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Change in β-glucosidase activity in root zone of ferns under toxic elements soil contamination

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Abstract: The influence of toxic elements, such as arsenic (As), cadmium (Cd), lead (Pb), and zinc (Zn), in the root zone of As-hyperaccumulator *Pteris cretica* cv. Albo-lineata and non-As-hyperaccumulator *P. straminea*, on the enzymatic activity of β-glucosidase, dissolved organic carbon (C) in soil, toxic element accumulation in fern roots, and root biomass were evaluated in a pot experiment. Ferns were cultivated in soils from the locality of Suchdol (control) and Litavka (high contamination) for six months. For all toxic elements, an increasing trend in their contents in the roots was observed with soil contamination for both ferns. Differences between ferns were observed in As and Zn accumulation. *Pteris cretica* had a significantly higher As accumulation than *P. straminea*. Zinc accumulation in the roots showed an opposite trend. A significant difference between ferns was confirmed in the dissolved organic C content. Our results showed a significantly higher content of dissolved organic C in the *P. straminea* root zone than in *P. cretica*. The significant effect of toxic elements in the soil on β-glucosidase activity was observed. Toxic elements inhibited β-glucosidase activity in the root zone of *P. cretica*, and an increase in *P. straminea* was determined in the Litavka soil. The results suggest a higher sensitivity of *P. straminea* to toxic element contamination in soil, leading to increased β-glucosidase activity and increased dissolved organic C content.

Keywords: pollution; heavy metal; soil enzyme activity; microorganism; Pteridaceae

Soil contamination with toxic elements affects soil microbiology and causes changes in the interactions between plants and microorganisms. It also significantly affects soil characteristics, plant growth, vegetation type, and agricultural land production (Wahsha et al. 2017, Zeng et al. 2019, Aponte et al. 2020a, Majumder et al. 2022). The accumulation of toxic elements by plants results in entry into the food chain, which then becomes hazardous to human health (Pande et al. 2022).

Soil enzymes produced extracellularly by microorganisms are key participants in soil nutrient cycles and functional sustainability. They are sensitive to changes in the soil environment, such as contamination by toxic elements (Wahsha et al. 2017, Nkongolo and Narendrula-Kotha 2020, Cui et al. 2021, Majumder et al. 2022). Due to these characteristics, they can be used as biological indicators for evaluating soil health (Aponte et al. 2020b, Skowrońska et al. 2020).

The mineralisation of carbon (C) in the soil is crucial for soil quality, as it increases soil fertility. However, this is influenced by plant root exudation, microbial activities, and soil pH (Yu et al. 2021). The zone of plant roots also influences the activity and

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composition of soil microbial communities through their exudates (Duan et al. 2022). The microbial utilisation of root exudates by soil microorganisms is reduced in soils contaminated with toxic elements, which is reflected in soil enzyme activity (Ciadamidaro et al. 2014). β-glucosidase is one of the most important soil enzymes involved in the C cycle. It plays a key role in the last phase of the cellulose degradation process by hydrolysis of cellobiose, resulting in glucose, an important source of energy for the growth and activity of soil microorganisms (Adetunji et al. 2017). In addition to other soil enzymes, the activity of β-glucosidase is influenced by soil properties, such as organic matter content and pH (Aponte et al. 2020b). Many studies have confirmed the sensitivity of β-glucosidase activity to toxic elements, so it can be a good indicator of the impact of toxic elements on soil health (Zimmer et al. 2012, Strachel et al. 2018, Haddad et al. 2019). In addition, understanding changes in β-glucosidase activity can help explain C metabolism and its cycle (Verma and Pandey 2022).

In this study, differences in root zones between the As-hyperaccumulator *Pteris cretica* cv. Albo-lineata and non-As-hyperaccumulator *P. straminea* were evaluated after six months of growth in non-contaminated soil and soil contaminated with toxic elements. This study aimed (1) to determine the effect of toxic elements on the activity of β-glucosidase and C content in the root zone and (2) to compare the accumulation of toxic elements in the roots of hyperaccumulating and non-hyperaccumulating species. This study improves the understanding of the processes in the root zone of plants accumulating significant amounts of toxic elements.

