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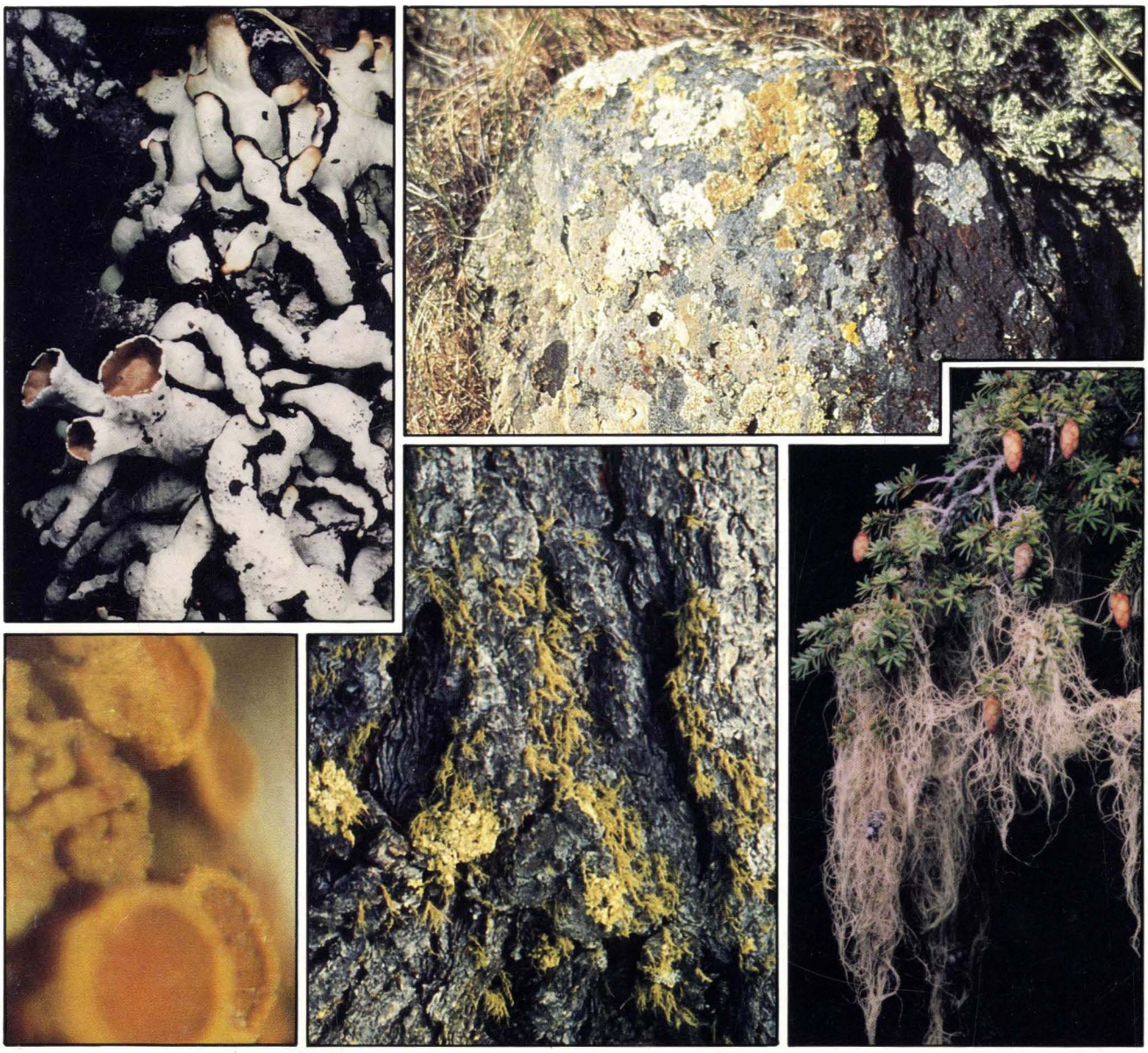
Forest Service

**Rocky Mountain
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Fort Collins,
Colorado 80526

**General Technical
Report RM-224**

Lichens as Bioindicators of Air Quality



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Abstract

This report is the result of a workshop held in Denver, Colorado on April 9-11, 1991. It summarizes the current literature and techniques for using lichens to monitor air quality. Experts in lichenology and ecology contributed information on lichen floristics, characterization of monitoring sites, lichen species and communities, identifying lichen species sensitive to pollutants, active monitoring with transplants, chemical analysis of lichens, and case studies as examples of lichen biomonitoring scenarios.

Keywords: lichens, air quality, biomonitoring, lichen floristics, lichen communities, gradient analysis, air pollution, sensitive lichen species, elemental analysis, study designs, quality assurance/quality control.

Descriptions of front cover photos, clockwise starting in the upper left corner:

F1. *Hypogymnia enteromorpha*, found on trees. Used in the Tongass National Forest Air Quality Biomonitoring Project, it accumulates higher concentrations of most ambient pollutants than other lichens analyzed for biomonitoring. **Photo by Sylvia Duran Sharnoff and Stephen Sharnoff.**

F2. *Lecanora melanophthalma* (Ram.) Ram., also known as *Rhizoplaca melanophthalma* (Ram.) Leuk and Poelt. This is the best pollution indicator of the xerophytic lichens, referred to in Rope and Pearson (1990). It is an umbilicate species usually found on basalt. It is easily pried loose from the substrate; its prominent and distinctive apothecia make identification easy and certain. It is very sensitive to gaseous pollutants such as sulfur dioxide and also accumulates heavy metals and other elements. This photo shows the habitat of *L. melanophthalma.* A number of other species are also pictured, including *muralis, christoi, calcarea, Lecidea tessilata, Candelariella rosulans, Caloplaca saxicola, Acarospora americana*, and others. **Photo by Lorenz Pearson.**

F3. *Alectoria sarmentosa* is the most common lichen on the Tongass National Forest and the only *Alectoria* in SE Alaska that drapes from trees. It provides important winter survival forage for black-tailed deer and mountain goats and was used by Northwest Coast Indians for bandages and baby diapers. *Alectoria sarmentosa* was collected for tissue analysis by Tongass Air Quality Biomonitoring Project. **Photo by Sylvia Duran Sharnoff and Stephen Sharnoff.**

F4. *Usnea hirta* (L.) Weber ex Wigg. is a common fruticose lichen in western conifer forests, growing mostly on partly sunny branches and trunks of trees. After five years of operation of a coal-fired power plant at Colstrip, Montana, *U. hirta* thalli within 15 km of the plant had significantly higher sulfur content than thalli at distances of more than 40 km, though no differences in physiology or appearance were noted. The yellowish foliose species is *Parmeliopsis ambiqua*, not known to be particularly sensitive to pollutants because it lives mostly at the bases of trees. **Photo by Sharon Eversman.**

F5. *Xanthoria polycarpa* (Ehrh.) Olive. on sagebrush bark. This species is tolerant to relatively heavy levels of atmospheric pollution. However, even though it survives in city environments and other polluted areas, it is less abundant where pollution is heavy. **Photo by Lorenz Pearson.**

Back-cover photos described on the inside back cover.

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Lichens as Bioindicators of Air Quality

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Preface

We are pleased to offer this document summarizing the recommendations of a group of conference participants on issues relating to air pollution and lichens. These recommendations were compiled at a workshop held in Denver, Colorado, on April 9-11, 1991. The workshop was organized and co-sponsored by the Washington Office, Watershed and Air Staff of the U.S. Forest Service and the Air Quality Division of the National Park Service. This workshop brought together many of the experts in the field of lichenology to discuss ways to improve our understanding of the effects of air pollution on lichens, lichen inventory and monitoring methods, and the range of uses of lichens as biomonitors. We appreciate the efforts of the organizers and contributors to this document and wish to especially thank the U.S. Forest Service, Rocky Mountain Forest and Range Experiment Station, whose staff graciously agreed to edit and publish this document.

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Introduction

Ken Stolte, Deborah Mangis, Robert Doty

THE WORKSHOP

The National Park Service, Air Quality Division (NPS-AQD) and the USDA Forest Service (USFS) convened a three-day workshop in April 1991, attended by federal land managers, regulatory experts, and researchers who specialize in the study of lichens (see Appendix I). The objectives of the workshop were: (1) to review what is known about the suitability of lichens as bioindicators of air quality; (2) to review existing methods and recommend standard research and monitoring procedures to study lichen populations; and (3) to assess the usefulness of data on lichen responses to air pollution within the regulatory arena.

RATIONALE

Lichens are a group of non-vascular plants composed of fungal and algal species growing in a symbiotic relationship. The fungi supply structural support to the organism, and the algae supply nutrition through photosynthesis. Lichens are an extremely diverse floral group, occupying ecological niches on varied physical and biological substrates such as soil, rocks, and the branches and boles of vascular plants. Lichens lack an epidermis, stomata and a waxy cutin, and consequently lack the control over gas exchange as vascular plants do (Larcher 1975).

Lichens were first recognized as organisms sensitive to high concentrations of gaseous pollutants such as sulfur dioxide (Skye 1968; Rose and Hawksworth 1981). For this reason they have been used as indicators of urban pollution and point-source emissions from uncontrolled combustion sources. They were also found to act as accumulators of elements, such as trace metals and sulfur (Ferrey et al. 1973; Nieboer and Richardson 1981; Puckett 1985; Tyler 1989).

The pollutant that most directly affects the health of lichen communities is sulfur dioxide (McCune 1988; Nimis et al. 1990). Lichens are often studied due to their ability to concentrate elements that may be present in low concentrations in the ambient air (Tyler 1989; Nieboer et al. 1972).

Lichens are relatively easy to collect, preserve, and analyze for pollutant-related studies. Because they can convert atmospheric nitrogen to a form usable by other terrestrial and aquatic plants, lichens are considered to be important contributors to nutrient cycling in some types of ecosystems (McCune 1992). For these reasons, a number of federal land management agencies such as the Forest Service, the National Park Service, the Fish and Wildlife Service, and the Bureau of Land Management, along with regulatory agencies and the regulated industries have had an ongoing interest in studying lichens to determine the presence and effects of air pollutants on natural ecosystems (Ryan and Rhoades 1991; Rhoades 1988; Wetmore 1983; Gough and Erdman 1971; Thomas and Rosentreter 1992; Showman and Hendricks 1989; Nash 1989; McCune 1988).

COMPLICATING FACTORS

A number of factors complicate the interpretation of field and laboratory studies of lichen response to air pollution. Recent studies of lichens in NPS units have raised questions about the completeness of species lists and the ability of field technicians to perform follow-up surveys in subsequent years to determine the existence of trends in species diversity. Since there are no standards for conducting lichen floristic studies or for calculating the completeness of a flora, it is difficult to determine if the absence of a species is due to anthropogenic influences or to the lack of a comprehensive baseline survey. We should also consider the possibility that changes in lichen communities are a function of changes in other environmental variables (see Chapter 3).

Rydzak (1969) argued that the disappearance of lichens from polluted cites at the turn of the century was due to lowered relative humidity, increased temperatures, and more frequent wet-dry cycles in the altered urban environments. This argument is

countered by evidence of the return of pollutant-sensitive species to cities as air quality has improved (Coppins 1973; Rose and Hawksworth, 1981).

DISCUSSION TOPICS

During the workshop we discussed the following topics, developed in more detail in Chapters 2-7 of this volume:

1. Lichen floristics (development of lichen species lists and estimates of their completeness).
2. Identification of lichen species that are sensitive to air pollutants and the pollutant concentrations that might affect these sensitive species.
3. Methods used to characterize the status of lichen communities and the ability of lichens to accumulate air pollutants.
4. Evaluation of the overall usefulness of lichens as biological monitors of air quality.

Listed below are the more general topics discussed during the workshop.

Lichen Floras

Topics included consideration of the protocols for conducting lichen floristic studies and methods of quantifying the completeness of any floristic study considering the range of habitats and substrates surveyed, the percentage of the land area (NPS unit, National Forest) covered, and the amount of survey time spent in each habitat. Other topics included methods to determine changes in lichen floras by comparing present lichen species with past species lists reconstructed from herbarium specimens.

Identification of Pollutant-Sensitive Species

During this session we considered the development of protocols for controlled pollutant-exposure (fumigations) of lichens under natural conditions and field studies conducted along pollutant gradients. Important considerations for identifying pollutant-sensitive species in chamber fumigations include exposure regimes, mixed pollutant interactions, environmental conditions within the fumigation chambers, and identification and quantification of lichen responses to air pollution exposures.

Some concerns in identifying pollutant-sensitive species in field gradient studies include homogeneity of sites along a pollution gradient, determination of pollutant exposures along the gradient, and assessing lichen responses to these air pollution exposures.

Lichen Responses to Air Pollutants

This discussion covered subjects relating to the determination of responses of lichens to air pollution, definition of unique symptoms, the variability of symptoms among individuals, and what symptoms can be realistically measured under field conditions. For example, depression of photosynthesis may be the initial sign of air pollutant stress identified in exposure studies, but it may be impractical to measure this parameter in the field in remote areas. We discussed possible responses to air pollution stress including chlorophyll degradation, changes in photosynthesis and respiration, alterations in nitrogen fixation, membranae leakage, accumulation of toxic elements, and possible changes in spectral reflectance, lichen cover, morphology, community structure, and reproduction.

Lichen Biomonitoring Plots

We discussed the usefulness of establishing and evaluating long-term lichen monitoring plots to differentiate natural and air-pollution caused changes in community composition, community structure (diversity and abundance), morphological condition, and elemental content. Objectives and experimental design must be well thought-out before setting up this type of monitoring plot.

Elemental Analysis

In this group we discussed protocols that might be used to analyze the chemical content of lichen tissue. Once the elements of interest are defined, it is important to understand the natural variability that might be expected due to the accumulation of elements from natural sources (e.g. sulfate from dust and sea spray). Important considerations for elemental analysis of lichen samples include identification of elements to be evaluated, sample preparation, methods of analysis, quality assurance and control (QA/QC) of analyses, comparison with herbarium samples, separation of anthropogenic

elements from natural-source elements (e.g. use of stable isotopes), and comparison with samples of host substrate (bark, soil, rock).

Transplants and Biomonitoring Gardens

We discussed the development of protocols for active manipulation or transplantation of lichen species. In these kinds of experiments it is important to consider performing reciprocal species transplants, establishing lichens near pollutant monitors for exposure-response studies, physiological analyses to evaluate effects of transplant shock, and comparing macro- and microhabitat conditions at host and transplant sites. Monitoring air pollution exposures at transplant sites with instruments should be done if air pollution is to be linked to effects on the transplanted species.

Spectral Analyses

We need to develop a field method to spectrally analyze lichen species and communities that will be able to quantify species composition and cover, compare community structure changes over time, and correlate spectral signatures with physiology or toxic element uptake in lichens. Considerable development work should be done before we can use spectral analysis to measure lichen responses or to provide QA/QC on visual estimates of lichen cover and morphological condition.

Sampling Design and Statistical Analysis

Topics covered during the discussion included development of sampling designs and methods of statistical analysis to address spatial and temporal variability in lichen community structure, elemental content, and vigor. Some important things to consider in experimental design and analysis are stratification of habitats, random versus systematic sampling, frequency of re-evaluation, sample sizes, types of data collected, and methods of analysis (e.g. parametric, non-parametric, regression). This activity is an important component of all aspects of lichen research and monitoring.

Quality Assurance and Quality Control of Data Collection

We discussed the need to develop QA/QC protocols to be used during the collection and analysis of data on community structure and vigor, elemental content, and evaluation of plots over time. QA/QC information is essential to providing credible data for regulatory proceedings.

Geographic Information Systems (GIS)

GIS has the potential to be used in the development of sampling designs, regional maps of precipitation, air pollution, land-use, and storm patterns. All of these abiotic variables can affect the distribution of lichen species. GIS can be used to map plot-level data on community structure, vigor, and elemental content.

Land Navigation Systems

We discussed the use of land navigation systems and global positioning systems (GPS) to locate sample points and to allow for accurate relocation during subsequent observations. GPS are becoming more accurate and cheaper and are now reasonably accessible to field researchers.

Herbarium Collections

These collections are valuable tools for comparing current lichen floras, community structure, and elemental concentrations with herbarium samples collected in pre-pollution eras. If such comparisons are to be made, it is important to obtain adequate amounts of tissue for analysis. Controlled experiments should be performed to determine the degree to which preserved specimens can be used to evaluate changes in pollution deposition over time and to identify signature elements of anthropogenic deposition.

Meteorology and Climate

We discussed ways that researchers can evaluate trends in meteorology and climate, particularly in temperature and precipitation patterns, which may affect lichen species and community structure and vigor. The usefulness of these databases depends on their accessibility at local and regional scales. When designing lichen fumigation studies, climate data are useful to approximate ambient environmental conditions.

Air Pollution Monitoring

Air pollutants of interest in lichen research and monitoring include sulfur oxides, nitrogen oxides,

fluorides, and toxic metals. To estimate the dose or exposure of lichen communities to air pollutants, the following methods can be employed: instrumental monitoring, passive monitors, or extrapolation of data from distant sites using techniques such as kriging.

Identification of Research Needs

We discussed emerging areas of environmental research, including the effects of global warming, elevated carbon dioxide concentrations, increased UV-b, and nitrogen fertilization. Researchers need to consider which of these stresses might have an effect on lichen species and communities.

Cooperation with Other Research/Monitoring Programs

Lichen monitoring should be investigated as a component of planned or ongoing environmental monitoring programs such as the USFS/EPA Forest Health Monitoring Program, the USFS Global Change Program, the NPS Global Change Program, the National Acid Precipitation Assessment Program II, and the EPA Clean Air Status and Trends Network. Coordination of lichen monitoring efforts among agencies will result in the comparability of databases.

THE PROCEEDINGS VOLUME

The workshop participants chose six areas for in-depth discussion and development into chapters to be included in the proceedings of the workshop. Each of the six working groups developed a detailed outline of the chapter, identified a lead author, and assigned writing tasks and milestones. Drafts of the chapters were sent out for peer review and the reviewer comments were incorporated into the final document.

This document summarizes much of the literature on the use of lichens as bioaccumulators of air pollutants, and describes possible responses of lichens to air pollutants, especially sulfur dioxide. The final chapter of this volume attempts to pull all this information together by describing seven different air pollution scenarios, and presenting case studies that can help guide a researcher or land manager in the design of a lichen monitoring program.

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Floristics

Thomas H. Nash III, Clifford M. Wetmore, William Anderson, Charis Bratt, William C. Denison, Sharon Eversman, Barbara Murray, Larry St. Clair

Floristic studies provide essential baselines for judging future changes. This chapter documents the essential resources, collection protocol, voucher specimen requirements, identification procedures, report requirements, and database compilations needed for thorough floristic studies.

INTRODUCTION

Floristics may be defined as a compilation of species present in an area and the distribution information for those species. A complete and accurate species list is an ideal basis for understanding the flora of the area being studied. It gives ecological information about the unit, provides information necessary for determining appropriate species for biomonitoring, and provides information concerning the resources of the unit. The value of floristic studies in lichen biomonitoring projects has recently been reviewed by Wetmore (1988).

Lichen floristic studies require the expertise of specially trained lichenologists because lichens possess many unique characteristics. Collection and identification of lichens involve recognizing habitats, substrates, and specimens in the field, and using appropriate collection, curation, and identification procedures. Quality assurance (QA) and quality control (QC) standards must be met, as legal challenges to a study are possible. Time constraints are frequently part of a study and its subsequent report; for example, a study and its reviewed report must be completed before construction of a polluting point source. For these reasons, beginners and amateurs in lichenology are not satisfactory personnel for a floristic study of a management unit unless they are working directly in association with experienced lichenologists. An additional QA/QC requirement is adequate peer review of proposals and reports, preferably by other lichenologists.

RESOURCES NEEDED

Literature

For aid in planning field work, literature should be available on any previous lichen studies as well as literature on the vegetation, geology, climate, and air quality studies in the area. These may be published papers or reports on file in the study area headquarters. All papers reporting lichens from the area must be consulted, including monographs and revisions.

Access to all of the pertinent monographs, revisions, and regional floras is essential for identification of the collections. In some areas, keys may be available for some of the lichens. If regional floras are not available, it may be necessary to obtain papers from other areas to aid identification. Only a well-equipped lichen library is likely to have adequate reference material for many study areas.

Herbaria

A good lichen herbarium is necessary for the identification of lichens. After a lichen species has been keyed out, it should be compared with reliably identified herbarium material for confirmation of the identification. The herbarium reference collections should contain specimens identified by authorities in the group and/or duplicates of specimens seen by the authorities.

Personnel

The researcher should be familiar with the other lichenologists who know the lichens of the management unit or the taxonomic groups found there. These lichenologists may be called upon to help with problem identifications and review of some of the identifications. Some ecologists may also be able to provide helpful ideas on collection design and field work.

Site-Specific Information

The managers of the study area (forest, park or land management unit) should be able to provide maps, air photos, climate data and other information needed for field work and report writing.

COLLECTION PROTOCOL

It is essential that well-trained professionals make the field collections. Because of their relatively small size, many lichens may not be noticed by amateur naturalists, and even individuals with initial training in lichenology will not be sensitive to the range of microhabitats in which lichens occur. Poorly trained individuals are not sensitive to the subtle morphological differences that distinguish different species or populations in decline. It is important to be aware of relatively obscure species as well as the more obvious ones, since some of the obscure ones may be more sensitive to air pollution (Wirth 1985).

SAMPLING DESIGN

Initially, sampling design strategies should be discussed with the appropriate federal land manager and resource people. Sampling should include areas representative of all major vegetation types (*e.g.*, alpine, spruce-fir forests, ponderosa pine forests, etc.). Microclimate variation is important in determining lichen distributions, and should be considered in site selection. Consequently, it is important to consider the effects of topography. USGS topographic maps are helpful. One should sample on north- and south-facing slopes as well as in riparian areas. Cliff faces, both in exposed and shaded localities, are frequently rich in lichens.

Geologic maps should be consulted as rock lichens may be specific to substrate (*e.g.*, serpentine, limestone, basalt, etc.). Similarly, soil lichens may be restricted to specific soil types. Knowledge of site history is also important. For example, areas that have been burned in the past few decades may have some lichens species that occur specifically on charred substrates. Similarly, insect outbreaks will open up canopies, change the local microclimate, and provide extensive wood substrates that are occupied by other lichen communities. The potential for aquatic lichens should also be considered.

Sites of about one hectare in size in major habitat types should be replicated if possible. Ideally, an initial reconnaissance of the study area would identify a number of potential sites in each major habitat type. A randomization procedure could then be used to select the replicate sites within each habitat type.

Tools

A geological hammer and coal chisel are essential for collecting rock lichens that are normally collected with a sliver of the substrate attached. A hunting knife and/or wood chisel are essential for collecting from trees. Lichens growing on loose soils require prompt impregnation of the soil with a dilute mucilage (*i.e.*, water soluble) glue after removal to insure that the specimen does not disintegrate during transport. All specimens should be stored in paper bags as lichens will quickly mold in plastic bags or other closed containers.

If it is not practical to determine elevations from USGS maps, then altimeters should be carried. Precise locations may be determined with a portable GPS instrument. A bottle of water is helpful to moisten umbilicate lichens to facilitate their removal from the substrate. A hand lens is essential to examine reproductive structures (*i.e.*, isidia, perithecia, etc.), particularly with crustose lichens where different species may appear superficially similar.

SAMPLING PROCEDURES

At each site, ecological notes on the composition and structure of the vascular plant vegetation community should be made. The full range of substrates within the sampling site should be investigated to insure as complete a species list as possible. For example, different lichens may be found on different tree species, at different heights on the tree, under shaded overhangs versus exposed locations, on mosses versus mineral soils, on

different rock types (size as well as geological composition), and on wood or bark in different stages of decomposition.

Unless a species is extremely rare, sufficient material should be collected to fill a 4" x 5" card (a size to fit a typical lichen storage packet). Replicate samples are recommended so that representative specimens may be deposited in more than one herbarium. Although some species are typically asexual, reproductive characters are important to correctly identify many lichens; consequently, specimens with "fruiting" bodies (apothecia, perithecia, etc.) should be sought. Particularly within the more common species, pay attention to potential morphological variation as air pollutant-induced morphological changes often occur prior to the actual disappearance of the species.

Label collecting bags clearly with, at minimum, substrate type, date, and location. Collection numbers should be assigned, if practical, in the field. In general, at least an hour should be spent at each site; frequently, several hours may be required to adequately sample the species present. If specimens are moist at the time of collection, they should be air dried as soon as possible to prevent molding. Specimens should be packed carefully for transport to insure that the more fragile specimens are not crushed.

VOUCHER SPECIMENS

A voucher specimen is an actual sample of one species taken from a study site at a particular time and deposited in an established publicly accessible herbarium. A voucher specimen of a lichen documents the presence of that species at the study site at the time of study. A collection of voucher specimens, appropriately prepared and identified, is an essential part of the quality assurance of any floristic study.

Note that a voucher specimen must consist of an actual lichen; it may not be a photograph. Photographs add to the value of a report but they are not acceptable substitutes for voucher specimens. Verification of a lichen's identity requires microscopic and chemical information not obtainable from a photograph.

A well-prepared voucher specimen may be of immense value. For example, during litigation, a voucher specimen provides evidence of a critical species at a given place and time. But a good voucher specimen is expensive to prepare. Contracts for floristic studies must make adequate budgetary allowance for handling and preparation of voucher specimens.

Furthermore, to be of use, a voucher specimen must be both protected and made available for responsible public use. That is, it must be entered into and maintained by an established, publicly accessible herbarium to insure that it is available into the indefinite future. Contracts for lichen floristic studies should stipulate which herbarium or herbaria have agreed to receive and maintain the voucher specimens.

At a minimum, the collection of voucher specimens which document a floristic study must consist of one voucher specimen for each species recorded. It is expected that if only one set of voucher specimens is prepared, then that set will be deposited in the herbarium of the collector. It is strongly recommended that duplicate sets of voucher specimens be deposited in another herbarium within one day's drive of the study area, as well as in the U.S. National Herbarium (Smithsonian Institution). The lichenologist responsible for the study usually distributes other duplicates to other herbaria.

When many small subareas are sampled in the course of a floristic study of a large unit, only one voucher specimen is required for each species recorded in the large unit. However, the investigator or the contracting agency may wish to retain a specimen of each species from each subarea.

Selection

Selection of appropriate samples begins in the field and continues in the laboratory. Selection involves choices based on the quantity of lichen needed, its quality, and its rarity. Collections from more than one site or time must not be pooled for voucher specimens. Ideally, a voucher specimen documenting a floristic study consists of enough material to include several to many lobes or branches and contains representative sexual or asexual fruiting structures. Depending on the species, the volume of lichen in a specimen will vary from one to ten cubic centimeters. In many instances, the sample will contain substrate -- bark, rock, moss, soil, etc. -- in addition to the lichen.

If the lichen is rare, collecting even for voucher specimens should be severely curtailed. In all cases, collections should be limited to those required for scientific purposes.

Processing

Some selection, sorting, and processing may be made in the field, but specimens normally arrive in the laboratory in paper bags and in need of further processing before they can be placed in paper packets for long term storage. The nature of the processing depends upon the growth form of the lichen and upon the amount and kind of substrate included with the lichen.

Often a sample will include more than one species of lichen. Where it is possible to do so, the species of choice should be freed of contaminants (*e.g.*, separated from mosses, most detritus, other lichens, etc., although retention of part of the substrate is preferable). In some instances, especially with crustose lichens, it will not be possible to eliminate all associated species.

In general, it is undesirable to rewet lichens in the laboratory. However, bulky fruticose or foliose species require moistening with water followed by pressing in a plant press to permit their enclosure in a packet and to prevent subsequent fragmentation of the specimens. Take care that moist lichens are pressed and redried promptly to avoid spoilage. To avoid alteration of the chemistry of the lichen, use distilled water rather than tap water, which can alter reactions.

Specimens growing attached to soil require treatment to consolidate the soil. Often this process is begun in the field. The soil is infiltrated with an adhesive solution which dries and binds it together. Without this treatment, soil and lichen disintegrate over time. Any process that adds extraneous solutions to the sample, such as moistening or soil consolidation, should be constrained by the realization that subsequent study of the specimen may require microchemical tests that may be affected by the content of the adhesive solution.

Lichens growing attached to fragments of rock, bark, or soil require special attention to prevent them from abrading each other in the packet. In some herbaria, the individual fragments are glued to a card, which is enclosed in the packet. In other herbaria, a card is covered on one side with a layer of surgical cotton. Fragmented samples will adhere to the cotton but can be removed for study.

Herbarium packets are folded from sheets of 100% rag papers in a variety of ways. A standard size is a 4" x 6" (10 x 15 cm) packet. A label is attached to the packet (see Hale 1979). Some herbaria glue the individual packets to standard herbarium sheets. In this case the packet must be folded so that it can be opened even when its back is attached to a sheet. Many herbaria, including the Smithsonian, routinely repacket specimens to their own standards.

Identification

Some lichens, mainly some of the larger ones, can be identified in the field. However, many lichens require the use of a microscope and chemical analyses to determine the species. The problem of identification is compounded by the lack of comprehensive manuals, regional or national. Consequently, an investigator proposing to undertake a floristic study should be required to establish:

1) expertise in the use of appropriate microscopic and chemical techniques;
2) access to equipment for performing these techniques;
3) familiarity with the world literature on lichen taxonomy;
4) access to a major library containing the relevant literature; and
5) access to a major lichen herbarium.

TECHNIQUES AND EQUIPMENT

Reference Materials

Reference materials essential to lichen identifications are of several types. One category is published literature, including books, monographs, and journal articles. Another is the collection of specimens in a major lichen herbarium. A third is the world-wide network of lichen systematists.

A substantial collection of literature relating to lichen identification is essential to lichen identification given the absence of comprehensive regional or local manuals. In rare instances, an investigator's personal library may be adequate, but in most instances, lichen identification requires a major research library. It is by no means certain that a large technical library will hold those specialized, and often foreign, books and journals needed for lichen identification. Among the journals, the last 25 years of *The Bryologist* and *Lichenologist* are essential. Additional important journals include *Bibliotheca Lichenologica, Bulletin of the British Museum, Cryptogamic Botany, Hertzogia, Journal of the Hattori Botanical Garden, Mycotaxon, Nordic*

Journal of Botany, Nova Hedwigia, Opera Botanica, and *Smithsonian Contributions to Botany.* Some of the important floristic treatments are listed in the appendix of this chapter.

Published descriptions, with or without illustrations, are often inadequate for reliable identifications. Under those circumstances, the investigator must have recourse to comparison of his or her collections with herbarium material determined and often annotated by experts. In a limited number of cases, an investigator may borrow a critical specimen from a remote herbarium just as one may borrow a book on interlibrary loan. However, serious taxonomic study of a large number of collections normally requires that the investigator have daily access to a lichen herbarium.

There are relatively few lichenologists, and they collaborate globally as well as nationally. Often a letter or phone call to a colleague is the most efficient way to resolve a difficult identification. A substantial body of not-yet-published "gray" literature is exchanged within this informal network. An investigator proposing to undertake a lichen floristic study should be prepared to demonstrate that he or she can make use of this resource.

For determination or verification, it is desirable in many instances to send specimens of species in a difficult genus to a colleague who is monographing the genus. Unfortunately, many of the most difficult genera have not been monographed and it may be that no one is presently specializing in the genus. An added drawback to sending specimens to an external authority is that it might be months and sometimes years before one receives a final determination. Providing a modicum of funding to specialists for specimen verification would alleviate this problem.

Equipment

Expertise in both microscopy and lichen secondary product determinations is necessary to adequately identify lichens. Much of the structure used in identifying lichens is microscopic. This is especially true of crustose lichens, but reference to microscopic features, such as algal cells, asci, ascospores, and tissue types are required for many foliose and fruticose lichens as well. An investigator proposing a lichen floristic study must demonstrate the routine availability of a research quality light microscope with oil immersion capability. Access to a scanning electron microscope (SEM) is highly desirable and may eventually be essential.

Lichen taxonomy depends heavily on determination of lichen chemistry because lichens produce an abundance of unusual and distinctive secondary metabolites. Some of these metabolites may be determined by relatively simple spot tests or microcrustal techniques, but increasingly, lichen chemistry is determined by thin-layer chromatography (TLC). The methods of TLC to identify specific lichen metabolite products (lichen chemistry) should be considered necessary to obtain a species determination. Standard techniques described by Culberson (1972), Culberson and Johnson (1982), and White and James (1975) are recommended. Other published methods of TLC may be substituted at the discretion of the principal investigator.

High-performance liquid chromatography (HPLC) may be used to support or better define the TLC results. An investigator proposing a lichen floristic study should be prepared to demonstrate familiarity with these techniques and access to appropriate equipment.

Use of an External Authority

There are two instances in which it is advisable to send voucher specimens to an expert other than the original investigator. One instance, mentioned above, is when there is a colleague specializing in species determinations in a difficult group. The second instance is when there is an actual or anticipated legal challenge to the identity of a species. Such an instance is likely to arise in cases where the species in question has been recognized as rare and/or endangered, but may also occur when a species has been established as having a specific sensitivity to a known pollutant. In any such case, discretion suggests the use of an external authority to substantiate the identification given by the original investigator.

New Species

During lichen floristic surveys of previously unexplored areas, it is inevitable that some lichens will be found that are initially perceived as new species. Usually such collections can be assigned to a genus and treated in reports as a numbered "taxonomic species" of that genus, *e.g.*, *Lecanora* taxonomic species 1. Ultimately, these preliminary identifications are resolved in one of two ways. Either the species is finally recognized as belonging to some described but obscure species, or it is described and published as a new species. In some

instances, publication of the new species may be undertaken by the original investigator, but in many instances it will be described by a specialist in that group of lichens. In sending material to an expert to be described as new, it should be understood that the type specimens and/or an adequate paratype will be returned to join the other voucher specimens. The author of the new species should provide the management agency and the herbarium housing the voucher specimens with reprints of the original description.

Labels

Voucher specimens must be accompanied by adequate labels. Ideally, labels are made from archival-quality paper and printed with ink that does not fade or chip off. If computers are used to print labels, it is important to find papers, inks, and printers that satisfy these requirements.

Investigators and herbaria will differ to some degree both on the information to be printed on labels and in the arrangement, but see Hale (1979) for a commonly used format. Measurements should be metric. The following elements should be considered in the design of labels:

1. State, county, governmental unit.
2. Name of lichen - the scientific (Latin) name of the *genus* and *species* followed by the appropriate authorities according to international convention.
3. Locality:
   a. Site specific name, *e.g.*, Smith Canyon
   b. Latitude and longitude/ or township, range and section/ or UTM reference/ or GPS notation.
4. Ecological site characterization:
   a. Physical features: slope, aspect, elevation, rock or soil type, etc.
   b. Biotic features: vascular plant vegetation
   c. Substratum on which the specimen was growing.
5. Name of collector(s).
   a. Date of collection
   b. Collector's number.
6. Name of determiner.

QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

The following are QA/QC concerns with respect to specimens collected in a floristic study:

1. Maintain adequate control associating a specimen with its field data.
2. Ensure quality storage for specimens from field to ultimate herbarium. Storage should be dry, cool, pest-free.
3. Ensure adequate care in species determination by use of all appropriate techniques and references.
4. When appropriate, submit samples for verification to external experts.
5. Deposit a complete set of vouchers for all species to an established institutional herbarium. Where possible, back up this set with a duplicate set in a regional herbarium.

REPORTS

Preparation and submission of effective written reports is central to the establishment of meaningful lichen biomonitoring programs. This section contains specific suggestions on format and preparation of written reports. Subsequent publication in peer reviewed scientific journals is strongly encouraged.

Format

Reports should follow a consistent format to facilitate review and analysis. Using the following outline will insure that the information essential for establishing a lichen biomonitoring baseline is always included:

1. **Abstract** - a brief overview of the project.
2. **Introduction** - a summary of current lichen air quality biomonitoring literature with any specific information about previous lichen surveys in the study area.
3. **Methods** - a description with adequate references of field and laboratory methods used.
4. **Site description** - a brief description of the major habitat types within the study area together with detailed information about

each specific collection locality. A general map of the study area, showing the location of each collection site, should also be included.

5. **Results** - the species list will comprise the major portion of this section. The following information should be included for each species:
   A. Genus, species, and authorities for each taxon.
   B. Collection site(s).
   C. Growth form.
   D. Substrate information.
   E. Relative abundance information (*i.e.*, rare, common, abundant, etc.).
   F. Pollution sensitivity status with appropriate documentation.
   G. Deposition of specimens (*i.e.*, name(s) of herbaria and collection number(s).
   H. Notation of species found in earlier studies.
   I. General comments concerning disjunct distribution patterns, new distributional records, unusual substrates, modified growth forms, unusual reproductive patterns, incidence of thallus bleaching or necrosis, or unusual nomenclatural considerations.

   Expanded reports may include elemental analyses and community data, such as transect surveys. (See Chapter 7 for details on elemental analyses and Chapter 4 for community analyses.) Taxonomic keys and color photographs of sensitive indicator species may also be included.

6. **Conclusions and recommendations** - This section of the report is diagnostic in nature and should be based on a critical interpretation of the data contained in the results section. This section essentially defines the baseline condition. An appropriate combination of the following may be included:
   A. Comments on lichen species diversity
   B. Comments on the general health of the lichen community
   C. Observations on the occurrence and abundance of sexual and asexual reproductive structures
   D. Comments on rare and/or endangered species
   E. Comments on "missing" species
   F. Comments on the occurrence and relative abundance of pollution sensitive species
   G. Comments on growth form frequency and distribution patterns
   H. Comments on significant substrate considerations
   I. Comparison of historical information or data from comparable sites with current data
   J. Evaluation of ecological data
   K. Assessment of pollutant concentrations in lichens

   This list is neither comprehensive nor absolute; other appropriate items may be included depending upon the specific characteristics of a study site and/or lichen flora.

Review of Reports

All technical reports should be evaluated by two peer reviewers who have appropriate technical expertise and familiarity with the habitats and flora of the study area. These individuals could be chosen from the national advisory board proposed below. Reviewers should be given copies of the project contract and any approved modifications. The primary responsibility of the reviewers is to determine whether appropriate techniques and procedures were followed throughout the project. They should also comment on the general presentation and organization of the report as well as offer any new or unique observations.

DATABASES

At the simplest level, a floristic database conveys what plants grow where. More complex floristic databases may encompass all data about the plants that grow in specific sites. When a written flora, or manual, is produced, other types of floristic data, morphological and chemical, etc., that make identification possible are also important.

