EFFECT OF CADMIUM STRESS ON THE UPTAKE AND DISTRIBUTION OF MICROELEMENTS COPPER AND ZINC IN PLANT PARTS OF BARLEY (*HORDEUM SATIVUM* L.)*

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Effect of Cd-stress caused by Cd^{2+} (1.10^{-6} mol.L^{-1}) in 32 days old barley plants cultivated hydroponically in special boxes under the same conditions as control plants were investigated for their microelement copper and zinc uptake from nutrient medium and distribution in roots, shoots and leaf blades. Cd-stress caused the increase of Cu by 27.9% in the whole plant and Zn content was nearly the same (increase by 2.1%) as compared with the control. Cd-stress caused the increase of Cu content in roots (by 48.2%) and leaf blades (44.8%) and the decrease in shoots (by 32.9%). Zn content increased under Cd-stress only in leaf blades (by 31.1%), meanwhile in roots and shoots decreased (by 6.1% and 4.5%, respectively). Cd-stress enhanced copper and zinc contents in leaf blades, meanwhile in shoots their contents decreased in comparison with the control. Differences were found in roots – an increase of Cu content from 15.6 mg.kg^{-1} DM to 23.4 mg.kg^{-1} DM and a decrease of Zn content from 109.6 mg.kg^{-1} DM to 102.9 mg.kg^{-1} DM in comparison with control.

barley; stress; cadmium; copper; zinc; uptake; distribution; roots; shoots; leaf blades

INTRODUCTION

In recent years the potential mechanism by which Zn antagonizes Cd toxicity has been studied (Aravind, Prasad, 2003). Accumulation and translocation of the environmental pollutants as cadmium, lead, mercury, chromium and micronutrients as copper and zinc is evaluated in different portions of the plants (Herrero et al., 2003). Although roots comprise usually only a little portion of whole plant biomass, they consistently contain 70–100% of the whole plant metal burdens (Windham et al., 2003). Wolterbeck, van der Meer (2002) confirm that the major part of an accumulated metal is retained in the plant roots. As Krauss et al. (2002) showed, the Freundlich-type function is suitable to predict Cd (grain = 0.71, leaf: $r = 0.86$, the log-transformed data), Zn concentrations (grain: 0.69, leaf: 0.68) and poorer for Cu (grain: $r = 0.44$) in wheat plants. As Green et al. (2003) confirm, wheat grown on cadmium uncontaminated soils can still potentially translocate unacceptable levels of Cd to grain, but even at high Cd^{2+} activities, Zn is effective in regulating Cd uptake and translocation in wheat. The uptake of heavy metals is dependent on pH of nutrient solution and soil matrix with higher values at lesser pH (4.5–7.1), as it was shown by Peralta-Videa et al. (2002). The maximum relative uptakes were found to be 26 times for nickel, 23 times for cadmium, 12 times for zinc, and 6 times for copper. As Kim et al. (2003) showed in *Polygonum thubergii*, soil lead, copper and zinc are correlated with each metal's accumulation in the plants (Pb: $r = 0.841$, $P < 0.005$; Cu: $r = 0.874$, $P < 0.001$; Zn: $r = 0.770$, $P < 0.005$). They found that lead content in roots and leaf blades was highly correlated ($r = 0.5529$, $P < 0.001$), as was lead content in roots and shoots ($r = 0.5425$, $P < 0.001$) with bioaccumulation coefficients 2.0 (Cd), 3.2 (Pb), 17.2 (Cu) and 13.1 (Zn) for whole plants. As Simmons et al. (2003) demonstrated, rice stalks, leaf blades and grains accumulate comparatively higher Cd than Zn and Fe in contrast to soybean. As it was shown by Vysloužilová et al. (2003) in pot experiments with seven clones of *Salix* spp., the As, Cd, Pb and Zn accumulation was significantly different among willow clones. The concentration of chromium, cadmium, copper, nickel, lead, strontium and zinc in garden pea shoots varied between pea genotypes (Belimov et al., 2003). Cajuste et al. (2002) found higher Cd and Ni concentrations in wheat seeds in comparison with leaf blades suggesting the absence of a physiological barrier in the transfer of metal from plant vegetative tissue to storage organ.

