Effects of different sulfur compounds on the distribution characteristics of subcellular lead content in Arabis alpina L .var. parviflora Franch under lead stress

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Research Article

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Abstract

Sulfur plays a vital role in the phytoremediation of lead-contaminated soil. Effects of different sulfur forms (S^0, Na_2S, Na_2SO_4) on Pb absorption in hyperaccumulator *Arabis alpina* L. var. *parviflora* Franch were studied by soil culture pot experiment. The subcellular sulfur and lead enrichment characteristics in *A. alpina* were studied by adding sulfur in different forms and concentrations (0, 75, and 150 mg/kg) to Pb-contaminated soil. The results showed that the root and shoot biomass increased by 1.94 times under Na_2S and Na_2SO_4 treatment, and the root-shoot ratio of *A. alpine* increased 1.62 times under three forms of sulfur treatments, compared with the control. Sulfur content in cell walls and soluble fractions of root and shoot of *A. alpine* significantly increased 3.35 ~ 5.75 times and decreased 5.85 and 9.28 times in the organelles under Na_2SO_4 treatment. Meanwhile, Pb content in the root and shoot cell walls of *A. alpine* significantly increased by 3.54 and 2.75 times, respectively. Pb content in the shoot soluble fraction significantly increased by 3.46 times, and the root organelle and soluble fraction significantly decreased by 2.72 and 2.46 times, respectively, while it significantly reduced by 3.78 times in the shoot organelle. Different forms and concentrations of sulfur had no regularity in the effect of Pb and sulfur content in the subcellular components of *A. alpina*, but the bioconcentration and translocation factors of *A. alpina* increased compared with the control. Under different concentrations of Na_2SO_4, there was a significant positive correlation between the contents of sulfur and Pb in the subcellular components of the root of *A. alpina* ($P < 0.05$). These results indicate that sulfur application can enhance the Pb resistance of *A. alpina* by strengthening the cell wall fixation and compartmentalization of vacuoles.

Introduction

Lead has a relatively large molecular weight and stable properties, and its geochemical cycle in the soil is largely affected by human factors. The analysis of lead pollution sources in the environment also confirmed that industry and agriculture are major contributors (Dong et al. 2015). Lead migrates from soil to plants and accumulates in organisms, and eventually endangers human health through the food chain. Many factors affecting the migration and transformation of lead in soil are reported, such as the form of lead, soil type, soil properties, soil nutrients, and fertilization (Hu et al. 2009). Sulfur application can change the growth condition of crops and promote soil remediation or reduce pollution as a non-metallic element, which positively affects plant adsorption of heavy metals (Sun et al. 2003). The cyclic transformation of sulfur in the soil can significantly affect heavy metals' biogeochemical process and play an essential biological role in the plant's physiological metabolism against heavy metal ion pollution and the detoxification of heavy metals (Yuan et al. 2016). The current research on the effect of sulfur on heavy metals is contradictory (Sun et al. 2014). One view is that sulfur can activate heavy metals in soil and increase their bioavailability. Applying S to soil can significantly increase the proportion of soluble copper (Cu) and zinc (Zn) in soil and increase Cu accumulation in roots and shoots of *Elsholtzia chinensis* (Wang et al. 2008). Another view is that sulfur can inactivate heavy metals in soil and reduce their bioavailability. Applying sulfur fertilizer to copper-contaminated farmland reduced the content of acid-extractable copper in the soil and increased residual copper, iron-manganese oxidized copper, and organically bound copper, which was different from the amount of sulfur fertilizer (Lu et al. 2013). Studies have shown that exogenous sulfur can reduce the absorption and toxicity of heavy metals to plants. Spraying Na_2S on rice leaves can increase rice yield by 6–30% and reduce cadmium content in rice grains by 28–50% (Liu et al. 2020). Na_2SO_4 treatment significantly reduced the Pb content in the shoot stalks and rice grains (Yang et al. 2019) The sulfur application significantly reduced the absorption of As in rice (Hu et al. 2007). The activation or deactivation of heavy metals by sulfur in soil may be related to the form of sulfur. Different sulfur forms and concentrations significantly affected the content of iron and manganese film in rice roots (Hu et al. 2005).

Under anaerobic conditions, S^{2-} in the soil is prone to precipitation with heavy metals to form insoluble sulfide, which leads to the decreased availability of sulfur and heavy metals (Hu et al. 2009; Li et al. 2019). However, it is easily oxidized under aerobic conditions, and heavy metals in sulfide are released, leading to an increase in the heavy metals absorbed by plants (Moreau et al. 2007). In addition, the change of sulfur valence state in the soil causes a change in soil pH, which directly affects the dissolution and adsorption of heavy metals (Zhou et al. 2018). Therefore, the sulfur form in the soil is closely related to the bioavailability of heavy metals.

In this paper, pot experiments were conducted to study the shoot and root biomass of *A. alpina* under different sulfur forms and treatments, the accumulation characteristics of lead, and the distribution of lead in subcellular components to reveal the influence of sulfur form on the uptake and enrichment of lead by hyperaccumulator plants. The response mechanism of *A. alpina* to lead stress under the action of sulfur and the mechanism of *A. alpina* enriched lead at the subcellular level was explored and used to provide theoretical and technical guidance for using hyperaccumulator plants *A. alpina* to remediate lead-contaminated soil.

Materials And Methods

Experimental design and materials

The test soil was taken from an abandoned slag pile in the Sanduoduo Village, Zhehai Town, Huize County, Yunnan Province (altitude 2463 ~ 2516 m, E: 103°03' ~ 103°55', N: 25°48' ~ 28°38'). The collected soil was 0 ~ 20 cm tillage soil of corn planted farmland near the slag heap. After air-drying, the soil was passed through a 2 mm sieve, accurately weighed (5.0 kg), and placed in plastic pots (length 44 cm × width 15 cm × height 19 cm) for later use. The physical and chemical properties of the soil are shown in Table 1.

