Dalip Kumar Upreti · Pradeep K. Divakar
Vertika Shukla · Rajesh Bajpai *Editors*

Recent Advances in Lichenology

Modern Methods and Approaches in Biomonitoring and Bioprospection, Volume 1

Springer

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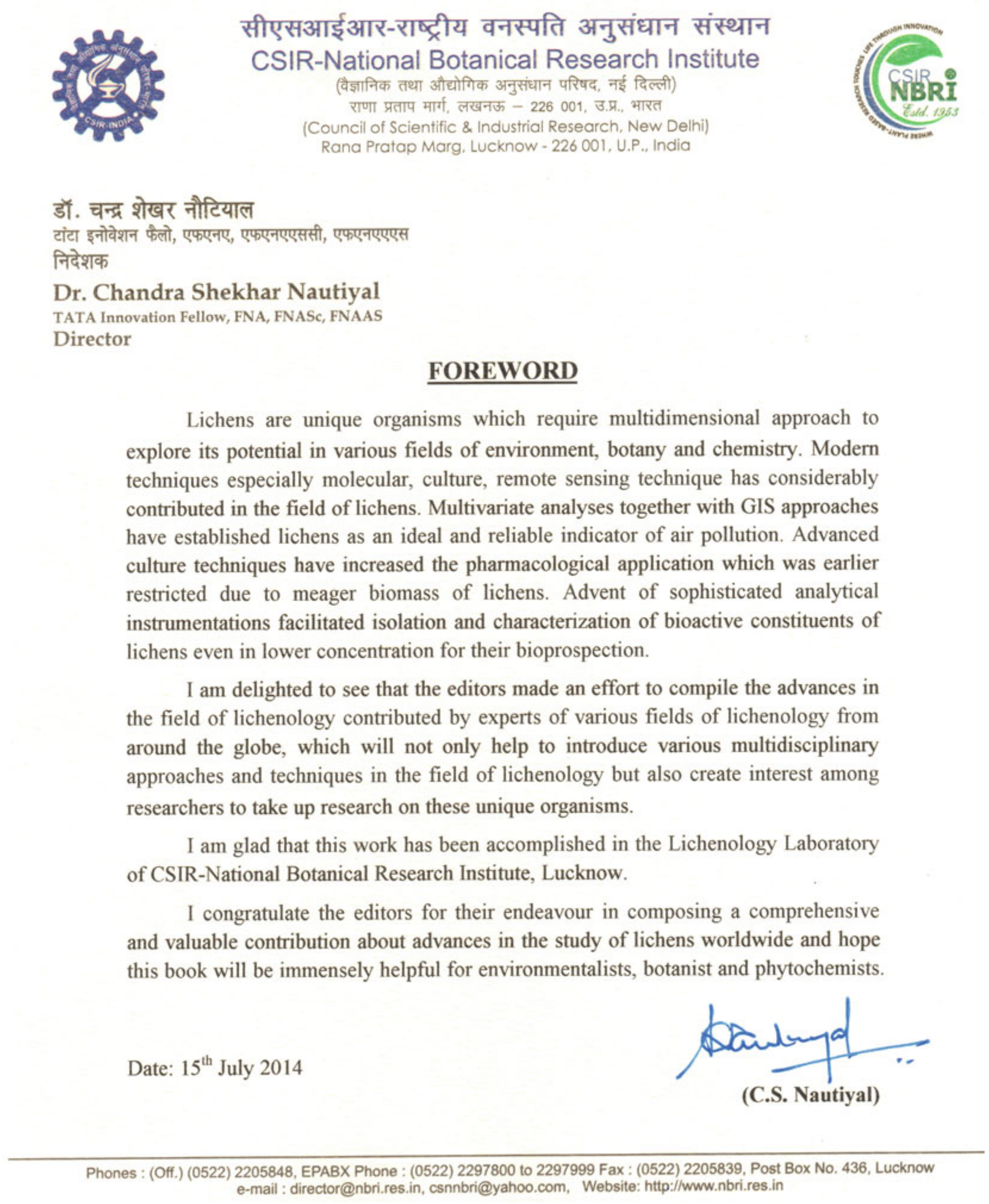
Dedicated to

Dr. D.D. Awasthi
Father of Indian Lichenology

Foreword



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निदेशक
Dr. Chandra Shekhar Nautiyal
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FOREWORD

Lichens are unique organisms which require multidimensional approach to explore its potential in various fields of environment, botany and chemistry. Modern techniques especially molecular, culture, remote sensing technique has considerably contributed in the field of lichens. Multivariate analyses together with GIS approaches have established lichens as an ideal and reliable indicator of air pollution. Advanced culture techniques have increased the pharmacological application which was earlier restricted due to meager biomass of lichens. Advent of sophisticated analytical instrumentations facilitated isolation and characterization of bioactive constituents of lichens even in lower concentration for their bioprospection.

I am delighted to see that the editors made an effort to compile the advances in the field of lichenology contributed by experts of various fields of lichenology from around the globe, which will not only help to introduce various multidisciplinary approaches and techniques in the field of lichenology but also create interest among researchers to take up research on these unique organisms.

I am glad that this work has been accomplished in the Lichenology Laboratory of CSIR-National Botanical Research Institute, Lucknow.

I congratulate the editors for their endeavour in composing a comprehensive and valuable contribution about advances in the study of lichens worldwide and hope this book will be immensely helpful for environmentalists, botanist and phytochemists.

Date: 15th July 2014

(C.S. Nautiyal)

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Preface

Lichens present an excellent example of symbiotic association. The unique composition of alga and fungi in lichens not only results in conferring differential sensitivity towards a range of environmental factors, but also makes them physiologically adapted and chemically diversed to combat various abiotic and biotic environmental stresses.

This book volume covers two very important aspects of lichens, i.e. biomonitoring and bioprospection. In order to understand the role of lichens as biomonitors, it is important to know the various factors which influence the growth of lichens in natural conditions.

Lichens have been known for long as bioindicators of air pollution and various studies have explored the biomonitoring potential of different lichen species. Recently, more standardised protocols have been adopted in Europe and America, which ensures the authenticity and reproducibility of lichen biomonitoring data.

Standardised methods for lichen diversity assessment not only authenticate classical approaches of lichen diversity changes related to ecological studies and air quality, but also provide an opportunity to extend to the studies related to climate change and land use changes. Lichen diversity studies coupled with remote sensing data can be used for mapping lichen species, which provide vital information regarding the present scenario and predict the future model of climate change or land use change. Applicability of lichen diversity-remote sensing studies further extends to estimating and mapping influence of pollutant in unsampled location based on sampled locations, which help to track the long-range transport of semi-volatile pollutants to high altitude ecosystems.

Lichens also play a major role in the functioning of the ecosystem by the process of soil formation (Lichens have the ability to grow on barren rock), but this characteristic is disadvantageous for conservation of ancient monuments as lichen growth results in biodeterioration. Studies on restricting lichen growth and preserving monuments are a subject of research and needs implementation of strategies for conservation of monuments.

Another aspect of lichens, which is gaining importance in recent climate change studies, is lichenometric studies, to observe glacier retreat phenomenon. As any change in shape and size of glacier provides vital information regarding climate change, therefore age of lichens growing on exposed moraines is a possible clue to retreat of glacier.

Apart from being excellent biomonitors, another not much explored aspect of lichens is their chemistry. For lichens their unique chemistry supplements physiological adaptation for sustaining in extreme climates, but for mankind lichens are a treasure house of pharmacologically important bioactive constituents capable of curing simple cough and cold to HIV and cancer.

The therapeutic potential of lichen secondary metabolites has been known since medieval times mainly through folklore and ethnobotanical uses. The main constraint which restricts its commercial utilisation is slow growth rate and low biomass of lichens. Recent advances in analytical instrumentation techniques have resulted in high throughput techniques for isolation, while highly sensitive detection techniques allow characterisation of bioactive compound even in very low quantity. Structural characterisation of bioactive compound paves the way for its synthesis in laboratory and further structural modifications.

Therefore, the book volume intends to introduce researchers to advancements in the field of lichenology with an aim to involve more active participation of multidisciplinary research in the study of lichens.

Contents

Editors and Contributors

About the Editors

Dalip Kumar Upreti FNASc, Head of the Lichenology Laboratory, CSIR-National Botanical Research Institute (CSIR-NBRI), Lucknow, has a Ph.D. (1983) in Botany on *Studies on the lichen family Cladoniaceae from India and Nepal* from Lucknow University under the guidance of Dr. D.D. Awasthi. Dr. Upreti has over 35 years of research experience and has published more than 300 research papers in peer-reviewed journals and co-authored eight books.

Apart from taxonomy, Dr. Upreti has also carried out extensive research on ecology, lichen chemistry, pollution monitoring, in vitro culture and biodeterioration studies and bioprospection of Indian lichens. Dr. Upreti was in Antarctica in 1991–1992. He is the Indian corresponding member to the British Lichen Society and International Association for Lichenology.

Pradeep K. Divakar has an M.Sc. in Botany and earned his Ph.D. (2002) on *Revisionary studies on the lichen genus Parmelia sensu lato in India* from Lucknow University. Dr. Divakar is Professor at the Department of Plant Sciences, Faculty of Pharmacy, Complutense University Madrid, Spain. Dr. Divakar has published two books and more than 70 papers in national and international journals. Currently, he is editor of *Biodiversity and Conservation*, *Mycokeys*, *Organisms Diversity and Evolution*, and *Lazaroa* (Iberian Journal of Botany).

His research activities centre on molecular systematics, genetic diversity, evolution, speciation events, gene mapping, gene flow, TCS estimation of gene genealogies and phenotypic character mapping, ancestral character state and genomics of fungi that form mutualistic symbioses with algae or cyanobacteria and mycobiont cultures.

Vertika Shukla holds an M.Sc. in Organic Chemistry and a Ph.D. (2003) on the *Chemical Study of Macrolichens of Garhwal Himalayas* from H.N.B. Garhwal University, Srinagar (Garhwal). She is currently working in the Lichenology Laboratory of CSIR-NBRI as a DST scientist and to date she has published more than 50 scientific articles.

Dr. Shukla has contributed to secondary metabolite chemistry, spatio-temporal behaviour of pollutants in high altitude ecosystems, lichen responses to climate change and the role of lichens in bioremediation of atmospheric fallouts.

Rajesh Bajpai who has an M.Sc. in Environmental Science and received his Ph.D. in 2009 from Babasaheb Bhimrao Ambedkar (Central) University, Lucknow on *Studies on lichens of some monuments of Madhya Pradesh with reference to Biodeterioration and Biomonitoring*, is working as a DST scientist in Lichenology Laboratory of CSIR-NBRI, Lucknow. Dr. Bajpai has published 30 research papers in various national and international journals.

Dr. Bajpai has carried out extensive research work on the interaction of lichens with the environment, climate-change modelling, arsenic pollution and biodeterioration.

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The Influence of Environmental Factors on the Growth of Lichens in the Field

1

Richard A. Armstrong

Contents

Abstract

The majority of studies of the effects of environmental factors on lichen growth have been carried out in the field. Growth of lichens in the field has been measured as absolute growth rate (e.g. length growth, radial growth, diameter growth, area growth, or dry weight gain per unit of time) or as a relative growth rate, expressed per unit of thallus area or weight, e.g. thallus specific weight. Seasonal fluctuations in growth in the field often correlate best with changes in average or total rainfall or frequency of rain events through the year. In some regions of the world, temperature is also an important climatic factor influencing growth. Interactions between microclimatic factors such as light intensity, temperature, and moisture are particularly important in determining local differences in growth especially in relation to aspect and slope of rock surface, or height on a tree. Factors associated with the substratum including type, chemistry, texture, and porosity can all influence growth. In addition, growth can be influenced by the degree of nutrient enrichment of the substratum associated with bird droppings, nitrogen, phosphate, salinity, or pollution. Effects of environmental factors on growth can act directly to restrict species distribution or indirectly by altering the competitive balance among different species in a community.

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1.1 Introduction

Lichens are influenced by many environmental factors including the long-term effects of climate, local changes in microclimate, and a variety of factors associated with the substratum such as type of rock, bark, or soil, substrate chemistry, and the degree of nutrient enrichment by birds, salinity, or pollution (Armstrong 1974; James et al. 1977). Studies of the effects of environmental factors on lichen growth are important for several reasons. First is to understand the factors determining the distribution of rare or interesting species and the structure of important lichen communities. Hence, a change in the environment to a less than optimal condition may inhibit lichen growth to such an extent that thalli rapidly fragment and disappear from a substratum. In addition, the environment can act more indirectly, subtle changes altering the competitive balance among different species in a community (Armstrong and Welch 2007). Second, knowledge of environmental growth effects is important in 'lichenometry', a technique which uses lichens to estimate the surface age of a substratum (Locke et al. 1979; Innes 1985; Matthews 1994; Benedict 2009). Third, it may be important to separate out growth effects due to natural factors, such as climate or microclimate, from those attributable to human activity, such as grazing by herbivores, pollution, or woodland management.

Two major problems have been encountered in studying lichen growth. First, studies have been limited by the relatively few facilities available for culturing whole lichen thalli in the laboratory for long periods of time. Hence, few 'factorial-type' experiments have been undertaken, in which the synergistic effects of different variables have been studied (Jones and Platt 1969; Armstrong 1994). As a result, most studies have been carried out in the field and therefore lack the control of the environment possible in a laboratory. Second, lichens are morphologically diverse consisting of crustose, foliose, placodiod, and fruticose forms, and they also grow on different substrata with contrasting properties such as rock, tree bark, and soil. Hence, different methods have been devised to measure growth, making it difficult to compare the results of different studies. This review considers various aspects of the influence of the environment on the growth of lichens as measured in the field including: (1) methods of measuring and expressing growth, (2) the influence of climate, microclimate, substratum factors, and nutrient enrichment on growth, and (3) the importance of the influence of growth on competition as a factor determining distribution and community structure (Armstrong and Welch 2007).

1.2 Measuring Lichen Growth

Different methods have been employed to measure lichen growth in the field depending on growth form and substratum type. Hence, foliose and crustose lichens both exhibit a flat dorsiventral thallus and when growing on relatively flat substrata such as rock and tree bark, similar methods can be used to measure their growth (Armstrong and Bradwell 2011). Current techniques enable measurements of foliose lichen growth to be made over relatively short-time intervals such as weeks and months (Hale 1970; Armstrong 1973) and are based on the original method described by Hale (1970, 1973). This involves measuring the advance of the thallus perimeter, either the tip of a lobe in foliose species or the edge of the hypothallus in many crustose species, with reference to fixed markers on the rock. Digital photography (Hooker 1980) and an image analysis system (Image-J) (Armstrong 2013) can then be used to measure growth increments. Measurements can be made over various time scales, viz. one month for faster-growing foliose (Armstrong and Bradwell 2011) and three months for slower-growing crustose species (Armstrong and Bradwell 2010).

Many studies have employed an absolute measure of growth such as radial growth, diameter growth, area growth, or dry weight gain. The most popular measure in many studies of foliose lichens has been rate of radial extension or radial growth rate (e.g. mm year^{-1}). A limitation of radial growth rate, however, is that it is strongly correlated with thallus size and exhibits a high degree of variability both within and between thalli (Armstrong and Bradwell 2010, 2011). As a consequence, measurement of relative growth rate has been used to compensate for these variations in size, growth being scaled to an existing thallus measure such as increase in area per unit of area in unit time (mm^2 mm^{-2}, time^{-1}) (Armstrong 1973, 1976a).

Fruticose lichens have a much more complex-branched morphology and different methods necessary to study their growth. Methods of quantifying growth of fruticose species have usually involved measuring length, internode length, diameter, thallus dry weight, or biomass (Karenlampi 1971). Karenlampi (1970) measured growth of thalli of *Cladonia alpestris* (L.) Rabenh. by dividing up thalli and weighing the various parts. The major growth zone was at the tip of the podetia, and relative growth values for length, diameter, and dry weight suggesting significantly higher values for younger than older portions of thalli. More recently, a non-destructive method of estimating biomass of *Usnea* species by harvesting a sample of sites and measuring dry weight gain, ratio of chlorophyll ‘a’ to ‘b’, and thallus area have been described, regression methods then being applied to calibrate biomass against the non-destructive measures (Bohuslavova et al. 2012).

1.3 The Influence of Climate on Growth

Beschel’s detailed lichenometric studies in the 1950s and 1960s were some of the first to demonstrate the important role played by climate on lichen growth (Beschel 1958, 1961). Beschel found that the same species of the crustose genus *Rhizocarpon* grew considerably more slowly in Greenland than in the Swiss Alps (Beschel 1958, 1961). Re-measurement at Beschel’s sites, 12 years after the original study (Tenbrink 1973), confirmed that growth of *Rhizocarpon geographicum* (L.) DC. was more than 50 % slower at those sites in Greenland furthest from the sea (6–7 mm/100 year compared with 17–18 mm/100 year). Subsequently, many lichenometric studies from the northern hemisphere have reported similar trends. Hence, Innes (1983) found a general decrease in growth rate of thalli of *Rhizocarpon* section *Rhizocarpon* from west to east in Scotland, UK. In addition, Bradwell (2001a) and Mathews (2005) found growth rate gradients across Iceland and southern Norway, respectively, probably reflecting reduced moisture availability associated with a diminishing maritime influence. Not all such studies, however, have found significant growth effects. For example, growth of transplanted specimens of *Dolichousnea longissima* (Ach.) Articus was studied in four contrasting habitats (Keon and Myer 2002), length growth or dry weight gain being measured after one year. Growth was similar at all sites studied including those in which the habitat was not predicted to be suitable for growth of this species. Similarly, dry weight gain of *Cladonia portentosa* (Dufour) Coem. was studied at five heathland sites differing in precipitation acidity, nitrogen deposition, and annual rainfall (Hyvarinen and Crittenden 1998), but no regional differences in growth were evident.

1.3.1 Rainfall

Evidence for the influence of climate on lichen growth has usually been obtained from studies of

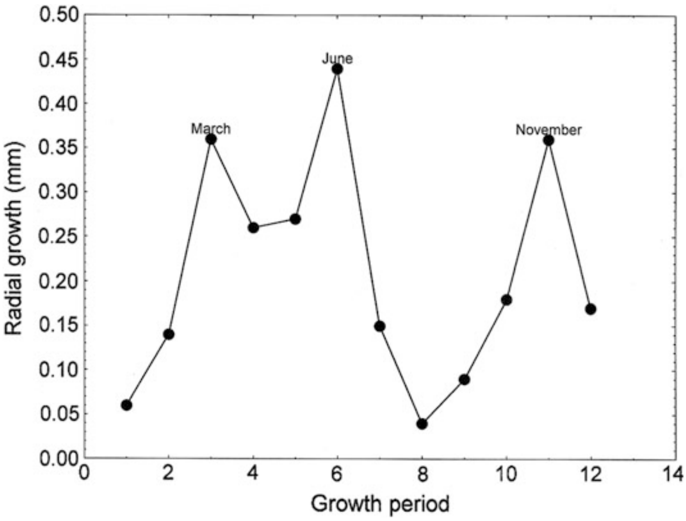

Fig. 1.1 Radial growth (RaG, mm) of the foliose lichen *Melanelia fuliginosa* ssp. *fuliginosa* (Fr. ex Duby) Essl. at a site in north Wales, UK, over 12 months. A large peak of growth occurred in June with smaller growth peaks in March and November corresponding to periods of maximum rainfall (data from Armstrong 1973)

annual or seasonal variations in growth (Rydzak 1961; Hale 1970; Armstrong 1973, 1993a; Showman 1976; Lawrey and Hale 1977; Fisher and Proctor 1978; Moxham 1981; Benedict 1990a). Hence, monthly relative growth rate of *Melanelia fuliginosa* ssp. *fuliginosa* (Fr. ex Duby) Essl. was measured over a period of one year in north Wales (Armstrong 1973, 1993a) (Fig. 1.1). A large peak of growth occurred in June with smaller growth peaks in March and November corresponding to periods of maximum rainfall. In general, monthly fluctuations in growth correlate best with average or total rainfall (Karenlampi 1971; Armstrong 1973; Golm et al. 1993), but linear regressions fitted to radial growth increments measured per month against total rainfall account for relatively small amounts of the total variance in growth (usually <40 %) (Armstrong 1988). For example, growth of *Xanthoparmelia conspersa* (Ehrh. Ex Ach.) Hale in each of 22 successive months in north Wales in relation to total monthly rainfall was studied by Armstrong (1988). A significant positive correlation between growth and rainfall over this period was evident ($r = 0.61$, $P < 0.001$), but the proportion of the variance accounted for by rainfall alone was low ($r^2 = 0.37$). In a study of *Cetraria* species, Paterson et al. (1983) found that moisture was the most important factor influencing growth, assimilation gains achieved during rainy days being sufficient to offset any carbon losses accrued over five dry days. In addition, mean daily rainfall in summer was an important determiner of growth in *Flavocetraria nivalis* (L.) Kärnefelt & A. Thell. although temperature was also considered an important factor (Karenlampi 1971). Similarly, in the high Arctic, the frequency of watering was the most important factor determining the relative growth rate of *Cetraria* species (Cooper et al. 2001). The effect of climate on growth on tree bark has frequently been studied using reciprocal transplants (Gaio-Oliveira et al. 2004). Hence, the relationship between thallus size and growth was studied in three successional forest stands across three boreal climate zones using transplants of *Lobaria pulmonaria* (L.) Hoffm. (Gauslaa et al. 2009). Stand-specific water availability was the most important factor increasing thallus area.

Because of significantly lower radial growth rates, fewer studies of the seasonal growth of crustose species have been carried out. Nevertheless, seasonal growth over three-month growth periods was studied in *R. reductum* Th. Fr. and *Lecidea tumida* Massal. growing on rocks in north Wales (Armstrong 1973). Both showed maximum growth between May and July, relatively little growth in November to January, and slow growth in August to October suggesting that a combination of higher rainfall and lower temperatures was not conducive to growth of these species.

One of the first lichenologists to study the relationship between growth and climate in fruticose lichens was Karenlampi (1971). At sites in Finland, relative growth rate of a number of fruticose species, measured as increase in weight or length, was correlated with mean daily rainfall in summer while temperature was inversely related to growth. In addition, growth of *Ramalina menziesii* (Taylor) was studied in California at coastal and inland sites (Boucher and Nash 1990), the primary determinant of growth appearing to be rainfall. However, the growth of a second species, viz. *R. farinacea*, (L.) Ach. was not be explained by any of the climatic variables.

1.3.2 Temperature

Although rainfall is the predominant variable in many regions, other climatic factors have also been identified as affecting lichen growth. Hence, Brodo (1965) studied annual variations in radial growth of the corticolous lichens *Lecanora chlarotera* Nyl. and *L. caesiorubella* Ach. and observed significantly greater growth during 1959–1961 compared with 1961–1962 largely attributable to differences in mean temperature. In addition, diameter growth of *R. geographicum* was studied at 47 sites covering 18 glaciers in south Norway. Annual growth was correlated with mean temperature and winter mean temperature but not with summer temperature and less correlation with rainfall was evident compared with other studies (Trenbirth and Mathews 2010).

In a study of the seasonal growth of *R. geographicum* (Armstrong 2006), radial growth rate of a sample of 20 thalli was measured in situ on a southeast-facing rock surface at three-month intervals over 51 months (Fig. 1.2). There were five periods of significant growth: July–September of 1993, 1994, and 1995, in January–March of 1996, and in April–June of 1997. In four of these periods, growth coincided with a mean temperature maximum over a three-month period exceeding 15 °C and three of the maxima also coincided with greater than 450 sunshine hours. In addition, two of the growth maxima coincided with three-month periods of total rainfall exceeding 300 mm and one with greater than 50 rain days.

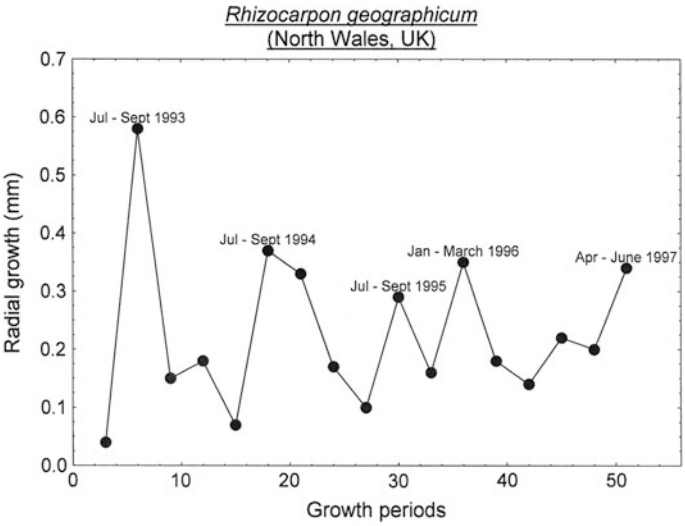

Fig. 1.2 Mean radial growth of thalli of *Rhizocarpon geographicum* measured in 17 three-month periods from 1993 to 1997 in south Gwynedd, Wales, UK. There were five periods of significant growth: July–September of 1993, 1994, and 1995, in January–March of 1996, and in April–June of 1997. Analysis of variance (two-way), between thalli, $F = 0.03$ ($P > 0.05$), between growth periods, $F = 3.86$ ($P < 0.001$) (data from Armstrong 2006)

There were no significant linear correlations between growth and individual climatic variables but significant nonlinear relationships were evident between growth and maximum temperature, mean temperature minimum, total number of air and ground frosts, and number of rain days, maximum temperature being the most significant (Armstrong 2006). Hence, in north Wales, maximum growth of *R. geographicum* may occur in any season, although July–September appeared the most favourable. Coxon and Kershaw (1983) studied changes in photosynthetic capacity in *R. superficiale* (Schaer.) Vain. through the season. This species showed a broad response to temperature and a high resistance to heat stress, characteristic of species living in 'boundary-layer' habitats, but there were no significant seasonal changes in photosynthetic capacity.

1.3.3 Frost Injury and Snowfall

At higher altitudes, the degree of winter frost injury and snowfall are important factors. Hence, Innes (1985) found that thalli of *Rhizocarpon* were smaller close to snow patches due to reduced growing season and ground instability. At some sites, however, the effects of increasing moisture balanced the negative influence of snow. In the Colorado Front Range (Benedict 1990b), frost damage, including 'spalling' of the upper cortex and algal layers, was evident especially in foliose species. Crustose species such as *R. riparium* Räsänen were less affected but some spalling did occur affecting the surface of areolae. Benedict (1990b) also demonstrated that, under snow, photosynthesis was limited due to impaired light penetration but thalli did continue to respire. To demonstrate this effect, lines of lichen-covered rocks were installed in two semi-permanent snow patches in the Colorado Front Range. Thalli of *R. geographicum* survived 5–8 years after transplanting when average duration of snow cover in the year exceeded 40–43 weeks. By contrast, *R. riparium* thalli survived the duration of the study and were clearly more snow tolerant than *R. geographicum*. The effect of snow on long-term lichen growth at high elevations has also been studied by measuring the size of *R. geographicum* along a transect traversing snow-free and snow-accumulation areas (Benedict 1991). No differences in maximum thallus diameter were observed despite large differences in duration of snow cover. Hence, zoned lichen communities, which are often observed adjacent to snow patches, are more likely to be attributable to episodic snow kill rather than to slow growth under seasonal cover.

1.3.4 Light Intensity

The influence of light on lichen growth in the field has been studied most frequently in woodland. Hence, in a transplant experiment, in which the influence of canopy structure on *L. pulmonaria* was studied, growth was strongly correlated with canopy light transmission, dry weight gain over 2 years being less than 5 % under canopies and rising to 20 % on branches associated with canopy gaps (Coxson and Stevenson 2007). In a further transplant experiment, the influence of canopy cover on growth was studied along a regional forest gradient in the boreal forest (Gauslaa et al. 2007). Dry weight gain was greatest in the Atlantic rainforest (36–38 %) but was reduced by low-light levels even in old forests and in most semi-exposed clear-cuts. The relationship between light intensity and growth of five macrolichens was studied in Sweden (Palmqvist and Sundberg 2000), a strong correlation being observed when thalli were moist. Hence, clear-cutting, which often creates an abrupt edge to forest stands, may affect foliose lichen growth. The effect of 'hard edges' and a less abrupt edge to the forest on growth was studied in British Columbia using transplants of *Lobaria retigera* (Bory) Trevis. (Stevenson and Coxson 2008). There was a high mortality of thalli and loss of biomass over 3 years at 'hard' edge sites but a significantly reduced loss when a less abrupt edge was present, suggesting that residual trees at the margin could reduce the impact of forest clearing on lichen diversity.

1.3.5 Interactions Between Factors

Factorial experimental designs have been relatively little used to study the synergistic effects of climatic and other factors on lichen growth. However, Jones and Platt (1969) studied the growth of *X. conspersa* using a three-factor experimental design incorporating four levels of radiation exposure, two contrasting climates, and two nutrient addition conditions. All factors significantly influenced radial growth, but climate had the most substantial effect. There were also significant interactions between climate, radiation exposure, and nutrient levels.

Stepwise multiple regression has also been used to analyse the various climatic factors determining seasonal trends in growth. Hence, Lawrey and Hale (1977) found that a multiple linear regression, which included maximum temperature, total rainfall, and per cent cloudy days as independent variables, explained 22 % of the variation in the growth of *Flavoparmelia baltimorensis* (Gyeln. & Fóriss) Hale. This method was also used to study the monthly radial growth of single lobes of *X. conspersa* in North Wales over 22 months (Armstrong 2009). Of eight climatic variables studied, the frequency of rain days, ground frosts per month, and average wind speed were selected as significant variables in total accounting for 49 % of the variance in the data.

1.4 The Influence of Microclimate on Growth

Investigation of lichen physiology suggests that interactions between light intensity, temperature, and moisture are likely to be important in determining local growth rates (Farrar 1973). Hence, the growth of the fruticose species *Cetrariella delisei* (Bory ex Schaer.) Kärnefelt & A. Thell. was measured in the high Arctic (Uchida et al. 2006). Positive net photosynthesis was recorded when thallus water contents were high, photosynthetic rates being lower on clearer days due to low thallus water contents. Net assimilation rate is influenced by the moisture content of the thallus (Harris 1971a, b, 1972; Kershaw 1972), reaching a peak between 65 and 90 % saturation, and falling at water contents near to saturation. In addition, in some species of temperate lichens, wetting the thallus results in losses of carbon by a release of carbon dioxide gas and re-saturation respiration (Smith and Molesworth 1973; Farrar 1973, 1976). Hence, to grow, thalli have to be wet sufficiently long in the light to overcome the carbon lost on rewetting, but not to be continuously saturated. An experimental demonstration of this effect was reported by Armstrong (1976b) who wetted thalli of three foliose species at different frequencies (Fig. 1.3). The radial growth of *M. fuliginosa* ssp. *fuliginosa* and *Phaeophyscia orbicularis* thalli (Neck.) Moberg declined with increased wetting frequency, while growth of *X. conspersa* increased with wetting frequency until 10 experimental wettings per month, declining at 15 wettings per month. Brown and Webster (1997) studied the growth of *Peltigera canina* (L.) Willd. transplanted onto garden soil. They found considerable linear growth of the transplants, especially when the soil was kept permanently watered, suggesting that a more continuous moisture regime may be beneficial in some species. Similarly, intact lichens grew most rapidly during periods of sustained moisture in the high Arctic (Cooper et al. 2001).

1.4.1 Aspect and Slope

Light, temperature, and rate of drying of the thallus may all vary with aspect (Armstrong 1975), slope (Sletvold and Hestmark 1998), rock and bark texture (Moxham 1981; Armstrong 1993b), and vertical location on a substratum (Harris 1971a; Armstrong 1978). The influence of aspect on the radial growth of *M. fuliginosa* ssp. *fuliginosa* at a site in north Wales was investigated by Armstrong (1975). In the periods September–October and March–July, growth of thalli on a northwest-facing rock surface was greater than on a south-facing rock surface. However, in the period January/February, growth on the south face was greater than on the northwest face. A physiological model which involved interactions between the levels of light, wetting

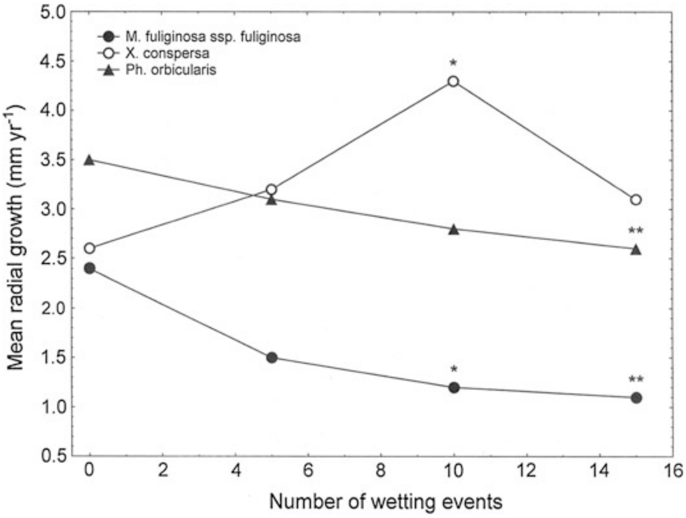

Fig. 1.3 The effect of frequency of wetting events on the radial growth (*RaG*) of three foliose lichens in south Gwynedd, Wales, UK. The radial growth of *Melanelia fuliginosa* ssp. *fuliginosa* and *Phaeophyscia orbicularis* thalli declined with increased wetting frequency while growth of *Xanthoparmelia conspersa* increased with wetting frequency until 10 experimental wettings per month, declining at 15 wettings per month. *Asterisk* indicates treatments significantly different from control (0) (data from Armstrong 1975)

regime, and rates of drying of the thalli was necessary to explain growth on the two surfaces (Armstrong 1975).

A number of studies have reported the effects of aspect on growth of crustose species. Hence, in the Brooks Range, Alaska, for example, radial growth of *R. geographicum* at sites with high to moderate light intensities was twice those at shaded locations (Haworth et al. 1986). Similarly, Bradwell (2001b) found that in Iceland, the largest thalli of *R. geographicum* were generally found on south-facing surfaces of boulders and are possibly a consequence of the longer thermal operating period and higher temperatures on southern surfaces. In the Cascade Mountains, Washington State, radial growth of *R. geographicum* on boulders was significantly greater on east-southeast and south-southeast facets compared with north-facing facets suggesting that at sites in the northern hemisphere, well-lit, southern facing surfaces are optimal for the growth of *R. geographicum* (Armstrong 2005) (Fig. 1.4). By contrast, variation in the slope of the facet appeared to have less influence on growth.

1.4.2 Vertical Height on Trees

Microclimatic differences also influence lichen growth with height on trees thus influencing vertical zonation. Hence, *Parmelia olivacea* (L.) Ach. em. Nyl. and *Parmeliopsis ambigua* (Wulf.) Nyl. differ in abundance and in vertical distribution especially on birch trees (Sonesson et al. 2011). During the cold season, *P. ambigua*, which was largely distributed on the lower trunk, was covered much of the time by snow, whereas *P. olivacea* occurred well above the snowline. The radial growth of *P. olivacea* was approximately twice that of *P. ambigua* but *P. ambigua* avoided competition by being adapted to the environment at the base of trees where snow accumulated and where *P. olivacea* could not survive. In addition, Harris (1971a, b, 1972) constructed a mathematical model to test the hypothesis that the vertical distribution of foliose lichens on trees in south Devon, UK, was a function of light intensity and water availability. Predicted vertical net carbon assimilation rates compared not unfavourably with the observed vertical distribution of the lichens (Harris 1972).

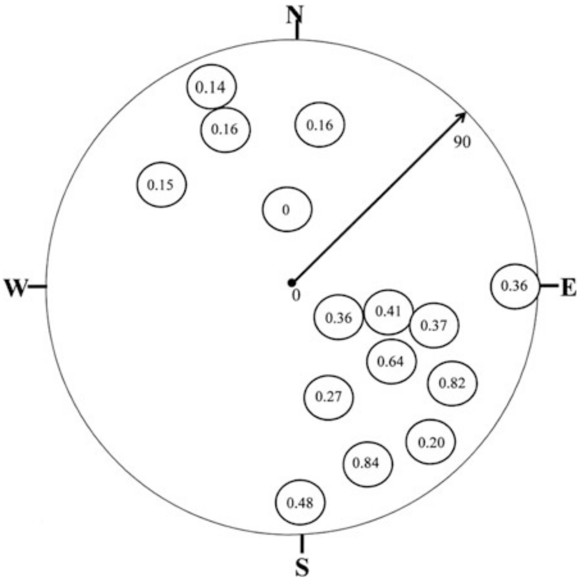

Fig. 1.4 Polar graph showing the influence of aspect and slope of the boulder facet on the radial growth (mm in 6 years) of *Rhizocarpon geographicum* in the north cascades, Washington State. Radial growth was significantly greater on east-southeast and south-southeast facets compared with north-facing facets suggesting that at sites in the northern hemisphere, well-lit, southern facing surfaces are optimal for growth. By contrast, variation in the slope of the facet appeared to have less influence on growth (data from Armstrong 2005)

1.5 The Influence of the Substratum on Growth

1.5.1 Physical Properties

The substratum may have several different effects on lichen growth and many of these factors could also interact with climate and microclimate. Hence, the relationship between the mechanical, chemical, and mineralogical properties of soil and the types of substratum chosen by terricolous lichens was studied by Garty et al. (1974). Species of *Squamarina* were found to be located on soils with the lowest shrinkage rates. When the shrinkage rate was above 5 %, however, the lichens grew on rock and moss instead suggesting that interactions between texture, porosity, rate of drying, and the physical attributes of the substratum on growth could have been responsible. In addition, *Xanthoria parietina* (L.) Th. Fr. can be affected by the texture of the substratum, thalli on rock, and smooth-barked surfaces having a higher radial growth than those on rough bark (Moxham 1981). Moreover, a combination of high water absorbing power and a high base content of the substratum favoured growth of lichens on roofs in suburban London (Brightman 1959). In addition, many lichens grow on moss which creates a very specific environment. In *Peltigera rufescens* (Weiss) Humb., for example, moss-associated thalli have a different morphology, have a 66 % higher net photosynthetic rate, and have a carbon dioxide concentration measured beneath the thalli more than 25 % greater than thalli growing on non-moss substrata (Colosie et al. 2012).

Relatively few studies of the growth of crustose lichens have been carried out on different types of substrata. *R. geographicum*, however, appears to be able to grow on a wide variety of siliceous rocks (Innes 1985). In addition, observations suggest *R. geographicum* may exhibit faster radial growth on sandstone than gneiss (Belloni 1973) and on andesite compared with granodiorite (Porter 1981).

The growth of the fruticose *Cladonia alpestris* (L.) Rabenh. and *C. rangiferina* (L.) Web. was studied by Kershaw and Rouse (Kershaw and

Rouse 1971). A strong correlation was observed between podetial length, diameter, internode length, and soil moisture; the level of soil moisture essentially integrating the effects of net radiation and drying rates of the lichen canopy as well as the relative metabolic activity of the lichen.

1.5.2 Rock Chemistry

The lichen flora of lime-rich rocks substantially differs from that of lime-poor, siliceous rocks and relatively few species are able to grow successfully on both types of substrata (James et al. 1977). To test the hypothesis that rock chemistry has a substantial effect on growth, fragments were cut from the perimeters of foliose lichens and glued onto different substrata, slate, granite, cement, and asbestos (Armstrong 1993b). The data for two foliose species are shown in Fig. 1.5. Radial growth of *X. conspersa* was inhibited on the calcium-rich compared with the siliceous substrates, while *X. parietina* would only grow successfully on a calcium-rich substratum, the fragments becoming discoloured and fragmented on lime-poor substrates.

A requirement for calcium may explain many aspects of the distribution of *Xanthoria parietina* in the UK. Hence, this species occurs on limestone rocks and walls, wall mortar, asbestos, tree bark where stemflow is enriched with calcium, and siliceous rocks near the sea (Armstrong 1990). By contrast, *Parmelia saxatilis* (L.) Ach. is virtually restricted to acid substrata, thalli of this species adopting a 'crescent-shaped' form after treatment with calcium carbonate, after which the thalli fragment and disappear (Armstrong 1990). Treatment with a 0.250 mM solution of calcium as calcium chloride, however, had little effect on growth of *P. saxatilis* (Fig. 1.5) suggesting that this species may tolerate mildly alkaline conditions. Gilbert (1971) found, for example, that *P. saxatilis* could occur at more alkaline sites in polluted environments. Zinc, copper, and mercury in rocks may also influence lichen growth as they have been shown to affect the chlorophyll content of lichen algae (Backor and Djubai 2004).

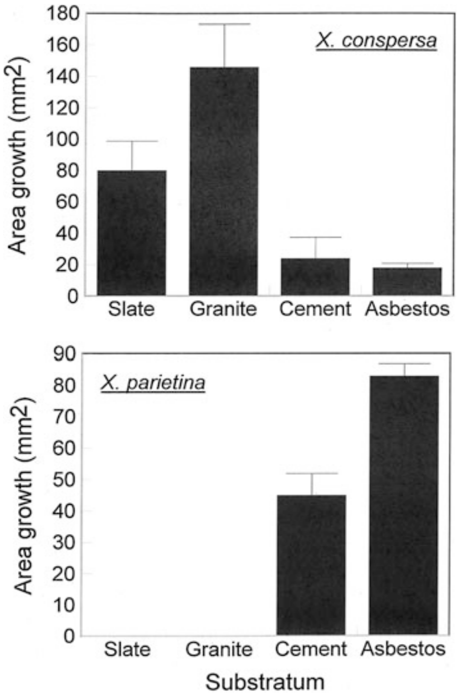

Fig. 1.5 The influence of the substratum on the radial growth (RaG mm year^{-1}) of two foliose saxicolous lichens in north Wales, UK. Analysis of variance (*ANOVA*): Radial growth of *Xanthoparmelia conspersa* was inhibited on the calcium-rich compared with the siliceous substrates while *Xanthoria parietina* would only grow successfully on a calcium-rich substratum. Between substrates $F = 11.09^{***}$, *post hoc*: Asbestos = cement, Slate/Granite > Asbestos/Cement; *Xanthoria parietina*, Asbestos versus Cement $t = 4.71^{**}$, $^{***}P < 0.001$, $^{**}P < 0.01$ (data from Armstrong 1993b)

1.6 The Effect of Nutrient Enrichment on Growth

1.6.1 Bird Droppings

Several studies suggest that the degree of nutrient enrichment of a substratum especially by bird droppings can have a significant influence on lichen growth and community composition (Hale 1967). Bird droppings may influence growth by smothering the thalli, altering pH, or adding inhibitory and stimulatory compounds (Armstrong 1984). Hence, droppings from a variety of birds were applied as a thick paste (12 applications in one year) and as a suspension in deionized water (24 applications in one year) to several foliose lichens with differing distributions on and off bird perching stones (Armstrong 1984). Treatment with bird droppings increased the radial growth of *X. conspersa*, a species common on well-lit nutrient-enriched rocks. The paste and to a lesser extent the suspension inhibited growth of *P. saxatilis* (L.) Ach. a species which is relatively rare on bird perching stones. Uric acid, the most abundant nitrogenous component of bird droppings, did not influence growth when applied as a suspension to either species (Armstrong 1984). Hence, growth response to bird droppings was likely to be attributable to either increased pH or inorganic chemicals in the bird droppings.

Few direct studies of the influence of nutrient enrichment on growth of crustose species have been carried out. In a long-term study at Signy Island (Maritime Antarctic), however, growth of lichens at sites enriched by nitrogenous compounds derived from seabirds was rapid, especially that of *Acarospora macrocyclos* Vain. and species of *Buellia* and *Caloplaca* (Smith 1995). In addition, Wootton (1991) compared different maritime lichen communities with and without overhead seabird colonies. When guano was present, the 'orange' zone dominated by *Caloplaca marina* Wedd. was elevated further up the shore, and the grey *Physcia* zone eliminated in the supralittoral zone. Moreover, in the splash zone, *Verrucaria maura* Wahlenb. Ex Ach. decreased as a result of enhanced competition with the green alga *Prasiola*.

1.6.2 Nitrogen and Phosphorus

The response of *X. parietina* to varying nitrogen concentrations was studied by Gaio-Oliveira et al. (2005). Thallus specific weight was similar in all thalli without any significant effect of added nitrogen suggesting that this species may respond more to changes in pH than to nitrogen consistent with previous data (Armstrong 1990). The relationship between nitrogen concentration and growth was also studied in *Nephroma arcticum* (L.) Torss. and *Peltigera aphthosa* (L.) Willd. (Sundberg et al. 2001). After 4-month growth, control and nitrogen-fertilised thalli of *Nephroma arcticum* increased in area by 0.2 m^2 m^{-2} (mean relative growth rate = 3.8 mg gm^{-1} d^{-1}) and *Peltigera aphthosa* by 0.4 m^2 m^{-2} (mean relative growth rate = 8.4 mg gm^{-1} d^{-1}). Slower growth in *Nephroma arcticum* was explained by lower nitrogen and chlorophyll a concentration and, as a consequence, substantially lower light-energy conversion efficiency. The interaction between nitrogen availability and light exposure was also studied in *Platismatia glauca* (L.) WL Culb. & CF Culb. (Palmqvist and Dahlman 2006). Dry weight gain was significantly enhanced by nitrogen supply, variations in growth being most significantly accounted for by chlorophyll a concentration. Hence, *Platismatia glauca* may respond to increasing nitrogen concentration by increasing growth rate and carbon assimilation capacity through encouraging the production of algal cells. Phosphorus may also be important in some cyanobacteria lichens such as *Lobaria pulmonaria* (McCune and Caldwell 2009). Hence, this species doubled its annual biomass after a single 20-min immersion in a phosphorus solution suggesting that phosphorus is an important stimulant to growth.

Growth of the fruticose lichen *Cladonia stellaris* (Opiz) Brodo was studied in relation to levels of fertilisation, and growth not responding to nutrient additions suggesting that some fruticose species may not respond to short-term changes in the availability of nutrients (Hyvarinen et al. 2003). In a further study of *C. stellaris*, Makkonen et al. (2007) found that both nitrogen and phosphate could limit the growth of fruticose lichens with phosphate having a greater effect than nitrogen. Moreover, nitrogen and phosphorus enrichment may have differential effects on the algal and fungal partners (Makkonen et al. 2007). In addition, nitrogen was added to two lichen communities in south-west Norway dominated by species of *Cladonia* and *Cetraria,* respectively, (Fremstad et al. 2005). There was a decrease in lichen size and overall cover, and later, thalli became discoloured, the most sensitive species being *Alectoria nigricans* (Ach.) Nyl. and *Cetraria ericitorum*.

1.6.3 Salinity

Additional chemical factors may be important in determining growth at maritime sites (Fletcher 1976). Although calcium was shown experimentally to be an important ion in these environments, salinity exerted little effect, the species responding more to periods of immersion (Fletcher 1976). By contrast, Ramkaer (1978) found that the response of four different lichen fungi to salinity correlated well with the zonation on maritime rocks. No studies appear to have been published on the influence of chemical treatments on the radial growth of species in situ on maritime rocks. The radial growth of *X. parietina*, a common species of the submesic zone of the suppralittoral (Fletcher 1976) and many nutrient-enriched sites inland (Brodo 1973), however, was inhibited when transplanted to an inland site (Armstrong 1990). In addition, the transplanted thalli grew successfully inland when calcium carbonate was added as a paste to the thalli at intervals over a year, treatment with a 0.250 mM solution of calcium chloride at similar intervals having less effect. A relatively constant supply of calcium may therefore be necessary for successful growth of this species. This hypothesis is also supported by experiments showing that *X. parietina* thalli lost potassium ions when treated with distilled water and an application of a 0.250 mM solution of calcium to the medium prevented this loss in the light (Fletcher 1976). By contrast, addition of calcium carbonate to the calcifuge species *P. saxatilis* inhibited its growth which may explain its absence from lime-rich rocks.

1.6.4 Pollution

Despite the reinvasion of many urban sites by lichens in the last 20 years, polluted sites have significantly lower lichen diversity than rural sites, a testament to the powerful effect of air pollution on growth. Samples of foliose and crustose species were transplanted on rock fragments from north Wales to a city centre site in Birmingham, UK; no measurable growth was recorded, all thalli exhibiting evidence of fragmentation within 7 days of transplantation and all had disappeared completely within one month (Armstrong unpublished data). Gilbert (1971) directly measured the effects of air pollution on the growth of *Parmelia saxatilis*. As the level of sulphur dioxide increased, thalli became crescent-shaped, the thalli persisting for several years before flaking off the substratum. During this period, however, actively growing lobes were found on the outside margin and radial growth did not appear to be affected. In addition, both *P. saxatilis* and *X. parietina* developed an intense orange or green colour within the medulla, respectively, representing the breakdown of lichen acids (Gilbert 1971). In the West Riding of Yorkshire, UK, there was greater uniformity in radial growth of *Lecanora muralis* (Schreb.) Rabenh. as measured from year to year, than observed in rural environments (Seaward 1976). In addition, at nine sites within the WNW to NNE quadrant of Leeds, radial growth varied from 2.84 to 6.5 mm year^{-1} increasing with distance from the city centre. The study also suggested that urban environmental factors have a greater detrimental effect on growth during winter months suggesting that toxicity, due to air pollution, rather than drought was the most important factor.

1.7 Ecological Significance of Growth Effects

Ecological consequences may result from direct or indirect action of environmental factors on growth. A direct effect results from a significant change in an environmental factor which has such a detrimental effect on lichen physiology so as to completely inhibit growth. In such a circumstance, lichen thalli often exhibit the characteristic signs of degeneration, i.e. they become discoloured, crescent-shaped, or exhibit fragmentation of the thallus centre, ultimately disappearing from a substratum (Armstrong and Smith 1997). In many situations, however, growth effects are more subtle and indirect, changes in growth being manifest as alterations in the competitive balance among different species in a complex community (Armstrong and Welch 2007).

Few studies have examined the interaction between growth and competition in determining the composition of lichen communities (Armstrong and Welch 2007). In north Wales, for example, a complex relationship exists between growth and aspect of *R. geographicum* (Armstrong 1974, 2002). Hence, *R. geographicum* is abundant on south-facing surfaces at these sites but exhibits significantly greater growth on some northwest-facing surfaces (Armstrong 2002). The size frequency distributions of the thalli also differed between these two habitats, a higher percentage of thalli in the smallest size classes and a more restricted range of size being observed on the north-west surfaces. The rate of mortality of the thalli also appeared to be greater on north-west surfaces. It was concluded that the radial growth of *R. geographicum* did not predict its aspect distribution in north Wales but that the differing competitive environments on the two surfaces could explain its aspect distribution rather than the direct effects of environmental factors. In addition, Bradwell (2010) studied the radial growth of *R. geographicum* in a maritime subpolar environment in northern Scotland. The fastest radial growth of thalli greater than 10 mm in diameter at the two sites was 0.67 and 0.29 mm year^{-1}, the difference being attributable to the level of competition.

Armstrong (1991) studied the influence of rock surface aspect on competition between four foliose lichen species in experimental plots. In south-facing plots, the growth of *X. conspersa* was not reduced by the presence of any of its competitors and, *X. conspersa* is frequently found on well-lit, south-facing rock surfaces at the site (Armstrong 1974). By contrast, in north-facing plots, the growth of *X. conspersa* was reduced by *P. saxatilis*, thus reducing its competitiveness and possibly allowing *M. fuliginosa* ssp. *fuliginosa* to coexist with *X. conspersa*.

In a further experiment (Armstrong 2000), four foliose species were grown in well-lit plots with and without nutrient enrichment by added bird droppings. The results of the experiment for one of the species, *M. fuliginosa* ssp. *fuliginosa*, are shown in Fig. 1.6. Without addition of bird droppings, area growth of *M. fuliginosa* ssp. *fuliginosa* was reduced by each of the other three species, most notably by *X. conspersa*. In addition, performance in the 3-species mixture was not predictable from the 2-species mixture. Hence, *M. fuliginosa* ssp. *fuliginosa* survived better in the *Ph orbicularis/P. saxatilis* and *X. conspersa/Ph. orbicularis* mixtures than predicted but was completely eliminated in the *X. conspersa/P. saxatilis* mixture. In addition, the performance of *M. fuliginosa* ssp. *fuliginosa* in the 4-species mixture was not predictable from the 3-species mixture, this species surviving better than expected. In plots with bird droppings added, however, *M. fuliginosa* ssp. *fuliginosa* was not significantly reduced by *P. saxatilis* but was substantially reduced by *Ph. orbicularis* and *X. conspersa* and eliminated completely in the *X. conspersa/P. saxatilis*, *X. conspersa/Ph. orbicularis*, and *X. conspersa/P. saxatilis/Ph orbicularis* mixtures. Hence, addition of bird droppings altered the competitive balance between the species, reducing the effectiveness of *P. saxatilis* as a competitor, but increasing the competitive effect of *X. conspersa*. Hence, *M. fuliginosa* ssp. *fuliginosa* survives better in more complex mixtures but only under conditions where there is little nutrient enrichment.

Addition of nutrients, by increasing the growth of some species and reducing the growth of others, could increase the chance of competitive exclusion of a species and therefore could reduce species diversity (Lawrey 1981; Vagts and Kinder 1999). By contrast, Welch et al. (2006) grew lichen fragments attached to roofing slate in various combinations and sprayed them at different frequencies with a nutrient solution. At medium application rates, there was increasing thallus growth and an increase in the number of ‘overlaps’ between adjacent thalli compared with controls. In addition, where nutrients were added, *Flavoparmelia caperata* (L.) Hale and *X. parietina* were equally strong competitors as also were *Parmelia reddenda* Stirt. and *F. caperata*. Where no nutrients were added, this balance between the pairs of species was lost with *F. caperata* outcompeting *X. parietina* and

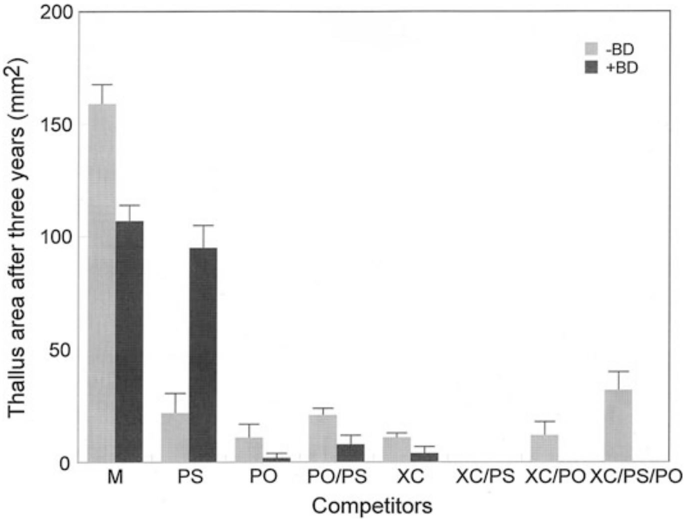

Fig. 1.6 The influence of nutrient enrichment by bird droppings (*+BD*) on the pattern of competition between *Melanelia fuliginosa* ssp. *fuliginosa* and three foliose saxicolous lichens (*M* growth of *M. fuliginosa* ssp. *fuliginosa* in monoculture, *PS Parmelia saxatilis*, *PO Phaeophyscia orbicularis*, *XC Xanthoparmelia conspersa*). Without addition of bird droppings, area growth of *M. fuliginosa* ssp. *fuliginosa* was reduced by each of the other three species, most notably by *X. conspersa*. In addition, performance in the 3-species mixtures was not predictable from the 2-species mixture. Hence, *M. fuliginosa* ssp. *fuliginosa* survived better in the *Ph orbicularis/P. saxatilis* and *X. conspersa/Ph. orbicularis* mixtures than predicted but was completely eliminated in the *X. conspersa/P. saxatilis* mixture. In addition, the performance of *M. fuliginosa* ssp. *fuliginosa* in the 4-species mixture was not predictable from the 3-species mixture, this species surviving better than expected. In plots with bird droppings added, however, *M. fuliginosa* ssp. *fuliginosa* was not significantly reduced by *P. saxatilis* but was substantially reduced by *Ph. orbicularis* and *X. conspersa* and eliminated completely in the *X. conspersa/P. saxatilis*, *X. conspersa/Ph. orbicularis*, and *X. conspersa/P. saxatilis/Ph orbicularis* mixtures (Data from Armstrong 2000)

P. reddenda outcompeting *F. caperata*. Hence, nutrient additions may not always reduce species diversity of a lichen community and further experiments of this type would be useful to understand the role that nutrient enrichment may play in determining the structure of lichen communities.

1.8 Conclusions

Despite the difficulties of growing lichens under controlled conditions and the challenges of field experimentation, there is a substantial literature on the effects of environmental factors on lichen growth. The major conclusions that can be drawn from these studies are as follows:

1. Seasonal fluctuations in radial growth often correlate best with average or total rainfall, the number of rain days, or rainfall in a specific season. Temperature may also an important climatic factor in some regions.
2. Interactions between microclimatic factors and especially light intensity, temperature, and moisture are important in determining local annual growth rates and differences in growth with aspect and slope of the substratum or with height on trees.
3. The physical and chemical nature of the substratum can affect growth especially the effects of texture, porosity, and rate of drying.
4. Whether the substratum is lime-rich or lime-poor and the degree of nutrient enrichment or maritime influence can have profound effects on growth both directly and by altering the competitive balance between species.
5. Effects of environmental factors on growth can alter the competitive balance on a substratum

ultimately influencing the composition of lichen communities.

6. Factorial experiments are needed to investigate the synergistic influence of different environmental factors and between the environment and competition and would lead to a more realistic understanding of the interactive processes influencing lichen growth in the field.

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Sampling and Interpreting Lichen Diversity Data for Biomonitoring Purposes

2

Paolo Giordani and Giorgio Brunialti

Contents

Abstract

Although lichen diversity values are broadly used as bioindicators, mainly for air pollution, lichen communities can be substantially influenced by other ecological factors, such as tree species and forest structure, and microclimatic conditions. In particular, species composition may be a suitable indicator for climate and land-use effects as well. For effective utilization of lichen diversity data in biomonitoring studies including air pollution, ecosystem functioning, and forestry studies, standardized sampling procedure and avoiding sampling and non-sampling errors are the important aspects to be considered. Further interpretation of lichen diversity data requires careful data analysis for providing affirmative results related to ambient air quality. In any lichen biomonitoring program, expected deliverables are based on a hypothesis, which may be achieved by standardization of the sampling procedures based on the functional requirement of the dependent environmental variables. The chapter discusses the procedures and methodology for sampling and interpreting lichen diversity data for biomonitoring purposes.

Keywords

Air pollution · Ecosystem functioning · Quality assurance · LDV

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2.1 Introduction

Due to their physiology, lichens are sensitive to a series of environmental parameters (namely light, air humidity, UV-B radiation, temperature, and airborne chemicals such as SO_2 and NO_x making them useful indicators for air pollution and climate change (Poikolainen et al. 1998; Tarhanen et al. 2000; Cornelissen et al. 2001; Kricke and Loppi 2002; Castello and Skert 2005). Biomonitoring methods based on the diversity of epiphytic lichens are among the most used worldwide. Apart from assessing the effects of gaseous pollutants, these approaches were recently extended to a suite of other anthropogenic disturbances (Nimis et al. 2002). Several aspects of lichen diversity (e.g., species richness and abundance, species composition, indicator species, functional traits and groups) are usually considered, each of them for a particular reason (Nimis et al. 2002). In simplified terms, it is possible to identify three main purposes to perform lichen monitoring:

1. Air quality monitoring. The sensitivity of lichens to phytotoxic gases (mainly SO_2 and NO_x) underlies their use to assess and monitor the effects of atmospheric pollution (Hawksworth and Rose 1970; Richardson 1993). They have been used as long-term biomonitors of air pollution both for small-scale (Nimis et al. 1990; Loppi et al. 2004; Giordani 2007) and large-scale surveys (Nimis et al. 1991; Van Dobben and DeBakker 1996; Bennett and Wetmore 1999; Frati and Brunialti 2006; Giordani 2006). Lichen biomonitoring surveys are often used to integrate instrumental data of atmospheric pollution (Nimis et al. 1990; Giordani et al. 2002; Giordani 2007; Pinho et al. 2004) and for developing forecasts in connection with human health (Cislaghi and Nimis 1997).
2. Sustainable forestry. Epiphyte diversity is related to forest structure and dynamics. Forest management, directly or indirectly, affects several environmental factors relevant to dispersal, establishment, and maintenance of lichen species. Studies on lichen diversity clearly demonstrate dramatic losses of species caused by forest management in European temperate and boreal forests (Hauck et al. 2013). In general, higher lichen diversity is related to lower management intensity, even though in some cases non-intensively managed forests may provide better conditions for epiphytic lichens than recently abandoned forests. For instance, several studies suggest that selective cutting is less detrimental to forest lichens than the shelterwood system or clear cutting (Nascimbene et al. 2013b).
3. Ecosystem functioning. Epiphytic lichens play an important role in both the forest water cycle, increasing canopy interception of precipitations and forest–nutrient cycling, above all for N-fixing ‘cyanolichens’ (lichens with a cyanobacterial symbiont; for a review see Ellis 2012). In addition to nutrient cycling, lichens are a focal point in forest food webs. They influence the ecological success of forest-dwelling animals in a wide variety of ways, e.g., for macrofauna in nest building, and as forage (Ellis 2012). In some cases, indicator species have been proposed as proxies for lichen species richness more generally. As an example, forest stands with the flagship species *Lobaria pulmonaria* tended to have more red-listed species and more continuity indicator species than stands without this lichen (Nilsson et al. 1995), tentatively pointing to covariation among continuity indicators. Similarly, comparing stands with different abundance and fertility attributes for *L. pulmonaria*, these attributes were associated with higher tree and stand-level macrolichen species richness and with significant differences in species composition among *L. pulmonaria* site-types, i.e., cyanolichens and rare species skewed to the ‘*L. pulmonaria* stands’ (Nascimbene et al. 2010).

In this chapter, we intend to explore a series of key issues on the topic in order to provide a framework of the subject with an overview of recently published researches.

In this regard, Fig. 2.1 provides a step-by-step flow diagram of operations required to address a lichen monitoring program. As a rule, these are

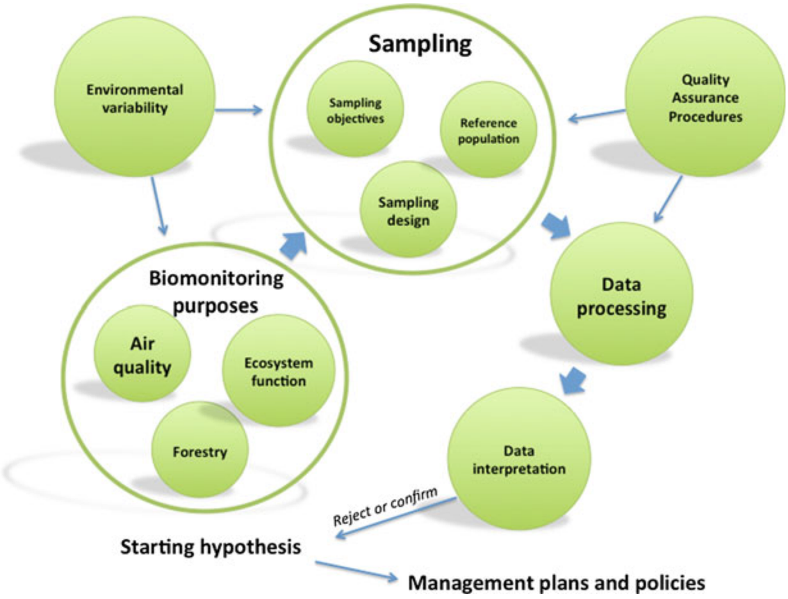

Fig. 2.1 Schematic diagram of the operations required to address a lichen monitoring program

the typical questions to be answered: does air quality affect lichen diversity in our study area? Or else, does forest management (or fragmentation) influence lichen communities in our investigated forests? Or even, do lichen diversity play a role in ecosystem function of our natural park?

The first step in the organization of a lichen monitoring program is to establish the initial null versus alternative hypotheses. For example, we may state the basic hypothesis that the expected lichen diversity is equal for impact and control sites (with respect to air pollution or to forest management). However, this is where environmental variability comes into play. Thus, the second step is to qualify, quantify, and control the main ecological factors that can affect our response variable, both including those of our interest and also the ones which may represent a source of noise (Brunialti et al. 2010a). Indeed, the effect of large-scale (such as climate) or local scale (as tree substrate) natural factors may represent relevant sources of variability and noise in relation to the phenomenon we are studying (Giordani et al. 2013; Ellis 2012). Also, some of these effects may be cumulative (Jovan and McCune 2004; Pinho et al. 2004; Giordani 2006; Caruso and Thor 2007). For instance, we should study air pollution effect on lichen diversity in forest sites with different forest management: We will have to set the study in order to disentangle the effect of individual factors and determine which of the two acts as main driver affecting lichen diversity or the presence/abundance of indicator species (Giordani 2007; Giordani et al. 2014a).

Our opinion is that this issue may have a central role in biomonitoring studies. For this reason, an entire Sect. 2.2.1 of the chapter focuses on these aspects, where we explore the main sources of variability affecting lichen diversity at different spatial scales. This variability being considered to adequately respond to our initial question, and the third step of our scheme concerns the adoption of an appropriate sampling design, from the selection of objectives and target population to the development of an adequate sampling strategy. We address this topic in Sect. 2.2.2, where we explore the main standardized methods for lichen diversity assessment, and analyze their suitability for various purposes.

Section 2.2.3 focuses on quality assurance procedures that are closely linked to sampling design and should be taken into account to obtain reproducible, reliable, and defensible data (Ferretti 2011). In this respect, we must remember that there is a strong link between data quality, information quality, and decision-making processes (Crumbling 2002).

The fourth step is then the phase of data processing. A broad range of statistical tools may be adopted to test the initial hypothesis. A subsequent interpretation of the results is essential to verify that the statistical significance of the test has a meaning even from an ecological point of view (Sect. 2.3). Starting from raw data, it is possible to adopt a series of interpretative devices that allow analyzing various aspects of the phenomenon under investigation. Only in this way, we may obtain useful information for management and decision making.

In this regard, the last step of our process involves the rejection or acceptance of the starting null hypothesis. On the basis of this result, we get essential information to proceed with the process of planning and management (policies to reduce air pollution, forest and landscape management, etc.). Indeed, without reliable information on changes to ecological systems and on the causes of those changes, decision making cannot deal effectively with these issues (Ferretti 2011).

The main objectives reported above are addressed in most cases with management and decision-making intent. In this perspective, if biomonitoring should be taken as a serious basis for decision making, it needs to produce robust, defensible data and documented quality (Brunialti et al. 2004). With this in mind, it is essential to approach the issue by means of a suitable sampling design and also appropriate statistical and interpretative tools that should be carefully selected to address properly each of these purposes (Ferretti and Erhardt 2002).

2.2 Sampling Lichen Diversity

There is no doubt that ecological monitoring has a fundamental role in providing baseline data on the status and trend of environmental resources. However, as underlined by Legg and Nagy (2006), in several cases, ecological conservation programs suffer from the lack of details of goal and hypothesis formulation, survey design, data quality and statistical power at the start. As a result, sometimes, they are likely to fail to reach the necessary standard of being capable of rejecting a false null hypothesis with reasonable power. This is mainly related to the adoption of an unsuitable sampling design, and this is basically the reason why sampling has frequently been considered as the weakest point of monitoring programs. This is unfortunate because failure to provide convincing evidence of the overall quality of environmental information can have serious consequences in environmental decision making. Indeed, we must consider that the defensibility of the data supporting the decision is fundamental (Ferretti 2011). To avoid this and to promote a correct process, the selection of a suitable sampling design represents the first step to reduce data variability due to sampling error. When selecting the proper design, the objectives (biomonitoring purposes, Fig. 2.1) of the survey and environmental variability should be taken into account carefully (Brunialti et al. 2004).

With respect to lichen biomonitoring, environmental variability acting at different spatial scales has been taken into account during the standardization process of the sampling protocols (Giordani et al. 2001; Brunialti and Giordani 2003; Nimis et al. 2002), thereby providing useful information for the selection of suitable sampling designs.

This section provides useful insights on several issues relevant to environmental variability, sampling design, and quality assurance procedures, which are the basis of a proper environmental monitoring program.

2.2.1 Variability of Lichen Diversity Across Scales

Variability is an inherent property of ecological systems and every attempt to measure and interpret the environment should consider it (Brunialti et al. 2004). Thus, in the assessment of environmental quality by means of biomonitors, it is important to understand the environmental processes driving the variability of biological data, since this may affect the forecasting precision of these techniques (Laskowky and Kutz 1998). Environmental factors such as geomorphology, climatic variables, and substrate could have a great impact on the ecosystem property being studied in order to assess environmental quality, such as the rate of indicator species, the biodiversity of a community, or the presence of injuries on organisms. In simple terms, environmental heterogeneity may control the variability of lichen diversity and the composition of lichen communities at least at three spatial scales: (1) tree level; (2) plot level, and (3) landscape level. In this section, we highlight the possible effect of these ecological factors on lichen diversity by referring to the main studies conducted so far.

2.2.1.1 Spatial Scale of Variation in Lichen Monitoring Data

Quantifying spatial and temporal variation is a key element for understanding mechanisms and processes that structure the species assemblages for assessing the environmental impact and for identifying an appropriate scale of sampling (Levin 1992; Underwood et al. 2000). Nonetheless, biodiversity assessments are strongly dependent on observation scales (Gaston 2000; Purvis and Hector 2000), and natural spatial variation or the spacing of sampling units may decisively affect results (Dungan et al. 2002).

Although several studies have investigated spatial variation in plants (see, e.g., Palmer 1990; Gunnar and Moen 1998), lichens are still poorly investigated from this point of view (Ferretti et al. 2004), even though descriptors of lichen diversity are widely used in applicative studies for assessing alterations of the environment (Nimis et al. 2002), covering areas ranging from some dozen to some thousands square kilometers (e.g., Giordani et al. 2002). In particular, bioindication techniques usually focus on between-site variability in epiphytic lichen diversity using a high sampling density and do not sufficiently consider possible within-site variability, probably because this would imply sampling more trees, thus increasing the cost of the survey (Ferretti et al. 2004; Brunialti et al. 2010a). Controlling the spatial variation at macroscales is necessary but not sufficient, if the variation at more detailed levels (e.g., considering areas <1 km^2) remains unknown.

A recent study explored the variability of lichen composition throughout the spatial levels using sound sampling protocols, along biogeographic, climatic, and pollution gradients (Giordani et al. 2013). The authors showed that the distribution of variation in lichen diversity was impressively similar across the spatial scales (Giordani et al. 2013). In particular, they found that it was fairly constant despite climatic variation, landscape complexity, air pollution, and general anthropogenic pressure. Moreover, the predictability of lichen bioindication methods, as estimated by the between-plot variability/total variability ratio, was limited to less than 40 %, thus calling for some refinements in sampling and interpretation phases (Fig. 2.2). This finding suggests the adoption of specific experimental designs, such as a stratified random sampling or a tree-based sampling to improve the detection of the sources of variation.

2.2.1.2 From Tree-level to Landscape-level Variability

Epiphytic lichens distribution depends on a complex set of environmental and substrate-related explanatory variables, acting from the tree to the landscape level (Nimis et al. 2002). Several tree-level environmental factors are important for the growth of epiphytic lichens and drive the diversity and composition of their communities (Ellis 2012). Figure 2.3 summarizes the main ecological factors acting at this level on a hypothetical tree with a different canopy in winter and summer periods or a deciduous and an evergreen tree, respectively.

We can distinguish at least two main categories of ecological factors that have been explored by many studies (see Ellis 2012): microclimatic variables (light availability, temperature, and relative humidity) and bark properties (pH, texture, exfoliation, water-holding capacity). In this respect, both substrate eutrophication, due to the grazing of cattle and wild animals and also to soil re-suspension (Loppi and De Dominicis 1996; Frati et al. 2007) and circumference, and

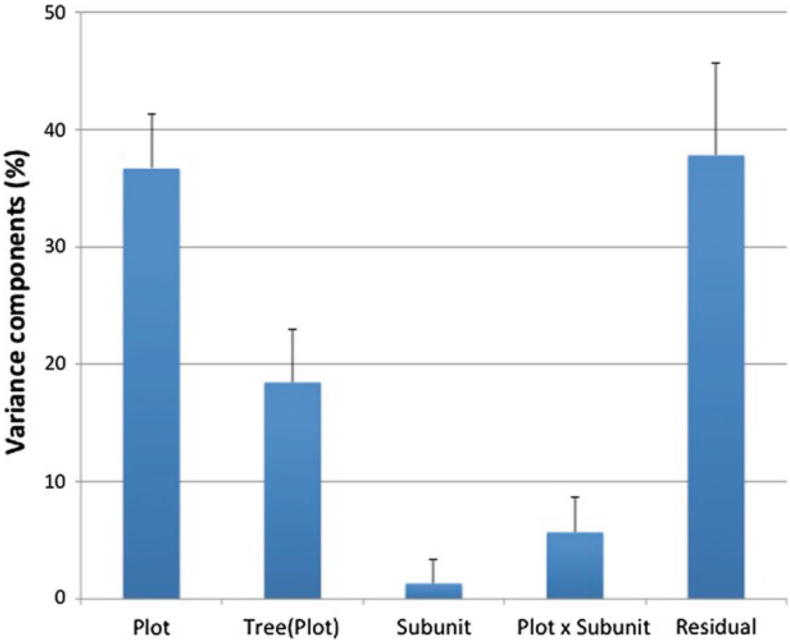

Fig. 2.2 Estimated components of variance (%) in lichen diversity values, calculated for each spatial scale (modified from Giordani et al. 2013)

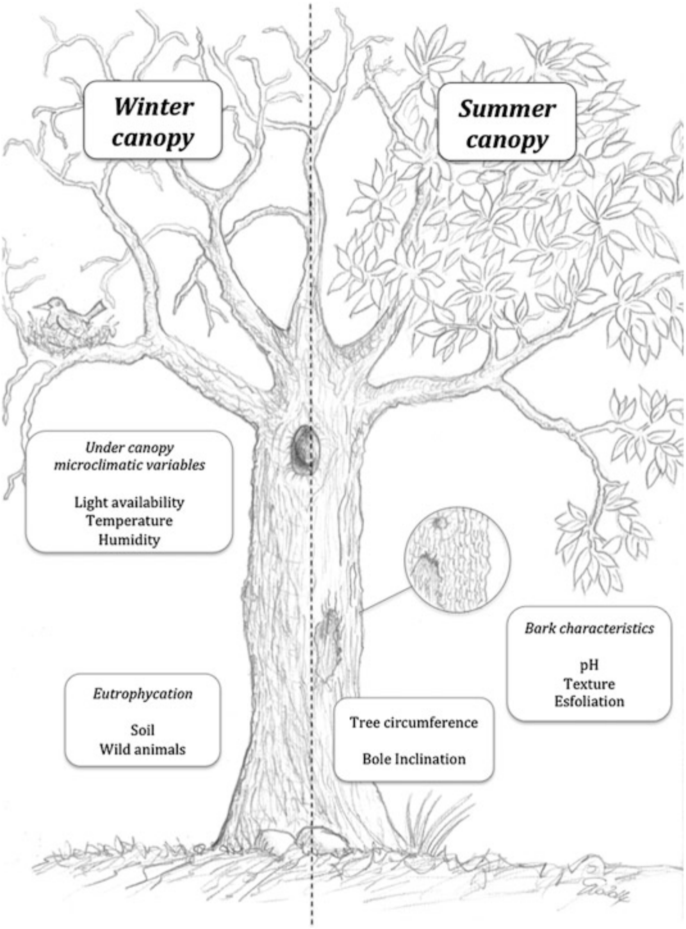

Fig. 2.3 Conceptual model illustrating the main ecological factors potentially affecting lichen communities under the canopy of a broad-leaved tree in winter and summer periods (see explanation in the text)

inclination of the bole may contribute indirectly to modify these ecological factors (see, e.g., Giordani et al. 2001).

Figure 2.4 summarizes the main ecological factors affecting lichen communities at plot and landscape levels. They are mainly related to the distribution and density of trees (e.g., the gradient from isolated trees to near-natural forest stands) and to the land-use and geomorphological variability (e.g., altitudinal range, agricultural, or forested landscapes). Light and humidity availability generally play the role of direct factors in this context.

Basically, all these aspects are very interconnected and show that the effects largely covariate. Canopy structure decisively controls light conditions at the lower bole (Moe and Botnen 1997; Fritz et al. 2009), and this may result as a negative effect of canopy shading on species richness, both at tree level (Loppi and Frati 2004) and at plot level (Brunialti et al. 2010b). While focusing on edge effects in fragmented boreal and Mediterranean forests, several authors pinpointed light as a main driving factor for lichen colonization, affecting lichen abundance, diversity, and composition at tree level (Esseen and Renhorn 1998; Belinchón et al. 2007; Boudreault et al. 2008; Brunialti et al. 2012b). In particular, higher lichen species richness was found at the side of the trunk facing the edge (Brunialti et al. 2012b), or in correspondence with the first light peak (Belinchón et al. 2007). Loppi and Frati (2004) found higher lichen diversity values on *Tilia platyphyllos* compared to *Quercus ilex.* These findings were explained by significantly higher winter light conditions and water-holding capacity of the bark for *Tilia* trees. Bark-pH was not found as discriminant parameter among the two tree species. On the contrary, many studies have demonstrated a strong epiphyte response to bark-pH, explaining epiphyte community variation compared among tree species in both boreal and temperate systems (Hyvärinen et al. 1992; Gauslaa 1995; Kuusinen 1996; Jüriado et al. 2009; Lewis and Ellis 2010; Leppik et al. 2011). Moreover, intraspecific studies, among boles of the same tree species, have indicated that bark-pH/nutrient status may be modified by an interaction with the soil environment (Gauslaa 1995; Gustafsson and Eriksson 1995; Kermit and

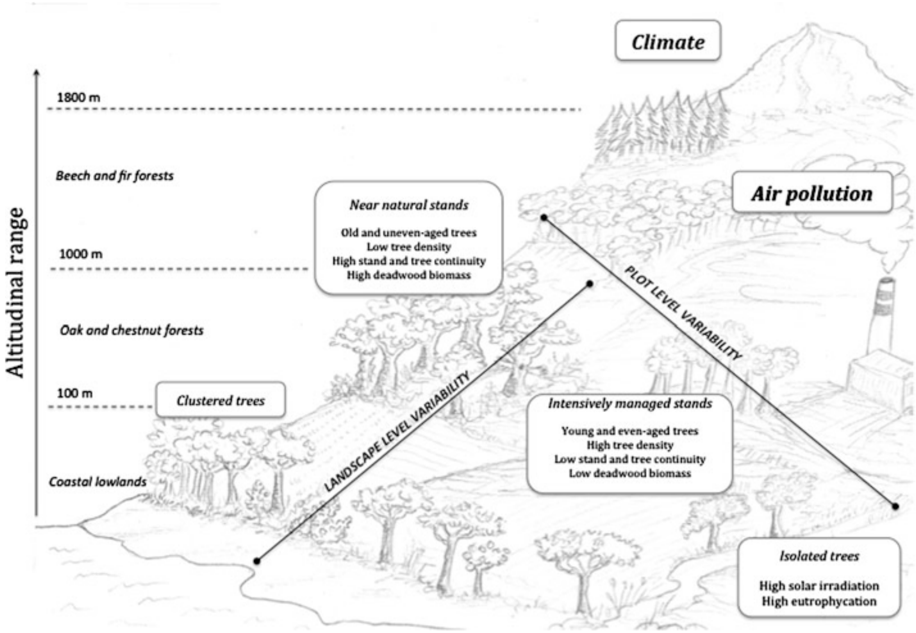

Fig. 2.4 Conceptual model illustrating the main ecological factors potentially affecting lichen communities at plot and landscape levels (see explanation in the text)

Gauslaa 2001) and with a subsequent effect on epiphyte community composition. Interestingly, differences in tree age/size (dbh) correlate with bark-pH among individual boles of the same species, though the direction of this relationship cannot be generalized. Bark-pH may decrease with increasing tree circumference (Bates 1992; Kuusinen 1994) and/or age (Ellis and Coppins 2007), or pH may increase with circumference (Jüriado et al. 2009) and/or tree age (Fritz et al. 2009). Also, bark texture was found to be an important tree species factor influencing lichen diversity (Bates 1992; Cácares et al. 2007; Ranius et al. 2008; Fritz et al. 2009). This is subject to an effect of tree age/size (dbh) and an interaction with vertical height on the trunk (Johansson et al. 2007; Ranius et al. 2008; Fritz 2009), and it is related with a species-specific relationship between bark roughness and tree age/size (Uliczka and Angelstam 1999).

