SIMILARITIES IN THE ALLOCATION OF METALLIC ELEMENTS IN DIETARY PLANTS

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Abstract. The accumulation of metals in different plant parts may show species-specific patterns, possibly influenced by soil properties. The carrot (*Daucus carota*) and zucchini (*Cucurbita pepo var. giromontia*) were studied in five sampling areas with different soils in Hungary. We wanted to know how the different soil types affected the uptake and the allocation of the examined heavy metals within the plants. The accumulation and allocation of Co, Cu, Fe, Mn, Ni and Zn have been assessed within these agricultural plants. We found inconsistency between allocation pattern of the plants and the metal content of the soils. We tried to reveal which among the total heavy metal contents of the soils and the attributes (pH, $CaCO_3$-content, humus-content, granulometric composition) plays the most important role in the heavy metal uptake and those allocation within the plants. We found that the allocation of these elements in the selected dietary plants showed species-specific response. The two studied vegetables have their own allocation pattern, but both accumulate more elements in the leaves, than in the edible parts. We supposed that the examined plants would give different physiological answers in the cases of soils with different attributes. The Cu and Ni showed low variation within the plant, while Co and Zn had particular variation among the samples. The Fe and Mn patterns may be influenced by the different soils. In this study we revealed that the similarities found in the response of the plants bear functional character, thus they are tending to avoid the high accumulation of metals in the reservoirs/propagules regardless their species-specific purposes.

Keywords: carrot, Hungary, metals, soil types, zucchini

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Introduction

The widespread use of agrochemicals enhances agricultural productivity and provides protection against pests, weeds and pathogens (Carvalho, 2006). However, due to adaptation and resistance of pests to agrochemicals the latter must to be used in ever increasing amounts what poses a serious problem as many of agrochemicals end up as environmental pollutants. The high concentration of metals (like Cu, Cd and Hg) in the environment could be the footprint of agrochemical use. There is a positive correlation between the content of metals in the soils and their uptake by plants (Whatmuff, 2002; McBride, 2003). This can be a direct link to the high probability of gastrointestinal diseases in humans (Turkdogan et al., 2002), because most people are exposed to metal contamination through their food. There is an attempt to improve the food safety for agricultural products worldwide (Carvalho, 2006; Farsang et al., 2007) through the control/monitoring of the metal content and uptake in soil-plant systems. Soils normally contain low or moderate concentrations of metals such as nickel (Ni), which is the essential micro-nutrient for optimum growth in higher plants (Brown et al., 1987; Szalai, 1998; Sengar et al., 2008; Zeng et al., 2008; Vandenhove et al., 2009). Although iron (Fe) is the fourth most abundant element in the earth crust, its availability is far below that required for optimal growth (Marschner, 1995). Other metals in suboptimal concentrations in agricultural soils include cobalt (Co; Bakkaus, 2005), copper (Cu; Brun, 1998), zinc (Zn; Brekken and Steinnes, 2004; Sharma et al., 2008), and manganese (Mn; Schubert, 1992; Kitao *et al*., 2001).

Agricultural intensification is targeted at increase of the availability of such elements in the soils, but this carries the risk of differential accumulation in plant parts, creating a potential health hazard in the case of food plants (Yang *et al*., 2009). Previous studies assessed the effects of metal accumulation in plants of some cardinal elements (e.g. Kawada *et al*., 2002; Verma *et al*., 2007; Szabó *et al*., 2008; Szalai, 2008a, b; Yang et al., 2009; Singh and Agrawal, 2010), but these studies concentrate on edible parts only. Only few studies which attempted to investigate the whole plants (including vegetative parts) with their fruits or reservoirs (Finster *et al*., 2004). Kawada *et al*. (2002) have found that the Cu content showed some regional variation regardless of the soil type; they only used the edible taproots. However Verma *et al*. (2007) and Yang *et al*. (2009) found that there was positive relationship between the Cd content of the plant and the soil. Finster *et al*. (2004) found that the edible part of the fruits or the fruiting vegetable plants (zucchini, tomatoes, peppers) avoid to accumulate the metals (e.g. lead) in their edible parts, while the dietary leafy vegetables and edible roots (e.g. carrot, radish, onion) were found to have high levels of hazardous elements.