MATERIAL AND METHODS

Design of the experiment and plant material. A pot experiment was performed with 5 kg of Haplic Chernozem and Gleyic Fluvisol from two localities in the Czech Republic (Table 1). Each soil was replicated six times. Three pots of each soil contained *Pteris cretica* (L.) cv. Albo-lineata (As-hyperaccumulator), and another three pots contained *P. straminea* (Mett. ex Baker; non-As-hyperaccumulator). The used soils were fertilised with N, P and K (0.5 g N/kg soil in the form of NH_4NO_3 and 0.16 g P, and 0.4 g K/kg soil in K_2HPO_4). Each pot contained one plant. Plants were grown under greenhouse conditions (natural photoperiod; day/night temperature 22–24 °C/15–18 °C; relative humidity ~60%) (Zemanová et al. 2022). Plants and soil were collected after 180 days. The roots were rinsed with demineralised water, dried with filter paper, weighed, and dried in an oven to a constant weight (three days at 40 °C) and homogenised for element analysis. Samples of the root zone (defined as soil adhering to the roots after shaking) were collected during plant harvesting (soil was shaken off roots, homogenised and two subsamples were col-

Table 1. Basic characteristics and toxic element content of experimental soils

	Suchdol (50°8'8''N, 14°22'43''E)	Litavka (49°43'N, 14°0'E)
Soil type	Haplic Chernozem	Gleyic Fluvisol
Soil texture	silt loam	sandy loam
Sand (%)	26.0	56.5
Silt (%)	71.8	34.8
Clay (%)	2.2	8.7
Bulk density (g/cm^3)	1.57	1.33
pH_{H_2O}	7.1 ± 0.1	5.4 ± 0.1
CEC (mmol$_+$/kg)	230.1 ± 5.0	109.0 ± 31.9
C_{org} (%)	1.8 ± 0.3	3.6 ± 0.4
DOC (mg/kg)	153.0 ± 3.4	317.3 ± 19.3
As_{total} (mg/kg)	18.1 ± 1.0	283.9 ± 7.7
Cd_{total} (mg/kg)	0.4 ± 0.01	37.4 ± 1.1
Pb_{total} (mg/kg)	32.1 ± 0.7	2 361.2 ± 32.4
Zn_{total} (mg/kg)	85.5 ± 2.5	3 496.6 ± 60.2

CEC – cation-exchange capacity; DOC – dissolved organic carbon

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lected). Before analysis, the soil was sieved (< 2 mm), homogenised, lyophilised, and stored at –20 °C.

Plant analysis. The content of As, Cd, Pb, and Zn in the roots was determined using an Agilent 720 inductively coupled plasma optical emission spectrometer (ICP-OES; Agilent Technologies Inc., Torrance, USA) after low-pressure microwave digestion. Homogenised dry plant material (0.5 ± 0.05 g) was digested in 10 mL of a 4:1 (*v*/*v*) mixture of HNO_3 and H_2O_2 in an Ethos 1 device (MLS GmbH, Leutkirch im Allgäu, Germany). After cooling, the digested sample was diluted to 50 mL with demineralised water. Certified reference material (CRM NIST 1573a Tomato leaves) was mineralised under the same conditions for quality assurance.