The term database refers not only to the above types of data but also to the form in which they are gathered and stored. The simplest floristic database could be a field notebook containing plant names and

collection information that is associated with each name. With the advent of readily available computers and sophisticated database software, the possibilities for storing and presenting floristic data are almost limitless. However, limitless possibilities and numerous, different computer systems require the standardization of data collection in order to facilitate transfer and exchange of data. Taxonomists and others interested in floristic and associated data are developing such standards, including minimum requirements, for data collected for floristic, monographic, and collections management purposes. Standardization makes it possible to transfer data between databases made on different hardware and software. Minimum standards ensure adequate data collection, and flexibility in database design permits special-purpose data collection while retaining the minimum standards agreed upon for all floristic work.

Minimum standards for floristic data should include the names of plants and the authors of those names, as well as information on the localities in which the plants are found. These data can be placed in two general categories: taxon-related and specimen-related. Taxon-related data that must be included in floras are scientific names at the rank of genus and below as well as the authors of these names. Required specimen-related data include the information that is traditionally included on specimen labels (scientific name and authority, locality of collection, collector, collection number, collection date). With these data, it is possible to produce the simplest kind of flora -- a list of what plants grow where.

Possible standards for data collection for lichen floristic studies may include:

Names:

- Genus
- Species
- Authority(ies)
- Infraspecific rank (if appropriate)
- Infraspecific name
- Authority(ies)

Specimens:

- Genus
- Species
- Authority
- Infraspecific rank (if appropriate)
- Infraspecific name
- Authority
- State
- County
- Land management unit
- Physiographic unit
- Easily mappable locality
- Specific locality
- Latitude
- Longitude
- UTM grid or GPS notation
- Degree of accuracy of mapping points
- General habitat
- Specific habitat
- Substratum
- Elevation (meters)
- Collector(s)
- Collection number
- Date of collection
- Determiner (if not collector)
- Herbarium where deposited

Recently, computers have been used to store, manipulate, and report pertinent data. As can be seen from the list of possible data standards for names and for specimens, one item is common to both sets of data: scientific names. This common element relates the two data sets, since the two data files share common fields. With the advent of sophisticated relational databases, it is possible to relate numerous fields, which simplifies data entry but also enables complex analyses and reports.

When different names have been used for the same organism in the area covered by the flora, it is necessary to keep track of this information by listing the synonyms. An accepted name, usually taken from an accepted list such as the Checklist of North American Lichens (Egan 1987), is used in floras,

with synonyms used in the flora area listed also. Other information that pertains to genera or species are often also available (family, growth form, etc.).

NATIONAL ADVISORY BOARD

The Floristics Group at the 1991 workshop recommends that a National Advisory Board be established to advise federal and other agencies on all lichen biomonitoring projects, including floristic studies. The functions of the Advisory Board should be:

1) To provide technical and protocol advice on biomonitoring projects.
2) To provide information concerning qualified personnel for specific projects.
3) To provide QA/QC control and help ensure legally defensible work, especially with regard to the Clean Air Act (CAA) and Prevention of Significant Deterioration (PSD).
4) To provide liaison among lichenologists.
5) To recommend research priorities. It is suggested that the Advisory Board be composed of five lichenologists representing different regions of the United States and various areas of expertise, plus one representative from each appropriate federal agency. Suggested terms are three to five years, with staggered appointments and no consecutive terms.

In promoting liaisons, the National Advisory Board could also be instrumental in coordinating efforts from various parts of the country that will culminate in the production of a national lichen flora. This would include cooperative work among lichen herbaria.

It was suggested that federal agencies arrange agreements with and retain experts who may be called upon for completing assignments and needed tasks such as identifications, verifications, surveys, and other lichen-related work.

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White, F.J. and P.W. James. 1985. A new guide to microchemical techniques for the identification of lichen substances. British Lichen Society Bulletin 57: 1-41.

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Chapter 2 Appendix

Some important floristic treatments for North America

General References:

Fink, B. 1935. The Lichen Flora of the United States. Ann Arbor. (Largely out-of-date for most genera, but still the only source of information for a number of genera.)

Hale, M.E., Jr. 1979. How to Know the Lichens. Dubuque. (Limited to selected macrolichens for the lower 48 states, but contains many useful illustrations.)

For the American Arctic:

Thomson, J.W. 1984. American Arctic Lichens I. The Macrolichens. New York.

For the West Coast:

Hale, M.E., Jr. and M. Cole. 1988. Lichens of California. California Natural History Guides. 54: 1-254. (Limited to the macrolichens.)

For the North Central Region:

Wetmore, C.M. 1968. The Lichens of the Black Hills of South Dakota and Wyoming. Publication Museum. Michigan State University Biology Series 1:369-452. (Contains keys to all groups.)

For the Northeast:

Brodo, I.M. 1965. The Lichens of Long Island, New York: A Vegetational and Floristic Analysis. New York State Museum Science Service Bulletin 410: 1-330. (Contains keys to all groups.)

Brodo, I.M. 1988. Lichens of the Ottawa Region. Ottawa Field-Naturalists Club Special Publication 3: 1-115. (Covers all species; obtainable from the National Museum of Natural Sciences, Ottawa.)

Gowan, S.P. and I.M. Brodo. The lichens of Fundy National Park, New Brunswick, Canada. Bryologist 91: 255-325. (Obtainable as a separate reprint from *The Bryologist.*)

For the Southeast:

Harris, R.C. 1990. Some Florida Lichens. New York. (Published by the author, but available through the New York Botanical Garden. It contains valuable keys for all groups.)

Moore, B.J. 1968. The macrolichen flora of Florida. Bryologist 71: 161-266.

Site Characterization

Ken Stolte, Sherry Pittam, Roger Rosentreter

The growth, reproduction, and survival of lichens are influenced by the physical and biological environments in which they are found. The primary physical influences are insolation, temperature, moisture, chemistry, soils, and wind. The biological factors that most affect lichens include the moisture and chemical characteristics of host substrates and biological modification of the physical environment. To differentiate the effects of air pollutants from other physical and biological environmental factors, it is necessary to quantify, through measurement or estimation, the environs in which the lichen species are found. This chapter provides detailed methods for describing the environments in which lichen studies are conducted.

INTRODUCTION

The purpose of this chapter is to identify, quantify, and document the physical, chemical, and biological factors at sites where lichen studies are conducted. These factors can be chronic, influencing physiological processes, or acute, affecting immediate survivability. They have a substanal influence on the condition, growth, and survival of lichens. Since lichens lack stomata and have no control over gas exchange, they are responsive to changes in their physical and chemical environments. There is also some evidence that lichens respond to changes in their host substrates (Prussia and Killingbeck, 1991).

The goal of evaluating site conditions is to quantify biotic and abiotic factors so that changes in lichen species and communities due to air pollution can be reasonably separated from other environmental causes. We describe physical and chemical variables that should be measured or estimated at lichen study sites.

The physical factors controlling lichen growth and composition of lichen communities include geology, air, and water (Oosting, 1956; Ferry et al., 1973). Biological factors include host species, canopy cover, and herbivores (Skye, 1968). These factors are important at both macroenvironmental and microenvironmental scales. The macroenvironment refers to the climate and soil characteristics of a region which influence the nature of the vegetation (Bailey, 1991). The microenvironment is generally considered to be the environment immediately adjacent to the earth's surface, i.e., the zone below the height of 4.5 feet, the height of standard weather instruments. Microenvironments for lichens are often highly specialized habitats such as rock crevices, tree trunk surfaces and crevices, and anthropogenic materials.

The macroenvironmental factors most likely to affect lichens are:

- insolation (intensity and quality)
- wind (speed, frequency)
- temperature (maximum, minimum, seasonality)
- moisture (rain, snow, fog, relative humidity)
- atmospheric chemistry (nutrients, toxins, acidity)

The microenvironmental factors that may affect lichen condition are:

- host substrate
  - topography
  - chemistry
  - stability
  - moisture
  - type
    - rock
    - tree bark
    - mosses
    - herbs
    - anthropogenic materials

- soil
  - pedogenic (structure and chemistry of soil)
  - edaphic (plant and soil interactions)
- atmospheric (regional gases and gases whose concentration changes within a plant canopy, e.g., carbon dioxide, ozone)

INSOLATION

Insolation refers to the radiant solar energy that reaches earth. Of particular interest is the portion of the electromagnetic spectrum available to plants for photosynthesis, between 460 and 640 nanometers. These wavelengths of light are called photosynthetically active radiation (PAR). The insolation reaching the earth is affected by substances in the atmosphere and the angle of incidence with the earth. Consequently, insolation varies with air quality, latitude, elevation, seasons, and topography. Tree canopies alter insolation and can reduce the intensity of PAR by 85% or more. Ultraviolet light may have a deleterious effect on some lichen species, and becomes increasingly important at higher elevations and northern latitudes.

Site Measurement of Insolation

Precise quantification of annual PAR at a site involves multiple samples with an integrated radiometer throughout the year (Isebrands et al. 1992). This is usually not feasible, and in most cases even a one-shot measurement of transmitted PAR is difficult. The intensity and duration of the insolation can be quantified into broad classes of exposure based on factors that decrease maximum insolation. The primary physical and biological site factors needed to measure or estimate insolation are:

Physical

- latitude
- aspect
- slope
- elevation
- microrelief

Biological

- plant community type
  - forest type (density of crowns)
  - understory vascular and non-vascular flora
- canopy strata (layers and total height)
- canopy opening (percent)

TEMPERATURE

Ambient temperature ranges have a pronounced influence on the distribution of all plants. Temperature effects are demonstrated by vegetation changes with elevation on mountain slopes; decreases of approximately 1.5° C for every 1000 feet of increase in elevation have a marked impact on the distribution of vegetation communities. Temperatures below freezing can result in the formation of ice crystals within cells and subsequent cell plasmolysis. Extremely high temperatures (50° C) can lead to desiccation of plant tissue and protein denaturation. Lichen species distribution is usually controlled by temperature. Temperature extremes may affect either the algal component or the fungal component or both equally (Lange 1953, in Skye 1968). There is usually a good correlation between habitat-type for lichen species and their heat tolerance (Levitt 1980a).

Site Measurement of Temperature

It is relatively easy to quantify the maximum and minimum temperatures at a site for any time interval, and for some lichen transplant studies quantifying temperatures at the site may be important in the interpretation of the results. For example, death of lichen transplants at new sites may be due to differences in temperature ranges between sites, rather than to differences in air quality. Daily extremes of temperature and relative humidity can be obtained with relatively low-maintenance hygrothermographs or maximum-minimum thermometers. For floral, elemental, or community studies involving surveys or plots, where many sites are visited briefly, it is not possible to measure temperature in any meaningful way. Relative temperature differences among sites and microsites can be estimated from the following site characteristics:

Physical

- latitude and longitude
- elevation
- aspect
- topographic position
- snow line (depth; duration of cover)

Biological

- canopy cover (percent)

- plant communities
  - forest type
  - understory vascular flora

MOISTURE

Moisture has a substantial effect on the vigor of lichens and the diversity of lichen communities. Lichens can photosynthesize and respire only when hydrated to a species-specific threshold, and they tend to reflect the moisture regime of their atmospheric and substrate environments (Farrar 1973, in Ferry et al. 1973). Moisture assimilated by lichens comes from the atmosphere in the form of rain, relative humidity, fog, dew, and snow melt, and from the host substrate. Lichens can persist for long periods when dried to moisture levels as low as only 1 - 15% of their dry weight.

Lichens are adapted to different moisture zones within habitats, and their physiological rates reflect their moisture zones. Most lichens that have a high tolerance for heat also have a high tolerance to drought. They are usually, but not always, characteristic of hot, arid regions (Levitt 1980b). Some shade-tolerant lichens have similar adaptations to high heat and drought. Lichens appear to be more tolerant of dry air pollutants when they are not hydrated.

Site Measurement of Moisture

Moisture can be measured directly at lichen sites where detailed quantification is desired, as in transplant and fumigation studies. Moisture availability can be estimated from the following site variables:

Physical

- elevation
- topographic position
- snow depth and duration
- aspect
- slope
- soil texture (clay vs sand)
- wind (speed and relative humidity)

Biological

- vascular plants
  - flora
  - general foliar morphology (leaf size)
- non-vascular plants
  - mosses

ATMOSPHERIC ELEMENTS

When the air, precipitation, or substrate is contaminated with anthropogenic chemicals, lichens will be contaminated somewhat proportionally with the same chemicals, especially the foliose and fruticose forms. The degree of contamination depends largely on the hydration state of the lichens.

Toxic elements can affect lichens by direct toxicity (e.g., SO^2), or more indirectly through accumulation (e.g., toxic metals). Metals accumulate in lichens and then become a source of additional metal input to ecosystems as the lichens decompose. Toxic elements known or suspected to negatively affect lichens include sulfur dioxide, hydrogen fluoride, nitrogen oxides, metals (e.g. Cu, Ni, As, Cd, Pb), ozone, and radionuclides. (See Chapters 5 and 7 for details.)

Site Measurement of Atmospheric Elements

Site measurement of most atmospheric elements is difficult and costly, and in most cases is limited to specific toxic elements of concern at intensive study sites. Toxic elements may be derived from anthropogenic sources (e.g., power plants, metal refineries), or from natural sources, particularly soil particles. In some cases toxic elements, sulfur oxides, nitrogen oxides, fluorides, and ozone can be measured directly with passive pollutant monitors [e.g. sulfation and nitration plates (Noel et al. 1989); or passive ozone monitors (Grosjean and Hisham 1992)]. In the absence of active or passive pollutant monitors, measurement of the following site characteristics and chemistry will give some information on nutrient and toxic element deposition at a site:

Physical

- parent material chemistry
- soil chemistry (horizon gradients)
- type and nearness of pollution source(s)
- air and precipitation chemistry
- snow melt chemistry

Biological

- symptoms in vascular and non-vascular plants
- chemistry of vascular and non-vascular plants

SOILS

Soils are important components of ecosystems and have both direct and indirect influences on lichen communities. The physical and chemical characteristics of soils are the results of both the inorganic parent material and associated biological communities; consistent relationships exist between major vegetation groups (tundra, desert, forest), major climatic types (wet, semi-arid), and major soil groups.

Some lichens grow on soils, absorbing soil moisture and nutrients, toxins, and other chemicals directly. Lichens growing on vascular plants may also be affected by soil chemistry as chemicals are absorbed by plants and leached out through bark or leaves. Even characteristics such as soil texture and color may affect lichens through wind-blown particle abrasion, water capacities, reflection of PAR, or absorption of heat. Soil characteristics help quantify the ecology of a site and reflect past climate and biota as well as anthropogenic pollution.

Site Measurement of Soils

Physical

- chemistry (pH, toxins, nutrients)
- general particle size
- color
- available water capacity
- bareness (percent rock and plant cover)

Biological

- ground cover
- duff depth

CASE STUDY EXAMPLE OF MEASUREMENT OF SITE CHARACTERISTICS

Numerous site variables were measured or estimated by Skye (1968) to evaluate the effects of air pollutants from Stockholm, Sweden on lichen species and communities. Host substrate was considered very important (Skye 1968). The following site data were recorded:

- age of host plant
- condition of the tree crown
- condition of the bole
  - growth habit
  - length of trunk
  - bark appearance
- host exposure
  - location (isolated, in rows, dense stand, etc.)
  - distance from host to nearest tree, building, etc.
  - distance to nearest bush
  - percent vegetation cover

DATA FIELDS AND DATA SHEETS

To aid in analysis of site characteristics, we suggest the following data-fields, which produce two forms: one to be addressed by the agency or group supporting the lichen study (Part 1) and the second to be filled out by the lichen expert, the principal investigator (PI) conducting the study (Part 2). Part 1 details the background information on the general physical, chemical, and biological factors that should be considered prior to conducting the field work. Part 2 details the information that should be collected at each of the sites during field work. The format for the field data collection form (Part 2) comes from the ECODATA procedures developed by the USFS, Northern Region (Jensen et. al. 1992). It has been modified and expanded in this document for lichen field studies.

The information in Part 1 is important for choosing study sites and determining associated work constraints and potential problems. The information from Part 2 is useful for analysis and describes the present condition of the site and the distribution patterns of lichen species. It records current conditions for reference in future studies, particularly long-term monitoring for community or elemental analysis. Part 2 can help differentiate the effects of air pollution on lichens from the effects of natural or anthropogenic influence.

Part 1

This information may be provided to the Principal Investigator by the contracting agency, and should be included in requests for bids. It is intended to give a general overview of the area where the lichen studies are to be conducted and to aid the PI in calculating necessary resources needed to conduct the study, including budgets, personnel, logistics, and safety.

Part 2

We recommend that the Principal Investigator provide the following information for each site where lichen studies are conducted. It is left up to the PI to determine which of the following 106 data fields are most important or relevant for a particular study. These data fields were modified from the USDA Forest Service's ECODATA method (Jensen et al, U.S. Forest Service, Northern Region, 1992).

PART I
MANAGEMENT UNIT

A. Heading
   1. Unit name
   2. Managing agency
   3. Purpose of study

B. Geographic location of study
   1. State
   2. County
   3. Approximate boundary of unit by Lat./Long., and description (if appropriate).

C. Physical environmental factors
   1. Geology/parent material
   2. Soils (short description or soils map)
   3. Landform(s)/topography
      a. Terrestrial
      b. Aquatic
   4. General climatic factors
      a. Annual precipitation
      b. Length of growing season
      c. Range of elevations
      d. Mean annual T°, humidity, insolation, etc.
   5. Air chemistry
      a. Types of known pollution and sources
      b. Location of nearest mechanical air quality monitoring station and type of monitor(s)
      c. Locations of known pollution sources relativ to site

D. Biological factors
   1. Biome type(s)
   2. Significant disturbance history
      a. Natural
      b. Anthropogenic
   3. Unique plant/animal species

E. Any other unique features

PART II
LICHEN SITE-SPECIFIC DATA

Fields 1-8 - Key ID - Record Identifier

Field 1 - AG - Agency (2 characters)
Enter one of the following codes to describe the agency conducting the study:

CODE	DESCRIPTION
AR	Agricultural Research Station
BI	Bureau of Indian Affairs
BL	USDI, Bureau of Land Management
CH	Champion International Lands
FG	State Fish and Game Department
FA	USDA, Forest Service (Air Resource Manag
FR	USDA, Forest Service Research Station
FS	USDA, Forest Service (ECODATA Plot)
FW	USDI, Fish and Wildlife Service
NC	The Nature Conservancy
NP	USDI, National Park Service
PV	Other private lands
SC	USDA, Soil Conservation Service
SL	Department of State Lands
UR	University Research
OT	Other (explain in Comments, Form GF)

For additional codes coordinate with the USDA, Forest Service (Northern Region, Regional Office, Range, Air, Watershed and Ecology Staff Unit).

Field 2 - St - State (2 characters)

Enter the two-character Postal Service abbreviation for the state in which the plot is located (e.g., Montana = **M T**). Use **U N** when the state is unknown. For countries other than the U.S., enter a two-digit country code and explain code in Comments.

Field 3 - NF - National Forest (2 numeric)
Enter the number corresponding to the U.S. Forest Service's National Forest being sampled (See USDA Forest Service for National Forest codes). Users may develop their own coding conventions for other land units, e.g., National Parks, in this subfield - explain in Comments.

Field 4 - Yr - Year (2 numeric)

Enter the last two digits of the calendar year (e.g., for 1990, enter **9 0**).

Field 5 - Plt - Plot Number (3 numeric)

Enter sequential plot numbers for each examiner through the calendar year, up to 999. If one examiner samples more than 999 plots in one year, a new examiner code should be assigned and the individual encouraged to take a vacation.

Example - Fields 1-5: A USFS plot (AG=FS) in the Northern Region of Montana (St=MT), on the Helena NF (NF=12), during 1986, by Ilike Lichens, which is the 45th plot for I. Lichens in calendar year 1986, would be entered as:

1 Ag F S 2 St M T 3 NF 1 2 4 Yr 8 6
5 Plt 0 4 5

Field 6 - Mo - Month (2 numeric)

Enter one of the following codes to describe the calendar month of sampling:

CODE	MONTH
01	January
02	February
03	March
04	April
05	May
06	June
07	July
08	August
09	September
10	October
11	November
12	December

Field 7 - Day (2 numeric)

Enter the calendar day of sampling.

Field 8 - Name - Name of Examiner (20 characters)

Enter the first initial followed by a period (.), then the last name of the person doing the majority of the species identification, data measurements, and evaluations (e.g., I.LICHENS).

SAMPLE SYSTEM DATA

Fields 9-13 - Sample Forms - Codes for Sample Forms Collected at a Plot (Up to five 2-character fields).

Various types of sampling procedures may be used to describe characteristics of the site being sampled (e.g., community grid, lichen line intercept cover). Identify the different types of sampling methods used at the site by entering up to 5 of the following codes (**Note**: The coding conventions used below are suggested for some of the more common lichen studies; develop additional codes as needed and explain in Comments):

CODE	DESCRIPTION
LF	Lichen floristics
LC	Lichen community
LE	Lichen elements
LT	Lichen transplant
LG	Lichen gradient
LP	Lichen plots
LB	Lichen biomonitoring garden
LX	Lichen fumigation exposures

Example: A macroplot on which lichen community and lichen chemistry analysis were done, would be entered as:

Sample Forms: 9 L C 10 L E 11 __ __ 12 __ __
13 __ __

The above indicates that Lichen Community analysis and samples of lichens for Lichen Elements (chemistry) were conducted at the site.

Field 14 - Unit - Unit of Measurement (1 character)
Enter one of the following codes to describe if the units of measurement entered are English (E) or Metric (M). Either type of measurement may be used at a plot; however, metric is preferred.

Note: Lichen methods, whenever possible, will use metric (M) units for all standard inventory and monitoring projects.

Field 15 - Permanent Plot ID (15 characters)
Enter the Key ID recorded in Fields 1-7 if this plot is to be used as a permanent plot. If the plot sampled is a repeat measurement of a permanent plot, enter the Key ID corresponding to the first time the plot was sampled.

Required for permanent plots only.

Field 16 - PRI - Plot Remeasurement Interval (2 numeric)
For a permanent plot, enter the number of years since the last measurement. For example, 0 0 indicates that the plot is a permanent plot and was not yet remeasured; 0 1 means the plot was measured last year; 0 2 means it was measured 2 years ago; 0 9 means it was measured 9 years ago, and - 1 means the remeasurement interval since the last measurement is unknown.

Required for permanent plots only.

Field 17 - Comparison Plot ID (15 characters)
If appropriate, enter the Key ID for a plot to be used as a comparison with this plot (e.g., lichen transplant study).

Optional.

Field 18 - Potential Vegetation Form - Potential Vegetation Formation (2 characters)
Enter one of the following codes to describe the potential vegetation formation of your plot.

CODE	DESCRIPTION
AQ	Aquatic
NV	Non-vegetated terrestrial (sand dunes, scree, rock)
CF	Coniferous upland forest
CW	Conifer-dominated wetland
BF	Broadleaf upland forest
BW	Broadleaf-dominated wetland
SW	Shrub-dominated alpine
HW	Predom. herbaeous (graminoid, forb, fern) wetland
SA	Shrub-dominated alpine
SU	Shrub-dominated upland
HA	Herbaceous dominated alpine
HU	Herbaceous dominated upland
ML	Moss/Lichen-dominated
OT	Other Type-specify in comments
X	Unable to assess

EXISTING VEGETATION CLASSIFICATION DATA

Fields 19 - 22 - Existing Vegetation Strata

Field 19 - LF - Dominant Live Life Form (1 character)
Enter one of the following codes to describe the dominant (based on canopy volume) live life-form present on the plot. (Note: Dominant life form = life form with the greatest canopy volume; i.e., height x cover.)

CODE	DESCRIPTION
A	Aquatic species dominate
B	Broadleaf trees dominate
C	Conifers dominate
H	Herbaceous species dominate (graminoid/forb/fern mixture)
K	Krumholz
M	Moss or lichens dominate
P	Agricultural cropland
S	Shrubs dominate
O	Other (explain in Comments)
X	Unable to assess

Field 20 - LSC - Live Life Form Size Class (2 characters)

Enter one of the following codes to describe the size class of the dominant live life form specified in Field 19. (Note: Size class is selected to describe the dominant layer component of a life form; i.e., the size class with maximum canopy volume.)

If the dominant live life form is shrubs (S) use:

CODE	DESCRIPTION
LS	Low (< 2.5 ft average height) dominated
MS	Medium (2.5 to < 6.5 ft average height) dominated
TS	Tall (6.5+ ft) dominated

If the dominant live life form is conifer (C) or broadleaf (B) trees, use:

CODE	DESCRIPTION
SE	Seedling (< 1.0 in. DBH) dominated
SA	Sapling (1.0 - 4.9 in. DBH) dominated
PT	Pole tree (5.0 - 8.9 in. DBH) dominated
MT	Medium tree (9.0 - 20.9 in. DBH) dominated
LT	Large tree (21.0 - 32.9 in. DBH) dominated
VL	Very large tree (33.0+ in. DBH) dominated

For other life form types, enter **N** (i.e., not applicable). If you are unable to make this assessment, enter an **X** in this field.

Field 21 - DSC - Dead Life Form Size Class (2 characters)

Enter one of the following codes to describe the size class of the dominant dead life form on the plot (i.e., trees or shrubs). If the dominant dead life form is shrubs, the size class chosen should have 1% canopy cover of dead stems and branches. If the dominant dead life form is trees, the size class chosen should have 1% canopy cover of dead stems and branches.

If the dominant dead life form is shrubs (S), use:

CODE	DESCRIPTION
LS	Low shrub (<2.5 ft average height) dominated
MS	Medium shrub (2.5 to < 6.5 ft average height)
TS	Tall shrub (6.5+ ft) dominated

If the dominant dead life form is conifer (C) or broadleaf (B) trees, use:

CODE	DESCRIPTION
SE	Seedling (1.0 in. DBH) dominated
SA	Sapling (1.0 - 4.9 in DBH) dominated
PT	Pole (5.0 - 8.9 in DBH) dominated
MT	Medium tree (9.0 - 20.9 in. DBH) dominated
LT	Large tree (21.0 - 32.9 in. DBH) dominated
VL	Very large tree (33.0+ in. DBH) dominated

For other types or when no dead life form exists, enter **N** (i.e., not applicable). If you are unable to make this assessment, enter an **X** in this field.

Field 22 - CC - Live Canopy Cover Class (1 character)

Enter one of the following codes to describe canopy cover (CC) of the dominant live vegetation life form identified in Field 20:

CODE	DESCRIPTION
N	No cover (≤ 9% CC)
L	Low cover (10-39% CC)
M	Moderate cover (40-69% CC)
H	High cover (70-100% CC)
X	Unable to assess

Fields 23 - 28 - Upper, Middle, and Lower Layer Vegetation Zones

Note: Fields 23 through 28 are used to describe characteristics of the upper layer (above 6.5 ft.), middle layer (2.5-6.5 ft.), and lower layer (below 2.5 ft.) vegetation zones. A minimum of 5 percent canopy cover must be present within a layer to distinguish it in the database. Dominant and codominant species must each have at least 5 percent canopy cover within a layer to distinguish them in the database. If no single species has at least 5 percent cover within a layer (yet collectively the layer has 5 percent cover), enter a representative code to describe the dominant life form within that layer (e.g., **T R E E S H R U B**, **G R A S S** , **F O R B**). If a layer has no vegetation, enter **N P** to indicate "not present."

Field 23 - UL Dom Spl - Dominant Species in the Upper Layer (8 characters)

Enter an eight-character code to describe the dominant species in the upper layer (above 6.5 ft. tall), if present. If no upper layer exists, enter **N P**. If you are unable to make this assessment, enter **X.**

Field 24 - UL Dom Sp2 - Codominant Species in the Upper Layer (8 characters)

Enter an eight-character code to describe the codominant species in the upper layer (above 6.5 ft. tall), if present. If no upper layer exists or exists with no codominant species, enter **N P**. If you are unable to make this assessment, enter **X.**

Field 25 - ML Dom Spl - Dominant Species in the Middle Layer (8 characters)

Enter an eight-character code to describe the dominant species in the middle layer (2.5 - 6.5 ft. tall), if present. If no middle layer exists, enter **N P**. If you are unable to make this assessment, enter **X.**

Field 26 - ML Dom Sp2 - Codominant Species in the Middle Layer (8 characters)

Enter an eight-character code to describe the codominant species in the middle layer (2.5 - 6.5 ft in height), if present. If no middle layer exists or exists with no codominant species, enter **N P.** If you are unable to make this assessment, enter **X.**

Field 27 - LL Dom Spl - Dominant Species in the Lower Layer (8 character)

Enter an eight-character code to describe the dominant species in the lower layer (below 2.5 ft. tall), if present. If no lower layer exists, enter **N P.** If you are unable to make this assessment, enter **X.**

Field 28 - LL Dom Sp2 - Codominant Species in the Lower Layer (8 characters)

Enter an eight-character code to describe the codominant species in the lower layer (below 2.5 ft. tall), if present. If no lower layer exists or exists with no codominant species, enter **N P.** If you are unable to make this assessment, enter **X.**

SITE DATA

Field 29 - SpFea - Special Feature Information (2 characters)

Enter one of the following special feature codes to describe the dominant environment of the plot. These codes are hierarchical in design; consequently, you may choose from the more generic codes (e.g., **A C**, **N S**, **S E**)or from the more detailed codes (e.g., **A N**, **S W**, **B E**).

CODE	DESCRIPTION
N	Not Applicable
AC	Avalanche chute
AN	With non-scoured surface (soil not being eroded)
AS	With scoured surface (soil eroded, rocks exposed)
CV	Cave
CL	Cropland
CD	Dryland
CI	Irrigated crops
CR	Scree (sheet of stones/rocky debris mantling slope)
TA	Talus (rock fragments derived from and positioned at the base of a cliff)
SC	Streamside community
SB	Stream bar community
NS	Nonstream riparian communities
SW	Shrub Wetland (e.g., willows and birches)
WM	Wet meadow dominated by graminoids (e.g., sedges, grasses)
PE	Peatland (e.g., willows and birches)
FL	Floating or quaking bog or fen mat
SA	Swale (concave or bench area with no surface water, but moist surface soil produces vegetation reflecting a moist environment)
SE	Seep and/or spring
BE	Concave bench with surface water creating moist or wet soils that support vegetation reflecting a moist site
SS	Side Slope seep (not on bench or concavity)
LA	Lakeside communities

TE	Terrace (in valley bottom)
SN	Snow catchment area (retains snow cover 2-4 weeks longer than 90% of the surrounding areas of same aspect due to deeper snow accumulation on a different physiographic location, includes nivational hollows)
CA	Cold air drainage or frost pocket
WB	Wind blasted environments where vegetation is maintained in a deformed state
UW	Upslope warm air flow; area that is in the direct path of afternoon warm upslope flow from an adjacent deep valley bottom or a thermal belt above a cold air inversion area
WU	Windswept upper slopes that do not accumulate snow and are relatively dry due to snow loss compared to adjacent areas
RI	Ribbon forest
RR	Ridgetop ribbon forest
RS	Snow-caused ribbon forest
RG	Geologically caused ribbon forest
O	Other (Explain in Comments)
X	Unable to assess

Coordinate with the USDA Forest Service for additional codes.

Fields 30-32 - Landform - Geomorphic Landform (three 2-character fields)

Enter one set of the following codes to describe the landform where the site/plot is located. The codes presented are hierarchical in that they describe general-to-specific landform settings following Holdorf and Donahue (1990). The first two-character field (i.e, Landform 1) is always recorded; descriptions of Landforms 2 and 3 are optional, yet highly recommended.

LANDFORM 1	CODE 1	LANDFORM 2	CODE 2	LANDFORM 3	CODE 3
Glaciated Mountains	**GM**	Ridgetops	RT		
		Mountain Slopes	MS	Undissected	UD
				Dissected	DI
		Cirques	CI	Walls	WA
				Floors	FL
		Trough Walls	TW	Undissected	UD
				Dissected	DI
		Trough Bottoms	TB		
		Avalanche Debris	AD		
Unglaciated Mountains	**UM**	Mountain Slopes	MS	Undissected	UD
				Dissected	DI
		Nivation Hollows	NH		
		Structurally Controlled Complexes	SC		
		Plateaus	PL		
		Structural Benches	SB		
		Dip Slopes	DS		
		Landslide Deposits	LB		
Breaklands	**BR**	Structural Breaks	SB		
		Stream Breaklands	ST	Undissected	UD
				Dissected	DI
				Drainage Heads	DH
Hills	**HI**	Rolling Uplands	RU	High Relief	HR
				Low Relief	LR
Valleys	**VA**	Moraines	MO		
		Kames, Kettles	KK		
		Stream Bottoms	SB		
		Alluvial Basins	AB		
		Fans, Toeslopes	FT		
		Terraces	TE		
Other	**O**				
Not Applicable	**N**				
Unable to Assess	**X**				

Fields 33-35 - PMat - Surficial Geology (three 2-character fields)

Enter up to three of the following codes to describe the type of parent material present at the plot. The first code is required, the second code is highly recommended, and the third code is usually specific to individual project needs. If an undefined third code is used, keep a listing of coding conventions and describe such codes in Comments.

*These types indicate situations where more than one type of parent material may be influencing plant growth. If appropriate, further specify the types of parent material present on the plot by listing appropriate rock type codes in the Rock Type 2 and 3 fields. For example, a site with volcanic ash over sandstone would be entered as: **M X/A S/S A**; a site with mixed sedimentary rocks such as limestone and dolomite would be entered as: **M S/L I/D O.**

ROCK TYPE 1	CODE 1	ROCK TYPE 2	CODE 2
SEDIMENTARY	SE	Limestone	LI
		Dolomite	DO
		Sandstone	SA
		Siltstone	SI
		Shale	SH
		Conglomerate	CO
		Other type	OT
METAMORPHIC	ME	Argillite	AR
		Siltite	SI
		Quartzite	QU
		Slate	SL
		Schist	SC
		Gneiss	GN
		Other type	OT
GNEOUS	IG	Basalt	BA
		Andesite	AN
		Diorite to gabbro	DI
		Latite	LA
		Quartz monzonite	QM
		Trachyte & syenite	TS
		Rhyolite	RH
		Granite	GR
		Welded tuff (Tufa)	WT
		Scoria (porcellanite)	SO
		Other type	OT
ALLUVIUM	AL	Gravelly alluvium	GA
		Sandy alluvium	AA
		Silty alluvium	SI
MIXED ALLUVIUM*	MA		
MIXED SEDIMENTARY*	MS		
MIXED METAMORPHIC*	MN		
MIXED IGNEOUS*	MI		
MIXED FROM MORE THAN TWO ROCK TYPES*	MX		
GLACIAL TILL*	GT		
ASH*	AS		
LOESS*	LO		
SAND*	SA		
OTHER	O	(Explain in Comments)	
NOT APPLICABLE	N		
UNABLE TO ASSESS	X		

Fields 36-37 - Pos - Plot/Site Position (two 2-character fields)

Enter up to two of the following codes to describe the position of the plot or site. The codes presented are hierarchical in that they allow general (i.e., Position 1) to specific (i.e., Position 2) descriptions of plot/site position.

PLOT POSITION 1	CODE 1	PLOT POSITION 2	CODE 2
Narrow Valley Bottom (<100 ft. wide)	NV	Stream channel	SC
		Stream bar	SB
		Levee (narrow flood plain)	LE
		Colluvial deposit (fan)	CF
		Terrace	TE
Moderate Valley Bottom (100-300 feet wide)	MV	Stream channel	SC
		Flood plain	FP
		Abandoned meander	AM
		Oxbow	OX
		Backwater slough	BS
		Terrace	TE
		Alluvial fan (toeslope)	AF
		Other type	OT
Wide Valley Bottom (>300 feet wide)	WV	Stream channel	SC
		Flood plain	FP
		Abandoned meander	AM
		Oxbow	OX
		Backwater slough	BS
		Terrace	TE
		Alluvial fan (toeslope)	AF
		Other type	OT
Alluvial Fan	AF	Lower slope (fan skirt)	LS
		Mid-slope	MS
		Upper-slope	US
Mountain Slopes	MS	Lower slope (fan skirt)	LS
		Mid-slope	MA
		Upper-slope	US
		Short slope, neither upper nor lower	SS
Benches	BE	Narrow (<100 ft wide)	NW
		Wide (>100 ft wide)	WI
Shoulders	SH	Narrow (<100 ft wide)	NW
		Wide (>100 ft wide)	WI
Ridges	RI	Narrow (<100 ft wide)	NW
		Wide (>100 ft wide)	WI
Rolling Uplands	RU	Level to rolling plains	FL
		Upland ridge	UR
		Upland swell	US
		Upland knoll	UK
Badland Breaks	BK	Upland breaks	UB
		River breaks	RB
		Plateau, mesa tops	PL
		Toe slopes, alluvial/colluvial fans	TS
Other	O	(Explain in Comments)	
Not Applicable	N		
Unable to Assess	X		

Field 38 - VerPS - Vertical Plot Shape (1 character)

Enter one of the following codes to describe the slope shape perpendicular to the contour of the slope at the plot or site:

CODE	DESCRIPTION
S	Straight or even
R	Rounded or convex
D	Depression or concave
P	Patterned (microrelief of hummock and swales within several feet)
U	Undulating pattern of one or more low relief ridges or knolls and draws within plot area
O	Other (Explain in Comments)
N	Not Applicable
X	Unable to assess

Field 39 - HorPS - Horizontal Plot Shape (1 character)

Enter one of the following codes to describe the slope shape parallel to the contour of the slope at the plot location:

CODE	DESCRIPTION
S	Straight or even
R	Rounded or convex
D	Depression or concave
P	Patterned (microrelief of hummock and swales within several feet)
U	Undulating pattern of one or more low relief ridges or knolls and draws within plot area
O	Other (Explain in Comments)
N	Not applicable
X	Unable to assess

Field 40 - Elevation - Plot Elevation (5 numeric)

Enter the elevation of the plot above Mean Sea Level in meters or feet. Use a topographic map or altimeter to estimate elevation.
Accuracy Standards - ± 100 meters

Field 41 - Aspect - Slope Aspect (3 numeric)

Enter the declination-corrected azimuth of the plot's slope aspect to the nearest degree. Enter flat areas as 0 0 0.
Accuracy Standards - ± 5 Percent.

Field 42 - Slope - Slope Percent (3 numeric)

Enter the average percent slope of the terrain on which the sample plot is located. Enter flat areas as 0 0 0.
Accuracy Standards - ± 5 Percent.

