Chapter 3
Use of Lichens in Biological Monitoring of Air Quality

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Abstract This chapter focuses on biomonitoring of air quality using lichens in the industrial, urban and suburban areas in cities and in the vicinity of pollution sources, mainly based on the studies carried out in the last decades. Also lichen diversity studies in natural areas and in polluted sites, analytical methods and statistical analyses used in these studies are discussed. In addition, the text covers complementary information on the subject, for instance, environmental and anthropogenic factors which are effective on pollution sensitivity of lichen communities, negative effects of pollution on structure of lichen, metal uptake mechanisms and comparative analysis of data relating to changes in lichen vitality parameters. In particular, it is emphasized how to utilize the lichens featuring bioindicators and biomonitors to determine air quality in terms of quantities and impacts of airborne pollutants such as sulphur dioxide, heavy metals, particulate matters and radionuclides. With respect to lichen biomonitoring, the appropriate biological methods, their advantages and disadvantages, past to present studies on this subject in the world, the assessment of the relevant literature and the reliability of the obtained results are reviewed from a broad perspective. It is envisaged that this compilation will serve as a guiding source for biologic monitoring of air quality and creation of management and conservation strategies with lichens today.

Keywords Air quality · Air pollution · Bioindicator · Biomonitor · Lichens · Heavy metals

3.1 Introduction

Despite the relatively low concentration of sulphur dioxide in recent years due to the discouraging the use of fossil fuels, pollutants in the atmosphere together with global climate change still pose a serious threat to human health. The effects of air

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pollutants on human health can be listed as the reduction of the immune function of the body, the diminution of lung function, changes in the respiratory and circulatory system and the induction and promotion of human allergic diseases, respiratory diseases and other diseases (Cen 2015). Constant and serious precautions must be taken to monitor the sources of pollution. In order to be able to reach sustainable air quality, it is necessary to first determine the level of air pollution and then find solutions to reduce pollution rates. Instantaneous or ongoing air pollution levels can be measured in several ways, directly or indirectly.

Lichens are successful examples of the coexistence of some fungi (mostly *Ascomycota*) and green algae (*Chlorophyta*) and/or blue-green algae (*Cyanobacteria*), represented with approximately 20,000 species in the world. This common life, "symbiosis", is a mutualistic association (which both partners benefit), as it is often accepted. According to Vernon Ahmadjian's opinion, the mushroom is a controlled parasitic strain on algae (Ahmadjian 1982). The terms "mycobiont" for the fungal partner and "photobiont" for the photosynthetic partner are frequently used, while the symbiotic life partners in lichens are referred to as "symbiont". This symbiotic unity forms a common "thallus" without roots and cuticles (waterproof outer cover) and is essentially the intake of minerals from the atmosphere. These properties of lichens, when combined with their extraordinary abilities to evolve over a wide geographical range and their accumulation of much more than the needs of mineral elements, have put them among the best biological indicators of air pollution (Garty 2001; Wolterbeek 2002).

A variety of chemical, physical and biological techniques have being applied to determine the level and environmental impact of pollutants deposited in the atmosphere. Studies over the past three decades have drawn considerable attention to the relationship between lichens and air pollution and their role in monitoring air pollution in terms of particulate matter (Rossbach et al. 1999; Garty 2001; Adamo et al. 2008; Freitas et al. 2011; Malaspina et al. 2014), trace elements (Wolterbeek 2002; Bargagli et al. 2002; Uğur et al. 2003; Balarama Krishna et al. 2004; Conti et al. 2012; Caggiano et al. 2015; Will-Wolf et al. 2017), radionuclides (White et al. 1986; Gaare 1987; Başkaran et al. 1991; Biazrov 1994; Pipiška et al. 2005; Ramzaev et al. 2007; Yazıcı et al. 2008; Iurian et al. 2011) and persistent organic contaminants (Augusto et al. 2013; Ratier et al. 2018).

In this chapter, lichen biomonitoring studies are discussed in terms of contributions to air quality management. Examination of studies on lichen monitoring contributes to awareness of the environmental change caused by air pollution, measures to be taken and new approaches to conservation and management of the environment.

3.2 Biological Advantages of Lichens as Bioindicators

Lichens are plantlike organisms that develop on various types of "substrates" such as tree (epiphytic), rock (epilithic) and soil (epigeic) in most ecosystems on earth. Similar to plants, thanks to its chlorophyll partner capable of photosynthesis, lichen can produce food for itself. In fact, lichens also called "lichenized fungi" are included in the kingdom of *Fungi*, not the plants (Plantae), in the classification based on the phylogeny of the fungal partner. In thallus, at least two members from three kingdoms, fungi, algae (Protista) and cyanobacteria (*Monera* or *Bacteria*), can live together at the same time. Fungal species that cannot produce their own nutrients come together with certain microscopic algae or cyanobacteria species (such as *Trebouxia*, *Trentepohlia* or *Nostoc*) that can live freely in nature but often prefer to form lichen (Nash 2008).

Lichen species also have a wide variety of morphological diversity. Thallus types with very different shapes, sizes and colours may be "fruticose" (1 m long hanging from one point on trees or standing upright) or "foliose" spreading in the form of a rosette with large and small lobes on the substrate or "crustose" which can be as small as 1 cm deep buried in the substrate or 10 cm wide on the surface.

Because of many reasons, lichens benefit as biological monitoring tools in atmospheric deposition studies. Lichens are allowed to be compared at a universal level since they have a wide geographical spread. As an additional advantage, lichens, which are perennial and slow-growing organisms, show a single morphology that does not change over time, rather than like the flowering plants seen by season. So the morphological changes in lichens themselves are the effects of time-dependent accumulation.

Lichens are "poikilohydric" organisms whose metabolic activity is limited by atmospheric humidity. The absence of stomas and cuticles in lichens in high plants means that pollutants in the air are absorbed by the entire surface of the tallus. Perhaps most important is the ability of lichens to accumulate many elements in much greater quantities than their physiological needs (Nash 2008). It is reported that lichens absorb 100 times more sulphur dioxide than vascular plants (Winner et al. 1988). When compared with flowering plants in terms of air pollution indicator role, thalline plants such as lichens and mosses are more prominent. Because, when it comes to monitoring the quality of the air, the choice of lichens living as a whole tallus (without spilling leaf or flower) for many years gives longer term and reliable results.

The high susceptibility of lichens to pollution is closely related to their biology. Long-living lichens exposed to pollutants throughout the year as living perennial organisms have to maintain the symbiotic balance. All lichen species are not sensitive to pollution at the same time. But in general they can only tolerate pollution at a certain tolerance limit. For this reason, they have gained importance in monitoring the quality of the air. Because of the variety in the lichen communities, distribution maps based on the frequency of certain species come at the beginning of this work.

The second approach is to examine the morphological and anatomical changes of lichen species in response to pollution. The third way is to examine the physiological response (membrane integrity, CO_2 gas exchange, chlorophyll, pigment destruction, N_2 fixation and enzyme activity). Some of the changes that occur in pollution-damaged lichens include morphological and anatomic symptoms, fine-structure symptoms, membrane system disorders, chlorophyll fluorescence disorder, physiological disorders and reproductive-developmental and growth rate disorders (Nash 2008). While some of these changes (morphological and physiological) can be observed in the field, some changes can be observed with transplantation studies or controlled laboratory studies.

3.3 Air Pollution Monitoring Related Terms and Definitions

The term "monitoring" can be defined as the process of gathering information at different points about system state variables in time in order to evaluate the status of the system and to be inferred about the changes over time (Yoccoz et al. 2001). While focusing on the monitoring of biological diversity, that is, "biomonitoring", the systems of interest are typically ecosystems or components of such systems (communities and populations), and relevant variables are quantities such as species richness, species diversity, biomass and population size (Upreti et al. 2015).

By combining various definitions, "air pollution" can be defined as follows: the presence of contaminants or substances (chemicals, particulate matter (PM) or biological materials) in the air that interfere with human health or welfare, and other living organisms, or cause other harmful environmental effects on ecosystems. Air pollution exists at all scales, from personal to global. Ambient air pollution scales may be further subdivided into local, urban, regional, continental and global. The spheres of influence of the air pollutants themselves range from molecular (e.g. gases and nanoparticles) to planetary (e.g. dispersion of greenhouse gases throughout the troposphere). It is stated that when the local scale shows a spatial dimension about 5 km radius on the world surface, the urban scale has a radius of about 50 km, and the regional scale has a radius of 50–500 km. The continental scales range anywhere from 500 to 1000 km but are often driven by wind, because this road is passed by the pollutant. Of course, the global scale extends worldwide. Air pollution arises from numerous sources and processes. Air pollution and other atmospheric data include information about the air pollutants, the media (layers of the atmosphere and components of the hydrosphere), the modifiers (physical, chemical and biological substances that transform and transport the agents within the media) and the receptors (human individuals, human populations and ecosystems and their components) (Vallero 2008).

