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LICHEN ACCUMULATION OF SOME HEAVY METALS FROM ACIDIC SURFACE SUBSTRATES OF COAL MINE ECOSYSTEMS IN SOUTHEASTERN OHIO¹

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Surface coal mining results in the formation and release of sulfuric acid and the mobilization of large quantities of heavy metals. Terricolous lichens, particularly those of the genus *Cladonia*, are important invaders on spoil material left after mining in Ohio, and are known to be significant accumulators of various elements. We analyzed lichen samples and samples of the underlying substrate for P, K, Ca, Mg, Mn, Fe, Cu, Zn, Al, and Mo and found that lichens accumulated metals to many times the concentrations found in their substrates. *Cladonia cristatella* samples washed with water contained significantly less metals (Al, Fe, Mo) than the samples which were left untreated. Lichen material extracted with acetone showed no significant drop in metal content compared with untreated lichens. The accumulating nature of lichens in strip mine ecosystems makes them potential research tools in mineral cycling studies.

Surface coal mining involves the removal of the substrate overlying a coal seam which in the eastern U. S. is called overburden. It is usually high in sulfur-bearing pyritic compounds and subsequent weathering results in oxidation of the pyrites to sulfates. The resultant low pH conditions contribute to in-

creased solubility of numerous substrate elements, particularly metals (Grim and Hill, 1974). The high concentrations of heavy metals found in some strip mine spoil materials are thought to be significant in limiting the successful re-establishment of vegetation, natural or introduced, in these strip mine habitats (Barnhisel and Massey, 1969; Berg and Vogel, 1973).

Lichens appear to be among the more important early invaders of some primary bare areas following surface mining. Huey (1973) sampled the lichen vegetation which had colonized bituminous surface mines of various ages in Pennsylvania. She found that lichens, particularly *Cladonia* spp., were an important component of the ground cover very soon (as early as two years) after strip mines were abandoned and graded. Tuominen and Jaakkola (1973) reviewed much of the current literature regarding lichens as accumulators of minerals and radionuclides and indicated that they were important accumulators of specific elements. In arctic ecosystems where nutrients are frequently limiting, lichens have been implicated as important traps for elements (Williams *et al.*, 1975, in press).

The mechanism by which lichens accumulate metals is an interesting problem. One important role of lichens in nature was considered to be rock weathering and soil formation. Lichens were thought to be significantly involved in breaking down rock and contributing to primary soil development and plant succession. Whether or not this hypothesis is true, it stimulated research into the mechanisms by which lichens are able to

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bind substrate elements. The lichen compounds, those chemical compounds which are uniquely characteristic of lichen species and used extensively in lichen systematics, were thought to be involved. These compounds often contain polar groups (such as -OH, -CHO, -COOH) which are capable of donating electrons. Two such groups when in *ortho* positions favor chelation of cations (Iskandar and Syers, 1972; Williams and Rudolph, 1974). The water solubility of the lichen compounds is also determined by the number of these polar donor groups. Iskandar and Syers (1971), indicated that the solubility of these compounds is very low in water but they believe that the water solubility of these compounds is sufficient to allow them to participate in rock weathering. Williams and Rudolph (1974) showed that squamatic acid from *Cladonia squamosa* chelated iron, to a certain extent, from southern Ohio sandstone. Fumarprotocetraric acid from *Parmelia conspersa*, has been found to have a chelating capacity (Syers, 1969) and Iskandar and Syers (1972) showed that significant amounts of Ca, Mg, Fe and Al were complexed by lichen acid compounds. Syers and Iskandar (1973) recently reviewed the current research on metal accumulation by lichens

STUDY AREA

We collected lichens in NE sec 16, Clayton township, Perry Co., Ohio, a portion of a large (1782 ha.) reclamation area presently owned by the State of Ohio. Extensive strip mining activities from 1948 to 1960 have left a large portion of the area barren of all but scattered and transient vegetation. In those sections which have become secondarily reforested, black locust (*Robinia pseudo-acacia*) is dominant over other less numerous deciduous tree species. In various places where original revegetation attempts were successful, red pine (*Pinus resinosa*) is now dominant. Blackberries (*Rubus* spp.) are the dominant shrub species in all places. Lindsay (1974) examined the elemental content of numerous vascular plant species from this area to determine the suitability of strip mine habitats for wildlife. She found that

numerous important forage species contained high amounts of metals. Although lichens, mosses and some herbaceous plants are commonly abundant, approximately 15% of the total surface area is primarily bare and devoid of all vegetation (Bookhout *et al.*, 1967).

