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PREFACE

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All papers were subject to anonymous double reviews and the category of papers were determined by the editors based on the recommendation of the reviewers.

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Original scientific paper

The content of metals and metalloids in bulbs of different genotypes of *Allium* species

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Abstract

In this research, we examined the effect of 15 genotypes of selected *Allium* species: *A. sativum* L. (10 genotypes), *A. ampeloprasum* L. var. *ampeloprasum* (3) and *A. cepa* L. (2), on the content of metals and metalloids in bulbs. Determination of the content of elements was performed using the method of atomic absorption spectroscopy and ICP-OES method (mg/kg of dry matter).

This research found that all tested genotypes differed statistically significantly in the content of Na, K, Ca and Mg both from each other and within the species. The highest content of Na, K, Ca and Mg was established in *A. sativum*, while the lowest content were observed in onion (*A. cepa*). Genotypes of *A. ampeloprasum* var. *ampeloprasum* contained moderate amounts of Na, K, Ca and Mg. The content of certain metals was the same in all genotypes of the tested *Allium* species and was < 0.01 mg/kg (Hg) and < 0.5 mg/kg (Hg, Co, Ni and Mo). The species *A. ampeloprasum* var. *ampeloprasum* showed the highest affinity for Cd accumulation in bulbs, followed by *A. sativum* and *A. cepa*. According to the content of Fe and Zn, *Ljubičasti sredbrenjak* as onion variety, it stood out in relation to all genotypes of tested *Allium* species. The highest content of Cd, Mn and Se was noticed in genotypes of *A. ampeloprasum* var. *ampeloprasum*. The presence of potentially toxic elements (Pb, Hg, As) was also determined in the bulbs of tested species, but in the safe levels. Considering the fact that species of the genus *Allium* show the ability to accumulate elements that are important for human health, especially Fe, Zn and Se, future research should be directed to enriching popular species from this genus

with these elements by applying simple and cost effective agrotechnical measures, such as biofortification.

Key words: Allium species, genotypes, metals, metalloids

Introduction

The genus *Allium,* from family Alliaceae, is one of the largest plant genera, which includes more than 800 species (Fritsch et al., 2010). All members of this genus are annual, biennial or perennial geophytes with underground stems known as bulbs (Li et al., 2010). The most economically important species from this family are: onion (*Allium cepa* L.), garlic (*A. sativum* L.), chives (*A. schoenoprasum* L.), shallots (*A. cepa* var. *aggregatum*), leek (*A. ampeloprasum* L. var. *porrum*) and bunching or Welsh onion (*A. fistulosum* L.).

Onion a very commonly used vegetable, ranks third in the world production of major vegetables, with 104 million tons per year. It is grown in almost all countries of the world, and the largest producer is China with a share of 25% in total world production. Garlic is the second most important species of the genus *Allium* with a production of 28 million tons per year, and China is also the world's largest producer with a 75% of total world production. In Europe, total production of onions is around 10 million tons and the largest producers are Russia, the Netherlands, Ukraine and Spain, while, in the case of garlic, the largest producers are Spain, Ukraine and Russia with total production with 870 thousand tons (FAO, 2020).

Allium species contain a large number of different compounds that have a positive effect on human health. These species are source of numerous bioactive compounds together with many vitamins (B1, B2, C, E, K), micro and macronutrients (especially K and S). Recent research has shown that onion and garlic extracts can prevent cardiovascular and other diseases, so they are increasingly used in traditional medicine (Zeng et al., 2017). Therapeutic effects as well as the smell and taste of *Allium* species are associated with a high content of essential oils in edible parts (Block, 1985).

The chemical composition of plants, especially the content of macro and microelements, as well as their dislocation in plant tissues largely depends on the chemical composition of the soil. Research conducted by Gambelli et al. (2021) indicates that agroecological conditions, but also varieties, significantly affect the mineral composition of garlic.

In general, the dry matter of plants contains about 45% of C, 45% of O, 6% of H, 1.5% of N and 2.5% other elements. Depending on the importance for the plants, all elements are divided into essential (C, H, O, N, P, K, Ca, Mg, S, Fe, B, Mn, Cu, Zn, Cl, Mo and Ni), beneficial (Na, Si, Co, Se, and more recently Al) and others (all other elements found in plants) (Kastori, 2006). In addition to mentioned elements, toxic elements, in the literature often known as heavy metals, can often accumulate in plant tissues, as: Hg, Pb, As, Ni, Cd. Research by Soudek et al. (2009) performed on four *Allium* species (onion, garlic, leek and chives), which are related to the absorption of toxic elements from nutrient solution and their distribution and accumulation in plant parts, showed that increasing their content through nutrient solution, increases their content in plant tissues. The same authors point out that toxic elements have mainly accumulated in the root system of plants. Considering the total amount of accumulated Cd, 75% was contained in the root, while in the case of Co this percentage varied from 40-90% (Soudek et al., 2009). According to Ke et al. (2011) the presence of heavy metals in the soil negatively affects the germination and growth of garlic.

