Floristic versus Single Species Analysis in the Use of Epiphytic Lichens as Indicators of Air Pollution in a Boreal Forest Region, Northern Finland

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Summary

The influence of air pollution from an iron and steel works upon the surrounding vegetation is studied by two parallel methods, one based on the epiphytic lichen flora on pines, and the other on measurements of certain reactions of the lichen *Hypogymnia physodes* (L). Nyl. (*pH*, conductivity, and the chlorophyll, total S and iron content of the thallus, and the reactions of the algal cell component). Comparison of the results suggests that a clear picture of the nature and spread of air pollution may still be obtained from morphological examinations and various measured parameters in a single commonly occurring lichen species. This method is especially practical in the boreal coniferous zone, where the epiphytic lichen vegetation is limited.

1. Introduction

Conditions in the northern coniferous forest zone differ sufficiently from those in Central Europe, for instance, that experiences obtained in the latter area with the use of lichens as indicators of air pollution cannot be applied directly to this more northerly region. A reliable impression of the degree of air pollution may be attained in the maritime areas of Central Europe simply by examining the species composition of the epiphytic lichens on certain deciduous trees at mutually comparable sampling sites (De Wit 1976), the resulting distribution of the lichen species then allowing a zonation to be constructed which conforms well with the mean sulphur dioxide concentrations in the air, for example (Hawkesworth and Rose 1970). In Northern Europe, however, the only lichenbearing trees available are the conifers, the pine and spruce, for it is only these which appear in sufficient numbers in the countryside to account for all possible directions in which a pollutant may spread. Since these species carry an extremely poor epiphytic lichen flora, the detailed mapping of air pollution on a floristic basis alone may well prove impossible, as is emphasized by Ahti (1977). In such a case the necessary extra information on the nature and spread of impurities in the air may be supplied by a study of the morphological and physiological reactions and pollutant content of individuals of a certain selected species.

The present study of the environmental hazards posed by an steel works is based on a close examination of specimens of *Hypogymnia physodes* (L.) Nyl., the most common epiphytic lichen found on the bark of pine-trees. Being a relatively resilient species, it is capable of surviving in areas close to a source of pollution, and thus en-
ables varying degrees of pollution to be ascertained. The information obtained by this means is compared here with that afforded by the floristic approach, i.e. by examining the species composition of the epiphytic lichens on the pines, and additional observations on the general condition of the trees and measurements of the sulphur content of the needles are also reported. The general aim is thus to discover rapid, easily learnable methods for use in the practical study of air pollution which would take advantage of the information afforded by the epiphytic lichens.

2. The study area and its sources of pollution

The study area, the administrative district of the town of Raaha, is located in Northern Finland, on the coast of the Gulf of Bothnia, lat. 64° 40' N (Fig. 1). By far the most important source of impurities in the air is the integrated iron and steel works of Rautaruukki Oy. The first blast furnace of this complex came into operation in August 1964, and the steel plant and rolling mill started production in the autumn of 1967. Since 1977 it has been operating at a production capacity of 1.7 mil. tons (metric) of steel per annum. Up until the end of 1975 the works released some

Fig. 1 Location of the study area and sites. The area depicted in the subsequent maps is indicated by the broken line. The shaded portion is the factory area and the stippled portions the main housing areas in the vicinity. — . . — State border.
6,000—7,000 tons of sulphur dioxide a year into the atmosphere, a figure which increased after that date to around 20,000 tons/yr (being at this latter level in summer 1976, when the present research was carried out). The majority of this SO$_2$ is emitted from the blast furnace through a chimney 110 m in height. Large quantities of dust (exact figures not known) are also released into the air, containing oxides of iron, calcium compounds and a certain amount of heavy metals. The majority of the dust falls within the factory area or thereabouts, within a radius of a couple of kilometers. Since this dust is alkaline in character, it is capable of neutralizing the acid sulphur compounds in the immediate vicinity of the works, and thus serves to reduce their toxic effects (ESTLANDER 1971).

The other sources of pollution in the area, such as light industries, domestic heating and traffic exhaust are of little more than local significance, but taken together they constitute an areal influence which exercises a stress upon the vegetation which is felt through the built-up area of some 16,000 inhabitants (in 1976) situated to the north-east of the iron works (Fig. 1). Elsewhere the countryside has a sparsely populated rural character.

As a result of the coastal location of the steel works, the effluent can, of course, always disperse freely over the sea, but in the other directions, too, the countryside is relatively flat, with the greatest variations in relief only of the order of 10 m or so, so that there are no pronounced valleys in which impurities might be expected to accumulate.

3. Material and Methods

3.1. Sample plots

The research is based for the most part on a network of 25 sample plots distributed over a total area of approx. 80 km$^2$ surrounding the steel works (Fig. 1). These sites were selected so as to be as closely similar as possible in all respects except their degree of pollution, special attention being paid to

— similarity of forest type. Those forests which proved the most suitable in these experiments were the dry heath forests with pine dominant, the Empetrum-Vaccinium type in the Finnish forest typology (KALELA 1961),

— an even, fairly open distribution of pines (Pinus sylvestris L.) of medium age,

— open country towards the source of pollution, and no topographical features likely to interrupt the passage of the effluent,

— avoidance of sites actually bordering onto open areas, however,

— location at a sufficient distance from any houses, fields, or roads.

