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EFFLUX OF URANIUM FROM FOUR MACROLICHENS DUE TO AQUEOUS WASHING

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Abstract. It was previously established that uranium concentrations in lichens may be altered by aqueous washing, and that disproportional loss occurs from different thallus regions. In this study, uranium retention was compared in four species of macrolichens before and after wash treatments. In regard to individual parts of the thallus, the greatest differences in uranium concentration between species were observed prior to washing, and significant interspecific differences were maintained after aqueous treatments. In respect to whole thalli, differences between species were also observed in starting uranium concentrations, but these were not maintained after treatment with water or dilute acid solutions. The lack of post-wash differences in whole thalli was apparently related to the fact that all thallus zones were not represented to the same extent in the intact lichen, and the efflux of uranium differed between zones. In any case, exposure to water had an equalizing effect on overall U levels in the four lichen species tested.

Abbreviations: U = uranium; EDX = energy dispersive x-ray analysis, SEM = scanning electronmicroscope, PH = photobiont; MD = medulla; UC = upper cortex; LC = lower cortex or lower region of compactly arranged conglutinate hyphae; RZ = rhizines

Introduction

It is sometimes assumed that metallic impurities are not only incorporated by lichens, but accumulated on a long-term basis. However, the elemental content of contaminant heavy metals decreases with time after metal input ceases (Walther et al. 1990, Fahselt et al. 1995). Because heavy metals may effect both physiological functioning and genetic properties, they are relevant to population studies of lichens, and it is thus important to investigate the question of retention. An understanding of the relative propensities of lichen species to relinquish metal ions is also valuable in connection with pollution monitoring that involves lichens as indicator species.

Naturally-occuring radionuclides of uranium (U) may be locally dispersed into vegetational communities near active mines, and concentrations in lichens along transects near Elliot Lake and Agnew Lake, Ontario, Canada, were first documented by Beckett et al. (1982). Uranium can be taken up as particulate matter, such as dust generated during ore-processing and, if such particulates become associated with the thallus surface, they may be lost due to the expansion and contraction that occurs during wetting and drying (Brown & Brown 1991). However, some uptake is in a soluble form, especially as the uranyl ion, with soluble ions being released, for example, from entrapped particulates through biogeochemical and biogeophysical degradation (Nieboer et al. 1978, Brown & Brown 1991). In the dissolved form U may be transported to the interior of the thallus, like small molecules or other dissolved ion, through the apoplastic space in cell walls (Honegger & Peter 1994). Although there is limited information available on the egress of metals from lichens, movement is presumably outward as well as in. If the uranyl ion is displaced by another dissolved cation, perhaps it can be leached out of the thallus following contact with natural moisture such as rain. Uranium is lost from some thallus regions of lichens wetted experimentally (Trembley et al. 1997, Fahselt et al. 1997); unbound uranyl ions presumably are removed by water washing and bound ions by exhange with cations in wash solutions.

Quantification of metals in lichens is generally accomplished using neutron activation analysis or X-ray fluorescence spectroscopy and concentrations reported on a $\mu g\ g^{-2}$ basis in whole lichens; this has mainly been the case for uranium (e.g., Beckett et al. 1982, Jeran et al. 1990, Fahselt et al. 1995). However, scanning electron microscopes (SEM) equipped with energy-dispersive X-ray (EDX) analyzers have made it possible to accurately determine concentrations of elements such as U in specific regions of a thallus. Assessing the radionuclide content of lichens is time-consuming and costly, and there are limits to the number of analyses that can be performed in any given study site. Thus, it is practically useful to determine whether generalizations are possible concerning the quantities of U incorporated and retained by various lichens. Nevertheless, little effort has been made to compare U retention in different species under controlled conditions, either in the whole thallus or in its component parts.

The purpose of the present study was to compare on the basis of EDX analysis the concentrations of U in four ecologically important macrolichens after thalli, which have received the same opportunity to incorporate uranium, are subjected to aqueous washing. Interspecific comparisons will first be made on the basis of anatomically similar thallus regions that are common to all species and then on the basis of whole thalli.