The above mentioned facts prompted us to investigate the allocation of six elements' (Co, Cu, Zn, Mn, Fe, Ni) in carrot and zucchini from intensively cultivated fields in eastern Hungary. Carrot (*Daucus carota*) and zucchini (*Cucurbita pepo* var. *giromontia*) were selected, because carrot provides 5 % of the yearly vegetable consumption in Hungary, while zucchini is frequently used in (conventional) food production (e.g. baby food) as a pulp material in Hungary (Central Agricultural Office, 2007). During this study, we hypothesized that the plant accumulates metals in their vegetative parts (e.g. leaves, nodes), in order to protect their reproductive parts and reservoirs (fruits and propagules) from higher, damaging concentrations of these elements. This was called the protective accumulation hypothesis. We also wanted to know whether the studied dietary plant species have species-specific response to metal content in the soils, in order to improve our knowledge about the allocation pattern of metallic elements within plants.

These two approaches might have a direct impact on the choice of cultivars/plant species made by producers. The first would help to address food safety issues in management, the second one provides a basis for the proper choice of cultivars/species related to local conditions (e.g. land use features, soils, micro-climatic issues).

Materials and methods

Sampling sites and methods

We sampled private gardens in five different locations in eastern Hungary, used as gardens consistently in the past two decades (Fig. 1). We used the names of the villages as identifiers of the sampling sites: 1.) Pusztafalu is located in North-East Hungary, where the typical soil type is Luvisol; 2.) Tiszavasvári is in the outskirts of the town with Cherozem soil; 3.) Hajdúnánás also is characterized by Chernozem with lime incrustation, but the soils here contain more silt and clay (Table 1); 4.) Debrecen is a suburban zone in the eastern part of the city, where the dominant soil type is Arenosol with a relatively high humus content (Table 1); 5.) Berettyóújfalu sites are characterized by Gleysol. Carrot was sown in March and the full-grown plants were sampled in mid July; while the zucchini was planted in May and sampled in mid July. At every site, six different locations were selected *ad hoc*, and from every location, three carrot (taproot and leaf), three zuc-

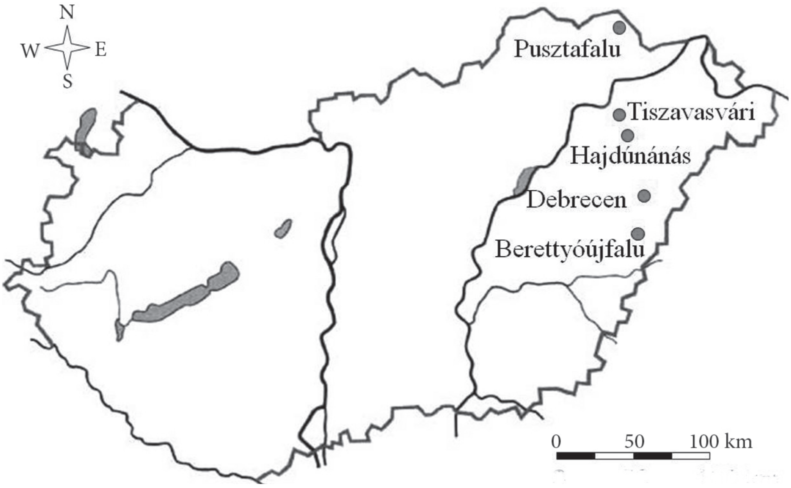

Fig. 1. Map of the sampling sites denoted by full circles in Hungary

Table 1. Characteristics of soil samples in relation to the sampling locations in Eastern Hungary