Actually, toxicity is mainly related to the amount of an element, but this range varies greatly for each individual element. In the case of fresh vegetables, the maximum allowed concentration prescribed by Regulation (28/2011) for Pb is 1 mg/kg, Cd 0.05 mg/kg, Hg 0.02 mg/kg and As 0.3 mg/kg of fresh matter.

Research conducted by Vadalà et al. (2016) which analyzes the concentration of elements in different varieties of garlic from Spain, Tunisia and Italy indicate that the concentrations of the considered elements can be used as geographical indicators to distinguish the origin of garlic samples. Namely, it was determined that samples of garlic from Tunisia and Spain had a high level of Ni, while a relatively high content of Se was detected in the garlic variety *Nubia Red Garlic* from Italy. As *Allium* species have a high ability to accumulate Se in edible parts, they can be used as an effective tool to increase Se levels in human nutrition. In this case, the doses of selenium fertilizers as well as the methods of their application should be studied in order to avoid negative effects. In this work was examined the effect of 15 genotypes of three *Allium* species: *A. sativum* L. (10 genotypes), *A. ampeloprasum* L. var. *ampeloprasum* (3 genotypes) and *A. cepa* L. (2 varieties), on the content of metals and metalloids in bulbs. Determination of the content of elements: sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), mercury (Hg), cadmium (Cd), iron (Fe), zinc (Zn), cobalt (Co), nickel (Ni), manganese (Mn), chromium (Cr), molybdenum (Mo), lead (Pb), aluminum (Al), arsenic (As) and selenium (Se) was performed using the method of atomic absorption spectroscopy (AAS) and ICP - OES method. The aim of the research was to determine the mineralogical composition of selected species of the genus Allium, with special reference to elements that are useful or toxic to human health.

Materials and Methods

Source of plant material

Plant material and experimental field Rimski Šančevi used in this study were provided by Institute of Field and Vegetable Crops (Novi Sad). In the experiment were used following genotypes: *A. sativum* – autumn garlic (JBL 3/17, JBL 7, JBL 8/17, Ranko, Bosut)*, A. sativum* – spring garlic (Sedef, PBL 37-3, PBL 95, PBL 101, Labud), *A. ampeloprasum var. ampeloprasum* - elephant garlic (Živa, 30A, 17A) and *A. cepa* - onion (Ljubičasti srebrenjak and Kupusinski jabučar). Standard agrotechnical measures were applied during experiment. Bulbs of autumn garlic, elephant garlic and Ljubičasti srebrenjak were planted in October of 2018, and harvested in Jun of 2019. Nevertheless, the crops that consisted rest of the *Allium* species were formed in March of 2019, while plant material was collected in July of the same year. For purposes of chemical analysis each genotype counted 10 samples of healthy, properly formed bulbs.

Chemicals

All of the chemicals used in this research were of analytical purity grade. Nitric Acid (67-69 % for trace metal analysis, VWR BDH Prolabo chemicals, Canada) and hydrogen peroxide (30% for traces analysis, VWR BDH chemicals, France) have been used for the digestion of samples

Sample preparation technique for content determination of metals via AAS and ICP-OES methods

Solid samples of the plant material, previously minced, were prepared using the microwave digestion method to investigate the level of metals. Respectively, triplicates of samples were weighted 0.4 g and transferred into cuvettes. Subsequently, 7 mL of nitric acid (67-69% HNO_3) and 1.5 mL of hydrogen peroxide (30% H_2O_2) were added. In the same manner, blank was prepared, excluding the sample.

Vessels then stood for 10 minutes, before they were sealed and relocated to microwave oven (TITAN MPS Microwave Sample Preparation System). Digestion lasted for 56 minutes at constant temperature and pressure that was evenly amplified from 0 to 30 bar (Banule and Ajwa, 1999; Block 1995; Liang et alm 2019). The content of cuvettes cooled down before transferred to 50 mL volumetric flasks and diluted with distilled water up to mark. All samples were made in triplicate

Atomic absorption spectroscopy (AAS)

The toxic metal concentration (Pb, Cd, As) was determined by electrothermal atomization using a graphite furnace Atomic absorption spectroscopy (GF-AAS), PinAAcle 900T. Adjusted wave lengths for elements were respectively 283.3 nm, 228.8 nm and 193.7 nm. The accuracy and precision, LOD (limits of detection) and LOQ (limits of quantification) of the method were tested by the above mentioned standard reference material. By validating the method, it was determined that all validation parameters are satisfied, that the method is accurate and that it can be used to determine the mentioned metals.

