Original Research

Experimental Studies on the Potential of Two Terrestrial Mosses in Monitoring Water Contaminated by Cd and Cr

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Received: 19 December 2011
Accepted: 11 April 2012

Abstract

The terrestrial mosses *Hypnum hamulosum* and *Brachythecium brotheri*, which could live in water under experimental conditions, were treated with Cd2^{+} at 0, 20, 50, 100, 150, and 200 mg·L^{-1}, and Cr^{3+} at 0, 10, 20, 30, 50, and 100 mg·L^{-1}. The Cd^{2+} and Cr^{3+} contents in *H. hamulosum* and *B. brotheri* were measured by ICP-AES after they had been submerged in the treatment solution for 7, 14, 21, and 28 days.
The results showed:

(1) Both moss species have a high ability in absorb Cd and Cr.
(2) With the increase of Cd^{2+} (or Cr3^{+}) concentration in the culture solution, the Cd (or Cr) contents both in *H. hamulosum* and *B. brotheri* increased linearly
(3) With the extension of the treatment time, their Cd and Cr content increased logarithmically.
(4) *Hyphnum hamulosum* and *B. brotheri* differ in their ability to absorb Cd^{2+} and Cr^{3+}; the former has a higher ability to absorb Cd^{2+}, but lower ability to absorb Cr^{3+} than the latter.

Keywords: bryophyte, cadmium, chromium, water quality monitoring

Introduction

Bryophytes have been widely used in monitoring environmental pollution in the world due to their specific structure and physiological characters [1, 2]. Aquatic mosses have been widely used to assess water quality in different areas and countries such as Belgium [3, 4], Chile [5], Dominica [6], England [7], France [8], North America [9], Portugal [10, 11], and Spain[12]. At present, the mosses used for monitoring water pollution are mostly aquatic and hydric species, including *Platyhypnidium riparioides* (Hedw.) Dixon [12, 13], *Fontinalis antipyretica* Hedw. [10, 14-16], *Fontinalis dalecarlica* Bruch & Schimp. [10, 14, 17], *Fontinalis squamosa* Hedw., *Amblystegium riparium* (Hedw.) Schimp. [7], and *Vittia pachvloma* (Mont.) Ochyra [5].

Shanghai, the biggest city in China, and its adjacent regions have large areas of freshwater as rivers and lakes. Water quality varies greatly among different environments in the studied region owing to local urbanization and industrialization, and water pollution has become more and more severe. We tried to select suitable mosses for monitoring water pollution. The climate of the Shanghai area is subtropical and thus mosses that have been used for monitoring of environmental pollution in other areas and countries, mostly in temperate climates, were not suitable. Therefore, we selected two terrestrial mosses, *Hypnum hamulosum* Froel. and *Brachythecium brother* Paris, to conduct the present research.

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Hypnum hamulosum is widely distributed from northern to southern provinces such as Anhui, Guizhou, Hebei, Heilongjiang, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Jilin, Nei Mongol, Shaanxi, Sichuan, Xinjiang, Yunnan, and Zhejiang in China, and also is found in Russia, Europe, and North America [17]. *Brachythecium brotheri* is recorded from Chongqing, Shaanxi, Yunnan, and Shanghai in China as well as from Japan [18]. In trials, both remained healthy after immersion in fresh water for 30 days and were chosen to investigate their potential as bioindicators. The purpose of this paper is to determine the efficacy of *H. hamulosum* and *B. brotheri* in absorbing Cd and Cr in water, and to elucidate their potential for monitoring aquatic heavy metal pollution and environmental remediation.

Materials and Methods

Experimental Material

Hypnum hamulosum and *Brachythecium brotheri* were collected from eastern China on March 12-13, 2009; the former was collected from hillside ground in Mt. Beishan (29º13'19" N, 119º37'56"E, elevation ca. 1180 m) in the suburb of Jinhua city, Zhejiang province, and the latter from the grounds of the botanical garden (31º09'51"N, 121º24'50"E, elevation 3 m) on the campus of Shanghai Normal University. The mosses were cleaned and dried by ventilation for the following experiment.

Voucher specimens in the Herbarium of Shanghai Normal University (SHNU) include: *Hypnum hamulosum* – Zhejiang, Jinhua, Beishan Mt. 12 Mar. 2009, Guo Shuiliang 20090312001, 20090312002; and *Brachythecium brotheri* – Shanghai, the botanical garden of Shanghai Normal University, 13 Mar. 2009, Guo Shuiliang 20090313001.

Three g dry gametophyte material was put into a nylon bag (12 cm high, diameter 6 cm, mesh 5 mm in diameter). A total of 144 moss bags were made for each moss species. To determine the influences of Cd concentration and submersion time on Cd content of the mosses, 24 plastic buckets (each with a volume of 12 L) were prepared for the treatment. Each bucket contained 10 L treatment solution (10% Knop nutrient solution with cadmium chloride). The 24 buckets were divided into four groups. Each group contains six buckets, with cadmium chloride at concentrations of Cd^{2+} ($mg \cdot L^{-1}$, calculated with pure Cd) at 0, 20, 50, 100, 150, and 200 $mg \cdot L^{-1}$, respectively. Six moss bags (three for *B. brotheri*, three for *H. hamulosum*) were submerged into the treatment solution in each bucket. The submersion time of the mosses in groups 1, 2, 3, and 4 is 7, 14, 21, and 28 days, respectively.

The experiment with Cr was designed in the same way as that with Cd, only the concentration of Cr^{3+} ($mg \cdot L^{-1}$) using Chromium chloride was 0, 10, 20, 30, 50, and 100 $mg \cdot L^{-1}$.

During the experiment, the buckets with the treatment solutions were kept in an air-conditioned chamber at 20ºC with a 12-h light (ca. 200 $\mu mol\ m^{-2} \cdot s^{-1}$) / 12-h dark regime.

Determine Method

Treatment of Moss Plants

Hypnum hamulosum and *B. brotheri* treated with heavy metal Cd (or Cr) were removed with a wooden clamp and cleaned by tap water（note: the concentrations of Ca, Cd, and Cr are 29.1500 $mg \cdot L^{-1}$, 0.0450 $mg \cdot L^{-1}$, 0.0082 $mg \cdot L^{-1}$, respectively), then washed by distilled water three times (The concentrations of Cd and Cr in the distilled water were too low to be determined by ICP-AES). The cleaned moss material was put in clean cultural containers in an electric blast oven and dried at 80ºC for 12 hours until it reached constant weight. The moss material was ground into powder and kept in a clean, dry, plastic bag.

Nitrification Treatment of Samples

Cleaned and dried moss material (0.6 g) was accurately weighed and digested in $HClO_4$ and HNO_3 ($HClO_4$:HNO_3 = 1:4) for about 48 h. The filtered solution was dried on a hotplate and the resulting white powder dissolved in distilled water and made up to 25 ml in a volumetric flask for chemical analysis.

Chemical Analysis of the Samples

The heavy metal content of the moss material was determined with Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) [19].

Data Analysis

The content of Cd (or Cr) of the three moss bags in the same bucket for the same species was averaged, and the data used to analyze the relationship of the content of Cd (or Cr) in the moss with the treatment concentration and treatment time.

The Cd and Cr content absorbed in *H. hamulosum* and *B. brotheri* were determined and the enrichment coefficient of heavy metal = average content of one element in the plant/mean content of the element in the treatment solution.

Results

Background Content of Cd^{2+} and Cr^{3+} in *B. brotheri* and *H. hamulosum*

The mean value from three sample analyses for background content of Cd^{2+} and Cr^{3+} in *B. brotheri* was determined to be 1.2845 $mg \cdot kg^{-1}$ and 1.2555 $mg \cdot kg^{-1}$, respectively, while that of *H. hamulosum* was 1.7100 $mg \cdot kg^{-1}$ and 1.6845 $mg \cdot kg^{-1}$, respectively. Both mosses thus have low background values of Cd^{2+} and Cr^{3+}.

Influence of Cd^{2+} and Cr^{3+} Concentrations and Treatment Times on Metal Content in *B. brotheri*

The regression equations reveal the relationship of Cd content in *B. brotheri* with Cd^{2+} concentration and are shown in Fig. 1, and those with the length-of-treatment time in Fig. 2.

Fig. 1 shows that with the increase of Cd^{2+} concentration in the solution, *B. brotheri* absorbs Cd^{2+} linearly during the same length of treatment time, and Fig. 2 shows that the species absorbs Cd^{2+} logarithmically with extension of the treatment time at the same Cd^{2+} concentration, indicating that Cd content of *B. brotheri* gradually approaches a saturation value with the extension of the treatment time.

The change of Cr content in *B. brotheri* with the increase of Cr^{3+} in the solution, or with the extension of treatment time is similar to that of Cd and is shown in Figs. 3 and 4, respectively.

B. brotheri has a high enrichment capacity for Cr; all the coefficients are higher than 3 (Table 1). The Cr enrichment coefficient of *B. brotheri* increased logarithmically with the increase of Cr^{3+} concentration in the solution. With the exception of the 10 mg·L^{-1} Cr^{3+} treatment, and increasing submergence time in the Cr^{3+} solution, the enrichment coefficient for Cr in *B. brotheri* also increased logarithmically.

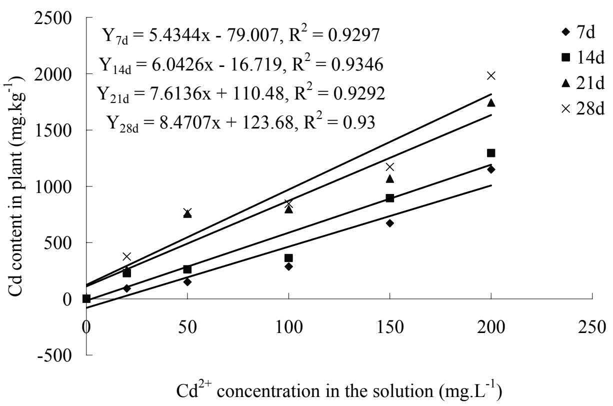

Fig. 1. Change of Cd content in *B. brotheri* treated with Cd^{2+} at six concentrations for 7, 14, 21, and 28 days, respectively, revealing the influence of treatment Cd^{2+} concentrations on Cd content in the plant.

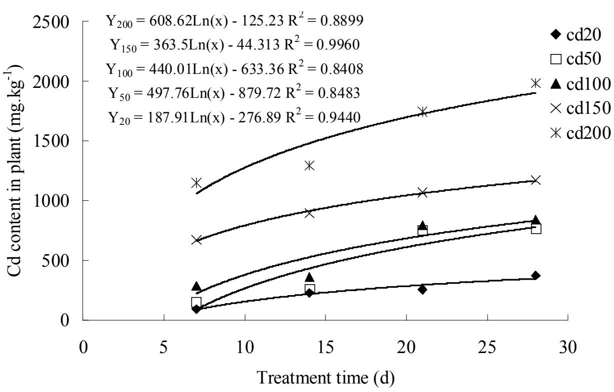

Fig. 2. Change of Cd content in *B. brotheri* treated with Cd^{2+} at five concentrations for 7, 14, 21, and 28 days.

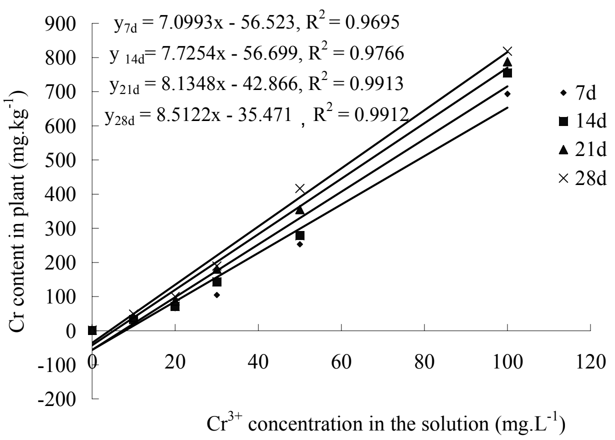

Fig. 3. Change of Cr content in *B. brotheri* treated with Cr^{3+} at six concentrations for 7, 14, 21, and 28 days.

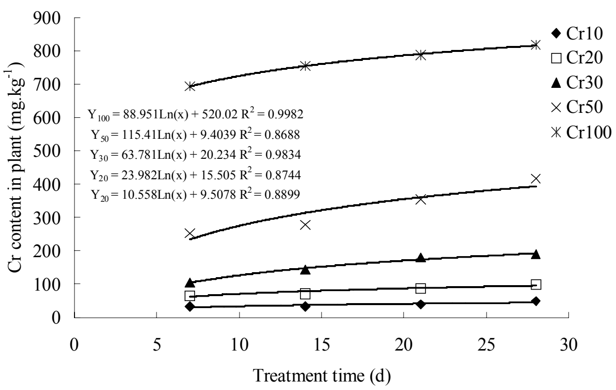

Fig. 4. Change of Cr content in *B. brotheri* treated at five Cr^{3+} concentrations for 7, 14, 21, and 28 days.

Table 2 shows that *B. brotheri* also has a high enrichment capacity for Cd, the enrichment coefficient is as high as 18.7 after being cultured in Cd^{2+} solution at 20 mg·L^{-1} for 21 days. With the extension of the submerged time in the solution, the enrichment coefficient of *B. brotheri* linearly increased, as shown in Table 2. With the increase of the treatment concentration of Cd^{2+}, the enrichment coefficient of *B. brotheri* first decreased, then increased, but there was no apparent trend change compared to the Cr^{3+} response (Table 2).

Influence of Cd^{2+} or Cr^{3+} at Different Concentrations and Treatment Times on Metal Content in *H. hamulosum*

The changes of Cd content in *H. hamulosum* treated with Cd^{2+} at six different concentrations for 7, 14, 21, and 28 days are shown in Figs. 5 and 6. As for *B. brotheri*, increasing the Cd^{2+} concentration results in absorbance of Cd linearly (Fig. 5), and with the extension of treatment time at the same Cd^{2+} concentration, the Cd content of *H. hamulosum* gradually increased logarithmically (Fig. 6).

Table 1. Cr enrichment coefficients (Y) of *B. brotheri* treated with Cr^{3+} at five concentrations (x_1) for different lengths of treatment time (x_2) ($mg \cdot kg^{-1}$).

	Treatment time				
	7d	14d	21d	28d	
10	3.29	3.26	4.89	3.93	—
20	3.30	3.58	5.00	4.37	Y_{20}=1.2018Ln(x_2)+0.769, R^2=0.876
30	3.49	4.78	6.31	6.02	Y_{30}=2.1282Ln(x_2)-0.6822, R^2 = 0.9831
50	5.06	5.57	8.33	7.10	Y_{50}=2.3093Ln(x_2)+0.1865, R^2=0.8699
100	6.94	7.55	8.19	7.88	Y_{100}=0.8889Ln(x_2)+5.2041, R^2=0.9982
Relation between enrichment coefficient (Y) and Cr concentration (x_1)	Y_{7d}=1.6458*	Y_{14d}=1.9068*	Y_{21d}=1.8841*	Y_{28d}=1.7368*	
	Ln(x_1)-1.2509	Ln(x_1)-1.6179	Ln(x_1)-0.6278	Ln(x_1)+0.5635	
	R^2=0.8195	R^2=0.9384	R^2=0.9414	R^2=0.8371	

Table 2. Cd enrichment coefficient (Y) of *B. brotheri* treated with Cd^{2+} at five concentrations for different lengths of treatment time (x) ($mg \cdot kg^{-1}$).

	Treatment time				
	7d	14d	21d	28d	
20	4.62	11.39	18.77	12.83	Y_{20}=0.627x+0.93, R^2=0.9506
50	3.00	5.23	15.40	15.12	Y_{50}=0.6727x-2.085, R^2=0.8748
100	2.87	3.62	8.44	7.96	Y_{100}=0.3007x+0.46, R^2=0.888
150	4.48	5.96	7.82	7.12	Y_{150}=0.1597x+3.55, R^2=0.9761
200	5.75	6.47	9.92	8.72	Y_{200}=0.2109x+4.025, R^2=0.9654

With the increase of Cr^{3+} concentration in the solution, the content of Cr in *H. hamulosum* increased linearly (Fig. 7), and with the extension of treatment time at the same concentration, Cr content in *H. hamulosum* increased logarithmically (Fig. 8), which is similar to the comparative treatment in *B. brotheri*.

With the extension of the submerged time in Cd^{2+} solution, the Cd enrichment coefficient of *H. hamulosum* linearly increased, as shown in Table 3, which is similar to that of *B. brotheri*. With increasing Cd^{2+} concentration, the enrichment coefficient did not change significantly (Table 3), which is different from that of *B. brotheri*.

With the increase of Cr^{3+} concentration, the Cr enrichment coefficient of *H. hamulosum* first increased and then decreased following a quadratic model as shown in Table 4. This differs from that of *B. brotheri*, where enrichment coefficient follows a logarithmic model. With the extension of treatment time in the Cr^{3+} solution, the coefficient linearly increased, except for the Cr^{3+} treatment at 100 $mg \cdot L^{-1}$, which followed a logarithmic model.

Discussion

Aquatic plants have been widely used to monitor and purify polluted water. Bryophytes differ from higher plants in the way they utilize mineral nutrients. They can absorb pollution elements directly through their surface because their leaves are composed of a single or only a few layers of cells and have large surface areas, and they lack the thick cuticle layer of the vascular plant leaf surface. Bryophytes appear not only sensitive to environmental change but may also efficiently absorb heavy metals in the water.

Bryophytes have strong ion exchange properties because of their special physiological structure and can pas-

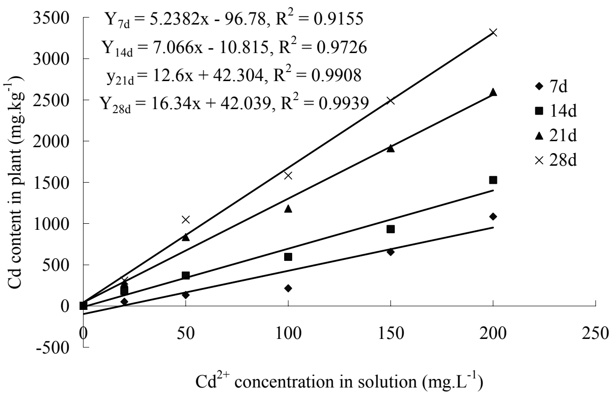

Fig. 5. Change of Cd content in *H. hamulosum* treated with Cd^{2+} at six concentrations for 7, 14, 21, and 28 days.

sively absorb the ions from the environment [20]. Moss plants start ion exchange in a few minutes without energy input after being put into a metal salt solution [21] because they lack a thick cuticle and lignified vascular tissue and can absorb ions directly over the leaf surface [22].

Our studies show that the uptake of Cd (or Cr) in these two mosses was closely related to the concentration of the relevant element in the treatment solution and could be modeled using a linear equation. Both *H. hamulosum* and *B. brotheri* have great ability to absorb Cd and Cr.

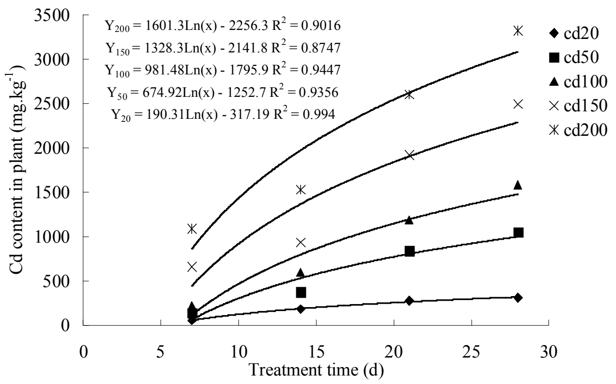

Fig. 6. Change of Cd content in *H. hamulosum* treated with Cd^{2+} at five concentrations for 7, 14, 21, and 28 days.

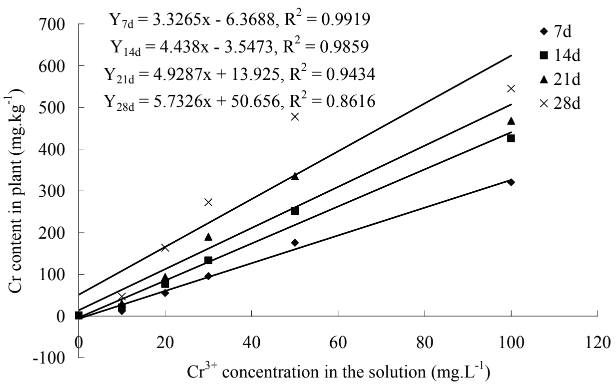

Fig. 7. Change of Cr content in *H. hamulosum* treated with Cr^{2+} at five concentrations for 7, 14, 21, and 28 days.

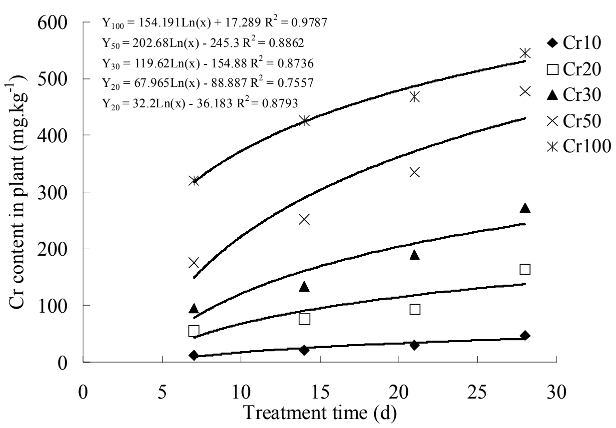

Fig. 8. Change of Cr content in *H. hamulosum* treated with Cr^{3+} at five concentrations for 7, 14, 21, and 28 days.

When cultured in Cd^{2+} solution at 200 mg·L^{-1} for 14 days, *H. hamulosum* and *B. brotheri* could absorb as much as 3317.84 mg·kg^{-1} and 1983.52 mg·kg^{-1}, respectively. When cultured in Cr^{3+} solution at 100 mg·L^{-1} for 14 days, *H. hamulosum* and *B. brotheri* could absorb as much as 545.10 mg·kg^{-1} and 818.76 mg·kg^{-1}, respectively, and both their Cd and Cr enrichment coefficients are larger than 3. *Hypnum hamulosum* and *B. brotheri* have different abilities to absorb Cd^{2+} and Cr^{3+}; the former a higher ability to absorb Cd^{2+}, but a lower ability to absorb Cr^{3+} than the latter. Moreover, their enrichment coefficients of Cd and Cr increased with the increasing concentration of Cd and Cr, which means that these two mosses have a potential in the remediation of aquatic environments contaminated by Cd and Cr.

Cadmium is a non-essential element for humans and is known as one of the most hazardous heavy metals [23]. The normal range of Cd concentration in leaf tissue (dry weight) of some plant species is 0.05-0.2 mg·kg^{-1} [24]. The standard Cd content of a hyperaccumulation plant is higher than 100 mg·kg^{-1} [25]. Under some conditions, the Cd content of *H. hamulosum* and *B. brotheri* could reach as high as 3317.84 and 1983.52 mg·kg^{-1}, respectively, their enrichment coefficient being 16.58 and 9.92, respectively. Therefore, these two mosses meet the criterion of hyperaccumulation plants according to their ability to absorb the element.

Wei et al. [26] found that *Hypnum revolutum* has great ability in absorbing Cd and Pb. The content of Pb and Cd in plants increased with the increasing pollution concentration either in single or combined treatments, especially under high Cd concentration pollution. The Cd content of *Hypnum revolutum* also reached 27480 mg·kg^{-1}, which is similar to that of *H. hamulosum* in this study.

The criterion for Cr^{3+} content of a hyperaccumulation plant is over 1000 mg·kg^{-1}, and only three Cr hyperaccumulation plant species have been reported in the world [27]. Among these, *Leersia hexandra* Sw (Poaceae), reported from Guangxi near an electroplate factory, is an aquatic Cr hyperaccumulator, its Cr content reaching 1786.9 mg·kg^{-1} [28]. Under some conditions, the Cr content of *B. brotheri* and *H. hamulosum* reached 818.76 and 545.1 mg·kg^{-1}, respectively. Though lower than the hyperaccumulation criterion, they have the possibility to be used in the monitoring and remediation of aquatic environments contaminated by Cr.

There are other criteria of hyperaccumulation plants:

(1) heavy metal content of the above-ground part of a plant is 100 times that of other common plants in the same living conditions according to natural concentrations of different elements in the soil and plants
(2) plant shows no obvious injury symptoms and its enrichment coefficient is larger than 1.

An ideal hyperaccumulator species should possess such characters as rapid growth and large biomass, and accumulate two or more elements at the same time [29, 30]. Because the enrichment coefficient of Cr^{3+} and Cd^{2+} in *H. hamulosum* and *B. brotheri* under most experimental conditions exceeded 3 (some as high as 18.7 in this study), these two mosses could be regarded as hyperaccumumla-

Table 3. Cd enrichment coefficient of *H. hamulosum* treated with Cd^{2+} at five concentrations for different time lengths. (mg·kg^{-1}).

	Treatment time				
	7d	14d	21d	28d	
20	2.63	9.05	13.74	15.45	Y_{20}=0.6164x-0.57, R^2=0.9430
50	2.65	7.41	16.72	20.97	Y_{50}=0.9181x-4.13, R^2=0.9778
100	2.15	5.96	11.82	15.82	Y_{100}=0.6696x-2.78, R^2=0.993
150	4.37	6.22	12.76	16.60	Y_{150}=0.6176x-0.82, R^2=0.9617
200	5.43	7.64	12.99	16.59	Y_{200}=0.5547x-0.955, R^2=0.9782

Table 4. Cr enrichment coefficient of *H. hamulosum* treated with Cr^{3+} at five concentrations for different days. (mg·kg^{-1}).

	Treatment time				
	7d	14d	21d	28d	
10	1.20	2.08	2.99	4.68	Y_{10} = 0.1621x_2-0.1, R2 = 0.971
20	2.77	3.83	4.68	8.19	Y_{20}=0.2444x_2+0.59, R2=0.8844
30	3.18	4.46	6.33	9.09	Y_{30}=0.28x_2+0.865, R2 = 0.9721
50	3.51	5.04	6.71	9.56	Y_{50}=0.1621x_2-0.1, R2=0.9712
100	3.20.	4.25	4.68	5.45	Y_{100}= 3.89Ln(x_2)-7.0653, R2=0.9129
Relation between enrichment coefficient (Y) and Cr concentration (x_1)	Y_{7d}=-0.0014x_1^2+ 0.1424x_1+0.1617 R^2=0.9771	Y_{14d}= -0.0011x_1^2+ 0.1362x_1+1.1473 R^2 = 0.9287	Y_{21d}= -0.0016x_1^2+ 0.1945x_1+1.4246 R^2 = 0.9465	Y_{28d}= -0.0024x_1^2+ 0.2621x_1+3.0219 R^2 = 0.9074	

tion plants of Cd and Cr based on that criterion. In fact, *Scopelophila cataractae* (Mitt.) Broth. (Pottiaceae), a terrestrial moss, is able to completely remove Cu at a concentration of 100 mg·L^{-1} from wastewater by suspended cultivation [31]. In view of their low biomass and growth rates, *H. hamulosum* and *B. brotheri* are more useful in the assessment and monitoring of water contaminated by Cd and Cr than in their remediation of contaminated water. Both *H. hamulosum* and *B. brotheri* are useful in the assessment and monitoring of water contaminated by Cd and Cr.

Acknowledgements

We are grateful for financial support by the Science and Technology Commission of Shanghai Municipality, China (No. 10JC1412100; 11391901200, 08390513800), Shanghai Leading Academic Discipline Project No. S30406 and Leading Academic Discipline Project of Shanghai Municipal Education Commission, China (No. J50401).

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