BIOLOGICAL INDICATORS IN ENVIRONMENTAL PROTECTION

Margit Kovács
(Editor)

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This book demonstrates how living organisms are used to detect the presence of environmental pollutants, e.g. sulphur dioxide, hydrogen fluoride, and heavy metals in air and in water. It presents recent results of research in this field, using standard methods for the determination of heavy metals in the air of cities and industrial regions. It is a comprehensive review of plant and fungus species for the detection of pollution, surveying various species of fungi, lichens, mosses and flowering plants and the reactions of pollutants.

Based on practical real-life studies, the authors' work demonstrates means of selecting species for the detection of microelements, showing how to discover toxic substances hazardous to man amongst these elements. Tables help the reader to select the species most suitable for pollution detection. The book shows the physiological, cytological and histological changes used in bioindication, and demonstrates that environmental contamination is indicated by various physiological phenomena.

Readership: Environmental and biological scientists in both human and veterinary science. Agricultural and forestry science. Urban and city planning.

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AKADÉMIAI KIADÓ, BUDAPEST 1992

This book is the up-dated English version of the Hungarian *A környezetszennyezést jelző és mérő élőlények* published by Mezőgazdasági Kiadó, Budapest 1986

Translated by
Ákos Máthé, Zoltán Tuba (Ch. 7, 12), *Jan L. D. Meenks* (Ch. 7), *Zsolt Csintalan* (Ch. 12)

Translation revised by
László Nagy and *János Podani*

Joint edition published by
AKADÉMIAI KIADÓ, Budapest, Hungary
and
ELLIS HORWOOD LIMITED
Market Cross House, Cooper Street,
Chichester, West Sussex, PO19 1EB, England

ISBN 963 05 6027 5

Printed in Hungary by Akadémiai Kiadó és Nyomda Vállalat, Budapest

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1.1 THE CONCEPT OF BIOLOGICAL INDICATORS

The indicator values of soil-indicating plants (chemical response, N content, depth of groundwater, etc.) are known (compare: Ellenberg, 1979; Zólyomi et al., 1967).

On the basis of phytosociological studies as well as vegetation maps, it is possible to make deductions as to the environmental conditions.

Many plant and animal species can also be used to indicate air, water or soil pollution. The indicators react to both natural and anthropogenic effects.

Biological indicators are those organisms (or populations) of which their occurrence, vitality and responses change under the impact of environmental conditions.

Each organism (just like an open system) responds to environmental change as it would respond to a specific stimulus. The absorbed stimuli bring about reactions that provide information on both the changes and the level of pollution of the environment.

The various species respond on a variable scale, in a most sensitive, sensitive or less sensitive (resistant) way. Resistant species can often be considered as accumulating indicators.

It is a requirement that biological indicators occur in sufficient number (i.e., abundant) and possess specific reactions to the environment.

Certain plant species are especially suitable to indicate air pollution.

The response of plants to pollutants depends on their:

- genetic make-up;
- stage of development;
- environmental conditions, and
- the concentration of pollutants.

Although physical and chemical measurements provide quantitative data on the presence and levels of different pollutants, they do not provide an exact image of the extent of pollution reaching the living organisms, nor of their effects.

The data provided by indicator organisms can be used to estimate the degree of environmental impact and its potential danger for other living organisms (e.g. man).

1.2 THE CLASSIFICATION OF BIOLOGICAL INDICATORS

Indicator species can be classified into the following groups (Steubing, 1978; Bünau et al., 1979; Bick, 1982; Arndt, 1982; Ehmke, 1982; Huber and Huber, 1984; Arndt et al., 1987) (Table 1).

Indicator species: occurrence or absence indicates the effect of certain defined factors (environmental factors). Species with a low tolerance to a given environmental factor (stenoecious species) are suitable. These species can be positive indicators by their occurrence, distribution or abundance. For example, the intensive growth of blue-green algae (e.g. *Aphanizomenon flos aquae, Anabaena flos aquaticae*) indicate the level of eutrophication of waters.

Table 1 — Classification of biological indicators (Nobel et al., 1983)

Indicator type	Indication	Example
Indicator organisms	passive presence or absence	floristic and vegetation study, floral inventory, mapping
Monitoring organisms	passive reaction	ecological sequence of species groups, the measurement of the degree of damage
	accumulation	determination of the chemical composition of the species
	active reaction	transplantation; the measuring of the degree of damage in the species exposed
	accumulation	transplantation; the determination of the chemical composition of the species exposed
Test organisms	active reaction and accumulation	loading analysis under laboratory or natural-like conditions; toxicity test

Negative indicators indicate environmental changes by their absence or disappearance: (e.g. so-called lichen deserts, areas devoid of lichens, might develop due to the impact of sulphur dioxide).

Species living under varied environmental conditions in general are species of broad distribution (euryoecious species), they have only a limited usage as indicators, since by tolerating wide variations in the given environmental factors, they manifest a delayed reaction compared with stenoecious species.

Monitoring species indicate the presence of pollutants either in a quantitative or qualitative way.

Sensitive monitors are highly susceptible to various pollutants, and consequently are suitable to indicate both acute and chronic exposures. The damage suffered is usually displayed in the form of external symptoms. Their vitality as well as growth is reduced. The symptoms often indicate a specific pollutant exposure. For example, exposure to fluorine leads to various necrotic symptoms on the leaves of certain monocots e.g. gladiolus, freesia and tulip.

From experiments, i.e., by studying the symptoms caused by exposure to pollutants, the quality of environmental pollution can often be identified.

Accumulating indicators are generally plants with a resistance to pollutants since they can accumulate large amounts of pollutants (e.g. heavy metals) in their plant tissues generally without any harm.

In plants, the rate of accumulation of various air pollutants may vary. Information obtained by the observation of a given species can seldom be related to other species.

Accumulating indicators can be:

– passive indicators—naturally occurring species are used to detect various pollutants;

– active (experimental) indicators—the indicating species produced under standardized conditions are exposed to the pollutants of specific areas. As a rule, these are used to indicate short-term loads.

Test organisms. These species are suitable for the toxicological study of a given pollutant. They respond to exposure either in a quantitative or a qualitative way. Many species belong to this group; most frequently they are plants and bacteria but sometimes vertebrates, such as rats or mice, are also used.

1.3 THE EVALUATION OF BIOLOGICAL INDICATORS

Biological indicators can be evaluated in the following ways (Steubing, 1978):

1) On the basis of visible damage (macroscopic symptoms).

Leaf chlorosis. This symptom is brought about by the long-term (chronic) exposure to small amounts of pollutants. In the leaves the appearance of yellowish-green, later on reddish spots, can be observed. The green pigments (chlorophyll) decompose, while other pigments appear. The chlorosis is frequently associated with the aging of leaves.

Leaf necrosis. With exposure to the pollutant the cells and tissues die off. This symptom can be brought about by a single episode of air pollutants at high concentration; e.g., during photochemical "smog". The characteristic symptoms appear on the leaves of petunia within 24 hours, first appearing at the leaf margin, and later expanding to the tissue of the internerval region. In coniferous species the brownish coloration appears at the tip of the needle.

Changes in the growth (anomalies). With exposure to pollutants different symptoms can be observed in sensitive tissues. For example, sulphur dioxide or fluorine pollution cause the green leaves to fall, even to the extent that the trees can lose their entire foliage.

The impact of ozone is shown for example in a decrease in the height of tomato plants and in the size of leaves. The number of flowers and as a consequence the yields are frequently decreased.

2) Microscopic symptoms (cytological damage). Cell damage is indicated by disorders in the plasma movement or the plasmolysis of cells. The cell contents shrink and the chloroplasts are deformed.

In deciduous trees the palisade parenchyma is damaged and the discoloration of cell walls is commonly observed.

3) Physiological and biochemical, chemical changes.

Ecophysiological symptoms. Air pollution affects gas exchange, and the rate of photosynthesis of plants.

The water balance is also affected together with the function of stomata resulting in an increase in the rate of transpiration.

Biochemical symptoms. With exposure to pollutants cell permeability, osmotic properties and buffering capacity will also be affected. Changes take place in the metabolism of amino acids as well as in the activity of enzymes and coenzymes.

Chemical changes. The various pollutants (e.g. sulphur, fluorine, heavy metals or their residuals) are accumulated.

Larger amounts of heavy metals are generally accumulated by resistant ecotypes. For example such a resistant ecotype has evolved in the case of rye grass *(Lolium perenne)*.

The resistant, accumulating indicators are especially suitable for monitoring of the conditions of certain source areas of pollution. In areas where sensitive plant species can no longer survive, or where they have diminished, these indicators are suitable for the indication of pollutants in the long term.

Plant resistance can be specific either to species or variety. It is also a function of the stage of plant development. In general, younger plants are more susceptible to the pollutants. This property is used in some monitoring studies e.g. employing tobacco plants with only 2–3 leaves, where the stomata have not yet fully developed and are most susceptible to ozone.

Conditions of the habitat also influence the resistance.

Weather conditions, such as high humidity of the air, and light that stimulates stomatal opening can reduce resistance. The physical, chemical and biological properties of soils can also play an important role. Nutrient status can either increase or reduce the resistance. With the use of different fungicides, plants can tolerate a higher level of photochemical "smog".

1.4 THE PROGRAMME OF UNESCO

One of the aims of the UNESCO programme "Man and Biosphere" (MAB) is to identify those organisms that can serve as important indicators of the pollution stress of various natural resources (air, water, soil). The MAB programme states that in the study of ecosystems, as well as physical and chemical measurements, it is essential to use biological indicators, too.

It is necessary to identify those species that are susceptible to changes in the environment and to the changes taking place in natural ecosystems.

In the MAB programme, the advantages to be expected from the use of biological indicators can be summarized as follows:

– bioindicators reflect the complex effect of environmental factors, the entire environment;

– they supersede the difficult task of making physical and chemical measurements with biological effects;
– they help to visualize the rate and direction of environmental changes;
– they locate those compartments of the ecosystem where polluting and toxic matters accumulate.

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2 Monitoring system

Monitoring is a system of regular observations, both temporal and spatial, that provides information on the state of the environment. It aims to make comparisons between past and present states. Data collected by monitoring are expected to be useful in predicting future changes that are important for man. Consequently, monitoring is indispensable in attacking the problems of enrivonmental control.

The basic tasks of monitoring can be summarized as follows (MAB report, 1974):

1) The measurement of the concentration of pollutants in the inorganic medium (water, air, soil).

2) The measurement of physical variables (e.g. temperature, soil structure, rainfall, river flow).

3) The estimation of the frequency and the level of impact detrimental to man (mortality, morbidity, structural and functional changes), and the study of correlation between these symptoms and the physical and chemical variables of the environment.

4) The inventory and classification of large-scale damage arising from human activity (e.g. deforestation, desertification).

2.1 BASELINE STATE

With the main emphasis being placed on environmental changes and their measurement, data can only be informative and suitable for assessment if observations can be related to some standard of comparison. In an ideal case, this standard is perfectly free from anthropogenic effects. It is well known, however, that intact natural conditions exist almost nowhere in the world. Areas situated thousands of kilometers from emission sources may also suffer some damage. Consider for example acid rain in North America and Europe, which has destroyed the fauna and flora of lakes. Similarly, the detrimental effect of pollutants reaching the seas may also extend to large areas due to oceanic currents.

Usually, we must therefore accept a relatively intact basis for comparison. It is particularly true for small and relatively densely populated countries, like Hungary, with a fairly developed industrial background. No doubt that monitoring has a special importance in these countries because they have practically no areas without air, water and soil pollution. Fortunately, records obtained some decades ago are often available and can be used for comparison with the current situation. In many cases, however, the basic state of the environment can only be judged from conditions observed relatively recently.

2.2 PHYSICAL AND CHEMICAL MONITORING

Monitoring is often simply used to detect changes in the physical and chemical properties of the abiotic environment; for example, monitoring stations for analyzing atmospheric sulphur dioxide concentration. Nonetheless, data provided by physical and chemical monitoring are indispensable for interpreting the ecological damage.

2.3 BIOMONITORING

The goal of monitoring is to provide data on the relationship between environmental conditions and the living world, so as to develop an effective control programme. The measurement of physical and chemical variables alone is clearly inadequate; biological monitoring is also necessary to detect changes in the environment. Further arguments supporting the need for biological monitoring include:

1) The direct measurement of certain physical and chemical variables may be very expensive, requiring well-equipped laboratories and trained staff. The indirect methods of biomonitoring may replace physical and chemical analyses in many cases.

2) A more substantial reason is that the joint impact of two or more pollutants can only be evaluated by studying of biological effects. The level of a single pollutant in many cases may not reach critical levels but the synergistic effect of two or more substances can be substantial. For example, Applegate and Durant (1969) found that exposure to ozone greatly amplifies the effect of sulphur dioxide on plants.

Biomonitoring is not as broadly applicable as physical or chemical measurements, however. Organisms and communities seldom have universal occurrence, so the comparison of different areas based on biological observations is problematic. Prior to applications of a biomonitoring programme it is admissible to perform pilot studies to establish the usefulness of different biological materials for detecting environmental pollution.

Biomonitoring takes one of two approaches:

1) Direct monitoring. The quantity of pollutants is monitored directly in the organisms by applying a continuous sampling design. The only difference between biological and chemical monitoring is that the first samples are obtained from the living organisms rather than from the environment. The variables measured (criterion variables, e.g. concentrations of toxic substances) are similar.

In general, measurements of concentrations are applied where the location and time of the expected impact are unknown. Therefore, a large sample size is necessary to record damage. Also, long-term investigations are required to select plant and animal species that are resistant to relatively large amounts of toxic materials while accumulating them in their tissues. Control studies should also be performed to establish the baseline concentration of the substances measured (e.g. heavy metals) in the unpolluted samples of the organism. As the measured concentration of the pollutant in the organism can also be influenced by physiological factors, direct biological monitoring should be complemented by physiological analyses. Consequently, knowledge of the mechanisms of absorption and excretion becomes crucial.

2) Indirect biological monitoring. In the strict sense, biological monitoring involves the examination of biological variables to detect environmental change. The principle is that any changes taking place in the environment have a significant effect on the biota. Knowledge of these environmental changes and the response of target organisms can therefore facilitate the assessment of environmental conditions. Thus, organisms indicate the environment while our task is merely to interpret and understand these biological signals. Soil indicating plants provide a typical example. Their mere presence implies certain soil properties (e.g. nitrogen content, pH value). Being extremely sensitive to air pollutants, epiphytic and epilithic lichens are suitable indicators of air pollution.

Indirect monitoring has three basic types; their distinction is important for theoretical and practical reasons.

a) The study of morphological, physiological and cytological responses to the environment is one of the main approaches to biomonitoring. Monitored species should be selected to give a specific response to a given environmental change. The study of correlation between annual wood rings and climatic conditions for the examination of overall climatic changes in the past is a good example (Haugen, 1967). A quantitative assessment of air pollution levels on angiosperm leaves can be directly detected by measuring the extent of the necrotic leaf surface (Naveh et al., 1979).

b) In biomonitoring at the population level the criterion variable is based on the characteristics of a given plant or animal species, for example, its presence/absence, abundance or biomass. The most commonly used variable type is the presence/absence of a species. The occurrence of at least one individual of a species in the study area implies that at least the minimum living conditions for the species are present. Thus, the occurrence provides significant information on the state of environment at the site. Obviously, for this type of monitoring species of narrow ecological tolerance are preferred. The situation is more difficult when the absence of species is used to make conclusions on the state of environment. The absence of a species can be attributed to several factors (Green, 1979; Cairns, 1979):

– the species is absent because the conditions in the study area are unfavourable, or

– the species could survive in the site but it does not occur for biogeographical and historical reasons, or

– the abiotic conditions are acceptable for the species but it is competitively excluded, or

– the individuals of the species are overlooked in the field, or they are missing from the record due to inadequate sampling design, or

– the species formerly occurred in the study area but has become extinct as a result of pollution.

Unfortunately, it is usually impossible to decide which of these factors explains the absence of a species. An exception is the long-term study of an area in which disappearance of a species is observed during a relatively short period (e.g. extinction of lichens in cities). In such cases disappearance of species can be attributed to high certainty to environmental deterioration. In general, however, the absence of a species cannot be considered as decisive in biomonitoring.

Any change of abundance should also be considered carefully. A considerable increase of abundance is decisive only if the species is known to react in this way to the change of certain environmental factors (e.g. nitrophilous species to an excess of nutrient). Decreases in abundance may also refer to pollution, although competition and genetic changes in the population may also have similar outcomes.

c) Where the criteria of biomonitoring comprise variables pertaining to an assemblage of two or more species, rather than to the population of a single species, we are concerned with biomonitoring at the community level. Although a given species may be susceptible to several pollutants, it could be at the same time tolerant to others. Therefore, overall changes in the environment are expected to occur only at the community level (Cairns, 1979).

d) When the characteristics of the abiotic components, the development of life stages, the processes of energy flow, the nutrient cycles, food webs, and the impact of organisms on the environment are also considered in addition to community variables, we reach the ecosystem level. In general, the term ecosystem is used to refer to an open "ecological system" possessing a high degree of equilibrium which can be clearly distinguished from other systems (Reichle and Auerbach, 1972). The numerous and often circular definitions of the ecosystem, such as that given above, show that it is a concept far from being completely clarified. The reader is referred to Odum (1962) and the problem-oriented discussion in Juhász-Nagy (1970), for a more detailed treatment of the problem.

The possibilities of ecosystem and community level bioindication and monitoring are discussed in Chapter 4. These two levels should not be separated, partly because of difficulties with the existing definitions of the ecosystem concept.

3 International networks for monitoring environmental pollution

As the example of acid rain illustrates, environmental pollution is not confined to a single country. International cooperation is therefore desirable in a monitoring programme. Recognizing this need, United Nations urged the establishment of an international monitoring network. UN organizations have undertaken many initiatives in developing monitoring programmes such as the MAB programme (Man and Biosphere). Although the MAB programme does not contribute directly to international monitoring or participate in environmental protection control, its role is important. MAB focuses on the following tasks (MAB Report, 1974):

1) Elaboration of the scientific principles of monitoring.

2) The selection of pollutants to be measured and monitored, the selection of study areas and the measurements to be made.

3) The establishment of criteria of environmental quality. These criteria will be considered later together with the elaboration of standards.

The first task of the MAB programme is to study the impact of pollutants on the structure and function of aquatic and terrestrial ecosystems. These studies should select the large-scale measurements to be taken in international monitoring networks. Furthermore, suitable measurement techniques should be developed and tested in the field during pilot activities.

The results of fundamental research within the framework of MAB are used by the Global Environmental Monitoring System (GEMS) coordinated by the United Nations Environmental Protection (UNEP) programme. The objective of GEMS is to organize an international network for monitoring environmental changes. The recommendations of the UNEP conference in 1974 specified the goals of GEMS as well as the principles of international cooperation as follows:

Tasks

To provide information necessary to the protection of human health, welfare, security and freedom; the maintenance of the natural state of the environment and the reasonable utilization of natural resources. Included are:

a) The collection of data on environmental changes of natural and anthropogenic origin, and their impact on man.

b) The study of the dynamic equilibrium of environment for a better understanding of underlying processes.

c) The forecasting of significant environmental changes (including natural catastrophes) and effective preventive measures.

d) The development and implementation of the technical basis necessary for efficient monitoring.

Basic principles invoked

a) Cooperation between governments should incorporate existing national and international systems and reduce their potential differences.

b) Specialist UN agencies should provide an institutional basis for launching and coordinating monitoring programmes.

c) Global and regional problems of multinational concern should have priority in international monitoring systems.

d) Exchange of information is of primary importance even in cases that are internationally less significant.

e) On the global scale special attention should be paid to selected pollutants. These are listed in current order of importance in Table 2.

f) Development of more efficient methods for measurement of substances for which currently available techniques are inadequate or inefficient.

Table 2

	Pollutant	Medium recommended for its study
1	Sulphur dioxide and aerosols	air, soil, water, aquatic ecosystems
2	Radioactive isotopes (Sr_{90}, Cs_{137})	food
3	Ozone	air
4	DDT and other chlorinated hydrocarbons	living organisms
5	Cadmium and its compounds	food, man, drinking water
6	Nitrates and nitrites	drinking water, underground water, food
7	Nitrogen oxide and dioxide	air
8	Mercury and its compounds	food, water
9	Lead	air, food, water
10	Carbon dioxide	air
11	Carbon monoxide	air
12	Mineral oil and its residues	sea water
13	Fluorides	drinking water
14	Asbestos	air
15	Arsenic	drinking water
16	Mycotoxins	food
17	Microbial pollutants	food
18	Reactive carbohydrates	air

g) Countries participating in the programme will exchange data and results, especially in cases where natural catastrophes and pollution involving several countries occur.

h) The economic status of less developed countries must not prevent their participation in monitoring networks. Developing countries should be aided by other countries in educating specialists and providing equipment.

i) The countries involved assume responsibility to establish monitoring systems outside the authority of governments (e.g. oceans, space, arctic regions).

4 Biological indication at the community and ecosystem levels

Bioindication and monitoring at the individual and population level are generally confined to a particular toxic agent or to a small group of chemicals of similar structure and/or impact. This has both advantages and disadvantages. Although specificity leads to high reliability at these levels, the general state of the environment cannot be detected by such methods. In principle, one could simultaneously study several species susceptible to different pollutants but this cannot be realized in practice. It is impossible to find a single specific indicator for each known or unexpected polluting agent. Consequently, in order to characterize the environment sufficiently, all species present in the study site, rather than selected indicator organisms, should be considered. Factors affecting individuals and populations also manifest themselves at the level of communities and ecosystems. Another reason for considering high level monitoring is that even inclusion of all separate species does not necessarily provide a practical method of monitoring since communities and ecosystems are not simple "assemblages" of their constituent elements. Clearly, at every level of organization new characteristics arise as a result of interactions and other relationships among the constituent elements (Reichle and Auerbach, 1972; Wilson et al., 1974).

Many fundamental problems of environmental pollution can be attacked only through biomonitoring at the level of communities and ecosystems. These include changes of the living world caused by the increased CO_2 concentration in the atmosphere and by changes in nitrogen cycling. From the viewpoints of economics it seems extremely important to survey how pastures, forests and surface waters are changed under the influence of human activity. Changes in the natural communities which are not economically exploited also bear influence on the quality of human life and are receiving increased attention.

In spite of these reasons, individual and population level investigations continue to dominate monitoring studies, and it appears that this situation is not likely to change markedly in the near future. At the level of population some most important factors are registered such as growth, reproduction and death (Matthews et al., 1982) but these investigations need to be extended to higher levels of organization.

In monitoring the state of communities and the direction of changes, several basic types of responses can be distinguished (Hirsch, 1979):

1) The study of structural degradation and measurement of its rate. As degradation can be caused by a multitude of factors (e.g. drainage of marshes, river regulation, mining, deforestation, grazing), monitoring is not confined to the impact of pollutants alone. The classification of communities and ecosystems, the preparation of inventories and comparison with a baseline state, are essential tasks.

2) An alternative type of monitoring is the study of the impact of selected pollutants within a limited area for a long period of time. For example: studies of the impact of dust in the environs of cement works, and the analysis of relationships between sewage discharges and the structure of algal communities. Surveys of this kind are not rare, and in most cases no sharp distinction is made between the population and community level.

3) Detection of relatively small-scale changes in comparable ecosystems of large regions. Well-known examples include the acid rain in North America and Europe and ocean pollution by crude oil arising from tanker catastrophes. Monitoring in this case should be extended to areas not directly exposed to the impact.

4) In the most complex type of monitoring at the community and ecosystem level, the kinds of impact to be expected are unknown. In this case, we must ensure that the ecosystem is in an intact state, or else we must detect all the possible pollutants of primary concern.

4.1 STRUCTURE AND FUNCTIONING OF BIOLOGICAL SYSTEM

Biological monitoring at the population level is concerned with changes in both the structure and function of communities. In this regard, the available literature is quite unbalanced. Many studies focus on the taxonomic analysis of communities (species lists, comparisons based on abundance data). This is the easier part of the job. The structural analysis of communities is far less time-consuming than the tedious and long-lasting experiments on nutrient cycling or other dynamical aspects of community and ecosystem organization. Observations pertaining to the taxonomic structure are usually easy to compare with data derived from various studies but this is not so with measurements of mineral cycling, for example. The most fundamental problem is, however, the relative imprecision of methods suitable for the study of functioning and the lack of uniform standards. Studies of relatively undisturbed communities have provided most of the available information of functioning but these results can find limited application in biological indication (Matthews et al., 1982).

According to Odum (1962), the structure of an ecosystem is characterized by the composition of assemblages of living organisms (species, abundance, biomass, spatial distribution) and its temporal changes; and the quality and quantity of abiotic materials (e.g. water, nutrients).

The functioning of an ecosystem includes: 1. the direction of energy flow, and production and respiration rates; 2. the cycling of organic materials (biogeochemical cycles); and 3. biological and ecological regulatory mechanisms (e.g. photoperiodism) and the interaction between organisms and environment (e.g. nitrogen-fixing bacteria).

Whereas methods of biological monitoring may be discussed separately for structure and functioning in case of communities, such a separation is inappropriate at the ecosystem level.

4.2 BIOINDICATION AND BIOMONITORING BASED ON COMMUNITY STRUCTURE

Community structure is characterized by variables pertaining to species composition and to their abundance, biomass and spatial distribution. Evaluation of such data is potentially meaningful if they are available from many sample sites within the study area. Such data are summarized in matrices or tables where, for example, the rows represent species and the columns are sample sites. Such a table conveys inher information on community structure which may not be obvious from visual tion of isolated values. Interpretation is facilitated, however, if the data are formed" into some derived variables and are analyzed through statistical analyses.

Calculations are not required if only the descriptive characteristics of communities, such as the number of species, are considered. The general view is that a decrease of species number (abundance) indicates deterioration of the environment. Nevertheless, changes in species abundance should be interpreted with care. Since the number of species can be influenced by several factors independently of pollution exposure, comparisons of polluted and intact areas on this basis may not produce reliable results. In the long-term study of a site, however, changes in species number can provide an acceptable basis for characterizing community response to pollutants. Successful applications of this approach are found in March (1976), Harman (1972), Green (1979) and Cairns et al. (1971).

In addition to species abundance, the total number of individuals of a given taxonomic group (Davies and Gamble, 1979), changes in total plant cover and biomass at a site (Grodzinski and Yorks, 1981) and total biomass of bacteria (Bartsch, 1967) can be used as bioindicators. The advantage of such an approach is that no species identifications are required.

4.3 SPECIES ABUNDANCE CURVES

Raunkiaer (1934) pioneered the quantitative description of community structure. He calculated the percentage frequency of plant species in samples and established five frequency categories: 1–20, 21–40, 41–60, 61–80 and 81–100%. He analyzed the distribution of species richness over these categories for various communities and illustrated the results as bar diagrams. Typical distributions, resembling an inverted letter "J", is shown in Fig. 1. This led to the elaboration of the "frequency law" which states that the majority of species are relatively rare in the community (i.e., they occur in relatively few sample plots). There are fewer species with medium frequency while the number of species in the category of high frequency again increases. This observation has been verified in many phytosociological studies.

Raunkiaer's five categories are, however, too imprecise to provide a suitable means for expressing abundance relationships. Preston (1948) suggests that a drawback of

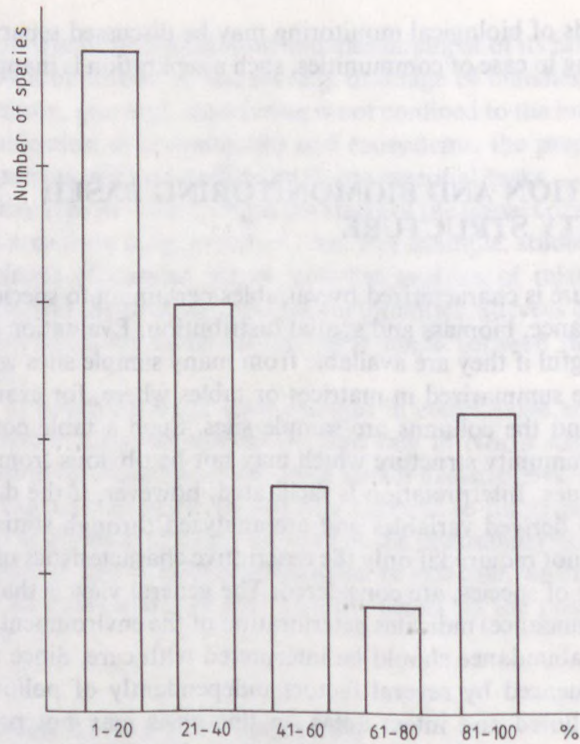

Fig. 1 — Frequency distribution of species according to Raunkiaer (1934).

Fig. 2 — Frequency distribution of species according to Preston (1948).

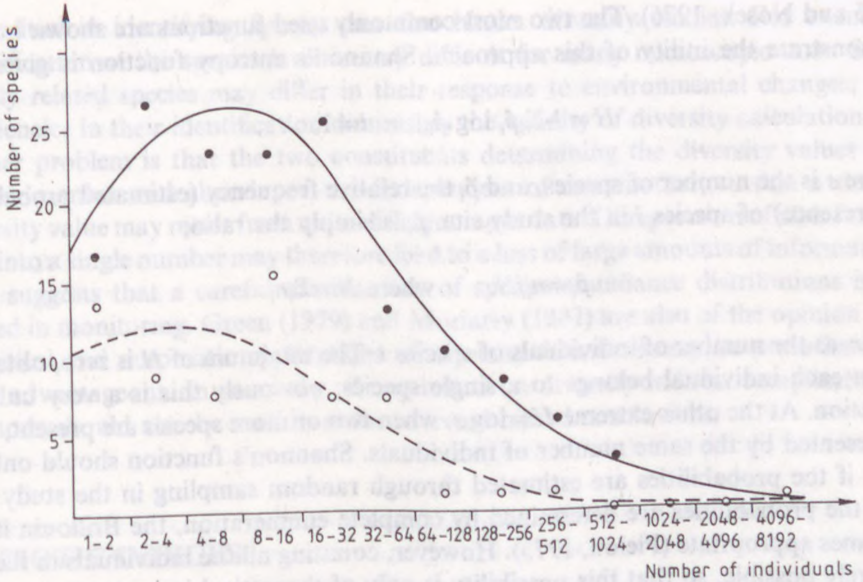

Fig. 3 — Frequency distribution of diatom species according to investigations by Patrick (1954) (empty o = in polluted water, black o = in unpolluted water).

using Raunkiaer's frequency classes is that the two extreme categories are in fact not commensurable with the others. Therefore, it is more straightforward to create an alternative system in which the number of species for each category is half of the next (i.e., 1–2, 2–4, 4–8, 8–16, etc.). The distribution of number of species in such categories approximates the lognormal (Fig. 2). A striking difference from Raunkiaer's "frequency law" is that the extreme categories of the lognormal distribution contain very few species, whereas the medium categories are the dominant.

Patrick (1954) suggested that Preston's "law" can be applied successfully to biological indication in a study of diatom assemblages in clean and polluted waters. The species abundance curves (Fig. 3) obtained for the two situations are markedly different. In clear water most species represent intermediate abundance categories, whereas in polluted water the curve is flattened and the range of manifested abundance categories is wider. Deterioration of water quality resulted in the disappearance of many species, and caused an enormous increase in the abundance of a few species that tolerate pollution. Further examples for the biological application of species/abundance curves are reported in Gray and Mirza (1979) and Gray (1981).

4.4 DIVERSITY

In the past decades many attempts have been made to express species/abundance relationships as a single number. Diversity indices are functions of both the number of species and the number of individuals for each species. A number of diversity indices have been described in the literature (for details the reader is referred to Pielou, 1975 and Nosek, 1976). The two most commonly used functions are shown here to demonstrate the utility of this approach. Shannon's entropy function is given by,

$$H = -\Sigma_i \hat{p}_i \log \hat{p}_i, \qquad i = 1, \ldots, s$$

where s is the number of species, and $\hat{p}_i$ the relative frequency (estimated probability of presence) of species i in the study site. $\hat{p}_i$ is simply the ratio,

$$\hat{p}_i = n_i / N, \qquad \text{where } N = \Sigma_i n_i$$

and n_i as the number of individuals of species i. The minimum of H is zero, obtained when each individual belongs to a single species; obviously this is a very unlikely situation. At the other extreme $H = \log s$, when two or more species are present, each represented by the same number of individuals. Shannon's function should only be used if the probabilities are estimated through random sampling in the study site.

If the probabilities are determined by complete enumeration, the Brillouin index becomes appropriate (Pielou, 1975). However, counting all the individuals in the site is rarely possible, so that this possibility is only of theoretical importance.

Simpson (1949) suggested the following diversity index:

$$D = 1 - \Sigma_i n_i (n_i - 1) / N(N-1)$$

The value of D corresponds to the probability that two individuals selected at random belong to different species. The maximum of D is 1, obtained when every individual represents a different species. D is zero when a single species is present only.

Diversity indices have been widely used in ecology. Wilhm and Dorris (1968) reflect the dominant opinion that "these indices express the relative importance of individual species, they have no dimension and are independent of the number of samples analyzed . . . Environmental pollution brings about a decrease in diversity." Cairns et al. (1972), Williams et al. (1969) and Briand (1975) present successful applications of diversity indices which support this viewpoint. However, diversity values are influenced by sample size (Pielou, 1975), and many investigators now feel that the relationship between diversity and environmental quality is more complex than earlier thought. For example, Morris (1971) demonstrated that fewer annuals and small herbs were found when grazing ceased in a grassland community. At the same time, however, diversity of certain insect groups increased. Consequently, the overall diversity of the grassland community hardly changed, since the decrease in plant species was compensated for by an increase in the insects. This demonstrates that the restricted use of diversity measures may give misleading results as to the nature of the environmental change.

Although environmental quality does have an influence on diversity, other factors should also be considered. For example, diversity was found to display seasonal variation (Holland and Polgar, 1976; Menge and Sutherland, 1976). Diversity can also be influenced by trophic relationships (Paine, 1966) and random perturbation of natural conditions (Hendricks et al., 1974).

One might expect that different indices will respond similarly to changes in species abundances. But, as pointed out by Hellawell (1978), this is not always the case.

Difficulties in identifying species can also hinder diversity studies. For example, identification at the species level is very difficult for many microscopic taxa. Since closely related species may differ in their response to environmental changes, any deficiencies in their identification diminishes the validity of diversity calculations. A further problem is that the two constituents determining the diversity values (i.e. species number and abundance) are independent of one another, so that a similar diversity value may result from quite different input data. Compression of all information into a single number may therefore lead to a loss of large amounts of information. This suggests that a careful examination of species/abundance distributions is required in monitoring. Green (1979) and Moriarty (1982) are also of the opinion that uncritical and automatic applications of diversity functions may be more harmful than advantageous. In summary, if one insists on diversity indices, complimentary methods should also be used in order to avoid biased conclusions.

4.5 BIOTIC INDICES

Diversity indices give equal weight to every species and every individual in the community. For bioindication purposes however, an optimal index should make distinction between taxa with known ecological requirements and others for which no information is available. Also, "generalist" species are much less useful for bioindication than species with narrow ecological tolerance. Biotic indices were suggested to solve these difficulties, particularly in hydrobiological investigations.

The use of biotic indices can be traced back to the beginning of the century, when sewage was considered as the main pollutant of surface waters. Sewage causes considerable changes in the oxygen concentration of the water which in turn greatly influences the distribution of organisms. Kolkwitz and Marsson (1902) distinguished three oxygen concentration zones, the polysaprobic, mesosaprobic and the oligosaprobic, and published a list of species characterizing these zones. Later, the saprobity system was refined by adding further groups (Sladecek, 1973). Species categorization may be used to characterize environmental quality by a single number. In addition, samples taken at different sites and times can be directly compared. As an example, Pantle and Buck (1955) suggested the following index of saprobity,

$$T = \Sigma_i \, b_i \, h_i \, / \, \Sigma_i \, h_i$$

where Σ_i is the number of species, b_i is a value assigned to species i, indicating the zone to which this species is specific. By dividing the mesosaprobic zone, these authors distinguished four saprobity zones so that b_i can have the value of 1, 2, 3 or 4 (from the oligosaprobic to the polysaprobic). h_i denotes the abundance of species i on the following scale: 1 = rare, 3 = uncommon, 5 = very abundant. It is obvious that low T values indicate the oligosaprobic state while higher values indicate a shift towards polysaprobicity. Additional indices were proposed by Dittmar (1959), and Zelinka and Marvan (1961). A review of biotic indices is found in Hellawell (1978).

Cook (1976) suggests that biotic indices to be used in bioindication should satisfy the following basic requirements:

– sensitivity to pollution;
– general validity;
– ability to indicate environmental quality on a linear scale ranging from the natural to the heavily polluted state;
– independence of sample size;
– simplicity of data collection and calculations.

Obviously, it is difficult to develop indices that would satisfy all these conditions. A further problem is that the indices are sensitive to the categorization of organisms, and therefore the use of biotic indices is not recommended for classifications of organisms relying on a subjective basis (Moriarty, 1982; Herricks and Cairns, 1982).

4.6 MULTIVARIATE METHODS

Diversity and biotic indices offer a very simple way to summarize environmental and biological data. The underlying and less obvious information in data tables however, can only be revealed by the methods of multivariate analysis. Since there are many books presenting these procedures for biologists (Orlóci, 1978; Green, 1979; Pielou, 1984; Digby and Kempton, 1987), we summarize only the basic aspects here.

Most multivariate methods start from a data table (or matrix) in which the rows are, say, variables or attributes, and the columns are observations or individuals. For example, a data matrix may have sampling stations as the columns and various heavy metals as the rows. Then, each value, x_{ij}, in the matrix, is the concentration of element i at sampling station j. In many environmental studies data tables have species in rows and sampling units in colums but there are many possibilities. If we wish to analyze relationships among different sampling units, the first step usually involves their pairwise comparison by some appropriate function. For example, the similarity index proposed by Czekanowski (1909) may be used,

$$C_{jk} = 2\ \Sigma_i \min\,(x_{ij}, x_{ik}) / \Sigma_i\,(x_{ij} + x_{ik}).$$

If the data matrix contains species abundances, then the nominator is the sum of the abundances of all species in both sampling units, whereas the denominator takes the double of the sum of minima, i.e., the sum of abundances that are common to both sampling units. If the two units completely agree in species abundances, the similarity is 1; whereas on the other extreme we obtain a value of zero if the two units being compared have no species in common. Another commonly used formula is the Euclidean distance given by,

$$d_{jk} = [\Sigma_i (x_{ij} - x_{ik})^2]^{1/2}$$

which is zero if the two sampling units have identical species with identical abundances. d_{jk} has no theoretical upper limit.

In other cases the data comprise only presences and absences to which the Jaccard coefficient (Jaccard, 1901), one of the oldest index in ecology, can be applied,

$$s_{jk} = a/(a+b+c)$$

where we do not indicate the original values of the data matrix. Instead, we simply denote by *a* the number of species present in both sampling units, by *b* the number of species occurring only in j, and by *c* the number of species present only in unit k. s_{jk} is 1 (complete similarity) if both sampling units have the same species composition, and 0 if they have no species in common. Obviously, this index gives equal weight to all the species. Sneath and Sokal (1973) give an exhaustive review of presence/absence coefficients.

To express relationships among variables, the product-moment correlation coefficient may be used,

$$r_{hi} = \Sigma_j (x_{hj} \bar{x}_h)(x_{ij} - \bar{x}_i) / (\Sigma_j (x_{hj} - \bar{x}_h)^2 \, \Sigma_j (x_{ij} - \bar{x}_i)^2)^{1/2}$$

which ranges from −1 through 0 to 1. The extreme values indicate that the two variables are perfectly correlated either negatively or positively, whereas values close to 0 indicate lack of correlation.

When the data comprise observations from sites representing baseline conditions and those representing different levels of pollution, the usual objective is to reveal structural changes in communities attributable to environmental impact. Such a study requires large numbers of sampling units, because species abundances may greatly vary even within a relatively homogeneous area. The pairwise similarity or distance coefficients may be collected in a new matrix, termed similarity or distance matrix, respectively. This matrix will be the starting point for future analyses. Basically, we may proceed in two ways: clustering and ordination.

Methods of cluster analysis will reveal natural groupings in the data which can be used for classification purposes. The objective is to arrange objects into classes (groups, clusters) so that our hypotheses on the set of objects may be tested, and their description facilitated (e.g. for vegetation mapping in polluted and unpolluted areas). Clustering is an automatic process; the classes are created during the analysis based on, say, the similarities or distances among objects (e.g. Anderberg, 1973; Hartigan, 1975; Williams, 1971). Nonhierarchical classifications represent simple partitions of observations into a certain number of groups; no hierarchical relationships between groups are revealed. In hierarchical classifications most similar objects are assigned to clusters, similar clusters are combined into larger clusters and so on. The hierarchy is illustrated by a dendrogram. An example is shown in Fig. 4 which represents a hierarchical classification of algal assemblages in Lake Balaton, Hungary (Padisák, 1980). The classification is in good agreement with the spatial arrangement of sampling units as well as with the water quality conditions within areas of the lake. The sampling units taken from the relatively more polluted Keszthely Bay are distinct from the others which were collected in less polluted zones. Sandiland's study (1970) also provides a good example for the evaluation of relationships between classifications and environmental conditions. Based on species composition data, he classified the benthic assemblages of polluted and unpolluted lakes. The lakes were also classified on the basis of the chemical and physical variables of the water. The comparison of the alternative classifications facilitated interpretation of structural changes of benthic communities in terms of environmental change. Green and Vascotto (Green, 1979) classified zooplankton samples and subjected the groups thus obtained to a discriminant analysis of water quality variables. The authors were then able to reveal main factors influencing zooplankton structure.

Ordination methods replace the observed 'real' variables (e.g. abundance of species) by artificial variables in order to reduce dimensionality of the data and to facilitate visualization of inherent data structure. Ordination methods include principal component analysis, correspondence analysis and metric and nonmetric multidimensional scaling procedures (Orlóci, 1978; Digby and Kempton, 1987; Pielou,

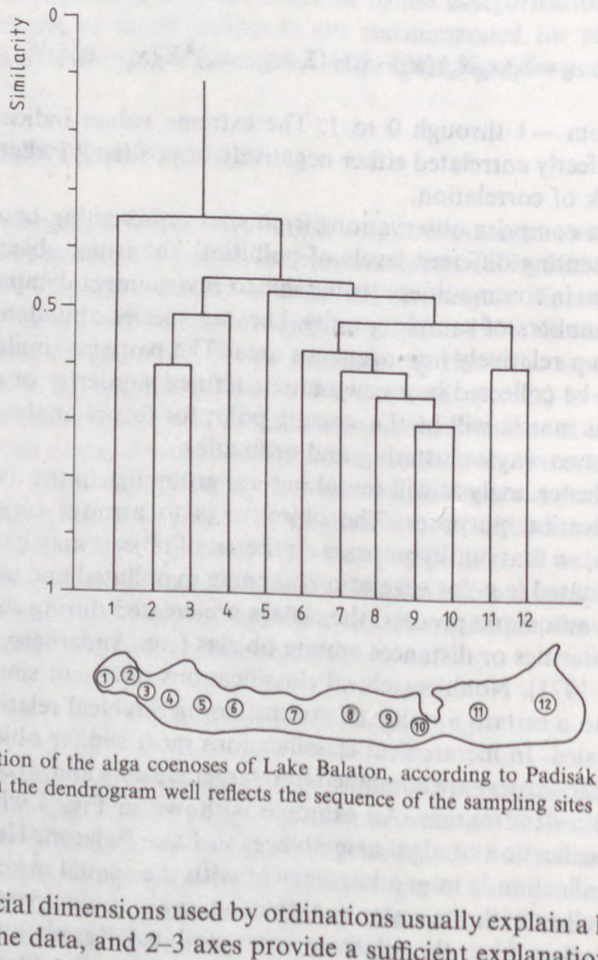

Fig. 4 — Classification of the alga coenoses of Lake Balaton, according to Padisák (1980). The sample sequence in the dendrogram well reflects the sequence of the sampling sites in the lake.

1984). The artificial dimensions used by ordinations usually explain a high proportion of variation in the data, and 2–3 axes provide a sufficient explanation of distance or similarity structure of the samples. For two axes at a time, ordination results may be displayed in the plane using a Cartesian coordinate system. The example in Fig. 5 illustrates the ordination of nematode assemblages in rivers (Callahan et al., 1979). In the space of the first two dimensions, the 16 samples are assigned to 3 groups (A, B and C). Groups A and B originate from areas of high organic matter concentration, whereas group C corresponds to a more intact environment. In the habitat of B both water flow and the particle size of the substrate of the riverbed are the smallest. Consequently, as seen in the ordination scattergram, axis 1 is closely correlated with nutrient content, while axis 2 can be identified as water flow/particle size. This analysis suggests that community structure is most significantly influenced by concentration of organic matter than by the two physical variables used.

Indeed, such a clear correspondence between ordination axes and environmental variables is not always demonstrable *a posteriori*. Green (1979) suggests the use of canonical correlation analysis (see e.g. Pimentel, 1979; Digby and Kempton, 1987) which considers two sets of variables, one usually containing species abundances and the other comprising environmental information. Two main axes result from this

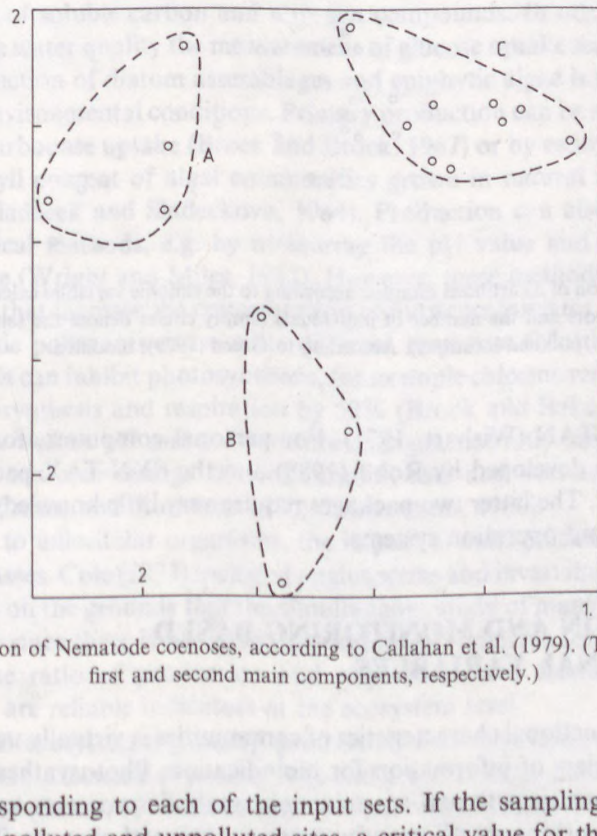

Fig. 5 — Ordination of Nematode coenoses, according to Callahan et al. (1979). (The axes represent the first and second main components, respectively.)

analysis, corresponding to each of the input sets. If the sampling units have been collected from polluted and unpolluted sites, a critical value for the abundance axis can be determined as a threshold to determine if new samples came from polluted or unpolluted areas. Green (1979) gives a simple artificial example to illustrate this. Fig. 6 shows the ordination of 36 sampling units for the first canonical axis for environmental variables (x axis) and abundances (y axis). A value of approximately −1.6 on the x axis is a threshold beyond which sampling units collected in polluted sites predominate. Using the canonical functions it is possible to locate further sampling units in this diagram.

The analysis of species/environment relationships by numerical classification and ordination requires computers and appropriate software. Published listings of programmes are presented in Anderberg (1973), Cooley and Lohnes (1971), Hartigan (1975), Orlóci (1978) and Podani (1980). Commercial programme packages usually available at large computer centres include SPSS (Nie et al., 1975), BMDP (Dixon,

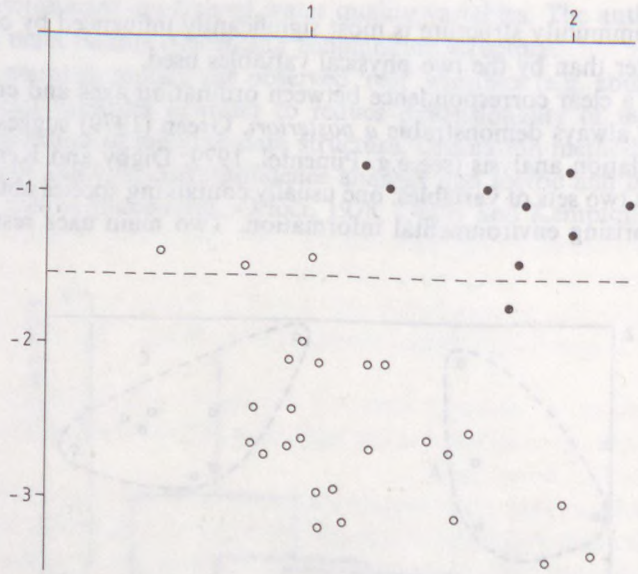

Fig. 6 — The ordination of 35 artificial samples, according to the canonic variables calculated on the basis of environmental factors and the number of individuals (empty circles denote the samples taken at the polluted locations). According to Green (1979), modified.

1975) and CLUSTAN (Wishart, 1975). For personal computers, for example, the NT-SYS package developed by Rohlf (1988) and the SYN-TAX package (Podani, 1989) can be used. The latter two packages require very little knowledge of programming languages and operation systems.

4.7 INDICATION AND MONITORING BASED ON FUNCTIONAL VARIABLES

The number of functional characteristics of communities is virtually unlimited and all are potential carriers of information for bioindication. Photosynthesis, respiration, primary production, growth and reproduction, anabolic processes, nutrient cycling, food chain relationships are all sensitive to environmental changes. The analysis of these functions is promising for biological indication since this level of monitoring is still unexploited.

Changes in life functions caused by environmental stress have been studied mainly at the level of species and individuals, whereas there have been relatively few papers devoted to the similar problem at the community and ecosystem level. This is illustrated by a recent bibliography on the effects of sulphur dioxide pollution (Liggon and Laugenroth, 1980), in which only 18 of the 1730 references deal with changes at the ecosystem level. This is unreasonable if we consider that many studies dealing with a single species can easily be extended to the entire community and, on the other hand, assessment at the ecosystem level is indispensable to obtain a realistic picture on the state of the environment.

The simplest extension to functions at the population level is the application of selected taxa for indication. For example, bacterial tests are used to indicate water quality which can be measured by biological oxygen demand (BOD, Bott, 1973). Enhanced bacterial activity, i.e., increased by excessive amounts of nutrients, is manifested as increased oxygen consumption. Experiments with radioactive carbon isotopes are suitable to measure increased bacterial activity resulting from organic pollution and to study the mechanism of nutrient absorption. Albright and Wentworth (1973) studied the absorption of glucose labelled with radioactive carbon in several fresh waters. They found significant correlations between the maximum rate of uptake and the number of heterotrophic bacteria per unit volume as well as the concentration of soluble carbon and nitrogen compounds. In other words, in order to characterize water quality the measurement of glucose uptake seems sufficient. The primary production of diatom assemblages and epiphytic algae is also suitable as an indicator of environmental conditions. Primary production can be measured either by C^{14} labelled carbonate uptake (Brock and Brock, 1967) or by estimating the biomass and chlorophyll content of algal communities grown in natural and artificial substrates (e.g. Sladecek and Sladeckova, 1964). Production can also be estimated by physico-chemical methods, e.g. by measuring the pH value and concentrations of carbon dioxide (Wright and Miles, 1967). However, these methods are suitable only for pollutants that increase the concentration of nutrients (nitrates, phosphates, etc.). As a rule, toxic pollutants cause quite different responses (Matthews et al., 1982). Toxic materials can inhibit photosynthesis, for example chlorine reduced the intensity of both photosynthesis and respiration by 50% (Brook and Baker, 1972). Zinc and copper ions as well as pH and temperature changes, not only inhibit the metabolic processes but can also change the colonization rate and succession of protozoan assemblages (Cairns and Ruthven, 1970; Cairns et al., 1980).