The present study is aimed at obtaining an insight into accumulation and distribution of Cu and Zn micronutrients in barley roots, shoots and leaf blades under control and Cd-stress plant conditions.

MATERIAL AND METHODS

Cultivation of spring barley

Experiment was carried out under controlled conditions of a climate-controlled room at the Department of

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Plant Botany and Physiology of the Czech University of Agriculture in Prague. Spring barley variety Kompakt was selected as the experimental material. Caryopses germinated in redistilled water at laboratory temperature (25 °C) during 4 days. Then the seedlings were replanted into special boxes with nutrients medium. Two variants of the cultivation experiment were carried out. The control variant without added cadmium and the experimental variant with the addition of cadmium in the form of $CdCl_2.2H_2O$ into nutrient medium in concentration 1.10^{-6} $mol.L^{-1}$. Each variant was cultivated in 8 boxes. Number of cultivated plants varied from approx. 100 to 125 according to dimensions of the used boxes. Plants were cultivated in the conditioning plastic boxes for 28 days and nutrient medium was exchanged one time per week. Young plants were grown at stable light fluency rate (300 μmol photon.$m^{-2}.s^{-1}$) with day period 16 hours, at temperature 22 °C and air humidity 60–80%. Nutrient media for the both variants differed only in cadmium. Basic nutrients were supplied in the form of Knop's nutrient solution diluted into half concentration (Table 1), which is useful for spring barley, and microelements were supplied in the form of Shive's solution diluted into half concentration (Table 1). After cultivation, the plants were separated into roots, leaf blades and shoots and plant material was subsequently freeze-dried using a Lyovac GT 2 (Leybold-Heraeus, Germany) freeze-drier.

Determination of Cd, Cu and Zn content by a method of atomic absorption spectrometry (AAS). Freeze-dried samples (250–300 mg of leaf blades, 150–250 mg of shoots and 50–100 mg of roots) were decomposed according to the standard operational procedure described by M a d e r et al. (1998). They were charred on a hot plate in temperature range 350–500 °C and then ashed in a muffle furnace at 350–500 °C. Non-decomposed organic residues were oxidised with conc. HNO_3 and decomposed at 500 °C. Obtained white ash was dissolved in 1.5% HNO_3, the dissolving was accelerated by sonication. Cadmium concentration in the digests of the control samples was measured using AAS with electrothermal atomisation (ET-AAS) with standard deviation less than 5% and detection limit 0.028 mg $Cd.kg^{-1}$ dried material. Cd concentration in the digests prepared from plant samples cultivated in presence of cadmium salt and Cu and Zn contents in all samples were determined in the acetylene-air flame (FAAS) using a Varian SpectrAA 110 spectrometer (standard deviation of measurement less than 1%, detection limits were < 1.8 mg $Cu.kg^{-1}$ dried material, < 1.2 mg $Zn.kg^{-1}$ dried material and < 2 mg $Cd.kg^{-1}$ dried material). Determination of Cd concentration in the control samples was carried out in argon atmosphere in a pyrolytic graphite tube with platform using a spectrometer Varian SpectrAA 400 with graphite atomiser GTA-96 with compensation of non-selective absorption using deuterium corrector. Temperature of pyrolysis was 450 °C and that of atomisation 1900 °C. Injected volume was 20 μL. Analyte concentration was measured in the digests as well as in the calibration solutions in two replicates, and results were evaluated from calibration curve constructed by successive dilution of standard calibration ASTASOL solution with 1.5% HNO_3. All plant samples were analysed in three parallel determinations and the quality of the results obtained was assessed by parallel analysis of standard reference material CRM 12-02-03 (Lucerne) and internal reference material with known Cu, Zn and Cd contents BIOMA 3 (*Chlorella*) with the results given in Table 2. Parameters of measuring of all elements by both techniques are given in Table 3.

