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Original Research

Phytoextraction Potential of *Solanum nigrum* L. and *Beta Vulgaris* L. *Var. Cicla* L. in Cd-Contaminated Water

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Abstract

The potential of two Cd-hyperaccumulators, *Solanum nigrum* L. (*SN*) and *Beta vulgaris* L. *var. cicla* L. (*BV*), as phytoremediation plants to remove Cadmium (Cd) from contaminated water was evaluated in hydroponics. The results showed that Cd exposure induced chlorosis and inhibited biomass growth in *SN*. However, these symptoms did not appear in *BV*. In *BV*, the Cd concentrations increased from 14.2 to 314.7 mg kg^{-1} in the shoots and 241.1 to 4547.9 mg kg^{-1} in the roots when the Cd concentration in the nutrient solution increased from 0.5 to 50 μM. The corresponding increase in *SN* was from 7.4 to 100.6 mg kg^{-1} in the shoots and 30.2 to 2010.7 mg kg^{-1} in the roots. Generally, *BV* showed a higher tolerance and accumulation of Cd, while *SN* was more efficient for Cd removal due to its higher biomass.

Keywords: water contamination, Cd-hyperaccumulators, hydroponics

Introduction

Toxic metals and metalloids, such as cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As), are constantly released into the environment by mining, industrial processes, and agriculture, threatening environmental and human health. In China, urban wastewater has been considered one of the most important freshwater resources and has been used for agricultural irrigation since the 1950s [1, 2]. Unfortunately, as one of unexpected side effects, large areas of rivers and soils have been contaminated by heavy metals, especially Cd, which is the main harmful, toxic element in China and may represent a potential hazard to human health due to exposure through the food chain [3, 4].

There is an urgent need to develop low-cost, effective, and sustainable methods for Cd removal or detoxification. Recent research has shown that phytoextraction may be an effective method for removing and detoxifying heavy metals and metalloids from contaminated soil and water [5-8]. Two groups of plant species have been proposed for phytoextraction: hyperaccumulators species able to accumulate and tolerate extraordinary metal levels, and high biomass-producing species compensating for lower metal accumulation by high biomass yields [9]. Several high biomass-producing species, including *Colocasia antiquorum, Ipomoea aquatica, Arundo donax, and Phragmites australis* (Cav.) Trin. and *Typha orientalis* Presl, have been fully studied for their phytoextraction potential in hydroponics [7, 10-12]. However, non-hyperaccumulators will yield a metal-poor, large-volume biomass, which will be more uneconomical than the hyperaccumulators to recover metals and safely dispose of them [8]. Among the Cd-hyperaccumulators, *Solanum nigrum* L. (*SN*) and

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Beta vulgaris L. *var. cicla* L. (*BV*) [13, 14] may be good candidates for phytoextraction due to their fast growth, easy propagation, and simple mangement. These two plants have shown a substantial potential for phytoextraction of Cd in the wastewater-irrigated soils [15, 16]. However, there are limited data on the capacity of *SN* and *BV* for the phytoremediation of contaminated water bodies.

The objective of this study is therefore to evaluate the potential of *SN* and *BV* for phytoremediation in Cd-contaminated water. A series of hydroponic tests was conducted to verify the capacity of these two plants for phytoextraction of Cd. The visible toxic symptoms, shoot and root dry biomass, Cd accumultion, and Cd removal by these two plants were determined in response to increasing Cd levels to assess the potential of these two plants for application in the phytoremediationof Cd-contaminated water.

