the limit of phytotoxic effects (30) for non-essential heavy metals, the concentrations of As (0.019-0.116 mg kg^{-1}), Cd (0.04-1.52 mg kg^{-1}), Cr (below detection limit), and Pb (0.08-2.22 mg kg^{-1}) were below the respective plant toxicity thresholds, and the concentrations of Hg (0.0020-0.0215 mg kg^{-1}) were below the limit of phytotoxic effects in food crops (Table 1).

DISCUSSION

To summarise, the concentrations of Cd and Pb exceeded the maximum levels stipulated in the regulations (27, 28) in five of the 28 samples of vegetables analysed. In these five samples, six detected concentrations were above the maximum levels: one for Cd (Figure 1b) and five for Pb (Figure 2c).

Lončarić et al. (31), for example, investigated heavy metals (Fe, Mn, Zn, Cu, Ni, Mo, Cd, Pb, and Cr) in 29 soil and 147 vegetable samples from vegetable gardens in the urban areas of Osijek and its surroundings. A very close relationship was established between high Pb soil concentrations and exceedingly high concentrations of Pb in vegetables (detected in 19 of the 147 samples): a high number of such samples was detected in root vegetables (12 of 46). Although no exceedingly high concentration of Cd in soil was recorded, exceedingly high concentrations of Cd were detected in vegetables at three localities. Next, Puntarić et al. (32) detected concentrations of heavy metals (Pb, Cd, As, and Hg) in 100 samples of leafy vegetables, including lettuce (22 samples) and white cabbage (11 samples), and 16 samples of soil in gardens in the urban area of Zagreb. The concentrations of heavy metals exceeded the regulated maximum permitted levels in nine vegetable samples (9 %): for Pb in three samples, for Cd in one sample, and for Hg in five samples. The findings from Osijek and Zagreb both support the results of our study. In the vegetable samples from the Zagreb area, high Hg content was also observed, which was not the case in the vegetable samples from Varaždin. The authors hypothesised that elevated Hg content in eastern Zagreb might be due to thermal-heating plant and a battery factory, while in northern Zagreb culprits might be crematorium and the dominant winds which seem to transport contaminants from the industrial eastern parts to the residential northern parts of the city (32). Furthermore, Vitali et al. (33) compared the concentrations of heavy metals (Cd, Pb, Hg, As, Zn, and Cu) in white cabbage and potato samples in and out of war-affected regions in Croatia. No significant differences were found between the areas affected by war and those unaffected. All cabbage samples were below the regulated levels for Cd and Pb, all potato samples were below the regulated levels for Cd, and eight (of 20) potato samples exceeded the regulated levels for Pb. These findings are similar to the results of our study, since all cabbage samples were below the maximum regulated levels for all heavy metals analysed, and the Pb content exceeded the maximum level in potato. Sapunar-Postruznik et al. (34) analysed data on the levels of Pb and Cd in foodstuffs (22 groups for Pb and 19 for Cd) available on the Croatian market during a five-year period (from 1988 to 1993). In the group of vegetables, the mean concentrations of Pb and Cd in 510 samples were 94 μg kg^{-1} and 24 μg kg^{-1}, respectively. In our study, the mean concentrations of Pb and Cd in vegetables were higher (140 and 50 μg kg^{-1}, respectively). Of note, the number of samples analysed in our study was considerably lower.

In 2013, Croatia became a Member State of the European Union (EU), and the Commission Regulation (EC) 1881/2006 on setting maximum levels for certain contaminants in foodstuffs (with amendments) became valid (27, 28), while the previous regulation, the Ordinance on maximum levels of certain contaminants in foodstuffs (35) was suspended. Further below, the information provided in the European Food Safety Authority (EFSA) reports on investigated heavy metals is discussed.

Although there are no official maximum levels permitted at the EU level for As, EFSA provided an estimate of daily dietary exposure to inorganic As in the European population to be between 0.09-0.45 μg kg^{-1} (minimum - maximum of lower bound, LB) and 0.24-1.37 μg kg^{-1} (minimum - maximum of upper bound, UB) b.w. (36). In comparison with EFSA's measurements of As content in vegetables, the mean concentrations of As (total) in our study were higher in red potato and similar in white potato (13 and 6 μg kg^{-1} compared with 1.6-7.2 μg kg^{-1}) and lower in lettuce (5 μg kg^{-1} compared with 6.9-10.8 μg kg^{-1}). Of note, in our study the concentration of As was below the limit of detection in all onion, carrot, bean, and cabbage samples (Figure 1a).

