Bioaccumulation and translcation of metals in selected plants from fam Apiaceae

Gorica Djelic[1]*, Sinisa Timotijevic, Zoran Simic[2]

[1]*University of Kragujevac, Faculty of Sciences,* Department of Biology and Ecology
R. Domanovic 12, 34000 Kragujevac, Serbia
[21]*University of Kragujevac, Faculty of Sciences,*Department of Chemistry*,*
R. Domanovic 12, 34000 Kragujevac, Serbia

** Corresponding author: Gorica Djelic, e-mail* gorica.djelic@pmf.kg.ac.rs

Abstract: *The paper analyzes heavy metals (Mn, Ni, Fe, Cu, Zn, Ca, Mg) in plant species Petroselinum crispum Mill., Daucus carota L., Conium maculatum L. and in the land on which they grow. The aim of the research is to determine the differences in the acceptance, distribution, and accumulation of metals between the investigated species, based on the content of metals in the plant organs (root, tree, leaf, and fruit), based on the bioaccumulation coefficient, bioconcentration and translocation factor. The atomic absorption spectrophotometer determines the amount of metal in the soil and plant material. Results showed that in the investigated soil there were the highest Fe levels, but in quantities not exceeding the maximum permitted concentrations. The content of Mn, Fe, Cu in all analyzed plants is elevated. Follows the sequence of heavy metals on the basis of total quantity in the species P. crispum Ca>Mg>Fe>Mn>Zn>Ni>Cu, and for the species D. carota, C. maculatum, Ca>Mg>Fe>Zn>Mn>Cu>Ni. There is a significant intraspecies difference in the distribution of the examined elements.*

Keywords: *Apiaceae, metals, bioaccumulation, bioconcentration, translocation*

I. INTRODUCTION

At the beginning of the 20th century, contamination of an environment with heavy metals rapidly increased and represented a major ecological and health problem worldwide. [1]. Natural, heavy metals are widely represented in the Earth's crust and originate from metamorphic walls [2] [3]. Although natural constituents of the geological substrate, the higher level of heavy metals in the substrate are also caused by human activities and is a far more serious problem. Every type of heavy metal pollution has a negative impact on plants, animals, and human health. Heavy metals in the environment are often carcinogens and mutagens [3].

Plant species can absorb accumulated harmful substances (including heavy metals) from the soil. Therefore, they play an important role in soil Plant species can absorb accumulated harmful substances (including heavy metals) from the soil. Therefore, they play an important role in soil bioindication[4].

The uptake potential of heavy metals in plants depends on the amount of metals in the soil, their bioavailability [5] [6]. They develop mechanisms (accumulation and exclusion) that protect them from the toxic effects of metals. Adopted pollutants are retained at plant roots and/or translocated to the aboveground parts [7]. Due to the ability to absorb heavy metals from the external environment, plants have found great application in biotechnologies for remediation of polluted habitats. [7]. However, due to the accumulation of heavy metals, the use of plants from contaminated terrains in nutrition and treatment is not allowed.

The aim of the research is to determine the differences in the absorption, distribution, and accumulation of heavy metals (Mn, Ni, Fe, Cu, Zn, Ca, Mg) in *Petroselinum crispum* Mill., *Daucus carota* L., *Conium maculatum* L., based on quantitative content of Mn, Ni, Fe, Cu, Zn, Ca, Mg in the underground and above-ground organs of species, bioaccumulative factor (BAF), bioconcentration factor (BCF) and translocation factor (TF) for all metals in the investigated species.

II. MATERIAL AND METHODS

2.1 Study area

The plant material (*Petroselinum crispum* Mill., *Daucus carota* L., *Conium maculatum* L.) and the soil were collected from the Ovčar-Kablar Gorge site (43 ° 55'07 "NL; 20 ° 13 ' 17 "IGL, Fig.1.) from the year 2017 to 2019. Samples were collected twice (June, August) every year.

