

- Conf. Ecol. Impact Acid Precip., Norway 1980, SNSF project, Oslo-Aas 1980.
- 18 Honegger, R. E., Threatened amphibians and reptiles in Europe. Akademische Verlags Gesellschaft, Wiesbaden 1981.
- 19 Huckabee, J. W., Goodyear, C. P., and Jones, R. D., Acid rock in the Great Smokies: Unanticipated impact on aquatic biota of road construction in regions of sulfide mineralization. *Trans. Am. Fish. Soc.* 104 (1975) 677–684.
- 20 Johnson, R. E., Ed., Acid rain/Fisheries. Proc. Int. Symp. Acid Precip. Fish. Imp., American Fisheries Society, Bethesda, Maryland 1982.
- 21 Karns, D. R., Toxic bog water in northern Minnesota peatlands: ecological and evolutionary consequences for breeding amphibians. Ph. D. Thesis, University of Minnesota 1983.
- 22 Lenders, A. J. W., Het voorkomen van de Knoflookpad (*Pelobates fuscus* [Laurenti]) in relatie met de zuurgraad van het voortplantingswater. *Natuurh. Maandbl.* 73 (1984) 30–35.
- 23 Leuven, R. S. E. W., and Schuurkes, J. A. A. R., Effecten van zure, stikstof- en zwavelhoudende neerslag op kalk en voedselarme wateren, vol. 47, pp. 1–131. Ministry of Housing, Physical Planning and Environment. Publikatiereeks Lucht 1985.
- 24 Leuven, R. S. E. W., Hartog, C. den, Christiaans, M. M. C., and Oyen, F. G. F., Effects of acid precipitation on amphibians and fish, in: Proc. Acid. Precip. Symp. 's-Hertogenbosch, pp. 184–186. Eds E. H. Adema and J. van Ham, Pudoc, Wageningen 1984.
- 25 Nielsen, C. L., Pough, F. H., and Gorn, J. A., The effect of acid precipitation on reproduction in salamanders: egg transplantation studies. *Am. Zool.* 17 (1977) 947.
- 26 Overrein, L. N., Seip, H. M., and Tollan A., Acid precipitation – effects on forest and fish. Final report of the SNSF project 1972–1980. Oslo–Aas 1981.
- 27 Petranka, J. W., Just, J. J., and Crawford, E. C., Hatching of amphibian embryos: the physiological trigger. *Science* 217 (1982) 257–259.
- 28 Peterson, R. H., Daye, P. G., and Metcalfe, J. L., The effects of low pH on hatching of Atlantic salmon eggs, in: Ecological Impact of Acid Precipitation, p. 328. Eds D. Drablos and A. Tollan. Proc. Int. Conf. Ecol. Impact Acid Precip., Norway 1980, SNSF project, Oslo–Aas 1980.
- 29 Pierce, B. A., Hoskins, J. B., and Epstein, E., Acid tolerance in Connecticut wood frogs (*Rana sylvatica*). *J. Herpetol.* 18 (1984) 159–167.
- 30 Pough, F. H., Acid precipitation and embryonic mortality of spotted salamanders, *Ambystoma maculatum*. *Science* 192 (1976) 68–72.
- 31 Pough, F. H., and Wilson, R. E., Acid precipitation and reproductive success of *Ambystoma* salamanders. *Water, Air Soil Pollut.* 7 (1977) 531–544.
- 32 Prescott, I., Cooke, A. S., and Corbett, K. F., in: The Changing Flora and Fauna of Britain, pp. 229–254. Ed. D. L. Hawksworth. Systematics Association special volume No. 6, Academic Press, London 1974.
- 33 Roelofs, J. G. M., Impact of acidification and eutrophication on macrophyte communities in soft waters in The Netherlands. I. Field observations. *Aquat. Bot.* 17 (1983) 139–155.
- 34 Roelofs, J. G. M., Schuurkes, J. A. A. R., and Smits, A. J. M., Impact of acidification and eutrophication on macrophyte communities in soft waters. II. Experimental studies. *Aquat. Bot.* 18 (1984) 389–411.
- 35 Runn, P., Johansson N., and Milbrink, G., Some effects of low pH on the hatchability of eggs of perch, *Perca fluviatilis*. *L. Zoon* 5 (1977) 115–125.
- 36 Saber, P. A., and Dunson W. A., Toxicity of bog water to embryonic and larval anuran amphibians. *J. expl Zool.* 204 (1978) 33–42.
- 37 Salthe, S. N., Increase in volume of the perivitelline chamber during development of *Rana pipiens* Schreber. *Physiol. Zool.* 38 (1965) 80–98.
- 38 Salthe, S. N., and Mecham, J. S., Reproductive and courtship patterns, in: Physiology of the Amphibia, vol. II, pp. 310–521. Ed. B. Lofts. Academic Press, New York 1964.
- 39 Schlichter, L., Low pH affects the fertilization and development of *Rana pipiens* eggs. *Can. J. Zool.* 59 (1980) 1693–1699.
- 40 Schoots, A. F. M., and Denucé, J. M., Purification and characterization of hatching enzyme of the pike (*Esox lucius*). *Int. J. Bioch.* 13 (1981) 591–602.
- 41 Stribosch, H., Habitat selection of amphibians during their aquatic phase. *Oikos* 33 (1979) 363–372.
- 42 Tome, M. A., and Pough, F. H., Responses of amphibians to acid precipitation, in: Acid Rain/Fisheries, pp. 245–254. Ed. R. E. Johnson. Proc. Int. Symp. Acid Precip. Fish. Imp., Am. Fish. Soc., Bethesda, Maryland 1982.
- 43 Urch, U. A., and Hedrick, J. L., Isolation and characterization of the hatching enzyme from the amphibian, *Xenopus laevis*. *Archs Biochem. Biophys.* 206 (1981) 424–431.
- 44 Vangenechten J. H. D., Puymbroeck, S. van, Vanderborcht, O. L. J., Bosmans, F., and Deckers, H., Physico-chemistry of surface waters in the Campine region of Belgium, with special reference to acid moorland pools. *Archs Hydrobiol.* 90 (1981) 369–396.
- 45 Witschi, E., Development of Vertebrates. Sounders Company London 1956.