Field 43 - EroStat - Erosion Status (2 characters)

Enter the most appropriate code based on indicators of soil cover, sheet erosion, rill erosion, bank stability, gullying, etc. Instability includes situations where human activity has increased the rates of disturbances above that which would occur naturally.

CODE	DESCRIPTION
ST	Soil surface stable and no evidence of accelerated erosion.
X	Unable to assess because examiner cannot determine stability or instability compared to undisturbed conditions.
UC	Soil surface is unstable because of compact (weight per unit volume natural).
UD	Soil surface is unstable because of displacement and/or churning of the soil.

Field 44 - EroType - Erosion Type (2 characters)

Enter one of the following codes to describe the dominant erosion process present on the plot.

CODE	DESCRIPTION
N	Not Applicable
SE	Sheet erosion
RE	Rill erosion
GE	Gully erosion
DE	Deposition
WE	Wind erosion
SC	Soil creep
SL	Slump
TD	Terrace development
SD	Slide
SP	Splash erosion/soil crust
O	Other (Explain in Comments)
X	Unable to assess

Fields 45-52 - GCov - Ground Cover (eight 2-number fields)

Enter a cover class code from the list below for each of the following ground cover categories to indicate cover at the soil surface plane.
(Note: Foliar canopy cover above the soil surface plane is not considered to be ground cover.)

Field 45 - BS - Bare Soil (1/16 in. diameter soil particles).
Field 46 - Gr - Gravel (1/16 - 3 in. diameter).
Field 47 - Ro - Rock (3 in. diameter).
Field 48 - LD - Litter, Duff and Ash: charred and uncharred litter, duff and scat; litter includes freshly fallen leaves, needles, twigs, fecal material, bark, and fruits; duff is the fermentation and humus sections of the organic layer.
Field 49 - Wo - Wood: charred and uncharred dead wood that is greater than 1/4 in. diameter. This includes standing dead trees.
Field 50 - ML - Moss/lichen/fungi/algae
Field 51 - BV - Basal Vegetation: the soil surface taken up by the live basal or root crown portion of **vascular** plants; this includes live trees, *Lycopodium,* and *Selaginella.* This is not foliar cover of plants; typical values for basal plant cover range between 10 and 20 percent, e.g., 30 percent is a very high value that is rarely encountered in the Northwest U.S.

Field 52 - Wa - Water: that portion of the plot's area which is covered by standing water at the time of the sampling.

Use the following standard cover class codes to quantify ground cover by the preceding categories:

CLASS CODE	RANGE OF CLASS	CLASS MIDPOINT
00	0 cover	0
01	> 0 - < 1% cover	0.3%
03	1 - < 5% cover	3.0%
10	5 - < 15% cover	10.0%
20	15 - < 25% cover	20.0%
30	25 - < 35% cover	30.0%
40	35 - < 45% cover	40.0%
50	45 - < 55% cover	50.0%
60	55 - < 65% cover	60.0%
70	65 - < 75% cover	70.0%
80	75 - < 85% cover	80.0%
90	85 - < 95% cover	90.0%
98	95 - 100% cover	97.5%

Accuracy Standards - $\pm$ 1 Class

Note: Percentages for the eight ground cover categories should total approximately 100 percent. Since cover classes are entered, the sum of the class codes may vary from 90 to 110 percent.

Field 53 - FLC - Fuel Loading Class (2 numeric)

Fuel loading classes are from the fire behavior fuel models of Anderson (1982) and Albini (1976)(both cited in Jensen et. al. 1992). Enter the appropriate code from the following list. Classes are general and describe the fuels that will drive the fire behavior model. If you are not familiar with this system be sure to reference Anderson (1982) and obtain assistance from a qualified fire behavior specialist. If you do not have Anderson's (1982) publication or have not been trained to identify fuel loading classes, enter 0 0 in this field.

CODE	DESCRIPTION
00	Unable to assess
01	Fine, porous and continuous herbaceous fuels of grasslands, savannas, grass-tundra and grass-shrub types.
02	Fine herbaceous fuels with some litter and dead stemwood in habitat types with open shrub and forest overstories.
03	Tall, thick graminoid-dominated stands.
04	Forest or shrub stands with a continuous overstory that contain much flammable woody material.
05	Forest or shrub stands with light surface fuels and slightly flammable shrub and woody fuels.

06 Open forest with shrubs or shrubs that have moderate amounts of flammable woody material.
07 Closed forest stands and understory shrub layer with flammable materials in both layers.
08 Closed conifer stands with low flammability and a compact litter layer.
09 Closed stands of pine with a thick litter layer.
10 Closed forest types with heavy fuel loading of down woody material.
11 Light logging slash, varying in continuity.
12 Moderate, continuous logging slash.
13 Heavy, continuous logging slash.

Field 54 - FD - Average Fuel Depth (3 numeric)

Determine the average depth of all live and dead ground fuels on the plot to the nearest 1/10-foot. Fuel depth is the vertical distance from the bottom of the litter layer to the highest fuel particle (below 6.5 ft. height) that is capable of carrying a ground fire under moderate fuel moisture and wind conditions. This value is averaged over the plot (e.g., envision a sheet draped over the ground fuels and live and dead shrub/herb layer on the plot, and determine the average height of that sheet.) This calculation does not include the crowns of overstory trees or any species greater than 6.5 ft. height.

Accuracy Standards - ± 0.5-Foot.

Field 55 - Down Log Dia - Down Log Average Diameter (3 numeric)

Enter the average diameter of the down woody material. Down woody material is identified as any sound or rotten log which is distinguishable as a log, including logs not in direct contact with the ground but lying at an angle with the ground of no greater than 20 degrees. Count all such dead woody material that occurs below a height of 6.5 feet. Entries are to the nearest inch.

Accuracy Standards - ± 6 Inches.

Field 56 - Dom. Layer Ht. - Average Height of the Dominant Layer (3 numeric)

Enter the average height to the nearest foot of the dominant vegetation layer on the plot (i.e., the one with the greatest canopy volume).

Accuracy Standards - ± 5 Feet.

VEGETATION DATA

Live Trees

Field 57 - DBH - Average DBH of Live Trees (3 numeric)

Estimate (from several measurements and an ocular evaluation) the average diameter at breast height of the dominant live tree layer (i.e., the tree layer that has the greatest canopy volume).

Accuracy Standards - ± 6 Inches.

Field 58 - Height - Average Height of the Dominant Live Tree Layer (3 numeric)

Estimate the average height to the nearest foot of the dominant live tree layer (i.e. with the greatest canopy volume).

Accuracy Standards - ± 5 feet

Dead Trees

Field 59 - DBH - Average DBH of Dead Tree (3 numeric)

From several measurements and an ocular evaluation, estimate the average diameter at breast height of the upper dead tree size class layer (i.e., the size class in the tallest layer that has 1% dead stem and foliage cover).

Accuracy Standards - ± 6 Inches.

Field 60 - Height of the Dominant Dead Tree Layer (3 numeric)

Estimate the average height to the nearest 5 feet of the dominant dead tree layer (i.e., tallest layer that has 1% dead stem and foliage cover).

Accuracy Standards - ± 5 feet

Fields 61-67 - Tree Cover (seven 2-number fields)

Estimate the percent canopy cover for trees as a life form and by size class. This estimate is the horizontal percent cover of the vertical projection of trees and does not account for overlap. Do not include trees less than 6 inches tall in this estimate. The following percent cover class codes are used to record tree cover:

CLASS CODE	RANGE OF CLASS	CLASS MIDPOINT
00	0 cover	0
01	0 - < 1% cover	0.3%
03	1 - < 5% cover	3.0%
10	5 - < 15% cover	10.0%
20	15 - < 25% cover	20.0%
30	25 - < 35% cover	30.0%
40	35 - < 45% cover	40.0%
50	45 - < 55% cover	50.0%
60	55 - < 65% cover	60.0%
70	65 - < 75% cover	70.0%
80	75 - < 85% cover	80.0%
90	85 - < 95% cover	90.0%
98	95 - 100% cover	97.5%

The above cover class codes are used to categorize the following variables:

Field 61 - Tot - Total tree cover (do not include overlap)
Field 62 - Seed - Seedling size cover (≥ 6 in. - < 0.1 in. DBH)
Field 63 - Sap - Sapling size cover 90.1 - 4.9 in. DBH)
Field 64 - Pole - Pole size cover (5.0 - 8.9 in. DBH)
Field 65 - Med - Medium size cover (9.0 - 20.9 in. DBH)
Field 66 - Large - Large size cover (21.0 - 32.9 in. DBH)
Field 67 - VLarge - Very large size cover (33.0+ in. DBH)

Accuracy Standards ± 1 Cover Class.

Fields 68-71 - Shrub Cover (four 2-number fields)

Estimate the percent canopy cover for shrubs as a life form and by size class. This estimate is the horizontal percent cover of the vertical projection of shrubs and does not account for overlap. Enter the appropriate percent class using the standard cover class codes presented in Fields 61-67.

Field 68 - Tot - Total shrub cover
Field 69 - Low - Low size shrub cover (< 2.5 ft. tall)
Field 70 - Med - Medium size shrub cover (2.5 to 6.5 ft. tall)
Field 71 - Tal - Tall size shrub cover (> 6.5 ft. tall)

Accuracy Standards - ± 1 Cover Class.

Fields 72-75 - Herb Cover - Herbaceous Cover (four 2-number fields)

Enter the percent canopy cover for each of the following herbaceous components using the cover class codes presented in Field 61-67. (These estimates do not count species overlap within an herbaceous component.)

Field 72 Gram - Graminoid cover
Field 73 Forb - Forb cover
Field 74 - Fern - Fern and fern allies cover (includes *Lycopodium spp.* and *Selaginella spp.* at ≤ 6.5 ft.)
Field 75 - Moss - Mosses, lichens, fungi and other bryophytes

Accuracy Standards - ± 1 Cover Class.

DISTURBANCE DATA

Fields 76-78 - Ground Cover Disturbance: M, F, A - Ground Cover Disturbance: Mechanical, Fire, Animal (three 1-character fields)

Enter one of the following codes to describe the intensity of disturbance caused by mechanical equipment (M), fire (F), and animals (A) on the plot:

CODE	DESCRIPTION
N	No disturbance (ground cover in stable condition)
L	Low (if mechanical or animal disturbance, 5-20 percent of ground cover removed exposing bare soil and pavement; if fire, most fine fuels burned, some charring of 3+ in. fuels, woody plants scorched but not burned to the ground; uneven patch burning of duff and litter)

CODE	DESCRIPTION
M	Moderate (if mechanical or animal disturbance, 20-40 percent of ground cover removed exposing bare soil and pavement; if fire, nearly all fine fuels consumed, some consumption of 3+ in. fuels, some woody plants consumed; patch distribution of duff and litter consumption)
H	High (if mechanical or animal disturbance, 40-100 percent of ground cover removed exposing bare soil and pavement; if fire, many areas of exposed mineral soil, many woody plants consumed, most in. fuels charred and many consumed; fairly even distribution of duff and litter consumption)
X	Unable to assess.

Fields 79-84 - Animal Evidence 1, 2, 3, 4, 5, 6 - Animal Use Evidence (six 2-character fields)

Enter the following codes to describe evidence of animal use on the plot, in decreasing order of use and effect on vegetation and site characteristics. Evidence may include tracks, browse, caches, beds, wallows, scat, antler rubs, nests, dens, etc. Record up to six animal use evidence codes, in decreasing order of evidence.

CODE	DESCRIPTION
NO	No evidence found
AN	Antelope
BB	Black Bear
BE	Bear (species not known)
BS	Bighorn sheep
BV	Beaver
CA	Cattle
CO	Coyote
DE	Deer
DO	Dog
DS	Domestic sheep
EL	Elk
GB	Grizzly bear
GS	Ground squirrel
HO	Horse
HU	Human
MG	Mountain Goat
ML	Mountain Lion
MO	Moose
PG	Pocket gopher
PM	Porcupine
PW	Pileated woodpecker
RA	Rabbit
RT	Raptor
TS	Tree Squirrel
UB	Upland birds
WO	Wolf
O	Other (Explain in Comments Data)
X	Unable to assess

Consult the U.S. Forest Service for additional codes.

HOST CHARACTERISTICS

Fields 85-106: General Characteristics of Host Plant

Field 85-86 - Condition of the Tree Crowns - (two 2-number fields)

Estimate the average condition of the crowns of the host tree(s), using an estimate of the live crown ratio (0-100%) and crown density (0-100%) of the tree crowns, in 10% classes (listed below). For example, a tree with a 100% live crown ratio has a live crown extending from the top of the tree to the ground. Similarly, a tree with a 100% crown density has a completely dense crown with no sunlight visible through the crown.

Field 85 - LCR - Live Crown Ratio

Estimate the average live crown ratio of the host trees where the lichens were collected. The live crown ratio is the percentage of the tree bole that supports living branches.

Field 86 - CD - Crown Density

Estimate the average crown density of the host trees where the lichens were collected. The crown density is estimated relative to the amount of sunlight that is blocked out by the entire tree crown including the bole, branches, foliage, and reproductive structures, as outlined by the branches on the tree. Therefore, even a dead tree with no foliage has some amount of crown density, even though live crown ratio would be zero. A tree with no sunlight visible through the crown has a crown density of 100%.

Use the following percentage classes to quantify live crown ratio and crown density:

CLASS CODE	RANGE OF CLASS
00	0%
10	> 0 - 10%
20	11 - 20%

CLASS CODE	RANGE OF CLASS
30	21 - 30%
40	31 - 40%
50	41 - 50%
60	51 - 60%
70	61 - 70%
80	71 - 80%
90	81 - 90%
99	91 - 100%

Accuracy Standards - ± 1 Class

Field 87-89 - Condition of the Tree Boles

Field 87 - GH - Growth Habit - (two 2-character fields)

Growth habit refers to the general morphology of the tree trunk (bole). Two fields should be recorded describing the average condition of the tree(s) where the lichens are collected or identified - one field describes the average branching of the stem below breast height (1.47 meters); the second field describes the average inclination of the tree(s).

CODE	CLASS
	Branching:
SV	Simple, undivided trunk
DT	Divided trunk (2 or more stems)
	Inclination:
NI	No incline, ± vertical trunk
SI	Slightly inclined trunk (11 - 30 degrees)
MI	Moderately inclined trunk (31 - 60 degrees)
HI	Highly inclined trunk (61 - 90 degrees)

Field 88 - BTL - Bare Trunk Length

Estimate the length of the tree trunk in feet/meters that is bare (from the ground to the beginning of living branches) and open to colonization of lichens.

Accuracy Standards - ± 1.5 feet/0.5 meters

Field 89 - BAL - Bole Azimuth of Lichens

Record the common azimuth of the lichens found on the tree boles, in classes of 60 degrees. Record up to six classes for lichens that are colonizing the entire bole, i.e., lichens are found on all sides of the tree bole.

CODE	RANGE OF CLASS
00	No lichens on bole
06	> 0 - 60 degrees
12	61 - 120 degrees
18	121 - 180 degrees
24	181 - 240 degrees
30	240 - 300 degrees
36	301 - 360 degrees

Accuracy Standards - ± 1 Class

Fields 90-93 - BHL - Bole Height of Lichens

Record the height of bole that contains lichens (all species = Field 90), whether the lichens were collected or identified from this entire area or not. This refers to the maximum height of bole colonization on the tree, even if it is restricted to one azimuth. Estimate the height from the ground to the highest point in the tree where lichens can be observed from the ground. Record height for all species in Field 90, or record height for up to 3 individual species in Fields 91-93 (record species ID in Comments).

CODE	RANGE OF CLASS
NA	Not Applicable
00	No lichens on bole
05	0-0.5 m above ground
10	0.6-1.0 m above ground
15	1.1-1.5 m above ground
20	1.6-2.0 m above ground
25	2.1-2.5 m above ground
30	2.6-3.0 m above ground
35	3.1-3.5 m above ground
40	3.6-4.0 m above ground
45	4.1-4.5 m above ground
50	4.6-5.0 m above ground
10	5.0-10.0 m above ground
99	> 10.0 m above ground

Accuracy Standards - ± 1 Class

Field 94-97 - BCHL - Bole Collection Height of Lichens

Record the height on the bole from the ground where all the lichen species were collected or identified (all species = Field 94), or the height range on the bole where up to 3 specific lichen species were collected (Fields 95-97). These may be the same values as in Fields 90-93 if all the lichen species are restricted to the lower bole where collection or species identification usually occur, from ground level to about 2.5 meters above ground.

CODE	RANGE OF CLASS
NA	Not Applicable
00	No lichens on bole
05	0-0.5 m above ground
10	0.6-1.0 m above ground
15	1.1-1.5 m above ground
20	1.6-2.0 m above ground
25	2.1-2.5 m above ground
30	> 2.5 m above ground

Accuracy Standards - ± 1 Class

Field 98-103 - Appearance of Bark

Record the general characteristics of the tree bark of the host species, with particular emphasis on bark morphology and bark color. If more than 1 host species is involved in collection or evaluation of lichen species, record the bark characteristics of the 3 most common species.

Field 98-100 - BM - Bark Morphology

Record the general morphological characteristics of the bark of the 3 most common host tree species. Of primary importance is the general nature of the bark (e.g., broad flat plates, deep fissures, etc.), and the relative flakiness of the bark (e.g., flaky, persistent, etc.). Record up to 3 codes for each tree species, e.g., broad flat plates (BP), shallow fissures (SF), with flaky bark (FB).

CODE	DESCRIPTION
NA	Not Applicable
BP	Broad, flat plates
SP	Small, flat plates
NP	No plates, bark essentially smooth
TF	Tiny fissures in bark (1.0 cm)
SF	Shallow fissures in bark (1.0 - 2.5 cm)
MF	Moderate fissures in bark (2.6 - 4.0 cm)
DF	Deep fissures in bark (4.0 cm)
FB	Flaky bark; freely exfoliating when touched
LB	Loose bark; pulled off with slight effort
PB	Persistent bark; firmly attached

Fields 101-103 - BC - Bark Color

Record the general color (hue) of the tree bark of the 3 most common host tree species. Combine up to two codes to suggest lighter shades of any one hue, with the first code indicating the predominant shade of the hue.

CODE	DESCRIPTION
NA	Not Applicable
WH	White
BL	Black
BN	Brown
RD	Red
GN	Green
TN	Tan

Fields 104-106: Exposure of Host Species

Field 104 - HTL - Host Tree Location

Record the general location of the host tree(s) where the lichens are collected, e.g., isolated trees, in rows/orchards, dense natural tree stands, surrounded by buildings, etc. Add additional codes if necessary and explain in Comments.

CODE	DESCRIPTION
N	Not Applicable
IT	Isolated trees
RO	Rows of trees
TS	Tree stands (natural)
SB	Surrounded by buildings

Field 105 - DNLO - Distance to Nearest Large Object

Estimate the average distance to the nearest object that is greater than 3 meters in height. This is most relevant for hosts in transplant studies and lichen plots, but should be estimated for studies involving collection of lichens from multiple host trees.

Accuracy Standards - ± 1 meter.

Field 106 - DNSO - Distance to Nearest Small Object

Estimate the average distance to the nearest object that is less than 3 meters in height, such as bushes. This is most relevant for hosts in transplant studies and lichen plots, but should be estimated for studies involving collection of lichens from multiple host trees.

Accuracy Standards - ± 1 meter.

DATA MANAGEMENT AND ANALYSES

We suggest the variables mentioned above be coded for entry first onto a hard-copy data sheet or into a personal data recorder. Whenever possible, the method of quatifying the site variables should be constructed with subsequent data analyses in mind. It may be desirable to construct continuous variables to reflect site factors to relate them to the condition of the lichen species.

This can be done by correlation analyses (e.g., percent canopy opening and number of lichen species), regressions (e.g., elevation and lichen cover), principal component analyses, and covariate analyses. The quantification of site factors will aid in mapping lichen distribution and abundance using conventional or geographic information systems mapping.

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Chapter 3 APPENDIX

PART 1 - GENERAL DATA FORM

MANAGEMENT DATA FORM

Management Unit Data Sheet

A. **Heading**

1. Unit name _____

2. Managing agency _____

B. **Geographic location**

1. State _____

2. County _____

3. Land management unit _____

4. Nearby large city 1 (distance and direction) _____

_____

Nearby large city 2 (distance and direction) _____

_____

5. Nearby topographic features (Mt. Ranges) _____

_____

6. USGS Quad name, 7.5' or 15' _____

7. Approximate boundary of unit by Latitude and Longitude, or Universal Transverse Meridian (UTM), or Township range and section; and description (if appropriate)

_____

_____

C. **Physical environmental factors**

1. Geology/parent material (general) _____

_____

2. Soils (short description or soils map) _____

_____

3. Landform(s) (general)

   a. Terrestrial _____

   b. Aquatic _____

4. General cliamatic factors. Specify the following climatic factors.

   a. Annual precipitation (10-yr mean) _____

   b. Length of growing season _____

   c. Range of elevations _____

   d. Other factors _____

5. Air chemistry

   a. Types of known pollution sources within 100 km of study area. Specify all known sources of air pollution and approximate distance to study area.

Source 1: _____
Location 1: _____

Source 2: _____
Location 2: _____

Source 3: _____
Location 3: _____

Source 4: _____
Location 4: _____

Source 5: _____
Location 5: _____

   b. Type and Location of nearest continuous or passive air quality monitoring station.

Type of Monitor 1: _____
Location of Monitor 1: _____

Type of Monitor 2: _____
Location of Monitor 2: _____

Type of Monitor 3: _____
Location of Monitor 3: _____

Type of Monitor 4: _____
Location of Monitor 4: _____

D. **Biological Factors**

1. Biome type(s): _____
_____
_____

2. Significant disturbance history

a. Natural: _____

_____

b. Anthropogenic: _____

_____

_____

Unique animals: _____

_____

_____

E. **Any other Unique Features**

_____

_____

_____

_____

_____

_____

_____

_____

PART 2 - SITE-SPECIFIC DATA FORM
PRINCIPAL INVESTIGATOR DATA FORM

Key ID, Field 1-8

1 Ag __ __ 2 St __ __ 3 NF __ __ 4 Yr __ __ 5 Plt __ __ __ 6 Mo __ __ 7 Day __ __

8 Name __

Sample System Data, Field 9-18

Sample Forms: 9 __ __ 10 __ __ 11 __ __ 12 __ __ 13 __ __

14 Unit __ 15 Permanent Plot ID __ __ __ __ __ __ __ __ __ __ __ __ __ __ __

16 PRI __ __ 17 Comparison Plot ID __ __ __ __ __ __ __ __ __ __ __ __ __ __ __

18 Potential Vegetation Form __ __

Existing Vegetation Classification, Field 19-28

Existing Vegetation Strata: 19 LF __ 20 LSC __ __ 21 DSC __ __ 22 CC __

Vegetation Layers:

23 UL Dom Sp1 __ __ __ __ __ __ __ __ 24 UL Dom Sp2 __ __ __ __ __ __ __ __

25 ML Dom Sp1 __ __ __ __ __ __ __ __ 26 ML Dom Sp2 __ __ __ __ __ __ __ __

27 LL Dom Sp1 __ __ __ __ __ __ __ __ 28 LL Dom Sp2 __ __ __ __ __ __ __ __

Site Data, Field 29-56

29 SpFea __ __ 30-32 Landform __ __ / __ __ / __ __ 33-35 PMat __ __ / __ __ / __ __

36-37 Pos __ __ / __ __ 38 VerPS __ 39 HorPS __ 40 Elevation __ __ __ __ __

41 Aspect __ __ __ 42 Slope __ __ __ 43 EroStat __ __ 44 EroType __ __

45-52 GCov __ __ (BS) + __ __ (Gr) + __ __ (Ro) + __ __ (LD) + __ __ (Wo) + __ __ (ML) + __ __ (BV) + __ __ (Wa) 53 FLC __ __

54 FD __ __ . __ 55 Down Log Dia __ __ __ 56 Dom. Layer Ht. __ __ __

Vegetation Data, Field 57-75

Live Tree: 57 DBH __ __ __ 58 Ht. __ __ __ Dead Trees: 59 DBH __ __ __ 60 Ht. __ __ .

Tree Cover: 61 Tot __ __ 62 Seed __ __ 63 Sap __ __ 64 Pole __ __

65 Med __ __ 66 Large __ __ 67 VLarge __ __

Shrub Cover: 68 Tot __ __ 69 Low __ __ 70 Mid __ __ 71 Tal __ __

Herb Cover: 72 Gram __ __ 73 Forb __ __ 74 Fern __ __ 75 Moss __ __

Disturbance Data, Field 76-84

Ground Cover Disturbance: 76 M __ 77 F __ 78 A __

Animal Evidence: 79 (1) __ __ 80 (2) __ __ 81 (3) __ __

82 (4) __ __ 83 (5) __ __ 84 (6) __ __

Microsite/Host Data, Fields 85-106

Crown/Bole Features: 85 LCR __ __ 86 CD __ __

87 GH __ __ / __ __ 88 BTL __ __ 89 BAL __ __ / __ __ / __ __ / __ __ / __ __ / __ __ /

90 BHL (all) __ __ 91 BHL (1) __ __ 92 BHL (2) __ __ 93 BHL (3) __ __

94 BCHL (all) __ __ 95 BCHL (1) __ __ 96 BCHL (2) __ __ 97 BCHL (3) __ __

BM: 98 BM (1) __ __ / __ __ / __ __ 99 BM (2) __ __ / __ __ / __ __ 100 BM (3) __ __ / __ __ / __ __

Bark Color: 101 BC (1) __ __ __ __ 102 BC (2) __ __ __ __ 103 BC (3) __ __ __ __

Host Tree Location: 104 HTL __ __ 105 DNLO __ __ 106 DNSO __ __

Comments/Code Explanations

1. _____

2. _____

3. _____

4. _____

5. _____

6. _____

Species and Communities

Cliff Smith, Linda Geiser, Larry Gough, Bruce McCune, Bruce Ryan, and Ray Showman

This chapter recommends sampling techniques for air pollution studies that are appropriate for lichen species and community monitoring. The techniques evaluate the condition of and changes in lichen vegetation, use lichens to estimate air pollution concentrations, and measure responses of lichens to air pollutants in a manner that will meet quality assurance (QA) and quality control (QC) requirements established by federal agencies. Some options are provided, along with guidelines for making necessary choices.

We discuss design principles that must be considered in conducting any sampling or monitoring program using lichens to evaluate air pollution. Three sections follow on the recommended approaches: general lichen survey, indicator species, and quantitative community surveys. The general lichen survey is a relatively simple and inexpensive biomonitoring technique but is best used around point sources. It is not useful for monitoring small changes in pollution or where relocation of study sites is difficult. The use of lichen indicator species is an effective, sensitive, and inexpensive means to monitor lichen responses to air pollutants. Unfortunately, the air pollution responses of only a few species are adequately known. Quantitative community surveys provide detailed evaluations of many species. The techniques can be time-consuming, expensive, and generally require highly trained personnel. All approaches can be used for a single sample or for long-term monitoring programs. We conclude with some recommendations for reporting results.

DESIGN PRINCIPLES

Funding and time constraints are often cited as excuses for not conducting a well-designed research program. In most instances, projects carried out without adequate attention to QA/QC are not worth the time, money and effort spent. We recommend three different approaches that can be fine-tuned to evaluate air quality using lichens. If the requirements of these approaches cannot be met, obtain professional assistance. Many of the authors of this handbook are available for consultation (see Appendix I).

There are two opposite approaches to assessing and monitoring air pollutants: source-based models and receptor-based models. Source-based models use emission inventories and dispersion predictions, while receptor-based models use enrichment factors, chemical element balances, factors analysis, element concentration-distance trends, and stable isotope ratios to identify anthropogenic and natural atmospheric elemental emission sources. Source-based models suffer from uncertainties or inaccuracies in the emission inventories and dispersion models, whereas receptor-based models suffer from the difficulties inherent in identifying contributions from multiple sources at a receptor site. See Chapter 5, Sensitive Species, and Chapter 7, Chemical Analysis, for details on monitoring and analysis techniques.

Several hypothetical models are shown in figure 1 along with possible explanations for the concentration of an element in a receptor versus distance from a point source (Gough et al. 1986).

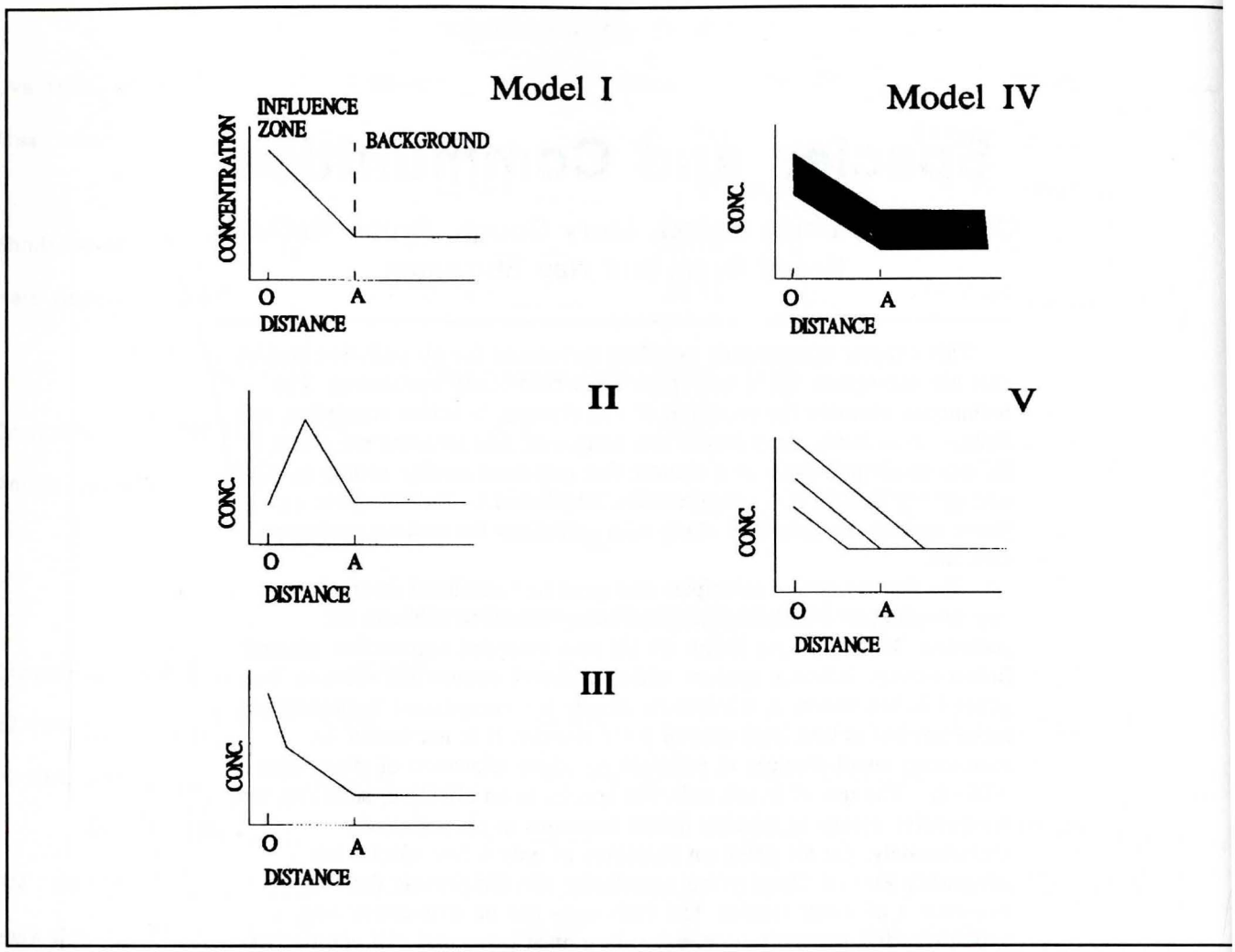


Figure 1. -- Hypothetical receptor-based models of the concentration (C) of an element in a receptor versus distance (D) from a point source contamination.

In Model I, a point source emits an element M. All receptor sites are sampled at one instant in time and are assumed to be equally efficient collectors. In this model there is a negative correlation with the concentration of M versus the distance from the source, up to the point at which the background concentration is reached. This distance (O to A) defines a region of influence for the emitting source.

Model II illustrates a situation in which chemical conversion of the emitted species occurs during transport away from the source, and the receptor only collects the reaction product. An example of this situation might be oxidation of an emitted species during transport.

Model III demonstrates a similar situation to model I; however, two point sources, or pseudo-point sources with different regions of influence, emit the same elemental species (e.g. fine particulates) from the same point. An example of this model would be a point source situated on the ocean coastline.

In Model IV, a less distinct negative correlation between concentration and distance may be found if the background concentration of M varies greatly.

Model V demonstrates the influence and importance of the sampling time. If the emissions vary by the source, or there are climatic, diurnal, or seasonal influences on dispersion or on the receptors, variations in the regions of influence around a point source can be expected. Differences in the regions of influence might be obtained based on the selection of the receptor used, such as soil versus vegetation sampling, or sampling of different plant species. Hence, regions of influence determined by this technique should only be considered as the minimum

region of influence, and caution should be used in interpreting results where competing emission sources may be involved.

Contamination gradients are often suspected but their magnitude, spatial and temporal trends, and deviation from some norm are unknown. See Chapter 5 for a discussion of Gradient Analysis. Over the last fifteen years, a protocol has evolved involving both gridded and non-gridded study designs which help define the sources of variability in biogeochemical data. These estimates help establish baseline element concentration values.

What is "Baseline"?

The terms "baseline" and "background" differ. Background is intended to represent natural concentrations, which ideally exclude human influence. A baseline represents the concentration measured at some point in time, a "snap shot", and is typically not true background. A background represents an idealized situation and is typically more difficult to measure than a baseline.

A true background may be obtained by sampling and analyzing tree rings, glacial ice cores, or varves from lake or ocean sediments. Biogeochemical baselines are best defined as a range of values representing 95% expected range. Following log transformation to normalize data, the mean becomes the geometric mean (GM) and the deviation becomes the geometric deviation (GD). The 95% expected range is the range defined by the GM/GD^2 to the $GMxGD^2$ (Tidball and Ebens 1976), where GM and GD are calculated from data generated by nested ANOVA designs (see Chapter 7).

So-called "working" baselines can be approximated when examining the distance vs. concentration data generated by studies using transects that radiate away from a contamination point source. A well-defined trend will show an inflection point, the asymptote of which can be used as a working baseline.

Stability Through Time

Investigations of temporal variability in air quality studies are few, and a thorough understanding of the proper use of study designs and statistical methods is lacking (see, for example, Jackson and Gough 1991).

Repeatability of field studies and laboratory procedure should be of major concern in the planning of a study. Identical field and laboratory procedures must be maintained through time. Some things to watch out for: (1) once a study has begun, do not change laboratories or analytical procedures, (2) avoid changes in personnel performing the field work, (3) sample at the same time of the year under similar weather conditions, and (4) do not change sample handling and storage. If procedures vary, the error associated with this variability must somehow be measured. This is not an easy task.

Green's 10 Principles

R.H. Green (1979) presents 10 principles of study design and data interpretation that should be reviewed by anyone beginning an air quality-related study. They are paraphrased here.

1. Be sure to state concisely the question you are asking.
2. Take replicate samples within each combination of time, location, and any other control variable. Differences among variables can only be demonstrated by comparison to differences within.
3. Take an equal number of randomly allocated replicate samples for each combination of controlled variables.
4. To test whether a condition has an effect, collect samples both where the condition is present and where the condition is absent but all else is the same.
5. Carry out some preliminary sampling to provide a basis for evaluation of sampling design and statistical analysis options.
6. Verify that your sampling device or method is actually sampling the population of interest, and with equal and adequate efficiency over the entire range of sampling conditions to be encountered.
7. If the area to be sampled has a large-scale environmental pattern, break the area up into relatively homogeneous subareas and allocate samples to each in proportion to the size of the subarea.
8. Verify that your sample unit size is appropriate to the size, density, and spatial distribution of the organisms you are sampling.
9. Test your data to determine whether the error variation is homogeneous, normally distributed, and independent of the mean.

10. Having chosen the best statistical method to test your hypothesis, stick with the result.

Quality Assurance/Quality Control

The level of quality assurance and quality control (QA/QC) will vary between studies depending on the objectives of the study and the available funding. While minimum levels are outlined here, more rigorous levels are provided in EPA manuals. The more rigorous approaches are appropriate if study results could be involved in litigation. The study objectives and the potential uses of the study results should guide in the choice of QA/QC level within budget constraints. In general, QA/QC requirements add an additional 25 percent to the total budget.

Vouchers

The collection and preservation of voucher specimens is an important aspect of all studies, even those which are not purely floristic. These activities generate key reference material for later studies, promoting accuracy and consistency between studies. In some cases the specimens may be required as evidence for litigation purposes.

The collection, storage, organization and availability of voucher specimens is discussed fully in Chapter 2, Floristics.

Permanent Markers

Re-location and re-sampling of specific sample units are facilitated if several quadrats or transects are located in close proximity of each other. In some study areas, especially wildernesses, it may not be permissible or desirable to use conspicuous permanent markers. Plots located far away from trails or campgrounds are less likely to be disturbed, and also less subject to vandalism, but they are more difficult to re-locate during follow-up studies. Global Positioning System technology is becoming cheap enough that most marker relocation problems can be minimized.

Metal Nails or Tags

These should be located so that water runoff does not pass through the sample unit and affect the results.

Plots on Bark or Wood

On living trees, permanent markers should be chosen to minimize damage and defacement of the bark. Aluminum nails and tags are preferable to other kinds of metal markers.

Plots on Rock

The corners or endpoints of plots on rock should be marked on the rock with small daubs of red or orange paint which are clearly visible but unobtrusive. Car touch-up paint in a small tube with a brush attached to the inside of the lid is convenient, as is nail polish. The paint will stick better if it is placed in a slight indentation in the rock made with a hard nail. On crumbly or exfoliating surfaces, daubs of paint may not be permanent, although the outline of a quadrat can be reconstructed if at least two of the corners can be re-located. Repainting plot corners may be necessary; an adequate paint supply should be taken each time the plots are measured.

Alternatives to the use of paint dots, such as drilling holes into the substrate, may be difficult. Aluminum tags, with the sample identification and other information scratched into them, can be affixed to rocks with silicone or nailed to nearby trees.

Plots on Soil or Moss on the Ground

Although stakes can driven into the ground to mark plots, a better method is to place tags on rocks or trees to mark one end of a transect or one corner of a plot (with the other points being located using a compass and tape). See Chapter 6, Active Monitoring, for precautions on permanently marking plots.

