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Discrimination of lichen genera and species using element concentrations

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Abstract: The importance of organic chemistry in the classification of lichens is well established, but inorganic chemistry has been largely overlooked. Six lichen species were studied over a period of 23 years that were growing in 11 protected areas of the northern Great Lakes ecoregion, which were not greatly influenced by anthropogenic particulates or gaseous air pollutants. The elemental data from these studies were aggregated in order to test the hypothesis that differences among species in tissue element concentrations were large enough to discriminate between taxa faithfully. Concentrations of 16 chemical elements that were found in tissue samples from *Cladonia rangiferina*, *Evernia mesomorpha*, *Flavopunctelia flaventior*, *Hypogymnia physodes*, *Parmelia sulcata*, and *Punctelia rudecta* were analyzed statistically using multivariate discriminant functions and CART analyses, as well as *t*-tests. Genera and species were clearly separated in element space, and elemental discriminant functions were able to classify 91–100% of the samples correctly into species. At the broadest level, a Zn concentration of 51 ppm in tissues of four of the lichen species effectively discriminated foliose from fruticose species. Similarly, a S concentration of 680 ppm discriminated *C. rangiferina* and *E. mesomorpha*, and a Ca concentration of 10 436 ppm discriminated *H. physodes* from *P. sulcata*. For the three parmelioid species, a Ca concentration >32 837 ppm discriminated *Punctelia rudecta* from the other two species, while a Zn concentration of 56 ppm discriminated *Parmelia sulcata* from *F. flaventior*. Foliose species also had higher concentrations than did fruticose species of all elements except Na. Elemental signatures for each of the six species were developed using standardized means. Twenty-four mechanisms explaining the differences among species are summarized. Finally, the relationships of four species based on element concentrations, using additive-trees clustering of a Euclidean-distance matrix, produced identical relationships as did analyses based on secondary product chemistry that used additive-trees clustering of a Jaccard similarity matrix. At least for these six species, element composition has taxonomic significance, and may be useful for discriminating other taxa.

Key words: *Cladonia rangiferina*, *Evernia mesomorpha*, inorganic chemistry, lichen products, parmelioid lichens, taxonomy

Introduction

It is well established that secondary chemical metabolites are unique to lichen species and have become diagnostic characters in lichen taxonomy (reviewed by Elix 1996). Hundreds of these distinctive compounds, all of them organic, have been identified; they allow for consistent discrimination of lichen species not only across their geographic ranges, but also across habitats. Organic chemicals have been used to define taxonomic groupings not only among species, but also for populations up through the genus level. It is now recognized that chemical traits are under genetic control because of constancy even when populations and individuals are sympatric.

In contrast to this well-developed understanding of differences among species in organic chemistry, the possibility that lichen species might differ in terms of their elemental composition is recognized (Brown 1976; Haas & Purvis 2006) but poorly understood in terms of taxonomy. Elements may exist in lichen tissues in the form of entrapped

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particles or in ionic form (as anions and cations). Generally speaking, inorganic element concentrations are believed to be less tightly controlled genetically but instead are determined more by environmental factors (Cornelissen *et al.* 2007). Consequently, some of these compounds act as nutrients, moisture regulators (e.g. Ca oxalate), or sun screens. Both quantitative and qualitative aspects of inorganic compounds are important for lichen function and survival.

In addition, the inorganic elemental chemistry of lichens is widely used in biomonitoring and the study of air pollution impacts. In these studies, the quantity and identity of elements are studied to determine external influences on lichen populations and to monitor elemental contamination. Hundreds of studies using lichens to map or describe the patterns of heavy metals in the environment exist in the literature (Garty 2001). Most of these are based on sampling one or a few species, and only a few compare several species in the same study.

In an earlier study it was discovered that four lichen species (*Cladonia rangiferina*, *Evernia mesomorpha*, *Hypogymnia physodes* and *Parmelia sulcata*) sampled from the Boundary Waters Canoe Wilderness in northern Minnesota, USA, differed significantly in multivariate element space to such an extent that almost no overlap between species was observed (Bennett & Wetmore 1999). The effects of sample locality and year of collection over an 11-year time span did not differ significantly in element space and overlapped considerably. Using principal component analysis (Manly 1994), it was noted that the foliose and fruticose species were significantly discriminated by one of the components, suggesting that the biology of these species somehow confers special elemental affinities to these lichens. This study was based on 2774 data points from the one area.

For the past 23 years, I have collected samples of 11 lichen species within 13 protected areas (i.e., national parks, wilderness areas) in the northern Great Lakes ecoregion of the United States and analyzed their elemental chemistry for environmental studies (summarized in Bennett 2006). In addition to the Boundary Waters study, the same four species were collected for elemental analyses from ten other areas (Table 1), resulting in over 35 000 records of concentrations of elements in lichen tissues. Using these data, it became possible to examine the species in elemental space using much larger sample sizes. In this paper element concentrations in four lichen species (one species from each of four currently recognized genera) are analyzed to test the hypothesis that element concentrations were significantly different between species and could be used to discriminate taxa. In addition, because three other species sampled (*Flavopunctelia flaventior*, *Parmelia sulcata* and *Punctelia rudecta*) are closely related and used to be considered as congeners within *Parmelia* (Brodo *et al.* 2001), the resolving power of the methods at the level of species within a genus (*sensu lato*) were tested.

Materials and Methods

Detailed methods of the individual studies are described in numerous publications (e.g. Bennett 1995; Bennett & Wetmore 1999, 2003) and the metadata that are available at the *NPElement* website: www.nwhc.usgs.gov/our_research/np_element.jsp. The six species selected for analyses in this study were sampled at 11 national and state parks and two national forests in three states: Michigan, Wisconsin and Minnesota (Table 1). These species were selected because they are common and abundant in this region, easy to collect and analyze for element concentrations, and occur in all the study areas. Investigations occurred during 1983–2005 and included sampling at a total of 162 localities. Lichens were sampled in each year during this period except for 1989, 1994, and 1996. The numbers of lichen samples are shown in Table 1. The numbers in each cell are the total number of lichens sampled across all localities and years of sampling for each species and study area. These numbers approximate closely the number of observations for each element measured, although some vary slightly because of missing data, values below detection limits, or other factors.

