The hyperaccumulating of *Scirpus tabernaemontani* to Cd and its potential for phytoremediation

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Abstract: Cadmium phytoextraction can be economically feasible only when the developed systems high biomass plant can accumulate greater than 100 mg/kg Cd on dry weight. In this experiment, *Scirpus tabernaemontani*, a perennial herb, was used to demonstrate its capability at accumulating Cd, more than 280 mg/kg Cd in its above-ground parts. This hydroponics experiment showed the effects of two factors, solution pH and medium Cd concentration, on the plant biomass growth and Cd-extraction in 30 days. The herb could bear high level of Cd (30 mg/L) and widespread pH value shifting. The highest Cd-extractions are 264.71 mg/kg (above-ground parts) and 234.39 mg/kg (underground parts), with the average translation coefficient 1.13. It demonstrated the potential of this herb for phytoremediation.

Key words: hyper accumulator; *Scirpus tabernaemontani*; cadmium; phytoremediation

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Cadmium concentration is increasing rapidly in the environment because of its increasing used by human society. Alarming concentrations of the metal have been reported in water and land near main area and industrial waste disposal sites. The contamination originates mainly from mining, metallurgy, chemical industry and heavy metal recycling industry (Guo, 1994). Cd not only affects plant growth and productivity but also enters into the food chain, which will cause health hazards to man and animals (Seaward *et al.*, 1993; Li *et al.*, 2004). Remediation of Cd contaminating soil and water is necessary for protecting both human's life and agricultural production.

There are many conventional treatments for remediation of Cd-contaminated sites like absorption, ion exchange, co-precipitation, membrane process, electrodialytic and treatment etc (Yang *et al.*, 2002). Among the new strategies for removal of heavy metals from contaminated sites, phytoremediation is growing interest because of its low environmental impact and cost-effectiveness, even if a longer time is required for treatment. The term hyperaccumulation was first used to describe plants containing more than 1000 mg/kg Zn on dry weight (Seaward *et al.*, 1993). It now extends to represent a concentration of about 100 times greater than that of the largest values to be expected in non-accumulating plants (Brooks *et al.*, 1998).

Hyperaccumulators are usually small, native plants, such as those belonging to genus *Thlaspi* and several others (Brown *et al.*, 1997). Conversely, the plant, *Scirpus tabernaemontani*, not only produces large biomass production, but also fulfills distinct prerequisites such as metal tolerance and metal accumulation via extraction translocation (from underground parts to above-ground parts) and sequestration. And the herb is easy to harvest. So it is a plant whose species is efficient for phytoextraction of heavy metals (Reeves *et al.*, 2000). The plant biomass and cadmium extraction of *Scirpus tabernaemontani* depend on environmental factors such as soil type, nutrient supply and medium pH. In all fac-

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tors, medium pH and Cd concentration are very important ones influencing plant growth and Cd-extraction. It was expected that the analysis of the influences of these two factors and their combinations on plant growth and Cd-extraction could obtain the optimum combination of pH and Cd to achieve maximum plant growth and Cd-extraction of Softstem Bulrush.

1 Materials and methods

1.1 Seedling collection and pro-treatment

Seedlings were obtained from fertile fronds of Softstem Bulrush growing in the effluent of Yongzhou lead-zinc mine, Hunan Province (26°22′ E, 111°63′ N). The local soil contains Cd 74.52 mg/kg, Zn 7 653.75 mg/kg, Pb 157.90 mg/kg, Hg 0.74 mg/kg. The pH value is 6.57.

To make the plants according the requirement of the hydroponics experiment, uniform seedlings were picked out and washed under running tap water for 30 min. Then they were surface-sterilized with 0.2% mercuric chloride for 5 min, followed by rinsed three times with distilled water. After that, every four seedlings, placed in separate holes on a Styrofoam sheet (as a plant supporter), were transferred to a plastic pot, containing 500 mL of Hoagland's nutrition solution, and allowed to grow for 2 weeks. The pH value of nutrient solution was adjusted with dilute HCl and NaOH. All chemicals were of analytical reagents.

The solution was vigorously aerated and replaced every 3 days. The environment was climate-controlled at a cycle of 24/20 ℃, light cycle at 16h light/8h dark, relative humidity 70%. When the seedlings attained a higher of 4～11 cm in 2 weeks, they were ready for the following experiments.

1.2 Plant growth experiment

Seedlings with fresh weight of 7～10 g per plant and similar size of roots were selected for the experiment. All of them were washed with tap water for 30 min and then rinsed for 3 times with distilled water. All samples were weighted for their fresh weight, and then ten of them were sampled to determine basic content of Cd. After that, two groups of hydroponics experiments were carried out.