Table 1
Physical and chemical properties of soil

pH	Available sulfur (mg/kg)	Pb content (mg/kg)	Organic matter (g/kg)	Available phosphorus (mg/kg)	Available nitrogen (mg/kg)	Available potassium (mg/kg)	Cation exchange capacity (cmol/kg)
6.25	10.67	2618.74	16.87	12.08	116.34	28.77	16.45

A. alpina seeds were sown in a seedbed with the substrate. Seedlings with good growth and uniform size after sown for two months were transplanted in the pot experiments. The planting method was to set 2 plants in one hole and 18 plants in 9 holes in each pot. The plants were watered with 1/2 Hoagland nutrient solution once every two days. Samples were collected for the experiment three months later.

The sulfur concentration was determined based on the effect of sulfur nutrition on lead accumulation in *A. alpina* (Wang et al. 2011). The concentrations of three different sulfur forms (S^0, Na_2S, and Na_2SO_4) were set as 0 mg/kg, 75 mg/kg, and 150 mg/kg, respectively. Each treatment was set with three parallel pots, with a total of 21 pots.

Determination Of Biomass

The soil was bought to the semi-humid state with water, and the whole plant was pulled out from the soil. Plant roots were quickly rinsed with tap water to remove impurities attached to the roots and then rinsed with deionized water. Plant surface moisture was absorbed using filter paper and then evenly divided into two parts. One was deactivated at 105°C for 30 min and then dried at 70°C to a constant weight. The dry matter was weighed. Another fresh sample was kept to measure the subcellular sulfur and lead content.

Determination Of Subcellular Sulfur And Lead Content

Differential centrifugation was used to separate the subcellular components: the underground and above-ground parts of the prepared *A. alpina* fresh samples were separated. Accurately 1 g of fresh samples in a mortar was weighed. Pre-cooled subcellular extract (DTT: 1 $mmol·L^{-1}$, sucrose: 0.25 $mol·L^{-1}$, Tris-HCl: 50 $mmol·L^{-1}$, pH = 7.4) (10 mL) was added and quickly placed in a mortar. Then ground in an ice bath to a homogenate shape, transferred to a 50 mL centrifuge tube, centrifuged at 1000 $r·min^{-1}$ for 10 min, removed the supernatant, and the precipitate contained the cell wall component. The removed supernatant was centrifuged in a high-speed refrigerated centrifuge (TGL-20M, Thermo) at 12000 $r.min^{-1}$ at 4°C for 45 min. The supernatant is the soluble fraction of the cell, and the precipitate is the organelle component (Pan et al. 2016). Each component was added with HNO_3-$HClO_4$, heated and digested with an adjustable electric heating plate until it was clear, transferred to a 50 mL volumetric flask, and the lead content was determined by an atomic absorption spectrophotometer (TAS-990, Agilent). Coupled plasma emission spectrometer (ICAP 6300, Thermo Elemental) was used to determine the sulfur content.

Determination Of Total Lead Content In The Plant And Soil

The Cd content of the plants was digested with HNO_3-$HC1O_4$ (V: V = 1:4) and determined using atomic absorption spectrophotometry (TF-4520, Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China). Similarly, the total Cd content of the soil was digested with HNO_3-H_2O_2 (V: V = 1:4) and determined using atomic absorption spectrophotometry (Liu et al. 2021).

Statistical Analyses

Bioconcentration factor (BCF) = Pb content in the shoot of plant (mg/kg)/Pb content of soil (mg/kg);

Translocation factor (TF) = Pb content in the shoot of the plant (mg/kg)/Pb content in the root of the plant (mg/kg). The ratio of root biomass to shoot biomass = root biomass of *A. alpina* (g)/shoot biomass of *A. alpina* (g). The data were collected and analyzed using Microsoft Excel 2010. One-way analysis of variance (one-way ANOVA) and significance test (Duncan's method, $P = 0.05$) were done using IBM SPSS Statistics 22. The data obtained are expressed as mean ± standard deviation (n = 3), while figures were drawn using Origin 9.1.

Results And Analysis

The effect of different forms of sulfur on the biomass of A. alpina

As shown in Table 2, Na_2S and Na_2SO_4 treatments significantly changed the root and shoot biomass of *A. alpine* compared with the control. Na_2S treatment at 75 mg/kg and 150 mg/kg increased the root biomass 1.83 and 2.33 times, Na_2SO_4 increased 3.00 and 3.28 times, respectively. Compared with the control, 75 mg/kg and 150 mg/kg Na_2SO_4 treatments significantly increased the shoot biomass of *A. alpina* by 1.59 and 2.01 times. Different forms of exogenous sulfur increased the root-to-shoot ratio of *A. alpine* (except for 75 mg/kg elemental sulfur treatment), indicating that sulfur promotes the growth of the root system of *A. alpina*, which is beneficial to increase the contact of *A. alpina* with lead-contaminated soil.

Table 2
Effects of different forms of sulfur on the biomass of *A. alpina* under lead stress

Sulfur forms	**Treatment concentration (mg/kg)**	**Root (g/pot)**	**Shoot (g/pot)**	**Ratio of root biomass to shoot biomass**
CK	0	0.17 ± 0.06 d	0.57 ± 0.10 d	0.30
S	75	0.20 ± 0.05 d	0.70 ± 0.06 d	0.29
	150	0.24 ± 0.03 d	0.58 ± 0.07 cd	0.41
Na_2S	75	0.32 ± 0.03 c	0.71 ± 0.02 cd	0.45
	150	0.40 ± 0.05 b	0.77 ± 0.06 c	0.53
Na_2SO_4	75	0.52 ± 0.03 a	0.91 ± 0.04 b	0.57
	150	0.57 ± 0.04 a	1.16 ± 0.18 a	0.49

CK: the control of non-treatment, S: S^0 treatment, Na_2S: Na_2S treatment, Na_2SO_4: Na_2SO_4 treatment.

