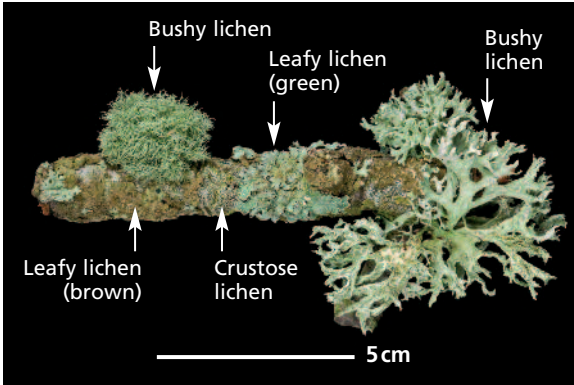


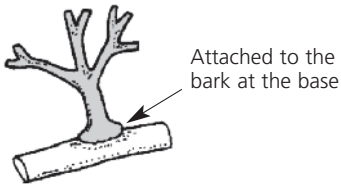
Guide to using a lichen based index to nitrogen air quality

Indicator lichens



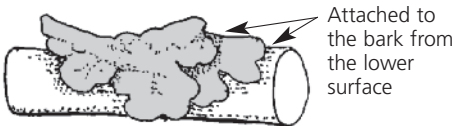
Indicator lichens used in this guide fall into three growth forms:

1. Bushy lichen



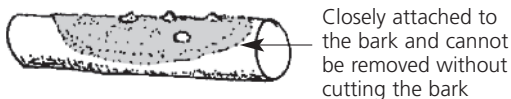
Branched and shrub-like, attached to the bark at the base. Branches rounded in section or strap-like with a distinct upper and lower surface.

2. Leafy lichen



Leaf-like lobes closely or loosely attached to the bark from the lower surface.

3. Crustose or granular lichen

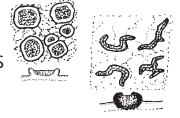


Glossary

Thallus – the main vegetative body of the lichen.

Cilia – hair-like outgrowths from the margins of the lobes.

Sexual reproduction occurs in rounded to elongate fruiting bodies in which spores are produced.



Many lichens reproduce asexually from propagules these include:

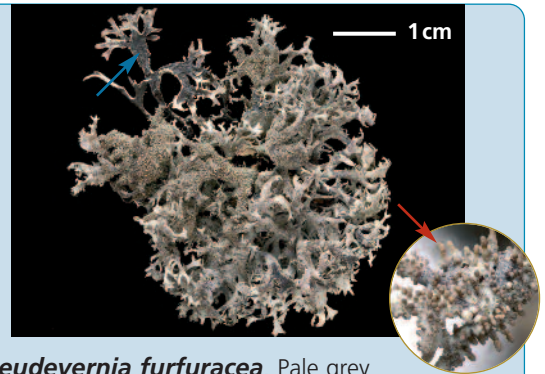
Isidia – pin-like projections from the thallus, often darker than the thallus.



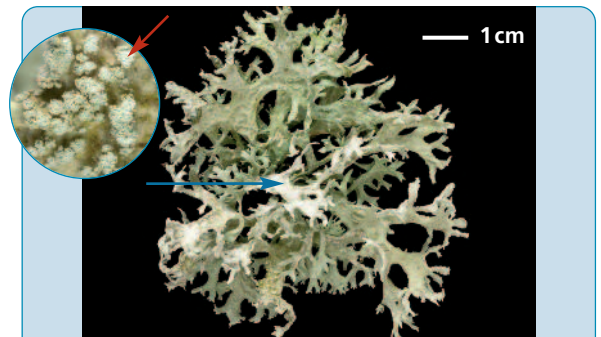
Soredia – paler powdery granules produced from round or elongated ruptures in the thallus surface.



Lobes strap-like in section



Pseudevernia furfuracea. Pale grey upper surface, black lower surface (except on young lobes), with numerous pin-like isidia on the upper surface.



Evernia prunastri. Lobes pale green on the upper surface and whitish on the lower surface; strap-like, flattened; with soredia present on upper surface of well-developed specimens.