Briefly, individual lichen thalli were collected from multiple substrata at each locality and aggregated into a bulk sample placed in a perforated spun polyethylene bag which allowed the sample to dry. Each bagged collection was then manually cleaned of debris and foreign material, and ground unwashed, to a fine powder using both stainless steel and polytetrafluoroethylene types of mills. Grinding times were short enough to avoid element contamination. The powdered samples

TABLE 1. *Number of samples of two fruticose and two foliose lichen species collected within each of 11 protected areas. Not listed are two foliose species*, Flavopunctelia flaventior *(n=21 samples, at Voyageurs National Park only) and* Punctelia rudecta *(n=54 samples, at St. Croix National Scenic River only)*

Study area	*Cladonia rangiferina*	*Evernia mesomorpha*	*Hypogymnia physodes*	*Parmelia sulcata*	Total
Apostle Islands National Lakeshore, Wisconsin	41	32	32	23	128
Boundary Waters Canoe Area Wilderness, Superior National Forest, Minnesota	84	64	64	54	266
Grand Portage National Monument, Minnesota	15	12	12	12	51
Isle Royale National Park, Michigan	62	108	108	99	377
Keweenaw National Historical Park, Michigan	14	22	22	22	80
Pictured Rocks National Lakeshore, Michigan	28	23	27	0	78
Rainbow Lake Wilderness, Chequamegon-Nicolet National Forest, Wisconsin	3	18	18	18	57
St Croix National Scenic River, Minnesota and Wisconsin	9	9	10	27	55
Sleeping Bear National Lakeshore, Michigan	23	8	12	8	51
Tahquamenon Falls State Park, Michigan	3	3	0	0	6
Voyageurs National Park, Minnesota	222	183	105	186	696
Total	504	482	410	449	1845

were stored in plastic bags until elemental analyses. Most analyses were done by inductively-coupled argon plasma spectrometry (ICP, either optical emission or mass spectrometry), or infrared absorption (for sulphur) at either the University of Wisconsin Soil and Plant Analysis Laboratory or the University of Minnesota Research Analytical Laboratory. Sample streams included internal laboratory checks or spikes by standard reference materials for quality assurance. Recoveries varied, and are reported for the individual studies.

Although originally these studies included analyses of 29 elements plus ash content, some of the elements were not analyzed in all studies and hence have smaller sample numbers. In order to base this study on the largest possible sample sizes, tissue concentrations for only the following 16 elements were used: Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S and Zn. Sample numbers are presented in Tables 1, 2 and 5.

Concentrations of many, but not all, of the elements were log-normally distributed and skewed to the right (indicating possible contamination—see below), but the number of high concentration values was extremely small. Differences between means and medians were 3–6% on average and ranged from 2% for K up to 36% for B. This is important to point out because high concentrations of anthropogenic elements that may be due to pollution should not be included in a study of this type. Including such data would obviously bias the results away from true biological differences in favour of environmental causes. Instead, element patterns in species should reflect concentrations in unaffected environments, where the lichens grow normally without external influences. The large sample sizes in this study are primarily from areas that are not influenced greatly by external influences, and the concentrations probably represent normal elemental levels for these species.

Of the >35 000 data points, less than 0·2% were below detection limits of the measurement equipment. When the number of below-detection-limit values for any single species by study and year dataset was less than 30% of the values they were substituted with 0·7 times the detection limit. This was done to maintain as high a sample number as possible. When above this threshold the below detection limit values were omitted. A similar number of values (<1%) were analyzed as laboratory splits to determine the precision of the data and were averaged. Finally, the accuracy of the data was checked in every sample run by analyzing standard reference materials and comparing the results with the certified values. Information on these results is available for every study, either in published accounts (see references) or in the metadata referenced above.

I first tabulated the means of the elements by species. The entire data matrix was then subjected to a multivariate analysis to derive discriminant functions to separate the species by element concentrations. Unlike principal component analysis, discriminant analysis uses dependent variables to predict the members of a grouping variable (Gerdol *et al.* 1985). This was followed by Classification and Regression Trees (CART) analyses (Breiman *et al.* 1984; Urban 2002) to derive threshold concentrations that separated species. Next, element means by species were standardized to standard deviation units within elements (thereby scaling the elements similarly) to derive signature element patterns that were unique to each species. Finally, to compare similarities of taxa based on secondary chemistry and elemental compositon, mathematical distances among species were computed. The secondary chemistry of the first four species was tabulated from the data in Culberson & Culberson (1970), and scored in terms of presence or absence in a matrix of 22 substances.

TABLE 2. *Mean element concentrations (ppm) in two fruticose and two foliose lichen species, sampled at 11 protected areas in the western Great Lakes ecoregion during 1983–2005*

Element	*Cladonia rangiferina*	*Evernia mesomorpha*	*Hypogymnia physodes*	*Parmelia sulcata*
Al	337·6	628·1	558·9	767·1
B	2·63	5·76	4·91	5·94
Ca	802·7	2591·6	22 973·9	4160·1
Cd	0·17	0·29	0·98	0·49
Cr	0·63	1·20	1·19	1·24
Cu	2·05	3·46	5·39	6·49
Fe	373·1	843·4	713·4	862·1
K	1541·1	2500·5	3335·7	3494·5
Mg	323·8	406·2	859·7	644·2
Mn	39·4	43·5	214·6	160·3
Na	31·3	45·8	34·7	31·8
Ni	0·83	1·54	3·36	2·06
P	478·1	661·1	867·6	1336·6
Pb	2·63	6·29	14·9	15·0
S	457·5	1208·4	1015·5	1156·0
Zn	16·7	33·6	75·4	89·9
Number of samples	432	443	386	436

The distances between taxa based on organic versus elemental chemistry were then computed as Jaccard (from the organic chemistry presence/absence matrix) and Euclidean matrices (from the original elemental chemistry concentration data) respectively, and analyzed by additive-trees clustering (Sattath & Tversky 1977). These analyses were conducted using *Systat 11* and *JMP 6*.

Results

Differences among the two foliose and two fruticose species

The mean element concentrations and the sample numbers (mean=424 samples) for the comparisons of two foliose and two fructose species are presented in Table 2. It is obvious that concentrations of particular elements differ remarkably among species. Rather than assessing statistical significances of differences among species for each element individually, this study focused instead on patterns of differences across many elements.

For some of the elements in Table 2, the means were greater than the medians, because of the distributions being lognormal. Only K, S and Zn were consistently normally distributed in all four species. For elements with lognormal distributions, medians more accurately depict central tendencies. However, due to the huge number of observations around the means, the differences between the means and medians were relatively small. The log-normally distributed elements were right-skewed, and the outlier observations ranged from 1–8% of the total number of samples, depending on species, and averaged 3% of the total dataset. These few outliers did not influence the statistical analyses significantly, and were not removed from the dataset.

In the *H. physodes* samples, tissue concentrations of Ca were 6474 ppm (i.e., 39%) above the enrichment threshold for this species (Bennett 2000). Similarly, tissue concentrations of two other elements, K and Mn, were only 1% and 4% below the enrichment thresholds for those elements. Concentrations of all other elements were significantly below enrichment thresholds. As discussed below, the Ca concentrations in *H. physodes* are consistently high in this region, compared to the values in the species across the rest of its geographic range.

Discriminant analysis of the four species extracted four classification functions, one for each species, and then classified the original samples to one of the four

TABLE 3. *Classification matrix of samples into four lichen species**

	Cladonia	*Evernia*	*Hypogymnia*	*Parmelia*	Actual total	% correct
Cladonia	427	5	0	0	432	99
Evernia	17	426	0	0	443	96
Hypogymnia	0	3	374	9	386	97
Parmelia	1	5	2	428	436	98
Total classified	445	439	376	437	1697	98

*Numbers in each cell represent the actual samples for each species in the rows that were classified by the classification functions into predicted species in columns. For example, 427 actual *Cladonia* samples were correctly classified as *Cladonia*, but 5 were incorrectly classified as *Evernia*.The species are listed only by their generic names for the sake of brevity.

species based on their tissue concentrations (Table 3). The functions correctly classified 96–99% of the samples. Three discriminant factors were calculated to separate the four species, and the factors were plotted against one another in Fig. 1A. The canonical means of the factors are given in Table 4, showing which genera were separated by each factor by the size of the means. The most important factor (Factor 1), which accounted for 59% of the data variance, clearly separated the fruticose from the foliose species. The second most important factor, which accounted for 28% of the data variance, clearly discriminated between *H. physodes* and *P. sulcata* samples. The last factor, which accounted for the remaining 13% of the data variance, discriminated *C. rangiferina* from *E. mesomorpha*. This can be seen in the groupings in Fig. 1A and in the canonical means (Table 4).