The data in the table is the mean ± standard deviation of 3 replicates, the different lowercase letters indicate significant differences among treatments ($P < 0.05$), respectively.

Effects of Different Forms of Sulfur on the Sulfur Contents in Subcellular part of A. alpina

It can be seen from Table 3 that the three sulfur forms significantly changed the sulfur content in the subcellular components of *A. alpina*, but different forms of sulfur had different distributions of sulfur content in the root and shoot cell walls, organelles, and soluble components of *A. alpina*. Compared with the control, 75 mg/kg and 150 mg/kg elemental S^0 treatments significantly reduced the sulfur content in the root and shoot organelles of *A. alpina* by 58.33% and 48.80%. Na_2S treatment significantly increased the sulfur content of the root cell wall and the soluble component of *A. alpina* by 115.53% and 183.33% and significantly reduced the organelle sulfur content by 67.54% (Fig. 1). Further, the shoot cell wall and soluble component sulfur content increased by 82.28% and 90.08%, respectively, and the sulfur content of organelles was significantly reduced by 84.13%. Na_2SO_4 treatment significantly increased the sulfur content of the root and shoot cell walls and soluble components of *A. alpine* by 3.65, 5.75, 4.58, and 3.35 times, respectively, and significantly reduced the organelle sulfur content to 82.89% and 89.22%.

Table 3
Effects of different forms of sulfur on the Sulfur Contents in different Subcellular Components of *A. alpina*

Treatment		**Root (g/kg)**			**Shoot (g/kg)**		
Sulfur forms	Concentration (mg/kg)	Cell wall	Organelle	Soluble fraction	Cell wall	Organelle	Soluble fraction
CK	0	1.03 ± 0.06 d	2.28 ± 0.56 a	0.12 ± 0.13 e	0.79 ± 0.16 d	3.34 ± 0.23 a	1.31 ± 0.07 e
S	75	1.74 ± 0.09 cd	1.24 ± 0.14 b	0.14 ± 0.08 e	1.03 ± 0.12c d	1.71 ± 0.49 b	1.54 ± 0.11 de
	150	1.30 ± 0.05 d	0.95 ± 0.07 bc	0.17 ± 0.09 de	1.07 ± 0.11 cd	0.86 ± 0.13 c	1.87 ± 0.09 d
Na_2S	75	1.68 ± 0.27 cd	0.80 ± 0.04 bc	0.22 ± 0.21 d	1.39 ± 0.19 c	0.67 ± 0.07 cd	2.45 ± 0.35 c
	150	2.22 ± 0.19 c	0.74 ± 0.06 bc	0.34 ± 0.37 c	1.44 ± 0.09 c	0.53 ± 0.03 cd	2.49 ± 0.45 c
Na_2SO_4	75	3.04 ± 0.48 b	0.48 ± 0.08 c	0.47 ± 0.20 b	2.41 ± 0.25 b	0.46 ± 0.02 cd	3.34 ± 0.23 b
	150	3.76 ± 0.32 a	0.39 ± 0.05 c	0.69 ± 0.85 a	3.62 ± 0.44 a	0.36 ± 0.04 d	4.39 ± 0.42 a

CK: the control of non-treatment, S: S^0 treatment, Na_2S: Na_2S treatment, Na_2SO_4: Na_2SO_4 treatment.

The data in the table is the mean ± standard deviation of 3 replicates, the different lowercase letters indicate significant differences among treatments ($P < 0.05$), respectively.

Effects of Different Forms of Sulfur on Lead Content in Subcellular part of A. alpina

From Table 4, compared with the control, different forms of sulfur treatment significantly changed the lead content in the root and shoot subcells of *A. alpina*. Under Na_2SO_4 treatment, the lead content in the root and shoot cell walls of *A. alpina* significantly increased by 3.55 and 2.75 times, respectively. S^0 and Na_2SO_4 treatments at 75 mg·kg^{-1} and 150 mg·kg^{-1} increased the lead content in the root cell wall of *A. alpina* significantly by 1.78 times, but there was no significant difference between the two sulfur forms and concentration treatments. Under S^0, Na_2S, and Na_2SO_4 treatments, the lead content in the root and shoot organelles of *A. alpina* was significantly reduced by 0.71 and 0.57 times, 0.36 and 0.78 times, 0.41 and 0.26 times, respectively (Fig. 2). In addition, the soluble components in the root significantly reduced by 0.76, 0.58, and 0.41 times in sequence, and the soluble components in the shoot significantly increased by 1.74, 2.38, and 3.46 times in sequence, respectively.

