https://doi.org/10.17221/8/2025-PSE

Effects of silicon on the transport, subcellular distribution, and chemical forms of lead in *Salix viminalis* L.

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Citation: Zhai F.F., Yin M.L., Mao J.M., Liu J.X., Li H.D., Zhang Y.X., Zhang S.W., Sun Z.Y. (2025): Effects of silicon on the transport, subcellular distribution, and chemical forms of lead in *Salix viminalis* L. Plant Soil Environ., 71: 249–258.

Abstract: Lead (Pb) is a harmful heavy metal that threatens ecosystems and plant growth. Silicon (Si) plays a crucial role in plant responses to heavy metal stress. In this study, the effects of Si on Pb^{2+} content and transport, subcellular distribution, and chemical forms in *Salix viminalis* L. under Pb stress were analysed, aiming to elucidate the detoxification mechanism of Si in *S. viminalis* under such conditions. Results showed that Si reduced Pb^{2+} in aboveground parts and increased it in roots, lowering its movement to leaves and stems. Analysis of the subcellular distribution of Pb^{2+} revealed that Si application promoted the transfer of Pb^{2+} to vacuole-dominated soluble components (F4) and cell wall components (F1), which increased the binding capacity of the cell wall and the vacuolar storage compartmentalisation for Pb^{2+}. Changes in the chemical forms of Pb^{2+} indicated that Si significantly decreased the proportion of more mobile, ethanol-extractable Pb^{2+} (FE) and deionised water-extractable Pb^{2+} (FW) while increasing the proportion of less mobile Pb^{2+} forms, such as NaCl-extractable (FNaCl), HCl-extractable (FHCl), and acetic acid-extractable (FHAc) Pb^{2+}, thereby reducing its mobility. This study provides empirical support for the application of Si in the phytoremediation of heavy metal-contaminated soils.

Keywords: heavy metal; toxic element; toxicity; accumulation; detoxification

With the rapid advancement of urbanisation and the intensification of agricultural and industrial production activities, soil heavy metal pollution has become increasingly severe. Among them, lead (Pb) is a widely distributed and harmful heavy metal in the environment, which has been classified by the World Health Organisation as one of the top ten chemicals causing major public health problems. Pb is not easily degradable and tends to accumulate in the surface layers of soil, affecting plants' metabolic processes and causing severe plant death (Zulfiqar et al. 2019). Additionally, Pb can significantly threaten human health through the food chain (Raj et al. 2023). Pb is one of the main heavy metal pollutants in agricultural soil. Therefore, the remediation of Pb pollution is urgent and essential. Silicon (Si) is beneficial for plant growth and, as an exogenous regulatory substance, has been found in many stud-

Supported by the National Natural Science Foundation of China, Project No. 31700533; by the Young Backbone Teacher Foundation of Henan Polytechnic University, Project No. 2020XQG-19, and by the Innovative Talent Support Program of Henan Open University, Project No. PDGC-2023-007.

https://doi.org/10.17221/8/2025-PSE

ies to positively alleviate biotic and abiotic stress in plants (Khan et al. 2021). Meanwhile, the application of silicon fertilisers for the remediation of heavy metal pollution is cost-effective and environmentally friendly and has garnered widespread attention for controlling soil heavy metal contamination.

Previous research has shown that Si influences the absorption and transport of heavy metals in plants by enhancing both avoidance and tolerance mechanisms, thereby mitigating heavy metal stress (Etesami and Jeong 2018). Through avoidance mechanisms, Si alters the rhizosphere environment by increasing soil pH, promoting organic secretion, and facilitating metal co-precipitation, thus modifying the chemical forms of heavy metals and reducing their absorption by plants (Huang et al. 2021, Xiao et al. 2021). Xiao et al. (2021) found that Si application transformed cadmium forms in soil, lowering extractable and reducible cadmium levels while promoting cadmium oxide formation and residual cadmium, which ultimately reduced cadmium uptake in *Oryza sativa* L. Regarding tolerance mechanisms, Si can chelate heavy metals within root cells, thereby reducing the transport of these metals to the aboveground parts of the plant (Keller et al. 2015). Si can precipitate as silica in roots and accumulate in the endodermis and pericycle, impeding heavy metal transport and accumulation in aboveground tissues (Bhat et al. 2019). The transport coefficient is defined as the ratio of heavy metal content in the aboveground parts of the plant to that in the underground parts, and it often serves as a measure of the plant's ability to translocate heavy metals (Mirecki et al. 2015). Previous studies have demonstrated that under heavy metal stress, Si can significantly reduce the ability of plants to transport metals such as chromium (Cr) (Ashfaque et al. 2017), aluminium (Al) (Pontigo et al. 2017), and cadmium (Cd) (Cai et al. 2022), thereby lowering their transport coefficients.