	Sampling sites				
	Pusztafalu	Tiszavasvári	Hajdúnánás	Debrecen	Berettyóújfalu
	(mean ± SD)				
pH (H2O)	7.09 ± 0.36	7.21 ± 0.27	7.19 ± 0.33	6.67 ± 0.44	7.22 ± 0.07
CaCO3 (%)	5.12 ± 0.40	4.99 ± 1.40	5.32 ± 1.62	4.08 ± 0.78	5.65 ± 0.67
Humus (%)	3.97 ± 1.50	3.11 ± 0.45	4.05 ± 0.74	2.52 ± 0.37	3.02 ± 0.88
	Granulometric composition (%)				
Coarse sand	16.0 ± 4.3	3.8 ± 2.0	4.5 ± 1.4	12.7 ± 2.1	5.6 ± 5.1
Fine sand	27.5 ± 4.2	38.6 ± 7.0	45.3 ± 2.7	76.8 ± 5.4	54.5 ± 6.5
Silt	32.8 ± 4.5	42.3 ± 8.0	39.3 ± 3.7	8.1 ± 5.2	29.8 ± 2.5
Clay	23.7 ± 8.3	15.3 ± 2.9	10.9 ± 3.8	2.4 ± 1.4	10.1 ± 3.2

chini (fruit and leaf) plants were sampled, and were stored in plastic bags in a refrigerator. After taking the plant sample, a corresponding soil sample (20 cm radius around the plant, 15 cm deep, volume, 200 ml in volume, using a soil corer) was collected at every site. Soil samples were put into plastic bags, and transported to the laboratory for analysis.

In the laboratory, the soil samples were homogenized and dried at 80°C and treated by acid digestion (65 % nitric acid and 30 % hydrogen peroxide). The metal content for the soils was determined according to the Hungarian standards (Hungarian Standards Institution, 1989). In order to consider the effect of the soil type on the metal content some major soil parameters were determined: granulometric composition by Köhn-pipette (Hungarian Standards Institution, 1978b), the humus content (after Tyurin's scheme (Hungarian Standards Institution, 1977), the pH (Hungarian Standards Institution, 1978a) and the calcium carbonate content by Scheibler-calcimeter (Ballenegger and Di Gléria, 1962).

The plant samples were cleaned and washed in fresh water without any detergent and rinsed by distilled water, sliced up and then the different plant parts were separated into sub-samples and dried at 80°C; the dried samples were homogenized and treated by acid digestion. We added 2 ml 65 % nitric acid and 2 ml 30 % hydrogen peroxide to 1 g sample then it was being digested on 130°C for one and a half an hour in a Gerhardt SMA 2000 trace metal digestion system. After that, they were filtered with filter paper (pore size 288 μm) and diluted to 30 ml. The metal content for all samples was determined with a Perkin-Elmer 3000 (FAAS) spectrophotometer and every sample was measured in two independent manners.

Data analysis

Principal component analysis (PCA; Mardia *et al*., 1979; Gaunch, 1984; Becker *et al*., 1988; Venables and Ripley, 2002) was chosen to compensate for multicollinearity and study which variables explained the observed treatment patterns best.

In order to study the dependences among the studied elements and soil parameters, Spearman rank correlation (Sokal and Rolf, 1995) was applied for the dataset. We tested the potential interdependences within soils (elements and soil parameters); plants and soil parameters; the elements in the plants and soils.

Generalized linear mixed-effect models based on the Gaussian distribution were used to study the relationship between single metal elements, their allocation in the plant and the location of the samples. In these models, the log-transformed element content (mg kg^{-1}) was the response variable and two explanatory variables were included: 1.) the plant species and its parts (leaves, edible parts) combined into one variable based on PCA results; 2.) sampling sites and sample identity as a random factor to control the differences among the samples and replicates. In order to avoid the heterogeneity in the variance caused by the different sampling intensity, the log-transformed sample number was added to the linear predictor as a known coefficient (1). The differences among the levels of the tested factors were revealed by multiple comparisons (with Tukey computed contrast matrices for several multiple comparison procedures). The allocations of the elements were also checked by Cleveland's dot plots as a graphical interpretation (Cleveland, 1993). The analyses were carried out in R 2.11.1 (R development core team, 2010) using package MASS (Venables and Ripley, 2002) for PCA, package *agricolae* (Mendiburu, 2010) for Spearman rank correlation tests, package *lme4* (Bates and Maechler, 2010) for linear mixed models, package *multcomp* (Hothorn et al., 2008) for multiple comparisons and package *lattice* (Deepayan, 2008) for dot plot panel graphs.