An attempt was also made to take local factors into account when examining the results and determining the pollution zones. In view of the stringent restrictions placed upon their choice, however, the sites could not be arranged so as to be located regular distances apart. Such a standardization of sites is nevertheless essential to ensure an adequate level of comparability in the results.

3.2. Methods for the study of epiphytic lichens

A close inspection was made only of the lichens on the trunks of the pine-trees, the occurrence and percentage cover of each being both estimated by eye and measured by the "point comb method". In order to speed up this work, only the foliaceous and fruticose lichens were included in the survey, and then only determined to species in the case of those easily recognizable in the field. Thus no distinctions were made within the genera Alectoria, Cladonia and Usnea, even though each contains both species sensitive to air pollution and more resistant ones. These genera are ranked alongside the individual species in the evaluation of the results.
Since the aim of the estimates was to form a general impression of the mean incidence of the lichen species within each plot, all the trunks lying within a 5 m radius of a given point were examined up to a height of 3 m and the abundance of each species expressed according to the following scale:

1 = extremely poor cover, only one or two examples in the whole plot,
2 = poor cover, found on many trees, but not constituting any real cover,
3 = fairly poor cover, mean under 3%,
4 = patchy, mean 3–10%.
5 = numerous patches, mean 10–25%.
6 = good cover, mean 25–50%.
7 = very good cover, mean over 50%.

The estimates were made separately for the sides of the trunks facing the source of pollution and the protected sides. It is rare for so many classes to be used in a survey of this kind (cf. SKYE 1968), but in view of the small number of species growing on the pines it was thought desirable to attempt to apply a more detailed scale.

The "point comb method" has proved a reliable means of tracing long-term changes in the species composition and percentage cover of lichens. Three trees with maximally straight trunks of diameter at least 20 cm were selected at each site and examined at heights of 0.5, 1.0 and 1.5 m on both the exposed and protected side. Using an adaptation by M. KAUPPI (Fig. 2) of the "point quadrat frame" of HARRIS (1971), the percentage cover of each lichen species was calculated from the number of hits scored by the teeth. The trees and areas of the bark examined were then marked, so that the experiment could be repeated at exactly the same points some time in the future.

3.3. Calculation of IAP values

Index of Atmospheric Purity values based on the epiphyte lichen flora have been used in many instances to express areal differences in the level of pollution, since if based on carefully selected criteria, these prove sufficient in themselves for this purpose. In the present work the IAP indices
were calculated from the incidence values for the various lichen species and their percentage cover using the following formula (LeBlanc and Desloover 1970):

\[
IAP = \frac{1}{n} \sum (Q \times f) / 10,
\]

where \( n \) is the number of species at a given site, \( f \) the abundance of each species at each site, and \( Q \) the average number of epiphytes present concurrently with a given species at all sites.

Since the aim here was to ascertain what kind of data an air purity index should be based on in order to provide the best indication of the distribution of air pollution in relation to the results achieved by other methods, it was decided to calculate the IAP values separately using the percentage cover figures estimated by eye and secondly those obtained by the point comb method, and also separately on the basis of either certain selected indicator species or all the species occurring at the site in question. Thus the sets of alternative values for each site comprised the results of calculations based on the following:

- the indicator species and their abundance as estimated from the exposed face of the tree (the side towards the source of pollution),
- the indicator species and their mean estimated abundance for the site (i.e. including both the exposed and protected face),
- all species and their mean abundance for the site,
- the indicator species and their percentage cover as determined by the point comb method from the exposed face,
- the indicator species and their mean percentage cover as determined by the point comb method from both faces of the tree,
- all species and their mean percentage cover as determined by the point comb method from both faces of the tree.

The choice of suitable indicator species may in itself be a difficult task, and it is one which requires great care in research of this kind (cf. Hawksworth and Rose 1976). Those eventually chosen for the present purpose included some more resilient species (the nomenclature according to Poelt 1969), Hypogymnia physodes, Parmeliopsis ambigua and Alectoria spp., and some which are sensitive but are common and abundant in nonpolluted areas in the region, i.e. Usnea spp., Parmeliopsis hyperopta and P. aleurites.

3.4. Condition of the trees

Since conifers are relatively susceptible to the chronic effects of air pollution, the condition of the trees was examined at all the sites and even beyond those in the area adjacent to the steel work itself. The aim in each case was to estimate the mean condition class which would describe the state of the trees best, using the following scale (cf. Jokinen 1972):

I
- healthy, undamaged trees
- needles green and growing thickly, very few brown needles

II
- first signs of damage visible
- a number of needles turning brown or yellow just at the tip
- needles growing less thickly

III
- clear signs of damage
- crowns of the trees dead up to as much as half the length of the branch
- needles remaining only on recent years' growth
- high proportion of needles turning brown, especially towards the tip
IV
- extremely serious damage
- very large numbers of needles turning brown or shrivelling up
- few live branches left
- needles very sparse