The term "air pollution" is not synonymous with "air quality". "Air pollution", pollutants and "air quality" are defined by the effects of pollutants including humans, animals, inorganic substances, monuments, etc. (Garty 2001). "Air pollution" refers to atmospheric smoke, mineral-rich dust, sulphur dioxide (SO_2), nitrogen oxides (NOx), sulphur (S) and nitrogenous (N) compounds, and fluorine (F), and photooxidants (such as ozone and PAN) refers to air toxic substances.

Many terms used for organisms in environmental pollution have different meanings. "Bioindicator" or biological indicator refers to the ability of organisms to show the presence and amount of pollutants in the atmosphere in relation to the response to different levels of pollutants. Biological indicators that provide qualitative information at the level of air pollution are called “bioindicators”, while biological monitors that allow quantitative data to be identified by time are defined as “biomonitors”. The practice in which biomonitors are used is the “biological monitoring” or “biomonitoring” phenomenon. “Bioaccumulator” term is used for biological agents holding metals in the air. “Bioremediators (phytoremediators)” means biological remedies, so they should exhibit the properties of removing, eliminating or protecting the contaminants from the environment. Bioremediators are short-lived living organisms that accumulate high amounts of toxic substances with large biomass. Although lichens are good bioindicators and bioaccumulators, they are not considered to be air remedial and environment protective bioremediators. The reason is that it is difficult to elucidate the true source of the metals that accumulate in the large amount of lichen biomass that comes from the very slow growth (Shukla et al. 2014). The terms “biological indicator” and “biological accumulator” cannot be used interchangeably.

For the term “biomonitor”, Markert et al. (1997) used a definition of “informative organisms or communities of organisms comprising certain elements and substances and/or on the quantitative effects of environmental changes or environmental quality, including morphological, histological or cellular structures, metabolic-biochemical events, behaviour, population structures and changes in these parameters”.

Seaward (1995) has introduced a contemporary approach to the monitoring of some contaminants with living materials and the assessment of human technology in the biosphere. However, it does not replace the measurement of pollutants physically and chemically. Biological monitoring is recommended as a complementary or alternative method in extensive and inexperienced comprehensive research that requires extensive instrumentation. Thus, besides physical and chemical data, physiological data showing viability are also provided.

The qualities required for an organism to be a “biomonitor” are listed by Garty (2001) as follows:

1. The organism should have the ability to accumulate metal in measurable quantities.
2. The organism or its parts should be appropriate in terms of quality and distribution on the earth and sample collection is feasible.
3. The study should be reproducible with the same qualifications.
4. The expenditures required for the collection and analysis should be acceptable (Wolterbeek 2002).

3.4 The Role of Lichens in Atmospheric Pollution Assessments

General atmospheric pollutants classified by Hutchinson are as follows:

1. Primary pollutants: SO_2, NO_2 and F compounds that remain in the same chemical form in the atmosphere

2. Secondary pollutants: resulting from chemical reactions of primary pollutants during transport in the atmosphere, such as sulphuric acid (H_2SO_4) and nitric acid (HNO_3) occurring in acid rains, also (O_3) ozone and peroxyacetyl nitrate (PAN)
3. The third group of pollutants: industrial organic compounds that contain toxins in the air, agricultural pesticides, trace metals and metalloids

Usually, sulphur and nitrogen compounds are found in the form of gaseous in the atmosphere, whereas heavy metals are found attached to particulate matter (PM). Some particles come naturally from volcanoes, dust and sandstorms, forest and pasture fires, live vegetation and sea spray effects. In addition, various human activities such as power plants, industrial activities and the use of fossil fuels in motor vehicles produce significant amounts of particles. They cause an increase in cancer, heart problems, respiratory diseases and infant mortality (Garty 2001).

Lichen diversity, spatial and temporal distribution of species, is one of the most valuable biological tools for environmental evaluation particularly on the air pollution. Lichen diversity value (LDV) based on epiphytic taxa is the European method developed for environmental stress/quality indication (Svoboda 2007).

Lichens have been used as indicators around the city pollution and emission sources since their susceptibility to gas pollutants such as SO_2 (sulphur dioxide) have been noticed in the 1960s. With their ability to accumulate the elements found in low concentrations in the air, the lichens have become a frequent topic from that year until today. There are hundreds of articles on this (Henderson 2000), among which the oldest are about lichen communities interacting with pollutants in the gas state (Gilbert 1965, 1970; Hawksworth and Rose 1970; LeBlanc and De Sloover 1970; Showman 1975; Türk and Wirth 1975).

In addition to being sensitive to air pollutants, lichens are also good metal accumulators. Mineral nutrients, metals and heavy metals are found in rainfall and dust, as well as in natural and anthropological substrates (shell, soil, rock). The elements Pb, Ni, Hg, Cr, Zn, Ti and V are among important metal pollutants (Hutchinson et al. 1996). The term “heavy metal”, which is often used in biological studies, refers to elements that are toxic to living organisms when they are physically present in high amounts in the atmosphere, whether metal, transition metal or semimetal.

3.4.1 Bioindicator Role of Lichens in Air Pollution

Bioindicators are living organisms that react to environmental pollution with their life functions. Because of the biological properties, lichens, which have potency to reflect air pollution, are at the forefront of bioindicators. The features that allow lichens to be reliable indicators of atmospheric changes are listed Branquinho et al. (2015) as follows:

1. Lichen species differ in their susceptibility to atmospheric changes.

2. Lichens are slow-growing organisms, and their morphologies do not show seasonal changes.
3. Lichens have a wide distribution and can be found in almost all terrestrial biomes.

The distribution of lichen species in a region can provide estimates of atmospheric pollution, especially the level of sulphur dioxide. Since the susceptibilities of species to air pollution are different, the distribution of epiphytic lichen species in some European countries is zoned by the level of air pollution according to the winter average SO_2 values (Nimis et al. 2002). Thus, it is possible to estimate the SO_2 level in that region by looking at which lichen species are present in a region.

The first qualitative zoning scale established by Hawksworth and Rose (1970) for this purpose indicates ten regions described in England based on estimates of sulphur dioxide pollution. Region 1 is defined as the absence of epiphytic lichens when the average winter SO_2 level exceeds 170 $\mu g\ m^{-3}$, whereas region 10 refers to the region where the highest number of lichens is present when the SO_2 level is less than 10 μgm^{-3}. For example, *Lobaria pulmonaria*, *L. amplissima* and *Usnea florida* can be present below level of 30 $\mu g\ m^{-3}$ SO_2, while *U. articulata* and *U. filipendula* only in regions with pure air. Due to the impacts of climatic and geographical conditions in the lichen distribution, different regions do not have the same lichen species. Therefore, it is not possible to apply exactly the same chart to the estimation of the air pollution level of another country. This chart is specific to that country as it is done in the UK, and similarly for new regions or countries where the natural lichen flora is designated, new lichen zoning scales can be created based on their lichen species.

3.4.2 Lichens as Biomonitors of Air Quality

Environmental assessments have been carried out on lichen populations for long-term pollution effects. For instance, over a period of 15 years, changes in lichen communities under the influence of climate and pollution have been investigated in terms of deposition of nitrogen (N) and sulphur (S) compounds in Norway by Evju and Bruteig (2013), where the largest change in species composition was found in the site with the biggest reduction in sulphur deposition. The degree to which the lichen species, physiology and morphology and symbiotic life partners are individually affected by air pollutants has been questioned (Bačkor et al. 2010; Piovar et al. 2011; Vannini et al. 2017). For instance, physiological and ultrastructural effects induced by acute exposure to ozone (O_3) were investigated in *Xanthoria parietina* by Vannini et al. (2017). They put forward that the hydration state may play a major role in determining the extent of the damage, and the presence of parietin may support the recovery.

Due to their slow growth and long life, lichens are important living things used in monitoring the environmental changes of a region depending on the air quality.

Numerous studies have been carried out using lichens (Conti and Cecchetti 2001) or mosses (Harmens et al. 2010; Behxhet et al. 2013) as biological tools.

Majority of the recent lichen biomonitoring studies is documented on the spatial distribution of elements or radionuclides in urban or suburban areas (İçel and Çobanoğlu 2009; Freitas et al. 2011; Rani et al. 2011; Doğrul Demiray et al. 2012; Conti et al. 2012; Shukla et al. 2012; Sujetoviene and Sliumpaite 2013; Malaspina et al. 2014; Paoli et al. 2015; Petrova et al. 2015; Çobanoğlu and Kurnaz 2017); in forests or natural sites (Blasco et al. 2011; Klimek et al. 2015); in vicinity of pollution resources such as factories, mills, mines or thermal power plants, etc. (Uğur et al. 2003; Boamponsem et al. 2010; Behxhet et al. 2013; Paoli et al. 2015; Protano et al. 2015; Lucadamo et al. 2016; Boonpeng et al. 2017); and in the Arctic (Singh et al. 2012). A large number of publications on biomonitoring of air quality with lichens have been screened worldwide in the literature, and selected publications from the last decade are listed in Table 3.1.