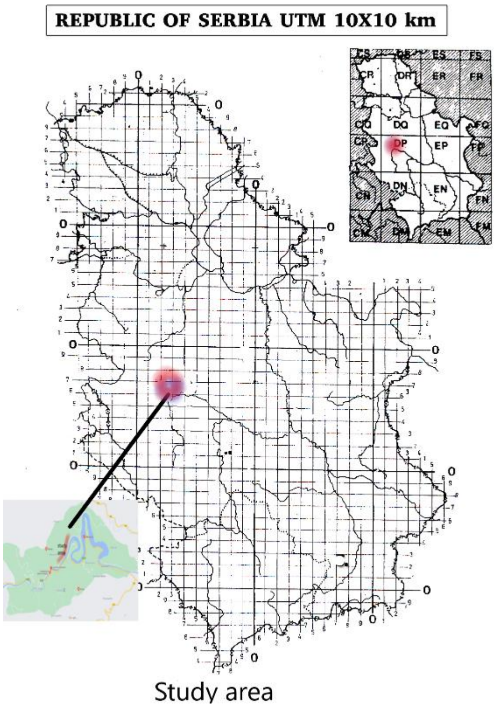

Figure 1. Study area

The identification of plant material was made using appropriate equipment in the laboratory of the Institute of Biology and Ecology, Faculty of Science, Kragujevac, with the help of standard keys for determination of plants: Javorka and Csapody [8], Flora of the Republic of Serbia ([9] and Flora Europe [10].

2.2 Sample analysis

Soil samples and plant material were dried in the air to an airy dry state. After drying of plant specimens and soil samples, a certain mass of prepared material (3 g of soil and 2 g of plant material) was measured on the analytical scale, followed by a standard procedure for the preparation of samples for chemical analysis [11],[12].
For the chemical processing and analysis, it was used 2 g of plant material and 5 g of soil. The soil sample was prepared by a standard procedure (using HCl). The destruction of the plant material was carried out using concentrated HNO3 and HClO4. In the samples of soil and plant material, the concentrations of metals: Mn, Ni, Fe, Cu, Zn, Ca, Mg were determined on the Atomic Spectrophotometer (Perkin Elmer 3300) on the principle of atomic absorption flame of photometry at the Faculty of Science and Mathematics, University of Kragujevac. Each sample is read in five repetitions.

2.3 Phytoextraction ability

The mean value, the standard deviation, the bioaccumulation factor (BAF), bioconcentration factor (BCF), and the translocation factor (TF) were determined. The bioaccumulation factor is a plant's ability to accumulate a

certain metal relative to its concentration in the substrate [13], [14], [15], and it is calculated as the ratio of the concentration of certain metal to the root and its concentration in the soil. The translocation factor is used to estimate the relative translocation of metals from the underground organs to the above-ground organs and it is calculated as the ratio of metal concentration in the above-ground organ and its concentration at the root [16], [17]. An effective translation of the metal takes place when TF>1.
The biological concentration factor, BCF, was calculated as the ratio of the metal concentration in roots to that in soil [18]:

$$BCF= \text{concentration metal root/ concentration metal soil} \quad (1)$$

2.3 Statistical analysis

The differences in the concentration of metals between the soil in plants, as well as the differences in concentrations between plants, were determined by the method of variance analysis (One-WayAnova) and the Kruskal-Wallis test of factorial observation and the significance level r<005, r<0.005, r<0.001. Statistical significance was determined using Pearson's correlation coefficient (r). The correlation coefficient (r) is evaluated as: 0-0.3: no correlation; 0.3-0.5: poor correlation; 0.5-0.7: medium correlation; 0.7-0.9: high correlation; 0.9-1.0: a very high statistically significant correlation [19], [20], [21]. Statistical processing of measurement results was carried out using the SPSS (Chicago, IL) statistical software package [22].

III. RESULTS AND DISCUSSION

3.1 Metals content in soil

The soil of the Ovčar-Kablar gorge from which the collected plants were gathered for the analysis belongs to the brown-ore soil on the limestone. Results of the study (Table 1) show that soil contains the highest iron concentrations (21.717,7 mg/kg) among all tested metals, hence the color of this soil (brown-ore). However, the quantities of the analyzed metals in the soil do not exceed the permissible quantities prescribed by the regulations on the permitted quantities of hazardous and harmful substances in soil and irrigation water and the methods of their examination [23]. The content of calcium in the soil sample is 21.501 mg/kg, which can be explained by the fact that this land is formed on limestone. Magnesium is 1.8 times less than the calcium concentration. Such a relationship is considered favorable for the development of many plant species.

Based on the determined quantity (Table 1) in the soil, all investigated metals can be compared to the series Fe>Ca>Mg>Mn>Zn>Cu>Ni.