V
- trees dead

3.5. Laboratory tests and observations on Hypogymnia physodes

One of the main aims of the work was to ascertain what information on the spread of impurities in the air could be obtained from studying the reactions of a single lichen species. *Hypogymnia physodes* was selected for this purpose as it was the most common epiphytic lichen in the area and, being relatively resilient, was still to be found growing close to the sources of pollution. The following observations and tests were employed:

- examination of the outward appearance of the samples
- testing of the condition of the algal cells by fluorescence microscopy
- measurement of the chlorophyll content, pH, conductivity and total sulphur and iron content of the thallus

(— also measurement of the total sulphur content of the pine needles)

The equipment used for fluorescence microscopy was a Leitz Dialux microscope with Pleem Opak epi-illumination (see v. Ploek 1969) and a HBO 50 W mercury vapour lamp. The blue excitation light was obtained using a KP 500 filter and the appropriate dichromatic mirror. The heat filter was a 4 mm BG 38 and the barrier filter a K 530. In order to obtain consistent samples, the preparations were always made from the tip of the thallus lobe. Since their thickness is of little importance in epi-illumination fluorescence microscopy, the sections were cut by hand directly from the fresh material.

For chlorophyll measurement the dark fungal hyphae were removed from the undersurface of the lichens and the young outer edges of the thallus from as many lichen specimens as possible were mined together to form a single sample. Extraction into acetone was carried out following the method of Hill and Woolhouse (1966) and extinction values measured on a Hitachi 139 spectrophotometer. Chlorophyll content was expressed in mg/g of dry matter. The method also enabled the proportions of chlorophyll a and b to be determined.

For the conductivity and pH determinations lichen powder was extracted for 12 h in ten times the amount of water (w/w) and the measurements performed with a Radiometer CMD 2d conductivity meter and Beckman 76A pH meter. The conductivity values were corrected for pH and temperature.

The chemical analyses were as follows: for sulphur, Na₂O₃ — Na₂CO₃ fusion, suspension in water, filtering and precipitation as BaSO₄; for iron, atomic adsorption spectrophotometry.

4. Results

4.1. Floristic studies

4.1.1. Grouping based on species composition of epiphytic lichens

A total of 18 epiphytic lichen species were identified, the estimated abundances of which are indicated for each site in Table 1. Both the species composition of the epiphyte flora and the number of species present (normally 8—14) proved to be essentially the same at the least polluted sites as may be found in an entirely unpolluted
area, the species most typically occurring on the bark of pine-trees in areas of Finland with clean air (Koskinen 1955; Kauppi et al. 1977) being:

— regularly occurring, plentiful species: *Hypogymnia physodes*, *Parmeliopsis ambigua* and *Alectoria spp.*

— regularly occurring, but in smaller amounts: *Cetraria pinastri*, *C. chlorophylla*, *Usnea spp.*, *Cladonia spp.*, *Parmeliopsis aleurites* and *P. hyperopta*.

— irregular and generally in small amounts: *Parmelia sulcata*, *Platismatia glauca* and *Pseudevernia furfuracea*.

The most plentiful species also proved the most resistant to air pollution, while the most susceptible of those species occurring regularly are *Parmeliopsis aleurites* and *P. hyperopta*. The other more sensitive species are of less value as indicators, as they generally only appear on pine trunks in places where a slight fertilization effect has been achieved, e.g. *Evernia prunastri*, *Hypogymnia tubulosa*, *H. bitterana* and *Cetraria sepincola* (see also Skye 1968; Skye and Hallberg 1969).

The area studied here featured a lichen desert of some 1.5 km², on the edges of which some of the more durable species, *Hypogymnia physodes*, *Parmeliopsis ambigua* and *Alectoria sp.*, were to be found in protected crevices and at ground level. Determination to species was in any case difficult in such cases, as the thallus was so small and had suffered so much morphological alteration. As the influence of pollution diminished, these species were augmented by *Cetraria pinastri*, *C. chlorophylla*, *Usnea (U. hirta)* and *Parmelia sulcata*.

The plots may be arranged in the following groups on the basis of their species composition and the distribution of the lichens on the trunks (see Tables 1 and 2):

A — no lichens at all, some struggling specimens of the more resilient species in crevices in the bark on the protected sides of the trees (a zone extending approx. 0.5—0.8 km from the source of pollution)

B — practically no epiphytic lichens on the exposed sides of the trees, but reasonable numbers of the more resilient species on the protected sides (approx. 0.8—1.6 km)

C — only the more resilient species occur, and these are very much more abundant on the protected sides of the trees (approx. 1.5—2 km)

D — the more sensitive species also occur, and no difference appears between the exposed and protected sides of the trees, species composition as under unpolluted conditions.

Only the first three of these zones may be distinguished purely on the numbers of lichen species present (Table 2c), there being no clear distinction in this respect between zones C and D.

Reference will be made to this zonation below when reporting the results of the measurements carried out on material from the sample plots.
Table 1. Relative abundance of lichen species on the exposed and protected sides of pine trunks of accompanying species, from the most resilient to the least. The left-hand figure in the pair for (− = species absent). For explanation of the site groups A–D, see text.

