



Cardamine violifolia as a potential Hg hyperaccumulator and the cellular responses[☆]



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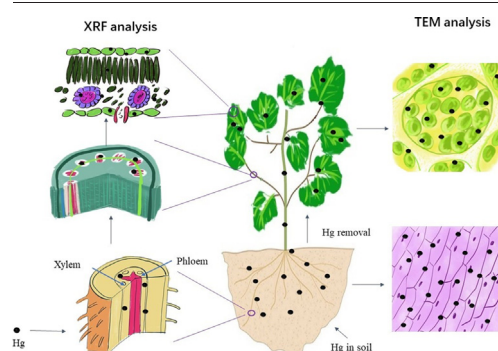
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HIGHLIGHTS

- *C. violifolia* accumulated mercury (Hg) in roots and aboveground parts up to 6000 µg/g.
- Hg crossed the root casparian.
- Then was transported to aboveground parts via vascular cylinder.
- *C. violifolia* root cells were tolerant to Hg.
- *C. violifolia* is a promising Hg hyperaccumulator.

GRAPHICAL ABSTRACT



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ABSTRACT

Cardamine violifolia belongs to the Brassicaceae family and is a selenium (Se) hyperaccumulator found in Enshi, China. In this study, *C. violifolia* was found to accumulate mercury (Hg) in its roots and aboveground parts at concentrations up to 6000 µg/g. In the seedling and mature stages, the bioaccumulation factors (BAF_s) of Hg reached 1.8–223, while the translocation factor (TF) for Hg reached 1.5. We observed a significant positive correlation between THg concentrations in plant tissues and those in the soil ($r^2 = 0.71–0.84$). Synchrotron radiation X-ray fluorescence with focused X-ray (µ-SRXRF) showed that Hg was translocated from the roots to shoots through the vascular bundle and was transported through the leaf veins in leaves. Transmission electron microscopy showed that root cells were more tolerant to Hg than leaf cells. These findings provide insights into the mechanisms of Hg hyperaccumulation in *C. violifolia*. Overall, we demonstrated that *C. violifolia* is a promising Hg hyperaccumulator that may be used for phytoremediating Hg-contaminated farmlands.

[☆] Dedicated to Prof. Zhifang Chai on the occasion of his 80th birthday.

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1. Introduction

Mercury (Hg) is one of the most toxic metals and is transported long distances in the air (Hsu-Kim et al., 2013; Qiu et al., 2005; Selin and Noelle, 2010). The occurrence of heavily Hg-polluted soils is mainly associated with natural deposition and anthropogenic dispersion from Hg mining areas (Li et al., 2015a, b; Zhang et al., 2010). Mercury is bioaccumulated and biomagnified in ecosystems. In particular, Hg contamination of farmlands poses a major threat to the safety of agricultural products and human health (Antoniadis et al., 2016; Lavoie et al., 2013; Li et al., 2018a, b). Therefore, minimizing Hg contamination in soil is of great significance for ensuring the quality and safety of crops, and maintaining ecological safety.

Conventional remediation technologies for Hg-contaminated soils can be physical, chemical, and biological (Asfa et al., 2020; Xu et al., 2015). Phytoremediation is an effective, low-cost, and environmentally friendly bioremediation approach for transferring or stabilizing all toxic metals in polluted soils (Cherian and Oliveira, 2005; Hussein et al., 2007; Liu et al., 2020; Wang et al., 2017; Yaashikaa et al., 2022), and it has been carried out on large scales and has been proven effective in many countries (Bonanno et al., 2017; Chandra et al., 2017; Cox et al., 1996; Eid and Shaltout, 2016; Zhang et al., 2015; Wang et al., 2019). Certain plants, called hyperaccumulators, are good candidates for phytoremediation, particularly because of their tolerance, absorption, accumulation, and translocation of metals (Ent et al., 2012; Salt et al., 1995; Yadav et al., 2021). In the past 20 years, more than 200 plant species have been studied and their ability to accumulate and transfer Hg has been tested. Although a few plant species, such as *E. ciliaris*, *E. polymnioides*, *A. ageratoides*, *B. ampestris*, *D. stramonium*, and *T. subterraneum*, are defined as “potential Hg hyperaccumulators,” no Hg hyperaccumulators have been reported thus far (Chamba et al., 2017; Liu et al., 2020; Lomonte et al., 2010; Mbanga et al., 2019; Qian et al., 2018). Therefore, finding Hg hyperaccumulators is highly desirable.