Group one was an experiment to determine the effect of medium pH on plant Cd-extraction potential and the plant biomass of *Scirpus tabernaemontani*. The pH of the nutrient solution, with the fixed concentration of Cd (20 mg/L), was varied as the following values: 3.7, 4.7, 5.7, 6.7, and 7.7.

Group two was to investigate the plant's toleration ability of Cd and uptake of Cd influenced by medium Cd concentration. The basal medium, Hoagland's nutrition solution, which pH value was adjusted at 5.6, was supplemented with $Cd(NO_3)_2$ at the following concentrations: 5, 10, 15, 20, 25 and 30 mg/L.

Each level of treatments was repeated 3 times (3 pots) with 4 plants in every pot. The solution was aerated vigorously and renewed every 3 days. During this period, the solution pH was checked and adjusted at least once or as necessary. The plants were allowed to grow for 30 days until the effects of experimental treatments were fully displayed.

The herbs fostered in the normal conditions (without cadmium in nutrient solution and at pH 5.63) were designed in all experiments, so as to determine the changes in the plants treated by Cd.

1.3 Cd analytical methods

All plant samples were weighted and washed with tap water followed by rinsing in deionized water three times. Then herbs were separated into above-ground parts (stalks) and underground parts (roots plus rhizomes) and oven-dried at 80 ℃ to weight the dry biomass of stalks and roots. After that, plant samples were digested with $HNO_3/HClO_4$ in a microwave digestion system (MARS 5, USA) and determined Cd concentrations by a Flame Atomic Absorption Spectrophotometer (Agilent 3510, Shanghai).

2 Results

2.1 The growth and Cd-extraction of wild Softstem Bulrush

The field investigation showed that *Scirpus tabernaemontani* could adapt seeper and the poverty of the soil. Advanced analysis indicated that it could absorb Cd from contaminative soil: The above-ground parts accumulated 78.97 mg/kg (dry weight); The underground parts were 106.45 mg/kg (dry weight); The translation coefficient was 0.72.

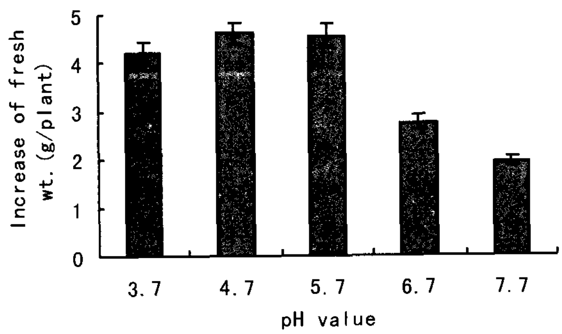

Fig. 1 Effect of different medium pH on fresh weight of *S. tabernaemontani* grown in Cd calcareous solution in 30 days
Data represent mean ±S. D. of three independent replications. The same bellow.

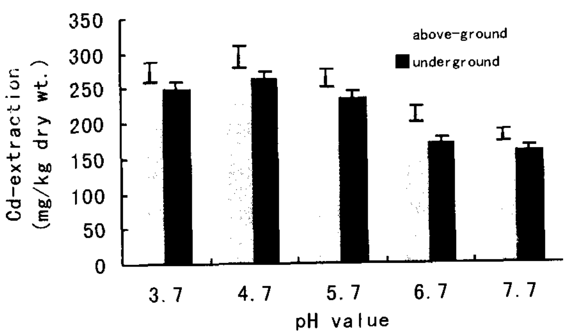

Fig. 2 Effect of different medium pH on Cd-extraction of *S. tabernaemontani* grown in Cd calcareous solution in 30 days

2.2 The influence of nutrient solution pH

The increase of Softstem Bulrush seedlings' biomass and Cd-extraction were influenced by nutrient solution pH distinctly.

Seedlings produced relatively high biomass (4.62 g/plant) at pH 4.7. But it reduced to 2.76 g/plant at pH 6.7, and the minimum (1.94 g/plant) at pH 7.7 (Fig. 1). Many seedlings leaves shriveled and turned yellow at that pH value. One of the probable reasons was the high pH value effects seedlings to counteract the toxic properties of Cd.

The maximal seedlings Cd-extractions were 295.23 mg/kg (above-ground part) and 261.76 mg/kg (underground part) at nutrition solution pH 4.7. After that, the Cd-extractions were descent with the revising of pH value (Fig. 2).

2.3 The influence of solution Cd-concentration

S. tabernaemontani seedlings could tolerate high levels (30 mg/L) of Cd when grown in modified Hoagland's nutrient medium under sterile conditions (pH5.6). 25 mg/L of Cd could enhance the growth of seedlings effectively (4.97 g/plant). But seedlings were poisoned at Cd concentration 30 mg/L (Fig. 3).

