

Cleaning up of heavy metals-polluted water by a terrestrial hyperaccumulator *Sedum alfredii* Hance

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Abstract *Sedum alfredii* Hance is a terrestrial zinc/cadmium (Zn/Cd)-hyperaccumulating and lead (Pb)-accumulating plant. Previous studies on *S. alfredii* were mostly focused on its physiological mechanism of heavy metal uptake and the application in phytoextraction of metals from contaminated soils. In this study, we evaluated the application potential of *S. alfredii* in the cleanup of heavy metals from contaminated lake water. Our research revealed that changing pH in lake water would not make particular difference on the final accumulation amount of heavy metals, because the acidic water environment negatively affected plant growth compared with the neutral and alkaline environments, but was more conducive for heavy metal absorption and accumulation. In addition, *S. alfredii* showed an increase of approximately 2.2-fold in dry weight (DW) when cultured with lake water for 25 d. At the same time, it accumulated approximately 5.0 mg/kg DW of Cd and 41.4 mg/kg DW of Pb. The absorption of heavy metals was highly effective during the first 10 d of culture. Also, the quality of lake water was greatly improved after only 2-d cleanup by *S. alfredii*. In general, this hyperaccumulator exhibits great potential for application in the cleanup of heavy metals-polluted waters.

Keywords heavy metals, hyperaccumulator, lake water, phytoremediation, *Sedum alfredii*

Introduction

The presence of heavy metals in aquatic ecosystems, resulting from industrial and urban discharges, is one of the most important environmental concerns of scientists. Heavy metals are severely hazardous to human health and lead to ecological damages at levels higher than toxic concentrations. Appropriate technology for removing heavy metals from contaminated water is thus crucial. Different methodologies are used for the removal of heavy metals, such as electro dialysis, reverse osmosis, and adsorption, but these are quite costly and energy intensive (Singh et al., 1996). Phytoremediation is the application of plants for *in situ* or *ex situ* treatment/removal of contaminated soils, sediments, and water (Salt et al., 1998). It offers a cost-effective, non-intrusive, environmentally sustainable, and safe alternative to conventional cleanup techniques (Lombi et al., 2001; Santos et al., 2006). Plant

species selection is a critical management decision for phytoremediation. Some species are highly metal specific, have a small biomass, grow slowly, and require careful management for multiplication, which are not suitable for commercial application (Yang et al., 2002; Sarma, 2011). Therefore, the identification of novel plant species or exploration of existing plant species with a large biomass and high capability of accumulating multiple heavy metals has become an important aspect of phytoremediation research.

Sedum alfredii Hance is a terrestrial zinc (Zn)/cadmium (Cd) hyperaccumulator native to China (Yang et al., 2002, 2004) and later proved to be also a lead (Pb)-accumulating species (He et al., 2002). In addition to its extraordinary ability to tolerate and accumulate high concentrations of Cd, Pb, and Zn, it is characterized by its large biomass, fast growth rate, asexual propagation, and perenniality (Chao et al., 2008). Previous studies on *S. alfredii* mostly focused on the physiological mechanism of heavy metal uptake and the application in phytoextraction of metals from contaminated soils (Yang et al., 2006; Li et al., 2007; Lu et al., 2008; Tian et al., 2009; Li et al., 2011), leaving the application potential of

Received April 24, 2013; accepted July 8, 2013

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this plant for phytoremediation of heavy metal-polluted waters, such as urban lakes or rivers, largely unexplored. Some aquatic species such as duck weed, water hyacinth, and calamus, were proved to be effective in the removal of heavy metals from polluted water (Prasad and Freitas, 2003), but they can only tolerate and accumulate relatively low concentrations of heavy metals. Land plants also have good capability for cleaning up heavy metals from contaminated water, such as Canna lily, sunflower, maize, and even *Taxodium ascendens* (Feng et al., 2003; Rajakaruna et al., 2006; Sun et al., 2008; Liu et al., 2010). However, these land plants grow slowly. The planting of them and subsequent heavy metal recovery increase the management costs. Thus, the future application of *S. alfredii* in the cleanup of heavy metals-polluted waters is highly expected.

