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Zinc and Copper Accumulation and Distribution in the Tissues of Nine Crops: Implications for Phytoremediation

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Abstract

Field experiments were carried out near Kraków (Poland), between 1999 and 2001, with nine crops (red beet, field pumpkin, chicory, common bean, barley, white cabbage, maize, alfalfa, common parsnip) to determine zinc and copper accumulation and their distribution in the plants' tissues. Based on the results, species suitable for phytoremediation were selected. Field pumpkin and red beet were characterized by the highest zinc accumulation, especially in the leaves (119.14 and 64.97 mg·kg^{-1} d. w. respectively). In the case of alfalfa, field pumpkin, and barley the maximum Cu content was found in the roots (35.81, 25.74, 15.92 mg·kg^{-1} d. w. respectively). The field pumpkin, chicory, and red beet were characterized by the highest zinc concentration ratios (shoots/roots): 2.8, 2.2, 2.0. Concentration ratios for copper were within the range: 0.1 (barley) – 1.4 (common parsnip).

Keywords: metals, phytoextraction, vegetables, barley, alfalfa

Introduction

Plant-based remediation techniques appear to hold a great deal of promise. Their most basic uses may be to provide cost-effective and environmentally sound mechanisms for remediation of heavy metals in contaminated soils [1]. Advances of phytoremediation are likely to result from more efficient plant variety selection and soil amendments as well as optimizing agronomic practices used for plant cultivation [2]. Studies on heavy metal-plant interactions should improve the understanding of the mechanisms of ion uptake, accumulation and resistance of plants to excess of toxic elements in tissues [3].

Metals such as Pb and Cd are often cited as primary contaminants in the environment, but zinc and copper are also problematic in some sites. Ebbs and Kochian [4] investigated the toxicity of Zn and Cu in three species from *Brassica* (*B. juncea, B. rapa,* and *B. napus*) genus. In terms of heavy metal removal, *Brassica spp*. were more effective at removing zinc from nutrient solution than Cu. The extent of Zn and Cu removal was reduced when both metals were present, as compared to the single heavy metal treatments. In further investigations Ebbs et al. [5] found that *Brassica spp*. are the most effective in removing Zn from the contaminated soils, due to the high biomass production in comparison to *Thlaspi caerulescens* – a zinc hyperaccumulator. The results suggest that for phytoremediation to be successful, a strategy should be considered that combines rapid screening of plant species possessing the ability to tolerate and accumulate heavy metals with agronomic practices that enhance shoot biomass production and/or increase metal bioavailability in the rhizosphere [5].

The extremes of physiological responses of plants to heavy metals are accumulators and excluders. Excluders contain large amounts of metal in their roots and prevent

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metal from entering their aerial parts over a broad range of metal concentration in the soil. They are characterized by a concentrations ratio (shoots/roots) <1. Hyperaccumulators concentrate metals in above-ground tissues in levels exceeding those in the soil (concentration ratio > 1) [6].

Based on the cited results, the possibility of using common crops (red beet, field pumpkin, chicory, common bean, barley, white cabbage, maize, alfalfa, common parsnip) to remove heavy metals from the soil was investigated. In the previous publication it was shown that the most effective species in Cd, Mn, Cu, Ni, Pb, and Zn extraction were field pumpkin, Cr – maize, and Fe – alfalfa [7]. The phytoremediation efficiency of the investigated crops depended both on biomass production and the ability of metal accumulation in above-ground tissues. In subsequent publications we demonstrated the Pb, Cd, Mn, and Ni accumulation and distribution in the tissues of the mentioned crops [8, 9].

The aim of the present investigation was to determine zinc and copper accumulation and distribution in the tissues of nine crops. The obtained results will allow the selection of species for further investigations in order to determine agronomy-based optimizations as a tool for metal phytoextraction improvement.