The factors in Fig. 1A are composed of the chemical elements and each element is weighted on the factor according to its importance. The elements that were most important on Factor 1 were Ca, K, Mg and Zn (weightings not presented). Since these were negatively weighted, it appears that the fruticose species, which appear to the right on this factor, are low in these elements, while the foliose species are high in them, as can be seen in Table 2. Factor 2 is positively weighted with Al and P, and negatively weighted with Ca and Mg. *Parmelia sulcata* is higher in tissue concentrations of Al and P than is *H. physodes*, while the opposite is true for Ca and Mg (Table 2). Factor 3 is positively weighted with Al and P, and negatively weighted with K and S. *Evernia mesomorpha* is higher in all four of these elements than is *C. rangiferina*, and the discrimination between the two species, while effective, is not as great as Factor 2's discrimination of the two foliose species. Factors 2 & 3, which together accounted for 41% of the variance, effectively discriminated between all four species, while Factor 1, which accounted for 59% of the variance, discriminated between the thallus types.

Using recursive partitioning, CART analyses identified Zn as the most important element for the first separation: that between foliose and fruticose species (Fig. 2A). Tissue concentrations of Zn <51 ppm occurred in the two fruticose species, whereas foliose species had Zn concentrations greater than 51 ppm. The two foliose species were then separated at a Ca concentration of 10 436 ppm, and the two foliose species were separated at a S concentration of 680 ppm. The actual sample numbers in the terminal nodes were close to those in Table 2. The four species can arguably be separated by just two elements, Ca and S. A sample with low tissue concentrations of both Ca and S is from *C. rangiferina*; one with low Ca and high S is *P. sulcata*, one with high Ca but low S is *E. mesomorpha*, and finally one with high Ca and S is a *H. physodes*. In this set of four species, Zn is an element that discriminated thallus types, whereas Ca and S discriminated between species (Fig. 2A).

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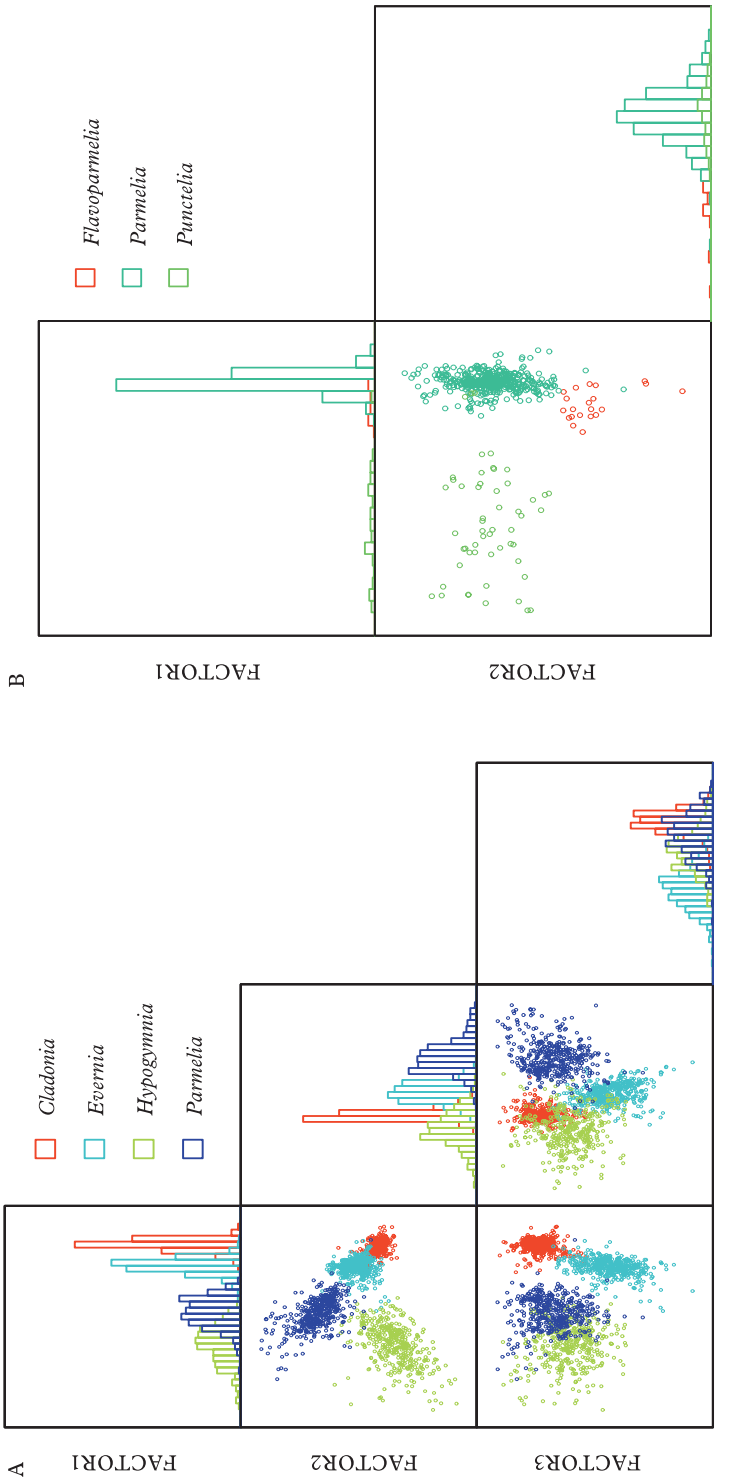

FIG. 1. The separation of lichen species using discriminant factors based on elemental chemistry. A, four species from diverse genera; B, three parmelioid species. See text for explanation. Each factor is plotted against the others with the four species shown in colour. Histograms show score distributions for each species. Species are labelled with generic names only for the sake of brevity.

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TABLE 4. *Canonical means of four lichen species resulting from the discriminant analysis**

Species	Factor 1	Factor 2	Factor 3
Cladonia (C)	3·286	− 1·217	1·37
Evernia (E)	1·751	0·347	− 2·003
Hypogymnia (H)	− 3·895	− 2·298	− 0·087
Parmelia (P)	− 1·586	2·887	0·755
Genera separated	C,E vs H,P	H vs P	C vs E

*Each number is the canonical mean of the classified data for each species and factor and shows by its magnitude and sign how the factor separates species, shown by letters after the generic names in column 1. For example, Factor 1 has positive values for *Cladonia* and *Evernia*, and negative values for *Hypogymnia* and *Parmelia*, thus separating them. Factor 2 has the largest means for the two foliose species, and Factor 3 has the largest means for the two fruticose species, and in each case they are subdivided by the signs of the means. The species are listed only by their generic names for the sake of brevity.

