

Phytoremediation potential of sweet flag (*Acorus calamus* L.), an emergent macrophyte, to remove inorganic arsenic from water

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Abstract

In this study, the emergent macrophyte sweet flag (*Acorus calamus* L.) was used to remove different concentrations (200, 500, and 1,000 $\mu\text{g L}^{-1}$) of arsenate [As(V)] and arsenite [As(III)] from water. The removal efficiencies of As(V) and As(III) reached more than 95%. As(III) could be removed by *A. calamus* L. more efficiently than As(V). In both As(V)- and As(III)-exposed *A. calamus* L., the arsenic contents were much higher in the root than in the stem and leaf. The translocation factors of As(V) and As(III) were no more than 0.152. Both As(V) and As(III) were found in the whole plant, whereas dimethylarsinic acid (DMA, 0.06–0.13 mg kg^{-1}) was only present in the aboveground part (leaf). As(V) was the main species in the As(V)-exposed plants (45.86%–70.21%). As(III) was the main species in the stem and leaf of As(III)-exposed plants (55.76%–85.52%), while As(V) was still dominant in the root. *A. calamus* L. could keep its green leaves during the 31 days of inorganic arsenic (iAs) exposure. However, As(V) had a little inhibitory effect on height growth, and As(III) could slightly inhibit the weight gain. The concentrations of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), as well as the activity of catalase (CAT) were significantly higher in the root than those in the stem and leaf. The oxidative stress response of *A. calamus* L. to As(III) was more than that to As(V). Our findings indicated that *A. calamus* L. was regarded as a promising material for the removal of iAs.

Introduction

Arsenic is widely distributed in the Earth's crust mostly as minerals and in inorganic forms. Arsenic may enter the air, water, and soil through various geochemical processes and anthropogenic activities such as mining, smelting, fossil fuels burning, and the application of pesticides and herbicides. Human exposure to arsenic is primarily a result of ingestion of arsenic-contaminated drinking water or food, inhalation of arsenic dust in the air, and dermal absorption. However, ingestion is the predominant type of arsenic exposure (Moreira et al., 2021). The ingestion of arsenic-contaminated drinking water can cause various health problems including skin, lung, liver, and bladder cancers (Sodhi et al., 2019). Given its toxicity, the International Agency for Research on Cancer (IARC) classified arsenic as a Group I carcinogen. The World Health Organization (WHO) has set a provisional guideline limit of 10 $\mu\text{g L}^{-1}$ for arsenic in drinking water. In China, more than 19 million people are exposed to arsenic-rich water (Xu et al., 2022). Thus, it is urgent to remediate the arsenic-contaminated water by effective methods.

Arsenic can occur in four different oxidation states (−3, 0, +3, +5) in the environment. However, the inorganic forms as pentavalent arsenate [As(V)] and trivalent arsenite [As(III)] are mostly found in natural waters, with As(V) more predominant and thermodynamically stable than As(III). Various methods have been developed to remove inorganic arsenic (iAs) from water, e.g., chemical precipitation, ion exchange, membrane filtration, electrocoagulation, adsorption, and biological treatments (Jiang et al., 2022). The physicochemical methods are usually expensive, labor-intensive, and time-consuming. Biological treatments (phytoremediation and bioremediation) are now widely used as an alternative to the equivalent physicochemical methods owing to their low-cost and eco-friendly advantages (Wei et al.,

2021). Phytoremediation is the use of plants to reduce the environmental contaminants in surface water and sediments/soils. In nature, there are several plants existing with the ability to remove toxic metals from the surrounding environment as a survival strategy. The well-studied strategies are phytoextraction (or phytoaccumulation), phytofiltration (or rhizofiltration), phytostabilization, phytovolatilization, and phytodegradation. The other desirable characteristic permitting phytoremediation as an ideal method is arsenic biomethylation by plants. Tape grass (*Vallisneria natans* (Lour.) Hara) was found to be able to metabolize the iAs to dimethylarsinic acid (DMA) and arsenobetaine (AsB) (Li et al., 2018). In general, organic arsenic (oAs) is less toxic than iAs, and complex organoarsenicals (e.g., AsB) are considered to be nontoxic. Hence, phytoremediation is emerging as an attractive option for the cleanup of arsenic-contaminated environments.

Aquatic macrophytes (including emergent, floating-leaved, submerged, and floating macrophytes and hygrophytes) are key components of wetland ecosystems as they help in degrading and removing organic and inorganic contaminants. Emergent macrophytes are rooted in sediments and undergo photosynthesis above the water. Thus, they can absorb toxic metals from water and sediments (Lin et al., 2018). Some emergent macrophytes have been studied as adsorbents to remove toxic metals, for instance, sweet flag (*Acorus calamus* L.), oriental cattail (*Typha orientalis* Presl), reed (*Phragmites australis*), and purple loosestrife (*Lythrum salicaria* L.) (Sesin et al., 2021). *A. calamus* L. is a perennial herbaceous plant with rhizome that is indefinitely branched, smooth, pinkish or pale green, and widely distributed in China. Although *A. calamus* L. is a highly valued medicinal plant, it has been used for the removal of contaminants such as hexavalent chromium and divalent lead ions (Shooto, 2020), leachate (Bhagwat et al., 2018), microcystin-LR (Chen et al., 2019), and algae (Zhang et al., 2016). Nevertheless, less is known about the strategy for arsenic removal from water by *A. calamus* L.. A better understanding of the arsenic removal mechanism by *A. calamus* L. will help to develop the phytoremediation potential of emergent macrophytes.

Given the above-mentioned reasons, *A. calamus* L. was selected as a case (1) to compare the removal efficiency of As(V) and As(III); (2) to investigate the biotransformation of iAs to oAs; and (3) to clarify the physiological responses to the iAs stress. This study aimed to provide experimental evidence for the application of *A. calamus* L. to the phytoremediation of arsenic-contaminated water.

