

**ADDENDUM
TO ORIGINAL REPORT**

BY

**LARRY L. ST. CLAIR
ASSISTANT PROFESSOR**

OF

BOTANY

BRIGHAM YOUNG UNIVERSITY

PROVO, UTAH 84602

4 JANUARY 1988

1. REGARDING: Compilation of a pollution sensitive list of lichen species for the James River Face Wilderness Area.

RESPONSE: All pollution sensitive species lists for lichens have been prepared on the basis of growth form and substrate. With corticolous, foliose and fruticose species generally considered to be the most pollution sensitive. This assumption is based on the general observation that species from these two growth forms and from bark substrates generally are the first lichens to disappear from areas where air quality has been severely impacted. This phenomenon appears to be related to the increased surface area of these two growth forms as well as the increased exposure of epiphytic species to pollutants and the poor buffering capacity of most corticolous substrates. Unfortunately, there are no studies where the selection of pollution sensitive species has been based on controlled fumigation experiments using several species from several substrates. Consequently, following the pattern as well as the same line of reasoning used in other similar studies (including Hale's study in the Flat Tops Wilderness Area) I have identified six species of lichens (see Table 1), from the James River Face Wilderness Area, which due to growth form and substrate are sensitive to both lead and sulfur. All of these species have either appeared on other pollution sensitive lists, come from "recognized" pollution sensitive genera or demonstrate a growth form or occur on a substrate which has been designated pollution sensitive. Until we are able to conduct controlled fumigation studies to absolutely determine actual pollution sensitivity all we can do is subjectively compile lists of pollution sensitive species. However, it is important to point out that I am totally confident that the species we have selected are indeed hypersensitive to both lead and sulfur and that fumigation studies would simply verify our choices. My decision to select species from other substrates (one each from soil and rock) was to simply provide additional coverage. Note that four of the species from the list come from corticolous substrates and that all the species are either foliose (umbilicate fits into this general category) or fruticose. Note also that thallus sulfur concentrations (the predominant pollutant) and membrane leakage rates were consistently high in all of the sensitive species at all sites (except for the sulfur content in Cladina subtenuis).

There are inevitably other lichens from the study area which are as sensitive or possibly even more sensitive to lead and sulfur pollution than those species selected, including: Lobaria pulmonaria, Nephroma helveticum, Ramalina fastigiata, Platismatia tuckermanii, Sticta fuliginosa, and Pseudevernia consocians. However, in every case none of these species were common enough to use effectively. The rarity of the above mentioned species may in and of itself reflect air pollution related damage to the lichen flora of the James River Face Wilderness Area. Unfortunately, there is no way to document the validity of this statement because of a lack of previous baseline information.

At your request I have also prepared and submit for your consideration a list of lichen species which I have determined would not be as sensitive to lead and sulfur pollution. Included in this list are the following species:

<u>Cladonia</u> sp.	<u>Graphis scripta</u>
<u>Dimelaena oreina</u>	<u>Lecanora subfusca</u>
<u>Lecanora varia</u>	<u>Lecidea albocaerulescens</u>
<u>Lecidea scalaris</u>	<u>Lepraria</u> sp.
<u>Thrombium epigaeum</u>	

2. REGARDING: Levels of lead and sulfur which are known to cause injury or death to sensitive species.

RESPONSE: Toxicity of specific thallus concentrations of air pollutants (e.g. lead, sulfur and fluoride) to lichens is generally known. For example, thallus concentrations of sulfur in excess of .2%, and concentrations of lead in excess of 1000 ppm and fluoride in excess of 50-70 ppm are considered toxic to lichens. These values are based on general observations concerning the viability of lichen communities where thalli of selected species demonstrate levels of pollutants in the before mentioned concentrations. Viability of the lichen community is generally assessed in terms of species richness and abundance of lichen cover, with occasional references to the condition of specific physiological parameters (e.g. photosynthetic rate, respiration rate, membrane leakage). Actual toxicity of specific concentrations of pollutants to certain lichen species must be based on detailed, controlled laboratory studies. Frankly, very little of

this type of work has been done. However, general information concerning the toxicity of thallus concentrations of specific pollutants to certain lichen growth forms and substrates is available (see information above and "response" section of question #1).

As far as the effects of ambient levels of pollutants on lichens is concerned again very little is known. What we do know generally comes from two sources: 1) field observations which demonstrate the disappearance of certain growth forms and substrate types when pollutant concentrations reach certain levels, and 2) laboratory fumigation studies which may or may not have application to real field situations.

At this point most air quality-related, management decisions for the James River Face Wilderness will have to be based on the information we have concerning actual thallus concentrations of certain pollutants (giving special consideration to sulfur) and their relationship to community viability and membrane leakage. As I pointed out in the initial report the data show that the indicator species from the study area have been impacted and are demonstrating varying degrees of pollution related stress.

3. REGARDING: Concerns about Table 2.

RESPONSE: I have adjusted Table 2. to more effectively present the information. See attached copies of modified Table 2.

4. REGARDING: Questions concerning the paucity of Cetraria oakesiana and Heterodermia squamulosa in the study area.

RESPONSE: The fact that both of these species were collected in the study area suggests that suitable habitat is available. Rarity of these two species could be related to a number of factors including air pollution impact. As far as I can see the available information suggests that the scarcity of several species (see response to question #1) including the two species mentioned above is likely related, at least in part, to diminished air quality in the study area. However, without previous baseline information all we can do is speculate on this question.

5. REGARDING: Suspected occurrence of other pollution sensitive lichen species in the James River Face Wilderness Area.

RESPONSE: Until I have been able to process all of the material from the study area and our species list is compiled it will not be possible to give you a complete answer to this question. However, as I indicated specifically in my response to questions #1 and #4 I am convinced that there are several species which "should" have been more abundant and more broadly distributed in the study area. Furthermore, in a general sense the data suggest that discrepancies in the expected and actual abundance and distribution for some species has likely been affected by toxic levels of specific air contaminants (mainly sulfur). Again, so much of this is at best hypothetical because of the absence of a previous benchmark.

6. REGARDING: Significance and meaning of sulfur concentrations.

RESPONSE: Thallus sulfur concentrations in excess of .2% are considered toxic (see the response to question # 2 for further information). Therefore, comments in the original report concerning "high" sulfur concentrations are related to toxicity levels or values near or in excess of .2%. Differential accumulation of pollutants by lichens is related directly to two factors: 1) growth form, with foliose and fruticose lichens having greater surface area and thus greater potential for accumulating airborne contaminants, and 2) substrate, with trees generally providing more aerial exposure and less buffering capacity.

Concentrations of specific pollutants in lichens must be correlated with other parameters in order to accurately determine real pollution tolerance. In other words, as you suggest in your question, thallus concentrations of pollutants are meaningless unless they are put into a context of actual pollution impact such as changes in community structure or physiological activity. Pollution tolerance is most effectively assessed by correlating physiological parameters such as photosynthetic rate or membrane leakage with actual thallus concentrations of the pollutant in question.

The advantage of using physiological parameters to determine pollution tolerance relates to the fact that the earliest signs of pollution damage, and thus the clearest indication of real pollution tolerance, are best established by measuring changes in ultra-sensitive physiological activities such as photosynthetic rate or membrane leakage.

This project was designed to establish correlations between contaminant concentrations and pollution impact by assessing community structure, species abundance and distribution as well as membrane leakage in selected pollution sensitive lichen species.

7. REGARDING: Reference levels for membrane permeability values and sulfur content for lichen thalli.

RESPONSE: I have some unpublished membrane leakage data from several locations in the United States for Parmelia caperata and also for a closely related corticolous, foliose species from Japan (Parmelia tinctorum). I also have some sulfur content information from two sites in Japan for Parmelia tinctorum. I feel that this information will provide you with a valuable context for evaluating the data from the James River Face Wilderness Area. However, please be sensitive to the fact that this information has not been published and consequently should be used discretely. I am also including some information concerning thallus sulfur and lead content in several species from Hale's study in the Flat Tops Wilderness Area in Colorado.

I have been working on several projects using Parmelia caperata as a pollution sensitive species. I currently have membrane leakage data from three different sites, two in Massachusetts and one in Colorado. One of the sites in Massachusetts has been heavily impacted by pollution while the other is a "clean" air area. The mean leakage value for the material from the polluted site was .7344 umhos/ml. While the mean leakage rates for the material from the two "clean" air sites was .392 umhos/ml and .292 umhos/ml for Massachusetts and Colorado respectively. Comparing these values with the mean leakage data for Parmelia caperata from the James River Face Wilderness Area (.8216 umhos/ml for all sites) the similarity between the James River study area and the "poor" air quality site in Massachusetts becomes apparent. Unfortunately, at this point I do not have any sulfur content

data for either the Massachusetts or Colorado material. I do, however have both membrane leakage and sulfur content data for Parmelia tinctorum a closely related corticolous, foliose species from two sites in Japan (This species also occurs in the southeastern United States). One of the sites in Japan has "poor" air quality while the second site has relatively "clean" air. The mean leakage rate for the material from the "clean" air site was .271 umhos/ml and the average sulfur content was .13%. On the other hand the mean leakage values for the material from the polluted site was .778 umhos/ml with an average sulfur content of .19%. It is particularly interesting to note the similarities between the values for Parmelia tinctorum from the polluted site in Japan and the corresponding values for Parmelia caperata in the James River Face (membrane leakage .8216 umhos/ml and sulfur content .21%).

Generally speaking, leakage values less than .350 umhos/ml are considered low and indicate minimal pollution impact. There is some variability in leakage rates between species, growth forms and substrates, however the trend toward increasing leakage with increasing pollution appears to be universal.