The standardization process of air quality biomonitoring method took into account all these sources of variability acting on lichen diversity at tree level, making it possible to isolate environmental gradients in a simplified system (Asta et al. 2002; EN 16413 2014; see paragraph interpreting lichen diversity). For this reason, the standard features of the sampling trees (slope, pH of the bark, etc.) have been selected carefully (Table 2.1).

With respect to plot-level variability, Giordani (2006) carried out a hierarchical evaluation of the effects of substrate- and environmental-related variables on lichen diversity. Elevation, mean annual temperature, and latitude were the main factors influencing epiphytic lichen distribution, together with disturbances (such as atmospheric pollution, forest fires and agricultural practices) and habitat heterogeneity that covary along the strongest gradient of lichen community composition. Lichen species richness was positively associated with mean annual rainfall and longitude, negatively associated with harvesting, and positively associated, though weakly, with the occurrence of past forest fires (these burnt sites were characterized by recolonization processes).

In a study of epiphytic lichens of forest ecosystems of Tuscany, Loppi et al. (1999) concluded that habitat characteristics are more important than phorophyte properties and there is evidence that the epiphytic lichen vegetation of deciduous *Quercus* trees follows a distribution which is related to elevation and climate, with great differences in community structure along the altitudinal gradient (Loppi et al. 1997).

As for the aspects affecting lichen distribution at landscape level, many authors pointed out the role of climatic factors (McCune et al. 1997; Goward and Spribille 2005; Hauck and Spribille 2005; Giordani 2006, 2007). In this respect, Giordani and Incerti (2008) found that the distribution of more than 30 % of epiphytic species was associated to macroclimatic variables. A significant subset of epiphytic lichens in the study area has been proved to be efficient bioclimatic indicator for montane, humid sub-Mediterranean, and Mediterranean units.

Further, also land-use intensity drives the local variation of lichen diversity, both in Mediterranean (Giordani et al. 2010) and in boreal ecosystems (Holt et al. 2008). Land-use intensity

Table 2.1 List of features to of a standard tree (see EN 16413 2014; Asta et al. 2002)

Feature	Description
Suitable tree species	The sampling tree belongs to one of the groups with similar bark physicochemical properties (EN 16413 2014; Asta et al. 2002). Indicatively, species belonging to the same group can be used interchangeably
Trunk circumference	The sampling tree has a trunk circumference (at 130 cm from the ground level) between 50 and 250 cm
Trunk inclination	Each exposition (N, E, S, W) has an inclination (at the center of each grid) <20°
Bark damage	The area of the trunk that is unsuitable for recording (damage, decortication, branching, knots and/or other epiphytes or climbing plants such as ivy, preventing growth of lichens) within each of the 4 grids when summed <20 %

was detected as a good proxy for describing the lichen distribution and abundance under anthropogenic pressures. In particular, land-use categories, mainly based on vegetational features, differed in epiphytic and epilithic lichen communities, and the strongest differences were observed among the forested sites versus managed agroforestry lands. These latter were mainly characterized by common lichen vegetation (mainly xerophytic–nitrophytic species).

Similarly, in Scotland, lichen communities differed between natural ancient pine forests and those of trees within managed sites both in forest and agricultural areas (Wolseley et al. 2006). The alteration was mainly due to increasing nitrophytes, most evident for epiphytes, but also significant for saxicolous communities. According to Bergamini et al. (2005), the trend of modification of lichen communities under changing land use is nearly constant along large latitudinal gradient, ranging from northern Europe to the Mediterranean. Also, at this large scale, strong differences were detected between the forested and the more open land-use types, especially for epiphytic crustose lichens.

2.2.2 Standardizing Lichen Diversity Sampling

Differences between methods, difference in the application of the same method, measurement error, sampling and non-sampling error, and errors related to model applications are all terms of the whole error budget that inevitably affects environmental surveys (Gertner et al. 2002). In this perspective, the extent to which the objective of the survey is matched depends very much on the ability to manage the various sources of variability (Khol et al. 2000; Wagner 1995) by adopting suitable standard operating procedures (Brunialti et al. 2004, 2012b).

A recent example of standardization process relates to the sampling protocol to assess epyphitic lichen diversity for air quality. In fact, the European Committee for Standardization (CEN, Comité Européen de Normalization) has recently published an European standard, reporting the sampling protocol for lichen diversity assessment (EN 16413 2014). The process of standardization started in 2007 and took into account the previous European and national guidelines (Asta et al. 2002; VDI 2005; AFNOR 2008). In the meantime, some field tests have been performed to obtain information on the type and size of errors and the uncertainty of the methodologies under standardization (Brunialti et al. 2012a, Cristofolini et al. 2014). To fill this gap, the tests dealt with the entire process from survey design to field measurements. In particular, the comparative tests, consisting in multiple exercises, were run with the aim of comparing the results obtained by different, well-experienced operators faced with the same problem, at the same time, under the same field conditions and following the same standard operating procedures (SOPs).

A similar standardization sampling procedure has been adopted in forest monitoring. An example is represented by the sampling protocol of the EU project ‘Forest BIOdiversity Test phase Assessments’ (ForestBIOTA), carried out by 12 European countries in the framework of the ICP Forests Expert Panel on Biodiversity and Ground Vegetation Assessments (Fisher et al. 2009). In this context, Stofer et al. (2003, 2012) prepared a standardized sampling protocol for lichen diversity assessment that takes into account all the main steps of the sampling from the tree- to the stand level.

2.2.2.1 Sampling Objectives

A clear definition of objective is important; otherwise, any statement about data quality remains elusive (Ferretti 2011). As a consequence, although it is not a typical source of error per se, ambiguous monitoring objectives can be great promoters of errors: On the one hand, the monitoring design cannot be properly addressed if the objective is not clear, and incorrect design may jeopardize the whole monitoring. For this reason, objective must be explicit with respect to target population and its attributes, spatial and temporal domains, desired precision level and minimum detectable change, and type I and II acceptable error rates.

With respect to the CEN standard for biomonitoring ambient air, the sampling objective is to obtain an estimate of the parameter of the response variable (e.g., mean species richness or mean lichen diversity value, LDV) over the study domain with a given precision (EN 16413 2014). The precision level should be expressed in terms of confidence intervals for a defined probability level. It is required that the sampling objective is defined for each study. For example, we may want to obtain an estimate of the mean LDV for the study domain with a confidence interval ±10 % of the mean value at a probability (*P*) level of 95 %.

Since the computation of estimates and confidence intervals depends on the sampling design adopted, each study shall define precision and probability levels, taking into account the requirements of the study framework and considering the available resources (EN 16413 2014).

2.2.2.2 Target Population

In statistical terminology, the target population refers to a different concept than that associated with the community, population, individual, and genetic concept of biological systems. The target population is the collection of elements about which information is wanted (Cochran 1977). Both the 'target population' for which information is wanted and the 'elements' that make up the target population must be rigorously defined. As clearly stated, target population should be carefully selected before starting each monitoring program. It must be representative of the whole population in order to extend the results obtained by the selection of a sample of it to the entire population. In fact, we must consider that data obtained outside a formal sampling design cannot be considered representative for the entire population, and therefore, conclusions cannot be extended without making assumptions about it (Ferretti 2011). Similarly, models built upon non-representative data can be seriously biased. A classical example is the biodiversity data collected by taxonomists who are inclined to concentrate their efforts in the localities that guarantee success in the collection of as many species as possible, thus resulting in species-richness bias (Sastre and Lobo 2009).

For instance, a recent comparative test to identify critical issues in lichen biomonitoring demonstrated that different teams may select different target populations when planning the work (Brunialti et al. 2012a). Although all of them may be formally correct, these differences are a source of inconsistency in the results and could potentially lead to differing conclusions. It is therefore extremely important that every step of the design process is properly documented and reported in survey documents so that future repetitions may be made.

2.2.2.3 Sampling Design and Sampling Schemes

For broader applications (e.g., at the European level), a standard protocol should be flexible enough to preserve the representativeness of the data under different ecological conditions. In general, it is difficult, if not impossible, to find a single sampling design that is suitable for each situation. Different options of sampling schemes may be adopted, mainly in relation to the complexity of the survey area and with the distribution of the trees to be sampled (Elzinga et al. 2001; EN 16413 2014; Ferretti and Erhardt 2002). With respect to air monitoring pollution, the recent CEN standard proposes the adoption of different schemes in relation to ecologically homogeneous and heterogeneous areas. In particular, in the first case, we may have tree options:

1. When standard trees are abundantly and homogeneously distributed over the study domain, a simple random or systematic design is recommended. Plot sampling is recommended, with sample plots allocated according to a regular grid, with the starting point of the grid chosen at random (e.g., Giordani et al. 2002).
2. When standard trees are abundantly scattered in clusters over the study domain, tree-based cluster sampling or two stage sampling is recommended. A criterion to identify clusters should be initially set, then identify, and list

all the clusters and obtain a random sample of them. It should be noted that a two-stage sampling requests a subsampling of the sampling unit, and it introduces a further source of variability which may affect the quality of the data. It is important to take this into account when performing statistical analysis of the data (EN 16413 2014).

3. When standard trees are infrequently scattered over the study domain, a simple tree-based random sampling is recommended. It is possible to obtain a list of the individual trees on the basis of the aerial photo and to select randomly the sample trees.

Similar options may be selected also in ecologically heterogeneous areas. In this case, however, a stratified random sampling design is recommended both with standard trees homogeneously distributed and scattered in clusters over the study domain. A tree-based stratified random design is suitable when we have standard trees infrequently scattered over the study domain.

Notwithstanding this great variability of solutions, it is not so obvious that each of these is properly taken into account when a monitoring program is designed, since the experts in the field may be more influenced by examples from the past (seen as best practices) that by newly suggested options. The results of a comparative test carried out of operators on the same area and with the same standard procedures by five independent groups partly confirm this statement. In this study, in fact, most teams adopted a stratified random sampling (Brunialti et al. 2012a). Although they largely agreed on selection of the sampling scheme, considerable differences occurred in subsequent steps of the sampling design: For example, the number of selected land cover categories ranged from 2 to 8 and the sampling density 23–43. The authors observed that training courses on sampling design and basic statistics may be successful in reducing this source of variability in routine field studies.

2.2.2.4 Sampling Units

As reported above, both plot-based and tree-based sampling schemes can be adopted. According to the latter option, the sampling units are represented by the standard trees available in our survey area. Instead, when considering the plot-based sampling, the selection of the optimal shape and size of the plot remains an open question. In this case, the goal is to find an acceptable trade-off between a good representativeness of the study area and a cost-effective sampling effort.

Concerning the shape of sampling units, circular or square plots are used in most cases. In general, the former are suggested in air pollution monitoring programs (Giordani et al. 2002), while the latter are mainly adopted in forest surveys (Giordani 2006). The basis for a proper selection of the shape of sampling units relies both on probabilistic issues and on practical questions (Elzinga et al. 2001). For rectangular sampling units, the associated edge error is larger than for circular plots. Moreover, a circular plot requires only one measurement (radius) to be installed. In special cases, such as for the assessment of the effect of forest fragmentation, rectangular transects were taken into account, as they could fit better with the natural shape of forest fragments, which were further divided into subplots (Brunialti et al. 2012b).

As for the plot dimensions, McCune and Lesica (1992) found trade-offs between species capture and accuracy of cover estimates for three different within-site sample designs for inventory of macrolichen communities in forest plots. On average, whole-plot surveys captured a higher proportion of species than did multiple microplots, while giving less accurate cover estimates for species. The reverse was true for microplots, with lower species captures and much better cover estimates for common species. Belt transects fell between the over two sampling designs.

Ravera and Brunialti (2013) showed that a probabilistic sampling based on the selection of only three trees within small circular plots (14 m diameter) can be effective for assessing the occurrence of species of conservation concern in old growth forests in an Italian National Park. They found that most of the species were present in a few sampling sites and only a small group of species were common to more than 50 % of the plots. This may suggest that the adopted sampling design allowed not only to determine the most local common species but also to detect the presence of sporadic species (Ravera and Brunialti 2013). To confirm this, their lichen list reported several new and interesting species and represented 30 % of the indicators of forest continuity known from the region. This is a considerable result if we consider the nature of the adopted sampling and also the fact that regional floras mainly report records from preferential surveys. We must keep in mind, however, that floristic surveys, mainly carried out by means of a preferential sampling, are biased by the fact that they are primarily carried out to discover rare species in specific habitats or ecological niches. Humphrey et al. (2002) obtained similar results in a study carried out to compare lichen and bryophyte communities between planted and seminatural stands. A high percentage of species was recorded only once, and very few species were common to more than half the plots. This 'local rarity' phenomenon has been noted in other studies (Vitt et al. 1995; Collins and Glenn 1997; Qian et al. 1999; Humphrey et al. 2000) and is partly related to sampling area. The authors of that study observed that it is possible that a 1-ha sampling plot used is too small to capture a representative sample of lower plant diversity in forest stands. For example, Rose (1993) recommends a minimum sampling area of 1 km^2, but this depends on the objective of the survey. Hence, these findings suggest to adopt one of the following approaches for future investigations: (1) extend the surveys to plot with a broad area and, at the same time, detect a higher number of trees and/or substrates (rocks, soil, etc.). Although this approach could be very time consuming, it should ensure the finding of a large number of species; (2) improve the number of small plots in the study area. In this way, with a lower sampling effort at the individual plot, it is possible to obtain information on a wider territory. Moreover, this second approach should be mostly useful for preliminary lichen surveys in poorly studied wild areas (Ravera and Brunialti 2013).

2.2.2.5 Sampling Density

Optimal sampling density is an essential requirement of lichen biomonitoring surveys for obtaining precise and unbiased estimates of population parameters and maps of known reliability (Ferretti et al. 2004). This aspect is often a sore point of biomonitoring projects and should be carefully addressed to respond correctly to the sampling objective and to select a sample with a proper sample size to avoid jeopardizing the effectiveness of the investigation. Apart from catching a sufficient amount of variability, a decision on a proper sample size forcely considers the sampling effort and its costs and it often happens to reduce the number of sampling sites in order to limit the costs.

Both the ecological complexity of a given area and the desired level of precision (in terms of confidence interval) drive the sampling design. In heterogeneous areas, a greater number of sampling units and more detailed land cover stratification are needed to obtain accurate estimates. For broader application (e.g., at the European level), a standard protocol should be flexible enough to preserve the representativeness of the data under different ecological conditions (Brunialti et al. 2012a). In this respect, Ferretti et al. (2004), starting from the results of two large-scale surveys undertaken in Italy, carried out a study on the effects of different sampling densities (the number of sampling units in the study area) and on the reliability (in terms of confidence intervals and relative error in the mean values) of the estimates of the lichen diversity values of a given area and of lichen diversity maps. An iterative approach was taken into account to generate subsets with lower sampling density with respect to the original sampling units (ordinary kriging interpolations). Obviously, a higher sampling density may lead to a low error rate but may be financially unsustainable. A very low sampling density, on the other hand, may provide uncertain data as to be of no real use (Khöl et al. 1994; Ferretti and Erhardt 2002). Their findings suggested that a large-scale lichen diversity spatial pattern can be detected with a much more relaxed grid density than those originally applied (Ferretti et al. 2004). This is extremely important, since reducing the sampling effort can result in considerable savings in resources that can be diverted to additional, more detailed investigation with denser sampling in those areas identified as problematic by the study based on the low sampling density. These results were also confirmed by a similar study (Frati and Brunialti 2006), within a long-term monitoring program, that showed the possibility to reduce the sampling effort in future monitoring surveys, resulting in a considerable reduction of the sampling effort maintaining a good data quality.

2.2.2.6 Lichen Survey

As discussed in the previous sections, a series of micro- and macroenvironmental variables can affect the composition and diversity of epiphytic lichen communities. For this reason, a standardized sampling strategy to count lichen diversity is extremely important to ensure comparable and accurate results. The recently developed European standards for lichen biomonitoring (Stofer et al. 2012; EN 16413 2014) have been developed as the result of a standardization process carried out in the last few years starting from the Index of Atmospheric Purity (IAP) approach (Hawksworth and Rose 1970; Nimis et al. 1990, 1991) and upgrading it with previous guidelines (Asta et al. 2002; VDI 3957 2005). Furthermore, also field experience of several European researchers and the results of recent comparative tests (Brunialti et al. 2012a; Cristofolini et al. 2014) were useful to obtain the current sampling strategy for lichen survey. At the end of this process, to reduce the effect of several possible sources of error (e.g., different size of explored area on the trunk, subjectivity in the positioning of the sampling grid, etc.), several parameters have been standardized. Hence, the abundance of each lichen species is currently sampled by means of a sampling grid consisting of a 10 × 50 cm ladder divided into 5 10 × 10 cm quadrants. This ladder grid is placed systematically on the N, E, S, and W side of the bole of each tree (4 per tree), with the top edge 1.5 m above ground, following the standards suggested by Asta et al. (2002). Summary measurements of species richness and abundance are usually calculated for each plot: mean number of species per tree; total number of species within the plot; and mean lichen diversity value (LDV—Asta et al. 2002) calculated as the sum of the abundance of each species within the sampling grids on a tree, averaged for all trees within a plot.

2.2.3 Quality Assurance

Biomonitoring investigations are subjected to a variety of error sources that need to be acknowledged and documented in order to be managed properly (Brunialti et al. 2004). The quality of the data originating from biological measurements depends heavily on at least three factors (see, e.g., Kovacs 1992; Brunialti et al. 2004): (1) variability of the biomonitoring organisms (interactions between the organisms and environmental factors); (2) type of sampling (sampling design, density of sampling points); and (3) operators involved in assessing lichen diversity, which is a method requiring a relatively high taxonomic knowledge.

The first issue has already been discussed above (Sect. 2.2.1), and we have seen that this source of variability can be controlled with a good knowledge of the ecological characteristics of biological indicators considered and adopting proper sampling designs. Regarding the type of sampling, the sampling errors are closely associated with the sampling design adopted and its quality. As for the third factor, the effect of operators' subjectivity and expertise has been widely addressed within lichen biomonitoring so as to obtain useful information to further improve standardized protocols.

To respond to these issues and to take all the steps of the monitoring survey into account, the adoption of quality assurance procedures is strongly recommended. QA is an organized group of activities defining the way in which tasks are to be performed to ensure an expressed level of quality. The main benefit of a QA plan is the improved consistency, reliability, and cost-effectiveness of a program through time (Ferretti 1998). A QA plan is essential since it forces program managers to identify and evaluate most of the factors involved in the program. In addition, the assessment of data quality enables mathematical management of uncertainty due to the method used (Ferretti 1998; Cline and Burkman 1989).

It is therefore important that environmental biologists and field ecologists consider QA as a key attribute of their work in order to provide robust and defensible data to decision makers (Brunialti et al. 2004).

2.2.3.1 Sampling Errors

Sampling errors are generated by the nature of the sampling itself and by the degree of variability in the target population. As reported above, such kinds of errors always occur but can be controlled by appropriate sampling design (Cochran 1977; Köhl et al. 2000; Ferretti and Erhardt 2002).

A good sampling design is essential either to collect data amenable to statistical analyses and to control errors in relation to the costs (Brunialti et al. 2004). An important issue, in this respect, is selectivity, which seems particularly important in ecological measurement. A protocol is selective if the response provided as a measurement depends only on the intended ecosystem property (Olsen et al. 1999). Regarding this aspect, Yoccoz et al. (2001) suggest that quantitative state variables characterizing the system well should be privileged. For example, when defining management objectives in terms of changes of densities of indicator species, the program should incorporate tests to ensure that selected species are indeed indicators of the process and variables of interest (Yoccoz et al. 2001). For this reason, it is important to establish a priori the variables of interest in a sampling protocol. The criteria for this selection should be based on data quality, applicability, data collection, repeatability, data analysis and interpretation, and cost-effectiveness. In particular, the use of quantitative state variables is recommended in order to reduce the error in data collecting due to the subjectivity of the operators.

2.2.3.2 Non-sampling Errors

Non-sampling errors include measurement, classification, and observer errors, which are rooted in how the standard operating procedures (SOPs) are prepared and applied and how well-trained and skilled the field crews are (Ferretti 2009). In general, non-sampling errors can occur when the methodology is poorly standardized, when teams have insufficient skills or insufficient care is taken in applying the method, or when there are problems with instrument calibration (Ferretti and Erhardt 2002). Many papers have focused on these topics and have shown that non-sampling errors can be a significant source of variability in monitoring studies (Gertner and Köhl 1992; McCune et al. 1997; Giordani et al. 2009; Francini et al. 2009; Gottardini et al. 2009; Marchetto et al. 2009; Sastre and Lobo 2009).

In the specific case of epiphytic lichen assessment, non-sampling error may basically occur at two stages (Brunialti et al. 2012a): the identification of standard trees and the counting of lichen species. As for the former case, an imprecise definition of suitable trees in the SOPs might be one of the main reasons for differences in the number of suitable trees found by the different teams. As for the counting of lichen species, floristic knowledge is a crucial issue that needs to be addressed in lichen biomonitoring where the protocol is based on assessment of all lichen species, including groups of lichens which are hard to identify in the field, such as crustose lichens (Asta et al. 2002). Differences in the floristic skills of the teams can cause serious errors (Brunialti et al. 2002; Giordani et al. 2009). Brunialti et al. (2012a) confirmed that considerable underestimation of species richness may occur even when sampling within an a priori positioned grid. Floristic skill is even more important when assessing crustose-dominated communities, where poorly developed thalli often occur (Giordani et al. 2009). Undoubtedly, variability among crews could be reduced with intercalibration courses and harmonization procedures (McCune et al. 1997; Brunialti et al. 2002, 2004). In fact, there is evidence that operators often improve in accuracy during the same test and that their accuracy improves with taxonomic training and, above all, continuous fieldwork (Brunialti et al. 2002).

As far as precision is concerned, very high levels are usually registered among operators, suggesting a high reproducibility of the lichen diversity counts (Brunialti et al. 2002). This is extremely important for correct evaluation of time series in biomonitoring studies. However, it should be borne in mind that changes in operators in long-term monitoring of permanent plots can give misleading results (McCune et al. 1997) and should be carefully addressed.

2.3 Interpreting Lichen Diversity

As described in the previous paragraphs, a considerable effort has been made in the recent years for standardizing the sampling design and strategies of lichen biomonitoring. In the following paragraphs, we will describe some of the most used approaches for interpreting lichen diversity data in terms of effects of various anthropogenic disturbances. In general, the interpretation of geographic patterns and temporal trends of lichen diversity may be assisted by using ecological indicator values (Hawksworth and Rose 1970; Wirth 2010; Nimis and Martellos 2001, 2002), multivariate statistics, such as numerical analysis of matrices of species (Giordani et al. 2002; Giordani 2006), nonparametric models (McCune and Mefford 2004; Giordani 2007), or other statistical tools.

2.3.1 The Concepts of Biomonitoring

We can define monitoring as the process of gathering information about some system–state variables at different points in time for the purpose of assessing the state of the system and making inferences about changes in state over time (Yoccoz et al. 2001). If our focus is on the monitoring of biological diversity, the systems of interest to us are typically ecosystems or components of such systems (communities and populations), and the variables of interest include quantities such as species richness, species diversity, biomass, and population size.

2.3.1.1 Lichen Diversity Value, α-Diversity

According to the recently developed standards for lichen biomonitoring (Stofer et al. 2012; EN 16413 2014), the basic results of lichen diversity sampling are aggregated matrices of the species frequencies at nested spatial levels of sampling, i.e., a matrix of species at aspects of each tree; at trees of each sampling units; and, finally, at sampling unit level. Several diversity indices are calculated a posteriori basing on these basic matrices. This recommendation comes to the fact that several interpretative tools may be applied to basic data, which are used in a various manner in different countries.

As a simple but effective approach, α-diversity (number of species at plot level) is a robust parameter for interpreting patterns of epiphytic lichen communities along gradient of anthropogenic effects, such as pollution or forest management.

Among the possible descriptors obtained from the basic species × sampling unit matrix, the lichen diversity value (LDV) by Asta et al. (2002) is by far one of the most used in applicative biomonitoring surveys. The value for a given sampling unit is calculated as the sum of the frequencies of all lichen species found on each tree within the unit and averaged by the number of sampled trees. Relevant differences in lichen growth may be expected on different cardinal aspects of the trunks; therefore, it is suggested that frequencies are also summed separately for each aspect, and possibly, additional analysis might be carried out in this sense. LDV in its basic definition has been extensively used in applicative studies all over Europe (Paoli et al. 2006; Svoboda et al. 2010; Giordani et al. 2014a), and its relationships with pollution and other environmental factors had been analyzed in details. In the Mediterranean, together with pollution, the mean annual rainfall of the survey area seems to be the best predictor for LDV variability (Giordani 2006). However, the decisive variables affecting the lichen diversity are apparently different in urban versus forested areas (Giordani 2007). In these latter, harvesting and forest fires showed a predominant effect. Contrarily, in urban areas, air pollutants, mainly SO_2, are the main limiting factors, even if this relationship is lowering under ameliorating conditions of atmospheric pollution. Similarly, Svoboda et al. (2010) found that lichen diversity in Central Europe responded differently to environmental predictors depending on different human impact. These authors observed that in industrial regions, air pollution was the strongest factor affecting lichen diversity, whereas in agricultural to highly forested regions, LDV was mainly influenced by forest age and forest fragmentation.

Starting from the basic species × sampling unit matrix, further parameters can be derived from the data set of the species frequencies, including the relative LDVs of morphofunctional groups of lichens associated to particular sources of atmospheric pollution (e.g., nitrophilus versus acidophilus species—see Sect. 3.2.3).

2.3.1.2 Rapid Biodiversity Assessment (RBA) Based on Morphospecies

The application of biomonitoring methods based on high levels of taxonomic knowledge, such as the recently standardized lichen biomonitoring method (EN 16413 2014), requires an adequate number of specialists that are not always available, especially in large-scale biodiversity assessments (Wilkie et al. 2003). The use of guilds or morphological groups as indicators of changes in ecosystem function has been considered by several authors as a good compromise between the need for specialized knowledge and rapid field procedures employing non-specialist technicians, thus providing a possible shortcut in assessing total species richness (see, e.g., Pharo et al. 2000; Giordani et al. 2009). This issue has been considered in several ecological monitoring fields to explore the possibility of using surrogate species for estimate total biological diversity. As an example, several studies have been performed to assess the congruence among vascular plants, vertebrate, invertebrate, bryophytes, and lichens within large-scale biomonitoring surveys using simplified assessment methods (see Oliver et al. 1998; Pharo et al. 2000; Wilkie et al. 2003; Santi et al. 2010). However, some conflicting results obtained from the works listed above suggest that this is not always the ideal solution and several critical issues emerge: Among the others, there are sources of variation coming from the fact that the communities do not always behave in a linear and unambiguous manner. Also, the data quality in surveys involving non-specialist crews may vary at such a level which could drastically compromise the reliability of the results. In this respect, Giordani et al. (2009) carried out a study to compare data obtained by non-specialists through simplified methods based on morphospecies (RBA), with those collected by specialists using the lichen diversity value (LDV) method. They found that lichen diversity estimated by means of Rapid Biodiversity Assessments (i.e., based on morphospecies) showed good correlations with the results of a classical, systematic identification of species only when performed by operators with high taxonomic knowledge. Furthermore, the use of sampling lists based on highly simplified morphospecies did not lead to significant advantages in terms of time needed for fieldwork (Fig. 2.5).

In some other cases, Rapid Biodiversity Assessments of lichen biodiversity led to interesting results even at large scale. Recently, citizen science approaches have been applied to lichen biomonitoring of the effects of atmospheric pollution. These are voluntary schemes engaging members of the public in the collection of scientific information. The OPAL Air Survey in the UK (Davies et al. 2011) used presence and abundance data for 9 selected lichens, collected by more than 4,000 volunteers in a public survey of lichens on trees, to examine the response of individual species and groups of indicator lichens to air pollution and climate drivers on a national scale. The use of these macrolichen indicators has shown to have robust relationships with

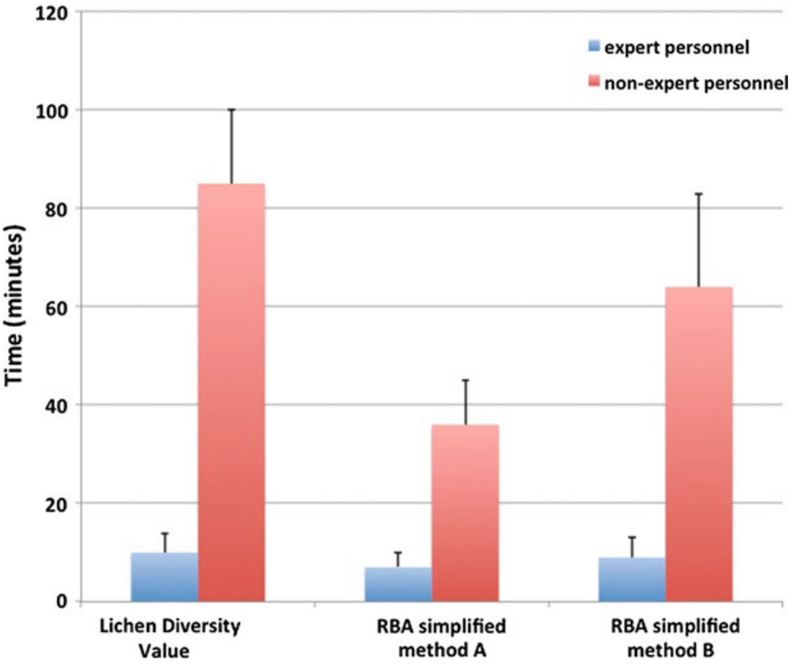

Fig. 2.5 Time (minutes) required for each lichen diversity assessment (tree level), using different sampling lists. Lichen diversity value (sampling of lichens at species level) was compared with two different simplification methods of rapid biodiversity assessment (*RBA*, simplified methods A and B). Data from Giordani et al. (2009)

modeled nitrogenous pollutants at the national scale (Seed et al. 2013). In the USA, Casanovas et al. (2014) proposed a citizen scientist-based survey methodology for macrolichen diversity in which parataxonomic units (PUs), as identified in lichen photographs, served as species surrogates to estimate lichen diversity. Although in most cases the authors showed that the observed and estimated cumulative richnesses from both techniques were not statistically significantly different from each other, the extensive use of these approaches in biomonitoring surveys should be carefully evaluated, as misidentifications of morphologically similar species could led to wrong interpretation of data, e.g., in terms of relative abundance of functional groups. At this regards, it has been shown that the use of electronic devices and identification tools may help to increase the quality of RBA. The project Dryades developed interactive identification keys in the form of applications for mobile devices. Keys were generated from databases of morphoanatomical characters. The applications were tested Europe wide during the project KeyToNature and have proved to be useful in education and in projects of citizen science (Nimis et al. 2012).

2.3.1.3 β-Diversity

Most biomonitoring applicative surveys and researches using epiphytic lichens have focused on analyzing plot-level species richness (e.g., alfa-diversity), abundance, and/or composition patterns along ecological gradients. Recently, it has been suggested that also analyses of β-diversity may provide insights into mechanisms and drivers influencing lichen communities, thus contributing to a better interpretation of the results. Beta-diversity has been interpreted mostly as the extent of change in community composition (Whittaker 1960). Basically, beta-diversity patterns are originated from two distinct processes, the replacement and the loss of species (Carvalho et al. 2012). With the aim of differentiating the relative influences of these components on beta-diversity, various measures have been proposed, which relied on an additive rather than multiplicative approach (Baselga 2010). As a first application to lichen communities, Nascimbene et al. (2013a) used the conceptual scheme by Podani and Schmera (2011) to evaluate the relative importance of β-diversity, nestedness, and agreement in species richness in presence–absence data matrices via partitioning pairwise gamma diversity into additive components. Podani and Schmera (2011) considered three complementary indices that measure similarity (S), relative species replacement (R), and relative richness difference (D) for given pairs of observations. In particular, according to these authors, β-diversity is defined as the additive result of R and D, whereas other descriptors, namely nestedness and richness agreement, result from the additive effects of similarity with the other two complementary components (S + D and S + R). By analyzing the diversity in *L. pulmonaria* communities in Italian forests, Nascimbene et al. (2013a) showed that both species replacement and similarity were generally associated with forest structure predictors, such as the number of trees in the plot and the distance between trees, while richness difference was mainly associated with geographic predictors, with special reference to longitude and altitude. Giordani et al. (2014b) coupled the analysis of β-diversity with the approach based on the relative abundance of functional groups. These authors explored the shift in functional groups for nitrogen tolerance along a gradient of increasing cattle load in epilithic lichen communities of alpine pasturelands. An increasing cattle load caused a decreasing replacement of oligotrophic species and consequently a decrease in β-diversity. Conversely, when considering a data set with only N-tolerant species, there was very high pairwise similarity among sampling plots, irrespectively by the cattle load gradient to which they were exposed.

2.3.1.4 Indicator Species

Detecting species that best characterize some set of sites is an important step in evaluating classifications in community ecology. With reference to lichen biomonitoring, this approach has been mostly applied for detecting the effects of forest management or to assess the ecological continuity of ancient woodlands. In some cases, lists of indicators species were compiled on the basis of expert assessments (e.g., Rose 1976). As for other biologically based approaches, also for biomonitoring, the use of proper methods for measuring the explanatory power of species is essential, whenever priority must be given to species that best reflect environmental quality (Dufrêne and Legendre 1997; Podani and Csányi 2010).

Whittet and Ellis (2013) tested 29 indicator species of forest continuity, as proposed by the current suite of British lichen indicators (Coppins and Coppins 2002) for different biogeographic regions in the UK. In accordance with previous studies (e.g., Sætersdal et al. 2005; Giordani and Incerti 2008), these authors confirmed that indicator species are likely to have a restricted geographic scope. Moreover, they suggested that only some of the studied taxa could be actually accurate indicators of ecological continuity through a dependency on the long-term persistence, whereas in other cases, several species may possibly be associated with specialist microhabitats under a sub-optimal climate, whereas they would not be significantly associated with ancient woodlands. Giordani (2012) compared the performance of four potential indicators for monitoring the effects of forest management on epiphytic lichens in broadleaved Mediterranean forests. Indicators included total lichen diversity (LDV) and the abundances of species associated with intensive management, species associated with aged coppiced woodlands and indicator species ratio (ISR). ISR was defined as the ratio between the difference between the species associated to aged coppice forests and those associated to intensively managed forests and the total abundance. At each of 50 sampling sites, the four indicators were calculated using indicator value analysis (Dufrêne and Legendre 1997) and compared through correspondence analysis. By balancing the partial information provided by both sensitive and resistant species, ISR was shown to be a more effective indicator, being independent of floristic composition and the occurrence of rare species. The main drawback of the indicator species approach is that evaluation of the effects of a given stress (e.g., forest management) is possibly biased due to the fact that lichen species are strongly threatened by several anthropogenic disturbances occurring at the same time (e.g., high levels of air pollutants or forest fires).

2.3.2 Interpretative Tools

2.3.2.1 Interpretative Scales of Alteration

Following the definition of Nimis (1999), biomonitoring techniques estimate the degree of alteration from normal conditions resulting from the effects of pollution on the reactive components (e.g., lichens) of the ecosystems. However, defining 'normal conditions' in ecology is extremely critical and can only rely on an operational basis. At this regard, some authors proposed an interpretation of lichen diversity data in terms of percentile deviations from an observed maximum diversity. Loppi et al. (2002a, b) sampled lichen diversity on about 3,000 trees in northern Tyrrhenian, Italy, and considered as 'natural' those values ≥ the 98° percentile of their frequency distribution. The average of these values was taken as an operational definition of 'naturality,' which represents a 'maximum potential lichen diversity' in a given area. These authors considered that a 25 % deviation from 'normal conditions' could still be regarded as 'natural,' owing to natural fluctuations of lichen diversity. Starting from this point, an interpretative scale of naturality/alteration was build basing the different degrees of naturality/alteration on progressive 25 % deviations from 'normal conditions.'

As lichen distribution strongly depends on macro- and mesoclimatic factors (see Ellis 2012), such interpretative scales of lichen diversity in terms of deviation from maximum potential conditions information should be referred to a regional level. Thus, direct comparisons between biomonitoring surveys carried out in different bioclimatic regions are often poorly informative and can also led to misleading interpretations. A similar approach of regionalization has been followed and debated in the case of other biomonitoring techniques (e.g., Moog et al. 2004). For example, the EU Water Framework Directive suggested qualitative reference for evaluating the ecological status of water bodies, assessing the highest potential quality, based on the composition of aquatic communities. It was possible to predict how the response of diatom communities to anthropogenic pressure in each French hydro-ecoregion was predicted (Tison et al. 2007), in relation to the topology of running waters and validated with benthic macroinvertebrates from reference sites (Wasson et al. 2002).

The delimitation of eco-regions or bioclimatic regions bases upon differences in the composition of their lichen flora. At this regards, Giordani and Incerti (2008) applied a nonparametric multiplicative regression model to the lichen flora of a climatically heterogenous area and detected 59 species which were significantly associated to macroclimatic variables (i.e., annual rainfall and temperature). A cluster analysis grouped the taxa into four subsets that were related to different climatic niches (warm–humid, cold–humid, mesothermic–humid, warm–dry) corresponding to distinct bioclimatic regions.

As an example of regionalization of interpretative scales, Castello and Skert (2005) provided evaluation scales of environmental alteration based on lichen diversity in the North Adriatic sub-Mediterranean bioclimatic region. These authors sampled deciduous oaks in 11 reference sites in open stands within natural woods or near very small isolated villages in rural or natural areas, far from large urban areas, industrial zones, and long-distance transport of air pollutants. As a result of relatively dry condition occurring in North Adriatic, the LDV threshold for naturality class was slightly lower than the one calculated for the more humid Thyrrenian Italy (Giordani 2004). Regionalized interpretations are also strongly recommended by the German guidelines (VDI 3957 2005), which states that a comparison of surveys of different regions is only possible if the surveyed areas have a similar climate and, therefore, a comparable lichen flora, as is the case with Central Europe (excepting the Alps) for which the evaluation scale was calibrated.

In some cases, ad hoc interpretative scales have been calculated for local situations. Due to the lack of an interpretative scale for semiarid Mediterranean bioclimatic region, Paoli et al. (2006) developed a calibrated scale for a small area (36 km^2) according to the protocol suggested by Loppi et al. (2002a, b) for the assessment of environmental deviation from natural conditions. Macroregional- and local-based interpretations of lichen diversity are not mutually exclusive and can provide integrate information on the actual environmental conditions.

Thought that nitrogen is becoming one of the most relevant limiting factors for lichen communities, especially in Central Europe, the German standard for lichen biomonitoring (VDI 3957 2005) proposed an interesting integrate approach for interpretation, which is basically based on the relative diversity of functional groups for nitrogen requirements. According to the German guidelines, nitrophytic species which respond positively and oligotrophic species which respond negatively to eutrophication (referred to as ‘reference species’) are calculated separately. The two partial LDVs of a sampling unit are combined to form the ‘air quality index’. The LDV of the reference species is entered along the ordinate axis, whereas the LDV of the indicators of eutrophication is entered along the abscissa. The quality class assigned to the field in which the crossing point of the lichen diversity values comes to lie gives the evaluation of the air quality of the sampling unit. According to the German approach, the thresholds of the air quality classes in the matrix are chosen so that the class width is approximately equal to three times of standard deviation of the LDV in the study area. In this way, the lichen cover of sampling units belonging to different air quality classes is significantly different if the respective classes are separated by at least one other class.

The use of interpretative scales of lichen diversity based on deviation from maximum potential diversity also presents some weak points. From a theoretical point of view, it is well known that natural situations are not necessarily associated with maximum values of biodiversity. According to influential ecological theories, such as the intermediate disturbance hypothesis (Connell 1978), intermediate levels of disturbance, in terms of frequency and/or intensity of the phenomena, will maximize species diversity. The question has been shown to be more complex and less generalizable than this, with recent studies demonstrating that diversity could show monotonic, unimodal, or even flat response to disturbance, depending on the studied organisms and on the disturbance aspects considered (Hall et al. 2012). In an applicative perspective, the lacking of undisturbed reference situations in some eco- or bioclimatic regions (e.g., the Po Plain in Italy or many anthropized areas of Central Europe and North America) makes difficult to define properly the values associated to the highest classes of the scale, thus affecting the entire interpretation process.

2.3.2.2 Mapping Lichen Diversity

Mapping lichen diversity is an attractive approach, which allows an immediate representation of the results. Showing spatial distribution patterns of the studied descriptors, maps of lichen biodiversity, or abundance enable a quick and clear identification of areas with different levels of disturbance (Pinho et al. 2004; Asta et al. 2002). Spatial mapping of lichen diversity or associated measurements had been extensively used both in research and applicative lichen biomonitoring works (Giordani et al. 2002; Pinho et al. 2004, 2008a; Geiser et al. 2010). A large suite of GIS softwares provide tools for interpolating (i.e., estimating) the values of the response variable (e.g., lichen diversity) in non-measured parts of the survey area, basing on the data collected at sampling sites. In this chapter, we do not review the technical aspects of this approach. Basically, it follows the principle of geostatistical modeling theory originally developed for applied geology and more recently applied to ecology (see, e.g., Perry et al. 2002 for a detailed description). However, when applying these techniques, lichenologists should be aware that incorrect settings of the geostatistical model could lead to misleading results and interpretation and that the error associated to a poor spatial model might be also larger than those imputable to other sources of errors, such as sampling design or taxonomic misidentification.

Estimated values in lichen biodiversity maps are related to sampling densities (the number of sampling units in the study area), which in turn affect the reliability of the collected data in terms of confidence intervals and relative error in the mean values (Geiser and Neitlich 2007). By analyzing two large surveys of lichen mapping carried out in Italy (Nimis et al. 1991; Giordani et al. 2002), Ferretti et al. (2004) showed that even a considerable reduction (up to 50 %) of the original sampling effort led to a much smaller increase in mapping errors (<18 %). These data suggested that reducing the sampling effort ensures a considerable saving in resources that can be diverted to additional and/or more detailed investigation with denser sampling in those areas identified as problematic by the study based on the low sampling density.

Despite the fact that the interactions of pollution with other confounding factors in determining the distribution of lichens has been largely ascertained (Nimis et al. 2002), surprisingly, this source of variation was not always explicitly considered when building a spatial model for lichen biomonitoring data. A good example of integrate interpretation of lichen data, accounting the variability of both pollution and climate, was provided by Geiser and Neitlich (2007). Analyzing the effects of air quality and climate on epiphytic lichens in the US Pacific Northwest, they produced a kriged map of air scores, which were derived by the coordinates on the main axis of an NMS ordination of sampling plots located along gradients of pollution and climate. The estimated values of the air scores in non-sampled cells of the study area were calculated using the Gaussian semi-variogram model and a variable search radius including 20 sampled points. The spatial scale of analysis of a geostatistical model is often determined a priori. Pinho et al. (2008a) warned that, using this approach, some relationships between indicator and environment may be overlooked. Ribeiro et al. (2013) faced this problem investigating the relationships between ecological indicators and underlying environmental factors in Portugal. They used a multivariate geostatistical method, a linear model of coregionalization, to analyze the joint distribution of biodiversity variables in their study area. They were able to assess the strength of the relationship between each environmental factor and ecological indicator at several spatial scales. Basically, they related information on land cover and climatic variables with the abundance of fruticose lichen species, which were expected to be very sensitive to multiple environmental drivers. Their analysis implied the calculation of a nested variogram function to quantify the intensity and direction of correlations between the abundance of fruticose lichens and environmental factors at relevant spatial scales. These authors found that at medium scales (c. 15 km), open-space areas (considered as a proxy variable for particle emissions) were more important for shaping the abundance of this lichen group, whereas at larger scales (c. 45 km), open artificial areas (as a proxy for gaseous pollutants) and climate were preponderant.

2.3.2.3 The Use of Lichen Functional Groups to Detect Critical Loads and Critical Levels of Pollutants

'Functional groups' is the term used to describe sets of species exerting a comparable effect upon a particular process or responding in a similar manner to changes in their external constraints (Lévêque and Mounolou 2003). In particular, species' functional groups have been proved to be a valuable tool for comparing lichen diversity across diverse regions where high levels of floristic variation may occur (Giordani et al. 2012). Interestingly, recent revisions of the critical loads (CLOs) and critical levels (CLEs) for a large number of ecosystems are based on the response of lichens to main pollutants (e.g., Cape et al. 2009; Fenn et al. 2008). CLOs and CLEs have been defined to set sustainable thresholds for the protection of ecosystems from the effects of pollutants. The CLO is defined as "a quantitative estimate of deposition of one or more pollutants below which significant harmful effects on specified elements of the environment do not occur according to present knowledge" (Posthumus 1988), whereas the CLEs refer to the concentration of pollutants in the atmosphere above which adverse effects occur (Cape et al. 2009). Pinho et al. (2014) recently proposed a new tool to calculate CLEs by stratifying ammonia concentrations into classes and focusing on the highest diversity values. Based on the significant correlations between ammonia and biodiversity, the CLE of ammonia for Mediterranean evergreen woodlands was found to be 0.69 μg m^{-3}, below the currently accepted pan-European CLE of 1.0 μg m^{-3}.

Concerning the detection of CLOs, the occurrence of oligotrophic lichen species provided information on the actual impact of reduced nitrogen compounds (mainly ammonia) in the forest plots of the European network ICP Forests (Giordani et al. 2014a). The critical load causing a significant change of the expected composition of epiphytic lichen vegetation occurred at nitrogen deposition = 2.4 kg N ha^{-1} $year^{-1}$. Lichen functional groups for eutrophication and/or, more specifically, nitrogen tolerance have been extensively used to assess the critical level and critical load of nitrogen compounds in several forest ecosystems all over the world (Fenn et al. 2008; Geiser et al. 2010; Pinho et al. 2008b, 2011). Interestingly, the results for European plots were in accordance with those of other areas of the world. For example, in conifer forests of the Pacific North West of USA, Geiser et al. (2010) found, for wet deposition, a critical load ranging from 0.7 to 4.4 kg ha^{-1} $year^{-1}$, depending on the amount of precipitation. The concept of critical load for lichen communities has been also applied to other sources of disturbance. Giordani et al. (2014b) established the cattle critical load in alpine pasturelands, in terms of Adult Cattle Units (ACU) per hectare per year. These authors showed that the relative frequency of oligotrophic epilithic lichen species significantly decreased as ACU increased. The cattle critical load was set for ACU = 0.12 ACU ha^{-1} $year^{-1}$.

2.4 Open Questions

Despite the relevant number of researches in the field of lichen biomonitoring carried out in the last 25 years, there are still open points which have not yet been sufficiently addressed. In some cases, these questions are far from being minor and responses are urged in order to make the interpretation of lichen biomonitoring more robust. Among the others, the insufficient knowledge on the timescale of the response of epiphytic lichen communities to disturbances and the lacking of an adequate integration about lichen biomonitoring and other standard biomonitoring techniques particularly call for more attention by researchers and stakeholders. According to its technical definition, monitoring is the collection and analysis of repeated observations or measurements to evaluate changes in condition and progress toward a management objective (Elzinga et al. 2001). Thus, also for lichen biomonitoring studies, an accurate knowledge on the temporal variation of the observed phenomenon is crucial for getting reliable results. However, despite the huge literature on physiological effects of disturbances on lichen thalli, the time span between the disturbance (e.g., pollution) and the alteration on epiphytic lichen communities in terms of species loss, recover, or changes in species composition has not been fully explored. Such effects result from complex interactions between temporal trends of limiting factors (e.g., phytotoxic gases) and natural dynamics of the communities, which include re-colonization processes driven by dispersal, substrate availability, establishment of propagules, and intra- and interspecific competition (Werth et al. 2006).

Data from long observation periods showed clear trends in lichen diversity (Lisowska 2011). The diversity of epiphytic species in London has continued to increase from the 1970s to 2004 as a response to decreasing NO_x atmospheric concentrations (Hawksworth and Rose 1970; Davies 2007). In the urban area of Turin (N-Italy), contrasting trends of the numbers of both lichen species presence and abundance were observed over a period of 200 years as a result of changing pollution scenarios (Isocrono et al. 2007). A dramatic species loss was detected in seminatural broadleaved forests in northwestern Germany from the nineteenth century to date (Hauck et al. 2013). Up to 70 % of the species became rarer during the 100- to 150-year long observation period, and an extinction rate of 28 % was estimated.

Against these robust evidences of long-term variations, information on short-term trends of lichen diversity and composition are surprisingly scarce and urge more detailed researches. Loppi et al. (2004) carried out 5 repeated surveys of lichen from 1993 to 2000 with time spans ranging from 1 to 3 years. They showed that despite their slow growth rate, lichens respond rapidly to decreasing concentrations of air pollutants, allowing annual changes to be detected. Total species richness increased from 1993 to 1999 and then decreased again in 2000, while the mean number of species per station increased from 1993 to 1999 and remained constant in 2000. The β-diversity decreased linearly from 1993 to 1999, indicating that sampling stations became floristically more similar in time.

From an applicative perspective, when the aim is the evaluation of change between subsequent measurements, there are several implications related to the statistical analysis for detecting changes, which should carefully considered (EN 16413 2014). Among them, one should decide whether to make the sampling units temporary or permanent. When sampling units are temporary, the random sampling procedure is carried out independently at each sampling period (Elzinga et al. 2001). The principal advantage of using permanent instead of temporary sampling units is that the statistical tests for detecting change from one time period to the next in permanent sampling units are much more powerful than the tests used on temporary sampling units.

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Lichens and Particulate Matter: Inter-relations and Biomonitoring with Lichens

3

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Abstract

In the last three decades, considerable attention was paid to the inter-relations of lichens and particulate matter. This chapter highlights the determinant factors of the entrapment of airborne particles by the lichen, e.g., the growth form of the thallus. The soil which provides the greater part of the particles adhered-to and/or entrapped-in the thallus should be considered and characterized in biomonitoring studies which traditionally focus on airborne elements derived from anthropogenic activity, with an emphasis on heavy metals and radionuclides. Both visual data and analyses of coefficients of variation (CVs) contribute to knowledge of the nature of particulate matter entrapped by lichens. To date, a considerable number of laboratories studying the elemental content of lichens avoid the pre-treatment of thallial samples. A rough estimation based on a review of the relevant literature revealed that about 75 % of the laboratories used only dry cleaning and do not wash thalli prior to elemental determination. Other laboratories include in their protocol washing, sometimes several consecutive washings, combined with an ultrasonic treatment and/or shaking. This treatment has the potential to remove deposited material of natural and anthropogenic origin and induces the loss of elements. Lichenologists/bryologists need to face the dilemma of pre-treatment. In this context, scientists should determine whether the constituents of lichen are just and only algal and/or cyanobacterial cells and fungal hyphae, and particulates are "aliens." The location of biomonitoring studies is

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of crucial importance: the increasing number of annual desert-dust storms especially in the Mediterranean basin and in southern Europe is the result of climate change. The temporal and spatial dimensions of bulk deposition are discussed, with relevance to change observed in biomonitoring studies.

Keywords

Lichens · Particulate matter · Entrapment · Soil · Dust · Biomonitoring

3.1 Introduction

Traditional definitions of lichens adopted for the greater part a biological ("organic") point of view focusing on the main components of the thallus. Writers on the subject defined lichens as stable, mutualistic, symbiotic, perennial, slow-growing associations of heterotrophic fungi (mycobionts), and photosynthetic green algae or cyanobacteria (photobionts). The fungal partner was described as belonging to a wide range of classes and orders, thus comprising an ecological non-systematic group. The greater part of this group consists of ascomycetes, whereas some are basidiomycetes (Gadd 2007). This is the accepted concept of a bipartite lichen. *Hypogymnia physodes*, for example, is a bipartite chlorolichen comprised of a fungus as mycobiont and a unicellular green alga as photobiont (Paul and Hauck 2006). *Nephroma helveticum* and *Leptogium saturnium* also contain a fungus as mycobiont and a *Nostoc* cyanobacterium as photobiont (Hauck et al. 2006) in a bipartite association.

The symbiotic association of one mycobiont and one or two photobionts forms a common thallus. The mycobiont receives fixed carbon produced by the population of photobiont cells. This thallus does not possess roots or a waxy cuticle. As a result of its high surface:volume ratio, its simple anatomy and the absence of a cuticle, lichens accumulate nanoparticulate matter derived from different natural sources, e.g., mineral dust (with a lithospheric signature), or organic dust from soil surface erosion and occasional wildfires, in addition to anthropogenic sources of pollution. Transport of pollutants is either long or short ranged. The factor of deposition, either dry or wet, is of relevance.

The reason for the adaptability of lichens to biomonitoring studies of air pollution is their extraordinary capability to accumulate mineral elements far above their needs, and their occurrence at a large geographical range. Lichens adjust to any terrestrial environment: an estimated percentage of 6 % of the Earth's land surface is covered by lichen-dominated vegetation (Haas and Purvis 2006; Gadd 2007).

Many publications dealt with the utilization of lichens as monitors of trace elements and accumulators of macro-nutrients in varying geographic areas, representing all continents. Lichens exhibit long-term deposition patterns and do not reflect necessarily the short-term deposition measured by most atmospheric scientists. The advantages of biomonitoring with lichens over instrumental monitoring are that lichens accumulate most of the elements of the periodic table, are usable at low expense, do not depend on electricity for their operation, do not need treatment, and are easy to hide, thus discouraging vandalism. Inexpensive biomonitoring systems also provide a great deal of information on the impact of heavy metals on physiological processes.

The greater share of the publications of the last five decades reports on the results of biomonitoring experiments of trace elements, radionuclides and macroelements, including nutrients. In the last three decades, however, considerable attention was paid to inter-relations between lichens and particulate matter. The present chapter deals specifically with the origin and mode of interception and incorporation of particulate matter with an emphasis on metal-containing particles. Of the large number of lichen species, only a few were used as biomonitors of trace-elemental deposition. However, the study of these few species yielded hundreds of publications.

3.2 Efficient Entrapment of Particulate Matter: Determinant Factors

Morphological and structural features of the lichen thallus determine the entrapment of airborne particles, in addition to the relevant features of surface and degree of attachment. The greater adaptability of foliose and fruticose lichens for biomonitoring over and above crustose lichens derives from ease of sampling. Since the interception of atmospheric particulates is dependent on a high ratio of surface to mass (Tretiach et al. 2007; Adamo et al. 2007), foliose lichens accumulated in certain cases more than twice the amount of Cu than fruticose lichens. St. Clair et al. (2002a) related the morphological features of the lichens to environmental conditions to explain this finding. Foliose thalli are continuous in space (within the same plane) and therefore more efficient at the interception of atmospheric deposition. In contrast, fruticose species with their three-dimensional open structure accommodate a greater flow-through of atmospheric deposition and their capability to entrap airborne elements is smaller.

A nationwide interspecies survey confirmed the higher affinity of the foliose *Flavoparmelia caperata* to Cd and Zn detected in a local study performed in northeast Italy by Nimis et al. (2001). The higher affinity of the foliose *Xanthoria parietina* to Al and Fe, on the other hand, did not coincide with nationwide data and was related to local factors and to the characteristics of the species. Previous studies suggest that Al and Fe are deposited for the greater part in the form of large particles. *Xanthoria* is assumed to endure a higher rate of eutrophication than *Flavoparmelia* and to be bound to communities with a strong accumulation of dust. Thus, the high Al and Fe content of *Xanthoria* is related to a study area with widespread deposits of Terra Rossa in northeast Italy, largely made up of Al- and Fe-hydroxides, and subject to strong, dry winds in winter. Physiological differences are thus assumed to account for the interspecies difference of affinity.

A different capability to entrap particles of lichens with a similar growth form, apparent in a study of two foliose lichens *F. caperata* and *Lobaria pulmonaria*, was attributed to ecological characteristics (Loppi and De Dominicis 1996). According to St. Clair et al. (2002b), elemental accumulative patterns are not the same for fruticose species either: for example, the epilithic *Usnea amblyoclada* and the epiphytic *Usnea hirta* present different accumulation patterns. Consequently, it is confirmed that analyses of elemental accumulation involve the consideration of variables other than growth form: the substrate and the inter-relation of growth form and substrate. This is true also for foliose species such as the epilithic *F. caperata* and the epiphytic *Flavopunctelia flaventior*: the growth form variable was found to be significant for K, Ca, Ti, B, Fe, Ni, Cu, Zn, Rb, and Sr, whereas the substrate variable was found to be significant for K, Ti, Mn, Fe, Ni, Rb, and Sr. The accumulation of S, on the other hand, appeared to be independent of both growth form and substrate (St. Clair et al. 2002b).

Foliose lichens such as *Parmelia sulcata* and *X. parietina*, both epiphytic, contained more ^{137}Cs than filamentose or fruticose epiphytic lichens in the same biotope in Macedonia, northern Greece (Sawidis et al. 1997). The authors suggested that the large area of attachment to the substrate of the foliose species may have helped to accumulate more ^{137}Cs from the tree canopy or from stemflow leachates, whereas filamentous or fruticose forms are attached to the substrate either at one or at a few points. For both filamentous and fruticose species, the accumulative capacity was found to accord with a high ratio of surface to biomass, e.g., *Usnea* sp. (Sawidis et al. 2010). Table 3.1 provides data of elemental content of lichens with different growth forms, while Table 3.2 presents the elemental content of lichens with similar growth forms.

Table 3.1 Elemental content of lichens with different growth forms

	Foliose lichens	Fruticose lichens	Elemental content	Remarks according to cited reference
Lawrey and Hale (1981)	*Flavoparmelia baltimorensis* *Xanthoparmelia conspersa*	*Cladonia subtenuis*	Higher in the foliose lichen	The prostrate growth form of the foliose lichens makes for a large, flat, particle-trapping surface. *C. subtenuis* is lesser structured to trap particles
Gough et al. (1988)	*Hypogymnia enteromorpha*	*Usnea* spp.	Higher in the foliose lichen	
Glenn et al. (1995)	*Parmelia subrudecta*	*Anaptychia cilliaris*	Higher in the foliose lichens	
	Parmelia sulcata			
Calvelo et al. (1997)	*Candelariella vitellina*	*Protousnea magellanica*	Higher in the foliose lichens	Findings are related to the greater capability of foliose lichens to entrap particulate matter
	Hypotrachyna brevirhiza			
	Parmelia cunninghamii	*Usnea fastigiata*		
	Physcia adscendens			
Aslan et al. (2004)	*Xanthoparmelia somloensis*	*Ramalina capitata*		Structural factors relevant to metal uptake are wider thallial surface, a thin or nonexistent upper cortex, a wide cellular space and a high cell permeability in the foliose lichens
	Dermatocarpon miniatum	*Pseudevernia furfuracea*		
Bennet and Wetmore (2003)	*Hypogymnia physodes*	*Evernia mesomorpha*	Higher in the foliose lichen	The *Hypogymnia* accumulated more Ca, Cd, Mg, and Mn of atmospheric origin, whereas the *Evernia* accumulated mostly soil elements
Garty et al.(1996a)	*Hypogymnia physodes*	*Usnea hirta*	Higher content of Fe, Zn, Ca, Mg, Cu, and Ni in the foliose lichen	
Käffer et al. (2012)	*Parmotrema tinctorum*	*Teloschistes exilis*	Higher content of Pb, Cr, Cd, and Zn in the foliose lichen	
Bergamaschi et al. (2007)	*Hypogymia physodes*	*Usnea hirta*	Similar accumulation capacity in the four lichens	
	Parmelia sulcata	*Pseudevernia furfuracea*		
Kahraman et al. (2009)	*Cladonia foliacea*	*Ramalina pollinaria*	Higher content of K, Zn, Rb, and Pb in *R. pollinaria*; higher Mn and Sr content in *C. foliacea*	
Bosserman and Hagner (1981)	*Parmelia* spp.	*Usnea* spp.	Higher content of dustfall elements such as P, Si, Al, Fe, and K in *Parmelia* spp. Higher content of Na, Ca, and Mg dominant in precipitation in *Usnea* spp.	
Singh et al. (2013)	Names of spp. not given	Names of spp. not given	The variation in the metal content was insignificant	
Bajpai et al. (2009)	*Foliose lichens*	*Leprose lichens*	Higher arsenic (As) in the foliose and the leprose lichen species than in squamulose and crustose lichen species	
	Phaeophyscia hispidula	*Lepraria lobificans*		
	Parmotrema praesorediosum	Squamulose lichens		
	Crustose lichens	*Peltula euploca*		
	Caloplaca subsoluta	*Phylliscum indicum*		
	Diploschistes candidissimus			

Table 3.2 Elemental content of lichens with similar growth forms

	Foliose lichens	Foliose lichens	Elemental content
Nimis et al. (2001)	*Flavoparmelia caperata*	*Xanthoria parietina*	Higher content of Fe and Al in *X. parietina*. Higher content of Cd and Zn in *F. caperata*
Bartók (1988)	*Xanthoparmelia conspersa*	*Peltigera canina*	Higher elemental content in *X. conspersa*
Upreti and Pandey (1994)	*Umbilicaria decussata*	*Umbilicaria aprina*	Higher content of Pb and Fe in *U. descussata*
Loppi and De Dominicis 1996	*Flavoparmelia caperata*	*Lobaria pulmonaria*	Higher ^{137}Cs in *L. pulmonaria*
Koz et al. (2010)	*Physcia adscendens*	*Flavoparmelia caperata*	Highest content of Cr, Mn, Ni, Zn, Sn, Ba, and Pb in *P. adscendens*; highest content of Cu and Co in *F. caperata*; highest content of Ti in *Xanthoparmelia conspersa*; highest content of Fe in *Parmotrema perlatum*
	Xanthoparmelia conspersa	*Parmotrema perlatum*	
Aslan et al. (2004)	Crustose lichens	Crustose lichens	*R. melanophtalma* accumulated more of K, Ca, Sr, and Ba than *R. crysoleuca*
	Rhizoplaca melanophtalma	*Rhizoplaca cryrsoleuca*	

3.3 Soil Particles Entrapped by Lichens: Apportionment and Characterization

Particulate matter of soil origin is very often adhered-to and/or entrapped-in the lichen thallus (e.g., Garty et al. 1979; Garty and Delarea 1987; Markert 1993; Dongarà et al. 1995; Dongarà and Varrica 1998; Bergamaschi et al. 2004; Giordano et al. 2013). Correlations of mineral elements such as Al and Fe accumulated in lichens are indicative of small entrapped aerosols (Nakajima et al. 2013) and of soil particulates (Doğrul et al. 2012). Particulate deposition can lead to damage in the lichen thallus. Some particulates are chemically inert, whereas others are highly reactive (Purvis and Pawlik-Skowrónska 2008). In the literature referring to lichens as biomonitors of air pollution, different definitions of soil particles are in use. Table 3.3 presents different definitions of particulate matter. An examination of the elemental composition of lichens should consider their dependence on the soil surface. A principal component analysis (PCA) of data obtained in Greenland of elemental concentrations in soil, humus, moss (*Rhacomitrium lanuginosum*), and lichens *(Flavocetraria nivalis)* indicated the soil–dust factor as representing one of three principal components explaining 87 % of the total variation. Dominant constituents of the soil-dust factor were Fe, Al, V, Ni, Cr, Cu, and Pb (Riget et al. 2000).

Data obtained in a monitoring survey on a national scale in The Netherlands were applied for the purpose of source apportionment (Sloof 1995b). A Monte Carlo-assisted factor analysis yielded 10 factors, interpreted in terms of 10 sources. One of these factors strongly depended on concentrations of Th, Sc, Al, Cr, Fe, and Cs, indicative of crustal material. Other significant constituents of this factor were La, Co, As, Se, and V, common trace elements in uncontaminated Dutch topsoils. Kuik and Wolterbeek (1995) indicated Sc, Sm, Ce, La, Th, Al, Fe, Cr, As, Co, and V as important constituents of crustal material accounting for 25 % of the total variance in the greater part of data obtained in 1992. Similarly, Reis et al. (1996) used the lichen *P. sulcata* to set up a database incorporating data for 43 elements, subjected to a Monte Carlo-aided target transform factor analysis (MCATTFA). Of nine factors obtained in this manner, four were interpreted as representing natural sources. Three were further interpreted by means of their Fe/Si concentration ratio, as limestone, basalt, and shale. Another factor, initially identified as anthropogenic, was found to

Table 3.3 Definitions of mineral elements originating in rock and soil and remarks given in biomonitoring studies using lichens

	Definition	Remarks
Loppi et al. (1999a)	Lithogene elements	Al, Fe
Loppi et al. (1999b)	Lithogene elements	Al, Fe, and Ti. In the Mediterranean environment the content of trace elements of unwashed samples is greatly affected by soil contamination
Nimis et al. (2001)	Terrigenous elements	Al, Fe
Loppi and Pirintsos (2003)	Terrigenous material	Al, Fe
Brunialti and Frati (2007)	Terrigenous material	The relationship of the terrigenous Al and Fe with Ni and Cr confirmed the presence of anthropogenic impact factors suggesting soil particulates trapped in the lichen
Ribeiro Guevara et al. (1995)	Lithophile elements	Al, Sc, Th, Hf, and Fe. Indicators of clastic inputs. Detritical particulate material could be trapped inside lichen tissues
Bargagli et al. (2002)	Lithophile elements	Al, Cr, Fe, Mn, Ni, and Ti
Bargagli (1995)	Lithophile elements	Al, Fe, Ba, Cr, and Mn. Lithophilic elements were higher at the end of the dry season, due to the quantities of soil particles trapped in the samples
Monna et al. (2006)	Lithophilic elements	Th, Sc, La, and Fe
	Chalcophilic elements	Pb, Zn, and Cu. Detected in mine dump and coals
Singh et al. (2013)	Lithophiles	Al, Cr, Cs, and V
	Siderophiles	Co, Fe, Mn, and Ni
	Chalcophiles	As, Cd, Cu, Pb, and Zn
Adamo et al. (2008a)	Lithophilic elements	Al, Fe, and K
Takala et al. (1998)	Geogenic element	Zn
Matschullat et al. (1999)	Geogenic elements	Ba, Mn, Rb, Sr
	“Intermediates”	Cr,Mo, Sn, V
	“Anthropogenically emitted”	Cd, Pb, Zn
Giordano et al. (2013)	Geogenic and anthropognic elements	Al, Ba, Ca, Cr, Cu, Fe, Ni, Pb, Ti, and Zn
		The high and significant correlations between geogenic and anthropogenic elements suggest that the polluted urban soil is a massive source of elements
Bermudez et al. (2009)		Mn is related to agricultural activity and Fe to industry and soil particles
Getty et al. (1999)		Fe is an important constituent of eolian dust
Pignata et al. (2007)		High levels of As were related either to As-rich loessic soil or to long-range transport of industrial contamination

resemble a soil factor. Its high P and K content in addition to the presence of S, Cu, and Zn referred to the resuspension of particles of viticultural origin. Freitas et al. (1999) using *P. sulcata* for a similar study in Portugal applied the elements Cr and Sb as markers of soil particles.

A comparison of the elemental content of the epiphytic foliose lichen *F. caperata* with that of the moss *Hypnum cupressiforme* found greater amounts of lithophile elements (Al, Cr, Fe, Mn, Ni, and Ti) in the moss, whereas the lichen contained a greater share of elements such as Hg, Cd, Pb, Cu, V, and Zn (Bargagli et al. 2002): the elemental composition of the moss was affected to a greater extent than that of the lichen by the geochemical features of the study area, in a former mining district in Italy. Another comparison of the metal bioaccumulation capacity of the epiphytic moss *Scorpiurum circinatum* and the epiphytic fruticose lichen *Pseudevernia furfuracea* exposed in bags in an urban area in S. Italy by Basile et al. (2008) revealed the following: (1) both species accumulated the entire range of the metals Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Ti, V, and Zn and (2) the moss, however, had the highest bioaccumulation capacity for all metals and showed a more constant, linear accumulation trend. These findings agree with a previous study located in Naples which used the lichen *P. furfuracea* (Giordano et al. 2005). The particulate matter contained Al, Si, P, S, Cl, Ca, Fe, and Cu deriving mostly from abrasion processes activated by vehicles: trams, railways, and cars. Additional studies performed by Sorbo et al. (2008) and by Aprile et al. (2010) confirmed that high values of Cu and Zn derive from abrasion as a result of vehicular activity. The varying results of similar studies using both an epiphytic lichen and an epigeic moss were ascribed by Basile et al. (2008) to the morphology and ecophysiology of the species: epigeic species were found to be susceptible to lithophile elements to a greater extent than epiphytic species, which are strictly dependent on atmospheric deposition. As contended previously by Bargagli et al. (2002), mosses and lichens cannot, apparently, be used interchangeably as biomonitors of trace metal accumulation. Moreover, in a comparative analysis of the elemental content of oak leaves (*Quercus pubescens*), epigeic mosses and epiphytic lichens in a former mining district in Italy, Bargagli et al. (2003) found that oak leaves were less affected by the elemental contribution of adsorbed soil particles, thus facilitating the evaluation of atmospheric inputs.

Two groups of elements were identified in lichens in the vicinity of Mt. Etna and on Vulcano island (Sicily, Italy) by Varrica et al. (2000). Of the lithophile elements derived from local crustal material, the average concentrations of Al, Ca, Mg, Fe, Na, K, P, and Ti were the most substantial. Typical crustal elements were found to account for over 95 % in weight of the total elements. On the other hand, enriched elements (Sb, Br, Zn, Au, and Cu) forming volatile compounds were found to originate mostly in anthropogenic sources and/or volcanic gases.

A clear distinction between “lithophilic” and “anthropogenic” elements was indicated by a cluster analysis following a study of trace elements in the lichen *P. furfuracea* and the moss *Sphagnum capillifolium* exposed in bags in the city of Naples (Italy). It was suggested that lithophilic elements were supplied by entrapped particulate matter circulated by erosive winds. The marked upward trend detected in *S. capillifolium* and *P. furfuracea* for elements such as Pb, Zn, Cr, Cu, As, Fe, Mo, and V was related to anthropogenic sources (Adamo et al. 2003). A comparative analysis of elemental levels in lichens *(H. physodes*) and mosses (*H. cupresiforme)* and/or *Pleurozium schreberi* collected in the same location yielded mean values of about 9 % soil-derived or lithophilic elements such as Cr, Fe, La, and Sc in *H. cupressiforme.* On the other hand, anthropogenic elements such as As, Br, Cd, and Zn attained significantly higher values in *H. physodes* (Jeran et al. 2003).

The contamination of lichen samples by soil particles is an apparent issue to be dealt with, the more so in studies focused on the use of cryptogams as biomonitors (Bargagli 1995; Bargagli et al. 1995; Loppi et al. 1999b; Bergamaschi et al. 2002, 2004; Loppi and Pirintsos 2003). To eliminate the contribution of local soil in calculations of the “net” elemental content of lichens used as biomonitors of anthropogenic pollutants, several studies analyzed both the elemental content of the lichen thallus and of the upper layer of soil in the adjacent collection/transplantation sites. Studies in this category should be classified as follows:

1. Descriptive studies of the elemental content of lichens, soil, and additional media performed to indicate the general status of the environment or to establish a set of markers on a local or regional scale: several studies in this category compared the elemental content of both soil and lichens (Garty 1985; Bargagli 1990a;

Garty et al. 1995, 1996b; Dillman 1996; Loppi 1997; Rizzio et al. 2001; Bačkor and Fahselt 2004; Bargagli et al. 2007; Leonardo et al. 2011; Grangeon et al. 2012); these studies showed mostly a high level of coincidence. Chemical analyses of both soil and the epiphytic foliose lichen *X. parietina* sampled in Luxemburg (Hissler et al. 2008) determined fractionated ratios of Sm/Nd as a function of distance from a steelworks. In remote areas, the lichens had Sm/Nd ratios which were comparable to those of corresponding soils. Lichens collected closer to steelwork sites showed decreasing Sm/Nd ratios.

2. Analytic studies determining significant direct correlations of elemental content of soil and lichens (Bargagli et al. 1987, 1993, 1999; Loppi et al. 1999a; Bari et al. 2001; Walker et al. 2006, 2009): in certain cases, significant correlations of metal content, such as Cu in soil and in lichens, are applicable as a tool for biogeochemical prospecting; Chettri et al. (1997) determined that lichens may be used to locate mining areas; other cases, however, of correlations of elemental content in both lichens and soil did not yield significant results (Rencz et al. 2003).

A comparison of the elemental content of soil, epiphytic lichens, tree bark, and leaves (or needles) has the potential to yield interesting results: according to Migaszewski et al. (2005), air and soil are the probable source for trace elements. The variability of S and trace metals in soil and bioindicators was found to be primarily governed by bedrock lithological factors in addition to anthropogenic factors. Another study (Purvis et al. 2008) showed highly significant correlations for Mn/Ca ratios in a lichen (*P. sulcata*), versus oak-tree bark, for soil versus bark of the same tree, and for soil versus epiphytic lichens growing on the same tree. This study confirmed the existence of a cycling process via the soil–tree–lichen system.

Analyses of enrichment factors (EF) are rather helpful in an attempt to distinguish the contribution of air pollutants from that of crustal components associated with windblown soil particles (Nyarko et al. 2006). The calculation of EF was designed to discriminate between anthropogenic and natural sources of trace metals measured in lichens. Enrichment factors were calculated by a comparative analysis of the elemental composition of lichens and crustal rock or soil. Puckett and Finegan (1980) normalized these elemental levels to obtain a reference element considered an unambiguous indicator of the source of the material. The general formula presented by Puckett and Finegan to calculate the enrichment factor for any element (X) in the lichen relative to crustal rock or soil is as follows:

$$\mathrm{EF} = \frac{(X/\text{reference element})\text{ in lichen}}{(X/\text{reference element})\text{ in crustal rock or soil}}$$

Scandium (Sc) was commonly used as a crustal indicator by Puckett and Finegan and in following biomonitoring studies of air pollutants with lichens in different regions in the world (e.g., Olmez et al. 1985; Sloof 1995a; Ribeiro Guevara et al. 1995; Grasso et al. 1999; Bergamaschi et al. 2002, 2004; El-Khoukhi et al. 2004; Nyarko et al. 2006; Freitas et al. 2007). Puckett and Finegan determined that enrichment factors near unity imply non-enriched elements. Low EF values ranging from 1 to 5 obtained for Al, Cr, Fe, Na, Ti, and V negated the enrichment of the lichen.

Several authors applied Al as a crustal indicator (Bargagli 1990b; Wiersma et al. 1992; Calvelo et al. 1997; Varrica et al. 2000; Carignan et al. 2002; Cloquet et al. 2006; Rodushkin et al. 2007; Merešová et al. 2008; Boamponsen et al. 2010). In certain cases, Al is not available as a determinant factor of crustal identity and Mn is selected as a crustal reference (Machado et al. 2004). Manganese is unquestionably lithophilic, with negligible marine sources (Nriagu 1989).

Titanium was also used as a crustal indicator (Bargagli 1998; Bennett and Wetmore 1999; Purvis et al. 2006, 2007). Certain studies used titanium as an alternative to Al (Bettinelli et al. 2002; Minganti et al. 2003). Further applications of the EF factor, in addition to its use in lichen studies, were reported for mosses (Aničić et al. 2009) and trees: the reference element selected for sweet chestnut leaves was Mg, according to the following equation: (Martin et al. 2009)

$$\mathrm{EF} = \frac{(Y/\mathrm{Mg})\ \mathrm{plume}}{(Y/\mathrm{Mg})\mathrm{lava}}$$

Another reference element used for the calculation of elemental enrichment was zirconium (Zr): Dolgopolova et al. (2006) calculated the Pb and Zn enrichment relative to the local geological background using the following equation:

$$\mathrm{EF}_x = (X/\mathrm{Zr})\ \mathrm{sample}/(X/\mathrm{Zr})\,\mathrm{host}$$

As: X is Pb or Zn, (X/Zr) sample is the ratio obtained for the individual sample, and (X/Zr) is the ratio obtained for host rocks representative of the local background.

Rare earth elements (REE) are applicable reference elements for the calculation of elemental enrichment of lichens. According to Chiarenzelli et al. (2001), the advantageous properties of REE relevant to environmental studies are transport in the particulate phase, lack of significant anthropogenic sources, coherent group geochemistry, robust concentrations, and upper crustal signatures. The authors calculated the REE enrichment factor relative to the local geological background following the equation:

$$\mathrm{EF}_{\mathrm{REE}} = (\mathrm{Metal\ sample}/\mathrm{REE\ sample}/\mathrm{Metal\ UCC}/\mathrm{REE\ UCC})$$

As UCC = upper continental crust.

REE analyses may be helpful for the identification of particulate deposition related to anthropogenic activities and enrichment by other elements originating in biogenic processes.

Although the calculation of EF is used widely to identify and quantify the influence of anthropogenic activity with respect to global elemental cycles, several authors (Reimann and De Caritat 2000; Simonetti et al. 2003) demonstrated the intrinsic flaws of the concept in studies of environmental geochemistry. The attempt to normalize elemental concentrations to an average value for the total crust was found to be rather theoretical, due to its neglect of the following: (1) The natural fractionation of elements upon transfer from the crust to the atmosphere, (2) the differential solubility of mineral elements upon alteration in the environment, and (3) the impact of biogeochemical processes.

3.3.1 Dependence of Soil-particle Contamination on Distance from Ground Level

Several factors should be considered when selecting the height above ground for sampling and exposure. If epigeic lichens are used, the material grows directly either on soil or on top of other lichens, mosses, or organic matter located on the forest floor. Lichen samples collected from a substrate like this are contaminated by the greatest amount of soil particles. Epilithic lichens located on substrates near the ground are also subject to soil particles to a great extent. This was the case for the epilithic desert lichen *Ramalina maciformis* growing on flintstones in the Negev Desert, Israel, and found to contain large amounts of mineral elements deriving partly from local soil (Garty 1985; Garty et al. 1995, 1996b, 2002b, c, 2007). Its transfer and relocation was done with its substrate and its new location in polluted sites was on the ground. In comparison, other studies using the epilithic lichen *Usnea amblycolada* growing near ground level detached it from its substrate (rock), put it in bags, and tied it to posts in biomonitoring sites at a height of 3 m, thus neglecting the relevance of the height factor (Carreras and Pignata 2002; Carreras et al. 2005, 2009a, b). Table 3.4 presents papers published in the years 2001–2014, referring to height above ground of collection and transplantation. In many cases, the data for height above ground for the transplants are not given (not only in Table 3.4) leaving the reader with incomplete data with reference to contamination by soil particles.