After standardization to scale the concentrations similarly, the means for each species are presented in Fig. 3. *Cladonia rangiferina* is low in every element compared to the other three species, but is particularly low in tissue concentrations of Al, B, Cr, Cu, Fe, K, Ni, Pb, S and Zn. *Evernia mesomorpha* has distinctive tissue concentrations for only one element: it is unusually high in Na. *Hypogymnia physodes* is high in five elements: Ca, Cd, Mg, Mn and Ni. In contrast, *P. sulcata* is high in Al, Cu, P and Zn. Each species thus has a unique signature of elements.

Differences among the three parmelioid species

Mean concentrations of the various elements in lichen tissues collected during 1983–2005 and the sample numbers for the three parmelioid species in the second analysis are presented in Table 5.

This discriminant analysis resulted in three classification functions, which then classified the samples into the three species 91–100% correctly (Table 6). Two discriminant factors were then calculated to separate the three species, and these are plotted in Fig. 1B. The canonical means show how each factor separated the species in Table 7. The first factor accounted for 91% of the dispersion of data and the second factor accounted for the remainder (9%). Hence the most important factor clearly discriminated tissue concentrations of *Punctelia rudecta* from those of the other two species, while the second factor discriminated *Parmelia sulcata* from *Flavoparmelia flaventior.* Except for a few outliers, the discrimination of species was excellent.

Factor 1 is dominated by negatively weighted Ca, and since *Punctelia* occurs at the negative end, this corresponds with the high concentrations seen in this species in Table 5. There is a tendency for Al and P to be positively weighted on this factor as well, both of which are lower in *Punctelia* than the other two species. Factor 2 is dominated by positive weightings of Al and maybe K, Mg and Zn, and negative weighting of Fe and possibly Na. These correspond with the higher concentrations of these elements in either of the two other species, indicating that this factor was not as powerful in discriminating between them as the first factor was in discriminating them from *Punctelia*.

The CART tree results are shown in Fig. 2B. The first branch separated all 51 *Punctelia* samples from the other two species at Ca concentration of 32 837 ppm, which agrees with the mean values in Table 5. The next branch separated *P. sulcata* from the *Flavoparmelia* at Zn concentration of 56·2 ppm.

After standardization to scale the concentrations similarly, the means for each species are presented in Fig. 4. It can be seen that *Punctelia* is characterized by high Ca and Cd, and low Al, Fe, Ni, P, Pb and S compared to the other two species. *Parmelia sulcata* is characterized by high B, Cu, K, Mn and Zn, while *Flavoparmelia* is characterized by high Cr, Fe, Na and low Mg.

Discussion

This is not the first study to document differences among species in element concentrations. However, it may be the first to

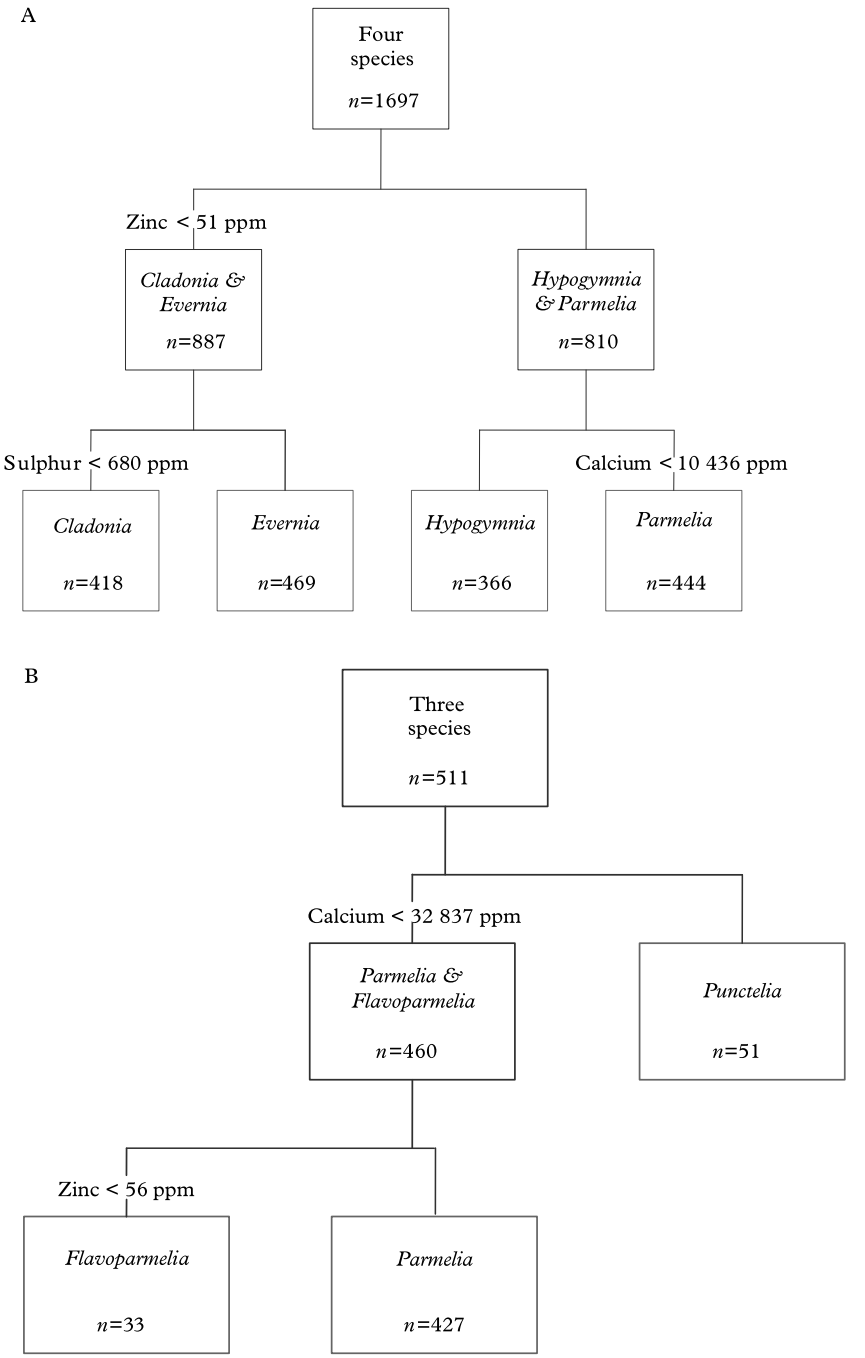

FIG. 2. CART classification tree separating lichen species* based on inorganic element concentrations. A, four species from diverse genera; B, three parmelioid species. Sample size is indicated at the bottom of each box. The concentration separating any pair is given on one arm of the branch with a < symbol, and the other arm represents concentrations > the given concentration.
*Species are labelled with generic names only for the sake of brevity.