Experimental Procedures

A field experiment was conducted at the Agricultural Academy Research Station near Kraków (Poland), between 1999 and 2001, on soil classified as *Eutric Cambisols*, with loess as the basement complex, pH_{KCl} 4.8, 1.2% organic carbon. The soil contained 43.0 $mg \cdot kg^{-1}$ d. w. of exchangeable (187.4 $mg \cdot kg^{-1}$ d. w. of total) Zn and 7.4 $mg \cdot kg^{-1}$ d. w. of exchangeable (12.5 $mg \cdot kg^{-1}$ d. w. of total) Cu. To determine exchangeable Zn and Cu content, soil samples (10 g) were treated with 100 cm^3 0.01 M $CaCl_2$ and shaken for 2 hours. After filtration of the solids, Zn and Cu were obtained by AAS method, using Varian-SpectrAA 20 and air/acetylene flame under standard operating conditions. Total Zn and Cu content was determined by AAS method, after wet-mineralization of soil samples (1.000 g) in an MDS 2000 CEM microwave oven, in the presence of 30% HNO_3. The pH was measured using a potentiometric method in 1 mol dm^{-3} KCl solution. The organic carbon content was determined using Tiurin's method, based on the oxidizing of C to CO_2, using potassium dichromate as an oxidant [10].

The objects of investigation were nine crops selected according to their different taxonomy position and information cited in literature about their remediation efficiency: red beet (*Beta vulgaris var. cicla* L.) – 'Wodan F_1', field pumpkin (*Cucurbita pepo* L. *convar. giromontiana* Greb.) – 'Astra F_1', chicory (*Cichorium intybus var. foliosum* Hegi) – 'Rubello F_1', common bean (*Phaseolus vulgaris* L.) – 'Tara', barley (*Hordeum vulgare* L.) – 'Stat', white cabbage (*Brassica oleracea var. capitata* L.) – 'Krautman F_1', maize (*Zea mays* L. *convar. saccharata* Koern.) – 'Trophy F_1', alfalfa (*Medicago sativa* L.) – 'Vela', and common parsnip (*Pastinaca sativa* L.) – 'Półdługi Biały'.

Crops were grown under standard agronomic conditions on experimental plots (plot area – 9 m^2) in four replications, using the random blocks method in 1999-01. Crops were harvested at the stage of harvest maturity. The fresh weight and Zn and Cu content were determined in the morphological tissues of the investigated crops: red beet – roots and leaves; chicory – roots, rosette leaves, and head; field pumpkin – roots, stem, leaves, and fruits; common bean – roots, stem, leaves, and pods; barley – roots, straw, and grain; white cabbage – roots, stem, rosette leaves, and head; maize – roots, stem, leaves, husks, shank, and grain; alfalfa – roots and shoots; and common parsnip – roots and leaves. Plant samples were rinsed in demineralized water, dried at 105°C, and ground using a colloidal grinder (Retsch). Analyses of Zn and Cu content were carried out using the AAS method, following prior dry-mineralization of 5.000 g plant samples at 500°C, in the presence of 20% HNO_3 [10].

The average concentrations of heavy metals in aerial parts of investigated plants were calculated according to the formula: mean metal conc. = sum of (metal conc. in each plant part x fraction; each part contributes to total shoot biomass). Averages were used to calculate the concentration ratios for Cu and Zn (Table 2).

The results were statistically evaluated using analyses of variance, significant differences between means were calculated by the Student t test at $p = 0.05$. Differences between years of the experiment were not significant; results were presented as averages for three years.

Coefficients of simple correlation were calculated between the amount of metals in particular plants tissues (N=12) at $p = 0.001$.

Results and Discussion

Red Beet

In the case of red beet, most Zn was accumulated in the leaves (64.97 $mg \cdot kg^{-1}$ d. w.), whereas the amount of this metal in the roots was twice lower (32.68 $mg \cdot kg^{-1}$ d. w.) (Fig. 1). There were no statistical differences in the level of copper in the tissues of the presented species – the distribution of this element was proportional between tissues (Fig. 2). A significant correlation coefficient was found between the amount of copper in the roots and leaves of red beet (r = 0.860) (Table 1). In the conditions of a pot experiment, Gambuś [11] compared the capacity of heavy metals uptake in 7 species of vegetables. He found that red beet accumulated two times more Cu in the roots in comparison to the leaves, but the distribution of Zn was different: more of this metal was accumulated in the leaves. In the present experiment shoots/roots concentration ratio for Zn was 2.0 (Table 2).

Table 1. Coefficients of simple correlation (r) between the amount of metals in mentioned plants tissues (* – r significant at p = 0.001, N = 12).

