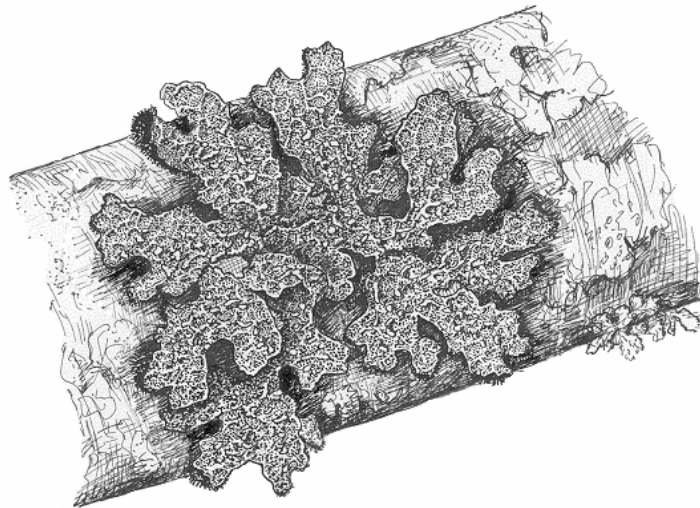




**Air Pollution-Related Lichen Monitoring in
National Parks, Forests, and Refuges:
Guidelines for Studies Intended for Regulatory and
Management Purposes**



**National Park Service Air Resources Division
U.S. Forest Service Air Resource Management Program
U.S. Fish and Wildlife Service Air Quality Branch**

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Air Pollution-Related Lichen Monitoring in National Parks, Forests, and Refuges: Guidelines for Studies Intended for Regulatory and Management Purposes

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Cover Illustration of *Parmelia sulcata* by Alexander Mikulin

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Introduction

This guidance document is intended to serve as a resource for national park, forest, and refuge staff when considering lichen studies to address air quality concerns. It provides background regarding the use of lichens as air pollution indicators, their sensitivities to various air pollutants, and the effects of air pollution on lichen physiology, communities, and tissue chemistry. It discusses the types of information and objectives that can optimize the utility of lichen studies from an air management and air regulatory perspective. It also provides a checklist of questions to consider when designing or evaluating the potential of a lichen study to address air pollution issues on federally managed lands. Lichen studies may be conducted for a variety of other reasons unrelated to air quality (e.g. inventory and monitoring, biological diversity assessment, evaluating habitat quality) but those types of studies are not discussed in detail here.

Background

Air Resource Management and Air Quality-Related Values

The National Park Service (NPS), U.S. Forest Service (USFS), and Fish and Wildlife Service (FWS) have responsibilities under the Clean Air Act, the Wilderness Act, and their respective agency organic acts to protect air quality-related values (AQRVs) on lands that they manage. AQRVs are defined as resources that may be adversely affected by a change in air quality (FLAG 2000) and may include vegetation, wildlife, water quality, soils, and visibility. Both lichens and vascular plants have been the subject of numerous studies to assess air pollution effects. These studies often assist land managers in determining whether lichens and plants should be considered AQRVs for a specific park, forest, or refuge. The term AQRV originated in the Clean Air Act Amendments of 1977 in the provisions called “Prevention of Significant Deterioration” (PSD). Under PSD, federal land managers in the NPS, USFS, and FWS are given specific responsibilities to review and provide recommendations to state or federal air regulators on pollution emissions permits for many types of large “point source” facilities. The Clean Air Act specifies that “the state may not issue a PSD permit if the federal land manager demonstrates to the satisfaction of the State that the emissions from such a facility will have an adverse impact on the air quality-related values (including visibility) of Class I lands.” The PSD process requires land managers to *predict* AQRV changes that would likely occur if a pollution source were built with the pollutant emissions levels proposed in the permit. This predictive requirement presents a challenge in using ecosystem-based AQRVs, such as lichens, in the PSD process because no models are available that quantitatively predict how incremental changes in air chemistry can affect site and species-specific lichen condition or viability in the future. Situations in which general or circumstantial inference about future impacts of air pollutants on lichens might be used in PSD processes are discussed in more detail later in this guidance.

In addition to the requirements in the Clean Air Act, the National Park Service Organic Act and the 1964 Wilderness Act contain legislative requirements protecting park and wilderness resources to leave them “unimpaired” for the future. The National Wildlife System Improvement Act of 1997 requires the FWS to manage refuge lands to “ensure that the biological integrity, diversity and environmental health of the System are maintained for the benefit of present and future generations of Americans.” Because of these requirements, NPS, USFS, and FWS are concerned about air pollution effects on AQRVs including lichens in national park, forest, and refuge ecosystems. Effects of poor air quality on sensitive organisms have implications for management of sustainable ecosystems in North America. Lichens and bryophytes, for example, not only contribute to biodiversity but also play integral roles in nutrient and hydrological cycles, and are valuable sources of forage, shelter, and nesting material for mammals, birds and invertebrates (Brodo et al. 2001, McCune and Geiser 1997). Generally, loss of biological diversity or population within or across groups of organisms contributes to a decline in ecosystem stability, functionality and productivity (Eldredge 1998, Novacek 2001). Intact natural ecosystems are increasingly rare, and are valued for the

many ecosystem services they provide, including oxygenating the air, cleansing and storing water, productive soils, habitat for fish and wildlife, and esthetic value (Daily 1997). Air pollution is one of many potential stressors that can adversely affect lichen health. Well-designed and implemented studies can help land managers determine whether air pollution is linked to any changes in lichen habitat, condition, or viability.

In addition to assessing lichen condition as an indicator of ecosystem health, another potential use of lichen studies by air managers is to use lichens that are relatively insensitive to air pollutants as “passive monitors” of air pollution. This type of study generally does not yield information directly useful to air regulators, because regulators are required to use federally approved methods and precision instruments to determine if federal or state air quality standards are being violated. Hourly, daily and annual air concentrations are used to evaluate compliance with air quality standards. Pollutant concentrations estimated from passive monitors (including lichens) are not usually thought to be of high value by air regulators. This is because the values are not precise enough to compare with equipment-monitored concentrations, and the time periods of accumulation in the lichen are either unknown or are difficult to correlate with the monitoring time periods required by laws and regulations (e.g., 24-hr standards, annual standards). If the desired outcome is to know what concentrations of pollutants are in the air, then the best strategy is to monitor the air rather than using plants as a surrogate. However, studies using lichen as passive monitors of air pollution can confirm that a pollutant is present in the environment and show us the relative amounts of pollutants between locations. Lichen information can then be used to identify areas at risk from air pollution, or to select sites (e.g., “hot spots”) for subsequent instrument monitoring by providing spatial distributions of pollutant concentration in lichen tissue over broad areas. In general, “passive monitoring” lichen studies are of most value as a screening mechanism for establishing a subset of sites where follow-up work (such as instrument monitoring) should be done, and of limited value where the follow-up work is not conducted.

Land managers often face challenges when using information collected in air pollution-related lichen studies to “protect” ecosystems from existing or future adverse impacts. This is because it is often difficult to establish a direct “cause and effect” between air pollution and adverse effects on lichens. Therefore there is little chance studies not specifically designed to make these linkages can be used effectively by managers. This document will describe some of the ways in which lichen studies can be strengthened by careful planning and design to collect and present the best possible information useful for protecting resources in parks, forests, and refuges.