Table 3.4 Epiphytic lichens collected and transplanted: height above ground

	Collection (m)	Transplantation (m)
Adamo et al. (2003)	0.1–1.2	4–8
Yildiz et al. (2008)	Not given	above 3 m
Garty et al. (2001a)	3–4	2–3
Doğrul Demiray et al. (2012)	At least 1	–
Yenisoy-Karakaş and Tuncel (2004)	1–2	–
Affum et al. (2008)	2	approx. 1.5
Leonardo et al. (2011)	1.5	–
Farinha et al. (2009)	1.5	1.5
Sorbo et al. (2008)	Not given	5.0
Monna et al. (2006)	1–3	–
Boamponsen et al. (2010)	1.5–2	–
Basile et al. (2008)	Not given	4.0
Nyarko et al. (2006)	Approx. 1.5	–
Migaszewski et al. (2001)	0.25–2	–
Rosamilia et al. (2004)	Above 1	–
Sawidis et al. (2010)	1.5–2.5	–
Salo et al. (2012), Paoli et al. (2014)	0.5–2	–
Dzubaj et al. (2008)	1–1.5	–
Minganti et al. (2003)	1–1.7	–
Häffner et al. (2001)	Not given	1.5–2
Bari et al. (2001)	Not given	2
Nimis et al. (2001)	Above 1.2	–
Scerbo et al. (2002)	Approx. 1	–
Kularatne and de Freitas (2013)	Not given	approx. 2
Ayrault et al. (2007)	At least 1.5	1.5
Viera et al. (2004)	Approx. 1.5	–
Paoli and Loppi (2008)	1	2–2.5
Culicov and Yurukova (2006)	Not given	21
Otnyukova (2007)	2–5, 15–22	–

3.3.2 Particulate Matter Entrapped by Lichens: Visual Data and Coefficients of Variation (CV)

Mosses and lichens are preferable as biogenic dust collectors for detailed studies of airborne pollutants (Salo et al. 2012). Microscopic evidence of the entrapment of particulate matter by lichens is nowadays abundant. A large portion of the metal burden of lichens is found to occur as particulate matter trapped in the intercellular space of the medulla and/or on the cortex of the lichen thallus (Garty et al. 1979; Garty and Delarea 1987; Purvis et al. 2000; Clark et al. 2001; Bettinelli et al. 2002; Bačkor and Fahselt 2004; Vingniani et al. 2004; Williamson et al. 2004; Giordano et al. 2005, 2010; Ayrault et al. 2007; Adamo et al. 2008b; Bubach et al. 2012; Chaparro et al. 2013) and Fig. 3.1. The above-mentioned publications yield direct, visual evidence indicating that a certain part of the mineral elements, accumulated in the lichen thallus, displays indeed a particulate nature. The visual documentation of the presence of particulates, continuously provided by optical instrumentation, is complemented by an elaboration of quantitative data obtained by common chemical analyses of elemental content. Garty et al. (1977)

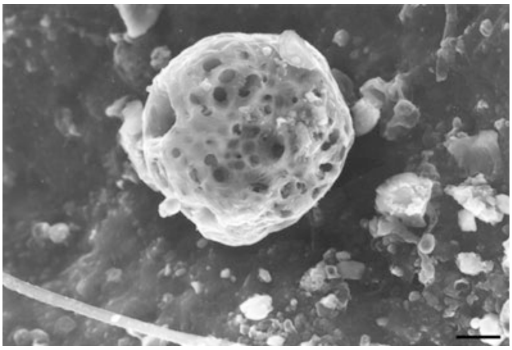

Fig. 3.1 A large particle, derived from heavy fuel combustion, detected on an unwashed thallus of the epiphytic lichen *Ramalina lacera* growing on *Faidherbia albida* trees close to the local power plant and the oil refineries, Ashdod, S.W. Israel. Scale bar = 1 µm (Garty et al., unpublished)

suggested that the occurrence of metal-containing particles in the lichen thallus is indicated by high coefficients of variation (CV) obtained by the ratio of values of standard deviation (SD) and mean metal content: high CVs indicate the probability of unevenly distributed particles in the thallus. Low CVs were assumed to indicate a low variation due to an even suspension of metal particles. CVs were further applied to assess the size of deposited/entrapped particles (Garty 1985, Garty and Ammann 1987; Loppi et al. 1994, 1997a; Garty et al. 1995, 1996b, 1997a, b, 1998a, b; Carreras and Pignata 2002; Purvis et al. 2004).

In a study, biomonitoring airborne heavy metals along a major road in Accra, Ghana, the CV values calculated for lichens (*Parmelia* sp.) were high for both sides of the road, indicating an irregular distribution. The very high CVs for Cr on both the eastern and western side (0.95 and 1.18, respectively) and for Ni on the eastern side (0.94) suggest an irregular pattern of pollution (Affum et al. 2008).

Lippo et al. (1995) applied CVs in a nationwide survey of atmospheric deposition in Finland. High CVs were found to reflect both regional differences in background areas and local emission sources. The CVs calculated for lichens in volcanic areas in Sicily, Italy, were high for several elements such as Au (1.48), Sb (1.30), Zn (1.07), Cu (1.00), and Pb (0.95) (Varrica et al. 2000). These ratios were not, however, related in this study to either particulate size or any other feature of particulate matter. In a survey of elemental deposition in the Aegean region of Turkey, Yenisoy-Karakaş and Tuncel (2004) applied CVs to determine local variation: morphology, size, and substrate of the lichen (*X. parietina*) and local pollution and negate its capability to interfere with regional elemental data.

To test the impact of pre-treatment, Adamo et al. (2008a) applied CVs in a study of the adaptability of the lichen *P. furfuracea*, growing on branches in NE Italy, for biomonitoring studies. The basal portion of washed thalli yielded lower CV ratios than unwashed basal parts for Al, Fe, Hg, K, Mn, Na, Pb, and V. The washing of this fruticose lichen not only made for a statistically significant decrease of Al, Fe, and Mn levels in the basal portion, which is assumed to contain high concentrations of lithophilic elements, relative to the lobes of the thallus, but also reduced the variability, as expressed by lower CVs following the washing treatment. CVs were proved to differentiate peripheral from central parts of foliose lichen thalli (Loppi et al. 1997b): the trace-elemental content of peripheral (younger) and central (older) parts of thalli of *F. caperata* in an unpolluted site in Central Italy was highly variable. Elements of limited metabolic significance such as Al, Cd, and Pb attained higher concentrations in the central part, thus suggesting their being trapped in the medulla; the CVs were 0.71 for Al, 0.54 for Cd, and 0.73 for Pb; the corresponding CVs for peripheral parts of the lichen were 0.48, 0.51, and 0.46, respectively. In a retrospective study performed by Loppi et al. (2004) in Toscana (Central Italy), the reduced use of leaded gasoline was found to produce in 1999 a significant decrease of Pb content of *F. caperata*, relative to 1993. The mean ± SD of Pb concentrations were 35.3 ± 24.9 $\mu g\ g^{-1}$ in 1999 versus 66.7 ± 51.3 $\mu g\ g^{-1}$ in 1993. The CVs obtained for 1999 (0.71) were a little lower than those obtained for 1993 (0.77). These CVs suggest a stability of environmental emissions. A similar stability was observed in an analysis of elemental content of the epiphytic *Bryoria fremontii* and *Letharia vulpina* investigated in Yellowstone National Park, Wyoming, USA, by Bennett and Wetmore (1999). The CVs for most of the elements were found to be equal or less than 0.50, indicating fairly constant values. According to Bergamaschi et al. (2004), the elemental content of different lichen species growing in three mountainous regions (Himalaya, Kenya and Italy) was quite similar, and the greater part of the CVs were around 0.50 or less, whereas some attained CVs of 0.70 or more. The average CVs obtained for 23 elements in the lichens in Kenya, Himalaya, and Italy were 0.65, 0.62, and 0.47, respectively. Since these lichen samples were collected in unpolluted areas, the different values obtained for elemental concentration should be attributed to the different composition of the soil, as the vegetative cover in these regions is sparse and the soil is exposed to wind erosion.

Windblown dust represents an important source of mineral elements in lichens (Bargagli 1995; Loppi et al. 1999b; Garty et al. 2002c, 2007, 2008; Bergamaschi et al. 2004; Jiries et al. 2008). Alterations in the annual number of dust storms are to be expected as a result of climate change, reflected by divergent parameters of which the increased number of dust storms originating in the great deserts of the world, like the Sahara and the Sahel in Africa is a prominent example. Data presented by Avila and Peñuelas (1999) in northern Spain demonstrated the increased frequency of rainfall loaded with mineral dust (red rain) originating in North African deserts in the last decades: for the period 1983–1997, a clear upward trend of the annual median pH of rainwater was observed in Montseny, in addition to a double frequency of weeks with red rain, from 2.3 in 1944–1974 to 5.6 in 1983–1997, thus reflecting an increased transport of dust from Africa (Fig. 3.2) to southern Europe. Similarly, Ganor et al. (2007) and Garty et al. (2008) reported on an enlarged number of days with dust events in Israel, per year, from 5 days in 1958 to 30 days in 2006 (Figs. 3.3, 3.4, 3.5 and 3.6).

The average CVs calculated for the high mountains in Italy (0.47) based on the data obtained by Bergamaschi et al. (2004) were compared with the average CVs calculated by Pacheco et al. (2004) in 29 littoral-north sites and 29 littoral-center sites in mainland Portugal. The average CV of 0.58 determined for 17 elements detected in *Parmelia* spp. was a little higher than the average CV obtained for the elevated sites in Italy. The highest CV was determined for Al (0.87), a typical marker of soil contamination, whereas the lowest was obtained for K (0.30) which is reported consistently to be included in the thallus in a non-particulate form and attains very often low CVs. An additional case of low CVs for K was observed for *Ramalina lacera* in the North-Eastern part of Israel which has a Mediterranean climate. The CVs obtained for K in this lichen growing in an unpolluted site (HaZorea) were 0.10 (Garty et al. 1997a), 0.07 (Garty et al. 1998b), 0.08 (Garty et al. 2001a), and 0.14 (Garty et al. 2002a). Another study (Garty et al. 1998a) yielded a CV of 0.09 for K, whereas the CVs for lithophilic elements such as Al, Si, Ti, and Fe were 0.27, 0.27, 0.25, and 0.21, respectively. Thus, the status of entrapped elements such as Al, Si, Ti, and Fe as markers of soil-derived particulate matter was further established.

Fig. 3.2 A dust storm in the Sahara Desert, Erfoud, an oasis town, Morocco, April 2005

Fig. 3.3 Saharan dust over Hod Hasharon, W. Israel, March 1998

Fig. 3.4 Saharan dust over Petach Tiqwa, W. Israel, April 2010

The CV obtained for a different *Ramalina* species, *R. maciformis*, collected in an unpolluted site in the Negev Desert, was 0.10 for K (Garty et al. 1996b), whereas Fe and Mn attained a CV of 0.17 and 0.24, respectively (Garty et al. 1995). A low CV for K (0.08) was obtained also in the epiphytic fructicose lichen *Protousnea magellanica* in a pristine, unpolluted area in Northern Patagonia, in the Argentine Andean Mountains, whereas the CVs obtained for Al, Mn, Ti, and Fe were 0.33, 0.79, 026, and 0.35, respectively. An additional subject investigated in this study was the distribution of the elements within the thallus. A comparison of the CVs for K in primary branches of the thallus versus secondary branches yielded ratios of 0.07 versus 0.03, respectively, whereas Al attained a CV of 0.20 for primary branches versus 0.27 for secondary branches. Based on additional data obtained in this study, the elemental distribution was found to be mostly homogenous. Na, however, attained a CV of 0.43 for primary branches and 0.73 for secondary branches (Bubach et al. 1998). Low CVs for K and higher CVs for lithophilic elements obtained

Fig. 3.5 Saharan dust over Wadi Ara, C. Israel, April 2011

Fig. 3.6 Saharan dust over Tel Aviv University, Tel Aviv, W. Israel, March 2012

for *R. lacera* in Israel and for *P. magellanica* in Patagonia are attributed to the difference between soluble and insoluble particulates.

In transplant studies, it is apparent that high CVs refer to short exposures. Based on the assumption that lichens capture a great deal of their metal content by particulate impaction and retain most of their metals by physical entrapment, Gailey and Lloyd (1986) contended that short exposures yield low values for metal content (near the detection limit) and high CVs. It was assumed that a lichen needs time to adapt to a new environment and reach an equilibrium at which it accumulates metals efficiently and evenly.

Another study calculated CVs for unwashed lichen samples (*F. caperata*) to provide evidence for the presence of soil particles. Soil contamination, associated with an enlarged Al or Fe content, was indeed indicated by variable concentrations in lichens, tree bark, and leaves. The CVs ranged from 0.43 to 1.81. The CVs obtained for the lichens were 0.50 (Al), 0.43 (Fe), 0.77 (Mn), and

0.60 (Mo). Thus, crustal material was found to interfere with baseline concentrations and with inter-relations of trace elements (Loppi et al. 1997a).

3.4 Pre-treatment of Lichen Thalli Prior to Elemental Determination

Although lichens were used for biomonitoring for five decades, a standardized protocol for the preparation of thalli for elemental determination is still missing. There is, however, some consistency in the literature with regard to the cleaning of lichen samples. It is generally accepted that detritus should be removed. Many laboratories report on a procedure of dry cleaning which leaves a cover of dust, yet separates remains of the substrate, mineral particles, foreign lichens, mosses, insects, and litter.

Markert (1993) defines "cleaning" as performing mechanical steps, such as using a dry/damp cloth, shaking, blowing, bursting, or washing, either in solvents (common for vascular plants and less for lichens and mosses) or in distilled water. This pre-treatment has, however, the potential to remove deposited material of natural and anthropogenic origin (Wadleigh and Blake 1999). This factor is particularly relevant in the case of stable isotope studies, as removed material might be of a different isotopic composition and could bias the interpretation of sources. The washing procedure was found to induce the loss of elements in *X. parietina* (Bargagli et al. 1985) but not in *Cladonia* (Nieboer et al. 1972). Washing was proved to remove nitrogen from *Physcia adsendens,* which according to Gombert et al. (2003), is a part of the nitrogen deposited on the surface of thalli, e.g., as fine particles adsorbed by the lichen surface or as gaseous nitrogen compounds, such as HNO_3 which is also adsorbed but not assimilated.

The washing of exposed lichen material led to a substantial loss of elements such as Al, Cr, Mn, and Ni in washed *P. furfuracea* following a transplantation experiment (Adamo et a. 2007). Water-washing made for a statistically significant decrease of Al, Fe, and Mn in the basal portion of the lichen that contains normally higher concentrations of lithophile elements (Adamo et al. 2008a). Water-washing was found also to lead to a statistically significant decrease of C and N in the lobes of the lichen, explained by the washing away of adsorbed organic or carbonate particles and/or the extraction of water soluble compounds (Adamo et al. 2008a). It was found also that concentrations of F decreased significantly in washed lichen thalli (Palomäki et al. 1992). The washing procedure induced also a loss of elements in *Evernia prunastri* in urban and highly industrialized areas in and around Padova, Italy. In the vicinity of a steel works, however, the loss was lower than in a site located near a crossroads (Caniglia et al. 1994).

Certain elements such as Pb and S which are tightly bound to cell components are little changed by washing (Prussia and Killingbeck 1991; Richardson et al. 1995). Indeed, Pb (together with Cu, Fe, and Zn) was retained in thalli of *P. furfuracea*, washed upon exposure, in considerable quantities (ca. 60–80 %) in all lichen material (Adamo et al. 2007). Unwashed samples of *Canoparmelia texana* presented elemental concentrations at the same magnitude or slightly higher than the washed samples (Saiki et al. 1997). However, the results were statistically insignificant, with the exception of Al, K, Na, Se, and Ti. These results indicate that the washing procedure can be used to eliminate adhering material.

In the absence of any form of washing, great amounts of airborne dust and soil particles will be left on the thallial surface. As much as the collection site is exposed to dust storms and/or emissions of industrial or vehicular dust, the determination of elemental content is influenced by external contamination. If a period of dry windy weather precedes collection, significant surface dust should be expected, whereas rain preceding collection removes most of the dust. According to Richardson et al. (1995), differences of weather conditions preceding collection provide an unwanted source of variability of elemental levels in mosses, lichens, and hairy leaf samples.

A survey of literature including hundreds of publications dealing with lichens as biomonitors of air quality revealed a very wide range of pretreatments prior to determination of elemental content, ranging from no cleaning to wet cleaning. The use of dry cleaning is established in laboratories applying lichens washed in the field by rain and snow which is fitting in Nordic countries. In quite dry and relatively unpolluted terrestrial ecosystems of the Mediterranean, the elemental composition of unwashed epigeic lichens was affected to a greater extent by soil and rock dust than that of unwashed epiphytic lichen samples (Bargagli et al. 2002). Lithophile elements dominate in mosses, whereas atmophile elements dominate in epiphytic lichens in central Italy (Bargagli 1995; Loppi and Bonini 2000; Bargagli et al. 2002). In Mediterranean countries, levels of airborne soil dust rise in summer and precautions should be taken in order to gather comparable data at different times (Bargagli 1998; Bettinelli et al. 2002).

In the last two decades (1994–2014), the greater part of studies in this field applied a pretreatment of dry cleaning. The reasons cited for the omission of a washing procedure were to avoid the leaching of soluble substrates (Carreras and Pignata 2002), to avoid losing particles trapped on the lichen surface (Bergamaschi et al. 2007), and to avoid unpredictable alterations of the chemical composition of the thalli (Bettinelli et al. 1996; Paoli et al. 2014). Jia et al. (2006) further explained that the lichen samples were not washed in order to measure the elements that were physically trapped on the surface of the thallus, as well as those chemically bound to cell walls.

The objective of washing is to produce initial, homogeneous results (Tretiach et al. 2007; Adamo et al. 2008a; Ares et al. 2012). Ares et al. (2012) evaluating the degree of standardization for mosses, suggested that the outcome of the washing procedure is determined by the following parameters: (1) number of washes; (2) duration of washing; (3) use (or not) of shaking; (4) type of water; and (5) relation between the weight of the moss and the volume of water. Data referring to these parameters were presented in one of the papers reviewed, whereas in 42 % of the papers, none of these were specified.

In lichens, it seems that the number of protocols is close to the number of laboratories that use lichens as biomonitors. The variability is such that it is difficult to compare results of elemental determination even if they are collected in the same location. Adamo et al. (2003) submitted lichen and moss samples to seven consecutive washings with distilled water prior to exposure in bags and performed an additional washing after exposure. The washing treatment prior to transplantations of cryptogams is not very common in biomonitoring studies (e.g., Gonzáles et al. 1996, 1998, 2003; Godinho et al. 2009a; Spagnuolo et al. 2011; Giordano et al. 2013). In certain cases, lichen and moss materials, collected in the field, were rehydrated overnight in a humid chamber and washed four times. Following this procedure, bags were prepared, exposed in transplantation sites, and collected for vitality assessment and for a determination of total content of C, N, and S (Tretiach et al. 2007). Adamo et al. (2007) applied washing for the determination of trace-elemental content of lichens and mosses in addition to a washing in distilled water post exposure, in order to evaluate the contribution of surface particulate matter. Water-washing of exposed material leached similar amounts of most elements from mosses and lichens, thus indicating a similar mechanism. Water-washings of *P. furfuracea* prior and post transplantation were applied also in Ankara, Turkey, by Yildiz et al. (2008). Upon collection, the transplants were washed in tap water and then in distilled water. This study, however, did not compare the impact of washing prior and post transplantation.

Table 3.5 presents selected studies describing the wet cleaning of lichen samples prior to elemental determination. It is apparent that almost every laboratory treats the lichens arriving from the field in a different manner. Standardization of procedures (Seaward 1995) is called for, in order to attain comparable results. Wolterbeek and Bode (1995) contended that problems of comparability are introduced as soon as more than a single sampling-method and/or sample pre-treatment/

Table 3.5 Washing procedures applied in lichen biomonitoring studies (2001–2012)

	Washing procedure
Leonardo et al. (2011)	Cleaning in distilled water to remove dust, bark, and insects
Backor et al. (2009)	Rinsing in deionized water
Dowdal et al. (2005)	Rinsing in distilled water to remove detritus and remaining soil
Fenn et al. (2007)	Sonicating five times in one liter of distilled water, 1 min, then air drying to remove mineral debris trapped externally between overlapping lobes
Farinha et al. (2009)	Washing, 30 s, in deionized water
Freitas et al. (2007)	Two washing cycles in deionized water with agitation
Nyarko et al. (2006)	Washing approx. one min. in deionized water
Sorbo et al. (2008)	Five consecutive washings in distilled water
Garty et al. (2001a, b, 2002a, b)	Three consecutive rinsings, 5 s each
Godinho et al. 2004, 2008, 2009a, b)	As above
Paoli and Loppi (2008)	As above
Pignata et al. (2007)	Washing in double distilled water and leaving to dry at room temperature in a clean area
Golubev et al. (2005)	After collection, washing in bi-distilled water
Affum et al. (2008)	Washing in water, 30 s
Giordano et al. (2005)	Removing soil particles and water washing
Monna et al. (2006)	Rinsing in Milli-Q water in an ultrasonic bath, 1 min
Doğrul Demiray et al. (2012)	Stirring clean samples in deionized water, 1 min to remove dust
Boamponsen et al. (2010)	Washing approx. 60 s in deionized water to remove dust and sand
Yenisoy-Karakaş and Tuncel (2004)	Floating and stirring lichens and substrate in a glass beaker approx. 30 s to remove soil particles and debris
Basile et al. (2008)	Seven consecutive washings in distilled water (50 mL), 5 min, to remove soil particles

analysis is applied. The authors suggested a single laboratory should take care of all pre-treatments. It seems that a unification of pre-treatment is problematic to a greater extent than the standardization of a qualitative determination of accumulated mineral elements.

The International Atomic Energy Agency (IAEA) supports the biomonitoring of air pollution since 1997 (Smodiš 2003; Smodiš and Bleise 2002). Members of the working group, representing 14 countries and different biomonitors, suggested a procedure for sample preparation that includes the cleaning and separation of samples from the substrate. Smodiš and Bleise (2002) suggested the following steps: (1) dry cleaning (2) rinsing in distilled water for 5–10 s (3) drying at 40 °C for 48 h (or freeze-drying), and (4) grinding using liquid nitrogen. We recommended that the rinsing (washing) procedure will be done by handshaking the samples with double distilled water at a temperature of 20 °C for 15 s. The rinsing procedure has to be repeated three times of 5 s each in order to minimize the loss of water soluble elements. We suggest to apply additional washes in the same manner if the water used for the third rinsing is still unclear. The above-mentioned procedure used in Israel yielded low coefficients of variation (CV) for Ca (an important constituent of desert dust and soils) calculated for the lichen *Ramalina lacera*: 0.10–0.26 (Garty et al. 1997a, b, 1998a, 2000, 2001a, b, 2003, 2007, 2008).

3.5 Elemental Content of Lichens and Bulk Deposition: Temporal and Spatial Dimensions

The validity of lichens as environmental biomonitors depends on a positive correlation of the elemental content of the thallus and the average concentration of atmospheric deposits. Jenkins and Davies (1966) detected a linear positive correlation for elemental content of lichens and average concentration of ashed atmospheric deposits, thus paving the way for subsequent studies. Today, the notion that the chemical composition of lichens reflects the availability of trace elements in the environment is established (Bačkor and Loppi 2009; Paoli et al. 2012). In the present section, we deal with lichens applied as biological receptors in addition to man-made receptors, collectors, or filters designed or applied to accommodate bulk deposition. Bulk deposition is defined in the relevant literature either as "airborne particulates" (Bari et al. 2001), "air particulate matter" (Costa et al. 2002), "airborne particulate matter" (Carreras et al. 2009a), aerosol samples (Farinha et al. 2009), dust samples (Rossbach et al. 1999), particulate matter (Farinha et al. 2009), and dry dust deposition (Olszowski et al. 2012).

The ratio of concentrations of certain elements such as Cr, Zn, and Fe in dust collected on air conditioners of large hotel buildings and in samples of *Usnea* spp. was found to be comparatively high relative to the ratio of concentrations of Ca, Rb, and Sr in the same media (Rossbach et al. 1999): data of this kind imply the necessity to complete the available information relating to the span of concentrations and address modifying factors. A comparison of the uranium content of *H. physodes* and air sampled in filters, on the other hand, yielded good results for the lichen-indication method (Golubev et al. 2005), enabling the diagnosis of a long-term atmospheric uranium contamination and an identification of its source.

Another study demonstrated the applicability of continuously operating particulate samplers set up near transplanted lichens from an uncontaminated area. The use of this instrumental apparatus in combination with the fruticose epiphytic lichen *P. furfuracea* exposed in bags yielded significant correlations for atmospheric concentrations and for the greater part of the metals (Cd, Pb, Zn, Fe, Cu, and Ni) accumulated in the thalli. This study thus corroborated the assertion that correlations of this kind are indicative of the efficacy of the lichen and its ability to represent atmospheric contamination (Bari et al. 2001). However, several groups of lichenologists operating in different study areas encountered the issue of modified availability of airborne particulate matter. For example, the observed ^{206}Pb/^{207}Pb range for urban and industrial aerosols in Sicily, closely matched the anthropogenic local signature, whereas the range obtained for the lichens was closer to the compositional field of ^{206}Pb rich geogenic sources (Monna et al. 1999). Following analyses of elemental content of lichens (*P. sulcata*), filters, and collectors, it was possible to state that lichens do not function as a measuring instrument for many elements available in the atmospheric environment. The information presented by lichens with reference to availability is definitely biased by biological effects (Reis et al. 2002). This modified availability termed equivalent constant availability (ECA) represents a cluster of properties similar to the equivalent aerodynamic diameter for airborne particles' size. It is apparent that attempts to establish correlations for lichen content and bulk deposition involve the consideration of two dimensions: (a) the temporal dimension and (b) the spatial dimension.

(a) The temporal dimension

The following study, referring to the temporal dimension, presented a compilation of data obtained in a rural area in Brasil (Dias da Cunha et al. 2004). Analyses of elemental content of the lichen *Ramalina gracilis* in addition to dust collected in diverse man-made receptors yielded information referring to human health issues in a span of 15 years. The analysis of lichen content indicated that in this period, prior to the investigation, the health of the inhabitants of a certain village in the region was endangered by monazite particles. Another study applying lichens to testify of the atmospheric chemistry on a temporal axis was that performed by Cloquet et al. (2006) in Metz, France. Lichens sampled in the city close to traffic roads displayed $^{206}Pb/^{207}Pb$ ratios of ca. 1.13, whereas urban aerosols displayed homogenous $^{206}Pb/^{207}Pb$ ratios of 1.153 ± 0.003. The ratios obtained for lichens thus suggested that the Pb was reflective of “old Pb pollution.”

The capability of lichens to tell of episodic contamination periods in the past is further illustrated by the model of Reis et al. (1999): the performance of the lichen is viewed as a dynamic process of uptake and release which operate simultaneously and continuously. Reis et al. (2002) introduced the concept of “remembrance time” which describes the time span in which lichens reflect the environmental availability of heavy metals at a certain point in time, based on the rate of uptake and release. It was shown (Wolterbeek et al. 2003) that lichens need time to “follow” a change of availability: the rate of uptake and release is conclusive for the time span needed to “forget” previous conditions. Reis et al. (1999) suggested a mathematical approach to calculate the “memory-loss” function. The model suggests that every alteration of rate-constant will lead to a change of both lichen equilibrium levels and remembrance times.

Transplanted lichens may bear witness to extended periods of elemental deposition, relative to the limited period of transplantation, apparently preserving background information. On the other hand, lichens may reflect shorter periods than the total transplantation time or sub-periods of exposure (Godinho et al. 2008). The foliose lichen *F. caperata* is rather much in use in biomonitoring studies (Loppi et al. 2004; Aslan et al. 2006, Pacheco et al. 2007, 2008; Baptista et al. 2008; Godinho et al. 2009a, b). Godinho et al. (2008) used it applying the cross-transplant technique to determine “lichen remembrance” along different exposure periods. The lichens were sampled every two months. The ultimate period of exposure was 12 months. The atmospheric bulk deposition was measured concurrently. The data thus obtained, differentiated between short periods of exposure and cumulative exposure-periods suggesting the presence of acclimatization patterns. The study concluded that the content of the lichen transplants does not unequivocally represent the average or cumulative environmental availability along the exposure period. The above-mentioned study included in addition to chemical analyses of elemental content of both lichens and bulk deposition, physiological parameters of viability such as membrane integrity (see reviews Garty 2000, 2001, 2002; Conti and Cecchetti 2001; Wolterbeek et al. 2003; Bačkor and Loppi 2009). Other applications of physiological parameters of vitality were described in additional studies. Godinho et al. (2008) concluded that the elemental content of lichens is dependent on both elemental availability and the physiological condition of the lichen. Recent studies comparing the accumulation of trace elements in the peripheral and central parts of epiphytic foliose lichens transplanted in a polluted site in Portugal (Godinho et al. 2009a, b) suggested that the distribution data of the elements demonstrate the biological regulation of internal concentrations. For thallus parts, this internal translocation should be taken into account as an additional factor affecting “memory length.”

How long is the time span needed to “forget” contaminating events under semiarid conditions? A retrospective study performed in Israel (Garty et al. 2009) compared the Pb content of *Ramalina lacera* in an unpolluted site in the NE of the country along the years 1974–2002. Relative high Pb values were detected in the lichen in this site in July 1982, subsequent to an extraordinary large range of vehicular activity, mostly using leaded gasoline, in June of the same year. The Pb values remained relatively high until 1993. Only in the years 1994–2002, the Pb values decreased to a level resembling the first series of data obtained in the years 1974–1981. It is apparent that a decade and more were needed to “forget” the polluting event and that the data obtained in this period (1982–1993) were irrelevant to the availability of Pb pollutants.

The lichen *X. parietina* was taken from a herbarium to compare early twentieth century and present-day atmospheric pollution in SW France. The atmospheric environment characterized by a high dust-content was impacted in the beginning of the twentieth century mainly by coal combustion, indicated by findings of As, Pb, and Cd of both local and long-range origins. Recently, however, a different mixture of pollutants, such as Sb, Sn, Pb and Cu originating in local factories and in car traffic has emerged (Agnan et al. 2013).

(b) The spatial dimension

The pattern of increase near the source of metal/ash content and of decrease away from it refers to the particulate nature of the metals accumulated in the lichen. A significant decrease correlating with distance from point emission sources refers to coarse particles that carry to a limited extent. In many cases, the decrease occurs at a distance of less than 100 m from the source of emission (Nieboer et al. 1982). In other cases, coarse particles were found to carry to a greater distance (Garty, 2001; Sensen and Richardson 2002; Branquinho et al. 2008). In certain cases, the bulk deposition of trace metals such as Cu or Ni increases exponentially toward a large source of pollution, i.e., a smelter. Biomonitors, however, accumulate these metals at a different rate. Salemaa et al. (2004) detected the highest trace metal concentrations in the vicinity of a large Cu–Ni smelter in Finland in bryophytes, whereas lichens and vascular plants accumulated lower quantities.

The relevance of temporal and spatial factors is accentuated by environmental factors such as the interference of humidity with atmospheric bulk deposition. Walker et al. (2003) investigated the chemical composition of snow and terricolous lichens in the USA river basin in the northeastern part of European Russia. Both mediae provided evidence for pollution gradients on a spatial scale, produced by local deposition and by the long-range transport of pollutants. However, data obtained in analyses of wet media such as rainwater and snow do not always coincide with data of elemental content of lichen thalli in the same study area. A comparison of plant and precipitation chemistry in catchments with different levels of pollution on the Kola Peninsula, Russia (Reimann et al. 1999) detected the highest levels of Cr, Fe, Mg, S, in lichens, mosses and vascular plants near a nickel roaster. This study detected also the highest concentrations within the impact zone for Ba, Bi, Cd, and Na in mosses and lichens. The importance of studies of precipitation chemistry derives from their capability to point to the well-represented elements in each plant group. It appears that mosses react most strongly to precipitation, whereas lichens are probably the most efficient "integrators" and the vascular plant *Emperetum nigrum* reflects best the particulate input. Of the total atmospheric input by precipitation, nickel appeared to be the element reflected directly by the three plant groups. The relevance of these findings appears to be restricted to the above-mentioned study area, as it involves three representatives of the local biota. It is to be expected that the use of other media will yield different results. Aubert et al. (2006), for example, found a similar distribution of rare earth elements (REE) in snow, lichens, and peat, in the Black Forest, Germany, suggesting that the complete range of atmospheric signals is present in the peat profile. A comparative analysis of total deposition samples (wet plus dry) and metal content of lichens may yield interesting results: Carreras et al. (2009b) found that the lichen *Ramalina celastri* attained the highest correlation rates (in comparison with other epiphytes) with samples of total deposition in Córdoba City, Argentina. It appears that measurements of metal content of bulk deposition and/or biological receptors are also valuable for assessments of human risk in cases of exposure. The significant correlations observed in this case for the metal content of both *Ramalina celastri* and the flowering epiphyte *Tillandsia capillaris* and respiratory diseases in children accentuated the applicability of biomonitoring systems, in spite of their characteristic shortcomings.

Søndergaard et al. (2010) performed a study of Pb isotopes as tracers of mining-related Pb in lichens, seaweed, and mussels along a temporal-spatial axis. The lichen used, *F. nivalis*, growing on dead organic matter near an abandoned mine in West Greenland was contaminated by atmospheric deposition, spreading Pb as dust. Following the determination of stable Pb isotope ratios and total Pb concentrations in lichens, the natural background Pb ($^{207}Pb/^{206}Pb$: 0.704–0.767) and the Pb originating from the mine ore ($^{207}Pb/^{206}Pb$: 0.955) were found to possess a distinct isotopic fingerprint, applicable as an identification tool. The Pb in *F. nivalis* was found to amount to 633 mg kg^{-1} dry weight and was shown to be comprised of natural background Pb and of mining-related ore-Pb. A significant linear correlation between metal concentrations in transplanted thalli of *F. nivalis* and atmospheric metal deposition in Greenland was found by Søndergaard et al. (2013), thus obtaining data to estimate the dust deposition/dispersion period in the snow-free period of the year. The quantification of mining-related Pb provides the temporal dimension, valuable in the absence of baseline information. The applicability of Pb isotope-data is made evident by their capability to determine the spatial variation in Pb mine-related contamination in accordance with distance from the mine. For additional information on Pb isotopes as tracers of anthropogenic activities, see the review by Komárek et al. (2008). Fine particulates containing potentially harmful metals such as Pb detected in street sediments are possibly re-suspended in the air, as suggested by their presence in lichens. Previously, particulates containing Pb in street sediments were attributed to vehicular exhaust and break wear. However, yellow road paint containing $PbCrO_4$ as a pigment was mostly ignored (LeGalley et al. 2013).

Lichens offer an additional advantage for the detection of both spatial and temporal change produced by airborne Cr, Cu, Pb, and Zn. The lichen *Parmotrema reticulatum* accumulated these metals even when they were present in the air in very low concentrations which is the case in different study areas in New Zealand (Kularatne and de Freitas 2013).

3.6 Radionuclides in Lichens and in Soil: Sources of Pollution

Studies performed in the last decades yielded several publications focused on both radioactivity in lichen biomonitors and soil contaminated by radionuclides. In cases of radioactive fallout as a result of nuclear tests or nuclear accidents such as Chernobyl, emissions of several short-living radionuclides are assumed to make for a readily available distinction between the overall natural nanoparticular atmospheric load and pollution by anthropogenic sources in addition to enrichment factors. The sources of radionuclides as reported for both lichens and soil may be categorized as follows.

3.6.1 Global Background Activity

The testing of nuclear weapons led to the introduction of large amounts of radionuclides into the environment. Samples of lichens, mosses, soil, and air collected since 1961 in Greenland, Svalbard, Iceland, the Faroe Islands, Sweden, and Denmark testified to the presence of ^{207}Bi, a nuclide not reported previously as part of the worldwide fallout (Aarkrog et al. 1984). It was assumed to be produced by thermonuclear test explosions and in particular by the 55 Mt detonation of October 30, 1961, at Novaya Zemlya. With regard to the same part of the world, Nifontova (1995) presented data referring to the ^{90}Sr and ^{137}Cs content of moss–lichen vegetation and other components of the soil–vegetation cover of the Yamal Peninsula, Russia. The last fifteen years yielded decreased concentrations of both irradiants in mosses and lichens, as well as a lowered degree of radionuclide pollution of the superficial soil layer. The present data were found to be applicable as a base for further assessments of the presence of radionuclides in the Far North.

Following the intensive testing of nuclear weapons which started in the early years of 1950, efforts were made to detect the deposition of radionuclides in other parts of the world, i.e., in the Antarctic Peninsula area. Analyses focusing on ^{210}Pb, ^{137}Cs, $^{239+240}Pu$, ^{238}Pu, and ^{241}Am in lichens, mosses, grass, and soil samples in the South Shetland Islands in the Antarctic revealed a significant difference of $^{137}Cs/^{239+240}Pu$ ratios between lichens and moss/grass/soil (Roos et al. 1994), related to the effect of melt water and submerging. In other parts of the Antarctic region, King George Island and other South Shetland Islands, mosses accumulated ^{137}Cs to a greater extent than lichens, thus enlarging their relative applicability as bio-indicators (Godoy et al. 1998).

3.6.2 Coal-fired Power Plants

Several studies dealt with coal-fired power plants as sources of pollution (Daillant et al. 1996; Uğur et al. 2003, 2004). The amount of natural radionuclides discharged into the atmosphere by power plants depends on factors such as concentration in coal, ash content, temperature of combustion, partitioning of bottom and fly ash, and efficiency of the emission-control device. Uğur et al. (2003) observed a maximum of ^{210}Po and ^{210}Pb activity in lichens in the vicinity of ash stacks of power stations. The concentrations of ^{210}Pb in the soil decreased in accordance with increasing depth, as a result of the excessive deposition of atmospheric ^{210}Pb on the surface soil. The ratio $^{210}Pb/^{226}Ra$ referring to activity in soil indicated that the main source of ^{210}Pb, in addition to the radioactive decay of ^{226}Ra, is the atmospheric deposition of ^{210}Pb produced by coal combustion. Uğur et al. (2004) observed a correlation of ^{210}Po activity and Pb and Fe content in lichens around coal-fired power plants, attributed to the emissions produced in the process of combustion of fossil fuels. Sheppard et al. (2008) determined the activity ratio $^{210}Po/^{210}Pb$ for Canadian background sites indicating unity (1.0) in soils and lichens, whereas annual plant tissue diverged to an extent of 0.6. Lichens and mosses attained as expected, exceptionally high plant/soil concentration ratios (CRs) for ^{210}Pb, ^{210}Po, and ^{226}Ra, as cryptogams retain atmospheric particles rather effectively. Coal used for the generation of electric power apparently affects the distribution of different radioactive elements. As the combustion of coal is supposed to increase in the next century, it is rather important to observe the enlarged amounts of natural radionuclides, e.g., ^{210}Pb around coal-fired power plants near populated areas (Sert et al. 2011).

3.6.3 Redistribution of Radioactive Fallout by Fire

Dry summers with numerous lightening storms inevitably turn forested areas into zones of high fire-frequency. Shcherbov et al. (2008) investigated the radioactive fallout of the Chernobyl accident, transferred by smoke plumes, in a study of the ecogeochemical role of forest fires in the Baikal region. Paliouris et al. (1995) investigated the ecological implication of ^{137}Cs cycling in ecosystems in the Canadian boreal forest. The effect of fire was found to be decisive for the redistribution of fallout ^{137}Cs. Shcherbov et al. (2008) contended that forest fires accompanied by the airborne migration of several heavy metals (Cd, Hg, Pb) and artificial nuclides produce pollution in adjacent areas: up to 40 % of ^{137}Cs and ^{90}Sr and to a lesser extent $^{239,240}Pu$, originally contained in the soil-vegetation cover in the burned area, were deposited in forest litter, mosses, lichens, and tree needles in its vicinity. Paliouris et al. (1995) observed relatively high concentrations of ^{137}Cs in the organic surface soil of burned stands. In the unburnt stands on the other hand, the highest ^{137}Cs concentrations were identified in epiphytic lichens and in mosses. The fire was assumed to mobilize part of the ^{137}Cs bound to the above-ground matter and concentrate it in the ash layer of burned surface soil. Forest fires presumably make for the loss of part of the ^{137}Cs, through volatilization and fly ash or surface runoff, thus contaminating other ecosystems, initially unaffected, as well as previously contaminated ecosystems.

3.6.4 Mining and Metal Processing

A study performed in Brasil indicated significantly high mean values for ^{210}Pb concentrations in lichens and bryophytes in the vicinity of uranium mining and milling plants relative to the control region (Santos et al. 1993): soil and vegetables from kitchen gardens located near the mills contained much higher concentrations of ^{210}Pb than those observed for other regions. A similar study performed in Brazil (Dias da Cunha et al. 2004) detected ^{210}Pb and ^{226}Ra in aerosols and in samples of the lichen *Ramalina gracilis* in the exclusion area of a mineral sands processing plant and in the adjacent village.

To evaluate the applicability of lichens as bioindicators of air pollution by analyses of radionuclides and REE in the vicinity of a tin and lead industry in Brazil, Leonardo et al. (2011) used the large foliose epiphytic (or epilithic) lichen *Canoparmelia texana*. The authors examined the composition of the piles disposed in open air to obtain the fingerprint of the pollutant and the composition of the raw material used in the industry. As the lichen is assumed to derive nutrients mainly from wet- and dry precipitates, it was postulated that the re-suspension of soil-dust particles and the emissions from the stacks will accumulate in the lichen. Lichen, soil, raw material, and residue samples were analyzed for uranium, thorium, ^{226}Ra, ^{228}Ra, ^{210}Pb, and REEs. Lichen samples were found to concentrate radionuclides in amounts that were 15-fold higher than the background on average, whereas REEs attained amounts that were 10-fold higher on the average.

An example of a radioecological investigation under different climatic conditions is the study performed in the vicinity of prospected uranium mining sites in a subarctic environment (Northern Sweden). Radionuclides in the uranium series were detected in both soil, epigeic lichens (*Cladonia* sp.) and epiphytic lichens (*Alectoria* sp.). Aerial dispersion led to ten times higher concentrations of uranium, thorium, and radium in lichens in the vicinity of the mineralizations. At different distances from the mineralizations, the relevance of ground surface contamination was apparent. Plant-to-soil-activity ratios for *Alectoria*, *Cladonia,* and the mosses *Polytrichum* and *Sphagnum* varied to a great extent. The quotients of plant-to-soil ratios for ^{226}Ra and plant-to-soil ratios for ^{238}U averaged 1.7, 11.4, and 2.6 for *Alectoria*, *Cladonia,* and mosses, respectively. The higher quotient for *Cladonia* was produced by a higher transfer of radium (relative to uranium) from the soil. In the case of the mosses and the epiphytic *Alectoria*, the transfer of soil particles was insignificant (Pettersson et al. 1988).

Nineteen years after the closure of a lead–zinc mine in West Greenland, elevated concentrations of Pb in transplants of *F. nivalis* after one year of exposure indicated that the dispersal of contaminated dust still occurred within a distance of 20 km from the mine: dust is considered to be redeposited continuously. Additional dust is generated by the weathering of the waste rock left on the steep mountain sides (Søndergaard et al. 2011).

3.6.5 Underground Nuclear Explosion Sites

Plutonium (Pu) is a toxic radioactive element dispersed widely in the environment since 1945, mostly through testing of nuclear weapons and nuclear reactor accidents (Bu et al. 2013). Nuclear underground explosions were conducted in Sakha (Yakutia), North East Siberia, in the years 1974–1987. Consequently, radioactive products were discharged into the environment leading to residual radioactive contamination. Geodonov et al. (2002) investigated nuclear explosion sites and determined the concentrations and activity ratios for ^{137}Cs, $^{239+240}$Pu, and ^{238}Pu in lichens and soil. In a contaminated area, the $^{239+240}$Pu in soil in two sites exceeded the limit of 0.37 Bq/g determined for material, thus defined as radioactive waste. Clean-up efforts of these sites were found to be inadequate.

3.6.6 The Chernobyl Accident

The fallout of the Chernobyl accident on April 26, 1986, was subject to many studies investigating the distribution of radionuclides. Studies focused on the presence of radionuclides in both lichens and soil in the wake of the Chernobyl accident reported mostly on the detection of ^{137}Cs. The presence of radionuclides such as

^{137}Cs and others (for plutonium see review of Mietelski 2001) in soil and in epiphytic lichens was reported by van den Berg et al. (1992); Barci-Funel et al. (1995); Heinrich et al. (1999); Yoshida et al. (2004); Celik et al. (2009). Additional studies focused on radionuclides such as ^{137}Cs in soil and in both epigeic and epiphytic lichens (Steinnes and Njåstad 1993; Strandberg 1994). The sequence of accumulation of radionuclides in epiphytic lichens and its relevance to the radioactivity of soil, reported in the literature, was studied by Adamo et al. (2004). Following the Chernobyl accident, lichens sampled in Italy in October 1986 were found to contain lower concentrations of radionuclides than soil. Of the total ^{137}Cs measured in the lichens, only 14 % was attributed to the fallout of the 1950s and 1960s nuclear tests. A report presented by Mitchell et al. (1998) of different radionuclides such as curium (Cm) detected in soil and in lichens excluded, however, the relevant data of lichen identity and substrate. Several studies dealt with radionuclides in epigeic lichens providing forage, and in soil, in Nordic countries (Varskog et al. 1994; Pálsson et al. 1994; Outola et al. 2003; Skuterud et al. 2005; Dowdall et al. 2005; Korobova et al. 2007; Lehto et al. 2008).

The behavior of radioactive Cs in soil–plant systems was extensively studied in the literature, motivated by ^{137}Cs being one of the major long-lived constituents of the fallout from the atmospheric nuclear weapons testing in the 1950s and 1960s (Varskog et al.1994). Although correlating results referring to lichens and soil appear to corroborate the assertion that the contribution of soil particles is decisive, several studies pointed to the atmospheric origin of metal particles common to both media. In a study conducted in Norway in the years 1987–1989, Varskog et al. (1994) analyzed soil and lichen (*F. nivalis*) samples with respect to the total amount of radiocesium (^{137}Cs and ^{134}Cs), Rb, stable Cs and exchangeable ^{137}Cs (in soil only), K, Ca, and Mg. The radiocesium activity in lichens decreased by at least 40 % in the sampling period. This decrease was explained by the growth of new low-activity material at the top of the lichen in 1988 and 1989, thus diluting the radioactivity in the living part of the lichen. The fast decrease of activity in the lichen indicates that some of the radiocesium, accumulated on the outer surface, was washed out by rain. The results of the average vertical distribution of ^{137}Cs in the soil profile sampled in the above-mentioned study did not show a downward movement of ^{137}Cs. These findings are in accordance with the slow movement of ^{137}Cs in soil observed, for example, by Squire and Middleton (1966).

The activity of radionuclides in soil depends on its composition. Strandberg (1994) investigated a podsolic soil which consists mostly of sand and is covered by a ~5-cm-thick layer of decomposed organic matter with a low clay content of 0–2 %. This study, performed in Denmark, found that the Chernobyl cesium is distributed as follows: 20 % in the litter layer and 80 % in the upper 5 cm of the soil profile. The insignificant penetration observed for lower soil layers should be attributed to a certain form of fixation or binding to soil compounds. Since clay is almost completely absent, the author suggested that humus compounds are responsible for this binding of cesium. Similarly, Yoshida et al. (2004) found that the ^{137}Cs in soil was the highest in near-surface organic layers sampled in Belarus, decreasing with depth in the mineral layers, whereas the concentrations of stable Cs were almost constant in the entire soil profile.

Certain factors referring to the ^{137}Cs budget in the environment, such as industrial activity in regions subjected to the fallout of radionuclides, make for rather complex analyses of results. Such is the case in a study investigating the impact of a major Cu–Ni smelter in Harjavalta, SW Finland (Outola et al. 2003): the authors contended that the greater part of the ^{137}Cs bound to different environmental media, derived from the Chernobyl accident. The atmospheric pollution produced by the smelter damages the microbial population in its vicinity, thus inhibiting the decomposition of litter into organic material. This inhibition of decomposition produced a decrease of ^{137}Cs activity in the organic soil layer in the immediate vicinity of the smelter, whereas away of it the ^{137}Cs activity increased. The ^{137}Cs concentrations in lichens were supposed to increase in accordance with their proximity to the smelter in the same way they did in the litter layer. As the opposite situation was apparent, it is suggested that large amounts of trace metals accumulating in the lichens result in the removal of ^{137}Cs. Several studies demonstrated an enlarged loss of potassium following an exposure of lichens to trace elements (Puckett 1976; Nieboer et al. 1979; Tarhanen et al. 1999; Hyvärinen et al. 2000; Cabral 2003; Mrak et al. 2007) or to SO_2 (Puckett et al. 1977; Tomassini et al. 1977). Low K contents were observed also for lichens in environments polluted by trace metals and other contaminants (Garty et al. 1985, 1997c, 1998a, 2002c; Adamo et al. 2003; Pacheco et al. 2004; Williamson et al. 2004; Tretiach et al. 2007). Studies comparing the K content of lichen thalli and total elemental deposition, measured by air samplers, reveal sometimes a strong inverse correlation (Marques et al. 2009).

Circumpolar environmental conditions were illuminated by comparative analyses of the uptake of anthropogenic radionuclides such as ^{137}Cs and natural radionuclides such as ^{40}K, ^{238}U, ^{226}Ra, and ^{232}Th. Lichens, mosses, vascular plants, and soil in a High Arctic location (Svalbard) were analyzed for their comparative content of the above-mentioned elements. According to Dowdall et al. (2005), the levels of ^{137}Cs in vegetation generally followed the order: mosses > lichens > vascular plants. This sequence was explained by the different mechanism of uptake of this radionuclide. The concentration in vegetation showed little correlation with associated soil concentrations, whereas the uptake of ^{238}U, ^{226}Ra, and ^{232}Th by vascular and non-vascular plants was generally low. Plant species forming important components of Arctic food chains in the Yenisey Estuary (Russia) were ranked according to their ^{137}Cs activity in the following sequence: grasses < alder, willow (leaves), lichens < mosses (upper part) < mosses (lower part), litter (Korobova et al. 2007): the ^{137}Cs contamination of soil indicated both regional and global sources of anthropogenic pollution. The association of radionuclide activity in soil and epigeic lichens was not considered. Studies performed in Denmark (Strandberg 1994), Iceland (Pálsson et al. 1994), and Norway (Skuterud et al. 2005), however, demonstrated the implausibility of the transfer of ^{137}Cs from soil to lichens. The concentrations of ^{137}Cs activity in lichens declined due to washout, dilution by growth, and removal of contaminated parts by grazing reindeer (Skuterud et al. 2005). Lichens were the primary source for ^{137}Cs in reindeer in winter until 1998–1999. Since then, vascular plants became a major source for ^{137}Cs in winter (Skuterud et al. 2005).

A comparison of the ^{137}Cs concentrations in two lichens grazed by reindeer in Iceland found higher concentrations in *Cetraria delisei* than in *Flavocetraria islandica* (Pálsson et al. 1994). Although similar in structure and appearance, *C. delisei* was observed to be smaller and to have consequently a larger surface area per unit of mass. A relatively efficient collector of deposition, *C. delisei* apparently accumulated higher concentrations of ^{137}Cs.

Lehto et al. (2008) used epigeic lichens to analyze the activity of radionuclides in lichen-soil columns in order to determine the transfer of radionuclides from lichen to soil and from the organic soil layer to the rock surface in order to predict the performance of radionuclides in lichen carpets. This information was needed to determine, for example, the period of time at which the lichen is representative of total deposition values. Four different procedures were determined for radionuclides to be removed or transferred downward in lichen-soil columns: (1) Particles attached to the lichen surface removed mechanically by rain and wind, (2) radionuclides taken up into the lichen along with nutrients and transported within the lichen, (3) radionuclides, present in surface-attached particles, dissolved and transported toward the underlying soil, and (4) radionuclides transferred in a process of decomposition and growth of lichens. Twenty years after the Chernobyl accident, epilithic species in W. Macedonia, N. Greece, were contaminated to a greater extent by ^{137}Cs than epiphytic species (Sawidis et al. 2010). Epiphytic lichens depend on airborne nutrients, whereas lichens inhabiting rock or soil depend on the proximity of their substrate. Thus, the epilithic lichens presumably reflect conditions in the soil.

A study performed in France was designed to follow the transfer of radionuclides from soil to trees with lichens on them. The radionuclide activity in the tree rings and in the deposition of fallout did not correlate and no direct track soil–tree–lichen could be detected (Barci-Funel et al. 1995). Another study found that the location of epiphytic lichens on tree trunks is a determinant of the degree of activity of radionuclides in the thallus. According to Heinrich et al. (1999), the epiphytic fruticose lichen *P. furfuracea* growing on dead trunks in the Styria Province, Austria, was contaminated about three times as much at the top-end relative to the lower section of the tree. This was explained by the vertical rainfall making for a greater rate of absorption at the upper end. In certain parts of Stỳria, a high degree of ^{137}Cs contamination was detected in soil relative to the ^{90}Sr soil contamination, following the Chernobyl accident. The assumption that the greater part of the radionuclide contamination derives from atmospheric deposition was corroborated by others (Strandberg 1994), whereas the possible contribution of soil contamination was largely dismissed.

In Turkey, Celik et al. (2009) determined the sequence of concentrations of ^{137}Cs activity in soil, moss, and epiphytic lichens in the following descending order: ^{137}Cs moss > ^{137}Cs lichen > ^{137}Cs soil explained by factors such as removal from soil by washing-off versus a continued fixation of radionuclides in cryptogams. Indeed, a strong correlation was obtained for concentrations of ^{137}Cs activity in lichens and moss samples. According to van den Berg et al. (1992), the averaged levels of ^{137}Cs of atmospheric origin in lichens (*P. sulcata*) around Chernobyl were generally in proportion to soil surface deposition unlike higher soil ^{137}Cs activity classes (>500 kBq m^{-2}).

The radionuclide activity of epiphytic lichens depends also on the characteristics of the sampled tree: coniferous trees and birches yield different results. According to Steinnes and Njåstad (1993), the epiphytic foliose lichen *H. physodes* growing on birch in Norway and collected in July 1986, shortly after the Chernobyl accident, was exposed to a greater extent to the precipitation that carried the radioactive fallout than the deciduous leafless birch. On coniferous trees, on the other hand, a significant fraction of the radionuclides was fixed to the tree needles. The use of *H. physodes* to biomonitor Cs was therefore discouraged.

3.6.7 Depleted Uranium in the Balkan War Zone

Lichens are known to accumulate uranium emitted by various sources (Gough and Erdman 1977; Beckett et al. 1982; Boileau et al. 1982; Looney et al. 1985; Sheard 1986a, b; Pettersson et al. 1988; Singh and Wrenn 1988; Fahselt et al. 1995; Jeran et al. 1995, 1996, 2002, 2003, 2007; Daillant et al. 1996; Trembley et al. 1997; Gueidan et al. 1997; Horvat et al. 2000; Golubev et al. 2005; Purvis et al. 2006). In the war fought in the Balkan area in the years 1995–1999, depleted uranium (DU) was emitted by anti-tank ammunition fired by NATO aircrafts. DU is the by-product of the industrial process applied to enrich natural uranium for use in nuclear reactors and nuclear weapons (Jia et al. 2006). DU is an extremely dense (19.05 g cm^{-3}), hard, autopyrophoric, relatively cheap metal used for military applications such as piercing of munitions and enhancement of armor protection (Jia et al. 2005). Soil analyses performed in the Kosovo area indicated that U concentrations exceeding normal environmental values (~2–3 mg/kg) were due to DU contamination (Danesi et al. 2003a). An additional study of the same area yielded results which did not permit a legitimate extrapolation for all war sites hit by DU in the conflict, but indicated the probability of "spots" where hundreds of thousands of particles are present in a few milligrams of DU-contaminated soil (Danesi et al. 2003b), About 50 % of the DU particles collected in the Kosovo war zone were characterized as UO_2, whereas the remaining DU particles were either U_3O_8 or a mixture of oxidized forms (Salbu et al. 2003).

Following the Kosovo conflict, Loppi et al. (2003) observed that the average U concentrations found in lichens collected in the Balkan were in line with values reported for lichens in other countries. The measurement of uranium concentrations in lichen thalli, in addition to a calculation of $^{235}U/^{238}U$ isotopic ratios, denied the presence of depleted uranium in the Balkan area. These findings were supported by Di Lella et al. (2003) who detected species-specific differences of total uranium concentrations in the lichens *X. parietina, Phaeophyscia orbicularis, Physcia adscendens,* and *Physcia biziana* contaminated by soil particles. The results of another study (Di Lella et al. 2004) suggested that the use of DU ammunitions in Kosovo did not cause a diffuse environmental contamination capable of producing a detectable U enrichment in lichens. Isotopic $^{235}U/^{238}U$ did not indicate the presence of DU particles in lichens either. Sansone et al. (2001), however, reported on a large degree of variability in the concentrations of uranium activity in the surface soil. As DU was detected in lichens, their role as sensitive bioindicators of DU dust or aerosols, formed at targets hit by DU ammunition, was accentuated. Uranium isotopes detected in lichens and tree bark in Bosnia-Herzegovina (Rosamilia et al. 2004) still bore evidence, eight years after the conflict, of past airborne contamination by depleted uranium in target sites. Similarly, Žunić et al. (2008) detected traces of DU in lichens and soil in target sites in the Balkan and recommended the use of lichens as bioindicators for the presence of DU in the environment.

3.7 Conclusions and Open Questions

The traditional definition of lichens describes different organisms living in a stable symbiotic association. Recent knowledge indicates, however, that an important share of additional, non-cellular constituents is integrated in the lichen thallus. Mineral particulate matter originating in the environment apparently derives from both crustal and anthropogenic sources. The significance of trace metals produced by anthropogenic activity cannot be overstated: many trace metals are toxic for living organisms. The historical reason to apply lichens as biomonitors was their capability to absorb and accumulate airborne anthropogenic particulate matter, whereas the inclusion of soil particles entrapped-in the thallus was considered to be of lesser significance.

Lichenologists focused in the 1960s and the 1970s on the capacity of lichens as active or passive biomonitors to yield data referring to air quality based on analyses of elemental content. The 1980s and 1990s of the last century introduced, however, innovative studies including, in addition to chemical analyses of lichen samples, data referring to the physiological status of the lichen. Lichen physiology and cell biology of the thallus were extensively studied also in the first decade of the new millennium. In the last 20 years, combined chemical and physiological studies of lichens used as biomonitors, with an extensive use of collectors, were designed and operated to detect particulate matter derived from both crustal sources and anthropogenic activity. Combined studies of this kind increase the validity of lichen biomonitoring, as chemical analyses of particulate matter collected by samplers yield corroborating data. The significant progress made in recent years makes for a better understanding of the capability of lichens to demonstrate alterations and enhance the applicability of bioaccumulators and bioindicators.

Our understanding of the strategy applied in the domain of chemical analysis and biomonitoring is at a challenging junction. It appears that the second decade of the new millennium will yield several comparative studies investigating chemical content and physiological status in addition to a detailed description of the composition of the particulate matter trapped by collectors. Future studies should be dedicated also to an effort to quantify the share of soil in the composition of epigeic lichens, in order to accomplish an evaluation of the upward flow of soil particles into lichen carpets. The immediacy of this subject is accentuated by herbivores (reindeer and others) grazing in areas infested by radionuclides.

Lichenologists/bryologists need to face the dilemma of pre-treatment: to wash or not to wash? Particulate matter adhered and/or included in the plant material provides data referring to the environmental quality and pre-treatment by washing eliminates an important constituent of the lichen or the moss. Furthermore, is it legitimate to ask: what is the organism we want to analyze? Are the constituents of a lichen just and only algal and/or cyanobacterial cells and fungal hyphae? Is there an organism which does not contain any mineral component? And what about calcium oxalate crystals (Fig. 3.7), sometimes produced in great amounts in and on the thallial surface (Garty et al. 2002a)? What about calcium sulfate crystals (Fig. 3.8) that emerge from the thallial surface of lichens exposed to SO_2 in polluted industrial sites (Garty et al. 2002a)? Do we and can we eliminate such structures by 5–8 washings, sonication, and shaking in an aquatic medium and other drastic treatments and then determine chemical elements located in a "pure" organic association?

It appears improbable to expect a definite conclusion: is the lichen indeed the sum of its "pure," "traditional" components and is there room for particulate matter as a third component? Should scientists refer to particulates as "aliens" or are these mineral structures legitimate members of the symbiotic association? Finally, Table 3.4 and other references indicate that epiphytic lichens are collected in many cases close to ground level. The issue of pre-treatment by washing (Table 3.5) needs to be addressed especially in these cases. Of crucial importance in this context is also the increasing number of annual desert-dust storms, especially in the Mediterranean basin, in southern Europe and probably in other parts of the world.

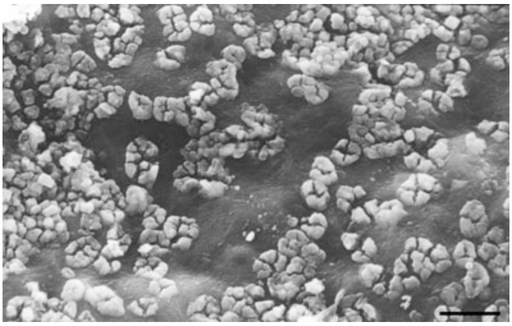

Fig. 3.7 Crater-like structures on thallial surface of in situ samples of *Ramalina lacera* collected in HaZorea Forest, N.E. Israel. Significant amounts of calcium were demonstrated by EDX analyses. Infrared spectroscopy of powders obtained by scraping the thallial surface found crystals of calcium oxalate monohydrate (whewellite: $CaC_2O_4 \cdot H_2O$) and calcium oxalate dihydrate [weddellite: $CaC_2O_4 \cdot (X + 2)H_2O$]. Scale bar = 10 μm (Garty et al., unpublished)

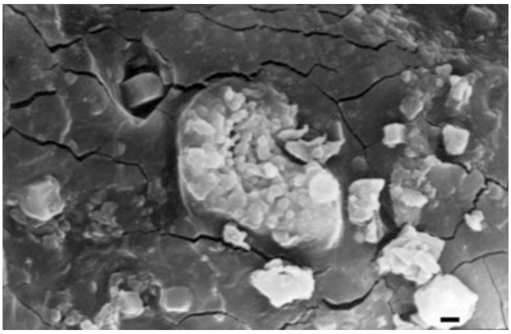

Fig. 3.8 Edge of *Ramalina lacera* thallus collected in HaZorea Forest, transplanted and retrieved after 10 months of exposure near the oil refineries in Ashdod, SW Israel, covered by crater-like structures. EDX spectra showed these structures to consist mainly of Ca and S. Powder samples of scraped thalli revealed crystals of whewellite, weddellite, and gypsum (calcium sulfate dihydrate—$CaSO_4 \cdot 2H_2O$). Scale bar = 1 μm (Garty, Kunin, Delarea, Weiner, unpublished)

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Monitoring Lichen as Indicators of Atmospheric Quality

4

Gintarė Sujetovienė

Contents

Abstract

Bioindicators are living organisms that react to environmental pollution with their life functions. Lichens are reviewed for their potential to reflect air pollution. This chapter highlights the usefulness of lichens as bioindicators of air pollution and recent advances in usage of lichens in the biomonitoring studies. The attention is focused on lichens biological performance at physiological and biochemical levels, the accumulation of substances and on community level. Laboratory approaches related assessment of lichen reactions along with results from field studies point out the main potential characteristics of lichens as indications of air pollution. Discussed data on the lichen studies indicated that analysis of some physiological parameters has given clear picture about air quality of site. Recently, more studies analyse changes at biochemical level that lichens gives more reasonable clarification of induced changes in lichens by pollutants. Review on lichen monitoring contributes to our understanding of change in environmental conditions caused by air pollution.

Keywords

Bioindicator · Biomonitoring · Heavy metals · Lichens · Nitrogen · Persistent organic pollutants · Pollution · Sulphur

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4.1 Introduction

Air pollution is an essential problem in many urban, industrialized and densely populated areas. Pollution is increasing as a consequence of combustion for transport power generation and other human activities. Combustion process produce a mixture of pollutants including gaseous sulphur and nitrogen compounds in association commonly have high concentrations of heavy metals, organic compounds and others. Air pollution is a major environmental-related health treat. In order to protect living organisms from exposure to environmental hazards requires a better understanding of the relationship between environmental conditions and response of organisms.

As environmental contamination becomes actual problem, scientists have to find methods of detecting and quantifying atmospheric pollution. An automated monitoring system is a useful tool for the determination of levels of atmospheric contamination. One of the limitations of such system is large number of sampling sites that leads to high cost. Alternatively, many recent studies on atmospheric pollution have used biological samples such as lichens or mosses (Conti and Cecchetti 2001; Wolterbeek et al. 2003; Seaward 2004). Biological monitoring is based on the assumption that changes in environment affect the biota and these changes can be effectively used as an early warning signal in order to detect environmental changes (Garty 2001). The advantage of the biomonitors usage is the monitoring of several sites at low cost. In addition, their reaction reflects the effects of integrated pollutants impact over long period.

Lichens are one of the most sensitive and valuable biomonitors of atmospheric pollution and can be used as indicators of air pollution at community-level mapping lichen species present in an area, transplanting healthy lichens and measuring deterioration in thallus, assessing the contaminants accumulated in lichen thallus. Lichens have been effective biomonitoring tools for decades because they absorb substances directly form the air and are sensitive to them.

Many lichen communities are affected by various pollutants deposition which resulted alteration in the community structure. The most sensitive species tend to disappear from the urban and industrial areas, whereas tolerant species exist in moderately polluted areas (Ranta 2001; Davies et al. 2007; Larsen et al. 2007). At individual-level biomonitoring studies on the physiological effects of pollution has often emphasized the inhibition of photosynthesis, respiration, fluorescence processes, chlorophylls content, membrane damage and oxidative stress (Garty et al. 2001; Weissman et at. 2006; Bermudez et al. 2009; Bajpai et al. 2010; Oztetik and Cicek 2011; Paoli et al. 2011; Majumder et al. 2013; Karakoti et al. 2014). As lichens absorb elements through their entire surface, even in quantities above their metabolic requirements (Gonzalez et al. 1998; Adamo et al. 2003; Bergamaschi et al. 2007; Shukla and Upreti 2009; Bajpai and Upreti 2012; Kularatne and de Freitas 2013; Sondergaard 2013; Stamenkovic et al. 2013). The absorbed substances are stored for a long time and can be used for bioaccumulation studies. This allows to assess the different level of accumulated certain elements in an altered environment. Due to their accumulation capacity and their sensitivity to various pollutants, lichens can be used either as accumulation indicators or as sensitive indicators of environmental quality.

The review presented in the chapter deals with response of lichens to pollution as indicators of air quality.

4.2 Lichens as Bioindicators of Air Pollution

Lichens are widely used in the biomonitoring studies as an effective tool for mapping spatial and temporal changes of atmospheric contamination (Nimis et al. 2002). Due to the unique biology and their sensitivity to pollutants, lichens respond to variations in atmospheric composition and may be a useful measure for monitoring environmental quality. Lichens might be as a warning indicator of the changes in the environmental parameters and the exceedance of critical levels of pollutants in the atmosphere before the damage become evident. Sensitive lichen species respond rapidly to pollution and helps in detection of unrecognized pollution sources.

Most of the studies have been found that epiphytic lichens are the first to be affected by environmental contamination and the high sensitivity of lichens are related to its biology (Conti and Cecchetti 2001; Giordani et al. 2002; Nash 2010). Lichens have no deciduous parts of their structure, thus, can not avoid damaged or accumulated parts by shading them. Besides, they are slow growers and lacking any root, cuticle (lipid surface layer) and stomata. This means that the unique way for their nutrition is through atmospheric deposition as lichens absorb water and nutrients including pollutants through entire thallus surface.

Lichens do not react to a particular pollutant but the reaction includes the general effect of complex of pollutants. Lichens also have different sensitivity to specific pollutants. Tolerant lichen species may persist in polluted environment, while sensitive species would become extinct. Such differential sensitivity of lichens is very useful in biomonitoring studies (Seaward 2004; Hauck 2008; Mayer et al. 2009). However, the sensitivity can be influenced by different morphological and biochemical properties. Blasco et al. (2011) found that foliose lichen *Lobaria pulmonaria* was more enriched in PAHs compared with *Parmelia sulcata* due to the fact that the former was more aerohydrophytic—absorbing more water and air moisture and consequently more airborne PAHs. The sensitivity to different pollutants varies among different species, and the response to different pollutants and substrates may vary even within a species (Sigal and Nash 1983; Nash 2010).

Other features that contribute to the success of lichens as biomonitors are related to their morphology which is constant around the year, which allows collecting them at any season and are also widely distributed over the Earth. Lichens are long-lived perennial organisms that accumulate pollutants and are subject of cumulative effects of pollutants over decades thus being indicators of long-term and complex atmospheric pollution (Nash 2010).

4.3 Biomonitoring Methods with Lichens

Lichens may be used for biological monitoring studies in two ways: (a) by mapping species in an area, this technique include distribution maps of individual species or the whole lichen flora; (b) lichen species sampling growing naturally or by transplanting lichens from relatively clean environment to a contaminated one, evaluating the accumulation of pollutants, morphological and/or physiological parameters changes (Conti and Cecchetti 2001).

The composition of species in an area reflects the quality of atmosphere. Therefore, changes in lichen communities are correlated with changes in levels of atmospheric pollution. The mapping of lichen diversity is quick and inexpensive method, provides an estimate of the biological impact of air pollution and gives an integrative picture of air quality. Quantitative information could be obtained by calculating different indices to estimate air quality. The most frequently used are the index of atmospheric purity (IAP) and index of lichen diversity (ILD). The elemental composition of lichen thalli both naturally growing and/or transplanted could indicate the accumulation of certain elements providing means of the sources of pollution. More recently and widely physiological parameters have been tested in order to assess the vitality of lichens.

Index of Atmospheric Purity. In order to assess the quality of air pollution, one of approaches could be the use of the IAP: $\mathrm{IAP} = \frac{1}{10} \times \left(\sum_{1}^{n} \frac{Q \times f}{n}\right)$ (where n—the number of species, Q—degree of toxiphoby and f—the frequency or abundance of each species) (LeBlanc and De Sloover 1970). IAP is a quantitative approach combining the number, frequency and tolerance of the lichens species present in a study area with their sensitivity to environmental stress. Recently, the IAP was reviewed by Kricke and Loppi (2002).

The index values obtained may be plotted to obtain air quality map. Several diverse modifications from the original formula of the IAP were proposed in many studies (Yule and Lloyd 1984; Batic and Mayrhofer 1996; Asta and Rolley 1999; Fernandez-Salegui et al. 2006b). For example, the index of poleotolerance was developed in Estonia (Trass 1973)—a similar approach to the IAP but with a value of poleotolerance: $\mathrm{IP} = \sum_1^n \left(a_i \times \frac{c_i}{C_i}\right)$ (where n—the number of species, a_i—species-specific factor expressing tolerance to pollutants (scale from 1 to 10), c_i—degree of cover for each species (scale from 1 to 10) and C_i—degree of cover for all species.

A number of IAP studies have been applied in different regions of the world, around a paper mill (Das et al. 2013), thermal plants (Romeralo et al. 2012), geothermal power plants (Loppi 1996; Loppi and DeDominicis 1996; Loppi and Nascimbene 1998), municipal solid waste incinerator (Loppi et al. 1995), industrial area (Sim-Sim et al. 2000) and in the National Park (Gibson et al. 2013). A large number of IAP studies were performed in urban areas (Siena, Italy: Monaci et al. 1997; Thessaloniki, Greece: Vokou et al. 1999; Grenoble, France: Gombert et al. 2004; San Carlos de Bariloche and Villa Regina, Argentina: Calvelo and Liberatore 2004; Ohio, USA: Washburn and Culley 2006; Linz, Austria: Zechmeister and Hohenwallner 2006; Aragon, Spain: Blasco et al. 2008; Bariloche, Argentina: Calvelo et al. 2009; Dimitrovgrad, Serbia: Stamenkovic et al. 2010; Knjazevac, Serbia: Stamenkovic and Cvijan 2010; Viamao, Brazil: Kaeffer et al. 2011; Blace, Serbia: Stamenkovic et al. 2013). Some IAP studies have involved a larger geographical areas (Finland and Russia: Mayer et al. 2009; Mediterranean area: Giordano et al. 2004; Slovenian forests: Jeran et al. 2002). The results of the studies suggest that the IAP may be a useful air pollution indicator tool in combination with modelled data, even in relatively clean regions and it was strongly correlated with sulphur and nitrogen concentrations (Zechmeister and Hohenwallner 2006; Mayer et al. 2009). IAP values were compared with data of multi-elemental composition of lichen thalli and the resulted in negative correlation indicated that the IAP is an accurate, reliable and relatively inexpensive method to detect and evaluate atmospheric pollution (Calvelo and Liberatore 2004; Blasco et al. 2008).

The IAP value also showed no clear relation with sources pollution, such as roads or industry, and was nor correlated with element concentrations in the atmosphere (Gombert et al. 2004). It initially created to characterize atmospheric pollution or atmospheric purity by means of the lichen flora and was shown to be influenced by other parameters such as environmental and landscape factors. At higher IAP values, lichen frequencies did not seem to be affected by air pollution, and variations in the epiphytic lichen communities presumably depended on other environmental factors (Loppi et al. 1995).

Other quantitative methods were also proposed for monitoring the effects of air pollution in Germany. The VDI method (1995) is based on the calculation of the frequency of species within the ladder of 10 units (each 10 × 10 cm) positioned on the trunks of trees. In Italy, the biodiversity of epiphytic lichens was calculated as the sum of frequencies within the sampling ladder of 10 quadrats (each 15 × 10 cm) (Nimis et al. 1990). A standardized methodology was proposed by Asta et al. (2002) which provides a general picture of environmental quality. This method supplies information on the long-term effects of air pollutants, eutrophication, anthropization and climatic changes on lichens. The objective method (sampling design, procedure and data interpretation) allows comparing results throughout the different studies (Asta et al. 2002).

The qualitative scale for estimating SO_2 pollution was developed by Hawksworth and Rose (1970). The basic element is the species, each of them having a range of tolerance to pollution. The tolerance can be expressed as ecological indicator value. This scale has been widely applied in different countries (Bates et al. 1990; Khalil and Asta 1998; Svoboda 2007; Lisowska 2011). However, such scale designed to assess SO_2 may no longer be accurate indicator of atmospheric pollution because SO_2 levels in many areas have declined meanwhile nitrogen deposition in urban and agricultural areas is the main driver of lichen communities changes (Larsen et al. 2007; Mayer et al. 2009; Rogers et al. 2009; Grandin 2011; Johansson et al. 2012; Evju and Bruteig 2013; Sparrius et al. 2013). Zone scales as the biomonitoring approach allow predicting the level of pollution by sulphur dioxide from the assemblages of lichen species typical for unaltered habitat. Scales of lichen sensitivities have been published by Hawksworth and Rose (1970), de Wit (1976), and Wirth (1991).

Index of lichen diversity. ILD is calculated as the sum of frequencies of epiphytic lichens in a sampling grid (Giordani 2007). To interpret the IDL values in terms of air pollution, Loppi (2004) suggested using derivative ILD values. For example, ILD_{NN} value without the frequency of nitrophytic species as reflection of air pollution by phytotoxic gases (SO_2, NO_x); ILD_N calculated only with frequency of nitrophytic species, expected to reflex eutrophication and ILD_{SN} calculated only with the frequency of strictly nitrophytic species as reflection of ammonia pollution (Loppi and Frati 2006). For each index, a different interpretative scale of environmental alteration was devised (Table 4.1).

Nitrophytic species are those which has an indicator value for eutrophication of four (rather high eutrophication) or five (very high eutrophication) according to Nimis (2003). Strictly nitrophytic species are those with an indicator value for eutrophication from four to five (Loppi and Frati 2006). Lichen diversity values with only the frequency of nitrophytic species could be taken as rough estimates of eutrophication, mainly nitrogen deposition.

Despite the decrease in the main lichen abundance affecting pollutant SO_2, decreased lichen diversity over time and space are still detected (Slaby and Lisowska 2012; Hauck et al. 2013b; Mayer et al. 2013). Various anthropogenic variables are responsible for lichen decline along with successive processes of lichen communities. In the complex ecosystems with the high climatic variability and sharp environmental gradients, the factors are particularly complex and hard to detect (Brunialti and Giordani 2003; Giordani 2007). Bates et al. (2001) proposed that methods based totally on the total lichen diversity may not be appropriate for future monitoring urban air pollution. According to Giodani (2007), the relationship between lichen diversity and environmental variable changes under different ecological conditions, air pollutants are the main limiting factor in non-forested areas, whereas harvesting or fires predominate in forested areas.

The transplant method. In areas with low pollution levels where lichens are not killed by contaminants, the biomonitoring studies could be performed using native lichen species. When biomonitors are insufficient in the sites to be monitored or when the aim is to assess pollution deposition and accumulation rates, the use of transplants is a very useful tool in terrestrial and aquatic environment (Augusto et al. 2013a).

The lichen thalli are collected from relatively non-polluted site and transplanted to the study sites to be monitored under the exposure of pollution. The use of transplanted lichens allows assessing the response of organisms exposed to different atmospheric conditions including pollutants over fixed exposure period. This method also allows to determine threshold levels of toxic substances in a thallus (Mikhailova 2002). The transplant method is used in bioaccumulation

Table 4.1 Interpretative scale of environmental alteration using different ILD (Loppi and Frati 2006)

ILD_{NN}	ILD_N	ILD_{SN}	Environmental alteration
0	>36	>12	Very high
1–18	25–36	9–12	High
19–36	13–24	5–8	Moderate
37–54	1–12	1–4	Low
>54	0	0	Negligible

studies and analysing morphological, physiological and ultrastructural damage. Samples can be taken periodically in order to evaluate vitality of lichen thalli. The transplantation studies are used often in order to assess air pollution in urban and industrial areas (Garty et al. 1996; Gonzalez and Pignata 1997; Canas and Pignata 1998; Zambrano and Nash 2000; Gonzalez et al. 2003; Carreras et al. 2005; Cloquet et al. 2009; Oztetik and Cicek 2011; Aslan et al. 2013).

Physiological and biochemical parameters to assess the vitality of lichens exposed to air pollution. Due to different sensitivity of lichens to air pollution, they are potentially useful for air monitoring. In order to assess pollution induced stress effects in lichens, the methodologies have to be sensitive and reliable (Garty et al. 2000). The physiological reactions of lichens to atmospheric contamination have been evaluated by means of changes in photosynthesis and fluorescence rates, the degradation of chlorophylls and decrease in the cell membrane integrity.

The measurement of modulated chlorophyll *a,* fluorescence in lichens is one of several valid tools in biomonitoring studies (Jensen and Kricke 2000; Tretiach et al. 2007b). The chlorophyll fluorescence parameter ratio of variable fluorescence to maximal fluorescence (Fv/Fm) has been widely used to assess the state of the photosynthetic apparatus in response air pollution (Bjerke et al. 2005; Fernandez-Salegui et al. 2006a; Tretiach et al. 2007a; Paoli et al. 2010; Piccotto et al. 2011; Karakoti et al. 2014).

It has been shown that air pollution reduces both photosynthesis and respiration rates in lichens (Haffner et al. 2001; Nash and Gries 2002; Zambrano and Nash 2000; Boonpragob and Nash 1991). This parameter may provide diagnostic information on the state of photosystems, being related to the environmental factors and pollution. The results of recent transplantation studies showed that the pulse amplitude modulation fluorimetry applied may become an important tool in biomonitoring studies. In such cases, particular care should be taken in sample collection, selection and exposure (Tretiach et al. 2007b).

With increasing levels of air pollution, lichens show a transformation of chlorophyll *a* into phaeophytin *a*, followed by a reduction of chlorophyll content (Gonzalez and Pignata 1994; Zambrano et al. 1999; Gauslaa and Solhaug 2000; Zambrano and Nash 2000; Hauck et al. 2003; Backor et al. 2004; Godinho et al. 2004; Pignata et al. 2004; Shukla and Upreti 2007; Frati et al. 2011; Bajpai and Upreti 2012; Majumder et al. 2013; Karakoti et al. 2014). A loss of chlorophyll finally could lead to extinction of lichen species.

Significant damage to lichen cell membranes has also been shown as a result of exposure to pollutants (Adamo et al. 2003; Alebic-Juretic and Arko-Pijevac 2005; Spagnuolo et al. 2011; Majumder et al. 2013; Sujetoviene and Sliumpaite 2013). In the case of mycobiont, content of ergosterol, the main sterol of fungal membranes, is the marker of the response to pollution in the environment (Backor et al. 2006; Vantova et al. 2013).

