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Environmental Pollution 114 (2001) 471–492

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Biological monitoring: lichens as bioindicators of air pollution assessment — a review

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Received 24 March 2000; accepted 1 November 2000

"Capsule": *Lichen species may be used as biological monitors for environmental prevention programs.*

Abstract

Often as part of environmental impact studies and, above all, to obtain authorisations in accordance with prescriptions from the Ministry for the Environment (Italy), surveys and controls that use biological indicators are required. This is because such indicators are valid instruments for evaluating the quality of the air ensuing from the subject (often an industrial plant) of the Environmental Impact Assessment (EIA). In this context, this paper aims to analyse some of the theoretical aspects of biological monitoring and to provide a progress report on the use of lichens as bioindicators of air quality, with a particular eye to the situation in Italy. The object of this paper is that of pointing out the most important lines in the current state of knowledge in this field, evaluating the methodological applications and their advantages/disadvantages with respect to traditional surveying methods.
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Keywords: Lichens; Biological monitoring; Air pollution; Environmental impact assessment; Italy

1. Biological monitoring

The use of cosmopolite organisms to assess pollution has developed notably during the last few decades. Such organisms assume environmental contaminants and may be used as indicators of the bioavailability of a given contaminant over time, allowing, in certain cases, comparison between contamination levels in geographically different areas.

It is in this context that OECD countries have taken many initiatives for examining potentially dangerous products by proposing general programmes for the monitoring and evaluation of environmental impact (Tessier et al., 1980; Connell, 1986; Herman, 1987; Krumgalz, 1989; Bero and Gibbs, 1990).

From an ecotoxicological perspective, we can consider as contaminants or producers of environmental stress, all chemical compounds that are fundamentally released into the environment as a result of human activities, and which cause damage to living organisms (Moriarty, 1999).

In general, bioindicators are organisms that can be used for the identification and qualitative determination of human-generated environmental factors (Tonneijk and Posthumus, 1987), while biomonitors are organisms mainly used for the quantitative determination of contaminants and can be classified as being sensitive or accumulative.

Sensitive biomonitors may be of the optical type and are used as integrators of the stress caused by contaminants, and as preventive alarm systems. They are based upon either optical effects as morphological changes in abundance behaviour related to the environment and/or upon chemical and physical aspects as alteration in the activity of different enzyme systems as well as in photosynthetic or respiratory activities.

Accumulative bioindicators have the ability to store contaminants in their tissues and are used for the integrated measurement of the concentration of such contaminants in the environment. Bioaccumulation is the result of the equilibrium process of biota compound intake/discharge from and into the surrounding environment.

The first studies of bioindicators date back to the 1960s. Beginning with the theoretical calculations of

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0269-7491/01/$ - see front matter © 2001 Elsevier Science Ltd. All rights reserved.
PII: S0269-7491(00)00224-4

Stöcker (1980) and Phillips (1977, 1980), we can define the main characteristics of a bioaccumulator.

Bioaccumulators must:

1. accumulate the pollutant without, however, being killed by the levels with which it comes into contact;
2. have a wide geographical distribution;
3. be abundant, sedentary, or of scarce mobility, as well as being representative of the collection area;
4. be available all year round and allow for the collection of sufficient tissues for analysis;
5. be easy to collect and resistant to laboratory conditions, as well as being usable in laboratory studies of contaminant absorption, if necessary;
6. have a high concentration factor for the contaminant under study, and thus allow direct analysis with no prior increase in concentration;
7. have a simple correlation between the quantity of contaminant contained in the organism and the average contaminant concentration in the surrounding environment; and
8. have the same contaminant content level correlation with the surrounding environment in every site studied and under any condition. This must be true for all organisms examined.

2. The problems of biomonitoring

For a variety of reasons, it is of fundamental importance to define the reference levels for pollutants in an ecosystem when making biological monitoring studies to:

1. evaluate the state of conservation or degradation;
2. predict the incidence of possible future human activities in order to establish the necessary interventions; and
3. control evolution over time, using monitoring programmes, if necessary.

To correctly evaluate the degree of contamination in an ecosystem, or to carry out biomonitoring operations, it is necessary to first establish the background level of the contaminant, both in the environment (air, water, soil), and in the organisms. The background level may be interpreted in different ways: it may be understood as a pre-industrial level (prior to any human activity); as a natural level (the average conditions of an area or a region where there may be human activity, but which is in a good state of conservation); a standard level (based upon global geographical references); or even a zero level (the concentration of an element in the environment or in an organism prior to the development of a particular activity that is independent of the degree of conservation; Carballeira et al., 2000; Cecchetti and Conti, 2000).

Once the background level has been established according to Carballeira et al. (2000), the contamination factor may be used to evaluate the state of conservation of an ecosystem, or to monitor its state. This is the relationship between the level of a contaminant found in the biota or environment and a reference value that represents a determined stage (pre-industrial, natural, zero):

$$\mathrm{CF_b} = C_\mathrm{b}/\mathrm{BL_b} \quad \text{or} \quad \mathrm{CF_a} = C_\mathrm{a}/\mathrm{BL_a}$$

where CF = the contamination factor for the biota ($_\mathrm{b}$) or the environment (air, water, soil) ($_\mathrm{a}$); C = the concentration of contaminant in the biota ($_\mathrm{b}$) or in the environment ($_\mathrm{a}$), respectively; BL = the background level of the pollutant in the biota ($_\mathrm{b}$) or in the environment ($_\mathrm{a}$), respectively.

If the background level is a reference of the zero phase, it will allow us to observe the evolution of a pollutant (in terms of both space and time), during a contamination process. This concept may also be used to observe the decontamination rate in an ecosystem (positive impact).

The CF is said to have been corrected when:

$$\mathrm{CF_{corrected}} = \mathrm{Cb_2}/\mathrm{Cb_1}$$

where $\mathrm{Cb_1}$ = the concentration of pollutant present in the biota at the time or the point 1; $\mathrm{Cb_2}$ = the concentration of pollutant present in the biota at the time or the point 2;

This signifies that the CF has been corrected when, during comparisons between different environmental situations, no data is available for the background levels (BL).

The system for environmental classification is realised by starting with the *Contamination Factors* obtained for each contaminant present in the environment or organisms. When evaluating the CFs obtained, it is also necessary to take into account the uncertainties that derive from the following: sampling; space and time variations for the samples; the age and condition indexes of the organisms, etc. In general, a CF that is above a given number (generally 1.5, 2, or 3 times the BL), is taken to be the minimum level under which it is no longer possible to refer to certain contamination. The qualification of a contamination situation may follow a linear scale, or, in high-level pollution conditions, a scale of the exponential type.

3. Lichens as bioindicators of air pollution

Lichens are considered the result of a symbiotic association of a fungus and an alga. More precisely the term "alga" indicates either a Cyanobacteriae or a

Chlorophyceae; the fungus is usually an Ascomycetes, although on rare occasions it may be either a Basidiomycetes or a Phycomycetes.

In this association, the alga is the part that is occupied with the formation of nutrients, since it contains chlorophyll, while the fungus supplies the alga with water and minerals. These organisms are perennial and maintain a uniform morphology over time. They grow slowly, have a large-scale dependence upon the environment for their nutrition, and, differently from vascular plants, they do not shed parts during growth. Furthermore, their lack of cuticle or stoma means that the different contaminants are absorbed over the entire surface of the organism (Hale, 1969, 1983).

As far back as 1866, a study was published on epiphytic lichens used as bioindicators (Nylander, 1866). Lichens are the most studied bioindicators of air quality (Ferry et al., 1973). They have been defined as "permanent control systems" for air pollution assessment (Nimis et al., 1989).

During the last 30 years, many studies have stressed the possibility of using lichens as 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 (Brodo, 1961; Rao and LeBlanc, 1966; Schönbek, 1968; Hawksworth, 1971; Gilbert, 1973; Mendez and Fournier, 1980; Lerond, 1984; St Clair and Fields, 1986; St Clair et al., 1986; Galun and Ronen, 1988; Showman, 1988; Nimis, 1990; Oksanen et al., 1991; Loppi et al., 1992a; Seaward, 1992, 1996; Halonen et al., 1993; Gries, 1996; Loppi, 1996; Hamada and Miyawaki, 1998). For indeed, many physiological parameters are used to evaluate environmental damage to lichens, such as: photosynthesis (Ronen et al., 1984; Calatayud et al., 1999); chlorophyll content and degradation (Kardish et al., 1987; Garty et al., 1988; Balaguer and Manrique, 1991; Zaharopoulou et al., 1993); decrease of ATP; variations in respiration levels (Kardish et al., 1987); changes in the level of endogenous auxins; and ethylene production (Epstein et al., 1986; Garty et al., 1993).

Furthermore, laboratory exposure to SO_2 causes relevant membrane damage to lichen cells (Fields and St Clair, 1984). Many studies show a positive correlation between the sulphur content of lichens and SO_2 present in the atmosphere (Takala et al., 1985; Rope and Pearson, 1990; Silberstein et al., 1996).

Various authors report that the concentration of chlorophyll $a+b$ is altered by vehicle traffic pollution (LeBlanc and Rao, 1975; Ronen and Galun, 1984; Carreras et al., 1998), and by urban emissions (Zambrano and Nash, 2000). In general, lichens that are transplanted into areas with intense vehicle traffic show an increase in chlorophyll $a+b$ concentration that is proportional to increases in emissions. Such effects are generally caused by traffic emissions and in particular, sulphur and nitrogen oxides. In areas with intense vehicle traffic and elevated levels of industrial pollution, high values are obtained for Chl b/Chl a ratios.

Air traffic, and in particular the effects of kerosene and benzene, seems to have a lesser effect on the lichen population than vehicle traffic. This has been demonstrated in a study of Hamburg airport (Rothe and Bigdon, 1994).

Lichens may be used as bioindicators and/or biomonitors in two different ways (Richardson, 1991; Seaward, 1993; Gries, 1996):

1. by mapping all species present in a specific area (method A); and
2. through the individual sampling of lichen species and measurement of the pollutants that accumulate in the thallus; or by transplanting lichens from an uncontaminated area to a contaminated one, then measuring the morphological changes in the lichen thallus and/or evaluating the physiological parameters and/or evaluating the bioaccumulation of the pollutants (method B).

4. Lichens in the control of environmental contamination

4.1. The index of atmospheric purity (IAP)-method (method A)

The compositional changes in lichen communities are correlated with changes in levels of atmospheric pollution. The application of method A allows the elaboration of an IAP. This method (LeBlanc and De Sloover, 1970) makes it possible to map out the quality of the air in a determined area. The IAP gives an evaluation of the level of atmospheric pollution, which is based on the number (n), frequency (F) and tolerance of the lichens present in the area under study. There are twenty different formulae for IAP calculation, and these are able to predict, to a good level of approximation, the degrees of eight atmospheric pollutants measured using automatic control stations (SO_2, NO_x, Cl, Cd, Pb, Zn, and dusts; Amman et al., 1987).

The formula with the highest correlation with pollution data is that which considers as a parameter only the frequency (F) of the lichen species present in a sampling network comprising 10 areas:

$$\mathrm{IAP} = \sum \mathrm{F_i}$$

F is the frequency (max. 10) of every ith species that is calculated as the number of rectangles in the grid (a rectangle of the dimensions 30×50 cm, subdivided into 10 areas measuring 15×10 cm each), in which a given species appears (Herzig and Urech, 1991). It has been shown that the frequency method makes it possible to predict pollution levels with a certainty of over 97% (Lo Porto et al., 1992; Gottardini et al., 1999).

Method A foresees a choice of sampling stations on the basis of the presence of suitable trees on which it is possible to observe lichens. The difficulty of this method lies in finding the same tree species in the study sites so as to enable homogenous observations to be made. For example, in Italy trees of the *Tilia*, *Acer*, *Quercus* and other species are used. In the event that the species are not totally homogenous, observations can be made using other, different species of tree. When selecting suitable trees, it is necessary to take into account the state of damage to the bark as well as the inclination of the trunk (this must be $<10\%$) and the circumference (min. 70 cm).

Periodic notations are made of all lichen species present within the network (made on a weekly and monthly basis, etc.). A frequency value (F) is given for each species noted and this corresponds to the number of sub-units within the network in which it is present (minimum = 1, maximum = 10). The IAP is then computed for each tree and each station.

The values obtained may be plotted in order to create an air quality map. The IAP values are grouped into five quality levels which are given in Table 1 (Kommission Reinhaltung der Luft im VDI und DIN, 1995).

The main part of the studies that concern air quality in Italy deal fundamentally with atmospheric pollution in towns and cities or in larger geographical areas, where different sites with different impacts are compared. Of the numerous works, we quote as examples data collected in different Italian sites: the city of Isernia (Manuppella and Carlomagno, 1990); the province of Potenza (Lo Porto et al., 1992); the cities of Trieste (Nimis, 1985), Udine (Nimis, 1986), Pistoia (Loppi et al., 1992b), Siena (Monaci et al., 1997), Montecatini Terme (Loppi et al., 1997a), Trento (Gottardini et al., 1999), and La Spezia (Nimis et al., 1990; Palmieri et al., 1997); the Veneto region (Nimis et al., 1991); the Valle del Susa in Piedmont region (Piervittori, 1998); the city of Teramo (Loppi et al., 1998b), the volcanic areas of Italy (Grasso et al., 1999); the city of Pavia (Brusoni et al., 1997), and the province of Viterbo (Bartoli et al., 1997).

Cislaghi and Nimis (1997) report a high degree of correlation between lung cancer and the biodiversity of lichens as a result of atmospheric pollution. These conclusions are based upon thousands of observations made in 662 sites in the Veneto region (northern Italy). These high correlation levels have been found for the more common atmospheric pollutants, such as SO_2, NO_3, dusts and SO_4^{2-}, which is, respectively: $r^2=0.93$, 0.87, 0.86 and 0.85: $P<0.01$ in all cases. Of the many lichen species present (Nimis and Tretiach, 1995), *Physcia tenella* is among the most common in Italy, above all below the mountain areas. It is a species that is considered to be toxin-tolerant, even if experts are in disagreement as to its sensitivity to sulphur dioxide. In urban environments or cultivated areas, it is possible to find toxin-tolerant species that belong — from a phytosociological viewpoint — to the lichen association category of *Xanthorion parietinae* (Nimis, 1987). The more toxin-tolerant species include *Pheophyscia orbicularis* and *Candelaria concolor*; this latter being present at the limit of the "lichen desert".

Different lichen species react to different pollutants in different ways, and various authors give lists that classify them according to sensitivity (Nimis and Tretiach, 1995). The classification of lichen species is one of the most discussed points in literature. In particular, sensitivity to SO_2 is the base factor for most classifications. Several authors, however, suggest classification on the basis of a scale of semiquantitative characteristics (Wirth, 1991); while others classify lichen species according to SO_2 sensitivity on a scale that distinguishes between "acid" and "eutrophic" bark (Hawksworth and Rose, 1970).