Statistic evaluation. The results (mean values from three parallel determinations) were statistically evaluated with Statgraphics programme by the analysis of variance with multiple grouping. More detail evaluation was performed by Scheffé's test.

Table 1. Composition of nutrient media-basic Knop's solution and modified Shive's solution with microelements used for barley cultivation

Knop's solution	
$Ca(NO_3)_2$ anhydr.	0.572 $g.L^{-1}$
KNO_3	0.143 $g.L^{-1}$
KCl	0.071 $g.L^{-1}$
KH_2PO_4	0.132 $g.L^{-1}$
$MgSO_4$ anhydr.	0.143 $g.L^{-1}$
$FeCl_3.H_2O$	1 drop of 5% solution to 1 L of nutrient medium
Shive's solution	
H_3BO_3	1.43 $mg.L^{-1}$
$MnCl_2.H_2O$	0.90 $mg.L^{-1}$
$ZnSO_4.7H_2O$	1.00 $mg.L^{-1}$
$CuSO_4.5H_2O$	0.08 $mg.L^{-1}$

RESULTS AND DISCUSSION

The Cd-stress caused an increase by 27.9% Cu and 2.1% Zn in the whole plants. On the other hand different changes of Cu and Zn contents were found in individual plant parts. In the leaf blades an increase of average Cu content from 4.79 $mg.kg^{-1}$ DM to 6.94 $mg.kg^{-1}$ DM (Table 3) and from 44.2 $mg.kg^{-1}$ DM to 58.0 $mg.kg^{-1}$ DM of average Zn content (Table 4) was found. This suggests their increased transport from roots to leaf blades. An increase of Cu content was found also in roots – from 15.8 $mg.kg^{-1}$ DM to 23.4 $mg.kg^{-1}$ DM. On the contrary, a decrease of average Cu content in shoots was found – from 6.89 $mg.kg^{-1}$ DM 4.62 $mg.kg^{-1}$ DM as well as of average Zn content in shoots from 56.2 $mg.kg^{-1}$ DM to 53.7 $mg.kg^{-1}$ DM and roots (from 110 $mg.kg^{-1}$ DM to 103 $mg.kg^{-1}$ DM).

Cadmium addition to the medium decreased Zn content in roots (by 6.10%) and shoots (by 4.46%, Fig. 2, Table 5) and Cu content in shoots (by 32.9%) as compared with control plants (Fig. 1). This is in accordance with the results obtained by W u et al. (2003) that cad-

Table 2. Assessment of obtained results by parallel analysis of standard and internal reference material

CRM/IRM	**Cd_{found} (mg.kg^{-1} DM)**	**$Cd_{certified/known}$ (mg.kg^{-1} DM)**
CRM 12-02-03	0.131 ± 0.044	0.136 ± 0.065
BIOMA 3	556 ± 17.8	573 ± 15.1
CRM/IRM	**Cu_{found} (mg.kg^{-1} DM)**	**$Cu_{certified/known}$ (mg.kg^{-1} DM)**
CRM 12-02-03	10.9 ± 0.7	11.7 ± 0.8
BIOMA 3	54.6 ± 2.35	55.2 ± 1.78
CRM/IRM	**Zn_{found} (mg.kg^{-1} DM)**	**$Zn_{certified/known}$ (mg.kg^{-1} DM)**
CRM 12-02-03	32.4 ± 0.7	33.2 ± 1.0
BIOMA 3	556 ± 17.8	199 ± 8.1

Table 3. Parameters of measuring of all metals

Analyte	Cadmium (ETA)	Copper	Zinc
Wave length (nm)	228.8	423.8	213.9
Width of spectral interval (nm)	0.5	0.5	1.0
Supply current of deuterium lamp (mA)	4	4	5
Background correction	yes	no	yes
Total time of reading (s)	1.7*	12	12
Number of replications from measuring vial	2*	3	3
Measuring of signal	peak area*	integral	integral
Results determination from	calibration curve	calibration curve	calibration curve