Hale, in the Flat Tops Wilderness Area measured sulfur and lead content in several lichen species including Parmelia (Xanthoparmelia) cumberlandia, a saxicolous foliose lichen and Usnea sp. (probably a different species from the one we are using in Virginia) a corticolous, fruticose lichen. Sulfur concentrations in Parmelia cumberlandia ranged from .11-.16% and from .13-.15% in Usnea sp. While lead values in Usnea sp. ranged from 14-28 ppm and from 27-119 ppm in Parmelia cumberlandia. In this case thallus concentrations for both lead and sulfur were quite low, suggesting essentially unpolluted conditions in the Flat Tops.

8. REGARDING: Extent of bleaching and necrosis of lichens.

RESPONSE: The data sheets from our transect studies have notations concerning bleaching and necrosis of corticolous lichens from two transects, Matts Creek and Piney Ridge. These two sites seem to have more bleached and necrotic material than any of the other sites, however this observation is strictly subjective, we did not make any attempt to

quantify either bleaching or necrosis. The decision to include the other transects (except Belfast Trail) in this category was based on observations made while processing material brought back to the laboratory.

9. REGARDING: Relative pollution sensitivity of Cetraria halei and Parmelia caperata.

RESPONSE: Pollution sensitivity was determined by assessing both membrane permeability and sulfur accumulation. Both Cetraria halei and Parmelia caperata consistently demonstrated high leakage rates and relatively high concentrations of sulfur (mean leakage rates of 6.105 and .8216 umhos/ml, and mean sulfur concentrations of .19% and .21% respectively). Both Parmotrema stuppeum and Umbilicaria papulosa had higher concentrations of sulfur (mean values of .22% and .24% respectively), but demonstrated lower membrane leakage rates (mean rates of .460 and .417 umhos/ml respectively).

Obviously, there are significant differences in both membrane leakage rates and pollutant accumulation capacities between all of the indicator species. This is probably related to a number of factors including: substrate, growth form (surface area) and inevitably inherent differences in the species as well as differences in microclimate. In spite of this, membrane leakage rates are consistently high (above the .350 umhos/ml level) for all of the designated pollution sensitive species.

10. REGARDING: Relationship between collecting time and species diversity at study sites.

RESPONSE: At this point the species list for Sulfur Springs Trail is a little short. However, I feel this problem will be remedied as soon as we are able to finish processing the rock material we collected just down from the junction of Sulfur Springs and Piney Ridge Trails. If you remember, we spent quite a bit of time collecting at two fairly diverse rock sites near the junction, and from that material we should be able to add a substantial number of species to the list. In spite of the fact that we were tired toward the end of that day, I feel confident that we were able to obtain a representative

collection of the lichens along that trail. However, I will carefully reevaluate this when we finalize the species list.

11. REGARDING: Comparison of hypersensitive species between study sites in the wilderness.

RESPONSE: See the response to question # 1.

12. REGARDING: Statement in final report concerning lumbering in the wilderness.

RESPONSE: I misunderstood concerning the amount of lumbering that had taken place in the area prior to the establishment of the wilderness. I am including with this letter three copies of page six with the following correction: "... some lumbering had occurred along the periphery of the general study area."

GENERAL QUESTIONS CONCERNING RECOMMENDATIONS

1. REGARDING: Purpose for specifically analyzing bleached and necrotic material.

RESPONSE: Direct analysis of bleached and necrotic material will allow us to specifically correlate actual concentrations of sulfur with membrane leakage values in material showing visible signs of damage. This will also allow us to more directly assess relationships between pollution impact, pollutant accumulation and thallus bleaching and necrosis. At this point it is generally assumed that bleaching and necrosis are outward signs of pollution-related damage to lichens. As far as I have been able to determine there have been no in situ, studies to document this widely held view. As you have suggested in your letter, both necrosis and bleaching could be caused by other non-pollution related factors, this procedure should allow us to document actual relationships.

2. REGARDING: Timing for retesting sensitive species for sulfur accumulation and membrane permeability.

RESPONSE: With existing levels of sulfur in the six pollution sensitive species I think it is imperative that the indicator species be monitored on an every other year basis. In my studies in Massachusetts I have detected almost a doubling in membrane leakage rates (from .7344 umhos/ml in 1986 to 1.197 umhos/ml in 1987) in Parmelia caperata from the exact same site in a single year. Fairly rapid changes can happen over short periods of time. I think we are in a very strong position to effectively document the point in time when threshold pollution levels in several of the indicator species are exceeded.

3. REGARDING: Analysis of sample cores from chestnut oak for specific elements.

RESPONSE: My intent in recommending analysis of xylem tissues from chestnut oak was not as a replacement for elemental analysis of lichen tissues. In fact, the purposes are quite different, analysis of lichen tissue yields direct information concerning concentrations of airborne contaminants, whereas elemental analysis of cores from chestnut oak would document cause and effect relationships between pollution impact and the mobility of certain elements in the soil. It is an established fact that acid rain (a real consideration in the wilderness due to the concentrations of sulfur found in the lichen indicator species) can dramatically reduce the pH of acidic soils. In turn reduced soil pH greatly increases the mobility of certain soil minerals (some of which are quite toxic) thus resulting in the increased uptake of these elements by vascular plants.

This procedure would simply provide us with another parameter for assessing the condition of the wilderness ecosystem relative to air quality.

FINAL REPORT CONCERNING:
THE ESTABLISHMENT OF AN AIR QUALITY BIOMONITORING
PROGRAM USING VARIOUS LICHEN PARAMETERS IN THE
JAMES RIVER FACE WILDERNESS AREA, JEFFERSON
NATIONAL FOREST, VIRGINIA

SUBMITTED TO
THE UNITED STATES FOREST SERVICE
JEFFERSON NATIONAL FOREST
ROANOKE, VIRGINIA
2 4 0 0 1

BY
LARRY L. ST. CLAIR
ASSISTANT PROFESSOR OF BOTANY AND RANGE SCIENCE
BRIGHAM YOUNG UNIVERSITY
PROVO, UTAH 84602

CONTRACT NUMBER:
53-3395-00033

NOVEMBER 16, 1987

TABLE OF CONTENTS

Project objectives	1
Project summary	1
Review of pertinent literature	2
General information	2
Information related to Virginia	4
Site description	5
General information	5
Establishment of reference sites	6
Methods	8
Collection, preparation and identification of material for voucher collection	8
Collection of ecological data	8
Collection of material for laboratory analyses	9
Determination of lead, sulfur and fluoride in thallus tissues	9
Determination of membrane leakage	9
Statistical analyses	
Ecological data	9
Laboratory data	10
Results and Discussion	10
Floristic survey	10
Ecological survey	10
Laboratory analyses	
Data concerning accumulation of pollutants	11
Membrane permeability data	12
Observations and Recommendations	12
Literature cited	14
Appendix A (Color photographs from permanent ecological transects)	
Appendix B (Raw data sheets from permanent ecological transects)	
Appendix C (Raw elemental concentration data, and membrane leakage values)	

PROJECT OBJECTIVES

1. Collect, identify and provide voucher specimens of the lichen species which occur within the study area.
2. Establish five permanent reference sites within the study area.
3. Determine those species of lichens, from the study area, which according to the available literature are hypersensitive to lead, sulfur and fluoride.
4. Analyze replicate thallus samples of each of six pollution hypersensitive lichen species, from each of the permanent reference sites, for total lead, sulfur and fluoride content.
5. Review current literature concerning the effects of various levels of air pollutants, especially lead, sulfur and fluoride, on lichen species found in the study area.

PROJECT SUMMARY

During the months of July and August, 1987 a comprehensive air quality biomonitoring program using various lichen parameters was established in the James River Face Wilderness Area. The purpose of this project was two fold: 1) assess the current condition of the lichen flora in the study area and 2) establish an effective baseline for future assessment of air quality changes in the wilderness area using lichens. These purposes were accomplished by: 1) conducting a survey of the lichen flora of the study area, 2) establishing five permanent ecological transects, on corticolous substrates, in various parts of the wilderness, 3) determining those species of lichens which tend to be hypersensitive to lead, sulfur and fluoride, 4) analyzing replicate thallus samples of six pollution hypersensitive lichen species for total lead, sulfur and fluoride from each of the permanent transect sites, and 5) reviewing the current literature concerning the effects of various levels of air pollutants on lichen species found in the study area.

Five reference sites within the study area were established. At each reference site the following was accomplished: 1) all substrates and habitat types were carefully examined for lichen species and a representative collection was made, 2) an ecological transect was established to evaluate the structure of the corticolous lichen community, 3) material from six lichen species from three different substrates was obtained in order to assess total lead, sulfur, and fluoride accumulation in

thallus tissues as well as membrane leakage, and 4) a permanent photographic record of 12 study quadrats was made.

Analysis of corticolous lichen community structure revealed several factors which appear to be influencing species distribution patterns namely: substrate differences, and composition, density and maturity of the vascular plant community. Several species of lichens tended to be cosmopolitan while other species demonstrated definite substrate and habitat preferences. Throughout the study area Quercus prinus L. (Chestnut Oak) consistently supported the most diverse and well developed lichen flora.

To date a total of 94 species in 36 genera have been identified from the study area. All lichen growth forms are well represented in the flora. Parmelia rupestris, Parmelia caperata and Parmotrema stuppeum are the most ubiquitous corticolous species, with Cladonia subtenuis the most common terricolous species and Parmelia plittii, Umbilicaria pustulata, Umbilicaria mammulata and Umbilicaria pensylvanica representing some of the more common saxicolous species.

Analysis of thallus tissues for lead, sulfur and fluoride accumulation as well as membrane leakage revealed significant trends and interactions in terms of species, substrates and sites. The data indicate that sulfur is a critical pollutant in the wilderness with both lead and fluoride generally occurring in relatively low concentrations.