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The biological indication of SO₂

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Key words. Air pollution; SO₂; biological indicator; environment monitoring; plant sensitivity.

Introduction

In the course of the so-called 'acid rain' debate the question of the direct effects of SO₂ and other gaseous pollutants has gained increasing attention. Obviously, the total reaction of ecosystems is a result of the combined impact of direct effects of air pollution and of indirect ones, like those due to changes in soil properties – overlaid by climate variation and cultivation practices. Measurements of air pollutants like SO₂, NO₂ and O₃ in

rural areas have been scarce until now; this complicates the task of deducing the resulting impact on e.g. terrestrial vegetation from a dose-response kind of approach, as the immission values are normally too crude or are nonexistent. Consequently, an alternative means of assessment of direct effects separately was looked for, and the application of biological indicators of SO₂ and other pollutants came into the picture. It is well-known that some plant groups possess a high sensitivity to changes in air quality, and some species react quite spe-

cifically towards e.g. SO_2 ⁷. The purpose of this chapter is to present the biological indicators of SO_2 and the methods used to characterize effects, and finally to discuss this approach in relation to methods of environmental monitoring not oriented towards effects.

Biological indicators of SO_2

Biological indication means characterization of recipient – air, soil or water – quality by means of various biological parameters, usually a measure of adverse effects on particularly sensitive living organisms. The range of experimental approaches with the above heading is, however, very wide and comprises transplantation of dead plant material to be measured for uptake of specific air pollutants over a period of time as well as description of historic changes in planktonic algal communities from analysis of layers of lake sediments, followed by correlation of these changes with pollution trends. With respect to biological indication of air pollution with SO_2 , the methods described in the literature are based on observation of the performance of plants covering a wide range of sensitivity to SO_2 ; mainly lichens and bryophytes (mosses). A large body of information makes probable connections between geographic and historic variation in other groups of organisms and changes in SO_2 -levels over space and time; most of these observations have not resulted in the development of biological indication methods.