Materials And Methods

Plant material and treatment conditions

A. calamus L. was obtained from an aquatic plant nursery in Suqian City, Jiangsu Province, China. All plants were washed with running tap water to thoroughly remove attached soil particles and rinsed three times with ultrapure water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$). Then, the clean plants with similar size ($30.0 \pm 2.0 \text{ cm}$ in height and $6.7 \pm 0.3 \text{ g}$ in weight for As(V) treatment, $39.5 \pm 2.0 \text{ cm}$ in height and $11.0 \pm 1.0 \text{ g}$ in weight for As(III) treatment) were selected and acclimatized for 7 days in laboratory conditions (15% Hoagland's solution, pH 7.0 ± 0.5 , $112 \mu\text{mol m}^{-2} \text{ s}^{-1}$, 12L:12D, $22 \pm 1^\circ\text{C}$). After acclimatization, the plants were

washed using ultrapure water, and then treated with different concentrations of As(V) or As(III) (200, 500, and 1000 $\mu\text{g L}^{-1}$) under the above-mentioned laboratory conditions. Experiments were set up in triplicate and each replicate contained three plants of similar size. The treatment without arsenic was used as the blank control. Ultrapure water was replenished every day due to evaporation losses. Water samples were taken every 2 days and filtered through a 0.22- μm filter (Xinya Purify Device Company, Shanghai, China). For arsenic analysis, the water samples were acidified with 1% HNO_3 solution (v/v) and immediately cooled to 4°C. The plant samples were collected on day 31 of the treatment. The collected plants were first washed with running tap water and then with ultrapure water to remove apoplastic arsenic. After being dried with filter papers, the plant samples were used to measure height and fresh weight, and then divided into root, stem, and leaf. Some fresh samples were frozen at -70°C for subsequent determination of lipid peroxidation level and antioxidant enzyme activity. Some were freeze-dried and grounded in an agate mortar. The powdered dry samples were sealed in PE bags and stored in a desiccator before arsenic analysis.

Digestion and analysis of total arsenic

Each plant sample (0.2 g of leaf or 0.1 g of root) was digested using 4.0 mL of 70% HNO_3 ($\geq 99.999\%$ metals basis) and 1.0 mL of 30% H_2O_2 (guaranteed reagent grade) in a microwave-assisted digestion system (MDS-6G, Sineo Microwave Chemistry Technology Co. Ltd, Shanghai, China). The digestion procedure was conducted in three steps: 15 min to 120°C , 15 min to 190°C , and 30 min at 190°C (Li et al., 2018). After cooling down, the digested solution was diluted to 25 mL with ultrapure water, passed through a 0.22- μm filter, and kept at 4°C before analysis. Total arsenic in both water samples and digested solutions were determined by an Agilent 7700x ICP–MS (Agilent, Tokyo, Japan). The same method was used to analyze a certified reference material of celery (GBW-10048) from the CRM/RM Information Center of China (Beijing, China). A method blank was conducted during each batch of samples to confirm the blank value had no statistical significance. The recoveries for total arsenic in spiked samples ranged from 90–110%.

Extraction and speciation of arsenic

Arsenic species were extracted from the plant samples using a microwave-assisted extraction method. 0.2 g of leaf or root powder was weighed into a Teflon digestion vessel and 10 mL of 1% HNO_3 solution (v/v) was added. The digestion vessel was placed in a microwave-assisted digestion system at 90°C for 90 min. After cooling, the extracted solution was centrifuged at 9,000 rpm for 30 min. The supernatant was then filtered through a 0.22- μm filter and stored at 4°C before analysis. Arsenic speciation analysis in plant samples was performed using Agilent 1260 HPLC (Agilent, Tokyo, Japan) with Agilent 7700x ICP–MS. The extraction efficiency of arsenic was validated by analyzing the certified reference material GBW-10048. The extraction efficiency was 69–98%.

Determination of lipid peroxidation and antioxidant enzyme activities

About 0.3 g of leaf or root sample was homogenized in 2.7 mL of precooled (4°C) 0.9% saline solution in an ice-bath. The homogenate was then centrifuged at 4,000 rpm for 10 min at 4°C. The sample solution for the measurement of H₂O₂ concentration was centrifuged again at 10,000 rpm for 10 min. The obtained supernatant was used for further analysis. Malondialdehyde (MDA) content was determined using the thiobarbituric acid (TBA) reaction. H₂O₂ was measured in the reaction mixture by the molybdcic acid (MA) method. Catalase (CAT) activity was assessed in the reaction with ammonium molybdate. All the kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Statistical analysis

All values are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with Duncan's multiple range test (DMRT) was run to test the differences among groups. Values of $P < 0.05$ were considered statistically significant. All statistical analysis was performed with SPSS 25.0 (SPSS Inc., Chicago, IL, USA). Graphics were generated using Origin 2018C (OriginLab, Inc., Northampton, MA, USA).

Results And Discussion

Comparison of As(V) and As(III) removal by *A. calamus* L.

The removal efficiencies of As(V) and As(III) by *A. calamus* L. were compared during a 31-day exposure. As shown in Fig. 1, the remaining concentrations of As(V) and As(III) in water showed a trend of continuous decrease. Differently, the remaining concentration of As(III) dropped more quickly than that of As(V). For As(V) treatment, the arsenic removal efficiency increased first slightly for 5 or 7 days and then sharply from 28.39%±0.72% (200 µg L⁻¹), 6.13%±1.60% (500 µg L⁻¹), 10.66%±2.14% (1,000 µg L⁻¹) to 96.50%±0.33%, 95.03%±0.13%, 97.09%±0.36%, respectively. For As(III) treatment, the removal efficiency of arsenic presented a linear increase since the beginning of the experiment and then kept nearly invariant between the 13th and 31st day. The removal efficiency of arsenic reached 97.10%±0.67% (200 µg L⁻¹), 97.41%±0.08% (500 µg L⁻¹), and 95.45%±0.40% (1,000 µg L⁻¹), respectively. In general, *A. calamus* L. could remove more than 95% of As(V) or As(III) from water. Compared with As(V), As(III) could be removed by *A. calamus* L. more efficiently.

