Essential and toxic microelement profile of walnut (*Juglans regia* L.) cultivars grown in industrially contaminated area – Evaluation for human nutrition and health

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Summary

The levels of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se and Zn were determined in nuts of twelve walnut cultivars grown in a lead metallurgy-contaminated area in Bulgaria. The average microelement contents varied in the following ranges, depending on the cultivar: Cd, 0.013–0.045 mg·kg^{-1}; Co, 0.05–0.38 mg·kg^{-1}; Cr, 0.032–0.076 mg·kg^{-1}; Cu, 15.4–20 mg·kg^{-1}; Fe, 28–33 mg·kg^{-1}; Mn, 31–36 mg·kg^{-1}; Ni, 0.64–1.62 mg·kg^{-1}; Pb, 0.08–0.20 mg·kg^{-1}; Sb, 0.006–0.01 mg·kg^{-1}; Se, 0.034–0.047 mg·kg^{-1} and Zn, 28–36 mg·kg^{-1}. For comparison, the same analytes were determined in nut samples from seven unpolluted regions in Bulgaria. An overall trend of increased contents for Cd, Co, Cr, Ni and Pb at the polluted sites was observed. However, mean intakes of Co, Cr, Cu, Fe, Mn, Ni, Se and Zn from 50 g "polluted" walnuts were below the toxicological reference values. Likewise, the maximum walnut exposure to Cd, Pb and Sb represented only 10.5%, 4.7% and 0.1% of the respective tolerable maximum weekly intakes. Therefore, walnuts can be grown even in a polluted area without losing their nutritional qualities.

Keywords

walnuts; microelements; toxicity

Determination of the contents of essential and toxic microelements in foods is important for assessment of their nutritional and toxicological characteristics. The elements Co, Cr, Cu, Fe, Mn, Ni, Se and Zn are considered essential micronutrients in human nutrition. The United States National Academy of Sciences recommended for these essential microelements safe ranges for adults daily intake, which are necessary for human health yet far below their toxic action [1–3]. On the other hand, Cd, Pb and Sb are toxic elements without any wholesome biological functions in living organisms. Therefore, the European Food Safety Authority's Panel on contaminants in food chain set a new reduced tolerable weekly intake (TWI) for Cd of 2.5 μg per kilogram of body weight (bw), but keeping the TWI levels for Pb at 25 μg·kg^{-1} bw [4, 5]. Concerning antimony, the tolerable daily intake (TDI), as recommended by the World Health Organization (WHO), is 6 μg·kg^{-1} bw [6], and hence 42 μg·kg^{-1} bw TWI for Sb. So, the TDI or TWI are the levels at which no adverse effects are expected.

Walnuts are widely used in confectionery, baking, candy and chocolate products. They are also a good source of the essential omega-3 and omega-6 fatty acids (mainly linolenic and linoleic acids), of vitamin E, of many essential B-complex group vitamins and other bioactive ingredients [7]. Also, they are rich in essential microelements associated with an improved health status when consumed at doses beyond those necessary to pre-

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vent deficiency states. Thus, consumption of walnuts may be beneficial for heart health, diabetes, cholesterol level and bone health [8–12].

Walnuts are considered to be a good source of dietary minerals. However, in spite of the known contents of important essential microelements like Cu, Fe, Mn, Se and Zn [13–17], the literature data about contents of the trace elements Cd, Co, Cr, Ni, Pb and Sb in walnuts are scarce [18, 19]. The European Commission regulation 466/2001 stated the maximum admissible levels of the toxic Cd and Pb in walnuts to be 0.05 $mg \cdot kg^{-1}$ and 0.1 $mg \cdot kg^{-1}$, respectively [20].

Growing of walnuts at area polluted by lead metallurgy would cause accumulation of toxic elements due to air and soil pollution. To the best of our knowledge, no investigations have been reported on accumulation of microelements in nuts grown in a polluted area. Determination of the microelement profile in walnuts is very important in order to estimate the microelements impact on human nutrition and health. Therefore, the purpose of the present study was: 1. to obtain data on the microelement contents of 12 walnut cultivars grown in an industrially contaminated area; 2. to compare these results with the mineral contents of walnuts harvested in uncontaminated areas, and 3. to discuss the significance of the levels of these elements for human nutrition and health.

