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The contents of free amino acids and elements in As-hyperaccumulator *Pteris cretica* and non-hyperaccumulator *Pteris straminea* during reversible senescence

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ABSTRACT

Pavlíková D., Zemanová V., Pavlík M. (2017): The contents of free amino acids and elements in As-hyperaccumulator *Pteris cretica* and non-hyperaccumulator *Pteris straminea* during reversible senescence. Plant Soil Environ., 63: 455–460.

The objectives of this study were to analyse the relationship between the contents of elements and free amino acids (AAs) in fronds of As-hyperaccumulator *Pteris cretica* cv. Albo-lineata (PC) and non-hyperaccumulator *Pteris straminea* (PS) during reversible senescence. The time-course effect on senescence was also investigated. The two ferns were grown in a pot experiment with soil containing 16 mg As_{total}/kg soil for 160 days. The contents of elements and AAs in both ferns and in individual sampling periods differed. The highest accumulation of elements and AAs was measured in PS fronds after 83 days; however, the accumulation of As, Ca, Cu, Fe, Mg, P and asparagin in PC fronds was highest after 160 days. The results of principal component analysis showed more rapid senescence of PS compared to PC. This was caused by changes in the relationship between the contents of elements (cofactors of metalloenzymes, stress metabolites) and AAs (transport of NH_2 group and stress metabolites). The hyperaccumulator plant (PC) was more resistant than the bioindicator plant (PS) to the conversion from reversible to irreversible senescence.

Keywords: metallophytes; anabolic and catabolic processes; stress metabolism plants; nitrogen assimilation

Several species of the genus *Pteris* including *P. vittata* and *P. cretica* (PC) have been identified as arsenic hyperaccumulators (Luongo and Ma 2005). On the other hand, Meharg (2003) first reported *P. straminea* (PS) as a non-hyperaccumulator plant. The phytochelatins play a key role in As (arsenic) detoxification in non-hyperaccumulators (Raab et al. 2004). In contrast to non-hyperaccumulators, hyperaccumulators complex only 1–3% As with phytochelatins (Zhao et al. 2003, Vetterlein et al. 2009), because As detoxification is associated with the conversion of As^{V} to As^{III} and As methylation (Gonzaga et al. 2006). The low As content in *Pteris* hyperaccumulators promotes plant growth; a high content inhibits growth (Cao et al. 2004). This finding confirms that As induces a hormetic response in these plants. A high level of detoxified As leads to its re-oxidation and to demethylation and As becomes toxic after degradation of complexes that substituted $As^{III/V}$ species with CH_3 in cells (Tu et al. 2003). The accumulation of As declines during senescence (Kertulis-Tartar et al. 2006). The result of senescence process is an irreversible senescence ending in apoptosis. A lack of

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information limits our ability to characterize the metabolic processes of reversible and irreversible senescence in plants (Ding et al. 2005, Kobayashi et al. 2013). The effect of toxic elements including As on the metabolism of free amino acids (AAs) is connected with senescence (Pavlík et al. 2010, 2012, Pavlíková et al. 2014a,b, Zemanová et al. 2016) and also with CH_3 metabolism. CH_3 metabolism is determined by the transmethylation cycle (Zemanová et al. 2017), which involves the folate and methionine cycle as a donor for the biosynthesis of antioxidative metabolites (polyamines, tocopherols, phenylpropanoid metabolites including flavonoids, isoflavonoids, stilbenes etc.), cytokinins and mainly for substituted $As^{III/V}$ species with CH_3. The profiling AA content in *Pteris* is unknown, although the AA content is significantly affected by As content in hyperaccumulating ferns *Pityrogramma calomelanos* (Campos et al. 2016). To determine the non-stress metabolism of AAs in plants it is necessary to define reversible senescence in the *Pteris* genus. It is assumed that such senescence during the growing period differs between hyperaccumulator plants (PC) and non-hyperaccumulators (PS). For this reason, this study investigated AAs that are important for transport, stress and adaptive responses and also the changes in contents of selected elements during the growing period.

