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MARC

BIOMONITORING OF AIR QUALITY USING PLANTS

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REPORT 10

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MARC
Monitoring and Assessment Research Centre
King's College, London
– WHO Collaborating Centre for Monitoring and Assessment –

in co-operation with

Federal Environmental Agency, Berlin
– WHO Collaborating Centre for Air Quality Management and Air Pollution Control –

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Air Hygiene Report 10

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Biomonitoring of Air Quality Using Plants

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PREFACE

Biological monitoring with plants comprises low-cost, effective methods to estimate levels of air pollutants and their impact on biological receptors. Plants show an integrated response to contamination and other environmental factors. For many years now, they have been used to evaluate the uptake and enrichment of air pollutants in biota, plant injury and damage and crop losses. In particular, plants relevant for human nutrition and animal food were exposed to air pollution in addition to indicator plants such as lichens and mosses. Biological monitoring with plants is of practical value in assessing exposure and risks caused by various air pollutants. The uptake of pollutants in plants does not only affect plant life but also nutrition and food cycles. Contamination of nutrition and food influences human uptake of compounds and contributes - in addition to air pollutants - to the total human exposure.

Total human exposure can only be estimated by looking at the individual exposure routes - air, food, drinking water, dermal contact. The critical organs in the human body - the organs were adverse effects are first observed - are usually not accessible for investigation in living individuals. Therefore, knowledge of the uptake in edible plants and of the contamination of animal food can give a hint to potential exposure risks for humans. On the other hand, indicator plants such as lichens and mosses possess efficient accumulation capacity for many air pollutants. Their exposure, therefore, can lead to detailed statements on the integral air pollutants.

This report is an update of the MARC Report No. 32 "Biological Monitoring" and a first volume referring to a WHO project on biological monitoring. The monograph reviews comprehensively the existing literature on biological monitoring of air quality with plants. This review includes consideration of all plant species that are currently, or have a potential of, being used as bioindicators of air pollution. This review is intended to serve as a background paper for the derivation of guidelines for the use of biological monitors in air pollution control.

The report was prepared at the Monitoring and Assessment Research Centre, MARC, at King's College in London, a WHO Collaborating Centre for Environmental Monitoring and Assessment. The work was supported by the United Nations Environment Programme (UNEP), the Division of Operational Support and Environmental Health at the WHO, and the Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) of Germany.

A forthcoming report within the WHO project will provide a review of the methodological guidance for the drawing-up of guidelines on the use of biomonitors (plants) with respect to their relevance to human health impacts.

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I INTRODUCTION

1 Background

In 1986, the Monitoring and Assessment Research Centre, MARC, at King's College London - University of London, supported by United Nations Environment Programme, prepared and published a technical report on Biological Monitoring of Environmental Contaminants Using Plants (Burton, 1986). This report highlighted in detail the increasing awareness of the effects of pollutants on plants and their potential as biomonitors/bioindicators of air pollutants in the terrestrial environment.

Physico-chemical measurement of air pollution levels is an objective and accurate method. It is essential for the accumulation of air quality data for the analysis of standards, interrelating effects, source reductions and general air pollution control. This enables the formulation of policies and regulations necessary for the protection of humans, animals and plants.

The response of plants to elevated concentrations of air contaminants is modified by other environmental factors and by the physiological status of the plant. Monitoring the plants directly assesses the integrated effects of these factors and contamination. Tingey (1989) emphasised that "there is no better indicator of the status of a species or a system than the species or system itself". Physical and chemical methods do not provide sufficient information on the risk associated with an exposure.

In contrast, biological methods allow direct assessment of risk from an exposure. Biological data can be used to estimate the environmental impact and potential impact on other organisms including humans. Often biological data have not necessarily been collected continuously, instead this can be performed periodically. Biological monitoring is generally less expensive than other methods and is thus particularly suitable for long-term monitoring over large areas without deploying sophisticated and high maintenance equipment. Biological monitoring has been defined as "the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in a quality control programme. These changes often are due to anthropogenic sources ..." (Matthews, 1982). Biological monitoring of air pollutants can be passive or active. Passive methods observe plants growing naturally within the area of interest. Active methods detect the presence of air pollutants by placing test plants of known response and genotype into the study area.

A distinction is increasingly being made between using organisms as bioindicators and biomonitors in air pollution studies. According to Tingey (1989) "a bioindicator is an organism or biological response that reveals the presence or absence of an air pollutant by the occurrence of typical symptoms or measurable responses. A biomonitor provides information on the presence of the pollutant and attempts to provide additional information about the amount and intensity of the exposure." Similar definitions were outlined by Market *et al.* (1997).

The concept of bioindicators is of central importance in biological monitoring. Certain plant species are highly sensitive to particular air pollutants and show specific responses to pollution effects (for example the formation of brown upper surface speckle by ozone). These indicator species can be used to detect, recognise and monitor the presence or absence of pollutants. Considerations in bioindicator selection are summarised by Tingey (1989) and are presented in Box 1.1.

Box 1.1 Selection of bioindicators

The selection of bioindicators should consider the following factors:

- be easily measured and describe responses of concern within the ecosystem;
- have a distinct response which is capable of predicting how the species or ecosystem will respond to the stress;
- measure the response with acceptable accuracy and precision;
- be based on knowledge of the pollutant and its characteristics;
- consider the final use of data.

Bioaccumulative indicators are frequently regarded as biomonitors. Plants can also act as bioaccumulative indicators by accumulating air pollutants from their surroundings without necessarily displaying an obvious response. Analysis of their tissues provides an estimate of environmental concentrations of the pollutants (for example mosses are frequently used to monitor heavy metal deposition).

The earliest and currently most recognised effects of atmospheric pollutants on plants was revealed by correlations between plant species distributions with particular pollutants. A common example was the discovery of 'lichen deserts' in parts of the UK.

2 Project scope

The current project builds on these aspects of biological monitoring and updates and reviews progress in this topic over the past ten years. Activity in this field has accelerated over the last decade with the development of internationally co-ordinated monitoring networks, unilateral long-term monitoring research programmes in addition to new legislation. In general, investigations, which examine effects, which are not suitable for monitoring purposes, have not been considered in this review. There is a growing awareness of the importance of particular plant groups and communities in their own right and a vast amount of literature is concerned with impact assessments of air pollution on plants. The significance of such studies is appreciated but the current review focuses on biological monitoring as a practical management tool in detecting and assessing air pollution.

The review divides each chapter by pollutant type since methods used are generally dependent on the type of pollution under investigation. For this purpose, air pollutants are categorised into metals, gaseous and organic compounds. Heavy metals are generally non-acidic particulates and include lead, zinc etc. Gaseous pollutants included nitrogen oxides, sulphur dioxide, ozone (volatile organic compounds and nitrogen oxides, once emitted, undergo chemical transformation in the atmosphere in the presence of sunlight to form ozone) and fluoride. Organic and synthetic chemicals include substances such as dioxins, polycyclic aromatic hydrocarbons and organochlorines. Radionuclides and the indirect impact of air pollution - acidification of soil and water, will not be considered in the current review.

Each chapter is subdivided into plant groups. The review includes all plant species, which are currently or have potential of being used as biomonitors/bioindicators of air pollution. These will primarily be lichens, bryophytes (i.e. mosses), higher plants (such as trees, shrubs and crops), algae and fungi. Where applicable, comments on monitoring design are included in each section.

The review focuses exclusively on terrestrial environments omitting freshwater or marine habitats.

3 References

Burton, M.A.S. 1986 Biological monitoring of environmental contaminants (plants). MARC Report Number 32. Monitoring and Assessment Research Centre, King's College London, University of London.

Matthews, R.A., Buikema, Jr. A.L., Cairns, Jr. J. and Rodgers, Jr. J.H. 1982 Biological monitoring. Part IIA. Receiving system functional methods, relationships and indices. *Water Research*, **16,** 129-139.

Market, B., Oehlmann, J. and Roth, M. 1997 General aspects of heavy metal monitoring by plants and animals. In: *Environmental monitoring: Exposure, assessment and specimen banking*, Subramanian, K.S. and Lyengar, G.V. (eds.), ACS Symposium series 654. American Chemical Society.

Tingey, D.T. 1989 Bioindicators in air pollution research - applications and constraints. In: *Biologic markers of air pollution stress and damage in forests,* Committee on biological markers of air pollution damage in trees. National Research Council, National Academy Press, Washington D.C.

II HEAVY METALS

1 Bryophytes

1.1 Introduction

Bryophytes include mosses and liverworts but most literature on air pollution monitoring centres on mosses. Bryophytes can indicate the presence of elements and their concentration gradients. The use of bryophytes constitutes an effective method in air pollution monitoring for many reasons:

- Many species are widely distributed and grow in a range of habitats.
- Bryophytes are small and easy to handle.
- Most of them are evergreen and can be surveyed all year round.
- Bryophytes lack a cuticle and a root system and obtain nutrients as particulates and in solution directly from atmospheric deposition. They have good bioaccumulating ability, particularly for heavy metals, where metal concentrations reflect deposition without the complication of additional uptake via a root system.
- Comparisons of fresh samples with herbarium specimens enable retrospective analysis of metal pollution.
- The ability of bryophytes to accumulate elements in very high concentrations aids chemical analyses of the tissues and may facilitate the detection of elements present in very low concentrations in the environment.
- The annual growth increment is usually easier to detect in mosses than lichens and mosses are often believed more desirable for temporal studies (this is particularly true of *Hylocomium splendens*).

Some studies have been carried out to compare the effectiveness of different biological samplers as biomonitors. Kansanen and Venetvaara (1991) compared the capability of two mosses, an epiphytic lichen, pine bark samples, pine needle litter, earthworms, and moths in assessing airborne chromium and nickel dust near a ferrochrome and stainless steel works in Finland. The two mosses and the lichen were the most effective biomonitors at low and moderate depositions. None of the biomonitors worked effectively at high deposition loads. Moss and epiphytic lichens were found to be the best indicators for zinc (Zn) and aluminium (Al) in a study of biological indicators around the Rautaruukki steel works in northern Finland (Mukherjee and Nuorteva, 1994).

Most methods in heavy metal monitoring employ bryophytes as bioaccumulators and involve sample collection followed by laboratory analysis techniques to detect actual levels. Bioindication of heavy metal deposition by the use of bryophyte distribution techniques and physiological effects is rare.

This section is subdivided depending on the geographical scale of the survey, into multi-national, national, regional and urban/industrial areas. National studies are mostly part of wider multi-national monitoring programmes. Urban/industrial area studies deal with urban areas and point source emissions. Spatial studies and temporal studies are considered.

Table 2.1 summarises the surveys reviewed in this section. The type of survey, species used and metals analysed are compared.

The majority of studies have been multi-element investigations and few have been restricted to a particular metal except where different methods of analysis were required. Transplantation techniques are discussed separately. Comment is limited to monitoring metal deposition from the atmosphere using bryophytes and will not examine in detail the effects of metals on bryophytes.

Since Burton's review in 1986, approaches to moss monitoring of heavy metal deposition have changed little in principle. Refinements have been made in monitoring design, in terms of standardised sampling and reduction of error and variance. As may be expected new developments in chemical analysis have occurred.

Table 2.1 Comparison of parameters used in various aerial heavy metal studies using mosses as biomonitors

Scale of survey	Species	Elements	No. of sites	Type of survey	Author
Multi-national	*Hylocomium splendens* *Pleurozium schreberi*	As, Cd, Cr, Cu, Fe, Ni, Pb, V, Zn	Denmark - 185 Finland - 534 Norway - 522 Sweden - 839	Spatial and temporal	Rasmussen *et al.* 1988
Multi-national	*Hylocomium splendens* *Pleurozium schreberri*	As, Cd, Cr, Cu, Fe, Ni, Pb, V, Zn	As above	Spatial and temporal	Ruhling 1995
Multi-national	*Hylocomium splendens* *Pleurozium schreberi*	As, Cd, Cr, Cu, Fe, Ni, Pb, Zn	Standard reference sites	Spatial and temporal	UN ECE 1993
National (Sweden)	*Hylocomium splendens* *Pleurozium schreberi*		Approx. 1000 forest inventory plots	Spatial and temporal	Bernes 1990
National (Norway)	*Hylocomium splendens*	Ag, Al, As, Br, Cd, Cl, Co, Cr, Cs, Cu, Fe, I, La, Mn, Mo, Na, Ni, Pb, Rb, Sb, Sc, Se, Sm, Th, V, Zn	512	Spatial and temporal	Schaug *et al.* 1990
National (Norway)	*Hylocomium splendens*	Al, As, Br, Cd, Co, Cr, Cu, Fe, I, Ni, Pb, Sb, Sc, Se, V, Zn	523	Spatial and temporal	Steinnes *et al.* 1994
National (Norway)	*Hylocomium splendens*	Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, Hg, La, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sb, Sr, Te,Th,Ti,U,V,Y, Zn	495	Spatial and temporal	Berg *et al.* 1995

Table 2.1 (continued from page 15)

National (Norway)	*Hylocomium splendens*	Hg	198	Spatial and temporal	Steinnes and Andersson 1991
National (Netherlands)	*Pleurozium schreberi*	Al, As, Ba, Br, Ca, Ce, Co, Cr, Cs, Cu, Fe, Hg, I, K, La, Mg, Mn, Na, Ni, Pb, Rb, Sb, Sc, Se, Sm, Th, V, Zn	66	Spatial and temporal	Kuik and Wolterbeek 1995
Regional (Nigeria)	*Polytrichum juniperinum*	Cd, Cu, Mn, Pb, Zn	56	Spatial	Kakulu 1993
Regional (Poland)	*Pleurozium schreberri Hylocomium splendens*	Pb, Cd, Ni, Zn, Cu	2	Spatial	Godzik and Grodzinska 1991
Urban and industrial (India)	*Plagiothecium denticulatum,* *Bryum argenteum,* *Sphagnum sp.*	Cd, Cu, Fe, Mn, Ni, Pb, Zn	9	Spatial	Gupta 1995
Urban and industrial (Sweden)	*Hypnum cupressiformae*	Cr	Approx 30	Spatial and temporal	Bernes 1990
Urban and industrial (Canada)	*Isothecium stoloniferum*	Cd, Cr, Mn, Ni, Pb, Zn	62	Temporal	Pott and Turpin 1996
Transplants (Greenland)	*Sphagnum girgensohnii*	Cd, Ce, La, Nb, Nd, Pb, Th, U, Zn	12	Spatial	Pilegaard 1993
Transplants (Quebec)	*Pleurozium schreberri*	Hg	4	Spatial	Evans and Hutchinson 1996
Transplants (Scotland)	*Sphagnum*	Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn	47	Spatial and temporal	Gailey and Lloyd 1993

1.2 Monitoring design

The importance of planning when initialising a biomonitoring programme of trace-element air pollution is emphasised by Wolterbeek and Bode (1995). Successful cryptogram monitoring is achieved when contaminant burden is readily distinguished from background levels in the plants. Certain important parameters have been considered in heavy metal monitoring utilising bryophytes, which are discussed below. It is noteworthy that many of the following comments are also applicable to the monitoring of aerial deposition of heavy metals using lichens unless specificity to mosses is expressed.

1.2.1 Method selection

A major choice lies between using techniques which employ indigenous species or transplanted species. This will ultimately affect the type of species selected and to some extent the chemical analytical techniques employed.

Comparisons of techniques utilising indigenous and transplanted samplers are summarised in Table 2.2 (taken from Gailey and Lloyd, 1993).

Factors which should be considered in methodology selection include finance and resources, desired accuracy of results, study time-scales, size of study area, extent and type of pollution.

1.2.2 Species selection

In metal deposition biomonitoring, species selection criteria include the availability of the species, its tolerance, its bioaccumulation characteristics and ease of sampling (Wolterbeek and Bode, 1995). Additionally, the species utilised and its effectiveness will depend to an extent on the elements to be monitored.

Puckett (1988) reviewed the applicability and mechanisms of mosses and lichens as biomonitors of metal deposition. Ectohydric mosses with no differentiated water conducting system, enabling direct absorption over the entire plant surface, are more appropriate than endohydric mosses possessing differentiated water conducting systems and cuticle-like surfaces. The use of epigeic mosses (mosses growing naturally on the ground) has been recommended in Scandinavia for assessment of heavy metal deposition on a regional scale (Steinnes *et al.,* 1993). Pleurocarpous species, otherwise known as the carpet-forming mosses or feather mosses, are probably the most commonly utilised group (Table 2.1). However, element concentrations in carpet forming mosses may be elevated by soil-blown dusts. Such contamination and possible misinterpretation of results would be particularly heightened in seasonally arid countries (Ruhling, 1995).

Table 2.2 Comparisons of indigenous samplers and transplants in heavy metal deposition monitoring (from Gailey and Lloyd, 1993)

Indigenous (in situ) sampling	**Transplants**
Results of pollution patterns can be obtained within a few days.	A survey period of a year is required to allow for effects of seasonal variation.
Results demonstrate pollution in previous years.	Results illustrate pollution only over the sampling period.
Accumulation levels are usually above detectable levels due to longer exposure time.	Concentrations of accumulated levels may be undetectable over shorter sampling period.
Minimal supervision and risk from vandalism.	Potential risk from vandalism.
Costs acquired from transport to sites and chemical analyses.	Additional costs from materials, increased transport and more sampler preparation.
Potential shortage of indigenous samples.	Density of sampling sites, samplers and their position under the control of investigators.
Pollution deposition rates difficult to estimate.	Deposition rates calculated from controlled exposure time.
Metal concentrations reflect influences from other factors such as age of plant, metal content of substrate and local contamination.	Pollutant concentrations in plants can be more directly related to airborne pollution.
Plants may be stressed or undergo morphological/physiological changes, which affect uptake, by long-term exposure to certain pollutants.	Plants come from relatively ‘clean’ environments.

Markert and Weckert (1989) investigated the suitability of *Polytrichum formosum* as a passive biomonitor of heavy metal deposition. Moss samples from the forest under study showed seasonal variation in metal content. The authors concluded that sample collection of this species should be undertaken in the last week of September if comparable results between regional surveys are to be obtained. This study highlighted that species type and sampling period should be considered prior to initiating a moss monitoring programme.

The signal-to-noise ratio was suggested by Wolterbeek *et al.* (1996) as a means of assessing the quality of a biomonitoring survey. The authors used large-scale biomonitoring surveys of trace-element air contamination to illustrate the utility of such an approach. The investigators used survey variance as the signal and the survey noise was defined by measurement of local variance per site. The most significant conclusion from this study was that the 'selection of the biomonitor species should be based on minimisation of the signal-to-noise ratio rather than on minimisation of the noise level of the survey'.

1.2.3 Site selection

The density and location of sampling sites will depend very much on the type of survey. Larger scale surveys covering larger areas will obviously require more sites than studies investigating point emission sources (Table 2.1). In the latter, sites are frequently spaced along transects or gradients in relation to the pollution source. Intensity of sampling sites should be adequate to detect gradual changes along the study area. If indigenous species are to be utilised, the number and location of sites will depend on the natural distribution of the species. If transplantation techniques are used, choice of sites is at the discretion of the investigator.

At the sampling site, attention should be given to the substrate since this may affect the elemental composition of the study species. Other considerations include safety and ease of access of the site.

To overcome noise variation the development of strict criteria in site selection is necessary. For example in large-scale surveys in Europe, sampling sites were selected in areas remote from roads, large population centres and industrial plants in order to identify areas susceptible to long-range transported pollutants (Ruhling, 1995). Samples from forest ecosystems are usually taken from openings in the canopy, not directly exposed to throughfall precipitation. However, some species are found in sheltered areas where they may not be freely exposed to aerial deposition and measured levels in the sample may not adequately indicate pollutant levels. This highlights the need to choose locations where exposure to atmospheric pollutants is not reduced.

Sources of heavy metals other than atmospheric deposition which can contribute to metal concentrations in moss samples include (Steinnes, 1992):

- Natural cycling process, particularly the deposition of emissions from marine sources.
- Root uptake in vascular plants and subsequent leaching from the tree canopy, understorey vegetation and leaf litter.
- Windblown mineral particles.

Ideally an accurate interpretation of results from large-scale moss surveys should include an assessment of the contribution from other sources (Brumelis and Nikodemus, 1995). This can be undertaken using multivariate analyses techniques such as factor analysis (e.g. Sloof and Wolterbeek, 1991).

In bioaccumulation monitoring studies, the standardisation of sample collection, preparation and analytical techniques has been recommended (Puckett, 1988). In terms of collection this could include the general area of collection (e.g. forest), the specific area of collection (e.g. position on tree) and the quality and quantity of sample. Sample preparation varies for example in washing and drying procedures. Differences also exist in the analytical chemical methods adopted.

1.2.4 Chemical analyses

Results are more useful when background elemental levels are obtained (Seaward, 1995). Generally, a large number of elements is chosen for analysis because the benefits of obtaining large amounts of data outweigh the extra effort, especially when the extent of fieldwork is independent of the number of elements chosen for analysis (Wolterbeek and Bode, 1995). Contamination during collection should be avoided. Replication of samples is recommended for accurate results. Consistency of measurement units aids comparative studies.

The choice of analytical method will depend on the purpose of the respective survey. Some analytical methods are non-destructive (e.g. neutron activation) and are useful for repetitive surveys such as baseline studies. Samples can also be archived and used at a later date for additional analysis. Destructive techniques include atomic absorption spectrometry and inductively coupled plasma analysis.

Steinnes *et al.* (1993) compared methods previously used in heavy metal deposition studies in Norway (instrumental neutron activation analysis (INAA) and atomic absorption spectrometry (AAS)) with other multi-element techniques. The alternative analytical techniques investigated were inductively coupled plasma emission spectrometry (ICP-ES) and inductively coupled plasma mass spectrometry (ICP-MS). INAA produced much higher values for Na, Al and Fe compared with ICP-ES and ICP-MS. The former technique measures total content while the other techniques are based on leaching procedures. On consideration of the methods available for moss monitoring in Norway, Steinnes *et al*. conclude that ICP-ES works well for Fe, Zn, Pb and Cu, to a lesser extent for V and Ni and but is not satisfactory for Cr, Cd and As. ICP-MS analysis proves a good method for all of the above except As and Cd where less satisfactory results were observed. In conclusion, ICP-MS was regarded as a valid alternative to INAA/AAS analysis of *Hylocomium splendens* samples within the Norwegian monitoring programme at the time.

A similar study was conducted by Frontasyeva *et al.* (1994). Epithermal neutron activation analysis (ENAA) was compared with conventional INAA

and
ICP-MS. ENAA produced promising results for expansive multi-element analysis of mosses used in monitoring atmospheric deposition.

1.3 Multi-national surveys

Techniques using indigenous moss populations to identify and monitor geographical patterns in heavy metal atmospheric pollution are well established in Europe. Precise element concentrations are often not reported and techniques are applied as a practical tool in establishing and characterising deposition sources. Such long-term, larger scale monitoring is extremely useful and also enables transboundary ameliorative action to be taken. Most programmes are ongoing, allowing comparisons over time and space to be made.

In a joint Nordic project, Rasmussen *et al.* (1988) used moss analysis as a means of identifying sources of airborne pollutants and mapping metal deposition in northern Europe (Denmark, Finland, Norway and Sweden). In 1985 *Hylocomium splendens* and *Pleurozium schreberi* samples were collected from openings in coniferous forest or young plantations, not directly exposed to throughfall precipitation. The three youngest fully developed segments of the moss were used for analysis. Atomic absorption, neutron activation or
ICP techniques were used to determine various metal concentrations. Lead (Pb), arsenic (As), cadmium (Cd) and vanadium (V) concentrations in samples showed a steep gradient from south to north Fennoscandia, with highest levels in the south decreasing towards the north. Nickel (Ni), chromium (Cr), copper (Cu) and iron (Fe) and to an extent zinc (Zn) concentrations showed weaker gradients. This pattern was attributed to long-range transport of air pollutants from the densely populated areas in the south. In non-forested areas such as alpine and agricultural regions, metals originating from soil dust such as As, Cr, Cu, Fe and V were present in high concentrations in the collected moss samples. The importance of larger local emission sources was also revealed.

Ruhling (1995) reported on a comparable study in northern Europe carried out in 1990-91 as part of a large-scale heavy metal monitoring programme covering 21 European countries. By mapping results, conclusions on heavy metal deposition in northern Europe were drawn and comparisons with past studies made. Sources of long-range transported air pollution were identified andregional atmospheric deposition of heavy metals was characterised. Almost ten locally important emission sources of heavy metals and the extent of these emissions were established. Metals showed similar gradients from south to north as indicated by Rasmussen *et al*. (1988). Heavy metal concentration levels have displayed a decrease over the last two decades. The authors attribute this to improved filter techniques and response to new legislation. These studies clearly emphasised the effectiveness of moss survey technique as a biological tool in long-term, large-scale monitoring initiatives.

The UN ECE Convention on Long-range Transboundary Air Pollution (1993) produced a manual for integrated monitoring with a programme phased between 1993 to 1996. The overall purpose of the programme is monitoring and assessing effects from air pollutants in the environment. Numerous countries are involved in the programme but Sweden was appointed lead country and Finland took responsibility for data handling. Nordic countries have a high profile within the framework particularly in the development of methods. Monitoring metal concentration of mosses is included in the programme. Detailed sampling procedures were prescribed in the manual. A general outline is provided below:

- Three composite samples should be collected from either *Pleurozium schreberi* or *Hylocomium splendens* species.
- Samples should be retrieved from areas not exposed to direct throughfall water - ideally in forests or open heathland or peatland.
- Sampling period should be early summer.
- Contamination should be minimised during sampling.
- Only green shoots from the three most recent years should be included in the analysis.
- Chemical analysis should be undertaken using AAS /ICP/neutron activation.
- Metals As, Cd, Cr, Cu, Fe, Ni, Pb, Zn should be monitored.

1.4 National surveys

Sweden is very experienced in moss monitoring techniques and has been using mosses as a means of studying heavy metal deposition every five years since 1975 (Bernes, 1990). Sampling and analysis is carried out in much the same way as the studies mentioned above. Mapping levels of metals in mosses has illustrated the geographical distribution and long-term changes in metal fallout. The amount of fallout is quantitatively estimated from the growth rate of *Hylocomium*, which grows at the same rate in northern and southern Sweden according to the equation:

$$\text{metal deposition (mg m}^{-2}\text{ y}^{-1}) = \text{metal content in moss (mg kg}^{-1}) \times \text{average growth rate (0.15 kg m}^{-2}\text{ y}^{-1})$$

This assumes, however, that mosses possess 100% efficiency in metal uptake from the atmosphere. Comparisons of fallout estimates with heavy metal values in precipitation were good for Pb, Cd, Cu, Zn and V. Correlations with levels of Cr and Ni were less satisfactory and this was attributed to mosses concentrating these metals from other sources besides atmospheric deposition (Bernes, 1990). The author concluded that in point emission source studies, where metals tend to be deposited in particulate form rather than through precipitation, moss analysis provides an adequate picture of total fallout.

Schaug *et al.* (1990), Steinnes *et al.* (1994) and Berg *et al.* (1995) used the moss technique for mapping atmospheric deposition patterns of metals in Norway. Pilot studies carried out in the 1970s determined the most appropriate species and analytical techniques. *Hylocomium spendens* was chosen for a number of reasons, most of which are mentioned in Section 1.1. The investigators reported on results from national surveys carried out in 1977, 1985 and 1990. Elements monitored, number of sites and date of study are provided in Table 2.1. As the studies progressed the sampling network was altered to enable more accurate descriptions of areas of high deposition rates (i.e. the south). The Norwegian surveys differed from the multi-national ones discussed earlier in that many more elements were investigated. This was achieved by modification of the analytical techniques.

This ongoing programme enabled national spatial and temporal patterns in heavy metal deposition to be presented as isopleth maps. Although some variation occurred over the survey years, statistical analysis enabled the following pollution sources to be defined (elements in brackets derived their main contribution from some other factor) (from Berg *et al.*, 1996):

- Long-range transported pollutants from other parts of Europe: V, (Cu), Zn, (Ga), As, Cd, Mo, Cd, Sb, Se, Ag, Hg, Tl, Pb, Bi.
- Mineral particles, mainly windblown dust from local soil: Li, Al, (V), Cr, Fe, (Co), Ga, Y, La, Th, U.
- Local point sources: Co, Ni, Cu.
- Marine environment: (Li), B, Na, Mg, Ca, Sr.
- Root uptake in vascular plants from soil, and subsequent transfer to mosses by leaching from living or dead plant material: (Mg), (Ca), Mn, (Cu), (Zn), Rb, (Sr), Cs, Ba.

Most long-range transported elements present in southern Norway had decreased by 1990 to 70 to 50% of 1977 levels.

Spatial and temporal trends of atmospheric deposition of mercury (Hg) in Norway were investigated using moss samples and peat samples, respectively (Steinnes and Andersson, 1991). The distribution of Hg in moss is different from the previously studied elements in that it did not appear to be associated with long-range atmospheric transport or point sources. Hg showed a much less pronounced south-north gradient than other elements and the authors suggested that dry deposition of Hg may be important at northern latitudes.

Moss techniques were reported by Kuik and Wolterbeek (1995) in the Netherlands as part of a larger European study. This 1992 study was compared to previous accounts using epiphytic lichens samples from the same area in 1986 to 1987. Average moss concentrations in the 1992 study were significantly lower than those observed in lichens in 1986/87. Application of Monte Carlo-Assisted Factor Analysis to the data proved an effective method of determining and characterising different heavy metal sources throughout the country. Major sources were classified as:

- Crustal material: Al, Ce, Cr, Fe, La, Sc, Sm, Th.
- Sea aerosol: Na, Br, I.
- Zinc industry: Zn, Pb, Hg, As, Co, Sb.
- Metallurgical processes or refuse incineration: Sb, V, Se, Co, Pb, Hg, Fe, Ni, La.
- Oil combustion and processing of oil products: Cr, Ni, Co, Fe, V.

1.5 Regional surveys

More intensive regional based investigations using natural growing mosses as biomonitors of heavy metal pollution have been undertaken. Results tend to be classified into zones within the region allowing identification of sources. Description of the heavy metal status within the region can be made.

The epiphytic moss *Polytrichum juniperinum* and the bark of *Azardirachta indica* produced comparable results when used to determine the atmospheric metal in a north-eastern region of Nigeria (Kakulu, 1993). Three pollution zones for Pb and Zn (high, medium and low) were evident within the region (Table 2.3). Pb and Zn levels in moss samples showed ranges of 10 to 241 µg g^{-1} and 28 to 123 µg g^{-1} dry weight, respectively. Cd, Fe and Mn moss levels where highest in the big towns. For example, mean Cd, (µg g^{-1}) Mn, (µg g^{-1}) and Fe (mg g^{-1}) in the high pollution zones were 0.41, 97 and 12.7, respectively, whereas mean metals concentrations in dry weight moss samples in the low pollution zones were 0.1, 37.8 and 5.6, respectively. Ni and Cu did not show significant concentration gradients between the larger towns (Jos, Maiduguri and Bauchi) and smaller towns and villages. The author concluded that Pb, Zn and Fe were responsible for the greatest heavy metal pollution burden in the study area. Sources of these and other metals in the high pollution zone were attributed to fossil fuel burning due to industrialisation, automobile exhaust emissions and incineration of domestic wastes due to urbanisation. Vehicle emissions, small metal works and incineration of domestic wastes accounted for the presence of metals in the less polluted areas.

Table 2.3 Mean Pb and Zn levels in mosses in sites of different pollution zones within the north-eastern region of Nigeria (from Kakulu, 1993)

Site	Zone	Concentration in µg g^{-1} dry weight	
		Pb	Zn
Jos	High	201	84
Maiduguri	High	241	123
Bauchi	High	190	98
Potiskum	Medium	106	49
Gombe	Medium	89	56
Wikki	Low	10	28
Gubi	Low	16	33

Godzik and Grodzinska (1991) used *Pleurozium schreberri* and *Hylocomium splendens* to gauge the heavy metal burden in Mazurian Landscape Park, Mazurian Lake District, Poland, in comparison to the relatively polluted Ojcow National Park and the 'cleaner' Bialowieza National Park. Metal levels in Mazurian Landscape Park were significantly lower than in the other parks. For example, the *Pleurozium* from the Ojcow National Park was found to accumulate 14 times as much lead and cadmium. The authors concluded that the relatively unpolluted character of the landscape park in combination with its unique flora and fauna is justification for its promotion to a higher conservation, national park status. This is a primary example of the application of moss monitoring techniques as a practical management tool. Another relevant outcome of this study was the observation of *Hylocomium splendens* as a better copper accumulator than *Pleurozium schreberri*.

Winner (1988) reviewed studies related to metal concentrations and mosses in North America. These studies were mainly regionally based.

1.6 Urban and industrial surveys

Moss techniques have been applied to measure heavy metal levels and trends within and around urban and industrial areas. These studies can analyse temporal and/or spatial trends in heavy metal deposition and results are generally expressed as pollution gradients. Within the gradient, metal levels in the local moss populations are seen to decrease with increasing distance from the suspected source.

In Sweden, long-term metal fallout, particularly of Cr has been mapped using moss analyses as part of the monitoring programme operated by Vänersborg-Trollhättan Regional Air Quality Association. Samples of the carpet moss *Hypnum cupressiformae* from approximately thirty sites in the vicinity of the works have been collected every three years since 1973 (Bernes, 1990). In the early 1970s chromium levels at Trollhättan reached 20,500 mg/kg dry weight moss in the immediate vicinity of the plant. Less polluted areas of Sweden showed moss levels of 3 mg/kg at this time. Chromium emissions from Trollhättan fell in the late 1970s but this coincided with the initiation of chromium alloy manufacture at Vargön. Since then, high emissions from Vargön have been reduced and the Trollhättan plant has been closed down. Bernes (1990) reported other similar monitoring programmes in Sweden.

Gupta (1995) analysed three moss species in an assessment of non-point sources of heavy metal contamination in Shillong, Meghalaya State, north-eastern India. *Plagiothecium denticulatum* (from stones and cemented surfaces) and *Bryum argenteum* (from asbestos roofs) were collected from four sites within the urban area and four from the outskirts. *Sphagnum sp.* was collected from one site where it was found in a forest edge, two km away from the city centre. Results (Table 2.4) showed that *Sphagnum* sp. reflected Cd and Zn concentrations better than the other two even in the suburban areas. *P. denticulatum* appeared to be the best accumulator of lead. All species accumulated manganese effectively. An urban-suburban gradient was obtained for lead and zinc in *P. denticulatum* and for cadmium in *B. argenteum.* This study provided a good baseline dataset for future moss monitoring in India, where data is lacking. It will allow comparative studies between other urban areas.

Tissue analysis of the woodland epiphyte *Isothecium stoloniferum* was used to report atmospheric trace-element deposition in the Fraser Valley, B.C. Canada from 1960 to 1993 (Pott and Turpin, 1996). By studying herbarium samples available from the 1960s, significant reductions of varying degrees of Cd, Cr, Pb, Ni and Zn levels in moss samples from 1960 to 1993 were observed. The authors provided several reasons for the decline. There had been an obvious shift in the area from a resource and manufacturing based economy in the 1970s towards a service-based economy in the 1990s. The closure of heavy industries, new emission control legislation, reduction in fossil fuel combustion and significant decreases in leaded petrol consumption contributed to the reduction in metal deposition. Only manganese (Mn) showed a significant increase over the survey years. This was attributed to the introduction of methylcyclopentadienyl manganese tricarbonyl as an anti-knock additive to petrol in the 1970s.

Table 2.4 Metal concentrations in three moss species in urban and suburban sites in Shillong, India (from Gupta, 1995)

Element	Concentrations ($\mu g\ g^{-1}$)				
	P. denticulatum		*B. argenteum*		*Sphagnum*
	Urban	Suburban	Urban	Suburban	Suburban
Cd	1.25	1.08	1.98	1.30	2.01
Cu	45.38	37.73	30.70	24.68	25.18
Mn	397.19	503.6	308.54	333.17	639.08
Pb	66.38	52.27	40.74	35.07	28.42
Zn	40.05	24.36	15.28	14.97	92.34

1.7 Transplants

Transplantation is an experimental technique where study plants are transferred, along with their original substrate, from unpolluted control areas to suspected or known polluted areas. The effects and responses of the transplants are subsequently examined after a measured time scale and compared to the control areas. Conclusions on pollution levels and/or nature of the pollutants with respect to exposure time can be composed. In terms of aerial metal monitoring using transplants, levels in tissues rather than effects are measured before and after the exposure period.

Pleurozium schreberri showed elevated Hg concentrations when transplanted from control sites to Roundtop Mountain and Mt. Tremblant in southern Quebec despite their distance from known point sources of mercury (Evans and Hutchinson, 1996). Mercury values in the Roundtop Mountain and Mt. Tremblant summit sites were 248.3 and 174.0 ng g^{-1} representing increases of 129 and 61% respectively. This was attributed to long-range transported Hg deposition.

A similar approach is the moss-bag technique which involves using *Sphagnum* or *Rhynchostegium* species in nylon or muslin (0.07 to 0.9 mesh cm^{-1}) bags. Exposure times are usually shorter with this method prior to elemental analysis. This method, although applied to the terrestrial environment, is used more extensively in the detection of heavy metals in the aquatic environment. The technique is based on the high cation exchange capacity of mosses.

The effects of exploration activities associated with a niobium-mineralisation in Sarfartoq, south-west Greenland were illustrated using the moss-bag technique (Pilegaard, 1993). Samples of *Sphagnum girgensohnii* were collected from a remote unpolluted area in southern Sweden. Metal concentrations in the pre-exposed bags were measured (i.e. background levels). Metal levels were determined from moss bags at twelve sites, set at three different periods: before major dust producing activities; during intensive drilling activity; and after work was completed. Concentrations of Nb in particular, especially in sites close to the outcrop, were elevated during the period of highest dust production. However, examination of the indigenous flora indicated that pollution existed near the outcrop prior to drilling, with elevated concentrations in Nb, La, Ce, Th and U. This study emphasised the importance of pre-operational monitoring in assessing the scale of effects at such sites. It also provided good baseline data for further exploration works and acted as a scoping study for the sensitivity and appropriateness of methods.

Gailey and Lloyd (1993) compared the suitability of four different biomonitors in assessing short-distance and short-term changes in airborne metal contamination in Armadale, central Scotland. This formed part of an environmental epidemiology study of respiratory cancer. *Sphagnum* moss bags produced more consistent results than the transplanted lichen *Hypogymnia physodes* attached to its twig substrate. Indigenous *Hynum cupressiforme* provided better results than the indigenous lichen *Lecanora conizaeoides*. Sampling sites were positioned along a gradient from a steel foundry in the town. Metals investigated are shown in Table 2.1. All samplers showed a general decrease in metal content from the foundry, indicating a declining pollution gradient from this source. Statistical analyses of temporal data implied that meteorological factors and the steel foundry were more important pollution sources than the brickworks, the other main industry in the town.

Kirchhoff and Rudolph (1989) described a sandwich technique for the continuous monitoring of air pollutants with the bryophyte *Sphagnum magellanicum,* collected from a bog in Germany. The sample was washed and transferred to the field sandwiched between two layers of plastic screen and suspended, by the edges only, in a plastic holder. The method allowed the installation of a heater in the holder, permitting winter use. Results were compatible with those obtained from rainfall trapped at the same sites.

2 Lichens

2.1 Introduction

Lichens are effective biomonitors of metal deposition in that they possess many similar characteristics to bryophytes (Section 1.1). Lichens are slow growing and assimilate metals at a rapid rate but release them at a low rate. Metal concentrations in lichen thalli have been shown to correlate with atmospheric levels (Burton, 1986). Lichens were first used as bioaccumulative indicators in relation to point emission sources, where decreasing metal concentrations in species correlated with increasing distance from the source (Burton, 1986). Lichens have also been used to assess deposition patterns and heavy metal burdens for larger scale monitoring purposes. Their application as bioaccumulators on the multi-national and national scale is not as established as it is for bryophytes. The role of lichens in multi-national monitoring programmes is primarily in relation to gaseous air pollutants.

Some important aspects in the design of programmes utilising lichens for the biomonitoring of airborne heavy metal pollution are considered briefly at the beginning of this section. Their application on national, regional and urban/industrial scales are presented. Transplantation techniques are also discussed. The uptake, retention and toxicity of heavy metals in lichens are not treated in detail here. A comprehensive review of these aspects is provided in Tyler (1989) and Tyler *et al.* (1989).

2.2 Monitoring design

Most of the comments in Section 1.2 are applicable to lichen monitoring for metal deposition. Some additional remarks are presented below.

2.2.1 Species selection

In lichens, metals can accumulate to high levels by trapping insoluble particles (Tyler, 1989), extracellular ion exchange processes (Richardson, 1988), adsorption and active uptake (Král *et al.*, 1989). Sloof (1995a) also indicated the importance of passive processes in the uptake and release of Co and Zn. Burton (1986) reviewed some of these mechanisms in different species. The author stressed the need to consider the differences in morphology and ion exchange properties between different lichen species when selecting a species for atmospheric heavy metal monitoring.

The nature and form of the metals under study is important in the selection of species in that this often determines whether the lichen will die, show symptoms or accumulate without apparent harm (Richardson, 1991). The chemical properties of an element and its associated particles affect its accumulation by a biomonitor. The sensitivity of lichens to elevated tissue concentrations of heavy metals varies greatly between species, populations and elements (Tyler, 1989). Obviously species with the ability to bioaccumulate high metal concentrations without apparent damage are more beneficial in biomonitoring studies.

In metal deposition studies, fructose (shrub-like) lichens have been recommended since these forms are easier to separate from the substrate in comparison to foliose (leaf-like) and crustose (crust-forming) lichens (Puckett, 1988). For example, foliose *Parmelia* was chosen over crustose *Lecanora* species in the Netherlands (Wolterbeek and Bode, 1995).

Box 2.1 Lichen species useful for the indication of heavy metals (from Kovács, 1992a)

Acarospora strigata	*C. squamosa*	*Parmelia polydactyla*
Alectoria capillaris	*C. uncialis*	*P. rudecta*
A. nigricans	*Cornicularia aculata*	*P. sulcata*
A. ochroleuca	*C. divergens*	*P. saxatilis*
A. sarmentosa	*C. muricata*	*P. taractica*
A. tremonti	*Dermatocarpon miniatum*	*Peltigera canina*
Caloplaca aurantia	*Evernia mesomorpha*	*P. rufescens*
C. trachyphylla	*E. prunastri*	*Pseudoevernia furfuracea*
Cetraria cuoullata	*Hypogymnia enteromorpha*	*Ramalina duriaei*
C. delisei	*H. physodes*	*R. farinacea*
C. islandica	*Lasallia papulosa*	*R. stenospera*
Cladonia alpestris	*Lecanora alphoplaca*	*Rhiyoplaca melanopthalma*
C. arbuscula	*L. conizaeoides*	*Sphaerophorus fragilis*
C. convoluta	*L. frustulosa*	*Stereocaulon evolutum*
C. chlorophaea	*L. novomexicana*	*S. nanodes*
C. cristatella	*Letharia vulpina*	*S. pascale*
C. deformis	*Micarea trissepta*	*Umbilicaria grisea*
C. furcata	*Parmelia borrei*	*U. hirsuta*
C. gonecha	*P. caperata*	*U. mammulata*
C. impexa	*P. chlorochroa*	*U. polyphylla*
C. mitis	*P. conspersa*	*U. pustulata*
C. rangiferina	*P. fuliginosa*	*U. sporodochroa*
C. stellaris	*P. plittii*	*Varrucaria nigrescens*
C. sylvatica		

As mentioned previously (Section 1.2.2), availability of species within study areas often determines the selection of a species. Sloof and Wolterbeek (1993) discussed the possibility of interchangeability of lichen species within polluted areas by interspecies calibration.

Several lichen species are appropriate for the bioindication of heavy metal exposure (Box 2.1 from Kovács, 1992a).

2.2.2 Sample collection and preparation

Various sampling strategies are employed during collection. Some sampling strategies are more specific than others. Standardisation of collecting methodologies would allow more appropriate comparisons to be made between surveys.

In epiphytic lichen studies, some investigators collect only from one tree species, others from numerous tree species within a specified range. Some investigators specify collection height of lichens from trees e.g. 1.5 to 2 metres above ground. Others believe that sampling from all around the tree is important while others do not. Some samplers specify composite lichen volumes while some do not.

Wolterbeek and Bode (1995) discussed the possible substrate contributions to lichen elemental concentrations. This may vary depending on species, substrate type and element. In the Netherlands lichens are sampled from protruding rims of rough bark in an attempt to minimise effects of excessive stem flow and build up of soil and bark particles at lichen attachments.

Bargagli (1990a) demonstrated that the younger, more external part of the thallus (corresponding to biomass produced the previous year) contained lower concentrations than the older, more internal part. In a study of abandoned mines in Italy, Bargagli (1990b) used the outermost three to five mm part of *Parmelia sulcata* thalli for the following reasons:

- It is easily distinguished by its colour and lack of rhizinae.
- It has known age (about one year).
- The outermost zone is almost completely devoid of rhizinae and as a result has little connection with the bark.
- Physiologically, it is the most active portion of the thallus.

Similarly in another lichen biomonitoring study in Italy, Loppi *et al.* (1994) used only the external part of *Parmelia caperata.*

Variations in cleaning procedures can yield different results. For example, Bennett *et al.* (1996) confirmed that failure to remove excess matter from lichen samples affected elemental determinations.

2.2.3 Chemical analysis

The need for method standardisation has been expressed above (Section 1.2.3). For the provision of accurate results and error minimisation, the Standards, Measurements and Testing Programme of the European Commission has proposed the use of lichen certified reference materials. This is the first stage of a certification procedure as part of overall quality control in the analysis of lichen material throughout Europe (Quevauviller *et al.*, 1996).

Other aspects of quality control such as experience of investigator, sample replication and awareness of local variations should also be applied. Wolterbeek and Bode (1995) treat these aspects in detail.

2.3 National surveys

The gradient method, based on decreasing metal concentrations in species with increasing distance to source must be be applied with caution in large-scale surveys as unknown sources may contribute to metal content in lichens.

In the Netherlands, elemental concentrations in *Parmelia sulcata* obtained during two national lichen surveys undertaken over a five year period were presented as maps of geographical concentration patterns (Sloof and Wolterbeek, 1991). A standard sampling technique was utilised throughout. Healthy samples were taken from pH neutral bark substrates such as ash, alder, elm, oak, poplar and willow. Lichens were collected from all around the tree at heights between 1 to 2.5 m above ground. Samples were collected from open locations removed from farms and motorways. Application of target transformation factor analysis enabled the determination of elemental source profiles and source contribution. For example factor group Al, Cr, Fe, Mn, Sc and Th were associated with crustal material, Ni, V and Co with oil combustion processes and As, Cd, Sb, W and Zn with zinc smelters and/or electronic industry. Changes in the aerial heavy metal levels and their distribution were evident over the five-year period. Sloof (1995a) recommended the use of Monte-Carlo-assisted factor analysis (Kuik *et al.*, 1993) as a refinement to data analysis of the 1986/87 lichen data in terms of source apportionment.

2.4 Regional surveys

Herzig *et al.* (1989) evaluated passive monitoring using *Hypogymnia physodes* in the Swiss Midlands. This method was compared to the Calibrated Lichen Indication Method (which uses an index called IAP18, Chapter III, Section 2.3) as a qualitative and quantitative measure of individual air pollutants. Pb, Fe, Cu, Cr, total sulphur (S), Zn and phosphorus (P) concentrations in lichens showed good correlations with IAP18 values. Good correlations between Pb and Cu deposition gradients and Pb and Cu accumulation in *Hypogymnia physodes* were observed. The investigators recommended the combined use of the Calibrated Lichen Indication Method and passive biomonitoring method as an integrated biological air pollution monitoring system in Switzerland. By concentrating on human-toxic trace-elements measurements of risk to human health could be assessed. Both methods complement each other and can be applied to spatial and temporal monitoring on larger scales.

Analyses of indigenous lichen species, *Parmelia praesorediosum* and *Ramalina stenospora*, in south-west Louisiana demonstrated spatial and temporal changes in airborne heavy metal levels and distribution throughout the area (Thompson *et al.*, 1987 and Walther *et al.*, 1990a). The first survey took place in 1983/84 (Thompson *et al.*, 1987) and the second in 1987/88 (Walther *et al.*, 1990a). Contour maps, graphs and three-dimensional plots representing mean concentrations over the eighteen stations indicated higher element concentrations near the metropolitan and industrial areas than in the background sites. The results also demonstrated dramatic decreases in metal levels and distribution over time, which were attributed to reductions in industrial activity from 1982 to 1988. Discriminant analysis obtained statistical differences in element concentrations in lichens between stations up to 10.4 km from source (group 1) and stations beyond this distance (group 2). The authors showed a straight-line relationship for Fe and Zn concentrations with the distance from industrial corridor and concluded that their similar slopes imply a common origin and similar rate of washout for these elements (Thompson *et al.*, 1987). Enrichment factors for each element were investigated by dividing the mean concentrations of group 1 stations by the mean concentration of the background stations.

Residence times for Al, Fe, Hg and Zn were calculated according to the equations:

$$C_t = C_0 e^{-\lambda t}$$

where: C_t = concentration at time t

C_0 = original concentration

λ = loss rate constant

$$T_R = \ln 2/\lambda$$

T_R = Residence time assuming loss rate from lichens was dependent upon total concentration in the lichen and not upon time.

Residence times were postulated to be independent of background values and dependent on original concentrations. The mean life residence times for Al, Fe, Hg and Zn were 1.7, 2.0, 2.3 and 3.5, respectively, which correspond to mean residence times of two to five years proposed by Nieboer and Richardson (1981). This suggests that lichens are expected to show a decrease in elemental content a year or two after exposure to an emission source ceases.

2.5 National park surveys

Increasing research has been conducted into the effects of air pollution on national parks in the US. This probably relates to concern regarding the threat of air pollution from nearby cities. Furthermore, such relatively unpolluted areas represent background sites and provide good baseline data. The application of lichens as bioaccumulators of heavy metals in this type of monitoring is illustrated below.

Observations of *Hypogymnia physodes* and *Evernia mesomorpha* at eighteen localities in the Isle Royale National Park, Michigan between 1983 and 1992 demonstrated the temporal and geographical pattern of elements in the area (Bennett, 1995). Results of sixteen elemental analyses were compared to the sequence of element concentrations in the earth's crust. Zn, Pb, Se and sulphur (common air pollutants) for example ranked higher in lichen tissue than they did in the earth's crust. Heavy metal elements associated with atmospheric deposition increased over the nine-year study period to a greater extent than non-metallic elements. Geographic distributions of anthropogenic elements were elevated around known local pollution sources and some elements (e.g. sulphur) reached toxic levels in lichens. The author recommended this method, i.e. employing more than one species and analysing elemental data in aggregate rather than individually, as an appropriate indication of early stages of air pollution within less polluted areas.

Frenzel_*et al.* (1990) observed higher burdens of As, Cd, Cu and Zn in *Alectoria sarmentosa* in Mount Rainier National Park, Washington, than in a control site (Olympic National Park). Industrial sources near to Mount Rainier National Park were thought to be the cause. In contrast, higher levels of Pb were obtained in Olympic National Park, which were attributed to dispersed regional and global sources.

Comparisons of lichen tissue metal levels in the aforementioned national park studies are presented in Table 2.5. Despite slight differences in methodologies and different lichen species, Isle Royale National Park showed consistently higher tissue element levels than the other two parks.

2.6 Urban and industrial surveys

Urban and industrial studies generally entail spatial biomonitoring such as distribution/fall-out patterns and correlations with distance from source.

Table 2.5 Comparison between three national parks in US in terms of mean lichen element concentrations (ppm) from all sampling sites.

Ele-ment	Mt. Rainier	Olympic	Isle Royale	
	Alectoria sarmentosa	*Alectoria sarmentosa*	*Hypogymnia physodes*	*Evernia mesomorpha*
As	0.43 (0.39-0.47)	0.26 (0.22-0.29)	3.96	-
Cu	0.99 (0.96-1.03)	0.73 (0.69-0.76)	7.91 [5.19-10.63]	4.20 [2.65-6.25]
Cd	0.03 (0.03-0.03)	0.01 (0.01-0.02)	0.65 [0.27-1.04]	0.45 [0.27-0.64]
Pb	6.75 (6.35-7.18)	7.70 (7.31-8.11)	29.7 [19.5-40.0]	10.5 [5.3-15.7]
Zn	14.99 (14.5-15.5)	10.2 (9.7-10.6)	89.1 [82.3-101.6]	32.9 [29.1-35.2]

(value) denotes range of values from sample points
[value] denotes range of mean values from three years of study

2.6.1 Urban sources

The application of lichen biomonitoring in three urban surveys in different continents is compared in Table 2.6. Species utilised, number of sampling sites, type of analyses and thalli metal concentrations are expressed. Details of each study are provided in the text where the increasing importance of statistical analyses in such studies is apparent.

A distinct difference in metal concentrations in lichen tissue between industrial and urban zones was evident in the Baton Rouge area, Louisiana (Walther *et al.,* 1990b). Levels decreased with increasing distance from the city. Concentration ranges (μg g^{-1}) are shown in Table 2.6. Discriminant analysis indicated insignificant differences in metal levels between the two lichen species, and average values of the two species were used to construct three dimensional and contour plots of metal concentrations in the area. Industrial activity and urban traffic were responsible for the observed heavy metal trends.

Levels of atmospheric Cd, Cr, Cu, Hg, Ni, Pb and Zn around Pistoia in central northern Italy were assessed using the widely distributed indigenous lichen *Parmelia caperata* (Loppi *et al.,* 1994). Concentration ranges (μg g^{-1}) are presented in Table 2.6. Distribution maps and cluster analysis indicated similar distribution patterns and fall-out patterns for Cd, Pb and Zn, with maximum values observed in the centre of Pistoia. Cd was a by-product of lead and zinc smelting industry in the area. Fertiliser and pesticide use in plant nurseries explained correlation between Zn and Cd south east of town.

Table 2.6 Comparisons of lichen biomonitoring studies in three urban areas from three continents - **A**. Baton Rouge, Louisiana (Walther *et al.,* 1990b), **B.** Pistoia, Italy (Loppi *et al.,* 1994) and **C**. Kampala, Uganda (Nyangababo, 1987).

Parameter	**Study areas**		
	A	**B**	**C**
Lichen species	*Parmelia praesorediosum* *Ramalina stenospora*	*Parmelia caperata*	*Calyrneferes usambaricum*
No. of sampling locations	11	30	10
Sample collection	Oak tree - collection of all sides, differing heights	Oak tree >80cm diameter, collection at height 1.5-2.0 m	Not specified
Chemical analyses	Atomic absorption spectrometry	Atomic absorption spectrometry	Atomic absorption spectrometry
Data analysis	Univariate (ANOVA) Multivariate (discriminant analysis) Contour maps/three dimensional plots	Univariate (correlations, coefficients of variation) Multivariate (cluster analysis) Distribution maps using SURFER programme	Univariate (correlations) Synthetic Pollution Indices
Mean metal concentrations (μg g^{-1})			
Al	120-7237		
Cd		0.24-0.95	2.31-4.94**
Cr		0.94-4.07	
Cu	3.0-53	5.40-15.30	
Fe	170-10105		8360-13400**
Hg		0.05-0.18	
Ni		1.19-4.59	71.30-79.6**
Pb	10.0-342	5.50-24.80	148-246**
Zn	56-421	43.0-134.7	

** - range represents the mean concentration in rural area in comparison with mean concentration in urban area

Correlations, cluster analysis and distribution maps demonstrated an association between Cr and Ni, which was attributed to metal-plating industries in the southern part of the study area. The authors used coefficients of variation for each element (Garty and Ammann, 1985) as a relative measure of dispersion of particles. Cu showed a low coefficient of variation implying it was dispersed relatively consistently as small particles over the study area. Overall, the analysis indicated that most metal levels were modest in comparison to heavily polluted areas. Fertilisers and pesticides, which produced the relatively high zinc concentrations, posed the biggest pollution threat in the area.

The degree of heavy metal contamination by heavy industry and motor vehicles in Kampala, Uganda was indicated by metal bioaccumulation in the lichen *Calyrneferes usambaricum* (Nyangababo, 1987). Heavy metal concentrations in lichen tissue were higher in the rural area than in the urban area (Table 2.6). Pb levels were higher than those obtained two metres from a highway in northern Nigeria (Kapu *et al.*, 1991), suggesting a heavy metal burden from traffic within the study area. The author applied Grodzinska's (1978) synthetic pollution indices for mosses to the lichen data. This used standardised metal values divided by the number of elements to calculate a pollution index at each site. The author used these values to classify the sites into clean and heavily contaminated, which corresponded to the metal concentration gradient. However, it is debatable whether these methods would identify intermediately polluted sites as distinctly.

The species mentioned in Table 2.6 appear to be adequate biomonitors of heavy metal pollution patterns. The extent of data analysis is important. Multivariate analyses are useful in 'grouping' sites and metals, making conclusions easier. The surveys varied in their choice of metals for analysis, which ultimately depends on knowledge of the local area and pollution sources.

2.6.2 Line point sources

Kapu *et al.* (1991) used the bioaccumulative properties of *Parmelia* sp. to assess the aerial fallout of heavy metals from traffic in Zaria, northern Nigeria. Metal concentrations in epiphytic lichens, with the exception of Fe, decreased significantly with distance from Zaria/Samaru-Sokoto Highway but showed no significant difference along the residential road in the Samara Campus, Ahmadu Bello University, Zaria (Table 2.7). The former area was therefore suffering from aerial heavy metal dispersion and contamination. Such studies are vital in developing countries where heavy metals pose a serious health risk.

Table 2.7 Mean concentrations ($\mu g\ g^{-1}$) of heavy metals in lichens along **A.** Zaria/Samaru-Sokoto Highway and **B.** residential road in the Samara Campus, Ahmadu Bello University, Zaria.

Element	**Study area**									
	A (distance from highway (m))					**B (distance from road (m))**				
	2	*10*	*30*	*45*	*60*	*2*	*10*	*30*	*60*	*90*
Cr	50.0	11.0	5.0	5.0	1.6	3.3	5.0	5.3	5.3	5.0
Cu	8.5	11.0	2.9	2.3	1.6	6.0	5.3	4.8	6.6	6.3
Fe	308	294	307	310	296	307	316	318	311	315
Pb	115	88.8	8.4	6.9	2.5	3.0	6.3	3.8	2.5	1.3
Zn	9.0	6.0	5.0	5.3	4.6	4.6	6.3	5.4	4.5	4.9

2.6.3 Industrial point sources

(i) Species distribution

Lichen distribution studies in relation to metals are rare in comparison to their use in gaseous air monitoring. St. Clair *et al.* (1995) compared lichen communities in two sections of Anaconda-Pintler wilderness area, Montana. Only 21 species, of which two were regarded as sensitive, were observed in the sites located in the area between a defunct copper smelter and the wilderness boundary. In comparison, 161 species and 17 indicator species were found in the sites across the rest of the wilderness area. Wind blown dust and contaminated soil containing Ni, As, Cu and Pb from the smelter were thought to be responsible.

(ii) Bioaccumulation

Nash and Gries (1995) summarised various studies utilising lichens as bioaccumulators around point sources in arctic/boreal localities. All examples showed significantly lower lichen concentrations in sites removed from the industrial point source.

In a study of trace-element concentrations in *Parmelia caperata* and *Parmelia rudecta* in the vicinity of a coal-fired power plant near Washington D.C. no significant differences in concentrations were apparent in lichens at distances ranging from 1.6 to 20 km from the plant (Olmez *et al.*, 1985). Various reasons for this apparent anomaly were proposed, e.g. tall stacks, and preference of lichens to collect large airborne particles of similar composition to their crustal material.

In their comparison of the suitability of a variety of plant species as bioindicators of steel works in coniferous forests in Raahe, Finland, Mukherjee and Nuorteva (1994) found that Grodzinska's (1978) pollution index system could be applied using the lichen *Hypogymnia physodes*. The pollution index of site j (S_j) was calculated according to the following equation:

$$S_j = \sum_{1}^{7} K_{ij}$$

$$\text{where: } K_{ij} = \frac{X_{ij} - X_i}{X_i}$$

K_{ij} = content of i toxic metals in site j.

X_i = mean of i toxic metals in all sites.

Calculated indices were correlated with forest condition and lichen quality. For example, at a distance of one km from the steel works, a pollution index of 4.39 corresponded to extremely serious forest damage and lichen poor areas. In contrast, at a distance of 10 km an index of -0.63 was found, in accordance with a 'healthy forest' and lichens 'slightly affected by air pollution'.

The pattern of air pollution in the vicinity of an aluminium smelter in Yugoslavia was investigated using epiphytic and lithophytic lichens (*Hypogymnia caperata*, *Diploicia canescens and Lecanora expallens*) (Jovanovic *et al.*, 1995). Epiphytic lichens were absent from the immediate zone around the smelter. Correlations existed between the elemental composition in the lichens and air pollutants associated with the works. Metal concentrations in lichen tissue decreased with increasing distance from the smelter. On comparison with other biological indicators (grasses and pine needles), lichens proved more effective in terms of bioaccumulative characteristics. This work provided good baseline data for future biomonitoring programmes in this area.

Relatively low levels of trace-metals were found in *Parmelia caperata* samples around the Travale-Radicondoli geothermal area in Italy (Loppi and Bargagli, 1996). High correlations of many elements with Al, a common indicator of crustal material, implied soil dust was a source of these metals. B and Hg, common geothermal pollutants, were correlated to distance from geothermal sources. B concentrations ranged from 5.1 to 22.1 µg g^{-1} and Hg from 0.062 to 0.555 µg g^{-1}. The other major contaminant associated with geothermal pollution, As, appeared to be derived from power plant sources and neighbouring thermal springs.

In Idaho, inverse relationships between Cd, Cr, Zn and P concentrations in *Rhizoplaca melanophthalma* and distance from phosphate refineries were obtained (Dillman, 1996). More intensive monitoring of this semi-arid area was recommended.

The epiphytic lichen *Parmotrema madagascariaceum* was used to assess atmospheric trace-element contamination to Venezuelan cloud forests (Gordon *et al.*, 1995). Increasing Pb from leaded gasoline was proposed as the greatest risk to the forests due to the lack of abatement procedures such as those adopted in temperate regions.

Several studies have concentrated on the specific biomonitoring of aerial mercury. These are discussed below.

In an abandoned mining area around Mount Amiata, Italy, mercury levels in the indigenous lichen *Parmelia sulcata* were used to assess mercury emissions to enable subsequent environmental reinstatement of the area (Bargagli, 1990b; Bargagli *et al.*, 1989 and Ferrara *et al.*, 1988). Mercury concentrations in lichen tissue within the study area ranged from 0.31 $\mu g\ g^{-1}$ to 7.80 $\mu g\ g^{-1}$. Mercury concentrations in the lichen had a highly significant exponential relationship ($p < 0.001$) with distance from one of the plants and a very highly significant relationship with mercury content in surface soil ($p < 0.0005$). The latter suggested that lichens accumulated Hg from degassing of nearby soils. Aluminium assays (indicator of crustal derived material) implied that soil particles are trapped by *P. sulcata* but did not show a correlation with mercury. Lichen, soil and air sampling concluded that the main sources of gaseous mercury were large piles of roasted cinnabar, geothermal power plant emissions and ventilation systems of mineshafts. *P. sulcata* was recommended for further monitoring in the area. Other metals did not show similar patterns to Hg. Zn levels in the lichen were possibly a result of long-range atmospheric transport. Fe and Mn levels were attributed to accumulation of soil particles.

Dispersion of Hg from volcanic eruptions in Hawaii was investigated by measuring concentrations of the element in the lichen *Stereocaulon vulcani* (Davies and Notcutt, 1996). Mercury concentrations ranged from < 8 $\mu g\ g^{-1}$ to 59 $\mu g\ g^{-1}$ with most values below background atmospheric levels for the island. Elevated mercury levels (twice background levels) were associated with two local irregular sites. This data proved more beneficial in assessing dispersion and sources of Hg than previous physico-chemical air monitoring.

2.7 Transplants

Transplantation exercises are rarely undertaken on a large scale and are mainly focused around point source emissions. Examples of their application in various countries are illustrated below.

Vestergarrd *et al.* (1986) compared transplantion data from 1977 and 1982 to assess the effect of changing from oil-fired open-hearth furnaces to electric-arc furnaces in a Danish steel factory. *Hypogymnia physodes* samples removed from *Pinus* sp. 7.6 km from the works were exposed for seven months at a height of 1.5 m on wooden stands at twelve transplant sites in the vicinity of the works in 1977 and in 1982. Regression analysis was used on the data.

Metal concentration was related to distance from source by following equation:

$y = ax^b + c$ where
- y = concentrations in lichen ($\mu g\ g^{-1}$)
- x = distance from pollution source (m)
- c = background concentration ($\mu g\ g^{-1}$) i.e. before transplantation
- a,b = constants

Metal concentration in lichens and bulk precipitation were related by following equation:

$y = ax^b$ where
- y = concentrations in lichen ($\mu g\ g^{-1}$)
- x = concentrations in fallout ($\mu g\ g^{-1}$)
- a,b = constants

Lichens showed a decrease in metal content with increasing distance from source. Comparison of regression lines showed that Cr, Cu and Pb had decreased significantly between survey years 1977 and 1982. Relationships between lichen and bulk precipitation data showed that metal uptake of lichen was not proportional to the concentrations measured in bulk precipitation although direct proportionality was observed when each station was considered individually. The difference in relative uptake in lichens between 1977 and 1982 was attributed to changes in the particle size distribution of the emission.

In Israel, Garty and Hagemeyer (1988) carried out a similar impact assessment study by comparing metal content in lichens transplanted within the vicinity of a coal-fired power plant before (1979/80) and after (1983/84) initiation of operation of the plant. Twigs containing *Ramalina duriaei* were suspended from a variety of local trees at ten sampling locations, ranging from four to thirty km from the plant. In comparison to the previous example, this survey covered a much greater area and spanned urban, agricultural and rural territories. Furthermore, slightly different statistical analyses were employed. Duncans' multiple range tests compared metal concentrations in lichen thalli between sampling locations in each study period. Correlations and two-way analysis of variance (ANOVA) showed that Cr levels in the region had increased, regional Cu and Zn concentrations had decreased and regional Ni remained the same. However, some local elevations in metal concentrations in lichens were apparent (e.g. Ni levels increased from 7.7 $\mu g\ g^{-1}$ to 33.6 $\mu g\ g^{-1}$ at a local nature reserve site, 9.4 km away from the plant). Reduction in Cu and Zn levels were attributed to changes in agricultural practises in the region.

Potential improvement in air quality in Indiana Dunes National Lakeshore Park was examined by transplanting *Hypogymnia physodes* (Bennett *et al.*, 1996). This species was formerly present in the park and its impoverishment has been attributed to air pollution. The lichens, attached to small branches, were positioned on artificial trees at four sampling locations across 333 km north to south. Tissue concentrations of 20 elements were analysed every year for three years. ANOVA and Tukey's Single-Degree-of-Freedom Test for Non-Additivity showed that the transplanting process itself did not effect lichens. Most elements showed elevated concentrations in lichens at Indiana Dunes compared to control sites and some elements increased significantly over the three-year study period. Increased mortality between the second and third year was attributed to the deleterious effects of significant increases in a number of elements, synergism of Zn, Cd and K and exceedance of maximum concentrations of some elements. Although transplant density was not intensive, the study was able to illustrate general trends in the area.

Biological parameters other than element bioaccumulation in thalli have been performed to assess heavy metal contamination in transplants. In Israel, inverse relationships were obtained between Pb and Cu content and ATP concentration in transplanted lichen thalli. Additionally, the degradation of chlorophyll-a to phaeophytin-a, expressed as a ratio, was inversely correlated to levels of Cu, Pb and Zn in lichen (Garty *et al.*, 1988).

3 Fungi

3.1 Introduction

Burton (1986) found that the use of macrofungi in monitoring atmospheric deposition was rare, however a few metal bioaccumulation studies using macrofungi in relation to urban and industrial sources had been reported. Burton (1986) also concluded that the use of microfungi for monitoring contamination was limited due to the time consuming processes involved.

Very few accounts of monitoring of heavy metal deposition using fungi were found in the current review. However, interest in their use in monitoring gaseous pollutants appears to have increased (Chapter III, Section 3).

3.2 Bioaccumulation

Kovács (1992a) dedicated a chapter to fungi as environmental indicators. This is summarised in the following paragraphs.

The following fungal groups have been used in heavy metal accumulation studies, the most effective listed first:

- organic matter (compost) decomposers (e.g. *Agaricus arvenis, Lycoperdon giganteum*)
- mycorrhiza forming fungi (e.g. *Amanita rubescens, Boletus edulis*)
- wood decomposers (e.g. *Pleurotus ostreatus, Polyporus betulinus*).

In a study in a forest in Hungary, species from the above three groups were examined in terms of their elemental composition. Wood decomposers contained lower concentrations than representatives from the other groups.

Certain heavy metals are 'excluded' by fungi and are absorbed only in small quantities, depending on the species. A study was carried out in an area exposed to iron containing spoil banks in the north-eastern-central mountains, Hungary. Various heavy metal concentrations were examined in spoil samples, *Coprinus comatus* stems and *Coprinus comatus* caps. A selection of the results is presented in Table 2.8.

Table 2.8 The chemical composition of *Coprinus comatus* (μg g^{-1} dry weight) (from Kovács 1992a)

Element	Red spoil	*Coprinus comatus* stems	*Coprinus comatus* caps
Cd	16.1	0.3	0.8
Fe	92962	1646	918.1
Ni	87.7	1.4	1.4
Pb	48.1	0.0	0.0

Results showed that only iron was present in high concentrations in the spoil and in the fungi. Cadmium, nickel and lead were present in high concentrations in the spoil but were in very low to non-detectable concentrations in the fungi.

Although many fungi species may be suitable for heavy metal indication, certain indicator species should be chosen for comparability between regions. Knowledge of the species requirements, accumulating abilities and habitat is desirable and its distribution should be widespread.

In an intensive study of total mercury content in vegetation in Ontario, Hg levels were higher in mushrooms than in the other plant types studied (i.e. mosses, lichens and tree species). Mushrooms accumulated 144.39 ng g^{-1} Hg in comparison to 37.03 ng g^{-1} in lichens and 75.32 ng g^{-1} in pleurocarpous mosses.

3.3 Species distribution

Little published literature exists with regard to fungi distribution patterns in response to aerial metal contamination. However, in Finland, Helander (1993) reported on the responses of pine needle endophytes to air pollutants, Ni, Cu and sulphuric acid emitted from an industrial complex at Harjavalta. The number of pine needles infected with endophytes and also the number of needles infected specifically with *Cenangium ferrucinosum* were significantly higher at increasing distances from the factories.

4 Higher plants

4.1 Introduction

Higher plants have appeal as indicators in air pollution monitoring in highly polluted areas where lichens and mosses are often absent. Higher plants act as biomonitors in the assessment of aerial heavy metal contamination by means of their bioaccumulative properties. Therefore, mainly analytical approaches are used in monitoring of metals.

Metal aerosols pollute soil and plants. Higher plants not only intercept pollutants from atmospheric deposition but also accumulate aerial metals from the soil. Aerial heavy metal deposit are taken up from the soil by plants via their root system and translocated to other regions of the plant.

Particle deposition on leaf surfaces may be affected by a variety of factors, including particle size and mass, wind velocity, leaf orientation, size, moisture level and surface characteristics (Bache *et al.*, 1991). The deposited particles may be washed by rain into the soil, resuspended or retained on plant foliage. The degree of retention is influenced by weather conditions, nature of pollutant, plant surface characteristics and particle size (Harrison and Chirgawi, 1989). Harrison and Chirgawi (1989) demonstrated experimentally the significance of foliar accumulation and translocation of air derived metal pollutants. The foliar route was found to be of similar importance to the soil-root pathway.

Heavy metal absorption is governed by soil characteristics such as pH and organic matter content (Csintalan and Tuba 1992; Jones 1991). Thus, high levels of heavy metals in the soil do not always indicate similar high concentrations in plants. The extent of accumulation and toxic level will depend on the plant and heavy metal species under observation. In an investigation of Cd, Cu, Ni and Pb uptake from air and soil by *Achillea millefolium* (milfoil) and *Hordeum vulgare* (barley) in Denmark, Pilegaard and Johnsen (1984) concluded that Cu and Pb plant concentrations correlated with aerial deposition but not with soil concentrations. In contrast, Ni and Cd content in the plants correlated with deposition and soil content. The distribution patterns and budgets of heavy metals within forest trees growing at contaminated sites in Germany were investigated by Truby (1995).

The interpretation of analytical data is therefore complicated by many factors. However, metal accumulation in plants can reflect the relative extent of the burden and its dispersal.

Plants also demonstrate morphological and physiological responses to heavy metal pollution, some of which may be utilised in bioindication.

4.2 Monitoring design

4.2.1 Species selection

For convenience, species selection can be separated into two groups: herbs/grasses and trees/shrubs. Sensitive species are more appropriate where the measurement of plant effects and responses are to be used as bioindication of air pollutants. Accumulative bioindicators tend to be more tolerant to metal loading.

4.2.1.1 Herbs and grasses

Kovács (1992b) recommended the use of ruderal plants as bioaccumulative indicators due to their ability to accumulate metals in high quantities without visible injury. Ruderals are also widespread plants enabling comparison between regions. Pilegaard and Johnsen (1984) chose to study metal uptake by milfoil (*Achillea millefoilium*) because of its large surface area.

Some plant species may be more efficient in retaining atmospheric metal particles than others. A measure of this efficiency can be resolved by calculating air accumulation factors (AAF) according to the following equation:

$$\text{AAF } (\text{m}^3\text{g}^{-1}) = \text{PAc } (\mu\text{g g}^{-1} \text{ dry weight})/\text{CA } (\mu\text{g m}^{-3})$$

Where: PAc = atmospheric contribution of the metal in plants

CA = concentration of the metal in the atmosphere.

In their study of tropical plants growing in an industrial area of India, Rao and Dubey (1992) discovered that the degree of accumulation differed substantially between the five species under study. Bache *et al.* (1991), in their study of metal concentrations in grasses in relation to a municipal refuse incinerator, suggested that the nature and area of the leaf surface would affect foliar deposition. The same authors recommended that this is grounds for the use of consistent sampling of the same plant species at similar times of year and same life cycle stage during plant biomonitoring surveys.

4.2.1.2 Trees and shrubs

Both coniferous and deciduous trees can be used in the detection of aerial heavy metal pollution. Coniferous trees indicate pollution over a longer time period. Growth rings may reflect annual variations in metal concentrations in the surrounding environment.

Broad-leaved tree species regarded as sensitive to metal contamination include *Betula pendula, Fraxinus excelsior, Sorbus aucuparia, Tilia cordata* and *Malus domestica* (Kovács, 1992c). Numerous bioaccumulative indicators exist. Some examples include *Ailanthus glandulosa, Celtis occidentalis, Salix alba, Tilia tomentosa, Sambucus nigra, Quercus robur* and *Fagus silvatica* (Kovács, 1992c). *Populus nigra* sp. *Italica* (Italian poplar) has been recommended as a particularly suitable bioindicator of heavy metal burden in Europe (Kovács, 1992c). Amongst many of its appropriate features, this species is genetically homogeneous, easily identifiable and ubiquitously distributed. *Robinia pseudoacacia* (black locust tree) was recommended as a suitable bioindicator of heavy metal contamination in Hungary (Kovács, 1992c).

The leaves of *Rosa rugosa* have been reported as effective detectors of rare elements (Kovács, 1992b).

Coniferous trees are often regarded as better temporal bioindicators of environmental contamination, as their wood type reduces the lateral transfer of contaminants between rings (Zayed *et al.*, 1991)

As with herbs and grasses, tree leaf surfaces may govern the extent of accumulation of particles. In Greece, Sawidis *et al.* (1995) studied a selection of tree species as biomonitors of Zn and Cu. The investigators discovered that the strongest metal accumulators possessed rougher surfaced leaves which gave rise to the effective trapping and retention of particles.

4.2.2 Site selection

The size of the area under study will generally determine the spatial distribution of sampling sites. The depth of the study will determine the density of sampling locations. Site selection will also depend on the type of sampler. Site selection criteria should be consistent throughout a survey.

In the Netherlands tree-bark samples were collected from locations arranged along six straight line transects across the country (Kuik and Wolterbeek, 1994). This can be achieved relatively easily for tree sampling because tree/forest locations are readily identified.

In larger scale surveys, for example on the national scale, location of sites near point sources of air pollution is avoided. For example, in a national study in Poland, Dmuchowski and Bytnerowicz (1995) chose sampling sites at least two km away from direct emission sources and at least 300 m from highways.

4.2.3 Sampling period

For comparative studies it is important that sampling is undertaken at the same time of the year to reduce variability. Chemical composition of foliage varies with season and rainfall (Taylor *et al.*, 1990). This is important when sampling annuals and deciduous trees.

Standard sampling of heavy metal accumulation in *Populus nigra* in central Europe is carried out in August. For most deciduous species this is the time of year when metal content in leaves will be highest. Sawidis *et al.* (1995) found higher mean Cu and Zn concentrations in autumn compared to spring in the foliage of a variety of tree species. Only the evergreen species, *Ligustrum japonicum,* which possesses a two year leaf ageing process, showed insignificant seasonal differences in Cu and Zn content.

4.2.4 Sample collection

Throughout a monitoring programme, sample collection should be standardised. The same plant parts from consistent plant heights from the same species of similar height should be utilised.

4.2.4.1 Plant parts

Metal content will vary depending on which part of the plant is sampled. For example, in herbaceous plants, roots and leaves retain higher metal concentrations than stems and fruits (Kovács, 1992b; Csintalan and Tuba, 1992). The extent of accumulation in different plant parts will vary with species and the nature of the element. Chemical composition varies not only with the age of the plant itself but also with the age of the leaf/needle. Second year needles of balsam and spruce contained significantly higher Hg levels than first year needles during an intensive sampling study in Ontario, Canada (Rasmussen *et al.,* 1991).

In trees, metal concentrations in needles/leaves have been recorded which are three times that in twig tissue of the same branch (Rasmussen *et al.,* 1991).

In coniferous trees two-year-old pine needles are commonly analysed.

Tree bark is appropriate in indicating longer term air pollution. Bark is exposed to air pollutants either directly from the atmosphere or from stemflow. The changes in the chemical composition of the surface layers can be documented. Kuik and Wolterbeek (1994) proposed the use of tree bark samples as biomonitors of heavy metal pollution in the Netherlands. Their use was recommended for larger scale surveys because of their greater availability compared to lichens and mosses. The collection of a large number of samples is more beneficial for the analysis of data by factor analysis (Kuik and Wolterbeek, 1994). The same authors suggested that field sampling procedures of tree bark were simpler and less time-consuming than those practised for lichens and mosses. Bark flakes of about five mm thickness at 1.5 m above ground level were cut. Bark sampling also does not damage the tree (Poikolainen, 1997).

Zayed *et al.* (1991) used an incremental corer to remove xylem samples from black spruce for Al analysis. By dividing wood samples into two-year sections possible temporal changes may be determined. However, in a study of distribution patterns of heavy metals in forest trees on contaminated sites in

Germany, Truby (1995) found no relationship between the radial distribution in the tree rings and the historical heavy metal deposition in the area. The author therefore recommended that xylem rings should not be utilised in the determination of air pollution history at forest sites.

4.2.4.2 Collection procedures

Kovács (1992c) reported on standard sampling methodology for *Populus nigra* accumulation studies in central Europe. Eight branches are removed at height 5.5 m of solitary unshaded trees. Three one-year shoots are cut from each branch and one leaf is removed from each of these. This results in a total of 24 leaves for analysis of heavy metal content. Similar standard procedures are conducted on *Robina pseudoacacia* in Hungary.

Lin *et al.* (1995) used twenty one-year old shoots from branches about five to six m above ground for analysis of metal concentration in balsam fir foliage in Quebec.

4.2.5 Analysis

Soil often contains higher metal concentrations than exposed parts of herbaceous plants and thus root analysis is frequently recommended during contamination assessment exercises (Kovács, 1992).

Replication is vital since pollutant concentration can vary even within species (Taylor *et al.,* 1990).

An assessment of within-site variation in metal content in the study has been recommended by some authors (Rasmussen *et al.,* 1991; Lin *et al.,* 1995). In southern Quebec, Lin *et al.* (1995) tested variability in the elemental concentration in balsam fir needles between individual trees at the same site. Coefficients of variation of < 50% for all elements were regarded as acceptable.

Great variation exists between sample preparation in terms of washing procedures. Priority attention to this issue has been given to leaf samples in particular. Analysis of washed leave samples provides elemental concentration in leaf tissue. Alternatively, elemental content of unwashed leaves will reflect leaf surface and leaf tissue content.

Analytical procedures are similar to those applied to moss and lichen samples, e.g. atomic absorption spectrometry (AAS), neutron activation analysis (NAA) and inductively coupled plasma mass spectrophotometers (ICP-MS). Standard quality control procedures are regularly applied to these laboratory techniques. For example Lin *et al.* (1995) used standard reference material, NBS-SRM 1575 (pine needle) during NAA measurements of metal burden in forests.

4.3 National surveys

The following paragraphs discuss three national surveys constituting very different sampler types and approaches. Studies are concerned with sources and patterns of pollution rather than actual metal concentrations.

In the Netherlands, analysis of metal content in tree-bark samples indicated trends in heavy metal concentrations in the country (Kuik and Wolterbeek, 1994). Application of factor analysis supported evidence regarding pollution sources highlighted in previous lichen surveys (Sloof and Wolterbeek, 1991). A criticism of bark sampling in aerial heavy metal deposition monitoring is in the lack of distinction between contributions from soil and airborne sources. In the Netherlands study, factor analysis established metal contribution from soil. Generally, elemental concentrations in bark were lower than those observed in lichens in the 1986/87 survey. For example, Ni mean concentration in bark was 11.0 ppm, which represented a bark/lichen ratio of 0.68. However, some elements displayed higher concentration in bark than lichen samples. For example, Cd showed a mean bark content of 3.1 ppm and a bark/lichen ratio of 1.07. Until fairly recently, the use of tree bark in biomonitoring had been restricted to smaller scale urban and industrial areas. This Dutch study demonstrated the potential of this biomonitor on a larger scale.

A national survey of trace-metal content in the leaves and roots of *Taraxacum officinale* (dandelion) in Poland demonstrated the value of this plant as a bioindicator of airborne contamination (Kabata-Pendias and Dudka, 1991). Performance of Analysis of Variance, Tukey's test of significance and multiple regression on the data revealed distribution patterns in dandelion metal content throughout the country. In general, trace-metal concentrations in leaves and roots were higher in the industrialised south-western part of Poland than in the rural north-east. Calculation of the ratios of metal concentration in leaves and roots in the whole country, the south-west and north-east provided an indication of which metals originated from airborne pollution. All ratios where greater than 1 and statistically significantlly higher element concentrations were observed in dandelion leaves compared to root samples. Ratios increased from north-east to south-west for Cd, Pb and Zn, implying a significant aerial input of these metals as opposed to root uptake from soil and translocation to leaves.

A different approach was used in a later Polish survey where analysis of Scots pine needles were used to characterise metal contamination in the country as a whole and in the city of Warsaw (Dmuchowski and Bytnerowicz, 1995). Three zones of Cu pollution were mapped throughout the country, four zones were apparent for Pb, five geographical zones of pollution were established for Zn and Cd and six zones represented As pollution. Zones are summarised in Table 2.9. This survey did not utilise statistical techniques but the use of digital mapping and database production proved an effective alternative in evaluating possible threats to humans and the environment.

Table 2.9 Zones of heavy metal pollution in Poland - mean metal levels (ppm) and percentage of Poland represented by that zone.

Zone	Zn		Cd		Pb		Cu		As	
	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%
I	<70	62.0	<0.5	91.2	<10	94.1	<5	99	<0.3	50.7
II	71-100	29.6	0.5-1.0	4.8	10-20	5.1	5.1-10		0.3-1.0	42.8
III	101-130	4.2	1.01-2.5	2.9	20-30		>10	1	1.1-2.0	4.5
IV	130-250	3.6	2.51-5.0	1.1	>30	0.2			2.1-3.0	1.5
V	>250	0.6	>5	0.3					3.1-4	0.45
VI									>4.1	0.1

An array of vegetative types were used to establish regional and temporal changes in atmospheric deposition patterns of Zn, Cu, Pb and Cd in Norway over the decade 1982 to 1992 (Berthelsen, 1995). The study concentrated on forest and ombrotrophic bog sites located in southern and central Norway. Temporal and spatial variations were determined by the calculation of ratios between metal concentrations in 1992 and 1982, supplemented by t-test analysis. All element levels in plants were elevated in southern Norway in comparison to the central region of the country.

Ratios of approximately 1 and the lack of significant t-tests suggested little change in Zn, Cd and Cu levels in plants over the study period. However, this did not reflect the decrease in deposition of these metals in southern Norway between 1982 and 1992. This was explained by enhanced root uptake of these metals from long-term contaminated soils subjected to heavier air pollution in the south. In contrast, Pb levels in plants strongly reflected both decreased atmospheric Pb deposition from 1982 to 1992 and increased deposition in southern Norway. Therefore changes in Pb concentration were reflected much more quickly than changes in concentrations of other atmospheric contaminants.

This study demonstrated the aptness of even the simplest statistical analyses in drawing conclusions on a national scale. Only the exposed plant parts were analysed in the study and it might have proved advantageous to also analyse roots to support inferences regarding the role of roots in element uptake.

4.4 Regional surveys

Most regional surveys are associated with forest ecosystems over large areas. Tree bark and foliage are commonly used as bioindicators of heavy metal contamination in forest studies.

Poikolainen (1997) used bark samples from Scots pine collected along seven transects in northern Finland (Lapland) to illustrate heavy metal distribution trends in the area. Heavy metal concentrations were reasonably low throughout the region, and high Cu, Ni and Cr concentrations were only associated with emissions from the Kola Peninsula and industry in south-west Lapland.

Huhn *et al.* (1995) analysed bark samples from 60-year-old Scots pines to evaluate heavy metal deposition in forest ecosystems in central Germany in comparison to background forest sites. Correlations and factor analyses revealed four metal groups. Fe, Ni, Cr, Cu and Pb were emitted as industrial dust and fly ash and accumulated well in bark. Zn and Cd were associated together and Mn and Hg formed the other two groups. The authors proposed the methodology as a diagnostic tool in monitoring air pollution damage to forests.

Metal analysis of balsam fir foliage demonstrated that trace-metal damage to forests in southern Quebec was not severe (Lin *et al.*, 1995). Trace-element content varied with forest location and elevation.

4.5 Urban surveys

By analysing Pb and Cd levels in rose-bay leaves (*Nerium oleander*), Hernandez *et al.* (1987) characterised the city of Madrid, Spain into four different areas of contamination. Levels of Pb ranged from 13.63 ppm in the lowest contamination zone to 74.25 ppm in the very high contamination zone.

A selection of tree species were sampled from 12 sites throughout Thessaloniki city, Greece, to assess their value as biomonitors of heavy metal pollution (Sawidis *et al.*, 1995). Trees nearer the city centre contained higher metal levels than trees 15 km away. Cu showed a narrow mean concentration range from 5 to 10 mg kg^{-1} and showed little variation between tree species. Pb content ranged from < 1.5 to 4.5 mg $kg^{-1}$and was highest in *Populus alba* (white poplar) and *Populus niger* (black poplar). Poplar leaves also contained the highest Zn concentrations. Overall Zn displayed mean concentrations from 19 to 85 mg kg^{-1}.

Gradients of pollution of Cu, Pb and Fe were demonstrated in Naples, Italy, by analysing metal content in leaf surfaces and tissue of *Quercus ilex* (Holly oak) (Alfani *et al.,* 1996). Metal concentrations were significantly higher in leaves from roadside sites than in leaves collected from town squares, which in turn were significantly higher than concentrations measured in urban park trees. Positive correlations between Pb, Cu and Fe concentrations in leaf tissue and leaf surface demonstrated the significance of deposition to leaf tissue content. This supports the theory that aerial deposition to leaves is an important source of metal contamination in leaves. Further evidence was the lack of correlation between metal content in leaves and in soil. Soil samples possessed a higher metal burden than leaf samples. The authors postulated that soil was an appropriate indicator of long-term metal deposition but its utility is limited in the assessment of metals which are highly mobile or major components of soil.

The epiphytic monocot of the genus *Tillandsia* has been used as an effective biomonitor of trace-metal pollution in Latin America. *Tillandsia* species are common throughout Latin America and are similar to lichens and mosses in that their roots do not play an absorptive role. Instead, *Tillandsia* absorbs water and nutrients directly from the air by means of trichomes present on the leaf surface. *Tillandsia caput-medusae* was analysed for Cu, Pb and Cd bi-monthly in San José city, Costa Rica to provide an overview of the atmospheric heavy metal burden in the city (Brighigna *et al.*, 1997). Metal concentrations were higher in the urban sampling area and external leaves were more exposed to air than internal ones. Highest metal concentrations corresponded to the dry season. The extremely high Pb content in leaves in comparison to the other two metals confirmed that vehicular traffic was the most important source of air pollution within the city.

4.6 Line point surveys

Pb accumulation in leaves is a direct reflection of its deposition level. It is a non-essential plant element and deposition and accumulation on plant leaves is its primary route of uptake. Therefore analysis is not complicated by the root uptake and translocation processes apparent for other metals. Furthermore, analysis for Pb is made easier by the fact that concentrations are often unaffected by washing procedures. For example, Hernandez *et al.* (1987) discovered that the amount of Pb measured in samples of rose-bay leaves showed no difference when the samples were washed or unwashed prior to analyses.

In Madrid, Spain, Hernandez *et al.* (1987) found significant positive correlation between lead levels in rose-bay leaves and traffic density at sites. However, Sawidis *et al.* (1995) in Thessaloniki reported that Pb levels were not proportional to traffic density, and higher levels of Pb were associated with sites near road junctions.

Other specific studies have been undertaken directly in relation to highways and their effects on plants. Albasel and Cottenie (1985) found that Cu, Zn and Pb concentrations in plants decreased with increasing distance from major highways in Belgium. Concentrations of Pb were particularly pronounced in comparison to control plants in rural areas. In Finland, Ylaranta (1995) found that the lead concentration of wheat, Italian rye grass and lettuce was 1.5 to 3 times higher 22 m from the roads under study than in plants 200 m from the roads.

4.7 Industrial surveys

Several studies have been undertaken in relation to heavy metal burdens and zones of influence with respect to industrial point sources. Trees and forests, particularly in association with forest decline, appear to be examined more frequently than other higher plants. Sampling stations are generally situated along transects at increasing distances from the point source in known wind directions.

In a study of heavy metal burden in air, soil and plants around a zinc smelter in India, Agrawal *et al.* (1988) did not observe statistically significant correlations between heavy metal content in the air, soil and plants. Local topography and microclimate of the study area also played a role in the dispersion of heavy metals. However, in general the zone of influence of the smelter extended to seven km. The leaves of the four plant species analysed (*Mangifera* spp., *Acacia* spp., *Triticum* spp. and *Brassica* spp.) showed great variation in heavy metal concentration depending on species, metal type and sampling site.

A variety of bioindicators were used to determine heavy metal fallout from a steel works in northern Finland along two sampling transects (Mukherjee and Nuorteva, 1994). Although the authors concluded that *Hypogymnia physodes* and *Pleurozium schreberi* were more appropriate bioindicators for most of the selected metals, the May-lily (*Maianthemum bifolium*) was a highly effective bioaccumulator of Cd from the iron and steel works. Other bioindicators under investigation included birch (*Betula pubescens*), Scots pine *(Pinus sylvestris*), Norway spruce (*Picea abies*) and *Vaccinium* sp. These showed generally higher Al, Fe and Zn concentrations in sampling areas within close vicinity to the steel works in comparison to sampling sites at a distance of > 6 km.

In another Finnish study, copper showed a pollution gradient along Scots pine (*Pinus sylvestris*) forest stands growing 0.5, 4 and 8 km from a copper-nickel smelter in Harjavalta (Helmisaari *et al.*, 1995). Mean copper concentrations in one-year old pine needles in 1992 were 8.74, 20.04 and 210.53 mg/kg at distances of 8, 4 and 0.5 km from the smelter respectively. Analysis of *Pinus sylvestris* fine root samples implied that root uptake from the soil was the primary route of copper. Copper accumulation was significantly higher in the roots than in the leaves and copper concentrations in the leaves elevated significantly when the roots appeared to become saturated. This study demonstrated the significance of long-term heavy metal accumulation in the soil and its effects on soil processes and vegetation. The same smelter was investigated by Koricheva and Haukioja (1995) who discovered an exponential decrease in Cu, Ni, Fe and Zn concentrations in the leaves of *Betula* sp. with distance from the factory.

In terms of point sources of air pollution and vegetation, less work has focused on incinerators than on other industrial emission sources. Elemental analysis of grasses in the vicinity of a municipal refuse incinerator in the United States found that concentrations of Cd, Fe, Hg, Mo, Pb and Zn were highest within 100 m of the incinerator (Bache *et al.*, 1991). Of these metal species, all but Hg showed inverse relationships with logarithmic distance downwind. Hg showed a linear relationship with distance downwind. However, no soil or root analysis was undertaken during the survey, which makes it difficult to distinguish between the contribution of metals from root uptake and from direct aerial deposition. This study is of significance in that incinerators are often located in rural areas where contaminated vegetation may be used for pasture or edible crops.

The primary source of Hg in the aerial parts of a plant is, like Pb, generally thought to be via aerial deposition and not via translocation and root uptake (Zhang *et al.*, 1995; Bache *et al.*, 1991). Plant parts accumulate Hg from interception of wet and dry precipitation and absorption of gaseous Hg.

4.8 Response methods

It has been observed that aerial heavy metal pollution has biochemical/ physiological effects on plants. Effects on growth have also been detected in response to air pollution. Many parameters can be affected by heavy metals. Toxic heavy metals may effect germination, young or old trees, stem growth, leaf formation, root growth, flowering/fruiting, plant growth rate and biomass, photosynthesis, transpiration, mineral nutrition and secondary metabolism etc. (Breckle and Kahle, 1992; Csintalan and Tuba, 1992). Some of these responses may be useful in the bioindication of aerial heavy metal contamination where elemental analysis proves too expensive or time consuming.

Most response techniques used in bioindication are in relation to gaseous pollutants but some emphasis has been placed on physiological responses with regard to metal pollutants. Most studies are in relation to industrial sites where a combination of air pollutants may be emitted. It is often difficult to determine if single aerial pollutants such as sulphur dioxide (SO_2) or a combination of pollutants including metals are more important in producing observed deleterious effects on plants. Effects may be additive, synergistic or antagonistic.

Examples of the limited number of studies which include plant responses to aerial metal depositions in the field are discussed briefly below. Most of these are controlled field study or *in vitro* experiments whereby seeds are sown in known contaminated areas or artificially enhanced contaminated locations. It is assumed that high levels of heavy metals in the soil medium will produce similar effects as atmospheric heavy metal pollution. Frequently such culture experiments are primarily aimed at determining threshold toxicity of pollutants to plants.

In the industrial area of Shoubra Elkheima near Cairo, heavy metal contamination of soil and vegetation has occurred. In a study of the area, seeds of clover (*Trifolium pratense*) and Egyptian mallow (*Malva parviflora*) were sown in pots which were placed in nine fields at each of three sampling locations at increasing distances from the industrial pollution sources (Ali, 1993). A selection of injurious responses were observed in plants at the two locations within the vicinity of the industrial area in comparison to the control station. These include: a reduction in chlorophyll content; an increase in the number of plants with visible damage and in the area of injured leaves; a reduction in leaf number; a reduction in plant growth and weight. Much of this visible injury may be attributed to SO_2, NO_x and ozone pollution. However, Cd and Pb were present in the vegetative parts of the crops in elevated quantities and may be partly responsible for the observed effects. The lack of specificity of the aforementioned responses limits their utility as diagnostic tests of heavy metal contamination. According to Turcsanyi (1992), changes in chloroplast number and volume represent typical plant damage due to salts of heavy metals. The same author describes other cellular effects exerted by the salts of heavy metals.

In Slovakia, Kodrik (1994) measured root biomass and length in Norway spruce (*Picea abies*) at four sites of varying emission regimes. Although destructive, this method is appropriate as a measure of ecosystem health.

The combined effects of acidic and trace-elements on *in vitro* pollen germination and tube growth in a variety of higher plant species was investigated by Cox (1988). This acts as a potential bioindication method of the effect of air pollutants on reproduction processes.

Breckle and Kahle (1992) reported on a range of growth responses, mineral uptake and transpiration rates of beech seedlings to increasing exposure of Cd and Pb in isolation and in combination in the soil medium. Dose-response levels were comparable to metal concentration levels in German forests. Direct uptake of heavy metals through the leaf after deposition is an important route, especially for Pb, although this investigation was primarily concerned with effects of Pb and Cd on trees after root uptake. However, the measurable effects discussed in this paper, such as root elongation and architecture and leaf development, may still be appropriate in biomonitoring.

Toxic symptoms in response to Ni exposure to roots were displayed by cultured tomato plants under controlled experimental conditions in a study undertaken by Prokipcak and Ormrod (1986). The leaflets nearest the main stem appeared to be the most sensitive to Ni treatment. In a study of the response of a selection of crop plants transplanted to the vicinity of a copper smelter in Poland, older leaves were affected first (Fabiszewski *et al*., 1987). Typical Ni-injury in tomato plants was visible as interveinal chlorosis followed by necrosis distinguishable as 'small beige patches of dead tissue surrounded by darker brown or purple pigmented tissue'. It is noteworthy that chlorosis is often not specific and can vary with the time of year (Saxe, 1996). Decreases in growth response parameters such as leaf, stem and root weight, leaf area and plant height were also observed with increased Ni exposure to the roots.

4.9 Transplants

Transplantation exercises are not as common using higher plants as they are utilising lichens and mosses. However, a limited demonstration of their use as transplants is provided below.

Pilegaard and Johnsen (1984) used transplantation to study the extent of heavy metal uptake by *Achillea millefolium* (milfoil) and *Hordeum vulgare* (barley) through their leaves and roots. Plants were grown in pots and exposed at areas of different aerial heavy metal deposition for 75 days prior to analysis.

Horse bean (*Vicia faba minor*), blue lupine (*Lupinus angustifolius*), oat (*Avena sativa*) and red fescue (*Festuca rubra*) were grown in a greenhouse prior to transplantation 800 m from a copper smelter for two weeks. Acute chlorosis associated with a reduction in chlorophyll-a and chlorophyll-b in leaves was significantly different from the control plants remaining in the greenhouse. Red fescue was the most sensitive species under investigation.

5 Conclusions

Plants have the ability to indicate the presence of elements and their concentration gradients. Mosses and lichens are particularly effective biomonitors of aerial heavy metal contamination because of their bioaccumulative properties. These plant groups are amenable to biomonitoring because they are widespread, easy to handle and they lack a cuticle and root system thus reflecting directly aerial heavy metal deposition. Most surveys use passive monitoring and active monitoring is used infrequently. Analysis of indigenous mosses are currently used in international and national monitoring programmes, particularly in Europe. Techniques are applied as a practical tool in establishing and characterising deposition sources. Such long-term, larger scale monitoring is extremely useful and also enables transboundary ameliorative action to be taken. Both lichens and moss techniques have been applied to measure heavy metal levels and trends within and around urban and industrial areas. These studies can analyse temporal and/or spatial trends in heavy metal deposition and results are generally expressed as pollution gradients. Within the gradient, metal levels in the local moss populations are seen to decrease with increasing distance from the suspected source.

The use of fungi in the monitoring of heavy metal pollution is limited but some fungal groups are better bioaccumulators than others.

Higher plants have appeal as indicators in air pollution monitoring in highly polluted areas where lichens and mosses are often absent. They are primarily used as bioaccumulators. The use of higher plants in the assessment of aerial heavy metal contamination is hampered by their inherent ability to absorb metals from the soil. Interpretation of results should therefore be handled with caution. However, metal accumulation in plants does reflect the relative extent of the pollution burden and its dispersal. Analysis of leaves and needles are common techniques but tree bark has also been used in heavy metal biomonitoring. It is often useful to include analysis of plant roots as a means of assessing pollution contributions from soil.

The use of physiological and biochemical parameters in the bioindication of heavy metal contamination is not routinely practised. Some responses of higher plants to heavy metal contamination have potential. Very few examples of using species distribution and mapping methods in the assessment of heavy metal pollution exist.

It is important during metal biomonitoring programmes that background concentrations are established. The design of a monitoring programme will involve the selection of appropriate species, sampling locations, sample collection, sampling frequency, metals to be analysed, chemical technique and data analysis.

6 References

Agrawal, K.M., Sharma, H.C. and Aggarwal A.L. 1988 Heavy metal pollution of air, soil and plants around a zinc smelter: A case study. *Indian Journal of Environmental Health*, **30**, 3, 234-241.

Albasel, N. and Cottenie, A. 1985 Heavy metal contamination near major highways, industrial and urban areas in Belgian grassland. *Water, Air and Soil Pollution*, **24**, 1, 103-110.

Alfani, A., Batroli, G., Rutigliano, F.A., Maisto, G. and Virzo De Santo, A. 1996 Trace metal biomonitoring in the soil and the leaves of *Quercus Ilex* in the urban area of Naples. *Biological Trace Element Research*, **51**, 1, 117-131.

Ali, E.A. 1993 Damage to plants due to industrial-pollution and their use as bioindicators in Egypt. *Environmental Pollution*, **81**, 3, 251-255.

Bache, C.A., Gutenmann, W.H., Rutzke, M., Chu, G., Elfving, D.C. and Lisk, D.J. 1991 Concentrations of metals in grasses in the vicinity of a municipal refuse incinerator. *Archives of Environmental Contamination and Toxicology*, **20**, 4, 538-542.

Bargagli, R., Barghigiani, C., Siegel, B.Z and Siegel, S.M. 1989 Accumulation of mercury and other metals by the lichen, *Parmelia sulcata*, at an Italian minesite and a volcanic area. *Water, Air and Soil Pollution*, **45**, 3-4, 315.

Bargagli, R. 1990a Assessment of metal air pollution by epiphytic lichens: the incidence of crustal materials and possible uptake from substrate barks. *Studia Geobotanica*, **10,** 97-103.

Bargagli, R. 1990b Mercury emission in an abandoned mining area: Assessment by epiphytic lichens. *Encyclopedia of Environmental Control Technology: Hazard Waste Containment and Treatment*, **4**, 613.

Barghigiani, C., Ristori, T. and Bauloeo, R. 1991 *Pinus* as an atmospheric mercury biomonitor. *Environmental Technology*, **12**, 12, 1175-1182.

Bennett, J.P. 1995 Abnormal chemical-element concentrations in lichens of Isle-Royale National-Park. *Environmental and Experimental Botany*, **35**, 3, 259-277.

Bennett, J.P., Dibben M.J. and Lyman, K.J. 1996 Element concentrations in the lichen *Hypogymnia Physodes* (L.) Nyl. after three years of transplanting along Lake Michigan. *Environmental and Experimental Botany*, **36**, 3, 255.

Berg, T., Pedersen, U. and Steinnes, E. 1996 Environmental indicators for long-range atmospheric transported heavy-metals based on national moss surveys. *Environmental Monitoring and Assessment*, **43**, 1, 11-17.

Berg, T., Royset, O., Steinnes, E. and Vadset, M. 1995 Atmospheric trace-element deposition - Principal component analysis of ICP-MS data from moss samples. *Environmental Pollution*, **88**, 1, 67-77.

Bernes, C. (ed.) 1990 Environmental monitoring in Sweden. Monitor 1990. Swedish Environmental Protection Agency Informs, Solna.

Berthelsen, B.O., Steinnes, E., Solberg, W. and Jingsen, L. 1995 Heavy-metal concentrations in plants in relation to atmospheric heavy-metal deposition. *Journal of Environmental Quality*, **24**, 5, 1018-1026.

Breckle, S.W. and Kahle, H. 1992 Effects of toxic heavy metals cadmium and lead on growth and mineral nutrition of beech, *Fagus sylvatica* L. *Vegetatio*, **101**, 1, 43-53.

Brighigna, L., Ravanelli, M., Minelli, A. and Ercoli, L. 1997 The use of an epiphyte (*Tillandsia caput-medusae* morren) a bioindicator of air pollution in Costa Rica. *Science of the Total Environment*, **198**, 2, 175-180.

Brumelis, G. and Nikodemus, O. 1995 Biological monitoring in Latvia using moss and soil: Problems in the partitioning of anthropogenic and natural effects. In: *Bioindicators of environmental health*, Manawar, M., Hanninen, O., Roy, S., Munawar, N., Kärenlampi, L. and Brown, D.H. (eds.), Ecovision World Monograph Series, SPB Academic Publishing, Amsterdam.

Burton, M.A.S. 1986 Biological monitoring of environmental contaminants (plants). MARC Report Number 32. Monitoring and Assessment Research Centre, King's College London, University of London.

Cox, R. M. 1988 The sensitivity of pollen from various coniferous and broad-leaved trees to combinations of acidity and trace metals. *New Phytologist* **109**, 193-201.

Csintalan, Z. and Tuba, Z. 1992 The effect of pollution on the physiological processes in plants. In: *Biological indicators in environmental protection*, Kovács, M. (ed.), Ellis Horwood, New York.

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

Dillman, K.L. 1996 Use of the lichen *Rhizoplaca melanophthalma* as a biomonitor in relation to phosphate refineries near Pocatello, Idaho. *Environmental Pollution*, **92**, 1, 91-96.

Dmuchowski, W. and Bytnerowicz, A. 1995 Monitoring environmental-pollution in Poland by chemical-analysis of Scots Pine (*Pinus-Sylvestris* L.) needles. *Environmental Pollution*, **87**, 1, 87-104.

Evans, C.A. and Hutchinson, T.C. 1996 Mercury accumulation in transplanted moss and lichens at high-elevation sites in Quebec. *Water, Air and Soil Pollution*, **90**, 3-4, 475-488.

Fabiszewski, J., Brej, T. and Bielecki, K. 1987 Plant reactions as indicators of air pollution in the vicinity of a copper smelter. *Acta Soc Bot Pol,* **56**, 2, 353-363.

Ferrara, R., Maserti, B.E. and Bargagli, R. 1988 Mercury in the atmosphere and in lichens in a region affected by a geochemical anomaly. *Environmental Technology (Letters*), **9**, 7, 689.

Frenzel, R.W., Witmer, G.W. and Starkey, E.E. 1990 Heavy metal concentrations in a lichen of Mount Rainier and Olympic National Parks, Washington, USA. *Bulletin of Environmental Contamination and Toxicology,* **44**, 1, 158.

Frontasyeva, M.V., Nazarov, V.M. and Steinnes, E. 1994 Moss as a monitor of heavy-metal deposition - Comparison of different multi-element analytical techniques. *Journal of Radioanalytical and Nuclear Chemistry-Articles,* **181**, 2, 363-371.

Gailey, F.A.Y. and Lloyd, O.L. 1993 Spatial and temporal patterns of airborne metal pollution: The value of low technology sampling to an environmental epidemiology study. *Science of the Total Environment,* **133**, 3, 201.

Garty, J. and Ammann, K. 1985 The amounts of Ni, Cr, Zn, Pb, Cu, Fe and Mn in some lichens growing in Switzerland. *Environmental and Experimental Botany*, **27**, 127-138.

Garty, J. and Hagemeyer, J. 1988 Heavy metals in the lichen *Ramalina duriaei* transplanted at biomonitoring stations in the region of a coal-fired power plant in Israel after three years of operation. *Water, Air and Soil Pollution,* **38**, 3-4, 311-324.

Garty, J., Kardish, N., Hagemeyer, J. and Ronen, R. 1988 Correlations between the concentration of ATP chlorophyll degradation and the amounts of airborne heavy metals and sulphur in a transplanted lichen. *Archives of Environmental Contamination and Toxicology,* **17**, 5, 601-612.

Godzik, B. and Grodzinska, K. 1991 Heavy metals in mosses of Mazurian Landscape Park Poland. *Ochr,* **49**, (1), 81-86.

Gordon, C.A., Herrera, R. and Hutchinson, T.C. 1995 The use of a common epiphytic lichen as a bioindicator of atmospheric inputs to two Venezuelan cloud forests. *Journal of Tropical Ecology,* **11**, 1-26.

Grodzinska, K. 1978 Mosses as bioindicators of heavy metal pollution in polish National Parks. *Water, air and soil pollution*, **9**, 83-97.

Gupta, A. 1995 Heavy metal accumulation by three species of mosses in Shillong, north-eastern India. *Water, Air and Soil Pollution*, **82**, 3-4, 751-756.

Harrison, R.M. and Chirgawi, M.B. 1989 The assessment of air and soil as contributors of some trace metals to vegetable plants. I. Use of a filtered air growth cabinet. *Science of the Total Environment*, **83**, 1-2, 13-34.

Helander, M.L. 1993 Responses of pine needle endophytes to air-pollution. *New Phytologist* **131**, 2, 223-229.

Helmisaari, H.S., Derome, V.J., Fritze, H., Nieminen, T., Palmgren, K., Salemaa, M. and Vanha-Majamaa, I. 1995 Copper in Scots pine forests around a heavy-metal smelter in south-western Finland. *Water, Air and Soil Pollution,* **85**, 3, 1727.

Hernandez, L.M., Rico, C., Gonzalez, J. and Hernan, A. 1987 Environmental contamination by lead and cadmium in plants from an urban area of Madrid, Spain. *Bulletin of Environmental Contamination and Toxicology,* **38**, 2, 203-208.

Herzig, R., Liebendorfer, L., Urech, M., Ammann, K., Cuecheva, M. and Landolt, W. 1989 Passive biomonitoring with lichens as a part of an integrated biological measuring system for monitoring air pollution in Switzerland. *International Journal of Environmental and Analytical Chemistry,* **35**, 43.

Huhn, G., Schulz, H., Staerk, H.J., Toelle, R. and Scheuermann, G. 1995 Evaluation of regional heavy metal deposition by multivariate analysis of element contents in pine tree barks. *Water, Air and Soil Pollution,* **84**, 3-4, 367-383.

Jones, K.C. 1991 Contamination trends in soils and crops. *Environmental Pollution,* **69**, 4, 311-326.

Jovanovic, S., Carrot, F., Deschamps, C., Deschamps, N. and Vukotic, P. 1995 A study of the air-pollution in the surroundings of an aluminum smelter, using epiphytic and lithophytic lichens. *Journal of Trace and Microprobe Techniques,* **13**, 4, 463-471.

Kabata-Pendias, A. and Dudka, S. 1991 Trace metal contents of Taraxacum officinale dandelion as a convenient environmental indicator. *Environmental and Geochemical Health,* **13**, 2, 108-113.

Kakulu, S.E. 1993 Biological monitoring of atmospheric trace metal deposition in north-eastern Nigeria. *Environmental Monitoring and Assessment,* **28**, 2, 137.

Kansanen, P.H. and Venetvaara, J. 1991 Biological collectors of airborne heavy metals near ferrochrome and steel works. *Water, Air and Soil Pollution*,

60,
3-4, 337-360.

Kapu, M.M., Ipaye, M.M., Ega, R.A.I., Akanya, H.O., Balarabe, M.L. and Schaeffer,D.J. 1991 Lichens as bioindicators of aerial fallout of heavy metals in Zaria, Nigeria. *Bulletin of Environmental Contamination and Toxicology,* **47**, 3, 413-416.

Kirchhoff, M., and Rudolph, H. 1989 A sandwich technique for the continuous monitoring of air pollutants with the bryophyte sphagnum. *J hattori bot lab,* **67**, 423-431.

Kodrik, M. 1994 Distribution of root biomass and length in *Picea abies* ecosystem under different emission regimes. *Plant and Soil*, **167**, 1, 173-179.

Koricheva, J. and Haukioja, E. 1995 Variations in chemical composition of birch foliage under air pollution stress and their consequences for Eriocrania miners. *Environmental Pollution,* **88**, 1, 41-50.

Kovács, M. (ed.) 1992a Biological indicators in environmental protection. Ellis Horwood, New York.

Kovács, M. 1992b Herbaceous (flowering) plants. In: *Biological indicators in environmental protection,* Kovács, M. (ed.), Ellis Horwood, New York.

Kovács, M. 1992c Trees as biological indicators. In: *Biological indicators in environmental protection*. Kovács, M. (ed.), Ellis Horwood, New York.

Král, R., Kryzova, L. and Liska, J. 1989 Background concentrations of lead and cadmium in the lichen, *Hypogymnia physodes*, at different altitudes. *Science of the Total Environment,* **84**, 1, 201.

Kuik, P., Sloof, J. E. and Wolterbeek; H.T.H. 1993 Application of Monte Carlo-assisted factor analysis to large sets of environmental pollution data *Atmospheric Environment*. **27A**, 13, p1975-1983

Kuik, P. and Wolterbeek, H.T.H. 1994 Factor-analysis of trace-element data from tree-bark samples in the Netherlands. *Environmental Monitoring and Assessment,* **32**, 3, 207-226.

Kuik, P. and Wolterbeek, H.T. 1995 Factor analysis of atmospheric trace-element deposition data in the Netherlands obtained by moss monitoring. *Water, Air and Soil Pollution,* **84**, 3-4, 323-346.

Lin, Z.Q., Schuepp, P.H., Schemenauer, R.S. and Kennedy, G.G. 1995 Trace metal contamination in and on balsam fir (*Abies balsamea* (L) Mill.) foliage in southern Quebec, Canada. *Water, Air and Soil Pollution,* **81**, 1-2, 175-191.

Loppi, S., Chiti, F., Corsini, A. and Bernardi, L. 1994 Lichen biomonitoring of trace metals in the Pistoia area (central northern Italy). *Environmental Monitoring and Assessment,* **29**, 1, 17.

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

Markert, B. and Weckert, V. 1989 Use of *Polytrichum formosum* (moss) as a passive biomonitor for heavy metal pollution (cadmium, copper, lead and zinc). *Science of the Total Environment,* **86**, 3, 289.

Mukherjee, A.B. and Nuorteva, P. 1994 Toxic metals in forest biota around the steel works of Rautaruukki Oy, Raahe, Finland. *Science of the Total Environment,* **151**, 3, 191.

Nash, T.H. & Gries, C. 1995 The use of lichens in atmospheric deposition studies with an emphasis on the arctic. *Science Of The Total Environment,* **161** 729-736.

Nieboer, E. and Richardson, D.H.S. 1981 Lichens as Monitors of atmospheric deposition In: *Atmospheric pollutants in natural waters*, Eisenreich (ed.), Ann Arbor Science Publ., p339.

Nyangababo, J.T. 1987 Lichens as monitors of aerial heavy metal pollutants in and around Kampala. *Bulletin of Environmental Contamination and Toxicology,* **38**, 1, 91.

Olmez, I., Gulovali, M.C. and Gordon, G.E. 1985 Trace element concentrations in lichens near a coal-fired power plant. *Atmospheric Environment,* **19**, 10, 1663.

Pilegaard, K. 1993 Biological monitoring of particulate pollutants during exploration work at a niobium mineralization in Greenland. *Environmental Monitoring and Assessment,* **27**, 3, 221.

Pilegaard, K. and Johnsen, I. 1984 Heavy metal uptake from air and soil by transplanted plants of *Achillea millefolium* and *Hordeum vulgare.* Ramussen, L. (ed.), *Ecological Bulletins* (NFR) **36**. (Ecotoxicology: 3rd Oikos conference), 97-102.

Poikolainen, J. 1997 Sulphur and heavy metal concentrations in Scots pine bark in northern Finland and the Kola Peninsula. *Water, Air and Soil Pollution,* **93**, 395-408.

Pott, U. and Turpin, D.H. 1996 Changes in atmospheric trace element deposition in the Fraser Valley, B.C., Canada from 1960 to 1993 measured by moss monitoring with *Isothecium stoloniferum*. *Canadian Journal of Botany,* **74**, 8, 1345-1353.

Prokipcak, B. and Ormrod, D.P. 1986. Visible injury and growth responses of tomato and soybean to combinations of nickel, copper and ozone. *Water, Air and Soil Pollution,* **27**, 3-4, 329-340.

Puckett, K.J. 1988 Bryophytes and lichens as monitors of metal deposition. In: *Lichens, bryophytes and air quality*, Nash, T.H. and Wirth, V. (eds.) J.Cramer, Berlin.

Quevauviller, P., Herzig, R. and Muntau, H. 1996 Certified reference material of lichen (Crm-482) for the quality control of trace-element biomonitoring. *Science of the Total Environment*, **187**, 2, 143-152.

Rao, M.V. and Dubey, P.S. 1992 Occurrence of heavy metals in air and their accumulation by tropical plants growing around industrial areas. *Science of the Total Environment,* **126**, 1-2, 1-16.

Rasmussen, L., Pilegaard, K. and Ruhling, A. 1988 Atmospheric heavy metal deposition in northern Europe measured by moss analysis. In: *Air pollution and ecosystems*, Mathy, P. (ed.), Proceedings of an international symposium held in Grenoble, France, 1987. D. Reidel Publishing.

Rasmussen, P.E., Mierle, G. and Nriagu, J.O. 1991 The analysis of vegetation for total mercury. *Water, Air and Soil Pollution,* **56**, 379.

Richardson, D.H.S. 1988 Understanding the pollution sensitivity of lichens. *Botanical Journal of the Linnean Society,* **96**, 31-43.

Richardson, D.H.S. 1991 Lichens as biological indicators - recent developments. In: *Bioindicators and environmental management.* Jeffrey, J.W. and Madden, B., Academic Press Ltd., London.

Ruhling, A. 1995 Atmospheric heavy metal deposition in Europe estimated by moss analysis. In: *Bioindicators of environmental health*, Manawar, M., Hanninen, O., Roy, S., Munawar, N., Kärenlampi, L. and Brown, D.H. (eds.), Ecovision World Monograph Series, SPB Academic Publishing, Amsterdam.

Sawidis, T., Marnasidis, A., Zachariadis, G. and Stratis, J. 1995 A study of air-pollution with heavy-metals in Thessaloniki City (Greece) using trees as biological indicators. *Archives of Environmental Contamination and Toxicology,* **28**, 1, 118-124.

Saxe, H. 1996 Physiological and biochemical tools in diagnosis of forest decline and air pollution injury to plants. In: *Plant responses to air pollution*, Yumus, M. and Igbal, M. (eds.), John Wiley and Sons Ltd.

Schaug, J., Rambaek, J.P., Steinnes, E. and Henry, R.C. 1990 Multivariate analysis of trace element data from moss samples used to monitor atmospheric deposition. *Atmospheric Environment*, **24A**, 10, 262.

Seaward, M.R.D. 1995 Use and abuse of heavy metal bioassays in environmental monitoring. *Science of the Total Environment,* **176**, 129-134.

Sloof, J.E. 1995a Pattern recognition in lichens for source apportionment. *Atmospheric Environment,* **29**, 3, 333.

Sloof, J.E. 1995b Lichens as quantitative biomonitors for atmospheric trace-element deposition, using transplants. *Atmospheric Environment,* **29**, 1, 11-20.

Sloof, J.E. and Wolterbeek, H.T. 1991 Patterns in trace elements in lichens. *Water, Air and Soil Pollution,* **57-58**, 785.

Sloof, J.E. and Wolterbeek, B.T. 1993 Interspecies comparison of lichens as biomonitors of trace-element air-pollution. *Environmental Monitoring and Assessment,* **25**, 2, 149-157.

St. Clair, L.L., Newberry, C.C. and Hatch, A.S. 1995 Residual effects of a defunct copper smelter on lichen communities in the Anaconda-Pintler Wilderness Area, Montana. *American Journal of Botany,* **82**, 6 (suppl.), 6.

Steinnes, E. and Andersson, E.M. 1991 Atmospheric deposition of mercury in Norway: Temporal and spatial trends. *Water, Air and Soil Pollution,* **56**, 391-404.

Steinnes, E, Rambaek, J.P. and Hanssen, J.E. 1992 Large scale multi-element survey of atmospheric deposition using naturally growing moss as a biomonitor. *Chemosphere*, **25,** 735-752.

Steinnes, E., Johansen, O., Royset, O. and Odegard, M. 1993 Comparison of different multi-element techniques for analysis of mosses used as biomonitors. *Environmental Monitoring and Assessment,* **25**, 2, 87-97.

Steinnes, E., Hanssen, J.E., Rambaek, J.P. and Vogt, N.B. 1994 Atmospheric deposition of trace elements in Norway: Temporal and spatial trends studied by moss analysis. *Water, Air and Soil Pollution,* **74**, 1-2, 121-140.

Taylor, H.J., Ashmore, M.R. and Bell, J.N.B. 1990 Air pollution injury to vegetation. IEHO, London.

Thompson, R.L., Ramelow, G.J., Beck, J.N., Langley, M.P., Young, J.C. and Casserly, D.M. 1987 A study of airborne metals in Calcasieu Parish, Louisiana, using the lichens, *Parmelia praesorediosa* and *Ramalina stenospora*. *Water, Air and Soil Pollution,* **36**, 3-4, 295.

Truby, P. 1995 Distribution patterns of heavy metals in forest trees on contaminated sites in Germany. *Angewandte Botanik,* **69**, 3-4, 135-139.

Turcsanyi, G. 1992 Plant cells and tissues as indicators of environmental pollution. In: *Biological indicators in environmental protection*, Kovács, M. (ed.) Ellis Horwood, New York.

Tyler, G. 1989 Uptake, retention, and toxicity of heavy metals in lichens: a brief review. *Water, Air and Soil Pollution*, **47**, 321-333.

Tyler, G., Balsberg Pahlsson, A.M., Bengtsson, G., Baath. E. and Tranvik, L. 1989 Heavy-metal ecology of terrestrial plants, microorganisms and invertebrates. *Water, Air and Soil Pollution,* **47**, 3-4, 189.

UN ECE Convention on Long-Range Transboundary Air Pollution 1993 *Manual for integrated monitoring, programme phase 1993-1996*. Environmental Data Centre, National board of Waters and the Environment, Helsinki.

Vestergarrd, N.K., Stephansen, U. Rasmussen, L. and Pilegaard, K. 1986 Airborne heavy metal pollution in the environment of a Danish steel plant. *Water, Air and Soil Pollution,* **27**, 3-4, 363.

Walther, D.A., Ramelow, G.J., Beck, J.N., Young, J.C., Callahan, J.D. and Marcon, M.F. 1990a Temporal changes in metal levels of the lichens *Parmotrema praesorediosum* and *Ramalina stenospora*, Southwest Louisiana. *Water, Air and Soil Pollution,* **53**, 1-2, 189-200.

Walther, D.A., Ramelow, G.J., Beck, J.N. Young, J.C., Callahan, J.D. and Marcon, M.F. 1990b Distribution of airborne heavy metals as measured in the lichens *Ramalina stenospora* and *Parmotrema praesorediosum* in Baton Rouge, Louisiana. *Water, Air and Soil Pollution,* **50**, 3-4, 279-292.

Winner, W.E. 1988 Responses of bryophytes to air pollution. *Bibliotheca Lichenologica* **30**, 141-173. Wolterbeek, H.T. and Bode, P. 1995 Strategies in sampling and sample handling in the context of large-scale plant biomonitoring surveys of trace-element air-pollution. *Science of the Total Environment,* **176**, 1-3, 33-43.

Wolterbeek, H.T., Bode, P. and Verburg, T.G. 1996 Assessing the quality of biomonitoring via signal-to-noise ratio analysis. *Science of the Total Environment,* **180**, 2, 107-116.

Ylaranta, T. 1995 Effect of road traffic on heavy metal concentrations of plants. *Agricultural Science in Finland,* **4**, 1, 35-48.

Zhang, L., Planas, D. and Qian, J.L. 1995 Mercury concentrations in black spruce (*Picea mariana* Mill. B.S.P.) and lichens in boreal Quebec, Canada. *Water, Air and Soil Pollution* **81**, 1-2, 153**.**

Zayed, J., Andre, P. and Kennedy, G. 1991 Variation of aluminium levels in black spruce (*Picea mariana*). *Water, Air and Soil Pollution,* **55**, 3-4, 337.

III GASEOUS POLLUTANTS

1 Bryophytes

1.1 Introduction

Bryophytes are generally easier to identify and are as equally susceptible to air pollution as lichens, yet less attention has been paid to their use in gaseous air pollution monitoring. A reason for this may be the larger number of lichen species (particularly epiphytic species) available for air pollution monitoring (Adams and Preston, 1992). Most studies are associated with regional and urban sulphur dioxide (SO_2) contamination. On a national and multi-national monitoring scale, bryophytes are used more as bioaccumulative indicators of aerial metal contamination than as bioindicators of gaseous air pollution.

Most literature regarding gaseous pollutants and bryophytes is concerned with measuring and assessing impacts of pollution on bryophyte communities as a valuable ecological group in their own right. Hallingback and Tan (1996) discussed the IUCN/IAB Bryophyte Committees' commitment to endangered bryophytes and presented a skeleton action plan for these species. The conservation and protection of these 'key-stone' species was emphasised and air pollution was listed as one of the major threats to species. Biomonitoring of air pollution impacts on bryophytes therefore plays a role in biodiversity and conservation. Most of the published literature on research with regard to bryophytes and gaseous air pollutants concentrates on pollutant effects and harm and little is available in terms of their role as biomonitors and bioindicators. However, such work may still be used to ascertain pollution problems and indicate potential threats to other biological systems including humans.

Standard practices such as sampling, analysis and species selection in bryophyte monitoring are underdeveloped in comparison to lichen monitoring.

1.2 Sulphur dioxide (SO_2)

1.2.1 Species distribution

1.2.1.1 Regional

Adams and Preston (1992) presented comprehensive evidence of long-term effects of gaseous pollutants, particularly SO_2, on bryophyte distribution in the UK. The methods, patterns and correlations discussed highlight air pollution trends and much of the information is highly applicable to gaseous pollutant biomonitoring using bryophytes. In order to draw their conclusions, the authors collated evidence from various sources. Analysis of the quaternary sub-fossil record demonstrated that the reduction in *Sphagnum* cover in peat profiles corresponded with the presence of soot deposits due to the advent of the industrial revolution. Bryophyte distributions determined from the British Bryological Society's mapping scheme from 1960 onwards and herbarium collections at national and local scales appeared to be correlated with long-term direct air monitoring data.

The national recording scheme revealed bryophyte species which had been adversely affected by atmospheric pollution on a national scale (Table 3.1). More intensive distribution studies in the heavily polluted London and Essex areas enabled the development of an epiphytic bryophyte sensitivity scale (Table 3.2), similar to the highly recognised lichen zonation scale drawn up by Hawksworth and Rose (1970). It is important to note that the position of a species on the scale will vary with humidity in the area and acidity of the bark substrate. Epiphytic species may be indirectly affected by the soil through influences on the bark chemistry, as demonstrated by Gustafsson and Eriksson (1996) in aspen *Populus tremula* in Sweden. Some species may prefer limestone to trees. In addition, it would be difficult for this scale to consider the effects of events such as short seasonal elevations in SO_2 associated with prevailing winds. It is difficult to distinguish between the response of moss communities subjected to a few severe air pollution events or continued chronic exposure. It would prove difficult to develop a scale to include mosses in polluted environments, which may be protected against the effects of air pollutants if located in sheltered areas such as deep valleys (Winner, 1988). (Details of Hawksworth and Roses' scale are discussed in Section 2). Although not as detailed or as accurate as the lichen scale, the proposed bryophyte scale lends itself as a positive biomonitoring tool.

National and local lists (Table 3.1 and 3.2) showed relatively good correlation. Disparities may be due to factors other than $SO_{2,}$ which may cause local variation. Furthermore, local species decline due to localized high SO_2 levels may not be reflected on the national scale.

The same authors also reported similar reductions in the distribution of terrestrial and saxicolous bryophytes in relation to high SO_2 levels.

As is the case with lichens, recent declines in SO_2 levels are reflected in bryophyte recolonisation to formerly polluted areas. However, the sequence of decline in species associated with SO_2 pollution may not be as marked in bryophyte recolonisation for several reasons:

- Inconspicuous species may have simply been missed in previous studies.
- Bryophytes have shown a slower response to reduced SO_2 levels than lichens.
- Species may colonise areas where they were not previously found.
- Species may not recolonise areas where they were previously found because of changes in habitat. In a study of epiphytic bryophytes in Groningen in the

Netherlands, (van Zanten, 1992) certain species (e.g. *Leucodon sciuroides* and *Orthotrichum lyelli*) became scarce in areas of relatively good air quality due to Dutch Elm Disease. These species rely on vulnerable *Ulmus* sp. for survival, illustrating the importance of available habitats even in improved air pollution conditions.

- Increasing levels of other atmospheric pollutants such as nitrogen oxides and ozone may reach threatening levels.
- Recolonisation processes depend on the mobility of the species and the proximity of source populations.

Table 3.1 Bryophyte species which appear to have been most adversely affected by atmospheric pollution in terms of distribution at a national scale, UK (from Adams and Preston, 1992)

Epiphytes	Epiliths	Species which can grow as epiphytes or epiliths
Cryphaea heteromalla	*Grimmia affinis*	*Antitrichia curtipendula*
Frullania dilatata	*G. decipiens*	*Leucodon sciuroides*
Neckera pumila	*G. laevigata*	
Orthotrichum lyellii	*G. orbicularis*	
O. obtusifolium	*G. ovalis*	
O. schimperi		
O. speciosum		
O. stramineum		
O striatum		
O. tenellum		
Tortula laevipila		
Ulota crispa var. Crispa		
U. crispa var. Norvegica		

Changes in bryophyte cover in forest ecosystems in response to air pollution have been reported. In Estonia, two groups of moss in Scots pine forests were observed: moss whose distribution depended on the level of air pollution and those whose distribution was more dependent on other factors such as climate and soil (Vilde and Martin, 1996). Bryophyte cover also increased in thickness along a pollution gradient from the polluted north-east to less polluted south-west. In a study of bryophyte communities in two Atlantic forests in Brazil, higher percentage cover and biomass of bryophytes were observed in the least polluted forest (Rebelo *et al.,* 1995).

1.2.1.2 Urban and industrial

In a Finnish study of effects of metal, chemical and fertiliser plants on forest floor vegetation in the vicinity of the plants, the common forest bryophytes *(Hylocomium* sp. *Pleurozium dicranum*) were more sensitive than lichens. Further away from the factories *Ceratodon purpureus* and *Pohlia nutans* became frequent (Vaisanen, 1986).

Huber (1992) found that bryophyte species distribution in the Swiss Canton of Basel-Stadt was similar to that in the proximity of a cellulose factory. Furthermore, the area of suspected good air quality in the Canton contained similar species distribution to an area 200 m above the cellulose factory.

In Romania, the zone of influence on bryoflora of the industrial Alba district extended to a distance of 20 km (Plamada, 1986). The main atmospheric burden was from SO_2.

Winner (1988) reviewed the responses of bryophytes to air pollution by presenting an overview of North American studies in this field. Types of studies include determination of impact zones, Indices of Atmospheric Purity (IAP) methods, bioaccumulation and physiological responses to gaseous air pollution.

IAP methods have been applied to moss communities in much the same way as to lichens (Section 2.2.1.2). IAP values have been calculated and mapped with respect to urban areas and point emission sources. Zones have been determined representing changes in bryophyte communities relative to the location of an air pollution source. According to Winner (1988), IAP values should be regarded tentatively and cannot be compared between different sites for the following reasons:

- All changes in moss communities are too often attributed to air pollution alone, whereas other environmental factors such as elevation, humidity and temperature may have an effect.
- Cities generally have higher temperatures and fewer appropriate substrates than rural areas.
- IAP may not be an accurate measure of the complexity of bryophyte communities.

Table 3.2 Epiphytic bryophytes that showed poor or restricted growth during the maximum phase of SO_2 pollution, or were exterminated, with their approximate equivalent SO_2 thresholds (taken from Adams and Preston 1992)

Mean winter SO_2 (µg m^{-3})	**Approximate pollution zone***	**Species**	**Last or only date recorded**	
			Epping forest	Outer Essex
Pure	9-10	*#Antitrichia curtipendula*	c 1800	1874
	9	*Orthotrichum sprucei*	-	1866
<30	9	*O. schimperi*	-	1873
	9	*O. tenellum*	-	1870
	9	*Ulota crispa var. crispa*	c 1800	1874
	9-8	*Orthotrichum. striatum*	c 1800	1870
c 35	8	*Zygodon conoideus*	1885	1886
	8	*Neckera pumila*	1890	1874
	8-7	*Tortula papillosa*	-	1874
	7	*Ulota crispa var. norvegica*	-	
c 40	7	*#Anomodon viticulosus*	1932	
	7	*#Radula complanata*	c 1890	
	7-6	*Leucodon sciuroides*	1885	
	6	*Orthotrichum lyellii*	1898	
	6	*Cryphaea heteromalla*	c 1800	
c 50	6	*Frullania dilatata*	1923	
	6	*#Homalia trichomanoides*	1973	
	6	*Porella platyphylla*	c 1890	
	6-5	*Isothecium myurum*	1885	
	5	*Tortula laevipila*	c 1980	
	5	*Neckera complanata*		
c 60	5	*Zygodon viridissimus*		
	5	*Orthotrichum affine*		
	5	*O. diaphanum*		
	5-4	*Homalothecium sericeum*		
	4	*Hypnum mammillatum*		

Table 3.2 (continued)

Mean winter SO_2 ($\mu g\ m^{-3}$)	Approximate pollution zone*	Species	Last or only date recorded	
			Epping forest	Outer Essex
c 70	4	*Hypnum cupressiforme var. cupressiforme*		
	4	*Dicranum scoparium*	extant	
	4	*Isothecium myosuroides*		
	4	*Bryum capillare*		
	4-3	*Dicranoweisia cirrata*		
c 125	3	*Hypnum cupressiforme var. resupinatum*		
	3	*Lophocolea heterophylla*		
c 150	2-3	*Ceratodon purpureus*		

Species which may be limited by factors other than specific sensitivity to SO_2
* Hawksworth and Rose (1970)

1.2.2 Transplants and effects

The biochemical and physiological effects of SO_2 on bryophytes will not be discussed here. Recent reviews in this area are presented by Brown (1995), Kovács (1992a) and Winner (1988). A recent review of transplantation exercises using bryophytes can be found in Brown (1995). These studies are concerned with assessing effects of air pollutants on bryophytes rather than their use as biomonitors.

A technique that was not mentioned by Burton (1986) was bryometers. Bryometers were developed in Japan to measure phytotoxic air pollution (Taoda, 1973). Mosses were placed in small, transparent plant chambers and two chambers were placed at a study site. One chamber was filled with ambient air and the other exposed to filtered, pollution free air. In this way the presence or absence of ambient air pollutants could be detected.

1.3 Nitrogen and its compounds (N, NO_x and NH_3)

Much of the published literature in relation to inorganic nitrogen (N), acid rain and bryophytes is concerned with the effects of this type of deposition on bryoflora. This research is gaining profile in response to the coincidence of reduced atmospheric SO_2 levels and increases in nitrogen deposition and acid rain. However relatively few attempts have been made to use bryophytes to directly monitor nitrogen deposition.

The following paragraphs attempt to highlight aspects which may be suitable for monitoring purposes in the future. Due to their dependence on atmospheric inputs of nutrients, total N content in bryophyte tissues can reflect atmospheric inputs of N.

Press *et al.* (1986) chose two ombrotrophic mires dominated by bryophytes to study the ecological significance of increased atmospheric nitrate deposition in the UK *Sphagnum* sp. transplanted to a relatively polluted site from a non-polluted site showed an increase in tissue nitrogen concentration and reduced growth in the polluted site. The authors concluded that elevated nitrogen deposition in the polluted area might be affecting the growth and metabolism of ombrotrophic *Sphagnum* sp.

Comparison of bryophyte tissue N content in herbarium samples and field samples collected in 1989 illustrated temporal N deposition trends in the UK (Pitcairn *et al.,* 1995). The study demonstrated increasing N deposition levels apparent throughout the UK and potential harm to the ecosystems themselves. Percentage increase in tissue N content at a range of sites of varying pollution climates corresponded with atmospheric increases in N levels. Certain sites remained unpolluted over the thirty-year period (e.g. Beinn Eighe National Nature Reserve in Scotland) and showed insignificant elevations in bryophyte tissue concentrations. A 62% increase in tissue N content of ombrotrophic *Sphagna* at Moor House, Cumbria, is of concern. The relationship between tissue N content with atmospheric N inputs was expressed as: $N_{Bry} = 0.62 +0.022\ N_{Dep}$ i.e. tissue N increases at 0.022 mg g^{-1} dry weight per kg total N deposited. The authors recommended the application of this equation as a rough measure of atmospheric N deposition in areas where monitoring equipment is impractical.

Mäkipää (1995) proposed changes of forest floor moss biomass as an early warning indicator of atmospheric N and S deposition in boreal forests. In a study of acidic deposition, experimental plots of boreal forest in Finland were exposed to annual treatments of ammonium sulphate over a four year period. The forest floor mosses, dominated by *Pleurozium schreberi* and *Dicranum polysetum*, were more sensitive to N and S deposition than vascular plants. Biomass of bryophytes decreased by 60% over the study period and N content in moss tissues was greater than in the control areas. Although this experiment was not undertaken under natural conditions, such experiments are valuable in enhancing our knowledge of the response of bryophytes to N deposition if they are to be used as biomonitoring tools.

Woolgrove and Woodin (1996) analysed tissue-N content in the late snowbed bryophyte, *Kiaeria starkei,* collected from sites in the Scottish Highlands. Results corresponded with the atmospheric N loads to which the bryophyte was exposed, which due to acid flushes during snowmelt and the sensitivity of the species pose a threat to these snowbed species.

Urban and industrial point source studies in relation to nitrogen oxide pollution gradients are limited. A study of the distribution of epiphytic bryophytes in Naha City in Japan showed that few species were found in the city centre where NO_2 levels were > 0.02 ppm (Inui and Yamaguchi, 1996). An interesting observation of this study was the lack of correlation between IAP values and NO_2 concentration. If bioindication of NO_2 pollution using bryophytes and lichens is to develop, additional research into the applicability of indices and methods used in SO_2 monitoring should be encouraged.

1.4 Other gases

In contrast to Burton's (1986) review, little published literature was found in relation to fluoro-compounds and bryophytes. With regard to ozone (O_3), literature is concerned with the effects of this gas on bryophytes since they are regarded as an important ecological group. An open chamber fumigation experiment of the effects of elevated O_3 concentrations on forest floor moss cover was undertaken by Stanosz *et al.* (1990). Significant negative correlations between moss cover (predominantly *Ditrichum pusillum*) and O_3 concentrations were demonstrated. In the UK, *Sphagnum recurvum* and *Polytrichum commune* were exposed to long-term chronic O_3 concentrations (Potter *et al.,* 1996). Both species showed a reduction in growth when exposed to O_3 in comparison to control experiments. *Sphagnum* appeared to be more sensitive to fumigation than *Polytrichum*. Fumigation studies will enable effects to be determined which could possibly be used as bioindication responses, since bryophytes may be more sensitive to O_3 than higher, economically important plants.

2 Lichens

2.1 Introduction

Lichen communities growing on tree bark (corticolous species) and walls and rocks (saxicolous species) show changes in response to air pollutants, particularly sulphur dioxide (SO_2), fluoro-compounds (F), deposition of nitrogen compounds and ozone (O_3). Lichens are particularly useful in indicating pollution loads over long periods (Richardson, 1988).

Literature on lichens and air quality is vast, to the extent that the Lichenologist publishes updated abstracts of the literature periodically (e.g. Henderson, 1994; 1995; 1996a; 1996b). Seaward (1993) examined the history and future of field studies concerned with lichen and SO_2 air pollution. A useful overview of recent developments in the use of lichens as bioindicators up to 1990 was provided by Richardson (1991). Numerous books have been published in this area (Richardson, 1992; Nash and Wirth, 1988).

Will-Wolf (1988) undertook a review of North American air pollution monitoring studies using lichens and/or bryophytes. The review revealed that most studies focused on lichens on trees in eastern deciduous and coniferous forests around a point source of low-moderate level pollution.

Burton (1986) found that the use of lichens in gaseous pollutant monitoring was based mainly on species distribution observations and to a lesser extent on chemical analyses and lichen transplants. Recent developments show an increased emphasis on the use of biochemical and physiological responses as indicators of air pollution, probably due to technological advancement.

This section initially highlights some aspects of monitoring design in gaseous air pollution monitoring using lichens. Few regional monitoring surveys have been undertaken using lichens and surveys are very much centred around urban and industrial areas.

2.2 Monitoring design

2.2.1 Monitoring method selection

Several gaseous air quality monitoring methods utilising lichens are available. Monitoring can be qualitative or quantitative and employ single indicator species or community changes. The choice of method depends on the purpose of the survey, the size of study area, resources available and the desired detail of the output. Air quality assessments, which observe species distribution patterns, are well recognised. Data on changes in lichen occurrence and abundance at species and community level are subjected to varying degrees of analyses and used to produce maps, identify zones and/or indices of air quality. Other methods reviewed are physiological/biochemical responses as indicators of air pollution, and lichen health has been used as an indicator of air quality degradation. By analysing element content from lichen samples at different distances from a pollution source, the type of pollution and the size of the fallout zone can be determined. Transplantation techniques are frequently used to assess air quality impact in an area.

The basis of these methods is presented in the following paragraphs. Their application and development on multi-national, national, regional and urban scales are illustrated in Sections 2.3 to 2.6.

2.2.1.1 Mapping

Most species distribution investigations involve mapping. Distribution mapping of common and sensitive lichen species is a relatively simple and inexpensive method of air quality monitoring. The method distinguishes areas with varying degrees of pollution. Studies can be in relation to point emission sources such as power plants and smelters, a general source area such as an urban area or industrial complex, or as a means of producing baseline data of a previously unsurveyed site or in pre-development appraisals (Showman, 1988). Species distribution monitoring can investigate spatial or temporal patterns. It is more useful with prior knowledge of lichen sensitivity to air pollutants and historical and/or natural or control data of the study area.

Distribution patterns can include presence or absence of species in response to a pollution gradient, reductions in cover of species and recolonisation of species associated with improved air quality. Distribution studies often include investigations of the health of the lichen flora.

Showman (1988) reviewed North American lichen mapping research up to 1986. This review advised on aspects of study area determination, reconnaissance, site selection, data collection and final distribution mapping. These considerations are paramount in the effective execution of a biological monitoring programme.

A major difficulty in lichen distribution studies in air quality monitoring is the fact that the measured parameters (e.g. abundance/presence/absence/diversity) can be attributed to several factors other than changes in air quality. Other factors include unfavourable habitat conditions, historical reasons, competition pressures, or anthropogenic impacts other than air pollution, such as landscape changes.

A practical progression of species distribution maps was the development of zone mapping. Zone maps classify areas using the number and type of lichen species present, which indicate the extent and/or distance from the pollution source. Burton (1986) reviewed the earliest zone mapping studies. Hawksworth and Roses' (1970) qualitative scale of relating mean winter SO_2 concentrations ($\mu g\ m^{-3}$) with certain epiphytic lichen assemblages on acidic and basic tree bark remains the key paper on zonal mapping and is cited and utilised extensively in the literature. Ten zones were devised, with zone 1 species indicating SO_2 levels > 170 µg m^{-3} and zone 10 representing 'purity'. Although the scale has proved effective in monitoring the spread and extent of SO_2, doubts have been cast on its application in areas encountering reduced levels or qualitative changes in air quality (Richardson, 1988).

2.2.1.2 Index of Atmospheric Purity

Calculations of Indices of Atmospheric Purity (IAP) examine the effects of a pollutant source on lichen communities. It is a quantitative phytosociological approach requiring the collection of data such as frequency and/or percentage cover and a factor of tolerance to toxicity. IAP values generally increase as communities become more complex further from the pollution source. These values can also be plotted on a map, which in turn can be used to determine IAP zones. Burton (1986) reviewed the use of Indices of Atmospheric Purity in air quality monitoring.

Showman (1988) highlighted the importance of site selection in this type of survey to ensure that lichen patterns are due to air quality differences rather than lichen substrate variations. Will-Wolf (1988), in her review of quantitative approaches to air quality studies, concluded that IAP indices rely on overall linear declines of species in communities affected by air pollutants. Therefore their efficacy as measures of community change are limited when pollutant gradients are shallow, where species dominance shifts are as important as linear declines. On comparison with other phytosociological methods, Wirth (1988) suggested that due to potential error associated with Q (refer to Section 2.3.1.4), regions which are inherently species poor with the same emission impact as lichen rich regions will have lower IAP values. The same author believes that the IAP method is not adequate in differentiating between pollution and climatic impacts.

Modifications of the IAP and developments of new indices are discussed below (Section 2.3.1).

2.2.1.3 Phytosociological methods

Phytosociological methods in air pollution monitoring study plant communities rather than single indicator species. Air pollution alters community structure, which is reflected by changes in the community composition and coverage. Wirth (1988) reviews the advantages and disadvantages of the phytosociological approaches to monitoring temporal and spatial changes in air quality. The method lends itself better to temporal monitoring than spatial. It is adept at differentiating between climatic/edaphic and air quality impacts on lichen species/community distribution. However, quantitative data collection is inherently more labour intensive, especially over large study areas. The same review presented a phytosociological sensitivity scale (1 to 14) to (acidic) air pollution in southern Germany (Box 3.1), but tolerance values for particular air pollutants were not available. This method is not recommended in obtaining actual pollutant concentrations, but more as an early warning system in environmental protection and in revealing relative changes in air quality over time.

2.2.1.4 Response methods

Additional information in lichen monitoring studies can be gained from observing morphological changes and the health status of lichens. Quantifiable physiological changes can be examined as a measure of pollution stress. The advancement of laboratory techniques has aided the development of such procedures.

Box 3.1 A phytosociological scale for estimation of relative (acid) air pollution in southern Germany. Relative sensitivity scale (after Wirth, 1988), 1: resistance low, 14: resistance high

1	*Lobarietum pulmonariae subass of Lobaria amplissima*
1	*Nephrometum laevigati*
2	*Gyalectetum ulmi*
3	*Usneetum florido-neglectae*
3-4	*Ramalinetum fastigiatae*
4	*Parmelietum acetabuli with Anaptychia ciliaris*
5	*Usneetum filipendulae*
5-6	*Physietum adscendentis with Physconia distorta, Physica stellaris, Ph. aipolia*
6	*Bacidia rubella - Aleurodiscus-ass.*
6	*Leprarietum candelaris*
7	*Pertusarietum hemisphaericae*
8	*Parmelietum caperatae (Flavoparmelia caperata damaged, if present)*
8	*Pyrenuletum nitidae*
9	*Opegraphetum vermicelliferae*
9-10	*Porinetum aeneae*
10	*Hypogymnia physodes-Parmelia sulcata-comm*
11	*Chaenothecetum ferrugineae*
12	*Bullietum punctatae*
13	*Lecanoretum conizaeoides*
14	*Pleurococcetum vulgaris*

2.2.1.5 *Element bioaccumulation*

Various studies have demonstrated good correlations between non-metallic elemental content in lichens and atmospheric deposition levels of these elements (Burton, 1986). For example, sulphur concentrations in lichen tissue have been correlated to SO_2 contamination in the atmosphere (Garty, 1985). Monitoring elemental accumulations in lichens does not monitor actual effects but can indicate areas of lesser or greater deposition. This method can indicate areas of impact in advance of other detectable effects.

2.2.1.6 *Transplants*

Transplantation exercises are frequently used to demonstrate or assess effects of gaseous air pollution on lichens transplanted from a clean to a polluted environment. The transplantation concept has been discussed in Chapter II with regard to heavy metal contamination. Most of the same principles apply to gaseous air pollutant assessment. The use of transplants requires some prior knowledge of the influence of transplantation on the measured response.

2.2.2 *Species selection*

Different lichen species are chosen for air pollution monitoring depending on their tolerance to air pollutants. The sensitivity of lichens to air quality has facilitated their utilisation as important tools in air quality monitoring. Kovács (1992a) summarised the physiological and morphological features of lichens, which make them more sensitive to air pollutants than higher plants. Features include the absence of a cuticle, low chlorophyll content and lack of excretion.

Awareness of the sensitivity of lichens is important in their function as bioindicators, particularly in mapping studies and transplantations. Richardson (1988) claimed that 'the best way to convince non-biologists of their (lichens) value is by establishing how, why, and at what levels, air pollutants are harmful to lichens'. Sensitivity coefficients are frequently used in the calculation of pollution related indices (Brakenhielm and Qinghong, 1995).

Steubing (1983), cited by Kovács (1992a), summarised the critical SO_2 concentrations for various lichen species published by different authors. In general these authors show comparison with Hawksworth and Roses' (1970) qualitative lichen sensitivity scales (discussed below). These scales of sensitivity have been supported and correlated with the results of controlled laboratory fumigation results (Nash, 1988).

Empirical laboratory and field fumigation exercises to establish actual sensitivity values of lichens to air pollution have often proved unsuccessful (Richardson, 1988). This is due to a combination of maintaining the metabolic activity of lichens in the lab, the need for sophisticated monitoring equipment and simulating low doses over a long period of time. The advent of open top fumigation chambers used for air pollution effects on field-grown plants show promise for lichenologists (Richardson, 1988). These systems overcome many fumigation problems in allowing the control of air quality by adding and subtracting pollutants without creating unacceptable environmental changes. In this way absolute values of lichen tolerance and relative importance of particular gaseous pollutants over longer time periods in field situations can be determined. Bates *et al.* (1996) used open-air fumigation to investigate lichen colonisation in a newly planted forest subjected to SO_2 and O_3. Small environmental changes may still be observed in open top chambers. These include higher than ambient temperatures, reduced rainfall and unnatural wind speeds (Colls, 1997).

A comprehensive review of the physiological responses of lichens to laboratory air pollutant fumigations is presented in Fields (1988). Various gaseous pollutants were investigated which affected lichen physiological processes in the apparent order of sensitivity: nitrogen fixation > potassium ion efflux/total electrolyte leakage > photosynthesis, respiration > pigment status. This may aid the selection of parameters in monitoring programmes but the application of laboratory results to actual field studies should still be used tentatively.

The sensitivity of a species may vary to an extent on the substrate type. In response to air pollution, species growing on basic substrates will persist longer than species growing on acidic substrates (Hawksworth and Rose, 1976). In the Liphook forest fumigation project, Bates *et al.* (1996) found that the three lichen species under investigation (*Evernia prunastri* (L.) Ach., *Hypogymnia physodes* (L.) Nyl. and *Lecanora conizaeoides* Nyl.) showed an obvious preference for Norway spruce over Scots pine, the latter containing the most acidic bark. The sensitivity of lichen species may be attributed to other factors such as the tolerance of the contained algal strains, morphology and water relations (Richardson, 1988).

Eight ecological groups of lichens are presented in Kovács' (1992a) review of lichens' role in environmental protection. Attributes of these groups may account for the tolerance of different lichens. Additionally, different reproductive stages of epiphytes show varying pollution sensitivities.

It has been hypothesized that the sensitivities of epiphytic lichens vary between regions. For example, Richardson (1988) suggested that the wetter climates of Sweden and Ireland make species more sensitive to SO_2 than they would be in England.

2.2.3 Sampling methods

Various sampling methods are available in lichen monitoring, depending on the nature of the method deployed and desired output. Some sampling methodologies used to estimate or measure lichen presence and/or abundance are presented below.

2.2.3.1 Lichen cover

Estimation of lichen cover in relation to pollution patterns is a common procedure and may involve total lichen cover or individual species cover. Some exercises are more time-consuming than others.

Quantification of lichen cover has been undertaken using quadrats and/or photographs. Addison (1984), in a study into the influence of oil sands extraction and processing emissions on lichen cover of transplanted communities, used a 15 x 20 cm gray-card quadrat and photography. In this study lichens were also moistened to minimise errors arising from different lichen hydration states at the time of sampling. The author concluded that when practicing this technique, lichen cover must be high to overcome measurement errors.

Bates *et al.* (1996) used two methods to estimate percentage thallus cover of the bark in their assessment of lichen colonisation in the Liphook Forest fumigation project, Hampshire, England. For *Hypogymnia physodes* and *Evernia prunastri*, thallus area was determined from thalli numbers and diameters, and for *Lecanora conizaeoides* a scale of one to five corresponding to percentage thallus cover of the bark was used.

Global average cover (GAC) is sometimes estimated as a measure of lichen abundance. This is measured as the average cover of lichen species on all trees sampled (Legrand *et al.*, 1995).

Sampling of trunk epiphytes was detailed in the manual of integrated monitoring (UN ECE 1993). Three methods have been prescribed for the estimation of epiphyte cover.

- **Line method**. Lichen cover is read at four levels (60, 90,120 and 150 cm) in relation to trunk circumference. This is achieved by tying a measuring tape (mm) horizontally around the tree with zero to the north and figures increasing clockwise. Overall cover of each species is the mean of individual levels values.
- **Point method**. A permanent transparent plastic frame (e.g. 30 x 40 cm) divided into uniformly spaced points is placed on either side of the tree trunk. Reported cover is the mean of the percentage of points occupied by each species at each sampling location.
- **Visual estimate**. Visual estimates of lichen cover can be taken between selected trunk heights.

Correlations between percentage cover of total lichen vegetation and a particular SO_2 level may be erroneous due to the inclusion of toxitolerant species. Selection, observing and/or mapping of either toxiphobous or toxitolerant species associated with a particular SO_2 level would be more successful in terms of pollution monitoring (Seaward, 1993).

2.2.3.2 Lichen frequency

Kovács (1992a) suggested a method for estimating lichen species frequency on a tree trunk. An area of 30 x 130 cm at height approximately 150 cm above ground is chosen. By dividing the area into forty subsections, the number of sections in which the species is observed is determined. A score can be applied to this data.

Lichen frequency on its own is rarely correlated to pollution contamination. This parameter is usually determined for other index calculations such as IAPs. Species frequencies are easily expressed as maps of the study area.

2.2.3.3 Lichen diversity

Some authors have demonstrated correlation of species diversity with pollutant concentrations (Seaward, 1993). In the Netherlands, Van Dobben and De Bakker (1996) obtained stronger correlation between the number of epiphytic lichen species per 5 x 5 km^2 grid square and measured SO_2 pollution than with number of species per sample point or species abundance measurements and SO_2 concentration. Species richness is easily obtained from distribution and/or IAP mapping studies.

Other parameters used in lichen distribution and mapping studies in particular include species density (i.e. number of species per unit squared) and luxuriance-density values (Showman, 1988).

2.3 Sulphur dioxide (SO_2)

2.3.1 Species distribution

2.3.1.1 Multi-national surveys

Epiphytic lichen monitoring is prescribed in the manual: *UN ECE International Co-operative Programme on Integrated Monitoring under the Convention on Long-range Transboundary Air Pollution*. The onitoring programme was initially in response to increasing awareness of acid rain but now covers other air transported pollutants. Lichen coverage (refer to Section 2.2.3.1) and thalli vitality (Section 2.3.3.1) were proposed as bioindication parameters of 'noxious gases and chemistry of precipitation, throughfall, bark and stemflow'. The manual provides prescriptive details on the recommended methodology to be carried out at one to five year intervals (UN ECE, 1993). Some key considerations are summarised below.

- During tree plot selection extreme habitats such as sheltered depressions or wind-exposed heights should be avoided.
- At least one widely distributed species should be utilised within a country or a region for temporal and spatial comparability.
- Select sample trees that are visibly healthy and have stable bark.
- Observations should be made at a trunk height of 50 to 200 cm. All species types within this area should be noted in addition to those receiving a cover value.
- Surveys are supplemented with measurements of certain pendulous lichens, which are a useful gauge of pollutant levels.

The manual defined the following lichen Pollution Sensitivity Index (PSI):

$$\mathrm{PSI}_i = \sum_{j=1}^{n} \{D_{ij} Q_j\}$$

where: D = mean cover per tree

Q = empirical sensitivity factor for each lichen species

i = plot or area

j = lichen species

High index values indicate a high abundance of sensitive species, which implies a low pollution effect.

Standardised site selection criteria, sampling methods, data presentation and quality control measures as prescribed in this manual are key to integrated multi-national air pollution monitoring programmes. This is a reflection of the increasing role of biomonitoring in air pollution monitoring.

2.3.1.2 National surveys

Sweden is particularly experienced in the use of lichens in air pollution monitoring. Long-term monitoring within the Swedish National Environmental Monitoring Programme (PMK) is undertaken in much the same way as the UN ECE collaborative studies. Lichen cover is estimated by the line method described in Section 2.2.3.1. Permanent marking of sample trees and observation points enable continuous monitoring of the same lichens.

Poor correlations between lichen occurrence and measured pollutants in an area imply that other factors may be responsible for lichen trends. In this way the importance of air pollution in an area in relation to other environmental factors can be assessed. An array of statistical approaches has been implemented to assess such situations. Brakenhielm (1996) and Brakenhielm and Qinghong (1995) reported on PMK results and applied First Principal

Components Analysis (PCA) and partial Redundancy Analysis (RDA) to the data. Weighted Mean Sensitivity (WMS) was used as the most effective index of air quality:

$$WMS = \frac{1}{4}\sum_{i=1}^{4}\sum_{j=1}^{s}[(K_j * n_{ij}) / N_i]$$

where: K_j= sensitivity coefficient for the j^{th} species.

n_{ij} = number of individuals of the j^{th} species along a circle at the i^{th} level;

j = 1,2,….s, the number of species along the circle;

N_i = total number of individuals of all species at the i^{th} level.

Each species was allocated a sensitivity coefficient (on a ten point scale) based on its tolerance to pollutant deposition (Hultengren *et al.*, 1991). For example, the relative tolerance *Hypogymnia physodes* was assigned a K_j value of 2 whereas the more sensitive *Alectoria sannentosa* had a K_j value of 7.

Lichens were more strongly influenced by climate and geographic location than pollutant deposition. The different growth rates of *Pinus sylvestris* between north and south may be responsible. Bark loss due to faster growth rates in the south favours rapid colonisers (e.g. *Hypogymnia physodes*) over slower colonisers such as *Bryoria* and *Usnea*. The authors believed that refinements to Swedish sampling methodology should be made.

Increasingly, species distribution studies are concerned with mapping re-colonisation in areas. This is in response to a decline in SO_2 levels since the 1970s and increases in other pollutants such as NO_x and NH_3. In 1988/89 Van Dobben and De Bakker (1996) updated previous surveys of epiphytic lichens in the Netherlands. Only 17% of the previous area was covered but sampling design was such that a representative picture of lichen trends since the 1970 to 1973 study (De Wit, 1976) was obtained. Sophisticated statistical analyses such as RDA were employed in the recent Dutch study enabling more quantitative relationships between direct air quality measurements to be determined. Two major differences between surveys were apparent:

- Increases in species diversity in the later survey, attributed to decreases in SO_2 levels.
- Increase in the number of nitrophytic species such as *Physcia caesia* in 1988/89, probably due to bark pH and/or NH_3.

2.3.1.3 Regional surveys

The Calibrated Lichen Indication Method is a standard method of evaluating biological effects and total air pollution in the Swiss midlands (Herzig *et al.*, 1989). It evolved as the result of comparisons between twenty variations of the IAP calculation and technical emission data. The model, IAP18 was selected because it produced the highest correlations. The model is based on the frequency of forty selected lichen species. Five zones of effect have been categorised which correspond to five zones of total air pollution (Table 3.3).

Table 3.3 Five classification zones corresponding to degree of injury to lichen flora and level of total air pollution (Herzig *et al.,* 1989)

Lichen zones	**Emission zones**
Lichen desert	Critical air pollution
Inner struggle zone	High air pollution
Outer struggle zone	Medium air pollution
Transition zone	Low air pollution
Normal zone	Very low air pollution

Osaka plain in Japan was divided into three areas for the purpose of assessing lichen distribution patterns in relation to air pollution (mainly SO_2 and NO_2) in the region (Hamada *et al.*, 1995). Frequency and cover of five indigenous lichen species were mapped. Lichen frequency was measured as the percentage of trees possessing a specific lichen species of all trees in each grid unit. Lichen cover was categorised according to three classes: > 1/3, > 1/10 to 1/3 and ⦸ 1/10. *Phaeophyscia limbata* displayed relatively high tolerance. Frequencies of this species peaked at intermediate SO_2 concentrations (7 to 8 ppm for this region) and were depressed at low and high pollution areas. In contrast *Lecanora pulverulenta* proved a better bioindicator, decreasing in frequency with increasing distance from the central, polluted areas. Conclusions were easily drawn with respect to SO_2 pollution. However, this study revealed the comparative lack of knowledge in terms of NO_2 contamination, despite NO_2 levels reaching three times that of SO_2 in the Osaka plain.

2.3.1.4 Urban and industrial surveys

Despite some regional studies, lichen distribution in relation to urban studies is still the most common application. Additionally, lichens continue to be frequently used to estimate air pollution influences of industrial sources.

Muir and McCune (1988) undertook comparisons of lichens, tree growth and foliar symptoms in detecting air pollution in a polluted area of Illinois and a 'cleaner' area in Indiana. Differences between the two sites were most pronounced in lichen results, which reflected several years of air pollution. Foliar symptoms tended to indicate conditions of the current year. Species richness, total cover and species composition were greater in the least polluted site.

Despite criticism, IAP methods have been practiced extensively in air pollution monitoring, particularly in mapping studies. In Thessaloniki, Greece (Diamantopoulos *et al.*, 1992), IAPs for twenty one sites were calculated according to Leblanc and DeSloovers' (1970) formula:

$$\mathrm{IAP} = \frac{1}{10}\sum_{1}^{n}(\mathrm{Q\ x\ f})$$

where: n = number of species present at a site

f = frequency (cover) of the species (scale of 1 to 5)

Q = ecological index of each species i.e. the mean number of other lichen species growing with the species under study in the surveyed area

In association with this, a site-by-species matrix was subjected to Detrended Correspondence Analysis (DCA) (Hill and Gauch, 1980) and Polythetic Divisive Technique Two-way Indicator Species Analysis (TWINSPAN) (Hill, 1979). The results of the ordination and classification analyses and IAP calculations were comparable, enabling separation of lichen species into three groups and sites into four zones. Zone A represented the most polluted sites, with Zone D depicting least polluted sites. IAPs ranged from 0 at zone A to 37 at the least polluted site within zone D. Lichen groups 1 to 3 succeeded each other and were characterised by varying degrees of pollution. Zone A showed an absence of epiphytic lichen flora. Zone B was characterised by group 1 lichen species dominated by *Physcia adscendens* and *Xanthoria parietina* and lacked group 3 species. The relative frequency of group 1 species is lower and group 3 species are still absent in zone C. In zone D, lichen group 3 species dominated.

Few physico-chemical measurements were available for the study area and conclusions on pollution levels were based on the distribution of lichen zones. A noteworthy observation was the association of the foliose lichen *Hypogymnia physodes* with only the least polluted sites. In contrast this species has been documented as having moderate tohigh tolerance to SO_2 pollution (Hawksworth and Rose, 1970).

Ammann *et al.* (1987) discussed a slightly modified form of the IAP, sometimes referred to as the Ammann method. Herzig and Urech (1991) cited by Loppi *et al.* (1996) illustrated the reliability of this method in monitoring total air pollution and recommended its use in alliance with standard sampling procedures practiced in Italy. Kumer *et al.* (1991) applied this methodology in a study of air pollution in the Ferrara area (north-east Italy) and Loppi and Corsini (1995) adopted it in a study of air quality in the town of Montecatini Terme, Central Italy. Loppi *et al.* (1996) applied it in Arezzo in central Italy and Loppi (1996) used it in a study of geothermal air pollution in central Italy. The method was as follows:

- 1 to 4 trees were chosen at each sampling station
- a 30 x 50 cm grid, divided into 10 units of 10 x 15 cm, was placed on the trunk of each tree at a height of 120 to 200 cm, in the part of the bole with the highest lichen density
- all the lichen species were noted together with their frequency (F), i.e. the number of grid units in which the species was present
- IAP was calculated for each grid as the sum of all the frequencies present (IAP = $\sum$F)
- Reported IAPs for each station were taken as the maximum value calculated.

Zone maps of air quality were constructed either by a function equation using maximum and minimum IAP values (Kumer *et al.*, 1991) or by the plotting programme SURFER (Loppi and Corsini, 1995). Kumer *et al.* (1991) detected seven classes corresponding to different levels of air pollution. Loppi and Corsini (1995) and Loppi *et al.* (1996) detected four zones - Zone A: very high pollution, Zone B: high pollution, Zone C: moderate pollution and Zone D: low pollution. Lichen sensitivities in these studies showed compliance with similar urban studies. Both studies suggested that vehicular traffic was the main source of air pollution in the study areas. This emphasised the transition from SO_2 based pollution to NO_x and CO in towns and cities, and the effectiveness of the Ammann method in detecting other pollutants besides SO_2.

Improvement in air quality in north-west London in 1989 was reflected by recolonisation of some epiphytic lichen species since 1980 (Hawksworth and McManus, 1989). Recolonisation in response to decreased mean winter SO_2 levels of 130 µg m^{-3} to 29 to 55 µg m^{-3} appeared not to follow the normal sensitivity scale sequence (Hawksworth and Rose, 1970). Instead the recolonisation process exhibited 'zone skipping', where species did not return in the sequence that they disappeared. An assemblage of zone 4 to 5 species failed to colonise where more sensitive zone 5 to 7 species had. SO_2 levels had fallen so rapidly over the study period that the conditions necessary for invasion of zone 4 to 5 species did not occur.

A twelve year study of epiphyte recolonisation of *Quercus robur* in south-east England revealed that recolonisation of oaks was lower than for other tree species such as *Salix* (Bates *et al.,* 1990). This was attributed to the high bark acidity of older London oaks preventing recolonisation of even the relatively tolerant species. Other trees composed of basic bark possessed higher buffering capacities.

Recolonisation surveys in response to ameliorating SO_2 levels in urban areas are complicated by an array of factors. Recolonisation is affected by the rate of SO_2 decrease, nature and age of the substratum, lichen form (foliose species appear to be more successful in recolonisation than crustose species), competitive nature of lichen, extent of agrochemicals and other pollutants in the area (Seaward, 1993).

A detailed study of lichen communities in the vicinity of a coal power station at La Robla, Spain, revealed three areas of contamination (Alfonso and Rodriguez, 1994). Little air pollutant data was available but the three areas were characterised below:

- Area I represented the most polluted area. A total of 38 different lichen species were observed. For *Quercus pyrenaica*, IAP, global average coverage (GAC) and species richness index ranges were calculated at 3.0 to 6.0 x 10^{-1}, 6.0 to 11.0 and 0.2 to 0.5, respectively. Species development was poorer than in the other areas.
- Area II contained 57 lichen species. This area obtained intermediate indices values in comparison to the other areas.
- Area III was the least polluted area, supporting 64 well-developed lichen species. Highest indices were also observed. IAP values fell in the range 11.9 to 15.8 x 10^{-1}, GAC ranged from 21.0 to 26.2 and species richness from 0.7 to 1.0.

For future monitoring of the area, the authors recommended a selection of valuable bioindicators for three phorophytes used in the study.

2.3.2 Sulphur content

Lichen sulphur (S) concentrations are often measured in association with trace element accumulation studies.

Bruteig (1993) studied atmospheric sulphur and nitrogen deposition in Norway as part of an ongoing programme established to monitor deposition and effects of long-range transported pollutants on various ecosystems and organisms. The author highlighted the importance of sampling strategy and presented detailed procedures for sample collection and analysis, which took place in 1990. Sulphur content in *Hypogymnia physodes* ranged from 0.046 to 0.183% sulphur of lichen dry weight. Regression analysis demonstrated that sulphate deposition accounted for most of the variation in measured sulphur concentration.

Burton (1986) concluded that sulphur isotopic ratios provided a useful method of assessing main sources of S in lichens. Takala *et al.* (1991) undertook a large-scale sulphur isotope study of epiphytic and terricolous lichens in non-polluted areas in Finland. The lichen species *Hypogymnia physodes* showed highly significant correlation between S isotope composition and S content.

A regional gradient in lichen S content was observed by an intensive study in eastern Canada (Zakshek *et al.,* 1986). Highest concentrations (959 $\mu g\ g^{-1}$) were observed near a smelting complex, and decreased progressively heading east to 329 $\mu g\ g^{-1}$ within the remoter areas of the region. Thalli S content displayed agreement with sulphate deposition measurements in the study area. Lichen S concentrations in eastern Canada in comparison to north-western Canada reflected the higher pollution burden in the east.

2.3.3 Response methods

2.3.3.1 General lichen health

Reduction in fertility, injured thalli and absence of young thalli are indicative of air pollution degradation (Wetmore, 1988). Other external changes listed by Kovács (1992a) included change in thallus colour, reduction in thallus size and changes in the thickness of the thallus.

The contained algal part of lichens is responsible for lichen sensitivity between species (Richardson, 1988). Increases in the number of dead and plasmolyzed algal cells and decreases in size and number of regenerative algal cells have been used to measure lichen injury in response to air pollution (Kovács, 1992a).

The same author also presented a table where the rate of degeneration of lichen thalli due to SO_2 pollution was correlated to damage to higher plants. For example, a rate of lichen degeneration of 10 to 35% represented chlorosis and necrosis of the leaves of conifers and cultivated plants. Increasing rates of degeneration implied greater impacts on agriculture, sylviculture and horticulture.

Many European countries utilise vitality scales in assessing the health of lichen thalli (UN ECE, 1993). Five vitality classes are expressed:

1. normal
2. slight damage
3. distinct damage
4. severe damage
5. dead.

Caution should be exerted in lichen health studies to ensure that damage is the result of air pollution and not of other environmental factors.

2.3.3.2 Physiological and biochemical effects

Some comments on physiological and biochemical effects of air pollutants on lichens were mentioned in Section 2.2.2. Physiological and biochemical responses of lichens can be assessed by laboratory and field studies. Field studies under natural conditions are necessary in determining long-term effects of air pollution on lichen metabolism. The basis of laboratory experiments is to confirm under controlled conditions the implied effects observed in the field. Brown (1995) details the interpretive problems associated with cryptogam physiology when measuring characters such as gas exchange, chlorophyll stability and free radical protective systems in the laboratory. Fields (1988) provided a comprehensive overview of the physiological responses of lichens to air pollutant fumigations. Brown (1995) details the interpretive problems associated with field fumigation studies. A whole spectrum of literature is available with respect to air pollutant damage to lichens. This includes Richardson (1988, 1991), Kovács (1992a), Seaward (1993) and Calatayud *et al.* (1996). The following paragraphs will concentrate on effects on lichens, which are applicable to biomonitoring in the field.

In Rijeka City, Croatia, the extent of cell membrane damage in lichens was correlated with established lichen pollution zones (Alebic-Juretic and Arko-Pijevac, 1989). The laboratory procedures are detailed in the paper and entailed measuring electrolyte leakage (mainly K^+ ions) from the prepared lichen specimens, and specific conductivity in leachate when immersed in deionised water. *Parmelia tiliacea* collected from sites in the lichen desert and struggle zones showed the highest specific conductivity and K content in the leachate. Comparison of lichen species at a coastal control site, 25 km away from Rijeka, showed that the most sensitive species, *Parmelia perlata*, exhibited the most membrane damage whereas the most resistant species, *P. Saxatilis,* was regarded as healthy. Results were tentatively correlated with SO_2 concentrations. Humidity plays a role in lichen damage measured as electrolyte leakage, which is significant in semi-arid and arid locations (Rope and Pearson, 1990).

A similar investigation was undertaken within the city of Biel, Switzerland (von Arb and Brunold, 1990). Various physiological responses were measured in naturally growing lichens collected from sites within five defined pollution zones. Annual growth rate of *Parmelia sulcata* was seven times higher and statistically greater in the low pollution zone than in the critical total air pollution zone. Transfer of C-assimilates from algal to fungal part of the lichen decreased significantly (fifteen times less) between the urban and suburban zones. Chlorophyll-a content in lichens increased significantly within the higher pollution zones in the city and was attributed to elevated NO_x emission from vehicles. Marked changes in the rate of net photosynthesis and dark respiration were not as evident between varying pollution levels.

Silberstein *et al.* (1996a) used a variety of physiological parameters to distinguish relative sensitivities of two lichen species (*Xanthoria parietina* and *Ramalina duriaei*) in Israel. By transplanting *R. duriae* from clean locations to polluted areas where *X. parietina* persists, comparisons between the species were made. The possible protective mechanisms of *Xanthoria parietina* are discussed in Silberstein *et al.* (1996b). The different physiological responses observed between the two species may provide further use of lichens as bioindicators. These are listed below:

- Red autofluorescence in photobiont cells indicate healthy cells. Changes in colour to brown, orange and then white under the UV microscope indicates increased pollution damage to the algal cells. *X. parietina* in the polluted and clean environments exhibited red autofluorescence, whereas only *R. duriae* from the clean site demonstrated red autofluorescence.
- Chlorophyll degradation was represented by the ratio of optical density at 435 and 415 nm (OD 435/415) in pigment extracted with dimethyl sulphoxide (Ronen and Galun, 1984). *R. duriae* showed a reduction in OD 435/415 ratio from the clean to polluted site. This parameter has been suggested as more appropriate than chlorophyll-a content (Kardish *et al.*, 1987).
- A significant decrease in photosynthetic rate (measured by photo-acoustic spectrophotometry, Ronen *et al.*, 1985) was observed in transplanted *R. duriae.* This was not consistent with results obtained by von Arb and Brunold (1990).
- Electrolyte and potassium leakage from lichen membranes can also be used as an indicator of SO_2 contamination as mentioned previously (Alebic-Juretic and Arko-Pijevac, 1989). Silberstein *et al.* (1996a) found a significant increase in electrolyte leakage in *R. duriae* from the clean to polluted location.
- ATP content (Silberstein *et al.* 1990) decreased significantly only in transplanted *R. duriae.* Kardish *et al.* (1987) discovered similar trends when *R. duriae* was transplanted from a clean site to a selection of monitoring sites. ATP content in lichen thalli decreased with increasing pollution at one site. In the same study ATP content proved a much more sensitive indicator of pollution damage than chlorophyll degradation, despite the more time-consuming procedures involved.

The benefit of the latter two parameters is that they are related to the whole lichen and not just the photobiont. The other parameters are indicative of changes in the algal part only (Silberstein *et al.*, 1996a).

The production of stress-ethylene by lichens is another potential physiological response which could be used in bioindication of air pollution in communities in their natural habitats or as a measure of damage in lichen transplants.

In conclusion, alterations to biochemical and physiological mechanisms are useful early detectors of air pollution. However, natural conditions may also affect many internal processes and the inclusion of healthy specimens within the monitoring programme is always advisable.

2.3.4 Transplants

The following section addresses recent lichen transplantation studies only in the context of bioindication and/or biomonitoring of air pollution.

In the city of Cordoba, Argentina, the lichen *Ramalina ecklonii* was transplanted to 24 different urban sites along three transects of varying traffic density (Levin and Pignata, 1995). Lichen samples collected from a 'clean' site north-west of the city were hung in nylon bags at a height of three m for eight weeks, prior to analysis. Chlorophyll, phaeophytin, conjugated dienes concentration, soluble protein content and thalli sulphur content were used as bioindication of air pollution in the study area. Assessment of the effectiveness of each parameter would have been useful. Although differences between transects were observed, no indication of decreasing effects from distance from pollution sources or pollution gradient was apparent. A potentially useful Pollution Index (PI) for each transplant was employed in the study defined as follows:

$$PI = (Pa/Ca + S_t/S_c) * CD_t/CD_c$$

where: Pa = phaeophytin-a concentration (mg g^{-1} dry mass)

Ca = chlorophyll-a content (mg g^{-1} dry mass)

S_t = sulphur content of transplant (mg g^{-1} dry mass)

S_c = sulphur content of control lichen (mg g^{-1} dry mass)

CD_t = conjugated diene in transplant (mmol g^{-1} dry mass)

CD_c = conjugated diene in control (mmol g^{-1} dry mass)

On the basis of the above results, a later more intensive study focusing on one sector of the city of Cordoba, Argentina was undertaken by Gonzalez *et al.* (1996). The investigation obtained more detail of the traffic and industry related pollution in the area. Sulphur content in lichens was considered a good indicator of heavy traffic sites and PIs proved effective in distinguishing between sites with heavy industrial activity. Vehicular traffic was regarded as the most significant source of pollution in the study area.

A recent development in lichen transplant methodology was 'the culturing of epiphytic lichens on inert non-absorbent materials' which enabled 'easy and repetitive determination of biomass growth rates' (McCune *et al.*, 1996). These authors proposed effective modifications of this technique and discussed biomass growth rate calculations. The developments play an important role for air pollution monitoring using transplants, enabling other parameters such as biomass growth rate to be compared between clean and polluted areas.

2.3.5 Combined methods

Wetmore (1988) proposed a combination of lichen methods for the rapid assessment of air quality in large areas, such as national parks in the United States. The so called 'floristic method' involved collection of samples of all lichen species at each chosen site for identification and elemental analysis, and notation of the health of the lichens at each locality. Lichen health was assessed by observation of symptoms associated with air pollution damage: dead or injured thalli, abnormal growth patterns and frequency of fertile thalli. Details of lichen habitat such as substrate and habitat disturbance were also included in the fieldwork programme. Lichen data was compared with historical records of the area, with flora of the same region in a known area of clean air and the natural geographical distribution and habitat requirements. This established absent species which would normally occur in the study area. Wetmore (1988) postulated that if these species were regarded by the literature as sensitive to air pollution, this could be a possible explanation for the lack of such species. A distribution map of the most sensitive species could then be produced and compared with the distribution of thalli with elevated element concentrations. Wetmore (1988) concluded that the floristic method could provide useful baseline data for the whole lichen flora in a large area, particularly in the United States where lichen flora data is limited in comparison to Europe. The author also stressed its benefit as a screening tool to determine areas requiring further investigation. In these respects the floristic method would be useful in the creation of databases for long-term monitoring of air quality changes, the application of which is growing in importance for internationally and nationally protected areas.

2.4 Fluoro-compounds

Available data was limited with regard to the assessment of fluoro-compound contamination utilising lichens.

Most studies are concerned with accumulation of fluoro-compounds in relation to a point emission source. However, distinct visible injury of lichens in response to hydrogen fluoride (HF) has been observed (Kovács, 1992a). Lichens turn grayish-white in colour, colony size is reduced and finally colonies separate.

Rope and Pearson (1990) reported levels of fluorine in *Lecanora melanophthalma* two to four times greater at the Idaho National Engineering Laboratory sampling sites than at two reference sites approximately 60 km away.

In Anglesey in Wales, monitoring of lichens in 58 permanent quadrats in the vicinity of an Aluminium works had been conducted since the 1970s (Perkins, 1992). Response of saxicolous lichens was slower than observed in corticolous species. Fluoride concentration in thalli reached 396 µg g^{-1} at well-exposed sites 0.6 km from the works. Percentage cover and fluoride content of *Ramalina* sp. varied depending on exposure of sites and distance from works. Percentage cover and fluoride content in lichen thalli were closely correlated. Using regression analysis it was predicted those fluoride concentrations of 300 µg g^{-1}, 100 µg g^{-1} and 50 µg g^{-1} would correspond to 46, 15, and 10% loss in lichen cover per year. This work is an example of a coherent long-term data set enabling the most appropriate bioindicator species to be sought, exposure of long-term trends and prediction models to be determined.

2.5 Nitrogen oxides and ammonia (NO_x and NH_3)

Nitrogen deposition is increasing in many areas primarily due to increased road traffic intesity and further laboratory and field fumigation experiments and reasearch are needed to confirm the effects of NO_2 on lichens.

Symptoms in response to NO_2 exposure include the production of dark bodies in the vacuoles of algal and fungal parts of lichens (Richardson, 1988). Field fumigations in Sweden found that nitrogen fixation in the nitrogen fixing lichen *Peltigera aphthosa* increased when exposed to nitrogen in neutral solution, and a combination of ammonium and sulphuric acid had deleterious effects on the lichen (Hallingback and Kellner, 1992).

In the Norwegian study of nitrogen and sulphur deposition in the epiphytic lichen *Hypogymnia physodes* mentioned previously, Bruteig (1993) discovered nitrogen content levels in the lichen thalli in the range 0.42 to 1.96% lichen dry weight. This value was ten times that of thalli sulphur content. N/S ratios were higher in southern Norway and correlated well with the estimated N/S deposition ratio in Norway. Regression and correlation analyses indicated that most of the variation in lichen N content was attributed to annual mean nitrate concentration, annual mean concentration of ammonium in precipitation and annual wet deposition of ammonia. The aim of the survey was to provide a large-scale spatial picture, and results showed that long-range transported nitrogen from central Europe and Britain contributed significantly to the nitrogen content in *Hypogymnia physodes*. In addition, this study emphasises the growing importance of nitrogen compounds in air pollution over the past fifteen years.

2.6 Ozone (O_3)

There was also paucity in the literature in relation to lichen bioindication and O_3. Some fumigation exercises have been reported.

The effects of CO_2 and O_3 on green-algal lichens (*Parmelia sulcata*) under controlled laboratory conditions were investigated by Balaguer *et al.* (1996).

Fumigation of two lichen species with SO_2, O_3 and a combination of both, suggested that O_3 was more phytotoxic than SO_2. The combination of gases produced different ultrastructural changes than exposure to either pollutant alone (Eversman and Sigal, 1987).

In the Liphook forest fumigation project mentioned previously, effects of O_3 on lichen colonisation were also investigated (Bates *et al.*, 1996). No marked changes in the abundance of colonising lichens in response to O_3 fumigations were observed.

3 Fungi

3.1 General

Burton (1986) found that available information concerning fungi and air pollutants was sparse compared to lichens and bryophytes, was limited to plant pathogens and was based largely on short-term SO_2 fumigation studies. This remains the situation, but since 1986 a few authors have discussed fungi as potential bioindicators of air pollution damage in forests (Fellner, 1989; Marx and Shafer, 1989).

Mycorrhizae (root symbioses) are responsive to effects of atmospheric deposition on forests and ectomycorrhizae in particular may be the first line of biological defense against stress for trees (Marx and Shafer, 1989). In their review of fungal and bacterial symbioses as potential biologic markers of effects of atmospheric deposition on forest health, Marx and Shafer (1989) concluded that the lack of standard techniques to assess these symbiotic associations and the paucity of base-line data on healthy forests limited their utility.

Fellner (1989) and Fellner and Pešková (1995) proposed mycorrhiza-forming fungi as potential bioindicators of air pollution by indicating the disturbance of forest ectotrophic stability in the Czech Republic. The paper is brief but two myco-bioindication methods were proposed:

- Myco-indication through mycocoenoses (assemblages)

  This combined the evaluation of the ratio of mycorrhiza-forming fungi (fungus-root) to all macromycetes within the study area with an examination of the degree of pauperization of ectomycorrhizal mycocoenoses. Three stages of impoverishment have been identified which are also linked to three stages of disturbance of ectotrophic forest stability and enhancement of lignicolous mycocoenoses (Table 3.4) (Fellner and Pešková, 1995). Fellner and Pešková (1995) debated the utility of fruiting bodies as representative measures of the occurrence or decline of mycorrhizal fungi. The authors demonstrated that the proportion of proper mycorrhizal active tips was positively correlated with the proportion of mycorrhizal species and could be used to replace fruiting body counts.

- Myco-bioindication through selected sensitive fungi

  The presence and/or abundance of *Russula mustelina* was recommended as a suitable myco-bioindicator in mountain and submountain spruce forests of central Europe.

Table 3.4 The system of forest deterioration as a consequence of air pollution (after Fellner and Pešková, 1995)

Ecotrophic forest stability disturbance (degrees)	Ectomycorrhizal mycocoenoses impoverishment (phases)	Lignicolous mycocoenoses enrichment (phases)
Latent - The percentage of species of ectomycorrhizal fungi in the total count of macromycetes decreases to 40%, while the percentage of lignicolous species tends to reach more than 30%	Inhibition of sporocarp production (accompanied with a decline of highly sensitive species, e.g., hydnaceous fungi)	Stimulation of sporocarp production
Acute - Ectomycorrhizal species contribute constantly less than 40% of the total number of macromycetes, while lignicolous species as a rule exceed 40%	Reduction of species diversity (with a continuous inhibition of sporocarp production)	Increase of species diversity (with a continuous stimulation of sporocarp production)
Lethal - Ectomycorrhizal species contribute constantly less than 20%, while lignicolous species as a rule exceed 55% of all macromycetes	Partial to total destruction of mycorrhizal mycocoenoses	Expansion of mycocoenoses

In the Netherlands, Schaffers and Termorshuizen (1989) obtained strong negative correlations between the number of mycorrhizal fungal species on field stands of *Pinus sylvestris* L. and the occurrence of fruit bodies on these species with levels of NH_3 and SO_2. The authors were not aware if air pollution affected tree vitality which consequently altered the mycoflora or vice versa. If air pollution influences in the mycoflora modify tree vitality these associations could potentially be used as early indicators of air pollution impacts on tree vitality in forests.

The number of fungal endophytes isolated from birch leaves in Lapland decreased significantly in response to simulated acid rain treatments (Helander *et al.,* 1993). Such sensitivity enhances the potential of these endophytes as bioindicators of air pollution.

An open chamber fumigation study in California used percentage frequencies and diversity indices to measure the effects of O_3 and SO_2 on leaf colonising fungi of three tree species (Fenn *et al.,* 1989). Chronic exposure of the trees to either pollutant reduced the fungal populations and, to a lesser extent, reduced fungal diversity on the leaves.

Other studies concentrate on effects of air pollutants on host/pathogen combinations. These are generally impact assessments associated with economic crop and forest species rather than in the context of air pollution monitoring (Lorenzini *et al.*, 1992; Wookey and Ineson, 1991; Khan and Kulshrestha, 1991; Tiedemann *et al.*, 1991; Singh and Bharat, 1990).

3.2 Leafyeasts and sulphur dioxide (SO_2)

Dowding and Richardson (1990) demonstrated the suitability of leafyeasts for assessing air quality in both urban and rural areas of the non-Mediterranean countries of Europe. Leafyeasts are found on a wide variety of leaves in temperate regions and actively discharge spores at night. These properties enabled the development of a simple and effective methodology for collecting and isolating *Sporobolomyces roseus*, the most common leafyeast. Dowding (1994) detailed an extremely clear and concise description of a method designed for school children in a co-ordinated survey. Other advantages of using leafyeasts for air pollution monitoring are their sensitivity to SO_2, their ability to provide a current assessment of air pollution and results can be obtained within one week. Furthermore, sampling work in Hamburg established quantitative relationships between leafyeast numbers and SO_2 levels, where a regression line was derived between the natural logarithm of counts and mean SO_2 concentration for the previous four days. Yeasts respond to SO_2 levels in the range 0 to 100 µg m^{-3} (Dowding and Richardson, 1989). The disadvantages of the leafyeast method are its reliance on weather and seasonal conditions. Leafyeast growth is restricted to the time of year when deciduous trees are in leaf and show variations with time of year, which will vary with location and country. Sporulation is enhanced in wet conditions such that weather conditions on days immediately prior to collection affect numbers significantly.

4 Algae

Burton (1986) was sceptical towards the use of algae as biomonitors other than as an indicator of urban boundaries. Although literature on the use of algal biomonitoring of air pollution is rare, some recent developments have occurred in this area.

An increase in epiphytic algal growth usually coincides with a reduction or disappearance in epiphytic lichens (Hanninen *et al.,* 1993; Brakenhielm and Qinghong, 1995). Green algae respond positively to increased nitrogen deposition (Bates *et al.,* 1990). Algae have been included in epiphyte recolonisation studies (Bates *et al.,* 1990), where *Desmococcus viridis* decline was postulated to be a consequence of decreasing SO_2 levels in London, UK.

The use of epiphytic algae as bioindicators of air quality has been hindered by the lack of a non-destructive, non-laborious sampling method. Hanninen *et al.* (1993) proposed a reliable, replicable method, which overcomes these obstacles and allows time series analysis. This method was based on photography and digital image processing used to estimate the chlorophyll density of the algal layer. Detailed methodology along with calibration and testing aspects are outlined in the paper.

Since 1986 aerial green-algal monitoring has been carried out annually in about twenty reference sites as part of the National Swedish Environmental Monitoring Programme (PMK). The abundance and colonisation of mainly *Pleurococcus vulgaris* (*Protococcus viridis*) growing on needles of Norway Spruce (*Picea abies*) were related to atmospheric deposition. Detailed methodologies are found in Brakenhielm (1996), Brakenhielm and Qinghong (1995) and Bernes (1990), and are summarised in the following paragraphs.

- Sample sites consisted of 20 small trees and on each of them algae on needles of three branches at eye height were observed using a magnifying glass.
- Algal thickness was categorised into thin, medium or thick classes.
- Algal colonisation rate was estimated as the inverse of age (1/A) of the youngest annual branch (A = age) which had been colonised.
- Climatic, geographic and sulphur and nitrogen deposition data were collected.

In a study of spatial and temporal patterns of epiphytes in relation to low level, long-range pollutant deposition in Sweden (Brakenhielm and Qinghong, 1995), spatial distribution of algal colony thickness and colonisation rate correlated well with climatic conditions and pollutant deposition gradients in the country. However, the statistical analysis was not capable of distinguishing between the natural and anthropogenic effects. The four-year algal data set from 1989 to 1993 was not sufficient to draw conclusions about temporal variations. Colonisation rate was thought to be a more reliable and objective sampling tool than colony thickness.

Brakenhielm (1996) obtained comparable results with the above study. Refinements such as partial Redundancy Analysis (RDA) and modeling were applied to the data in an attempt to clarify signal and noise relationships. The author concluded that climatic factors were of primary importance in green-algal distribution and deposition of S and N were of secondary importance. However under similar climatic conditions, epiphytic green algae are potentially good bioindicators of S and N deposition.

UN ECE (1993) prescribed sampling methods for aerial green algae in their manual for integrated monitoring programme phase 1993 to 1996. The basis is similar to the Swedish format but the following modifications/additions are provided: precise details on tree selection; a four point scale is used in assessing algal thickness; the type of annual shoots to be used in the assessment, and recommended sampling time is July to September. All of this information is used in the assessment.

5 Higher plants

5.1 Introduction

Literature related to higher plants (i.e. herbs, grasses, trees and shrubs) and air pollution is vast. There is a growing concern of the effects of air pollutants, particularly ozone (O_3) on crops and forests and literature in these areas are particularly extensive. Bennett (1996) prepared a floristic summary of plant species in the air pollution literature derived from the BIOLEFF database (Bennett and Buchen, 1995). Smidt (1996) summarised the type of assessments used in the evaluation of air pollution stress on forest ecosystems.

This section is directed towards published literature focusing on monitoring of gaseous air pollutants and no attempt has been made to cover the extensive publications on the effects of air pollutants on higher plants. Bioindicator plants have been used to indicate the relative air quality of specific areas and regions and can provide unique information as to the ambient air quality within a particular area.

The most common method of bioindication is visible injury. Prior to the establishment of the Environment Agency in England and Wales, UK, a guidance manual on the diagnosis of air pollution injury to vegetation was produced by HM Industrial Air Pollution Inspectorate (HMAPI) (Taylor *et al.*, 1990). This is a comprehensive volume, which successfully attempted to provide a guide to aid personnel in identifying the range of factors, which may lead to particular symptoms in the field, particularly in association with industrial sources. The text covers most major pollutants and a wide range of plant types and plant parts. This manual is unique in that it extends beyond reporting pollutant effects on vegetation in that it is structured to enable the user to detect the pollution (i.e. bioindication).

Generally, visible injuries are typically non-specific and may indicate various stresses on the plants. Increasingly physiological, structural and biochemical effects are being used in biomonitoring studies. These responses not only occur prior to visible injury and therefore represent early detectors but also are regarded as more precise and objective parameters.

Turcsanyi (1992) reviewed the use of plant cells and tissues as indicators of environmental pollution. The effects of acid gases such as NO_2, SO_2, and HF on cells and tissues were discussed. Saxe (1996) provided an excellent overview of bioindicative methods involving biochemical, physiological and ultrastructural processes. This was a sequel to a previous review by the author (Saxe, 1991). The extensive 1996 review focused primarily on forest decline and air pollution injury and considered laboratory and field based conditions. The mechanisms discussed included photosynthesis and stomatal conductance, leaf pigmentation, chlorophyll fluorescence, element content, metabolite content, enzyme activity, morphological analyses, ultrastructure and histopathology and genetical analysis. This literature is used extensively in the succeeding sections.

5.2 Critical loads/levels

The concept of critical loads and levels is gaining profile world-wide. Critical loads and levels are not directly related to biomonitoring using plants as such but do warrant recognition in this report. Bioindication using sensitive species plays a role in establishing critical levels and loads.

The basis of the critical levels/loads approach is that that once the sensitivity of an ecosystem to acid deposition or to gaseous pollutants is established a critical load of wet deposition or a critical level of gases is calculated and set. Below this value the ecosystem will not be significantly damaged. These values are then compared with actual or estimated concentrations at the locations under study. Load and level exceedances can then be established and emission reductions and controls can be initiated. This is regarded as a more scientific, quantifiable method of assessing effects and planning emission controls compared with the previous arbitrary percentage reduction approach. Critical loads and levels are being mapped for countries in Europe and North America. To date, critical levels have been set for the effects of SO_2, O_3, NO_x, and NH_3 on crops, trees and natural vegetation by UN ECE (1993). Values were based on literature of the effects of these pollutants on vegetation. The next stage is to map and/or model critical level exceedance.

It is beyond the scope of the report to appraise methods, models and approaches to setting and determining exceedance of critical levels and loads. Instead the reader is referred to the following useful pieces of literature. Bull (1991) reviewed some different approaches to the calculation of critical loads and levels. Sanders *et al*. (1995) discussed specifically how critical levels for the effects of air pollution on crops, trees and natural vegetation. Exceedance of critical levels of O_3 in Europe was treated by Benton *et al.* (1995). The use of critical load maps in estimating the impact of air pollution on environmentally valuable sites in the UK was discussed by Brown *et al*. (1995). More recently, results of exceedance mapping of O_3 for crops and trees in the Netherlands were presented by de Leeuw and van Zantvoort (1997). Setting air quality standards, particularly O_3 to protect vegetation in the US were discussed by Lefohn and Runeckles (1987), Lefohn and Foley (1993) and Larsen *et al*. (1993).

5.3 Monitoring design

5.3.1 Species selection

Ideally plants which show specific responses to specific pollutants are most suitable as bioindicators. Sensitive species are more useful as bioindicators and tolerant species are more appropriate as accumulative indicators. Agrawal *et al*. (1991) used the air pollution index (APTI) to examine the susceptibility of a number of plant species growing in an urban/industrial region of India. APTI considered levels of total chlorophyll, ascorbic acid, leaf pH and relative water content. Plants were classified into three categories of sensitivity:

- APTI $\leq$ 10, sensitive;
- APTI 10 - 16, intermediate;
- APTI $\geq$ 17, tolerant.

The authors demonstrated that plants classed as sensitive to SO_2 under laboratory conditions were also ranked as the most sensitive species by APTI calculation under field conditions. Kalyani and Charya (1995) calculated the APTI of 54 plant species under natural conditions in Warangal City to establish which species were the most suitable for biomonitoring purposes.

Species selection primarily involves choosing herbs and/or grasses or tree species.

5.3.1.1 Herbs/grasses

Kovács (1992b) and Taylor *et al*. (1990) listed sensitive and accumulative plant indicators of gaseous air pollutants. Seedlings, semi-mature and mature plants have been used in air monitoring studies. The age of the leaves used in bioindication is important. Apparently young leaves of cultivars of the genus *Petunia* are particularly effective indicators of SO_2 pollution. *Gladiolus gandavenis* is an excellent bioindicator by showing marked responses to fluoride pollution. Kozuharov (1986) reviewed the appropriateness of plants as bioindicators. The author suggested that in annual plants, younger specimens were more sensitive than older ones. Such a distinction in the different stages of perennials is not as obvious.

5.3.1.2 Trees

Air pollution studies in relation to trees may involve tree parts, individual trees, tree stands or entire forests. Tree foliage reflects changes in pollution conditions within a relatively short time (two or three years). By contrast, air pollution impacts on entire tree stands are detected in the much longer term. Tree stands are commonly used in the assessment of forest decline of which air pollution is believed to be an important contributing factor.

Suitable tree bioindicators of air pollutants were listed in Kovács (1992c) and Taylor *et al*. (1990). Coniferous trees are regarded as more sensitive indicators of air pollutants than deciduous trees. This can be attributed to their continual exposure to air pollution all year round, their longevity and their response to low levels of contaminants.

5.3.2 Site selection

Site selection will depend on the extent and purpose of the survey. For the EC and UN ECE programme on the assessment of forest condition in Europe, the transnational survey is based on a 16 x 16 km transnational grid of sample plots. The national surveys are conducted on a national grid basis.

During biomonitoring programmes using higher plants it is often important to consider other environmental conditions at the study site.

5.3.3 Sampling

Sampling procedures should be consistent throughout the study period and area. Jones *et al*. (1991) emphasised that if a bioindicator is used in different locations for comparison of responses, then the same variety and seed source should be used. In addition only plants at similar stages of plant development should be compared. Plant parts of the same age should be used consistently throughout a survey.

Sample frequency will depend on the plant under investigation. Some plant species may be restricted by time of year or even time of day. Generally the greater the sample frequency the better. However, this is in many cases limited by resources. In addition the collection of extensive amounts of data is fruitless without effective programme planning and design in the offset. It is pointless to concentrate efforts on collating large amounts of random data, which provide us with little more information than a smaller, focused dataset.

Some countries have adopted standard, national methods for sampling. For example, in a Finnish study of S content in pine needles sampling, preparation and analyses were undertaken in accordance with Finnish standard method SFS-5669 (Haapala *et al*. 1996).

5.4 Sulphur dioxide (SO_2)

Many factors affect the sensitivity of plants/trees to SO_2 (Taylor *et al*. 1990). Young fully expanded leaves/needles are more sensitive than older needles and generally seedlings are more sensitive than older plants. Drought, low temperature, N, S, and P deficiency all reduce SO_2 sensitivity whereas high relative humidity, wind, K and Ca deficiency increase sensitivity.

5.4.1 Species distribution surveys

Changes in species distribution of higher plants are rarely used in biomonitoring in comparison to lower plants. Many established methods are available to assess species diversity, cover etc. but these are generally used as conservation tools not as biomonitoring tools. Cape (1989) suggested that changes in composition of sensitive herbaceous species would be appropriate bioindication of air pollution in forest ecosystems. Similar decreases in the floristic composition of the herbaceous layer were observed in zones of increasing pollution around Shaktinager Power Plant in India (Agrawal *et al*. 1991).

Sweden undertakes extensive vegetation surveying at national reference areas as part of their national monitoring programme (PMK). Vegetation is surveyed every twenty years at transects situated in one square kilometre catchments (Bernes, 1990). More frequent sampling is taken in circular subplots of radius ten metres along the transects. The development of trees and tree stands (e.g. their status, trunk diameter, fallen trees) are recorded. Within these circular plots more intensive surveying of all vegetation is carried out in smaller 'tree plots'. In the vicinity of the tree plots more intensive sampling of tree, shrub, field and ground layers is undertaken. This includes estimations of cover and fertility. Even more thorough monitoring of ground and field layers takes place in 'intensive square plots' containing 0.5 x 0.5 m quadrats.

$$H' = -\sum P_i * \log_2(p_i)$$

In 1996 the Swedish Environmental Protection Agency produced a report on the impacts of air pollutants on processes in small catchments which forms part of Sweden's integrated monitoring network. (Brakenhielm, 1996). Results from intensive plot monitoring were reported. Estimates of percentage cover, species diversity and the composition of indicator groups of lichens, mosses and vascular plants were recorded. Species diversity was calculated by the Shannon-Weiner (H′) index according to the following formula:

$$ATI = \sum(R_i * p_i)$$

$$NDI = \sum(N_i * p_i)$$

Where: $p_i =$ $n_i/\sum n_i$

$n_i =$ cover of the ith species

The composition of the indicator groups was expressed by two indices, the Acid Tolerance Index (ATI) and the Nitrogen Demand Index (NDI) where:

Where: $R =$ soil acidity indicator value of the ith species

$N =$ nitrogen indicator of the ith species

$p_i = c_i/\sum c_i$ where: c_i =cover of the ith species in a group

$\sum P_i = 1$

Both indices range from 1 to 9.

These were calculated for each site and were based on the values of sensitivity of each species to acidification and eutrophication. The data was subjected to Principal Component Analysis (PCA) and regression analysis. The NDI appeared to be the best parameter for detecting N deposition-induced impact on forest understorey vegetation.

5.4.2 Visible injury

Measurements of plant injury can be very uncomplicated (e.g. injured vs. non-injured) or more elaborate such as the application of numerical values or indices as measures of severity. Both systems are discussed below. Plant injury symptoms generally indicate acute damage to plants.

5.4.2.1 Multi-national surveys

Most multi-national monitoring programmes are concerned with forests and tree stands. One of the most comprehensive ongoing programmes in Europe is the monitoring of forest condition. According to Lorenz *et al.* (1997) and Lorenz (1995) 'In response to growing concern about forest damage caused by air pollution, in 1985 the United Nations Economic Commission for Europe (UN ECE) under its Convention on Long-range Transboundary Air Pollution (CLRTAP) established the International Co-operative Programme on the Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests). In 1986 the Member States of the European Union (EU) agreed upon the European Union Scheme on the Protection of Forests against Atmospheric Pollution. Since then ICP forests and the European Commission (EC) have been monitoring forest condition in close co-operation.'

This programme is not aimed exclusively at SO_2 but includes air pollution in general (e.g. O_3, NO_x). For convenience general air pollution studies will be included in this section.

Defoliation and discolouration are the major assessment parameters of crown condition in the transnational survey. Defoliation is assessed by 5 to 10% stages in comparison to a standard reference tree. This has replaced the traditional five-class system given in Table 3.5. Discolouration is also evaluated by a five-class system (Table 3.5). Additional parameters reported include country, plot number, plot co-ordinates, altitude, aspect, water availability, humus type, soil type, mean age of dominant storey, tree numbers, tree species, observations of easily identifiable damage. Crown condition is not a specific indication of air pollution damage since many other conditions such as climate, drought or disease may cause similar symptoms. However, by undertaking correlation analysis with the aforementioned parameters some factors, which show no correlation, may be eliminated. Analyses of results indicate that crown condition has deteriorated over the 11 year study period, particularly in central Europe. Although some discrepancies in sampling work have occurred between countries, this is an example of an effectively co-ordinated, large-scale, biomonitoring programme. The collection of a long-term dataset will allow the determination of spatial and temporal patterns of forest ecosystems and individual tree species. This integrated approach will also aid in the identification of more specific cause-effect relationships.

Table 3.5 Defoliation and discolouration classes according to UN ECE and EU classification (from Lorenz *et al.,* 1997)

Defoliation class	Needle/leaf loss	Degree of defoliation
0	up to 10%	None
1	>10-25%	Slight (warning stage)
2	>25-60%	Moderate
3	>60-<100%	Severe
4	100%	Dead
Discolouration class	**Foliage discoloured**	**Degree of discolouration**
0	up to 10%	None
1	>10-25%	Slight
2	>25-60%	Moderate
3	>60-<100%	Severe
4		Dead

5.4.2.2 National surveys

The UN ECE programme mentioned above incorporates national surveying and reporting currently from 33 countries (Lorenz *et al.* 1997). The national surveys vary in intensity depending on country. For example, in Finland five variables are used in the assessment of tree vitality: defoliation, crown degeneration, needle and leaf discolouration, cone yield and male flowering on conifers and abiotic and biotic damage. Whereas other countries employ the transnational 5% class method alone.

The most recent synopsis of tree health as a consequence of air pollution in the UK was printed in 1993 (DoE, 1993). The results indicated localised areas of decline in crown condition in the UK.

5.4.2.3 Regional surveys

In response to increased acid deposition from S and N in south-east Norway, a local monitoring network of Norway spruce stands growing on soils of poor neutralising capacity (Solberg and Tørseth, 1997). By using crown density as an indicator of tree health, surveyors found no evidence to support the hypothesis that S and N deposition are having deleterious effects on crown condition. The authors stipulated that other environmental factors might be concealing any limiting effects that deposition may be exerting.

An earlier study on the effects of air pollutants (mainly S and N) on ecological forest condition in the eastern part of the Gulf of Finland was conducted by Haapala *et al*. (1996). Bioindication methods were based specifically on collected needles and more generally on tree specimens.

Twigs were removed from the sample plots and the number of needle age classes counted in addition to the number of needles in each age class. A sample of 100 needles from each plot were analysed as follows:

- chlorosis symptoms;
- necrosis damage;
- measurement of yellow or dry tips;
- coverage of green spots;
- needle length;
- five needle damage classes were established;
- needle damage index (DI) = $(n_1{*}1 + n_2{*}2 + n_3{*}3 + n_4{*}4 + n_5{*}5)/n$

where: n_1-n_5 = the number of needles in each damage class (1-5)
n = is the total number of needles (usually 100).

This index of damage based on a qualitative five-point scale has been used in other studies. For example in the Czech Republic (Tichy, 1996).

The following measures of pine tree condition were assessed:

- needle defoliation in the crown into five damage classes
- the number of needle age classes in the top third of the crown
- the percentage of chlorotic and necrotic needles.

Needle damage parameters did not show great statistical variation between sampling areas but some localised acute damage to needles was recorded. Needle age structure differed between areas, with general lowest needle longevity in the polluted regions. Defoliation was not regarded as severe and no significant differences between sites were obtained. By contrast epiphytic lichen distribution was seriously affected by pollution. The authors concluded that a whole array of abiotic and biotic factors affect tree stands and the effects of air pollution stress were not as apparent as observed for lichens.

The Canadian Forest Service have been monitoring the condition of Canadian boreal forests since 1984 under the auspices of the Acid Rain National Early Warning System (Hall, 1995). Two classification systems are applied: conifer tree crown and hardwood tree crown classification systems. The former is based on the amount (expressed in nine percentage classes) of defoliation in the crown. Hardwood crowns are classified into 13 classes according to the visible parts of the outer crown, the quantity and quality of foliage in the outer crown and the percentage of the outer crown that contains bare twigs and dead branches. By noting any observed effects of climatic conditions, nutrient deficiencies, air pollution, insects and diseases, poor tree condition and mortality was not attributed to long range transport of air pollutants.

SO_2 fumigation of 41 seedlings of herbaceous species in England resulted in reductions in dry mass of plants, roots and shoots in many of the species (Ashenden *et al.*, 1996). Antagonistic effects were more apparent on root growth than shoot growth. The former could possibly be used in bioindication of SO_2.

5.4.2.4 Industrial surveys

Taylor *et al*. (1990) review of the symptoms of acute SO_2 injury in vegetation will be summarised in the succeeding paragraphs. The authors also present in tabular form, concentrations of SO_2 known to cause injury in a variety of plants. Dose-response concentrations will depend on exposure time. Short, high doses are probably more representative of industrial emissions and/or accidents. It is also important to note that many of these concentrations will be based on experimental conditions and more field evidence is required.

Herbs/grasses/crops

The symptoms of acute damage (SO_2 >1 ppm) according to Kovács (1992b) are necrosis on the upper and lower leaf surfaces, at the apices, margins and between the veins. The tissues around the stomata may also decompose. Taylor *et al.* (1990) also reported watersoaked appearances on leaves in many species. Specific coloured leaf necrosis is common. For example light brown necrosis in daffodil, grey necrosis in geranium and black necrosis in broad beans. Tip necrosis on sepals has been observed in marigold and gladiolus.

Necrosis on awns of grasses and cereals has been reported. Barley, bracken and clover are regarded as very sensitive species to SO_2 exposure.

Observation of visible symptoms was used to assess air pollution burden in three locations in Egypt (Ali, 1993). The extent of chlorosis, necrosis, red pigmentation and growth parameters such as height, leaf area and stem diameter in clover and Egyptian mallow plants was generally related to pollution load.

To test the suitability of a variety of plant species as bioindicators, Agrawal *et al*. (1991) subjected the plants to two hours a day of 0.15 ppm SO_2 for a period of 30 days under controlled laboratory conditions. Most plants showed bifacial interveinal chlorosis and necrosis but the authors concluded that the use of foliar symptoms alone was not a specific enough method of bioindication unless one air pollutant dominated.

Coniferous trees

Acute visible injury affecting various parts of coniferous trees has been observed in response to SO_2. Young needles show chlorosis and are poorly developed and stunted. Middle aged needles often show yellow then red/brown discoloration succeeded by necrosis. Necrosis commonly affects the tip first but in larch and spruce the tip is often not the most sensitive part of the needle. Abscission of older needles in fir trees is frequently an immediate response but generally occurs after several months SO_2 exposure in pine trees. SO_2 exposure may bleach stems of young shoots.

Deciduous trees and shrubs

Larch is viewed as a particularly sensitive tree species to SO_2. Taylor *et al*. (1990) reported solely on leaf damage in broad-leaved trees and shrubs. Symptoms include interveinal chlorosis, irregular interveinal necrosis and abscission in many species. Necrosis characterised by brown/orange colouring has been revealed in lime, beech and hazel trees while black necrosis has been observed in pear trees. Distortion, puckering and curling of leaves has been detected in birch and maple. A scale to assess the extent of necrotic spots in birch (*Betula pendula*) was devised by Jäger (1980) as cited by Kovács (1992c). Observations of reduction in annual shoot growth in broad-leaved trees have been identified (Kovács, 1992a).

5.4.3 Sulphur content

Sulphate from external SO_2 can be accumulated in plant shoots, a portion of which may be transported to the root (Rennenberg *et al*., 1996). Miller (1989) reported on a few American studies of decreased S foliar tissue content with increasing distance to industrial works.

Manninen and Huttunen (1995) found very good correlations between S content in young Scots pine needles and SO_2 load. The authors concluded that needles under pollutant stress are extremely influenced by high short term SO_2 doses which have implications for the setting of critical levels to forest ecosystems. S concentration in Scots pine needles from forest of the Gulf of Finland correlated with S emissions in the region (Haapala *et al*., 1996). Regression analysis demonstrated pollution gradients in pine needle content with increasing distance from known pollutant sources. S content in two *Ligustrum* species at a variety of sites in Argentina was elevated in those sites with high traffic density (Carreras *et al*., 1996).

Foliar analysis of S and N was used to assess the air pollution burden in national parks and landscape protection areas in Slovakia (Mankovska, 1997). S content ranged from 0.72 to 6.77 g kg^{-1} in hardwoods and from 0.98 to 4.3 g kg^{-1} in softwoods. Results indicated cause for concern in some areas.

5.4.4 Biochemical/physiological response methods

5.4.4.1 Introduction

Plant responses depend not only on the characteristics of a species but also on the stage of development, age and nutritional status of the plants (Kovács, 1992b). The biochemical/physiological and ultrastructural changes discussed in the following paragraphs represent chronic damage to plants. The following information summarises and supplements the extensive review in this area undertaken by Saxe (1996). Much of the work on the effects of pollutants on plants is under controlled laboratory or field conditions using fumigation chambers. There is paucity in natural field based research or even correlations between laboratory and fieldwork. Open top chambers have tried to overcome some of the pitfalls of laboratory experiments but results should still be interpreted with caution.

The lack of availability of simple methods is a potential drawback to using responses at cellular/molecular levels for the bioindication of air pollutants. However, these techniques may often provide additional information on causal factors.

Agrawal *et al*. (1991) showed that different plant species showed varied responses to different parameters. For example, a species which showed the highest reduction in relative water content in response to SO_2 did not show the greatest reduction chlorophyll content. The choice of physiological/ biochemical/ultrastructural method will depend on the species.

This section will focus on plant responses, which can be used in bioindication. Areas of activity such as the mechanisms of air pollution effects on plants, dose-responses and gas absorption are beyond the scope of this text. Hippeli and Elstner (1996) and Winner (1994) treat some of these aspects.

5.4.4.2 Photosynthesis/stomatal conductance/transpiration

Measurement of photosynthesis and stomatal conduction are common measures of gaseous air pollution damage in that they respond quickly to air pollutants and can be measured by non-destructive techniques (Winner, 1989). They are obvious measures of gaseous air pollution in that the primary function of stomata in plants is gas exchange.

Csintalan and Tuba (1992) and Saxe (1996) reviewed published experiments of the effects of SO_2 on photosynthesis, stomata functioning and transpiration. Depending on dose, exposure time and species and other abiotic and biotic factors, SO_2 can increase or decrease photosynthesis or open or close the stomata.

Disruption of hloroplast metabolism has been implicated in the inhibition of photosynthesis due to SO_2 exposure under controlled conditions (Veeranjaneyulu et al., 1990). Stomatal conductance in association with S and N foliar content was used to assess the air pollution burden in national parks and landscape protection areas in Slovakia (Mankovska, 1997).

5.4.4.3 Chlorophyll

In their study of suitable biomonitoring parameters, Agrawal *et al*. (1991) suggested that determination of total chlorophyll level could be a good bioindicator of chronic SO_2 conditions. Total chlorophyll is measured as part of the APTI mentioned in Section 5.3.1.

Chlorophyll reduction directly relates to damage in plants (Heath, 1989). However it is often regarded, like chlorosis, as a non-specific indication of SO_2 stress. Chlorophyll content was lower in one year old needles of damaged spruce trees in comparison with healthy specimens when studied in three different sites in northern Germany (Godbold *et al*., 1993). No correlation with a specific pollutant was made. Total chlorophyll determination in potted plants transferred to three different locations in Egypt showed correlation between pollution burden at the sites (Ali, 1993). Both clover and Egyptian mallow indicated up to 29% reduction in total chlorophyll levels in plant leaves grown in the most polluted sites. Also under field conditions Pandey and Agrawal (1994) found reductions in chlorophyll content in leaves of three woody perennials in an urban area of India. Chlorophyll levels were correlated with ambient SO_2 concentrations.

The usefulness of chlorophyll fluorescence has been discussed in Section 2.3.3.2. This method is regarded as a more sensitive measure of photosynthetic activity than pigment content and more specific than the measurement of photosynthetic rate (Saxe, 1996). Chlorophyll fluorescence assessment is non-destructive and can be achieved with relatively little effort in the field (Saarinen and Liski, 1993). Chlorophyll fluorescence using a portable fluorometer in the field and pigment (chlorophyll a and carotenoid) content determined by laboratory analysis in Scots pine needles were mapped around the vicinity of an oil refinery in Finland by Saarinen and Liski (1993).

5.4.4.4 Metabolite content

Saxe (1996) provides several examples of metabolic changes to plants induced by air pollutants. These include changes in amino acid, polysaccharides and ATP/ADP ratios. A recent study by Julkunen-Titto and Lavola (1995) demonstrated changes in the production of phenolic secondary chemicals and soluble sugars by willow species in response to 0.11 ppm SO_2 fumigation for three weeks.

5.4.4.5 Enzyme activity

Enzyme activity has been used as a biochemical stress bioindicator of air pollutants. In Germany, acid phosphatase and peroxidase activity in needles of healthy Norway spruce trees was generally lower than in damaged trees (Godbold *et al*., 1993). However, the authors could not relate these effects to specific stress factors. Saxe's (1996) review summarised similar findings.

5.4.4.6 Ultrastructure

Saxe (1996) and Berg (1989) documented varying responses of leaf cuticles to pollution stress in the published literature and concluded it was not a very specific bioindication tool. By applying electron microscopy, Manninen and Huttunen (1995) observed that the epicuticular wax structure of Scots pine needles was very badly degenerated in trees in close vicinity to an oil refinery in southern Finland. The destruction rate of the needle surface wax decreased with decreasing S content in needles.

5.4.4.7 Tree ring analysis

Cook and Innes (1989) reviewed the value of tree ring analysis in assessing the impact of lower-level regional air pollution on forests. Tree ring patterns offer a long-term, baseline dataset whereby changes in growth (displayed as annual tree-ring increments) in response to pollution stresses can be detected. This method is complicated by the fact that air pollution effects on ring increments are not necessarily distinct and may be prone to misinterpretation. The potential of tree-ring analysis as a bioindication method in air pollution diagnosis is obvious. However, further research in this field is necessary to determine the credibility and future of the technique.

Currently, increments can be quantified non-destructively or destructively. Non-destructive analysis measures the radial ring widths from cores taken at breast height of a tree. Detailed stem analysis is destructive but provides a more accurate measure of annual volume increment and complete growth layer profiles.

A more recent review of the application of tree-ring analysis in air pollution studies is presented by Turcsanyi (1992).

5.4.4.8 Root growth and functioning

By effecting photosynthesis and translocation, gaseous pollutants may reduce carbon allocation to roots. This in turn may reduce root growth, turnover and capacity for water and nutrient uptake. Richards (1989) reviewed the techniques used to measure these responses in their application in the monitoring of gaseous air contaminants.

5.4.4.9 Multivariate methods

Any response method is complicated by the fact that several factors may cause the same or similar reaction. Specificity is enhanced if a number of these responses are observed together (Saxe, 1996; Miller, 1989). Combining several response parameters into multivariate indices is a promising tool recommended by Saxe (1996).

Four pollution zones around a power plant in India were observed by analysing changes in a selection of physiological and biochemical parameters in plant leaves (Agrawal *et al*., 1991). Correlations between ambient SO_2 concentrations and decreases in the levels of chlorophyll, ascorbic acid and specific leaf area and increased S content were obtained. The authors recommended this type of monitoring as an essential companion to chemical monitoring in India.

5.4.5 Transplants

Little published literature appears to exist in relation to transplantation of higher plants to polluted sites. A method known as grass cultures was discussed by Weinstein and Laurence (1989). This is a system predominately utilised in Germany whereby self-watering rye grass cultures are established and samples removed at specific intervals. Samples are analysed for SO_2, F or heavy metals. More details of the system are provided in Kovács (1992a).

Specimens of the tropical shrub *Carissa carandas* grown in controlled pot conditions were transplanted to a selection of sites of varying pollution load in India for a period of two years. At four monthly intervals plant growth and morphological parameters such as height, leaf number, extent of chlorosis and necrosis and basal diameter were observed. The aim of the study was not primarily for biomonitoring purposes but to establish the adaptational responses of the shrub.

5.5 Fluoro-compounds

5.5.1 Introduction

The most phytotoxic and best studied fluoro-compound is hydrogen fluoride gas. Other gases include silicon fluoride (SiF_4) and fluorine (F_2). In the following discussions fluoride as reported in the literature is thought to represent the total fluoro-compounds measured unless otherwise stated. Fluorides differ from other gaseous air pollutants in that they are readily accumulated in tissues. Therefore, chronic exposure over long periods of time can result in translocation to leaf tips and margins where visible symptoms are exhibited.

Many of the factors which affect plant sensitivity to SO_2 are applicable to fluorides. An interesting observation in relation to fluoride is that different cultivars of the same plant species react in very different ways to the pollutant. For example, there is a large variation in the sensitivity of different cultivars of gladiolus and tomato plants. The susceptibility of gladiolus varies with flower colour.

5.5.2 Visible injury

5.5.2.1 Herbs/grasses/crops

The following paragraphs summarise the reviews by Kovács (1992b) and Taylor *et al*. (1990) on fluoro-compounds (measured as total fluoride content) and air pollution injury. The characteristic symptoms of fluoride damage are tip and marginal chlorosis which later extend to the inter-venial areas. This is succeeded by tip and marginal necrosis which gradually covers the whole leaf area. Loss of leaf may result. Ivory necrosis has been observed on tomato, wheat and oats, red/brown in St. Johns Wort and black in dahlia. Tip necrosis has been recorded on awns and bracts and marginal necrosis on sepals and petals.

Kovács (1992b) presented a scale devised by Arndt *et al*. (1984) which relates the magnitude of leaf necrosis in vine leaves to fluoride pollution (Table 3.6).

Table 3.6 Scale relating vine leaf necrosis to fluoride pollution (from Arndt *et al*., 1984)

Damage class	Symptoms
No recognizable damage	Slight apical and spot necrosis on every tenth leaf No difference in growth vigour
Slight damage	Recognizable necrosis on every fifth leaf Necrosis damage approx. 2 to 3 cm^2 Growth of axillary shoots normal
Medium damage	Every second leaf is necrotic Growth of entire plant reduced
Extensive damage	Assimilating surface of every leaf is reduced Entire marginal area is necrotic Tendrils are shorter
Most extensive damage	Each leaf damaged Abscission occurs Tendrils stunted

Total damage	>80% of leaves are entirely necrotic Axillary shoots and tendrils are missing Growth is greatly reduced

Gladiolus cv. White Friendship and *Hemerocallis* cv. Red Moon were cultivated and exposed to twelve sites in the region of Cubato and the Serra do Mar at varying distances from hydrogen fluoride (HF) emission sources for 28 days (Klumpp and Klumpp, 1994). HF induced injury, estimated by measuring the area of tip and margin necroses on the sample plants, was most severe at sites nearest the fluoride-emitting fertiliser industries.

5.5.2.2 Coniferous trees

In coniferous trees, young chlorosis in young needles is a common symptom of fluoro-compound contamination, followed by red/brown discolouration. Tip burn may occur which usually results in necrosis of the whole needle. Frequently necrosis appears as red/purple bands distinctive from healthy tissue.

5.5.2.3 Deciduous trees and shrubs

The leaves of deciduous trees and shrubs show marginal and tip necrosis which turn into sharply defined red/brown bands between necrotic and healthy tissue. Necrosis in fruiting parts of trees has also been observed.

5.5.3 Fluoro-compound accumulation

Miller (1989) reported on a few American studies of decreased fluoride foliar tissue content with increasing distance to industrial works. As part of an active biomonitoring scoping study in Brazil, Klumpp and Klumpp (1994) found that foliar fluoride concentrations in transplanted *Gladiolus* cv. White Friendship and *Hemerocallis* cv. Red Moon correlated with severity of fluoride-injury. Plants at close vicinity to fluoride-emitting fertiliser industries contained fluoride content of 80 to 120 µg g^{-1} dry weight compared to content of >10 µg g^{-1} dry weight at reference sites. Chemical analysis of transplanted standardised grass cultures of *Lolium* showed that it was a much more effective bioaccumulator of fluoride than the two foregoing plant species.

5.5.4 Biochemical/physiological response methods

Photosynthesis is inhibited at relatively low hydrogen fluoride (HF) concentrations and this may be a potential bioindicative response. Plant stomatal conductance probably shows the greatest variation in response to HF than any other gaseous air pollutants (Csintalan and Tuba, 1992).

5.6 Nitrogen oxides and ammonia (NO_x and NH_3)

5.6.1 Introduction

Nitrogen content in plants is regarded as a questionable bioindicator of NO_x and NH_3 because it so easily translocated throughout the plant (Saxe, 1996). However, some crop species such as bean, leak and pea are regarded as very sensitive to nitrogen oxides. Kovács (1992b) lists sensitive and accumulating plant indicators of nitrous gases. Young leaves and needles are more sensitive to NO_x than older ones. High relative humidity and N deficiency increase plant sensitivity to $NO_{x,}$ whereas N excess and drought conditions decrease sensitivity. NH_3 has received less attention in the literature than NO_x.

5.6.2 Visible injury

Relatively higher concentrations of NO_2 are needed to produce acute symptoms on plants in comparison to SO_2. Different plant groups react differently to NO_x and NH_3 (Taylor *et al*., 1990). Concentrations of these two pollutants known to cause injury in a variety of plant species are found in Taylor *et al*. (1990). Most reports of acute pant injury in response to NH_3 are in relation to accidental release or spillage.

Herbs/grasses/crops

NO_x

Many species show a watersoaked appearance on the leaves followed by necrosis in response to acute NO_x exposure. Leaf glazing has been observed in annual poa, cabbage and spinach. Necrotic streaking and interveinal necrosis has been recorded in many narrow-leaved and broad-leaved species. Legumes among many other species show ivory necrosis while some species display yellow, orange or brown necrosis. Tip necrosis has been observed on other plant parts such as awns, bracts and sepals.

NH_3

Yellow discolouration, watersoaked appearance, glazing and bleaching have been observed on leaves in response to NH_3. Red/purple discolouration of leaf upper surfaces in cereals is common. Ivory necrosis, reddish necrosis and brown/black necrosis are typical NH_3-injury symptoms in certain species.

Coniferous trees

NO_x

Chlorosis of young needles is a common symptom in response to NO_x. Tip burn of older needles is often observed in coniferous species. Pine trees display bleaching followed by sharply defined red/brown bands between necrotic and healthy tissue in older needles. Immediate abscission of older needles occurs in spruce.

NH_3

Red/yellow discolouration of young spruce needles has been observed. Many coniferous species display black discolouration and tip burn of older needles as NH_3-injury symptoms.

Deciduous trees and shrubs

NO_x

Herringbone necrosis in the older leaves of beech, hazel and apple trees have been observed. Ivory necrosis, red/brown necrosis and black necrosis have been recorded in certain species.

NH_3

Yellow discolouration of leaves has been observed in sycamore species. Other symptoms recorded in many species include watersoaked appearance on leaves, intercostal necrosis and finally desiccation and abscission of damaged leaves.

5.6.3 Biochemical/physiological response methods

Published literature with respect to the effects of NO_x and NH_3 is limited and literature on the use of biochemical and physiological responses as bioindicators is even sparser.

Plant photosynthesis and stomatal conductance are thought to be relatively tolerant to NO_x exposure (Csintalan and Tuba, 1992).

Nitrate reductase activity in plants has been measured in response to nitrogen deposition. Addition of ammonium nitrate to experimental grassland plots in the UK increased nitrate reductase activity in vascular plants (Morecroft *et al*., 1994). Krywult *et al*. (1996) found higher nitrate reductase activity in ponderosa pine needles correlated with sites characterised by higher nitrate deposition to branches. However, the authors concluded that nitrate reductase activity was not a sensitive enough parameter to be used in bioindication studies because it is influenced by many other biotic and abiotic factors.

Other enzymes may have potential as useful response parameters in nitrogen deposition bioindication studies. The possible role of the enzyme glutamate dehydrogenase in the adaptation of plants to ammonia assimilation with respect to air pollution was discussed by Schlee *et al*. (1994). The activity of this enzyme may be used in bioindication of nitrogen deposition but substantial further work would be required. Glutathione reductase and ascorbate peroxidase activity in red spruce needles was enhanced by exposure to acidic mists (Chen *et al*., 1991).

Soares *et al*. (1995) proposed the use of multivariate analysis of physiological and biochemical parameters such as enzyme activity and total nitrogen content of woody and herbaceous plant species as a means of assessing plant susceptibility to acid rain.

5.7 *Ozone (O_3)*

Currently tropospheric O_3 is probably the gaseous pollutant of most concern to plant scientists. Estimates suggest that tropospheric O_3 concentrations have increased substantially over the last few decades. The available literature on the adverse effects of O_3 on forest and crops has accelerated over the last 10 to 15 years. Much effort has been placed on finding suitable cultivars for bioindication purposes.

In the assessment of O_3 on plants de Leeuw and van Zanvoort (1997) claimed that the spatial distribution of O_3 over a particular area is of more interest than concentrations recorded at monitoring stations. Plant biomonitoring techniques are particularly useful in establishing the distribution of air pollutants in a study area.

Ozone is not bioaccumulated in plants and can only be detected by sensitive plants. The major effects of O_3 on terrestrial vegetation include visible foliar injury, reductions in growth and productivity, changes in crop quality and increased sensitivity to either abiotic or biotic stresses.

5.7.1 *Visible injury*

5.7.1.1 Introduction

With respect to O_3, most attention in the published literature has been placed on observations of visible injury in plants and trees. Much of the work reported here is from field based studies but fumigation studies are still necessary.

Taylor *et al*. (1990) presented typical acute O_3 injury symptoms. As before these can be divided into plant type. O_3 symptoms tend to be exhibited in plant species after a relatively short exposure time.

Herbs/grasses/crops

Most visible O_3 injury symptoms have been described for crop species. In general, white, fawn, tan, grey and brown necrotic streaks on the upper surface of leaves are typical of O_3 injury. Kovács (1992b) presented typical O_3 injury symptoms on certain crop species. For example, bean plants show foliar browning and chlorosis, cucumber plants display white stipple, onion plants show white flecks and tip dieback while spinach plants exhibit grey to white flecks on leaves.

Coniferous trees

Chlorotic flecks later becoming pink lesions followed by orange-red tip necrosis have been observed on current year pine needles in response to O_3.

Deciduous trees

A variety of O_3 injury symptoms have been observed in deciduous trees and shrubs. Ash and maple show dense purple or reddish stipple on upper leaf surface. Many species including lime and apple show leaf bronzing while some species such as birch display leaf bleaching. Leaf curling and tip drying have been observed in lilac. Fruits are often affected and may prematurely drop
(e.g. citrus trees).

5.7.1.2 Multi-national surveys

At one time, elevated concentrations of O_3 were thought to be restricted to urban areas but currently larger scale biomonitoring surveys of O_3 are in the increase. Visible damage to vegetation and agricultural crops over wide areas is becoming an important problem. Medium and long-range transport of O_3 precursors and O_3 itself have resulted in increased concentrations in rural areas. Bioindicators have been used to evaluate the effects of O_3 pollution in a number of impact areas.

The United Nations Economic commission for Europe (UN ECE) Convention on Long-range Transboundary Air Pollution includes an international co-operative programme to evaluate the effects of air pollution on agricultural crops. This programme has three main aims:

- evaluate the effects of ambient concentrations of air pollution on crop production;
- assess the importance of environmental conditions in affecting crop response to air pollutants;
- determine the influence of different aspects of pollutant exposure (e.g. combinations of pollutants, concentration peaks and means) on crop response.

An initial stage of this project was to select a single species which could be used as a convenient bioindicator of phytotoxic effects of pollutants. Jones *et al*. (1991) reported on the potential of radish (*Raphanus sativus* L. cv. cherry belle) as a bioindicator for this programme. Radish seedlings were subjected to one of three treatments – placed in unfiltered, open top chambers, placed in filtered chambers or treated with ethylenediurea (EDU protects plants from O_3). Results demonstrated a reduction in radish plant yield (measured as shoot and root dry weight and leaf area) in the unfiltered chambers in the month of July. The authors concluded that cherry Belle radish plants were sensitive enough bioindicators to be used in a co-ordinated programme to evaluate the effects of O_3 on crop plants.

5.7.1.3 Regional surveys

From the published literature, Tobacco plants (*Nicotiana tabacum*), in particular variety Bel-W3, are the most widely used bioindicators of O_3 for the following reasons (Kovács, 1992b; Koppel and Sild, 1995):

- the plants are easy to grow;
- they are susceptible to air pollution;
- they show a relatively clear reaction to oxidants;
- symptoms are easily identifiable (specific necrotic flecks);
- the sensitivity of leaves is a function of leaf age, so that new damage can be distinguished from previous damage;
- leaf injury in Bel-W3 can be quantified using Leaf Injury Indices (LII) (reviewed by Burton, 1986).

The drawbacks of using tobacco plants are their susceptibility to diseases and pests and it can only be used during a limited period of the year. A review of the use and origin of tobacco cultivars until 1990 is presented in Heggestad (1991). A variety of techniques using tobacco have been employed in O_3 biomonitoring on a regional context.

Schenone and Lorenzini (1992) used three techniques to study the effects of regional air pollution on crops in northern and central Italy. The extent of ivory interveinal flecks on *Nicotiana tabacum* cv. Bel-W3 correlated with ambient
O_3 concentrations indicating that levels exceeded the threshold for phytotoxic effects. Ambient air pollution exclusion experiments with open-top chambers demonstrated air pollution effects on crop productivity. Furthermore in controlled SO_2 fumigation chambers plant growth and physiology was only affected at concentrations above those measured at rural sites in Italy. This collaborated work confirmed that O_3 was the most important phytotoxic gas at a regional level in northern and central Italy. Further work in this area has continued over the past five years.

By estimating the percentage increase in injured leaf area (necrosis) of cultured semi-mature *Nicotiana tabacum* cv. Bel-W3, Mignanego *et al*. (1992) calculated LIIs at 23 sites in northern Italy over a six month period. Leaf injury was most pronounced in the summer months. This is the time of year when photochemical smog (of which O_3 is a major constituent) is generally at its highest. LII monthly and seasonal averages were calculated. The authors were able to characterise sites depending on their seasonal indices (SI) values.

Lorenzini *et al*. (1995a) reported on a modification of the procedures undertaken by Mignanego *et al*. (1992). An easily transportable miniaturised kit utilising two week old ozone supersensitive tobacco (Bel-W3) seedlings was used to monitor O_3 at 27 sites of varying pollution burdens in Tuscany, Italy. Design details are discussed in detail by Lorenzini (1994). In summary, tissue culture plates were used and seedlings were grown in an ozone-free environment before being transplanted to the monitoring sites for one week. The severity of O_3 pollution was assessed by recording the percentage area of injured cotyledon and leaf compared to control plants. Previous pilot fumigations studies validated that visible O_3 foliar injury in younger leaves, characterised by grey water-soaked marks (flecking), was analogous to those observed in mature leaves. Cotyledon injury showed good correlation with a number of O_3 parameters. This method was also successfully used to map O_3 distribution across a wide geographical area over a land-sea transect in Italy (Lorenzini *et al*., 1995b). The authors recommended the application of this simple, cost effective method for biomonitoring in large areas in developing countries.

Another approach using tobacco to estimate the phytotoxic effects of existing O_3 levels in two regions over a three year period in Spain was reported by Gimeno *et al*. (1995a). Three tobacco cultivars, Bel-W3, Bel-C and Bel-B of increasing sensitivity to O_3 were grown in greenhouses to the fourth true leaf stage before being transferred to sampling locations spread over the two regions. Ozone induced visible injury was assessed and mapped according to the classifications described in Table 3.7. This study demonstrated the need to consider environmental conditions in O_3 biomonitoring and in the calculating critical levels. At coastal sites the effects of relative humidity appeared to enhance O_3 injury in the sample plants. The use of the foregoing three cultivars was recommended by Heggestad (1991).

Table 3.7 Characterisation of O_3 phytotoxicity using three tobacco varieties (from Gimeno *et al*., 1995b)

Ozone phytotoxicity	Observation
Low	O_3 injury recorded on Bel-W3 cultivar only
Medium	O_3 injury recorded on Bel-W3 and Bel-C cultivars
High	O_3 injury recorded on Bel-W3, Bel-C and Bel-B cultivars

Two varieties of tobacco, BelW3 (indicator) and Samsun (control) were used to study O_3 contamination in four locations in Estonia over a two year period (Koppel and Sild, 1995). Plants were grown in chambers for approximately 3.5 months prior to transplantation to the sampling areas. By observing leaves >10 cm in length, necrotic leaf indices (NI) were estimated as the percentage of the leaf blade covered with necrotic flecks. The mean daily increment of the necrotic index for the period between two observations (typically 6 to 14 days) was calculated for each plant (NII_{plant}) and for the site (NII_{site}) where the later was used to indicate the variability of O_3 episodes during the growing period. Highest NII_{site} values corresponded to the site closest to major thermal plants and cities in Estonia. As may be expected O_3 injury was greatest in the year with the warmest summer. Temperature data is therefore a further consideration in O_3 biomonitoring and the setting of critical levels.

Other crop species besides tobacco have been used in O_3 biomonitoring studies. Watermelon (*Citrullus lanatus*) was used as a passive bioindicator of O_3 contamination in eastern Spain (Gimeno *et al*., 1995b). This study differed from the foregoing investigations in that it did not involve transplantation of plants grown from seed. Commercial fields of watermelon were visited and O_3 injury was evaluated using the classification outlined in Table 3.8. O_3 injury was widespread throughout the study area but varied in intensity. The authors recommended that all the considerations mentioned in Section 5.3.3 above should be adhered to during field sampling. Furthermore, plant canopy structure and the agricultural practices that affect it should be considered. This passive method of biomonitoring is simpler than transplantation exercises. However a drawback of using this approach over different areas is that the sample plants may be affected by other conditions unknown to the observer such as contaminated soil or drought.

Table 3.8 Classification of O_3 injury in watermelons in eastern Spain (from Gimeno *et al*., 1995b)

Class*	Injury description
I	< 10% of foliar area affected brownish-red spots diffuse.
II	between 10 and 40% of foliar area affected increase in number of spots.
III	between 40 and 80% of area affected most of upper surface of leaf covered by brownish-red spots
IV	> 80% injury white necrotic areas covering most of leaf

* Injury classes are a function of both O_3 concentration and duration of exposure.

Measurements of foliar injury in trees and shrubs have been used in the evaluation of regional O_3 pollution. The incidence and severity of O_3 injury in black cherry (*Prunus serotina*) was used to assess the impact and extent of O_3 pollution in Great Smoky Mountains National Park (Chappelka *et al*., 1997). Incidence was defined as the number of individuals with visible foliar symptoms of O_3 injury. Severity was measured as the percentage of foliage injured per plant and as the percentage of leaf area injured for the injured leaves. Statistical analysis was used to determine significant injury levels within different park areas. Skelly *et al*. (1997) also used black cherry in a study of the Desierto de Los Leones National Park in Mexico City. A sample of mature trees were subjected to an intensive survey, where the extent of surface stipple, leaf reddening and premature senescence was recorded. A more general survey estimated the percentage of trees exhibiting O_3 induced injury.

Miller (1989) reported the common visual symptoms used in ozone-injury detection in coniferous forests in the United States:

- stimulated removal of older foliage;
- barren branches near the bole;
- lower branches possess fewer and shorter needles;
- crown deteriorates from inside-out and the bottom-up.

The same paper reviewed some pine tree injury indices caused by ozone. Crown density, extent of chlorotic mottle on each whorl, needle length and extent of needle retention appear to be useful parameters in the calculation of combined indices.

5.7.1.4 Urban/industrial surveys

At present, most O_3 injury based investigations are concerned with crops and forests over wide areas and urban and industrial point source investigations are limited.

De Bauer and Krupa (1990) summarised observations of typical O_3 injury symptoms to assess the air quality burden on vegetation in Mexico city over the past twenty years. Allegrini *et al*. (1994) favoured integrated evaluation of tropospheric O_3 pollution in urban and semi-rural areas in Italy using physico-chemical and biological monitoring. Reduction in biomass and leaf area of radish plants (*Raphanus sativus* L. cv. Cherry Belle) correlated negatively with ambient O_3 concentrations. The sensitivity of this cultivar favours their use as early warner systems to indicate that injurious levels of O_3 had been reached.

Kovács (1992b) presented a table indicating potential dangers to humans from O_3 exposure by correlating effects with O_3-injury symptoms displayed by tobacco. This is duplicated as Table 3.9.

5.7.1.5 Experimental studies

Literature with respect to laboratory and field studies using different types of chambers under ambient and fumigation conditions is extensive. Such studies are essential in establishing plant thresholds and responses prior to adaptation to natural conditions. For example, open top chamber experiments have been used to determine the most sensitive plant species to be utilised in biomonitoring studies. Such studies have been useful in classifying quantitative responses of plants to actual O_3 exposure.

Gimeno *et al*. (1995b) classified watermelon injury into four classes depending on the number of diurnal hours O_3 concentrations > 40 ppb using open top chamber experiments. This classification was then applied in a passive biomonitoring programme to determine the intensity and extent of crop damage in eastern Spain.

The chemical ethylenediurea (EDU), when applied to plants as either a soil drench or to foliage, can protect the plant against elevated O_3 concentrations. Hence the response of EDU-treated and untreated plants can be compared. However, the effectiveness of EDU is variable and this procedure should be used with caution (Jones *et al*., 1991).

Table 3.9 The effect of O_3 on humans and tobacco plants (from Kovács, 1992b)

Short-term O_3 concentration	**Physiological effect on:**	
	humans	**tobacco (Bel-W3)**
0.13 - 0.23	Decreased performance in sports (the partial pressure of blood oxygen decreases)	At high temperature and humidity the first flecks appear on the leaves
0.4 - 0.6	The mouth and throat become dry during sport; chest pains develop leading to asthmatic symptoms	The extent of flecks amounts to 50%
> 0.7	Without physical effort respiratory problems occur	Leaf fleck >90%
1 - 1.2	Premature death of ill and aged persons	Decay of plants

Most experimental studies are in relation to effects of O_3 on crops and in establishing dose-response relationships but the information generated is often useful in determining sensitive species and potential bioindicators. Table 3.10 summarises some recent experimental procedures used to assess the response of certain plant species and cultivars to ambient and elevated O_3 exposure.

This table is purely an illustrative guide to some examples of the published literature in different countries and is by no means exhaustive.

Table 3.10 Use of chamber experiments to investigate O_3-injury in a variety of plant species

Reference	Location	Experimental procedure	Species and cultivar	Response
Brennan *et al*., 1990	USA	*EDU treated and untreated plots	Soybean (*Glycine max* L. Merr.)	Foliar damage
Carey and Kelley, 1994	USA	Open top chamber using ambient air, carbon filtered air and non-filtered air plus extra O_3	Loblolly pine seedlings (*Pinus taeda)*	Growth parameters
Clarke *et al*., 1990	USA	EDU treated and untreated plots	White potato (*Solanum tuberosum*)	Foliar damage
Kasana, 1991	England	Closed-chamber fumigation	Chick pea (*Cicer arietinum*), Black-gram (*Vigna mungo*), fenugreek (*Trigonella foenumgraecum*)	Growth parameters
Keller, 1988	Switzer-land	Closed chamber fumigation	American aspen (*Populus tremuloides* Michx.)	Growth parameters
Kobayashi *et al*., 1994	Japan	Closed chamber fumigation	Rice (*Oryza sativa* L.)	Yield and growth parameters
Kraft *et al*., 1996	Germany	Open-top chamber using charcoal filtered air and charcoal filtered air supplied with O_3	Spring wheat (Triticum aestivum cv. Turbo), white clover (Trifolium repens cv. Karina), maize (Zea mays cv. Bonny)	Foliar damage symptoms and spectral reflectance measure-ments

Table 3.10 (continued)

Maggs *et al*., 1995	Pakistan	Open-top chamber using ambient and charcoal-filtered air	Wheat and rice	Yield parameters e.g. grain weight
Meier *et al*., 1990	USA	Closed chamber fumigation	Loblolly pine (*Pinus taeda*)	Growth parameters
Paakkonen *et al*., 1997	Finland	Open-top chamber using charcoal filtered air and charcoal filtered air supplied with O_3	Betula pendula, Betula pubescens	Foliar damage and growth parameters
Pleijel *et al*., 1991	Sweden	Open top chamber using ambient air, charcoal filtered air and non-filtered air plus extra O_3	Spring wheat (*Triticum aestivum* L., cv. Drabant)	Yield and grain quality parameters
Renaud *et al*., 1997	Canada	Open top chamber using ambient and filtered air	Alfalfa (*Medicago sativa* L.)	Yield and growth parameters
Runeckles *et al*., 1990	Canada	Zonal Air Pollution System (ZAPS) simulating various exposure regimes	Processing peas	Yield parameters
Soja and Soja, 1995	Austria	Closed-chamber fumigation	Winter wheat	Yield parameters

* EDU- Ethylenediurea (protective chemical against O_3 exposure)

5.7.2 *Biochemical/physiological response methods*

5.7.2.1 Photosynthesis/stomatal conductance/transpiration

Stomatal response to O_3 varies between species and even cultivars. Some species show an increase in stomatal conductance, some show a decrease and some are unaffected. However at concentrations above 200 ppm O_3, most plants close their stomata (Csintalan and Tuba, 1992).

Under controlled conditions, loblolly pine seedlings exposed to 0.12 ppm O_3 for seven hours per day, five days a week for 12 weeks, displayed a 16% reduction in photosynthesis in comparison with plants exposed to charcoal-filtered air (Spence *et al.*, 1990). The same plants failed to exhibit visible injury under the elevated O_3 conditions. Schenone *et al*. (1994) used open top chambers to compare field grown bean plants under ambient conditions and filtered air. Net photosynthesis was less in ambient air. Visible injury was not detected. These experiments demonstrate the potential of such parameters as early warning bioindication techniques.

A drawback of using photosynthesis, stomatal conductance and transpiration in biomonitoring of O_3 is their lack of specificity.

5.7.2.2 Chlorophyll

Chlorophyll levels are a direct measure of leaf damage but again are not a specific measure of plant damage to pollutant type.

Tenga and Ormrod (1990) measured total chlorophyll concentration tomato plant leaves using a leaf greenness meter. Chlorophyll levels decreased with increased O_3 exposure at levels too low to cause visible damage. Smith *et al*. (1990) and Fernandez-Bayon *et al*. (1993) also reported reductions in chlorophyll content prior to the onset of visible injury.

5.7.2.3 Metabolite content

Metabolite content has been recommended as a potential response parameter in air pollution biomonitoring (Saxe, 1996). Fumigation of Aleppo pine with O_3 resulted in delayed rate of ethene emissions, accumulation of total polyamines and increase pool sizes of reduced glutathione and ascorbate in current year needles (Wellburn et al., 1996).

5.7.2.4 Enzyme activity

All major air pollutants effect enzymes and their activity (Saxe, 1996). O_3 fumigation reduced light regulation of Rubisco activity in barley leaves (Machler *et al*., 1995). Alternatively, Ranieri *et al*. (1994) demonstrated increased peroxidase-catalase detoxification in response to exposure to ambient air in open-top field chambers in Italy.

5.7.2.5 Ultrastructure

Turcsanyi (1992) reviewed plant cells and tissues as bioindicators of environmental pollution and found that ultrastructural responses to O_3 were not very specific. The effects of peroxy-acetyl-nitrate and O_3 are often indistinguishable.

Degeneration of needle wax layer in response to SO_2 has been demonstrated (Section 5.4.4.6) but Barnes *et al*. (1990) discovered that O_3 did not significantly alter the needle wax layer in Norway spruce.

5.8 Complex studies

In nature pollutants often occur in combination with other pollutants. It is therefore necessary to investigate the effects of pollutant mixtures on plants. Responses which allow the identification of pollutant mixtures would be extremely useful in bioindication. Integrated usually involve fumigations and open or closed chamber systems. It is often necessary to rank pollutants according to their degree of damage. Obviously, studies using most natural conditions and pollutant levels are more useful. The more information obtained from complex studies the easier it would be to ascribe different responses to different sources and emission types.

Generally, plants are more severely affected by mixtures of pollutants than individual pollutants. For example, the threshold limit of tobacco Bel-W3 to O_3 is reduced if low concentrations of SO_2 are present (Heggestad, 1991). Closure of stomata occurs at lower concentrations of O_3 if O_3 and SO_2 are present together (Csintalan and Tuba, 1992). Pollutant mixtures may produce synergistic, additive or antagonistic responses. A combination of O_3 and HF enhanced senescence in maize which was not apparent when similar concentrations of the pollutants on their own were applied (MacLean, 1990).

Heck (1989) summarised a number of studies involving pollutant mixtures including exposure information and plant responses. Most work has been in relation to mixtures of O_3 and SO_2 (for example, Fialho and Bucker, 1995; Mcleod, 1995). To a lesser extent SO_2 and NO_2 combinations have been investigated (Kasana and Lea, 1994) and sometimes a combination of all three are studied (for example, Bucker and Guderian, 1994).

Some studies have investigated metal and gaseous pollutant mixtures (Dueck *et al*., 1987; Keller and Matysseck, 1990; Edwards *et al*., 1992). Others have focused on the effects of gaseous pollutants and acid rain mixtures on vegetation (Blank *et al*., 1990; Blaschke and Weiss, 1990; Ashenden *et al*., 1996; Shan *et al*., 1996).

6 Conclusions

The majority of published literature with regard to gaseous air pollution is concerned with SO_2. However in response to a decline in SO_2 levels since the 1970s focus has increased with regard to other pollutants such as NO_x , NH_3 and $O_{3.}$.

Lichens are the most widely recognised plant group in air pollution monitoring and will probably continue to be in the near future. Lichens are particularly useful in indicating pollution loads over long periods. Lichen surveying is routinely included as part of international and national monitoring programmes. Urban and industrial pollutant monitoring generally involves classification and mapping of pollutant zones and calculation of pollution indices. Responses of lichen species and communities has been related quantitative to air pollutant concentrations but often this would still require measurement of physio-chemical data. Recent developments show an increased emphasis on the use of biochemical and physiological responses of lichens as indicators of air pollution, probably due to technological advancement. A multivariate approach using a number of lichen parameters such as community changes, visible injury, physiological effects and element content would provide an integrated approach to air quality assessment.

Most bryophyte studies are associated with regional and urban sulphur dioxide (SO_2) contamination. On a national and multi-national monitoring scale, bryophytes are used more as bioaccumulative indicators of aerial metal contamination than as bioindicators of gaseous air pollution.

The use of fungi in air pollution monitoring is generally limited in comparison to lichens and bryophytes but recent developments have proposed their use in the assessment of pollution impact on forests. Leafyeasts have also been proposed as bioindicators of SO_2 pollution in urban and rural areas.

An increase in epiphytic algal growth usually coincides with a reduction or disappearance in epiphytic lichens since green algae respond positively to increased nitrogen. This group therefore have potential as bioindicators with regard to the recent increases in N deposition. Aerial green algae are currently used in international and national monitoring programmes in this respect.

In their comparisons of SO_2 absorption capacities between vascular plants, mosses and lichens in a range of habitats, Winner *et al.* (1988) concluded that the cryptograms are much stronger SO_2 sinks and therefore more sensitive to this pollutant than vascular plants. However for O_3 the use of higher plants as bioindicators of crop and forest injury looks more promising. The use of higher plants in bioindication is relatively recent in comparison to lower plants. Generally the exposure time of higher plants to a pollutant is known because observation is normally restricted to the vegetation period. The detection of visible symptoms is the most widely recognised bioindication method using higher plants. However, symptomatic bioindication utilising higher plants may not always assist in the protection of vegetation. This is because air pollution may affect plant species at levels less than those resulting in visible injury. Furthermore visible injury is not specific to particular environmental stresses. Most studies using higher plants employ a bioassay approach whereby plants from standard genetical origin and state of development are utilised. This ensures that plant responses indicate pollution damage and does not reflect previous natural abiotic or biotic conditions of the plant.

Biochemical, physiological and structural bioindication methods are better developed for higher plants than lower plants. This is probably due to increased interest on the effects of air pollution on higher plants because of the economic implications for example in relation to crops and forests. For example, a special issue of the journal Environmental Pollution was dedicated to the response of crops to air pollutants. This was reported on an international conference on assessment of crop loss from air pollutants in October 1987. However, these responses are often not specific and careful assessment is required in identifying cause and effects relationships. One attempt to overcome this problem would be to use multivariate approach where several response parameters are combined into multivariate indices.

7 References

Adams, K.J. and Preston, C.D. 1992 Evidence for the effects of atmospheric pollution on bryophytes from national and local recording. In: *Biological recording of changes in British wildlife*, Harding, P.T. (ed.), ITE Symposium 26.

Addison, P.A. 1984 Quantification of branch dwelling lichens for the detection of air pollution impact. *Lichenologist*, **16**, 3, 297.

Agrawal, M., Singh, S.K., Singh, J. and Rao, D.N. 1991 Biomonitoring of air pollution around urban and industrial sites. *Journal of Environmental Biology*, **12**, 211.

Alebic-Juretic, A. and Arko-Pijevac, M. 1989 Air pollution damage to cell membranes in lichens: Results of simple biological test applied in Rijeka, Yugoslavia. *Water, Air and Soil Pollution*, **47**, 1-2, 25.

Alfonso, A.T. and Rodriguez, E.B. 1994 Estimation of air-pollution in the area of influence of the coal power-station at La Robla (Leon, northwest Spain) using epiphytic lichens as bioindicators. *Cryptogamie Bryologie Lichenologie*, **15**, 2, 135-151.

Ali, E.A. 1993 Damage to plants due to industrial-pollution and their use as bioindicators in Egypt. *Environmental Pollution*, **81**, 3, 251-255.

Allegrini, I., Cortiello, M., Manes, F. and Tripodo, P. 1994 Physico-chemical and biological monitoring as integrated tools in evaluating tropospheric ozone in urban and semi-rural areas. *Science of the Total Environment*, **141**, 75.

Ammann, K., Herzig, R., Liebendörfer, L. and Urech, M. 1987 Multivariate correlation of deposition data of 8 different air pollutants to lichen data in a small town in Switzerland. *Advances in Aeriobiology*, **51**, 401-406.

Arndt *et al*. 1984 cited by Kovács, M. (ed.) 1992. In: *Biological indicators in environmental protection*. Ellis Horwood, New York.

Ashenden, T.W., Bell, S.A. and Rafarel, C.R. 1996 Interactive effects of gaseous air pollutants and acid mist on two major pasture grasses. *Agriculture Ecosystems and Environment*, **57**, 1, 1-8.

Ashenden, T.W., Hunt, R., Bell, S.A., Williams, T.G., Mann, A., Booth, R.E. and Poorter, L. 1996 Responses to SO_2 pollution in 41 British herbaceous species. *Functional Ecology*, **10**, 4, 483-490.

Balaguer, L., Valladares, F., Ascaso, C., Barnes, J.D., De-Los-Rios, A., Manrique, E. and Smith, E.C. 1996 Potential effects of rising tropospheric concentrations of CO_2 and O_3 on green-algal lichens. *New Phytologist*, **132**, 4, 641-652.

Barnes, J.D., Eamus, D., Davison, A.W., Ro-Poulsen, H. and Mortensen, L. 1990 Persistent effects of ozone on needle water loss and wettability in Norway spruce. *Environmental Pollution*, **63**, 345-363.

Bates, J.W., Bell, J. N. B. and. Farmer, A. M. 1990 Epiphyte recolonization of oaks along a gradient of air pollution in south-east England, 1979-1990. *Environmental Pollution* **68**, 1-2, 81.

Bates, J.W., McNee, P.J. and McLeod, A.R. 1996 Effects of sulphur dioxide and ozone on lichen colonization of conifers in the Liphook Forest Fumigation Project. *New Phytologist*, **132**, 4, 653-660.

De Bauer, L.I. and Krupa, S.V. 1990 The valley of Mexico: summary of observational studies on its air quality and effects on vegetation. *Environmental Pollution*, **65**, 109-118.

Bennett, J.P. 1996 Floristic summary of plant species in the air-pollution literature. *Environmental Pollution,* **92**, 3, 253-256.

Bennett, J.P. and Buchen, Mj. 1995 Bioleff - 3 databases on air-pollution effects on vegetation. *Environmental Pollution,* **88**, 3, 261-265.

Benton, J., Fuhrer, J., Skarby, L. and Sanders, G. (1995). Results from the UN ECE ICP-Crops indicate the extent of exceedance of the critical levels of ozone in Europe. *Water, Air and Soil Pollution,* **85**, 3, 1473-1478.

Berg, V.S. 1989 Leaf cuticles as potential markers of air pollutant exposure in trees. In: *Biologic markers of air pollution stress and damage in forests,* Committee on biological markers of air pollution damage in trees, National Research Council, National Academy Press, Washington D.C.

Bernes, C. (ed.) 1990 *Environmental Monitoring in Sweden. Monitor 1990*. Swedish Environmental Protection Agency Informs. Solna

Blank, L.W., Payer, H.D., Pfirrmann, T., Gnatz, G., Kloos, M., Runkel, K.H., Schmolke, W. and Strube, D. 1990 Effects of ozone, acid mist and soil characteristics on clonal Norway spruce (*Picea abies* (L.) Karst.) – an introduction to the joint 14 month tree exposure experiment in closed chambers. *Environmental Pollution*, **64**, 189-207.

Blaschke, H. and Weiss, M. 1990 Impact of ozone, acid mist and soil characteristics on growth and development of fine roots and ectomycorrhiza of young clonal Norway spruce. *Environmental Pollution*, **64**, 3-4, 255-263.

Brakenhielm, S. 1996 Impacts of air pollutants on processes in small catchments. Integrated monitoring 1982-1995 in Sweden. Swedish Environmental Protection Agency Report 4524.

Brakenhielm, S. and Qinghong, L. 1995 Spatial and temporal variability of algal and lichen epiphytes on trees in relation to pollutant deposition in Sweden. *Water, Air and Soil Pollution* **79**, 1-4, 61.

Brennan, E.G., Clarke, B.B., Greenhalgh-Weidman, B. and Smith, G. 1990 An assessment of the impact of ambient ozone on filed grown crops in New Jersey using the EDU method: Part 2 - soybean (*Glycine max* (L.) *Merr*.). *Environmental Pollution*, **66**, 361-373.

Brown, D.H. 1995 Physiological and biochemical assessment of environmental stress in bryophytes and lichens. In: *Bioindicators of environmental health*, Manawar, M., Hanninen, O., Roy, S., Munawar, N., Kärenlampi, L. and Brown, D.H. (eds.), Ecovision World Monograph Series, SPB Academic Publishing, Amsterdam.

Brown, M.J., Dyke, H., Wright, S.M, Wadsworth, R.A., Bull, K.R., Farmer, A., Bareham, S., Metcalfe, S.E., Whyatt, D. and Powesland, C. 1995. Estimating the impact of air pollution on environmentally valuable sites. *Water, Air and Soil Pollution*, **85**, 2589–2594.

Bruteig, I.E. 1993 The epiphytic lichen *Hypogymnia physodes* as a biomonitor of atmospheric nitrogen and sulphur deposition in Norway. *Environmental Monitoring and Assessment* **26**, 1, 27.

Bucker, J. and Guderian, R. 1994 Accumulation of myoinositol in populus as a possible indication of membrane disintegration due to air-pollution. *Journal of Plant Physiology,* **144**, 1, 121.

Bull, K.R. (1991). The critical loads/levels approach to gaseous pollutant emission control. *Environmental Pollution,* **69**, 105-123.

Burton, M.A.S. 1986 Biological monitoring of environmental contaminants (plants). MARC Report Number 32. Monitoring and Assessment Research Centre, King's College London, University of London.

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

Cape, J.N. 1989 The use of biomarkers to monitor forest damage in Europe. In: *Biologic markers of air pollution stress and damage in forests,* Committee on biological markers of air pollution damage in trees, National Research Council, National Academy Press, Washington D.C.

Carey, W.A. and Kelley, W.D. 1994 Interaction of ozone exposure and Fusarium subglutinans inoculation on growth and disease development of loblolly pine seedlings. *Environmental Pollution,* **84**, 1, 35.

Chappelka, A., Renfro, J., Somers, G. and Nash, B. 1997 Evaluation of ozone injury on foliage of black cherry (*Prunus serotina*) and tall milkweed (*Asclepias exaltata*) in Great Smokey mountains National Park. *Environmental Pollution,* **95**, 1, 13-18.

Chen, Y., Lucas, P.W. and Wellburn, A.R. 1991 Relationship between foliar injury and changes in antioxidant levels in Red and Norway spruce exposed to acidic mists. *Environmental Pollution,* **69**, 1-15.

Clarke, B.B., Greenhalgh-Weidman, B. and Brennan, E.G. 1990 An assessment of the impact of ambient ozone on filed grown crops in New Jersey using the EDU method: Part 1 - White potato (*Solanum tuberosum*). *Environmental Pollution,* **66**, 351-360.

Colls, J. 1997 Air pollution. An introduction. E & FN Spon, London.

Cook, E. and Innes, J. 1989 Tree-ring analysis as an aid to evaluating the effects of air pollution on tree growth. In: *Biologic markers of air pollution stress and damage in forests,* Committee on biological markers of air pollution damage in trees, National Research Council, National Academy Press, Washington D.C.

Csintalan, Z. and Tuba, Z. 1992 The effect of pollution on the physiological processes in plants. In: *Biological indicators in environmental protection*, Kovács, M. (ed.), Ellis Horwood, New York.

De Leeuw, F.A.A.M. and van Zantvoort, E.D. G. 1997 Mapping of exceedances of ozone critical levels for crops and forest trees in the Netherlands: preliminary results. *Environmental Pollution*, **96**, 1, 89-98.

De Wit, A. 1976 Epiphytic lichens and air pollution in the Netherlands. *Bibliotheca Lichenologica,* **5**, Cramer, Vaduz.

Dempster, J.P., Manning, W.J. (1988). Response of crops to air pollutants. *Environmental Pollution, Special issue,* **53**.

Diamantopoulos, J., Pirintsos, S., Laundon, J.R., and Vokou, D. 1992 The epiphytic lichens around Thessaloniki (Greece) as indicators of sulphur dioxide pollution. *Lichenologist*, **24**, 63-71.

Department of Environment (DoE) 1993 Air pollution and tree health in the UK. HMSO, London.

Dowding, P. 1994 Leafyeast survey for air pollution monitoring. In: *Biological monitoring of the environment* by Salanki, J., Jeffrey, D. and Hughes, G. M. (eds.), IUBS Methodology series, CAB International, Oxon, UK.

Dowding, P. and Richardson, D. H. S. (1989) Final report on leafyeasts as indicators of air quality in Europe. Project No. B-71-58 (08081886-004373). Report to DGXL European Commission, Brussels.

Dowding, P. and Richardson, D. H. S. (1990) Leafyeasts as Indicators of Air Quality in Europe. *Environmental Pollution,* **66** 3, 223-235.

Dueck, T.A., Wolting, H.G., Moet, D.R. and Pasman, F.J.M. 1987 Growth and reproduction of *Silene cucubalus* Wib. intermittently exposed to low concentrations of air pollutants zinc and copper. *New Phytologist*, **105**, 4, 633-646.

Edwards, N.T., Edwards, G.L., Kelly, J.M. and Taylor, G.E. Jnr. 1992 Three year growth responses of *Pinus taeda* L. to simulated rain chemistry, soil magnesium status and ozone. *Water, Air and Soil Pollution,* **63**, 1-2, 105-118.

Eversman, S. and Sigal, L.L. 1987 Effects of SO_2, O_3, and SO_2 and O_3 in combination on photosynthesis and ultrastructure of two lichen species. *Canadian Journal of Botany*, **65**, 1806.

Fellner, R. 1989 Mycorrhiza-forming fungi as bioindicators of air pollution. *Agriculture Ecosystems and Environment,* **28**, 1-4, 115.

Fellner, R. and Pešková, V. 1995 Effects of industrial pollutants on ectomycorrhizal relationships in temperate forests. *Canadian Journal of Botany,* **73,** S1 E-H Sie- S1310-S1315.

Fenn, M.E., Dunn, P.H. and Durall, D.M. 1989 Effects of ozone and sulfur dioxide on phyllosphere fungi from three tree species. *Applied Environmental Microbiology,* **55**, 2, 412.

Fernandez-Bayon, J.M., Barnes, J.D., Ollerenshaw, J.H. and Davison, A.W. 1993 Physiological effects of ozone on cultivars of watermelon (*Citrullus lanatus*) and muskmelon (*Cucumis melo*) widely grown in Spain. *Environmental Pollution*, **81**, 199-206.

Fialho, R.C. and Bucker, J. 1996 Changes in levels of foliar carbohydrates and myoinositol before premature leaf senescence of *Populus-Nigra* induced by a mixture of O_3 and SO_2. *Canadian Journal of Botany,* **74**, 6, 965-970.

Fields, R.F. 1988 Physiological responses of lichens to air pollutant fumigations. In *Lichens, bryophytes and air quality*, Nash, T.H. and Wirth, V. (eds.),
J. Cramer, Berlin.

Garty, J., Ronen, R. and Galun, M. 1985 Correlation between chlorophyll degradation and the amount of some elements in the lichen *Ramalina duriaei* (De Not.) Jatt. *Environmental and Experimental Botany*, **25**, 1, 67-74.

Gimeno, B.S., Penuelas, J., Porcuna, J.L. and Reinert, R.A. 1995a Biomonitoring ozone phytotoxicity in eastern Spain. *Water, Air and Soil Pollution*, **85**,3, 1521-1526.

Gimeno, B.S., Salleras, J.M. Porcuna, J.L., Reinert, R.A., Velissariuo, D. and Dawson, A.W. 1995b The use of watermelon as an ozone bioindicator. In: *Bioindicators of environmental health*, Manawar, M., Hanninen, O., Roy, S., Munawar, N., Kärenlampi, L. and Brown, D.H. (eds.) Ecovision World Monograph Series, SPB Academic Publishing, Amsterdam.

Godbold, D.L., Feig, R., Cremer-Herms, A. and. Huttermann, A. 1993 Determination of stress bioindicators in three Norway spruce stands in northern Germany. *Water, Air and Soil Pollution,* **66**, 3-4, 231.

Gonzalez, C.M., Casanovas, S.S. and Pignata, M.L. 1996 Biomonitoring of air pollutants - from traffic and industries employing *Ramalina ecklonii* (Spreng.) Mey. and Flot. in Cordoba, Argentina. *Environmental Pollution*, **91**, 269-277

Gustafsson, L. and Eriksson, I. 1996 Factors of importance for the epiphytic vegetation of aspen *Populus tremula* with special emphasis on bark chemistry and soil chemistry. *Journal of Applied Ecology*, **32**, 412-424.

Haapala, H., Goltsova, N., Seppala, R., Huttunen, S., Kouki, J., Lampp, J., Popovichev, B. 1996 Ecological condition of forests around the eastern part of the Gulf of Finland. *Environmental Pollution*, **91**, 2, 253-265.

Hall, J.P. 1995 Forest health monitoring in Canada: How healthy is the boreal forest? *Water, Air and Soil Pollution*, **82**, 77-85.

Hallingback, T. and Kellner, O. 1992 Effects of simulated nitrogen rich and acid rain on the nitrogen- fixing lichen *Peltigera aphthosa (*L.*) Willd*. *New Phytologist*, **120**, 1, 99-103.

Hallingback, T. and Tan, B.C. 1996 Towards a global action plan for endangered brophytes. *Anales del Instituto de Biologica Universidad Nacional Autonoma de Mexico Serie Botanica*, **67**, 213-221.

Hamada, N., Miyawaki, H. and Yamada, A. 1995 Distribution pattern of air-pollution and epiphytic lichens in the osaka plain (Japan). *Journal of Plant Research*, **108**, 1092, 483-491.

Hanninen, O., Ruuskanen, J.I. and Oksanen, J. 1993 A method for facilitating the use of algae growing on tree trunks as bioindicators of air quality. *Environmental Monitoring and Assessment*, **28**, 3, 215.

Hawksworth, D.L. and McManus, P.M. 1989 Lichen recolonisation in London under conditions of rapidly falling sulphur dioxide levels, and the concept of zone skipping. *Botanical Journal of the Linnean Society*, **100,** 99-109.

Hawksworth, D.L. and Rose, F. 1970 Qualitative scale for estimating sulphur dioxide air pollution in England and Wales using epiphytic lichen. *Nature*, **227**, 145-148.

Hawksworth, D.L. and Rose, F. 1976 Lichens as pollution monitors. *Institute of Biology's Studies in Biology*, 66, Edward Arnold Publishers Ltd., London.

Heath, R.L. 1989 Alteration of chlorophyll in plants upon air pollutant exposure. In: *Biologic markers of air pollution stress and damage in forests*, Committee on biological markers of air pollution damage in trees, National Research Council, National Academy Press, Washington D.C.

Heck, W.W. 1989 Assessment of crop losses from air pollutants in the United States. In: *Air pollutant's toll on forests and crops*,Mackenzie, J.J. and Mohmed, T.E. (eds.), Vail-Bauou Press, NY.

Heggestad, H.E. 1991 Origin of Bel-W3, Bel-C and Bel-B tobacco varieties and their use as indicators of ozone. *Environmental Pollution*, **74**, 264-291.

Helander, M.L. 1993 Responses of pine needle endophytes to air-pollution. *New Phytologist* **131**, 2, 223-229.

Henderson, A. 1994 Literature On Air-Pollution And Lichens 39. *Lichenologist* 26, Pt2 193-203

Henderson, A. 1995 Literature On Air-Pollution And Lichens 42. *Lichenologist* 27, No- Pt5 395-404

Henderson, A. 1996a Literature On Air-Pollution And Lichens 43. *Lichenologist* 28, No- Pt3 279-285

Henderson, A. 1996b Literature On Air-Pollution And Lichens 44. *Lichenologist* 28, No- Pt6 603-612.

Herzig, R. and Urech, M. 1991 Flechten als Bioindikatoren, integriertes biologisches Meßsystem der Luftverschmutzung für das Schweizer Mittelland. *Bibl. Lichenologica*, **43**, 1-283.

Herzig, R., Liebendorfer, L., Urech, M., Ammann, K., Cuecheva, M. and Landolt, W. 1989 Passive biomonitoring with lichens as a part of an integrated biological measuring system for monitoring air pollution in Switzerland. *International Journal of Environmental and Analytical Chemistry* **35**, 43.

Hill, M.O. 1979 TWINSPAN - a FORTRAN program for arranging multivariate data in an ordered two-way table by classification of the individuals and attributes. Ithaca, NY Cornell University.

Hill, M.O. and Gauch, H.G. 1980 Detrended correspondence analysis, an improved ordination technique. *Vegetatio*, **42** 47-58.

Hippeli, S. and Elstner, E.F. 1996 Mechanisms of oxygen activation during plant stress - Biochemical effects of air-pollutants. *Journal of Plant Physiology*, **148**, 3-4, 249-257.

Huber, H. 1992 Epiphytic mosses in the vicinity of Basel and their potential suitability as indicators of the air quality. *Bauhinia*, **10**, 181-190.

Hultengren, S., Martinsson, P-O. and Stenstrom, J. 1991 Lichens and air pollution. Sensitivity classification and index calculation of epiphytic lichens. Report 3967, Swedish Environmental Protection Agency, Solna.

Inui, T. and Yamaguchi, T. 1996 Epiphytic bryophytes in Naha city (subtropical urban area in Okinawa Island, southern Japan), with special references to air pollution. *Hikobia*, **12,** 161-168.

Jäger E.J. 1980 cited by Kovács, M. (ed.) 1992. Biological indicators in environmental protection. Ellis Horwood, New York.

Jones, M.B., Booth, C.E. and Shanahan, E. 1991 The use of radish as a bioindicator in an international programme for evaluating the effects of air pollution on agricultural crops. In: *Bioindicators and environmental management*, Jeffrey, J.W. and Madden, B. (eds.), Academic Press Ltd. London.

Julkunen-Titto, R. Lavola, A. and Kainulainen, P. 1995 Does SO_2 fumigation change the chemical defense of woody plants: The effect of short-term SO_2 fumigation on the metabolism of deciduous *Salix Myrsinifolia* plants. *Water, Air and Soil Pollution*, **83**, 195-203.

Kalyani, Y. and Charya, M.A.S. 1995 Biomonitoring of air pollution in Warangal City, Andhra Pradesh. *Acta Botanica Indica*, **23**, 1, 21-24.

Khan, M.W. and Kulshrestha, M. 1991 Impact of sulphur dioxide exposure on conidial germination of powdery mildew fungi. *Environmental Pollution* **70**, 1, 81.

Kardish, B.N., Ronen, R., Bubrick, P. and Burrick, P. 1987 The influence of air pollution on the concentration of ATP and on chlorophyll degradation in the lichen, *Ramalina Duriaei* (De Not.). *New Phytologist*, **106**, 687.

Kasana, M.S. 1991 Sensitivity of three leguminous crops to O_3 as influenced by different stages of growth and development. *Environmental Pollution*, **69**, 131-149.

Kasana, M.S. and Lea, P.J. 1994 Growth-responses of mutants of spring barley to fumigation with SO_2 and NO_2 in combination. *New Phytologist*, **126**, 4, 629-636.

Keller, T. 1988 Growth and premature leaf fall in American aspen as bioindicators for ozone. *Environmental Pollution*, **52**, 183-192.

Keller, T. and Matyssek, R. 1990 Limited compensation of ozone stress by potassium in Norway spruce. *Environmental Pollution*, **67**, 1, 1.

Khan, M.R. and Khan, M.W. 1994 Single and interactive effects of root-knot nematode and coal-smoke on okra. *New Phytologist*, **126**, 2, 337-342.

Klumpp, A., Klumpp, G. and Domingos, M. 1994 Plants as bioindicators of air-pollution at the Serra-Do-Mar near the industrial-complex of Cubatao, Brazil. *Environmental Pollution*, **85**, 1, 109-116.

Kobayashi, K., Okada, M. and Nouchi, I. 1994 A chamber system for exposing rice (*Oryza-Sativa* L) to ozone in a paddy field. *New Phytologist*, **126**, 2, 317-325.

Koppel, A. and Sild, E. 1995 Bioindication of ozone in Estonia by using the tobacco variety Bel W3. *Water, Air and Soil Pollution*, **85**, 3, 1515-1519.

Kovács, M. (ed.) 1992a. Biological indicators in environmental protection. Ellis Horwood, New York.

Kovács, M. 1992b Herbaceous (flowering) plants. In: *Biological indicators in environmental protection*, Kovács, M. (ed.), Ellis Horwood, New York.

Kovács, M. 1992c Trees as biological indicators. In: *Biological indicators in environmental protection*. Kovács, M. (ed.), Ellis Horwood, New York.

Kozuharov, S.I. 1986 Plants as bioindicators. In: *Biological monitoring of the state of the environment*, Salanki, J. (ed.), IUBS Monograph Series, No.1. IRL Press limited, Oxford, UK.

Kraft, M., Weigel, H.J., Mejer, G.J. and Brandes, F. 1996 Reflectance measurements of leaves for detecting visible and non-visible ozone damage to crops. *Journal of Plant Physiology*, **148**, 1-2, 148-154.

Krywult, M., Karolak, A. and Bytnerowicz, A. 1996 Nitrate reductase activity as an indicator of ponderosa pine response to atmospheric nitrogen deposition in the San Bernardino mountains. *Environmental Pollution*, 93, 2, 141-146.

Kumer, E., Bonalberti, L., Piccoli, F. and Garasto, G. 1991. Lichens as monitors of air-pollution. *Grana*, **30**, 1, 48-50.

Larsen, R.I., McDonnell, W.F. and Coffin, D.L. 1993 An air quality data analysis system for interrelating effects, standards, and netted source reductions: part 12. Effects on man, animals, and plants as a function of air pollutant impact. *Journal of Air and Waste Management Association*. December 1993, 1585.

Leblanc, F. and DeSloover, J. 1970 Relations between industrialization and the distribution and growth of epiphytic lichens and mosses in Montreal. *Canadian Journal of Botany*, **48**, 1485-1496.

Lefohn, A.S. and Foley, J.K. 1993 Establishing relevant ozone standards to protect vegetation and human health: Exposure/dose-response considerations. *Journal of Air and Waste Management Association*, **43**, 1, 106.

Lefohn, A.S. and Runeckles, V.C. 1987 Establishing standards to protect vegetation. Ozone exposure/dose considerations. *Atmospheric Environment*, **21**, 3, 561.

Legrand, I. and Asta, J. 1995. Epiphytic lichen flora and bark characteristics (pH conditions) in relation to forest decline in the northern Alps. In: *Forest decline and atmospheric deposition effects in the French mountains*, Landmann, G. and Bonneau, M. (eds.), Springer, Germany.

Levin, A.G. and Pignata, M.L. 1995 Ramalina Ecklonii as a bioindicator of atmospheric-pollution in Argentina. *Canadian Journal of Botany,* **73**, 8, 1196-1202.

Loppi, S. 1996 Lichens as bioindicators of geothermal air-pollution in central Italy. *Bryologist*, **99**, 1, 41-48.

Loppi, S., Corsini, A. 1995 Lichens as bioindicators of air quality in Montecatini Terme (central northern Italy). *Ecologia Mediterranea,* **21**, 3-4, 87-92.

Loppi, S., Francalanci, C., Pancini, P., Marchi, G. and Caporali, B. 1996 Lichens as bioindicators of air quality in Arezzo (central Italy). *Ecologia Mediterranea,* **22**, 1-2, 11-16.

Lorenz, M. 1995 International cooperative program on assessment and monitoring of air-pollution effects on forests - ICP Forests. *Water, Air and Soil Pollution*, **85**, 3, 1221-1226.

Lorenz, M., Augustin, S., Becher, G. and Förster, M. *1997* Forest condition in Europe, results of the 1996 crown condition survey. 1997 Technical Report, EC-UN ECE, Brussels, Geneva.

Lorenzini, G. 1994 A miniaturized kit for ozone biomonitoring. *Applied Biochemistry and Biotechnology*, **48**, 1, 1-4.

Lorenzini, G., Farina, R. and Guidi, L. 1990 The effects of sulphur dioxide on the parasitism of the rust fungus *Uromyces viciae-fabae* on *Vicia faba*. *Environmental Pollution*, **68**, 1-2, 1.

Lorenzini, G., Nali, C. and Biagioni, M. 1995a An analysis of the distribution of surface ozone in Tuscany (Central Italy) with the use of new miniaturized bioassay with ozone-sensitive tobacco seedlings. *Environmental Monitoring and Assessment*, **34**, 1, 59.

Lorenzini, G., Nali, C. and Biagioni, M. 1995b Long-range transport of photochemical ozone over the Tyrrhenian sea demonstrated by a new miniaturized bioassay with ozone-sensitive tobacco seedlings. *Science of the Total Environment,* **166**, 1-3. 193-199.

Lorenzini, G., Panattoni, A Guidi, L. and Schenone, G. 1992 On the effects of exposure to realistic sulfur dioxide levels on six host/pathogen combinations. *Journal of Environmental Science and Health - Environmental Science* A27, **7,** 1863.

Machler, F., Wasescha, M.R., Krieg, F. and Oertli, J.J. 1995 Damage by ozone and protection by ascorbic-acid in barley leaves. *Journal Of Plant Physiology,* **147**, 3-4, 469-473.

MacLean, D. 1990 Joint action of ozone and hydrogen fluoride on foliar senescence in maize. *Environmental Pollution*, **63**, 283-292.

Maggs, R., Wahid, A., Shamsi, S.R.A. and Ashmore, M.R. 1995 Effects of ambient air pollution on wheat and rice yield in Pakistan. *Water, Air and Soil Pollution*, **85**, 2, 1311-1316.

Mäkipää, R. 1995 Sensitivity of forest-floor mosses in boreal forests to nitrogen and sulphur deposition. *Water, Air and Soil Pollution,* **85**, 3, 1239-1244.

Mankovska, B. 1997 Variations in sulphur and nitrogen foliar concentration of deciduous and coniferous vegetation in Slovakia. *Water, Air and Soil Pollution*, **96**, 329-345.

Manninen, S. and Huttunen, S. 1995 Scots pine needles as bioindicators of sulphur deposition. *Canadian Journal of Forest Research,* **25**, 10, 1559-1569.

Marx, D.H. and Shafer, S.R. 1989 Fungal and bacterial symbioses as potential biological markers of effects of atmospheric deposition on forest health. In: *Biologic markers of air pollution stress and damage in forests,* Committee on biological markers of air pollution damage in trees, National Research Council, National Academy Press, Washington D.C.

McCune, B., Derr, C.C., Muir, P.S., Shirazi, A., Sillett, S.C. and Daly, W.J. 1996 Lichen pendants for transplant and growth experiments. *Lichenologist*, **28**, 2, 161-169.

Mcleod, A.R. 1995 An open-air system for exposure of young forest trees to sulfur-dioxide and ozone. *Plant Cell and Environment,* **18**, 3, 215-225.

Meier, S., Grand, L.F., Schoeneberger, M.M., Reinert, R.A. and Bruck, R.I. 1990 Growth, ectomycorrhizae and nonstructural carbohydrates of loblolly pine seedlings exposed to ozone and soil water deficit. *Environmental Pollution*, **64**, 11-27.

Miller, P.R. 1989 Biomarkers for defining air pollution effects in western coniferous forests. In: *Biologic markers of air pollution stress and damage in forests.* Committee on biological markers of air pollution damage in trees, National Research Council, National Academy Press, Washington D.C.

Mignanego, L., Biondi, F. and Schenone, G. 1992 Ozone biomonitoring in northern Italy. *Environmental Monitoring and Assessment,* **21**, 2, 141.

Morecroft, M.D., Sellers, E.K. and Lee, J.A. 1994 An experimental investigation into the effects of atmospheric nitrogen deposition on 2 seminatural grasslands. *Journal of Ecology,* **82**, 3, 475-483.

Muir, P.S. and McCune, B. 1988 Lichens, tree growth, and foliar symptoms of air pollution: Are the stories consistent. *Journal of Environmental Quality,* **17**, 3, 361.

Nash, T.H. 1988 Correlating fumigation studies with field effects. In: *Lichens, bryophytes and air quality,* Nash, T.H. and Wirth, V. (eds.), J. Cramer, Berlin.

Nash, T.H. and Wirth, V. (eds.) 1988 *Lichens, bryophytes and air quality*, J. Cramer, Berlin.

Paakkonen, E., Holopainen, T. and Karaelampi, L. 1997 Variation in ozone sensitivity among clones of *Betula pendula* and *Betula pubescens*. *Environmental Pollution*, **95**, 1, 37-44.

Pandey, J. and Agrawal, M. 1994 Evaluation of air-pollution phytotoxicity in a seasonally dry tropical urban environment using 3 woody perennials. *New Phytologist*, **126**, 1, 53-61.

Perkins, D.F. 1992 Relationship between fluoride contents and loss of lichens near an aluminium works. *Water, Air and Soil Pollution,* **64**, 3-4, 503.

Pitcairn, C.E.R., Fowler, D. and Grace, J. 1995 Deposition of fixed atmospheric nitrogen and foliar nitrogen-content of bryophytes and *Calluna-Vulgaris* (L) *Hull. Environmental Pollution, **88***, 2, 193-205.

Plamada, E. 1986 the effect of atmospheic pollution on the bryoflora of the Zlatna industrial zone Alba District, Romania. *Stud. Cercet. biol. Ser. Biol. Veg*. **38** 57-67.

Pleijel, H., Skarby, L., Wallin, G. and Selden, G. 1991 Yield and grain quality of spring wheat (*Triticum aestivum* L., cv. Drabant) exposed to different concentrations of ozone in open-top chambers. *Environmental Pollution,* **69**, 151-168.

Potter, L., Foot, J.P., Caporn, S.J.M. and Lee, J.A. 1996 The effects of long-term elevated ozone concentrations on the growth and photosynthesis of *Sphagnum recurvum* and *Polytrichum commune*. *New Phytologist,* **134**, 4, 649-656.

Press, M., Woodin, S.J. and Lee, J.A. 1986 The potential importance of an increased atmospheric nitrogen supply to the growth of ombrotrophic Sphagnum species. *New Phytologist,* **103**, 45-55.

Ranieri, A., Schenone, G., Lencioni, L. and Soldatini, G.F. 1994 Detoxificant enzymes in pumpkin grown in polluted ambient air. *Journal of Environmental Quality*, **23**, 2, 360-364.

Rebelo, C.F., Struffaldi-De Vuono, y. and Domingos, M. 1995 Ecological study of epiphytic bryophyte communities of Paranapiacaba Biological Reserve, SP, at forest areas subject to air pollution influence. *Revista Brasileira de Botanica*, **18**, 1-15.

Renaud, J.P., Allard, G. and Mauffette, Y. 1997 Effects of ozone on yield, growth, and root starch concentrations of two alfalfa (*Medicago sativa* L.) cultivars. *Environmental Pollution*, **95**, 273-281.

Rennenberg, H. Herschbach, C. and Polle, A. 1996 Consequences of air-pollution on shoot-root interactions. *Journal of Plant Physiology,* **148**, 3-4, 296-301.

Richards, J.H. 1989 Evaluation of root growth and functioning of trees exposed to air pollutants. In: *Biologic markers of air pollution stress and damage in forests,* Committee on biological markers of air pollution damage in trees, National Research Council, National Academy Press, Washington D.C.

Richardson, D.H.S. 1988 Understanding the pollution sensitivity of lichens. *Botanical Journal of the Linnean Society,* **96**, 31-43.

Richardson, D.H.S. 1991 Lichens as biological indicators - recent developments. In: *Bioindicators and environmental management,* Jeffrey, J.W. and Madden, B. (eds.), Academic Press Ltd. London.

Richardson, D.H.S. 1992 Pollution monitoring with lichens. Richmond Publishing, Slough, England.

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

Ronen, R., Canaani, O., Garty, J., Cahen, D., Malkin, S. and Galun, M. 1985 Photosynthetic parameters in *Ramalina duriaei* in vivo studied by photoacoustics. In: *Lichen physiology and cell biology*, Brown, D.H. (ed.), London Plenum Press.

Rope, S. K. and Pearson, I. C. 1990 Lichens as air-pollution biomonitors in a semiarid environment in Idaho. *Bryologist,* **93**, 1, 50-61.

Runeckles, V.C., Wright, E.F., and White, D. 1990 A chamberless field exposure system for determining the effects of gaseous air pollutants on crop growth and yield. *Environmental Pollution*, **63**, 61-77.

Saarinen, T. and Liski, J. 1993 The effect of industrial air-pollution on chlorophyll fluorescence and pigment contents of Scots pine (*Pinus-Sylvestris*) needles. *European Journal of Forest Pathology,* **23**, 6-7, 353-361.

Sanders, G.E., Skarby, L., Ashmore, M.R. and Fuhrer, J. 1995 Establishing critical levels for the effects of air pollution on vegetation. *Water, Air and Soil Pollution*, **85**, 189-200.

Saxe, H. 1991 Photosynthesis and stomatal responses to polluted air, and the use of physiological and biochemical responses for early detection and diagnostic tools. *Advanced Botanical Research*, **18**, 1-128.

Saxe, H. 1996 Physiological and biochemical tools in diagnosis of forest decline and air pollution injury to plants. In: *Plant responses to air pollution*, Yumus, M. and Igbal, M. (eds.), John Wiley and Sons Ltd.

Schenone, G. and Lorenzini, G. 1992. Effects of regional air-pollution on crops in Italy. *Agriculture Ecosystems and Environment,* **38**, 1-2, 51-59.

Schenone, G., Fumagalli, I., Mignanego, L., Montinaro, F. and Soldatini, G.F. 1994 Effects of ambient air-pollution in open-top chambers on bean (*Phaseolus-vulgaris* L). 2. Effects on photosynthesis and stomatal conductance.
New Phytologist, **126**, 2, 309-315.

Schlee, D., Thoringer, C. and Tintemann, H. 1994 Purification and properties of glutamate-dehydrogenase in Scots pine (*Pinus-Sylvestris*) needles. *Physiologia Plantarum* **92**, 3, 467-472.

Seaward, M. R. D. 1993 Lichens and sulphur dioxide air pollution: Field studies. *Environmental Review,* **1**, 2, 73.

Shan, Y., Feng, Z., Izuta, T., Aoki, M. and Totsuka, T. 1996 The individual and combined effects of ozone and simulated acid rain on growth, gas exchange rate and water-use efficiency of *Pinus armandi Franch*. *Environmental Pollution*, **91**, 3, 355-361.

Showman, R. 1988 Mapping air quality with lichens, the North American experience. In: *Lichens, bryophytes and air quality*, Nash, T.H. and Wirth, V. (eds.), J.Cramer, Berlin.

Silberstein, L., Siegel, S.M., Keller, P., Siegel, B.Z. and Galun, M. 1990 A new method for the extraction of ATP from lichens. *Bibliotheca Lichenologica*, **38**, 411-418.

Silberstein, L., Siegel, B.Z., Siegel, S.M., Mukhtar, A., and Galun, M. 1996a Comparative studies on *Xanthoria parietina*, a pollution-resistant lichen, and *Ramalina duriaei*, a sensitive species: 1. Effects of air pollution on physiological processes. *Lichenologist*, **28**, 4, 355-365.

Silberstein, L., Siegel, B.Z., Siegel, S.M., Mukhtar, A. and Galun, M. 1996b Comparative studies on *Xanthoria-Parietina*, a pollution-resistant lichen, and *Ramalina-Duriaei*, a sensitive species: 2. Evaluation of possible air pollution-protection mechanisms. *Lichenologist*, **28**, 4, 367-383.

Singh, A.K. and Bharat, R. 1990 Effect of SO_2 and NH_3 on growth behavior of some phylloplane fungi of wheat in in vitro. *Water, Air and Soil Pollution* **49**, 3-4, 343**.**

Singh, A.K. and Rai, B. 1990 Effect of SO_2 and NH_3 on growth behavior of some phylloplane fungi of wheat in vitro. *Water, Air and Soil Pollution*, **49**, 3-4, 343.

Skelly, J.M., Savage, J.E., De Bauer, M. and Alvarado, D. 1997 Observations of ozone-induced foliar injury on black cherry (*Prunus serotina*, var. *capula*) within the Desierto de los Leones National Park, Mexico City. *Environmental Pollution*, **95**, 155-158.

Smidt, S. 1996 Assessment of air pollution stress on forest ecosystems by the example of the northern tyrolean limestone alps. *Journal of Plan Physiology*, **148**, 287-295.

Smith, G., Neyra, C. and Brennan, E. 1990 The relationship between foliar injury, nitrogen metabolism and growth parameters in ozonated soybeans. *Environmental Pollution*, **63**, 79-83.

Soares, A., Ming, J.Y. and Pearson, J. 1995 Physiological indicators and susceptibility of plants to acidifying atmospheric-pollution - A multivariate approach. *Environmental Pollution,* **87**, 2, 159-166.

Soja, G and Soja, A. 1995 Wheat as an ozone sensitive crop. *Acta Phytopathologica Et Entomologica Hungarica*, **30**, 59-70.

Solberg, S. and Tørseth, K. 1997 Crown condition of Norway spruce in relation to sulphur and nitrogen deposition and soil properties in southeast Norway. *Environmental Pollution*, **96**, 1, 19-27.

Spence, R.D., Rykiel, E.J. and Shrape, P.J.H. 1990 Ozone alters carbon allocation in loblolly pine: assessment with carbon-11 labeling. *Environmental Pollution*, **64**, 93-106.

Stanosz, G. R., Smith V. L. and Bruck; R. I. 1990 Effect of ozone on growth of mosses on disturbed forest soil. *Environmental Pollution,* **63**, 4, 319.

Steubing, Y., Kirschbaum, U., Poss, F. and Cornelius, R. 1983 Monitoring mittels Bioindikatoren in Belastungsgebieten. *Umweltforschungsplan des Bundesministers des Innern*. Umlandverband Frankfurt.

Takala, K. Olkkonen, H. and Krouse, H. R. 1991 Sulphur isotope composition of epiphytic and terricolous lichens and pine bark in Finland. *Environmental Pollution*, **69**, 4, 337-348.

Taoda, H. 1973 Bryo-meter, an instrument for measuring the phytotoxic air pollution. *Hikobia*, **6,** 224-228.

Taylor, H.J., Ashmore, M.R. and Bell, J.N.B. 1990 Air pollution injury to vegetation. IEHO, London.

Tenga, A.Z. and Ormrod, D.P. 1990 Diminished greenness of tomato leaves exposed to ozone and post-exposure recovery of greenness. *Environmental Pollution*, **64**, 29-41.

Schaffers, A.P. and Termorshuizen, A.J. 1989 A field survey on the relations between air pollution, stand vitality and the occurrence of fruitbodies of mycorrhizal fungi in plots of *Pinus sylvestris*. *Agriculture, Ecosystems and Environment*, **28**, 449-454.

Tichy, J. 1996 Impact of atmospheric deposition on the status of planted Norway spruce stands: a comparative study between sites in southern Sweden and the northeastern Czech Republic. *Environmental Pollution*, **93**, 3, 33-312.

Tiedemann, A.V., Weigel, H.J. and Jager, H.J. 1991 Effects of open-top chamber fumigations with ozone on three fungal leaf diseases of wheat and the mycoflora of the phyllosphere. *Environmental Pollution*, **72**, 3, 205.

Turcsanyi, G. 1992 Plant cells and tissues as indicators of environmental pollution. In: *Biological indicators in environmental protection*, Kovács, M. (Ed.) Ellis Horwood, New York.

UN ECE Convention on Long-Range Transboundary Air Pollution 1993 Manual for integrated monitoring, programme phase 1993-1996. Environmental Data Centre, National board of Waters and the Environment, Helsinki.

Vaisanen, S. 1986 Effects of air pollution by metal, chemical and fertiliser plants on forest vegetation of Kokkola, West Finland. *Ann. Bot. Fenn*, **23**, 305-316.

Van Dobben, H.F. and De Bakker, A.J. 1996 Re-mapping epiphytic lichen biodiversity in The Netherlands - effects of decreasing SO_2 and increasing NH_3. *Acta Botanica Neerlandica*, **45**, 1, 55-71

Van Zanten, B.O. 1992 Distribution of some vulnerable epiphytic bryophytes in the north of the province of Groningen, The Netherlands. *Biological Conservation*, **59**, 205-209.

Veeranjaneyulu, K., Charlebois, D., N'soukpoe-Kossi, C.N. and Leblanc, R.M. 1990 Effect of sulphur dioxide and sulfite on photochemical energy storage of isolated chloroplasts - a photoacoustic study. *Environmental Pollution,* **65**, 127-139.

Vilde, R. and Martin, J. 1996 Air pollution deposition impact on the structure of bryophyte cover in Scots pine forests in Estonia. *Proceedings of the Estonian Academy of Sciences Biology*, **45**, 181-191.

Von Arb, C. and Brunold, C. 1990 Lichen physiology and air pollution. I. Physiological responses of in situ Parmelia sulcata among air pollution zones within Biel, Switzerland. Canadian *Journal of Botany,* **68**, 35-42.

Weinstein, L.H. and Laurence, J.A. 1989 Indigenous and cultivated plants as bioindicators. In: *Biologic markers of air pollution stress and damage in forests,* Committee on biological markers of air pollution damage in trees, National Research Council, National Academy Press, Washington D.C.

Wellburn, F.A.M., Lau, K.K., Milling, P.M.K. and Wellburn, A.R. 1996 Drought and air-pollution affect nitrogen cycling and free-radical scavenging in *Pinus-Halepensis* (Mill). *Journal of Experimental Botany,* **47**, 302, 1361-1367.

Wetmore, C.M. 1988 Lichen floristics and air quality. In: *Lichens, bryophytes and air quality*, Nash, T.H. and Wirth, V. (eds.), J. Cramer, Berlin.

Will-Wolf, S. 1988 Quantitative approaches to air quality studies. In: *Lichens, bryophytes and air quality*, Nash, T.H. and Wirth, V. (eds.), J. Cramer, Berlin.

Winner, W.E. 1988 Responses of bryophytes to air pollution. In: *Lichens, bryophytes and air quality*, Nash, T.H. and Wirth, V. (eds.), J. Cramer, Berlin.

Winner, W.E. 1989 Photosynthesis and transpiration measurements as biomarkers of air pollution effects on forests. In: *Biologic markers of air pollution stress and damage in forests,* Committee on biological markers of air pollution damage in trees, National Research Council, National Academy Press, Washington D.C.

Winner, W.E. 1994 Mechanistic analysis of plant-responses to air-pollution. *Ecological Applications,* **4**, 4, 651-661.

Winner, W.E., Atkinson, C.J. and Nash, T.H. 1988 Comparisons of SO_2 absorption capacities of mosses, lichens, and vascular plants in diverse habitats. In *Lichens, bryophytes and air quality*, Nash, T.H. and Wirth, V. (eds.), J.Cramer, Berlin.

Wirth, V. 1988 Phytosociological approaches to air pollution monitoring with lichens. In: *Lichens, bryophytes and air quality*, Nash, T.H. and Wirth, V. (eds.), J.Cramer, Berlin.

Wookey, P.A. and Ineson, P. 1991 Combined use of open-air and indoor fumigation systems to study effects of SO_2 on leaching processes in Scots pine litter. *Environmental Pollution,* **74**, 4, 325.

Woolgrove, C.E. and Woodin, S.J. 1996 Current and historical relationships between the tissue nitrogen- content of a snowbed bryophyte and nitrogenous air-pollution. *Environmental Pollution*, **91**, 3, 283-288.

Zakshek, E. M., Puckett, K. J. and Percy, K. E. 1986 Lichen sulphur and lead levels in relation to deposition patterns in eastern Canada. *Water, Air and Soil Pollution*, **30**, 1-2, 161-169.

IV ORGANIC COMPOUNDS

1 Introduction

Organic compounds include polycyclic aromatic hydrocarbons (PAHs), chlorinated hydrocarbons, polychlorinated biphenyls (PCBs) and ethylene (C_2H_4). PAHs result from activities such as fossil fuel combustion and aluminium and coke production. Common PAHs in the published literature include fluoranthene and benzoperylene. Chlorinated hydrocarbons, such as benzohexachloride (BHC), are characterised by different pollution sources than PAHs and show a high mobility in the air. The emissions of PCBs such as tri- and tetrachlorobiphenyls have reduced in recent decades but in some countries the long-range transport of these chemicals to rural areas is still regarded an important problem. Ethylene is primarily emitted from the combustion or processing of petroleum and its products and can easily accumulate to levels injurious to plants.

Biomonitoring of organic compounds using plants is not as widely established as it is with metal or gaseous air pollutants. Biomonitoring of organic compounds has the advantage in that background concentrations can be assumed to be zero because persistent organic compounds are anthropogenic in origin. Most biomonitoring of organic compounds involves plants which have the ability to bioaccumulate these persistent trace pollutants. This may entail passive monitoring where natural growing vegetation is observed or active monitoring which detects the presence of air pollutants by placing test plants of known response and genotype into the study area.

Analysis of tissues is undertaken in most biomonitoring studies of organic compounds. It is important that any standard analytical procedures are adhered to. Contamination of samples during collection and preparation should be avoided and sample replication is recommended for reliable interpretation of results.

2 Bryophytes

At present, mosses are probably the most widely used plant group in relation to the assessment of airborne organic compounds.

2.1 Passive monitoring

Thomas (1986) found that mosses were suitable in the monitoring of the chlorinated hydrocarbon, benzohexachloride (BHC) and polycyclic aromatic hydrocarbons (PAH) in Europe. Different moss samples from across Europe were analysed for their trace substance residue content by a variety of research groups in Germany, the Netherlands, Denmark, Norway and Iceland. *Hypnum cupressiforme* was analysed at most sites. The effective biomonitoring properties of this species in aerial heavy metal monitoring has previously been recognised (Chapter II, Section 1). *Hylocomium splendens* and *Rhacomitrium lanuginosum* were analysed at a few sites in the study. Both these species have also been utilised in the assessment of heavy metal contamination (Chapter II, Section 1).

Results showed a clear PAH concentration gradient in mosses, which was high in the industrial centres in middle Europe and low in northern Europe. The content of fluoranthene (1.0 ng g^{-1}) and benzoperylene (0.6 ng g^{-1}) in moss samples in Iceland were regarded as background levels. However, the ratio of fluoranthene to benzoperylene was lower at these sites than at industrial sites due to a high concentration of fluoranthene in the gaseous phase being transported longer distances to remote areas. The distribution of BHC content in moss samples does not show a clearly defined gradient due to the high mobility of this substance in air. Mosses in the remote sites accumulate comparable concentrations of BHC as industrial sites.

An important component of Thomas's (1986) work was the derivation of multiple regression equations relating PAH concentrations in mosses with particulate air quality data. Such models are crucial since they allow quantifiable predictions in air quality monitoring to be made. However, no significant models were established for chlorinated hydrocarbons because mosses are more appropriate for measuring environmental chemicals deposited in particulate form.

In Finland, analysis of 26 *Sphagnum* moss samples collected from 12 locations over a period of ten years indicated substantially higher concentrations of PCBs in the industrial south compared to the non-industrialised northern part of the country (Himberg and Pakarinen, 1994).

2.2 Active monitoring

Transplantation of plants to other areas for the purposes of pollution assessment is not restricted by the current distribution of a species at the sites of interest and allows the time of exposure to be defined.

Sphagnum moss bags were used to assess organic contamination (PCB and organochlorine pesticides) in three regions of Canada (Strachan and Glooschenko, 1988). Sheltered moss bags retained higher levels of compounds than bags exposed to wash off from rainwater. In conclusion, the authors did not recommend the use of such bags as monitors of atmospheric levels of persistent organic contaminants. Material in the commercially available polypropylene mesh bags was lost due to wind and the use of finer, air permeable mesh bags for surveying were suggested. However, the bags provided qualitative relative assessments and can act as early warning indicators for further action.

Wegener *et al*. (1992) transferred *sphagnum* moss samples in nylon bags from a rural area in Ireland to a sampling location at one km distance from an aluminium production plant in Botlek, the Netherlands. Samples were also exposed to three rural areas of the country approximately 20 to 30 km away, to provide reference data. Exposure time was 30 days. Concentrations of PAHs in moss bags near the production plant were approximately 30 times higher than concentrations in bags in the rural areas. Concentrations in the rural areas were not significantly different.

3 Lichens

Lichens are recognised monitors of heavy metal deposition (Chapter II, Section 2). The same properties have assisted in their use of biomonitors of organic compounds.

The lichen, *Usnea barbata* was used to assess the chlorinated hydrocarbon burden at two sites on the mountains facing the Mediterranean Sea, near Monaco (Villeneuve *et al*., 1988). Results were comparable with chlorinated hydrocarbon values observed in industrial areas in Italy and Norway. Concentrations of insecticides and toxaphene were higher at the site of highest altitude and were thought to have been transported from agricultural areas to this exposed site. PCBs were unaffected by altitude which enabled bioconcentrations factors between lichen PCB content and the atmosphere to be calculated.

Muir *et al*. (1993) analysed samples of the lichen, *Cladina rangiferina* at a number of locations over a three-year period in Ontario, Canada. Concentration gradients were observed for DDT, chlordane and dieldren, with significantly higher concentrations in lichens in south-central Ontario locations than in northern and north-western locations. Multivariate statistical analyses, such as principal components analysis was applied to the data to examine dominant patterns of all individual organochlorine components, their variation with geographical location and establish relationships between lichen and air precipitation data. Data was insufficient to infer any conclusions regarding temporal trends.

4 Higher Plants

Herbs and trees have been used in biomonitoring both as bioaccumulators and bioindicators by exhibiting symptoms of visible injury.

4.1 Visible injury

Taylor *et al*. (1990) reported acute injury symptoms and growth abnormalities of ethylene. Most of this information has been obtained from laboratory studies. The age of tissue, presence of other pollutants and temperature will affect plant sensitivity to ethylene.

In conifers, yellow tips on needles are a common response to ethylene. Necrosis and abscission of cones has also been recorded. Abscission of leaves and flowers in broad-leaved trees usually occurs. Loss of bark has been observed in elm.

Various effects have been observed in herbs, grasses and crops. These include curling and twisting of leaves prior to abscission in lily, tulip and hyacinth. Chlorosis followed by necrosis has been detected in rose plant leaves while beet and radish have exhibited red/purple discolouration. With regard to flowers, delayed opening, premature opening, inhibition of flowering, promotion of flowering, flower close, loss of petals, necrosis and male flowers becoming female flowers have all been observed in different species. Bushy plants and promotion of senescence are general features observed in many herb, grass and crop species in response to ethylene exposure.

Felsot *et al*. (1996) used injury symptoms in pea, bean and corn seedlings to assess the exposure of non-target crops to atmospheric deposition of herbicide residues from drift or localised transport of herbicide residues. The most frequently observed symptom was chlorotic spots on the upper leaf surfaces on exposure to sulfonylurea herbicides. Exposure to phenoxyacetate herbicide caused stem kinking and abnormal twisting with leaf cupping. Bright green veins appeared on leaves exposed to the herbicide aminophosphonate while discrete necrotic circular spots characterised exposure to bipyridilinium.

4.2 Bioaccumulation

Most studies involving plants as bioacccumulators involve passive monitoring.

In France, Granier and Chevreuil (1992) used tree leaves from the plane tree *Plantanus vulgaris* as bioindicators of aerial organochlorines. Plants absorb PCBs mainly from the surrounding atmosphere and not from the roots. However this may not be true for other organic compounds. The authors calculated air/leaf bioconcentrations factors of 10^5 for PCBs and 10^4 for lindane. PCBs showed spatial and temporal variation throughout Paris. The authors recommended the use of tree leaves in both point source emission assessment and on larger scale studies.

Kylin *et al*. (1994) developed an analytical method for the determination of PCB in pine needle wax. This was applied to pine needle samples collected as part of a mapping distribution programme of organochlorines in Europe. The importance of this technique is that it allows specific and precise measurement of PCBs in needles of varying age classes, enabling not only spatial but temporal trends to be established.

Herbs have also been recognised in organic biomonitoring. Goldenrods, a widely distributed indigenous weed, are excellent accumulators of PCB (Weinstein and Laurence, 1989).

5 Conclusions

Literature with regard to biomonitoring of organic compounds is somewhat sparse in comparison to other air pollutants. Direct comparison of the literature is difficult due to the different techniques used. Differences exist between the use of natural or transplanted plants, the type of species used, exposure time, sampling location, sampling procedures including sampling height and sample collection and chemical techniques used.

Mosses and lichens appear to be very effective bioaccumulative tools in the detection of organic compounds. The same properties that make mosses and lichens suitable monitors of heavy metal deposition also appear to make them appropriate biomonitors of organic pollutants. These include slow growth, large surface areas, lack of a cuticle, the lack of internal transport mechanisms and a dependence on the atmosphere for nutrients.

Indigenous mosses have been used on the multi-national and national scale. Transplanted mosses appear to be more effective in the assessment of point sources of pollution but have been used in larger scale surveys. Quantifiable models correlating concentrations in moss samples to air quality data have been established for some compounds.

No literature relating to fungi and organic compounds was available at the time of this study.

Higher plants also have potential in the biomonitoring of organic micropollutants. They have an advantage over lower plants in that they display recognisable, distinguishable and often quantifiable injury symptoms to these compounds. Higher plants, particularly tree leaves and needles, can also be used as bioaccumulative indicators but as is the case for heavy metals, interpretation of results may be complicated by uptake of chemicals via the root system.

Further work in relation to the mechanisms and the accumulation of micropollutants in plants is required. This should improve the use and interpretation of results in biomonitoring of these compounds.

6 References

Felsot, A.S., Bhatti, M.A., Mink, G.I. and Reisenauer, G. 1996 Biomonitoring with sentinel plants to assess exposure of nontarget crops to atmospheric deposition of herbicide residues. *Environmental Toxicology and Chemistry,* **15**, 4, 452-459.

Granier, L. and Chevreuil, M. 1992 On the use of tree leaves as bioindicators of the contamination of air by organochlorines in France. *Water, Air and Soil Pollution*, **64**, 3-4, 575.

Himberg, K.K. and Pakarinen, P. 1994 Atmospheric PCB deposition in Finland during 1970s and 1980s on the basis of concentrations in ombrotrophic peat mosses *(Sphagnum)*. *Chemosphere*, **29**, 3, 431.

Kylin, H., Grimvall, E.and Östman, C. 1994 Environmental monitoring of polychlorinated biphenyls using pine needles as passive samplers. *Environmental Science and Technology*, **28**, 7, 1320-1324.

Muir, D.C.G., Segstro, M.D., Welbourn, P.M., Toom, D., Eisenreich, S.J., Macdonald, C.R. and Whelpdale, D.M. 1993 Patterns of accumulation of airborne organochlorine contaminants in lichens from the Upper Great Lakes Region of Ontario. *Environmental Science and Technology,* **27**, 6, 1201.

Strachan, W.M. and Glooschenko, W.A. 1988 Moss bags as monitors of organic contamination in the atmosphere. *Bulletin of Environmental Contamination and Toxicology*, **40**, 3, 447.

Taylor, H.J., Ashmore, M.R. and Bell, J.N.B. 1990 Air pollution injury to vegetation. IEHO, London.

Thomas, W. 1984 Statistical models for the accumulation of PAH, chlorinated hydrocarbons and trace metals in epiphytic *Hypnum Cupressiforme*. *Water, Air and Soil Pollution*, **22**, 4, 351.

Thomas, W. 1986 Representativity of mosses as biomonitor organisms for the accumulation of environmental chemicals in plants and soils. *Ecotoxicology and Environmental Safety* 11, 3, 339.

Villeneuve, J.P., Fogelqvist, E. and Cattini, C. 1988 Lichens as bioindicators for atmospheric pollution by chlorinated hydrocarbons. *Chemosphere* **17**, 2, 399.

Wegener, J.W.M., van Schaik, M.J.M. and Aiking, H. 1992 Active biomonitoring of polycyclic aromatic hydrocarbons by means of mosses. *Environmental Pollution* **76**, 1, 15.

Weinstein, L.H. and Laurence, J.A. 1989 Indigenous and cultivated plants as bioindicators. In: *Biologic markers of air pollution stress and damage in* *forests*, Committee on biological markers of air pollution damage in trees, National Research Council, National Academy Press, Washington D.C.

V FINAL CONCLUSIONS

1 Plant groups

From the foregoing chapters it is evident that plants have a role in the biomonitoring of air pollution. A wide range of plant groups, species and techniques are available. Analysis of plant tissue provides direct quantitative information on relative concentration loads. Alternatively, observing plant responses is a simpler and less expensive technique and can be used as early warning systems.

Lichens, bryophytes, fungi, algae and higher plants have been used in biomonitoring of air pollution. Lichens and bryophytes are the most widely used plant groups in air pollution monitoring. These groups contain tolerant species with effective bioaccumulating properties and sensitive species which show pronounced responses to air pollutants. Passive and active air quality monitoring using these groups can include quantitative evaluations allowing relative pollution assessments to be made. Fungi may have a potential role in the assessment of air pollution impact on forest ecosystems. Algae are useful in the monitoring of nitrogen deposition.

In higher plants the assessment of foliar symptoms are probably the most widely used bioindication techniques. This is particularly true for ozone. The presence or absence of foliar injury has been used to establish zones of impact, while the type of foliar injury has been used to discriminate among various possible air pollutants. However, visible damage is not always specific to a particular pollutant or other environmental stress. In addition not all species have been exposed to all known pollutants to establish their symptom expression.

Traditionally, biomonitoring programmes have been developed in relation to local and industrial sources of pollution. Bioindication programmes on the local scale require less effort due to a relatively easily located point source from which contamination generally follows a gradient. In this instance cause and effects relationships are often obvious. In large-scale surveys other factors such as uneven spatial distribution and pollutant mixtures become more significant. However such large-scale standard monitoring programmes are important in providing data on long-term temporal and spatial trends of air pollutants.

Biomonitoring using plants can be a simple and inexpensive process which lends itself as a potential, adaptable method of assessing air quality in developing countries. However, due to climatic and edaphic differences additional considerations may be necessary. For example, in arid areas cryptogamic flora may be less sensitive to air pollution because of low humidity. Biological monitoring becomes highly applicable in remote areas where continuous, direct air sampling is expensive and impractical.

Laboratory and/or artificial field investigations are often necessary to establish the role of individual pollutants, the synergistic effects of pollutant mixtures, biological responses and tolerances. These studies can be used to establish parameters of biological monitoring programmes conducted under natural conditions.

2 Design of monitoring programmes

A well designed monitoring programme will aid the determination of cause and effect relationships. There is often a need for pilot studies to determine usefulness of different biological materials and approaches in detecting environmental pollution. As demonstrated in the preceding chapters several important factors therefore need to be considered.

2.1 Method selection

When devising an air quality biomonitoring programme using plants a major choice lies between using passive or active biomonitoring. Passive monitoring is generally quicker, simpler and may allow the assessment of long-term pollution exposure. In contrast, active methods such as transplantation exercises generally represent a pollution regime over a short period of time but may allow quantitative assessments such as deposition rates to be made. In addition the genetic state and physiology of the plant is known and results can be more reliably related to air pollution. However transplantation monitoring requires the use of control plants for comparison.

As illustrated in the above chapters several air quality biomonitoring methods are available. Monitoring can be qualitative or quantitative and can employ single indicator species or use community changes. Other methods include physiological/biochemical plant responses or visible injury as indicators of air pollution. By analysing element content from plant tissue samples at different distances from a pollution source, the type of pollution and the size of the fallout zone can be determined.

The choice of method depends among other factors, on the purpose of the survey, the size of study area, the resources available and the desired detail of the output.

2.2 Species selection

Careful selection of plant bioindicator species during the design of a monitoring programme enables not only the identification of the pollutants but can supply approximate estimates of the pollutant dose, the strength and location of the polluting source and assist in the demarcation of the spatial and temporal distribution of the pollutant.

The sensitivity and tolerance of plant species is fundamental to their selection. Bioaccumulative indicators tend to be tolerant to the pollutants under investigation, whereas sensitive species indicate air pollutants by showing recognisable responses. The selection of a species will depend on the techniques employed and the objectives of the monitoring programme. For example, if the sole reason for a study is to determine whether air pollution is having an impact or not, then it may be useful to observe all sensitive species.

Alternatively, the most sensitive species with the widest distribution are the preferred bioindicators in mapping sources of pollution. Species selection will also depend on whether an array of air pollutants are the suspected contaminants or a single pollutant is responsible. In metal deposition biomonitoring the species utilised and its effectiveness will depend to an extent on the elements to be monitored, certain species being better bioaccumulators of a particular element than others..

In general, bioindicators should show a distinct, easily measured response to a pollutant and the response should be measured with an acceptable accuracy and precision. Other factors which affect plant species selection include the availability of the species and ease of sampling.

2.3 Site selection

The density and location of sampling sites will depend very much on the type of survey required by the monitoring programme. Larger scale surveys covering larger areas will obviously require more sites than studies investigating point emission sources. In the latter, sites are frequently spaced along transects or gradients in relation to the pollution sourceIn general intensity of sampling sites should be adequate to detect gradual changes along the study area. If indigenous species are to be utilised, the number and location of sites will depend on the natural distribution of the species whereasif transplantation techniques are used, choice of sites are at the discretion of the investigator.

The collection of additional environmental data at a sampling location is often necessary and will often aid the interpretation of results.

2.4 Data analyses

Biomonitoring surveys can generate large quantities of data. Data analyses and interpretation methods should be addressed at the earliest stages of sampling design. Semi-quantitative and quantitative indices are a useful summary tool and allow comparisons between datasets. Indices often involve less fieldwork and provide good baseline data (Miller, 1989). A good biological index system is ideally simple, rapid, robust and user friendly but based on sound mathematical reasoning. According to Muir and McCune (1987) the ideal index uses quantitative information which is equally weighted or carefully weighted.

The application of statistical analytical methods should be considered. For example multivariate analysis techniques have successfully been used to assess the importance of air pollution in causing plant responses in relation to other environmental factors. For example, multivariate analyses techniques such as factor analysis have been used in the accurate interpretation of results from large-scale moss surveys by including an assessment of the contribution from other sources.

2.5 Chemical analysis

Chemical analysis of plant tissue is generally applicable to bioaccumulative monitoring studies. Analysis of tissues for sulphur, nitrogen, heavy metals or organic compounds has been used in air quality biomonitoring.

Over the years a number of lessons have been learned. These include:

- contamination during collection should be avoided;
- replication of samples is recommended for accurate results;
- consistency of measurement units aids comparative studies;
- results are more appropriate when background elemental levels are obtained.

The choice of analytical method will depend on the purpose of survey. Some analytical methods are non-destructive and are useful for repetitive surveys such as baseline studies. Such samples can also be archived and used at a later date for additional analysis. Alternatively although destructive techniques result in the loss of the plant they may be more effective in achieving the desired results.

When estimating elemental content in plant tissue, the reliability of procedures can be assured by including measures such as: use of standard solutions; use of blanks; reanalysis of selected samples and the standard solutions used in calibrating the instrument after analysis of every five samples for atomic absorption spectrophotometry (Rao and Dubey, 1992).

2.6 Quality assurance

Effective quality assurance during a biomonitoring programme is essential to the production of high quality data. Quality assurance procedures are required for two reasons:

- to ensure that the method is being followed properly;
- to ensure that the required levels of accuracy and precision are being achieved at each step in the process of sampling, processing, data generation, interpretation and archiving.

Quality assurance is of particular importance in situations where data may be exposed to legal challenge, or when regional, national or international comparisons of monitoring results are required.

The application of general quality assurance and analytical quality control is more widespread and comprehensive for chemical methods than for biological ones, although it is of equal importance for both disciplines.

At its most basic, quality assurance procedures should take the form of a predetermined level of random repeat surveys, analyses, measurements or identifications (as appropriate to the individual method), with a pre-set action level for disparity. Training provision, inter-laboratory calibration exercises and accreditation schemes for individual methods should all be considered in the overall planning of quality assurance. Minimisation of the bias introduced by different surveyors employing the same method needs to be minimised. This may be partly achieved by training and the application of statistical measures of assurance. If standard methods of biomonitoring are to be developed, formal training procedures are necessary. The United Nations Economic Commission for Europe (UN ECE) monitoring programme mentioned regards training of surveyors an important element of international co-operation.

Throughout the published literature, quality assurance procedures were seldom addressed, although some standard national and international (e.g. UN ECE) protocols ensure that a consistent approach is taken during surveys. The spects of quality assurance addressed by certain authors during compilation of this report are provided here. In their study of O_3 distribution using tobacco plants in northern Italy, Mignanego *et al.* (1992) described how measurements at sites were always undertaken by the same recorder. In addition, inter-calibration exercises were stressed and samples were sent off to external auditors on a monthly basis. More recently Chappelka *et al.* (1997) detailed specific quality assurance procedures employed during a survey of O_3 plant injury in Great Smoky Mountains National Park. Sampling teams were trained in species recognition, O_3 symptom recognition and quantification before embarking on fieldwork. Survey teams were also evaluated against an 'expert system' for testing the ability to rate injured foliage using herbarium samples. An acceptable score was defined as 75% of the leaves correctly estimated to within one class of the actual amount of foliar injury.

In biomonitoring studies, the standardisation of sample collection, preparation, analytical techniques and data analysis is recommended. This will ensure consistency and comparability between different sampling sites, surveys and between different regions.

3 References

Chappelka, A., Renfro, J., Somers, G. and Nash, B. 1997 Evaluation of ozone injury on foliage of black cherry (*Prunus serotina*) and tall milkweed (*Asclepias exaltata*) in Great Smokey Mountains National Park. *Environmental Pollution*, **95**, 1, 13-18.

Mignanego, L., Biondi, F. and Schenone, G. 1992 Ozone biomonitoring in northern Italy. *Environmental Monitoring and Assessment*, **21**, 2, 141.

Miller, P.R. 1989 Biomarkers for defining air pollution effects in western coniferous forests. In: *Biologic markers of air pollution stress and damage in forests*, Committee on biological markers of air pollution damage in trees, National Research Council, National Academy Press, Washington D.C.

Muir, P.S. and McCune, B. 1987 Index construction for foliar symptoms of air pollution injury. *Phytopathology*, **71**, 558-565.

Rao, M.V. and Dubey, P.S. 1992 Occurrence of heavy metals in air and their accumulation by tropical plants growing around industrial areas. *Science of the total Environment*, **126**, 1-2, 1-16.