* For flame determinations these values are consistent with Cu and Zn values

Table 4. Average Cu content in barley plant parts

Variant	Part of plant	Average Cu content (mg.kg^{-1} DM)	Change (%)	Standard deviation
Control	roots	15.8		4.49777
Control	shoots	6.89		1.71430
Control	leaf blades	4.79		1.07200
Cd 1.10^{-6} mol.L^{-1}	roots	23.4	48.3	9.17406
Cd 1.10^{-6} mol.L^{-1}	shoots	4.62	–32.9	1.03835
Cd 1.10^{-6} mol.L^{-1}	leaf blades	6.94	44.8	1.55701

Table 5. Average Zn content in barley plant parts

Variant	Part of plant	Average Zn content (mg.kg^{-1} DM)	Change (%)	Standard deviation
Control	roots	110		40.12091
Control	shoots	56.2		8.15147
Control	leaf blades	44.2		7.26306
Cd 1.10^{-6} mol.L^{-1}	roots	103	–6.10	36.24847
Cd 1.10^{-6} mol.L^{-1}	shoots	53.7	–4.46	10.33440
Cd 1.10^{-6} mol.L^{-1}	leaf blades	58.0	31.1	14.83245

mium addition to the medium significantly decreased Zn concentrations in barley tissues and inhibited its translocation from roots to shoots. Significantly negative correlation between Zn, Cu, or Mn concentrations and Cd concentration in different barley parts suggests the possibility of alleviating Cd accumulation in barley plants through application of these microelements. Decrease of Zn content in barley roots and shoots confirms the fact that Zn antagonizes Cd toxicity. Aravind, Prasad (2003) found that Cd uptake was suppressed by Zn and simultaneously, Zn concentration increased in the *Ceratophyllum demersum* L. after treatment with Zn. Results indicated the efficient antioxidative and reactive oxygen species scavenging activity by Zn against Cd-induced

Table 6. Increase of Cd content in barley in the experimental variants

Variant	Part of plant	Average Cd content ($mg.kg^{-1}$ DM)	Increase (%)	Standard deviation
Control	roots	2.29		4.0201
Control	shoots	0.32		0.2769
Control	leaf blades	0.22		0.1316
Cd 1.10^{-6} $mol.L^{-1}$	roots	240	10 400	106.3873
Cd 1.10^{-6} $mol.L^{-1}$	shoots	16.3	4 990	4.5022
Cd 1.10^{-6} $mol.L^{-1}$	leaf blades	5.78	2 580	1.3320

Table 7. Statistical evaluation of the effect of investigated parameters on Cd, Cu and Zn contents

