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Arsenic-induced response in roots of arsenic-hyperaccumulator fern and soil enzymatic activity changes

*Veronika Zemanová[1,2], Daniela Pavlíková[1], Milan Novák[1], Petre I. Dobrev[3], Tomáš Matoušek[4], Václav Motyka[3], Milan Pavlík[1,2]**

[1]*Department of Agro-Environmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*
[2]*Isotope Laboratory, Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic*
[3]*Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic*
[4]*Department of Trace Element Analysis, Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic*
**Corresponding author: pavlik@biomed.cas.cz*

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Abstract: In a pot experiment, arsenic-hyperaccumulating *Pteris cretica* cv. Albo-lineata plant ferns were cultivated and exposed to low and high doses of arsenate (20 and 100 mg As/kg, respectively) for six months. Physiological and morphological changes of roots, as well as changes in soil quality of the root zone and bulk soil (water-soluble fraction of elements and activity of soil enzymes), were determined. The results showed that the accumulation of inorganic As, mainly in the form of As^{3+}, did not significantly affect the yield of roots, but caused changes in root morphology (deformation of root cell walls due to lignification) and metabolism (decrease of auxin indole-3-acetic acid and 2-oxoindole-3-acetic acid contents). Although the soil quality results varied according to the As dose, there was a clear difference between the root zone and the bulk soil. The activities of enzymes in the root zone were greater that those in the bulk soil. The results showed a significant influence of the high dose of As (100 mg As/kg), which decreased the activity of arylsulfatase, nitrate reductase, and urease in the root zone, while a decrease in acid phosphatase and nitrate reductase was observed in the bulk soil. The water-soluble fractions of As, organic nitrogen, nitrate nitrogen and organic carbon were significantly affected by the high dose of As.

Keywords: phytohormone; contamination; manganese; metalloid; risk/toxic element

Arsenic (As) a non-essential metalloid is considered as one of the environmentally hazardous metals, which is notoriously toxic to plants. Furthermore, As is an important and complicated element to investigate because of its redox activity and severe toxicity to organisms, and its proclivity to be methylated. Despite its non-essentiality, plants can freely absorb As through different transporters, resulting in its transfer from the soil to the food chain (Ali et al. 2022). According to these authors, in recent years, significant progress has been made in understanding the molecular mechanisms of plant As uptake and detoxification. Plants can selectively assimilate specific As forms *via* distinct pathways and transporters. In addition, in *Pteris cretica* ferns (cvs. Albo-lineata or Parkerii), two important genes have been identified. These genes are generally associated with the tolerance of As-hyperaccumulators to As. The

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gene, arsenate reductase (Pc*ACR2*) detoxifying the reduction of As^{5+} to As^{3+} has been identified in both cultivars. The second one was an arsenite transporter (Pc*ACR3*) that translocating reduced As from the roots through the xylem to the above-ground parts (fronds) of ferns (Popov et al. 2021). The cellular detoxification of As^{3+} in non-hyperaccumulation plants involves complexation with phytochelatin proteins and storage of these complexes in vacuoles (Modal et al. 2022). In both hyperaccumulating cultivars, only one phytochelatine (PC2) was identified from all phytochelatine analogues. The amount of As bound by PC2 was low and it was an insignificant portion of the total As content accumulated in the roots and the fronds. The PC2 content was higher in the roots than in the fronds, and its content was 200 times higher in cv. Parkerii in contrast to the cv. Albo-lineata. The concentration of PC2 increased in both cultivars with an As dose and growing period (Popov et al. 2021). Organic forms of As were also identified as mono-, di-, and trimethylarsenite/arsenate. These methylated forms were determined only in the cv. Parkerii and their contents very fluctuated (Popov et al. 2021). These results showed that the As reduction and the accumulation of methylated As forms in *P. cretica* take place in the roots.

Arsenic stress in plants can trigger various harmful effects at morphological, biochemical, physiological, and molecular levels (Mondal et al. 2022). It also inhibits the rate of photosynthesis and transpiration by reducing the content of photosynthetic pigments and gaseous exchange parameters that eventually induce leaf senescence (Ali et al. 2022). The effect of As accumulation on photosynthetic parameters of *P. cretica* was clarified in previous papers (Pavlíková et al. 2020, Zemanová et al. 2021a,b). Arsenic doses generally showed a decrease of net photosynthetic rate, stomatal conductance, transpiration rate, the maximum quantum yield of PSII, and photosynthetic pigments. Additionally, a significant increase of amino acid glycine (precursor of cytokinins) was observed in fronds of *P. cretica*. The ratio of amino acids glycine (Gly)/serine (Ser) is a marker for the rate of photorespiration because these amino acids are the only two directly in the photorespiratory C recycling pathway. This ratio increased with net CO_2 uptake. In a study by Zemanová et al. (2021a), the Gly/Ser ratio increased in *P. cretica* fronds, while the net photosynthetic rate decreased.

