

Accumulation Mechanisms of Uranium, Copper and Iron by Lichen *Trapelia involuta*

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Abstract

To understand a metal accumulation mechanism by a lichen, we examined the lichen *Trapelia involuta* growing directly on secondary uranyl minerals and U-enriched Fe oxide and hydroxide minerals. Uranium, Cu and Fe in *T. involuta* are concentrated in the exciple and epithecium, and the distribution of U, Cu and Fe and that of the melanin-like pigments are strongly correlated to one another. TEM observations revealed that the presence of U, Cu and Fe in the exciple and epithecium was not due to mineral particulates or organic crystals. The characteristic formation of the melanin-like pigments and the distribution of U, Cu and Fe only in the exciple and epithecium suggest that the accumulation of U, Cu and Fe is related to the melanin-like pigments but probably not to polysaccharides or cells present in the whole apothecium.

Key words: lichens, uranium, toxic metals, melanin, bioaccumulation

Introduction

Lichens are symbiotic organisms composed of fungi (mycobiont) and green algae or cyanobacteria (photobiont). They are highly diverse and successful organisms, occurring in extreme environment worldwide in all major ecosystems apart from deep sea and forming the dominant biomass in the Arctic and the Antarctic regions. Lichen-dominated vegetation covers approximately 8% of the earth surface, giving lichens a globally important role in plant ecology, and C, N, and P cycles (e.g., Nash, 1996).

Lichens can occur in extreme metalliferous environments (Purvis and Halls, 1996) and accumulate high amounts of potentially toxic metals (e.g., Richardson, 1995). This leads to their use for biomonitoring uranium discharge from mining activities in Canada (Boileau *et al.*, 1982; Richardson *et al.*, 1985; Fahselt *et al.*, 1995) and radionuclide fall-out from nuclear weapon testing and nuclear accidents (e.g., Feige *et al.*, 1990). These studies have utilized the high metal accumulation and widespread distribution of lichens but not necessarily focused on accumulation mechanisms.

McLean *et al.* (1998) have suggested that U is adsorbed to melanin-like pigments in the outer apothecial wall of the lichen *Trapelia involuta* growing directly on secondary uraniferous minerals at the South Terras mine, England.

The purposes of the present study are (i) to localize toxic metals in the lichen *T. involuta* at the South Terras mine, and (ii) to discuss on the possible mechanisms of the metal accumulation.

Samples and Methods

T. involuta is a dominant lichen growing directly on metatorbernite $[\text{Cu}(\text{UO}_2)_2(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}]$, metazeunerite $[\text{Cu}(\text{UO}_2)_2(\text{AsO}_4)_2 \cdot 8\text{H}_2\text{O}]$ and U-enriched Fe oxide and hydroxide minerals at the South Terras mine, Cornwall, SW England.

A schematic diagram of *Trapelia* apothecium is shown in Figure 1. A thallus is organized as layered tissues comprising algae and a medulla consisting of long-celled, loosely interwoven hyphae with high internal air space. Algae extend into the exciple. An apothecium is composed of four parts, an exciple, an

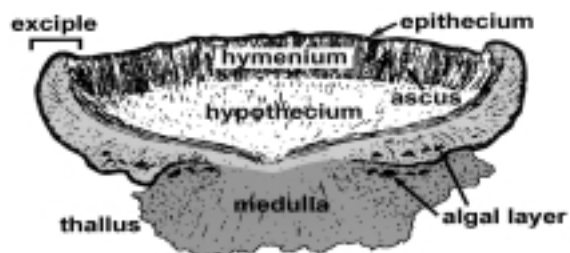


Fig. 1 Schematic diagram of a *Trapelia* apothecium

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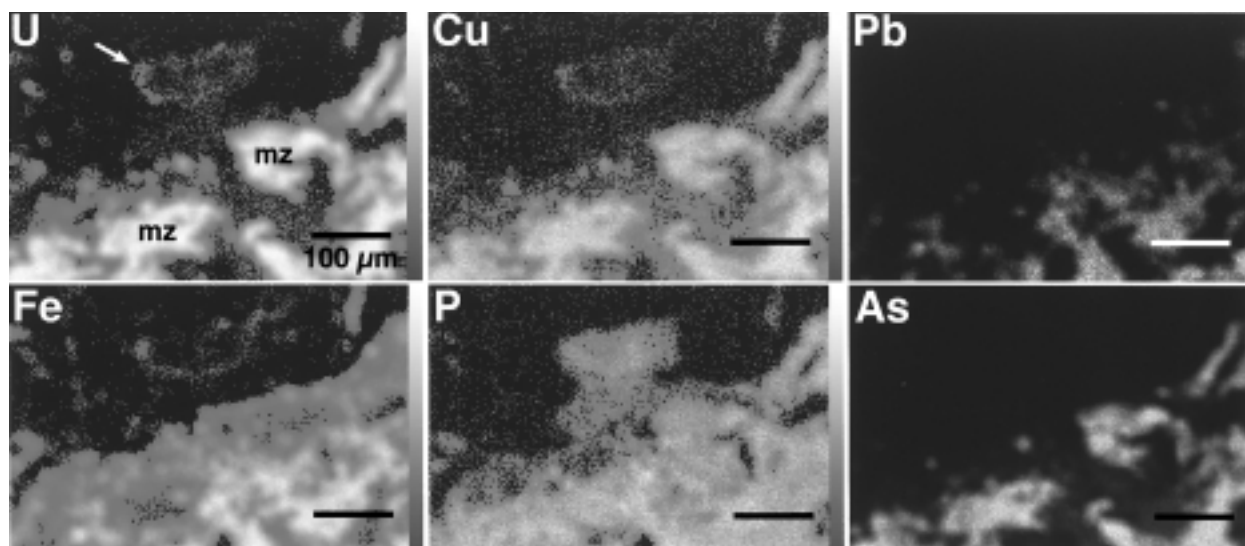