The research on biological indication and monitoring of air pollutants like SO_2 has been initiated as a result of the recognition among ecologists of a need for effect-oriented studies of environmental quality. The physico-chemical measurement of a limited number of pollutants does not in itself provide the necessary basis for statements about damage or injury to living organisms. As the dose-response relations even towards a single pollutant may be complicated, and as several pollutants always occur together in the air, the estimation of, say, crop losses on basis of pollutant concentrations is very diffi-

cult and often impossible. Only when the combined physico-chemical (analytical) and biological characterization of the environment tells the investigator or administrator that adverse effects are not likely to occur, is it possible to be really sure that the environment is in satisfactory state. It is necessary to apply both methods, as they are complementary to each other; see the table below.

More or less specific reactions in certain organisms have been used in the biological indication of a number of air pollutants, e.g. heavy metals, ozone, PAN, fluorides, SO_2 etc. In the following, biological indication of SO_2 is described in more detail.

The influence of SO_2 on plants

Sulphur is an element essential to all organisms, and whether sulphur is taken up as sulphide, sulphite or sulphate, the plant cell possesses the necessary ability to use sulphur in these different oxidation states in the synthesis of the sulphur containing amino acids (cysteine, cystine and methionine). Most sulphur is taken up through the roots as sulphate, but some direct exchange of gaseous sulphur compounds, mainly H_2S and SO_2 , does also occur. Obviously, plants without a root system, like lichens and mosses, cover their total sulphur demand exclusively by uptake from the air or precipitation (supplied with water soluble sulphur compounds from the air). Even for these plants, the supply of sulphur from the atmosphere is – and has probably always been – more than sufficient for their growth; this is due to the large amount of H_2S formed by natural processes under anaerobic conditions and SO_2 by volcanic eruptions. The problem for some of these species is that the concentration of SO_2 has risen dramatically since the industrial revolution firstly in the cities, and later – mainly during the last 2 decades – in rural regions. If the influx of SO_2 into the plant cells becomes too large in relation to the available buffering capacity (pH and redox buffer capacity), a toxic effect will occur giving rise first to invisible and then to visible injury.