Cardamine violifolia is an annual or perennial plant that belongs to the *Brassicaceae* family, and it is found in a typical seleniferous area of Enshi, Hubei, China. It grows rapidly, has a long growth cycle, and has large plant biomass (up to 400 g strain⁻¹) (Rao et al., 2021; Zhu et al., 2016). The roots, shoots and leaves of *C. violifolia* contain an average of 2985, 3329 and 2491 mg/kg Se DW, respectively, so it is considered a Se hyperaccumulator (Cui et al., 2018). A previous study found that *C. violifolia* was also a hyperaccumulator of cadmium (Cd) and can be effectively applied for the remediation of Cd-polluted soils (Liu et al., 2018). Hg and Cd are in the same group in the periodic table. Therefore, we hypothesized that *C. violifolia* could accumulate Hg from contaminated soils.

Plants have many complex mechanisms for minimizing damage caused by metal exposure (Li et al., 2018a, b); however, their mechanisms of detoxification are poorly understood. In particular, studies on Hg stress response mechanisms have not been performed in *C. violifolia*. The toxic effects of metals on plants are determined by a series of parameters, including metal absorption sites, distribution sites, and competition for metal binding sites in plant cells (Israr and Sahi, 2006; Zhao et al., 2008). Therefore, it is important to know where Hg is localized, and how Hg is transported in plant tissues. Synchrotron radiation-based X-ray fluorescence (SRXRF) is a useful tool for studying the spatial distribution of elements of interest (Kopitke et al., 2018; Li et al., 2010; Zhao et al., 2013). Furthermore, cell ultrastructure changes form the cytological basis for a series of physiological changes in plants (Kang et al., 2015; van Doorn and Papini, 2013). Thus, investigations of the translocation and transformation of Hg in *C. violifolia* and identification of ultrastructural changes in response to Hg stress are critical steps toward revealing the mechanisms of Hg tolerance in plants.

In this study, the concentration of Hg in the roots and aboveground parts of *C. violifolia* was measured after the plants were cultured in soils containing different levels of Hg. The spatial distribution of Hg in the roots, shoots and leaves of *C. violifolia* was studied using synchrotron radiation-based μ -XRF. Moreover, ultrastructural changes in the roots, shoots and leaves were studied by transmission electron microscopy (TEM). The

information from these techniques is fundamental for gaining a better understanding of the mechanisms of Hg accumulation in *C. violifolia*. To the best of our knowledge, this is the first report to show that *C. violifolia* is a promising Hg hyperaccumulator. Further studies are warranted for applying *C. violifolia* as a hyperaccumulator plant to real Hg-contaminated soils.

2. Materials and methods

2.1. Cultivation experiment

C. violifolia seeds were disinfected with 1 % (V/V) sodium hypochlorite (NaClO) solution for 15 min and then washed thoroughly with deionized water (18.2 M Ω ·cm). The seeds were germinated in moist perlite at 28 °C in the dark. The plant incubator relative humidity was controlled in the range of 70 % to 80 % (Cui et al., 2014). After germination, the one-month-old seedlings were transferred to soils containing 0.1, 0.5, 1, 5, 10, 50, 100, and 500 μ g/g HgCl₂ (Sigma, USA). The greenhouse temperature was controlled at 25 \pm 3 °C for 16 h under light and 18 \pm 3 °C for 8 h in the dark. The plants were watered every 4 days to maintain soil moisture. Sixty-day-old seedlings and mature plants were selected for Hg concentration analysis.

The harvested *C. violifolia* were washed thoroughly with deionized water to remove soil and dust and were completely dried at room temperature. Triplicate roots, shoots, and leaves were separated by plastic scissors and placed into separate plastic bags. The dried samples were ground into powders using a plant grinder prior to further analysis (Chang et al., 2020; Cui et al., 2014).

2.2. Mercury concentration analysis

For THg analysis, approximately 50 mg of the roots and aboveground parts of each group were weighed and placed a digestion tank. The samples were digested with 5 mL nitric acid (BV-III) and 0.5 mL hydrogen peroxide (MOS level) and kept overnight at room temperature after complete mixing. The predigested samples were heated on an electric heating plate for 5 h (160 °C). Then, the samples were heated at 90 °C until the transparent solution reached a volume of approximately 1 mL. All the samples were cooled to room temperature and then diluted to 4 mL with 2 % nitric acid (containing 0.1 % β -mercaptoethanol) for analysis (Li et al., 2006; Li et al., 2017; Zhao et al., 2014a, b). THg concentrations were measured by inductively coupled plasma–mass spectrometry (ICP–MS, X7, Thermo Elemental, USA) following a previously reported method (Li et al., 2015a, b). The working conditions for ICP–MS were optimized with a 5 % nitric acid solution containing 1 μ g/L Be, Co, In and U. A standard curve was prepared for Hg (0, 0.5, 1, 2, 5, 10, and 50 μ g/L) with Hg standard stock solutions (GBW 08617, National Research Centre for CRMs, China). The limit of detection for THg by ICP–MS was 0.1 μ g/L.