MATERIAL AND METHODS

Two fern species were cultivated in pots in a temperature- and humidity-controlled greenhouse (a 16 h day/8 h night cycle and a temperature cycle of 26°C day/18°C night, the soil moisture – 60% maximum water-holding capacity): *Pteris cretica* (L.) var. Albo-lineata (PC) and *P. straminea* (Mett. ex Baker) (PS). For cultivation of plants, 4.5 kg of modal chernozem soil (Prague – Suchdol, Czech Republic; pH_{KCl} = 7.4, cation exchange capacity (CEC) = 258 $mmol_+/kg$, C_{org} = 1.83%, As = 16 ± 1.7 mg/kg) was mixed with 0.5 g N, 0.16 g P and 0.4 g K per 1 kg of soil (applied in the form of NH_4NO_3 and K_2HPO_4). Plants were harvested 35, 83 and 160 days after planting.

Total element contents were determined after microwave-assisted low pressure acid-digestion (HNO_3:H_2O_2, 4:1, v/v) by ICP-OES (Agilent 720, Agilent Technologies Inc., Torrance, USA) and by AAS (Varian SpectrAA-280, Mulgrave, Australia) for K. Certified reference material (SRM 1515, apple leaves, Analytika, Prague, Czech Republic) was mineralized under the same conditions for quality assurance.

The contents of free AAs were determined by GC-MS (Hewlett Packard 6890N/5975 MSD, Agilent Technologies, Torrance, USA) according to Pavlík et al. (2012) after their derivatization in extracts (1.0 g of fresh biomass, 15 mL of methanol and double distilled H_2O (7:3, v/v), 24 h) by EZ:faast set (Phenomenex, Santa Clara, USA).

The statistical analyses were performed using CANOCO 4.5 (ter Braak and Šmilauer 2002).

RESULTS AND DISCUSSION

The profile of free AAs and macro- and microelements in ferns is a significant indicator of reversible senescence. In our experiment, some AAs in *Pteris* plants were below the detection limit of gas chromatography (GC) or at very low concentrations during reversible senescence. The levels of proteinogenic AAs (methionine, histidine, and arginine) were below the detection limit for all sampling periods and in both ferns. Hydroxyproline, which is generated after protein degradation during senescence, was not detected. Similarly, sarcosine, which is found in some hyperaccumulators, was not detected. This AA is linked with methyl metabolism and affects epigenetic changes induced by oxidative stress (Nikiforova et al. 2006, Zemanová et al. 2017). The contents of other identified AAs (γ-aminobutyric acid, lysine, α-aminoadipic acid, ornithine, tryptophan, valine and tyrosine) oscillated around the detection limit and therefore they were not evaluated using the principal component analysis (PCA).

The physiological processes of senescence affect the element content in plants and the regulation of plant metabolism (catabolism and anabolism of AAs), thus determining the contents of free AAs (Figures 1–3). Senescence and oxidative stress are affected by toxic elements in associated processes connected with the metabolism of fatty acids (Pavlík et al. 2017) and with changes in phytohormone contents. Regulation of the expression of cytokinin genes affects the proteolysis of proteins, photosynthetic activity, gas exchange parameters, element uptake and AAs (Pavlíková et al. 2014a,b).

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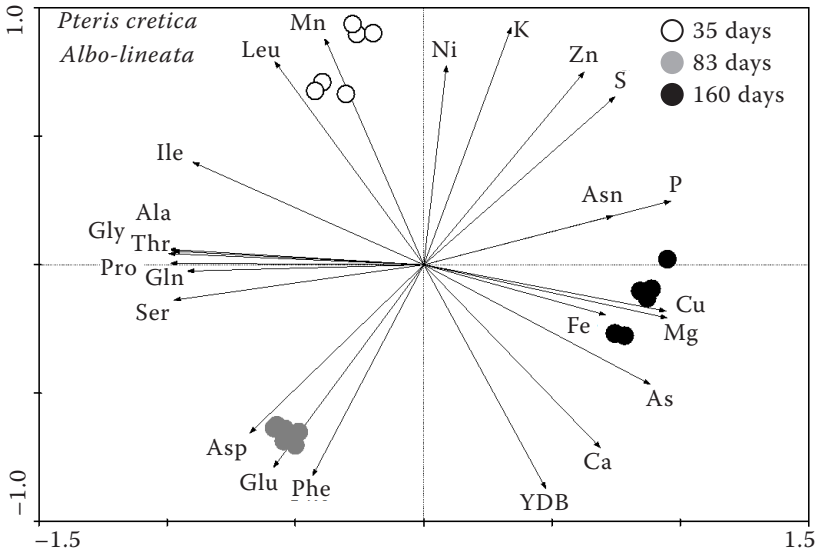