RESULTS

Soil characteristics and metals

The Arenosol from Debrecen was characterized by moderate acidity and high proportion of fine sand fraction, but low humus content (Table 1). The Luvisol from Pusztafalu was moderately rich in clay, silt and humus. The Chernozem from Tiszavasvári and Hajdúnánás was characterized by high amount of silt and humus, while the clay fraction was moderate. The distribution of the studied metals showed particular differences among the soils (Table 2); Ni, Co, Fe and Mn content were higher in the Luvisol (Pusztafalu) and the Chernozem (Tiszavasvári and Hajdúnánás) than in the Arenosol.

Allocation of metals in the plants and soils – global approach

The PCA for sample locations (Fig. 2A) showed that the soil types separated from each other sufficiently; the cumulative variances explained by the first two axes were 0.40 and 0.17 respectively. Pusztafalu was characterized by the Mn and clay, while Debrecen by the sand and fine

Table 2. Distribution of the studied metals in the soil samples according to the sampling locations in Eastern Hungary

Elements mg kg^{-1}		Sampling sites				
		Berettyóújfalu	Debrecen	Hajdúnánás	Pusztafalu	Tiszavasvári
Ni	mean	18.03	17.08	23.9	28.45	30.87
	SD	0.88	4.97	4.31	6.4	3.36
Cu	mean	26.1	24.37	68.9	12.75	15.3
	SD	6.34	16.56	58.12	5.87	2.71
Co	mean	11.75	5.57	12.92	14.4	12.88
	SD	0.63	1.51	0.82	1.82	1.3
Zn	mean	74.57	53.06	125.83	55.46	56.76
	SD	51.35	18.76	86.19	13.68	5.85
Mn	mean	430.12	144.75	468.5	1060	554.25
	SD	109.72	50.82	62.68	262.57	76.36
Fe	mean	12 975	4 387	20 687	15 025	26 237
	SD	1 840	1 568	16 914	2 691	18 314

sand components. Hajdúnánás and Berettyóújfalu were comparable in the Cu and Zn content, while Tiszavasvári was characterized by the sediment components (e.g. silt). The PCA for the element allocations in the plants (Fig. 2B) did not fully correspond to the above patterns. For the combined variables of plant parts and species, the cumulative variances explained by the first two axes were 0.25 and 0.17 respectively. The leaves, regardless of the plant type or sample location, accumulated more Co, Mn, Fe and Ni than other parts; especially in carrot.

