# **LICHENS AND AIR QUALITY**

IN

# **RAINBOW LAKE WILDERNESS**

OF THE

# **CHEQUAMEGON NATIONAL FOREST**

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### **ABSTRACT**

This study of the lichens of Rainbow Lake Wilderness was designed 1) to collect lichens for a lichen species list, 2) to collect lichens for elemental analysis, 3) to study the health and distributions of species most sensitive to air pollution, and 4) to assess the effects of air quality on lichens. Twenty seven localities were studied throughout the wilderness. Samples of three species were collected at three localities and one species was collected at one locality for elemental analysis.

The lichen flora is quite diverse. There were 190 species present including seven species very sensitive to sulfur dioxide. The distributions of these sensitive species do not show patterns that would suggest directional air quality problems. All of the lichens found were in good health and with normal fertility. The lichens studied by elemental analysis show levels of all elements comparable to other clean areas. ANOVA and pairwise comparisons of the elemental data showed somewhat higher element levels at the northern edge of the RLW and no singificant differences between the Boundary Waters Canoe Area and RWL. Therefore, there seem to be no indications of air quality problems (mainly sulfur dioxide) in the wilderness.

Recommendations are for periodic (5 year) restudy of the lichens by elemental analysis. A complete lichen restudy of the lichen flora should be done every 10-15 years. If construction or maintenance activities are planned within the wilderness, a lichenologist should be consulted to prevent loss of species.

### **PREFACE**

Under a contract from the USDA National Forest Service a lichen study was performed in the Rainbow Lake Wilderness Area (RLW) of the Chequamegon National Forest. The objectives were to survey the lichens of the wilderness area, produce an inventory of the lichen flora, collect and analyze lichens for chemical contents, and evaluate the lichen flora with reference to the air quality. This establishes baseline data to determine the future change in air quality. All work was done at the University of Minnesota with consultation with Mr. Manfred Mielke, and with personnel on the Forest.

The Forest Service personnel have been very helpful during the field work which has contributed significantly to the success of the project. The study was made possible by funds from the U. S. Forest Service, Chequamegon National Forest and NAS & PF Forest Health Protection. Dave Rugg, statistician with the NCFES did the statistical analysis. The assistance of all of these is gratefully acknowledged.

### INTRODUCTION

Lichens are composite plants composed of two different types of organisms. The lichen plant body (thallus) is made of fungi and algae living together in a symbiotic arrangement in which both partners are benefited and the composite plant body can grow in places where neither component could live alone. The thallus has no protective layer on the outside, such as the epidermis of a leaf, so the air in the thallus has free exchange with the atmosphere. Lichens are slow growing (a few millimeters per year) and remain alive for many years and so they must have a habitat that is relatively undisturbed in order to survive. Lichens vary greatly in their ecological requirements but almost all of them can grow in places that only receive periodic moisture. When moisture is lacking they go dormant until the next rain or dew-fall. Some species can grow in habitats with very infrequent occurrences of moisture while others need high humidity and frequent wetting in order to survive. This difference in moisture requirements is very important in the distribution of lichens.

Lichens are known to be very sensitive to low levels of many atmospheric pollutants. Many are damaged or killed by levels of sulfur dioxide, nitrogen oxides, fluorides or ozone alone or in various combinations. Levels of sulfur dioxide as low as 13 µg/cubic meter (annual average) will cause the death of some lichens (LeBlanc et al., 1972). Other lichens are less sensitive and a few can tolerate levels of sulfur dioxide over 300 µg/cubic meter (Laundon, 1967, Trass, 1973). The algae of the thallus are the first to be damaged in areas with air pollution and the first indication of damage is discoloring and death of the algae causing bleached lobes, which quickly leads to the death of the lichen. After the lichen dies it disappears from the substrate within a few months to a year as it disintegrates and decomposes (Wetmore, 1982).

Lichens are more sensitive to air pollution when they are wet and physiologically

active and are least sensitive when dry (Nash, 1973, Marsh & Nash, 1979) and are more sensitive when growing on acid substrates.

Contrary to some published reports (Medlin, 1985) there is little evidence that most lichens are good indicators of acid precipitation. However, Sigal & Johnston (1986) have reported that one species of <a href="Umbilicaria">Umbilicaria</a> shows visible damage due to artificial acid rain. They also report that similar symptoms were found in collections from various localities in North America. Lechowicz (1987) reported that acid rain only slightly reduced growth of <a href="Cladina stellaris">Cladina stellaris</a> but Hutchinson et al. (1986) reported that extremely acid precipitation (less than pH 3.5) killed or damaged some mosses and lichens. Scott & Hutchinson (1987) showed temporary reduction of photosynthesis in <a href="Cladina stellaris">Cladina stellaris</a> and <a href="C. rangiferina">C. rangiferina</a> after artificial acid rain.

Lichens are able to accumulate chemical elements in excess of their metabolic needs depending on the levels in the substrate and the air, and, since lichens are slow growing and long lived, they serve as good summarizers of the environmental conditions in which they are growing. Chemical analysis of the thallus of lichens growing in areas of high fallout of certain elements will show elevated levels in the thallus. Toxic substances (such as sulfur) are also accumulated and determination of the levels of these toxic elements can provide indications of the sub-lethal but elevated levels in the air.

The Rainbow Lake Wilderness (RLW) is about 6388 acres and is located in northern Wisconsin, about 65 miles ESE of Superior, Wisc. The land is rolling glacial till with numerous low areas with lakes or bogs. The driest ridges have oaks (Quercus) and pines (Pinus) but most of the land is a mixed hardwood forest of maple (Acer), basswood (Tilia americana), quaking aspen (Populus tremuloides) and white birch (Betula papyrifera). In a few localities there are stands of eastern hemlock (Tsuga canadensis). The lower areas have balsam fir (Abies balsamea) and spruce (Picea). There are numerous black spruce bogs (Picea mariana) and an occasional low area with white cedar (Thuja occidentalis). Much of the area was probably logged near the turn of the

century. Between the 1930's and 1985 only limited timber harvest or other forest management activities occurred. The area was designated Wilderness in 1985. Rocks are rare within the wilderness but a few are exposed along roads and trails.

There has been no lichen collecting in the wilderness prior to this study and no literature references to lichen collections from the wilderness have been found.