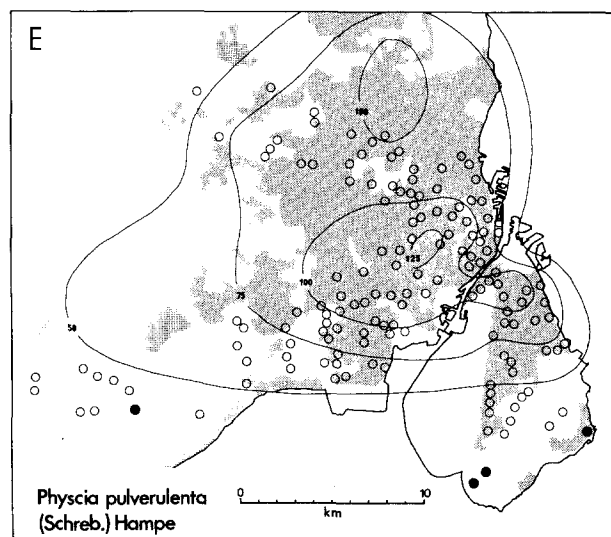
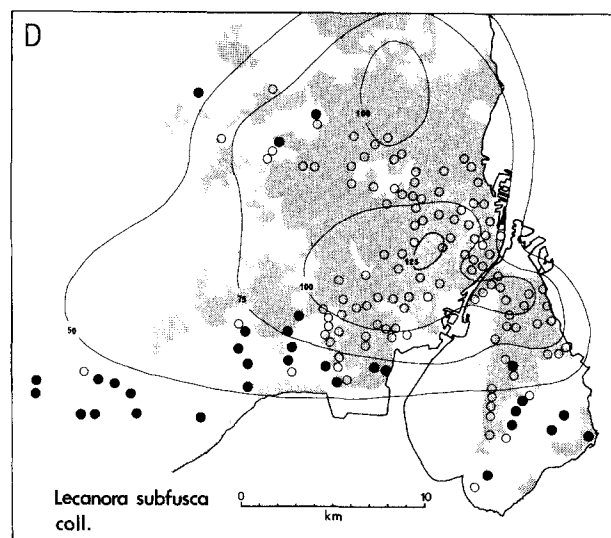
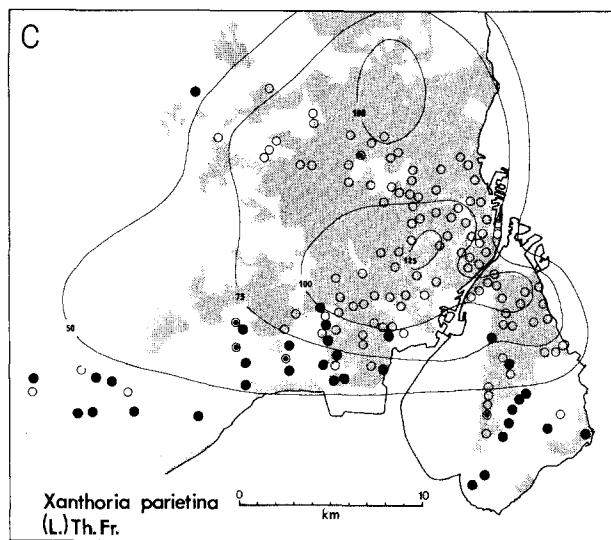
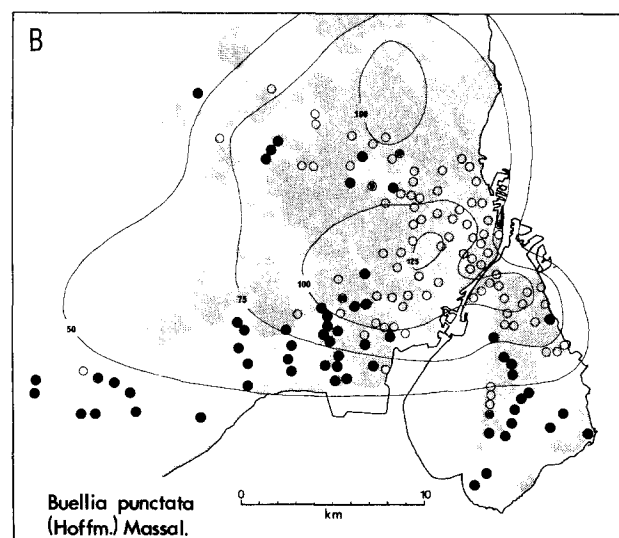
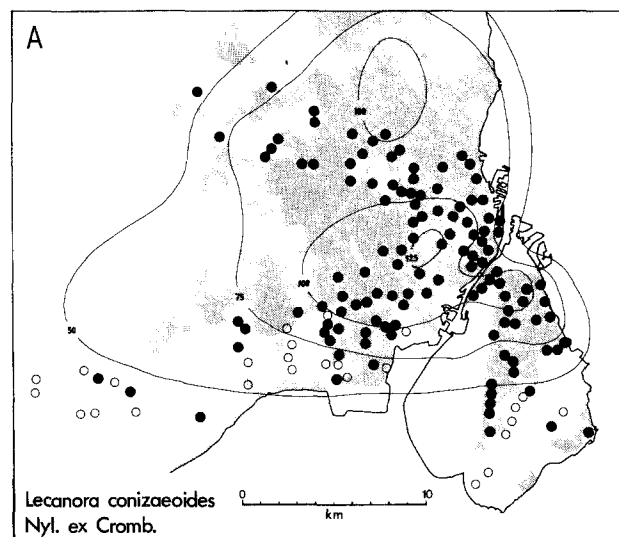
Approach in characterization of environmental pollution

