Growth and Antimony Bioconcentration Characteristics of Wild Ramie (*Boehmeria nivea*) under Sb Stress in Different Valence States

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Abstract [**Objectives**] To explore the effects of antimony (Sb) in different valence states on the growth and Sb bioconcentration and transfer of wild ramie (*Boehmeria nivea*). [**Methods**] A pot experiment was conducted to study the effects of Sb (III) and Sb (V) stress on plant height, biomass, leaf SPAD value, Sb contents in various organs, Sb bioconcentration and transfer factors, and other indexes. [**Results**] Both kinds of Sb treatments showed a trend of "first increasing and then decreasing" in plant height as Sb concentration increased. The plant heights in Sb (III) treatments with a concentration lower than 1 000 mg/kg and Sb (V) treatments with concentrations lower than 4 000 mg/kg were significantly higher than that of the control check (CK); and the Sb (III) treatment of the high concentration (4 000 mg/kg) resulted in a significant decrease in plant height, while the 8 000 mg/kg Sb (V) treatment still showed an increase in plant height, indicating that the toxicity of Sb (V) to wild ramie was significantly lower than that of Sb (III). The Sb (III) treatments with a concentration lower than 2 000 mg/kg had little effect on the dry weight of the aboveground part, while the treatment with a higher concentration showed a significant decrease; and the dry weights in various concentrations of Sb (V) treatments showed no significant differences, but they were significantly higher than the CK and corresponding concentration of Sb (III) treatment. Both types of Sb stress could promote the increase of SPAD value in wild ramie leaves, and the performance was significantly higher than the CK. As the concentration of Sb treatment increased, the Sb content in both the aboveground and underground parts showed a significant increase; and the comparison of Sb content under corresponding concentrations of the two types of Sb treatments showed that in the aboveground part, Sb (III) was higher than Sb (V), while in the underground part, the opposite was true. The bioconcentration factor (BF) of Sb decreased with the increase of Sb treatment concentration overall, and the value of Sb (V) was smaller than that of Sb (III), but both kinds of Sb stress were significantly smaller than the CK. The transfer factor (TF) of Sb in wild ramie showed a trend of "increasing first and then decreasing" with the concentration of Sb treatment, and various treatments of Sb (III) were higher than the CK, with significant differences, while among various treatments of Sb (V), except treatment B3, the reduction of which was not significant, other Sb (V) treatments showed significant decreases. Comparing the two types of Sb treatments, the BF and TF values of Sb in wild ramie under Sb (III) treatments were higher than those under Sb (V) treatments, and the TF reached a significant level, indicating that the Sb transport ability of wild ramie under Sb (III) treatments was stronger. [**Conclusions**] This study provides a theoretical basis for the mining of wild ramie Sb restoration genes and the application of large-scale cultivation in ecological restoration.

Key words Antimony, Wild ramie, Growth, Antimony bioconcentration

1 Introduction

Antimony (Sb) is a non-essential element for plant growth and development. Excessive absorption of Sb can inhibit germination and growth, affect photosynthesis and other physiological characteristics, reduce the absorption of certain essential elements and the synthesis of certain metabolites, resulting in reduced yield and quality[1-2]. Plants usually have defense mechanisms to reduce Sb toxicity, such as efficient antioxidant systems and the ability to fix Sb in the cell wall or separate Sb in the cytoplasm[1]. Human beings or animals can come into contact with Sb and its compounds through skin, respiration, and food chain, causing imbalance of enzyme activity in the body, disruption of intracellular ion balance, metabolic disorders, and organ damage, resulting in various diseases such as cancers, cardiovascular diseases, liver diseases, and respiratory system diseases[3-6]. Sb is a scarce and non-renewable resource widely used in semiconductor devices, batteries, ammunition, and fire-resistant materials. Its increasing demand has led to the large-scale mining of Sb minerals. The natural weathering of Sb minerals, combustion of Sb fuels and the use of Sb compounds have caused serious environmental pollution[7-8]. The Xikuangshan in Hunan Province is known as the "antimony capital of the world", and the soil around the mining area has a Sb content up to 100 – 5 045 mg/kg, and the Sb content in downstream farmland soil reaches 1 565 mg/kg, resulting in Sb contents in rice roots and grains reaching 225 and 5.79 mg/kg, respectively[9-12].

