

## Lichens as an indicator for Air Pollution: A Review

GBG Pananjay Kartikey Tiwari

Department of Environmental Sciences, Vira College of Engineering, Bijnor, U.P 246701  
(E-mail: [pananjay\\_gbg@rediffmail.com](mailto:pananjay_gbg@rediffmail.com))

### Abstract

During recent years a number of different species of lichens have been found (species richness) and the presence of indicator species has been studied to characterize regional air quality impact on lichen populations. The following review describes some of the pollutants affecting lichens, mechanisms of pollutant accumulation by lichens, physiological responses to pollution, and methods of study utilizing lichens.

### 1. Introduction

Lichens are a group of non-vascular plants composed of fungal (mycobiont) and algal (photobiont) species growing in a symbiotic relationship. Lichens are classified and named according to the fungal partner, with many thousands of species of lichens sharing a much smaller number of photobiont species. Lichens are an integral and important component of many ecosystems. They contribute to nutrient and hydrological cycles (Harper and Marble, 1988), biological diversity (McCune and Geiser, 1997), and they are critical sources of forage, shelter and nesting material for many mammals, birds and invertebrates (Maser *et al.* 1986). The largest biomass of forage and nitrogen-fixing lichens occurs in late-successional and old-growth forests with clean air quality (McCune, 1993). An effect of deteriorating air quality on lichens and other sensitive, ecologically critical organisms is an important management concern all over the world.

Lichens were recognized as potential indicators of air pollution as early as in the 1860's in Britain and other countries in Europe. Since then, lichens have played prominent roles in air pollution studies throughout the world because of their sensitivity to different gaseous pollutants, particularly sulfur dioxide. They have also been found to act as accumulators of elements, such as trace metals, sulfur, and radioactive elements (Ahmadjian, 1993). During the period 1973-1988, approximately 1500 papers were published on the effects of air pollution on lichens (Ahmadjian 1993), and many general reviews of lichens and air pollution have been compiled (Ahmadjian, 1993). Lichens have been used often as receptor-based bio-monitors in air quality studies. Lichen characteristics measured in air pollution studies include morphological, physiological, and population. Historically, lichens have been mostly used in a qualitative way serving as indicators of pollution. In the last few decades quantitative measurements of the chemical content of lichens and sensitive physiological processes have increasingly been used to indicate pollutants.

Possible responses to air pollution stress include chlorophyll degradation, changes in photosynthesis and respiration, alterations in nitrogen fixation, membrane leakage, accumulation of toxic elements, and possible changes in spectral reflectance, lichen cover, morphology, community structure and reproduction. The most widely used methods to measure these responses are fumigation and gradient studies (Stolte *et al.* 1993).

In the past ten years scientists in Sweden, Great Britain, Canada and United States have studied the effects of airborne pollution on lichens and have developed ways to use lichens to detect pollution. These scientists have found that some kinds of lichens are more easily killed by air pollution than others. Thus, in places where air is very dirty, no lichens survive; in areas with slightly cleaner air, one or two very resistant lichens can grow; in cleaner areas five or six species are found, while in the cleanest areas, a dozen or more species of lichen thrive. By learning which types of lichens are most sensitive to air pollution and which ones are most resistant, one can judge the quality of air by examining the lichens. However, lichen technique can not tell the amount of a particular pollutant present in the air for which other techniques need to be used.

### 2. Major Lichen Sensitive Air Pollutants

There is a large variety of elements and chemical compounds present in the atmosphere that affect lichen growth and distribution. Primary pollutants include sulfur dioxide (SO<sub>2</sub>), nitrogen dioxide (NO<sub>2</sub>) and fluoride (F) compounds. These pollutants remain in the same chemical form after they are emitted into the atmosphere. Besides these, there are secondary pollutants that result from chemical reactions involving primary pollutants during their transport in the atmosphere. This class of pollutants includes ozone (O<sub>3</sub>).

peroxy acetyl nitrate (PAN), and acid rain components such as sulphuric (H<sub>2</sub>SO<sub>4</sub>) and nitric (HNO<sub>3</sub>) acids. Finally, there is a third mixed category of 'air toxics' that include industrial organic compounds, agricultural pesticides, trace metals, and metalloids. Information on effects of these compounds on lichens is not extensive (Belnap, *et al.*, 1993).

#### **i) Sulfur Dioxide**

Sulfur dioxide is a by-product of coal or fuel oil combustion, ore reduction, paper manufacture, many industrial processes, and vehicle exhaust. Natural background concentration is 0.28 to 2.8 µg/m<sup>3</sup>, but values near pollution sources can rise to as high as 200 µg/m<sup>3</sup> or more. Most lichens cannot survive extended periods of SO<sub>2</sub> exposure above 60 µg/m<sup>3</sup> (Hale 1981).

#### **ii) Oxides of Nitrogen**

The dominant anthropogenic source of NO<sub>2</sub> is the combustion of fossil fuel by stationary sources and vehicles. Although NO<sub>x</sub> thresholds for lichens have not been established, NO<sub>x</sub> and other nitrogenous compounds are components of acid rain and photo-oxidants.

#### **iii) Fluorides**

Fluoride is an important pollutant around smelters, brickworks, glass making factories and fertilizer plants and is also released in large amounts by volcanic eruptions (Richardson 1992). Many lichens are sensitive to this pollutant as it can get concentrated in hydrated lichens to more than 200 times its ambient concentrations. It has been found that obvious damage to lichens begins at internal concentrations between 30-70 ppm (Perkins *et al.*, 1980).