Arsenic is a non-essential metalloid that is highly toxic to organisms including plants. Phytoremediation is a sustainable, natural, and eco-friendly approach to environmental decontamination (Amiri et al., 2020). Thus, more information is needed to identify the suitable plant species for the phytoremediation of arsenic-contaminated water. Aquatic macrophytes can be a good candidate for phytoremediation of metals. Several floating macrophytes have been investigated for the removal of metals. Water lettuce (*Pistia stratiotes* L.) had the highest total Mn removal from the water (Hua et al., 2012). Both duckweed (*Lemna minor*) and water hyacinth (*Eichhornia crassipes*) were also effective in the removal of metals (i.e., Cu) (Rezania et al., 2016). Azolla (*Azolla pinnata*) could remove 65–95% of applied metals (Zn, Cu, Pb, Cr, and Cd) from wastewater (Akhtar et al., 2021). Compared to floating macrophytes, little information has been gathered to date regarding arsenic removal by emergent macrophytes. In this study,

more than 95% of the total arsenic was removed from As(V)-/As(III)-contaminated water. Nevertheless, de Souza et al. (2019) reported that *Lemna valdiviana* was able to remove 82% of its initial As(III) concentration under optimal conditions. It was suggested that *A. calamus* L. could be an effective plant for phytoremediation. On the other hand, the pre-oxidation of As(III) to As(V) is a desirable process for the effective removal of iAs from water by using physicochemical methods. Our results showed that *A. calamus* L. could efficiently remove As(III) as well as As(V) without a pre-oxidation process. Accordingly, *A. calamus* L. could be suitable for iAs phytoremediation.

Accumulation and transformation of arsenic species by *A. calamus* L.

Accumulation of arsenic in *A. calamus* L. was presented in Fig. 2. After 31-day exposure, the total arsenic in different vegetative organs of *A. calamus* L. increased with the increase of initial As(V) concentration. About $34.24 \pm 0.49 \text{ mg kg}^{-1}$ ($200 \mu\text{g L}^{-1}$), $46.76 \pm 5.39 \text{ mg kg}^{-1}$ ($500 \mu\text{g L}^{-1}$), and $292.77 \pm 36.18 \text{ mg kg}^{-1}$ ($1,000 \mu\text{g L}^{-1}$) of arsenic have been found to be accumulated in the underground part (root) of *A. calamus* L.. Only $1.31 \pm 0.06 \text{ mg kg}^{-1}$ ($200 \mu\text{g L}^{-1}$), $6.12 \pm 0.10 \text{ mg kg}^{-1}$ ($500 \mu\text{g L}^{-1}$), and $3.71 \pm 0.15 \text{ mg kg}^{-1}$ ($1,000 \mu\text{g L}^{-1}$) of arsenic were accumulated in the aboveground part (stem and leaf). Thus, it can be seen that the arsenic contents were much higher in the root than in the stem and leaf. After As(III) treatment, *A. calamus* L. accumulated $82.14 \pm 11.51 \text{ mg kg}^{-1}$ ($200 \mu\text{g L}^{-1}$), $26.08 \pm 6.36 \text{ mg kg}^{-1}$ ($500 \mu\text{g L}^{-1}$), and $62.38 \pm 7.97 \text{ mg kg}^{-1}$ ($1,000 \mu\text{g L}^{-1}$) of arsenic in the roots, respectively. Similarly, a small amount of arsenic was accumulated in the stem and leaf under As(III) exposure. It was indicated that iAs was mainly accumulated in the root of *A. calamus* L.. This conclusion was supported by the observation that the translocation factors (TFs) of As(V) and As(III) were no more than 0.044 (see Table 1).

Various arsenic species were identified and quantified in the vegetative organs of *A. calamus* L. (Fig. 3). For As(V) treatment, As(V) and As(III) were found in the whole plant, whereas DMA ($0.09\text{--}0.13 \text{ mg kg}^{-1}$) was only present in the aboveground part. As(V) was the main species in the As(V)-exposed plants (45.86%–70.21%). Both the content and percentage of As(III) significantly increased with the increase in the concentration of As(V) treatment. But the percentage of As(III) was no more than 51.23%. It was suggested that some of the As(V) absorbed into the root system was reduced to As(III) and further methylated to DMA in the stem and/or leaf. For As(III) treatment, As(V) and As(III) were also identified in the whole plant, but DMA (0.06 and 0.07 mg kg^{-1}) was detected in the leaf under the treatment of 500 and $1,000 \mu\text{g As(III) L}^{-1}$. Correspondingly, As(III) was the main species in the stem and leaf of As(III)-exposed plants (55.76%–85.52%), while As(V) was still dominated in the root under 200 and $500 \mu\text{g As(III) L}^{-1}$ treatment (64.8% and 58.42%). Although the As(V) content increased with increasing the concentration of As(III) treatment, the percentage of As(V) decreased. It was indicated that a large amount of As(III) absorbed could be oxidized to As(V) in the root system, and its oxidation efficiency was limited by the As(III) content in cells. The methylation of As(III) to DMA seems to occur mainly in the leaf of emergent macrophyte.

Table 1
Bioaccumulation factors (BCF) and translocation factors (TF)
of *A. calamus* under different arsenic concentrations.