REVIEW OF PERTINENT LITERATURE

General Information

The use of lichens as bioindicators of air quality is a well documented procedure (Skye 1979; Richardson and Nieboer 1981; Fields and St. Clair 1984). Hale (1983) noted that lichens have been used in three basic ways to monitor air pollution: 1) elemental analysis of thallus tissues, 2) mapping of all (or selected) lichen species found in areas adjacent to pollution sources, and 3) transplant studies. The most commonly used approaches are floristic surveys (Wetmore 1981), analysis of species distribution patterns and community structure (Rushforth et al. 1982; Sigal and Nash 1983) or a combination of the two (Pyatt 1970; Marsh and Nash 1979). It has also been shown that lichens effectively accumulate a wide variety of pollutants washed from the atmosphere by precipitation. Thus, lichens provide an effective record of the types and quantities of materials being put into the atmosphere by various pollution sources (Gough and Erdman 1977; Schutte 1977). Elemental analysis of lichen tissues as a pollution monitoring device has progressed to the point that it is now possible to measure pollution patterns for specific elements over extended periods. This is done by determining thallus growth rates and excising and

analyzing annual growth increments of tissue for specific elements (Lawrey and Hale 1981). Hale and Lawrey (1985) have also been able to document actual accumulation rates of lead by the foliose lichen Parmelia baltimorensis.

Laboratory studies have verified the adverse effects of a variety of pollutants on several lichen metabolic functions. Specifically, the effects of sulfur dioxide on photosynthetic rates, respiration rates, and membrane integrity have been evaluated by Fields and St. Clair (1984). Puckett (1976) studied the impact of several heavy metals on potassium efflux, chlorophyll content and photosynthetic rates in lichens. The effects of ozone on photosynthesis have also been assessed (Nash and Sigal 1979). Sigal and Taylor (1979) have fumigated lichen thalli with peroxyacynitrates and evaluated the effects of this common pollutant on lichen photosynthetic activity. Generally, laboratory studies have shown that photosynthetic rate, respiration rate and membrane integrity are all very sensitive to a wide range of common air pollutants. Indeed lichen physiological processes appear to provide an indication of pollution damage long before any visible thallus necrosis or changes in community structure can be detected (Sundstrom and Hallgren 1973; Fields and St. Clair 1984).

Unfortunately, field studies assessing the effects of air pollutants on lichen metabolic activities are rare. Eversman (1978) examined the impact of low level sulfur dioxide fumigation in the field on the respiration rate of two lichen species. A similar field study conducted in the Arctic (Moser et al. 1980) showed a significant decline in both respiration and photosynthetic rates in lichens following fumigation with sulfur dioxide. In another field study (Pearson and Rodgers 1982) the effects of sulfur dioxide on membrane integrity in two corticolous lichens were evaluated. This study was conducted over a period of several weeks and lichen material from several sites downwind from a sulfur burner was examined. Shortly after the initial exposure there was a significant increase in electrolyte leakage, followed by discoloration, bleaching and necrosis of specimens closest to the sulfur burner. This study showed that membrane integrity in lichens is severely impacted following exposure to sulfur dioxide.

St. Clair Et al. (in review) conducted an in situ air pollution-related field study where membrane integrity, chlorophyll content and elemental analysis for selected pollutants in thalli of the foliose, pollution-tolerant lichen Parmelia tinctorum were compared from two sites in Japan. The first site (Miyajima Island) is a National Park located approximately two km downwind and off the coast of Hiroshima, a large industrial complex which generates substantial amounts of air contaminants. The second site is located in a relatively pollution free, rural area east of Kochi City on

Shikoku Island. This study showed that the normal appearing Miyajima material demonstrated significantly greater electrolyte leakage, significantly less chlorophyll and substantially more sulfur, lead and fluoride than the Kochi material. Thus lending further credibility to the idea that physiological properties of lichens reflect air pollution-related damage much earlier than changes in thallus morphology or community structure.

Pollution sensitive species lists have commonly been compiled in conjunction with many floristic and ecological surveys (Wetmore 1981; Rushforth et al. 1982; Hale 1982). These lists have been prepared in an effort to monitor air quality by assessing the vitality of lichen species which are theoretically hypersensitive to air pollution. The rationale behind this approach is based on the premise that certain lichen growth forms as well as species from certain substrates are more sensitive to airborne contaminants.

Information related to Virginia

Prior to the mid 1960s very little was known about the lichen flora of Virginia (Culberson 1955). In the 1940s and 1950s three brief papers concerning various aspects of lichen floristics in the state were published (Allard and Leonard 1944; Luttrell 1954; Allard 1957). A review of the literature since that time shows that several papers have been written concerning the lichens of Virginia and adjacent states, these basically fall into two broad categories: general floristic surveys of given localities or specific habitats (Culberson 1965; Dey 1978), as well as some monographic work (Culberson 1966; Culberson and Culberson 1968). These papers provide useful information concerning the flora in general, as well as specific information regarding habitat and substrate preferences of individual species and groups. Dey's work on the high mountain fruticose and foliose lichen of the southern Appalachians (Dey 1978) has been particularly useful during the course of this project.

A similar project using lichens as bioindicators of air quality has been underway in Shenandoah National Park in northern Virginia since 1983 (Lawrey 1984; 1985; 1987). Basically, the participants in this project have been examining patterns of lead and sulfur accumulation over space and time in the two foliose lichens Parmelia baltimorensis (a saxicolous species) and Parmelia caperata (a corticolous species). Data generally indicate that there are no distinct patterns concerning lead accumulation except that there seems to be an overall decline in lead accumulation over time with some apparent localized road effect (Lawrey 1985). On the other hand, sulfur concentrations in both species were consistently high, with samples from the northern district of the Park demonstrating the highest concentrations. Due to higher concentrations of sulfur in Parmelia caperata over the central and southern sections of the Park it was

concluded that this species may be a more effective accumulator of sulfur (Lawrey 1985). It was suggested that differences in accumulation patterns between the two species may be related in part to substrate and habitat differences.

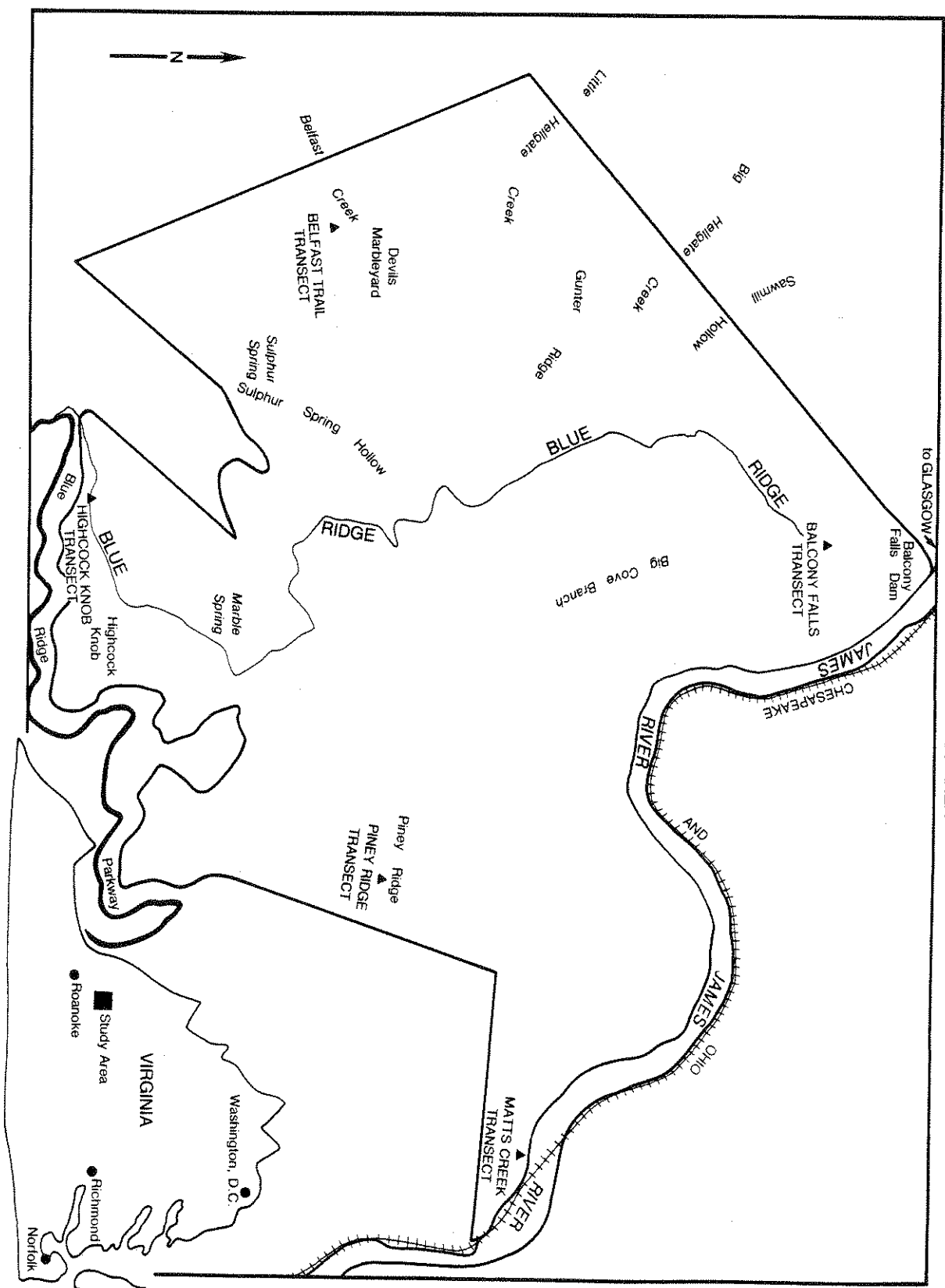
More recently Lawrey has evaluated distribution patterns of three suspected pollution-sensitive corticolous lichen species (Cetraria oakesiana, Usnea spp. and Heterodermia squamulosa) throughout the Park. Results from this portion of the study indicated that the three indicator species were consistently missing from the more polluted northern district of the Park. Whereas in the southern and central districts Heterodermia squamulosa and Cetraria oakesiana were abundant with Usnea spp. generally restricted to the southern district (Lawrey 1987). Thus indicating that increasing levels of sulfur dioxide pollution in certain areas of the Park may have resulted in the elimination of at least some species of lichens. These three studies represent the extent of the information available concerning the role of lichens as bioindicators of air quality in the state of Virginia. However, this information has been instrumental in the organization and design of the lichen biomonitoring program for the James River Face Wilderness Area. For example two of our indicator species (Usnea subfloridana and Parmelia caperata) were also used in the Shenandoah National Park studies. The other species used in Lawrey's studies (Parmelia baltimorensis, Heterodermia squamulosa and Cetraria oakesiana) were only found rarely in the James River Face Wilderness Area and therefore could not be effectively included in the various analyses.

