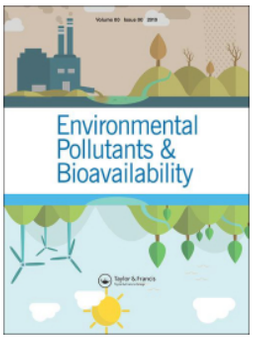

Taylor & Francis
Taylor & Francis Group

Environmental Pollutants and Bioavailability

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tcsb21

The potential of berula erecta in vitro for As bioaccumulation and phytoremediation of water environments

Jana Ambrožič-Dolinšek, Anja Podgrajšek, Nik Šabeder, Zdenka Mazej Grudnik, Andreja Urbanek Krajnc, Biljana Todorović & Terezija Ciringer

To cite this article: Jana Ambrožič-Dolinšek, Anja Podgrajšek, Nik Šabeder, Zdenka Mazej Grudnik, Andreja Urbanek Krajnc, Biljana Todorović & Terezija Ciringer (2023) The potential of berula erecta in vitro for As bioaccumulation and phytoremediation of water environments, Environmental Pollutants and Bioavailability, 35:1, 2205010, DOI: 10.1080/26395940.2023.2205010

To link to this article: https://doi.org/10.1080/26395940.2023.2205010

Published online: 26 Apr 2023.

ENVIRONMENTAL POLLUTANTS AND BIOAVAILABILITY
2023, VOL. 35, NO. 1, 2205010
https://doi.org/10.1080/26395940.2023.2205010

Taylor & Francis
Taylor & Francis Group

The potential of berula erecta in vitro for As bioaccumulation and phytoremediation of water environments

Jana Ambrožič-Dolinšek[a,b,c], Anja Podgrajšek[d], Nik Šabeder[e], Zdenka Mazej Grudnik[f], Andreja Urbanek Krajnc[c], Biljana Todorović[c] and Terezija Ciringer[a]

[a]Department of Biology, Faculty of Natural Sciences and Mathematics, University of Maribor, Maribor, Slovenia; [b]Department of Elementary Education, Faculty of Education, University of Maribor, Maribor, Slovenia; [c]Department of Botany and Plant Physiology, Faculty of Agriculture and Life Sciences, University of Maribor, Maribor, Slovenia; [d]Faculty of Medicine, Institute of microbiology and immunology, University of Ljubljana, Ljubljana, Slovenia; [e]Department of Forestry and Renewable Forest Resources, Biotechnical faculty, University of Ljubljana, Ljubljana, Slovenia; [f]Eurofins ERICo Slovenija, Inštitut za ekološke raziskave doo, Velenje, Slovenia

ABSTRACT

A potential plant species suitable for As bioaccumulation and phytoremediation of water environments could be the macrophyte Berula erecta. The objective of the present study was to investigate the effect of arsenate (As(V), C2H6AsNaO2·3 H2O), predominant in freshwater systems, on the growth, development and low molecular weight thiols of *Berula erecta* under controlled tissue culture conditions *in vitro*. Uptake of total arsenate increased with increasing arsenate treatments, at a higher percentage in the roots than in the aboveground parts of the plants. Lower concentrations of As(V) (0.1, 1, 10 mg L−1) had a positive effect on growth, dry weight, length of roots and shoots and number of buds. High concentrations of arsenate (50 and 100 mg As(V) L−1) significantly inhibited all growth parameters and decreased the photochemical efficiency of PSII. Evaluation of thiols revealed the critical As level (146 µg g −1 DW; 50 mg As(V) L−1 treatment) above which the As concentration can be toxic.

ARTICLE HISTORY
Received 17 January 2023
Accepted 15 April 2023

KEYWORDS
Arsenic; arsenate; uptake; accumulation; thiols; apiaceae; lesser water-parsnip

Introduction

Arsenic (As), a naturally occurring element, a semimetal in the earth's crust. It occurs in trace amounts and may be present in soil, water, and air. It is estimated that one-third of As is present in the environment in relatively high concentrations due to natural conditions, and the rest is due to anthropogenic activities [1,2]. Polluted areas exist both worldwide and in Slovenia [2–5]. Pollution studies in the Zasavje region of central Slovenia revealed elevated As levels of in the sediments (median 29.5 mg kg^{-1}) and in water samples (0.8 µg L^{-1}) [3]. In the Šaleška Valley, arsenic was the only heavy metal studied with values above the European background concentration of 2.0–10.0 mg kg^{-1}, ranging from 4.9 to a maximum of 20.3 mg kg^{-1}. Higher concentrations of As were also found in samples of lichens, forest fruits, and macrofungi [4]. Arsenic contamination also occurs in mining areas and steel industry sites in the municipalities of Celje, Idrija, Jesenice, and Mežica with median concentrations ranging from 20 to 21 mg kg^{-1}, whose values range from 6.0 to a maximum of 387.0 mg kg^{-1} [5]

To the public arsenic is synonymous with poison, and in high concentrations, inorganic arsenic can cause death [6]. Increasing arsenic contamination worldwide poses a growing threat to the environment and humans [2,6,7]. Even low concentrations of As can be highly toxic and potentially carcinogenic. As can attack cellular organelles and their components in biological systems [8]. A major health problem in the world, mainly due to anthropogenic activities, is the As contamination of drinking water [2,9]. In natural waters, As occurs in inorganic and organic forms [2]. The inorganic form, which is the most abundant and toxic in the environment, is the predominant form of the two found in polluted waters, and occurs in two oxidation states: Arsenite (As(III)) and Arsenate (As(V)) – depending on pH and redox conditions. The former (As(III)) is predominant under reduced conditions in anaerobic environments, while As(V) is predominant under oxidizing conditions in aerobic environments [10].

It is known that thiols play a crucial role in the detoxification of As [11]. The authors reported a number of glutathione (GSH)-related genes involved in synthesis and metabolism of GSH in rice seedlings exposed to As(V) [11–13]. This reflects a higher requirement for GSH under As stress. Glutathione is involved in the non-enzymatic reduction of As(V) to As(III). Consequently, oxidation of GSH occurs through the formation of a di-sulphide bond, resulting in a glutathione di-sulphide dimer that can be rapidly recycled into two GSH molecules by GSH reductase [14]. The reduced As(III) is further detoxified either by forming a complex with thiol-rich

CONTACT Jana Ambrožič-Dolinšek jana.ambrozic@um.si

peptides such as reduced cysteine, GSH and phytochelatins (PC) or by vacuolar sequestration [15], or by a combination of both. Glutathione not only plays a direct role in As detoxification, but also participates as an antioxidant in the oxido-reductive processes of photosynthesis [16], which are negatively affected by As treatment. Previous studies on rice have shown that As-induced damage to the photosynthetic apparatus is modulated by hydrogen peroxide and the activity of superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), and glutathione reductase (GR) have confirmed that oxidative stress is generated in rice [13]. Moreover, glutathione S-transferase, glutathione reductase, S-nitrosoglutathione reductase, glutathione-conjugated transports and genes of sulphate-metabolising proteins were reported to be upregulated in rice during As(V) stress [12,17,18], reflecting the versatile role of glutathione in As detoxification. In addition, cysteine and GSH are precursors of PC, which are known to be primary chelators of As [11].

To address the problems associated with As, various aquatic plants that can bioaccumulate arsenic have been tested. Arsenic was bioaccumulated by *Lemna valdiviana* Phil [19], *Eichhornia crassipes* (C.Mart.) Solms, *Lemna minor* L [20], *Spirodela intermedia* W. Koch [21] and *Eleocharis acutangula* [22]. In this regard, *Eichhornia crassipes* has proven to be a more suitable and reliable alternative for bioremediation of arsenic from aquatic environments. The fern group has been shown to be a successful heavy metal remediator. *Pteris vittata* L. is known to be the first fern to hyperaccumulate arsenic [23]. There are also other ferns, namely, *Nephrolepis cordifolia* (L.) C. Presl., *Hypolepis muelleri* N.A.Wakef., *Pteris umbrosa* R.Br., *Marsilea quadrifolia* and *Pteris cretica* L. that can accumulate arsenic in their leaves [24–27]. In addition to a large number of studies on Pteridaceae, there are also studies done on aquatic, semi-aquatic and submerged plants [28]. There are some studies on the accumulation of heavy metals in Apiacea [29–33], two of them on *B. erecta* [32,33], but none on the removal of As.

