

## Bioaccumulation of metals by lichens: Uptake of aqueous uranium by *Peltigera membranacea* as a function of time and pH

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### ABSTRACT

Uranium sorption experiments were carried out at ~25 °C using natural samples of the lichen *Peltigera membranacea*. Thalli were incubated in solutions containing 100 ppm U for up to 24 h at pH values from 2 to 10. Equilibrium sorption was not observed at less than ~6 h under any pH condition. U sorption was strongest in the pH range 4–5, with maximum sorption occurring at a pH of 4.5 and an incubation time of 24 h. Maximum U uptake by *P. membranacea* averaged ~42 000 ppm, or ~4.2 wt% U. This appears to represent the highest concentration of biosorbed U, relative to solution U activity, of any lichen reported to date. Investigation of post-experimental lichen tissues using electron probe microanalysis (EPM) reveals that U uptake is spatially heterogeneous within the lichen body, and that U attains very high local concentrations on scattered areas of the upper cortex. Energy dispersive spectroscopic (EDS) analysis reveals that strong U uptake correlates with P signal intensity, suggesting involvement of biomass-derived phosphate ligands or surface functional groups in the uptake process.

### INTRODUCTION

The threat of environmental pollution from the release and dispersal of uranium and related radionuclides into the biosphere has stimulated extensive research focusing on the geochemical properties of radionuclides and the interaction of U minerals and dissolved species with biological systems. The strong tendency of U to form stable aqueous complexes and precipitates with organic ligands and compounds is well known (e.g., Nash et al. 1981; Turner et al. 1993; Landais 1993; Read et al. 1993; Jamet et al. 1993) and indicates that U-biotic interactions could play an important role in radionuclide cycling at the Earth's surface. This hypothesis is supported by recent work elucidating the profound influence exerted by microbial communities on element cycling in the biosphere.

Experimental and field studies by Mann and Fyfe (1985), Milodowski et al. (1990), Lovely et al. (1991), and Macaskie et al. (1992) demonstrate that algae and bacteria can facilitate the precipitation of solid U phases directly from solution, through adsorption of aqueous U onto bacterial cell walls and subsequent nucleation of hydrous uranyl phases (Macaskie et al. 1992) or through U adsorption followed by reduction of  $\text{UO}_2^{2+}$  ( $\text{U}^{6+}$ ) to relatively insoluble  $\text{U}^{4+}$  at the cell wall interface (Lovely et al. 1991). Clearly, the effects of bioaccumulation by bacteria or fungi must be acknowledged in attempts to un-

derstand radionuclide cycling and dispersal. Many basidiomycete fungi are efficient accumulators of radionuclides and may form mycorrhizae with vascular plants. Such symbiotic relationships can lead to radionuclide uptake by the vascular plant hosts (Shaw and Bell 1994). Herbivorous grazing of bioaccumulating lichenized fungi in arctic and subarctic habitats can promote radionuclide cycling into higher trophic levels, resulting in widespread distribution of toxic metals from originally localized sources (Sheard 1986a, 1986b; Thomas et al. 1994).

In terrestrial environments, fungi can exist in a free-living state, as symbioses, e.g., with vascular plant roots (mycorrhizae), or else forming intimate associations with green algae or cyanobacteria. This latter type of association comprises the lichens, or lichenized fungi. Lichens are highly diverse and successful organisms, occurring in extreme environments worldwide in all major ecosystems apart from the deep sea and forming the dominant biomass in Arctic and Antarctic regions. Indeed, lichen-dominated vegetation covers approximately 8% of the Earth's land surface, giving them a globally important role in plant ecology and carbon, nitrogen, and phosphorus cycling (e.g., Nash 1996; Knops et al. 1991). However, the role of lichens in micronutrient and trace metal cycling in many settings remains largely unquantified.

Lichens lack roots, a protective outer cuticle, are long-lived and depend on sorption of nutrient elements often

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over their entire surface. Lichenized fungi bioaccumulate a range of both essential and non-essential elements through a combination of mechanisms including surface complexation, biomineralization, and physical trapping of dust and soil particulates (Brown 1991; Richardson 1995; Wilson 1995; Purvis 1996a, 1996b). Although their perennial nature and capacity to sorb high metal contents has led to widespread use of lichens as pollution indicators (Richardson 1992), significantly less attention has been focused on specific accumulation mechanisms.

Where lichens form the dominant biomass component, metal accumulation by these lithobiotic communities may have a direct and significant bearing on radionuclide cycling through higher trophic levels. Sheard (1986a, 1986b) found elevated U and daughter isotope concentrations in lichens and mosses near U mines in northern Saskatchewan. These elevated U concentrations were attributed to airborne transport of U-bearing dust particles trapped by lichens growing at the surface. Thomas et al. (1994) interpreted measurable concentrations of  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  in northern Canadian lichen, caribou, and wolf biomass, as evidence of trophic cycling of radionuclides derived from the Chernobyl nuclear disaster of 1986. Similar results are reported in Richardson (1992) for bioaccumulation and trophic cycling of radionuclides following nuclear weapon tests. On a more local scale, McLean et al. (1998) report on U accumulation from underlying uraniumiferous substrata in the lichen *Trapelia involuta*.