MATERIALS AND METHODS

Materials

The walnut cultivars Tehama, Adams, Hartley, Pedro, UC 61-25, Shejnovo, Slavejnovo, Oreshinovo, Drjanovsky, Izvor-10, Kuklensky and Proslavsky were obtained from the Agricultural Experimental Station in Kardzhali, South-Eastern Bulgaria, in 2011. The region is industrially polluted due to activities of a lead/zinc smelter located near the orchard. In addition, walnut samples from seven pure regions in Bulgaria uninfluenced by anthropogenic activities (Simeonovo, Dragalevtsi, Gorna Banja, Izvor and Bistritsa in Western Bulgaria, and Elena and Yablanitsa in Central Bulgaria) were collected as control samples for comparison.

The air-dried walnut samples (1500 g from each cultivar randomly collected) were kept unshelled in a control cabinet (at 10 °C with relative humidity of 60–65%) and were analysed within three months after harvest. Right before analysis, approximately 300 g were randomly selected from the respective boxes for each cultivar, the walnuts were shelled, kernels were milled and subjected to sample preparation.

All chemical reagents and standards used were of analytical reagent grade and were obtained from Merck (Darmstadt, Germany). Deionized water was used throughout the experiments.

Methods

A modified USGS Test Method B-9001-95 was applied to the determination of the microelement contents [21]. Approximately 1 g of a milled nut sample was accurately weighed into a 50 ml beaker and firstly digested with 20 ml concentrated nitric acid for 12 h at room temperature (beaker was covered with a watch glass). Then the samples were placed on a sand bath and heated at 150 °C until the removal of nitrogen oxides. After cooling, cover glass was removed, 5 ml of 0.3 $g \cdot ml^{-1}$ H_2O_2 in water were added and the solution was again heated on a sand bath until the volume reached approximately 5 ml. The digest was quantitatively transferred into a 25 ml volumetric flask with deionized water prior to analysis. From each walnut cultivar, three independent analytical portions [22] were weighed and procedural blanks were run during the procedure.

The microelements Cu, Fe, Mn and Zn were determined in an air-acetylene flame by an AAnalyst 400 Perkin Elmer spectrometer (Perkin Elmer, Norwalk, Connecticut, USA) against appropriate aqueous standard solutions. The limits of detection (*LOD*) were 0.25 $mg \cdot kg^{-1}$, 1 $mg \cdot kg^{-1}$, 0.5 $mg \cdot kg^{-1}$ and 0.1 $mg \cdot kg^{-1}$ for Cu, Fe, Mn and Zn, respectively. The relative standard deviations (*RSD*) were in a range of 3–7%. Near the detection limit, *RSD* reached 12%.

The microelements Cd, Co, Cr, Ni, Pb, Sb and Se were determined by electrothermal atomic absorption spectrometry (ETAAS) using Perkin Elmer Zeeman 3030 spectrometer with an HGA-600 graphite furnace and standard addition calibration mode. The limits of detection for Cd, Co, Cr, Ni, Pb and Se were 0.001 $mg \cdot kg^{-1}$, 0.04 $mg \cdot kg^{-1}$, 0.005 $mg \cdot kg^{-1}$, 0.05 $mg \cdot kg^{-1}$, 0.01 $mg \cdot kg^{-1}$ and 0.02 $mg \cdot kg^{-1}$, respectively. *RSD* were between 4% and 9%. However, *RSD* up to 23% were registered for samples with element contents close to the detection limit. For Sb, a preliminary pre-concentration by extraction of its dithiocarbamate complex at pH 1 into xylene according to Serafimovska et al. [23] was performed, achieving a detection limit of 0.001 $mg \cdot kg^{-1}$.