Our study reports on the elemental composition of *Cladonia* spp. collected in an abandoned, severely disturbed acid surface mine area in southeastern Ohio. The available amounts of heavy metals in the subjacent substrates were analyzed and some preliminary evidence is presented concerning the role of lichen acids in binding metal cations by *Cladonia cristatella*.

METHODS

We collected samples of representative terricolous lichens of the genus *Cladonia*, including *C. verticillata* (Hoffm.) Schaer., *C. subcariosa* Nyl., *C. capitata* (Michx.) Spreng., *C. cristatella* Tuck., and *C. subtenuis* (Abb.) Evans. These were handled with care to avoid contamination. Healthy podetia were clipped in the field, to remove them from their substrates, and were kept in paper envelopes. The substrate immediately subjacent to each lichen sampled was collected to a depth of 1 cm and placed in paper bags. Lichen material was oven dried 24 h at 105° C and ground in a Wiley mill (20 mesh screen). The concentrations of all elements reported here were determined from plant-ash solutions by spark-emission spectroscopy by a method described by Jones and Warner (1969). The available concentrations of Mn, Fe, Zn and Cu in the substrate samples were obtained by atomic absorption spectroscopy.

In order to determine the nature of the metal-accumulating mechanism of these strip mine lichens, samples of the most numerous lichen from our study area, *Cladonia cristatella*, were collected for laboratory treatment. The lichen acids characteristic of *Cladonia cristatella* (usnic, barbatic and didymic acids) are more soluble in acetone than in water (Asahina and Shibata, 1954). We extracted acetone-soluble and water-soluble substances, and then analyzed the washed lichen material that remained. All samples were air dried for 24 h at 25° C. One gram samples were then ground in a mortar and placed in 100 ml flasks. Half the flasks had 50 ml of acetone added, and to the other half, 50 ml of double distilled water was added. The flasks were shaken continuously for six h at 25° C. The lichen material was then filtered (Whatman #1 paper) and washed with 25 ml portions of either acetone or water. Then, all samples were oven dried for 24 h at 105° C, ground in a Wiley mill (20 mesh), and analyzed by emission spectroscopy. Data obtained from the lichens washed with acetone or water were then compared with

data from samples which had not been subjected to an extraction treatment. Statistical evaluation by *t* test was done for the *C. cristatella* data.

RESULTS AND DISCUSSION

Elemental analysis of five commonly occurring *Cladonia* spp. in the Perry Co. surface mine habitat (table 1) showed

composition. The two lichen species collected under a *Pinus resinosa* canopy, *Cladonia verticillata* and *C. subtenuis*, contain lower amounts of Mo.

Total amounts of metal elements in the lichens were compared with available amounts of the metals found in the substrate underlying each lichen collected

TABLE 1
Total accumulated selected elements in *Cladonia* species from acidic surface coal mine habitats in Perry Co., Ohio.

Species	P	K	Ca	Mg	Mn	Fe	Cu	Zn	Al	Mo
OPEN HABITAT										
<i>Cladonia cristatella</i>	1600*	3000	1000	300	27	1529	8	27	1682	5.26
<i>Cladonia capitata</i>	1500	3800	1000	300	27	1315	8	33	1829	10.31
<i>Cladonia subcariosa</i>	1500	3000	1000	300	59	385	18	37	1936	9.01
PINUS HABITAT										
<i>Cladonia verticillata</i>	1500	3000	1000	300	76	1085	7	36	1356	1.54
<i>Cladonia subtenuis</i>	1300	3000	1000	300	40	788	7	42	1293	1.38

*All data are expressed in ppm of oven dried weight.

that concentrations of the macronutrients P, K, Ca, Mg were extremely low. This is not surprising considering the small amounts of these elements found in strip mine substrates (Plass and Vogel, 1973). The concentrations of trace metal cations, particularly Fe and Al, were high relative to those observed for vascular plants in the same area (Lindsay, 1974). Molybdenum was also high in some species, a factor which may explain the low concentration of Cu, to which Mo is probably antagonistic. The species sampled show a general similarity in elemental

(Table 2). In all species sampled, there are greater concentrations of each metal cation found in the lichen than available in the subjacent substrate. This suggests that these lichen species accumulated Mn, Fe, Zn and Cu from the strip mine substrates. We were not able to determine available Al for the substrates, but we would assume that the lichens also accumulated Al to greater concentrations than are in available form in the substrate. The substrate pH data (table 2) indicate that these 15–25 year old surface mine habitats are still being adversely

TABLE 2
Total accumulated trace metal content and available substrate metal content for *Cladonia* species and their substrates.