Determination of mercury using Mercury Analyze instrument

In order to establish the content of Hg, samples of onion genotypes underwent examination on Mercury Analyze instrument, FIMS 100, Perkin Elmer, serial number: 101S14121001. SRM used for this purpose is: Reference Standard Hg - Mercury standard traceable to SRM from NIST in HNO_3 (5%), 10 mg/L, Perkin Elmer, N9300253, CL9-136HGY1.

Inductively coupled plasma optical emission spectrometry (ICP-OES)

Instrument used to analyze the remaining content of metals and metalloids was ICP-OES (Optima 8000), whilst SRM utilized: Instrument calibration standard 2, 100 µg/mL, Perkin Elmer, Ag, Al, Ca, Co, Cr, Fe, K, Mg, Mn, Mo, Na, Ni, Se, Zn, catalogue number N9301721, lot: CL3-191MKBYl.

Chemical analyses of soil

A Pye glass electrode pH—meter—potentiometer (W.G. Pye, Cambridge) was used to measure the pH value (in 001 M KCl). The humus content was determined by oxidation with the KMnO4 solution (according to Kotzman), and total nitrogen content by the Kjeldahl method. Available P and K were determined by extraction with Al solution, and P and K by colorimetry with molybdate and flame photometry, respectively (Egner et al., 1960). The EDTA extractable concentrations of heavy metals were determined by the EDTA extraction protocols for IRMM BCR reference materials CRM-484 (Milenković et al., 2015).

Statistical analysis

The obtained results were analysed according to the model of the one-factor analysis of variance, and the individual comparison of groups was performed by the subsequent LSD test ($p<0.05$ and $p<0.01$). The data were processed using various mathematical and statistical softwares (Excel 2010, DSAASTAT) and results are presented in the tables.

Results and Disscusion

Chemical properties of soil

Since soil and its richness in chemical composition play a significant role in defining the chemical composition of plants that grew on it (Banuelos and Ajwa, 1999; Němeček, 2001), Table 1 shows the results of basic agrochemical properties of soil, while Table 2 shows content of total and content of accessible microelements and potentially toxic elements, detected in soil.

Table 1. Basic agrochemical properties of soil

Basic properties								
pH (KCl)	**pH (H_2O)**	**$CaCO_3$ %**	**Humus %**	**Total N %**	**P_2O_5 (Al)**	**K_2O (Al)**	**Total salts %**	**EC (mS/cm)**
7.44	8.22	16.59	0.98	0.06	1.78	9.5	0.03	0.34

According to obtained results of the basic agrochemical analysis, the soil on which *Allium* species were grown was: the carbonate, alkaline reaction, poor in humus content and poor in content of accessible P and K (Table 1).

Table 2. The content of total and easy accessible microelements and toxic elements in soil (mg/kg)

Element	**Cu**	**Zn**	**Mn**	**Fe**	**Mo**	**Co**	**Pb**	**Cd**	**Cr**	**Ni**
Total content (HNO_3)	19.93	56.23	368.33	23264.96	10.27	17.79	30.41	0.61	28.6	41.97
Accessible content (EDTA)	0.86	0.132	2.784	5.024	0.464	0.464	2.07	0.106	0	0.932

The results of the content of elements in the tested soil shown in Table 2 indicate that their total content is appropriate, while their accessibility for the plant is lower, which is attributed to the pH value as well as the mechanical composition of the soil.

Elemental content of plant material

The content Na as beneficial element for plants, and the content of K, Ca and Mg as essential elements for growth determined in *Allium* genotypes was shown in table 3.

The obtained results indicate that the highest content of Na and K was observed in genotypes of *A. sativum*, and the lowest in *A. cepa*. Namely, the content of Na was the highest in genotypes

JBL 7, *Ranko* and *Bosut*, while the the highest content of K was achieved in *Ranko*, *Bosut* and *JBL 8/17*. In the case of both elements, the contents achieved in the mentioned genotypes were statistically significantly higher compared to contents observed in other genotypes of all tested *Allium* species (Table 3).