Materials and Methods

Hydroponic Experiment

Solanum nigrum L. (*SN*) and *Beta vulgaris* L. *var. cicla* L. (*BV*) were used for the hydroponic experiments. The seeds were sterilised in 75% ethanol for 10 minutes and washed several times with sterile distilled watr. Then the seeds were germinated on filter paper moistened with distilled water in a thermostat for 7 days. After one week of incubation, the seedlings with similar biomasses were transferred to a hydroponic culture. The seedlings of the plants were placed through a perforation in a plastic platform in a 250 ml glass jar containing 200 ml Hoagland's nutrient solution [17] so that the root was immersed in liquid medium and the shoot was above the platform. Each glass jar was used for one plant. In the first week, these plants were grown in quarter-strength Hoagland's solution, and then the cultures were changed to a half-strength solution in the second week. The solutions were renewed once evey seven days. In the seventh week, the seedlings were exposed to different Cd concentrations (μM) with three replications. The Cd concentrations (prepared from Cadmium nitrate) tested were 0 (control), 0.1, 0.5, 5.0 10.0, and 50.0 μM. The solution was aerated continuously. The level of the solution was maintained by adding distilled water to maintain a volume of 200 ml in all treatments. The temperature of the greenhouse was approximate 22ºC during the day and 15ºC at night throughout the growth period. After a growth period of three weeks in the Cd-enriched nutrient solution, the plants were then harvested and separated into roots and shoots and washed with distilled water. After that, they were drie at 100ºC for 10 min and thn at 70ºC in an oven until completely dry. After measuring the dry weight, the plants were round.

Determining of the Plant Cd Content

The plant samples were digested with a solution of 3:1 HNO_3:$HClO_4$ (v/v). The concentration of Cd was determined by flame atomic absorption spectrometry (FAAS, Varian SpectrAA 220, Australia). To evaluate the phytoextraction potential of the plants, the enrichment factor (EF = metal concentration in plant / habitat) and the translocation factor (TF = metal concentration in shoots / roots) were calculated [18, 19].

Statistical Analysis

All treatments were replicated three times. The means and standard deviations (SD) were calculated using SPSS13.0. One-way analysis of variance was carried out with SPSS10.0. When a significant ($p<0.05$) difference was observed between the treatments, multiple comparisons were made by the LSD test.

Results and Discussion

Visible Toxic Symptoms and Plant Growth

The symptoms of Cd toxicity have been studied in several plant systems under various conditions. Visible signs of Cd toxicity include growth inhibition, brown margin to leaves, chlorosis, curled leaves, brown stunted roots, redis veins, and petioles [20, 21].

In this study, the visible symptoms of Cd toxicity on the young leaves included chlorosis when *SN* was grown in a Cd concentration ranging from 1 to 10 μM. This symptom did not appear in *BV* for any of the treatments. Moreover, the Cd treatments caused a statistically significant inhibition of biomass production in *SN* (Fig. 1a-1b). Both the shoot and root biomass of *SN* decreased with the increasing concentration of Cd in the nutrient solution. The growth of *SN* was decreased significantly due to the Cd treatments, even at a low concentration of Cd (0.5 μM) in the nutrient solution. This suggested a toxic effect of Cd on *SN*. This deleterious effect was not observed in *BV* at up to 5 μM Cd in the growth medium. The biomass of the shoots showed a downward trend at Cd concentrations of 10.0 and 50.0 μM in the nutriment solution, but the difference was not statistically significant. This result indicates that the *BV* has higher tolerance to Cd than *SN*. For all Cd treatments, *SN* showed a higher shoot and root dry biomass than *BV*. The biomass of the shoots of *SN* was approximately 3 times that of *BV*; meanwhile, the biomass of the roots of *SN* was approximately 6 times of that of *BV*. These results suggested that *SN* had a greater biomass, while *BV* had a higher Cd tolerance. Metal tolerance is a prerequisite for metal accumulation and hence phytoremediation [22]. The degree of basal Cd tolerance is presumably found in all plant species [21]. It is important to select a phytoextration plant with high Cd tolerance capability. This type of plant would be more efficient for the application in phytoremediation the the plant selected solely based on high accumulation of Cd.