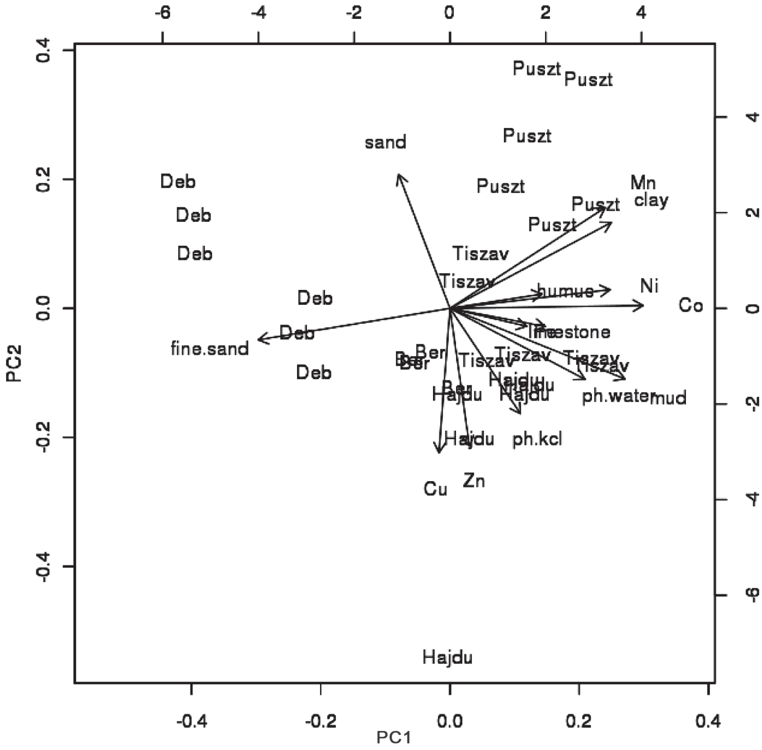

Fig. 2. PCA biplot for soil metal contents/parameters and the sampling locations in Eastern Hungary. Abbreviations denote the sampling sites: Ber – Berettyóújfalu, Deb – Debrecen, Hajdu – Hajdúnánás, Puszt – Pusztafalu, Tiszav – Tiszavasvári

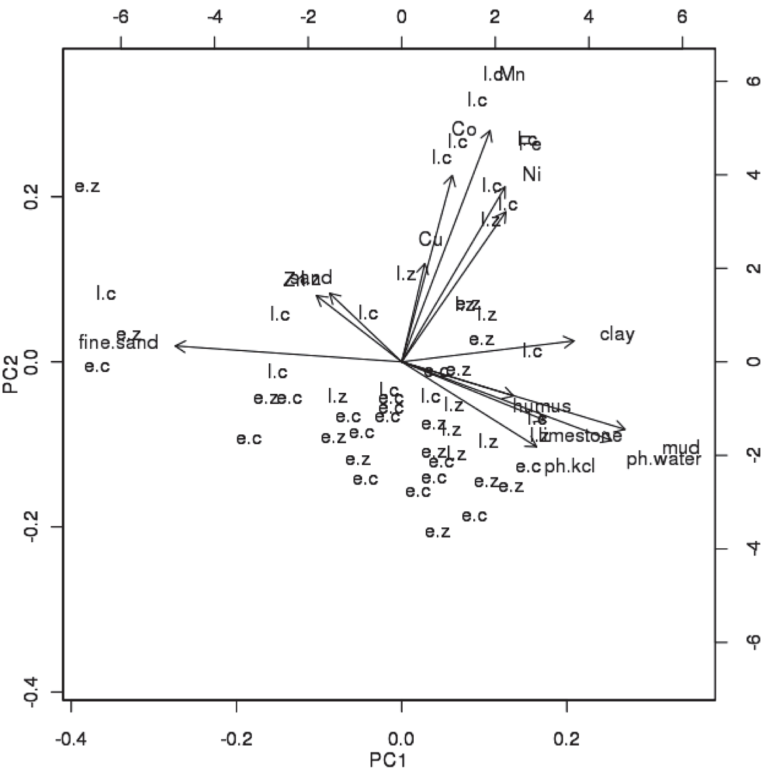

Fig. 3. PCA biplot for metal content in plants with soil parameters in relation to plant parts in Eastern Hungary. Abbreviations denote parts of the studied plants: l.c. – leaves carrot; e.c. – edible carrot; l.z. – leaves zucchini; e.z. – edible zucchini

Correlation patterns of elements

Based on the results of Spearman correlation, we found that the Ni positively correlated with most of other studied elements in the soils. Cu was the least affected by other elements; showing a positive correlation ($R = 0.5$; $p < 0.01$) with the Zn content only. The element content in the soils was positively affected by pH, while their correlation with the portion of fine sand in the soil was negative. Cu was the only exception as it showed a positive correlation ($R = 0.38$; $p < 0.05$) with this component. We found no consistent patterns in the relationship of the element content of the plants and the studied soil parameters. The relationship of the soil-plant element content showed that there was a positive relationship among the Zn content in the plant and the Ni ($R = 0.34$; $p < 0.01$), Co ($R = 0.43$; $p < 0.001$), Zn ($R = 0.28$; $p < 0.05$) and Fe ($R = 0.31$; $p < 0.05$) content in the soils. The Ni ($R = 0.42$; $p < 0.001$) and Mn ($R = 0.37$; $p < 0.01$) content in the plants were positively affected by the Cu content in the soils.