2.3. Quality control

Reference materials and reagent blanks were used for analytical quality control. The standard reference material GBW10020 (citrus leaf) and sample replicates were included during the THg analysis. The average THg concentration of the standard reference materials was 0.16 \pm 0.01 μ g/g (n = 6), which was comparable to the certified value of 0.15 \pm 0.02 μ g/g. Duplicate analyses of plant tissue samples were conducted every ten samples, and the relative standard deviations of all duplicate samples were within 5 % (n = 20).

2.4. Bioconcentration factors and translocation coefficient of Hg in *C. violifolia* tissues

Bioconcentration factors (BAFs) and translocation factors (TF) for Hg by *C. violifolia* were used in this study to reflect the absorption and transport capabilities of Hg. BAFs were defined as the ratio of Hg concentration in

the roots or aboveground parts to the Hg concentration in the soil using the following equation:

$$BAF_{tissue} = \frac{C_{tissue}}{C_{soil}} \quad (1)$$

TF was defined as the ratio of Hg concentration in the aboveground parts to the Hg concentration in the roots using the following equation:

$$TF = \frac{C_{aboveground\ part}}{C_{root}} \quad (2)$$

2.5. Analysis of the spatial distribution of Hg by μ -XRF

The roots and shoots were first immersed in an embedding agent (Sakura Tissue-Tek OCT), frozen at -80°C and then cut into $40\ \mu\text{m}$ -thick slices with a freezing microtome (CM1850, Germany). The sections were fixed onto Mylar films (polycarbonate). The leaves were clamped between two pieces of cellophanes to keep them flat. The leaf samples were also fixed onto Mylar films. All samples were stored at -20°C until analysis with μ -SRXRF (Li et al., 2020a, b; Zhao et al., 2013).

The spatial distribution of Hg in the different *C. violifolia* tissues was measured at the 4W1B beamline in the Beijing Synchrotron Radiation Facility (BSRF, China). The μ -SRXRF analysis employed polychromatic excitation with an energy of 10–18 keV. The storage ring ran at 2.5 GeV and at a current intensity of 200–300 mA. The incident X-ray was focused to $50\ \mu\text{m} \times 50\ \mu\text{m}$ (Li et al., 2020a, b). The plant samples were mounted on an XYZ translation stage, and the sample platform was moved with a 2D stepping motor along the X/Z directions at $50\ \mu\text{m}$ each step. The count time was 10 s per pixel. The elemental fluorescence intensities and the Compton scattering intensities were normalized to the collection time and changes in I_0 (Lin et al., 2021).

2.6. Transmission electron microscopy

After seed germination in a plant incubator, one-month-old seedlings that had similar heights ($8.5 \pm 0.3\ \text{cm}$) and had four fully expanded leaves were exposed to Hg by immersing the roots in soils with 0 (control) and $5\ \mu\text{g/g}$ HgCl_2 (Sigma, USA). After 3 days of Hg exposure, the roots, shoots and leaves were excised, pooled, and rinsed with deionized water. Sample sections from the tips of the longest root (1–3 mm in length, 2–3 mm behind the apex) and the middle portion of the last developed leaf ($1\ \text{mm}^2$) were excised and fixed in cold 4% (v/v) glutaraldehyde in a 0.1 M potassium-phosphate buffer (PBS, pH 7.2), were vacuum-infiltrated until the material sank, and were left overnight at 4°C . The samples were then dehydrated in a graded alcohol series and embedded in resin (Spurr, 1969). Sample sections of $70\ \text{nm}$ thickness were generated using an LKB11800 Pyramitome (Sweden), and then examined using a transmission electron microscope (model 7650; Hitachi, Tokyo, Japan) at 80 kV. At least five sections from each treatment were examined (Zheng et al., 2018).

3. Results and discussion

3.1. Mercury accumulation in *C. violifolia* at the seedling stage and mature stage

The highest total Hg concentration in the soil was reported to be $790\ \mu\text{g/g}$ in the Wanshan Hg mining area of Guizhou Province (Yin et al., 2016). Therefore, a range of 0.1 – $500\ \mu\text{g/g}$ Hg was used in the soil in this study. THg concentrations of the aboveground tissues and roots of *C. violifolia* at the seedling stage and mature stage are shown in Fig. 1. At the seedling stage, the accumulated Hg concentration in the roots reached $6499\ \mu\text{g/g}$ when *C. violifolia* was exposed to a Hg concentration of $500\ \mu\text{g/g}$ in the soil. Meanwhile, the aboveground tissues accumulated $1115\ \mu\text{g/g}$ Hg. At the mature stage, Hg concentrations also increased in the roots and aboveground tissues after exposure, and Hg concentrations increased in the soils. When *C. violifolia* was exposed to Hg concentrations of $500\ \mu\text{g/g}$ in the soil,