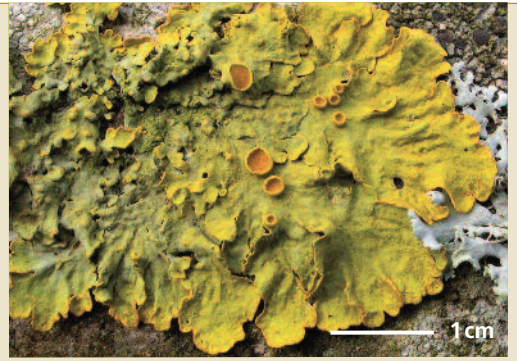
1. Bushy – branched and shrub-like, attached to the bark at the base

▶ Branches rounded in section



Usnea species. Beard lichens – greenish-grey, with a central core (visible when the branch is stretched); often pendent.

▶ Lobes yellow to yellow-green (greener in



Xanthoria parietina. Lobes broad (to 7 mm wide), spreading yellow to greenish, orange fruiting bodies often present.

▶ Lobes grey when dry to greenish when



Bryoria species. Hair lichens – green-brown to brown; hair-like and fine without central core; pendent when well developed.



Hypogymnia species. Lobes swollen, hollow when cut ↗; without rhizines.



Sphaerophorus globosus. Green-grey to brownish; branches erect, solid, with many digitate branchlets at the tip.



Punctelia subrudecta. Lobes leaf-like, ± appressed with soredia in white spots (● red), lower surface pale tan.

ched to the bark at the base

2. Leafy – leaf-like lobes closely or loosely

shaded conditions e.g. underside of branches)

▶ Thallus grey to whitish

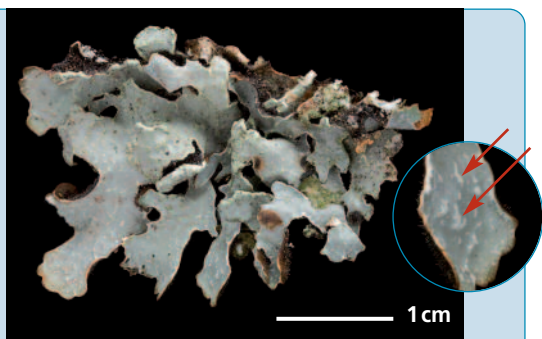


Xanthoria polycarpa **A** and *X. ucraïnica* **B**. Lobes narrow (to 1 mm wide), erect. *X. polycarpa* **A** has abundant fruiting bodies; *X. ucraïnica* **B** has marginal soredia.

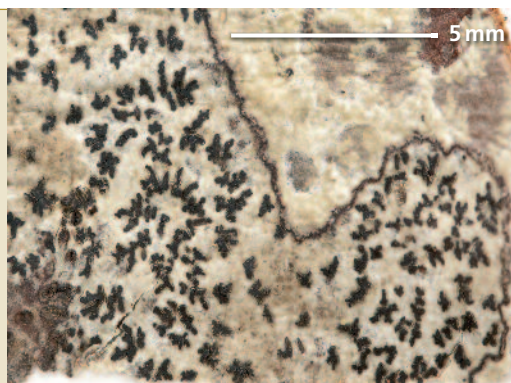


Graphis species. Script lichens – thallus whitish grey, fruiting bodies elongate with raised lip-like margins.

wet



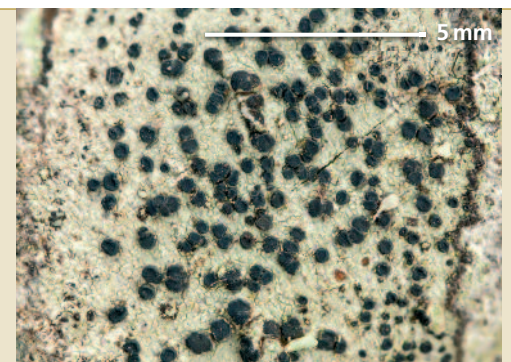
Parmelia species. Lobes leaf-like; overlapping with a network of pale lines that become sorediate in *P. sulcata* while *P. saxatilis* has isidia on the upper surface.



Arthonia radiata. Thallus whitish, fruiting bodies brown to black; irregular to branched; not raised above the surface.