SITE DESCRIPTION

General information

The James River Face Wilderness Area is a relatively small tract of land consisting of approximately 3445 hectares of mountainous forestland. The wilderness is located in the Jefferson National Forest approximately 75 km northeast of Roanoke, Virginia (Figure 1). It is bounded on the north and east by the James River and on the south by the Blueridge Parkway. Most of the wilderness is located in Bedford County with the balance in Rockbridge County. Elevation in the wilderness ranges from 246 m on the east side to over 923 m on the south side. A wide variety of habitat types are found within the wilderness including mature deciduous forestland, pine forests, rock outcrops and some riparian communities. Composition of the deciduous forest community varies but is commonly dominated by Quercus prinus L. (Chestnut Oak). Other common components of the deciduous forest include: Carya spp. (Hickory), Acer rubrum L. (Red Maple), Quercus coccinea Muenchh. (Scarlet Oak), Quercus alba L. (White

AIR QUALITY BIOMONITORING SITES IN THE JAMES RIVER FACE WILDERNESS AREA



Oak), and Nyssa sylvatica Marsh. (Black gum). The pine forest varies depending on the major species, with either Pinus virginiana Mill. (Virginia Pine), Pinus pungens Lamb. (Table-mountain Pine) or Pinus rigida Mill. (Pitch Pine) dominating this community type. Prior to the establishment of the wilderness, some lumbering had occurred along the periphery of the general study area. The wilderness currently supports a diverse and well developed lichen community.

Establishment of reference sites

Five permanent reference sites were established at various points in the James River Face Wilderness Area (Figure 1). These sites were intentionally placed along the periphery of the study area in order to provide a more effective approach to biomonitoring air quality in the wilderness.

The first permanent study site was set up at the top of the Balcony Falls Trail on the north side of the wilderness, 2.3 km east of Natural Bridge Station at an elevation of 569 m (37° 36' 49" north latitude and 79° 26' 53" west longitude). This site is dominated by Quercus prinus (Chestnut Oak) and Carya sp. (Hickory). Lichens were collected all along the trail beginning at the entrance to the wilderness to the top of the ridge. All substrates including soil, rocks, shrubs, and a variety of trees were examined for lichen species. The transect is located at the top of Balcony Falls Ridge. Point #1 is marked with a metal stake 9 m (29 ft.), 143° south by southeast from the path (see color photographs in Appendix A).

The second permanent study site was established west of Highcock Knob on the southwest side of the wilderness, 3.9 km southeast of Natural Bridge Station at an elevation of 738 m (37° 33' 39" north latitude and 79° 23' 29" west longitude). This study site was established in a mature deciduous forest consisting of Quercus prinus, (Chestnut Oak) Nyssa sylvatica (Black Gum), Quercus coccinea (Scarlet Oak), Carya spp. (Hickory), and Acer rubrum (Red Maple). Lichens were collected all along the trail beginning at the entrance to the wilderness to a point just west of Highcock Knob. All substrates including soil, rocks, shrubs, and a variety of tree species were examined for lichen species. The transect is located 203 m (660 feet) up the path from the large James River Face Wilderness Area sign on the south side of the trail. Point #1 is marked with a metal stake 7 m (23 ft.), 167° south by southeast from the path (see color photographs in Appendix A).

The third permanent study site was established along Belfast Trail west of the Devil's Marbleyard on the west side of the wilderness, 2.55 km south of Natural Bridge Station at an elevation of 425 m (37° 34' 38" north latitude and 79° 26' 33" west longitude). The vascular plant community at this site was dominated by Quercus prinus (Chestnut Oak) and Pinus

virginiana (Virginia Pine). Lichens were collected all along the trail beginning at the entrance to the wilderness to a point where Belfast Trail intersects with the Appalachian Trail above Devil's Marblyard. All substrates including soil, rocks, shrubs, and a variety of tree species were examined for lichen species. The transect is located 62 m (200 ft.) down the trail from the stream crossing, above a prominent rock outcrop. Point #1 is marked with a metal stake 15 m (50 ft.), 120° north by northeast from the path (see color photographs in Appendix A).

The fourth permanent study site was established along Piney Ridge Trail, south of Piney Ridge on the south side of the wilderness, 4.3 km southeast of Natural Bridge Station at an elevation of 548 m (37° 34' 51" north latitude and 79° 27' 14" west longitude). The vascular plant community at this site was dominated by Quercus prinus (Chestnut Oak) Quercus coccinea (Scarlet Oak) and Pinus rigida (Pitch Pine). Lichens were collected all along the trail beginning at the wilderness boundary of to a point where Piney Ridge Trail intersects Sulphur Springs Trail. All substrates including soil, rocks, shrubs, and a variety of tree species were examined for lichen species. The transect is located 154 m (500 ft.) west along the trail from the wilderness boundary sign. Point #1 is marked with a metal stake 11 m (36 ft.), 176° south by southwest from the path (see color photographs in Appendix A).

The fifth permanent study site was set up along Matts Creek Trail on the east side of the wilderness, 5.05 km southeast of Natural Bridge Station at an elevation of 311 m (37° 35' 29" north latitude and 79° 28' 50" west longitude). The vascular plant community at this site was dominated by Quercus prinus (Chestnut Oak), Quercus alba (White Oak) Nyssa Sylvatica (Black Gum), Quercus coccinea (Scarlet Oak), and Acer rubrum (Red Maple). Lichens were collected all along the trail beginning at the boundary to the wilderness to Matts Creek shelter. All substrates including soil, rocks, shrubs, and a variety of tree species were examined for lichen species. The transect is located 500 m (1623 ft.) from the intersection of the Appalachian Trail and second curve of the old logging road, south of the path. Point #1 is marked with a metal stake 7 m (22 ft.), 200° south by southwest from the path (see color photographs in Appendix A).

In addition to the lichen collections made at each of the permanent study sites extensive collections were made along the Gunter Ridge Trail in the northeast portion of the wilderness and along Sulphur Springs Trail which is located in the southwest quadrant of the wilderness. All substrates including soil, rocks, shrubs and a variety of tree species at these two sites were examined for lichen species.

METHODS

Collection, preparation and identification of lichen specimens

Due to the fact that the distribution of lichen species is directly related to substrate, moisture conditions and insolation patterns, all available substrates and habitats within the general vicinity of the five reference sites were examined. All herbarium material was collected by removing small sections of bark, soil or rock, with the attached specimen, using a knife or hammer and chisel. All specimens were placed in paper sacks which were labeled as to site, date and substrate for transport back to Lichen Herbarium at Brigham Young University for further processing and identification. Upon returning to the laboratory all specimens were thoroughly washed, dried and pressed and placed in permanent herbarium packets with detailed labels indicating collection locality, habitat type and substrate. Species identifications have been accomplished using standard keys and taxonomic works in the field of lichenology. Standard chemical spot tests as well as thin-layer chromatography techniques were used to finalize many species designations.

All material, whenever possible, was collected in duplicate. One complete set will be deposited in the U.S. Forest Service Regional Center in Atlanta, Georgia as a reference collection, and the second set will remain in the Lichen Herbarium at Brigham Young University, Provo, Utah as a voucher collection. These collections will provide a duplicate record of the current lichen flora of the James River Face Wilderness Area. This record will serve as an effective baseline for future floristic surveys in the study area.

Collection of ecological data

Five permanent reference sites were established around the periphery of the study area (Figure 1). Location of these sites was based on various factors including: 1) elevation, 2) vegetation type, 3) potential, local pollution sources, and 4) overall coverage of the study area. Each transect was permanently marked, compass bearings were taken and specific locations were noted on 7.5 minute topographic maps (Snowden Quadrangle, Virginia).

A ten point transect was established at each reference site using the quarter method (Phillips 1959). The quarter method is a proven plotless sampling technique in which points along a line transect are identified. In our study the transect points were located every 10 m along the transect. The area around each point was divided into four quadrants and the nearest tree in each quadrant was sampled. Due to the potential for aspect induced differences in corticolous lichens (St. Clair et al. 1986), north, east, south and west exposures on all trees were sampled separately. A 10 cm

by 20 cm clear plastic quadrat was used to visually estimate percent cover of all lichen species. Quadrats were consistently placed at a point 1 m above the ground. Color photographs of all quadrats on the first, fifth and tenth trees were taken (Appendix A). Corticolous communities were selected for this portion of the study due to the fact that most of the more pollution sensitive lichen species occupy corticolous substrates.

Collection of thallus material for laboratory analyses

In light of Lawrey's work in Shenandoah National Park, and after carefully considering the following factors: abundance, substrates, growth forms, documented/suspected pollution sensitivity, and distribution patterns of the lichens in the wilderness, six taxa were selected as test species for all laboratory analyses (Table 1).

At each of the permanent reference sites adequate material from all six pollution sensitive species was collected for conducting laboratory analyses including: Pb, S and F assessments, as well as membrane permeability. Test material was stored dry in paper sacks and transported back to the lichen laboratory at Brigham Young University.