Certified reference material (CRM) NCS ZC73011 Soy bean (LGC Standards, Łomianki, Poland) was digested and measured with each batch of walnut samples in order to check the accuracy of the analytical procedure. Differences between the certified and obtained values were not significant ($p > 0.05$). The bias calculated from the means of certified and found values was 1.8% for Cu, 2.2% for Fe, –3.4% for Mn, –5.7% for Pb and 2.5% for Zn. The obtained mean value for Cd was 12.2 mg·kg^{-1} Cd at an indicative value of 11 mg·kg^{-1}.

The statistical significance (*t*-test: two sample equal variance, using two-tailed distribution) was performed using Microsoft Excel (Microsoft, Redmond, Washington, USA). Differences at $p < 0.05$ were considered to be significant. Correlation analysis was performed using Statistica 7.0 (StatSoft, Tulsa, Oklahoma, USA) and correlations were estimated as significant at $p < 0.05$.

RESULTS AND DISCUSSION

Eight essential (Co, Cr, Cu, Fe, Mn, Ni, Se, Zn) and three toxic (Cd, Pb, Sb) microelements were determined in walnut cultivars grown in a lead metallurgy-contaminated area, and the results are given in Tab. 1. The contents of essential elements Cu (15.4–20 mg·kg^{-1}), Fe (28–33 mg·kg^{-1}), Mn (31–36 mg·kg^{-1}), Se (34–47 μg·kg^{-1}) and Zn (28–36 mg·kg^{-1}) were comparable to those reported in the literature about walnuts available on markets [13, 15–18]. Similar content ranges were obtained for these analytes also in the control samples of walnuts ($p > 0.05$) from pure areas in Bulgaria (Tab. 2).

In order to gain a more complete evaluation of the industrial pollution on the bioaccumulation processes, an accumulation coefficient C_{acc} was calculated as a ratio between the mean analyte content in walnuts from the polluted area to its mean content in the control samples. The results are presented in Fig. 1. As can be seen, the accumulation coefficients for Cu, Fe, Mn, Se and Zn ranged from 0.9 (Fe) to 1.04 (Cu). It can be concluded that the accumulation of these elements in walnuts was not affected by the industrial activity near the orchard, thus preserving the nutritional and health benefits of walnuts.

The amount of Co was found to be between 0.052 mg·kg^{-1} and 0.38 mg·kg^{-1} in the studied walnut cultivars (Tab. 1). Significant difference ($p < 0.05$) existed between the mean contents of Co in the control samples (0.09 mg·kg^{-1}, Tab. 2) and in walnuts from the contaminated area (0.17 mg·kg^{-1}, Tab. 1), giving an accumulation coefficient of 1.9 (Fig. 1). For comparison, Rodushkin et al. [19] found a mean content of 0.045 mg·kg^{-1} Co in 6 walnut samples from the Swedish market. Despite the relatively higher content of Co in wal-

Tab. 1. Essential and toxic element contents of walnut cultivars grown in a lead metallurgy-contaminated area.

Walnut cultivar	Content of elements [mg·kg^{-1}]										
	Co	Cr	Cu	Fe	Mn	Ni	Se	Zn	Cd	Pb	Sb
Tehama	0.052	0.062	16.6	32	32	0.87	0.038	36	0.015	0.10	5.8
Adams	0.20	0.060	15.9	28	34	0.64	0.041	29	0.018	0.08	6.0
Hartley	0.068	0.057	17.6	29	35	0.96	0.044	29	0.017	0.09	6.4
UC 61-25	0.20	0.038	16.3	30	36	1.57	0.043	31	0.015	0.09	8.3
Pedro	0.074	0.076	16.5	29	34	0.96	0.047	28	0.045	0.20	9.6
Oreshinovo	0.13	0.056	15.4	31	32	0.88	0.036	28	0.013	0.10	5.6
Shejnovo	0.13	0.040	15.5	28	35	1.10	0.036	28	0.018	0.09	7.4
Izvor 10	0.15	0.078	15.4	28	35	1.20	0.040	29	0.042	0.09	5.8
Slavejnovo	0.26	0.032	16.2	32	31	1.62	0.038	33	0.015	0.12	5.6
Drjanovsky	0.26	0.055	18.0	31	32	0.96	0.034	34	0.015	0.18	5.8
Kuklensky	0.38	0.053	15.6	32	33	1.18	0.036	35	0.014	0.12	6.8
Proslavsky	0.15	0.052	20.0	33	35	1.05	0.034	36	0.020	0.13	7.2
Minimum	0.052	0.032	15.4	28	31	0.64	0.034	28	0.013	0.08	5.6
Maximum	0.38	0.076	20	33	36	1.62	0.047	36	0.045	0.20	9.6
Mean	0.17	0.055	16.6	30	34	1.08	0.039	31	0.021	0.12	6.7
SD	0.01	0.003	0.7	1.0	1.0	0.07	0.003	1.0	0.004	0.01	0.6
SEM	0.095	0.014	1.4	1.8	1.6	0.28	0.004	3.3	0.011	0.04	1.25