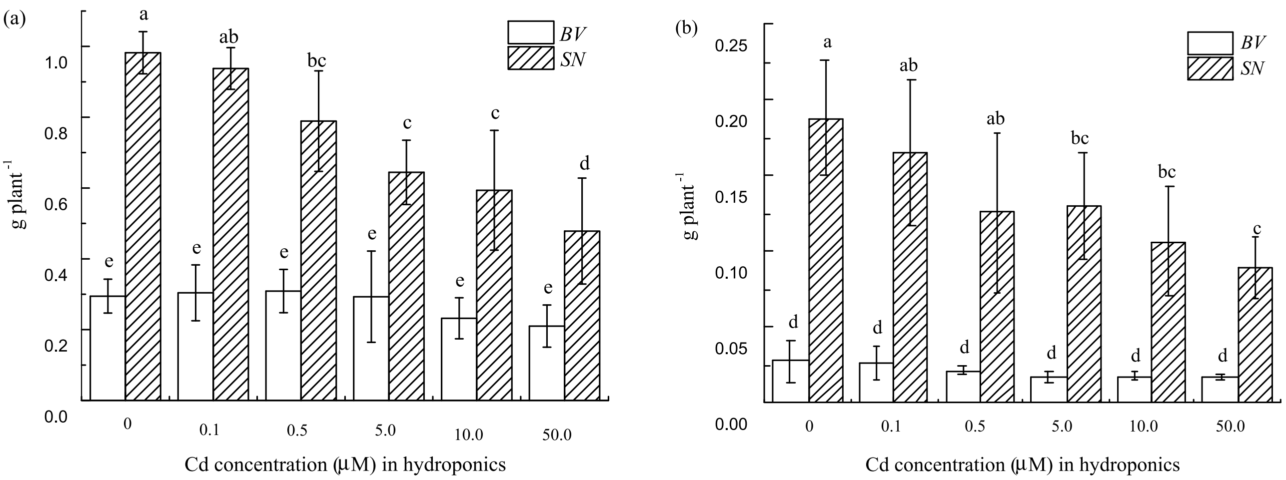

Fig. 1. Plant dry biomass (g plant^{-1}) of *Solanum nigrum* L. and *Beta vulgaris* L. *var. cicla* L. grown in hydroponics treated with different cadmium concentrations (μM). (a) Shoots, (b) Roots, (*BV*) *Beta vulgaris* L. *var. cicla* L., (*SN*) *Solanum nigrum* L. Error bars represent the standard deviations (n = 3). Different letters above each column indicate significantly different means ($p<0.05$) by the least significant difference (LSD) test.

Cd Concentrations in Plants

The concentrations of Cd in the tissues of *SN* and *BV* were much higher than their corresponding concentrations in the nutriment solution (Figs. 2a-2b). This indicates that these two plants accumulated Cd from the nutriment solution, indicating that the conditions were appropriate for the removal of Cd from water [23]. The potential of a plant to be used for phytoremediation is dependent on the general characteristics of the plant. The hyperaccumulators are generally recognised as the most effective phytoremediation plants. The criteria for Cd hyperaccumulars are Cd content>100 mg kg^{-1}, enrichment factor (concentration in plant/habitat)>1, and a translocation factor (Cd concentration in shoots/roots)>1, and high tolerance to Cd [18, 19]. In soil culture experiments, both *SN* and *BV* were able to meet the above criteria for a hyperaccumulator with a Cd treatment of 20 mg kg^{-1} [13, 14].

In the hydroponics, these two plants also showed a strong Cd accumulation ability, as presented in Figs. 2a-2b. The Cd concentration in the control plants was generally below or near the detection limit and is therefore not shown in Figs. 2a-2b. The Cd concentration in the two plants markedly increased with increasing Cd concentrations in the nutrient solution, but the magnitude of Cd varied among the plant parts and species. In *BV*, the Cd concentrations increased from 6.9 to 314.7 mg kg^{-1} in the shoots and 6.9 to 4547.9 mg kg^{-1} in the roots when the Cd concentration in the nutrient solution increased from 0.1 to 50 μM (Fig. 2). The corresponding increase in *SN* was from 2.8 to 100.6 mg kg^{-1} in the shoots and 3.1 to 2010.7 mg kg^{-1} in the roots. The shoots and the roots of

