A preliminary study of the factors affecting the kinetics of cadmium uptake by the liverwort *Dumortiera hirsuta*

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Intra- and extracellular Cd uptakes by the liverwort *Dumortiera hirsuta* (SW) Nees. were investigated. While intracellular Cd uptake was linear with time and displayed Michaelis–Menten kinetics, extracellular Cd uptake displayed saturation kinetics. K_m and V_{max} were 149 ± 19 μM and 11.0 ± 0.8 μmol g^{-1} h^{-1} respectively. Intra-, and to a lesser extent, extracellular uptakes were temperature dependent. Light stimulated intracellular Cd uptake by *c.* 15%. The rate of intracellular Cd uptake in the dark was probably not directly linked to the supply of respirable reserves, because storage for 20 days in the dark did not affect it. Incubating the liverwort concurrently with Cd and equimolar concentrations of Ca, Mg or Zn all reduced intra- and extracellular Cd uptake, which suggests that the Cd uptake systems had low specificity. Pretreatment of plants with 80 mM KNO_3 stimulated Cd uptake, apparently because the K removed potentially competing ions from the cell wall. Results are compared with similar investigations on other cryptogams.

Keywords: Bryophytes, cadmium, heavy metal uptake, liverworts.

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Introduction

Acquisition of elements by bryophytes has received increased attention in recent years because of improved analytical techniques and the relevance of this work in pollution monitoring (Brown 1984). Terrestrial bryophytes acquire elements through both dry and wet deposition, and from the substratum (Brown & Bates 1990). However, the exact proportions originating from each source are unknown. Many workers argue that because bryophytes lack well-developed cuticles and elaborate rooting systems, most mineral uptake is from the atmosphere and not the substratum (Brown 1984). Where aerial deposition occurs, bryophytes trap much of the accumulated metal as particulate matter. The large extracellular cation exchange capacity of bryophytes may also provide the basis for explaining metal uptake (Brown 1984). Elements associated with bryophyte cells occur in four possible cellular locations: (a) trapped as particulate matter; (b) in an intercellular, soluble fraction; (c) extracellular, bound to exchange sites; and (d) intercellular.

While some laboratory investigations of heavy metal uptake by bryophytes measured only total uptake, Brown and his co-workers (e.g. Brown & Beckett 1985a; Wells & Brown 1990; Wells *et al.*, 1995) investigated the intracellular uptake of Cd by the grassland moss *Rhytidiadelphus squarrosus*. Although it may be numerically smaller than extracellular uptake, intracellular uptake is probably of major importance for plant survival. Elements located within the cell potentially have an immediate impact on metabolism, and may be of more biological importance to the bryophyte than elements held on external sites. At present, no information is available on the uptake of heavy metals by liverworts under laboratory conditions. The aim of this study was to compare the kinetics of Cd uptake by the thalloid liverwort *Dumortiera hirsuta* with the results obtained from studies on the moss *R. squarrosus*, and members of the lichen genus *Peltigera*.

Materials and Methods

Plant material

The thalloid liverwort *Dumortiera hirsuta* (SW) Nees: was collected from rocky boulders forming a water fall at Ferncliffe, Pietermaritzburg, KwaZulu Natal Province, Republic of South Africa. The material was washed with tap water to remove debris, and stored moist in a culture room under continuous fluorescent lighting (75 μmol m^{-2} s^{-1}) at 22 ± 1°C. The plant material was re-washed in deionized distilled water before use. Representative material was fixed in gluteraldehyde, embedded in resin and 1-μm sections were cut and stained with Ladd's multiple stain.

Metal uptake and determination of cellular location

Sufficient disks (10 mm in diameter) were cut for each experiment, and for each treatment randomly selected samples comprising 25 disks were incubated in 1 litre of 100 μM $CdSO_4$ at 20 ± 1°C, under sodium lamp lighting at 125 μmol m^{-2} s^{-1} (light saturating for photosynthesis). All the disks needed for an experiment were initially cut, then disks for a particular treatment were randomly sampled. Compressed air was continuously bubbled through the treatment solutions to prevent the formation of unstirred boundary layers. After 1 h the discs were filtered from the solution, divided into five replicates of five disks, and the cellular location of the Cd was determined using the method of Brown and Buck (1979). This involved washing the material in deionized distilled water (10 ml) for 0.5 h to displace intercellular Cd. Extracellular Cd was displaced by shaking the material twice with 20 mM $NiCl_2$ (10 ml) for 0.5 h each. The material was then shaken in 1 M HNO_3 for 1 h to displace intracellular Cd and finally washed in deionized distilled water for 0.5 h. The material was oven-dried at 80°C and weighed. All elements were determined by atomic absorption spectrophotometry in an air/acetylene flame.