Furthermore, Pavlíková et al. (2020) showed that increasing As hyperaccumulation increased the content of $N\text{-}NO_3^-$ and the sum of bioactive cytokinins (bCKs) as well as storage forms of amino acids (sAAs). This study compared the As-hyperaccumulator and non-As-hyperaccumulator *Pteris* ferns and as first showed that increased accumulation of bCKs correlated with an increase in accumulation of $N\text{-}NO_3^-$ and especially sAAs (glutamine and asparagine). Several studies have revealed that As can trigger epigenetic DNA modifications such as methylation and demethylation which can regulate the expression of potential stress-resilient genes (Ali et al. 2022). A comparison of the effect of increasing As accumulation in young and old leaves in relation to photosynthetic parameters and DNA demethylation is discussed in detail in the paper by Zemanová et al. (2020). The authors explained the reasons why demethylation was decreased more significantly in older leaves than in young leaves in connection with the increasing As content in fronds of *P. cretica*. These results showed the main role of salicylic acid as a demethylating agent of DNA (Kiselev et al. 2015) and as a regulator of jasmonic acid. Jasmonic acid determines the activation of the biosynthesis of glutathione (Wasternack and Hause 2013), which is the source of the formation of phytochelatins and is an essential component of the ascorbate-glutathione cycle. However, many gaps remain in the fundamental understanding of the As perception and the signal transduction pathways in plants (Ali et al. 2022).

In plants, the root is the first tissue to be exposed to As, where the metalloid prevents its expansion and proliferation (Ali et al. 2022). The root zone is the site of great activity between roots, soil particles, and the soil microbial community. Roots influence soil structure, aeration, and microbial activity (Bertin et al. 2003). Root systems exhibit phenotypic plasticity as they develop in a heterogeneous soil environment and plants adapt to different environmental conditions by changing root architecture. Root zone processes play an important role in nutrient cycling and C sequestration. Optimal N supply can promote the increase in root density, quality, and quantity, but a large amount of nitrogen in the soil inhibits root growth (Xiong et al. 2021). Singh et al. (2009) found that *Pteris vittata* L. (As-hyperaccumulator) and *P. ensiformis* Burm. (non-As-hyperaccumulator) differed in arsenic accumulation in the plant and the effects of As contamination on root growth. The reduction in root biomass was greater in *P. ensiformis* than in *P. vittata* plants. This difference in the decrease in root growth could be related to the difference in nitrate uptake between the two species.

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The results of Krishnamurthy and Rathinasabapathi (2013) confirmed the inhibition of primary root elongation and lateral root initiation by As. Both processes are regulated by auxin, thus the authors suggested a significant role of auxin in root response to As. Regulation of auxin homeostasis, such as indole-3-acetic acid (IAA), optimises root formation and developmental processes, allowing them to overcome abiotic stress (Piacentini et al. 2020). The IAA homeostasis includes biosynthesis, transport (Tripathi et al. 2021), inactive IAA conjugation with amino acids or sugars (Magidin et al. 2003), and IAA oxidation processes induced by H_2O_2 or peroxidases (Savitsky et al. 1999). Phylogenetic analyses have shown that selection pressure from As-contaminated soils resulted in a change in the bacterial profile in the root zone of *P. vittata* by increasing the number of As-resistant bacteria compared to soils without hyperaccumulators (Abou-Shanab et al. 2020). Isolates of these bacteria reduced As content by 95% and produced the growth phytohormone auxin IAA from the amino acid tryptophan.

Soil microbes are the source and sink of plant nutrients and are used in nutrient cycling (Das et al. 2017). Soil enzyme activity is a sensitive parameter of microbial nutrient cycling and can be used as a significant indicator of metal/metalloid pollution in soil (Xian et al. 2015). The changes in the root zone and bulk soil of *P. cretica* are expected to affect soil quality and nutrient cycling due to As-toxicity. In this study, the differences in root zone and bulk soil quality and changes in roots of As-hyperaccumulating fern *P. cretica* cv. Albo-lineata (*Pc*-Al) grown under two different As treatments during the six-month growing season were evaluated. The objectives of the study were to (1) characterise the root biomass and morphological changes of *Pc*-Al; (2) determine the activity of soil enzymes in the root zone and bulk soil of *Pc*-Al, and (3) determine the water-soluble fraction of selected elements in the root zone and bulk soil of *Pc*-Al under two As doses (20 and 100 mg/kg) selected for low and high soil contamination. This study improves the understanding of the effects of As on As-hyperaccumulating ferns and associated soil quality.