#### **iv) Photo-Chemical Toxins**

Photochemical toxins are generally associated with automobile exhaust in cities and are formed from the burning of fossil fuels. In this group are ozone and peroxy acetyl nitrate (PAN). Photochemical threshold for lichens is a controversial topic (NAPAP, 1990). Part of the difficulty in establishing lichen sensitivity to ozone has been in finding a study area where ozone concentration gets elevated at time of the day when lichens are metabolically active.

#### **v) Metals**

Lichens are laced with mineral nutrients and heavy metals. These pollutants get deposited through precipitation, fallouts, dust, and underlying substrate from both natural and anthropogenic sources.

### **3. Mechanisms of Accumulation**

Lichens accumulate substances from their environment by a variety of mechanisms, including particulate trapping, ion exchange, extra cellular electrolyte absorption, hydrolysis, and intracellular uptake (Nieboer *et al.*, 1978).

#### **i) Particulate Trapping**

Particles of various elements, such as iron, titanium, aluminum, chromium, and uranium can become embedded in the lichen thallus in the algal and/or fungal layer under moist or dry conditions (Nieboer *et al.*, 1978). Morphological characteristics that contribute to particulate trapping include micro-topography of the lichen surface and a large surface area-to-volume ratio of the lichen, which can result from thin thallus, branching, or projections from the thallus such as isidia and phyllocladia (Richardson & Nieboer, 1980).

#### **ii) Ion Exchange**

Ion exchange requires that lichen must be moist to make elements in the ionic form. Uptake of metal ions by ion exchange is passive and rapid and obeys principals of mass and charge balance. Extra cellular binding sites at cell walls are most likely involved.

Lichen species vary in their capacity for ion-uptake as well as on the efficiency of particulate trapping of their surface morphology. This underlines the importance of correct identification of lichen samples used for monitoring purposes and demonstrates the desirability of collecting the same species for analysis from all sampling sites.

#### **iii) Accumulation of Metals**

The action of rain, surface water, and passive upward diffusion from the substrate are likely to bring dissolved minerals in contact with lichen thalli (Nieboer *et al.*, 1978), while the amount of each type of metal

ion that can be accumulated by a lichen is dependent upon the uptake characteristics of that particular species and the amount and availability of metal ions in the surrounding environment (Tyler, 1989).

Lichens are very efficient accumulators of Lead through aerosols, particulate metal fallout, or acid rain. The toxic effect of Lead on lichens is minimal. Some species can accumulate up to about 2000 ppm, after which concentrations do not increase, indicating a degree of physiological turnover (Lawrey & Hale 1981).

Ni is taken up by algal cells in lichens.

Mercury is readily accumulated by lichens. Background values were measured at approx. 0.223 ppm while specimens 8 km from a chlor-alkali plant contained 0.53 ppm (Lodeni, 1979).

Schutte (1977) has reported that Chromium accumulation in lichens at Ohio has increased two to ten-fold in concentrations over turn of the century.

Nieboer *et al.* (1979) have documented two phases of Cu uptake in *Umbilicaria muhlenbergii*. In the first phase copper ions get bonded to receptor sites on algal cells. In the second phase, copper ions bind to the fungal cells as evidenced by  $K^+$  efflux.

Zinc is found in fairly large concentrations (200-600 ppm) in lichens near zinc smelters.

#### 4. Application / Effect of Physiological Responses to Pollution

##### a) Lichens as Pollution Monitors

Lichens possess a number of characteristics that make them suitable bio-monitors for air pollution. Many lichen species have large geographical ranges, allowing study of pollution gradients over long distances. Lichen morphology does not vary with season and so accumulation of pollutants can occur throughout the year. Lichens are usually very long lived.

Lichens are very sensitive to a large variety of pollutants. Several factors contribute to this sensitivity. Water and gas are exchanged over the entire lichen thallus. Lichens do not have access to soil nutrient pools because they lack roots and depend on deposition, water seeping over substrate surfaces, and atmospheric and other comparatively dilute sources of nutrients. Lichens also lack the protective tissues or cell types necessary to maintain constant internal water content. Many lichens pass through multiple wetting and drying cycles during a day. When hydrated, nutrients and contaminants are absorbed over the entire surface of the lichen. During dehydration, nutrients and many contaminants are greatly concentrated by being converted to slow-releasing forms, i.e., absorbed to cell walls, cloistered inside organelles or crystallized between cells. During heavy rains, nutrients and pollutants are gradually leached. A dynamic equilibrium thus exists between atmospheric nutrient/pollutant accumulation and loss, which makes lichen analysis a sensitive tool for the detection of changes in air quality (Huckaby, 1993). As a result of the above stated combined factors that are not well-understood, different morphological types of lichens exhibit differing levels of sensitivity to pollution. In general sensitivity increases in the following series: crustose < foliose < fruticose.

There are **four kinds of lichens**. Foliose lichens are leaf-like; Fruticose lichens are bushy or shrubby; Squamulose lichens are scaly and may have stalks called podetia; and Crustose lichens are crusty. In general, the following table is a yardstick for measuring the local amount of sulphur dioxide ( $SO_2$ ), and hence the local air quality:

- Heavily polluted air - No lichen, only green algae
- Polluted air - Crustose lichen, e.g. Lecanora
- Moderate air - Squamulose and Podetial lichen
- Clean air - Foliose and Fruticose lichen
- Very clean air - Sensitive species such as Ramalina, Usnea, and Lobaria

However, to get an accurate indication of pollution one needs to identify specific species, for example, *Hypogymnia physodes* is tolerant of  $SO_2$  and ozone but not fluoride, *Lobaria pulmonaria* is sensitive to  $SO_2$  but mildly tolerant of ozone, and *Lecanora conizaeoides* is a very toxictolerant bioindicator in general.