Table 1. The content of investigated metal [mg kg^{-1}] in the soil

metal	soil	**MDK**[a]
Mn	335±3,62	2 000
Ni	29,5±0,38	50
Fe	**21 717,7**±169,59	50 000
Cu	47,4±0,4	100
Zn	71,9±0,9	300
Ca	**21 501**±65	
Mg	12 193,4±65,3	

[a] Official Gazette of RS, No. 23/94

3.2 Metals content in plant parts

The order of metals in *P. crispum* based on their amount in the root and stem is: Ca>Mg>Fe>Zn>Mn>Ni>Cu, and in leaves and fruit Ca>Mg>Fe>Mn>Ni>Zn>Cu. In the species *D. carota*, based on the content in the root and stem, the metals can be arranged in series Ca>Mg>Fe>Zn>Cu>Mn>Ni, in leaves

Ca>Mg>Fe>Mn>Zn>Cu>Ni and in fruit Ca>Mg>Fe>Mn>Ni>Zn>Cu. In all plant organs in *C. maculatum* the order of metals, is Ca>Mg>Fe>Zn>Mn>Cu>Ni.

The content of the investigated metals in the studied species was different and it depended on the plant organ and the type of metal. The analysis found that all species in the largest quantities accept Ca, *D. carota*, and *C. maculatum* least adopt Ni, and *P. crispum* least adopts Cu (Table 2).

According to Le Bot (1996), toxic concentrations of Mn are in the range from 80 to 5,000 mg/kg [24]. In the investigated plants, Mn content ranges from 5.4 mg/kg -53.9 mg/kg, which is within the limits (up to 300 mg/kg) that are characteristic of Mn content [25]. Analysis of the content and distribution of Mn showed that the highest concentrations of this metal are found in the leaf of species: *D. carota* (44.4 mg/kg) and *C. maculatum* (29.7 mg/kg) while *P. crispum* fruit contains a maximum amount of Mn (41.9 mg/kg).

Nickel (Ni) is found in plants at very low concentrations of 1-10 mg / kg, predominantly in the divalent form [26]. *P. crispum* is a species that contains the largest amounts of Ni compared to other examined species. The highest concentrationsof Ni in *P. crispum* are found in the fruit, in speacies *C. maculatum* at root, while in *D. carota* the amount is uniform (Table 2). Carrot's uptake of heavy metals depends on environmental conditions [27]. Stasinos and Zabetakis, (2013) showed that carrots do not absorb large amounts of Ni and Cr if it is watered with water containing Cr I Ni and grows on unpolluted soil [28].

The total Fe content in most of the land is about 3.2% [30]. The concentration of Fe kills has a lethal effect in the range of 50-1000 mg/kg [29]. In all examined plant species, the increased concentration of this metal is constant. In all examined plants, the highest Fe concentrations are at the root of *P. crispum* at a concentration of 2093 mg/kg, at *C. maculatum* at a concentration of 1684 mg/kg, at *D. carota* at a concentration of 1650.9 mg/kg. The lowest Fe concentrations are found in the stems of all investigated species.

In plants, the concentration of copper ranges between 5 to 30 mg/kg [30], and the content of copper from 30 to 100 mg/kg present elevated concentrations and can cause toxic effects [25]. The largest amounts of Cu are found in the roots of the species *P. crispum* (22.4 mg/kg) and *C. maculatum* (15.4 mg/kg), and in *D. carota* fruit (10 , 2 mg / kg).

Zinc is a macroelement that is present in the Earth's crust in a small concentration of 0.02%, but that is a necessary amount for the growth and development of plants ([31]. The content of Zn in plants is low, concentrations are in the range of 0.6-83 mg/kg [25]. Our research has shown that the species *C. maculatum* accumulates the largest amounts of Zn and *D. carota* accumulates the least quantity. The largest amounts of Zn are found in *C. maculatum* (50.4 mg/kg) and *D. carota* (37.4 mg/kg) in the fruit; and *P. crispum* at the root (38.4 mg/kg).

Table 2. The content of investigated metal [mg kg^{-1}] in species *Petroselinum crispum* Mill., *Daucus carota* L., *Conium maculatum* L.