MATERIAL AND METHODS

Experimental soil, plant material and experimental design. The experiment was performed in plastic pots, each filled with 5 kg of Haplic Chernozem from a non-polluted area in Prague-Suchdol, Czech Republic (total organic carbon 18.3 g/kg, cation-exchange capacity 258 $mmol_+$/kg, pH_{KCl} 7.1, pseudo-total As 19 mg/kg, the fraction of water-soluble As 0.19 mg/kg, and As extraction efficiency 20%). Each kilogram of soil was mixed with 0.5 g N, 0.16 g P, and 0.4 g K (in the form of NH_4NO_3 and K_2HPO_4). Arsenic (20 and 100 mg/kg soil) was artificially added to the soil in an aqueous solution of Na_2HAsO_4. A control treatment was maintained in which As was not added. Each treatment was replicated three times and arranged in random blocks.

The spiked soil was used for a six-month growth period of *Pteris cretica* (L.) cv. Albo-lineata. Plants were purchased at the 15-frond stage from the Tulipa Praha Garden Center (Czech Republic) and grown under greenhouse conditions (natural photoperiod; day/night temperature 22–24 °C/15–18 °C; relative humidity ~60%). Each pot contained one plant. After 185 days of growth, *Pc*-Al plants were harvested. The roots were detached and collected, and the soil adhering to the roots after shaking was defined as the root zone, while the bulk soil was defined as the unvegetated soil adjacent to the plants. The moist soil was sieved (< 2 mm), homogenised, and stored at 4 °C for analysis. Roots were rinsed with demineralised water and blotted dry with filter paper. After the root samples were weighed, they were divided, and one part was immediately frozen in liquid nitrogen and stored at –80 °C until analysis for phytohormones, while the other part was oven-dried (three days at 40 °C) until constant weight and homogenised for analysis of the elements.

To investigate possible morphological changes in roots caused by As, cross-sections through an adventitious root were performed using a Nikon E 200 microscope equipped with a DS camera head and the NIS-Elements application (Nikon Instruments, Inc., Melville, USA).

Plant and soil analysis. In the roots, As and Mn content was determined using an Agilent 720 inductively coupled plasma optical emission spectrometer (ICP-OES; Agilent Technologies Inc., Santa Clara, USA) after low-pressure microwave digestion according to Pavlíková et al. (2020). Arsenic species in roots were determined as described previously (Popov et al. 2021). Extraction, purification, and quantification of auxins in roots were performed according to the previously published method (Zemanová et al. 2019).

Determination of soil enzyme activity was modified for acid phosphatase (AP), arylsulfatase (ARY), urease (UR) and nitrate reductase (NR) in 2.0, 1.0, 2.5, and 2.5 g soil, respectively. The *p*-nitrophenol

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was determined spectrophotometrically after hydrolysis of *p*-nitrophenyl phosphate and *p*-nitrophenyl sulfate. Urease activity was determined spectrophotometrically by a modified indophenol reaction. We determined soil enzymes activities according to previously published methods (Balík et al. 2007, Kotková et al. 2008, Kandeler et al. 2011), which calculated individual enzyme activity at a different time – 1 h for AP and ARY, 24 h for NR and 2 h for UR. Therefore, we used the same units for our results. The activity of all enzymes was measured using a Lambda 25 UV/VIS spectrophotometer (PerkinElmer Inc., Waltham, USA).

The water-soluble fraction of the elements was extracted with demineralised water (1 : 5, *w*/*v*; 30 min shaking; 12 h equilibration; centrifugation at 5 000 rpm). The As content in the supernatant was determined by ICP-OES (Agilent 720; Agilent Technologies Inc., Santa Clara, USA), and the contents of soluble organic C and N and nitrate-nitrogen ($N\text{-}NO_3^-$) were determined by segmented flow analysis with infrared detection on a SKALARplusSYSTEM (Skalar Analytical B.V., Breda, the Netherlands).

Statistical analysis. Statistical processing of the results was performed using Statistica 12.0 (StatSoft, Tulsa, USA) program. All data were tested for homogeneity of variance and normality (Levene and Shapiro-Wilk test). The data are presented as the mean ± standard deviation ($n = 3$). One-way ANOVA followed by post-hoc comparison with Fisher's *LSD* (least significant difference) test ($P < 0.05$) was used to identify significant differences between (1) treatments (lower-case) and (2) root zone and bulk soil of each treatment (asterisks). Correlation analysis was performed using Pearson's linear correlation (r; $P < 0.05$).