The latter method, basically qualitative, considers the degree of atmospheric pollution varying from 10 (zero pollution pure air) to 0 (strongest pollution) as a function of SO_2 levels. Each level is defined by various ephyphytic lichens of broad ecological amplitude grouped in different communities according to the acidic or alkaline character of the bark. This method, due to its rapidity and sensitivity, can be applied to draw cartographic representations of pollution indexes on ample geographic areas, also based on absolute values, provided the lichen flora is comparable to that used for the original reference study in United Kingdom. A partial drawback of this method is that the knowledge of 80 lichens species is required (Deruelle, 1978).

Van Haluwyn and Lerond (1986) proposed a qualitative method based on lichenosociology. The authors suggest the use of a 7-level scale (indicated by letters A to G) defined on the basis of easily recognizable species. According to this method, letters A to E refer to strongly polluted, and letters F and G to less polluted, areas. Studies performed on Northern France showed that the two group of zones relates to SO_2 levels higher and lower than 30 $\mu g/m^3$, respectively. According to this method, even the presence of one species only can be

Table 1
Quality levels of index of atmospheric purity (IAP)

Level A	$0 \leqslant IAP \leqslant 12.5$	Very high level of pollution
Level B	$12.5 < IAP \leqslant 25$	High level of pollution
Level C	$25 < IAP \leqslant 37.5$	Moderate level of pollution
Level D	$37.5 < IAP \leqslant 50$	Low level of pollution
Level E	$IAP > 50$	Very low level of pollution

sufficient to characterize one zone. One of the major advantages of this method is that it is not directly correlated to the levels of SO_2 only, but it is based on the overall response capacity of epyphytic communities as a whole (Lerond et al., 1996).

The IAP method is the most commonly adopted in Italy. Despite the quantitative information that it can supply, this method also presents some disadvantages, primarily among them the fact that a deep knowledge of lichen flora is required, and that it refers to a specific group of environmental pollutants (Amman et al., 1987).

Another quantitative method is the index of poleotolerance (IP) proposed by Trass (1973) and subsequently reviewed by Deruelle (1978). This method, developed and applied in Estonia, allows to draw a map of pollution on the basis of a mathematical index that, in turns, is obtained following observation carried out in predefined conditions. This method considers trees of different age and species. Every observation is performed taking into account the area of the bark covered by lichens, which is related to a graded reference scale varying from 1 to 10 according to the percent of covered surface. Indeed, every species is classified according to the IP, calculated as:

$$\mathrm{IP} = \sum_{1}^{n} a_i \times c_i / C_i$$

where n represents the number of considered species, a_i the degree of tolerance of each species, c_i the corresponding level of covering and C_i the overall degree of covering of all species as a whole.

According to this method, an IP value of 10 refers to a zone of lichen desert, while normal condition correspond to level 1–4. IP can also be correlated with SO_2 levels, where an IP of 1–2 corresponds to zero SO_2 and an IP of 10 to SO_2 concentration higher than 300 $\mu g/m^3$.

Deruelle (1978), Van Haluwyn and Lerond (1988), Lerond et al. (1996) critically reviewed the main advantages and disadvantages of the above-mentioned qualitative and quantitative methods. The two qualitative methods are also compared by Khalil and Asta (1998), in a French study considering the recolonization by pollution sensitive lichens of the Lyon area.

Some authors (Insarov et al., 1999) propose a methodology for biomonitoring climatic changes by measuring the lichen communities of calcareous rocks and for determining the Trend Detection Index with which to verify the sum of lichen species, allowing variability coefficients to be applied to lichen communities that are sensitive to average annual temperature changes of up to 0.8°C. This application is of great interest, above all taking into account that realistic predictions for planetary global warming should be in the range of 2.5°C for the end of the 22nd century.

4.2. Use of native lichens and the transplant method (method B)

In areas where lichens are not killed by contaminants, it is possible to make biomonitoring studies through the direct analysis of contaminants in the thallus. Method B, which consists of transplanting lichen thalli, has the great advantage of being applicable even in "lichen desert" areas (in areas that are unsuited to lichen survival due to high pollution levels), or it can be used in areas where there are no suitable substrata.

The lichen thalli used are taken from tree bark in areas of low pollution and then fixed to suitable surfaces (e.g. cork) and exposed in monitoring areas where samples are taken periodically in order to evaluate the health of the thalli and their degree of damage. Lichen damage is expressed as a percentage of necrotised lichen surface.

The main problem with this method is that found in the difficulty in providing a valid interpretation of transplanted thalli damage percentages. There are also methods that allow identification of necrotic areas, defining them on photographs of lichen thalli. A certain error margin, due to the subjective interpretation of the images, has also been found for this procedure. Possible tendencies to over- or underestimate may be corrected through use of statistics tests (χ^2, t student).

The transplant method is also used in classical bioaccumulation studies that analyse contaminants in tissue. Numerous works regarding this method are concerned with trace elements and in particular, bioaccumulation, absorption, retention, localisation and release, tolerance and toxicity (James, 1973; Nieboer et al., 1978; Burton et al., 1981; Brown and Beckett, 1984; Burton, 1986; Nash and Wirth, 1988; Puckett, 1988; Richardson, 1988, 1992, 1995; Nash, 1989; Brown, 1991; Deruelle, 1992; Garty, 1992, 1993; Sloof, 1995; Bargagli et al., 1997; Garty et al., 1997, 1998a; Benett and Wetmore, 1999; Freitas et al., 1999).

4.3. Heavy metals

The accumulation of metals in plants depends upon many factors, such as the availability of elements; the characteristics of the plants, such as species, age, state of health, type of reproduction, etc.; and other such parameters as temperature, available moisture, substratum characteristics, etc. (Baker, 1983). Contaminants deposit on lichens through normal and indirect (occult) precipitation. This latter includes mist, dew, dry sedimentation and gaseous absorption (Knops et al., 1991). Indirect precipitation occurs in highly stable atmospheric conditions and contains higher nutriment and contaminant concentrations of different orders of size when compared to normal precipitation (Nash and Gries, 1995).

In general, three mechanisms have been put forward with regard to the absorption of metals in lichens (Richardson, 1995):

1. intracellular absorption through an exchange process;
2. intracellular accumulation; and
3. entrapment of particles that contain metals.

Many experts have attempted to increase knowledge of these bonding processes — that is, the interaction between lichen and metal — using various analytical techniques, such as nuclear magnetic resonance, electron paramagnetic resonance, and luminescence. It should, however, be noted that knowledge regarding the understanding of the entire process that is responsible for metal absorption and accumulation in lichens is still scarce. A new approach has recently been attempted (Antonelli et al., 1998), where metal-lichen interaction is studied by applying microcalorimetric techniques with the aim of obtaining enthalpic measurement data. To carry out these tests and to process the microcalorimetric data, the metal-lichen complex is considered as an overall co-ordinating agent, given that it is not possible at this time to know which particular molecule is responsible for co-ordination with the metal. Considering the constant towards equilibrium and the enthalpy trend for *Evernia prunastri*, the following trend has been found: $Pb \gg Zn > Cd \approx Cu \approx Cr$; which indicates a good correlation between the metal bond and the enthalpy values in the absorption process (metal uptake).

Lichens are also excellent bioaccumulators of trace elements, since the concentrations found in their thalli can be directly correlated with those in the environment (Andersen et al., 1978; Herzig et al., 1989; Sloof and Wolterbeek, 1991; Herzig, 1993; Bari et al., 1998).

Studies made of transplanted *Evernia prunastri* highlight the fact that the capacity for Pb accumulation expressed as the relationship between the concentration in the latest sample and the initial concentration value, is 10.2 in the Fontainbleau site (France), 3.7 for the Würzburg site (Germany; Deruelle, 1992), and 4.4 for the city of Rome (Italy; Bartoli et al., 1994).

In Italy, different biomonitoring studies carried out using lichens have shown that Pb is still very widespread in spite of the introduction of lead-free petrol. This indicates that high levels of this metal are still released (and/or re-suspended) by vehicle traffic (Cardarelli et al., 1993; Deruelle, 1996; Monaci et al., 1997). Vehicle traffic seems to be the main source of atmospheric Cr, Cu and Pb in the central Italian sites (Loppi et al., 1998b).

Climatic factors most probably play an important role in the bioaccumulation of heavy metals, even if this role is as yet unclear. The direction in which pollutants are transported by the wind is most surely fundamental in determining their main fallout points. Nimis et al. (1989) correlates pollution from an industrial pole (northern Italy) with that at a distant agricultural centre, situated in the predominant wind direction.

It is well known that heavy metal content in lichen thalli tends to alternate over time in phases of accumulation and subsequent release. The causes of these differences may lie in the incidence on this phenomenon of acid rain. Deruelle (1992) indicates that the periodic releases of Pb that occur in lichens may depend upon lixiviation induced by acid precipitation. Indeed, laboratory experiments show that lixiviation does not occur at pH 7 (Nimis et al., 1989). Heavy metals do, in any case, influence water loss in lichen thalli, and the accumulative effect of Pb, Cu and Zn on water loss, after absorption of a mix of metals in solution, has been observed in the laboratory (Chettri and Sawidis, 1997).

Altitude seems to play an important role in Pb and Cd concentrations, as studied on *Hypogymnia physodes* (Kral et al., 1989). In particular, Pb concentration increases in a linear fashion as altitude increases, while Cd increases in the same way up to altitudes of 900–1100 m. For higher altitudes, Cd concentrations follow a decreasing trend. What is more, *Hypogymnia physodes* is one of the most suitable bioindicators in the study of the bioaccumulation of trace elements (Jeran et al., 1996) in view of its high-tolerance capacities.

In general, the higher accumulation of heavy metals in the thallus found after the summer period, may be due to the increased hydration that results from autumn rainfall (Nieboer et al., 1978). In Mediterranean climates, the trace element content in lichens as they are (unwashed), is strongly influenced by soil dust contamination (Loppi et al., 1997a). Loppi et al. (1999) in spite of high correlation levels of Al, Fe and Ti in *Parmelia sulcata* does not find any linear correlation for these elements with their concentration levels in the soil. This would lead to the supposition that contamination through dust is highly variable and probably depends upon the local characteristics of the sites under study.

Cd is considered to be particularly toxic for various lichen species (Nieboer et al., 1979; Beckett and Brown, 1984). Concentration intervals of 1. 26–5.05 and 1.56–6.40 $\mu g\ g^{-1}$ have been found for *A. ciliaris* and *L. pulmonaria* respectively. These values (considering average values) are considered to be close to the appearance of toxicity symptoms. Furthermore, Cd has a high negative correlation with protein and reducing sugar content (Riga-Karandinos and Karandinos, 1998).

Lichens from the *Usnea* species have been used to evaluate heavy metal deposition patterns in the Antarctic (Poblet et al., 1997). The activities carried out in the different scientific stations could be potential sources of pollution and contribute to the circulation of trace metals in this site.

The relationship between cationic concentrations in lichens, as shown for *Cladonia portentosa*, can be used as an index of acid precipitation. In particular, the

K^{+}/Mg^{++} ratio and the (extracellular) Mg^{++}/(intracellular) Mg^{++} in lichen apexes is strongly correlated to H^{+} concentrations in precipitation. High concentrations of H^{+} that are found in acid rain cause increases in extracellular Mg^{++}. In general, the variation in Mg^{++} concentration in lichens can be considered to be a good indicator of acid rainfall (Hyvarinen and Crittenden, 1996).

Acid-moisture depositions containing heavy metals can significantly reduce lichen survival in affected geographical areas. In lichens (*Bryoria fuscescens*) exposed to simulated acid rainfall containing two levels of Cu^{++} and Ni^{++} only or combined with acid rain (H_2SO_4) at pH 3 for 2 months in addition to environmental rainfall, it was observed that the alga and fungus components respond in different ways to pH levels and that they have a specific interaction that is correlated to the toxicity of the metals. In particular, the alga component is the more sensitive to acid rain and to the mix of heavy metals and, as a result, it has a higher quantity of degenerated cells, which causes significant changes in membrane permeability. Critical concentrations of heavy metals in alga thalli were >50 μg g^{-1} for Cu and >7 μg g^{-1} for Ni in the presence of acidity and >20 μg g^{-1} for Ni in absence of acidity (Tarhanen, 1998; Tarhanen et al., 1999).

Another recently developed field of application for biomonitoring with lichens is that of indoor pollution and in particular, the analysis of air particulates. Rossbach et al. (1999) found a high ratio between the concentrations of Cr, Zn, and Fe in air particulate samples taken from the filters of air conditioning systems in different hotels in different cities and in *Usnea* spp. samples found in the conditioned environments.

Table 2 reports some bibliographical data on heavy metal bioaccumulation on lichens. For over 20 years, lichens have been used as bioindicators and/or biomonitors in environment quality evaluations for such industrial realities as iron foundries and fertiliser manufacturing plants (Kauppi, 1976; Laaksovirta and Olkkonen, 1977; Palomaki et al., 1992); steel works and iron foundries (Pilegaard, 1978, 1979; Pilegaard et al., 1979); oil extraction plants (Addison and Puckett, 1980); sites contaminated by the petrochemical industry (Pakarinen et al., 1983); areas surrounding zinc foundries (de Bruin and Hackenitz, 1986); areas surrounding nickel foundries (Nieboer et al., 1972); coal-fired power stations (Olmez et al., 1985; Garty, 1987; Freitas, 1994); power stations in high-density industrial areas (Gonzalez and Pignata, 1997). Garty et al. (1997), in a study of a heavy oil combustion plant in the south-west of Israel (Ashdod area), carried out using transplanted of fruticose epiphytic lichens (*Ramalina duriaei*) found high concentrations of S, V and Ni in thalli that were transplanted to the industrial area. These values can be correlated with environmental measurements of SO_2 and V made in the same region. Furthermore, the high V/Ni ratio in the lichens may be an indicator (tracer) of pollution in the area caused by heavy oil combustion plants. The same authors (Garty et al., 1998a) found a high Pb, V, Ni, Zn and Cu bioaccumulation potential for *Ramalina lacera* in the same site.

Lichen are also used to study copper dust emissions from mines. *Ramalina fastigiata* has been used as a bioindicator of the impact of a coal mine in Portugal. The threshold concentration of intracellular Cu above which total inhibition of photochemical apparatus occurs, is approximately 2.0 μmol g^{-1} (Branquinho et al., 1999). *Neophuscelia pulla* and *Xanthoparmelia taractica* were used to study the bioaccumulation of heavy metals in abandoned copper mines in Greece, where a significant correlation ($P<0.05$) was found between the copper content in the soil and that of the lichen thalli (Chettri et al., 1997). *Hypogymnia physodes* has been used as a bioindicator of the presence of mercury and methylmercury in metal extraction areas in a site in Slovenia, where its excellent bioaccumulation capacities were confirmed (Lupsina et al., 1992). Riga-Karandinos and Karandinos (1998) took three native lichen species (*Anaptychia ciliaris*, *Lobaria pulmonaria*, and *Ramalina farinacea*) directly from 22 sites distributed over an area of 250 km^2 in Peloponnesus, Greece, where there is a coal-fired power station. There they found levels of Cd that were close to levels of toxicity for lichens (respectively, 3.09, 3.42, and 3.80 μg g^{-1}) and below-toxic levels of Pb (respectively, 8.60, 9.76, and 11.18 μg g^{-1}).