Methods

Lichen species selected for the study were three fruticose lichens, *Cladina rangiferina, C. mitis* and *Stereocaulon saxatile,* and a foliose lichen, *Umbilicaria mammulata.* Each was collected from within a 1 x 1 m^2 plot in an area free of U contamination, *C. rangiferina* near Mallorytown, Ontario, Canada (44°29'N, 75°53'W) on April 27, 1996, and the other three species near Gravenhurst, Ontario, Canada (45°55'N, 79°27'W) on February 2, 1996. Samples were examined under a microscope to ensure that no visible contaminants were present, and checked by EDX-SEM to ensure that essentially no U was detectable in field-collected material. Samples of all species were prepared for charging with U using solutions of identical strength. Each sample of *C. rangiferina* and *C. mitis* consisted of a small group of randomly chosen podetia weighing 0.25 g. Fifteen such samples were selected per species. Two or three small branches of *S. saxatile* were similarly combined to produce each of 15 samples weighing 0.25 g each, and a 0.50 g sample was taken from near the margin of each of 15 randomly chosen thalli of *U. mammulata.* All thalli were then immersed for 1 hr in separate beakers in 8.4 mM solutions of uranyl nitrate hexahydrate ($UO_2(NO_3)_26H_2O$), which yields the uranyl cation. Five samples of each species were then allowed to dry at room temperature and treated no further, while five of the remaining samples were subjected to washing with ddH_2O for 1 hr and the other five were treated to 1 hr immersion in an acidic solution of pH 4.0. In the case of *C. rangiferina* the acid used was acetic acid and with the other three species, H_2SO_4. During infusion and washing, samples were maintained under a light source with photon flux density of 150 $\mu mol\ m^{-2}\ s^{-1}$.

Because of the propensity for U retention to differ between zones of the thallus, U content was measured separately in each thallus region. Samples were sectioned transversely by hand and coated to 100-200 Å thickness with carbon using an Edwards 306 carbon evaporative processor. Specimens were examined at the Surface Science Center at the University of Western Ontario under an ISI-DS 130 dual stage scanning electronmicroscope equipped with an EDX analyzer. Details of the method are found in Trembley et al. (1997). An acceleration voltage of 15 kV was used, and measurements were made using the EDX probe in three randomly chosen locations within each of the major thallus regions in each of the 15 sections examined per species. Thus, three regions, the photobiont region where the green algal symbiont formed layers or clusters (PH region), the medulla where fungal hyphae were loosely-arranged (MD) and the lower layer or central axis where conglutinate hyphae were compactly-arranged (LC), were examined in all species. In addition, *U. mammulata* was sampled in the continuous layer of conglutinate hyphae over the upper surface (the upper cortex or UC) as well as, when possible, in rhizines (RZ) on the lower surface of the thallus.

The relative volume occupied by each of the five thallus regions was estimated on the basis of areas in five transverse views of each species. In any given species the mean area of each region, thus determined, was expressed as a proportion. The U concentration in a region was then multiplied by this value, a procedure used to adjust the U concentrations of each thallus region to represent the amounts ($\mu g\ g^{-1}$) contributed to the whole thallus. Finally, the calculated contributions of all thallus regions were combined to indicate the total U concentration for each of the 15 samples of each species.

Uranium concentrations were compared between species, both before and after the two types of aqueous washes. Each of three thallus regions in each species was analyzed separately. Data were arcsin transformed and subjected to analysis of variance (ANOVA) for balanced data and pairwise comparisons among species were made using Tukey's method, with family error rate of 0.05, in conjunction with one-way ANOVA (Minitab Version 11, Minitab Inc., State College, PA). The same analyses were performed on total whole thallus data. To compensate for missing data neutral values were generated.

Results

Highly significant differences (P= 0.01) in initial U concentrations were observed between species in respect to each of the three thallus regions that were comparable among all lichens (Table 1). Highly significant interspecific differences were found also after washing with water or with acid, although the magnitude of discrepancies between species were less, particularly for PH and MD regions. There were also highly significant differences between sample thalli in respect to LC regions, and interactions were evident between thalli and species. Because *U. mammulata* was the only lichen examined with an upper cortex or rhizines, interspecific comparisons were possible for neither of these two thallus regions, but U concentrations in the UC were found to be 25.35 ± 6.79 $\mu g\ g^{-1}$ initally, 15.81± 4.93 $\mu g\ g^{-1}$ after water washing, and 16.12 ± 3.35 $\mu g\ g^{-1}$ after immersion in acid (n = 5 in each case).