RESULTS AND DISCUSSION

Total As content in roots of *Pc*-Al was treatment-dependent (Figure 1A). Arsenic content increased with increasing As dose in the soil, as shown by the correlation ($r = 0.94$, $P < 0.001$). Its accumulation in roots of the As100 treatment was 44-fold higher compared with the control and exceeded 1 000 mg As/kg dry weight, which is the limit for identifying plants as As-hyperaccumulators (Ma et al. 2001). The results of As species determination show that As^{3+} is both a detoxified and the major As storage form in the roots of *Pc*-Al (Figure 1B), with a range of 76.5–99.4% and a treatment-dependent increase ($r = 0.82$, $P < 0.01$). The proportion of the other identified As storage form, As^{5+}, decreased with increasing soil As dose ($r = -0.82$, $P < 0.01$). Our results are consistent with the study by Popov et al. (2021), which showed that the ratio of As^{3+} to As^{5+} in *Pc*-Al roots gradually increased as a function of As exposure. The study by Duan et al. (2005) with the As-hyperaccumulator *P. vittata* also documented that imported As^{5+} was mainly reduced in roots. It was reported that in *Pteris* spp., As^{3+} and As^{5+} were the major As storage forms in As-hyperaccumulating plants exposed to high As concentrations (Chen et al. 2016).

The dry weight of *Pc*-Al root biomass was negatively affected by As20 and As100 treatments, but the 16.5% and 25% decreases, respectively, were not statistically significant compared with the control. Our results suggest that *Pc*-Al roots have a high tolerance to low and high As doses in soil due to their ability to hyperaccumulate As, especially As^{3+} (Chen et al. 2016). The results are partially consistent with the study by Popov et al. (2021). These

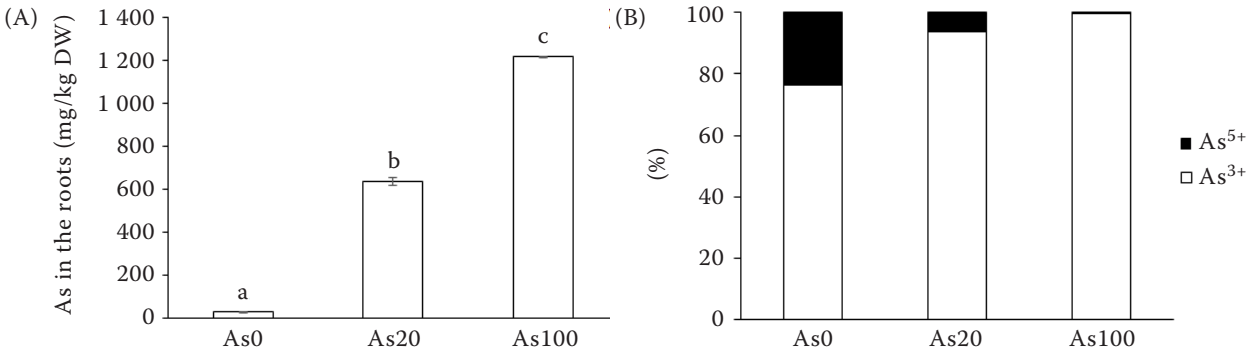

Figure 1. (A) Arsenic (As) accumulation and (B) speciation in roots of *Pteris cretica*. Lower-case letters indicate significant differences by Fisher's *LSD* (least significant difference) test ($P < 0.05$). As0 – control; As20 – 20 mg As/kg soil; As100 – 100 mg As/kg soil; DW – dry weight

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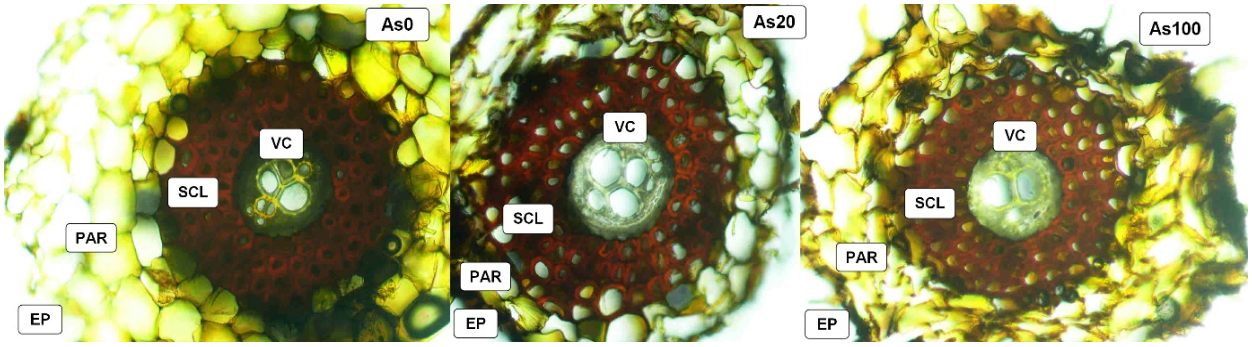