The chemical forms of heavy metals within plants are closely associated with their biological functions. Different extracting agents can yield various forms of heavy metal compounds, which exhibit differing degrees of mobility and toxicity within the plant (Zhao et al. 2015). Studies indicate that ethanol-extractable fraction (FE) and deionised water-extractable fraction (FW) exhibit the highest activity and toxicity, followed by the sodium chloride-extractable fraction (FNaCl). In contrast, the acetic acid-extractable fraction (FHAc), hydrochloric acid-extractable fraction (FHCl), and residual fraction (FR) display relatively low activity and toxicity (Yang et al. 2018). Existing research indicates that heavy metal bioactivity within plants is related to their chemical forms (Huang et al. 2018). Typically, when plants are subjected to heavy metal stress, the application of Si can facilitate the conversion of heavy metals into less bioactive chemical forms and restrict their mobility within the plant, thereby alleviating the toxicity associated with heavy metals (Vaculík et al. 2012). Pan et al. (2024) found that, following Si application, the chemical forms of cadmium in *O. sativa* were altered, with a significant increase in the proportion of low-activity chemical forms such as the sodium chloride-extractable fraction, acetic acid-extractable fraction, and residual fraction, while the proportion of high-activity chemical forms such as the ethanol-extractable fraction and hydrochloric acid-extractable fraction significantly decreased.

Si can influence the subcellular distribution of heavy metals within plants, alleviating the damage caused by heavy metal stress. Research has shown that heavy metal ions complex with Si^{4+} or form co-precipitates, which adsorb onto the cell wall or are transferred into vacuoles, effectively reducing the bioactivity of heavy metals and thereby mitigating damage to cellular ultrastructures, such as chloroplasts (Guo et al. 2018). Specifically, Si can enhance cell wall binding capacity for various heavy metals, including Zn (Zajaczkowska et al. 2020), Cd (Zhao et al. 2022), Al (Xiao and Liang 2022), and Pb (Sun and Luo 2018). Si and heavy metals form insoluble complexes in the cell wall, thereby reducing the toxicity of heavy metals (Vaculík et al. 2020). Additionally, Si can promote the silicification of the cell wall, increasing its mechanical strength and reducing the likelihood of heavy metals entering into the cytoplasm (Zheng et al. 2019). When cell wall-binding sites become saturated, intracellular heavy metals are transferred to vacuoles, resulting in vacuolar sequestration and reducing their toxicity to the plant (Krämer 2000). Further research indicates that vacuolar membrane transport proteins can move heavy metal complexes (e.g., Cd-PCs and As-PCs) into vacuoles, facilitating heavy metal compartmentalisation, decreasing cytoplasmic concentrations, and thus effectively reducing toxicity to the plant (Lv et al. 2022). The study of plant detoxification mechanisms for Al and Zn revealed that Si binds with heavy metals to form insoluble Zn/Al-Si precipitates, which are abundantly distributed in the cell wall and vacuoles of the plant,

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thereby alleviating the toxicity of heavy metals to some extent (Kopittke et al. 2017).

Numerous studies have shown that Si plays a significant role in plants' transport, subcellular distribution, and chemical forms of heavy metals. However, the mechanisms by which Si operates can vary among different plant species. Currently, research on the role of Si in alleviating heavy metal damage primarily focuses on crops such as *O. sativa* (Qiu et al. 2024), *Zea mays* L. (Kollárová et al. 2019), and *Hordeum vulgare* L. (Rhimi et al. 2024). However, the effects of Si on the absorption and transport of heavy metals, along with their subcellular distribution and chemical forms in woody plants, require further investigation.

S. viminalis is widely used in soil phytoremediation and biomass energy development among woody plants due to its rapid growth, high biomass production, and strong heavy metal accumulation capacity (Mleczek et al. 2010). In this study, *S. viminalis* was used as the test material, and a treatment approach was employed in which Pb stress was applied first, followed by the addition of Si. The effects of Si on the transport, subcellular distribution, and chemical forms of Pb in *S. viminalis* under Pb stress were investigated. Based on the existing research foundation and the experimental design of this study, it is hypothesised that silicon can alleviate lead toxicity in *S. viminalis* through three mechanisms: inhibiting the translocation of lead to the aboveground parts, subcellular compartmentalisation and inducing the conversion of lead into low-activity chemical forms. This research aims to elucidate the mechanisms by which Si mitigates Pb stress in *S. viminalis*, thus providing a scientific basis for Si application in the phytoremediation of heavy metal-contaminated soils.