	Copper	Zinc
	Red beet	
Roots – leaves	0.860*	0.614
	Field pumpkin	
Roots – leaves	-0.334	-0.088
Roots – stem	-0.431	0.558
Roots – fruits	-0.203	-0.187
Leaves – stem	0.558	-0.705
Leaves – fruits	-0.055	0.908*
Stem – fruits	0.166	-0.799*
	Chicory	
Roots – rosette	-0.015	0.556
Roots – head	-0.492	0.701
Rosette – head	0.122	0.980*
	Common bean	
Roots – leaves	0.501	-0.725
Roots – stem	0.532	0.977*
Roots – pods	0.187	0.796*
Leaves – stem	-0.050	-0.689
Leaves – pods	-0.237	-0.644
Stem – pods	0.324	0.877*
	Barley	
Roots – straw	0.701	0.875*
Roots – grain	-0.186	0.828*
Straw – grain	0.237	0.733
	White cabbage	
Roots – stem	0.850*	0.773
Roots – rosette	0.108	0.854*
Roots – head	0.947*	-0.248
Rosette – stem	-0.035	0.865*
Rosette – head	-0.015	0.081
Stem – head	0.846*	0.110

	Copper	Zinc
	Maize	
Roots – stem	-0.100	0.266
Roots – leaves	0.444	0.752
Roots – husks	0.089	0.462
Roots – shank	0.574	-0.253
Roots – grain	-0.353	0.274
Leaves – stem	0.236	0.654
Leaves – husks	-0.038	0.236
Leaves – shank	-0.410	-0.061
Leaves – grain	0.461	-0.320
Stem – husks	0.359	0.445
Stem – shank	-0.152	-0.319
Stem – grain	0.305	-0.256
Husks – shank	0.079	-0.046
Husks – grain	0.015	0.157
Shank – grain	-0.764	0.397
	Alfalfa	
Roots – shoots	0.024	0.017
	Common parsnip	
Roots – leaves	0.061	-0.593

Field Pumpkin

The distribution of the investigated metals within the field pumpkin was as follows: the roots contained the most of copper (25.74 mg·kg^{-1} d. w.). The stem, leaves, and fruits contained about two times less of this element (average 11.05 mg·kg^{-1} d. w.). The leaves were the most contaminated with Zn (119.14 mg·kg^{-1} d. w.), fruits contained about two times, roots and stem – three times less of the mentioned metal (Fig. 1, 2). The level of Zn in fruits was proportional to leaves (r = 0.908) and stems (r = -0.799) (Table 1) and the concentration ratio (shoots/roots) for this element was the highest among the investigated species (Table 2). The research suggests the unique status of *Cucurbita pepo* L. in its ability to accumulate the pollutants in its roots and to transport them to aerial tissues [12]. The results of the first part of our studies involving nine mentioned crops, presented previously [7], showed that field pumpkin is the most effective in Cd, Mn, Cu, Ni, Pb, and Zn remediation [7]. The main factor affecting remediation efficiency was the high fresh weight of harvestable parts of this species.

Chicory

The roots of chicory contained the lowest amount of both Zn and Cu (21.19 and 7.20 mg·kg^{-1} d. w. respectively). The leaves of rosette contained more Zn than the head (Fig. 1). In the case of Cu, the differences between leaves of rosette and those formed in the head were not significant (Fig. 2). Chicory was characterized by the intensive accumulation of Zn in above-ground tissues (shoots/roots concentration ratio 2.2). In the opposite, Cu was distributed similarly between tissues (shoots/roots concentration ratio 1.2) (Table 2). A significant correlation coefficient was found between the level of Zn in rosette leaves and the head of chicory (r = 0.980) (Table 1). Liao et al. [13] examined the uptake and distribution of copper in chicory plants grown in a Nutrient Film Technique System. They found that a large proportion of total Cu uptake was retained by roots. Copper retention by roots limited Cu translocation to xylem and shoots.

Common Bean

Sanchez et al. [14] reported a soilless culture experiment to examine heavy metal accumulation in *Phaseolus vulgaris* and its impact on growth and yield. At harvesting, roots contained the greatest amounts of all investigated metals (i. e. Cu and Zn). In the present experiment within the common bean the level of Zn declined in the order: stem> pods> leaves> roots, and the level of Cu: leaves> stem> pods> roots (Figs. 1, 2). Both metals were characterized by similar concentration ratios within the common bean (1.2 for Zn and 1.3 for Cu) (Table 2). The level of zinc in the roots of common bean was

Table 2. Zinc and copper accumulation in roots and shoots of the investigated crops, averages for 1999-01 (mg·kg^{-1} d. w.).