Pollutants in the atmosphere can be sourced both from nature and from human activities. Lichen surveys for biomonitoring include the following pollution parameters:

- Heavy metals and/or trace elements (many documents, see Table 3.1)
- Atmospheric nitrogen and sulphur (Van Herk et al. 2003; Evju and Bruteig 2013)
- Particulate matter (PM) (Adamo et al. 2008; Freitas et al. 2011; Malaspina et al. 2014)
- Polycyclic aromatic hydrocarbon (PAH) concentrations (Blasco et al. 2011; Shukla and Upreti 2009)
- Radionuclides (Iurian et al. 2011)
- Nanoparticle emissions (no study with lichens yet but with bryophytes (Walser et al. 2013)

Besides lichen monitoring studies, there is also a number of moss surveys in this regard (Balarama Krishna et al. 2004; Ramzaev et al. 2007; Harmens et al. 2010; Behxhet et al. 2013).

3.5 Environmental Factors Affecting Lichen Diversity

One of the most important factors that the lichens show sensitivity is “atmospheric pollution”, which is directly or indirectly due to human activities. Biotic and abiotic factors are influential in this sensitivity. In biological monitoring studies, physical environmental conditions and human-induced factors should also be considered.

Lichens are used as an important indicator for evaluations in terms of hemeroby (the impact and grade of human activities in ecosystems). Indicator species are determinants of vegetations that are damaged at various degrees. For example, while well-preserved forests contain the Xanthorion communities, the Lobarion communities are only available in forests away from hemerobic effects (Zedda 2002). In a variety of studies conducted on roadside lichens (soil, rock and tree

Table 3.1 Lichen species diversity in the biomonitoring studies in the last decade are listed chronologically

Lichen species	Growth form	Substrate	Publication	Region, Country	Method, Pollutants
Pseudevernia furfuracea	Fruticose	Epiphytic	Adamo et al. (2008)	Naples, Italy	Bag technique, multi-element analysis, PM_{10}
Hypogymnia physodes	Foliose	Epiphytic	Williamson et al. (2008)	Karabash, Russia	Transplantation technique, multi-element analysis
Phaeophyscia hispidula	Foliose	Epilithic- Epiphytic	Shukla and Upreti (2009)	Dehradun City, India	PAHs, Soxhlet apparatus
Parmelia sulcata	Foliose	Epiphytic	Boamponsem et al. (2010)	Tarkwa, Ghana	Sb, Mn, Cu, V, Al, Co, Hg, As, Cd, Th
Evernia prunastri	Fruticose	Epiphytic	Blasco et al. (2011)	The Pyrenees, Spain and France	PAHs, DSASE
Lobaria pulmonaria	Foliose	Epiphytic			
Parmelia sulcata	Foliose	Epiphytic			
Pseudevernia furfuracea	Fruticose	Epiphytic			
Ramalina farinacea	Fruticose	Epiphytic			
Usnea sp.	Fruticose	Epiphytic			
Favoparmelia caperata	Foliose	Epiphytic	Freitas et al. (2011)	Porto, Portugal	Lichen diversity value (LDV), CO, CO_2, SO_2, NO_2, O_3, PM_{10}
Parmotrema chinense	Foliose	Epiphytic			
Punctelia subrudecta	Fruticose	Epiphytic			
Cladonia fimbriata	Fruticose	Epigeic	Iurian et al. (2011)	Salzburg, Austria	^{137}Cs, gamma spectrometry
Cladonia squamosa	Foliose	Epigeic			
Pseudevernia furfuracea	Fruticose	Epiphytic			
Hypogymnia physodes	Foliose	Epiphytic			
Phaeophyscia hispidula	Foliose	Epiphytic	Rani et al. (2011)	Uttarakhand, India	Cu, Pb, Ni, Zn, Fe, Cr, Hg, Cd
Usnea barbata	Fruticose	Epiphytic	Conti et al. (2012)	Patagonia, Argentina	Multi-element analysis
Xanthoria parietina	Foliose	Epiphytic	Doğrul Demiray et al. (2012)	Kocaeli, Turkey	Multi-element analysis
Pyxine subcinerea	Foliose	Epiphytic	Shukla et al. (2012)	Uttarakhand, India	PAHs, Soxhlet apparatus

(continued)

Table 3.1 (continued)

Lichen species	Growth form	Substrate	Publication	Region, Country	Method, Pollutants
Cladonia amaurocraea	Fruticose	Epigeic	Singh et al. (2012)	Ny-Ålesund, Arctic	Multi-element analysis
Cladonia mediterranea	Fruticose	Epigeic			
Cetraria fastigiata	Fruticose	Epigeic			
Flavocetraria nivalis	Fruticose	Epigeic			
Physcia caesia	Foliose	Epigeic			
Pseudophebe pubescens	Fruticose	Epigeic			
Umbilicaria hyperborea	Foliose	Epigeic			
Xanthoria elegans	Foliose	Epigeic			
Hypogymnia physodes	Foliose	Epiphytic	Evju and Bruteig (2013)	Norway	N, S
Melanelia olivacea	Foliose	Epiphytic			Monitoring lichen species composition
Evernia prunastri	Fruticose	Epiphytic	Sujetoviene and Sliumpaite (2013)	Kaunas, Lithuania	Transplantation technique, Cd, Cu, Pb
Evernia prunastri	Fruticose	Epiphytic	Malaspina et al. (2014)	Genoa, Italy	Transplantation technique, multi-element analysis, PM_{10}
Platismatia glauca	Foliose	Epiphytic	Caggiano et al. (2015)	Agri Valley, Italy	Bag technique, multi-element analysis
Evernia prunastri	Fruticose	Epiphytic			
Ramalina fraxinea	Fruticose	Epiphytic			
Pseudevernia furfuracea	Fruticose	Epiphytic			
Hypogymnia physodes	Foliose	Epiphytic	Klimek et al. (2015)	Beskidy Mountains, Poland	Cd, cu, Ni, Pb, Zn, AAS
Evernia prunastri	Fruticose	Epiphytic	Paoli et al. (2015)	Molise, Italy	Transplantation technique, multi-element analysis
Pseudevernia furfuracea	Fruticose	Epiphytic	Petrova et al. (2015)	Plovdiv, Bulgaria	Bag technique, multi-element analysis

(continued)

Table 3.1 (continued)

Lichen species	Growth form	Substrate	Publication	Region, Country	Method, Pollutants
Pseudevernia furfuracea	Fruticose	Epiphytic	Protano et al. (2015)	Latium region, Italy	Transplantation/ bag technique, As, Cd, Ni, Pb, 12 PAHs, 17 PCDDs and PCDFs, 27 PCBs
Pseudevernia furfuracea	Fruticose	Epiphytic	Lucadamo et al. (2016)	Calabria region, Italy	Transplantation technique, multi-element analysis
Parmotrema tinctorum	Foliose	Epiphytic	Boonpeng et al. (2017)	Map Ta Phut, Thailand	Multi-element analysis
Physcia adscendens	Foliose	Epiphytic	Cobanoglu and Kumaz (2017)	Istanbul, Turkey	Multi-element analysis
Evernia mesomorpha	Fruticose	Epiphytic	Will-Wolf et al. (2017)	Wisconsin, USA	Multi-element analysis
Flavoparmelia caperata	Foliose	Epiphytic			
Physcia aipolia/ stellaris	Foliose	Epiphytic			
Parmelia sulcata	Foliose	Epiphytic			
Punctelia rudecta	Foliose	Epiphytic			
Xanthoria parietina	Foliose	Epiphytic	Ratier et al. (2018)	Provence, France	Multi-element analysis

species), it has been found that dust and traffic density in the form of wind scattered in the metal content of soil profiles and vegetation on the highway are effective. Motor vehicles emit nitrogen oxides (NOx), carbon monoxide (CO), sulphur dioxide (SO_2), hydrocarbons, volatile organic compounds, airborne particles and various metals. Lead (Pb) is a common indicator of anthropogenic (human-induced) activity in atmospheric accumulation in industrialized countries. It is possible to use lichens and mosses as a monitor of the change in lead (Pb) pollution from motor vehicles (Garty 2001).

A variety of studies have been carried out to examine the respiratory tract cancer cases in relation to the material properties of metal-containing particles in airborne dust. Airborne association of particulate matter and gaseous pollutants is closely related to human respiratory tract diseases. In a study conducted at Armadale (Central Scotland), values of lichens were shown with their supporting roles in interpreting respiratory tract infections and the highest lung cancer mortality seen in Scotland between 1963 and 1973 (Garty 2001). In a study conducted by Cislaghi and Nimis (1997), lung cancer in young male individuals in certain regions of Italy showed very highly positive correlation with lichen variability ($r = 0.95$; $P < 0.01$). In the case where the common anthropogenic pollutants SO_2, NO_3^-, dust and SO_4^{-2} are positively related ($r = 0.93$, 0.87, 0.86 and 0.85, $P < 0.01$), an association with non-anthropogenic substances (such as Cl^-, Ca^{+2}, Mg^{+2}, HCO^{-3}, K^+ and Na^+) has not been achieved. Such studies clearly demonstrate the effects of anthropological activities.

