EUROPEAN GUIDELINE

FOR MAPPING LICHEN DIVERSITY AS AN INDICATOR OF ENVIRONMENTAL STRESS

Based on the German VDI Lichen Mapping Guideline (VDI, 1995) and the Italian Guideline of ANPA (Nimis, 1999), with several important modifications (see Asta et al. 2002 for an abridged version).

It was prepared by:

J. Asta (France), W. Erhardt (Germany), M. Ferretti (Italy), F. Fornasier (Italy), U. Kirschbaum (Germany), P. L. Nimis (Italy), O.W. Purvis (UK), S. Pirintsos (Greece), C. Scheidegger (Switzerland), C. Van Haluwyn (France) and V. Wirth (Germany)

Contents

Introduction

Lichens are extremely sensitive to environmental stress, especially concerning atmospheric pollution, eutrophication, and climate change (e.g. see Galun, 1988; Richardson, 1992; Nash, 1996, Nimis et al., 2002). The main reasons are: a) a delicate symbiotic association exists between the lichen partners and the fungus can rarely exist on its own; b) unlike higher plants, lichens have no cuticle (protective layer) and pollutants can readily penetrate to the fungal and algal cells; c) the uptake of substances occurs mainly from the atmosphere; d) lichens have an increased metabolic rate, especially when moist; e) lichens continue to metabolize at low temperatures and are susceptible to damage during the winter months; f) lichens grow slowly and injuries cannot be quickly restored.

Lichen diversity is an excellent indicator of pollution by phytotoxic gaseous substances (e.g. see Hawksworth & Rose, 1970; Ferry et al., 1973; Nash & Wirth, 1988; Richardson, 1992, Cislaghi & Nimis, 1997; Purvis, 2000, Nimis et al., 2002). Lichens respond relatively fast to a deterioration in air quality and can re-colonize urban and industrial environments as a consequence of improved conditions within a few years, as recorded in many parts of Europe (e.g. Rose & Hawksworth, 1981; Kandler & Poelt, 1984; Seaward & Letrouit-Galinou, 1991, Seaward, 1997, Kricke & Loppi, 2002). Lichens are also sensitive to other types of environmental alteration, a well-known example being eutrophication (see e.g. van Dobben & De Bakker, 1996; van Herk, 1999; van Dobben et al., 2001, van Haluwyn & van Herk, 2002). Lichens have also been used to estimate the ecological continuity of forests as they are also very sensitive to changes in woodland management (Rose, 1976; McCune, 2000, Rose & Coppins, 2002), and to establish networks to monitor climate change (e.g. see Insarov & Schroeter, 2002; van Herk et al., 2002).

This guideline proposes a standardized method to assess lichen diversity on tree bark. Results are shown in a map indicating zones with different lichen diversity. The guideline is largely based on the German VDI Lichen Mapping Guideline (VDI, 1995) and the Italian Guideline (Nimis, 1999), with several important modifications. These were agreed upon during a meeting of the authors in Rome (November 2000), sponsored by the Italian National Agency for the Environment (ANPA). The main modifications concern several elements of subjectivity in the sampling process which were present both in the VDI and in the Italian guidelines. An abridged version of this guideline was published by Asta et al. (2002).
The method proposed here determines the actual state of lichen diversity before or after long-term exposure to air pollution and/or to other types of environmental stress. The interpretation of geographic patterns and temporal trends of lichen diversity in terms of pollution, eutrophication, climatic change, etc., may be assisted by using ecological indicator values (e.g. Wirth, 1992; Diederich & Scheidegger, 1996; Kirschbaum & Hanewald, 1998; Nimis & Martellos, 2001, 2002), and a numerical analysis of a matrix of species and relevés (Gombert, 1999; van Haluwyn & Lerond, 1988).

1. Principles of the Procedure

This guideline is based on the fact that epiphytic lichen diversity is impaired by air pollution and environmental stress. The frequency of occurrence of lichen species on a defined portion of tree bark is used as an estimate of diversity, and as a parameter to estimate the degree of environmental stress. The following procedure provides a rapid, low cost method to define zones of different environmental quality. It provides information on the long-term effects of air pollutants, eutrophication, anthropization and climatic change on sensitive organisms. It can be applied in the vicinity of an emission source to proove the existence of air pollution and to identify its impact, or, on a larger scale, to detect hot-spots of environmental stress. Repeated monitoring at the same sites enables assessment of the effects of environmental change. Data quality largely depends on the uniformity of growth conditions: the more uniform, the more reliable are the results. A high degree of standardization in sampling procedures is therefore necessary.

The procedure is universally applicable. Interpretation of the results, however, has to be adapted to the regional characteristics of the lichen flora and to the prevalent types of environmental stress. An example is monitoring acidic gaseous emissions caused by fossil fuel combustion. Different methods may be used to solve particular problems, or in particular areas. Examples include monitoring eutrophication in the

Netherlands (van Herk, 1999, 2002), lichens on twigs to detect environmental change (Wolseley & Pryor, 1999; Wolseley 2002), and the French phytosociological approach (van Haluwyn & Lerond, 1986 and 1993, van Haluwyn & van Herk, 2002).

Investigations performed according to this guideline require personnel or institutions having the necessary expertise. Quality assurance standards should be followed, and National Authorities must ensure that operators are properly trained and inter-calibrated.

2 Sampling design

2.1 Objective

Sampling design provides rules to objectively select monitoring sites (hereafter termed "sampling units") within a survey area and comprises sampling strategy and sampling tactics. Briefly, sampling strategy defines how sampling units are selected and located, whereas sampling tactics regulates how many trees are selected for sampling within each sampling unit and how they are selected. Both depend on the aim of the study, and should be defined in relation to its geographical scale. Both sampling design and data quality were extensively discussed by Ferretti & Erhardt (2002) in relation to lichen biomonitoring studies.

2.2 Sampling strategy

Investigating the effects of environmental stress for a particular region depends on an even distribution of sampling units, which are best located within a grid (Van Meirvenne, 1991). Each sampling unit is regarded as being representative for a certain portion of the survey area, and must receive equal attention in terms of monitoring effort to avoid bias.
When monitoring the effects of a point/linear emission pollution source, the sampling units should be located in a pattern corresponding to the expected distribution of pollutants, (taking into account distance from source, prevailing wind direction and other source characteristics; see AK BW, 1999). (Fig. 1).