Contamination of urban or suburban lakes with multiple heavy metals affects the safety of drinking water, fish, and vegetables. In urban lakes of Wuhan City, China, heavy metals, such as mercury, Zn, Cd, and Pb, are present in surface water, sediments, and fish (Liu et al., 2006; Qiao et al., 2007; Tang et al., 2009). Notably, the concentrations of two typical heavy metals Cd and Pb in surface water near the shores are above toxic levels in several urban lakes of Wuhan City (Table 1). Therefore, a distinct advantage of the application of *S. alfredii* in the cleanup of contaminated waters would be its efficient accumulation of multiple heavy metals from surface water near the shores. In this study, we aimed to examine the application potential of *S. alfredii* in the cleanup of heavy metals-polluted lake water by (1) evaluating the influence of various pH values on the growth and heavy metal accumulation of *S. alfredii* in standard culture solution, because various lakes may have different pH levels and even in the same lake, the pH value usually changes with the shift of seasons, and (2) assessing the growth and heavy metal accumulation of *S. alfredii* during the culture process with lake water in an outdoor environment.

Table 1 Contents of three heavy metals in surface water near the shores of urban lakes of Wuhan City, China

Lakes and criterion	Content ($\mu\text{g/L}$)		
	Cd	Pb	Zn
Donghu Lake	8.60	97.97	66.44
Moshui Lake	12.32	79.33	35.41
Jinying Lake	8.34	83.10	21.52
GB3838-2002 (III)*	5	50	1000

*The environmental quality standard for surface water of China. Class III: applies to Grade-II protective zone of surface water resource area of drinking water, wintering grounds and migration channels of fish and shrimp as well as aquaculture grounds and swimming areas.

Materials and methods

Plant material

S. alfredii Hance was collected from an old Pb/Zn mining area

in Quzhou City, Zhejiang Province, China, in 2005 and grown in the campus of Central China Normal University. This plant was continuously regenerated by asexual propagation, and new plants showed no heavy metal accumulation. Healthy and uniform shoots were chosen and grown in liquid Murashige and Skoog (MS) (1962) medium to regenerate roots as described by Zhou and Qiu (2005). After pre-culturing for 20 d, flourishing root systems were regenerated.

Growth conditions

For evaluating the influence of changing pH (acidic or alkaline environment) on the growth and heavy metal accumulation of *S. alfredii*, we cultured the plants in liquid MS solution at pH 5.8, 7.0, or 8.0. The cultures were performed in a greenhouse with controlled temperature ($25 \pm 2^\circ\text{C}$) and a 14-h light/10-h dark photoperiod. The light intensity was $90 \mu\text{mol photons}/(\text{m}^2 \cdot \text{s})$. The culture solution was aerated continuously with an aquarium air pump and replaced every 2 d.

For evaluating the influence of lake water on the growth and heavy metal accumulation of *S. alfredii*, we grew the plants with flourishing root systems in water collected from Donghu Lake, as shown in Fig. 1. *S. alfredii* plants with equal fresh weights were chosen for treatment. Plants grown in a solution containing 10% MS and 90% tap water were used for comparison. The pH of the 1/10 MS solution was adjusted to 5.8 every 2 d with 0.1 M NaOH or 0.1 M HCl, and the lake water was replaced every 2 d. This experiment was performed in an outdoor environment during March and April 2011. The outdoor temperature varied between 2 and 24°C .

Plant growth assay

The above-ground parts (shoots and leaves) were separated from the harvested *S. alfredii* plants. Then the separated parts (above-ground parts and roots) were respectively oven-dried at 70°C for 48 h and cooled in a desiccator to constant weight. Dry weights (DWs) were determined with an electronic balance. For detecting the DWs of plants at the start of the experiment, plants with equal fresh weights were used for treatment. In this study, plant growth was calculated based on the difference in DW at the start and that at the end of the experiment.

Determination of heavy metals

The above-ground parts of *S. alfredii* plants were dried as mentioned above. Dried samples were digested in 5 mL of boiling concentrated nitric acid (70%) until clear. The digested solutions were diluted to 25 mL with deionized water. The concentrations of heavy metals in the diluted solution were determined by flame atomic absorption spectrometry (Purkinje General Instrument 990, China) as described by Zhou and Qiu (2005). For the determination of



Figure 1 *S. alfredii* plants cultured with lake water in this experiment.