The concentration of Cd required for half the maximum uptake rate (K_m) and the maximum uptake rate (V_{max}) were calculated by the method of Wilkinson (1961). The effect of dark storage on uptake was investigated by measuring uptake in plants stored on moist filter paper under continuous fluorescent lighting (75 μmol m^{-2} s^{-1}) or in the dark at 22 ± 1°C for 20 days.

The effect of K pretreatment on Cd uptake

This was tested by incubating thallus disks in 1 litre of deionized distilled water or 80 mM KNO_3. Disks were stored overnight in the

light on moist filter paper as described above, then Cd uptake was measured in K pretreated and untreated plants. The Ca and Mg concentrations of the nickel fractions (extracellular) were measured, and the additional Ca and Mg present in the uptake solutions in the non-K pretreated plants was deduced from the difference in concentrations. These amounts of Ca and Mg were then added to the uptake solutions of replicate water and K pretreated plants, and the Cd uptake was measured.

Toxicity of Cd to *Dumortiera*

In preliminary experiments, the toxicity of Cd to *D. hirsuta* was tested by measuring the effect of Cd on K leakage and photosynthesis. Discs (25 of 10-mm diameter) of liverwort thallus were incubated in 1 litre of deionized distilled water, 100 μM $CdSO_4$ or 300 μM $CdSO_4$ as described above. After 1 h the discs were filtered from the solution, divided into five replicates of five disks and gross photosynthesis was determined by the method of Brown *et al.* (1981). The extra- and intracellular concentrations of Cd and the intracellular concentration of K were then determined as described above. In addition, replicate samples of liverwort tissue were treated as above, and left for 24 h on moist filter paper under continuous fluorescent lighting (75 μmol m^{-2} s^{-1}) at 22 ± 1°C. Gross photosynthesis, the extra- and intracellular concentrations of Cd, and the intracellular concentration of K were determined as above.

Results and Discussion

Anatomy of *Dumortiera*

Figure 1 presents a vertical section through the thallus of *Dumortiera hirsuta*. Air chambers, filaments, pores and scales, found in members of the closely related genus *Marchantia*, were absent, in agreement with the description of the plant by Arnell (1963). The average thickness of the thallus was 0.25 mm or about eight cells thick. Chloroplasts were largely restricted to the smaller-celled upper and lower epidermis, and the thallus appeared almost isobilateral. The cuticle was extremely thin, suggesting that it offered little resistance to water and nutrient uptake.

Toxicity of Cd

Treatment of *D. hirsuta* for 1 h with 100 or 300 μM $CdSO_4$ did not affect photosynthesis or intracellular K concentration measured immediately after exposure (Table 1). However, after 24 h of storage following treatment, both Cd concentrations reduced intracellular K, and 300 μM $CdSO_4$ reduced photosynthesis. The reduction in photosynthesis was similar to that observed by Wells and Brown (1987) when they studied the effects of increasing concentrations of Cd on the moss *R. squarrosus*. Interestingly, during the 24 h of storage, intracellular Cd concentrations increased, while extracellular Cd concentrations decreased. This indicates that during storage Cd moved from the cell wall into the cell. Brown and Beckett (1985b) obtained similar results with a range of lichen species. The main conclusion, however, from the toxicity experiment was that Cd was not toxic to *D. hirsuta* during the 1 h over which Cd uptake was measured.