Determination of Pb, S and F concentrations in thallus tissues

Upon returning to the laboratory all samples were washed thoroughly to remove surface debris and dust, samples were then allowed to air dry. Ten 500 mg replicates for each species at each reference site were then weighed out and oven dried. Samples for lead and sulfur determination were then wet ashed using perchloric acid. Total lead content was then determined by atomic absorption, and total sulfur content was assessed turbidimetrically using barium chloride. Samples for fluoride determination were digested using nitric acid and potassium hydroxide and then total fluoride was determined using an ion selective electrode.

Determination of membrane leakage

For membrane permeability measurements, uniformly sized pieces (100 mg dry weight) of lichen thalli were placed in a humidity chamber for 12 hours to allow for establishment of resaturation rate. Replicate samples were then immersed in 75 ml of deionized water for 5 minutes then removed. Conductivity of the deionized water both before and after immersion of each lichen sample was measured using a YSI model 32 conductance meter with a #3402 cell (Pearson and Rodgers 1982) and recorded as net conductivity gain. Net leakage was calculated by multiplying the net conductivity by 75 ml (volume of deionized water) and then dividing the product by the weight of the sample.

Statistical methods: ecological data

Evaluation of distribution patterns of corticolous lichens was accomplished by performing various statistical analyses on the quadrat data. Stand similarity indices were calculated using the methods of

Table 1. Pollution-sensitive lichen species from the James River Face Wilderness Area with substrate and growth form information.

SPECIES	SUBSTRATE	GROWTH FORM
<u>Cetraria halei</u>	Twigs of Mountain Laurel	Foliose
<u>Cladina subtenuis</u>	Soil along paths	Fruticose
<u>Parmelia caperata</u>	Bark of trees	Foliose
<u>Parmotrema stuppeum</u>	Bark of trees	Foliose
<u>Umbilicaria papulosa</u>	Rocks	Umbilicate
<u>Usnea subfloridana</u>	Bark of trees	Fruticose

Ruzicka (1958). Cluster analysis was used to compare each stand with each other stand using arithmetic averages (Sneath and Sokal 1973). Importance values for each species were also calculated by multiplying frequency by average cover (Warner and Harper 1972). The total number of species on each substrate type and at each site was determined and Shannon-Weaver diversity indices for each substrate and site were calculated (Shannon and Weaver 1963).

Statistical methods: laboratory data

Analysis of variance was used to determine significant effects due to species, substrates, sites and interactions among these variables.

RESULTS AND DISCUSSION

Floristic survey

To this point a total of 94 species in 36 genera have been identified from the James River Face Wilderness Area (Table 2). The lichen flora is diverse and well developed on all substrates at each of the permanent reference sites. All lichen growth forms are also well represented at each of the study sites. Parmelia rupecta, Parmelia caperata, and Parmotrema stuppeum are the dominant corticolous species, being found commonly on the bark of most tree species throughout the study area. Cladonia subtenuis is the most common terricolous species; with Parmelia plittii, Umbilicaria pustulata, Umbilicaria pensylvanica, and Umbilicaria mammulata representing some of the more common saxicolous species.

Ecological survey

Results of the overall cluster analysis of study sites, based on evaluation of corticolous communities, revealed a very low degree of similarity (29% average similarity) between sites (Figure 2). This indicates substantial differences in community structure among the five permanent reference sites with two very loose cluster groups, Belfast Trail with Highcock Knob and Matts Creek comprising group I (34% similarity) and Balcony Falls and Piney Ridge making up group II (24% similarity).

Importance values for all corticolous lichens were calculated (Table 3). Data indicate that Parmelia rupecta (IV of 9.02), Parmelia caperata (IV of 2.23), Physcia sp. (IV of 0.95), Cladonia sp. (IV of 0.64), Parmotrema stuppeum (IV of 0.20), and Pertusaria amara (IV of 0.10) were the most important species. Species importance values for each corticolous substrate were also calculated (Table 4). Dominant lichen species on Virginia Pine (Pinus virginiana) were Cladonia sp. (IV of 4.90), Physcia sp. (IV of 0.51), Parmelia caperata (IV of 0.33), and Cetraria halei (IV of 0.32). Dominant species on White Oak (Quercus alba) included: Parmelia rupecta (IV of 2.20), and Lecanora varia (IV of 0.16). The Black Gum (Nyssa sylvatica) lichen flora was dominated by Parmelia rupecta (IV

Table 2. Substrate, abundance and distribution information for the lichen species of the James River Face Wilderness Area, Jefferson National Forest, Virginia.

SPECIES	SUBSTRATE	MATS CREEK	PINEY RIDGE	BELFAST TRAIL	BALCONY FALLS	HIGHCOCK KNOB	GUNTER RIDGE	SULFUR SPRINGS
Anaptychia palmatula	Bark		R*	R-C**	R	R	R-C	
Baeomyces absolutus	Soil	R-C		R				
Baeomyces fungoides	Soil	C	R	R-C	R		R	
Candelaria concolor	Bark							R
Cetraria aurescens	Bark			R	R		R	R
Cetraria fendleri	Bark	R			R		R	
Cetraria halei	Bark	R-C	C	C	R-C	R	C	
Cetraria oakesiana	Bark				R	R	R	
Cladina rangiferina	Soil	R	R				R	
Cladina subtenuis	Soil	C-A+	R-C	C	C-A	R	C	
Cladonia apodocarpa	Soil			R				
Cladonia capitata	Soil			R				
Cladonia cariosa	Soil	R	R	R				
Cladonia chlorophaea	Soil	C	R-C	R-C	C	R-C	R-C	
Cladonia clavulifera	Soil			R				

SPECIES	SUBSTRATE	MATS CREEK	PINEY RIDGE	BELFAST TRAIL	BALCONY FALLS	HIGHCOCK KNOB	GUNTER RIDGE	SULFUR SPRINGS
Cladonia cristatella	Soil/deadwood		R-C	R-C		R	R-C	
Cladonia cylindrica	Soil		R	R	R			
Cladonia furcata	Soil				R			
Cladonia papillaria	Soil	R-C	R	R-C			R-C	
Cladonia parasitica	Bark	R						
Cladonia piedmontensis	Soil	R						
Cladonia pyxidata	Soil			R				
Cladonia squamosa	Soil	R-C		R-C		R	R-C	
Cladonia uncialis	Soil			R				
Coccocarpia cronia	Bark			R			R	
Collema subflaccidum	Bark			R		R	R	
Dimelaena oreina	Rock	R		R-C	R			
Graphis scripta	Bark		R-C	C		C	R-C	
Heterodermia albicans	Bark						R	
Heterodermia hypoleuca	Bark					R		
Heterodermia leucomelaena	Bark			R			R	
Heterodermia obscurata	Bark						R	

SPECIES	SUBSTRATE	MATTS CREEK	PINEY RIDGE	BELFAST TRAIL	BALCONY FALLS	HIGHCOCK KNOB	GUNTER RIDGE	SULFUR SPRINGS
Heterodermia propagulifera	Bark/deadwood						R	
Heterodermia squamulosa	Bark			R				
Hypogymnia physodes	Bark			R				
Hypotrachyna imbricatula	Moss				R			
Hypotrachyna virginica	Bark		R	R			R	
Lecanora chrysoleuca	Rock					R		
Lecanora subfusca	Bark	R-C	R-C	C	C	C	R-C	R-C
Lecanora varia	Bark	R-C	R-C	R-C	R	R-C	R-C	R
Lecidea albocaerulescens	Rock	C	C	C	C	C	C	C
Lecidea scalaris	Bark	C	C	C	C	C	C	C
Lepraria candelaris	Bark			R-C	R	R	R	
Lepraria chlorina	Rock			R				
Leptogium cyanescens	Bark	R				R	R	
Lobaria pulmonaria	Bark			R-C				
Lobaria quercizans	Bark			R				
Nephroma helveticum	Bark						R	
Normandina pulchella	Foliose Lichens	R		R				

SPECIES	SUBSTRATE	MATTS CREEK	PINEY RIDGE	BELFAST TRAIL	BALCONY FALLS	HIGHCOCK KNOB	GUNTER RIDGE	SULFUR SPRINGS
Pannaria leucosticta	Bark			R			R	
Parmelia appalachensis	Bark							R
Parmelia baltimorensis	Rock	R	R	R-C	R-C	R	R-C	R
Parmelia caperata	Bark	A	A	A	A	A	A	A
Parmelia caroliniana	Bark			R			R	
Parmelia conspersa	Rock	C	C	C	C	C	C	C
Parmelia dissecta	Rock						R	
Parmelia galbina	Bark						R	
Parmelia obsessa	Rock			R-C				
Parmelia omphalodes	Rock			R				
Parmelia plittii	Rock	C	C	C-A	C-A	C	C-A	C
Parmelia rudecta	Rock/bark	C	C	C	C	C		C
Parmelia saxatilis	Rock/bark			C	R-C	R-C	C	
Parmelia subrudecta	Twigs/deadwood				R	R	R	
Parmelia sulcata	Bark				C		C	
Parmelia taractica	Rock							R
Parmeliopsis aleurites	Bark/deadwood	C	C-A	C-A	C	C	C-A	C

SPECIES	SUBSTRATE	MATTS CREEK	PINEY RIDGE	BELFAST TRAIL	BALCONY FALLS	HIGHCOCK KNOB	GUNTER RIDGE	SULFUR SPRINGS
Parmeliopsis placododia	Bark/twigs	R	R	R	R-C	R	R-C	R
Parmotrema hypotropum	Bark	C	C	C-A	C	C	C-A	C
Parmotrema perlalum	Bark	R-C	R-C	C	R-C	R-C	C	R-C
Parmotrema reticulatum	Bark	C	C	C-A	C	C	C-A	C
Parmotrema stuppeum	Bark	C-A	C	C-A	C	C	C	C
Parmotrema xanthium	Bark	R						
Peltigera malacea	Soil	R		R			R	R
Pertusaria amara	Bark	C	C-A	C-A	C-A	C	C	C
Pertusaria multipuncta	Bark	C	C	C	C	C-A	C	C
Phaeophyscia imbricata	Bark							R
Phaeophyscia rubropulchra	Deadwood/moss	R					R	
Physcia americana	Bark/deadwood						R	
Physcia ciliata	Bark					R		
Physcia millegrana	Bark					R-C	R-C	
Physcia stellaris	Bark							R
Physcia syncolla	Bark							R