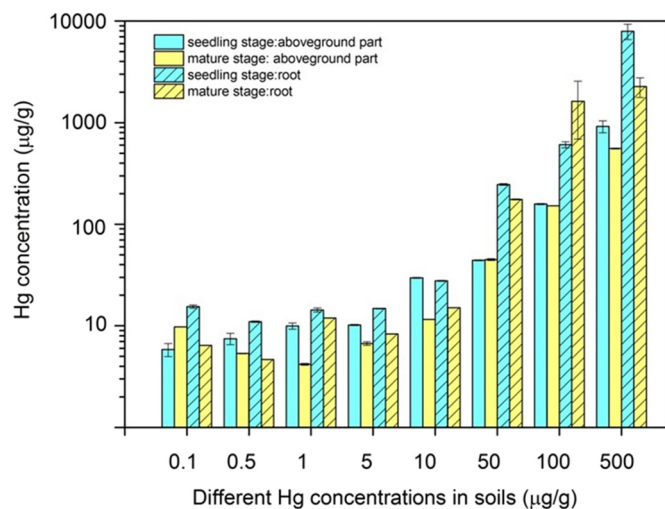


Fig. 1. THg concentrations of aboveground tissues and roots in *C. violifolia* at seedling stage and mature stage ($\mu\text{g/g}$).

the Hg concentrations in the roots and aboveground tissues were $2266\ \mu\text{g/g}$ and $557\ \mu\text{g/g}$, respectively.

In general, Hg is very toxic to plants. Exposure to excessive levels of Hg disrupts the plant oxidative stress system and photosynthesis system and inhibits plant growth (Azevedo et al., 2018; Calgaroto et al., 2010). However, the biomass of *C. violifolia* did not decrease when they grew in the Hg-contaminated soils. Moreover, there were no visual toxicity symptoms, such as wilting and water loss, observed during the entire *C. violifolia* growing stage, even when the plants were exposed to $500\ \mu\text{g/g}$ Hg.

In this study, at both the seedling and mature stages, the THg concentrations in the roots and aboveground tissues showed significant positive linear correlations with the soil, despite the wide range (0.1 – $500\ \mu\text{g/g}$) (Fig. 5), indicating that Hg readily translocated among plant tissues and that Hg uptake by *C. violifolia* was not limited by the Hg exposure concentrations. This indicated that *C. violifolia* had the ability to accumulate and translocate Hg from the contaminated soils.

In this study, Hg accumulation in the *C. violifolia* roots and aboveground parts was higher than that in other plants used for phytoremediation. For example, *Rumex induratus* and *Marrubium vulgare* were reported to accumulate Hg from soils, with phytoextraction yields of 12.9 and $27.6\ \text{g ha}^{-1}$, respectively (Moreno-Jiménez et al., 2006). Although thiosulfate promoted an increase in the concentration of Hg in three plants, *Brassica juncea* var. LDZY, *Brassica juncea* var. ASKYC and *Brassica napus* var. ZYYC, the Hg accumulated in the plants was lower than that in *C. violifolia*, indicating that *C. violifolia* is a promising Hg hyperaccumulator for phytoremediation (Wang et al., 2014; Wang et al., 2012). Moreover, in the seedling stage and mature stage, the roots accumulated the most Hg. This finding is in agreement with results from other studies, and it probably occurs due to the high affinity of the roots for Hg, which trap most of the bioavailable Hg (Marrugo-Negrete et al., 2015; Molina et al., 2006). This is generally because the roots are directly exposed to the Hg present in the soils, and Hg is mostly accumulated in the cell walls to avoid toxic effects to the aerial parts (Marrugo-Negrete et al., 2016).

3.2. The BAF and TF of Hg in *C. violifolia* in the seedling stage and mature stage

BAF is an important index for measuring the ability of plants to accumulate metals, while TF indicates the relative ease with which Hg is translocated from the roots to aboveground parts (Chang et al., 2020). To evaluate the ability of *C. violifolia* to translocate Hg from the roots to the aboveground parts of the plant, the TF and BAF were calculated, and these are shown in Table 1. Briefly, the BAF of Hg in *C. violifolia* roots at the seedling stage was significantly higher than that in the mature stage. At the seedling stage, the root BAF, aboveground part BAF and TF were

Table 1
Hg BAFs and TF of *C. violifolia* (n = 3) at seedling stage and mature stage in Hg exposed soils.