	Treatment concentration ($\mu\text{g L}^{-1}$)	BCF	TF
As()	200	1.32	0.043
	500	1.44	0.036
	1000	0.81	0.044
As()	200	8.30	0.007
	500	1.90	0.044
	1000	2.29	0.032

In this study, iAs was more accumulated in the root than in the stem and leaf of *A. calamus* L. at each treated concentration. Our result was similar to the results of several submerged macrophytes. *V. natans* (Lour.) Hara accumulated more arsenic in the roots ($187.11\text{--}248.65\text{ mg kg}^{-1}$) than in the leaves ($19.10\text{--}39.52\text{ mg kg}^{-1}$) after 14 days of treatment (Li et al., 2018). Arsenic was also more accumulated in the roots of alternate watermilfoil (*Myriophyllum alterniflorum* DC.) ($156\pm 22\text{ mg kg}^{-1}$) than in its shoots ($104\pm 12\text{ mg kg}^{-1}$) (Krayem et al., 2016). *Hydrilla verticillata* (L.f.) Royle has been shown to accumulate > 73% of arsenic in the root (Xue and Yan, 2011). Additionally, the highest concentration of arsenic was found in the roots of two free-floating macrophytes [*E. crassipes* (Mart.) solms and water spinach (*Ipomoea aquatica* Forsk)] and one emergent macrophyte [narrow-leaved cattail (*Typha angustifolia* L.)] growing in the wastewater treatment ponds (Nateewattana et al., 2010). It was proved that the root of aquatic macrophytes acted as a barrier to limit arsenic translocation to the stem and leaf. This idea was further evident from the low TF values ($0.007\text{--}0.044$) of arsenic in *A. calamus* L..

Some organisms, such as bacteria, archaea, fungi, algae, and animals (including humans), are able to methylate As(III) to various oAs species (e.g., monomethylarsonic acid (MMA) and DMA) (Zhao and Wang, 2020). In organisms, As(V) can be first reduced to As(III), followed by methylation to MMA, and then methylation to DMA (Wu et al., 2015). However, few higher plants appear to have the ability to methylate As(III). In this study, DMA could be detected in the aboveground part (i.e., leaf) of *A. calamus* L. at each concentration of As()/As(III) treatment. This appears to be the first demonstration of DMA formed in higher plants. DMA is a major metabolite of iAs in many organisms including humans (Cullen et al., 2016). In general, methylation of iAs has been regarded as a detoxification mechanism because oAs is supposed to be less toxic than iAs (Zhen et al., 2020). The presence of DMA in the aboveground part (i.e., leaf) of *A. calamus* L. was considered to be a means of detoxification.

iAs-induced changes in growth status of *A. calamus* L.

The effect of iAs concentration on the growth status of *A. calamus* L. was studied to evaluate its stress resistance. As illustrated in Table 2, the height growth rates of As(V)-exposed plants were lower than the

control group, but the weight gain rates were slightly higher than the control group (except for the 500 µg As(V) L⁻¹ condition). On the contrary, the height growth rates of As(III)-exposed plants were higher than the control group, while the weight gain rates were lower than the control group. These results indicated that As(V) had a little inhibitory effect on height growth, and As(III) could slightly inhibit the weight gain. In addition, *A. calamus* L. could keep its green leaves during the 31 days of iAs exposure (Fig. 4), suggesting that low content of iAs in leaves had no obvious inhibitory effect on the chlorophyll synthesis of emergent macrophyte. *A. calamus* L. has a certain resistance to arsenic pollution in water.

Table 2
Effect of iAs concentration on growth status of *A. calamus* L. during 31 days of exposure.

Concentration (μg L ⁻¹)	Height ₀ (cm)	Height ₁ (cm)	Height growth rate* (%)	Weight ₀ (g)	Weight ₁ (g)	Weight gain rate* (%)	
As()	0	30.3	73.3	142	6.91	16.68	142
	200	30.7	71.0	131	6.75	16.39	143
	500	31.3	61.1	96	6.59	12.45	89
	1,000	28.8	67.0	132	6.50	16.13	148
As()	0	41.5	71.3	72	10.93	20.97	92
	200	39.5	69.4	76	11.14	19.75	77
	500	40.3	70.4	75	10.53	19,82	88
	1,000	37.4	68.4	83	10.72	19.97	86
*Height growth rate=[(Height ₁ –Height ₀)/Height ₀]×100%							

Weight gain rate=[(Weight₁–Weight₀)/Weight₀]*100%

In nature, few plant species are capable of accumulating or detoxifying extraordinarily high levels of arsenic (Souri et al., 2017). Arsenic can affect the morphological, physiological, biochemical, and metabolic attributes of plants like root-shoot length and biomass, chlorophyll content, and photosynthetic rate (Bali & Sidhu, 2021). Singh et al. (2019) reported As(V) treatments (100 and 200 µM) led to significant reduction in root and leaf biomass. Arsenic treatment also reduced the fresh weight by 25.8% and dry weight by 31.0% in *Artemisia annua* L. as compared to the respective control (Naeem et al., 2020). In this study, As(V) was found to slightly decrease the height growth rate of *A. calamus* L.. This may be related to the decrease of nutrients (e.g., Ca, Mg, and P) uptake and transport caused by As(V) (Gusman et al., 2013). However, As(III) was more inclined to slightly reduce the weight gain rate of *A. calamus* L.. This phenomenon may be caused by the reduction in the photosynthesis rate under As(III) treatment, which leads to reduced organic matter content (Gupta et al., 2021).

iAs-induced changes in lipid peroxidation and oxidative stress of *A. calamus* L.

MDA, a marker of lipid peroxidation, was determined in the As(V)/As(III)-exposed plants. As demonstrated in Fig. 5a&d, the MDA concentrations in the root were significantly higher than those in the stem and leaf. For As(III) treatment, the MDA concentrations showed an upward trend with the increase of treatment concentration. For As(V) treatment, the MDA concentrations in *A. calamus* L. with 500 $\mu\text{g As(V) L}^{-1}$ treatment were the highest. H_2O_2 is a normal by-product of cellular metabolism that in higher concentrations can induce oxidative stress. Similarly, the concentrations of H_2O_2 were the highest in the root of As(III)-exposed plants in each treated concentration (Fig. 5f). But the amount of H_2O_2 generated in response to As(V) was lower than that responding to As(III). CAT is involved in H_2O_2 catabolism, which is important in defense against oxidative stress. The activities of CAT in the As(III)-treated groups were the lowest in the leaf, and increased with the increase of the As(III) concentration (Fig. 5e). The highest CAT activity in As(V)-exposed plants was found in the root at 500 $\mu\text{g As(V) L}^{-1}$ (Fig. 5b).