Figure 2. Cross-section through an adventitious root of *Pteris cretica*. As0 – control; As20 – 20 mg As/kg soil; As100 – 100 mg As/kg soil; EP – epidermis; VC – vascular cylinder; SCL – sclerenchymatous inner cortex; PAR – parenchymatous outer cortex

authors observed no significant changes in *Pc*-Al roots by high As dose (100 mg/kg soil), while the higher As dose (250 mg/kg soil) showed a significant reduction in root biomass, indicating a limitation in the As hyperaccumulation capacity of these ferns. Despite the tolerance of *Pc*-Al roots to As toxicity, cross-sectional analysis through adventitious roots showed morphological changes due to As (Figure 2). Lignification deformed the cell walls of the roots, especially in the parenchymatous outer cortex due to the increased As dose. Compared to the control, the roots of the As treatments showed thinning of the sclerenchymatous inner cortex as well as a reduction in the average tracheid metaxylem in the vascular cylinder. Similar results were observed by Zemanová et al. (2020) in *Pc*-Al roots grown at two As doses – 100 and 250 mg/kg soil.

Among auxins, the active form IAA and its major primary catabolite 2-oxoindole-3-acetic acid (OxIAA), were determined in the *Pc*-Al roots (Figure 3A, B), and the OxIAA level was higher than that of IAA in all treatments. The toxicity of a high dose of As in the soil negatively affected the levels of both auxin forms, as shown by our results. The IAA levels decreased by 49% compared with the control (Figure 3A), whereas OxIAA levels decreased by 83% compared with the control (Figure 3B). These trends are consistent with the negative correlation between soil As dose and IAA content ($r = -0.95$, $P < 0.001$) as well as OxIAA content ($r = -0.87$, $P < 0.01$).

The high portion of reduced As^{3+} from As^{5+} (Figure 1B) causes oxidative stress (Abbas et al. 2018), which induces the plant peroxidases of class III. These peroxidases catalyse lignification, thus increasing root adaptation to abiotic stresses including As (Kidwai et al. 2019), and also inducing catabolism of auxins (Cosio and Dunand 2009). These findings are consistent with our results (Figures 1 and 3) that show that IAA decreases due to oxidative stress after induction of stress metabolism. The decrease in IAA and OxIAA concentration was related to Mn content, as shown in the results in Table 1. This indicates

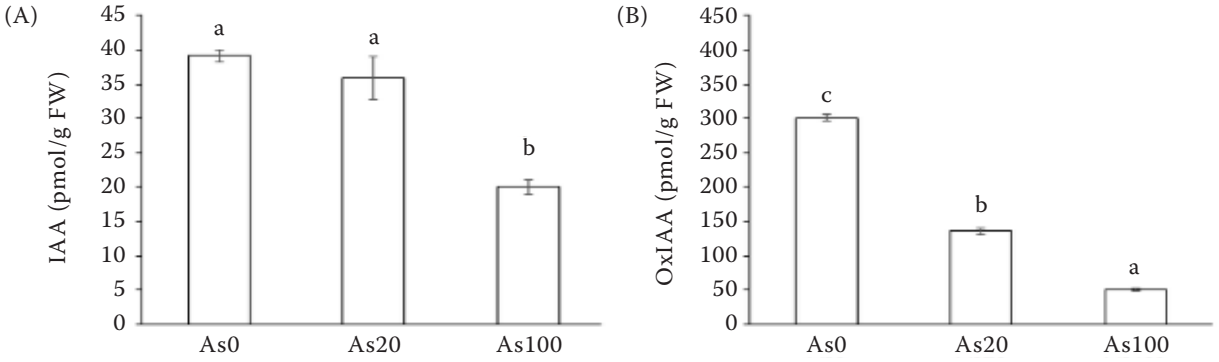

Figure 3. (A) Levels of indole-3-acetic acid (IAA) and (B) 2-oxoindole-3-acetic acid (OxIAA) in roots of *Pteris cretica*. Lower-case letters indicate significant differences by Fisher's *LSD* (least significant difference) test ($P < 0.05$). As0 – control; As20 – 20 mg As/kg soil; As100 – 100 mg As/kg soil; FW – fresh weight

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Table 1. Content of manganese (Mn) in roots of *Pteris cretica* and correlation with selected parameters

Treatment	Mn (mg/kg DW)	*r* (As × parameter)				
As0	12.6 ± 1.5[a]	IAA	OxIAA	As dose	As	As^{3+}
As20	17.2 ± 2.7[a]	–0.87**	–0.81**	0.96***	0.88**	0.76*
As100	43.1 ± 7.3[b]					