##### b) Effects of Pollution on Lichens

A myriad of pollution effects on lichens have been described. At the level of the whole plant, investigators have described decreases in thallus size and fertility, bleaching and convolution of the thallus, restriction of

lichen occurrence at the base of vegetation and mortality of sensitive species (Sigal & Nash, 1983). Microscopic and molecular effects described include reduction in the number of algal cells in the thallus (Holopainen, 1984), ultra structural changes of the thallus (Hale 1983, Holopainen, 1984), degradation of photosynthetic pigments (Garty *et al.*, 1993), altered photosynthesis and respiration rates (Sanz *et al.*, 1992), and elevation in the content of heavy metals in the thallus (Lawry 1986). Huebert *et al.*, (1985) concluded that peak exposures may be of primary importance in determining the survival of lichens in industrial areas.

### ***i) Effect of Sulphur Dioxide***

SO<sub>2</sub> is considered to be the primary factor causing the death of lichens in most urban and industrial areas. It has been reported that fruticose lichens are more susceptible to SO<sub>2</sub> than many foliose and crustose species (Seaward 1987). The observed effects of SO<sub>2</sub> include decrease in respiration and photosynthesis, increase in membrane permeability, increases in K<sup>+</sup> influx and ions lost, and ultra structural changes (Belnap *et al.*, 1993). Damage to the algal component of the thallus is evidenced by its discoloration. The entire thallus dies soon after algal cells are damaged (Wetmore, 1985). Low pH increases toxicity due to SO<sub>2</sub> (Farmer *et al.*, 1992).

There are three general mechanisms by which SO<sub>2</sub> exerts its toxic effects upon lichen thallus. At low pollution levels, enzyme systems within thallus may become activated. This can be observed in the form of increase in activity in glucose-6-phosphate dehydrogenase, or increase in the levels of glutathione and total protein. Heightened enzyme activity can be used as a bioindicator for the non-visible injury metabolism and detoxification of absorbed SO<sub>2</sub>. At higher levels SO<sub>2</sub> deactivates enzymes by chemical modification (sulfitolysis) leading to reduced metabolic activity and loss of membrane integrity. It also binds to central metal atoms of enzymes, and thereby adversely affects membrane function and cell osmolality. In addition, SO<sub>2</sub> competitively inhibits carbonate (HCO<sub>3</sub><sup>-</sup>) and phosphate (H<sub>2</sub>PO<sub>4</sub>) interactions with enzymes (Nieboer *et al.*, 1979) (Table 1).

**Table 1: General Mechanisms of SO<sub>2</sub> Toxicity**

<b>Type of Reaction</b>	<b>Observed/Expected Response or Injury</b>	<b>References</b>
<b>Enzyme Deactivation:</b> (e.g. sulfitolysis) -chemical modification -binding to metal centers (Vit B <sub>12</sub> ; Fe) - inhibition	Reduced metabolic activity; loss of membrane integrity, membrane function and cell osmolality, (competition with HCO <sub>3</sub> <sup>-</sup> , H <sub>2</sub> PO <sub>4</sub> )	Nieboer <i>et al.</i> , 1979.
<b>Stimulation of Enzyme Systems:</b> (often in response to low pollution levels) -Glucose-6-phosphate dehydrogenase, -Increases in glutathione and total protein SH	Use of increases in enzyme activity as a bio-indicator for non-visible injury and detoxification of metabolism absorbed SO <sub>2</sub>	Nieboer <i>et al.</i> , 1979.
<b>Reaction with Reactive Bio-molecules:</b> -chemical (bi-sulfite adducts) - redox (acts as electron acceptor/donor at pH 7)	Modification of metabolic precursors and products; interference with electron flow in photosynthetic and respiratory electron-transport chains.	Puckett <i>et al.</i> , 1980, Nieboer <i>et al.</i> , 1979.

### ***ii) Effect of Acid Rain***

Natural and anthropogenic variations of substrate and water acidity are a major factor governing the composition and health of lichen communities (Farmer *et al.*, 1992). At the level of the individual thallus acidity affects nitrogen fixation, photosynthesis, growth, and cellular ultra structure (Belnap *et al.*, 1993). Besides these direct effects on physiological processes, environmental acidity can affect cryptogamic communities indirectly by altering substrate chemistry, thereby affecting species diversity and composition at the community level.

### ***iii) Sensitivity of Lichens to Sulfur Dioxide vis a vis Acid Rain***

Species tolerant to acid rain need not be the same as those tolerant of SO<sub>2</sub>. *Usnea* species intolerant to SO<sub>2</sub> grow on bark of low pH, while *Parmelia sulcata* is tolerant of SO<sub>2</sub>, but not of acid rain (Gilbert, 1986).