It is known that the average concentration of sulphur dioxide (SO_2), especially winter, increases with population density, especially with smoke, industrial and commercial centres and residential areas. It has been reported that lichens are more sensitive to SO_2 than to smokers (Gilbert 1970). The epiphytic crustose lichen known as “the most pollutant species” in Western and Central Europe is *Lecanora conizaeoides*, and the amount of sulphur is a limiting factor for this species since it is colonized by the level of SO_2. In a study conducted by Hauck et al. (2001) in the Harz Mountains of Germany, it has been reported that this sulphur-loving lichen species also decline in the case of falling sulphur values in tree barks, and it is also stated that competition with other pollution-tolerant epiphytic species is also an important element in the distribution of the species. Hauck et al. (2011) reported the populations of *L. conizaeoides*, one of the most common epiphytic lichens that have been adapted to very low pH (about 3) and high SO_2 values in Europe, have decreased significantly in the last 20 years. A slight increase in acidity (0.4 pH units) has been stated as the cause of this, with the decrease in sulphur dioxide values in the recent years.

Like other plants, lichens are mentioned to be sensitive to pH. Tree bark pH and susceptibility to toxic substances are the primary factors affecting epiphytic lichen composition (Van Herk 2001). Most nitrogen-loving species (nitrophytes) show low sensitivity to the toxic effects of sulphur dioxide, and their only requirement is high bark pH. The increase in bark pH has led to a large increase in nitrophytic species in Switzerland and the disappearance of acidophytic species in the last decade. It has been revealed that there is an almost linear relationship between measurements of ammonia (NH_3) concentration in air and the number of nitrophytes on *Quercus*. The abundance of nitrophytes is not related to the SO_2 concentration. Most of the acidophytes were highly sensitive in areas with concentrations of 35 μgm^{-3} or more NH_3, and all acidophytes disappeared (Van Herk et al. 2003). Accordingly, it has been argued that current methods that use species diversity to monitor or estimate SO_2 air pollution require some modifications; otherwise air quality may be incorrectly assessed in relatively good regions with high NH_3 levels.

SO_2 is harmful to lichen species at lower pH values, becoming more severe as acidity increases. However, when the pH is low, this does not always lead to the poor lichen flora, because well-developed lichen communities can be found in regions with low SO_2 (Gauslaa 1985). The calcareous substrata are more suitable for lichen colonization, such that the high pH of the substrate, such as in lime mortar and cement, can reduce the toxic effect of acidity, allowing the lichen to survive the pollution. Acid rain affects the lichens directly or indirectly by increasing the acidity of the substrate. It is also the case for epiphytes. For example, *Parmeliopsis ambigua*, which is prevalent especially in bark-free conifers, has been reported to have spread in recent years in regions with moderate pollution in the UK, possibly in response to rising bark pH. On the other hand, pollution-sensitive species such as *Lobaria pulmonaria* have largely disappeared in tree barks such as *Quercus* (Seaward 1989).

In a study intended to show a vertical change of bark pH in the *Picea abies* forest unaffected by acid rain, bark pH was found declining from ground level to upper branches and high again in the terminal branches, resulting in an unusual species composition. It has been reported that the high-bark pH-dependent Lobarion communities developed on *P. abies*. Species have their own pH requirements; it has been noted that acid rain, which is stated to have a pH value at almost industrial level, affects the distribution of lichen in situations where pH of the substrate changes (Kermit and Gauslaa 2001).

Normally the pH of the rains is 5.6, because CO_2 is balanced with carbonate and bicarbonate ions when dissolved in water. But today, the pH of the rains is 4–5 and sometimes lowers. The fog versus rain occurs normally under stationary atmospheric conditions and a much higher concentration of ions. In this case, the pH of the fog was recorded as 2–3. There are not only direct acid effects of acid rain and fog on lichens but also indirect effects which cause substrate conditions to change. For example, it has been reported that the acidity of tree bark, both in and around cities and copper mines, has increased (Nash and Gries 1991).

The distance to the source of pollution, elevation, wind direction, air temperature and moisture are the most influential factors in the degree of pollution. It is stated that the physiological activity of the lichens is dependent on the amount of water and that the conversion of sulphur into a less toxic form is regulated accordingly. Thus, lichens in polluted areas, if the humidity is high, will be adversely affected, and their drying will slow down as the process of converting pollutants to less toxic form increases (Gilbert 1970).

The "habitat" characteristics that are effective in lichen development and spread are "macrohabitat factors" (sunlight, wind, temperature, humidity, chemistry of atmospheric air) and "microhabitat factors" (substrate-type, tree, rock, man-made material, etc., soil structure and chemistry, forest canopy, concentration changes of atmospheric gases and so on). In atmospheres with fluctuating pollutant content and levels, the importance of microclimate and microhabitat conditions (such as shadow, light, humidity) is noticeably evident when lichens are able to respond to pollution and even survive (Huckaby 1993). In the regression models created for a forest ecosystem, the ecological variables such as pure or mixed stand, tree species, tree diameter and number of lichen species are stated as the most expressive environmental factors (Sevgi et al. 2016).

Altitude is one of the factors that influence the spread of lichens. It is emphasized that since the SO_2 is spreading and rising in the air, as the altitude increases from the ground, the lichens are more affected by pollution (Showman 1975). In a study of lichens on the periphery of pollution source, it was shown that the first factor affecting the degree of susceptibility of lichens to pollution was the distance to the source, and the altitude affects the lichen frequency positively (Oksanen et al. 1991). According to this, in the near vicinity of the source, the number of the taxa is low, and the values are close to each other. As the distance increases, the number of the taxa increases, and this increase becomes even faster as the altitude increases.

Studies of the dependence of metal content on lichens on topography show that chemistry (H^+, NH_4^+, Na^+, K^+, SO_4^{-2}, NO^{3-}) and associated acidity, which accumulate significantly in the high forests, are another determinant of the metal composition of lichens exposed to fog and clouds at high altitudes. In lichens, the content of caesium (^{137}Cs) increases with altitude. Lead (Pb) is highest in the middle elevations (200–400 m). Mercury (Hg) content is almost twice as high as in the high forest in the tundra below 400 m. It has been stated that the level of mercury drops as it moves away from the sea edge. Antarctica is seen as the "cleanest" area in the world due to its geographical location. Natural and artificial radioactivity is the lowest level here (Garty 2001). Furthermore, it has been reported that the presence of thick lichen-moss cover on the soil inhibits the permeation of Cs-radionuclides (^{137}Cs) into the soil (Ramzaev et al. 2007). Lichens can be suggested as biological monitors of radionuclides, which are caused by nuclear accidents and nuclear accidents, with high concentrations of radionuclides that they acquire from the atmosphere as a result of slow growth and long life.

Secondary metabolites unique to lichens provide them with the advantage of being able to survive in an ecological niche by giving them habitat adaptability. Many environmental factors (biotic and abiotic) affect the expression of PKS genes involved in the production of these metabolites (Deduke et al. 2012). Therefore, it appears that air quality is an important factor that directly affects lichen chemistry and secondary pathway of metabolite synthesis, in relation to the level of heavy metals and other pollutants in the air.

3.6 Metal Accumulation in Lichens

A major portion of the metal content of lichens has atmospheric origin (Garty 2001), but at the same time, it is documented in many studies that they have captured particles from the substratum (Bačkor and Loppi 2009). These particles can be stored for long periods unchanged by being held on the lichen surface or in the intercellular spaces. The lichens thus accumulate heavy metals in quantities exceeding their requirements and tolerate them in complexity with extracellular crystals or lichen acids. The toxicity of the metals in the retained and stored particles is determined by the chemical and physical factors such as the amount of metals, their chemical form and their solubility in water, pH and temperature. The metals in the air are taken up by dissolving or catching particles in the lichen thallus. Soluble metals tend to settle in extracellular or intracellular regions. Classical and modern histochemical methods used to determine the location of metals within the lichen thallus have been reported comparatively (Rinino et al. 2005).

There are three main mechanisms of metal accumulation (Nash 2008):

1. Capture of solid particles
2. Extracellular binding with exchange sites on the cell walls of symbionts
3. Intracellular uptake

In the third, PM adsorbed onto thallus surface and penetrates into intercellular spaces (Shahid et al. 2016). Element accumulation in lichen thallus depends on various factors, such as the nature of the element, environmental parameters, production of secondary metabolites, growth form of lichen, part of thallus and its specific morphological/anatomical properties (Garty 2001; Bačkor and Loppi 2009). For instance, in *Cladonia* species, crystals are accumulated in intensively in thallus deformations and in granular parts (Rola et al. 2016; Osyczka et al. 2018).

Metal accumulation in the lichen is a dynamic process, and it has been observed that it was taken to the thallus quickly – in a few hours – when it was immersed in metal solutions in the investigations.