Arsenic is a redox-active element that causes oxidative stress by generating reactive oxygen species (ROS) in the plant tissues from its conversion of As(V) to As(III), leading to non-specific oxidation of biomolecules, enzyme inactivation, and membrane damage (Adhikary et al., 2022). ROS are reactive derivatives of O_2 metabolism, including superoxide radical ($\text{O}_2^{\cdot-}$), singlet oxygen ($^1\text{O}_2$), hydroxyl radical ($\cdot\text{OH}$), and H_2O_2 . Out of all the ROS molecules, H_2O_2 has the longest stability (half-life of 10^{-3} s) within plant cells (Huang et al., 2019). H_2O_2 is considered to be an important signaling molecule that responds to various stimuli. In *A. calamus* L., the accumulation of H_2O_2 was greater in the root than in the stem and leaf. The concentration of H_2O_2 was obviously related to the concentration of arsenic absorbed. On the other hand, plants have evolved a suite of antioxidant system to protect against oxidative stress by scavenging ROS (Singh et al., 2017). CAT is one of the crucial antioxidant enzymes that mitigates oxidative stress to a considerable extent by metabolizing H_2O_2 to H_2O and O_2 . Corresponding to H_2O_2 , CAT activity increased significantly upon As(III) treatment. As described by Liu et al. (2017), a higher activity of antioxidant enzyme generally exhibited better resistance to heavy metal stress. It was suggested that *A. calamus* L. could well resist As(III) stress. In addition to the enzymatic antioxidant defense systems, plants synthesize the non-enzymatic antioxidants such as MDA and glutathione (GSH) which are also involved in scavenging ROS (Ahire et al., 2021). MDA is generated by the peroxidation of membrane polyunsaturated fatty acids, which is the most commonly used marker for the determination of the antioxidant status. In this study, both As(V) and As(III) stress caused a significant increase in MDA levels in the root of *A. calamus* L.. At the same time, the level of MDA increased with increasing arsenic concentration. These results were in agreement with the findings of Banerjee and Roychoudhury (2022) and Chu et al. (2022), suggesting that *A. calamus* L. was equipped with an efficient antioxidant mechanism against iAs-induced oxidative stress.

Conclusions

This study quantified the contribution of emergent macrophyte *A. calamus* L. in inorganic arsenic removal from water. *A. calamus* L. exhibited a satisfactory removal efficiency for As(V) and As(III). iAs

could be absorbed into *A. calamus* L. and mainly accumulated in its root. Meanwhile, *A. calamus* L. favored the methylation of As(III) to DMA in the leaf. Plant growth was nearly not affected by As(V) and As(III). iAs treatment could cause an increasing generation of ROS (i.e., H₂O₂) in *A. calamus* L., which was effectively scavenged by its enzymatic and non-enzymatic antioxidants (CAT and MDA). Our present work concluded that *A. calamus* L. had a high level of iAs resistance in the tested conditions. In summary, *A. calamus* L. can be considered a promising plant for the phytoremediation of iAs-contaminated water.

Declarations

Author contributions Shuhui Li: Experiment conducting, Formal analysis, Writing—original draft. Ting Zhang: Experiment designing, Supervision, Writing—review & editing, Funding acquisition. Guangyi Liu: Funding acquisition.