Table 4
Effects of different forms of sulfur on the subcellular lead content of *A. alpina*

Treatment		**Root**			**Shoot**		
Sulfur forms	Concentration (mg/kg)	Cell wall	Organelle	Soluble fraction	Cell wall	Organelle	Soluble fraction
CK	0	87.39 ± 6.6 d	244.66 ± 33.6 a	457.65 ± 30.5 a	297.29 ± 39.3 d	256.12 ± 27.8 a	192.05 ± 46.3 f
S	75	137.90 ± 21.7 c	174.89 ± 28.3 b	406.93 ± 18.5 ab	381.42 ± 24.2 cd	260.02 ± 24.2 a	260.75 ± 33.0 ef
	150	173.99 ± 27.3 c	170.61 ± 16.4 b	349.73 ± 28.3 b	471.15 ± 82.8 c	199.28 ± 23.2 b	334.71 ± 43.3 de
Na_2S	75	133.61 ± 17.8 c	142.72 ± 52.4 bc	263.92 ± 38.8 c	634.60 ± 52.0 b	144.05 ± 13.7 c	374.52 ± 26.5 d
	150	175.52 ± 20.6 c	134.36 ± 14.1 c	277.19 ± 55.5 c	706.78 ± 19.4 ab	104.58 ± 9.8 d	458.35 ± 34.1 c
Na_2SO_4	75	241.33 ± 27.9 b	134.18 ± 74.3 c	218.64 ± 31.8 cd	798.60 ± 67.2 a	80.17 ± 12.2 de	539.99 ± 56.0 b
	150	309.92 ± 38.0 a	89.85 ± 14.8 d	185.70 ± 17.5 d	817.66 ± 101.1 a	67.80 ± 5.8 e	664.89 ± 54.9 a

CK: the control of non-treatment, S: S^0 treatment, Na_2S: Na_2S treatment, Na_2SO_4: Na_2SO_4 treatment.

The data in the table is the mean ± standard deviation of 3 replicates, the different lowercase letters indicate significant differences among treatments ($P < 0.05$), respectively.

Effects of Different Forms of Sulfur on the Characteristics of Lead Accumulation in A. alpina

From Table 5, different forms and concentrations of sulfur increased the enrichment coefficient and transport coefficient of *A. alpina*. The enrichment coefficient and transport coefficient of S^0 treatment increased by 1.29 and 1.43 times on average, Na_2S treatment increased by 1.64 and 2.28 times on average, and Na_2SO_4 treatment increased by 2.02 and 2.67 times on average, respectively. The effects of different forms of sulfur on the enrichment and transport of lead in *A. alpina* were $Na_2SO_4 > Na_2S > S^0$.

Table 5
Effects of different forms of sulfur on the characteristics of lead accumulation in *A. alpina*

Treatment		Root (mg/kg)	Shoot (mg/kg)	BCF	TF
Sulfur forms	**concentration (mg/kg)**				
CK	0	789.7	745.46	0.28	0.94
S	75	719.72	902.19	0.34	1.25
	150	694.33	1005.14	0.38	1.44
Na_2S	75	540.25	1153.17	0.44	2.13
	150	587.07	1269.71	0.48	2.16
Na_2SO_4	75	594.15	1418.76	0.54	2.38
	150	585.47	1550.35	0.59	2.64

CK: the control of non-treatment, S: S^0 treatment, Na_2S: Na_2S treatment, Na_2SO_4: Na_2SO_4 treatment.

Correlation of Sulfur Content and Lead Content in Subcellular parts of A. alpina

It can be seen from Table 6 that there is a significant positive correlation between the sulfur content of S^0 and Na_2SO_4 under the treatments of 75 $mg·kg^{-1}$ and 150 mg/kg and the lead content of each subcellular part of *A. alpina* ($p < 0.05$, the same below). Na_2S under 150 $mg·kg^{-1}$ treatment had a significant positive correlation with the cell wall and organelles of the *A. alpine* root. It showed that the sulfur in *A. alpina* is closely related to the absorption of lead by the roots. Except for Na_2SO_4 at 75 mg/kg and 150 mg/kg, there was a significant positive correlation between the sulfur content and the shoot cell wall of *A. alpina*. However, there was no significant correlation between the sulfur content and lead content in other shoot subcellular parts.

Table 6
Correlation between lead and sulfur contents of subcellular in *A. alpina*

Treatment		**Root**			**Shoot**		
Sulfur forms	Concentration (mg/kg)	Cell wall	Organelle	Soluble fraction	Cell wall	Organelle	Soluble fraction
CK	0	0.751	0.692	0.633	0.512	0.044	0.479
S	75	0.968*	0.971*	0.994*	0.281	0.455	0.822
	150	0.997*	0.997*	0.982*	0.929	0.436	0.651
Na_2S	75	0.671	0.835	0.967*	0.422	0.536	0.178
	150	0.996*	0.956*	0.864	0.649	0.195	0.112
Na_2SO_4	75	0.975*	0.984*	0.989*	0.999*	0.517	0.495
	150	0.991*	0.973*	0.962*	0.964*	0.197	0.728

CK: the control of non-treatment, S: S^0 treatment, Na_2S: Na_2S treatment, Na_2SO_4: Na_2SO_4 treatment. '*' indicates the significant at the 0.05 probability level ($P < 0.05$).