Lower-case letters indicate significant differences by Fisher's *LSD* (least significant difference) test ($P < 0.05$). As – arsenic; IAA – indole-3-acetic acid; OxIAA – 2-oxoindole-3-acetic acid; As0 – control; As20 – 20 mg As/kg soil, As100 – 100 mg As/kg soil; DW – dry weight; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

that the increase in Mn content is related to its role in oxidation-reduction processes as a cofactor of metalloenzymes, including peroxidases (Broadley et al. 2012). Mn^{2+} oxidation catalysed by manganese peroxidases (Sutherland and Aust 1996) partially enables the maintenance of oxidation-reduction (redox) homeostasis in the plant cell, which was caused by the high As^{5+} reduction to As^{3+} in *Pc*-Al roots (Figure 1). In plant cells, Mn played another role in auxin catabolism. IAA oxidation or oxidative decarboxylation is either induced by enzymes, when Mn is a cofactor of peroxidases, or non-enzymatically in the presence of Mn or reactive oxygen species, i.e. H_2O_2 (MacLachlan and Waygood 1956, Savitsky et al. 1999).

The effect of As dose on soil quality was determined in terms of the activities of soil enzymes ARY, AP, NR, and UR (Figure 4) and the water-soluble fraction of As, N, C, and N-NO_3^- (Figure 5). The activities of soil enzymes such as ARY, AP, and UR are sensitive to the presence of risk elements (Xian et al. 2015). Our results of soil enzyme activities showed consistent results in terms of the effect of the soil part that showed significantly higher activity of all

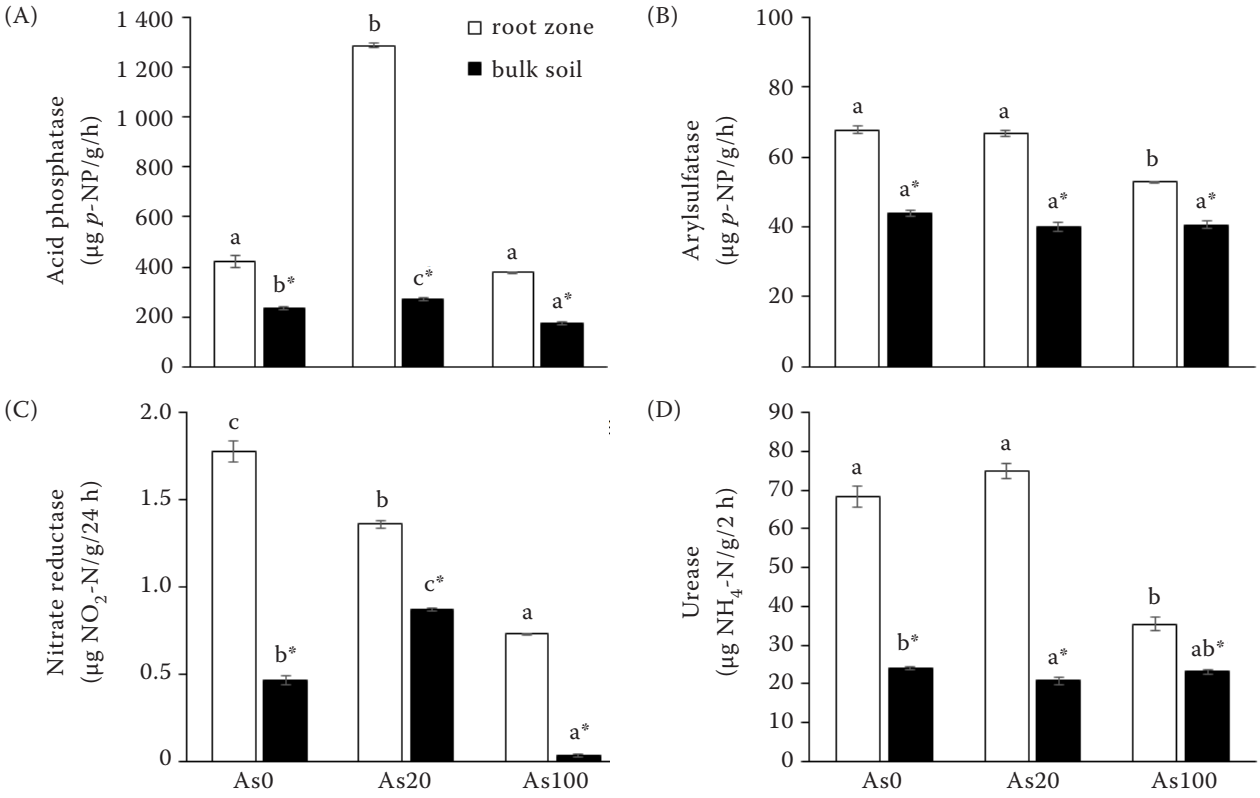

Figure 4. The activity of (A) acid phosphatase; (B) arylsulfatase; (C) nitrate reductase, and (D) urease for the root zone and bulk soil of *Pteris cretica*. Lower-case letters (treatments) and asterisk (soil part) indicate significant differences by Fisher's *LSD* (least significant difference) test ($P < 0.05$). As0 – control; As20 – 20 mg As/kg soil; As100 – 100 mg As/kg soil; *p*-NP – *p*-nitrophenyl