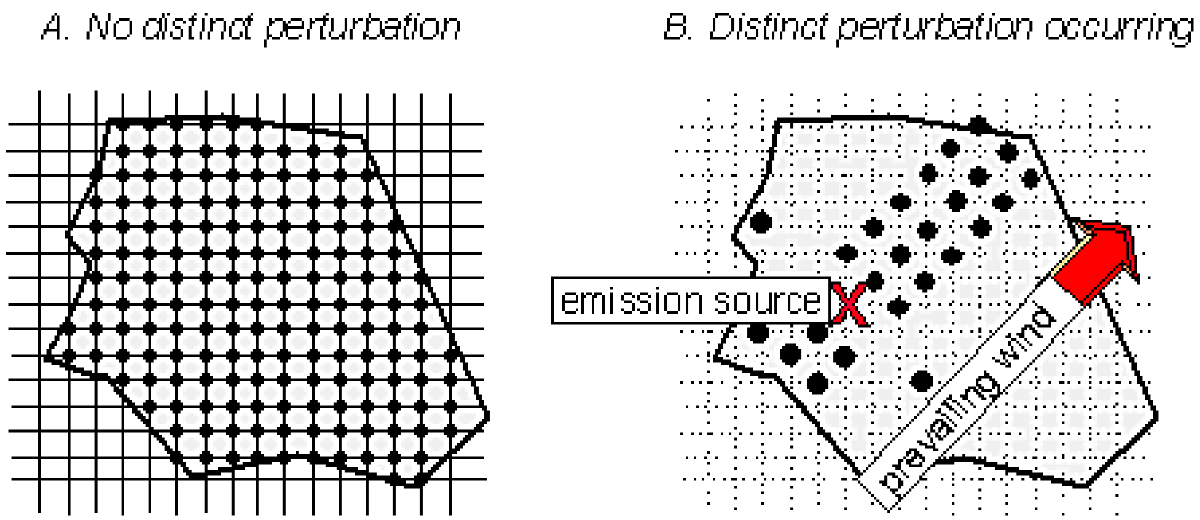


Fig. 1 – Example of sampling units placement in the general investigation of a region (A), and in the vicinity of a point source (B): In case A the sampling units are located at the intersections of the gridlines. In case B they are located around the emission source, where the highest pollution gradients are expected, and at two points where no influence of the point source is to be expected (to obtain information on background levels).

2.3 Sampling density

Sampling density corresponds to the number of sampling units in a given area and depends on grid size. Appropriate sampling density can be defined according to the following formula (Van Meirvenne, 1991):

(1)

$$n = \left(t_{\alpha} \frac{CV}{E} \right)^2$$

where:

n= number of sampling units;
t= Student t at defined probability level 1-α (for large sample sizes – normal distribution - and $\alpha = 0.05$, t=1.96);
CV= coefficient of variation, given by $CV = \frac{s}{X} 100$, where *s* is the standard deviation and *X* the sample mean;
E= desired level of relative error (% of mean).

Equation (1) is independent of the size of geographical area. However, it needs an *a priori* knowledge about the expected variability of lichen data, which is in part dependent on environmental conditions. An appropriate sampling density should ideally be established by carrying out a preliminary survey. When this is not possible, information from other studies carried out under similar environmental conditions may be used. In this case, it is better to adopt a conservative approach (i.e. a larger coefficient of variation in equation (1) and – at the same time – to consider whether it can be sustained by the available financial support.

When information about data variability is not available, and when it is impossible to carry out a preliminary survey, Tab. 1 suggests sampling densities appropriate at different geographical scales and type of study.

Table 1 –Practical grid densities for different geographical scales and type of study (Data are in km).

	<5 km²	**>5 - 100 km²**	**>100 - 1000 km²**	**>1000 km²**
Distinct perturbation occurring (gradient studies)	0.25 x 0.25	0.5 x 0.5 to 6 x 6	1 x 1 to 12 x 12	Unusual
Before-After	0.25 x 0.25	0.5 x 0.5 to 6 x 6	1 x 1 to 12 x 12	Unusual
No distinct perturbation occurring	0.25 x 0.25 to 0.5 x 0.5	0.5 x 0.5 to 6 x 6	3 x 3 to 12 x 12	>9 x 9

2.4 Sampling tactic

Sampling tactic concerns the size of the sampling units, the number of trees to be sampled, and their selection within the sampling units.

2.4.1 Sampling unit size

The size of sampling units depends on the grid size and hence on the geographical scale of the study. With sampling units of 0.25 x 0.25 km, 0.25 km is the maximum grid density. For the same reason, a 1 x 1 unit can be sampled every 1, 2, 3, ...*n* km according to the survey needs. Sampling units larger than 1 x 1 are not recommended, as they can cause a number of practical problems.

2.4.2 Number of trees per sampling unit

The number of trees per sampling unit depends on its size, on the within-unit data variability, and on the availability of suitable trees (see section 3.1). For practical reasons, the number of trees can vary between 3 and 12 (Tab. 2): The numbers suggested in Tab. 2 take into account both the problem of finding sufficient suitable trees in small areas, and the need for an objective selection procedure (see 2.4.4).

Table 2 – Recommended number of trees for sampling units of different sizes.

Size of sampling unit	**0.25 x 0.25 km**	**0.5 x 0.5 km**	**1 x 1 km**
Number of trees	3 - 4	4 - 6	6 - 12

If the minimal number of trees is not available, the sampling unit has to be shifted according to the rule described in section 2.4.3.

The following cases can occur taking units of 1 x 1 km as a model, the most suitable sampling unit for studies at large geographical scales (Ferretti & Erhardt 2002):
1) The number of suitable trees per sampling unit is less than 6 - In this case, the sampling unit cannot be used, and another unit must be selected, if possible, according to the procedure described in section 2.4.3.
2) The number of suitable trees is between 6 and 12 - Ideally, all trees should be sampled. When this is not possible, the selection procedure of section 2.4.4 applies.
3) The number of suitable trees is more than 12 (or more than the actual sampling possibilities) - In this case, sample trees must be selected according to the selection procedure outlined in section 2.4.4.

The same procedure can be readily adapted to smaller sampling units.

2.4.3 Shifting the sampling unit in case of insufficient trees

Where there are too few trees, another sampling unit should be selected according to a standard procedure, e.g. moving to the nearest unit to the North and then moving clockwise to the next according to the scheme in Fig. 2 (Ferretti & Erhardt, 2002). A new sampling unit is installed as soon as the sampling requirements (at least *n* suitable trees) are met. If none of the surrounding potential sampling units will fulfil the requirements, the sampling unit must not be selected for monitoring purposes.

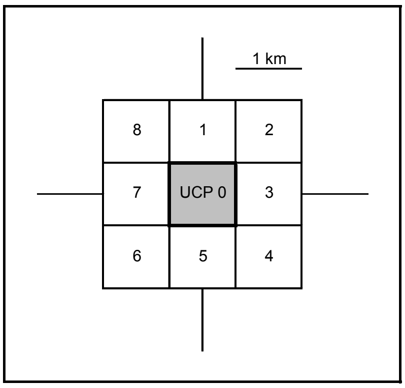


Fig. 2 - Scheme for selecting a new sampling unit when suitable conditions are not found at the originally identified sampling unit (UCP 0). Numbering of sampling units corresponds to shifting priority (after Asta et al., 2002).

2.4.4 Tree Selection procedure

Where more trees occur in a sampling unit than the number chosen for sampling, it is important to select trees according to a statistically valid method. Various objective procedures can be used including:

a) Selecting suitable trees closest to the centre of the sampling units, regardless of their position in the unit (Fig. 3a),
b) Dividing the mapping units into four quadrants, and selecting 3 trees per quadrant, either considering the distance from the centre of the unit (Fig. 3b) or,
c) Using sub-plots (Fig. 3c).

Selecting trees in each of the quadrants is recommended as a practical method to distribute the trees throughout the sampling unit.