	Red beet	Field pumpkin	Chicory	Common bean	Barley	White cabbage	Maize	Alfalfa	Common parsnip
	Zinc								
Roots	32.68	30.18	21.19	31.08	40.82	31.35	35.88	36.13	22.77
Shoots	64.97	86.03	46.96	39.09	26.83	19.84	18.31	44.15	29.22
Concentration ratios (shoots/roots)	2.0	2.8	2.2	1.2	0.6	0.6	0.5	1.2	1.3
$LSD_{0.05}$ for species	10.163								
$LSD_{0.05}$ for tissues	4.791								
$LSD_{0.05}$ for interaction	14.373								
	Copper								
Roots	9.84	25.74	7.20	6.55	15.92	6.54	9.74	35.81	6.30
Shoots	10.29	11.08	8.65	8.54	2.44	4.54	3.98	12.11	8.58
Concentration ratios (shoots/roots)	1.0	0.4	1.2	1.3	0.1	0.7	0.4	0.3	1.4
$LSD_{0.05}$ for species	2.319								
$LSD_{0.05}$ for tissues	1.093								
$LSD_{0.05}$ for interaction	3.280								

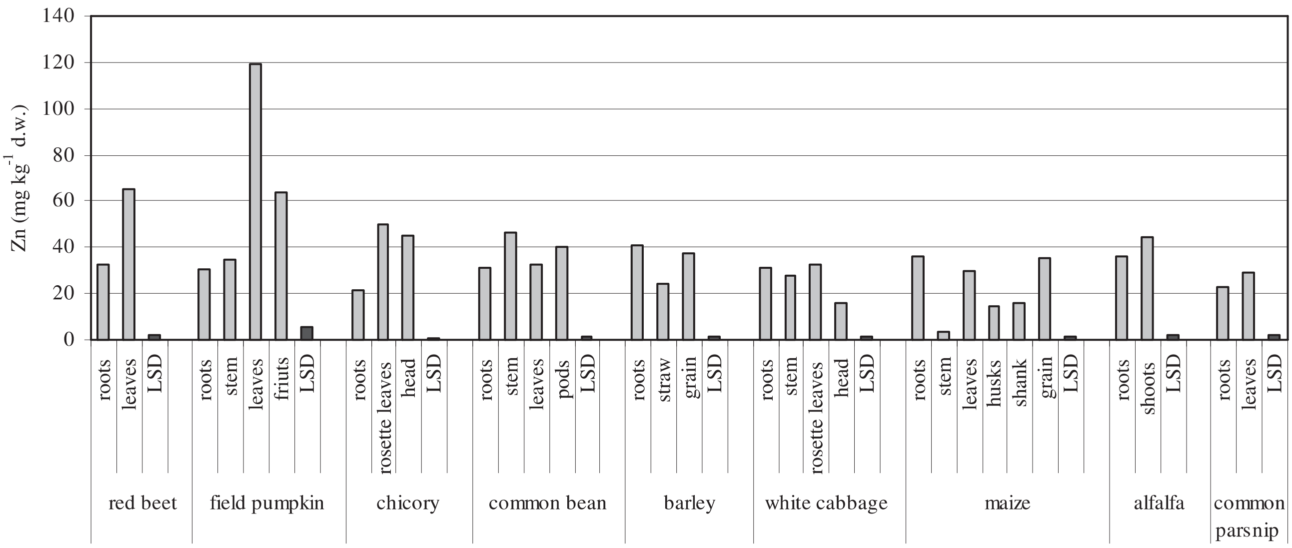

Fig. 1. Zinc distribution among the tissues of the investigated crops, average for 1999-01 (mg·kg^{-1} d. w.).

proportional to the stem (r = 0.977), and pods (r = 0.796) (Table 1).