Mechanisms by which lichenized and non-lichenized fungi accumulate trace metal cations from aqueous solutions have been investigated by several authors (see reviews by Brown 1991; Richardson 1995; Purvis 1996a, 1996b). Experimental studies by Puckett et al. (1973) revealed that uptake of Cu by *Cladonia mitis* and *Umbilicaria muhlenbergii* was rapid and monotonic, reaching equilibrium concentrations in about 1 h. The authors modeled these results in terms of rapid saturation of active surface-complexation sites on the lichen biomass. Nieboer et al. (1976) concluded, based on experimental studies of Ni uptake by *Umbilicaria muhlenbergii*, that short-term accumulation of metals by lichens could be described as an "ion exchange" process involving carboxyl or hydrocarboxyl sites on external cell walls or structural macromolecules. Gadd (1993) proposed that other functional groups may also influence uptake, including amine, hydroxyl, and phosphate groups. However, there is limited experimental data elucidating quantitatively the biogeochemical properties of fungal or lichen biomass or the identities of functional groups active in uptake.

As an approach to understanding the role of lichenized and non-lichenized fungi in U cycling in natural settings, and to investigate U bioaccumulation over short time scales, we conducted a series of experiments designed to measure the U-uptake capacity of a widely distributed foliose cyanobacterial lichen, *Peltigera membranacea*, under controlled conditions as a function of time and fluid

pH. *P. membranacea* grows directly on soil, on the surfaces of exposed rocks, and on carpets of moss that grow on soil surfaces (Vitikainen 1994). Previous studies have demonstrated the strong capacity of the lichen genus *Peltigera* to sorb metals from solution (Goyal and Seaward 1981, 1982; Richardson and Nieboer 1983; Brown and Beckett 1983; Beckett and Brown 1984), although none have yet been carried out on uranium.

## EXPERIMENTAL PROCEDURES

### Lichen materials

Samples of *P. membranacea* thalli (plant bodies) used in uptake experiments were collected in June 1996 from a non-uraniferous location near the town of Blair Atholl, Caithness, Scotland (UK grid reference 27/875657). Thalli were collected together with their moss-leaf litter substrate from an earth bank. All visible extraneous leaf, soil, and mossy material was removed from thalli by hand picking under a binocular microscope. Cleaned thalli were washed with distilled deionized water and allowed to air dry. Dried thalli were cut into smaller sections that were used in U uptake experiments. Thallus sections were cut to fairly similar sizes using a stainless steel press, forming disks of roughly 1 cm diameter. Sections were prepared in this way to minimize errors or uncertainties arising from variations in sample preparation, and to standardize as much as possible the masses and surface areas of samples used in experiments.

Discolored sections, extreme marginal sections, or regions containing fruiting bodies were avoided. Lower surfaces of *Peltigera* thalli commonly possess projecting hyphal structures called rhizines, which serve as anchoring structures and may aid in nutrient uptake (Nash 1996). Numbers of rhizines projecting from lower cortical surfaces of cut thallus sections were counted. Although sample mass of cut thallus sections varied from ~3.0 up to ~15 mg, this variation is largely attributable to differences in rhizine number and length. Additional variation in sample mass results from varying numbers of short rhizines, which typically decorate lower cortices of *Peltigera* lichens, and form anastomosing networks of dense hyphae on the undersides of thalli. Cut thallus sections were allowed to air dry at 30 °C overnight prior to weighing.

### Experimental methods

Stock solutions of dissolved U were prepared in two ways: (1) by dilution of plasma grade AA standard solution of 10 000 ppm U in 5%  $\text{HNO}_3$  to final U concentrations of 100 ppm U in distilled deionized water, and (2) by dissolution of an appropriate mass of reagent grade  $\text{UO}_2(\text{NO}_3)_2$  (VWR Scientific Products) crystals in distilled deionized water to attain a fluid concentration of 100 ppm U. Stock solutions were titrated to appropriate pH values by addition of concentrated NaOH and  $\text{HNO}_3$ . Titrant volume was less than ~1% of overall fluid volume.

Dry lichen samples were wetted thoroughly with distilled deionized water immediately prior to experimental

incubation to minimize osmotic concentration of U in lichen biomass. Wetted lichen samples were immersed in 5 mL of U-bearing solution at the desired pH and incubated under gentle agitation at 25 °C in a heated shaking circulating water bath. Experiments were performed at incubation times ranging from 5 min up to 24 h.