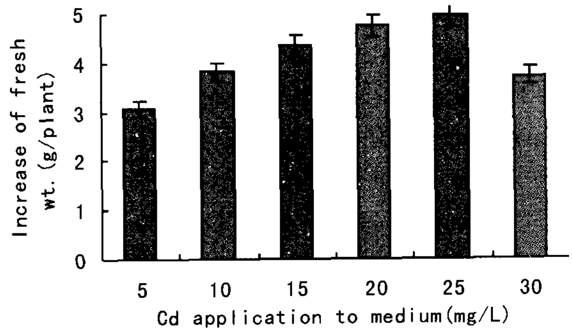

Fig. 3 Effect of different Cd application on fresh weight of *S. tabernaemontani* grown in Cd calcareous Hoagland's nutrition solution in 30 days

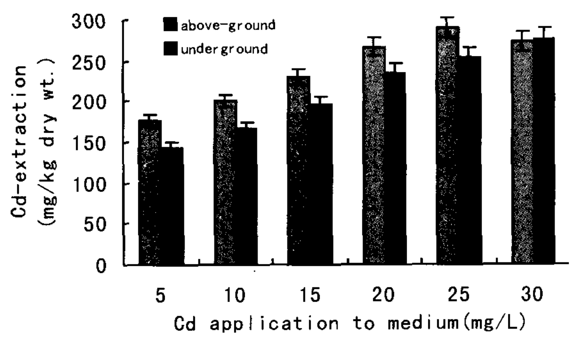

Fig. 4 Effect of different Cd application on Cd-extraction of *S. tabernaemontani* grown in Cd calcareous Hoagland's nutrient solution in 30 days

The Cd-extraction after 30 days of growth was positively correlated with Cd concentration in the solution until 30 mg/L Cd (Fig. 4). When the concentration of Cd in solution was more than 25 mg/L, the Cd-extraction in the above-ground stem did not enhance further while that of the underground part still increased. The *Scirpus* seedlings could uptake the most amount of Cd in shoots (289.74 mg/kg in dry weight) when the Cd concentration was 25 mg/L in the medium, and in roots

(275.67 mg/kg in dry weight) with Cd at 30 mg/L.

There were visible conclusions that optimum conditions for *Scirpus* Cd-extraction were low pH and high Cd concentration in nutrient medium, with the maximum point at Cd 25 mg/L and pH 4.7 for above-ground part and Cd 30 mg/L and pH 4.7 for the underground part. The amount of Cd assimilated by above-ground parts in this species is far greater than that of the known Cd-hyperaccumulator, *Brassica juncea*, which accumulates 107.63 mg/kg Cd in dry weight when grown in nutrient solution (Jonathan *et al.*, 2004).

2.3 Translation coefficient

Translation coefficients means the above-ground part of heavy metal extraction divided by underground part. If a hyperaccumulator is with high translation coefficients, it will accumulate heavy metal from soil effectively. The translation coefficients of *Scirpus tabernaemontani* seedling were more than 1 in most conditions. They were influenced by medium pH and Cd concentration.

The translation coefficient ranged between 1.11～1.24 with medium pH value changed between 3.7～7.7 (Table 1), and ranged between 0.99～1.24 with Cd concentration changed between 5～30 mg/L (Table 2).

Table 1　Effect of medium pH on translation coefficient of *Scirpus tabernaemontani*

pH value	Above-ground	Underground	Translation coefficient
3.7	273.15	248.27	1.10
4.7	295.23	261.76	1.13
5.7	264.71	234.39	1.13
6.7	211.64	170.53	1.24
7.7	179.82	157.92	1.14

Table 2　Effect of medium Cd application on translation coefficient of *Scirpus tabernaemontani*

Cd	Above-ground	Underground	Translation coefficient
5	176.43	142.28	1.24
10	200.83	165.98	1.21
15	229.31	195.99	1.17
20	267.24	234.42	1.14
25	289.74	253.48	1.14
30	273.35	276.67	0.99

It seemed that pH6.7 and 5 mg/L Cd in nutrition solution would be the most suitable conditions to achieve the highest translation coefficient (1.24).

3　Discussion

Scirpus tabernaemontani G. seedlings tolerated high levels (30 mg/L) of Cd and large-scale of pH values (3.7～7.7) when growing in modified Hoagland's nutrient medium. It can uptake Cd into its tissue more than 200 mg/kg in dry weight, which is as much as twice to the recognized standard of typical cadmium hyperaccumulator (David *et al.*, 1999).