Cd, Pb, and Zn concentrations in lake water, approximately 500 mL of surface water was immediately mixed with 50% HCl to adjust the pH to 2.0 after collection (Levei et al., 2008). The acidified lake water was concentrated in a beaker by heating and then mixed with concentrated nitric acid in a volume ratio of 1:3 for digestion. The digested solution was filtered with filter paper and set to a constant volume for determining heavy metal concentrations.

Quality assay of lake water

Lake water at approximately 0.10 m underneath the surface was collected near the Shuanghu Bridge of Donghu Lake. Approximately 35 L of surface water was taken back to the laboratory for plant culture every week during March and April 2011. The quality of lake water was assessed by a multifunction water quality analyzer (United States Hash Company HYDROLAB DS5). Orthophosphate in lake water was determined using the ammonium molybdate spectrophotometric method (Wetzel and Likens, 2000).

Results

Influence of pH on the growth of *S. alfredii*

The pH value of lake water generally changes along with the shift of seasons. For example, the pH of Donghu Lake water was approximately 5.4–5.8 in November 2010 and approximately 7.8–8.3 in April 2011. To study the possible influence of pH on the growth of *S. alfredii*, we detected DW changes in *S. alfredii* plants after they were cultured in MS nutrient

solution at different pH values (Fig. 2). The DWs of the above-ground parts showed no significant difference between the neutral and alkaline media after 20-d culture (Fig. 2A), whereas their DWs were higher than those in the acidic medium. For root system, the DW increase in the neutral medium was approximately 1.9- and 1.4-fold higher than those in the acidic and alkaline media, respectively (Fig. 2B). Therefore, the neutral and alkaline environments were relatively more effective in promoting the growth of *S. alfredii*.

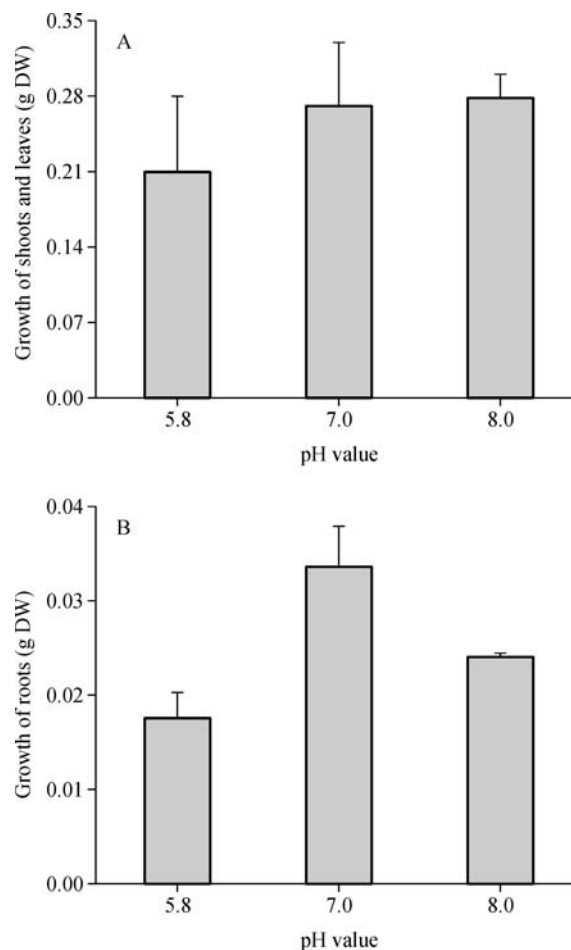


Figure 2 Growth changes in the above-ground parts (A) and roots (B) of *S. alfredii* in MS solution at various pH values for 20 d. Data are expressed as mean ± SD ($n = 4$).