Lichens are excellent bioindicators of atmospheric pollution from geothermal power stations and in particular, of the pollutants that are correlated with this phenomenon, such as mercury (Bargagli and Barghigiani, 1991), boron (Koranda, 1980), radon (Matthews, 1981) and other metals (Connor, 1979). Several authors (Loppi, 1996; Loppi et al., 1998a) give data on atmospheric pollution from geothermal power stations in central Italy using the method of mapping lichen communities, where they found minimum IAP values within 500 m of the power station and progressive increases in frequency in line with increases in distance from the power stations themselves. Pollutants that are typically associated with geothermal activity are As, B, Hg, and H_2S (Loppi, 1996; Loppi and Bargagli, 1996). Nonetheless, it is not clear if the drop in the richness of the species in those areas close to geothermal power stations is due to the action of a single contaminant or to the combined actions of all contaminants. In any case, the author considers that the worst damage to lichen thalli is caused by H_2S, which is a highly toxic gas (Beauchamp et al., 1984) and which is continuously present in high levels as a local contaminant in areas surrounding geothermal power stations.

Different studies have established correlation factors between chlorophyll damage and the concentrations of

Table 2
Selected references of heavy metals (μg/g dry weight) studied on lichens species from different geographical areas (mean values and ranges of concentrations[a]

Species	Site	Note	Cd	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn	References
Anaptychia ciliaris (N)	Southern Greece	Area of 250 km^2 of Peloponnesus where a lignite-burning power plant is located (RM)	3.09 (1.26–5.05)		4.06 (1.10–5.60)	2153 (1359–3092)	43.87 (15.6–92.1)		8.60 (3.57–12.6)		31.22 (23.3–41.9)	Riga-Karandinos and Karandinos, 1998
Lobaria pulmonaria (N)	Southern Greece	idem c.s.	3.42 (1.56–6.4)		6.85 (4.6–12.3)	1103 (339–2180)	65.32 (17.7–137.1)		9.76 (3.87–21.1)		28.16 (16.9–59.4)	Riga-Karandinos and Karandinos, 1998
Ramalina farinacea (N)	Southern Greece	idem c.s.	3.80 (2.18–7.06)		3.63 (1.70–5.80)	748 (409–1222)	52.35 (28.7–81.4)		11.18 (5.10–19.5)		19.46 (15.8–25.6)	Riga-Karandinos and Karandinos, 1998
Ramalina duriaei (N)	Israel	SC			5.5							Kardish et al., 1987
Ramalina duriaei (T)	Israel	AC			12							Kardish et al., 1987
Ramalina duriaei (T)	Israel	Urban (U), rural (R) and sub-urban (SU) sites that are 4.5–24.5 km from a power plant		(U) 11.7 (R) 12.8 (SU) 23.8	(U) 13.6 (R) 12.9 (SU) 14.2			(U) 49.5 (R) 14.5 (SU) 20.0	(U) 165.4 (R) 30.3 (SU) 43.4		(U) 59.6 (R) 49.4 (SU) 59.0	Garty, 1988
Ramalina duriaei (N)	HaZorea (Northeast Israel)	SC		10.8	10			20.1	22.6		33.1	Garty, 1988
Ramalina duriaei (N)	Israel	Urban- indus. area						14.3		23.1		Garty et al., 1997
		Suburban area						15.3		31.7		
		Rural area						9.9		6.4		
Ramalina lacera (T)	Israel	Southwest (AC)		6.53 (5.4–8.5)	7.34 (5.2–12.4)	1505 (1235–1954)	32.51 (26.4–40.9)	6.84 (2.1–12.2)	34.95 (10.4–155)	14.1 (6.7–26.2)	60.1 (38–113)	Garty et al., 1998
Evernia prunastri (T)	Rome	Area of 300 km^2 of the big annular connection (AC)		14.05	13.37				40	5.2	57.45	Bartoli et al., 1994
Parmelia sulcata (T)	Bern (Switzerland)	Urban area		28.5	47.5	833.5	18	106	189		259	Garty and Amman, 1987
Parmelia sulcata (T)	Biel, Champagne Allee (Switzerland)	Urban area		22	84	890	21	22	172		191	Garty and Amman, 1987
Parmelia sulcata (T)	Lauenen, (near Gstaad, Switzerland)	Suburban area		526	18	4613	48	667	29		192	Garty and Amman, 1987
Parmelia sulcata (N)	Tuscany (provinces of Siena and Grosseto) (RM)	SC		3.6	9.1	1800	38.2		15.9	2.32	65.7	Loppi et al., 1999
Parmelia sulcata (N)	Portugal	228 sites along the Atlantic coast and iterior of the country		5.77 (1.53–32.3)				3.92 (0.52–33.1)	18.4 (2.0–142)	14.5 (1.83–130)		Freitas et al., 1999
Hypogymnnia physodes (N)	Village of Gusum (Sweden)	Surroundings of a brass foundry	1.1 (0.4–1.7)		28.2 (11–79)	832 (290–1300)		2.6 (1.7–3.9)	22.6 (14–33)		232 (93–450)	Folkeson, 1979
Hypogymnnia physodes (T)	Bern (Switzerland)	Urban area		n.d.	23	864	n.d.	30	315		224	Garty and Amman, 1987
Hypogymnnia physodes (T)	Biel (Switzerland)	Urban area		30	41	1311	13	12	111		159	Garty and Ammann, 1987

Table 2 (*continued*)

Species	Site	Note	Cd	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn	References
Hypogymnnia physodes (T)	Slovenia	The sites were located at least 300 m away from main roads and at least 100–200 m away from dwelling (RM)	1.05 (0.31–5.42)	5.78 (2.33–21.8)		1253 (492–3756)					90.2 (47.3–151)	Jeran et al., 1996
Parmelia caperata (T)	Washington (near to the Potomac River)	The locations are near the Dickerson power plant	1.2 (1.1–1.4)	3.8 (2.0–5.1)		1400 (750–2090)	240 (140–380)				64 (55–80)	Olmez et al., 1985
Parmelia caperata (T)	Travale-Radicondoli (central Italy)	Area of 15 km^2 near a geothermal power plant (RM)	0.329 (0.11–0.69)	4.51 (1.25–8.41)	10.8 (4.5–25.4)	1019 (275–2370)	85.8 (10.9–280)	4.41 (1.65–8.18)	6.3 (2.1–19.7)		43 (22.2–63.8)	Loppi and Bargagli, 1996
Parmelia rudecta (T)	Washington (near to the Potomac River)	The locations are near the Dickerson power plant		4.6 (2.8–73)		1620 (780–3090)	230 (86–365)				68 (34–100)	Olmez et al., 1985
Cetraria cucullata (N)	Northwest of Canada					478.8	47.7	2.5	4.2	1.78	24.1	Puckett and Finegan, 1980
Cetraria nivalis (N)	Northwest of Canada					257.7	84.5	2.7	5.6	1.2	25.0	Puckett and Finegan, 1980
Cladina stellaris (N)	Northwest of Canada					568.7	30.2	2.9	4.3	3.98	15.9	Puckett and Finegan, 1980
Cetraria islandica (N)	Switzerland (Devos)	Rural area		8	8	149	43	7	40		60	Garty and Amman, 1987
Usnea filipendula	Slovenia	Surroundings of a brass foundry	0.6 (0.4–1.0)		22.4 (7–48)	614 (250–1400)		2.6 (1.7–4.1)	27.0 (15–40)		182 (82–200)	Folkeson, 1979
Usnea sp. (T)	Switzerland (Devos)	Rural area		18	14	402.5	26	32	120		72.5	Garty and Amman, 1987
Pseudovernia Furfuracea (N)	Slovenia	Surroundings of a brass foundry	0.6 (0.4–0.9)		35 (15–71)	926 (504–1640)		2.8 (1.9–3.9)	37.3 (20–53)		237 (125–444)	Folkeson, 1979
Pseudovernia Furfuracea (T)	Switzerland			41.8	34.7	3423	44.8	74.5	135.5		159.5	Garty and Amman, 1987
Cladonia rangiferina (N)	Slovenia	Surroundings of a brass foundry	0.5 (0.1–1.0)		14.5 (4–40)	442 (160–1200)		1.5 (0.8–2.3)	22.8 (11–36)		102 (51–204)	Folkeson, 1979
Umbilicaria deusta (N)	Sudbury District (Northern Ontario, Canada)	Surroundings of a copper foundry			65	1470		37				Tomassini et al., 1976
Umbilicaria muhlenbergii (N)	Sudbury District (Northern Ontario, Canada)	Surroundings of a copper foundry			30	920		16				Tomassini et al., 1976
Stereocaulon paschale (N)	Sudbury District (Northern Ontario, Canada)	Surroundings of a copper foundry			43			26				Tomassini et al., 1976
Cladonia alpestris (N)	Mackenzie Valley (Ontario, Canada)	Boreal forest			12.6	164						Tomassini et al., 1976
Cladonia alpestris (N)	Sudbury District (Northern Ontario, Canada)	Surroundings of a copper foundry			15	320		11				Tomassini et al., 1976

(Table continued on next page)

Table 2 (*continued*)

Species	Site	Note	Cd	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn	References
Cladonia deformis (N)	Mackenzie Valley (Ontario, Canada)	Boreal forest			1	70						Tomassini et al., 1976
Cladonia deformis (N)	Sudbury District (Northern Ontario, Canada)	Surroundings of a copper foundry			21	220		10				Tomassini et al., 1976
Cladonia mitis (N)	Mackenzie Valley (Ontario, Canada)	Boreal forest			20.6	170						Tomassini et al., 1976
Cladonia mitis (N)	Sudbury District (Northern Ontario, Canada)	Surroundings of a copper foundry			19	260		10				Tomassini et al., 1976
Cladonia uncialis (N)	Sudbury District (Northern Ontario, Canada)	Surroundings of a copper foundry			19	210		10				Tomassini et al., 1976
Cladonia uncialis (N)	Mackenzie Valley (Ontario, Canada)	Boreal forest			4	120						Tomassini et al., 1976
Diploschistes steppicus (N)	Sede Boquer region (Negev desert, Israel)	In this area no industry exists, we may assume that the heavy metals derive partly from car traffic		17.2	66.9		66.0	23.6	63.0		33.2	Garty, 1985
Teloschistes lacunosus (N)	Sede Boquer region (Negev desert, Israel)	idem c.s.		15.2	4.7		33.5	12.8	19.4		30.3	Garty, 1985
Squamarina crassa (N)	Sede Boquer region (Negev desert, Israel)	idem c.s.		18.0	14.4		68.6	21.2	31.5		41.9	Garty, 1985
Ramalina maciformis (N)	Sede Boquer region (Negev desert, Israel)	idem c.s.		10.3	8.1		8.1	11.9	39.6		20.9	Garty, 1985
Caloplaca ehrenbergii (N)	Sede Boquer region (Negev desert, Israel)	idem c.s.		20.0	8.7		42.4	14.4	35.3		28.8	Garty, 1985

[a] T, transplanted; N, natives; AC, contaminated area; SC, control site; n.d., no detectable; RM, reference material used.

several elements in lichens. Garty et al. (1998a) found that chlorophyll integrity is inversely correlated with concentrations of Cr, Fe, Mn, Ni, Pb, and B. K concentrations, however, have a positive correlation with chlorophyll integrity (Kauppi, 1976; Garty et al., 1998a). In a study of *Cladina stellaris* samples that were transplanted to an area near to a fertiliser factory in Finland, Kauppi (1976) found that the high concentrations of K in the lichens corresponded to increases in chlorophyll content and in particular, to the chlorophyll *a*/chlorophyll *b* ratio.

Garty et al. (1998a), found differences of approximately factor 2 in electrical conductivity in lichens from industrially polluted sites (Israel) compared with those from rural sites. This indicates a process of cell membrane damage. Membrane integrity is highly correlated with the presence of Ca (Beckett and Brown, 1984), which is a macronutrient and has regulatory functions in sites of extracellular interchange on the surface level of alga cell walls or hyphae, and intracellular interchanges with proteins. Concentrations of several elements (S, B, Al, Cr, Fe, Si, Ti, and Zn) are positively correlated with cell membrane damage for *R. duriaei* (Garty et al., 1998b).

Various studies report that exposure of lichen thalli to chemicals in laboratory and using fumigation experiments, reveals subsequent damage to cell membranes corresponding to an increase in water electrical conductivity caused by the loss of electrolytes (Pearson and Henriksson, 1981; Hart et al., 1988; Garty et al., 1998b). In general, the loss of K is related to cell membrane damage and is inversely correlated with electrical conductivity (Garty et al., 1998b; Tarhanen et al., 1999). The extent of cell membrane integrity may be evaluated by measuring electrical conductivity.

Another field of study using lichens as biomonitors is that involving volcanic areas (Grasso et al., 1999) and in particular, the release of mercury. In the Hawaiian islands, Hg has been found in the interval of 8–59 μg g^{-1}, meaning that bioaccumulation is therefore more widely distributed in areas that are strongly affected by volcanic activity (Davies and Notcutt, 1996).

An important problem — as far as concerns the determination of trace elements — is the quality control of analytic methods as well as of the sampling strategies and treatment of samples (Wolterbeek and Bode, 1995). In the last few years, some 2000 articles on the analysis of lichens have been published. The high variability of the data found may not only be caused by the different distribution of pollution patterns, but it may also be the result of possible errors in analysis. It is in this sense that the European Commission, through its Standards, Measurement and Testing programme has developed a new certified reference material (CRM) of lichen (CRM 482) to determine and remove the major sources of error in lichen analysis (Quevauviller et al., 1996). In addition to this, the International Atomic Energy Agency (IAEA) has recently initiated an interlaboratory study with developing countries on the reference material IAEA 336 lichen (Smodis and Parr, 1999).

It should be noted that the problem of sample pretreatment and collection methods, as well as the standardisation of analytical methods, is of fundamental importance above all when comparing lichens from different geographical areas (Puckett, 1988; Jackson et al., 1993). Literature contains little information above all about the techniques for washing lichens. Different lichen washing strategies may cause relevant changes in metal or sulphur content (Wadleigh and Blake, 1999) or in fluorides (Palomaki et al., 1992), as against unwashed samples.

Furthermore, the enormous developments in analytical techniques (atomic absorption spectroscopy, ICP-AES, neutron activation, etc.) should be taken into consideration. These developments have, in the last few decades, notably improved instrument detection limits, allowing increasingly accurate analyses and eliminating possible sources of error. When determining trace elements in lichens, some interesting analytical applications, such as the use of short-life radionuclides in neutron activation (Grass et al., 1994) and X-ray fluorescence spectrometry (Caniglia et al., 1994; Richardson et al., 1995), can supply valid information, in particular for the determination of macronutrients (K and C), or of trace metals (Cu, Pb, Zn) and non-metals (S).