### **METHODS**

Field work was done during August, 1992 when 1170 collections were made at 27 localities. A complete list of collection localities is given in Appendix I and these are indicated on Fig. 1. Collection localities, about 2 acres in size, were selected first to give a general coverage of the wilderness, second, to sample all vegetational types, and third, to be in localities that should be rich in lichens. Undisturbed as well as disturbed habitats (such as roadsides and trails) were studied. At each locality voucher specimens of all species found were collected to record the total flora for each locality and to avoid missing different species that might appear similar in the field. At some localities additional material of selected species was collected for chemical analysis (see below). While collecting at each locality observations were made about the general health of the lichens. Lichen health was evaluated by looking for damaged or dying lichens on all of the trees where collections were made(at least 100 trees). The presence of many dead, dying, or abnormal thalli of particular species at a locality would indicate poor health, but an occasional damaged thallus is not significant.

Identifications were carried out at the University of Minnesota with the aid of comparison material in the herbarium and using thin layer chromatography for identification of the lichen substances where necessary. The original packet of each collection has been deposited in the University of Minnesota Herbarium. All specimens deposited at the University of Minnesota have been entered into the herbarium computerized data base maintained there.

## **LICHEN FLORA**

The following list of lichens is based on my collections. Species found only once are indicated by "Rare". In the first columns the letters indicate the sensitivity to sulfur dioxide, if known, according to the categories proposed by Wetmore (1983): S=Sensitive, l=Intermediate, T=Tolerant. S-I is intermediate between Sensitive and Intermediate and I-T is intermediate between Intermediate and Tolerant. Species in the Sensitive category are absent when annual average levels of sulfur dioxide are above 50 µg per cubic meter. The Intermediate category includes those species present between 50 and 100 µg and those in the Tolerant category are present at over 100 µg per cubic meter. Those species without sensitivity designations have unknown sensitivity.

### **SPECIES LIST**

Acarospora fuscata (Nyl.) Arn. :Rare

Anaptychia palmulata (Michx.) Vain.

Arthonia caesia (Flot.) K"orb.

Arthonia didyma K"orb.

Arthonia fuliginosa (Schaer.) Flot. :Rare

Arthonia punctiformis Ach. :Rare

I <u>Arthonia radiata</u> (Pers.) Ach.

1 additioal unidentified species of Arthonia

Bacidia polychroa (Th. Fr.) K"orb.

I Bacidia rubella (Hoffm.) Mass.

Bacidia sabuletorum (Schreb.) Lett.

Bacidia schweinitzii (Tuck.) Schneid.

Bacidia suffusa (Fr.) Schneid.

1 additioal unidentified species of Bacidia

Biatorella microhaema Norm.

Biatorella ochrophora (Nyl.) Arn.

- S Bryoria furcellata (Fr.) Brodo & Hawksw.
- S <u>Bryoria trichodes</u> (Michx.) Brodo & Hawksw.

Buellia arnoldii Serv. & Nadv.

Buellia dialyta (Nyl.) Tuck.

Buellia polyspora (Willey in Tuck.) Vain. :Rare

Buellia schaereri De Not.

I Buellia stillingiana Steiner

Calicium parvum Tibell

Calicium trabinellum Ach.

Caloplaca camptidia (Tuck.) Zahlbr. :Rare

S-I <u>Caloplaca cerina</u> (Ehrh.) Th. Fr.

Caloplaca chrysophthalma Degel.

- I <u>Caloplaca holocarpa</u> (Hoffm.) Wade
- S-I <u>Candelaria concolor</u> (Dicks.) B. Stein

Candelaria fibrosa (Fr.) M"ull. Arg.

Candelariella efflorescens R. Harris & Buck

1 unidentified species of Catillaria

Cetraria halei W. & C. Culb.

I <u>Cetraria pinastri</u> (Scop.) Gray

Cetrelia olivetorum (Nyl.) W. & C. Culb. :Rare

Chaenotheca brunneola (Ach.) M"ull. Arg.

Chaenotheca chrysocephala (Turn. ex Ach.) Th. Fr.

I <u>Chaenotheca ferruginea</u> (Turn. ex Sm.) Mig. :Rare

Chaenotheca stemonea (Ach.) Zw. :Rare

Chaenothecopsis debilis (Turn. & Borr. ex Sm.) Tibell

Chaenothecopsis pusilla (FI"orke) Schmidt :Rare

I Chrysothrix candelaris (L.) Laund. :Rare

Cladina mitis (Sandst.) Hustich

Cladina rangiferina (L.) Nyl.

Cladonia bacillaris Nyl.

Cladonia caespiticia (Pers.) Fl"orke

Cladonia cenotea (Ach.) Schaer.

Cladonia chlorophaea (Fl"orke ex Somm.) Spreng.

I <u>Cladonia coniocraea</u> (Fl"orke) Spreng.

Cladonia cornuta (L.) Hoffm. :Rare

I Cladonia cristatella Tuck.

Cladonia cryptochlorophaea Asah.

Cladonia digitata (L.) Hoffm. :Rare

Cladonia gracilis (L.) Willd.

Cladonia grayi G. K. Merr. ex Sandst.

Cladonia merochlorophaea Asah. :Rare

Cladonia parasitica (Hoffm.) Hoffm. :Rare

Cladonia pyxidata (L.) Hoffm.

Cladonia rei Schaer. :Rare

Cladonia ramulosa (With.) Laund.

Cladonia scabriuscula (Delise in Duby) Nyl. :Rare

Cladonia squamosa (Scop.) Hoffm.

Cladonia verticillata (Hoffm.) Schaer. :Rare

Collema conglomeratum Hoffm. :Rare

Collema subflaccidum Degel.

Conotrema urceolatum (Ach.) Tuck.

Cyphelium lucidum (Th. Fr.) Th. Fr.

Eopyrenula leucoplaca (Wallr.) R. Harris

I <u>Evernia mesomorpha</u> Nyl.

I Graphis scripta (L.) Ach.

Haematomma elatinum (Ach.) Mass.

Haematomma pustulatum Brodo & W. Culb. :Rare

Heterodermia galactophylla (Tuck.) W. Culb.

Heterodermia hypoleuca (Muhl.) Trev.

Heterodermia speciosa (Wulf.) Trev.