In addition to unicellular organisms, the impact of eutrophication can be studied with higher classes. Cole (1973) included angiosperms and invertebrates as well as fish, in his analyses on the grounds that the simultaneous study of many phenomena is the most plausible since there is no universally applicable criterion for bioindication. He found that the ratio of production and respiration, and fluctuations in oxygen concentration are reliable indicators at the ecosystem level.

In terrestrial ecosystems, primary production and environmental pollution are clearly correlated. Decrease of productivity and growth rate as the effect of pollutants is often reported (Smith, 1974; Guderian, 1977). Grodzinski and Yorks (1981) suggest that for indicating air pollution it is sufficient to monitor changes of production for dominant plant species. Such observations do not have general validity, however.

Steps of nutrient cycling are thought to be particularly sensitive to pollutants and can be used as indicators at the ecosystem level (Jackson and Watson, 1977). With exposure to sulphur dioxide and heavy metals (especially lead, zinc, copper and cadmium), decomposition of decayed plant parts slows down as frequently observed in grasslands and forests (Smith, 1974; Freedman and Hutchinson, 1980). Partial explanation of this is the decreased microbial activity (Hutchinson, 1980), i.e., the inhibition of dehydrogenase, phosphatase and nitrase enzymes. Changes in the assemblages of soil Articulata are also important (Freitag et al., 1973). Inhibition of decomposition causes increased accumulation of litter so that a large proportion of organic matter remains excluded from nutrient cycling for a long time. This may have consequences for the entire ecosystem (Strojan, 1978).

4.8 SIMULTANEOUS STUDY OF STRUCTURE AND FUNCTIONS

The structure and functioning of ecosystems cannot be dissociated, even though some suggest that functions may be modified without apparent structural changes and vice versa (e.g. Cairns, 1977). However, limitations of research facilities lead to the separate treatment of functioning and structure, without a fairly good synthesis of the results. There is no doubt that an ideal case would be a simultaneous analysis of structure and functioning but it requires well-trained and organized working groups. Nevertheless, in some cases the structural and functional factors are combined in the same simple index. For example, Rodgers and Harvey (1976) proposed a measure to express the rate of microbial production and biomass.

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Biological indicators
5 Fungi

Natural growing fungi that spread out a network of mycelia over large soil surfaces are suitable—as accumulative indicators—for the indication of heavy metal pollution (Gast et al., 1988).

The soil in which forest fungi proliferate generally contains high amounts of organic matter capable of accumulating higher amounts of heavy metals. (There is a positive correlation between the organic matter and heavy metal content of the soil.)

The heavy metal residues in soils are derived from:

– geochemical sources (chemical composition of the underlying rock);
– the deposition of heavy metals from atmosphere to the soil surface;
– the deposition of heavy metal pollutants (e.g. spoil banks, mud) directly to the soil surface;
– increasing acidity of the soil.

The heavy metal content of fungi is determined by:

– the organic matter content of the substrate and its chemical composition (Gast et al., 1988);
– the selective cation absorbing capacity of fungi (Fleckstein, 1979).

Based on the extent of heavy metal accumulation (e.g. Cd, Hg, Pb) the following sequence of fungi can be established (Fleckstein, 1979):

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

Organic matter decomposers and mycorrhiza-forming species are most helpful as pollutant indicators.

Lycoperdon gemmatum, Mycena pura and *Collybia* species accumulate the mercury from humus while the epiphytic fungi retain relatively small amounts (Byrne et al., 1976; Seeger and Nützel, 1976).

Hymenomycetales growing in the parks of large towns (e.g. Helsinki) have the following amounts of heavy metals (Laaksovirta and Alakuijala, 1978; Laaksovirta and Lodenius, 1979):

Cd: 0.17–10.3 $mg \cdot kg^{-1}$ lowest and highest values measured *Lyophyllum connatum*
Hg: 0.12–72 $mg \cdot kg^{-1}$ lowest and highest values measured *Agaricus* sp.
Pb: 2.2–41 $mg \cdot kg^{-1}$ lowest and highest values measured *Lyophyllum connatum*
Zn: 24–345 $mg \cdot kg^{-1}$ lowest and highest values measured *Boletus edulis*

Table 3 — Heavy metal contents ($mg \cdot kg^{-1}$ dry matter) of the different types of fungi in Helsinki (urban area) and in rural areas (Kuusi et al., 1981)

	Pb			
	Urban area		Rural areas	
	N	Mean + S.D.	N	Mean + S.D.
Lawn decomposers	177	6.4 + 8.2	8	3.0 + 4.8
Lawn decomposers *Agaricus* spp. excluded	123	5.1 + 8.3	16	1.8 + 2.8
Mycorrhizal symbionts	50	2.7 + 3.0	58	1.4 + 1.7
	Cd			
	Urban area		Rural areas	
	N	Mean + S.D.	N	Mean + S.D.
Lawn decomposers	177	5.3 + 14.2	18	2.9 + 7.6
Lawn decomposers *Agaricus* spp. excluded	124	2.8 + 3.7	16	1.1 + 0.9
Mycorrhizal symbionts	50	2.7 + 5.0	58	0.9 + 0.8
	Mg			
	Urban area		Rural areas	
	N	Mean + S.D.	N	Mean + S.D.
Lawn decomposers	191	6.6 + 10.8	23	1.7 + 1.9
Lawn decomposers *Agaricus* spp. excluded	137	3.6 + 4.5	21	1.4 + 1.5
Mycorrhizal symbionts	48	0.7 + 2.7	69	0.2 + 0.3

Kuusi et al. (1981) found a higher Pb, Cd and Hg contents of fungi growing in urban areas (Helsinki) than is rural zones (Table 3).

Investigations in southern Germany show that 236 species have Hg contents between 0.04–21.60 mg·kg^{-1} dry weight (Seeger and Nützel, 1976).

Generally, saprophytic fungi contained higher amounts of lead, cadmium and mercury than the symbionta (mycorrhiza-fungi, see Lodenius et al., 1981; Gast et al., 1988).

The heavy metal content of fungi is also influenced by the heavy metal content of the soil. Laboratory analyses show that higher amounts of Cd and Hg in the soil are accompanied by higher heavy metal values in *Agaricus bisporus* (Enke et al., 1979; Dietl et al., 1987).

The accumulation of toxic heavy metals in edible fungi can reach such a high level that they are unsuitable for consumption (Alsen et al., 1977).

Table 4 — The chemical composition of *Coprinus comatus* (μg·g^{-1} dry weight)

	1	2	3
Al	818.4	456.8	317.0
As	0	0	0
B	0	6.5	8.7
Ba	26.2	14	3.7
Ca	9972	6933	2940
Cd	16.1	0.3	0.8
Co	7.3	0.5	0.3
Cr	282.1	1.3	1.1
Cu	27.9	28.8	81.1
Fe	92962	1646	918.1
Ga	62.6	0	0
Hg	0	0	0
K	152.8	34459	51994
Li	0.7	1.5	0.7
Mg	5840	2210	2005
Mn	577.3	22.6	26
Mo	0	0	0
Na	0	1347	1534
Ni	87.7	1.4	1.4
P	254.6	2667	5063
Pb	48.1	0	0
Se	0	0	0
Si	3731	321.6	190.5
Sr	15.2	25.9	9.6
Ti	41.8	22	9.7
V	0	0.8	0.7
Zn	620.3	46.5	85.3

1: Red spoil (Borsodnádasd)
2: *Coprinus comatus* stem
3: *Coprinus comatus* cap

Not every heavy metal is accumulated by fungi to the same extent. Certain heavy metals are absorbed only in small quantities, i.e., they are excluded. Depending on species, the elements excluded comprise Pb, Fe, Mn (Tyler, 1982).

Coprinus comatus, a common species, was collected in the North-Eastern-Central Mountains (at Borsodnádasd), Hungary in a territory exposed to the impact of mainly iron containing spoil banks. In addition to iron, the soil contains large concentrations of Cd, Cr, Ni, Mn, Pb and Zn (Table 4).

In the fungi, only iron could be detected in high concentrations and quantities, Cd and Pb were below levels of detection.

According to our investigations in a *Quercetum petraeae-cerris* forest in the North-Eastern Mountain Range (Mátra Mountains) both decomposers (excluding wood decomposers) and mycorrhiza fungi contain high concentrations of heavy metals such as Cd, Cu, Ni, Pb and Zn (Tables 5–8).

Wood decomposers contained lower concentrations of heavy metals e.g. Pb, Cd (Table 9).

The threshold concentration of Pb and Cd in dried vegetables set by health authorities is 2.0 and 0.3 $mg \cdot kg^{-1}$, respectively.

Of the investigated species, the Pb and Cd contents of some edible fungi exceeded that limit.

According to our present knowledge, many fungi species are suitable for the indication of heavy metals which occur in regions and to obtain internationally comparable data selection of a few fungi species occurring frequently and in large

Table 5 — The chemical composition of *Russula* species (mycorrhiza-fungi) ($\mu g \cdot g^{-1}$ dry weight)

	1		2		3	
	T	K	T	K	T	K
Al	39	101	197	189	28.0	47.4
Ca	2 030	2 945	1 560	1 895	1 740	1 835
Cd	2.3	—	1.4	1.7	1.4	1.3
Co	0	1.5	0	0	0.3	0
Cr	0	0	0	0	0	—
Cu	64.1	123.1	31.8	56.7	6.3	23.6
Fe	39	101	164	291	16.1	36.1
K	46 590	44 505	49 055	39 420	12 855	63 650
Mg	3 100	4 820	2 600	3 550	2 795	3 555
Mn	8.9	31.6	13.1	34.5	5.3	13.3
Mo	11.3	21.2	7.9	7.9	18.6	7.3
Na	319.5	233.4	1 950	—	21 370	28 830
Ni	6.4	7.1	6.0	7.3	4.7	4.8
Pb	16.6	15.1	11.9	8.3	6.5	11.6
Zn	155	219	123	105	23.1	79.1

1: *Russula xerampelina*
2: *R. cyanoxantha*
3: *R. delica*
T = stem
K = cap

Table 6 — The chemical composition of mycorrhiza-fungi (μg·g^{-1} dry weight)

	1		2	
	T	K	T	K
Al	60.1	212.9	54.4	0
Ca	2 580	2 185	1 490	1 225
Cd	1.6	2.6	1.8	2.4
Co	0.6	0.3	0	0
Cr	0	0	0	0
Cu	20.9	90.6	16.9	28.7
Fe	71.5	148.5	98	54
K	19 860	37 910	29 040	30 840
Mg	2 495	3 905	2 605	—
Mn	31.5	15.1	11.2	8.5
Mo	16.6	11.9	16.3	10.7
Na	225	229.4	235.4	206
Ni	3.9	6.1	7.0	6.6
Pb	8.9	10.5	11.2	10.6
Zn	154	635	195	426

1: *Xerocomus rubellus*
2: *Boletus aestivalis*
T = stem
K = cap

Table 7 — The chemical composition of mycorrhiza-fungi (Mátraháza) (μg·g^{-1} dry weight)

	1		2		3	
	T	K	T	K	T	K
Al	408.6	71.1	410.7	168.6	31.4	21.2
Ca	4 335	4 335	560	985	1 100	1 240
Cd	1.5	1.5	1.7	1.2	1.1	1
Co	0	0	0	0.1	0	0
Cr	0	0	1.2	3.2	0	0
Cu	41.5	21.8	63	347	28.6	10.3
Fe	298	94	270.5	130.5	95.5	50.5
K	45 225	35 400	67 485	77 310	35 625	23 280
Mg	3 490	2 295	1 450	5 530	7 000	3 680
Mn	35.1	13.2	25.3	9.1	19.6	27.6
Mo	9.1	12.3	1.2	10.9	5.3	6.9
Na	420	307	315	477	256	210
Ni	1.5	6.2	7.5	5.1	4.9	5.3
Pb	12.0	12.9	14.5	12.1	15.0	12.5
Zn	129	116	239	132	185	80

1: *Amanita vaginata*
2: *A. rubescens*
3: *Lactarius quietus*
T = stem
K = cap

Table 8 — The chemical composition of organic matter decomposing fungi
(µg·g⁻¹ dry weight)

	1		2		3	
	T	K	T	K	T	K
Al	12.6	12.2	121.5	81.8	—	125
As	—	—	0	0	—	—
B	—	—	3.5	5.1	—	—
Ba	—	—	2.5	2.7	—	—
Ca	1 545	1 316	457	773	1 603	1 955
Cd	—	—	0.3	1.1	1.3	1.0
Co	0.7	0.9	0	0	0.3	0
Cr	0	1.8	0.9	1.4	0	0
Cu	118	195	101	137	—	—
Fe	151	71.5	108	147	25	119
Ga	—	—	0	0	—	—
K	25 415	49 895	46 867	44 733	8 995	28 800
Li	—	—	0	0	—	—
Mg	5 395	2 740	1 283	1 935	1 650	2 825
Mn	23.7	10.1	21.2	10.5	3.2	17.7
Mo	12.6	12.2	0.5	0.4	10.4	11.9
Na	870	166	216	219	326	468
Ni	6.4	7.8	0.5	0.9	3.9	6.1
P	—	—	11 116	7 162	—	—
Pb	15.4	15.4	3.1	6.1	7.3	11.4
Se	—	—	4.8	6.6	—	—
Si	—	—	146	97.3	—	—
Sr	—	—	1.5	1.9	—	—
Ti	—	—	1.8	1.1	—	—
V	—	—	0.3	0.3	—	—
Zn	193	240	72.8	121.9	15.1	62.9

1: *Agaricus arvensis*
2: *Lepiota procera*
3: *Oudemansiella radicata*
T=stem
K=cap

numbers within a certain region (e.g. in Europe) are needed. The fungus *Mycena pura*, (Dietl, 1987; Dietl et al., 1987), recommended for an international indicating network, is an organic matter "decomposer", a cadmium accumulating species growing in both deciduous and coniferous forests.

Since the concentration of heavy metals detected in the cap, hymen and stem may vary, only the cap of the mushroom is used for chemical analysis.

The indication of radioactive contamination

Fungi are also suitable for the indication of radioactive pollutants. Studies carried out in Sweden (Mascanzoni, 1988), in Germany (Elstner et al., 1987), in Yugoslavia (Byrne, 1988) following the Chernobyl accident, revealed an increase of radioactive isotopes (103Ru, 106Ru, 134Cs) in fungi.

Table 9 – Chemical composition of wood decomposing fungi

	1	2
	K	K
Al	37.8	106.5
As	0	0
B	0.63	0.46
Ba	4.18	4.85
Ca	625.6	1 283
Cd	0	1.59
Co	0	0
Cr	0	1.01
Cu	11.41	11.13
Fe	40.63	172.0
Ga	0	0
K	55 841	14 182
Li	0	0
Mg	2 212	1 369
Mn	7.0	15.8
Mo	0	0
Na	131.6	164.2
Ni	0.9	0.7
P	4 150	4 364
Pb	0	0
Se	0	0
Si	104.7	113.0
Sr	1.3	3.6
Ti	1.5	2.3
V	0	0.2
Zn	37.2	71.7

1: (MG-13) *Fistulina hepatica*
2: (MG-14) *Ganoderma lucidum*
K = cap

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6 Lichens

6.1 THE SENSITIVITY OF LICHENS

Lichens are specialized organisms in which a fungus and an alga form a nutritional and physiological unit. The autotrophic alga supplies nutrients for both itself and the heterotrophic fungus. Being an obligate parasite, the fungus symbiont never occurs in nature without the alga. In contrast algae—the less specialized components of the lichen symbiosis can also occur separately. Should the life conditions of the two organisms not reach the optimum, the equilibrium within the lichen colony is unstable. In this symbiosis, the viability of the alga that is "forced" to supply the fungus with nutrients is at the edge of subsistence. Any further deterioration in conditions is intolerable for the alga. This extreme state for the assurance of its own supply as well as that of the fungus explains the hypersensitivity of lichens towards adverse environmental factors. Challenging changes can take place in humidity and irradiation

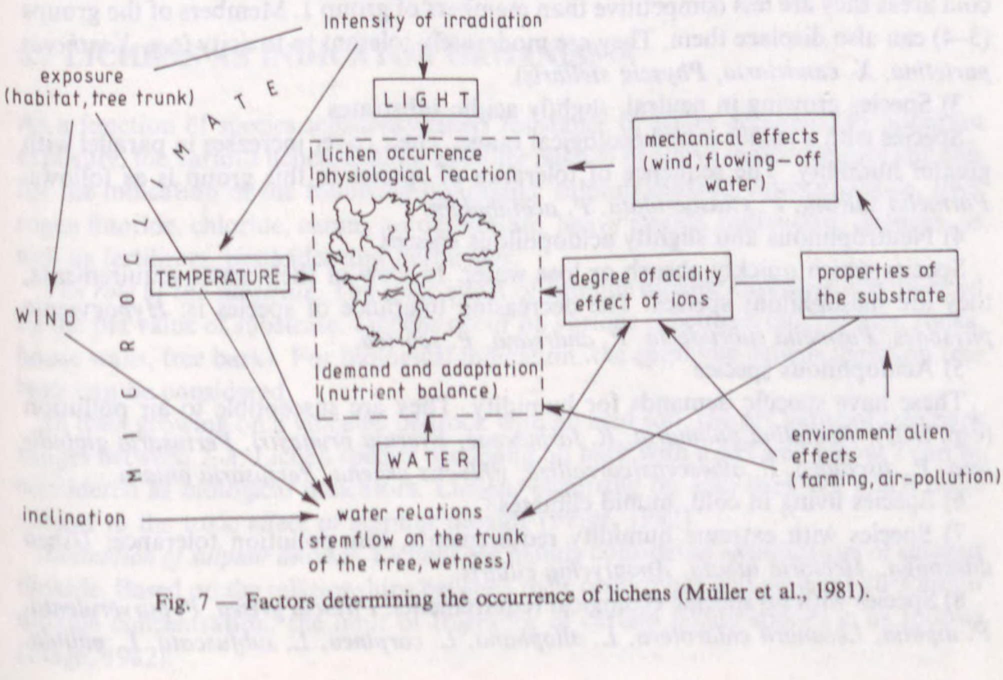


Fig. 7 — Factors determining the occurrence of lichens (Müller et al., 1981).

conditions, temperature and air pollution (Fig. 7). If the lichen has sufficient water, the symbiosis of fungus and alga continues to be harmonious.

Lichens react to the pollutant emissions. Their sensitivity to various air pollutants —in comparison with higher plants—can be ascribed to both morphological and physiological differences. These include:

– low chlorophyll content which results in low rates of photosynthesis and metabolism, slow growth and limited capacity to regenerate;
– in the absence of a cuticle, pollutants find an easier access into the thallus;
– corticolous lichens absorb both water and nutrients directly from the air;
– the water balance of lichens is almost entirely a function of humidity or precipitation (polikilohydrical plants), so that their opportunity for assimilation and regeneration is limited;
– lichens accumulate various materials without selection;
– the materials once absorbed will accumulate since there is no excretion;
– activity of lichens is greater in winter, when the sulphur dioxide content of air is generally higher than in summer.

Ecological groups of lichens:

The tolerance of lichens to toxic materials is also a function of the ecological requirements of species (Schmidt and Kreeb, 1975).

The following ecological groups can be distinguished:

1) Neutrophilous species

Species of low water demand, high tolerance to toxicity, living in dry habitats (e.g. *Physcia ascendens, Candelariella xanthostigma*).

2) Species of neutral, slightly alkaline substrates

Species occurring mainly in habitats rich in nutrients, in warm, dry areas. In humid, cold areas they are less competitive than members of group 1. Members of the groups (3–4) can also displace them. They are moderately tolerant to toxicity (e.g. *Xanthoria parietina, X. candelaria, Physcia stellaris*).

3) Species growing in neutral, slightly acidic substrates

Species with a rather broad ecological range. Their cover increases in parallel with greater humidity. The sequence of tolerance of species in this group is as follows: *Parmelia sulcata, P. exasperulata, P. acetabulum.*

4) Neutrophilous and slightly acidophilous species

Species which quickly absorb or lose water. In view of their water requirements, they are mesophilous species. The decreasing tolerance of species is: *Hypogymnia physodes, Parmelia subrudecta, P. andreana, P. scortea.*

5) Acidophilous species

These have specific demands for humidity. They are susceptible to air pollution (esp. SO_2): *Ramalina pollinaria, R. farinaceae, Evernia prunastri, Pertusaria globulifera, P. discoidea, P. albescens/corralliza, Phlyctis argena, Pertusaria amara.*

6) Species living in cold, humid climates

7) Species with extreme humidity requirements, low pollution tolerance: *Usnea dasypoga, Alectoria iubata, Anaptychia ciliaris.*

8) Species with no specific ecological requirements: *Physcia grisea, P. purverulenta, P. aipolia, Lecanora chlarotera, L. allophana, L. carpinea, L. subfuscata, L. pallida.*

The effects of air pollution:

With exposure to air pollution changes can be observed or measured for example in *Hypogymnia physodes* (Klee, 1970; Schmidt and Kreeb, 1975; Punz, 1979; Kauppi and Mikkonen, 1980).

External changes:

- change of the thallus colour;
- decrease in thallus size;
- increase in the thickness of thallus.

Anatomical changes:

- increase in the number of dead and plasmolyzed alga cells;
- decrease in size and the number of regenerative alga cells.

Physiological changes:

- changes both in the living and dead alga cells;
- decrease in net carbon dioxide absorption and respiration;
- changes in the water content of thallus;
- decrease in nitrogen fixation;
- decrease in phosphatase enzyme activity;
- changes in the chemical concentration;
- increase in concentration of pollutant residues;
- decrease in chlorophyll content, a/b ratio, pH value changes;
- leaching of potassium and magnesium from the thallus.

6.2 LICHENS AS INDICATOR ORGANISMS

As a function of species sensitivity (their resistance or water demands) to pollutant exposure, the various lichen species, as either passive or active indicators, are suitable for the indication of the following pollutants: sulphur dioxide, nitrogen oxide, hydrogen fluoride, chloride, ozone, peroxi-acetate, heavy metals, radioactive isotopes, as well as fertilizers, pesticides and herbicides.

The role of the substrate. The toxic effect of sulphur dioxide exposure is influenced by the pH value of substrate. Lichens occur on various substrates (soil, stones, rocks, house walls, tree bark). For biological indication, the epiphytic lichens living on tree bark can be considered.

In trees growing on a volcanic bedrock with an acid soil, the pH value of tree bark ranges between 2–4. Lichen species occurring on bark with a pH well below 7 can be considered as biological indicators. Lichens occurring on acid bark are much more subject to the toxic effect of sulphur dioxide (Feige, 1982).

Indication of sulphur dioxide. Lichens are mainly considered as indicators of sulphur dioxide. Based on the relationships between their occurrence and ambient air sulphur dioxide concentration, the limit of tolerance of certain lichen species is as follows (Feige, 1982):

at 170 $\mu g \cdot m^{-3}$ SO_2 – above this value no lichens exist;
at 150 $\mu g \cdot m^{-3}$ SO_2 *Lecanora conizaeoides;*
at 70 $\mu g \cdot m^{-3}$ SO_2 *Xanthoria parietina;*
at 60 $\mu g \cdot m^{-3}$ SO_2 *Ramalina farinacea;*
at 40 $\mu g \cdot m^{-3}$ SO_2 *Anaptychia ciliaris;*
at 30 $\mu g \cdot m^{-3}$ SO_2 *Ramalina fraxinea;*
at 0 $\mu g \cdot m^{-3}$ SO_2 *Lobaria amplissima.*

At locations where the sulphur dioxide content of the air exceeds 170 $\mu g \cdot m^{-3}$ no lichens can survive or their survival can be observed only when for example limestone dust forms on the surface of bark, thus raising the pH value above 7. Relict colonies of certain lichen species of high tolerance survive on these trees, e.g. *Lecanora conizaeoides* even when the concentration of sulphur dioxide is extremely high. The species mentioned is insensitive to sulphur dioxide loading (Feige, 1982).

Lichen species greatly differ in their sensitivity and tolerance to SO_2, and the presence of various lichen species follows the annual means of sulphur dioxide concentration (see: Tables 10, 11).

The degree to which lichen colonies are damaged may be informative as to the potential damage to other plants. These data can be utilized in agri-, sylvi-, as well as horticulture (Prinz and Scholl, 1978; compare Table 12).

Table 10 — SO_2-sensitivity of different epiphyte lichens (Steubing et al, 1983). Critical SO_2 concentrations of ambient air ($mg \cdot m^{-3}$) for different species published by various authors

	United Kingdom winter mean SO_2 concentration	Copenhagen winter mean SO_2 concentration	Northern Ruhr Region annual mean SO_2 concentration	Lower-Rhein Westphalien annual mean
Lecanora varia	above 0.150	above 0.110	above 0.150	above 0.150
Buellia punctata	0.125	0.090–0.110	0.100	0.100
Parmelia sulcata	0.070	0.090–0.110	0.100	0.100
Physcia tenella/ ascendens	0.070	0.080–0.090	0.110	0.100
Hypogymnia physodes	0.070	0.90–0.110	0.106	0.100
Lecanora subfusca	0.60	0.070–0.080	0.065	0.070
Parmelia exasperatula/glabra	0.060	–	0.075	0.070
Evernia prunastri	0.060	–	0.065	0.060
Plastismatia glauca	0.060	–	0.060	below 0.060
Pseudoevernia furfuracea	0.050	–	–	–
Ramalina sp	0.035	–	–	below 0.060
Anaptychia ciliaris	0.040	0.040	–	–
Trees where lichens were found	oak, ash and elm	maple, ash, elm and lime	apple	apple
Author (cit in Steubing et al., 1983)	(Hawksworth et al., 1970)	(Johnsen et al., 1973)	(Heidl, 1974)	(Kirschbaum et al., 1974)

Table 11 — Sensitivity of epiphytic lichens to the SO_2 content of air in N.W. France (Lerond, 1984)

Species	SO_2 content of air, $\mu g \cdot m^{-3}$	
	winter	summer
	average	
Pleurococcus viridis	170	120
Lecanora conizaeoides	150	100
L. expallens	125	80
Buellia punctata	70	50
Diploicia canescens		
Lecidella elaeochroma		
Lepraria incana		
Parmelia sulcata		
Physcia ascendens	60	40
P. tenella		
Xanthoria parietina		
Evernia prunastri		
Hypogymnia physodes		
Pertusaria amara		
Parmelia acetabulum	40	30
Phaeophyscia orbicularis		
Physconia grisea		
Ramalina fastigiata		
Parmelia caperata		
Pertusaria pertusa		
P. subrudecta		
Ramalina farinacea		
Anaptychia ciliaris	40	30
Physcia aipolia		
Ramalina fraxinea		
Parmelia perlata		
P. revoluta		

Table 12 — Rate of degeneration of lichen thalli due to SO_2 pollution and the possible related damage to higher plants (Prinz and Scholl, 1978; Arndt et al., 1982)

Rate of lichen degeneration	Possible damage in higher plants
10–35%	Chlorosis and necrosis of the leaves of conifers and cultivated plants
35–60%	Plant cultivation is limited, the sensitive decorative plants as well as conifers are damaged
60–85%	Limited cultivation of sensitive decorative plants, deciduous and coniferous trees, horticultural and agricultural crops
Above 85%	Limited cultivation of the less sensitive decorative plants, deciduous and coniferous trees, horticultural and agricultural crops

The most sensitive lichen species to sulphur dioxide are *Lobaria* and *Usnea* spp.

Parallel with increase in sulphur dioxide load the number of corticolous lichen species present decreases. On this basis, it is possible to assess both air quality and sulphur dioxide concentration.

With higher sulphur dioxide concentration the following lichen species have less value in indication: *Xanthoria parietina, Grimmia pulvinata, Parmelia saxatilis, P. sulcata, P. physodes.*

A greater level of air pollution is also damaging to species living in acid soils. As an example, species of *Cladonia* occur in soils with extremely low buffering capacity. Under the impact of cement dust, on the other hand, the acidophilous lichens (e.g. *Hypogymnia physodes*) would disappear (Jürging, 1972).

Sulphur accumulation can be detected in some lichen species: *Cladonia sylvatica, C. arbuscula, C. mitis, Hypogymnia physodes, Pseudoevernia furfuracea, Peltigera aphthosa* (Pakarinen, 1981; Takala et al., 1985).

The sulphur pollution level of eastern Canada has been estimated by Zakshek et al. (1986) on the basis of the sulphur content of tissues of *Cladonia rangiferina.*

The indication of hydrogen fluoride pollution

With exposure to hydrogen fluoride the colour of lichens becomes grayish-white, the size of colonies decreases, and later, the colonies fall apart.

As a result of fluoride exposure the coverage of lithophytic (saxicolous) lichens would greatly decrease. This phenomenon is most apparent in the case of the "fruticose" and "foliose" types (Perkins and Millar, 1987).

Several lichen species accumulate fluorine (Asta and Garrec, 1980; Perkins et al., 1980; Davies, 1982, 1986; Perkins and Millar, 1987). Examples are: *Alectoria iubata, Anaptychia fusca, Cladonia pyxidata, Evernia prunastri, Hypogymnia physodes, Letharia vulpina, Parmelia omphalodes, P. glabratula ssp. fuliginosa, P. furfuracea, P. physodes, P. saxatilis, P. sulcata, Peltigera canina, Ramalina farinacea, R. fastigiata, R. siliquosa, R. subfarinacea, Usnea muricata, Xanthoria parietina.*

The fluorine content of lichen colonies is also a function of air humidity (Perkins et al., 1980; Table 13).

Transplanted lichens are also suitable for indication of fluorine exposure (Fig. 8). Sensitive species are *Pseudoevernia furfuracea, Parmelia physodes* and *P. sulcata;* a less sensitive or indifferent species is *Parmelia acetabulum.*

Table 13 — Fluorine content of lichen thalli ($\mu g \cdot g^{-1}$) after 4 days' exposure to HF. The F concentration in air is 5 $\mu g \cdot m^{-3}$ (Perkins et al., 1980)

Species	Relative air humidity		
	40%	63%	87%
Cladonia cristatella	10	32	82
Parmelia caperata	11	26	89

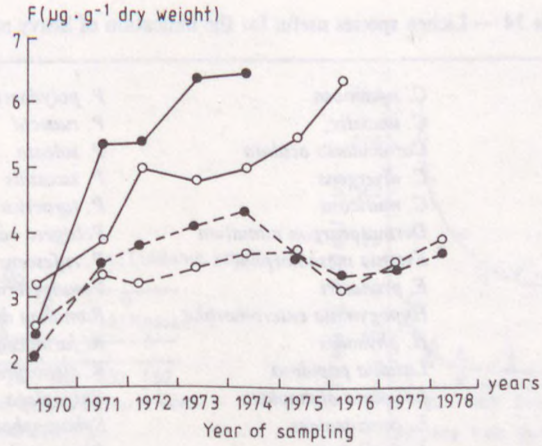


Fig. 8 — Fluoride concentrations (F µg·g^{-1}) in corticolous (filled o) and saxicolous (empty o) lichens, sampled in successive years, either 0.55–1 km (—) or 3.2–4.9 km (---) from the aluminium reduction plant in Anglesey (Perkins et al., 1980).

The indication of heavy metals

Several lichen species are suitable to indicate heavy metal exposure (Table 14).

The lead content of *Parmelia physodes* decreases proportionally with distance from highways (Deruelle, 1981; Table 15 and Fig. 9).

Corticolous, saxicolous and terricolous lichens are equally suitable to indicate lead exposure (Table 15). In corticolous lichens there is a correlation between the elemental concentrations of the bark of the host tree and the lichen (de Bruin and Hackenitz, 1986).

Cladonia rangiferina and *C. nitei* can be applied as accumulation type indicators of uranium, iron, lead and titanium (Boileau et al., 1982; Beckett et al., 1982; Nieboer et al., 1982; Figs 10–12).

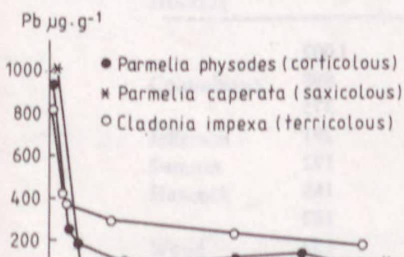


Fig. 9 — Pb contents of lichen species occurring on various substrates, as a function of their distance from the highway (Deruelle, 1981).

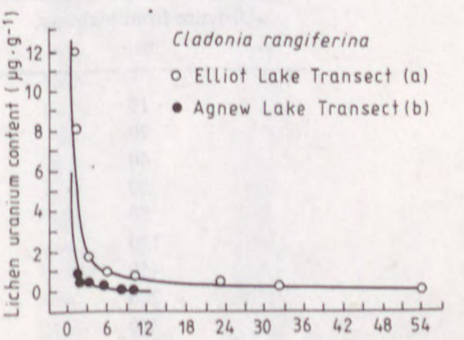


Fig. 10 — Uranium contents of the lichen *Cladonia rangiferina* as a function of distance along Elliot Lake (curve a) and Agnew Lake (curve b) macrotransects (Beckett et al., 1982).

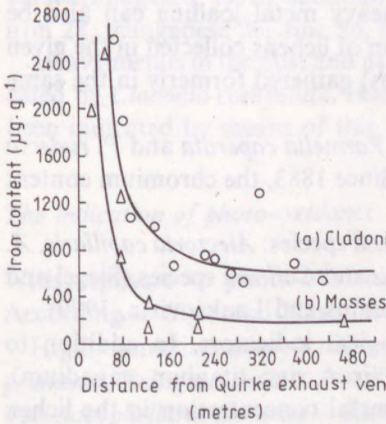
Table 14 — Lichen species useful for the indication of heavy metals

Acarospora strigata	*C. squamosa*	*P. 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. stenospra*
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*	*Verrucaria nigrescens*
C. sylvatica		

After Bossermann and Hagner, 1981; de Bruin and Hackenitz, 1986; Folkeson, 1979, 1981; Fuchs and Garty, 1983; Gailey and Lloyd, 1986; Garty et al., 1977, 1979, 1988; Garty and Amman, 1987; Garty 1987, 1988; Garty and Hagemeyer, 1988; Gough et al., 1988; Goyal and Seaward, 1981–1982; Laaksovirta and Olkkonen, 1977, 1979; Lodenius and Laaksovirta, 1979; Lorch and Weber, 1985; Mueller et al., 1987; Nash, 1975; Nash and Sommerfeld, 1981; Olmez et al., 1985; Pilegaard, 1979; Saeki et al., 1975; Schutte, 1977; Seaward et al., 1978; Solberg and Selmer-Olsen, 1978; Takala and Olkkonen, 1981; Vestergaard et al., 1986.

Table 15 — The lead content of *Parmelia physodes* in the proximity of a highway, in the Paris basin (Deruelle, 1981)

Distance from highway, m	Pb, $\mu g \cdot g^{-1}$ dry weight
15	1 002
20	898
40	375
50	291
80	192
120	188
150	182
200	152
300	156
400	112
500	89
600	65



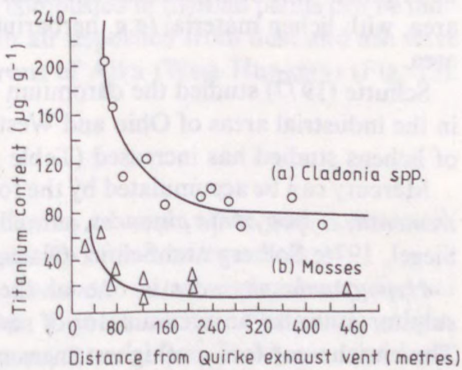


Fig. 11 — Iron content of *Cladonia* spp. and mosses (*Polytrichum commune* + *Sphagnum* spp.) as a function of distance from the horizontal exhaust vent at Quirke-1E mine at Elliot Lake, Ontario. Solid curves correspond to the following equations: curve a, $C_{Fe}=(5.8\times10^6)\ d^{-2}+575$; curve b, $C_{Fe}=(3.0\times10^6)\ d^{-2}+160$.

Fig. 12 — Titanium content of *Cladonia* spp. and mosses (*Polytrichum commune* + *Sphagnum* spp.) as a function of distance from the horizontal exhaust vent at Quirke-1E mine at Elliot Lake, Ontario. Solid curves correspond to the following equations: curve a, $C_{Ti}=(5.0\times10^5)\ d^{-2}+76$; curve b, $C_{Ti}=(8.0\times10^4)\ d^{-2}+12$.

Table 16 — The chromium content of *Parmelia caperata* and *P. rudecta* determined in herbarium specimens (Ohio and West Virginia) (Schutte, 1977)

County	Year	Thallus, $\mu g \cdot g^{-1}$ dry weight
Franklin	1883	4.78
	1961	8.85
	1976	22.00
Cuyahoga	1903	6.16
Butler	1907	7.81
	1961	9.01
	1976	15.30
Hocking	1933	8.53
	1961	9.64
	1975	12.80
Columbiana	1959	18.30
	1975	26.80
Jefferson	1959	13.20
Summit	1959	27.60
Hancock	1962	14.80
	1975	31.20
Wood	1962	15.50
	1975	33.20
Lucas	1962	8.21

By the use of lichens, the temporal trend of heavy metal loading can also be determined. One compares the chemical composition of lichens collected in the given area, with lichen material (e.g. herbarium specimens) gathered formerly in the same area.

Schutte (1977) studied the chromium content of *Parmelia caperata* and *P. rudecta* in the industrial areas of Ohio and West Virginia. Since 1883, the chromium content of lichens studied has increased (Table 16).

Mercury can be accumulated by the following lichen species: *Alectoria capillaris, A. tremontii, Hypogymnia physodes,* as well as *Cladonia* and *Collema* species (Siegel and Siegel, 1976; Solberg and Selmer-Olsen, 1978; Lodenius and Laaksovirta, 1979).

Hypogymnia physodes is one of the best biological indicators. In addition to sulphur, it is also an accumulator of several metals (iron, zinc, titanium, vanadium). The enrichment factors (highest measured heavy metal concentration in the lichen divided by corresponding values from control areas) for individual heavy metals as measured in the environs of steel and iron works in Denmark showed the following

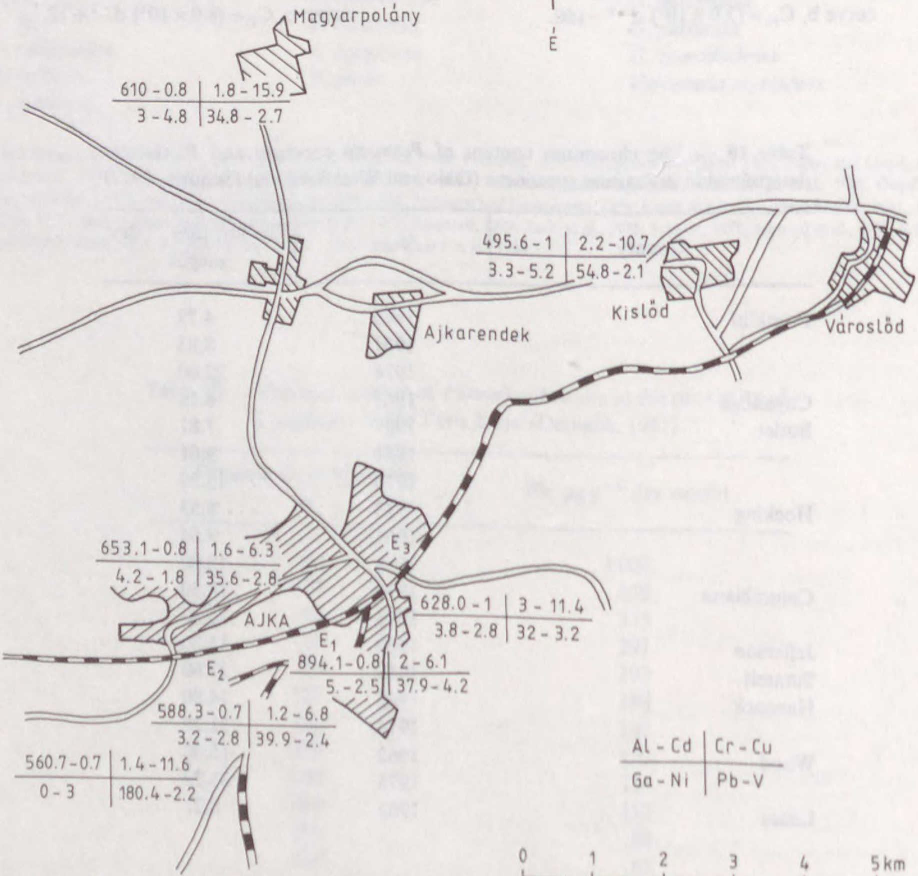


Fig. 13 — Al, Cd, Cr, Cu, Ga, Ni, Pb and V content of *Cladonia convoluta* at Ajka (West Hungary). Emitters: E_1 = coal power station; E_2 = aluminium works; E_3 = glassworks.

values (Pilegaard, 1978): cadmium 74, lead 62, copper 35, chromium 34, vanadium 31, iron 28, manganese 28, zinc 26, nickel 9.

Heavy metals in the dust and ash from coal combusted in thermal plants can be indicated by *Cladonia cornvoluta.* Heavy metals in air deposited from dust and ash have been indicated by means of this in the environs of Ajka (West Hungary) (Fig. 13).

The indication of photo-oxidants

With exposure to photo-oxidants, lichen colonies become white and compacted. According to Sigal and Nash (1983) the sensitivity of lichens is as follows:

Highly sensitive: *Brorya abbreviata, B.* cf. *fremontii, Cetraria canadensis, Evernia prunastri, Peltigera canina, P. collina, P. spuria, Physcia sciastra, Platismatia glauca, Pseudocyphellaria anthraspis, Ramalina farinacea, R. menziesii, Xanthoria candelaria.*

Sensitive: *Cetraria merrillii, Collema nigrescens, Leptogium californicum, Parmelia sulcata, P. quercina, Peltigera rufescens, Physcia ciliata, P. orbicularis, Polychidium alboliciadum, Usnea sp.*

Moderately tolerant: *Hypogymnia enteromorpha, Parmelia glabrata, P. elegantula, P. subolivacea, Xanthoria polycarpa.*

Tolerant: *Letharia vulpina, Physcia bisiana, P. tenella, Physconia grisea, Xanthoria fallax.*

Cladonia rangiferina is suitable for the indication of the following radioactive elements (Ellis and Smith, 1987): ^{141}Ce, ^{144}Cs, ^{103}Ru, ^{106}Ru, ^{95}Zr, ^{137}Cs, ^{40}K, $^{239,270}Pu$, ^{210}Pb, ^{54}Mn, ^{7}Be, ^{238}Pu.

Polychloride-biphenyl (PCB) can be indicated by transplanted *Caloplaca aurantia* and *Ramalina duriaei* (Garty et al., 1982).

In the northern parts of Sweden chlorinated hydrocarbons accumulating in *Cladonia alpestris* are also introduced into man (Lapps) through the food chain (Villeneuve et al., 1985):

The indication of herbicides

Lichens can also be applied in the indication of various herbicides. According to investigations by Hällbom and Bergman (1979), the nitrogenase activity of *Peltigera praetextata* is reduced under the impact of herbicide loading.

6.3 THE INDICATION OF AIR POLLUTION BY LICHENS

The mapping of lichen distribution

The geographical distribution of lichen species distinguishes areas with different degrees of air pollution.

According to Nylander (1866) lichens were absent in the city of Paris, as early as in 1866. Owing to higher temperatures in the town centre, the relative humidity of air is lower, and both sulphur dioxide and other air pollutants are found in higher concentrations. These areas are almost totally void of lichens (lichen deserts).

On the basis of the occurrence and the distribution of bark lichens, it is possible to make deductions as to the presence of various air pollutants.

To date, lichen maps have been compiled for almost every large city of the world. In these, generally, three or five zones can be distinguished:

1) Lichen desert: areas with high sulphur dioxide concentrations where no lichens occur. This area is also called the zone of "critical total loading" (Herzig et al., 1987). In this area, the annual or daily average concentration of SO_2, NO_2 and other pollutants in air is near or above the determined limit values with high probability.

In Switzerland, the annual limit values are as follows: SO_2: 30 $\mu g \cdot m^{-3}$, NO_2: 30 $\mu g \cdot m^{-3}$, particulate materials: 70 $\mu g \cdot m^{-3}$, deposited dust: 200 $\mu g \cdot m^{-2} \cdot d^{-1}$, Pb: 100 $\mu g \cdot m^{-2} \cdot d^{-1}$, Cd: 2 $\mu g \cdot m^{-2} \cdot d^{-1}$, Zn: 400 $\mu g \cdot m^{-2} \cdot d^{-1}$, ozone: 98% of the average of one month 1/2 h.s. 100 $\mu g \cdot m^{-3}$.

2) "Struggling zone": certain resistant lichen species still occur but the colonies of sensitive species have suffered damage. Within the "struggling zone" the following subzones are also frequently distinguished:

– inner struggling zone: the species studied can be found on 10% of the trees;
– intermediate struggling zone: the species studied can be found on 25% of the trees;
– outer struggling zone: the species studied can be found on 50% of the trees.

Fig. 14 — Lichen map of Budapest, Hungary (Farkas, 1982).

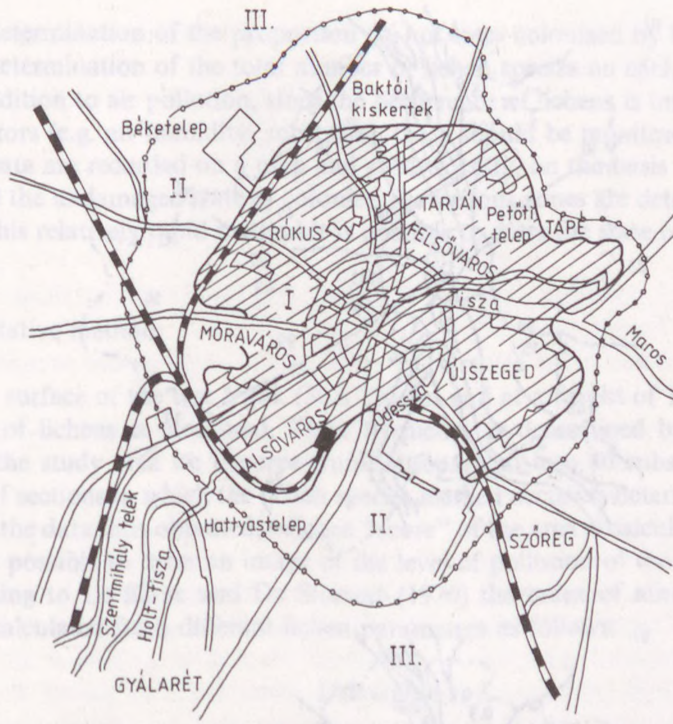


Fig. 15 — Lichen map of Szeged (Gallé, 1979). I. lichen desert, II. the lichen colonies are damaged, III. the lichen colonies are normal.

3) Normal zone: air pollution has no effect, the development of lichens is normal. Between the outer and normal zones, another, so-called transitional zone is often distinguished.

In the maps of Szeged and Budapest (Figs 14, 15) the occurrence of lichen deserts coincides with the presence of high concentration of sulphur dioxide. The lichen desert of Budapest is especially large covering almost the entire town. The normal zone is rather small. It is confined to a small part of the Buda Mountains. The Figure reflects both the dry local climate and the bad air quality of the area.

By use of a transplanted lichen *(Hypogymnia physodes)*, the lead impact in the vicinity of Budapest has also been demonstrated (Fig. 16).

In Hungary, lichen maps have been compiled for Debrecen (Felföldy, 1942), Szeged (Gallé, 1979) and Budapest (Farkas, 1982).

In order to compile such a map several thousand trees are inspected, for example, the lichen map of Leipzig has been compiled on the basis of 13 000 trees.

In the observed area, the lichens found on the bark of trees (in parks, alleys) are recorded. (The bark itself and its acid reaction also plays an important role in the occurrence of lichens.)

In the course of mapping, attention should be paid to the following factors: the colonies to be studied on each tree should belong if possible to the same lichen species, they should be of identical age and occur in an undamaged and free state.

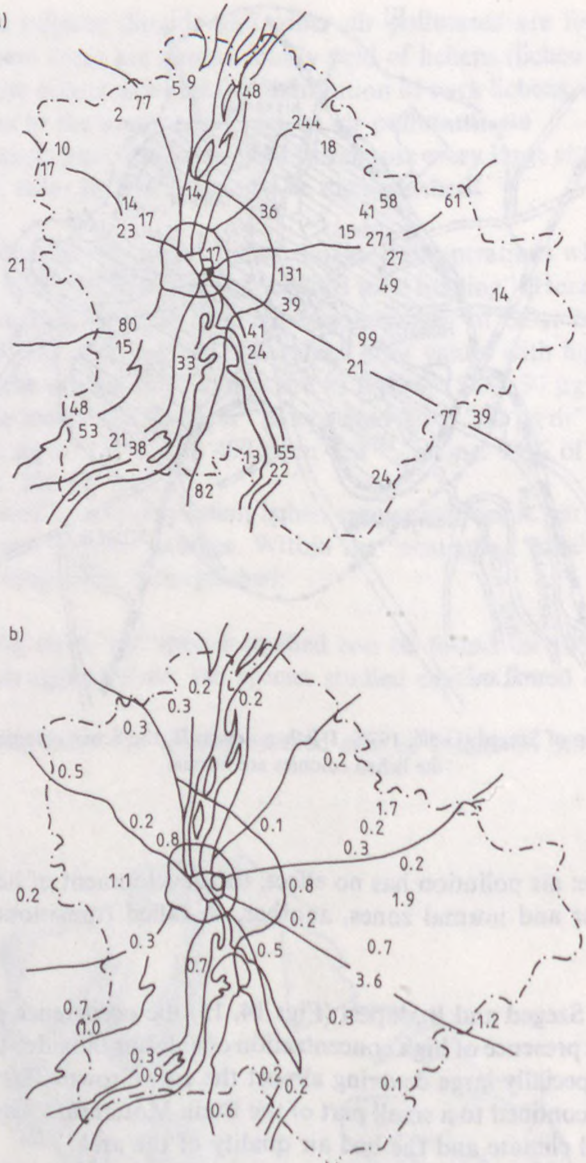


Fig. 16 — Lead (a) and cadmium (b) accumulation ($\mu g \cdot g^{-1}$) in *Hypogymnia physodes* transplanted samples (Farkas et al., 1985).

The ambient levels of air pollution of forests in large areas can also be determined by lichen surveys (Bartholmess et al., 1987).

The mapping procedure is as follows:

Qualitative method:

– the determination of the coverage of all lichen species occurring in the assemblage;

– the determination of the proportion (%) of trees colonized by lichen;

– the determination of the total number of lichen species on each tree;

– in addition to air pollution, since the occurrence of lichens is influenced also by other factors (e.g. air humidity, substrate), these should be monitored;

– the data are recorded on a map and subsequently, on the basis of lichen occurrence and the undamaged state of colonies, the various zones are determined. On the basis of this relatively rapid method it is possible to asses the state of environmental pollution.

Quantitative method:

On the surface of the tree trunk (30 × 130 cm and at a height of 120–170 cm) the coverage of lichens is measured. Their frequency is determined by the following method: the study area on the tree trunk is separated into 40 subsections and the number of sections in which the lichen species studied occurs is determined (Fig. 17). Based on the data thus obtained, a lichen "score" of the area is calculated. This value renders it possible to form an image of the level of pollution of the given area.

According to Le Blanc and De Sloovar (1970) the index of atmospheric purity (IAP) is calculated from different lichen parameters as follows:

$$\mathrm{IAP} = Q \cdot \mathrm{f}$$

where Q = factor of tolerance to toxicity, indicating the sensitivity of the species towards pollutants is deduced on the basis of the number of observed species. A hierarchical sequence of species occurring in the study area is based on their increasing sensitivity. A low value of Q indicates only a few accompanying species present.

f = frequency, of % coverage of the species studied in the study area. It is evaluated according to a 0–5 scale.

A disadvantage of this method is that the value of sensitivity of individual lichen species is determined repeatedly for each experimental area.