Archiving Data

An appendix of the final report should be produced for each study. This appendix should include the actual data used in the analysis, any photographs used in the analysis, and any maps and/or other site location information relevant to the study. If the inclusion of this material generates an undesirably long report, the appendix can be published separately from the report and fewer copies produced, perhaps in a less expensive format. These can then be included in the distribution of the report only when appropriate. This approach assures that multiple copies of this material will be in circulation, promoting the availability and future accessibility of this reference material. The second archive location is a "local" location, that is the office or administrative location of relevance to the study site.

The third is a central location, which can serve as a nation-wide reference center for this type of material. The following types of reference material should be included in this discussion:

1) actual field notes, lab sheets, etc.;
2) raw data such as the data initially entered in a computer database;
3) analysis data, the data as it was actually used in the analysis;
4) general maps (geological, topographic, etc.);
5) site location information which may include maps;
6) photographs.

Not all of these types of reference material necessarily exist in all studies. These reference materials and their recommended archive locations are listed in Table 1.

Table 1. — Archive locations.

Reference material	Appendix	Local	Central
Field notes, lab sheets, etc.		X	
Raw data (database)		X	X
Analysis data (database)	X	X	X
General maps		X	X
Specific site location information	X	X	X
Photographs	X	X	X

In this system, the appendix serves to make the general study reference material readily available to anyone who might request it. After the initial distribution of a study, the appendix and report should be available by request from both the central and local locations. The central location will serve as a clearinghouse to anyone interested in obtaining information from a number of studies and/or sites. The local location will serve as a repository of all the material relevant to that management unit.

Program Continuity

The consistent use of methods, systematic documentation of work to date, and proper archiving of reference material will enable other researchers to pick up a study and move it forward with the least down time or methods errors. Systematic documentation is also applicable to a wide range of activities discussed throughout this manual.

Documentation should contain **general background information** including (but not limited to) names of contact persons who are knowledgeable concerning some aspect of the study, a schedule of research activities, site locations, field personnel (names and addresses), descriptions of equipment used, and so forth.

Secondly, an explicit **description of methods** or a reference to an easily accessible document or publication of the methods should be provided. Any deviation from these methods should be annotated in the field notes or research management notes. Finally, **raw data** in any form (field notes, lab sheets, photos, etc.) should be available in sufficient detail to enable a researcher to trace back to the original observation any datum of interest. These data should also be annotated with any observations that might have implications regarding later analysis of the data.

GENERAL LICHEN SURVEY

The general lichen survey is a relatively simple biomonitoring method designed to indicate air quality differences over fairly large areas. Repeat studies can also track changes in air quality over time. On a scale of increasing structure and complexity, the general survey bridges the gap between the floristic method of Wetmore (1988, 1990) and the more complex quantitative methods of Case (1980), McCune (1988), Will-Wolf (1980), and others.

The survey method is similar to the classical species mapping approach used by numerous European workers (see Ferry et al. 1973 for good examples). In the United States, this method has been used extensively by Showman (1973, 1975) to indicate air quality around coal-burning power plants in the Ohio Valley region. Re-surveys of the study sites can also document changes in air quality (Showman 1981, 1990). Metzler (1980) used the survey method to map species distribution and condition over the entire state of Connecticut.

Description of Method

The survey method can be used to study lichens on any substrate. Usually some preliminary reconnaissance is advisable to determine substrate availability and distribution in a study area. In the eastern U.S., it is generally accepted that corticolous lichens are the best indicators of air quality. Thus study sites are chosen to contain several to many

unshaded trees. Sites can be further standardized to tree species or bark type, but this may severely limit the number of available sites. Showman (1975) utilized hardwood trees in open situations such as churchyards and cemeteries. These sites typically contain 3-6 trees and occupy an area of 0.1-0.2 hectares (0.25-0.5 acres). In some regions trees may be absent, and saxicolous or terricolous lichens must be studied instead. Sites containing rock or soil substrate should be standardized to whatever extent possible. A careful record should be kept of site selection criteria.

The number of study sites used in a survey will depend on the size of the study area and the desired sampling density. Biomonitoring studies by Showman average about 10 sites per 7-1/2 minute topographic map quadrangle. This approximates one site per 13 square kilometers (5 square miles). A large study area with a low pollution gradient could be covered by a lower site density, while a small area with a steep pollution gradient would require a greater site density.

For a valid temporal study, it is necessary to relocate the same study sites precisely at a later time. In populated areas, this can be accomplished by accurately marking the site location on a 7-1/2 minute topographic map. However, in remote areas site relocation may be more difficult, and some permanent marker may be necessary.

The general data recorded at each site should include the date, site identification number, and the study area description. See Chapter 3, Site Characterization, for more details and for characterization using ECODATA forms. The lichen species present are then recorded. Notes on thallus condition, i.e., bleached, discolored or fragmented, fertility, and size may also be recorded.

Data Analysis

Data from the general lichen survey are usually presented cartographically. Some general aspects of mapping are discussed by Showman (1988). Distribution maps are prepared for the common species and then compared to known or suspected pollution gradients. A discrete void in the distribution of one or more species downwind from a pollution source could indicate an air quality effect. A random pattern of absences would suggest some other factor, such as differences in site ecology. Species distribution maps might also include other information such as injury or fertility. Metzler (1980) presented distribution maps which distinguished between healthy thalli and thalli with obvious necrosis. By using different symbols, several different kinds of data may be presented on the same map.

Species richness (number of species) is also a good indicator of air quality (Showman 1988). Richness values can be easily obtained from data collected by the general survey method. Richness values can be mapped to detect anomalies in distribution within a study area. Data can also be summarized in terms of total richness and mean richness per site for comparison between study areas. Showman and Long found species richness to be a simple yet sensitive indicator of differences in deposition between forested areas of Pennsylvania (Long 1990).

Discussion

The general lichen survey method is applicable where the purpose of the study is to determine large overall patterns of air quality. The method is very appropriate around point sources and in areas of complex terrain and ecology where site standardization is difficult. This is a "quick and dirty" method best suited for use in areas with medium to high pollution levels and in populated areas.

The primary advantage of this method is that it is simple and fast. Site standardization is not as critical as for the quantitative methods so a broader range of sites can be used. A survey of 15-25 sites per day can usually be accomplished, allowing a larger area to be covered at a moderate cost. Data analysis and presentation are also relatively simple and can be done on a day-to-day basis.

The primary limitation of the survey method is that it is not as sensitive to small changes in air quality as the quantitative methods are. It is also less appropriate for remote wilderness areas where non-vehicular travel and difficulties in site location/relocation negate the primary advantage of this method.

INDICATOR SPECIES

The indicator species method can be used to assess changes in growth or other parameters in one or more known air pollution-sensitive lichens using quantitative methods.

The indicator species approach is useful when particular target species are known to occur in the study area. Such species are usually ones for which information on pollution sensitivity is available from previous studies, and ones with other characteristics suitable for using the species as monitors. The most suitable species are relatively easy to identify in the field, have individual thalli with fairly discrete boundaries and reasonably rapid growth rates, and occur frequently over a large portion of the study area. Species with distinctive colors that contrast strongly with the background and with other lichens are especially desirable, particularly if video monitoring techniques are used.

Although indicator species are usually ones likely to be sensitive to particular pollutants, species that are tolerant may also be useful: an increase in their abundance can indicate increased pollution.

Advantages

Once a limited number of indicator species are identified, only minimal training is required to recognize them in the field and follow changes in their growth and condition over time. The indicator species method gives efficient focus on the air pollution problem. If high-tech methods are not applied, the indicator species approach is inexpensive, involving little time on the part of personnel, and while it does not require complex analyses, it is more sensitive to changes in air quality than the general survey method.

Limitations

On the other hand, the indicator species approach presupposes knowledge of which species are sensitive. This is a problem in most areas of the United States, especially in the West, where the pollution responses of few, if any, species are known. See Chapter 5 for more information. Even when some data on sensitivity are available for a species, considerable caution must be used in applying that information to a particular study area. More data are needed on the sensitivity of species.

Another apparent limitation of the indicator species approach is that it gives information on only a few species and says nothing about the others. In presenting the results to decision-makers or to the public, there is a danger of the "snail darter syndrome", i.e., people may ask "who cares about loss of this species?", especially when in most cases little if anything is known about the potential economic or ecological significance of any lichen.

If indicator species monitoring is the only approach used, there is the risk that the particular species chosen may not show any detectable changes that can be ascribed to the influence of air pollution. However, such "negative" results can still provide reliable conclusions, since this is analogous to using an instrument and reporting that air pollutant concentrations are below the detection limit of the instrument.

Choice of Indicator Species and Response Variables

Determination of suitable indicator species involves evaluation of many features, as discussed in more detail in Chapter 5, Sensitive Species Responses. However, after walking through the study area to determine which lichen taxa are present, the investigator can make a preliminary assessment of likely indicator species, based on growth form and photosynthetic partner, and on information from the literature when available.

Variables to measure in determining the response of indicators include cover, elemental or other chemical content, and others as discussed in Chapter 5.

Sample Units

Selection of permanent sample units requires the investigator to consider number, size and arrangement, and tradeoffs between number and size (see discussion of quantitative community sampling later in this chapter). The choice of plot type depends on growth form of the lichen, kind and topography of the substrate, and other factors.

Lichen Growth Forms

The growth form of the thallus affects whether photographic or other methods of determining growth changes are used. Growth forms should be considered in choosing specific methods of measuring or estimating cover or other growth parameters.

Many of the lichen species which are known to be sensitive to pollutants and are the most useful as indicators have a fruticose or foliose thallus. The growth of such taxa may be difficult to measure by photographic means because of the three-dimensional form of the thallus. Pendulous (beardlike, hanging) lichens, especially those hanging from branches and

mostly free from the substrate (e.g., many *Bryoria* and *Usnea* species), present a different set of problems from other forms, since growth is primarily in length, rather than in area or diameter. The growth of caespitose (shrublike) fruticose lichens, and large, loosely attached and suberect or subpendulous foliose lichens (e.g., *Lobaria* spp.) consists of increases in both diameter and thickness.

Growth changes in lichens composed at least partly of numerous small, scattered subunits (areoles or squamules), and species with the thallus mostly or entirely immersed in the substrate are usually very difficult to follow. Often frequency (presence/absence) is the only parameter that can be measured for such species. Crustose and appressed foliose lichens, forming flat, solid patches or rosettes, with growth entirely in the radial direction, are the easiest ones to measure by photographic or other methods of determining cover, but few of these species are known to be sensitive to air pollution. Another potential problem with crustose species is the difficulty of obtaining voucher specimens, which must be collected with pieces of the substrate.

Substrate and Habitat Considerations

Lichens on Trees or Shrubs

At many sites, the majority of species growing on bark or wood (including several of the most sensitive lichens) are abundant mainly on the branches and in the canopy rather than on the boles of trees, and are not accessible from the ground. These situations require special methods, including climbing the tree, or special plotless methods to estimate abundance. Likewise, shrubs may be a major substrate for lichens, requiring development of special techniques.

Lichens on Rock

It is difficult to follow long-term changes in the growth of lichens found on exfoliating or unstable surfaces, or in areas likely to be covered by litter or overgrown by other plants. Lichens on small or unstable rocks likely to roll down hill should be avoided. Many sensitive lichens preferentially grow on steep or overhanging surfaces, or in crevices and shaded areas where photography and other methods are difficult to use. Although aquatic or semi-aquatic species may be potentially useful monitors of acidification, it is difficult to study them and to establish permanent quadrats or transects for them.

Lichens on Soil or Moss

Some of the most abundant and potentially useful indicator lichens (sensitive, easily identified, and rapid growing) occur on soil or moss, especially in alpine or desert areas. Such species have rarely been included in permanent monitoring studies related to air pollution, but the methodology for doing so can be found in other types of studies on lichens, and in studies on vascular plants.

Methods of Using Plots

Photographic Methods

Photographic methods are most useful for following changes in two-dimensional lichens (crustose and most foliose forms) growing on two-dimensional surfaces, especially at angles less than 90 degrees from horizontal. The more variation in surface features of the substrate, the more depth-of-field and focus problems will occur. Photographing quadrats, especially on steep or overhanging surfaces, or in partial shade, is sometimes a rather awkward process, involving two people. One person holds the frame in place or holds up something to give uniform lighting or shading, while the other person takes the pictures.

For easier identification of species and thalli in the photographs, it is helpful to restrict the number of species within the sample unit to one or a few kinds, preferably ones with distinct colors that contrast with each other and with the background. Video methods with digitizing capabilities are very useful both in enhancing contrast and in determining the area covered by the thalli, especially species composed of scattered units, but such methods may be prohibitively expensive.

Cover and Frequency Determinations

Measurements or estimates of cover and frequency can be done in any situation, including cases where the lichen or surface or both are three-dimensional. They can also be combined with photography, including video techniques, in making the determinations. In cases where lichens do not contrast much with the substrate or with other species as in shaded areas, cover determinations may require use of a hand lens or even a flashlight to see the boundaries of the thalli more clearly.

Line-Intercept Transects

Tape Methods

Ryan and Nash (1991a) used a 20 m long flexible transect tape to determine cover and frequency of lichens on rock, for community analyses. Though their transects were not designed to be permanent, the same method could be adapted for permanent transects focusing on one or a few indicator species. This method is generally more difficult and less reliable than quadrats or very short transects, and is probably most useful in studying lichens growing on very large, contiguous patches of rock or soil. Denison (1990) sampled lichens on branches by wrapping a flexible tape in a spiral along the length of the branch. Such an approach may be useful in some cases, but not for making permanent plots, because even on a tree trunk it is difficult to place the tape in exactly the same place each time the sampling is done.

Pin Intercept Methods

For lichens growing on trees with large, straight trunks, the "dotiometer", a moveable pin attached to a meter stick, may be useful. This method is discussed by Ryan (1990) and is described in the 1988 U.S. Forest Service Region 5 draft protocol. If such a method is used, it is important that the meter stick is oriented nearly vertical, so that the transect will not be greatly affected by changes in the diameter of the tree. Frames with fixed rows of pins that can be moved up and down may also be used. Pin methods have the advantage of being easier to use in situations where the substrate, lichens, or both, are strongly three-dimensional. However, in most situations a quadrat method may work as well as, if not better than, pin methods.

Quadrats

Various methods of establishing permanent photographic quadrats for sampling lichens on rock have been used in a large number of studies, including the "quadpod" described in the 1988 U.S. Forest Service Region 5 draft protocol and used by Ryan (1990). Other studies (e.g., Skorepa and Vitt 1976) have used various types of photographic quadrats for lichens on trees. The method of Looney and James (1990) using a wire grid, is recommended for most situations, and should work well not only on tree trunks, but also on rock and soil. Video techniques with digitizing may also be useful if they are financially feasible.

Various non-photographic quadrat methods have also been used in many studies. Ryan and Nash (1991b) located quadrats randomly along a transect line across a field of small boulders and estimated percent cover within each quadrat. Such methods may be useful where it is desirable to make a relatively rapid survey of a large area with numerous replications. However, when monitoring changes in indicator species over time, it is desirable to make such quadrats permanent and to increase the repeatability of the cover estimates by using a grid of squares or pins within the quadrats.

Plotless Methods

Examples of several plotless methods for estimating the abundance of lichens are given by Ryan and Nash (1991b). In that study, the individual sampling units were not permanent, since they were selected so that it is impossible to return to the same trees or rocks to record changes. Plotless methods generally do not produce the precision of plot methods, but in some situations, as where lichens are growing mainly on inaccessible branches of trees, they are the quickest and most efficient way of sampling.

Computerized video techniques may allow the use of entire trees or rocks or parts of them as permanent sampling units, which could be considered a plotless approach.

Analysis of elemental or other chemical content can be done with plotless methods. This subject is discussed in detail in Chapter 7, Chemical Analyses.

Data Analysis--Indicator Species

The type of data analysis needed depends on the sampling designs and methods used and the kinds of data to be analyzed. Although various studies have reported establishment of permanent plots to follow indicator species over time, few have presented analyses of the results.

Data Storage and Retrieval

Data should be stored in an appropriate relational database, as described by Looney and James (1990). Files to be established include the following:

1) Sample unit information: name and code, location, date, notes, etc. for each unit;

2) Cover and frequency data, coded to site, sampling unit, and year;

3) Photographic records;

4) Maps and aerial photographs;

5) Locations and collection numbers of voucher specimens, and

6) Information on species observed at each site other than the target species.

Basic Analyses of Data

Some response variables, such as physical condition or fertility, can at best be coded by semi-quantitative scales. In the simplest cases, changes in cover and frequency (or other quantitative parameters) can be analyzed by graphically and visually comparing trends over time or space for each plot or site. Scatterplots may be useful for analyzing some kinds of patterns. Map series with data for a given species can be useful in detecting changes over time as well as space.

When sufficient data on pollutant concentrations or other physical-chemical parameters are available, these data can be compared with cover/frequency data to determine whether correlations exist between data patterns and non-pollution physical or biological factors. Such correlations may provide a basis for determining to what extent these factors influence the lichens.

Standard Forms -- Indicator Species

Detailed site characterization in the ECODATA format is described in Chapter 3. Here, we suggest standard forms for recording more generalized plot descriptions and detailed transect measurements. Examples of some forms that have been used for permanent plots or transects are given in appendices 1 and 2 of this chapter. These are only examples, and the exact format of the form is not critical as long as the form provides certain types of information to be recorded. It may be desirable to develop generalized forms for a wide variety of specific sampling techniques, as well as for community analysis approaches (see section on Community Analyses).

In addition to forms for specific sampling methods, the general site description form should be used and cross-referenced on the specific form. Plenty of room should be allowed on any specific form for describing the location of the sample unit, describing reference photos, and comments or sketches, especially ones that will facilitate reproducibility of the sampling.

Looney and James (1990) recommended using percent cover data to calculate relative growth rate (RGR), which allows initial size differences to be discounted and the net changes or performance of individuals to be compared.

Analysis of photographic or other cover data for monitoring indicator species requires a solid understanding of the structure, growth, and population dynamics of the particular taxa involved. For example, several photos may show approximately equal cover by a given species, but may represent quite different situations, such as numerous tiny thalli vs. a few large ones, or young, single-layered thalli vs. older multi-layered thalli. Other factors, such as competitive or other interactions among thalli of the same or different species, or the effects of distribution of suitable areas of the substrate (e.g., fissures or depressions) also need to be considered to understand the patterns observed. Special types of analyses may be required for the computerized video techniques currently being developed.

Statistics

Regression analyses can be used for studying trends over time and space. If the appropriate experimental design is used and the statistical assumptions are met, various parametric or non-parametric univariate approaches may be useful. Principal components analyses or other multivariate techniques are likely to be useful in detecting patterns of variability. Standard statistical texts should be consulted (see Chapters 5 and 7 for references).

Interpretation of Data

As with other monitoring approaches, the basic principles of design must be applied for interpretation of the results to be valid. The challenge is to distinguish patterns that can be attributed to pollution. With indicator species, perhaps the most important consideration is the quality and quantity of data available about the species, including both its basic biology/ecology and its response to particular pollutants or other factors. If the results of the indicator approach are ambiguous, they may suggest the need for further testing of the species' response (e.g., by fumigation, transplants, or studies in areas with known levels of pollution -- see Chapter 5), a different method of determining growth or other parameters, or even choice of a different species.

QUANTITATIVE COMMUNITY ANALYSES

The objective of quantitative sampling of lichen communities for air quality monitoring is the accurate and sensitive detection of changes in lichen communities through time. In most cases, another objective to be met by the same sampling is the description of differences in lichen communities along a pollution gradient. Methods somewhat different from those discussed below should be used for a comprehensive characterization of lichen communities in an area.

Quantitative community sampling is useful when response information is desired for a large number of species. This is often the case when setting up a baseline or when there is uncertainty about species sensitivities or about specific threats to air quality. However, such sampling is also useful in any other circumstance involving a point source of pollution. If some modification is requested and/or made to a point source of pollution, quantitative community sampling may detect responses to the modification. Quantitative community data will likely produce other useful spinoffs, such as improving our understanding of basic lichen ecology. Furthermore, these studies produce information on abundance and distribution relevant to other kinds of management problems involving lichens (e.g., ecosystem nutrient balances and food for caribou, deer, and small mammals).

Advantages

Quantitative community data methods are highly sensitive to changes in air quality. In areas with multiple direct monitoring stations, the opportunity exists to produce calibrated monitoring studies, i.e. quantitative estimation of air pollutant concentrations, particularly sulfur dioxide, based on lichen data (Ammann et al. 1988, Liebendorfer et al. 1988, McCune 1988, Nimis et al. 1990).

Disadvantages

This class of methods can be more time-consuming than other methods. Special taxonomic training is required for field workers. Most seasonal, temporary workers will not have the background needed to quickly develop the required field expertise. Site standardization is critical for these methods, since lichen communities are sensitive to many factors other than air pollution (see Chapter 3). Although extraneous factors can be removed to some extent in the analysis phase, training in multivariate analysis is required to isolate differences due to air quality from variation caused by other factors.

General Design Considerations

In most cases, sampling designs will be hierarchical, with subsampling at a number of individual sites within the study area. In some cases, as discussed below, a single large plot will be used at each site. Typically, sites or "stands" are considered to be areas that, at the scale of the dominant vegetation, are essentially homogeneous in vegetation, environment, and history. Sample units in the subsample are typically plots (quadrats) or points.

Designing a study thus requires decisions about the number, size, and arrangements of sample units at the site level and within sites. Site selection will depend on study objectives (e.g., transects along gradients, stratification among several habitats, and standardization to restrict extraneous environmental or historical factors). The goals of subsampling sites are to (1) determine accurate abundance values at the site level and (2) characterize the within-site variation, to allow stronger comparisons among sites and through time.

Site Selection

Sites should be selected according to the general study design principles outlined in the Design Principles section and in Chapters 5 and 7. To summarize, one must define the population of interest, then ideally use a sampling strategy to locate permanent study sites. The number of sites depends on the complexity of the vegetation and the goals of the study (McCune and Lesica 1992). With some preliminary sampling, one can use standard statistical techniques to determine the sample size required to achieve some specified degree of accuracy. In practice, however, such decisions are often made without good information based on preliminary sampling. In those cases, one can use a rule of thumb (slightly modified from Tabachnik and Fidell 1989) that for every important controlling factor or gradient, 20 additional sites are needed. For example, to determine community differences along a gradient from a point source on a homogeneous plain, 20 sites along a transect would be reasonable. If two substrates were involved and there were elevational differences, then 60 sites would be desirable (3 important factors x 20 sites/factor = 60 sites).

Table 2.— Tradeoffs between few-and-large and many-and-small sample units (McCune and Lesica 1992).

Variable	Few-and-large	Many-and-small
Bias against cryptic species	Higher. There is a hazard that some species, particularly cryptic species, are inadvertently missed by the eye.	Lower. Small sample units force the eye to specific spots, reducing inadvertent observer selectivity in detection of species.
Degree of visual integration	High. The use of visual integration over a large area is an effective tool against the normally high degree of heterogeneity, even in "homogeneous" stands.	Low. Minimal use is made of integrative capability of eye, forcing the use of very large sample size to achieve comparable representation of the community.
Inclusion of rare to uncommon species	High. Visual integration described above results in effective "capture" of rare species in the data.	Low. Unless sample size are very large, most rare to uncommon species are missed.
Accuracy of cover data on common species	Lower. Cover classes in large sample units result in broadly classed cover estimates with less accuracy and precision than that compiled from many small sample units.	Higher. More accurate and precise cover estimates for common species.
Sampling time	Varies by complexity and degree of development of vegetation. No consistent difference from many-and-small.	Varies by complexity and degree of development of vegetation. No consistent difference from few-and-large.
Analysis time	Faster. Data entry at site level leads directly to site-level-analysis.	Slower. Point data or microplot data require initial data reduction (by hand, calculator, or computer) to site-level abundance estimates.
Analysis options	No estimates of within-site variance restricts analysis to individual sites as sampling units.	Within-site variance estimates not possible unless sample units are larger than points.
Recommendations	The extreme case (single large plot) is most useful with extensive inventory methods.	The extreme case (point sampling) is most useful when rare to uncommon species are of little concern and accurate estimates are desired for a common species. In most cases a compromise by using a smaller number of larger sample units is better.

Number and Size of Sample Units

There are tradeoffs between number and size of sample units (table 2). The extremes are single large quadrats to characterize a stand (e.g. releves or habitat type plots) vs. many tiny sample units (point sampling). All intermediates are possible.

Arrangement of Subsample Units

The initial sampling should follow a carefully designed sampling plan. Subsequent samplings should revisit as exactly as possible the same sample units. Most statistical texts advocate random or stratified random sampling. However, systematic sampling will usually make relocating the permanent plots easier. In most cases, evenly spaced (systematic) sample units are acceptable because they will not produce results that differ from strictly randomized sampling. The specific plan for arranging the sample units will depend on the substrate (table 3).

Many air-quality studies have used lichen communities on tree trunks. Only a few examples are listed here, selected on the basis of proven ability to detect differences in lichen communities among sites with relatively subtle differences in air quality.

Abundance Measures

The most commonly used abundance measure, excluding presence-absence, is percent cover. For a review of abundance measures used to estimate epiphyte biomass, see Stevenson and Enns (1992). Abundance measures are also discussed in Chapters 2 and 5 of this document. Cover excels as an abundance measure in speed, repeatability, comparability between different estimation methods, and the ability to measure it nondestructively (Hermy 1988). Conversion to biomass estimates is possible but requires additional data collection for calibration (Forman 1969, McCune 1990, Stevenson and Enns 1992). This conversion is not normally necessary for studies of lichens as indicators. Frequency measures (proportion or percent of sample units containing a given species) should be avoided because frequency, unlike cover, is highly dependent on the size of the sample unit. Because there is usually little standardization in the size of sample units, the use of frequency measures restricts opportunities for comparison with other studies.

Table 3.—Examples of designs for subsampling individual sites for percent cover of lichen species. Studies using frequency in quadrats are not included because of problems with comparison with other studies.

Substrate Class	Specific case	Sample unit number, size, and arrangement
Branches	Lower outer branches of open-grown trees	5 5-yr and 5 10-yr branch segments on oak and ash (Denison and Carpenter 1973)
	Large, low branches of open-grown trees	100 equidistant points at 3 cm intervals on tape spiraled around branch (Denison and Sillett 1990)
	Lower- to mid-canopy branches in conifer forests	24 1-m branch lengths cut from midpoint of branches closest to points at 4 m intervals along 3 parallel transects (Lesica et al. 1991)
Rock	High lichen cover, stone walls and natural outcrops	Point sampling on 10 cm grid, variable number of points per rock face (1.1m), three rock faces per station (John 1989)
Ground	Mostly sparse flora on forest floor	100 10x30 cm quadrats/site, regular intervals on parallel transects (McCune and Antos 1982); 45 20x50 cm quadrats at regular intervals on parallel transects (Lesica et al. 1991)
Trunks	Mostly sparse flora near cities or large point sources	1 m cylindrats, 10 trees per tree species per site; certain tree species used (McCune 1988; Muir and McCune 1988); 2 m cylindrat, 10 trees per site (Johnson 1979).
	Both rich and sparse floras in a regional study	British acidification study: 100 evenly spaced grid points in 18x27 cm quadrat with wire grid; subjective quadrat placement, permanently marked, certain tree spp. (Quercus and Fraxinus preferred); variable height and aspect; variable number of quadrats/site (1-20) (Looney and James 1990; Wolseley and James 1990).
	Fairly rich flora in deciduous forest	25x25 cm quadrat on NE aspect at 1.4m on 10 trees per host species per site (Will-Wolf 1980).

Cover Classes

Using cover classes rather than attempting to estimate cover to the nearest one percent tends to speed sampling and data entry. Cover classes do not pretend to more accuracy than is realistically achievable; they yield statistical results that are similar to unclassed data, provided that the classes are not too broad (Sneath and Sokal 1973). Cover classes have been shown to be effective surrogates for direct biomass measurement (Hermy 1988, McCune 1990), and are good detectors of community changes through time (Mitchell et al. 1988). A disadvantage of cover classes is the potential for consistent differences between observers (Sykes et al. 1983).

The most useful and commonly used cover classes are narrow at the extremes and broad in the middle. These approximate an arcsine-squareroot transformation, which is generally desirable for proportion data. The cover classes can thus be analyzed directly, improving normality and homogeneity of variances among groups, without converting to percentages. Many cover class schemes have been devised. Some of the most common and/or logical are listed in table 4. Note the high degree of similarity among the systems.

Other Abundance Classes

Methods for field scoring lichen abundance that are not based on cover have also been used. Several of these are summarized in table 5. Use of these scales is probably most appropriate for broad-scale studies using single large plots as sample units, without subsampling the large plots.

A Broad-Scale Survey Method

A gradient strategy of regional sampling is given in Chapter 5. Here we present an extreme case of the "few-and-large" sampling strategy using a single large plot without subsampling. The method presented below is an example of this strategy. This method would be most useful in a broad-scale survey of lichen communities. The advantages and disadvantages of this general approach are summarized in table 2. The method was tested in 1991-1992 (McCune and Lesica 1992) as part of the

Table 4.—Cutoff points for cover classes. Question marks for cutoff points represent classes that are not exactly defined as percentages, but substitute another criterion, such as number of individuals. Cutoffs in parentheses are additional cutoffs points used by some authors.

Name	Cutoff points, %	Notes/References
Arcsine square-root	0, 1, 5, 25, 50, 75, 95, 99	Designed to approximate an arcsin squareroot transformation of percent cover (McCune 1988; Muir and McCune 1987, 1988).
Braun-Blanquet	0, ?, ?, 5, 25, 50, 75	Uses two categories of low cover not exactly defined as percents. Commonly used in Europe (Braun-Blanquet 1965, Mueller-Dombois and Ellenberg 1974).
Daubenmire	0, (1), 5, 25, 50, 75, 95	Widely used in western U.S. in habitat-typing efforts by U.S. Forest Service and many other studies (Daubenmire 1959, Denison and Carpenter 1973).
Domin	0, ?, 1, 5, 10, 25, 33, 50, 75	One category of low cover not exactly defined as percent (Krajina1933; Mueller-Dombois and Ellenberg 1974).
Hult-Sernander Modified	(.02, .05, .10, .19, .39, .78); 1.56, 3.13, 6.25, 12.5, 25, 50, 75...	Based on successive halving of the quadrat (Oksanen 1976).

Table 5.—Abundance classes not based on cover estimates.

Name	Description of classes	References
Canadian System	1 = Rare, a single individual 2 = a few sporadically occurring individuals 3 = a single patch or clump of a species 4 = several sporadically occurring individuals 5 = a few patches or clumps 6 = several well-spaced patches or clumps 7 = continuous uniform occurrence of well-spaced individuals 8 = continuous but with a few gaps 9 = continuous and dense	Canadian Ministry of Forests (1988) in Geiser et al. (1989)
FHM pilot	1 = Rare (3 individuals) 2 = Uncommon (4-10 individuals) 3 = Common (10 individuals but less than half of the boles or branches have that species present) 4 = Abundant (more than half of boles or branches have the subject species present)	McCune (1992)
Henderson System	1 = very rare (1% cover, approximately) 2 = uncommon (2-10%) 3 = common (11-25%) 4 = abundant (26-50%) 5 = very abundant (50%)	Henderson et al. (1989)

pilot studies for Forest Health Monitoring (FHM) under the EMAP program (general information: Messer et al. 1991). The FHM method was designed to be "user-friendly" and usable by a field crew with minimal training in lichenology. Key components of the method are that the field crew distinguishes among species and assigns coarse abundance classes in the field, collects vouchers of each species in each plot, and defers the naming of species to a specialist.

The first objective of the field sampling is to make a collection of voucher specimens for identification by a specialist. The collection represents the species diversity of macrolichens on woody plants in the plot as fully as possible. The population being sampled consists of all macrolichens occurring on woody plants, excluding the 0.5 m basal portions of trees and shrubs. Second, an estimate of abundance of each species is made in the field. Note that the crew member responsible for this task need not be able to accurately assign species names to the lichens (that is done later by a specialist), but must be able to make distinctions among species.

Recommended Field Procedures (about 1-1.5 hr for 1 person):

- The area to be sampled (henceforth the "Lichen plot") is a circular area with 36.6 m (120 ft) radius.

- Take a reconnaissance walk through the lichen plot, locating lichen epiphytes on woody plants, and collecting voucher samples and estimating abundances as you go.
- Collect lichen species with fruticose and foliose growth forms (i.e. macrolichens).
- Inspect all trees and shrubs 0.5 m tall within the lichen plot for lichens. Also inspect branches collected for the destructive samples (the same trees used for foliar nutrient analyses, branch and foliage visible symptoms, and tree cores) for lichens.
- Be careful to inspect the full diversity of substrates present: trunks and branches, fallen branches, hardwoods and conifers, large shrubs.
- Be careful to inspect the full range of microhabitats present: shaded and exposed, upper branches and lower branches, and trees in particular topographic positions (for example, in a draw, on an otherwise uniform slope, so long as the draw occurs within the lichen plot).
- Record relative abundances within the lichen plot. Relative abundance for each species is estimated using the following Abundance Code:

  1. Rare (3 individuals in area)
  2. Uncommon (4-10 individuals in area)
  3. Common (10 individuals in area but less than half of the boles and branches have that species present)
  4. Abundant (more than half of boles or branches have the subject species present.

Sample Procurement

1. Collect a palm-size (about 5 cm in diameter) sample of fruticose and foliose growth forms. This includes all species that are three-dimensional or flat and lobed. Even minute fruticose and lobate forms should be included. In many cases, a much smaller sample will be obtained because of the scarcity of the species.
2. Place in #2 brown paper bag labelled with appropriate codes:
   - species number (sequentially as collected)
   - plot ID code
   - relative abundance (revise as collection proceeds)
3. Place all voucher bags from one plot in a larger brown paper bag. Record plot ID code and date. Staple or tape top of large bag closed.
4. Store bags in a dry place. Specimens must be thoroughly air dried to avoid fungal decay. If specimens were wet when collected, the individual bags should be spread out and dried inside or in the sun as soon as possible.

How to Handle Uncertainties

The field crew will frequently have uncertainties about the classification of an organism. The following rules for the field crew are designed to put the responsibility for classification on the specialist, not the field crew.

1. When in doubt, assume it is a lichen.
2. When the growth form is in doubt, assume it is a macrolichen.
3. When in doubt, assume that two different forms are different species.

Once the samples are collected, mail the bags to the lichen specialist. Bags should be packed closely, but without excessive crushing, in sturdy cardboard boxes.

The purpose of these rules is to encourage the field crew to make as many distinctions in the field as possible. The specialist can later adjust the data by excluding specimens that are not macrolichens and by combining forms that were considered separately by the field crew but are actually conspecific.

Upon receiving the samples, the specialist should open the boxes immediately and check for damp lichens. If some are damp, they should be thoroughly air dried. The specialist should then identify the contents of each bag by species. In the case of mixed collections, see the special instructions in McCune (1992). The specialist should then record species name(s) on the bag and on the data sheet (appendix 5 of this chapter) and return the contents to the original bag. The bags and lichens should be stored for future reference.

Data Analysis - Quantitative Community Data

Analysis of quantitative data on lichen communities relative to air pollution can have several components: descriptive summary statistics, description of major gradients and community differences associated with those gradients, and isolation of differences related to air pollution from differences related to other sources. Each of these topics is considered below.

Descriptive Summary Statistics

1. Site by species table -- A straightforward summary of community data is a table of species abundances for each site with row and column totals or averages. Species abundances may be site-level averages if sites were subsampled.
2. Diversity statistics -- The site by species table can be supplemented with columns or rows representing species richness, such as average number of species per sample unit and total number of species recorded at the site.
3. Components of variance -- A helpful but infrequently used method for describing scales of variation within a hierarchical sampling scheme is to partition the total variation of a response variable like species richness into variation contributed at different scales. The calculations can be done using nested ANOVA, available in most standard statistical packages. The method can be used to describe the relative importance of different sources of variation or to formally test the hypothesis of no difference at a given level, based on the F-ratio.

Analysis of Spatial Gradients

Historically, the most common technique for relating lichen community data to air quality is to calculate an "Index of Atmospheric Purity" (IAP). Many different indexes have come under this rubric, but most are site scores based on the average diversity associated with the species occurring on a site. The IAP is calculated as a weighted-average ordination with many variants on the weighting scheme. Although in some cases this method can be used effectively, it cannot be recommended in general for reasons listed below:

1. Differences in IAP values will be found in any data set, regardless of the presence or absence of differences in air quality. Therefore, the name "Index of Atmospheric Purity" is potentially misleading.
2. The method does not attempt to separate community variation caused by differences in air pollution from other sources of community variation affecting species diversity. In an area thought to have pristine air quality, differences in community composition can be attributable to variation in environmental factors and disturbance history. In areas with a gradient in air quality, community differences due to variation in air quality must be separated from differences due to other factors. This is usually done by extracting the dominant compositional trends in the data using an ordination technique, then determining if any of those trends are related to air quality.

The basic steps in this procedure are described in Ludwig and Reynolds (1988), Greig-Smith (1983), and Gauch (1982). Briefly, the steps are:

Preliminary Data Adjustments

Perform any needed data standardizations and transformations.

Ordinate the Samples in Species Space

Reduce the n sample unit by p species matrix to a smaller number (typically 1-3) of composite variables representing much of the variance in the original n x p matrix.

Interpret Ordination

Relate sample scores on each dimension of the ordination space to species abundances and site characteristics. These relationships are elucidated by correlation, regression, and graphical overlays.

There is a wide menu of ordination techniques available. The choice of technique becomes important as the heterogeneity of the data set increases. Methods were reviewed by Will-Wolf (1988). Currently the most popular ordination techniques for plant community data are Detrended Correspondence Analysis (DCA), Nonmetric Multidimensional Scaling (NMS), and Bray-Curtis ordination (BC). NMS is a robust, relatively assumption free method that is

generally suitable for community data, using the Sorenson group of distance measures. Computer programs to conduct these analyses are available. Chapter 5, Sensitive Species, also explores gradient analysis and ordination.

Community Changes Through Time

Two data sets from the same community taken at two different times will always differ to some degree. Three components of observed differences through time are: (1) actual changes, (2) visual estimation error, and (3) spatial variability. Actual community changes may be due to changes in environment or interactions among species. Visual estimation error is unavoidable, but can be measured by repeated sampling close in time, and hopefully will be small. Spatial variability in communities is an intrinsic part of even the most seemingly uniform communities. With repeated random sampling, some of the observed differences through time will be derived from spatial variability. This source of variation can be virtually eliminated by using permanent plots.