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Figures

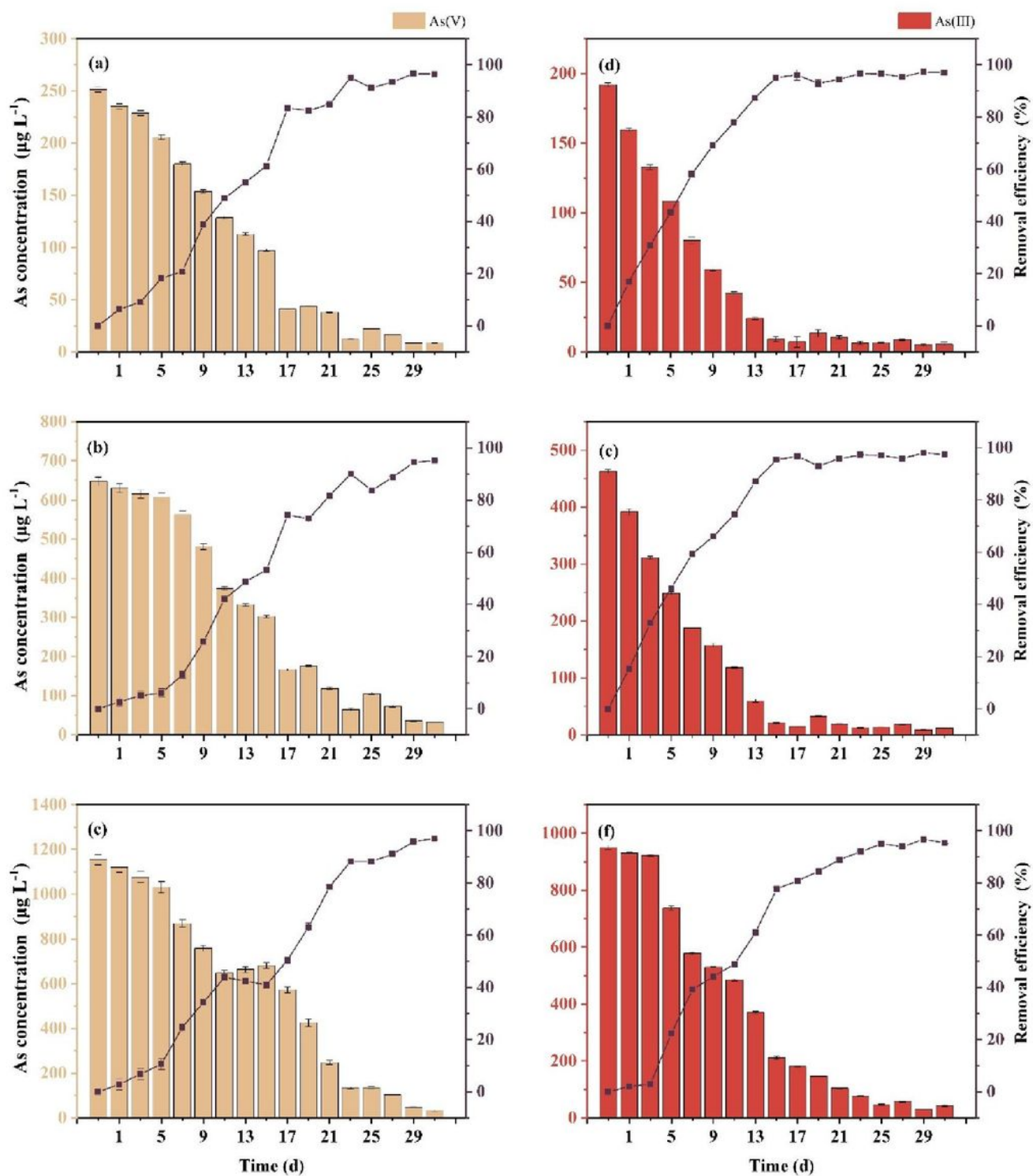


Figure 1

Arsenic removal by *A. calamus* L. treated with different concentrations of As() (a–c) and As() (d–f).

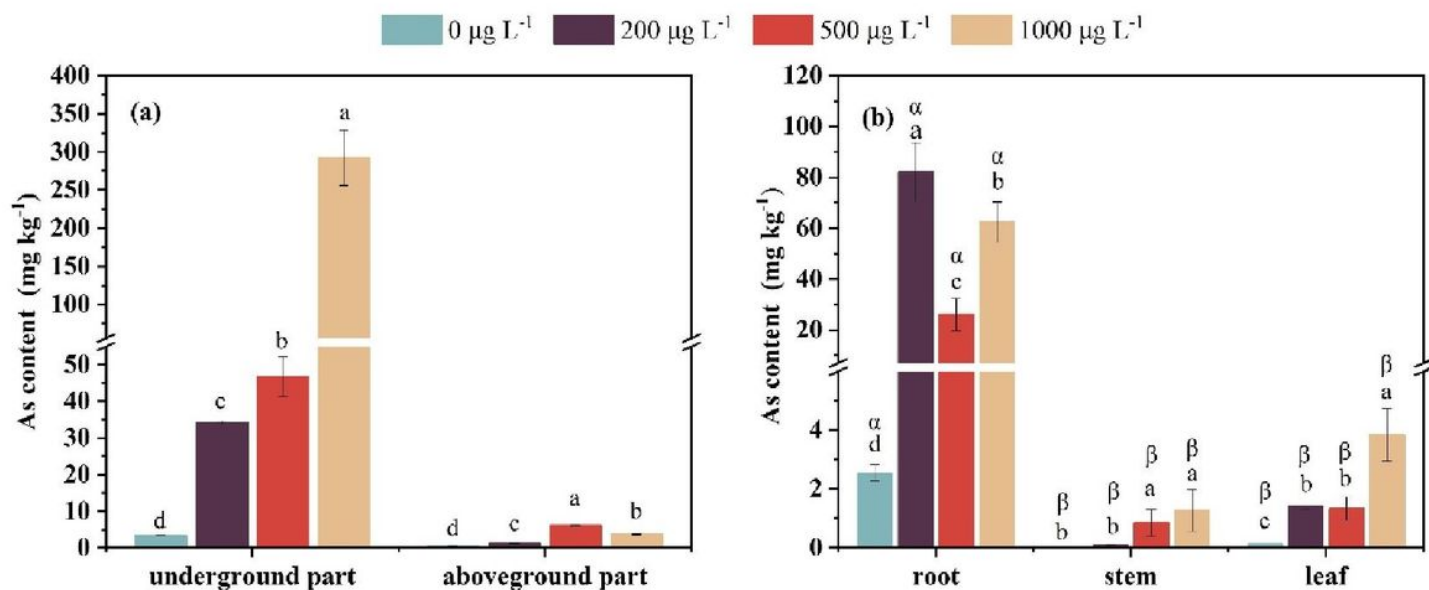


Figure 2

Accumulation of arsenic in *A. calamus* L. treating with different concentrations of As(V) (a) and As(III) (b). Different English letters (a, b, c, and d) indicate significant differences ($P < 0.05$) among each treatment group, and Greek letters (α, β, and γ) represent the significant differences between vegetative organs. Data are expressed as the mean $\pm$ SD ($n=3$).