	Physico-chemical (i.e. analysis of pollutant concentrations in the environment)	Biological indication/monitoring (i.e. observation of effects in organisms occurring in or transplanted to the area under investigation)
Observed/measured parameter	Pollutant concentrations; results depending on analytical methods, averaging times and performance of equipment	Effects of various nature e.g. changes in population pattern, injury to individuals etc.
Informations value with respect to pollutant concentrations	'Exact' with respect to the measured pollutants. Knowledge of variations of concentration with time often insufficient	Approximate levels can be estimated; relation to concentrations of single pollutants sometimes complicated, as the observed effect often is a result of combined action of a number of the actually occurring pollutants
Information value with respect to e.g. crop loss assessments and forest damage	Dose-response relations normally not easy to calculate; basically no effect value obtained	Transfer of results obtained from biological indicators may reflect reactions in plants of economic importance; information about the potential effects to vegetation obtained
Basic importance of approach	Necessary in environmental management, which is based on regulations of single compounds	Important in order to make reasonable injury and damage assessment (due to occurrence of antagonism/synergism and unforeseen injury from pollutants not measured); provides an early warning system which is particularly valuable in remote areas

Lichens as monitors of SO₂

Lichens are symbiotic organisms with a fungal and an algal partner in a mutually beneficial relationship. They are dominant under conditions where seed-plants cannot grow, e.g. in the arctic, deserts etc. In a temperate climate they are, consequently, found on substrates where competition from faster growing plants like seed plants is at a minimum, such as tree trunks, stones and extremely poor sandy soils. Lichens on vertical tree trunks are most suitable as air pollution indicators, as the influence of substrate properties is at a minimum, although not without some importance.

These plants exhibit different degrees of sensitivity towards SO₂, and many lichen species are among the plants which are the most sensitive towards SO₂. They are excellent biological indicators of SO₂⁹ and their reaction gives valuable information about long-term average values of SO₂-immission. For short-term values, other plants may be used.



The distribution of the indicator lichen species in the investigation area. ○, Sampling station; ●, the species present above 30 cm from the ground; ○, the species present only below 30 cm from the ground. (Source: *Oikos* 24 (1973) 347).

Investigations of air pollution using lichens have fallen into two groups: one group deals with the vegetation present^{3,6} on trees in the area under investigation, another group with transplantation¹ of lichens on twigs or bark from rural, pristine areas to the investigation area. It has been possible using both these methods, to depict a detailed picture of the SO₂-emission in a large number of European and North-American city areas. An example of such a study, from Copenhagen⁶, is given below.

A number of methods have been applied in biological indication of SO₂ pollution.

1) *Mapping studies*^{3,6}. The geographic distribution of epiphytic lichens within the investigation area is analyzed, and the resulting maps give information about the distribution limits for lichen species with different SO₂ sensitivities. Within the area of distribution of one species, the SO₂ immission level must be lower than the tolerance level for the species in question. Of course, it is problematic to draw safe conclusions from the absence of a species, as this may have a number of causes; this emphasizes the need for a particularly careful search for the indicator species in the so-called 'struggle zone', where the lichen becomes increasingly rare. When the distribution limits have been determined, knowledge of the tolerance levels for the indicator species allows the construction of a picture of the SO₂ immission – typically yearly or half-yearly average levels – in the area being investigated. The above method is based on simple observation of the presence or absence of a species in the investigation localities, but various 'importance values' for the lichen vegetation have been suggested as well. These importance values comprise e.g. analysis of relative cover or frequency of occurrence of the species on the trunks studied.

2) *Studies of injury*^{1,2}. A number of adverse effects on the indicator species may be used to characterize the air quality. The severity of the measured effect over a particular period of exposure time and/or the length of time needed to produce a certain effect in the indicator plant, usually transplanted to the investigation area, are both a function of the SO₂ level at the site. As the observed effects may occur due to other environmental changes than SO₂ pollution, the most reliable results from such studies have been obtained when comparisons were made between areas which only differed with respect to air quality. The effect parameters used in this approach are e.g. discoloration (bleaching of thallus lobes), formation of fruit bodies (apothecia), sulphur content (accumulation in thallus), chlorophyll content (degradation in the algal partner) and composition (ratio between chlorophyll a and b; phaeophytin formation). The time scale varies from a few days to months.