Soil analysis. β-glucosidase activity in the root zone was determined following Hřebečková et al. (2019) with several modifications. The lyophilised soil (0.2 ± 0.002 g) was mixed with 20 mL phosphate buffer (molar concentration 50 mmol/L, pH 7.0). The mixture was homogenised for 30 s at 8 000 rpm. Homogenised samples before measurement were stored for 24 h in a refrigerator. To determine the activity of β-glucosidase, solutions of 10 mL of dimethyl sulfoxide and 9.30 mg 4-methylumbelliferyl-β-D-glucopyranoside (molar concentration 2.75 mmol/L) were prepared. The homogenised suspension (200 μL), a substrate solution (40 μL), and additional dimethyl sulfoxide (20 μL) were pipetted into the relevant wells in the microplate. The microplate was placed in a Robbins Scientific® 2000 micro hybridisation incubator (SciGene, Sunnyvale, USA) at 40 °C for 5 min. Fluorescence was then measured using the Tecan Infinite® M200 (Tecan Austria GmbH, Salzburg, Austria). The microplate was again placed in the incubator for 120 min, and fluorescence was measured. Enzymatic activity was calculated from the difference between the initial and final values (μmol/h/g).

The dissolved organic C was extracted with demineralised water (1:5, *w*/*v*; 30 min shaking; 12 h equilibration; centrifugation at 5 000 rpm) and determined by segmented flow analysis with infrared detection on a SKALAR^{plus}SYSTEM (Skalar Analytical B.V., Breda, the Netherlands).

The pH was measured in water extracts (1:5, *w*/*v*; 60 min shaking; 1 h equilibration) using a pH 8+ DHS meter (*XS* Instruments, Carpi, Italy).

Statistical analysis. Statistical processing of the results was performed using the programme Statistica 12.0 (StatSoft, Tulsa, USA) and the CANOCO 4.5 programme (ter Braak and Šmilauer 2002). To determine significant differences among soils and plants, a one-way analysis of variance (ANOVA) was performed, followed by a posthoc comparison with Fisher's *LSD* (least significant difference) test ($P < 0.05$). A Pearson's linear correlation (r; $P < 0.05$) was performed to assess the relationships between root biomass and toxic elements in the soil. Data of technical replicates (three per analysis) were averaged for three independent biological repeats (pots). The data of roots and soil were presented as the average of three independent repeats with standard deviation. The data of β-glucosidase activity was presented as the average of two subsamples and three independent repeats (pots) with standard deviation.

RESULTS AND DISCUSSION

Pteris cretica had a significantly higher root biomass yield compared to *P. straminea* (Figures 1–2). There was an increasing trend in *P. cretica*, with a 65% increase in Litavka soil, in contrast to Suchdol soil. In contrast to these results, Zemanová et al. (2022) indicated no statistically significant differences in the root yield of *P. cretica* grown on As-contaminated soils compared with the control. Popov et al. (2021) confirmed no significant changes in the roots of this fern with an As dose of 100 mg/kg soil, while root yield on soil contaminated with a higher As dose (250 mg/kg soil) was significantly reduced. The correlations of root biomass yield with As ($r = 0.95$, $P < 0.001$), Cd ($r = 0.94$, $P < 0.001$), Pb ($r = 0.95$, $P < 0.001$), and Zn ($r = 0.94$, $P < 0.001$) were determined. In contrast

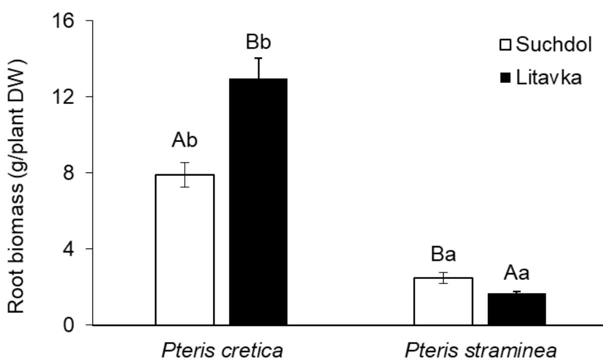

Figure 1. Root biomass yield of *Pteris cretica* and *P. straminea* in Suchdol (control) and Litavka (high contamination). The data represent the mean ± standard deviation ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among soils (uppercase letters) and plants (lowercase letters). DW – dry weight