Metal	Plant	**root**	**stems**	**leaf**	**fruit**
Mn	*Petroselinum crispum* Mill.	37,2 ± 0,46	8,2±0,04	32,7±0,42	41,9±0,63
	Daucus carota L.	8,3±0,04	5,4±0,05	44,4±0,51	34,4±0,40
	*Conium maculatum*L.	21,3±0,16	6,2±0,11	23,7±0,42	19,5±0,36
Ni	*Petroselinum crispum* Mill.	28,5±0,42	7.9±0,04	32±0,42	40,9±0,6
	Daucus carota L.	2,2±0,04	1,3±0,03	2,6±0,03	2,4±0,03
	*Conium maculatum*L.	9,2±0,04	1,5±0,04	1,2±0,04	1,6±0,07
Fe	*Petroselinum crispum* Mill.	2093,5±18,24	147,2±4,96	287,7±6,52	519,3±4,84
	Daucus carota L.	1650,9±31,86	442,8±6,09	863,9±8,92	539,8±7,65
	*Conium maculatum*L.	1684±19,97	474,8±3,69	949,5±13,19	633,2±7,33
Cu	*Petroselinum crispum* Mill.	22,4±0,41	6,8±0,07	8,9±0,05	15,6±0,42
	Daucus carota L.	8,6±0,11	4,1±0,05	9,1±0,05	10,2±0,07

	*Conium maculatum*L.	15,4±0,36	5,9±0,04	6,8±0,05	11,6±0,31
Zn	*Petroselinum crispum* Mill.	38,4±0,3	21,8±0,59	23,3±0,40	33,6±0,35
	Daucus carota L.	21,6±0,26	14,5±0,42	37,5±0,60	37,4±0,46
	*Conium maculatum*L.	24,5±0,35	15,5±0,44	24,3±0,41	50,4±0,7
Ca	*Petroselinum crispum* Mill.	8807,8±60,11	1938,9±40,14	19096,4±77,67*	11824,5±24,84
	Daucus carota L.	7925,9±20,66	5932,9±40,97	21191,6±81,16*	17645,7±73,19
	*Conium maculatum*L.	18452±136,01	8760,9±24	24126,7±116,29*	5952,8±31,33
Mg	*Petroselinum crispum* Mill.	8187,2±36,4	978,3±6,66	8150,9±55,48	5142,9±46,96
	Daucus carota L.	1651,7±27,89	976,9±7,64	4477±42,77	6343,8±34,98
	*Conium maculatum*L.	4518,4±73,48	1958,5±15,83	5883,8±108,59	3945,1±30,12

In examinated species, in all parts of the plant, high concentrations of Ca are constant. Our study showed that the highest Ca concentration is in *P. crispum* (19096.4 mg/kg), *D. carota* (21191.6 mg/kg), and *C. maculatum* (24126.7 mg/kg) in leaves. The minimum quantities of Ca are found in stems in all tested species except for *C. maculatum* in which the fruit contains the smallest amount of Ca.

The concentration of Mg in plants varies from 0.09-0.70% at the dry weight. The results of the study indicate that Mg is accumulated in the fruit in the *D. carota*, at the root of *P. crispum*, and in the leaves of *C. maculatum*.

3.3 Bioaccumulation, Bioconcentration and translocation factors

Bioaccumulation, bioconcentration, and translocation factors can be applied in the estimation of the potential of the plant species for its application in phytoremediation. However, they also point out that plants that have high values for BAC and TF must not be used in human nutrition and treatment.

The values of bioaccumulation coefficient show that species *P. crispum*, *D. carota* and *C. maculatum* in leaves accumulate Ca because BAC is greater than 1 (Table 3) and that species *P. crispum* accumulates Ni at the root. Bibi and et al. (2016) examined the effect of Hg on primrose growth [32]. They found that primrose accumulates large amounts of Hg and has phytoremediation potential (bioaccumulation factor and translocation factor increased with increasing Hg concentration in the medium). Parsley is characterized by the ability to absorb and accumulate a large quantity of Cr [33]. For these reasons, authors recommend that primrose, if used in nutrition and treatment, should not be grown on soil rich in Hg and Cr. We add that it also shouldn't be grown on soil rich in Ni.