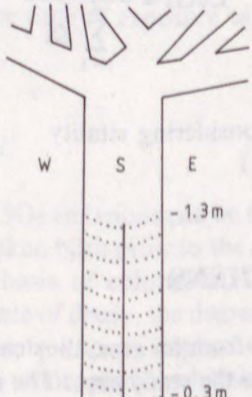


Fig. 17 — The study of the occurrence of lichen species on a tree trunk. W = west; S = south; E = east.

As a consequence, the IAP values of neighbouring areas cannot be compared.

Herzig et al. (1987) use the following formula:

$$IAP = \frac{Q \cdot C \cdot F}{v \cdot s}$$

where

Q = as above;

F = frequency [number of units (1–10) in the grid laid over the tree trunk where the observed lichen species occurred];

C = cover, it indicates the value of coverage of the lichen species occurring in the subsection (0, 1, 2, 3, 4, 5);

v = vitality, characterizing the state of health and growth of the colony, on the basis of a three-grade scale (good, medicore, underdeveloped);

s = the degree of damage estimated on the basis of visible symptoms (chlorosis, necrosis), according to a three-grade scale (no damage, somewhat damaged, strongly damaged).

The IAP methods may replace the tedious work of mapping. The principle of the method is the following: at each site the performance of lichens is generally correlated with air quality. In the case of low impact, both the number of lichens and the value of coverage are high. Based on the number of species and the value of coverage the IAP index is calculated. Thus, air quality is characterized by a single number.

The disadvantages of the IAP method are eliminated by Rabe's (1987) air quality index (LuGI = Luftgute Index). The "sensitivity" of each lichen species is determined (at present only for SO_2). The index of sensitivity is determined on the basis of the maximum SO_2 concentration tolerated by the lichen species without damage. The index value of the most susceptible lichen species, *Lecanora conizaeoides*, is of unity. This provides a standard of comparison for all the other values of sensitivity.

The air quality index is calculated as follows:

$$LuGI = \frac{\sum_{i=1}^{n} Di\, E_i}{\sum_{i=1}^{n} Di}$$

Di = the cover of species i, considering vitality

E_i = the sensitivity of species i

6.4 TRANSPLANTED LICHENS

Even though lichens are absent from an area, they can still be utilized in the form of active monitors, transplanted to the study area. The internationally accepted, standard method is as follows (Schönbeck, 1969):

A sensitive, bark-colonizing (corticolous) lichen, most frequently *Parmelia (Hypogymnia) physodes*, is collected in an area void of S emissions. In areas of unpolluted

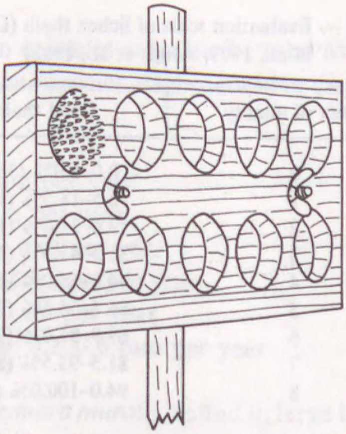


Fig. 18 — Exposition table for lichen species.

air the lichen forms rosette-like colonies. The transplants should be collected possibly from the trunk of a recumbent oak tree. By means of a metal borer 15 mm thick disks of 42 mm diameter are cut out from the trunk. When collecting the lichen, special care should be taken that the individual colonies should not be damaged, i.e., on the surface of the disk the colony should remain perfectly undamaged. If the disk is taken from the trunk of living trees, the wound should be covered by wound wax. The disks are placed on a 29 × 12 × 2.5 cm exposure plate (Fig. 18). In the plate 10 holes of 45 mm diameter and 15 mm depth are prepared. The trunk disks are fixed by using resin. The exposure plates are fixed on a pole, in the direction of chimney emissions of SO_2 in towns or industrial centres in open areas at a height of 150 cm.

In addition to *Parmelia physodes,* the following species are also suitable for transplantation: *Parmelia sulcata, P. caperata, P. cortea, P. furfuracea, Xanthoria parietina, Evernia prunastri.* The species *Ramalina duriaei* is used in Israel (Garty and Fuchs, 1982; Fuchs and Garty, 1983; Garty, 1988; Garty et al., 1988).

The transplanted lichens indicate the level of air pollution within a short time (e.g. the exposure period for *Parmelia physodes* is 10 weeks). Other transplants are exposed for 4, 12, or 15 weeks. The time of exposure can be affected by the season of the year.

Evalution of exposed lichen thalli

The degree of damage caused by SO_2 emissions can be recorded also by photography. Colour photographs should be taken both prior to the exposure and at the end of the exposure period. Thus, on the basis of coloration, the degree of damage can be measured exactly. Based on the rate of decay, the degree of damage and the measured chlorophyll content, the response to known sulphur dioxide concentrations can be assessed. As a result of damage to chlorophyll a whitish-brown coloration is frequent.

The degree of lichen damage can be determined also by means of a scale of values (Table 17) based on the proportion of dead thalli. A zero value is given at the beginning of the exposure, and a final evaluation is made at the end of the exposure.

Table 17 — Evaluation scale of lichen thalli (Dreyhaupt et al., 1979; Arndt et al., 1982)

Class of quality	Dead thallus
0	0.0–6.0% (3%)
1	6.5–18.5% (12.5%)
2	19.0–31.0% (15.0%)
3	31.5–43.5% (37.5%)
4	44.0–56.0% (50.0%)
5	56.5–68.5% (62.5%)
6	69.9–81.0% (75.0%)
7	81.5–93.5% (87.5%)
8	94.0–100.0% (96.9%)

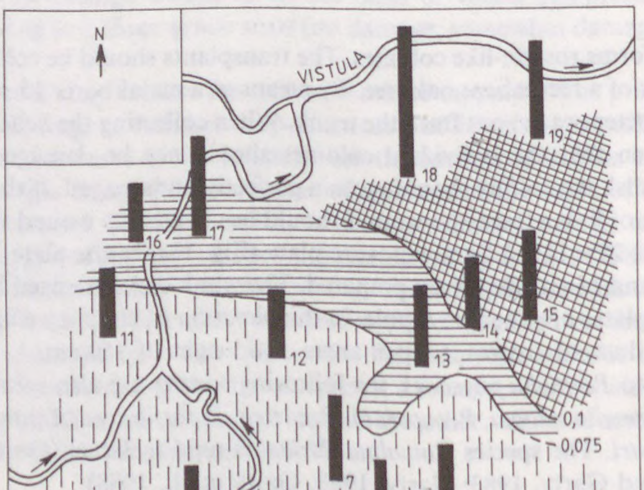


Fig. 19 — SO_2 concentrations of the air and the S content of *Parmelia physodes* growing on tree bark in the environs of the aluminium works Skawina. B = power station (Swieboda and Kalemba, 1978).

Using the scale, the rate of increase of dead lichen material is estimated and expressed as a percentage of the initial value (Dreyhaupt et al., 1979).

The sulphur content of lichens is correlated with the level or time of exposure to sulphur dioxide (Fig. 19).

The growth rate of lichen colonies can also be used as an indicator. The average annual growth of certain lichen species, as measured in unpolluted areas (Feige, 1982) is the following:

Peltigera species 10–30 mm per year
Physcia caesia 0.8–1.1 mm per year
Parmelia saxatilis 1.7–3.2 mm per year
Cladonia rangiformis 2.0–5.0 mm per year
Lecanora muralis 0.8–2.3 mm per year
Rhizocarpon geographicum 0.2–0.6 mm per year

The circular colony of *Lecanora muralis,* found in large towns, is an especially good target for study. In towns it grows on calcareous substrates (stone, concrete) with a pH value exceeding 8.

Lichens living on alkaline substrates deeply invade the lichen desert area of Szeged. Such species are: *Lecanora, Caloplaca, Physcia spp.* (Gallé, 1979). Parallel with any decrease in the sulphur dioxide emissions, the recolonization of corticolous lichens *(Lecanora muralis, L. conizaeoides, L. hageni)* is occurring in cities and urban areas (Rabe and Wiegel, 1985; Kandler, 1987; Henderson-Sellers and Seaward, 1979).

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7 Bryophytes

Bryophytes are invaluable constituents of many different ecosystems, in spite of their small size and relatively low total biomass. Their abundance and significance in pioneer successional stages have long been widely recognized. In pioneer vegetations terrestrial bryophytes (especially acrocarpous moss species) are important for soil fixation and humus accumulation. Bryophytes also play a major role in climax communities. In this respect we refer to their importance in controlling the water balance of tropical rain forests (Pócs, 1982).

Bryophytes are widely used as bioindicators due to their specific indicative properties. Certain mosses give valuable information about the soil features of forests, whereas the presence of others in *Sphagnum* bogs indicates the water level and pH value of the bog. The occurrence of some water mosses in streams and lakes is thought to be closely connected with the eutrophication level and Ca content of the water (Ando and Matsuo, 1984). In Europe, special attention has been paid to mosses for classifying different forest types (Cajander, 1926). Bryophytes have also been found to be valuable indicators of climatic conditions (Pospisil, 1975; Piippo, 1982).

During the last two decades the role of bryophytes as environmental indicators has been emphasized. Due to some specific characters, bryophytes are especially suitable for biological monitoring. Thus, at one of the first international conferences completely devoted to air pollution (Wageningen, The Netherlands, 1968), it was recommended to use bryophytes and lichens for this purpose (Rao, 1982). We indicate below why bryophytes are more useful for biological monitoring than flowering plants.

Because of their small size, bryophytes are easy to handle which is obviously an advantage for all sorts of experiments, e.g. chemical analysis. They are evergreen and (with a few exceptions) perennial plants, thus they can be utilized throughout the year. Many species have a wide geographical distribution and grow in a wide range of habitats, which is beneficial for comparative studies. Most bryophyte species do not possess a cuticle and, therefore, can take up water over the entire plant surface. As a consequence, they obtain their nutrients directly from atmospheric deposition, i.e., dustfall and precipitation. Vascular plants, on the other hand, take up their nutrients from the soil by means of their roots, which might have a more or less tempering effect on possible harmful factors. Their accumulation ability makes bryophytes good indicators for the detection of certain elements, for instance, heavy metals. By comparing fresh specimens with herbarium specimens we can also perform retrospective studies on heavy metal pollution. In addition, there are some specific features of certain bryophyte species which make them extremely useful for biomonitoring studies. There are mosses showing typical characteristics after rehydration following a period of desiccation. These tend to be rather constant and thus very useful for comparative studies. Especially useful are some species (e.g. *Hylocomium splendens*) that produce distinct annual segments. By analyzing these different segments we may obtain a fairly good insight into the state of our environment during a certain period.

Notwithstanding the fact that biomonitoring with bryophytes has been commonly applied and generally is recognized as a simple method, some conceptual problems may arise. Unfortunately, in many studies of this sort only one aspect is considered. Consequently, in overemphasizing such aspects (and at the same time underestimating or neglecting others!) subjective or even misleading conclusions may be drawn. In this context, the following three questions must be asked: (1) which bryophyte species should we use?; (2) what does the indicator species indicate?; (3) how does it indicate environmental factors? Only by this approach may we be succesful in drawing well-balanced conclusions from biomonitoring experiments.

7.1 APPLICATION OF BRYOPHYTES

Bryophytes (together with lichens) have long been used to assess the quality of the environment. Initially this was merely in a more or less descriptive way. That is to say, by determining the bryoflora in an area or by mapping the presence of a certain bryophyte species one tries to draw conclusions about the state of the environment. A pioneer study in this field was carried out by Barkman (1958). Undoubtedly, such studies have proved their value in the past and will remain important in the future. However, there was also a need to measure environmental changes by a more experimental approach.

A frequently utilized method to evaluate the quality of our environment is the transplantation experiment. Le Blanc and Rao (1966) first used bryophytes for this purpose. During a transplantation experiment the study plants are transferred, along with their original soil substrate, from an unpolluted area (the control site) to potentially polluted places. Favourite sites for transplants are those with high concentrations of gaseous pollutants (chiefly SO_2, HF or O_3), for instance, in and around industrial areas. Sites with considerable amounts of (heavy) metals are also used for this purpose. After a certain exposure time (which may range from a few weeks to several years) the different responses of the transplants are examined and compared with those in the control plants. Subsequently, conclusions can be drawn as to the pollution level of a certain area.

The so-called moss-bag technique is useful, too, for monitoring environmental pollution. *Sphagnum*, *Fontinalis* or *Rhynchostegium* species are put into bags made of nylon or muslin (0.07–0.9 mesh·cm^{-1}). Upon a relatively short exposure time (only a few hundred hours for water mosses!) the element content of the mosses can be determined. This method which is rather popular in Great Britain (e.g. Kelly et al., 1987; see also literature therein), is in particular suitable for the detection of heavy metals in aquatic environment.

In Japan, a "bryometer" was developed for recording air pollution (Taoda, 1973). Bryophytes are placed in small, transparent plastic plant chambers. One of the chambers is filled with urban air and the control chamber with clean air. Then, the different reactions of the test plants are compared. The thalloid liverwort *Marchantia polymorpha* has been a reliable test plant in bryometers.

In another type of experiment bryophytes are exposed to a certain pollutant under controlled laboratory conditions. SO_2, NO_2 (single and in combination) or certain heavy metals are frequently used. From this sort of experiment important conclusions may be drawn about the specific impact of the different pollutants.

7.2 BRYOPHYTES AND AIR POLLUTION

The number of bryophyte species has been much reduced in urban areas, industrial centres and their environs due to the sensitivity of these plants to air pollution. A great number of species are extinct, while others which were earlier common and widespread, have been reduced in number and are now rarely found. In The Netherlands, for example, 15% of the terrestrial bryophytes and 13% of the epiphytic bryophytes fell victim to pollution during the last century (Barkman, 1969).

The two major pollutants in cities are industrial smoke and car exhaust. The first is mainly sulphur dioxide, carbon monoxide and soot, whereas the components of the latter are carbon monoxide, nitrogen oxides, sulphur dioxide, lead, aldehydes and carbohydrogens. In industrial areas the most frequently occurring pollutants are sulphur dioxide, hydrogen fluoride and ozone.

The harmful effect of SO_2 on both lichens and bryophytes was first described by Rao and LeBlanc (1966) and Coker (1967). They observed a considerable breakdown of the chlorophyll and an impairment of cell structure and function through plasmolysis, when the sulphur dioxide concentration exceeded 5 ppm. The destruction of the chloroplasts means the cessation of assimilation which eventually brings about the death of the whole organism. When sulphur dioxide is present in the plant, it increases the free H^+ concentration which in turn facilitates the transformation of chlorophyll-a into phaeophytin-a. Since sulphur dioxide turns to sulphuric acid under moist conditions, and this can impair the plants, it is the ambient water content in the moss which determines the extent of chlorophyll breakdown. SO_2 pollution initially makes respiration more intensive, however, after the appearance of necrotic spots on the leaves the intensity is reduced (Gilbert, 1968; Syratt and Wanstall, 1969). The general symptom of sulphur dioxide pollution is fading. First, the apical leaves, which are more exposed, and later the basal parts can also be discoloured. Completely discoloured mosses are usually not able to recover, even after being placed in a clean environment.

It seems that sulphur dioxide and other pollutants have a great effect on the reproductive capacity of bryophytes. It became clear from several studies that an enhanced level of pollution is correlated with a decrease in sexual reproduction (e.g. De Sloover and LeBlanc, 1970; Longton, 1985; Raeymaekers and Glime, 1986; Sérgio, 1987). On the other hand, there are some reports of stimulated asexual reproduction of bryophytes by air pollution (Comeau and LeBlanc, 1971; Sérgio, 1987). Sérgio found an enhanced production of terminal leaf from gemmae in the moss *Tortula laevipila* in areas with high SO_2 concentrations. She concluded that this is apparently an adaptation to stress caused by air pollution. Gilbert (1971) examined the reproductive potential of some common bryophyte species (among others *Bryum argenteum, Ceratodon purpureus* and *Marchantia polymorpha*) in polluted urban areas. These species showed an abundant production of spores and gemmae, which had a high degree of fertility. Thus, it seems that there are at least some contrasting figures about the reproductive capacity of bryophytes in relation to air pollution. Nevertheless, it is widely understood that (with the exception of some widespread and common species) air pollution is negatively correlated with sexual reproduction. A phenomenon worth mentioning here is that the total reduction of biomass and the disappearance of certain species may reflect the enhanced effect that SO_2 has, in particular on the protonema stage of bryophytes. It has been shown in laboratory experiments that protonemata are much more sensitive to sulphur dioxide than the adult life stage (Gilbert, 1968; Nash and Nash, 1974; Ferguson and Lee, 1979). This agrees well with observation that the protonema stage of SO_2-tolerant species in general is short-lived and that budforming is rapidly initiated (Le Blanc and Rao, 1974).

The reaction of bryophytes fo fluoride pollution, which mainly occurs in the environs of aluminium foundries, is similar to that of the flowering plants. The hydrogen fluoride absorbed on the leaf surface is translocated into the apices of the leaves or phylloids causing the typical damage symptoms. The extent of damage is proportional to the amount of HF and the duration of exposition, the so-called factor of exposure (concentration × time). In the leaves of *Funaria hydrometrica,* for example, exposed with a factor 780 (65 ppb HF × 12 hours) the apical areas died, the chloroplasts were destroyed, and the cells became plasmolitic. It was found that after a three-week recovery period the F concentration in the leaves was reduced by 26–36% of that accumulated during the exposure period.

A characteristic example of the harmful effect of HF on bryophytes was provided by LeBlanc et al. (1971). They transplanted moss species from clean areas and moved them near to an aluminium foundry, 40 km from the control site. Investigation of the mosses after a year revealed that their chlorophyll was fully decomposed, the plants had turned brown, and plasmolysis and other damage were detectable in their cells. One of the transplanted species, *Orthotrichum obtusifolium* contained an F concentration of 600 ppm while in the control sample only 20 ppm was measured.

Bryophytes also show different levels of tolerance to fluoride. Epiphytes are much more sensitive than species living on earth substrate. The latter comprise some rather tolerant species such as several bog mosses (*Sphagnum* spp.), *Leucobryum glaucum, Polytrichum commune, Rhytidiadelphus squarrosus* and numerous acrocarpous moss species as well as some of the smaller liverworts (Gilbert, 1971).

Ozone is a much more toxic substance than those gases which have a part in increasing ozone levels, for example nitrogen oxides and some hydrocarbons. Ozone causes acute damage and early senescence. Small concentrations of O_3, however, may stimulate growth in mosses (Comeau and LeBlanc, 1971).

7.3 BRYOPHYTES AND RADIOISOTOPES

Radioactive substances are also accumulated in greater quantities in bryophytes than in flowering plants. For instance, cushions of *Pleurozium schreberi* extensively absorbed Zr, B, Ba and La from fallouts after nuclear tests (Svensson and Liden, 1965). This resistance to ionizing radiation is probably because of the small size of the bryophytes' nuclei (4–150 μm).

The ability of mosses to accumulate radioisotopes, and consequently their usefulness as bioindicators, was recently demonstrated following the Chernobyl reactor accident. Daróczy et al. (1988) utilized three common moss species (*Ceratodon purpureus, Tortula ruralis* and *Bryum argenteum*) for mapping of the long-lived ^{137}Cs in Hungary. Furthermore, they paid attention to some specific methodological problems encountered when using mosses for monitoring fallout nuclides. For example, one of the questions they put forward was whether it is possible to transform by the same constant, the cesium concentration found in mosses to that for the contamination of the soil for the whole area concerned. Indeed the SA (specific activity) of the ^{137}Cs isotope, obtained from the mosses collected in different parts of Hungary, correlated well with comparative data for the ground surface contamination in the country.

In this regard the paper of Kwapulinski and Sarosiek (1988) should also be mentioned. They determined the $^{226}Ra/^{228}Ra$ ratio in dustfall, air and in a *Hypnum* species nearby, and around a power station in Poland. This work suggests that mosses can be used as bioindicators of radium poisoning and radium contamination in the environment. In particular, they may provide useful information on previous levels of airborne radioactivity.

7.4 BRYOPHYTES AND HEAVY METALS

Depending on their concentration in the environment, heavy metals may be toxic to plants. Nevertheless, some of them are indispensable as micronutrients. In this respect, we refer to the importance of copper and zinc as constituents of metalloenzymes. All heavy metals occur naturally in our environment but along with industrialization their concentrations have steadily increased. Heavy metals are mainly released from mining areas, metallurgic industries and through the combustion of fossil fuels. Only recently some measures have been taken to limit their emission to the environment. Such measures are, for instance, smoke-washing or electric filters at power stations or the use of lead-free petrol and introduction of catalyzators for cars.

So far only a few experiments have been carried out on the effects of metals on bryophytes (e.g. Coombes and Lepp, 1974; Lepp and Roberts, 1977; Simola, 1977; Meenks, 1990). It was found that the metal toxicity sequence was similar to that found for flowering plants; i.e., from most toxic to less harmful: Hg, Pb, Cu, Cd, Cr, Ni, Zn (cf. also Nieboer and Richardson, 1980). It is important to underline that environmental pollution may induce additive or synergistic effects while there is often a simultaneous contamination by several heavy metals.

Much has been written about the relationship between bryophytes and heavy metals in the environment. One aspect is the occurrence of bryophyte species which are pretty tolerant to high concentrations of certain metals in the environment. These metal-tolerant species are, therefore, good indicators of the presence of iron or copper in the soil or atmosphere. There even exist communities which almost exclusively consist of so-called "copper mosses", mainly species from the genera *Dryoptodon, Gymnocolea, Merceya* and *Mielichoferia* (e.g. Persson, 1956). There have been many speculations about the physiological background of this phenomenon but a satisfactory explanation has not been postulated so far. The presence of certain bryophytes on Cu-rich substrates suggests a requirement for this element, perhaps with some additional ecophysiological factors as well. Support for this view was provided by Brown and House (1978) who detected a copper-tolerant ecotype ("microspecies") of the liverwort *Solenostoma crenulatum*. It turned out that the photosynthesis of these plants was stimulated in the presence of copper which indicates a specific need for Cu. More recently, tentative studies on this subject were carried out by Shaw and his co-workers (Shaw, 1987a, 1987b; Shaw et al., 1987; Shaw and Anderson, 1988; Shaw et al., 1989). Shaw (1987a) studied the copper moss *Scopelophila cataractae* at six localities in the eastern United States. Chemical analysis of their substrates showed that all but one population grew on copper-enriched soil. Specimens of these populations were grown experimentally on four soil types, ranging from highly to not contaminated, and all grew best on the soil contaminated with copper, lead and zinc. However, no variation in growth between the different populations with respect to the three metals was found. These findings contrast with the situation in angiosperms and probably reflect the absence of sexual reproduction of this moss species in North America. Another species of the same genus, *S. ligulata* which generally was also thought to be a "copper moss", turned out to be not so (Shaw and Anderson, 1988). From the above it is evident that much more research work must be done before the intricate ecophysiological position of the "copper mosses" can be fully understood.

Another feature which should be emphasized is the ability of many bryophyte species to accumulate heavy metals in extremely high concentrations (for example Lee et al., 1977; Sarosiek et al., 1978; for more references see also Maschke, 1981). LeBlanc et al. (1974) made an interesting comparison between the accumulation capacity of some vascular plants and the mosses *Hylocomium splendens* and *Pleurozium schreberi* originating from the copper mine area at Murdochville, Canada. Especially *H. splendens* turned out to be a very good accumulator of Pb, Cd, Cu and Zn. For example, at the most polluted location they found a lead concentration of 17 320 ppm in the moss *H. splendens*, whereas the corresponding values in the *Picea* and *Clintonia* species were 349.5 and 548.5 ppm, respectively. In aquatic environments the moss *Fontinalis antipyretica* is often used as a test plant to analyze its element content. Dietz (1972) investigated this moss in the Ruhr river, Germany, and found concentration factors (=ppm moss/ppm milieu) of 3200 for lead and 9400 for zinc. Similar results were reported by Kovács et al. (Kovács and Podani, 1986) for Hungary and by Empain (1977) for Belgium. An illustrative example was given by Empain (1988) who investigated several moss species along the polluted Sambre river in Belgium. Thus, by plotting the copper concentration in the water against that in bryophytes (Fig. 20), one obtains a clear picture about this accumulation phenomenon. Furthermore, it is shown that the copper concentration in the mosses is correlated with the copper concentration in the water. However, there is a large ratio between these concentrations. Practically, this means that analytical estimates

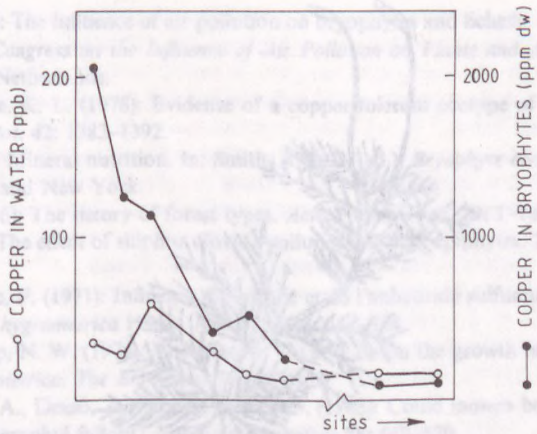

Fig. 20 — Comparison of copper concentrations in water (white dots) and aquatic mosses (black dots) of the River Sambre — Belgium) (After Empain, 1988).

with mosses are higher, by more than 3 orders of magnitude, compared with the trace levels in the water. Consequently, aquatic bryophytes are very useful for monitoring heavy metal pollution.

The ability of bryophytes to accumulate elements in extremely high concentrations may also facilitate the detection of elements present in very low concentrations in the environment. In some common bryophyte species Shacklette (1965) found very rare elements such as Ag, Bi and Sn, which were undetectable in the substrate. This specific property is particularly important in case of elements that may cause severe damage at very low concentrations. In this context we mention cadmium, which is thought to have a carcinogenic effect on organisms.

A further benefit for species which accumulate heavy metals is that they enable us to follow changes over a certain period. By examining herbarium specimens we can describe the level of pollution in the past, or at least we can make comparisons. An interesting comprehensive retrospective was performed by Rao et al. (1977). They determined the amounts of heavy metals inmosses from Mount Royal, Montreal, Canada, from the beginning of our century onwards. They observed a steady, and sometimes even dramatic increase of heavy metal amounts over this period, most likely caused by urbanization and industrialization. A specific problem involved with retrospective studies is to ascertain whether the compared herbarium specimens do originate from the same locality. Therefore, it is recommended to use only specimens which undoubtedly came from the same site. The establishment of an "environmental specimen bank" might be a step into the right direction. In this respect a very elegant example was given by Johnsen and Rasmussen (1977) who studied the epiphyte *Pterogonium gracile* not only from the same locality but even from the same sporosphyte!

A special case of retrospective investigations is the determination of heavy metal levels in peat profiles (e.g. Lee and Tallis, 1973; Pakarinen and Tolonen, 1976, 1977). Important considerations in this context are the homogeneity of the deposit and its degree of decomposition. Homogeneous deposits are preferable for making com-

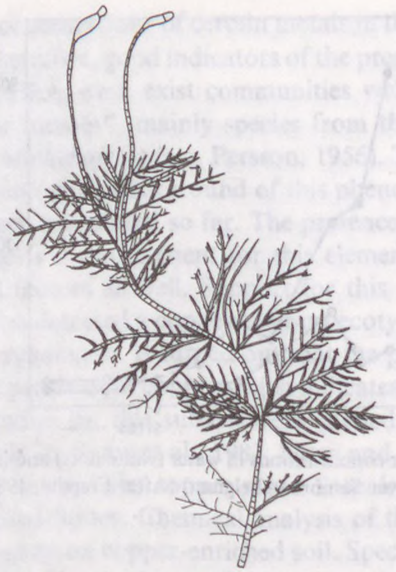

Fig. 21 — The moss species, *Hylocomium splendens*.

parisons, and the better decomposed the peat the less chance of metals leaching downward in the peat profile.

Especially useful for assessing the quality of environment are moss species that have a layered habit and produce distinct annual segments. An often utilized test plant for this is the moss *Hylocomium splendens* (Fig. 21). On the basis of the analysis of these annual segments of this species, Mäkinen (1987) provided detailed information on the pollution of a power station in Finland between 1978–1981. Also, most biomonitoring studies on heavy metal pollution in the Nordic countries have been performed with *H. splendens* (see Rühling et al., 1987).

In this chapter some attention should also be paid to the actual phenomenon of acid rain. As a matter of fact, acid rain enhances the solubility of metal salts and increases their uptake by bryophytes (Brown, 1982). Compared to higher plants, however, surprisingly few published accounts have been available on this topic, most studies being carried out in the USA (Raeymaekers 1986, 1987; Raeymaekers and Glime, 1986) and Canada (Klein and Bliss, 1984; Rochefort, 1987; Rochefort and Vitt, 1988; Hutchinson and Scott, 1988). From all these experiments in turned out that simulated acid rain has a disastrous effect on the main physiological processes, as was reflected by reduced photosynthesis, lower biomass production and decreased sporophyte density.

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8 Herbaceous (flowering) plants

8.1 SENSITIVE INDICATORS

In addition to the various characteristics of species (genetic conditions, resistance), plant response also depends on the stage of development, physiological activity, the age and nutritional state of individual organs and on the ecological factors affecting plants. Depending on the concentration of gaseous pollutants and on the duration of the impact, the damage suffered by plants can be either acute or chronic. In the case of acute damage high concentrations of gaseous pollutants exert their impact for a short period. Characteristic external symptoms, e.g. necrosis (the dying off of leaf tissues) develop on the plant. Chronic damage refers to the long-term impact of low concentrations of gaseous pollutants. As a rule, the plants do not develop external symptoms (e.g. changes in colour). Should the pollutants accumulate in the leaves, this would damage cells: plant growth is retarded and leaf surface area cannot increase.

Some frequent gaseous pollutants
Sulphur dioxide

Larger quantities of sulphur dioxide are produced by the incineration (household and industrial heating, power stations, etc.) of sulphur containing fossil fuels (coal, oil). Sulphur dioxide is emitted by petrochemical works, iron and steel processing plants, cementworks, brick factories, the ceramic industry, glassworks and refuse burning plants, etc.

The symptoms of acute damage (above an SO_2 concentration of 1 ppm) can be observed in the form of necrosis located, both on the upper and lower surface of the leaves, at the apices, the margins and between the veins. The appearance of necrotic spots scattered over the entire leaf surface is frequent. The tissues that surround the stomata may decompose.

In the case of chronic damage (sulphur dioxide concentration above 0.5 ppm) the appearance of leaf chlorosis (acidification, whitish colour) is frequent. Both plant and leaf growth as well as plant production are reduced.

Table 18 — Sensitive and accumulating (A) indicators of air pollution (Bünau et al., 1979; Posthumus, 1980; Temmerman, 1979, 1980)

SO_2	
Anagallis arvensis	*Medicago sativa*
Aster bigelovii	*Phaseolus vulgaris*
Avena sativa	*Plantago major* (A)
Beta vulgaris	*P. lanceolata*
B. vulgaris var. *cicla*	*Poa annua*
Brassica oleracea var. *gemmifera*	*Raphanus sativus*
B. oleracea var. *acephala*	*Rheum rhaponticum*
Chelidonium majus (A)	*Secale cereale*
Cichorium endivia	*Solidago canadensis* (A)
Fagopyron esculentum	*Spinacia oleracea*
Gossypium hirsutum	*Trifolium repens* (A)
Helianthus sp.	*Triticum* sp.
Hordeum vulgare	*Trifolium* sp.
Lactuca sativa	*Verbena canadensis*
Lathyrus odoratus	*Viola* sp.
Lepidium sativum	*Zinnia elegans*
Lolium perenne (A)	

Table 19 — Concentrations of cadmium, lead, copper and manganese in plant material with and without simultaneous sulphur dioxide fumigation after a total application of 5.2 $mg \cdot m^{-2}$ Cd, 488 $mg \cdot m^{-2}$ Pb, 40.8 $mg \cdot m^{-2}$ Cu, 72.8 $mg \cdot m^{-2}$ Mn as a dust mixture (Krause and Kaiser, 1977)

Plant species	Treatment	Mean concentration of heavy metals in ppm*			
		Cd	Pb	Cu	Mn
Lactuca sativa L.	Control	0.8a	7.3a	4.8a	108.3
	Control + SO_2	0.8a	8.3a	4.2a	106.6
Leaf	Dust	12.0b	163.5b	32.7b	106.2
	Dust + SO_2	11.8b	173.8b	30.3b	110.0
Raphanus sativus	Control	0.7a	7.4a	8.4a	158.9
oleifera L.	Control + SO_2	1.0a	9.7a	7.9a	139.0
Leaf	Dust	23.2a	494.2b	60.4b	214.2
	Dust + SO_2	24.3b	391.3b	61.7b	184.9
Setaria italica L.	Control	0.4a	11.7a	8.9a	47.3
	Control + SO_2	0.7a	12.5a	8.3a	40.5
Leaf	Dust	20.8b	232.6b	34.9b	76.3
	Dust + SO_2	32.0b	261.9b	39.0b	109.7
Raphanus sativus	Control	0.5a	3.4a	nd a	21.2
radicula L.	Control + SO_2	0.3a	2.5a	nd a	17.9
Root	Dust	1.8b	45.6b	nd a	19.8
	Dust + SO_2	1.5b	39.1b	nd a	10.3

* Means followed by a different letter within one plant species differ significantly ($P < 0.05$), nd = not detectable

A number of plant species are suitable to indicate sulphur dioxide pollution. Their list is given in Table 18. Sensitive indicators are among others *Medicago sativa* var. *Du Puits* (Posthumus, 1983), *Lupinus sativus* (Steubing, 1978).

Owing to their chemical composition, the young leaves of the following cultivars of the genus *Petunia* are especially capable of indicating sulphur dioxide pollution: Capri, White, Magic, White Cascade (Elkiey and Ormrod, 1981). The following plant species under the impact of sulphur dioxide pollution (in the Northern Great Plains, Montana, USA) accumulated larger quantities of sulphur than in the unpolluted environment: *Andropogon scoparius, Agropyron spicatum, Artemisia frigida, A. tridentata, A. cana, Gutierrezia sarothraea* (Rice et al., 1984).

When loaded with sulphur dioxide some plants absorb higher quantities of heavy metals (e.g. *Lactuca sativa, Raphanus sativus oleifera, Setaria italica, Raphanus sativus radicula, Tagetes* sp.) (Krause and Kaiser, 1977; Table 19).

Fluorine compounds

The most frequent sources of fluorine (HF, F_2, H_2Si, SiF_4) emission are brick factories, power stations (by the burning of coal), iron, steel, aluminium and china industries, smelteries, glass factories, cementworks and refuse burners, fertilizer producing plants (phosphate), etc. When exposed to atmospheric humidity, gaseous fluorine immediately forms hydrogen fluoride (HF), an extremely toxic compound for plants.

The characteristic symptoms of fluorine damage are discoloration of leaves (chlorosis) that appear in the marginal and apical regions and expand to the inter-veinal areas. Later on the leaf turns brown (necrosis). Frequently, leaf length and leaf area are also reduced.

Table 20 — Sensitive and accumulating (A) indicators of air pollution (Bünau et al., 1979; Posthumus, 1980; Temmerman, 1979, 1980)

Fluorine	
Anagallis arvensis	*Lolium multiflorum* var. *italicum* (A)
Allium cepa	*Majanthemum bifolium*
A. porrum	*Narcissus poeticus*
Begonia tuberhybrida	*Paeonia officinalis*
Colchicum autumnale	*Phleum pratense*
Convallaria majalis	*P. pratense* (A)
Crocus sp.	*Polygonatum amphibium*
Dactylis glomerata	*P. odoratum*
Fagopyron esculentum (A)	*Silene vulgaris*
Fragaria vesca (A)	*Sinapis alba* (A)
Fresia sp.	*Trifolium* sp.
Gladiolus gandavensis (A also)	*Trifolium incarnatum*
Hypericum perforatum (A also)	*Tulipa gesneriana*
Hordeum vulgare	*var.* Blue Parrot (A also)
Iris sp.	*var.* Preludium (A also)
Lilium candidum	*Zea mays*

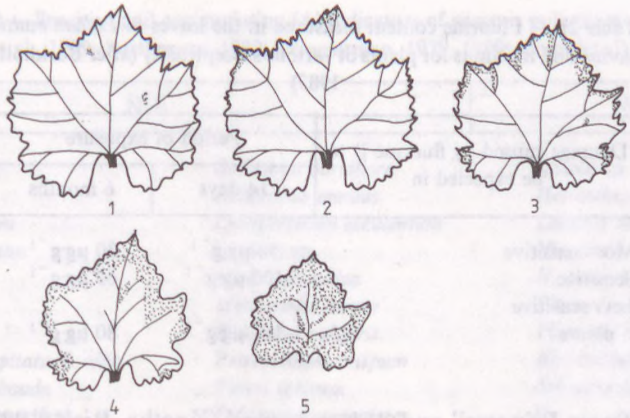

Fig. 22 — Fluorine necrosis of vine leaves, a 1–5 damage-scale (Arndt et al., 1984).

The gaseous forms of fluorine compounds penetrate through the stomata and the cuticle into the leaf. Having reached the intercellular spaces of the mesophyll, fluorine is translocated by the transpirational flow to the marginal regions of the leaf, where the transpiration rate is the highest.

The fluorine containing, water-soluble dust particles, that settle down on the surface of leaves, are dissolved by the atmospheric humidity. They then enter the leaf through the epidermis.

In polluted areas fluorine is also absorbed from the soil by the roots.

Fluorine pollution is indicated by numerous plants (Table 20). Grape is a rather susceptible crop plant. Based on the extent of leaf necroses, one can estimate the degree of pollution. Arndt et al. (1984; Fig. 22) have elaborated the following evaluation scale:

1) No recognizable damage. Slight apical or spot necrosis on every tenth leaf, at the most. There is no difference between the growth vigour of plants in the polluted and unpolluted areas.

2) Slight damage. Recognizable necrosis on every fifth leaf, on an average. The extent of each necrosis is approximately 2–3 cm^2. The growth of axillary shoots is normal, however, the growth of tendrils is limited.

3) Medium damage. On average every second leaf is necrotic. The growth of the entire plant is reduced.

4) Extensive damage. The assimilating surface of almost every leaf is reduced, and the entire marginal area is necrotic. Tendrils still occur, though in a partially shortened form.

5) Most extensive damage. Each leaf is damaged, abscission occurs, the internodes become shorter. The tendrils are stunted.

6) Total damage. More than 80% of the leaves are entirely necrotic. The axillary shoots and tendrils are missing. Growth is greatly reduced.

Gladiolus *(Gladiolus gandavensis)* is one of the most sensitive reaction type indicators. Its susceptibility to fluorine varies according to variety. The most sensitive

Table 21 — Fluorine content measured in the leaves of *Lolium multiflorum* and the limits for plants of various susceptibility (after Bockholt, 1987)

Damage caused by fluorine is to be expected in	Period of exposure	
	14 days	6 months
Most sensitive	50 $\mu g \cdot g^{-1}$	30 $\mu g \cdot g^{-1}$
Sensitive	100 $\mu g \cdot g^{-1}$	60 $\mu g \cdot g^{-1}$
Less sensitive plants	160 $\mu g \cdot g^{-1}$	80 $\mu g \cdot g^{-1}$

varieties are "Snow Princess" and "Flowersong" (Kostka–Rick, 1988). The various gladiolus varieties are not merely sensitive but also accumulating indicators. Increases in the extent of leaf damage are also accompanied by increasing fluorine accumulation (Steubing, 1978). Based on the necroses appearing on the leaves, as well as on the fluorine content of the plants, *Tulipa gesneriana* var. Blue Parrot and var. Preludium can also be considered as both sensitive and accumulating indicators. As compared with the unloaded (unpolluted) environment, under the impact of fluorine pollution (e.g. Northern Great Plains, Montana, USA) significantly higher amounts of fluorine can be detected in the following plant species: *Agropyron spicatum, Andropogon scoparius, Artemisia cana, A. frigida, A. tridentata* and *Gutierrezia sarothraea* (Rice et al., 1984). It can be determined on the basis of fluorine content measured in the leaves of *Lolium multiflorum,* what kind of crops can be grown with respect to fluorine sensitivity (Table 21). The degree of risk of damage to species can be determined and ranked with respect to the various classes of resistance. According to Scholl (1975), should the F content of *Lolium multiflorum* reach the value of 80 µg. g^{-1} after a 6 months' exposure period, it would mean that this degree of pollution is already detrimental to the cattle grazing in the pasture.

Nitrous gases

Emitters of the nitrous gases ($NO_x = NO_2$, N_2O_3, N_2O_4, NO) are fertilizer plants (nitric acid), vehicles (automobiles), power stations, the iron, steel and petrochemical industries, household and industrial heating and refuse burners. As with sulphur dioxide and hydrogen fluoride, the "acid" gases reacting with water form acids. Even short periods of exposure to nitrogen oxides cause wilting in cereals. On the leaves of dicots the appearance of reddish-brown or black-brown spots can be observed, and in a short time the leaves dry up. In certain plants (e.g. on the leaves of beans) white, transparent spots appear. In the case of chronic damage the mesophyll cells are reduced in volume.

The list of species susceptible to nitrous gases is given in Table 22.

Emitters of hydrogen chloride and chlorine are power stations (the burning of fossil fuels), soda, fertilizer, plastic and china factories, the glass industry, refuse burners (especially plastics), etc. Gaseous hydrochloric acid is not transported by air as great a distance as gases such as sulphur dioxide. With water vapour (atmospheric humid-

Table 22 — Sensitive and accumulating (A) indicators of gaseous pollutants (Bünau et al., 1979; Posthumus, 1980; Temmerman, 1979, 1980) (completed)

NH_3		NO_x
Allium sp.	*Galinsoga parviflora*	*Azalea* sp.
Apium graveolens	*Helianthus annuus*	*Helianthus annuus*
Arctium tomentosum	*Lycopersicum esculentum*	*Lactuca sativa*
Arrhenatherum elatius	*Lupinus* sp.	*Nicotiana glutinosa*
Artemisia vulgaris	*Medicago sativa*	*N. rustica*
Atriplex hortensis	*Mercurialis annua*	*Phaseolus vulgaris*
Brassica oleracea	*Phaseolus vulgaris*	*Plantago major*
B. oleracea var. *capitata*, f. *alba*	*Petroselinum crispum*	*Rhododendron* sp.
B. oleracea var. *sabauda*	*Pisum sativum*	*Spinacia oleracea* var. Subito
B. oleracea var. *gemmifera*	*Polygonum aviculare*	
B. oleracea var. *botrytis*	*P. persicaria*	*S. oleracea* var. Dynamo
Calendula officinalis	*Scorzonera hispanica*	
Calystegia sepium	*Sonchus asper*	
Cichorium intybus var. *foliosum*	*Solanum tuberosum*	
Convolvulus arvensis	*Tagetes erecta*	
Cucurbita maxima	*Taraxacum officinale*	
Cucumis sativus	*Trifolium* sp.	
Dactylis glomerata	*Tussilago farfara*	
Dahlia pinnata	*Urtica dioica*	
Daucus carota	*Viola tricolor*	

Table 23 — Sensitive and accumulating (A) indicators of air pollution (Bünau et al., 1979; Posthumus, 1980; Temmerman, 1979, 1980)

HCl	Cl_2
Beta vulgaris	*Allium cepa*
Begonia tuberhybrida	*Coleus* sp.
Calendula sp.	*Fagopyrum esculentum*
Fragaria vesca	*Helianthus annuus*
Lolium multiflorum var. *italicum* (A)	*Medicago sativa*
Lupinus luteus	*Nicotiana tabacum*
Medicago sativa	*Raphanus sativus*
Phaseolus vulgaris	*Stellaria media*
Prunus sp.	*Tulipa* sp.
Solanum lycopersicum	*Zea mays*
S. tuberosum	*Zinnia* sp.
Spinacia oleracea	
Stellaria media	
Vitis vinifera	
Zinnia sp.	

ity) it forms a dense fog which rapidly condenses on the ground. The impact of gaseous hydrochloric acid is similar to that of sulphur dioxide. In the case of acute damage, the appearance of marginal and apical leaf necroses can be observed. These soon cover the entire leaf surface. The damaged leaves abscind precociously. In the case of chronic damage, the chloroplast is decomposed, the cells plasmolyse. The list of species susceptible to hydrochloric acid is given in Table 23.

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8.2 INDICATORS OF PHOTOSMOG

In central Europe the so-called London type smog is frequent. It occurs at low temperature, when the sulphur dioxide content of air is high.

The Los Angeles type smog can be observed on dry, warm days of summer months, when the air contains the components of combustion gases, petrol and diesel oil in significant quantities.

Both types of smog develop only in the presence of a specific inversion.

The photosmog develops at locations with intensive circulation, in the environs of refineries and in petrochemical industrial districts. In contrast to sulphur dioxide and carbon monoxide, the so-called aggressive photo-oxidants develop under the impact of solar radiation, as a result of photochemical processes. The main components of oxidative smog are ozone, peroxi-acetyl-nitrate (PAN) and aldehydes. In the presence of sulphur dioxide the influence of photo-oxidants is enhanced.

For a long time it was believed that photosmog could develop only in Los Angeles or in cities lying in the same latitude. However, in consequence of increased photosmog due to heavy traffic and air pollution, this phenomenon has been observed in every part of the USA, in Israel, Greece, England, the Netherlands, Germany (in the

Rhine and Neckar valleys, in Stuttgart, Munich and Frankfurt) and in Yugoslavia (Zagreb).

In 1983, in certain regions of Budapest, the ozone concentration exceeded the permitted limit. The occurrence of photosmog can be expected not only in Budapest but also in the environs of Lake Balaton and certain industrial centres.

When the concentration of ozone and PAN exceed 0.04–0.05 and 0.014 ppm respectively, various plants suffer injuries, according to their susceptibility.

When the concentration of ozone and PAN exceed 0.04–0.05 and 0.014 ppm respectively various plants suffer injuries, according to their susceptibility.

The detrimental effect of photosmog on plants was first recognized in California, in the 1950s. In the 50s and 60s a plant disease was observed in numerous regions of the USA. The symptoms included necrosis at the top of needles, and necrotic spots on tobacco leaves.

Photosmog also damages agricultural and horticultural crops. In California the damage caused to crops by ozone exceeds 100 million dollars a year. The total financial loss caused by ozone is estimated to amount of 1–2 billion dollars in the USA (Skärby and Sellden, 1984).

Indicator plants

In plants the primary pollutants e.g. sulphur dioxide, fluorine, heavy metals can be detected by chemical analyses (accumulating indicators). Ozone pollution can only be detected by sensitive indicators.

Only those sensitive plants have a value in indication, the leaves of which develop coloured spots or other colour-change symptoms under the impact of photo-oxidants.

Frequently, the species itself is resistant and only one of its specific subspecies, cultivated variety, or cultivar is suitable for indication (Table 24).

On the basis of symptoms to be observed in plants, one can infer the presence of photo-oxidants and the degree of pollution. The following plant species are indicators of photo-oxidants:

Tobacco is a frequent indicator (Braun, 1977; Floor and Posthumus, 1977; Scholl and van Haut, 1977; Ro-Poulsen et al., 1980; Arndt and Linder, 1981; Mortensen and Weisberg, 1981; Rademacher, 1987). It is suitable for indication because:

– it is rather susceptible to air pollution;
– shows a relatively clear reaction to oxidants;
– the symptoms can be easily identified;
– the sensitivity of leaves is a function of leaf age, so that new damage can be distinguished from past damage. The formation of leaves is continuous in the course of the entire period.

Table 24 — Sensitive indicators of photosmog
(Bünau et al., 1979)

Dianthus caryophyllus	*Ph. vulgaris*
Glycine max	*Poa annua*
Nicotiana tabacum	*Spinacia oleracea*
Petunia hybrida	*Urtica urens*
Phaseolus coccineus	

Table 25 — Crops commonly affected by ozone, and typical symptoms (Krupa and Manning, 1988)

Plant	Foliar symptoms
Bean *(Phaseolus)*	browning and chlorosis
Cucumber *(Cucumis)*	white stipple
Grape *(Vitis)*	red to black stipple
Morning glory *(Ipomoea)*	chlorosis
Onion *(Allium)*	white flecks and tip dieback
Potato *(Solanum)*	grey fleck and chlorosis
Soybean *(Glycine)*	red-bronzing and chlorosis
Spinach *(Spinacia)*	grey to white fleck
Watermelon *(Citrullus)*	grey fleck

Bean *(Phaseolus vulgaris)* varieties "Pinta", "Hilds Maja", "Sanalac", ev. Gintebo and "Seafarer", "Tempo" are also suitable for indication (Steubing, 1978. Amiro et al., 1984).

Table 26 — Visual symptoms caused by ozone and SO_2 on the leaves of *Petunia hybrida*

0	no visible injury
1	1–10% of leaf area injured
2	10–25% of leaf area injured
3	25–50% of leaf area injured
4	50–75% of leaf area injured
5	75–90% of leaf area injured
6	90–99% of leaf area injured
7	100% of leaf area injured

As a result of ozone pollution light yellowish-green, necrotic, frequently reddish-brown pigmented flecks appear on the upper surface of leaves, within a very short period of time (Kohut and Laurence, 1983; Krupa and Manning, 1988) (Table 25).

Bean plants are also susceptible to peroxi-acetyl-nitrate pollution. On the lower surface of young leaves silvery-white coloured flecks can be observed with a metallic lustre (Bünau et al., 1979).

In *Petunia* species (*Petunia hybrida, P. nyctaginiflora, P. multiflorum*) and varieties ("Snowstorm", "White Joy", "Blue magic", "Red magic", "White magic", "White cascade", "Capri") ozone pollution brings about the appearance of silvery-white coloured, bright flecks on the lower surface of leaves (Elkiey and Ormrod, 1979).

The above mentioned species are also suitable for the indication of SO_2, i.e., they indicate these two pollutants simultaneously. Following a 3-day period of exposure, the symptoms appearing on the leaves are evaluated on the basis of a 7-grade scale (Table 26).

The first damage caused by atmospheric O_3 to the leaves of *Petunia hybrida* cv. "White ensign" appears at a concentration of 0.20 ppm (Nouchi et al., 1984, Table 27).

The garden carnation *(Dianthus caryophyllus)* indicates low concentrations of ozone (0.05–0.09 ppm). Characteristic symptoms are: apical necrosis and tiny buds.

Table 27 — Effect of 4-hour simultaneous exposures to ozone and PAN at various concentrations on *Petunia* plants (Nouchi et al., 1984)

PAN concen-tration, ppm	Type of injury	Percentage of leaf injury*				
		0	0.10	0.20	0.30	0.40
0	ozone	0	0	9+8	26+8	47+27
	PAN	0	0	0	0	0
0.010	ozone	0	0	1+1	9+7	46+15
	PAN	0	0	0	0	0
0.020	ozone	0	0	0	7+4	35+24
	PAN	34+12	22+9	16+7	1+1	0
0.030	ozone	0	0	0	7+5	18+10
	PAN	43+14	27+10	26−14	3+7	0
0.040	ozone	0	0	0	0	11+7
	PAN	56+15	49+	48+13	36+16	11+7

* Data represent the mean and standard deviation of leaf injury for each exposure based on 8 plants (4 plants at a time for 2 replicates). Ozone injury was evaluated on the upper surface of leaves while PAN injury was evaluated on the lower surface.

Poa annua responds primarily to peroxi-acetyl-nitrate. It is less susceptible to ozone. The symptoms of pollution appear in the form of transverse, light coloured stripes (Claussen, 1975).

The cotyledons of soybean *(Glycine max)* respond sensitively to ozone pollution.

The leaves of spinach (*Spinacia oleracea,* var. Matador) manifest symptoms comparable to those observed on tobacco, although spinach is less sensitive (Floor and Posthumus, 1977).

Tomato *(Lycopersicum esculentum)* is rather susceptible to ozone; its symptoms are similar to those of tobacco.

Nettle *(Urtica urens)* is equally responsive to ozone and peroxi-acetyl-nitrate. Light coloured elongate necrotic flecks appear on the leaves (at the margin, later on on the lower surface). The 3rd and 4th leaf pairs are especially susceptible (Posthumus, 1983; Rademacher, 1987; Cornelius and Markan, 1984).