Figure 1. Ordination diagram showing the results of principal component analysis (PCA) analysis of selected parameters in hyperaccumulator plant (PC) fronds. The first axis of the PCA analysis explained 66%, the first two axes 91%, and the first four axes together, 99.9% of the variability of all analysed data. Two vectors were not positively correlated if the angle between them was larger than 90°. A long vector for particular parameters indicates a strong effect on the results of the analysis, and vice versa. YDB – yield of fronds in dry biomass; Ni, As, Ca, Cu, S, Fe, P, Zn, Mg, K and Mn – total content of elements; concentration of free amino acids (AAs): Asp – aspartic acid; Asn – asparagine; Pro – proline; Glu – glutamic acid; Gln – glutamine; Ser – serine; Leu – leucine; Ile – isoleucine; Gly – glycine; Thr – threonine; Phe – phenylalanine; Ala – alanine

Antioxidative enzymes and metabolites containing assimilated C, N, S and P are important for plants in overcoming stress. Reductions in element assimilation as well as photosynthetic activity have a strong influence on the biosynthesis of stress metabolites, which accelerates the conversion from reversible to irreversible senescence. The depletion of assimilated metabolites leads to

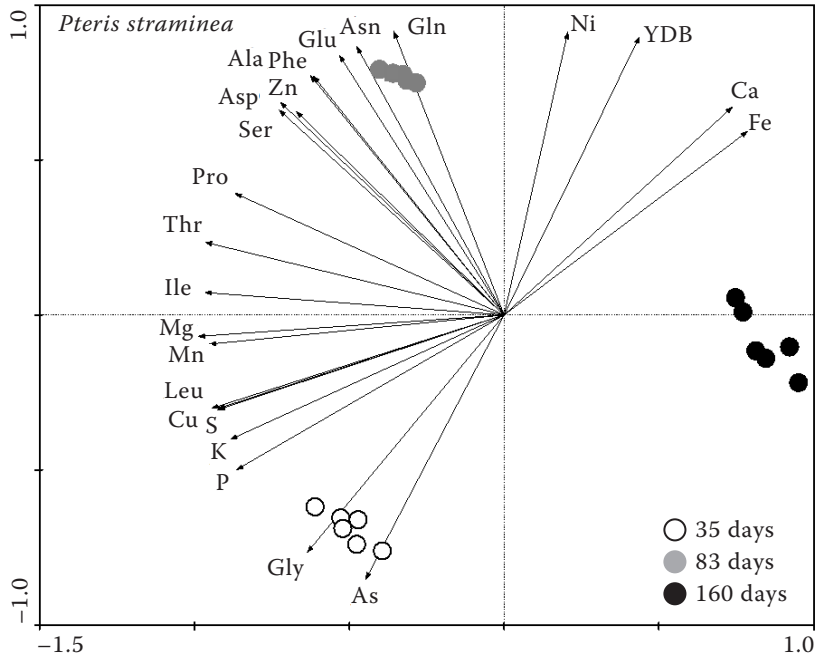

Figure 2. Ordination diagram showing the results of principal component analysis (PCA) analysis of selected parameters in non-hyperaccumulators (PS) fronds. The first axis of the PCA analysis explained 59%, the first two axes 96%, and the first four axes together, 100% of the variability of all analysed data. For note and parameter abbreviations see Figure 1

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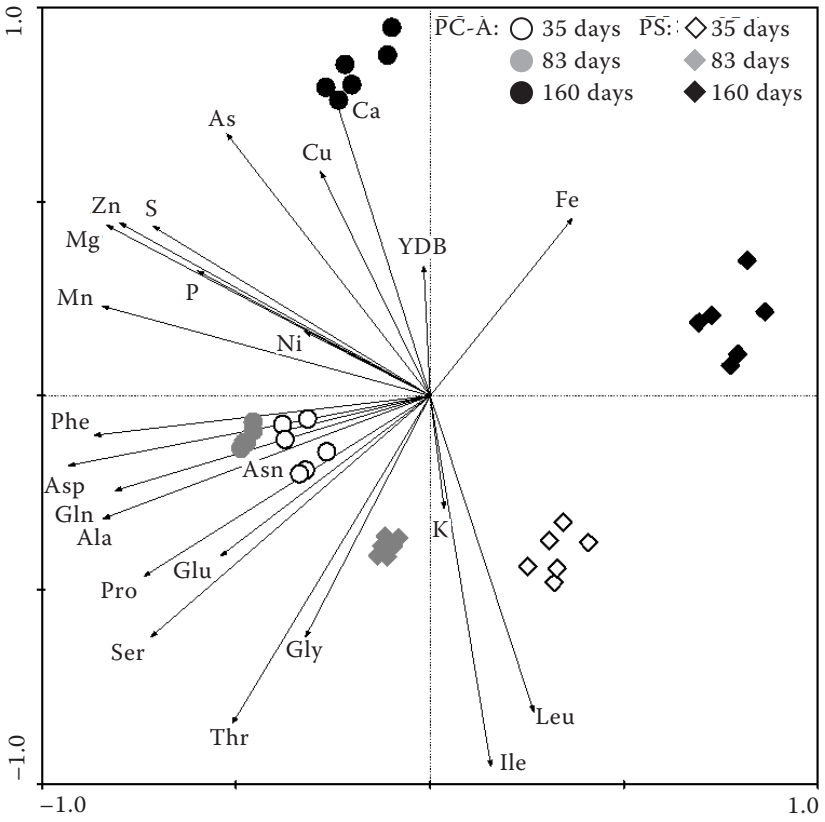