Influence of pH on Cd accumulation of *S. alfredii*

For *S. alfredii*, heavy metals are absorbed by root system and then transferred to and accumulated in shoots and leaves (Yang et al., 2002). Cd is a non-essential trace element and one of the most toxic heavy metals (Das et al., 1997). The influence of pH on Cd accumulation efficiency in *S. alfredii* plants was evaluated (Fig. 3). At pH 5.8, Cd accumulation in the above-ground parts obviously increased with higher concentration of Cd treatment; by contrast, at pH 7.0 and 8.0,

such increase was not very obvious. However, at either high or low level of Cd treatment, Cd accumulation in the acidic condition was higher than those in the neutral and alkaline conditions. These results suggested that an acidic environment was more beneficial for heavy metal absorption and accumulation in *S. alfredii*.

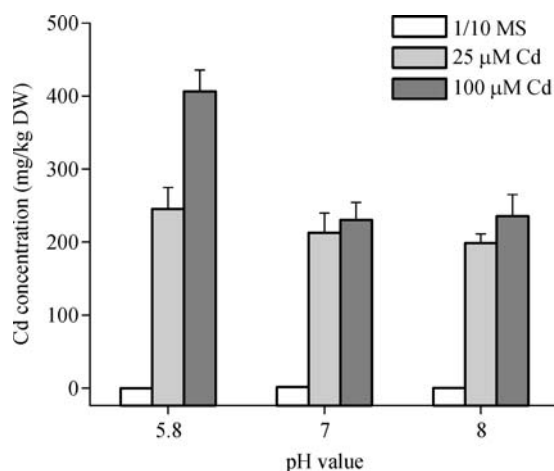


Figure 3 Cd accumulation in the above-ground parts of *S. alfredii* after being exposed to low (25 µM) and high (100 µM) Cd solutions with different pH values for 16 d. Data are expressed as mean±SD ($n = 4$).

Growth of *S. alfredii* with lake water

In this experiment, *S. alfredii* plants were cultured with Donghu Lake water in an outdoor natural environment. The DW changes of *S. alfredii* were assayed at different time points (Fig. 4). In both conditions, the DWs of plants gradually increased with the extension of culture time, and plants showed a similar growth rate during the first 18 d. Nitrogen (N) and phosphorus (P) concentrations in 1/10 MS medium were approximately 84 and 4 mg/L, respectively, whereas their values in fresh lake water were around 0.66 and 0.28 mg/L (Table 2). We thus speculate that other unknown nutrient components in lake water counteracted the possible slow growth resulting from the lower levels of N and P sources. After 18 d, the growth rate of lake water-cultured plants decreased, which implied that prolonged exposure to lake water was not beneficial for plant growth.

Accumulation of heavy metals from lake water by *S. alfredii*

Heavy metal accumulation efficiency is another important aspect for evaluating the application potential of *S. alfredii* in water remediation. Thus, the accumulation of heavy metals by *S. alfredii* plants was evaluated (Fig. 5). A sharp increase in Cd accumulation was observed at 10 d, whereas a sharp increase in Pb accumulation occurred at 4 d. Thus, the accumulation of heavy metals from lake water was most

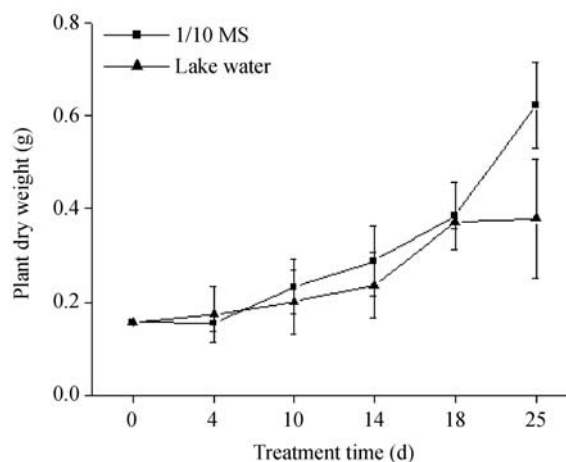


Figure 4 Growth of the above-ground parts of *S. alfredii* after being cultured with 1/10 MS medium and lake water for 25 d. Data are expressed as mean±SD ($n = 3$).