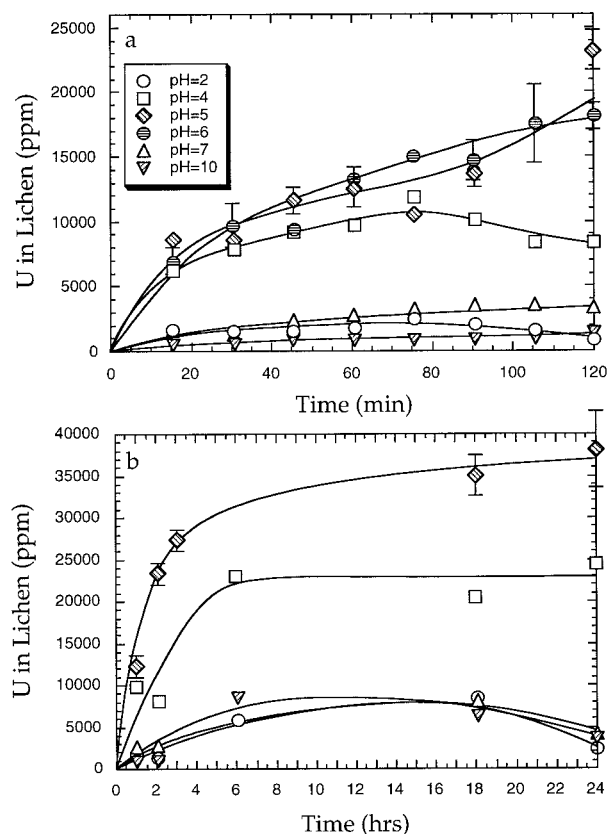
At the end of the experiments, lichen samples were extracted from supernatant fluids using stainless steel forceps and washed repeatedly with distilled deionized water for approximately one minute to remove any U-bearing solution retained through surface tension. Lichen samples were then allowed to air dry in clean test tubes overnight. This was followed by desiccation of the samples in a drying oven at ~30 °C for at least 1 h. Post-experimental lichen samples were digested in 0.5 mL concentrated HNO<sub>3</sub> at 90–100 °C until dry. Visible digestion of lichen biomass typically occurred in less than five minutes. Residues were taken up into 0.5 mL of concentrated HNO<sub>3</sub> and diluted to 2 mL total volume with distilled-deionized water. Resulting solutions were analyzed for U using inductively coupled plasma atomic emission spectroscopy (ICP-AES) at the Natural History Museum in London and at the University of North Carolina at Charlotte. Post-experimental lichen samples were examined using a Phillips model XL 30 FEG SEM with a Robinson BSED, and a Hitachi S2500 scanning electron microscope with a Link AN10000 EDS attachment, both at the Natural History Museum in London.

## RESULTS

### Time dependence of uptake

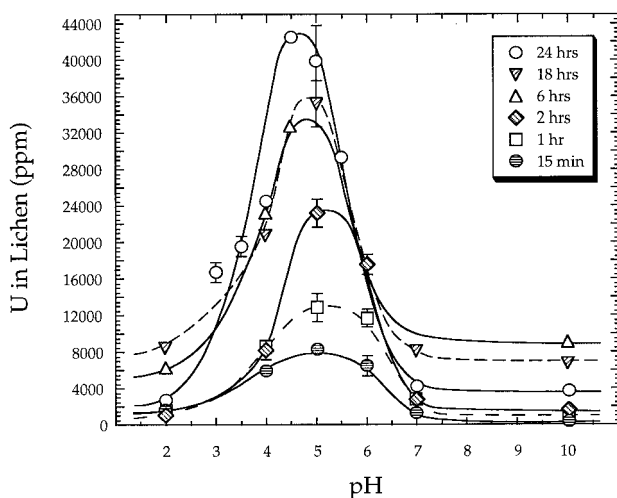
In contrast to the results of previous studies of lichen bioaccumulation (Puckett et al. 1973; Boileau et al. 1985), our results (Fig. 1a) indicate that uptake of U by *P. membranacea* does not achieve equilibrium in less than 2 h, nor does monotonic uptake of aqueous U as a function of time adequately describe the uptake process at all pH conditions. In Figure 1a, time-dependent U sorption at pH 4 reaches a maximum followed by a decrease in U content of the lichen biomass at longer incubation times. At a pH of 4, uptake of U by *P. membranacea* reached an average (5 replicates) of 11 823 ± 940 ppm U (~1.2 wt%) at 75 min. The uncertainty presented in association with this and other reported experimental values reflects the standard deviation of replicate analyses. Analytical uncertainties associated with analysis of fluid U concentrations, based on replicate analyses of standard solutions, was approximately ±1%.

After 120 min at a pH of 4, U concentration dropped to an average (5 replicates) of 8140 ± 1530 ppm. After 2 h, maximum U sorption was observed, with *P. membranacea* accumulating an average (2 replicates) of 22 970 ± 2870 ppm U (~2.3 wt% U) at a pH of 5. Values at a pH of 5 display a trend of increasing U uptake throughout the experiments. At pH values of 2 and 10, uptake appears to cease after 2 h, but at a pH of 7 there is a trend of slowly increasing U sorption as a function of time.



**FIGURE 1.** Results of U sorption experiments with *P. membranacea* thalli, expressed as U concentrations (ppm) sorbed by lichen samples as a function of time. (a) Results for incubation times of 15 to 120 min. (b) 1 to 24 h. Best-fit curves are shown for experimental results at fluid pH values of 2, 4, 5, 6 (part a only), 7 and 10. Error bars reflect one standard deviation of the mean of replicate analyses. No error bars are provided for values having uncertainties equal to or smaller than marker sizes.