The content of Ca and Mg in tested species was as follows in ascending order *A. cepa* < *A. ampeloprasum* < *A. sativum*. Genotype *Bosut* (*A. sativum*) had the highest content of Ca (183.5 mg/kg) and that content was statistically significantly higher compared to other genotypes of *A. sativum*. Observing the content of Mg, genotypes *Bosut* and *Ranko* was contained statistically significantly higher content of Mg than other *A. sativum* genotypes. The lowest content of Ca (68.0 mg/kg) and Mg (97.9 mg/kg) was observed in variety *Kupusinski jabučar* (*A. cepa*) (Table 3).

Table 3. The content of N, K, Ca and Mg in bulbs of selected genotypes of *Allium* sp. (mg/kg of dry matter)

***Allium* species**	**Genotype**	**Element**			
		Na	**K**	**Ca**	**Mg**
A. sativum	Sedef	31.46	3133.1	142.6	183.6
	PBL 37-3	16.15	3077.7	130.9	197.1
	PBL 95	15.83	3628.5	164.4	200.5
	PBL 101	67.73	3514.5	137.8	202.4
	Labud	36.59	3827.2	95.6	200.7
	JBL 3/13	32.40	2811.5	106.0	224.6
	JBL 7	50.61	3560.8	121.0	209.8
	JBL 8/17	25.39	4145.0	128.8	228.0
	Ranko	72.85	4018.4	128.0	265.4
	Bosut	84.68	3992.5	183.5	348.8
A. ampeloprasum	30 A	17.84	2899.5	167.0	285.1
	Živa	16.61	2505.7	100.8	314.6
	17A	17.49	2871.2	74.1	228.7
A. cepa	Ljubičasti srebrenjak	19.49	1378.6	107.4	105.0
	Kupusinski jabučar	44.21	1517.4	68.0	97.9
	$\bar{x}$	**36.6**	**3125.4**	**123.7**	**219.5**
	Min	**15.8**	**1378.6**	**68.0**	**97.9**
	Max	**84.7**	**4145.0**	**183.5**	**348.8**
	LSD 0.05	12.50	410.5	11.4	19.1
	LSD 0.01	17.44	770.2	16.2	25.7

The content of microelements (Fe, Zn, Ni, Mn, Mo), beneficial (Co, Se, Al) and potentially toxic elements (Hg, Cd, Cr, Pb, As) determined in bulbs of genotypes of selected *Allium* species was shown in table 4.

Table 4. The content of microelements and potentially toxic elements in bulbs od selected genotypes of *Allium* species (mg/kg per dry matter)

***Allium* species**	**Genotype**	**Element**												
		Hg	**Cd**	**Fe**	**Zn**	**Co**	**Ni**	**Mn**	**Cr**	**Mo**	**Pb**	**Al**	**As**	**Se**
A. sativum	Sedef	< 0.01	0.017	1.56	4.40	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.3	0.5
	PBL 37-3	< 0.01	0.021	0.69	4.86	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.3	2.18
	PBL 95	< 0.01	0.015	0.74	4.38	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.3	0.5
	PBL 101	< 0.01	0.016	1.33	6.92	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.1	0.66	< 0.3	0.5
	Labud	< 0.01	0.015	0.56	4.66	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.3	1.95
	JBL 3/13	< 0.01	0.017	5.35	5.54	< 0.5	< 0.5	1.51	< 0.5	< 0.5	0.41	5.93	< 0.3	1.62
	JBL 7	< 0.01	0.024	3.47	3.75	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	0.52	17.43	< 0.3	2.62
	JBL 8/17	< 0.01	0.029	4.44	4.05	< 0.5	< 0.5	0.58	< 0.5	< 0.5	0.21	2.99	< 0.3	2.79
	Ranko	< 0.01	0.013	10.07	8.00	< 0.5	< 0.5	1.48	0.59	< 0.5	< 0.1	8.09	< 0.3	0.5
	Bosut	< 0.01	0.025	11.62	8.83	< 0.5	< 0.5	2.59	< 0.5	< 0.5	< 0.1	15.55	< 0.3	0.5
A. ampeloprasum	30 A	< 0.01	0.032	6.49	4.39	0.86	< 0.5	4.29	< 0.5	< 0.5	< 0.1	6.58	< 0.3	6.39
	Živa	< 0.01	0.022	6.57	4.28	0.79	< 0.5	3.68	< 0.5	< 0.5	< 0.1	8.95	< 0.3	4.57
	17A	< 0.01	0.037	6.72	3.56	0.99	< 0.5	2.47	< 0.5	< 0.5	< 0.1	6.42	< 0.3	4.45
A. cepa	Ljubičasti srebrenjak	< 0.01	< 0.01	405.71	10.53	0.88	< 0.5	2.26	< 0.5	< 0.5	< 0.1	1.97	< 0.3	0.5
	Kupusins ki jabučar	< 0.01	< 0.01	2.82	1.46	0.78	< 0.5	< 0.5	< 0.5	< 0.5	< 0.1	4.66	< 0.3	1.46
	$\overline{x}$	**< 0.01**	**0.022**	**31.21**	**5.31**	**0.86**	**< 0.5**	**2.30**	**0.59**	**< 0.5**	**0.38**	**7.20**	**< 0.3**	**2.07**
	Min	**-**	**0.013**	**0.56**	**1.46**	**0.78**	**-**	**0.58**	**0.59**	**-**	**0.21**	**0.66**	**-**	**0.50**
	Max	**-**	**0.037**	**405.71**	**10.53**	**0.99**	**-**	**4.29**	**0.59**	**-**	**0.52**	**17.43**	**-**	**6.39**
	LSD 0.05	-	0.005	2.11	3.26	-	-	0.46	-	-	0.09	2.92	-	0.8
	LSD 0.01	-	0.010	3.42	5.48	-	-	1.39	-	-	0.14	4.10	-	1.5