Phytoremediation has many advantages such as being highly economic, high efficiency, and environmental protection and good safety, and is now widely used and studied by countries all over

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the world. Plant extraction refers to the method which achieves the remediation purpose by planting enriched plants in heavy metal-contaminated areas to absorb and accumulate heavy metals through the ground part and then removing them. Plant stabilization is to fix heavy metals in the roots and rhizosphere by planting enriched plants, so as to reduce the mobility of soil heavy metals. Ramie (*Boehmeria nivea*), also known as "Chinese grass", is a perennial herbaceous perennial plant with well-developed roots, large biomass, and strong resistance to adverse environments. The Ministry of Water Resources has listed ramie as a preferred plant for soil and water conservation in China. Studies have shown that wild ramie is widely distributed in multiple heavy metal mining areas, indicating that wild ramie has strong resistance to Sb and good soil remediation effects[13]. However, few studies have been conducted on the tolerance potential and bioconcentration ability of wild ramie to Sb at present. Therefore, in this study, with wild ramie from Xikuangshan, Lengshuijiang City as a material, the effects of Sb (III) and Sb (V) on its growth, biomass, leaf SPAD value, Sb contents in various organs, and bioconcentration and transfer were investigated, aiming to explore the response changes of growth and Sb bioconcentration of wild ramie to Sb stress and the potential of wild ramie for remediation of Sb-contaminated soil and provide a theoretical basis for the mining of wild ramie Sb restoration genes and the application of large-scale cultivation in ecological restoration.

2 Materials and methods

2.1 Experimental materials The experimental ramie was wild and collected from the slag heaps of Hunan Xikuang Guangrong Antimony Industry Co., Ltd. (111.486° E, 27.764° N). Trivalent antimony (Sb^{3+}), potassium antimony tartrate hemihydrate ($KSbOC_4H_4O_4 \cdot 1/2H_2O$), and pentavalent antimony (Sb^{5+}), potassium pyroantimonate ($K_2H_2Sb_2O_7 \cdot 4H_2O$), were analytical reagents produced by Xilong Science Co., Ltd.

2.2 Experimental design The experiment was conducted in the Agricultural Laboratory, College of Agriculture and Biotechnology, Hunan University of Humanities, Science and Technology. The test soil was collected from Jiu'er Modern Industrial Park (112°1′ E, 27°46′ N) in Loudi City, Hunan Province, and its physical and chemical properties were as follows: total N 1.77 g/kg, total P 1.06 g/kg, total K 15.79 g/kg, available N 128.53 mg/kg, available P 23.63 mg/kg, available K 185.15 mg/kg, organic matter 30.34 g/kg, pH 7.28, and Sb content 3.57 mg/kg. After natural air drying, the soil was ground, and sieved through a 4 mm sieve and added in PVC basins with a diameter of 15 cm and a height of 13 cm, each weighing 2 kg. Different concentrations of contaminated soil were prepared using potassium antimony tartrate and potassium pyroantimonate, and the treatment without adding heavy metals was used as the control check (CK). The concentration settings were shown in Table 2. Various treatments were repeated three times, with labels A and B representing Sb (III) and Sb (V) treatments, respectively. The reagent of each treatment was dissolved in water first, then slowly added to pre-screened fine soil with stirring, and aged for 30 d for later use. Ramie plants 5 cm in length with consistent growth and size were transplanted according to 3 plants per pot, and cultivated in a greenhouse (temperature 26–28 ℃, light for 12 h, humidity 60%–80%) for 6 weeks, during which Hoagland's nutrient solution was supplemented once every other week.

Table 1 Setting of antimony concentration

Valence of antimony	Sb Concentration // mg/kg				
	CK	1	2	3	4
Sb^{3+}	0	500	1 000	2 000	4 000
Sb^{5+}	0	1 000	2 000	4 000	8 000

2.3 Determination items and methods Plant height: The plant height in each treatment was measured with a ruler, and the average value was taken.

Dry weight: Samples were subjected to deactivation of enzymes in an oven at 105 ℃ for 0.5 h, and then baked at 80 ℃ for 24 h to constant weight. After being taken out, the samples were weighed.

Relative chlorophyll content (SPAD): On the 30th d after plant transplantation, the chlorophyll content (SPAD) was measured using a portable chlorophyll analyzer (SPAD-520Plus, produced in Japan). The third newly-unfolded leaf at the top of plant was selected, and the average value was taken. During measurement, attention should be paid to avoiding leaf veins and selecting healthy and undamaged leaves.

Sb element: The content of Sb element was determined according to previous research methods[14–15].

The calculation formulas for root bioconcentration factor (BF) and transfer factor (TF) were as follows[10]:

BF = Sb content in aboveground part of ramie/Sb content in soil (1)

TF = Sb content in aboveground part of ramie/Sb content in roots (2)

2.4 Data processing The data of this experiment were recorded using WPS office xlsx, subjected to analysis of variance using SPSS 26 software, and plotted using GraphPad Prism 8 software.