In the so-called fumigation experiments (4000 ppb O_3, temperature 23–30 °C, relative humidity 50–60%) lasting 4 hours, the leaves of the 6-week-old *Urtica urens* suffered 75–100% damage.

According to recent investigations ozone is also responsible for the deterioration of various forest tree species (Naveh et al., 1980; Noble and Jensen, 1980; Arndt et al., 1982; Ashmore et al., 1985).

On the leaves of susceptible conifers ozone pollution causes the appearance of whitish-grey, frequently silvery-white coloured spots. Later on these merge. The appearance of needlepoint sized necroses can also be observed, mainly on the upper leaf surfaces. Later on the point necroses merge and also expand downwards to the lower surface.

In the case of *Pinus strobus,* even low concentrations of ozone bring about the appearance of silvery-white to yellowish flecks on the stoma-bearing side of the needle. At higher concentrations the top of the needle dies and a red-brown, later a grey colour develops. Needles of the current year indicate acute damage, whereas the

Table 28 — Incidence of foliar oxidant symptoms on woody vegetation in New Jersey and southeastern Pennsylvania, 1973–1979 (Rhoads et al., 1980)

Ailanthus altissima	*P. trichocarpa* x *maximowicyii*
Cornus florida	*Prunus serotina*
Crataegus crusgalli	*Rhus radicans*
Fraxinus americana	*Tilia americana*
Liquidambar styraciflua	*T. europea*
Morus alba	*T. heterophylla*
Pinus strobus	*T. petiolaris*
Platanus x *acerifolia*	*Vitis vinifera*
Populus tremuloides	*Zelkova serrata*

Table 29 — The effect of ozone on man and tobacco (Otto and Daines, 1969; Theil, 1976, in Ehmke, 1982)

Short-term ozone concentration	Physiological effect on	
	man	tobacco (Bel-W-3)
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 spasms	the extent of flecks amounts to 50%
above 0.7	without physical effort respiratory problems occur	leaf fleck exceeding 90%
1–1.2	premature death of ill and aged persons	decay of plants

flecks caused by chronic damage can be observed on the older needles. *Pinus resinosa* (Bünau et al., 1979), *Pinus ponderosa* and *Pinus virginiana* (Smidt, 1978) are further examples of ozone sensitive species.

In southeastern Pennsylvania, Rhoads et al. (1980) observed damage from ozone pollution in 18 tree and shrub species (Table 28).

Syringa vulgaris L. is also considered to be a sensitive indicator of photosmog (Steubing, 1978).

Several plant species display a more sensitive response to photo-oxidants then man or animals. Based on the damage caused by ozone to the leaves of tobacco, we can estimate the state of atmospheric hygiene and the potential dangers menacing human health (Table 29).

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8.3 ACCUMULATING INDICATORS OF HEAVY METALS

The heavy metal impact can be detected mainly by means of accumulation indicators. According to recent studies, several, naturally occurring plant species seem to be suitable for this purpose.

Owing to their high resistance most ruderal plants are capable of accumulating larger quantities of pollutants (including also the heavy metals) without the appearance of external symptoms.

The ruderal plants are common and often cosmopolitan plants, therefore they are suitable for the comparative evaluation over wide areas.

In comparative studies it is important that samples be taken at the same time (suggested date: August).

Different plant organs contain different amounts of heavy metals. Highest quantities can be detected in roots and leaves, whereas stems, inflorescences and fruits contain only low amounts.

Root analysis is recommended, especially in those areas, where the soil contains higher amounts of heavy metals.

The leaf indicates both soil pollution and floating dust loads. The washing of plant samples is a frequently debated issue. Should we wish to determine the element content accumulating only in plants, it is necessary to apply washing.

Washing should be carried out for the same period of time, for example the author washes plants for half a minute. For samples used in so-called food chain studies (e.g. plants →herbivores) the washing should be omitted.

Indicator species

Lolium perenne and *L. multiflorum* are suitable as exposure (active) indicators. In parks, alongside the roads they are frequent. In addition to S and F they can also be used in the indication of heavy metals. The chemical composition of their leaves indicates the heavy metal load of a given area (Table 30).

Table 30 — Heavy metal content of soil and *Lolium perenne* ($\mu g \cdot g^{-1}$) at Nagytétény, Hungary

	1			2		
	Soil	Root	Leaf	Soil	Root	Leaf
Cd	1.9	3.5	2.5	16.5	0	0
Co	9.1	0	0	10.5	0.2	4.6
Cr	17.1	32.0	7.5	13.7	1.0	1.0
Cu	67.0	47.5	7.0	4030	94.3	60.0
Fe	26900	5350	1000	2700	—	626
Mn	1700	1700	148	992	62.7	38.9
Mo	486	6.0	0	107	10.5	15.8
Ni	28.4	16.0	12.0	41.2	4.0	3.0
Pb	171	85.0	13.5	4740	149	120
Zn	1270	337	67.5	9600	150	812

1 Dunaújváros: Fe-loading
2 Nagytétény: Pb- and Zn-loading

Table 31 — Pb content of *Lolium perenne* ($\mu g \cdot g^{-1}$) as a function of its distance from the emittant (battery manufacturing plant) at Sülysáp, Hungary

Plant organs	Distance from the battery manufacturing plant			
	50 m	100 m	150 m	1000 m
Ear	106	90	29	0
Leaf	1 034	345	103	14
Stem	68	23	9	0
Root	1 809	485	860	38

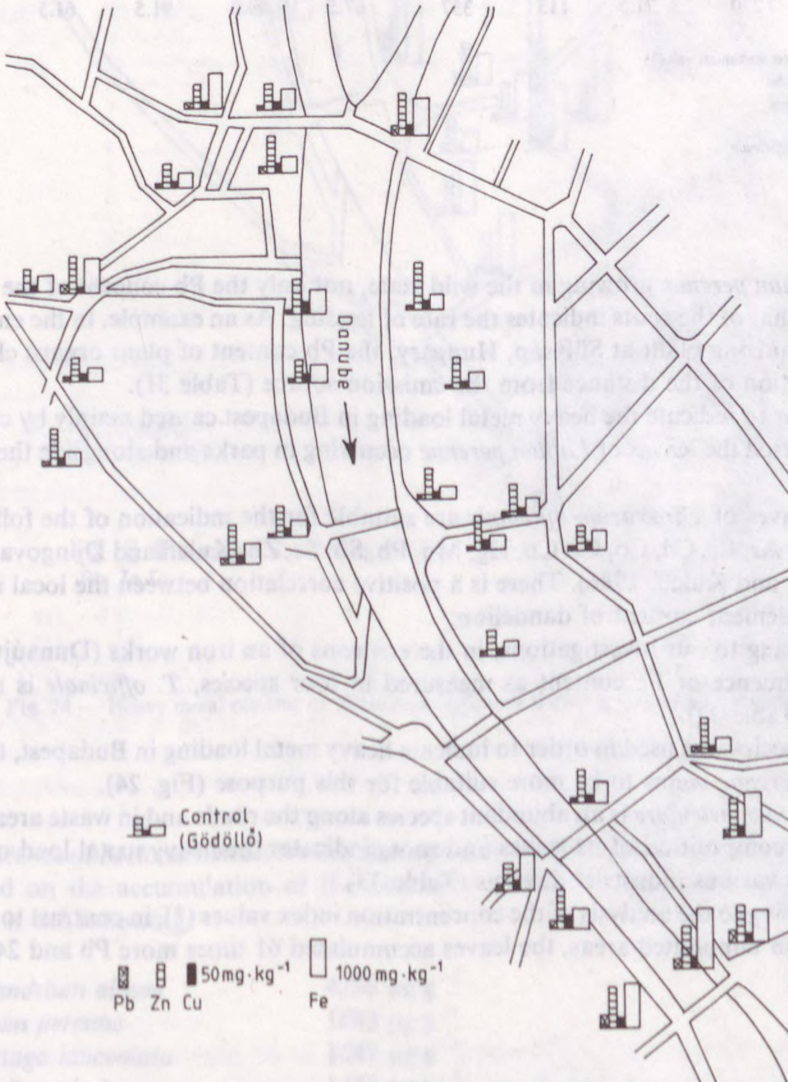

Fig. 23 — Heavy metal content of *Lolium perenne* leaves in Budapest, Hungary.

Table 32 — Heavy metal content of soil and weeds ($\mu g \cdot g^{-1}$) at Dunaújváros, Hungary (Fe-loading)

	S	1		2		3		4	
		a	b	a	b	a	b	a	b
Cd	1.9	25	3.0	3.5	2.5	3.0	3.0	2.5	9.9
Co	9.1	0	0	0	0	0	0	0	0
Cr	17.1	4.5	15.0	32.0	7.5	7.0	9.0	11.5	12.5
Cu	67.0	3.5	6.5	47.5	7.0	34.0	18.0	19.5	18.5
Fe	26 900	175	2 475	5 350	1 000	775	975	2 100	1 650
Mn	1 700	34.5	148	1 770	148	96.5	153	138	188
Mo	486	1.5	7.0	6.0	0	16.0	31.5	2.0	1.0
Ni	28.4	10.0	12.5	16.0	12.0	11.5	12.0	11.0	11.5
Pb	171	5.0	10.5	85.0	13.5	12.0	21.0	8.5	10.0
Zn	1 270	71.5	113	337	67.5	60.0	91.5	61.5	82.0

S: soil (measured maximum value)
1: *Lepidium draba*
2: *Lolium perenne*
3: *Reseda lutea*
4: *Taraxacum officinale*
a: root, b: leaf

In *Lolium perenne* growing in the wild state, not only the Pb concent of the leaves but also that of the roots indicates the rate of loading. As an example, in the environs of a galvanizing plant at Sülysáp, Hungary, the Pb content of plant organs changes as a function of the distance from the emission source (Table 31).

In order to indicate the heavy metal loading in Budapest caused mainly by circulation, we used the leaves of *Lolium perenne* occurring in parks and alongside the roads (Fig. 23).

The leaves of *Taraxacum officinale* are suitable for the indication of the following elements: As, Br, Cd, Co, Cr, Cu, Hg, Mn, Pb, Sb, Se, Zn (Kuleff and Djingova, 1984; Djingova and Kuleff, 1986). There is a positive correlation between the local impact and the element content of dandelion.

According to our investigations, in the environs of an iron works (Dunaújváros), in the sequence of Fe content as measured in four species, *T. officinale* is ranked second (Table 32).

This species was used in order to indicate heavy metal loading in Budapest, though *Lolium perenne* seems to be more suitable for this purpose (Fig. 24).

Polygonum aviculare is an abundant species along the roads and in waste areas. The chemical composition of its leaves and roots indicates the heavy metal load of road sides and various industrial districts (Table 33).

According to the analysis of the concentration index values (*), in contrast to plants growing in unpolluted areas, the leaves accumulated 61 times more Pb and 24 times more Zn.

* Concentration index (Ci) = the concentration of the element in the plant under loading divided by the concentration of the element in the "normal" plant (Cottenie and Verlov, 1984).

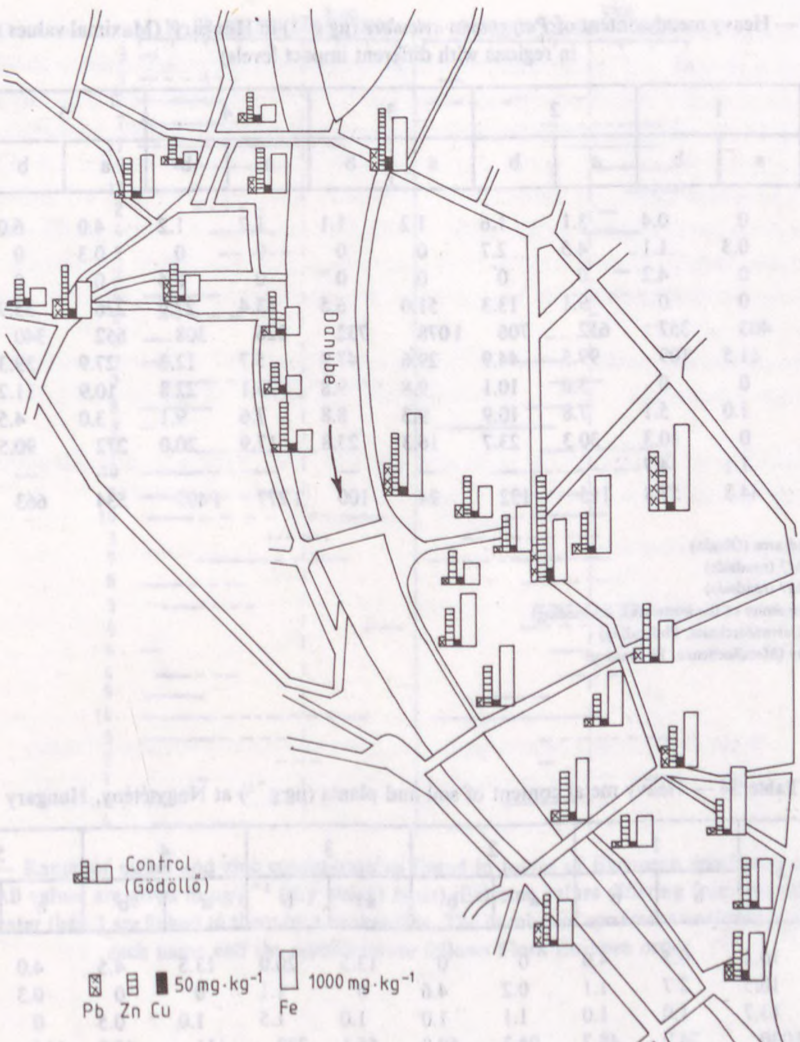

Fig. 24 — Heavy metal content of *Taraxacum officinale* leaves in Budapest, Hungary.

The leaves of *Melandrium album* are also useful for the indication of lead (Table 34).

Based on the accumulation of the 10 heavy metals measured, the sequence of species is the following:

Melandrium album	4286 $\mu g \cdot g^{-1}$
Lolium perenne	1683 $\mu g \cdot g^{-1}$
Plantago lanceolata	1547 $\mu g \cdot g^{-1}$
Lepidium draba	1437 $\mu g \cdot g^{-1}$
Polygonum aviculare	1190 $\mu g \cdot g^{-1}$

Table 33 — Heavy metal content of *Polygonum aviculare* ($\mu g \cdot g^{-1}$) in Hungary. (Maximal values measured in regions with different impact levels)

	1		2		3		4		5		6
	a	b	a	b	a	b	a	b	a	b	b
Cd	0	0.4	3.1	1.6	1.2	1.1	1.2	1.2	4.0	6.0	0.9
Co	0.3	1.1	4.5	2.7	0	0	0	0	0.3	0	1.4
Cr	0	4.2	0	0	0	0	0	0.4	0	0	2.1
Cu	0	0	9.1	13.3	51.0	6.5	53.4	27.9	226	35.9	84
Fe	403	357	612	706	1 078	732	226	308	652	340	2 348
Mn	11.5	109	99.5	44.9	29.6	47.9	5.7	12.3	27.9	39.3	110
Mo	0	0	3.6	10.1	9.8	9.8	23.1	22.8	10.9	11.2	0
Ni	1.0	5.1	7.8	10.9	9.3	8.8	7.6	9.1	3.0	4.5	4.1
Pb	0	10.3	30.3	23.7	16.3	23.8	17.9	20.0	272	90.5	631
V	1.1	4.7	—	—	—	—	—	—	—	—	
Zn	44.8	57.8	115	132	94	100	1 277	1 407	534	663	123

1: Unpolluted area (Óbuda)
2: Freeway M3 (roadside)
3: Freeway M7 (roadside)
4: Csepel (proximity of the ironworks, Zn-loading)
5: Sülysáp (Galvanotechnics, Pb-loading)
6: Nagytétény (Metallochemia, Pb-loading)
a: root
b: leaf

Table 34 — Heavy metal content of soil and plants ($\mu g \cdot g^{-1}$) at Nagytétény, Hungary

	S	1		2		3		4		5	
		a	b	a	b	a	b	a	b	a	b
Cd	16.5	0.5	4.0	0	0	13.5	20.0	13.5	4.5	4.0	6.0
Co	10.5	2.7	1.1	0.2	4.6	0	1.1	0	0	0.3	0
Cr	13.7	1.0	1.0	1.1	1.0	1.0	1.5	1.0	0.5	0	0
Cu	4 030	24.9	48.7	94.3	60.0	65.1	233	111	35.9	116	35.9
Fe	2 700	312	545	—	627	339	797	534	797	652	340
Mn	992	17.1	89.8	62.7	38.9	23.4	88.5	31.5	22.9	27.9	39.3
Mo	107	26.0	31.0	10.5	15.8	23.2	20.7	23.2	26.7	10.9	11.2
Ni	41.2	2.0	7.0	4.0	4.0	8.5	9.5	8.5	4.5	3.0	4.5
Pb	4 740	53.5	70.5	149	120	214	483	214	123	272	90.5
Zn	9 600	310	639	150	812	2 540	2 610	2 270	532	534	663

S: Soil (maximum value measured)
1: *Lepidium draba*
2: *Lolium perenne*
3: *Melandrium album*
4: *Plantago lanceolata*
5: *Polygonum aviculare*
a: root
b: leaf

Members of the family *Caryophyllaceae* seem to be useful for indicating heavy metal loading (e.g. *Silene cucubalus*) (Lolkema et al., 1986 and our investigations, see below).

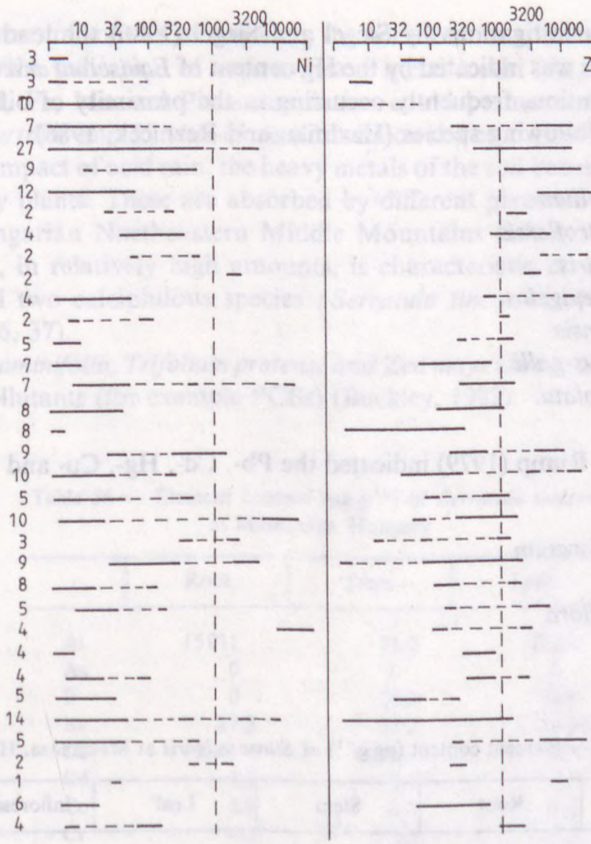

Fig. 25 — Range of nickel and zinc concentrations found in leaves of European specimens of *Thlaspi* species. All values are given in $\mu g \cdot g^{-1}$ (dry weight basis). Extreme values differing from the others by a factor greater than 3 are linked to them by a broken line. The number of specimens analyzed is given after each name and the nomenclature follows Flora Europea order.

Members of the genus *Thlaspi* (as hyperaccumulators) are especially suitable to indicate Ni and Zn loading (Fig. 25).

Species living in serpentine soils and in substrates rich in Ni are capable of accumulating even 1000–3500 $\mu g \cdot g^{-1}$ Ni (Reeves and Brooks, 1983).

Solidago canadensis can be used in the indication of lead and fluorine loads. As an example, near highways its leaves contained 110 $mg \cdot kg^{-1}$, whereas 6.4–21.8 $mg \cdot kg^{-1}$ lead was measured farther away (Faensen et al., 1977, Rebele, 1986).

In Berlin, Rebele (1986) used the following species in the indication of the heavy metal loading of various areas:

Artemisia vulgaris
Calamagrostis epigeios
Chelidonium majus
Plantago major
Poa annua.

According to investigations by Siegel and Siegel (1986) the load caused by the volcano St. Helens was indicated by the Hg content of *Equisetum arvense* shoots, too.

Vanadium pollution, frequently occurring in the proximity of oil refineries, was indicated by the following species (Hartman and Reznicek, 1986):

Achillea millefolium
Amaranthus retroflexus
Artemisia vulgaris
Calamagrostis epigeios
Conysa canadensis
Echinochloa crus-galli
Plantago lanceolata.

Höllwarth and Rump (1979) indicated the Pb-, Cd-, Hg-, Cu- and Zn-load by the following species:

Hypericum perforatum
Hedera helix
Impatiens parviflora
Urtica dioica.

Table 35 — Element content ($\mu g \cdot g^{-1}$) of *Silene vulgaris* at Mátraháza, Hungary

	Root	Stem	Leaf	Inflorescence
Al	4899	155.1	685.8	252.5
As	0	0	0	0
B	9.9	11.5	40.6	22.6
Ba	116.6	63.0	83.0	20.1
Ca	9419	4623	20302	5537
Cd	1.3	0.5	0.8	0
Co	1.7	0	0.3	0
Cr	2.2	0	0.8	0
Cu	4.9	1.7	3.8	3.2
Fe	3520	125.5	482.3	303.3
Ga	5.5	0	0	0
K	9790	27444	53581	16147
Li	2.6	0	0.5	0
Mg	1653	1557	7731	2679
Mn	472.8	77.7	512.6	115.1
Mo	0	0	0	0
Na	115.2	153.7	123.6	121.8
Ni	2.0	0	0.5	0.3
P	1311	1643	2302	1479
Pb	9.4	3.4	4.5	3.2
Se	0	0	0	0
Si	175.8	171.7	304.6	267.2
Sr	48.3	25.6	55.8	20.2
Ti	155.4	3.5	12.7	3.7
V	8.4	0	0.6	0
Zn	66.6	34.7	43.0	22.2

In the environs of sources of emission, the herbaceaous species of forests can also be applied in the indication of various heavy metal loads. (As an example, near a zincworks Cd, Fe, Mn and Pb accumulation could be measured in the leaves of *Vaccinium myrtillus* and *V. vitis-idaea* (Czuchajowska et al., 1980).

Under the impact of acid rain, the heavy metals of the soil become both mobile and absorbable by plants. These are absorbed by different plants in different amounts.

In the Hungarian Northeastern Middle Mountains (Mátra) the occurrence of heavy metals, in relatively high amounts, is characteristic of *Silene vulgaris (cucubalus)*, and two calciphilous species *(Serratula tinctoria, Waldsteinia geoides)* (Tables 35, 36, 37).

Solidago graminifolia, Trifolium pratense and *Zea mays* are accumulation indicators of organic pollutants (for example PCBs) (Buckley, 1982).

Table 36 — Element content ($\mu g \cdot g^{-1}$) of *Serratula tinctoria* at Mátraháza, Hungary

	Root	Stem	Leaf
Al	15011	71.2	222.0
As	0	0	0
B	0	20.3	54.0
Ba	87.2	57.2	43.65
Ca	6321	6078	16449
Cd	2.2	2.1	1.5
Co	5.8	0.3	0
Cr	4.5	1.3	2.2
Cu	12	4.9	7.9
Fe	10341	136.1	364.8
Ga	17.9	0	0
Hg	—	0	0
K	7745	31692	41397
Li	6.4	0	0.2
Mg	1590	1343	4952
Mn	764.9	174.9	227.3
Mo	0	0	0
Na	241.3	163.5	174.9
Ni	4.4	3.8	3.6
P	915.4	1057	1445
Pb	19.4	0	0
Se	0	0	0
Si	122.5	126.0	145.6
Sr	42.1	47.1	65.9
Ti	231.9	1.4	3.5
V	22.1	0.4	0.3
Zn	65.9	34.9	52.4

Table 37 — Element content ($\mu g \cdot g^{-1}$) of *Waldsteinia geoides* at Mátraháza, Hungary

	Root	Leaf		Root	Leaf
Al	3 894	671	Mg	3 238	7 580
As	0	0	Mn	487.9	277.5
B	16.8	45.7	Mo	0	0
Ba	104	63.6	Na	146	147
Ca	9 200	16 562	Ni	2.6	0.4
Cd	1.3	0.9	P	1 777	1 651
Co	1.2	0	Pb	7.6	4.2
Cr	2.7	0.9	Se	0	0
Cu	9.8	5.6	Si	116	228
Fe	2 456	551	Sr	49.2	58.4
Ga	4.2	0	Ti	62.9	12
K	8 910	23 033	V	5.5	1.0
Li	1.6	2.8	Zn	110	101

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8.4 THE EXPOSURE OF STANDARDIZED PLANT SPECIES

Both plants growing in the wild and cultivated species can be used as active indicators. In order to determine the emission load, plants grown under identical conditions (soil, humidity, temperature) are transplanted to the site of experiments for a definite period of time. At the end of exposure time (whose duration depends on the pollutant and plant species) the sensitive symptoms or elements accumulating are determined. Thus the extent of pollution can be estimated. *Lolium multiflorum* (varieties "Odstein", "Lema", Barnoldi") is one of the indicators used most frequently. This species, as accumulating indicator, is suitable in the detection of S, F and heavy metal pollution (Scholl, 1975, 1987; Denaeyer-de Smet, 1975).

The method of indication is as follows (Arndt et al., 1982, Kostka-Rick and Arndt, 1987): *Lolium* seeds are sown, into soil filled pots of 14–18 cm diameter, and grown for 6–8 weeks. The seedlings are watered with deionized water. Plants at a height of 8–10 cm are cut back to a height of 4 cm.

For exposure special containers are used (Fig. 26). The lower part of the container (with a basic area of 350 cm^2) is filled with nutrient solution (liquid nutrient).

Above this is placed the ceramic pot with the *Lolium* (darnel) plants inside. The nutrient solution (5 l) and the ceramic pot are connected by a glass fibre wick (lampwick) or by a ceramic plug incorporating a tube supplying water to the soil. At the experimental site the containers are fixed to a stand at a height of 150 cm. The exposure period normally lasts for 14 days. Should the investigation last longer, the containers with the turf are changed at 14 days intervals. It is also necessary to supplement the nutrient solution. The darnel plants growing in the ceramic pot are

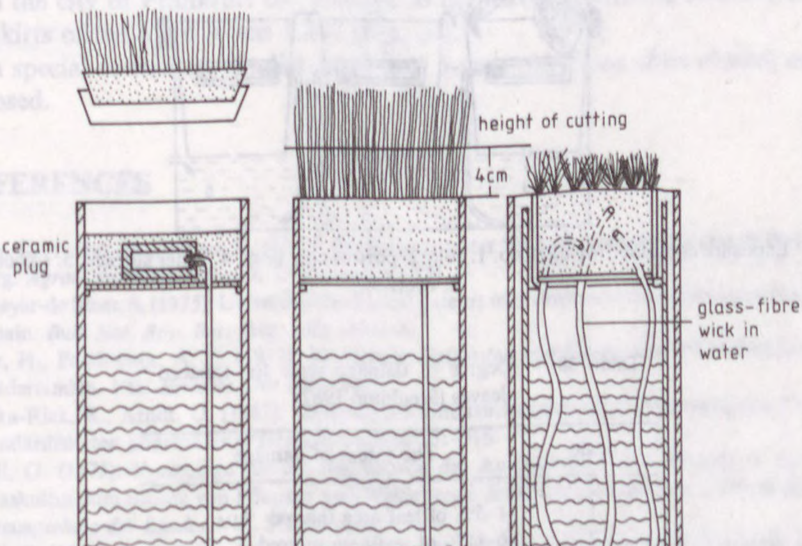

Fig. 26 — An exposure container for *Lolium perenne* (Arndt et al., 1982).

cut, dried and ground. The sample is suitable for the detection and indication of the following elements: sulphur, fluorine, chloride, lead, zinc, copper, cadmium, chromium, cobalt, mercury, nitrogen and vanadium.

Frequently, sensitive gladiolus varieties are also transplanted as indicators to the polluted area. 5–6 gladiolus bulbs are placed in each pot and covered by a 6 cm thick soil layer. Prior to the exposure the number of plants is reduced to four. The plants with 4–5 leaves are generally exposed for a period of 4 weeks. Necrosis appears at the apices and margins of the leaves. On the basis of this, leaf growth and the degree of damage are determined. The standard method for the indication of O_3 pollution is the following (Floor and Posthumus, 1977):

Variety applied: *Nicotiana tabacum* Bel-W-3 seeds are sown into a pot and placed in a climatic chamber (with an air temperature of 20–22 °C) for a period of 14 days. The seedlings are transplanted and kept at 20–22 °C in a glasshouse for 14 days. They are then transplanted into compressed peat pots or plastic flower pots, taking care not to damage the roots. The plantlets are kept in a glasshouse until exposure. The 6 (occasionally 7–8) weeks old, 5–6 leaved plants are transplanted to the site of exposure (Fig. 27). The young plants are susceptible to ozone and react to pollution with well defined symptoms. On the surface of tobacco plants spotlike, whitish, yellow-brown flecks, in a network-like array, appear. Later on the formation of parchment-like necroses can be observed. Mainly, the chlorophyll-rich palisade parenchyma is damaged. The exposure period is 4 weeks. At 4-weeks' intervals new plants are exposed.

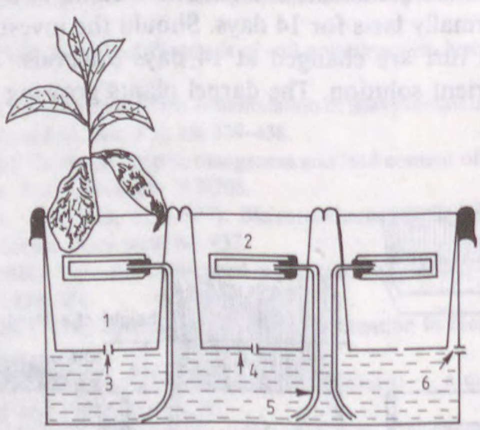

Fig. 27 — Exposure container for tobacco. 1. soil, 2. porous clay plug, 4. water surface, 5. silicon "slag", 6. overflow.

Table 38 — Degree of damage scale for tobacco leaves (Steubing, 1982)

Scale	The extent of damage
1	1–5% of leaf area injured
2	6–15% of leaf area injured
3	16–30% of leaf area injured
4	31–60% of leaf area injured
5	more than 61% of leaf area injured

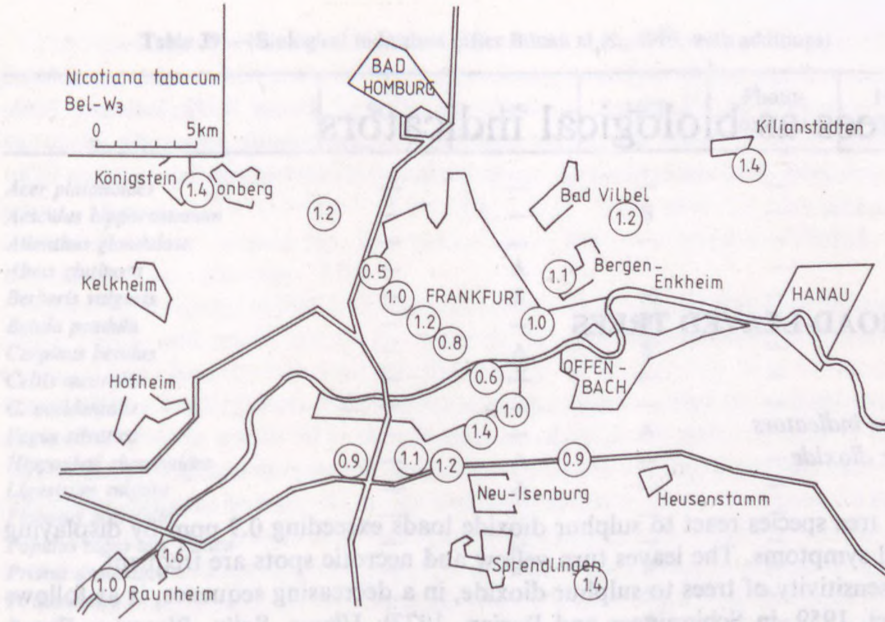

Fig. 28 — Mean values of the extent of leaf damage observed on the tobacco plants exposed, on the basis of the damage-scale. Frankfurt, 1. VI.–1. X., 1978 (Steubing, 1982).

At each location, the necrotic symptoms are determined on the basis of 6 + 6 plants. The evaluation is carried out using a degree of damage scale (Steubing, 1982; Table 38). This is calculated as follows:

$$\text{average damage per plant} = \frac{\text{number of damaged leaves}}{\text{total number of leaves}} \times 100$$

In the city of Frankfurt the damage to the leaves amounted to 0.7–1.2%, on the outskirts of the town it was 1.4% (Fig. 28).

In special containers smaller trees (e.g. 5-year-old *Picea abies* clones) can also be exposed.

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9 Trees as biological indicators

9.1 BROAD-LEAVED TREES

Sensitive indicators
Sulphur dioxide

Several tree species react to sulphur dioxide loads exceeding 0.9 ppm by displaying external symptoms. The leaves turn yellow and necrotic spots are frequent.

The sensitivity of trees to sulphur dioxide, in a decreasing sequence, is as follows (Wentzel, 1959, in Schinninger and Burian, 1977): *Ulmus, Salix, Platanus, Fagus, Alnus, Populus* and *Acer* species.

Betula pendula has a mediocre sensitivity to the direct impact of sulphur dioxide. Under the impact of higher sulphur dioxide loading (200 $\mu g \cdot m^{-3}$), annual shoot growth is reduced by fifty per cent. The extent of necrotic spots appearing on the leaves can be assessed by the help of a damage scale (Jäger, 1980; Fig. 29).

In resistant species the load caused by sulphur can be indicated also on the basis of the chemical composition of leaves. As an example, in Mediterranean areas laurel (*Laurus nobilis;* Alfani et al., 1983) is suitable for this purpose. Indicators of sulphur dioxide loading are summarized in Table 39.

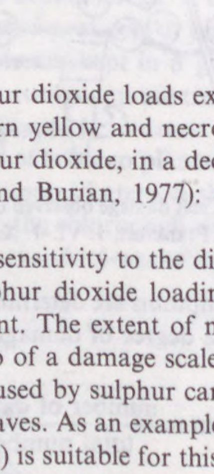

Fig. 29 — Damage-scale of the leaf necroses of birch *(Betula pendula)* (Jäger, 1980).

Table 39 — Biological indicators (after Bünau et al., 1979, with additions)

	SO_2	HF	HCl	Photo-oxidant	Heavy metals
Acer platanoides	—	—	—	—	A
Aesculus hippocastanum	—	—	S	—	A
Ailanthus glandulosa	—	—	—	—	—
Alnus glutinosa	—	A	—	—	—
Berberis vulgaris	—	S	—	—	—
Betula pendula	—	—	S	—	—
Carpinus betulus	—	A	S	—	A
Celtis australis	—	—	—	—	A
C. occidentalis	—	—	—	—	A
Fagus silvatica	—	—	A	—	A
Hippophaë rhamnoides	—	A	—	—	—
Ligustrum vulgare	—	A	—	—	—
Platanus acerifolia	—	—	—	—	A
Populus nigra ssp. *italica*	—	—	—	—	A
Prunus armeniaca	—	—	S	—	—
P. domestica	—	—	S	—	—
P. spinosa	—	—	S	—	—
Quercus robur	—	—	—	—	A
Ribes nigrum	—	A	—	—	—
Robinia pseudoacacia	—	—	—	—	A
Rosa rugosa	—	—	—	—	A
Salix fragilis	—	A	—	—	—
Sambucus nigra	—	S	—	—	A
Sophora japonica	—	S	—	—	A
Syringa vulgaris	—	—	S	—	—
Tilia cordata	—	S	S	—	A
T. tomentosa	—	S	S	—	S

A = accumulation indicator
S = sensitive indicator

Hydrogen fluoride

As a result of fluoride loading, the leaves of trees and shrubs turn scarlet, yellowish-red or brownish-red (Koeller, 1979). According to Horning and Mitchell (1982) Australian plants can be divided into the following groups of fluoride sensitivity:

– sensitive: visible injury, when foliar fluoride content is less than 100 $\mu g \cdot g^{-3}$ dry weight. For example Myrtaceae *Chamelaucium uncinatum;*

– intermediate: visible injury, when foliar fluoride content varies between 100 and 250 $\mu g \cdot g^{-1}$ dry weight. For example Myrtaceae: *Acmena smithii, Agonis flexuosa;*

– resistant: visible injury at foliar fluoride content above 250 $\mu g \cdot g^{-1}$ dry weight. For example Myrtaceae: *Acacia aulocarpa, A. implexa, Eucalyptus botryoides, E. camaldulensis, E. globulus, E. robusta, E. tereticornis, E. viminalis, E. gomphocephalus.*

The *Eucalyptus* species enumerated are planted in the Mediterranean region.

Fluoride loading can be indicated also on the basis of the chemical composition of leaves. As an example, *Sambucus nigra* (Höllwarth and Rump, 1979; Rebele, 1986), and *Syringa vulgaris* leaves are suitable for this purpose. In the proximity of aluminium works, the fluoride content of leaves varies as a function of the distance from the emittant.

Accumulative indicators
The detection of heavy metals

In the environs of certain industrial plants and of high density road traffic larger amounts of heavy metals are introduced into the air. The leaves absorb the heavy metals either directly from the air or from the soil, by way of root absorption. The chemical composition of leaves varies with their age. At the end of summer, the heavy metal content is generally higher; therefore this seems to be the appropriate period for sampling. Under the impact of these pollutants, a decrease can be observed in the rate of tree growth and leaf size which occasionally can result in precocious leaf fall.

The sensitivity of trees to heavy metal loading is as follows:

Most sensitive:

Betula pendula, Fraxinus excelsior, Sorbus aucuparia, Tilia cordata, Malus domestica.

Table 40 — Average concentration of elements in the leaves of *Fagus silvatica* (as % of the starting value) in various years (values of the pilot sampling in 1971/73 = 100%) (Keller, 1986)

	1971/73	1977		1984/85	
	ppm	ppm	%	ppm	%
Cl	1 500	24 100	1 607	18 850	1 258
Al	62	74	121	70	114
Ca	12 268	15 661	128	10 867	89
Cd	0.07	0.58	830	0.09	128
Co	0.20	0.31	150	0.16	80
Cr	1.51	1.80	120	1.12	74
Cu	7.31	7.84	107	7.02	96
Fe	121	135	112	150	124
K	7 022	6 294	89	6 295	90
Na	25	45	180	25	100
Ni	2.25	2.88	128	1.71	96
P	974	961	99	912	94
Pb	7.5	19.5	260	4.7	63
S*	853	1 013	118	829	85
Sb	0.33	2.2	670	0.34	103
Sn	0.35	2.3	660	0.40	114
V	0.52	0.98	189	0.56	108
Zn	27	46	171	27	100

* SO_4–S

Accumulating indicators are mainly species with possible resistance, e.g. *Ailanthus glandulosa, Celtis occidentalis, C. australis, Elaeagnus angustifolia, Koelreuteria paniculata, Populus canadensis, Robinia pseudoacacia, Salix alba, Sophora japonica, Tilia tomentosa, Thuja occidentalis, Sambucus nigra.*

The following species are also regarded as accumulating indicators: *Carpinus betulus, Quercus robur* (Lerche and Breckle, 1974), *Fagus silvatica* (Sawicka, 1987), *Acer saccharum, A. platanoides, Quercus palustris, Platanus xavecifolia* (Smith, 1972), *Carpinus betulus* (Thomas, 1977).

According to Keller (1980, 1986) heavy metals can be indicated by tree species with a large crown and broad leaves. He reported the element content of *Fagus silvatica* measured in a clean environment and the subsequent accumulation of elements; thus indicating the loading both before and after the functioning of a refuse treatment works (Table 40).

Tree leaves in large towns as well as in their environs contain significantly larger quantities of heavy metals, than do the trees in rural areas (compare Table 41). According to studies these are suitable for the detection of heavy metal loading: *Ailanthus glandulosa, Aesculus hippocastanum, Tilia tomentosa, Sophora japonica, Celtis occidentalis, Robinia pseudoacacia.*

In Brussels, the leaves of *Tilia platyphyllos* and *Platanus acerifolia* were analyzed in order to detect the presence of Cu, Co, Ni, Pb, Fe, Mn and Zn (Delcarte and Impens, 1976).

In leaves, Mn and Fe accumulation can take place as a result of higher ground water levels. In large towns, however, this might result from gas pipeline damage. Natural gas decreases the oxygen content of soil. Soilborne bacteria derive their oxygen from the reduction of manganese dioxide, the Mn^{2+} is in turn absorbable for plants.

Heavy metals and various micro- and ultramicroelements, are most readily indicated by *Rosa rugosa* (Table 42). Owing to the morphological properties of its leaves, it is one of the most efficient dust absorbing plants. As a consequence of this, its leaves contain large numbers and relatively large amounts of rare elements. It is suitable for the detection of such rare elements as uranium, thorium, bismuth, wolfram, circonium, and the lanthanoids (La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy; Fig. 30).

Table 41 — Heavy metal content (Fe, Mn, Pb, Zn, Cu) in tree leaves ($\mu g \cdot g^{-1}$) in an urban and rural environment (Kovács et al., 1982)

	Urban environment		Rural environment
	average	maximum	average
Ailanthus glandulosa	1027	2112	190
Aesculus hippocastanum	874	1483	300
Tilia tomentosa	806	2458	240
Sophora japonica	754	2076	233
Celtis occidentalis	565	1239	214
Robinia pseudoacacia	596	1194	262
Acer platanoides	451	1101	289
Platanus acerifolia	399	660	193

Table 42 — Trees and shrubs indicating micro- and ultramicroelements based on the chemical composition of leaves

Ag	*Robinia pseudoacacia*
B	*Rosa rugosa, Acer campestre, Aesculus hippocastanum, Morus alba, Platanus hybrida, Salix alba, Sambucus nigra*
Bi	*Robinia pseudoacacia*
Ce	*Thuja occidentalis, Aesculus hippocastanum, Koelreuteria paniculata*
Co	*Sophora japonica*
Cr	*Koelreuteria paniculata*
Cs	*Rosa rugosa*
Dy	*Rosa rugosa, Thuja occidentalis*
Eu	*Rosa rugosa, Thuja occidentalis*
F	*Sophora japonica*
Ga	*Rosa rugosa, Thuja occidentalis*
La	*Rosa rugosa*
Mo	*Robinia pseudoacacia*
Nd	*Rosa rugosa, Thuja occidentalis, Aesculus hippocastanum, Sophora japonica*
Ni	*Rosa rugosa, Sophora japonica, Thuja orientalis, Aesculus hippocastanum, Koelreuteria paniculata, Morus alba*
Pr	*Thuja occidentalis*
Sb	*Thuja occidentalis, Aesculus hippocastanum*
Sm	*Rosa rugosa, Thuja occidentalis*
Sn	*Thuja occidentalis*
Tb	*Rosa rugosa, Thuja occidentalis*
Th	*Rosa rugosa, Thuja occidentalis*
U	*Rosa rugosa, Thuja occidentalis*
V	*Rosa rugosa, Thuja occidentalis, Robinia pseudoacacia, Aesculus hippocastanum*
W	*Robinia pseudoacacia*
Y	*Robinia pseudoacacia*
Zr	*Robinia pseudoacacia*

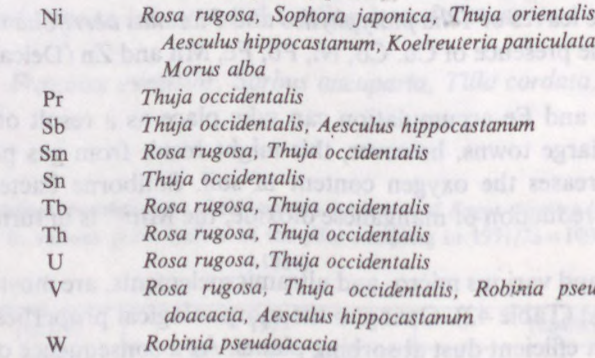

Fig. 30 — Occurrence of lanthanoides in the leaves of *Rosa rugosa*.

Radionuclides (plutonium-239–240) can be indicated by the leaves of beech, ash, oak, lime, plane and whitebeam (Dienstbach et al., 1983).

Various pollutants of organic origin (e.g. PCB) can be indicated by the leaves of *Populus tremuloides* and *Rhus typhina* (Buckley, 1982).

The indication of heavy metal load by Populus nigra

Populus nigra ssp. *italica* is suitable for the indication of heavy metal loading over a wide range of habitats (Wagner, 1984, 1987; Claussen, 1987).

This species has the following features:

– owing to its vegetative propagation it is genetically homogeneous;
– in the temperate belt it has a universal distribution;
– it is equally frequent in conurbations and agricultural areas;
– it has high ecological tolerance, being resistant to pollutants, de-icing salts and insect pests;
– it can be easily located and identified;

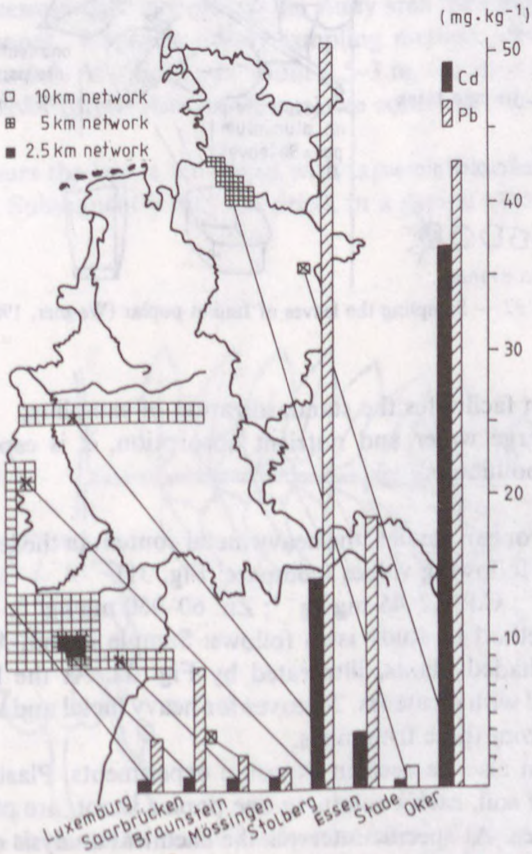

Fig. 31 — Occurrence of Cd and Pb in the leaves of Italian poplar (Wagner, 1984).

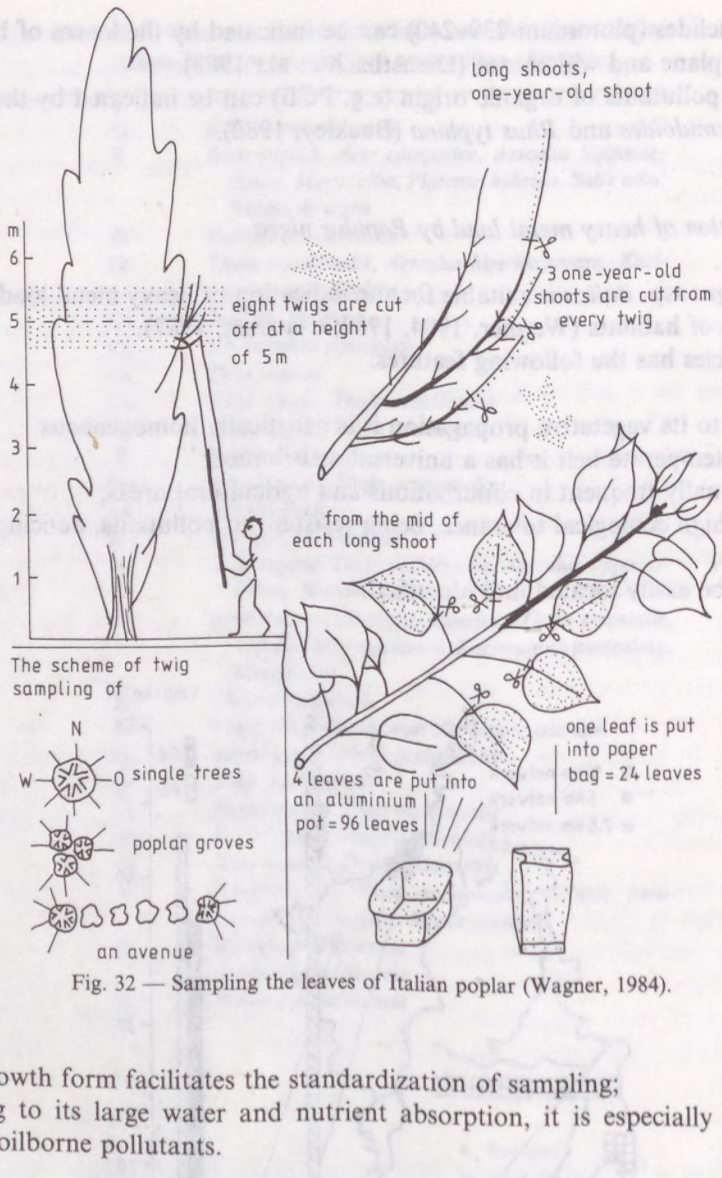

Fig. 32 — Sampling the leaves of Italian poplar (Wagner, 1984).

– its growth form facilitates the standardization of sampling;
– owing to its large water and nutrient absorption, it is especially suitable to indicate soilborne pollutants.

According to European studies, the heavy metal content in the leaves of this species varied between the following values (compare: Fig. 31):
Pb: 1–51 $mg \cdot kg^{-1}$; Cd: 0.2–45 $mg \cdot kg^{-1}$; Zn: 60–980 $mg \cdot kg^{-1}$.
The standard method of study is as follows: Sample in mid-August. If possible select solitary, unshaded plants, illustrated by Fig. 32. At the height of 5.5 m 8 branches are pruned with secateurs. 24 leaves for heavy metal and sulphur determination are collected from these trimmings.

Poplar clones can also be used in exposure experiments. Plastic bags filled with mixture of peat and soil, each containing one poplar shoot, are placed in the soil of the experimental area. At specific intervals the chemical analysis of leaves is carried out.

The indication of air pollution by Robinia pseudoacacia

In the indicator network to be established in Hungary and Central North Europe *Robinia pseudoacacia* (the black locust tree) is a recommended passive indicator species.

This species of North American origin was introduced to Europe in 1601, and in the 1700s to Hungary. It is now distributed throughout the entire country and occupies 18.2% of the total forest area. In Europe it can be found also in France, Germany, Switzerland, Italy, Austria, Czechoslovakia, Romania and the Soviet Union. It occurs also in the countries of Asia Minor, in Japan, China, South America, Africa, Australia and almost throughout the world (Keresztesi, 1988).

It is a readily regenerating species of high growth rate. It can be propagated by vegetative methods, so that genetically homogeneous plant material can be produced and be planted throughout the indicator network.

It grows in various soil types and under different climatic conditions. It is frequent in settlements and beside roads. It tolerates various air and soil pollutants (e.g. de-icing salts). Its leaves are good accumulating indicators (Fidora, 1972; Majerus and Denaeyer-de Smet, 1974; Kovács et al., 1986; Rebele, 1986; Rebele and Werner, 1987).

In *Robinia,* larger amounts of heavy metals can be detected in late August and early September. It is necessary that throughout the study area the sampling be carried out in a consistent manner. Wagner's (1984) sampling method, developed for Italian poplar, is recommended. At a height of about 2.5–3 m, the level of tree crown, 1 kg (fresh weight) of leaves (preferably sun-leaves) are collected into a plastic bag (Fig. 33).