<table>
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<td>−(2)</td>
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<td>−3</td>
<td>−4</td>
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<td>26</td>
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<td>Parmeliopsis ambiguа</td>
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<td>−(1)</td>
<td>23</td>
<td>−3</td>
<td>−2</td>
<td>−3</td>
<td>11</td>
<td>13</td>
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<tr>
<td>Alectoria spp.</td>
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<td>−(1)</td>
<td>−1</td>
<td>−1</td>
<td>−3</td>
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<td>14</td>
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<tr>
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<td>−</td>
<td>−1</td>
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<td>−2</td>
<td>11</td>
<td>12</td>
<td>12</td>
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<tr>
<td>C. chlorophyllа</td>
<td>−</td>
<td>−</td>
<td>11</td>
<td>−1</td>
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<tr>
<td>Usnea spp.</td>
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<td>11</td>
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<td>Parmelia sulcata</td>
<td>−</td>
<td>−</td>
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<td>−4</td>
<td>−1</td>
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</tr>
</tbody>
</table>

No. of named species (or genera) at the site:

|   | 3 | 4 | 6 | 6 | 6 | 9 | 7 | 8 |

Since the number of epiphytic lichens growing on pine trunks is limited, and the most typical species are also those which withstand the effects of pollution best, it is not possible to attempt a zonation of the same degree of detail as the zones 0–10 used by Hawksworth and Rose (1970), for instance, simply on the strength of species composition mapping. As it is, some sites subject to slight toxic effects from pollution and dust, e.g. plot 6, may even appear from their species composition and abundance data to possess better conditions for lichen growth than those in the entirely unpolluted areas (cf. also Jürging 1976, p. 22; Gilbert 1976). At sites such as these the present age cover and number of species may have even increased in spite of obvious morphological changes in the lichens. In such cases it is essential to examine the morphology and chemistry of the lichens if one wishes to form a more precise picture of the spread of pollutants.
The aim of these accurate, systematic assessments of the percentage cover of the lichen species was to obtain answers to such questions as whether pollution zones can be distinguished on the basis of such data. The measurements were performed in the manner described in section 3.2., so that a total of 18 readings was obtained at each site, 9 from the exposed sides of the trunks and 9 from the protected sides. The mean values for the percentage cover of each species at each site are detailed in Table 3. These results point to distinct differences between the species, the cover of the most common species, which normally occur in abundance, declining markedly with proximity to the source of pollution. This is particularly true of Hypogymnia physodes, and also of Parmeliopsis ambiguia and Alectoria spp., although it is admitted that
Table 2 Location of sites, numbers of epiphytic lichens on pines, their percentage cover and IAP values

- a = distance from source of pollution (km)
- b = direction from source of pollution
- c = no. of epiphytic lichen species on exposed side of pines/whole site
- d = total percentage cover of the lichen species studied as determined by the point comb method from the exposed sides of the trunks/mean for whole site
- e = IAP values calculated from estimated mean abundance of selected indicator species on exposed sides of trunks
- f = IAP values calculated from estimated mean abundance of selected indicator species, including both sides of trunks (values also presented in Fig. 3)
- g = IAP values calculated from estimated mean abundance of all identified species, including both sides of the tree

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Fig. 3 IAP value (upper figure), condition class of Hypogymnia physodes/algal cell layer (centre figure), and condition class of trees (lower figure) for the sites examined. The inner zone indicated by the dotted line comprises the sites with severe pollution damage, including those with no lichens (IAP < 2.5, condition of Hypogymnia physodes and pines the poorest of all), while the outer zone is that still bearing obvious marks of air pollution. The factory area and the sites lying beyond the map are distinguished by broken lines.
considerable fluctuations can be found in the percentage cover of these species even in unpolluted areas. Alterations in the cover of those species which occur in smaller quantities could not be detected reliably by this method, as it was mostly a matter of chance whether or not the small individual thalli would coincide with the teeth of the comb. The total cover (Tables 3 and 2d) is similarly determined largely by the behaviour of the most resilient species. Corresponding total cover values for the epiphytic lichens on pine trees at unpolluted sites otherwise comparable with those studied here were generally in the range 40 (30)–70 (80)% (Kauppi and Mikkonen 1975; Kauppi et al. 1977). Since the influence of local factors independent of pollution is reflected so clearly in the percentage cover results obtained from the lichens, it would be difficult to delimit zones of varying degrees of pollution damage. The principal value of the point comb method in general indeed lies precisely in its objectivity and repeatability.

4.2. IAP values

Since IAP values alone serve to reflect the epiphytic lichen flora at each site, this index provided a relatively simple means of comparing the plots studied, while the calculation of parallel values from the primary data according to a variety of criteria enabled an assessment to be made of the influence of the criteria selected upon the results obtained. As it is intended to publish the results obtained by this method separately at a later date, these will only be reviewed briefly in the present paper. It appears that the use of the percentage cover data given by the point comb method tends to emphasize the role of the most resilient species, and to give rise to a considerable scatter in the values that prevents any clear zonation from emerging. A more clearcut situation is obtained by basing the calculations on the estimated abundance
Figures are the means for the $3 \times 6$ measurements taken at each site. No point comb readings were

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</table>

of the lichens (Table 2e - g), although the zone boundaries still proved difficult to delimit if all the species present were included, as the occasional species tended to increase the scatter (Table 2g). Thus the clearest picture of all of the relative extent of air pollution was provided by the selected indicator species, taking the percentage cover of these on both faces of the tree into account (Table 2f, Fig. 3). The resulting IAP values did not alter to any appreciable extent, however, if only the exposed face of each tree was included (Fig. 2f), although in this case the effect of the direction of the site from the source of pollution exercised a greater influence on the results.