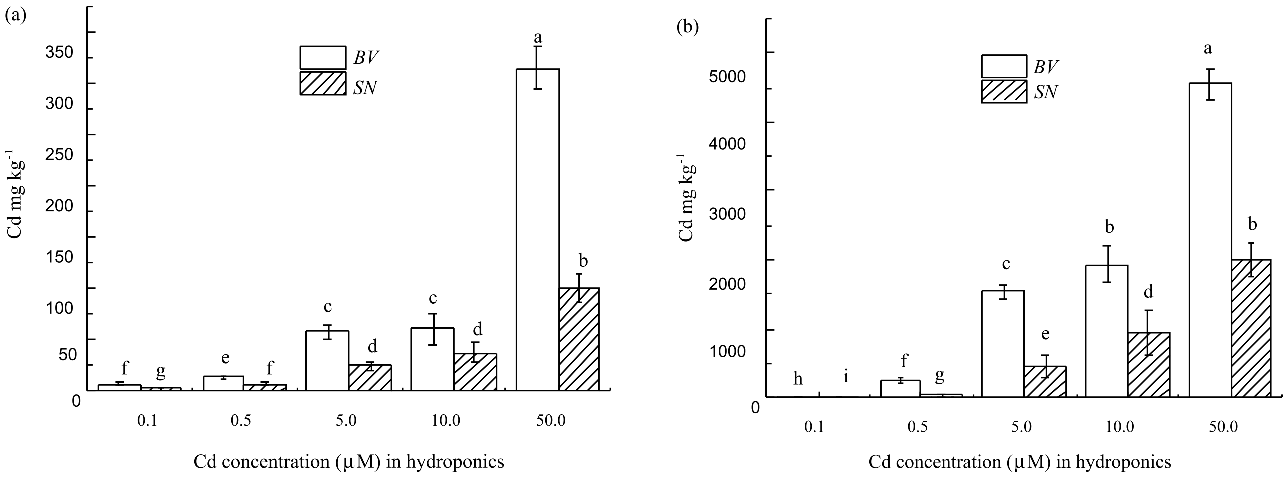

Fig. 2. Cd concentrations in dry plant tissues (mg kg^{-1}) of *Solanum nigrum* L. and *Beta vulgaris* L. *var. cicla* L. grown in hydroponics and treated with different Cd concentrations (μM). Error bars represent the standard deviatos (n = 3). (a) Shoots, (b) Roots. (*BV*) *Beta vulgaris* L. *var. cicla* L., (*SN*) *Solanum nigrum* L. Different letters above each column indicate significantly different means ($p<0.05$) by the least significant difference (LSD) test.

BV accumulated higher concentrations of Cd compared to those of *SN,* indicating its higher Cd uptake and efficient transport to the shoots.

Enrichment Factor (EF) and Translocation Factor (TF) of Plants

The enrichment factor (EF), which measures the degree of metal transfer from the water to the plant roots and shoots for Cd, was comparatively assessed, and the results are presented in Table 1. Generally, the EF was much greater than 1, which is required for phytoremediation plants. Moreover, the EF in the hydroponics was much greater than that in the soil cultures, which indicated the plants' high ability to intercept, absorb, and accumulate metals in both their roots and shoots in hydroponic conditions. Previous studies have reported that the success of phytoextraction of metals depends on factors such as the degree of contamination, the plant's ability to absorb and accumulate metals, the metal availability for uptake into the rots and, ultimately, the interaction between the plant habitat, the metals, and the plant [24]. The high bioavailability of the metals that are already in the aqueous phase for easy uptake by the plant and a favourable interaction between the metals, the plant, and the aquatic habitat where the plant grows are important factors [23].

Another important feature of phytoremediation plants is their ability to translocate the metals from the root to the shoot. The translocation factors of the plants are also presented in Table 1. At a Cd concentration of 0.1 μM, the TF of *BV* and *SN* were 1.0 and 0.9, respectively. However, the TF of the plants decreased substantially with the increasing concentration of Cd in hydroponics. This result indicated the lower plant's ability to translocate Cd from the roots to the shoots in hydroponics with higher Cd levels. Low translocation of metal from roots to shoots also was observed in many hydroponic studies [10, 11, 25]. This may partly be an artifact of the short-term exposure to metals because several days of metal exposure in hydroponic systems may not be enough to permit metal accumulation in aerial tissues [10].

Table 1. Enrichment factor (EF) and translocation factor (TF) of plants grown in hydroponics treated with different Cd concentrations (μM). (*BV*) *Beta vulgaris* L. *var. cicla* L., (*SN*) S*olanum nigrum* L.