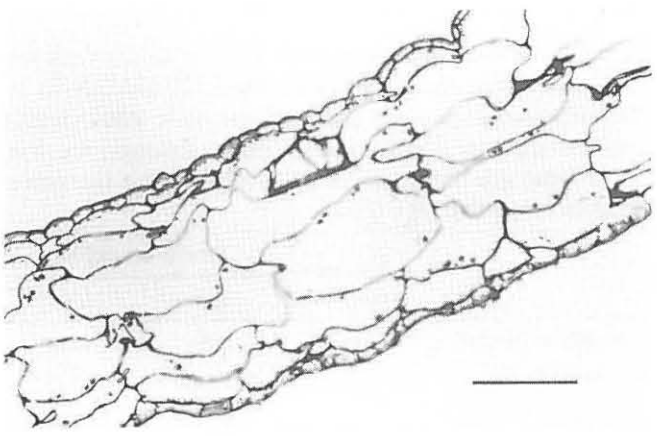


Figure 1 Vertical section through the thallus of *Dumortiera hirsuta*. Bar line = 100 μm.

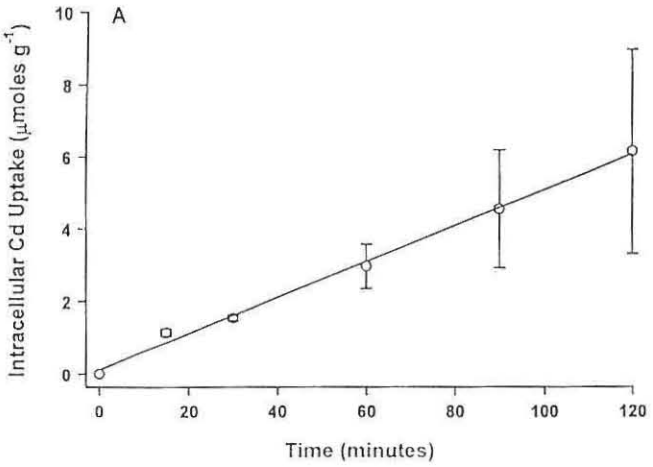
Cd uptake as a function of time

Using 20 mM $NiCl_2$, it was possible to differentiate between exchangeable cell wall-bound Cd (extracellular) and Cd remaining after displacement (intracellular). The rate of intracellular Cd uptake was linear with time (Figure 2a), suggesting that 20 mM $NiCl_2$ had effectively displaced extracellular Cd. The calculated rate of intracellular Cd uptake was 3.0 ± 0.3 μmol g^{-1} h^{-1}, but it varied between experiments, ranging from 1 to 10 μmol g^{-1} h^{-1}. No obvious reasons existed for this, (e.g. times of the year that plants were collected or the amount and nature of existing ions on the cell wall). In this study uptake rates are expressed based on the weight of the plant material following incubation in 1 M HNO_3, which removed the protoplasm and reduced pre-acid dry weights in all collections by *c.* 20%. The uptake rates reported here must be reduced by this factor when comparing results with those of Brown and his co-workers who usually calculated them using dry weights measured before acid treatment.

Intracellular Cd uptake rates were similar or higher to those reported by Beckett and Brown (1984a) for the lichen *Peltigera membranacea* (1.8 ± 0.2 μmol g^{-1} h^{-1}), and generally lower than those reported by Brown and Beckett (1985a) for the moss *R. squarrosus* (11.9 ± 2.3 μmol g^{-1} h^{-1}). The rate of extracellular Cd uptake declined with time (Figure 2b). Using a double-reciprocal transformation, a straight line could be fitted to extracellular Cd uptake as a function of time. The maximum extracellular Cd uptake was 64 ± 6 μmol g^{-1}, and the time required to reach half the maximum uptake was 26 ± 6 min. This was less than the 39 min reported by Beckett and Brown (1984a) for the lichen *P. membranacea*, but much more than the 4 min reported by Brown and Beckett (1985a) for the moss *R. squarrosus*. Brown and Beckett (1985a) concluded that the difference between the lichen and the moss was due to the multi-layered structure of the lichen.