Within 24–36 hours the leaves are rinsed with tap and then distilled water, for 30 seconds with each. Subsequently they are dried, in a drying oven, at approximately

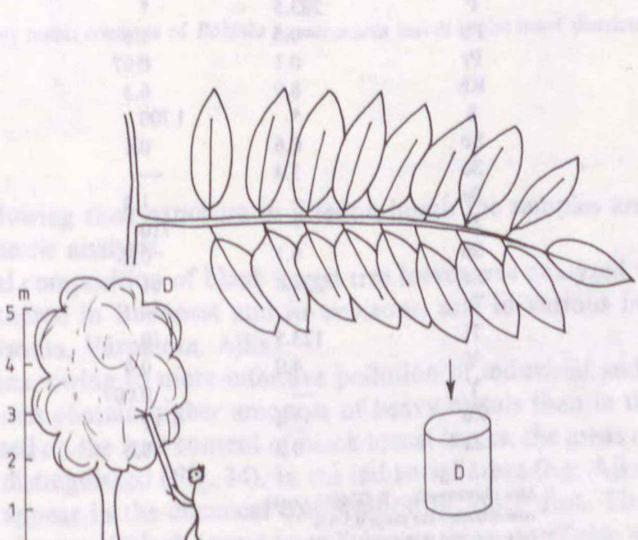

Fig. 33 — Sampling the leaves of black locust for heavy metal analysis. 3–4 twigs are collected at a height of approx. 3 m on the southern side of the tree. Approx. 1.5 kg pinnate leaves are needed.

Table 43 — Chemical composition of *Robinia pseudoacacia* leaves (μg·g^{-1} dry weight) (Analysis by the Laboratory for Mass-Spectrometry of KFKI AEKI, Budapest, Hungary, 1984)

	1	2
Ag	0.4	0.3
Al	34.4	110
As	0.3	3
Ba	4.4	6
Bi	—	3.4
Br	4.4	1.3
Ca	**	**
Cd	0.4	—
Ce	0.1	0.1
Cl	162	86
Cr	5.4	2.5
Cs	0.02	0.01
Co	0.4	0.9
Cu	72.9	35
Fe	*	280
Ga	—	15
I	—	—
K	*	4900
La	—	0.1
Mg	344.3	**
Mn	64.8	53
Mo	125.3	0.24
Na	22.3	93
Nb	0.2	0.003
Nd	—	0.2
Ni	10.1	3.5
P	283.5	*
Pb	0.5	1.6
Pr	0.1	0.07
Rb	8.9	6.3
S	*	1700
Sb	0.6	0.1
Sc	3.4	—
Se	—	1.3
Si	*	710
Sn	9.1	0.8
Sr	222.8	61
Te	—	—
Ti	123.5	10
V	4.0	0.4
Y	—	0.007
Zn	32.4	17
Zr	0.2	0.6

1: Ajka (downtown) — 2: Gödöllő (park)
* concentration the range 0.5–1%
** concentration in the range 1–10%

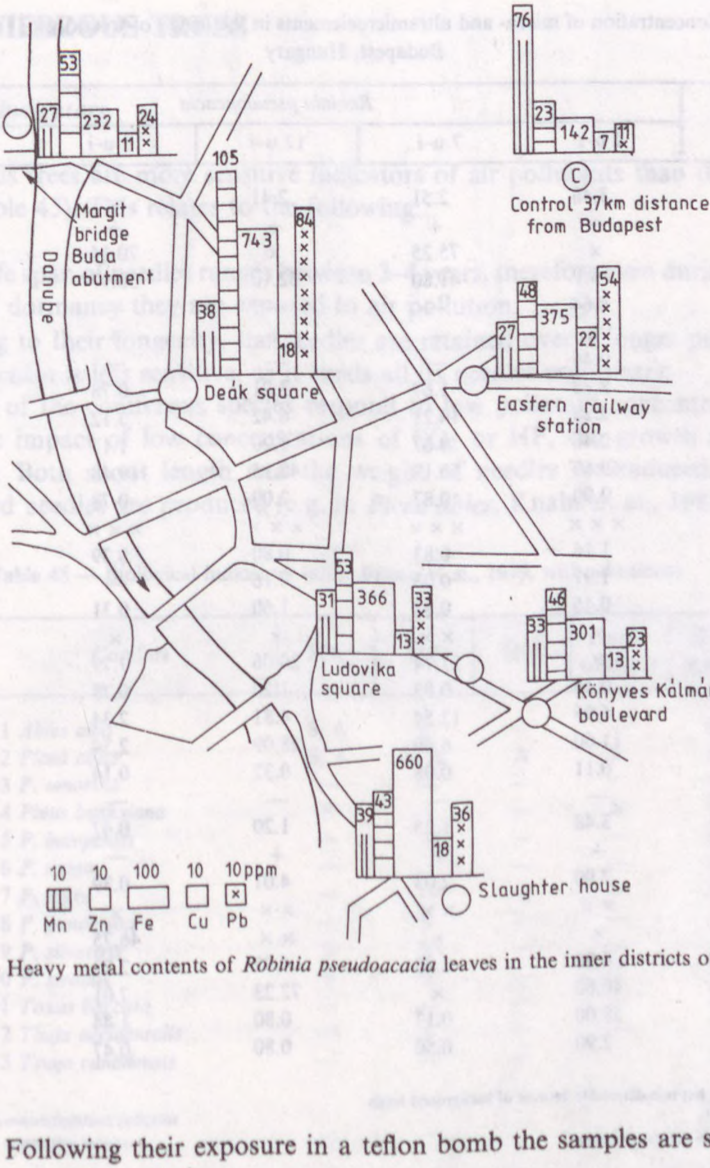

Fig. 34 — Heavy metal contents of *Robinia pseudoacacia* leaves in the inner districts of Budapest.

60–80 °C. Following their exposure in a teflon bomb the samples are submitted to spectrophotometric analysis.

The chemical composition of black locust tree leaves was analyzed by the authors in samples gathered in Budapest and its environs, and in various industrial areas (Gyöngyös, Visonta, Várpalota, Ajka).

In large towns, owing to more intensive pollution of industrial and traffic origin, the leaves of trees contain higher amounts of heavy metals than in the countryside (Table 43). Based on the lead content of black locust leaves, the areas of heavy traffic can be clearly distinguished (Fig. 34). In the industrial areas (e.g. Ajka), also several rare elements appear in the chemical composition of loose dust. They can be also detected in the leaves of black locust trees living in this area (Table 44).

In one of the industrial districts of Budapest, *Acer platanoides* and *Robinia pseudoacacia* grow side by side, near a candescent lamp factory. Relatively large amounts

Table 44 — Concentration of micro- and ultramicroelements in the leaves of trees (μg·g^{-1} dry matter) in Budapest, Hungary

	Robinia pseudoacacia				
	2 u–i	7 u–i	12 u–i	15 u–i	C
Ag	3.48	2.51	2.41	—	—
As	+	+	+	+	—
B	×	75.25	×	70.14	44.63
Ba	×	41.80	32.10	38.97	×
Bi	4.64	—	—	—	—
Br	+	+	+	+	—
Ca	0.46	—	—	—	—
Ce	2.32	1.67	3.21	0.78	0.48
Cr	9.28	14.21	6.42	3.12	1.41
Co	0.46	0.67	1.20	1.17	0.94
Cu	69.60	50.17	48.15	19.48	30.19
F	2.90	0.87	2.09	0.78	1.21
Fe	× × ×	× × ×	× × ×	× × ×	× ×
Ga	1.16	0.83	0.80	0.39	0.60
I	1.97	0.03	0.16	—	—
La	0.46	0.83	1.60	0.31	0.24
Mn	×	× ×	×	×	× ×
Mo	×	21.74	20.06	7.79	6.04
Nd	0.46	0.83	1.20	0.78	—
Ni	6.96	12.54	4.81	2.34	7.24
Pb	11.60	6.69	28.09	2.34	1.21
Pr	0.11	0.08	0.32	0.14	0.12
Rb	—	—	—	—	—
Sb	3.48	1.25	1.20	0.47	1.81
Se	+	+	+	—	—
Sn	2.90	2.03	4.01	0.39	0.60
Sr	× ×	× ×	× ×	×	× ×
Ti	×	×	× ×	46.75	72.46
V	4.06	2.92	1.52	2.73	1.81
Zn	40.60	×	72.23	7.01	21.74
W	58.00	0.17	0.80	1.44	—
Y	2.90	0.50	0.80	0.47	0.24

+ = present but non-detectable because of background levels
× = 0.1–0.2%
× × = 0.2–1.0% estimated values
× × × > 1.0%
C = control (rural areas)
u-i = urban-industrial areas
Sampling areas: 2 = Váci Road–Megyeri Road
7 = Erzsébet Street–Chinoin Street
12 = Istvántelki Street–Elem Street
15 = Rákospalota–Újpest Railway Station

of W (58 mg·kg^{-1}) and Zr (27.8 mg·kg^{-1}) were found in the latter's leaves. By contrast, leaves of *Acer plantanoides* contained 8 mg·kg^{-1} W and 2.5 mg·kg^{-1} Zr (compare: Table 44). Evidently *Robinia pseudoacacia* more readily accumulates these two elements.

9.2 CONIFEROUS TREES

Sensitive indicators

Coniferous trees are more sensitive indicators of air pollutants than the deciduous trees (Table 45). This relates to the following:

– the life span of needles ranges between 3–4 years, therefore even during the winter period of dormancy they are exposed to air pollution;
– owing to their longevity, the needles are retained over a longer period of time (*Larix decidua* is less sensitive, as it sheds all its needles each year);
– most of the coniferous species respond to low pollutant concentrations. Even under the impact of low concentrations of SO_2 or HF, the growth rate of trees decreases. Both shoot length and the weight of needles are reduced. Frequently malformed needles are produced (e.g. in *Picea abies,* Knabe et al., 1982).

Table 45 — Biological indicators (after Bünau et al., 1979, with additions)

Conifers	SO_2	HF	HCl	Photo-oxidants	Heavy metals
1 *Abies alba*	S, A	S	—	—	A
2 *Picea abies*	S, A	S, A	A	S	A
3 *P. omorika*	—	—	—	—	—
4 *Pinus banksiana*	S	—	—	S	—
5 *P. halepensis*	—	—	—	—	A
6 *P. mugo*	—	—	—	—	A
7 *P. nigra*	—	—	—	—	A
8 *P. ponderosa*	—	S	—	—	—
9 *P. silvestris*	S	S	—	—	—
10 *P. strobus*	S	S	—	S	—
11 *Taxus baccata*	—	—	—	—	A
12 *Thuja occidentalis*	—	—	—	—	A
13 *Tsuga canadensis*	—	—	—	—	A

A = accumulation indicator
S = sensitive indicator

Sulphur dioxide

Sulphur dioxide concentrations over 1 ppm in the air cause reddish-yellowish-brown discolouration at the needle tips. Beside the stomata water-saturated pitted spots appear. The needles fall, the crown becomes thinner, and growth is reduced.

The extent of chronic damage (when the sulphur dioxide content of the air exceeds 0.05 ppm frequently or for long periods) can normally be determined only on the basis of microscopic symptoms.

Occasionally chlorosis, the necrosis of certain leaf parts, as well as reduced production also occur. The extent of leaf damage can be determined, e.g. by means of a 6-grade scale of necrosis, too (Jäger, 1980; Fig. 35).

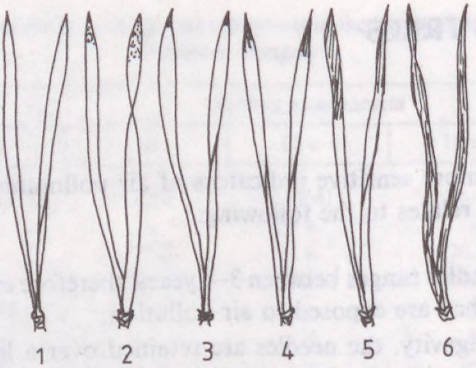

Fig. 35 — Damage values (from 1 to 6) for the heavy metal content of *Pinus silvestris* needles (Jäger, 1980).

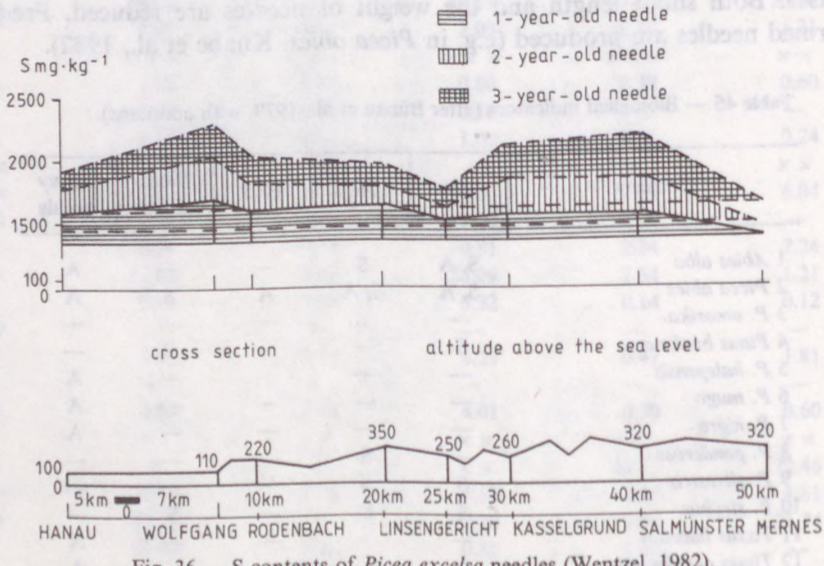

Fig. 36 — S contents of *Picea excelsa* needles (Wentzel, 1982).

The following species are sensitive to sulphur dioxide:
Abies alba, Picea excelsa, Pseudotsuga menziesii, Pinus silvestris, P. strobus, P. nigra, Biota orientalis, Larix decidua.

The sulphur, fluoride and heavy metal load can be indicated also on the basis of the chemical composition of needles.

In the needles of *Picea abies*, the sulphur content varies with the season, attaining higher values in October than in May. In the older needles the rate of accumulation is higher. Near industrial plants, the older needles of *Picea abies* contained 2000 ppm while the younger needles only 200–300 ppm sulphur.

Based on the sulphur content of pine needles, the extent of imission load can be determined for large areas (Wentzel, 1982; Fig. 36).

Fluoride loading, in addition to the symptoms (whitish-grey-reddish-brown necrotic spots), can be indicated also by means of chemical analysis, e.g. in the needles of

Picea abies (Rudolph and Halbwachs, 1983). Near sources of emission, the fluoride content of two-year-old spruce needles can reach 500 µg·kg^{-1} (Braun, 1974).

According to data by Taylor et al. (1982), in the USA the older needles of various *Pinus* species *(taeda, echinata, virginiana)* contained 20% more fluoride than the younger ones.

Accumulating indicators

Several coniferous species are accumulation indicators of heavy metals, for example *Taxus baccata* (Höllwarth, 1975, 1984; Lötschert and Grosch, 1984). In the course of sampling, care should be taken that only two-year-old needles be analyzed. By sampling successive years changes in heavy metal loading can be determined (Table 46).

Table 46 — Heavy metal content of the needles of *Taxus baccata*, in Darmstadt, between 1975–1982 (average values in mg·kg^{-1}) (Höllwarth, 1984).

	Pb	Cu	Cd	Ni	Cr	Hg
1978	45	16	0.31	4.5	1.4	0.07
1980	33	16	0.11	1.3	1.7	0.11
1982	29	9	0.13	2.5	2.2	0.00

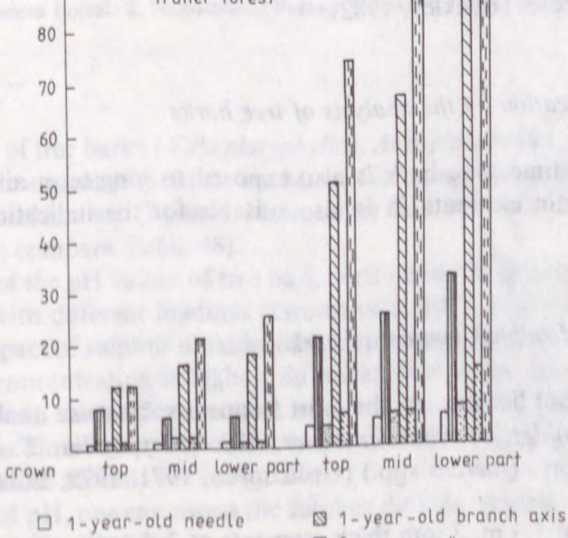

Fig. 37 — Pb contents of *Picea excelsa* leaves in a forest and along the Brenner motorway (Schinner, 1980).

Table 47 — Heavy metal content in the needles of *Picea abies* (μg·g^{-1}) in the Rhine–Main region (after Grünhage and Jäger, 1988)

		1972	1976	1982
Pb	Minimum	25	2.6	0.8
	Maximum	35.9	14.8	7.1
Cd	Minimum	0.11	0.15	0.17
	Maximum	0.92	1.72	0.55

The lead content of *Picea abies* needles changes proportionally with the extent of loading (Schinner, 1980; Fig. 37; Grünhage and Jäger, 1988; Table 47).

In the proximity of a lead glass factory the lead content of one-year-old *Picea abies* needles can be as high as 16.3 mg·kg^{-1} (Wandtner and Lötschert, 1978).

The conifers are suitable for the indication of not only air pollution, but also of heavy metal contamination of soil. However, the cation absorbing capacity of soils has also to be considered. In the soil of a spoil bank rich in heavy metals, the concentration of heavy metals accumulating had the following quantitative sequence: Zn, Cd, Cu, Pb (Hurrle, 1980).

With increasing soil acidity, as well as at low phosphorus and calcium content, the absorption of heavy metals increases.

Picea abies, Pinus silvestris and *Pseudotsuga menziesii* are suitable for the indication of iron, manganese, copper, lead, zinc, cadmium and silver.

In the Mediterranean regions, lead-loading can be indicated by the analysis of the needles of *Pinus halepensis* (Nakos, 1979).

PCB (poly-chlorinated-biphenyl) can also be indicated by the chemical analysis of *Pinus strobus* needles (Buckley, 1982).

Air pollution indication by the analysis of tree barks

In the course of time, tree bark is also exposed to long-term air pollution and it accumulates certain elements. It is also suitable for the indication of special pollutants.

The pH value and sulphur content of bark

Tree barks, without lichens, are the most frequent subjects of analysis (*Pinus silvestris, Acer platanoides, Fraxinus excelsior, Tilia platyphyllos, T. cordata, Carpinus betulus, Ulmus* spp., *Quercus* spp.) (Grodzinska, 1971, 1979; Lötschert and Köhm, 1979).

At the height of 1.3 m, 3 mm thick segments of 2–5 cm^2 surface are excised from the tree bark. 20 ml distilled water is added to the air-dried samples and, after shaking for 24 hours, the pH value is measured with glass electrodes.

Table 48 — pH values of tree barks in Frankfurt am Main, Germany, and in the Taunus Mountains (Köhm and Lötschert, 1972)

Locality	*Acer platanoides*	*Fraxinus excelsior*	*Tilia platyphyllos*
Centre of Frankfurt			
City	3.42	3.12	2.72
Grüneburg park	3.54	3.36	2.83
Taunus mountains			
Kronberg	4.10	3.83	3.39
Königstein	4.22	3.89	3.50
Falkenstein	4.35	4.21	3.74

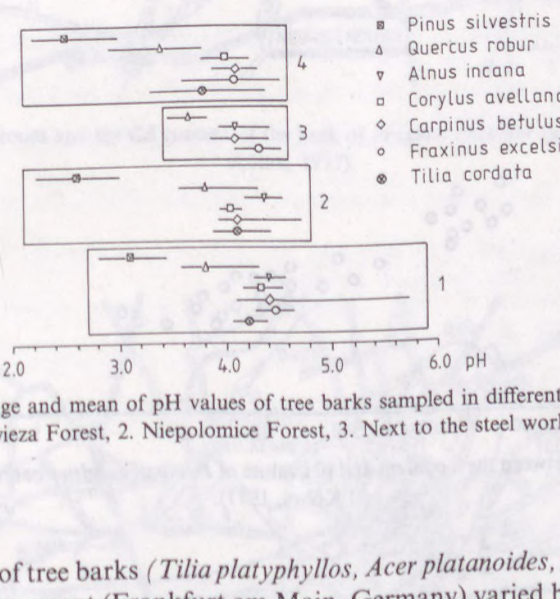

Fig. 38 — Total range and mean of pH values of tree barks sampled in different localities (Grodzinska, 1971). 1. Bialowieza Forest, 2. Niepolomice Forest, 3. Next to the steel works, 4. Cracow City.

The pH value of tree barks *(Tilia platyphyllos, Acer platanoides, Fraxinus excelsior)* in an urban environment (Frankfurt am Main, Germany) varied between 2.7–3.5 but in an area with clean air (Taunus Mountains) laid between 3.4–4.4 (Köhm and Lötschert, 1972; compare Table 48).

On the basis of the pH values of tree bark, well-definable distinctions may be made between areas with different loadings (Grodzinska, 1971; Fig. 38).

Under the impact of sulphur dioxide load, sulphur can be detected also in tree bark (Table 49). Its concentration is highest on the surface of the bark. There is a linear correlation between the sulphur content of tree bark and the pH value. The impact of higher sulphur dioxide concentrations is manifested in the increasing acidity of tree bark (Lötschert and Köhm, 1977; Fig. 39).

On the basis of pH, one can assess the sulphur dioxide concentration of air and the extent of sulphur dioxide load.

In water extracts of bark, electrical conductivity can be measured. The latter is a function both of water soluble calcium content and of dust loading.

Table 49 — Sulphur content in the barks of three tree species in localities with different SO_2-loading around Frankfurt am Main, Germany

Tree species	Locality	S content (µg·cm^{-3}) in different depths		
		0–3 mm	3–6 mm	6–9 mm
Norway maple				
(Acer platanoides)	Grüneburg park	199	130	71
	Kronberg	72	35	7
Common ash				
(Fraxinus excelsior)	Grüneburg park	180	100	40
	Kronberg	51	32	5
Lime				
(Tilia platyphyllos)	Grüneburg park	234	115	52
	Kronberg	58	43	18

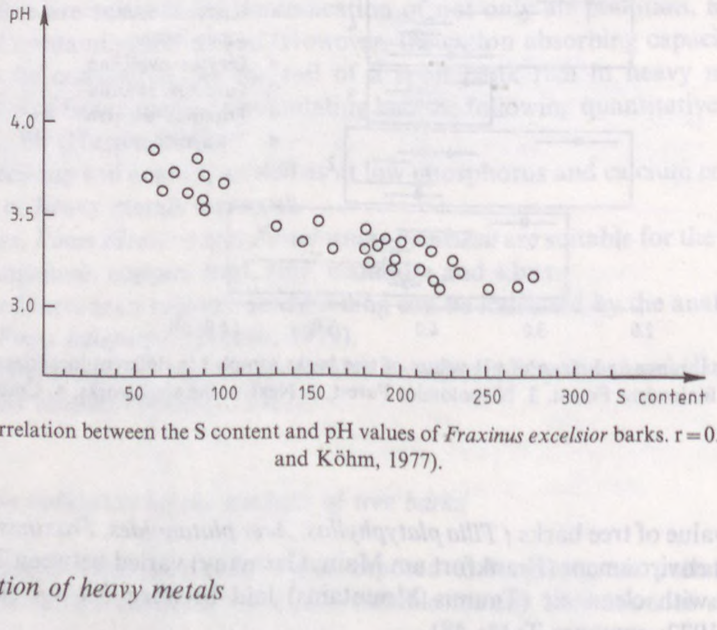

Fig. 39 — Correlation between the S content and pH values of *Fraxinus excelsior* barks. r = 0.93 (Lötschert and Köhm, 1977).

The indication of heavy metals

Tree barks absorb air pollutants. As an example, in Frankfurt the extent of heavy metal loading was determined by the lead, cadmium and zinc content of apple trees. Correlations could be established between the lead and cadmium content of lime, maple and ash trees and the traffic density (Figs 40, 41).

The bark of *Pinus silvestris* is also suitable for the indication of lead.

The bark of various trees *(Acer, Betula, Fagus, Quercus)* is suitable for the indication of radioactive pollutants (e.g. ^{7}Be, ^{137}Cs, ^{40}K) (Brownridge, 1985).

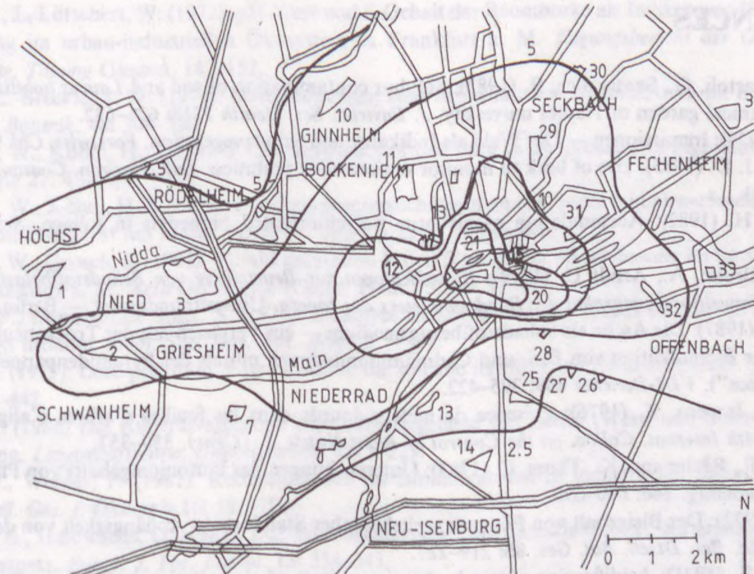

Fig. 40 — Emission zones and the Cd content of the bark of *Fraxinus excelsior* (μg·cm^{-2}) (Lötschert and Köhm, 1977).

Fig. 41 — Emission zones and the Pb content of the bark of *Fraxinus excelsior* (g·cm^{-2}) (Lötschert and Köhm, 1977).

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10 Biological indicators of water pollution

Under the impact of increasing industrial and municipal pollution the nitrogen, phosphorus, sodium, chloride and heavy metal content of waters has been increasing. Aquatic plants (macroalgae, mosses, seaweeds) can indicate water pollution:

– as indicator species (species groups or their communities). Presence or absence indicates the degree of pollution, e.g. increasing eutrophication;
– as monitoring organisms (reaction or accumulating type indicators). They can be exposed in a variety of aquatic sites;
– as test organisms they can be used in ecotoxicological tests under laboratory conditions.

10.1 INDICATOR SPECIES

The presence and frequency of various aquatic plants (algae, mosses, various aquatic weed species as well as higher plants in the coastal zone) indicates specific characteristics of the water (Haber and Kohler, 1972; Janauer, 1982; Dykyjova et al., 1985). From the presence of these species one can infer the trophic status, chemical characteristics, ion content, etc. of water (Table 50). Pietsch (1982) established a 5-grade rating for estimation of the level of pollution, using 21 chemical characteristics (pH value, total hardness, HCO_3 content, chloride-acid absorbing ability, dissolved CO_2, Ca, total salt, absolute ion, relative anion, Na, K, Mg, Mn, Fe, SO_4, Cl, Si_2O_3, PO_4, NH_4, NO_3, total dissolved organic matter and O_2 content).

Plant species indicating water pH can be characterized by the following reaction numbers:

Reaction number 1—water of extreme acidity (pH 1.8–4.5),
Reaction number 2—acid water (pH 4.6–6.8),
Reaction number 3—water of moderate acidity or neutral chemical reaction (pH 6.1–7.5),
Reaction number 4—water of varying alkalinity (pH 6.0–9.0),
Reaction number 5—alkaline water (pH 7.1–10.0).

Table 50 — Classification of aquatic plants based on trophic status (Haslam, 1982)

Lake types	Aquatic plants
Dystrophic brown coloured humus containing water, poor in nutrients, lime is absent (or in low concentrations)	*Sphagnum* sp. *Eriophorum angustifolium* *Menyanthes trifoliata* *Ranunculus flammula* *Sparganium minimum*
Oligotrophic poor in nutrients	*Caltha palustris* *Eleocharis palustris* *Juncus bulbosus* *Nymphaea alba*
Semioligotrophic transition to the next type	*Batrachium aquatile* *Glyceria fluitans* *Phalaris arundinacea*
Mesotrophic (acidic) with average nutrient content	*Batrachium fluitans* *Polygonum amphibium* *Potamogeton natans* *Petasites hybridus*
Mesotrophic calciferous	*Catabrosa aquatica* *Hippuris vulgaris* *Lemna trisulca* *Mentha aquatica* *Sium erectum* *Solanum dulcamara* *Sparganium erectum* *Veronica anagallisaquatica* *V. beccabunga*
Semieutrophic transition to the next type	*Alisma plantago-aquatica* *Batrachium trichophyllum* *Carex acutiformis* *Elodea canadensis* *Glyceria maxima* *Myriophyllum spicatum* *Phalaris arundinacea* *Phragmites communis* *Potamogeton crispus* *P. densus* *P. lucens* *P. perfoliatus* *Typha latifolia* *Zannichellia palustris*
Eutrophic rich in nutrients	*Butomus umbellatus* *Ceratophyllum demersum* *Epilobium hirsutum* *Potamogeton pectinatus* *Rorippa amphibia* *Rumex hydrolapathum* *Sagittaria sagittifolia* *Scirpus lacustris*

In clean (unpolluted) waters with low concentration of organic matter, the presence of *Charophyceae* (*Nitella* and *Chara* species) is characteristic. Lakes rich in *Charophyceae* are generally shallow, oligotrophic, and the benthos is composed of both dead *Charophyceae* and their lime cover. The species *Chara aspera, Ch. contaria, Ch. tomentosa* and *Nitellopsis obtusa* occur primarily in waters in which the orthophosphate content seldom exceeds a value of 0.12 $mg \cdot l^{-1}$. As a result of nutrient accumulation the *Charophyceae* disappear (exceptions are *Chara fragilis, Ch. vulgaris* and *Nitella mucronata* species living in eutrophic waters) (Krause, 1981).

As a result of nutrient accumulation the "Chara-lakes" are transformed into pondweed *(Potamogeton)* containing mesotrophic lakes.

This process has been observed in several European lakes (Krause, 1981; Melzer, 1981). In addition to pondweed water milfoil *(Myriophyllum spicatum, M. verticillatum)* and Canadian pondweed *(Elodea canadensis)* are also frequent. Increasing nitrogen content is accompanied by the proliferation of hornwort *(Ceratophyllum submersum, C. demersum)*.

When defining four water quality categories the hornwort *(Ceratophyllum demersum)*, Canadian pondweed and Ivy-leaved duckweed *(Lemna trisulca)* can be ranked in class no. 1; while fennel pondweed *(Potamogeton pectinatus)* is characteristic of 2–3 class waters (Bock and Scheubel, 1979).

Changes in the nutrient content of Lake Balaton were indicated by the mass proliferation of certain aquatic weed species (Tóth, 1972; Kárpáti, 1977; Fig. 42).

Among indicator species green algae *(Chlorophyceae*, especially *Cladophora)* may become dominant. It can be used as an accumulating indicator, by means of transplants.

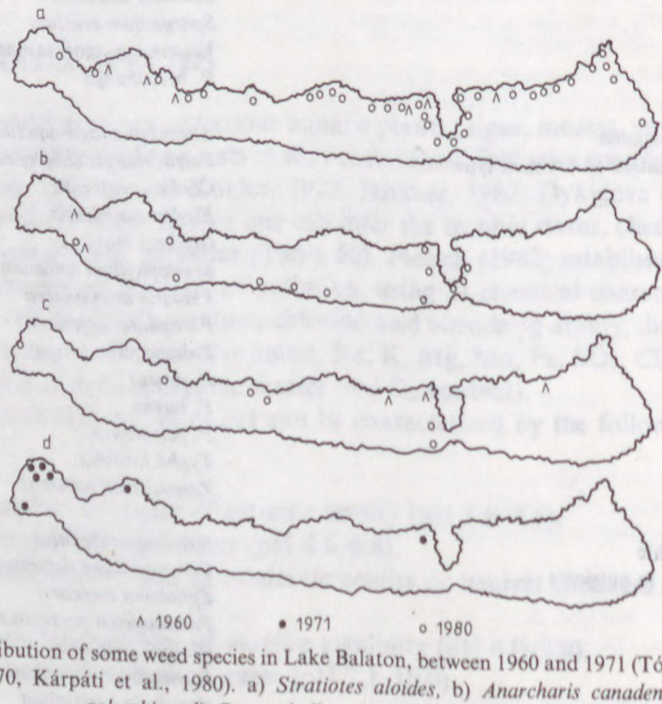

Fig. 42 — Distribution of some weed species in Lake Balaton, between 1960 and 1971 (Tóth, 1972; Kárpáti and Varga, 1970, Kárpáti et al., 1980). a) *Stratiotes aloides*, b) *Anarcharis canadensis*, c) *Spirodella polyrrhiza*, d) *Ceratophyllum demersum, C. submersum*.

10.2 MONITORING SPECIES

a) Aquatic mosses. The following moss species are suitable for the indication of saprobity and various elements (Empain, 1976a, b; Say et al., 1981; Wehr and Whitton, 1983; Wehr et al., 1983): *Amblystegium riparium, Cinclidotus nigricans, Eurhynchium riparioides, Fontinalis antipyretica, Fontinalis squamosa, Hygramblystegium irriquum, Rhynchostegium riparioides, Scapania undulata.*

Correlations can be established between the presence of aquatic mosses and the degree of saprobity. *Cinclidotus danubicus* is suitable to indicate polychlorinated biphenyl and hexachlorocyclohexane (Mouvet et al., 1985).

Mosses are susceptible to the phenolic content of water. Should the concentration of phenolics exceed 0.05 $mg \cdot l^{-1}$, the more susceptible moss species disappear.

When *Leptodyctium riparium* is used as an indicator species the following limit values can be established (Frahm, 1977):

Cl max. 300 $mg \cdot l^{-1}$,
Na max. 200 $mg \cdot l^{-1}$,
Fe max. 5 $mg \cdot l^{-1}$,
MH_4 max. 5 $mg \cdot l^{-1}$,
PO_4 max. 2 $mg \cdot l^{-1}$,
O_2 max. 4 $mg \cdot l^{-1}$.

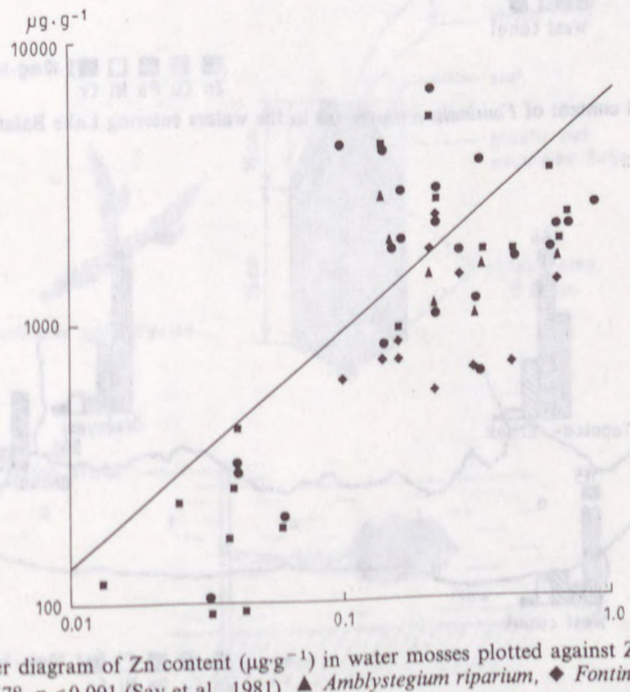

Fig. 43 — Scatter diagram of Zn content ($\mu g \cdot g^{-1}$) in water mosses plotted against Zn content in water ($mg \cdot l^{-1}$). $r = 0.6678$, $p < 0.001$ (Say et al., 1981). ▲ *Amblystegium riparium*, ◆ *Fontinalis antipyretica*, ■ *Fontinalis squamosa*, ● *Rhynchostegium riparioides*.

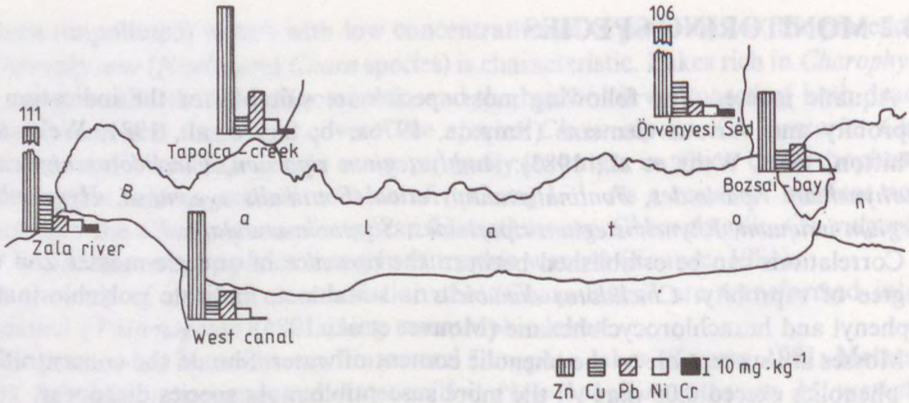

Fig. 44 — Element content of *Fontinalis antipyretica* in the waters entering Lake Balaton in May, 1984.

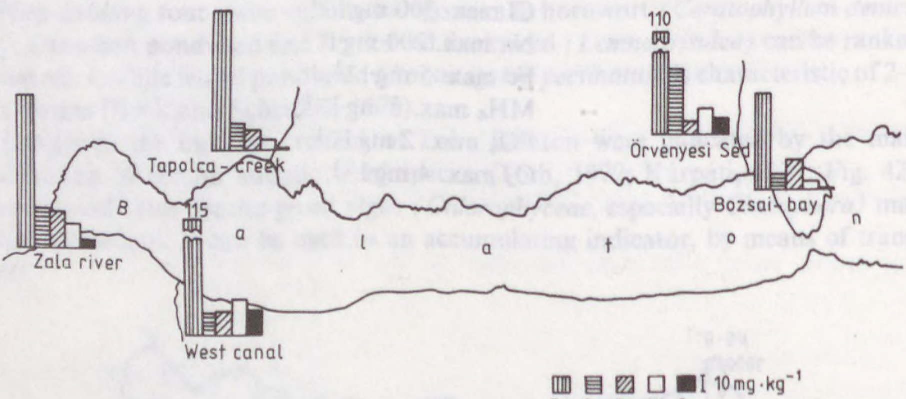

Fig. 45 — Element content of *Fontinalis antipyretica* in the waters entering Lake Balaton in June, 1984.

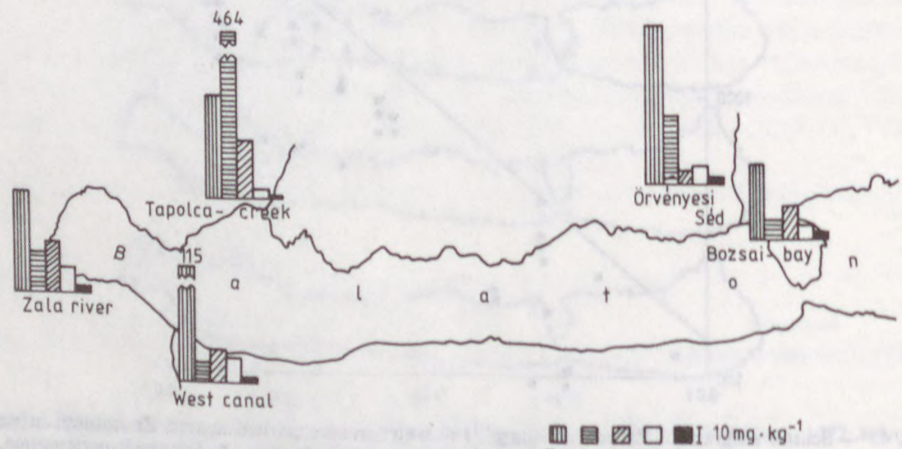

Fig. 46 — Element content of *Fontinalis antipyretica* in the waters entering Lake Balaton in July, 1984.

Most frequently *Fontinalis antipyretica* is used as an active indicator (Empain, 1976a, b, 1978; Burton, 1979; Say et al., 1981; Say and Whitton, 1983; Wehr et al., 1983; Mouvet, 1984).

Fontinalis antipyretica is primarily suitable to indicate the heavy metal pollution of waters, but also the following elements: Al, B, Cu, Fe, Mn, Ni, Pb, Ti, V, Zn, Co, Cd, Cr, etc. This species is capable of accumulating these elements in large quantities. Pb and Zn concentration in samples taken from the river Ruhr was 3200 times and 9400 times higher, respectively, than in water samples, themselves.

The amount of an element absorbed is proportional to the concentration present in the water (Say et al., 1981; Fig. 43).

According to our own investigations the heavy metal pollution of waters (rivers, brooks, canals) flowing into Lake Balaton can be indicated by the chemical composition of *Fontinalis antipyretica* (Figs 44–46).

The following method was used in our investigations: Samples of approximately 500 g fresh weight of living moss were collected in unpolluted waters (e.g. in the Bozsai Bay near Tihany, Lake Balaton) and placed in containers made of plastic mesh with 2.5 × 3.5 mm pore size. They were then fixed under water, at a depth of 10–20 cm (Fig. 47).

b) Aquatic weeds, as passive indicators, indicate the element content of waters by their chemical composition.

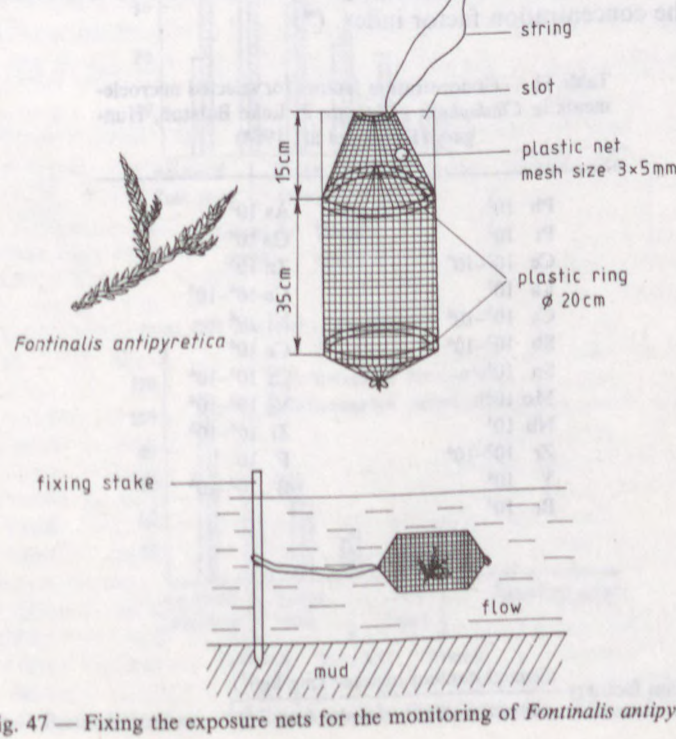

Fig. 47 — Fixing the exposure nets for the monitoring of *Fontinalis antipyretica*.

As a result of increasing eutrophication the malate, starch, hexose, potassium and chloride content of coloured pondweed *(Potamogeton coloratus)* decreases. By contrast, an increase can be detected in the concentration of citrate, sucrose, certain amino acids as well as phosphates and nitrates.

The preferential accumulation of the following elements is characteristic of the following, most frequently occurring, aquatic weed species of Lake Balaton (Kovács and Tóth, 1979).

Potassium accumulating species: pondweed *(Ceratophyllum submersum)*, water soldier *(Stratiotes aloides)*. (It is characteristic of water soldier that it accumulates both alkaline and alkaline earth metals.)
Sodium accumulating species: frogbit *Hydrocharis morsus-ranae*.
Magnesium accumulating species: *Ceratophyllum submersum, Stratiotes aloides.*
Phosphate accumulating species: *Hydrocharis morsus-ranae.*
Nitrogen accumulating species: *Ceratophyllum submersum, Hydrocharis morsus-ranae, Utricularia vulgaris.*
Zinc accumulating species: *Ceratophyllum submersum, Hydrocharis morsus-ranae, Utricularia vulgaris.*
Strontium accumulating species: *Potamogeton pectinatus, P. perfoliatus, P. crispus.* (Presumably Sr accumulation is one of the characteristic features of the genus *Potamogeton.*)
Lead accumulating species: *Hydrocharis morsus-ranae, Potamogeton pectinatus.*
Copper accumulating species: *Hydrocharis morsus-ranae.*

The element accumulation of floating aquatic weeds can also be well estimated, on the basis of the concentration factor index. (*)

Table 51 — Concentration factors for selected microelements in *Cladophora glomerata* in Lake Balaton, Hungary (Kovács et al., 1984)

Pb	10^3	As	10^2
Pr	10^3	Ga	10^3
Ce	10^3–10^4	Zn	10^5
La	10^3	Cu	10^4–10^5
Cs	10^3–10^4	Ni	10^4
Sb	10^3–10^4	Co	10^4
Sn	10^4	Cr	10^2–10^4
Mo	10^2	V	10^3–10^4
Nb	10^4	Ti	10^4–10^5
Zr	10^3–10^4	F	10
Y	10^4	B	10^3–10^4
Br	10^3		

$$* \text{ Concentration factor} = \frac{\text{element content of weed (mg·kg}^{-1})}{\text{element content of water (mg·l}^{-1})}$$

The following concentration factors have been determined for the aquatic weeds of Lake Balaton:

$$Na10^2, N10^4, Fe10^3, Mg10^2, P10^5,$$
$$Zn10^3, K10^3, Cu10^3, Mn10^3–10^4$$

The aquatic weed species can also be used to detect various microelements, e.g. bismuth, lead, neodimium, prazeodimium, cerium, iodine, antimonium, tin, molibdenum, niobium, circonium, ittrium, strontium, rubidium, bromine, arsenic, gallium, nickel, cobalt, chromium, vanadium, titanium, aluminium, scandium, fluorine and boron (Kovács et al., 1984).

According to investigations carried out at Lake Fertő, Hungary, *Najas marina* ssp. *minor* can also be considered as an accumulator of zinc (Kárpáti et al., 1979).

According to data by Raghi-Atri (1983) aquatic weeds contain higher amounts of the following elements:

Copper: *Anacharis canadensis, Potamogeton* species.

Zinc: *Potamogeton, Myriophyllum, Ceratophyllum* species and *Lemna minor* is capable of absorbing higher amounts of thallium as well (concentration factor 10^3, Kwan and Smith, 1988).

Molibdenum: *Ceratophyllum demersum.*

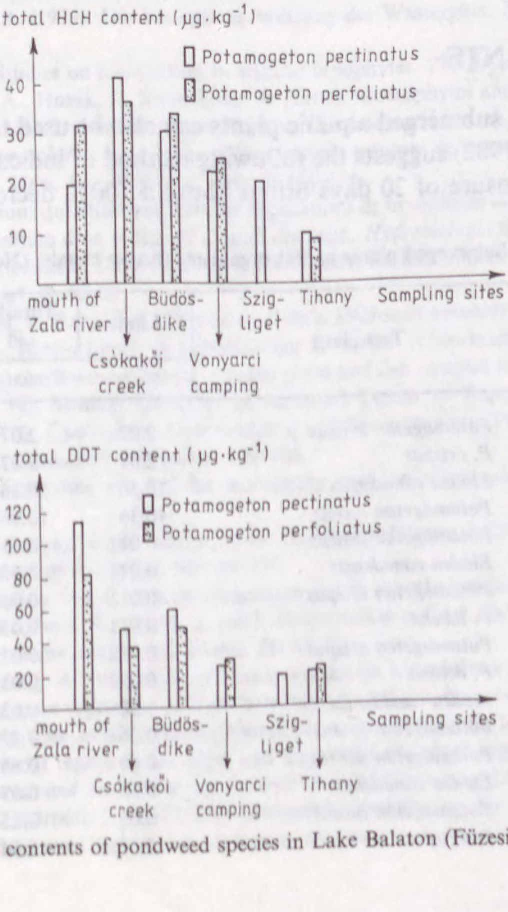

Fig. 48 — Pesticide contents of pondweed species in Lake Balaton (Füzesiné et al., 1980).

Boron: *Myriophyllum, Potamogeton* and *Lemna* species.

Relatively high amounts of mercury are absorbed by *Potamogeton perfoliatus, P. natans, Nuphar lutea, Myriophyllum alterniflorum* (Lodenius, 1980).

In eutrophic fresh waters *Cladophora glomerata* occurs very frequently and in abundance. It is suitable for the indication of various heavy metals (Stokes et al., 1983).

Mercury pollution in water can be detected by *Ceratophyllum demersum* (Suckharoen, 1979).

Certain macroscopic algae (e.g. *Cladophora glomerata*) accumulate the various microelements in relation to the water's concentration by factors of 10^4–10^5 in Lake Balaton (Table 51).

According to Abo-Rady (1980) the following quantitative sequence could be established for the heavy metals accumulating in *Cladophora glomerata:*

$$Pb > Zn > Hg > Ni > Cu > Cd.$$

In certain aquatic weed species, e.g. *Potamogeton pectinatus, P. perfoliatus, Myriophyllum spicatum, Ceratophyllum demersum* and *Trapa natans* a significant accumulation of chlorinated carbohydrates can be observed. Thus these are suitable indicators of these compounds (DDT, HCH, Fig. 48).

10.3 TEST PLANTS

As test organisms, submerged aquatic plants can also be used to indicate anionactive tensides. Kohler (1982) suggests the following method of indication: under aquarium conditions an exposure of 20 days brings about a 100% decrease in the rate of net

Table 52 — Submerged plants as test organisms (heavy metals) (Nobel et al., 1983)

Heavy metal	Test plant	Limit	Thresh-old	Semi-lethal	Lethal
		Concentration, ppm			
Lead (Pb–NTA)	*Potamogeton densus*	2.07	2.07		
	P. crispus	2.07	2.07		
	Elodea canadensis	10.36	10.36		
	Potamogeton lucens	10.36	10.36		
Cadmium (Cd–NTA)	*Potamogeton densus*	0.011	0.011	0.56	
	Elodea canadensis	0.011	0.056	0.56	0.56
	Potamogeton crispus	0.011	0.056	0.56	0.56
	P. lucens	0.011	0.056	0.56	0.56
Copper (Cu-NTA)	*Potamogeton crispus*	0.032	0.032	0.064	0.32
	P. densus	0.032	0.032	0.064	3.2
	Elodea canadensis	0.0064	0.032	0.32	0.32
	Potamogeton lucens	0.064	0.32	0.32	
Zinc (Zn-NTA)	*Potamogeton densus*	0.65	0.65	1.625	65.4
	Elodea canadensis	0.65	0.65	3.25	32.5
	Potamogeton lucens	0.65	3.25	6.54	32.5
	P. crispus	4.875	6.54	6.54	65.4

Table 53 — Submerged plants as test organisms (Nobel et al., 1983)

Salt	Test plant	Limit	Threshold	Semilethal	Lethal
		Concentration, ppm			
Chloride (NaCl)	*Potamogeton alpinus*	50	50	500	2 500
	Elodea canadensis	100	150	1 000	1 500
	Myriophyllum alterniflorum	800	1 000	1200	
	Potamogeton crispus	300	300		

photosynthesis. The plants to be used (in order of susceptibility) are the following: *Potamogeton coloratus, P. lucens, P. densus* and *Anacharis canadensis*.

In the aquatic weed species one can determine the limit for values of pollution, the semilethal and lethal concentrations, e.g. for a certain salt or heavy metal (Nobel et al., 1983; Tables 52, 53).

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11 Plant cells and tissues as indicators of environment pollution

11.1 THE ROLE OF PLANT ANATOMY IN THE INDICATION OF ENVIRONMENT POLLUTION

Advantages and disadvantages

At the ecological level the interrelationship between indicator (i.e., the population of a species or a community of species) and indicandum (e.g. a pollutant) is mediated by several subordinate levels. Among them the molecular, the cellular, the organ and the whole organism levels are the most important. It is primarily the superimposition of these levels that makes it difficult to establish the relationships between the ecological phenomena, such as changes in the spatial and temporal characteristics of the populations or the vegetation and the environment itself. This was aptly expressed by Juhász-Nagy (1984) who stated that "everything is subject to indication". He went on to emphasize the limits of our analytical possibilities and to underline that no matter how substantial the data available, it is still only possible to assess selected indicandum phenomena.