4.3. Condition of the trees

The classification of the trees according to their estimated condition is presented in Fig. 3. Those within the area of the steel works itself, which had suffered very badly, were generally assigned to classes III or IV. The crowns of the trees and the tips of their branches were totally or partially dead, and there was a striking absence of needles from earlier years and an abundance of needles which had turned brown. The most serious reason for this damage appears to have been the large quantity of dust settling on the needles, since fine dust will block the stomata and stifle the tree (HAVAS 1971). Further away from the source of pollution, where the proportion of dust in the air becomes less, the influence of gaseous pollutants, principally sulphur dioxide, increases. The amounts of sulphur dioxide emitted had remained fairly small up to 1976, however (see section 2 above), and it is perhaps for this reason that three damage has so far been confined to a relative restricted area. The obvious correlation between tree damage, IAP values and the various estimates and measurements indicative of the condition of the Hypogymnia physodes lichens (section 4.4.) may be appreciated from Fig. 3.
4.4. External appearance of Hypogymnia physodes specimens and condition of the algal cells

The external appearance of lichens reflects the mean level or air pollution to the extent that it is possible to trace the distribution of impurities on this basis alone especially in the vicinity of a point source (Hållgren et al. 1978). Healthy thalli of Hypogymnia physodes are smooth, an even greyish-green in colour, and with long lobes, while pollution damage leads to the appearance of surface cracks, and various colour changes, etc. Lichen samples from both the exposed and protected sides of the trees at the various sites were studied together in the laboratory for comparison purposes, and the impression of the condition of the lichens gained in this way was complemented with notes made on collection concerning the occurrence of the lichens on the tree trunks. This comparison enabled the samples, and thus also the sites, to be divided into four classes as follows (Table 4 and Fig. 3):

1. Lichens healthy and in good condition on both the exposed and protected faces of the trees.
2. Lichens on the exposed face slightly affected, but those on the protected face more or less healthy in appearance.

Table 4. External appearance of Hypogymnia physodes at each site and results of fluorescence microscopy

<table>
<thead>
<tr>
<th>Macroscopic observations</th>
<th>Fluorescence microscopy</th>
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<td>Site</td>
<td>Appearance and colour of thallus</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>surface cracked wrinkled, sooty black</td>
</tr>
<tr>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>surface grainy, wrinkled, black-brown</td>
</tr>
<tr>
<td>4</td>
<td>lobes absent, surface grainy, black-brown</td>
</tr>
<tr>
<td>12</td>
<td>lobes, small, surface uneven, grey-brown</td>
</tr>
<tr>
<td>11</td>
<td>lobes small, grey-brown</td>
</tr>
<tr>
<td>5</td>
<td>lobes absent, surface grainy, dark brown</td>
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Table 4 (continued)

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<th>Fluorescence microscopy</th>
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<tr>
<td>Site</td>
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<tr>
<td>13 furrowed, grey</td>
<td>3</td>
</tr>
<tr>
<td>20 slightly wrinkled, grey-green</td>
<td>1</td>
</tr>
<tr>
<td>6 lobes small, surface uneven, grey-green</td>
<td>3</td>
</tr>
<tr>
<td>8 lobes practically absent, wrinkled, grey-green</td>
<td>3</td>
</tr>
<tr>
<td>14 lobes smooth, surface even, grey</td>
<td>2</td>
</tr>
<tr>
<td>19 lobes smooth, surface even, grey</td>
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</tr>
<tr>
<td>7 lobes small, grey-green</td>
<td>2</td>
</tr>
<tr>
<td>25 slightly furrowed, grey-green</td>
<td>2</td>
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<td>18 lobes small, surface uneven, grey-green</td>
<td>2</td>
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<tr>
<td>21 slightly wrinkled, grey-green, healthy</td>
<td>1</td>
</tr>
<tr>
<td>22 lobes smooth, grey-green, healthy</td>
<td>1</td>
</tr>
<tr>
<td>17 lobes smooth, grey-green, healthy</td>
<td>1</td>
</tr>
<tr>
<td>10 slightly wrinkled, grey-green</td>
<td>2</td>
</tr>
<tr>
<td>9 slightly wrinkled, colour variegated</td>
<td>2</td>
</tr>
<tr>
<td>24 lobes smooth, grey-green, healthy</td>
<td>1</td>
</tr>
<tr>
<td>23 lobes small, partially wrinkled, variegated</td>
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1) — = no samples
2) ++++ = very large amount, +++ = large amount, + + = moderate amount, + = little, (+) = very little
3. Few lichens on the exposed face, and these in poor condition. More on the protected face, but these still obviously in difficulties.
4. No lichens present, or only a few at the base of the trunks, with rugged thalli.