Table 1 The toxicity of Cd to *Dumortiera hirsuta*. Plants were incubated in $CdSO_4$ solutions for 1 h and Cd uptake, intracellular K concentration and gross photosynthetic rate measured immediately or after storage for 24 h. Figures in each column followed by the same letter are not significantly different using Duncan's multiple range test ($P < 0.05$)

Cd concentration (μM)	Time of measurement after exposure to Cd (h)	Intracellular Cd uptake (μmol g^{-1})	Extracellular Cd uptake (μmol g^{-1})	Intracellular K after uptake and sequential elution (μmol g^{-1})	Gross photosynthetic rate (μmol CO_2 g^{-1} s^{-1})
0	0	0	0	16.4a	2.87bc
0	24	0	0	16.6a	3.31c
100	0	11.3 a	30.5b	15.3a	3.05bc
100	24	25.5c	17.6a	10.0b	2.45b
300	0	19.0b	32. 0b	16.7a	2.50 b
300	24	33.9d	18.4a	11.5b	1.56 a



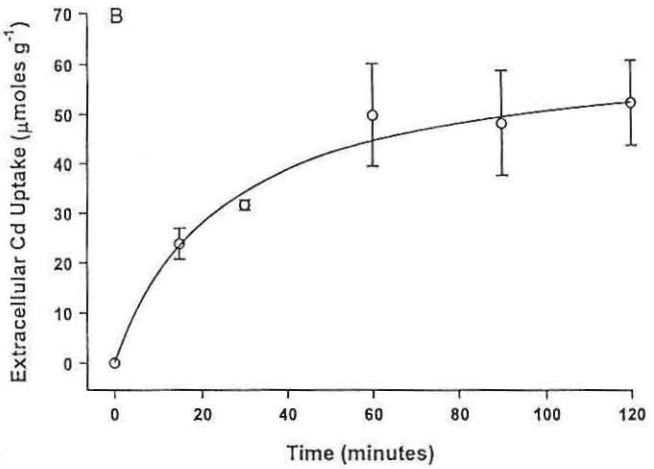
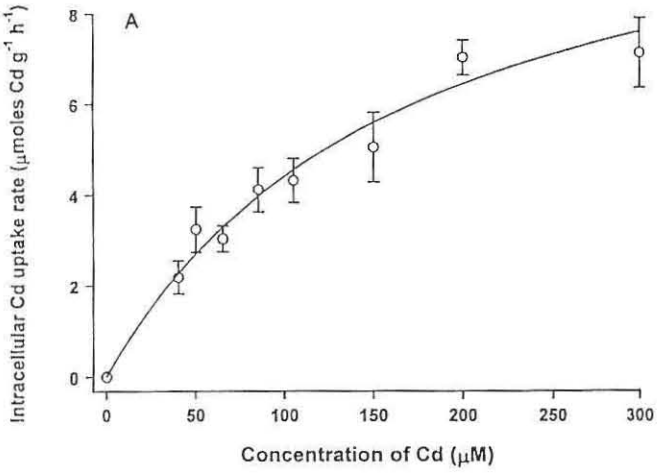


Figure 2 Intra- (A) and extracellular (B) Cd uptake from 100 μM $CdSO_4$ as a function of time in *Dumortiera hirsuta*. In this and all following graphs error bars represent 95% confidence limits.



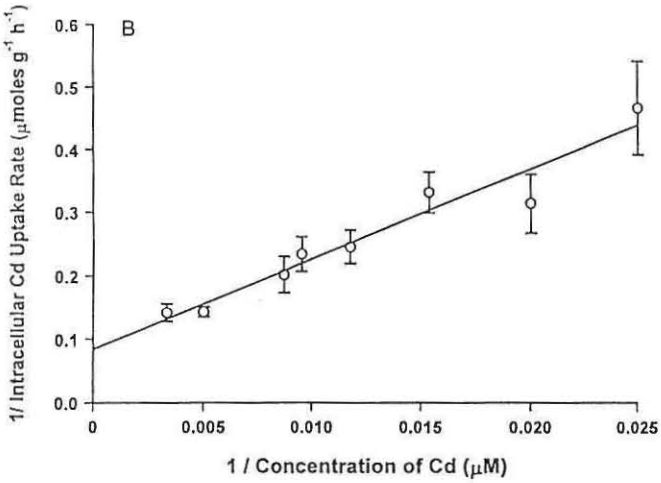


Figure 3 A, intracellular Cd uptake rate as a function of concentration, and B, double-reciprocal plot of intracellular Cd rate in *Dumortiera hirsuta*.