Discussion

Elemental sulfur cannot be directly absorbed and utilized by plants. It must be oxidized into sulfate in the soil through the following pathways S^0-$S_2O_3^{2-}$-$S_4O_6^{2-}$-SO_4^{2-} to be absorbed by the plant (Mahar 2018). The addition of different forms and concentrations of sulfur from external sources lowers the soil pH, and the circulation of sulfur enhances the activity of microorganisms in the soil (Bai et al. 2010), significantly increasing the biomass of the plant's shoot and root (Xu et al. 2016). For example, foliar spraying of nano-sulfur significantly promoted the growth of tomatoes and zucchini (Salem et al. 2015; Salem et al. 2016; Salem et al. 2016). The addition of different forms of sulfur such as SNPs (nano sulfur), SBPs (large particle sulfur), and sulfate under Pb stress significantly increased the biomass of rapeseed (Yuan et al. 2021). In this study, different concentrations of Na_2S treatment under Pb stress significantly increased the underground biomass of *Arabis alpina* L. Na_2S treatment at 150 mg/kg had a significant effect on the shoot biomass. This is consistent with the significant increase in the rice root system and biomass with Na_2S treatment (Guan et al. 2021). Na_2SO_4 treatment significantly increased the root and shoot biomass of *Arabis alpina* L. Conversely, the effects of different concentrations of Na_2SO_4 on the shoot were not significant. It is speculated that the oxidation of Na_2S reducing substances in the rhizosphere affects the microenvironment, Eh (soil redox potential) of the rhizosphere soil, which in turn affects the activity of lead. The presence of SO_4^{2-} can reduce the excessive heavy metals toxicity (Mikkelsen et al. 1990), SO_4^{2-} in the soil accumulates on the root surface of plants, which affects the adsorption, desorption, absorption, and antagonism of lead ions, which affects the bioavailability of lead. In this paper, Na_2SO_4 treatment significantly increased the biomass of *Arabis alpina* L., and the root-to-shoot ratio of the three forms of sulfur treatment increased compared with the control.

There have been many literature reports on the regulatory role of sulfur in the phytoremediation of soil heavy metals (Muhammad et al. 2010). For example, different forms of sulfur reduced the absorption of Pb by rapeseed, but the Pb content of different tissues was significantly higher than the corresponding tissues treated with the same concentration of SNPs (Yuan et al. 2008). It is speculated that SNPs have a large specific surface area and strong adsorption capacity to adsorb and fix Pb ions as PbS, thereby reducing the absorption and transport of exchangeable and free Pb ions by rape roots. In this paper, different forms and concentrations of sulfur treatment had no regularity in the distribution of lead and sulfur content in the root and shoot subcellular components of *Arabis alpina* L. On the one hand, it is speculated that it is related to the form of sulfur redox in the soil, leading to significant differences in the amount of *Arabis alpina* L. absorbed and stored in the body. Under different concentrations of Na_2SO_4, there was a significant positive correlation between the content of sulfur and lead in each component of the root Subcellular of *Arabis alpina* L. ($p < 0.05$). For example, the copper activity of different forms of ammonium sulfate and sulfur-coated urea was affected by reducing and oxidizing conditions. Ammonium sulfate promoted the coordinated migration of copper and sulfur in the soil, while the effect of sulfur-coated urea on copper was closely related to the flooding period (Zhu et al. 2014).

On the other hand, it is speculated that the sulfur-containing compounds formed by different chelating forms of sulfur into the plant changed the subcellular distribution of lead. Lin and Shi (2010) used K-edge X-ray absorption spectroscopy (XANES) and found that the sulfhydryl functional groups that chelate metals in the soil have significant differences between rhizosphere and non-rhizosphere soils. The K-edge XANES of sulfur indicated that the content of high-valent sulfur in rhizosphere soil was higher than in non-rhizosphere soil, and the valence of heavy metals from rhizosphere soil to plants was also changing, indicating a detoxification mechanism for heavy metals in plants. Synchrotron radiation X-ray micro-area fluorescence spectroscopy (μ-XRF) showed that Cu and S in the soil showed a specific correlation with the increase of sulfur fertilizer and the extension of the flooding period (Zhu et al. 2014).

Plant cell wall retention and vacuolar compartmentalization play a major regulatory role in the detoxification and tolerance of hyperaccumulator plants to heavy metals (Küpper et al. 2000). Plants participate in the metabolic processes of cysteine (Cys), Methionine (Met), small molecule glutathione (GSH), and phytochelatins (PCs) during the stress response (Sun et al. 2013). The sulfhydryl substances in the metabolic process were combined with heavy metals in the cells and then transferred and stored in vacuoles, thereby alleviating the toxic effects of heavy metals on plants (Das et al. 2020). Studies have shown that both exogenous and endogenous GSH promoted the absorption, transport, and accumulation of copper ions by wheat seedlings, and GSH was highly positively significantly correlated with the accumulation of wheat sulfur nutrients under copper stress ($P < 0.01$) (Peng et al. 2012). The sulfur application under cadmium stress significantly reduced the content of hydrogen peroxide and malondialdehyde in plant leaves, increased the activity of key enzymes in the ASA-GSH cycle, the content of antioxidants and PCs, and the increased PC content can chelate Chinese cabbage Excessive cadmium in the body promoting the transport of cadmium from the root to the shoot (Sun et al. 2013). The sulfur application significantly increased cadmium content in the cell wall and soluble components of *Sedum crassipes* (Li et al. 2009). In this paper, adding Na_2SO_4 to *Arabis alpina* L., the shoot cell wall lead and sulfur content significantly increased by 2.75 and 4.58 times, and significantly reduced in the organelles by 3.77 and 9.27 times, respectively. Thus, this showed that sulfur application strengthened the solidification of lead on the cell wall and vacuole compartmentalization is the main way for sulfur to enhance the lead tolerance of *Arabis alpina* L. Our results are consistent with applying sulfur to increase the content of non-protein sulfhydryl (NPT) groups in rice leaves, increase the distribution of cadmium in the soluble part of cells, including vacuoles, and enhance the tolerance of rice to cadmium (Pan et al. 2016).

It is speculated that a part of S^0 and Na_2S directly reacts with lead in the soil to form PbS, which affects the bioavailability of lead in *Arabis alpina* L., and part of the process that affects the microbial activity through sulfur redox reactions changes the physical and chemical properties of soil such as pH, Eh, and indirectly affects the behavior of lead (Qu et al. 2022). *Arabis alpina* L. directly absorbs the SO_4^{2-} in Na_2SO_4 to generate sulfur-containing functional groups that combine with lead to form high-affinity and stable compounds and enhance the lead tolerance of *Arabis alpina* L. Regarding how different forms of sulfur affect the characteristics of lead-enrichment in *Arabis alpina* L., it is necessary to use the near-side X-ray absorption fine structure absorption spectrometer to understand the geometric configuration of sulfur and lead atoms and reveal how sulfur regulates lead absorption and enrichment in plants.