Hg concentrations in soil ($\mu\text{g/g}$)	Seedling stage			Mature stage		
	Root BAF _s	Aboveground part BAFs	TF ($C_{\text{aboveground}}/C_{\text{root}}$)	Root BAF _s	Aboveground part BAFs	TF ($C_{\text{aboveground}}/C_{\text{root}}$)
0.1	223 \pm 7.7	59 \pm 8.6	0.3 \pm 0.0	128 \pm 0.7	196 \pm 0.8	1.5 \pm 0.0
0.5	44 \pm 0.7	28 \pm 2.7	0.6 \pm 0.1	18 \pm 0.1	21 \pm 0.2	1.2 \pm 0.0
1	29 \pm 1.3	19 \pm 1.1	0.7 \pm 0.0	24 \pm 0.1	8.4 \pm 0.2	0.4 \pm 0.0
5	6.0 \pm 0.1	5.6 \pm 0.1	1.0 \pm 0.0	3.3 \pm 0.0	2.7 \pm 0.1	0.8 \pm 0.0
10	5.5 \pm 0.1	3.0 \pm 0.0	0.5 \pm 0.0	3.0 \pm 0.0	2.3 \pm 0.0	0.8 \pm 0.0
50	10 \pm 0.2	1.8 \pm 0.0	0.2 \pm 0.0	7.0 \pm 0.1	1.8 \pm 0.0	0.3 \pm 0.0
100	12 \pm 0.7	3.2 \pm 0.0	0.3 \pm 0.0	11 \pm 1.3	3.0 \pm 0.0	0.3 \pm 0.0
500	14 \pm 1.9	1.8 \pm 0.2	0.1 \pm 0.0	4.5 \pm 1.0	2.2 \pm 0.0	0.5 \pm 0.0

5.5–223, 1.8–59 and 0.1–1.0, respectively. At the mature stage, the root BAF, aboveground part BAF and TF were 3.0–128, 1.8–196 and 0.3–1.5, respectively. When the Hg concentration in the soil was 5 $\mu\text{g/g}$, the TF was highest in all the groups.

In general, metal hyperaccumulators are considered for phytoextraction when both the BAF and TF are greater than one (TF and BAF > 1) (Yoon et al., 2006; Zhao et al., 2014a, b). In this study, the root BAF and aboveground tissue BAF were both > 1. The BAF value reached the reference value for Hg hyperaccumulators (Hannah et al., 2017; Liu et al., 2007). Moreover, studies have been reported that hyperaccumulating plants have intrinsic adaptive regulatory mechanisms to hyperaccumulate metals

in their aboveground tissues. In this study, the BAF of Hg in the aboveground tissues was >1.8 in the seedling stage and mature stage, indicating that *C. violifolia* could absorb and enrich Hg from soils and could accumulate it in the aboveground parts.

3.3. Distribution and translocation of Hg in roots, shoots and leaves

The normalized X-ray fluorescence intensities were scaled from blue (minimum) to red (maximum). These images visually demonstrated the distribution and accumulation of Hg in the roots, shoots and leaves of *C. violifolia*, as shown in Fig. 2.