They argued that the structure of the lichen may have increased the size of the unstirred boundary layer through which metal ions must diffuse before being bound. The results of the present study possibly show that the liverwort *D. hirsuta*, which is on average eight cells thick, is intermediate in this regard between the moss *R. squarrosus* and the lichen *P. membranacea*. As for intracellular uptake, variation existed in extracellular uptake between collections. Experiments are currently being carried out to investigate this problem further, but it should be noted that Wells and Brown (1987) observed similar, but less extreme, variations in intra- and extracellular Cd uptake between different populations of the moss *R. squarrosus*.

Cd uptake as a function of concentration

Intracellular Cd uptake displayed typical Michaelis–Menten kinetics when measured over a range of Cd concentrations (Figure 3a). K_m was 149 ± 19 μM and V_{max} 11.0 ± 0.8 μmol g^{-1} h^{-1} (Figure 3b). K_m was high compared with the values of around 25 μM reported by Brown and Beckett (1985a) and Wells *et al.* (1995) for the moss *R. squarrosus* and 65 to 100 μM reported by Beckett and Brown (1984a) and Wells *et al.* (1995) for the lichen genus *Peltigera*. This suggests that the transporting system of *D. hirsuta* had a low affinity for Cd. The V_{max} was similar to that of the moss (10 to 15 μmol g^{-1} h^{-1}) and higher than that of the lichen (2 to 4 μmol g^{-1} h^{-1}). Extracellular Cd uptake also followed saturation kinetics. However, kinetic constants could not be calculated because uptake was not constant throughout the experimental period.

The effect of light on uptake

Light stimulated intracellular uptake by *c.* 15% (Table 2). By contrast, Brown and Beckett (1985a) reported little effect of light on intracellular Cd uptake in *R. squarrosus*. It is interesting that workers have often reported that light stimulates the membrane potential and enhances K^+ uptake in other thalloid liverworts (see

Table 2 Effect of light and dark storage for 20 days on intra- and extracellular Cd uptake from 100 μM $CdSO_4$ in *Dumortiera hirsuta* in the light and the dark. Within intra- and extracellular Cd uptakes, figures followed by the same letter are not significantly different using Duncan's multiple range test ($P < 0.05$)

	Treatment conditions	Storage conditions	
		light	dark
Intracellular Cd uptake rate (μmol g^{-1} h^{-1})	light	1.19a	1.18a
	dark	1.02ab	0.95b
Extracellular Cd uptake after 1 h (μmol g^{-1})	light	8.99a	10.41b
	dark	8.52a	9.09a