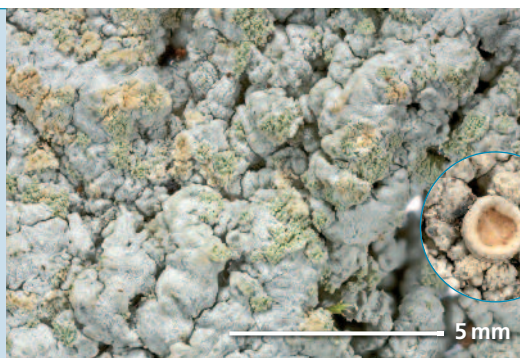
Physcia adscendens or *P. tenella*. Lobes small narrow (c. 1 mm wide) with white lower surface and conspicuous black-tipped cilia along the margins of the lobes. Both have soredia at the lobe ends.



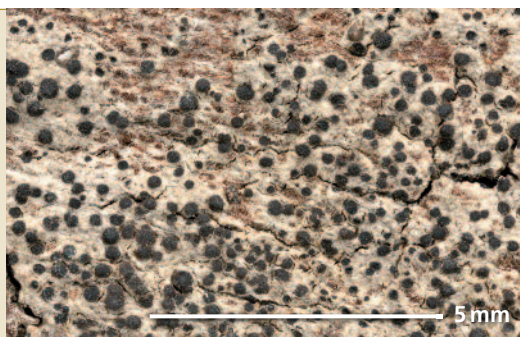
Lecidella elaeochroma. Thallus grey with black margin, often forming a mosaic on smooth bark; fruiting bodies black (c. 1 mm diameter); disc-like, concave with distinct black margin (orange).

attached to the bark from the lower surface

3. Crustose or granular – closely attached



Ochrolechia androgyna. Thallus white to cream with soredia in conspicuous rounded clusters and occasional whitish fruiting bodies ↗ with a thick margin (thallus and soredia 📌 red)



Amandinea punctata. Thallus grey-green with pale margin, fruiting bodies small, (c. 0.5 mm diameter); rounded, convex without distinct margin (📌 negative).

▶ Thallus yellow



Candelariella reflexa. Sorediate granules (<1 mm) often with yellow fruiting bodies (📌 negative).

Key

- ▶ Main feature of the lichen
- 🟡 N-tolerant species are shown in **yellow** boxes
- 🟢 N-sensitive species are shown in **blue** boxes
- 📌 A chemical test can be used to help confirm identification, see below.

📌 Chemical test using household bleach

This can help confirm species identifications. Some lichens change colour when a drop of bleach is applied.

To test the colour reaction of the lichen, scrape away a small part of the upper surface (cortex) of the lichen and apply a small drop of fresh household bleach to the exposed paler medulla (eyedropper bottles are ideal for this task).

Watch for a pink to red colour change where you have applied bleach to the lichen medulla. This colour change may fade rapidly. If the colour changes this is called a + reaction.

Safety warning

- ⚠ Take care when doing this test.
- ⚠ Use 'Milton' (available from a chemist) or the cheapest supermarket bleach without additives.
- ⚠ Carry a small amount in a clearly labelled leak proof dropper bottle.
- ⚠ Avoid contact with skin and clothing.
- ⚠ Read the manufacturers safety warnings on the bottle prior to use.

as if pressed on the bark

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Guide to using a lichen based index to nitrogen air quality

By **Centre for Ecology and Hydrology, Natural History Museum** and **University of Nottingham**

What does the guide do?

- Provides a simple, effective and robust method for determining concentrations of gaseous nitrogen (N) pollution that can be used by anyone interested in the impacts of gaseous N on sensitive habitats in the UK.
- Includes a simple identification key to epiphytic lichens growing on oak and birch trees that show distinct sensitivity to, or tolerance of, atmospheric N pollution.
- Provides a simple robust method to determine levels of risk to a habitat from gaseous nitrogen pollution by calculating a standardised nitrogen air quality index (NAQI).
- Indicates nitrogen air quality at the location of the trees sampled.

Field manual

The information supporting the protocols underpinning this guide and suggested recording sheets can be found in the Field Manual at www.apis.ac.uk/nitrogen-lichen-field-manual.