#### **iv) Effect of Substrate Interactions**

Evidence from field observations, intensive studies of the environment at contrasting sites, and field and lab experiments (Farmer *et al.*, 1992) show that acid rain can affect lichens both directly and indirectly by the acidification of substrata. Seaward (1987) writes, "Regional pollution over wide areas has reduced lichen diversity, and has favored the expansion of a relatively small number of species formerly having narrower ecological requirements and/or more restricted distributions such as *Buellia punctata*, *Lecanora conizaeoides*, *Lecanora muralis*, *Parmelia incurva*, *Parmeliopsis ambigua*, *Phaeophyscia orbicularis*, *Scolisciösporium chlorococcum*, *Xanthoria candelaris*, and *Xanthoria elegans*." Pollution greatly affects lichen species sensitive to high concentrations of  $H^+$  and  $Al^{3+}$ . In addition, when pollution occurs in poorly buffered environments, species such as *Lobaria* which normally thrive on mildly acidic to neutral substrate can also be negatively affected (Farmer *et al.*, 1992).

#### **v) Effect of Acid Fog**

Fog, which often increases with increasing elevation, contains higher levels of dissolved ions, including  $H^+$ , than precipitation rain or snow. Although the effects of occult deposition from fog on epiphyte communities have not yet been critically examined, acid fog has been implicated in changes to terricolous cryptogamic floras (Wolseley & James, 1992). Desert lichens, which obtain most of their water from fog and dew, are particularly vulnerable to air quality and weather pattern changes (Nash, 1996).

#### **vi) Effect of Nitrogen Enriched Acid Rain**

The nitrogenous component of acid rain can produce a fertilizer effect on lichens and cause floristic changes. Sochting (1990) surveyed the tissue nitrogen content of reindeer lichens in Denmark. In unpolluted areas, tissue levels were 0.26-0.49 % while in areas of wet deposited acidity, values were 0.70- 0.73 % and visible injury could be found. However, the problem of defining critical loads for nitrogen has not been resolved. Presently, this is determined by the biological response of the system, and is very hard to quantify, compared to  $H^+$ , which is measured by buffering capacity (Farmer *et al.*, 1992).

#### **vii) Effect of Acid Rain on Lichen Structure and Physiology**

The effects of acid rain on lichen structure and physiology include a decrease in nitrogen fixation, photosynthesis, and growth, and changes in cellular ultra structure (Belnap *et al.*, 1993). Hutchinson *et al.*, (1986) treated *Cladina rangiferina* and *Cladina stellaris* with artificial acid rain for more than five years. They found reduced photosynthesis and chlorophyll levels and decreased dry weight and podetial height of thalli of both the species receiving pH 2.5. No effects were observed at pH 3 and above. Nitric acid applied alone at pH 2.8 caused dry weight increase of 62% over controls in *C. rangiferina* with an increase in tissue nitrogen levels. Sulphuric acid applied alone caused a significant reduction in dry weight.

#### **viii) Effects of Fluorine**

The ability of lichens to accumulate F is a function of relative humidity, which determines the moisture conditions of thallus. In general, obvious damage to lichens begins at levels of 50-70 ppm. The effects of fluorine include a decrease in respiration and photosynthesis, an increase in membrane and thallus permeability with a concomitant loss of ions and changes in cellular ultra structure (Belnap *et al.*, 1993). Lichens exposed to ambient F at 4 mg F/m<sup>3</sup> were found to accumulate F within their thalli, surpassing eventually the critical concentration of 30-80 ppm (Perkins *et al.*, 1980). Both in the field and in the lab, whenever the level of F within the thallus exceeded 80 ppm, chlorosis was observed. Subsequently, all the photosynthetic pigments were degraded and the lichen thalli disintegrated. In the field, wherever chlorotic transplants were found, high levels of F on lime filter papers were also found, suggesting that ambient F was the cause of lichen injury. Lichens used in estimating F are *Cladonia cristellata*, *C. polycarpoides* and *Parmelia plittii* (Nash, 1989).

#### **ix) Effects of Photochemical Toxins**

Ozone, PAN and nitrogen oxides are toxic to lichens in sufficient concentrations. Ozone effects noted by researches have included decrease in photosynthesis, reduction in geographical distribution of species, and morphological and ultra structural changes. PAN can also affect photosynthesis and cause ultra structural changes, while NO<sub>x</sub> effects are expressed in the loss of chlorophyll pigment (Belnap *et al.*, 1993).

## **x) Effects of Metals**

Limited field data is available concerning threshold concentration of metals in lichens. Tolerance mechanisms mainly involve immobilization of the toxic metal ions in biologically inactive forms. Tolerance to metals may be phenol-typically acquired, but sensitivity of lichens to elevated tissue concentrations of metals varies greatly between species, populations, and elements (Tyler, 1989).

## **5. Methods of Study**

Lichens have been used to assess deposition and air quality in hundreds of studies reported worldwide. Reviews of the literature on this topic include Stolte *et al.* (1993), and Nash (1989). *The Lichenologist* publishes an ongoing series "Literature on air pollution and lichens". Below are some of the different approaches reported in the literature.

### **a) Using Studies Related to Naturally Occurring Lichens**

#### **i) Distribution Mapping**

Distribution mapping is the classic field method for studying lichens. It was also the first method used to indicate air quality with lichens (Showman, 1988). Different kinds of maps can record the total number of species per site (richness), the percentage of the quadrants in which particular species can be found (frequency), presence or absence of indicator species, and the estimated or measured cover.