One of the potential plant species suitable for the removal of As from contaminated environments could also be the macrophyte lesser water-parsnip, *Berula erecta* (Huds.) Coville, Apiaceae. Narrow-leaved stonecrop is a creeping perennial that grows vegetatively gaining weight rapidly and reaching a height of up to 40 cm. The species occurs in various freshwater habitats such as bogs, swamps, springs, lakes, puddles, streams and ditches. It is a subcosmopolitan species of the Northern Hemisphere and native to Slovenia [34,35]. Since it grows well in tissue culture under controlled conditions (temperature, humidity, light) *in vitro* in the tissue culture medium Murashige and Skoog [36], without the need for additional growth regulators [32], it is a suitable species for research and cultivation.

Our objective was to determine whether *B. erecta* has potential for As bioaccumulation and phytoremediation of water environments and whether this plant can be used for detoxification and phytoremediation of waters contaminated with As. This potential was investigated under controlled conditions in tissue culture *in vitro* by exposure to various concentrations of the water-soluble form of arsenate (As(V)). In addition to the known toxic effects of As, application of a low dose of As may also have a stimulatory effect on plant growth and development [37], although the metabolism responsible for the stimulation is not clearly understood. Therefore, another objective was to investigate the stimulatory and toxic As dose-dependent effects on growth, development and metabolism of *B. erecta*.

Material and methods

Plant material and growth conditions

To study the response of plants to arsenic in the form of arsenate (As(V)), in vitro propagated shoots of *Berula erecta* (Huds.) Coville (Apiaceae) were placed on 20 mL of Murashige and Skoog [36] solid medium (MS) without growth regulators. The MS medium, supplemented with 0.8% Difco – Bacto agar and 3% sucrose, was adjusted to a pH of 5.7–5.8 before autoclaving. Two shoots, each approximately 90 mg each, were placed on the surface of the MS medium in 175 mL jars with transparent lids and cultured in a growth chamber at 23 ± 2°C and 50% relative humidity with a photoperiod of 16 h at 38—50 µmol m^{-2} s^{-1} (Osram L 58W/77 - Fluora). After two weeks of root induction on the MS medium, the vessels were filled with an additional 20 mL of arsenate (As(V), $C_2H_6AsNaO_2{\cdot}3\,H_2O$) (Dimethylarsenic acid Sodium salt, 99%, Fluka, BioChemika, Packed in Sigma-Aldrich®, Steinheim, Switzerland) in concentrations of 0.1, 1, 10, 50 and 100 mg L^{-1}. Distilled water (20 mL) was used for control treatment. Growth, development, bio-chemical and physiological parameters were measured weekly over a period of three weeks on As(V) exposed plants and compared with controls. The experiment with six replicates per treatment was repeated twice. Both similar results were included into the statistical analysis.

Growth and development

Growth and development parameters were measured every 7 days during the three-week experimental period. Determined were: Fresh and dry weight, shoot and root length, and number of shoots and stolons. Parameters were measured at the beginning of the experiment on 20 plant samples of each As(V) concentration treatment to obtain baseline values, and then after 7, 14 and 21 days on 12–14 plant samples for each As(V) treatment.

Photochemical efficiency of photosystem II

Chlorophyll fluorescence was measured *in situ* using Handy PEA fluorometer (Hansatech, Kings Lynn, UK). Chlorophyll fluorescence measurements were made after 10 min of darkness using dark adaptation clips on 12–14 samples for each As treatment. Fluorescence, excited with ultra-bright red LEDs is optically filtered to a peak wavelength of 650 nm at 3000 µmol m^{-2} s^{-1} of 0.8 s. The potential photochemical efficiency is described by the abbreviation Fv/Fm [38].

Determination of photosynthetic pigments

Samples of 6–8 plants from each As(V) treatment were used to determine chlorophylls *a* and *b* and carotenoid content. Pigment samples were extracted with 100% acetone. Pigment content was determined using a UV/VIS spectrometer (Varian Cary 100 Bio UV-Visible Spectrophotometer, SpectraLab Scientific Inc. Canada) according to the method of Lichtenthaler and Buschmann [39]. Samples from 6 to 8 plants were used to determine anthocyanin content. They were extracted (methanol: HCl = 99:1) and measured as described by Drumm and Mohr [40].

Determination of total As

For As analysis, samples, belowground roots and aboveground parts of plants were separateed, freeze-dried (LIO-5 P Kambič, Slovenia) and ground (Fritsch pulverisette 14). The amount of As was measured using Agilent method 132 ICP-MS 7500c, SIST EN ISO 17,294–2: 2005. The detection limit of the method (LOD) was 0.050 mg kg^{-1} and the quantification limit was 0.020 mg kg^{-1}. The measurement uncertainty was 25%. Each sample of the experiment was analyzed twice. The accuracy of the method was checked using the reference material CRM NIST 1515 'Apple leaves'.

Determination of thiols

Eight samples from each As(V) treatment were used to determine thiols. Each sample was divided among aboveground parts and belowground roots of three in vitro plants and immediately frozen in liquid nitrogen and stored. The frozen material was freeze-dried and ground (Fritsch pulverisette 14) before analysis.

Total glutathione, oxidized glutathione, total cysteine, and cystine were determined quantitatively as described by Tausz [41]. Thiols were separated and determined using a Waters 2695 HPLC system, a Waters 2475 Multi fluorescence detector (excitation: 380 nm; emission: 480 nm), Spherisorb S5 ODS2 250 × 4.6 mm column (solvent A: 0.25% (v/v) acetic acid in water with 5% methanol, pH 3.9; solvent B: 90% (v/v) methanol in water). Gradients were 5% to 15% of solvent B, 20 min, 100% solvent B, 6 min, 5% solvent B, 8 min. Flow rate was 1 mL min^{-1}.

Statistical analysis

All measured parameters, growth, development, pigments, As content, photochemical efficiency and thiols were represented by means and standard deviations (±SD) and were statistically analysed by one-way analysis of variance (ANOVA) using SPSS 27 software (SPSS Inc., Chicago, IL, U.S.A). Significant differences between means were determined using the Kruskal–Wallis test followed by Dunn's post hoc test. Different letters indicate significant differences ($P < 0.05$) between means.

Results

Growth and development

During the first two weeks of the experiment, fresh weight and dry weight increased slowly but not significantly in controls (Figure 1(a,b)) and in individual plants exposed to lower As concentrations (0.1–10 mg L^{-1}). By the third week of the experiment, fresh weight and dry weight increased significantly in controls and in plants exposed to all lower As concentrations (0.1–10 mg L^{-1}), with no significant differences between them, although As exposure increased weights slightly. At the higher As concentrations (50, 100 mg L^{-1}), fresh weight and dry weight remained unchanged during all three weeks of the experiment and were comparable to the control from the beginning of the experiment.

The same negative effect of high As(V) concentrations on *B. erecta* growth and development is also clearly seen in Figure 2 after a three-week experiment. Exposure to concentrations between 0.1 and 10 mg As(V) L^{-1} significantly stimulated the growth of *B. erecta* shoots, roots and stolons compared to the control (Figure 2). Negative effects of higher As(V) concentrations occurred only after the third week of the experiment (Figures 1, 2). The highest concentration (100 mg L^{-1}) had the most noticeable negative suppression effect (Figures 1, 2).

Photochemical efficiency of photosystem II

Low As concentrations had no effect on photochemical efficiency in all three weeks of the experiment (Figure 3). The highest As concentration decreased photochemical efficiency values as early as the second week at a concentration of 100 mg As per L^{-1} and in the third week at the two highest concentrations (50 and 100 mg L^{-1} As(V)). The values at the highest concentrations decreased by up to 30% (Figure 3).