4.4. Sulphur compounds

The effect of sulphur compounds on lichens have been extensively studied. For indeed, many studies are generally concerned with the effects of SO_2 fumigation of exposed lichens (Rao and LeBlanc, 1966; Henriksson and Pearson, 1981; Fields, 1988; Balaguer and Manrique, 1991; Gries et al., 1995), or with the effects of simulated acid rainfall (Scott and Hutchinson, 1987; Holopainen and Kauppi, 1989; Sanz et al., 1992; Tarhanen, 1998). Other works deal in the respiration rate (Baddeley et al., 1972), photosynthesis (Richardson and Puckett, 1973; Kauppi, 1976; Lechowicz, 1982) and chlorophyll fluorescence (Calatayud et al., 1996, 1999; Deltoro et al., 1999). For the most part, these studies aim to evaluate the effects of sulphur compounds on the physiology of lichen thalli and/or on the integrity of photobiont chlorophyll.

Chlorophyll analysis is usually carried out following the method proposed by Ronen and Galun (1984). Phaeophytin is a product of chlorophyll degradation. The variation in the normal chlorophyll–phaeophytin ratio is an indication of suffering in lichens. This method foresees the extraction of chlorophyll using 5 ml of solvent (DMSO). The ratio between chlorophyll *a* and phaeophytin *a* is measured using a spectophotometer

(OD 435 nm/OD 415 nm); and this is considered to be an appropriate index for measuring the impact of high concentrations of SO_2 in lichens, or for evaluating the effects of heavy metal pollution in transplanted lichens (Garty, 1987). A ratio of 1.4 indicates that chlorophyll is unchanged. Any reduction in this value indicates chlorophyll degradation with ensuing stress to the organism (Boonpragob and Nash, 1991; Gonzales and Pignata, 1994, 1997; Levin and Pignata, 1995; Gonzales et al., 1996, 1998; Silberstein et al., 1996). Kardish et al. (1987) report a value of 1.44 for the Chl/Ph ratio of *Ramalina duriaei* in the control site, while for a polluted site with high levels of vehicle traffic, they found a value of 0.80. In general, an alteration of the Chl/phaeophytin ratio has been found, indicating the toxic effect of a combination of gaseous and non-gaseous pollutants.

Sulphur content is determined by transforming elementary sulphur into SO_4^{2-} ions, which occurs through the acid suspension method using barium chloride (Gonzales and Pignata, 1994; expressed in mg g^{-1} dry weight).

Some authors (Levin and Pignata, 1995; Gonzales et al., 1996; Carreras et al., 1998) report that data relating to sulphur accumulation and obtained indirectly from the bioindicator, seem to show that the influence of SO_2 from industry (Còrdoba, Argentina), is rather restricted compared to that which comes from vehicle traffic.

Sensitivity to SO_2 and to other atmospheric pollutants in general, varies according to species (Insarova et al., 1993). *Lobaria pulmonaria* is considered to be one of the most sensitive species according to the scale of Hawksworth and Rose (1976): 30 μg m^{-3} for average winter concentrations of SO_2. This species' high degree of sensitivity is probably due to the presence of isidia, which is a vegetative structure on the upper surface of the thallus and which plays a role in asexual reproduction. The isidia increases the absorption surface of the thallus per unit of mass (Riga-Karandinos and Karandinos, 1998). *Hypogymnia physodes* is, on the contrary, a species that is particularly resistant to SO_2. Indeed, it has been seen that exposure of this species to H_2SO_4 in highly acid conditions, produces no effect (Garty et al., 1995). *H. physodes* has also been used in the area surrounding a fertiliser plant in Finland, where sulphur levels of 3000 ppm had been found (Palomaki et al., 1992). Typical ultrastructural damage caused by the action of sulphur on photobiont cells is seen within the first two weeks of the transplant, without the sulphur concentration levels being particularly high.

Another interesting field of research is that which correlates sulphur content and the composition of sulphur isotopes (Case and Krouse, 1980; Krouse and Case, 1981; Takala et al., 1991). A recent study (Wadleigh and Blake, 1999) reports the spatial variation of the sulphur isotope composition of 83 epiphytic lichen samples (*Alectoria sarmentosa*). The study reveals a positive correlation between isotope composition and different sources of sulphur emission in the site under study (Newfoundland Island, Canada). It is interesting to note the fact that lichens are also sensitive to the sulphur salts that come from the sea. Indeed the degree of sulphur concentration has been seen to decrease in lichens, the further they are from the coastal to internal areas of the island.

The role of sugars in alga–fungus interaction is most important in lichen biology. Chronic SO_2 fumigation of lichens may cause interference in the flow of such nutrients as carbohydrates, thus creating symbiont damage. SO_2 causes reducing sugars to increase and non-reducing sugars to decrease. This effect is probably due to a breakdown in the polysaccharides that are rich in reducing sugars. Reducing sugars are determined by extracting 10 mg of lichen thallus with 1 ml of d-H_2O and centrifugation at $2000\times\boldsymbol{g}$ for 10 min in an Eppendorf vials. A mix of two solutions (4 ml; sodium potassium tartrate and an indicator) are added to the supernatant and the vials were bathed in darkness at 100°C for 3 min. After the proper cooling period, solution absorbance is measured at 660 nm (Riga-Karandinos and Karandinos, 1998).

Spectroscopic measurements, carried out to study changes in the spectral reflectance response of lichen thalli have been exposed in contaminated sites as against those exposed in control sites (Satterwhite et al., 1985; Garty et al., 1997). As a rule, lichen scanning revealed, as for the higher plants of uncontaminated sites, a significant drop of between 600 and 700 nm (which corresponds to the absorption region of the chlorophyll), and a net increase in spectral reflectance around 700 nm (red edge) together with a continual and relatively high reflectance in the near infrared between 700 and 1100 nm. The near infrared plateau is the result of differences between the varying refractive indexes of the internal components of the thallus (cell walls, chloroplast, air, water content, etc.). In spectra of lichens transplanted to contaminated sites, the red edge (700 nm) is much less pronounced and the plateau is very low. This indicates a clear situation of organism stress (Garty et al., 1997). In extreme cases, for example in plants that are subjected to high stress levels, or which are already dead, the spectrum shows a continuous line that rises gradually.

Membrane proteins may be damaged by the presence of SO_2, which may cause a reduction in protein biosynthesis in some lichens; or there may be negative effects on the nutritional interchange between symbionts with, as a consequence, an alteration of their delicately balance. To determine protein content, 100 mg of tissue are extracted using 3 ml of a phosphorous buffer solution at pH 7 and the extract is centrifuged at $1600\times\boldsymbol{g}$ for 5 min. The extracted solution (100 μl) is added to 5 ml of Bradford solution (Bradford, 1976) and absorbance at 595 nm is taken and compared with a calibration curve made following with protein standard (e.g. bovine serum albumin-BSA; Riga-Karandinos and Karandinos, 1998). The structural proteins found in cell membranes and the lichens enzymes can have considerable damage in the presence of high levels of SO_2 concentration. These processes concern the delicate interchange of nutrients between symbionts and can damage the delicate equilibrium of the association (Fields and St. Clair, 1984).

Thus, the damage to cell membranes can be used as an indicator of environmental stress. Indeed, it has been demonstrated that SO_2, such as O_3 and NO_2, are powerful catalysts of lipid membrane peroxidation (Menzel, 1976; Gonzalez and Pignata, 1994; Gonzalez et al., 1996). Experiments where lichens were exposed to 1 ppm of SO_2 in aqueous solution show a slight reduction in the overall content of phospholipids and an increase in unsaturated fatty acids. This latter type of response to SO_2 may be considered to be of the adaptive type (Bychek-Guschina et al., 1999).

The effect of SO_2 can also be evaluated by dry weight/fresh weight ratio. This ratio has been proposed as an indication of the influence of the environment on the bioindicator. It has indeed been observed that in highly polluted areas (e.g. where traffic is intense), there is a tendency in lichens to lose moisture (Levin and Pignata, 1995).

Finally, the production of ethylene is another indicator of stress in lichens. Lichens exposed to solutions containing sulphur in an acid environment have different levels of ethylene production. In general, these solutions increase the solubility of the particles containing heavy metals that are trapped within the hyphae. This phenomenon may lead to an increase in the production of endogenous ethylene in lichens when they are exposed to chemical agents containing sulphur, to acid rain and to air polluted with heavy metals (Garty et al., 1995).

4.5. Nitrogen and phosphorous compounds

Although lichens have already been proposed as bioindicators of NH_3 (De Bakker, 1989), only in the last few years has a clear positive correlation been established between nitrophytic lichens and atmospheric NH_3 concentrations; even if responses are always greater to SO_2. Tree bark analyses in sites in Holland demonstrate that nitrophytic lichen species do not respond directly to nitrogen levels found in the environment, but that they are favoured by the high pH values in the bark, which are related to the high levels of NH_3 in the environment (Van Dobben and Ter Braak, 1998).

Cladonia portentosa is an excellent bioindicator for the study of precipitation chemicals and nitrogen and phosphorous concentrations. Hyvarinen and Crittenden (1998a, b) have found concentrations in the range of 0.08–1.82% for nitrogen and 0.04–0.17% for phosphorous (per unit of dry weight) in apexes (5 mm top part) and thalli (bases) in various comparison sites. The concentration levels found for these elements are 2–5 times higher in the apexes than at the bases and furthermore, both the apexes and base parts show a high positive correlation between elements. The correlation between N deposition and the nitrogen accumulated in the lichens is positive; becoming higher when referred to concentrations found in the thalli rather than in the apexes. On the other hand, nitrogen concentrations in the thallus are little correlated with the N values in precipitation. The nitrogen found in thalli is, however, highly linked to moist nitrogen deposits, but it is also correlated positively with the NO_2 present in the air. As well as *C. portentosa*, *H. physodes* has also been proposed as a bioindicator of nitrogen total deposition (dry and wet; Sochting, 1995) as well as of nitrogen and sulphur (Bruteig, 1994).

High levels of SO_2 and NO_x can cause a reduction of pH of lichen thalli (see for example data respective to Peloponnesus area [Greece], Riga-Karandinos and Karandinos, 1998). To this respect, it shall be highlighted that atmospheric pollution of this kind has led to extinction of *L. pulmonaria* and *R. farinacea*. The measurement of the pH of lichen thalli can supply information with regard to the state of pollution of a site. To determine pH levels, 50 mg of lichen thallus is homogenised in liquid nitrogen and 2 ml of d-H_2O. After centrifugation at $100\times\boldsymbol{g}$ for 10 min, the pH value for the supernatant is read. Various authors report that *L. pulmonaria* is endangered in some sites subject to acid rain, and pH = 5 has been indicated as a threshold value below which lichen is unable to survive (Gauslaa, 1985; Gilbert, 1986).

4.6. Ozone

O_3 and NO_2 (see also Section 4.4) are powerful catalysts of lipid membrane peroxidation: the main effect of O_3 on lichens is indeed the damage of cell membrane.

It has been demonstrated that in biological systems the presence of oxidation products such as malondialdehyde is directly correlated to the start of the peroxidation of unsaturated fatty acids (Mehelman and Borek, 1987). Egger et al. (1994), reports an increased production of both malondialdehyde and superoxide dismutase in *Hypogymnia physodes* that was transplanted to highly polluted sites with monthly O_3 concentrations in the range of 20–198 μg m^{-3} (10–100 ppb). These compounds are products of lipid peroxidation and are indicators of oxidative damage to membranes and to the enzyme systems that protect against oxidation in plants.

Oxidation products are estimated by determining malondialdehyde (MDA), which is measured using the colorimetric method (Heath and Packer, 1968). MDA is determined using the extinction coefficient of 155 mM^{-1} cm^{-1} (Kosugi et al., 1989). The results are expressed in μmol g^{-1} of dry weight.

Other important peroxidation products are hydroperoxy conjugated dienes (HPCD) which can be isolated by solvent extraction. Concentration is calculated using the molar extinction coefficient $\epsilon = 2.64 \times 10^4$ M^{-1} m^{-1}. Results are expressed in mmol g^{-1} of dry weight.

O_3 damage to the photochemical apparatus of lichens after repeated exposure to real doses has been well-documented (Scheidegger and Schroeter, 1995). In particular, this kind of damage has been studied in other species such as, for example, clover, where pollution causes typical and easily recognisable leaf damage (Karlsson et al., 1995). Furthermore, O_3 is the subject of monitoring and controls made by the European Community (Benton et al., 1995). Ross and Nash (1983), report a study where *Flavoparmelia caperata* was fumigated with O_3 for brief periods (10 and 12 h) and in quantities of 200 μg m^{-3} (100 ppb). This caused a 50% net decrease in photosynthesis. *Usnea ceratina* was fumigated for 6 h per day for a period of 5 days with concentrations of 100–200 ppb of O_3, causing a notable reduction in net photosynthesis (Zambrano and Nash, 2000).

4.7. Fluorides, chlorides and other atmospheric pollutants

Literature regarding the bioaccumulation of fluorides is scarce. Asta and Garrec (1980) have demonstrated that fluoride concentration in the lichen thallus is dependent on both the lichen species and the environmental F levels. Fluoride levels above 360 ppm were found in lichens (*H. physodes* and *Bryoria capillaris*) transplanted to areas near to a fertiliser factory and mine (Palomaki et al., 1992). Fluoride accumulation in these sites reached maximum levels during the summer followed by decreases in the autumn. Already at levels of 30–40 ppm of dry weight it is possible to see the typical ultrastructural damage in the photobiont cells, caused by exposure to fluorides. It has also been found that fluoride content in lichens is inversely correlated to the distance from an aluminium processing plant. The losses in the lichen populations around this plant show a high level of correlation ($r^2 = 0.90$) with their F content (Perkins, 1992).

Studies inherent to the impact of chlorides are also rather scarce. To this regard, a biomonitoring study using the lichen *Parmelia sulcata* Tayl. and several mosses, carried out in 26 sites, shows a spatial and temporal correlation between chloride bioaccumulation and the environmental impact trend of a waste incineration plant in the city of Grenoble, France (Gombert and Asta, 1997).

For other atmospheric contaminants, such as polychlorinated dibenzodioxins and polychlorinated dibenzofurans ($PCDD_s/PCDF_s$), bioindication studies using lichens are very scarce. In general, the available studies of PCDDs and PCDFs report the temporal variations of these pollutants in samples of different types (e.g. plants) taken in the vicinity of urban waste incineration plants (Schuhmacher et al., 1997; Schuhmacher et al., 1998). Furthermore, for example, the eggs of several bird species and in particular, those of the herring-gull, seem to be good bioindicators of the presence of $PCDD_s/PCDF_s$ (Oxynos et al., 1997).