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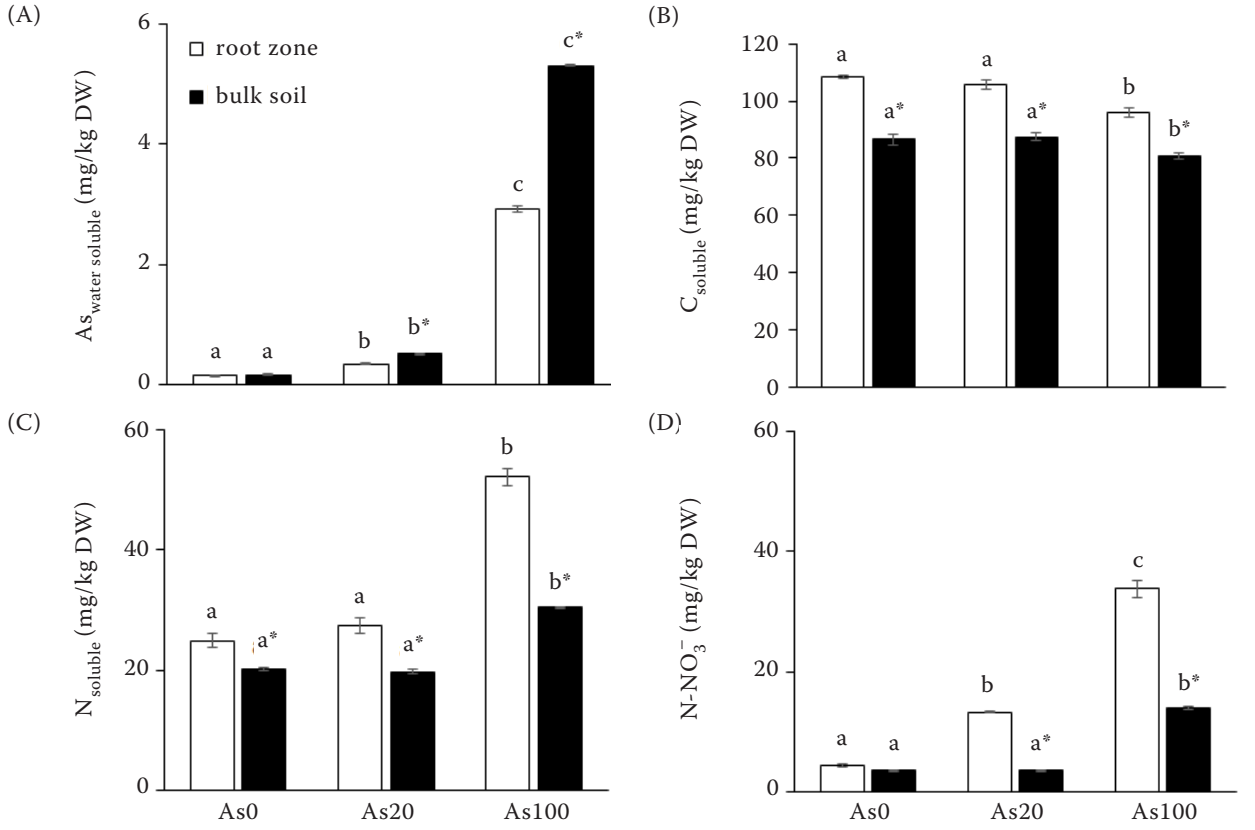

Figure 5. (A) Water-soluble arsenic (Asú fraction; (B) soluble carbon; (C) soluble nitrogen, and (D) nitrate nitrogen content in the root zone and bulk soil of *Pteris cretica*. Lower-case letters (treatments) and asterisk (soil part) indicate significant differences by Fisher's *LSD* (least significant difference) test ($P < 0.05$). As0 – control; As20 – 20 mg As/kg soil; As100 – 100 mg As/kg soil; DW – dry weight