SPECIES	SUBSTRATE	MATTS CREEK	PINEY RIDGE	BELFAST TRAIL	BALCONY FALLS	HIGHCOCK KNOB	GUNTER RIDGE	SULFUR SPRINGS
Physconia detersa	Deadwood						R	
Physconia enteroxantha	Bark			R		R	R	R
Platismatia tuckermanni	Bark/rock	R			R			R
Pseudevernia consocians	Bark/deadwood			R	R			R
Ramalina fastigiata	Bark			R			R	
Ramalina pollinaria	Rock			R				
Sticta fuliginosa	Bark				R			
Thrombium epigaeum	Soil	R-C	R	R	R-C	R	R	
Umbilicaria mammulata	Rock	C	C	C-A	C-A	C		
Umbilicaria papulosa	Rock	C	C	C-A	C-A	C		
Umbilicaria pennsylvanica	Rock/deadwood	C	R	C-A	C-A	C		R-C
Usnea subfloridana	Bark/rock	C	C	C-A	C-A	R-C		

*R = rare

**C = common

+ A = abundant

[illegible]

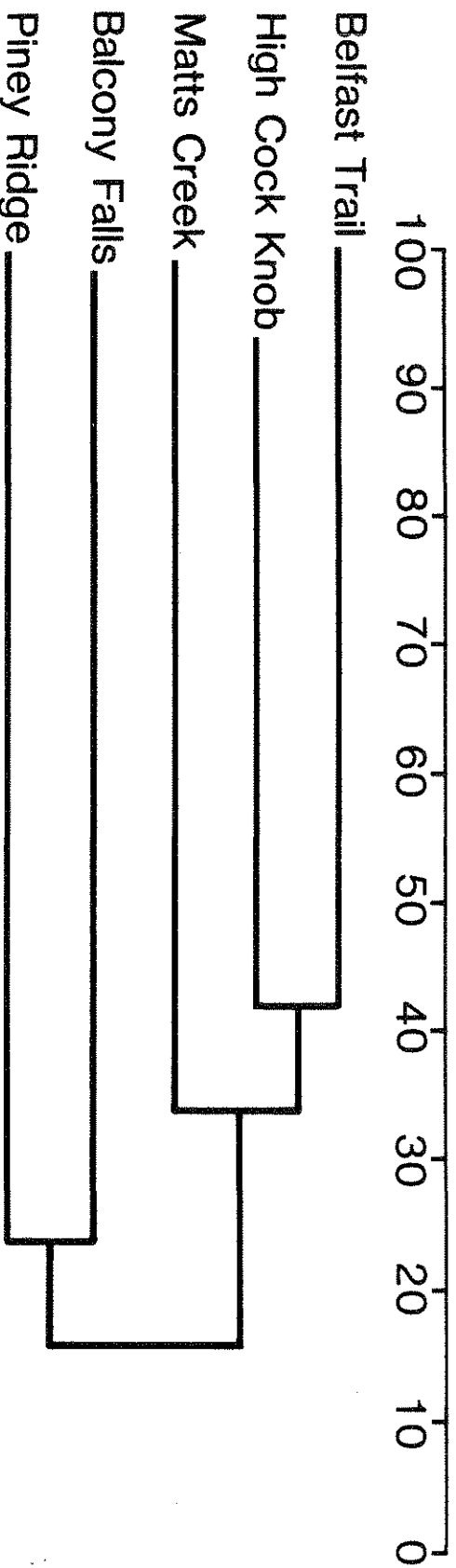


Table 3. List of corticolous lichen species with importance values greater than 0.01 collected from five sites in the James River Face Wilderness Area. Importance values were determined by multiplying average relative density of a species by its frequency of occurrence.

SPECIES	IMPORTANCE VALUE
<i>Parmelia rudecta</i>	9.02
<i>Parmelia caperata</i>	2.23
<i>Physcia</i> sp. (Gray)	0.95
<i>Cladonia</i> sp.	0.64
<i>Parmotrema stuppeum</i>	0.20
<i>Pertusaria amara</i>	0.10
<i>Parmeliopsis aleurites</i>	0.04
<i>Lecanora varia</i>	0.03
<i>Parmotrema hypotropum</i>	0.03
<i>Lecidea scalaris</i>	0.03
<i>Graphis scripta</i>	0.02
<i>Lecanora subfusca</i>	0.01
<i>Lecidea</i> sp.	0.01

Table 4. List of species importance values (greater than 0.01) for corticolous taxa by substrate. Importance values were determined by multiplying relative density of a species by its frequency of occurrence.

Species	CORTICOLOUS SUBSTRATES					
	Virginia Pine	White Oak	Black Gum	Chestnut Oak	Hickory	Scarlet Oak Red Maple
<i>Cetraria halei</i>	0.32					
<i>Parmelia rudecta</i>	0.16	2.20	6.67	17.19	22.66	2.45
<i>Lecanora subfusca</i>	0.16		0.06			0.08
<i>Parmeliopsis aleurites</i>	0.08					
<i>Lecidea scalaris</i>	0.04					
<i>Lecanora varia</i>	0.04	0.16	0.25	0.04		0.03 0.04
<i>Cetraria fendleri</i>	0.04					
<i>Cladonia</i> sp.	4.90			1.57	0.09	0.76
<i>Physcia</i> sp. (gray)	0.51		1.03	2.02	0.58	2.65
<i>Parmelia caperata</i>	0.33		0.25	7.18	0.43	3.36
<i>Candelaria concolor</i>		0.08	0.19			0.16
<i>Pertusaria amara</i>			0.13	0.19	0.26	0.06
<i>Parmotrema stuppeum</i>			0.02	1.11		0.16
<i>Parmotrema hypotropum</i>				0.14		0.03
<i>Lecanora</i> sp.				0.01		0.04
<i>Graphis scripta</i>					0.15	0.34
<i>Crustose</i> sp. 1 (green)					0.02	
<i>Lecidea</i> sp. 1					0.01	0.23 0.32

of 6.67), Physcia sp. (IV of 1.03), Lecanora varia (IV of 0.25), and Parmelia caperata (IV of 0.25). The dominant lichens on Scarlet Oak (Quercus coccinea) were Parmelia caperata (IV of 3.36), Physcia sp. (IV of 2.65), Parmelia rudecta (IV of 2.45), Cladonia sp. (IV of 0.76) and Graphis scripta (IV of 0.34). Dominant lichens on Red Maple (Acer rubrum) included: Lecidea sp. (IV of 0.32), Parmelia caperata (IV of 0.16), and Physcia sp. (IV of 0.16). The important species on Hickory (Carya sp.) were Parmelia rudecta (IV of 22.66), Physcia sp. (IV of 0.58), Parmelia caperata (IV of 0.43), and Pertusaria amara (IV of 0.26). Finally, the dominant lichens on Chestnut Oak (Quercus prinus) included: Parmelia rudecta (IV of 17.19), Parmelia caperata (IV of 7.18), Physcia sp. (IV of 2.02), Cladonia sp. (IV of 1.57), and Parmotrema stuppeum (IV of 1.11). Similarity indices were used to cluster corticolous lichen species (Figure 3). Cluster analysis revealed several distinct and relatively tight species groups. Group I was composed primarily of species from Balcony Falls and to a much lesser extent species from other sites. Species included in this group were Lecanora sp 1., Physcia sp. (small, gray), Candelaria concolor, crustose sp.1 (green), Graphis scripta, and Lecidea sp. 1. The second group was made up of broad ranging species. Species in this group included Parmotrema stuppeum, Physcia sp. (gray), Pertusaria amara, Parmelia rudecta, Parmelia caperata, Cladonia sp. and Parmotrema hypotropum. Group III was comprised of species primarily from the Belfast Trail reference site. Lichens within this group were Lecanora sp. 2, Pannaria leucosticta, Buellia sp., crustose sp. 2 (yellow), crustose sp. 3 (brown), Usnea subfloridana, Cetraria fendleri, and Lecanora varia. The fourth group consisted of species found almost exclusively at the Piney Ridge reference site. Species in this group were Physcia sp. (fine), and Physcia sp. (gray, sorediate). Group V was composed of species found on Virginia pine (Pinus virginiana), principally at the Belfast Trail reference site. Species in this group included Parmeliopsis aleurites, Lecanora subfusca, Lecidea scalaris, Physcia sp. (tan apothecia), and Cetraria halei. The last group (VI) was comprised of rare species. Included in this group were Physcia sp. (brown, sorediate), Physcia sp.1, and Physcia sp. 2.

Shannon-Weaver diversity values were calculated for each reference site (Table 5). Matts Creek demonstrated the greatest species diversity (1.7493), followed by Belfast Trail (1.4276), while Balcony Falls had the lowest (0.6627). Diversity values were also calculated for each corticolous substrate (Table 6). Scarlet Oak (Quercus coccinea) had the greatest species diversity (1.7111), followed by Chestnut Oak (Quercus prinus) at 1.5522 and Black Gum (Nyssa Sylvatica) at 1.4136. Red Maple (Acer rubrum) had the lowest diversity (.8628).