Since it is probable that the changes induced by impurities in the air will be reflected more readily in the physiology of the lichens, and especially in the algal cell reactions, the condition of the algal cell layer was determined by fluorescence microscopy, which has proved from experience to be a very much more rapid method than the measurement of CO₂ assimilation (Kauppi and Kauppi 1976; cf. also Arnold and Kreeb 1976). The application of this method to the study of pollution is based on the observation that the fluorescence from healthy algal cells in lichens differs from that from damaged or dead cells when excited by an appropriate form of short wave radiation. Thus fluorescence microscopy offers a viable means of determining the condition of lichens, largely the living or dead status of the algal cells, and an alternative to the procedure whereby this status is evaluated from the reactions of the cells to neutral red and TTC (LeBlanc et al. 1971, 1976; Brown 1974). Details of the equipment used for this purpose and the preparation of the samples are provided above in section 3.4.

Healthy algal cells emit a powerful red radiation, due to the emission capacity of chlorophyll a in the second light reaction of photosynthesis (Arnold and Kreeb 1976), while damage to the cells results in a change in the colour of the fluorescence from red through brown and organe to yellow, and finally white (Arnold and Kreeb 1976; Kauppi and Kauppi 1976). Thus an impression of the degree of damage suffered by a given sample may be obtained by calculating the percentages of the cells emitting the various types of fluorescence. The samples examined here were then classified into four groups on the basis of their condition as reflected in the results of fluorescence microscopy (Table 4 and Fig. 3), placing particular emphasis upon the numbers of dead cells emitting a white fluorescence, although also taking into account the colour changes in the other cells present. The classes are denoted by Roman numerals, as follows:

I Algal cell layer entirely healthy and even, with less than 5% dead cells.
II Algal cell layer in reasonable condition, with less than 10% dead cells and few other colour changes.
III Algal cell layer variable, with large numbers of cells emitting brown or orange fluorescence and as many as 30% dead cells.
IV Algal cell layer entirely destroyed in places, an average of over 50% dead cells.

Although the use of MPV photometer (microfluorometer, Leitz, Wetzlar, Germany) would have enabled the colour changes to be indicated numerically (Kauppi and Kauppi 1976), this was not done here, as a visual classification of the kind outlined above is usually quite adequate for practical purposes. A certain amount of deviation is found between samples from the same site, and even from the same thallus, as far as the numbers of dead cells are concerned, for instance. In order to minimize this effect, the sections were taken only from the edges of the thallus, which will eliminate
any changes resulting simply from the normal aging process. Similarly, at least five fluorescence microscopy samples were run from each site. In spite of this necessary duplication of work, the method proved a relatively rapid one, so that an experienced research assistant could examine 10 or 20 samples an hour, and in addition to providing evidence on the destruction of algal cells, it also enabled the quantity of dust particles present on the thallus to be estimated.

Table 4 depicts the good correlation achieved between the classification based the visual estimates of the condition of the Hypogymnia physodes specimens and that based on the fluorescence microscopy results. The generally assumed superiority of fluorescence microscopy over macroscopic observations does not emerge particularly
Fig. 5. Values for the pH (upper figure), conductivity (μS, centre figure) and chlorophyll content (mg/g, lower figure) of *Hypogymnia physodes* at each site. The limit of the severe effects of dust (pH 4.1, conductivity 310 μS) is shown by the dotted line.
Fig. 6. Values for the sulphur content of the pine needles (mg/g, upper figure), and of *Hypogymnia physodes* (mg/g, centre figure) and the iron content of *Hypogymnia physodes* (mg/g, lower figure) at each site. Inner zone border: lichen sulphur content approx. 115 mg/g, iron approx. 30 mg/g; outer zone border: sulphur approx. 1.0 mg/g, iron approx. 15 mg/g.
strikingly here, as fertilizer ions are also present in the atmosphere of the area, and these affect the algal cells in a different manner from sulphur dioxide, for instance (Kauppi 1976, Table 3). The correlation between the morphological data and the other measurements and evaluations may be seen from the maps in Figs. 3, 5 and 6.

4.5. Chlorophyll content of Hypogymnia physodes

One property of the oxide of sulphur and of the fluorides and other compounds contained in the air of urban areas is that they decompose chlorophyll (Rao and LeBlanc 1966). This takes place in a reaction which is to a great extent dependent on pH; the lower the pH, the more rapidly the chlorophyll will oxidize (Pickett et al. 1973). Chlorophyll a is particularly susceptible to the action of sulphur dioxide, and the influence of this compound in the environment should thus be evident in a reduction in the chlorophyll content of the lichens, and in particular that of chlorophyll a. On the other hand, fertilizer ions are capable of preventing the decomposition of chlorophyll and even of causing an increase in its concentration in lichens (Kauppi 1976). Chlorophyll measurements will thus supply information on the distribution of impurities in the air and even on the various pollutants involved.

The results of the chlorophyll measurements performed here are presented in Fig. 4, in which the results of this and other measurements performed on the material from each site are arranged vertically to facilitate comparison, and also in map form in Fig. 5. The chlorophyll concentrations measured in the lichens were distinctly higher in the immediate vicinity of the factory than further away, with the highest figures of all, 3.1–2.5 mg/g, being recorded to the north, at sites 3–6. Very much lower values were obtained at the same distance to the south, 1.8–2.3 mg/g, and the lowest concentrations of all were of the order of 1 mg/g. The ratio chlorophyll a/b varied in the range 2.5–3. Chlorophyll concentrations of 0.6–0.8 mg/g and an a/b ratio of 2.0–2.5 have been recorded in entirely unpolluted areas in Finland (Kauppi and Mikkonen 1975). Both the higher concentrations found here and the increase in the chlorophyll a/b ratio point to the presence of fertilizer dust in the vicinity of the factory, which appears to have obliterated any influence which sulphur dioxide have had upon the lichens, or else protected them from the toxic effects of this compound by forming a dust layer on their surface (see Türk and Wirth 1975; Gilbert 1976).