Lichens as Air Pollution Indicators

Lichens are composite organisms formed by a fungus and a green alga and/or a blue-green bacterium. Lichens have been used worldwide as air pollution monitors because relatively low levels of sulfur, nitrogen, and fluorine-containing pollutants (especially SO₂ and F gas, and acidic or fertilizing compounds), adversely affect many species, altering lichen community composition, growth rates, reproduction, physiology, and morphological appearance. Lichens are also used as pollution monitors because they concentrate a variety of pollutants in their tissues. More than 1,500 scientific articles have been published on the topic of lichens and air pollution. The British Lichen Society journal, *The Lichenologist*, publishes an on-going series, “Literature on Air Pollution and Lichens,” tracking recent publications. Articles from this series and other lichen-related literature can be searched on-line at: http://www.toyen.uio.no/botanisk/bot-mus/lav/sok_rll.htm. Reviews of the literature and methods regarding air quality assessment using lichens include Nash and Gries (2002), Nimis et al. (2002), Garty (2000 and 2001), Hyvärinen et al. (1993), Stolte et al. (1993), Richardson (1992), Nash (1989), and Nash and Wirth (1988).

The most commonly used lichen biomonitoring methods are community analysis, lichen tissue analysis, and transplant studies. In the U.S., the Forest Inventory and Analysis program, and the Forest Health Monitoring program (developed under the auspices of the U.S. Forest Service and the U.S. Environmental Protection Agency) use lichen communities as indicators of air quality and climate change in most forested parts of the U.S. (McCune et al. 1997; methods documents and other reports available on-line at

<http://www.fia.fs.fed.us/program-features/indicators/lichen/> Species composition of lichen communities has also been used to demonstrate the improvement of air quality in the Ohio Valley (Showman 1990 and 1997), to show oxidant air pollutant gradients in southern California (Nash and Sigal 1998), and to show SO₂ gradients in Seattle (Johnson 1979), the Indianapolis vicinity (McCune 1988), and other locations (Showman 1988). Lichen survey data exist for the majority of parks and forests, ranging from species lists to studies specifically related to air quality (see history section below). Tissue analysis has also been widely conducted using lichens from national forests and parks of the U.S. (see Figures 1 and 2) and a large body of information is developing regarding the elemental content of lichen tissue, both in natural states and under pollution stress (Rhoades 1999, Garty 2000).

Lichens are long-lived and can be monitored, field conditions permitting, in any season. Many lichens have extensive geographical ranges, allowing study of pollution gradients over large areas. These properties make them useful for spatial and temporal evaluation of pollutant accumulation in the environment. Epiphytic lichens (those that grow on trees or plants) are often best suited to the study of air pollution effects on lichen communities, lichen growth or physiology, and to the study of pollutant loading and distribution. Because they lack roots and are located above the ground, epiphytic lichens usually receive greater exposure to air pollutants and do not have access to soil nutrient pools. Because they depend on deposition, water seeping over substrate surfaces, atmospheric gases, and other comparatively dilute sources for their nutrition, tissue content of epiphytic lichens largely reflects atmospheric sources of nutrients and contaminants. Lichens on soils and rock substrates are more likely to be influenced by elements and chemicals from these substrates, but otherwise share morphological and physiological characteristics of epiphytes.

Under certain conditions, lichen floristic and community analyses can be used in conjunction with measured levels of ambient or depositional pollutants accumulated by lichens to detect effects of changing air quality on vegetation. This information can demonstrate whether air pollutants cause undesirable changes in species composition or presence/absence of lichen species within terrestrial plant communities. It is important that any alternative hypotheses (e.g., drought, grazing, habitat alteration) for changes observed in species condition or composition (in addition to air pollution) are discussed and evaluated when using lichen floristics and community studies in an air pollution context. Lichens exhibit differing levels of sensitivity to pollution. In general, air pollution sensitivity increases among growth forms in the following series: crustose (flat, tightly adhered, crust-like lichens) < foliose (leafy lichens) < fruticose (shrubby lichens), though there are exceptions to this gradation. Some of the most sensitive lichens in parks, forests and refuges are likely to be epiphytic macrolichens from the genera *Alectoria*, *Bryoria*, *Ramalina*, *Lobaria*, *Pseudocyphellaria*, *Nephroma*, and *Usnea* (McCune and Geiser 1997). Declines in the condition and biomass of these genera would be an expected outcome of harmful levels of nitrogen- and sulfur-containing deposition or exposure to sulfur dioxide and fluorine gases.

The concentrations at which nitrogen, sulfur, or metals are considered “harmful” differ greatly among lichen species and sometimes between controlled laboratory studies and field conditions. The USFS has developed a web site that lists what is known about the levels of nitrogen and sulfur at which effects have been documented, and lichens have been shown to be tolerant or intolerant (disappear) for each of a large variety of species (<http://www.nacse.org/lichenair>).

This web site also lists “provisional element analysis thresholds” above which lichen tissue levels of elements might be considered “elevated” (based on species and background levels of air pollutants found in the Pacific Northwest). *Hypogymnia physodes* is a relatively commonly occurring lichen for which baseline levels of heavy metals have been established using data from the species collected worldwide (Bennett 2000).

One of the challenges of linking pollutant concentrations in air to concentrations in lichen tissue is to correlate the time period over which pollutants are monitored in the air with the age of the tissue sampled. If the time of deposition is important, then species with visible annual growth increments (Peck et al. 2000) such as the moss, *Hylocomium splendens*, can be used (Bargagli 1998). Alternatively, lichens can be collected from substrates of determinable age such as twigs, or mean tissue concentrations of selected species can be compared over time. However caution should be used in such correlations. Garty’s (2001) review of a dozen studies of age-related differences in lichen thalli (vegetative bodies) revealed that differences are not always significant, nor always size-related, and vary with growth rate, target element,

that pollutant mixes can have synergistic, protective, or adverse effects on lichens, and that individual species differ in their sensitivity to these pollutants and their response to pollutant mixes (Hyvärinen et al. 1992, Gilbert 1986, Farmer et al. 1992).

During the past 20 years, much data have been collected concerning metal tolerance and toxicity in lichens (Garty 2001). Metals can be classified into three groups relative to their toxicity in lichens (Nieboer & Richardson 1981):

1. Class A metals: K^+ , Ca^{2+} , and Sr^{2+} are characterized by a strong preference for O_2 -containing binding sites and are not toxic.
2. Ions in the B metals class: Ag^+ , Hg^+ , Cu^+ tend to bind with N- and S-containing molecules, and are extremely toxic to lichens even at low levels.
3. Borderline metals: Zn^{2+} , Ni^{2+} , Cu^{2+} , Pb^{2+} are intermediate to Class A and B metals. Borderline metals, especially those with class-B properties (e.g., Pb^{2+} , Cu^{2+}), may be both detrimental by themselves and in combination with sulfur dioxide. This provides a good rationale to monitor both metal and sulfur/nitrogen containing pollutants simultaneously if possible.