The effects of medium pH and Cd concentrations on plant biomass increase indicated that low pH and high Cd concentrations enhanced plant growth. It is possible that plant biomass increase affected by medium pH is more beneficially than Cd concentration (Cd≤25 mg/L). However, shoots showed symptoms of some toxicity at and above 30 mg/L, while the roots could tolerate higher Cd and without any stunted growth apparently. The most common effect of Cd toxicity in plants shown earlier is stunted growth, leaf chlorosis and alteration in the activity of many key enzymes of various metabolic pathways (Godbold *et al.*, 1985; Arduini *et al.*, 1996).

Our results indicated that the beneficial Cd concentrations for *Scirpus* uptake cadmium were greater than those of other typical Cd hyperaccumulator e.g. *Thlaspl.* and *Brassica*, (Levent *et al.*, 2003; Qadir *et al.*, 2004). This observation suggests that *S. tabernaemontani* is well equipped with Cd tolerance mechanisms. The findings also suggest that the processes causing metal hyperaccumulation trait in *S. tabernaemontani* also cause this plant species to be sensitive to Cd deficiency.

The effects of Cd concentrations and medium pH on Cd-extraction were exhibited from Fig. 3 and Fig. 4. The *Scirpus* seedlings uptake cadmium efficiently when the medium Cd concentrations were above 10 mg/L (shoot contained more than 200 mg/kg of Cd on dry weight). However, there was little decline of Cd concentration in shoot when medium Cd attained reached 30 mg/L. But the Cd in plant root moved upward with the same Cd concentrations (Fig. 4). This finding suggests that *Scirpus* root is adapted high Cd concentration better than its above-ground parts. It is possible that a new polypetides is synthetised in root under Cd pollution. It is proved that the mechanism of cadmium resistance in hyperaccumulator may involve different strategies: retention in root, accumulation in intercellular deposition on cell wall, compartmentalization in vacuole, insoluble Cd-binding components and Cd-binding protein (Nishizono, 1987; Wang, 1991).

Consideration of pH is crucial to phytoremediation strategies since pH varies greatly in Cd polluted soils and waters. Although there was no significant effect of pH on plant biomass where the medium pH was below 5.7 (Fig. 1), high plant biomass occurred at Cd≥20 mg/L (Fig. 2). The agreement between the cadmium uptake and the proton release during the accumulation process indicates an equivalent ion exchange between Cd^{2+} and H^{+}. The active binding groups reside in the cell wall and the initial sorption rate is accelerated due to its large surface.

High translation coefficient of hyper accumulator is one of the most important influence facts to any phytoremediation progress waltz carry through (Nedelkoka *et al.*, 2000). It is the ratio of above-ground part Cd concentrations to underground part in dry weight. High translation coefficient results in more cadmium ion being uptake from underground to above-ground (Tu *et al.*, 2003). In our study, medium pH influenced plant translation coefficient from 1.11 to 1.24. Rising of medium pH value enhanced the plant translation coefficient basically, meanwhile, the translation coefficient increased with the decrease of medium Cd concentration, thus was opposite to the effects of medium pH and Cd concentration on plant biomass and Cd-extraction. So it is a dilemma to obtain high biomass, Cd- extraction and translation coefficient with same medium pH and Cd concentration.

In summary, *Scirpus tabernaemontani* fresh biomass, and Cd-extraction were influenced by medium pH and Cd concentration under hydroponic conditions. Cd concentrations at 20～25 mg/L was beneficial to plant growth and Cd extraction. Such effects of Cd were influenced by pH due to the significant interactions between them. The results suggested that optimum plant growth could be achieved by adjusting pH based on Cd concentrations, whereas maximum Cd hyperaccumulation by maintaining solution pH≤5.7. Our results should be useful for developing strategies to Cd-phytomining and remediate Cd-contaminated water and soil using Softstem Bulrush.

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水葱对镉的超富集作用及其用于植物修复的潜力

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摘 要：野外观察与研究发现水葱（*Scirpus tabernaemontani* G.）可以耐受土壤中高浓度的重金属污染，并对镉有很高的生物富集量。实验室水培试验研究了两个主要因素，营养液 pH 值以及镉含量，对其生物量以及镉富集效果的影响。结果表明，水葱可耐受的高浓度 Cd (30 mg/L) 和大范围 pH 值变化 (3.7～7.7)。当营养液 pH 值为 4.7，Cd 含量为 25 mg/L 时，水葱富集的 Cd 达到最大值：地上部分 264.71 mg/kg，地下部分 234.39 mg/kg，平均转运系数 1.13。这显示了它用于植物修复镉污染土壤的巨大潜力。

关键词：地超富集植物；水葱；镉；植物修复

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钙向皮肉的扩散微弱，推测果核向果肉的主动运输困难。

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