Mapping lends itself well to longitudinal studies that document temporal change. Using this method Sigal and Nash (1983) compared lichen communities in the San Bernadino and San Gabriel Mountains of California in 1983 to epiphytic macro lichens on conifers reported by Hasse in the same area in 1913. A number of species were no longer found: *Nodobryoria abbreviata*, *Bryoria fremontii*, *Nodobryoria oregana*, *Alectoria sarmentosa*, *Calicium viride*, *Cetraria canadensis*, *Platismatia glauca*, *Evernia prunastri*. Other species, such as *Cetraria merrilli*, *Parmelia quercina*, *Ramalina farinacea*, and *Cladonia* spp., were found in only trace amounts. Species that were present included *Hypogymnia enteromorpha*, *Melanelia elegantula*, *Melanelia subolivacea*, and *Letharia vulpina*.

One disadvantage of presence/absence distribution mapping is that a species must disappear before an effect can be registered. To address this shortcoming, De Sloover & Leblanc (1968) developed the Index of Atmospheric Purity (IAP). At each site, this method assigns numerical values to characteristics such as frequency and cover. Then, using a mathematical formula, it reduces these values to a single IAP value for each site. The IAP values are then mapped. The primary limitation of IAP values is that they are not comparable from one geographic region to another. Even in the absence of pollution, climatic differences between regions can produce changes in the lichen communities that underlie the IAP values.

#### **ii) Gradient Studies**

Available pollution exposure data are correlated with observations of visible injury, species richness and species abundance through studies of gradients and the requisite regression equations are developed.

Taylor and Bell (1983) used regression to show that at 95% confidence level there was a functional relationship between distance from a smelter in Tacoma, Washington and total lichen cover, with an increase in cover with increasing distance from the smelter. Compared to data collected in 1976, SO<sub>2</sub> emissions from the smelter and the mean concentrations of sulfur found in leaves of alder trees at the study sites had both decreased. This suggested that correlation between lichen growth and distance from the smelter was more or less established during earlier periods of greater ambient SO<sub>2</sub> levels.

### **b) Laboratory Measurements**

Laboratory methods used to study the chemical, morphological and physiological responses of lichens to pollution include elemental analysis, physiological measurements, transplant studies, fumigation studies and photography.

#### **i) Elemental Analysis**

Methods of elemental analysis include atomic absorption spectrophotometry, neutron activation analysis, and X-ray fluorescence spectrometry. Commonly analyzed elements include: S, N, Zn, F, Pb, Ni, Cu, Fe, Mg, Mn, Cr, Cd, Zn, Ca, and several rare earth elements. A large body of information is developing on elemental content of lichens both in natural states and under pollution stress (Nash 1996). Hale (1981) recommended that every baseline study should include elemental analysis of lichen indicator species for future reference.

## **x) Effects of Metals**

Limited field data is available concerning threshold concentration of metals in lichens. Tolerance mechanisms mainly involve immobilization of the toxic metal ions in biologically inactive forms. Tolerance to metals may be phenol-typically acquired, but sensitivity of lichens to elevated tissue concentrations of metals varies greatly between species, populations, and elements (Tyler, 1989).

## **5. Methods of Study**

Lichens have been used to assess deposition and air quality in hundreds of studies reported worldwide. Reviews of the literature on this topic include Stolte *et al.* (1993), and Nash (1989). *The Lichenologist* publishes an ongoing series "*Literature on air pollution and lichens*". Below are some of the different approaches reported in the literature.

### **a) Using Studies Related to Naturally Occurring Lichens**

#### **i) Distribution Mapping**

Distribution mapping is the classic field method for studying lichens. It was also the first method used to indicate air quality with lichens (Showman, 1988). Different kinds of maps can record the total number of species per site (richness), the percentage of the quadrants in which particular species can be found (frequency), presence or absence of indicator species, and the estimated or measured cover.

Mapping lends itself well to longitudinal studies that document temporal change. Using this method Sigal and Nash (1983) compared lichen communities in the San Bernadino and San Gabriel Mountains of California in 1983 to epiphytic macro lichens on conifers reported by Hasse in the same area in 1913. A number of species were no longer found: *Nodobryoria abbreviata*, *Bryoria fremontii*, *Nodobryoria oregana*, *Alectoria sarmentosa*, *Calicium viride*, *Cetraria canadensis*, *Platismatia glauca*, *Evernia prunastri*. Other species, such as *Cetraria merrilli*, *Parmelia quercina*, *Ramalina farinacea*, and *Cladonia* spp., were found in only trace amounts. Species that were present included *Hypogymnia enteromorpha*, *Melanelia elegantula*, *Melanelia subolivacea*, and *Letharia vulpina*.

One disadvantage of presence/absence distribution mapping is that a species must disappear before an effect can be registered. To address this shortcoming, De Sloover & Leblanc (1968) developed the Index of Atmospheric Purity (IAP). At each site, this method assigns numerical values to characteristics such as frequency and cover. Then, using a mathematical formula, it reduces these values to a single IAP value for each site. The IAP values are then mapped. The primary limitation of IAP values is that they are not comparable from one geographic region to another. Even in the absence of pollution, climatic differences between regions can produce changes in the lichen communities that underlie the IAP values.

#### **ii) Gradient Studies**

Available pollution exposure data are correlated with observations of visible injury, species richness and species abundance through studies of gradients and the requisite regression equations are developed.