Background

Air pollution has had widespread impacts on sensitive organisms since the industrial revolution. In the 1970s and 1980s, sulphur dioxide (SO₂) was the principal pollutant causing concern. Following the substantial reduction in sulphur dioxide emissions, the main threat to sensitive vegetation now comes from reactive nitrogen compounds in the atmosphere.

Nitrogen sources

Reactive nitrogen comes in two chemical forms: oxidised and reduced. These forms occur in two

physical states: gaseous (contributing to dry deposition) and dissolved (in rainfall, cloud and dew) contributing to wet deposition. Oxides of nitrogen (NO_x) are mostly produced through combustion, from power stations and motor vehicles. These are emitted in gaseous form and some will be dry deposited locally, whereas most will be transported in the atmosphere to be washed out in wet deposition mostly as nitrate. Reduced forms of nitrogen (NH_x) have a more rural source, mainly from emissions associated with farming i.e. arable and livestock; cattle, pigs and poultry. These are emitted as ammonia gas (NH₃), some of which will be dry deposited locally, with a larger fraction being transported in the atmosphere as aerosols and washed out in precipitation as ammonium ions (NH₄⁺).

Lichens as indicators of nitrogen air quality

Lichens are composite organisms comprising a symbiotic relationship between a single species of fungus and one or more species of algae. The fungal partner provides structure and protection for the algae, which through photosynthesis provides energy and assimilates for the fungal partner.

As organisms without roots, lichens obtain their nutrients from the atmosphere and so are highly susceptible to changes in atmospheric chemistry. In Europe, lichens have been used as sensitive bioindicators of air quality for more than a century. Recent research on oak and birch trees across the UK has identified lichens that are sensitive to, or tolerant of, increasing concentrations of nitrogenous pollutants in the atmosphere. In the field, the response to increasing atmospheric nitrogen pollution can be measured by the decrease in N-sensitive lichens and the increase in N-tolerant lichens.

Emphasis has been placed on the use of indicator lichens that do not require identification at the microscopic level and that are least likely to be confused with other species. In some cases, all species in a genus are known to be sensitive (e.g. species of *Usnea* – the beard lichens). In other cases, an individual species within a genus may be an indicator (e.g. *Lecidella elaeochroma*).

The Lichen Indicator Score (LIS)

Using easily identified indicator species we can calculate a lichen indicator score (LIS) based on the presence or absence of N-tolerant and N-sensitive

lichens on three aspects of the trunk (Fig. 1) and on three sections of a branch of oak or birch trees (Fig. 2). The assessment method is repeated on at least five trees of the same species to obtain a LIS (Fig. 3). Using the LIS the NAQI for your site can be derived (Fig. 4).

Not all of the listed species will be present at a site, but utilizing the presence or absence of any of the N-tolerant or N-sensitive indicator lichens minimizes any bias in the LIS index due to regional variation in species distributions.

In conditions where concentrations of gaseous N compounds are increasing the older bark of trunks may continue to support N-sensitive species while the younger bark of the branches may be colonised by N-tolerant species. If possible survey both trunks and branches to find out about on-going changes in your environment.

The survey

Essential equipment to take with you

- This fold-out guide.
- Recording sheet, preferably waterproofed (download from Field Manual website).
- Tape measure.
- Pencil or waterproof pen.
- Map to record location and GPS device if available.
- x 10 hand lens as some lichen features are small.
- Compass (or GPS) for locating trunk aspect.

Establish suitability of site for survey

- Identify five or more oak or birch trees (not a mixture of the two) growing under similar environmental conditions (not densely planted or shaded).
- Trees that are covered in ivy should not be used.
- Trees should be single stemmed (standard) with a straight trunk, > 40 cm in girth.
- Look for availability / accessibility of branches.
- Avoid sites on calcareous soils. Determine the calcium carbonate (CaCO₃) soil levels in your location at <http://maps.bgs.ac.uk/soilportal/wmsviewer.html>