Table 2 Changes in the quality of water from Donghu Lake after cleanup by *S. alfredii* plants

	Fresh lake water	Cleaned lake water	Tap water
pH	7.92±0.04	7.41±0.12	7.07±0.09
ORP (mV)	382±11	414±7	412±7
TDS (g/L)	0.4±0.0	0.3±0.0	0.2±0.0
DO (g/L)	7.50±0.08	7.67±0.10	8.10±0.02
NH ₄ ⁺ (mg/L N)	0.30±0.02	0.07±0.01	0.04±0.00
NO ₃ ⁻ (mg/L N)	0.36±0.01	0.26±0.02	0.22±0.01
Cl ⁻ (mg/L)	60±2	50±2	40±1
PO ₄ ³⁻ (mg/L P)*	0.28±0.05	0.19±0.10	0.02±0.01

Data, except PO₄³⁻, were obtained using a water quality analyzer in April 2011, and approximately 3 L of lake water was treated by six plants for 2 d. *PO₄³⁻ was analyzed using the ammonium molybdate spectrophotometric method in November 2011.

efficient for *S. alfredii*, which may be due to its high capability for Cd and Pb absorption. After 10 d, the accumulation rates for both heavy metals slowed down. At 25 d, the plants accumulated approximately 5.0 mg Cd/kg and 41.1 mg Pb/kg in dry above-ground parts, which are only 137% and 123% higher than those observed at 10 d, respectively. Therefore, a short-term application (e.g., 10 d) for water cleanup seemed appropriate and cost-effective.

Improvement of water quality after cleanup by *S. alfredii*

The fresh untreated water was yellow-green and cloudy and emitted an unpleasant stench, and there are many visible small insects in it. The water quality was evaluated after 2-d cleanup by *S. alfredii* (Fig. 6; Table 2). The lake water became clearer after the cleanup (Fig. 6). Also, small insects were no longer observed and the unpleasant stench disappeared. After 2-d cleanup, the pH of lake water decreased from 7.92 to 7.41 closer to that of tap water. NH₄⁺ was rapidly consumed and

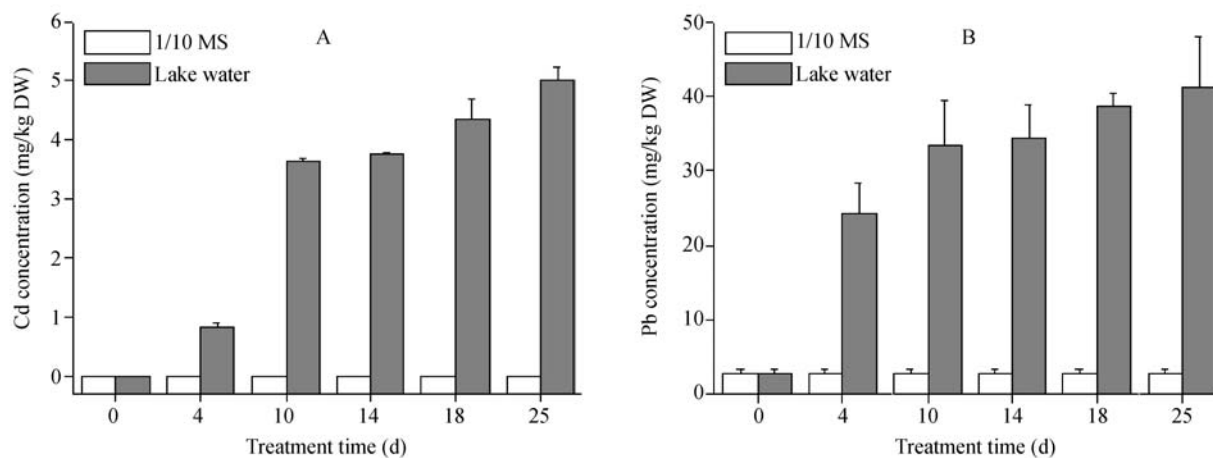


Figure 5 Accumulation of Cd (A) and Pb (B) in the above-ground parts of *S. alfredii* after being cultured with lake water. 1/10 MS medium was used as control solution. Data are expressed as mean \pm SD ($n = 4$).