MATERIAL AND METHODS

Plant material. The cuttings of *S. viminalis* branches were selected from the experimental site of the Chinese Academy of Forestry. The upper end of the cuttings is flat, the bottom end is inclined, and the size of the cuttings is 15 cm × 1.5 cm (length × diameter). The cuttings were initiated on April 1, 2024. Each cutting was planted in a 5 cm × 15 cm (diameter × height) nutrient pot, with one plant per pot. The cultivation substrate used was quartz sand with a mesh size of 16 to 18. During the seedling period, the average temperature was 23.29 °C, and the average humidity was 50.88%. Normal watering and fertilisation were applied to the seedlings throughout the growing period. On the 12th day after the cuttings of *S. viminalis* were planted, a bud thinning treatment was performed, retaining one shoot per cutting. On the 60th day, uniform and healthy seedlings were selected for cultivation in a nutrient solution. Every 2 days, the plants were watered with a half-strength Hoagland's nutrient solution (pH 6.0) until water began to drain from the bottom of the pots. The nutrient solution was prepared following the method described by Islam et al. (2011). The pH was adjusted to 5.5–5.7 using 0.1 mol/L HCl or 0.1 mol/L NaOH. On the 7th day of nutrient solution cultivation, treatments were applied using $Pb(NO_3)_2$ and $Na_2SiO_3·9\ H_2O$ (analytical grade). Plant samples were collected from the leaf, stem, and root tissues of *S. viminalis*. Leaf samples were taken from mature leaves in the upper-middle section, with the leaf tips and bases removed, stem samples from mature stems in the middle section, and root samples from the lateral roots.

Experimental design. The experiment employed a treatment method involving Pb stress followed by the application of Si, with Pb stress applied for 10 days followed by Si treatment for 30 days, resulting in a total duration of 40 days. Based on preliminary gradient experiments, the Pb concentration was set at 1.8 mmol/L, a level that significantly inhibits the morphological characteristics of *S. viminalis*. The Si concentration was set at 1.5 mmol/L, which has been shown to promote the growth of *S. viminalis* significantly. This experiment comprised two treatments: (1) Pb treatment (control): 1.8 mmol/L $Pb(NO_3)_2$ treatment for 10 days, followed by 30 days of nutrient solution treatment; (2) Pb + Si treatment: 1.8 mmol/L $Pb(NO_3)_2$ for 10 days, followed by 1.5 mmol/L $Na_2SiO_3·9\ H_2O$ treatment for 30 says. The experiment was conducted using a completely randomised block design with four blocks.

Measurement methods

Measurement of Pb content. A 0.500 g sample of *S. viminalis* was thoroughly ashed in a microwave muffle furnace (CEM Phoenix, Matthews, USA). The resulting ash was digested with 10 mL of *aqua regia* ($HCl:HNO_3$ = 3:1) until a clear solution was obtained. The solution was filtered and then diluted to 50 mL with deionised water. Pb content was measured using flame atomic absorption spectrometry with an AA-7000 spectrophotometer (Beijing East and West Analytical Instruments Co., Ltd., Beijing, China).

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Separation of subcellular components. Subcellular components were separated using differential centrifugation. A fresh sample of 0.500 g was accurately weighed and mixed with 20 mL of extraction solution (0.25 mol/L sucrose, 50 mmol/L Tris-HCl (pH 7.5), 1 mmol/L DTE ($C_4H_{10}O_2S_2$, Sigma D8255)), ground to a homogenate, and centrifuged at 300 × *g* for 30 s in a refrigerated centrifuge to obtain the precipitated cell wall fraction (F1); the supernatant was then centrifuged at 2 000 × *g* for 15 min to isolate the nucleus and chloroplast fraction (F2); the supernatant was further centrifuged at 10 000 × *g* for 20 min to isolate the mitochondrial fraction (F3), and the remaining supernatant representing the vacuole-enriched soluble fraction (F4) (Wu et al. 2005). All operations were conducted at 4 °C. After separation, the samples were evaporated to near dryness on a hot plate, and 10 mL of a mixed acid solution (HNO_3: $HClO_4$ = 4:1) was added. Following complete digestion, the solution was heated until clear, transferred to a 50 mL volumetric flask and diluted to volume with 10% nitric acid. The Pb content was measured using flame atomic absorption spectrometry with an AA-7000 spectrophotometer (Beijing East and West Analytical Instruments Co., Ltd, Beijing, China).