Species	Mn		Fe		Zn		Cu		pH substrate
	total lichen	avail. substr.	total lichen	avail. substr.	total lichen	avail. substr.	total lichen	avail. substr.	
OPEN HABITAT									
<i>Cladonia cristatella</i>	27*	8	1529	58	27	2	8	1	3.3
<i>Cladonia capitata</i>	27	10	1315	89	33	6	8	2	3.4
<i>Cladonia subcariosa</i>	59	21	385	12	37	6	18	5	3.5
PINUS HABITAT									
<i>Cladonia verticillata</i>	76	14	1085	69	36	6	7	1	3.7
<i>Cladonia subtenuis</i>	40	13	788	98	42	4	7	1	3.6

*All data are expressed as ppm of oven dried weight.

affected by acid production. Thus, heavy metal mobilization is a continuing problem. The pH levels of the substrates beneath the *Pinus resinosa* canopy (for *C. verticillata* and *C. subtenuis*) are acidic, and unfavorably low for most plants.

These results indicate that *Cladonia* spp. are locally important invaders and residents in strip mine habitats with highly acidic substrates. Lichens are significant heavy metal sinks capable of accumulating certain metal cations to many times the concentrations found in their associated substrates. Such lichens may be important in trapping mobilized metals and removing them from surface run-off in disturbed ecosystems.

The total amounts of metals in *Cladonia cristatella* samples after washing and extracting with acetone or water are shown in table 3. The untreated

The pH of the lichen-water mixtures after washing ranged from 4.7 to 6.0, but there was no correlation between these pH levels and amounts of metals extracted. Less metal was found in the material washed with water, suggesting that the lichen acids are not contributing significantly to the accumulation of metal elements by *Cladonia cristatella*, but that metal cations are either adsorbed on the cell surfaces, or are loosely bound inside the cells.

There is contradictory evidence in the recent literature concerning the elution of metals from lichen material with distilled water. Subbotina and Tomotéeff-Ressovskii (1961) found that crustose lichens which had accumulated radioactive Fe and other metals released significant amounts of Fe in water solutions over a period of eight days. These data support our findings. Brown and

TABLE 3
Total trace metal content of strip mine *Cladonia cristatella* samples
after extraction with either acetone or water.

	Mn	Fe	Cu	Zn	Al	Mo
<i>Cladonia cristatella</i> untreated	29± 6*	1226±406	12±4	37±9	1498±173	3.04±1.69
washed six hours with acetone	20± 1	1140±378	12±1	101±21	1529±172	2.32±0.61
washed six hours with water	20±11	661±157***	14±5	64± 8	1078± 99**	1.11±0.15

*All data expressed as ppm ± standard deviation of oven dried weight (Sample Size N=4).

**Significantly less than untreated sample of 1% significance level.

***Significantly less than untreated sample at 5% significance level.

samples contain concentrations of metals comparable to those found in the *Cladonia* spp. from the original analyses (table 1). Those samples which were washed with acetone do not contain significantly lower amounts of metal than the untreated samples. The samples washed with water exhibit concentrations of Al, Fe and Mo which are significantly lower than the untreated samples. Zinc exhibited extreme variability from one thallus sample to another and appears to accumulate unevenly. Zinc has been reported to have a wide range (up to 1000×) of concentrations in lichens from the same locality (Shimwell and Laurie, 1972; Tuominen and Jaakkola, 1973).

Slingsby (1972), however, found that distilled water did not elute appreciable amounts of Pb or K from lichen material and Puckett *et al.* (1973) were unable to elute significant amounts of metal ions (Fe, Cu, Ni, Zn, Pb) from several lichen species, including *Cladonia* spp. They contended that ion exchange, rather than electrolyte sorption, was the most important process involved in the accumulation of metals by lichens. Our data do not necessarily contradict their contention, but do indicate that under certain circumstances, water does elute significant amounts of certain metals from lichens.

The fact that heavy metals may be

removable by water is somewhat contradictory to our suggestion that lichens are metal accumulators in strip mine habitats. The natural situation, however, is very different from our artificial laboratory situation. In the strip mine habitat under low pH conditions, heavy metals become soluble. In this form they are continually available for uptake by lichens, especially during water movement. Under field conditions, the only water which participates in extraction is due to natural precipitation. Rain water, particularly low pH rain water, probably removes small amounts of the heavy metals which were previously accumulated by lichens. Lichen accumulation of heavy metals could occur concomitantly with mobilization and release of these metals during periods of heavy precipitation. We suggest that the position lichens assume in the cycling of minerals through these ecosystems makes them important components in the analysis and modeling of such systems.

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