Conclusion

1. The biomass of *Arabis alpina* L. with different forms of sulfur increased significantly, and the increase in root was significantly higher than the shoot.
2. Na_2SO_4 treatment of *Arabis alpina* L. significantly increased the sulfur content in the root and shoot cell walls and soluble components, and reduced in organelles. The shoot cell wall and soluble components had significantly increased lead content. However, The score was significantly reduced in root organelles and soluble components.
3. There was a significant positive correlation between the sulfur and lead content in the subcellular components of the root of *Arabis alpina* L. treated with different concentrations of sodium sulfate.

Declarations

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Author contribution All authors contributed to the study conception and design**. Cui Xu,** Data curation, Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **Qin Li,** Data curation, Formal analysis**,** Writing – review & editing**. Yan-qun Zu**, Writing – review & editing. **Yuan Li**, Investigation, Resources, Supervision, Project administration, Funding acquisition, Supervision. **Ji-xiu Wang**, Conceptualization, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Availability of data and materials The datasets used and analyzed during this study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References

1. Bai YN, Lai HX, Wen XL, Li XP (2010) Study on microflora in the remediation of saline-alkali soil by addition of sulfur. Journal of Northwest A & F University (Natural Science Edition) 38(2): 153–157.
2. Das U, Rahman MA, Ela EJ, Lee KW, Kabir HA (2020) Sulfur triggers glutathione and phytochelatin accumulation causing excess Cd bound to the cell wall of roots in alleviating Cd-toxicity in alfalfa. Chemosphere 262:128361. https://doi.org/10.1016/j.chemosphere.2020.128361
3. Dong LR, Hu WY, Huang B, Liu G, Qu MK, Kuang RX (2015) Source appointment of heavy metals in suburban farmland soils based on positive matrix factorization. China Environmental Science 35(7): 2103–2111. https://kns.cnki.net/kcms/detail/detail.aspx?FileName=ZGHJ201507031&DbName=CJFQ2015
4. Guan D, Wu JM, Liu ZB, Chen S, Ji XH (2021) Effects of exogenous sodium sulfide on cadmium migration and accumulation in soil and rice plant systems. Journal of Agro-Environment Science. http://kns.cnki.net/kcms/detail/12.1347.S.20210414.1305.004.html
5. Hendrik K, Lombi E, Zhao FJ, Mcgrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis helleri*. Plant 212: 75–84. https://doi.org/10.1007/s004250000366
6. Hu ZY, Hanelalus S, Cao ZH, Ewald S (2005) Chemical behavior of soil sulfur in the rhizosphere and its ecological significance. Landbauforschung Volkenrode Special issue 283:53–60.
7. Hu ZY, Xia X, Wu CYH, Fan JL (2009) Chemical Behaviors of Sulfur in the Rhizosphere of Rice and Its Impacts on Heavy Metals Uptake in Rice. Soils 41(1): 27–31. Hu ZY, Zhu YG, Li M, Zhang LG, Cao ZH, Smith FA (2007) Sulfur (S)-induced enhancement of iron plaque formation in the rhizosphere reduces arsenic accumulation in rice (*Oryza sativa* L.) seedlings. Environmental Pollution 147(2): 387–393. https://doi.org/10.1016/j.envpol.2006.06.014
8. Hu ZY, Xia X, Wu CYH, Fan JL (2009) Chemical Behaviors of Sulfur in the Rhizosphere of Rice and Its Impacts on Heavy Metals Uptake in Rice. Soils 41(1): 27–31. https://kns.cnki.net/kcms/detail/detail.aspx?FileName=TURA200901006&DbName=CJFQ2009
9. Liu JH, Hou H, Zhao L, Sun ZJ, Li H (2020) Protective Effect of Foliar Application of Sulfur on photosynthesis and antioxidative defense system of Rice under the Stress of Cd. Sciences of Total Environment 710:136230. https://doi.org/10.1016/j.scitotenv.2019.136230
10. Lu ZH, Tan XM, Zhu MY, Wu JF, SHI QH, Pan XH (2013) Effect of Sulfur and Silicon on the Forms of Soil Copper and Absorption of Copper in Rice. Journal of Agro-Environment Science 32(9): 1806–1813. https://kns.cnki.net/kcms/detail/detail.aspx?FileName=NHBH201309016&DbName=CJFQ2013
11. Li HH, Yang XE (2009) Effects of sulfur on accumulation, subcellular distribution and chemical forms of cadmium in hyperaccumulator-Sedum alfredii Hance. Plant Nutrition and Fertilizer Science 15(2): 395–402.