Cellular injury may be caused by free radicals which are induced upon exposure to air pollution. Exposure to pollution increases the production of reactive oxygen species (ROS), including superoxide radicals, hydroxyl radicals and hydrogen peroxide along with disbalance of cellular redox status. Under the high pollution, algal cells are injured because the capacity of cellular antioxidant protection system is not able to cope with high ROS levels. ROS can react with cellular components (lipids, proteins, pigments and nucleic acids) to cause lipid peroxidation (del Hoyo et al. 2011). To protect themselves from potentially damaging effects of the oxidative stress induced by ROS, living organisms have enhanced some antioxidant enzymes [superoxide dismutases (SOD), catalases (CAT) , peroxidases (POD) and glutathione S-transferases (GSTs)] and non-enzymatic antioxidants (glutathione (GHS), ascorbate (APX) and phenolic compounds) as defence mechanisms (Oztetik and Cicek 2011). Superoxide dismutase (SOD) catalyses the dismutase of superoxide radicals ($O_2^{\cdot -}$) to yield hydrogen peroxide (H_2O_2) and oxygen (O_2). The enzyme is important as defence mechanism against oxygen radical-mediated toxicity. Thus, the induction of SOD can be as indicator of enhanced $O_2^{\cdot -}$ production. It has been shown the good correlation between SOD activity and atmospheric O_3 and SO_2 concentrations in transplants of *Hypogymnia physodes* (Egger et al. 1994). An increase in the activity of SOD by exposure to pollutants has been documented in *H. physodes*, *Xanthoria parietina* and *Ramalina farinacea* (Egger et al. 1994; Silberstein et al. 1996b). Declines in the activity of SOD, POD and CAT in pollution sensitive *Ramalina duriaei* were documented (Silberstein et al. 1996b). The activity of all the antioxidant enzymes assayed was also decreased in *Evernia prunastri* by SO_2 exposure (Deltoro et al. 1999). It is possible that pollutant accumulation exceeded the rate of detoxification processes in sensitive lichen species with the result in H_2O_2 accumulation (Hippeli and Elstner 1996). The increase in glutathione (GSH) concentrations and glutathione S-transferase (GSTs) activities was observed under the exposure of air pollutants (Oztetik and Cicek 2011).

The peroxidation of membrane lipid is one of the most important aspects of oxidative stress induced by the reactivity of oxygen free radicals. Malonedialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation. An increase in free radicals causes the surplus production of MDA. Therefore, the content of MDA can be used as indicator of the degree of oxidative stress. This is one the most frequently used indicator of oxidative stress in lichens (Gonzalez et al. 1996; Gonzalez and Pignata 1997; Carreras et al. 1998; Gonzalez et al. 1998; Kong et al. 1999; Cuny et al. 2004; Monnet et al. 2005; Carreras and Pignata 2007; Sujetoviene and Sliumpaite 2013).

During lipid peroxidation conjugation of the ethylenic groups of polyunsaturated fatty acids may be observed which results in an increase in hydroperoxy conjugated dienes (HPDC). HPDC as peroxidation product seems to be a better estimator of damage because no changes were detected in the MDA content under the exposure of urban atmospheric pollutants (Rodriguez et al. 2007). HPDC was successfully applied in the studies of transplanted lichens to urban areas (Canas et al. 1997; Gonzalez and Pignata 1997, 2000; Carreras et al. 1998; Gonzalez et al. 1998, 2012; Carreras and Pignata 2002; Pignata et al. 2004).

For the evaluation of air quality under the mix of contaminants (e.g. urban areas), the use of pollution index (PI) was proposed by Gonzalez and Pignata (1994) and is used in the biomonitoring studies (Levin and Pignata 1995; Gonzalez and Pignata 1997; Carreras et al. 1998; Pignata et al. 2007). The index includes such changes in the transplanted lichens parameters as content of chlorophyll *a* and *b* and sulphur content in lichen thallus, and the concentration of HPDC compared with the control. The PI was proposed as a good indicator of global damage to lichen and of different air quality (Levin and Pignata 1995; Gonzalez et al. 1996; Gonzalez and Pignata 1997). The PI values were highest in lichens transplanted to sites with high industrial activity and traffic.

Despite used changes in physiological and biochemical parameters as biomarkers determining the level of environmental stress on lichens, extracellular and intracellular content of nitrogen compounds or heavy metals is also used. It is suggested that intracellular rather than total concentrations must be considered (Backor and Loppi 2009). In the intracellular spaces accumulated particles can remain unaltered for a long time. Because the negatively charged anionic sites of the cell walls of symbionts in lichens present, the extracellular content also plays important part of compound uptake in lichens. These binding sites are carboxyl, phosphate, amine and hydroxyl groups (Backor and Loppi 2009). Because elements bounded to cell wall are readily exchangeable, it is suggested that extracellular amounts and proportions reflects environmental input (Brown 1987).

Lichens can accumulate and retain many heavy metals in quantities above their physiological requirements. The lichens continuously accumulates pollutants from the air until equilibrium is reached; thus, transplanted lichens are useful for monitoring air pollution concentrations overtime (Kularatne and de Freitas 2013). The more polluted area, the longer the equilibrium time. Transplantation studies showed that lichens respond to changes in heavy metals concentrations within a period of several months (Kularatne and de Freitas 2013; Cloquet et al. 2009), after one year (Mikhailova and Sharunova 2008) or several years (Sondergaard 2013).

4.4 Evidence from Experiments and Filed Studies: Lichen Diversity in Response Pollution and Physiological Response of Lichens

4.4.1 Nitrogen Compounds

Large quantities of nitrogen (N) compounds produced naturally exceed anthropogenic emissions. Anthropogenic inputs from power generation, industrial activity, transport and agriculture have disrupted this balance. Other atmospheric contributions of nitrogen compounds come from non-combustion processes, for example nitric acid manufacture, welding processes and the use of explosives. The main two categories of nitrogen compounds are oxidized [nitric oxide (NO) and nitrogen dioxide (NO_2)] and reduced nitrogen forms [ammonia (NH_3)]. The atmospheric deposition of nitrogen has increased about threefold from pre-industrial times in 1995, and by 2050, it is projected to redouble (Galloway et al. 2004). Nowadays, the rates of nitrogen deposition are steadily increasing and becoming a big problem along with increase in world population and its demands (Galloway et al. 2008).

Nitrogen deposition can eutrophy and acidify the ecosystems (Stevens et al. 2011). The impact of N deposition on a species depends on several factors, including the duration of exposure, total amount and form of nitrogen and the sensitivity of species (Bobbink et al. 2010; Erisman et al. 2013). In order to monitor atmospheric pollution by nitrogen compounds with lichens, three approaches are used: (1) changes in lichen species composition, (2) changes in physiological parameters and (3) accumulation of nitrogen in lichens.

Species composition. Unlike most other pollutants, nitrogen is an essential nutrient. Increased N availability usually leads to increase growth by investing more in their photosynthetic capacity. As nitrogen concentration in lichen thalli increase in order to balance their carbon-to-nitrogen ratio, the growth rate and carbon assimilation capacity increase (Palmqvist and Dahman 2006; Johansson et al. 2011). The higher growth rate could be characteristic to fast-growing species which may out compete the slow-growing ones. Thus, changes in nitrogen availability could induce changes in lichen species composition. Johansson et al. (2012) proposed that those community responses could be due to physiological responses of the individual species rather than changes in competitive interactions.

Adding nitrogen to the environment tends to shift lichen community composition. Lichen responses to nitrogen are different due to their sensitivity to their ionic environment. Increase in nitrate nitrogen can acidify the substratum while enrichment in the form of ammonium results in higher pH levels. NO_x has a strong effect on lichen diversity (Davies et al. 2007), its community composition and frequency and dispersal (Larsen et al. 2007). In addition, lichen population declines under high NO_x concentration (van Dobben et al. 2001; Giordani 2007). A number of studies have observed a positive relationship between the proximity to livestock farms and abundance of nitrophilous lichen species (Sanchez-Hoyos and Manrique 1995; van Dobben and DeBakker 1996; van Dobben and ter Braak 1998; van Herk 1999; Ruoss 1999). Despite the negative effects, some lichen species such as *Lecanora dispersa* and *Phaeophyscia orbicularis* are NO_x tolerant (Davies et al. 2007). Phytotoxic effect of NO_2 on lichens that were fumigated with 4 ppm (7,520 μg m^{-3}) for six hours in a laboratory experiment was confirmed by Nash (1976) and suggested that the pollutant would probably not be harmful to lichens since the NO_2 concentration detected in natural environment was usually less than 1 ppm. Thus, the effect of NO_x on lichens seems controversial and unclear.

Some N tolerant species are favoured by N addition improving their physiological status and a luxury consumption of the extra N supplied (Ochoa-Hueso and Manrique 2011). It was concluded that the tolerant lichen *Cladonia foliacea* could be used as biomarker of responses to nitrogen. In contrast, other species adapted to nutrient poor habitats and acidophytic (sensitive to eutrophication) species abundance and distribution are negatively correlated with nitrogen enrichment (Gaio-Oliveira et al. 2004; Friedel and Muller 2004; Davies et al. 2007; Franzen-Reuter and Frahm 2007; Pilkington et al. 2007; Pinho et al. 2008). High levels of nitrogen cause a decrease in lichen abundance and overall homogenization of the lichen species with remaining the most tolerant species (Liska and Herben 2008). The loss of ground lichen species adapted to nutrient poor conditions were observed when studying eutrophication effects in pine forests over 45 years period (Reinecke et al. 2014).

Direct nitrogen fertilization highlights the potential threat to lichens. The disappearance of lichen *H. physodes* and *Lepraria incana* following 21 months of fertilization was observed by Franzen-Reuter and Frahm (2007). The lichen community composition responded to fertilization even at the lowest nitrogen application level —6 kg N ha^{-1} $year^{-1}$ (Johansson et al. 2012). The data suggest that those community responses could be used as sensitive bioindicators in order to detect low levels of N pollution. As most studies have focused on the total amount of N deposition, it is very important to investigate the effects of different ratios of oxidised and reduced N forms on lichen communities. The form of N deposition has important consequences on lichen community response to N enrichment.

Changes in physiological parameters. Green algae–lichens are solely depended on atmosphere nitrogen directly deposited on their thallus surfaces to meet their nitrogen demand. Lichen pigment concentration can reflect N deposition. The lichen transplanted from an environment relatively low in nitrogen, used nitrogen as a nutrient, and in response chlorophyll *a* content could increase. It was suggested that chlorophyll synthesis could be stimulated at the urban sites due to higher concentrations of nitrogen compounds, which are beneficial to lichens (Boonpragob and Nash 1991). Such trend was found in studies with transplants in urban areas—with *P. sulcata* transplanted to the urban area of Biel city (von Arb and Brunold 1990), with *Usnea amblyoclada* transplanted at the centre of Cordoba city (Carreras et al. 2005; 2009) and with *E. prunastri* exposed in the urban parks and residential sites (Sujetoviene and Sliumpaite 2013). The concentration of chlorophyll was also positively affected by the traffic level: the content of chlorophylls increased in parallel with the level of traffic density and pollutants emitted by transport (Carreras et al. 1998; Satya and Upreti 2009) and under the impact of agricultural activities (Fig. 4.1) (Frati et al. 2011). Increase in chlorophyll *a* concentration was recorded along with accumulated nitrogen under the fertilization experiment (Dahlman et al. 2003; Gaio-Oliveira et al. 2005a). Short-term (24-, 48- and 96 h) exposure of N excess do not cause chlorophyll degradation in *X. parietina* (Munzi et al. 2009).

The increase in photosynthetic pigments was not always characteristic because the algal partner could suffer under the high nitrogen deposition. Degradation of photosynthetic pigments is commonly used as an indicator of lichen vitality. The rate of degradation of chlorophyll *a* into phaeophytin is closely parallel to environmental pollution (Garty et al. 2000), which may be indicated by decreased concentrations of chlorophylls content, carotenoids and chlorophyll degradation to phaeophytin as effects of NO_2 and NH_3 from agricultural activities and road traffic (Fig. 4.1) (Frati et al. 2006, 2011). Samples of nitrophytic *X. parietina* also showed loss of chlorophyll when exposed to increasing ammonium concentrations (Gaio-Oliveira et al. 2004). Experimental evidence supports the hypothesis that high concentrations of nitrogen have toxic effects on lichens (Munzi et al. 2010; Meysurova et al. 2011). Different physiological responses were observed nitrophytic and oligotrophic lichens treating with combination of oxidised and reduced nitrogen (Gaio-Oliveira et al. 2005a). Chlorophyll *a* and ergosterol concentrations

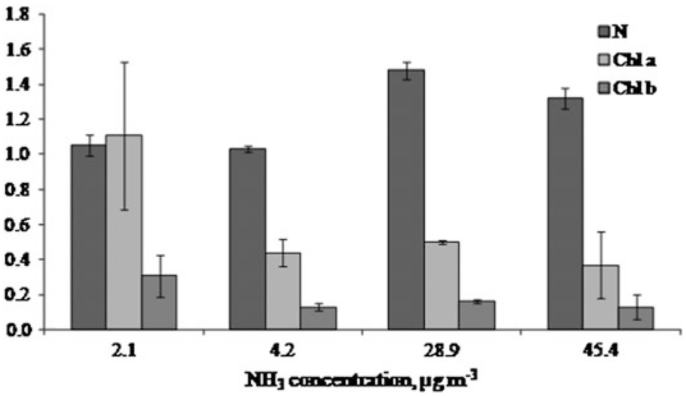

Fig. 4.1 The concentrations of total nitrogen (N, % dry weight), chlorophyll *a* (Chl *a*, mg g^{-1}) and chlorophyll *b* (Chl *b*, mg g^{-1}) in *Evernia prunastri* thalli transplanted for 2 months at plots with different levels of agricultural activities (Frati et al. 2011)

remained unaltered in nitrophytic *X. parietina* irrespective of N dosage, while ergosterol decreased with increasing N uptake in acidophytic *E. prunastri*. The latter species had accumulated a large pool of ammonium at the highest N dosage, while in *X. parietina* showed a significant nitrate concentration.

At the molecular level, Tretiach et al. (2007b) reported that a large amount of NO$_x$ can damage the photobionts of transplanted *Flavoparmelia caperata*, hindering photosynthesis probably due to the increased reactive oxygen species (ROS), as high concentration of NO$_2$ in the cells forms nitrous and nitric acid, which acidifies cytoplasm and results in protein denaturation, deamination of amino acids and nucleic acid. When exposed to excessive nitrogen supply, the higher damage was observed for mycobiont than the photobiont (Dahlman et al. 2002, 2003; Gaio-Oliveira et al. 2004).

Ergosterol a component of the fungal plasma membrane is used as a marker of mycobiont respiration. The significant decrease in ergosterol with increasing N uptake was observed in *E. prunastri* (Gaio-Oliveira et al. 2005b), suggesting that increasing nitrogen uptake has caused more damage to the fungal partner than to the photobiont. Chitin a component of the fungal cell wall is being correlated with amount of mycobiont biomass. Increase in photobiont investment took parallel by a subsequent decrease of investments in the mycobiont was proved by the increased ratio chlorophyll to ergosterol and chlorophyll to chitin (Dahlman et al. 2003).

Different degrees of lichen sensitivity are related with different mechanisms of avoiding excess of nitrogen uptake. Some nitrogen sensitive species are able to avoid toxicity and are able to avoid the excess of nitrogen uptake while others are able to neutralize it once when it entered the cell. The latter is related with lichen ability to assimilate N into non-toxic forms such as arginine (Silberstein et al. 1996a; Dahlman et al. 2003). The accumulation of arginine could be a way to avoid toxic effects of surplus NH$_4^+$ assimilation. The lichens may be able to adapt to the increased N supply either through uptake regulation or through increased growth (Johansson et al. 2010). Recent studies have shown that some species have repairing systems involved in its metabolism. The neutralization of nitrogen excess was observed when species produce or use polyamines for that (Pirintsos et al. 2009). Studies have indicated some species (e.g. *E. prunastri*) could not avoid nitrogen entrance into the cell due to its high cation exchange capacity (Gaio-Oliveira et al. 2001). Lower cation exchange capacity prevents ion absorption within lichen thallus, and this could explain why some species (e.g. *X. parietina*) are more adaptable and react different under high N availability (Gaio-Oliveira et al. 2005a).

Cell membrane damage. A common sign of N compounds toxicity is the decline of essential cations in the lichen thallus. Ammonium supply resulted in a decrease in intracellular K^+ and Mg^{2+} of *X. parietina* (Munzi et al. 2011). An excessive ammonium load affected the cell membrane integrity until rupture and as a consequence, a loss of cytoplasmatic material occurred that can be confirmed by intracellular K^+ leakage. Electrical conductivity indicated cell membrane damage in the cases of acute, high N stress episodes (Munzi et al. 2009) but no significant differences were detected during the four weeks experimental conditions (Munzi et al. 2012).

Photosynthesis and fluorescence. Under the non-toxic increase in N availability, lichens showed the response by investing more N in their photosynthetic and carbon assimilation capacity (Palmqvist et al. 2002; Ra et al. 2005) and finally to increase in growth rate (Palmqvist and Dahlman 2006). The increased N supply favours photosynthesis with loads of N as high as 20 kg N ha^{-1} $year^{-1}$ (Ochoa-Hueso and Manrique 2011) and above this threshold, the improved photosynthesis was not observed.

It is thus clear that the excess nitrogen might negatively affect the N pathway and the photosynthesis process of lichens. High NO_x levels induce deleterious effects in the photosynthetic apparatus of the photobionts of transplanted lichens (Tretiach et al. 2007b). It is suggested that nitrogen oxides interrupt the formation of the trans-thylakoid proton gradient as showed heavily affected non-photochemical quenching.

According to Paoli et al. (2010) samples of *E. prunastri* transplanted to close proximity of a point source of NH_3 showed a marked decrease in the potential quantum yield of PSII after 15 days. Nitrogen sensitive lichen *E. prunastri* exposed to NH_3 showed decrease in Fv/Fm above 3 μg m^{-3}, suggesting direct adverse effects of NH_3 on its photosynthetic performance (Munzi et al. 2014). By contrast, nitrogen tolerant *X. parietina* showed decreased capacity of fluorescence above 50 μg m^{-3} NH_3. Smaller chlorophyll *a* fluorescence variation in pollution-resistant lichen *X. parietina* was also observed (Piccotto et al. 2011). The results of effect of ammonia on lichens clearly indicate that the ecological success and species-specific tolerance of *X. parietina* at ammonia-rich sites or urban areas might be related to indirect effects of increased nitrogen availability.

Accumulation. Lichens are largely dependent on the atmosphere for acquisition of major nutrients including nitrogen. Nitrogen concentration in lichen thallus can respond to N from the atmosphere and can be a good indicator which reflects the content of it in the atmosphere (Hyvarinen and Crittenden 1998; Olsen et al. 2010; Boltersdorf and Werner 2013). Nitrogen concentrations in *Usnea lapponica* were strongly correlated with nitrogen deposition (McMurray et al. 2013). Nitrogen accumulation was observed in transplants as a response of anthropogenic N enrichment by traffic activity (Gombert et al. 2003; Frati et al. 2006; Sujetoviene 2010). Lichen N accumulation patterns matched throughfall N deposition patterns (Fenn et al. 2007). Experimentally nitrogen deposition was simulated by irrigating trees over a 3 yr period with NH_4NO_3, providing N loads ranging from ambient to 50 kg N ha^{-1} $year^{-1}$ (Johansson et al. 2010). The experiment results showed that the thallus showed increased N concentration in *Alectoria sarmentosa* and *Platismatia glauca* with increasing N load.

Lichens when transplanted in an agricultural area with high atmospheric NH_3 concentrations have responded to NH_3 air pollution accumulating nitrogen in its thalli (Søchting 1995; Ruoss 1999; Frati et al. 2011) and showed maximum content of nitrogen in *X. parietina* after one month of exposure but at sites with background concentrations, it took several months to reach statistically significant uptake (Olsen et al. 2010). The excessive nitrogen supply applying fertilizers also induced the increase in lichen thallus N concentration (Dahlman et al. 2003).

Root et al. (2013) presented a new monitoring approach that estimates throughfall inorganic N deposition from N concentration in lichens. It was concluded that lichen N concentration is a cost-effective way to monitor N deposition, and its N concentrations can be used to estimate throughfall N deposition and such lichen monitoring allows identification of areas that may exceed N critical loads.

The identification of early stages of biological effects of nitrogen pollution is essential to prevent consequences for a whole ecosystem. Lichen physiological changes are successfully used as an early indicator of environmental stress (Vingiani et al. 2004). Composition or cover changes in lichen communities indicate exceedance of critical loads for terrestrial ecosystems.

Using these approaches, a threshold value was suggested for nitrogen compounds. A threshold value was defined as a concentration of atmospheric NH_3 above which changes in species diversity or physiology occurs. Concentration of 3 μg m^{-3} NH_3 in the atmosphere was suggested which cause adverse effects on lichens photosynthetic performance—a decrease in lichen species frequency and Fv/Fm of oligotrophic *E. prunastri* (Munzi et al. 2014). Empirical data suggest that the critical load for epiphytic lichens in boreal forests is c. 10–15 kg N ha^{-1} $year^{-1}$, although lower critical loads (as low as 3 kg ha^{-1} $year^{-1}$) have been suggested (Fenn et al. 2008).

4.4.2 Sulphur Compounds

Sulphur dioxide originates from both natural and anthropogenic sources in the environment. Natural sources of the sulphur in the atmosphere are volcanic activity, fires and bacterial activity, and the sea and ocean. The main anthropogenic source of SO_2 is industrial activity that contains sulphur, e.g. the fossil fuel combustion, smelting, manufacture of sulphuric acid, incineration of refuse and production of elemental sulphur. Anthropogenic emissions constituted about 90 % of the total sulphur input to the atmosphere (Whelpdale et al. 1997). Sulphur emissions have been increasing steadily since the year 1850, now mainly in Asian countries by developing industries. According to Europe's Environment Agency assessment (2012), in Europe, sulphur emissions have decreased by 74 % between the years 1990 and 2011.

Sulphur dioxide (SO_2) was formerly viewed as the most important phytotoxic pollutant and until the early 1980s was the subject of the greatest research in this field. Now, it has attained less attention because of its declining concentrations as emissions have been reduced and also because nitrogen gases and ozone have been found to be of increasing significance.

Sensitivity of lichens to sulphur compounds has been investigated for a long time and is the basis of the use of lichens as bioindicators. High sensitivity of lichens to sulphur compounds lies on the acidic characteristics of sulphur dioxide which is the main source of sulphur in the atmosphere (Batic 2002). Damaging effects of sulphur compounds are related with proton formation along with oxidation of sulphite to sulphate and free radical generation during the oxidation process.

The main tools for assessing the response of lichens to sulphur pollution is changes in their distribution and frequency and changes in physiological parameters. It was proved that lichens are very suitable bioindicators as they are sensitive to atmospheric sulphur dioxide, and sulphur dioxide considered as a primary factor causing death of lichens in most urban and industrial areas (Giordani 2007). Fruticose species are more sensitive to sulphur compounds than many foliose and crustose species (Owczarek et al. 1999; Tretiach and Ganis 1999).

The summarized historical evidence of lichen decline with increasing burning of fossil and rising concentrations of SO_2 along with lichen species decline are provided by Bates (2002). The disappearance of lichens from polluted urban and industrialized areas was recorded under high pollution with sulphur dioxide (LeBlank and De Sloover 1970; LeBlanc et al. 1972). In recent decades, concentrations in the atmosphere decreased markedly and as consequence, the air quality improved quite rapidly and remarkable in some polluted areas. Recent trends towards lower concentrations of sulphur dioxide lead to changes in lichen occurrence and recolonization of lichen desert (Rose and Hawksworth 1981; Hawksworth and Mcmanus 1989; Letrouitgalinou et al. 1992;

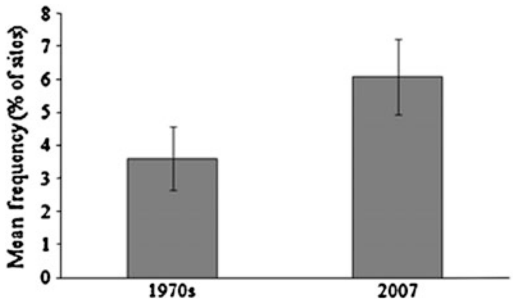

Fig. 4.2 Changes in number of epiphytic species observed in Skawina, in the 1970s and in 2007, in the urban-industrial area of southern Poland. The increase in species number showed lichen recolonisation process as a result of air quality improvement (Lisowska 2011)

van Dobben and De Bakker 1996; Ranta 2001; Loppi and Corsini 2003; Loppi et al. 2003; Hultengren et al. 2004; Adamska 2011; Lisowska 2011; Slaby and Lisowska 2012; Evju and Bruteig 2013; Fig. 4.2). However, in the case of heavy polluted areas ,species recolonisation cannot be expected and lichens in such area shows growth of toxitolerant species (Upreti et al. 2005). Modest SO_2 levels, interacting with low bark pH, still inhibit recolonization of sensitive lichens in urban areas (Bates et al. 2001; Batty et al. 2003).

Individual lichen species differ in their sensitivities to sulphur dioxide. Such differences in sensitivities lead to biomonitoring approach to divide polluted areas into zone according to lichen species assemblages. According to measurements of ambient sulphur dioxide and the IAP the surveyed area may be divided into pollution zones (Gilbert 1968, 1970; Hawksworth and Rose 1970).

Sulphur dioxide may be adsorbed on a thallus and then absorbed when the thallus are wet. The sensitivity of lichens to sulphur dioxide depends on their ability to absorb and retain water and on morphological characteristics of a lichen thallus such as a thick cortex that prevent or impedes the penetration of sulphur dioxide into the thallus (Vernon 1993). Because of the high solubility of SO_2 in water, lichen absorb SO_2 even when not metabolically active and absorption leads to higher total sulphur levels in lichen thallus (Nash 2010). Accumulation of sulphur appeared to be independent of growth form (St Clair et al. 2002). There is a positive relationship between the concentration of sulphur dioxide in the atmosphere and content of sulphur found in lichen thallus (LeBlanc and Rao 1973; Pyatt 1973; LeBlanc et al. 1974; Pakarinen 1981; Takala et al. 1985; Garty et al. 1988; Showman and Long 1992; Oztetik and Cicek 2011). The sulphur content in lichens has been associated quite well with the exposure to sulphur dioxide time (Carreras et al. 1998; Gonzalez and Pignata 2000; Wiseman and Wadleigh 2002; Vingiani et al. 2004; Tretiach et al. 2007a). In some cases, it is difficult to correlate SO_2 exposure and sulphur content in lichens because injured lichens lose their ability to accumulate sulphur due to increased membrane permeability.

The severity of injuries of SO_2 has been related to the concentrations of SO_2: the higher is concentration, the greater symptoms of injuries. Over time lichens could be affected under the SO_2 exposure and the morphological changes occur. Sulphur dioxide affects the growth of lichens: the smaller, more compacted thalli and decrease in cover were recorded (Massara et al. 2009). Sulphur compounds caused reduction or absence of fruiting bodies. Under the exposure, the colour of the lichen thallus could also be changed. For example some lichens could be pigmented or turn to brown. More sensitive species degrade more or less rapidly. Degradation includes discolourations, mostly bleaching due to damage of the photobionts. Some of lichen could become white and detached from the substrate (Saunders 1970).

A destructive effect of SO_2 on lichens was indicated by a statistically significant negative correlation between $\delta^{13}C$ values in the whole lichen and atmospheric sulphur dioxide concentrations (Batts et al. 2004). $\delta^{13}C$ values in lichens in wet environments, in the presence of high SO_2 concentrations, were more negative than that in samples from drier sites. An explanation of this effect may relate to an increase in carboxylation resistance resulting from damage to chlorophyll and cell structure by SO_2 (Batts et al. 2004).

The observed effects of sulphur dioxide on lichens also involve decline in nitrogen fixation (in cyanolichens lichens with bacteria and actinomycetes), decreases in photosynthesis rate, increase in membrane permeability and ultrastructural changes. Correlation between the S content and physiological markers of SO_2 injuries were observed in lichens under the sulphur pollution (Boonpragob and Nash 1990; von Arb et al. 1990; Gonzalez and Pignata 1994). The nitrogen fixation proved to be more susceptible than photosynthesis (Hällgren and Huss 1975; Henriksson and Pearson 1981). Nitrogen fixation rates of *Peltigera canina* decreased when the lichen was exposed to sulphur dioxide (Henriksson and Pearson 1981). Photosynthesis is one of the first processes to be affected by SO_2 pollution (Malhotra and Hocking 1976). Photosynthesis was found to be up to five times more sensitive than respiration to sulphur dioxide (Beekley and Hoffman 1981). Since under the moderate pollution conditions, the opposite results were observed—SO_2 showed the highest positive correlation with net photosynthesis along with increase in chlorophyll content (von Arb et al. 1990). Under the SO_2 fumigation, net photosynthesis rates were more negatively affected in *E. prunastri* than in *R. farinacea* (Deltoro et al. 1999). At low concentration, inhibition of photosynthesis occurs and a prolonged exposition to hydrogen sulphide seems to cause permanent damage to the photosynthetic apparatus, with a decrease in photosynthetic capacity, causing a drastic decrease in thallus growth (Tretiach and Baruffo 2001).

An application of pulse amplitude method (PAM) to measure fluorescence in order to monitor the status of photosystem II (PSII) in lichens exposed to gaseous SO_2. The results indicated rapid induction kinetics of fluorescence upon fumigation, which indicated the inhibition of PSII by SO_2 (Gries et al. 1995). Fluorescence was negatively correlated with S content (Garty et al. 2003).

One of the direct effects of sulphur dioxide on the metabolism of lichens is destruction of photosynthesis pigments. The loss of chlorophyll finally could lead to extinction. With the increasing sulphur dioxide concentrations, a transformation of chlorophyll *a* into phaeophytin along with a reduction of chlorophyll content was observed (Hällgren and Huss 1975; Kong et al. 1999). Chlorophyll reduction in fruticose lichen *E. prunastri* was observed only after severe damage to photosynthesis (Sanz et al. 1992). The experimental results were in accordance with pigment losses reported under the field conditions (Gonzalez and Pignata 1994; Gonzalez et al. 1998; Silberstein et al. 1996a; Garty et al. 1998). The field evidence has suggested that chlorophyll degradation as a result of SO_2 could be affected under the moderate stress conditions (Satya and Upreti 2009).

Lichens exposed to a range of SO_2 concentrations display ultrastructural alterations. Ultrastructural changes in *Cladina stellaris* cells which was affected by SO_2 included lipid accumulation and membrane thickening (Plakunova and Plakunova 1987). Symptoms are characteristic when thallus is ageing. Ultrastructural injuries in exposed *H. physodes* involve swelling of the mitochondria and stretching of thylakoids (Holopainen and Kauppi 1989). At the biochemical level, reduction of protein and lipid synthesis was recorded (Bychek-Guschina et al. 1999). The main effect of SO_2 on cells is acidification, which influence enzymatic reactions (Grube 2010). Lichen sensitivity to acidification could be related to the buffering capacity of secondary metabolites produced by lichens. Usnic acid producing species makes lichens vulnerable to acidity and such species would be rare in areas of high pollution levels (Hauck and Jürgens 2008).

Damage to cell membranes. Lichens under the sulphur stress have been experienced loss of electrolytes (Pearson and Rodgers 1982; Grace et al. 1985). Leakage of electrolytes is a result of damage to cell membranes of the bionts. Exposures of lichens to SO_2 have induced ion efflux, indicating changes in membrane integrity under laboratory conditions (Puckett et al. 1977; Pearson and Henriksson 1981; Fields and St. Clair 1984) or in field studies (Fuchs and Garty 1983; Alebic-Juretic and Arko-Pijevac 1989; Boonpragob and Nash 1990; Garty et al. 1993). The increase in conductivity values in *P. sulcata* was positively related to the increases in SO_2

values in the atmosphere (Marques et al. 2005). The highest cell membrane damage was observed in lichen thallus collected at sites with highest winter SO_2 levels (Alebic-Juretic and Arko-Pijevac 2005). The conductivity of leachates was positively correlated with modelled atmospheric SO_2 concentrations and with bark S (Tarhanen et al. 1996). On the basis of sensitivity, low cost, portability and rapidity, the membrane permeability test has been recommended for evaluation of air pollution damage to lichens (Garty et al. 1993).

Oxidative stress. The response to SO_2 in lichens is an increase in activities of enzymes as a defence against increased oxidant formation. The tolerant lichen *X. parietina* showed increase in peroxidise activity more than that of the sensitive *Ramalina duriaei,* and the activity was higher at polluted than at unpolluted sites (Silberstein et al. 1996b). SO_2-induced lipid peroxidation in biomembranes as indicated by its positive correlation with the MDA content (Egger et al. 1994).

In polluted areas, sulphur dioxide is often in conjunction with other pollutants. Therefore, it is difficult to distinguish the effects of sulphur along with mixture of other pollutants. As it was proved that the direct toxic effects to lichens occur at much lower concentrations than those causing damage to higher plants; thus, lichens is valuable indicator setting critical level for sulphur in the environment. Lichens are useful bioindicators of sulphur pollution because they can absorb and accumulate toxic sulphur compounds and have different sensitivities to them.

4.4.3 Ozone

Ground level ozone is not emitted into the atmosphere but is created by chemical reactions between oxides of nitrogen and volatile organic compounds. Ozone is the most important air oxidant causing injuries to vegetation (Fuhrer 2002). In general, lichens are not considered as sensitive as vascular plants to ozone (Nash and Sigal 1998; Bertuzzi et al. 2013). Comparing historical and recent lichen sampling data, different sensitivity lichen species against ozone were listed by Sigal and Nash (1983). Sensitive species such as *E. prunastri*, *Peltigera* spp. *Pseudocyphellaria* spp. and *Ramalina* spp. disappeared as consequence of this oxidant impact in the Los Angeles. Sensitive species (*Usnea* spp., *Collema nigrescens*) are found in protected areas, and moderately tolerant Hypogymnia enteromorpha are in reduced abundance and show morphological anomalies (thallus bleaching and decay).

Physiological studies showed conflicting results in relation to ozone toxicity. A number of recent experiments with O_3 confirm the statements that it negatively affects lichens. The experimental fumigation with O_3 revealed a significant decline in the efficiency of PSII photochemistry, the collapse of a high number of photobiont cells and as a consequence—bleaching of the thallus (Scheidegger and Schroeter 1995). The decrease in photosynthesis rate was also observed (Ross and Nash 1983; Zambrano and Nash 2000). Under the field conditions among other potential factors, regional ozone levels during the course of the transplant period were sufficiently high to have contributed to the adverse effects on lichens, i.e. reduced photosynthesis and chlorophyll *b* degradation (Zambrano et al. 1999; Zambrano and Nash 2000). Ozone has direct, deleterious effect on lichens through the fast formation of reactive oxygen species—O_3 concentration showed a positive correlation with the contents malondialdehyde and superoxide dismutase) in *H. physodes* (Egger et al. 1994), which is a product of lipid peroxidation and are indicators of oxidative damage to membranes and to the enzymes that protect against oxidation.

By contrast, Calatayud et al. (2000) found that exposure to O_3 for 4 h per day for 14 days did not induced changes in the efficiency of PSII as measured by fluorescence in all of the five lichen species studied. Additionally, neither photosynthetic CO_2 assimilation nor xanthophylls cycle activity was affected by high concentrations of ozone. Exposure of *P. sulcata* to ozone also did not have any effect on Fv/Fm (Balaguer et al. 1996). Under the field conditions, the content of chlorophyll in transplanted *H. physodes* was positively correlated with O_3 concentration (Egger et al. 1994). The results of the fumigation studies suggest that the functionality of photosystems was not impaired by ozone (Calatayud et al. 2000; Riddell et al. 2010, 2012; Bertuzzi et al. 2013).

It is well evident that the effects of ozone under experimental and field conditions included biophysical and physiological, as well as structural impairment in the lichens studied. Despite the evidence of O_3 effects, some researchers suggest that lichens are not good indicators of ozone as no significant consequence to O_3 exposure were detected (Ruoss and Vonarburg 1995; Riddell et al. 2010; Bertuzzi et al. 2013). It is suggested that lichens have significant capacities to withstand oxidative stress induced by high ozone concentrations (Calatayud et al. 2000; Bertuzzi et al. 2013).

4.4.4 Heavy Metals

Heavy metals originate from naturally and anthropogenic sources in the environment. The natural sources of these elements are volcanic eruptions, dusts, fires and salt spray. Anthropogenic sources include emissions from different anthropogenic activities—mining, industrial production (smelters, oil refineries, petrochemical plants and chemical industry) and untreated sewage sludge and from diffuse sources—coal combustion and traffic. The problem is that the amount naturally occurring heavy metals is relatively very small as compared with anthropogenic loads of these elements.

There are some detailed reviews which have referred to the entrapment, uptake and accumulation of heavy metals by lichen thalli, made apparent by parameters of lichen vitality and stress (Garty 2001; Wolterbeek 2002; Szczepaniak and Biziuk 2003; Backor and Loppi 2009).

The main approach identifying pollution by heavy metals with lichens has been the accumulation of metals in lichen thallus. The accumulation process depends on many factors such as environmental conditions (temperature, humidity, wind velocity etc.), characteristics and health of lichen and availability of heavy metal in the environment (Folkenson 1979). It is no doubt that lichens are excellent bioaccumulators of heavy metals because the concentrations found in their thalli have been directly correlated with concentrations in the environment (Pawlik-Skowronska et al. 2008; Conti et al. 2009; Aprile et al. 2010; LeGalley et al. 2013; Hauck et al. 2013a). The metal concentrations in lichens are depended primarily on what metal are in the environment and the chemical and physical properties of the metal containing particles (size and acidity) (Folkenson 1979). The different lichen uptake rates were observed for some heavy metals, and this evidenced the existence of a selective metal uptake. The differences observed in metal uptake are probably due to the composition and type of cell wall binding sites leading to a differential absorption and/or cation exchange (Carreras and Pignata 2007). Metals with a higher affinity for binding sites than divalent cations could saturate the absorption capacity of lichen thalli and thus block the incorporation of other cations (Gailey and Lloyd 1993).

The tolerance of lichen to accumulate elements varies greatly between species and elements (Folkeson 1979). Species-specific information on accumulation capacity is scarce. One method for the evaluation of the accumulation capacity is the index of exposed-to-control ratio (E/C) (Frati et al. 2005; Bergamaschi et al. 2007; Aprile et al. 2010), which helps to compare on the same scale the responses of the different biomonitors after exposure. Some lichen species could be considered as “severe accumulators”. Transplantation studies have highlighted that lichen could accumulate metals by several times comparing with the initial concentration in its thalli: the E/C ratios investigated in all sites show “severe accumulation” for *F. caperata,* while for *Parmotrema chinense* they indicate “accumulation” (Aprile et al. 2010). *H. physodes*, *P. furfuracea* and *Usnea hirta* transplanted to an urban site of Italy have a similar accumulation capacity, while that of *P. sulcata* is lower (Bergamaschi et al. 2007).

The accumulation capacity could be different for some elements. The accumulation capacities of two species—*X. parietina* and *Parmelia tiliacea*

—differed only for the elements Ca, Cl, Cu and Pb (Karakas and Tuncel 2004). A relative high concentration of K, Fe, Mg, Zn, Mn, Ph, and Cu was found in thalli of *H. physodes,* and Mg, Zn, Pb, Cu, and Cd in *U. hirta* transplanted to traffic sites (Garty et al. 1996). Other species could have low accumulation capacity and could be considered as weak accumulators. The concentrations of heavy metals in *Cladonia* spp. thalli were relatively low considering the frequently enormous substrate contamination and for example such species could have ecological importance as stable and resistant pioneer in anthropogenically affected sites (Osyczka and Rola 2013).

Some lichen species are considered to be more suitable for transplanting and heavy metal accumulation. The lichen species with finely divided or densely sorediate thalli usually showed a greater affinity for collecting particulate matter (Garty 2001; Aprile et al. 2010). Surface development strongly influenced accumulation capacity of the biomonitors (Adamo et al. 2007). The accumulating capacity of *P. tiliacea* was higher than that of *X. parietina* and this difference proposed would be due to morphological differences in lichen features (Sloof 1993; Karakas and Tuncel 2004). Other properties such as cation exchange capacity and amount of ligands could be also important (Folkenson 1979). A lower incorporation of some metallic cations could be due to the formation of weak complexes with cell wall ligands that can consequently be easily removed and replaced by cations with a higher affinity for the cell wall binding sites (Carreras and Pignata 2007).

Some heavy metals induce inhibition of chlorophyll synthesis. Chlorophyll *a* was sensitive to the presence of copper and mercury but chlorophyll *b* increased in response to copper and zinc exposure (Backor and Dzubaj 2004). As a result, a significant decrease of the chlorophyll *a/b* ratio was found, while the total chlorophyll content was almost unchanged. Thus, the total content of chlorophyll is not suitable indicator of physiological damage. Chlorophyll *a* was more sensitive to metals than chlorophyll *b*, and its decrease was attributable to conversion of chlorophyll *a* to chlorophyll *b* by copper (Chettri et al. 1998). It has been suggested that the Chl *b*/Chl *a* ratio is a useful parameter to determine the physiological conditions of lichens and photobionts subjected to air pollution and heavy metals (Chettri et al. 1998; Carreras and Pignata 2007).

High negative correlations between Al and pigment concentrations were found (Nakajima et al. 2013) which suggests that Al stress reduces pigment concentrations. Accumulation of Al, Cr, Fe, Pb and Zn in the thallus suppressed the concentrations of pigments such as chlorophyll *a*, chlorophyll *b* and total chlorophyll, however, enhanced the level of protein (Bajpai et al. 2010). Short-term exposure to high Cu^{2+} concentrations (40 and 400 mu mol g^{-1}) under non-complexing conditions caused a dose-dependent decrease in chlorophyll *a* content; chlorophyll *b* and total carotenoid contents remained constant (Pawlik-Skowronska et al. 2006). It has been observed that Pb and Cd are mainly complexed to the fungal cell wall (Di Toppi et al. 2005; Sarret et al. 1998) and thus do not have any detrimental effects on physiological parameters in lichens. These ions could be tightly bound to binding sites of the mycobiont cell wall forming stable complexes (Carreras and Pignata 2007).

Some studies showed that there is correlation between chlorophyll integrity and the concentrations of heavy metals. The chlorophyll integrity decreased with increase in amount of Cu, Pb and Zn (Shukla and Upreti 2008). The integrity of chlorophyll correlated with the concentration of K and correlated inversely with the concentration of Cr, Fe, Mn, Ni, Pb and B (Garty et al. 1998). The chlorophyll integrity was especially affected following treatment of the thalli with Cu, Zn, Mn, Pb and Fe salts under acidic conditions (Garty et al. 1992). The chlorophyll degradation as well as alteration in the pigment content was the most sensitive parameters to assess the vitality of lichen thallus against polluted environment (Karakoti et al. 2014).

The lichen vitality under the metal stress was examined using the potential quantum yield of photosynthesis expressed as fluorescence ratio Fv/Fm. The Fv/Fm ratios were correlated with Al, Cd, Cr and Cu elements in lichens transplanted or naturally growing at sites with different degree of pollution level (Garty et al. 2000; Karakoti et al. 2014). Significantly positive correlations between Fv/Fm and element contents (Pb, Cu, Fe and Cr) were found in naturally growing lichen *Pyxine cocoes* (Karakoti et al. 2014). Garty et al. (2000) found that Fv/Fm ratios in transplanted *Ramalina lacera* were correlated with Al, Cd, Cr and Cu elements. The decreased Fv/Fm values of experimentally treated lichens and their bionts with different metal concentrations were also reported (Backor et al. 2004; Unal et al. 2010; Vantova et al. 2013). Cadmium induced the decrease in Fv/Fm values indicating damage to PSII (Backor et al. 2007).

Calcium, Cu and Sr showed an inverse correlation with the Fv/Fm ratio expressing the potential quantum yield of PSII (Garty et al. 2002). In *Ramalina fastigiata* intracellular Cu concentrations above ca. 4.0 mu mol g^{-1} were linked to a decline in chlorophyll fluorescence. The fluorescence parameter Fv/Fm was shown to be useful in determining the sensitivity of the lichens to Cu uptake (Branquinho et al. 1997a).

Pb uptake, particularly in cyanobiont lichens, caused a decrease in photosystem II (PSII) photochemical reactions, measured as a change in the ratio Fv/Fm (Branquinho et al. 1997b). Lichen PSII photochemical reactions were more sensitive to Pb than cell membrane damage since, with increasing Pb concentrations, no clear alterations in lichen membrane permeability, as measured by the loss of intracellular K and Mg, were observed.

Regarding the effects of the cations studied on the physiological parameters measured in *U. amblyoclada*, the following sequence could explain their toxicity: $Cu^{2+} > Pb^{2+} > Ni^{2+} > Zn^{2+}$ (Carrearas and Pignata 2007). Metal cations appeared to be ionically bound within the cell wall in an exchangeable form with binding affinities of Pb > Cu > Zn (Chettri et al. 1998).

Among the two bionts, the algal partner appears to be more susceptible to Cd stress, probably because of the presence of delicate and sensitive components such as the chloroplast and photosynthetic pigments (Di Toppi et al. 2005). Heavy metals have induced oxidative stress as indicated enhancement of GSH concentrations and GST and SOD activities (Cuny et al. 2004; Oztetik and Cicek 2011; Fig. 4.3). Monnet et al. (2006) documented an increase in antioxidant activities (SOD, CAT and APX) with increased Cu concentrations. Concentrations of stress-ethylene correlated positively with Al, Ba, Pb, S and V content and negatively with Cu and Sn (Garty et al. 2003).

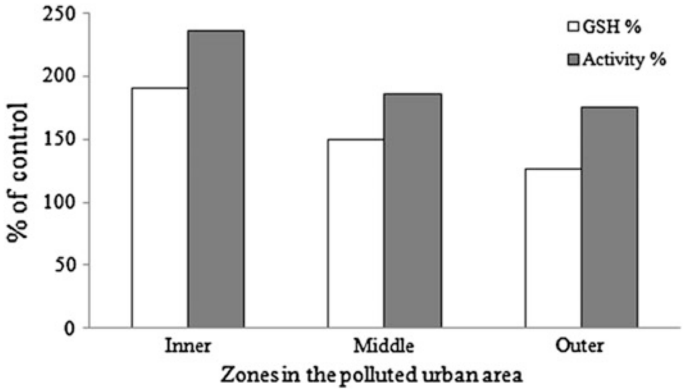

Fig. 4.3 Effects of air pollution on glutathione (*GSH*) concentrations and on the glutathione S-transferase (*GST*) enzyme activities in transplanted epiphytic lichen *Pseudevernia furfuracea*. The values represent the % difference from the control. The oxidative stress biomarkers were related with higher level of heavy metals in the inner zone as a result of heating houses, exhaust gases and industrial activities (Oztetik and Cicek 2011)

Various studies showed that cell membrane damage are related with the concentrations of elements. The extent of cell membrane integrity may be evaluated by measuring electrical conductivity. Electric conductivity values correlated positively with B, Fe, Mg, Mn, Na, Pb, Sn and Ti content (Garty et al. 2003). Calcium, Cr, Cu, Mg, Na and Sr showed a positive correlation with the electrical conductivity corresponding with cell membrane disintegration (Garty et al. 2002). High concentrations of Ni, Mg and B coincided with damage caused to cell membranes (Garty et al. 1998). Membrane integrity was highly correlated with the presence of Ca (Beckett and Brown 1984).

4.4.5 Persistent Organic Pollutants (POPs)

High toxicity, bioaccumulation and long persistence in the environment is characteristic for the POPs. The main pollution sources are related with human activities—industrial processes, domestic heating and traffic (Boström et al. 2002). Recently, a detail review of biomonitoring POPs using lichens was given by Augusto et al. (2013a).

The evaluation of the impact of POPs is receiving increasing attention because of their mutagenic and carcinogenic properties. Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs) are important in environmental health studies due to their carcinogenic and potentially toxic effects, even in very low concentration (Augusto et al. 2007). PAHs persist in the environment for long periods due to their relatively stable molecular structure and tend to accumulate in organisms.

PAHs pollution present in the ecosystem can be efficiently detected by lichen biomonitoring techniques, and a large number of PAH accumulation in lichens from different regions of the world are available (Guidotti et al. 2003; Blasco et al. 2006, 2011; Augusto et al. 2009a; Shukla et al. 2010, 2013; Nascimbene et al. 2014; Protano et al. 2014).

Lichens accumulate PAHs over detection limits with highest values being during winter and the lowest—during summer (Augusto et al. 2013b). Interception and accumulation of POPs by lichens are dependent on the structure of the lichen thallus. Fruticose lichens accumulate higher concentrations of POPs than foliose lichens (Augusto et al. 2009a; Blasco et al. 2011). The results of the latter study showed that foliose lichens such as *E. prunastri*, *Usnea* sp. or *R. farinacea* accumulated more low molecular weight POPs (3- and 4-ringed PAHs) than foliose lichen *P. sulcata* which was enriched in high molecular weight POPs (5 and 6-ringed PAHs). Lichen biomonitoring studies indicated that PAHs with 2–4 rings are dominated in lichen thallus (Augusto et al. 2010; Blasco et al. 2011; Bajpai et al. 2013; Shukla et al. 2013; Nascimbene et al. 2014; Protano et al. 2014) while in the case of PCDD/Fs, the lichen accumulated mostly from TCDD/Fs to PeCDD/Fs (Augusto et al. 2007). Lichens accumulated higher PAH concentrations when compared with other environmental indicators (Fig. 4.4) and its concentrations were significantly and linearly correlated with concentrations of PAHs in soil (Augusto et al. 2010). The accumulation process was more intensive when lichens were hydrated than when they were desiccated (Kylin and Bouwman 2012). Accumulated airborne PAHs in lichens was proportional to traffic density (Guidotti et al. 2009; Shukla and Upreti 2009; Blasco et al. 2011).

Regarding the physiological changes, the chlorophyll degradation and protein content were found to be the most sensitive parameters to assess the vitality of lichen thallus against PAHs pollution (Bajpai et al. 2013).

It was argued that PCDD/F in lichens may be used as spatial estimators of the potential risk of inhalation by the population present in the area (Augusto et al. 2007). Using spatial models of PAHs accumulated in lichens, it was possible to fingerprint multiple sources of atmospheric PAHs at regional scale (Augusto et al. 2009b). When comparing several POPs in lichens, spruce needles and passive air sampling devices, it was found that lichens accumulated the highest

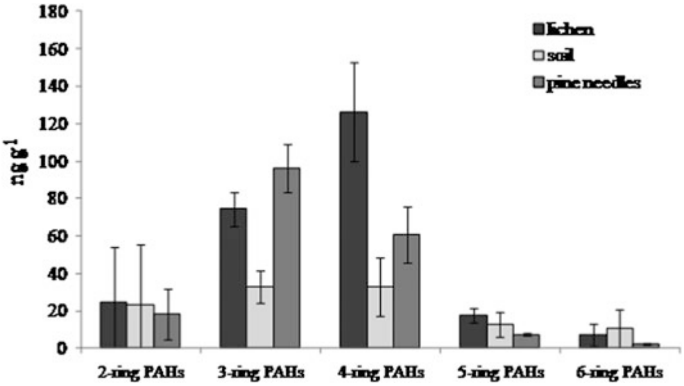

Fig. 4.4 PAH concentrations (ng g^{-1}) in lichens, soil and pine needles. Bars represent standard deviations (Augusto et al. 2010)

content (Ockenden et al. 1998; Migaszewski et al. 2002; Schrlau et al. 2011). Temporal variation of levels of PAHs in air and in lichens is in accordance (Augusto et al. 2013a).

4.5 Conclusions

In order to develop an early warning system and to avoid detrimental effects of pollutants on living organisms, lichens can be used as an excellent bioindicators to monitor atmospheric quality. Various studies have highlighted the relevance of both physiological and biochemical properties of lichens, which attributes to the biomonitoring potential of lichen species. In the present review, characteristic physiological attributes and lichen response to different pollutants have been discussed in detail.

The changes in physiological and biochemical parameters as a response to pollution are important for assessment of ambient air quality changes, but lichen vitality and diversity are influenced by number of other factors. Changes could be induced by microclimatic changes as different light regimes, temperature, drought or humidity, substrates and other environmental factors. Therefore, in order to draw any conclusion on the effect of pollution on lichens, these environmental parameters especially microclimatic changes should be taken into consideration.

Reproducibility of the data and comparison of various studies or datasets can be ensured by following standardized protocol for sampling and preservation of the lichen samples.

Lichens occur in all available substrata and geographical conditions; therefore, they are exposed to different ambient stresses too. In order to withstand such stresses, lichens have evolved complex mechanism of exclusion or avoidance which is still not well understood. Lichens respond differently to different pollutants/abiotic/biotic stresses depending on the type and nature of pollution and the lichen species involved. Therefore, it is very important to clarify and classify the causes of differential sensitivity of different lichen species towards various pollutants.

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Bio-monitoring in Western North America: What Can Lichens Tell Us About Ecological Disturbances?

5

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Contents

Abstract

Throughout modern history, many habitats across western North America have faced continuous anthropogenic disturbances. Mining, forestry, agriculture, grazing, industrial and residential development, and air pollution have altered natural habitats to varying degrees. Lichens have been considered 'a canary in the coalmine' and can provide important insights into the biological impact of human-related disturbances. Here, we discuss the role of lichens as bio-monitors in the intermountain region of western North America.

Keywords

Air pollution · Bio-accumulation · Bio-indicator · Climate change · Eutrophication · Mining

5.1 Introduction

Lichens are listed among a suite of ecosystem health indicators, which also includes some terrestrial and aquatic invertebrates (Hodkinson and Jackson 2005), nematodes (Bongers and Ferris 1999), bryophytes (Frego 2007; Pesch and Schroeder 2006), vascular plants (Coulston et al. 2003), and other sensitive species and/or communities. Rather than abiotic metrics, bio-indicators are likened to canaries in a coalmine, serving as a direct surrogate for assessing disturbances on biological communities. Lichens

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are particularly useful as bio-indicators due to the fact that many live and grow continuously for decades, or even hundreds of years, showing cumulative responses to changes in atmospheric pollution levels, land management practices, and climate change. Others are inherently sensitive to some particularly important pollutants, and their presence or absence can be indicative of current ecological conditions. Measurable responses of individual lichen thalli (e.g., differential accumulation of atmospheric pollutants) and lichen communities (e.g., changes in community composition and population density) can provide a means to quantitatively assess ecosystem health (McCune 2000) and provide a record of the kinds, and relative quantities, of air pollutants (Loppi and Pirintsos 2003; St. Clair et al. 2002b; Hawksworth 1971).

As lichens have no vascular system, they obtain mineral nutrients and co-occurring contaminants through either wet or dry deposition, rather than from their substrates (Agnan et al. 2014). Dry deposition involves the deposition of airborne gases or other particles due to increasing influence of gravity with decreasing wind speed. In contrast, wet deposition involves airborne aerosols and particles that are deposited on lichen thalli during precipitation events (i.e., fog, dew, snow, and rain) (Fenn et al. 2003; St. Clair et al. 2002b).As a result, the general indiscriminant accumulation of atmospheric outwash by lichens also makes them useful as bio-monitors of air quality (Hawksworth 1971; Henderson and Seaward 1979; Geiser and Neitlich 2007; Jovan 2008).

Monitoring air pollutant concentrations in lichen thalli can thus be used as an indirect measure of the potential impact of air pollution on other less sensitive biological systems. Patterns of element accumulation by lichens can also be used to document the occurrence and potential biological impact of specific sources of air pollution (Table 5.1). While in some cases lichens may simply accumulate and concentrate atmospheric pollutants with little noticeable physiological impact, in other cases, pollutants may reach toxic concentrations and the most sensitive lichens tend to disappear from highly polluted areas (Nash 1975; Branquinho et al. 1999). For example, the lichen *Hypogymnia physodes* was completely absent in close proximity to a heavily polluted smelter town in the Ural Mountains, Karabash, Russia, and occurrences of the lichen in nearby regions consistently showed elevated concentrations of heavy metals (Purvis et al. 2004). Furthermore, healthy lichen transplants set up near the smelter showed visible damage within three months, correlated to high concentrations of K, S, and toxic metals (Purvis et al. 2004).

Documenting the impact of air pollution on biological systems using lichens as bio-indicators generally involves four basic approaches: (1) elemental analysis of potential pollutants accumulated by lichen thalli; (2) assessing the presence or absence of pollution-sensitive, or conversely, pollution-tolerant, indicator species; (3) transplant studies assessing changes in appearance, physiology, growth rates, etc.; and (4) ecological assessments of lichen community structure (Nimis et al. 2002; St. Clair 1999; McCune and Geiser 2009). Lichens have been used to monitor a variety of atmospheric pollutants, including sulfur dioxide, ozone, nitrous oxides, peroxyacetyl nitrates (PAN), fluorides,

Table 5.1 A list of major sources of pollution and associated pollutants that are commonly monitored using lichens

Pollutant source	Characteristic pollutants
Coal-fired power plants	Hg, S, $\delta^{34}S$, SO_2, NO_X
Mining	Ore metals
Agriculture	N (NH_3), S, and phosphate
Vehicle exhaust	Cr, Ni, N (NO and NO_2), V, and T
Tire and brake wear	Ti, Fe, Cu, Sb, Zr, ZnO, and Ba
Nuclear radiation	^{106}Ru, ^{137}Cs, and ^{144}Ce
Aluminum manufacturing	F

Sources (Adachi and Tainosho 2004; Thorpe and Harrison 2008; McCune and Geiser 2009; Nimis et al. 2002) aromatic compounds, various metals, and radioactive nuclides (St. Clair 1999; Nash 2008; Nimis et al. 2002; McCune and Geiser 2009).

While bio-monitoring studies evaluating airborne element accumulation patterns relative to a specific pollution source are common (Rose and Hawksworth 1981; Seaward 1993; Naeth and Wilkinson 2008; Aznar et al. 2008; Perlmutter 2010), directly correlating element concentrations in the air with lichen-accumulated elements are confounded by a number of factors (Jovan 2008; Riddell et al. 2011; Will-Wolf et al. 2006; Will-Wolf 2010). For example, although data suggest that the lichen genus *Parmelia* may be a useful indicator of atmospheric particulates in the city of Sendai, Japan, attempts to definitively correlate element air concentrations and lichen-accumulated elements were largely unsuccessful (Saeki et al. 1977). Not surprisingly, lichen morphology can influence, to some degree, the rate at which lichens accumulate elements from the atmosphere, and at least in some cases, it appears that foliose lichens may more effectively accumulate airborne pollutants than fruticose species (St. Clair et al. 2002a, b; Lawrey and Hale 1981). Similarly, different lichen species respond differently to air pollution. Some lichens are sensitive to specific pollutants and relatively insensitive to others. Differential sensitivity is likely related to several factors, including growth form, substrate chemistry and pH, and other lichen-specific physiological differences. Ultimately, attempts to correlate air pollution effects with a specific response are often difficult (Riddell et al. 2011), and data from lichen air quality bio-monitoring surveys require cautious interpretation and limited extrapolation (Nash 2008; Will-Wolf et al. 2006).

In this chapter, we discuss the roles of lichens in assessing air quality and ecological health in the intermountain region of western North America. Geographically, the Intermountain West is located between the rocky mountains on the east and Cascade and Sierra Nevada ranges on the west and extends north from Arizona and New Mexico into British Columbia, Canada. In the USA, the region contains a diverse array of habitats, ranging from low elevation arid deserts (e.g., the Mojave Desert) to inland temperate rainforests (e.g., portions of the Cabinet Mountains Wilderness Area in western Montana) to alpine tundra habitats (e.g., High Uinta Wilderness Area in northeastern Utah) (Dellasala et al. 2011). Throughout modern history, many habitats across western North America have faced continuous anthropogenic disturbances, and bio-monitoring with lichens has the potential to offer valuable insights into the impact of historic, contemporary, and projected disturbances in the region.

5.2 Bio-monitoring with Lichens in the Intermountain West

Throughout western North America, forestry, grazing, mining, energy development and processing, and recreation have played a major role in determining land-use strategies and economic development of federal, state, and private lands. To varying degrees, these land-use practices have had a substantial ecological impact, including habitat and community alteration, increased levels of pollutants, and the introduction and spread of non-native species (Fleischner 1994; Miller et al. 1989). Additionally, air pollution, altered fire regimes, and other broadscale disturbances have been shown to impact ecological health throughout the intermountain region (Smith et al. 2000; Miller et al. 1989). In spite of historic and ongoing disturbances, large tracks of quality habitat have been protected and managed by federal, state, and private agencies. Public lands—including state-owned, Bureau of Land Management (BLM), United States Department of Agriculture, Forest Service (USFS), National Park Service (NPS), National Wildlife Refuge (NWR), and military lands—comprise a substantial percentage of the total land area in the Intermountain West, ranging from ca. 35 % in Montana to over 81 % in Nevada. Effectively monitoring the ecological impact of anthropogenic disturbances can play an important role in assessing general ecological health while providing informed direction for future land-use strategies in western North America (Dillman 1996; Hyvärinen and Crittenden 1998).

Altitudinal, geological, and climatic variation create diverse habitats supporting distinct and varied lichen communities throughout the intermountain region of western North America. For over thirty years, lichens have been used to assess spatial and temporal patterns of atmospheric pollution in the Intermountain West. As part of a long-term bio-monitoring program, we have established over 350 reference sites throughout the intermountain region. Reference sites are selected to monitor both broadscale and more localized disturbances. The majority of our reference sites are found on land managed by the USFS, but the program also includes reference sites on lands managed by the BLM and NPS. For each reference site, we compile a comprehensive checklist of all lichens occurring at the site, document the presence (or absence) of pollution-sensitive indicator species, and assess mean concentrations of over 25 potential pollutant elements accumulated by documented sensitive lichen species. Due to the fact that certain lichen species are inherently sensitive to airborne contaminants, air pollution-related changes can be effectively documented by periodic reviews of reference sites and comparisons to original baseline data. Overall, our goal is to establish valuable baseline data and periodically review reference sites to effectively monitor ecological health throughout the Intermountain West using lichens. Generally, we plan to re-evaluate vulnerable reference sites on a three- to five-year cycle and more stable sites every 5–10 years; and the vast majority of our sites have been resampled at least once.

In recent decades, a number of policy changes have been implemented to mitigate the environmental impact of atmospheric pollution and other anthropogenic disturbances. In spite of these changes, both historic and contemporary disturbances continue to impact lands throughout western North America. While contemporary data and onetime assessments can provide critical insights into the general ecological health of a region or site, the strength of any bio-monitoring program is related to consistent reviews and long-term assessments of subsequent reviews compared back to the baseline data. Our ongoing lichen air quality bio-monitoring program at sites throughout the intermountain region offers a unique and valuable perspective into the ecological health across the region and affords opportunity for future long-term research.

5.2.1 Lichens as Air Quality Bio-indicators in the Intermountain West

A striking example of the role of lichens as bio-indicators of air quality in western North America is reflected in the major changes observed in lichen communities in southern California's South Air Basin, which includes the heavily urbanized Los Angeles and Orange Counties (Riddell et al. 2011). Historic records from the early 1900s and more recent surveys in the 1970s and then again in 2008 reveal an ongoing pattern of declining species ranges and community shifts. For example, high levels of N deposition have been documented in the Los Angeles Basin over the last 30 years, and several nitrophilous lichen genera, *Physcia*, *Physconia*, and *Xanthomendoza*, have steadily increased in abundance. Conversely, one of the most pollution-sensitive lichens remaining in the basin region, *Melanohalea subolivacea*, showed significant decline between 1976 and 2008, and no sensitive indicator species have returned since the 1976 inventory (Riddell et al. 2011).

In the intermountain region, the most common air pollution-sensitive indicator lichens used for elemental analyses include the following: *Alectoria sarmentosa* (epiphyte), *Bryoria fremontii* (epiphyte), *Letharia vulpina* (epiphyte), *Rhizoplaca melanophthalma* (saxicolous), and various *Xanthoparmelia* species (saxicolous and terricolous). *Xanthomendoza montana* (epiphyte) has also been collected and analyzed from a number of bio-monitoring reference sites; however, this species actively accumulates nitrogen and sulfur and consistently shows unusually elevated levels of these two elements. Additional research may ultimately provide important insights into varying elemental accumulation patterns among acidophytes, neutrophytes, and nitrophytes.

Various analytical techniques, including atomic absorption spectrometry, inductively coupled plasma emission spectroscopy (ICP), X-ray fluorescence spectrometry, and proton-induced X-ray emission technology (PIXE), have been used to determine pollutant element concentrations in lichen thalli (St. Clair 1999; Nimis et al. 2002). Care must be taken when collecting, preparing, and analyzing lichen material for elemental analysis. Samples should be collected with a ceramic knife to avoid metal contamination, and additional care must be taken to avoid other potential contaminants during collection, processing, and storage. Samples should be completely dried and stored in sterile plastic bags, avoiding the use of commonly used paper bags for more general collecting. Nasco Whirl-Pak 18 oz. plastic bags prevent contamination of samples and offer an effective and relatively inexpensive container for long-term storage of elemental analysis samples (St. Clair 1999).

An alternative, or often complementary, approach to elemental analysis for assessing air quality is tracking the presence or absence of sensitive indicator species—usually the larger, conspicuous macrolichens (McCune and Geiser 2009). A floristic survey at bio-monitoring reference sites, including collecting and identifying lichen species from as many habitats and substrates in the study area as possible, provides a general list of the lichens occurring at each site, and from the general list of lichens, a second list restricted to the documented sensitive indicator species and their relative abundance is compiled (St. Clair 1999). Periodic re-evaluations of the lichen flora, and particularly sensitive indicator lichens, at reference sites are central to documenting temporal changes in the relative abundance of indicator species and assessing the impact of changing air quality through time. Air quality ratings for many lichens occurring in western North America are found in McCune and Geiser (2009), St. Clair (1999), and the USDA Forest Service "National Lichens and Air Quality Database and Clearinghouse" (http://gis.nacse.org/lichenair/), providing important resources for qualitatively assessing the impact of air quality based on the occurrence of sensitive indicator lichens. Sensitive lichens should be assessed for air pollution-related changes, including bleaching of the upper cortex, thallus discoloration, necrotic spots, hyper-production of asexual reproductive propagules, or other abnormalities in thallus morphology. Consistent patterns in thallus damage can be indicative of air pollution-induced stress. The establishment of lichen air quality bio-monitoring references sites is limited by a number of practical constraints, most notably, the significant investment of time and the high level of training and expertise required. Given the environmental heterogeneity of lands in western North America and the species-specific microhabitat conditions required to support lichens, the absence of sensitive indicator species may not necessarily reflect poor air quality. Therefore, subjectively selecting reference sites that are likely to support sensitive indicator species, incorporating elemental analysis of lichen thalli to determine levels of atmospheric pollutants, and consistent periodic reviews are essential requirements for effectively monitoring air pollution-related impacts over time.

Assessing ecological health and recovery may be particularly important in areas that have been severely impacted by air pollution. However, impacted sites generally lack baseline floristic surveys and information about pollutant element concentrations in lichen thalli (McMurray et al. 2013; Riddell et al. 2011; Howe and Lendemer 2011). In these cases, transplant studies may provide important insights concerning the current health and recovery potential of damaged sites. Ideally, lichens thought to be native to the damaged site are transplanted from adjacent clean-air sites, and changes to physiological parameters (ex. photosynthetic activity, membrane leakage, etc.) are evaluated over time (Riddell et al. 2011; Nimis et al. 2002). Transplant samples may also be analyzed to evaluate patterns of atmospheric pollutant accumulation (Conti and Cecchetti 2001). Data obtained from transplant studies can then be used as an artificial baseline for assessing future air quality-related impact or recovery in habitats that have previously sustained significant air pollution-related damage (St. Clair 1999).

Thorough ecological surveys and community analyses likely yield the most comprehensive assessment of air pollution-related impact (McCune and Geiser 2009). This type of study generates data that can be directly used for evaluating air pollution-related effects on lichen species diversity, air quality, and climate, although they require high levels of training and expertise, a substantial investment of time and manpower, and the application of sophisticated statistical techniques to effectively separate the major factors influencing lichen community composition and structure. The USFS Forest Inventory and Analysis program (FIA) includes lichens among a suite of other forest health indicators (Stolte 2001; Will-Wolf 2010). The FIA program uses specially trained field crews to periodically survey epiphytic macrolichen communities to evaluate and monitor biodiversity, air quality, and climate (Will-Wolf 2010). In western North America, this approach has provided detailed insights into the responses of epiphytic lichens and lichen communities to air pollution and climate change (Geiser et al. 2010; Hyvärinen and Crittenden 1998; Jovan 2008; Jovan and Carlberg 2007). Although the FIA program provides an effective strategy for indicating particular environmental conditions in specific lichen communities (Will-Wolf 2010), interpretation of lichen species and community composition should not be generalized beyond the geographic and ecological scale of the observation (Will-Wolf et al. 2006). In the intermountain region of western North America, the FIA program may be difficult to implement for a number of reasons, including the dominance of arid and semiarid habitats with vegetation types that do not fit the FIA model. In addition, many intermountain area forests are relatively depauperate of epiphytic macrolichens due to climatic conditions rather than poor air quality or environmental health-related issues, and incorporating saxicolous sensitive indicator lichens is not a part of the FIA sampling protocol.

One of the best known examples of the use of lichens as bio-monitors of air pollution involves efforts to monitor sulfur dioxide (SO_2) levels (Nash and Gries 2002; Seaward 1993). In the Intermountain West, the major sources of SO_2 pollution include coal-burning power plants, diesel consumption, petroleum processing, and ore smelting. However, beginning in the 1970s, SO_2 emission levels have continuously declined in North America and Europe due to legislation regulating SO_2 emissions (Mylona 1996; Ellerman and Montero 1998). This dramatic decrease in SO_2 emissions has resulted in the recolonization of many SO_2—sensitive lichens into urban areas in Europe formerly impacted by poor air quality (Henderson and Seaward 1979; Rose and Hawksworth 1981; Seaward and Letrouit-Galinou 1991). However, recovery patterns associated with decreasing SO_2 emissions have not generally been observed in the intermountain region of western North America, although this may be due to a combination of factors including a more recent history of industrialization, a general lack of pre-industrialization baseline studies of lichen species diversity, and generally lower human population densities.

Anthropogenic-related increases in the levels of atmospheric sulfur (S) and nitrogen (N) are known to have potentially damaging effects on both terrestrial and aquatic ecosystems (Fowler et al. 2013). For example, even small increases in N deposition can alter ecosystem processes in N-limited environments, and previous studies suggest that current levels of N deposition are predicted to adversely affect a range of sensitive ecosystems in the western North America (Fenn et al. 2008; McMurray et al. 2013; Geiser et al. 2010; Glavich and Geiser 2008; Hyvärinen and Crittenden 1998; Jovan and Carlberg 2007). The primary nitrogen-containing atmospheric pollutants are nitrogen oxides and ammonia, but may also include further oxidized forms including, ammonium, nitrates, and nitric acid vapor

(McCune and Geiser 2009). While fossil fuel combustion, forest fires, and combustion of other forms of biomass release nitrogen oxides, ammonia waste is a common by-product of agriculture (i.e., fertilizers and animal waste). Nitrogen deposition rates are largely unknown for most areas in the Intermountain West, but the available data indicate that over much of the region, N deposition rates range from 1–4 to 30–90 kg/ha/year downwind of major air pollution sources, particularly agricultural and urban areas (Fenn et al. 2003). Rapid expansion of natural gas drilling throughout the intermountain region has raised important concerns about the potential ecological impact of increased N deposition associated with oil and gas extraction and processing. Analysis of lichen thalli for total nitrogen is known to accurately document N deposition (McMurray et al. 2013; Fenn et al. 2008; Hyvärinen and Crittenden 1998; Root et al. 2013).

Long-term bio-monitoring networks will be crucial for evaluating the impact of increased N deposition associated with oil and gas development, urban development, increased vehicular traffic, and large concentrations of domestic animal feeding operations. Root et al. (2013) proposed a simple model to monitor N deposition across broad spatial and topographical regions in western North America using widespread, easily recognized lichens. This approach offers particular utility to federal land managers for assessing N critical load exceedance through a cost-effective approach for assessing air quality in their management units, identifying vulnerable areas for more intensive monitoring, and directly assessing the impact of potential sources of air pollution (e.g., vehicular traffic).

5.2.2 Monitoring Past Disturbances

Pollutants associated with abandoned or uncontrolled areas pose significant risk to both ecosystems and human health at hundreds of sites throughout the Intermountain West. For example, heavy metals and arsenic waste from abandoned mining operations are artifacts of the prominent mining history of western North America. The impact of mining and smelting activities on lichens is well documented (Howe and Lendemer 2011; St. Clair et al. 2002a; Bargagli et al. 2002; Rusu et al. 2006). The US Federal Superfund program, operated under the management of the US Environmental Protection Agency, identifies, analyzes, and remediates hazardous waste sites in an attempt to improve the overall health of the region. In western Montana, 19 sites have been listed on the National Priorities List (NPL) and are eligible for long-term remedial action through the Superfund program (Fig. 5.1). In western North America, the majority of these NPL Superfund sites are the direct result of historic mining and smelting operations.

Beginning in 1992, a total of 49 lichen air quality bio-monitoring reference sites were established in or near eight wilderness areas in western Montana and the Idaho Panhandle (Fig. 5.1), including sites in the Anaconda-Pintler (including the Mount Haggin Wildlife Management Area), Bob Marshall, Cabinet Mountains, Gates of the Mountains, Gospel Hump, Mission Mountains, Scapegoat, and Selway Bitterroot wilderness areas (St. Clair et al. 2013). These bio-monitoring reference sites have been systematically reviewed and offer an ongoing perspective into the ecological health of western Montana. A number of air quality bio-monitoring reference sites have been established in and near the Mount Haggin Wildlife Management Area and the Gates of the Mountains Wilderness Area in western Montana. Several of these reference sites were established in close geographic proximity to decommissioned smelters which are now Superfund sites, including the Anaconda copper smelter, operated from 1919 until 1981, and the East Helena lead smelter (1888–2001) (Fig. 5.1).

At most bio-monitoring reference sites in western Montana, the lichen communities appear to be diverse and healthy, suggesting generally positive ecological conditions in the wilderness areas in western Montana (St. Clair et al. 2013). Additionally, the number of sensitive indicator species per reference site has remained high in

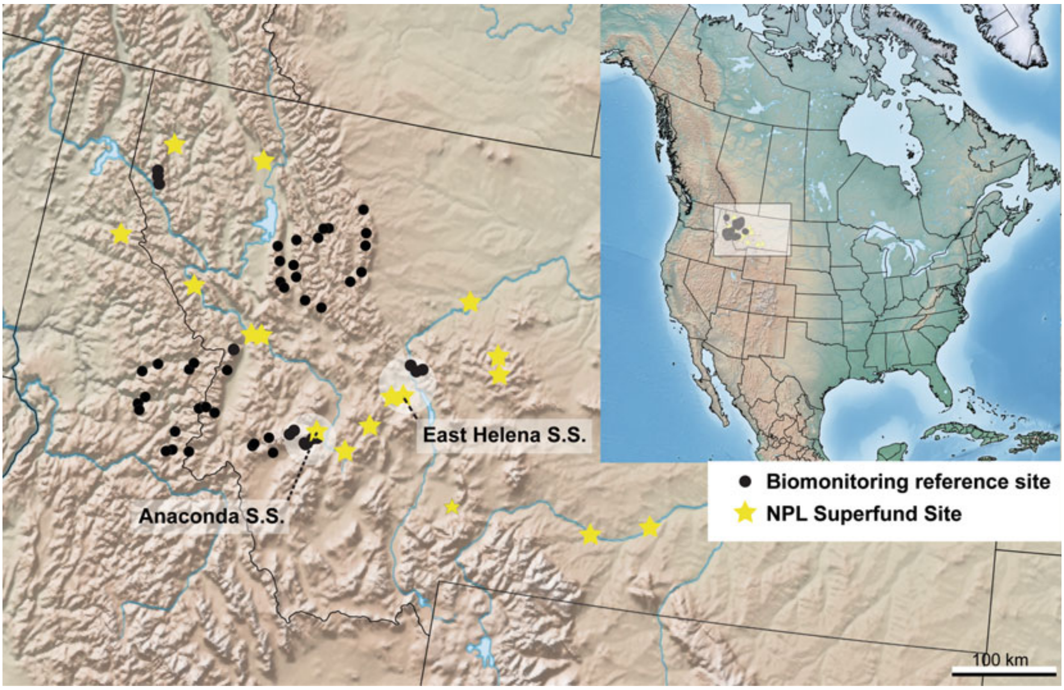

Fig. 5.1 Distribution of air quality bio-monitoring reference sites in western Montana and the Idaho Panhandle, USA. National Priority List (*NPL*). Superfund sites in Montana are indicated with *yellow stars*, and the Anaconda copper smelter and East Helena smelter Superfunds sites are highlighted on the map

comparison to other similar sites in the Intermountain western USA. While elemental analysis data over the entire study period have shown some minor fluctuations in the concentrations of some pollutant elements, the lichen flora of western Montana generally remains free from air pollution-related impact. However, the impact of historic mining operations and their subsequent decommissioning and remediation have been documented at a number of reference sites near the Anaconda and East Helena Superfund sites.

Distinct improvements in lichen communities have been observed at sites in close proximity (ca. 8 km) to the decommissioned Anaconda copper smelting operation (St. Clair et al. 2013). Closure of the copper smelter in the early 1980s, followed by remediation efforts, has resulted in significant improvements in local air quality, marked by an increase in overall species diversity as well as the number and abundance of sensitive indicator species. When the original bio-monitoring reference sites in the Anaconda-Pintler Wilderness Area and eastward sites in the Mount Haggin Wildlife Management Area were established in the early 1990s, a total of 143 lichen species, in 65 genera, were reported for the Anaconda-Pintler Wilderness Area, while only 21 species were reported for the sites between the Anaconda copper smelter and the eastern boundary of the wilderness area. Over the last 22 years, reference sites in and near the Mount Haggin Wildlife Management Area have shown significant improvement in response to declining levels of As, Cu, Ni, and Pb (Fig. 5.2). Likewise, Cu/Zn ratios from samples in the wildlife management area have also declined over the same time period (1.15 → 0.683) further documenting reduced levels of airborne Cu. The re-establishment and increase in relative abundance of a number of sensitive lichen species (*Bryoria fucescens*, *Letharia vulpina*, and *Usnea lapponica*), coinciding with the general decline in concentrations of atmospheric pollutants in the thalli of sensitive indicator species, suggests that

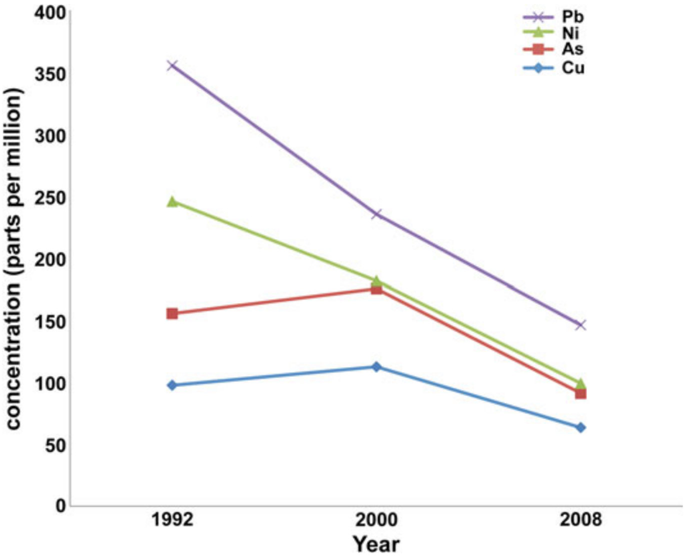

Fig. 5.2 Declining concentrations of four potential air pollutants, arsenic (As), copper (Cu), lead (Pb), and nickel (Ni), in lichen samples at a bio-monitoring reference site in Deerlodge County, Montana near the Anaconda copper smelter NPL Superfund site

decommissioning of the smelter operations and remediation of the slag debris and flu dust have had a significant positive impact on the adjacent biological communities. The average thallus size of several air pollution sensitive species at the eastern (closer to the Anaconda Superfund site) reference sites was smaller, suggesting recent re-establishment of these species at these sites. Continued monitoring is crucial to monitor recovery in this region. In other nearby reference sites in the Anaconda-Pintler Wilderness Area, 25–60 km west and upwind of the Anaconda Superfund site, pollutant element concentrations have generally remained within background levels and the number of sensitive indicator species per reference site has remained high throughout the sampling period. By all parameters, the lichen flora in the western portion of the Anaconda-Pintler Wilderness Area is healthy and free from air pollution-related impact, while the eastern reference sites between the eastern boundary of the wilderness area and the Anaconda Superfund site are showing evidence of significant recovery.