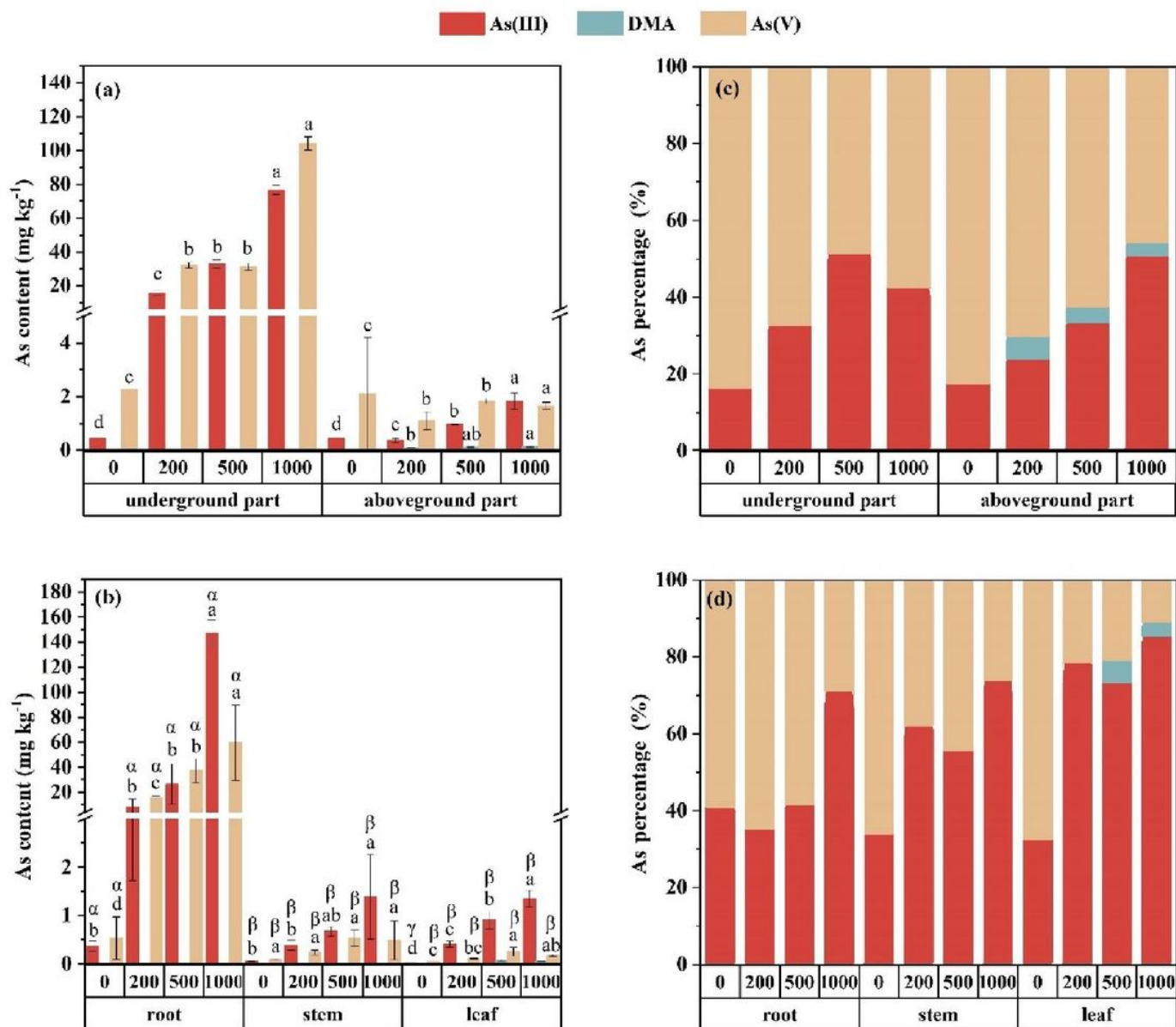


Figure 3

Arsenic species in *A. calamus* L. under different concentrations of As(V) (a, c) and As(III) (b, d). Different English letters (a, b, c, and d) indicate significant differences ($P < 0.05$) among each treatment group, and Greek letters (α , β , and γ) represent the significant differences between vegetative organs. Data are expressed as the mean $\pm$ SD ($n=3$).