3 Results and analysis

3.1 Effects of Sb (III) and Sb (V) on plant height of wild ramie Fig. 1 shows the response changes in the plant height of wild ramie to Sb (III) and Sb (V) stress. Both kinds of Sb treatments showed a trend of "first increasing and then decreasing" in plant height as Sb concentration increased. For Sb (III), the plant heights of A1 and A2 were significantly higher than that of the CK, while A4 was significantly lower than the CK, with a decrease of 25.71%, indicating that treatment A4 had a significant inhibitory effect on the growth of wild ramie, resulting in a significant reduction in plant height. All four treatments in Sb (V) showed a plant height higher than that of the CK, and treatments B1, B2 and B3 reached a significant level, indicating that Sb (V) had a promoting effect on the growth of wild ramie under the experimental concentrations. The average plant height of the Sb (V) treatments was 9.56% higher than that of Sb (III), and the plants grew well under various treatments with concentrations twice those of Sb (III), indicating that the toxicity of Sb (V) to wild ramie was significantly lower than that of Sb (III).

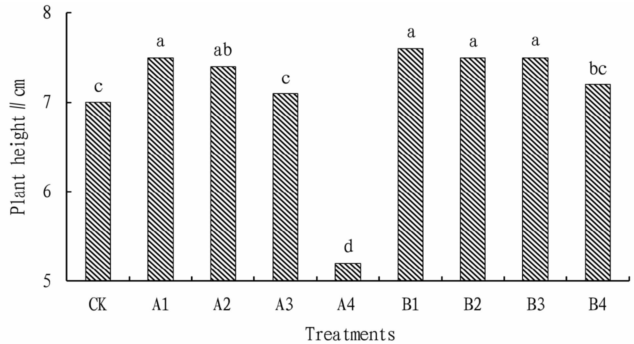

Fig. 1 Changes in plant height of ramie under Sb stress

3.2 Effects of Sb (III) and Sb (V) on dry weight of wild ramie The amount of biomass can reflect the remediation effect of plants on heavy metals, while higher biomass can improve the remediation efficiency of plants. From Fig. 2, it can be seen that the stress with Sb (III) and Sb (V) had a certain impact on the biomass of wild ramie. In the Sb (III) treatments, stress at concentrations below 2 000 mg/kg had little effect on the dry weight of the aboveground part, while the 4 000 mg/kg Sb treatment significantly reduced it. Except for A1, the dry weight of the underground part of which was significantly higher than that of A4, other Sb treatments showed no significant differences in the dry weight of the underground part from the CK. The Sb (V) treatments of various concentrations exhibited no significant differences in the dry weights of the aboveground and underground parts and the total dry weight, but they were all significantly higher than the CK and the Sb (III) treatments of various concentrations. It indicated that Sb (V) stress and the Sb (III) treatments of the high concentrations (above 4 000 mg/kg) had a significant impact on the biomass of wild ramie. Sb (V) could significantly promote the increase of biomass, while high concentrations of Sb (III) could lead to a significant decrease in biomass.

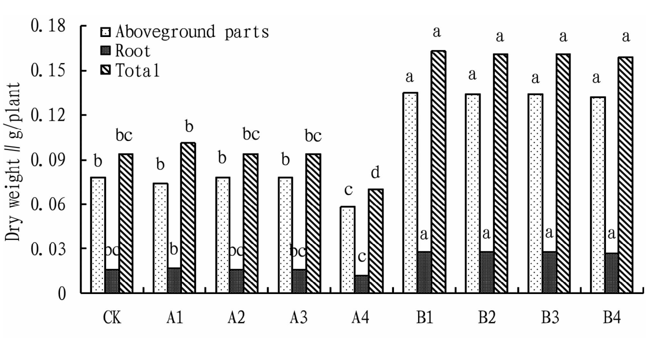

Fig. 2 Changes in dry weight of ramie under Sb stress

3.3 Effects of Sb (III) and Sb (V) on SPAD value of wild ramie The SPAD value of crop leaves can reflect the relative content of chlorophyll in crop leaves. The increase of SPAD value indicates an increase of chlorophyll content, which helps to improve the photosynthetic efficiency of crops and is of great significance to improve crop yield[16]. From Fig. 3, it can be seen that under the stress of Sb (III) and Sb (V), the SPAD values of wild ramie showed significantly better performance than the CK, indicating that Sb treatments had a promoting effect on the SPAD value of wild ramie leaves. Under the treatment with Sb (III), the SPAD value significantly decreased with the concentration increasing, indicating that the promoting effects of high Sb concentrations were significantly weakened. The differences in the SPAD value among different concentrations of Sb (V) were not significant, with an average increase of 16.26% compared with the CK. It indicated that Sb (V) still maintained a good promoting effect on the SPAD value of wild ramie at high concentrations, so it was beneficial for plant growth and development.