Taylor and Bell (1983) used regression to show that at 95% confidence level there was a functional relationship between distance from a smelter in Tacoma, Washington and total lichen cover, with an increase in cover with increasing distance from the smelter. Compared to data collected in 1976, SO<sub>2</sub> emissions from the smelter and the mean concentrations of sulfur found in leaves of alder trees at the study sites had both decreased. This suggested that correlation between lichen growth and distance from the smelter was more or less established during earlier periods of greater ambient SO<sub>2</sub> levels.

### **b) Laboratory Measurements**

Laboratory methods used to study the chemical, morphological and physiological responses of lichens to pollution include elemental analysis, physiological measurements, transplant studies, fumigation studies and photography.

#### **i) Elemental Analysis**

Methods of elemental analysis include atomic absorption spectrophotometry, neutron activation analysis, and X-ray fluorescence spectrometry. Commonly analyzed elements include: S, N, Zn, F, Pb, Ni, Cu, Fe, Mg, Mn, Cr, Cd, Zn, Ca, and several rare earth elements. A large body of information is developing on elemental content of lichens both in natural states and under pollution stress (Nash 1996). Hale (1981) recommended that every baseline study should include elemental analysis of lichen indicator species for future reference.

Puckett (1988) reported a method of calculating "enrichment factors" to compare the concentration of metal within a plant with potential sources in the environment. The equation is:

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

In Switzerland, Herzig *et al.* (1990) used multivariate analysis to compare element concentrations in *Hypogymnia physodes* to total air pollution as assessed by lichen communities using the IAP index and an instrumented monitoring network. Four groups were discerned, namely:

- Group 1: Ca. Calcium was the only element which increased with improving air quality.
- Group 2: Pb, Fe, Cu, Cr, S, Zn and P. Concentrations of these elements decreased in distinct curvilinear gradients with decreasing total air pollution. For example, the concentration of Pb was reduced six-fold in the "very low pollution" compared to the "critical air pollution" zone. These elements were strongly correlated with annual average atmospheric deposition measurements detected by the instrument network.
- Group 3: Li, Cd, Co. Concentrations of these elements were lower in the "very low pollution" zone than in the "critical air pollution", but the gradients were not strictly curvilinear.
- Group 4: Al, B, K, Mg, Mo, Na, Ni, Sn, Cl and organic S. These elements showed no clear gradients to the total air pollution.

### ii) Physiological Responses

The physiological responses of lichens to pollution can be observed and measured directly. Plasmolysis of cells of the algal component of the thallus can be measured microscopically. Respiration rates have also been measured. Von Arb *et al.*, (1990), observed changes in the fine structure of chloroplasts. Chlorophyll content, as a measure of SO<sub>2</sub> damage, was determined spectroscopically or by microfluorometry by Gries *et al.*, (1995), (Table 2).

**Table 2: The Physiological Responses of Lichens after Exposure to Fumigated Pollutants (Farmer *et al.*, 1992).**

Response	Pollutant
Reduced photosynthesis	SO <sub>2</sub> , NaHSO <sub>3</sub> , Na <sub>2</sub> S <sub>2</sub> O <sub>5</sub> , O <sub>3</sub> , PAN.
Reduced respiration	SO <sub>2</sub>
Decreased chlorophyll content	SO <sub>2</sub> , NO <sub>2</sub> , HF
Increased electrolyte leakage, loss of K	SO <sub>2</sub> , HF
Reduced nitrogen fixation	SO <sub>2</sub> , H <sub>2</sub> SO <sub>4</sub> > loss w/< pH > exposure, NaHSO <sub>3</sub> , NaF

### iii) Transplants

Lichen transplants are used to assess air quality in areas where lichens are absent or sparse. Richardson (1992) reviews the use of transplants to assess air quality in urban environments and to monitor contaminants in air and water. Pearson (1993) discusses advantages and limitations of transplant methods. Ideally, healthy lichens are transferred from an area where they occur naturally to the test area. Changes in physiology or element accumulation as a result of exposure to pollution are then studied. Physiological studies are most likely to be successful when using species well within their normal range of adaptation both at the source and at the test area.

### iv) Fumigation

Fumigation studies involve the controlled introduction of pollutants to lichens in either a closed chamber that allows for air movement, or in a chamber-free field environment. The purpose of this kind of study is to develop quantitative relationships between concentrations of air pollutants and various anatomical and physiological lichen responses. The anticipated outcome of air pollution fumigation experiments is a clear exhibition of dose-response relationships. Thus, fumigations for a long period of time at a low pollutant level would be expected to have the same effect as a shorter fumigation at a higher concentration where the dose, measured as concentration multiplied by time, is equal for each of the fumigations (Sanz *et al.*, 1992). The most commonly measured responses are physiological, such as photosynthesis, respiration, nitrogenase



activity in blue green phycobionts, K<sup>+</sup> efflux and/or total electrolyte leakage from the thallus, and pigment status.

Researchers evaluating fumigation experiments also need to remain mindful of the ecological conditions under which the studies are carried out (Farmer *et al.*, 1992). Dry lichens absorb far less SO<sub>2</sub> than moist ones and are resistant to SO<sub>2</sub> fumigation (Coxson, 1988). The low humidity used in some studies (Showman 1972) could have completely dried the thalli. Thus the finding that responses in some studies appear to be independent of exposure time is not surprising (Huebert *et al.*, 1985). With poikilohydric plants such as lichens, it is difficult to maintain water status and hence metabolic activity of a sample for more than a few hours. Complex laboratory equipment is necessary to successfully conduct fumigation studies

The order of sensitivity of lichen physiological processes to fumigation appears to be:

**N<sub>2</sub> fixation > K<sup>+</sup> efflux/total electrolyte leakage > photosynthesis and respiration > pigment status**

as shown in Table 2.