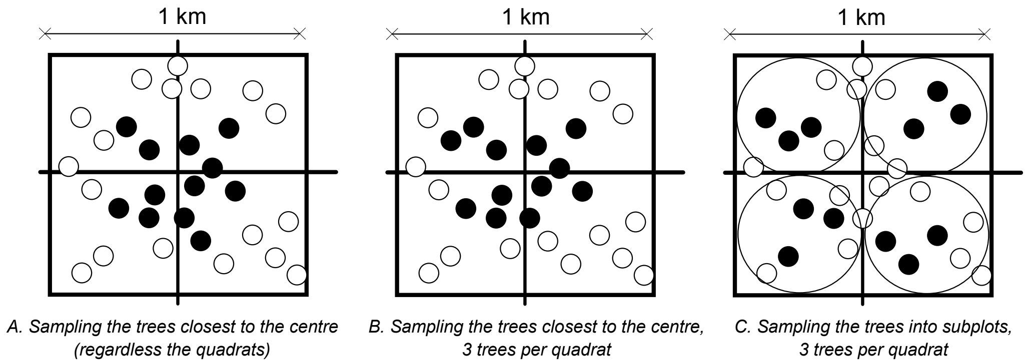


Fig. 3 – Effects of different sampling tactics on the spatial distribution of sample trees selected from a population within a sampling unit. A: the trees closest to the centre are sampled, regardless of their location in the sectors; B: 3 trees per sector are selected, those closest to the centre of the unit; C: 3 trees per sector are selected, those closest to the centre of a subplot installed within each sector (Ferretti & Erhardt, 2002).

To select sample trees for each of the quadrants the following procedure can be adopted:
1) Define the centre and divide the sampling unit into four sectors (Fig. 4).
2) Number the sectors clockwise from 1 to 4, starting from the upper right sector.
3) First (operation 1), for each sector search for the 3 suitable trees which are closest to the centre of the sampling unit (Fig. 4) . Two cases may arise:
(i) At least 3 suitable trees occur per sector. This is the ideal situation, and 12 trees can be sampled.
(ii) Some sectors have less than 3 suitable trees and others more than 3. In this case (operation 2) come back to the first sector with more than 3 trees (sector 2 in Fig. 4). Trees not yet selected during operation 1 are now selected until a total of 12. If this is not possible, move to the next sector and so on, until the number of 12 is reached. For operation 2, the tree closest to the centre of the unit should also be considered.

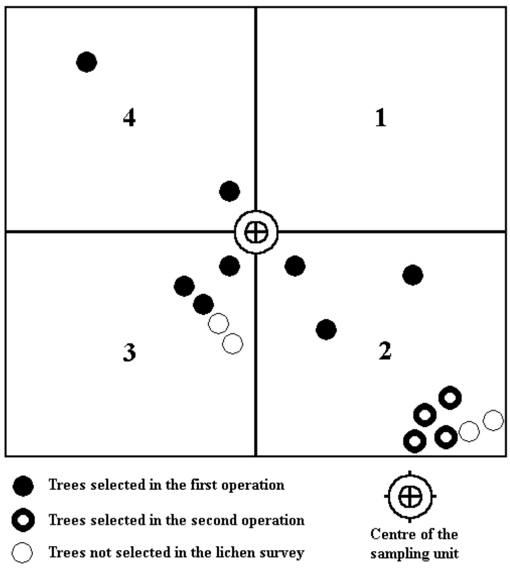


Fig. 4 – Example of selection of trees in a sampling unit (after Asta et al., 2002)

3. Sampling procedure

3.1 Selecting tree species

Tree species must be selected after a reconnaissance of the study area, in order to verify the frequency/distribution of suitable trees.

Free-standing trees should be selected, i.e. those whose trunks receive direct solar radiation for at least part of the day (ca. 15 m distance between trunks). Trees from forest stands should be used only for particular purposes (e.g. monitoring environmental change in forests). However, the use of both free-standing trees and of those in closed-canopied stands must be avoided in a single survey.

It is advisable to select only trees of a single species within a survey because epiphytic lichen growth strongly depends on bark properties (e.g. see Türk & Wirth, 1975; Kricke, 2002), as well as on the age and shape of trees. If this is not feasible, tree species can be chosen with similar bark properties (e.g. pH, water storage capacity, nutrient contents). National Authorities should prepare lists of trees with similar physico-chemical bark properties based on the national tree floras. The list should not include trees with unknown ecological bark properties. Examples are provided in the German (VDI 1995) and Italian (Nimis 1999) guidelines. Data from very different tree species cannot be used interchangeably.

Trees with a readily peeling bark, such as *Platanus*, should not be used for sampling.

Table 3 – Tree species with similar physico-chemical properties which can be used interchangeably.

Group I	**Group II**

The circumferences of the trunks must not be less than 40 cm, and it is preferable to select trees with a circumference of at least 70 cm. Within the same survey, trees of similar size should be used.
Injured trees are not suitable. Trees visibly affected by actions such as liming, removal of the bark by humans or of the lichens by grazing animals are also not suitable. Mapping of trees in fruit-plantations affected by fungizides is not permitted.
The inclination of trees must not exceed 10° from the vertical.

3.2 Surveying Lichen Diversity

A monitoring quadrat consisting of four independent quadrat segments of five 10 x 10 cm squares each, as shown in Fig. 5, is attached vertically to the trunk so that the lower edge of each segment is 1 m above the highest point of the ground. In particularly arid areas, and especially in city centres, the lichen cover is often restricted at the base of the trees (higher humidity and nutrient enrichment; see Pirintsos et al., 1993). In such cases the survey may be carried out at heights under 100 cm. However these data cannot be processed together with those obtained from other surveys using quadrats at the recommended height, but may be used separately, in the most convenient form, in order to define further zonations within the survey area (be aware of the problem of eutrophication by dogs in this case!).

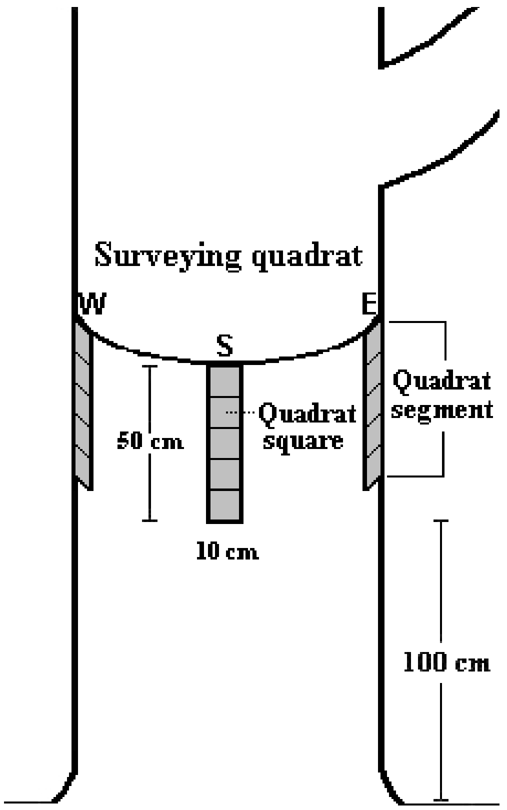
The four segments of the sampling quadrat must be placed to correspond with the 4 aspects (NSEW) of the tree trunk.