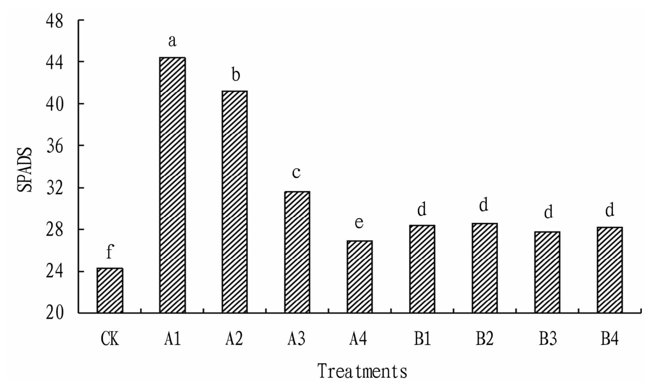

Fig. 3 Changes in leaf SPAD of ramie under Sb stress

3.4 Effects of Sb (III) and Sb (V) on Sb contents in various organs of wild ramie Fig. 4 shows the changes in Sb content in the aboveground and underground parts of wild ramie under the two kinds of Sb stress. From the figure, it can be seen that as the concentration of treatment increased, the Sb content in both the aboveground and underground parts showed a significant increase, but the increase of Sb content varied under Sb treatments of different valence states. For the aboveground part, the Sb contents in the Sb (III) treatments increased by 1.09 – 9.15 times compared with the CK, while the Sb contents in the Sb (V) treatments increased significantly less than those in the Sb (III) treatments, being 0.52 – 4.35 times that of the CK. It indicated that the absorption of Sb (V) in the aboveground part was still relatively lower than that under corresponding gradient of Sb (III) stress. In the underground part, the increases in Sb contents in the Sb (V) treatments were significantly higher than those in the Sb (III) treatments, and 1.13 – 5.85 times higher than the CK, while the Sb (III) treatments were only 0.52 – 3.70 time higher than the CK, indicating higher Sb contents in the underground part under the Sb (V) treatments.

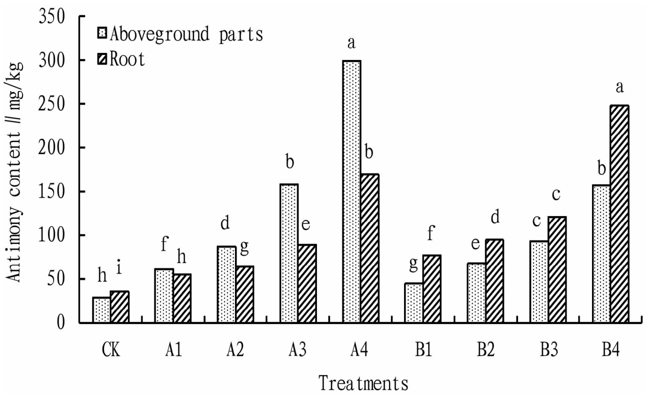

Fig. 4 Changes in Sb Content of ramie under Sb stress

3.5 Changes in Sb bioconcentration factor (BF) and transfer factor (TF) under different Sb (III) and Sb (V) stress The bioconcentration factor (BF) and transfer factor (TF) reflect the ability of plants to absorb heavy metals and the ability of plants to transport heavy metals from the underground part to aboveground part, respectively. A larger BF indicates a stronger ability of plants to absorb heavy metals, while a larger TF indicates a stronger ability of plant roots to transport heavy metals[17]. From Fig. 5, it can be seen that the BF of Sb in wild ramie decreased with the increase of Sb treatment concentration, and the value of Sb (V) was smaller than that of Sb (III), and both kinds of Sb stress treatments were significantly smaller than the CK, indicating that the bioconcentration ability of Sb in wild ramie plants was limited under Sb treatments; and the TF of Sb in wild ramie showed a trend of "first increasing and then decreasing" with the concentration of Sb treatment, and various treatments of Sb (III) were 38.27% – 119.75% higher than the CK, with significant differences, while various treatments of Sb (V) showed varying degrees of reduction compared with the CK, and except treatment B3, the reduction of which was not significant, other Sb (V) treatments showed significant differences. Comparing the two types of Sb treatments, the BF and TF values of Sb in wild ramie under Sb (III) treatments were higher than those under Sb (V) treatments, and the TF reached a significant level, indicating that the Sb transport ability of wild ramie under Sb (III) treatments was stronger.