Effects of Specific Air Pollutants on Lichens

A myriad of pollution effects on lichens have been described in studies to date. At the level of the whole plant, investigators have described decreases in thallus size and fertility (Kauppi 1983, Sigal & Nash 1983), bleaching and convolution of the thallus (Kauppi 1983, Sigal & Nash 1983), restriction of lichens to the base of vegetation (Sigal & Nash 1983, Neel 1988), and mortality of sensitive species (DeWit 1976). Microscopic and molecular effects include reduction in the number of algal cells in the thallus (Holopainen 1984), ultrastructural changes of the thallus (Hale 1983, Holopainen 1984, Pearson 1985), changes in chlorophyll fluorescence parameters (Gries et al. 1995), degradation of photosynthetic pigments (Kauppi 1980, Garty et al. 1993), and altered photosynthesis and respiration rates (Sanz et al. 1992, Rosentreter & Ahmadjian 1977). The first indications of air pollution damage from SO_2 are the inhibition of nitrogen fixation, increased electrolyte leakage, and decreased photosynthesis and respiration followed by discoloration and death of the algae (Fields 1988). More resistant species tolerate regions with higher concentrations of these pollutants, but may exhibit changes in internal and/or external morphology (Nash and Gries 1991, Will-Wolf 1980).

Elevation in the content of heavy metals in the thallus has also been documented in many cases (Garty 2001; Addison & Puckett 1980; Carlberg et al. 1983; Gailey & Lloyd 1986a, 1986b, and 1986c; Gough & Erdman 1977; Lawry 1986), but it is not always easy to establish what specific effect these elevated levels will have on lichen condition or viability. Tolerance to metals may be phenotypically acquired, but sensitivity of lichens to elevated tissue concentrations of metals varies greatly among species, populations, and elements (Tyler 1989). The toxicity of metal ions in lichen tissue is the result of three main mechanisms: the blocking, modification, or displacement of ions or molecules essential for plant function. Metal toxicity in lichens is evidenced by adverse effects on cell membrane integrity, chlorophyll content and integrity, photosynthesis and respiration, potential quantum yield of photosystem II, stress-ethylene production, ultrastructure, spectral reflectance responses, drought resistance, and synthesis of various enzymes, secondary metabolites, and energy transfer molecules (Garty 2001).

Use of Chemical Analysis of Lichens to Indicate Air Quality

A dynamic equilibrium exists between atmospheric nutrient/pollutant accumulation and loss that can make lichen tissue analysis a sensitive tool for the detection of changes in air quality of many pollutants (Boongaprob and Nash 1990, Farmer et al. 1991, Ottonello et al. 2000). All lichens lack the protective tissues or cell types necessary to maintain constant internal water content. Water and gas are exchanged over the entire lichen thallus. In many locations, lichens pass through multiple wetting and drying cycles during a day. When hydrated, nutrients and contaminants are absorbed over the entire surface of the lichen. During dehydration, nutrients and many contaminants concentrate by absorption to cell walls, cloistering inside organelles, or crystallizing between cells (Nieboer et al. 1978). During rain events, nutrients and

pollutants are potentially leached. Lichens and bryophytes (mosses, liverworts and hornworts) often accumulate sulfur, nitrogen, and metals from atmospheric sources better than plants. The relationship between tissue content and depositional pollutants is the subject of many studies (Bargagli 1989, Evans and Hutchinson 1996, Garty 2001, Palomäki et al. 1992, Rühling 1994)

Lichens can accumulate pollutants quickly (Palomäki et al. 1992). In one case of lichen transplantation near a large source of agricultural nitrogen, within five months of exposure, 29% of the lichen's dry tissue weight consisted of accumulated nitrogen (Søchting, 1995). Values of up to 13% total dry weight for sulfur in lichen tissue have been observed from urban/industrial areas (Nieboer et al. 1978). By contrast, lichens from clean sites in forests of the Pacific Northwest contain less than 0.15% S and 2.5% N (Rhoades 1999, Geiser et al. 1994, USFS data at <http://www.fs.fed.us/r6/qa>).

The residence time that contaminants and nutrients remain in lichen tissue differs among elements (Pucket 1985). Macronutrients, such as nitrogen, sulfur, potassium, magnesium and calcium are comparatively mobile and easily leached and therefore measurable changes in tissue concentrations can occur over weeks or months with seasonal changes in deposition (Boongaprob et al. 1989). In one study, mobile elements reached the same levels in transplants as the indigenous lichens within four to six months (Palomäki et al. 1992). Trace and toxic metals such as cadmium, lead, and zinc, are more tightly bound or sequestered within lichens and therefore more slowly released (Garty 2001). However, metals can stay in the environment for twenty years or more after their deposition, and elevated levels in lichens reveal this. Furbish et al. (2000) demonstrated the presence of very high levels of lead and zinc in lichens of Klondike National Historic Monument and the city of Skagway decades after over-ground rail transport of crushed ore and its transfer to open barges at Skagway harbor had ceased. Levels at all sites were higher than baselines established at over 120 sites on the surrounding Tongass National Forest (Geiser et al. 1994).

If air quality improves, levels of metals will decrease over time, and changes in air quality can be detected in lichen tissue over a period of years (Bargagli and Nimis 2002). While it may take decades to return to background levels, changes may be observable from one year to the next as new growth takes place and metals are leached from older tissues. For most air quality assessment purposes, collection of a large enough sample size, comprised of many individuals, should be sufficient to determine the average tissue concentration for that population. The same collection method can then be used to track changes over time, where careful study design provides for similar methodology among sampling periods.

History of Lichen Studies on Federal Lands in the United States

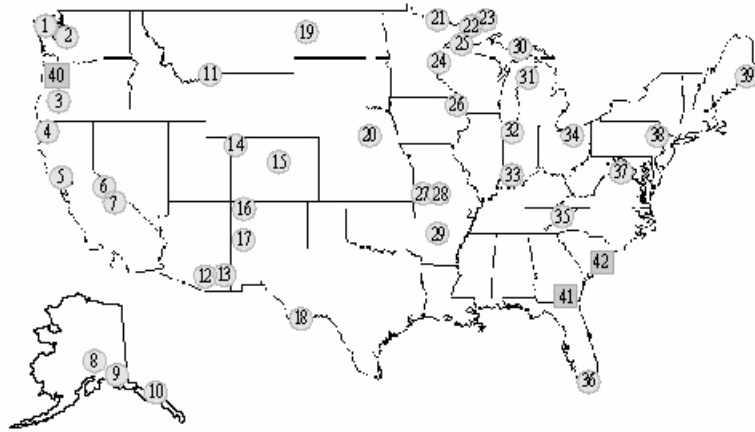
Lichens have been used to study air pollution chemistry in national parks and forests since the 1980s (Figures 1 and 2). There have also been a few lichen studies on national wildlife refuges. Most of the studies have been floristic studies, reports of baseline concentrations of elements in lichen tissues and, occasionally, trends in these concentrations. Figure 1 shows park and refuge locations with tissue chemistry data. USGS Biological Resources Division maintains a web site listing lichens known from each of the national parks shown on the map (<http://www.ies.wisc.edu/nplichen>). Results from these studies have been reported in numerous publications and reports, including Bennett 1995; Bennett and Banerjee 1995; Bennett et al. 1996; Bennett and Wetmore 1997, 1999a and b, 2000a and b; Crock et al. 1992; Crock et al. 1993; Ford and Hasselbach 2001, Furbish et al. 2000, Gough et al. 1994, Gough and Crock 1997; Rhoades 1988; Wetmore 1986, 1989, 1991; and Wetmore and Bennett 2001 a and b. Studies that describe concentrations of various elements in lichen tissue are most useful where they also relate those concentrations to levels at which change in health of a sensitive lichen species would be expected, or where spatial patterns or lichen tissue concentrations are correlated with known spatial patterns of pollutant emissions or monitored pollutant concentrations.