Immersion experiments were also carried out at incubation times of 6, 18, and 24 h. Incubation times of greater than 24 h were not attempted to avoid complications arising from bacterial growth or decomposition of the lichen biomass after fluid immersion for extended periods. For incubation times that were longer than 2 h (Fig. 1b), lichen samples generally accumulated higher concentrations of U. Maximum uptake at 24 h was observed at pH = 4.5, averaging (2 replicates) 42 169 ± 1022 ppm U (~4.2 wt% U). Less U accumulated at more acid and more basic fluid pH values. These results demonstrate that although initial uptake of U by *P. membranacea* is rapid, reaching 7030 ± 79.4 ppm U (2 replicates) at pH = 5 after only 5 min (~24% of total U accumulation after 24 h at pH = 5), uptake continues at a slower rate for significantly longer periods of time. A best-fit curve through U uptake values at pH = 4 and incubation times up to 24 h indicates that a steady-state is obtained, relative to the ambient fluid, after about 6 h. However, at pH = 5 uptake does not appear to attain steady-state levels



**FIGURE 2.** Results of U sorption experiments with *P. membranacea* thalli, expressed as U concentrations (ppm) sorbed by lichen biomass as a function of fluid pH. Separate best-fit curves are depicted for experimental results at each incubation time. Error bars reflect one standard deviation of the mean of replicate analyses. No error bars are provided for values having uncertainties equal to or smaller than marker sizes. For clarity, dashed lines connect data for incubation times of 1 h and 18 h.

within 24 h. A best-fit curve through the experimental data at pH = 5 suggests that equilibrium is approached at the end of 24 h, but that attainment of fluid/lichen equilibrium under these conditions may require incubation periods of 48 h or longer. In none of the experiments was all available U accumulated by the lichen samples. At pH values of 2, 7, and 10, U uptake in our experiments appears to plateau after 6 to 18 h, after which U levels in the lichens drop to lower values at 24 h incubation. Based on the limited data set it is not clear if uptake maxima actually occurred at pH values of 2, 7, and 10 at incubation times of 6–18 h. Alternatively, absolute levels of uptake among individual thalli may be sufficiently variable to produce the observed range of values.

#### pH dependence of U uptake

The strong pH dependence of U uptake by *P. membranacea* is depicted more directly in Figure 2. Maximum uptake occurred at all incubation times at an initial fluid pH of 4–5, and precipitously decreased at more acidic and more basic pH values. The curves in Figure 2 display similar overall shapes, exhibiting uptake maxima for all incubation times in the pH range 4 to 5 and much lower U uptake at more acidic and basic pH values. Minimum uptake is observed at pH = 2 and pH = 10. Absolute uptake capacity under these pH conditions appears to vary complexly as a function of time, up to 24 h, but this variation may be an artifact of variations in uptake potential among individual thalli. Such variations would likely occur as a result of differences in reactive surface area, physical condition of the thallus, relative propor-

tions of surface functional groups, and absolute concentrations of surface functional groups.