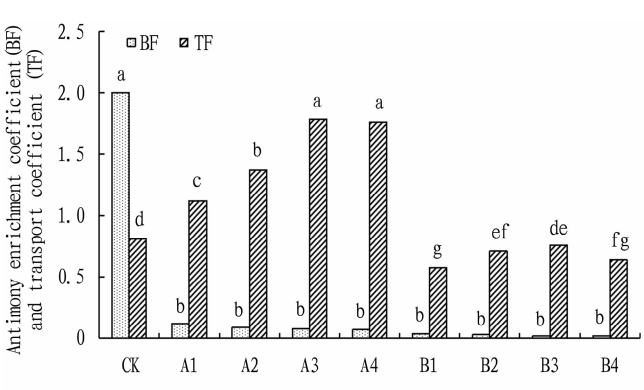

Fig. 5 Changes in BF and TF of Sb in ramie under Sb stress

4 Discussion

At present, there are many studies on the effects of heavy metals such as lead and cadmium on the growth, physiological and biochemical characteristics, yield and quality of ramie both at home and abroad, but few studies have been conducted on the effects of heavy metals such as Sb on ramie. The Sb pollution in Xikuangshan, Hunan is severe, causing a significant impact on the environment and residents. Studies have found that Sb, Cd and Hg in the soil are extremely high ecological risk factor in Xikuangshan area, and their average values are far higher than the background values of the soil in Hunan Province, with the exceeding rate reaching 100%, and 62.50% of the sample points have very high ecological risks[18]. It was also reported that the average contents of Sb, As and Cd in the wastewater and dust of the mining area exceeded the background values of Hunan Province, and Sb was the main ecological risk factor[19]. An investigation on 609 workers in the Sb mining area of Xikuangshan, Hunan Province showed that about 1/5 of the workers were suffering from pneumoconiosis, and the hair Sb content of residents in the mining area reached 15.9 mg/kg[20]. Crops such as rice and vegetables grown in Xikuangshan have varying degrees of enrichment in Sb and As, posing health risks to local residents[21]. According to statistical reports, the total area of heavy metal-contaminated farmland in the Xikuangshan area is 2 054.9 ha, of which the heavily-polluted soil area that is no longer suitable for use as farmland is 1 112.3 ha[22]. Hyperaccumulators have a high ability to absorb, accumulate, and enrich certain heavy metals, and thus a good effect on the remediation of contaminated soil. Currently, research has found highly-enriched crops such as rice for Sb and ramie for Cd, and a large number of studies have been carried out to strengthen ecological remediation measures, achieving good remediation results.

Sb has an inhibitory effect on the growth and development of the aboveground part of plants. As the treatment concentration increased, the inhibitory effect increased, mainly manifested as plant height becoming shorter, leaf color turning yellow, tiller number decreasing, biomass decreasing, and yield decreasing. Studies have found that Sb (III) poses a greater threat to the aboveground part of rice plants than Sb (V). At a Sb concentration in the range of 1 – 1 000 mg/kg, the harm intensified as the treatment concentration increased; and after transplanting for 10 d, it was found that the leaves of rice treated with Sb in the range of 300 – 1 000 mg/kg turned significantly yellow and grew slowly, and after 20 d, the rice plants became significantly shorter, and during the maturation stage, the Sb (III) treatment of 1 000 mg/kg showed plant height significantly reduced, leaves turning yellow, no tillers, and low seed setting rate, resulting in a significant decrease in yield[23]. The results of this study showed that the toxic effect of Sb (III) on wild ramie was significantly greater than that of Sb (V). Under the treatment of 4 000 mg/kg Sb (III), the plant height and total biomass were significantly reduced, while under the treatment of 8 000 mg/kg Sb (V), the plant height was still higher than the CK, and the total biomass was significantly higher than the CK. It indicated that wild ramie could maintain good growth and development under high concentrations of Sb (V). Under the two kinds of Sb stress, the Sb contents in both the aboveground and underground parts increased with the increase of treatment concentration; the Sb content and TF of the aboveground part were higher in the Sb (III) treatments than in the Sb (V) treatments, while the Sb content of the underground part was higher in the Sb (V) treatments than in the Sb (III) treatments. It indicated that the Sb transport ability of wild ramie under Sb (III) treatments was stronger, resulting in higher concentrations of Sb (III) being more toxic to the aboveground part than Sb (V), leading to decreases in plant height and biomass. Wild ramie has a strong transport advantage for Sb (III), which can alleviate the toxicity of roots to heavy metal Sb, and facilitate the absorption and accumulation of Sb in the aboveground part. Hence, it can be widely used for ecological restoration in polluted areas.

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