In transplantation studies, lichens reacted to atmospheric heavy metal changes within a few months. The duration of many elements in the lichen tall is 2–5 years (Bačkor and Loppi 2009).

There is considerable intercellular space in the lichens, which is evidence that particles of dust, soil and metal are trapped in these spaces (Nash 2008). Information on the contents of particles displayed by SEM (scanning electron microscope) on cross-sections and surface was obtained by energy-dispersive X-ray (EDX) analysis method.

The metal content of lichens depends on the texture and its morphological structural properties. Most of the lichens requiring metal-rich surfaces belong to the genera *Acarospora*, *Aspicilia*, *Lecanora*, *Lecidea*, *Porpidia*, *Rhizocarpon* or *Tremolecia* (Bačkor and Fahselt 2004). The amount of metal varies in the same parts of different species. In foliose lichens (*Flavoparmelia baltimorensis* and *Xanthoparmelia conspersa*), the amount of metal is found more than in fruticose lichens (*Cladonia subtenuis*). Also, metal amount is greater in *Hypogymnia enteromorpha* than in *Usnea* and similarly in *Parmelia sulcata* than in *Anaptychia ciliaris*. Again it is in decreasing order in the foliose *Xanthoparmelia conspersa* and *Peltigera canina* and crustose *Lecanora subfusca*. Character of the lichen thallus surface (mucilage, cilia, hairs, holes, isidia and roughness) influences the particle retention. For example, in *Usnea* and *Alectoria*, the feature of holding more particles than *Umbilicaria* is striking (Garty 2001).

Al, Fe, Mg and Mn elements are abundant in the earth's crust. Unwashed lichen thalli have high metal content due to dust and soil. High-contrast contaminated dust samples collected with equipment placed in hotel ventilation in the city centre by Rossbach's method were compared with lichen samples affected by trace contaminants transportable to remote areas (Rossbach et al. 1999). In *Usnea* species and dust, the element concentrations are highest for Cr, Zn and Fe and lowest for Ca, Rb and Sr.

Sections taken from lichens have shown that the central parts generally contain more metals than the edge parts. Also, the average contents of Fe, Pb, Zn, Mn, Cr and Al were found higher in the inner parts of various *Parmelia* species than in the outer parts. This is related to the age of lichen. In the case of these saxicolous foliose lichens, the inner parts are older, and the edges are younger. On the other hand, the slowly growing epilithic crustose lichen *Protoparmeliopsis muralis* has accumulated more Pb in the edge regions than in the thallus centre (Garty 2001).

Lichen potential contribution of habitat on the metal content of the thallus is another issue to be considered. Substrates, metallic rocks and metal-containing soils that the lichens live on should be considered in determining the metal content of the lichen. In fact, most of the metal content of lichen is of atmospheric origin. Zn, Cu and Cd concentrations of lichen, moss and snow samples were found to be in the same order in snow samples and cryptogams under snow, but in flowering plants, Pb contents were found to be 10 times higher than in the snow sample (Garty 2001). Using the methods of EDX-microanalysis (Energy-Dispersive X-ray) and X-ray mapping, the accumulation of elements in *Lecidea lithophila* and *Rhizocarpon oederi* lichens which developed on the old copper mine residues for centuries has been compared with the amount in the substrate (Bačkor and Fahselt 2004). According to this, Al, Si, and K are found at very low concentrations in the apothecium in both species compared to the substrate, while C is higher than in substrate as expected, and O, Na and Mg are at the same level.

In the study on the rocky coastal area of Baikal Lake, aquatic species of *Verrucaria*, which cover the rocks in the depth of 1.5 m, were examined for their chemical composition. In *Verrucaria* species, the same elements (Ca > K > Fe > Al > Mg > P > S > Na > Mn > Sr > Ba) were dominant that are often found in the rocks. Compared to the element structure of the water layer near the bottom, the lichens are dense with elements slowly passing to the water: Gd > Sm > Pr > Nd > Al > La > Dy> Tb > Y > Lu > Ce > Yb > Be> Tm > Co > Nb > Mn > Zn. Compared with the compound of rock, *Verrucaria* thalli were enriched by Hg > As> P > Zn > Li > S > U > Mo > Se > Cd > Ca > Tl > Sr > Pb > Be. In relation to the rock surface, it was noted that the water lichens accumulate As> P > Zn > Li > S > U > Mo > Se > Cd > Ca > Tl > Sr > Pb > Be in the most intense order, respectively (Kulikova et al. 2011).

Many lichenologists acted to monitor the atmospheric mercury (Hg) toxic metal, which is not only collected in aquatic but also in terrestrial ecosystems. Uptake of Hg in lichens and their accumulation in particulate form have been studied in volcanic and geothermal fields and in mine areas. Lichens in urbanized and industrial areas are also effective markers for Hg in the air (Garty 2001). Study results around the thermometer factory have shown that mercury contamination can be transformed into a form retained by some chemical binding agents on its main elemental form, on lichen or moss surface, or may be diffused into lichen/moss cells (Balarama Krishna et al. 2004). It has also been proposed that lichen and moss can be used as sorbent material for the purification of mercury-methylene from inorganic aqueous solutions.

3.6.1 The Extracellular Metal Exchange

Lichens absorb the metal ions from the rainwater through the extracellular absorption, while they release the H^+ ions or weakly bound metal ions to carry out ion exchange. Regarding the biomonitoring application of metal contaminants in the air, advantages of these characteristics of lichens are utilized. Various types of organic materials such as dead biomass, bacteria, filamentous fungi, algae and higher plants contain metals bound to carboxyl, aldehyde, hydroxyl, sulphide, phosphoryl or amine groups. In metal uptake in lichens, the mycobiont is active rather than the photobiont. The chitin material, which is the polymer of acetyl-D-glucosamine in the fungal cell walls, is the main binder in this case (Garty 2001).

The biosorption of Cu and Co metal ions by infrared spectroscopy (IR) was investigated in the biomass of *Penicillium cyclopium* from free-living fungi, and the hydroxyl groups were identified as the main group binding heavy metals. Amides and carboxyls are the least bonded groups. The uptake of Cu and Co metals is realized as the result of the ion-exchange mechanism with K^+, Mg^{++} and Ca^{+2} (Tsekova et al. 2006).

Branquinho and Brown (1994) by the method they use found that when the element Pb is treated with thiol-rich chemicals, it is replaced by cations such as Cu and left to extracellular exchange areas, and they showed that this led to significant intracellular K ion loss due to membrane integrity deterioration.

It has also been shown that pH is also an effect in the metal bonding. For example, in a study by Akçin et al. (2001), dry lichen samples were mixed with different concentrations of metals and adjusted to pH 2–10 with nitric acid and ammonium hydroxide. In aqueous acid solutions, metal ions competed with H^+ ions to bind to the cell walls. It is stated that the metal bonding is performed optimum at pH 4 and reaches the maximum level.

In natural *Hypogymnia physodes* samples, the relationship between time, temperature, pH, and inhibitor (formaldehyde) was assessed by caesium (^{137}Cs) bioaccumulation in controlled laboratory conditions (Pipiška et al. 2005). In the study, it was noted that caesium intake was achieved at an optimum 20 °C and a pH range of 4.0–5.0 and was lower in the presence of metabolic inhibitors.

3.6.2 The Intracellular Metal Uptake

The content of intracellular metals in lichen thallus has been reported to be relatively stable over time, and at the same time, the binding of extracellular metals and particle capture may continue (Bačkor and Loppi 2009). Experiments made on *Ramalina* for Cu (Branquinho et al. 1999) and for *Peltigera* on Cd (Brown and Beckett 1984) have shown that the intracellular metal uptake is much slower and less in contrast to the extracellular uptake.

Intracellular metal uptake has been shown to be stimulated by light and closely related to metabolism. It has been reported that the intracellular Cd uptake in the dark decreases very rapidly. Light-induced Cd uptake represents active entry into the algal cells, but it is unclear as to which symbiont plays a role in the intake and whether requires energy in the dark. Intracellular uptake is also dependent on the species. Metals in the live and heat-killed lichen trusses also differ according to species (Garty 2001).

Interactions between lichens and heavy metals have been examined in the Cd^{2+}- and Ni^{2+}-containing solutions of cyanolichen *Peltigera rufescens* and green algal lichen *Cladonia arbuscula* subsp. *mitis* growing on old copper mine-spoil heaps in Slovakia by Bačkor et al. (2010), in terms of ultrastructural changes as well as physiological parameters such as membrane integrity, pigment composition, chlorophyll a fluorescence, photosynthesis, respiration, contents of ATP, amino acids, ergosterol, ethylene, nonprotein thiols, activity of antioxidant enzymes and expression of stress proteins. The results of the study showed that toxicity of the non-redox active metals Cd and Ni was lower than the redox-active metal Cu on these lichen species, with physiological findings containing no significant sensitivity.