Three lichen air quality bio-monitoring reference sites were established during the summers of 2000–2001 in the Gates of the Mountains Wilderness Area, located ca. 25 km northeast of Helena, Montana (St. Clair et al. 2013). Elemental analysis data from the initial sampling period showed elevated levels of S, As, Ni, and Pb from all three reference sites (Table 5.2). The East Helena Superfund site represents a potential contributor to the elevated pollutant levels in the lichen samples collected from the Gates of the Mountains Wilderness Area. For over 100 years, the East Helena smelting operation deposited lead, arsenic, copper, zinc, cadmium, and other contaminants into the soil, surface water, and groundwater of Helena Valley. The East Helena smelting operation was decommissioned in 2001, and the area was subsequently listed as an NPL Superfund site. However, following closure of the smelter, concentrations of several critical pollutant elements continue to fluctuate at elevated levels. In spite of the high concentrations of pollutant elements, the overall species diversity as well as the number and abundance of sensitive indicator species per site in this wilderness area suggests that the lichen community is generally healthy. Ongoing monitoring of pollutant element concentrations in lichen samples from the Gates of the Mountains Wilderness Area will be essential for documenting full recovery of the biological systems following remediation of the East Helena Superfund site. Our data from bio-monitoring reference sites near the Anaconda superfund site suggest that a

Table 5.2 Mean concentrations of potential pollutant elements in sensitive indicator species from air quality bio-monitoring sites in the Gates of the Mountains Wilderness Area

Species and collection site	Elements (ppm except where indicated)													
	As		Cu		Ni		Pb		Zn		S %		N %	
	2000–2001	2009	2000–2001	2009	2000–2001	2009	2000–2001	2009	2000–2001	2009	2000-2001	2009	2000–2001	2009
Usnea sp. Meriwether Canyon, sample #808, 1192	**6.00**	**6.61**	8.00	6.30	2.00	1.62	**11.0**	**8.11**	**55.0**	**50.81**	**0.1752**	**0.148**	n.a.	**1.38**
Letharia vulpina, Meriwether Canyon, sample #809, 1191	**4.00**	**4.38**	6.00	6.71	2.00	1.30	**44.0**	**14.98**	**73.0**	**45.75**	**0.1278**	**0.148**	n.a.	**1.92**
Letharia vulpina, Refrigerator Canyon, sample #883, 1195	**4.29**	**7.85**	5.84	8.23	2.50	**4.26**	**43.6**	**14.55**	**66.9**	41.17	**0.1752**	**0.097**	n.a.	0.58
Rhizoplaca melanophthalma, Refrigerator Canyon, Sample #884, 1197	**10.40**	**9.59**	13.50	12.06	**3.85**	1.82	**41.9**	**106.10**	42.3	42.45	**0.1374**	**0.103**	n.a.	0.69
Usnea sp. Refrigerator Canyon, sample #885, 1196	**4.38**	**4.96**	5.26	9.06	1.31	3.21	12.8	**15.13**	56.7	42.03	**0.1594**	**0.087**	n.a.	**0.85**
Letharia vulpina, Hunter's Gulch, sample #886, 1194	**3.87**	**5.68**	7.16	7.50	1.86	**5.78**	**32.3**	**9.55**	**49.1**	42.03	**0.2142**	**0.149**	n.a.	**1.35**
Usnea sp. Hunter's Gulch, sample #887, 1193	**4.95**	**5.36**	7.47	6.55	2.40	1.30	**30.8**	9.11	57.1	39.13	**0.3213**	**0.106**	n.a.	**1.1**

Values for 2000–2001 and 2009 collections are shown; **bolded** values indicate values above provisional threshold of background levels (see: http://gis.nacse.org/lichenair/index.php?page=cleansite). All voucher material is housed at the Brigham Young University Herbarium of Nonvascular Cryptogams (BRY), Provo, Utah, USA reduction in pollutant elements to background levels will likely require decades following the decommissioning and cleanup of the smelter operation.

Similar to the results from our study near the decommissioned Anaconda copper smelter, Howe and Lendemer (2011) documented the re-establishment of a formerly degraded lichen community downwind of the decommissioned Palmerton zinc smelter (also a NPL Superfund site) in Pennsylvania. A number of lichen bio-monitoring reference sites in the Gila National Forest in southwestern New Mexico and the Chiricahua National Monument in southeastern Arizona are located in close proximity to some of the most intensive copper mining and smelting operations in the world (St. Clair et al. 2002a). Lichen samples from these sites had copper concentrations >100 µg/g dry weight (background copper concentrations in other intermountain area locations are generally <20 µg/g dry weight) and Cu/Zi ratios ≥1 (Cu/Zn ratios > 0:6 suggest accumulation of airborne copper from emissions associated with copper processing activities) (St. Clair et al. 2002a). In southern Idaho, concentrations of heavy metal pollutants and P in rock posy lichens were found to be negatively correlated with the distance of sample sites from phosphate refineries (Dillman 1996).

Notwithstanding the potential use of lichens for monitoring degraded sites associated with mining activity, the appropriate sample design for accurately characterizing ecological health using lichen indicators remains challenging. For example, both the temporal impact and scale of disturbances may vary widely across specific sites, and effective sampling design is crucial to assessing the impact of the disturbance (Loppi et al. 2004; Lisowska 2011; Aznar et al. 2008). Establishing bio-monitoring reference sites along a gradient, relative to potential pollutant sources, and serial sampling can aid in characterizing the spatial and temporal impact at disturbed sites. For example, serial sampling of bio-monitoring reference sites near NPL Superfund sites in western Montana (Anaconda copper smelter) and eastern Pennsylvania (Palmerton zinc smelter) revealed a gradual recovery of lichen communities and decrease in elemental pollutants in sensitive lichen indicators approximately 30 years after the smelting operations were decommissioned (Howe and Lendemer 2011; St. Clair et al. 2013). In other cases, elevated heavy metal pollutants can be observed in otherwise health lichen communities, including references sites established in the Gates of the Mountains Wilderness Area, near the recently decommissioned East Helena smelter (St. Clair et al. 2013). In a separate case, one of six references sites established in the Flaming Gorge National Recreation Area in northeastern Utah during the summers of 2010 and 2011 showed elevated levels for As (2.8 ppm), Ni (3.39 ppm), and Pb (218.6 ppm) in the sensitive indicator species *Xanthoparmelia-coloradoënsis* (St. Clair and Leavitt 2013). The Pb concentrations were of particular concern—representing some of the highest values reported for the intermountain area. In spite of the elevated levels of heavy metal pollutants, the overall diversity of lichen species, and particularly the number of sensitive indicator species, at the site suggests that at some temporal scales lichens may effectively accumulate airborne pollutants without any apparent impact on community composition or species diversity.

In conclusion, a combined approach, including assessing overall lichen diversity, the abundance of sensitive indicator species, and elemental analysis of potential pollutants, is central to monitoring the impact of historic disturbances in long-term lichen bio-monitoring programs.

5.2.3 Monitoring Future Disturbances

Climate change is projected to promote major ecological shifts worldwide (Araújo and Rahbek 2006; Parmesan and Yohe 2003; Sykes et al. 1999). Shifting biomes due to climate change may lead to adjustments in species distributions and potential extinctions if populations are unable to tolerate or adapt in situ to altered environmental conditions or migrate to suitable habitats (Aitken et al. 2008; Jackson and Overpeck 2000; Beck et al. 2011; Bradshaw and Holzapfel 2006). Some components of cryptogamic communities have been shown to be particularly sensitive to climatic shifts (McCune 2000; Jägerbrand et al. 2006; Cornelissen et al. 2001; Bjerke 2011), and these communities, due to their sensitivity, may play an important role in monitoring the potential impacts of climate change. Although lichens are well-known indicators of air quality, recent studies indicate that they may also be useful in assessing ecological shifts related to climate change (Cornelissen et al. 2001; Rogers and Ryel 2008; Bjerke 2011).

On a global scale, macrolichens in climatically milder arctic ecosystems may decline if and where global changes cause vascular plants to increase in abundance (Cornelissen et al. 2001). In the UK, the southern elements of Britain's lichen flora, and other lichen species adapted to warmer climates, are projected to expand northward, while the montane species appear to be disproportionally threatened by climate change (Ellis et al. 2007). Other data suggest that a warmer, humid climate in Norway will likely be beneficial for the generalist species *H. physodes*, but detrimental to the subalpine birch specialist *Melanohalea olivacea* (Evju and Bruteig 2013). These studies highlight the importance of continued research on specific responses of lichen species and communities to changing climate. However, only by consistently coupling efficient quantitative methods with accurately characterized species- and/or community-specific responses to changing climatic conditions will we be able to effectively document climate change-related impact on vulnerable lichen communities.

In general, specific responses of most species, including non-vascular cryptogams, to rapid climate changes in vulnerable habitats remain uncertain, and detailed, long-term monitoring will be essential to accurately assessing biologically meaningful shifts in community composition and species distributions (Manel et al. 2010; Eaton and Ellis 2012).

Due to the limited number of experts, time constraints, funding, and the need for long-term monitoring, simple field methods that can be applied consistently over multiple years are crucial to adequately monitor shifts in population density and community composition resulting from climate change (Leavitt and St. Clair 2011; Nimis et al. 2002). While a number of effective methods have been developed for assessing community structure in corticolous lichen communities (Nimis et al. 2002), distance methods provide a scalable alternative for assessing population density and community composition in some types of terricolous and saxicolous lichen communities (Leavitt and St. Clair 2011). Distance methods are less prone to significant variation among observers, are generally less labor and time intensive, and generally minimize damage to the study area (Benavides and Sastre-De Jesús 2009; Kunze 1980; St. Clair et al. 2007; Dahdouh-Guebas and Koedam 2006). The population density of *Xanthoparmelia* communities in six long-term reference sites in subalpine habitats in the southwestern USA has been determined using distance methods, although the specific response of subalpine *Xanthoparmelia* communities to changing climate remains unknown (Leavitt and St. Clair 2011). Lichens and bryophytes are commonly important components of high-altitude/high-latitude ecosystems (Longton 1997; Jagerbrand et al. 2006) and Arctic/alpine lichens have been shown to be sensitive to a warmer and more fluctuating winter climate (Bjerke 2011). In addition, winter icing events may have a substantial effect on lichen-dominated ecosystems (Bjerke 2011). A number of studies on the impact of climate change on lichen communities in Greenland reveal the potential for dynamic shifts in lichen communities, where a warmer climate will likely result in more favorable conditions for some lichens in specific habitats and unfavorable conditions for others (Leavitt et al. 2014; Hansen 2010, 2011a, b, 2012). Non-vascular cryptogams are an important component of alpine tundra communities in western North America, and effective monitoring of alpine tundra lichen communities has the potential to make significant contributions to conservation and management of some of the most vulnerable biological communities in the Intermountain West (Conti et al. 2009; St. Clair et al. 2007; Ustin et al. 2009; Leavitt et al. 2014).

Alpine tundra habitat in the western USA is comprised of a variety of specialized vegetative communities adapted to severe climate. Of the estimated 3 million hectare of alpine tundra habitat in North America, nearly one-third lies in Colorado (Fig. 5.3). Historically, livestock grazing, mining, and recreation have resulted in significant disturbances in alpine habitats that may take decades to centuries to recover (St. Clair et al. 2007). Now global climate change provides an additional threat to sensitive alpine tundra communities through the encroachment of forest communities and increased variation in winter temperatures and precipitation.

Among the specialized Arctic-alpine lichens, two species from the genus *Flavocetraria* (Parmeliaceae), *F. cucullata* and *F. nivalis*, are important components of Colorado's alpine tundra community and have been collected historically from a number of alpine tundra communities throughout the state (Fig. 5.4). Evidence from recent field surveys suggests that lichen communities in Colorado's alpine tundra habitats have likely been impacted by climate change, along with other disturbances. Due to the specific habitat conditions required to support *F. cucullata* and *F. nivalis* and their restricted distribution in alpine tundra habitats in the continental USA, *Flavocetraria* spp. may potentially serve as effective bio-indicators for monitoring the impact of climate change on biological communities in alpine tundra habitats in the intermountain region.

During the summer of 2013, we established baseline estimates of population density for *F. nivalis* at four long-term, bio-monitoring sites in alpine tundra habitats in Colorado: (1) Niwot Ridge Long-Term Ecological Research Site, ca. 15 km northwest of Nederland in north-central Colorado; (2) vicinity of Loveland Pass, ca. 95 km west of Denver; (3) vicinity of Red Mountain Pass in the San Juan National Forest, along US Route 550; and (4) Cottonwood Pass in

Fig. 5.3 Alpine tundra habitat in Colorado, USA. The *panel on the left* shows Kiowa (4,047 m.a.s.l.) and Navajo (4,087 m.a.s.l.) peaks from the Niwot Ridge Long-term Ecological Research site; *panel on right* shows a habit shot of *Flavocetraria nivalis* at Loveland Pass, Colorado (scale bar = 1 cm)

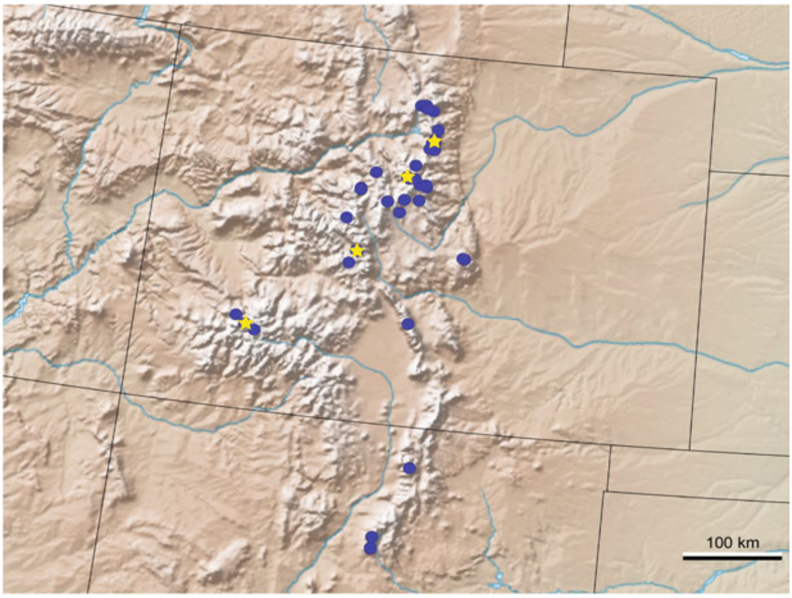

Fig. 5.4 Southernmost distribution of *Flavocetraria* species in North America: *blue circles* indicate all known occurrences of *Flavocetraria* species in Colorado and New Mexico, and *yellow stars* indicate sites established for long-term monitoring of *Flavocetraria nivalis* population densities (see Table 5.3)

south-central Colorado, ca. 30 km west of Buena Vista. *Flavocetraria nivalis* population density was estimated at the four sites following previously described methods, and baseline estimates are provided in Table 5.3 (Leavitt and St. Clair 2011; Zhu and Zhang 2009). Herbarium collections document that *F. nivalis* has historically occurred in abundance at each of these sites. In the recently established long-term plots, *F. nivalis* occurs in patchy, locally common populations at the Cottonwood Pass, Loveland Pass, and Niwot Ridge sites; however, *F. nivalis* was rare at the Red Mountain site (Table 5.3). *F. nivalis* populations at Red Mountain may be particularly vulnerable due occurrence near the southern-most extent of its range and low population density.

Recent records of *Flavocetraria* spp. are relatively sparse throughout Colorado. Only 13 collections made between 1990 and 2014 are reported in the Consortium of North American Lichen Herbaria, compared to 149 collections made between 1950 and 1990 (data accessed from http://lichenportal.org/portal/collections/index.php on March 11, 2014). Our baseline estimates of population density for *F. nivalis* populations and long-term bio-monitoring of these established plots will provide an important framework to help document the impact of climate change and other human-related disturbances on alpine tundra communities in the Intermountain West. Furthermore, revisiting additional sites where *Flavocetaria* spp. have historically been collected throughout the intermountain region may provide important perspectives about general patterns across the region. In particular, alpine tundra habitats in New Mexico represent the furthest south occurrences of *F. nivalis*, and these, along with occurrences at lower elevations further north, are likely vulnerable to a much higher probability of decline. However, in addition to observational studies, experimental manipulation studies will be essential to adequately characterize the response of *Flavocetraria* spp. to altered climate regimes. Other lichens restricted to alpine tundra habitats may also serve as important indicators of the impact of a warming climate on these sensitive habitats, and we advocate ongoing observational and experimental studies to develop effective strategies for monitoring climate change.

Table 5.3 Initial *Flavocetraria nivalis* population density estimates at four long-term reference sites in Colorado, USA

Site	Lat. lon. elevation	Abundance	Estimated population density 2013	Notes
Cottonwood Pass: Gunnison National Forest, Sawatch Range, just southwest of Cottonwood Pass on prominent hilltop	38° 49.441′N 106° 24.989′W 3,810 m.a.s.l.	Locally abundant	3.5 ± 0.02 individuals/m^2	Site receives heavy recreational traffic and moderate vehicle traffic during the summer months
Loveland Pass: Arapaho National Forest, Front Range, along US Route 6, vicinity of Loveland Pass	39° 39.875′N 105° 52.682′W 3,670 m.a.s.l.	Locally common	0.17 ± 0.09 individuals/m^2	Site is in close proximity to US Interstate 70 and receives heavy vehicle and recreational traffic year round
Niwot Ridge: Roosevelt National Forest, Niwot Ridge Long-Term Ecological Research Station, ca. 3 km west of Niwot Mountain	40° 03.190′N 105° 35.250′W 3,514 m.a.s.l.	Locally common	0.83 ± 0.10 individuals/m^2	Site is located within the Niwot Ridge Long-term Ecological Research Site, administered by the University of Colorado at Boulder
Red Mountain: San Juan National Forest, south of Red Mountain Pass on US Highway 550, east of County Road 16, between Big Horn and Browns Gulches	37° 52.422′N 107° 42.339′W 3,750 m.a.s.l.	Rare	$\ll$0.1 individuals/m^2	Recreational and vehicle traffic are negligible

These sites are distributed across the southernmost distribution of *Flavocetraria* species in North America

5.3 Conclusions

Our lichen bio-monitoring program, including over 350 reference sites, represents the largest air quality bio-monitoring program in western North America and provides crucial baseline data across a wide range of habitats. In order to exploit the value of this research program, there is a critical need for ongoing periodic reviews of the existing reference sites. Ongoing reviews of the lichen flora, and particularly sensitive indicator lichens, and elemental analyses of potential pollutants at reference sites will be central to effectively assessing ecological health and the impact of changing air quality over time. Similarly, establishing lichen air quality bio-monitoring programs in and around important as of yet unprotected airsheds—including, national parks, wilderness areas (all classes), and other vulnerable undesignated wildland landscapes—should be a central focus of ongoing air quality bio-monitoring research. In light of expanding energy extraction and processing activity in western North America, there is a pressing need to establish additional lichen air quality bio-monitoring programs on lands that are most likely to be impacted by oil and natural gas development (McMurray et al. 2013). This is particularly important in light of the closing window relative to the opportunity for effectively establishing baseline, pre-impact programs. Additional research is needed, particularly in vulnerable and sensitive landscapes (alpine/Arctic tundra and warm deserts), to determine if sensitive indicators of climate change impact are in decline. It is imperative that we collect the data while it is still available—future opportunities for systematically exploring and characterizing the lichen flora of these rare and unique systems may be limited. In the case of *F. nivalis* in the Intermountain West, examination of historical distribution records, followed by determination of current population densities of *F. nivalis* across additional sites, would provide a more extensive baseline for monitoring climate change-related impact on alpine tundra systems. Furthermore, critical assessments of other potentially sensitive components of subalpine, alpine, and alpine tundra communities are desperately needed.

In arid regions of the Intermountain West, lichens are often important components in many biological soil crust communities. BSC play a major ecological role in arid and semiarid regions worldwide. BSC often provide 40–100 % of the ground cover in areas with relatively sparse vascular plant cover (St. Clair et al. 1993). They also effectively reduce wind and water erosion, significantly increasing soil fertility, and are generally important in ecosystem function (Belnap and Eldridge 2003). Soil crusts in general and the lichen component in particular are very sensitive to human-related perturbation. Over the last 150 years, a significant portion of the soil crust communities of the Great Basin and Colorado Plateau has been heavily damaged (Zhao et al. 2010; Chambers et al. 2014; St. Clair et al. 1993). Much of the disturbance is due to intensive grazing by cattle and sheep, urban and agricultural development, altered fire regimes, and off-road recreation (St. Clair et al. 1993; Belnap and Eldridge 2003). Today, novel combinations of disruptive factors, including climate change, invasive species, and changes in land use strategies, threaten these vulnerable soil crust communities [86]. Given the overall ecological characteristics of biological soil crust communities, including diversity and physiological status, these communities can also potentially serve as effective early indicators of ecosystem change or decline due to their rapid response to altered environmental conditions (Zhao et al. 2010; Chambers et al. 2014; Eldridge 2000). Both observational and experimental manipulation studies will be essential to appropriately characterizing the response of soil crust lichens to altered climate regimes and the development of effective bio-monitoring approaches.

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6 Community Metrics Under Air Pollution Stress Condition

Pulak Das, Jayashree Rout and Santosh Joshi

Contents

Abstract

The chapter deals with the pattern of changes in the diversity components of lichen communities around a paper mill in northeastern Indian region. Four indices (Shannon index, Pielou index, Margalef index, and Simpson index) are calculated for the seventeen sites situated within a radius of 25 km from the mill. The indices are studied vis-a-vis the distance (from the mill) and elevation. Positive correlation is found between number of species and distance, while a negative correlation is found between numbers of species and the elevation. Changes in the pattern of indices are seen along three distance categories; <10, 10–20, and >20 km. As expected, the dominance index increased (54 %) and the diversity, richness, and evenness index decreased near the mill; maximum decrease being for Shannon index (>14 %) of diversity. It is observed that neither of the indices showed significant correlation with the altitude or distance. The Shannon, Margalef, and Simpson index showed significant correlation with the number of species. The effect of stress on biodiversity is depicted with the help of changes in the shapes of Odum's model of 'concave' curve. It is observed from the graphical representation of the 'number of species' and 'number of individual per species' that the nearness to the mill converted the 'concave' hollow curve into a flattened one and ultimately into a 'convex' curve.

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Lichen · Diversity indices · Paper mill · Odum's concave curve

6.1 Introduction

An index is usually a count statistic that is obtained in the field and carries information about a population (Wilson et al. 1996a, b). The index is used when individuals of the species in question are difficult to observe and count, or capture and tag, or when a formal abundance estimate is too expensive or time-consuming (Gaines et al. 1999). In ecology, diversity in a sample, or an area sampled, is typically measured as either the number of species or an index of their relative abundance (Magurran 1988). Various indices and models have been developed to measure diversity within a community (Magurran 1988). In general, three main categories of measures are used to assess species diversity: (1) species richness indices, which measure the number of species in a sampling unit, (2) species abundance models, which have been developed to describe the distribution of species abundances, and (3) indices that are based on the proportional abundances of species such as the Shannon and Simpson indices (Magurran 1988). Numerous biodiversity indices (such as Shannon–Wiener and Margalef indices) based on the proportional abundance of species (Wilson et al. 1996a, b) characterize the biological community within an area as a whole (i.e., in terms of averages over the area).

Diversity indices are one of the favorite tools of ecologist world over to see, either the continuity of the naturalness of any environment or the perturbations occurred to it due to any internal or external factor. From last couple of decades, researchers have used special indices of species diversity as a means of indicating perturbations in almost all types of habitats and environments using different organisms (Adams and Barrett 1976; Bulan and Barrett 1971; Karr 1958; Wilham and Dorris 1968). A number of examples of the application of indices to assess diversity of species within a community are mentioned in the literature. Thiollay (1992) used species richness, the Shannon index, evenness index, and the Simpson index to assess the influence of selective logging on bird species diversity. Cushing and Gaines (1989) used the species richness, the Shannon index, and an equitability or evenness index to compare diversity of aquatic insects in three cold desert streams and to evaluate the effects of winter spates on diversity. Walker et al. (2006) in a study used species richness, Pielou evenness index, Shannon index, etc., of plant communities including lichens across the Tundra Biome to study the trend of climate warming.

Lichens, the unique and peculiar organism, are one of the most reliable and used worldwide for monitoring studies. High or low lichen diversity can result from various factors such as certain types of air pollution, changes in forest management or stand structure, diversity of plant substrates available for colonization, climate favorability, periodicity of fire, and so on (Jovan 2008). Lichens are sensitive to changes in atmosphere and micro-climate conditions, including changes in forest structure, and have long been used as environmental bioindicators for air pollution and environmental changes in tropical regions (Wolseley and Aguirre-Hudson 1997).

The present research work as a case study is an attempt to understand the species structure of the lichen communities by studying the different mathematical indices around a paper mill in northeastern region of India. It is presumed that the study around a potential pollution source is more likely to produce a differentiated pattern of the community structure. A comparative assessment of the indices was made to evaluate the effectiveness of the tool.

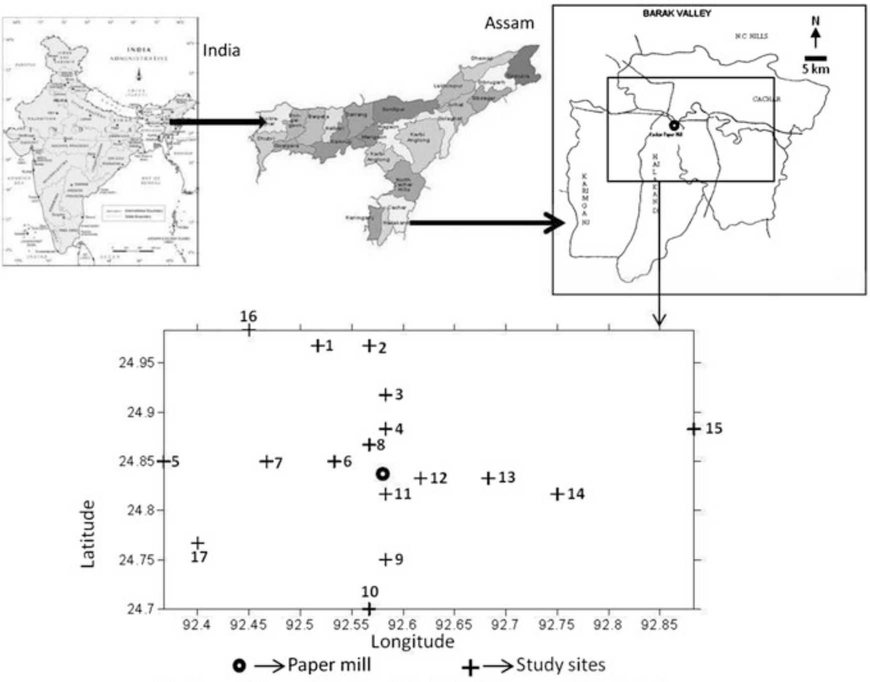
6.2 Materials and Methods

6.2.1 The Study Area

The study was conducted around a paper mill situated in Barak Valley (consists of three districts of Cachar, Hailakandi, and Karimganj, Fig. 6.1) in northeastern region of India. The paper mill (Latitude 24° 52′N and Longitude 92° 36′E) which was set up in the year 1973–1974 has a manufacturing capacity of 100,000 mega tones/year of writing and printing paper. The bamboo resource which is abundant in this region is used as a raw material. The Barak Valley is a swampy plain interspersed with low hills. The valley experiences subtropical climate with high humidity during the rainy seasons. The temperature drops to 6°–8° during the winter and reaches a maximum of 35°–38° during summer. The wind generally blows from the northeast in the morning and from the southeast in the afternoon. The yearly rainfall (mm) ranges from around 3,000 to as high as over 4,000.

6.2.2 Sampling Sites and Strategy

The sampling sites (seventeen) selected for the study are radially distributed (Fig. 6.1) around the mill up to a maximum distance of 24.1 km and minimum distance of 2.4 km. Out of all the sites, seven sites (Site nos.: 12, 11, 13, 8, 6, 14, and 4) are situated within 10 km distance from the mill, six sites (Site nos.: 7, 9, 3, 2, 1, and 5) are situated at a distance between 10 and 20 km from the mill, and four sites (Site nos.: 10, 17, 15, and 16) are situated beyond 20 km. The air



Site Nos.: 1-Gumra, 2-Dumkur, 3-Barotal, 4-Mohanpur, 5-Elongjuri, 6-Umarpur, 7-Bhanga, 8-Devendranagar, 9-Bornogod, 10-Lokhirbond, 11-Uttarkanchanpur, 12-Kalinagar, 13-Ghagrapar, 14-Sangjurai, 15-Udharbond, 16-Jalalpur, 17-Kaliganj

Fig. 6.1 Map of Barak Valley (consisting three districts of Cachar, Hailakandi, and Karimganj), showing the paper mill and seventeen sampling sites spread around it quality measured during the year 2002–2005 inside the paper mill revealed that the concentration ($\mu g/m^3$) of SPM (Gradient: 0.011) and SO_2 (Gradient: 0.001) showed an increasing trend, while the concentration of NO (Gradient: −0.038) showed a decreasing trend. Site nos. 5 and 14 are situated adjacent to urban area (Karimganj and Silchar town, respectively) with moderate to heavy vehicular activities. Although the former is more close to the town, Site No. 15 is situated in an area amidst a dozen of stone crushers. Site No. 16 is comparatively an undisturbed area away from urban influence and vehicular activities. All the other sites have more or less similar characteristics. A single host tree species (*Artocarpus heterophyllus*) was selected to avoid the differences in species structure pattern due to the differences in bark characteristics. At each site, five numbers of host trees are selected with more or less similar height and girth measurements, and conditions of light, temperature, and humidity. Lichens are collected during the year 2005, up to a height of two meters from the base. All the other required information for the calculation of the indices was collected.

6.2.3 Calculation of Indices of Species Structure

The indices of species structure (Odum 1996) or single-number indices (Costello et al. 2001) used are as follows:

Shannon Diversity Index (H)

$$H = -\sum(\mathrm{ni}/N)\log(\mathrm{ni}/N)$$
or
$$-\sum(\mathrm{Pi})\log(\mathrm{Pi})$$

where

ni importance value for each species
N total of importance values
Pi importance probability for each species.

Margalef index of species richness (d)

$$d = (S-1)/\log N$$

where

S number of species
N number of individuals.

Pielou evenness index (e)

$$e = H/\log S$$

where

H Shannon index
S number of species.

Simpson index of dominance (c)

$$c = \sum(\mathrm{ni}/N)^2$$

where

ni importance value for each species
N total of importance value.

The Shannon–Wiener index gives equal weighting to rare and common species, while Simpson's dominance index gives more weight to common species and less weight to rare species (Magurran 1988). Thus, using both the Shannon–Wiener index and Simpson's dominance index provides measures of species heterogeneity. Heterogeneity is greater for communities with more species that are equally abundant compared to communities with fewer species that are not equally abundant. O'Neill et al. (1988) and Turner (1990) proposed indices of dominance (along with some other indices) as standard measures of landscape pattern. According to Gaines et al. (1999), dominance is an index of vegetation-type composition and equitability. Evenness was calculated to provide an estimate of the equitability of species abundances within each community.

6.2.4 Graphical Representation of the Species and Species Abundance

The general relationship between the organisms and the habitat conditions is demonstrated using the shape of the curves (Odum 1996) plotted against number of species and number of individual per species in *y*-axis and *x*-axis, respectively. According to Odum (1996), the hollowness or the 'concavity' of the curve depicts the trend of natural environment, but the stress factor tends to flatten the curve.

6.3 Result and Discussion

The seventeen sampling sites (Fig. 6.1) located around the paper mill are grouped into three categories; <10, 10–20, and >20 km. The numbers of sites in each category are seven, six, and four, respectively (Table 6.1). The altitude of sampling sites ranged from 17.1 m at site number 5 to 27.1 m at site number 15. The study revealed 53 species distributed among 13 families and 23 genera. The dominant family is observed to be the Graphidaceae consisting of 22 species followed by Pyrenulaceae with 17 species and Arthoniaceae with 11 species. Out of 23 genera, *Graphis* dominates with 10 species followed by *Pyrenula* and *Arthonia* with 5 species each. Out of the two growth forms (crustose and foliose), crustose consists of around 91 % of the lichen flora, whereas remaining is contributed by the foliose growth form. The foliose growth form is contributed by three families; Physciaceae (60 %), Parmeliaceae, and Biatoraceae (20 % each) consisting of five different genera (*Dirinaria*, *Parmotrema*, *Physcia*, *Phyllopsora*, and *Pyxine*).

Table 6.1 Indices of diversity and dominance (Shannon index of general diversity *H*, Margalef index of species richness *d*, Pielou index of evenness *e*, and Simpson's index of dominance) along with the number of species, distance, and altitude at each site around the paper mill

Site No.	Distance (km)	No. of species (S)	Altitude (m)	*H*	*d*	*e*	c
12	2.4	5	17.4	0.559	2.11	0.800	0.325
11	4.8	11	19.2	0.779	4.31	0.748	0.247
13	5.2	10	20.7	0.711	3.89	0.711	0.308
8	5.6	10	22.6	0.884	5.20	0.884	0.160
6	7.2	10	20.7	0.832	4.39	0.832	0.190
14	8.4	24	19.5	1.033	8.61	0.749	0.177
4	8.8	18	21.6	1.065	7.42	0.848	0.128
7	11.3	12	25.3	0.991	5.12	0.918	0.121
9	12.1	18	22	1.053	6.71	0.839	0.109
3	13.7	20	18.3	1.199	9.61	0.922	0.078
2	16.1	13	19.5	0.977	5.82	0.877	0.127
1	16.9	12	21	0.861	5.54	0.798	0.196
5	19.3	11	17.1	0.799	4.50	0.768	0.234
10	20.1	15	25.6	1.063	6.43	0.904	0.106
17	20.1	16	21.3	1.051	5.97	0.873	0.115
15	23.3	9	27.1	0.779	4.18	0.816	0.233
16	24.1	17	19.2	1.037	5.42	0.842	0.114

Table 6.2 Mean values and standard deviation of the indices of species structure in three distance categories

Distance category (km)	*N*	*H*	*d*	*e*	*c*	*S*
<10	7	0.838 ± 0.178	5.13 ± 2.21	0.796 ± 0.063	0.219 ± 0.076	12.57 ± 6.32
10–20	6	0.980 ± 0.142	6.22 ± 1.82	0.853 ± 0.063	0.853 ± 0.063	14.33 ± 3.72
>20	4	0.982 ± 0.137	5.50 ± 0.97	0.859 ± 0.038	0.142 ± 0.061	14.25 ± 3.59
From <10 to >20	17	**0.922 ± 0.164**	**5.60 ± 1.81**	**0.831 ± 0.063**	**0.175 ± 0.073**	**13.58 ± 4.74**

Bold indicates mean values and standard deviation of indices of species structure for the entire study area (<10 to >20)

The number of epiphytic lichens ranged (Tables 6.1 and 6.2) from 5 at Site nos. 12–24 at Site No. 14 with an average of 13.6. The numbers ranged from 5 to 24 within first 10 km from the mill with an average of 12.6, 11–20 within 10–20 km category with an average of 14.3, and from 9 to 17 beyond 20 km with an average of 14.3. The number of species/site showed (Fig. 6.2) an increasing trend along with the increase in distance from the mill. The number of species is showing a decreasing trend along with the increase in altitude.

The Shannon index of diversity (*H*) in the study area ranged from 0.559 to 1.199 with a mean of 0.922. Within different distance categories, its value ranged from 0.559 to 1.065 (Mean: 0.838) within 10 km category, from 0.799 to 1.199 (Mean: 0.980) (10–20 km category), and from 0.779 to 1.063 (Mean: 0.982) (>20 km category).

Index of species richness (*d*) of lichen community in the study area ranged from 2.108 to 9.607 with a mean of 5.60. The *d* value ranged from 2.108 to 8.607 (Mean: 5.13) in <10 km category, from 5.118 to 9.607 (Mean: 6.22) in 10–20 km category, and from 4.180 to 6.434 (Mean: 5.50) in >20 km category.

The mean value of evenness index (*e*) in the study area was observed to be 0.831 and it ranged from 0.711 to 0.922. Within the three different distance categories, the *e* value ranged from 0.711 to 0.884 (Mean: 0.796) in <10 km category, from 0.768 to 0.922 (Mean: 0.853) in 10–20 km category, and from 0.816 to 0.904 (Mean: 0.859) in >20 km category. The Simpson dominance index (*c*) ranged from 0.078 to 0.325

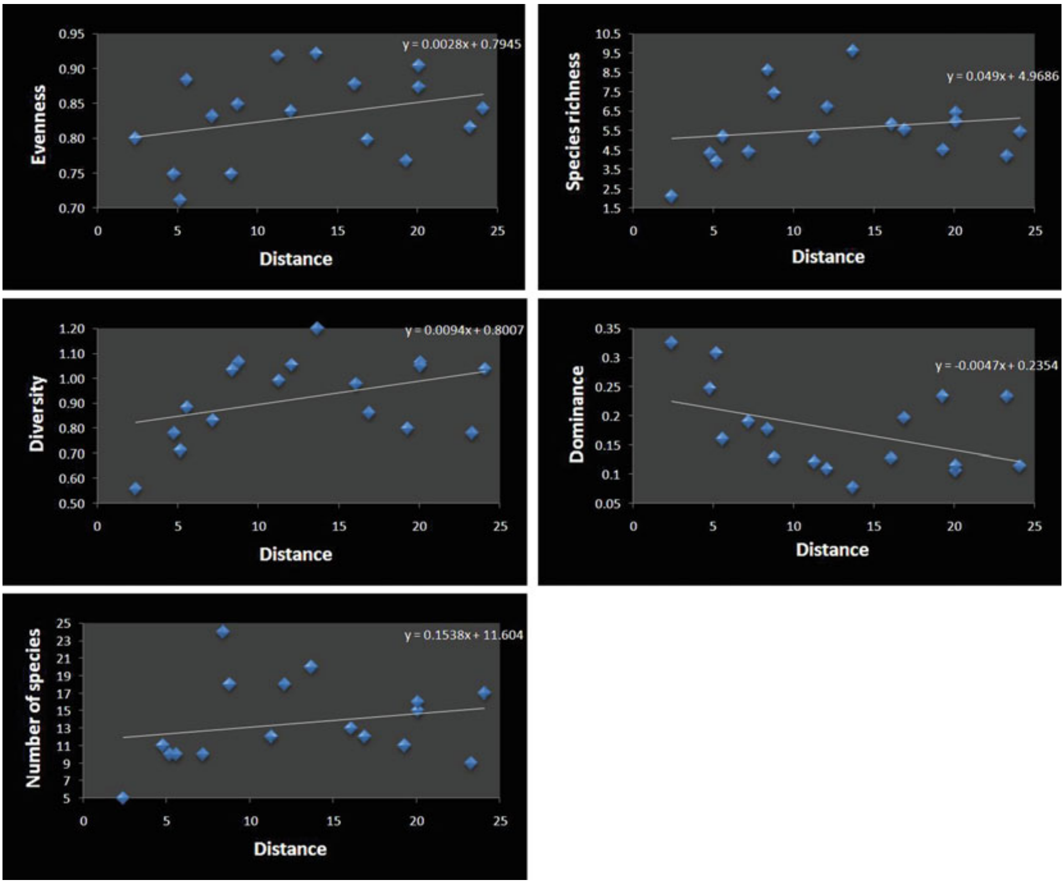


Fig. 6.2 Trend lines of different indices along the distance from the paper mill

with a mean value of 0.175 around the study area. The '*c*' value ranged from 0.128 to 0.325 (Mean: 0.219), from 0.078 to 0.234 (Mean: 0.144), and from 0.106 to 0.233 (Mean: 0.142) at three different distance categories of >10, 10–20, and >20 km category, respectively.

The three indices, diversity (*H*), evenness (*e*), and richness (*d*), showed (Table 6.3) a positive increasing trend with the increase in distance. With the increase in elevation, '*H*' and '*e*' showed an increasing trend, but the '*d*' value showed a decreasing trend. The number of lichen species showed an increasing trend with the distance and a decreasing trend along with the altitude (Table 6.3).

Relationship between the number of species and number of individuals/species around the paper mill is shown by using scatter plot diagram (Fig. 6.3a) as described by Odum (1996). Three 'curves' are obtained for three different distance categories. The curves are parabolic in nature with the axis of parabola being the *y*-axis (number of species).The thicker solid curve ($y = 0.009x^2 - 0.2576x + 7.2636$) is the trend line for category >20 km category. The thinner solid curve ($y = -0.019x^2 - 0.4214x + 4.2323$) and the thinner dotted one ($y = -0.0051x^2 - 0.1691x + 3.9163$) are the curves for 10–20 and <10 km category, respectively (Fig. 6.3a). Both the curves are convex in nature.

Diversity, richness, evenness, and abundance also called as community metrices (Therriault 2002) or single-number indices or heterogeneity indices or univariate indices can be the useful tools for summarizing patterns in biodiversity. These biodiversity indices, which are the functions of the number of species and the number of individuals in each species, reflect the effect of habitat conditions on them. Both spatial and temporal changes are of significance to understand the local variability in the habitat conditions.

In the present study, four indices, Shannon, Pielou, Simpson, and Margalef indices, are studied with respect to the changes in distance from the paper mill. The categorization of the distance into three types helped in more detailed analysis of the above-mentioned parameters (Table 6.2). The Shannon, Pielou, and Simpson indices revealed a continuous gradient with increasing distance. The Shannon diversity index increased from 0.838 at <10 km distance to 0.980 at 10–20 km and to 0.982 at >20 km distance with an overall increase of about 17 %. The evenness index increased about 7 %. The Simpson index decreased by about 34 % from <10 km distance category to >20 km category. The Margalef index increased from near to the remote distance. A similar correlation was noted at a coal-fired power plant where variation in distances (11–24 km) from the pollution source resulted in a decrease in the number of species, Margalef richness, and Shannon diversity by 33, 27, and 1 %, respectively, and an increase in evenness index and dominance index by 22 and 20 %, respectively, in Southeastern Ohio and West Virginia (Stefan and Rudolph 1979). Compared to this, in the present study, change in distance from >20 to <10 km reduced the number of species, species richness, Shannon diversity

Table 6.3 Correlations (*r*)

	H	*e*	*d*	*c*	*S*	*D*	*E*
H	1	**0.630**	**0.896**	**−0.951**	**0.858**	0.396	0.161
e	**0.630**	1	0.381	**−0.791**	0.194	0.313	0.375
d	**0.896**	0.381	1	**−0.751**	**0.922**	0.187	−0.006
c	**−0.951**	**−0.791**	**−0.751**	1	**−0.688**	−0.444	−0.251
S	**0.858**	0.194	**0.922**	**−0.688**	1	0.224	−0.077
D	0.396	0.313	0.187	−0.444	0.224	1	0.283
E	0.161	0.375	−0.006	−0.251	−0.077	0.283	1

Bold values are significant at $P < 0.05$

H Shannon index, *e* Pielou index, *d* Margalef index, *c* Simpson index, *S* number of species, *D* distance from paper mill, *E* elevation index, and evenness index by 12, 7, 15, and 7 %, respectively, and an increase in dominance index by 54 %. The trends of the four indices and the number of species at different distances from the mill are quite interesting (Fig. 6.2). Except the Simpson index, all the other showed an increasing trend away from the paper mill. The highest gradient was observed for the number of species (0.1538), followed by Margalef index (0.049), Shannon index (0.0094), and Pielou index (0.0028). Simpson index has a gradient of −0.0047.

Researchers have studied the changes in such parameters considering different organisms and in different stress conditions. Barrette (1969) found a decrease in species richness while an increase in evenness of arthropod population nearer to the environmental stress (acute insecticidal effect). A decrease in the number of species and Shannon value was observed for the nematodes due to increase in the Pb content in their habitat (Zullini and Peretti 1986). Wilhm (1967) demonstrated the changes in the Shannon value of the benthos downstream of a pollution outfall. Junshum et al. (2008) have shown the changes in the Shannon index value of algal diversity in water bodies in power plant. Wilhm (1975) has even classified the condition of water on the basis of Shannon index. Washburn and Cully (2006) found significant differences in the indices between various urban and non-urban sites in Ohio. Although some workers found little correlation between the *H* value and the extent of perturbation (Stefan and Rudolph 1979; Boyle et al. 1990), Therriault (2002) found only temporal differences within the community metrices and not due to differences in habitat conditions. Godfrey (1978) believes that the large evenness component within a community may mislead the interpretation of *H* value.

The *H*, *d*, and *c* value showed highly significant correlation ($P < 0.05$) with the number of species (Table 6.3). Evenness (*e*) does not exhibit a significant correlation with the number of species. Giordano et al. (2004) in a study found that the biodiversity is highly correlated with the total number of species ($r = 0.88$, $P < 0.05$). Most univariate indices and mathematical models, however, are designed to show greater diversity when abundance is more evenly distributed among the species sampled and when the number of species in a sample increases (Costello et al. 2001). In the present study, no significant correlation was found between evenness and richness ($r = 0.381$) although correlation between diversity and evenness ($r = 0.63$) and diversity and richness ($r = 0.896$) was statistically significant. Richness and evenness are not necessarily correlated always (Edgar 1983; Costello and Myers 1987; Mackey and Currie 2001), but richness and species diversity are often positively correlated with some exceptions (Hurlbert 1971).

The number of species showed negative correlation ($r = -0.077$, $P > 0.05$) with the elevation, while the Shannon index exhibit positive correlation ($r = 0.161$, $P > 0.05$) with the elevation. Earlier workers have found both positive (Insarov et al. 1999) and negative (Jovan and McCune 2006) correlations of number of species and diversity with the elevation. Unimodel relationship of Shannon diversity with the altitude has been studied in a lichen biodiversity study in northeast India (Pinokiyo et al. 2008). A positive correlation of number of species ($r = 0.224$, $P > 0.05$) and Shannon diversity ($r = 0.396$, $P > 0.05$) with the distance is observed in the present study. This, however, may not be similar for everywhere (Stefan and Rudolph 1979).

Present study encompassing the region around the paper mill with a maximum distance of over 20 km revealed a variation of different indices (Table 6.1). The Shannon index showed a small range from 0.559 to 1.199, Pielou index ranged from 0.711 to 0.922, the Margalef index exhibited a high range from 2.108 to 9.607, Simpson index ranged from 0.078 to 0.325, and the number of species ranged from 5 to 24. In a study conducted on epiphytic lichens of some European countries, Stofer (2006) found a varied range and differences of the indices within countries. For instance, Shannon index ranged from 1.49 to 3.1 in Switzerland, from 0.28 to 2.4 in Germany, and from 0.99 to 3.26 in Italy. Simpson index ranged from 0.06 to 0.33 in Switzerland, from 0.14 to 1 in Germany, and from 0.05 to 0.43 in Italy. Pielou index ranged from 0.57 to 0.88 in Switzerland, from 0.2 to 0.89 in Germany, and

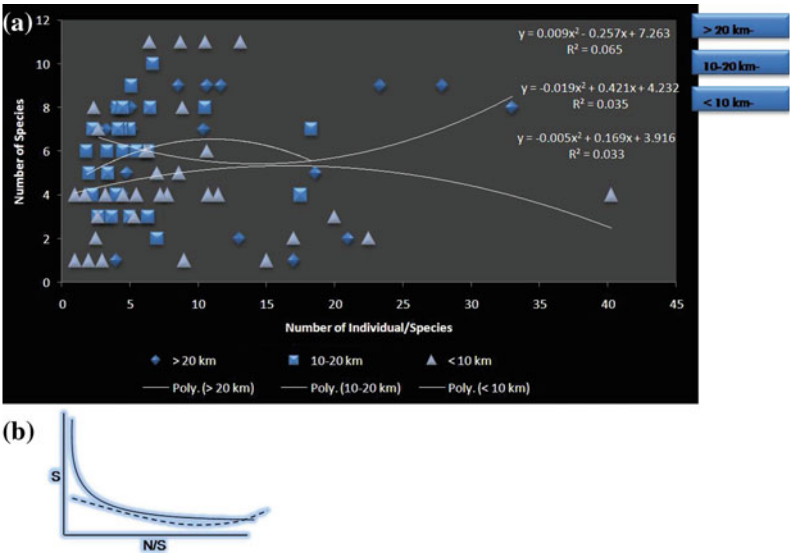


Fig. 6.3 Relationship between species (S) and number of individual per species (N/S) of lichens at three distance categories around the paper mill (**a**), from Odum (1996) (**b**)

from 0.64 to 0.87 in Italy. The number of species per plot (12 trees) ranged from 9 to 40 in Switzerland, from 1 to 24 in Germany, and from 4 to 47 in Italy.

Nearness to the pollution, source (paper mill) decreases the evenness and increases the dominance (Table 6.3; Fig. 6.2). Low evenness index value reveals dominance of opportunistic species or a species that can adapt better to the present ecological condition (Ramaharitra 2006) which also indicates a change in the dominance structure of the plant communities where fewer species produce a larger proportion of the cover. This response may represent a first step toward local species extinction (Walker et al. 2006).

Most natural communities contain few species with a large number of individuals (dominant species), and many species represented by a few individuals (rare species) (Odum 1996). The relationship can be pictured as a 'concave' curve (solid line, Fig. 6.3b). Rigorous physical environment (e.g., pollution) tends to flatten the curve as shown by the dotted line. To understand the concept more clearly, the distance from the pollution source in the study area has been divided into three categories (<10, 10–20, and >20 km) and the relationship between the number of species and number of individuals per species has been plotted (Fig. 6.3a). The curves do not reveal the characteristics of the natural communities. The curve for the third category (>20 km) is flattened (similar to the dotted curve of Fig. 6.3b), as the rare species (with less number of individuals) declines, indicating stress. The curves for the first two categories (<10 and 10–20 km) are quite interesting; it has gone beyond flattening assuming a 'convex' form. To be more specific, all the three curves are parabolic in nature having quadratic equation of the form '$ax^2 + bx + c$.' The 'a' value (indicating direction of the parabola) is positive (0.009) for the >20 km category, while it is negative and for 10–20 km category (−0.019) and for <10 km category (−0.005). In comparison with >20 km category, further declination of the rare species is observed in the remaining two categories. This decline is more pronounced in <10 km category as compared to the 10–20 km category.

The pattern of the community exhibits few number of both common/abundance and rare individuals species possessing large (common/abundant) and less (rare), while the species with moderate number of individuals are many. The stress (air pollution) has reduced the rare species and also the abundant species in both 'convex' curves in comparison with the '>20 km category' curve.

Graphis inamoena, *G. persulcata*, *G. chloroalba*, *Sarcographa subtricosa*, *Phaeographis dendritica*, *P. albolabiata*, and *Pyrenula fuscoolivaceae* are the abundant lichen species in

>20 km category. Some of the rare species in the same category are *Sarcographa leprieurii*, *Graphis assamensis*, *Arthothelium nigrodiscum*, *A. abnorme*, *Graphis subassimilis*, *G. scripta*, *G. persulcata*, *Arthonia arctata*, *Arthopyrenia indusiata*, *Chiodecton leptosporum*, *Bacidia inundata*, *Pyrenula costaricensis*, *Trypethelium albopruinosum*, *Dirinaria aegialita,* and *Thecaria austroindica*. In 10–20 km category, the abundant species are *S. subtricosa*, *P. fuscoolivaceae*, *D. aegialita*, *Cryptothecia lunulata*, *G. chloroalba*, etc., and the rare species are *A. indusiata, C. lunulata, G. inamoena, Arthothelium chiodectoides*, *Lecanora achroa*, *Graphis capillaceae*, *Platygramme pudica*, *Graphis nigroglauca*, *Porina subhibernica,* etc.

In the distance category nearest to the mill (<10 km), the abundant species are *D. aegialita*, *G. capillaceae*, and *Graphis subasahinae* and the rare species are *Trypethelium macrosporum*, *T. albopruinosum*, *P. subhibernica*, *Parmotrema saccatilobum*, *Arthonia catenatula*, *A. recedens*, *P. dendritica*, *A. chiodectoides,* and *Platygramme wattiana*.

It is interesting to note that in comparison with the species of >20 km category, the general abundance is greater (although not of the same species) in <10 km category. The species which contributed in the lowering of right arm (abundant species) of the curve from >20 km category to <10 km category are *A. indusiata*, *G. inamoena*, *P. albolabiata*, *P. dendritica*, *Pyrenula brunneae*, *Pyxine cocoes*, *and S. subtricosa* which are highly abundant in the former category, but its number reduced drastically in the latter.

Some of the rare species which are present in the >20 km category but are not found in <10 km category are *A. abnorme*, *A. nigrodiscum*, *B. inundata*, *C. leptosporum, G. assamensis*, *G. scripta*, *G. subassimilis*, *Physcia dilatata*, *Pyrenula oxysporiza*, *S. leprieurii*, *T. austroindica*, and *Trypethelium endosulphurium*. The rare species can be regarded to be locally extinct in the vicinity of the paper mill. Faith and Norris (1989) suggested that rare and common species respond differently to different environmental variables with rare species being more sensitive to changes in habitat condition. The species sensitivity depends on physiology, while abundant species may have greater tolerance for environmental change compared to rare species, a condition that allows them to become habitat generalists by dominating communities.

6.4 Conclusion

The diversity indices can be used as an effective tool to monitor changes due to the effect of pollution on the living organism around any pollution source. The result can be correlated to the result found by Stefan and Rudolph (1979), Barrette (1969), Zullini and Peretti (1986), Wilhm (1967), and Junshum et al. (2008). In the present study, it was found that Shannon diversity, Pielou evenness, and Margalef richness showed a reduction, while Simpson dominance index exhibits an increase as one approaches near the paper mill. Further, it is observed that the graph between number of species and number of individual per species (Odum 1996) precisely describes the effect of stressed environment on the richness and dominance factor of lichen community through the changes in the shape of the graph. The study also describes the lichen diversity pattern around a paper mill using four commonly used diversity indices. Application of Odum's graphical way of depicting diversity helps in understanding the effect of stress on the lichens and changes in lichens preferences to different levels of stress.

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7

Remote Sensing and GIS for Biodiversity Conservation

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Contents

Abstract

This chapter focuses on the application of space-borne remote sensing and GIS for biodiversity conservation in the context of the state-of-the-art technology which has enhanced the classical approach. It reviews currently available instruments, i.e. space-borne or satellite sensors providing data which can be used without analysis or interpretation for studying individual organisms, species assemblages or ecological communities on ground. Subsequently, the image processing and GIS techniques developed to derive information from the captured satellite data are reviewed, and finally, this chapter concludes by reviewing the use of remote sensing and GIS techniques for mapping, monitoring and modelling lichens and their habitats.

Keywords

Remote sensing · GIS · Space-borne sensors · Biodiversity mapping · Lichen species richness

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7.1 Introduction

Biogeographers are continuously researching methods to map species distributions and diversity that can have significant applications for conservation planning (Foody 2003; Whittaker et al. 2005). Exhaustive ground surveys and inventories of species in field are the precise ways to accumulate biogeographical data on species distributions. However, such ground-based methods require skilled individuals and significant amount of time in the field, making them highly expensive. Also, even for relatively well-studied areas, different field data sources can lead to dissimilar or biased results of species distributions and diversity (Graham and Hijmans 2006; Moerman and Estabrook 2006; Pautasso and McKinney 2007); in areas such as the tropics, species occurrence and distribution data are relatively coarse and not well collected (Phillips et al. 2003; Schulman et al. 2007), and in areas with high biodiversity but inaccessible terrain, surveying for mapping vegetation and other forest cover using conventional techniques is complex and time-consuming (Lele and Joshi 2009). Moreover, the traditional field ecological data do not translate readily to regional or global extents, and models derived purely from such local data are unlikely to predict the global consequences of human activities. Thus, ecologists and conservation biologists are turning to remote sensing techniques and data sources for mapping species diversity and for preparing scientific responses to environmental change such as management techniques and various decision-making (Kerr and Ostrovsky 2003).

Given the difficulties associated with field-based data collection, use of remote sensing for estimating environmental heterogeneity and species diversity is appropriate since it provides a synoptic view of an area with a high temporal resolution (Loarie et al. 2008), offering an inexpensive way of deriving complete spatial coverage of environmental information for large areas in a consistent manner that may be updated regularly (Muldavin et al. 2001; Duro et al. 2007). Because of the potential capacity for systematic observations at various scales, remote sensing technology extends possible data archives from present time to over several decades back (Xie et al. 2008). Also, it is not as labour-intensive and time-consuming as the field-observation-based studies. Remote sensing has considerable potential as a source of information on biodiversity at landscape, regional, continental and global spatial scales (Nagendra 2001; Willis and Whittaker 2002; Turner et al. 2003).

Satellite remote sensing's process of obtaining information about earth from space and then effectively processing it (collection, storage, analysis and dissemination) has swept the fields of biodiversity conservation and its management in recent years (Table 7.1). The latest advances in RS technologies strengthen its use in capturing/measurement of biodiversity on earth and in addressing in situ and ex situ biodiversity

Table 7.1 Advantages and disadvantages of remote sensing as compared to traditional in situ methods

Advantages	Disadvantages
Provides a continuous, repetitive, large-scale synoptic view relative to traditional point-based field measurements	Instruments used in satellite remote sensing are expensive to build and operate
Practical way to obtain data from dangerous or inaccessible areas	Remote sensing data are not direct samples of a phenomenon and so must be calibrated against reality
Relatively cheap and fast method of information acquisition over large geographical areas	Remote sensing data need to be geometrically corrected and georeferenced in order to be useful as maps, which is an intricate process
Easy to analyse with the computer and combine with other geographical data	Remote sensing data interpretation needs subject experts having knowledge of the phenomena being studied
Data acquisition from present time to over several decades back	Most sensors can monitor only features that can be viewed from above; characteristics of the understory must be inferred rather than directly observed

conservation efforts by adding value to the detection of species, ecological communities and patterns of species richness.

This chapter focuses on the space-borne remote sensing in the context of state-of-the-art technology, including instruments and techniques. The following section of this chapter reviews currently available instruments, i.e. space-borne or satellite sensors providing data that can be used without analysis or interpretation for studying individual organisms, species assemblages or ecological communities on ground. Subsequently, the image processing and GIS techniques developed to derive information from the captured satellite data are reviewed, and finally, this chapter reviews the use of remote sensing and GIS techniques for mapping, monitoring and modelling lichens and their habitats.

7.2 Sensors (Passive or Active) Used to Assess and Model Biodiversity

There has been a remarkable increase in earth observation satellites and sensors over the last few years that are being used to measure and model biodiversity from space (Table 7.2) (Gillespie et al. 2008). Advances in the resolutions of space-borne sensors available for ecologists are making it increasingly possible to distinguish species assemblages or identification of species of individual trees (Turner et al. 2003).

Passive sensors, which record reflected (visible and infrared wavelengths) and emitted energy (thermal wavelengths), are most frequently used in biodiversity studies. The highest spatial resolution data come from commercial satellites such as WorldView, QuickBird and IKONOS which contain visible and infrared bands used in species mapping. The NASA Landsat series is the most widely used sensor for biodiversity studies due to the ease in which the data can be obtained, long time series and low cost. MODIS and AVHRR sensors have provided extremely useful data for regional, continental and global studies of land-cover classification and diversity models. These sensors also provide data on temperature, precipitation and fire that have been incorporated into biodiversity studies (Gillespie et al. 2008). Other satellites and sensors such as IRS, SPOT and ASTER are also becoming common.

Active sensors, unlike passive sensors, can penetrate cloud cover, providing imagery of both day and night, regardless of weather conditions. Light detection and ranging (LIDAR) offers new and improved capabilities for vertical and structural characterization of objects, such as plant height and different growth stages/strata. The stand height and structural information derived from the LIDAR imagery contribute greatly to the characterization of biodiversity through vertical stratification (Dees et al. 2012). The Shuttle Radar Topography Mission (SRTM) provides 30–90-m resolution data on elevation and topography that has been used in species and diversity models. Radar backscatter from RADARSAT-1 has been used in land-cover classification and diversity models. ALOS PALSAR sensors have been shown to have very high level I accuracy when classifying several different forested regions (Kellndorfer et al. 2008). LIDAR pulses from the orbiting ICESat GLAS satellite have provided a wealth of data for forest biomass mapping and validation (Phlugmacher et al. 2008).

Based on the current state of the art in remote sensing instruments and their existing applications in the literature, we review four types of instruments: hyperspatial, hyperspectral, thermal infrared and LIDAR sensors with their applications in biodiversity conservation.

7.2.1 Hyperspatial Remote Sensing

Recently, many fine spatial resolution systems have been launched which allow ecosystems to be characterized over a range of scales and consider queries that were previously impractical to study from space or on the ground to be addressed (Table 7.3).

High spatial resolution imagery greatly increases the accuracy of identification and characterization of small objects at spatial scales which were previously only available from airborne

Table 7.2 Popular satellites and sensors used to measure and model biodiversity from space

Sensor	Launch date	Source	Spectrum	Details and global imagery (GI) extent
Passive				
Landsat 5 TM	1984	USA	V-TIR, 7 bands	Operating beyond expected lifetime. Continuous GI to 1985, spotty 1985 to present
IRS-1C/1D LISS-III, WIFS, PAN	1995, 1997	India	V-SWIR, 4 bands, 2 bands, V-NIR, 1 band	Constellation twin satellites, each hosts two moderate-resolution sensors. Pointable, panchromatic camera
IKONOS	2000 Commercial	USA	V-NIR, 5 bands	Pointable, stereo capability. Spotty GI, developing continuous GI
QuickBird	2001 Commercial	USA	V-NIR, 5 bands	
SPOT-5 HRVIR	2002 Commercial	France	V-SWIR, 5 bands	Pointable. Indonesia and Thailand use with Landsat data
RESOURCESAT-1 LISS-4	2003	India	V-NIR, 4 bands	Pointable and mounted with two moderate-resolution sensors
GeoEye-1	2008 Commercial	USA	V-NIR, 5 bands	Pointable, pan imagery released at 0.5 m. Spotty GI, developing continuous GI
WorldView-2	2009 Commercial	USA	V-NIR, 9 bands	Eight bands (+PAN) in VNIR, pointable
Active				
AVHRR	1978–2005	USA	V-TIR, 4–6 bands	A long-term satellite constellation, most recent is AVHRR/3. Continuous GI
MODIS Terra & Aqua	2000, 2002	USA	V-TIR, 36 bands	Mounted on two satellites. Vegetation bands are 250 m in resolution. Thermal bands used to detect fires. Continuous GI
SRTM	2000	USA	InSAR. X band, C band (Quadpolar)	Result of a 10-day shuttle mission
ENVISAT ASAR	2002	EU	SAR. C band. Quad-polar	Pointable. Continuous GI, with temporal interferometry in select areas
ICESAT GLAS	2004	USA	LIDAR	Tracks currently spaced at 15 km (equator) and 2.5 km (80° latitude)
RADARSAT-2	2006	Canada	SAR. C band. Quad-polar	Pointable. Continuous GI, with temporal interferometry in select areas
ALOS PALSAR	2006	Japan	SAR, L band. Quad-polar	Resolution and swath vary depending on mode. Continuous GI
TerraSAR-X	2007 Commercial	Germany	SAR, X band. Quad-polar	Commercial, variable resolution, and first in a satellite constellation

Adapted from Fagan and DeFries (2009)

platforms (Turner et al. 2003; Gillespie et al. 2008). With the availability of high-resolution imagery, the focus on mapping specific land-cover classes such as tree species rather than broad land-cover classes has gained significant attention (Boyd and Foody 2011). The derived information from mapping specific land-cover classes can aid both biodiversity assessment and conservation (Landenberger et al. 2003; Wilson et al. 2004).

The availability of data from high spatial resolution sensors has opened up new opportunities for the development of operational mapping and monitoring of small features such as individual tree crowns (Hurtt et al. 2003; Levin et al. 2007; Rocchini 2007); mapping of fine-scale habitat elements, e.g. trees, at a landscape scale (Pitkanen 2001; Culvenor 2002; Hirschmugl et al. 2007; Rocchini 2007); mapping of invasive species; and

Table 7.3 Main space-borne hyperspatial sensors currently available for biodiversity studies

Sensor	Spatial grain (pixel size)	Spatial extent (swath width)	Spectral grain (no. of bands)	Launch date
IKONOS	1 m PAN, 4 m MSS	11.3 km	5	September 1999
QuickBird	0.65 m PAN, 2.6 m MSS	16.5 km	4	October 2001
SPOT-5 HRVIR	2.5–5 m PAN, 10 m MSS, 20 m SWIR	60 km	5	May 2002
RESOURCESAT-1 LISS-4	5.8 m	70 km PAN, 23.9 km MSS	3	October 2003
WorldView-1	0.5 m PAN, 2 m MSS	17.6 km	1	September 2007
GeoEye-1	0.5 m PAN, 2 m MSS	15.2 km	4	September 2008
WorldView-2	46 cm PAN, 1.84 cm MSS	16.4 km	8	October 2009
SPOT-6	1.5 m PAN, 8 m MSS	60 km	5	September 2012

development of species-level distribution maps along with structural information on dominance, canopy diameters and age–class distribution (Singh et al. 2010). Turner et al. (2003) have pointed out the applicability and feasibility of direct identification of certain species and species assemblages ; Gillespie et al. (2008) provide several examples of accurately identifying plant species based on the high spatial resolution imagery.

IKONOS, QuickBird, GeoEye-1 and Satellite Pour l'Observation de la Terre-5 (SPOT-5) are the commonly used systems. IKONOS imagery with a spatial resolution of 1 m (panchromatic) and 4 m (multispectral) enables the study of local-scale features from space (Read et al. 2003); QuickBird system from Digital Globe with multispectral imagery at resolutions of 2.4–2.8 m and panchromatic imagery 0.6–0.8 m makes direct identification of certain species and species assemblages feasible; WorldView-2 (WV-2) imagery combines high spatial resolution (0.5 m), sufficient spectral resolution (8 bands) and a Red-Edge detector (705–745 nm) for conducting vegetative analyses to reveal plant type, age, health and diversity in unprecedented detail. It is anticipated that the joint use of these data sets can be used for a variety of natural resource management applications.

Recent studies (Omar 2010; Kanniah 2011) have reported that the spectral bands of WV-2 ranging from 400 to 1,040 nm are suitable for discriminating tree species. IKONOS imagery has been used in conjunction with Landsat to map the expansion of an invasive plant species (Gillespie et al. 2008); to study forested environments at the scale of individual tree crowns (Hurtt et al. 2003; Clark et al. 2004); and for quantification and evaluation of the spatial structure of critical habitats and its effect on endemic species (Perotto-Baldivieso et al. 2009). Recently, Eckert (2012) explored the potential of WV-2 data for biomass and carbon estimation of tropical humid rainforests; the results on linking biomass and carbon inventory data for tropical humid rainforests with very high-resolution WV-2 satellite data are promising.

High spatial resolution imagery can be employed to assess the accuracy of remote sensing precuts derived from moderate or coarse spatial resolution imagery. For instance, Wabnitz et al. (2008) assessed the accuracy of LANDSAT-based large-scale seagrass mapping against patterns detectable with very high-resolution IKONOS images.

High-resolution data are also being increasingly used for ex situ biodiversity conservation; for example, scientists at the Royal Botanical Garden, Kew, discovered some previously unknown species in a remote highland forest in Mozambique, identified using very high-resolution (VHR) imagery from Google Earth (Timberlake et al. 2007). High-resolution images are being keyed to tabular data for providing additional dimensions of access to specimens (Bisby 2000; Edwards et al. 2000; Oliver et al. 2000),

Table 7.4 Main space-borne hyperspectral sensors currently available for biodiversity studies

Sensor	Satellite	Spatial grain (pixel size)	Spatial extent (swath width) (km)	Spectral grain (no. of bands)	Spectral grain (bandwidth)	Spectral resolution (nm)
Compact high-resolution imaging spectrometer (CHRIS)	Project for on-board autonomy (PROBA)	19–36 m	14	Up to 62, programmable	1–12 nm	Up to 410–1,050
Hyperion EO-1	Earth observing-1	10 m PAN, 30 m all other bands	7.7	220	10 nm	356–2,578
Global imager (GLI)	Advanced earth observing satellite-II (ADEOS-II)	250 m—6 bands corresponding to Landsat, 1 km—all other bands	1,600	36	12–2,985 nm	380–12,000
Medium-resolution imaging spectrometer (MERIS)	ENVISAT	250 m–1 km	1,150	15	2.5–12.5 nm, programmable	390–1,040
Moderate-resolution imaging spectrometer (MODIS)	TERRA	250 m VNIR, 500 m VNIR-SWIR, 1 km TIR	2,330	36	10–15 nm VNIR-SWIR	405–14,385

and these data sets are being made available through the Internet, e.g. New York Botanical Garden, Museum of Vertebrate Zoology, University of California at Berkeley, Missouri Botanical Gardens and Instituto Nacional de Biodiversidad, Costa Rica.

Although high spatial resolution satellite remote sensing is a very useful source of data, Nagendra and Rocchini (2008) have rightfully pointed out that it is the most potentially powerful yet underutilized source for tropical research on biodiversity and stimulating discussion on its possible applications should be the first step in promoting a more extensive use of such data.

7.2.2 Hyperspectral Remote Sensing

Hyperspectral remote sensors acquire images across many narrow contiguous spectral bands throughout visible, near-infrared and mid-infrared portions of electromagnetic spectrum and measure the reflected spectrum at wavelengths between 350 and 2,500 nm using 150–300 contiguous bands of 5–10 nm bandwidths (Table 7.4). According to Shippert (2004), several recently launched hyperspectral sensors are acquiring imagery from space, including Hyperion sensor on NASA's EO-1 satellite, CHRIS sensor on European Space Agency's PROBA satellite and FTHSI sensor on US Air Force Research Lab's MightySat II satellite. Many airborne hyperspectral sensors, including NASA's AVIRIS sensor, are also available to collect data. Of these sensors, the first-civilian and most commonly used data are derived from the Hyperion, which is operated by the Earth Resources Observation and Science (EROS) at a relatively low cost to the general public.

Hyperspectral data can discriminate fine-scale, species-specific land cover (Turner et al. 2003), such as vegetation categories or soil types which make remarkable contribution to any study regarding biodiversity patterns. These data are well suited for vegetation studies since reflectance/absorption spectral signatures from individual species as well as more complex mixed-pixel communities can be better differentiated from the much wider spectral bands of hyperspectral imagery (Varshney and Arora 2004). Nagendra and Rocchini (2008) summarize that hyperspectral data have been successfully applied in recording information regarding critical plant properties (e.g. leaf pigment, water content and chemical composition), in discriminating tree species in landscapes and in fairly accurate distinction between different species. Xie et al. (2008) have emphasized how vegetation extraction from hyperspectral imagery has been increasing; recent studies imply that this imagery is capable of separating plant species, which may be difficult using multispectral images.

Recent applications of Hyperion hyperspectral imagery mainly include ecology and biodiversity in forest, grassland (Guerschman et al. 2009), agriculture (Bannari et al. 2008), vegetation (Walsh et al. 2008), fragmented ecosystem and ecosystem succession and coastal environment (Lee et al. 2007). Vegetation types and densities have also been classified using Hyperion data to support of wildfire management (Keramitsoglou et al. 2008). Foster et al. (2008) state that hyperspectral imagery from EO-1 Hyperion is capable of mapping low-lying woody lianas, which are critical to tropical forest dynamics; Pignatti et al. (2009) successfully analysed the capability of Hyperion data for discriminating land cover according to the structure of current European standard classification system (CORINE Land Cover 2000). Besides the application of land-cover classification, the relationships between LAI and spectral reflectance were studied (Twele et al. 2008) using narrowband (EO-1 Hyperion) and broadband Landsat-enhanced thematic mapper plus (ETM+) remotely sensed data. Integration of Hyperion and IKONOS imagery has also been tested successfully for differentiation of subtle spectral differences of land-use/land-cover types on household farms with an emphasis on secondary and successional forests (Walsh et al. 2008). The MODIS vegetation continuous fields product pictures heterogeneous vegetation more realistically and improves the detection of changes in complex landscapes (Pfeifer et al. 2011).

There are many drawbacks associated with hyperspectral remote sensing as its high cost of acquisition data and complex technical aspects of processing make the whole process outside the expertise of most ecologists, and also the huge volume of data require a large data storage capacity and can be time intensive to process. Nagendra and Rocchini have preliminarily discussed strengths and drawbacks of hyperspatial (i.e. high spatial resolution) and hyperspectral data (Nagendra and Rocchini 2008).

7.2.3 Thermal Remote Sensing

Thermal remote sensing detects the energy emitted from Earth's surface in thermal infrared (TIR, 3–15 μm), which can be radiated by all bodies above absolute zero. Theoretically, TIR sensors measure surface temperature and thermal properties of targets (Canada Centre for Remote Sensing 2007), which are essential for developing a better understanding and more robust models of land surface energy balance interactions. TIR remote sensing is capable of uncovering the principles of ecological patterns of structure and function due to the development of ecological thermodynamics (Quattrochi and Luvall 2009).

The well-known sensors with TIR bands include the advanced VHR radiometer (AVHRR) on board the Polar Orbiting Environmental Satellites (POES), Landsat Thematic Mapper (TM) and ETM+, the advanced space-borne thermal emission and reflection radiometer (ASTER) on Terra Earth observing satellite platform (Quattrochi and Luvall 2009).

There have been several successful attempts in using TIR remote sensing for biodiversity conservation; for example, biophysical variables have been derived from thermal and multispectral remote sensing data and coupled with a Soil-Vegetation-Atmosphere-Transfer (SVAT) model (Gillies and Temesgen 2004); Duro et al. (2007) have illustrated that TIR region is important to study environmental disturbance because of negative relationship between vegetation density and land surface temperatures (LST), and Mildrexler et al. (2007) have successfully applied disturbance detection index using Moderate Resolution Imaging Spectroradiometer (MODIS) 16-day Enhanced Vegetation Index (EVI) and 8-day LST to detect continental-scale disturbance events such as wildfire, irrigated vegetation, precipitation variability and incremental process of recovery of disturbed landscapes. Another good use of TIR remote sensing data is to measure evapotranspiration, evaporation and soil moisture; for example, Crow and Zhan have analysed the continental-scale performance of surface soil moisture retrieval algorithms depending on satellite passive microwave, scatterometer and thermal remote sensing observations (Crow and Zhan 2007), and Petropoulos et al. (2009) have reviewed surface temperature/vegetation index remote sensing-based methods for the retrieval of land surface energy fluxes and soil surface moisture.

7.2.4 LIDAR Remote Sensing

LIDAR, also called laser altimetry, is an active remote sensing technology that utilizes a laser to illuminate a target object and a photodiode to register the backscatter radiation (Lim et al. 2008; Hyyppa et al. 2009). The longer wavelength pulses of radars can penetrate clouds, and the longest radar wavelengths (i.e. L band and beyond) penetrate tree canopies or, in cases of bare and loamy soil, the surface of the earth to depths of a metre or more (Ulaby et al. 1982). LIDAR sensors use the return signals to detect the height of the canopy top, ground elevation and the positions of leaves and branches in between, giving it the ability to penetrate forest canopies and making it a potential tool for measuring biomass and determining vegetation structure (Turner et al. 2003). LIDAR is particularly useful for measuring height (Van der Meer et al. 2002), which may then be incorporated into further ecological analysis.

Besides airborne LIDAR with limitations of large data volumes, footprint size and high costs (Duncanson et al. 2010), space-borne LIDAR has come through with launch of Ice, Cloud, and land Elevation Satellite/Geoscience Laser Altimeter System (ICESat/GLAS), which is the first laser-ranging instrument for continuous global observations. Space-borne LIDAR focuses on the forest vertical structure, especially forest canopy height and aboveground biomass estimation. Lefsky et al. (2007) have estimated forest canopy height with an RMSE of 5 m in varied forest types including evergreen needle leaf, deciduous broadleaf and mixed and tropical evergreen broadleaf stands. The height and aboveground biomass of mangrove forests have also been successfully measured and mapped based on SRTM elevation data, GLAS waveforms and field data (Simard et al. 2008). Pflugmacher et al. (2008) have compared GLAS height and biomass estimates with reference data from the Forest Inventory and Analysis (FIA) program of the US Forest Service at a regional scale to obtain promising results. Helmer et al. (2009) have combined Landsat time series and GLAS to estimate the biomass accumulation of Amazonian secondary forest to have results in agreement with ground-based studies. Duncanson et al. (2010) successfully tested simulated GLAS data for areas with dense forests, high relief or heterogeneous vegetation cover to demonstrate the capability of GLAS waveforms as supplemental model input to improve estimates of canopy height. The application of LIDAR technology to marine biodiversity conservation also shows promise for detecting habitats (Turner et al. 2003). Large-footprint LIDAR information is fused with MODIS data to generate forest height maps (Lefsky 2010), and the P band of the synthetic aperture radar (SAR) shows good agreement with boreal forest biomass.

In many recent studies, the detection of individual vegetation objects has been facilitated by increasing spatial resolutions of remotely sensed imagery, LIDAR and use of object-based approaches to data classification (Falkowski et al. 2006; Koch et al. 2006; Zhang et al. 2010).

In ex situ conservation efforts also, LIDAR seems to have found a use; for example, professionals at Montgomery Botanical Center, Florida, were able to improve the assessment of their property with deeper evaluation of the collections and natural resources using remote sensing imagery and data. By adding LIDAR imagery to maps and employing techniques normally used at larger regional scales, new information was discovered about the garden and its collections. (http://arnoldia.arboretum.harvard.edu/pdf/articles/2011-69-1-remote-sensing-as-a-botanic-garden-tool.pdf).

7.3 Satellite Image Processing and GIS Techniques Used to Measure and Model Biodiversity

Several researchers have pursued cutting-edge studies on biodiversity conservation using remote sensing and GIS approaches, some of which have been presented in Table 7.5.

Existing studies of biodiversity conservation using remote sensing and GIS can be grouped into three categories:

- Biodiversity mapping (individual species, species assemblages and habitats)
- Biodiversity monitoring
- Biodiversity modelling.

7.3.1 Biodiversity Mapping for Individual Species, Species Assemblage and Habitats

The improved spatial, temporal and spectral resolution of satellite data has made it possible to generate information at diverse scales on major climatic, physiognomic vegetation types as well as region-specific vegetation types depicting local gregarious formations and distinctive habitats. The higher-resolution imagery shows great potential in identification of tree species and canopy attributes, with several studies undertaking targeted mapping to estimate the variety, type and extent of specific species; for example, economically and medicinally important species in remote and inaccessible areas have been mapped by several researchers (Behera et al. 2000; Roy et al. 2001; Porwal et al. 2003; Joshi et al. 2005) using Indian Remote Sensing (IRS) satellite data; QuickBird data have been used to map giant reed (Arundo donax) in southern Texas with 86–100 % accuracy (Everitt et al. 2006); high-resolution data have been used to identify mangrove species (Dahdouh-Guebas et al. 2006; Wang et al. 2004). Mapping of species assemblages has been attempted using satellite data; for example, evergreen forests/vegetation of Mouling National Park, Arunachal Pradesh, India, was mapped by integrating a digital elevation model (DEM) with Linear Imaging Self-scanning Sensor (LISS) III multi-band data (Singh et al. 2005); seven species of trees were classified with an overall accuracy of 86 % in temperate forests (Carleer and Wolff 2004).