4.6. Electrical conductivity and pH of Hypogymnia physodes

The effects of air pollution are reflected in increased values for both pH and electrical conductivity (Figs. 4 and 5), while the close correlation between the conductivity and chlorophyll results further confirms the above assumption concerning the action of fertilizer dust. The area most obviously affected by the dust, which contains iron and calcium compounds (see section 2), is that immediately surrounding the factory itself, as represented by the lichenless sites 1 and 2, and by sites 3–6, and the influence of this dust continues to be felt strongly up to the limit indicated with the dotted line
in Fig. 5, and could still be detected well beyond this. The dust had been carried furthest from the factory in a northern and north-eastern direction (sites 3—6 and 8), representing the quarter of prevailing winds, and the effect of exposition may be seen from the fact that the samples from the protected sites gave the lowest pH and conductivity values in relation to distance and direction from the factory. The pH values for Hypogymnia physodes lichens in unpolluted areas of Finland are of the order of 3.6—3.7, and the electrical conductivity 150—200 μS (Kauppi and Mikkonen 1975). Only three of the present sites, nos. 7, 17 and 22, all amongst the most sheltered, gave values within these limits, thus providing evidence that even the most distant sites studied here fell within the range of the pollutant dust.

4.7. Analysis of sulphur and iron concentrations

Since impurities from the air tend to accumulate within plants gradually over a long period of time, analyses based on plant samples can provide an indirect indication of the mean pollutant concentrations in the air, whilst it would usually require lengthy series of tests to obtain comparable information by direct measurements. The performing of chemical analyses on plants involves limitations of its own, however. For instance, the natural concentrations of the ions in question and the deviations to be expected from these need to be taken into account in interpreting the results. Similarly, such analyses do not indicate the extent to which the concentrations detected may be detrimental to the plant, as they do not provide any information on the form in which the substances are to be found. The different sulphur compounds, for instance, vary greatly in toxicity. At the same time, such macro-scale methods do not show whether the impurities have reached the level of the plant cells. For these reasons it is essential to back up any chemical analyses with a study of the reactions of the plants, and in this way chemical analysis and methods concentrating on the effects of pollution tend to complement each other. The only chemical tests to be performed here were determinations of sulphur and iron concentrations, the former to ascertain the spread of SO₂ and other sulphur compounds, and the latter chiefly to demonstrate the presence of dust (Figs. 4 and 6).

The sulphur content of the pine needles varied in the range 0.69—1.48 mg/g, compared with a range of 0.8—1.9 mg/g obtained six months earlier in X-ray spectrometry experimentes commissioned by the steel works itself (Hakala 1976). As was to be expected, the highest concentrations were to be found at the sites in and around the area of the factory itself. The natural sulphur content of pine needles fluctuates from one experiment ot another, but may be said to lie within the range 0.3—1.0 mg/g (Laaksovirta and Olkkonen 1977), a level which is exceeded at the majority of the present sites.

The sulphur content of the lichens varied in the range 0.63—1.9 mg/g, as compared with values of 0.5—0.9 mg/g obtained previously in unpolluted areas (Kauppi and Mikkonen 1975; Kauppi et al. 1977), and about the same figures are mentioned in connection with other experiments as representing the lowest natural concentrations
in *Hypogymnia physodes* (Laaksovirta and Olkkonen 1977). A close correlation is to be found between the analyses from the lichens and pine needles, although the rise in concentrations with proximity to the factory is very much more pronounced in the case of the lichens (Fig. 6).

Extremely high iron concentrations were found in the lichens at all the sites studied, again testifying to the surprisingly widespread distribution of the iron and calcium dust emitted by the factory. The increase was greatest just around the factory itself, where the greater part of the dust falls, but even the lowest values recorded, approx. 7 mg/g, are many times greater than those reported under natural conditions, approx. 0.6—1.7 mg/g (Laaksovirta and Olkkonen 1977). The high iron concentration thus constituted that effect which could be detected farthest away from the factory. These chemical analyses also serve to confirm that the majority, at least, of the dust visible on the thalli of the lichens in fluorescence microscopy (Table 4) does indeed originate from the steel works.