It is possible to relocate monitoring quadrat segments by a maximum shift of 20° in a clockwise direction to avoid parts of the trunk which are not suitable for sampling (e.g. wounds, knots, etc).

At least three segments should be placed on a given trunk; if this proves to be impossible, another tree must be selected.
The following situations must be avoided when locating the quadrat segments, even if a high lichen cover is present:

- damaged or decorticated parts
- knots,
- seepage tracks
- parts where the bryophyte cover is higher than 25% (however, muscicolous lichens must be considered when calculating lichen diversity values).

It is advisable to mark the location of the areas examined on the trunk accurately and durably if a repetition of the inventory is planned, for which prior approval of the landowner may be necessary.



The four segments of the monitoring quadrat are attached to the tree with a rubber band

Fig. 5 - Recording quadrat composed of four quadrat segments each with 5 squares.

All lichen species present within each quadrat segment are recorded using a form (see Appendix) and the frequency of occurrence of each species in the 5 squares of each quadrat segment noted. The list of species with their frequency values in one segment constitutes a relevé of lichen vegetation.

All species are suitable for the calculation of Lichen Diversity Values (LDV). However, a few small crustose lichens are particularly difficult to identify and/or are easily overlooked. Where the identification of certain thalli is troublesome both in the field and/or in the laboratory, it is advisable to include them in the calculation of diversity as "*Sp. nr. x*", having established that they are not damaged or poorly developed forms of species already occurring in the monitoring quadrat.

No specimens of red-listed species should be removed.
No lichens should be removed from within the monitoring quadrat if a future survey is planned.
Lichens are identified according to the methods described in the published literature (see references: determination keys).

4. Data Analysis

4.1 Calculation of Lichen Diversity Values (LDV)

The Lichen Diversity Value (LDV) of a sampling unit is a statistical estimator of the environmental conditions in that unit.

The first step in calculating the LDV of a sampling unit (j) is to sum the frequencies of all lichen species found on each tree (i) within the unit. Since substantial differences in lichen growth may be expected on different sides of the trunks, the frequencies have to be summed separately for each aspect. Thus, for each tree there are four Sums of Frequencies (tree i: SF_{iN}, SF_{iE}, SF_{iS}, SF_{iW}).

Next, for each aspect the arithmetic mean of the Sums of Frequencies (MSF) for sampling unit j are calculated

$$MSF_{Nj} = (SF_{1Nj}+SF_{2Nj}+SF_{3Nj}+SF_{4Nj}+\ldots..+SF_{nNj})/n$$

….

where
MSF: Mean of the sums of frequencies of all the sampled trees of unit j
SF: Sum of frequencies of all lichen species found at one aspect of tree i
N, E, S, W: north, east, south, west
n: number of trees sampled in unit j

The Lichen Diversity Value of a sampling unit j (LDV_j) is the sum of the MSFs of each aspect

$$LDV_j = (MSF_{Nj}+MSF_{Ej}+MSF_{Sj}+MSF_{Wj})$$

Table 4 - Example of Calculation of the Lichen Diversity Value

Tree 1, Unit j:

	N	**E**	**S**	**W**
Lichen species 1	0	5	4	2
Lichen species 2	1	3	3	2
Lichen species 3	1	2	5	2
Lichen species 4	0	0	0	0
Lichen species 5	0	5	1	5
Lichen species 6	0	1	2	5
Lichen species 7	0	4	1	4
Lichen species 8	0	4	2	1
Lichen species 9	0	1	0	5
Lichen species 10	1	1	1	0
Sums of Frequencies (SF)	**3**	**26**	**19**	**26**

Tree 2, Unit j:

	N	**E**	**S*)**	**W**
Lichen species 1	0	0	-	2
Lichen species 2	1	2	-	5
Lichen species 3	0	0	-	0
Lichen species 4	1	3	-	5
Lichen species 5	1	4	-	4
Lichen species 6	0	0	-	0
Lichen species 7	1	5	-	4
Lichen species 8	1	4	-	3
Lichen species 9	1	1	-	4
Lichen species 10	0	2	-	1
Sums of Frequencies (SF)	**6**	**21**	**-**	**28**

....

....

Tree n-1, Unit j:

	N*)	**E**	**S**	**W**
Lichen species 1	-	5	5	4
Lichen species 2	-	4	2	1
Lichen species 3	-	2	3	3
Lichen species 4	-	0	3	4
Lichen species 5	-	0	0	0
Lichen species 6	-	5	4	3
Lichen species 7	-	2	2	3
Lichen species 8	-	4	0	3
Lichen species 9	-	2	3	3

Tree n, Unit j:

	N	**E**	**S**	**W**
Lichen species 1	0	5	5	5
Lichen species 2	0	3	1	4
Lichen species 3	1	4	5	2
Lichen species 4	0	3	5	3
Lichen species 5	1	4	4	4
Lichen species 6	0	3	3	4
Lichen species 7	0	0	0	0
Lichen species 8	0	3	2	0
Lichen species 9	0	0	0	0

Lichen species 10	-	3	1	2	Lichen species 10	0	0	1	3
Sums of Frequencies (SF)	**-**	**27**	**23**	**26**	**Sums of Frequencies (SF)**	**2**	**25**	**26**	**25**

	N	E	S	W
Sums of Frequencies Tree 1	3	26	19	26
Sums of Frequencies Tree 2	6	21	-	28
....	....	....	....	....
....	....	....	....	....
Sums of Frequencies Tree n-1	-	27	23	26
Sums of Frequencies Tree n	2	25	26	25
Means of Sums of Frequencies (MSF)	**3,7**	**24,8**	**22,7**	**26,3**
LDV of unit j		**77,3**		

*) data omitted to simulate the case when one aspect was unsuitable for sampling .

4.2 Determination of Lichen Diversity Classes (LDC)

LDV values should be grouped into classes, sufficiently wide to reflect statistically and environmentally significant differences among sampling units to interpret and present results. On the other hand the classes should not be too wide, to avoid loss of information on geographic patterns. This is best achieved if the class width is linked to the range of error of results. Thus, the quality of the results (e. g. availability and quality of sampling trees, number of trees per unit, ecological homogeneity of the area, work precision) determines how many different classes, and as a consequence, how many zones of different environmental conditions can be distinguished in a given mapping project.

The accuracy of Lichen Diversity Values is best described by their standard errors. If these are large, the classes will be broad and no fine differentiation between various degrees of lichen diversity is possible; if standard errors are small, a finer distinction of lichen diversity values is possible.

A reasonable classification is achieved, if LDVs of non-adjoining classes differ in a statistically significant way (e.g. if the LDVs of class 1 differ significantly from those of class 3). As the best estimator, the median of the standard errors of the sampling units is chosen as the basis for classification.

Two groups of data may be considered to be significantly different, if the distance between their means is equal to 3 standard errors, which means that the width of a class should be equal to 3 standard errors.

In order to calculate the standard error of the LDVs, the standard deviation of the sums of frequencies must be determined. For this, the systematic differences among lichen frequencies at each aspect have to be taken into account. This is either done by separately determining the standard deviations for each aspect in each sampling unit and then averaging them, or by following the example given below.