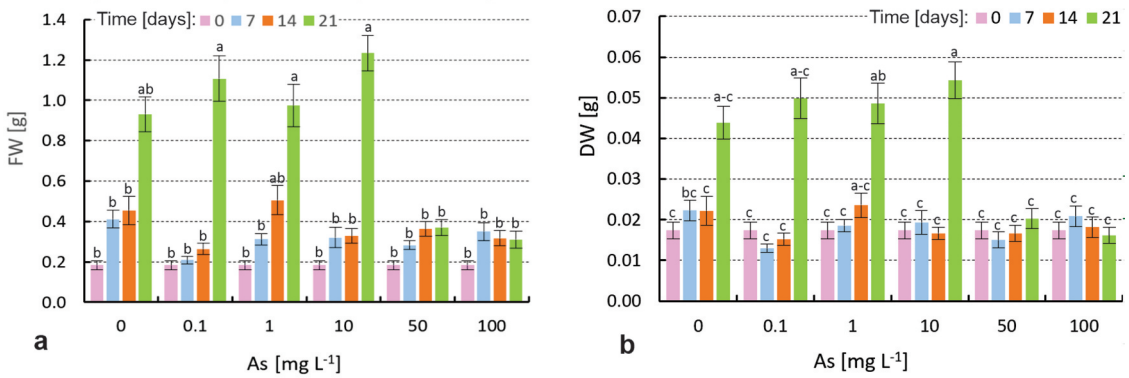

Figure 1. The effect of As(V) exposure (0.1, 1, 10, 50 and 100 mg As(V) L^{-1}) of B. erecta in vitro on (a) fresh weights (FW) and (b) dry weights (DW were determined at the beginning and after 7, 14 and 21 days of the experiment. Means ±SD (n = 11–12) are shown. Significant differences are indicated by different letters (Kruskal–Wallis test, Dunn's post hoc, $P \leq 0.05$).

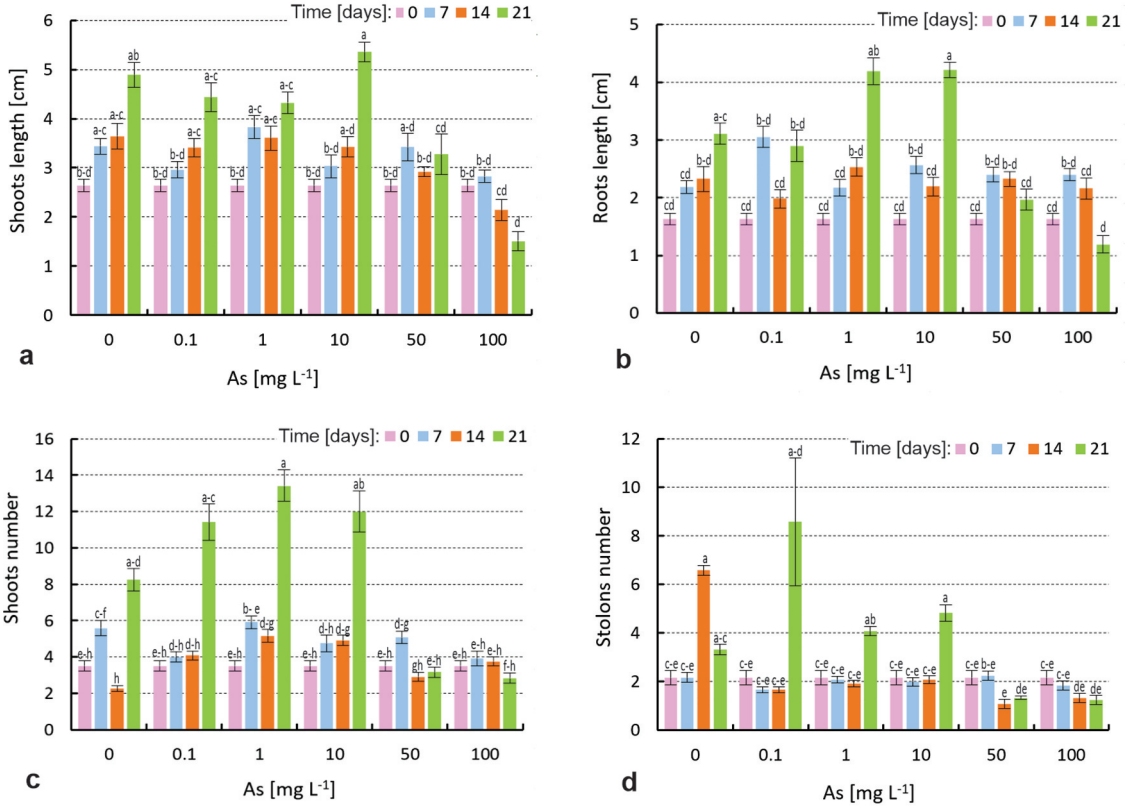

Figure 2. The effect of As(V) exposure (0.1, 1, 10, 50 and 100 mg As(V) L^{-1}) of B. erecta in vitro on (a) the plant heights, (b) the roots lengths, (c) the average shoot numbers (d) the average stolon numbers were determined at the beginning and after 7, 14 and 21 days of the experiment. Means ±SD (n = 12) are shown. Significant differences are indicated by different letters (Kruskal–Wallis test, Dunn's post hoc, $P \leq 0.05$).

Pigments

As(V) affected the amount of pigment synthesized (Figure 4). Exposure to concentrations between 0.1 and 10 mg As L^{-1} after one week actually slightly stimulated pigment synthesis in *B. erecta* compared to the control (Figure 4), especially at concentrations lower than 10 mg As L^{-1}. This effect was not observed after the second week of the experiment. Chlorophylls *a* were slightly reduced at the highest As concentrations after the second week of the experiment and reduced at all As concentrations after the third week of the experiment, most strongly at 100 mg As L^{-1}. The same tendency was observed for the amount of carotenoids. The same tendency was observed for chlorophyll b, except that the highest concentration of 100 mg As L^{-1} did not decrease as much as chlorophyll a and carotenoids after three weeks of the experiment. Anthocyanins decreased over the course of the experiment in control plants and plants exposed to As. The effect was most pronounced after three weeks of the experiment at the two highest concentrations, i.e. 50 and 100 mg As L^{-1}.

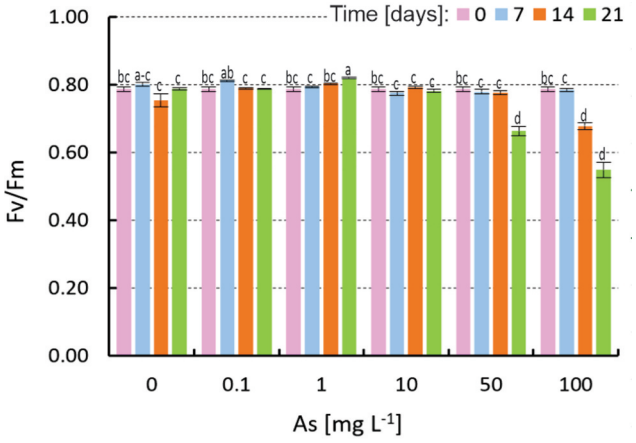

Figure 3. The effect of As(V) exposure (0.1, 1, 10, 50 and 100 mg As(V) L^{-1}) of B. erecta in vitro on the values of maximal photochemical efficiency were determined at the beginning and after 7, 14 and 21 days of the experiment. Means ±SD ($n = 12$) are shown. Significant differences are indicated by different letters (Kruskal–Wallis test, Dunn's post hoc, $P \leq 0.05$).