For Cd, a tolerable weekly intake (TWI) of 2.5 μg kg^{-1} b.w. has been established (37, 38). EFSA estimated the mean dietary exposure to Cd in the adult population to be between 1.9 and 3.0 μg kg^{-1} b.w. per week (37). In comparison with EFSA's measurements, the mean concentrations of Cd in our study were higher in white and red potato (0.05 and 0.06 mg kg^{-1} compared with 0.02 mg kg^{-1}), carrot (0.06 mg kg^{-1} compared with 0.02 mg kg^{-1}), beans (0.04 mg kg^{-1} compared with 0.01 mg kg^{-1}) and lettuce (0.06 mg kg^{-1} compared with 0.02 mg kg^{-1}), and almost the same in cabbage (0.02 mg kg^{-1} compared with 0.02 mg kg^{-1}) (Figure 1b). A comparison for onion was not possible. Also, EFSA found that 63 samples (2.6 %) of stem and root vegetables and no leaf vegetables (without spinach) exceeded the maximum level defined in the regulations (37). In our study, only one sample of red potato exceeded the maximum level for Cd (3.6 %).

For Hg, a TWI for methylmercury of 1.3 μg kg^{-1} b.w. and a TWI for inorganic mercury of 4 μg kg^{-1} b.w. have been established, both expressed as mercury, and no maximum levels for Hg in vegetables are currently established at the EU level (39). EFSA estimated the mean dietary exposure to methylmercury in the overall European population between 0.06 and 1.57 μg kg^{-1} b.w. per week, and to inorganic Hg between 0.13 and 2.16 μg kg^{-1} b.w. per week (39). In comparison with EFSA's measurements of Hg content in vegetables, the mean concentrations of Hg (total) in our study were higher in white and red potato and beans (2.3, 2.2, and 12.5 μg kg^{-1} compared with 0.1-2.1 μg kg^{-1} for potatoes and potato products and 1.4-2.2 μg kg^{-1} for dried beans), and similar in onion, carrot, lettuce, and cabbage (1.1, 0.8, 0.7, and 1 μg kg^{-1}, respectively, compared with 0.1-2.3 μg kg^{-1} for bulb vegetables, 0.4-2.6 μg kg^{-1} for root vegetables, 0.5-3.8 μg kg^{-1} for leaf vegetables and 0.4-1.3 μg kg^{-1} for *Brassica* vegetables) (Figure 1d).

For Pb, EFSA concluded that the previously established PTWI of 25 μg kg^{-1} b.w. was no longer appropriate, due to critical endpoints for which no safe threshold could be established, and calculated benchmark doses and corresponding dietary intakes of 0.50 μg kg^{-1} b.w. per day for developmental neurotoxicity in children, and 1.50 μg kg^{-1} b.w. per day for cardiovascular and 0.63 μg kg^{-1} b.w. per day for nephrotoxic effects, both in adults (40). EFSA estimated the mean dietary exposure to Pb in the adult European population to be between 0.36 and 1.24 μg kg^{-1} b.w. per day (40). In comparison with EFSA's measurements of Pb content in vegetables, the mean concentrations of Pb in our study were higher in white and red potato, carrot, beans and cabbage (0.08, 0.15, 0.09, 0.58, and 0.09 mg kg^{-1}, respectively, compared with 0.02-0.03 mg kg^{-1} for potatoes other than peeled, 0.05-0.06 mg kg^{-1} for root vegetables, 0.02-0.04 mg kg^{-1} for legumes and 0.01-0.03 mg kg^{-1} for *Brassica* vegetables), and similar in lettuce (0.06 mg kg^{-1} compared with 0.05-0.06 mg kg^{-1}) (Figure 2c). A comparison for onion was not possible. EFSA found that the Pb content was above 1 mg kg^{-1} in 771 samples (40). In our study, five samples (two samples of red potato, one sample of carrot and two samples of beans) exceeded the maximum regulated level for Pb (17.9 %).