Table 5 - Calculation of standard deviation of the sums of frequencies:

	N	E	S	W		N	E	S	W
Sums of Frequencies Tree 1	3	26	19	26	absolute deviations from MSF	-0,7	1,3	-3,7	-0,3
Sums of Frequencies Tree 2	6	21	-	28	absolute deviations from MSF	2,3	-3,8	-	1,8
....	....	....	....	....		....	....	....	....
....	....	....	....	....		....	....	....	....
Sums of Frequencies Tree n-1	-	27	23	26	absolute deviations fromMSF	-	2,3	0,3	-0,3
Sums of Frequencies Tree n	2	25	26	25	absolute deviations from MSF	-1,7	0,3	3,3	-1,3
Means Sums of Frequencies (MSF)	**3,7**	**24,8**	**22,7**	**26,3**					
					Standard Deviation	**2,127**			

The standard errors of the LDVs is then calculated according to the formula:

standard error of unit j = standard deviation of unit j/root(n_j-1)

where
s_j: standard deviation of sums of frequencies in unit j
n_j: number of trees sampled in unit j

The width of the LDV classes is then:

width of the LDV classes = 3*standard error

4.3 Mapping

Maps can be constructed in two different ways:

1) The *sampling grid is* plotted onto the map: The LDV values of the sampling units are assigned to Lichen Diversity classes (see above) and sampling units coloured according to the respective class.
2) Automatic mapping programs can be used which calculate interpolations from adjoining points. It is important to consider whether the survey area topography and sampling density are appropriate to allow use of such algorithms.

5. Data Interpretation

5.1 Scales to interpret lichen diversity

Lichen growth may vary between regions because of floristic, ecological and climatic differences. Lichen diversity values should therefore be interpreted according to specific regional scales developed from the results of several mapping projects both to ensure comparability and to critically evaluate projects. Such scales should assign verbal evaluations and colour codes to characterise different levels of lichen diversity, according to their deviation from the "normal/natural" conditions prevailing in the region.

In this guideline, diversity is defined as "very high - high - moderate - low - very low " following the scale proposed for Germany (VDI 1995). Similar scales were proposed by Nimis (1999) for submediterranean Italy, and by Loppi et al. (2002) for Tyrrhenian Italy. These scales, however, were developed on the basis of different sampling grids, and in the following they will be used as mere examples. Interpretation scales based on the method presented here are being developed in different parts of Europe.

Geographic patterns of LDV can be interpreted in terms of general environmental alteration (deviation from background conditions) on the basis of the interpretation scales. If no such scale is available, interpretations can be based on the differences between maximum and minimum LDV values within the survey area. In this case, however, patterns of environmental alteration can be detected, but their magnitude cannot be properly assessed.

LDV classes are assigned to the interpretation scale so that they match the best suitable verbal expressions and colour codes (Figs. 6 and 7). If LDV classes fall into two categories, the verbal expressions of both categories are combined, e.g. "moderate to high diversity", and hatched colour codes (e.g. green hatching on yellow background) are used. If several LDV classes fall into one category they are separated by black hatching. The density of the hatching decreases toward the range of higher diversity.

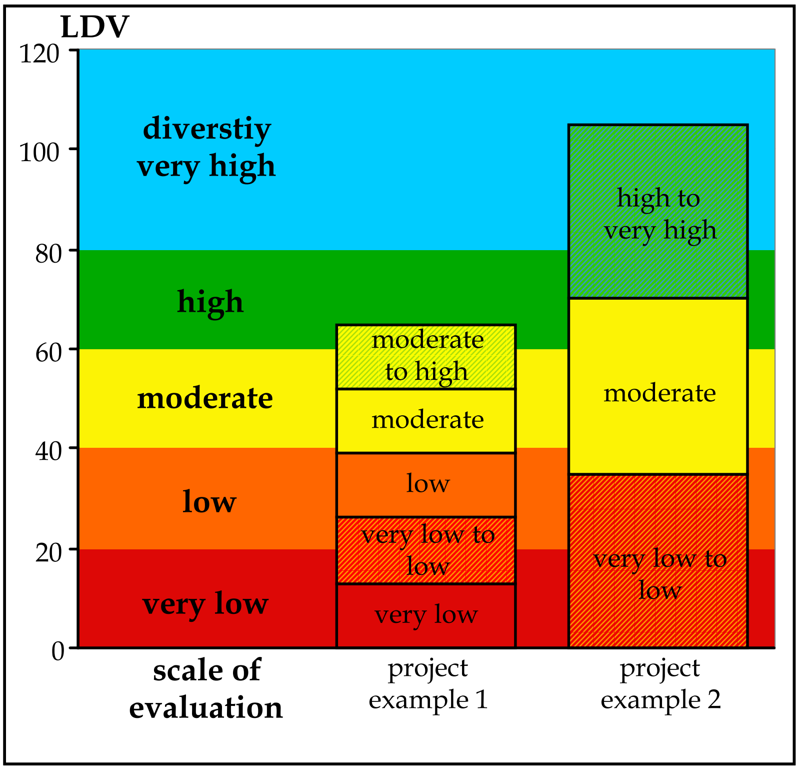


Fig 6: Examples for the scale

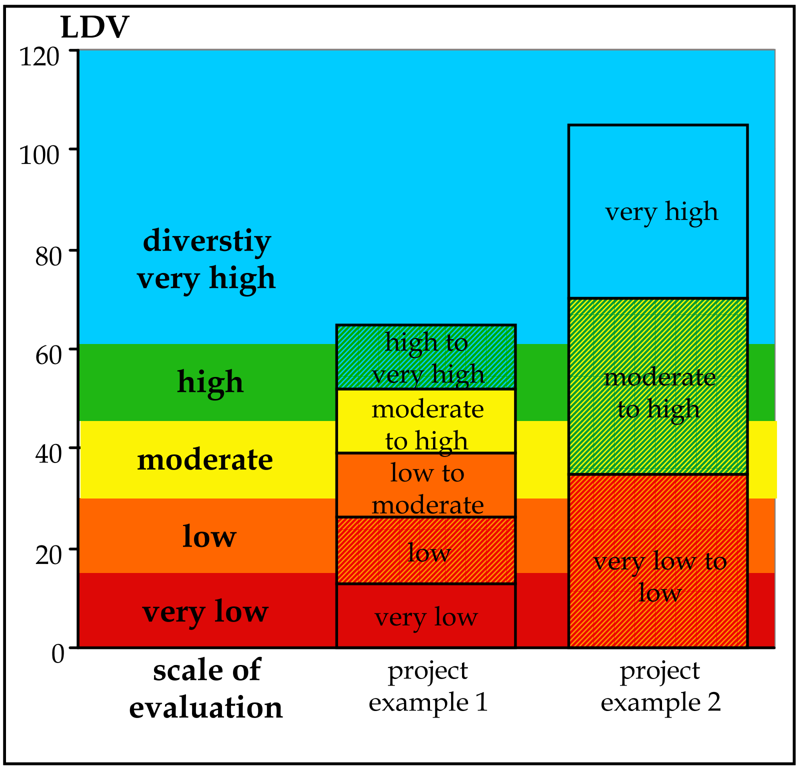


Fig 7: Examples for the scale

A mathematical procedure for assigning the LDV classes to the interpretation scales was deliberately avoided not to give the impression that an accurate distinction is feasible.