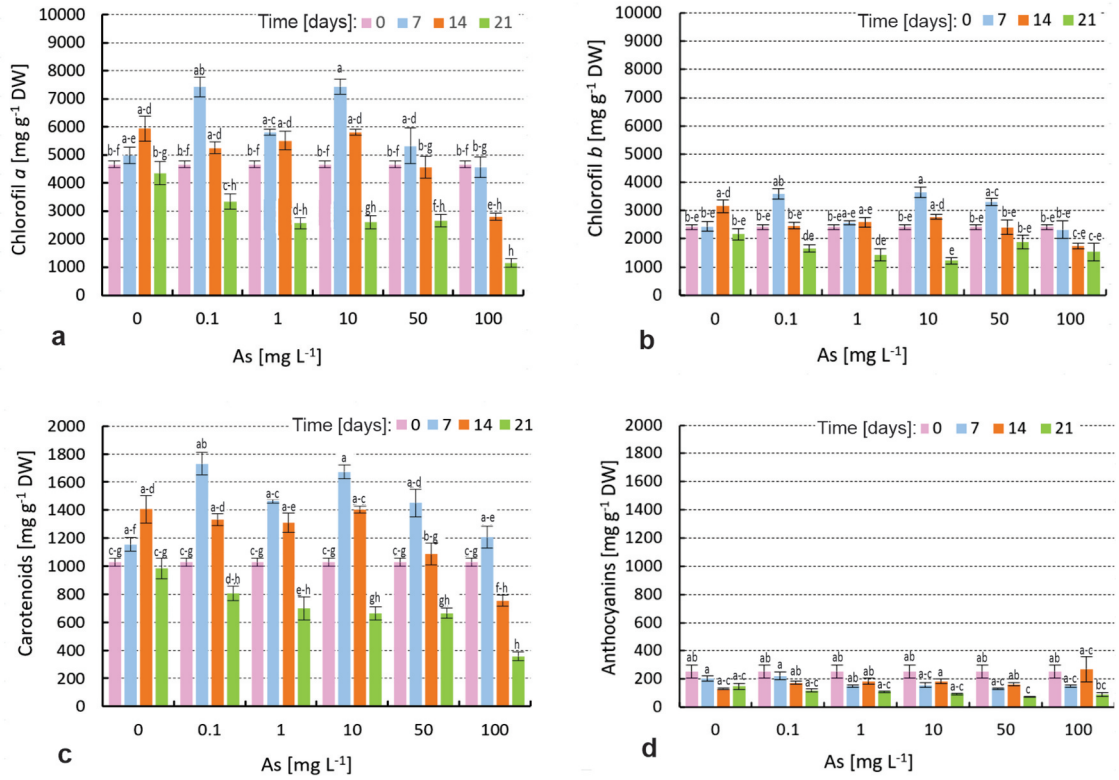

Figure 4. Effects of As(V) exposure (0.1, 1, 10, 50 and 100 mg As(V) L^{-1}) of B. erecta in vitro on content of (a) chlorophyll a, (b) chlorophyll b, (c) carotenoids and (d) anthocyanins. were determined at the beginning and after 7, 14 and 21 days of the experiment. Means ±SD ($n = 6–8$) are shown. Significant differences are indicated by different letters (Kruskal–Wallis test, Dunn's post hoc, $P \leq 0.05$).

Arsenic

The results show that *B. erecta* absorbs As in both belowground roots and aboveground parts of the plant (Table 1). The lowest As content was detected in the controls and the plants treated with 0.1 mg As L^{-1}, and the highest in the plants treated with 100 mg As L^{-1}.

Uptake of As from the solution was more effective after exposure to lower As concentrations than after exposure to higher As concentrations (Table 1). The bioconcentration factor (or BCF; bioconcentration factor calculated on DW; plant/surrounding medium) for plants exposed to the lowest 0.1 mg As(V) L^{-1} was 4.5% in the belowground and 2% in the aboveground part of the plants. BCF for plants exposed to 1 and 10 mg As(V) L^{-1} was 1.7% in the belowground and between 1.7% and 1.8% in the aboveground part of the plants. BCF for plants exposed to concentrations greater than 10 mg As(V) L^{-1} ranged from 0.5% to 0.6% in the belowground roots and

Table 1. Content of As(V) ($n = 2$) in Berula erecta, exposed to As(V) in vitro.

	Belowground parts		Aboveground parts		
Treatment	As content roots [μg g^{-1} DW] *	BCF Absorbed As from solution into roots [%]	As content aboveground [μg g^{-1} DW]*	BCF Absorbed As from media into plant [%]	TF Above/below concentration of As [%]
control	<0.2				
0.1 mg As(V) L^{-1}	0.9 ± 0.2	4.5	0.4 ± 0.1	2.0	45
1 mg As(V) L^{-1}	3.5 ± 0.8	1.7	3.6 ± 0.9	1.7	102
10 mg As(V) L^{-1}	32.4 ± 8.1	1.8	31.8 ± 7.95	1.7	98
50 mg As(V) L^{-1}	139.0 ± 34.8	0.6	146.0 ± 36.5	0.6	105
100 mg As(V) L^{-1}	280.5 ± 70.0	0.5	198.5 ± 49.5	0.3	71

DW, dry weight; BCF, bioconcentration factor; TF, translocation factor; * Average ± measurement uncertainty; BCF; Plant/medium concentration of As in DW; TF; Above/below concentration of As in DW.

between 0.6% and 0.3% in the aboveground parts of the plants. In addition to the high BCF for As, *B. erecta* accumulated As in the below and aboveground parts of the plant, with translocation factors (TF; above/below concentration of As in DW) ranging from 45% to 105% for As treatments (1–10 mg L^{-1}).

Thiols

The effects of As(V) treatment on thiols were studied in the aboveground parts and roots of *B. erecta* plants grown in vitro. Total GSH concentrations in plants, treated with 0.1 to 1 mg As(V) L^{-1}, were comparable to those of the controls. After treatment with 10 mg As(V) L^{-1}, increased total GSH concentration were measured in the aboveground parts (+25%) and in the roots (+28%) and compared with those of the controls (Figure 5(a,b)). In addition, treatment with 50 mg and 100 mg As(V) L^{-1} resulted in a significant increase of 258% and 240%, respectively, in the total GSH concentration in the aboveground parts, which was accompanied by a significantly higher level of glutathione disulphide (GSSG) (Figure 5(a)). In the roots, total GSH concentration increased by 143% after treatment with 50 mg As(V) L^{-1}. In roots of plants treated with 100 mg As(V) L^{-1}, the increase was less pronounced (43%), while the GSH redox state shifted toward a more oxidized value (Figure 5(b)).

Cysteine content was significantly altered by treatments with more than 0.1 mg As(V) L^{-1}, but at a higher percentage in aboveground parts and at a lower percentage in the roots (Figures 6(a,b)), whereas cysteine content and redox state in the roots were less affected by the treatments. After treatment with 50 mg As(V) L^{-1}, the total cysteine content in the aboveground parts increased by 94%, which was accompanied by a higher percentage of cystine content (Figure 6(a)). In roots treated with 50 mg As(V) L^{-1}, the increase in total cysteine was 70% (Figure 6(b)), while the percent cystine remained at the control level. In contrast, the increase was less pronounced in the plants treated with 100 mg As(V) L^{-1}. In aboveground parts of the plants treated with 100 mg As(V) L^{-1}, the cysteine content was 76% higher than in the controls (Figure 6(a)), and the percent of cystine decreased by less than 10%. In the roots, the cysteine content was slightly increased (14%), while the percent of cystine content (40%) shifted to the more oxidized value of 40% (Figure 6(b)).

Discussion

Growth and development responses

Growth and development of *B. erecta* were stimulated by low As concentrations and inhibited by higher As concentrations. The stimulatory effect was observed during the first two weeks of the experiment. It appeared as a slow but non-significant increase in all growth and development responses of *B. erecta* control plants (Figure 1) and plants exposed to lower As concentrations (0.1–10 mg L^{-1}). It was clearly visible after the third week of the experiment. The general trend was also that higher concentrations of 50 and 100 mg L^{-1} significantly suppressed the growth and development of this plant, which again was visible after the third week of exposure to higher As concentrations. The effect was observed in all measured parameters, dry weight, shoot height and root length, number of newly developed shoots and stolons. The optimal promotive As(V) concentration in *B. erecta* ranged from 0.1 to 10 mg As L^{-1}, with 10 mg As L^{-1} appearing to be the limiting concentration that still promoted growth and development of this plant. The stimulatory effect of low-dose As on plant growth and development was also observed in some previous studies [37]. This interesting contradictory effect of otherwise toxic As was also observed in *P. vittata* [42] and *Arabidopsis thaliana* [43]. Results obtained under culture conditions, as in the case of our study, are the result of plant metabolism or interaction with plant nutrients rather than interaction with other organisms of the rhizosphere [37,43]. The mechanism of growth benefits is thought to be due to the stimulation of Pi uptake by As [37,44]. As(V) is an analogue of inorganic phosphate (Pi) and is