Use of permanent plots changes the way differences through time should be analyzed. T-tests or standard analysis of variance (ANOVA) using time as one of the factors are not appropriate, and are less powerful than a repeated-measure ANOVA. Repeated measures ANOVA (also known as within-subjects designs) focuses on the differences through time for each sample unit.

Assuming that changes in permanent plots are found that exceed differences expected from measurement error alone, the direction of the changes should be interpreted. Interpretations of observed differences must be made on the basis of many sources of information, including data or observations on changes in air quality or other environmental variables, successional changes, disturbance history, and known ecological characteristics of the species showing the changes (i.e., sensitivities to air pollutants).

Isolating Air Pollution Effects

Lichen community monitoring provides correlative results rather than proof of causal relationships, unless the monitoring is part of a controlled, replicated experimental manipulation of air quality. Because that is rarely the case, we must rely on inferences based on the literature on lichen responses to air pollutants. These inferences must be linked to a careful attempt to separate community differences, whether spatial or temporal, into a component related to air quality and components related to other sources of variation. There are only a few basic tools for separating potential air pollution effects from other factors affecting lichen communities, but there are many variations on those tools. Six main tools are listed below, along with examples of their use.

1. Remove factors unrelated to air quality by specifying them as covariates in an ANOVA or by removing them first in a multiple regression. For example, if species diversity on trunks is related to trunk diameter and air quality, the portion related to trunk diameter can be controlled first, using that variable as a covariate or preceding the entry of air quality variables into a regression (e.g. McCune 1988).
2. Separate the main compositional trends (axes) in the data using an ordination technique (see also Chapter 5), then determine which, if any, of the axes are related to air quality by using other tools listed here.
3. Interpret results based on known relative sensitivities of species to air pollutants. Depending on the study area, much or virtually nothing will be known about the particular species. Existing compilations of species sensitivities (Wetmore 1983, Ryan and Rhoades 1991) contain only a small fraction of North American species, reflecting the preponderance of data from Europe and, to a lesser extent, eastern North America. Nevertheless, in most study areas, enough of the species will have been studied before to allow one to infer whether or not a compositional trend is related to air quality.
4. Correlation with direct, instrumental air quality data at sampling points (e.g. McCune 1988) is ideal, but this approach has seldom been used because so few of these stations exist in most areas. Direct instrumental monitoring is so expensive that, outside of urban/suburban areas, an investigator is lucky to have even one or two direct monitoring stations as reference points.

5. The spatial pattern of community differences relative to pollution sources is one of the most common approaches to make inferences about potential effects of air quality on lichen communities.

6. Temporal patterns relative to known changes in air quality can also be used. When air quality changes rapidly, as with the startup or closure of a point source, strong inferences can be made with a Before/After /Treatment/Control design. See Stewart-Oaten et al. (1986) and Eberhardt and Thomas (1991) for an in-depth discussion of this class of designs and potential problems with pseudoreplication. Repeated Measures ANOVA can also be used to make inferences about changes through time.

Determining Species Sensitivities

Where there is a clear gradient in air quality, ordination of species in sample space can be used to assess relative species sensitivities to the air quality gradient. Species ordinations are provided automatically by the ordination method DCA. Alternatively, with other ordination methods (e.g. BC, NMS) one can construct a separate ordination of species in sample space. The resulting species ranks or scores on axes that are strongly related to the air quality gradient can be used as a measure or ranking of species sensitivity. See Muir and McCune (1988) for an example.

REPORT REQUIREMENTS

All reports should meet the standards established by biological journals. Most follow **The Chicago Manual of Style**, published by the University of Chicago Press. Pay particular attention to fully describing the methods and materials used. Full details of locations and statistical techniques used should be included in an appendix. All study area locations should be described in great detail and marked on topographic maps or copies of the relevant sections thereby enabling anyone to relocate plots in later years. Make sure that the map includes suitable landmarks for easy reference. UTMs or latitude/longitude references for each site must be provided. Photographs of each study site should also be provided; again, make sure that distinctive landmarks are present to aid relocation.

Statistical techniques should list the statistical package used, version, etc. Original data, on field data sheets and in computer files, should also be included in the appendix. Any data collected but not used in subsequent analyses should be marked in some way with a brief explanation of why it was not used.

Future Work

This section should include recommendations on additional data that needs to be collected or sites that should be studied. The frequency of future monitoring should also be discussed.

Management Recommendations

If future work is being recommended, the cost and timing should be included here with a succinct rationale. This information will help managers to program budgets or seek funds if they are not immediately available. If management options are provided, they should be stated concisely and correlated with evidence presented in the report. Provide managers with evidence and rationales useful in preparing the environmental assessment of the management action if funds were available to implement it.

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Chapter 4 Appendix 1

TRANSECT DATA SHEET

TRANSECT DESCRIPTION

Direction of reading (e.g., Left to Right, or Top to Bottom) _____

Measuring device set points (positions of nails or stakes along the tape or meter stick, holding it in place):
A _____ B _____

Transect set points: Beginning _____ End _____ Transect length _____

INTERCEPT LENGTHS (in centimeters)

SPECIES_____	Total	SPECIES_____	Total
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
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_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
TOTALS	_____		_____

% COVER total cover x 100% = FREQUENCY

transect length

FOOTNOTES:

COMMENTS:

Chapter 4 Appendix 2

LICHEN PLOT DATA SHEET

SITE NAME DATE TIME

PLOT ID (alphanumeric code) SURVEYOR(S) (include affiliation)

LICHEN SPECIES (for each species, give scientific name or acronym, collection number of voucher, color and other features used in field identification)

SUBSTRATE (record kind or name, size or diameter, condition, surface features, and modifying factors, e.g., rain tracks, or adjacent branches)

PLOT TYPE (e.g., dotiometer transect, photographic quadrat, etc.)

POSITION OF PLOT (height above ground, inclination, slope and aspect, etc.)

SITE DESCRIPTION (beyond information on general site description form)

DIRECTION OF PLOT (compass bearings, landmarks, trail references, markers)

PHOTOGRAPHS (roll and frame numbers; subject; direction; where taken from)

COMMENTS (include diagram of plot and measurements of selected thalli)

Chapter 4 Appendix 3

Recommendation -- The following dichotomous key can be used as a decision tree for recommended sampling methods. However, it is important to retain more flexibility than can be indicated in this tree, since many situations will dictate an approach different than that recommended here. The letter following each entry in the key corresponds to the rows in Appendix 4, "Synopsis of Recommended Methods."

1a. Rapid, broad-scale survey method needed, to be done by field crew with minimal lichen training
Whole plot/voucher method -- A

1b. Intensive, more precise method needed, to be done by field crew with good lichen identification skills.
2a. Sampling on trees or shrubs
3a. Trunks
4a. Communities mostly sparse
Trunk Cylindrats -- B

4b. Communities mostly dense
5a. Objectives require random or otherwise non-subjective sampling
Trunk quadrant pairs -- C

5b. Objective is to monitor well-developed examples of target communities
British fixed-point method -- D

3b. Branches
6a. Open-grown trees and shrubs
7a. Objectives allow small datable branches
Equal-aged branchlets -- E

7b. Objectives require study of larger branches
Branch centers -- F

6b. Forest trees
8a. Tall forests without easy branch access
Twig pickup -- G
or Litter pickup -- H

8b. Good access to lower- or mid-crowns
Branch centers -- F

2b. Sampling on ground or rock
9a. Mostly sparse cover
Simple quadrat -- I

9b. Dense cover
Simple quadrat -- I

or Fixed points, ground -- (application to lichen biomonitoring not known)

Chapter 4 Appendix 4

Synopsis of recommended methods. Use the preceding key as a guide to which methods might be appropriate given particular study objectives.

Name	Sample Unit (SU)	Arrangment of SU's	Typical # of SU's per site	References
A. Whole plot/ voucher	Circular Plot, 36.6 m (120 ft) radius	Single plot, random location in homogeneous stand	1	McCune 1992
B. Trunk cylindrat	t2, 1-m half cylindrats per tree, 0.5-1.5m high on tree. Average both values from half cylindrats	Randomized within specific tree selection criteria (e.g. particular tree species or size range)	10 per target tree species or 24 per mixed species stand	McCune 1988, Muir & McCune 1988
C. Trunk quadrat pairs	2, 0.2 x 0.5 m flexible quadrats on 2 opposite sides. Average the 2 quadrats	Randomized or nearest trees to pre-determined points along parallel transects	10 per target tree species of 24 per mixed species stand	Lesica et al. 1991
D. British fixed point	Grid point in 18 x 27 cm quadrat	Points in fixed-location grid on trunk, subjective grid placement	10-20 grids per site, 100 points per grid	Wolseley and James 1990; Looney and James 1990
E. Equal-aged branchlets	Variable-Length branch segment of known age	Randomized, one branchlet per tree or (original method) subjective selection of twigs with most lichen cover, all branches from same tree	20 or 10 (original method)	Denison & Carpenter 1973
F. Branch centers	1 m long branch segment centered on midpoint of branch	Branches 1 m long and 2-8 m above ground, choosing branch nearest to vertical line from pre-determined points on parallel transects	10 per target tree species or 24 per mixed-species stand	Lesica et al. 1991
G. Twig Pickup	Fallen twigs or branches 10 cm long	Branch with highest lichen cover 4 x 1 m box centered and equidistant on eight 13 m radii		Geiser 1991
H. Litter pickup	Lichen Litter picked up in circular plot, 2m radius, sorted & weighted	Randomized along parallel transects; standardize sampling to season when violent storms are rare	10-20 depending on canopy uniformity	McCune 1991
I. Simple quadrat	0.2 x 0.5 m or 1 x 1 m quadrat; smaller quadrat for more dense communities	Equal or randomized intervals on parallel transects	45	Lesica et al. 1991

Chapter 4 Appendix 5

Lichen Identification Data Sheet

Plot No._____ Date:_____

Lichen Specialist_____

A = Abundance rating # = Bag number													
	Species Name	Data to Enter		Data from collecting bags									
		Sp. code	A	#	A	#	A	#	A	#	A	Comments	
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
15													
16													
17													
18													
19													
20													

Abundance codes: 1 = rare (≤ 3 individuals seen), 2 = occasional (4-10 individuals seen), 3 = common (>10 individuals seen), 4 = abundant (more than half of the branches seen have this species).

Species codes: Refer to master list for 4-character lichen species code names.

Identification of Sensitive Species

Jayne Belnap, Lorene Sigal, William Moir, and Sharon Eversman

We describe procedures for determining the responses and sensitivities of lichen species to air pollutants. Pollutant chemicals in their primary forms and as derivatives such as ozone or acid rain can cause acute and chronic injury to lichens and other cryptogamic plants. Fumigation studies are designed to reveal plant responses to air pollutants under controlled conditions in enclosed exposure systems, generally through changes in physiological processes. Gradient studies are designed to show responses at predetermined locations along an air pollution gradient. They usually measure visible damage and are done around existing or proposed pollutant sources. Both types of study have limitations, but are useful for detecting air pollution effects and tracking changes in existing or proposed pollution sources, as well as for understanding natural succession and variability.

OVERVIEW

Air pollutants can cause both acute and chronic effects on lichens and other organisms such as liverworts, mosses and cyanobacteria. Atmospheric deposition and concentrations of air pollutants great enough to cause acute injuries to vegetation, such as necrosis, morbidity, and mortality, are usually found only around point sources. Examples of large point sources include fossil-fuel fired steam electric generating plants, gas purification plants, metal smelters, aluminum production plants, cement plants, chemical plants, and pulp mills. Historically, sulfur dioxide (SO_2) and fluorides have received the most study, and acute effects to lichens and other vegetation around point sources of these pollutants are well documented (Shriner et al. 1990; Nash 1988, 1972; Ferry et al. 1973; Gilbert 1973).

Primary pollutants such as SO_2 and fluorides are of biological concern in the same chemical form as they are emitted. Secondary pollutants are created as a result of chemical reactions involving primary pollutants during transport in the atmosphere. Ozone (O_3), peroxyacetyl nitrate (PAN), and acid rain are examples of secondary pollutants. Ozone and PAN are formed in photochemical reactions involving nitrogen oxides (NO_x) and hydrocarbons mostly emitted by vehicles. Acid rain is formed as SO_2 and NO_2 are oxidized to sulfuric acid (H_2SO_4) and nitric acid (HNO_3) during atmospheric transport. Visible injuries such as bleaching and necrotic spots due to acute and chronic ozone exposure occur in the Los Angeles basin (Sigal and Nash 1983), the central Sierra Nevada, and in the eastern United States (Shriner et al. 1990). Acute foliar injury to vegetation from acid rain is rare, with few documented cases (Shriner 1986).

Other types of pollutants, commonly referred to as "air toxics" (e.g., industrial organics, agricultural pesticides, trace metals, and metalloids) are of concern as well (Moser et al. 1992), although there is limited information on the effects of these compounds. Semi-volatile and persistent organics are transported and deposited via atmospheric processes. High concentrations of toxic organics and pesticides have been found in fog and in lichen tissues (Glotfelty 1987; T. Moser, personal communication). Studies have demonstrated that various trace metals affect lichens (Nash 1972, 1975).

A number of other pollutants, known to be present but for which there is little or no information, are of less interest for one or more of the following reasons:

1) pollutants are widely distributed, but seldom cause damage because ambient exposures have mean ratios and mean concentrations that are too low to affect lichens (e.g., ethylene, carbon monoxide);

2) pollutants are not widely distributed, occurring only as accidental releases (e.g., chlorine, ammonia); and/or

3) pollutants are widely distributed, but seldom cause damage to vegetation because the high concentrations necessary to cause damage are rare (e.g., hydrogen sulfide, hydrogen chloride) (Shriner et. al. 1990).

Chronic injury develops after long-term or repeated exposure to sub-acute concentrations of multiple air pollutants associated with urban development, such as small stationary sources and large numbers of mobile sources. Chronic injury is less likely to involve death of tissues, but is likely to involve nonspecific symptoms of plant stress such as chlorosis (loss of chlorophyll, yellowing) and reduced growth. At the community level, sensitive species may disappear from the population. Lists of species known to be sensitive to pollutants under certain conditions have been published (Nash and Greis 1991).

In this chapter we review procedures for determining the responses and sensitivities of lichen species to air pollutants. The most widely used methods are gradient studies and fumigation studies. Historically, gradient studies usually involved observations of visible injury, species richness, or species abundance correlated with pollutant exposure, when exposure data were available. However, these studies did not address proof of cause/effect relationships.

More recently, fumigation studies involving some variation of a closed chamber with air movement through the chamber, or chamberless field fumigations, have been used to develop quantitative relationships between concentrations of air pollutants and various plant responses, thereby establishing cause/effect relationships. Controlled fumigation studies coupled with gradient studies give the best information. For SO_2, scales of sensitivity have been independently established based on controlled laboratory fumigation results and on field observations (Nash 1988). By non-parametric correlation analysis, significant agreement among the various scales is shown and this strongly supports the inference that lichens actually do respond to SO_2 (Nash 1988). For other air pollutants, data are currently insufficient to establish precise sensitivity scales. However, pollutant concentrations measured in the field are high enough to adversely affect species that are demonstrably sensitive to fumigation experiments with air pollutants such as ozone, hydrogen fluoride, and zinc (Nash 1988).

The information collected from gradient and fumigation studies can be used in relation to applications for Prevention of Significant Deterioration (PSD) permits, to show that estimated emissions from a proposed facility may unacceptably affect resources of a Class I area. For areas in which there are no data, the information provides baseline or control data to which subsequent data on effects of new pollution can be compared. For areas where there are historic data, the information may allow comparisons over time that contribute to understanding successional patterns, the natural variability of species, and the effects of existing air pollution. Following permitting, construction, and operation of air pollution sources, continued measurements can show the magnitude and significance of changes in air quality.

FUMIGATION STUDIES

Fumigation studies are designed to show measurable responses to air pollutants, singly and in combination, under controlled conditions in more or less enclosed exposure systems such as continuously stirred tank reactors (CSTRs), open-top chambers, branch chambers, and miniature cuvette chambers. Pollution studies are occasionally done in the field without chambers, using techniques such as Zonal Air Pollutant Study (ZAPS) and simulated acidic rain exposures.

The most commonly measured responses are selected physiological processes. Lichen physiological processes sensitive to fumigation appear to be: nitrogenase activity, K^+ efflux/total, electrolyte leakage, photosynthesis, and respiration pigment status, in order of sensitivity (Fields 1988). There are still problems with fumigation studies because sensitivity depends on such factors as concentration and duration of exposure, environmental conditions, and status of the thallus during exposure (Fields 1988). In addition to the very important influence of thallus hydration level, it

is known that different portions of a thallus vary in their physiological response (Nash et al. 1980, Karenlampi 1970; Moser and Nash 1978; Moser et al. 1983).

Long-term laboratory fumigation studies to determine chronic injury are not currently considered feasible because of problems in maintaining specimen viability in growth chambers. However, short-term fumigation studies (less than a month) are valuable for explaining the mechanisms of plant response and establishing exposure-response relationships. Since lichen material does not generally do well for even short times in artificial chambers, attention must be paid to the treatment of specimens and their physiological condition throughout the study (Link and Nash 1984, Pearson and Benson 1977, Pearson 1970).

Although air pollution is a complex phenomenon involving multiple contaminants, most fumigation studies have used only single pollutants under given sets of conditions. Most laboratories are not equipped to perform the factorial experimental designs necessary to study the responses of plants to pollutant mixtures because of limitations in the number of exposure chambers available. However, additive, antagonistic, and synergistic responses to two or more pollutants are known for vascular plants (Shriner et. al. 1990), and there is no reason to think that lichen responses to multiple pollutants are any less complex.

Exposure Systems

Gaseous and particulate exposure systems range from plastic bags (Anderson 1976) to elaborate chamber systems with automatic control of environmental parameters and pollutant concentrations (Heagle and Philbeck 1984). Over time, systems for studying the effects of single or multiple pollutants have become more sophisticated (Lange et al. 1984). However, there is no perfect all-purpose exposure system. Essential requirements for plant exposure systems, whether they be controlled environment, greenhouse, or field, are listed below in order of their importance (Heagle and Philbeck 1984):

- uniform pollutant concentration throughout chamber and between chambers;
- uniform environment throughout chamber and between chambers;
- non-reactive surfaces;
- precise control of pollutant concentrations; and
- environment resembling ambient conditions.

In addition, the accurate determination of pollutant concentrations and environmental parameters within and between chambers requires acceptable calibration procedures which should be followed before and during an experiment. Properly designed and controlled experimental techniques and comprehensive reporting are important for other researchers working in related areas and for regulatory agencies in developing dose-response curves for the establishment of air quality standards (Drummond and Pearson 1979). The chamber systems described below have the advantage of multiple replicates, but are usually too expensive to build for individual lichen studies. However, it may be possible to conduct experiments at existing facilities. Chamber studies are most useful for determining the effects of pollutants on lichen physiology (e.g., photosynthesis, nitrogenase activity, respiration) and ultrastructure.

Controlled Environment Chambers

Controlled exposure conditions can be attained in three ways:

- by converting commercially available controlled-environment chambers to pollutant exposure capability;
- by putting smaller exposure chambers (plexiglass boxes, bell jars or even plastic bags) within a single controlled-environment chamber; or
- by adding environmental controls to exposure chambers.

Currently, the most widely accepted system is the continuously stirred tank reactor (CSTR) system, which ensures rapid mixing of pollutants injected into the system and uniformity of conditions within the chambers. The primary concern is how well the results reflect plant response under field conditions, since the dynamic nature of field environmental conditions (e.g., temperature, light, relative humidity) is not reproduced in these systems. (Shriner et al. 1990).

Mini-Cuvettes

An additional form of exposure chamber used successfully for a number of years is a cuvette which encloses a branch or a portion of a branch in a mature tree canopy (Legge et al. 1977, Lange et al. 1984, Amundson et al. 1992). This approach permits the measurement of mechanistic plant tissue

responses, such as photosynthesis, **in situ** in a canopy, eliminating many of the concerns regarding the effects of controlled exposure chambers. In small chambers it is possible to introduce pollutant gases in sub-parts per million as part of the inflow using the flow-through air exchange principle. However, this type of pollution exposure has not yet been used with lichens.

Open-Top Field Chambers

Open-top chambers used in the field duplicate the ambient environment as closely as possible while allowing control of pollutant concentration within the chambers. Appropriate experimental designs for using open-top chamber systems include pollutant-free and ambient chambers as well as non-chambered control plots to estimate any chamber effects. The open-top chambers are the best currently available experimental technique for developing functional relationships useful for predictive purposes (Shriner et al. 1990).

ZAPS Systems

The acronym ZAPS stands for Zonal Air Pollution Systems, referring to means of delivering air pollutants in the field for relatively long-term testing with no control over environmental conditions. The two systems described dispensed only SO_2.

Moser et al. (1980) used a small system at Anaktuvuk Pass in Alaska to fumigate caribou forage lichens *Cladina stellaris*, *C. rangiferina*, and *Cetraria cucullata*. Anhydrous SO_2 was dispensed through paired holes along an aluminum pipe. Sulfate concentrations were measured at intervals upwind and downwind from the pipe.

One large system (ZAPS I) was operated by the US Environmental Protection Agency from 1975-1979 in Custer National Forest in southeast Montana to predict possible effects of emissions from new coal-fired power plants on components of a grassland ecosystem. A duplicate system (ZAPS II) operated from 1976-1979. Both ZAPS I and ZAPS II had four plots, each with different SO_2 concentrations. In 1978, sulfation plates were set at 10, 50, and 100 cm above the soil surface at five locations per plot, adjacent to transplanted lichens. The plates demonstrated slight differences in sulfation levels with elevation above ground level. In general, less SO_2 was recorded at the lowest level, 10 cm, than at the upper two levels, indicating little or no pooling of SO_2 at ground level and vertical variations in SO_2 concentrations within plots (Eversman 1979).

Acid Rain Simulation

Contaminated rainfall has been simulated in the field and laboratory using a variety of approaches, from watering cans to back-pack sprayers and irrigation sprinklers, to very elaborate systems designed to permit variation of all of the major components of dose on a programmable basis (Shriner 1979). Rain simulators have been used in combination with a ZAPS-type fumigation system and are being used with open-top chambers to study the combined effects of acid rain and gaseous pollutants. The more sophisticated systems can reproduce the physical and chemical characteristics of natural precipitation including:

- rainfall rate (intensity controllable between 0.5 and 2.7 cm/hr);
- droplet size range (0.1 - 3.2 mm);
- chemical composition, through metering pumps which supply carefully controlled concentrations of ions to a deionized water stream.

GRADIENT ANALYSIS

The gradient analysis method assumes that measurable attributes of affected plants vary along causative environmental gradients. This assumption is illustrated in figure 1, which shows unimodal relationships among the importance values of three lichens and one moss along a moisture gradient (Flock 1978).

Species are best represented at their environmental optima, and will not be found at limits outside their tolerances. In addition to air pollution, other gradients influencing lichens include climate and substrates, as well as gradients of disturbance factors such as grazing intensity, fire regimes, or trampling.

Gradient analysis can be designed to show measurable responses of lichens at predetermined locations along an air pollution gradient. Studies are usually done around existing or projected sources of contaminants. The sources can be point sources like power plants or diffuse sources like the Los Angeles basin. The most commonly measured response variables are visible injuries such as bleaching and thallus deformation, changes in community structure such as species richness or cover, and physiological

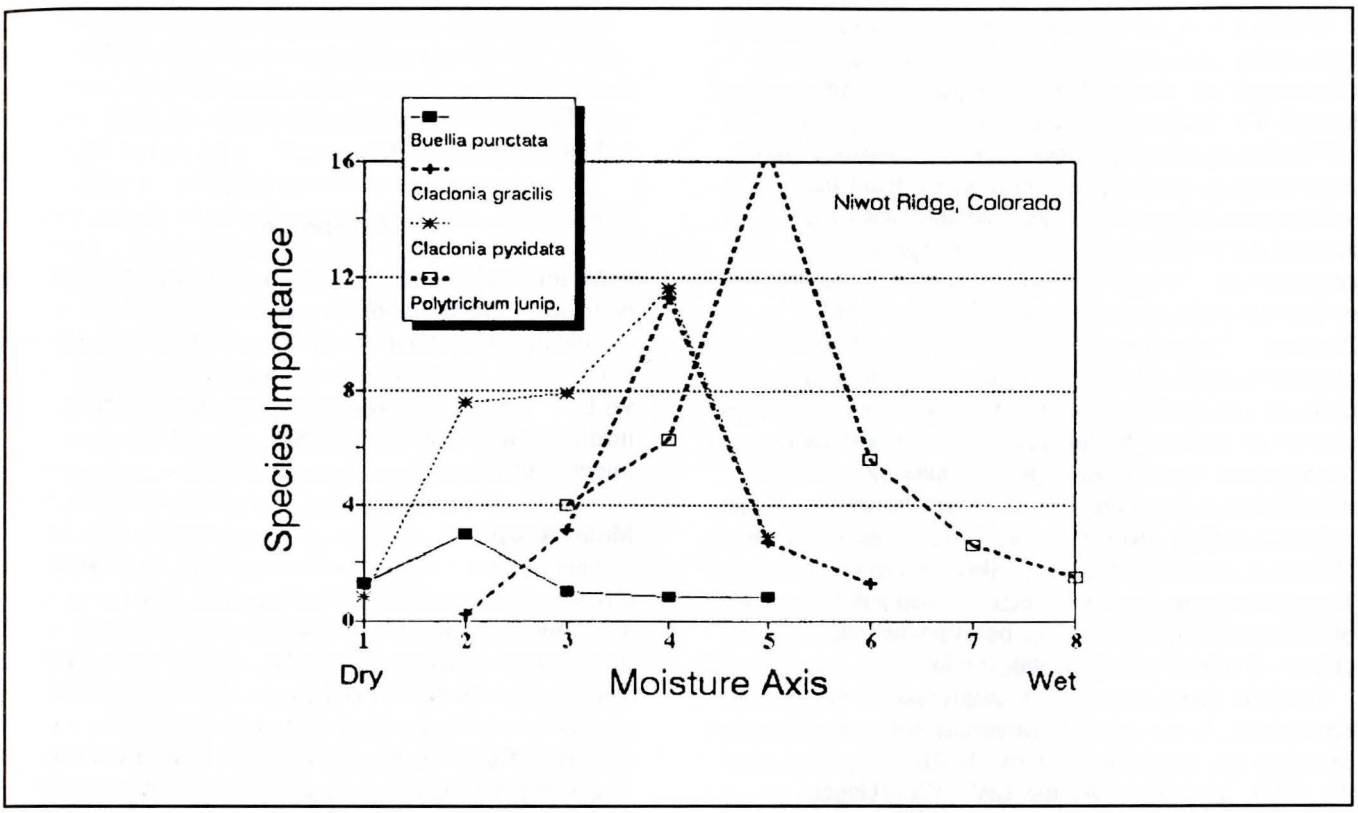


Figure 1.—Importance of 4 cryptogams along an indexed moisture gradient from dry to wet (from Flock 1978).

processes such as photosynthesis, nitrogenase activity, element uptake, membrane integrity (electrolyte leakage), pigment quantity, degradation, and fluorescence.

Gradient analysis is appropriate for studies of the effects of air pollutants on lichens, since pollutant loadings often vary with distance from point sources. It is not necessary for other environmental factors to vary continuously in the real world in order to use gradient analysis. There are often sharp discontinuities in spatial or temporal dimensions. Gradient analysis is also useful for analysis of regional effects derived from non-point sources. In this case, the regional airshed is assumed to be large enough that there is wide latitude in pollutant exposure levels.

The use of lichens in gradient studies has some limitations of which the researcher should be aware (Johnson 1976). These include:

- difficulties associated with identification of species;
- determination of the best indicator species for the particular study; and
- demonstration that the observed distribution patterns reflect pollution stress and not other biotic and/or abiotic factors.

This last problem can be especially vexing, since pollution impacts on lichens may be minor relative to more direct effects of microclimate, substrates, trampling, or extremes of weather variability.

The theory of gradient analysis is well developed (Ter Braak 1987). Measurements are taken across a variety of environments, and differences among the measures are assumed to be controlled by environmental differences between the sites. Data can be analyzed in several ways, which are summarized here from the treatise by Ter Braak and Prentice (1988). Readers are referred to Ludwig and Reynolds (1988) for mathematical details.

Direct gradient analysis is used where lichen abundances, probabilities of occurrence, or morphological or physiological symptoms are described as a function of measured environmental variables. Each sample can be associated with its elevation, soil pH, on-site air chemistry measurements, or long-term exposures to pollutants as given, for example, by air quality maps. Measured attributes of lichens or other cryptogamic plants are plotted along an elevation, soil pH, or pollution loading axis. The relationship between the lichen variable and the environmental quantity can be determined by regression.

Gradient analysis is also applied by using community composition as a measure of environmental values when the regressions are known. Ter Braak and Prentice refer to this as a calibration problem. If environmental variables are not measured, one can use information from the species assemblage to compute abstract axes that account for much of the variation in species composition. This is indirect gradient analysis, an ordination problem according to Ter Braak and Prentice. Ludwig and Reynolds (1988) define ordination as "a set of techniques in which sample units are arranged in relation to one or more coordinate axes such that their relative positions to the axes and to each other provide maximum information about their ecological similarities". Indirect gradient analysis is most useful when lichens of known sensitivities to air pollutants are present. The method may be less effective when subtle pollution effects are likely to be overwhelmed by effects of other environmental variables.

Gradient analysis is widely employed by ecologists. A variety of multivariate software packages are available, such as ORDIFLEX, PC_ORD, DECORANA, and CANOCO (Gauch 1977, Hill 1979, Ter Braak 1988). Which of the various methods to use on a particular data set, and interpretations derived from the various multivariate analyses, are discussed by Ter Braak and Prentice (1988) and Ludwig and Reynolds (1988).

ANALYTICAL TECHNIQUES

Many analytical techniques are available for examining the effects of air pollutants on lichens. Which techniques are appropriate for a given study will depend on the type and levels of pollutants involved, the lichen species used, the nature of the research (gradient studies as opposed to fumigation work, for example) and the resources available.

The analytical techniques used most often are listed below. These are generally applicable to both field and laboratory work, unless otherwise indicated. Using any of these techniques will require additional literature research to determine their suitability for the proposed work, instrumentation needed, specific procedural protocols and other recommendations, including quality assurance, quality control, levels of injury and levels of exposure.

Characteristics measured in air pollution studies include individual morphological and physiological characteristics and population characteristics. Specific limitations and/or advantages of each technique are noted here.

Morphology

Morphological analyses detect change to the form or structure of an organism. A disadvantage to morphological analyses is that not all pollution injury will produce a response that can be distinguished visibly. Morphological changes can be analyzed macroscopically, without optical aids, or microscopically.

Macroscopic

This is the unaided visual assessment of lichen characteristics such as coloration, size, and appearance of the species chosen. An advantage to macroscopic analyses is that they can be done with limited instrumentation and funds. These types of analyses can also be done with still or video cameras. Comparisons can be made before and after treatments, throughout time at the same sites, and/or along a distance gradient from a known pollution source.

Microscopic

Microscopic analyses allow for observation of cellular injuries that may or may not be otherwise visible. Two types of microscopes are used in analysis of injury assessment in lichens: light microscopes and transmission electron microscopes. Light microscopy has been useful in estimating the effects of pollutant stress, especially of SO_2 on algal cells. Eversman (1978) and Will-Wolf (1980) estimated the percentage of plasmolyzed algal cells in fresh mounts of lichens exposed to SO_2, showing that the percentage of plasmolysis increased significantly with increasing exposure time. Observations of fresh mounts use color and shape of chloroplasts, which comprise most of the algal cell, to estimate the algae's health. Healthy cells are green and round; plasmolyzed cells are yellowed and shrunken. Kauppi (1980) demonstrated the usefulness of fluorescence microscopy in determining the status of algal cells.

An alternative method to determine dead and plasmolyzed algal cells uses fixed and embedded sections of lichens stained with toluidine blue (Holopainen and Kauppi 1989). Empty cells are

interpreted as dead; cells with shrunken contents are considered plasmolyzed. Again, increased plasmolysis was observed with SO_2 fumigation.

Some cells are too small to accurately observe with light microscopy. Transmission electron microscopy is a helpful tool to visualize or verify cellular effects when physiological effects have been observed. Many characteristics of chloroplasts and mitochondria change in intermediate stages of injury; severe injury results in near collapse of all recognizable cell structure (Eversman and Sigal, 1984, 1987; Holopainen and Kauppi 1989). Some specific changes recorded are: deformation of thylakoids and pyrenoglobuli of the chloroplast, swelling followed by degeneration of mitochondria, unusual accumulation of starch granules and lipid bodies, and disappearance of recognizable organelles and membrane structures.

Since cellular changes due to pollutant stress can mimic ordinary seasonal stress or normal senescence, it is important to have fresh material and to control conditions under which samples are stored before fixation so that moisture and light conditions of the control and treated samples have been identical. Unfortunately, all pollutants seem to cause the same ultrastructural changes in algal cells so one cannot distinguish among pollutants. It does seem, however, that the oxidant pollutants such as O_3 and PAN cause cellular damage at lower concentrations and in less time than SO_2 and acid treatments (Eversman and Sigal 1987, unpublished data).

Physiology

Physiological analyses detect alteration to the normal functioning of an organism or any of its parts. There are many techniques available to determine physiological changes resulting from air pollution; the more commonly used ones are listed here. All require some level of instrumentation. Most are sensitive to seasonal or geographical variation, which should be taken into account when sampling. It is important to recognize that lack of homogeneity within a lichen thallus has been documented for various characteristics (e.g., photosynthesis, nitrogenase activity, chlorophyll concentrations), and care must be taken if experimental material does not include the entire thallus (Karenlampi 1970, Nash et al. 1980, Moser and Nash 1978, Moser et al. 1983).

Pigments

Chlorophyll and its degradation products are the pigments most often used to assess pollution injury. Studies have shown chlorophyll levels to be significantly affected by pollutants (Beekley and Hoffman 1981, Belnap and Harper 1990, Garty et. al. 1985, Henriksson and Pearson 1981, Kardish et. al. 1987, LeBlanc and Rao 1973, Nash 1973, Nash 1976, Ronen and Galun 1984, Eversman 1980, Moser et al. 1980). Researchers have traditionally reported effects on chlorophyll as total chlorophyll concentrations, percent reduction in chlorophyll concentrations, chlorophyll a:b ratios, or the ratio of chlorophyll to its degradation products.

There are several means of quantifying chlorophyll and degradation products. The more commonly used method is extraction of these pigments in dimethyl sulfoxide (DMSO). Extracts are then spectrophotometrically scanned to determine levels of chlorophyll at optical density 435 nm and degradation products at optical density 415 nm (Ronen and Galun 1984). It is important to reduce chlorophyll degradation during sample analysis; techniques to do so include the use of red light during sample preparation, cold storage, and standardization of time between extractions and analysis. For further details on these techniques, see Brown (1980), Brown and Hooker (1977) and Vernon (1960).

Nitrogenase Activity

Lichens with cyanobacterial phycobionts are capable of "fixing" atmospheric nitrogen, converting it into a form of nitrogen usable by vascular plants. These lichens may be important contributors of nitrogen to the ecosystems in which they occur (West and Skujins 1978, Evans and Ehlringer unpublished data, Denison 1973). Atmospheric pollutants have been shown to affect nitrogenase activity levels (Belnap 1990, Denison et. al. 1977, Hallbom and Bergman 1979, Hallgren and Huss 1975, Henriksson and Pearson 1981, Kallio and Varheenman 1974, Sheridan 1979, Sigal and Johnston 1986).

Measurement of nitrogen fixation is difficult and costly. Measuring nitrogenase activity, however, is fairly simple and cheap. In the presence of the nitrogenase enzyme, acetylene is converted to ethylene. Consequently, nitrogenase activity levels are reflected in the amount of ethylene that is produced. Levels of ethylene and acetylene can be measured on a gas chromatograph. Incubation in an acetylene atmosphere can be done in the field or the laboratory, using gas-tight containers with an

atmosphere of approximately 10% acetylene under standard conditions of light and temperature in the lab, or ambient conditions in the field (see above references). In the field, portable chambers can be placed over the experimental material, and vacutainers can be used to transport samples (Rychert and Skujins 1974).

Respiration (Gas Exchange)

A significant decrease in respiration rates of lichens exposed to increasing pollutant levels has been demonstrated repeatedly in the literature (Baddeley et al. 1971, Eversman 1978, 1979, 1980; Fields and St. Clair 1984a, 1984b). The most common methods of measuring respiration rates employ an oxygen electrode to measure O_2 absorption or an infrared gas analyzer to measure CO_2 evolution in the absence of light.

Photosynthesis

Photosynthetic rates have been determined using chambers and gaseous $^{14}CO_2$ (Fields and St. Clair 1984a, Hallgren and Huss 1975, Ross and Nash 1983, Sigal and Johnston 1986, Lechowicz 1982), immersion in liquid $^{14}CO_2$ (Fields and St. Clair 1984a) or measured as the rate of CO_2 absorption or O_2 evolution (Beekley and Hoffman 1981). Determining net gas exchange in either flow-through or closed chamber systems depends on the availability of an infrared gas analyzer (IRGA), while estimating gross photosynthesis with $^{14}CO_2$ labeling techniques requires a liquid scintillation counter.

Chlorophyll fluorescence is a set of response variables associated with the light reactions of photosynthesis, particularly those associated with photosystem II. It is a convenient, sensitive, rapid, and non-invasive way to assess photosynthetic efficiency. The fluorescent behavior of photosynthetic systems most commonly analyzed is the kinetics of the fluorescence rise to maximum levels at saturating light. This induction curve is markedly altered after exposure to air pollutants. Fluorescence is measured using a chlorophyll fluorometer, or a modified gas exchange system (T. Nash, personal communication).

All methods which measure photosynthesis in lichens have found decreased photosynthetic rates with exposure to pollutants. Determining which methods to use, (instrumentation required, replication and portability for field work) is discussed in Link et al. (1984).

Membrane Integrity

Exposure to sulfur dioxide and trace metals has been shown to compromise membrane integrity in lichens. This has been demonstrated using measurements of potassium efflux, total electrolyte leakage, and scanning electron microscope work (Beckerson and Hofstra 1980, Hart et al. 1988, Pearson 1985, Pearson and Rodgers 1982, Belnap and Harper 1990, Fields and St. Clair 1984b, Moser et al. 1983). Membrane leakage can be estimated by measuring the conductivity of de-ionized water before and after immersion of the lichen. Instrumentation is limited to a conductivity meter, which is both cheap and portable.