Extraction of Pb chemical form. A stepwise extraction method using chemical reagents was employed. A fresh sample weighing 0.500 g was accurately measured and combined with 20 mL of extraction solvent. The mixture was homogenised and placed in a 50 mL plastic centrifuge tube. After incubation at 25 °C with constant shaking for 22 h, it was centrifuged at 2 500 × *g* for 10 min, and the supernatant was decanted. An additional 10 mL of extraction solvent was added, and after shaking at 25 °C for 1 h, the mixture was centrifuged again at 2 500 × *g* for 10 min, and the supernatant was collected. Both supernatants were then combined. The extraction was performed sequentially using the following five extractants: 80% ethanol, deionised water, 1 mol/L sodium chloride solution, 2% acetic acid solution, and 0.6 mol/L hydrochloric acid solution, with the final residue representing the residual fraction (Wu et al. 2005). According to the order of extraction solvents used, the FE solution primarily extracted inorganic salts and amino acid salts; the FW solution mainly extracted water-soluble organic acids and phosphates; the FNaCl solution was primarily used to extract pectates and heavy metals in adsorbed or protein-bound forms; the FHAc solution mainly extracted insoluble heavy metal phosphates; and the FHCl solution primarily extracted oxalates (Fu et al. 2011).

Data analysis. The data were organised in Microsoft Excel 2019 (Redmond, USA), analysed on the SPSSAU data science analysis platform (Qingsi Technology Co., Ltd., Beijing, China), and graphs generated in SigmaPlot 12.5 (Systat Software, Inc., San Jose, USA).

RESULTS

Regulation of Pb^{2+} accumulation and distribution in *S. viminalis* by Si. Figure 1 illustrates the distribution of Pb^{2+} within *S. viminalis*. Under Pb treatment, the Pb^{2+} content in *S. viminalis* accounted for 13.35% of the total in the leaves, 8.72% in the stems, and 77.93% in the roots; after Si application following Pb stress, the proportion of Pb^{2+} in the leaves decreased by 54.77%, in the stems by 0.93%, and in the roots increased by 76.12%. These results indicate that Pb^{2+} is primarily distributed in the roots of *S. viminalis* and that after Si application following Pb^{2+} stress, the proportion of Pb^{2+} in the leaves of *S. viminalis* significantly decreased, while it significantly increased in the roots (P < 0.05). However, the proportion in the stems declined; this difference was not significant (P > 0.05).

Figure 2 shows the changes in the Pb^{2+} transport coefficients in the leaves and stems of *S. viminalis* under various treatments. Compared to Pb treatment alone, the application of Si under Pb stress signifi-

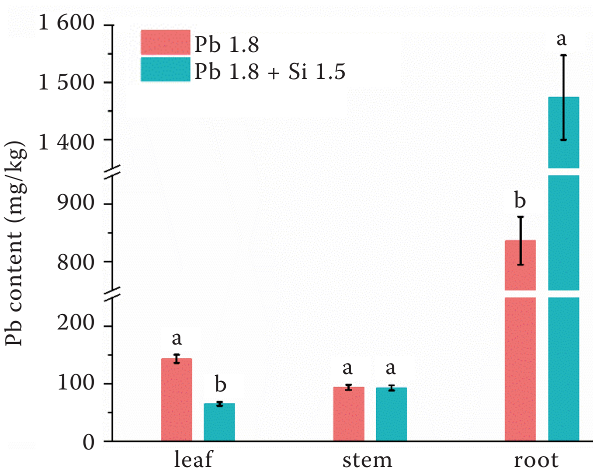

Figure 1. The distribution of Pb^{2+} in *Salix viminalis* under silicon (Si) treatment after Pb^{2+} stress. Lead (Pb) content is expressed in dry weight (DW) to eliminate moisture interference. Data are the mean ± standard error; different small letters indicate significant differences in treatments (P < 0.05)