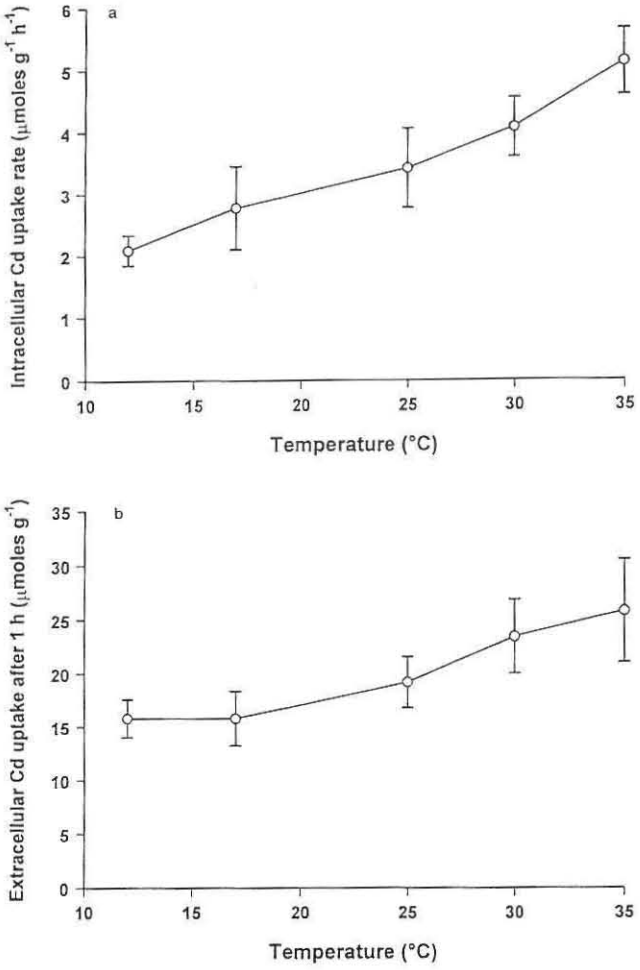


Figure 4 Intracellular Cd uptake rate (A) and extracellular Cd uptake after 1 h (B) from 100 μM $CdSO_4$ as a function of temperature in *Dumortiera hirsuta*.

Brown 1984 for review). Presumably, light stimulates proton pumping, creating a more favourable electrochemical gradient for entry of Cd into the cell. Surprisingly, storing plants in the dark for 20 days had little effect on intracellular uptake (Table 2). The maintenance of similar uptake rates even following long periods of dark storage indicates that either uptake is not active, or, more likely, intracellular transport receives high priority in the energy budget of the plant. Dark storage slightly increased extracellular Cd uptake.

Effect of temperature on uptake

Intracellular Cd uptake increased with increasing temperature (Figure 4a), suggesting that uptake is at least in part an active process. Brown and Beckett (1985a) obtained similar results for Cd uptake by the moss *R. squarrosus*. In *D. hirsuta*, extracellular Cd uptake also increased with increasing temperature (Figure 4b), although the ratio of extra- to intracellular uptake declined from 7.5 to 1 at 12°C and 5 to 1 at 35°C. Again, the moss *R. squarrosus* gave similar results, and in this species, extracellular Cd increased more with increasing temperature when uptake was measured after 0.5 h than after 1 h. From this, Brown and Beckett (1985a) concluded that increased extracellular Cd uptake probably represented a change in the rate of equilibration between cell wall exchange sites and the metal in solution, rather than a temperature-induced alteration in the total Cd uptake capacity. Similar considerations may also apply for the results obtained in the present study.

Table 3 Effect of thallus portion sampled on intra- and extracellular Cd uptake from 100 μM $CdSO_4$ in *Dumortiera hirsuta*. Within intra- and extracellular Cd uptakes, figures followed by the same letter are not significantly different using Duncan's multiple range test ($P < 0.05$)

	Distance from the apex (mm)		
	0	10	20
Intracellular Cd uptake rate (μmol g^{-1} h^{-1})	1.55a	1.11b	0.99b
Extracellular Cd uptake after 1 h (μmol g^{-1})	33.70a	29.70a	29.65a

The dependence of Cd uptake on plant age

Intracellular Cd uptake was highest in disks cut 0 mm from the apex (Table 3), and declined by 28% and 35% in disks cut 10 mm and 20 mm from the apex respectively. Presumably the growing apical regions were metabolically more active than the older parts of the thallus. Extracellular Cd uptake declined by 11% and 12% in disks cut 10 mm and 20 mm from the apex respectively, but the differences were not significant. When carrying out uptake experiments, replicates were prepared from randomly sampled disks from all parts of the thallus. Clearly, this contributed to the variability of the data, but limited availability of material precluded the sampling of disks from selective parts of the thallus.

Effect of competing ions on Cd uptake

In this experiment, the concentration of Cd used (300 μM) was chosen to be high enough for the liverwort to display near maximal intracellular uptake rates when supplied with Cd only (see Figure 3a). This allowed clear observation of the effect on uptake of competing cations, supplied at an equimolar concentration. Mg was added as sulphate, and Ca and Zn were added as nitrates. Cd was added as the same salt as the competing ion. Supplying Cd as nitrate rather than sulphate gave higher rates of intracellular Cd uptake (Table 4). No obvious explanation exists for this result, but Brown and Beckett (1985a) also noted that supplying

Table 4 Effect of Mg, Ca and Zn on intra- and extracellular Cd uptake in *Dumortiera hirsuta*. In the Table below, – refers to Cd uptake when Cd was supplied on its own, and + when Cd was supplied with a competing ion. Within intra- and extracellular Cd uptakes, figures followed by the same letter are not significantly different using Duncan's multiple range test ($P < 0.05$)