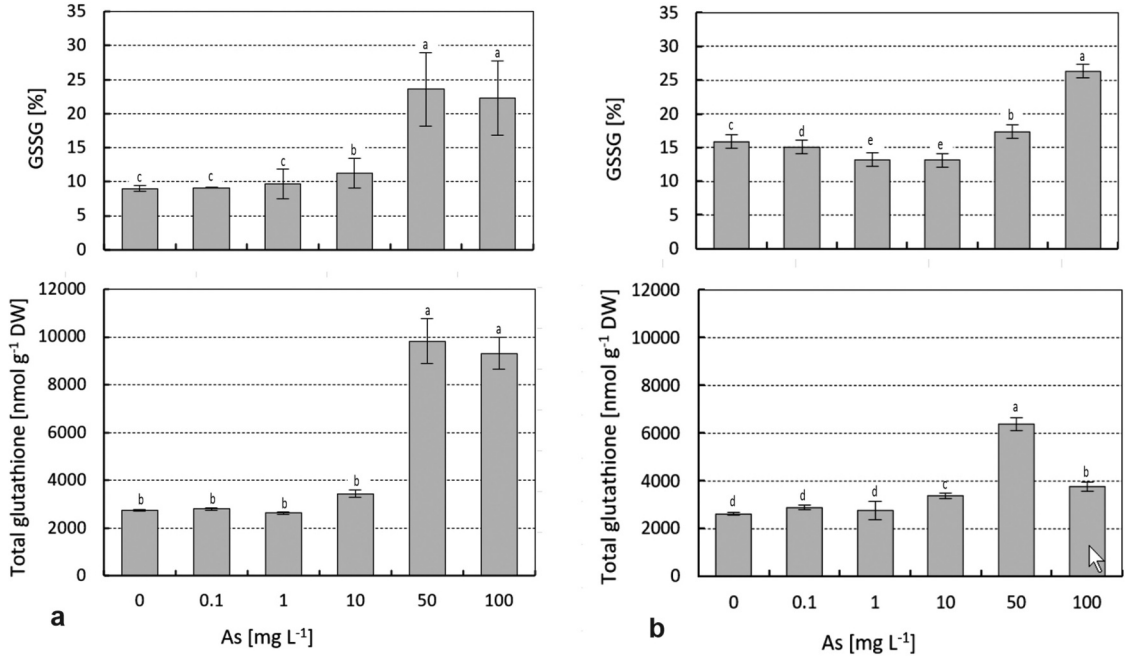

Figure 5. Effects of As(V) exposure (0.1, 1, 10, 50, 100 mg As(V) L^{-1}) on total GSH (total glutathione) and percentages of GSSG (oxidized glutathione) (a) in aboveground parts and (b) in roots of B. erecta in vitro were determined after 21 days of the experiment. Means ±SD ($n = 6–8$) are shown. Significant differences are indicated by different letters (Kruskal–Wallis test, Dunn's post hoc, $P \leq 0.05$).

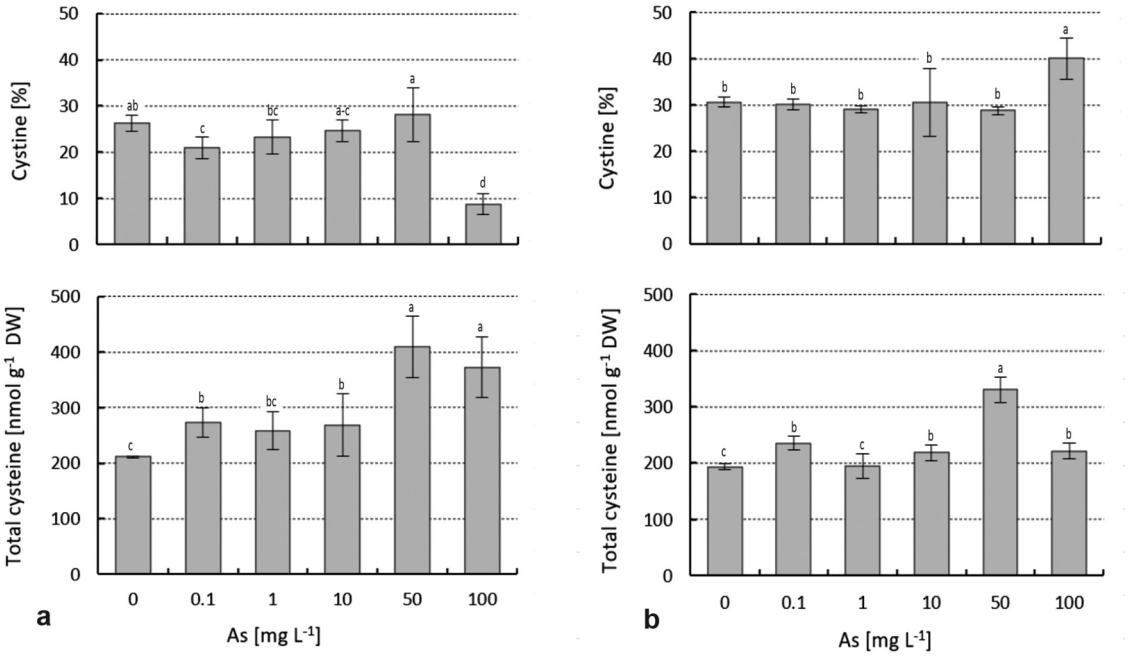

Figure 6. Effects of As(V) exposure (0.1, 1, 10, 50 and 100 mg As(V) L^{-1}) on cysteine and percent-age of cystine (a) in aboveground parts and (b) in roots of B. erecta in vitro were determined after 21 days of the experiment. Means ±SD ($n = 6–8$) are shown. Significant differences are indicated by different letters (Kruskal–Wallis test, Dunn's post hoc, $P \leq 0.05$).

transported through the plasmalemma by Pi transporter proteins (PHT). As(V) and Pi compete for uptake by the same transport systems. Therefore, As(V) may amplify Pi deficiency symptoms under low Pi conditions and, conversely, Pi fertilization may protect the plant from As(V). This competition between Pi and As(V), which is responsible for stimulation, has not yet been fully understood [37].

The same promoting effect of low As concentrations on plant growth and development has already been observed in *Berula erecta* and *Apium repens* (Jacq.) Lag. exposed to low concentrations of another metalloid selenium (Se(IV)) [45,46]. The growth-promoting effect of the otherwise toxic metalloid As might be related to detoxification and impaired upregulation of the antioxidant defense system, which boosts other metabolic

pathways and leads to promotion of growth and development.

As concentrations greater than 10 mg As L^{-1} generally suppressed the growth and development of *B. erecta*. There are several data on the suppressive effects of As on plants, especially on crops. The suppression of growth of sunflower (*Helianthus* sp.), chickpea (*Cicer arietinum* L.) [47], bean (*Phaseolus vulgaris* L.) [48], horseradish (*Armoracia rusticana* L. Britton) [49], rice (*Oryza sativa* L.) [50–52], and wheat (*Triticum* sp.) [53] germination have been reported and are explained by the induction of numerous metabolic disorders in plants [37,54,55]. After As treatment, carbon assimilation decreases and secondary metabolism increases, which is due to the reduction process of As, its detoxification, and the defense response against increased ROS. The induced accumulation of secondary metabolites (antioxidants, phytochelatins) and sequestration into the vacuole is energetically expensive and rapidly depletes local carbohydrate reserves and must be supported by photosynthates translocated to the site of de novo biosynthesis, limiting energy and carbon requirements for growth [11,15]. In contrast to the promoting effect of As, its toxicity may be the result of slow detoxification or disruption of the antioxidant defense system, leading to disruption of primary metabolism, which is also associated with growth and development. A better understanding of the mechanisms responsible for As resistance and toxicity in plants is needed. A possible outcome of a better understanding would also be the production of As-resistant plants for phytoremediation and safe cultivation [37].