Laboratory analyses: pollutant accumulation

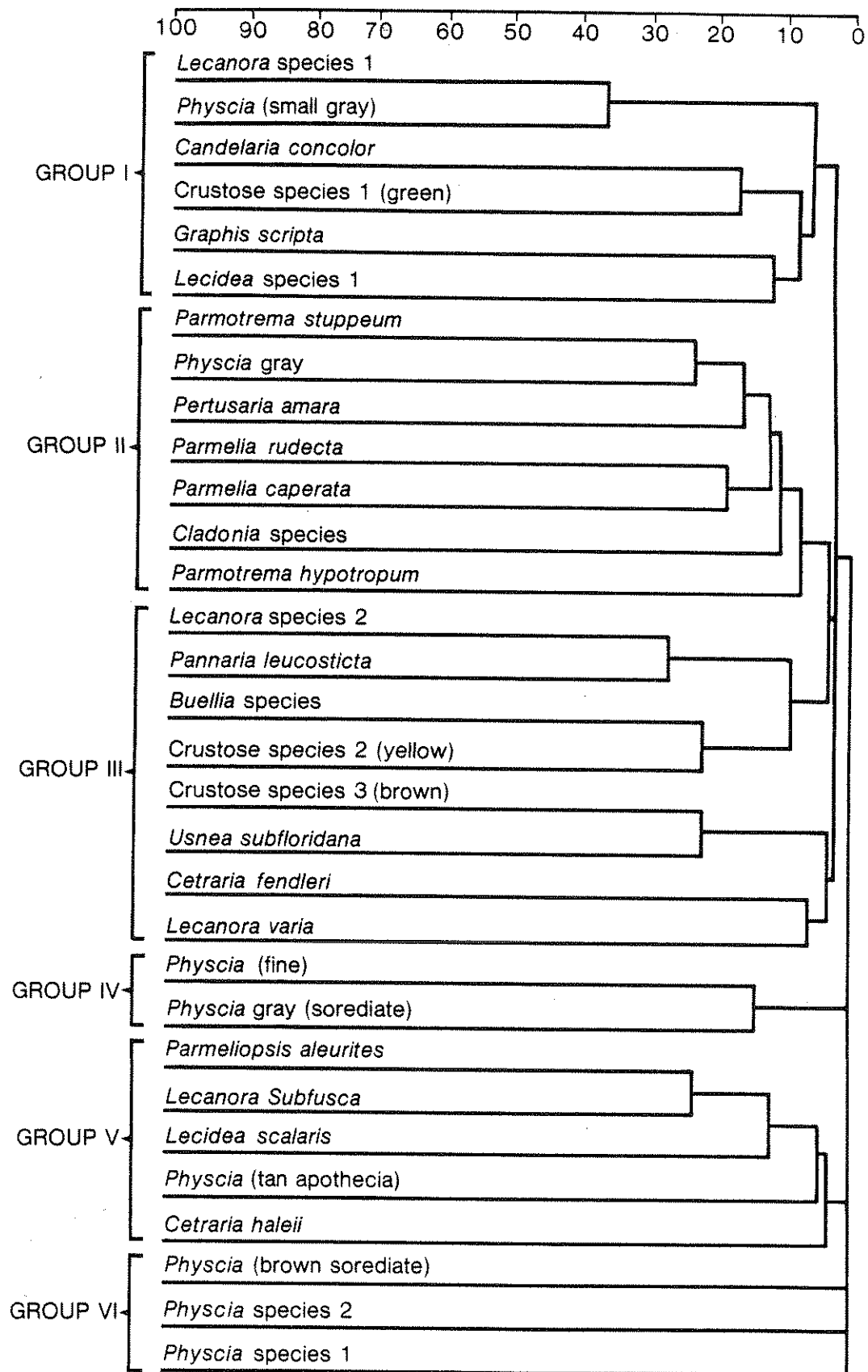


Table 5. Shannon-Weaver species diversity values for lichen species at each reference site in the James River Face Wilderness Area, Jefferson National Forest, Virginia.

SITE	SHANNON-WIENER DIVERSITY VALUE
Balcony Falls	0.6627
Belfast Trail	1.4276
Highcock Knob	1.2688
Matts Creek	1.7493
Piney Ridge	1.0084

Table 6. Mean Shannon-Weaver species diversity values (including high and low values) for lichen species by corticolous substrate in the James River Face Wilderness Area, Jefferson National Forest, Virginia.

CORTICOLOUS SUBSTRATE	SHANNON-WEAVER DIVERSITY VALUE
Carya sp. (Hickory)	.9399 (.0000-2.3535)
Acer rubrum (Red maple)	.8628 (.0000-2.5216)
Quercus coccinea (Scarlet oak)	1.7111 (.0000-2.5547)
Quercus alba (White oak)	1.0034 (.0000-1.8424)
Nyssa sylvatica (Black gum)	1.4136 (.0000-2.000)
Pinus virginiana (Virginia pine)	1.0644 (.0000-2.000)
Quercus prinus (Chestnut oak)	1.5522 (.0000-3.1115)

Lead, Fluoride and Sulfur concentrations in thalli of the six test species were calculated and recorded by species and reference site (Table 7). Lead concentrations in all six test species were consistently low, exceeding 100 ppm in only one out of 300 replicates. Fluoride concentrations were also low, exceeding 40 ppm in only one replicate. Levels of lead above 1000 ppm (Lawrey 1987) and levels of fluoride between 50 and 70 ppm (Hale 1983) are generally considered to be harmful to lichens. Lichen material from Piney Ridge demonstrated the highest lead values, while material from the Matts Creek site had the highest fluoride values. Belfast Trail material consistently demonstrated the lowest lead and fluoride levels.

Sulfur concentrations were consistently high at all reference sites, exceeding .20% at most sites. Levels of sulfur above .20% are generally considered to be detrimental to lichens (Lawrey 1987). Cladina subtenuis and Usnea subfloridana demonstrated the lowest sulfur values (0.046-0.178%), with Umbilicaria papulosa, and Parmotrema stuppeum yielding the highest values (0.104-0.301%). The highest sulfur values were found in the material from Balcony Falls, followed by Highcock Knob and Piney Ridge with the lowest values in the material from Belfast Trail.

All two way interactions between substrates, species and sites were highly significant.

Laboratory analyses: membrane permeability test

Membrane permeability values for each of the six test species were calculated and recorded by species and reference site (Table 7). Leakage values were consistently high for all species at all sites. Cetraria halei consistently demonstrated the highest leakage values, followed by Parmelia caperata, and Cladina subtenuis with Usnea subfloridana yielding the lowest rates. Material from Piney Ridge yielded the highest leakage rates, followed by Matts Creek, and Balcony Falls. The Belfast Trail material consistently had the lowest leakage values.

All two way interactions between substrates, sites and species were highly significant.

OBSERVATIONS AND RECOMMENDATIONS

Observations: floristic and ecological surveys

1. Lichen species diversity, on all substrates, is high at all five reference sites.
2. Corticolous lichen community structure is complex and well developed at all study sites.
3. All lichen growth forms, foliose, fruticose and crustose are well represented at all study sites.
4. Chestnut Oak (Quercus prinus) is the single most common

Table 7. Mean membrane leakage, Pb, F and S content values and standard deviations for six species of lichens from five sites in the James River Face Wilderness Area, Jefferson National Forest, Virginia.

Species	Membrane Permeability	Pb	F	S
Sites (substrates)	(μ mhos/ml)	(ppm)	(ppm)	(%)
<u>Cetraria halei</u> (Bark)				
Balcony Falls	4.578(1.649)	25.760(5.723)	27.380(1.328)	0.181(0.020)
Belfast Trail	1.639(0.464)	13.300(5.148)	21.240(0.723)	0.205(0.045)
Highcock Knob	5.289(1.495)	24.500(2.819)	21.320(1.421)	0.190(0.024)
Matts Creek	5.360(2.707)	16.926(4.797)	28.580(0.494)	0.164(0.031)
Piney Ridge	7.553(2.142)	45.066(7.636)	27.500(0.613)	0.200(0.026)
<u>Cladonia subtennis</u> (Soil)				
Balcony Falls	0.412(0.246)	13.496(1.548)	26.800(2.960)	0.139(0.017)
Belfast Trail	0.895(0.444)	9.492(0.646)	20.560(0.858)	0.134(0.037)
Highcock Knob	0.595(0.240)	5.460(2.509)	21.800(0.618)	0.174(0.027)
Matts Creek	0.462(0.152)	17.122(1.831)	28.500(0.575)	0.064(0.014)
Piney Ridge	0.648(0.088)	4.452(1.450)	28.300(1.965)	0.046(0.017)
<u>Parmelia caperata</u> (Bark)				
Balcony Falls	0.696(0.069)	35.686(3.324)	30.780(3.102)	0.208(0.017)
Belfast Trail	0.739(0.197)	22.750(5.806)	24.260(2.106)	0.111(0.038)
Highcock Knob	0.579(0.181)	57.680(2.862)	34.700(2.368)	0.228(0.024)
Matts Creek	0.773(0.240)	35.938(3.324)	28.920(0.492)	0.170(0.037)
Piney Ridge	1.321(0.342)	59.990(9.945)	30.680(0.944)	0.320(0.106)

Table 7. (continued)

Species Sites (substrates)	Membrane Permeability ($\mu\text{mhos/mL}$)	Pb (ppm)	F (ppm)	S (%)
<u>Parmotrema stuppeum</u> (Bark)				
Balcony Falls	0.359(0.135)	25.242(7.444)	26.320(0.773)	0.273(0.026)
Belfast Trail	0.622(0.270)	17.990(1.309)	32.940(1.799)	0.104(0.035)
Highcock Knob	0.217(0.052)	51.240(8.957)	29.660(1.038)	0.263(0.032)
Matts Creek	0.690(0.495)	26.796(5.977)	35.540(1.016)	0.239(0.045)
Piney Ridge	0.411(0.188)	59.923(26.164)	23.540(1.987)	0.231(0.055)
<u>Umbilicaria papulosa</u> (Rock)				
Balcony Falls	0.562(0.258)	8.792(0.656)	24.960(0.804)	0.301(0.016)
Belfast Trail	0.225(0.035)	8.456(2.200)	23.620(1.030)	0.207(0.023)
Highcock Knob	0.209(0.106)	29.120(1.104)	23.760(0.898)	0.216(0.024)
Matts Creek	0.690(0.257)	10.318(1.233)	30.720(1.799)	0.227(0.029)
Piney Ridge	0.401(0.195)	11.382(2.496)	20.700(0.474)	0.241(0.019)
<u>Usnea subfloridana</u> (Bark)				
Balcony Falls	0.229(0.049)	30.856(3.780)	24.900(0.756)	0.178(0.034)
Belfast Trail	0.203(0.074)	25.354(0.899)	16.280(1.152)	0.137(0.016)
Highcock Knob	0.243(0.159)	11.340(3.057)	27.680(1.059)	0.157(0.028)
Matts Creek	0.316(0.105)	16.156(3.922)	24.760(1.975)	0.100(0.023)
Piney Ridge	0.415(0.103)	44.926(3.980)	26.500(2.163)	0.170(0.025)

tree species in the study area.