5.2 Analysis of relevés

A much finer level of data interpretation is possible by carrying out multivariate analyses of the relevé data on each sampled tree since each relevé consists of a list of species occurring in a monitoring quadrat segment, each with an associated frequency value. Relevés should be organized into a matrix of species and their associated frequencies. Multivariate classification of this matrix groups species with a similar ecological behaviour, and groups of relevés with a similar floristic composition (communities). Identification of communities can be made with reference to the available phytosociological literature (see e.g. Barkman, 1958; James et al., 1977; Lerond, 1981). Used in conjunction with physico-chemical and other environmental data, ordination programs allow the main environmental gradients responsible for the observed floristic variation in the survey area to be determined.

The distribution of lichen communities around the trunks may provide useful information on the spatial and/or temporal variation of environmental conditions and help identify the main sources of environmental stress, as in the case of the alkaline dust effect arising from quarrying activities (e.g. Gilbert 1976) where characteristic assemblages may develop on different aspects of the trunk according to the prevailing wind direction.

Information associated with each species can be used to interpret the results in terms of different types of environmental alteration. Examples include:

a) the grouping of species into ecological groups (e.g. nitrophytic/non nitrophytic to identify alteration due to eutrophication),
b) the use of sensitivity values to quantify temporal and spatial changes in air pollution (e.g. see Hawksworth & Rose, 1970; Trass, 1973; van Dobben & Ter Braak, 1999),
c) the use of ecological indicator values (e.g. for pH, to reveal acidification etc.; Wirth, 1992; Nimis & Martellos 2001, 2002).

It should be stressed that indicator and sensitivity values should not be uncritically adopted in floristically and climatically very different areas from those for which they were originally developed. Finally, the average frequencies of selected indicator species in each sampling unit can be used to draw distribution maps of those species, as suggested for the LDV.

5.3 Comparison with other studies

The LDV of the sampling units (average of the LDVs of all trees sampled in a unit) may be used to compare results of surveys from various areas, or to study the temporal variation of environmental stress in the same area,

If the survey areas are very different, a comparison is only feasible provided certain requirements are met:

- the survey areas are located in the region where the same interpretation scale was proposed (i.e. they are climatically and floristically similar),
- or, the results of mapping are correlated with physico-chemical air quality measurements and calibrated,
- the examined trees belong to species that can be compared with each other.

For all areas, the following pertains:

In areas with very low LDV values, pollution may be the main source of environmental alteration in which case measures to improve air quality are urgently needed. A minimum level of air quality should be a target. The success of air pollution control measures becomes apparent when higher LDVs are obtained in a repeated survey. No measures to improve air quality are required in areas having very high LDV values, where healthy specimens of fruticose lichens *(e.g. Usnea, Bryoria* and *Ramalina)* are consistently present. A marked decline of LDV values, compared with earlier studies, signals increased environmental alteration (e.g. deterioration in air quality).

6. Data pool

Each study should present the following basic data in a table:

- operator(s)
- geographical name of the survey area,
- size of the sampling units,
- tree species surveyed,
- total number of trees surveyed,
- mean number of trees surveyed per sampling unit,
- an exact description of the location of the examined trees,
- frequencies of occurrence of individual lichen species on the examined trees (matrix of species and relevés for each tree, and LDV of the tree),
- standard deviation of the LDVs in the sampling units,
- confidence limits of the LDVs,
- width of LDV classes,
- every sampled tree georeferenced.

References

AA.VV. (2001). "I.B.L. Indice di Biodiversità Lichenica". ANPA, Serie Manuali e Linee Guida 2/2001, Rome.

AK BW (1999). Arbeitskreis Bioindikation/Wirkungsermittlung der Landesämter und –anstalten für Umweltschutz: Empfehlungen zum emittentenbezogenen Einsatz von pflanzlichen Bioindikatoren. – UWSF-Z. Umweltch. Ökotox. 11 (1999), 4, 207-211.

Asta, J., Erhardt, W., Ferretti, M., Fornasier, F., Kirschbaum, U., Nimis, P.L., Purvis, O.W., Pirintsos, S., Scheidegger, C., van Haluwyn, C. & Wirth, V. (2002). Mapping lichen diversity as an indicator of environmental quality. - In: Nimis, P.L. et al. (eds.): Monitoring with Lichens – Monitoring Lichens. NATO Science Series, IV, vol. 7. Kluwer, Dordrecht, pp. 273 -279.

Barkman, J. J. (1958). Phytosociology and ecology of cryptogamic epiphytes – van Gorcum, Assen.

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

Dietrich, M. & C. Scheidegger (1996). Diversität und Zeigerwerte von epiphytischen Flechten der häufigsten Baumarten: Ein methodischer Ansatz zur Beurteilung von Umweltveränderungen im Wald und im Freiland. – Bot. Helv. 106, 85-102.

Ferretti, M. & Erhardt, W. (2002). Key issues in designing biomonitoring programmes. – In: Nimis, P.L. et al. (eds.): Monitoring with Lichens – Monitoring Lichens. NATO Science Series, IV, vol. 7. Kluwer, Dordrecht, pp. 111-139.

Ferry, B.W., Baddeley M.S. & Hawksworth, D.L. (1973). Air pollution and lichens – The Althone Press, London.

Galun, M. (ed.) (1988). CRC Handbook of Lichenology. 3 vol. – CRC Press. Boca Raton, Florida.

Gilbert, O.L. (1976). An alkaline dust effect on epiphytic lichens. – Lichenologist 8, 173-178.

Gombert, S. (1999). Utilisation de la bioindication lichénique dans l`estimation de la qualité de l`air de l`agglomération grenobloise: étude à différents niveaux d`organisation biologique. – Thesis Univ. Grenoble, Grenoble.

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

Herzig, R. & Urech, M. (1991). Flechten als Bioindikatoren. Integriertes biologisches Meßsystem der Luftverschmutzung für das Schweizer Mittelland. – Bibliotheca Lichenol. 43. Berlin, Stuttgart: Cramer.

Insarov, G.E. & Schroeter, B. (2002). Lichen monitoring and climate change. – In: Nimis, P.L. et al. (eds.): Monitoring with Lichens – Monitoring Lichens. NATO Science Series, IV, vol. 7. Kluwer, Dordrecht, pp. 183-201.

Insarov, G.E., Semenov, S.M. & Insarova, I.D. (1999). A system to monitor climate change with epilithic lichens. – Environmental Monitoring and Assessment, 55, 279-298.

Kricke, R. (2002). Measuring bark pH. – In: Nimis, P.L. et al. (eds.): Monitoring with Lichens – Monitoring Lichens. NATO Science Series, IV, vol. 7. Kluwer, Dordrecht, pp. 333-336.

Kricke, R. & Loppi, S. (2002). Bioindication: the I.A.P. approach. – In: Nimis, P.L. et al. (eds.): Monitoring with lichens – Monitoring Lichens. NATO Science Series, IV, vol. 7. Kluwer, Dordrecht, pp. 21-37.