Remote sensing-based habitat maps and information on species habitat associations are generally being used to derive information on the distribution of species. Satellite data can be combined with field-based habitat data, landscape structure and species abundance information to identify the habitat boundaries and biophysical characteristics of species (Kerr et al. 2001; Scribner et al. 2001). Satellite data classification has long been used to link species distributions with vegetation types and associated habitat preference (Nagendra 2001; Gottschalk et al. 2005; Leyequien et al. 2007). Way back in 1994, Franklin et al. carried out an analysis using satellite imagery to differentiate compositionally distinct vegetation communities, and in a later study, the preliminary observations in a tropical forest in northern India suggested that supervised classification of satellite data permits the discrimination of vegetation types with distinct species composition (Ravan et al. 1995). In a detailed analysis in tropical forests of Western Ghats of India, Nagendra and Gadgil (1999) mapped a landscape into seven habitat types ranging from secondary evergreen forests to paddy fields, using supervised and unsupervised classification of IRS satellite imagery. Vegetation maps as a surrogate for habitat preference have provided insights into the distributions of birds (Peterson et al. 2006), herpetofauna (Raxworthy et al. 2003) and insects (Luoto et al. 2002).

Table 7.5 Some important biodiversity conservation studies, techniques employed and data sources

Issue addressed	Technique used	RS data used	References
Biodiversity mapping	Individual species mapping, species assemblage mapping	IRS LISS-III, QuickBird MSS	Franklin et al. (1994), Behera et al. (2000), Roy et al. (2001), Porwal et al. (2003), Carleer and Wolff (2004), Dahdouh-Guebas et al. (2006), Wang et al. (2004), Joshi et al. (2005), Singh et al. (2005), Everitt et al. (2006), Kandwal et al. (2009), Kimothi et al. (2010)
	Habitat mapping	IRS LISS-III, Landsat ETM+	Ravan et al. (1995), Nagendra and Gadgil (1999), Peterson et al. (2006), Raxworthy et al. (2003), Luoto et al. (2002), Giriraj et al. (2008, 2009), Roy and Tomar (2001), Amarnath et al. (2003), Roy et al. (2012)
Biodiversity monitoring and assessment	Monitoring habitat modification/ degradation	IRS LISS-II, LISS-III, Landsat TM/ETM/MSS, SPOT MSS	Nagendra and Gadgil (1999), Singh et al. (2004), Joshi et al. (2005), Jha et al. (2005), Reddy et al. (2007), Sharma et al. (2002), Giriraj et al. (2009), Behera and Roy (2010), Roy (2011), Joseph et al. (2011), Roy et al. (2013)
	Monitoring human-induced habitat threat	LISS-III, Landsat TM/ ETM/MSS, IKONOS, DMSP-OLS, IRS P6-AWIFS	Roy and Tomar (2001), Roy (2003), Gupta et al. (2004), Talukdar (2004), Kiran Chand et al. (2006), Kandwal et al. (2009), Kimothi et al. (2010), Nagendra et al. (2010), Thakur et al. (2011), Sudheesh and Reddy (2013), Krishna and Reddy (2012), Badrinath et al. (2011), Reddy et al. (2012), Saranya et al. (2014)
	Monitoring vegetation versus climate change	IRS LISS-III, LISS-IV, Landsat TM/MSS	Panigrahy et al. (2010), Dudley et al. 2010, Bharti et al. (2012), Volante et al. 2012, Caride et al. 2012
Biodiversity modelling	Species distribution modelling	NA	Engler et al. (2004), Araujo et al. (2005), Akcakaya et al. (2006), Hamann and Wang (2006), Austin (2007), Botkin et al. (2007), Carvalho et al. (2010), Luoto et al. (2007), Williams et al. (2009), Adhikari et al. (2012), Yang et al. (2013), Giriraj et al. (2008), Kumar and Stohlgren (2009), Irfan ulah et al. (2006), Saran et al. (2010a, b), Nativi et al. (2009), Richardson et al. (2010), Barik and Adhikari (2011), Lorenzen et al. (2011)
	Species diversity modelling	MODIS, QSCAT, SRTM	Gould (2000), Oindo and Skidmore (2002), Foody and Cutler (2006), Tuomisto et al. (2003), Fairbanks and McGwire (2004), Waser et al. (2007), Rocchini (2007), Stickler and Southworth (2008), Lucas and Carter (2008), He and Zhang (2009), Rocchini et al. (2009), Oldeland et al. (2010)

Giriraj et al. (2009) applied data generated from RS and GIS to categorize habitats and determined the relationship between habitat categorizations and species distribution patterns in tropical rainforests of southern Western Ghats, India.

In order to spatially delineate habitats, remote sensing-derived habitats were analysed in conjunction with landscape metrics, species assemblages, microhabitats such as slope, topography, species endemism and proportion of core and edge species (Giriraj et al. 2008). Under a national-level project on biodiversity characterization at landscape-level, vegetation-type maps were analysed in conjunction with climate and topography using geographical information system (GIS) to identify habitats a priori and determine the relationship between remotely sensed habitat categories and species distribution patterns (Roy and Tomar 2000; Amarnath et al. 2003; Roy et al. 2012). Giriraj et al. (2009) have used data generated using remote sensing and geographical information systems to categorize habitats and determine the relationship between habitat categorizations and species distribution patterns. Nandy and Kushwaha (2010) have attempted habitat classification of mangroves in Sunderban Biosphere Reserve (SBR) in West Bengal province of India using IRS satellite data using different classification approaches to delineate four distinct mangroves classes. Habitats with clear boundaries (e.g. grassland and agriculture) can generally be mapped with greater accuracy (Bock et al. 2005; Lucas et al. 2007; Forster and Kleinschmit 2008). SAC (2001) attempted grassland mapping in Gujarat using remote sensing and GIS techniques. Recently, Rahman et al. (2013) used Landsat ETM+ data for mapping and inventory of mangrove forest in Sundarbans using different classification methods.

New mapping approaches such as object-oriented classification (Collingwood et al. 2009), decision tree, support vector machine, multilayer perception and radial basis function neutral networks significantly improve classification accuracy (Dalponte et al. 2009; Mukhopadhyay and Maulik 2009; Kumar et al. 2007; Foody and Mathur 2004; Sanchez-Hernandez et al. 2007a; Boyd et al. 2006). Remote sensing may be used to monitor a habitat of interest with a one-class classification approach adopted to focus effort and resources on the class of interest (Boyd et al. 2006; Sanchez-Hernandez et al. 2007b), and there is a considerable scope for different types of classification methods such as soft or fuzzy classifications which allow the study of environmental gradients, transition zones and subpixel land cover (Jensen et al. 2009; Lu and Weng 2007; Plourde et al. 2007; Rocchini and Ricotta 2007). Integration of radar data improves classification accuracy (Saatchi et al. 2001; Boyd and Danson 2005; Li and Chen 2005), and LIDAR data demonstrate the potential for mapping emergent tree species and subcanopy layers that are important indicators of stratification for forest bird species. Further research is underway on information extraction techniques such as image classifier development for derivation of thematic maps.

More advanced techniques have examined the variability of spectral signals in satellite imagery which has been demonstrated to have an intrinsic power in evaluating species diversity (Palmer et al. 2002) since it is expected that the higher the spectral variability is, the higher the habitat and species variability will be (Carlson et al. 2007; Rocchini et al. 2007).

7.3.2 Biodiversity Monitoring

Effective monitoring is critical to evaluate and improve biodiversity conservation practice and a well-conceived, designed and implemented biodiversity monitoring agenda should (i) deliver information on trends in key aspects of biodiversity; (ii) provide early warning of problems that might otherwise be difficult or expensive to reverse; (iii) generate quantifiable evidence of conservation successes and failures; (iv) highlight ways to make management more effective; and (v) provide information for return on conservation investment (Lindenmayer et al. 2012).

Ecological studies increasingly require biophysical and habitat data through time and over significant areas, and also background rate and direction of change in ecological systems are essential to detect the signature of anthropogenic impacts. These prerequisites are generating a lot of interest in methods to aid tracking and interpretation of biodiversity changes through time to produce monitoring data sets. Technologies such as remote sensing and GIS can facilitate rapid collection of huge amount of large-scale data (Youngentob et al. 2011), establish baselines of the extent and condition of habitats and associated species diversity as well as quantify losses, degradation or recovery associated with specific events or processes more effectively as compared to more traditional, field-based methods (Nagendra et al. 2013; Kerr and Ostrovsky 2003).

In current times, when the satellite record is around four decades old, it can provide potential means to study impacts of environmental change. Remote sensing can be applied to habitat inventory and evaluation, assessment of habitat attributes and identification of suitable sites for protected areas (Kamat 1986; Panwar 1986). Mostly, the use of high-to-moderate spatial resolution data, such as provided by the Landsat and the Indian Remote Sensing (IRS) satellite, may be sufficient to capture the broad extent and spatial patterns of habitats (Lucas et al. 2007, 2011). Satellite data sets from IRS, Landsat and Système Pour l'Observation de la Terre (SPOT) have been used effectively in monitoring and analysing biodiversity (Nagendra and Gadgil 1999; Joshi et al. 2005; Behera and Roy 2010; Roy 2011). In recent years, the advent of VHR satellites has provided opportunities for more detailed mapping and studies of changes in habitat coverage, landscape fragmentation and human pressure.

Habitat modification and degradation are prevalent even in intact landscapes, so developing methods to quantify and monitor changes in habitats is critical. Many researchers have been working in this direction; for example, Singh et al. (2004) highlighted the application of satellite remote sensing in assessment and monitoring of mangrove habitats along Goa state coastline, India, by analysing the changes in mangrove forest cover using IRS data; Reddy et al. (2007) performed periodic assessment and monitoring of mangroves of Bhitarkanika Wildlife Sanctuary, Orissa, India, using temporal multisensor satellite data to find changes in mangrove and other land-cover categories during the last 30 years. Disruption of habitat connectivity due to landscape fragmentation can impact species dispersion, habitat colonization, gene flows, population diversity, species mortality and reproduction. Quantitative analyses of changes in landscape structure can provide early warnings of habitat degradation. Sharma et al. (2002) analysed the spatial patterns of different attributes and explored the extent and patterns of forest fragmentation in a Himalayan landscape to find that out of total landscape in study area, 41 % was fragmented. Fragmentation led vegetation changes in Vindhya hills over a decade, and the resultant impact on local biodiversity was studied using remote sensing data (Jha et al. 2005). Giriraj et al. (2009) studied remnant intact patches of evergreen forest using multitemporal satellite data and explored the likelihood of their sustenance in coming decades with respect to vegetation composition, changes in patch characteristics and regeneration potential in Kalakad Mundanthurai Tiger Reserve, Tamil Nadu (India). Joseph et al. (2011) reviewed the state of remote sensing technology in characterization of tropical forest degradation by describing the factors responsible and its likely impacts. The authors conclude by requesting an additional momentum in research to answer numerous unresolved questions of tropical forest degradation. A recent exemplary work by Roy et al. (2013) used a moving window approach to identify potential areas of forest fragmentation in Indian landscape to assess the impact of anthropogenic pressures and cultural practices on forest fragmentation that provides critical inputs for prioritization and conservation of forests and associated biodiversity.

There can be many types of threats to habitats depending on the landscape, context and time period of focus, and more common types include urbanization, road construction, mining, logging, agriculture, fire, invasion by alien species, hunting, grazing and drought (DeFries et al. 2005; Nagendra 2008). Remote sensing data sets of medium-to-fine spatial resolution can provide important information on signature of human pressure related to land use, management and other disturbances (Fuller et al. 2007). Satellite sensors such as AWiFS, LISS-III, ETM+, MODIS, OCM, AVHRR and MODIS provide synergistic data sets that have potential in forest fire detection and monitoring (Sudheesh and Reddy 2013). The forest fire detection capabilities of Indian Defense Meteorological Satellite Program Operational Linescan System (DMSP-OLS) satellite data for effective monitoring of forest fires have been evaluated with positive outcomes (Kiran Chand et al. 2006; Badrinath et al. 2011). The state of forests, methodology, models and case studies of forest fire risk and degradation assessment in the context of Indian forests have been discussed by Roy (2003), emphasizing the utility of geospatial techniques as powerful tools to assess the forest fire risk and degradation assessment. The critical spatial information for determination of forest burnt areas has been achieved using IRS P6 AWIFS satellite data which emphasize the need of remote sensing-based time series analysis in forest fire management (Reddy et al. 2012; Krishna and Reddy 2012). Sudheesh and Reddy (2013) have highlighted the utility of multitemporal satellite data in effective planning and conservation of forest resources by analysing the fire-prone areas in Nagarjunasagar–Srisailam Tiger Reserve (NSTR), Andhra Pradesh, India. Saranya et al. (2014) have accentuated that spatial databases offer excellent opportunity to understand the ecological impact of fires on biodiversity and are helpful in formulating conservation action plans by locating and estimating the spatial extent of forest burnt areas and fire frequency covering decadal fire events in Similipal Biosphere Reserve.

Satellite measurements of broadscale trends in vegetation also provide direct estimates of habitat loss, increasing the power of applied ecological studies to detect changes in species distributions or to model extinction rates. Roy and Tomar (2001) analysed the modification of natural landscape due to anthropogenic activities using temporal IRS and Landsat Multi-spectral Scanner (MSS) data by studying the land-cover dynamics pattern of biologically rich landscapes of Meghalaya, India. The linkages between socio-economic drivers and consequent forest loss in Nicobar group of islands have been investigated on the basis of indicators derived from LULC mapping of satellite remote sensing data (Gupta et al. 2004). Geostatistical analysis of forest cover changes in Meghalaya, India, has been attempted using LULC trends for 1980, 1989 and 1995, revealing the incessant impact of shifting cultivation and mining by generation of the likely landscape pattern for year 2025 (Talukdar 2004). The detection, mapping and patch information extraction of invasive weeds using high-resolution satellite data have also been attempted revealing the suitable seasons for weed detection and developing of a new technique by analysis of 29 vegetation indices (VIs) for discrimination of invasive weeds (Kandwal et al. 2009; Kimothi et al. 2010). Thakur et al. (2011) analysed cumulative impacts of human-induced processes such as livestock grazing and fuel wood extraction on forest cover using satellite data and field observations and revealed a significant loss of forest cover around the fringes of Kedarnath Wildlife Sanctuary in the last 29 years. Nagendra et al. (2010) used Landsat TM and ETM+ imagery to find a clear signal of forest fragmentation and deforestation at the periphery of an Indian tiger park because of extraction by local residents of villages outside the boundary.

Remote sensing data can provide insights into the impacts of climatic variability through analysis of changes in the extent and condition of vegetation (e.g. phenological shifts and species range shifts). Species in transition zones are especially vulnerable to climate change, as they have limited scope to move further (ICIMOD 2010) like in Indian Himalayan region, a unique habitat for distinct biological assemblages, and native and endemic, floral and faunal species, economically important species (Dhar 2000); it is speculated that under continuing climate change, tree species will be affected in different ways and ranges will adjust at different rates and by different processes (Subedi 2009). Many researchers are focussing their efforts in this direction; for example, Panigrahy et al. (2010) reported an upward shift of timberline vegetation by 300 m and a considerable reduction in snow cover in the Nanda Devi Biosphere Reserve (NDBR), Garhwal Himalaya, India. More recently, Bharti et al. (2012) studied temporal changes in the timberline ecotone vegetation of Nanda Devi National Park (NDNP), Indian Himalaya, on the basis of remote sensing analysis of Landsat MSS and TM images, over a period of 30 years. Satellite-derived data along with field data can be effectively used both to estimate and to monitor the level of provision of ecosystem services over large areas (e.g. He et al. 2005; Nosetto et al. 2005). Remotely sensed data and modelling techniques can provide estimates of carbon-related services for forest regional quantification of carbon gains in protected areas to support their protection given that they help address ecological problems related to climate change issues (Volante et al. 2012; Caride et al. 2012; Dudley et al. 2010). Though numbers of such studies are increasing, the full potential of remote sensing is yet to be explored in terms of monitoring forest health and monitoring range shift.

7.3.3 Biodiversity Modelling

Techniques that can greatly aid ecological modelling activities in support of conservation efforts are becoming increasingly available to the ecological community (Santos et al. 2006). Remote sensing and GIS have been used to predict species distributions and risks to biodiversity (Spens et al. 2007), to aid visualization, exploration and modelling of data on species distributions (Lopez-Lopez et al. 2006; Vogiatzakis et al. 2006; Zhang et al. 2007) and to study the effect of major variables such as disturbance events (Pennington 2007).

7.3.4 Species Distribution Modelling, Ecological Niche Modelling or Habitat Suitability Modelling

Species distribution modelling, ecological niche modelling or habitat suitability modelling has been growing at a striking rate in the recent years. Species distribution or environmental (or ecological) niche models (ENMs) provide a tool for addressing lack of species distribution data and can be used to fill knowledge gaps by projecting habitat suitability in areas with few or no occurrence records. Species distribution modelling (SDM) can combine georeferenced occurrence data points (latitude, longitude) with different data sets that characterize the environment where the focal species occur. These sets are combined and analysed aiming to build a representation of ecological requirements of the focal species, i.e. a representation of their ecological niche. The final result can be projected in geographical space, indicating areas suitable for focal species that can be potentially occupied by it (Giannini et al. 2011).

Usually, the data sets used are comprised by abiotic features such as temperature, precipitation and altitude, which describe the environment of species occurrence. But SDM can also include data about occurrences of interacting species (biotic features) responsible for shaping geographical distribution and species dispersal capacities, to estimate their capability of occupying new environments. Intermingled with these environmental (abiotic) and interaction (biotic) features, geographical distribution is also related to species evolutionary trends, determining patterns of genetic diversity and trait variation across space. These models utilize presence, absence or abundance data from museum vouchers or field surveys and environmental predictors to create probability models of species distributions within landscapes, regions and continents (Guisan and Thuiller 2005). These models can forecast potential future impacts of environmental changes on species distributions, allowing practitioners to assess alternative policies and actions to plan for the change (Nielson et al. 2008). It also estimates the relative suitability of habitat known to be occupied by the species, relative suitability of habitat in geographical areas not known to be occupied by the species, changes in the suitability of habitat over time given a specific scenario for environmental change and the species niche (Warren and Seifert 2011).

After years of ongoing development and testing, species distribution models are now offering valuable contribution to facilitation in site selection for reintroduction of critically rare and endangered species (Williams et al. 2009); design of their management plans (Adhikari et al. 2012); modelling the distribution and abundance of medicinally important species (Yang et al. 2013); and economically important species (Giriraj et al. 2008) or threatened and endangered species (Kumar and Stohlgren 2009; Irfan-ullah et al. 2006; Saran et al. 2010a). These models are extremely helpful in studying impacts of climate change on spatial distribution of biodiversity to facilitate future conservation activities (Skov and Svenning 2004; Nativi et al. 2009; Richardson et al. 2010; Araujo et al. 2005; Akcakaya et al. 2006; Hamann and Wang 2006; Austin 2007; Botkin et al. 2007) using bioclimate envelope models (Berry et al. 2002; de Garzon et al. 2007). The outcomes may be used to project future distribution of species under a set of climate change scenarios (Pearson and Dawson 2003; Luoto et al. 2007) which can provide a valuable initial assessment of likely climate change impacts. A variety of other applications also use these models such as invasive species management and prediction of their geographical distribution (Barik and Adhikari 2011); elucidation of paleodistributions (Lorenzen et al. 2011); design of field surveys (Engler et al. 2004); or design and selection of reserves (Carvalho et al. 2010).

New approaches are necessary to analyse the importance of these complex features. Recently, Pavoine et al. (2011) suggested a framework based on a mathematical method of ordination to analyse phylogeny, traits, abiotic variables and space in a plant community. Another example can be found in Diniz-Filho et al. (2009) proposing an integrated framework to study spatial patterns in genetic diversity within local populations, coupling genetic data, SDM and landscape genetics. The new trends on SDM, regarding the impacts of global changes on species diversity, are niche evolution and phylogeographic and phylogenetic research (Zimmermann et al. 2010).

Many taxonomic groups, however, are highly specious with little information on distribution or other attributes of every component species, and more new species are still being discovered. In such situations, SDM is limited in its ability to predict occurrences for all species in a taxon and hence in predicting patterns for biodiversity as a whole (Mokany and Ferrier 2011). Community-level modelling has the capacity to complement species-level approaches by predicting spatial patterns in biodiversity for highly diverse, poorly studied taxa. It may confer significant benefits for applications involving very large numbers of species, particularly where a sizeable proportion of these species is rarely recorded in the data set. Unlike species-level modelling, for which species with too little data are usually excluded from further analysis (for statistical reasons), many community-level modelling strategies make use of all available data across all species, regardless of the number of records per species. Among the broad range of community-level modelling approaches (Ferrier and Guisen 2006), species richness prediction of communities (alpha-diversity) and dissimilarity in community composition between pairs of sites (beta-diversity) are the most commonly employed ones. Alpha-diversity accounts for local species richness or abundance within each sampling unit, whereas beta-diversity is related to species compositional turnover among sampling units.

The prediction of diversity has substantially relied on simple univariate regression or multiple regression models appropriately scaling sensor imagery to field data on vascular plants (Fairbanks and McGwire 2004; Rocchini 2007; Stickler and Southworth 2008), lichens (Waser et al. 2007) and mammals (Oindo and Skidmore 2002). While these approaches provide a basic understanding of patterns and can be used to create predictive diversity maps for a landscape, region or continent, increasingly more sophisticated statistical and spatial techniques are being examined and developed to model patterns of diversity (Foody 2004, 2005) such as use of hyperspectral imagery for predicting species richness and abundance using univariate statistics (Lucas and Carter 2008; Oldeland et al. 2010); explicit mapping of uncertainty in species diversity prediction by spectral variability (Gould 2000; Oindo and Skidmore 2002); use of neural networks for predicting species diversity (Foody and Cutler 2006); or use of spectral distances between sampling units for estimating turnover in species composition (Tuomisto et al. 2003; He and Zhang 2009; Rocchini et al. 2009). Spatial statistics such as geographically weighted regression analyses have also resulted in improved models of diversity (Foody 2005). Furthermore, increased accuracy of predictions can be obtained using more complex approaches such as neural networks (Foody and Cutler 2006). There has also been an increasing interest in the combination of passive and active sensors to improve species diversity models, e.g. using a combination of passive sensors (MODIS) and active sensors (QSCAT, SRTM) to model tree diversity for the entire Amazon Basin (Saatchi et al. 2008).

7.4 Limitations of Using Remote Sensing and GIS for Biodiversity Conservation

Using remote sensing data for biodiversity mapping, monitoring and modelling requires a trade-off between data availability, spatial resolution and coverage, spectral resolution, timing of image acquisition, practicality of ground validation combined with overall cost of imagery and analytical effort. Any of these criteria can potentially limit the use of RS data for biodiversity conservation (Biodiversity report, 2012); hence, there are several limitations to using satellite images for biodiversity conservation, including

i. Possible end of key satellite programs; that is, the uncertainty in long-term continuity of remote sensing data restrains individual and organizations to invest in biodiversity monitoring projects utilizing remote sensing data.
ii. Except LIDAR and RADAR sensors, other sensors can monitor only features viewed from above, and characteristics of understory are unable to monitor/sense.
iii. Restricted data dissemination policy and low investment by public organizations lead to assignment of satellite development to private sector, making remote sensing data expensive and unaffordable. Many space agencies and countries are now offering free and open data access to their satellite data (e.g. Landsat, MODIS), but most data products especially high spatial resolution imagery have expensive and restricted access, making financial cost a major challenge to most biodiversity researchers and conservation practitioners (Leidner et al. 2012) (Table 7.6).
iv. Low temporal resolution leading to long temporal repeat cycle and short time series for trend analysis limits the sensitivity of remote sensing products to detect surface changes. The time-composited satellite products are insensitive to some natural phenomena which occur on finer timescales such as phenological changes (Cleland et al. 2007).
v. Technical expertise required to handle satellite imagery and other data products remains a challenge for ecologists and conservation biologists. Application of ecological models that can convert remote sensing data products into actual knowledge of species distributions and richness also requires additional software and analytical and technical skills.

7.5 Prospects

Key areas of research identified by Task Force on Mountain Ecosystems (Planning Commission of India 2006) were to understand the relationship between climatic patterns and species response to predict future distribution of biota, especially in subalpine and alpine habitats, to understand the

Table 7.6 Current cost of most common and popular satellite data

Satellite (sensor)	Pixel size (m)	Minimum order area (km^2)	Approximate cost ($)
NOAA (AVHRR)	1,100	Free	No cost
EOS (MODIS)	250, 500, 1,000	Free	No cost
SPOT-VGT	1,000	Free	No cost
Landsat	15, 30, 60, 100, 120	Free	No cost
ENVISAT (MERIS)	300	Free	No cost
ENVISAT (ASAR)	150	Free	No cost
SRTM (DEM)	90	Free	No cost
EO-1 (Hyperion)	30	Free	No cost
EOS (ASTER)	15, 30, 90	3,600	100
SPOT-4	10, 20	3,600	1,600–2,500
SPOT-5	2.5, 5, 10	400	1,300–4,000
SPOT-6	1.5, 6.0	500	1,000–3,000
RapidEye	5	500	700
IKONOS	1, 4	100	1,000–2,000
QuickBird	0.6, 2.4	100	2,500
GeoEye	0.25, 1.65	100	2,000–4,000
WorldView	0.5, 2, 4	100	2,600–7,400

Source IKONOS, QuickBird, GeoEye, WorldView and RapidEye: Landinfo. SPOT-4 and SPOT-5: Astrium EADS. Aster: GeoVAR. SRTM DEM, Landsat, Hyperion, MERIS, ASAR, AVHRR, SPOT-VGT and MODIS: NASA, ESA and Land Cover Facility

current distribution of sensitive species, to identify species having better carbon dioxide sequestration potential for cold deserts and to understand the impact of retreating glaciers at the species and ecosystem levels.

There is an acute paucity of information on the fringe habitats in high-altitude regions, such as timberline and snowline zones, which are under direct influence of climate change (Planning Commission of India 2006). Some key/gap areas for further ecological research as identified by Singh et al. (2010) include macroecological studies to understand environment and species richness, habitat/species transitions and losses, landscape-level solutions to adaptation and mitigation strategies to climate change (Singh et al. 2010).

The ongoing and persistent loss of biodiversity is the main challenge of today, which can be addressed and mitigated by targeted approach in conservation. The site, amount and kind of biodiversity to be conserved should be the focus of research, which relates very much to the contemporary developments of remote sensing. The recently launched satellites and many in various stages of development can provide us with tools and methods to address these challenges. To make progress, ecologists, evolutionary biologists and conservation biologists must bring their data sets on species distributions, levels of species richness, areas of endemism and so on to the table and combine them with the global, regional and local data sets of, for example, primary productivity and climate, which have been generated by remote sensing researchers.

7.6 Exploring Lichen Species Richness Using Remote Sensing and GIS

Various possible applications of remote sensing and GIS have barely been explored; here, we discuss their potential use in detection, mapping and modelling for lichens. Niche modelling approaches are gaining acceptance for lichen habitat mapping but needs comprehensive and robust database on environmental parameters.

Lichens are fixed components of almost all known ecosystems, but despite the diversity of their morphological and ecological forms as well as their capability of colonizing extreme habitants, they remain generally little known. They are natural sensors of our changing environment: the sensitivity of particular lichen species and assemblages to a very broad spectrum of environmental conditions, both natural and unnatural, is widely appreciated. Lichens disappear or die out as a result of the environmental changes, which directly or indirectly are anthropogenic in nature. Lichen-dominated landscapes are among the most sensitive to mechanical damage, and they are affected by various forms of anthropogenic disturbance such as agricultural and forest management (Scheidegger and Goward 2002), atmospheric pollution and climate change (Nash 1996; Nimis et al. 2002). Lichens are therefore used increasingly in evaluating threatened habitats, in environmental impact assessments and in monitoring environmental perturbations, particularly those resulting from a disturbingly large and growing number of chemical pollutants.

In the year 1866, a study was published on the use of epiphytic lichens as bioindicators (Nylander 1866), and since then, many studies have stressed the possibility of using lichens as bioindicators or biomonitors of air quality in view of their sensitivity to various environmental factors, which can provoke changes in some of their components and/or specific parameters (Gilbert 1973; Nimis 1990; Loppi 1996). Remote sensing technology, owing to its synoptic coverage and repeatability, can be used in mapping of lichen species and characterization of lichen land cover (Richardson 1991; Seaward 1993). Also, the changes in reflectance and in ecophysiological responses, such as chlorophyll levels, gaseous exchange and water absorbance, caused by anthropogenic disturbances to lichen-dominated communities, can be detected in remotely sensed images (Petzold and Goward 1988; O'Neill 1994; Karnieli et al. 2001).

Way back in 1976, researchers used lichens as indicator of pollution extent, their presence and abundance being related to pollution level (Kuliyev 1977, 1979; Kuliyev and Lobanov 1978). In a similar assessment later, Virtanen et al. (2002) attempted evaluation of lichen bioaccumulation to resolve principal impact zones, where satellite images provided evidence of long-term cumulative pollution impacts by revealing dramatic changes in plant species composition, most notably reduction of lichens. Nordberg and Allard (2002) used change in lichen cover as a tool for monitoring disturbed ecosystem; Waser et al. (2007) attempted ecological modelling for predicting species richness of lichens by combining remote sensing data and regression models; Olthof et al. (2008) studied 20 years of vegetation-specific responses to northern climate warming, revealing that lichen-dominated communities exhibit lower trends than those dominated by vascular plants; Gilichinsky et al. (2011) utilized NFI data as training data in mapping lichen classes and demonstrated high classification accuracy of SPOT imagery for classification of lichen-abundant and lichen-poor areas.

Lichen habitat mapping using remote sensing imagery was initiated for understanding of antelope population dynamics and spatial behaviour for development of adequate wildlife management and conservation plans (Crittenden 2000). Several such studies were conducted successfully in the late 1990s and early 2000s; for example, Nordberg (1998) developed a Normalized Difference Lichen Index (NDLI), derived from Landsat TM spectral bands. Later, Nordberg and Allard (2002) found Normalized Difference Vegetation Index (NDVI), to be a better predictor of lichen cover than NDLI. Dahlberg (2001) argued that NDVI might be better representative of land-cover classes than lichen biomass and recommended topography or other ancillary data to be used together with NDLI or NDVI to achieve better estimates of lichen biomass. Normalized Difference Moisture Index (NDMI) first introduced by Wilson and Sader (2002) also proved to have a potential for lichen biomass detection (Rees et al. 2004).

Measurement of lichen ground cover from satellite images largely relies on a variety of supervised and hybrid supervised classification methods to distinguish among a few rough classes of abundance, yielding a good classification accuracy for most lichen-dominated vegetation classes (Colpaert et al. 2003; Gilichinsky et al. 2011; Nordberg and Allard 2002; Tømmervik et al. 2003). Theau et al. (2005) used Landsat TM imagery for mapping lichen and evaluated results from enhancement–classification method (ECM) and spectral mixture analysis (SMA) for their suitability to characterize lichen land cover. In more recent works, Nelson et al. (2013) attempted modelling of lichen abundance by capitalizing on unique spectral characteristics of specific lichens using Landsat 7 ETM+ imagery; Falldorf et al. (2014) developed Lichen Volume Estimator and used it for obtaining a continuous prediction of lichen volume based on two previously developed indices derived from Landsat TM: NDLI and NDMI with promising results.

Like remote sensing, the number of ecological publications using GIS has grown very rapidly, and it has been used to predict species distributions and risks to biodiversity (Spens et al. 2007), to aid the visualization, exploration and modelling of data on species distributions (Lopez-Lopez et al. 2006; Vogiatzakis et al. 2006; Zhang et al. 2007) and to study the effect of major variables such as disturbance events (Pennington 2007). GIS data and its derivatives are being increasingly used now for lichen-related studies owing to their capability to capture and analyse the spatial patterns of lichen cover.

Berryman and McCune (2006) estimated biomass of epiphytic macrolichen by developing regression models based on GIS-derived topographic variables, stand structure and lichen community data; Holt et al. (2008) used GIS-based categorical variables to explore scaling effects on vegetation using lichens and identified potentially useful stratifying variables such as remote sensing-derived land-cover types for future studies; Lattman et al. (2014) used GIS for species classification while surveying a selection of lichen species on trees to show the effect of urbanization on species number and cover of lichens.

Relationship between lichen diversity and environmental factors can be even better characterized by analysing them at different scales using geostatistical methods which use preliminary GIS data. Geostatistics allows development of spatial models of variables used in interpolation with known uncertainty (Store and Jokimaki 2003) along with providing generalization and scaling-up methods (Burrough 2001). Geostatistical modelling theory is being used in basic and applied ecology (Perry et al. 2002; Liebhold and Gurevitch 2002) to provide models for studying spatial pattern of biodiversity variables and is being increasingly seen as important tools for biomonitoring studies.

Several authors have related lichen variables to neighbourhood land cover considering land-cover types as potential pollutant sources (Tommervik et al. 1998; Aarrestad and Aamlid 1999; Augusto et al. 2004); other authors have considered distance to roads and traffic intensity (Gombert et al. 2003) and distance to farms and cattle grazing (Ruoss 1999; van Herk 1999, 2001; Wolseley et al. 2006) and associated NO_2 and NH_3 (Frati et al. 2006). Most popular and studied types of neighbourhood land cover that may induce changes in lichen diversity are urban and industrial as observed by Kapusta et al. (2004), who analysed the spatial pattern of lichen species richness in a forest ecosystem impacted for 50 years by industrial emissions using geostatistical tools to identify factors influencing it. Pinho et al. (2008) used geostatistical modelling to describe spatial relations between biodiversity data, land-cover categories and atmospheric pollutant concentrations. Ribeiro et al. (2013) applied a multivariate geostatistical method to describe relationships between abundance of a lichen species used as an ecological indicator and environmental factors at multiple spatial scales.

Intensive lichen monitoring is a necessary component of any programme aimed at effective long-term observation of environmental disturbances, both natural and man-made. The proper use of lichens as indicators and samplers of ambient conditions is a valuable resource for the environmentalist for appraisals and impact studies (Seaward 2004). Studies of changes in lichen zonation through detailed ground truthing coupled with remote sensing and GIS techniques for comprehensive mapping can form the basis for large-scale monitoring of quantitative and qualitative changes in environmental regimes, ranging from air pollution to climatic disturbances/global warming (e.g. van Herk et al. 2002; Belnap and Lange 2005). In this review, we have extensively examined biodiversity conservation studies using GIS and remote sensing and we recommend that future biodiversity conservation research prioritizes macroecological studies such as lichen cover monitoring using GIS and remote sensing.

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Reflectance Spectra of High-altitude Lichens Based on In Situ Measurements

8

C.P. Singh and C. Prabakaran

Contents

Abstract

The unique chemistry together with typical physiology, morphology and anatomy enables lichens to survive in any harsh environment and same forms the basis for their unique spectral signatures. In this study, five lichen species found at Ravanshila, Tungnath in Garhwal Himalaya region were studied for their spectral characteristics. The reflectance measurements were taken using ASD Fieldspec 3 portable spectroradiometer at every 1 nm from 350 to 2,500 nm. The discrimination analysis was performed by band ratios and stepwise discriminant analysis (SDA). The band ratios of 400/685 and 773/685 nm distinctly provided the spectral characteristics of different colour and growth forms of lichen species. A set of 11 wavelengths were selected using SDA, and most of the variances in the spectra were observed in the NIR and SWIR regions. The maximum variability between lichen species was found at 950 nm (F = 7,572.86; L = 0.00). It was also observed that all lichens showed a more gradual increase through 400–1,300 nm with a maximum inflection point at about 680 nm (red edge). Spectra of all lichens were similar in shape beyond 700 nm, but the magnitude of reflectance varied among them. Between 1,500 and 1,800 nm, all lichens exhibited similar pattern, but with difference in the level of reflectance. Study on spectral properties of high-altitude lichens will be helpful in developing methods for classifying and monitoring lichens using hyperspectral remote sensing.

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Lichens • Himalaya • Hyperspectral • Discriminant analysis

8.1 Introduction

Lichens are symbiotic organisms which consist of a fungal and an algal component. The fungal component provides a structure and algal component provides food. Lichens are distributed worldwide and represented by about 17,000 species (Hale 1983). The Indian Himalayan region exhibits luxuriant growth of different plant groups including lichens. The lichens form dominant terrestrial vegetation in higher alpine habitats in the Himalaya. The higher alpine habitats in the Himalaya also exhibit dominant terrestrial vegetation of lichens together with other herbaceous plants (Upreti 1998). The unique chemistry together with typical physiology, morphology and anatomy enables lichens to survive in any harsh environment. The adaptability of the lichens in the harsh environments resulted in different growth forms such as crustose, foliose, fruticose, leprose and squamulose forms. Lichens have an ability to colonize on any perennial substrate such as bark, rock, soil and many man-made artefacts. Lichens are slow growing organisms and need stable conditions for habitat establishment. Though lichens have great ability to survive in harsh environment but are most sensitive to microclimate changes, thus they act as a natural sensor and successfully utilized as biological indicator of air quality and climate change studies. To understand the changing environmental condition of an area the monitoring of lichen habitat cover, their distribution and change in distribution pattern provide useful data. Hyperspectral remote sensing methods play a major role in the study of lichen characterization. The Ager and Milton (1987), Petzold and Goward (1988), Nordberg and Allard (2002), Bechtel et al. (2002), Rees et al. (2004) and Zhang et al. (2005) studied the spectral properties of lichens worldwide. Most of the spectral properties of lichens from high latitude lichens, especially from arctic and subarctic regions, are available. Many researchers used hyperspectral images to retrieve the lichen spectra. However, lichens are growing in and around many substrates, the discriminating lichens from others become inevitable, and many methods have been used. Zhang et al. (2005) used a derivative spectral unmixing method by combining spectral mixture analysis (SMA) and derivate spectra to discriminate the lichens from the rocks. Bechtel et al. (2002) proposed a band ratio of 400/685 and 773/685 nm to discriminate lichen species and 2,132/2,198 and 2,232/2,198 nm to distinguish features between rocks with OH bearing minerals and lichens. Spectral discrimination of lichens was done using the technique of principle components analysis (PCA) by Rees et al. (2004). This method constructs a variance–covariance matrix for the spectra to identify correlations between different wavelengths which identify the set of wavelengths containing most of the information in the spectra.

The present study is an attempt to investigate the spectral properties of high-altitude lichens of Indian Himalaya and to explore the common features and variations among the lichen species.

8.2 Study Site

The study site, Ravanshila peak (30° 29′ 09.8″ N, 79° 12′ 38.5″ E) at Tungnath, is situated at 3,433 m above mean sea level in Rudraprayag district of Uttarakhand, India. The in situ spectral measurements of lichens were collected during post-monsoon season in the month of October 2013. The study site is completely covered by snow during winter period from late November to March. The area comes under the alpine habitats above the tree lines in the western Himalayan region, and the lichens are exposed to various climatic stress factors such as low atmospheric pressure, low precipitation, high UV

Table 8.1 Lichen species sampled for their spectral measurements

Lichen species	Growth form	Colour	Total observations
Umbilicaria indica Frey	Foliose	Greyish green	25
Porpidia macrocarpa (DC.) Hertel and Hertel and A. J. Schwab	Crustose	Greyish green	25
Cladonia furcata (Huds.) Schrad.	Fruticose	Greyish green	25
Stereocaulon foliolosum Nyl.	Fruticose	Greyish green	25
Ioplaca pindarensis (Räsänen) Poelt and Hinter.	Crustose	Yellowish orange	25

radiation and high wind speed with limited nutrient resource and less growing season. Five lichen species growing luxuriantly and having different growth form were selected for studying the reflectance spectra (Table 8.1).

8.3 Experimental Approach

The measurements were taken using ASD Fieldspec 3 portable spectroradiometer that operates in the spectral region between 350 and 2,500 nm, with a spectral resolution of 3 nm at 700 nm, 10 nm at 1,400 nm and 10 nm at 2,100 nm, and the sampling intervals for the spectral region of 350–1,000 nm and 1,000–2,500 nm are 1.4 and 2 nm, respectively (Analytical Spectral Devices 2010).

The spectroradiometer instrument was battery operated and connected with Lenovo notebook laptop through Ethernet cable. Spectral measurements were collected using a fibre optic (FOV 25°) sensor. Spectroradiometer was calibrated with white reference panel made up of polytetrafluoroethylene (PTFE) and cintered halon, before taking sample measurements. The sensor height was adjusted depending on the specimen size in order to preclude viewing a mixture of lichen and rock. Measurements were viewed and recorded taken by using RS3 software and stored in the laptop memory in ‘asd’ format. These measurements were recorded in reflectance mode, and 25 samples were taken for each lichen specimen to calculate an average spectrum. Later, ‘asd’ files were viewed and exported as reflectance in text format by View-Spec Pro software (Analytical Spectral Devices 2008) for further analysis. The reflectance measurements were taken at every 1 nm from 350 to 2,500 nm. Each sample data was subsampled at 10-nm interval from 400 to 2,500 nm, thus making each sample by up to 211 values of the spectral reflectance. Water absorption wavelengths were excluded from the analysis to reduce the noise from the data. An average endmember spectrum was created by taking average of 25 samples to characterize the spectral properties of each lichen species (Table 8.1).

8.3.1 Discrimination of Lichen Spectra

The discrimination of lichens was performed by band ratio and stepwise discriminant analysis (SDA) methods using Wilks’ lambda. Following the Bechtel et al. (2002) method, lichen species at Ravanshila peak of Tungnath, Garhwal Himalaya were studied for their spectral characteristics. Ratio of reflectance at 400/685 and 773/685 nm was adopted to discriminate among the lichen species and other materials such as rock from lichens. The ratio distinctly provided the spectral characteristics of different colour, types and species of lichens. In another method, discriminant analysis was performed using SPSS 16.0. Total of 125 samples (10-nm subsamples) were grouped into five according to species for the analysis.

8.4 Results

8.4.1 Spectral Features of Lichens

From the reflectance features of five lichen species (Fig. 8.1), *Porpidia macrocarpa* showed a reflectance between 17 and 22 % in the visible

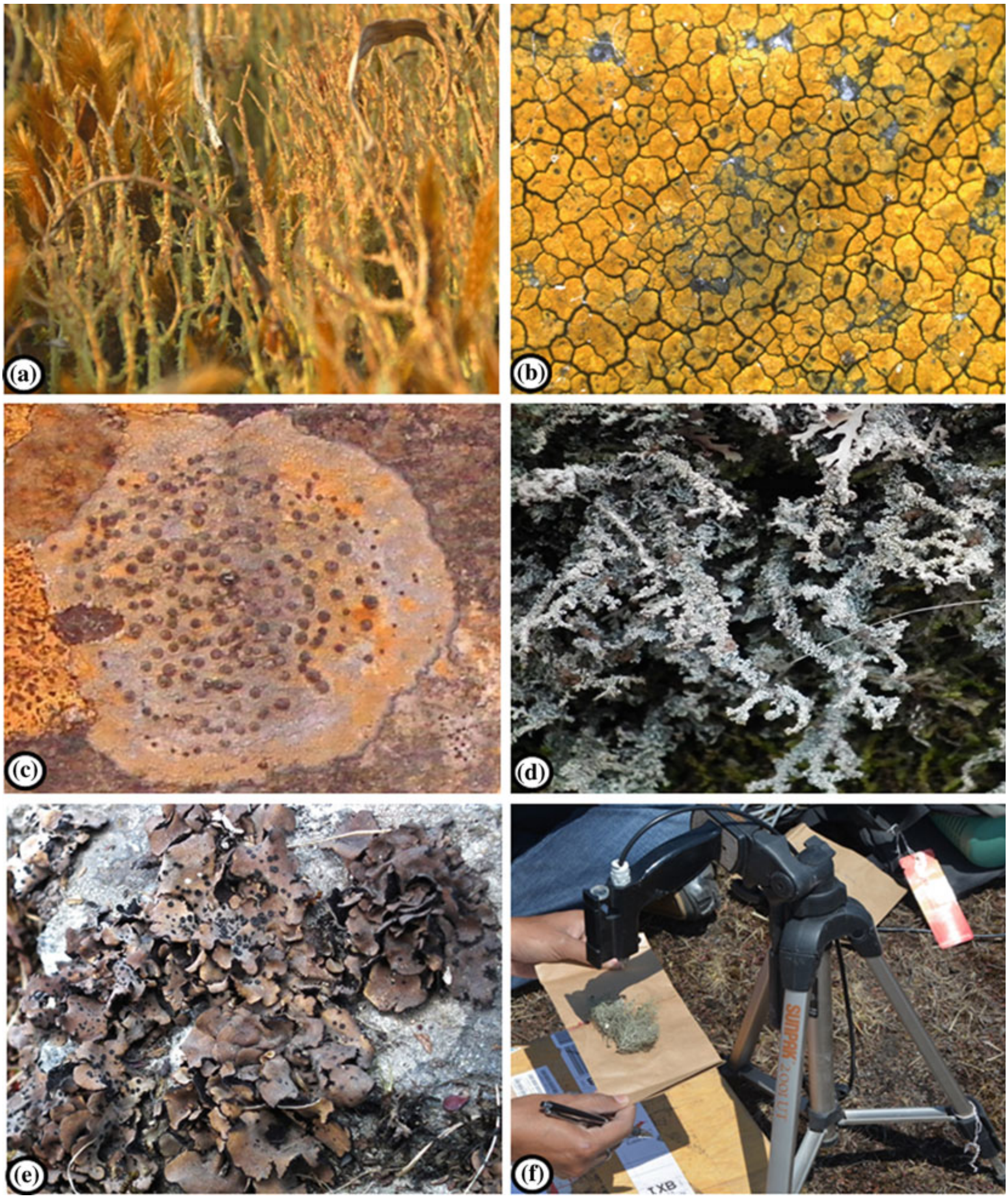

Fig. 8.1 Reference lichen species selected for spectral signatures in Chopta–Tungnath region of Garhwal Himalaya. **a** *Cladonia furcata*, **b** *Ioplaca pindarensis*, **c** *Porpidia macrocarpa*, **d** *Stereocaulon foliolosum*, **e** Umbilicaria *indica* and **f** spectral measurement of lichen thallus being taken in field

region, while other lichens showed a reflectance between 4 and 16 % in the visible region. *Ioplaca pindarensis* exhibited 4–5 % of reflectance at 400–500 nm and quickly rose to approximately 6–16 % from 500 nm until the chlorophyll absorption at approximately 680 nm. *Cladonia furcata, I. pindarensis* and *Stereocaulon foliolosum* showed a strong absorption feature at 680 nm which is attributed to the presence of chlorophyll, and a very weak absorption feature

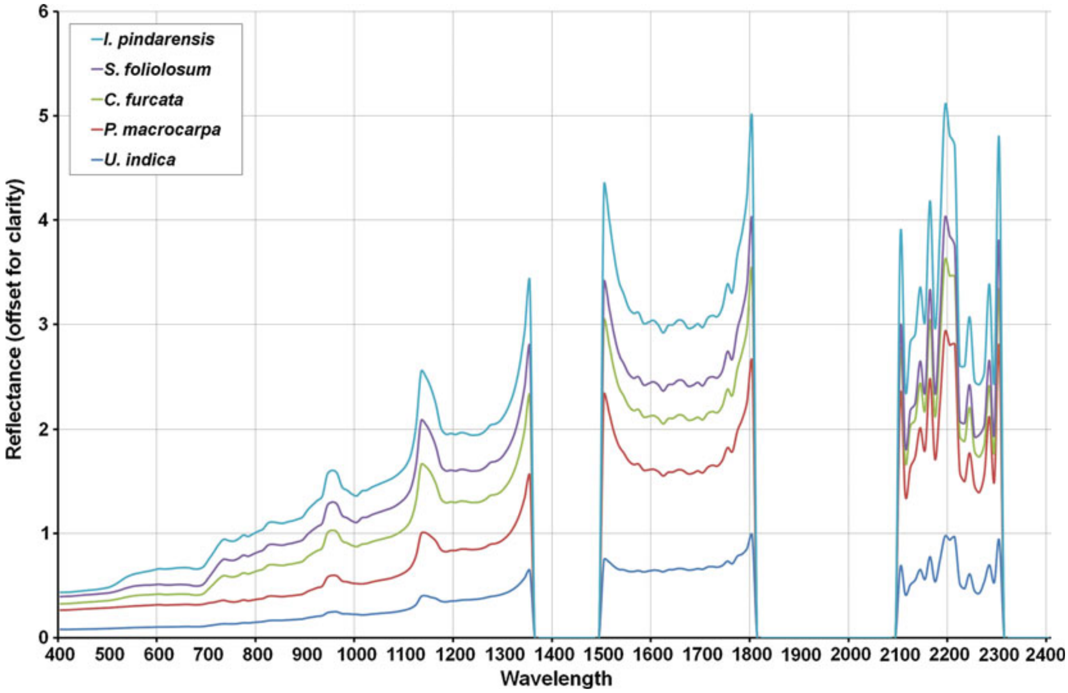

Fig. 8.2 Reflectance spectral profile of five lichen species in Tungnath area of Garhwal Himalaya (reflectance values in *y*-axis were offset for clarity. The value of second profile can be obtained by subtracting the first profile and so on)

observed in the spectra of *P. macrocarpa* and *Umbilicaria indica* at 680 nm. *C. furcata* reflected a greater proportion of the NIR. *U. indica* and *P. macrocarpa* exhibited a strong absorption in visible wavelengths and a gradual increase thereafter. All the lichen species showed a similar pattern of reflectance between 720 and 1,320 nm. A weak absorption feature was observed in the water absorption band at 950 nm in all lichen spectra, except that foliose lichen, *U. indica* which showed a moderate absorption feature.

All lichen species had a gradual increase in reflectance to 1,380 nm followed by water absorption feature centred near 1,430 nm. Reflectance features in the region from 1,500 to 1,800 nm were same for all the lichen species. A minimum absorption at 1,600–1,800 nm centred around 1,700 nm, attributed to absorption by cellulose, which was observed in all samples, though less absorption found in foliose and crustose lichens (Fig. 8.2).

8.4.2 Discrimination of Lichen Species Using Band Ratios

The discrimination of lichen spectra was performed by a plot of the ratio of reflectance at 400/685 and 773/685 nm (Fig. 8.3). The reflectance values at 400 nm were less than 6 % except *P. macrocarpa* and strong absorption at 680 nm due to the presence of chlorophyll pigments. All lichen species were showing high reflectance at NIR (773 nm), yet with low variations among species (Fig. 8.2). *I. pindarenesis*, a yellowish orange lichen, had very low value for both 400/685 and 773/685 nm ratios, thus forming a separate group than other lichens which were having a different colour (greyish green). Fruticose lichens, *C. furcata* and *S. foliolosum,* were having high values, while crustose lichens have less values for 773/685 nm ratio and moderate for 400/685 nm ratio. Foliose lichen was found to be somewhere between fruticose and crustose, for both ratios. The high and low reflectance values

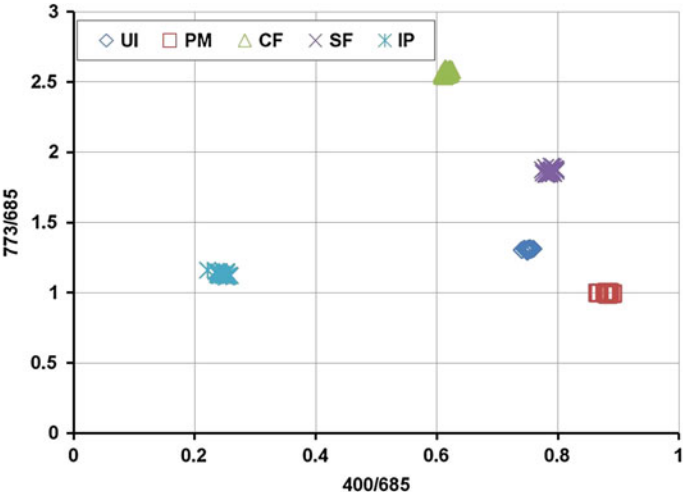

Fig. 8.3 The band ratios of 400/685 nm against 773/685 nm were showing the separation of different lichens species (UI: *Umbilicaria indica*, PM: *Porpidia macrocarpa*, CF: *Cladonia furcata*, SF: *Stereocaulon foliolosum*, IP: *Ioplaca pindarensis*)

at 773 nm may be explained by the structural nature of fruticose lichens and in crustose, respectively. Values for 400/685 nm ratio were mainly influenced by the amount of chlorophyll present in the lichens. Since reflectance values at 400 nm were low and less variation among lichen species, denominator value of 685 nm played a significant role in the discrimination. Among greyish green lichens, *P. macrocarpa* had high value for 400/685 nm ratio which stated the presence of more chlorophyll concentration and yellowish orange lichen *I. pindarensis* had less chlorophyll concentration (Fig. 8.3).

8.4.3 Discrimination of Lichens Using SDA Method

SDA was performed, and a set of 11 wavelengths were selected for discriminating lichens spectra. Most of the variances in the lichen spectra were accounted in the NIR and SWIR regions. The maximum variability between lichen species was found at 950 nm (F = 7,572.86; L = 0.00) (Table 8.2). Variability in green wavelength band at 520 nm showed because of the colour variation among them. Rest of the wavelength sets were having minimum difference in Wilks' lambda value.

Table 8.2 Stepwise discriminant analysis (SDA), combination of selected wavelengths, Wilks' lambda and F value in each steps of SDA

Step	Wavelengths	Wilks' lambda (L)	F value
1	950	1.2e-011	7,572.862
2	520, 950	1.3e-005	7,999.789
3	520, 950, 1,060	1.4e-011	8,460.630
4	520, 950, 1,060, 1,670	8.9e-012	9,709.012
5	520, 950, 1,060, 1,500, 1,670	1.0e-011	11,438.580
6	470, 520, 950, 1,060, 1,500, 1,670	2.1e-011	13,454.240
7	470, 520, 740, 950, 1,060, 1,500, 1,670	6.2e-008	13,739.930
8	470, 520, 740, 860, 950, 1,060, 1,500, 1,670	1.7e-011	14,242.570
9	470, 520, 710, 740, 860, 950, 1,060, 1,500, 1,670	2.7e-011	17,890.660
10	470, 520, 550, 710, 740, 860, 950, 1,060, 1,500, 1,670	1.2e-010	18,536.340
11	470, 520, 550, 710, 740, 860, 880, 950, 1,060, 1,500, 1,670	7.1e-010	21,988.300

8.5 Discussion

8.5.1 Spectral Features of Lichens

The spectral reflectances of lichens are differed from that of vascular plants in the visible region as a result of lichens' symbiotic character and consequential photosynthetic system (Petzold and Goward 1988). Lichens are having much lower values than that of green vegetations in NIR region (Nordberg and Allard 2002) because of anatomical differences. Lichen spectrum showed a little (in yellowish orange colour lichen) or no reflectance peak (in greyish green colour lichens) in green light (500–600 nm). Petzold and Goward (1988) observed a similar spectral reflectance in the medium dark brown lichen (*Cetraria ericetorum*) which exhibited strong absorption in visible region (400–700 nm). According to Nordberg and Allard (2002), the darker colour lichen (*Cetraria islandica*) exhibits much lower reflectance in visible region as compared to pale counterpart, which helps to discriminate clear difference between pale and darker lichen species spectral responses.

In the present study, it was observed that all lichens showed a more gradual increase through 400–1,300 nm with a maximum inflection point at about 680 nm (red edge). Similar to studies carried out by Nordberg and Allard (2002) on lichens under laboratorial condition, the three lichen species (*C. furcata*, *S. foliolosum*, *I. pindarensis*) of the study area exhibit highest reflectance in red band of visible region. Reflectance differences among lichens in the visible regions are mainly due to variable pigmentation of the lichens (Petzold and Goward 1988) and type of fungal components in the lichens which are responsible for the pigments colouration and secondary metabolic by-products (Hale 1983).

Similar to studies carried out by Rees et al. (2004) on subarctic lichens, *P. macrocarpa a c*rustose lichen in the study area exhibit a more gradual increase in reflectance up to 1,300 nm, while *I. pindarensis* showed a week absorption feature at 950 nm, indicated that lichens were having lower intercellular water reserves in their thallus and measurements were performed during post-monsoon season a more or less dry winter period. Lichens have no root system and cannot receive water from soil, and they dry quickly after rainfall (Petzold and Goward 1988). Peak reflectance at 1,380 and 1,800 nm followed by water absorption features was reported in lichen species by Zhang et al. (2005), Rivard and Arvidson (1992) and Ager and Milton (1987). Cellulose presence is observed by the absorption at 1,700 nm, but Ager and Milton (1987) identified three cellulose absorption features near at 1,730, 2,100 and 2,300 nm. However, weak and strong cellulose absorption at 1,700 and 2,300 nm were observed, respectively, in the present study.

8.5.2 Discrimination of Lichen Species

The band ratio of 400/685 and 773/685 nm showed an interesting result in discriminating different coloured lichens. Yellowish orange lichen has low value than that of other lichens having greyish green colour and having higher concentration of chlorophyll. The band ratio placed fruticose lichens in upper level and crustose in lower level in the scatter plot (Fig. 8.2) due to high reflectance in NIR region which attributes to internal structures of thallus. The foliose lichens exhibited a moderate value between fruticose and crustose forms. Thus, the band ratio method can be used to discriminate various growth forms of lichens. However, Bechtel et al. (2002) reported that band ratio method cannot be helpful to distinguish all lichen species. According to Elvidge's (1990), lichens can be clearly distinguished from non-photosynthetic vegetation in the visible region. The present studies clearly indicate that the band ratio method can be useful to discriminate the lichens in terms of colour and growth forms, but not as individual species.

The SDA brought out a total of 11 wavelengths out of 211 wavelengths which can be used to distinguish the five lichen species. Most of the variation between the lichen spectra could be accounted between 700 and 1,100 nm region. Spectra of all lichens were similar in shape beyond 700 nm, but the magnitude of reflectance varied among them. A similar pattern was reported by Zhang et al. (2005). Two green wavelengths at 520 and 550 nm and NIR bands at 710 and 740 nm can be used to distinguish the colour differences and structural differences among lichens, respectively. Petzold and Goward (1988) observed that light coloured and dark coloured lichens were having spectral difference in NIR and MIR reflectance wavelengths (Nordberg and Allard 2002). Two MIR bands—1,500 and 1,670 nm—can be helpful in discriminating the wet and dry lichens. Between 1,500 and 1,800 nm, all lichens exhibited similar pattern, but with difference in the level of reflectance.

8.6 Conclusion

This present study was attempted to investigate the spectral properties of high-altitude lichens from Indian Himalaya. Spectral analyses of five lichen species were carried out. Greyish green and yellowish orange lichens exhibited different patterns in the visible region. All lichens had a similar reflectance beyond 700 nm. Colour and growth forms of lichen species can be distinguished using 400/685 and 773/685 nm band ratio. This method was applicable to colour and growth forms, but not to distinguish each species. The individual species of lichens can be used to discriminate by a set of 11 wavelengths from SDA. Study on spectral properties of high-altitude lichens will be helpful in developing methods for classifying and monitoring lichens using hyperspectral remote sensing.

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Lichens as Agents of Biodeterioration

9

M.R.D. Seaward

Contents

Abstract

One of the major roles lichens play in shaping the natural world, both physically and biologically, is as agents in soil development: formerly considered only in a geological context, recent research has shown that they are capable of biodeteriorating stone substrata within a relatively short timescale. Chemical alteration of the substratum is achieved by the disruptive action of many species, particularly those capable of producing an oxalate at the thallus–substratum interface. The oxalate contributes significantly to the bulk and composition of the thallus itself and persists as an obvious encrustation after the lichen's death. In the past, these disfiguring oxalate residues on ancient monuments have been variously interpreted as resulting from former mechanical/chemical renovation treatments, atmospheric pollution, and climatic weathering. The origin and nature of oxalate accretions, the factors involved in pedogenesis, and the development of lichen mosaics are reviewed. Aesthetic disfigurement *versus* physical damage to stonework is considered, and various aspects of stonework conservation are discussed.

Keywords

Biological weathering · Pedogenesis · Calcium oxalate · Biofilms · Bioprotection · Stonework conservation

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9.1 Introduction

Although earlier literature provided an insight into the uniqueness of lichens, it gave little hint of the major role these organisms play in the shaping of the physical and biological environment of our planet and their importance in maintaining its equilibrium. Their role as biological weathering agents in the development of soils, for example, was formerly considered in a geological context only, but it has been shown that lichens are capable of biodeteriorating stone substrata within a relatively short timescale. Lichens also have the capacity to accumulate elements, such as nitrogen, phosphorus, and sulphur, thereby increasing their potential bioavailability to successive life forms. Organic material derived from lichen decomposition together with detached particles of the substratum and atmospherically derived dusts trapped by thalli all contribute to the development of primitive soils. As well as mineral cycling through biodeterioration processes (Wierzchos and Ascaso 1996; De los Rios et al. 2005), even in a stony littoral zone (Kulikova et al. 2009), lichens often contribute substantial biomass and support a high biodiversity of organisms, creating complex food webs and adding significantly to energy flow. The disappearance of lichens, due to many aspects of human interference in the natural world, leads inexorably to environmental impoverishment (Seaward 2008).

The role of lichens in biodeterioration processes has been investigated worldwide in habitats ranging from polar to desert, at low to high altitudes, in human influenced environments, and involving every conceivable rock type and substrate. However, little attention has been given to the impact of lichens in zones subjected to periodic inundation by marine or fresh water (Gómez-Pujol et al. 2007). In a natural setting, biodecomposition of rock is accepted as normal and even desirable, but in a landscape modified by human activity, biodeterioration of monuments, buildings, artwork, statues, and gravestones is regarded as a serious problem. Even in natural settings, culturally significant prehistoric and historic rock art is subject to the same processes of biodeterioration. However, there are often significant differences in the particular lichen assemblages, and therefore the biodeterioration processes, between those monuments which are composed of stones translocated to the study site (the classic example being Stonehenge in England) and those monuments created on in situ rocks or from locally occurring rocks.

In the case of translocation, studies which compare the differences between lichen colonization of the natural rock, as for example in quarries, and similar material when used in the construction of the monument, such as that demonstrated by Ascaso et al. (2004), are of relevance. In the case of in situ lichen colonization, the detailed study of El Morro Inscription Rock in New Mexico, USA, a large sandstone formation which has become a natural repository for more than 700 years of comments and notations recorded by passing travellers (Knight et al. 2004) is of particular interest. Rather interestingly, the lichen assemblages on monuments are often significantly different in composition to those on in situ rocks close to where they have been constructed, no doubt due to special, sometimes devotional, practices the latter have received over the centuries (Fig. 9.1).

Over the past half-century, considerable interest has been shown in the preservation of our cultural heritage from a biological standpoint, with an extensive and growing literature on the subject, particularly in respect of lichens (Nimis and Monte 1988; St. Clair and Seaward 2004a) which, unfortunately, as demonstrated even a decade ago is to be found in a wide variety of published sources, some aimed at a very specific readership (Koestler and Vedral 1991; Piervittori et al. 2004). It should be recognized that a wide range of organisms are implicated in biodeterioration processes (Paine et al. 1933; May et al. 1993; Scheerer et al. 2009). Of particular significance has been the proliferation of published material on the role of lichens in biodeterioration processes in terms of natural rocks, particularly pedogenesis (Syers and Iskandar 1973; Jones et al. 1981; Jones and Wilson 1985; Jones 1988), ancient monuments (Nimis et al. 1992; Piervittori et al. 2004),

Fig. 9.1 Prehistoric dolmen, Browne's Hill, Ireland—some of the lichen assemblages differ significantly from those on similar local in situ rocks, more particularly by the presence of a maritime species, *Anaptychia runcinata*, despite being more than 50 km from the sea (Seaward 1976)

and man-made materials such as cement, concrete, and brick (Brightman and Seaward 1977; Ariño and Saiz-Jimenez 2004).

Modern electron microscopy and chemical techniques have made it possible to recognize that many lichens contribute to the deterioration of a wide range of materials, particularly rocks and stonework, as a result of physical and/or chemical processes (Seaward 1997a). In the past, attention was drawn to the possible effect of dissolved carbon dioxide, derived from lichen respiration, attacking the substratum to produce pits and channels for easier penetration of hyphae, with attendant loosening of mineral particles and their incorporation into lichen tissue. Until recently, such effects, although important on a geological timescale, have been considered to be minimal in terms of the life of stone buildings and monuments.

Many lichen species create microclimatic effects at thethallus–substratum interface, particularly in terms of water retention, which undoubtedly lead to mechanical damage to stonework on a short timescale of ten, or even fewer, years; it has also been shown that raised temperatures are generated at the interface (Carter and Viles 2004). A wide variety of endolithic, crustose, and squamulose lichens are implicated, their aggressive behaviour no doubt promoted by particular man-made environmental conditions, but under special circumstances, foliose (Ascaso et al. 1976) and fruticose (Adamo and Violante 1991; Ariño et al. 1996) species are also effectiveweathering agents. The forces generated by climatic wetting and drying of lichen thalli cause them to expand and contract in conjunction with the chemical breakdown of substrata by lichen acids, which, despite their relatively low solubility (Iskandar and Syers 1971), are effective chelators (Schatz et al. 1954; Schatz 1963), forming metal complexes with silicates, etc., derived from the substratum. It would appear that lichens having the capacity to fix nitrogen, such as *Xanthoparmelia mexicana*, can enhance organic acid release, thereby leading to further solubilization of the mineral substrate (Seneviratne and Indrasena 2006). X-ray powder diffraction and transmission electron microscopy have clearly demonstrated the presence of characteristic alteration products at the interface between rocks and various lichens, and experiments involving pure lichen acids or lichen fragments incubated with different types of rock have confirmed these observations.

9.2 Biogeochemical Processes

The major chemical agent in biodeterioration processes is oxalic acid secreted by the mycobiont (Jones et al. 1980), which is extremely soluble in water and acts as a chelator of metal ions, the oxalates formed at the thallus–substratum interface being closely related to the chemical composition of the rock (Purvis 1996). Thus, species growing on serpentinite, mainly composed of magnesium silicate, form magnesium oxalate dihydrate at the interface (Wilson et al. 1981). Other alteration products have been shown to be incorporated into the thallus and/or precipitated at the lichen–substratum interface, such as manganese oxalate (Wilson and Jones 1984) and copper oxalate (Purvis 1984) on manganese-rich and copper-rich rocks, respectively.

However, the commonest oxalate found in lichens is that of calcium (Seitz and Schade 1976; Wadsten and Moberg 1985), the oxalic acid reacting with an underlying substratum containing calcium carbonate to form the insoluble compound calcium oxalate. The calcium oxalate can accumulate on the surface of thalli, often forming distinctive pruina, within lichen thalli, and/or at the thallus–substratum interface (Ascaso and Wierzchos 1995; Seaward and Edwards 1997); however, calcium carbonate derived from neighbouring substrata or atmospherically can also be sourced by the lichen. The thallial content of calcium oxalate ranges from 1 to 50 % according to the species and its underlying substratum (Syers et al. 1967; Edwards et al. 1994). It should be noted, however, that the compound exists in two hydrated forms, monoclinic monohydrate(whewellite) and tetragonal dihydrate(weddellite). The monohydrate form is the major biodeterioration product and the equilibrium (ratio between monohydrate and dihydrate forms) is dependent on various environmental factors (Edwards et al. 1992).

After the death of the lichen, residues of calcium oxalate often remain on such substrata, leaving significant and often unsightly white deposits. This phenomenon can be particularly serious in the case of delicate and intricate stone monuments, as calcium oxalate deposits often obscure the detail and historical significance of such structures (Seaward and Edwards 1995). Oxalic acid has also been shown to solubilize magnesium silicates (Jones 1988).

It has been shown that lichens are capable of producing calcium oxalate on a wide variety of substrata (Edwards et al. 1997); for example, *Dirina massiliensis* forma *sorediata* has the capacity to produce encrustations of various thicknesses, with different concentrations of calcium oxalate and a variable chemistry derived from the following range of substrata: gypsum/calcite underlying a fresco, stucco of a church wall, a Roman brick wall, a lead–glass interface of a church window, mortar, and acidic stone. However, it was also noticeable that the effectiveness of this lichen in degrading its particular substratum was determined by the various environmental conditions obtaining in the different habitats and geographical settings studied. The ability of lichens to produce significant levels of calcium oxalate, even when growing on non-calcareous substrata, is particularly interesting. FT-Raman spectroscopic studies of lichens on granitic monuments in Spain, for example, have shown that not all species are capable of producing calcium oxalate, and those that do have significant levels derive some of the calcium for its production from the atmospheric or from leachates of neighbouring substrata (Prieto et al. 2000).

Undoubtedly, many lichens known to contain calcium oxalate cause extensive damage to a range of rock substrata, but it is the production of calcium oxalate dihydrate by thalli, which is a measure of a lichen's capacity to biodeteriorate its substratum; its production is related to microclimatic conditions such as temperature and humidity of the air, as well as the chemical and physical nature of the substratum. It would appear, for example, that warmer and drier sites are more conducive to the production of calcium oxalate dihydrate by *Diploicia canescens* (Edwards et al. 1995) and by *Ochrolechia parella* (Prieto et al. 1999); this accords with the findings of Wadsten and Moberg (1985) who observed that lichens growing on humid sites produced calcium oxalate monohydrate, whereas those from drier sites produced a mixture of the monohydrate and dehydrate forms.

9.3 Biogeophysical Processes

The natural process of soil formation, pedogenesis, whilst inherently slow, is nevertheless relentless, usually operating on a geologic time scale and involving a complex and interactive combination of physical, chemical, and biological activities. Some aspects of the process are strictly abiotic, such as wetting/drying, heating/cooling, and freezing/thawing, whilst others are clearly biological in nature, such as the encroachment of roots, rhizines and hyphae into cracks and fissures in rock surfaces, the biomediated chemical erosion of cementing agents, and the biotransformation of the molecular structure of rock substrata.

Both biological and abiotic factors operate together, the effects of one accentuating the effects of the other, relentlessly breaking down rocks, large and small, to form the mineral component of soil. The nature and rate of pedogenesis are mainly dictated by the chemical and physical attributes of the rock (Favero-Longo et al. 2005; Rajakaruna et al. 2012), including elemental composition, molecular structure, cementing agents, density, porosity, pH and nature of origin, the composition and degree of development of the epilithic and endolithic biological community, and local and regional climatic patterns.

The dynamics of rock decomposition apply to both natural rocks and man-made 'stone' substrata, but the onset of human-induced changes to the environment, especially changes in air and water quality, have altered the dynamics and rates of rock degradation. More specifically, increasing levels of air pollutants accompanied by acidification of precipitation have dramatically altered, or in some cases eliminated, some biological communities, particularly in urban/industrial settings; therefore, although environmental degradation has reduced the impact of the biological components on rock decomposition, it has dramatically enhanced the impact of some abiotic factors.

It is highly probable that lichens were early colonizers of terrestrial habitats on our planet, coping with harsh environments and contributing to the evolution of atmospheres more suited to a much wider variety of life-forms. However, their major long-term role has been as biologicalweathering agents, with a pedogenic action which is both physical and chemical in nature (Syers and Iskander 1973; Jones 1988). Historically, their contribution to the breakdown of rock substrata was perhaps somewhat overrated; however, today it is often seriously underestimated (St. Clair and Seaward 2004b). Frequently, lichens are the first living things to occupy newly exposed rock surfaces, their invasion being dictated by several factors, including proximity of native lichen communities on similar substrata, reproductive strategies (sexual and/or asexual) of local lichens, and dispersal capacity of local lichens (Bailey 1976). The ability of reproductive propagules to succeed on what appears to be an inhospitable substratum such as a rock is remarkable. However, although the discharge, dispersal, and establishment of reproductive propagules in fungi have been extensively studied, these processes in lichenized fungi are still under-researched.

Efficient scattering of reproductive propagules in no way guarantees their successful establishment and development: suitable substrata and environmental conditions are necessary when propagules come to rest following one or more deposition processes involving sedimentation, gravitation, and wash-out by rain. Propagule impact on various surfaces leading to successful development of thalli is accounted for by efficient trapping, and indeed electrostatic charges generated by lichen ascospores have been suggested as the cause for this phenomenon in some species (Garrett 1972). The importance of aerobiology in understanding how different propagules are dispersed and transported in the air and how they impact on surfaces cannot be underestimated (Mandrioli et al. 2003; Piervittori 2004).

Lichens are capable of colonizing a wide range of substrata (Brodo 1973), including not only natural rock surfaces, but also stone imported for the construction of monuments and buildings, supplemented by other man-made materials, such as cement, concrete, asbestos-cement, brick, roofing tiles, metals and wood (Brightman and Seaward 1977), and even glass (Mellor 1923; Krumbein et al. 1991; Kossowska and Węgrzyn 2009), all of which are subject to biodeterioration. Field experiments have shown the importance of surface texture and micro-topography for the successful attachment and survival of propagules (Armstrong 1988). Although surface topography may provide a favourable environment for germination, it is likely that inter- and intra-specific competition as a result of a build-up of propagules in such niches will be intense, their interactions determining the ultimate composition of the local lichen flora.

Successful germination and establishment of the various types of lichen propagule are dependent not only on characteristics of the available substratum, such as texture, pH, nutrient status, and microtopography, but also on numerous other environmental factors such as humidity and temperature (Barkman 1958; Brodo 1973; Topham 1977). In addition, competition with established lichens and other organisms for space, light, and nutrients by the newly developing propagule can be considerable; such competition may arise as a consequence of mechanical action, inhibition of gaseous exchange, reduction in light intensity, or allelopathic or toxic chemicals. The possibility of intra-specific allelopathic effects determining the growth and spatial distribution of lichens has been postulated by Seaward (1982). All the above examples refer to mature thalli, but it is reasonable to suppose that actively germinating ascospores and other propagules are all capable of producing chemicals with similar inhibitory properties.

The factors which control morphogenesis are poorly understood; for example, little is known of the growth rate of thallus initials, a phase crucial to both the establishment and competitive ability of the new thalli, or how long a period must pass before lichens establish themselves on a particular substratum, how long before this can actually be detected, and how successful are the thallus initials. However, once established, their growth rates can be easily determined and a wealth of literature on this branch of lichenometry is available, the dating curves for particular species, for example, providing a guide to the date of their establishment and to their potential rate of colonization.

Many saxicolous lichen communities undergo regular patterns of successional change; for example, one assemblage of species may occupy a given rock surface for several years, steadily altering the substratum in ways that eventually better accommodate a new combination of species. Many species inhabit large areas for a considerably longer time than could be expected from the lifespan of a single thallus; this fact may be responsible for some exaggerated ideas about the age of individual thalli. With the passage of time, different lichen species and assemblages are subsumed by other lichen cover as a consequence of a particular succession, in many cases eventually giving way to bryophytes and vascular plants unless interrupted by a controlling factor, more than likely man-induced, that prevents the sequence. Although the ecology of the later phases of such successions has been widely studied and extensively cited in classic textbooks, changes in the development of the different lichen phases, spatially and temporally, have received less attention, particularly in respect of biodeterioration.

Lichen associations play an important role in energy-budgeting, modelling, etc., and should therefore not be underestimated. Energetics of lichen faunas are poorly understood due to the wide variety of data from disparate sources: information on ingestion, assimilation, egestion, respiration, growth and death rates, numbers and biomass needs careful co-ordination and interpretation (Seaward 2008). Of special interest to our understanding of biodeterioration processes is the pedogenetic study of Clarens sandstone, South Africa, (Wessels and Schoeman 1988) involving lichenophagous bagworm larvae (Wessels and Wessels 1991). The sandstone is weathered by endolithic lichens which dissolve the cementing material, thereby loosening quartz crystals which are then used by the larvae to construct the bags in which they live. It is estimated that the larvae contribute 4.4 kg of weathered sandstone per hectare per year to the area; furthermore, the larvae utilize the lichens as a food source, the resulting faeces providing 200 g of organic material per hectare per year and contributing to mineral cycling. In general, the impact of endolithic lichens on stonework has been underestimated (Matthews and Owen 2008) and there are relatively few detailed investigations on their role in biodeterioration and biomineralization (Bungartz et al. 2004; De los Rios et al. 2005; Favero-Longo et al. 2009a, b; McIlroy de la Rosa et al. 2012a, b). In their study of *Lecidea auriculata* thalli, Matthews and Owen (2008) found that the rapid initial weakening of rock surfaces is indicative of rates of biological weathering by endolithic lichens that may be 200–300 times faster than physico-chemical weathering alone.

Despite early controversy concerning the pedogenic significance of lichens, their effectiveness in the biodeterioration of rocks has been clearly demonstrated by recent research, which has revealed that substantial quantities of the substratum can be degraded even over relatively short periods of time. Furthermore, lichens have the capacity to accumulate elements such as N, P, and S, thereby increasing the letters' potential bioavailability to successive life forms which may replace lichens during soil development. Organic material derived from lichen decomposition, together with detached particles of the substratum and atmospherically derived dusts trapped by thalli, all contribute to the development of soils.

The weathering action of saxicolous lichens can be physical and/or chemical. Lichens contribute to mechanical weathering of rocks in four ways: (1) penetration of mycobiont hyphae (up to 15–20 mm) and rhizines into naturally occurring crevices and cracks in rock surface;, (2) expansion and contraction of lichen thalli with daily and seasonal changes in ambient temperature and humidity; (3) swelling action of organic salts produced by lichens; and (4) fracturing and incorporation of mineral fragments by lichen thalli (Chen et al. 2000).

Lichens chemically weather rocks by means of carbon dioxide (internally and/or externally derived), oxalic acid, and the complexing action of lichen substances. The latter have a low but significant solubility in water, forming soluble metal complexes under laboratory conditions when they react with minerals and rocks, particularly limestone (Syers and Iskandar 1973). Water is essential for many of the chemical reactions associated with the breakdown of rock substrata; since lichens are able to absorb water in either the liquid or vapour phase, chemical (and indeed) physical weathering processes are expedited. The mixing of respiratory carbon dioxide with water in lichen tissues results in the formation of carbonic acid, which also enhances the solubility of rock surfaces by lowering the pH of the substratum microenvironment adjacent to lichen thalli (Chen et al. 2000). Oxalic acid, formerly considered of minor importance in the biodeterioration process, has been proved otherwise; the nature of the thallus–substratum interface generated and the chemical disruption of the substratum are significant components of the weathering process. An ecological approach to our understanding of the genesis of calcium oxalate patinas on stone monuments is provided by Caneva (1993).

Detailed spectroscopic studies have demonstrated the highly destructive properties of calcium oxalate produced by lichen thalli, particular attention being directed towards the dramatic effects caused by the action of certain aggressive lichen species on historic monuments and other works of art, where biodeterioration processes have been shown to be devastatingly destructive within a surprisingly short timescale (Seaward and Edwards 1997). Such action on natural substrata is clearly of significance in a pedogenetic context, since lichens are usually regarded as weathering agents on a geological timescale.

The impact of lichen weathering of rocks on a global scale has been, and continues to be, important in terms of climatic consequences and the habitability of our planet: their disappearance from particular ecosystems would be critical over major areas. Lichens form such an important component of the complex web of life that their disappearance affects the balance of nature to a surprising degree, particularly in tundra zones, high altitudes, cold deserts, dune systems, semi-arid lands and deserts, and even urban areas, where they are, for example, important in community development and succession on rocks and soils.

There are areas of the globe where the results of lichen denudation are now being detected by means of remote sensing. Such losses may well have climatic repercussions and exert a measurable influence on global warming, as, for example, in the case of the disappearance of epilithic lichens over a very large area of the Canadian shield as a direct consequence of atmospheric pollution formerly emanating from the smelting operations at Sudbury (Seaward 1996); fortunately, this has subsided due to pollution abatement measures, allowing the barren rock surfaces to be recolonized by lichens, thereby restoring their light-absorbing ability (Rollin et al. 1994). It has been noted that if today's weathering were to take place under completely abiotic conditions, dramatic increases in global temperature would result (Schwartzman and Volk 1989).