Hymenelia lacustris (With.) Poelt & Vezda :Rare

Hypocenomyce anthracophila (Nyl.) P. James & G. Schneid.

Hypocenomyce friesii (Ach. in Lilj.) P. James & G. Schneid.

I Hypocenomyce scalaris (Ach. in Lilj.) Choisy

I <u>Hypogymnia physodes</u> (L.) Nyl.

I <u>Imshaugia aleurites</u> (Ach.) S. F. Meyer

Julella fallaciosa (Stizenb. ex Arn.) R. Harris

Lecania dubitans (Nyl.) A. L. Sm.

I <u>Lecanora</u> allophana Nyl.

Lecanora caesiorubella Ach.

Lecanora carpinea (L.) Vain.

I Lecanora circumborealis Brodo & Vitik.

Lecanora hybocarpa (Tuck.) Brodo

Lecanora impudens Degel.

I Lecanora pulicaris (Pers.) Ach.

Lecanora strobilina (Spreng.) Kieff.

Lecanora symmicta (Ach.) Ach.

Lecanora thysanophora Harris ined.

Lecanora wisconsinensis Magn.

1 additioal unidentified species of Lecanora

Lecidea albohyalina (Nyl.) Th. Fr.

Lecidea berengeriana (Mass.) Nyl.

Lecidea elabens Fr.

Lecidea helvola (K"orb. ex Hellb.) Oliv. :Rare

S <u>Lecidea nylanderi</u> (Anzi) Th. Fr.

Lecidea plebeja Nyl.

Leptogium cyanescens (Rabenh.) K"orb.

Leptogium saturninum (Dicks.) Nyl. :Rare

Leptogium teretiusculum (Wallr.) Arn. :Rare

Leptorhaphis epidermidis (Ach.) Th. Fr. :Rare

S <u>Lobaria pulmonaria</u> (L.) Hoffm.

Lobaria quercizans Michx.

Micarea denigrata (Fr.) Hedl. :Rare

Micarea melaena (Nyl.) Hedl.

Micarea prasina Fr.

1 additioal unidentified species of Micarea

Mycocalicium subtile (Pers.) Szat.

Ochrolechia arborea (Kreyer) Almb.

Ochrolechia mexicana Vain.

Ochrolechia trochophora (Vain) Oshio

S-I Opegrapha varia Pers.

Pachyospora verrucosa (Ach.) Mass.

Pachyphiale fagicola (Hepp ex Arn.) Zw.

Parmelia aurulenta Tuck.

I Parmelia caperata (L.) Ach.

Parmelia crinita Ach.

Parmelia flaventior Stirt.

Parmelia galbina Ach.

Parmelia margaritata Hue :Rare

I <u>Parmelia rudecta</u> Ach.

I <u>Parmelia septentrionalis</u> (Lynge) Ahti

Parmelia soredica Nyl.

S Parmelia squarrosa Hale

I-T Parmelia subargentifera Nyl.

S-I <u>Parmelia subaurifera</u> Nyl.

I Parmelia subrudecta Nyl.

Parmelia subtinctoria Zahlbr.

I-T Parmelia sulcata Tayl.

I <u>Parmeliopsis ambigua</u> (Wulf. in Jacq.) Nyl.

I <u>Parmeliopsis</u> <u>hyperopta</u> (Ach.) Arn.

Peltigera didactyla (With.) Laundon :Rare

Peltigera elisabethae Gyeln.

Peltigera evansiana Gyeln.

Peltigera membranacea (Ach.) Nyl.

Peltigera neckeri Hepp ex M"ull. Arg.

Peltigera polydactyla (Neck.) Hoffm.

Peltigera praetextata (Fl"orke ex Somm.) Zopf

I Pertusaria amara (Ach.) Nyl.

Pertusaria macounii (Lamb) Dibb.

I <u>Pertusaria multipunctoides</u> Dibb. :Rare

Pertusaria ophthalmiza (Nyl.) Nyl. :Rare

Pertusaria trachythallina Erichs.

Pertusaria velata (Turn.) Nyl.

3 additioal unidentified species of Pertusaria

Phaeocalicium polyporaeum (Nyl.) Tibell :Rare

Phaeophyscia chloantha (Ach.) Moberg

Phaeophyscia ciliata (Hoffm.) Moberg

Phaeophyscia hispidula (Ach.) Essl. :Rare

Phaeophyscia imbricata (Vain.) Essl.

I <u>Phaeophyscia orbicularis</u> (Neck.) Moberg

Phaeophyscia pusilloides (Zahlbr.) Essl.

Phaeophyscia rubropulchra (Degel.) Essl.

- Phlyctis argena (Spreng.) Flot.
- I Physcia adscendens (Th. Fr.) Oliv.
- I <u>Physcia aipolia</u> (Ehrh. ex Humb.) F"urnr.

Physcia americana G. K. Merr. in Evans & Meyrow.

- I <u>Physcia millegrana</u> Degel.
- I <u>Physcia stellaris</u> (L.) Nyl.
- I <u>Physconia detersa</u> (Nyl.) Poelt

Placynthiella icmalea (Ach.) Coppins & James

Plagiocarpa hyalospora (Nyl.) R. Harris

Platismatia tuckermanii (Oakes) W. & C. Culb.

Porpidia crustulata (Ach.) Hert. & Knoph :Rare

Psilolechia lucida (Ach.) Choisy: Rare

Pyrenula pseudobufonia (Rehm.) R. Harris

Pyxine sorediata (Ach.) Mont.

S Ramalina americana Hale

Rinodina archaea (Ach.) Arn.

Rinodina ascociscana Tuck.

Rinodina efflorescens Malme

Rinodina subminuta Magn.

Rinodina thujae (Magn.) Sheard

Sarea resinae (Fr. ex Fr.) Kuntze :Rare

I Scoliciosporum chlorococcum (Graewe ex Stenh.) Vezda

Sphinctrina turbinata (Pers.) De Not. :Rare

Stenocybe pullatula (Ach.) B. Stein. :Rare

Stereocaulon paschale (L.) Hoffm. :Rare

Stereocaulon saxatile Magn.

Strigula submuriformis (R. Harris) R. Harris: Rare

Trapelia obtegens (Th. Fr.) Hert. :Rare

Trapeliopsis granulosa (Hoffm.) Lumbsch.