For Ni, a tolerable daily intake (TDI) of 2.8 μg kg^{-1} b.w. has been established, and no maximum levels are currently established for Ni in food at the EU level (41). EFSA estimated the mean chronic dietary exposure to Ni in the European population between 2.0 and 13.1 μg kg^{-1} b.w. per day (41). In comparison with EFSA's measurements of Ni content in vegetables, the mean concentrations of Ni in our study were higher in cabbage (190 μg kg^{-1} compared with 59-79 μg kg^{-1} for *Brassica* vegetables) and lower in carrot, beans, lettuce, and white and red potato (40, 2450, 40, 70, and 170 μg kg^{-1} compared with 160-170 μg kg^{-1} for carrots, 2900 μg kg^{-1} for dried beans, 110-120 μg kg^{-1} for leaf vegetables and 260-270 μg kg^{-1} for main-crop potatoes) (Figure 2b). Of note, in our study the Ni content was below the limit of detection in all onion samples.

For Cr^{3+}, a possible essential trace element for humans, a TDI of 300 μg kg^{-1} b.w. has been established, while no TDI has been established for toxic Cr^{6+}, and no maximum levels for Cr in food at the EU level are currently established (42, 43). EFSA estimated dietary exposure to Cr^{3+} in the European population between 0.6 and 5.9 μg kg^{-1} b.w. per day and to Cr^{6+} in drinking water between 0.7 and 159.1 ng kg^{-1} b.w. per day (43). The content of Cr in our study was below the detection limit in all samples analysed.

For Cu, an essential element for humans, a tolerable upper intake level of 5 mg per day has been established, and an adequate intake of 1.6 mg per day for men and 1.3 mg per day for women has been proposed, both in adults (44, 45). EFSA estimated dietary intake of Cu in the adult European population between 1.15 and 2.07 mg per day (45). In a Total Diet Study (TDS) in France, the mean concentrations of Cu (in fresh weight) were 0.853 mg kg^{-1} in potato-based products, 0.674 mg kg^{-1} in vegetables and 2.45 mg kg^{-1} in dried vegetables (46). In a TDS in the United Kingdom, the mean concentrations of Cu (unspecified whether in fresh or dry weight) were 0.580 mg kg^{-1} in green vegetables, 1.12 mg kg^{-1} in potatoes, and 0.808 mg kg^{-1} in other vegetables (47). In comparison, the mean concentrations of Cu in our study in white and red potato (1.9 and 1.5 mg kg^{-1}, respectively) were higher than in France and the United Kingdom, which might be attributed to the fact that the potatoes in our study were not peeled. The mean concentration of Cu in onion (1.1 mg kg^{-1}) was lower than in France and higher than in the United Kingdom. The mean concentrations of Cu in carrot (1.0 mg kg^{-1}) and beans (9.2 mg kg^{-1}) were higher than in France and the United Kingdom. High content of heavy metals in bean seeds is discussed further below. The mean concentration of Cu in lettuce (0.5 mg kg^{-1}) was lower than in France and similar to that in the United Kingdom. The mean concentration of Cu in cabbage (0.3 mg kg^{-1}) was lower than in France and the United Kingdom.

For Mn, a tolerable upper intake level has not been set, and an adequate intake of 3 mg per day in adults has been proposed (48, 49). EFSA estimated dietary intake of Mn in the adult European population between 2 and 6 mg per day (49). In a TDS in France, the mean concentrations of Mn were 1.03 mg kg^{-1} in potato-based products, 1.47 mg kg^{-1} in vegetables, and 4.40 mg kg^{-1} in dried vegetables (46). In a TDS in the United Kingdom, the mean concentrations of Mn were 2.06 mg kg^{-1} in green vegetables, 1.58 mg kg^{-1} in potatoes, and 1.54 mg kg^{-1} in other vegetables (47). In comparison, the content of Mn in our study was below the limit of detection in all potato samples. The mean concentrations of Mn in onion (0.3 mg kg^{-1}) and carrot (0.2 mg kg^{-1}) were lower than in France and the United Kingdom. The mean concentration of Mn in beans (4.1 mg kg^{-1}) was higher than the concentration of Mn in vegetables (but lower than the concentration of Mn in dried vegetables) in France and higher than in the United Kingdom. The mean concentration of Mn in lettuce (2.7 mg kg^{-1}) was higher than in France and the United Kingdom. The mean concentration of Mn in cabbage

(1.8 mg kg^{-1}) was higher than in France and lower than in the United Kingdom.