The Forest Service has also sponsored multiple studies utilizing lichen tissue chemistry (Geiser and Williams 2002, Figure 2). Data and draft thresholds for enhanced levels (amounts considered to be elevated above "clean" background site concentrations) of elements for ten regional species for the Alaska and the Pacific Northwest Regions are available online from the USFS Air Resource Program lichens and

air quality website at <http://www.nacse.org/lichenair>. Lichen data and PDF files of historic reports from other Forest Service regions are also available from this web site.

The USFS Forest Inventory and Analysis (FIA) program seeks to assess the condition and trend of the forests of the U.S. FIA recently assumed responsibility for all former Forest Health Monitoring program (FHM) plot work on a national level, and is currently active in 32 states. Lichen community monitoring was included in FIA in order to address key assessment issues such as the impact of air pollution on forest resources, spatial and temporal trends in biodiversity, and the sustainability of timber harvesting. The Lichen Community Indicator component of FIA collects data on the epiphytic lichen community (i.e., those species growing on trees, shrubs and standing dead wood) on a subset of permanent forest plots in the nationwide FIA grid. Lichen species richness scores are used to make a general spatial assessment of air quality, after taking into account other environmental factors (e.g., climate, forest age, or stand structure) that can affect lichen community composition. This lichen monitoring program dates back to 1994, and the program intent is for long-term observation of lichen community change to provide an early indication of improving or deteriorating air quality (see <http://www.fia.fs.fed.us/program-features/indicators/lichen/>).

Collaboration among federal agencies and air program managers about data collection, sharing, analysis, and production of analytical tools is valuable. In the early 1990s, a workshop sponsored by the USFS, NPS, and EPA led to the creation of a handbook for air managers using lichens as bioindicators of air quality (Stolte et al. 1993). In 2001, an interagency/multi-academic institution workgroup was formed to produce and share information that can be used in decision-making processes by federal air managers. The web site <http://ocid.nacse.org/research/airlichen/workgroup> contains the group mission, results of a recent workshop and descriptions and slide presentations of current lichen monitoring programs employed on federal lands. Members of this workgroup are developing an integrated database to store and to provide public access to lichen community, element analysis, and other related data, analysis tools, and reports sponsored by both the USFS and NPS at <http://www.nacse.org/lichenair>.



National Park Service Units

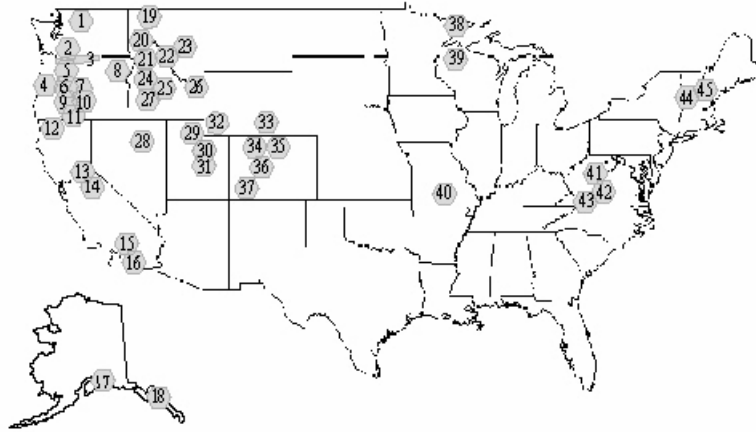
Acadia, ME	39	Klondike Gold Rush, AK	10
Apostle Islands, WI	25	Lincoln Boyhood, IL	33
Big Bend, TX	18	Mount Rainier, WA	2
Chaco Culture, NM	16	Olympic, WA	1
Chiricahua, AZ	13	Oregon Caves, OR	3
Cuyahoga Valley, OH	34	Pictured Rocks, MI	30
Delaware Water Gap, PA	38	Point Reyes, CA	5
Denali, AK	8	Redwood, CA	4
Dinosaur, CO	14	Rocky Mountain, CO	15
Effigy Mounds, IA	26	Saint Croix, MN & WI	24
El Morro, NM	17	Saguaro, AZ	12
Everglades, FL	36	Sequoia, CA	7
George Washington Carver, MO	28	Shenandoah, VA	37
Grand Portage, MN	22	Sleeping Bear Dunes, MI	31
Great Smoky Mountains, TN	35	Theodore Roosevelt, ND	19
Homestead, NE	20	Voyageurs, MN	21
Hot Springs, AR	29	Wilson's Creek, MO	27
Indiana Dunes, IN	32	Wrangell-St. Elias, AK	9
Isle Royale, MI	23	Yellowstone, WY	11
Kings Canyon, CA	6		

National Wildlife Refuges

Cape Romain, SC	42	William L. Finley, OR	40
Okefenokee, GA	41		

Figure 1. National Park Service Units and Wildlife Refuges with lichen chemistry data.

(Figure courtesy of James P. Bennett, USGS.)



National Forests

Angeles, CA	15	Monongahela, WV	41
Bitterroot, MT	22	Mt. Baker-Snoqualmie, WA	1
Boise, ID	27	Mt. Hood, OR	5
Bridger-Teton, WY	32	Nez Perce, ID	21
Chequamegon, WI	39	Payette, ID	24
Chugach, AK	17	Roosevelt, CO	35
Clearwater, ID	20	Routt, CO	34
Cleveland, CA	16	Salmon-Challis, ID	25
Columbia River Gorge, OR	3	San Juan-Rio Grande, CO	37
Deerlodge, MT	23	Siuslaw, OR	4
Deschutes, OR	7	Stanislaus, CA	14
Eldorado, CA	13	Superior, MN	38
Fremont, OR	10	Targhee, ID	26
George Washington, VA	42	Tongass, AK	18
Gifford Pinchot, WA	2	Uinta, UT	30
Green Mountain, VT	44	Umpqua, OR	9
Humboldt-Toiyabe, NV	28	Wallowa-Whitman, OR	8
Jefferson, VA	43	Wasatch-Cache, UT	29
Klamath, CA	12	White Mountain, NH	45
Kootenai, MT	19	White River, CO	36
Manti-La Sal, UT	31	Willamette, OR	6
Mark Twain, MO	40	Winema, OR	11
Medicine Bow, WY	33		

Figure 2. National Forests with lichen chemistry data.

(Figure courtesy of James P. Bennett, USGS.) In addition to the forests listed above, the Pisgah and Nantahala National Forests were the sites of a lichen study (*Gymnoderma lineare*, the only lichen on the federal endangered species list; Martin et al. 1996). There is also unpublished lichen tissue data from Larry St. Clair for the Gila NF that was not included on this map.