4.8. Radionuclides

Lichens are good bioaccumulators of radionuclides (Notter, 1988). This application concerns an important sector of research into the evaluation of the fallout of radionuclides, above all after the Chernobyl incident (Barci et al., 1988; Seaward et al., 1988, Mihok et al., 1989; Livens et al., 1991; Sloof and Wolterbeek, 1992; Hofmann et al., 1993; Triulzi et al., 1996; Sawidis et al., 1997). In lichens in several areas of Norway, after the Chernobyl incident of 1986, levels of Cs 134 and Cs 137 of two orders of size larger than those in vascular plants were found (Bretten et al., 1992). This phenomenon has caused a significant increase in the average concentration of radiocesium in reindeers, whose major food source are lichens (Jones et al., 1989; Rissanen and Rahola, 1989). *Parmelia sulcata* has been used as a bioindicator for the presence of radionuclides in areas close to Chernobyl where I 129 and Cl 36 have been measured. Regional distribution patterns of these radionuclides have shown a positive correlation with accumulated concentrations (Chant et al., 1996). One study reports values of the natural decontamination of radionuclides in lichens (Topcuoglu et al., 1995). The average biological life-span of Cs 137 is 58.6 months in *Xanthoria parietina*, which has shown itself to be the best bioindicator of radioactive fallout as against mosses.

It has been demonstrated that plutonium concentrations in *Xanthoria* spp. gathered in the vicinity of a nuclear arms deposit are inversely correlated with the distance from the contamination site, and that there is a direct correlation between concentrations of Pu 239 and Pu 240 with concentrations found on the soil surface ($r^2 = 0.767$; $P < 0.001$; Thomas and Ibrahim, 1995). Altitude is an important factor that is correlated with concentrations of Ra 226 and Ra 228 studied in lichens of the *Umbilicaria* species (Kwapulinski et al., 1985). Altitude is also correlated with levels of Cs 137 found in 1993 in samples taken in Italy in the province of Parma (Triulzi et al., 1996).

5. Concluding comments

The analysis of atmospheric pollutants using conventional analytical procedures allows data to be interpreted directly and results to be obtained rapidly. A summary of the most common conventional analytical techniques for the analysis of various environmental pollutants is given in Table 3.

On an ecological level, however, air quality studies using these methods may present the following problems:

1. space-time fluctuations could lead to sampling errors;
2. low concentrations of several microcontaminants (which could also change over time), could lead to difficulties in methodology;
3. it is also difficult to ascertain either the intermittent or sporadic emission of contaminants;
4. in this way the biological tolerance limits of the species concerned might not be taken into consideration; and
5. often the dose-effect ratio does not have linear response and it is thus possible to run into interpretation problems in evaluating damage to organisms and ecosystems.

Table 3
Analytical techniques for the analysis of environmental pollutants

Pollutant	Reference instrumental methods
SO_2	Flame photometric
	Gas chromatography with flame photometric detector
	Spectrophotometric (pararosaniline wet chemical)
	Electrochemical
	Conductivity
	Gas-phase spectrophotometric
O_3	Chemiluminescent
	Electrochemical
	Spectrophotometric (potassium iodide reaction, wet chemical)
	Gas-phase spectrophotometric
NO_2	Chemiluminescent
	Spectrophotometric (azo-dye reaction wet chemical)
	Electrochemical
	Gas-phase spectrophotometric
	Conductivity
Fluorides	Potentiometric method
PAH	Gas chromatography associated with high resolution mass spectrometry
PCDD	Gas chromatography associated with high resolution mass spectrometry
PCDF	Gas chromatography associated with high resolution mass spectrometry
Metals	Atomic absorption spectrometry
	Atomic emission spectrometry
	Inductive coupled plasma emission spectrometry
	Inductive coupled plasma optical emission spectrometry
	Inductive coupled plasma mass spectrometry
Chlorine and hydrochloric acid	Volumetric method; spectrophotometric analytical method
Phosphorus and its compounds	Gas chromatography with Nitrogen/Phosphorous detector; X ray spectroscopy

The above points highlight the fact that traditional environmental monitoring methods require numerous and extensive samples to be taken in the areas under study and that these samples must also be taken over prolonged periods of time.

Furthermore, the use of mathematical models of contaminant dispersion in the environment should also be pointed out (Benedini and Cicioni, 1992). These models, based on physical and chemical properties, have produced excellent results in the last few years, especially as far as predictions of contaminant dispersion and potential bioavailability are concerned. However, these methods are little developed, above all with regard to effects on species and ecosystems (Conti, 1996).

These models of pollutant propagation and transport usually, however, concern punctual sources of contamination and they also require large quantities of information if they are to be applied. From this stems the importance of biomonitoring when establishing contaminant levels in organisms and to use in ascertaining possible toxicity in relation to organism placement within the ecosystem.

From an ecotoxicological viewpoint, it is not possible, starting from chemical analysis, to establish a valid model for toxicity capable to foresee the bioavailability and various complex synergies that run between the organisms present in an ecosystem.

The ability to predict the incidence of many human activities with regard to a species and above all, to an ecosystem, is very limited. The difficulty in establishing a cause-and-effect relationship derives above all from the systematic lack of information on the "state of health" of the environment being studied or from the nature of its biological processes (which do not have linear characteristics and which are discontinuous through space and time). From here stems the importance of environmental monitoring plans and biomonitoring plans that, if properly applied, can supply an overall complete picture of the possible interventions that may be required.

Although lichens are important sources for control and environmental biomonitoring, it is necessary to take various precautions when using lichens as a quantitative measure of a single contaminant. Bioindicators of air pollution can most certainly supply information of a qualitative type; nonetheless, correlation studies using environmental data from the sites concerned and taken over a prolonged period of time (months–years), can supply information about semiquantitative aspects.

Through IAP calculation, lichens allow us to evaluate air quality as far as regards the presence of different environmental contaminants. As already mentioned, lichens do not react specifically to a particular contaminant, but rather to the overall toxic effect of a mix of contaminants (Amman et al., 1987). Levin and Pignata (1995) proposed the use of Pollution Index (PI) for the evaluation of air quality. PI is determined using the equation cited by Levin and Pignata (1995). This enables the evaluation of which of the biomonitored areas has the better air quality by measuring lichen reactions to atmospheric pollutants.

$$PI = [(Pha/Chla) + S_p/S_c](HPCD_p/HPCD_c)$$

The phaeophytin *a*/chlorophyll *a* ratio can be changed to chlorophyll *b*/chlorophyll *a*:

$$PI = [(Chlb/Chla) + S_p/S_c](HPCD_p/HPCD_c)$$

Chl *b* and Chl *a* express chlorophyll *a* and *b* concentrations in mg g^{-1} of dry weight. *S* is the sulphur content of lichens expressed as mg g^{-1} of dry weight, while HPCD expresses the concentration of hydroperoxy conjugated dienes in mmol g^{-1} of dry weight. The sub-index p indicates concentrations measured in samples transplanted to contaminated sites, while sub-index c indicates those measured in lichens transplanted to the control site.

Biomonitoring studies using lichens make it possible to verify, with our current state of knowledge, air quality and any improvements thereof. This is what happened in the case of the progressive improvement in air quality over the years (1989–1994) in several Italian cities (La Spezia; Palmieri et al., 1997). This improvement was due to the reduction in SO_2 emissions, which was partly linked to the increase in the use of methane gas for domestic heating and to the closure of a coal-fired power station. The city of Montecatini Terme (central Italy) has also improved its environmental situation: new lichen species have been found and the previous "lichen desert" situation has disappeared (Loppi et al., 1997b). This phenomenon has been correlated with low SO_2 emission levels (approx 15–20 μg m^{-3} from 1993 to 1996) and NO_x, which passed from 150 μg m^{-3} in 1993 to 100 μ m^{-3} in 1996. A similar marked improvement caused by decrease in environmental SO_2 levels was also found in Paris in the 1980s (Luxembourg Gardens) where lichen species from the previous century began to reappear (Seaward and Letrouit-Galinou, 1991; Letrouit-Galinou et al., 1992).

In general, lichen distribution in northern Italy seems mainly to be regulated by SO_2 pollution (Nimis et al., 1990, 1991; Bargagli et al., 1991). As far as regards central Italy, a study of *Parmelia caperata* made by Loppi et al. (1992a) found a strong correlation ($r^2 = 0.93$; $P < 0.05$) between IAP values and the total heavy metal content (Cd, Cr, Cu, Hg, Ni, Pb, Zn).

Techniques for drawing up air quality maps using lichens, or the use of the transplant method, allow us to obtain information about a vast area in a short amount of time and at contained costs.

These methodological approaches, although they cannot be considered as replacements for standard atmospheric pollution monitoring carried out using control stations, are without a doubt valid environmental biomonitoring instruments in different cases:

1. as a preliminary evaluation, or rather as an estimate of the base impact in a set area, with the aim of preventing future human-derived impact;
2. to monitor an already-compromised environmental situation; and
3. to control the quality of reclamation efforts already carried out.

Application of the system approach to the solving of problems regarding atmospheric pollution is doubtless valid and fundamentally requires an evaluation of the progress made in the areas of study considered, the identification of pollution sources, and the cause/effect correlation of the same (Conti, 1996). Of course, from that mentioned in the above points 1–3, it can be seen that for this reason, necessary interventions must have three main objectives:

1. environmental prevention: with the aim of intervening at the impact source and thus in advance of the pollutant event;
2. environmental protection: to eliminate the effects of pollutant actions or to tend to minimise these effects; and
3. environmental restoration: with the aim of removing damages caused by previous actions.

The necessity to increase our knowledge of bioindication studies using lichens remains a fundamental point in the development of research. It is possible to say that for a large majority of pollutants and their effects upon lichens, our knowledge is at an advanced stage in its development in terms of both the quantity and quality of information.

Nonetheless, it is possible to point out that in a significant part of bioindication studies of lichens, there is a tendency to study the environmental effects of situations that have already been compromised. This signifies a scarce propensity to carry out studies that fundamentally have an eye to aspects of environmental prevention.

Acknowledgements

The authors wish to thank Dr. Francesco Botrè for the useful suggestions. We would also like to thank Dr. Marzia Facchini for her valuable assistance.

References

Addison, P.A., Puckett, K.J., 1980. Deposition of atmospheric pollutants as measured by lichen element content in the Athabasca oil sands area. Can. J. Bot. 58, 2323–2334.

Amman, K., Herzig, R., Liebendörfer, L., Urech, M., 1987. Multivariate correlation of deposition data of 8 different air pollutants to lichen data in a small town in Switzerland. Advances in Aerobiology 87, 401–406.

Andersen, A., Hovmand, M.F., Johnsen, I., 1978. Atmospheric heavy metal deposition in the Copenhagen area. Environmental Pollution 17, 133–151.

Antonelli, M.L., Ercole, P., Campanella, L., 1998. Studies about the adsorption on lichen *Evernia prunastri* by enthalpimetric measurements. Talanta 45, 1039–1047.

Asta, J., Garrec, J.P., 1980. Etude de l'accumulation du fluor dans les lichens d'une vallee Alpine polluee. Environmental Pollution (series A) 21, 267–286.

Baddeley, M.S., Ferry, B.W., Finegan, E.J., 1972. The effect of sulphur dioxide on lichen respiration. Lichenologist 5, 284–291.

Baker, D.A., 1983. Uptake of cations and their transport within the plants. In: Robb, D.A., Pierpoint, W.S. (Eds.), Metals and Micronutrients: Uptake and Utilization by Plants. Academic Press, London, pp. 3–19.

Balaguer, L., Manrique, E., 1991. Interaction between sulphur dioxide and nitrate in some lichens. Env and Exp Botany 31 (2), 223–227.

Barci, G., Dalmasso, J., Ardisson, G., 1988. Chernobyl fallout measurements in some Mediterranean biotas. Sci. Total. Environ. 70, 373–387.

Bargagli, R., Barghigiani, C., 1991. Lichen biomonitoring of mercury emission and deposition in mining, geothermal and volcanic areas of Italy. Env. Mon. Ass. 16, 265–275.

Bargagli, R., Nimis, P.L., Monaci, F., 1997. Lichen biomonitoring of trace element deposition in urban, industrial and reference areas of Italy. J. Trace Elem. Med. Biol. 11 (3), 173–175.

Bargagli, R., Gasparo, D., Lazzarin, A., Lazzarin, G., Olivieri, S., Tretiach, M., 1991. Lichens as indicators and monitors of atmospheric pollutants in NE Italy, preliminary data on the integrated testing system. Botanica Chronica 10, 977–982.

Bari, A., Minciardi, M., Troiani, F., Bonotto, F., Paonessa, F., 1998. Lichens and mosses in air quality monitoring: a biological model proposal. Govt. Reports Announcements & Index, Issue 16.

Bartoli, A., Cardarelli, E., Achilli, M., Campanella, L., Massari, G., 1994. Biomonitoraggio dell'aria di Roma: accumulo di metalli pesanti in trapianti di licheni. Ann. Bot. LII (11), 239–266.

Bartoli, A., Cardarelli, E., Achilli, M., Campanella, L., Ravera, S., Massari, G., 1997. Quality assessment of the Maremma Laziale area using epiphytic lichens. Allionia (Turin) 35, 69–85.

Beauchamp, R.O., Bus, J.S., Popp, J.A., Boreiko, C.J., Andjelkovich, D.A., 1984. A critical review of the literature on hydrogen sulphide toxicity. Critical Review in Toxicology 13, 25–97.

Beckett, R.P., Brown, D.H., 1984. The control of cadmium uptake in the lichen genus *Peltigera*. J. Exp. Bot. 35, 1071–1082.

Benedini, M., Cicioni, G., 1992. I modelli matematici e le loro potenzialità. IRSA (Istituto di Ricerca sulle Acque), Atti della giornata di studio: modelli matematici per il Bacino del fiume Po, Parma, 3 June, 1992. Quad. IRSA 95, 1.1–1.30.

Bennett, J.P., Wetmore, C.M., 1999. Changes in element contents of selected lichens over 11 years in northern Minnesota, USA. Environmental and Experimental Botany 41 (1), 75–82.

Benton, J., Fuhrer, J., Sanders, G., 1995. Results from the UN/ECE ICP-crops indicate the extent of exceedance of the critical levels of ozone in Europe. Water, air and soil pollution 85 (3), 1473.

Bero, A.S., Gibbs, R.J., 1990. Mechanisms of pollutant transport in the Hudson estuary. The Science of the Total Environment 97/98, 9–22.

Boonpragob, K., Nash III, T.H., 1991. Physiological responses of the lichen *Ramalina menziesii* Tayl. to the Los Angeles urban environment. Environ. Exp. Bot. 31, 229–238.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Branquinho, C., Catarino, F., Brown, D.H., Pereira, M.J., Soares, A., 1999. Improving the use of lichens as biomonitors of atmospheric metal pollution. Sci. Total Environ. 232 (1–2), 67–77.