In my view, these problems can mainly be ascribed to the fact that the changes in the environment are only indirectly reflected at any one level of organization. It is partly because of this that the interpretation of data relating to the ecological indication of environmental pollution can be improved by considering the analytical, biochemical, physiological, cytological, histological and morphological data whose effects are ultimately reflected at the ecological level. On the basis of such data it is a most challenging task to reveal the interrelationships between changes in the environment and those observed at the different levels of organization.

Such studies have been carried out by many scientists under the label ecophysiology (e.g. Tuba and Fekete, 1986). Perhaps it is time to add the labels ecocytology, ecohistology and molecular ecology (the latter label being proposed by György Borbély, personal communication). The ecophysiological and other "lower" level investigations significantly differ. While the molecular biological processes, or those at the tissue level, cannot be easily interpreted (at least at present) at the supraindividual level, this can be done with the majority of physiological processes (Zoltán Tuba, personal communication). Thus one can talk about the photosynthetic production of a population or about its salt-tolerance.

Whether biological indication is attempted at the molecular, cytological, histological, organ or organism level, it is important that there should be a close correlation between what is measured and the environmental factor that has to be monitored

(Schubert, 1985). At lower levels the reactions are more specific as they tend to indicate the direct cause of modification observed at higher levels. Each adverse environmental factor brings about at least one biochemical change in the living organism. When a factor brings about more than one change, this alone complicates the situation substantially. Regrettably, this may be characteristic; as with light and high temperature effects. It can also be the case that several factors cause the same or a similar reaction. Nevertheless, by locating the site of an effect, it will be easier to correlate the latter with changes in ecological factors. Should these changes exceed a limit, the effects will be displayed at additional levels. This probably holds true for all levels. However, in contrast to lower levels, the reactions at higher levels (e.g. growth retardation, changes in reproductivity), are likely to be influenced by many more factors. As a result, the complexity of the interrelationships can be portrayed by graphs which oversimplify the real situation. At present we lack both the appropriate methods and sufficient knowledge to allow a more complete model of reality.

A further advantage of the study of lower levels lies in the fact that they more rapidly indicate environmental changes. This implies that under environmental loading, biochemical changes are the first to take place in the organism. Subsequently but prior to the manifestation of histological and morphological abnormalities, cytological symptoms develop. The modification, occasionally the deterioration, of several cells can lead to histological damage. Only extended tissue damage leads to morphological abnormalities (implying reduced growth or reproduction). The morphological abnormalities influence competitive and host-parasite interactions, etc. by weakening and by causing the decay of the organism. Which organism gains dominance is principally determined by processes at the molecular level.

The lack of observable damage on a plant does not necessarily mean that it has not suffered from the impact of a certain environmental factor. Ayres (1984) emphasized that environmental impacts causing a plant not to achieve the maximum growth characteristic for its age and genotype can be regarded as stressors. According to Ayres (1984), all persistent modifications of the plant caused by unfavourable environmental factors, including decreased growth, should be regarded as damage. When the stress and damage occur simultaneously, this is classed as direct, primary damage. Thus acid rain accumulating on the surface of leaves can cause direct, primary damage in the cells of the leaf. As a result of this impact the supply of apical meristems with products of photosynthesis will be reduced, which in turn inhibits the processes of cell division and cell elongation. This latter is classed as indirect, secondary damage. Indirect, primary damage is caused by a persistent "elastic load" (a loading factor the damage of which disappears without trace when the stressor is eliminated). For example, SO_2 concentration in the air that causes no damage to the leaf cells, nor does it evoke harmful physiological processes, can slow down photosynthesis to such an extent that both the frequency of cell division and cell elongation in the apical meristems will be reduced. In practice it is most frequently an indirect damage to growth processes that causes most serious concern. Consequently, when evaluating the impact of environment pollution, it is not only the observable abnormalities (e.g. deformation of cell components and leaves, the formation of chlorosis and necrosis) that should be considered but also reduced growth. As an example, dendrochronological studies offer an excellent possibility for the observation of the latter effect.

In a comprehensive work, Krause (1985) reviewed the effects of environment pollutants at the electron microscopic level. According to him, most studies indicate characteristic changes in cell structure prior to the appearance of symptoms visible to the naked eye. Should this be a universal fact, electron microscopic methods could be applied in forecasting the undifferentiated dangers. After the appearance of visible symptoms their role is to help identify underlying causes. Similarly with certain lower level indicators, the indication of environmental pollution by plant cells also faces difficulties. The major problem is that at these levels the traits are invisible to the human eye. The main precondition for the widespread application of biological indicators is the availability of a simple method. However, with most studies of scientific value it is difficult to comply with this requirement. Especially with the lower levels one requires specific items of equipment linked to computerized evaluation. Consequently, it is more labour-intensive and more expensive than those at the higher levels. Indeed, the less perceivable is the changing of a certain indicator-trait to the human eye, the more expensive is its widespread application. On the other hand, the more perceivable are the changes in a certain trait, the less specific is the underlying reaction.

The advantage of biological indication by intact plants is that mostly it does not require special techniques and it is rapid. However, cytological and histological investigations indicate damage manifested well in advance of that visible to the naked eye. In certain cases they may also provide useful additional information as to the extent and nature of causal factors.

Cytological and histological changes brought about by environmental pollution can also be useful in indicating the persistence of the latter. For this purpose, it is necessary to determine if the changes increase in the short term, within one generation; or whether they are the result of longer-term adaptations taking place over several generations (e.g. changes in the hairiness, or the fine pattern of leaves) and which can be ascribed to genetic selection. The latter tends to be brought about by persistent impacts.

Most probably it is the labour-intensive nature of cytological and histological methods that has prevented their widespread application, compared with the use of intact plants. However, the more easily applicable methods (e.g. the study of annual rings and wax erosion on leaf surfaces) have been more widely used. Therefore our aim is not to provide readily applicable recipies for the quantitative and qualitative detection of a wide range of pollutants. By surveying the impacts on cells and tissues, our aim is to outline possibilities. Our present knowledge makes it seem plausible that appropriate biological indicators (in the form of cells and tissues) are to be expected to emerge from the biotechnological revolution, such as the utilization of cell and tissue cultures (e.g. Huang et al., 1987).

11.2 PLANT CELLS AS INDICATORS OF ENVIRONMENT POLLUTION

Regrettably, when using plant cells for the indication of environmental pollution, one faces the very same problems as in indication processes at other lower levels. Thus it is difficult to select both the appropriate indicator organisms and methods. Consequently the choice of well-established methods is limited. In most instances one is only able to refer to published correlations between environmental pollutants and their anatomical impacts. The lack of real specificity in reactions, even at the anatomical level, also makes the situation more complex. Indeed numerous environmental facts cause similar and difficult to interpret changes in plant cells and tissues. To separate the impacts of simultaneously active pollutants presents further problems. As emphasized by electron microscopic studies, the discovery of rapid, easily applicable, widely used methods is not yet to be expected. In the following, an attempt is made to outline the impact of individual factors separately and then when in various combinations.

Effects caused by the salts of heavy metals

According to numerous data in the literature, the internal structure of chloroplasts can be significantly modified by changes in the mineral nutrition, especially by the excess uptake of the salts of heavy metals. According to Barber (1976), differences in the cation composition of the supporting medium can influence not only the buildup (stacking) of thylakoids but also the volume of intrathylakoidal space, including even the thickness of the thylakoid membrane. Modifications in chloroplast number and volume is also a characteristic trait of plant damage caused by salts of heavy metals. Nonetheless, most studies have been carried out with isolated chloroplasts. It is uncertain, if the concentrations used in these experiments could reach the chloroplasts of intact plants in similar quantities. For these reasons, real situations (i.e., in intact plants) can only be simulated (modelled) in such experiments. The following type of experiment is recommended. For example, Dudka et al. (1983) studied changes in the chloroplasts of intact tomato plant grown in a nutrient solution containing cadmium ions (Table 54). According to their data, in the 1–20 μM cadmium solution treatment chloroplast volume was reduced to approximately 57% of the control. The number

Table 54 — Chlorophyll content, number of chloroplasts per unit leaf area, and chloroplast volume of tomato plants (Dudka et al., 1983)

Parameters studied	Control	Cd treatment	Cd and Mn treatment
Chloroplast volume (μm^3)	162.7±6.4 (100)	93.6±7.78 (57)	140.7±9.19 (87)
Chloroplast number ($\times 10^{11} \cdot m^{-2}$)	11.0±1.39 (100)	7.7±0.93 (70)	11.2±1.38 (102)
Chlorophyll amount per chloroplast ($\times 10^{-8} \cdot mg$)	2.4±0.08 (100)	1.7±0.03 (70)	1.8±0.05 (75)
Chlorophyll content ($g \cdot m^{-2}$)	0.263±0.026 (100)	0.132±0.022 (50)	0.201±0.017 (76)
Total volume of chloroplasts (%)	28.5±2.29 (100)	21.6±1.66 (76)	24.4±1.95 (86)

Note: the control plants were not treated while the others were grown in a nutrient solution of 20 μM cadmium content for 14 days or in addition to 20 μM cadmium received 500 μM Mn after the 9th day. The values indicate the means of three independent measurements per each of three parallel treatments, as well as standard deviation of chloroplasts per unit leaf area and chlorophyll content was increased by low concentrations of cadmium (1–5 μM) while in the 10–20 μM range the values gradually decreased (at 20 μM cadmium concentration they amounted to 70 and 50% of the control, respectively).

The results obtained by above authors also prove that the changes in the chloroplasts were greatly dependent on some factors other than the pollution. Consequently such changes must be used in indication with appropriate circumspection. The effects of cadmium on chloroplast properties could partially be reversed by adding 500 μM manganese to the solution. Thus when the total volume of chloroplasts treated with cadmium was 24% less than that of the control, the addition of manganese reduced the difference to 14%. Moreover, the modified chloroplast volume returned to nearly the original value when, from the 9th day on, cadmium was applied jointly with manganese. Apart from its disadvantages from the viewpoint of indication, this phenomenon might have certain positive implications. It is encouraging that some environmental damage can be eliminated by an application of chemicals.

Baszynski et al. (1980) have also established that in tomato the *in vivo* application of cadmium reduced the activity of the photosystem 2, and the amount of both chlorophylls and carotenoids. Simultaneously, the number and size of plastoglobules increased. In the extreme, the inner structure of chloroplasts might also disintegrate. They also realized that cadmium treatment significantly changes the volume of chloroplasts. According to them all these changes resemble the symptoms of senescence. Indeed, one frequently finds that, as a result of environmental pollution, whole plants (not just individual cells) display the symptoms of senescence.

It has not been possible to find an appropriate explanation for either the biochemical or biophysical foundations of phenomena observed. It is known that cadmium accumulates in the cytosol of the cell and that it binds to proteins or to groups of proteins of low molecular weight. In the form of free ions it is hard to detect, indicating that its effect on chloroplast volume is not caused by osmotic factors.

The effect of cadmium on the fine structure of wheat plastids of different developmental stages was studied by Wrischer and Kunst (1981). In detached leaves, a 2 days' long 1 mM cadmium chloride treatment strongly inhibited chloroplast formation from etioplasts. As remnants of the prolamellar bodies, the plastids contained tubular complexes and prothylakoids and bent, compressed stacks of thylakoids. Surprisingly, the fine structure of chloroplasts in green leaves was not affected by the cadmium concentration applied. On the basis of this it can be assumed that wheat chloroplasts are more resistant to cadmium than are the similar cell organelles in tomato. It is also possible that the deformations observed in tomato (Dudka et al., 1983) are the result of certain, indirect, external impacts on the leaves (the protecting effect of manganese seems to indicate this).

Applying a similar method, Wrischer and Meglaj (1980) also established that, in etiolated leaves, 1–5 mM concentrations of lead compounds (lead chloride and lead nitrate) also inhibit the plastid differentiation. Plastids exposed to lead remain in the early differentiation stage of etioplasts. The ultrastructure of chloroplasts in green leaves was, however, less affected by lead; although, as with cadmium, it reduced both the chlorophyll content and photosynthetic efficiency of chloroplasts (in such a case one speaks of indirect, primary damage).

Other authors report on the localization of certain metals within the protoplast. From the point of view of indication, these results are more promising. By knowing the site of localization, on the basis of an EDXA analysis (X ray microanalysis) one can assess the loading of living organisms. As an example, Morimura et al. (1978) established that in root cells of onion aluminium binds to the nucleus. De Filippis and Pallaghy (1975) also observed that lead is mostly bound by the nucleus. However, in the form of methylplumbumchloride, this toxic element can also be detected in the cytosol and vacuoles in large amounts. Remarkably, with the intracellular localization of zinc De Filippis and Pallaghy (1975) obtained different results depending on the method of fixation. (This is a further problem indicating similar dangers in the study of other elements.)

Ernst (1974) reported that more than 90% of heavy metals showing high affinity to the carboxyl groups of the cell wall (especially mercury, chrome and lead) is bound in the cell walls of cortex. Even the mobile heavy metals (such as zinc, nickel, copper, cadmium and manganese) accumulate up to 80% at these sites. Comparative studies on heavy metal tolerant and heavy metal sensitive populations of the families Poaceae, Caryophyllaceae and Lamiaceae suggest that the mechanism of tolerance can at least partly be attributed to such a distribution of heavy metals in the plant (i.e, its absorption in the apoplast). Consequently, it can be concluded that in an environment loaded with heavy metals, it is the cell wall of cortex cells that displays the greatest differences in the composition of elements. It can also be assumed that by comparing the older and younger cortical cells of the same woody plant one can trace the changes of environmental pollution in time (e.g. the heavy metal mobilizing effect of acid depositions). Methods such as the comparison of annual rings, of leaves of subsequent years in conifers and of subsequently differentiating leaves or internodes of herbaceous species (as with the potassium-argon or the radio-carbon method) are suitable for pursuing changes taking place over periods of different length.

Barcelo et al. (1988) applied light-, transmission- and electron microscopy in the study of the effect of a growth inhibiting concentration (5 $\mu g \cdot ml^{-1}$) of cadmium on kidney beans. They found that neither the structural nor the ultrastructural changes had any correlation with the average Cd concentration of organs. Thus the structure of plastids hardly changed in the roots, although these organs displayed the highest Cd concentration. By contrast, in the aboveground organs severe chloroplast damage occurred. In comparison to the primary leaves, both chloroplast synthesis and ultrastructure suffered greater damage in the trifoliate leaves.

Data on the effects of aluminium on cell division, chromosomes, ribosomes, protein synthesis, Golgi apparatus and endoplasmic reticulum are provided in a comprehensive review by Roy et al. (1988). The available data indicate that in toxic concentration this element inhibits cell division, causes chromosome aberrations and affects the functioning of ribosomes, the Golgi apparatus, as well as the endoplasmic reticulum.

The joint impact of pH and certain heavy metals on pollen germination was studied by Cox (1988). On the basis of pollen susceptibility to acidity the species could be ranked as follows: deciduous trees, trees and bushes of lower layers, plants of the grass (ground) layer and conifers. Consequently, the susceptibility to acidity was a function of both systematic affinities (coniferous v. nonconiferous) and localization in the ecosystem. The individual heavy metals exerted varying impacts on the pollens.

The effects of acids and acid gases
(hydrogen chloride, sulphur dioxide, nitrogen dioxide, hydrogen fluoride)

During recent decades the amount of acid gases has significantly increased in the atmosphere. While significant amounts of some of these (e.g. sulphur dioxide, nitrogen oxides, hydrogen fluoride) occur in the atmosphere of the entire world, others are of merely local significance. As to the indication possibilities we have to make a distinction between the two types. In the case of factors of local significance (which can generally be recognized by the fact that these pollutants are caused by easily located point sources) the indication is easier. Starting from the source, there is usually an easily detected gradient of symptoms. There is a totally different situation with pollutants which are widely dispersed. Because of the critical importance of individual sensitivity, uneven spatial distribution, the occurrence of mixtures of difficult-to-identify pollutants and other factors, their indication is much more difficult. Consequently, in the case of point-sources, the number of factors (e.g. dose of point-like emission, its distribution in time, wind direction, etc.) to be taken into consideration is less. In addition to this the symptoms are unambiguous enough to facilitate the establishment of clear-cut correlations between cause and effect. Consequently many plant anatomists attempt to utilize the results obtained in the laboratory or in the vicinity of point-sources (the latter can be regarded as "laboratory experiments" carried out in the field) in the evaluation of complex situations.

Gaseous HCl may be a pollutant of local origin. Gaseous hydrochloric acid is produced in the course of PVC wrap production and heating coal. It is also one of the major components of the exhaust-gas of rockets. Consequently it may greatly damage vegetation in the vicinity of PVC factories and rocket launching bases.

The literature on the ultrastructural impact of gaseous hydrochloric acid is limited. The available results indicate that it is the chloroplasts within intact cells that are damaged in the first place. As a result of hydrochloric acid treatment the number of grana and plastoglobuli increases in these organelles or else the stroma-lamellae and granal ends get swollen and chrystalline structures are formed in the stroma.

Endress and Taylor (1981) subjected *Tagetes erecta* and spinach plants to hydrochloric acid treatment, in order to obtain more detailed information on the cellular effects of the gas. Their study revealed that the ultrastructure of cells suffered greater damage than was evident to the naked eye. The initiation of regenerative processes, upon hydrochloric acid treatment, is also a further important observation. This is underlined by changes in the size and number of chloroplast plastoglobuli (Table 55),

Table 55 — Number of plastoglobuli in an electron microscopic chloroplast section of spinach following a 20 min treatment with 30.4 mg·m^{-3} HCl (Endress and Taylor, 1981)

Treatment	Hours after treatment	Mean ± standard deviation	Limits
Control	0.75	11.6 ± 6.8	1–32
HCl	0.75	34.3 ± 26.1	2–105
	2.0	18.4 ± 15.4	5–57
	6.0	13.5 ± 4.5	7–22
	24.0	19.4 ± 14.8	6–36

as well as the deformations of granum components. All this implies that it is of primary importance to know how much time has elapsed between the onset of damage and its use for indication. Remarkably, other pollutants also lead to similar abnormalities in the fine structure of cells. Seemingly, the acid gases, such as hydrogen chloride, nitrogen dioxide and sulphur dioxide, bring about the same response processes in the chloroplasts in different species. This response is largely independent of the strength of the impact.

Swiecki et al. (1982) also used the primary leaves of young bean seedlings. At both cellular and tissue level they found substantial differences between the effects of gaseous hydrochloric acid and liquid acids (hydrochloric acid, nitric acid and sulphuric acid). The differentiating trait developing as a result of acidic solutions was in the form of both necrotic spots on the leaf surface and conspicuous damage of tissues of the leaf veins. It is obvious that accumulation of liquid acids in leaf hollows cause the greatest damage. On the other hand, gaseous hydrochloric acid (like draught) enhances the formation of crystalline incrustations in chloroplasts. These different symptoms manifested themselves despite the changes visible with the naked eye being the same in each case. Thus, both gaseous and liquid hydrochloric, nitric and sulphuric acids caused vitrification and the formation of necrotic spots on leaf surfaces. The physiological foundation was the plasmolysis and subsequent collapse of both epidermis and the underlying mesophyll. In all cases, the cell damage was also accompanied by similar cytoplasmic damages. Thus, in cells of leaves exposed to either gaseous hydrochloric acid or acidic solutions the appearance of both vesicles and other particles could be observed in the larger vacuoles.

The effect of sulphur dioxide, a widespread pollutant, on plant cells has been much studied. For example, Paul and Huynh-Long (1975) exposed bean plants to sulphur dioxide treatments of various lengths and concentrations. The lower (abaxial) leaf epidermis was studied by scanning electron microscopy, especially the cells of the hairs and stomata.

Leaf hairs, like root hairs, form a large surface area in contact with the environment. Probably, this is why they react more readily to environmental changes than any other cells. The build up of concretions can often be observed around these cells. Under the influence of sulphur dioxide, the extent of such concretions increases, especially along the veins.

All these observations pertain to leaves on which the symptoms caused by sulphur dioxide have not become visible to the naked eye, such as with necroses between the veins. The latter are caused by the desiccation of palisade parenchyma and neighbouring tissues. Such symptoms are sure to appear when the plants are exposed to 8 hours of 2 $mg \cdot m^{-3}$ air sulphur dioxide.

When a higher concentration of sulphur dioxide (4 $mg \cdot m^{-3}$ air during 8 hours) was applied, modification of stoma guard cells was observed. This modification was characterized by both a decrease in the turgescence of guard cells, causing permanent stomatal opening, and by the cells becoming abnormally sunken. This phenomenon was primarily observed on the necrosis-free parts of leaves on which the visible symptoms of sulphur dioxide toxicity had already been observed.

Remarkably, the strongly damaged guard cells of stomata are the only chlorophyll-containing cells in the epidermis. Moreover, it is in the chlorophyll-containing palisade parenchyma that the necroses brought about by sulphur dioxide establish themselves. Evidently sulphur dioxide pollution attacks the chlorophyll-containing cells first. These data and other data in the literature serve to verify that among cell organelles it is the chloroplast that reacts first to sulphur dioxide pollution.

Among anatomical features, it is the wax layer of leaf surfaces that has proved to be most useful in the indication of changes in the environment. It is known, that in the course of leaf senescence this layer undergoes a natural process of degeneration (so-called erosion), causing a decrease in its resistance to parasites, as well as the leaching out of nutrients. The erosion of this wax layer can be accelerated by several gaseous pollutants (of mainly acidic reaction). Indeed the study of this layer can provide useful information on the state of environment and the tolerance of higher plants exposed to environmental changes. The susceptibility of the wax layer towards environmental changes is related to it having the closest contact with the air. The efficiency of investigations can be increased by the comparison of needles formed in subsequent years. The best method is provided by scanning electron microscopy.

Krause and Houston (1983) studied 22 sulphur dioxide tolerant and sensitive *Pinus strobus* clones in order to find if there were any correlations between the degree of sulphur dioxide sensitivity and wax layer differences. In the case of all 10 sulphur dioxide tolerant clones they established the presence of continuous wax bundles above the stomata. In the sulphur dioxide sensitive clones this covering layer above the stomata was longitudinally split. The latter resembled very much the appearance of stomata observed by Trimble et al. (1982). They studied the epicuticular wax layer of ozone-tolerant and -sensitive clones of *Pinus strobus*, though in their photos they did not identify the degree of sensitivity of tissues. In all of the 12 sulphur dioxide sensitive clones studied by Krause and Houston (1983), independently of the stage of development of needles, the epistomatal wax layer was unambiguously split.

These observations indicate that characteristics of the epistomatal wax layer in *Pinus strobus* can be used as markers of sulphur dioxide tolerance in the breeding and selection programmes.

Koziol and Cowling (1981) also used scanning electron microscopy in the study of sulphur dioxide effects on the epicuticular wax layer in perennial ryegrass *(Lolium perenne)*. Surprisingly, they observed that when exposed to higher concentrations of sulphur dioxide the leaves were covered by a thicker wax layer. Above the white necrotic spots the thickness of wax layer was especially conspicuous. Therefore, it seems that, by enhanced wax formation, not only the subsequent generations but also in the course of its ontogenesis even an individual plant adapts itself to environmental changes. Of course, such modifications have a different indicator value than does the erosion of the surface wax layer. In the former case the living plant plays an important part in the formation of the layer while in the latter case it is merely passively subjected to the detrimental effect of gaseous pollutants.

Cape (1983) invented a simple and practical method to measure the degree of wax layer erosion. He characterized the degree of erosion by placing a drop of water on the surface of the leaf and by measuring the angle between the surface of the water drop and leaf surface (Fig. 49). According to his observations, in contrast to the younger ones, on older leaves the water drops are more expanded. Under the impact of sulphur dioxide pollution, the flattening of water drops was also observed on the needles of conifers.

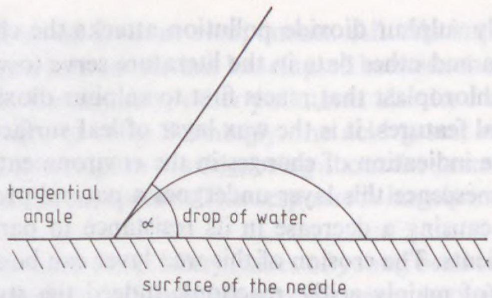

Fig. 49 — Method for measuring the angle between water drops and leaf surface (Cape, 1983).

Fridvalszky (1980) studied the effects of sulphur dioxide pollutants on the ultra-structure of the cell wall. Applying polarization techniques, he established that in the wall of subprotodermal cells of onions the direction of greatest refraction value is perpendicular to the longitudinal axis of cell. This would imply a perpendicular position of microfibrils. From quantitative measurements it was shown that the extent of ultrastructural organization of the wall most significantly increases during the formation of protodermal stoma mother cells, as well as at the initial phase of stomata formation. This "organization" refers to the intensive synthesis of secondary cell wall. In sulphur dioxide containing atmosphere, however, the increment of submicroscopic organization was apparently less, and in the course of differentiation the difference between the treatment and control became more and more conspicuous. Evidently, an abnormal sulphur dioxide content of air inhibits cell wall formation. As this effect manifested itself in a decrease of anisotropy, it can be assumed that sulphur dioxide exerts the greatest negative impact on the formation and orientation of cellulose structure.

Effects of ozone (O_3) and peroxy-acetyl-nitrate (PAN)

In a comprehensive paper by Claussen (1975) it is mentioned that ozone initially damages the chloroplasts. Just a few hours after treatment the stroma of chloroplasts becomes granular. According to Claussen (1975), this is the first stage of ozone damage while the general structure of cytoplasm is still intact. Later on the cytoplasm precipitates in the central zone of the cell and the cell plasmolyses. Also the vacuola disintegrate and the swollen cell organelles bundle up in the centre of the cell. Both the envelope of chloroplasts and their inner membrane structure get totally destroyed, when the cytosol has completely bundled. Simultaneously, the previously undamaged mitochondria also swell and an electron-impermeable layer is formed around them. Finally, these cell organelles also collapse, with unidentifiable, electron-dense substances remaining in the cell. The deterioration of cytosol components indicates the second stage of ozone damage, a symptom that is very likely to be observed at the tissue level, too.

Claussen (1975) also reviews Rich and Tomlinson's most ingenuous method, by which ozone damaged cells were distinguished from undamaged ones. 10–12-day-old

Phaseolus vulgaris plants were exposed to damaging ozone concentrations. The plants were then kept in glasshouses for 5 days; this being sufficient for the symptoms to fully develop in the primary leaves. This was followed by a 36-hour dark incubation. Then 2–5 tissue segments were prepared and embedded in phosphorus dioxane, a fixing substance. The impregnation was enhanced by the application of a vacuum. After two days the tissue segments had lost their pigments and become transparent. They were then thoroughly washed with water, and then dyed with a J-KJ solution (0.2 g iodine dissolved in a 2% potassium-iodide solution.)

The palisade and spongy parenchymatic cells destroyed by ozone preserved their starch grains which were stained blue colour by the J-KJ dye. Those cells, however, that survived the effect of ozone, had consumed their starch grains in the dark period, and so took up practically no stain. Any living stoma guard cells of primary leaves had not lost their starch grains, following the 36-hour dark treatment, and so were well stained. This method allowed the determination of the position of damaged palisade and spongy parenchyma cells in relation to the stomata. The method also proved to be suitable for the detection of leaf cells that had been previously attacked by pathogenic organisms. In these cells the starch grains had remained undigested. Thus this method is also not able to separate environmental pollution from other impacts.

Any consideration of indication must consider what, by what and how it is indicated (Juhász-Nagy, 1987). Practical considerations lead us to favour easy, unambiguous, cheap and rapid methods. Preference is given to the use of widespread organisms, likely to gain widespread application. While compromise is often inevitable, there are at least two means by which the correlation between indicandum and indicator can be successfully revealed. First, changes of the indicator can very well be correlated with the changing of indicandum. An example is when indicandum gradients build up around a point source. Unambiguous correlations can also be established in laboratory experiments. Secondly, one can hope to find indicators that are sensitive and specific to the changes of the given indicandum but it has proved difficult to find an indicator trait such as the various histological deformations caused by ozone and PAN (see later).

Returning to the cytological effects of ozone and PAN, it seems that the structure of cells damaged by these compounds is not significantly different. As with ozone, the acute effects of PAN also bring about a primary granulation of the stroma of chloroplasts, with the other cell components temporarily remaining intact. Later on the presence of PAN causes plasmolysis and the general collapse of cytosol. Indeed all the cell organelles are damaged by peroxy-acetyl-nitrate, except the membrane system of the chloroplasts remains intact. The symptoms of both ozone and PAN damage include the accumulation of darker pigments in the cell walls.

This similarity in the effect of these two toxic compounds reflects their common property of a strong oxidizing effect. Nonetheless, the similarity is surprising, since the histological symptoms caused by these compounds are different. PAN damages mainly the spongy parenchyma while ozone effects can be observed primarily in the palisade parenchyma (see section on histology).

As at the cellular level the effects of both ozone and PAN are the same, according to Claussen (1975) it is probable that the specificity of their response reactions to these compounds is not a function of cell components but much more of intercellurar factors or cell wall structure. Peculiarities of this type may lead to differences in the diffusibility of the two gases. According to Claussen (1975), it is also true that by developing limited specific symptoms, the plant has a limited ability to react differently to all the numerous gaseous pollutants and nutrient deficiencies. Therefore, the diagnosis of damage caused by gases can only rarely be established from a single symptom.

The symptoms of ozone damage observed by Evans and Miller (1972) in *Pinus ponderosa* do not resemble those observed in other species. The plasmalemma is only slightly detached from the cell wall, and the aggregation of cell organelles and cytoplasm in the central parts of the cell is not observed. Should the cells not plasmolyze in the course of fixation and the dehydration process, the vacuola remain intact. Consequently, the cytological effect of ozone can vary according to the species.

The intact parenchyma cells of *Pinus ponderosa* contain a number of randomly localized chloroplasts. In Evans and Miller's (1972) experiment, as a result of a 3–4 days' ozone treatment the chloroplasts aggregate in the branches of mesophyll cells, well before the appearance of visible symptoms. Simultaneously, they become less numerous in the central cell parts. Depending on the degree of damage, the plastids aggregate in amorphous structures. Subsequently the abnormal wrinkling and twisting of cell walls can be observed in the mesophyll. Cell wall deformation is more frequent in the thin-walled cells than in the thick-walled ones. This process results in the elongation of cells in all directions, causing greater detachments in the branches of damaged palisade parenchyma intercellulars. There is also a further decrease in cell surfaces connected with branches of other cells. Should the cytosol become absorbed, the walls might totally collapse. Extended cell wall deformations taking place in the mesophyll bring about the total collapse of leaf parts outside the endodermis. On the 5th day of treatment, the staining method applied could not indicate the presence of carbohydrates in the chloroplasts. The distribution of protein dyes became uneven in the treated cells also. Peripheral parts of the cells accumulated more dyes than the central parts did. Frequently the greatest degree of protein accumulation occurred in the immediate proximity of plasmalemma. After their total collapse the cells showed no response to staining.

In comparison with the treated ones, cells of the control plants contained less acidic-phosphatase. After the 7th day of the treatment this enzyme could be detected mainly in the second and central layers of the mesophyll. In the innermost layer the enzyme activity was low. Adaxial and abaxial layers of the mesophyll showed a more intensive dying with acidic-phosphatase-dyes than the lateral layers in the proximity of resin ducts. The acidic-phosphatase activity was observed in clusters of 3–5 cells while in the untreated plants only sporadic single cells displayed signs of reaction. While the dying was observed in the same cell types the intensity of dying was higher in plants treated with ozone than in the control. Within the cell the reaction could be observed primarily at the plasmalemma and cell peripheries. The fully destroyed cells did not indicate further enzyme activity.

As to the succinate-dehydrogenase activity and the intensity of DNA synthesis, no significant effect caused by ozone could be observed. In contrast to this, in damaged mesophyll cells, the dyes indicating nucleic acid precipitated in small clusters in the entire cytoplasm. In contrast to protein and carbohydrate aggregates, however, the nucleic acids were independent of the cell peripheries and could be well detected, even after the changing of other compounds.

As seen from the above examples, thorough investigation can lead to the selection of appropriate markers in the detection of ozone damage. It is a special factor in Evans and Miller's (1972) studies that while both cytological and histological studies indicated ozone damage within 5 days of treatment, visible symptoms in the form of chlorotic spots appeared only 2–3 weeks after the commencement of treatment.

Dijak and Ormrod (1982) studied physiological and anatomical peculiarities of ozone-sensibility in the pea. Neither ozone sensitive nor ozone resistant varieties displayed any difference in the epicuticular wax layer and the anatomical features of stoma guard cells. Similarly, in a scanning electron microscopic study of the needles of *Pinus strobus* clones, Trimble et al. (1982) found no differences in the structure of wax layers.

Complex effects

Miyake et al. (1984) exposed spinach plants to continuous effect of 0.5 $\mu l \cdot l^{-1}$ ozone and 1.0 $\mu l \cdot l^{-1}$ sulphur dioxide, both separately and simultaneously. The leaf tissues were regularly analyzed by an electron microscope until the date of appearance of necrosis on the leaves. The first sign of ozone damage was the swelling of thylakoids in chloroplasts that was followed by the swelling of dictyosomes, endoplasmic reticulum and nuclear membrane. The intermembrane spaces of mitochondria became smaller. Later on chloroplast deformation could be observed. The primary effect of sulphur dioxide was the swelling of stoma and the deformation of chloroplasts. It was only after this that the enlargement of thylakoids occurred. The final result of both treatments was the collapsing of cells and the precipitation of cell contents. When ozone and sulphur dioxide were applied simultaneously the appearance and development of symptoms were much more rapid. Nonetheless, the symptoms manifesting themselves in the cells resembled much more those of the sulphur dioxide damage.

Frolov and Goryshina (1982) studied the leaf anatomy and photosynthetic apparatus of *Quercus robur, Tilia cordata* and *Ulmus laevis* in different habitats (forests, suburban and municipal parks and an industrial estate) in Leningrad. They found that with increasing urbanization the leaf structure assumed a xeromorphic character, with a decrease in chloroplast number, size, and chlorophyll content. All these changes took place still prior to the appearance of visible leaf damage. The symptoms observed indicate that urban trees have a lower photosynthetic capacity than their relatives in forests.

Mikkonen and Huttunen (1981) studied the effect of air pollution on *Vaccinium vitis-idaea* and *Empetrum nigrum*. As a result of air pollution of traffic origin, characteristic sediments of silicon and other metals, as well as of soot, appeared on the waxy surface of leaves. The above authors also found modifications in the normal wavy structure of the leaf surface.

The acute air pollution of the chemical industry primarily damaged the leaf cells of *Empetrum nigrum*, causing more intensive waviness of the leaf surface. This increased waviness also occurred as an effect of air pollution caused by traffic, and was accompanied by characteristic particle sedimentation on the leaf surfaces. As a result of a 5-week treatment, instead of the erosion of the wax layer it was the structure of epidermis cells that changed.

Huttunen and Laine (1983) carried out an electron microscopic study of the epicuticular wax layer of *Pinus sylvestris* needles. Samples were taken partly in an air polluted town and partly in a forest. The erosion of the wax layer by aging (senescence) accelerated only in the 4–5th year while in the case of urban trees this process started 2–5 times earlier. In the town, the peristomatal wax cover was entirely destroyed during the 1st or 2nd year. This wax layer, however, plays an important role in the regulation of the water regime of the needle.

In a 20-week-long scanning electron microscopic experiment Sauter et al. (1987) studied the epistomatal wax chrystals of spruce. In comparison to the control, they observed the enhanced degradation of wax chrystals. According to them, this structural degradation leads to the clogging of stomatal cavity.

Studying sections of decaying fir and spruce needles in the Black Forest, Parameswaran et al. (1985) became aware of the precocious and frequently total collapse of sieve tubes. In such cases there was no reformation of sieve tubes. Instead of normal cell division, the cells of the cambium, especially in the spruce, exhibit only slight growth. Also the Strasburger-cells of the spruce become larger. As witnessed by raster electron microscopic observations, the amount of calcium oxalate chrystals on the surface of mesophyll cells of damaged trees significantly decreases with the apparent erosion of peristomatal wax layer.

Jäger (1980) investigated the changes caused by gaseous pollutants in higher plants. His aim was to discover simple and applicable methods for the mapping of damage caused by air pollution. Owing to the high labour input, he rejected the application of microscopic analytical methods.

According to Volters and Martens (1987) both pollen germination and the growth of the pollen tube *in vitro* are very susceptible to toxic compounds; therefore these pollen parameters offer a better method in the indication of gaseous pollutants, than does visible leaf damage and other vegetative symptoms. In their comprehensive paper they discuss the possibilities and limits of pollen application in indication.

11.3 THE EFFECT OF ENVIRONMENT POLLUTION ON PLANT TISSUES

Root damage. The effects of heavy metals

Parallel with the acidification of soils and the growth of waste (derelict) land, frequently containing high amounts of heavy metals, the increasing solubility of heavy metals and other potentially toxic elements (e.g. aluminium) in the soil waters has become an ever larger problem. Impacts such as de-icing salt in winter or the compaction caused by too intensive usage may also lead to soil deterioration. These changes are reflected in anatomical modifications of plants (mainly roots). Paivoke (1983) studied the development of pea roots in the presence of lead and arsenic ions. 1.0 and 0.1 mM concentrations of arsenic did not always bring about the formation of triarch vascular bundles. When applied in the same concentrations, lead caused abnormal radial growth. In the presence of both of these toxic elements the endodermis frequently merged with its surroundings, and its cell walls became lignified. The application of 1.0 mM lead concentration brought about the lignification of cortical parenchyma, too. Toxic concentrations of the elements brought about a decrease in the extent of both root epidermis and cortex.

Jásik (1986) demonstrated the detrimental effects of vanadium in the meristematic root cells of bean. The nuclei formed lobe-like protrusions and inclusions could be detected in the cytosol. Also the shape of nuclei and mitochondria changed. The extent of above symptoms was a function of both vanadium concentration and duration of the effect. Kowalski (1987) established that the increasing extent of industrial emissions reduces the amount of root-mycorrhiza in trees. When compared with thin-netted or non-netted mycorrhiza, the mycorrhiza with a thicker net proved to be more resistant to air pollution. On the roots of the pine species studied it was the latter type, while on the majority of roots of deciduous trees it was the former type of mycorrhiza that occurred.

Using a specific staining method, Morselt et al. (1986) have shown that the heavy metal tolerance of ectomycorrhizae is based on the presence of metallothionein-like proteins. They also give evidence that the tolerance of fungi can be brought about by using sublethal concentrations of heavy metals. The formation of such metallothioneins in ectomycorrhizal fungi probably contributes to the protection of plants against heavy metals.

Stem damage

Den Outer and Boersma (1987) report the effects of acidic irrigation water on the xylem of maize internodes. They demonstrated an increase in the number of xylem elements, especially in those of the tracheids. The simultaneous addition of nitric acid to the irrigation water enhanced the effect. The radial walls of mesocotyl epidermis cells were twice as thick as those of the control plants.

Leaf damage

The effects of acid rain. It is known that emissions of oxides of nitrogen and sulphur react with atmospheric humidity to form acidic solutions. The increased acidity of rain has a detrimental effect on the plant canopy. In order to be able to interpret the damage caused to both natural and cultivated vegetations it is of primary importance to clarify the exact cause and mechanism of leaf damage brought about by gaseous pollutants (Evans and Curry, 1979). In order to identify the cause underlying a certain disease additional proofs are needed. The study of plant tissues can be looked upon as one of the appropriate diagnostic methods. This is what Evans and Curry (1979) tried to verify when they studied the effect of simulated acid rains on the leaves of a *Tradescantia* sp., *Pteridium aquilinum*, *Quercus palustris* and *Glicine max* clones. They established the relative sensitivity of the species mentioned to acid rains, and have also identified all those changes in the leaf surface and leaf anatomy that can be made use of in the diagnosis of acid rain damage. The plants were treated with simulated acid rains of 5.7, 3.4, 3.1, 2.9, 2.7, 2.5 and 2.3 pH values.

On the basis of their observations they concluded that the number and extent of leaf surface lesions was increased by both a pH decrease and a greater frequency of acid rain treatments. Other studies (Evans and Miller, 1972; Pell and Weissberger, 1976) have indicated that initially both ozone and other gaseous pollutants affect the mesophyll cells. By contrast, the initial effects of simulated acid rain (a liquid pollutant) were observed in tissues of the leaf surface. The underlying cause for this is probably the occurrence of natural hollows in the epidermis, both above the vascular bundles and at the base of leaf hairs, where acid rain can readily collect.

As an example, on the leaves of *G. max* the rain drops collected and dried up along the leaf veins and towards the leaf margin. It is possible that as soon as the water evaporated from the drops the concentration of sulphuric acid rose, and eventually this process led to the formation of an acidic solution of high concentration. Low amounts of environmental humidity together with leaf transpiration could serve as further solvent for this acid. The repeated accumulation of acid solutions could have preceeded leaf degeneration.

Leaves of various species gave differing reactions to acid solutions. In the case of *Phaseolus aquilinum,* as well as *P. vulgaris* and *Helianthus annuus,* the processes were remarkably similar (Evans et al., 1977). In these species the process started with the epidermis cells, eventually leading to damage in some of the inner tissues. As a rule, repeated acid rains affected both the neighbouring epidermal and the lower lying mesophyll cells. In turn, the tissue necroses of collapsed cells led to the formation of new cavities in the leaf surface. Once these had formed, they probably served as additional collecting "dishes" for further acid rain. The more acidic solutions gathered in these the greater was the damage to the surface epidermis, the palisade and spongy parenchyma.

In leaves of *Q. palustris,* however, the repeated application of acid rain brought about hypertrophic and hyperplastic reactions in the mesophyll. This led to the formation of leaf surface tumours. In the case of *Q. palustris,* the formation of lesions and tumours was similar to the process described earlier for clones of *Populus* sp. (Evans et al., 1978). Thus the initial damage was the collapse of surface epidermal cells, followed by the deformation of lower lying mesophyll tissues. The formation of leaf tumours in *Populus* sp. can equally be ascribed to hyperplasy and hypertrophy of palisade and spongy parenchyma cells. In *Q. palustris,* however, tumour formation was caused by the abnormal processes taking place in the spongy layer.

On the basis of these experiments, Evans and Curry (1979) stated that the different species can be classified in terms of their susceptibility to acid rain. In *P. aquilinum, P. vulgaris* and *H. annuus* leaf tissues the process of damage formation was similar (Evans et al., 1977). In these the appearance of the first small lesions could be observed within 24 hours of the first acid rain treatment of 2.7 and 2.5 pH. In the various clones of *Populus* sp. the first damage appeared only after 3 days of treatment, of 6 minutes duration, each with a 2.7 pH solution (Evans et al., 1978). In the tissues of *Q. palustris,* however, no lesion developed before applications of 2.5 pH acid rain over 13 days.

Numerous reports suggest that the needles of conifers are rather tolerant to simulated acid rain. After reviewing all results of experiments on *Betula* sp., *Acer* sp., *Populus* sp. and *Quercus* sp. Evans and Curry (1979) concluded that the foliage of deciduous trees suffers greater damage than that of the conifers. Authors suggest these results allow the assumption that the broad-leaved trees of North America will be more susceptible to acid rain than the Scandinavian forests consisting mainly of coniferous species.

Herbaceous species such as *P. aquilinum, G. max, P. vulgaris* and *H. annuus* (data on the latter two are from Evans et al., 1977) are highly sensitive to simulated acid rain. By contrast, leaves of *Tradescantia* sp. and *Populus* sp. (Evans et al., 1978) suffer less visible damage. Among all the species studied, the foliage of *Q. palustris* showed the least damage. This suggests that the leaves of herbaceous species are more susceptible to the simulated acid rains than the foliage of woody plants.

Evans and Curry (1979) represent the opinion that in *Q. palustris* the great degree of resistance to acid rain can be ascribed to the hyperplastic and hypertrophic reactions going on parallel with the collapse of palisade cells in the spongy parenchyma (Fig. 50). Enhanced cell division and cell enlargement lead to the formation of tumours that raise the leaf tissues above the former epidermis level. As a result, the acid precipitation tends to flow off from these surfaces. In *P. vulgaris, H. annuus* and *P. aquilinum* the hyperplastic and hypertrophic processes are weak or simply absent. In these species subsequent rain may collect in the hollows of the leaf surface (Fig. 51). This will tend to enhance the formation of lesions. All this implies that leaf tissue responses in different species play a significant role in the frequency of development and the extent of lesions.

Clearly the above authors have produced significant results in the selection of both species suitable for indication and symptoms characteristic of environmental impacts. In the indication at the anatomical level two observations are an advance. First, acid precipitation primarily affects the leaf surface while acid gases affect the mesophyll. Second, it can be assumed that the shape and surface of leaves significantly influence the susceptibility to acid precipitation. Because of differences in leaf shape herbaceous species are the most susceptible while the coniferous trees are the most tolerant to acid rain.

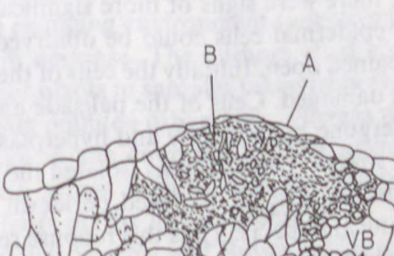

Fig. 50 — Tumour located next to the vascular bundle (VB) in the cross section of a *Quercus palustris* leaf. Both the epidermis (A) and palisade cells (B) are collapsed. Hypertrophy (C) and hyperplasy (D) of the cells of spongy parenchyma are apparent (after Evans and Curry, 1979)

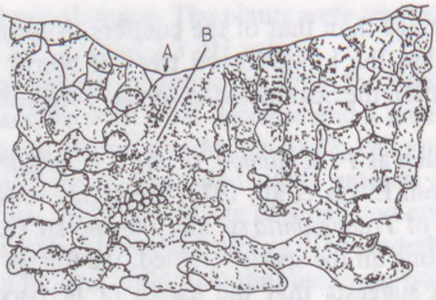

Fig. 51 — Lesion in the cross section of a *Pteridium aquilinum* leaf. The adaxial epidermis (A) is completely shrunk. The supporting tissues (B) above the vascular bundles—stained deep—have also died. The palisade and spongy parenchymatic, as well as vascular bundle tissues are intact (after Evans and Curry, 1979).

Papparozzi and Tukey (1983) studied the anatomical changes brought about by simulated acid rain in *Betula alleghaniensis* and bean. Following a two day 2.8 pH and a four day 3.2 pH simulated acid rain treatment, yellow and yellowish brown lesions appeared on the leaf surfaces of both species. Most lesions were between the smaller veins. The trichomes remained generally unaffected. The lesions were formed above the collapsed epidermal and strongly plasmolyzed palisade parenchyma. Above the damaged epidermal cells the epicuticular wax layer had remained intact.

The discovery of the mechanism of tolerance, and not the aspects of indication, increases the value of investigations into the histological characteristics of species tolerant to acid rain. Such studies allow one to sort out the basis of viability of positive indicator populations under such extreme conditions. For example Adams et al. (1984) studied the effects of simulated acid rain on the leaf tissues of *Artemisia tilesii*. This species is a herbaceous plant of the Northern Arctic. Its characteristic property is its increased tolerance to atmospheric acidity. Thus a simulated acid rain of 2.5 pH brought about only minor macroscopic modifications in its leaves. In the cross sections of leaves, however, there were signs of more significant differences. Lesions consisting of 1–3 collapsed epidermal cells could be observed, and in the damaged regions the stomata had remained open. Initially the cells of the upper epidermis, later the underlying tissues, were damaged. Cells of the palisade and spongy parenchyma around the lesions had undergone hypertrophy and hyperplasy, resulting a decrease in the extent of intercellular spaces. These regions isolated the affected domains from the neighbouring healthy tissues, just as damaged periderm protects itself against pathogenic fungi and mechanical impacts. Thus this reponse reaction might be one of the means of protection against acid rain.

Elliott et al. (1982) studied the effects of fluid fluoride on the foliage of *Cordyline terminalis*. Dissected, 10-node-long, terminal shoots of the plant were placed in ammonium hydrogen difluoride solutions of differring concentration. The raising of temperature and fluoride concentration enhanced the damage. At 29 °C and a fluoride concentration of 3 ppm the rate of damage was four times greater than at 18 °C. The deterioration of mesophyll, as with other acidic solutions, was preceeded by serious epidermal damage.

The effects of acidic gases (nitrogen dioxide, sulphur dioxide, hydrogen fluoride)

The first step in the investigation of the tissue-specific effects of gaseous pollutants was probably made by Solberg and Adams (1956). Their experiments showed that in leaves the primary damage caused by fluoride and sulphur dioxide can be observed in the cells of spongy parenchyma and the lower epidermis. This is followed by the deformation of the cells of palisade parenchyma.

Later on, owing to the increasing degree of forest deterioration, more and more researchers studied the impacts of air pollution on tree anatomy. As an example, Percy and Riding (1981) exposed the needles of *Pinus strobus* to sulphur dioxide of low concentration. Subsequently, the needles were submitted to histological and histochemical analysis. In line with Solberg and Adams' (1956) conclusions they also found that the cell damage was confined to the parenchyma of mesophyll. In the cells studied, the extent of protoplast death varied according to the extent of damage. In these cells the carbohydrates and plastids accumulated along the cell walls. The total protein content, especially the amount of proteins containing sulfhydryl groups, decreased. In the plasmalemma-cell wall region, the phospholipids gave a less intensive colouration.

Uzunova et al. (1981a) also carried out leaf anatomy investigations. Under laboratory conditions they studied the effect of sulphur dioxide on the mesophyll of *Picea abies* and *Pinus sylvestris* species. Then, as a comparison (Uzunova et al., 1981b), the experiment was carried out close to a metal works but this time on *Picea abies* and *Pinus nigra*. In the laboratory it was the mesophyll of *Pinus sylvestris* but in the vicinity of the works that of *Picea abies*, that proved more sensitive to gaseous pollutants.

Examples of plant adaptation to the environment can be cited from Sharma and Butler's (1973) investigations. The basis of such adaptations can be twofold. Either novel traits arise by mutations or rare genes are selectively favoured and increase in the population. Consequently, observations gained in the study of plant adaptations are suitable to indicate the effects of long-term environmental pollution. Nonetheless, the number of recognized cases of this type is relatively low. No doubt, most frequently a population adapts itself by way of its existing genetical resources. Traits (alleles) more adaptable to the changing environment (selective pressure) will increase while unfavourable traits will decrease. The authors cited studied the differences in the epidermis of white clover plants in samples taken from two differently polluted areas. One of them was a highly polluted urban area, whereas the other was the environs of a scarcely polluted lake in the countryside. The microscopic study involved the following measurements: stoma number and size, guard cell number, and the frequency and length of hairs on the lower epidermis.

It is also noteworthy that Sharma and Butler (1973) studied the characteristic fine pattern of the epidermis, a trait that readily reacts to environmental changes. Wolcsánszky (1972) and Turcsányi (1977) also showed that an unfavourable environment (e.g. nutrient or water deficiency or gamma-irradiation) brings about an increase in the stoma as well as hair number per unit area on a reduced leaf area. However, in the case under consideration the results indicated that the stoma frequency decreased in line with the increasing degree of pollution. In the polluted environment this change, no doubt, could be ascribed to an increase in the number of plant individuals "more efficient in the air exchange"; despite and because of having less stoma. In contrast to the effects of nutrient deficiency causing changes lasting merely one generation, this study revealed an adaptation persisting in subsequent generations. In Sharma and Butler's study (1973), the stoma density had decreased both on the upper and lower leaf surfaces. In the various regions, however, the size of stomata showed minor differences.

Hair frequency also displayed obvious correlations with air pollution. High hair frequency was observed primarily on the lower leaf surfaces of samples taken in the polluted industrial area while on the upper epidermis none of the samples showed such a significant number of formations. Presumably the principles of genetic adaptation are also valid in this case. The hairy leaf surface probably forms a filtering layer between the polluted air and the stomatal openings, thus inhibiting the penetration of pollutants into the mesophyll. Simultaneously, this layer exerts a shading effect on the cells, thus lowering the temperature of leaf tissues. This temperature decrease plus the direct shading effect could influence the rate of chemical reactions. This in turn may limit the damaging effect of environmental pollution on leaves.