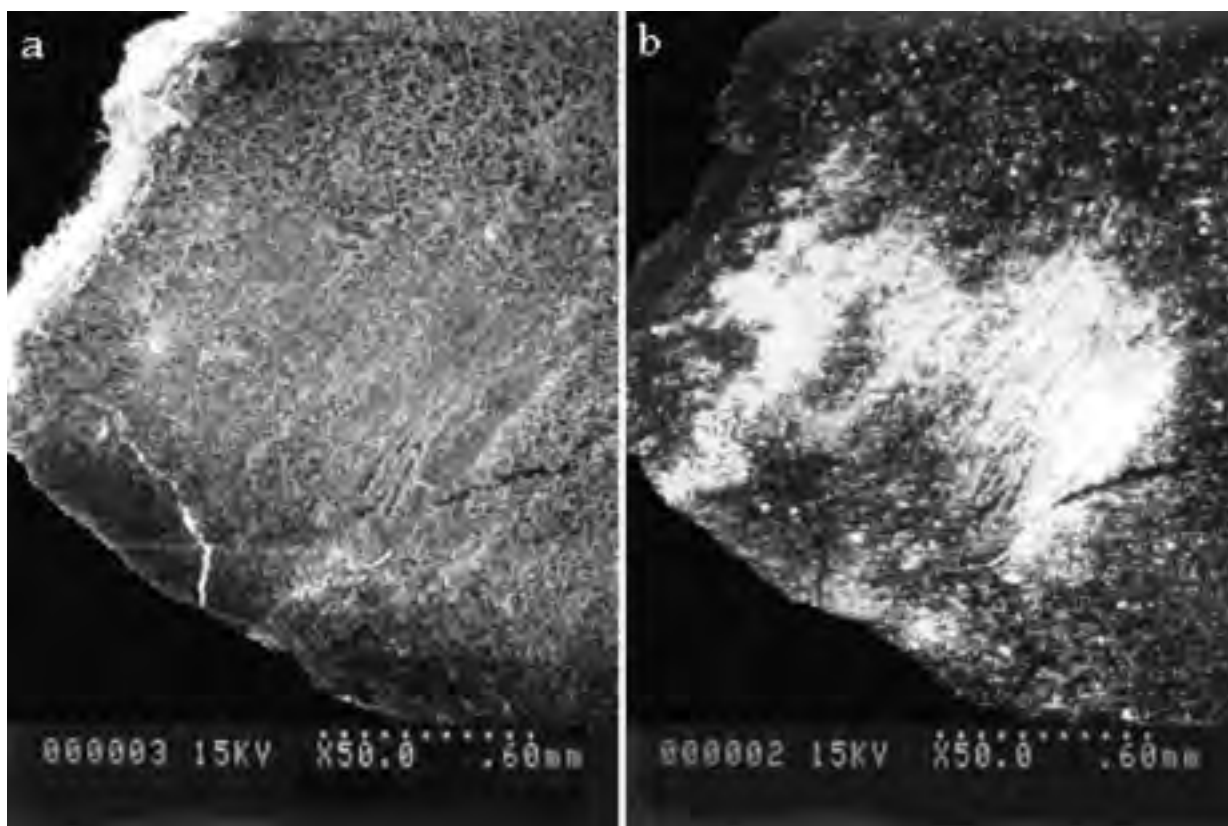
#### Probe studies of lichen samples

Areas of lichen that are highly enriched in U show high contrast in backscattered electron image (BEI), but not with secondary electrons (Fig. 3). Numerous tiny spots visible in Fig. 3b represent lithic material trapped by the interwoven mass of fungal hyphae. Lower-contrast areas of the upper cortex (Fig. 3b) are less enriched in U but typically display a detectable U EDS signal (Fig. 4). EDS spectra from control samples (Fig. 4a) illustrate the lack of detectable U concentrations in these materials prior to uptake experiments.

Upper cortical U-enriched areas of *P. membranacea* thalli display strong image contrast in BEI mode partially due to anomalously high electron densities relative to “background” biomass. These regions display significantly higher U to K signal intensity ratios than lower-contrast areas of the lichen surface. If K signal intensity is assumed to be fairly constant across the lichen surface, representing average concentrations of K in lichen biomass, and if matrix effects caused by heterogeneities in lichen biomass are assumed to be negligible, variations in U signal intensity relative to K should provide a qualitative indication of U enrichment. Comparison of cation U:K values indicates that uptake of U by *P. membranacea* biomass is heterogeneous across the cortical surface, such that some regions intensely accumulate U while other regions take up U to much lower local concentrations. This spatial heterogeneity could be the result of differences in the physical state of the lichen surface, or of compositional heterogeneities corresponding to differing concentrations of active surface functional groups, or both. Concentrations of specific surface functional groups may correlate with specialized anatomical structures. Specifically, highly intense U uptake within *P. membranacea* appears to correlate with the presence of a tomentum, or surface layer of cortical “hairs” arising from hyphal extensions on the upper cortex. The physiological function of a tomentum layer on lichens is unclear, but may involve trapping of airborne particulates or retention of water droplets by surface tension (Budel and Scheidegger 1996). Our findings demonstrate that the tomentum of *P. membranacea* is capable of accumulating high concentrations of U, relative to other parts of the lichen surface. These results suggest that the tomentum may also serve as a means of concentrating aqueous cations or nutrients from solution.

Strong U signals were also detected in rhizines, which are filamentous hyphal extensions of the lower medulla that serve as anchoring structures to the growth substrate. Rhizines probably also play a role in nutrient uptake from the substratum (Budel and Scheidegger 1996). Goyal and Seaward (1982) report that rhizines of the lichen *Peltigera canina* can accumulate higher concentrations of Ni, Cu, and Zn than the bulk thallus, although our experimental results do not confirm that rhizines of *P. mem-*





**FIGURE 3.** Electron photomicrographs of the upper cortex of a *P. membranacea* thallus after incubation in a solution containing 100 ppm U at a pH of 4 for 75 min. (a) Secondary electron image. (b) Backscattered electron image of the same area.

*branacea* accumulate higher concentrations of U than other parts of the thallus. The EDS spectrum in Figure 4d indicates that U uptake in the thalli and rhizines of *P. membranacea* are similar in magnitude.

EDS peak intensities for U and P in *P. membranacea* thalli appear to correlate where U signal intensities are strongest. As a test of this observation semi-quantitative values for U and P cation proportions were obtained by recalculation of EDS peak heights on an atom percent basis and normalizing to 100%. Our approach disregards light elements such as carbon and nitrogen, therefore our resulting values cannot be treated as quantitative measurements of U or P concentrations. Nonetheless, ratios of elemental concentrations determined on this basis may be used as a measure of relative abundance, assuming that light element concentrations are essentially similar from spot to spot and that matrix effects are negligible.

Semi-quantitative deconvolution of U and P spectra from areas of the lichen surface returning the strongest U signals reveals a U:P stoichiometry of approximately 1:1. In contrast, areas of the lichen surface that display relatively weaker U signals under BEI show higher relative U:P stoichiometries (averaging ~2.7:1 for spots analyzed). These systematic variations in relative U:P stoichiometry suggest that enrichment of the lichen in U may be controlled by differing surface functional groups, some

of which are rich in phosphorus while others are enriched in light elements such as C or N. Gadd (1993) proposes that uptake of cations by fungal biomass probably occurs through surface complexation reactions involving carboxyl, amine, hydroxyl, phosphate or sulphhydryl functional groups on biomass (Richardson 1995). Surface hydroxyl groups are unlikely to deprotonate under the mildly acidic conditions that obtained during the uptake experiments from which probed lichen samples derived. Sulphhydryl groups, if important to uptake, should produce a detectable sulfur signal that correlates with U, and no such correlation was observed in our study. Intense accumulations of U that correlate with P may be the result of surface complexation of aqueous uranyl species with active surface phosphate groups. Less intense uptake of U, correlating with lower P:U values, could indicate U surface complexation with functional groups poorer in P, such as carboxyl or amine. Uptake could also involve precipitation of multiple U-bearing phases or a single phase that differs in composition from adsorbed surface U complexes.