Bretten, S., Gaare, E., Skogland, T., Steinnes, E., 1992. Investigations of radiocesium in the natural terrestrial environment in Norway following the Chernobyl accident. Analyst 117 (3), 501–503.

Brodo, I.M., 1961. Transplant experiments with coricolous lichens using a new technique. Ecology 42, 838–841.

Brown D.H., 1991. Mineral cycling and lichens: the physiological basis. Lichenologist 23, 293–3,7.

Brown, D.H., Beckett, R.P., 1984. Uptake and effect of cations on lichen metabolism. Lichenologist 16, 173–188.

Brusoni, M., Garavani, M., Valcuvia Passadore, M., 1997. Lichens and air pollution: preliminary studies in the Oltrepo Pavese (Pavia, Lombardy). Archivio Geobotanico 3 (1), 95–106.

Bruteig, I.E., 1994. Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers. Gunneria (68), 1–24.

Burton, M.A.S., 1986. Biological Monitoring of Environmental Contaminants (Plants) (Marc-Report n. 32). Monitoring and Assessment Research Center, London.

Burton, M.A.S., LeSueur, P., Puckett, K.J., 1981. Copper, nickel and thallium uptake by the lichen *Cladina rangiferina*. Can. J. Bot. 59, 91–100.

Bychek-Guschina, I.A., Kotlova, E.R., Heipieper, H., 1999. Effects of sulphur dioxide on lichen lipids and fatty acids. Biochemistry 64 (1), 61–65.

Calatayud, A., Sanz, M.J., Calvo, E., Barreno, E., del Valle-Tascon, X., 1996. Chlorophyll *a* fluorescence and chlorophyll content in *Parmelia quercina* thalli from a polluted region of northern Castellon (Spain). Lichenologist 28, 49–65.

Calatayud, A., Deltoro, V.I., Abadia, A., Abadia, J., Barreno, E., 1999. Effects on ascorbate feeding on chlorophyll fluorescence and xanthophyll cycle components in the lichen *Parmelia quercina* (Willd.) Vainio exposed to atmospheric pollutants. Physiologia Plantarum 105 (4), 679–684.

Caniglia, G., Calliari, I., Celin, L., Tollardo, A.M., 1994. Metal determination by EDXFR in lichens. A contribution to pollutants monitoring. Biol. Trace Elem. Res. 43–45, 213–221.

Carballeira A., Carral E., Puente X., Villares R., 2000. Regional-scale monitoring of coastal contamination. Nutrients and heavy metals in estuarine sediments and organisms on the coast of Galicia (NW Spain). In: Conti, M.E., Botrè, F. (Eds.), The Control of Marine Pollution: Current Status and Future Trends. Special issue of International Journal of Environment and Pollution (IJEP), 13, 1–6, pp. 534–572.

Cardarelli, E., Achilli, M., Campanella, C., Bartoli, A., 1993. Monitoraggio dell'inquinamento da metalli pesanti mediante l'uso di licheni nella città di Roma. Inquinamento 6, 56–63.

Carreras, H.A., Gudiño Pignata, M.L., 1998. Comparative biomonitoring of atmospheric quality in five zones of Còrdoba city (Argentina) employing the transplanted lichen *Usnea* sp. Environmental Pollution 103, 317–325.

Case, J.W., Krouse, H.R., 1980. Variations in sulphur content and stable sulphur isotope composition of vegetation near a SO_2 source at Fox Creek, Alberta, Canada. Oecologia 44, 248–257.

Cecchetti, G., Conti, M.E., 2000. Monitoraggio biologico della qualità dell'aria: stato dell'arte. Ambiente, Risorse, Salute, Padova 73, 25–30.

Chant, L.A., Andrews, H.R., Cornett, R.J., Koslowsky, V., Milton, J.C., Van den Berg, G.J., Verburg, T.G., Wolterbeek, H.T., 1996. 129I and 36Cl concentrations in lichens collected in 1990 from three regions around Chernobyl. Appl. Radiat. Isot. 47 (9–10), 933–937.

Chettri, M.K., Sawidis, T., 1997. Impact of heavy metals on water loss from lichen talli. Ecotoxicol. Environ. Saf. 37 (2), 103–111.

Chettri, M.K., Sawidis, T., Karataglis, S., 1997. Lichens as a tool for biogeochemical prospecting. Ecotoxicol. Environ. Saf. 38 (3), 322–335.

Cislaghi, C., Nimis, P.L., 1997. Lichens, air pollution and lung cancer. Nature 387, 463–464.

Connell, D., 1986. Ecotoxicology — a new approach to understanding hazardous chemicals in the environment. Search 17 (1–2), 27–31.

Connor, J.J., 1979. Geochemistry of ohia and soil lichen, Puhimau thermal area, Hawaii. Sci. Tot. Env. 12, 241–250.

Conti, M.E., 1996. The pollution of the Adriatic sea: scientific knowledge and policy actions. Int. J. Environment and Pollution 6 (2/3), 113–130.

Davies, F., Notcutt, G., 1996. Biomonitoring of atmospheric mercury in the vicinity of Kilauea, Hawaii. Water Air and Soil Pollution 86 (1–4), 275–281.

De Bakker, A.J., 1989. Effects of ammonia emission on epiphytic lichen vegetation. Acta Bot. Neerl. 38, 337–342.

de Bruin, M., Hackenitz, E., 1986. Trace elements concentrations in epyphytic lichens and bark substrate. Environ. Pollut. (Ser. B) 11, 153–160.

Deltoro, V.I., Gimeno, C., Calatayud, A., Barreno, E., 1999. Effects of SO_2 fumigations on photosynthetic CO_2 gas exchange, chlorophyll *a* fluorescence amission and atioxidant enzymes in the lichens *Evernia prunastri* and *Ramalina farinacea*. Physiologia Plantarum 105 (4), 648–654.

Deruelle, S., 1978. Les lichens et la pollution atmosphérique. Bull. Ecol. 9 (2), 87–128.

Deruelle, S., 1992. Accumulation du plomb par les lichens. Bull Soc Bot Fr, 1139. Actual Bot. 1, 99–109.

Deruelle, S., 1996. The reliability of lichens as biomonitors of lead pollution. Ecologie (Brunoy) 27 (4), 285–290.

Egger, R., Schlee, D., Türk, R., 1994. Changes of physiological and biochemical parameters in the lichen *Hypogymnia physodes* (L.) Nyl. Due to the action of air pollutants-a field study. Phyton 35, 229–242.

Epstein, E., Sagee, O., Cohen, J.D., Garty, J., 1986. Endogenous auxin and ethylene in the lichen *Ramalina duriaei*. Plant Physiology 82, 1122–1125.

Ferry, B.W., Baddeley, M.S., Hawksworth, D.L., 1973. Air Pollution and Lichens. The Athlone Press, London.

Fields, R.F., 1988. Physiological responses of lichens to air pollutant fumigations. In: Nash III, T.H., Wirth, V. (Eds.), Lichens, Bryophytes and Air Quality. Bibl. Lichenol., Vol. 30. Cramer in der Gebruder Borntraeger Verlagsbuchhandlung, Berlin, pp. 175–200.

Fields, R.D., St. Clair, L.L., 1984. The effects of SO_2 on photosynthesis and carbohydrate transfer in the two lichens: *Colema polycarpon* and *Parmelia Chlorochroa*. Am. J. Bot. 71, 986–998.

Folkeson, L., 1979. Interspecies calibration of hevy metal concentrations in nine mosses and lichens: applicability to deposition measurements. Water, Air, and Soil Pollution 11, 253–260.

Freitas, M.C., 1994. Heavy metals in *Parmelia sulcata* collected in the neighborhood of a coal-fired power station. Biol. Trace Elem. Res. 43-45, 207–212.

Freitas, M.C., Reis, M.A., Alves, L.C., Wolterbeek, H.T., 1999. Distribution in Portugal of some pollutants in the lichen *Parmelia sulcata*. Environmental Pollution 106 (2), 229–235.

Galun, M., Ronen, R., 1988. Interaction of lichens and pollutants. CRC Handbook of Lichenology 3, 55–72.

Garty, J., 1985. The amounts of havy metals in some lichens of the Negev desert. Environmental Pollution Ser. B 10, 287–300.

Garty, J., 1987. Metal amounts in the lichen *Ramalina duriaei* (De Not.) Bagl. transplanted at biomonitoring sites around a new coal-fired power station after 1 year of operation. Environ. Res. 43, 104–116.

Garty, J., 1988. Comparisons between the metal content of a transplanted lichen before and after the start-up of a coal-fired power station in Israel. Can. J. Bot. 66, 668–671.

Garty, J., 1992. Lichens and heavy metals in the environment. In: Vernet, J.P. (Ed.), Impact of Heavy Metals on the Environment, Vol. 2. Elsevier, Amsterdam, pp. 55–131.

Garty, J., 1993. Lichens as biomonitors for heavy metal pollution. In: Markert, B. (Ed.), Plants as Biomonitors: Indicators for Heavy Metals in the Terrestrial Environment. VCH, Weinheim, pp. 193–263.

Garty, J., Amman, K., 1987. The amounts of Ni, Cr, Zn, Pb, Cu, Fe and Mn in some lichens growing in Switzerland. Environmental and Experimental Botany 27, 127–138.

Garty, J., Kauppi, M., Kauppi, A., 1995. Differential responses of certain lichen species to sulphur-containing solutions under acidic conditions as expressed by the production of stress ethylene. Environ. Res. 69 (2), 132–143.

Garty, J., Cohen, Y, Kloog, N., 1998a. Airborne elements, cell membranes, and chlorophyll in transplanted lichens. J. Environ. Qual. 27, 973–979.

Garty, J., Kloog, N., Cohen, Y., 1998b. Integrity of lichen cell membranes in relation to concentration of airborne elements. Arch. Environ. Contam. and Toxicol. 34 (2), 136–144.

Garty, J., Karary, Y., Harel, J., Lurie, S., 1993. Temporal and spatial fluctuations of ethylene production and concentrations of sulphur, sodium, chlorine and iron on/in the thallus cortex in the lichen *Ramalina duriaei* (De Not.) Bagl. Environmental and Experimental Botany 33 (4), 553–563.

Garty, J., Kardish, N., Hagemeyer, J., Ronen, R., 1988. Correlations between the concentration of adenosine triphosphate, chlorophyll degradation and the amounts of airborne heavy metals and sulphur in a transplanted lichen. Arch. Environ. Contam. Toxicol. 17, 601–611.

Garty, J., Kloog, N., Cohen, Y., Wolfson, R., Karnieli, A., 1997. The effect of air pollution on the integrity of chlorofill, spectral reflectance response, and on concentration of nickel, vanadium and sulfur in the lichen *Ramalina duriaei* (De Not.) Bagl. Env. Res. 74, 174–187.

Gauslaa, Y., 1985. The ecology of *Lobarion pulmonariae* and *Parmelion caperatae* in Quercus dominated forests in south-west Norway. The Lichenologist 17, 117–140.

Gilbert, O.L., 1973. Lichens and air pollution. In: Ahmadjian, V., Hale, M.E. (Eds.), The Lichens. Academic Press, New York, pp. 443–472.

Gilbert, O.L., 1986. Field evidence for an acid rain effect on lichens. Environmental Pollution (series A) 40, 227–231.

Gombert, S., Asta, J., 1997. Monitoring the chlorine pollution of a refuse incinerator using lichens and sphagnum mosses. Ecologie (Brunoy) 28 (4), 365–372.

Gonzàlez, C.M., Pignata, M.L., 1994. The influence of air pollution on soluble proteins, chlorophyll degradation, MDA, sulphur and heavy metals in a transplanted lichen. Chemistry and Ecology 9, 105–113.

Gonzalez, C.M., Pignata, M.L., 1997. Chemical response of the lichen *Punctelia subrudecta* (Nyl.) Krog transplanted close to a power station in an urban-industrial environment. Environmental Pollution 97 (3), 195–203.

Gonzàlez, C.M., Casanovas, S.S., Pignata, M.L., 1996. Biomonitoring of air pollutants from traffic and industries employing *Ramalina ecklonii* (Spreng.) Mey. And Flot. In Còrdoba, Argentina. Environmental Pollution 91 (3), 269–277.

Gonzalez, C.M., Orellana, L.C., Casanovas, S.S., Pignata, M.L., 1998. Environmental conditions and chemical response of a transplanted lichen to an urban area. J. Environ. Management 53 (1), 73–81.

Gottardini, E., Cristofolini, F., Marchetti, F., 1999. Biomonitoraggio della qualità dell'aria della città di Trento tramite licheni epifiti. Acqua&Aria aprile, 67–71.

Grass, F., Bichler, M., Dorner, J., Holzner, H., Ritschel, A., Ramadan, A., Westphal, G.P., Gwozdz, R., 1994. Application of short-lived radionuclides in neutron activation analysis of biological and environmental samples. Biol. Trace Elem. Res. 43–45, 33–46.

Grasso, M.F., Clocchiatti, R., Carrot, F., Deschamps, C., Vurro, F., 1999. Lichens as bioindicators in volcanic areas: Mt Etna and Vulcano Island (Italy). Environ. Geology (Berlin) 37 (3), 207–217.

Gries, C., 1996. Lichens as indicators of air pollution. In: Nash III, T.H. (Ed.), Lichen Biology. Cambridge University Press, Cambridge, pp. 240–254.

Gries, C., Sanz, M.J., Nash III, T.H., 1995. The effect of SO_2 fumigation on CO_2 gas exchange, chlorophyll fluorescence and chlorophyll degradation in different lichen species from western North America. Cryptogamic Botany 5 (3), 239–246.

Hale, M.E., 1969. How to Know the Lichens. Wm. C. Brown Company Publishers, Dubuque, Iowa.

Hale, M.E., 1983. The Biology of Lichens. E. Arnold, London.

Halonen, P., Hyvärinen, M., Kauppi, M., 1993. Emission related and repeated monitoring of element concentrations in the epiphytic lichen *Hypogymnia physodes* in a coastal area, W Finland. Annales Botanici Fennici 30, 251–261.

Hamada, N., Miyawaki, H., 1998. Lichens as bioindicators of air pollution. Jap. J. Ecol. 48, 49–60.

Hart, R., Webb, P.G., Biggs, R.H., Portier, K.M., 1988. The use of lichen fumigation studies to evaluate the effects of new emission sources on class I areas. JAPCA 38, 144–147.

Hawksworth, D.L., 1971. Lichens as litmus for air pollution: a historical review. Int. J. Environ. Stud. 1, 281–296.

Hawksworth, D.L., Rose, F., 1970. Qualitative scale for estimating sulphur dioxide air pollution in England and Wales using epiphytic lichens. Nature 227 (254), 145–148.

Hawksworth, D.L., Rose, F., 1976. Lichens as Pollution Monitors. Instituto of Biology. Studies in Biology, Vol. 66. E. Arnold, London.

Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acids peroxidation. Archives of Biochemistry and Biophysics 125, 189–198.