Guidelines

Lichen Monitoring Advantages and Limitations

Lichen monitoring has both advantages and limitations in terms of assessing the concentrations and impacts of air pollutants. These are briefly summarized below in Table 1.

Table 1. Lichen monitoring advantages and limitations

Topic	Advantages	Limitations
Assessing spatial and temporal status and trends in air quality	Evaluation of metal concentrations in lichen tissue can yield valuable information about presence or absence of metals in the environment and identify areas of high and low concentrations.	Some metals are not easily leached from lichen thalli and may remain concentrated for more than 10 years, making it difficult to evaluate when the pollutant was accumulated. To overcome this problem, transplants can be used, target species can be selected that have visible annual growth markers (e.g. the stair-step moss, <i>Hylocomium splendens</i>), or material can be collected from substrates of known age (such as within the last 3-5 terminal bud scars on host trees).
	Many measuring points can be made in a short time that summarize air quality over the past weeks, months or years, depending upon the pollutant.	To compare tissue analyses at different locations or across time in the same location, the same species must be located and used in the study. This is because individual species at the same locations or air quality conditions often have significantly different element profiles.
	Many lichens have wide geographical ranges making them suitable for a study over a large area.	Lichens may be difficult to find where acid rain, SO ₂ , or nitrogen deposition is a problem. In these cases using transplants or choosing species with relative tolerance to these pollutants may be necessary.
	Lichen tissue data can be used to map relative differences in air quality over a geographical area of interest or to track changes over time.	Individuals can vary widely in tissue contents of various contaminants at a single site or plot. Minimize within-site variability by collecting sufficient material to represent the population mean, i.e., collect a large number of individuals (suggest 60 g dry weight/ha) widely over the collection site. Replicate samples will establish deviation from the mean and can be used to adjust sample size and number of subsamples.
	Lichen community data can be used to map relative differences in air quality over a geographical area of interest or to track changes over time.	Lichen communities vary with ecoregion. The greater the climatic and elevational range within the study area, the more difficult it becomes to separate environmental influences from pollution influences on lichen communities. FHM is developing separate gradient models for different regions of the US to interpret community data.
Data integration	Deposition of sulfur, nitrogen, and metals can be estimated from lichen tissue levels if a sufficient number of instrumented sites are available to provide calibration.	Precipitation patterns and volumes influence element concentrations in lichen tissues. Calibration is easiest among sites with similar precipitation regimes, otherwise precipitation must be accounted for.
	Lichen monitoring data can compliment instrument measurements and other air quality information.	Because most air quality standards are based on ambient air concentrations, lichen monitoring data rarely can “stand alone” in a regulatory setting and is best used in conjunction with other types of data.
Documenting ecological effects of air pollution	Extensive comparison data exist for the Pacific NW, Alaska, Canada and arctic/boreal regions of the world for establishing “clean site” concentrations in common lichen species as well as concentrations at which species begin to disappear.	Lichen communities response (e.g., growth or decline) will be based on the total mix of acidifying, fertilizing and oxidizing pollutants, sometimes making it difficult to determine what impact element concentration in tissue is having on lichen condition or viability

	Lichen community composition (species richness, composition and abundance) can be used to demonstrate adverse effects to the terrestrial ecosystem from anthropogenic pollutants.	Multivariate analysis is usually needed to separate the pollution signal from other environmental variables that affect lichen communities (e.g., elevation, precipitation, forest continuity, relative humidity, available substrates). These environmental variables must be collected or the range of these variables within the study area must be restricted.
Establishing baselines	Lichen tissue analysis can be used to determine baseline tissue concentrations of pollutants in “clean areas” for comparison at the same site at a later date.	Optimally, baselines for tissue analyses should be established over 3-4 years (rather than the one-time studies often conducted) at the same time of the year, to define the current range in tissue concentrations for target chemicals over the intended study area. Follow-up studies must be designed carefully to ensure that field and lab methods comparable to the initial study are used.
Monitoring local conditions	Lichens are not mobile; therefore physiological, community, and tissue chemistry responses reflect local conditions.	Interference from local sources of dust can affect contaminant concentrations in lichens, especially metals in ground- or rock-dwelling lichens, and make it difficult to distinguish local from regional or point source pollutants. To overcome this problem, an enrichment factor could be calculated based on the ratio of aluminum to the target element in local soils compared to the aluminum to target element ratio in local lichens.
Monitoring remote locations	Lichens are useful as indicators of air pollutant presence in areas of rugged topography where modeling is inadequate or in remote areas where lack of power sources limit instrumented monitoring. Lichen data can be used to show areas of specific concern (high levels) for subsequent instrument monitoring or more intensive studies.	Regulatory personnel are often unfamiliar with lichen monitoring methods, and can therefore be unwilling to use lichen data. Communicate during monitoring design and implementation, or convert lichen data to units used by regulatory agencies through co-location of instrumented monitors and subsequent calibration
Pollutants indicated	Lichens are especially sensitive to sulfur dioxide, fluorine, acid rain, and fertilizing and alkalinizing pollutants. Weedy, nitrogen-loving species will increase with increasing availability of nitrogen and substrate alkalinization, notably from nitrates or ammoniacal forms of nitrogen. Lichen tissue chemistry is a good integrator of wet and dry deposition and multiple pollutants. For example total N in lichen represents contributions from ammoniacal and oxidized forms of nitrogen, and wet and dry deposition. It may be feasible to identify persistent organochlorines in lichen tissue to map their presence in the environment.	Depending on the pollutant(s) of interest and its concentration in the study area, use of other plants or biota may be required to achieve the desired sensitivity. For example, it will likely be easier to demonstrate adverse effects of low to moderate ozone levels on vegetation using sensitive vascular plants than using lichens. In areas with frequent precipitation, tissue analysis cannot differentiate wet from dry deposition, and may not be able to distinguish different forms of a single element (e.g. nitrate vs. ammoniacal forms of nitrogen). Lichen analysis for persistent organochlorines is technically challenging because concentrations are usually low and natural lichen substances interfere with analyses. But much base work has been completed for the North American arctic.
QA/QC	Tissue analysis of lichens can be used to indicate, monitor or assess inorganic pollutants containing sulfur, nitrogen, metals, radionuclides and organic pollutants such as organochlorines and aromatic hydrocarbons. Standardized lichen reference material is available to check laboratory precision and accuracy. Lichens are easy and inexpensive to collect for tissue analysis, because they are widespread and key species can be identified with minimal training.	Accumulation of these chemicals in lichen tissues can be used to show the presence of these pollutants in the ecosystem, but may not directly show adverse effects to the ecosystem if lichens are not sensitive to those pollutants. Lichen tissue chemistry studies must ensure that reference samples are used in the lab and results are reported for the specific study. A good quality assurance program and appropriate quality controls are needed to provide reliable measures of repeatability and maximize sensitivity to changes in air quality. While data is less expensive to obtain, data analysis is not necessarily less costly.

Source attribution	Source attribution of the pollutants found in lichen tissue is sometimes possible using multi-element analysis and/or stable isotope ratios.	Source attribution is difficult in areas where many similar types of sources are present, or where atmospheric transport and mixing are complex, e.g., the eastern U.S.
Training Requirements	Lichens for chemical analysis can be collected from a few key species by persons without specialized prior background in biology or lichenology.	Lichen community surveys require trained personnel, usually with an academic background in biology, including lichenology, knowledge of local plants and ecology, and approximately 1-2 weeks of training in, and practice of, field protocols.