Barley

The roots of barley contained 6.5 times more copper in comparison to the above-ground tissues (Fig. 2). The distribution of zinc was similar, but differences between tissues were smaller (24.42-40.82 mg·kg^{-1} d. w.) (Fig. 1). The level of zinc in roots was proportional to straw (r = 0.875), and grain (r = 0.828) (Table 1). Tlustos et al. [15] investigated the uptake of Cd, Zn, As, and Pb by chosen crops. They found a similar level of Zn in the straw and grain of barley (30.40 and 31.60 mg·kg^{-1} d. w., respectively), when total Zn content in the soil was about 154.0 mg·kg^{-1} d. w. A hydroponic screening of 22 grass species reported by Ebbs and Kochian [16] indicated that barley tolerated the high Zn concentrations present in the solution and also accumulated elevated concentrations of these metals in the shoot. The results of the cited authors show that barley has a phytoremediation potential equal to, if not greater than, that for *Brassica juncea* – a species commonly used in phytoremediation techniques. The present results do not support this thesis, because of very low concentration ratios of Cu and Zn in barley (0.1 and 0.6 respectively), resulting from low transport of both metals from roots to harvestable tissues (Table 2).

White Cabbage

The roots, stem and rosette leaves of white cabbage contained the highest amounts of zinc (31.35, 28.06, and 32.90 mg·kg^{-1} d. w., respectively). In the leaves of the head about two times less of this element was found (Fig. 1). The stem of white cabbage was characterized by the highest amount of Cu (7.68 mg·kg^{-1} d. w.). There were no statistical differences in the level of this element in the leaves of the rosette and head (average 4.53 mg·kg^{-1} d. w.) (Fig. 2). Significant correlations were found between the level of Cu in roots and head (r = 0.947), roots and stem (r = 0.850), stem and head (r = 0.846). The level of zinc in leaves of rosette was proportional to roots (r = 0.854) and stem (r = 0.865) (Table 1). Xian [17] investigated the effects of concentration and chemical forms of Cd, Zn, and Pb in soils on their uptake by cabbage plants. In the case of Zn he found the highest concentration of this element in the cabbage roots in comparison to shoots.

Maize

Antonkiewicz and Jasiewicz [18] analysed the influence of heavy metal pollution of soil, namely with Cd, Pb, Ni, Cu and Zn upon both the yield and the content of such elements in the studied plants. They found that maize has a possibility of heavy metals accumulation (i. e. Cu and Zn) in roots, for the highest tolerable level of heavy metal contamination of soil. Tissues most contaminated with Zn in the present work were roots (35.88 mg·kg^{-1} d. w.), grain (35.55 mg·kg^{-1} d. w.), and leaves (29.84 mg·kg^{-1} d. w.), in the case of Cu: roots (9.74 mg·kg^{-1} d. w.) and leaves (9.47 mg·kg^{-1} d. w.) (Figs. 1, 2). The stem of maize contained the lowest amounts of Zn and Cu. The concentration ratios were 0.4 for Cu and 0.5 for Zn (Table 2). Wenger et al. [19] examined Zn extraction potential of two common crop plants: *Nicotiana tabacum* and *Zea mays*. The cited authors found that only small amounts of Zn were translocated into the seeds of *N. tabacum* and cobs of *Z. mays*, despite the level of zinc in the soil. According to Gorlach [20], maize is not sensitive to the high level of heavy metals in the soil. In the conditions of pot experiments he found that, regardless of pH level, maize accumulated about