Trapeliopsis viridescens (Schrad.) Coppins & James

Usnea cavernosa Tuck. :Rare

- S <u>Usnea ceratina</u> Ach. :Rare
- S-I <u>Usnea hirta</u> (L.) Weber ex Wigg.

Usnea lapponica Vain.

- S-I <u>Usnea subfloridana</u> Stirt.
- S-I <u>Xanthoria fallax</u> (Hepp in Arn.) Arn.
- I Xanthoria polycarpa (Hoffm.) Rieber

### DISCUSSION OF FLORA

This list of species presents the first listing of lichens from the Rainbow Lake Wilderness and includes 190 species found during this study. There are also and additional 8 unidentified species, some of which are undescribed. The lichen flora is quite diverse for this area. Dr. John Thomson, Univ. of Wisconsin, Madison says that this list includes 21 new state records for Wisconsin (pers. comm.). Some of the most common species are Cladonia coniocraea, Evernia mesomorpha, Hypogymnia physodes, Parmelia aurulenta, P. caperata, P. squarrosa, Physconia detersa, and Xanthoria fallax.

None of the lichen distributions show unexpected patterns. Some of the species are found mainly on pines and were found only in the western part of the wilderness, where there are more pines. Many of the species prefer wetter areas, such as bogs, and were only found in these bogs. Some of the species found only once are rare wherever they are found throughout their distributional range and might be found at other localities with further searching; and, others may require special substrates that are rare in the wilderness. Some of the rare species are rare because of the scarcity of rock substrates. The vegetation and elevation on the RLW is fairly uniform overall and this provides few unusual habitats for rare lichens. The cases of rarity do not necessarily reflect sensitivity damage due from sulfur dioxide.

There were no cases where lichens sensitive to sulfur dioxide were observed to be damaged or killed. All species normally found fertile were also fertile in the wilderness. There are numerous species with blue-green algae, which are very sensitive to sulfur dioxide. These observations indicate that there is no air quality degradation in the wilderness due to sulfur dioxide that causes visible damage to the lichen flora.

This study found the following number of species in the different sensitivity categories.

Category	# of Species						
Sensitive	 7						
S/I	7						
Intermediate	35						
I/T	0						
Tolerant	0						

Most lichen species are unknown as to sensitivity category. The absence of species in the more tolerant categories in RLW indirectly indicates the lack of sulfur dioxide problems. In areas of high sulfur dioxide these categories would have more species and the most sensitive categories would have fewer species. The RARE species in RLW are not related to air quality (see above). The only way to determine past air quality impacts on the present lichen species inventory is by comparison with historical data (from before the presumed impacts occurred). Since there are no historical species lists from this area it cannot be determined whether the present lichen flora has changed prior to this study.

Another way of analyzing the lichen flora of an area is to study the distributions of the sensitive species within the wilderness to look for voids in the distributions that might be caused by air pollution. Showman (1975) has described and used this technique in assessing sulfur dioxide levels around a power plant in Ohio. Only the very common species have meaning with such a technique since the rare species may be absent due to other factors. This method of assessing air quality is weak but occasionally is useful in detecting directional effects in an area.

Many of the lichens in the wilderness have known sensitivity to sulfur dioxide according to the list presented in Wetmore (1983) and two of these are uncommon (occur more than once but less than in half of the localities). Species in the most sensitive category are usually absent when sulfur dioxide levels are above 50 µg per cubic meter average annual concentrations. The species that occur in the area in the most sensitive category are as follows.

Bryoria furcellata (Fr.) Brodo & Hawksw.

Bryoria trichodes (Michx.) Brodo & Hawksw.
Lecidea nylanderi (Anzi) Th. Fr.
Lobaria pulmonaria (L.) Hoffm.
Parmelia squarrosa Hale
Ramalina americana Hale
Usnea ceratina Ach.

The distributions of these species are shown in Fig. 2-8. Although these species are not found at all localities and two are not common, there is no indication that the voids in the distributions are due to high levels of sulfur dioxide. Some of the localities where collections were made do not have suitable habitats or substrates for some of these species. This is especially true for Lobaria pulmonaria that requires moist habitats.

#### **ELEMENTAL ANALYSIS**

An important method of assessing the effects of air quality is by examining the elemental content of the lichens (Nieboer et al, 1972, 1977, 1978; Erdman & Gough, 1977; Puckett & Finegan, 1980; Nash & Sommerfeld, 1981). Elevated but sublethal levels of sulfur or other elements might indicate incipient damaging conditions.

Four species of lichens were collected for elemental analysis in the RLW. At some localities not all species were present in quantities needed for the analysis.

### **METHODS**

Lichens were collected in spunbound olefin bags at three localities in different parts of the wilderness for laboratory analysis (Fig. 1). Species collected were <u>Cladina</u> rangiferina, <u>Evernia mesomorpha</u>, <u>Hypogymnia physodes</u>, and <u>Parmelia sulcata</u>. These species were selected because they are locally present in abundance and relatively easy to clean. <u>Cladina rangiferina</u> was present at only elemental analysis locality and was collected from the ground. The other three species were present at all three localities and were collected from conifer bark.

Three localities were selected for elemental analysis and are indicated on the map of collection localities (Fig. 1). These localities are: North Country Trail SE of Square Lake (6 Aug. 1992), N edge of Lee Lake (11 Aug. 1992), and N of Clay Lake (2 Aug.

1992). Full locality citations are given in Appendix I. Ten to 20 grams of each species were collected at each locality.

Lichens were air dried and cleaned of all bark and detritis under a dissecting microscope but thalli were not washed. Three samples (replicates) of each collection were submitted for analysis. Because of the scarcity of Cladina rangiferina in RLW, these samples were submitted along with the Boundary Waters Canoe Area (BWCA) samples, where adequate material was available for parallel analytical splits. Analysis was done for sulfur and multi-element analysis by the Research Analytical Laboratory at the University of Minnesota. In the sulfur analysis, a ground and pelleted 100-150 mg sample was prepared for total sulfur by dry combustion and measurement of evolved sulfur dioxide on a LECO Sulfur Determinator, model no. SC-132, by infra red absorption. Multi-element determination for Ca, Mg, Na, K, P, Fe, Mn, Al, Cu, Zn, Cd, Cr, Ni, Pb, and B were determined simultaneously by Inductively Coupled Plasma (ICP) Atomic Emission Spectrometry. For the ICP one gram of dried plant material was dry ashed in a 20 ml high form silica crucible at 485 degrees Celsius for 10-12 hrs. Crucibles were covered during the ashing as a precaution against contamination. The dry ash was boiled in 2N HCl to improve the recovery of Fe, Al and Cr and followed by transfer of the supernatant to 7 ml plastic disposable tubes for direct determination by ICP.