Photochemical efficiency

As in concentrations greater than 10 mg As L^{-1} are toxic to *B. erecta*. The undisturbed values of the first week of photochemical efficiency changed in the second and especially in the third week of As treatment in the two higher As treatments. For plants treated with 10 mg As(V) L^{-1} and less, photochemical efficiency remained within the range of control plants throughout the experimental period. The strong negative effect of the high As concentration became even more evident in the third week, when the mean value after treatment with 100 mg As(V) L^{-1} was 0.54. This indicates highly stressed plants, as the maximum values for non-stressed plants ranged from 0.80 to 0.83 [55]. The negative effect of high doses (>100 mg kg^{-1}) of As(V) on younger and older fronds was also observed in *Pteris cretica* L [26]. Arsenic affects the light-harvesting system of plants by reducing photosynthetic activity, resulting in a significant decrease in photosynthetic efficiency [55].

Pigments

As exposure decreased the amount of chlorophylls, carotenoids, and anthocyanins regardless of concentration. The effect was more pronounced with the amount of chlorophylls and carotenoids. Chlorophyll content is related to photosynthesis. The decrease in chlorophyll content and suppression of photochemical efficiency after concentrations of 50 mg As(V) L^{-1} and higher is evident in the second week and more pronounced and obvious in the third week for all As(V) treatments. Arsenic affects the light-harvesting system of plants through the structural deformation and degradation of chloroplast membranes and the reduction of chlorophyll content. The decrease in chlorophyll content is related to the competition of toxic arsenic ions with magnesium ions in the chlorophyll molecule, the substitution of which leads to the disruption of photosynthetic activity [55]. The decrease in chlorophyll content was also reported for the freshwater plant *Lemna valdiviana* Phil., and the crop species *Helianthus annuus* L: and *Brassica juncea* L [55].

Arsenic

To evaluate the effects of As on plant cell metabolism, it is important to know which As form is present in the surrounding media, whether it can enter plant cells, and whether the plant is able to convert one As form into another [37,56]. The results of our experiment showed that the accumulation of As increases with increasing As concentration in the surrounding medium, so *B. erecta* has an indicator accumulation potential. The accumulation of As in *B. erecta* is strongly dependent on the As conditions and the As concentration in the medium. The As content in the tissue gradually increased when the supply was increased from 0.1 to 100 mg As(V) L^{-1}. *B. erecta* showed a linear increase in As uptake with increasing As in the medium and can be described as an indicator species. The arsenate As(V) used in our experimental system would be rapidly reduced to arsenite As(III) upon entry in the plant cells, so that the final form of As in plants would be the trivalent form As(III). The transformation to arsenite occurs in the roots, is later translocated in the aboveground parts through the xylem, and is sequestered in the root and shoot vacuoles [42]. When it is removed from the roots, the main form is arsenate in the root and arsenite in the shoot.

In our experiment, the highest exposed concentration of As(V) was 100 mg L^{-1} DW. At this concentration, the maximum As concentration was 280 µg As g^{-1} DW, measured in the belowground part of the plants, which

is at least 10-times lower than the accumulation observed in the species *Pteris cretica* L. exposed to comparable 100 g As(V) kg^{-1} [26]. Although *Berula* does not achieve accumulation as high as this fern, its extremely rapid growth in aquatic environments would make it suitable for phytoremediation of As-contaminated sites (data not shown). The highest accumulation of As in plant tissue occurs in the species *Pteris vittate* L. This hyperaccumulating fern accumulates As in its fronds, up to 13,800 µg As(V) g^{-1} of DW. In the plant root, As accumulation is highest in *Populus nigra* L., it can reach more than 0.2 mg As g^{-1} DW of the plant root. Other plants mentioned that can accumulate As are ferns *Azolla caroliniana* Willd., and *A. filiculoides* Lam., *Oryza sativa* L., *Populus tremula* Michx., *Salix alba* L [56], *Vallisneria cenеseserrulata* L. and *Holcus lanatus* L [42].

Plants of *B. erecta* exposed to high As concentrations, accumulate As in below- and aboveground parts. The reason for this may be their submerged growth form, which allows uptake of elements through both roots and leaves. Although *B. erecta* is obviously not a hyperaccumulator like *P. vitatta* and *P. cretica*, it may be of interest for phytoremediation because it is a fast-growing plant, which accumulates As in the whole, above and belowground root parts of the plants. Exposure to 50 mg As(V) L^{-1} and above is toxic to *B. erecta*, while the compensatory mechanism stop functioning properly and leads to death. The accumulated As after treatment with 10 mg As(V) L^{-1} did not significantly affect photosynthesis or pigment content. Therefore, *B. erecta* could be a candidate for phytoremediation of As, but only when exposed to concentrations less than 10 mg As(V) L^{-1}.

Thiols

The effectiveness of glutathione and cysteine in metal homeostasis and antioxidant defense in the belowground roots and aboveground plant parts was evaluated. As exposure altered the glutathione/cysteine system of *B. erecta* depending on the As concentration in the medium. Plant tissue analysis showed that 90% of As was in the form of arsenite As (III), even when plants were originally exposed to the form of arsenate As (V) [57], reflecting the high affinity of plants to reduce As (V) to the As form (III). The reduction is usually carried out by enzymes, e.g. arsenate reductase (ACR) [11,58,59]. The non-enzymatic reduction of As (V) to As (III) is mediated by two glutathione molecules that are oxidized to glutathione disulfide, which can be rapidly recycled to reduced GSH molecules by GSH reductase [16]. The reduced As(III) is further detoxified either by complexation with thiol-rich peptides such as reduced cysteine, glutathione (GSH) and phytochelatins (PC) or by vacuolar sequestration [15] or a combination of both. Authors reported the upregulation of a number of genes or enzymes involved in glutathione synthesis and glutathione metabolism for As sequestration in rice seedlings exposed to As(V) [12,60]. This reflects a higher requirement for GSH under As stress. Jung *et al* [61]. reported that exogenous application of GSH in As-treated seedlings reduced As-induced oxidative stress, improved the antioxidant defense system by maintaining antioxidant and/or redox enzyme homeostasis, and increased As and GSH content. GSH application also increased As translocation from roots to shoots. The results suggest that exogenous GSH application should be a promising approach to improve As stress resistance in rice plants.

In the experiment discussed, there was a significant accumulation of GSH in the roots after 10 mg As L^{-1} treatment, while cysteine increased significantly when treated with 0.1 mg As L^{-1}. The percentage increase of total GSH in plants after As treatment was higher in aboveground parts than in the roots, indicating that the reduction process of As occurs mainly in photosynthetic tissues. The spatial dynamics of detoxification responses at the GSH level between aboveground and belowground plant parts in response to As treatment have not yet been investigated. Previous basic research on the spatial dynamics of the GSH system has shown that it largely depends on the efficiency of photosynthetic carbon fixation and the proportion of photorespiration that provides the glycine required for GSH synthesis [62]. In addition to direct role of glutathione in As detoxification, the higher sensitivity of the leaf glutathione system can be due its involvement in antioxidant processes to protect photosynthesis [16].

Higher As concentrations (50 and 100 mg L^{-1}) had negative effect on photochemical efficiency and photosynthetic pigment content. As a result, the increased formation of ROS causes a transition to a more oxidized redox state. Previous studies on SOD, APX, POD, and glutathione reductase (GR) have confirmed that the accumulation of As in rice causes oxidative stress [13]. Moreover, glutathione S-transferase, glutathione reductase, S-nitroso glutathione reductase, conjugated glutathione transporters, and sulphate metabolizing protein genes were reported to be upregulated in rice during As(V) stress [12,17,18], reflecting the versatile role of glutathione in detoxification of As.

Using the glutathione/cysteine system, a critical As content (approximately 146 µg g-1 DW) was determined for *B. erecta*, which was reached after treatment with 50 mg As(V) and above which the As concentration can be considered toxic. This can be assumed based on the lower total glutathione and cysteine levels after treatment with 100 mg As L^{-1} compared with 50 mg As L^{-1} and a more oxidized cysteine/GSH redox state. The results reflect a higher requirement of GSH and its precursor

cysteine for detoxification of reduced As(III) by complexation with reduced cysteine and GSH and by their conversion to phytochelatins (PCs), which are known as primary chelators of As [10,63]. Similarly, several authors [11,64–66] have reported induced PC levels in *Hydrilla verticillate* (L. f.) Royle, *Ceratophyllum demersum* L. and *Oryza sativa* L. under As stress.