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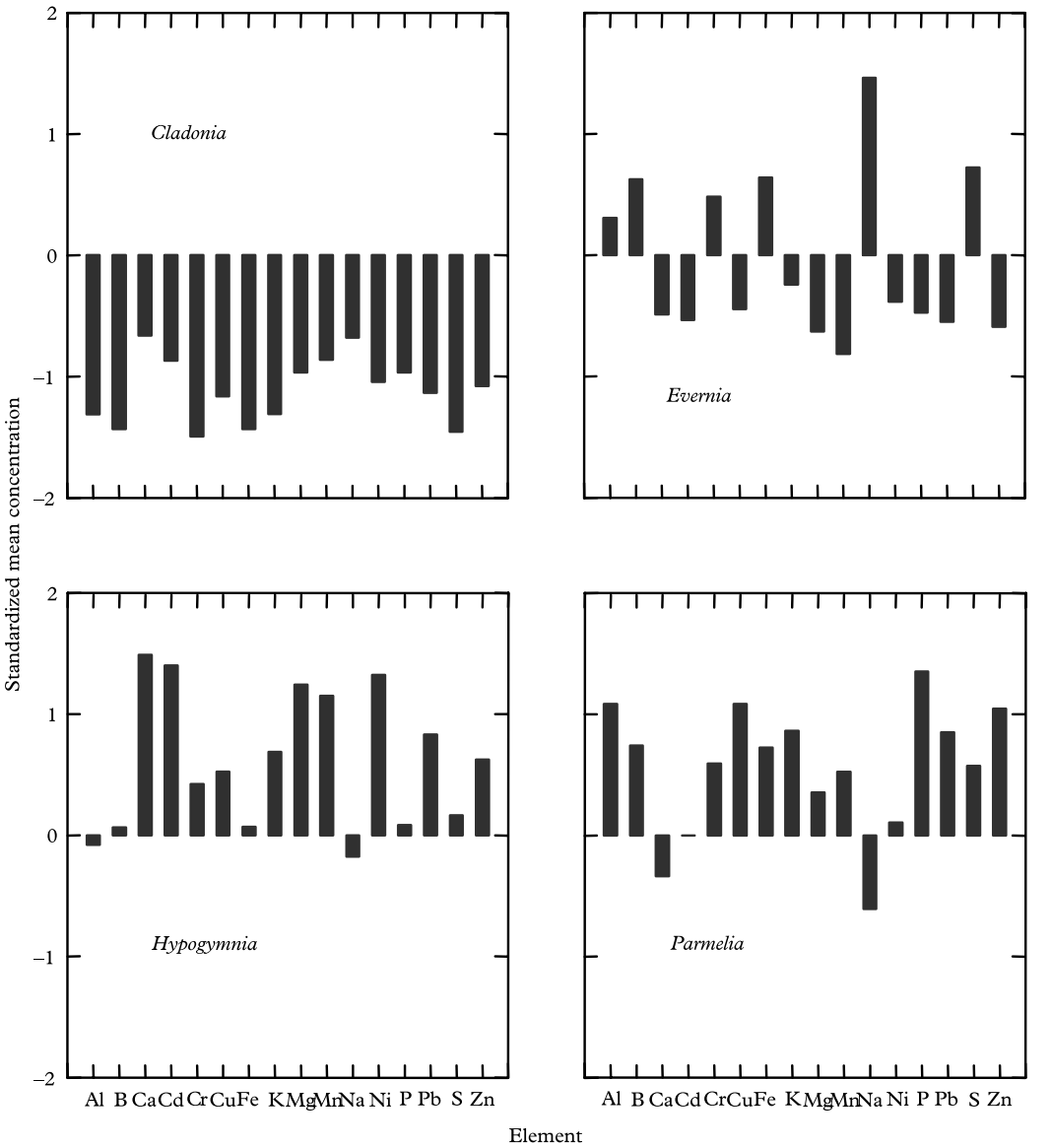

FIG. 3. Standardized mean concentrations of 16 elements in four lichen species* from diverse genera. Means were standardized by the standard deviations of each element for each species in order to scale them to standard deviation units (*y* axes) for comparisons among species. Standardized means >1 or < − 1 were used to describe element signatures for each species.
*Species are labelled with generic names only for the sake of brevity.

document such differences across a broad geographical range (three states) and over such a long time span (23 years). The differences in concentrations of elements in lichen tissues among species are greater than those between the states or years of the studies (to be presented in subsequent papers). This suggests that patterns of element concentrations among lichen taxa are relatively constant across time and space,

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TABLE 5. *Mean element concentrations (ppm) in three lichen parmelioid species sampled in 11 protected areas in the western Great Lakes ecoregion during 1983–2005*

Element	*Flavoparmelia flaventior*	*Parmelia sulcata*	*Punctelia rudecta*
Al	821·2	767·1	639·1
B	2·76	5·94	2·93
Ca	14 053·0	4160·1	75 588·3
Cd	0·50	0·49	0·66
Cr	1·65	1·24	1·15
Cu	3·70	6·49	4·45
Fe	1178·3	862·1	523·8
K	3001·5	3494·5	2737·0
Mg	521·5	644·2	651·9
Mn	70·9	160·3	55·6
Na	46·5	31·8	30·8
Ni	2·45	2·06	1·33
P	1227·5	1336·6	882·1
Pb	15·1	15·0	13·1
S	1165·2	1156·0	975·1
Zn	49·2	89·9	34·9
Number of samples	21	436	54

and therefore could be biologically meaningful. How reliable these patterns are for this purpose in other geographic areas, in other taxa, or under the influence of anthropogenic contamination remains to be studied further.

In the first analysis of the four taxa, the species are all in separate genera, so the use of the term ''species'' is interchangeable with ''genera''. The separation of the taxa therefore, is a combined separation of species and their genera. The taxa were separated with high accuracy (96–99% correct), but it is not possible in this analysis to determine whether the separation is at the genus or the species level. In the second analysis, however, the three taxa were originally all in one genus (*Parmelia)*, and they are separated with 91–100% accuracy. Of course they are now considered to be in three separate genera, so given this nomenclature the separation is also at the genus level. A rigorous test of the hypothesis at the species level, all in one genus, is still needed.

The interrelationship, if any, between inorganic and organic substances is probably most interesting to explore because organic chemistry is already used in species taxonomy. Based on organic chemistry (Fig. 5A), it is clear that the two foliose species are very similar to each other, having three substances in common (atranorin, physodic acid and calcium oxalate). In contrast, the two fruticose species appear very dissimilar. *Evernia mesomorpha* is the most dissimilar of the four as it has no secondary products in common with any of the other species. *Cladonia rangiferina* is intermediate, having atranorin in common with the two foliose species.

A similar analysis of the same four species based on elemental chemistry is shown in Fig. 5B, using Euclidean distances between the species calculated from the concentration data. The same pattern separating the two foliose species from the two fruticose species is observed. *Hypogymnia physodes* and *P. sulcata* are the closest species chemically, and *C. rangiferina* is the most dissimilar. The parallel separation of taxa by both organic and elemental chemistry is remarkable.