		Cd content ($mg.kg^{-1}$ DM)			
Effect	degrees of freedom	sum of squares of deviations	variance (average square)	*F*-test	*p* (level of significance)
Variant	1	268 991	268 991.0	131.8454	0.0000*
Part of plant	2	427 697	213 848.3	104.8173	0.0000*
Replication	2	604	301.9	0.1480	0.8626
Variant x part of plant	2	412 828	206 414.2	101.1735	0.0000*
Variant x replication	2	590	295.0	0.1446	0.8655
Part of plant x replication	4	1 494	373.6	0.1831	0.9468
Variant x part of plant x replication	4	1 447	361.8	0.1773	0.9497
Residual variance	126	257 065	2 040.2		
Total	143	1 370 717			
		Cu content ($mg.kg^{-1}$ DM)			
Effect	degrees of freedom	sum of squares of deviations	variance (average square)	*F*-test	*p* (level of significance)
Variant	1	225	224.5427	11.1118	0.0011*
Part of plant	2	6 068	3 034.0720	150.1454	0.0000*
Replication	2	0.9848	0.4924	0.0244	0.9759
Variant x part of plant	2	588	294.0100	14.5495	0.0000*
Variant x replication	2	3.9323	1.9661	0.0973	0.9074
Part of plant x replication	4	13.2361	3.3090	0.1638	0.9564
Variant x part of plant x replication	4	11.3120	2.8300	0.1400	0.9671
Residual variance	126	2 546	20.2076		
Total	143	9 456			
		Zn content ($mg.kg^{-1}$ DM)			
Effect	degrees of freedom	sum of squares of deviations	variance (average square)	*F*-test	*p* (level of significance)
Variant	1	83.066	83.0656	0.1392	0.7097
Part of plant	2	90 853	45 426.5223	76.1065	0.0000*
Replication	2	302	150.9347	0.2529	0.7770
Variant x part of plant	2	2 794	1 396.9116	2.3404	0.1005
Variant x replication	2	226	112.9861	0.1893	0.8278
Part of plant x replication	4	777	194.2951	0.3255	0.8604
Variant x part of plant x replication	4	990	247.4211	0.4145	0.7979
Residual variance	126	75 207	596.8808		
Total	143	171 232			

* significant at the level $p < 0.05$

free radicals and oxidative stress. Green et al. (2003) found in hard red spring wheat in the Cd series that Zn activity was $1.10^{-6.6}$ $mol.L^{-1}$, while Cd activity increased from $1.10^{-10.7}$ $mol.L^{-1}$ to $1.10^{-9.2}$ $mol.L^{-1}$; so high levels of Cd did not significantly affect the uptake and translocation of Zn in the roots of "Grandin" HRS-wheat.

Cd content increased in all barley parts in Cd added variants in comparison with control (Table 6). The highest increase was found in roots (from 2.29 $mg.kg^{-1}$

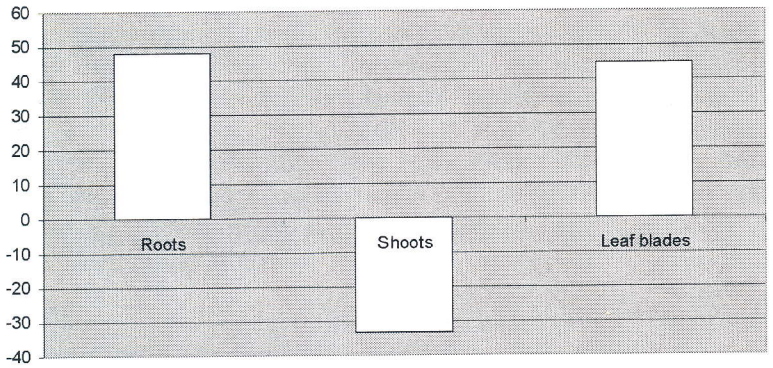

Fig. 1. Change of Cu content with Cd treatment (%)

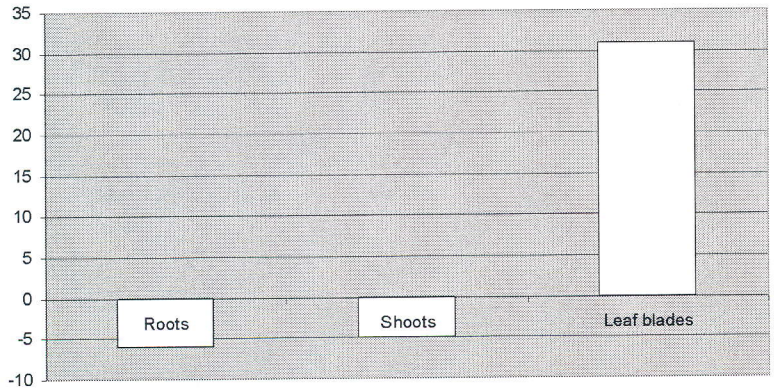

Fig. 2. Change of Zn content with Cd treatment (%)