Table 3. Bioaccumulation coefficient (BAC) **of species *Petroselinum crispum* Mill., *Daucus carota* L., *Conium maculatum* L.**

	P. crispum			***D. carota***			***C. maculatum***		
	stems	**leaf**	**fruit**	**stems**	**leaf**	**fruit**	**stems**	**leaf**	**fruit**
Mn	0,02	0,10	0,13	0,02	0,13	0,10	0,02	0,07	0,06
Ni	0,04	0,29	0,04	0,04	0,09	0,08	0,05	0,04	0,05
Fe	0,01	0,01	0,02	0,02	0,04	0,02	0,02	0,04	0,03
Cu	0,14	0,19	0,33	0,09	0,19	0,22	0,12	0,14	0,24
Zn	0,30	0,32	0,47	0,20	0,52	0,52	0,22	0,34	0,70

Ca	0,09	1,15*	0,55	0,28	1,11*	0,82	0,41	1,12*	0,28
Mg	0,08	0,67	0,42	0,08	0,37	0,52	0,16	0,48	0,32

*Bioaccumulation coefficient > 1

Table 4. Bioconcentration factor (BCF) of species *Petroselinum crispum* Mill., *Daucus carota* L., *Conium maculatum* L.

Metal	***P. crispum***	***D.carota***	***C. maculatum***
Mn	0,11	0,02	0,06
Ni	1,13*	0,07	0,31
Fe	0,0001	0,76	0,07
Cu	0,47	0,18	0,32
Zn	0,53	0,30	0,34
Ca	0,41	0,36	0,85
Mg	0,67	0,13	0,37

* Bioconcentration factor > 1

On the basis of obtained results (Table 4), BCF is not higher than 1, except for Ni in *P. crispum* which means that there is no effective accumulation of tested metals to the root of plants.

Table 5. Translocation factor of species *Petroselinum crispum* Mill., *Daucus carota* L., *Conium maculatum* L.

Metal	***P.crispum***			***D. carota***			***C.maculatum***		
	stems	**leaf**	**fruit**	**stems**	**leaf**	**fruit**	**stems**	**leaf**	**fruit**
Mn	0,22	0,87	1,12*	0,65	5,34*	4,14*	0,29	1,11*	0,91
Ni	0,04	0,29	0,03	0,59	1,18*	1,09*	0,16	0,13	0,17
Fe	0,07	0,13	2,48*	0,26	0,52	0,02	0,28	0,56	0,37
Cu	0,30	0,39	0,69	0,09	1,05*	1,18*	0,38	0,44	0,75
Zn	0,56	0,60	0,87	1,18*	1,73*	1,13*	0,63	0,99	2,05*
Ca	0,22	2,11*	1,34*	2,22*	2,67*	2,22*	0,47	1,30*	0,32
Mg	0,11	0,14	0,62	3,84*	2,72*	3,84*	0,43	1,33*	0,87

* Translocation factor > 1

Translocation factor (TF) is used to assess the potential of a plant to absorb metal ions from the soil, and to transport and accumulate them in aboveground organs. The results of the research (Table 5) show that for the fetus in *P. crispum* TF> 1 for Mn, Fe, Ca. Values of TF> 1 were found in C maculatum for Mn, Ca and Mg in the leaf and for Zn in the fruit. There is great mobility of almost all metals (except Fe) from the roots to the aboveground organs in *D. carota.*

3.3 Statistical analysis

The results obtained by the method of analysis of variance comparing the concentrations of metals in the soil and selected species show that there are statistically very significant differences in metal content between selected species and soil (Tabla 6).

Table 6. Analysis of variance between metal concentrations in soil and plant species

	Conium maculatum		***Daucus carota***		***Petroselinum crispum***	
	F	p	F	p	F	p
Mn	37284	***	36399,4	***	33741,1	***
Ni	24919	***	25435,8	***	15532	***
Fe	74384	***	72714,7	***	75759,8	***
Cu	18751,9	***	40585,4	***	12927,7	***
Zn	6857,3	***	6845,3	***	5852,1	***
Ca	41543,9	***	75197,4	***	97161	***
Mg	17209,4	***	62247,6	***	39740,4	***

The results indicate that there are statistically very significant differences in the content of metals in different plant organs of the selected species (Table 7). The results of variance analysis between the content of metal at the root and the leaf in the investigated plants (Table 7) showed that there is a very high statistical difference in the content of the examined metals at the root and the leaves in the analyzed species.

There is no statistically significant difference in the content of Cu in the leaves of examined plants. A more detailed Post Hoc analysis showed that there was no statistically significant difference in the iron concentration at the root of *D. carota* and *C. maculatum*, in the content of zinc in the leaves between *C. maculatum* and *P. crispum*; in the content of zinc in the stems between *C. maculatum* and *D. carota*; in the content of Mg in the stems between *P. crispum* and *D. carota*.