Henriksson, E., Pearson, L.C., 1981. Nitrogen fixation rate and chlorophyll content of the lichen *Peltigera canina* exposedo to sulphur dioxide. Am. J. Bot. 68, 680–684.

Herman, R., 1987. Environmental transfer of some organic micropollutans. Ecological Studies 61, 68–99.

Herzig, R., 1993. Multi-residue analysis with passive biomonitoring: a new approach for volatile multi-element contents, heavy metals and polycyclic aromatic hydrocarbons with lichens in Switzerland and the Principality of Liechtenstein. In: Markert, B., (Ed.). Plants as biomonitors — indicators for heavy metals in the terrestrial environment. VCH Publishers, Weinheim, pp. 286–328.

Herzig, R., Urech, M., 1991. Flechten als Bioindikatoren, integriertes biologisches Messystem der Luftverschmutzung für das Schweizer Mittelland. Bibliotheca Lichenologica 43, 1–283.

Herzig, R., Liebendörfer, L, Urech, M., Ammann, K., Guecheva, M., Landolt, W., 1989. Passive biomonitoring with lichens as a part of an integrated biological measuring system for monitoring air pollution in Switzerland. Intern. J. Environ. Anal. Chem. 35, 43–57.

Hofmann, W., Attarpour, N., Lettner, H., Turk, R., 1993. 137 Cs concentrations in lichens before and after the Chernobyl accident. Health Phys. 64 (1), 70–73.

Holopainen, T., Kauppi, M., 1989. A comparison of light fluorescence and electron microscopic observations in assessing the SO_2 injury of lichens under different moisture conditions. Lichenologist 21, 119–134.

Hyvarinen, M., Crittenden, P.D., 1996. Cation ration in *Cladonia portentosa* as indices of precipitation acidity in the British Isles. New Phytologist 132 (3), 521–532.

Hyvarinen, M., Crittenden, P.D., 1998a. Relationships between atmospheric nitrogen inputs and the vertical nitrogen and phosphorus concentration gradients in the lichen *Cladonia portentosa*. New Phytologist 140 (3), 519–530.

Hyvarinen, M., Crittenden, P.D., 1998b. Growth of the cushion-forming lichen, *Cladonis Portentosa*, at nitrogen-polluted and unpolluted heathland site. Environmental and Experimental Bot. 40 (1), 67–76.

Insarov, G.E., Semenov, S.M., Insarova, I.D., 1999. A system to monitor climate change with epilithic lichens. Environ. Mon. Ass. 55 (2), 279–298.

Insarova, I.D., Insarov, G.E., Semenov, S.M., Braakenhielm, S., Hultengren, S., 1993. Lichen sensitivity and air pollution — a review of literature data. Govt. Reports Announcements & Index Issue 17.

Jackson, L.L., Ford, J., Schwartzman, D., 1993. Collection and chemical analysis of lichen for biomonitoring. Govt. Reports Announcements & Index Issue 09.

James, P.W., 1973. The effect of air pollutants other than hydrogen fluorides and sulphur dioxide on lichens. In: Ferry, B.W., Baddeley, M.S., Hawksworth, D.L. (Eds.), Air Pollution and Lichens. The Athlone Press, London, pp. 143–176.

Jeran, Z., Jacimov, R., Batic, F., Smodis, B., Wolterbeek, H.T., 1996. Atmospheric heavy metal pollution in Slovenia derived from results for epiphytic lichens. Fresenius' Journal of Analytical Chemistry 354 (5–6), 681–687.

Jones, B.E., Eriksson, O., Nordkvist, M., 1989. Radiocesium uptake in reindeer on natural pasture. Sci. Total Environ. 85, 207–212.

Kardish, N., Ronen, R., Bubrick, P., Garty, J., 1987. The influence of air pollution on the concentration of ATP and on chlorophyll degradation in the lichen *Ramalina duriaei* (De Not.) Bagl. New Phytol. 106, 697–706.

Karlsson, G.P., Sellden, G., Pleijel, H., 1995. Clover as an indicator plant for phytotoxic ozone concentrations: visible injury in relation to species, leaf age and exposure dynamics. The New Phytologist 129 (2), 355.

Kauppi, M., 1976. Fruticose lichen transplant technique for air pollution experiments. Flora 165, 407–414.

Khalil, K., Asta, J., 1998. Les lichens, bioindicateurs de pollution atmosphérique dans la région Lyonnaise. Ecologie 29 (3), 467–472.

Knops, J.M.H., Nash III, T.H., Boucher, V.L., Schlesinger, W.L., 1991. Mineral cycling and epiphytic lichens: implications at the ecosystem level. Lichenologist 23, 309–321.

Kommission Reinhaltung der Luft im VDI und DIN, 1995. Messen von Immissionswirkungen. Measurement of Immission Effects, Dusseldorf.

Koranda J.J., 1980. Studies of boron deposition near geothermal power plants. US Department of Energy, interim report, UCID 18606. Berkeley.

Kosugi, H., Jojima, T., Kikugawa, K., 1989. Thiobarbituric acid-reactive substances from peroxidized lipids. Lipids 24, 873–881.

Kral, R., Kryzova, L., Liska, J., 1989. Background concentrations of lead and cadmium in the lichen *Hypogymnia physodes* at different altitudes. Sci. Total Environ. 84, 201–209.

Krouse, H.R., Case, J.W., 1981. Sulphur isotope ratios in water, air and vegetation near Teepee Creek gas plant, Alberta. Water, Air and Soil Pollution 15, 11–28.

Krumgalz, B.S., 1989. Unusual grain size effect on trace metals and organic matter in contaminated sediments. Marine Pollution Bulletin 20 (12), 608–611.

Kwapulinski, J., Seaward, M.R., Bylinska, E.A., 1985. Uptake of 226radium and 228 radium by the lichen genus *Umbilicaria*. Sci. Total Environ. 41, 135–141.

Laaksovirta, K., Olkkonen, H., 1977. Epyphytic lichen vegetation and element contents of *Hypogimnia physodes* and pine needles examined as indicators of air pollutiona at Kokkola, W. Finland. Ann. Bot. Fennici 14, 112–130.

LeBlanc, F., De Sloover, J., 1970. Relation between industrialization and the distribution and growth of epiphytic lichens and mosses in Montreal. Can. J. Bot. 48, 1485–1496.

LeBlanc F., Rao D.N., 1975. Effects of air pollutants on lichens and bryophytes. In: Mudd, B.J., Koziowski, T.T. (Eds.), Responses of Plants to Air Pollution. Academic Press, London–New York, pp. 237–271.

Lechowicz, M.J., 1982. The effect of simulated acid precipitation on photosynthesis in the caribou lichen *Cladina stellaris* (Opiz.) Brodo. Water Air Soil Pollut. 18, 421–430.

Lerond, M., 1984. Utilisation des lichens pour la cartographie et le suivi de la pollution atmospherique. Bull. Ecol. 15 (1), 7–11.

Lerond, M., Van Haluwyn, C., Cuny, D., 1996. Lichens et bioindication: réalisations concrètes et exigences éthiques. Ecologie 27 (4), 277–283.

Letrouit-Galinou, M.A., Seaward, M.R.D., Deruelle, S., 1992. On the return of epiphytic lichens to the Jardin du Luxembourg. Bull. Soc. Bot. Fr. Lett. Bot. 139 (2), 115–126.

Levin, A.G., Pignata, M.L., 1995. *Ramalina ecklonii* (Spreng) Mey. And Flot. As bioindicator of atmospheric pollution in Argentina. Canadian J. Bot. 73 (8), 1196–1202.

Livens, F.R., Horrill, A.D., Singleton, D.L., 1991. Distribution of radiocesium in the soil-plant systems of upland areas of Europe. Health Phys. 60 (4), 539–545.

Lo Porto, A., Macchiato, M., Ragosta, M., 1992. Bioindicazione della qualità dell'aria tramite licheni epifiti nella provincia di Potenza. Acqua e Aria 1 (92), 11–18.

Loppi, S., 1996. Lichen as bioindicators of geothermal air pollution in central Italy. Bryologist 99 (1), 41–48.

Loppi, S., Bargagli, R., 1996. Lichen biomonitoring of trace elements in a geothermal area (central Italy). Water Air and Soil Pollution 88 (1-2), 177–187.

Loppi, S., Pirintsos, S.A., De Dominicis, V., 1999. Soil contribution to the elemental composition of epiphytic lichens (Tuscany, Central Italy). Env Mon Ass 58, 121–131.

Loppi, S., Chiti, F., Corsini, A., Bernardi, L., 1992a. Preliminary data on the integrated use of lichens as indicators and monitors of atmospheric pollutants in central Italy. Giornale Botanico Italiano 126, 360.

Loppi, S., Corsini, A., Chiti, F., Bernardi, L., 1992b. Air quality bioindication by epiphytic lichens in central-northern Italy. Allionia 31, 107–119.

Loppi, S., Cenni, E., Bussotti, F., Ferretti, M., 1998a. Biomonitoring of geothermal air pollution by epiphytic lichens and forest trees. Chemosphere 36 (4–5), 1079–1082.

Loppi, S., Pacioni, G., Olivieri, N., Di Giacomo, F., 1998b. Accumulation of trace metals in the lichen *Evernia prunastri* transplanted at biomonitoring sites in central Italy. Bryologist 101 (3), 451–454.

Loppi, S., Nelli, L., Ancora, S., Bargagli, R., 1997a. Passive monitoring of trace elements by means of tree leaves, epiphytic lichens and bark substrate. Environ. Mon. Ass. 45 (1), 81–88.

Loppi, S., Giovannelli, L., Pirintsos, S.A., Putorti, E., Corsini, A., 1997b. Lichen as bioindicators of recent changes in air quality (Montecatini Terme, Italy). Ecologia Mediterranea 23 (3–4), 53–56.

Lupsina, V., Horvat, M., Jeran, Z., Stegnar, P., 1992. Investigation of mercury speciation in lichens. Analyst 117 (3), 673–675.

Manuppella, A., Carlomagno, C., 1990. Air pollution and zonation of epiphytic lichens in the city of Isernia. Ann. Ig. 2 (5), 335–341.

Matthews, K.M., 1981. The use of lichens in a study of geothermal radon emissions in New Zealand. Environmental Pollution 24, 105–116.

Mehelman, M.A., Borek, C., 1987. Toxicity and biochemical mechanisms of ozone. Environ. Res. 42, 36–53.

Méndez, A.O.I., Fournier, O.L.A., 1980. Los lìquenes como indicadores de la contaminaciòn atmosférica en el àrea metropolitana de San José, Costa Rica. Rev. Biol. Trop. 28 (1), 31–39.

Menzel, D.B., 1976. The role of free radicals in the toxicity of air pollutants (nitrogen oxides and ozone). In: Pryor, W.A. (Ed.), Free Radicals in Biology, Vol. 2. Academic Press, New York, pp. 181–203.

Mihok, S., Schwartz, B., Wiewel, A.M., 1989. Bioconcentration of fallout 137 Cs by funghi and red-backed voles (*Clethrionomys gapperi*). Health Phys. 57 (6), 959–966.

Monaci, F., Bargagli, R., Gasparo, D., 1997. Air pollution monitoring by lichens in a small medieval town of central Italy. Acta Botanica Neerlandica 46 (4), 403–412.

Moriarty, F., 1999. Ecotoxicology: The Study of Pollutants in Ecosystems. Academic Press, London.

Nash III, T.H., 1989. Metal tolerance in lichens. In: Shaw, A.J. (Ed.), Heavy Metal Tolerance in Plants: Evolutionary Aspects. CRC Press, Boca Raton, FL, pp. 119–131.

Nash III, T.H., Wirth, V. (Eds.), 1988. Lichens, bryophytes and air quality. Bibl. Lichenol., Vol. 30. Cramer in der Gebruder Borntraeger Verlagsbuchhandlung, Berlin.

Nash III, T.H., Gries, C., 1995. The use of lichens in atmospheric deposition studies with an emphasis on the Arctic. Sci. Total Environ. 160, 729–736.

Nieboer, E., Richardson, D.H.S., Tomassini, F.D., 1978. Mineral uptake and release by lichens: an overview. The Bryologist 81 (2), 226–246.

Nieboer, E., Ahmed, H.M., Puckett, K.J., Richardson, D.H.S., 1972. Heavy metal content of lichens in relation to distance from a nickel smelter in Sudbury, Ontario. Lichenologist 5, 291–304.

Nieboer, E., Richardson, D.H.S., Lavoie, P., Padovan, D., 1979. The role of metal-ion binding in modifying the toxic effects of sulphur dioxide on the lichen *Umbilicaria muhlembergii*. I. Potassium efflux studies. N. Phytol. 82, 621–632.

Nimis, P.L., 1985. Urban lichen studies in Italy. 1st: the Town of Trieste. Geobotania 5/85, 49–74.

Nimis, P.L., 1986. Urban lichen studies in Italy. 2nd: the Town of Udine. Geobotania 7/86, 147–172.

Nimis, P.L., 1987. I macrolicheni d'Italia, chiavi analitiche per la determinazione. Atti del Museo Friulano di Storia Naturale, Udine 8, 101–220.

Nimis, P.L., 1990. Air quality indicators and indices: the use of plants as bioindicators of monitoring air pollution. Colombo, AG and Premazzi, G, Ispra, Italy, JRC.

Nimis, P.L., Tretiach, M., 1995. The lichens of Italy — a phytoclimatical outline. Crypt. Bot. 5, 199–208.

Nimis, P.L., Castello, M., Perotti, M., 1990. Lichens as bomonitors of sulphur dioxide pollutions in La Spezia (Northern Italy). Lichenologist 22, 333–344.

Nimis, P.L., Lazzarin, A., Lazzarin, G, Gasparo, D., 1991. Lichens as bioindicators of air pollution by SO_2 in the Veneto region (NE Italy). Studia Geobotanica 11, 3–76.

Nimis P.L., Ciccarelli A., Lazzarin G., Barbagli R., Benedet A., Castello M., Gasparo D., Lausi D., Olivieri S., Tretiach M., 1989. I licheni come bioindicatori di inquinamento atmosferico nell'area di Schio-Thiene-Breganze (VI). In Bolletino del Museo Civico di Storia Naturale di Verona, 16. CO.GE.V. s.r.l., Verona, Ecothema s.r.l., Trieste.

Notter, M., 1988. Radionuclides in the environment around Swedish nuclear power stations, 1983. Govt. Reports Announcements & Index Issue 11.

Nylander, W., 1866. Les lichens du Jardin du Luxembourg. Bull. Soc. Bot. Fr. 13, 364–372.

Oksanen, J., Laara, E., Zobel, K., 1991. Statistical analysis of bioindicator value of ephyphytic lichens. Lichenologist 23 (2), 167–180.

Olmez, I., Gulovali, M.C., Gordon, G.E., 1985. Trace element concentrations in lichens near a coal-fired power plant. Atmos. Environ. 19 (10), 1663–1670.