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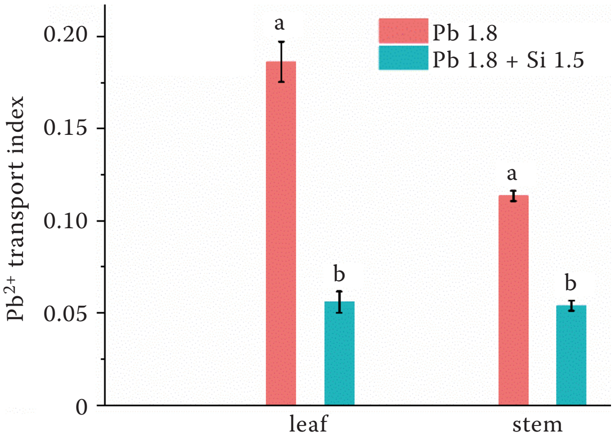
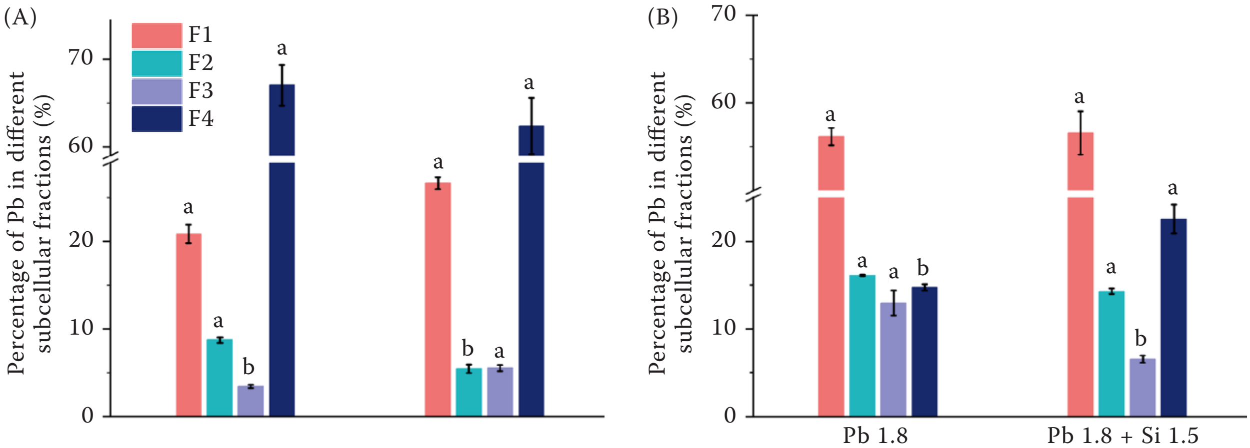


Figure 2. The change of Pb^{2+} transport index in *Salix viminalis* leaf and stem under silicon (Si) treatment after Pb^{2+} stress. Data are the mean ± standard error; different small letters indicate significant differences in treatments ($P < 0.05$)

cantly reduced the Pb^{2+} transport coefficients in the leaves and stems by 69.96% and 52.51%, respectively ($P < 0.05$). This indicates that Si significantly inhibited the transport of Pb^{2+} to the above-ground parts of *S. viminalis*.

Regulation of Pb^{2+} subcellular distribution in *S. viminalis* by Si. Si altered the subcellular distribution of Pb^{2+} in *S. viminalis* under Pb stress (Figure 3). According to Figure 3A, after Si application, the Pb^{2+} concentration in the leaves of *S. viminalis* increased by 27.77% in F1, which was not statistically significant ($P > 0.05$). In F2, Pb^{2+} levels decreased by 37.26%, a statistically significant change ($P < 0.05$). In F3, Pb^{2+} concentration increased by 63.08%, which was also significant ($P < 0.05$). In F4, the Pb^{2+} level decreased by 6.99%, which was not statistically significant ($P > 0.05$). The above results indicate that Si reduced the proportion of Pb^{2+} in F2 and F4 while increasing its proportion in F1, enhancing the cell wall binding capacity for Pb^{2+}. Additionally, the significant increase in Pb^{2+} content in F3 suggests that some heavy metals may have been transported to the mitochondria.

According to Figure 3B, after Si application, the Pb^{2+} concentration in F1 of the stems of *S. viminalis* increased by 0.69%, which was not statistically significant ($P > 0.05$). In F2, the Pb^{2+} level decreased by 11.20%, a statistically significant change ($P < 0.05$). In F3, Pb^{2+} decreased by 49.40%, which was also



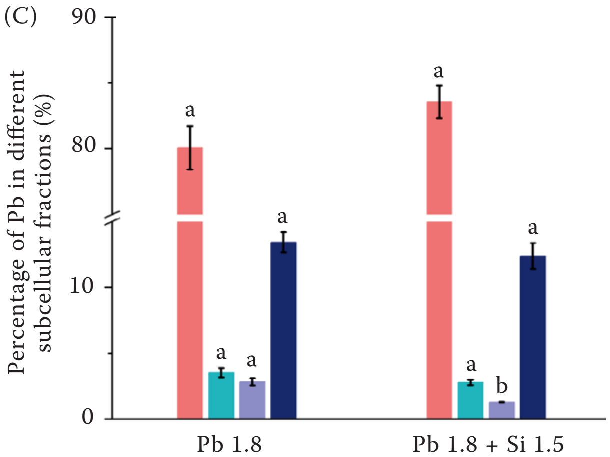


Figure 3. Subcellular distribution of Pb^{2+} in *Salix viminalis* (A) leaf; (B) stem, and (C) root under silicon (Si) cultivation after Pb^{2+} stress for 10 days. F1 – cell wall; F2 – nuclei and chloroplasts; F3 – mitochondria; F4 – soluble fraction