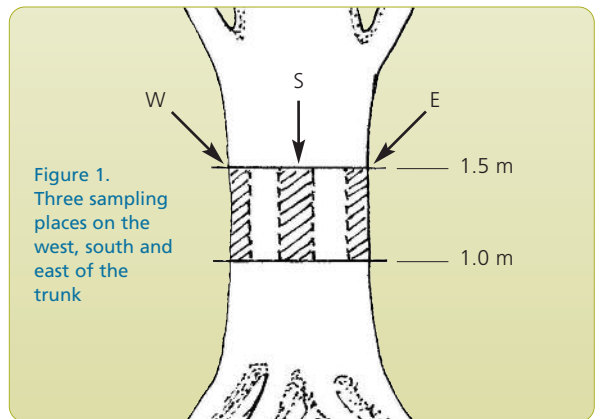
How to survey

- Record the site location, grid reference and altitude.
- Record the tree species you are surveying.

- Identify the three aspects (E, S and W using a compass) on the trunks that you will survey.

How to record indicator lichens on trunks

- Familiarise yourself with the nitrogen indicator (sensitive and tolerant) lichens, and their characteristic features, illustrated in this guide.
- Restrict your recording to only lichens listed in this guide.
 - Locate a 50 x 10 cm area on each of the three aspects of the tree between 1.0 and 1.5 m above ground level.
 - Record the presence of **N-sensitive** and **N-tolerant** species growing between 1 m and 1.5 m above ground level on each of the three aspects (E, S and W) within the 50 x 10 cm area (see Figure 3).



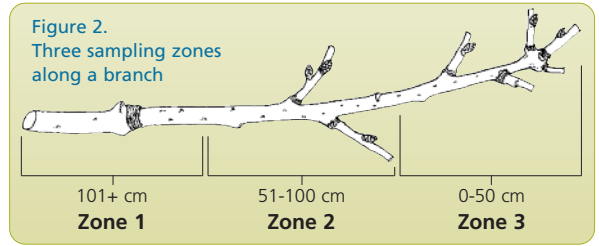
- Repeat this for each of the five trees.

There is no need to record the species present. However, recording the individual species can provide additional information about the site (see Field Manual).

Recording indicator lichens on branches

- Choose 3-5 branches on the same tree species (oak or birch) as used in trunk survey. Do not use fallen branches.
- If there are no suitable branches on these trees, branches from other trees of the same species can be used.
- Choose branches in an open aspect and within easy reach.
- Record the presence of N-sensitive and N-tolerant indicator lichens in the three zones represented by approximate distances along each main branch, measured from the growing tip (see Figure 2).

- You may have to modify these zones depending on the growth form of the trees that you are using. See Field Manual for further details.
- Use the same scoring procedure as you used for trunks substituting the three aspects for three zones along the branch.



Calculating your lichen indicator score (LIS) for trunks and branches

The LIS is based on the difference between the presence of N-tolerant and N-sensitive lichens on three aspects of the trunk or on three zones of the branch. Populate Figure 3 (worked example) by allocating a score of 1 (presence) for one or more of the listed **N-sensitive** species. Allocate a score of 0 if **N-sensitive** species absent. Repeat process for **N-tolerant** species.

- Count the number of aspects on all five trees that support tolerant species. i.e. a maximum value of 15 could be obtained if each of the five trees supported a tolerant species on each of the three aspects.

- Count the number of aspects on all five trees that support sensitive species i.e. a maximum value of 15 could be obtained if each of the five trees supported a tolerant species on each of the three aspects.
- Divide the count by the number of trees recorded to obtain an average value for both N-sensitive and N-tolerant lichens.
- Subtract the average for N-tolerant from the average for N-sensitive to obtain a lichen indicator score (LIS).

Figure 3. Worked example of LIS calculation for 5 trunks

	Tree 1			Tree 2			Tree 3			Tree 4			Tree 5			Count	Average
Aspect	W	S	E	W	S	E	W	S	E	W	S	E	W	S	E		$\frac{= \text{Count}}{\text{no. trees (5)}}$
N-sensitive	1	0	1	1	1	1	1	0	0	0	1	0	1	0	1	9	1.8
N-tolerant	0	0	0	1	0	0	1	1	1	1	0	0	0	0	1	6	1.2
Lichen indicator score (LIS) = (Average N-sensitive) – (Average N-tolerant)																0.6	

To estimate the LIS on branches, repeat the process as in Figure 3 for N-sensitive and N-tolerant species on the three zones on five sampled branches.