In regard to U determinations in RZ of *U. mammulata,* little data were available except from sections washed in acid. This was because it was difficult to cut sections through rhizines and maintain them attached to the thallus through the carbon coating process. However, the available RZ readings were extremely high in comparison to those in other parts of the thallus, thus could not be omitted from calculations of whole thallus U and were therefore estimated. Mean U concentration in RZ washed with acid (n = 5) was 46.40 ± 7.61 $\mu g\ g^{-1}$, and mean concentrations before washing must have been no less than this. Because neither water nor acid washing effectively removes U from the lower cortex (LC)

Table 1. U content (μg g^{-1}) in thalli of three comparable thallus regions in each of four lichens before washing and after washing with distilled water or weak acid. Thallus regions tested are: photobiont zone (PH), loosely-arranged hyphae or medulla (MD) and lower or inner zone of compact hyphae (LC). Letters after means and standard deviations (n=5) indicate homogeneous subsets within rows according to Tukey's test.

Treatment	Thallus region	Species			
		C. rangiferina	*C. mitis*	*S. saxatile*	*U. mammulata*
None	PH	4.26±2.48a	11.28±2.22b	13.50±3.71b	6.94±1.19a
	MD	10.80±2.09b	13.86±2.73bc	19.24±6.92c	2.88±0.79a
	LC	7.71±3.59a	18.00±5.08b	12.97±4.30b	17.93±9.09b
Water wash	PH	5.35±1.92a	9.07±1.96b	5.31±2.10a	4.56±2.22a
	MD	8.05±2.64b	9.77±2.59b	8.53±3.54b	2.96±2.51a
	LC	7.82±2.51a	14.10±3.55b	5.85±2.41a	19.46±6.72c
Acid wash	PH	4.05±0.49a	8.18±4.28b	5.85±1.23ab	5.45±1.08ab
	MD	8.98±2.77b	9.35±3.76b	7.61±1.91b	1.58±0.80a
	LC	8.98±4.11a	10.06±5.18ab	6.86±3.22a	14.49±6.41b

Table 2. Factors used to calculate U contributed to whole lichens by each of the constituent thallus regions. Values are based on mean areas in five transverse sectional thallus views of each species.

Species	Upper conglutinate region[1]	Photobiont layer	Medulla	Lower or inner conglutinate region[2]	Rhizines[3]
C. rangiferina	-	0.104	0.562	0.334	-
C. mitis	-	0.142	0.571	0.287	-
S. saxatile	-	0.127	0.725	0.148	-
U. mammulata	0.076	0.183	0.613	0.069	0.058

[1] Upper cortex, absent in the first three lichens.

[2] Includes lower cortex of *Umbilicaria mammulata*, inner podetial zone of *Cladina* spp. and central axis of *Stereocaulon*.

[3] Only *U. mammulata* was rhizinate

of *U. mammulata* (Fahselt et al. 1997), these treatments were probably ineffective with rhizines as well. Rhizines were assumed to be as retentive as the LC because they are likewise comprised of conglutinate hyphae, they are attached to the LC, and many of the hyphae are continuous between the two thallus regions. Thus, the assumption was made that U levels in RZ were the same before washing, as well as after washing with water, as they were following acid treatment. These values were used in the calculation of the U contributed by RZ to the whole thallus.

In terms of the volume of thallus occupied by each anatomically distinct region, the medulla was numerically most important, comprising well over half of the thallus in each of the species examined (Table 2). In contrast, the algal zone represented only 10-20% of the thallus interior. The remainder of the thallus, but no more than a third in any one species, was composed of conglutinate hyphae closely appressed to each other. In *Cladina* spp. these regions were internal, either as a solid or hollow supporting axis, while in *U. mammulata* they were located externally as thin upper and lower layers, the lower with rhizines attached. The highest observed U concentrations were often in conglutinate regions and, consequently, even though these constituted a relatively small proportion of the thallus, made an important contribution of U to the entire lichen.