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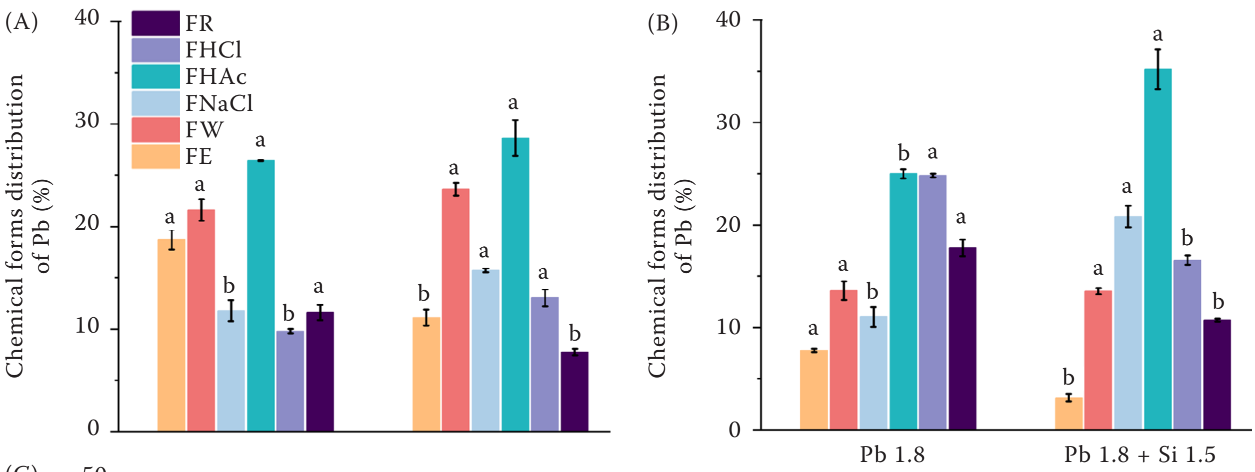
significant (P < 0.05). In F4, the Pb^{2+} concentration increased by 52.99%, which was significant (P < 0.05). These results indicate that Si significantly reduced the proportion of Pb^{2+} in F2 and F3 while significantly increasing its proportion in F4.

Figure 3C shows that in the subcellular distribution of Pb^{2+} in the roots of *S. viminalis*, the application of Si resulted in a 4.37% increase in F1. However, this change was not statistically significant (P > 0.05). In F2, Pb^{2+} decreased by 20.83%, which was also not significant (P > 0.05). In F3, Pb^{2+} levels decreased by 54.82%, a statistically significant reduction (P < 0.05). In F4, Pb^{2+} decreased by 7.84%, which was not statistically significant (P > 0.05). These results indicate that Si significantly reduced the distribution of Pb^{2+} in the F3 fraction, facilitating the transfer of Pb^{2+} to the less mobile F1 fraction. However, the amount transferred to F1 did not show a significant difference.

Regulation of Pb^{2+} chemical speciation in *S. viminalis* under Pb stress by Si. Figure 4 illustrates significant changes in the chemical speciation of Pb^{2+} in the leaves, stems, and roots of *S. viminalis* following Si application under Pb stress. According to Figure 4A, after Si application under Pb stress, the proportion of Pb^{2+} in the more active FE fraction decreased by 40.53%. In contrast, the proportions of FNaCl and FHCl fractions increased by 33.28%, while the proportion of the FR fraction decreased by 33.04%, and these differences were statistically significant (P < 0.05). These results indicate that Si facilitated the transformation of Pb^{2+} from the more active FE fraction to the less active FNaCl and FHCl fractions.

As shown in Figure 4B, under Pb stress following Si application, the proportions of the stems' FE, FHCl, and FR fractions decreased by 59.46, 33.26, and 39.57%, respectively. Conversely, the FNaCl and FHAc fractions increased by 88.60% and 40.68%, respectively, and these differences were statistically significant (P < 0.05). This indicates that Si promoted the conversion of Pb^{2+} in the stems to the less active FNaCl and FHAc fractions.