### **RESULTS AND DISCUSSION**

Table 1 gives the results of the analyses for all three replicates arranged by species. Table 2 gives the means and standard deviations for each set of replicates.

Values for National Bureau Of Standards Peach Leaves (NBS Peach) and a locally used lichen standard (Cladina stellaris) are also given. Lichens collected from hardwood bark sometimes have different accumulations than those collected from conifer bark. To reduce this substrate variable, all tree lichens are collected from conifer bark whenever possible. Different species may accumulate different amounts of elements and this is evident when comparing sulfur levels of the different species. Cladina rangiferina has

Species	Р	K	Ca	Mg	Al	Fe	Na	Mn	Zn	Cu	В	Pb	Ni	Cr	Cd	S	Locality
C. rangiferina	549	2135	853	393	328	355	27.8	78.8	24.5	3.1	2	2.3	0.7	0.7	0.2	745	Lee Lake
C. rangiferina	498	1964	867	362	304	315	26.7	75.1	21.7	2.9	2.1	2.6	0.7	0.7	0.2	697	Lee Lake
C. rangiferina	484	1878	871	362	320	349	27.4	75.2	21.1	3	2.2	2.7	8.0	8.0	0.2	712	Lee Lake
E. mesomorpha	639	2714	1211	408	675	744	32.2	62.2	41.4	4.4	3	6.8	1.3	1.4	0.2	1130	N. Country Trail
E. mesomorpha	685	2726	1154	414	585	650	30.5	73.1	47.5	4.1	3	5.8	1.1	1.3	0.2	1150	N. Country Trail
E. mesomorpha	716	2648	1212	444	811	932	35	60.5	42.2	4.5	3.4	7.9	1.4	1.6	0.2	1120	N. Country Trail
E. mesomorpha	491	1968	1116	320	410	436	29.2	51.7	46	2.7	2.2	4.8	0.5	0.9	0.2	988	Lee Lake
E. mesomorpha	451	1907	833	273	457	465	36	29.9	48	3	2.2	5.2	8.0	0.9	0.2	940	Lee Lake
E. mesomorpha	467	1919	744	288	447	470	35.9	30.7	43.6	2.9	2.3	5.1	8.0	1	0.2	968	Lee Lake
E. mesomorpha	457	1898	442	307	633	683	26.4	28.3	49.1	3.5	2.4	6.5	0.9	1.2	0.2	1110	Clay Lake
E. mesomorpha	478	1987	389	301	581	627	27.5	26	34.2	3.6	2.3	6.4	0.9	1.1	0.2	1070	Clay Lake
E. mesomorpha	440	1836	409	333	776	860	29.9	26.7	35.2	3.7	2.6	8.6	1.1	1.4	0.2	1080	Clay Lake
H. physodes	1003	3550	21655	727	528	618	27.9	389.5	75.7	5.2	2.8	15.4	0.7	1	0.6	1110	N. Country Trail
H. physodes	821	3174	17562	687	521	622	24.8	312.7	76.4	5.2	2.8	15.2	8.0	0.9	0.6	1050	N. Country Trail
H. physodes	739	3444	15976	667	481	569	26.2	288.7	77.4	5.1	2.6	14	0.9	0.9	0.6	1030	N. Country Trail
H. physodes	591	2489	18252	636	664	748	22.6	191.6	86.5	4.7	2.6	16.5	1.2	1.2	0.5	970	Lee Lake
H. physodes	928	3063	16683	711	648	731	29.2	234.1	87.6	5.1	3.3	15.2	1.2	1.2	0.5	1030	Lee Lake
H. physodes	996	3142	17015	744	674	751	31.3	221.6	90.5	5.4	3.5	15.5	1.4	1.4	0.6	1020	Lee Lake
H. physodes	715	2969	16925	754	409	446	31	353	68.4	4.7	2.3	10.3	0.9	1.1	8.0	931	Clay Lake
H. physodes	715	3322	15732	794	440	519	39.1	387.3	77.5	5.2	3	11.9	1	1.2	8.0	917	Clay Lake
H. physodes	672	2977	16121	801	451	529	29.4	340.3	65.9	5.1	2.7	14.6	1.1	1.2	8.0	967	Clay Lake
P. sulcata	1625	3910	2358	595	754	814	30.6	272.1	79.6	7	5.9	16.7	1.6	1.5	0.4	1130	N. Country Trail
P. sulcata	1351	3607	2300	535	762	841	28.7	246.1	80.9	6.4	5.2	16.5	1.5	1.4	0.3	1340	N. Country Trail
P. sulcata	1503	3785	3105	619	824	842	30.4	351.2	87.7	7.2	6.7	18.1	1.6	1.5	0.4	1200	N. Country Trail
P. sulcata	1569	3859	2897	617	560	580	26	208	82.4	6.2	5.9	13	1.5	1.3	0.4	1050	Lee Lake
P. sulcata	1263	3358	2558	525	543	564	26.3	188.1	82.7	5.9	5.4	12.6	1.5	1.3	0.4	1130	Lee Lake
P. sulcata	1270	3425	2847	522	550	576	19.7	208.2	95.2	5.2	4.7	11.3	0.9	8.0	0.3	1030	Lee Lake
P. sulcata	1104	3658	2624	562	859	861	24.4	248.8	86.8	5.8	3.2	12.9	1.1	1.2	0.3	995	Clay Lake
P. sulcata	1413	3593	2525	588	590	641	21.8	270.2	85.6	4.7	3.3	11.3	0.9	0.9	0.3	1020	Clay Lake
P. sulcata	1243	3206	2666	638	595	625	20.9	298.5	79.5	4.2	3.1	8.9	1	0.9	0.3	1090	Clay Lake
C. stellaris	200	673	231	286	451	573	80.3	20.2	18.3	2.6	1.2	14.3	1.3	1.2	0.2	429	Lichen std.
C. stellaris	201	681	239	291	467	578	81.6	20.8	17.9	2.5	1.3	14.8	1.2	1.1	0.2	437	Lichen std.
C. stellaris	194	621	224	280	440	540	80.2	19.4	17.3	2.7	1.3	14.7	1.3	1.2	0.2	427	Lichen std.
C. stellaris	197	652	228	282	455	555	82	19.9	17.1	2.8	1.6	15	1.5	1.3	0.3	416	Lichen std.
NBS-Peach	1226	3760	4442	1216	485	170	16.4	699.5	68.7	3.3	18	12.9	2.2	2.1	0.3	NA	NBS-Peach
NBS-Peach	1254	3799	4399	1224	481	171	17.6	692.6	67.7	3.2	18.3	12.1	2.1	2.4	0.3	NA	NBS-Peach
NBS-Peach	1190	3682	4332	1190	478	166	14.7	683.7	70.6	3.1	17.7	12.2	2.1	2.3	0.3	NA	NBS-Peach
NBS-Peach	1156	3493	4112	1143	449	169	16.1	641.2	72.4	3.1	16.9	12.3	2	2.3	0.3	NA	NBS-Peach
NBS-Peach	1234	3673	4338	1201	480	181	19.5	679.9	65.1	3.5	17.7	13.2	2.2	2.5	0.3	NA	NBS-Peach