Mean values are given, ($n = 3$). *SD* – standard deviation, *SEM* – standard error of the mean.

Tab. 2. Essential and toxic element contents of control walnut samples from pure areas.

Sampling place	Content of elements [mg·kg-1]										
	Co	Cr	Cu	Fe	Mn	Ni	Se	Zn	Cd	Pb	Sb
Simeonovo	0.12	0.030	16.6	36	32	0.40	0.036	34	0.006	< 0.01	< 2
Dragalevtsi	0.10	0.036	17.4	38	32	0.73	0.034	34	0.009	< 0.01	< 2
Gorna Banja	0.08	0.030	14.6	38	30	0.63	0.034	32	0.006	< 0.01	< 2
Izvor	0.07	0.038	15.3	28	32	0.96	0.047	34	0.005	0.04	< 2
Bistritsa	0.12	0.036	15.5	23	28	0.66	0.036	28	0.008	0.02	< 2
Elena	0.07	0.030	15.4	31	32	0.68	0.033	32	0.003	< 0.01	< 2
Yablanitsa	0.08	0.034	15.5	28	35	0.54	0.038	33	0.009	0.04	< 2
Minimum	0.08	0.030	15.3	23	28	0.40	0.034	28	0.005	< 0.01	< 2
Maximum	0.12	0.038	17.4	38	35	0.96	0.047	34	0.009	0.04	< 2
Mean	0.09	0.033	15.8	32	32	0.66	0.037	32	0.007	0.017	< 2
SD	0.01	0.002	0.6	1.0	1.0	0.07	0.003	1.0	0.001	0.008	–
SEM	0.022	0.003	0.9	5.8	2.2	0.17	0.005	2.2	0.002	0.017	–

Mean values are given, $n = 3$. *SD* – standard deviation, *SEM* – standard error of the mean.

nuts from the polluted area, the level of Co intake was lower than the dose (250 μg per day, Tab. 3) causing acute effects in humans [24].

The content of Cr varied between 0.032 mg·kg^{-1} and 0.076 mg·kg^{-1} for the analysed walnut varieties (Tab. 1). The mean Cr content was significantly ($p < 0.05$) higher in comparison with the control samples (0.033 mg·kg^{-1} Cr, Tab. 2), giving an accumulation coefficient of 1.7 (Fig. 1). Thus, the content range of Cr in Bulgarian walnuts is similar to that reported by Souci et al. [15], but is higher than the data (1.3 μg·kg^{-1} Cr) for walnuts from the Swedish market [19]. On the other hand, Cr content in Bulgarian walnuts was about 10 times (0.35 mg·kg^{-1} Cr) and 100 times (5.3 mg·kg^{-1} Cr) lower than the values reported for walnuts from Spain [18] and Romania [25, 26], respectively. These wide content ranges of Cr are probably caused by various factors such as soil and geographical conditions, agricultural management techniques or environmental and varietal attributes. However, no potential health risk due to the exposure to Cr exists when consuming 50 g per day [27] walnuts from the polluted area because, according to our results, a maximum of approximately 4 μg Cr could be obtained from such nuts and this is far below the toxic levels.