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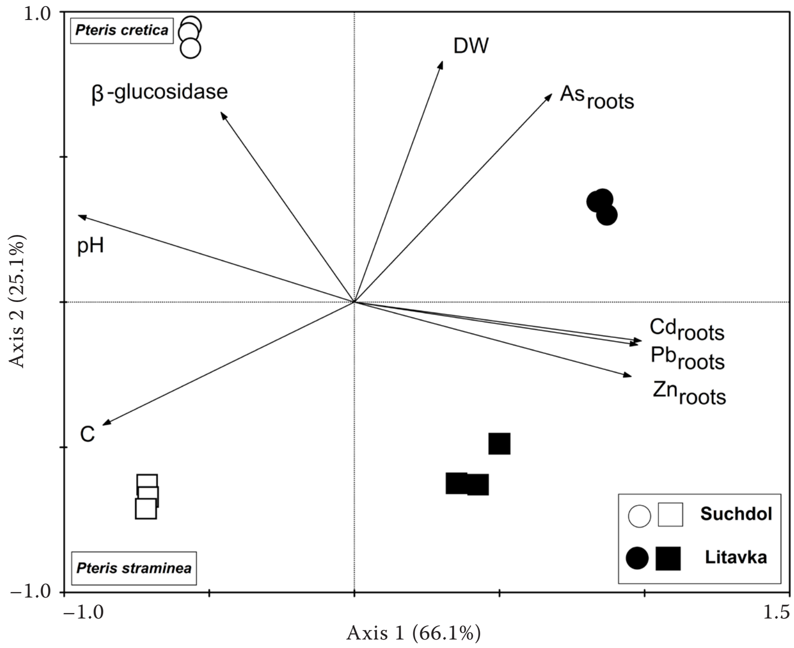

Figure 2. Ordination bi-plot of selected parameters in roots and the root zone of the As-hyperaccumulator *Pteris cretica* cv. Albo-lineata and non-As-hyperaccumulator *P. straminea* after a six-month growing cycle. The first axis of the PCA explains 66.1%, and both axes together explain 91.2% of the variability of all analysed data. The length and direction of the vectors indicate the strength of the vector effect and the correlation between the vectors, respectively. A long vector for a particular variable indicates that it greatly affects the results of the analysis, while the opposite is true for a short vector. An angle of < 90° between the vectors indicates that they are positively correlated. An angle of > 90° between two vectors indicates that they are not positively correlated. C – dissolved organic carbon; DW – yield of root biomass; pH – pH of soil water extracts

to *P. cretica*, a decreasing trend was observed for *P. straminea*, with a 32% decrease in Litavka compared to Suchdol (Figure 1). Correlations confirmed the impact of As ($r = -0.87$, $P = 0.02$), Cd ($r = -0.88$, $P = 0.02$), Pb ($r = -0.88$, $P = 0.02$), and Zn ($r = -0.88$, $P = 0.02$) on the formation of root biomass. Singh et al. (2009) described the difference in the effect of As soil contamination on root growth between *P. vittata* (As-hyperaccumulator) and *P. ensiformis* (non-As-hyperaccumulator), and root biomass yield was negatively affected mainly in *P. ensiformis* plants.

For all toxic elements (As, Cd, Pb, and Zn), an increasing trend in their content in the roots was observed with a dose in the soil for both ferns (Table 2, Figure 2). Significant differences between ferns were determined in As root content. The content of this element was 7.4–9.5 times higher in the roots of *P. cretica*. The results confirmed *P. cretica* as

Table 2. Toxic element content (mg/kg dry weight) in roots of *Pteris cretica* and *P. straminea* in Suchdol (control) and Litavka (high contamination)

	P. cretica		*P. straminea*	
	Suchdol	Litavka	Suchdol	Litavka
As	42.0 ± 2.9^{Ab}	143.2 ± 19.1^{Bb}	4.4 ± 0.1^{Aa}	19.3 ± 8.7^{Ba}
Cd	0.3 ± 0.04^{Ab}	14.4 ± 1.0^{Ba}	0.2 ± 0.05^{Aa}	13.4 ± 2.1^{Ba}
Pb	3.4 ± 0.6^{Aa}	480.5 ± 87.0^{Bb}	3.8 ± 0.002^{Aa}	225.1 ± 47.7^{Ba}
Zn	31.9 ± 3.3^{Aa}	1 315.9 ± 113.8^{Ba}	44.7 ± 0.7^{Ab}	1 570.3 ± 218.9^{Bb}