Table 7. Results of the variance analysis between the content of metals in the root, leaf, stems and fruit in the examinated plants

metal	**root**		**stems**		**leaf**		**fruit**	
	F	p	F	p	F	p	F	p
Mn	1957	***	3260,4	***	998,7	***	2327,5	***
Ni	17353,6	***	4656,6	***	101,8	***	760,4	***
Fe	1779,1	***	32812,3	***	7532,4	***	1098,8	***
Cu	3553,5	***	0,99	0,455	6117,6	***	311,9	***
Zn	4887,1	***	5171	***	1601,8	***	1314,3	***
Ca	34603,6	***	11539,3	***	35812,1	***	79359,3	***
Mg	15265,6	***	10001,4	***	8405,1	***	3591,2	***

The values of the correlation coefficient between the concentrations of metals in examined plant species (Table 8) showed that there is a strong positive correlation between the content Ni in the stems of the species *P. crispum* and fruit of *D. carota* species. The strong positive correlation of Fe metal was recorded only in the leaves of the species *P. crispum* a Cu in leaves and fruits of the species *C. maculatum*.

The negative strong correlation of Mn content with the root of *D. carota* species and root, leaf, and fruit of the species *C. maculatum* and the content of this metal in soil has been confirmed.

A statistically significant correlation between the concentration of Mn and the examined plant species was not found.

Table 8. Correlation coefficient values (r) between metal concentration in soil and plant species

Metal		Mn	Ni	Fe	Cu	Zn	Ca	Mg
		R	r	r	r	r	r	r
P.crispum	root	-0,68	-0,23	-0,19	-0,4	0,65	0,87*	-0,5
	stems	0,92**	0,88*	0,53	-0,58	-0,77*	0,96**	-0,52
	leaf	0,72*	-0,95**	0,92**	0,5	-0,1	-0,97**	-0,41
	fruit	0,35	0,47	-0,19	-0,67	-0,12	0,96	-0,36
D. carota	root	-0,71*	-0,56	-0,68	0,28	-0,19	0,36	0,21
	stems	0,36	-0,62	-0,36	0,4	-0,06	-0,08	0,42
	leaf	-0,68	0,58	-0,19	0,93	-0,32	-0,57	-0,61
	fruit	0,83	0,71*	0,16	-0,19	-0,19	0,67	0,07
C. maculatum	root	-0,94**	-0,88	0,4	0,23	0,7	-0,13	0,19
	stems	0,55	0,42	0,63	0,76*	-0,02	0,51	0,5
	leaf	-0,71*	-0,41	0,48	0,77*	0,41	0,13	-0,41
	fruit	-0,71*	0,28	0,68	-0,41	0,77*	0,67	0,24

r – Pearson- correlation coefficient: 0-0.3: no correlation; 0.3-0.5: poor correlation; 0.5-0.7: medium correlation; 0.7-0.9: high correlation *; 0.9-1.0: a very high statistically significant correlation **.

IV. CONCLUSIONS

Based on the analysis of the content of Mn, Ni, Fe, Cu, Zn, Ca, Mg in soil and plant organisms, the species *Petroselinum crispum* Mill., *Daucus carota* L., *Conium maculatum* L. we can conclude that examinated soils with abundant iron, also tested elements, on the basis of the concentrations in the soil, can be compared to the series: Fe> Ca> Mg> Mn> Zn> Cu> Ni;

The order of metals on the basis of the total quantity in the plant in the species *P. crispum* is Ca> Mg> Fe> Mn> Zn> Ni >Cu and for the species *D. carota*, *C. maculatum* Ca> Mg> Fe> Zn> Mn> Cu> Ni.

There is a significant intraspecies difference in the distribution of the examined elements, since, in *P. crispum* Mg, Fe, Zn, Ni, and Cu is most at the root, Ca in leaves, and Mn in the fruit; in *D. carota* Ca, Mn, Zn, Ni in leaves, of Mg and Cu in the fruit. In *C. maculatum* the root has the highest concentration of Cu and Ni, the leaves have the highest concentration of Ca, Mg, and Mn, and Zn is the most presented metal in fruit. For all types of Fe, it is most at the root.

The content of Mn, Fe, Cu in all analyzed plants is within the framework of toxic concentrations. Although the concentrations of these metals are elevated, the plants are growing on such soil, which tells us that the plants of the Apiaceae are tolerant to increased concentrations of these metals.

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