Total U content of thalli at the outset of the experiment as well as after application of washing treatments is shown in Table 3. Balanced ANOVA indicated highly significant differences between species and between treatments and also a highly significant interaction between the two (respective-

Table 3. Total U ($\mu g\ g^{-1}$) in four lichen species before and after washing calculated on the basis of proportional contributions of each thallus region and concentrations in each. Letters following means and standard deviations indicate homogeneous subsets within each column (n=5) according to Tukey's method.

Species	Number of thallus regions	Treatment		
		Initial	Water wash	Acid wash
C. rangiferina	3	9.09±2.31ab	7.68±8.26a	8.47±2.86a
C. mitis	3	14.68±3.53bc	10.91±1.70a	9.39±3.51a
S. saxatile	3	17.58±5.10c	7.72±2.80a	7.28±1.67a
U. mammulata	5	8.88±2.52a	7.88±2.21a	6.88±1.80a

ly, F = 6.97 (3 df), P = 0.001; 13.07 (2 df), P = 0.000; 3.14 (6 df), P = 0.012). One-way ANOVA's performed on total thallus U following individual treatments confirmed that species differed (P = 0.001) with respect to starting levels of U, but showed that differences between the four lichens species were insignificant after both water and acid washes. Tukey's method for comparing means indicated that the initial thallus content of U, representing the overall capacity of each species to take up uranyl ions from solution, was lowest in *U. mammulata* and highest in *S. saxatile*, and the differences between these two species were significant (P = 0.05). The mean U concentration in *C. mitis* was not significantly different from that of *S. saxatile*, while that of *C. rangiferina* was not significantly different from that of *U. mammulata* and the two species of *Cladina* were not significantly different from one another. Any interspecific distinctions were lost, however, after washing treatments.

Discussion

In nature lichens are repeatedly exposed to moisture and subject to airborne contaminants over an extended time. The time frame of the experiment was necessarily short compared to the life of a lichen, and exposure time to metal ions and thallus contact with water were both compressed. Nevertheless, some insight was provided into the relative abilities of different lichen species to retain or release U due to contact with water and acidified water.

It was previously known that U uptake differs from one thallus region to another (Trembley et al. 1997; Fahselt et al. 1997), and this study showed that initial accumulation in any particular region differed between species. For example, the capacity of the MD to take up U tended to be greater in ecorticate lichens than in *U. mammulata*, the one species which maintained a continuous cortical layer as the upper surface of the thallus. The upper cortex might be expected to block accumulation of U in the form of particulates, but the results observed with soluble U were more surprising. Field studies should be done as well, but based on the present evidence it appears that *U. mammulata* may offer fewer binding sites or a less efficient transport mechanism for U, rather than present a physical barrier. Another interspecific difference in U uptake capacity was the significantly low U concentration in the conglutinate region (LC) of *C. rangiferina*. This result also seemed unusual because *C. rangiferina* and *C. mitis* are similar lichens with similar anatomy and internal organization of podetia, but perhaps some aspect of chemistry or microstructure of cell walls differs between the two.

The absence of significant differences between whole thalli after washing with distilled water was probably a reflection of greater efflux of soluble U from some species than others. For example, greater loss of U from *S. saxatile* is understandable in view of the earlier report (Fahselt et al. 1997) that significant amounts are released into water by all thallus regions of this species.

The lack of significant differences in total thallus content of U among the three lichens washed with dilute sulfuric acid suggested that protons in acidic solutions displaced uranyl ions more effectively and enhanced efflux more markedly from *C. mitis* and *S. saxatile* than from *U. mammulata*. *Umbilicaria mammulata* may have had fewer uranyl cations that were bound exchangeably. Significant loss of U occurs from the lower cortex of *C. mitis* during acid treatment (Trembley et al. 1997), and from all thallus regions of *S. saxatile* (Fahselt et al. 1997), and such flux could account for the fact that U levels in these species were lowered to the same as in *U. mammulata*.

In any event, although the capacity for initial uptake of U by whole thalli differed between lichen species, relative concentrations were altered by wetting. Even unrelated lichens achieved similar concentrations under the experimental conditions tested, the levels in all four of those tested having been equalized by water washing. Boileau et al. (1982) reported earlier that the U content of *C. mitis* and *C. rangiferina* was similar under field conditions, but the present findings suggest that the two did not have achieve the same U level simply through similar uptake. Rather, correspondence may have been attained in spite of higher uptake by *C. mitis*, after greater loss by leaching from this same species.

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