## DISCUSSION

Maximum uptake of U by *P. membranacea* under the controlled conditions described in this work occurred at a pH of 4–5 and after incubation for 24 h in a solution

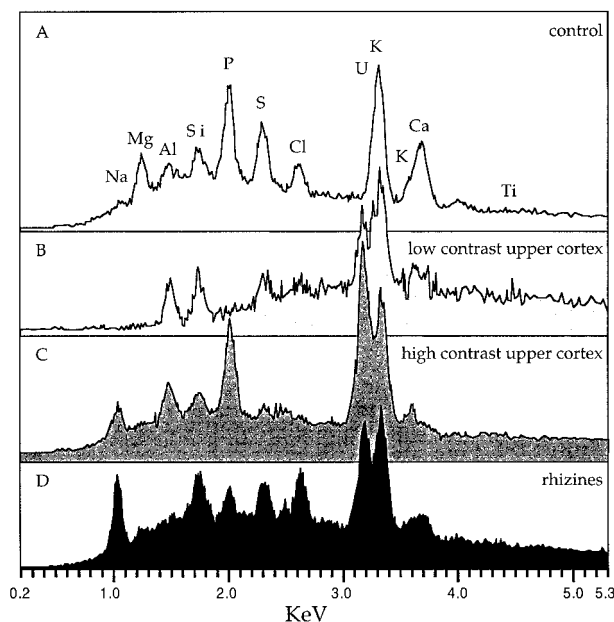


FIGURE 4. Energy dispersive X-ray spectra of *P. membranacea* thalli. (A) Control thallus, upper cortex. (B) BEI "dark" region of upper cortex of thallus incubated in a solution containing 100 ppm U at a pH of 4 for 75 min. (C) BEI "bright" region of upper cortex of thallus incubated in a solution containing 100 ppm U at a pH of 4 for 75 min. (D) EDS spectrum of a rhizine projecting from the lower surface of a thallus incubated in a solution containing 100 ppm U at a pH of 4 for 75 min.

containing 100 ppm U. Under these conditions, gross uptake of U by *P. membranacea* reached a maximum concentration of about 4.2 wt%. This appears to be the highest level of U uptake reported to date for any lichen, although higher absolute concentrations of U uptake from solution have been reported for free-living fungi (Gadd 1993). Table 1 reports values for maximum uptake of U by fungi and lichens for two other relevant studies. Comparison of our data with that of Boileau et al. (1985) demonstrates that U uptake by *P. membranacea* is significantly greater under similar experimental conditions than for other lichen taxa. It is noteworthy that the highest level of U uptake (14 494 ppm) reported by Boileau et al. (1985) for the lichen *Cladonia rangiferina*, after 1 h incubation in a solution containing 2000 ppm U at a pH of ~3.4, is similar in magnitude to our value of 11 283 ppm U accumulated after 1.25 h, at pH = 4.0, from a

solution containing only 100 ppm U. It is likely that this difference in reported U uptake capacity between two lichen species, *C. rangiferina* and *P. membranacea*, is real and represents a natural variation in composition and geochemical properties of different taxa. However, at present it is not possible to address this question rigorously because measurements of metal-uptake capacities, obtained under equivalent experimental conditions that allow for direct comparison, of different lichen taxa are largely unavailable.

We observe maximum uptake in the pH range of 4–5, which corresponds closely to the pH of many soils in temperate forested settings and in arctic, alpine and sub-alpine peat-bog and tundra biomes (Sparks 1995; Brady and Weil 1996). Trace element cycling in such settings is likely to be highly dependent on the rate of uptake of labile cationic and anionic species by extant flora, including vascular plants but also non-lichenized fungi existing as mycorrhizae or lichenized fungi growing on exposed surfaces. Consequently, quantitative data describing bioaccumulation by lichens and fungi at these pH conditions would appear to be crucial to more realistic modeling of element cycling in these settings.