3.6.3 Location of Metals in Mycobiont and Photobiont

Various studies using TEM (transmission electron microscope), SEM (scanning electron microscope) and EDX (energy-dispersive X-ray) have found that Cu and Zn are present in different parts of photobiont and mycobiont cells depending on the species. For example, dark rhizines and veins in *Peltigera* species contain high amounts of metal in metal-rich habitats. The rhizines in *Peltigera canina* are responsible for metal absorption, accumulation, displacement and regulation. The high metal content of this lichen's medulla and rhizines causes a considerable loss of potassium (K). The metal deposition capacity of the *Peltigera* species was found to be maximum level for Fe, Mn and Pb in their rhizines (mycobiont) and for Cu, Ni and Zn in the photobiont parts (Garty 2001).

X-ray microanalysis applications have shown that Fe deposition builds up in certain lichens (e.g. *Acarospora smaragdula*) an outer crust covering the upper cortex and accumulation gradually decreases from the upper cortex towards algae layer, medulla and lower cortex. When SEM combined with EDX analysis, visual and quantitative information about the locations of the elements in the thallus is provided. A study conducted in this way in *Hypogymnia physodes* showed that Fe and Al bind to the algal layer and algae-containing soredia more than medullary hyphae in the first stage (Farkas and Pátkai 1989).

Trapelia involuta, which is formed directly on secondary uranyl minerals and U-enriched iron oxide and hydroxide minerals, accumulation of U, Fe and Cu is concentrated on the apothecium exciple and epithecium. As a result of the TEM investigation, it is found that the distribution of U, Cu and Fe and that of the melanin-like pigments are strongly correlated to one another, not mineral particles or organic crystals (Kasama et al. 2003).

Piovar et al. (2011) assessed the long-term effect (14 days) of copper (Cu) on the levels of intracellular and total accumulation, growth, assimilation pigment composition, chlorophyll a fluorescence, soluble protein content and oxidative status (production of hydrogen peroxide and superoxide) in two algal species (free-living alga *Scenedesmus quadricauda* and the lichen alga *Trebouxia erici*). The presence of Cu negatively affected growth, assimilation pigments, chlorophyll a fluorescence, soluble protein content and oxidative status in both algae. However, *Scenedesmus* was much more sensitive compared to *Trebouxia*.

3.7 Methods Used in Biomonitoring with Lichens

Due to the rapid growth of the population, industry, agriculture and technology in the world, environmental pollution has inevitably come to the fore. In the settlement areas where atmospheric pollution is noticed, various methods applied locally have to be developed depending on the pollutant types (SO_2, radionuclides, heavy metals, etc.) and levels, and some of them are becoming more common nowadays. Since the emergence of the idea that measurable, long-term, cheap and reliable results can be obtained by means of biological methods, work has been increasingly carried out in the determination and monitoring of the quality of the environment. In most countries, a limited number of air pollution measurement stations in large cities and industrial areas record the concentration of particulate matter and SO_2 per day. However, it is not usually found in places with moderate pollution. Assessing the level of pollutants' effects on vegetation has been arised as an alternative approach in assessing air pollution. Estimates based on epiphytic vegetation show that lichens that grow very slowly and live for a long time may be much better indicators of quantitative levels of air pollution than quantitative chemical measurements made in a certain time period as being communities that can make more equilibrium with their environment.

In recent years, various methods were proposed for assessing environmental quality (air pollution) on the basis of lichen data. Qualitative methods can be converted into quantitative methods by quantifying the number of species in the region, the distributions and the frequency of species at the same time. Quantitative methods are generally applied by elemental analysis instruments and statistical analyses to determine the levels of pollutants (metals and radioactive substances) that accumulate in lichen thalli.

Atmospheric pollutants cause acute morphological and usually physiological damage on lichens. Chronic harms develop after prolonged or repeated exposure to pollution, which is a slowing, growth-related disorder of more species than damage to tissues. It results in the disappearance of susceptible species in population at the community level (Çobanoğlu 2015).

Lichen biomonitoring methods can be classified in different forms according to the purpose and content of the application. According to Huckaby (1993), two analytical methods can be used to quantitatively determine the response of lichens to airborne pollutants and to assess their susceptibility: (1) gradient analysis study and (2) fumigation study. In the gradient analysis study, harmful effects of contamination grades on lichens exposed to pollution, measurable environmental effects and quantities in species richness are analysed. It is usually studied around a pollution source. Species can best be examined in their own environment because not only air pollution but also climate and substrate-related properties (such as fire, grazing animals) also affect lichens. In the fumigation study, the quantifiable responses of the lichens exposed to the pollutants in a closed system under laboratory-controlled conditions are generally examined for physico-chemical events.

Various methods implemented within the scope of air quality monitoring studies using lichens up to date can be classified (Çobanoğlu 2015) mainly under the following headings:

A. Passive biomonitoring
   1. Method based on whole lichen floras (general lichen study and mapping method)
   2. Method based on bioindicator species and IAP (index of atmospheric purity) method
   3. Quantitative laboratory analysis (multi-element and radionuclide analysis)

B. Active biomonitoring
   1. Transplantation
   2. Controlled fumigation
   3. Culturing

While determining air quality, "active methods" result in controlled effects of pollutants directly on lichens under controlled conditions, whereas "passive methods" result in determining amounts accumulated in lichens or by monitoring the natural lichen floras, which are affected by pollutants (Hoodaji et al. 2012).

3.7.1 Passive Biomonitoring Methods

The first two passive methods are qualitative (indirectly quantitatively expressed) assessments based on the distribution of lichen species that constitute the general lichen flora or selected as bioindicators. The other can be based on quantitative analyses that can determine the quantities and effects of air pollutants in the lichen to be measured directly (Huckaby 1993). In principle, the first two methods for assessing contamination in a region can be applied alone or in combination with quantitative methods at the same time. However, in order to obtain more specific areas and levels of pollutant types, it is necessary to use the passive biomonitoring method which is applied to content analysis. Multi-element analysis is at the forefront of this (Çobanoğlu 2015).

For example, when the "Calibre Lichen Bioindication Method" is used together with the index of atmospheric purity (IAP) method, which is based on regression analysis, in the statistical evaluation of the frequency of lichen species selected in a region, the indication of the integrated air pollution problem can be detected early (air pollution early warning system) (Herzig et al. 1989). In Switzerland, where these two methods are applied comparatively, very common foliose lichen *Hypogymnia physodes* has been shown as the best bioindicator species for various emission components (SO_2, NO_x, O_3, heavy metals, pesticides and other organic compounds). The area is divided into five groups of lichens (lichen desert, internal strength zone, outer strength zone, transition zone and normal) parallel to five regions (critical, high, medium, low, very low air pollution) in terms of the total emission. Eventually Pb, Fe, Cu, Cr, Zn, total S and P elements decreased with decreasing pollution level and showed a negative relation with IAP. With the decrease in total air pollution, the only element that increased in lichen content was calcium (Ca) and showed a positive correlation with increasing IAP values.

1. Method based on whole lichen flora (general lichen study and mapping method). Repeated studies in the general lichen study show changes in species distribution in the same region. It is an easier method of monitoring than others, and it shows the change in the quality of the air over time in large areas.

The species distribution maps are performed by squaring. It is similar to the method of classical species mapping. Mostly corticolous (living on bark) lichens are studied, but if not available, saxicolous (living on rock) and terricolous (living on soil) species are studied. It requires an expert lichenologist to be based on all lichen flora. This method is suitable for the monitoring of air quality in a wider area, for a pollution-source environment and for ecologically variable and complex locations. “Mapping method” or “distribution map” is, according to many researchers, the best method of biomonitoring for areas with moderate-to-high air pollution (Gilbert 1970; Hawksworth and Rose 1970; Huckaby 1993; Showman 1988) and is still valid today.

Observations made independently of each other in England, Munich and Paris show that in the 1800s, the likeness disappeared in urbanized regions. The burning of coal to warm up homes and workplaces created smoke clouds over the cities, and this air pollution caused the lichens to disappear. Sernander’s classical work in Stockholm was followed up after the city influence was noticed in Europe during the 1900s (Sernander 1926). According to this, the city centre, which has almost no lichen, is called “lichen desert”. “Struggle zone” is a region in which some of the species live well. The “normal zone” is the area where species live without being affected by pollution. Such zoning studies have begun to spread around cities. The identification of species by mapping studies and quantitative techniques and the identification of air pollution communities have entered into a rapid development process. In 1930, the colourless gas “sulphur dioxide” (SO_2) was now well recognized as a phytotoxic agent. In the mid-1970s, experimental studies have shown that many lichens are SO_2 sensitive. Species such as *Lobaria pulmonaria* have disappeared in many areas. Species such as *Lecanora conizaeoides*, which are able to tolerate pollution, have been found to have spread widely (Nash and Gries 1991).

It has been argued that Hawksworth and Rose’s (1970) approach to creating general zones based on the number and quantity of species is a method that can be applied quickly considering the species richness, which does not require much information. The zone consisting of epiphytic lichen species indicates which species are distributed in SO_2 pollution at which point in the region. However, when the scale is being constructed with this method, care should be taken not to apply it in places close to other sources of pollution, such as keeping the number of examined trees as high as possible.