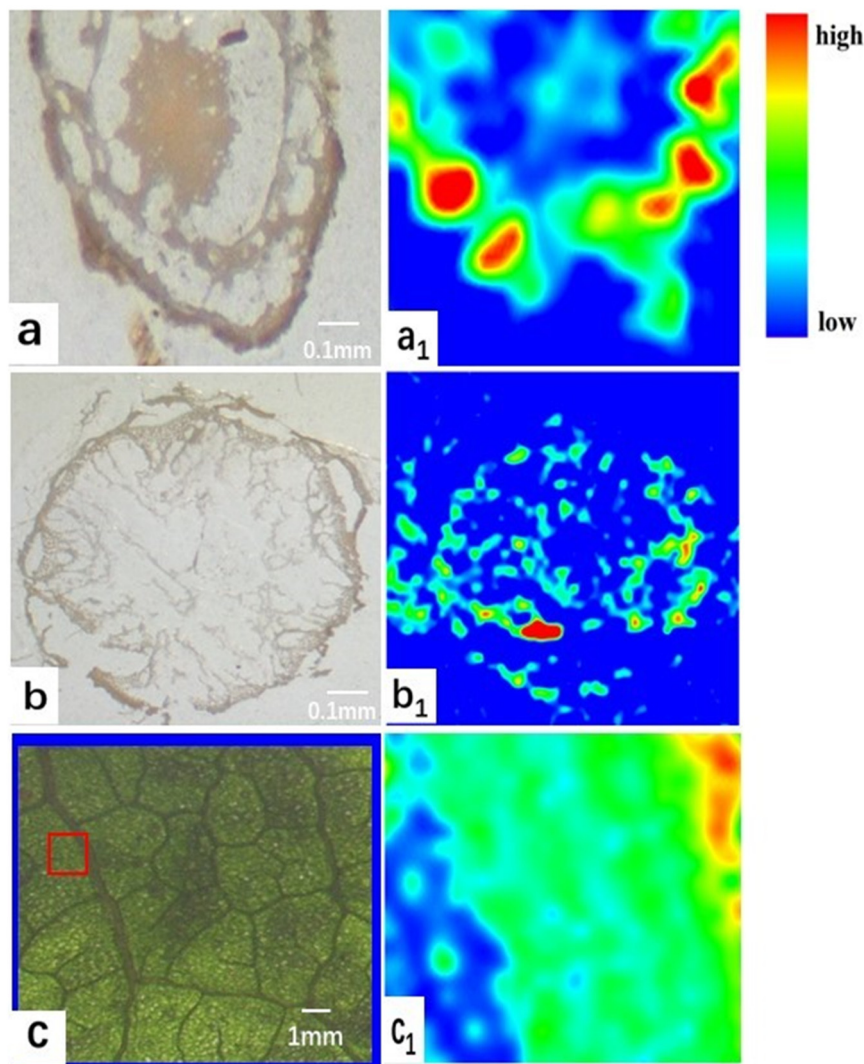


Fig. 2. The distribution of Hg in root, shoot and leaf of *C. violifolia* measured by μ -XRF. (a) The cross section of the root tip from *C. violifolia* under Hg exposure (a₁, Hg XRF image); (b) the cross section of the shoot from *C. violifolia* under Hg exposure (b₁, Hg XRF image); (c) the leaf from Hg exposed *C. violifolia* (c₁, Hg XRF image).

Generally, it is difficult for metals to reach the xylem vessels of the roots because the metals have to cross the endodermis and the suberized Casparian strips (Greger, 1999; Skinner et al., 2007). It has also been reported that the Casparian strip is a root barrier that excludes Hg, and thus, little Hg can be observed in the pericycle (Patty et al., 2009; White, 2012). Most of the accumulated Hg in plants remains in the roots, and only a small proportion is translocated to the shoots. Approximately 80 % of the Hg trapped in the roots is bound to the cell wall. However, metal hyperaccumulators can undergo active processes of uptake, accumulation, and translocation from the roots to aboveground tissues (Ahammad et al., 2018; Natasha et al., 2020; Greger, 1999; Wang and Greger, 2004). This was the case in this study, as shown in Fig. 2a₁ and b₁. Hg was mainly distributed in the epidermis and pericycle of the root, and a large amount was also found in the central cylinder of the shoot. The presence of Hg in the pericycle suggested that Hg was able to cross the Casparian strip and be transported from the roots to the aboveground parts through the vascular cylinder. Hg has been suggested to bind with phytochelatins (PCs) to form Hg-PC complexes that can be transferred to vacuoles, reducing Hg stress and acting as transporters in plants (Xu et al., 2017). PCs are chelators and are significant for metal detoxification in plants (Natasha et al., 2020; Park et al., 2012).

Moreover, Hg was dispersed in the leaves and was mainly located in the leaf vein due to transport from the roots and shoots, as shown in Fig. 2c₁. According to μ -XRF analysis, *C. violifolia* has the ability to absorb, accumulate and translocate Hg.

3.4. Ultrastructural changes in roots and leaves exposed to Hg

The processes of metal accumulation and transfer are complex in plant cells. In this study, TEM was used to analyze the impacts of Hg stress on the ultrastructure of plant cell organelles. Since the TF was the highest in all the

groups when the seedlings were exposed to 5 μ g/g Hg, Hg stress at 5 μ g/g was chosen for TEM analysis. TEM revealed that root cells of *C. violifolia* seedlings in the control group had smooth and continuous cell walls and well-developed mitochondria (Fig. 3a and c). After 3 days of Hg exposure, the cell walls in the roots were still intact (Fig. 3b). Generally, the cell wall is the first barrier against the entry of metals and has a strong ability to accumulate metal cations. Metal effects are often observed at the cell wall (Chandra et al., 2017; Feng et al., 2019; Feng et al., 2021; Liu et al., 2019; Wang et al., 2019). Additionally, Hg has a high affinity for the cysteine-rich domains of the major cell wall extension protein that resists Hg stress (Carrasco et al., 2011). Fig. 3d shows that the mitochondrial cristae and membrane were intact. A few large vesicles appeared in the mitochondria. Mitochondria are known to be much more resistant to metals than chloroplasts, and they remain undisturbed even at high metal concentrations (Heumann, 1987; Islam et al., 2007; Kleiner, 1974). This study showed that the mitochondrial ultrastructure was not affected and remained intact in Hg-exposed roots, indicating that the root cells of *C. violifolia* were tolerant to Hg (Fig. 3b and d).

TEM revealed that leaf mesophyll cells in the control group had well-developed cell walls, chloroplasts, chloroplast membranes, nuclear membranes, and thylakoid lamella in the leaves (Fig. 4a and c). However, considerably increased granum stacks were observed in the organelles of the leaf mesophyll cells when *C. violifolia* was exposed to Hg. In addition, the plasma membrane was detached from the cell wall. Plasmolysis was also found to be greater than in the control and irregularly distributed in the chloroplast (Fig. 4b and d), suggesting that leaf mesophyll cells were sensitive to Hg. Studies have reported metal granules in the cell wall and in vacuoles. Some studies have considered whether the plants have mechanisms to regulate their normal growth, and this is further discussed below (Wang et al., 2019).