Fig. 2 X-ray element maps for U, Cu, Pb, Fe, P and As of a transverse section through the lichen-rock interface of *T. involuta* on metazeunerite at South Terras (images provided by McLean *et al.* (1998) and O.W. Purvis and his co-workers)
mz: metazeunerite
The arrows indicate the apothecium of *T. involuta*.

epithecium, a hymenium and a hypothecium. An exciple, the outer wall of an apothecium, consists of conglutinate, short-celled and thin-walled hyphae. An epithecium on a hymenium has apices of paraphyses embedded in a gel-like matrix. A hymenium consists of spore-containing asci and paraphyses. A hypothecium is a generative layer of hyphal tissue situated below the hymenium. Part of the hymenium and hypothecium is related to the reproduction of a lichen.

Crystals and minerals present in the *T. involuta* were examined by X-ray powder diffraction (XRD, Rigaku RINT2000) and field emission scanning electron microscopy (FESEM, Hitachi S4500) with energy dispersive X-ray spectrometry (EDS). For the FESEM examination, some of the samples were gently broken and then coated with Pt-Pd to obtain high resolution images and detect the presence of C of organic acid crystals.

For detailed examination of mineral species and their distributions in the lichen was observed by transmission electron microscopy (TEM). The *T. involuta* samples were fixed with glutaraldehyde and osmic acid, and embedded in epoxy resin. After sectioning the samples by ultramicrotomy, thin films were stained with uranyl acetate and lead citrate solutions. TEM observation was carried out using a JEOL JEM2010 operated at 200 kV.

The samples of the lichens with underlying rocks were carefully broken into small blocks, embedded in epoxy resin, polished, and coated with C to examine element distribution in the lichen-rock interfaces by

electron microprobe analysis (EPMA, Cameca SX50) operated at 15 kV and 80 nA. Samples of the lichen-rock interfaces were prepared according to the methods described in Williamson *et al.* (1998).

Cross sections of the *T. involuta* samples were prepared by microtomy to localize areas containing dark brown to black pigments. The sections were studied by light microscopy (LM).

Results and Discussion

The constituent minerals and the textures of the rock substrates beneath the lichen to a depth of about 2 mm were examined in detail by FESEM (Kasama *et al.*, 2001). Sericite was present at the top of the rock substrates and quartz in the lower part. Between the quartz- and sericite-bearing parts, coronadite [$\text{Pb}(\text{Mn}^{4+}, \text{Mn}^{2+})_8\text{O}_{16}$] and P-bearing metazeunerite were scattered in hematite matrix.

X-ray element maps by EPMA indicated strong localization of Cu, Fe and P in the lichen (Fig. 2), which were provided by McLean *et al.* (1998) and O.W. Purvis and his co-workers. Uranium, Cu and Fe in the lichen were mainly present in the exciple and epithecium which work as a protective wall for the apothecium and positively correlated to one another. Phosphorous was distributed throughout the apothecium and thallus, and rich in the hypothecium and ascus where the reproductive ascospores are present. The accumulation of Pb and As was not observed probably due to their low concentrations in the rock substrates and/or no selective

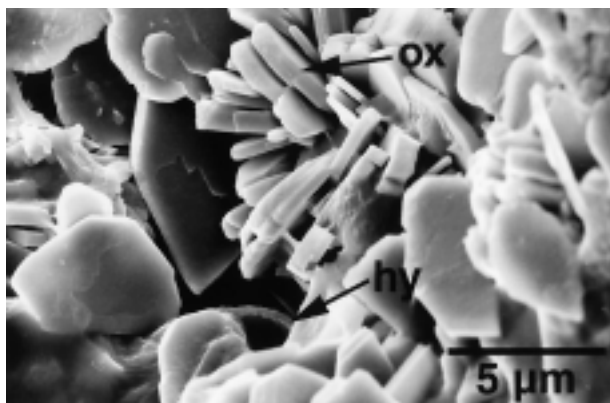


Fig. 3 SEM image outside of a *T. involuta* apothecium
Ca-ox: Ca-oxalate, hy: hyphae