James, P., Hawksworth D.L. & Rose, F. (1977). Lichen communities in the British Isles: A preliminary conspectus. – In: Seaward, M.R.D. (ed.): Lichen Ecology. Academic Press, pp. 295-413.

Kandler, O. & Poelt, J. (1984). Wiederbesiedlung der Innenstadt von München durch Flechten. – Naturwiss. Rundschau, 37, 90-95.

Kirschbaum, U. & Hanewald, K. (1998). Immissionsbezogene Flechtenkartierung in hessischen Dauerbeobachtungsflächen. – Angew. Bot., 72, 212-227.

Le Blanc, F., & De Sloover, J. (1970). Relation between industrialization and the distribution and growth of epiphytic lichens and mosses in Montreal. – Can. J. Bot., 48(7), 1485-1496.

Lerond, M. (1981). Les lichens épiphytes en Normandie Orientale, distribution, sociologie et application à la cartographie de la pollution atmosphérique – Actes du Museum de Rouen 1981, 1-295.

Loppi S., Giordani, P., Brunialti, G., Isocrono, D. & Piervittori, R. (2002). Identifying deviations from naturality of lichen diversity for bioindication purposes. - In: Nimis, P.L. et al. (eds.): Monitoring with Lichens – Monitoring Lichens. NATO Science Series, IV, vol. 7. Kluwer, Dordrecht, pp. 281-284.

Lorenz, R. J. (1988). Grundlagen der Biometrie. – G. Fischer-Verlag, Stuttgart.

McCune, B. (2000). Lichen communities as indicators of forest health. – Bryologist 103, 353-356.

Nash, T. (ed.) (1996). Lichen Biology. – Cambridge Univ. Press, Cambridge.

Nash III, T. & Wirth, V. (1988). Lichens, Bryophytes and Air Quality. – Cramer, Berlin.

Nimis, P. L. (1999). Linee guida per la bioindicazione degli effetti dell´inquinamento tramite la biodiversità dei licheni epifiti. – In: Piccini C., Salvati S. (eds.): Atti Workshop Biomonitoraggio Qualità dell´Aria sul territorio Nazionale. ANPA, Ser. Atti, 2, 267-277.

Nimis, P.L. & Martellos, S. (2001). ITALIC, A new information system on Italian Lichens. – Plant Ecology 157(2), 165-172.

Nimis, P.L. & Martellos S. (2002). Testing the predictivity of ecological indicator values. A comparison of real and "virtual" relevés of lichen vegetation. – Plant Ecology (in press).

Nimis, P.L., Scheidegger, Ch. & Wolseley, P.A. (2002). Monitoring with Lichens – Monitoring Lichens.

Pirintsos, S.A., Vokou, D., Diamantopoulos, J. & Galloway, D.J. (1993). An assessment of the sampling procedure for estimating air pollution using epiphytic lichens as indicators. – Lichenologist 25, 165-173. NATO Science Series, IV, vol. 7. Kluwer, Dordrecht, 408 pp.

Purvis, O.W. (2000). Lichens. – The Natural History Museum, London.

Richardson, D.H.S. (1992). Pollution monitoring with lichens. – Richmond, Slough, UK.

Rose, F. (1976). Lichenological indicators of age and environmental continuity in woodlands. – In: D.H. Brown, D.L. Hawksworth & R.H. Bailey (eds.): Lichenology: Progress and Problems. Academic Press, London pp. 279-307.

Rose, F. & Coppins, S. (2002). Site assessment of epiphytic habitats using lichen indices. – In: Nimis, P.L. et al. (eds.): Monitoring with Lichens – Monitoring Lichens. NATO Science Series, IV, vol. 7. Kluwer, Dordrecht, pp. 343-348.

Rose, C.I. & Hawksworth, D.L. (1981). Lichen recolonization in London`s cleaner air. – Nature 289, 289-292.

Seaward, M.R.D. (1997). Urban deserts bloom: a lichen renaissance. – Bibliotheca Lichenologica 67, 297-309.

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

Trass, H. (1973). Lichen sensitivity to air pollution and index of poleotolerance (I.P.). – Folia Cryptog. Estonica 3, 19-22.

Trümpener, E. (1926). Über die Bedeutung der Wasserstoffionenkonzentration für die Verbreitung von Flechten. – Beih. Bot. Centralblatt, Bd. XLII (3), 321-354.

Türk, R. & Wirth, V. (1975). The pH dependence of SO_2 damage to lichens. – Oecologia 19, 285-291.

Van Dobben, H.F. & De Bakker, A.J. (1996). Re-mapping epiphytic lichen biodiversity in the Netherlands: effects of decreasing SO_2 and increasing NH_3. – Acta Botanica Nederlandica 45, 55-71.
Van Dobben, H.F. & Ter Braak, J.F. (1999). Ranking of epiphytic lichen sensitivity to air pollution using survey data: a comparison of indicator scales. – Lichenologist 31, 27-39.
Van Dobben, H. F., Wolterbeek, H.T., Wameling, G.W.W. & Ter Braak, J. F. (2001). Relationship between epiphytic lichens, trace elements and gaseous atmospheric pollutants. – Environmental Pollution, 112(2), 163-169.
Van Haluwyn, C. & Lerond, M. (1988). Lichénosociologie et qualité de l`air: protocole opératoire et limites. – Crypto. Bryol. Lichénol., 9, 313-336.
Van Haluwyn, C. & Lerond, M. (1986). Les lichens et la qualité de l'air. Evolution méthodologique et limites. – Rapport n° 2130. Ministère de l'Environnement. SRETIE, 207pp.
Van Haluwyn, C. & Lerond, M. (1993). Guide des Lichens. – Lechevalier, 344pp.
Van Haluwyn, C. & van Herk, C.M. (2002). Bioindication: the Community Approach. – In: Nimis, P.L. et al. (eds.): Monitoring with Lichens – Monitoring Lichens. NATO Science Series, IV, vol. 7. Kluwer, Dordrecht, pp. 39-64.
Van Herk, C.M. (1999). Mapping of ammonia pollution with epiphytic lichens in the Netherlands. – Lichenologist 31, 9-20.
Van Herk, C.M. (2002). Epiphytes on wayside trees as an indicator of eutrophication in the Netherlands. – In: Nimis, P.L. et al. (eds.): Monitoring with Lichens – Monitoring Lichens. NATO Science Series, IV, vol. 7. Kluwer, Dordrecht, pp. 285-289.
Van Herk, C.M., Artproot, A. & van Dobben, H.F. (2002). Long-term monitoring in the Netherlands suggests that lichens respond to global warming. – Lichenologist 34(2), 141-154.
Van Meirvenne, M. (1991). Characterization of soil spatial variation using geostatistics. – PhDThesis, Univ. of Gent.
VDI-Richtlinie 3799, Blatt 1 (1995). Ermittlung und Beurteilung phytotoxischer Wirkungen von Immissionen mit Flechten: Flechtenkartierung. – VDI/DIN-Handbuch Reinhaltung der Luft, Bd. 1, Beuth-Verlag, Berlin.
Wirth, V. (1992). Zeigerwerte von Flechten. – In: Ellenberg, H. (Hrsg.), Zeigerwerte von Pflanzen in Mitteleuropa. Scripta Geobotanica XVIII, 215-237. Goltze-Verlag, Göttingen.
Wolseley, P.A. (2002). Using lichens on twigs to assess changes in abient atmospheric conditions. – In: Nimis, P.L. et al. (eds.): Monitoring with Lichens – Monitoring Lichens. NATO Science Series, IV, vol. 7. Kluwer, Dordrecht, pp. 291-294.
Wolseley, P. & Pryor, K.V. (1999). The potential of epiphytic twig communities on *Quercus petraea* in a welsh woodland site (Tycanol) for evaluating environmental changes. – Lichenologist 31, 41-61.