Figure 3. Ordination diagram showing the results of principal component analysis (PCA) analysis of selected parameters in hyperaccumulator plant (PC) and non-hyperaccumulators (PS) fronds. The first axis of the PCA analysis explained 37%, the first two axes 63%, and the first four axes together, 95% of the variability of all analysed data. For note and parameter abbreviations see Figure 1

increased catabolic activity in the plant, in turn causing the disintegration of chlorophyll and the development of necrosis. Irreversible senescence plays a significant role in the As accumulation of hyperaccumulator plants of the genus *Pteris* (Tu et al. 2003, Kertulis-Tartar et al. 2006), and therefore element contents and AAs were determined during reversible senescence. The results of our experiment verified that element contents and AAs differ in the two phenotypes and in reversible senescence and the growing period of both tested plants (Figures 1–3).

The results of PCA analyses showed a significant effect of sampling period (35, 83 and 160 days) on parameters in fronds of both *Pteris* plants (Figures 1 and 2), as indicated by the different locations of points in the diagram and the high percentage of variability explained by the ordination axes. In Figures 1 and 2, the length and direction of the vectors of the studied parameters indicate links among themselves with respect to the sampling period. In fronds of PC all AAs except Asn were found at higher levels after 35 and 83 days (Figure 1). Free AAs were divided into two groups (group 1: Ser (serine), Gln (glutamine), Pro (proline), Thr (threonine), Gly (glycine), Ala (alanine), Ile (isoleucine) and Leu (leucine); group 2: Asp (aspartic acid), Glu (glutamic acid) and Phe (phenylalanine)) and were positively correlated with each other as the angle between the vectors for them was < 90°, and negatively correlated with Asn as the angle between the vectors for AAs and Asn was > 90°. The major AAs accumulated in the different sampling periods were Leu after 35 days, Asp, Glu and Phe after 83 days and Asn after 160 days. In the PS fern, the major AAs accumulated in the fronds were Gly after 35 days and Asn, Gln and Glu after 83 days. Asparagine, Gln, Glu and Asp have N remobilization/transport/storage function in senescing leaf cell (McAllister et al. 2012). According to Figure 2, AAs were not accumulated after 160 days. All free AAs except Gly were positively correlated with each other as the angle between the vectors for them was < 90°. These results revealed differences in the element contents and AA contents in PS and PC between sampling periods. High levels of Gly and its negative correlation with Ser were only found in PS. Their biosynthesis pathways are linked (Nunes-Nesi et al. 2008, Campos et al. 2016) and they are also products of significant metabolites connected with reversible senescence (accumulated Ser initiates a senescence; Zhu et al.

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2005) and As metabolism – detoxified (reduced As), methylated and/or conjugated As with natural metabolites (Dembitsky and Levitsky 2004).

Vegetation growth reduced the element contents in fronds of the PS fern (Figure 2). On the other hand, the highest contents of all elements, especially As, Cu, Fe and Mg, were found in fronds of PC fern after 160 days. Copper and Fe are co-factors of the antioxidative metalloenzymes of superoxide dismutase and different cytochromes including Cyt P450 (Fernández-Ocaña et al. 2011, Fiore et al. 2012). The high level of Mg after 160 days showed that PC was at the start of reversible senescence (Figure 1). This finding was supported by the negative correlation between Mg and Glu contents. Glu is regulated by the feedback of Pro (Pavlíková et al. 2007). Its intermediate product glutamate-5-semaldehyde leads to chlorophyll biosynthesis *via* 5-aminolevulinate (Pavlík et al. 2012). Glu is a substrate not only for chlorophyll biosynthesis, but also for antioxidative and stress metabolites (glutathione and phytochelatins), and therefore the chlorophyll content and its degradation are associated with senescence (Meneguelli-Souza et al. 2016).