The richness of epiphytes in a locality suggests that the air there is clean, but the opposite may not be true (LeBlanc and De Sloover 1970). Gilbert (1970) thought that using groups instead of individual species for mapping would produce a more accurate picture. The distribution of lichen species in the region can be mapped by showing different colours (depending on pollution zones). Wirth (1988) suggests that the methods used to measure the susceptibility of lichens to air pollution should be addressed not only by monitoring changes in floristic species but also by a phytosociological approach, which will also be observed in lichen communities.

2. Method based on bioindicator species and IAP (index of atmospheric purity). Working with indicator species is the study of one or more of the known species susceptible to air pollution and other parameters by quantitative methods.

The advantages of this method are the identification of several indicator lichen species (especially fruticose and foliose) assigned to species and only the time-dependent development of these species in the field. In addition, these species will provide the most effective result in the problem of air pollution. It is a method that does not require advanced technology and complicated analyses, is inexpensive, has few staff, can be done in a short time and generally gives more reliable results. On the other hand, it should be well defined which species are sensitive indicator species in that region. For this purpose, gradient analysis studies and fumigation applications are made to select pollution-sensitive species correctly. A method based on measurements may also be applied in order to precisely and sensitively detect the change in pollution in time in the lichen communities. Thus, the species can be monitored for modification by quantitative analysis. In the quantitative analysis studies, tables are formed by giving the values according to frequency ratings. These values can then be converted to % ratios. However, some of the disadvantages of this method are that it is exhausting, the need for skilled workers for continuous and seasonal data during the year and the possibility that the results are influenced by many other factors (Huckaby 1993).

The index of atmospheric purity (IAP) established by LeBlanc and De Sloover (1970) is an ecologically based index, based on the incidence and frequency of lichen formation in the sample. The reliability of IAP studies increases the proportion of susceptible species recorded on the ground. The lower the proportion of susceptible species, the more trees should be examined in the area. However, if the total number of species in a field is low, more trees cannot be recovered by examining them. The IAP values of all fields are plotted on a draft map of the study area (Showman 1988). Values are usually grouped by IAP range. The completed IAP map, if any, can be compared to the emission points and air quality data of that zone. The IAP regions primarily reflect species richness and are very useful if the same tree types are studied within the study area (Garty 2001). Numerical values of species distribution by locality and frequency level can be obtained. Maps showing the pollution-related lichen distribution generated by such studies and index of atmospheric purity (IAP) maps are used (Herzig et al. 1989).

Each type of lichen separated by pollution levels has a bioindicator role and may naturally vary from region to region. However, it is also possible to make some generalizations. Especially because they are more sensitive, epiphytic lichens are often used as indicators. An industrial field of so-called epiphyte desert has been questioned as to whether it can be taken as an indicator (Van der Gucht and Hoffmann 1990). According to this, saxicolous species was found to be less susceptible than corticolous species, determined by distribution maps of *Lecanora dispersa* and *Xanthoria parietina* in the same areas. Zones were formed by a sequence in which saxicolous and epiphytic species coexist. As a result, distribution maps of corticolous and saxicolous lichens are very similar and thus reveal sources of pollution in the region. In another study (Showman 1975), *Flavoparmelia caperata* and *Punctelia rudecta* species were used as bioindicators, and distribution maps were presented. In different studies, the indicator lichen species also show differences.

In another study, two lichen species – *Xanthoria parietina* and *Ramalina canariensis* – compared in terms of biomonitor performance against atmospheric dioxins and furan (PCDD/Fs) toxic organic compounds yielded significantly different results. More chlorinated PCDD/Fs and metals were better captured and accumulated by *X. parietina* than *R. canariensis* (Augusto et al. 2009).

According to the survey in Italy (Nimis et al. 1990), *Flavoparmelia caperata* was reported to be the best indicator, whereas *Leprocaulon microscopicum*, *Lepraria incana* and *Haematomma ochroleucum* (probably due to waterproof crustose thalli) were the most tolerant species to air pollution, based on the relationship between sulphur dioxide (SO_2) pollution and IAP lichen diversity (frequency of the species in sample area).

Some lichen species belonging to fruticose *Usnea* and *Ramalina* and foliose species such as *Parmelia* and *Lobaria*, which have a relatively larger surface area, are the most sensitive species. In particular, sensitivity lists can be created for lichen species in some countries where the lichen flora is complete or nearly complete checklists and their distribution is determined.

3. Quantitative laboratory analysis (multi-element and radionuclide analysis). Analyses of species selected as biomonitor from lichens that have spread to a region are used to measure and monitor the accumulation of atmospheric elements in a given time interval.

Concentrations accumulated in lichen samples are considered to indicate air pollutant rates. Prior to analysis, samples are cleaned of dust and other substances, and then are passed through grinding and acid dissolution pre-treatments by being protected from contamination. Analytical measurement is then made available for devices such as atomic absorption spectrophotometer (AAS) or mass spectrometry (MS). IAEA-336 lichen (*Evernia prunastri*), developed by the International Atomic Energy Agency (IAEA), is frequently used as an international reference material in such air quality monitoring surveys with lichens (Conti and Cecchetti 2001). How to prepare the multi-element reference material used for environmental pollution studies was explained by Freitas et al. (1993). Spatial distributions of the element levels measured by multi-element analysis are shown on maps prepared with computer software such as ArcGIS, Grass GIS and Surfer using geographic information system (Doğrul Demiray et al. 2012; Bustamante et al. 2013; Paoli et al. 2015; Çobanoğlu and Kurnaz 2017).

Epiphytic macro-lichens, with generally common distributions, foliose or fruticose characters to have large broad surfaces, are among the most preferred species in biomonitoring studies. “Species specificity” is an important phenomenon in lichen biomonitoring surveys. The property of “widespread and continued growth” of the lichens was stated as the main reason for selecting the indicator taxa in environmental monitoring studies (Siddig et al. 2016).

Based on the worldwide current studies on biomonitoring with lichens (25 of the papers cited in this review) (Table 3.1), the number of how many times a lichen species used is as follows: *Pseudevernia furfuracea*, 7; *Evernia prunastri*, 5; *Hypogymnia physodes*, 4; *Parmelia sulcata*, 3; *Xanthoria parietina*, 2; *Phaeophyscia hispidula*, 2; *Favoparmelia caperata*, 1; and species of the genera *Cladonia*, 4; *Physcia*, 3; *Parmotrema*, 2; *Ramalina*, 2; and *Usnea*, 2, though it actually varies by country (Fig. 3.1). Accordingly, it is clear that epiphytic fruticose and/or foliose species as best biomonitoring agents are at majority.

Studies on radionuclides are usually made by measuring caesium and uranium concentrations with the gamma-ray spectrometer (GRS). Many studies have been conducted to evaluate the effects of the Chernobyl nuclear accident (White et al. 1986; Gaare 1987; Feige et al. 1990; Taylor et al. 1988; Başkaran et al. 1991; Biazrov 1994). From these studies, it is concluded that lichens may have important bioindicator roles that show the lasting effects of radioactive contamination.

In a radioactive monitoring study first made in Turkey (Topçuoğlu et al. 1992), measurements (caesium radionuclides, ^{134}Cs and ^{137}Cs) performed in a variety of lichen samples before and after the accident showed that radioactive contamination was large in all samples, especially at higher levels in the eastern Black Sea Region.

In a recent study in Salzburg, caesium (^{137}Cs) radionuclide were measured in *Cladonia fimbriata*, *Cladonia squamosa*, *Pseudevernia furfuracea* and *Hypogymnia physodes* lichens and some species of mosses by gamma spectrometry, and the rates were reported to be quite high even after 20 years after the accident (Iurian et al. 2011).

In an attempt to determine the level of environmental uranium in the Balkan countries, measurements were made on *Pseudevernia furfuracea*, *Evernia prunastri*, *Ramalina fastigiata* and *Cetraria islandica* lichen samples, which showed a very high concentration of *Evernia* collected from Greece but not a common contamination in the Balkans (Loppi et al. 2003).

Fig. 3.1 Cosmopolitan-spread lichen species that can survive moderate or even more air pollution, *Xanthoria parietina* and *Physcia* sp. (Photo G. Özyiğitoğlu)

3.7.2 Active Biomonitoring Methods

Active biomonitoring is the monitoring of air quality with lichens transported to a region suspected of pollution from the natural environment (with the response given by lichen) (Huckaby 1993). In some cases, active monitoring may be performed in nature or may be achieved by applying contaminated source-controlled fumigation (Hoodaji et al. 2012). It must be planned and repeated absolutely carefully.

1. Transplantation. Transplantation is taking the lichen from its natural location and transporting it to the area where air pollution will be monitored.