The separation of taxa using elemenal analysis chemistry is based on quantitative variation in concentrations of elements. There is precedent for this in secondary product chemistry in Culberson's chemosyndrome concept (Culberson & Culberson 1976; Egan 1986). Here the separation of taxa in several genera was based on the relative amounts of organic products, even though Hawksworth (1976) recognized no separation of taxa based on chemical concentrations. More recently Lumbsch (1998) has come out in favour of chemosyndromes, stating that different populations with strong chemical variation in concentration might justify taxonomic recognition.

If lichen species differ biologically in elemental composition, it follows that the identities of species can be portrayed in the form of elemental signature patterns. This is not to be confused with a pollution signature in lichens caused by high concentrations of pollutant elements (e.g. Purvis *et al.* 2005). Instead, the affinity of species for certain elements in a natural setting across the species range is a pattern that has biological

TABLE 6. *Classification matrix of samples into three parmelioid lichen species**

	Flavoparmelia flaventior	*Parmelia sulcata*	*Punctelia rudecta*	Actual total	% correct
Flavoparmelia flaventior	20	1	0	21	95
Parmelia sulcata	2	434	0	436	100
Punctelia rudecta	0	5	49	54	91
Total classified	22	440	49	511	98

*See Table 3 for an explanation of how to read this Table.

TABLE 7. *Canonical means of three parmelioid lichen species resulting from the discriminant analysis**

Species	Factor 1	Factor 2
Flavoparmelia flaventior (FF)	− 0·298	− 3·392
Parmelia sulcata (PS)	0·804	0·138
Punctelia rudecta (PR)	− 6·376	0·203
Species separated	PR vs FF, PS	FF vs PS

*See Table 4 for an explanation of how to read this Table.

and taxonomic meaning. In this study, the signatures were revealed after re-scaling the concentrations to standard deviation units. As more data become available it might prove possible to develop a standard reference lichen and fingerprint graphs for individual species (Markert 1992).

The role of Zn appears to be very important in separating species. In the first analysis it separated foliose from fruticose lichens, but in the second analysis it separated two foliose species at approximately the same concentration level, about 50–55 ppm. Nimis *et al.* (2001) also noted that Zn was important in separating *Xanthoria* from *P. sulcata*, the latter having concentrations above the same level of 50–55 ppm. They suggested this was due to higher chelation of Zn by the presence of more lichen substances in *P. sulcata*. This, however, does not explain why some species have such an affinity or what the function of Zn is in the tissues. Zn is thought to concentrate in the cells walls of the mycobiont, where it might be important structurally in the formation of filamentous proteins and/or the cross-linking of phenolic compounds, which are common in lichens (Frausto da Silva & Williams 2001). In vascular plants Zn is an essential element, so presumably it is for lichens also, and the role of Zn is well established (Broadley *et al.* 2007). It has been shown that >70% of the variation in Zn concentrations in plant shoots exists within genera and species, and in some species Zn concentration is a quantitative trait that can be mapped to quantitative trait gene loci (Broadley *et al.* 2007).

Calcium and S also appear to be important in separating species. Calcium, in the form of calcium oxalate, plays a major role in regulating hydration levels in tissues and maintaining K balance (Kuziel 1973; Flechter 1976). It is therefore important in determining the type of moisture habitat a species favours, i.e. dry versus wet. In the case of the species in this study, however, all are found in similar shady habitats (woodlands) with similar moisture regimes, so that other roles of Ca in these species should be explored. Different forms of Ca oxalate have been related to the medullary architectures that separate *H. physodes* and *Menegazzia terebrata* (Modenesi *et al.*, 1997) which might be a factor in this study. Sulphur is of course a macronutrient, and important for growth. In this study, *E. mesomorpha* was separated from *C. rangiferina* on the basis of higher S content. It also has a larger thallus, suggesting that perhaps it requires more S for growth.

If we accept these findings and agree there is a biological basis for elemental concentration differences among lichen species, it becomes necessary to explain what would cause them. Many mechanisms have already

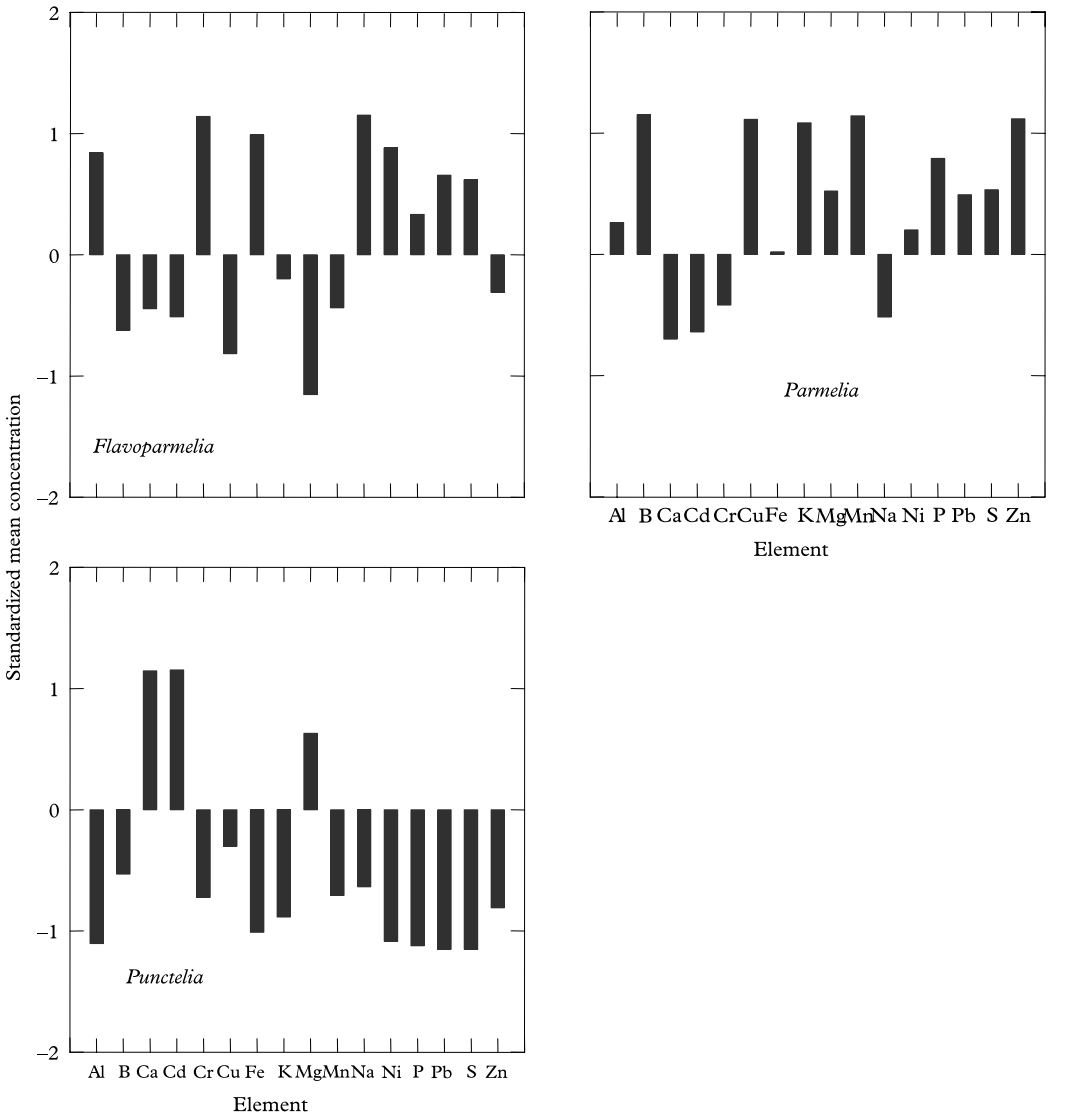

FIG. 4. Standardized mean concentrations of 16 elements in three parmelioid species (see Fig. 3 for explanation). Species are labelled with generic names only for the sake of brevity.