5. Bleaching and necrosis of thallus tissues in several corticolous, foliose lichen species was commonly observed at all sites except the Belfast Trail site.

Observations: accumulation of pollutants and membrane leakage

1. Lead concentrations in thalli of test species are not lethal (this correlates with Lawrey's observations in Shenandoah National Park).
2. Fluoride concentrations in thalli of test species are not lethal.
3. Sulfur concentrations in thalli of most test species are consistently high, (detrimental levels). This indicates that sulfur is more than likely the biggest single air pollution-related problem in the study area (this pattern also agrees with Lawrey's observations in Shenandoah National Park).
4. Sulfur is probably being transported on wind currents into the wilderness from industrialized area to the west and north.
5. Membrane leakage rates in all test species are consistently high.
6. The corticolous lichens Cetraria halei on Mountain Laurel and Parmelia caperata on Chestnut Oak appear to be the most pollution sensitive of the six test species.
7. High sulfur loads and high membrane leakage rates in the six test species indicates that the physiological condition of the test species has already been significantly impacted, even though species diversity and percent cover on most substrates seems high (see comment #5 under "Observations: floristic and ecological surveys").

Observations: overall

1. Based on membrane leakage, pollutant accumulation patterns and, various ecological parameters the lichens at the Piney Ridge site appear to have suffered more pollution damage than those at any other site, followed by the lichens at Balcony Falls, Highcock Knob and Matts Creek.
2. At this point, considering the factors listed in #1, the lichen community at the Belfast Trail reference site appears to be in the best condition.
3. Generally, the corticolous test species seem to be the most sensitive pollution indicators.

Recommendations

1. Membrane leakage and sulfur accumulation in the six test species, at all five reference sites, should be reevaluated at least every other year.
2. Sulfur accumulation and membrane permeability should be

- selectively evaluated in bleached and necrotic material from all sites.
3. In all follow-up studies chlorophyll content of all test species should be evaluated in addition to sulfur accumulation and membrane permeability.
 4. Species diversity and abundance at all permanent ecological transects should be reassessed every five years.
 5. Conduct a floristic survey every five years.
 6. Carefully evaluate the need for determining thallus concentrations of other potential airborne pollutants.
 7. Assess the accumulation of selected elements, over time, in cores of xylem tissue taken from Chestnut Oak (Quercus prinus) from all five reference sites.

LITERATURE CITED

- Allard, H. A. and E. C. Leonard. 1944. The Cladoniae of Bull Run Mountain, Virginia. *Castanea* 9(4):81-100.
- Allard, H. A. 1957. Occurrence of the lichen Cetraria islandica Ach. in Virginia and West Virginia. *Castanea* 22(2):106-109.
- Culberson, W. L. 1955. A guide to the literature on the lichen Flora and vegetation of the United States. Plant Disease Epidemics and Ident. Sect. Agr. Res. Serv., U.S. Dept. Agr., Spec. Publ. 7. 54 pp.
- Culberson, W. L. 1965. The foliose and fruticose lichens of the environs of Mountain Lake, Giles County, Virginia. *Castanea* 30:96-104.
- Culberson, W. L. 1966. Chemistry and taxonomy of the lichen genera Heterodermia and Anaptychia in the Carolinas. *The Bryologist* 69:472-487.
- Culberson, W. L. and C. F. Culberson. 1968. The lichen genera Cetralia and Platismatia (Parmeliaceae). *Constr. U.S. Natl. Herb.* 34:449-558.
- Dey, J. P. 1978. Fruticose and foliose lichens of the high-mountain areas of the southern Appalachians. *The Bryologist* 81(1):1-93.
- Eversman, S. 1978. Effects of low-level SO₂ on Usnea hirta and Parmelia chlorochroa. *The Bryologist* 81(3):368-377.
- Fields, R. D. and L. L. St.Clair. 1984. A comparison of methods for evaluating SO₂ impact on selected lichen species: Parmelia chlorochroa, Collema polycarpon and Lecanora muralis. *The Bryologist* 87(4):297-301.
- Gough, L. P. and J. A. Erdman. 1977. Influence of a coal-fired powerplant on the element content of Parmelia chlorochroa. *The Bryologist* 80(3):492-501.

- Hale, Jr., M. E. 1982. Lichens as bioindicators and monitors of air pollution in the Flat Top Wilderness Area. Final Report: Forest Service Contract No. RFPRZ-81-SP35.
- Hale, Jr., M. E. 1983. The Biology of Lichens. 190 pp. Edward Arnold Publishers, Ltd., London.
- Hale, Jr., M. E. and J. D. Lawrey. 1985. Annual rate of lead accumulation in the lichen Pseudoparmelia baltimorensis. The Bryologist 88(1):5-7.
- Lawrey, J. D. and M. E. Hale, Jr. 1981. Retrospective study of lichen lead accumulation in the northeastern United States. The Bryologist 84(4):449-456.
- Lawrey, J. D. 1984. Lichens as air pollution monitors in the Shenandoah National Park, Virginia. Final Report to the U.S. National Park Service, Denver, CO. Contract No. PX-0001-3-0694.
- Lawrey, J. D. 1985. Lichens as lead and sulfur monitors in Shenandoah National Park, Virginia. Final Report to the U.S. National Park Service, Denver, CO. Contract No. CX-0001-1-0114/PX-0001-4-1128.
- Lawrey, J. D. 1987. Lichens as indicators of atmospheric quality in the northern district of Shenandoah National Park, Virginia. Final Report to the U.S. National Park Service, Denver, CO. Contract No. CX-0001-4-0059.
- Luttrell, E. S. 1954. The Cladoniaceae of Virginia. Lloydia 17:275-306.
- Marsh, J. E. and T. H. Nash III. 1979. Lichens in relation to the Four Corners power plant in New Mexico. The Bryologist 82(1):20-28.
- Moser, T. J., T. H. Nash III, and W. D. Clark. 1980. A long-term field sulfur dioxide fumigation on Arctic caribou forage lichens. Canadian Journal of Botany 58(21):2235-2240.
- Nash III, T. H. and L. L. Sigal. 1979. Gross photosynthetic response of lichens to short-term ozone fumigations. The Bryologist 82(2):280-285.
- Pearson, L. C. and G. A. Rodgers. 1982. Air pollution damage to cell membranes in lichens. III. Field experiments. Phytion 22(2):329-337.
- Phillips, E. A. 1959. Methods of Vegetation Study. Holt, Rinehart and Winston, Inc., New York.
- Pyatt, F. B. 1970. Lichens as indicators of air pollution in a steel-producing town in South Wales. Environmental Pollution 1:45-46.
- Puckett, K. T. 1976. The effects of heavy metals on some aspects of lichen physiology. Canadian Journal of Botany 54(23):2695-2703.
- Richardson, D. H. S. and E. Nieboer. 1981. Lichens and air pollution monitoring. Endeavour, New Series 5(3):127-133.
- Ronen, R. and M. Galun. 1984. Pigment extraction from lichens with dimethyl sulfoxide (DMSO) and estimation of chlorophyll degradation. Environ. and Exper. Bot. 24(3):239-245.

- Rushforth, S. R., L. L. St.Clair, J. D. Brotherson and G. T. Nebeker. 1982. Lichen community structure in Zion National Park. *The Bryologist* 85(2):185-192.
- Ruzicka, M. 1958. Andwend mathematisch-statistischer methoden in der geobotanik. *Biologica (Bratislava)* 13:647-661.
- St.Clair, L. L., S. R. Rushforth, and J. D. Brotherson. 1986. The influence of microhabitat on diversity, distribution and abundance of corticolous lichens in Zion National Park, Utah and Navajo National Monument, Arizona. *Mycotaxon* 26:253-262.
- St.Clair, L. L. and R. D. Fields. A comprehensive approach to using lichens as bioindicators of air quality (in review).
- St.Clair, L. L., R. D. Fields, and M. Nakanishi. An in situ field study using lichens as bioindicators of air quality (in review).
- Schutte, J. A. 1977. Chromium in two corticolous lichens from Ohio and West Virginia. *The Bryologist* 80(2):279-283.
- Sigal, L. L. and O. C. Taylor. 1979. Preliminary studies on the gross photosynthetic response of lichens to peroxyacetylnitrate fumigations. *The Bryologist* 82(4):564-575.
- Sigal, L. L. and T. H. Nash, III. 1983. Lichen communities on conifers in southern California mountains: an ecological survey relative to oxidant air pollution. *Ecology* 64(6):1343-1354.
- Skye, E. 1979. Lichens as biological indicators of air pollution. *Annual Review of Phytopathology* 17:325-341.
- Sneath, P. H. and R. R. Sokal. 1973. *Numerical Taxonomy*. W. H. Freeman and Co., San Francisco.
- Sundstrom, K. R. and J. E. Hallgren. 1973. Using lichens as physiological indicators of sulfurous pollutants. *AMBIO* 2(1-2):13-21.
- Warner, J. H. and K. T. Harper. 1972. Understory characteristics related to site quality for aspen in Utah. *Brigham Young University Science Bulletin, Biological Series* 16(2):1-20.
- Wetmore, C. M. 1981. Lichens and air quality in Big Bend National Park, Texas. *The Bryologist* 84(3):426-433.