#### v) **Photography**

Some researchers have used photography as a way of monitoring air quality (Hale, 1981). Marked sites can be re-photographed over time to compare growth rates and condition of individual lichen colonies. Care must be taken to distinguish between air pollution and local habitat stresses.

## 6. Conclusion

The use of lichens for monitoring air quality is becoming routine in several countries since these organisms provide an estimate of the biological impact of air pollution and give an integrated picture of air quality by supplying information on the effects of all pollutants present in the atmosphere and of their reaction products. It is quick and inexpensive and provides results on which predictions for human health can be based. Lastly, due to the fact that lichens are slow-growers, they can be used as long-term bio-monitors of air pollution, i.e. summarizers of environmental conditions, constituting a well suited tool for monitoring air quality. However, it has to be remembered that use of Lichens in estimating air quality provides only a qualitative judgment and in no way quantitative conclusions can be deduced on any specific air pollutant i.e., Lichens as biomarkers provide indication that air pollution as a whole is high.

## 7. References

1. Ahmadjian V (1993) *The Lichen Symbiosis*. John Wiley and Sons, New York, NY. 250pp.
2. Belnap, J., et al. 1993. Identification of Sensitive Species. In: Huckaby, LS, et al. 1993. Lichens as bio monitors of air quality. Proc. of a workshop sponsored by the NPS and USDA-FS. USDA/USFS Rocky Mountain Forest and Range Exp. Sta. GTR RM-224.
3. Coxson, D.S. 1988. Recovery of net photosynthesis and dark respiration on re-hydration of the lichen, *Cladonia mitis*, and the influence of prior exposure to sulphur-dioxide while dessicated. *New Phytologist* 108:483-487
4. DeSloover, J., and F. LeBlanc. 1968. Mapping of atmospheric pollution on the basis of lichen sensitivity. *Proc. Symp. Recent Adv. Trop. Ecol.* 1968: 42-56.
5. Farmer, A.M., J.W. Bates, and J.N.B. Bell. 1992. Ecophysiological effects of acid rain on bryophytes and lichens. In: Bates, J.W., and A. M. Farmer (eds.). *Bryophytes and Lichens in a Changing Environment*. Clarendon Press, Oxford.
6. Garty, J., Y. Karary, and J. Harel. 1993. The impact of air pollution on the integrity of cell membranes and chlorophyll in the lichen *Ramalina duriaei* (De Not.) Bagl. transplanted to industrial sites in Israel. *Archives of Environmental Contamination and Toxicology* 24(4): 455-460.
7. Gilbert, O.L. 1986. Field evidence for an acid rain effect on lichens. *Environmental Pollution (Series A)*. 40: 227-231.
8. Gries, C., M.-J. Sanz, and T.H. Nash III. 1995. The effect of SO<sub>2</sub> fumigation on CO<sub>2</sub> gas exchange, chlorophyll fluorescence and chlorophyll degradation in different lichen species from western North America. *Crypt. Bot.* 5: 239-246.