measured soil enzymes in the root zone compared to the bulk soil (Figure 4). The significant difference between these soil parts is due to the fact that living plant roots are able to secrete a large amount of organic compounds that promote bacterial growth, resulting in a higher bacterial community, diversity, and enzyme activity in the root zone compared to the bulk soil (Smalla et al. 2001). The effect of As dose in soil was highly variable for all measured soil enzyme activities, with significantly different results depending on As treatment and soil part (Figure 4). Studies reported that enzyme activities are also influenced by a variety of factors such as soil nutrient availability and the type of risk elements (Lyubun et al. 2013, Wang et al. 2020). Although there was no significant difference in $As_{water\ soluble}$ (As_{ws}) content between parts of the soil, the effects of As treatments showed the same trend – an increase with increasing As dose in the soil (Figure 5A). Compared to the control, As_{ws} content in the root zone was 2.2 and 18.8-fold higher in the As20 and As100 treatments, respectively, while it was 3.2 and 33.7-fold higher in the bulk soil. These trends are consistent with the correlation between As dose in soil and As_{ws} content in the root zone as well as in the bulk soil ($r = 0.99$, $P < 0.001$). Bhattacharyya et al. (2008) found that the activities of soil enzymes in contaminated soils were strikingly negatively correlated with As_{ws} content. Aponte et al. (2020) also reported a decrease in enzyme activities due to the available form of risk elements, including As. Our results confirmed the effect of As_{ws} on the activities of ARY, NR and UR in the root zone ($r = -0.99$, -0.93 and -0.96, respectively; $P < 0.001$), whereas it affected only AP and NR in the bulk soil ($r = -0.88$ and -0.84, respectively; $P < 0.01$).

In the root zone and the bulk soil, the activity of AP was increased by the As20 treatment (206% and 16.5%, respectively), whereas the As100 treatment varied depending on the soil part (Figure 4A). The increase in the activity of AP at a low As the dose is consistent with the study of Lyubun et al. (2013),

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who suggested that the stimulation of phosphatase activity may depend on the similarities in the chemical composition of phosphate and arsenate ions.

Xian et al. (2015) and Aponte et al. (2020) reported that ARY is the most sensitive soil enzyme and can be used as an indicator to study the enzymatic toxicity of risk elements. However, in our study, the activity of ARY was affected by As dose only in the root zone of *Pc*-Al (Figure 4B). The effect of the As100 treatment resulted in a 22% decrease in ARY activity compared with the control and was confirmed by the correlation between As dose in soil and ARY activity in the root zone ($r = -0.98$, $P < 0.001$).

In the root zone, As caused a decrease in the activity of NR with increasing As dose in the soil (Figure 4C). Compared with the control, treatment with As20 and As100 decreased the activity of NR by 24% and 59%, respectively. This trend is consistent with the correlation between As dose in soil and activity of NR in the root zone ($r = -0.97$, $P < 0.001$). These results, together with those of $N_{soluble}$ and N-NO_3^- levels indicate changes in soil N cycling due to As stress, especially in the root zone. The As100 treatment resulted in an increase in $N_{soluble}$ content (root zone – 109%, bulk soil – 51%) compared to the control (Figure 5C). Correlation confirmed the relationship between As dose in soil and $N_{soluble}$ content (root zone: $r = 0.98$, $P < 0.001$; bulk soil: $r = 0.97$, $P < 0.001$). In the root zone, N-NO_3^- the content was clearly affected by increasing As dose in the soil. Compared with the control, this parameter was increased 3.1 and 7.9-fold in As20 and As100 treatments, respectively (Figure 5D). This trend is consistent with the correlation between As dose in soil and N-NO_3^- content ($r = 0.99$, $P < 0.001$). On the other hand, in the bulk soil, this parameter was affected only by the As100 treatment and was 3.9 times higher compared to the control. Moreover, in the bulk soil, the correlation between As dose in soil and N-NO_3^- the content was confirmed ($r = 0.98$, $P < 0.001$). The effect of As toxicity on nutrient availability and cycling was also observed for $C_{soluble}$, which showed consistent results in terms of the effect of As dose in the root zone and bulk soil, showing a decrease of 11.5% and 6.7% by As100, respectively, compared with the control (Figure 5B). The correlation confirmed the relationship between As dose in soil and $C_{soluble}$ content (root zone: $r = -0.94$, $P < 0.001$; bulk soil: $r = -0.78$, $P < 0.05$).

The change in soil N cycling by As stress also indicated the activity of UR affected by As100 treatment in the root zone of *Pc*-Al – 48% decrease compared to the control (Figure 4D). Correlation confirmed the relationship between As dose in soil and UR activity in the root zone ($r = -0.93$, $P < 0.001$). The opposite trend in the activity of this enzyme, which plays a key role in the transfer of organic N to ammonia nitrogen, was observed by Das et al. (2017) in the rhizosphere of *P. vittata* growing in As-rich soil.

Altogether, our results partially confirmed that As acts as an inhibitor of enzymes and also that the toxicity of As to enzyme activity is variable (Wang et al. 2020). In addition to reducing soil enzyme activities by directly inhibiting the catalytic activity of soil enzymes, the indirect effect of As on soil enzyme activities is associated with partial or complete inactivation of soil microbial enzymes, slowing enzyme synthesis or blocking microbial respiration and suppressing microbial growth (Lyubun et al. 2013).

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