The highest content of Fe (11.62 mg/kg) and Zn (8.83 mg/kg) was observed in garlic genotype *Bosut*, while the onion genotype *Kupusinski jabučar* had the lowest content of Fe (2.82 mg/kg) and Zn (1.46 mg/kg). All genotypes of elephant garlic had Fe and Zn in contents that did not differ statistically significantly.

The Mn content was ranged from < 0.5 mg/kg in most garlic genotypes to 4.29 mg/kg in elephant garlic genotype - *30A*. Similar results were observed in the case of Se, the lowest content (0.5 mg/kg) was noticed in garlic genotypes, while the highest content was achieved in genotype *30A*. In fact, all selected *A. ampeloprasum* genotypes (*30 A*, *Živa* and *17A*) had a statistically significantly higher content of Mn and Se compared to all tested *Allium* genotypes (Table 4).

The results indicate that in all tested *Allium* genotypes was achieved same content of Hg (< 0.01 mg/kg), Ni (< 0.5 mg/kg), Cr (< 0.5 mg/kg), Mo (< 0.5 mg/kg), As (< 0.3 mg/kg), while same content of Co (< 0.5 mg/kg) was observed only in *A. sativum* genotypes. The content of Cd in all *Allium* species was varies from < 0.01 to 0.037 mg/kg which is below of toxicity levels recommended by the WHO/FAO (0.2 mg/kg) (WHO 1986, 1989; Elbagermi et al., 2012). The content of Pb was < 0.1 mg/kg, except in the case of garlic genotypes, *JBL 3/13*, *JBL 7* and *JBL 8/17* which were contained 0.41, 0.52, 0.21 mg/kg, respectively. The safe value for Pb, in vegetables prescribed by the WHO is 0.3 mg/kg (Elbagermi et al., 2012). The highest content of Al was noticed in garlic genotypes *JBL 7* (17.43 mg/kg) and *Bosut* (15.55 mg/kg). In general, all genotypes analized in this stady had average content of Al 7.2 mg/kg which is in accordance with the values obtained in bulb crops (7.4 - 9.9 mg/kg) in similar studies (Liang, 2019).

Conclusion

Allium species have been valued in nutrition and folk medicine for centuries, due to their rich chemical composition. In this study, it was found that the genotypes of selected *Allium* species (*A. sativum*, *A. ampeloprasum* var. *ampeloprasum* and *A. cepa*) had a moderate content of the examined elements. Namely, genotype of *A. sativum Bosut* had the highest content of essential elements (K, Ca, Mg, Fe, Zn, Mn) and beneficial element (Na). All tested genotypes of *A. ampeloprasum* var. *ampeloprasum* (*30A*, *Živa* and *17A*) contained a similar level of all studied elements. Significantly higher content of iron and zinc was found in the onion varieties *Ljubičasti srebrenjak*. Content of toxic elements (Hg, As, Cd) was within safe limites recommended by WHO. Only in the case of garlic genotypes *JBL 3/13*, *JBL 7* the content of Pb was more than prescribed by the WHO.

Given that species of the genus Allium show a good ability to accumulate elements important for human health, especially Fe, Zn and Se, future research should focus on agro-technical measures that increase their content in plants (biofortification).

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