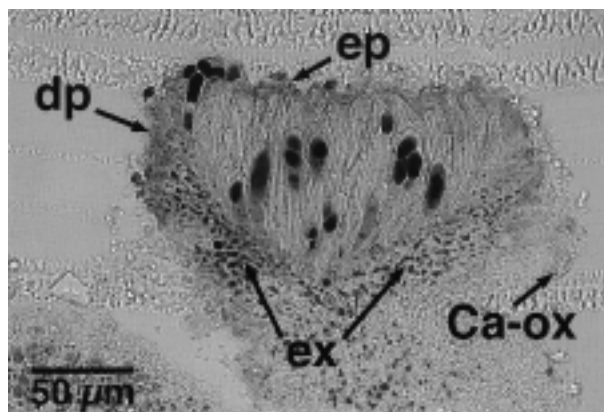


Fig. 4 LM image of cross sections of *T. involuta*
ex: exciple, ep: epithecium, dp: dark pigments, Ca-ox: Ca-oxalate

accumulation by the lichen. Therefore, the accumulation of Pb and As will be excluded from further discussion.

TEM observations revealed that at least nine mineral species including unidentified ones were distributed heterogeneously in the *T. involuta*. Sericite accompanied by an Fe-bearing mineral was found mostly in the medulla and occurred attached to and between hyphae and algae. Metazeunerite occurred in the medulla and on the epithecium but not inside the epithecium. Quartz was also found nearby metazeunerite on the epithecium. The minerals except for quartz are present in the intercellular space between loosely interwoven hyphae of the medulla that is in contact with the rock substrates. In contrast, U, Cu and Fe in the lichen are mainly distributed in the exciple and epithecium but not in the medulla (Fig. 2). Therefore, the presence of U, Cu or Fe mineral particulates such as metazeunerite can not account for the metal distribution in the lichen.

Lichens growing on mineralized substrata can make metal-lichen acid complexes such as Cu-norstic and -psoromic acid complexes (Purvis *et al.*, 1987; Purvis *et al.*, 1990). A gyrophoric acid that is a major lichen acid formed in *T. involuta* is present on and within the thallus (Coppins and James, 1984), but not in the exciple or epithecium that mainly contains U, Cu and Fe. This indicates that gyrophoric acid does not form any complexes with U, Cu or Fe and are not related to the accumulation of U, Cu or Fe.

XRD revealed that the crystals deposited on the exciple and epithecium were Ca-oxalates, weddellite [$\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$] and whewellite [$\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$]. Oxalates are formed by the primary metabolism of fungal hyphae. Purvis (1984) has found Cu-oxalates with some Ca-oxalates and silicate inclusions in the thallus of *Lecidea lactea* on a Cu-sulfide bearing rock by SEM. XRD and

FESEM with EDS have confirmed the presence of Ca-oxalates on the exciple (Fig. 3), but there is no evidence of precipitation of U-, Cu- or Fe-bearing oxalates. Therefore, the oxalate formation is not associated with the accumulation of U, Cu and Fe.

An apothecial disk in *Trapelia* is typically reddish to pinkish brown (Coppins and James, 1984) but *T. involuta* from South Terras has a dark brown to black apothecium attributed to the dark pigmented exciple and epithecium. The dark pigments are apparent even for the thin microtome section (Fig. 4). McLean *et al.* (1998) showed by decolorization by NaOCl and infrared analysis of the dark pigments that the dark pigments present in the exciple and epithecium of *T. involuta* from South Terras were melanin-like ones. They have suggested that the melanin-like pigments in *T. involuta* associated with the accumulation of U. Our results indicate that the distributions of U as well as Cu and Fe and that of the melanin-like pigments are strongly correlated to one another, i.e., they are mostly restricted in the exciple and epithecium (Figs. 2, 4). Melanin protects fungal hyphae from a wide range of environmental stresses including ultraviolet radiation (Bell and Wheeler, 1986) and metal contamination (e.g., Gadd, 1993). The role of melanin in fungal hyphae and the relationships of the distributions between U, Cu and Fe and the melanin-like pigments suggest that the melanin-like pigments in the exciple and epithecium have a high probability associated with the metal accumulation.

It is readily presumed that the apothecium and thallus of *T. involuta* contain polysaccharides because many lichenized fungi have neutral to acidic polysaccharides (e.g., Pinna *et al.*, 1998). Polysaccharides are negatively charged so that positively charged metal ions can be adsorbed onto their surfaces (e.g., Fortrin *et al.*, 1997).

Although we cannot completely exclude the possibility of the accumulation of U, Cu and Fe by polysaccharides or cells, polysaccharides and cells are present in the whole apothecium and thallus but U, Cu and Fe are distributed only in the exciple and epithecium of the apothecium. These suggest that either polysaccharides or cells is not probably responsible for the accumulation of U, Cu and Fe and that these metals are most likely to be bound to melanin-like pigments. The melanized exciple and epithecium seem to be an effective barrier protecting the reproductive ascospore by adsorbing the metals to the melanin-like pigments, which allows *T. involuta* inhabit even in extreme environments where other lichens are difficult to live.

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