According to Figure 4C, following Si application under Pb stress, the proportions of FNaCl and FHAc fractions in the roots increased by 15.02% and 21.55%, respectively. In comparison, the FW and FR fractions



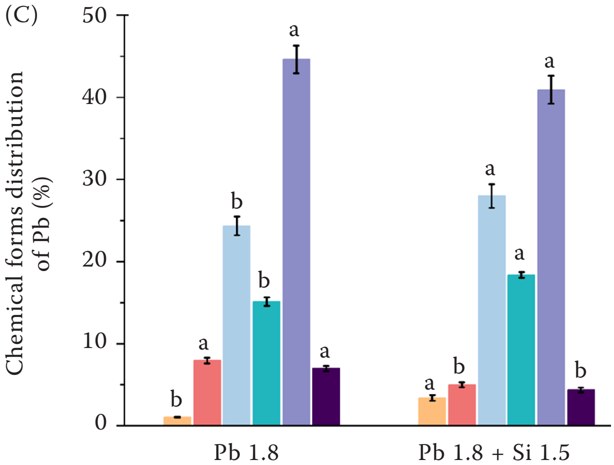


Figure 4. Chemical forms distribution of Pb^{2+} in *Salix viminalis* (A) leaf; (B) stem, and (C) root under silicon (Si) cultivation after Pb^{2+} stress for 10 days. FR – residual form; FHCl – HCl extractable form; FHAc – HAc extractable form; FNaCl – NaCl extractable form; FW – water extractable form; FE – ethanol extractable form

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decreased by 37.36% and 37.43%, and these differences were also statistically significant ($P < 0.05$). These results suggest that Si facilitated the transformation of Pb^{2+} in the roots to the less active FNaCl and FHAc fractions.

DISCUSSION

Inhibitory effect of Si on the transfer of Pb^{2+} to the aboveground parts of *S. viminalis*. When plants are subjected to heavy metal stress, Si application can effectively inhibit the translocation of heavy metals to the aboveground parts, thereby enhancing the plants' tolerance to heavy metals (Khan et al. 2021). The results of this experiment show that the Si application following Pb stress significantly reduced Pb^{2+} content in the leaves and stems while significantly increasing it in the roots, resulting in more heavy metals being immobilised in the roots of *S. viminalis* and thereby reducing the translocation factor of Pb^{2+} in the leaves and stems (Figures 1 and 2), which is consistent with previous studies. Liu et al. (2015) reported that Si inhibited the migration of Pb^{2+} from the roots to the aboveground parts in *O. sativa*, significantly reducing the Pb content in the aboveground biomass and grains. Wu et al. (2015) also discovered that Si significantly decreased the Cd^{2+} content in the shoots of *Solanum lycopersicum* L., thereby inhibiting the transfer of Cd^{2+} to the aboveground parts. In this study, Si inhibited the translocation of Pb^{2+} to the aboveground parts of *S. viminalis*, which may be one of the physiological mechanisms by which Si alleviates Pb^{2+} toxicity in *S. viminalis*. Through a study on the effects of Si on Cu^{2+} tolerance in *Triticum turgidum* L., Keller et al. (2015) found that Si hyperaccumulation in the root endodermis increased Cu^{2+} absorption at the root surface and immobilised it in the epidermis as organic and inorganic complexes, forming a physical barrier that restricted Cu^{2+} mobility. Further analysis revealed that hyperaccumulated Si in the endodermis produced an apoplastic obstruction, limiting the apoplastic transport of heavy metals (Pereira et al. 2018, Pang et al. 2024). Si can also combine with heavy metals to form silicates, which deposit in the plant cell walls, creating a physical barrier that inhibits the transfer of heavy metals (Bhat et al. 2019). Based on the above analysis, the inhibition of heavy metal transfer to shoot by Si may be a common mechanism in many plants, and the deposition of silica in the root endodermis and its surrounding areas could be a primary factor preventing the movement of heavy metals to the aboveground tissues.