For Zn, a tolerable upper intake level of 25 mg per day has been recommended, and a population reference intake range from 7.5 to 12.7 mg per day in women, and from 9.4 to 16.3 mg per day in men, both in adults, has been established (50, 51). EFSA estimated dietary intake of Zn in the adult European population between 8.0 and 14.0 mg per day (51). In a TDS in France, the mean concentrations of Zn were 2.49 mg kg^{-1} in potato-based products, 2.57 mg kg^{-1} in vegetables, and 9.79 mg kg^{-1} in dried vegetables (46). In a TDS in the United Kingdom, the mean concentrations of Zn were 3.26 mg kg^{-1} in green vegetables, 3.66 mg kg^{-1} in potatoes and 2.62 mg kg^{-1} in other vegetables (47). In comparison, the mean concentrations of Zn in our study in white and red potato (13.5 and 15.3 mg kg^{-1}, respectively) were higher than in France and the United Kingdom. The higher Zn concentrations could be attributed to potato peel, since the potatoes in our study were not peeled. The mean concentrations of Zn in beans (72.0 mg kg^{-1}), onion (5.2 mg kg^{-1}), carrot (8.3 mg kg^{-1}), lettuce (4.6 mg kg^{-1}), and cabbage (10.4 mg kg^{-1}) were higher than in France and the United Kingdom.

Overall, in our study only one sample of red potato exceeded the maximum level for Cd (3.6 %). On the other hand, five samples (two samples of red potato, one sample of carrot, and two samples of beans) exceeded the maximum regulated level for Pb (17.9 %). Therefore, red potato could represent a source of elevated exposure to Cd, while red potato, beans, and carrot could represent a source of elevated exposure to Pb, in the local population. Since there is no safe threshold for several critical endpoints of Pb exposure, including developmental neurotoxicity in children and nephrotoxicity and cardiovascular effects in adults (40), the overstepping of the maximum level in 17.9 % of the samples could be of concern for the local population, especially in children. Such a high percentage of contaminated samples calls for caution and, further, more comprehensive analysis.

The concentration of As was detectable in only five samples, while the content of Cr was below the detection limit in all the samples analysed. Therefore, no conclusions can be drawn for As exposure, while vegetables probably do not contribute to Cr exposure in the local population.

Interestingly, bean samples contained the highest concentrations of Cu, Hg, Mn, Ni, Pb, and Zn (Figure 1, Figure 2). All vegetable samples were bought at a local market, and the samples of beans were bought from different sellers (OPG-2, OPG-3, OPG-4 and OPG-7). Unlike the other vegetables, beans were sold already dried and out of the pod. Of note, EFSA found relatively high Ni concentrations in "legumes, nuts, and oilseeds", and dried beans contained the highest mean concentrations of Ni (41). Most importantly, developing seeds store essential heavy metals, e.g. Zn, Cu, Mn, and Ni, and may accumulate minerals at concentrations several times higher than those required for cell functioning (52-53). Since non-essential metals may also be transported to seeds by same mechanisms (14), this could explain the high content of Pb and Hg in bean seeds. Therefore, beans could represent a source of exposure to these metals in the local population.

Regarding the essential heavy metals, the concentrations of Cu detected in our study were either within or above the critical deficiency range (19) and below the critical toxicity range in all samples (Table 1). The concentrations of Cu in all cabbage samples (2.1-3.4 mg kg^{-1}) and one onion sample (3.6 mg kg^{-1}) were within the critical deficiency range (1-5 mg kg^{-1}). Various soil factors, including pH, external Cu supply, high organic-matter content, N availability, and Zn supply could have influenced the Cu concentration in vegetables (19). The concentrations of Mn were above the critical deficiency range (10-20 mg kg^{-1}) in lettuce. However, in cabbage (8.7-21.9 mg kg^{-1}), one sample was above the range, two samples were within (19.5 and 19.6 mg kg^{-1}), and one was below, as well as in all potato samples (below the detection limit), onion (1.2-3.5 mg kg^{-1}), carrot (1.4-3.0 mg kg^{-1}), and beans (3.1-5.4 mg kg^{-1}) (Table 1). This could be explained by the fact that the critical deficiency concentration for Mn was defined in fully-developed leaves (19), while the content of Mn in our study was detected in other plant organs. The concentrations of Mn in our study were below the critical toxicity concentrations (19) in all samples. The concentrations of Ni were within the adequate range and below the critical toxicity range. The concentrations of Zn were above the critical deficiency range in all samples and below the critical toxicity range in most of the samples. In two samples of lettuce (102.0 and 139.5 mg kg^{-1}) and one sample of cabbage (198.0 mg kg^{-1}) the concentrations of Zn were within the critical toxicity range (100-300 mg kg^{-1}) (19). High Zn supply may lower the concentration of Mn in plants (19). However, of the three samples with high Zn content, only in the sample of cabbage with the highest Zn concentration (198.0 mg kg^{-1}) was the concentration of Mn (19.5 mg kg^{-1}) within the critical deficiency range. Regarding the non-essential heavy metals, the concentrations of As, Cd, Cr, and Pb were below the corresponding plant toxicity thresholds (15) and the concentrations of Hg were below the limit of phytotoxic effects in food crops (30).