NO_3^- was partially consumed, which means that NH_4^+ is the preferred N source. P was also rapidly consumed during the culture. The reduction in N and P concentrations in lake water is advantageous for preventing the occurrence of algal blooms (Sellner et al., 2003; Abell et al., 2010). In addition, other indicators, such as oxidation reduction potential (ORP), total dissolved salts (TDS), and dissolved oxygen (DO), improved and were also closer to those in tap water after the cleanup, suggesting an overall improvement of water quality.



Figure 6 Water from Donghu Lake before and after cleanup by *S. alfredii*. (A) Fresh water collected from Donghu Lake. (B) Water after cleanup by *S. alfredii* for 2 d. (C) Still lake water stored for 2 d.

Discussion

In this study, we investigated the application potential of *S. alfredii* in the phytoremediation of heavy metals-contaminated water. Water from Donghu Lake, a representative urban lake in Wuhan city, was used. The first problem confronted by this application is the effect of changing pH in lake water on the phytoremediation efficiency. The pH in Donghu Lake water varies greatly. We used nutrient solutions

with various pH levels to treat *S. alfredii* plants, which indirectly reflected the influence of the changing pH in lake water on phytoremediation. We found that the acidic water environment negatively affected the growth of *S. alfredii* compared with the neutral and alkaline environments (Fig. 2) but seemed to be more conducive for the accumulation of heavy metals (Fig. 3). Therefore, changing pH in lake water would not make particular difference on the final accumulation amount of heavy metals in *S. alfredii*. *S. alfredii* is a kind of shade plant and cannot tolerate high temperature and strong sunlight. For example, it cannot survive when exposed to an outdoor temperature exceeding 37°C. Thus, if applied in the summer season, it should be placed at shady environment. The concentrations of heavy metals near the shores are usually higher than those in the center of lakes, which is to a certain extent attributable to the adsorption by small organisms and impurities gathered near the shores. Therefore, it should be more suitable to apply *S. alfredii* near the shores of lakes. Floating island (Stewart et al., 2008) is potentially good technology for the actual application of *S. alfredii* plants.

The second problem about this application is how effective *S. alfredii* is used to clean up contaminated lake water. *S. alfredii* not only survived in lake water, but also grew obviously in the 25-d culture (Fig. 4). Also, it could rapidly absorb and accumulate Cd and Pb during the first 10 d (Fig. 5). *S. alfredii* is reportedly capable of accumulating 11000 mg/kg DW of Cd and 514 mg/kg DW of Pb in the shoots (He et al., 2002; Zhou and Qiu 2005). By comparison, *S. alfredii* only accumulated 5.0 mg/kg DW of Cd and 41.4 mg/kg DW of Pb after 25 d of culture with lake water. Therefore, its high capability of heavy metal accumulation had not been fully exploited. However, its prolonged application is not cost-effective, because it showed a slow metal accumulation rate after the first 10 d (Fig. 5). Except for

excessive heavy metals, such organic pollutants as alkylbenzene, phthalate ester, and alkylphenols were also found in Donghu Lake (Wang et al., 2002). It is possible that complex water pollutants coupled with some biotic factors, such as small insects, negatively affected the growth and heavy metal accumulation of *S. alfredii* in lake water. Thus, we suggest that this plant is applied within a short-term cycle (e.g., 10 d) for the cleanup of lake water. After every 10 d of application, the poisoned roots of *S. alfredii* plants are cut off and allowed to regenerate in nutrient solution, after which the plants with flourishing new roots are used again. In addition to the enrichment of heavy metals, *S. alfredii* can also improve other quality indicators of lake water (Table 2). In general, *S. alfredii* plants exhibited great application potential in cleaning up heavy metals-polluted lake water, although the actual large-scale application of *S. alfredii* may confront new problems.

Acknowledgements

This work was supported by Wuhan Chenguang Project for Youth Scholar (Nos. 201150431110 and 20045006071-24) and the Natural Science Foundation of Hubei Province (No. 2008CDB073).

Compliance with ethics guidelines

Boxia Chen, Wenli Ai, Huan GONG, Xiang GAO, and Baosheng Qiu declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects by any of the authors.

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