been proposed, including the presence/absence of rhizines (Goyal & Seaward 1981, 1982*a*, *b*), presence/absence of isidia or soredia (Brown 1976; Garty 2001), thallus type (Garty 2001; Vieira *et al.* 2004; Kinalioglu *et al.* 2006; Cloquet *et al.* 2006), presence of organic substances (Brown 1976; Nimis *et al.* 2001; Carreras *et al.* 2005), presence of apothecia (Bačkor & Fahselt 2004), thallus colour (Sarrett *et al.* 1998; Baranowska *et al.* 2001), hydration state (Kuziel 1973), thallus size/area (Lawrey & Hale 1981; Goyal & Seaward 1981, 1982*a*, *b*), substratum (Doyle *et al.* 1973; Goyal & Seaward 1981, 1982*a*, *b*), exposure (Lambinon *et al.* 1964; Goyal & Seaward 1981, 1982*a*, *b*), metabolic rate (Erman *et al.* 2006), detoxification mechanisms (Sarrett *et al.* 1998), active vs.

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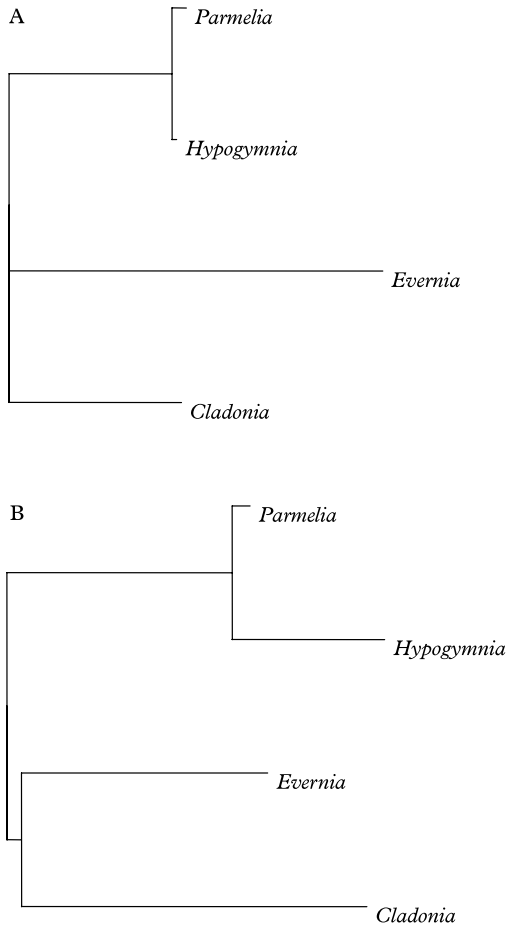

FIG. 5. Comparisons between four lichen species. A, Euclidean distances based on organic substances; B, Jaccard similarity distances based on inorganic substances. The distance between any two species is the sum of all horizontal lines connecting them. Species are labelled with generic names only for the sake of brevity.

passive uptake mechanisms (Brown 1976; Baranowska *et al.* 2001), deposition of elements to the surface or thallus interior (Baranowska *et al.* 2001), cation exchange capacity (Gaio-Oliveira *et al.* 2001), thallus thickness (Garty 2001; Nimis *et al.* 2001), pigment quantity (Hawksworth 1976), thallus acidity (Baranowska-Bosiacka *et al.* 2001), thallus age (Garty 2001; Nimis *et al.* 2001), presence/absence of necromass (Crittenden 1991; Hyvärinen *et al.* 2000), length of exposure (Bargagli 1987; Nimis *et al.* 2001; Culicov & Yurukova 2006), element competition/displacement (Carreras *et al.* 2005; Bačkor & Fahselt 2004; Vieira *et al.* 2004; Kuziel 1973; Goyal & Seward 1981, 1982*a*, *b*), and exposure to ultraviolet radiation (McEvoy *et al.* 2006). Detailed explanations of each of these with how each might explain the differences seen in this study are listed in the Appendix. Some of these mechanisms are obviously under genetic control, while others are clearly environmental, and still others are a combination of the two. Of course, many of these mechanisms are intertwined and correlated, and probably more than one operates for any particular species. For example, thallus age and size are obviously related, as are pigment quantity and quality. Further research is needed on these if elemental differences are used in taxonomy.

Elemental chemistry of lichens apparently has taxonomic significance. I do not propose that elemental chemistry be considered a primary diagnostic character; however, in instances where inorganic chemistry points out significant differences that cannot be detected with other taxonomic characters it may prove useful. Or, if the elemental differences correlate with other characters to support a proposed taxon they might be useful, similar to Lumbsch's proposal for organic secondary products (1998).

The implications of these findings for biomonitoring of trace elements and heavy metals are significant and severalfold. Studies that use multiple lichen species may yield results that differ significantly due to species differences rather than to environmental factors because the genetic component of chemical variation may contribute more strongly to lichen tissue concentrations than does the environmental component. That is, if not properly accounted for, environmental influences can be confounded by species-based differences in life history. Such studies should include comparisons between elemental signatures for each species in one or more control areas and in the impact area. Studies that use one species to calibrate another because of abundance differences may yield erroneous results due to species differences that are not

comparable. Careful calibration between species in control areas not impacted by pollution is essential. Finally, element specificity among species appears to be important, which would indicate that some species are better than others at biomonitoring certain elements. This should also be studied in pristine areas prior to biomonitoring polluted areas.

Note added in proof. In a newly published study of the *Lecanora dispersa* group in North America, Sliwa (2007) found differences between species within *Lecanora* in element composition of particular parts of the apothecia, using spectrometric microanalysis, lending further support to the ideas presented here (Sliwa, L. (2007) A revision of the *Lecanora dispersa* complex in North America. *Polish botanical Journal* **52:** 1–70).

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Appendix. Summary of element accumulation mechanisms in lichens

Rhizines. Species with rhizines have been observed to have higher concentrations of elements than species without rhizines, suggesting that rhizines store excess elements. Three of the six species studied have rhizines, while the other three do not.