Table 1. Analysis of Rainbow Lake Lichens Values in ppm of thallus dry weight

Table 1. Summary of Analysis of Rainbow Lake Lichens Values in ppm of thallus dry weight

					Value	55 III P	p 0.	tiiuiiuo	a. y	0.9							
Cladina rangiferina	Р	K	Ca	Mg	Al	Fe	Na	Mn	Zn	Cu	В	Pb	Ni	Cr	Cd	S	Locality
	510	1992	864	372	317	339	27.3	76.4	22.4	3	2.1	2.5	0.7	0.8	0.2	718	Lee Lake
Mean	35	131	9	18	12	21	0.5	2.1	1.8	0.1	0.1	0.2	0.1	0.1	<.1	25	
Std. Dev.																	
Evernia mesomorpha	Р	K	Ca	Mg	Al	Fe	Na	Mn	Zn	Cu	В	Pb	Ni	Cr	Cd	s	Locality
Mean	680	2696	1192	422	691	775	32.6	65.3	43.7	4.3	3.2	6.8	1.3	1.4	0.2	1133	N. Country Trail
Std. Dev.	39	42	33	19	114	144	2.3	6.8	3.3	0.2	0.2	1	0.2	0.2	<.1	15	-
Mean	470	1931	898	294	438	457	33.7	37.4	45.9	2.9	2.2	5	0.7	0.9	0.2	965	Lee Lake
Std. Dev.	20	32	194	24	25	18	3.9	12.4	2.2	0.1	0.1	0.2	0.1	<.1	<.1	24	
Mean	458	1907	413	314	663	723	27.9	27	39.5	3.6	2.4	7.2	1	1.3	0.2	1087	Clay Lake
Std. Dev.	19	76	27	17	101	121	1.8	1.2	8.3	0.1	0.1	1.2	0.1	0.2	<.1	21	
Hypogymnia physodes	Р	K	Ca	Mg	Al	Fe	Na	Mn	Zn	Cu	В	Pb	Ni	Cr	Cd	S	Locality
Mean	854	3389	18398	694	510	603	26.3	330.3	76.5	5.2	2.7	14.9	8.0	0.9	0.6	1063	N. Country Trail
Std. Dev.	135	194	2930	31	25	30	1.6	52.7	8.0	0.1	0.1	8.0	0.1	<.1	<.1	42	
Mean	838	2898	17317	697	662	743	27.7	215.8	88.2	5.1	3.1	15.7	1.2	1.3	0.5	1007	Lee Lake
Std. Dev.	217	356	827	55	13	11	4.5	21.8	2.1	0.3	0.5	0.7	0.1	0.1	0.1	32	
Mean	701	3089	16259	783	433	498	33.1	360.2	70.6	5	2.7	12.3	1	1.1	8.0	938	Clay Lake
Std. Dev.	25	202	608	26	22	45	5.2	24.3	6.1	0.2	0.3	2.1	0.1	<.1	<.1	26	
Parmelia sulcata	Р	K	Ca	Mg	Al	Fe	Na	Mn	Zn	Cu	В	Pb	Ni	Cr	Cd	s	Locality
Mean	1493	3767	2588	583	780	832	29.9	289.8	82.7	6.8	6	17.1	1.6	1.5	0.4	1223	N. Country Trail
Std. Dev.	137	153	449	43	38	16	1.1	54.7	4.3	0.4	8.0	8.0	0.1	0.1	<.1	107	
Mean	1367	3547	2767	555	551	573	24	201.4	86.8	5.7	5.3	12.3	1.3	1.1	0.3	1070	Lee Lake
Std. Dev.	175	272	183	54	9	8	3.7	11.5	7.3	0.5	0.6	0.9	0.3	0.3	0.1	53	
Mean	1254	3486	2605	596	682	709	22.4	272.5	84	4.9	3.2	11	1	1	0.3	1035	Clay Lake
Std. Dev.	155	244	72	39	154	132	1.8	24.9	4	8.0	0.1	2	0.1	0.2	<.1	49	
Cladonia stellaris	Р	K	Ca	Mg	Al	Fe	Na	Mn	Zn	Cu	В	Pb	Ni	Cr	Cd	s	Locality
Mean	198	658	231	286	453	564	80.7	20.1	17.8	2.6	1.3	14.6	1.3	1.1	0.2	431	Lichen std.
St. Dev.	4	33	7	6	14	20	8.0	0.7	0.5	0.1	<.1	0.3	0.1	0.1	<.1	5	Lichen std.
NBS Peach	Р	K	Ca	Mg	Al	Fe	Na	Mn	Zn	Cu	В	Pb	Ni	Cr	Cd	s	Locality
Mean	1223	3747	4391	1210	481	169	16.2	691.9	69	3.2	18	12.4	2.1	2.3	0.3	NA	NBS-Peach
St. Dev.	32	59	55	17	4	2	1.4	8	1.4	0.1	0.3	0.4	<.1	0.2	<.1	NA	NBS-Peach

lower levels of sulfur than the other species. None of the reported values are below the lower detection limits of the instruments.