Federal Land Managers' Objectives for Regulatory or Management Use of Lichen Data

NPS, USFS, and FWS managers want to utilize lichen or other ecosystem-related data to ensure that resources are adequately protected in accordance with policy, regulation, and law. In an air pollution context, this means that if air pollution can be shown to have a detrimental effect on lichen health, it is desirable to use this information to reduce emissions from pollution sources causing or contributing to this problem. Generally, federal land managers can only use lichen or other ecosystem-related data for recommendations to reduce existing source emissions in a regulatory setting (e.g., state or federal AQRV protection regulations such as the State of Colorado's AQRV Bill and EPA's setting of secondary standards) when certain conditions are met. Lichen-effects data must provide solid evidence for *current impacts* to lichen health that are clearly related to air pollution (e.g., documentation of a change in lichen condition or viability over space or time related to air chemistry or emissions data). Affecting pollution source emissions reductions based on documented ecosystem impacts is daunting because of the difficulty in establishing a cause-and-effect relationship between pollution concentrations and changes in vegetation condition or presence/absence of species, and because current air pollution standards are based on human health impacts rather than ecosystem impacts. In addition, legal or regulatory "windows of opportunity" for federal land managers to use ecosystem effects data to request emissions reductions are limited, sometimes occurring only every few years. For example, EPA generally only solicits information from federal land managers regarding pollutant impacts to AQRVs when they are considering changes to secondary pollutant standards, or developing emissions regulations. The Southern Appalachian Mountain Initiative (SAMI) was formed as a multi-year effort to determine the impacts of air quality of Class I resources in the area. These types of activities tend to occur infrequently and for limited duration.

Land managers may also wish to use lichen health-effects data in a Clean Air Act PSD context, however this is especially challenging. In this regulatory process, NPS, FS, and FWS review and provide comments on pollution source permit applications to states or EPA. These reviews must be based on an estimation of *future impacts* linked to a single point source (e.g., a smokestack) of air pollution. These comments could be used to determine if limiting future emissions from that source is warranted based on projected impacts to lichen health. In the PSD process, atmospheric models predict how air quality concentrations or deposition could increase in Class I parks or wilderness areas based on estimated source emissions. Increases in pollutant concentrations or deposition from any one source are usually very small, and it is difficult to estimate what change they might cause in lichen health. Cumulative emissions from multiple sources are, in many cases, more likely to be of concern than single sources in causing lichen health impacts, but cumulative source impacts to AQRVs are not often addressed within the context of PSD.

As noted above, it can be difficult to prove "cause and effect" when monitoring any type of stressor on an ecosystem component, including lichens. Most lichen studies attempting to link pollutant concentrations with lichen health rely on circumstantial or correlative evidence. Therefore, studies used to document current impacts or predict future impacts to lichens are usually most effective in a regulatory setting when used in conjunction with other impacts data (e.g., visibility impacts, water chemistry changes). This provides a more robust weight of evidence by demonstrating that several types of AQRVs would be impacted by a pollution source(s). Figure 3 provides a conceptual diagram showing the steps generally needed to link lichen health evaluations with regulatory endpoints.

Another type of lichen study often conducted on federal lands uses concentrations of elements in lichen tissue to establish spatial differences in element concentrations (i.e., lichens as passive air pollution monitors). State and federal regulators are often disinterested in this data because they cannot be used in a regulatory framework (as are instrument data), to determine whether national ambient air quality standards (NAAQS) have been violated. However, air regulators as well as land managers may have indirect uses for this data, because they can be used to identify areas with high air pollution concentrations where instrument monitoring or other types of follow-up studies should be located. Figure 4 provides a conceptual diagram showing the steps generally needed to link lichen pollution indicator study results with regulatory endpoints.

Outside the air regulatory setting, park, forest, and refuge managers may use data from air pollution related lichen studies to aid management decisions, conduct NEPA analyses, and provide information to the public about resource condition and impacts. To meet the requirements of the Wilderness Act, Organic Act, and National Wildlife System Improvement Act, federal land managers often subscribe to what is known as the “precautionary principle.” The precautionary principle states that “where an activity raises threats of harm to the environment or human health, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically.” In this context, federal land managers may choose to apply the precautionary principle to lichens, where data shows that lichens may be at risk for adverse impacts from air pollution but a strong cause-and-effect relationship cannot yet be established. Agencies may have a greater ability to exercise the precautionary principle to mitigate air pollutants emitted from their own activities (e.g., in-park or on-forest activities that may produce pollutants) than from air pollution outside their boundaries.

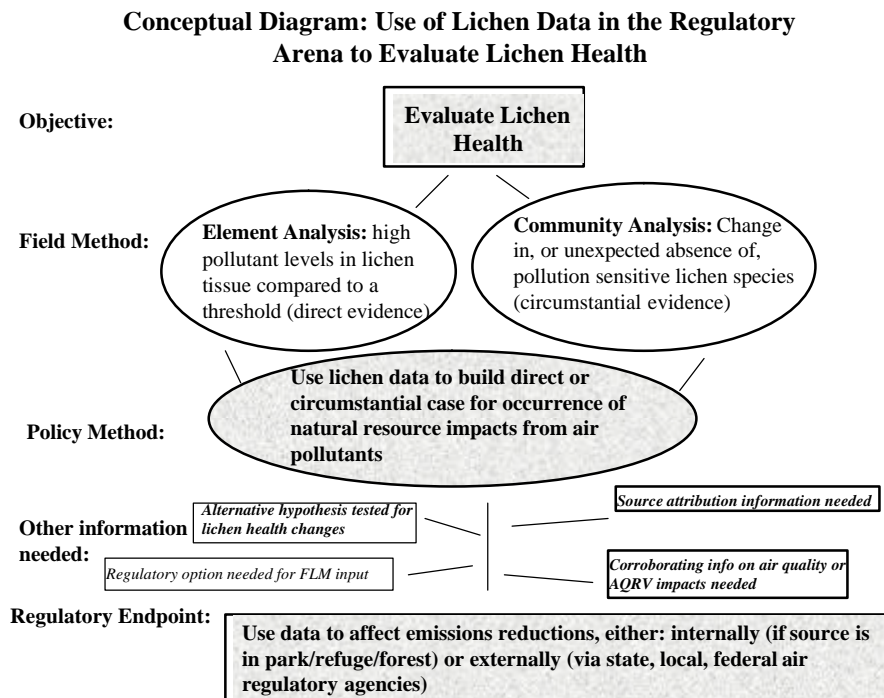


Figure 3. Conceptual diagram for the use of lichen data in the regulatory arena to evaluate lichen health.