The reduction in the explanation of variability in PCA analyses of both *Pteris* plants together and the location of points showed the significant effect of different species especially in the late sampling period (Figure 3). The effect of species was shown by the first ordination axis, which placed individual pots of PS (except at 83 days) on the right side and pots of PC on the left side of the diagram. The length and direction of the vectors of the studied parameters indicate the association of free AA content as well as element content with respect to the sampling period and fern species. As for the individual ferns, all AAs were found at the highest levels after 35 and 83 days. On the other hand, the highest levels of all elements other than K were found after 160 days. The As content was positively correlated with other elements except K, as the angle between the vectors for them was < 90°, while it was negatively correlated with the AAs Ile, Leu, Gly and Thr as the angle between vectors for these parameters was > 90°. A significant effect of species was evident in the contents of Cu, Mn (co-factors of antioxidative metalloenzymes; Fernández-Ocaña et al. 2011), S (antioxidative metabolite of glutathione – component ascorbate-glutathione cycle; Cao et al. 2004) and P (phospholipids – significant for semipermeability of membranes). These elements were accumulated more in PC compared to PS ferns (Figure 3). There were differences in the relationships between Glu and Mg between ferns, which were linked with chlorophyll content, gas exchange parameters and reversible senescence (Pavlíková et al. 2014a,b). These results confirmed the lower degree of adaptation of PS to As contamination and its lower effectiveness at senescence regulation compared to PC ferns. Both *Pteris* species are metallophytes, and, according to our results and the definition of Van der Ent et al. (2013), they can be classified as either a hyperaccumulator (PC) or a bioindicator (PS).

REFERENCES

Campos N.V., Araújo T.O., Arcanjo-Silva S., Freitas-Silva L., Azevedo A.A., Nunes-Nesi A. (2016): Arsenic hyperaccumulation induces metabolic reprogramming in *Pityrogramma calomelanos* to reduce oxidative stress. Physiologia Plantarum, 157: 135–146.

Cao X., Ma L.Q., Tu C. (2004): Antioxidative responses to arsenic in the arsenic-hyperaccumulator Chinese brake fern (*Pteris vittata* L.). Environmental Pollution, 128: 317–325.

Ding L., Wang K.J., Jiang G.M., Liu M.Z., Niu S.L., Gao L.M. (2005): Post-anthesis changes in photosynthetic traits of maize hybrids released in different years. Field Crops Research, 93: 108–115.

Dembitsky V.M., Levitsky D.O. (2004): Arsenolipids. Progress in Lipid Research, 43: 403–448.

Fernández-Ocaña A., Chaki M., Luque F., Gómez-Rodríguez M.V., Carreras A., Valderrama R., Begara-Morales J.C., Hernández L.E., Corpas F.J., Barroso J.B. (2011): Functional analysis of superoxide dismutases (SODs) in sunflower under biotic and abiotic stress conditions. Identification of two new genes of mitochondrial Mn-SOD. Journal of Plant Physiology, 168: 1303–1308.

Fiore A., Dall'Osto L., Cazzaniga S., Diretto G., Giuliano G., Bassi R. (2012): A quadruple mutant of *Arabidopsis* reveals a β-carotene hydroxylation activity for LUT1/CYP97C1 and a regulatory role of xanthophylls on determination of the PSI/PSII ratio. BMC Plant Biology, 12: 50.

Gonzaga M.I.S., Santos J.A.G., Ma L.Q. (2006): Arsenic phytoextraction and hyperaccumulation by fern species. Scientia Agricola, 63: 90–101.

Kertulis-Tartar G.M., Ma L.Q., Tu C., Chirenje T. (2006): Phytoremediation of an arsenic-contaminated site using *Pteris vittata* L.: A two-year study. International Journal of Phytoremediation, 8: 311–322.

Kobayashi N.I., Saito T., Iwata N., Ohmae Y., Iwata R., Tanoi K., Nakanishi T.M. (2013): Leaf senescence in rice due to magne-

doi: 10.17221/606/2017-PSE

sium deficiency mediated defect in transpiration rate before sugar accumulation and chlorosis. Physiologia Plantarum, 148: 490–501.