All of the levels found in the RLW lichens are within typical limits for similar lichens in clean areas and the levels within each species are fairly uniform across all localities. This shows that there is no point-source of pollution effecting one part of RLW.

The sulfur levels in lichens tested range from 697 to 1340 ppm for all samples and these values are near background levels as cited by Solberg (1967) Erdman & Gough (1977), Nieboer et al (1977) and Puckett & Finegan (1980) for other species of lichens. Levels may be as low as 200-300 in the arctic (Tomassini et al, 1976) while levels in polluted areas are 4300-5200 ppm (Seaward, 1973) or higher. The sulfur levels in RLW are well within typical levels for clean areas as reported in the literature.

All of the other elements show normal levels for areas with low pollution or relatively clean air.

## STATISTICAL ANALYSIS

# Introduction

Generally, one bag of lichens was collected from a site, cleaned, separated into groups (with different individuals in the groups), ground, and analyzed for chemical constituents. Occasionally, a composite sample was prepared and gound before being subsampled (=analytical splits). The questions of interest are:

- is variability among independent samples larger than variability among composite subsamples?
- I for each species, are there differences among locations? is there a geographical trend?
- l are there differences between the BWCA and Rainbow Lake?

# Variability

To analyze instrument error, samples of <u>Cladina rangiferina</u> were used from the 1992 BWCA data submitted along with the RLW samples. The whole samples (divided

into three replicates before grinding) + average of subsamples (ground before being divided into subsamples) as one data set, the subsamples as another. The MSE was computed for each chemical element in each data set, and then compared using a F-test. Nine of the 16 elements showed significantly (a = .05) higher variability among samples than among subsamples; for the other 7 elements there was no significant difference. Overall, these results provide strong evidence (P < 0.00001) that inter-sample variance is larger than within sample variance. This indicates that the instrumental results are accurate but individual thalli vary in accumulation. Perhaps more thalli of each species should be collected at each locality, all cleaned, and gound before partitioning into replicates. This would a better average for each species at each locality.

## Results -- Rainbow Lake 1992

The error terms used for these comparisons were the same as those used for the Year differences above. The localities are arranged from low on the left to high on the right. The results are and summarized below using a = 0.05. For all six analysis groups, the ANOVA showed a significant Species\*Locality interaction, so these results are broken down by species. Element group {P} showed no significant Species\*Location interaction; otherwise, the results are reported by species. The localities are listed from low on the left to high on the right.

More elemental groups had significantly higher levels at the North Country Trail locality than any other locality. The localities with lowest levels were both in the southern end of the wilderness. The actual differences in levels between localities is not great so there is probably no significant geographical gradient in air quality. All species

{P}

<u>Clay Lake Lee Lake</u> N. Country Trail Lee Lake N. Country Trail

### E. mesomorpha

{K, Zn, Cu, Pb, Ni}

Lee Lake Clay Lake N. Country Trail

{Ca, Mg, Mn, Cd}

Lee Lake Clay Lake N. Country Trail

{AI, Fe, Cr, S}

Lee Lake Clay Lake N. Country Trail

{<u>Na}</u>

Clay Lake N. Country Trail Lee Lake

{B}

Lee Lake Clay Lake N. Country Trail

# H. physodes

{K, Zn, Cu, Pb, Ni}

Clay Lake N. Country Trail Lee Lake

{Ca, Mg, Mn, Cd}

Lee Lake N. Country Trail Clay Lake

{AI, Fe, Cr, S}

Clay Lake N. Country Trail Lee Lake

<u>{Na}</u>

N. Country Trail Lee Lake Clay Lake Lee Lake Clay Lake

{B}

Clay Lake N. Country Trail Lee Lake

# P. sulcata

{K, Zn, Cu, Pb, Ni}

Clay Lake Lee Lake N. Country Trail

{Ca, Mg, Mn, Cd}

Lee Lake Clay Lake N. Country Trail

{AI, Fe, Cr, S}

Lee Lake Clay Lake N. Country Trail

{<u>Na}</u>

Clay Lake Lee Lake N. Country Trail

{B}

Clay Lake Lee Lake N. Country Trail

# Results -- BWCA 1992 vs. Rainbow Lake 1992

All four species were included in these comparisons. Data for each locality were averaged prior to analysis (note that this changes the error term from within location to

between locations). There were no element groups for which the 1992 compositions from the BWCA and Rainbow Lake differed significantly (a = .05).

These tables indicate that lichens of RLW are probably not adversely affected by sulfur dioxide or the other elements analyzed and there is no stong gradient of air quality across the RLW. Further, elemental accumulation in RLW lichens is not significantly distinct form the BWCA lichen accumulation of the same species.

### CONCLUSIONS

There is no indication that the lichens of RLW are being damaged by sulfur dioxide or the other elements studied. The lichen flora is diverse for such an area and there is no impoverishment of the lichen flora in any part of the the wilderness. There are seven species in the most sensitive category to sulfur dioxide in the wilderness but two of these are quite rare. This rarity seems to be due more to ecological and climatic conditions than pollution since these species are quite healthy when present. The maps of the distributions of the more sensitive species do not show any significant voids that are not due to normal ecological conditions. There is no evidence of damaged or dead lichens in any area where healthy ones are not also present. The elemental analyses do not show abnormal accumulations of polluting elements at any locality. There is only a slight geographical gardient of accumulations from north to south. Elemental levels are comparable to those in the BWCA.

#### **RECOMMENDATIONS**

Although there seem to be no sulfur dioxide effects or impacts from other elements monitored in RWL now, periodic restudy is recommended. Elemental analysis should be done every 5 years and compared to the levels reported in this study. A complete floristic restudy should be done every 10-15 years.

If plans are developed to do extensive trail or road construction or maintenance in the RWL, a lichenologist should be consulted to help design the work so that rare

lichens are not lost.

A study should be done at a few localities to determine the best way to collect and sample the lichens for elemental accumulation. The RLW may not be the best location for this study because of the less luxuriant lichen growth there.

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#### **APPENDIX I**

### Rainbow Lake collection Localities

Collection numbers are those of Clifford Wetmore. All collections are listed in ascending order by number and date of collection. All localities are in Chequamegon National Forest, located in Bayfield County, Wisc.