The concentrations of heavy metals in plants largely depend on the concentrations of heavy metals in the soil (9, 10). In our study, the overstepping of maximum levels for Cd and Pb in vegetable samples was most likely due to soil contamination with these heavy metals. The possible sources of soil contamination with Cd and Pb are similar, and include floodwater in the lowlands along rivers and streams, atmospheric deposition, proximity of traffic and industry, and use of artificial fertilisers and pesticides in agriculture (1, 54-56). Since the vegetables were bought at Varaždin market from local sellers, they might have been grown in Northwestern Croatia. The content of heavy metals in soil was not evaluated in this study. However, in several previous studies, authors have recorded increased concentrations of Cd and Pb in soils of the Varaždin area (54, 56-59).

The control of food contamination is crucial for the safety of consumers. A phytosanitary strategy has been created to set guidelines for the development of plant-health national policy in Croatia (60). Although various factors may contribute to elevated concentrations of heavy metals in food, soil contamination is one of the most important (9, 10). A programme for permanent monitoring of the soil in Croatia has been developed; however, data regarding the state of the soil has still not been collected (61, 62).

CONCLUSIONS

The concentrations of nine heavy metals (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Zn) were detected in the seven vegetables presumed most common in the diet of the continental region of Croatia (red and white potato, onion, carrot, beans, lettuce, and cabbage), bought at Varaždin market in 2013, using the AAS method. On the basis of the results obtained, the following conclusions can be drawn. Maximum regulated levels for Pb and Cd were overstepped in five samples. For Pb, transgression was detected in five samples (17.9 %): two samples of red potato, one sample of carrot and two samples of beans. For Cd, transgression was detected in one sample of red potato (3.6 %), which also contained an excessive concentration of Pb. Increased concentrations of Pb and Cd in the samples of vegetables were most likely due to contaminated agricultural soil. The possible sources of soil contamination with Pb and Cd include use of fertilisers and pesticides, floods, atmospheric deposition, proximity of traffic and other sources of contamination. Accordingly, increased concentrations of Pb and Cd in vegetables have also been detected in comparable studies previously conducted in Croatia. It was also previously shown that: heavy metal content in soil influences the concentration of heavy metals in vegetables, vegetables majorly contribute to dietary exposure to Pb and Cd, and increased concentrations of Pb and Cd may negatively affect human health. Therefore, monitoring of the concentration of heavy metals in both food and soil is necessary.

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Određivanje teških metala u čestim vrstama povrća na Varaždinskoj gradskoj tržnici

Cilj istraživanja bio je procijeniti sadržaj teških metala u povrću dostupnom na gradskoj tržnici jednog od gušće naseljenih hrvatskih gradova, Varaždina, te ispitati povezanost koncentracija teških metala s mogućim izvorima onečišćenja. Ukupno 28 uzoraka često konzumiranog povrća (crveni i bijeli krumpir, luk, mrkva, grah, salata i kupus) nasumično je kupljeno na Varaždinskoj tržnici u rujnu i listopadu 2013. godine. Koncentracije devet teških metala (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb i Zn) izmjerene su metodom atomske apsorpcijske spektrometrije. U pet od 28 ispitanih uzoraka povrća, šest je koncentracija teških metala bilo iznad najvećih dopuštenih količina: pet za Pb i jedna za Cd. Najveće dopuštene količine Pb prekoračene su u dva uzorka crvenog krumpira, dva uzorka graha i jednom uzorku mrkve (17,9 %), a Cd u jednom uzorku crvenog krumpira (3,6 %). Uzrok prekoračenja najvećih dopuštenih količina Pb i Cd u uzorcima povrća onečišćeno je tlo. Mogući uzroci onečišćenja tla uključuju promet, blizinu industrije, poplavne vode rijeka i potoka te upotrebu pesticida i mineralnih gnojiva u poljoprivrednoj proizvodnji.

KLJUČNE RIJEČI: *kadmij; olovo; najveće dopuštene količine; analiza hrane; atomska apsorpcijska spektrometrija*