12. Li XZ, Yu H, Sun XW, Yang JT Wang DC, Shen LF, Pan YS, Wu YC, Wang Q, Zhao Y (2019) Effects of sulfur application on cadmium bioaccumulation in tobacco and its possible mechanisms of rhizospheric microorganisms. Journal of hazardous materials 368: 308–315. https://doi.org/10.1016/j.jhazmat.2018.12.099
13. Lin HR, Shi JY, Chen XC, Yang JJ, Chen YX, Zhao YD, Hu TD (2010) Sulfate-reducing bacteria existed in the rice rhizosphere affected by lead and thiosulfate interaction. Soil Biology and Biochemistry 42(7): 1038–1044.
14. Liu M, Li ZR, Zhang GQ, Wang JX, Zu YQ (2021) Response of ascorbate-glutathione cycle in wild *Arabis alpina* L. to soil Cd and Pb stress. Journal of Agro-Environment Science 38(04):558-569. https://kns.cnki.net/kcms/detail/detail.aspx?dbcode=CJFD&dbname=CJFDLAST2021&filename=NHFZ202104004&uniplatform=NZKPT&language=CHS
15. Mahar A, Wang P, Ali A, Lahori AH, Awasthi MK Wang Z, Guo Z, Wang Q, Feng S, Li R, Zhang Z (2018) (Im)mobilization of soil heavy metals using CaO, FA, sulfur, and Na_2S: a 1-year incubation study. Chinese Journal of Soil Science. 38(2): 404–408. https://doi.org/10.1007/s13762-017-1427-7
16. Mikkelsen RL, Wan HF (1990) The effect of selenium on sulfur uptake by barley and rice. Plant and Soil 121(1): 151–153. https://doi.org/10.1007/BF00013109
17. Moreau JW, Weber PK, Martin MC, Gilbert B, Hutcheon ID, Banfield JF (2007) Extracellular proteins limit the dispersal of biogenic nanoparticles. Science 316(5831): 1600–1603. https://doi.org/10.1126/science.1141064
18. Muhammad S, Tseng MH, Stuiver CEE, Koralewska A, Posthumus FS, Venema JH, Parmar S,Schat H, Hawkesford MJ, De kok LJ (2010) Copper exposure interferes with the regulation of the uptake, distribution, and metabolism of sulfate in Chinese cabbage. Journal of Plant Physiology 167(6): 438–446. https://doi.org/10.1016/j.jplph.2009.10.016
19. Pan ZL, Li J (2016) The response of Non-Protein Thiols and Subcellular Distribution of Cadmium in Rice to the Exogenous Sulfur and Silicon. Chinese Journal of Soil Science 47(5): 1253–1258. https://kns.cnki.net/kcms/detail/detail.aspx?FileName=TRTB201605035&DbName=CJFQ2016
20. Peng XY, Chang B, Xu SR, Wu WL, Shi L (2012) Effects of Glutathione on Alleviation of Copper Toxicity and Its Correlation with Accumulation of Nitrogen, Sulfur, and Phosphorus in Wheat Seedlings. Journal of Agro-Environment Science 31(5): 867–873
21. Qu J, Yuan Y, Wang XM, Wang L, Tao Y, Zhao J, Yu H, Dong M, Zhang Y (2022) Stabilization of lead and cadmium in soil by sulfur-iron functionalized biochar: Performance, mechanisms and microbial community evolution. Journal Hazardous Materials 425:127876. https://doi.org/10.1016/j.jhazmat.2021.127876
22. Salem NM, Albanna LS, Awwad AM, Ibrahim QM, Abdeen AO (2015) Green synthesis of nanosized sulfur and its effect on plant growth. Journal of Agricultural Science 8(1): 188–194. https://doi.org/10.5539/jas.v8n1p188
23. Salem NM, Albanna LS, Abdeen AO, Ibrahim QI, Awwad AM (2016) Sulfur nanoparticles improve root and shoot growth of tomato. Journal of Agricultural Science 8(4): 179–185. https://doi.org/10.5539/jas.v8n4p179
24. Salem NM, Albanna LS, Awwad AM (2016) Green synthesis of sulfur nanoparticles using Punica granatum peels and the effects on the growth of tomato by foliar spray applications. Environmental Nanotechnology. Monitoring & Management 6: 83–87. https://doi.org/10.1016/j.enmm.2016.06.006

25. Sun HL, Lu JY, Jia SL (2013) Effects of Sulfur on Ascorbate-Glutathione Cycle and the Content of Phytochelatins in the Leaves of Pakchoi (*Brassica chinensis* L.) Under Cadmium Stress. Journal of Agro-Environment Science 32(7): 1294–1301. https://kns.cnki.net/kcms/detail/detail.aspx?FileName=NHBH201307004&DbName=CJFQ2013
26. Sun LJ, Duan DC, Peng C, He JY, Shi JY (2014) Influence of sulfur on the speciation transformation and phyto-availability of heavy metals in soil: A review. Chinese Journal of Applied Ecology 25(7): 2141–2148. https://kns.cnki.net/kcms/detail/detail.aspx?FileName=YYSB201407041&DbName=CJFQ2014
27. Wang JX, Tai GC, ZU YQ, Li Y, Zhan FD. (2011) Effects of Sulfur Application on Growth and Lead and Zinc Accumulation Contents in *Arabis alpinal Var. parviflora* Franch. Journal of Agro-Environment Science 30(6): 1064–1069.
28. Wang YP, Li QB, Wang H, Shi JY (2008) Effect of sulfur on soil Cu/Zn availability and microbial community composition. Journal of Hazardous Materials 159(2): 385–389. http://dx.doi.org/10.1016/j.jhazmat.2008.02.029
29. Xu Yao, Mu JM, Zhang GQ, Ma JJ, Xu J, Li J, Liu FJ, She XD (2016) Effect of Sulfur Availability on Nitrate Accumulation and Expression of Nitrogen and Sulfur Assimilation Related Genes in Non-Heading Chinese Cabbage. Scientia Agricultura Sinica 49(11): 2222-2233.
30. Yang JX, Liu ZY, Wan XM, Zheng GD, Yang J, Zhang HZ, Guo L, Wang XD, Zhou XY, Guo QJ, Xu RX, Zhou GD, Peters M, Zhu GX, Wei RF, Tian LY, Han XK (2016) Interaction between sulfur and lead in toxicity, iron plaque formation and lead accumulation in rice plant. Ecotoxicology and Environmental Safety 128: 206–212. https://doi.org/10.1016/j.ecoenv.2016.02.021
31. Yuan HY, Liu QQ, Zhang YX, Fu JH, Wang YJ, Sun YM, Tong HY (2021) Effects of sulfur nanoparticles on growth and Pb accumulation in *Brassica napus* L. seedlings under Pb stress. Journal of Agro-Environment Science 40(3): 517–524.
32. Yuan J, Zhang L, Jin MH, Ding ZH (2016) Effect of different forms of sulfur on Hg content in *Bruguiera gymnorrhiza* seedlings. Chinese Journal of Ecology 2016 35(6): 1525–1530.
33. Zhou J, Hao M, Liu YH, Huang GY, Fu QL, Zhu J, Hu HQ (2018) Effects of exogenous sulfur on growth and Cd uptake in Chinese cabbage (*Brassica campestris* spp. *pekinensis*) in Cd-contaminated soil. Environmental Science and Pollution Research 25(16): 15823-15829. https://doi.org/10.1007/s11356-018-1712-0
34. Zhu SH, Yang JJ, Sun LJ, Shi JY (2014) Effects of different sulfur fertilizer and flooding regimes on availability and distribution of copper in polluted paddy soil. Journal of Zhejiang University (Science Edition) 41 (3): 331–347.