Luongo T., Ma L.Q. (2005): Characteristics of arsenic accumulation by *Pteris* and non-*Pteris* ferns. Plant and Soil, 277: 117–126.

McAllister C.H., Beatty P.H., Good A.G. (2012): Engineering nitrogen use efficient crop plants: The current status. Plant Biotechnology Journal, 10: 1011–1025.

Meharg A.A. (2003): Variation in arsenic accumulation – Hyperaccumulation in ferns and their allies. New Phytologist, 157: 25–31.

Meneguelli-Souza A.C., Vitória A.P., Vieira T.O., Degli-Esposti M.S.O., Souza C.M.M. (2016): Ecophysiological responses of *Eichhornia crassipes* (Mart.) Solms to As^{5+} under different stress conditions. Photosynthetica, 54: 243–250.

Nikiforova V.J., Bielecka M., Gakière B., Krueger S., Rinder J., Kempa S., Morcuende R., Scheible W.R., Hesse H., Hoefgen R. (2006): Effect of sulfur availability on the integrity of amino acid biosynthesis in plants. Amino Acids, 30: 173–183.

Nunes-Nesi A., Sulpice R., Gibon Y., Fernie A.R. (2008): The enigmatic contribution of mitochondrial function in photosynthesis. Journal of Experimental Botany, 59: 1675–1684.

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.

Pavlík M., Zemanová V., Pavlíková D., Kyjaková P., Hlavsa T. (2017): Regulation of odd-numbered fatty acid content plays an important part in the metabolism of the hyperaccumulator *Noccaea* spp. adapted to oxidative stress. Journal of Plant Physiology, 280: 94–101.

Pavlíková D., Pavlík M., Procházková D., Zemanová V., Hnilička F., Wilhelmová N. (2014a): Nitrogen metabolism and gas exchange parameters associated with zinc stress in tobacco expressing an *ipt* gene for cytokinin synthesis. Journal of Plant Physiology, 171: 559–564.

Pavlíková D., Zemanová V., Procházková D., Pavlík M., Száková J., Wilhelmová N. (2014b): 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.

Pavlíková D., Pavlík M., Staszková L., Tlustoš P., Száková J., Balík J. (2007): The effect of potentially toxic elements and sewage sludge on the activity of regulatory enzyme glutamate kinase. Plant, Soil and Environment, 53: 201–206.

Raab A., Feldmann J., Meharg A.A. (2004): The nature of arsenic-phytochelatin complexes in *Holcus lanatus* and *Pteris cretica*. Plant Physiology, 134: 1113–1122.

ter Braak C.J.F., Smilauer P. (2002): CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5). Ithaca, Microcomputer Power, 500.

Tu C., Ma L.Q., Zhang W., Cai Y., Harris W.G. (2003): Arsenic species and leachability in the fronds of the hyperaccumulator Chinese brake (*Pteris vittata* L.). Environmental Pollution, 124: 223–230.

Van der Ent A., Baker A.J.M., Reeves R.D., Pollard A.J., Schat H. (2013): Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. Plant and Soil, 362: 319–334.

Vetterlein D., Wesenberg D., Nathan P., Bräutigam A., Schierhorn A., Mattusch J., Jahn R. (2009): *Pteris vittata* – Revisited: Uptake of As and its speciation, impact of P, role of phytochelatins and S. Environmental Pollution, 157: 3016–3024.

Zemanová V., Pavlík M., Pavlíková D., Hnilička F., Vondráčková S. (2016): Responses to Cd stress in two *Noccaea* species (*Noccaea praecox* and *Noccaea caerulescens*) originating from two contaminated sites in Mežica, Slovenia and Redlschlag, Austria. Archives of Environmental Contamination and Toxicology, 70: 464–474.

Zemanová V., Pavlík M., Pavlíková D. (2017): Cadmium toxicity induced contrasting patterns of concentrations of free sarcosine, specific amino acids and selected microelements in two *Noccaea* species. PLoS One, 12: e0177963.

Zhao F.J., Wang J.R., Barker J.H.A., Schat H., Bleeker P.M., McGrath S.P. (2003): The role of phytochelatins in arsenic tolerance in the hyperaccumulator *Pteris vittata*. New Phytologist, 159: 403–410.

Zhu Y.-R., Lü X.-Y., Wang S.-F., Wang N.-N., Wang Y. (2005): The relationship between photorespiration and senescence of *S. polyrrhiza* P143. Plant Science, 168: 1625–1632.

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