The significance of methionine, histidine and tryptophan in plant responses and adaptation to cadmium stress

V. Zemanová[1], M. Pavlík[2], D. Pavlíková[1], P. Tlustoš[1]

[1]*Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*
[2]*Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

ABSTRACT

Noccaea caerulescens (*NC*) and *Arabidopsis halleri* (*AH*) were studied to compare cadmiunm (Cd) accumulation and resistance. After 30, 60 and 90 days of plant cultivation in Cd contaminated soil (Cd1 = 30, Cd2 = 60 and Cd3 = 90 mg Cd/kg soil) amino acids were determined in plants. The comparison between both species showed that Cd stress resulted in different changes of amino acids levels playing a significant role in plant adaptation to Cd stress. Our analyses indicated higher accumulations of amino acids in the roots of *NC* compared to *AH.* Contrasting responses of plants to Cd contamination were confirmed in methionine metabolism. Methionine was determined only in roots of *AH* after 30 and 60 days of plant cultivation. Free methionine content decreased with increasing Cd contamination (Cd3 treatment – 40% decrease compared to the control treatment). Our results also showed that *NC* contains more than 10-fold higher content of histidine than *AH.* These observations indicated that this amino acid may be involved in Cd resistance and accumulation by reducing oxidative damage. Tryptophan plays a major role in the regulation of plant development and in defense responses. Its significant increase for *NC* treatments in contrast to *AH* treatments was determined.

Keywords: abiotic stress; amino acids; heavy metals; *Thlaspi caerulescens*

Cadmium (Cd) can have detrimental effects on plant growth and development even at very low concentrations. Leaf concentrations greater than 5–10 μg Cd/g dry matter (DM) are toxic to most plants (White and Brown 2010). Nevertheless, a few plant species have evolved the ability to accumulate and tolerate Cd concentrations that exceed 0.1% dry shoot mass without showing stunted growth and/or other toxicity symptoms (Koren et al. 2013). The term 'hyperaccumulator' was coined for plants that actively take up exceedingly large amounts of one or more heavy metals from the soil. Moreover, the heavy metals are not retained in the roots but are translocated to the shoot and accumulated in the aboveground organs, especially leaves, at concentrations 100–1000-fold higher than those found in non-hyperaccumulating species (Rascio and Navari-Izzo 2011, Pollard et al. 2014). Hyperaccumulators are tolerant to metals, but hyperaccumulation and tolerance are genetically independent traits.

According to Leitenmaier and Küpper (2011) hyperaccumulator plants have to store the taken up metal in a way that it does not harm important enzymes and especially not photosynthesis. It was shown that high amounts of metals are stored specifically in the vacuoles of large epidermal cells (Küpper et al. 1999, 2001), where no chloroplasts are located, and therefore, photosynthesis cannot be inhibited. According to Cappa and Pilon-Smits (2014) hyperaccumulators have enhanced levels of transporters (as a result of gene duplication) for uptake into the root and translocation within the

---

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, S grant.

plant. Hypertolerance and resistance mechanisms that were identified include enhanced levels of chelators or of enzymes that convert the element to less toxic forms.

Noccaea caerulescens and *Noccae praecox* are considered to be the most Cd-tolerant plant species, and they show the highest Cd hyperaccumulation capacities (Cosio et al. 2004, Vogel-Mikuš et al. 2005). In addition, *Arabidopsis halleri* can accumulate significant amounts of Cd without detrimental effects on plant growth and development (Zhao et al. 2006). According to Meyer et al. (2011) concentrations of phytochelatins in Cd-treated roots were the highest in *A. thaliana*, intermediate in *A. halleri* and the lowest in *N. caerulescens.* The comparison between hyperaccumulator with non-accumulator sister species (e.g. *A. halleri* with *A. thaliana*) suggests that hyperaccumulating features could reside in sequence mutations, gene copy number and/or in different expression levels of the proteins that contribute to metal tolerance (Gallego et al. 2012). According to Maestri et al. (2010) the two plant species *N. caerulescens* and *A. halleri* have evolved different mechanisms to control hyperaccumulation. The impact of trace elements on plant metabolism means that hyperaccumulator species must possess mechanisms for more efficient protein turnover. Proteomic analysis revealed the modulation or specific induction of several proteins involved in protein metabolism (DalCorso et al. 2013). The changes of amino acid levels can play a significant role in the physiological mechanism; therefore objectives of this study were to evaluate the differences of amino acid metabolism as expression of resistance to Cd soil contamination.

MATERIAL AND METHODS

The effect of Cd concentration on the levels of free amino acids was investigated in the pot experiment (Zemanová et al. 2013). Two species *Noccaea caerulescens* (formely *Thlaspi caerulescens* J. & C. Presl, FK Mey*)* ecotype cv. Ganges (southern France) (*NC*) and *Arabidopsis halleri* (O'Kane and AL Shehbaz) (northern France) (*AH*) were planted into pots (two plants per pot) containing 3 kg of soil (Chernozem modal, CEC 258 $mmol_+$/kg, C_{org} 1.83 %, pH_{CaCl_2} 7.2, total Cd content 0.42 mg/kg). Soil was thoroughly mixed with 0.3 g N, 0.10 g P, and 0.24 g K applied in the form of ammonium nitrate and potassium hydrogen phosphate for control treatment and with the same amount of nutrients plus Cd in the form of $Cd(NO_3)_2 \cdot 4\,H_2O$ in concentrations: 0 (control); 30 (Cd1); 60 (Cd2) and 90 (Cd3) mg/kg, for treated variants. Plants were harvested 30, 60 and 90 days after Cd application.

The amino acids from methanol + H_2O extracts from mature leaves were determined using EZ-faast amino acid analysis procedure (Phenomenex, Santa Clara, USA). Samples were analyzed for amino acid contents by GC-MS using the Hewlett Packard 6890N/5975 MSD (Agilent Technologies, Torrance, USA) (Pavlík et al. 2010).

Plant samples were decomposed using the dry ashing procedure in a mixture of oxidizing gases (O_2 + O_3 + NO_x) in a Dry Mode Mineralizer Apion (Tessek, Prague, Czech Republic). The ash was dissolved in 1.5% HNO_3. Aliquots of the certified reference material RM NCS DC 73350 poplar leaves (purchased from Analytika, Czech Republic) were mineralized under the same conditions for quality assurance. The Cd concentrations were analyzed by ICP-OES (Varian VistaPro, Varian, Mulgrave, Australia).

RESULTS AND DISCUSSION

Results of the pot experiment revealed the different effect of Cd on *NC* and *AH*. The yield of the aboveground biomass of *AH* was higher for all treatments in contrast to *NC* (Figure 1). Growing Cd doses were associated with the inhibition of above-ground and root biomass and with the enhancement of Cd content in leaves. The higher Cd content was determined for all Cd treatments of *NC* compared to Cd treatments of *AH* (Figure 2). The opposite effects were observed in control variants – higher Cd contents in *AH* in contrast to *NC* were determined. Our data correspond with those by Zemanová et al. (2013) and Procházková et al. (2012) who reported that excessive amounts of toxic elements in contaminated soil inhibited plant growth and development due to their phytotoxicity. The Cd content of roots was determined without replication, because there was a lack of biomass. The analyses showed values similar to Cd contents in the aboveground biomass (data is not shown here). Toxic Cd levels reduced incorporation of free amino acids into proteins. It caused the decline in protein content and therefore the decline of biomass accumulation (Solanki and

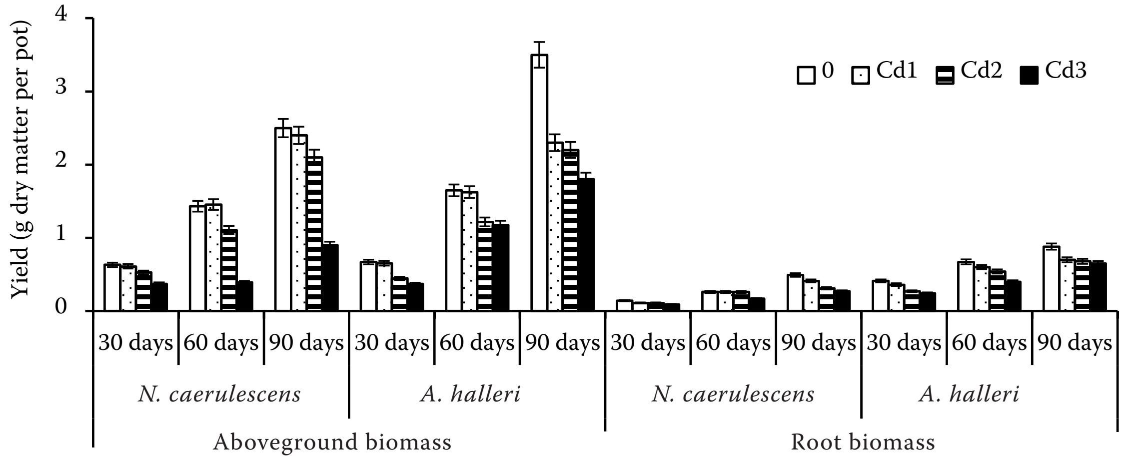

Figure 1. Effect of Cd on the aboveground biomass and root yield (g dry matter per pot) of *Noccaea caerulescens* and *Arabidopsis halleri*: 30, 60, 90 days from planting. Data are means ± S.E. ($n = 3$). Control – 0 mg Cd/kg soil; Cd1 – 30, Cd2 – 60 and Cd3 – 90 mg Cd/kg soil

Dhankhar 2011). This finding confirmed our results – higher Cd content in plant biomass and lower yield of *NC* in contrast to *AH*.

The changes of amino acid levels can play a significant role in mechanism of plant adaptation to Cd stress. Chaffei et al. (2004) suggested that an increase in the proportion of high N:C amino acids is a protective strategy in plants for preserving roots as a nutritional safeguard organ to ensure future recovery. Consistent with this hypothesis, our analyses indicated the accumulation of a large amount of amino acids in the roots of *NC* compared to *AC* (Figure 3). The highest accumulation of amino acids (AA) was determined on Cd2 treatment of *NC* after 90 days of cultivation. The high content of AA in roots of *NC* indicated high Cd accumulation and tolerance of this plant. The amino acid accumulation in *NC* roots also suggested that these Cd-chelating molecules are highly active in plant roots and that upon binding Cd, they may form a complex that can be translocated from the roots to the shoots (Couturier et al. 2010).

Methionine (Met) is one from AA with different content in *AH* in contrast to *NC*. Methionine originates from three convergent pathways: the carbon backbone deriving from aspartate, the sulfur atom from cysteine, and the methyl group from the β-carbon of serine. It is an amino acid that supports additional roles than simply serving as a building block for protein synthesis. This is because methionine is the immediate precursor of *S*-adenosylmethionine, which plays numerous roles of being the major methyl-group donor in

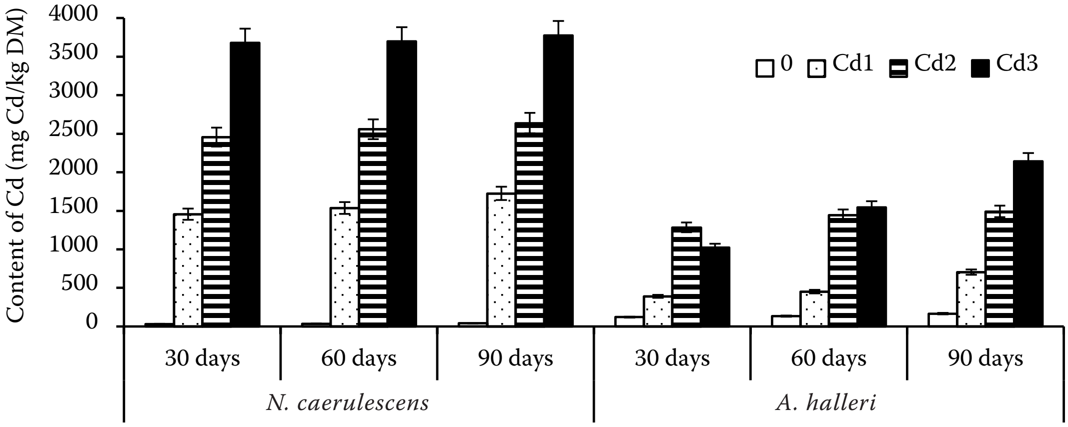

Figure 2. Total contents of Cd in the aboveground biomass of *Noccaea caerulescens* and *Arabidopsis halleri* exposed to increasing rates of Cd in soil. Data are means ± S.E. ($n = 3$). DM – dry matter; control – 0 mg Cd/kg soil; Cd1 – 30, Cd2 – 60 and Cd3 – 90 mg Cd/kg soil

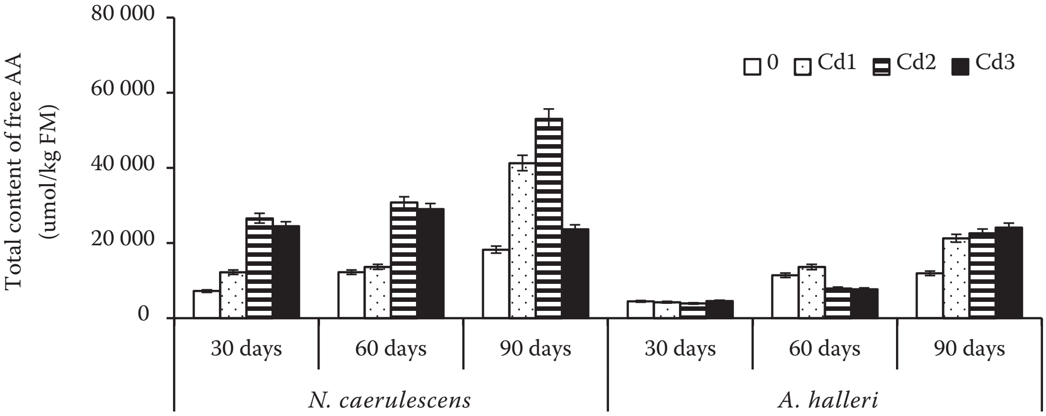

Figure 3. Total contents of free amino acids (AA) in roots of *Noccaea caerulescens* and *Arabidopsis halleri* exposed to increasing rates of Cd in soil. Data are means ± S.E. (*n* = 3). Control – 0 mg Cd/kg soil; Cd1 – 30, Cd2 – 60 and Cd3 – 90 mg Cd/kg soil; FM – fresh matter

transmethylation reactions and an intermediate in the biosynthesis of polyamines and of the phytohormone ethylene etc. (Ravanel et al. 1998). Met was differentially regulated between the tested plant species since its content was determined only in roots of *AH* after 30 and 60 days of plant cultivation (Figure 4), but it was not detected in *NC*. The effect of Cd contamination was confirmed after 60 days of plant cultivation, free Met content decreased with increasing Cd contamination (Cd3 treatment – 40% decrease compared to control treatment). Similar results were published for two lines of tobacco by Pavlíková et al. (2014). The results showed that *NC* regulated more effective Met compared to *AH*. This AA is quickly transformed into the required products or incorporated into a protein without increased accumulation in plant. The Met accumulation in *AH* plants in contrast to *NC* can be related to the oxidation of Met to methionine sulfoxide, which alters the activity and conformation of various proteins, can be reversed by methionine sulfoxide reductase (MSR). MSR participates in a protein repair system that is one of the defensive mechanisms that diminishes oxidative destruction (Li et al. 2012, Zagorchev et al. 2013). According to Ingle et al. (2005) Met biosynthesis is suppressed in *Alyssum lesbiacum*, indicating that thiol groups are diverted toward cysteine and glutathione biosynthesis. In contrast, methionine synthase was induced after metal treatment in *Phytolacca americana* (Zhao et al. 2011), suggesting there are diverse strategies for metal detoxification in hyperaccumulator species.

The observation of Holmes and Appling (2002) shows the possibility of a metabolic link between methionine and histidine (His) biosynthetic pathway through accumulation of 5'-amino 4-carboxamide ribonucleotide interfering with Met biosynthetic pathway. His was found to play an important role in regulation of biosynthesis of other AA, in chelation

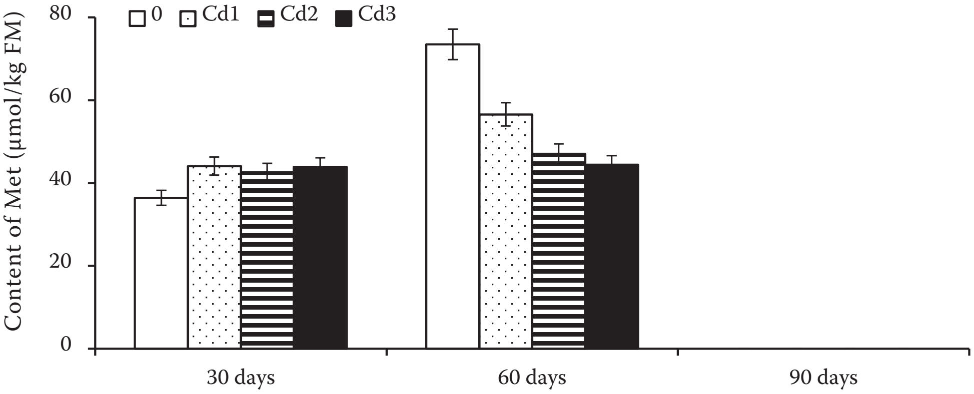

Figure 4. Total contents of free methionine (Met) in roots of *Arabidopsis halleri* exposed to increasing rates of Cd in soil. Data are means ± S.E. (*n* = 3). Values of Met analyzed after 90 days of plant cultivation were below detection limit of gas chromatography. Control – 0 mg Cd/kg soil; Cd1 – 30, Cd2 – 60 and Cd3 – 90 mg Cd/kg soil; FM – fresh matter

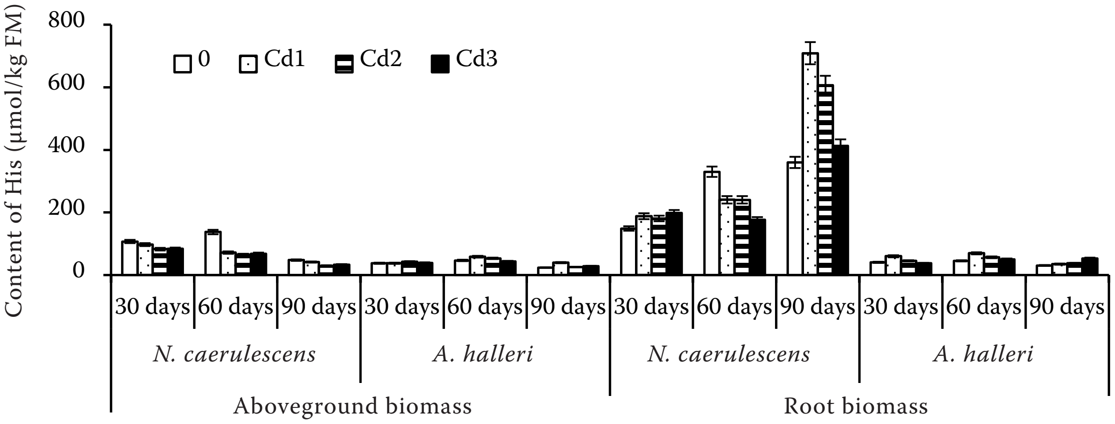

Figure 5. Total contents of free histidine (His) in the aboveground biomass and roots of *Noccaea caerulescens* and *Arabidopsis halleri* exposed to increasing rates of Cd in soil. Data are means ± S.E. (n = 3). Control – 0 mg Cd/kg soil; Cd1 – 30, Cd2 – 60 and Cd3 – 90 mg Cd/kg soil; FM – fresh matter

and transport of metal ions, and in plant reproduction and growth (Stepansky and Leustek 2006). Our result showed that content of His was accumulated during *NC* growing period, while significant changes were not observed in *AC* roots (Figure 5). The highest difference between *NC* and *AH* treatments was determined on Cd2. Accumulation of free His in *NC* roots of Cd2 treatment is more than 19-fold higher in contrast to Cd2 treatment of *AH*. These observations indicated that His may be involved in Cd resistance and accumulation by reducing oxidative damage. According to Xu et al. (2012) the high accumulation of His in plant promoted Cd uptake and improved root-to-shoot Cd transport, which thereby increased leaf Cd accumulation. Compared to other known low-molecular-weight metal chelators such as phytochelatins and nicotianamine, histidine is of relatively low metabolic cost. His biosynthesis does not involve the assimilation of sulfate as is required for the biosynthesis of phytochelatins, and it contains 6 C and 3 N atoms compared with nicotinamine (12 C and 3 N) or phytochelatins (approximately 18 or 36 C, 5 or 10 N, and 2 or 4 S) (Stepansky and Leustek 2006).

The histidine biosynthesis pathway is integrated with a number of other metabolic pathways including tryptophan (Trp). Tryptophan plays a major role in the regulation of plant development and defense responses and it is the precursor for indolacetic acid, a plant hormone necessary for cell expansion. Our results showed the significant increase of this AA for *NC* treatments (Figure 6). According to Pavlík et al. (2012) Trp biosynthesis is induced by stresses. However, the significant changes during growing period of *AH* were not confirmed. Little is known about Trp-mediated trace elements tol-

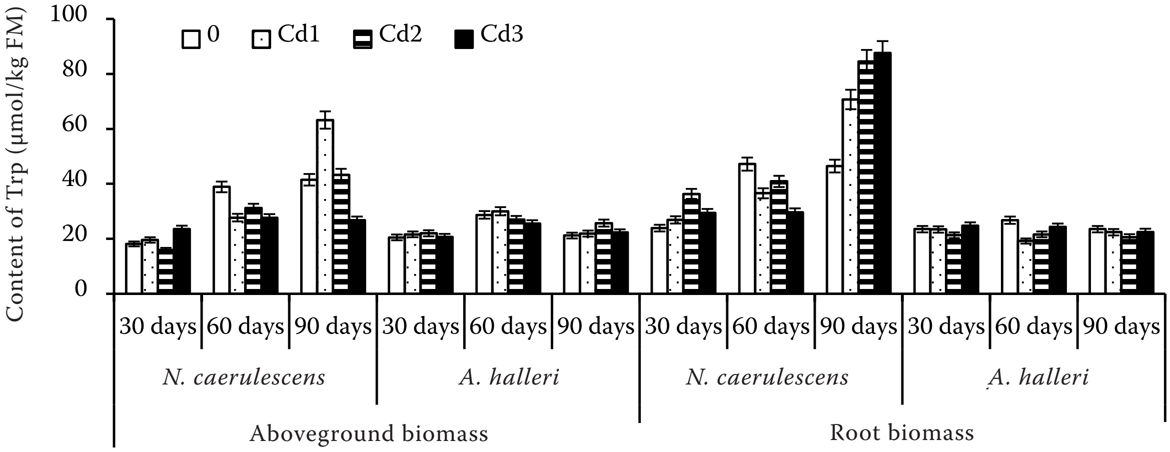

Figure 6. Total contents of free tryptophan (Trp) in the aboveground biomass and roots of *Noccaea caerulescens* and *Arabidopsis halleri* exposed to increasing rates of Cd in soil. Data are means ± S.E. (n = 3). Control – 0 mg Cd/kg soil; Cd1 – 30, Cd2 – 60 and Cd3 – 90 mg Cd/kg soil; FM – fresh matter

erance (Sanjaya et al. 2008). Sanjaya et al. (2008) reported that increased Trp levels make Cd less available to the plant, decrease Cd transport and thus reduce Cd accumulation. Metal ions and the bivalent Trp side chain indole were found to interact cooperatively (Li and Yang 2003).

REFERENCES

Cappa J.J., Pilon-Smits E.A.H. (2014): Evolutionary aspects of elemental hyperaccumulation. Planta, *239*: 267–275.

Chaffei C., Pageau K., Suzuki A., Gouia H., Ghorbel M.H., Masclaux-Daubresse C. (2004): Cadmium toxicity induced changes in nitrogen management in *Lycopersicon esculentum* leading to a metabolic safeguard through an amino acid storage strategy. Plant and Cell Physiology, *45*: 1681–1693.

Cosio C., Martinoia E., Keller C. (2004): Hyperaccumulation of cadmium and zinc in *Thlaspi caerulescens* and *Arabidopsis halleri* at the leaf cellular level. Plant Physiology, *134*: 716–725.

Couturier J., de Faÿ E., Fitz M., Wipf D., Blaudez D., Chalot M. (2010): PtAAP11, a high affinity amino acid transporter specifically expressed in differentiating xylem cells of poplar. Journal of Experimental Botany, *61*: 1671–1682.

DalCorso G., Fasani E., Furini A. (2013): Recent advances in the analysis of metal hyperaccumulation and hypertolerance in plants using proteomics. Frontiers in Plant Science, *4*: 280, doi: 10.3389/fpls.2013.00280.

Gallego S.M., Pena L.B., Barcia R.A., Azpilicueta C.E., Iannone M.F., Rosales E.P., Zawoznik M.S., Groppa M.D., Benavides M.P. (2012): Unravelling cadmium toxicity and tolerance in plants: Insight into regulatory mechanisms. Environmental and Experimental Botany, *83*: 33–46.

Holmes W.B., Appling D.R. (2002): Cloning and characterization of methenyltetrahydrofolate synthetase from *Saccharomyces cerevisiae*. Journal of Biological Chemistry, *277*: 20205–20213.

Ingle R.A., Smith J.A.C., Sweetlove L.J. (2005): Responses to nickel in the proteome of the hyperaccumulator plant *Alyssum lesbiacum*. Biometals, *18*: 627–641.

Koren Š., Arčon I., Kump P., Nečemer M., Vogel-Mikuš K. (2013): Influence of $CdCl_2$ and $CdSO_4$ supplementation on Cd distribution and ligand environment in leaves of the Cd hyperaccumulator *Noccaea (Thlaspi) praecox*. Plant and Soil, *370*: 125–148.

Küpper H., Zhao F.J., McGrath S.P. (1999): Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*. Plant Physiology, *119*: 305–312.

Küpper H., Lombi E., Zhao F.J., Wieshammer G., McGrath S.P. (2001): Cellular compartmentation of nickel in the hyperaccumulators *Alyssum lesbiacum*, *Alyssum bertolonii* and *Thlaspi goesingense*. Journal of Experimental Botany, *52*: 2291–2300.

Leitenmaier B., Küpper H. (2011): Cadmium uptake and sequestration kinetics in individual leaf cell protoplasts of the Cd/Zn hyperaccumulator *Thlaspi caerulescens*. Plant, Cell and Environment, *34*: 208–219.

Li Y., Yang C.M. (2003): A rationally designed novel receptor for probing cooperative interaction between metal ions and bivalent tryptophan side chain in solution. Chemical Communications, *23*: 2884–2885.

Li C.W., Lee S.H., Chieh P.S., Lin C.S., Wang Y.C., Chan M.T. (2012): *Arabidopsis* root-abundant cytosolic methionine sulfoxide reductase B genes *MsrB7* and *MsrB8* are involved in tolerance to oxidative stress. Plant and Cell Physiology, *53*: 1707–1719.

Maestri E., Marmiroli M., Visioli G., Marmiroli N. (2010): Metal tolerance and hyperaccumulation: Costs and trade-offs between traits and environment. Environmental and Experimental Botany, *68*: 1–13.

Meyer C.L., Peisker D., Courbot M., Craciun A.R., Cazalé A.C., Desgain D., Schat H., Clemens S., Verbruggen N. (2011): Isolation and characterization of *Arabidopsis halleri* and *Thlaspi caerulescens* phytochelatin synthases. Planta, *234*: 83–95.

Pavlík M., Pavlíková D., Staszková L., Neuberg M., Kaliszová R., Száková J., Tlustoš P. (2010): The effect of arsenic contamination on amino acids metabolism in *Spinacia oleracea* L.. Ecotoxicology and Environmental Safety, *73*: 1309–1313.

Pavlík M., Pavlíková D., Zemanová V., Hnilička F., Urbanová V., Száková J. (2012): Trace elements present in airborne particulate matter – Stressors of plant metabolism. Ecotoxicology and Environmental Safety, *79*: 101–107.

Pavlíková D., Zemanová V., Procházková D., Pavlík M., Száková J., Wilhelmová N. (2014): The long-term effect of zinc soil contamination on selected free amino acids playing an important role in plant adaptation to stress and senescence. Ecotoxicology and Environmental Safety, *100*: 166–170.

Pollard A.J., Reeves R.D., Baker A.J.M. (2014): Facultative hyperaccumulation of heavy metals and metalloids. Plant Science, *217–218*: 8–17.

Procházková D., Haisel D., Pavlíková D., Schnablová R., Száková J., Vytášek R., Wilhelmová N. (2012): The effect of risk elements in soil to nitric oxide metabolism in tobacco plants. Plant, Soil and Environment, *58*: 435–440.

Rascio N., Navari-Izzo F. (2011): Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? Plant Science, *180*: 169–181.

Ravanel S., Gakiére B., Job D., Douce R. (1998): The specific features of methionine biosynthesis and metabolism in plants. Proceedings of the National Academy of Sciences of the United States of America, *95*: 7805–7812.

Sanjaya, Hsiao P.Y., Su R.C., Ko S.S., Tong C.G., Yang R.Y., Chan M.T. (2008): Overexpression of *Arabidopsis thaliana* tryptophan synthase beta 1 (*AtTSB1*) in *Arabidopsis* and tomato confers tolerance to cadmium stress. Plant, Cell and Environment, *31*: 1074–1085.

Solanki R., Dhankhar R. (2011): Biochemical changes and adaptive strategies of plants under heavy metal stress. Biologia, *66*: 195–204.

Stepansky A., Leustek T. (2006): Histidine biosynthesis in plants. Amino Acids, *30*: 127–142.

Vogel-Mikuš K., Drobne D., Regvar M. (2005): Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox* Wulf. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. Environmental Pollution, *133*: 233–242.

White P.J., Brown P.H. (2010): Plant nutrition for sustainable development and global health. Annals of Botany, *105*: 1073–1080.

Xu J., Zhu Y., Ge Q., Li Y., Sun J., Zhang Y., Liu X. (2012): Comparative physiological responses of *Solanum nigrum* and *Solanum torvum* to cadmium stress. New Phytologist, *196*: 125–138.

Zagorchev L., Seal C.E., Kranner I., Odjakova M. (2013): A central role for thiols in plant tolerance to abiotic stress. International Journal of Molecular Sciences, *14*: 7405–7432.

Zemanová V., Pavlík M., Pavlíková D., Tlustoš P. (2013): The changes of contents of selected free amino acids associated with cadmium stress in *Noccaea caerulescens* and *Arabidopsis halleri*. Plant, Soil and Environment, *59*: 417–422.

Zhao F.J., Jiang R.F., Dunham S.J., McGrath S.P. (2006): Cadmium uptake, translocation and tolerance in the hyperaccumulator *Arabidopsis halleri*. New Phytologist, *172*: 646–654.

Zhao L., Sun Y.L., Cui S.X., Chen M., Yang H.M., Liu H.M., Chai T.Y., Huang F. (2011): Cd-induced changes in leaf proteome of the hyperaccumulator plant *Phytolacca americana*. Chemosphere, *85*: 56–66.

Received on July 2, 2014
Accepted on July 22, 2014

Corresponding author:

Prof. Ing. Daniela Pavlíková, CSc., Česká zemědělská univerzita v Praze, Fakulta agrobiologie, potravinových a přírodních zdrojů, Katedra agroenvironmentální chemie a výživy rostlin, Kamýcká 129, 165 21 Praha 6, Česká republika
phone: + 420 224 382 735, e-mail: pavlikova@af.czu.cz