Conclusion

The macrophyte *Berula erecta* is a potential plant species for As bioaccumulation and phytoremediation of waters less contaminated with As. Although *B. erecta* is not a hyperaccumulator like *P. vitatta* and *P. cretica*, it is still interesting for phytoremediation because it is a fast-growing plant with a submerged growth form that accumulates As in the above and belowground root parts. Exposure to 50 mg As(V) L^{-1} and above is toxic to *B. erecta*, but only at concentrations less than 10 mg As(V) L^{-1}.

In our *in vitro* study, we presented the dose-dependent response of *B. erecta* to different added As concentrations and showed that As has a stimulatory and toxic dose-dependent effect on growth, development and metabolism. Lower concentrations (0.1, 1, 10 mg L^{-1} As(V)) even had positive effects on growth, dry weight, root and shoot length, and number of buds and stolons. Higher concentrations (50 and 100 mg As(V) L^{-1}) inhibited all growth parameters and reduced the photochemical efficiency of PSII.

We have also presented the role of thiols in metal homeostasis and antioxidant defense under As stress. Evaluation of thiols and their redox state revealed a critical As content (146 µg g-1 DW; 50 mg As(V) L^{-1} treatment), above which the As concentration can be considered toxic. Moreover, the increase in total GSH after As treatment was higher in the aboveground parts than in the roots, indicating that the reduction process of As occurs mainly in the photosynthetic tissue. It can also be assumed that the lower concentration of cysteine and glutathione in *Berula* treated with 100 mg As L^{-1} compared with 50 mg As L^{-1} was due to the complexation of As and the conversion of thiols to PC.

Acknowledgments

The research was supported by The Slovenian Research Agency program Research for improvement of safe food and health (ARRS, Code P1-0164); and by the project Development of Research Infrastructure for the International Competitiveness of the Slovenian RRI Space—RI-SI-LifeWatch; and by the Republic of Slovenia, Ministry of Education, Science and Sport and the European Union from the European Regional Development Fund.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The work was supported by the Development of Research Infrastructure for the International Competitiveness of the Slovenian RRI Space—RI-SI-LifeWatch Republic of Slovenia, Ministry of Education, Science and Sport and the European Union from the European Regional Development Fund The Slovenian Research Agency program Research for improvement of safe food and health [ARRS, Code P1-0164]; ARRS, Code P1-0164, The Slovenian Research Agency program "Research for improvement of safe food and health " [ARRS, Code P1-0164].

References

[1] Ng J. Arsenic and arsenic compounds. 2nd ed. Geneva: World health organization; 2001.
[2] Ali W, Rasool A, Junaid M, et al. A comprehensive review on current status, mechanism, and possible sources of arsenic contamination in groundwater: a global perspective with prominence of Pakistan scenario. Environ Geochem Health. 2019;41(2):737–760.
[3] Teršič T, Miler M, Gaberšek M, et al. Vsebnosti arzena in nekaterih drugih prvin v potočnih sedimentih in vodah porečja medije, osrednja slovenija= contents of arsenic and some other elements in stream sediments and waters of the medija drainage basin, central Slovenia. Geologija. 2018;61(1):5–24.
[4] Mazej Z, Germ M. Trace element accumulation and distribution in four aquatic macrophytes. Chemosphere. 2009;74(5):642–647.
[5] Šajn R, Gosar M. An overview of some localities in Slovenia that became polluted due to past mining and metallurgic activities. Geologija. 2004;47(2):249–258.
[6] Chou CH, Harper C. Toxicological profile for arsenic. ATSDR case stud environ med: arsenic toxic. 2007;2009:1–124.
[7] Souri Z, Karimi N, Sandalio LM. Arsenic hyperaccumulation strategies: an overview. Front Cell Dev Biol. 2017;5:67.
[8] Tchounwou PB, Yedjou CG, Patlolla AK, et al. Heavy metal toxicity and the environment. In: Luch A, editor. Molecular, clinical and environmental toxicology [Internet]. Basel: Springer Basel; 2012[cited 2023 Jan 12]. p. 133–164. Available from: http://link.springer.com/10.1007/978-3-7643-8340-4_6
[9] Dastgiri S, Mosaferi M, Fizi MA, et al. Arsenic exposure, dermatological lesions, hypertension, and chromosomal abnormalities among people in a rural community of Northwest Iran. J Health Popul Nutr. 2010;28(1):14–22.
[10] Ansone L, Klavins M, Viksna A. Arsenic removal using natural biomaterial-based sorbents. Environ Geochem Health. 2013;35(5):633–642.
[11] Tripathi P, Mishra A, Dwivedi S, et al. Differential response of oxidative stress and thiol metabolism in contrasting rice genotypes for arsenic tolerance. Ecotoxicol Environ Saf. 2012;79:189–198.
[12] Norton GJ, Lou-Hing DE, Meharg AA, et al. Rice–arsenate interactions in hydroponics: whole genome transcriptional analysis. J Exp Bot. 2008;59(8):2267–2276.