- 70424- Near southwest corner of wilderness, NW of Wabigon Lake. On 70424 gentle hillside with maple, basswood, birch at top and white spruce and balsam fir at bottom. Sec. 12, T45N, R8W. 31 July 1992.
- 70474- Near road on west side of wilderness west of Anderson Lake. In area 70512 of white pine and some hardwoods. Sec. 2, T45N, R8W. 31 July 1992.
- 70513- In northwest corner of wilderness. On knoll with red oak, white pine 70555 and some jack pine and white birch. Sec. 26, T46N, R8W. 31 July 1992.
- 70556- Northwest of Reynard Lake in SE part of wilderness. In bog with 70597 black spruce, yellow birch and some white pines. Sec. 7, T45N, R7W. 1 Aug. 1992.
- 70598- At southwest end of Clay Lake. On hillside above shore with sugar 70652 maple, basswood and white birch. Sec. 8, T45N, T7W. 1 Aug. 1992.
- 70653- Northeast of Bufo Lake. On ridge with red oak, sugar maple and some 70690 white birch. Sec. 6, T45N, R7W. 2 Aug. 1992.
- 70691- North of Clay Lake. At edge of tamarack swamp with tamarack and 70725 red maple in the swamp and with sugar maple and some white pines on higher ground. Sec. 5, T46N, R7W. 2 Aug. 1992. CHEM. ANAL.
- 70726- In northeast part of wilderness southwest of Bellevue Lake. At edge of 70760 black spruce swamp with black spruce in swamp and pine-oak-maple on side. Sec. 31, T46N, R7W. 3 Aug. 1992.
- 70761- South of Bellevue Lake at northeast corner of wilderness. On knoll 70799 with red oak and sugar maple and few white birch. Sec. 32, T46N, R7W. 3 Aug. 1992.
- 70800- Northwest of Bellevue Lake. In and along edge of ash bog with black 70847 ash, red maple and some white pine. Sec. 30, T46N, R7W. 4 Aug. 1992.
- 70848- West of Bellevue Lake, north of Tower Lake. On ridgetop with sugar 70890 maple, basswood and some red oak. Sec. 25, T46N, R8W. 4 Aug. 1992.
- 70891- Northeast of Tower Lake at bend in trail. In young quaking aspen 70916 stand near stream with quaking aspen, some cherry and some old quaking aspen. Sec. 30, T46N, R7W. 4 Aug. 1992.
- 70917- In swamp at northwest side of Tower Lake. Ash bog with black ash, 70969

- some yellow birch and red maple. Sec. 25, T46N, R8W. 5 Aug. 1992.
- 70970- South of Frog Lake. On steep banks of gully with red oak, maples and 71006 white pine. Sec. 25, T46N, R8W. 5 Aug. 1992.
- 71007- West of Tower Lake at North Country Trail. On rolling upland with 71052 red oak, maples, white birch and some quaking aspen. Sec. 26, T46N, R8W. 6 Aug. 1992.
- 71053- At northwest corner of wilderness, southeast of Square Lake. In 71075 tamarack swamp west of North Country Trail with tamarack. Sec. 26, T46N, R8W. 6 Aug. 1992. CHEM. ANAL.
- 71076- On west side of wilderness northwest of Rainbow Lake. On side of 71125 ridge with red oak, white birch and some sugar maple. Sec. 35, T46N, R8W. 9 Aug. 1992.
- 71126- West of Rainbow Lake. In wet area with few tamarack, yellow birch, 71174 black ash, white pine and alders. Sec. 35, T46N, R8W. 9 Aug. 1992.
- 71175- At south end of Rainbow Lake. In bog with black spruce and tamar71211 ack. Sec. 36, T46N, R8W. 10 Aug. 1992.
- 71212- North of Rainbow Lake. In valley with basswood, sugar maple and 71247 some old quaking aspen. Sec. 36, T46N, R8W. 10 Aug. 1992.
- 71248- North edge of Lee Lake at southern edge of wilderness. In bog with 71288 black spruce and tamarack. Sec. 12, T45N, R8W. 11 Aug. 1992. CHEM. ANAL.
- 71289- On northeast side of Reynard Lake. On south facing hillside above 71343 bog with red oak, maples and white birch. Sec. 7, T45N, R7W. 11 Aug. 1992.
- 71344- West side of Beaver Lake. Along hillside above swamp with maple, 71398 white birch and hemlock. Sec. 32, T46N, R7W. 12 Aug. 1992.
- 71399- Northwest of Muck Lake and southwest of Beaver Lake. In bog area 71446 with some black ash, yellow birch, red maple and black spruce. Sec. 5, T45N, R7W. 12 Aug. 1992.
- 71447- Northeast of Anderson Lake. On ridge between trail and swamp with 71495 red maple, white birch and balsam fir. Sec. 1, T45N, R8W. 13 Aug. 1992.
- 71496- Near southwest corner of wilderness near Anderson Lake Trail. On 71537 level upland with sugar maple, white birch and red oak. Sec. 11, T45N, R8W. 13 Aug. 1992.
- 71538- At southeast corner of wilderness south of Wishbone Lake. In ash bog 71594 with black ash, yellow birch and red maple. Sec. 17, T45N, R7W. 14 Aug. 1992.

### **APPENDIX II**

# Species Sensitive to Sulfur Dioxide

Based on the list of lichens with known sulfur dioxide sensitivity compiled from the literature, the following species in the Rainbow Lake Wilderness Area fall within the Sensitive category as listed by Wetmore, 1983. Sensitive species (S) are those present only under 50 µg sulfur dioxide per cubic meter (average annual). Open circles on the maps are localities where the species was not found and solid circles are where it was found. Only the species in the Sensitive category are mapped.

Note: Refer to text for interpretation of these maps and precautions concerning absence in parts of the wilderness.

- Fig. 2 Bryoria furcellata (Fr.) Brodo & Hawksw.
- Fig. 3 Bryoria trichodes (Michx.) Brodo & Hawksw.
- Fig. 4. Lecidea nylanderi (Anzi) Th. Fr.
- Fig. 5. Lobaria pulmonaria (L.) Hoffm.
- Fig. 6. Parmelia squarrosa Hale
- Fig. 7. Ramalina americana Hale
- Fig. 8. <u>Usnea ceratina</u> Ach.