The results obtained in areas with high levels of SO₂ are not surprising and fit well with observations made on less sensitive plants like conifers. In relation to the question of acid deposition, the information about adverse effects in background areas far from SO₂ sources is of particular high importance. Firstly because immission measurements are and have been scarce in rural areas, where human health was not believed to be threatened, and secondly because effect evaluation at an early stage of impact from SO₂ is very difficult. The most sensitive biological indicators among the epiphytic lichens are suit-

able for this purpose. Only very few studies on trends in the lichen flora of remote areas have been conducted, however. In Scandinavia, the vegetation of epiphytes on free-standing trees (road trees) and twigs from the surface of the canopy of deciduous trees have shown a decline in the southernmost part, i.e. Denmark and Southern Sweden. This took place during a period when some medium sensitive lichen species were found to be extending their distribution towards the center of previously heavily SO₂-polluted urban areas. The explanation is naturally thought to be the following; the reduction of SO₂ in cities is often brought about through high stack emissions, which on the other hand will cause elevation of SO₂ levels in remote regions. The same phenomenon has been observed in the U.K.⁸.

The interspecific sequence of sensitivity towards SO₂ in lichens observed in the field has been verified in a number of cases through laboratory experiments. It must be realized, however, that the results of such laboratory experiments may not be directly applicable to field conditions. It is always important when using biological indicators of various pollutants to analyze carefully eventual changes in ecological conditions which may have taken place during the exposure period. An example of confusing evidence has occurred with respect to the relation between epiphytic lichen growth on conifers and the forest die-back syndrome in Western Germany. It was observed that in many stands of unhealthy conifers, the growth of leafy epiphytic lichens seemed to be improved. It was concluded that the direct effects of SO₂ were of minor importance among the various possible causes suggested for forest die-back, as you would have expected a negative trend for the epiphytic vegetation in that case. This conclusion may be wrong, firstly because of the improvement in growth conditions for epiphytic lichens in the dying stands with heavy needle loss in the canopies and thus better light penetration to the trunks and twigs otherwise strongly shaded, secondly because the species of lichens with increased abundance may belong to the less sensitive among the leafy lichens. In contrast to the above observations, there have been records of direct injury (discoloration, morphological anomalies) in lichens on trunks of conifers in the Schwarzwald and the Harz, where forest die-back has occurred. From the above field observations, it is obviously not possible to exclude that direct effects of SO₂ play a significant role in the overall explanation of the observed forest die-back in Western Germany – or indeed, in most of western Europe.

In order to interpret the results obtained by biological indicators, such as lichens, in terms of forest injury, a thorough understanding of the dose-response relations for these two very different plant groups is needed. Even if lichens are generally more sensitive to SO₂ than most trees, the fact that lichens react more slowly and are therefore less sensitive to short-term variations in SO₂ concentration than trees, illustrates the importance of the pattern of variation in, for example, the SO₂ concentration at the site. Similarly, it must be realized that a general no-effect level is very difficult to assess on the basis of biological indication. It will always be necessary to combine field observations of adverse effects with experimental fumigations of a range of indicator species/plant groups.

Mosses as biological indicators

Mosses or bryophytes are structurally simple small plants with leaves essentially one cell-layer thick and therefore without the stomata and vascular tissue present in seed plants. Most of the nutrients needed for bryophyte growth are taken up from the air, and in accordance with this uptake mechanism, cell walls of bryophytes possess extraordinarily strong ion-exchange properties. Especially positively charged ions (cations) are firmly bound in moss tissue, this means that mosses are among the best biological indicators of heavy metals, which may reach impressively high concentrations in mosses even with relatively low levels of heavy metal pollution from the air. With respect to biological indication of SO_2 , mosses are less suitable than lichens. Mosses are more sensitive towards prolonged drought periods than lichens, a fact that is illustrated by the scarcity of epiphytic moss vegetation in particular in cities with their dry climate. The gradient also observed in epiphytic moss vegetation when comparing rural with urban areas may thus merely reflect the climate difference, or, to be more precise, the climatic factor is very difficult to isolate from other possible cause-effect relation, which is not the case for the epiphytic lichen flora. In the periphery of cities and in remote regions, however, the epiphytic bryophytes may be used as indicators of air quality – e.g. the presence of SO_2 – in the same way as lichens. In Newcastle, U.K.³ mapping studies like those mentioned above have been conducted; the distribution limits have been identified and correlated with the SO_2 levels. In forests, the moss of deciduous trees includes species (genera: e.g. *Antitrichia*, *Neckera*, *Homalia*), which are considered particularly sensitive to SO_2 . These species, like the very sensitive lichens (genera: e.g. *Usnea*, *Bryoria*, *Lobaria*), form a group of biological indicators which may give an 'early warning' signal of potential air pollution injury to the ecosystem.