[13] Asgher M, Choudhary SA, Sehar Z, et al. Hydrogen peroxide modulates activity and expression of antioxidant enzymes and protects photosynthetic activity from arsenic damage in rice (*Oryza sativaL.)*. J Hazard Mater. 2020;401:123365.
[14] Foyer CH, Noctor G. Ascorbate and glutathione: the heart of the redox hub. Plant Physiol. 2011;155(1):2–18.
[15] Zhao FJ, McGrath SP, Meharg AA. Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. Annu Rev Plant Biol. 2010;61(1):535–559.
[16] Noctor G, Mhamdi A, Chaouch S, et al. Glutathione in plants: an integrated overview: glutathione status and functions. Plant Cell Environ. 2012;35(2):454–484.
[17] Chakrabarty D, Trivedi PK, Misra P, et al. Comparative transcriptome analysis of arsenate and arsenite stresses in rice seedlings. Chemosphere. 2009;74(5):688–702.
[18] Leterrier M, Airaki M, Palma JM, et al. Arsenic triggers the nitric oxide (NO) and S-nitrosoglutathione (GSNO) metabolism in *Arabidopsis*. Environ Pollut. 2012;166:136–143.
[19] Souza TD, Borges AC, Braga AF, et al. Phytoremediation of arsenic-contaminated water by *lemna valdiviana*: an optimization study. Chemosphere. 2019;234:402–408.
[20] Alvarado S, Guédez M, Lué-Merú MP, et al. Arsenic removal from waters by bioremediation with the aquatic plants water hyacinth (*eichhornia crassipes*) and lesser duckweed (*Lemna minor*). Bioresour Technol. 2008;99(17):8436–8440.
[21] da-Silva CJ, Canatto RA, Cardoso AA, et al. Arsenic-hyperaccumulation and antioxidant system in the aquatic macrophyte *Spirodela intermedia* W koch (lemnaceae). Theor Exp Plant Physiol. 2017;29(4):203–213.
[22] Ferreira AD, Viana DG, Pires FR, et al. Arsenic phytoremediation in contaminated and flooded soil: accumulation and translocation in two macrophytes. Water Air Soil Pollut. 2021;232(7):299.
[23] Vandana UK, Gulzar ABM, Singha LP, et al. Hyperaccumulation of arsenic by *Pteris vittata*, a potential strategy for phytoremediation of arsenic-contaminated soil. Environ Sustain. 2020;3(2):169–178.
[24] Praveen A, Pandey VC. Pteridophytes in phytoremediation. Environ Geochem Health. 2020;42(8):2399–2411.
[25] Zemanová V, Pavlíková D, Hnilička F, et al. Arsenic toxicity-induced physiological and metabolic changes in the shoots of pteris cretica and *spinacia oleracea*. Plants. 2021;10(10):2009.
[26] Zemanová V, Popov M, Pavlíková D, et al. Effect of arsenic stress on 5-methylcytosine, photosynthetic parameters and nutrient content in arsenic hyperaccumulator *pteris cretica* (L.) var Albo-lineata. Bmc Plant Biol. 2020;20(1):130.
[27] Singh S, Karwadiya J, Srivastava S, et al. Potential of indigenous plant species for phytoremediation of arsenic contaminated water and soil. Ecol Eng. 2022;175:106476.
[28] Roy D, Sreekanth D, Pawar D, et al. Phytoremediation of arsenic contaminated water using aquatic, semi-aquatic and submerged weeds. In: Biodegradation technology of organic and inorganic pollutants. IntechOpen.Publishing; 2020 [cited 2023 Jan 12]. pp. 159–186. Available from: http://link.springer.com/10.1007/978-3-030-34694-2_9
[29] Yap CK, Fitri M, Mazyhar Y, et al. Effects of metal contaminated soils on the accumulation of heavy metals in different parts of *centella asiatica*: a laboratory study. Sains Malays. 2010;39:347–352.
[30] Saadaoui W, Mokrani K, Tarchoun N. Assessment of lead, copper and cadmium tolerance by four vegetable species. Am Acad Sci Res J Eng Technol Sci. 2018;47:76–87.
[31] Tapia J, Cornejo J, Gutiérrez M, et al. Study of the copper, chromium, manganese and zinc contents in the species *azorella spinosa* (apiaceae), collected in the maule Region, Chile. J Environ Prot. 2019;10:601–613.
[32] Mechora Š, Sotler M, Krajnc AU, et al. How selenium affects *berula erecta*. Water Air Soil Pollut. 2016;227:451.
[33] Mechora Š, Rižnik T, Urbanek Krajnc A, et al. Response of *berula erecta* to lead in combination with selenium. Bull Environ Contam Toxicol. 2020;105:51–61.
[34] Berula Erecta IUCN: de Belair G & Lansdown RV. The IUCN red list of threatened species 2013: e. T164378A13575878 [internet]. Int Union Conserv Nature. 2012[cited 2023 Jan 12]. Available from: http://www.iucnredlist.org/details/164378/0
[35] Kuhar U, Gregorc T, Renčelj M, et al. Distribution of macrophytes and condition of the physical environment of streams flowing through agricultural landscape in north-eastern slovenia. Limnologica. 2007;37:146–154.
[36] Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant. 1962;15:473–497.
[37] Finnegan PM, Chen W. Arsenic toxicity: the effects on plant metabolism. Front Physiol. 2012[[cited 2023 Jan 12]];pp. 3. Available from: http://journal.frontiersin.org/article/10.3389/fphys.2012.00182/abstract
[38] Schreiber U, Bilger W, Neubauer C. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze ED Caldwell M, editors. Ecophysiol photosynth [internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 1995[cited 2023 Jan 12]pp. 49–70. Available from: http://link.springer.com/10.1007/978-3-642-79354-7_3
[39] Lichtenthaler HK, Buschmann C. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Curr Protoc Food Anal Chem. 2001;1:F4.3.1–4.3.8.
[40] Drumm H, Mohr H The mode of interaction between blue (uv) light photoreceptor and phytochrome in anthocyanin formation of the sorghum seedling. Annual of European Symposium Photomorphogenesis [Internet]. Elsevier; 1978 [cited 2023 Jan 12]. p. 241–248. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780080226774500253.
[41] Tausz M. Measuring antioxidants in tree species in the natural environment: from sampling to data evaluation. J Exp Bot. 2003;54:1505–1510.
[42] Zhang J, Hamza A, Xie Z, et al. Arsenic transport and interaction with plant metabolism: clues for improving agricultural productivity and food safety. Environ Pollut. 2021;290:117987.
[43] Chen W, Chi Y, Taylor NL, et al. Disruption of ptLPD1 or ptLPD2, genes that encode isoforms of the plastidial lipoamide dehydrogenase, confers arsenate hypersensitivity in arabidopsis. Plant Physiol. 2010;153:1385–1397.
[44] Tu S, Ma LQ. Interactive effects of pH, arsenic and phosphorus on uptake of as and P and growth of the arsenic hyperaccumulator *Pteris vittata* L. under hydroponic conditions. Environ Exp Bot. 2003;50:243–251.
[45] Mechora Š, Žerdoner Čalasan A, Felicijan M, et al. The impact of selenium treatment on some physiological

and antioxidant properties of *apium repens*. Aquat Bot. 2017;138:16–23.
[46] Rathoure AK, editor. Handbook of research on waste diversion and minimization technologies for the industrial sector. Hershey, PA: Engineering Science Reference, an imprint of IGI Global; 2021.
[47] Gunes A, Inal A, Bagci EG, et al. Combined effect of arsenic and phosphorus on mineral element concentrations of sunflower. Commun Soil Sci Plant Anal. 2010;41:361–372.
[48] Stoeva N, Berova M, Zlatev Z. Effect of arsenic on some physiological parameters in bean plants. Biol Plant. 2005;49:293–296.
[49] Kofroňová M, Hrdinová A, Mašková P, et al. Strong antioxidant capacity of horseradish hairy root cultures under arsenic stress indicates the possible use of *Armoracia rusticana* plants for phytoremediation. Ecotoxicol Environ Saf. 2019;174:295–304.
[50] Williams PN, Lei M, Sun G, et al. Occurrence and partitioning of cadmium, arsenic and lead in mine impacted paddy rice: hunan, China. Environ Sci Technol. 2009;43:637–642.
[51] Zhu YG, Williams PN, Meharg AA. Exposure to inorganic arsenic from rice: a global health issue? Environ Pollut. 2008;154:169–171.
[52] Ahmad P, Alyemeni MN, Al-Huqail AA, et al. Zinc oxide nanoparticles application alleviates arsenic (As) toxicity in soybean plants by restricting the uptake of as and modulating key biochemical attributes, antioxidant enzymes, ascorbate-glutathione cycle and glyoxalase system. Plants. 2020;9:825.
[53] Zhang F, Gu F, Yan H, et al. Effects of soaking process on arsenic and other mineral elements in brown rice. Food Sci Hum Wellness. 2020;9:168–175.
[54] Chandrakar V, Naithani SC, Keshavkant S. Arsenic-induced metabolic disturbances and their mitigation mechanisms in crop plants: a review. Biologia. 2016;71:367–377.
[55] Bali AS, Sidhu GPS. Arsenic acquisition, toxicity and tolerance in plants - from physiology to remediation: a review. Chemosphere. 2021;283:131050.
[56] Tangahu BV, Sheikh Abdullah SR, Basri H, et al. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng. 2011;2011:1–31.
[57] Zhao FJ, Ma JF, Meharg AA, et al. Arsenic uptake and metabolism in plants. New Phytol. 2009;181:777–794.
[58] Dhankher OP, Rosen BP, McKinney EC, et al. Hyperaccumulation of arsenic in the shoots of *Arabidopsis* silenced for arsenate reductase (ACR2). Proc Natl Acad Sci. 2006;103:5413–5418.
[59] Duan G, Zhou Y, Tong Y, et al. A CDC25 homologue from rice functions as an arsenate reductase. New Phytol. 2007;174:311–321.
[60] Ahsan N, Lee DG, Alam I, et al. Comparative proteomic study of arsenic-induced differentially expressed proteins in rice roots reveals glutathione plays a central role during as stress. Proteomics. 2008;8:3561–3576.
[61] Jung H, Kong MS, Lee BR, et al. Exogenous glutathione increases arsenic translocation into shoots and alleviates arsenic-induced oxidative stress by sustaining ascorbate–glutathione homeostasis in riceseedlings. Front Plant Sci. 2019;10:1089.
[62] Zechmann B. Diurnal changes of subcellular glutathione content in *Arabidopsis thaliana*. Biol Plant. 2017;61:791–796.
[63] Raab A, Ferreira K, Meharg AA, et al. Can arsenic-phytochelatin complex formation be used as an indicator for toxicity in *Helianthus annuus*? J Exp Bot. 2007;58:1333–1338.
[64] Srivastava S, Mishra S, Tripathi RD, et al. Phytochelatins and antioxidant systems respond differentially during arsenite and arsenate stress in hydrilla verticillata (L.F.) royle. Environ Sci Technol. 2007;41:2930–2936.
[65] Srivastava S, Suprasanna P, D'Souza SF. Redox state and energetic equilibrium determine the magnitude of stress in *Hydrilla verticillata* upon exposure to arsenate. Protoplasma. 2011;248:805–815.
[66] Mishra S, Srivastava S, Tripathi RD, et al. Thiol metabolism and antioxidant systems complement each other during arsenate detoxification in *Ceratophyllum demersum* L. Aquat Toxicol. 2008;86:205–215.