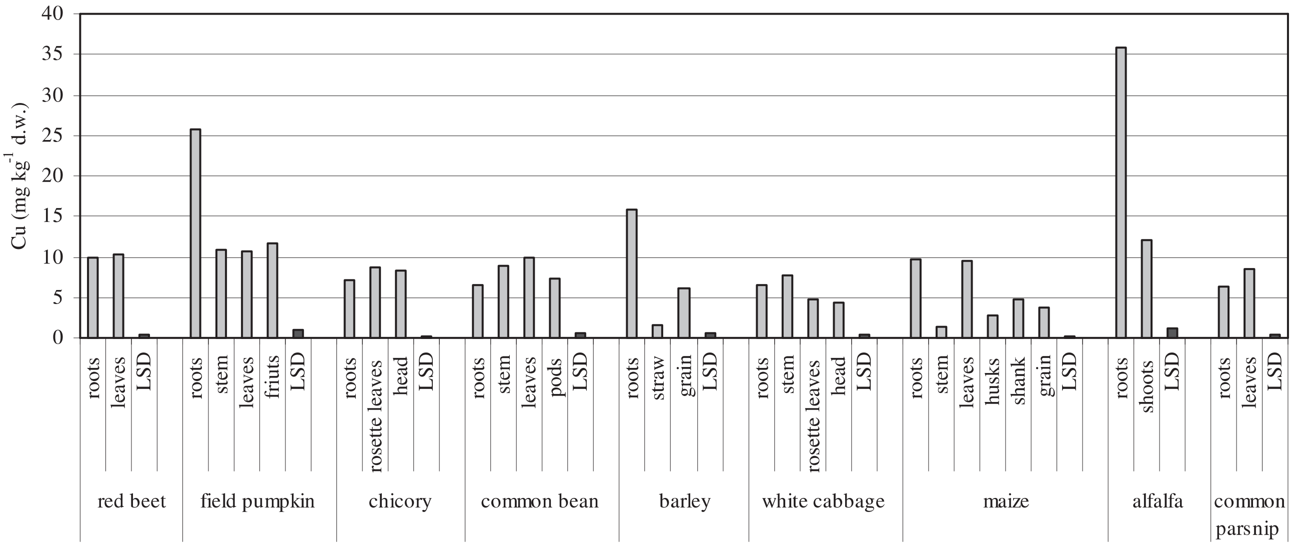

Fig. 2. Copper distribution among the tissues of the investigated crops, average for 1999-01 (mg·kg^{-1} d. w.).

two times more Zn in the roots in comparison to the above-ground tissues.

Alfalfa

It has been reported that alfalfa has the ability to accumulate concentrations of heavy metals well above the tolerance levels of other plants, which may be due to the presence of specialized chemical functional groups responsible for metal tolerance and accumulation. Based on this information, Gardea-Torresdey et al. [21, 22] performed column experiments to study the binding of Cd, Cr, Pb, and Zn to silica-immobilized African alfalfa shoots under flow conditions. These experiments showed that shoots of alfalfa were effective in removing metal ions from solution (an over 90% of the bound Zn). In the field conditions of the present experiment the alfalfa did not exceed the remaining species in Zn accumulation, but the level of Cu in its roots was highest among the investigated crops (Figs. 1, 2). The alfalfa contained 3 times more Cu in roots than in shoots. In the case of Zn, shoots contained only 1.2 times more of this element (Table 2).

Common Parsnip

Common parsnip accumulated more Cu and Zn in the leaves (8.58 and 29.22 mg·kg^{-1} d. w. respectively) (Figs. 1, 2). The concentration ratios (1.3 for Zn and 1.4 for Cu) confirmed the intensive transport of both metals from root to shoots of the common parsnip (Table 2).

As we showed, the use of the investigated species as Cu and Zn remediants should depend on the type of contamination and the possibility to accumulate metals on roots and transport to aerial tissues. According to Ebbs et al. [5], a greater shoot biomass of crops can more than compensate for lower shoot metal concentration in phytoextraction techniques. As we showed previously [7], among nine investigated species only barley produced biomass lower than that recommended for remediants (20 t·ha^{-1}). The most effective in copper and zinc remediation was field pumpkin, because of high biomass production. In conditions of the present experiment, it was possible to remove about 14.1 mg Cu and 136.3 mg Zn from 1 sq meter during 1 year of field pumpkin growth. A positive correlation between the amount of Zn and Cu removed from the soil and the fresh matter of field pumpkin and maize was found [7]. The cited and reported results show that the efficiency of cleaning soils polluted with Cu and Zn depends both on biomass production and possibilities of investigated species for metal accumulation in aerial tissues. In the case of crops that show a positive correlation between biomass production and remediation efficiency, it is advisable to use agrotechnical methods to increase the yield.

Conclusions

1. The use of the investigated species as remediants depends on the type of contamination and differences in metals accumulation and transport from roots to aerial tissues. The field pumpkin, chicory, and red beet were characterized by the highest zinc concentration ratios (shoots/roots): 2.8, 2.2, 2.0. Concentration ratios for copper were within the range: 0.1 (barley) – 1.4 (common parsnip).
2. The field pumpkin and red beet were characterized by the highest Zn accumulation, especially in the leaves (119.14 and 64.97 mg·kg^{-1} d. w., respectively). Within the alfalfa, field pumpkin, and barley the maximum Cu content was found in the roots (35.81, 25.74, 15.92 mg·kg^{-1} d. w., respectively).

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