Cd in hydroponic (μM)	*BV*			*SN*		
	EF (shoot)	EF (root)	TF	EF (shoot)	EF (root)	TF
0.1	613.7	615.0	1.00	244.7	273.0	0.90
0.5	252.0	4289.4	0.06	131.0	538.2	0.24
5	102.6	2757.2	0.04	43. 9	821.0	0.05
10	54.0	1721.5	0.03	33.6	849.9	0.04
50	56.0	809.2	0.07	17.9	357.8	0.05

Cd Removal by Plants

Cd removal by the two plants (%) was calculated as the product of the Cd concentration (mg kg^{-1}), the biomass (kg plant^{-1}), and the Cd content in the hydroponic solution, as shown in Table 2. Extracted Cd by both plants and plant parts varied significantly among the Cd treatments. In *BV*, the shoots extracted 73.4% of the total solution Cd in the 0.1 μM Cd treatment. However, for treatments of 0.5 μM to 50 μM Cd in the solution, a higher percentage of Cd was found in the roots. Similar to *BV*, the Cd proportion in the roots of *SN* was also first increased and then decreased with the increasing Cd concentration in the nutrient solution. The results suggested that the transfer of Cd from the roots to the shoots was less efficient in the solutions with a higher Cd concentration. We clearly observed that the roots were the major sinks of Cd accumulation in the hydroponics with high Cd levels. The root system of the plants acts as a powerful sampling mechanism as they collect solutions from a large volume of moist ground. Metals contained in the solutions are usually deposited in the plants' bodies. The higher proportion of Cd in the roots may be due to the immobilisation of Cd through precipitation and/or adsorption on the root surface and within the symplasm of root cells, as well as due to sequestration of Cd by phytochelatins in the vacuoles of root cells [26].

As presented in Table 2, at lower Cd concentrations in the solution the Cd uptake had a high ratio, and the uptake ratio was low at very high concentrations of Cd in the solution. Both *BV* and *SN* had the hihest Cd-extracted efficiencies in

Table 2. Cd removal (%) by *Beta vulgaris* L. *var. cicla* L. and *Solanum nigrum* L. grown in hydroponics treated with different cadmium concentrations (μM).

Cd in hydroponic (μM)	*BV* Extracted Cd (%)			*SN* Extracted Cd (%)		
	Shoots	Roots	Total	Shoots	Roots	Total
0.1	73.4±8.4	7.8±2.8	81.1±10.3	78.4±5.7	16.8±1.5	95.1±6.7
0.5	39.8±19.3	46.4±9.3	86.2±18.7	58.4±5.8	40.7±5.5	99.1±8.6
5	14.7±5.6	24.1±5.8	38.8±10.6	12.8±2.6	54.7±2.2	67.4±4.4
10	6.0±0.1	15.5±2.7	21.5±2.8	13.0±1.9	48.3±6.5	61.2±4.8
50	5.9±1.5	6.9±0.6	12.7±1.1	4.2±0.7	16.0±4.3	20.2±5.0

(*BV*) *Beta vulgaris* L. *var.cicla* L., (*SN*) *Solanum nigrum* L. Data are expressed as mean values ± standard deviation, n = 3.

the treatment with 0.5 μM Cd, and the efficiency decreased with the Cd supply levels. The Cd extraction efficiency of *BV* and *SN* were, respectively, 86.2% and 99.1% at a concentration of 0.5 μM Cd, while the corresponding values for a Cd concentration of 50 μM ere 12.7 % and 20.2%, respectively. Generally, *SN* was more efficient than *BV* for Cd extraction due to its higher biomass.

Conclusions

In this study, *Solanum nigrum* L. (*SN*) and *Beta vulgaris* L. *var. cicla* L. (*BV*) were found to accumulate Cd in both their roots and shoots to a high degree. Therefore, these two plants could serve as effective phytoextraction plants. In hydroponics, the roots of the plants were the major sinks of Cd accumulation. Generally, *BV* showed a higher tolerance and accumulation of Cd, while *SN* was more efficient for Cd removal due to its higher biomass.

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