9.4 Lichen Mosaics

Lichens are eminently successful and enjoy a worldwide distribution. Lichen species and assemblages are faithful to particular habitats and as such make ideal environmental indicators in biodeterioration studies. Their spatial andtemporal patterns are ecologically delimited by one or more factors. Lichen communities are often species rich and densely crowded with complex biotic interactions. In terms of determining the ecological processes in a community, spatial patterns need to be determined for habitats and ecosystems (e.g. an individual rock, a mountainside scree, and variously dated churchyard memorials). Lichens occur in almost every conceivable habitat and often play a key role in the operation of some natural systems, growing on a variety of substrata, including most natural ones as well as a host of human-manipulated or manufactured ones. Common natural substrata supporting lichens include all categories of rocks and common man-made substrata include fashioned stonework, asphalt, glass, concrete, cement, plaster, ceramic and terracotta tiles, bricks, processed wood products, and various types of metals (Brightman and Seaward 1977).

The presence of lichens on building materials, more particularly ancient monuments, is variously interpreted by the lay public and by specialists in different disciplines, whose attitudes are inevitably coloured by differing aesthetic and practical considerations. Undoubtedly, lichens are exceedingly sensitive to environmental change, and the diversity and composition of their assemblages can be a reliable indication of the level of atmospheric pollution, which in itself is one of the most serious factors in the deterioration of such stone surfaces.

The lichenologist regards the appearance of a lichen mosaic as a natural feature of ancient monuments, finding the diversity of species present aesthetically pleasing, besides being both taxonomically and ecologically interesting. Clearly, there is a direct correlation between the composition of the flora and the passage of time, the special technique of lichenometry being employed for dating surfaces (Innes 1985); furthermore, the different lichen communities established on stone surfaces not only reflect the various materials employed in their construction, but also can often be correlated with the chronology of successive building phases, thereby assisting in archaeological interpretation (Piervittori 2004). It should also be noted that many nationally or internationally rare lichen species that are to be found mainly or only on particular ancient monuments need to be considered in their own right as in need of conservation. In fact, some would argue that lichens can no longer be disregarded as aesthetical impairment or biodeterioration agents by architectural heritage preservation or cultural studies and that the 'conservation of a micro-flora is worthy of protection on historical monuments and its co-existence with the historically based elements' (Drewello and Drewello 2009).

It is ironic that in a bland, homogenous urban environment, where a lichen mosaic would be a welcome relief to the eye, the higher levels of air pollution prevent its establishment, only allowing the existence of a monotonous flora composed of a few algae and lichen crusts. Such a feature has proved problematic not only from an archaeological standpoint, but also architecturally in the development of modern buildings, in both cases being treated as a biodeterioration process. This lack of diversity and complexity in urban lichen communities is due to (a) local accumulation of high concentrations of interacting toxic airborne contaminants generated from a wide variety of urban and industrial activities and processes, (b) removal and/or replacement of natural substrata, and (c) alteration of natural hydrologic cycles. Prior to the onset of the industrial age, stone and wooden buildings, gates, fences and tombstones, as well as glass, bricks and tiles, in urban areas supported reasonably diverse lichen communities, as still witnessed in many rural settlements. As industrial activity developed in and around human population centres, lichen communities on natural substrata declined, but man-made substrata supported their own, albeit limited, lichen assemblages.

There is strong evidence that recent environmental changes have been conducive to increasingly detrimental invasion by certain aggressive lichens, as in the case of the establishment of nitrophilous species due to environmental hypertrophication (Deruelle 1988). Such evidence could help to explain why it is that monuments, undamaged for many centuries, appear in recent years to be vulnerable to lichen attack, in addition to the known problems resulting from air pollution (Pye and Schiavon 1989; Butlin 1991). Whilst acknowledging the recent effects of air pollution (especially acid rain) on monuments, it must also be recognized that *D. massiliensis* forma *sorediata*, for example, is a relative newcomer: its dramatic spread in Europe, and more particularly Britain, in recent years has been facilitated by new environmental regimes, including qualitative changes in air pollution, which have allowed it to dominate substrata in the wake of the rapid disappearance of other more pollution-sensitive species. *D. massiliensis* forma *sorediata* is by no means the only organism implicated in short-term deterioration processes: other lichens, and indeed other micro-organisms, capable of adapting to man-made environmental disturbances can be equally destructive when ecosystem equilibria are disrupted.

The urban ecosystem is of increasing interest to lichenologists, as well as ecologists and environmental scientists. For almost two centuries, urban areas, mostly in the developed world, have been of lichenological interest purely in terms of their lack or paucity of lichens, air quality being identified as the limiting factor, but the contributions of the many factors operating in a changing urban climate and complex landscape, although appreciated, were not fully evaluated. With the implementation of clean air policies in recent years, the urban environment is lichenologically unrecognizable: without the major limiting factor, a diverse landscape, in terms of habitats, substrata and chemistry (mainly nutrient-enrichment), is there to be exploited (Seaward 1997b). Some cities could soon be on a par with lichen-dominated ecosystems, since an urban landscape of man-made structures constructed from a very wide variety of natural and manufactured building materials can provide suitable substrata for the establishment of a diverse lichen mosaic, which in turn requires consideration from a biodeterioration perspective. Although it is pleasing to note that the undoubted improvement in air quality of many cities in recent years is reflected in the continued recovery of their lichen floras, there is no room for complacency, since other recent environmental changes brought about by human agencies have been conducive to increasingly detrimental invasion by certain aggressive microorganisms, as in the case of the establishment of nitrophilous species due to hypertrophication (Seaward and Coppins 2004).

However, historians, archaeologists, and fine art specialists concerned with the conservation of ancient monuments in both rural and urban settings view the encroachment of lichens from a very different standpoint from that of the lichenologist: inscriptions and fine details may be obscured, and depending on the nature of the substratum, and in some cases the ambient conditions, serious physical damage is often caused through lichen-induced biodeterioration. The lichen floras vary considerably according to the spatial differences in the chemical properties of stone surfaces, the micro-environmental conditions, and the overall influence of air pollution. Clearly, with all these conflicting issues, it is necessary to establish whether the particular lichens observed are merely causing aesthetically unpleasing disfigurement or are actually causing physical and/or chemical damage to their substrata.

9.5 Aesthetic Disfigurement Versus Physical Damage

It has been shown above that the role of lichens as biological weathering agents in the development of soils should no longer be considered in a geological context only, since such organisms are capable of biodeteriorating stone substrata within a relatively short timescale. Expert diagnosis of those species mainly responsible for this, and the conditions under which they thrive, is essential for the conservation of stonework. It is essential that the real causes of deterioration in any given situation are scientifically established before well-meaning, but uninformed, action is taken; for example, cleaning techniques may well accelerate deterioration in situations where lichens have not been reliably established as the prime cause of damage. Indeed, some species may well afford a protective cover, shielding the cement work from external weathering agents. It is therefore necessary to determine which species are disfiguring but intrinsically harmless and which cause actual physical damage (Lallemant and Deruelle 1978; St. Clair and Seaward 2004a). To this end, several studies aimed at determining the nature of the disfigurement and/or physical damage by particular lichens to various substrata have been undertaken in various parts of the world, a few of which are detailed below. These studies demonstrate some of the dilemmas facing conservationists: namely, do lichens damage the particular substratum or merely create a ‘patina of time’ (cf. ‘scialbatura’ in Del Monte and Sabbioni 1987) which is aesthetically unpleasing, and furthermore, do lichens afford some protection to the substratum and if removed without consolidation of the exposed surfaces, will further, or indeed more intensive, weathering ensue (St. Clair and Seaward 2004a; McIlroy de la Rosa et al. 2012a).

The exposure of Roman mosaics through excavation after centuries of being buried beneath soil, etc. poses just such a dilemma. Pioneer colonization by lichens, sometimes with a predilection for particular coloured tesserae (Fig. 9.2), detracts from the detail or overall

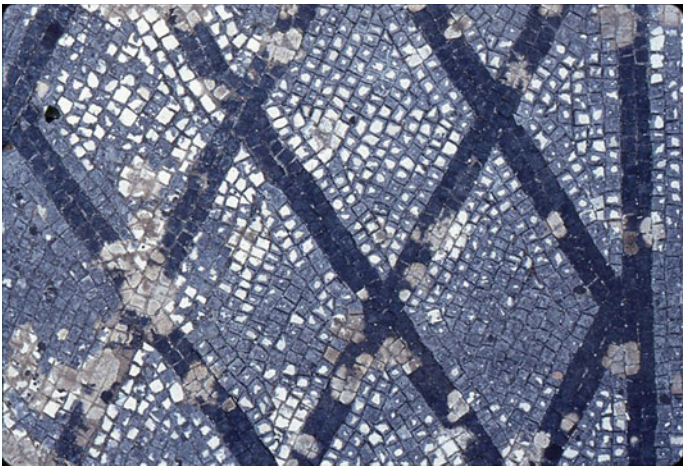

Fig. 9.2 An exposed Roman mosaic at Ostia Antica, Italy—the *light-coloured* lichens, such as *Aspicilia* spp. with a predeliction for the *dark-coloured* tesserae, clearly detracting from the overall effect of the original pattern

pattern of the mosaic, and it has been clearly demonstrated in a detailed study of exposed Roman mosaics in Italica, Spain that successional lichens, bryophytes, etc. have ultimately led to their complete destruction (Saiz-Jimenez et al. 1991). Common sense should prevail: clearly such archaeological exposures should either be re-covered after detailed recording, or suitably protected in situ againstweathering, or, if absolutely necessary, be carefully translocated to an appropriate museum setting.

In a study of archaeologically important monuments and artefacts constructed from a variety of materials in a grassed area surrounded by cloistered museum buildings of the Museo Nationale Romano in Rome, the role of the ambient urban climate and associated atmospheric pollutants had to be taken into consideration when interpreting the deterioration of these materials, particularly since the latter dramatically affects the lichen flora.

It was noted, for example, that several of the more toxi-tolerant lichen species (poleophiles) with an aggressive behaviour were actively colonizing stone monuments and other archaeological artefacts. A most dramatic case of lichen attack was observed in the case of several large terracotta pots scattered about the grassed area (Seaward 1988). The rims and shoulders of these pots were lightly colonized by relatively innocuous species such as *Lecanora dispersa* and *Candelariella vitellina*, but here and there, thalli of *Lecanora muralis*, mainly 4–7 cm in diameter, probably representing 6–15 years growth, were causing demonstrable damage. A section through one of the thalli clearly reveals the results of such damage: a central blister, created by the crowding of apothecia, pulled away a fragment of the substratum, two or more mm in thickness, over an area almost 12 cm^2 (Fig. 9.3). *L. muralis* appears to be a highly successful lichen in urban environments into which it has spread dramatically in recent years, due in part to lack of competition from other species; a change in the nature of air pollution in Rome in that period may be a contributory factor to this aggressive behaviour.

Fig. 9.3 Section through *Lecanora muralis* to show the blister created on the shoulder of a terracotta pot at the Museo Nationale Romano—not only has pottery been detached from the surface to a depth of 2–4 mm, but a chamber to a depth of 3–5 mm beneath this has created a microenvironment which influences other biodeterioration processes (Seaward 1988)

In another study, the deterioration of frescoes by Zuccari painted in the 1560s on the inner walls on ground and first floor levels of a circular cloister at the Palazzo Farnese, a beautiful mansion at Caprarola in central Italy built by Vignola in 1547–1549, was examined in detail. It was clear that the relatively recent alarming signs of biodeterioration of the water-based paintwork were the result of a single lichen species, *D. massiliensis* forma *sorediata*, its attack being very pronounced in many places and in its early stages of colonization clearly demonstrating a predilection for the brown and yellow pigments, rather than the red pigment which contained one or more metals antagonistic to its growth. When viewed in 1986, the distribution pattern of this lichen was clearly dictated not only by the colour of the paintwork, since it was obvious that some recent cleaning activity had distributed lichen propagules from existing thalli to create distinctive areas of lichen invasion across the frescoes (Edwards and Seaward 1993). More recently, lichen colonization was less clearly demarcated, and cleaning removed not only the lichen but also the underlying paintwork (Fig. 9.4); however, it is pleasing to the note that since restoration, involving the use of paints impregnated with biocides after the cleaning procedure, lichen reinvasion has ceased—but for how long?

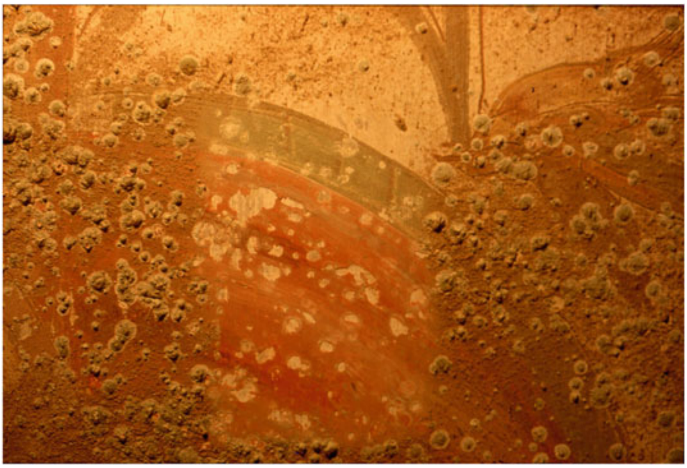

Fig. 9.4 Renaissance fresco in the Palazzo Farnese, Italy, attacked by *Dirina massiliensis* forma *sorediata*—its removal by light brushing from the central area has also removed the underlying paintwork

Fig. 9.5 Church wall, Fiskerton, England—the calcium oxalate coating generated by *Dirina massiliensis* forma *sorediata* suggesting a former surface rendering (Seaward and Edwards 1997)

Using FT-Raman spectroscopy, it was shown that *D. massiliensis* forma *sorediata* on these frescoes produced calcium oxalate encrustations at the thallus–substratum interface of almost 2 mm in thickness in less than 12 years. It was deduced that 1 m^2 of the fresco with a current 60 % obliteration by this lichen and the underlying substratum generated more than 1 kg of calcium oxalate over a similar time period; furthermore, with the incorporation of calcite and gypsum into the thallus encrustation, it is likely that more than four times this amount of the underlying substratum has been chemically and physically disturbed (Seaward and Edwards 1995, 1997).

The short-term biodeteriorative capacity of *D. massiliensis* forma *sorediata* is not specific to the above-mentioned frescoes and mural paintings (Nugari et al. 2009); for example, detailed studies of this lichen on exterior stonework of English churches, as demonstrated by a fourteenth-century church at Fiskerton in Lincolnshire, have shown its similarly destructive nature (Seaward and Edwards 1997). In the past, encrustations generated by this, and no doubt other lichen species, have been misinterpreted as the remaining traces of a whitish coating or rendering applied as a decorative or protective surface in a nineteenth- or twentieth-century restoration programme (Fig. 9.5). It is now clear that this is not so, since these ‘renderings’ consist essentially of calcium oxalate and more often than not evidence remains of the thalli producing them (Fig. 9.6). These encrustations are usually more than 0.5 mm in thickness and cover considerable areas of many church walls throughout England.

The impact of the paints and ‘renderings’ on the presence or absence of lichens in the above two examples raises an interesting issue: namely, would treatments of monuments in the past have intentionally or fortuitously discouraged lichen growth? Accepting the role of air pollutants in recent years, why, despite the ravages of various forms of weathering processes over centuries, has biodeterioration been shown to be of paramount importance in the deterioration of some of our cultural heritage, particularly in the Mediterranean region. A clue to this may be found on the Parthenon sculptures, the surviving traces of colour on them (Jenkins and Middleton 1988) representing an example of the widespread use of ancient polychromy, the applied paints, known to contain toxic metals, having biocidal properties. What other treatments have our monuments been subjected to by those who have revered either the structure or its (religious) function?

Undoubtedly, many of the major researchers in biodeterioration investigations have been, and continue to be, based in Italy. Their pioneer work and considerable output of published material have contributed significantly to our understanding of the characteristics of lichen assemblages on ancient monuments and their role in biodeterioration processes (for a review see Piervittori 2004). Valuable work has been undertaken in other Mediterranean countries, particularly Spain (Garcia-Rowe and Saiz-Jimenez 1991; Ascaso and Wierzchos 1995; Ascaso et al. 2004; Ariño and Saiz-Jimenez 2004; Silva and Prieto 2004).

Fig. 9.6 Calcium oxalate encrustation generated by in situ thalli of *Dirina massiliensis* forma *sorediata*

Contributors to our knowledge of biodeterioration processes based elsewhere, but not necessarily working solely on the cultural heritage of their own countries, include Antarctica, Australia, Britain, Canada, China, France, Germany, Holland, India, Israel, Japan, Korea, Norway, Poland, Portugal, South Africa, Sweden, Turkey, and USA. It is hoped that the review of lichens growing on historical monuments and buildings in India (Saxena et al. 2004; Bajpai and Upreti 2014) will stimulate others to investigate this under-researched subject in hot climates. Other than the occasional paper for Central and South America, south-east Asia and north-west Africa, relatively little is known of the impact of biodeteriorative processes on monuments in developing countries, much of the very considerable disfiguration observed in tropical and subtropical areas being due not only to lichens but also to cyanobacteria which create dark biofilms (Fig. 9.7).

9.6 Stonework Conservation: Practical Considerations

The above observations constitute a necessary first phase of any programme aimed at quantifying the actual role played by lichens in the deterioration of archaeological materials. Stone- and art-work in exposed and partially enclosed situations, giving rise to a variety of environmental conditions, need to be examined in detail. It is necessary to elucidate the relationships between particular lichen species and the physical and chemical nature of their substrata in order to determine the relative importance of those species in biodeterioration processes obtaining in specific circumstances. It is essential to determine, for example, which species are disfiguring but intrinsically harmless, and which cause actual physical damage (Fig. 9.8).

Fig. 9.7 Imam Bara Mosque, Lucknow, India—aesthetic disfiguration of building in a subtropical area, the dark biofilms created by both lichens and cyanobacteria

It is self-evident that baseline work is a prerequisite for laboratory research designed to establish the nature of the interface between problematic lichens and their substrata and field trials intended to test the relative effectiveness of differing techniques and treatments for the removal and discouragement of lichens from stonework. Any treatment should be selected with care, since although immediately effective, the long-term effects are highly likely to be deleterious. Other possibilities, mostly based on common sense, should also be investigated, as exemplified by the work 'towards a solution' on prehistoric rock art (Tratebas 2004). Recent work to quantify hyphal spread within the substratum (Gazzano et al. 2009a), for example, has shown that it may be possible to determine which particular lichens should be targeted in conservation measures. Currently, only tentative evaluations of lichen impact on stonework are available (Gazzano et al. 2009b).

Efforts to conserve our cultural heritage from deterioration are extensively practised worldwide. As would be expected, damage to stonework, more particularly limestones, is of international concern. Whilst accepting that both

Fig. 9.8 Limestone sculpture by F. Narvaez at the University of Caracas, Venezuela, showing lichen and cyanobacterial disfiguration about 40 years after its installation. (*Photo* M. Tabasso)

ancient monuments and more recent architectural structures of merit and/or importance are subjected to wind and water erosion, such damage is exacerbated by the chemical and mechanical effects of not only lichens but various other pioneer micro-organisms and mycota (Paine et al. 1933; Wee and Lee 1980; Danin and Caneva 1990; Ortega-Calvo et al. 1991; May et al. 1993; Burford et al. 2003), which in due course are succeeded by mosses and higher plants (Bech-Andersen 1984); each of these successional stages support other life forms as well as protista and invertebrates (Gerson and Seaward 1977), the complex ecosystem developed thereby creating a milieu for biodeterioration. Each of these successional stages generates its own problems for the conservationist to solve.

Various methods for controlling or eliminating lichen growth, and indeed other defacing micro-organisms, on rocks and stonework have been investigated, control measures ranging from mechanical removal to application of various types of biocides. Since many lichen species are able to regenerate from thallus fragments, mechanical removal of lichens may only temporarily reduce lichen coverage. Mechanical methods involving scraping and brushing, usually followed by washing, are tedious, damaging and often ineffective. Absorbed water may adversely affect the monument, particularly under fluctuating temperature regimes; although penetration can be minimized by the use of water repellents, entrapped water and rising damp can nevertheless prove highly destructive. Laser treatment of stonework to remove lichens has been tried, and although this could feasibly be adopted for small artefacts (such as those removed for display in a museum), it is too laborious for most of the cleaning tasks required. Lichen growth on buildings and monuments is often prevented, intentionally or indeed fortuitously, on stonework affected from downwash or adjacent to roofing, windows, guttering, and pipework (Fig. 9.9) employing metals, particularly lead, zinc, and copper.

Fig. 9.9 Church wall, Fiskerton, England—the absence of lichen assemblages to either side of a metal downpipe clearly demonstrated

Application of biocides has yielded mixed results, including: generally poor treatment response, changes in community dynamics, with surviving species aggressively exploiting open space, persistent dead thalli, which decompose slowly, especially in arid habitats, and good results on lichen removal but damage to substrate surfaces, with changes ranging from discoloration to structural issues requiring use of chemical consolidants (St. Clair and Seaward 2004a). A wide range of biocides have been tried (see literature cited in Martin and Johnson 1992), many of which have since been rejected due to side effects such as crystallization of soluble salts which have penetrated the stonework, staining, and discoloration of monuments where the chemicals used have interacted with particular metals present in the substratum, and the promotion of secondary biological growths, which may be even more unsightly than the primary growths.

Furthermore, regular treatments are likely to be necessary which are expensive both in terms of the chemicals used and the labour employed for the mechanical removal of only partially detached and brittle lichen growths which remain. As pointed out by Nascimbene et al. (2008), if restorations are not to be maintained, it would be better to retain a more historic, diverse, and complex lichen community than a simplified community of 'weedy' lichens that quickly cover almost the same area as the pre-restoration community did. The biocides employed may also be harmful to the operators and, not surprisingly, dangerous to wildlife. Some success has been achieved using Anatasa (Fonseca et al. 2010), Koretrel (Tretiach et al. 2007), and also organometallic compounds, quaternary ammonium compounds and borates, but the latter have proved problematic when used in air-polluted environments where, of course, many of the monuments it is desired to conserve are located. In a study by Prieto et al. (1995), it was found that biocides tested in the laboratory were highly effective in surface cleaning but remnants of crustose lichens and algae remained between the quartz and feldspar crystals; results obtained in the field, using the same substratum and biocides, proved to be unsatisfactory. Other approaches to solve the problems arising from standard biocides, such as the use of enzymes (Silver and Wolbers 2004), are under review.

After application of biocides, consolidants may be necessary, as in the case of eighth to eleventh-century structures at Xunanturick in Belize constructed of low strength, porous limestone composed of almost pure calcium carbonate. Clearly, the erosive forces of wind and water of the humid tropical climate have extensively weathered these monuments, but the impact of lichens and other organisms has also been significant (Kumar and Kumar 1999). To evaluate the effectiveness of possible stabilization treatments, tests were conducted on stone-penetrating consolidant solutions applied to the limestone under different environmental conditions over a period of 4 years. Several of these consolidants were capable of penetrating and stabilizing the moist limestone and, in combination with biocides, 'minimized' the growth of lichens and other local microflora (Ginell and Kumar 2004).

Consolidant testing was also undertaken to ascertain if lichen-damaged sandstone supporting rock art in Wyoming, USA, could be strengthened by chemical consolidation. To achieve this, sandstone samples with attached lichens were characterized by means of detailed laboratory analyses involving examination by reflected and transmitted light, water-absorption tests (i.e. porosity), ionic chromatography, X-ray diffraction, and environmental scanning electron microscopy (Silver and Wolbers 2004). One of the underlying objectives of this work was to determine if substrata exposed when lichens are removed by mechanical and/or chemical methods are far more vulnerable to weathering and therefore in need of consolidant treatment to restore their resistance to that of the adjacent hard-packed desert varnish surface (Silver and Wolbers 2004; Chiari and Cossio 2004; Tratebas 2004). It is clear that rock art, which depends upon an unaltered natural environment, presents different issues from the culturally constructed environment of historic buildings and monuments (Tratebas 2004).

The problem is further complicated by the fact that in some situations, lichen thalli may actually play an important role in consolidating and protecting the substrate surface. A protective effect by fungal hyphae for oolitic limestone buildings has also been noted (Concha-Lozano et al. 2011). However, when lichens are removed by mechanical and/or chemical methods, the structural integrity of the substratum can be reduced and is thereby far more vulnerable to weathering (Silver and Wolbers 2004; Tratebas 2004; McIlroy de la Rosa et al. 2012a). Furthermore, it has been shown that in certain situations lichens are bioprotective, shielding the underlying stonework from external weathering agents, such as rain and insolation (Viles and Pentecost 1994; Mottershead and Lucas 2000), and indeed from pollutants and salt deposition (Carter and Viles 2003, 2005). Increasing evidence has been assembled in recent years regarding the bioprotective role of lichens on stonework, particularly calcareous surfaces (McIlroy de la Rosa et al. 2012a). Therefore, there may be good reasons to retain the subtle colouration of a varied mosaic for its aesthetic appeal, provided it does not produce disfigurement or unduly obscure detail. Furthermore, in modifying the physical and chemical properties of, for example, asbestos-cement, lichens are acting as effective spontaneous bioattenuation agents (Favero-Longo et al. 2009a).

The ethical and conservation issues arising from remedial work aimed at combating biodeterioration of cultural monuments cannot be underestimated. Conflicting issues often result from the complexity of the problems involved in any restoration programme (Tratebas and Chapman 1996; Tratebas 2004). Recent work on the biodeterioration of ancient monuments has also shown that complex problems can arise which involve some combination of laws and ordinances enacted with the intent of protecting and maintaining the resource, altered local resource use patterns, which sometimes result in environmental conditions that enhance lichen growth and development on or near critical cultural resources, consideration of threatened and endangered and endemic lichen species, and religious and cultural concerns and expectations of aboriginal populations, which in some cases may run counter to government-mandated management programmes (St. Clair and Seaward 2004b).

There are instances where lichens are actually encouraged on new stone and cement surfaces, and various chemicals are employed to establish/promote their growth. Such practices are employed, for example, in the case of farm roofs (especially those constructed of asbestos-cement) where natural colonization by lichens can satisfy the design requirements regarding colour and reflectance specified by planning authorities and governmental agencies. Such ‘blending’ into the landscape of roofs, and sometimes other architectural features of new buildings and extensions to existing premises, such as garages, is not only aesthetically more pleasing but may be legally necessary in environmentally sensitive situations.

Restoration work on ancient monuments resulting in contrasting stonework may also require such chemical applications to tone down the differences (Fig. 9.10); furthermore, there are instances where new surfaces, such as mortar (=repointing), provide substrata conducive to lichen assemblages which contrast sharply with those on the original stonework and are the loci from which these alien lichens with a great potential for biodeterioration may spread onto the monument (Fig. 9.11). Furthermore, thalli established initially on the mortar subsequently invade the artefact in need of preservation such

Fig. 9.10 Lincoln Castle, England—the restoration of a wall demonstrating the need for remedial action through the promotion of a suitable lichen assemblage

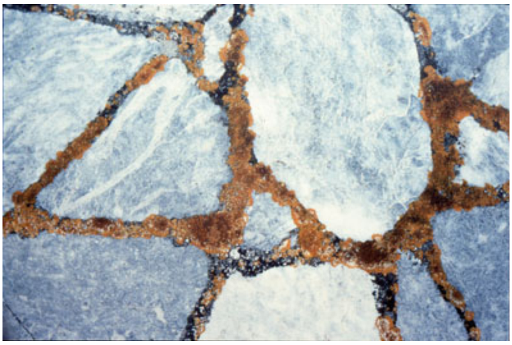

Fig. 9.11 Villa Adriano, Italy—the mortar of a reconstructed column providing loci for the colonization of distinctively coloured thalli of *Xanthoria* and *Caloplaca* spp. characteristic of calcareous substrata

Fig. 9.12 Ostia Antica, Italy—the mortared areas of a characteristically reticulated Roman wall clearly generating a distinctive lichen assemblage

as limestone tesserae (Saiz-Jimenez and Arino 2001) and reticulated Roman walls (Fig. 9.12). Why it is necessary to use such incongruent materials in the reconstruction of monuments (Fig. 9.13) when a supply of similar stonework is available?

On the other hand, there are instances where lichen recolonization should not be promoted by such practices; for example, where, for educational purposes, historical interpretation of building periods and architectural developments is highlighted or demarcated by the presence or nature of the lichen assemblages over time (Fig. 9.14).

It should also be noted that there are many instances where the actual lichens themselves are in need of conservation, since habitats generated by ancient monuments often represent ecological continuity. It is debatable whether habitats or species should be the primary concern: on the one hand, lichen conservation should aim at conserving habitats (their presence, for example, being a means of biomonitoring pollution) and, on the other, compiled regional, national, and international red lists of threatened species often include lichens known only from or mainly in such sensitive habitats (Fig. 9.15). Clearly, the maintenance of threatened species, lichen biodiversity, and our cultural heritage in general is dependent on good management practices.

Fig. 9.13 Bull's head monument, National Museum, Rome—the use of non-authentic material (cement) for the neck providing a locus for non-characteristic and unappealing lichens to spread onto the marble sculpture

Lichens are natural sensors of our changing environment and the presence or absence of particular species and the composition of assemblages on stonework are indicative of one or more identifiable factors. Information gained from our knowledge of how lichens respond to long-term perturbations and short-term upheavals

Fig. 9.14 Castle walls of Otranto, Italy—the area devoid of lichens demarcating the size and nature of a former earth embankment

Fig. 9.15 Gravestone, Baumber, England—*Ramalina capitata*, a very rare British lichen, currently found only in churchyards in two isolated areas of the country

in nature can be applied to the interpretation and monitoring of environmental changes and disasters brought about through a wide range of human activities. Lichens can often be used as an early warning system for other biota which without remedial action would subsequently suffer stress or indeed extinction through human mismanagement. Baseline information on lichen assemblages and ecosystems which are ecologically distinctive, such as those on prehistoric monuments (Aptroot and James 2002) and in churchyards, have proved invaluable in assessing human pressures on the environment. Clearly, long-term field investigations involving detailed inventories based on stringent ecological criteria are a necessary component of any programme aimed at interpreting environmental disturbances, both natural and man-made. Such work would be complementary to biodeterioration monitoring, many of the methods and measurement techniques for which are detailed in Mandrioli et al. (2003).

9.7 Conclusions

In the light of all of the above reasons, any decision to remove lichens from stonework must not be undertaken over-hastily or without very careful consideration of the wider implications of long-term effects. Unfortunately, it has to be acknowledged that the problem is under-researched and much of the work published to date is of a largely empirical nature, which has yet to be adequately substantiated by long-term experimentation. Biodeterioration of historical and culturally significant stone substrata is a complex problem: easy, risk-free solutions are simply not available. The dynamics of the problem are too complicated to address effectively with only one kind of expertise. Successful resolution of this issue will inevitably require a multidisciplinary effort, where lichenologists join with ecologists, geologists, geochemists, crystallographers, cultural conservators, archaeologists, and historians to recommend the most effective management scheme. The advantage of this approach is obvious: multidisciplinary management teams with good leadership can ask more appropriate questions whilst developing much more thoughtful and informed management decisions (St. Clair and Seaward 2004a).

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Lichen Growth and Lichenometry

10

Richard A. Armstrong

Contents

Abstract

Lichenometry is one of the most widely used methods available for dating the surface age of various substrata including rock surfaces, boulders, walls and archaeological remains. It depends on the assumption that if the lag time before colonisation of a substratum by a lichen is known and lichen age can be estimated, then a minimum date can be obtained by measuring the diameter (or another property related to size) of the largest lichen at the site. Lichen age can be determined by variety of methods including calibrating lichen size against surfaces of known age ('indirect lichenometry'), by constructing a growth rate–size curve from direct measurement of lichen growth ('direct lichenometry'), using radiocarbon (RC) dating and from lichen 'growth rings'. This chapter describes the following: (1) lichen growth rates and longevity, (2) methods of estimating lichen age, (3) the methodology of lichenometry and (4) applications of lichenometry. Despite its limitations, lichenometry is likely to continue to play an important role in dating a variety of surfaces and also in providing data that contribute to the debate regarding global warming and climate change.

Keywords

Lichen growth · Lichen growth rate–size curve · Direct lichenometry · Indirect lichenometry · Growth rings

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10.1 Introduction

Geological and climatic history is similar to many other disciplines, including history and politics, in the need to establish a timescale of events. In pre-history, timescales are often critical to understanding the evolution of biological, tectonic and climatic systems that have interacted to produce the contemporary surface of the earth. Many methods of establishing a timescale of events have been developed to allow elapsed time to be accurately estimated for timescales ranging from a few decades to millions of years.

One of the most widely used techniques to date surfaces over the last 500 years, a period in which radiocarbon dating is relatively inefficient, is ‘Lichenometry’, viz. the use of symbiotic fungi in the form of lichens, growing on rock surfaces or other suitable substrata, to obtain an approximate date of the deposition of the surface (Locke et al. 1979; Innes 1985; Matthews 1994; Benedict 2009). Lichenometry depends on the assumption that if the lag time before colonisation of a substratum by a lichen is known and lichen age can be estimated, then a minimum date can be obtained by measuring the diameter (or another property related to size) of the largest lichen present. This chapter describes various aspects of lichen growth and lichenometry including (1) lichen growth rates and longevity, (2) methods of determining the age of a lichen thallus, (3) the methodology of lichenometry and (4) discusses how the technique has contributed to the debate regarding global warming and climate change.

10.2 Lichen Growth Rates and Longevity

Many lichens, especially those which have a foliose or fruticose growth form, grow fairly rapidly and live for relatively short periods of time and are therefore less useful as lichenometric tools (Table 10.1). Hence, species such as *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale may live up to 30–40 years and *Cladonia rangiferina* (L.) Web for 25 years (Hale 1967). Of considerably more interest are the slow-growing crustose species, especially those in areas of the world in which very low radial growth rates (RaGR) have been recorded and exceptionally large individual thalli have been described. One of the first to investigate lichen growth rates in such regions was Roland Beschel, the ‘father of lichenometry’. In West Greenland, Beschel (1958, 1961) studied the yellow-green crustose lichen *Rhizocarpon geographicum* (L.) DC. (Fig. 10.1), one of the most useful lichenometric species, and found that for the first seven decades after colonisation of a rock surface, RaGR was slow and maximum size of thalli only occurred on moraines greater than approximately 1,000 years of age. At Disko Island in the same region, some individuals appeared to have not increased in size since 1896, whereas the fastest growing thalli had probably increased by a maximum of 1–4 mm in diameter. Beschel concluded from these data that some individuals of *R. geographicum* may be at least 4,500 years old. Similarly, Denton and Karlen (1973) studied

Table 10.1 Estimated ages for a selection of lichens

Site	Species	Estimated age (years)	Original data
North Canada	*Cladonia rangiferina*	10–25	Hale (1967)
Connecticut, USA	*Xanthoparmelia conspersa*	39	Hale (1967)
West Wales, UK	*X. conspersa*	30	Armstrong and Bradwell (2010a, b)
Utah, USA	*Caloplaca brachyphylla*	20	Hale (1967)
Cascade Mountains WA, USA	*Rhizocarpon geographicum*	1,428	Armstrong (2005a)
West Greenland	*R. geographicum*	4,500	Beschel (1958)
South Alaska	*Rhizocarpon alpicola*	9,000	Denton and Karlen (1973)

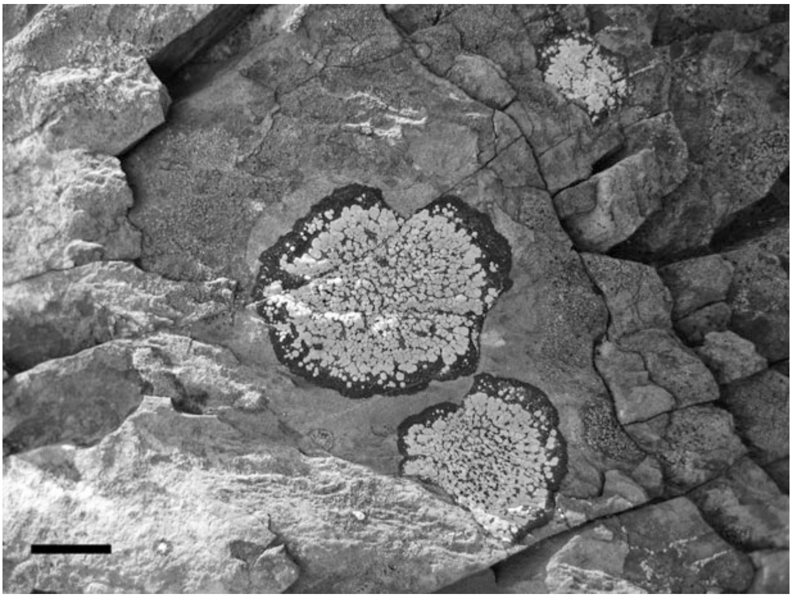

Fig. 10.1 A population of yellow-green thalli of the lichen *Rhizocarpon geographicum* on scree boulders in the Pacific Northwest, USA. The yellow-green islands of areolae can be clearly seen as well as the black fungal hypothallus at the margin (*bar* 10 mm)

Rhizocarpon alpicola Rabenh. at sites in the White River Valley, Skolai Pass, Southern Alaska. There was an initial, more rapid increase in diameter followed after a few centuries by a longer interval of nearly linear growth, a recorded RaGR of 3.4 mm 100 year^{-1} being typical. Extrapolating from these data, an individual of diameter 15.5 cm would be approximately 3,700 years old, and the largest recorded *R. alpicola* thallus found in the region, viz. 480 mm in diameter, potentially 9,000 years old!

The range of RaGR recorded in *R. geographicum* in various regions is shown in Table 10.2. Hence, in a study in the Cascade Mountains of Washington State, USA, an average RaGR of 0.1 mm year^{-1} was obtained for thalli of

Table 10.2 Annual growth rates (radial growth rate, RaGR, mm year^{-1}) for thalli of *Rhizocarpon geographicum* (L.) DC. from various habitats

Location	RaGR (mm year^{-1})	Author
North Cascades, USA	0.07	Armstrong (2005a)
West Greenland	0.05–0.1	Beschel (1961)
South Orkney Islands	0.1	Hooker (1980a)
Alaska	0–0.18	Haworth et al. (1986)
West Greenland	0.1–0.2	Ten Brink (1973)
Maritime Antarctic	0.25	Sancho and Pintado (2004)
Canada	0.05–0.29	Rogerson et al. (1986)
Tierra del Fuego	0.31	Sancho et al. (2011)
Scotland	0.14– 0.32	Bradwell (2010)
South Iceland	0.09–0.37	Bradwell and Armstrong (2007)
British Columbia, Canada	0.26–0.41	McCarthy (2003)
South Norway	0.21–0.43	Trenbirth and Matthews (2010)
Switzerland	0.5	Proctor (1983)
North Wales	0.67–0.81	Winchester and Chaujar (2002)
North Wales	0.03–0.94	Armstrong (2005b)

Rhizocarpon section *Rhizocarpon* measured over six years (Armstrong 2005a), while Beschel (1958, 1961) in West Greenland reported RaGR of 0.05–0.10 mm year^{-1}. Hooker (1980a) studied 63 thalli of *R. geographicum* in South Orkney Islands and found no measurable growth over a period of 3 years. However, small amounts of growth were recorded during a subsequent three-year period of 0.1 mm year^{-1}. A higher RaGR was recorded for *R. geographicum* by Rogerson et al. (1986) in northern Labrador (0.17 mm year^{-1}) and by Haworth et al. (1986) in the central Brooks Range, Alaska (0–0.18 mm year^{-1}).

Significantly higher growth rates have also been recorded from maritime regions in Antarctica and especially the Antarctic Peninsula. Hence, on Livingston Island, an RaGR of 0.25 mm year^{-1} was reported (Sancho and Pintado 2004). Hence, exceptionally, large thalli of *R. geographicum* in this region may be much younger than previously supposed. In addition, in Tierra del Fuego, average growth rates of *R. geographicum* were estimated to be 0.32 mm year^{-1} (Sancho et al. 2011).

In more temperate regions, significantly higher RaGR has been recorded. Hence, McCarthy (2003) reported an RaGR for *R. geographicum* in British Columbia of 0.26–0.42 mm year^{-1}. Higher rates of growth have also been reported from a glacier in Switzerland (maximum 0.5 mm year^{-1}) (Proctor 1983). In a detailed long-term study of a single thallus of *R. geographicum* (max. diam. 46.5 cm) measured over 26 years, the mean RaGR was 0.48 mm year^{-1} (range 0.02–1.54 mm year^{-1} in different years) (Mathews and Trenbirth 2011). In addition, in a study of 2,795 individuals of the subgenus *Rhizocarpon* measured at 47 sites from 18 glaciers in south Norway (Trenbirth and Mathews 2010), RaGR of 0.21–0.43 mm year^{-1} was recorded at different sites reflecting local habitat differences.

Some of the highest recorded growth rates have been reported in Europe and especially in the UK. Hence, Bradwell (2010) measured *R. geographicum* at a maritime subpolar site in Scotland. In thalli greater than 10 mm in diameter, growth rates were 0.67 mm year^{-1}, while thalli less than 10 mm grew at 0.29 mm year^{-1}. Winchester and Chaujar (2002) studied the growth of *R. geographicum* spp. *prospectans* on gravestones in North Wales and estimated RaGR to be 0.74 mm year^{-1}. Comparable results have been reported by Armstrong (1983, 2005b) in North Wales in which RaGR was in the range 0.03–0.94 mm year^{-1}, depending on thallus size.

10.3 Determining Lichen Age

Essential to lichenometry is the estimation of the age of the largest and potentially oldest lichen thallus present on a substratum. This section describes four methods of obtaining such ages: (1) by calibrating lichen size against surfaces of known age, (2) by establishing a lichen growth rate–size curve from direct measurement of lichen growth, (3) radiocarbon (RC) dating and (4) in a selective group of species, by measuring lichen ‘growth rings’.

10.3.1 Calibrating Lichen Size Against Age of Substratum

A variety of sources can provide information for dating a substratum and establishing a calibration curve of size versus age including gravestones (usually providing the most accurate dates), mine spoil heaps, abandoned farms or houses, stone walls and rock cairns (Innes 1983, 1985). In addition, natural deposits can be used that have been dated accurately from historical events, e.g. by RC dating or the use of tree growth rings (dendrochronology). Typical lichen growth curves relating lichen size and age are non-linear (Bradwell 2001a, b) and the relationship often fitted best by a third-order polynomial regression which describes the declining growth rates often apparent in larger thalli. This decline could be due to thallus senescence (Armstrong 1983, Armstrong and Smith 1997) or it could reflect the tendency for slower growth in the last 100 years.

10.3.2 Constructing a Growth Rate–Size Curve

A second method of obtaining an estimate of lichen age is by constructing a growth curve which describes how growth of a thallus may change with age. Because of very slow RaGR and consequent longevity of many crustose lichens, however, it has not been possible to measure the growth of any single individual throughout its life, even in relatively favourable environments. Hence, to obtain a growth curve, a 'cross-sectional' method has been employed, i.e. by studying individuals of different size and assuming a direct relationship between thallus size and age, the result being a growth rate–size curve. This type of study has three main limitations. First, there is no guarantee that the growth curve derived from a population of thalli of different size actually reflects the growth of any single individual throughout its life. Second, variation in RaGR between thalli of similar size, which are attributable in part to micro-environmental fluctuations and which can be considerable in foliose lichens (Fig. 10.2) (Armstrong and Smith 1992), can obscure changes due to size so that only major growth phases can be detected. Third, studies are often carried out over relatively short intervals of time, e.g. 1–3 years, and therefore ignore potential long-term effects of climate on growth rate by assuming 'average' climatic conditions over the lifespan of the thallus (Armstrong and Bradwell 2010a).

A number of studies have attempted to determine the growth rate–size curve of crustose lichens using a cross-sectional study (Bradwell and Armstrong 2007). In many of these studies, however, variability in RaGR combined with slow growth rates and small sample sizes has made it difficult to establish precisely the different growth phases (Proctor 1983; Haworth et al. 1986; Matthews 1994; McCarthy 2003; Armstrong 2005a). Furthermore, a major objective of this research has been to model the growth curve of crustose lichens as has been accomplished successfully for foliose species (Aplin and Hill 1979; Childress and Keller 1980). In foliose lichens, RaGR increases in smaller diameter

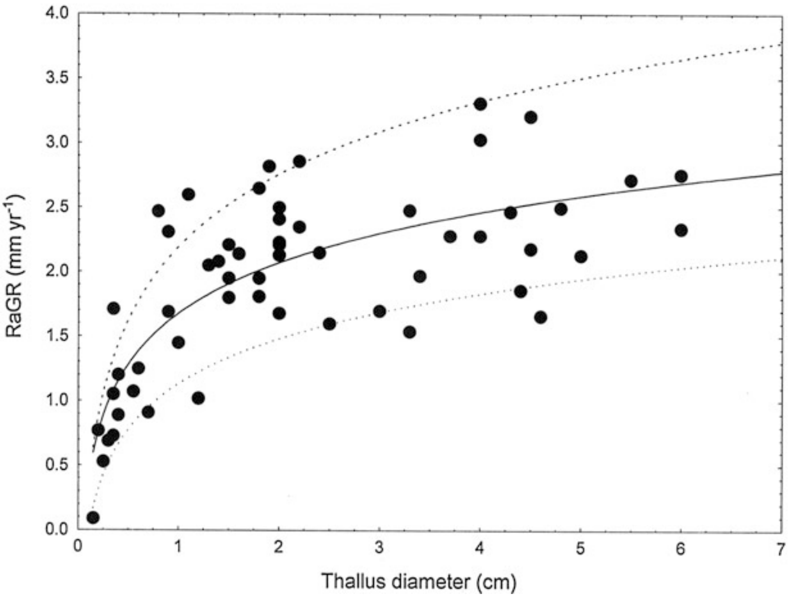

Fig. 10.2 Growth rate–size curve of the foliose lichen *Melanelia fuliginosa* ssp. *fuliginosa* (Fr. ex Duby) Essl. determined by a cross-sectional study. The *fitted curve* is a logarithmic function (data from Armstrong 1976). The *dashed lines* are logarithmic regressions fitted to the minimum and maximum RaGR values and indicate the range of variability in RaGR commonly observed in lichens

thalli becoming linear or asymptotic in larger individuals, the shape of the curve being approximately logarithmic (Fig. 10.2). There is no evidence for a declining growth phase in foliose lichens even during the later senescent phases when the thallus centre often degenerates and fragments (Armstrong 1973; Aplin and Hill 1979; Armstrong and Smith 1996, 1997; Armstrong and Bradwell 2011). This type of growth curve has been modelled based on rates of carbohydrate production within the thallus and the degree of translocation of materials between individual lobes and margin to the thallus centre (Aplin and Hill 1979; Childress and Keller 1980).

The earliest studies of the growth rate–size curves of crustose lichens often assumed a similarity to foliose lichens. Hence, Proctor (1977) studied the growth rate–size curve of the placodioid lichen *Buellia* (*Diploicia*) *canescens* (Dicks.) DNot. RaGR was assumed to be proportional to an area of thallus in an annulus of constant width at the growing margin, and therefore, the shape of the growth curve was essentially asymptotic. Similarly, in a study of *R. geographicum* on a glacier in Switzerland (Proctor 1983), the growth curve was similar to *B. canescens*, i.e. initial growth was logarithmic, rising asymptotically to a constant value. Proctor's data, however, did not include any thalli larger than 36 mm in diameter, and growth rates in thalli over 10 mm were assumed to be linear, the growth curve being extrapolated up to 60 mm without further measurement. Hence, although there was evidence that the growth rate–size curve of placodioid and foliose species were similar (Hill 1981, Benedict 2008), this may not be the case for many crustose lichens including the genus *Rhizocarpon* (Innes 1985).

In other growth studies (Bradwell and Armstrong 2007), variation in RaGR between individual thalli was often too great to establish the shape of the growth rate–size curve, and therefore, the data were not adequate to test the hypothesis that crustose and foliose lichens exhibited similar growth curves. Hence, Hooker (1980a) was unable to demonstrate any significant effect of thallus size on RaGR in South Orkney, while the studies of Rogerson et al. (1986) of seven thalli in northern Labrador shed little light on the relationship between growth and size. Similar conclusions were reported by Matthews (1994), Winchester and Chaujar (2002), Sancho and Pintado (2004) and Armstrong (2005a).

Brodo (1965) was one of the first to question whether crustose lichens grew differently to foliose species. He examined corticolous communities in Long Island, New York and observed lower growth rates of crustose thalli greater than about 40 mm in diameter compared with thalli 10–30 mm in diameter. Such a decline in RaGR in large thalli was also suggested by studies of the growth rate–size curve of *R. geographicum* in North Wales, UK (Armstrong 1983) (Fig. 10.3). Three phases of growth were identified as follows: (1) an early growth phase in which RaGR increased to a maximum, (2) a short phase in thalli 30–40 mm in diameter at which RaGR was maximal and (3) a phase in which RaGR declined in thalli greater than 50 mm in diameter. An inverse relationship between growth and size was also reported by Haworth et al. (1986) in Alaska. More substantial evidence for declining growth was obtained from a study of 134 thalli of four crustose lichen species growing in North Wales (Armstrong 2005b) and a study of *R. geographicum* in Iceland (Bradwell and Armstrong 2007). No evidence for an asymptotic growth rate–size curve was found in *R. geographicum*, *Rhizocarpon reductans*, Th. Fr. *Lecidea tumida* Massal. or *Buellia aethalea* (Ach.) Th. Fr. from North Wales or from *R. geographicum* in Iceland (Bradwell and Armstrong 2007). Instead, RaGR increased to the maximum and then declined in larger diameter thalli. In the absence of a growth model for crustose lichens, second-order polynomial curves have been fitted to growth data (Armstrong 2005b) (Fig. 10.3). More recently, Trenbirth and Matthews (2010) have proposed several models for crustose lichen growth in south Norway. At many of their sites, annual growth rates remained relatively constant or increased with size up to a diameter of 12 cm, the growth rate–size 'curve' being linear rather than parabolic or asymptotic,

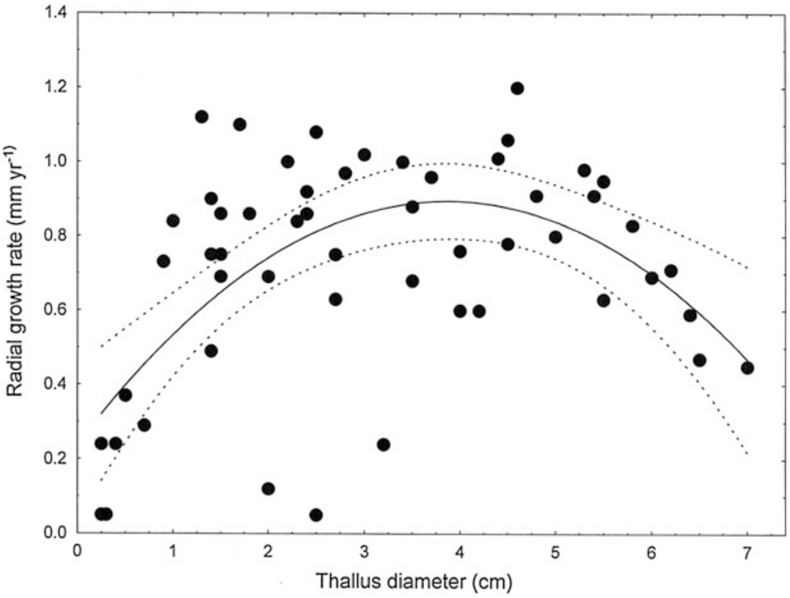

Fig. 10.3 The growth curve of the lichen *Rhizocarpon geographicum* (L.) DC. by direct measurement: a plot of radial growth rate (RGR) versus thallus diameter on rock surfaces in North Wales (data from Armstrong 2005b). Three phases of growth were identified as follows: (1) an early growth phase in which RaGR increased to a maximum, (2) a short phase in thalli 30–40 mm in diameter at which RaGR was maximal and (3) a phase in which RaGR declined in thalli greater than approximately 50 mm in diameter

raising the prospect that the growth curve of *R. geographicum* could vary at different sites or even within the same site in different years (Porter 1981).

That larger and presumably older crustose thalli may grow more slowly than younger individuals is also suggested by many traditional lichenometric studies. Porter (1981), for example, reviewed the lichen growth curves reported in the earliest lichenometric studies. Prior to 1980, growth curves were variously reported as linear (Andersen and Sollid 1971; Burrows and Orwin 1971), exponential (Mottershead and White 1972; Luckman 1977), exponential–linear (Benedict 1967; Denton and Karlen 1973) or ‘irregular’ (Curry 1969).

Lichenometric studies since 1980, however, have provided consistent evidence to support a declining phase of growth. Hence, Birkeland (1981) compared lichenometric and soil data from the Mount Cook region of New Zealand and found that the growth rate of *R. geographicum* decreased with time in a humid mountainous area. In addition, Bull and Brandon (1998) studied the growth of *Rhizocarpon* subgenus *Rhizocarpon* in the Southern Alps in New Zealand and observed that growth was non-linear. Hence, colonisation was followed by an exponential phase of growth for approximately the first 20 years succeeded by a phase of slower but more linear growth. The most similar type of growth rate–size curve of *R. geographicum*, provided by traditional lichenometry, to that obtained by direct measurement has been reported by O’Neal and Schoenenberger (2003). They revisited surfaces in the Cascade Range, USA originally measured by Porter (1981) and found that the growth curve was curvilinear with phases of increasing, constant and then decreasing RaGR, very similar in shape to the curve in Fig. 10.3.

10.3.3 RadioCarbon (RC) Dating

A number of authors have speculated whether RC dating could be used to age lichen thalli directly (Maguas and Brugnoli 1996; Clark et al. 2000; Bench et al. 2001). As a thallus grows radially, new tissue is produced at the margin which should be younger than the tissue in the centre. Hence, an RC date taken precisely at the centre could reveal the age of the thallus. Maguas and Brugnoli (1996) measured carbon isotope discrimination (d) in different individuals and with distance across thalli. Variation in ‘d’ was observed across the thalli, the highest values being observed at the margin and lowest in older, central tissues. It was concluded that variations in ‘d’ were caused by age-related changes in physiology in different regions of the thallus. In addition, the crustose lichen *Caloplaca trachophylla* (Tuck.) Zahlbr. was studied using C-14 accelerator mass spectrometry (AMS) (Clark et al. 2000). Sections of lichen were dated and exhibited a linear correlation with radial location, estimated ages also suggesting that RaGR was constant over long periods of time. The authors concluded that was potentially feasible to use AMS technology to date lichen tissues and to determine growth rates. However, Bench et al. (2001) measured radial C-14/C profiles across thalli *of R. geographicum* using AMS to determine whether C-14/C isotope ratio measurements could be used to estimate age. The C-14/C profile, however, was relatively flat, values of d-c-14 derived from modern RC standards for radial samples displaying a small spread. Hence, carbon cycling appears to occur within the thallus involving replacement and fusion of tissue as well as cellular and molecular replacement with age. It was concluded that lifespan estimates may not be routinely obtained from C-14/C profiles and that such methods were unlikely to provide an insight into lichen longevity or to be of use in lichenometry.

10.3.4 Lichen ‘Growth Rings’

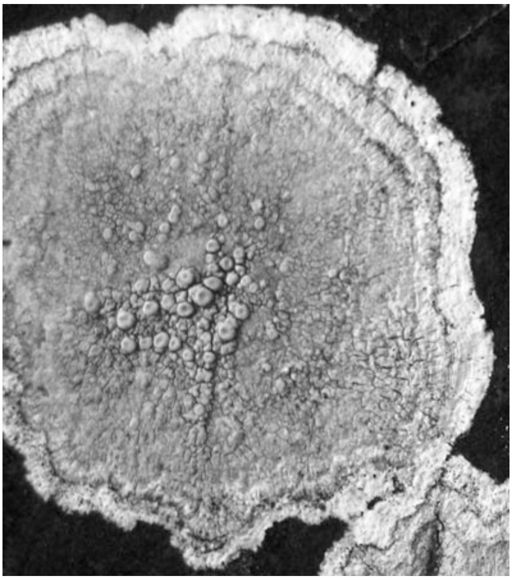

Fig. 10.4 Marginal rings in thalli of the crustose lichen *Ochrolechia parella* (L.) Massal. growing at a maritime site in North Wales, UK

Certain species of the lichen genera *Pertusaria*, *Lecanora* and *Ochrolechia* have concentrically zoned margins (Fig. 10.4), and in some species, there are alternating light and dark bands at the margin, the lighter bands which may represent rapid summer growth and the narrow dark zones growth in winter (Hale 1973). Each zone or ‘ring’ could therefore represent growth in 1 year and, if this assumption was correct, in some thalli, growth could be traced back 3–7 years. In a preliminary study, the widths of successive marginal rings in 25 thalli of *Ochrolechia parella* (L.) Massal. growing at a maritime site in North Wales were measured (Armstrong and Bradwell 2010b). Mean ring widths of all thalli varied from a minimum of 1.02 mm (the outermost ring) to a maximum of 2.06 mm (the third ring from the margin) suggesting yearly variations in growth. Marginal ring width and thallus size were also positively correlated, growth rates increasing in larger thalli. In a further study on recently exposed bedrock adjacent to Breidalon, SE Iceland, the potential for using marginal growth rings to estimate thallus age of a lichen tentatively identified as of the genus *Rhizocarpon* [possibly *R. concentricum* (Davies) Beltram.] was investigated to confirm the timing of surface exposure (c. 50 years). Collectively, these results suggested the following: (1) the measurement of marginal rings is a possible alternative method of studying the growth of crustose lichens, (2) *O. parella* may grow differently to other crustose species, exhibiting a rapidly increasing radial growth rate in thalli greater than 40 mm and (3) where lichens with marginal rings grow on recently exposed surfaces (<60 years), minimum age can be estimated using growth rings as an in situ indication of lichen growth rates.

A detailed study of marginal zonation, however, was carried out by Hooker (1980b) in Antarctica. He found that in *Buellia russa* (Hue.) Darb. zonation was present only in the non-lichenised hypothallus, and each concentric zone represented one year's growth. By contrast, *Buellia coniops* (Wahlenb.) Th. Fr. and *Caloplaca cirrochrooides* (Vainio) Zahbr. possessed 'pseudoannual rings' with each new ring representing two growth seasons. In addition, the rings of *Caloplaca* were not as distinct as those of the other species, and no new growth rings were formed during two subsequent growing seasons. Further research is therefore required before growth rings can be used as a reliable measure of growth in different habitats. Nevertheless, lichen growth rings potentially provide a previously unexploited, in situ lichenometric dating tool which could be used in areas where it is not possible to calibrate size and age (Armstrong and Bradwell 2010b).

10.4 Lichenometry

Lichenometry is a particularly useful method of dating surfaces in regions above and beyond the treeline and especially in Arctic–Alpine environments where lichens grow very slowly and have considerable longevity. In such environments, it may be possible to date surfaces and deposits up to thousands of years old, but in the majority of cases, the method has been most useful for dating over the past 500 years.

Although several species of lichen have been used in lichenometry, the majority have utilised a specific group of crustose lichens, viz. the yellow-green species of the genus *Rhizocarpon* (Fig. 10.1). This type of lichen is abundant in many environments from sea level to high elevations, grows very slowly (0.02–2 mm year^{-1}), especially in Arctic and Alpine environments (Table 10.2) and can live to a considerable age (Beschel 1958, 1961). Morphologically, the lichen comprises discreet areolae that contain the cells of the alga *Trebouxia*, located on a fungal medulla which is attached to the substratum and which extends into a black algal-free marginal zone around the thallus called the hypothallus (Armstrong and Smith 1987; Armstrong 2013). Within each areola, there is a cortical layer 15–80 μm thick, an algal layer and medullary tissue. The fungal hypothallus extends radially, and to grow, it relies on carbohydrate supplied from the areolae (Armstrong and Smith 1987). Primary areolae near the edge of the hypothallus may develop from free-living algal cells on the substratum that are trapped by the hypothallus, while secondary areolae may develop from zoospores produced within the thallus (Armstrong 2013). Different species of *Rhizocarpon* may also colonise a surface at different rates, e.g. species of the section *Rhizocarpon* may colonise a surface earlier than *R. alpicola*. However, *R. alpicola* may exhibit a faster RaGR than most members of the section *Rhizocarpon* and eventually may become the largest lichen on a substratum (Innes 1985).

10.4.1 Indirect Lichenometry

Traditional lichenometry, also known as 'indirect lichenometry', involves the dating of rock and stone surfaces using a calibration of size of lichen thalli present against known age of substratum (Locke et al. 1979; Innes 1985; Matthews 1994; Benedict 2009). Indirect lichenometry has been the most useful technique for dating and has the advantage that fluctuations in lichen growth that occur over long periods are taken into account. Several measurements of thallus size have been advocated as the most useful in indirect lichenometry. Hence, the use of the 'largest inscribed circle', i.e. the largest circle that can be drawn within an individual thallus, was suggested by Locke et al. (1979) and has been adopted by several workers and is equivalent to using the 'shortest diameter' of the thallus. By contrast, many workers have suggested that the largest diameter of the thallus is the most appropriate measure (Burbank 1981; Ellis et al. 1981; Proctor 1983). Other workers have averaged the longest and shortest axes or used both measurements to derive an estimate of the surface area of the thallus (Griffey 1977). In addition, various methods have been employed to sample a lichen population at a site. Either the single largest thallus at the site is measured and regarded as representative of age (Webber and Andrews 1973) or several of the largest thalli present are averaged (e.g. Mathews 1994), both methods being regarded as valid (Innes 1985). Some workers have used the frequency distribution of thallus size as an indicator of the age of the substratum (McCarroll 1993; Caseldine and Baker 1998; Dabski 2007), but is more controversial as size distributions can have different interpretations (Innes 1985).

A limitation of indirect lichenometry is that smaller lichen thalli could coalesce and therefore be recorded as a single individual (Armstrong 1984). In addition, thalli of the same and of different lichen species may fuse to form lichen mosaics, a feature of many lichen communities dominated by crustose species (Pentecost 1980; Armstrong and Welch 2007). The tendency for thalli to fuse without leaving evidence of the original thallus boundaries depends on species. For example, in North Wales, *R. geographicum* forms mosaics of sharply delimited individual thalli, while *R. lecanorinum* (Flörke ex Körb) Anders, thalli merge with one another at intraspecific contacts, leaving no visible trace of their initial boundaries (Clayden 1997). In *R. geographicum*, it is possible that there is somatic incompatibility between genotypes, whereas in *R. lecanorinum*, somatic compatibility may exist between genetically identical or closely related thalli allowing them to fuse more readily (Clayden 1997). Measurement of only circular or near-circular thalli has been advocated as one method of reducing this problem as fusion is likely to increase the degree of asymmetry of the thallus (Innes 1985).

10.4.2 Direct Lichenometry

In direct lichenometry, a lichen growth rate–size curve is constructed and then a lichenometric dating curve, from direct measurements of lichen growth (Armstrong 1976; Bradwell and Armstrong 2007; Trenbirth and Matthews 2010) and is potentially useful method where it is not possible to establish a calibration of size and age (Armstrong and Bradwell 2010a, b). Relatively few studies, however, have adopted the direct approach to lichenometry (Bradwell and Armstrong 2007, Trenbirth and Matthews 2010). Trenbirth and Matthews (2010) identified four main reasons for the neglect of the direct method: (1) high growth ratevariability, (2) lack of ecological data concerning the effects of environmental factors on growth, (3) small sample sizes and (4) the restricted number of years over which measurements were made.

Construction of a lichen growth curve usually involves dividing up the range of thallus size into suitable increments (Armstrong 1976). Hence, size classes of 1 cm were used to construct growth curves of *X. conspersa* (Armstrong and Smith 1996), *Melanelia fuliginosa* ssp. *fuliginosa* (Armstrong 1976) (Fig. 10.2) and *R. geographicum* (Armstrong 1983) (Fig. 10.3). RaGR of a sample of thalli is then measured within each size class, and RaGR plotted against thallus size to obtain the growth curve. The estimated age of a thallus of a diameter '*d*' cm is obtained by fitting a growth curve to the data and calculating the time taken to grow each size increment. A calibration of diameter against estimated age can then be constructed based on measured growth rates (Armstrong 1976). However, the within-thalli variation in RaGR results in large confidence intervals for estimated ages which accumulate with thallus size (Fig. 10.2). Nevertheless, it may be possible to model variation in RaGR on a single face using the normal distribution and, therefore, to estimate the number of thalli that should be measured in each size class to estimate RaGR within defined limits of error.

An example of an age/size curve for the foliose lichen *M. fuliginosa* ssp. *fuliginosa*

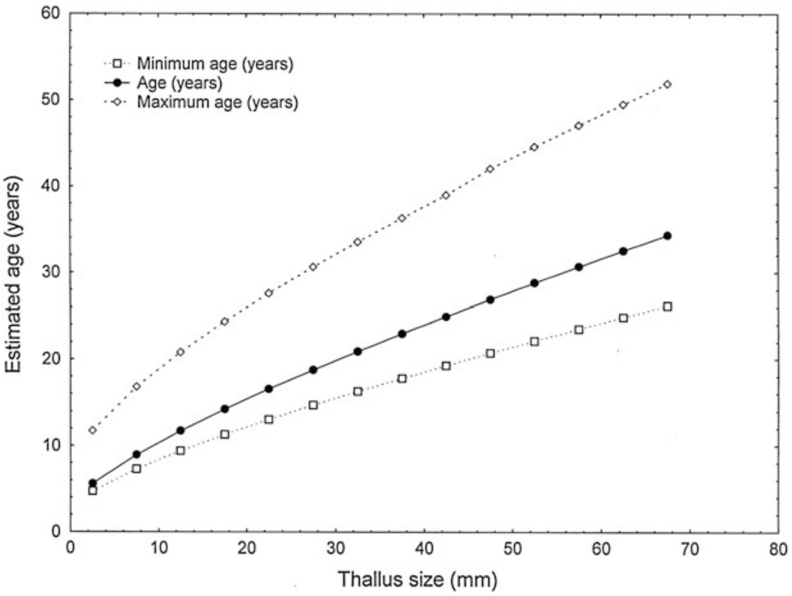

Fig. 10.5 Calibration of thallus age against size for the lichen *Melanelia fuliginosa* ssp. *fuliginosa* (Fr. ex Duby) Essl. in North Wales, UK by direct measurement for use in direct lichenometry. The data show the non-linear change in estimated age with thallus size and are best fitted by a second-order (quadratic) polynomial. In addition, the error limits for an estimated age are based on the logarithmic regressions fitted to the minimum and maximum values of RaGR in Fig. 10.2 and are asymmetrical, the upper error limit being greater than the lower. From this curve, a thallus of *M. fuliginosa* ssp. *fuliginosa*, 5 cm in diameter, would be estimated to be 28 years old (range 22–43 years)

derived from Fig. 10.2 is shown in Fig. 10.5. The data show a non-linear change in estimated age with thallus size which is best fitted by a second-order (quadratic) polynomial. In addition, the error limits for an estimated age, based on the maximum and minimum values of RaGR, are asymmetrical, the upper limit being greater than the lower (Fig. 10.2). From this curve, it was estimated that a thallus of *M. fuliginosa* ssp. *fuliginosa*, 5 cm in diameter, could be 28 years old (range 22–43 years), the large error reflecting the degree of variation in RaGR present in the original data. Hence, methods of controlling and reducing this variation are essential for the successful application of the direct method.

10.5 Applications of Lichenometry

Lichenometry has been used in many different environments and regions to date surfaces. However, it is the dating of the sequences of rocks that form glacial moraines that have been the most widely used application (Burrows and Orwin 1971). Porter (1981), for example, applied indirect lichenometry to the moraines of Mount Rainier in Washington State, USA and used historical evidence for the past marginal positions of the glaciers, including buildings, together with other structures in the national park, to provide surfaces of known age from 1857 to the present. The single largest circular *R. geographicum* thallus was used as a measure of lichen growth. The study indicated that the moraine probably stabilised 4 years earlier than had been previously suggested. Other applications of lichenometry include the dating of the stone images on Easter Island (Follman 1961), stone walls (Laundon 1980; Benedict 1967), river flooding (Gregory 1976), sea-level changes (Birkenmajer 1981) and the occurrence of landslides and avalanches (McCarroll 1993).

During the last 30 years, there has been increasing concern as to the environmental impact of global warming and climate change.

Lichenometric studies have played an important role in this debate (Bradwell 2001b). Hence, McCarroll (1993) used the size–frequency distribution of lichen populations to determine the ages of boulders resulting from avalanches in South Norway. Snow avalanches reflect periods of high winter snow and rapid spring melting rather than low temperatures. It was concluded that increasing avalanche activity in South Norway was attributable to global warming. Harrison and Winchester (2000), using a combination of lichenometry and dendrochronology, studied the nineteenth- and twentieth- century fluctuations of glaciers in South Chile. There was evidence for retreat of the glaciers following the 'little ice age' maximum between 1850 and 1880. Glacier retreat increased during the 1940s, and the degree of synchrony exhibited by different glaciers suggested a common climatic influence. This pattern has been repeated around the world. In Central Asia, there were small advances of the glaciers during 1908–1911, 1911–1934 and 1960–1977 (Narama 2002). However, significant recession also occurred in the 1900s, especially in the periods 1911–1934 and 1977–1978. Recession then accelerated after 1990 reflecting recent climatic warming in regions of inland Asia. Records of glacier fluctuation are now compiled by the World Glacier Monitoring Service and have derived estimates of global warming during the last 100 years (Oerlemans 1994). Examination of data from all over the world has confirmed that the retreat of glaciers is a common phenomenon over the globe and is likely to be explained by a linear warming trend of 0.66 K per century (Oerlemans 1994).

10.6 Conclusions

Several aspects of the biology and growth of lichens have implications for lichenometry (McCarthy 1999; Worsley 1981). First, there is uncertainty regarding the early stages of development of many lichens and especially the duration of a time lag between colonisation and the appearance of the first measurable thalli. In addition, small thalli may merge early in life to establish larger thalli than would be expected by their initial growth rates. There may also be substantial mortality of young thalli (Loso and Doak 2006) resulting in relatively few survivors of the first colonists on a mature rock surface. Therefore, studies of very early growth processes and mortality may enable a more accurate estimate of the time lag to be made and improve the accuracy of dating curves.

There is also uncertainty regarding the shape of the growth rate–size curve, especially in the *Rhizocarpon* group (Trenbirth and Matthews 2010). A major problem affecting most experimental growth data is the degree of between-thallus variability which is often larger than the growth–size fluctuations themselves. Different models have been proposed for the shape of the growth rate–size curve in *R. geographiucm* (Bradwell and Armstrong 2007; Trenbirth and Matthews 2010) including models suggesting parabolic, linear or increasing growth rates over time. It is possible that members of the *Rhizocarpon* group exhibit different growth curves at different sites or even at the same site in successive years. If this hypothesis is correct, it would limit the application of 'direct' lichen growth curves across different geographical regions.

R. geographicum is likely to exhibit seasonal patterns of growth (Armstrong 2006) and significant annual variation in RaGR. These variations may correlate with specific climatic factors (Armstrong 2006; Trenbirth and Matthews 2010), and further knowledge of such relationships could enable growth curves established in one region to be adjusted for use in others.

There has also been debate regarding the possible effects of competition in lichenometric studies. On older surfaces, there is likely to be increased competition leading to possible overgrowth and mortality, cessation of growth and the formation of lichen 'mosaics' (Armstrong and Welch 2007). Mature thalli of some species of *Rhizocarpon* may even merge imperceptibly, leading to the formation of much larger thalli than expected (Clayden 1997). Hence, Bradwell (2010) measured the growth of *R. geographicum* at two contrasting sites in Scotland and found that more intensive competition at one of the sites ruled out a meaningful comparison between the two. In addition, the rate of lichen growth may decline on older surfaces as a consequence of increased competition, a result which may have implications for dating older surfaces with considerable lichen cover (Gellatly 1982).

Lichenometry is one of a number of techniques now available for estimating the surface age of exposure of a substratum. Its advantages include an ability to date surfaces during the last 500 years, a time interval in which RC dating is least efficient and provides a quick, cheap and relatively accurate date for a substratum. Despite the continued lack of information regarding many aspects of lichen growth, lichenometry is likely to continue to play an important role in dating surfaces and especially in providing data that contribute to the debate regarding global warming and climate change.

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11

Analysis of Lichen Metabolites, a Variety of Approaches

Pierre Le Pogam, Gaëtan Herbette and Joël Boustie

Contents

Abstract

Lichens produce secondary metabolites which have been first considered as a chemical support fully involved in lichen taxonomy. As a consequence, analytical methods were developed and applied to these organisms from a long time, some of them being standardized. Then, lichen analysis benefitted from new developments and techniques applied for isolation and identification of secondary metabolites which are exposed and discussed herein. Some ancient techniques for lichen taxonomy are still used as spot tests, which involve application of specific reagents directly on the lichen thallus. TLC is also still extensively used with standardized protocols affording more accurate information on lichens' metabolic profiles. Identification of lichen compounds from the shape of crystals observed under microscope was facilitated as some major lichen metabolites are extracted in high yields. X-rays are now used in some cases where classical spectroscopic, UV, IR, MS, and NMR techniques do not allow unambiguous assignments. Using such techniques for isolated lichen compounds, some characteristic identification patterns of these substances are

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presented here. Hyphenated techniques, coupling separation and identification, are more and more used and broaden the analysis facilities of lichen compounds. They enable early dereplication and subsequent focusing on bioactive or original compounds. Other trends in lichenology involve in situ analyses thanks to specific and innovative NMR or MS techniques that yield valuable information directly from the natural complex matrix. Several advantages can be expected from those approaches: no extraction steps, qualitative and quantitative information in a few minutes or hours, and direct analysis of genuine compounds, avoiding chemical artefacts associated with extraction and purification processes (Table 11.2). Moreover, some of these methods pave the way for the development of imaging techniques that might help correlating metabolites to their specific ecological environment. Altogether, such enhancements might enable harnessing of lichens' unique chemo-diversity.

Keywords
Secondary metabolites · Characterization · Identification · Spectroscopic analysis · Phytochemistry · Natural structure

11.1 Introduction

The lichenous lifestyle is maintained by approximately 18,900 known species (Feuerer and Hawksworth 2007) resulting from the association between a fungus and an alga (chlorolichens). For 10 % of lichens, green alga is replaced or accompanied by a cyanobacterium, which can absorb atmospheric nitrogen due to specific structures named heterocysts. The fungus plays a role in water and mineral supply and in mechanical protection of the whole organism. Algal or cyanobacterial partners realize photosynthesis and provide their fungal associate organic compounds to be metabolized.

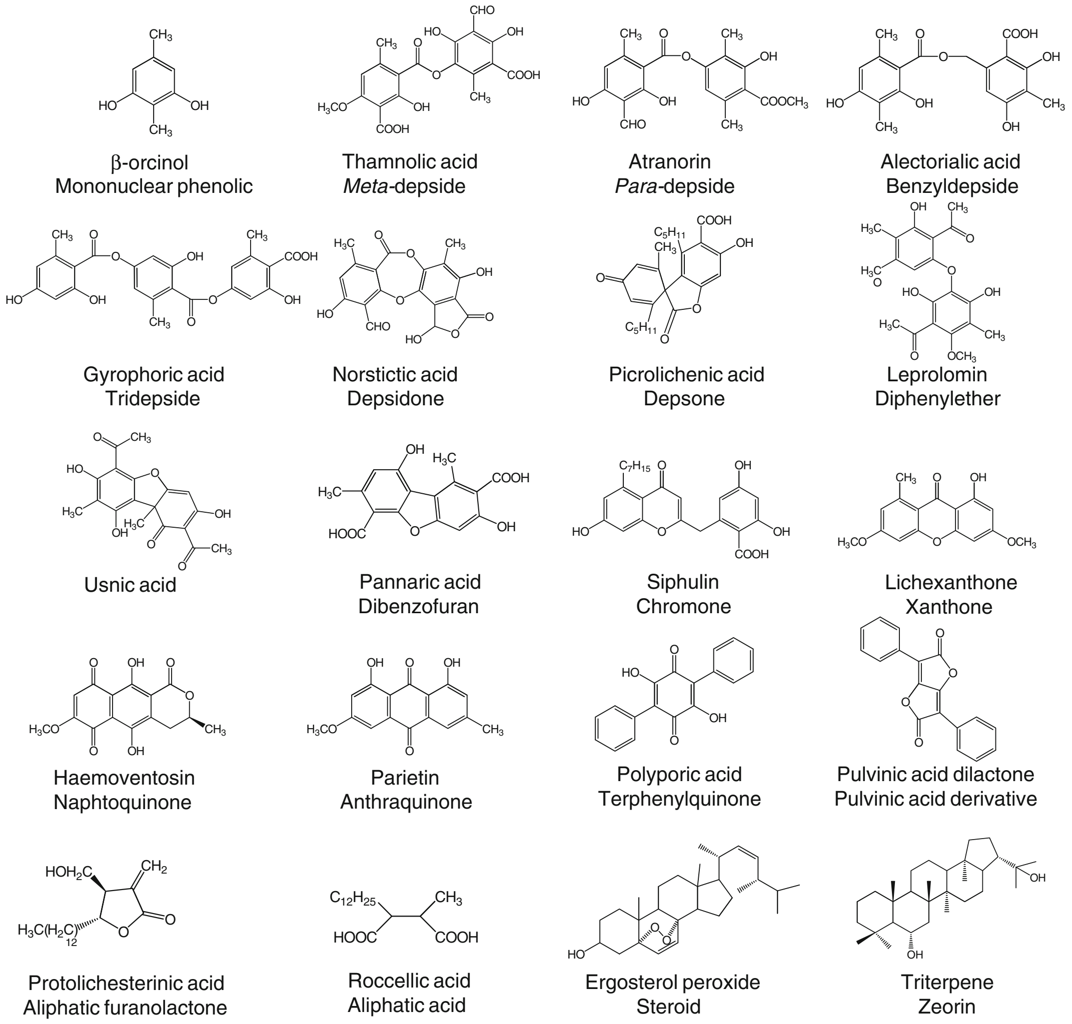
Irrespective of the shape and the size of the lichen thallus, the symbiotic thallus yields unique metabolites among all life forms (Fig. 11.1). Most lichen substances are phenolic orcinol and β-orcinol derivatives: dibenzofurans and usnic acids (pannaric acid and usnic acid), depsides (thamnolic acid and atranorin), depsidones (norstictic acid), depsones (picrolichenic acid), diphenylethers (leprolomin), benzyl esters (alectorialic acid), and tridepsides (gyrophoric acid). Other than specific and unique lichen metabolites, various other structural families can be recognized: aliphatic acids (roccellic acid) and related lactones, xanthones (lichexanthone), chromones (siphulin), pulvinic acid derivatives (pulvinic acid dilactone), quinones (haemoventosin, parietin), terpenes (zeorin), steroids (ergosterol peroxide), and carotenoids (astaxanthin). Cyanolichens mostly contain sugars and amino acid derivatives although original compounds such as mycosporines were detected recently (Torres et al. 2004; Roullier et al. 2011).

Unusual compound families can also be encountered as polypropionates, cyclic depsipeptides, brominated depsidones, and brominated acetylenic fatty acids, along with some glycosides (Boustie and Grube 2005). One should find a recent update of lichenic chemodiversity in the catalogue established by Elix (2014) (http://www.anbg.gov.au/abrs/lichenlist/Chem%20Cat%203.pdf). This diversity is also extended through the increasing number of metabolites isolated from the cultivated fungi and microflora associated with the lichen thallus (Grube et al. 2012).

Lichen metabolites considered in this chapter are mainly produced by the fungal partner and often released into the extracellular space to subsequently crystallize on or near the hyphal surface (Honegger 1986; Ozenda and Clauzade 1970; Hale 1983). Thus, large amounts of secondary metabolites can be detected in a lichen thallus. Extraction yields can rate from 1 to 25 % of dried lichen material, and composition is frequently characterized by one to three metabolites accumulated in high yields (over 80 % ratio) (Boustie et al. 2011).