Elemental Analysis

This is covered in Chapter 7.

Population Characteristics

These are species characteristics which can be measured in the field and require little or no instrumentation. Measurements are made using quadrats as samples. Variables such as reproductive characteristics, growth rates, mortality, presence/absence, cover, and biomass can be measured within quadrats. These measurements can be done by eye (Daubenmire 1959; Armstrong 1991; Karenlampi 1970, 1971) or by photography. Photography, whether done with a traditional camera or with video, can be computer-analyzed. Details are provided in Chapter 4. Table 1 provides an example of studies done with different pollutants and the effects of those pollutants on chosen lichen species.

EXPERIMENTAL DESIGN

The experimental design of studies addressing the sensitivity of lichen species to air pollutants should be similar to any research design. The choice of method should be determined by the nature of the problem, the questions to be answered, the anticipated use of the data, site characteristics and available resources. First, decide upon a set of broad initial hypotheses or working questions. Then do a literature search to determine the lichen species in the targeted area, the sensitivity of these species to the air pollutants in question, and other related work. Related research might include work with the same species in the same locality or with the same pollutants, and/or using the same analytical techniques.

Table 1. — Selected authors and papers describing effects of various pollutants on lichens. Source: Eversman and Sigal (1985).

Pollutant	Effect(s) observed
Sulfur dioxide	
Baddeley et al., 1973	Respiration decreased
Puckett et al., 1973, 1977	Membrane permeability increased
Tomassini et al. 1977	Permeability increased (ions lost)
Richardson & Nieboer, 1983	Review: physiology, ecology
Fields & St. Clair, 1984	Permeability increased, photosynthesis decreased
Holopainen & Karenlampi, 1984	Ultrastructural changes
Ozone	
Brown & Smirnoff, 1978	No effect on photosynthesis
Rosentreter & Ahmadjian, 1977	No effect on chlorophyll content
Nash & Sigal, 1979	Photosynthesis decreased
Ross & Nash, 1983	Photosynthesis decreased
Sigal & Nash, 1983	Distribution limited, morphological changes
Eversman & Sigal, unpub. ms.	Ultrastructural changes, photosynthesis decreased
PAN (peroxyacetyl nitrate)	
Sigal & Taylor, 1979	Photosynthesis decreased
Eversman & Sigal, 1984	Ultrastructural changes
NOx	
Nash, 1976	Loss of chlorophyll pigment
Fluoride	
Nash, 1971	Loss of chlorophyll pigment
LeBlanc, Comeau, Rao, 1971	Plasmolysis, color change, loss of chlorophyll pigment
LeBlanc, Rao,Comeau, 1972	Species die, disappear
Roberts & Thompson, 1980	Discoloration, morphological changes; species disappear
Takala, Kauranen, Olkkonen, 1978	Morphological changes; species disappear
Acid precipitation	
Denison et al., 1977	Nitrogen fixation decreased
Robitaille, LeBlanc, Rao, 1977	Loss of species
Lechowicz, 1982	Photosynthesis decreased
Lechowicz, 1984	Growth decreased
Sigal & Johnston, 1986	Photosynthesis decrease
Sigal & Johnston, 1986	Photosynthesis, nitrogen fixation decrease
Eversman & Sigal, unpub. ms.	Ultrastructural changes

Available resources should be identified. Resources include people, dollars, time, equipment, transportation, access to study areas, collaboration possibilities, and any other factor that may influence your capability to carry out the intended research.

From the collected information, develop a set of refined hypotheses. These hypotheses are a distillation of the initial broad hypotheses into questions that are feasibly answerable given available resources.

Sample design is the next stage. Sample design will vary greatly depending on geography, accessibility, species used, analytical techniques chosen, and resource availability. Elements common to most study designs include:

Species Selection

The species selected for study will depend on the questions being asked, the types of pollutants and species present, and available resources. Make a list of likely candidates from an inventory of available species and species known to be sensitive to the pollutants in question. Select species from the list based on the questions being asked and available resources. Another option is to run preliminary analyses on species that are selected for reasons other than known sensitivity, such as abundance or ease of collection, to ascertain whether they are suitably sensitive to the pollutants present.

Gradient Sampling

Gradient sampling can vary from survey data to more elaborate vegetation sampling techniques (Chapter 4). Lichens respond to natural gradients of moisture, temperature, light, nutrients, and biotic competition as well as to anthropogenic influences. The response of lichens to more direct natural or anthropogenic influences may overwhelm the subtle effects of pollution (Jackson and Gough 1990). Any secondary effect of pollution upon lichens is likely to be confounded with the influence of other variables. To minimize effects of confounding factors, we recommend a sample design which incorporates known pollution levels.

If the pollution source is local and fixed and the pollution "shadow" is known, sampling should be spatially explicit with respect to the source. Considerable environmental variation will still remain, which again may overwhelm possible secondary influences of the pollutant. Therefore, use a sample design that limits other sources of variation as much as possible. This can be done by narrowing the range of soil, topographic, grazing, or other variables across the widest range of pollutant loadings.

Direct measurements of pollution loadings should be obtained at as many of the sample sites as feasible. If only a few direct measurements can be obtained, they should be concentrated at places where the highest and lowest pollutant levels occur, with a few near the middle (to test for linearity). If no direct measurements of pollutant loadings are possible, then an attempt to measure pollution indirectly can be made by using known sensitive or accumulator species. This is the calibration problem referred to above. Samples of the accumulator species can be harvested for chemical analyses (Chapter 7). These samples should also be spread across the gradient, or if this is not practical, then near the high, low, and middle portions of the gradient.

If the pollution source is regional or non-local, we suggest using gradsect sampling (Austin and Heyligers 1989). This is a method of efficient sampling across the most important environmental features that control species distributions in a region. The concern is not so much with total floristic variation across a region as with floristic variation as affected by pollution. Therefore, gradsect sampling can be limited to one or several regionwide communities with known sensitive or accumulator species. If sensitive or accumulator species are unknown, sampling sites can be established across a wide range of cryptobiotic communities in different airsheds as discussed in Chapter 4. Particular attention should be given to the substrate and microclimate of the sample communities, since these may have a large effect on cryptobiotic species (Northrup Environmental Sciences 1987, Hawksworth and Rose 1976, McCune et al. 1987).

Sample Size

Sample size includes both the number of samples per site and the number of sites. Sample size depends on the variability of the characteristics being measured and the degree of confidence with which comparisons between samples can be made. Data from a pilot study are useful in determining variability and then computing adequate sample sizes to detect possible changes (testing null hypotheses).

Repeatability

Repeatability influences the timing of sampling and site selection. Factors to consider are the phenology of the organism, variability in pollutant levels, physiological condition of the organism, slope, aspect and substrate of sites, distance from influencing factors such as large bodies of water, roads, etc., and any other factors that may affect study results. Confounding variables that cannot be eliminated need to be identified.

Plot Protocol

Plot protocol determines the way in which current or future samples are taken. Permanent plots in which no destructive sampling takes place must be distinguished from temporary plots from which material is collected. Designation of sampling areas within temporary plots may be desirable so that the location of disturbance is known. Certain types of activities may need to be restricted at sites to prevent unwanted influences on materials. For example, indirect trampling may affect soil lichens. Methods for marking and mapping plots must be identified. Current options for establishing coordinates for study sites include topographic maps and global positioning systems.

Collection Techniques and Material Treatment

Collection techniques and handling, transportation, and storage of collected material depend on the end use of the material. In addition, requirements for different analytical procedures must be kept in mind. If fumigation work is to be done, pre-treatment protocol and the physiological condition of the material before and after experimental procedures must be considered.

Comparison with Past, Current, and Future Techniques

It is desirable to design studies so that results can be compared with past and current studies. To facilitate valid comparisons with future studies, field and analytical techniques should be kept as simple as possible. If new techniques are employed, find ways to compare data using statistical calibration and design studies with this in mind. Consider desired future studies and whether resources required for the current study will be available in the future. Create a monitoring manual with instructions for future monitoring personnel. Monitoring systems commonly fail because sites are lost or different analytical techniques are used that cannot be calibrated to previous techniques.

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Active Monitoring

Lorentz C. Pearson

Active monitoring of air quality using lichens may involve transplanting, controlled fumigation, or culturing of lichens. Transplanting involves moving organisms from areas where they occur in nature to localities where they are needed to monitor air pollution or other characteristics of the environment. Controlled fumigation consists of creating a point source of pollution that can be manipulated to test the effects of specific pollutants on lichens that occur in the area. Culturing is encouraging or enhancing the growth of lichens in areas where they occur in nature and/or developing methods of growing lichens in gardens, farms, or laboratory growth chambers under controlled conditions.

Active monitoring experimental treatments must be carefully planned, replicated, and randomized. The material to be transplanted must be healthy specimens of the biomonitor species. Methods of attaching the lichen material to the substrate must not be harmful to the lichens when it is impossible or impractical to move them with pieces of the substrate to which they are attached. We recommend that, where possible, lichens be transplanted to the same kind of substrate from which they were taken. We also recommend using a combination of evaluation methods to estimate the degree of environmental degradation caused by the suspected pollution source.

INTRODUCTION

Because lichens are especially sensitive to air pollution and can accumulate and concentrate toxic substances from the environment in their thalli, they are recognized by scientists as valuable biomonitors of atmospheric quality. Biomonitoring can be either passive or active. Passive biomonitoring is covered in other chapters in this book (See chapters 2, 4, and 5). Active monitoring of air quality with lichens involves transplanting lichens from a source area where they naturally occur to a target area where pollution is suspected (Brodo 1966, Hawksworth and Rose 1976, Skye 1979).

Active monitoring may also involve observing lichens in areas where they naturally occur following the installation of a factory or other source of pollution (Skye 1958, Will-Wolf 1980, Ryan 1990, Kandler 1987); controlled fumigation is sometimes employed as the source of pollution (Pearson and Rodgers 1982). In either case, periodic measurements of lichen characteristics are used to evaluate the quality of the environment in the target area. These measurements include observations of how well the lichens survive in the target area (Brodo 1961, 1966; Holopainen 1983, 1984; Skye 1958), various physiological responses of the lichens (Pearson and Skye 1965, Pearson 1980, Holopainen 1984), and accumulation of pollutants in the lichen tissues (Lawrey and Rudolph 1975, Nieboer et al. 1978, Rope and Pearson 1990).

Theoretically, active monitoring of air quality with lichens may also involve growing lichens in gardens or farms or encouraging more rapid or abundant growth by using culture techniques. At present, this is largely an unexplored field.

ADVANTAGES OF ACTIVE MONITORING

The chief advantages of using transplants to monitor air quality are:

- being able to plan and set up orthogonal experiments with sampling plots at all locations where it is desired to monitor air quality;
- having enough plant material at each location to run all of the desired tests;
- having uniform material collected from a common site at all of the sampling locations;
- being able to choose the best experimental design for the particular location and material;
- ascertaining how rapidly increased levels of pollution cause injury in lichens.

The ability to study the effects of different environmental conditions by planning where the samples are placed enables the researcher to judge more accurately whether a suspected pollution source is indeed causing the observed injury, and to evaluate more accurately the extent of any injury caused by a given source of pollution. The information gained can be supplemented by fumigation with a known pollutant. For example, Pearson and Rodgers (1982) placed four sulfur dioxide burners in areas of dense low-lying forest, dense upland forest, marsh with scattered trees, and open dry grassland. Planned levels of sulfur dioxide were produced by burning sulfur on days when the wind speed and direction were appropriate. Upwind sites provided the unpolluted controls.

An often overlooked advantage of transplant studies is that the investigator has a wide variety of experimental designs from which to choose. Often a randomized block design is best, but lattice designs, Latin and Greek squares, and other designs often not available to other types of field research can be employed in transplant studies. This is especially true in experiments involving fumigation. See Federer (1955) for descriptions of experimental designs and advantages and disadvantages of each.

Another advantage of active monitoring is the ability to observe rapid responses to pollution or other environmental changes. When abundant material has been placed at each monitoring site, subsamples can be harvested weekly, biweekly, monthly, or at other intervals. Some evaluation methods are sensitive enough that changes in injured lichens can be observed in a matter of weeks even at low levels of pollution, and in a matter of hours at extremely high levels.

When fumigation is used to supply a point source of pollution, it becomes possible to evaluate the effects of a variety of pollutants (sulfur dioxide, lead, mercury, copper, cadmium, zinc, ozone, carbon monoxide, hydrogen fluoride, or peroxyacyl nitrates) and to observe the nature of the resultant injury.

A number of problems may arise when conducting studies involving active monitoring. These must be anticipated and steps taken to avoid them. Some potential problems to consider with transplant studies include:

- insufficient supply of a test species of lichen;
- variation due to ecotype differences;
- transplant shock;
- seasonality of transplant;
- choice of species;
- the relationship between pollution level and the elemental content of the lichens;
- lack of baseline data or historical (herbarium) information on native lichen abundance in the area.

METHODS

Transplant methods may be classified in two categories:

1) Transfer of healthy lichens from an area where they occur naturally to an area for which they are adapted, to study possible changes in physiological processes or accumulation of heavy metals or other elements as a result of exposure to the pollution (Brodo 1966), and

2) Transfer of large amounts of healthy material to an area where pollutants are expected to occur, regardless of whether or not they are adapted to the area, for the purpose of accumulating pollutants in lichen tissues and observing physiological changes. This latter method is sometimes called the "moss bag" method.

In conducting transplant studies, we recommend:

- limiting the number of collection sources in order to minimize variability;
- choosing species that are easily collected, transplanted and observed;
- moving the lichen with substrate attached whenever possible, or transplanting to comparable substrate;
- if physiological studies are to be made, using species well within their normal range of adaptation both at the source and at the target area;

- using historical records, such as herbaria or check lists where available, to study the natural occurrence of species in the target area (Thomson 1970);
- where possible, using species of known sensitivity;
- whenever possible, collecting lichens from areas that are essentially identical to the transplant site in every way but air quality;
- measuring thallus dimensions, photographing each specimen at the time of transplant, and taking an initial sample for elemental baseline;
- if it is necessary to use glue, using a strong, non-toxic glue like araldite (Hawksworth and Rose 1976) or liquid nails; if it is desired to tie lichens to shrubs, trees, or posts, avoid string that rots when exposed to sun or water.

Generally, do not collect desert lichens for transplants in the high mountains. This will minimize effects of differences in microclimate between the collection and transplant locations.

Transplants may be in the form of bark plugs, branches, twigs, or rocks or pieces of rock with lichens attached. These may be attached to existing vegetation or placed on specially built poles. Specimens that blend with the natural vegetation are preferred to prevent vandalism, though they may be difficult to relocate.

Adhesives

For attaching clean specimens to objects in the target area, our first choice is always to take substrate to which the specimen is attached. If this is not possible, glue or string must be used. We recommend against cotton or paper string which rots, and against wire or string containing metal. Fishline seems to have no serious disadvantages, providing it is tied on with a knot that will not slip.

Glue must meet two criteria: it must hold well, even during rainy weather, and it must not give off toxic fumes. In a study conducted by Chris Warner, a student at Ricks College, "liquid nails," a silicone glue, met both criteria. Umbilicate lichens glued to basalt rock with epoxy showed visible signs of injury (change in color and much increased rate of electrolyte leakage), whereas those glued with liquid nails showed no sign of injury and remained attached through two winters and summers. Elmer's glue held better than expected, but did not survive the second spring thaw. Other silicone-type glues held well, but produced volatile substances that injured the lichens.

Measurement and Experimental Design

Ideally, lichen transplants should be measured and photographed every month for the first year. Later, the need for such observation should be assessed and modified if the data show that a longer or shorter frequency is more appropriate.

We also recommend that the number of experimental treatments be chosen carefully and adequate replication planned. We recommend at least 5 or 6 experimental treatments and at least 3 or 4 replications in most transplant experiments with a minimum of 25-30 experimental units. Adequate material must be transplanted, keeping in mind that some transplants may die, become detatched from their substrate, fertilized by birds or other animals, become victims of vandalism, or rubbed loose or eaten by animals. Based on estimates of potential losses, the amount of transplant material needed can be estimated.

To minimize transplant shock, we recommend the following:

- minimize the time from collecting to transplanting;
- allow specimens to air dry before transporting, and do not transport in plastic bags;
- attach specimens at the elevation above ground with the most similar microclimatic conditions to the elevation at which they were collected, keeping in mind that humidity and variability in humidity generally decrease with elevation above the ground, and average daily temperatures increase in the sequence N, E, W, S around the trunk of a tree or the sides of a rock;
- if the material cannot be transplanted immediately, refrigerate it.

If transplant studies are conducted for the purpose of evaluating accumulation of heavy metals or other elemental pollutants, we recommend keeping them away from metal objects during collection and transplanting.

Factorial designs involving both wind direction and distance from pollution sources can be used to set up the experimental treatments (Federer 1955). Windroses can help in choosing experimental transects.

At least one of the experimental treatments making up the independent variable should be a control treatment. Ecologists make use of four kinds of controls in conducting air pollution studies:

- transplanting some specimens at the source location to ensure that pollution effects and transplant shock are not being confounded (Brodo 1966);
- transplanting some specimens to sites upwind from the point source of pollution (Pearson and Rodgers 1982);
- reverse transplanting to ensure that observed injury is due to the increased pollution and not merely to a change in environment; and
- transplanting specimens close to mechanical monitoring devices if any are available.

Fumigation Studies

Field fumigation studies are especially valuable in monitoring air pollution (Pearson and Rodgers 1982) because they make possible direct observation of how specific physiological or morphological changes correlate with specific pollutants. If an industry claims that the substances they released are not the ones causing the problems observed, the claim can be tested by using controlled fumigation to identify the type of injury caused by a specific pollutant. If it is the same type of injury occurring at the site downwind from the suspected industry, there is strong evidence that the industry is indeed the polluter.

A related method consists of transplanting lichens into laboratory chambers where they are exposed to varying levels of sulfur dioxide, hydrogen fluoride, peroxyacyl nitrates, or other pollutants (Pearson and Henriksson 1981). See Chapter 5 for more methods. Pearson and Skye (1965) reported altered patterns of photosynthesis and respiration in lichens exposed to high levels of sulfur dioxide in stagnant air in laboratory flasks. Rydzak (1969), Coppins (1973), and others criticized the study because the levels of SO_2 were much higher than those occurring in nature, even in highly urbanized areas. However, the critics overlooked some factors that were pointed out in the report:

- because SO_2 is very soluble in water (Hodgman et al 1957), its levels in the flasks were not as high as the critics assumed;
- it was hypothesized on the basis of studies with other kinds of plants that high levels of pollution over a short period of time should reveal metabolic patterns similar to those obtained from lower levels over longer periods of time; and
- the symptoms observed in the lichens in the flasks were similar to those in lichens collected in urban areas where high levels of pollution occurred.

The study suggested that plastids are more sensitive to injury from high levels of SO_2 than are the mitochondria, which in turn are more sensitive than the peroxisomes. Photorespiration did not seem to be affected by the high levels of pollution. Improved chambers are available now in which the lichens may be exposed to air containing exactly measured amounts of known pollutants flowing over them, rather than to stagnant air. Studies by St. Clair (personal communication) and others indicate that exposure to high levels of pollution over a short period of time results in injury comparable to exposure at low levels over a long period of time.

Culture Methods

Very little work has been done on culturing of lichens. Under laboratory conditions, with whole lichens in growth chambers of various types, Tobler (1939), Kershaw and Millbank (1969),Pearson (1970), Pearson and Benson (1977), Dibben (1971), and Margot and Deslooves (1974) have had moderate success by ensuring periods of drying between periods of adding water. Ahmadjian et al. (1980), Ahmadjian and Jacobs (1982), and Jacobs and Ahmadjian (1971) have reconstituted whole lichens from isolated mycobiont and phycobiont cultures, and Lindstrom (personal communication) has obtained growth in *Peltigera* species from individual isidia. The results from these studies suggest that it should be possible to encourage lichen growth in the field by introducing lichens to study sites in large amounts, and encouraging dispersion of their propagules through experimental manipulation of the environment. Rosentreter (personal communication) has experimented with dispersal of lichens on BLM land in Idaho with some encouraging results, but much more research is needed.

It may be more rewarding, however, to encourage accelerated growth and reproduction of lichens in areas where they already occur. Adding water and/or fertilizer elements may speed up the growth and dispersal of species suitable for monitoring which are already in place. We know of no published research along this line.

SPECIES

In Europe, numerous studies have made it possible to classify lichen species into several categories ranging from the most tolerant to the most sensitive (Ferry, et al. 1973). Few comparable studies have been conducted in North America. Nevertheless, some progress has been made in some parts of the continent.

Lecanora conizaeoides is unanimously regarded as the most pollution-tolerant species in Europe. *Hypogymnia physodes*, *Parmelia sulcata*, and, according to some studies, *Evernia prunastri* are also tolerant. In increasing order of sensitivity, *Parmelia perlata, Usnea ceratina, Lobaria pulmonaria* and *Parmelia saxatilis* come next, the last four being extremely sensitive to atmospheric pollution. *P.sulcata, P.caperata, P.saxatilis, U.subfloridana*, and *U. ceratina* are widespread and common throughout most of North America. *E.prunastri* is common in the Pacific Coast states and *L.pulmonaria* is relatively common in mature forests of the northern tier of states, but is now rare in reforested areas. The other European indicators listed above are either rare or do not occur in North America. See Chapter 5 for more information on sensitive species.

EVALUATION

To measure the effects of pollutants on lichens, baseline data are needed and periodic observations must be made. Some of these observations involve destructive sampling. The amount of material needed for destructive sampling must be carefully estimated before the transplants are made. A rough yardstick for planning is to multiply the number of experimental treatments desired by the number of replications desired by the number of sampling dates, plus at least 10% to cover losses due to herbivory, vandalism, or other causes not related to pollution.

The simplest type of evaluation consists of measuring the survival rate of a species and expressing this as percent survival: how many of the transplants died showing symptoms of injury, how many died or disappeared as the result of causes not related to pollution, and how many survived relative to the original number. Related to this are observed morphological changes in color and thallus thickness, altered appearance of the apothecia or perithecia, width of lobes, etc. We recommend using a Munsell chart, available in many art textbooks and art sections of libraries, to aid in describing color changes. Photography using a fine grain black and white film can be used to measure growth of lobes and development of apothecia. In some cases, it may be desirable to have photographs taken monthly over a period of a year or two. Henriksson et al. (1978) found infrared photography especially useful because it indicates vitality of the organism.

Rates of photosynthesis, respiration, and nitrogen fixation can be measured in harvested material following exposure to air pollution for varying periods of time. Chlorophyll degradation can be studied with TEM, thin layer chromatography and/or spectroscopy (Chervin et al. 1968, Jacobs and Ahmadjian 1971, Holopainen 1983). Damage to cellular membranes can be measured directly with transmission electron microscopy (TEM) or indirectly with the potassium efflux method (Puckett et al. 1977) or the simpler electrolyte leakage method (Pearson 1980, 1985; Fields and St. Clair 1984). TEM can also be used to observe damage to plastids and other organelles. See Chapters 5 and 7 for more information on these methods.

For evaluating damage to lichens following exposure to air pollution, it now appears that TEM observation of the concentric rings and other organelles including the plasma membrane, and measurement of electrolyte leakage from the cells have the greatest potential for revealing injury following short time exposure at low levels, at least in areas where sulfur dioxide is the primary pollutant. Concentric rings appear to be visibly altered more rapidly following exposure to low levels of pollution than any of the other organelles.

Other physiological and biochemical measurements, such as chlorophyll degradation and photosynthesis rates and patterns, are also manifested early. For evaluating accumulation of toxic elements (Hale and Lawrey 1985, Laaksovirta and Lodenius 1979, Schwartzman et al. 1987, Steinnes and Krog 1977), spectroscopy can be used instead of traditional but more tedious direct chemical analyses (Pearson and Rope 1987). Energy-dispersive spectroscopy of X-rays is showing promise in microchemical analysis of lichens transplanted to areas near chemical processing plants.

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Collection and Chemical Analysis of Lichens for Biomonitoring

Larry L. Jackson, Jesse Ford, and David Schwartzman

This chapter discusses the interrelated aspects of biomonitoring using chemical analysis of lichens. Many unique aspects of study objectives, study design (including design tasks, considerations, and sampling schemes), sample collection, sample preparation, and sample analysis that are required for a successful biomonitoring program are emphasized. The advantages and disadvantages of common analytical methods suitable for chemical analysis of lichens are briefly discussed. Aspects of a quality assurance program and final contract reports are highlighted. In addition, some examples of studies using chemical analysis of lichens are discussed.

INTRODUCTION

Lichens have been used often as receptor-based biomonitors in air quality studies. Historically, they have been used in a qualitative way, in which their dwindling populations indicate poor air quality or chemical contamination. In the last few decades they have been used increasingly in a more quantitative fashion whereby their chemical content indicates poor air quality.

The objective of most studies focusing on the chemical content of lichens has been to determine the variability of a few selected elements or chemical species in a given lichen population through time and/or space. The paucity of published chemical data for lichens forces many of these studies to determine a chemical "baseline" or "reference point in time" for one or two lichen species within a politically or geographically defined region. The intent of such studies is typically to examine the long-term variability of a selected suite of elements or compounds by remeasuring the chemical content of the lichens at some point in the future. Spatial variability in the chemical content of lichens is commonly a confounding problem in such studies.

Other environmental studies have attempted to address only the spatial variability of the chemical content of lichens to define the influence of point or nonpoint sources of specific pollutants and to differentiate between natural and anthropogenic origins of these pollutants. Variability of chemical constituents in lichens with time may be a confounding problem in these studies. Hence, it is important to examine both temporal and spatial variability in the chemical content of lichens to provide interpretable, meaningful, and defensible data for use in air quality management decisions.

This chapter highlights the issues related to both temporal and spatial variability which must be considered in using the chemical analysis of lichens for biomonitoring. The interrelated aspects of such studies are illustrated in figure 1. Specific step-by-step instructions are not given because in any environmental study, decisions and compromises must be made based on the specific study objectives, the particular ecosystem, the project budget, and the laboratory facilities available, along with numerous other considerations. In addition, this chapter is not intended to examine those research studies where the chemical content of lichens has been used to identify and elucidate the biogeochemical processes affecting the chemical content or uptake of elements by lichens--studies that are much less numerous for lichens than for vascular plants.

Conceptually, in an air quality study it is easy to suggest that one should analyze the air, airborne particulates, and rainfall to define existing conditions or potential adverse impacts in an ecosystem. For a few chemical species this is possible, albeit expensive. Expense frequently restricts the number of measurement sites to only a handful within a large geographic region. For other potential airborne constituents, collection and measurement may be difficult, if not impossible, with current chemical technology. In addition, our understanding of the relationship between airborne chemical concentrations and adverse impacts on plant communities is limited. The chemical analysis of lichens offers a relatively inexpensive alternative to air sampling in which air contaminants can be indirectly measured. Many lichen species obtain much of their nutrients directly from the air and rainfall, thus serving as integrators of air quality. Because of their ubiquitousness, growth characteristics, and longevity, they may be used as passive collectors over time periods extending beyond just a few years. However, the practicalities of using lichens as living chemical sampling devices are fraught with difficulties that must be addressed to provide meaningful and interpretable results.

The major aspects of a biomonitoring study are interrelated, as shown in figure 1. For example, the study objectives directly influence the study design used, the species collected, the types of chemical analysis performed, and the nature of the quality assurance program. In turn, each of these aspects is controlled by the nature of the population to be studied in time and space and by the project budget. In addition, the planning aspects of the overall quality assurance program influence each part of a study. A successful biomonitoring program must give appropriate attention to all of these study aspects and how they interrelate.

STUDY OBJECTIVES

Most environmental studies examining the chemical content of lichens are used for *monitoring*, regardless of whether they address temporal or spatial variability, or both. Monitoring may be used to:

1) study conditions at a reference point in time, to determine a baseline against which future comparisons may be made to determine long-term trends,
2) study the extent of a single chemical release or spill,
3) determine the region of influence of point and nonpoint pollution sources,
4) assure that pollutant levels are within acceptable levels, and

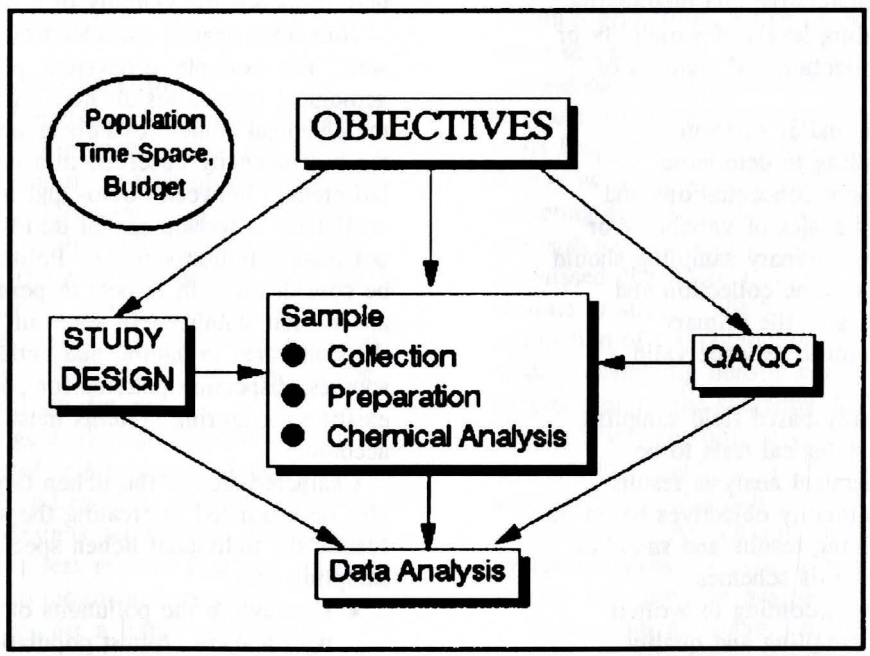


Figure 1. — Interrelated aspects of biomonitoring using chemical analysis of lichens.

5) determine those chemical or biological parameters that are measurable and/or changing temporally or spatially in order to define future monitoring programs.

Study objectives should be clearly defined and must consider the hypotheses to be tested and the target population to be sampled in both time and space. Study objectives must be tempered with the realities of sample collection, analysis, and budgetary constraints.

STUDY DESIGN

Design Tasks

A good study design enumerates a series of responsibilities and must meet the study objectives. The basic design tasks of an environmental monitoring study are (after Gilbert 1987):

- Define the target population in time and space.
- Understand the ecological and environmental setting of the target population.
- Define the samples to be collected and the field measurements to be made.
- Develop a quality assurance program for sample collection, chemical analysis, data analysis, and define the data quality objectives (i.e., qualitative and quantitative statements regarding levels of variability or uncertainty in collection and analysis of samples).
- Review literature and/or perform preliminary sampling to determine quantifiable element concentrations and estimate potential scales of variability or spatial trends. Preliminary sampling should be done using the same collection and analysis techniques as the primary monitoring program to produce valid conclusions.
- Develop statistically based field sampling plan and define statistical tests to be performed on chemical analysis results. Re-evaluate data quality objectives based on preliminary sampling results and sampling and statistical analysis schemes.
- Execute the study according to written protocols of the sampling and quality assurance plans.
- Evaluate the chemical analysis results and determine relevant temporal and spatial trends.
- Determine the uncertainty in the chemical and statistical results and evaluate them with respect to the data quality objectives.
- Determine if the study objectives have been met.

Design Considerations

Selection of Target Population

In developing the sampling plan, temporal considerations include seasonal aspects of plant physiology, natural cycles of weather such as wet and dry seasons or seasonal shifts in wind patterns, herbivore activity, and seasonal pollutant emission patterns. These considerations are important when comparisons with future collections are to be made. Aberrant seasonal conditions may artificially induce or negate chemical trends by introducing uncontrolled variability. In addition, the length of time required for sample collection must be considered to avoid uncontrolled temporal variability. For example, the chemical content of samples collected before and after a period of rain may be significantly different.

The stability of a habitat must also be examined in designing long-term studies. Potential human-caused changes such as logging or development, or natural changes caused by events such as fires or hurricanes, may influence the viability of a study design.

Numerous spatial considerations are important as well. For example, ecosystem, physiographic, and geological boundaries all may significantly influence the chemical content of lichens and the magnitude of the heterogeneity observed in a geographic region. Differences between micro- and macro-habitats may artificially introduce spatial trends unrelated to potential pollution sources. Political boundaries must be considered with respect to permission to sample and habitat stability. In addition, the relationship of the study area to natural and anthropogenic pollution sources, dispersion patterns for pollutants, and existing monitoring systems must be taken into account.

Characteristics of the lichen target population must also be evaluated in creating the study design. Ideally the individual lichen species chosen for sampling are:

- sensitive to the pollutants of interest, but with a stable, robust population (i.e. healthy and abundant)

- good passive collectors with measurable concentrations of the pollutants of interest
- common species present in monotypic stands on the same substrate throughout the study area
- easily identified in the field
- easily collected and cleaned in sufficient quantity
- sufficiently abundant that repeated sampling will not seriously impair the viability of local populations
- species with known physiological processes such as growth rate and nutrient uptake, and with previously measured chemical content from this study area and other regions.

In addition, the lichen substrate should have low concentrations of the elements and/or compounds of interest.

The considerations listed above are used to identify the specific target population that will become the sampled population, and to define it in time and space. To have a workable study design, other practical considerations must be weighed. Logistical considerations such as site accessibility, ability to precisely locate and re-find an individual sample site, ease of finding, collecting, and preparing the target species, sampling equipment required, and other issues of convenience all ultimately affect the sampling design. These logistical issues become an integral part of evaluating the cost-effectiveness of a particular sampling design.

Selection of Sampling Design

Whether a study objective is focused on temporal or spatial issues, population parameters such as concentration means and magnitude of variability are generally determined. The terms *bias*, the amount consistently measured above or below the true value, *accuracy*, how close the measured amount is to the true value, and *precision*, how close repeated determinations are to each other, are commonly used to describe analytical techniques. They may also be used to describe sampling designs.

Sampling designs generally fall into three categories: *judgmental or haphazard sampling*, *systematic sampling*, and *random sampling*. The latter two sampling designs can both be probability based, and hence have less relative bias and are more legally defensible than judgmental sampling. However, they may require a larger number of samples, and although they are generally preferable, they are not necessarily any more accurate than judgmental sampling.

Judgmental sampling may be as simple as collecting a few haphazard grab samples, or quite sophisticated and based upon known dispersion patterns and plant uptake of a specific pollutant. In general, these designs may be cost effective, but inferences are dependent entirely upon the knowledge and prejudices of the collector. Also, it is difficult to estimate the statistical bias or accuracy of conclusions. In the worst case, as in the collection of a few grab samples, no inferences may be made at all and the results may be used only for orientation purposes. A probability-based pilot study may be more effective and lead to more interpretable study results, even for orientation purposes.

Simple random sampling, which is not the same as haphazard sampling, offers the least relative bias of the sampling schemes, but homogeneity of the target population is assumed. Simple random sampling is not suitable if temporal or spatial patterns exist in the target population. Stratification of a heterogeneous target population into homogeneous subsets with random sampling within a subset may provide more accurate estimates of population means or other parameters. However, this depends on the ability to *a priori* pre-select sub-populations which are more homogeneous for the elements or compounds of interest.

Systematic sampling involves sampling at points within a grid, along a line or gradient, or by some other set pattern in space or time. Systematic designs can be judgmental or probability based if they use a random starting point. These designs are most frequently used for evaluating trends. However, it may be difficult to accurately estimate the sampling error in a systematic design, and unsuspected periodicity or patterns in the data may be missed depending upon the frequency of sampling in time or space. The temporal and spatial interaction of the population heterogeneity, and trends with the sampling density control the reproducibility or precision of the sampling design. For spatial studies where the objective is a map of a population characteristic, such as an element concentration, the reproducibility of a particular map is referred to as *map stability*. An unstable map produced from a systematic sampling scheme with insufficient sample density may produce misleading or incorrect results.

The various advantages and disadvantages, and the appropriate calculation aspects of each type of design and their combinations, are discussed in numerous

texts such as those by Gilbert (1987) and Keith (1988, 1991). More specific texts are also available on time series analysis or spatial statistics such as kriging and contouring (Davis 1986, Isaaks and Srivastava 1989).

Use of parametric versus nonparametric statistics must be considered in designing a study and in evaluating results. Whereas parametric statistics assume that the data will be normally distributed, non-parametric statistics make no assumption about the distribution of the data. Frequently, concentration data in plants are skewed and must be transformed in order to approximate normality. A logarithmic transformation is common. A "Chi Square" test or the non-parametric Kolmogorov-Smirnov test may be used to evaluate the goodness of fit for a log-normal distribution or other distributions.

Typically, the mean, either arithmetic or geometric, and its variance are estimated for chemical concentration data. The total variance is the sum of the variances for individual components. For example, if a series of samples is collected from a region, the total variance is the sum of the variability due to analytical measurement and sampling error, and to heterogeneity within a sample site and between sample sites. Analysis of variance may be used to estimate the variance attributed to the various sources of heterogeneity. Hierarchical or nested analysis of variance has been used in this fashion to estimate variability at various spatial scales (Leone et al. 1968, Miesch 1976, Severson and Tidball 1979, Klusman 1985).

No analysis of variance is possible without replication of measurements. It is especially important that the study design include replication so that the error contributed by the analytical measurement can be estimated. Estimates of population parameters may be erroneously interpreted without a proper estimate of the analytical error. For example, it is difficult to justify the calculation of baseline concentration ranges if the largest contributor to the variance is the analytical measurement, and it contributes more than 50% of the total variance. This situation is not uncommon when an element concentration approaches the lower limit of detection of the analytical method.

Thus, a study design must take into account replication of the analytical measurement and/or collection of samples. Unbalancing an analysis of variance design is a common approach to providing estimates of the variance components while maintaining a cost effective sampling scheme (Miesch 1976).

The Orientation Study

A good orientation study is invaluable in producing a successful and cost-effective primary study design. However, project budgetary constraints frequently do not permit a reasonable orientation study. Instead, the experience and *a priori* knowledge of a researcher is assumed to suffice. The result of such policies for studies of chemical concentrations in plants or lichens is all too commonly a primary research study that is little more than an expensive orientation study.