The two populations also differed in terms of hair length. Samples from the polluted area had very long, 95 μm trichomes while those, from the unpolluted area had 82 μm long hairs. The longer hairs might increase the shading effect and probably filter out foreign particles, too.

In samples from the polluted areas, both necroses and leaf chloroses were observed. However, these can be ascribed to the direct effect of air pollutants.

The effects of ozone and PAN

Evans and Miller (1972) exposed *Pinus ponderosa* clones growing under natural conditions to a 12 hour · day^{-1} · 0.45 ppm ozone treatment. In current year needles they observed histochemical modifications. The photosynthetically active (chloroplast-containing) palisade parenchyma cells proved to be most susceptible to ozone. Consequently the formation of a chlorotic pattern could be observed in the leaves within 35 days. In other cell types the impact of ozone could not be detected during the same period. Thus *P. ponderosa* differs from *P. strobus*, in the leaves of which Linzon (1967) observed the primary damage of the transfusion tissues. Within the mesophyll of the same leaf the extent of cell damage was different. The outer cells of mesophyll proved to be most vulnerable. In spite of this cells in the direct vicinity of stomata had not suffered greater damage than other cells of the mesophyll. On the abaxial side the damage appeared earlier than in the adaxial parts of the leaf. The cells that deteriorated last were located between the resin ducts and the endodermis. The greatest damage was suffered by cells in the tip of the needle.

Effects of ozone and PAN were also described in the comprehensive survey by Claussen (1975) which showed that in dicots the primary effect of ozone can be observed in the chlorophyll-rich palisade parenchyma. The neighbouring epidermal and spongy parenchyma cells remain intact for a long time. Ozone damage appears only when the deterioration of palisade parenchyma is extensive. In the presence of two or three palisade layers, it is most frequently the outer layer that is damaged.

The peculiarity of palisade cell damage lies in the fact that, as in the case of bifacial leaves, the ozone penetrates through the stomata of lower epidermis. This would mean that, in order to reach the palisade parenchyma, ozone first has to penetrate through the chlorophyll-poor spongy parenchyma. According to Claussen (1975) it is not yet fully understood why just the palisade cells are so susceptible to ozone.

The formation of chlorotic and necrotic spots is brought about by the collapsing of palisade cells. Should deformations of this type be also accompanied by the appearance of a dark spotting, this is probably caused by the colouration and thickening of cell walls.

In leaves of gramineous species, and other monocots, ozone damages first the perivascular parenchyma cells. Other cells of the mesophyll may be damaged also. In such cases the chloroses extend to the entire cross section and can be diagnosed as colorations on both sides of the leaf.

In almost complete harmony with Evans and Miller's (1972) observations, Claussen (1975) states that the histological damage of ozone in the needles of conifers commences with the collapsing of plicate parenchyma cells located in the vicinity of stomata. The only difference between the two observations is that Evans and Miller (1972) did not find the parenchymatic cells in the vicinity of stomata to be more susceptible than the rest. Nonetheless, Claussen (1975) also remarks that the plicate parenchyma cells in the vicinity of endodermis are most susceptible. He also observed the formation of "water saturated spots". The necrotic spots and stripes evolving from these, denote large groups of damaged plicate parenchyma cells. Initially, the transfusion tissue remains intact, later on it starts shrinking and becomes yellowish-brown.

Claussen (1975) first reported that PAN affects the spongy parenchyma first. As with ozone, the epidermis and vascular bundles are damaged last. Following staining with thionine, Sudan-III or Sudan-black this sequence can be studied in detail. The stains quickly penetrate damaged cells, leaving intact cells unstained.

The first visible "water saturated spots" develop from the epidermis, raising swellings on the abaxial side of the leaf. The origin of these swellings is that certain cells in the vicinity of stomata become water saturated thus pressing the stomata outwards. Simultaneously, the guard cells swell, making the stomatal apperture greater. Soon the entire leaf becomes turgescent. It can be concluded that, in contrast to ozone, the PAN penetrates the stomata, initially damaging the cells that surround the stomatal cavity. These cells absorb the total amount of PAN with such rapidity that the deeper lying cells remain intact.

Under certain conditions PAN affects the deeper lying parenchyma cells. It can be assumed that this is caused by the inactivation of plasmodesmata connecting spongy cells with the damaged parenchyma cells.

In species of the family Poaceae, the longitudinal stripes observed can be ascribed to the location of stomata. The phenomenon is evidently the result of necrosis in areas covered by stomata.

Two remarkable traits characterize the anatomy of fern leaves. First, they do not have palisade parenchyma. Second, owing to the size and branching of the cells of spongy parenchyma, the intercellulars are greatly enlarged over the whole leaf. Therefore, via the intercellulars, PAN spreads easily and rapidly, damaging all parts of the leaf.

We conclude that, for both PAN susceptibility and widespread usage in indication, two leaf factors should be considered. First, the presence of active (viable) stomata, second the abundance of intercellulars. These are best combined in ferns.

Complex effects

From the viewpoint of indication one has to pay special attention to the complex effects of gaseous pollutants on plant tissues. The main reason for this is that in nature the pollutants generally occur in complex combinations. Therefore, in order to indicate the complexity of their effects, to separate the effect of individual components, or to resolve controversial cases, it is frequently necessary to rank the pollutants according to their degree of destruction. Surprisingly, although technically less complicated than the cytological methods, histological studies have proved to be more useful in distinguishing the effects of different gaseous pollutants. This can be ascribed to two factors: the differing accessibility of tissues to gases and the differing sets of organelles in cells with their differential susceptibility.

Krol et al. (1982) carried out histological studies on *Melilotus albus* and *Plantago lanceolata* subjected to the combined impact of nitrogen dioxide, ozone and sulphur dioxide. In spite of the fact that both species have similar amphystomatic leaves with underdifferentiated mesophyll, the macroscopic and histological symptoms were distinctly different. The whitish necroses to be observed in the leaves of *Melilotus albus* differed significantly from the bright and bronze colouration of *Plantago* leaves.

These reactions were ascribed to different emission types. Thus, the white, spot-like necroses on the surface of the *Melilotus* leaves resembled the damages caused by ozone. This was verified by histological analysis. Initially chlorophyll-containing cells, mainly cells of the palisade parenchyma, were destroyed. In contrast the tissues of the vascular bundles, as well as the epidermis above the damaged mesophyll, had remained intact.

Symptoms of *Plantago lanceolata,* however, resembled much more those of the so-called PAN or oxidation symptom. Mainly mesophyll and the epidermis cells around the stomatal cavity (including the guard cells of stomata) were damaged. The process of differentiation of certain epidermis cells (e.g. stoma cells) was also disturbed. This eventually gave rise to leaf deformities. The oxidation syndrome is caused either by the photochemical reaction of NO_x and olefine or by the products of the dark reaction of ozone and olefines or by certain compounds of the PAN family. The results of Krol et al. (1982) indicate that in certain cases similar symptoms can also be caused by less complex gas mixtures.

Seemingly sulphur dioxide and nitrogen dioxide enhance the damaging effect of ozone. In plants with amphystomatic leaves, where the three gases have an access to the leaves from both sides, these symptoms are especially marked. Consequently, in such cases, more than the cells surrounding the stomatal cavity are damaged.

By applying anatomical methods Krol et al. (1982) failed to resolve the question as to the cause of difference in the response of *Melilotus albus* and *Plantago lanceolata.* Our current understanding is clearly incomplete.

In order to compare the visible and histological effects of sulphur dioxide and ozone, as well as the two in combination, Evans and Miller (1975) used the needles of a *Pinus ponderosa* clone susceptible to gaseous pollutants. The needles were in the phase of intensive elongation. In treatments lasting 9 hours, 0.45 ppm concentrations of the individual gases were applied both separately and simultaneously. The sulphur dioxide and sulphur dioxide plus ozone treatments were soon followed by both histological and visible damage in the proximal regions of the needles. Presumably, this damage is mainly the result of sulphur dioxide. The impact of ozone was, however, displayed in the precipitation of cytoplasm and cell organelles in the peripheral regions of damagad parenchymatic cells. The ozone damage that developed only a few days after the appearance of sulphur dioxide symptoms, was located mainly at a 10–15 mm distance from the distal end of needles. Sulphur dioxide dissolved the cell components of practically all cell types (parenchyma, epidermis, hypodermis, epithelial cells of resin ducts and vascular bundle tissues).

The results, of both these and other preliminary investigations carried out by the same lab, prove that in *Pinus ponderosa* the damage caused by gaseous pollutant and by winter can be distinguished both visibly and histologically. Among the gases, ozone only damages the parenchyma cells. It causes the compaction of cytoplasm in the peripheral regions of these cells. Up to the point when the entire mesophyll is fully destroyed, this gas is not indicated by specific reactions (Evans and Miller, 1972). In general, the first effects of ozone appear in the mesophyll cells of the adaxial side. Long-term gas treatments can in turn lead to damage in the adaxial part of the mesophyll.

According to Evans and Miller (1975) the needle damage caused by smog in the Los Angeles basin, California, cannot, either morphologically or histologically, be distinguished from that caused by ozone. Consequently, these symptoms are likely to have been caused by this gas. In sharp contrast to the symptoms characteristic of ozone, in the winter-damage formation of necrotic spots the endodermis, hypodermis and mesophyll cells are all involved. In addition, in needles showing such necrotic spot abnormalities may also appear in the vascular bundles (especially in the phloem). However, they never occur in the resin ducts or the ripe xylem elements. So these spots can be readily distinguished from those caused by air pollution.

Results by Solberg et al. (1955) indicated that the histological symptoms caused by fluoride differ from those described for ozone, sulphur dioxide, or winter damage. In contrast to ozone, before destroying the mesophyll, this compound damaged both the endodermis and the phloem. Similarly, fluoride damage, unlike sulphur dioxide damage, does not manifest as hypertrophy and hyperplasy. In these cells the gas dissolves the components of cytoplasm.

All these observations demonstrate that it is possible to develop histological methods by which damage caused by different air pollutants and by winter can be detected and distinguished, even before the appearance of visible symptoms.

The above conclusion seems to contradict observations made by Stölzer (1983) with *Tilia platyphyllos*. Under the impact of de-icing salt (Cl^- ions) application and SO_2 pollution, he observed differences in the development and shape of the leaves, whereas the cell damage was similar in both cases. The first unfavourable damage always appeared in the bundle sheath parenchyma.

Mishra (1982) studied the morphological aspects of environmental damage of industrial origin in the leaf epidermis of *Commelina benghalensis*. He found that in the districts studied the plants exhibited an increase in hair, as well as stoma frequency while the size of stomata had decreased. In our view, as with the changes observed by Sharma and Butler (1973), these symptoms probably result from the selective adaptation of plants growing in an environment near industry.

Frolov et at. (1984) compared the anatomical features and pigment content of urban (street) and forest trees. They established that unfavourable effects of the urban environment were reflected both in the anatomical structure, as well as chloroplast size and chlorophyll content of linden and oak trees but not to an equal extent. Leaf anatomy, above all the thickness of leaves, had not changed significantly. The most characteristic anatomical symptom was that the street trees were more xeromorphic than those growing in the forest. This is primarily indicated by the smaller intercellulars.

The most important conclusion to be drawn from the paper of Frolov et al. (1984) is that the formation of anatomical damage in leaves can be strongly influenced by the season and the age of trees. Therefore, these damage symptoms can only be used for indication with proper circumspection. The decomposition of chloroplasts accompanying leaf senescence was for example, enhanced by the unfavourable conditions prevailing in the street.

According to our unpublished observations, in *Robinia* trees growing in the vicinity of the pharmaceutical works Biogal, at Debrecen, Hungary, the cell layer underlying the upper and lower epidermis showed yellowish colouration. In a control sample collected in Gödöllő, Hungary, this colouration could not be observed. Seemingly, in the plants of Debrecen origin the protoplasm of the subepidermal parenchymatic cell layers had undergone an autolysis which was precocious for the season. The lumen of those cells, in which the organelles could be recognized, was also filled by vacuola.

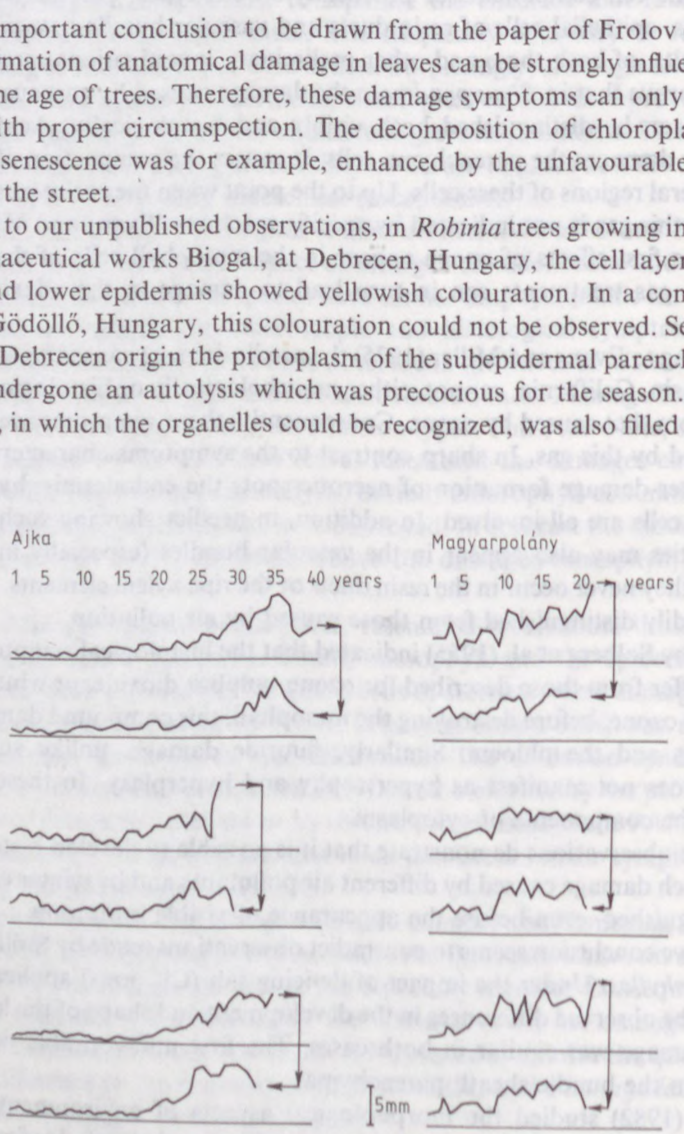

Fig. 52 — Annual ring diagram of 3–3 *Pinus nigra* individuals studied at locations with polluted (Ajka) and unpolluted air (Magyarpolány) (Turcsányi, 1986).

The mycelia appearing on the surface of the epidermis also showed either precocious senescence or greater susceptibility to pathogens.

In addition, the leaves of the plants growing in the vicinity of Biogal works were thinner and more compact than those gathered in Gödöllő. Also, under the lower epidermis a tightly-packed, palisade parenchyma-like cell layer could be observed. However, the underlying spongy layer was thicker than the corresponding layer in the Gödöllő sample.

A series of experiments carried out at Ajka (Turcsányi, 1986) has also highlighted the symptom of premature senescence. In these experiments we studied the effects of intensive, complex air pollution on the dendrochronological, leaf histological and morphological traits of *Pinus nigra*. Based on the study of annual rings (Fig. 52) it was established that in the last 20 years (even when assuming identical height) the urban trees assimilated approximately 40% less organic matter than the control trees growing in the countryside. The study of both leaf tissues and morphological traits proved that the leaves of plants exposed to intensive air pollution underwent a premature senescence and abscission. Also a proportion of young subsidiary branches had dried off prior to the appearance of green canopy. In the leaves the signs of senescence included the accumulation of crystals in the transitional cells as well as cell wall deformations in the transfusional cells. The fact that colouration started in the phloem of vascular bundles led to the conclusion that from among gaseous components of the air, the damage might have been primarily caused by fluoride.

Xylem damage in tree trunks

There is no other aspect of plant anatomy that has been applied more frequently in the indication, or the long-term survey, of environment pollution than the study of annual rings. Regularities in the formation of annual rings, indication possibilities of annual ring studies, their limits or the effect of environmental factors on the formation of annual rings, are discussed in detail by Schweingruber (1983). He stated that "The anatomy of annual rings is among others the reflection of the physiological processes of the trees. The shape and dimensions of cell walls, the number and size of cells, as well as the rate of individual elements furnish information on the growth conditions of the tree."

From the point of view of indication, the most important trait of the tree trunk is the variation in the thickness of annual rings. Depending on the habitat and environmental impacts, the thickness of annual rings varies. Their chronological sequence, like historical documents, indicates the previous growth conditions of the tree. For a long time the analysis of annual rings had been almost exclusively used in production studies in forestry or to assess the age of living as well as felled trees to be used by the building industry. It is only lately that the method has been extended both to the study of trees damaged by environmental factors, and to the documentation of damage in street trees caused by the environmental pollution.

In biological indication, however, it is not merely the analysis of annual rings that has found widespread application. According to Yokobori and Ohta (1983) and Schweingruber (1983) the methods used in the study of environmental effects on xylem structure can be grouped into 3 categories. The first category comprises the so-called dendrochronological studies, dealing exclusively with the thickness of annual rings. The second is the group of xylochronological observations carried out mainly by X ray densitometry. In addition to the thickness of annual rings, these measurements extend also to the density of wood of annual rings. Finally, the third method is the microscopic analysis of the anatomical structure of the tree. According to Keller (1980a) each method is able to track the changes taking place in the xylem, though none is able alone to explain the cause of these changes.

Annual ring studies provide an excellent, easily accessible means for the application of an anatomical method in a monitoring network for vast territories (mainly in the temperate belt and subarctic regions; see Schweingruber, 1983). This method faces the same problems as all other methods. Climate and soil have a great impact on the formation of annual rings, independently of any environmental pollution. The map-like illustration of the results of a monitoring network, as well as biometrical analyses can, nonetheless, provide great help in distinguishing the various effects.

It seems that environmental pollution affects annual ring formation by two routes, via the air and via the soil (Schweingruber, 1983). With regard to the air, so far mainly the effects of various gases and fumes have been studied. As to the soil, mainly the impacts of soil acidification, heavy metals, as well as de-icing salt application have been analyzed.

The effects of soil pollution

De-icing salts exert their effect on street trees through the root system. The sodium and chloride ions absorbed by roots are stored in the xylem, from where, at the time of budbreak and subsequently (especially at the time of the leaf elongation) they are transported into the canopy (Höster, 1979). Large amounts cause scalds (necroses) in the leaves. In turn, as a result of this damage, the cambial activity of the trunk and its tissue-differentiating activity is significantly damaged, too.

Annual ring diagrams of damaged trees clearly indicate a decrease in the annual ring thickness, resulting from the application of high salt doses. The impact of several simultaneously or consecutively acting, detrimental factors is frequent with street trees. The effects can be additive or greatly increased, leading (in extreme cases) to the death of the tree. The discovery of abrupt decreases in the annual ring thickness, might render it possible to forecast imminent damage before canopy damage is obvious. In addition, the curves of annual ring thickness might provide data on the intensity of damaging factors in former years.

According to observations in Germany, de-icing salts and other detrimental factors directly affect the two most frequent urban tree species, the linden (lime) and the maple. It has also been established that the horse chestnut is similarly susceptible (Höster, 1979). Significantly less damage occurs with the plane tree; while the oak, *Robinia* and *Sophora japonica* are highly resistant. Systematic studies of these species show that the former have diffuse-porous while the latter have ring-porous wood.

In trees with diffuse-porous wood (e.g. in linden and maple) the cambial activity starts 14 days after budbreak, at the earliest. In Germany in the environs of Münster, this means the middle of May, a time by which the leaves have already unfolded (Höster, 1979). Since at this time the enlarging leaves supply a relatively high auxin concentration, the inner tracheae of the annual rings are generally somewhat larger in diameter than those forming later. Starting in June, leaves of trees damaged by de-icing salts begin to turn yellow, with dark brown marginal necroses appearing. Consequently, from the middle of June (about two months prior to the usual date) the cambial activity is visibly decreasing. In undamaged trees it is in June and July that its mitotic activity culminates. Although the cells in the thin annual rings of damaged trees maintain their differentiating activity for a longer period, owing to the almost total cessation of assimilate transport from the tree crown, their walls remain unusually thin.

The histometric analysis of thin annual rings forming in a polluted environment has shown that in such annual rings the increase of water transport tissues is markedly greater than in normal cases. This increase can be ascribed to the significantly increased number of tracheae, though their diameter has decreased. The formation of these two traits (i.e., trachea number and trachea diameter) are unrelated. The tracheae are the products of the division of cambium, whereas their final size is determined by the available amount of auxin in the subsequent process of differentiation.

As the leaves remain smaller, and they also bear necroses, it is probable that their auxin production would soon diminish, thus advancing the formation of late xylem. Certain signs suggest that the increased number of tracheae indicates the reaction of cambium to stress.

In trees with diffuse-porous wood, as a result of the precocious curtailment of lateral-stem growth only 25% of the species-characteristic water-transporting surface is formed. Since it is the outermost 6–10 (–20) annual rings that participate in water transport, apart from a reduced growth which lasts only a few years, even a low rate (of 1–6 m·hour^{-1}) water transport does not cause significant problems in the water supply to the tree crown. It is only the long-lasting, subsequent formation of thin annual rings that may lead to an inadequate water-supply. We suggest it is this change in the annual rings, lasting several years (and associated with precocious leaf abscission), that could be effectively used in indicating environment pollution. Any change in the xylem structure of these tree species (e.g. a decrease in annual ring thickness or an increase in the rate of small pores) indicates a deterioration of favourable conditions for deciduous trees, and is therefore very suitable for indication.

Höster (1979) illustrated his ideas with observations on juvenile maple trees. With an undamaged juvenile tree the mean of annual ring thickness amounted to 4–6 mm. In the severe winter of 1962/63 a greater quantity of de-icing salt had been applied. This gradually reduced the mean of annual ring width to approximately 1.5 mm. Normally with such an annual ring width the renewal of the tree is generally still possible. The effect of salt loading, however, lasted longer, primarily because of the permanent accumulation of sodium chloride in the soil-plant system. Thus, during the last four years of the life of the trees the values of annual ring width decreased to 0.51 mm, 0.28 mm, 0.20 mm and finally 0.12 mm. At this stage the water supply collapsed, the accumulated nutrients had been consumed, and the tree died.

In contrast to this, trees with ring-porous wood (e.g. the oak and *Robinia*) have highly specialized transport tissues (Höster, 1979). In them the cambium becomes active at an early stage, about 14 days prior to sprouting (in the environs of Münster this is at the beginning of May). At the time of leaf unfolding the first water transport vessels are perfectly ready for functioning. By the end of May, i.e., 4 weeks later, about 60–70% of the total water transport surface of the normal annual ring width has been formed.

Although in these tree species it is always the youngest annual ring that serves water transport, owing to the width of early xylem bundles the rate of water transport is still about 10 times faster than in trees with diffuse pores. In the urban environment it is this high rate water transport that provides a significant advantage; provided the water supply of the soil is ensured.

It is a further favourable characteristic of trees with ring-porous wood that their root system penetrates deep into the soil. Should, in spite of this, these trees lack an adequate water supply for a longer period, as with a sudden fall of groundwater level (which might, for example, be the consequence of building activity) they will undergo significant damage or eventual death.

Apart from protecting against air-embolia, the storage function of the parenchyma envelope surrounding the tracheae of large volume (so-called paratracheal contact-parenchyma) to be found in trees with ring-porous wood performs a further important physiological function. Its cells are probably able to store a major proportion of sodium and chloride ions. Parenchyma envelopes of this kind are especially apparent in the xylem of *Robinia*. Towards the end of its period in foliage the large volume early vessels get clogged with thyllises, and no longer participate in transport processes. The ions stored in the parenchyma envelopes are hardly detectable in the water transport system of the subsequent year.

This might be one of the reasons for the observation that *Robinia* hardly suffers from salt damage. From this point of view plane trees, planted ever more frequently in the urban environment, have a transitional position. Although being trees with diffuse pores, they seem to resemble trees with "semiring-porosity". They are less resistant to de-icing salts.

Höster (1977) reviews a comprehensive study, by Aslanboga, carried out within the territory of urban Hannover. Aslanboga evaluated the results of annual ring investigations into 80 tree individuals of the genera *Acer, Tilia, Aesculus, Platanus, Fagus* and *Quercus*. The annual ring diagrams obtained were compared with similar data for undamaged trees in the vicinity. The detrimental factors she considered were mainly the following: the application of de-icing salts, the leakage of natural gas, soil compacting and soil flooding, as well as ditch digging near the root system.

In all trees damaged, they established a conspicuous decrease in the width of annual rings, frequently leading to the total deterioration of the tree. Typical was the case of a maple *(Acer pseudoplatanus)* growing in an area of turf near the city hall. Within the area of its root system there had been a large kiosk with an open storage facility, until 1964. The trunk-boring studies indicated that in 1964 the tree responded to the impact of soil compaction caused by the building activities (plus some root damage) by strongly reduced growth. In the subsequent years there was a slight increase in the radial growth, though the size of the annual rings never reached the former dimensions. The slow increase in the annual xylem formation indicated that the plant gradually adapted itself to the new growing conditions.

Höster's (1977) results unambiguously show that, in comparison with the thicker rings of trees in a normal area, in the narrower annual rings of damaged deciduous trees the frequency of tracheae and longitudinal parenchyma cells had visibly increased. Accordingly, in the annual rings the frequency of fibres is significantly less while the rate of ray cells shows slight variations. Perhaps, at the beginning of the period of renewed foliage, the cambium functioned normally while around June, the formation of necroses and leaf abscissions led to the precocious cessation of normal activities. As a result the late xylem, having more tightly packed tracheae and a higher frequency of fibres, had not developed. Höster (1977) considers it most important that in studies of this sort one should determine the beginning and end of cambial activity, of cell differentiation, and of the phenological phases.

Höster (1977) raised a further, most important, question. This concerns the generally applied height of sampling (1.30 m). In the case of street trees, it is at this height that trunk damage is the most frequent (e.g. as a result of car accidents or the sticking of bills). This damage may be followed by fungal infections. In addition, in the lower regions of the trunk of trees with diffuse- and ring-porous wood, the onset of cambial activity is different (in trees with diffuse-porous wood it generally commences later).

In the view of Petersen et al. (1982) it is primarily the effect of de-icing salts that is responsible for the large-scale damage to urban and highway trees in the last decade. In their investigations they measured annual ring width, xylem structure and the amount of sodium and chloride ions in the individual annual rings, in order to characterize the degree of vitality of trees growing in Hamburg. This method rendered it possible to establish the beginning, the course and the intensity of frost damage, and obtained information on the effect of salt application on the vital functions of the tree.

Though not an anatomical method, still the chemical analysis of annual rings as a means of indication is worth mentioning. While on the basis of the salt content of annual rings Petersen et al. (1982) drew conclusions as to the salt load of trees, Legge et al. (1984) used chemical analysis of the very same wood elements to evaluate assumptions on the soil response to acid air pollution. Based on the observations of a pine hybrid (*Pinus contorta* Loud. × *Pinus banksiana* Lamb.) the latter authors came to the conclusion that the concentration of certain elements (Si, Cl, As, Cu, Zn, S, Fe, Ni, Al and partly Cr) in the annual rings carries information on changes taking place in the soil, whether under the influence of natural environmental conditions or when especially affected by the deposition of the sulphuric acid from the air. Other elements (such as P, Mn, K, Rb, Ca and Sr) did not exhibit similar fluctuations. Its probable cause is that these elements are assumed to have a more significant biological role than their role in the soil's chemistry.

The effect of air pollutants

The investigation, by annual ring analysis, of the air polluting effects of unknown emittants throws up several problems (Abetz, 1985). Among the most important is the selection of appropriate controls. Both the species and the age, as well as the characteristics of the habitat, might also have a significant influence on the results.

Keller (1980b) applied the method of annual ring analysis in the study of potted, old spruce grafts. The plants treated permanently with sulphur dioxide (0.05, 0.1 and 0.2 ppm) were kept in boxes in the open. The sulphur dioxide treatment had been carried out for 10 weeks, from April to July. The CO_2 uptake was periodically monitored by an infrared-gas-analyzer. Following the treatment the plants were kept in a nursery until the end of November.

In the next phase, in order to carry out the X ray measurement of annual ring width, the author prepared sections from the stem. A density limit of 0.5 g·cm^{-3} was established to distinguish the early wood from the late wood (probably this explains why his results differ from those published by Grill et al., 1979, discussed later). In the course of the treatments he observed that the carbon dioxide uptake significantly decreased prior to the visible manifestation of damage. From the 7th through the 10th week of the treatment the relative carbon dioxide uptake and the number of cells in the radial rays of annual rings showed strong correlation. Under the impact of higher sulphur dioxide concentrations the annual ring width decreased. The spring treatment primarily reduced the amount and density of late wood (Fig. 53). The decrease of wood production could even be observed prior to the appearance of visible damage (Fig. 54).

According to Keller (1980b) the finding that, in comparison to late wood, the annual ring width and density of early wood differed only slightly from the control, can be readily explained. The formation of early wood is a function of both stored nutrients and more intensive photosynthetic activity at the beginning of the growth season. The fact that increased carbon dioxide uptake could be observed only after a few weeks' treatment also supports this interpretation. By this time the greater proportion of the wood had formed. Calculating wood production on the basis of average density and annual ring width, Keller (1980b) concluded that if one omitted the wood density, the decreased productivity due to sulphur dioxide application is slightly underestimated.

Consequently, sulphur dioxide concentrations that do not cause visible damage in spruce may already inhibit the uptake of carbon dioxide. Decreased cambial activity is also a symptom of reduced photosynthetic activity. All these factors indicate that in the analysis of the effect of air pollutants, the visible symptoms must not be regarded as the sole criteria.

Thompson (1981) compared five *Pinus monophylla* populations by the dendrochronological method. His aim was to distinguish the effects of climate from those of a copper smeltery on annual ring formation. Two of the populations

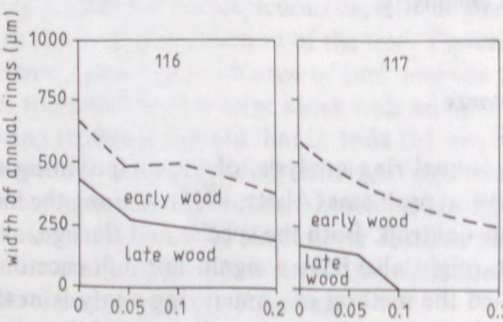

Fig. 53 — The effect of SO_2 treatment on the ratio of early wood and late wood, in the annual rings of two spruce clones. Broken line indicates the entire width of annual rings (Keller, 1980b).

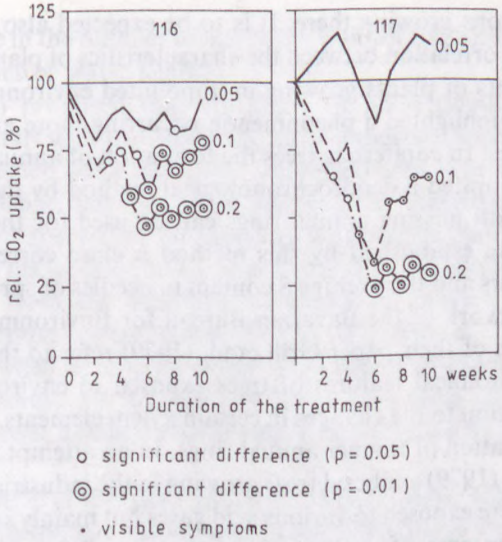

Fig. 54 — The relative CO_2 uptake of two spruce clones in the course of SO_2 treatment (100% stands for the control). The absolute values have been subjected also to a statistical analysis; these do not comply with the relative values indicated above (Keller, 1980b).

were in the vicinity of the smeltery while three situated at a distance served as control. Within a 320 km radius the copper smeltery was the sole source of air pollution. Among the gases emitted by the smeltery, sulphur dioxide and nitrogen oxides played the greatest role.

Using a biometric method Thompson (1981) proved that in the five *P. monophylla* populations the chronology of annual rings supplied information on the climate also. The rather high standard deviation values of annual ring width in all five populations, reflected this fact. Nevertheless, the first rank chronological autocorrelations in the two polluted populations (0.490 in the first, 0.429 in the second) were much higher than those in the control chronologies (0.315 in the third, 0.240 in the fourth and 0.258 in the fifth population). This indicated that the growth of the former group of trees might have been influenced by a long-term, nonclimatic factor.

Until 1908, the starting date of smelting, all chronologies show a rather close, positive correlation. Since that time, however, the correlation decreased between the chronology of trees of the nearest location and trees located at a distance from the smeltery (the 0.921, 0.780, 0.591 and 0.624 correlation coefficient values between the 1st population and populations 2–5 fell to 0.485, 0.432, 0.382 and 0.299 for the years 1908–1964). The analytical results, therefore, indicate that the growth of the population of the nearest location to the smeltery had been influenced by air pollution, too. However, the investigation had also made it clear that air pollution could significantly modify the growth-influencing effect of climate.

In our view, however, the results of the above investigations have a much greater significance from the viewpoint of indication. As compared to the autocorrelation of populations outside the affected area, in the polluted area the extreme modification of the environment no doubt increases the autocorrelation of several characteristic traits of the populations growing there. It is to be expected also in other cases that there will be a closer correlation between the characteristics of plants in polluted areas than between the traits of plants growing in unpolluted environments.

Elling (1987) has highlighted a phenomenon occurring more and more frequently in the last few decades. In coniferous trees the formation of annual rings is occasionally omitted. He elaborated a dendrochronological method by the help of which the frequency of trees with missing annual rings can be used for the estimation of the extent of damage. He established by this method a close correlation between the extent of damage in firs and the average S content in needles of spruce trees (measured by the indicating network of the Bavarian Bureau for Environment Protection).

In the introduction of their paper Grill et al. (1979) refer to the previous practice of reviewing the anatomical features of trees exposed to environmental pollution, without paying attention to the changes in certain xylem elements. Most authors only emphasized the formation of thinner annual rings. In an attempt to make up for this deficiency, Grill et al. (1979) analyzed trees growing in the industrial regions of Higher Styria. These trees were exposed to various acid gases but mainly sulphur dioxide. The species showing symptoms of extreme damage were: *Picea abies, Larix decidua, Quercus robur, Populus tremula* and *Betula pendula.*

Based on their results, they established that the decrease in annual ring width in Gymnospermae is brought about primarily by a reduction in the amount of early wood, and to a lesser extent by late wood. The consequence of this is that while in the damaged spruce trees early wood contributed, on average, 70% to the annual ring width, in all the Gymnospermae of the polluted area this value fell to about 35%.

They also established that, under the impact of air pollution, the conifers do not form transitional tissues between the autumn and spring wood. Thus, these two annual ring tracks were separated by a more or less conspicuous boundary line. As a result of the dominance of late xylem tracheae over early tracheae, in Gymnospermae the average number of tracheae had increased by 55%. As to the relative amounts of early and late wood in Angiospermae, no similar differences could be established. Only in oak had the number of macropores decreased to the value of one.

The length and diameter of tracheides in Gymnospermae, especially in the early wood, had in most cases become reduced. Primarily, it seems, the cell lumen was reduced along with a relative increase in cell wall thickness. In spite of this, compared to the control, a slight thickening could be observed in the outermost late wood of Gymnospermae that had suffered the greatest damage. The authors claimed that the wood elements forming under the influence of pollution resembled the xylem developing in the case of water deficit.

Among the wood elements of poplar and birch, the tracheae, tracheides and wood fibres had become shorter, and a simultaneous significant decrease in the diameter of these elements could also be observed. In oak, for example, the thickness of wood fibres exceeded by 30% the values for the control. Parallel with the increase of wall thickness the cell volume had decreased. Smaller cell diameters brought about an increase in the number of tracheae, tracheides and wood fibers per 1 mm^2.

The implication of the wood-anatomical changes discussed above are poorly known. Certainly the decrease in the frequencies of both early tracheides and macropores is likely to inhibit optimal water supply, just as this can be the consequence of wall thickening and the accompanying decrease in the lumen of transport vessels.

The slight increase in the number of pits in the walls of tracheides can only moderately ease the deficiencies of water supply.

Treshow (1968) proposed that the study of plant populations exposed to air pollution should start with the dominant, most important species. Thus Treshow and Steward (1973) carried out a study in a watershed area, where the dominant species were exposed to ozone damage. Their observations underlined the assumption that when the dominant species are damaged then the entire plant community is significantly impaired. This leads on to the possibility that, by applying appropriate mathematical models, from the behaviour of dominant species one can estimate the behaviour of the entire system as modified by air pollution. This idea was exploited by Knabe (1981), when he used the values of needle generations to infer the ecological condition of the forest. He was able to follow long-term changes in so far as the needles of spruce trees, dominant in the population affected by pollutants, represented at least 6 years.

Arndt and Wehrle (1982) also relied on these results, when in Siegerland they carried out investigations in the environs of an abandoned iron ore roasting works, in order to study the impact of sulphur dioxide on oak (*Quercus* sp.), the dominant species of the local forest. Using borings from different populations, they obtained heartwood for microscopic measurements to determine the width of annual rings. They used multiple-regression analyses, in which the raw ore production was tabulated as a function of the indirect degree of sulphur dioxide emission. They established that climate and sulphur dioxide emission accounted for about 80% of the variation in annual ring widths. The partial coefficients of regression also rendered it possible to estimate how much tree growth might have occurred in the absence of pollution. They concluded the emission had brought about a growth reduction of about 25%. They also discussed the correlations between the growth in thickness of recuperating oaks and the condition of the entire forest, as affected by the closure of the works in 1965.

Applying annual ring analysis, X ray densitometry, histometrical analysis and various biometric methods, Greve et al. (1985) have established that the HF sensitivity of spruce tree individuals is very variable. Changes in the main parameters of wood closely correlate with the degree of needle damage.

Kartusch and Halbwachs (1985) also studied the impact of HF on wood, in individual alders exposed to different levels of stress. Under stress the number of both rays and ray cells increased while the height of rays decreased.

Today annual ring analyses are applied in the indication of the detrimental effects of environmental pollution, on a large scale. Examples are provided by the following experiments. In a search for the causes of forest devastation in the Rhone Valley, Flueherl et al. (1981) also applied the method of annual ring analysis. 80% of the 177 trees studied showed abrupt and irreversible growth retardation. In the authors' view, in addition to drought, this retardation was due to a change in the fluorine emission after 1938.

Vins et al. (1982) also applied annual ring analysis in the study of emission effects in the Jizerske Mountains, Czechoslovakia. Similarly, Gemmill et al. (1982) made use of annual ring chronology in order to assess the detrimental effects of ozone air pollution on a South Californian forest. By dendrochronological analyses Eckstein et al. (1983) have succeeded in proving that in the southern parts of Germany fir devastation had not been caused by the dry summers. They suggest that long-term air pollution might have been the underlying cause of tree devastation. Yokobori and Ohta (1983) studied the relative annual ring thickness, the maximum and minimum wood density within the annual ring, and relative average wood density of *Pinus densiflora* in an area heavily contaminated with a mixture of air pollutants (sulphur dioxide, ozone and nitrogen oxide). These parameters indicated a close correlation with the degree of air pollution, at the level of 98% confidence.

Studying the "smog-illness" of *Pinus ponderosa* in the forests of California, Williams (1983) established a closer correlation between the abscission of needles and the rate of air pollution than between the latter and annual ring growth. According to him, the underlying cause was the consumption of storage nutrients.

In contrast to several other studies, are Keller's (1984) observations on the beech. A 0.075 ppm sulphur dioxide treatment brought about an immediate formation of large lumen water transport cells in the late wood, as the plant attempted to maintain its water supply in spite of the reduced water supply. According to the author, it is indisputable that a small tree with thin walls and large-lumened cells can be easily broken by snow. This can reduce the competitiveness of such individuals, and can lead to a shift in the age structure within the population of a species.

Greve et al. (1986) carried out a dendrochronological and radio-densitometric study of wood production and wood quality in spruce, at 28 habitats, in Northeastern Bavaria. They established that the emissions reduced wood growth. However, a deterioration of wood quality and thus a more restricted range of potential uses, could not be proven.

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12 The effect of pollution on the physiological processes in plants (The fundamentals of plant physiological indication of pollutants at individual level)

The indication of different pollutants (indicanda) at physiological level is based on the recording of the physiological responses to pollution (indicators) by plants. The effects of the most important pollutants on plant physiological processes are considered below. Our aim was to illustrate the characteristics of the changes in the main physiological processes caused by frequent pollutants. This approach did not necessitate a full account of all the works published in this rather extensive field and the space allotted did not allow this.

Pollutants through changing physiological processes in individual plants affect growth, development, production and reproduction, and also the tolerance to environmental and competition stresses. As a result these physiological changes, together with other biological changes manifested at individual level, will influence the ecological processes at the supra-individual level also (e.g. succession or degradation in communities). In fact it is the supra-individual level which provides reliable information about pollution. In order to obtain such data, however, one cannot leave out of consideration the background processes which occur at the individual level.

Air pollutants are partly of natural origin. They are emitted by volcanoes (different oxides, H_2S, ash), hot springs (S compounds), marshes and fens (carbohydrogens, H_2S), evergreen vegetation (photo-oxidants formed from terpenes in photochemical processes), bacteria (H_2S), decaying vegetation (H_2S), oceans (salt), lightning (ozone) and soil particles carried by wind is also a form of air pollution.

However, air pollutants of natural origin are of negligible importance if compared with the amount of pollution caused by human activities. The main polluters today are industry, power stations, households and cars using fossil fuels.

A large amount of sulphur dioxide is emitted by power stations, factories and households combusting fossil fuels (coal, petroleum) and also by the petrochemical industry, iron smelters, cement factories, glass foundries and waste-burning works, etc.

Nitrogen oxides are produced in great quantities by fertilizer mills, vehicles, power stations, industry and households, the petrochemical industry, iron smelters and waste-burning works, etc.

Power stations, iron smelters, aluminium and glass foundries, brick-works, cement factories, ceramics works and waste-burning works are responsible for emitting flourides into the atmosphere.

HCl is released by power stations, fertilizer mills, rubber works, petrochemical works and waste-burning works.

Petrochemical works and paper mills are the main H_2S emitters.

The main components of photosmog, ozone, PAN and aldehydes are formed in the presence of sunlight (UV radiation) from the combustion gases of petrochemical works and cars (CO, NO_2, unsaturated and aromatic carbohydrogens). This type of smog is called Los Angeles type while in the London type smog the main constituent is SO_2).

Some of the above-mentioned pollutants can directly harm plants, while gases such as SO_2 and nitrogen oxides are capable of forming acids, which will damage plants in the form of wet deposition, but partly through making the soil more acidic. SO_2 particles with other aerosol particles can also be deposited on dry days as "dry deposition".

Heavy metal pollution is also of atmospheric origin in most cases. Heavy metal is carried in the form of particles 1–10 μm in diameter from the emitters to the plants. Unlike gaseous pollutants and aerosols in the atmosphere, heavy metals are not carried over large distances, and their impact is mainly detected close to the polluting source.

12.1 SULPHUR DIOXIDE

Sulphur dioxide is considered one of the most important pollutants, since it is very common and can be transported over large distances and then deposited. In addition, SO_2 and its solute form play an important role in the S metabolism of plants.

Clean air contains approximately 2–8 ppb SO_2. The value for areas with a median SO_2 load is between 200–300 ppb (max. 500 ppb) while the concentration of SO_2 in heavily polluted industrial areas and in cities with heavy industry may reach values of 1400–1500 ppb.

Most of the research work aimed at investigating the damage to plants caused by SO_2 was carried out by exposing the plants to short-term (less than 8 hours) fumigation with high concentrations of SO_2 (200–700), not under conditions reflecting everyday *in situ* conditions (longer than one day and with lower concentrations of SO_2 of 25–250 ppb).

Stomatal response

SO_2 mainly enters plants through the stomata, significantly altering their normal function. According to the literature the changes in the function of stomata after short-term exposure to SO_2 are of various kinds. Sometimes it results in opening the stomata, thus increasing the amount of SO_2 absorbed (Majernik and Mansfield, 1971; Black and Black 1979; Black and Unsworth, 1980). In some cases SO_2 closes the stomata, decreasing the further uptake of the pollutant but also inhibiting CO_2 uptake (Winner and Mooney, 1980a,b; Carlson, 1983a; Taylor et al., 1986).

Changes in stomatal conductance in long-term fumigation experiments were not found (Klein et al., 1978; Rao et al., 1983; Chevone and Yang, 1985).

According to Black and Black (1979) stomatal guard cells are much more tolerant to SO_2 than the adjacent subsidiary cells. This is mainly because guard cells have a better protection of cuticular origin. Subsidiary cells are damaged, even at low concentrations of SO_2, to such an extent that they often die; causing a drop in turgor pressure and resulting in the opening of stomata. At high concentrations of SO_2 guard cells, together with other epidermal cells, are also damaged, and they ultimately lose their ability to function properly.

The impact of SO_2 on stomatal function depends on the species in question, the concentration of SO_2 (Majernik and Mansfield, 1971, 1972), and also on the relative moisture content of the air (Noland and Kozlowski, 1979) and on temperature (Taylor et al., 1985).

The higher sensitivity of C_3 plants to SO_2 is partly due to their higher stomatal conductance (Winner and Mooney, 1980c). Growing C_3 and C_4 plants in an environment containing higher concentration of CO_2, the stomatal resistance in C_3 plants will considerably increase while that of C_4 plants will remain largely unaffected. Therefore the tolerance of C_3 plants to air pollutants will increase and will reach and sometimes exceed that of the C_4 plants (Carlson and Bazzaz, 1982).

Sulphur metabolism

SO_2, after entering cells through stomata, dissolves and gradually forms sulphuric acid. This process is largely influenced by the pH of the protoplasm and also by its buffering capacity. The taking up of SO_2 results in the formation of H^+ and according to the prevailing pH, either HSO_3^- or SO_4^{2-} ions (Silvius et al., 1975). Thus SO_2 by causing pH to decrease inside the cell, also has an indirect damaging impact on the normal functioning of the cells because at low pH certain enzymes are inhibited and that may lead to the death of the cell.

Most of the absorbed and dissolved SO_2 is directly oxidized by the sulphite-oxidase formed in the mitochondria and then stored in the vacuoles. The sulphate content considerably rises in the shoots after 24 hours exposure to SO_2 while such change is not measurable in the roots even after long-lasting fumigation (Maas et al., 1987b). The accumulating amount of sulphate is in close correlation with the length of exposure and the concentration of SO_2 (Faller et al., 1970; Maas et al., 1987b). SO_2 uptake is also influenced by temperature, more sulphate is accumulated at higher temperature.

A small proportion of sulphite (10% at a maximum) is, equally rapidly, reduced to sulphide which is either emitted as sulphurated hydrogen (De Cormis, 1968) or accumulated by building into amino acids in the form of SH groups (Grill et al., 1979; Chiment et al., 1986; Maas et al., 1987b). This reduction is thought to take place in the chloroplasts with the help of an enzyme called sulphite-reductase. The process is assumed to affect the photosynthetic electron transport in plants. This view is supported by some researchers who found that H_2S was formed in the presence of light

(Silvius et al., 1976; Anderson, 1980). There is, however, a way independent of light since dark SO_2 fumigation can also increase the sulfhydryl content in plants (De Kok et al., 1981; Maas et al., 1987a).

In the presence of atmospheric SO_2 even short-term fumigation causes an increment in the amount of water-soluble, nonprotein sulphydryl compounds. Maas et al. (1987b) found that at a SO_2 concentration of 0.25 ppm the SH content trebled in spinach after 24 hours. In normal light conditions plants contain mainly glutathione (γ-L-Glu-Cys-Gly; GSH) but the amount of cysteine is also significant (De Kok et al., 1983, 1985, 1988; Chiment et al., 1986). Buwalda et al. (1988) found glutamyl-cysteine in relatively higher amounts in plants exposed to SO_2 fumigation in dark.

Accordind to Grill and Esterbauer (1973) the SO_2 induced GSH accumulation can be toxic in Norway spruce. However, later investigations did not find correlation between the amount of GSH in plants and the reduction in plant growth (Maas et al., 1985). It can be assumed that GSH serves as a temporary 'storage compound' for the excessive reduced S, thus preventing the toxic accumulation of cysteine (Rennenberg, 1984; De Kok et al., 1986).

Photosynthesis

The effects of SO_2 on photosynthesis become apparent after rather short periods of exposure. In most species a concentration of 200–400 ppb atmospheric sulphur dioxide content will inhibit CO_2 uptake. Inhibition at lower concentrations in most cases is due to the growing conditions applied. Taniyama (1972) in his experiments exposed *Hordeum vulgare* to SO_2 using fumigation chambers made of PVC. Inhibition was detectable at a concentration of 100 ppb. It should be noted that PVC emits dibutyl phthalate and chlorine in the presence of SO_2, which considerably influences the results (Hardwick et al., 1984). Black and Unsworth (1979) grew broad beans (*Vicia faba,* cv. Dylan) in growing chambers. They found that the intensity of photosynthesis was reduced after a mere two hours of SO_2 fumigation at a SO_2 concentration of 35 ppb. This increased sensitivity might have been caused by the low light intensity during the experiment. This is supported by Mansfield and Jones (1985) who found that in plants kept at lower light intensity the intensity of photosynthesis was more inhibited than in those grown at high light intensity. Darrall (1986) also used broad bean (cv. 'Blaze' and 'Three Fold White') in his experiment, and inhibition was detectable at a SO_2 concentration of 300 ppb only.

When the amount of sulphur readily available for the plant is limited, in the short run SO_2 fumigation at low concentrations can cause an increase in net assimilation, and the long-term effects can be manifested in higher crop yield. This is undoubtedly due to the improved supply of sulphur. The results of *in vitro* experiments showed that in isolated chloroplasts 1 mM sulphite increased both ferricyanide reduction and CO_2 fixation. An additional 2 mM sulphite still had a stimulating effect on ferricyanide reduction, however, CO_2 fixation suffered a check through the inhibition of the activity of the enzyme ribulose-1,5 biphosphate carboxylase/oxygenase (Libera et al., 1973). The maximum of CO_2 uptake was achieved at a sulphite concentration of 0.25 mM. The upper limit of SO_2 concentration which is still beneficial to the plant depends on a number of factors such as species, age and environmental conditions.

Few data concerning long-lasting fumigation are available. According to this kind of experiment, the effects are dependent on the concentration of SO_2, the length of treatment and, for repeated short-term applications, on frequency.

Continuous fumigation, just like short-term treatment, causes a drop in the intensity of photosynthesis if SO_2 exceeds the threshold value. The inhibition is more pronounced in the second photoperiod. Treatments repeated at a couple of hours intervals have an accumulated inhibitive effect (Matsuoka, 1978). This accumulative effect was not found in experiments, where plants were exposed to SO_2 for longer periods of time (days, weeks) (McLaughlin et al., 1979; Takemoto and Noble, 1982).

Takemoto and Noble (1982) found, in another experiment where *Pisum sativum* plants were treated twice a day, that net photosynthesis after the second exposure was higher in the second photoperiod than in that of the control plants. Taylor et al. (1986) studied the behaviour of sensitive and insensitive varieties of *Geranium carolinianum* in their fumigation experiments. The specimens were fumigated periodically for 196 days at a SO_2 concentration of 450 ppb. The sensitive variety responded with a 26% drop in net photosynthesis while in the insensitive plants CO_2 fixation increased by 28%.

It is characteristic of the effect of SO_2 fumigation that the inhibition of photosynthesis rapidly grows in the first two hours of the treatment then levels off (Matsuoka, 1978; Black and Unsworth, 1979). Thus the extent of damage is determined mainly by the concentration of SO_2 and it shows correlation with the time during the first two hours only. This also means that one cannot estimate the extent of the damage on the basis of the value of the calculated atmospheric dose (concentration × length of time).

Lichens exhibit high sensitivity to atmospheric SO_2 pollution (Hill, 1971, 1974; Puckett et al., 1973; Le Blanc and Rao, 1975). A mere 5 ppb can seriously damage some species, and even tolerant species cannot put up with more than 30–50 ppb SO_2. (The threshold value for flowering plants is 200–300 ppb.) It was assumed that the inhibitive effect of SO_2 in lichens was more pronounced than in flowering plants. However, ribulose-1,5 biphosphate carboxylase/oxygenase in lichens did not prove to be more sensitive to SO_2 than in spinach (Ziegler, 1977). Since tolerance is similar at the enzymatic level, the cause of the high sensitivity of lichens is probably due to morphological and physiological differences which abet SO_2 uptake.

Photosynthesis is more inhibited in C_3 plants than in C_4 plants. Sulphite with respect to bicarbonate can check carboxilase enzymes in both types of photosynthesis through competitive inhibition (Ziegler, 1972, 1973). Though PEP-carboxilase in C_4 plants has a greater affinity to bicarbonates than ribulose-1,5 biphosphate carboxylase/oxygenase in C_3 plants, so that SO_2 affects photosynthesis in C_4 plants less. For C_4 plants are capable of higher photosynthetic activity at lower stomatal conductivity than C_3 species, and their photosynthetic activity remains independent of the SO_2 induced stomatal changes until these changes considerably influence the amount of SO_2 entering the mesophyll.

Morphological differences in the leaves of C_3 and C_4 plants can also have an effect on the higher tolerance to SO_2 in C_4 plants: chloroplasts in C_3 species are dispersed throughout the mesophyll while in C_4 plants they are concentrated around the vascular bundles, which ensures an extra protection against atmospheric pollutants.

SO_2 affects both the initial and maximum values of the light-photosynthesis curve. The initial phase, which features quantum efficiency, is decreased in its growth and so is the maximum; so that it can be deducted that SO_2 pollution inhibits the activity of carboxilase enzymes and also the light gathering process (Matsuoka, 1978; Winner and Mooney, 1980b; Carlson, 1983a). There is also a difference between C_3 and C_4 plants in this respect. According to Winner and Mooney (1980c), both quantum efficiency and the maximum decreased in the C_3 *Atriplex triangularia* while in the C_4 *A. sabulosa* only the maximum was affected. The discrepancy was mainly due to the different bicarbonate affinity.

Air pollution is usually accompanied by chlorotic symptoms in the leaves. It should be borne in mind, however, that SO_2 fumigation does not have an effect on the chlorophyll content in the leaves (Arndt, 1971). The formation of phaeophytin *in vivo* can only be observed at toxic levels of SO_2 (LeBlanc, 1969). A drop in clorophyll content occurred in treatments with very high SO_2 concentration or long-lasting fumigation experiments only (Rabe and Kreeb, 1980). This observation is also true for other pollutants. According to Lichtenthaler and Buschmann (1983) the pigment-protein complexes in coniferous and deciduous trees are considerably damaged by both gaseous pollutants (SO_2, O_3, NO_2) and heavy metals. They measured chlorophyll fluorescence and found a drop in the photosynthetic activity during the light period, even in those cases when total pigment content did not decrease significantly. Thus it can be assumed that the chlorophyll-a/carotene-protein complexes of pigment systems I and II are more rapidly decomposed than chlorophyll-a/b-protein complexes which form the majority of light gathering pigments.

Thus while chlorophyll content remains almost constant the amount of carotenoids (β-carotene, xanthophylls) will decrease significantly as compared to that of the control plants even in the early phases of pollution, when visual symptoms are not yet apparent. This characteristic is a reliable indicator even in early stages of atmospheric pollution (including SO_2) (Arndt, 1971; Rabe and Kreeb, 1980).

Short periods of SO_2 fumigation do not result in chronic damage to photosynthesis. After the cessation of fumigation regeneration will promptly begin if the extent of inhibition is less than 20%, and photosynthesis will reach the original level in two hours. Muller et al. (1979) found a 20% increase to the original level of photosynthesis for 24 hours in *Glycine max* after a full regeneration of a 17% inhibition. After higher inhibition which still does not cause visual symptoms, the repair period can last as long as 24 hours (Bennett and Hill, 1973).

Observations concerning regeneration of photosynthesis after long-lasting fumigation indicated that regeneration after 5 days of exposure can be complete in Scotch pine (Hällgren and Gezelius, 1982) while a 4–5 week long treatment can result in irreversible changes (Saxe, 1983).

Respiration

Some studies showed an increase in dark respiration of SO_2 treated plants. According to Keller (1957) 1.6 ppm (!) atmospheric SO_2 content caused a 30% increase in respiration in pine trees, which was followed by a decrease later. Black and Unsworth (1979) found a strong dependence of an increase in the intensity of respiration on SO_2 concentrations (35–275 ppb) in their investigations. Respiration decreased to its normal level after one light period. Some workers did not find any difference in the values of respiration before, during and after fumigation (Takemoto and Noble, 1982).

Libera et al. (1975) found a considerable accumulation of glycollate, which might have been due to the inhibition of glycollate-oxidase by SO_2. Though there are not available data, it can be assumed that too high accumulation of glycollate can cause a drop in photorespiration or in extreme cases can fully inhibit it.

Transpiration

SO_2 affects transpiration through influencing stomatal processes. Thus in some species SO_2 increases tanspiration and in others decreases it (Klein et al., 1978; McLaughlin et al., 1979; Black and Unsworth, 1980; Winner and Mooney, 1980a). There was no significant difference between the levels of transpiration in SO_2 treated (250 ppb) and untreated spinach plants—neither at daily average, light period average, nor at dark period average levels (Maas et al., 1987a).

Plant growth

Sulphur dioxide at low atmospheric concentrations can stimulate plant growth (Faller et al., 1970; Faller, 1972), though, in experimental conditions, SO_2 in most cases damaged plants. SO_2 at high concentration or in sensitive plants causes a drop in plant growth and yield (Hällgren, 1978; Linzon, 1978).

The sensitivity of species with regard to inhibition of growth varies widely. A 2 week long SO_2 fumigation at a concentration of 250 ppb resulted in a 20% reduction in the growth of *Glycine max;* while *Spinacia oleracea, Phaseolus vulgaris* and *Trifolium pratense* did not suffer a check in growth or yield at all, and the fumigation did not affect the shoot system/root system ratio (Maas et al., 1987a, c).

12.2 OZONE

Ozone is also an important, secondary pollutant characterized by heavy oxidizing properties.

Stomatal response

Stomatal response to ozone in plants differs considerably under a concentration of 200 ppb. Some react with increase conductance, others close their stomata (Olszky and Tibbitts, 1981a, b; Reich and Lassoie, 1984; Keller and Häsler, 1986) and the third group is formed by those which do not react significantly. At approximately 200 ppb or above most plants close their stomata. This complete closure also occurs at lower concentrations when a mixture of ozone and sulphur dioxide is present (Beckerson and Hofstra, 1979a, b; Olszky and Tibbitts, 1981a, b).

Photosynthesis

The inhibition of photosynthesis in different plants was achieved at O_3 concentrations of 100–500 in experiments applying short-term ozone treatments (Bennett and Hill, 1973; Carlson, 1979).

The inhibition of photosynthesis occurred at lower concentrations (35–200 ppb) in long-lasting exposures (Reich, 1983; Reich et al., 1986; Taylor et al., 1986).

Trees are usually less sensitive to ozone than herbaceous plants.

A mixture of O_3 and SO_2 is much more toxic in most cases than they are separately. Photosynthetic activity was much more reduced in plants exposed to the gaseous mixture at concentrations close to the threshold values than when the gases were applied separately (Carlson, 1979). The rate of photosynthesis in *Acer saccharatum* decreased by 21% and 22% at the application of O_3 and SO_2 at concentrations of 200 ppb, respectively. The effect of applying the two gases together at concentrations of 200 ppb was manifested by a dramatic 74% drop in the rate of photosynthesis (Carlson, 1979).

Regeneration after treatment also requires more time (approximately 24 hours) when a mixture of the two gases is applied. When the concentration of ozone exceeds 200–300 ppb, the changes causing visible damage are hardly reversible (Black et al., 1982).

Respiration

Ozone at concentrations of 100 ppb or above was found to stimulate dark respiration (Barnes, 1972; Pell and Brennan, 1973; Reich, 1983; Reich et al., 1986).

12.3 NITROGEN OXIDES

Plants are capable of tolerating relatively high concentrations of NO_2. While concentrations of 500–700 ppb NO_2 are considered as limit values, NO at concentrations of 125–175 ppb can have inhibitive effects on physiological processes.

Plant response to nitrogen oxides is highly dependent on the N state of the plant.

Stomatal response

Few workers have given accounts of stomatal response to nitrogen oxides. Natori and Totsuka (1984) found that stomatal conductance increased in *Euonymus japonica* during fumigation (NO_2, 100 ppb). Most other plants do not exhibit any response. A drop in conductance can only be observed above a concentration of 1 ppm. A mixture containing SO_2 can decrease conductance in some species. The extent of decrease is usually higher than for separate application of the constituent gases.

Nitrogen metabolism

Nitrogen oxides can also be absorbed in plant leaves. Atmospheric NO_2 can serve as an extra N source in areas deficient in nitrogen (Troiano and Leone, 1977; Srivastava and Ormrod, 1984; Murray and Wellburn, 1985; Rowland, 1986).

After entering the cell, nitrogen oxides become dissolved in extracellular water forming H^+, nitrite and nitrate ions. These ions will take part in N metabolism, where they will be affected by the activity of nitrite and nitrate-reductase enzymes. Thus atmospheric NO_x-s (depending on the N state of the plant which basically determines both N uptake and the reduction of nitrate) can change the N balance of plants (Rowland et al., 1985).

Photosynthesis

Photosynthesis appears to be rather tolerant to NO_x: the concentration which inhibited the process in short-term fumigation experiments was between 500–700 ppb (Hill and Benett, 1970; Saxe, 1986), for long-term fumigation these values were around 250 ppb (Capron and Mansfield, 1976).

A mixture of NO_2 and SO_2 is more effective in reducing the rate of photosynthesis than when applied independently. A concentration of 200 ppb NO_2 had no effect on the photosynthesis of *Glycine max,* the same concentration of SO_2 reduced it by 10%, and the mixture of the two resulted in a 22% drop in the rate of photosynthesis (Carlson, 1983b).

Respiration

Any change in the pattern of respiration requires higher concentration of NO_x than in photosynthesis. The results of studying the impact of NO_x on respiration are conflicting—in some cases NO_x were found to inhibit it and in others they stimulated respiration. Under a concentration of 1 ppm no effects were detectable (Saxe, 1986).

12.4 HYDROGEN FLUORIDE

Hydrogen fluoride is a most poisonous gas occurring in relatively small quantities in the atmosphere. Very low concentration of HF can cause serious damage in sensitive species. In spite of this, the number of papers concerning HF fumigation is rather low.

Stomatal response

The stomatal response to HF is more varied in different plants than to other air pollutants. High HF concentration (14–84 ppb) increased significantly stomatal resistance in several species in short-term (4 hours) fumigation treatment (Navara and

Kozinda, 1967; Poovaiah and Wiebe, 1973). However, low concentration of HF (0.6 ppb) applied throughout the whole growing season had no effect on *Citrus* species (Thompson et al., 1967).

Photosynthesis

In comparison with other gaseous air pollutants HF inhibits photosynthesis at very low concentrations (14–44 ppb) while in sensitive species a mere 1–6 ppb can cause damage. McCune et al. (1976) reported an inhibitive effect of HF applied for two weeks at concentrations of 2–2.6 ppb on the activity of photosynthesis in *Sorghum vulgare* cv. Martin's. The damage was temporary, unlike after a too long exposure at concentrations of 4.2–6 ppb.

Respiration

Dark respiration, just like photosynthesis, reacts in a very sensitive way to hydrogen fluoride. HF at concentrations of 30–50 ppb was found to cause an increase in respiration in most species. The threshold value was much lower in sensitive plants (0.38–2.7 ppb) (Hill et al., 1959; McLaughlin and Barnes, 1975).

12.5 HYDROGEN SULPHIDE

H_2S is strongly reductive, i.e., it readly reacts with other molecules. Therefore H_2S soon loses its phytotoxicity after having been released.

The physiological and biochemical background of the phytotoxicity of this chemical is still not fully understood in spite of the several papers dealing with the problem.

The phytotoxicity of H_2S relative to that of the SO_2 is controversial. In general, SO_2 is considered more toxic (Linzon, 1978; Rennenberg, 1984), though results supporting those views who think H_2S is more toxic were also obtained (Krause, 1979; Maas et al., 1987c). Krause (1979) found H_2S twice as more toxic for plant growth as SO_2 in his comparative experiments. Since the damage caused by the toxicity of these compounds to plants varies from plant to plant according to their sensitivity to SO_2 and H_2S, their relative phytotoxicity is not easy to determine.

Stomatal response

The effect of H_2S on stomatal functions is yet to be understood. A short period of time in a fumigation chamber does not harm stomata. Several hours of exposure to high concentration (above 2 ppm) of H_2S can result in the opening of stomata. Long-lasting fumigation may develop a slight openness of the stomata if not applied during the dark period of photosynthesis.

Sulphur metabolism

The absorbed H_2S in plants has the same effect on the sulphur metabolism in plants as dissolved SO_2 does. This is evident even at a concentration of 0.03 ppm (De Kok et al., 1983). Both the amount of water-soluble nonprotein SH compounds (Brunold and Erismann, 1974, 1975; De Kok et al., 1983, 1985, 1986; Van Dijk et al., 1986; Von Arb and Brunold, 1986; Maas et al., 1987, a, b, c; Buwalda et al., 1988) and of sulphate (Brunold and Erismann, 1974; Maas et al., 1985, 1987a) grow in the shoots of the plants, depending on the concentration of H_2S and temperature. An increase, though, at a significantly lower level can also be detected in the S content of the roots. Unlike SO_2 which causes a rise in the sulphate concentration within a day, H_2S affects the level of SO_4^{2-} only after long-lasting fumigation.

Glutathione accumulates in largest quantities in the leaves (De Kok et al., 1983, 1985, 1986; Rennenberg, 1984; Maas et al., 1987b, c) but cysteine is also formed if in somewhat smaller amounts (Brunold and Erismann, 1974; Van Dijk et al., 1986; De Kok et al., 1988). γ-glutamyl-cysteine was also detected in the leaves of spinach exposed to H_2S in dark (Buwalda et al., 1988). Light-induced changes occur in the S metabolism of plants. For example, when dark-treated plants are put into light, the dipeptids soon disappear while glutathione is synthetized in almost equal quantity. The light-induced γ-glutamyl-cysteine change is yet to be explained (Buwalda et al., 1988).

According to Van Dijk et al. (1986), the amino acid concentration doubled 48 hours after H_2S treatment while serine content in the plants decreased by 70%. However, H_2S did not seem to have an effect on the levels of asparagic acid, glutamine and glutamine acid. On the other hand, the amount of cysteine can even increase by 1400% with a simultaneous drop in the amount of serine, the precursor for cysteine. As Rennenberg (1984) put it, this high concentration of cysteine can be toxic to plant cells.

Photosynthesis

Photosynthesis can only be affected by high concentration of H_2S (0.25 ppm) and long exposure to it (more than 1 day). Oliva and Steubing (1976) found that the exposure of spinach to H_2S at a concentration of 0.8 ppm for 3 or more days inhibited photosynthesis while the treatment at a concentration of 0.4 ppm for 9 days was ineffective. Common bean has also endured a treatment at a concentration of 0.74 ppm for 4 days without its photosynthetic activity being damaged (Coyne and Bingham, 1978). Maas and De Kok (1988) studied the effects of H_2S fumigation in spinach and found that photosynthesis was inhibited at a H_2S concentration of 0.25 ppm.

The inhibition of photosynthesis at higher light intensity was more pronounced (Maas and De Kok, 1988).

At light saturation the maximum rate of CO_2 fixation showed a significant decrease with time. This was 23% lower for treated spinach plants after 14 days than for untreated ones. There was not, however, a drop in the amount of chlorophylls and carotenoids, and Maas and De Kok (1988) found even a slight increase in the case of spinach. Therefore a change in photosynthetic pigment content cannot be blamed for decreased photosynthesis.

H_2S treatment alters the shape of the light-photosynthesis curve. On the one hand, the maximum rate of CO_2 fixation decreases and on the other at the initial end of the curve the pace of the increment in CO_2 uptake is somewhat slowed down.

H_2S treatment induces a change in chlorophyll fluorescence, the extent of the change is proportional to the length of fumigation. The maximum level of fluorescence (P) is lower for fumigated plants while steady state level (T) is lower for untreated plants. A significant difference is found between the measure of quenching ((P—T)/P) after the maximum. This quenching is much lower after fumigation and independent of light intensity. A damage to photosynthetic electron transport can be assumed in fumigated plants. It should be added, though, that electron transport was found to be inhibited *in vitro* in cyanobacteria and the tobacco plant at sulphide concentrations of 0.1 mM (Oren et al., 1979) and 0.25 mM (De Kok et al., 1983), respectively. This H_2S fumigation at a concentration of 0.25 ppm is unlikely to affect photosynthesis directly (Maas and De Kok, 1988). Studies concerning plant growth also support this latter (see below).

Respiration

H_2S fumigation does not affect the dark respiration of leaves (De Kok et al., 1986, Maas and De Kok, 1988). The amount of O_2 taken up remained constant after 24 and 48 hours of fumigation in plants treated at a concentration of 0.25 ppm (De Kok et al., 1986) and the same level of CO_2 release could be measured even 18 days after fumigation in treated and control plants (Maas and De Kok, 1988). In their experiment Oliva and Steubing (1976) could detect a decrease in the intensity of respiration after 5 days fumigation of spinach leaves at a H_2S concentration of 0.8 ppm only.

Transpiration

A short period of H_2S fumigation caused an increase in transpiration only when applied in high concentration. In the long term fumigation increased the transpiration in plants (Oliva and Steubing, 1976; Steubing, 1979; Maas et al., 1987a).

Maas et al. (1987a) found that H_2S fumigation applied at a concentration of 0.25 ppm for two weeks brought about only a slight increase in the daily average value of transpiration, when the treatment was carried out during the light period. Transpiration remained unaffected when the H_2S treatment occurred during the dark period.

The hourly average of transpiration at night was lower in the control, and in dark period treated spinach plants, than during the day. The difference between the averages decreased for light period treated plants and disappeared for continuously fumigated ones. In the latter the identical values in transpiration at night and during the day were due to the increase in night time transpiration (Maas et al., 1987a).

The increase in the transpiration of plants is not accompanied by an increase in water uptake, thus H_2S can affect water relations in plants as a whole.

Plant growth

Just like SO_2, H_2S will increase plant growth and yield in places where soil is deficient in S or contains very little. This is true only when H_2S fumigation is applied at a concentration less than 0.1 ppm. Thompson and Kats (1978) found similar results for lettuce, sugar beet and lucerne when they applied H_2S at a concentration of 0.03 ppm. Their findings were supported by De Kok et al. (1983) for sugar beet and by Maas et al. (1987c) for *Phaseolus vulgaris* (H_2S cc.: 0.25 ppm).

Conversely, a decline was measurable in plant growth when fumigation was applied at higher H_2S concentrations. The degree of decline is dependent upon the species, the concentration of H_2S, the length of exposure and various environmental factors. For example, a 2-week-long exposure to H_2S of 0.25 ppm brought about a drop in the growth of *Trifolium pratense* and *Spinacia oleracea*. The same treatment left unaffected the growth of *Glycine max* and resulted in an increase of the growth and yield of *Phaseolus vulgaris* (Maas et al., 1987c).

The different responses in the above experiment were due to the different amount of H_2S taken up by the plants. Thus the amount of H_2S was the highest in *Trifolium pratense* and *Spinacia oleracea* and the lowest in *Phaseolus vulgaris*. Transpiration showed a similar pattern in all species.

Relative air moisture content (McLaughlin and Taylor, 1981) and temperature (Maas et al., 1987a) were found to affect plant growth. For instance, lower daily and night temperature meant an advantage in growth for *Spinacia oleracea* as compared with the growth and yield attained at higher temperatures (Maas et al., 1985, 1987a).

H_2S fumigation applied either solely during the light period or the dark period causes a drop in growth and yield which means that light does not have an influence on the effects caused by H_2S. Thus photosynthesis is not directly affected by H_2S uptake (Maas et al., 1987a).

In general, the inhibition of CO_2 fixation results in narrower stem/root ratio caused by the decrease in the carbohydrate transport to the roots (Wareing and Patrick, 1975). Such change in the stem/root ratio was not measurable after H_2S fumigation, though the rate of photosynthesis declined. This latter also support the view of those who do not believe in the existence of a direct relationship between H_2S fumigation and inhibition of photosynthesis (Maas et al., 1987a).

12.6 HEAVY METAL POLLUTION

Cadmium, copper, nickel, lead and zinc are the most important heavy metal pollutants, of which lead and zinc are considered the most toxic.

The sources of heavy metal pollution include the metal industry, car fumes, power stations, the household burning of fossil fuels, sludge, paints containing lead, discarded tyres (Cd), organic manuring (pig manure contains high amounts of Cu and Zn) and certain chemicals used in agriculture such as phosphatic fertilizers.

Heavy metal particles are transported as part of the coarse-particled aerosol and are deposited, during their journey, continuously on the surface of the soil and plants in decreasing proportion with the distance form the emitter source. Deposition occurs in the form of both wet and dry deposition.

The uptake and accumulation of heavy metals

Heavy metals unlike gaseous pollutants are taken in by plants through their root system. Heavy metals deposited on the surface of the leaves are washed into the soil by rainwater. The acidity of both the soil and rainwater have a considerable effect on the solubility of heavy metal oxides, which ultimately affects their availability for plants. There is usually a positive correlation between the acidity of the soil and the uptake of heavy metals through the roots (Hagemeyer et al., 1985). Apart from pH, there are several factors influencing heavy metal absorption, such as the cation exchange capacity of the soil, phosphate content, soil temperature and organic matter content (Koeppe, 1977).

The absorbed heavy metals are translocated into different parts of the plants and accumulate in accordance with their concentrations in the soil. The measure of accumulation and the value of the toxic threshold concentration are highly dependent on the species or sometimes the variety in question. Bazzaz et al. (1974), for example, grew maize and soybean in identical conditions and maize accumulated more lead (450 ppm) than soybean (151 ppm).

The accumulation of heavy metals in different plant organs also varies widely in different species and varieties. According to Van Assche et al. (1986) and Van Assche and Clijsters (1987), who studied *Phaseolus vulgaris,* the accumulation of Zn, Ni, Co and Cr was mainly characteristic of the stem and their amount was always higher in primary leaves than in secondary ones. Conversely, Cu had accumulated in the roots and could not be measured in the stems, even after applying so high a concentration of Cu that it caused a 50% reduction in growth with the simultaneous appearance of visual symptoms. Cd was found to accumulate in both organs but in larger amounts in the roots. Wiegel and Jäger (1980) measured 700 $\mu g \cdot g^{-1}$ dry weight and 20 $\mu g \cdot g^{-1}$ dry weight Cd in the root and the stem of the same species, respectively. Eersels's (1986) corresponding data were 910 and 20 $\mu g \cdot g^{-1}$ dry weight Cd.

The existence of a so-far-unspecified morphological and/or physiological barrier can be assumed that it does not allow heavy metals to accumulate in fruits and seeds. However, this barrier is not perfect, i.e., small amounts of heavy metals can sometimes be found in seeds and fruits. Pieczonka and Rosopulo (1985) studied the seed of wheat (*Triticum aestivum* L. cv. 'Jubilar'). The highest amounts of heavy metals were found in the embryo and the aleuron layer of the endospermium. The embryo contained mainly Zn and Cd, and in the aleuron layer Cu was dominant.

Metabolic effects

The activity of several enzymes is affected by toxic metal ions which enter the cells. Metal concentrations exceeding the threshold value increase the capacity, per mg soluble protein, of the following: glucose-6-phosphate-dehydrogenase (G6PDH), NADP dependent glutamic-dehydrogenase (GlDH), NADPH dependent isocitrate dehydrogenase (ICDHO), glutamate-OAA transaminase (GOT), NADPH dependent malic enzyme (ME) and peroxidase (POD), (Matthys, 1975, 1977; Lee et al., 1976a; Wiegel and Jäger, 1980; Van Assche et al., 1984, 1988). The extent of change in enzyme activity and the concentrations of accumulated heavy metals in the different organs of the plants show a close correlation. Van Assche and Clijsters (1986a, b), carried out a comparative study of isoperoxidase activity in the leaves and roots of *Phaseolus vulgaris*. In accordance with their previous results, specific isoperoxidase activity induced by Zn was found in the leaves only. Cu induced peroxidase activity characterized the roots only, while Cd had an effect both in the leaves and roots. The significance of the increased enzyme activity caused by heavy metals is yet to be understood.

The key enzyme in the Calvin cycle, ribulose-1,5 biphosphate carboxylase/oxygenase (RuBisCo) is, however, strongly inhibited by Zn, Ni and Co (Wildner and Henkel 1979; Christeller and Laing, 1979; Robinson et al., 1979; Van Assche and Clijsters, 1986b). For heavy metals inhibiting carboxylase activity only the ratio of carboxylase/oxygenase significantly decreases. The inhibition ceased *in vitro* after Mg^{2+} was added, which suggests that possibly Zn^{2+} had in part substituted Mg^{2+} ions in the RuBisCo-CO_2-metal^{2+} complex, reducing this way the CO_2 affinity of the enzyme.

The increase in the amount of free amino acids and reducing sugars in heavy metal polluted plants can also be in association with enzymatic effects. Total amino acid content was 48% higher in the leaves of birch trees growing at a distance of 10 m from a motorway with heavy traffic than in those of growing four times as far (Flückiger et al., 1978). It is still unclear whether the increase in amino acid content is brought about by a higher rate of decomposition of proteins or is a result of more intensive synthesis.

According to Flückiger et al. (1978) the increment in the amount of reducing sugars is due to the inhibition of the processes after snythesis (e.g. polycondensation) which are possibly caused by the inactivation of catalytic enzymes. This is supported by the fact that pollution-affected plants contain less starch and cellulose (Flückiger et al., 1978).

Photosynthesis

In some cases photosynthetic activity was increased by treatments with concentrations of heavy metals under the threshold value (Bazzaz et al., 1974, 1975). Yet, apart from rare exceptions, photosynthetic processes in plants are adversely affected by heavy metals. The extent of inhibition depends on the species and also on the concentration of heavy metals in the specimen (Bazzaz et al., 1974a, b, 1975; Carlson et al., 1975; Austenfeld, 1979; Van Assche et al., 1979, 1980).

Photosynthesis is partly influenced by the inhibition of the RuBisCo enzyme mentioned above. The change in fluorescence in affected plants is also significant and indicates a check in the photosynthetic electron transport process and in the light processes.

Cu is prone to forming chelates and so does it with chlorophyll molecules by substituting Mg. This causes a drop in fluorescence, for the formed Cu complexes are completely inactive photosynthetically (Arndt, 1974).

Zn, Cd, and Pb pollution has a repressive effect on photosynthetic electron transport, increasing fluorescence this way (De Filippis et al., 1981; Van Assche and Clijsters, 1986a). Photosystem II is more susceptible than photosystem I. It was found in isolated chloroplasts that inhibition caused by zinc occurred close to the reaction centre, possibly at PQ level (Baker et al., 1982). Van Assche and Clijsters (1986a) found zinc to inhibit *in vivo* water splitting in *Phaseolus vulgaris*. This was indicated by the lack of loosely attached manganese to the water-splitting side of photosystem II, which was induced by Zn. It is highly likely that manganese ions were substituted for zinc ions, since a fivefold increase of zinc was measurable in the thylakoids. The discrepancy between the results of *in vitro* and *in vivo* experiments was in part due to the much higher toxic threshold value in the concentration of Zn *in vitro*, and on the other hand, the incubation period was also much shorter *in vitro*.

The capacity of cyclic photophosphorilation is also diminished when Zn treatment is applied as a result of the inhibition of the electron transport chain.

Cd^{2+} like Zn^{2+} was also found to decrease, reversibly, the amount of manganese.

Unlike gaseous pollutants, heavy metals affect both the carotenoid and chlorophyll content in plants. Trees flanking the roads and streets in cities absorb high amounts of copper and as a result of the chelate forming capacity of this element the chlorophyll content is considerably lower in these trees than in those growing in unpolluted control areas (Tuba et al., 1981).

Carotenoid content in the leaves is decreased by Pb pollution. Flückiger et al. (1978) found the carotene content 53% lower, and the Pb level ninefold, in birch trees growing on a roadside compared with those planted 40 metres away. This finding is supported by results of Tuba et al. (1981) on pigment analysis investigations in city trees in Hungary.

Dark respiration

According to the few published results, the effect of heavy metals on respiration is negligible (Lee et al., 1976a, b; Van Assche et al., 1979).

Transpiration

The number of reports considering the effect of heavy metal pollution on transpiration is rather limited. Concentrations of Pb not higher than 150 ppm did not affect transpiration in soybean, while those above decreased it. An increase in stomatal resistance might be responsible for the simultaneous drop in the rate of photosynthesis (Bazzaz et al., 1974). Cd solution treatment, depending on the concentration of the solution, decreased transpiration and increased stomatal resistance in beech saplings (Hagemeyer et al., 1985).

Heavy metals accumulating in the roots (e.g. Cd) adversely affect water uptake (Carlson et al., 1975).

In general, heavy metals reduce plant growth and yield by inhibiting most physiological processes. Plants become chlorotic at higher concentrations and permanent loading eventually causes their death.

12.7 CHANGES IN SECONDARY METABOLIC PRODUCTS

Environmental pollution, especially SO_2 emission, causes an almost universal damage to secondary metabolism in plants (Ziegler, 1975). Terpence production in Norway spruce becomes less intense (Dässler, 1965). Splaeny et al. (1965) found glycobrassicine to decrease in cabbage, in spite of the fact that part of the absorbed S was built in this compound. They also found glycoside to diminish in wheat and oats and the wax content to increase in Norway spruce needles (Splaeny et al., 1961).

12.8 CHANGES IN REPRODUCTION

Though most of the investigations carried out have concentrated on the effects of pollutants on the vegetative organs of plants, there were some among the pioneers (e.g. Döpp, 1931) who studied the responses of the reproductive organs to pollution. Recently we have been able to witness the proliferation of such works. It is known that pollution decreases the ability of seeds to germinate or, for instance, that pine trees produce smaller cones and yield is reduced (Paluch, 1968; Houston and Dochinger, 1977; Roques et al., 1980). Pollen germination suffers damage and the pollen of Scotch pine cannot be stored for long after exposure SO_2 emission (Beda, 1982). The growth of the pollen tube proved to be more susceptible to SO_2 treatment than the germination of the pollen grain (Varshney and Varshney, 1981). According to Flückiger et al. (1978) the growth of the pollen tube of the tobacco plant can be used as a sensitive indicator of pollutants of traffic origin.

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13 The application of the information supplied by living organisms

Impact survey entails the registration of atmospheric pollutants in a particular area. The impacts can be surveyed by using either passive or active biological indicators. Species are selected in which the level of the pollutant and the degree of damage or change in function are correlated. In addition, the impact survey is an information system, providing information not only about the conditions of atmospheric pollution in a given area (polluted and less polluted areas can be distinguished) but also about the extent to which the sensitive species are damaged. On the basis of accumulating indicators one can estimate the extent to which the various elements (e.g. heavy metals) accumulate in the living organisms.

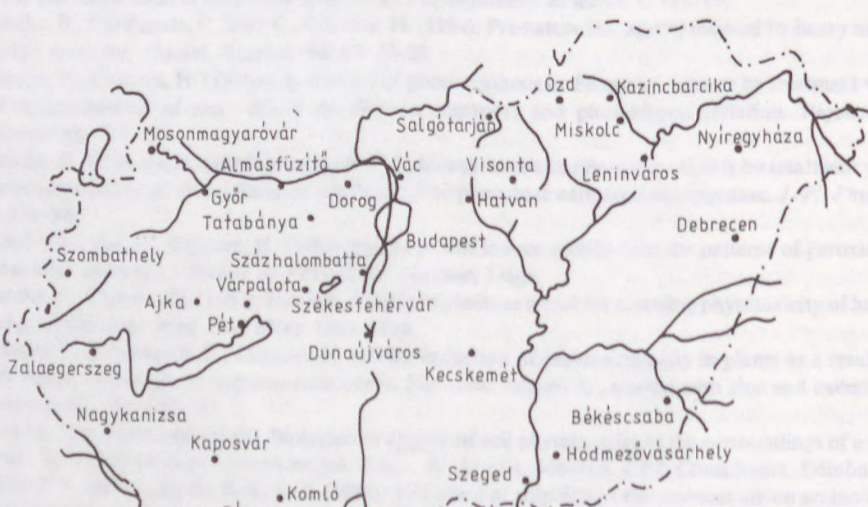

Fig. 55 — Regions with polluted air in Hungary.

According to the character of the pollutant suitable acceptors (plant or animal species) are employed in experimental exposures. Plant species occurring in the experimental area (passive indicators) can also be used. This is the simplest, cheapest and less labour-intensive method. Its disadvantage is that (in the case of accumulating indicators) in addition to air pollution the chemical composition of plants in influenced also by soil pollution as well as by the geochemical environment.

The advantage of investigations using selected experimental biological indicators is that these indicate atmospheric pollutants only. Furthermore, the experiments are reproducible and comparable. However, they are expensive and labour-intensive. In compiling impact surveys the UTM (Universal Transversal Mercators) system grids (scale 10 × 10 km or 5 × 5 km or 2.5 × 2.5 km) or subnetwork maps are used. The study of indicators is carried out at specific distances. In Hungary, in view of the density of industrial districts, iron works and large towns, approximately 10 000 km^2, more than 10% of the country's area is directly exposed to pollutants (Fig. 55). Monitoring points with a radius of 10 km (314 km^2) are recommended to be established around the sites located on the map. In an impact survey the influence of pollutants can be studied at three levels (Nobel and Michenfelder, 1987):

Level one
The determination of the dose of pollutants and its specific components.

By the use of standardized grass culture (*Lolium multiflorum* ssp. *italicum*) S, F, Pb, Cd, etc. can be detected.

The indication of photo-oxidants by the following species: *Nicotiana tabacum* "Bel-W 3", *Urtica urens, Phaseolus vulgaris* "Pinto", *Spinacia oleracea* "Dynamo",

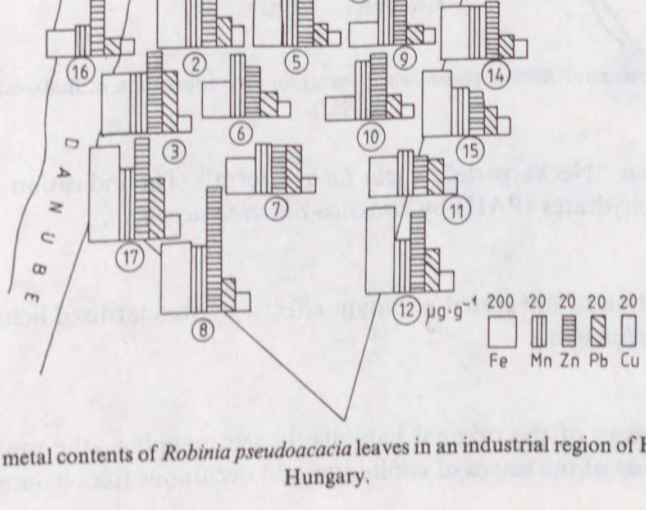

Fig. 56 — Heavy metal contents of *Robinia pseudoacacia* leaves in an industrial region of Budapest (Újpest), Hungary.

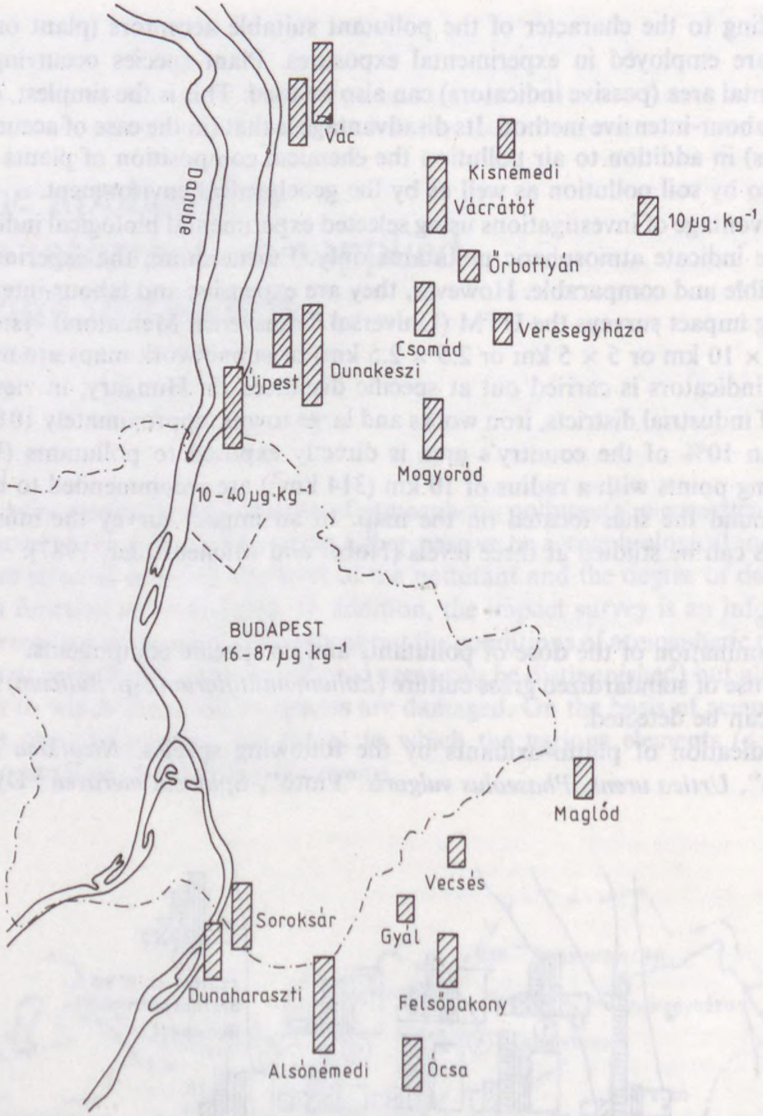

Fig. 57 — Lead content of *Robinia pseudoacacia* leaves, in the conurbation of Budapest (Kovács et al., 1982).

Raphanus sativus "Neckarperle", *Vicia faba* "Herra". The indication of polycyclic aromatic carbohydrates (PAH) by *Brassica oleracea acephala.*

Level two
The determination of integrated pollutant effects: by standardized lichen exposition *(Hypogymnia physodes).*

Level three
Vegetation surveys of the original habitats: lichen mapping—the ranking of damage—the analysis of the leaves of coniferous and deciduous trees (combined with the

chemical analysis of soils). In Hungary, the following easily transplantable and rapidly growing species of general distribution are recommended as being suitable (as accumulating indicators) for the indication of both sulphur and heavy metal pollution. *Robinia pseudoacacia* (Fig. 55), *Sambucus nigra* and *Lolium perenne* are common throughout the whole country, and in proportion to the degree of pollution, their leaves accumulate sulphur compounds and various heavy metals. In the large cities (e.g. Budapest) and in their environs, as well as in industrial regions, the areas polluted by heavy metals can be determined on the basis of the element composition of *Robinia* leaves. In Budapest, (Fig. 56) Deák Square and the surroundings of the Eastern

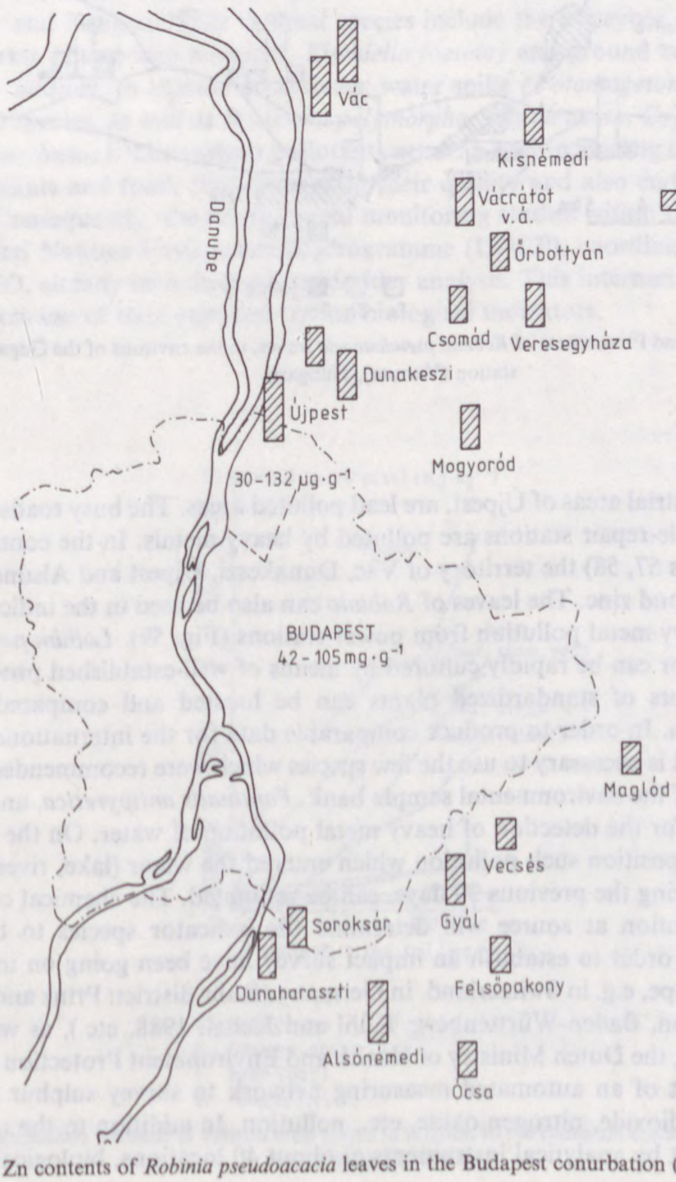

Fig. 58 — Zn contents of *Robinia pseudoacacia* leaves in the Budapest conurbation (Kovács et al., 1983).

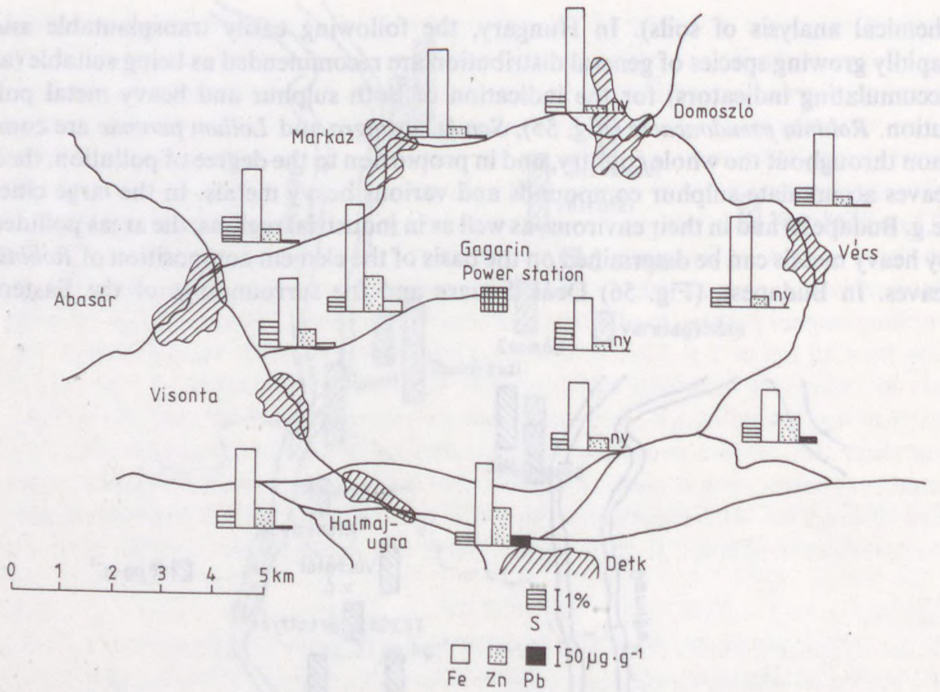

Fig. 59 — S, Fe, Zn and Pb contents of *Robinia pseudoacacia* leaves, in the environs of the Gagarin power station (Visonta), Hungary.

Station, the industrial areas of Újpest, are lead polluted areas. The busy roads and the environs of vehicle-repair stations are polluted by heavy metals. In the conurbation of Budapest (Figs 57, 58) the territory of Vác, Dunakeszi, Újpest and Alsónémedi is polluted by lead and zinc. The leaves of *Robinia* can also be used in the indication of sulphur and heavy metal pollution from power stations (Fig. 59). *Lolium perenne* as an active indicator can be rapidly cultured by means of well-established procedures. Adequate numbers of standardized plants can be located and compared in the experimental area. In order to produce comparable data for the international monitoring network, it is necessary to use the few species which were recommended within the framework of the environmental sample bank. *Fontinalis antipyretica,* an aquatic moss is suitable for the detection of heavy metal pollution of water. On the basis of its chemical composition such pollution which entered the water (lake, river, irrigation channel) during the previous 30 days, can be estimated. The chemical composition of the pollution at source will determine the indicator species to be used. Investigations in order to establish an impact survey have been going on in several countries in Europe, e.g. in Switzerland, in Germany (Ruhr district: Prinz and Scholl, 1975; Saarbrücken, Baden-Württenberg: Kühl and Keitel, 1988, etc.), as well as in Belgium. In 1970, the Dutch Ministry of Health and Environment Protection ordered the establishment of an automated measuring network to survey sulphur dioxide, ozone, nitrogen dioxide, nitrogen oxide, etc., pollution. In addition to the measurements carried out by analytical instruments at about 40 locations, biological indicators capable of indicating sulphur dioxide, ozone, nitrogen dioxide, carbon monoxide, PAN and ethylene were also exposed at approximately 40 locations. In Austria and Germany the needles of *Picea excelsa* are used as passive indicators of sulphur and fluorine. In the highly industrialized countries, such as the USA, Japan, France and Germany, a so-called environmental specimen bank was established, within the framework of the monitoring network.

The chemical composition of following plant and animal species is analyzed regularly: In the terrestrial ecosystems: wheat, barley, soybean, *Lolium multiflorum* (Italian ryegrass), moss *(Hypnum cupressiforme)*, lichens, various varieties of cabbage *(Brassica oleracea)*, carrot (*Daucus carota*, to indicate soil pollution), *Picea omorica*. Recently recommended further species are *Picea excelsa* and *Populus nigra italica* (Wagner and Kruger, 1982). Animal species include the honeybee *(Apis mellifera)*, earthworms *(Lumbricus terrestris, Eiseniella foetida)* and ground beetle species (e.g. *Carabus auratus*). In aquatic ecosystems: water spike *(Potamogeton)* and duckweed *(Lemna)* species, as well as *Dreissena polymorpha, Physia acuta, Cyprinus carpio* and pike *(Esox lucius)*. The various pollutants occur in ever increasing concentrations in fodder plants and food, thus decreasing their quality and also endangering human health. Consequently, the international monitoring studies within the framework of the United Nations Environmental Programme (UNEP), coordinated by the FAO and WHO, already include food and fodder analysis. This international programme also makes use of data supplied by the biological indicators.

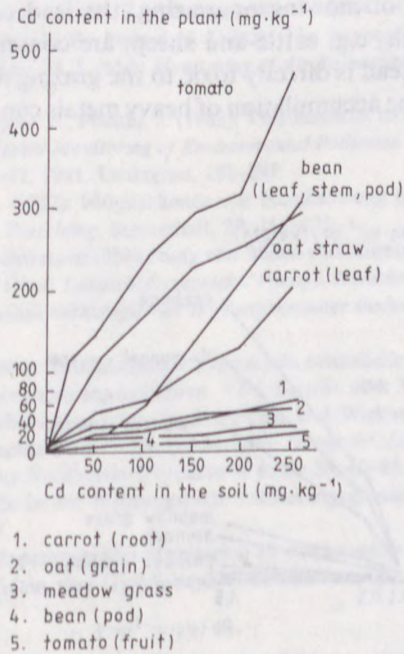

Fig. 60 — Cadmium contents of various crop plants in relation to the cadmium content of the soil (Kloke and Schenke, 1979).

13.1 THE UTILIZATION OF IMPACT SURVEYS IN AGRICULTURE

If the rate of air pollution cannot be reduced in polluted areas, such plant species should be grown which are resistant to the given pollutants and which do not accumulate harmful elements that are likely to be introduced into the food chain. The accumulation of fluorine, arsenic, cadmium, lead, benzpyrene, etc. should be monitored in the various fodder, fruit and vegetable crops. Based on the data supplied by the accumulating indicators and impact surveys it is possible to make preliminary estimates as to which elements or compounds were accumulated first in the region. Plants absorb cadmium, zinc and thallium from the soil. Depending on the cadmium content of soil this element accumulates primarily in the aboveground organs of plants, to a lesser extent in the fruits and seeds (Fig. 60). For example, polluted soils are not suitable for the cultivation of dwarf bean, as cadmium accumulates in the pods. The sensitivity of various vegetable crops to cadmium, in an increasing order, is the following: tomato (less sensitive) < broccoli < rye < cabbage < *Lolium perenne* < carrot < small radish < dwarf bean < spinach (rather sensitive) (Kloke and Schenke, 1979). Lead (as well as mercury and chromium) are absorbed primarily by the roots. Plants with a large leaf area contain higher quantities of lead, for example the leaves of field kale and mangel (Vetter, 1982; Früchtemicht and Vetter, 1982; Kowalewski and Vetter, 1982; Fig. 61). According to König and Krämer (1985) curly kale can accumulate Cr, Hg, Ni, Pb, Zn, sugar beet Pb and celery Cd. The heavy metal content of turf grasses changes in the course of the vegetation period. At the end of April, the beginning of May, when a large green mass in forming, the heavy metal content of plants abruptly decreases while in late autumn it increases again (Fig. 62). By determining the proper time of mowing or grazing, the lead content of fodder can be reduced. Grazing animals, e.g. cattle and sheep, are consequently indicators of the heavy metal pollution. Lead is directly toxic to the grazing animal but indirectly also to man. In areas where the accumulation of heavy metals can be detected by biological

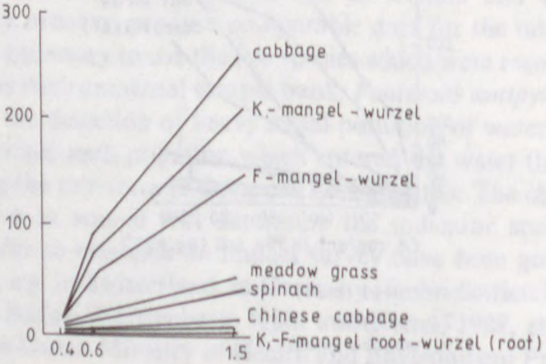

Fig. 61 — The influence of increasing lead content in the air upon the lead contents in different crops (Vetter, 1982).

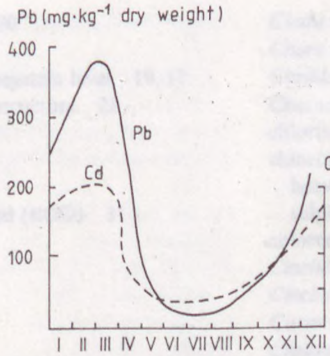

Fig. 62 — Changes in the lead and cadmium contents of a pasture vegetation, at 2–2.5 km distance from the factory in Nordenham, FRG (Vetter, 1982).

indicators, it is important to evaluate the possible risks to agricultural production. By selecting appropriate species and varieties it is possible to reduce the accumulation of various heavy metals in cultivated crops.

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Subject index

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BIOLOGICAL INDICATORS IN ENVIRONMENTAL PROTECTION