Conceptual Diagram: Use of Lichen Data in the Regulatory Arena to Determine “Hot Spots” of Air Pollution

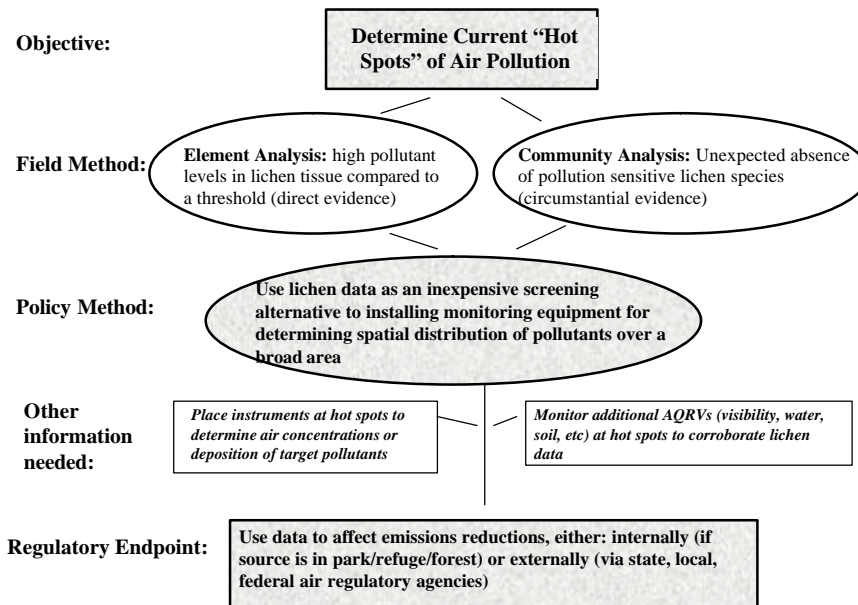


Figure 4. Conceptual diagram for the use of lichen data in the regulatory arena to determine hotspots of air pollution.

In order to use air pollution related lichen data to manage and protect parks, forests, and refuges, a study plan that incorporates one or more of the objectives below must be developed. The plan should clearly state which of these objectives the study will address, and it should identify all the types of information the study will provide, for a clear understanding of how the data can be used. If the information developed from the study is intended for the regulatory arena, in addition to meeting several of the objectives below, the data and reporting of them must be of very high quality. Generally this means meeting all or most of the criteria included in the checklist below, as well as peer-reviewed publication.

1. Document Existing Lichen or Ecosystem Health Impacts. To meet NPS/USFS/FWS management objectives (“to protect resources unimpaired for future generations”), it may be useful to determine if air pollutants (commonly sulfur, nitrogen, fluorine, ozone, and metals such as lead, mercury, and cadmium) adversely affect lichen or ecosystem health (individual plants, species, populations, communities). Species with known air pollution sensitivity could be selected for study to achieve this type of objective.

- It is important to *link element concentrations in lichen tissue with the specific effect* these concentrations may have upon lichen health or ability to survive (why are high concentrations in the tissue harmful for this lichen, or how might they be expected to affect the lichen in the future?)
- *Correlate adverse effects on lichen individuals or communities to pollution emissions or concentrations.* Seek to provide specific linkages between air pollution emissions, concentration, or deposition; and air pollution impacts to ecosystems. Correlation of lichen-effects data should be made with air pollution chemistry and/or emissions data.
- *Provide and test alternative hypotheses.* Ensure that alternative hypotheses are provided (and tested) for any changes observed in lichen health or condition (in addition to air pollution)

2. Document Pollutant Presence or Distribution. To assess spatial or temporal patterns of air pollutants, lichens may be used as “passive pollution samplers.” Lichen species insensitive to air pollution effects would usually be selected to achieve this objective. Information on air pollutant distribution could then be used to identify the best sites for future instrumented monitoring or for more intensive AQRV studies.

- *Identify the next steps.* It is important to identify what follow-up studies are necessary or planned to provide more specific information (what will managers or regulators do with the information about relative pollution concentrations?). In identifying “next steps,” a discussion should be included of specific types of monitoring (e.g., deposition, gaseous) or studies that would be appropriate. For example, if elevated levels of sulfur are found in lichens, it may be appropriate to place a deposition sampler in the area; it would not be appropriate in most cases to install a continuous SO₂ analyzer.

3. Predict Future Change in Lichen Condition or Viability. If intended for use in the Clean Air Act’s PSD regulatory process, (or NEPA documents in some cases), the study should predict future impacts of specific types and amounts of pollutant increases on lichens or other ecosystem components (e.g., via modeling). To do this, the following information is usually needed to assess the likelihood that emissions from a potential new source could adversely affect lichens: (1) identify pollution sensitive species that are present in the park/forest/refuge, (2) show pollution response thresholds for sensitive species present in the park/forest/refuge, and (3) compare lichen sensitivity thresholds to predicted pollutant concentrations or depositional increases from new source emissions.

- *Correlate to other AQRV impacts information.* Lichen data predicting (qualitatively or quantitatively) changes from emissions increases (or decreases) are generally used to greatest advantage in conjunction with other impacts assessments (e.g., visibility, water chemistry)

4. Establish Source Attribution. The determination of where a source of pollution affecting AQRVs originates can be a powerful and useful piece of information to federal land managers and air regulators. Isotope analysis and enrichment factors can provide evidence to identify potential contributors to site pollution. Reimann and Caritat (2000) discuss ways in which enrichment factors have been misused.

Air Quality-Related Lichen Studies Checklist

Lichens can be valuable indicators of biotic or abiotic effects due to air pollution in park, forest, and refuge ecosystems. However, because of the difficulties inherent in employing ecological data in a regulatory or policy setting, lichen studies are infrequently used by air managers and/or air regulators. This means studies must be designed very specifically (and carefully) to answer policy, management, and regulatory questions. Some past studies answered questions regarding lichen diversity or habitat requirements, but did not provide enough specific information about air quality impacts for federal land managers to effectively use. The following checklist was designed to assist managers in evaluating or developing lichen study proposals.

- _____ **Relevant Objectives.** Do the study objectives clearly describe the value of the anticipated results to federal land managers or air pollution regulators? The four objectives that are most relevant in answering common questions posed by park, forest, and refuge managers in the air pollution regulatory context are discussed in the previous section.
- _____ **Meaningful Study Design.** Is the study design clearly described and is it linked to the objectives? While it is beyond the scope of this guidance to thoroughly describe lichen study design alternatives, be aware that different methodologies for lichen sampling answer different questions. Examples of a few types of lichen study designs are discussed in Appendix 1 (also see Stolte et al. 1993).
- _____ **Alternative Hypotheses.** Are alternative hypotheses for changes in lichen presence or absence explored or at least described in the study? For example, there may be other reasonable explanations for changes in the distribution of a lichen species besides air pollution concentrations (e.g., herbivory, differences in site conditions, short- or long-term climatic variability.).

- _____ **Linkages to Air Pollution Chemistry.** Does the study use air pollution emissions or air chemistry data to explain variability in lichen chemistry or species distribution patterns? It is very valuable for managers and regulators to understand how spatial or temporal patterns in lichen chemistry or lichen species distribution are related to air chemistry concentrations or pollution emissions.
- _____ **Voucher Specimens.** Will the study collect “voucher specimens” to confirm the correct taxonomy and identification of species and save them for future reference? When a study characterizes lichen communities by describing all the species observed, it is common for voucher specimens to be collected for confirmation of lichen taxonomy by other experts. These samples are usually saved for long-term records.
- _____ **Temporal Methods Consistency.** When studies are used to detect change over time in lichen species composition or lichen chemistry, will steps be taken to ensure that studies done at different times (often by different researchers) be comparable to past or future field collection and chemical analysis and sample processing methodologies?
- _____ **Linkages Between Lichen Chemistry and Lichen Impacts.** When study objectives are related to assessing air pollution effects on lichen health, will lichen tissue chemistry concentrations be related to potential adverse effects (e.g., changes in photosynthesis, growth, viability) that might occur in lichen individuals, species, or populations; or in the ecosystems (e.g., lichens as a food source for other animals)?