Oxynos, S.K., Schmitzer, K., Marth, J., Kettrup, P., 1997. PCDD/F and other chlorinated hydrocarbons in matrices of the Federal Environmental Specimen Bank. Chemosphere 34 (9–10), 2153–2158.

Pakarinen, P., Kaistila, M., Hasanen, E., 1983. Regional concentration levels of vanadium, aluminium and bromine in mosses and lichens. Chemosphere 12, 1477–1485.

Palmieri, F., Neri, R., Benco, C., Serracca, L., 1997. Lichens and moss as bioindicators and bioaccumulators in air pollution monitoring. J. Environ. Pathol. Toxicol. Oncol. 16 (2–13), 175–190.

Palomaki, V., Tynnyrinen, S., Holopainen, T., 1992. Lichen transplantation in monitoring fluoride and sulphur deposition in the surroundings of a fertilizer plant and a strip mine an Siilinjarvi. Ann. Bot. Fenn. 29 (1), 25–34.

Pearson, L.C., Henriksson, E., 1981. Air pollution damage to cell membranes in lichens. II. Laboratory experiments. Bryologist 84, 515–520.

Perkins, D.F., 1992. Relationship between fluoride contents and loss of lichens near an aluminium works. Water Air Soil Pollut. 64 (3–14), 503–510.

Phillips, D.J.H., 1977. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments. A review. Environmental Pollution 13, 281–317.

Phillips, D.J.H., 1980. Quantitative aquatic biological indicators. Their use to monitor trace metal and organochlorine pollution. Applied Science Publ. Ltd, London.

Piervittori, R., 1998. Biomonitoring with lichens in the lower Susa Valley, Piedmont (Italy). Acta Horticulturae 457, 319.

Pilegaard, K., 1978. Airborne metals and SO_2 monitored by ephyphytic lichens in an industrial area. Environmental Pollution 17, 81–92.

Pilegaard, K., 1979. Heavy metals in bulk precipitation and transplanted *Hymogimnia physodes* and *Dicranoweisia cirrata* in the vicinity of a Danish steel works. Water, Air, Soil Pollut. 11, 77–91.

Pilegaard, K., Rasmussen, L., Gydesen, H., 1979. Atmospheric background deposition of heavy metals in Denmark monitored by ephyphytic cryptogams. J. Appl. Ecol. 16, 843–853.

Poblet, A., Andrade, S., Scagliola, M., Vodopivez, C., Curtosi, A., Pucci, A., Marcovecchio, J., 1997. The use of epiphytic Antartic lichens (*Usnea aurantiacoatra* and *U. antartica*) to determine deposition patterns of heavy metals in the Shetland Islands, Antarctica. Sci. Total Environ. 207 (2–3), 187–194.

Puckett, K.J., 1988. Bryophytes and lichens as monitors of metal deposition. In: Nash III, T.H., Wirth, W. (Eds.), Lichens, Bryophytes and Air Quality. Bibl. Lichenol., Vol. 30. Cramer in der Gebruder Borntraeger Verlagsbuchhandlung, Berlin, pp. 231–267.

Puckett, K.J., Finegan, E.J., 1980. An analysis of the element content of lichens from the northwest territores, Canada. Can. J. Bot. 58, 2073–2089.

Quevauviller, Ph., Herzig, R., Muntau, H., 1996. Certified reference material of lichen (CRM 482) for the quality control of trace element biomonitoring. Sci. Tot. Environ. 187, 143–152.

Rao, D.N., LeBlanc, F., 1966. Effects of sulphur dioxide on the lichen alga, with special reference to chlorophyll. Bryologist 69, 69–75.

Richardson, D.H.S., 1988. Understanding the pollution sensitivity of lichens. Bot. J. Linn. Soc. 96, 31–43.

Richardson, D.H.S., 1991. Lichens as biological indicators. Recent developments. In: Jeffrey, D.W., Madden, B. (Eds.), Bioindicators and Environmental Management. Academic Press, London, pp. 263–272.

Richardson, D.H.S., 1992. Pollution monitoring with lichens. Naturalists' Handbooks 19. Richmond Publishing, Slough, UK.

Richardson, D.H.S., 1995. Metal uptake in lichens. Symbiosis 18, 119–127.

Richardson, D.H.S, Puckett, K.J., 1973. Sulphur dioxide and photosynthesis in lichens. In: Ferry, B.W., Baddeley, M.S., Hawksworth, D.L. (Eds.), Air Pollution and Lichens. The Athlone Press, London, pp. 283–298.

Richardson, D.H.S., Shore, M., Richardson, R.M., 1995. The use of X-ray fluorescence spectrometry for the analysis of plants, especially lichens, employed in biological monitoring. Sci. Total Environ. 176 (1–3), 97–105.

Riga-Karandinos, A.N., Karandinos, M.G., 1998. Assessment of air pollution from a lignite power plant in the plain of Megalopolis (Greece) using as biomonitors three species of lichens; impacts on some biochemical parameters of lichens. Sci. Total Env. 215, 167–183.

Rissanen, K., Rahola, T., 1989. Cs-137 concentration in reindeer and its fodder plants. Sci Total Environ 85, 199–206.

Ronen R., Canaani O., Garhy J., Cahen D., Malkin S., Galun M., 1984. The effecto of air pollution and bisulfite treatment in the lichen *Ramalina duriaei* studied by photoacoustics. In: Advances in Photosynthesis Research, Proceedings of the 6th Congress on Photosynthesis, 1–6 August 1983, Brussels.

Ronen, R., Galun, M., 1984. Pigment extraction from lichens with dimethyl sulfoxide (DMSO) and estimation of chlorophyl degradation. Environmental and Experimental Botany 24, 239–245.

Rope, S.K., Pearson, L.C., 1990. Lichens as air pollution biomonitors in a semiarid environment in Idaho. The Bryologist 93, 50–61.

Ross, L.J., Nash III, T.H., 1983. Effects of ozone on gross photosynthesis of lichens. Env. Exp. Bot. 23, 71–77.

Rossbach, M., Jayasekera, R., Kniewald, G., Thang, N.H., 1999. Large scale air monitoring: lichen vs. air particulate matter analysis. Sci. Total Env. 232 (1-2), 59–66.

Rothe, H., Bigdon, M., 1994. Incidence of lichens in the area of the Hamburg airport. Gesundheitswesen 56 (10), 563–566.

Sanz, M.J., Gries, C, Nash III, T.H., 1992. Dose-response relationships for SO_2 fumigations in the *Evernia prunastri* (L.) Ach. and *Ramalina fraxinea* (L.) Ach. New Phytol. 122, 313–319.

Satterwhite, M.B., Ponder Henley, J., Carney, J.M., 1985. Effects of lichens on the reflectance spectra of grtanitic rock surfaces. Rev. Sens. Env. 18, 105–112.

Sawidis, T., Heinrich, G., Chettri, M.K., 1997. Cesium-137 monitoring using lichens from Macedonia, northern Greece. Can. J. Bot. 75 (12), 2216–2223.

Scheidegger, C., Schroeter, B., 1995. Effects of ozone fumigation on epiphytic macrolichens: ultrastructure, CO_2 gas exchange and chlorophyl fluorescence. Environmental Pollution 88, 345–354.

Schonbek, H., 1968. Influence of air pollution (SO_2) on transplanted lichens. Naturwissenshaften 55 (9), 451–452.

Schumacher, M., Domingo, J.L., Llobet, J.M., Müller, L., Jager, J., 1997. Levels of PCDD/F in grasses and weeds collected near a municipal waste incinerator (1996–1997). Sci. Total Environ. 201, 53–62.

Schumacher, M., Domingo, J.L., Llobet, J.M., Sunderhauf, W., Müller, L., 1998. Temporal variation of PCDD/F concentrations in vegetation samples collected in the vicinity of a municipal waste incinerator (1996–1997). Sci. Total Environ. 218 (2–13), 175–183.

Scott, M.G., Hutchinson, T.C., 1987. Effects of a simulated acid rain episode on photosynthesis and recovery in the caribou-forage lichens, *Cladina stellaris* (Opiz.) Brodo and *Cladina rangiferina* (l) Wigg. New Phytol. 107, 567–575.

Seaward, M.R.D., 1992. Large-scale air pollution monitoring using lichens. GeoJournal 28 (4), 403.

Seaward, M.R.D., 1993. Lichens and sulphur dioxide air pollution: field studies. Env. Reviews. 1, 73–91.

Seaward, M.R.D., 1996. Lichens and the environment. In: Sutton, B. (Ed.), A Century of Micology. Cambridge University Press, UK, pp. 293–320.

Seaward, M.R.D., Heslop, J.A., Green, D., Bylinska, E.A., 1988. Recent levels of radionuclides in lichens from southwest Poland with particular reference to cesium-134 and cesium 137. J. Environ. Radioact. 7 (2), 123–130.

Seaward, M.R.D., Letrouit-Galinou, M.A., 1991. Lichen recolonization of trees in the Jardin du Luxembourg, Paris (France). Lichenologist 23 (2), 181–186.F.

Showman, R.E., 1988. Mapping air quality with lichens — the North American experience. In: Nash III, T.H., Wirth, W. (Eds.), Lichens, Bryophytes and Air Quality. Bibl. Lichenol., Vol. 30. Cramer in der Gebruder Borntraeger Verlagsbuchhandlung, Berlin, pp. 67–90.

Silberstein, L., Siegel, B.Z., Siegel, S.M., Mukhtar, A., Galun, M., 1996. Comparative studies on *Xanthoria parietina*, a pollution-resistant lichen, and *Ramalina duriaei*, a sensitive species. Evaluation of possible air pollution-protection mechanisms. The Lichenologist 28, 367–383.

Sloof, J.E., 1995. Lichens as quantitative biomonitors for atmospheric trace-element deposition, using transplants. Atmos. Environ. 29, 11–20.

Sloof, J.E., Wolterbeek, H.T., 1991. National trace element air pollution monitoring survey using epiphytic lichens. Lichenologist 23 (2), 139–166.

Sloof, J.E., Wolterbeek, H.T., 1992. Lichens as biomonitors for radiocesium following the Chernobyl accident. J. Environ. Radioact. 16 (3), 229–242.

Smodis, B., Parr, R.M., 1999. Biomonitoring of air pollution as exemplified by recent IAEA programs. Biol. Trace Elem. Res. 71–72, 257–266.

Sochting, U., 1995. Lichens as monitors of nitrogen deposition. Cryptogamic Bot. 5 (3), 264–269.

St Clair, L.L., Fields, R.D., 1986. A comprehensive approach to biomonitoring of air quality using lichens. A field study. Am. J. Bot. 73 (5), 610.

St Clair, L.L., Fields, R.D., Nakanishi, M., 1986. Biomonitoring of air quality using lichens. A field study. Am. J. Bot. 73 (5), 610.

Stöcker G., 1980. Zu einigen theoretischen und metodischen. Aspekten der Bioindikation. In: Schubert, R., Schuh, J. (Eds.), Methodische und theoretische Grundlangen der Bioindikation (Bioindikation 1), Martin-Luther-Universität, Halle (Saale), GDR, pp. 10–21.

Takala, K., Olkkonen, H., Ikonen, J., Jaaskelainen, J., Puumalainen, P., 1985. Total sulphur contents of epiphytic and terricolous lichens in Finland. Ann. Botanici Fennici 2, 91–100.

Takala, K., Olkkonen, H., Krouse, H.R., 1991. Sulphur isotope composition of epiphytic and terricolous lichens and pine bark in Finland. Environmental Pollution 69, 337–348.

Tarhanen, S., 1998. Ultrastructural responses of the lichen *Bryoria fuscescens* to simulated acid rain and heavy metal deposition. Annals of Botany (London) 82 (6), 735–746.

Tarhanen, S., Metssarinne, S., Holopainen, T., Oksanen, J., 1999. Membrane permeability response of lichen *Bryoria fuscescens* to wet deposited heavy metals and acid rain. Environmental Pollution 104 (1), 121–129.

Tessier, A., Campbell, P.G.C., Bisson, M., 1980. Trace metal speciation in the Yamaska and St. François Rivers (Quebec). Canada Journal Earth Science 17, 90–105.

Thomas, R.S., Ibrahim, S.A., 1995. Plutonium concentrations in lichen of Rocky Flats environs. Health Phys. 68 (3), 311–319.

Tomassini, F.D., Puckett, K.J., Nieboer, E., Richardson, D.H.S., Grace, B., 1976. Determination of copper, iron, nickel, and sulphur by X-ray fluorescence in lichens from the Mackenzie Valley, Northwest Territories, and the Sudbury district, Ontario. Canadian Journal of Botany 54, 1591–1603.

Tonneijk, A.E.G., Posthumus, A.C., 1987. Use of indicator plants for biological monitoring of effects of air pollution: the Dutch approach. VDI Ber. 609, 205–216.

Topcuoglu, S., Van Dawen, A.M., Gungor, N., 1995. The natural depuration rate of 137 Cs radionuclides in a luchen and moss species. J. of Environ. Radioactivity 29 (2), 157–162.

Trass, H., 1973. Lichen sensitivity to the air pollution and index of poleotolerance (I.P.). Fol. Crypt. Est., Tartu 3, 19–22.

Triulzi, C., Marzano, F.N., Vaghi, M., 1996. Important alpha, beta and gamma-emitting radionuclides in lichens and mosses collected in different world areas. Annali di Chimica (86) 11–12, 699–704.

Van Dobben, H.F., Ter Braak, C.J.F., 1998. Effects of atmospheric NH_3 on epiphytic lichens in the Netherlands: the pitfalls of biological monitoring. Atmos. Environ. 32 (3), 551–557.

Van Haluwyn C., Lerond M., 1986. Les lichens et la qualité de l'air. Évolution méthodologique et limites. Ministère de l'environnement. SRETIE.

Van Haluwyn, C., Lerond, M., 1988. Lichénosociologie et qualité de l'air: protocole opératoire et limites. Cryptogamie Bryol. Lichenol. 9 (4), 313–336.

Wadleigh, M.A., Blake, D.M., 1999. Tracing sources of atmospheric sulphur using epiphytic lichens. Environmental Pollution 106, 265–271.

Wirth, V., 1991. Zeigerwerte von Flechten. Scripta Geobot. 18, 215–237.

Wolterbeek, H.T., Bode, P., 1995. Strategies in sampling and sample handling in the context of large-scale plant biomonitoring surveys of trace element air pollution. Sci. Total Environ. 176 (1–3), 33–43.

Zaharopoulou, A., Lanaras, T., Arianoutsou, M., 1993. Influence of dust from a limestone quarry on chlorophyll degradation of the lichen *Physcia ascendens* (Fr.) Oliv. Bull. Environ. Contam. Toxicol. 50 (6), 852–855.

Zambrano, A., Nash III, T.H., 2000. Lichen responses to short-term transplantation in Desierto de los Leones, Mexico city. Environmental Pollution 107, 407–412.

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