Lichen secondary compounds are mainly arising from four pathways of biosynthesis: polyketide path, mevalonic acid path, shikimic acid path, and photosynthetic products of the phycobionts as presented in Fig. 11.2.

Phenols are the most frequently produced lichen metabolites and generally found accumulated in high yields as extrolites, crystallized



β-orcinol
Mononuclear phenolic

Thamnolic acid
Meta-depside

Atranorin
Para-depside

Alectorialic acid
Benzyldepside

Gyrophoric acid
Tridepside

Norstictic acid
Depsidone

Picrolichenic acid
Depsone

Leprolomin
Diphenylether

Usnic acid

Pannaric acid
Dibenzofuran

Siphulin
Chromone

Lichexanthone
Xanthone

Haemoventosin
Naphtoquinone

Parietin
Anthraquinone

Polyporic acid
Terphenylquinone

Pulvinic acid dilactone
Pulvinic acid derivative

Protolichesterinic acid
Aliphatic furanolactone

Roccellic acid
Aliphatic acid

Ergosterol peroxide
Steroid

Triterpene
Zeorin

Fig. 11.1 Some typical secondary lichen metabolites illustrating a variety of structural classes

outside of the fungal hyphae. Oxidative coupling of orcinol and beta-orcinol derivatives result in the most typical lichen metabolites corresponding to depsides, depsidones, and dibenzofurane derivatives (Fig. 11.3). These lichen substances have indeed several possible biological roles that may impact biotic and abiotic interactions of lichens with their environment (Rikkinen 1995; Molnár and Farkas 2010).

Lichen secondary metabolites can act as allelochemicals, affecting the development and growth of lichens (Culberson et al. 1977; Whiton and Lawrey 1984), mosses (Heilman and Sharp 1963; Lawrey 1977), vascular plants (Pyatt 1967; Fisher 1979; Marante et al. 2003), and microorganisms that compete for space and light on a variety of substrates (Ranković et al. 2008; Schmeda-Hirschmann et al. 2008; Halama and Van Haluwin 2004). Strong experimental data support protection of lichens from herbivores grazing granted by lichen secondary metabolites (Asplund and Gauslaa 2008; Nimis and Skert 2006; Pöykkö et al. 2005; Dailey et al. 2008).

Ertl (1951) first observed that cortical accumulation of lichen compounds increased opacity of the upper cortex, restraining solar irradiance

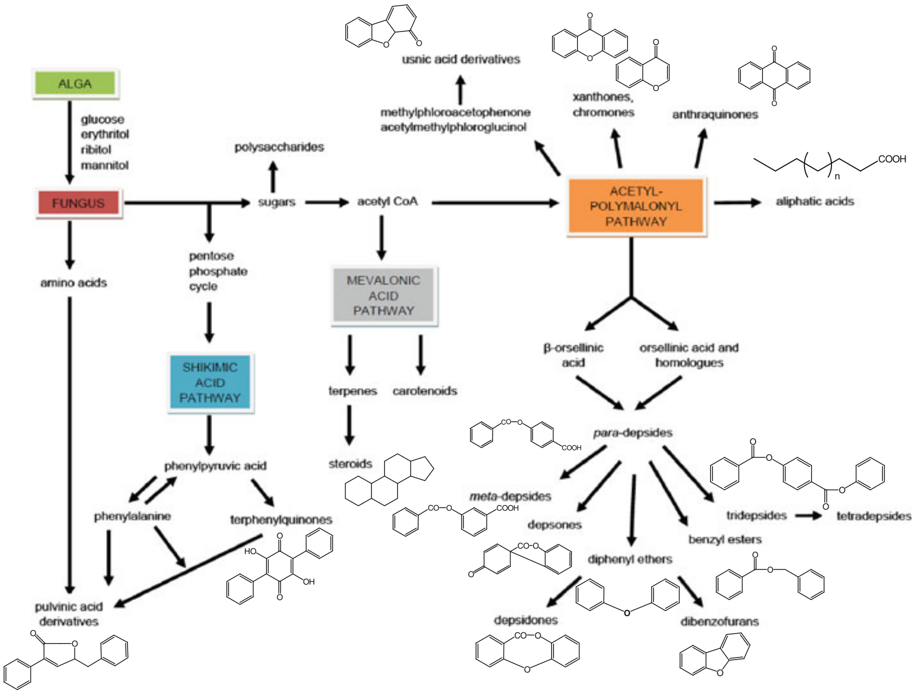


Fig. 11.2 Main metabolic pathways recognized in lichens [adapted from Elix (1996) and Stocker-Wörgötter (2008)]

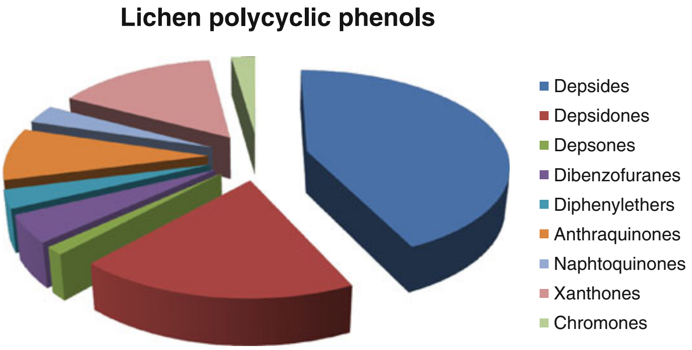


Fig. 11.3 Distribution of the main polycyclic lichen phenols arising from the acetyl-polymalonyl pathway (based on the 600 lichen polycyclic phenols compiled by Huneck and Yoshimura 1996)

reaching the light-sensitive algal layer. Various light-screening pigments display strong UV absorption abilities and might function as filters in order to prevent excessive UV-B irradiation (Nguyen et al. 2013). Some lichen phenolics have high ability to scavenge toxic free radicals generated by UV light (Russo et al. 2008; Odabasoglu et al. 2006; Karakus et al. 2009). Some other metabolites step in metal homeostasis and pollution tolerance (Hauck and Huneck 2007a, b; Hauck et al. 2009). Secondary metabolites quantification revealed tremendous changes in the concentrations of secondary metabolites from *Hypogymnia physodes* when thalli were transplanted to areas polluted with heavy metals and acidic inorganic sulfur compounds (Bialonska and Dayan 2005). As a result, the value of lichen metabolites in chemotaxonomy and systematics is questionable, but their isolation and identification is mandatory to recognize new active compounds and/or original physicochemical properties.

Most common biological activities deal with cytotoxicity and antibiotic activities (for a review see Shukla et al. 2010; Shrestha and St. Clair 2013). Additionally, miscellaneous other properties were described within several families of lichen products including antiviral or antiretroviral (Yamamoto et al. 1995; Pengsuparp et al. 1995), anti-inflammatory (Vijayakumar et al. 2000; Freysdottir et al. 2008), and analgesic activities (Okuyama et al. 1995). As natural plant-derived products have a less detrimental impact on the environment than synthetic chemicals, lichen metabolites also represent good candidates for new pesticides (Cetin et al. 2008) (Dayan and Romagni 2001). However, although studies carried on lichens to value their secondary metabolites are increasing, difficulties encountered in identification of the species, collection of bulk quantities, and the isolation of pure substances limit the number of tested compounds. Moreover, detection and isolation of minor compounds is generally complicated by the high abundance of redundant lichen compounds and techniques designed to bypass such a difficulty should allow findings of more specific and interesting compounds.

This chapter aims at presenting successive analytic methods developed during the history of lichens' secondary metabolites analysis. Arising from spot tests used in the first lichen taxonomy approaches, thin-layer chromatography (TLC) is still extensively used and standardized protocols have been established. Crystallography from crystal shapes to X-rays accurate measurements has been developed with lichen metabolites. The main classical spectroscopic techniques, UV, IR, MS, NMR used to identify isolated lichen compounds, are presented herein with a focus on some characteristic identification patterns of these substances. Early and refined dereplication approaches to eliminate already known compounds and to focus on species containing original or bioactive compounds have now to be used first. Accumulated spectroscopic data and hyphenated techniques, coupling separation and identification, are more and more used and broaden the analysis facilities of lichen compounds. Subsequent trends in lichenology involve in situ analyses due to more specific and innovative NMR or MS techniques that yield valuable information directly from the natural complex matrix.

Some of these methods are described in a second part as well as expected results they should yield. A special insight is given to some imaging techniques with high potential to dramatically improve our understanding of the metabolic activities revealed at the very root of the lichen symbiosis.

11.2 Oldest Analytical Methods

11.2.1 Spot Test (Reagents Directly Applied on the Lichen Thallus)

Color reactions should be considered as useful hints for the presence of functional groups or elements within lichen molecules and thus remain rather limited compared with classical spectroscopic analysis methods. Nevertheless, spot tests, involving application of tiny amounts of specific reagents directly to the lichen thallus or parts of this thallus (i.e., cortex, medulla, apothecia), have been applied since the nineteenth century (Nylander 1866) and can lead to distinguish varieties and even sometimes species according to any associated color change or not. This is particularly useful to distinguish some closely shaped species which could not be unambiguously discriminated on the basis of morphological characters. For instance, species of the *Ramalina siliquosa* complex are tricky to be separated and display a remarkable chemical plasticity, leading to six different chemotypes according to different thallus reactions related to the main compound as assessed with LC-DAD-MS analysis (Parrot et al. 2013). So, such colors obtained with given reagents are found in herbarium specimens since the method has been standardized.

Nowadays, spot tests remain either helpful or necessary to confidently discriminate between some lichens and are still met as a very important criterion in determination keys. However, color intensities which are to be interpreted are not easy to standardize, attribution to a given major compound is often hazardous, and this technique is poorly informative in analysis of pure compounds. Noteworthy, because different compounds are present in specific parts of the lichen, reference tests usually describe parts of the thallus which should be tested, which corresponds to a first attempt toward histo-localization (Fig. 11.4). Three main reagents used in color reactions are potassium hydroxide (commonly called K), sodium hypochlorite (abbreviated C), and paraphenylenediamine (mainly known as P or PD).

K (consisting of 10 % potassium hydroxide in water) is a useful reagent to differentiate between quinones and pulvinic acid derivatives as it turns quinones into a bright red to deep purple color. It also turns yellow and then red with most *ortho*-hydroxy aromatic aldehydes.

C (saturated aqueous $Ca(OCl)_2$ or commercial laundry bleach) is a reagent for *meta*-dihydroxy phenols, except for those substituted between the hydroxy groups with a –CHO or –COOH. This explains why arthothelin and thiophanic acid are C+, whereas lichexanthone does not react with C (Fig. 11.5). Different colors can be obtained ranging from light yellow to blood red. C turns green with dihydroxy dibenzofurans (Nash 2008).

In some cases, successive reagent application is required, and KC+ is pretreatment by KOH before having a coloration with C reagent. KC turns red with C– depsides and depsidones which undergo rapid hydrolysis to yield a *meta*-dihydroxy phenolic moiety. Blue color is obtained with dihydroxy dibenzofurans, whereas a yellow reaction appears with usnic acid (Nash 2008).

Although highly toxic, last routinely used macrochemical reagent is ethanolic solution of para-phenylenediamine (2–5 %) that gives yellow to orange colors with aldehyde-containing depsides and depsidones turned into a Schiff base according to the following reaction:

$$\begin{aligned} &R{-}CHO + H_2N{-}C_6H_4{-}NH_2 \\ &\quad \rightarrow R{-}CH{=}N{-}C_6H_4{-}NH_2 + H_2O \end{aligned}$$

Various other reagents can be found in literature, but their use is not common compared with those previously described. $FeCl_3$ can be used to

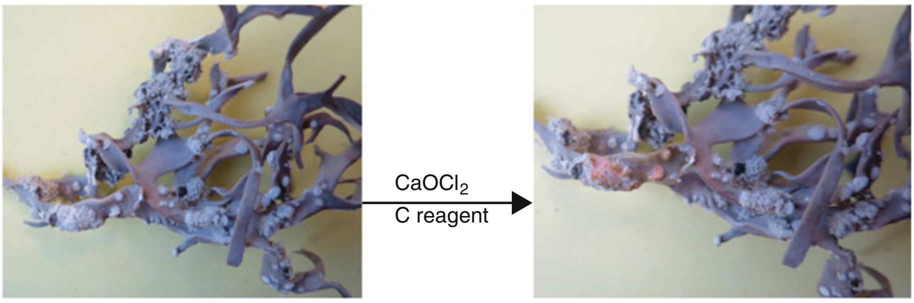


Fig. 11.4 Spot test with $Ca(OCl)_2$=C reagent drop on a thallus of the fruticose *Roccella fuciformis*. On the *right picture*, the C+ bright red reaction is limited to soralia (= white parts, corresponding to medulla as the gray thallus cortex is disrupted). This test is also confirming the distinction with the related species *Roccella phycopsis* where only the gray cortex is turning rose red C+

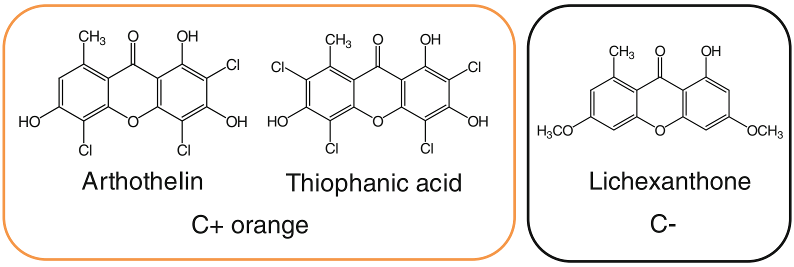


Fig. 11.5 Structures of chromones exemplifying the marked *orange* color obtained with $Ca(OCl)_2$ reagent =C spot test, indicating the presence of *m*-dihydroxyphenols in arthothelin and thiophanic acid compared to lichexanthone

detect phenolic groups (leading to red to violet coloration), quinones having free hydroxyl group in β-position are revealed with both 2 % magnesium acetate ethanolic solution (red or violet) and Dimroth's reagent, e.g., boric acid solved in acetic anhydride giving red to violine colors. Chlorinated metabolites can be detected thanks to Beilstein test which consists of bringing one milligram of the molecule on the tip of a glowed copper wire introduced into the flame of a Bunsen burner, halogenated compounds then transiently turn the flame into a deep green color (Huneck and Yoshimura 1996). Spot tests corresponding to a set of typical lichen products can be found below (Table 11.1). Chromones, xanthones, anthraquinones, and pulvinic acids are naturally colored compounds.

11.2.2 Crystal Tests

Microcrystallization was once a very if not the most important way to identify lichen metabolites in times without TLC and high-performance liquid chromatography (HPLC). Microcrystallization of lichen substances was mainly developed by Asahina and Shibata since 1950 (Shibata 2000). This approach was based on the formation of distinctive crystals from lichen extracts (Fig. 11.6).

The general procedure can be summarized as follows: the extract of a small piece of lichen is first filtered before being evaporated. Acetone is the most used solvent as it extracts compounds with a wide range of polarity. Dried residue is then transferred to a microscope slide, and a drop of proper microcrystallization reagent is added on it before capping with a cover glass. Different microcrystallization solutions were described, the most commonly used being GAW and GE, corresponding to H_2O/glycerol/ethanol 1:1:1 (v/v/v) and to acetic acid/glycerol 1:3, respectively. Slides using GE or GAW are further gently heated over microflame (other solvents do not need this heating step) and then let aside for progressive cooling, enabling the crystallization process to occur. Once formed, crystals are best observed under polarized light with a 200–1,000-fold magnification and present incredible diversity in both shapes and colors that was used to recognize the major metabolite in a given specimen. Although now outstood by modern analysis methods, this concept is still used nowadays after compound purification with great performances thanks to modern X-ray crystallography (Deschamps 2010).

This simple and rapid technique allowed to characterize the major metabolites in hundreds of lichen species but was obviously useless to detect minor substances and to analyze too complex mixtures of lichen substances.

11.2.3 Melting Point

Melting point was once an important criterion for identification of a pure lichen substance. It can be determined with a few crystals under a glass capillary or with a Köfler device. It should be noted that some compounds do not have a sharp melting point but decompose over a wide range

Table 11.1 Thalline reactions corresponding to a set of common lichen metabolites (Brodo et al. 2001)

Compound	K	C	KC	PD
Depsidones				
Psoromic acid	–	–	–	Light yellow
Salazinic acid	Dark red	–	–	Orange
Stictic acid	Yellow	–	–	Orange
Virensic acid	–	–	–	Orange/red
Benzyldepsides				
Alectorialic acid	Pale yellow	Red	Red	Dark yellow
Barbatolic acid	Yellow	–	–	Yellow
Dibenzofurans and related				
Didymic acid	–	Green	Green	–
Pannaric acid	–	Olive green	Olive green	–
Strepsilisin	–	Green	Green	–
Usnic acid	–	–	Yellow	–
Chromones				
Siphulin	Yellow brown	Violet	–	–
Xanthones				
Arthothelin	–	Orange	Orange	–
Lichexanthone	–	–	–	–
Thiophanic acid	–	Orange	Orange	–
Anthraquinones				
Nephromin	Red purple	–	–	–
Parietin	Red purple	–	–	–
Skyrin	Red purple	–	–	–
Triterpenoids				
Zeorin	–	–	–	–
Pulvinic acid derivatives				
Calycin	–	–	–	–
Rhizocarpic acid	–	–	–	–
Vulpinic acid	–	–	–	–

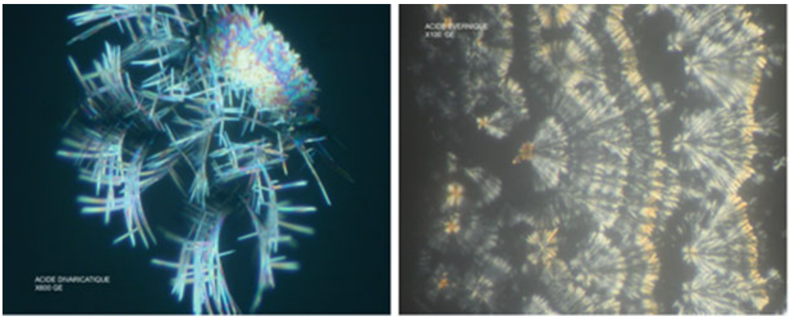


Fig. 11.6 Microcrystallizations of secondary lichen metabolites: divaricatic acid and evernic acid (both crystallized in GE from an acetone lichen extract then magnified 100-fold and observed through polarized light) (pictures from Jacques Lagabrielle)

of temperature. Decomposition of lichen substances may be accompanied with characteristic odors that can differ between aliphatic molecules, phenolic compounds, or triterpenes. Obviously, melting points can only serve as a hint to further confirm structural elucidation granted by other means. One could find a list of lichen substances' melting points in Hüneck and Yoshimura's reference book (Huneck and Yoshimura 1996).

11.3 Structure-elucidating Methods

General chemical procedure to study secondary metabolites is done from lichen extracts in organic solvent and further purification through crystallization or chromatography separation. Isolated compounds are then analyzed in most cases by NMR, MS, IR, and UV spectroscopy plus stereochemistry studies, if required.

11.3.1 NMR Spectroscopy (NMR, MAS-NMR, Innovative Methods)

As for any organic compound, NMR is an essential method for the structural determination of lichen secondary metabolites. Structural determinations for lower milligram samples could be made with data obtained in proton and carbon NMR. The assignments of signals can be achieved via crosspeak correlation observed in homonuclear 2D spectra of scalar couplings (COSY, TOCSY …) or dipolar couplings (NOESY, ROESY) and heteronuclear ^1J type (HSQC/HMQC …) and $^{2,3}J$ type (H2BC, HMBC …), 2D or 3D sequences to detect heteronuclear scalar couplings as HSQC-TOCSY, and HSQC-NOESY generally for complex structures.

However, most secondary lichen metabolites isolated so far belong to a limited variety of structural families as illustrated in Fig. 11.1. Some dedicated NMR studies related to several structural families were published: depsides (Cheng et al. 2013), tridepsides (Narui et al. 1998), depsidones and depsones (Sundholm and Huneck 1980, 1981), xanthones (Sundholm 1978, 1979), and triterpenes (Wenkert et al. 1978; Wilkins et al. 1990). A compilation of the published NMR data of lichen compounds is reported in the Huneck and Yoshimura (1996).

Solubilization of pure lichen compounds is often a challenge, but most of the NMR spectra are recorded after dissolution in $CDCl_3$ or DMSO-d_6. Some characteristic NMR features commonly found in NMR spectra of lichen compounds are selected here for monoaromatic or rearranged phenols corresponding to depsides, depsidones, depsones, or dibenzofuranes along with some NMR landmarks for aliphatic and related paraconic acids.

11.3.1.1 Phenolic Compounds

Chemical shifts are greatly influenced by inductive and mesomeric effects originating from other substituents that depend on their respective position. Some publications reported on the effect of substituents nature and respective position to predict corresponding chemical shift with the reference being the benzene chemical shift of 128.5 ppm (Ewing 1979). For orcinol, phenol-bearing carbons are deshielded (140–160 ppm) as well as the carbon that has two *meta*-hydroxy moieties (140 ppm), whereas ortho-hydroxy-located carbons are shielded (100–110 ppm). Aromatic methyls are usually found around 20 ppm. Noteworthy, for the closely related β-orcinol, the second aromatic methyl can be easily distinguished since it is strongly shielded due to two ortho-hydroxy groups (8 ppm). Side chains are evidenced through a variable number of signals arising in the 10–40 ppm range (divarinol, olivetol). Carbonyl functions are easily observable in ^{13}C NMR where shifts around 170–200 ppm have to be distinguished between a methyl ester from an acid function, e.g., methyl orsellinate at 170.3 ppm compared to orsellinic acid at 173.3 ppm, while shifts of aldehyde function are found around 194 ppm as of haematommic acid.

Depending on the substitution on the aromatic ring, ^1H NMR spectrum shows sometimes only one aromatic signal (singlet) due to the symmetry of the molecules with a signal at 6.09 ppm in ^{1}H NMR and at 106.7 ppm in ^{13}C NMR (β-orcinol). According to moieties present on the aromatic ring, other signals are methyl groups at 2.14 ppm (orcinol), 2.08 and 1.89 ppm (β-orcinol), 2.47 and 2.00 ppm (orsellinic acid), variable hydroxyl and acid shifts, or alkyl chains between 0.85 and 2.47 ppm as the pentyl moiety of the olivetol. Signals can also be found at 3.82 ppm indicating a *O*-methyl group (4-*O*-methylolivetolcarboxylic) or at 3.92 ppm for acid or ester (methyl-β-orcinolcarboxylate). A signal around 10.3 ppm is indicative for an aldehyde function (haematommic acid).

11.3.1.2 Dibenzofuranes

Most true dibenzofuranes exhibit two aromatic protons (6.6 and 6.9 ppm) while related compounds such as the commonly found and related usnic acid display a shielded aromatic signal at 5.92 ppm. Additional ^{1}H NMR signals correspond to the substitution of the dibenzofuran skeleton by a variety of substituents such as methyl, alkyl chain, hydroxyl, acid, ester, or ketone. The ^{13}C NMR of the aromatic ring signals is observed between 98.3 and 179.4 ppm, and those corresponding to the ketone function are at around 200 ppm and to methyl groups between 7.7 and 32.0 ppm (usnic acid).

A fourth cycle from a gamma-lactone (e.g., strepsilin) or a delta-lactone (e.g., haemophaein) may also occur with a ^{13}C NMR chemical shift around 170 ppm. Such an additional ring, which may be substituted by methyl (e.g., alectosarmentin), hydroxyl groups, and/or alkyl chains (e.g. haemophaein), is also found for some depsides and depsidones.

11.3.1.3 Depsides, Depsidones, and Depsones

Depsides are phenolic compounds bounded by ester linkage of two units (didepside), three units (tridepside), or four units (tetradepside). Barbatic acid, a didepside, shows two signals in ^{13}C NMR at 171.4 and 174.8 ppm for ester and acid function, respectively. Aromatic rings may be substituted by different groups: hydroxyl, aldehyde, methoxy, methyl, saturated or unsaturated alkyl chain with a ketone function, and halide (chlorine or bromine). Aromatic methyl groups' chemical shifts range from 7 to 30 ppm according to the neighboring substituents as previously discussed. Methoxyl groups can be easily recognized with chemical shifts ranging from 50 to 60 ppm, methylenic groups of alkyl chains have a chemical shift between 26 and 40 ppm, and methyls are encountered around 15 ppm. Noteworthy, one may recognize patterns of signals corresponding to the constitutive monomers within some depsides (e.g., isolecanoric acid and orsellinic acid, divaricatic acid and divaricatinic acid). Tri- and tetra-depsides obviously contain more carbon signals and can thus be easily distinguished from classical didepsides.

Benzyldepsides have an additional characteristic chemical shift at 5.60 ppm in ^{1}H NMR and 66.0 ppm in ^{13}C NMR of the methylenic group (alectorialic acid) due to a strong deshielding of the ester function and the aromatic ring in *alpha* position.

Depsidones are characterized by an ether linkage bridging the two aromatics moieties through a third ring including the C_7 carbonyl of the ether linkage which is shielded around 161 ppm while found around 169 ppm in depsides. The number of aromatic protons and substituents is consecutively reduced with regard to depsides, but substitutive patterns are often similar.

Depsones are usually picrolichenic acid derivatives which are cyclized depsides by a C_1–C_5 bond thereby forming a gamma-lactone with a signal at 170 ppm in ^{13}C NMR. A quaternary C_1 at 59 ppm and a cyclic ketone at 188 ppm are in most cases accompanied with signals of two alkyl chains.

11.3.1.4 Aliphatic Acids and Paraconic Acids

Aliphatic acids contain a long carbon chain whose chemical shifts are comprised between 10 and 40 ppm in ^{13}C NMR, and a variable number of carboxylic acid moieties located around 180 ppm. Identification of carbons belonging to the carbon chain is tricky since most of them are very close to one another. Double bond-containing compounds display a distinct chemical shift around 130–140 ppm (lichesterylic acid, fumaric acid), whereas molecules containing hydroxyl or methoxyl groups possess chemical shifts in the 60–80 and 50 ppm range, respectively.

Such aliphatic acids can cyclize, thus forming a gamma-lactone ring. The carbon bearing the lactone function displays a typical ^{13}C NMR chemical shift located between 174 and 178 ppm, whereas the carbon linked to the oxygen of the cycle is slightly deshielded at around 50 ppm. NMR is useful to characterize the length of the alkyl chain in C_{11} (nephrosterinic acid), C_{13} (lichesterinic acid), or C_{15} (muronic acid) as well as the substitution by a ketone (220.3 ppm in ^{13}C NMR, muronic acid), hydroxyl function (68.4 ppm in ^{13}C NMR, neuropogolic acid), a terminal acid group (168 ppm in ^{13}C NMR, protopraesorediosic acid), or acetoxy group (174.7 ppm in ^{13}C NMR, 19-acetoxylichesterinic acid). α, β-unsaturated-γ-lactones (e.g., neuropogolic acid) give signals in the range of ethylenic carbons (120–160 ppm) and deshields neighboring carbon (commonly found at 80 ppm). γ-lactones displaying an exocyclic methylenic function (e.g., protolichesterinic acid) are more shielded (125 ppm) than those comprising the double bond inside the lactone ring (135–145 ppm) with two characteristic signals in ^{1}H NMR at 6.39 and 6.03 ppm as doublets (J 3 Hz).

The gamma-lactone cycle may be substituted by a methyl group and an acid function as respectively observed at 14.4 and 177.3 ppm for (−)-dihydropertusaric acid. Structure of unsaturated lactones like isomuronic acid with two ethylenic carbons at 139.0 and 146.6 ppm in ^{13}C NMR or of lactones with an exocyclic ethylenic group can be resolved with NMR ^{1}H NMR characteristic signals (6.03 and 6.39 ppm as two doublets (J 3 Hz) in ^{1}HNMR and signals at 125.9 and 132.6 ppm in ^{13}C NMR).

Solid-state MAS-NMR (magic angle spinning) techniques enable monitoring the chemical composition in the solid state of intact biological samples (Miglietta and Lamanna 2006).

Identification of molecules from 5 *Cladonia* lichens, hardly differentiated on the basis of botanical characters, could be achieved by comparison of the ^{13}C CP-MAS-NMR (CP: Cross Polarization) spectrum of solid crushed lichens with the reference powder spectra of the three major secondary metabolites (fumarprotocetraric acid, perlatolic acid, and usnic acid) that enabled attributing the chemical content to different unambiguous NMR signals (Chollet-Krugler et al. 2008).

Likewise, ^{1}H HR-MAS-NMR (HR standing for high resolution) provided specific fingerprints that could be reliably assigned to different carbohydrates detected from several intact lichens (Alcantara et al. 2007).

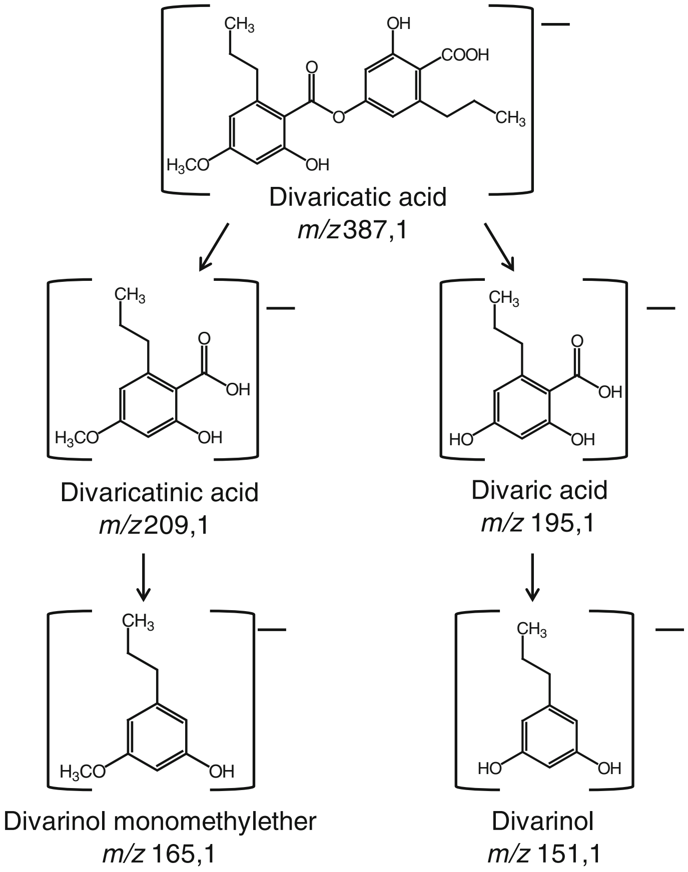
Recent NMR studies describe ^{1}H NMR spectra to achieve analysis under metabolomic conditions. Overall spectra from complete extract, with no further workup, display specific fingerprints enabling direct characterization of major compounds. Eisenreich et al. (2011) submitted methanol-D4 extract of chlorolichen *Xanthoria parietina* and cyanolichen *Peltigera horizontalis* to ^{1}H NMR. Those spectra comprise lichen-specific features as well as strong signals standing for mannitol, while those corresponding to sucrose are missing. Such methods seem interesting for both comparative studies (developing fingerprinting for given lichen species) or to assess metabolite modulations in response to environment changes (see Sect. 11.4).

11.3.2 Mass Spectrometry

The formula of an organic compound can be calculated from HR mass spectrometry data. Fractionations and skeletal rearrangements are also of particular significance in the application of mass spectrometry to structure elucidation. As an example, the fractionation of divaricatic acid is given below (Fig. 11.7).

Mass spectrometry also provides characteristic peaks regarding signature elements or specific

Fig. 11.7 Fractionation of divaricatic acid in negative-mode electrospray mass analysis



organic moiety, which hints regarding chemical structure of analyzed compounds. For instance, substances containing chloride give characteristic isotope pattern in their mass spectrum, indicating the number of chlorine atoms per molecule.

Huneck et al. (1968) first reported on mass spectra in both positive and negative mode corresponding to numerous depsides, depsidones, depsones, dibenzofuranes, and diphenylbutadienes. This paper described for the first time the main fragmentation route of depsides, consisting in the cleavage of its ester bond, enabling structural assignments to the carboxylic acid or phenolic parts of the molecule (Huneck et al. 1968). The specific cleavage of the ester bond as a typical process of didepsides can be followed by loss of water as the result of *ortho* interaction of the 2-hydroxybenzoic acid residue (Elix and Norfolk 1975; Addison 1985; Dmitrenok et al. 1987; Holzmann and Leuckert 1990). The classical fragmentation patterns of depsidones include neutral loss of CO_2 on their central ring to yield a dibenzofurane moiety alongside different elimination processes depending on the nature of the substituents (Parrot et al. 2013). Mass spectrum behavior of dibenzofuran and usnic acid derivatives was thoroughly discussed (Kutney et al. 1974; Schmidt et al. 1981). Hüneck and Schmidt (1980) described the fragmentation behavior of 16 usnic acid derivatives and established elimination mechanisms using deuterated analogues in both positive and negative modes. Characteristic rearrangements were also reported with some pulvinic acid derivatives (Letcher and Eggers 1967), polyporic acid derivatives (Grigsby et al. 1974), di- and tri-oxygenated stictane triterpenoids, and their trimethylsilyl derivatives (Holland and Wilkins 1979).

Non-hyphenated mass spectrometry was also applied to the screening of lichen compounds. Santesson developed a special technique, the so-called lichen mass spectrometry by introducing small lichen samples (sometimes less than 50 ng) into the direct inlet system of a mass spectrometer aiming to study xanthone pigments. The xanthones sublime as the temperature is increased (150 °C) under very low pressure, and the mass spectrum is recorded. Xanthones generally give prominent molecular ions, and the spectrum of the mixtures is often seen as additive of the individual components. However, this method remained limited to lichens containing only a few compound, otherwise giving spectra that are too complex to be interpreted (Santesson 1969).

Other mass spectrometry techniques relied on combination of soft-ionization negative-mode fast atom bombardment yielding intense $[M\text{-}H]^-$ ions that were subsequently analyzed by MS/MS. This enabled investigation of individual $[M\text{-}H]^-$ ions originating from crude extracts of lichen material and the selective study of the structure of components (Holzmann and Leuckert 1990). Tabacchi et al. (1991) also directly analyzed *Pseudevernia furfuracea* and *Lobaria pulmonaria* by tandem MS.

Recently, NMR and HRMS are the most commonly used structure-elucidating methods. Hyphenations of mass spectrometry with chromatographic techniques will be developed in Sect. 11.3 on dereplicative tools as well as recent innovations in the field of ambient and imaging mass spectrometry.

11.3.3 Infrared Spectroscopy

Like Raman spectroscopy, infrared spectroscopy is a technique based on the vibrations of the atoms of a molecule. The infrared spectrum is obtained by passing infrared radiation through a sample, in virtually any physical state, and further determining what fraction of the incident radiation is absorbed at a particular energy. Thus, infrared spectroscopy gives ability to associate patterns of absorption peaks with their corresponding functional groups (Stuart 2004). For example, carboxylic acid might be identified by a broad peak centered around 3,000 cm^{-1}, a strong peak around 1,720 cm^{-1}, and a broad medium intensity band around 920 cm^{-1} (Woodruff and Munk 1977). One might find pattern of absorption peaks corresponding to a wide variety of functional groups in Silverstein et al. (1998).

Infrared spectrometry is part of the classical set of structure-elucidating methods and IR data are thus most of the time available in Huneck and Yoshimura (1996).

Further enhancements in infrared spectroscopy now enable collecting space-resolved information, which has been discussed in detail in the Sect. 11.3.

11.3.4 UV Spectroscopy

As previously described, lichens are miniature ecosystems that can survive under very harsh conditions. Therefore, these stress-resistant organisms developed adaptive mechanisms to cope with such high light exposure including synthesis of UV-protectant metabolites (Nguyen et al. 2013). As most lichen compounds have chromophores, UV is useful for their detection and identification but remains most of the time associated with TLC or HPLC (see further).

Yoshimura et al. (1994) first divided lichen secondary metabolites into three groups according to their UV spectral pattern.

- depsides and monocyclic compounds
- depsidones
- dibenzofurans, pulvinates, xanthones, and anthraquinones exhibit typical UV spectra and are therefore easy to distinguish.

Then, mycosporines, more recently reported in lichens (Torres et al. 2004), are characterized through their UV spectrum absorbing in the 310–365 nm range with a strong molar extinction coefficient and a symmetrical shape. Detection through analytical techniques hyphenated with a PDA spectrophotometer offers a simple and easy method to screen them from lichen extracts

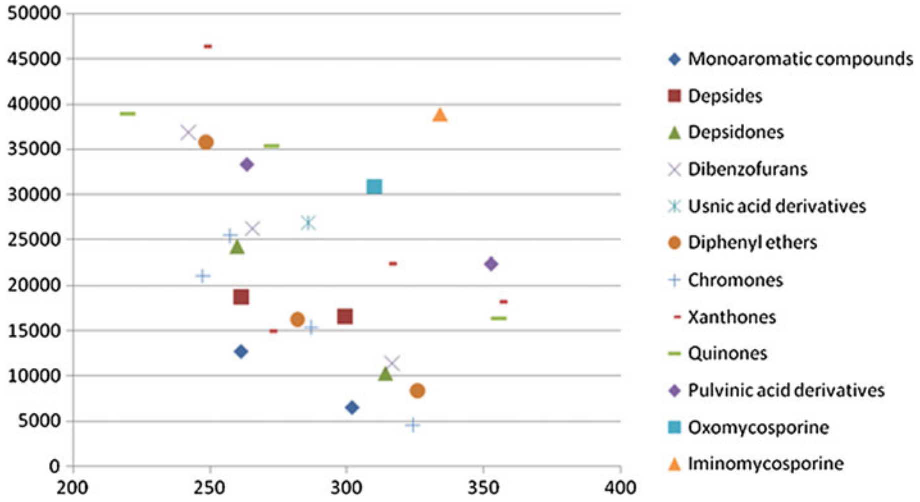


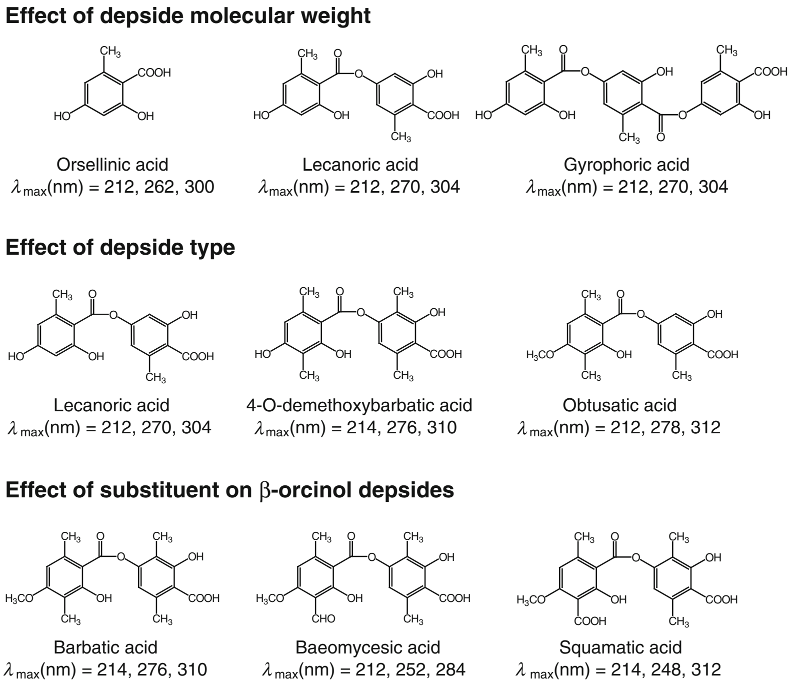
Fig. 11.8 UV properties of main structural families met within lichens (for quinones, additional absorptions are observed in visible range with medium λ_{max} of 458 and 529 nm) [adapted from Huneck and Yoshimura (1996)]

(Roullier et al. 2011). As an attempt, UV data of most lichen structural families are visualized in the Fig. 11.8:

Some relationships between chemical structures and UV spectra of depsides have been considered (Fig. 11.9) (Yoshimura et al. 1994).

First, it was noted that molecular weight affects UV spectra. For instance, comparison of orsellinic acid with lecanoric and gyrophoric acids, containing two and three orsellinic acid units, respectively, showed quite a similar spectrum, but both lecanoric and gyrophoric acids

Fig. 11.9 Some relationships between chemical structure and UV features of depsides [adapted from Yoshimura et al. (1994)]



displayed bathochromic shifts of long wavelength bands and lower absorbance. Moreover, monocyclic compounds (except 2-*O*-methyl derivatives) have broader UV second bands ($\lambda_{max} \sim 260$ nm).

Spectral differences were also linked to the depside type. Indeed, depsides are classically divided into three groups: orcinol type (e.g., lecanoric acid), β-orcinol type (having C_1 unit at 3 position of benzene ring, e.g., 4-*O*-demethylbarbatic), and mixed β-orcinol/orcinol type (e.g., obtusatic acid). Attachment of the methyl moiety has an influence on UV spectra as the second band of 4-*O*-demethylbarbatic acid and obtusatic acid is bathochromically shifted (+10 and +20 nm in comparison with lecanoric acid, respectively).

Influence of substitutions of β-orcinol depsides has also been studied. β-orcinol depsides may have CH_3, CH_2OH, CHO, or a COOH substituent at the 3 position of a benzene ring. Using barbatic acid (CH_3), baeomycesis acid (CHO), and squamatic acid (COOH), it was demonstrated that changing from CH_3 to CHO or COOH allows conjugation with the other ring and thus complicates the corresponding UV spectrum.

Hydroxyl group at the 2 position of depsides and monocyclic compounds is seldom methylated. Such 2-*O*-methylated compounds have shorter wavelength and lower intensity for their second band in comparison with their 4-*O*-methylated derivatives.

Methylation and alkyl chain length modifications of depsides do not affect their UV spectrum.

11.3.5 Stereochemistry Determination

Interests in determining the absolute stereochemistry of a chiral organic molecule originate from the widely admitted fact that stereochemistry determines important features regarding both chemical, biological, and physical properties.

X-ray crystallography remains the primary method to determine the absolute configuration of a molecule and originates from crystallographic studies that grant unambiguous, accurate, and reliable three-dimensional structural parameters (Deschamps 2010).

In a number of aromatic lichen substances, fully unsaturated structures do not justify the measurement of molecular optical activity. However, numerous lichen substances are optically active. Most associated optical rotations could be found in reference books (Huneck and Yoshimura 1996) and generally expressed through $[\alpha]^{DT}$ values according to the Biot formula. Nevertheless, accurate measurements require full solubilization and solutions in g/100 mL which are sometimes difficult to achieve.

Alternatively, optical rotatory dispersion (ORD) corresponding to the variation of circular birefringence as a function of wavelength (Brittain 1998) has been determined for lichen products, including different classes of compounds: macrolactone glycosides (Rezanka and Guschina 2001a), aliphatic acid derivatives (Rezanka and Guschina 2001b), aliphatic lactones (Aberhart et al. 1970; David et al. 1990), triterpenoids (Huneck 1976), quinones (Ejiri et al. 1975), and carotenoids (Czygan 1976).

Circular dichroism is another option when experimental circular dichroism of the molecule can be compared to either (i) a set of experimental spectra from related compounds, (ii) data from literature or (iii) calculated theoretical curves (Berova et al. 2007). Circular dichroism can also grant information regarding conformational features and was applied to a wide array of lichen compounds including aliphatic acids (Bodo and Molho 1980), cycloaliphatic compounds (Huneck and Himmelreich 1995; Polborn et al. 1995; Aberhart et al. 1970; Huneck and Höfle 1980; Huneck et al. 1986; Huneck and Takeda 1992), quinones (Mathey et al. 1980), chromones (Huneck 1972), carotenoids (Czeczuga et al. 1988), and dibenzofuran-related compounds (Millot et al. 2013).

Assignment of absolute configuration by NMR represents another approach. Most of such NMR techniques rely on previous derivatization of the substrate prior to its analysis into two different species. This is performed with enantiomeric substances known as chiral derivatizing agents (CDA). Most used CDA is Mosher reagent (also known as methoxy trifluoromethyl phenyl acetic acid—MTPA) (Seco et al. 2004).

Some reports stand with application of Mosher derivatization to elucidate stereochemistry of lichen metabolites (Rezanka and Dembitsky 1999a; Rezanka and Guschina 2001a, b).

11.4 Dereplicative Tools

Over the last three decades, considerable attention has been paid to the development of hyphenated techniques, which combine different separation techniques (TLC, LC, GC) with structure-elucidating methods (mass spectrometry, NMR, UV/Vis spectroscopy).

11.4.1 Thin-layer Chromatography (TLC)

Study of lichen metabolites moved one step forward with the advent of commercially available TLC at the end of the 60s leading to a marked decrease of Asahina's crystal tests.

In 1952, Wachtmeister first introduced paper chromatography for the separation and characterization of lichen substances (Wachtmeister 1952, 1956). A few relationship between chemical structure and chromatographic behavior has been studied (Mitsuno 1953).

Ramaut (1963a, b) applied TLC in the field of lichenology to separate depsides and depsidones. Then, because different authors used different solvent systems and chromatographic conditions (Bendz et al. 1967; Santesson 1967a), procedured methods for routine identification of lichen products by TLC were first attempted by Santesson who reported R_f values of 80 lichen metabolites (Santesson 1967b). Culberson and Kristinsson (1970) first described a TLC-based method to cope with R_f variations ensuring higher reproducibility and associating other identification methods to curtain identification possibilities. Indeed, to overcome R_f varying problems, internal standards atranorin and norstictic acid were chromatographed alongside samples of interest, and 8 R_f classes were defined according to the relative positions of each spot compared with atranorin and norstictic acid. TLC was developed in three solvent systems ("A": benzene/dioxane/acetic acid 90/25/4; "B": hexane/ethyl ether/formic acid 5/4/1; "C": toluene/acetic acid 85/15). Further identification possibilities among compounds that share same R_f classes for both 3 solvents were further narrowed according to (i) reaction after spraying H_2SO_4 and heating (ii) color reactions (as previously described), (iii) appearance of the spot under visible light, short- and long-wave UV light, and (iv) any other pertinent data available as solubility in different solvents. One hundred and four compounds were listed into R_f classes according to this approach (Culberson and Kristinsson 1970) Table 11.2. Due to diethyl ether fast evaporation, it was later replaced in solvent system B by a higher boiling ether with nearly identical chromatographic properties: Methyl tertiary butyl ether (MTBE) and corresponding changes in R_f of lichen metabolites were also published (Culberson and Johnson 1982). Another solvent system (referred to as solvent G: toluene/ethyl acetate/formic acid 139/83/8) was later introduced to separate polar compounds such as β-orcinol depsidones (Culberson et al. 1981).

Further enhancements of this previous method were later published. In particular, it was reported that acidic hydrolysis (with concentrated sulfuric acid) of a number of compounds difficult to distinguish under their intact form might yield fragments that chromatograph very well in standard solvent systems and thus enable identification of the unhydrolyzed fragments (Culberson 1972). Another simple derivatization step

Table 11.2 Various reagents used to detect the lichen substance

Reagent	After treatment	Identified compounds	References
UV light (254, 365 nm)	None	Most lichen substances	
		Specific set of wavelengthes can enable detection of compounds with characteristic UV profiles, e.g., mycosporines 310 nm	
Iodine vapor	Place the chromatogram into a chamber in which some crystals of iodine have been placed. Iodine vapor is more quickly generated by gently warming the chamber	General detection reagent	Brante (1949)
		All lichen compounds, brown spots	Munier and Macheboeuf (1949)
Sulfuric acid	Spray the chromatogram with one of these reagents, Allow to dry for 15 min in the air and heat to 110 °C until maximal visualization of the spots	All lichen compounds, gray, brown or blue spots	Jones et al. (1963)
A: Mix equal parts of 95 % sulfuric acid and methanol with cooling			Metz (1961)
B: 5 % ethanolic solution of 95 % sulfuric acid			
C: 5 % solution of 95 % sulfuric acid in acetic anhydride			
D: Mix equal parts of 95 % sulfuric acid and glacial acetic acid			
Chlorosulfonic acid dissolve 5 ml chlorosulfonic acid in 10 ml glacial acetic acid with cooling	Heat 5–10 min at 130 °C	All lichen compounds, wide array of colors, terpenoids violet	Tschesche et al. (1961)
			Tschesche and Wulff (1961)
			Takeda et al. (1963)
Sulfuric anisaldehyde	Heat to 100–105 °C until maximum visualization of the spots	Depsides, depsidones, diphenylethers, monophenolic compounds, pink, red, blue-violet	Stahl and Kaltenbach (1961)
A: anisaldehyde + 1 mL sulfuric Acid + 8.5 mL methanol or B: 0.5 ml anisaldehyde in			Lisboa and Diczfalusy (1963)
50 ml glacial acetic acid and 1 ml 97 % sulfuric acid			
5 % p-phenylene diamine		Aromatic aldehydes, yellow to red	Huneck and Yoshimura (1996)
Ninhydrin	Heat at 110 °C until maximal visualization of the spots	Aminoacids, peptides, proteins, yellow (secondary aminoacids) to purple (primary aminoacids)	Patton and Chism (1951)
			Fahmy et al. (1961)
0.2 % ninhydrin in ethanol or 0.3 mg ninhydrin in 100 mL 1-butanol and add 3 mL glacial acetic acid			Friedman (2004)

(continued)

Table 11.2 (continued)

Reagent	After treatment	Identified compounds	References
0.5 % magnesium acetate in methanol	Heat 5 min at 90 °C	Anthraquinones glycosides and related aglycones (pink to violet)	Shibata et al. (1950)
0.2 % primulin solution	Dip and wait for 30 min	Lipids, aliphatic compounds. White fluorescent spots at 366 nm	
Sulfuric thymol	Heat 15 min at 120 °C	Sugars. Pink spots	Adachi (1965)
Dissolve 0.5 g thymol in 95 ml ethanol and add 5 ml 97 % sulfuric acid with caution			
Diazotized benzidine + NaOH (solution A: 2.5 g benzidine + 7 mL conc.HCl in 500 ml H2O; solution B: 50 g NaNO$_2$ in 500 mL H$_2$O; equal volumes of A and B are mixed immediately before the application of the reagent)	The reagent is stable for a few hours	Depsides, depsidones, monophenolic compounds, red	Sherma and Hood (1965)
	Colors can appear quickly or develop long after depending on the nature of the phenol		

described is *O*-methylation using diazomethane that can be used as another identification hint. Eventually, acidic hydrolysis of *O*-methyl derivatives can provide further evidence for structure identification and to establish the order of linkage of phenolic acid units in simple depsides. As a consequence, R_f of hydrolyzed and *O*-methyl derivatives began being compiled, while an increasing number of lichen metabolites was also referenced (Culberson 1972). A useful tool for separation of complex mixtures is two-dimensional TLC as successfully applied for the separation of β-orcinol depsides from *Parmelia loxodes* and *P. verruculifera* (Culberson and Johnson 1976). TLC separation of (+) and (−) usnic acid was enabled by reaction with brucine as performed by Bendz et al. (1967).

Later, the use of high-performance thin-layer chromatography (HPTLC) in screening lichen substances was developed. HPTLC is more sensitive, allows the running of more samples in a shorter period of time, and requires smaller amounts of solvent (Arup et al. 1993).

As similar R_f value does not mean single compound, therefore, reliability to only R_f value remains a challenging and risky task. However, standardized methods listed above enable collecting valuable clues regarding chemical structures and might curtain identification possibilities.

Image-giving format of open, stationary phase of TLC can lead to numerous hyphenations. In particular, TLC can grant valuable information dealing with bioactivity and thus guide fractionation to focus on biologically active compound. For instance, bioautography is a microbial detection method developed on a planar chromatography plate and designed to recognize compounds with antibacterial and antifungal properties (Choma and Grzelak 2011). Thus, TLC bioautography was used to assess antibiotic properties of metabolites from *Ramalina farinacea* (Tay et al. 2004), anti-*Helicobacter* activity from various species of lichens (Luo et al. 2011) as well as fungitoxic compounds from *Lobaria kurokawae* (Wang et al. 2009). Biochemical detection can also be used, and for instance, screening of radical scavenging molecules can be evaluated using TLC by spraying stable free radical DPPH, which has already been performed on several lichen extracts (Bhattarai et al. 2008).

To sum up, TLC was one of the way to perform multiple detection in situ for (i) physical detection, (ii) microchemical detection, and

(iii) effect-directed analysis (either biochemically or microbiologically). Recent innovations in TLC allow successful hyphenations with different kinds of spectroscopic tools enabling proper in situ identification. Without any doubt, most developed hyphenated techniques are those combining TLC with mass spectrometry either online (Morlock and Schwack 2010) or off-line (Cheng et al. 2011). However, associating TLC with Fourier transform infra-red (FTIR) or Raman spectroscopy remain possible approaches. Furthermore, development of zone-collecting techniques (Luftmann et al. 2007) permits selective desorption and collection of molecules from a single TLC spot and further achievement of any structure elucidation technique as NMR (Morlock and Schwack 2010) (Fig. 11.10).

11.4.2 High-performance Liquid Chromatography

HPLC has become an important and efficient tool for the separation and identification of lichen substances, especially for compounds that cannot be studied by gas chromatography (GC) either because they are not volatile enough or are too unstable at high temperatures. First attempts were undertaken for chemotaxonomy with reversed-phase columns using methanol–water–acetic acid solvent system (Culberson and Culberson 1978).

Although such isocratic methods gave excellent results, gradient elution methods were later developed that better fit with complex lichen extracts containing a wide range of hydrophobic compounds (Culberson and Elix 1989). Gradient

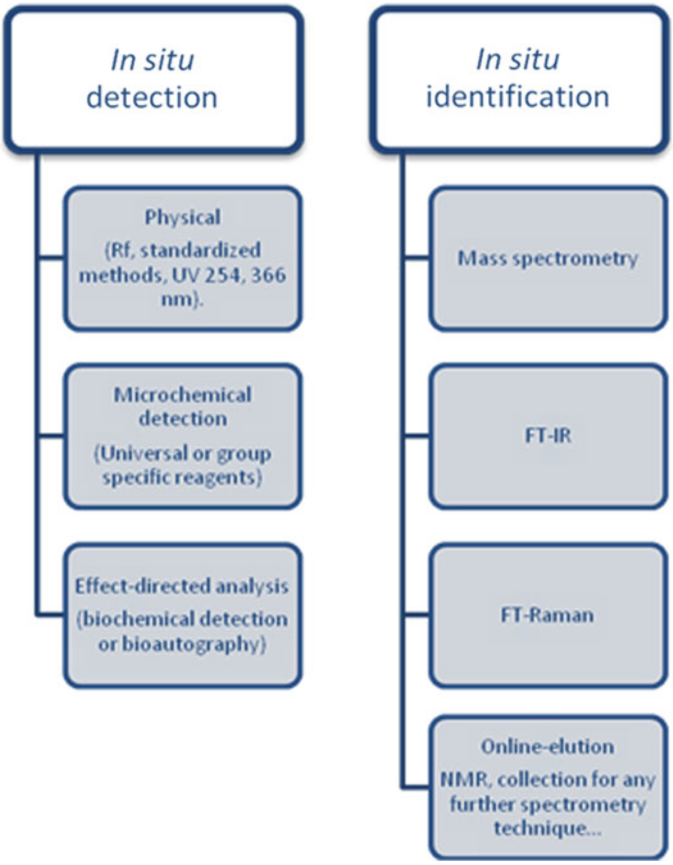


Fig. 11.10 Possible hyphenations with thin-layer chromatography [modified from Morlock and Schwack (2010)]

elution in lichenology was introduced by Strack et al. (1979) that separated 13 different lichen products (depsides, depsidones, dibenzofurans, and pulvinic acids) using a reversed-phase column with a linear gradient based on a water/methanol system. A first standardized method for separation of lichen aromatic products was described using different kinds of reversed-phase column on the basis of a methanol/orthophosphoric acid gradient system and including two internal standards: benzoic acid (low retention time) and bis-(2-ethyl-hexyl)phtalate (high retention time) (Huovinen 1987) according to which retention indices are defined ensuring better reliability. However, the latter standard was further replaced by solorinic acid (more hydrophobic) making the method suitable for the identification of lichen extracts containing chloroxanthones or long chain depsides as well. This second standardized reversed-phase HPLC method for identification of aromatic secondary metabolites from lichens was published by Feige et al. (1993) and is based on a retention index *I* calculated from the elution time of the appropriate peak compared with the standards benzoic and solorinic acids.

Such a relative index corresponding to 331 lichen compounds was determined by Feige et al. (1993), and this protocol was successfully applied to several genera of lichens (Lumbsch et al. 1993). Mietzsch et al. (1994) developed a computer program dedicated to lichen metabolites identification named Wintabolites. This software made use of HPLC-R_I values, TLC-R_f values, UV/visible colors of TLC spots, and lichen spot tests to identify molecules. The program database included 550 lichen compounds.

Derivatization techniques were further published to enable detection of aliphatic lichen acids (e.g., roccellic, rangiformic acids, lichesterinic acids) using the same method. Such metabolites are previously transformed into their phenacyl, para-nitrophenyl, or benzyl esters that display strong absorption in the UV (λ_{max} ca. 240 nm) (Huneck et al. 1994).

Later, hyphenation with photodiode array spectrophotometers (DAD), leading to complete UV–Vis spectra of corresponding peaks, greatly helped for the identification of molecules. Yoshimura et al. described detection of lichen metabolites according to their UV spectra features and relative retention time. HPLC was achieved using a reversed phase with an isocratic method ($MeOH/H_2O/H_3PO_4$). Retention times were expressed according to 2 standards: acetone and 1-phenyl-1-dodecanone.

However, relying on retention times only (even normalized with internal standard) and UV spectrum is not always sufficient to unambiguously identify a given compound. Nowadays, most liquid chromatography strategies rely on LC-DAD-ESI-MS that enable both (i) collection of UV–Vis spectrum and (ii) determination of the molecular weight associated with each peak of the chromatogram.

Hyphenated techniques are now commonly used as powerful tools of dereplication to focus on original or biologically relevant metabolites.

As an example, screening of mycosporines within different lichens was first based on their characteristic UV symmetrical shape while parallel ESI-MS helped focusing on undescribed molecules according to their molecular weight. Therefore, purification of a new molecule mycosporine hydroxyglutamicol from *Nephroma laevigatum* was achieved from 8 g of dried lichen material (Roullier et al. 2011). However, since the last standardized method described by Feige et al. and Yoshimura et al. in the nineties, no further publication updated extensively the behavior of lichen metabolites with hyphenated techniques such as LC-DAD-ESI/MS. Such a standardized method of reversed-phase LC-DAD-ESI/MS is described for the conspicuous reign of microfungi in which are described retention time, UV/Vis spectrum, and monoisotopic mass for 474 fungal metabolites (Nielsen and Smedsgaard 2003). Noteworthy, this database includes some fungal metabolites that can also be met within lichens (e.g., emodin, lichexanthone, skyrin …).

As previously discussed, NMR is arguably the most versatile analytical platform for complex mixture analysis (Corcoran and Spraul 2003). Indeed, successful interfacing of high-pressure liquid chromatography with parallel NMR and mass spectrometry were reported, yielding extensive structural data on each chromatographed compound (Exarchou et al. 2005). Wolfender et al. (2001) stated that coupling of HPLC with NMR is the one of the most powerful methods for the separation and structural elucidation of unknown compounds in mixture. However, to the best of our knowledge, no LC-NMR analysis was reported yet in the field of lichenology.

11.4.3 Gas Chromatography

GC found some limited applications in lichenology, since most lichen substances are polar and present low volatility. To cope with this problem, prior derivatization is usually required in order to yield volatile enough compounds to be analyzed by GC, most of the time as their trimethylsilyl, acetyl, methyl, or trifluoroacetyl derivatives. Derivatization procedures have been described by Nishikawa et al. (1973).

Using this method, Ikekawa et al. (1965) separated trimethylsilyltriterpene derivatives belonging to ursane, oleanane, and lupane groups (including compounds found within lichens as friedelin, taraxerol, taraxerone, and zeorin among others). Different C_{27}, C_{28}, and C_{29} sterols from the moss tree lichen *Pseudevernia furfuracea* were also characterized and quantified by GC-MS (Wojciechowski et al. 1973) after acylation from low polar organic solvent extractions. A few studies described GC analyses carried out on other structural families among which Furuya et al. (1966) demonstrated that retention time increases with the number of hydroxyl on the anthraquinone skeleton.

Low molecular weight carbohydrates from eight species of lichens were analyzed and quantified by GC as their acetyl, trifluoroacetyl, and trimethylsilyl derivatives in comparison with standard specimens. Arabitol, fructose, glucose, glycerol, erythritol, mannitol, sucrose, and trehalose were commonly found within tested samples alongside more specific sugars (as umbilicin, among others) (Nishikawa et al. 1973). Later publications reported on GC-MS characterization of *Ramalina celastri*'s mono and polysaccharides as their acetate derivatives (Stuelp et al. 1999). In the same way, acetylated saccharides from *Sticta* sp. were analyzed by GC-MS (Corradi da Silva et al. 1993) as well as carbohydrates from *H. physodes* and *Platismatia glauca* (Dahlman et al. 2003).

Fatty acid compositions of different *Parmelia* species were examined by capillary GC-MS. This enabled detection of 68 fatty acids as their methyl ester, trimethylsilyl, or oxazoline ethers (Dembitsky et al. 1992c). Fatty acid and phospholipid compositions were also reported within lichens growing in the Volga river basin using GC-MS (Dembitsky et al. 1992a), in the Tian Shan Mountains (Rezanka and Dembitsky 1999b), with different species of *Cladonia* (Dembitsky et al. 1991) or different lichens belonging to Lecanorales (Dembitsky et al. 1992b). More recently, fatty acid components—either free or combined—of the tropical lichen *Teloschistes flavicans* were analyzed by GC-MS of derived methyl esters and compared to those of separately cultivated symbionts. It was evidenced that the fatty acid compositions of the whole lichen strikingly differ from those of isolated symbionts. Moreover, changes were also observed according to the temperature. Likewise, capillary GC-MS demonstrated that seasonal changes also occurred in the fatty acid composition of two species of *Physcia* as well as in *Xanthoria parietina* (Bychuk 1993; Dembitsky et al. 1994).

In some cases, GC-MS analyses were performed for biomonitoring purposes as lichens are known to accumulate some given pollutants in their tissues (especially epiphytic lichens). Therefore, GC-MS air pollution assessment techniques were applied to detect polycyclic aromatic hydrocarbons within different Pyrenean lichens (Blasco et al. 2008) or within Italian alps-dwelling lichens (Nascimbene et al. 2014).

11.4.4 In Situ Detection

As discussed earlier, having access to the distribution of secondary metabolites could help understanding both fundamental facts about the symbiosis itself and enlightening relationships existing between the lichen and its environment. In many lichens, some metabolites are only present or occur in higher concentration in reproductive compared to somatic structures, giving support for optimal defense theory in lichens (Hyvärinen et al. 2000). Accordingly, it was shown that grazing pattern of snails inversely reflects the partitioning of the secondary compounds that have a documented deterring effect, avoiding reproductive parts of the lichen due to internal defense allocation (Asplund et al. 2010). Moreover, in situ characterization of compounds has always been considered as a very interesting approach since it eliminates complications and artifacts caused by time-consuming isolation procedures that are still required when structural analysis is achieved using conventional spectroscopic methods (Mathey et al. 1987).

11.4.4.1 Histochemical Reactions

First attempts of mapping metabolites' distribution within lichens used spot tests as described above. Indeed, specific pattern of distribution of some metabolites make thalline reactions organ specific (Kirschbaum and Wirth 1997). Honegger (1986) described ultrastructural studies of various *Parmeliaceae* using enhanced scanning electron microscopy sample preparation techniques to avoid dissolution of lichen substances. SEM images showed varying amounts of crystals covering the surface of both fungal and algal cells within different parts of the lichen thallus. Moreover, histochemical reactions (K, C, KC, PD) performed with thallus fragments or cryostats allowed localizing different secondary metabolites within the different layers of the thallus. The presence of mycobiont-derived crystalline lichen products on the surface of algae raises questions. One possible reason may be that such substances might slow down the metabolism of the photobiont to keep an harmonious growth pattern with a mycobiont whose growth abilities are limited (Kinraide and Ahmadjian 1970; Honegger 1986).

11.4.4.2 Laser Microprobe Mass Spectrometry

Along with thalline reactions, the first general method used to in situ analyze lichen substances on a microscale was laser microprobe mass spectrometry (LMMS also known as LAMMA) (Mathey 1981). Physical principle of this mass spectrometry method relies upon the irradiation of inorganic or organic samples with pulsed power UV laser under vacuum. Resulting ions are further analyzed using a time of flight or Fourier transform mass spectrometer (Van Roy et al. 1996). Microscale detection is enabled thanks to a concomitant light microscopic observation of thin cryosection that allows to focus laser beam on any sample detail (Wink et al. 1984). UV irradiation enables selective desorption and ionization of products with a chromophoric group. Indeed, a severe limitation of LMMS is its requirement for UV absorbing moieties.

As most lichen compounds have conjugated rings, this analysis method was the first showing ability to correlate analysis to given structures or substructures of the microscopical image. A large variety of lichen metabolites (especially lichen pigments) sublime when submitted to very low pressure, such as the vacuum existing in LAMMA-MS, especially lichen pigments (Mathey 1981). In such conditions, a mild elevation of temperature leads to vaporization of lichen metabolites that further yield mass spectra displaying very prominent parent ions, often corresponding to the base peak of the spectrum.

The tropical lichen *Laurera benguelensis* is characterized by yellow thallus warts that present a marked yellow fluorescence under UV radiation, originating from microcrystals located in the cortex (Mathey 1979). LAMMA spectrum obtained from single microcrystals displayed a characteristic lichexanthone profile while physcion and xanthorin, orange anthraquinone pigments, were distinctly found in the warts containing the fruiting bodies in additional *Laurera* species (Mathey et al. 1980, 1987, 1994).

LMMS was also successful in detection of a variety of lichen pigments belonging to different structural families (Mathey et al. 1987; Van Roy et al. 1996). However, LAMMA analysis only grants low mass resolution. Hence, unambiguous detection of compounds requires additional data from either NMR or HR mass spectroscopy.

11.4.4.3 Raman and FTIR Spectroscopy

First developed Raman analyses mostly dealt with biodeterioration of monuments and frescoes due to oxalic acid produced by encrusting microlichens, aiming at characterizing the physical and chemical nature of the lichen/substratum system (Edwards et al. 1991). Further works then moved to ecophysiological studies of lichen itself.

In situ Fourier transform Raman spectroscopy was used for identification of pigments and biodegradative calcium oxalate directly within thalli of viable epilithic lichens (*Acarospora* spp.) harvested in contrasted sites from Antarctic and Mediterranean (Holder et al. 2000). Raman-specific fingerprints could be used to recognize two photoprotective pigments: rhizocarpic acid and β-carotene. Near IR excitation (1,064 nm) eliminated possible fluorescence originating from pigments and insensitivity of Raman techniques to water enabled analysis of field fresh samples with a spatial resolution of about a 20 μm spot diameter. Hydration states of calcium oxalate could be differentiated with different signals between monohydrate (whewelite) and dihydrate (weddelite). Edwards et al. (1998) compared parietin contents of *Xanthoria* lichens harvested in Antarctic and temperate habitats using Raman spectroscopy. Other publications reported on scytonemin detection by Raman spectroscopy from cyanobacteria (*Nostoc* sp., *Chlorogloeopsis* sp., *Scytonema* sp., *Lyngbya* sp.) or from cyanolichen *Collema* species (Wynn-Williams et al. 1999).

Raman spectra of lichen samples colonizing active volcanic environment (Kilauea volcano, Kona, Hawaii) were acquired to decipher biomolecular protective strategies adopted in such extreme environments (Jorge-Villar and Edwards 2010). Raman profiles were acquired from 16 lichen specimens belonging to eight genera, growing on basaltic lava or on wood substrates. It was shown that chlorophyll and a carotenoid (most likely lutein or astaxanthin) were ubiquitous, but a wide range of protective pigments could be identified as atranorin, gyrophoric acid, parietin, pulvinic acid dilactone, and usnic acid (as well as calcium oxalate mono- and dihydrate in some cases) using specific patterns (Edwards et al. 2003a, b).

Lecanoric acid was characterized by FT-Raman spectroscopy in *Parmotrema tinctorium*, highlighting reliability of Raman spectroscopy for in situ detection of this metabolite from the whole lichen (de Oliveira et al. 2009).

FT-IR spectromicroscopy fits well to the examination of the conspicuous reign of filamentous fungi providing spatially resolved information on cellular biochemical content (Szeghalmi et al. 2007; Jilkine et al. 2008; Isenor et al. 2010).

More recently, Raman spectroscopy and FPA-FTIR were used to image the distribution of usnic acid within *Cladonia arbsucula*, *C. sulphurina,* and *C. uncialis* (Liao et al. 2010). This dibenzofuran-related metabolite was shown to be present in pycnidia and younger branches of *C. arbuscula* and *C. uncialis*, in *C. sulphurina*'s soredia as well as in the spore-forming region of *C. uncialis*' apothecia.

11.4.4.4 Ambient Mass Spectrometry

Recent technical innovations in mass spectrometry now allow detection of molecules in ambient conditions dealing with a large number of compounds in complex matrices and play an important role in various fields such as drug discovery, doping control, forensic identification, and food safety. Open-air sources especially revolutionized the way samples are introduced into the ion source for mass analysis and result in rapid acquisition, without special sample preparation enabling quick and sensitive dereplication. Among these open-air sources is especially worth quoting direct analysis in real-time mass spectrometry (DART-MS) that is based on the interactions of a sample in a water-containing atmosphere with a gas in a metastable state heated to a temperature up to 350 °C (Cody et al. 2005). DART's detection limit is comprised in the picomolar range and showed satisfying results for chemical fingerprinting of many plants (Kim and Jang 2009). Moreover, a number of lichenic compounds were shown to accumulate in upper cortex (Ozenda and Clauzade 1970). Such a distribution in the most superficial layers enables their detection using ambient mass spectrometry techniques as shown on the cyanolichen *Lichina pygmaea* using DART-MS (Le pogam et al. 2014).

Ambient mass spectrometry methods have to be tried with lichens to measure defense reactions as responses to environmental challenges as successfully applied to the red algae *Callophycus serratus* using DESI *imaging* (Lane et al. 2009). Other DESI imaging experiments reported on hydrolysis of hydroxynitrile glucosides along leaves wounds of *Lotus japonicus* (Li et al. 2013).

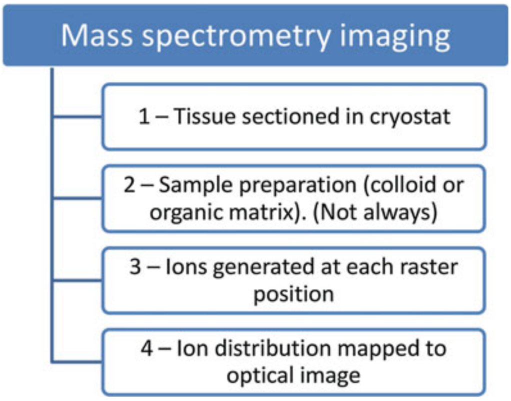


Fig. 11.11 Schematic outline of the workflow in a typical imaging mass spectrometry experiment [adapted from Greer et al. (2011)]

11.4.4.5 Imaging Mass Spectrometry

Imaging mass spectrometry allows to monitor the spatial distribution and abundance of metabolites. Experimental scheme for mass spectrometry imaging experiments can be defined as follows (Fig. 11.11). IMS involves rastering a laser or any other ionization source across a thin section of biological tissue by moving the sample stage in predefined *x*–*y* coordinates to generate thousands of position-dependent mass spectra. Sample preparation may be assisted to aid the ion ionization or can remain unassisted depending on both the mechanism of the ion source and the nature of the compounds to be analyzed. Then, data processing is accomplished by displaying ions of interest from a traditional *m*/*z* plot to a false color image eventually superimposed on a photograph of the analyzed sample (Greer et al. 2011).

Recently, three-dimensional imaging of metabolites in plant tissues was achieved thanks to LAESI-MS. LAESI-MS relies on tissue ablation with a single laser pulse of 2.94 µm wavelength. O–H vibrations of the native water molecules of the tissue samples absorb the laser pulse energy, leading to ejection of microscopic volumes of the most superficial layers of the sample (as neutral particulates and molecules) that enables establishing depth profiles. This plume is further intercepted by an electrospray that postionizes the ablated material (Nemes and Vertes 2012). 3D-LAESI-MS revealed specific distribution pattern of several molecules within the plant *Aphelandra* in correlation with tissue specificity (Nemes et al. 2009). Common ionization methods applied in imaging mass spectrometry are listed above (Table 11.3).

Although achieved on a wide range of organisms [for review see Esquenazi et al. (2009)], no ambient or imaging mass spectrometry was published until now on lichens to the best of our knowledge.

11.5 Deciphering Metabolic Networks and Partners' Cooperation at the Very Heart of Symbiotic Systems

Systematic analysis of the metabolite composition caused by cellular reactions is called metabolomics (Oliver et al. 1998). A metabolomic approach was developed on *Xanthoria elegans* in 2007 (Aubert et al. 2007). In this study, analysis

Table 11.3 Characteristics of most commonly used imaging mass spectrometry sources

Ionization source	Probe beam	Pressure regime	Spatial resolution (μm)
MALDI	Laser beam	Vacuum (most of the time) or ambient (AP-MALDI)	10–100
SIMS	Ion beam	Vacuum	0, 2–3
DESI	Solvent stream	Ambient	200
NIMS	Laser beam	Vacuum	15–20
LAESI	Infrared laser + electrospray	Vacuum	Lateral: 300–500
			Depth: 30–40

Adapted from Lee et al. (2012), Greving et al. (2011)

of polar metabolites was achieved from crude perchloric acid extracts using ^{13}C and ^{31}P NMR spectroscopy to compare chemical profiles of both dry and wet thalli. Such analysis methods afforded the detection of 30 metabolites from the raw extract with no further sample workup (mostly sugars, sugar phosphates, polyols, phosphoenolpyruvate, and nucleotides). The data implied that metabolite composition was affected by stress conditions. Aubert also described increased concentrations of ribitol and mannitol under dry conditions. Sugars are known protectors of cellular substructures when lichens have to survive under dryness (Crowe et al. 1992).

As previously discussed, lichens are able to synthesize unique secondary metabolites. However, the relative contribution of its partners in providing such original compounds remains quite unstudied. This question is especially interesting when both partners possess the enzymatic machinery required to biosynthesize one metabolite [e.g., mycosporine and cyanolichens (Balskus and Walsh 2010)]. Moreover, knowledge about biosynthesis pathway is also a prerequisite for natural compounds production by biotechnological means.

Most current methods used to decipher metabolic pathways are based on stable isotope labeling (Eisenreich and Bacher 2007). Typically, organisms are grown with labeled structures that are most of the time very general precursors (e.g., glucose, acetyl CoA, CO_2, NH_4, aminoacids) that will spread in all their possible metabolic fates. Subsequent analysis of enriched biomolecules by either quantitative NMR spectroscopy or mass spectrometry has shown to be a powerful method for unraveling the dynamics of metabolic networks (Fan et al. 2012). Indeed, according to the detected profiles, metabolic history of the studied metabolite can be traced and then compiled to a comprehensive map about metabolic pathways and fluxes occurring in the network.

Although initially focused on single organisms such as plants or bacteria [for review see Eisenreich and Bacher (2007) and Heinemann and Sauer (2010)], techniques of stable isotope labeling were later applied to multi-organismic systems (Götz et al. 2010).

To our knowledge, only a few labeling studies for biosynthetic-unraveling purposes were reported on lichens. Lines et al. (1989) investigated polyol metabolism in *Xanthoria calcicola* using a ^{13}C NMR spectroscopy approach. More recently, a proof on concept was published in 2011 on the chlorolichen *X. parietina* (Eisenreich et al. 2011). Photobiont cells (*Trebouxia decolorans*) were first harvested alone in a medium containing ^{13}C-glucose, which enabled satisfying labeling of most if not all metabolites according to the authors. In a second step, algae were combined with unmarked mycobiont to reconstitute lichen symbiosis. Therefore, detection of ^{13}C-labeled fungal metabolites is indicative for prior transfer and usage of algal biomass for biosynthetic purposes by the mycobiont. ^{13}C NMR of crude methanol extracts of marked photobionts alone and reconstituted symbiosis by cocultivation with unlabeled fungus bear striking differences that demonstrate substrate transfer and further metabolism by the mycobiont. Closer investigation of those signal patterns shall provide information regarding (i) the nature of transferred compounds, (ii) the rate of this transfer and eventually, (iii) subsequent transformations occurring within the second partner (Eisenreich et al. 2011).

Such technologies took advantage of tremendous advances in mass spectrometry that now enable microscale mapping of metabolites directly within producing organisms (Esquenazi et al. 2009). Coupling of stable isotope profiling with imaging mass spectrometry enables both tracing and mapping of molecules within its biological system. This approach is especially promising for interfaces or symbiotic systems in order to know the compartment in which a molecule undergoes biotransformation (Jones et al. 2013; Pernice et al. 2012).

11.6 Conclusion

The evaluation of lichen metabolites for therapeutic purposes is generating an increasing interest as their activities are varied and often significant (Boustie et al. 2011). Very few lichen compounds are commercially available, and the thousand known metabolites are only partly or not at all investigated for their medicinal potential (Stocker-Wörgötter 2008). Moreover, lichens appear to harbor a diversity of culturable microorganisms from which original active compounds were recently isolated (Grube et al. 2012).

As lichens grow slowly, both in nature and axenic conditions, analysis must deal with low amounts of crude material. The past few years have witnessed major developments in purification, dereplication, and structure elucidation of lichen compounds, enabling much faster access to sufficient amounts of pure compounds.

Although not described here, innovative strategies were also developed for extracting and separating compounds which facilitate detection and identification of lichen compounds. New extraction methods developed on lichens include supercritic fluid extraction (SFE) involving the so-called supercritic fluids (SCF) (Lisickov et al. 2002; Zizovic et al. 2012) that appears to be a powerful strategy. Although a specific device is required, the main advantages of these methods are that (i) they display solvating powers similar to liquid organic solvents with higher diffusivity and lower viscosity, (ii) they can provide quantitative or complete extraction (by forcing continuously the fluid to flow through the sample), (iii) the solvating power of the fluid can be adjusted by changing the pressure and/or the temperature, (iv) adding modifiers to a SCF can change its polarity, (v) they avoid solvent residuals and last but not least, (vi) they are chemically inert which avoid chemical artifacts that can be dread when using classical separation procedures (Lang and Wai 2001).

A light fluorous catch and release approach was another option used to separate three closely related paraconic acids from *Cetraria islandica* allowing the isolation and identification of roccellaric acid from this lichen for the first time (Horhant et al. 2007).

The recent innovations in hyphenated techniques combining separation (HPTLC, HPLC, GC) and detection tools (currently MS, UV, IR) have already had a substantial impact in shortening the timeline for dereplication, isolation, and structure elucidation of the lichen products present in crude extract. Once hampered by methods limitations, lichen study might benefit from tremendous advances in analytical chemistry (MAS-NMR, DART-MS, LC-NMR...) described herein. Consequently, sensitive dereplication of lichen chemodiversity should facilitate focusing on minor, and possibly unknown, compounds. Standardized methods to identify recurrent products and to remove them should be developed in this way. As illustrated, various methods can be performed on solid samples then bypassing chemical artifacts associated with purification processes, giving in some cases direct field information. Furthermore, improvements in imaging techniques pave the way for future investigations into metabolic interactions taking place in lichen miniature ecosystem and might help deciphering ecological relevance of lichens metabolites.

Coupling these approaches with advances in purification, structure elucidation, and streamlining of the screening processes, the timeline for lichen products drug discovery is shortened similar to that expected for synthetic compounds, now being compatible with the present regime of blitz screening campaigns.

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Index

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