Other SO_2 indicators

In a few cases, host-parasite relations may be used in biological indication of air quality. The best known example is tar spot disease¹⁰, *Rhytisma acerinum* on *Acer pseudoplatanus* (sycamore maple), which has been shown to correlate negatively with annual SO_2 average levels. The precise physiology behind this effect is not known, but it must be assumed that the most sensitive phase

occurs in spring during the few weeks where spore attack on the newly formed leaves take place. Direct observation of adverse effects in trees may be used for biological indication of SO_2 . This approach has the advantage that the observed reaction in the indicator may more easily be used to predict potential effects in forest stands. The trees most frequently used for biological indication of SO_2 are eastern white pine⁴ (*Pinus strobus*), Scots pine (*Pinus silvestris*) and hybrid poplar⁵ (*Populus tremuloides*).

Conclusion

The biological indication approach is necessary in environmental management, and should go hand in hand with conventional analytical-chemical techniques. It is essential that the characterization of environmental quality should also include the observations of skilled biologists. If not, we are likely in the future to face many unforeseen environmental problems at a very late stage of their development. The use of biological indicators represents one aspect of the biologist's characterization of environmental quality.

- 1 Brodo, I., Transplant experiments with corticolous lichens using a new technique. *Ecology* 42 (1961) 838–841.
- 2 Degelius, G., Biological studies of the epiphytic vegetation on twigs of *Fraxinus excelsior*. *Acta Horti gothoburg.* 27 (1964) 11–65.
- 3 Gilbert, O. L., The effect of SO_2 on lichens and bryophytes around Newcastle upon Tyne, in: *Air Pollution*, pp. 223–235. Centre for Agricultural Publishing and Documentation, Wageningen 1969.
- 4 Houston, D. B., Response of selected *Pinus strobus* clones to fumigations with SO_2 and O_3 . *Can. J. For. Res.* 4 (1974) 65–68.
- 5 Jensen, K. F., Growth analysis of hybrid poplar cuttings fumigated with O_3 and SO_2 . *Envir. Pollut. (A)* 26 (1981) 243–250.
- 6 Johnsen, I., and Søchting, U., Influence of air pollution on the epiphytic lichen vegetation and bark properties of deciduous trees in the Copenhagen area. *Oikos* 24 (1973) 344–351.
- 7 Mansfield, T. A. (ed.), *Effects of Air Pollutants on Plants*. Cambridge University Press, Cambridge 1976.
- 8 Rose, C. I., and Hawksworth, D. L., Lichen recolonisation in London's cleaner air. *Nature* 289 (1981) 289–292.
- 9 Skye, E., Lichens as biological indicators of air pollution. *A. Rev. Phytopath.* 17 (1979) 325–341.
- 10 Vick, C. M., and Bevan, R., Lichens and tar spot fungus (*Rhytisma acerinum*) as indicators of SO_2 pollution on Merseyside. *Envir. Pollut.* 11 (1976) 203–216.

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Effects of experimental acidification on freshwater environments

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Key words. Experimental acidification; freshwater; ecosystem; community; pH; alkalinity; sulfate; heavy metals.

Introduction

The major part of the research on the effects produced by acidic deposition in water bodies has, obviously, been

carried out in natural ecosystems in the areas which are most susceptible to damage; for example, Southern Scandinavia^{34,35}, the Precambrian Canadian shield⁷ and Northeastern USA²⁷.