9. Hale, M.E. 1981. Lichens as bio indicators and Monitors of Air Pollution in the Flat Tops Wilderness Area, Colorado. Final Report: USFS Contract No. ON RFP R2-81-SP35.
10. Hale, M.E. 1983 Cortical structure in *Physcia* and *Phaeophyscia* . *Lichenologist* 643-651
11. Harper, K.T., and J. R. Marble. 1988. A role of nonvascular plants in management of semiarid rangelands. In: *Vegetation Science Applications for Rangeland Analysis and Management*, ed. P. T. Tueller, pp. 135-60. London: Kluwer Academic Publishers.
12. Herzig, R., et al. 1990. Lichens as biological indicators of air pollution in Switzerland: passive bio monitoring as part of an integrated measuring system for monitoring air pollution. In: H. Lieth and B. Markert (eds.). Element Concentration Cadasters in Ecosystems Methods of Assessment and Evaluation. 141: VCH Verlagsgesellschaft, Weinheim. 317p.
13. Holopainen, T.H. 1984. Cellular injuries in epiphytic lichens transplanted to air polluted areas. Nordic J. Botany 4: 393-408.
14. Huebert, D.B., S.J. L'Hirondelle, P.A. Addison. 1985. The effects of sulphur dioxide on net CO<sub>2</sub> assimilation in the lichen *Evernia mesomorpha* Nyl. New Phytologist 100: 643-651.
15. Hutchinson, T.C., M. Dixon, and M. Scott. 1986. The effect of simulated acid rain on feather mosses and lichens of the boreal forest. Water, Air, & Soil Poll. 31: 409-416.
16. Lawry, J.D. 1986. Lichens as lead and sulfur monitors in Shenendoah NP, VA. Ann.Meeting of the Botanical Soc.of America. Amherst, MA.
17. Lawrey, J.D., and M. E. Hale, Jr. 1981. Retrospective studies of lichen lead accumulation in the northwestern United States. Bryologist 84(4): 449-456.
18. Lodenius, M., and Laaksovirta, 1979. Mercury content of Hypogymnia phsodes and pine needles affected by a chlor-alkali works at Kuusankoski, SE Finland. Annales Botanici Fennici 16:7-10
19. Maser, C., Z. Maser, J.W. Witt, and G. Hunt. 1986. The northern flying squirrel; a mycophagist in southwestern Oregon. Can. J. Zool. 64:2086-2089.
20. McCune, B. 1993. Gradients in epiphyte biomass in three *Pseudotsuga-Tsuga* forests of different ages in western Oregon and Washington. Bryologist 96(3): 405-411.
21. McCune, B., and L. Geiser. 1997. Macro lichens of the Pacific Northwest. OSU Press, Corvallis, OR. *In Press*.
22. NAPAP. 1990. Acidic Deposition: State of Science and Technology. Report 16: Changes in Forest Health and Productivity in the United States and Canada. The National Acid Precipitation Assessment Program, Washington, DC.
23. Nash, T. H. III. 1989. Metal tolerance in lichens. In: *Heavy Metal Tolerance in Plants: Evolutionary Aspects*, ed. A.J. Shaw pp 119-131. Boca Raton: CRC Press.
24. Nash, T. H. III (ed.). 1996. Lichen Biology. Cambridge University Press, Great Britain. 303 pp.
25. Nieboer, E.A., D.H.S. Richardson, and F.D. Tomassini. 1978. Mineral Uptake and Release by Lichens: An Overview. Bryologist 81(2):226-246.
26. Nieboer, E., D.H.S. Richardson, P. Lavoie, D. Padovan. 1979. The role of metal-ion binding in modifying the toxic effects of sulphur dioxide on the lichen *Umbilicaria muhlenbergii*. I. Potassium efflux studies. New Phytologist 82:621-632.
27. Pearson, L. 1993. Active monitoring. In: K. Stolte, D. Mangis, R. Doty, K. Tonnessen & L. S. Huckaby (eds.) *Lichens as Bio indicators of Air Quality*. U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station General Technical Report RM-224, Fort Collins, Colorado. Pp. 89-95.
28. Perkins, D.F. , R.O. Millar, and P.Neep. 1980. Accumulation of airborne fluoride by lichens in the vicinity of an aluminum reduction plant. Environmental Pollution (Series A) 21: 155-168.
29. Richardson, D.H.S. 1992. Pollution monitoring with lichens. Naturalists' Handbooks 19. Richmond Publishing Co., Ltd. Slough, England. 76 pp.
30. Richardson, D.H.S., and E. Nieboer. 1980. Cellular Interactions in Symbiosis and Parasitism Surface binding and accumulation of metals in lichens. In: C. B. Cook, P. W Pappas & E. D. Rudolph (eds.). *Cellular Interactions in Symbiosis and Parasitism* . Pp 75-94. Ohio State University Press, Columbus.
31. Sanz, M.J., C. Gries, and T.H. Nash III. 1992. Dose-response relationships for SO<sub>2</sub> fumigations in the lichens *Evernia prunastri* (L.) Ach. and *Ramalina fraxinea* (L.) Ach. New Phytol. 122: 313-319.

32. Schutte, J.A . 1977. Chromium in two corticolous lichens from Ohio and West Virginia. Bryologist 80: 279-283.
33. Seaward, M.R.D. 1987. Effects of quantitative and qualitative changes in air pollution on the ecological and geographical performance of lichens. *In*: Hutchinson, T. C. and K. M. Meema (eds.), Effects of Atmospheric Pollutants on Forests, Wetlands and Agricultural Ecosystems. 133: NATO ASI Series, Vol. G16. Springer-Verlag, Berlin-Heidelberg.
34. Showman, R.E. 1988. Mapping air quality with lichens, the North American experience. *In*: Nash, T.H., III, (ed.) Lichens, Bryophytes and Air Quality. Bibliotheca Lichenologica 30. J. Cramer, Berlin-Stuttgart.
35. Sigal, L.L., and T.H. Nash III. 1983. Lichen communities on conifers in southern California: an ecological survey relative to oxidant air pollution. Ecology 64:1343-1354.
36. Sochting, U. 1990. Reindeer lichens injured in Denmark. Bull. British Lichen Society 67:1-4.
37. Stolte, K., D. Mangis, R. Doty and K. Tonnessen (editors). 1993. Lichens as Bio indicators of Air Quality. Gen. Tech. Rep. RM-224. Fort Collins, Colorado. USDA-Forest Service, Rocky Mountain Forest and Range Experiment Station. 131 pp.
38. Taylor, R.J., and M.A. Bell. 1983. Effects of SO<sub>2</sub> on the lichen flora in an industrial area: Northwest Whatcom County, Washington. Northwest Science 57: 157-166.
39. Tyler, G. 1989. Uptake, retention and toxicity of heavy metals in lichens. A brief review. Water, Air, and Soil Pollution 47: 321-333.
40. Von Arb, C., et al. 1990. Lichen physiology and air pollution. II: Statistical analysis of the correlation between SO<sub>2</sub>, NO<sub>2</sub>, NO and O<sub>3</sub>, and chlorophyll content, net photosynthesis, sulfate uptake and protein synthesis of *Parmelia sulcata* Taylor. New Phytologist 115: 431-437.
41. Wetmore, C.M. 1985. Lichens and air quality in Isle Royal NP: Final Report. NPS Contract CX 0001-2-0034.
42. Wolseley, P.A., and P.W. James. 1992. Acidification and the Lobarion: a case for biological monitoring. Nature Conservancy Council Newsletter. *In*: Wolseley and James. 1991. The Effects of Acidification on Lichens 1986-1990. (CSD Report 1247). Nature Conservancy Council, Peterborough, UK.