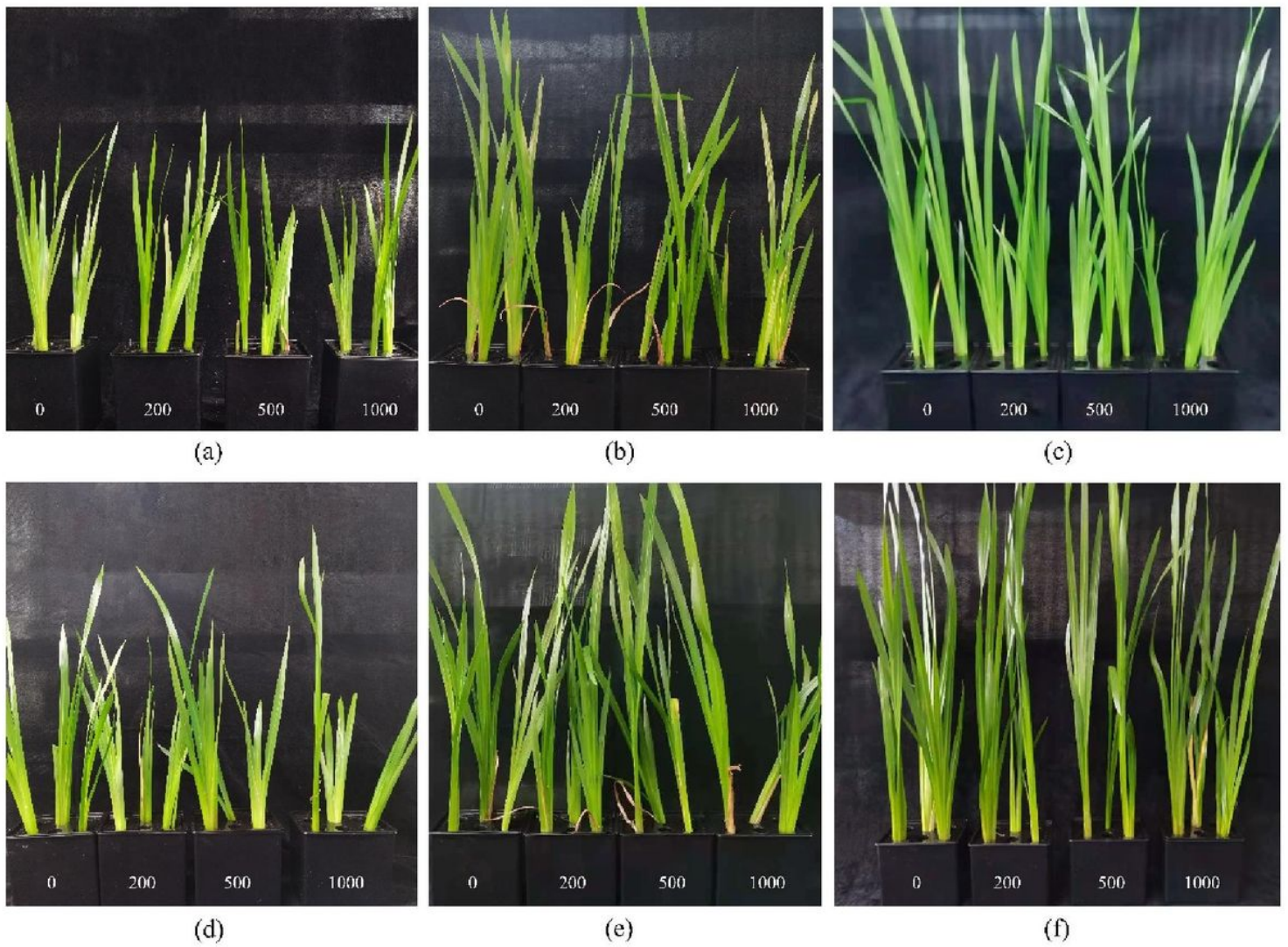


Figure 4

Growth status of *A. calamus* under different concentrations (0, 200, 500, and 1000 $\mu\text{g L}^{-1}$) of As(V) (a, b, c) and As(III) (d, e, f) on 1st day, 15th day, and 31st day.

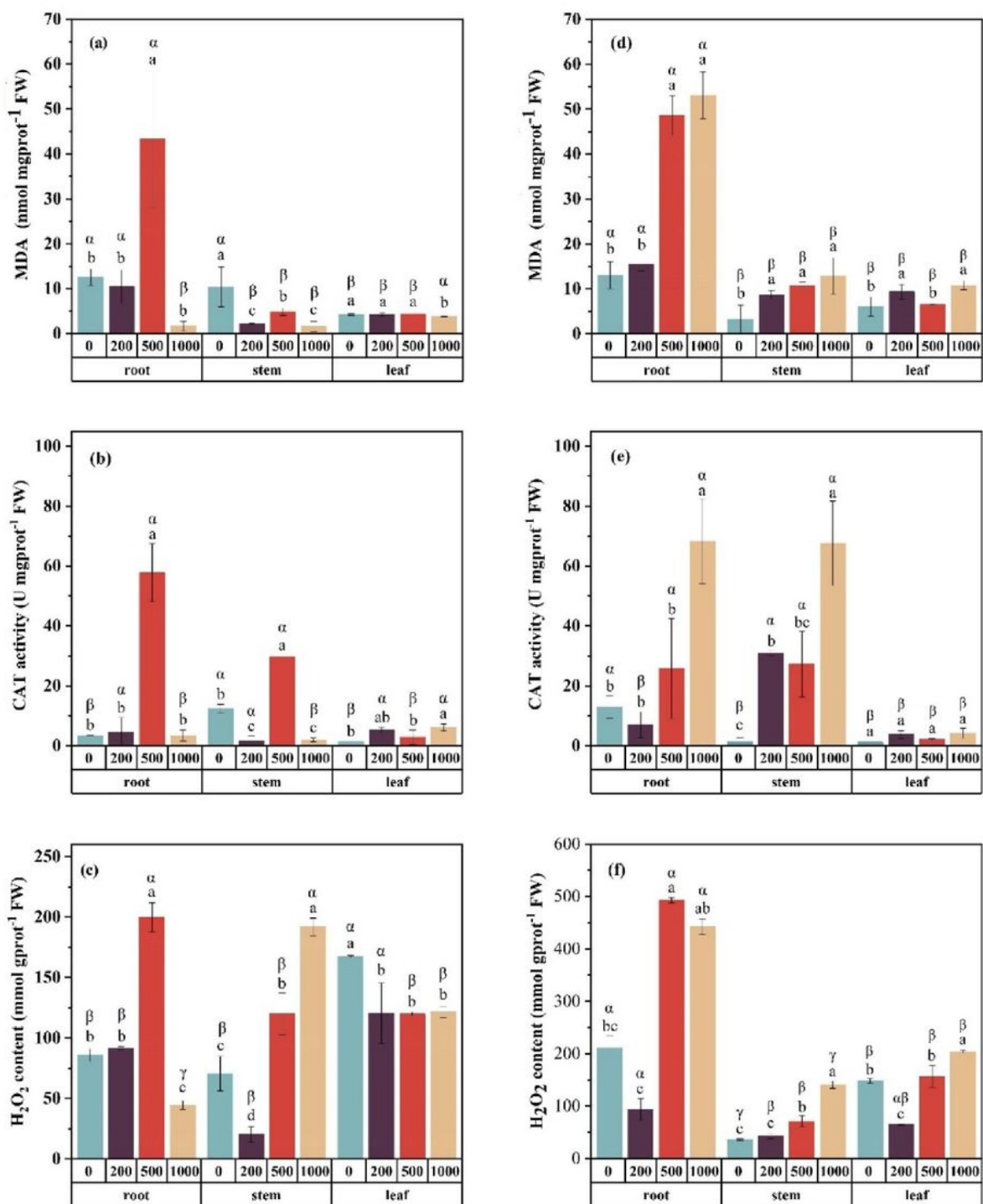


Figure 5

Antioxidant physiological indices (MDA, CAT, and H₂O₂) of roots, stems, and leaves of *A. calamus* L. exposed to different concentrations of As(V) (a, b, c) and As(III) (d, e, f) treatment groups. All values are expressed as mean $\pm$ SD ($n=6$). Different letters (a, b, c and d) indicate significant differences among treatment groups, and α , β , and γ indicate significant differences in antioxidant indicators in different parts of the plant.