The various aspects of an orientation study, which should represent about 10-15% of the field and analytical work, include:

- A comprehensive literature review.
- Ecological characterization of the region, habitats, and potential lichen species for collection.
- Evaluation of logistical considerations, convenience, accessibility, level of effort to find and collect target species.
- Necessary collection permits.
- Orientation study design and implementation compatible with potential primary study design.
- Development of quality assurance program for collection, chemical analysis, and data analysis which includes quality control samples.
- Coordination between funding agency (data users), contractor/researcher (samplers), and chemists.
- Analytical methods evaluation for required sample size, detection limits, accuracy, precision, and representativeness.
- Estimation of range of chemical concentration and magnitude of variability.

These tasks are necessary parts of a primary study, regardless of whether or not an orientation study is performed. In general, the orientation study should be performed using the same collection and analysis protocols to be used in the primary study. The orientation study results may dictate that changes be made in collection or analysis procedures for the primary research study.

The Primary Research Study

The primary study includes refinements of the tasks listed above, based on the results of the orientation study. An important facet of this process is evaluating the data quality needed compared to the quality attainable as estimated from the orientation study. This evaluation must include budgetary and resource constraints, with the potential outcome being a need for additional resources or a reduction in the quality or representativeness of the data.

During the primary study, strict control must be maintained over levels of stratification in both the space and time domains. To maximize the interpretability of the chemical concentrations in lichens, care must be taken to clearly identify in the sample plan the biological and physical or geographical stratification levels, if stratification is used. Spatial factors that may influence stratification include such things as macro- and micro-habitats, host/substrate for lichen (e.g., fir versus aspen, or tree versus soil), physical orientation (e.g. lower canopy, south-facing trunk, or site aspect), physiographical and geological setting, and proximity to pollution sources. Temporal factors, such as pollution events, phenological seasons, growth stages, and intra- or inter-annual climatic differences, may influence both initial sampling and re-sampling plans. In general, the better control maintained over stratification levels and the fewer the total number of levels, the easier study results will be to interpret. In addition, estimates of the sampling error and the analytical measurement error must be included. In creating the sampling design, a reasonable balance must be maintained between controlling for major sources of known or suspected variance while at the same time not biasing the design by inappropriate stratification.

An additional aspect of the primary study design is to explicitly define the working hypotheses and the statistical tests that will be used to test them. One part of this is evaluating whether the design created can be analyzed using existing statistical software. At this stage, the database for field measurements and chemical analysis results should be designed to identify the need for additional data and avoid potential problems in the reporting of results. Sample identification and tracking schemes must also be developed at this point.

Supplementary Sampling

A study design should incorporate at least the potential for additional sampling that would represent 10-15% of the field and analytical work. The supplementary sampling may involve collecting samples at additional sites or times, resampling specific sites, or reanalyzing specific samples. Supplementary sampling is usually needed to verify critical conclusions, clarify problems such as outlying values, estimate parts of residual variability not associated with laboratory variation, or explore unanticipated facets of the results.

SAMPLE COLLECTION

The goal of sample collection is to obtain samples that are representative, that will meet the study objectives, that are minimally contaminated with substrate (by the collection procedures, or during transportation to the laboratory), and that are uniformly collected throughout the duration of the study. Collection of single species as opposed to mixed-species specimens is preferred. Taxonomic vouchers should be collected to allow for expert verification, for archival purposes, and to address questions about growth form, biomass, etc.

The sampling plan should clearly identify the amount of sample to be collected to obtain a homogeneous, representative sample, to provide sufficient sample for analysis, and to allow for reanalysis to verify results. Compositing of lichens is commonly required to meet the laboratory needs. The nature of compositing should be uniform from site to site and should be documented. It may also be necessary to collect the sample substrate at each site to quantify its potential biochemical or geochemical influence.

Sample Handling

The sample collection and handling must be appropriate for the elements or chemical compounds of interest. The level of environmental pollutant expected dictates both the sample size and the nature of the contamination control that must be used in both the sample collection and analysis. Contamination from sampling tools or storage containers, or that occurs during transportation (fumes and particulates) may obscure the true chemical concentration trends. For example, for trace metal analysis of lichens, sample tools should be made of Teflon®, plastic, Teflon-coated metal, or stainless steel (in descending order of preference) to minimize metal contamination. Plastic tools may be inappropriate for organic pollutant studies, however. Oils or greases used to lubricate sample tools may

easily contaminate samples. Other potential sources of contamination include chemically treated wooden objects (e.g., Wolmanized® wood which may introduce high levels of As, Cr, and Cu), personal gear such as gloves and insect sprays, and activities such as smoking. For organic pollutant studies, insect repellents or other lotions or sprays should not be used in the field unless it can be demonstrated that the relevant chemicals will neither contribute an analytical interference nor serve as a contaminant source for target compounds. Finally, if the concentrations of the elements or compounds of interest are extremely low, special care must be taken to avoid contaminating the samples due to high background levels in the laboratory setting. This is particularly important for volatile species such as mercury and organic compounds (e.g., hexachlorobenzene and polychlorinated biphenyls (PCBs)).

Plastic bottles or bags, cloth bags, glass bottles, or paper envelopes are common sample containers. No one container is suitable for all studies because of contamination possibilities, cost, and transportability issues. The specific sample storage container must be selected to minimize contamination from the container itself (such as the potential addition of sulfur from Kraft paper bags or plasticizers from plastic bags or bottles that interfere with organic determinations) or during transportation (such as carrying cloth bags in the back of an open truck on a dusty road).

The nature of the study may dictate pre-cleaning the sample containers with acid or solvent washes using appropriate grade solutions. Containers and handling should not permit cross-contamination between samples. In addition, the sample handling and/or the container must not promote deterioration of the sample (such as by mold formation or rotting when wet samples are stored in plastic bags) or volatilization or transformation of the chemical compounds of interest.

Air-drying in the field may be beneficial if transportation time to the laboratory is long or contamination in the laboratory during the drying process is a potential concern. Air-drying in the field should not induce excessive loss of volatile constituents as long as drying temperatures do not exceed normal ambient temperatures. Refrigeration or freezing of samples is generally not required and may even have deleterious effects. However, freezing of samples may be required for long-term storage or to impede the degradation or volatilization of organic pollutants or of volatile elements such as mercury. Freezing samples immediately upon collection is possible using liquid nitrogen, but it is not common. Storage temperatures should be at -20° C or below to minimize loss of volatile chlorinated hydrocarbons or at -80° C if long-term storage on the order of years is planned.

Sample Collection Site

The sample collection site should meet the criteria dictated by the study design, such as presence of target lichen species, type of habitat, geographic location, proximity to confounding contamination sources (e.g., roads), and stability of habitat. Determination of the site location should be consistent with the objectives of the study and the available technology.

The ability to accurately locate a site is critical for studies in which the site must be resampled, and for some studies in which spatial statistics are used. The general sample location should be recorded on a topographic map at the appropriate scale such as the US Geological Survey Quadrangle series. Other locating techniques and devices with varying degrees of accuracy and precision may be required, such as the use of compass and chain, satellite Global Positioning System, Loran-C, or aerial photography.

Permanent marking of the site should be consistent with the degree of permanency required by the study and allowed by the property manager. Markers should not be obtrusive on the environment, but should be locatable in the future and should not invite vandalism. Metal markers are commonly used, but they should not be made of such material or positioned such that they would potentially contaminate future samples.

Field Documentation

In most studies, the field notes for a site are as valuable as the samples and should be recorded and protected with an appropriate degree of diligence. Whereas most researchers take adequate field notes for their own purposes, it should be kept in mind that these notes may be used by other researchers for the agency in the future. Hence the degree of thoroughness and legibility of the notes is important. In addition, referring to the field notes may be the only way to resolve problems in data interpretation.

The field notes for all sites should be uniform, and preprinted field data sheets should be used. All field notes have several aspects in common. They generally should include:

- site identifier, location information (e.g., site coordinates, hand-drawn site map, topographic sheet name),
- general information such as weather or other pertinent temporal conditions,
- habitat or community description,
- sample descriptions, samples collected, including vouchers and identifiers (field sample numbers),
- field measurements,
- miscellaneous comments on collection problems or deviations from sampling design,
- photograph numbers,
- date and time, and
- names of sample collectors, signature of field note transcriber.

If an encoded sample identifier scheme is used instead of a simple sequential numbering scheme, it should be preprinted on the field data sheet to minimize confusion and mislabeling by the field collectors.

Photographic documentation should be made of general site habitat, lichen growth habit and nature of substrate, thallus morphology, and sample collection and compositing methods. Where possible, sample site identifiers should be included in photographs.

SAMPLE ANALYSIS

Protocols for the chemical analysis of plants, and lichens in particular, have not been standardized. Whereas chemical analysis of plants and lichens has been used for some time for pollution monitoring, the majority of published methods on plant analysis address agricultural nutrient levels (Westerman 1990, Jones et al. 1991, Jones 1991). Very few publications describe chemical analysis procedures for lichens. In general, the analysis methods and issues of concern for lichens are the same as for agricultural crops.

Like every other aspect of lichen biomonitoring, the choice of preparation and analysis methods depends upon the study objectives, the concentration of elements in the lichens, and budgetary constraints. Quite frequently, the prejudices of the researcher and the laboratory facilities available also dictate the sample preparation and analytical methods used, the suite of elements determined, and the determination limits for the elements. Realistically, no one method can be defined as the only suitable method for most elements. Thus, in the following section, we highlight some of the primary analytical methods in current use and the issues of concern.

Lichen Sample Preparation

The various aspects of sample preparation that must be considered prior to chemical analysis of lichens are cleaning, washing, and drying of the raw sample, particle size reduction, homogeneous subsampling, and destruction of organic matter.

Cleaning and Washing

The objective in cleaning lichens is to remove foreign matter without leaching elements and/or compounds from the lichens themselves. Hand picking with the aid of a microscope may be done to remove adhering substrate such as bark or soil particles, extraneous plant species, and dead or decaying tissue. Whereas hand cleaning is not difficult, it is tedious and does not remove surface dust. Most plant surfaces are contaminated with windblown dust, fly ash, automotive particulates, agricultural sprays, or a variety of other airborne particulates. The efficacy of washing lichens or plants to remove such material is controversial. Several issues must be considered:

- Is surficial contamination present?
- Is the concentration of the elements or compounds of interest in the contamination significant with respect to their concentration in the lichen?
- Will a washing procedure remove a significant portion of the surficial contamination?
- Will a washing procedure leach significant amounts of the elements or compounds of interest from the lichen itself?

Numerous washing schemes have been used to clean plants: tap or distilled water rinses (with or without ultrasonication), mild detergent washes, and dilute acid washes (Jones and Case 1990, Little 1973, Saiki and Maeda 1982). Goyal and Seaward (1981), Lawrey and Hale (1981), and Jackson et al. (1985) observed leaching of metals and sulfur from lichens, but it is difficult to tell whether the decrease in element concentration with more aggressive cleaning (i.e., with additional washing cycles or more vigorous washes) is due to more effective removal of contaminants or leaching from the lichen. Little (1973) found that water washing removed the majority of the surficial deposits from foliar samples, but the total amount removed was element

dependent. Thus, the nature of samples and their contamination must be critically examined before deciding upon a washing procedure. The influence of any washing scheme on the final chemical results must be considered when providing the environmental interpretation.

Sample Drying

Drying the samples minimizes changes in the tissue once the sample is collected, and provides a uniform weight basis for analysis. Jones and Case (1990) reviewed various aspects of drying plant samples. They point out that temperatures above 60° C are generally required to stop enzymatic activity in a sample, but that excessive weight loss occurs at temperatures above 100° C. The National Institute for Standards and Technology (NIST, previously the National Bureau of Standards, NBS) formerly recommended drying standard reference materials (SRMs), such as SRM 1572 Citrus Leaves, for 2 hours in air in an oven at 85° C or vacuum drying at Room temperature for 24 hours. They now recommend that the new botanical reference materials, such as SRM 1547 Peach Leaves, be dried for 120 hours at room temperature in a desiccator with fresh anhydrous magnesium perchlorate or freezed dried for 24 hours at -5° C at a pressure of 13.3 Pa. The certificate for analysis for the new standards now states that "vacuum drying at room temperature and oven drying at elevated temperatures have resulted in excessive weight losses and therefore are **not** recommended." For standard reference materials drying in a desiccator or freeze drying is reasonable, but for large numbers or large quantities of samples this may be impractical.

An alternative method is to dry samples in air in an oven at 40° C for 48 hours (Gough et al. 1988a,b). These conditions do not drastically exceed the conditions to which samples may be exposed in the field in many geographic regions. Low drying temperatures (generally less than 35° C) would seem particularly appropriate when volatile species such as chlorinated hydrocarbons or mercury might be lost at higher temperatures. In cases where volatile species may be lost, analyses may be performed on un-dried material, and corrections to a dry-weight basis can be made by determining moisture on a separate subsample.

Sample Grinding

The objective in grinding a plant or lichen sample is to obtain a homogeneous sample of uniform particle size. This step is a prime source of contamination and/or loss of sample material. Various particle size reduction methods have been used, including hand grinding, kitchen or industrial blenders, Wiley mills, ball mills, and cyclone mills. The choice depends upon the amount of material, its morphology, the methods of chemical analysis, the sample size required, and the chemical species to be determined.

Frequently, only a small amount of lichen material is collected. Thus it must be conserved during the grinding process to have sufficient sample for analysis. The choice of mechanical grinder must take into account the amount of material lost during grinding. The morphology of the lichen thallus and the toughness of the different parts also influence the grinding method and the range of particle sizes that are produced (Gough et al. 1988a).

Segregation during sample handling and analysis may introduce an additional source of variability. The standard bench model Wiley mill typically produces particle sizes of 2mm (-10 mesh), 1mm (-18 mesh), or 0.5mm (-35 mesh). Jones and Case (1990) suggest that -20 mesh material (0.85mm) is sufficient for analysis of sample aliquots of 0.5g. Grinding to excessively small particle sizes may introduce additional metal contamination. Mechanical grinders may contaminate a sample with elements such as Al, Co, Cu, Cr, Fe, Mo, Ni, Si, W, and Zn. The use of agate ball mills or shatter boxes may reduce the metal contamination and provide a suitable particle size. In addition, excessive grinding and the heat produced may potentially volatilize some organic species.

The length of time between grinding and analysis is not particularly critical for most inorganic elements. However, organic compounds may be lost or converted due to enzymatic action after grinding disrupts cells (Spittler and Bourke 1988). Therefore, organic extractions should quickly follow grinding.

Following grinding, samples are often split prior to submission to the laboratory to obtain an estimate of analytical precision. To minimize between-sample heterogeneity, a "Jones" alternating-chute or riffle type splitter should be used. At the very minimum, "coning and quartering" should be done. Simply pouring a little sample into two or more bottles is inappropriate and may accentuate the chemical heterogeneity for the different particle sizes.

To split a sample by "coning and quartering," the entire ground sample is poured onto a sheet of paper. The sample is mixed by lifting the corners of the paper and rolling the sample over itself. After thoroughly mixing the sample, the cone is flattened

out and divided into quarters. The majority of sample in opposing quarters is transferred to one container and the material in the other two opposing quarters is transferred to another container. The process of mixing, quartering, and transferring is repeated until all residual sample is divided between the two containers.

Ashing of Samples

Prior to most inorganic chemical analysis procedures, the organic portion of the sample must be destroyed. This is generally done by wet oxidation with a mixture of acids such as HNO_3, $HClO_4$, or H_2SO_4, or a high temperature dry oxidation/ashing is performed. The choice of wet or dry oxidation is influenced by the nature of the analytical method and the volatility of the chemical species. For example, in acid digestions boron is typically volatilized, whereas in dry ashing As, Hg, Se, S, and various other elements may be lost. The procedures for wet oxidation are many and varied (Jones and Case 1990) and more and more include the use of microwave ovens and closed vessels. High-temperature ashing procedures are also varied and frequently depend upon the sophistication of the ashing furnace used.

Samples are generally ashed in muffle furnaces at about 500° C for 4 hours or more. Generally the temperature of the furnace is ramped up to 450-500° C over several hours and then held at that temperature for 4-8 hours or more. Programmable ashing furnaces with variable temperate ramping rates, holding times, and cooling stages are now in common use. They have added a degree of sophistication in temperature control and precision previously unattainable in sample ashing. Jones (1991) outlined a representative ashing procedure. Quartz or porcelain dishes or crucibles are typically used as ashing containers. Quartz or "Vycor" crucibles generally contribute less sample contamination. Material from the walls of the muffle furnace may be a major source of inorganic contamination during the ashing procedure.

Chemical Analysis Methods

Numerous instrumental analytical methods exist for the determination of most metals in lichens, each with a multitude of advantages and disadvantages. The choice of methods for the determination of Hg, semi-metals such as As and Se, and the nonmetals such as B, S, and halogens are more restricted. This is also true for the determination of many organic compounds. In choosing a method, many factors must be considered:

- the amount of sample required (typically, 1-10 g, or more)
- destructive or nondestructive sample preparation
- partial (i.e., extractable) or total determination
- measurable concentration range and limit of detection
- accuracy and precision of the method
- interferences
- the number of elements simultaneously determined
- availability of instrumentation, analysis time, and cost

The principles of most modern instrumental analysis techniques have been reviewed by Skoog and West (1980). In addition, Watson and Isaac (1990) have briefly discussed the principles, advantages, disadvantages, and sample preparation for many analytical techniques with regard to their use for plant analysis. Jones (1991) has reviewed the determination of micro-nutrients in plants.

Atomic Spectroscopy

Atomic emission, absorption, and fluorescence spectroscopy provide the basis for most techniques used for inorganic analysis of plants. Since the introduction of atomic absorption spectroscopy (AAS) by Walsh in 1955, it has been the most widely used method for the determination of metals in plants. Flame AAS has been used to determine elements such as Ca, Cu, Fe, K, Mg, Mn, Na, and Zn, among others. Each element is determined individually, a time-consuming procedure. In general, there are relatively few interferences, but the linear calibration region is usually small. Detection limits in the sample are commonly in the g g^{-1} range. Better detection limits by a factor of 10-1000 with very small amounts of sample are attainable using graphite furnace (flameless) AAS for elements such as Cd, Co, Cr, Mo, Ni, and Pb. Other flameless AAS techniques routinely used include cold-vapor AAS for Hg and hydride-generation AAS for the hydride-forming elements, As, Bi, Sb, Se, and Te. Flameless AAS techniques typically have detection limits in the sample in the ng g^{-1} range.

Historically, arc, spark, or flame emission sources have been used in photographic or direct-reading spectrographs for the determination of metals. These emission techniques and many AAS methods have

Table 1. — Example detection limits for the analysis of plant ash by ICP-AES (0.1 g plant ash/10 ml solution) (P. Briggs, USGS, personal communication).

Element	Detection Limit	Element	Detection Limit	Element	Detection Limit	Element	Detection Limit
Al %	0.1	Au $\mu g\ g^{-1}$	16	Ga $\mu g\ g^{-1}$	8	Sc $\mu g\ g^{-1}$	4
Ca %	0.1	Ba $\mu g\ g^{-1}$	2	Ho $\mu g\ g^{-1}$	8	Sn $\mu g\ g^{-1}$	20
Fe %	0.1	Be $\mu g\ g^{-1}$	2	La $\mu g\ g^{-1}$	4	Sr $\mu g\ g^{-1}$	4
K %	0.1	Bi $\mu g\ g^{-1}$	20	Li $\mu g\ g^{-1}$	4	Ta $\mu g\ g^{-1}$	80
Mg %	0.1	Cd $\mu g\ g^{-1}$	4	Mn $\mu g\ g^{-1}$	8	Th $\mu g\ g^{-1}$	8
Na %	0.01	Ce $\mu g\ g^{-1}$	8	Mo $\mu g\ g^{-1}$	4	U $\mu g\ g^{-1}$	200
P %	0.01	Co $\mu g\ g^{-1}$	2	Nb $\mu g\ g^{-1}$	8	V $\mu g\ g^{-1}$	4
Ti %	0.01	Cr $\mu g\ g^{-1}$	2	Nd $\mu g\ g^{-1}$	8	Y $\mu g\ g^{-1}$	4
Ag $\mu g\ g^{-1}$	4	Cu $\mu g\ g^{-1}$	2	Ni $\mu g\ g^{-1}$	4	Yb $\mu g\ g^{-1}$	2
As $\mu g\ g^{-1}$	20	Eu $\mu g\ g^{-1}$	4	Pb $\mu g\ g^{-1}$	8	Zn $\mu g\ g^{-1}$	4

been replaced with inductively coupled atomic emission spectroscopy (ICP-AES). In this technique, an argon plasma is used as the atomic emission source and many elements may be determined either simultaneously or sequentially. Simultaneous instruments generally require less sample than sequential instruments for the same number of elements determined. ICP-AES techniques usually have similar or better limits of detection than AAS techniques, but they have a wider dynamic concentration range (i.e., linear calibration over several orders of magnitude concentration). Table 1 lists detection limits typical of ICP-AES analysis of plant ash that has been acid digested. Detection limits may vary widely depending upon various aspects of the sample digestion and the ICP-AES instrumentation. Note that caution should be used in reviewing detection limit information for ICP-AES, as well as all other methods, to verify whether the detection limit given is for the raw plant material, ashed material, or digested material in solution. The precision of ICP-AES techniques is on the order of 5-10% relative standard deviation when the element concentration is well above the detection limit. Solid-sampling techniques have been used with ICP-AES, such as slurry-nebulization or laser ablation, but they are not in routine use, especially for plants. Whereas ICP-AES has commonly replaced AAS for multi-element determinations, it has not replaced flameless AAS techniques where lower detection limits are required, or for elements such as Hg, As, and Se.

In recent times ICP has been coupled with mass spectrometry (MS) to provide a technique with the advantages of ICP as an atomization source and MS as a sensitive detector. ICP-MS is becoming more common, albeit expensive. It offers better detection limits for some elements than ICP-AES, as well as isotopic distribution information. Mass spectral overlaps preclude the determination of some elements that are routinely determined by ICP-AES or AAS techniques.

X-ray fluorescence spectroscopy (XRF) with a wave-length or energy dispersive detector is also a technique used for the determination of metals and some nonmetals. Energy dispersive XRF (EDXRF) is probably more commonly used than wave-length dispersive XRF in plant analysis. Samples frequently are prepared nondestructively as a pressed powder, and elements in atomic number from sodium through uranium may be determined. Whereas detection limits in the sample in the $g\ g^{-1}$ range may be attained with EDXRF, sample heterogeneity and matrix matching of calibration standards pose distinct problems with this technique and may affect both accuracy and precision. The rapid nondestructive analysis and the large number of elements that may be determined with EDXRF make it an excellent screening technique.

Radiochemical Techniques

Numerous radiochemical analysis techniques may be used for plant analysis. Isotope dilution techniques have been used, though instrumental

neutron activation analysis (INAA) is more commonly used today. In INAA, a sample is irradiated with thermal neutrons and the induced radioactivity for many trace elements is measured with gamma-ray spectrometry. Plant samples can be analyzed nondestructively without any pre- or post-irradiation treatment. Typically, detection limits in the sample are in the g g^{-1} range for most of the 25-30 elements that can be routinely determined. However, lead, for example, is not routinely determined. Greater sensitivity and lower detection limits can be obtained for some elements by destructive preparation of the sample to remove various inter-element interferences. INAA can provide reasonably rapid determination of some elements; however, for a large suite of elements, analysis times of several months are more common. INAA is generally expensive and a limited number of reactor activation facilities exist in the U.S.

Mass Spectrometry

For the types of lichen analyses addressed in this chapter, mass spectrometry (MS) is generally confined to the determination of organic pollutants after a chromatographic separation, or to coupling with ICP for the determination of inorganic elements. In addition, stable isotope ratios may be used to help identify sources of elements such as Pb or S. Measurement of the stable isotope ratio for S^{34}/S^{32} has the potential for uniquely distinguishing the contribution of sulfur to an ecosystem from various natural and anthropogenic sources (Jackson and Gough 1989, Krouse 1989). Epiphytic lichens and mosses and atmospheric sulfur species have exhibited relatively similar sulfur isotope ratios (Krouse 1977, Winner et al. 1978, 1989; Case and Krouse 1980; Taylor and Bell 1983). However, the analysis is relatively expensive, generally not widely applicable to nonpoint source pollution studies, and the results can be complex and difficult to interpret.

Chromatographic Methods

Ion chromatography (IC) has been used to determine cations and anions in plant tissue. Basta and Tabatabai (1985) determined Ca, K, Mg, and Na in plant ash by IC, and Busman et al. (1983) determined total S and lC by the combustion/IC method. Other anions such as F^-, Br^-, NO_3-, and PO_43- are commonly determined by IC in water samples, but their determination in plants is complicated by difficulties in digesting the plant without volatilization losses or addition of interferences from the digestion procedure.

Other forms of chromatography have been used to qualitatively and quantitatively determine pollutants such as chlorinated hydrocarbons. Determination of these compounds is similar to the analysis of pesticides in plants, which involves extraction with a moderately polar solvent system (e.g., dichloromethane or an acetone:hexane mix), followed by removal of pigments using silica gel or Florisil. The choice of extraction solvent and its polarity, the type of clean-up and analysis columns and conditions, and the chromatographic detector used are dependent upon the expected concentration of the target compounds and the project objectives. Gas chromatography with an electron capture detector (GC-ECD) and with a mass spectrometer (GC-MS) are commonly used for the determination of chlorinated hydrocarbons and other organic pollutants. The choice between the two methods is largely governed by the desired detection limits and budgetary constraints. GC-ECD is a sensitive and relatively inexpensive method compared to GC-MS.

The two techniques both use high-resolution bonded phase GC columns for hydrocarbon separation. When using GC-ECD, sequential fractionation of PCBs from most other chlorinated hydrocarbons with eluents of differing polarities may be required. In GC-ECD analysis, peak identification is accomplished by comparison of retention times with chromatograms from standard solutions, and thus does not necessarily give unequivocal results. Confirmation is typically achieved by using a second column with a different polarity and retention time, or by confirmation of some portion of the samples by GC-MS.

Miscellaneous Other Methods of Analysis

Sulfur has been determined in plant material by a wide variety of techniques incorporating acid digestion or combustion methods followed by turbidimetric, colorimetric, and titrimetric quantitation. Jackson et al. (1985) examined in some detail the various aspects of washing, grinding, and analytical parameters for the determination of sulfur in a variety of plants and lichens by an automated combustion/IR method. They found that the combustion/IR method provides rapid and precise analyses with the analytical variance being much smaller than the natural variability of S in lichens from several different ecosystems.

Various electrochemical techniques such as potentiometry, coulometry, amperometry, and polarography have not found common usage in plant analysis. Of these techniques, potentiometric

methods using ion selective electrodes (ISEs) may be the only exception. Anions such as NO_3-, Cl^-, and F^- have been most widely determined using ISEs in extracts of plant tissue. Other ISEs are available for the determination of several cations and anions.

Reporting of Chemical Analysis Results

The reporting of chemical analysis results by the analyzing laboratory to the contractor/researcher and by the contractor to the contracting agency must conform to the standards of good laboratory practice. This requires that the following must be explicitly defined and appropriately documented:

- the analyte determined, e.g. total sulfur, water extractable SO_4^{2-}
- the reporting units, e.g. g g^{-1} Pb, wt% S
- the weight basis, e.g., ash weight, air-dried at 40° C
- the appropriate number of significant figures
- the analytical method used
- analytical method limits of detection
- problems encountered during analysis

Censored values (those results determined as below the limit of detection) should be reported as "less than a specified value (e.g., g g^{-1} Pb) and not as "zero" or "not detected." Questionable results should be independently verified with the laboratory to avoid misinterpretation or inappropriate conclusions in the final report. Transmittal of results by the laboratory or the contractor in an electronic form must be formatted so that no ambiguity exists with respect to the requirements listed above. This is especially true for significant figures. In general, reporting of more than two or three significant figures is an egregious misrepresentation of the analytical data by the laboratory or the contractor.

DATA ANALYSIS

The task of data analysis for environmental studies using the chemical analysis of lichens or plants typically has a number of common steps:

- database construction, data entry, and database management for field, laboratory, and QC data
- review and validation of field, laboratory, and QC data
- generation of summary statistics and identification of data distributions, trends, and outliers
- statistical evaluation, tests of significance, estimates of confidence levels
- ecological interpretation.

Each of these steps is important and should be well documented, especially where legal or regulatory issues are involved.

QUALITY ASSURANCE AND QUALITY CONTROL

Quality assurance and quality control (QA/QC) are no longer just associated with the production of widgets, but are an important mandatory part of every environmental study. The level of effort required for a QA program is commonly proportional to the legal ramifications of a project or the intended use of the data. However, even those ecological studies perceived as "pure research" need a good QA program to validate results.

Quality assurance incorporates quality control activities, and is a system that uses documentation to show that work is performed according to specific standards and data to show that measurements are produced to known levels of quality. Planning and documentation are the major components of the QA program. This includes planning and documentation of the study design, the field and laboratory methods, and data analysis procedures. In many environmental studies, the QA documentation is entirely lacking or completely inadequate, particularly with respect to details and descriptions of chemical analysis procedures.

A QA program should not be limited to the laboratory or contractor/researcher, but should be utilized and reviewed by the contracting agency as well. This may include submittal of blind standards to the laboratory by the contracting agency, reanalysis of samples by the contracting agency at a different laboratory, or external verification of data, data analysis, and taxonomic vouchers.

Quality control is defined as the operations and associated data to show that a field or laboratory method is in statistical control within known probability levels of accuracy and precision for a given time period. A QC program incorporates field collection, laboratory analysis, and data analysis aspects, such as duplicate sampling in the field by a second collector, analysis of replicate samples and standard reference materials, and spot checking of data entry and data analysis results.

Various aspects of QA programs directed at analytical chemistry laboratories have been discussed by Taylor (1987) and Dux (1986). In a laboratory QA program, documentation of the daily use and analysis of external or internal calibration standards, quality control samples, sample duplicates, blanks, and standard reference materials is mandatory. One particularly problematic aspect is the analysis of standard reference materials. NIST is the primary supplier of certified botanical reference materials in the U.S. A large number of determinations for certified and noncertified constituents in NBS 1571 Orchard Leaves has been published for many different analytical techniques. However, this SRM, along with its replacement NBS 1572 Citrus Leaves, is no longer available. Gladney et al. (1987) have compiled analytical results for these two SRMs and a number of other biological, geological, and environmental SRMs. The main botanical SRMs now available are NIST 1515 Apple Leaves, 1547 Peach Leaves, 1573 Tomato Leaves, 1575 Pine Needles, and 2695 Fluoride in Vegetation. Horwitz and Albert (1991) review the variability associated with concentration estimates for several of these SRMs. Unfortunately, these standards do not always have certified constituents in the same concentration range as lichens. Elements such as S are not certified, and noncertified values for various additional constituents have not been determined. This latter problem is especially true for organic pollutants, for which no botanical reference material exists. Lists of additional international biological reference materials and their suppliers have been compiled by Roelandts (1989a,b).

The cost of SRMs, as well as the lack of good matrix matching, has frequently served as an excuse to avoid the inclusion and analysis of SRMs in environmental studies. For any study currently involving trace element determinations in lichens, inclusion of one or more SRMs in the QA program should be mandatory. Taylor (1985) reviews numerous aspects of the use of SRMs in a QA program.

FINAL CONTRACT REPORTS

Final contract reports for environmental studies using chemical analysis of lichens for biomonitoring should conform to guidelines set by the agency requesting the report; for example, see Harbour (1987). These guidelines primarily concern style and format issues, not scientific content. A final contract report should thoroughly address the issues of study design, field methods, laboratory methods, data analysis, and ecological interpretation. This should not be done with the same brevity and lack of methods, details, or raw data typical of a regular journal article. This is especially true where a study may be repeated in the future by the same or another contractor. Minor changes in analytical methods due to incomplete documentation may artificially introduce biases or trends in the data, especially in the absence of an adequate QA program.

In addition to an understandable, concise description of a project's scientific conclusions, a final report should include clear, thorough descriptions of:

- study objectives and study design
- field and laboratory methods
- data analysis procedures and software used
- QA program plan

References to previously published descriptions of methods are acceptable if the original source provides sufficient detail and does not lead one on an endless wild-goose-chase of obscure and incomplete references. The report should include addenda with:

- all raw field and laboratory measurements conforming to the data reporting requirements outlined above,
- all QC data pertinent to verifying the quality of the raw data, such as the analysis of sample duplicates and SRMs

Without such documentation, independent validation of a report's conclusions or future repetition of a study without undetectable biases cannot be made.

CASE STUDIES ON THE USE OF CHEMICAL ANALYSIS OF LICHENS

The use of lichens and bryophytes as biomonitors of chemical contaminants, including metals, sulfur, fluoride, radionuclides, and organic pollutants, has been reviewed by Burton (1986)(see also Wilson, 1991). Other reviews which focus on lichens as indicators of metal pollution have been done by James (1973), Nieboer et al. (1977), Nieboer and Richardson (1981), Martin and Coughtrey (1982), Anderson and Treshow (1984), and Puckett (1988).

Most studies involving chemical analysis of lichens have focused on determining elemental baselines or examining spatial or temporal trends

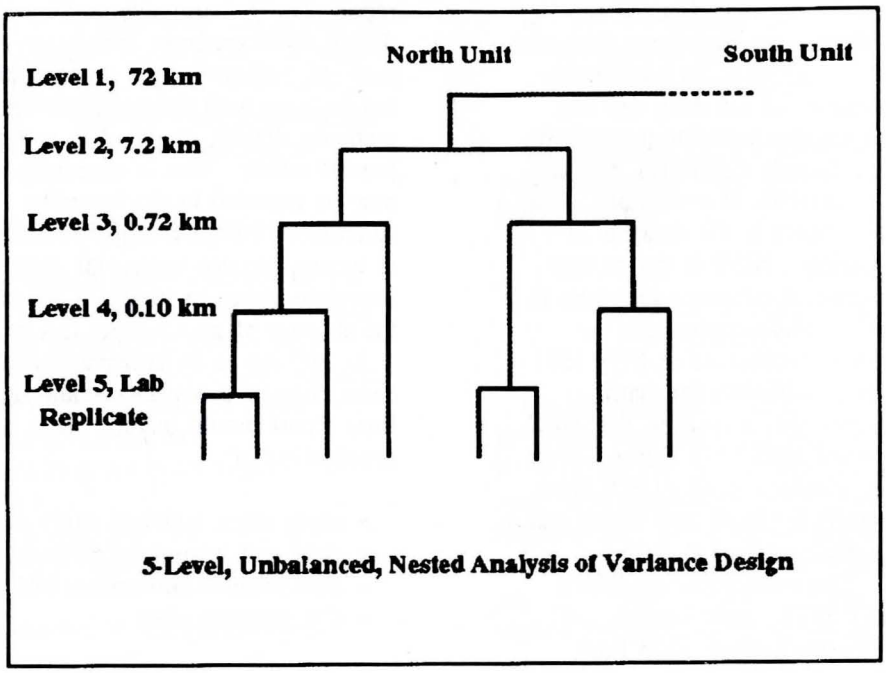


Figure 2. — Schematic of unbalanced, hierarchical ANOVA sampling design (data from Gough et al. 1988b).

with respect to pollution sources. Chemical baselines have been defined in various ways. Usually a baseline refers to a specific set of conditions and point in time (i.e. when the samples were collected) and not to historical or pre-industrial conditions. One definition of a baseline is the expected 95% range, which is the mean plus or minus two standard deviations. For lognormally distributed data (Tidball and Ebens 1976), the expected 95% range is:

$$(GM/GD^2) \text{ to } (GM \times GD^2)$$

where GM and GD are the geometric mean and deviation, respectively. Gough et al. (1988a,b) used the latter definition to determine baseline ranges for a number of elements in *Parmelia sulcata* at Theodore Roosevelt National Park, North Dakota, and in *Hypogymnia enteromorpha* and *Usnea* spp. at Redwood National Park, California. In both studies, spatial variability was estimated using an unbalanced nested ANOVA design. For example, in the Theodore Roosevelt National Park study, differences in elemental content in *P. sulcata* between park units (i.e. about 70 km apart) and within a park unit were examined with respect to the analytical measurement error (fig. 2). As shown in figure 3, for some elements a large proportion of the total variance was attributed to laboratory measurement error. This was typically the case when the mean concentration in the lichen was very near the limit of detection for the analytical methods and/or there was very little natural variability between samples, as in the case of sulfur concentration. When the laboratory error is large with respect to natural variability, it is unreasonable to calculate a baseline range. Gough et al. (1988a,b) chose not to express a baseline range for those elements in which the laboratory error contributed 50% or more of the total variance. They also found that the variance attributed to within-site variability (i.e. replicate samples at 10-100m) combined with the laboratory variance generally represented 70-100% of the total variance (fig. 4). As these studies point out, regional differences in element concentrations must be large with respect to the variability attributed to within-site differences and laboratory error to be detectable.

In numerous studies, elemental concentrations in lichens have been clear indicators of pollution. However, lichens will probably not give very reliable quantitative estimates of deposition without accompanying conventional atmospheric monitoring. Empirical correlations between heavy metal levels in lichens and mosses and actual measured deposition from bulk precipitation collectors have been observed in several studies (see review by Puckett, 1988). In the absence of conventional monitoring, the use of

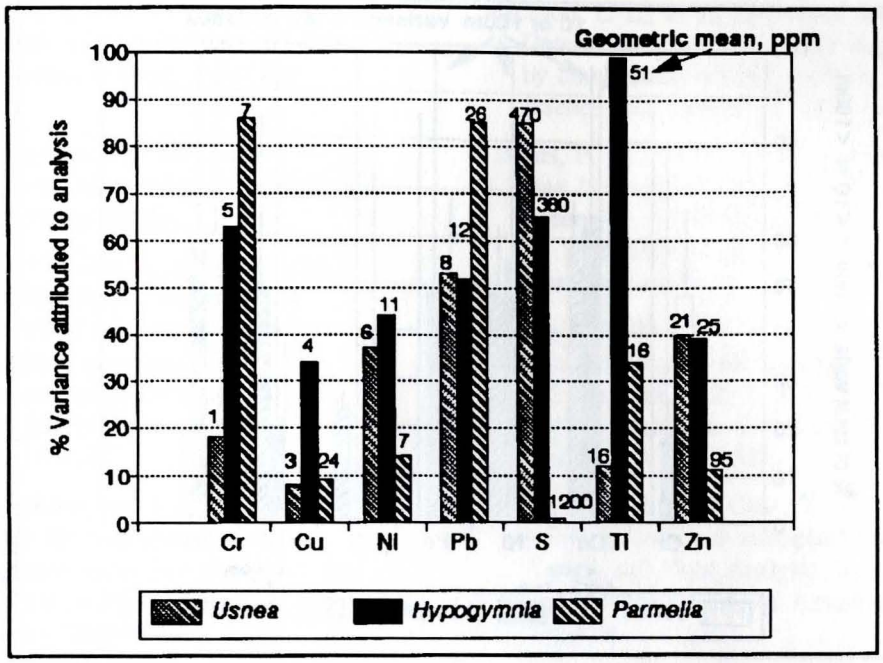


Figure 3. — Example of percentage of total variance attributable to laboratory error for the analysis of *Usnea spp.*, *Hypogymnia enteromorpha*, and *Parmelia sulcata* (data from Gough et al. 1988a,b).

inferred trapping efficiencies by lichens has the potential to provide retrospective quantitative monitoring information. The trapping efficiency is the percentage of metal ions in precipitation and/or particulates falling on a given surface area that are actually retained by the lichen. To estimate the trapping efficiency for an element, the total time of accumulation (i.e. the lichen age) is needed. The age of the thallus may be estimated from known growth rates or possibly from radionuclide measurements such as ^{137}Cs or $^{210}Pb/^{210}Po$ ratios.