In general, *C. violifolia* root cells are more tolerant to Hg than leaf cells, which may explain the higher concentration of Hg in the roots than in the

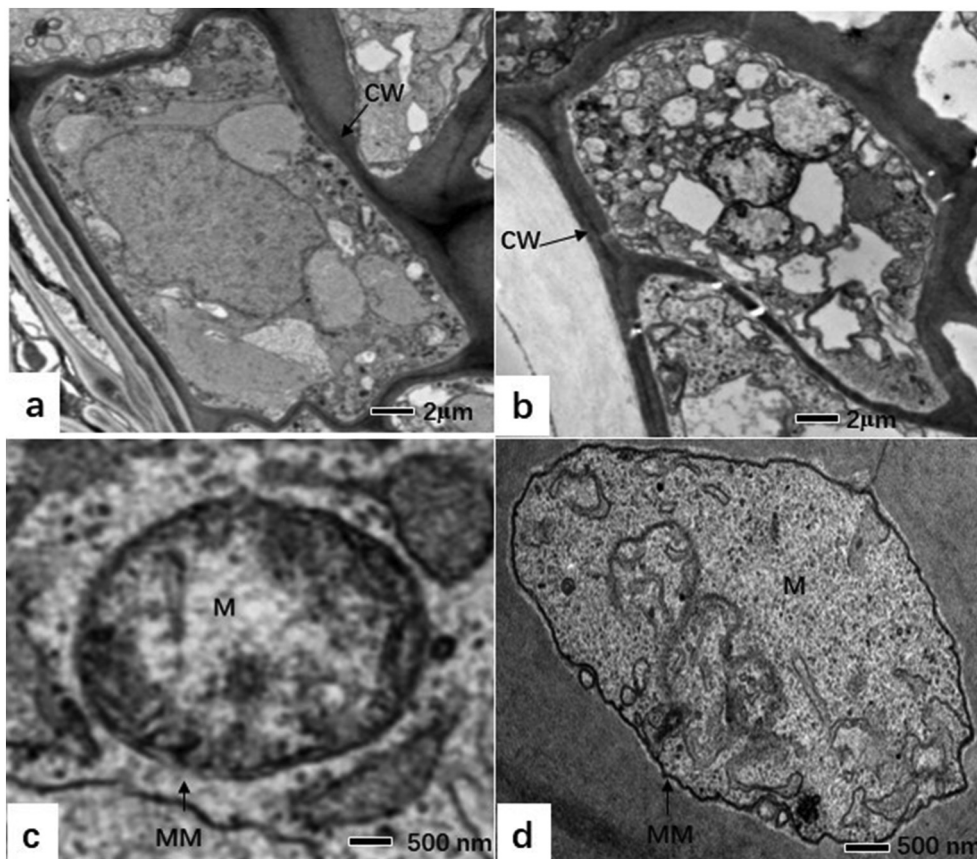


Fig. 3. Transmission electron micrographs of the root cells of *C. violifolia* seedlings exposed to 0 μ g/mg Hg (control) (A and C) and 2 μ g/mg Hg (B and D) for 3 days, respectively. A, B, C and D show single cell and mitochondria of control and Hg treatment plants, respectively. Bars: A = 2 μ m, B = 2 μ m, C = 500 nm, D = 500 nm. Labels: CW, cell wall; M, mitochondria; MM, mitochondria membrane.