Figures

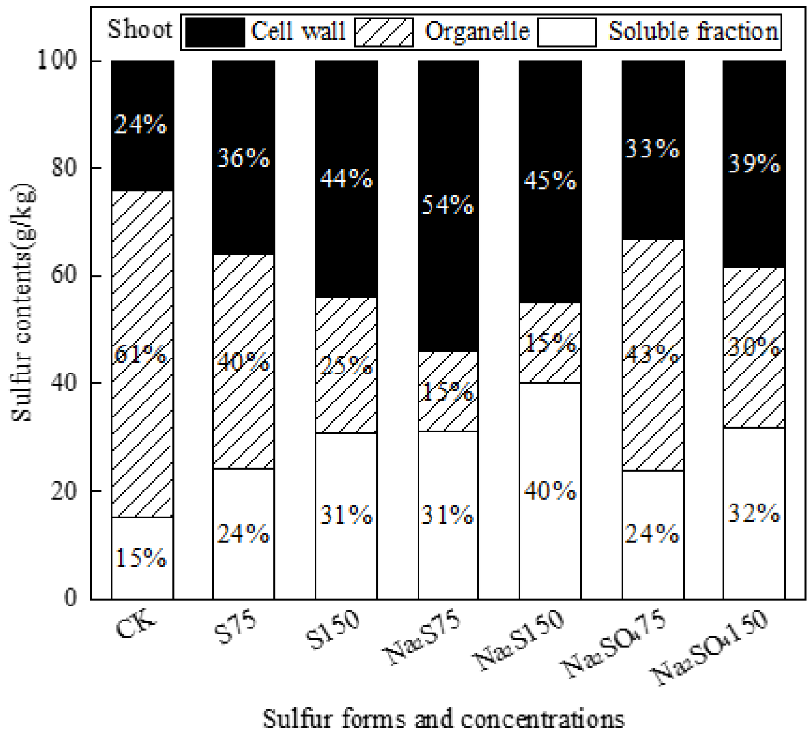

Figure 1

Percentages of subcellular distribution of sulfur in *A. alpina*

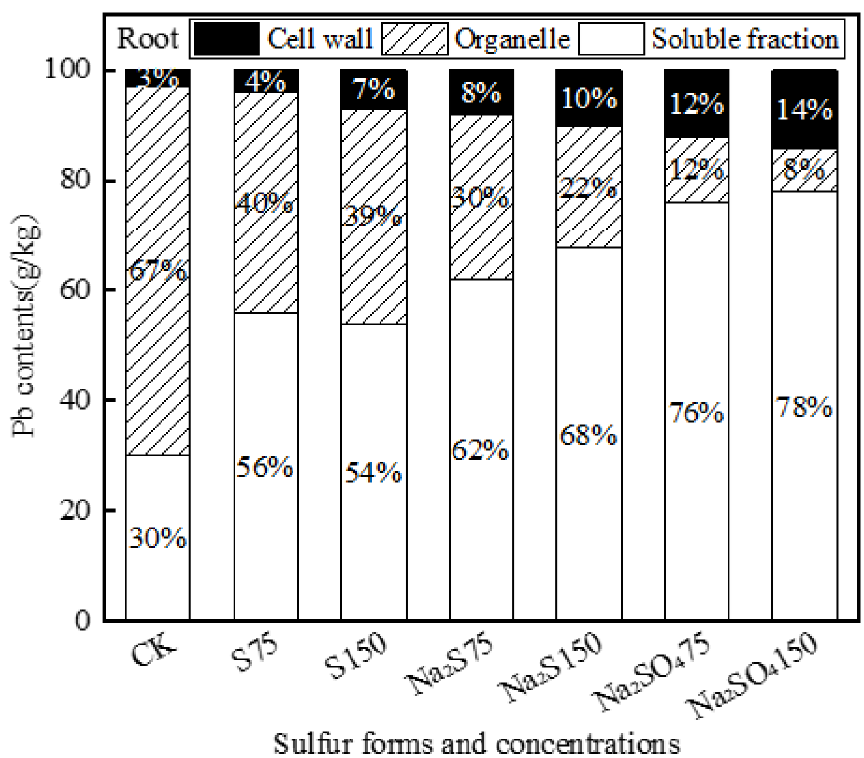

Figure 2

Percentages of subcellular distribution of Pb in *A. alpina*