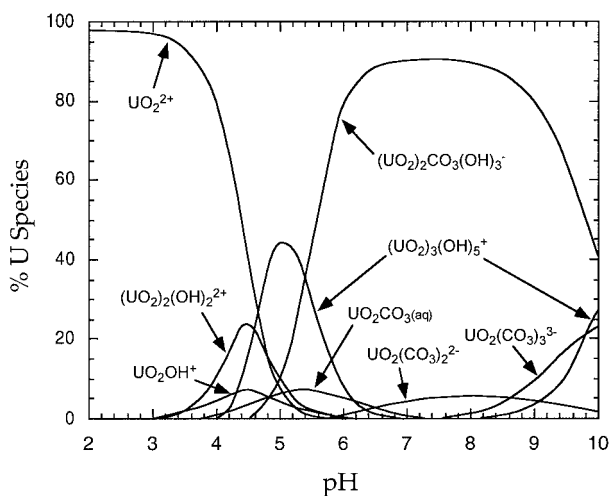
The pH range of maximum U uptake observed in our experiments is broadly consistent with results of previous experimental studies measuring metal uptake by lichens and fungi (e.g., Tobin et al. 1984; Tsezos and Volesky 1982; Fourest and Roux 1992; Strandberg et al. 1981; Puckett et al. 1973; Boileau et al. 1985). Our values are also consistent with limited surface titration studies of two lichen species by Tuominen (1967), who report acid dissociation constant ( $pK_a$ ) values in the range 2.8–4.4 and ~8.3 for bulk lichen biomass. Deprotonation of groups having  $pK_a$  values in the range ~2.8–4.4 would facilitate electrostatic attraction of aqueous  $UO_2^{2+}$  ions, leading to surface complexation or precipitation reactions at fluid pH values slightly more basic than the  $pK_a$ . Surface complexation or precipitation will also depend on the speciation of aqueous U.

In solution,  $UO_2^{2+}$  tends to form strong complexes with available carbonate, phosphate, hydroxyl, and carboxylate ligands under oxidizing conditions at 25 °C and 1 bar (Wagman et al. 1982; Tripathi 1984; Grenthe et al. 1992; Shock and Koretsky 1993; Giordano 1994; Ticknor et al. 1996). Using available thermodynamic values, the pH dependence of U speciation in our initial experimental solutions can be calculated. For a solution at 25 °C and 1

TABLE 1. Tabulation of experiment data describing maximum uptake of U by lichen or fungal biomass

Reference	Species (phylogeny)	Pretreatment	$t_{\text{INCUB}}$	pH	$U_{\text{INITIAL FLUID}}$	$U_{\text{LICHEN}}$
This work	<i>Peltigera membranacea</i> (lichen)	none	24 h	4.5	100	42 169
Boileau et al. (1985)	<i>Cladonia rangiferina</i> (lichen)	heat-killed (80 °C for 24 h)	1 h	3.3	2000	14 494
Boileau et al. (1985)	<i>Cladonia rangiferina</i> (lichen)	none	1 h	3.3	2000	11 662
Galun et al. (1983)	<i>Penicillium digitatum</i> (fungus)	5% KOH for 5 min	4 h	nv	61.7	9860

Notes: Pretreatment indicates whether the biomass was subjected to any unusual conditions prior to uptake experiments, other than washing in DI water and subsequent drying.  $t_{\text{INCUB}}$  represents incubation time in hours.  $U_{\text{INITIAL FLUID}}$  and  $U_{\text{LICHEN}}$  indicate U concentrations (ppm) in initial experimental solutions and by dry weight in lichen samples after experiments. The abbreviation "nv" indicates no value reported.



**FIGURE 5.** Calculated speciation of U in a solution at 25 °C and 1 bar containing 100 ppm U and  $10^{-3}$  molal  $\text{CO}_2(\text{aq})$ , as a function of fluid pH. Values were calculated using thermodynamic data for U aqueous complexes from Tripathi et al. (1984), and the computer code EQ3NR v.7.2b (Wolery 1992).

bar containing 100 ppm U, dissolved  $\text{Na}^+$  and  $\text{NO}_3^-$  (reflecting titration of solutions to appropriate pH using  $\text{NaOH}$  and  $\text{HNO}_3$ ), and  $\text{CO}_2$  fugacity reflecting equilibrium with the atmosphere ( $f_{\text{CO}_2} = 10^{-3}$ ), we have estimated the distribution of aqueous species, as a function of pH, using the computer program EQ3NR (Wolery 1992). The results of this calculation (Fig. 5) suggest that at pH values more acidic than  $\sim 4.4$ , the predominant form of aqueous U in this modeled solution is the aquo uranyl ion. Within the pH range of  $\sim 4.4$  to  $\sim 5.5$ , mixed cationic and neutral uranyl-hydroxide complexes are calculated to predominate, while at pH values greater than  $\sim 5.5$  but less than  $\sim 10.0$ , the mixed uranyl-hydroxycarbonate complex  $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$  is predicted to dominate. At pH values greater than  $\sim 10.0$ , anionic uranyl-hydroxide and uranyl-carbonate complexes appear to dominate. Our calculated speciation of a U-bearing solution is broadly consistent with our experimental findings of optimal U uptake by *P. membranacea* in the pH range 4–5. At pH values less than  $\sim 5.4$ , we estimate that uranyl speciation will be dominated by cationic aqueous species that will tend to be electrostatically attracted to surface functional groups that are active in the same range of pH. At pH values higher than  $\sim 5.4$ , stronger competition for uranyl cations by anionic aqueous species may tend to curtail complexation of uranyl ions with surface groups. This mechanism is analogous to competitive complexation reactions limiting uranyl adsorption onto mineral surfaces at basic pH conditions (Payne and Waite 1991; Waite et al. 1994).