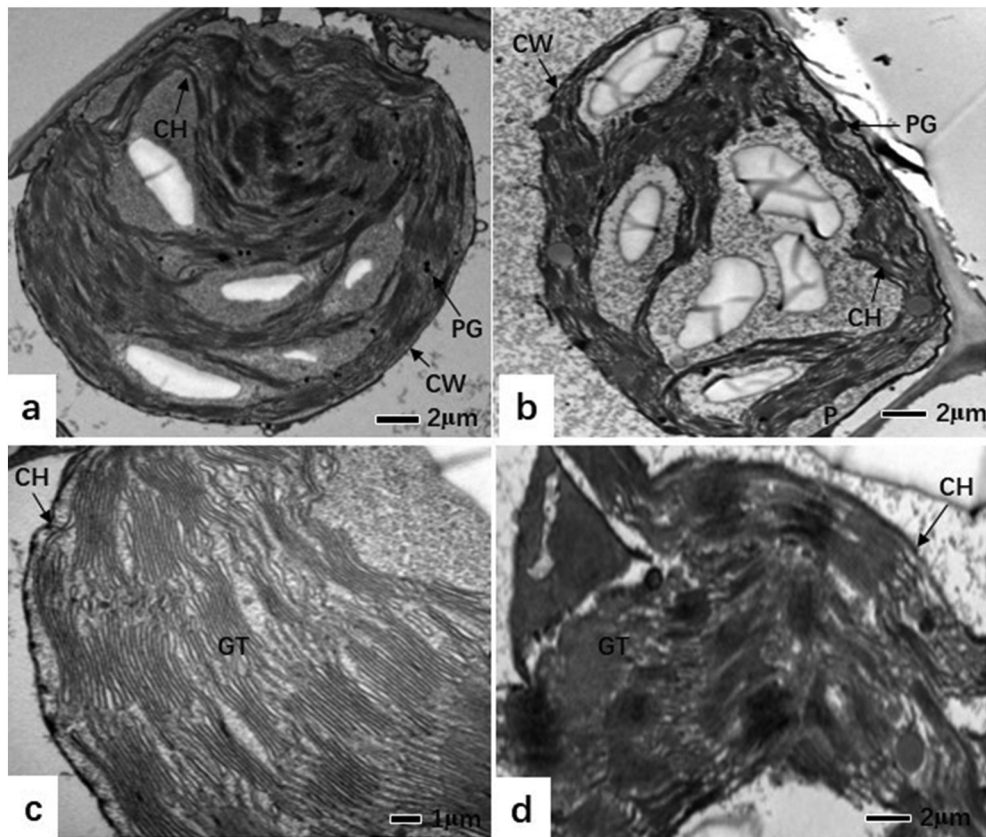


Fig. 4. Transmission electron micrographs of the leaf cells of *C. violifolia* seedlings exposed to 0 µg/mg Hg (control) (A and C) and 2 µg/mg Hg (B and D) for 3 days, respectively. Panels A and B, C and D show single leaf cell and chloroplast of control and Hg treatment plants, respectively. Bars: A = 2 µm, B = 2 µm, C = 1 µm, D = 2 µm, CH, chloroplast; CW, cell wall; GT, Grana thylakoid lamella; PG: plastoglobule; P, plasmolysis.

aboveground parts. In this study, ultrastructural study of the cells of *C. violifolia* provided detailed information on the mechanism of Hg accumulation.

4. Conclusions

Overall, this study highlights the accumulation, distribution and translocation of Hg and the changes in the cell ultrastructure of *C. violifolia*,

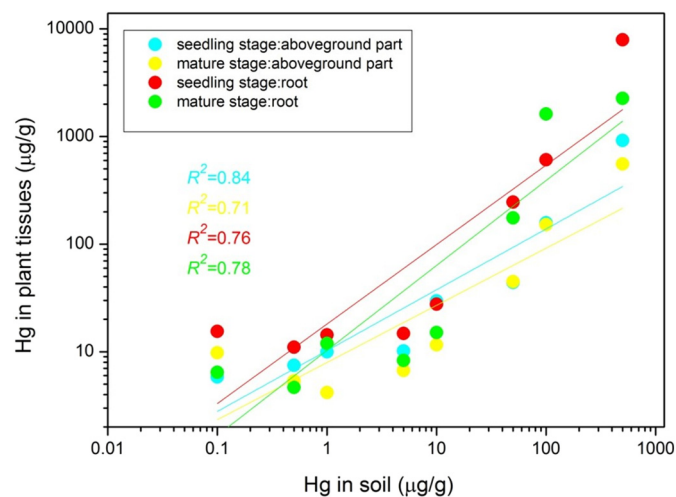


Fig. 5. Correlation between Hg concentrations in soil and in plant tissues from plant seedling stage and mature stage.

which is a promising Hg hyperaccumulator. The BAF was higher than 1 in *C. violifolia*. Furthermore, spatial distribution analysis of Hg revealed that Hg could cross the root Casparian strip and be transported from the roots to aboveground parts through the vascular cylinder. Ultrastructural changes indicated that the root tissue of *C. violifolia* seedlings was more tolerant to Hg than the leaves and confirmed a higher concentration of Hg in the roots than in the aboveground parts. To the best of our knowledge, this study reports for the first time that *C. violifolia* is a promising Hg hyperaccumulator. Further studies are warranted to apply *C. violifolia* as a hyperaccumulator plant to real Hg-contaminated soils to verify its capabilities.

CRedit authorship contribution statement

Liwei Cui: Conceptualization, Methodology, Writing – original draft preparation.

Xue Tian: Data curation, Software, Visualization.

Hongxin Xie: Data curation, Software, Visualization.

Xin Cong: Resources, Data curation.

Lihong Cui: Data curation, Formal analysis.

Han Wu: Data curation, Formal analysis.

Jianxu Wang: Visualization, Validation.

Bai Li: Visualization, Validation.

Jiating Zhao: Data curation, Software, Visualization.

Yanshan Cui: Visualization, Validation.

Xinbin Feng: Supervision, Visualization, Validation.

Yu-Feng Li: Funding acquisition, Writing – reviewing and editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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