Differences in metal uptake by plant parts

The location of the samples did not have a particular effect on the metal content in plants and their parts, except for Fe, whose amount was higher in the samples from Berettyóújfalu than in the samples from Debrecen (K-theta = -1.116, S.E. = 0.392, $p < 0.01$). The Ni, Cu and Co concentrations were higher in the leaves than in the edible parts (regardless the plant species, Table 3), but carrot leaves accumulated more Ni than those of zucchini; all differences were marginally significant. Zn was more intensively accumulated in the leaves of carrot than in its taproot. The Fe allocation was higher in the leaves than in the edible parts, but the carrot leaves accumulated more iron, than those of zucchini. The edible parts of carrot contained more Fe than those of zucchini. The Mn showed the same pattern like Ni, but the zucchini leaves accumulated significantly more Mn than its edible parts.

Table 3. Distribution of the studied elements in the plants and their parts in Eastern Hungary. The observed mean ± SD indicated the corresponding studied plant and their parts

Elements mg kg^{-1}		Edible, carrot	Edible, zucchini	Leaves, carrot	Leaves, zucchini	Comparison
Ni	mean	4.89	5.8	9.83	8.08	l.c > e.c.*; l.c. > e.z.**; l.c. > l.z.’
	SD	2.03	2.92	6.48	2.96	
Cu	mean	5.51	10.86	12.03	7.82	l.c. > e.c.**; l.c. > l.z.*
	SD	1.89	3.06	17.46	1.38	
Co	mean	2.49	3.04	4.73	4.95	l.c. > e.c.***; l.c. > e.z.**; l.z. > e.z.*
	SD	0.85	0.98	1.77	2.24	
Zn	mean	18.85	59.34	30.51	43.05	l.c > e.c.***
	SD	3.59	42.07	6.9	8.2	
Fe	mean	134.18	78.87	257.2	111.32	e.c > e.z.***; l.c. > e.c*;e.c > l.z*; l.c. > e.z.***; l.c.>l.z.***
	SD	53.92	39.48	183.32	38.51	
Mn	mean	14.18	24.34	65.58	38.64	l.c. > e.c***; l.c. > e.z***; l.z. > e.z.*; l.c. > l.z.***
	SD	4.42	13.38	34.74	18.45	

Legend: l.c. – leaves carrot; e.c. – edible carrot; l.z. – leaves zucchini; e.z. – edible zucchini; significance:*** $p <$ 0.0001; **$p < 0.001$; * $p < 0.01$; . ‘ $p < 0.1$

Discussion

Neither of the two vegetables showed a passive metal absorption pattern, and no characteristic pattern emerged. Both vegetables had their own allocation patterns, but both accumulated more metals in their leaves, than in their edible parts. Cu and Ni showed low variation within the plant, while Co and Zn had particular variation among locations. The Fe and Mn patterns may be biased by the locations of the samples.

Plants have the natural ability to extract ions from the soil and distribute towards the shoots. Within a certain concentration range, some heavy metals are essential for the growth of higher plants (Breckle, 1991). Our results revealed that the studied plants accumulate more metals in their vegetative parts (i.e. leaves) than in their functional reservoirs, such as taproot or fruits; therefore our accumulation hypothesis was supported. Similarly, Page *et al.* (2006) reported that there was strong retention in the roots system of wheat (*Triticum aestivum* L.) and lupin (*Lupinus albus* L.) for Co and Cd. Finster *et al.* (2004) also found similar issues for lead (Pb), but they also indicated that the lead contamination in leaves and the roots for carrot could be originated from the dust adhered to the plant surface as well as from the uptake from the soil. However Kawada *et al.* (2002) noted that the Cu accumulation in carrot does not always depend on the Cu content in the soils; the regional differences should be taken into account. Tahlil *et al.* (1999) also confirmed similar patterns for two zucchini cultivars, namely they accumulate more metals (i.e. Cd, Cu and Zn) in their roots than in their fruits. They also found some differences between the two cultivars: the Cu and Zn showed lower variation among the cultivars than Cd. They also emphasized that the physiological stress caused by metals should promote decline in the dry mass production of the plants. Verma *et al.* (2007) assessed the time component of the Cd accumulation and found that there is a progressive increase during the time (i.e. days), but after 100 days the accumulation rate approaches a steady-state, without any further progressive change in the accumulation.