Additional, more specific issues to be aware of when assessing lichen study components can be found in Table 1; “Lichen Monitoring Advantages and Limitations.”

Appendix 1. Examples of Air Quality-Related Lichen Study Objectives and Designs Useful to Land Managers

Calculating Enrichment Factors to Distinguish Regional from Local Sources of Metals

When both natural and anthropogenic sources contribute to the metals being measured, identifying the relative contribution from each source becomes a complex task. Puckett (1988) reported a method of calculating enrichment factors (EFs) to compare the concentration of metal within a plant with potential sources in the environment. The equation is

$$EF = \frac{x/\text{reference element in lichen}}{x/\text{reference element in crustal rock}}$$

Ford and Hasselbach (2001) analyzed the moss, *Hylacomium splendens*, mineral soils, and road dust along a highway used to transport lead- and zinc-enriched ore through Cape Krusenstern National Monument in arctic Alaska. Using aluminum as a reference element they convincingly demonstrated that dust composed of the roadbed material accounted for only a fraction of the substantially elevated lead, zinc, and cadmium concentrations observed along the road corridor and that levels of heavy metals, though not as high as sites within 200 m of the road, were still elevated at distances of 1,000-1,600 m away from the road. The use of enrichment factors is controversial however, because they can be highly variable in some circumstances (Reimann and Caritat, 2000)

Sulfur Isotope Analysis for Source Attribution and Correlation with other AQRV studies

Stable sulfur isotope ratios in combination with multi-element analysis of lichens were used to examine the influence of emissions from two coal-fired power plants in the Yampa Valley on pollutant deposition in the Mt. Zirkel Wilderness of northern Colorado (Jackson et al. 1996). Coal-fired power plants typically emit SO₂ with a stable isotope ratio ³⁴S/³²S characteristic of the coal combusted. Stable S-isotope ratios in the beard lichen, *Usnea*, were significantly heavier (more positive) in the wilderness and nearby sites compared to more distant regional sites and corresponded well with sulfur isotope ratio found in snow in the same area and average isotope ratios in coal used by the power plants. These data, combined with other AQRV impacts data in the wilderness, were used to convince the state and the utility companies to install additional emissions control equipment on the power plants. Sulfur isotope studies are most easily interpreted when the point source of concern is the predominant source of sulfur in the study area. In areas with many sulfur sources (e.g., most of the eastern U.S.) sulfur isotope studies are less useful because of issues of multiple sources and long-range transport.

Using Lichen Baseline Information, Lichen Effects Thresholds, and Air Quality Data to Assess Effect of Regulatory Increments.

Because air quality “increments” were exceeded in North Dakota in the early 1980s, the NPS was required under the Clean Air Act to make a determination as to whether or not the exceedance would be likely to produce an “adverse impact” to air quality-related values in the Theodore Roosevelt National Park. Lichen species determined to be present in the park (Wetmore 1983) and potentially sensitive to air pollutants were evaluated with regard to concentrations of air pollutants known to cause health impacts to these species. The analysis determined that the current and predicted concentrations of sulfur dioxide at the park were not anticipated to cause adverse effects on air pollution sensitive lichens in the park, based upon lichen-effects data available in the scientific literature at that time. The NPS subsequently certified that the sulfur dioxide levels would not be anticipated to cause adverse impacts to the park’s AQRVs even though air pollution increments (regulatory thresholds) were exceeded, giving the state the ability to proceed with permitting several new industrial sources in the state.

Gradients in Air Quality Detected by Lichen Community Surveys

Using the FIA/FHM Lichen Indicator methodology, a systematic sampling of epiphytic lichens at 203 sites in the southeast U.S. was used to produce a model linking gradients in air quality and climate to lichen community composition (McCune et al. 1997). Pollution-tolerant species and lower species richness were observed in urban and industrial areas, whereas pollution-sensitive species and high species richness were encountered in cleaner areas. Additional FIA/FHM models are being developed in the Pacific Northwest, California, northeast, and Colorado. These models can be used to score additional sites along an air quality gradient within the same study area, to monitor future changes in air quality, to rate relative sensitivity of regional species, and to document ecological effects of changing air quality.

Gradient Analysis in Lichen Tissue with Distance From a Pollution Source

Using the gradient method, element concentrations within the lichen are usually observed to increase as the distance to the suspected source decreases. Gough and Erdman (1977) used linear regression to evaluate the relationship between distance from a coal fired power plant and metal levels in *Xanthoparmelia chlorochroa*. However, as Puckett (1988) points out, concentrations of many elements will not reach zero at large distances from pollution sources because they have essential nutritional roles or are normal components of the lichen when growing in its natural environment. In the Mt Zirkel study mentioned above, three species of lichen were selected for chemical analysis at various sites within the wilderness area and at sites further away. The study found that *Xanthoparmelia cumberlandia* samples from within the Mt. Zirkel wilderness were elevated in sulfur, nitrogen, potassium, sodium, and phosphorus compared to the same species at more distant regional sites (>100 km). As with sulfur isotope studies, in areas with many pollution sources (e.g., most of the eastern U.S.) gradient studies are less useful.

Monitored Air Quality Data: Linkages to Lichen Element Concentrations

Determining relevant elements is an important part of any multi-element study. In Switzerland, Herzig et al. (1989 and 1990) used multivariate analysis to compare element concentrations in *Hypogymnia physodes* to total air pollution as assessed by lichen communities using the IAP index and an instrumented monitoring network. Four groups were discerned.

Group 1: Ca. Calcium was the only element that increased with improving air quality.

Group 2: Pb, Fe, Cu, Cr, S, Zn, and P. Concentrations of these elements decreased in distinct curvilinear gradients with decreasing total air pollution. For example, the concentration of Pb was reduced six-fold in the "very low pollution" zone compared to the "critical air pollution" zone. These elements were strongly correlated with annual average atmospheric deposition measurements detected by the instrument network.

Group 3: Li, Cd, Co. Concentrations of these elements were lower in the "very low pollution" zone than in the "critical air pollution" zone, but the gradients were not strictly curvilinear.

Appendix 2. Web Resources

Lichens and Air Quality Interagency Workgroup: <http://ocid.nacse.org/research/airlichen/workgroup>

USGS Biological Resources Division— National park sites for which lichen species lists are available: <http://www.ies.wisc.edu/nplichen/summary.php>

U.S. Forest Service Forest Inventory Analysis Lichen Indicator: <http://www.fia.fs.fed.us/program-features/indicators/lichen/>

U.S Forest Service Air Resource Management/Lichens and Air Quality Information Clearinghouse: <http://airlichen.nacse.org>

Search recent literature about lichens: http://www.toyen.uio.no/botanisk/bot-mus/lav/sok_rii.htm

American Bryological and Lichenological Society: <http://www.abls.org>

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