The values represent the mean ± standard deviation ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among soils (uppercase letters) and plants (lowercase letters)

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an As-hyperaccumulating fern (Meharg 2003, Eze and Harvey 2018). The results showed a higher Cd content in the root biomass of both ferns grown on Litavka soil, in contrast to Suchdol soil. A statistically significant difference between ferns was confirmed only for Suchdol soil. An increasing trend of Pb accumulation in the roots of *P. cretica* grown on Litavka soil compared to *P. straminea* was determined. Lead is passively absorbed into the root system, and structures within roots form barriers to Pb transport from roots to aboveground biomass (Tung and Temple 1996). Wan et al. (2014) tested the co-accumulation of As and Pb in *P. vittata* in a hydroponic experiment. The interaction between As and Pb in *P. vittata* was confirmed. According to these authors, Pb increased As absorption in the epidermis of *P. vittata* rhizoids, and more than 90% of Pb was restricted to the epidermis. Unlike congenital As accumulation, Pb accumulation in *P. vittata* may be an adaptive trait.

In both ferns, an increasing trend of Zn accumulation was confirmed; in *P. cretica*, the increase in Litavka compared to Suchdol was 41-fold. The same was the case with *P. straminea*, where there was a 35-fold increase. *Pteris straminea* accumulated significantly more Zn than *P. cretica* in both soils (Table 2, Figure 2). The results showed high Zn accumulation in the roots of both ferns and confirmed the findings of Cao et al. (2004). According to Roccotiello et al. (2010), the ferns *Polypodium cambricum* and *P. vittata* can uptake a high Zn quantity. The low translocation factor of both species indicated Zn sequestration in the root system. The content of Zn as a cofactor of the antioxidative enzyme superoxide dismutase is related to the reduction of oxidative stress induced by toxic elements (Cao et al. 2004). This explains the high Zn uptake by both ferns.

In the root zone of both ferns, the effect of toxic elements on soil quality was determined in relation to dissolved organic C and β-glucosidase activity. PCA analysis visualised in the PCA diagram (Figure 2) showed that the first ordination axis explained 65.8% of all analysed data variability and divided the Suchdol soil group on the left side from the Litavka soil group on the right side. This division indicated a large effect of soil and toxic element contamination on the studied parameters. For both soils, the location of fern species marks (circle and square) in the different parts of the diagram indicated a high effect of species on the recorded data. One-way ANOVA confirmed a significant difference between ferns in dissolved organic C content, which decreased in Litavka soil in contrast to Suchdol (Figure 3). In *P. cretica* and *P. straminea*, the decreases were 22% and 39%, respectively. Zemanová et al. (2020, 2022) confirmed that soil contamination affected physiological and morphological changes in *P. cretica* roots, as well as changes in soil quality of the root zone, and the dissolved organic C content decreased with increasing As soil contamination. In contrast to our results, Zhan et al. (2018) observed a significant increase in dissolved organic C in the root zone of the phytostabiliser *Athyrium wardii* in soils contaminated with Cd and Pb. Our results showed a significantly higher content of dissolved organic C in the *P. straminea* root zone compared to *P. cretica* (by 45% Suchdol, 13% Litavka). This can indicate higher production of exudates in the root zone of *P. straminea*. As mentioned previously, plants produce higher amounts of exudates in response to contaminated soil. Li et al. (2013) reported that the hyperaccumulator *Sedum alfredii* produced more exudates as a significant source of dissolved organic C due to higher Zn stress. A similar study was conducted by Wei and Twardowska (2013), who used the Cd-hyperaccumulator *Rorippa globosa* and the non-hyperaccumulator *R. palustris* to examine the effect of Cd contamination on dissolved organic C in the root zone. According to these authors, the hyperaccumulator had a significantly higher concentration of dissolved organic C in the root zone compared to the non-hyperaccumulator. These findings are in opposition to our results. We speculate that the source of the increased amount