At all analyzed pH values, our experimental data indicate that U uptake by *P. membranacea*, although initially rapid, nonetheless requires much longer than one or two h to attain a steady state. Experimental work by

Puckett et al. (1973) demonstrated that Cu uptake by the lichens *Cladonia mitis* and *Umbilicaria muhlenbergii* occurs rapidly and monotonically, and that after about 1 h uptake attains a maximum level relative to solution Cu activity. In contrast, our results illustrate that, although strong uptake can occur over incubation times less than 1 h, periods up to (and possibly exceeding) 24 h are required to reach apparent equilibrium. These results suggest that uptake may be governed by a rapid mechanism that can result in strong accumulation within minutes and a slower mechanism that requires much longer incubation times to attain equilibrium.

The mechanism of short term cation-uptake by lichens is generally regarded as an abiotic process governed by surface complexation of aqueous cations with exposed functional groups on the lichen biomass surface, or by the precipitation of solid phases on the exteriors of cell walls (Richardson 1995). Similar mechanisms have been invoked to explain accumulation of metals from solution by bacteria (Beveridge and Fyfe 1985; Mann and Fyfe 1985; Milodowski et al. 1990; Lovley et al. 1991; Macaskie et al. 1992; Fein et al. 1997; Daughney and Fein 1998), fungi, algae, and lichens (Brown 1991; Gadd 1993; Richardson 1995). According to this model, exposed acid-base functional groups, including carboxyl, phosphate, and hydroxyl groups (Gadd 1993) on the exterior surfaces of fungal hyphae, algal cell walls, and within an extracellular gel of mixed mucopolysaccharides characteristic of fungal and lichen biomass (Hale 1983; Budel and Scheidegger 1996), serve as sites of surface complexation with metal cations in solution. Adsorption can lead to precipitation under conditions of local supersaturation with respect to a given phase, and this effect has been reported for bacterial biomineralization of U (Macaskie et al. 1992). Abiotic uptake via surface complexation or precipitation mechanisms tends to be rapid (Nieboer et al. 1976; Richardson 1995), and it is likely that much of the U uptake observed during our experiments can be attributed to a combination of these processes.

However, intracellular exchange mechanisms involving metabolic processes may also facilitate cation uptake, especially over longer incubation times (Richardson 1995). Intracellular uptake of Cd, Zn, and Cu has been shown for the genus *Peltigera* (Brown and Beckett 1983; Beckett and Brown 1984; Brown and Avalos 1991, 1993). The contribution of intracellular metal uptake to total accumulation in the general case is unclear, although Beckett and Brown (1984) showed that for Cd uptake by *P. membranacea* under experimental conditions, intracellular uptake generally accounted for less than 10% of extracellular accumulation after incubation for 1 h. The importance of intracellular uptake at longer incubation times remains largely unquantified.

However, preliminary TEM results (Barker et al. 1998) show that intracellular U uptake by *P. membranacea*, localized mainly within cellular organelles known as “concentric bodies” (Griffiths and Greenwood 1972), occurs



after only 75 min. TEM investigations also reveal precipitation of acicular U and P-bearing nanocrystals along cell walls and within the extracellular gelatinous mucopolysaccharide matrix enclosing fungal cells. These findings clearly demonstrate that U uptake by *P. membranacea* is not accomplished through surface-complexation reactions alone. Precipitation of solid phases, albeit as nanometer-sized crystals, also occurs. It is likely that precipitation is preceded by surface complexation reactions involving acid-base functional groups, these reactions serving to concentrate U at the biomass surface and within the extracellular polysaccharide gel. Surface U complexes may then act as sites for heterogeneous nucleation of uranyl-molecular clusters, which in turn may grow into uranyl-bearing crystals. Macaskie et al. (1992) reported formation of  $\text{HUO}_2\text{PO}_4(\text{s})$  microcrystals on *Citrobacter* sp. cell walls following incubation in U-bearing solutions; the authors attributed this result to enzymatically mediated liberation of phosphate from bacterial cells.

Rapid uptake of U by *P. membranacea* appears to occur by a series of steps involving surface complexation of aqueous uranyl species by acid-base functional groups, followed by growth of heterogeneously nucleated U-bearing crystals at the cell wall and within a diffuse extracellular matrix. Slower uptake over longer incubation times may reflect continued precipitation of uraniferous phases within the extracellular matrix or on cell walls, after saturation of surface functional groups is achieved. Additional bioaccumulation occurs through intracellular uptake, primarily within concentric bodies (Barker et al. 1998). The functional role of these proteinaceous organelles is presently unclear, but they appear to strongly accumulate U, Os, Pb, and possibly other heavy metals (Peveling et al. 1985). Concentric bodies are uniquely associated with lichenized fungi. That these organelles strongly accumulate transition and heavy elements suggests that they may assist in cellular storage of micronutrients, and may therefore contain metallophilic compounds similar to siderophores. The identity(ies) of observed U-bearing phase(s) formed within *P. membranacea* concentric bodies, on cell walls and in extracellular exudates remains unclear. Do analogous phases form after the uptake of other heavy elements on cell walls, in extracellular gels, or in concentric bodies? How does this process vary among lichen taxa? At present it is not possible to directly compare the bioaccumulative properties of a wide variety of lichen, fungal and microbial taxa with respect to a broad range of metals, because the required experimental data are limited or unavailable. Such data could greatly facilitate studies of heavy metal and radionuclide speciation and transport at or near the Earth's surface.

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