Previous studies (e.g. Finster *et al.*, 2004; Page *et al.*, 2006; Sharma *et al.*, 2008) stressed that there is particular variation in the accumulation and distribution of metals within the plant species, what might be helpful in considering proper indicator species for risk assessments (Sipter *et al.*, 2008). Through their specific response we estimate the magnitude of the risk of individul pollutant. We hypothesized that the studied dietary plants provide species-specific response to the accumulation of metals. However functionally different plant parts were assessed, but the similarities/dissimilarities in their accumulation should prompt us to reconsider our current opinion about food safety. We found a slight difference in the response of the two dietary plants; thus our specific response hypothesis was partly supported. Tahlil *et al.* (1999) found that the some differences in the accumulation of Zn between squash (*Cucurbita maxima*) and carrot which accumulates more Zn. However Finster *et al.* (2004) proposed that the plants with edible leafy parts (i.e. carrot) accumulated more lead (Pb) than the plants with edible fruits (i.e. zucchini). Sharma *et al.* (2008) reported that the accumulation of Cu, Zn, Cd and Pb in vegetables might have shown some species-specific differences, but the higher probability of contamination can be reflected in the increased level of metals within the plants.

Conclusions

In this study, we revealed that dietary plants provide – at least slight – specific response to the exposure to metals, but more functional similarities were found in their response among the studied species. Namely, both vegetables had their own allocation patterns, and both accumulate more elements in their leafy parts than in their reservoirs (i.e. taproots and fruit). The leaves, regardless of the plant type or the sample location, accumulated more Co, Mn, Fe and Ni than other parts of the plants, especially in the case of the carrot. Thus our results revealed that the plants are tending to avoid the contamination of the functional reservoirs regardless of their species-specific purposes. This fact prompts us to stress the significance of the choice of cultivars/varieties for food production. If this trend can be generalized, the metal-contaminated areas could be risky for vegetables whose leaves are consumed. This study verifies those special literature sources which state that heavy metal uptakes within the carrot (*Daucus carota*) and zucchini (*Cucurbita pepo* var. *giromontia*) were not considerably affected by the soil types and the total heavy metal contents in the soils. The granulometric composition of soils had the most significant influence but this factor affects the uptake only of the half of the examined heavy metals (Ni, Mn, and Zn).

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SIMILARITIES IN THE ALLOCATION OF METALLIC ELEMENTS IN DIETARY PLANTS

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Abstract. The accumulation of metals in different plant parts may show species-specific patterns, possibly influenced by soil properties. The carrot (*Daucus carota*) and zucchini (*Cucurbita pepo var. giromontia*) were studied in five sampling areas with different soils in Hungary. We wanted to know how the different soil types affected the uptake and the allocation of the examined heavy metals within the plants. The accumulation and allocation of Co, Cu, Fe, Mn, Ni and Zn have been assessed within these agricultural plants. We found inconsistency between allocation pattern of the plants and the metal content of the soils. We tried to reveal which among the total heavy metal contents of the soils and the attributes (pH, $CaCO_3$-content, humus-content, granulometric composition) plays the most important role in the heavy metal uptake and those allocation within the plants. We found that the allocation of these elements in the selected dietary plants showed species-specific response. The two studied vegetables have their own allocation pattern, but both accumulate more elements in the leaves, than in the edible parts. We supposed that the examined plants would give different physiological answers in the cases of soils with different attributes. The Cu and Ni showed low variation within the plant, while Co and Zn had particular variation among the samples. The Fe and Mn patterns may be influenced by the different soils. In this study we revealed that the similarities found in the response of the plants bear functional character, thus they are tending to avoid the high accumulation of metals in the reservoirs/propagules regardless their species-specific purposes.

Keywords: carrot, Hungary, metals, soil types, zucchini.