Role of Si in the transfer of Pb^{2+} to cell wall and vacuolar compartments. When plants are subjected to heavy metal stress, they can regulate or prevent heavy metals from entering the protoplast. Once heavy metals have entered the plant, they mitigate toxicity by isolating these metals within cells, tissues, and organs through selective distribution and compartmentalised storage (Dalvi and Bhalerao 2013, Su et al. 2020). The results of this experiment indicate that the application of Si can promote the transfer of Pb^{2+} in the leaves and roots of *S. viminalis* to the less mobile F1 fraction and facilitate the movement of Pb^{2+} in the stems to the F4 fraction while reducing the Pb^{2+} content in the F2 fraction. This suggests that a substantial amount of Pb^{2+} is immobilised within the cell walls and vacuoles of *S. viminalis*, thereby alleviating, to some extent, the damage caused by Pb^{2+} (Figure 3), which is consistent with previous studies. Khlifi et al. (2024) discovered that under Cd stress, Si application facilitated the transfer of Cd^{2+} to metabolically inactive subcellular compartments (such as vacuoles and cell walls) in *H. vulgare*, achieving detoxification through compartmentalisation mechanisms. Si may also serve as a physiological mechanism to alleviate Pb^{2+} stress in *S. viminalis* by regulating the subcellular distribution of heavy metals. Further analysis indicates that the cell wall is a dynamic structure composed of polysaccharides (Głazowska et al. 2018). The hydroxyl groups in silicic acid can interact with the hydroxyl groups of cell wall polysaccharides, forming silica gels that bind with heavy metals to create complexes, thereby reducing the toxicity of these heavy metals (Guo et al. 2022). Wei et al. (2021) study found that, under the stress of Cd^{2+}, applying Si would help *O. sativa* of Cd^{2+} shift to the root cell wall; the accumulation and stabilisation of Cd in the cell wall is considered a key mechanism of cadmium resistance in plants, further analysis found that cell wall pectin (especially ion soluble pectin) is the combination of main components. Further analysis revealed that pectins in the cell wall, particularly ionically soluble pectins, are the main binding components. Although the cell wall has the capacity to retain heavy metals, it cannot completely prevent their entry into the protoplast. Heavy metals that enter the protoplast typically bind to compounds such as sulfur-rich peptides and organic acids and are subsequently transferred to the vacuole to mitigate damage to organelles (Pan et al. 2019). The above analysis indicates that Si can facilitate the binding of heavy metals to the cell wall and promote

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their compartmentalised storage in vacuoles, thereby alleviating heavy metal toxicity and enhancing the tolerance of *S. viminalis*. However, in this experiment, the significant increase in Pb^{2+} content in the leaves' mitochondrial fraction (F3) may be related to mitochondrial proliferation. Previous studies have found that mitochondria are relatively insensitive organelles within the cell and that stress conditions can increase mitochondrial numbers, thereby forming a robust energy supply system to enhance the plant's ability to withstand stress (Utrillas and Alegre 1997). Furthermore, mitochondrial proliferation has also been observed under various stressors, including Al (Konarska 2008), Cd (Gzyl et al. 2009), and Pb (Kaur et al. 2013).

Role of Si in the transformation of Pb^{2+} chemical speciation. When plants are subjected to heavy metal stress, the application of Si can facilitate the transformation of heavy metals from more active, extractable forms to less active forms within the plant, thereby inhibiting their migration and mitigating toxicity to plants (Xin et al. 2018). The results of this experiment show that Si facilitates the conversion of Pb^{2+} in *S. viminalis* leaves from FE to FNaCl and FHCl, in the stems from FE to FNaCl and FHAc, and in the roots from FW to FNaCl and FHAc ($P < 0.05$) (Figure 4), which is consistent with previous studies. Lu et al. (2017) discovered that Si application significantly increased the proportion of the FNaCl fraction for Cd^{2+} in the leaves and roots of *Amaranthus hypochondriacus* L. Therefore, the alteration of Pb^{2+} chemical speciation distribution by Si in *S. viminalis* may also be a physiological mechanism that alleviates Pb^{2+} stress. Substances such as proteins, pectates, and oxalates within plants can bind to heavy metals, altering their forms and consequently affecting their toxicity and mobility (Yang et al. 2018). Guo et al. (2023) also found that in *Lantana camara* L., heavy metals primarily bind with proteins, pectates, and insoluble phosphates, forming complexes with lower toxicity that enhance the plant's resistance. In summary, Si can alter the distribution of Pb^{2+} chemical speciation within *S. viminalis*, transforming heavy metals into less active and less toxic forms. Substances such as proteins, pectates, and oxalates are likely the primary binding components in this process.

Acknowledgement. We thank Shu-Hua Song for her assistance during plant cultivation and harvesting. We gratefully acknowledge the financial support from the National Natural Science Foundation of China, Henan Polytechnic University, and Henan Open University. We are grateful to the editor and anonymous referees for providing valuable comments.

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Received: January 6, 2025
Accepted: March 27, 2025
Published online: April 7, 2025