Interpreting your LIS in terms of exposure to gaseous Nitrogen Compounds

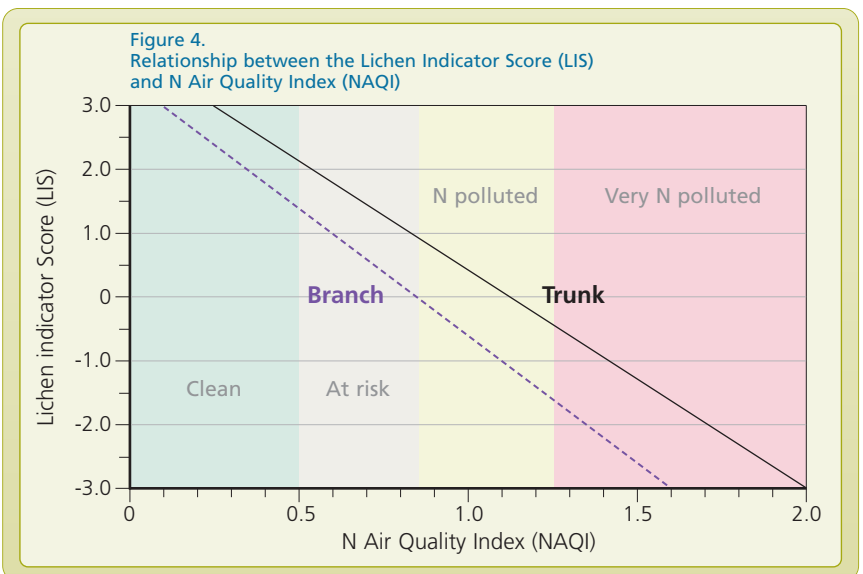
The fitted lines are derived from mean values taken from the UK wide survey. The LIS NAQI relationship for branches should be read from the broken line and for trunks from the solid line.

Sites that are designated as **clean** have an NAQI between 0 and 0.5,

at risk NAQI > 0.5-0.85,

N polluted NAQI 0.86-1.25,

very N polluted NAQI > 1.25.



Converting LIS into the N air quality index (NAQI)

- The range of lichen indicator scores (LIS) is given on the Y axis (from -3 to 3).
- Take the LIS you have just derived from your survey of either birch or oak trees based on trunks and/or branches.
- Read across the graph until you meet the broken line drawn for branches. Drop down a line perpendicular to the X axis and read off the NAQI. If the LIS has been derived from a survey of the trunks, use the solid line.
- A simple description of air quality is indicated by the coloured zones (e.g. a LIS of "2", based on branches, meets the line for branches in the green zone, which is indicative of a **clean** site with negligible gaseous N pollution; a LIS of "-2" meets the line for branches in the salmon pink zone, which is indicative of a **very N polluted** site).

Interpreting your score in a local and regional context

- The LIS for a site can vary from +3 for clean (low concentrations of gaseous N compounds) sites where only N-sensitive indicator lichens are present, to -3 for very high concentrations of gaseous nitrogen compounds sites where only N-tolerant indicator lichens are present.
- Transitional sites may have both N-tolerant and N-sensitive taxa present, especially where conditions are changing.
- If you compare the NAQI based on LIS for trunks with the LIS for branches and find that the LIS for branches is lower than that of the trunks, it suggests that conditions are deteriorating in your site.
- However, there may be sites where conditions are improving (e.g. sites formerly affected by acid rain where there is very little lichen cover on the trunk, and yet the branches have N-sensitive taxa present).

Further information

This guide was written by P.A. Wolseley, I.D. Leith, L.J. Sheppard, J.E.J. Lewis, P.D. Crittenden and M.A. Sutton, based on a PhD thesis "Biomonitoring for atmospheric nitrogen pollution using epiphytic lichens and bryophytes" by J.E.J. Lewis (University of Nottingham). Photographs are by Harry Taylor, Natural History Museum.

The Field Manual can be downloaded at:
www.apis.ac.uk/nitrogen-lichen-field-manual

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