Identification keys

Clauzade, G. & Roux, C. (1985). Likenoj de Okcidenta Europo. Illustrita Determinlibro – Bulletin de la Societé Bot. du Centre-Ouest. Royan.
Diederich, P. (1989). Les lichens epiphytiques et leurs champignons lichénicoles (Macrolichenes exceptes: lichens crustaces) du Luxembourg. Ministre des Affaires Culturelles. – Travaux scientifiques du Musee National D' Histoire Naturelle de Luxembourg XIV. Luxembourg.
Dobson, F.S. (2000). Lichens (an illustrated guide to the British an Irish Species). – The Richmond Publishing Co. LTD. Slough, UK.
Foucard, T. (1990). Svensk Skorplavs Flora (crustose lichens). – BTJ-Druck, Lund.
Jahns, H.M. (1987). Farne, Moose, Flechten. – BLV-Verlag, München.
Kirschbaum, U. & Wirth, V. (1995). Flechten erkennen - Luftgüte bestimmen. – Ulmer-Verlag, Stuttgart.
Kirschbaum, U. & Wirth, V. (1997). Les lichens bio-indicateurs – les reconnaître – évaluer la qualité de l'air. (Translated by C. van Haluwyn, J. P. Gavériaux, D. Cuny & M. Lerond). – Ulmer-Verlag, Stuttgart.
Krog, H., Østhagen, H. & Tønsberg, T. (1980). Lavflora. – Universitätsforlaget, Oslo.
Lorente, V.C. & Sánchez, M.J.S. (2001). Guia de liquenes epifitos. – Ministerio de Medio Ambiente. Secretaria General de Medio Ambiente. Madrid, ISBN: 84-8014-298-7.

McCune, B. (2000) Lichen communities as indicators of forest health. – Bryologist 103, 353-356.
Moberg, R. & Holmasen, I. (1992). Flechten von Nord- und Mitteleuropa. – G. Fischer-Verlag, Stuttgart, Jena, NewYork.
Nimis, P.L. (1987). I Macrolicheni d`Italia. Chiavi analitiche per la determinazione. – Gortania 8, 101-220.
Ozenda, P. & Clauzade, G. (1970). Les Lichens. – Masson, Paris.
Poelt, J. (1969). Bestimmungsschlüssel europäischer Flechten. – Cramer-Verlag, Lehre, Vaduz.
Poelt, J. & Vezda, A. (1977). Bestimmungsschlüssel europäischer Flechten. Ergänzungsheft I. – Bibl. Lichen. 9. Cramer-Verlag, Vaduz.
Poelt, J. & Vezda, A. (1981). Bestimmungsschlüssel europäischer Flechten. Ergänzungsheft II. – Cramer-Verlag, Vaduz.
Purvis, O.W., Coppins, B.J., Hawksworth, D.L., James, P.W. & Moore, D.M. (1992). The lichen flora of Great Britain and Ireland. – Natural History Museum Publications, London.
Schreiner, E. & Hafellner, J. (1992). Sorediöse, corticole Krustenflechten im Ostalpenraum. I. – Bibliotheca Lichenologica, 45. Cramer-Verlag, Berlin, Stuttgart.
Tønsberg, T. (1992). The sorediate and isidiate, corticolous, crustose lichens in Norway. – Sommerfeltia 14, 1-331.
Van Haluwyn, C. (1988). Essai de clé de détermination des lichens épiphytes crustacés stériles du nord ouest de la France. – Bull. Ass. Fr. Lichenol., 13(1), 5-14.
Van Haluwyn, C. & Lerond, M. (1993). Guide des lichens. – Lechevalier, Paris.
Wirth, V. (1995). Flechtenflora. – UTB-Verlag, Ulmer-Verlag, Stuttgart.
Wirth, V. (1995). Die Flechten Baden-Württembergs. – Ulmer-Verlag, Stuttgart.
Wirth, V. & Düll, R. (2000). Farbatlas Flechten und Moose. – Ulmer-Verlag, Stuttgart.

Appendix

Form 1: Information on the locality of the examined tree

Date: ....

Surveyor: ....

Mapping unit number: ....

Tree number: ....

Topographical map number: ....

General Data

☐ easting

☐ northing

altitude above sea level (in m) ☐

☐ GPS coordonates

☐ Main aspect of the community i.e. principally foliose, fruticose, crustose

☐☐ **Tree species** (Germany)

01 *Acer platanoides*
02 *Fraxinus excelsior*
03 *Juglans regia*

04 *Malus spec.*
05 *Populus spec.*
06 *Ulmus spec.*
07 *Acer pseudoplatanus*
08 *Pyrus communis*

09 *Robinia pseudacacia*
10 *Tilia cordata*
11 *Tilia platyphyllos*

12 *Alnus spec.*
13 *Betula pendula*
14 *Prunus avium*
15 *Prunus domestica*
16 *Ouercus spec.*

☐☐☐ circumference of tree trunk at 150 cm above ground level (in cm)

☐ **Age of lichens**

1 young and mature thalli together
2 predominantly young thalli
3 predominantly mature thalli

☐ **Lichen vitality**

1 predominantly healthy thalli
2 healthy and degraded thalli
3 predominantly degraded thalli

Tree description

☐ 1 exposed, 2 sheltered

☐ 1 unshaded, 2 shaded

☐ **Bark cracks:** 1 superficial, 2 moderately deep, 3 deep

Type of terrain

00 no statement
01 flat
02 valley
03 depression
04 slope
05 hill top
06 gap
07 mountain

(if not flat) Aspect

0 no statement
1 S
2 W
3 N
4 E

Site Landuse

00 no statement
01 urban area
02 rural area
03 village
04 industrial area
05 commercial area
06 park/grassy plot
07 memorial park
08 backyard
09 private garden
10 fallow land
11 grassland
12 pasture
13 farmland
14 forest
15 riparian vegetation

Influence by traffic

1 dirty road
2 asphalt road, low traffic
3 main road/highway, much traffic

Distance to the road

1 < 2 m
2 < 5 m
3 < 10 m
4 > 10 m

Other emission sources

01 domestic heating
02 chemical industry
03 power plant (fossil fuelled)
04 metallurgical plant
05 coking plant
06 incinerator
07 landfill
08 sewerage purification plant
09 intensive agriculture
10 lime emitting plant
11 Other emission sources

distance (km) and location of emission source(s) from examined tree

Location sketch / copy of site map showing tree location and number: