



## Short communication

Phytoaccumulation of cadmium through *Azolla* from aqueous solutionCai-yun Tan<sup>a</sup>, Xiao-quan Shan<sup>b</sup>, Guo-zhong Xu<sup>c</sup>, Yu-Man Lin<sup>a</sup>, Zu-liang Chen<sup>a,\*</sup><sup>a</sup> School of Chemistry and Material Sciences, Fujian Normal University, Fuzhou 350007, China<sup>b</sup> Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, PO Box 2871, Beijing 100085, China<sup>c</sup> *Azolla* Research Center at Fujian Academy of Agricultural Science, Fuzhou 350013, China

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## ABSTRACT

*Azolla*, which is an aquatic fern, has proved to be effective in the uptake and accumulation of metals from polluted waters. *Azolla* spp., namely *A. microphylla* cv. MH3 and *A. caroliniana* Willd, were chosen as model plants so that Cd(II) could be accumulated from aqueous solution. An increase in uptake time and the concentration of Cd(II) in aqueous solution resulted in more Cd(II) accumulation in both species. Modified Michaelis–Menten equation was employed to describe the concentration-dependent kinetics of Cd(II) uptake through the roots of *A. microphylla* cv. MH3, and the values of  $K_m$  and  $V_{max}$  were found to be 0.23 mg/L and 16.49  $\mu\text{g}/(\text{g}\cdot\text{f}\cdot\text{wt}\cdot\text{h})$ , respectively. Cd(II) uptake by *A. microphylla* cv. MH3 occurs partly through Ca(II) channels and has the potential to be mediated by Zn(II) transporters.

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## 1. Introduction

Phytoremediation, such as constructed treatment wetlands, is a technology that has the potential to solve pollution caused by heavy metals (Williams, 2002; Brix and Arias, 2005; Maine et al., 2006; Upadhyaya et al., 2007). However, choosing a plant species that can remove metal ions from polluted water depends on three variables in the environment: plant growth, biomass, and metal levels (Williams, 2002). *Azolla*, a floating aquatic fern, is found worldwide and it grows in all kinds of freshwater and wastewater. *Azolla* has high biomass productivity and a remarkable capacity to concentrate elements, including toxic heavy metals (Bennicelli et al., 2004). *Azolla* is easy to harvest and desiccate compared to other aquatic plants. These traits make it an ideal candidate for phytoremediation of Cd-contaminated wastewater (Arora et al., 2006). It has recently received attention as a potential plant for phytoremediation of heavy metals from polluted water. *A. caroliniana* has the capacity for taking up Hg(II) and Cr(III) (Bennicelli et al., 2004). A mechanistic study indicated that both apoplastic adsorption and symplastic storage were responsible for the overall accumulation of Pb(II) in the leaf of *A. filiculoides* (Benaroya et al., 2004). Living *A. filiculoides* can be used to remove Pb(II), Cd(II), Ni(II) and Zn(II); the accumulation fit the first-order kinetic model well (Rakhsaee et al., 2006). *A. filiculoides* grown in a high concentration of Cd(II)

was able to accumulate Cd(II) (Sela et al., 1989). However, it is necessary to understand the metal accumulation mechanism in order to assess the potential phytoaccumulation of Cd(II) from contaminated waters using *Azolla* species.

The objective of this study was to examine whether *Azolla* is able to accumulate Cd(II) from aqueous solution. Hence, a hydroponic culture method was used to study the accumulation of Cd(II) by *A. microphylla* cv. MH3 and *A. caroliniana* Willd. The roles of Ca(II) channel, and Zn(II) transporters in the uptake of Cd(II) using *A. microphylla* cv. MH3 were studied.

## 2. Materials and methods

2.1. *Azolla* species collection, cultivation and selection

*A. microphylla* cv. MH3 and *A. caroliniana* Willd were obtained from the *Azolla* Research Center at the Fujian Academy of Agricultural Sciences (Fuzhou, China) and cultured in a nutrient solution: 1.0 mM  $\text{CaSO}_4$ , 1.6 mM  $\text{MgSO}_4$ , 0.7 mM  $\text{NaNO}_3$ , 0.3 mM  $\text{KH}_2\text{PO}_4$ , 0.3 mM KCl, and 10.0  $\mu\text{M}$  EDTA–Na, 10.0  $\mu\text{M}$   $\text{FeSO}_4$ , 24.3  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 7.7  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4$ , and 0.2  $\mu\text{M}$   $\text{ZnSO}_4$  for 3 days. The nutrient solution was buffered to pH 6.0 with 2 mM Mes–Tris. Two species were grown in a plant growth chamber with operating conditions consisting of 12 h light/12 h darkness and a temperature of 30/22 °C. Two species were cultured in the nutrient solution for 3 days, then transferred to a 1.0 mg/L Cd(II) uptake solution for 1 day, and harvested, rinsed with ice-cold distilled water thoroughly. Then they were desorbed with 5 mM ice-cold  $\text{CaCl}_2$  solution for

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40 min. Preliminary experiments indicated that desorption in 5 mM ice-cold  $\text{CaCl}_2$  solution for 40 min was enough to desorb Cd(II) adsorbed onto the cell wall of fern roots. The results are illustrated in Fig. 2(a). After desorption, two species were rinsed with ice-cold distilled water thoroughly again, excised into roots and leaves, dried at  $80^\circ\text{C}$  for 2 days, then the dried plant material and digested were weighed with  $\text{HNO}_3\text{--HClO}_4$  for final determination of Cd(II). A usable *Azolla* species was employed in the subsequent experiments and all tested experiments were carried out in triplicate.

## 2.2. Time course and kinetics of Cd(II) uptake by *A. microphylla* cv. MH3

*A. microphylla* cv. MH3 grown in the nutrient solutions for 3 days was transferred to 1.0 mg/L Cd(II) uptake solution. At different time intervals of 5, 10, 15, 20, 25, 30, 40, 60 min, and 2, 4, 8 h, *A. microphylla* cv. MH3 was harvested. The digestion and extraction of Cd(II) have been stated previously.

This experiment was performed to test *A. microphylla* cv. MH3 uptake of Cd(II) at different Cd(II) concentrations. *A. microphylla* cv. MH3 was incubated with fresh nutrient solution containing various concentrations of Cd(II) (0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 5.0 mg/L). After 20 min of uptake, they were harvested, desorbed in 5 mM ice-cold  $\text{CaCl}_2$  for 40 min, excised into roots and leaves, dried with paper tissue and finally digested to determine Cd(II).

## 2.3. Effect of Ca(II) channel blocker and Zn(II) deficiency on Cd(II) influx into *A. microphylla* cv. MH3

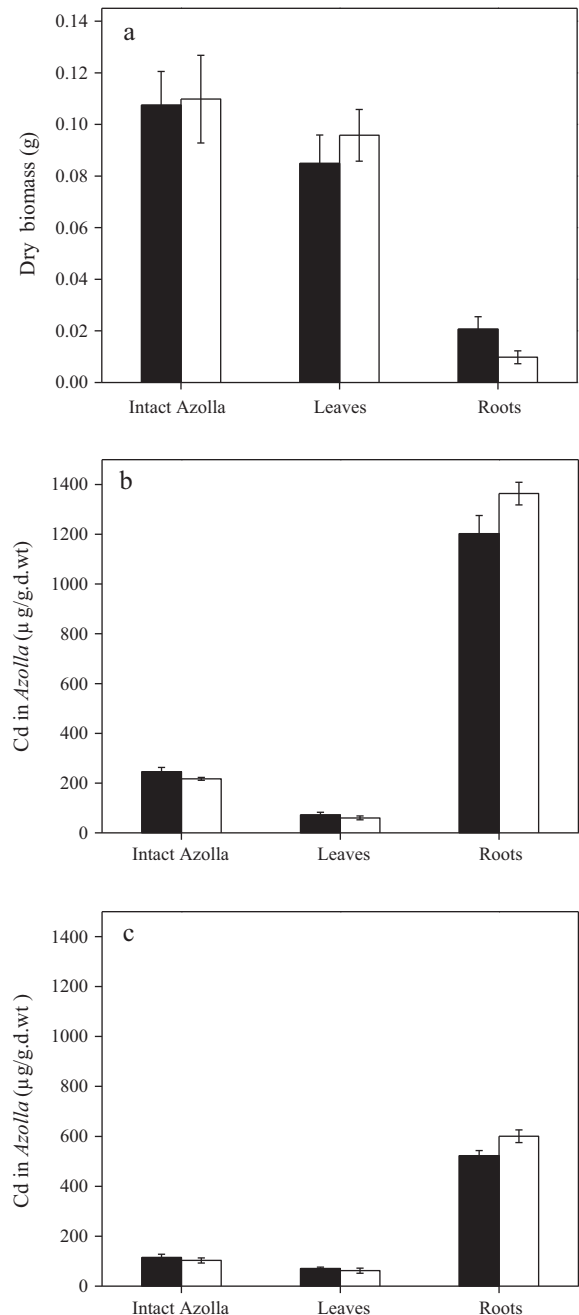
The experiments were performed to test the potential contribution of Ca(II) channels and Zn(II) transporters to Cd(II) influx into *A. microphylla* cv. MH3. A Ca(II) channel blocker, 0.2 mM  $\text{La}(\text{NO}_3)_3$ , was added to the Cd(II) uptake solution (White, 1997). Zn(II) deficiency was induced in *A. microphylla* cv. MH3 by replacing a full nutrient solution with a nutrient solution without Zn(II) for 3 days. Following this, *A. microphylla* cv. MH3 was incubated in a 1.0 mg/L Cd uptake solution without Zn(II). *A. microphylla* cv. MH3 grown in full nutrient solution and later incubated in 1.0 mg/L Cd(II) uptake solution with Zn(II) while a solution without  $\text{La}(\text{NO}_3)_3$  was used as control. After 4 h uptake, *A. microphylla* cv. MH3 was harvested and desorbed with 5 mM ice-cold  $\text{CaCl}_2$  for 40 min, excised into roots and leaves, and digested prior to Cd(II) determination.

Cadmium contents in the ferns were established using flame atomic absorption spectrometry (FAAS) (Varian AA240, Shanghai, China) after digestion of samples with  $\text{HNO}_3\text{--HClO}_4$  (3:1, v:v) on a hotplate. This continued until a clear solution or achromatous residue was obtained. After cooling, the residue was dissolved in 1%  $\text{HNO}_3$  prior to determining the amount of Cd(II). Cd contents in *Azolla* were calculated from firstly, the concentration and volume of the digested solution, and secondly, the plant's dry weight.

## 3. Results and discussion

### 3.1. Accumulation of Cd(II) by two *Azolla* species

Two species were used to compare the biomass and Cd(II) accumulation capability after culturing in the uptake solution for 1 day, and the results are shown in Fig. 1. In Fig. 1(a) there is no obvious difference in their dry biomass of the intact *Azolla* and leaves. Compared to *A. caroliniana* Willd (Fig. 1(c)), *A. microphylla* cv. MH3 roots have almost double the amount of biomass. As shown in Fig. 1(b), a much higher concentration of Cd(II) was found in the roots than in the leaves and intact ferns. For example, the Cd(II) contents in the roots of *A. caroliniana* Willd and *A. microphylla* cv. MH3 were



**Fig. 1.** A comparison of dry biomass of the intact *Azolla*, roots and leaves after culturing in the uptake solution for 1 day (a), Cd(II) contents in the intact *Azolla*, roots and leaves after Cd(II) uptake for 1 day without desorption (b), and after desorption with 5 mM ice-cold  $\text{CaCl}_2$  for 40 min (c). Black pillar for *A. microphylla* cv. MH3, and white pillar for *A. caroliniana* Willd.

1364 and 1206  $\mu\text{g/g}$ , respectively, while only 60 and 72  $\mu\text{g/g}$  were detected in the leaves of *A. caroliniana* Willd and *A. microphylla* cv. MH3, respectively. Those results suggested that neither species is a hyperaccumulator of Cd(II). This finding was supported by Kirkham (2006). Furthermore, after desorption in 5 mM ice-cold  $\text{CaCl}_2$  for 40 min, Cd(II) contents in the roots fell from 1364 to 600  $\mu\text{g/g}$  and from 1206 to 522  $\mu\text{g/g}$  for *A. caroliniana* Willd and *A. microphylla* cv. MH3, respectively. In contrast, the Cd(II) contents in the leaves remained virtually unchanged after desorption, implying that Cd(II) in the leaves was translocated from the roots, but not directly taken up by leaf surface cell walls (Fig. 2(a)). There is less

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