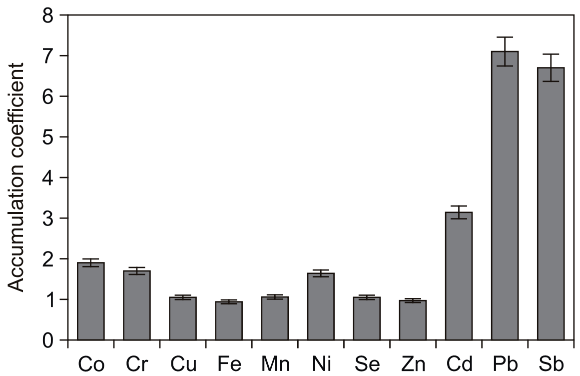

Fig. 1. Accumulation coefficients for essential (Co, Cr, Cu, Fe, Mn, Ni, Se, Zn) and toxic (Cd, Pb, Sb) elements in walnuts.

Accumulation coefficients were calculated as a ratio between the mean values of 12 walnut cultivars from a polluted area and the mean value of 7 control samples from pure areas.

Nickel contents in the samples were found to be between 0.64 mg·kg^{-1} and 1.62 mg·kg^{-1} (Tab. 1). These values are significantly ($p < 0.05$) higher than the contents in walnuts from the uncontaminated areas (0.4–0.96 mg·kg^{-1} Ni, Tab. 2), with the accumulation coefficient of 1.6 (Fig. 1). It should be noted that Ni contents reported for walnuts from Spain (1.8–2.0 mg·kg^{-1} [18]) and Sweden (1.6 mg·kg^{-1} [19]) were even higher than the amounts determined in nuts from the investigated polluted area in Bulgaria. Concerning the health benefits, Ni-enriched kernels help to improve health status by preventing a deficiency state. This element is thought to play an important role in Ca and Zn metabolism, as well as in the absorption of Fe. Ni is also a component of several enzyme systems. On the other hand, the Ni content of foods is very important since its larger amounts (0.6–5.6 mg per person per day) may cause Ni allergy [28, 29]. However, according to our results, the maximum intake of Ni when consuming 50 g walnuts daily is 0.08 mg Ni, which is far below the allergy-causing contents.

Statistical data processing revealed that the contents of microelements were correlated with each other only in three cases: a positive correlation ($p < 0.05$) determined between Cr and Cd ($r = 0.73$; $p = 0.007$) and between Fe and Zn ($r = 0.85$; $p = 0.001$), and inverse correlation found between Cr and Ni ($r = –0.58$; $p = 0.047$). The relative high correlation between iron and zinc ($r = 0.85$) contents shows that these two elements have similar plant uptake rates using different plant channels. The absorption of both cations takes place through different mechanisms, which do not interfere at the uptake by the roots.

Recommended dietary allowances for essential minerals

The results from the present study showed that Bulgarian walnuts contained significant amounts of essential microelements (Tab. 1) that are necessary to improve health status preventing a deficiency state. Regarding nutritional aspects, the percentage of recommended dietary allowances (*RDA*) or the adequate intakes (*AI*) for adults are given in Tab. 3. So, daily consumption of 50 g kernels supplied, depending on the walnut cultivar, 13–95% of Co, 4.3–114% of Cr, 86–111% of Cu, 7.8–9.2% of Fe, 14–16% of Mn, 4.6–11.6% of Ni, 5.7–10% of Se and 3.5–4.5% of Zn for *RDA*/*AI* for adults [3]. Slight overdoses of Cr and Cu were observed for some walnut cultivars. Nevertheless, slightly higher Cr and Cu contents could not cause unhealthy effects in humans [30] because they represented only 2% (Cr) to 10% (Cu) from the tolerable upper intake or therapeutic levels for these elements (Tab. 3).

Intake of the toxic elements Cd, Pb and Sb

Dietary exposure to Cd and Pb has been associated with toxic and adverse health effects [4, 5]. Pb may cause irreversible neurological damage as well as renal disease, negative cardiovascular effects and reproductive toxicity. Cd is primarily toxic to the kidney and has been classified as carcinogenic to humans by the International Agency for Research on Cancer. Concerning Sb, it possesses similar level of genotoxicity as As(III), and has carcinogenic potential [31]. There are no known requirements for Sb yet.