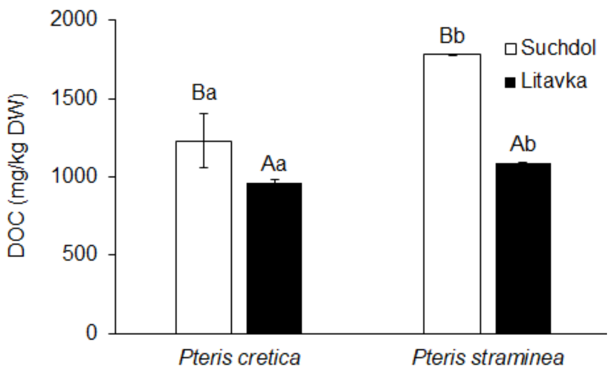

Figure 3. Dissolved organic carbon (DOC) in the root zone of *Pteris cretica* and *P. straminea* in Suchdol (control) and Litavka (high contamination) soil. The data represent the mean ± standard deviation (n = 3). Different letters indicate significant differences ($P < 0.05$) among soils (uppercase letters) and plants (lowercase letters). DW – dry weight

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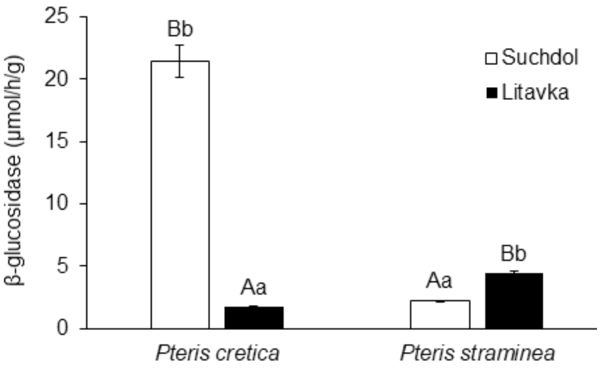

Figure 4. Activity of β-glucosidase in the root zone of *Pteris cretica* and *P. straminea* in Suchdol (control) and Litavka (high contamination) soil. Data represent the mean ± standard deviation (n = 3). Different letters indicate significant differences ($P < 0.05$) among soils (uppercase letters) and plants (lowercase letters)

of soluble organic C in the root zone of *P. straminea* is the dead cells of root hairs.

Changes in dissolved organic C in the root zone can affect the enzymatic activity of soil microorganisms (Jia et al. 2015). Huang et al. (2020) examined soil organic C fractions and their relationship to soil microbial properties in the rhizosphere of *Robinia pseudoacacia* seedlings in Cd-contaminated soils. They confirmed that β-glucosidase was related to soil organic C fractions because β-glucosidase produced by microorganisms plays a key role in the mineralisation process. β-glucosidase is involved in the degradation of cellulose, the main component of plant cell walls, in soil (Turner et al. 2002). This enzyme activity was significantly depressed in the root zone of *P. cretica* grown on Litavka soil, in contrast to Suchdol soil (Figures 2 and 4). The opposite trend was observed in the root zone of *P. straminea*. According to Ghori et al. (2019), plants markedly increase root exudate production in response to a contaminated environment. This subsequently increased the activity of the microorganisms, which then released more β-glucosidase for glucose hydrolysis (Cui et al. 2021). Similarly, Duan et al. (2022), who studied the activity of soil enzymes in the root zone of *Medicago sativa* growing in soils contaminated with Cd, Pb, Zn, and Cu, observed a significant increasing trend. We speculated that this increased β-glucosidase activity is not the result of increased exudate production in the root zone in response to contaminated soil, but it resulted from the decomposition of polysaccharides forming the structure of the roots.

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