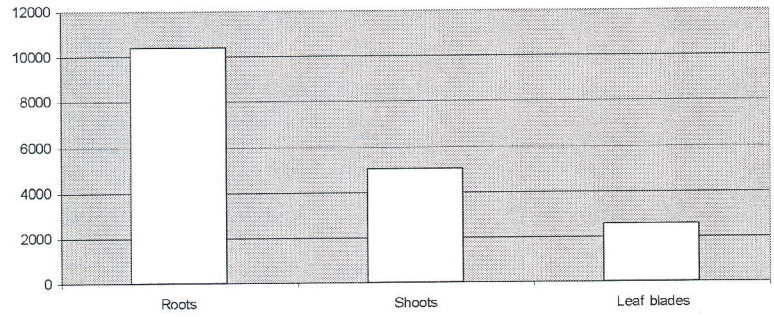

Fig. 3. Increase of Cd content with Cd treatment (%)

DM to 240 mg.kg^{-1} DM, less was in shoots (from 0.32 mg.kg^{-1} DM to 16.3 mg.kg^{-1} DM) and leaf blades (from 0.22 mg.kg^{-1} DM to 5.78 mg.kg^{-1} DM).

Our results confirmed that Cd-treatment in concentration 1.10^{-6} mol.L^{-1} caused Zn decrease in barley roots and shoots and that Zn and Cd are effective in regulation of their uptake and translocation in barley cv. Kompakt. From the Figs 2 and 3 it is clear that zinc and cadmium are antagonistic – the highest increase of cadmium in roots and shoots caused a decrease of zinc in these parts of the plant. It should be mentioned that significant differences among barley genotypes in uptake and translocation of cadmium, zinc and copper were found (W u et al., 2003).

CONCLUSION

Cd treatment of barley plants cv. Kompakt cultivated hydroponically caused the increase of Cu and Zn contents in leaf blades (by 27.9% and 2.1%, respectively) as compared with the control. On the contrary, the decrease of both micronutrients in shoots (by 32.9% and 4.46%, resp.) was found as well as the decrease of Zn in roots (by 6.10%). This confirms antagonist relationship between cadmium and zinc.

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Vliv stresu způsobeného kadmiem na příjem a distribuci mikroelementů mědi a zinku v rostlinných částech ječmene (Hordeum sativum L.).

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Byl sledován vliv stresu způsobeného kadmiem (1.10^{-6} mg.l^{-1} živného roztoku) u 32 dní starých rostlin ječmene, pěstovaných hydroponicky za stejných podmínek jako kontrolní rostliny, na příjem ze živného roztoku a obsah mikroelementů mědi a zinku v kořenech, nadzemních částech a listových čepelích. Kadmiový stres způsobil nárůst Cu o 27,9 % v celé rostlině a obsah Zn byl téměř stejný (nárůst o 2,1 %) ve srovnání s kontrolními rostlinami. Kadmiový stres měl za následek nárůst obsahu Cu v kořenech (o 48,3 %) a v listových čepelích (o 44,8 %) a jeho snížení v nadzemních částech (o 32,9 %). Obsah Zn se zvýšil za kadmiového stresu pouze v listových čepelích (o 31,1 %), zatímco v kořenech a nadzemních částech došlo ke snížení (o 6,1 % a 4,5 %). Stres způsobený kadmiem zvýšil obsah mědi a zinku v listových čepelích, zatímco v nadzemních částech došlo ve srovnání s kontrolou k jeho snížení. Rozdíly byly nalezeny u kořenů – obsah Cu se zvýšil z 15,6 mg.kg^{-1} sušiny na 23,4 mg.kg^{-1} sušiny a obsah Zn se snížil ze 109,6 mg.kg^{-1} sušiny na 102,9 mg.kg^{-1} sušiny ve srovnání s kontrolou.

ječmen; stres; kadmium; měď; zinek; příjem; distribuce; kořeny; nadzemní části; listové čepele

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