It is studied in a region that has already been affected by air pollution (Çobanoğlu 2015). This method is currently applied in many studies (Malaspina et al. 2014; Paoli et al. 2015; Lucadamo et al. 2016). The method of hanging transplanted specimens in locations exposed to pollution in nylon mesh bags (approximately 4 m above the ground) is referred to as "bag technique" (Adamo et al. 2008; Caggiano et al. 2015; Petrova et al. 2015).

The methodology of transplantation with *Hypogymnia physodes* around a copper mine in Russia and the distance effect to the source have been investigated (Williamson et al. 2008). Particle sources have been identified as mine-smelting and converter, flotation wastes, metallurgical slags, local road dusts and suspended particles in the air above and above the ground, and which were more effective was investigated. Accordingly, it was concluded that the mine melting furnace (Cu and

Fe is the highest) is the most effective source (<10 km) for transplants. The particulate matters, which remain longer in the atmosphere and higher in terms of Pb and Zn, have spread in a wider area as a powder from the converter. It has been determined that the mine has an area of influence of about 30 km.

Another study in which lichen *Usnea barbata* was used as a transplant showed that in the samples analysed at the end of 1 month and 1 year, of 26 elements, K, Mg and Mg were found at higher concentrations while Al, Ca, Co, Cr, Ni and Na with lesser levels. This lichen has demonstrated the ability to reflect the atmospheric level of the background in the selected field (Conti et al. 2012).

2. Controlled fumigation. The fumigation work shows the measurable response of the lichens exposed to pollutants in a closed system (with air circulation) under controlled conditions. For example, closed and controlled systems such as continuously stirred tank reactors, open-top cabinets, sectional cabinets and miniature tub cabinets are used.

The most common responses measured are selected from physiological events, for example, according to the order of sensitivity to pollution, nitrogenase activity, K + output/total, electrolyte flow, photosynthesis, respiration and pigment status, etc. (Huckaby 1993).

3. Culturing. This method is designed for the purpose of observing the effects of contamination at the controlled conditions by replicating cultured species more rapidly. However, mycobionts and photobionts isolated from lichens cannot be actively applied yet because they do not achieve the desired success in laboratory culture studies (since they do not show growth and development beyond a certain stage).

Culturing steps; first, the separate cultures of the algae and fungi partner, then the synthesis and development of the new lichen unit by bringing them together (Toma et al. 2001). Mycobiont can be derived from reproductive parts, from the spore or from the thallus propagules (Yamamoto et al. 2002). In vitro culture methods of lichen thalli and isolated symbionts (algae and fungi) can be improved according to the present scientific requirements, and it is possible to achieve better performance for different purposes.

3.8 Lichen Monitoring in Sustainable Air Quality Management

Urban air pollution with SO_2, NOx, CO, volatile organic compounds (VOCs) and particulate matter is still a serious problem in developed or developing countries. PM pollution refers to all solid and liquid particles in air which are harmful particularly due to trace metal content. In general, the main sources affecting air quality are vehicle traffic, domestic heating and industrial facilities, as well as agricultural activities.

Over the years, a great deal of forest ecosystems have been destroyed and even destroyed, despite some precautions, due to various human activities such as fires, tourism and construction. This leads to radical changes such as an increase in marginal effects in the forests, microclimate changes and loss of forest environment. The degradation of forest ecosystems affects forest biodiversity negatively after numerous reductions and extinctions. Biodiversity is an important component that governs ecosystem resistance, dynamism, balance and productivity (Shukla et al. 2014).

In order to address environmental issues and ensure sustainable development, an integrated effort should be made to identify local, regional and global resources and address a wide range of environmental issues. Sustainable development is a concept in which basic human needs are fulfilled conserving the natural environment for future generations without destroying or depriving their natural systems.

Biomonitoring is such an affordable and reliable method for monitoring ongoing environmental problems. It is now very well-known that lichens affected by atmospheric changes with time reflect the air quality. Consequently, understanding the temporal trends of biomonitoring and examination of the effects of urban pollutants on the lichen organism under stress conditions seems important and valuable.

Economically feasible, socially acceptable and environmentally sound, applicable management and monitoring methods should be developed for the determination and follow-up of air quality. In addition to periodic chemical measurements, biomonitoring studies with lichens, alone or in alternative and supportive applications, should not be neglected to monitor changes in air quality. Biological monitoring data indicate the presence and changes of pollutants in the environment. Biomonitoring species ensure data for the spatial distribution of the concentration of contaminants in areas.

3.9 Conclusions

After understanding that certain lichen species are susceptible to air pollution (SO_2) at different grades, the field of study and application of biological monitoring of air quality directly with living cells has been increasing steadily. “Natural living creatures for environmental quality monitoring” is a more naturalistic approach to the environment. Various methods have been developed to biologically measure by means of bioindicators such as lichen. Although it is not a complete standard for the application of these biological methods, it has been seen that, when the right selection is made, it can be used as an alternative to air pollution measuring stations, which are usually limited to large cities and industrial areas. Atmospheric concentrations of various pollutants (trace elements, heavy metals, radionuclides and organics), not only sulphur dioxide, can be determined by analysis on biomonitor lichens, so that periodic follow-up of air quality from the biologic route is possible.

Some of the important conclusions that can be drawn from the studies on the monitoring of the air quality with lichens mentioned here are as follows:

1. The biostructural properties of lichens are significantly effective at the rate for retaining elements from the atmosphere.
2. Selection and application of the most suitable biomonitoring method(s) to the working region are very important in reaching the accurate results.
3. While the likelihoods of selected lichens for analysis in one area are expected to reflect exactly the concentrations of atmospheric contaminants, the effects of the various microhabitat, substrate or climatic factors affected by the scratch and the instantaneous anthropological factors are ignored. In this respect, the average element rates obtained from a large number of samples may yield more reliable results. Factors affecting the susceptibility of lichens in the outcome evaluation of these studies should also be considered as a whole.
4. Qualitative observations are also necessary because it may be difficult to keep the variables in the areas where the quantitative analysis samples are collected. For example, identification of lichen species diversity in an area and tracking of changes in lichen flora for many years will provide supportive data in determining the level of pollution.
5. Careful study of the regulatory mechanisms in lichens for bioavailability, accumulation, toxicity and heavy metal detoxification is necessary.

The use of genotoxicity tests can improve the more efficient use of lichens as biomonitor when monitoring air pollutants. In-species genetic diversity analyses will clarify the responses of species to different environmental and experimental conditions and at the same time contribute to a better understanding of the variability of tallus metal content in a single species (Bačkor and Loppi 2009).

Physio-biological events such as respiration, photosynthesis and nitrogen fixation occur in the metal centre depending on various oxidative metalloproteins or metalloenzymes, for example, iron in cytochromes and haemoglobin, copper in amine oxidase and superoxide dismutase, manganese in photosystem II, molybdenum in nitrogenase, cobalt in vitamin B12 coenzyme and nickel in bacterial dehydrogenases. The oxidation state also governs the toxicity of the elements. On the other hand, it is known that metals are excreted by sweat, saliva and tear secretions through urinary tract, bile pancreatic and small intestinal evacuation routes. For most metals, the best defined one is the urine path, and there is little information about the others. The concentrations of most metals in blood (all blood, plasma, serum) and in urea reflect the exposure. Depending on the location within the body or the half-life, the elimination can be determined by these parameters; for example, some heavy metals such as Pb and Cd may remain in some tissues for years (Nieboer et al. 1999).

The decrease in the number of species of a live group suffering from air pollution in vegetation will certainly affect other living groups as well. If one of the circles in the food chain is affected by pollution, it ultimately reaches people by affecting others those connected to it (e.g. animals that feed on or feed from it and then other animals that feed on it). Thus, pollution will directly harm human beings both directly and through impairment of natural balance. In this respect, the methods developed by the approach of qualitative and quantitative determination of the level of pollution effects on vegetation can be used as an early warning system against pollution.

In Western Europe, an increase in epiphytic lichens was observed in response to global warming, whereas a decrease in terricolous ones was recorded. Rising numbers of *Trentepohlia* algae and southern-spread lichen species have been reported to be increased at a faster rate in the areas affected by pollution than the forests (Aptroot and Van Herk 2007). Changes in the lichen flora in 22 years (1989–1995) in Switzerland have been investigated in a study by Van Herk et al. (2002): during the years when the temperature increases became more evident, NH_3 increased compared to the decrease of SO_2. It has been concluded that subtropical species show a great increase (83%), while arctic-alpine/boreal species show a decrease of about 50%. Monitoring the long-term changes in the lichen flora will provide valuable evidence that not only will the air quality of the region be followed, but also the response of the nature against global warming, when the methods in which climatic data are combined in the following years.

Biological monitoring has a strategic precaution in nature-environment-human relationships. The use of biological monitors as supportive or alternative to expensive and instantaneous measurements will provide significant contributions to addressing the challenges posed by viable health, especially in urban and industrial space planning and the adverse effects of rapidly developing technology.

This is a comprehensive review of the role of lichens in biomonitoring environmental quality in the last 45 years ranging from the first studies that began in the 1970s to the present day, and it is a reference source for future work in this regard.

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