TABLE 8. *Mean concentrations (ppm) of elements in foliose and fruticose lichen species**

Element	Foliose species mean	Fruticose species mean	*t* value	*t* probability	Foliose sample size	Fruticose sample size
Ag	0·033	0·027	1·066	0·29	44	36
Al	**673·05**	**465·8**	15·99	0	905	978
As	1·43	1·31	0·456	0·65	212	174
Au	0·016	0·004	*not testable*		1	3
B	**5·3**	**3·99**	6·36	0	882	951
Ba	**35·11**	**6·44**	13·83	0	44	36
Ca	**16 753**	**1694**	22·16	0	905	978
Cd	**0·72**	**0·23**	33·92	0	905	926
Co	**0·624**	**0·346**	5·65	0	212	174
Cr	**1·22**	**0·89**	12·92	0	905	978
Cs	**0·133**	**0·102**	1·65	0·104	44	36
Cu	**5·95**	**2·7**	12·07	0	905	978
Fe	**781**	**586**	11·52	0	905	978
Hg	**0·128**	**0·111**	2·55	0·011	294	322
K	**3367**	**2006**	43·28	0	905	978
Lu	**0·004**	**0·002**	5·52	0	44	30
Mg	**739**	**363**	43·06	0	905	978
Mn	**175**	**42**	34·69	0	905	978
Mo	**0·295**	**0·3**	0·147	0·88	168	147
Na	**33·1**	**37·3**	5·42	0	896	969
Ni	**2·58**	**1·17**	12·65	0	905	929
P	**1099**	**568**	35·25	0	905	978
Pb	**14·9**	**4·62**	32·58	0	905	924
S	**1094**	**831**	16·61	0	907	980
Sb	**0·036**	**0·021**	2·63	0·01	44	36
Se	**1·9**	**1·52**	1·38	0·17	168	139
Ti	**22·32**	**17·15**	2·34	0·02	188	147
V	**1·81**	**0·99**	10·71	0	188	147
Zn	**80·2**	**25**	63·25	0	905	978
Ash	**5·31**	**1·48**	12·8	0	171	110

*Foliose species: *Flavopunctelia flaventior*, *Hypogymnia physodes*, *Parmelia sulcata*, *Punctelia rudecta*. Fruticose species: *Cladonia rangiferina*, *Evernia mesomorpha*. Means that are significantly different at $P<0{\cdot}05$ are in bold font.

Isidia/soredia. The presence of these structures on the cortex makes the surface rougher and with a greater surface area, thereby enhancing particle entrapment compared to smooth, shiny surfaces. All but one species in this study have these structures.

Thallus type. Many observations have been made showing that foliose species have higher element concentrations than fruticose species, independent of surface area, although this has not been rigorously tested. In this study the four foliose species did have higher concentrations of most elements compared to the fruticose types. Elemental means for foliose and fruticose types for all elements studied across the ecoregion are shown in Table 8. For foliose species, 21 elements plus ash content are greater than in fruticose species, while only one element, Na, is slightly greater in fruticose species. This mechanism is probably related to surface area, but it may also be a separate factor that we do not yet understand.

Lichen organic substances. Many lichen secondary products, e.g. parietin and usnic acid, are known to chelate metals, suggesting that species with higher concentrations of secondary products may also have higher metal loads. In this study, *Hypogymnia physodes* and *Parmelia sulcata* contain 7 and 8 secondary products respectively (Culberson & Culberson 1970†), about twice the number in the other species, and also have some of the highest element concentrations.

Apothecia. Some species are known to concentrate lichen substances and elements in fruiting structures, so species that produce many apothecia could be expected to contain greater amounts than those with few or none. In this study all but one species (*Evernia*) produce apothecia.

Colour. Some pigments complex metals, e.g. parietin complexes Pb and melanin complexes U, so it is thought that species with such pigments could contain more elements. Colour in the species in

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this study ranges from white to dark green and bluish grey, which could indicate the darker coloured species might contain higher levels of some elements.

Hydration. The moisture levels of the thallus affect the K/Ca ratio because these two elements interact to regulate water balance in tissues, primarily through the action of calcium oxalate. Species that are tolerant of drier habitats then are likely to have more Ca and a lower K/Ca ratio.

Size/area. Larger lichens have more surface area than small lichens, and could be expected to trap more elements and have higher concentrations. This appears to be the case with the parmelioid species in this study, all of which are larger than the other species.

Substratum. It is commonly thought that lichens growing on soil may have higher concentrations of soil elements than lichens growing elsewhere, although the evidence in support of this is contradictory. All but one species in this study grow on bark, however, so this is probably not a factor.

Exposure. Everything else being equal, it is thought that lichens on trees are more exposed to ambient wind carrying particulates than those on the ground. However, some authors suggest that the canopies of trees shelter lichens from aeolian deposition and that species exposed to the open air should be more exposed to ambient deposition. This hypothesis clearly needs more testing before a generalization can be made.

Metabolism. It is generally known, although not for lichens, that organisms with higher metabolic rates have higher uptake rates for nutrients and whatever else they are exposed to. We do not know the rates for the species in this study, but perhaps growth rate is an indication of metabolism. Perhaps some of these species have high growth rates that might explain high element concentrations.

Detoxification. It is known that species able to precipitate metals and other elements with oxalate have higher tissue concentrations of metals than species that use cell wall carboxylation to detoxify the metals. *Diploschistes* is an example of the former, while *Xanthoria* is an example of the latter. We do not know the detoxification mechanisms of the species in this study.

Active or passive uptake. A more recent paper has confirmed Brown's (1976†) hypothesis that passive uptake results in higher tissue concentrations than active uptake, presumably because there is no detoxification. This was found in *Hypogymnia physodes*, a species in this study.

Surface or interior. For whatever reason, some elements remain on the surface of the cortex while others penetrate to the interior of the thallus, as was found in *Hypogymnia physodes*. This question remains to be tested in the species used in this study.

Cation exchange capacity. Some lichens are known to have higher cation exchange mechanisms than other species, and this would lead to lower tissue concentrations of elements that are exchanged through this mechanism.

Thickness. Thinner lichens would be expected to have higher tissue concentrations than thicker ones because they have higher surface/volume ratios. The thallus thickness of species used in the present study is unknown.

Pigment quantity. Not only would the presence/absence of pigments affect element concentrations, but the quantity of pigments would also. Higher pigment concentrations would obviously lead to higher element concentrations.

Thallus acidity. Lichens with a lower thallus pH have been found to have increased elemental uptake, thereby leading to higher tissue concentrations. The thallus pH values of the species in this study are unknown.

Thallus age. Presumably older lichens will have higher elemental concentrations than younger ones because they have been exposed longer. This is an important factor in field studies. Some studies avoid this factor by sampling only the current year's growth. However, if elements leach out of older tissues, it is conceivable that younger tissues may have higher concentrations than older ones (Brown 1976†).

Necromass. Mat growing lichens accumulate dead tissue at the base, and probably accumulate higher concentrations of elements if this part of the mat is analyzed. Most studies of mat lichens analyze only the upper, live layers of the mat.

Length of exposure. A longer exposure, everything else being equal, will lead to higher tissue concentrations, and obviously prior exposure is important.

Element competition. Elements that are related in the periodic table often displace one another, e.g. Ca and Sr. The final tissue concentration of some elements may therefore depend on other elements already present.

Ultraviolet light. Lichens exposed to ultraviolet light increase their concentrations of usnic acid, which, in turn, would complex more elements. Therefore lichens in higher ultraviolet environments may have higher element concentrations than those in lower ultraviolet environments.

†See main reference list.