The contents of Cd, Pb and Sb in walnut kernels from the contaminated area are presented in Tab. 1. As can be seen, they are significantly higher in comparison with control samples (Tab. 2), having accumulation coefficients of 3.0, 7.1 and 6.7, respectively (Fig. 1). However, according to Cabrera et al. [18], walnuts from the Spanish market were found to contain even more Pb (0.20–0.26 mg·kg^{-1}) than the kernels from a contaminated area in Bulgaria (0.08–0.20 mg·kg^{-1}, Tab. 1). In contrary, much lower contents of Cd (0.65 μg·kg^{-1}) and Pb (0.25 μg·kg^{-1}) were determined in walnuts from the Swedish market [19].

In order to estimate nutritional and health effects of Cd, Pb and Sb as a result of walnuts consumption, weekly intakes of these elements were calculated assuming a daily consumption of 50 g kernels [27]. The results are presented in Tab. 4 and show that the maximum intake of Cd reached 15.8 μg per week, 70 μg per week for Pb and 3.4 μg per week for Sb. Thus, the highest percentage of Tolerable Maximum Weekly Intake was for Cd (10.5%) followed by Pb (4.7%). The maximum weekly intake of Sb when consuming walnuts was insignificant compared to the Provisional Tolerable Weekly Intake estimated by FAO/WHO

Tab. 3. Percentage of recommended dietary allowances or adequate intakes, and of tolerable upper intake level or therapeutic level for walnuts.

Element	*RDA* or *AI* [μg·d^{-1}]	*UL* or *TL* [μg·d^{-1}]	Intake from 50 g [μg·d^{-1}]	*RDA* or *AI* [%]	Maximum *UL* or *TL* [%]
Co	20	250	2.6–19	13–95	7.6
Cr	35 (*AI*)	200 (*TL*)	1.5–4	4.3–114 (AI)	2 (*TL*)
Cu	0.9	10	0.77–1.0	86–111	10
Fe	18	45	1.4–1.65	7.8–9.2	3.7
Mn	2.3 (*AI*)	11	1.55–1.8	14–16 (AI)	16
Ni	700	1000	32–81	4.6–11.6	8.1
Se	55	400	1.7–2.4	3.1–4.3	0.5
Zn	11	40	1.4–1.8	12.7–16.4	8

RDA – recommended dietary allowance, *AI* – Adequate intake, *UL* – Tolerable upper intake level, *TL* – Therapeutic level. Maximum values for 19–50 years females and males are given [3].

Tab. 4. Possible maximum intake of the toxic elements Cd, Pb and Sb by daily consumption of 50 g walnuts.

Element	Intake per week [μg]	*TMWI* [%]
Cd	4.6–15.8	3.1–10.5
Pb	28–70	1.9–4.7
Sb	2–3.4	0.09–0.13

TMWI – tolerable maximum weekly intake (60 kg body weight [4, 5]).

(6 μg per kg body weight or 360 μg per day per person [6]).

Hence, the results of this study suggest that despite the environmental pollution due to lead metallurgy, the exposure to toxic elements Cd, Pb and Sb mediated by the consumption of walnuts is low, and there is no risk of exceeding the Tolerable Maximum Weekly Intakes.

CONCLUSIONS

Walnuts are valuable horticultural products, which can be grown even in a polluted area without losing their nutritional qualities beneficial for human health. An important part of Co, Cr, Cu, Fe, Ni, Mn, Se and Zn requirements for the human healthy nutrition can be supplied by the consumption of 50 g walnuts daily. The slight overdoses of Cr and Cu observed for some walnut varieties can not cause adverse effects. In addition, exposure to the toxic elements Cd, Pb and Sb mediated by the consumption of walnut was found to be low despite their higher extent of accumulation in comparison to kernels from uncontaminated areas. Even when eating 50 g daily walnuts from a polluted area, the exposure to these toxic elements will not exceed the tolerable maximum weekly intake (60 kg adults) in an extent higher than 10.5% for Cd, 4.7% for Pb and 0.1% for Sb.

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