Because ^{210}Pb is a decay product of naturally occurring radon which diffuses into the atmosphere from the soil, and its deposition rate is known for specific geographic regions, the maximum theoretical concentration of ^{210}Pb in the lichen can be calculated. As a first approximation, the inferred trapping efficiency for lead is the ratio of the measured ^{210}Pb concentration in the thallus to the theoretical concentration. Using measurements of lead in thallus of varying ages, retrospective fallout rates can be estimated. However, because the thickness of foliose lichens varies with age, concentration must be determined per unit of surface area. Schwartzman et al. (1987, 1991) used measurements of atmospherically derived ^{210}Pb and its in-situ daughter ^{210}Po to infer trapping efficiencies in a saxicolous lichen, *Flavoparmelia baltimorensis*, from Great Falls, Maryland, and to estimate retrospective lead fallout rates. Their estimates agreed with levels expected for rural and suburban areas. They also found that the lead was apparently retained at ion-exchange sites in the foliose lichen.

Whereas lichens have frequently served as biomonitors of point and nonpoint source pollutants for metals, sulfur, and fluoride, they have only recently received attention as suitable monitors of organic pollutants. Carlberg et al. (1983), Thomas et al. (1984), Thomas (1986), Villeneuve and coworkers (1984, 1988), and Bacci and coworkers (Bacci et al. 1986, Focardi et al. 1991) have analyzed lichens from Norway, Spitzbergen, Sweden, France, and the Antarctic for chlorinated hydrocarbons and other organic pollutants. These studies indicate that DDT and its metabolites, PCBs, lindane and other hexachlorocyclohexane isomers, and hexachlorobenzene contaminate lichen tissue in at least ng g^{-1} levels even in the remote environments of the Arctic and Antarctic.

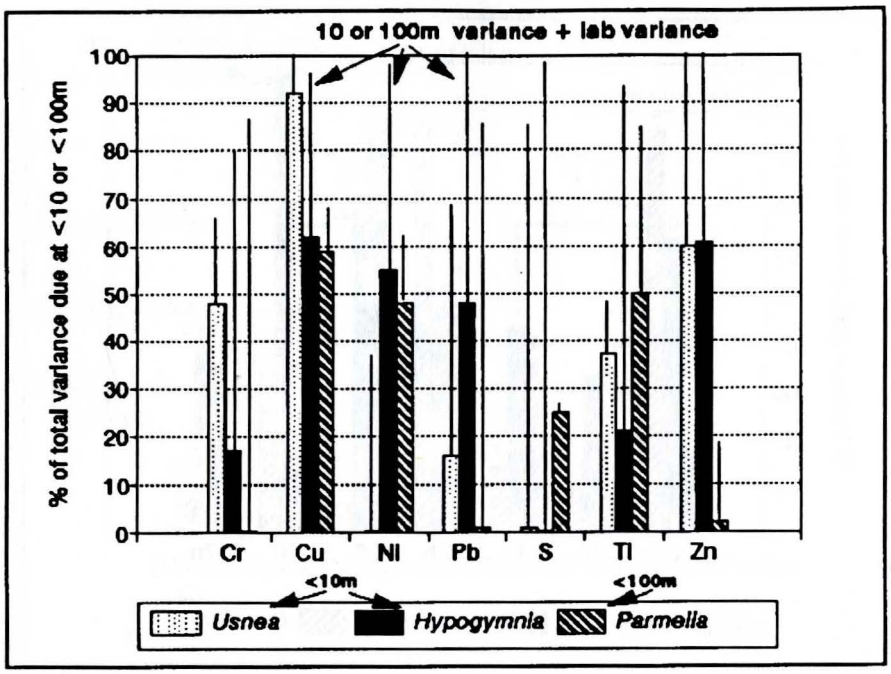


Figure 4. — Example of percentage of total variance attributable to spatial variance (closest spatial stratification level) and laboratory measurement variance (data from Gough et al. 1988a,b).

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Lessons from Seven Case Studies

Ken Stolte, Robert Doty, Kathy Tonnessen, Rich Fisher

This chapter provides a conceptual model for integrating the information provided in the earlier chapters of this report. The model is used to help analyze seven possible air resource management scenarios. Each scenario is illustrated with a case example of an actual air pollution study using lichens.

Lichen monitoring intended to directly support air resources management activities can be designed and conducted in many ways, depending on the specific air quality issue of interest. For example, the placement of plots and the frequency of sampling will differ depending on whether the air pollution stress is from a particular point source or a large urban area. Despite such differences, most lichen monitoring can be designed to follow a conceptual model.

The information in this chapter can be used to assist in deciding whether or not lichens can be used as bioindicators or bioaccumulators of air pollution.

THE CONCEPTUAL MODEL

The information presented in other chapters of this report can be interrelated as shown in figure 1, the Conceptual Model. The model uses air quality modeling and aerometric monitoring to characterize predicted and actual pollutant loadings, respectively. This, together with the problem description, is used to formulate a study design. See Chapters 2,4,5,6 and 7 for details. A characterization of the site (Chapter 3) is essential for establishing a foundation for specific lichen analyses. A community analysis (Chapter 4) is used to identify the grouping of lichen species and other environmental variables that define the terrestrial ecosystem. A floristics study (Chapter 2) determines the presence or absence of species. In many instances, this information is sufficient to identify sensitive species (Chapter 5) and to assess the air pollution effects on lichens. This information can be obtained from a lichen expert.

When the above steps do not yield conclusive results or when no previous information exists about the native species or their responses to air pollution, elemental analyses (Chapter 7) of the effects of air pollution on lichen chemistry and physiology can be performed. This work is more time consuming and expensive.

For areas already influenced by air pollution to the extent that species have been extirpated, active monitoring by studying transplants (Chapter 6) becomes necessary. The identification of indicator species is only needed when no previous information exists about the native species and their response to the air pollutant of interest. Elaborate fumigation facilities and well-designed exposure-response studies are necessary for this work.

POLLUTANTS OF CONCERN

The following list includes air pollutants which could affect lichens.

Primary

- Oxides of sulfur
- Hydrogen
- Oxides of nitrogen

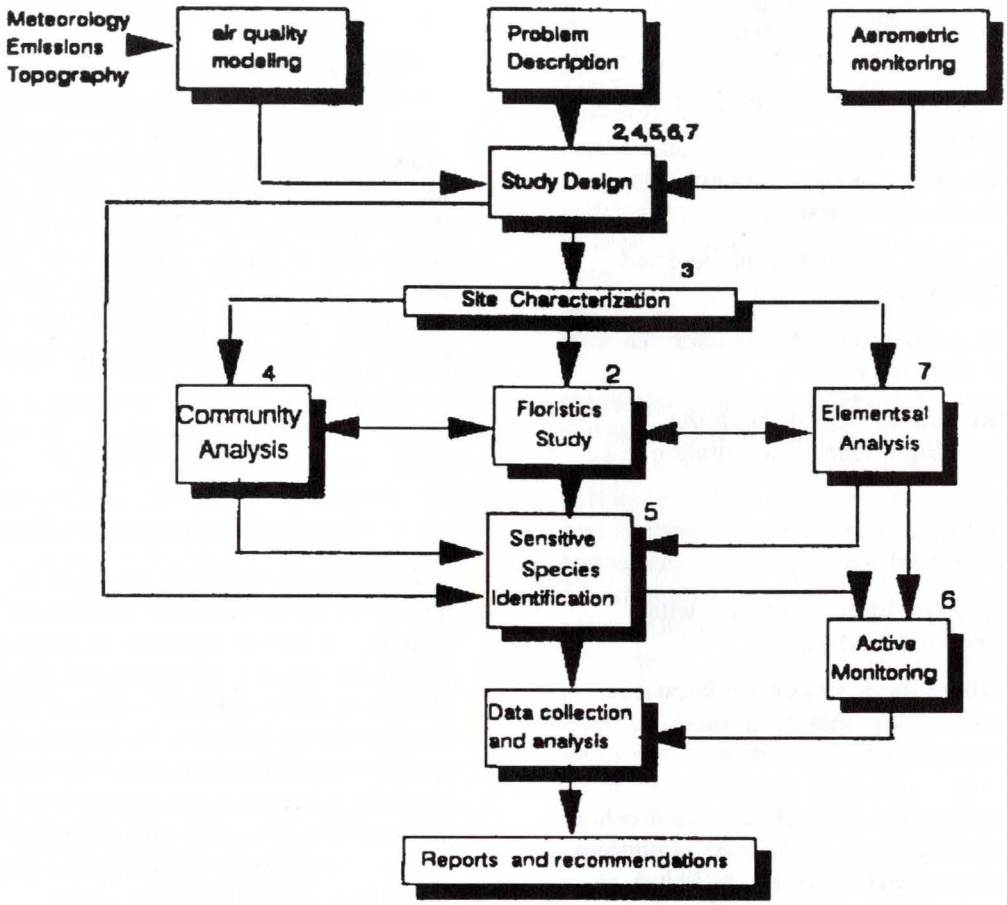


Figure 1.—Conceptual model for lichen-based air pollution monitoring. Numbers near the boxes refer to pertinent chapters in this report.

- Hydrogen fluoride
- PM10
- Toxics (metals, organics, radionucleides)
- Climate (especially temperature and humidity)

Secondary

- Sulfates (wet or dry)
- Nitrates (wet or dry)
- Ozone
- Ammonia

POLLUTANT EXPOSURE FACTORS

Important pollutant source characteristics include pollutant types, emission rates, operating schedules, and effective plume heights. Due to meteorological conditions, tall stacks and high effective plume heights can result in the fumigation of vegetation far from the point source. Some point sources operate sporadically, such as "peaking" power plants, which operate principally during periods of high demand.

Topography and geography also affect the level of lichen exposure to pollutants. Plumes that intersect elevated terrain increase the amount of pollutant the lichens experience. At night and often in winter, plumes can be channeled along valleys, allowing pollutants to accumulate in low spots. Vegetation canopies can absorb plumes, altering the exposure of lichens located close to the ground.

Wind direction is the most important meteorological variable affecting point-source pollutant exposure for a given lichen community. Mixing height and wind speed help determine plume concentration while temperature, humidity and sunlight contribute to the chemical transformation of pollutants.

SEVEN AIR RESOURCE MANAGEMENT SCENARIOS

Most air pollution situations fall into one of the following seven categories:

A) An existing point source that is not expected to change emissions.

B) An existing point source that is expected to reduce emissions.

C) An existing point source that is expected to increase emissions.

D) A new point source (e.g. through the Prevention of Significant Deterioration Process).

E) A region affected by air pollution with increasing regional loadings.

F) A region affected by air pollution with declining regional loadings.

G) A region where the loadings are known or thought to be so low that no adverse impact to resources has occurred.

Each of these scenarios is briefly discussed below in terms of the stressors (e.g., pollutants) of concern, the effect the stressor might have on the lichen species and/or communities, and research and monitoring methods that might be appropriate. A case example illustrates each scenario. A summary of the model components (the other chapters in this volume) illustrated in each of the seven scenarios is shown in table 1.

Table 1.—Scenarios of air pollution monitoring summarized in this chapter in relation to numbered model components of figure 1 and pertinent chapters in this report. (1) is Study Design and not a chapter.

		CHAPTER						
		(1)	2	3	4	5	6	7
SCENARIO	A	X	X	X		X		X
	B	X	X	X	X	X		
	C	X		X		X	X	X
	D	X	X	X		X		
	E	X				X		X
	F	X	X		X	X		
	G	X	X	X	X	X		X

CASE EXAMPLES

The case examples describe the use of lichens as bioindicators in past studies illustrating each scenario. Since each past and future situation is unique, these cases are presented only as examples; they are not necessarily the preferred solutions to the scenarios. When designing lichen biomonitoring projects, some, all, or none of the information from these examples may be useful along with details from the rest of this document. The case studies are brief summaries; refer to the original references for additional details.

SCENARIO A

Constant Point Source Emissions

A single existing point source of air pollution may already be affecting lichen populations in the vicinity. A loss or reduction in the number or vitality of sensitive genotypes and species due to chronic or acute exposure might be expected at some distance downwind from the source. A gradient of species composition and morphological condition might be expected as the ground-level pollutant concentration decreases with distance from the source. The greatest impact from a single source would be expected near that source.

Case Study of Constant Point Source Emissions

Roberts and Thompson (1980) investigated the effects of a phosphorus plant in Newfoundland, Canada, on the surrounding terrestrial and epiphytic lichens. This facility had been operating since 1969. The primary phytotoxic pollutants emitted were hydrogen fluoride (HF) and silicon tetrafluoride (SiF_4). By late 1974, damaged vascular vegetation around the plant covered 11,400 hectares, mostly northeast of the plant due to the prevailing winds. Sidhu and Roberts (1976) described the nature of the vegetation damage. They evaluated the composition of the lichen communities in four damage zones around the plant and at 12 similar control sites distant from the plant. Two of the most common species, the terrestrial lichens *Cladina rangiferina* (L.) Harm, and *Cladina stellaris* (Opiz.) Brodo., were analyzed for fluoride concentrations in the tissues. The morphological condition of the lichens was examined in each zone.

The concentration of fluoride in the samples was found to be inversely correlated with distance from

the plant in the direction of the prevailing southwest winds. Fluoride concentrations ranged from a peak of 2830 ppm (dry weight) in Zone 1 closest to the source to an average of 6.4 ppm in the control areas outside of Zone 4, about 9 km from the source. Fluoride concentrations in Zone 1 ranged from 525-2830 ppm. There was a strong correlation between the morphological appearance of the lichens and the accumulation of fluoride in the tissues. All individuals of the indicator species found in Zone 1 were amorphous (without structure) and discolored, while about 25% of the individuals of the indicator species in Zone 4 were only slightly discolored with some structural degradation. Fluoride concentrations in *C. rangiferina* ranged from 6.4 - 1338 ppm along the gradient from Zone 1 to the Control Zone. The researchers observed a similar pattern of fluoride accumulation in the 1974 needles of balsam fir (6.1 - 237.0 ppm), soil humus (2.1 - 24.2 ppm), terrestrial bryophytes (11.3 - 2255.0 ppm), and air (0.1 - 5.2 ug F/m^3).

Comparison with Conceptual Model

A) Pollutant Exposure: Ambient Fluoride (ug F/m^3); meteorological variables

B) Site Characterization: wind speed and direction; temperature and rainfall patterns; vegetation communities; tree species associated with forest types; other vegetation communities; and parent material and soil types. Control sites comparable to damaged sites.

C) Floristics: lichen floras from 4 damage zones; 1 control site

D) Indicator Species: *Cladina rangiferina* and *Cladina stellaris*

E) Community Analysis: No

F) Chemistry/Physiology: total fluoride in the two lichen indicator species and other epiphytic lichen species.

G) Transplants: No

SCENARIO B

Decreasing Point Source Emissions

Lichens have been used to indicate improvement in air quality as emissions of toxic elements from a point source decline. This reduction in emissions could result from improved technology to remove pollutants from the stack emissions, a switch to cleaner fuels, or a reduction in activity of the plant. The ecological consequences of reduced stack emissions should be favorable, and lichen species and communities downwind of the plant should be expected to show some improvement in condition under these circumstances. To measure improvement in lichen condition, we could measure the reduction in toxic element concentrations, improvement in morphological condition of existing lichens, and establishment of new or returning sensitive species.

Case Studies of Declining Emissions Around a Point Source

Showman (1975a) mapped the distribution of lichens in 1973 at 128 sites around the isolated coal-fired Muskingum River power plant in southeastern Ohio. He found that the lichen *Flavoparmelia caperata* was a particularly sensitive species and was absent from many sites near the power plant. Air quality monitoring in subsequent years showed a reduction in SO_2 emissions as a result of design changes to the power plant in 1972. Researchers reevaluated the 128 sites around the power plant annually; by 1976, *Flavoparmelia caperata* had recolonized a few sites, evidenced by small pieces of thalli in sheltered bole fissures at tree bases. By 1980, many more sites were recolonized with this species and there was no longer a recognizable void of this species near the power plant. A community-type analysis showed similar results: the number of corticolous species at sites within the 1973 void zone increased from 2.0 in 1973 to 3.8 in 1976 to 4.7 in 1980 (Showman, 1981). In this study the distribution of sensitive lichen species and overall lichen community structure were useful measures to delineate areas of poor air quality in 1973 and to monitor improvement in air quality following reductions in stack emissions.

Comparison with Conceptual Model

A) Pollutant Exposure: SO_2 monitoring around power plant

B) Site Characterization: Unshaded trees selected; elevation

C) Floristics: Initial flora to select sensitive species

D) Indicator Species: *Parmelia caperata* L. - SO_2 sensitive

E) Community Analysis: Quantified community analysis

F) Chemistry/Physiology: No

G) Transplants: No, natural recolonization occurred

SCENARIO C

Increasing Point Source Emissions

An increase in emissions from existing point sources can negatively affect existing lichens. If sensitive genotypes are eliminated from the population by chronic exposure to pollutants, only the more tolerant species and individuals will be present after the emissions increase. We can also expect to observe morphological deterioration, decreased number of species, increased mortality of less tolerant individuals, and expansion of the affected area around the point source.

Case Study of Increasing Point Source Emissions

Garty (1987) transplanted the common epiphytic species *Ramalina duriaei* (De Not.) Bagl., to 22 sites located near Sharonim and Tel Aviv, Israel. This study illustrates effective use of active monitoring of air pollution. Some of the biomonitoring sites were in heavily urbanized areas near Tel Aviv and other small cities; one was a control site. The Maor David, a 1400 MW coal-burning power station located near Sharonim, began operation in 1981. These were regarded as point sources because of their distance from affected areas, the small sizes of the urban areas, and the nature of their wind plumes.

Garty exposed lichens on transplanted twigs from February 1979 to March 1980 (Garty and Fuchs 1982, Fuchs and Garty 1983), and then exposed a second group of the same species from July 1981 - July 1982. He used X-ray fluorescence and atomic absorption spectrometry to compare the concentrations of the elements Ni, Cr, Cu, Zn, and Pb in the lichens among the sites and between the two time periods.

The thalli of *R. duriaei* nearest the urban centers in 1981-1982 were chlorotic after one year of exposure. Metal concentrations were higher at sites near the urban centers and the power plant than in tissues of lichens from the control site. Comparisons between 1970-1980 and 1981-1982 study periods indicated that Pb, Ni, and Cr were higher in 1981-1982, and that Zn and Cu were higher in 1970-1980. Correlation analyses among metal concentrations indicated the associations Pb/Ni, Pb/Cr, Pb/Mn, and Zn/Mn to be significant. For example, the Pb/Zn ratio increased from 0.19 in 1979-1980 to 2.11 in 1981-1982. Researchers attributed the reduction in Zn concentrations to reduced spraying of Zn in the citrus and pecan orchards during 1979-1980. They attributed Pb patterns to a 16% increase in the number of vehicles which still used leaded gasoline and an increase in miles driven during 1981-1982. High Cr and Ni concentrations found in lichen tissues in 1981-1982 were linked to the content of the coal burned at the plant during that period.

Comparison with Conceptual Model

A) Pollutant Exposure: metal content of fuels; amount of vehicular traffic; Zn spraying in orchards

B) Site Characterization: little information; host tree, number of replicates, and placement of transplants on the host tree were given

C) Floristics: no community analysis; only indicator species identification

D) Indicator Species: one common species (*Ramalina duriaei* (De Not.) Bagl.) selected for transplants

E) Community Analysis: not done (not purpose of the study)

F) Chemistry/Physiology: chemical analysis of toxic metals

G) Transplants: This method illustrates the effective use of transplants and the value of corroborative pollutant accumulation data.

SCENARIO D

New Point Source Emissions

Lichens can be used to map the distribution of air pollutants emitted from a newly installed industrial unit in a previously undisturbed area. A change from relatively good air quality conditions to an increase in pollutants in ambient air to phytotoxic concentrations can be observed in lichen communities through increased mortality, decreased morphological

condition of survivors, elevated element concentrations in tissues, decreased cover of sensitive species, and a general decline in species diversity. Under conditions of low moisture the magnitude of the impact will be lessened. The most severe effects usually occur within 1-5 kilometers of the source, depending on the size of the source and the height of the stack. The severity of effects usually declines with increasing distance from the source.

Case Study of New Point Source Emissions

Lichen species were evaluated on lands surrounding the Esso oil refinery at Fawley, Hampshire, England (Morgan-Huws and Haynes 1973). A small refinery was built in 1921; a larger unit was added in 1952. Sulfur dioxide and other air pollutants were emitted from the furnace stacks from 30-50 meters in height into the surrounding low-lying landscape of the Southampton Water area. Little smoke was emitted due to the high combustion temperatures. The areas surrounding the refinery were a mixed agricultural-heath-forest-residential landscape. No pre-1921 lichen records existed, but the lichen vegetation around the Fawley refinery in 1921 is presumed to have been similar to floras found in nearby areas of similar vegetation and habitats but without air pollutants. The town of Fawley is at low elevation on the Southampton Water, and consequently the area is influenced by maritime air-masses, with relative humidities from 95-100% common. There was some background influence of SO_2 and smoke from the ribbon-like urban development in the area.

Lichens were sampled from the trunk of a common tree in the area (*Quercus robur*) for comparison of sulfur dioxide effects. They examined the southwest-facing half-sector of the trunks to a height of 2 m above ground. This part of the trunk included the entire macrolichen flora for the area. Trees selected were well-established (dbh 0.5 meters), erect, undamaged, and free-standing (open-grown). Many sites included only a few trees each (4 trees), with the general approach patterned after Skye (1968). The sampling design was carefully devised to obtain statistical correlations relative to the pollution gradient. As a result, site replication was more dense in areas where ambient pollution exposure gradients changed abruptly.

Lichen floras were evaluated in 1966, after 45 years of exposure to the small source and 14 years of exposure to the larger source. There was a marked decrease in most lichen species around the emission site. Species present at the site, in order of sensitivity to the air pollutants, included: *Parmelia perlata*, *Usnea* spp., *Ramalina* spp., *Parmelia subrudecta*, *Parmelia glabratula*, *Parmelia caperata*, *Parmelia sulcata*, *Evernia prunastri*, *Hypogymina physodes*, and *Lecanora conizaeoides*. The first two species were particularly sensitive, and their distribution coincided approximately with the 40 ug/m3 SO_2 (annual average) pollution 'contours' recorded by SO_2 monitors in the area. Only *Lecanora conizaeoides* seemed relatively unaffected by the emissions and was still present at all sampling sites, even those close to the emission source.

Comparison with Conceptual Model

A) Pollutant Exposure: information on addition of SO_2 sources; isopleths of SO_2 around sources

B) Site Characterization: general landscape description; collection methods detailed

C) Floristics: detailed flora centered around one host species

D) Indicator Species: relative sensitivity of species inferred from field gradient analysis

E) Community Analysis: no abundance or cover of species recorded

F) Chemistry/Physiology: No

G) Transplants: No

SCENARIO E

Increasing Regional Air Emissions

Remote clean air areas of the western US are becoming increasingly dirty from the long range transport of pollutants from continually growing large urban areas. Small communities and rural areas in the West are also growing and adding to the increasing pollutant loading in remote areas. It is important to establish floristic and community baselines as soon as possible for trend comparison with future studies and analyses.

The transport of secondary pollutants, such as sulfates, nitrates, ozone, and particulate metals, has resulted in pollutant loadings in areas of the country relatively remote from large point sources of pollution such as power plants, refineries, or smelters. To determine the impact of these regional emission sources, lichens may be used as

bioaccumulators of certain elements. Historical concentrations of pollutants such as lead or sulfur can be measured in herbarium tissue samples of lichens from the same general area as the contemporary specimen. This is a situation in which "baseline" information is collected to determine the rate of degradation of an ecosystem in response in increasing inputs of regional pollutants to wilderness areas.

Case Study of Increasing Regional Emissions

In the Mid-Appalachian Mountains of western Virginia, Shenandoah National Park was created in 1936 to preserve second-growth hardwood forest ecosystems. Due to the proximity of this class 1 area to planned and projected sources of emissions, both point and non-point sources, the National Park Service Air Quality Division commissioned a study of the differences in the lead and sulfur concentrations between contemporary (1984-86) and historical specimens (1933-58) of the lichen *Flavoparmelia baltimorensis* (Lawrey and Hale 1988).

Lawrey and Hale (1988) located eleven sites within the park that had been sampled during the period of 1933-58. Tissue samples of the lichen *Flavoparmelia baltimorensis* found in the herbarium collection at the Smithsonian Institution were large enough to allow elemental analysis for lead and sulfur, using inductively coupled plasma techniques. Lead concentrations decreased at all sites during the time between collections, presumably as the result of the change in regional emissions of lead linked to changes in the burning of leaded gasoline in vehicles. However, sulfur concentrations in lichen tissues increased significantly at all but three of the sites, with those at high elevations (over 1000 m in elevation) showing the largest percentage change in sulfur (ug/g dry weight). This points to long-distance transport of sulfate as the source of the additional sulfur.

Comparison with Conceptual Model

A) Pollutant Exposure: Elemental analysis of lichen samples was used as a surrogate for instrumental monitoring. Gaseous pollutant and precipitation monitoring data are also available for this park.

B) Site Characterization: No information on site characteristics; the same sites were visited at different times to make the comparison.

C) Floristics: Only a single "indicator" species, *Flavoparmelia baltimorensis*, was used as a bioaccumulator of lead and sulfur.

D) Indicator Species: Researchers selected an indicator species that was common to the park to ensure that repeat surveys would yield specimens for chemical analyses.

E) Community Analysis: No, not part of the study plan. One would not expect detectable change to communities in "clean air" areas.

F) Chemistry/Physiology: Researchers analyzed tissue samples for lead and sulfur concentrations. Physiological changes were not noted because of the use of herbarium specimens.

G) Transplants: No

SCENARIO F

Decreasing Regional Emissions

The eastern United States, from the Ohio River valley to the Atlantic coast, is heavily populated and includes much of the nation's industrial development, both of which contribute to air pollution in this region. While the population continues to grow, traditional industries are being replaced by less-polluting forms of development. Furthermore, states and the EPA are implementing sections of the Clean Air Act Amendments of 1990 that call for the reduction of the total amount of sulfur dioxide and nitrogen oxide emissions. Lichen analyses can be used to measure the progress of these control activities. Documentation of the flora and the general health of lichen communities is needed to establish a baseline so we can detect vegetation improvements as air emissions are reduced.

Case Study of Decreasing Regional Emissions

Recolonization by epiphytic lichen species around the northwest areas of London, England was determined by evaluating species at 29 sites in 1980 (annual SO_2 averages around 130 ug/m^3), and re-evaluating the same sites in 1988 (annual SO_2 averages 29-55 ug/m^3) (Hawksworth and McManus 1989). This area includes parks, reserves, gardens, cemeteries, woods, and other natural areas. An additional 22 sites were added in 1988 to more

thoroughly document the spatial patterns of lichen recovery. The lichen flora occurring on trees at each site was determined during a .5-1.0 hour collection period per site from April-October 1988; therefore, the species lists were probably not complete. Researchers recorded the species identified, types of host trees, the relative frequency of occurrence on a 1-5 scale, and the diameter or length of the largest foliose and fruticose species at each site.

The lichen floras for both years were classified according to the species classes devied by Hawksworth and Rose (1970). By 1980, the lichen flora had shown improvement in diversity and vigor, compared to London floras established earlier (e.g., Laundon 1970), and it was postulated then that further improvements in lichen flora could be expected if the annual SO_2 levels fell to 40-50 ug/m^3 (Rose and Hawksworth 1981). [For reference, the annual average SO_2 concentrations found in class I areas in the eastern U.S. are in the range of 3.5-21.0 ug/m3 for the period 1989-91.]

The authors observed 26 species in the northwest area of London that had not been observed in 1980; and additional 11 species were found that had not been recorded by Laundon (1970). Eight species had not been recorded in the area for over 200 years. It is interesting to note that the recolonization of SO_2-sensitive species occurred at a faster rate than would have been expected from the Hawksworth and Rose species classes. This was attributed to the rapid improvement in air quality from 1980 to 1988.

This study illustrates that extremely high ambient SO_2 levels exert a pronounced influence on the occurrence of lichen species. Changes in microenvironmental conditions of temperature and moisture due to urban development are less of a factor, since environmental conditions within the city probably did not change dramatically in the last 20 years.

Comparison with Conceptual Model

A) Pollutant Exposure: characterized ambient mean winter SO_2

B) Site Characterization: host trees; time at each site

C) Floristics: intensive floristic characterization

D) Indicator Species: characterized species by sensitivity to SO_2

E) Community Analysis: relative abundance and size of species at each site; characterized flora relative to Hawksworth and Rose qualitative scale (1970)

F) Chemistry/Physiology: no

G) Transplants: no

SCENARIO G

Regional Air Pollution Emissions-Clean Areas

Recently, isolated regions of the United States, such as the Piceance Basin of Colorado and the Sublette Basin of Wyoming, have experienced sudden and dramatic increases in development, either through increasing industrial activity or related secondary growth. Because of their topographic locations and proximity to wilderness areas, these basins are the subject of concern about the effects of rapid increases in air pollution. The potential effects on lichen populations in regions such as these are similar to those anticipated in Scenario D, where we hypothesize the introduction of a new source to a previously clean area. In this case, however, the expected area ultimately affected by this regional energy development will be much larger than in Scenario D.

Case Study of Clean Air Areas

Ryan (1990) surveyed lichens in a number of wilderness areas in California. The Marble Mountain wilderness in Northern California is thought to be a clean area, with few air pollution sources upwind. The largest point source of emissions is a pulp mill located over 100 km from the wilderness boundary.

Comparison with Conceptual Model

A) Pollutant Exposure: this is not part of the report, but a state inventory of emissions does exist; no sources are identified in this inventory.

B) Site Characterization: a detailed mapping of the plant communities or ecotones was not performed, but the major land forms of the wilderness have been identified and the wilderness Air Quality Related Values (AQRVs) were listed for this region. This list included lichen species among the potentially sensitive AQRVs.

C) Floristics: Most of Ryan's work was

compilation of an inventory of lichen species; there was no existing information on the lichen flora of this wilderness area.

D) Indicator Species: Ryan identified sensitive and tolerant species based on other dose-response work. His analysis showed the need for additional work on the identification of indicator species.

E) Community Analysis: As part of the floristic survey, Ryan described species and their abundances.

F) Chemistry/Physiology: Samples were collected and sent to a lab for analysis of the baseline chemical constituents in the indicator species.

G) Transplants: No

CONCERNS

Using lichens to monitor air quality is not often straightforward and usually requires considerable expertise. Here are a few potential problems that should be kept in mind when conducting lichen air quality studies.

Mapping Community Cover

Because of the varied habitats that support often-diverse lichen communities, it may be difficult for a field researcher to devise a sampling scheme that allows a statistically robust estimate of species cover. Mapping community cover, if considered essential to monitoring, must be done very carefully to assure repeatability in future studies. It is also necessary to consider the spatial distribution of lichens in a given area relative to pollutant exposure.

Natural Variability in Characteristics

The use of the morphological status of lichens as an indicator of pollution stress is problematic. The natural variability in thallus geometry and chemistry should be measured in both baseline and treatment areas.

Anthropogenic vs. Natural Sources of Elements

When chemical analysis of lichen tissue is performed, it may be difficult to distinguish the relative contributions due to (1) contamination in collection, handling, and analysis, (2) natural background concentrations due to windblown dust, geologic substrate, and marine aerosols, and (3) anthropogenic air pollutants. The possibility of using sulfur isotopes to identify the source of this element has been raised by Nriagu and Glooschenko (1992) during their study of sulfur accumulation in mosses in different locations in Canada.

Taxonomy Update

Lichen surveys in wilderness areas often result in the discovery of new species or morphological variations of previously-named species. In recent years, floristic surveys and other lichen inventory efforts have also resulted in the identification of new species. In addition to the lack of good keys or guides to lichens in various areas, such discoveries result in difficulties in identifying lichens in the field for novice lichenologists or others who may have only a general background in botany.

Lack of Dose-Response Information

Most information on species sensitivities available in the literature is based on the exposure of common lichens to sulfur dioxide. There is some information on the susceptibility of lichens to ozone (Sigal and Nash 1983); however, many lichens have never been rigorously tested in controlled fumigation experiments to determine their responses to different ambient concentrations of gaseous air pollutants, toxics, and wet deposition.

There is also little information on the relationships between the genetic differences of subspecies and their susceptibilities to known concentrations of air pollutants. Much of what has been asserted about lichen sensitivities to air pollutants has been inferred from a few statistically designed fumigation studies. To address these gaps in knowledge, monitoring pollution and research on dose responses may need to be combined into the same effort.

Corroboration from other sources of monitoring information may be necessary to make decisions when statistical results are suggestive but not significant at 1% or 5% levels. For example, changes in the physiology of a single lichen species may suggest a pollution-related cause, but may not be statistically significant. Other, non-pollution causes may be possible explanations, such as aging or drought. However, information on changes in community floristics coupled with known increased pollution loadings in the area can help support the weak inference from the fumigation study, and the combination of evidence may be persuasive.

Table 2. — Cost Estimates for Conducting Various Lichen Studies.

METHOD	UNIT	# of SITES (per veg type)	SCALE	COST ($) (per unit)
Site characterization	study	-	area	300
Floristics	site	30+	all species	200-400
Community analysis	site	20-60+	all species	300-500
Indicator species				
fumigation	study	-	1-10 species	20,000-50,000
gradient	site	10+	many species	15,000-25,000
Transplants	site	6-20	1-few species	1,000-3,000
Chemical analysis	sample	20-100	multiple elements/sample analysis: 25-200 collection: 50-300	

COST ESTIMATES

The previous chapters describe methods and techniques for using lichens as bioindicators of air quality. Table 2 gives a range of costs associated with each method. The detail with which each method is applied, the remoteness of the project area, the specific objectives of the study, and many other factors will result in a wide range of projected costs. The costs presented here should be considered very rough estimates and should be used only as a guide when choosing among methods or specifying the level of detail of analysis to be applied.

Cost should not be the sole or even the major determinant for developing a monitoring program. Rather, the scientifically best method(s) should be chosen and scaled to an affordable level. Authors of the chapters in this manual state that monitoring studies are worthless unless they are done well, and a good study is usually worth the cost. They also emphasize that in most cases, a preliminary study should be done before costs are estimated, to make decisions about how many sites are needed, which indicator species are appropriate and where they are found. If a community analysis is planned, a preliminary study is essential to determine the number and location of homogeneous sites. Costs can be cut by combining data collection efforts. For example, floristics data, community data and tissue specimens can be collected simultaneously in the field.

SUGGESTED FURTHER RESEARCH

The idea that lichens are sensitive to air pollution is historical, since lichens are mostly sensitive to SO_2 pollution, and most air pollution was accompanied by SO_2 emissions earlier in this century and in the last century. Organic air toxics, heavy metals, nitrogen oxides, radionculeides, PAN, and ozone are major modern pollutants to which lichens are not necessarily sensitive. Howeve,r lichens can still be used as accumulators of pollutants; it is possible to measure pollutant concentrations in lichen tissues and correlate this information with ambient air pollution concentrations. There are many gaps in our knowledge about how lichens react to air pollution. We suggest the following areas for further research to begin to full those gaps:

1. Fumigation studies to determine if there are indicator species and bioaccumulation potential for specific pollutants: nitrogen oxides, ozone, carbon dioxide, and toxic air contaminants.
2. Video/photographic techniques that can be used for community analyses.
3. Standard methods and QA/QC procedures for chemical analyses.
4. Development of herbaria/libraries of useful lichen species that are accessible to researchers and relevant to different habitats.
5. Development of easy-to-use field and laboratory guides to lichen taxonomy that can be used for routine floristic surveys and are kept up-to-date as new species are identified.

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Descriptions of back-cover photos, clockwise from the upper left corner:

B1. *Lobaria oregana*, found on tree branches and trunks, provides winter forage for deer and mountain goats. Internal cephalodia contribute significant amounts of fixed nitrogen. Collected for tissue analysis in Tongass Air Quality Biomonitoring Project. **Photo by Sylvia Duran Sharnoff and Stephen Sharnoff.**

B2. *Parmelia exasperatula* Nyl. on *Juniperus scopulorum* bark. This species and its nearly identical twin, *P. elegantula* (Zahlbr.) Szat. (transferred to Melanella by Essl.) are not sufficiently discriminating to be useful as indicators; they are also difficult to remove from the bark. *P. exasperatula* was used by Rope and Pearson 1990, and they found it exasperating. **Photo by Lorenz Pearson.**

B3. *Hypogymnia enteromorpha* (Ach.) Nyl. in the foreground and *Usnea spp.* in the background on Douglas-fir in the Little Bald Hills ultramafic region of Redwood National Park. These two species were used to measure baseline element concentration ranges in Redwood National Park. **Photo by Larry Jackson.**

B4. *Cladina rangiferina*, reindeer or caribou lichen. This and other *Cladina* species are the primary food of caribou and occasionally of people during times of famine. *C. rangiferina* is common among mosses in muskegs. This species was collected for tissue analysis by Tongass Air Quality Biomonitoring Project. **Photo by Sylvia Duran Sharnoff and Stephen Sharnoff.**

B5. *Hypogymnia enteromorpha* (Ach.) Nyl. This species was used to measure baseline element concentration ranges in Redwood National Park (Gough et. al. 1988). **Photo by Larry Jackson.**

B6. *Usnea* spp. This species was used to measure baseline element concentration ranges in Redwood National Park (Gough et. al 1988). **Photo by Larry Jackson.**

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