### 5. General Picture of the Distribution of Air Pollutants Provided by the Vegetational Indicators

The reactions of the vegetation and of certain specific plants have been utilized in the above in a variety of ways in order to obtain the most reliable possible estimate of the spread of impurities in the air and of their influence upon the environment. Both the floristic work and the analyses performed on samples from the sites studied demonstrate conclusively that the iron and steel plant of Rautaruukki Oy is by far the most prolific source of air pollution in the area. The vegetation of the area surrounding this factory shows clear indications of air pollution damage, an impoverished epiphytic lichen flora, a significant decline in the physical condition of the pine trees and damage to the thalli of *Hypogymnia physodes* all being common features. Fig. 7 is intended as a synthesis map depicting the general outlines of the spread of air pollution and the extent of its effects, in which the zone boundaries are obtained by interpolation from the areal patterns for the various sets of measurement results. The area in which changes could actually be observed is a relatively limited one, extending only up to about 2 km from the blast furnace in the south and 6.5—7 km in the north and northeast, the outer limit of zone 4 in Fig. 7. This configuration corresponds to the long-term distribution of prevailing winds in the area (Estlander 1971, pp. 171—174), although admittedly the pollution effect of housing in the north has shifted the boundary further outwards at that point.

Essentially identical pictures of the areal distribution of pollution effects were obtained from the floristic surveys on the epiphytic lichens of the pine, including the IAP indices, on the one hand, and the laboratory tests on samples of *Hypogymnia physodes* on the other, and comparison of the maps derived from the various sets of results (Figs. 3, 5 and 6) serves to demonstrate that these measurements complement each other in a logical sense. The influence of pollution on the lichens themselves is borne out by changes in the morphology of the thallus, irregularities within the algal
Fig. 7. Air pollution zones.
1. Lichen desert, conifers gradually dying
2. Badly polluted, severe damage to the vegetation
3. Clear influence of air pollution, considerable damage to the vegetation
4. Slight influence of air pollution, effects of pollutants on the vegetation still visible
5. Practically unaffected, influence of pollution only detectable by chemical and physical analyses.
cell layer, an increase in chlorophyll content, rises in pH and electrical conductivity and increases in the sulphur and iron concentrations. The chemical analyses proved capable of detecting the influence of the pollution source at greater distances away, even in a zone where the condition of the vegetation appeared to be quite normal (zone 5 in Fig 7).

The above-mentioned lichen measurements show a clear mutual correlation (Fig. 4), and indicate that the outstanding pollution component is the alkaline dust emitted from the factory, which earlier immission studies employing purely technical methods (Estlander 1971) have shown to be composed principally of iron oxides, calcium in various forms, and sulphates. This exercise largely a stifling effect on the plants, clogging up the stomata on the needles of the conifers, and endangering gas exchange, for instance. This dust layer is similarly reported to create difficulties in assimilation and transpiration for lichens Jürging 1975, p. 46). It is difficult at present to state the part played by sulphur dioxide in the vegetation damage, as it is largely masked by the dust effect. The emission of this compound has increased considerably since the introduction of a new ore in 1976, however, and it is expected that the vegetation damage area will extend outwards very rapidly as a consequence, unless measures are taken to reduce these emissions.

6. Discussion

The aim of this work was to explore the extent to which a number of measurement carried out on a single lichen species, Hypogymnia physodes, could be used to complement and adjust the air pollution picture obtainable by means of a floristic lichen survey in the boreal coniferous forest zone, in which the natural lichen flora is severely limited. The results showed a close correspondence between the zonation constructed on floristic grounds, including IAP values, and that based on the reactions noted in specimens of Hypogymnia physodes, suggesting that the latter method alone would suffice for the purpose of tracing the spread of pollutants. Indeed, the analyses performed here on the lichens also furnished valuable additional information on the nature of the impurities involved and their mode of action upon the plants.

The analyses used here are nevertheless no more than a selection of the ways in which the reactions of lichens to pollution could be monitored, and it depends very much on local conditions such as the nature of the impurities, the lichen species chosen for examination, the equipment available, etc., as to which of the many possible reactions it will be most profitable to concentrate upon in each case. At least the following changes have been observed in lichens as a consequence of air pollution:

External changes

changes in thallus colour
decrease in thallus size, length of the lobes, thallus reflectivity, thallus adhesion and development of soredial and isidial structures
increase in thallus thickness, surface cracks and deposition of extracellular substance in the thallus
Anatomical changes
increase in the number of dead and plasmolyzed algal cells (as in this paper)
decrease in the size of the algal cells, in the number of dividing algal cells and in
the contact between the symbiotic partners

Physiological changes
changes in the living or dead status of algal cells (as in this paper)
decrease in net CO₂ assimilation and respiration, including a possible temporary
increase in respiration (Türk et al. 1974)
decrease in nitrogen fixation (Kallio and Varherrmaa 1974; Häggren and
Huss 1975) and in relative growth rate (Häggren et al. 1978)

Changes in chemical concentrations
increase in concentrations of pollutants and phaeophytin in acid conditions
decrease in total chlorophyll and the chlorophyll a/b ratio, changes in pH and
conductivity
potassium efflux (Puckett et al. 1977) and Mg²⁺ release by the thallus

The majority of these reactions are mentioned in one connection or another in the
review „Air pollution and lichens“ (Ferry et al. 1973), and a number of later examples
of research in the field are cited in the lists above. Some of the reactions are revealed
most clearly when studied in lichen transplants (see Kauppi 1976; LeBlanc et al.
1976). In areas affected by alkaline immission the directions of the changes may be the
opposite of those quoted above, which apply mostly to SO₂ immission (see Kauppi
1972, 1976). The use of a number of parallel but mutually independent methods thus
remains all the more necessary, the more complex the case of pollution to be examined.

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