		Intracellular Cd uptake rate (μmol g^{-1} h^{-1})		Extracellular Cd uptake after 1 h (μmol g^{-1})	
Cd salt	Competing ions	–	+	–	+
300 μM $CdSO_4$	300 μM $MgSO_4$	16.80c	13.39b	27.98c	17.16b
300 μM $CdNO_3$	300 μM $CaNO_3$	22.27d	13.71b	29.01c	17.41b
300 μM $CdNO_3$	300 μM $ZnNO_3$	22.27d	8.03a	29.01c	13.52a

Table 5 The effect of K pretreatment and amendment of uptake solutions with Ca and Mg on intra- and extracellular Cd uptake from 100 μmol $CdSO_4$ in *Dumortiera hirsuta*. Ca and Mg were supplied as nitrates at 3 μM and 0.6 μM respectively. See text for details. Within intra- and extracellular Cd uptakes, figures followed by the same letter are not significantly different using Duncan's multiple range test ($P < 0.05$)

Pretreatment	Competing ions	Intracellular Cd uptake rate (μmol g^{-1} h^{-1})	Extracellular Cd uptake after 1 h (μmol g^{-1})
H_2O	None	9.1 b	60.6 b
80 mM KNO_3	None	13.4 c	85.5 c
H_2O	Ca + Mg	7.2 a	45.2 a
80 mM KNO_3	Ca + Mg	8.3 ab	55.9 b

R. squarrosus with Cd and different anions caused small differences in intracellular uptake.

Mg, Ca and Zn reduced intracellular Cd uptake to 80%, 62% and 36% respectively of the rate when Cd was supplied on its own (Table 4). This suggests that the Cd transporting systems had low specificity. Wells and Brown (1990) obtained similar results for *R. squarrosus*, although in the lichen *Peltigera*, Mg was more effective at reducing intracellular Cd uptake (Beckett & Brown 1984b; Brown & Avalos 1993). All competing cations reduced extracellular Cd uptake, and again Zn was the strongest competitor.

Effect of K pretreatment on Cd uptake

Wells and Brown (1990) and Wells *et al.* (1995) showed that pretreating lichens and bryophytes with K can increase the subsequent rate of intracellular Cd uptake. During an experiment, Cd taken up onto the cell wall will displace existing divalent cations, such as Ca and Mg, into the uptake solution, where they may compete with Cd for intracellular uptake and therefore reduce these uptake rates. Wells and Brown (1990) suggested that K pretreatment increases intracellular Cd uptake by removing existing divalent cations, thus preventing this competition from occurring.

In the present experiment, pretreating *D. hirsuta* with 80 mM KNO_3 significantly increased Cd uptake (Table 5). As outlined in Materials and Methods, measuring the amount of Ca and Mg in the nickel fractions (extracellular) enabled estimation of the additional Ca and Mg present in the uptake solutions of the non-K pretreated plants. Addition of these amounts of Ca and Mg to the uptake solutions of water and K pretreated plants reduced the intracellular Cd uptake rate of K pretreated plants to a similar value to that of non-K pretreated plants (Table 5). This suggests that K pretreatment does indeed stimulate intracellular Cd uptake by removing cell wall-bound divalent ions that Cd may subsequently displace during uptake experiments. These divalent ions can obviously then compete for intracellular uptake. The results of this experiment support the contention of Wells and Brown (1990) that workers should be careful when comparing bryophytes from different localities, as uptake rates may vary because of differences in ions bound to the cell wall rather than inherent differences in transporting systems.

Conclusions

The aim of this preliminary study was to characterize the factors affecting the kinetics of Cd uptake in the liverwort *Dumortiera hirsuta*. Results showed that intra- and extracellular uptake rates had different kinetics and that time, concentration, plant age, light, temperature and competing cations all affected uptake rates. In general, results obtained were comparable to those reported by Brown and Beckett (1985a) for the moss *Rhytidiadelphus squarrosus*. However, in *D. hirsuta*, rates of intracellular Cd uptake and the amounts of extracellular uptake appeared to vary more between collections than those of *R. squarrosus*. We are currently carrying out experiments to investigate this problem further.

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