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 disease[10], *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 pine[4] *(Pinus strobus)*, Scots pine *(Pinus silvestris)* and hybrid poplar[5] *(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.

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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 shield[7] and Northeastern USA[27].

A body of fresh water may receive acidic substances from dry and wet deposition, and from its tributaries and watershed. To evaluate the relative contribution from each of these sources to the total acidic loading of a water body is not easy. An estimation of the importance of each source, and of variations of the loading rates during the years preceding the study is rather difficult to obtain. In a freshwater ecosystem it is practically impossible to separate the effects due to acidic substances present in depositions, from those produced by other pollutants (e.g. heavy metals) and by nutrients associated with them. In addition, acidic deposition increases the leaching of some heavy metals from the soil of the watershed as well as the release of metals and other substances from the lake sediments. Indeed, there is evidence that the increase of some metals in acidified waters of Scandinavia and the eastern area of Northern America is due to acid deposition[25]. These processes, if they are quantitatively consistent, may mask the actual effects of hydrogen ion concentration increase. The amount of metals in acid depositions can be easily evaluated, but, unfortunately, it is very hard to separate the contribution of metals from the soil from that from the sediments.

To gain better knowledge of these and other processes involved in water body acidification, during the past few years investigations have been carried out with experimental acidification. These studies are generally carried out in areas with a low rate of acid deposition, in order to reduce to a minimum its interference with the effects produced by the acid experimentally added to the natural or artificial environment. Experimental acidification simulates only the direct acid deposition loading (with or without metals, organic pollutants and nutrients associated), and excludes the acid contribution from the tributaries and the watershed. Together with these limitations there is the advantage of being able to separate the effects of direct acidification from those produced by other sources. Other advantages are the precise knowledge of the date at which the acidification has been started, and the amount of acid introduced into the environment, and also the possibility of regulating the pH value, and of recording the effects which occur early in the acidification process, when buffering capacity is abolished but the pH values are still high.

These experiments may be divided into two groups: experimental acidification of natural water bodies (streams and lakes) and acidification of 'outdoor experimental channels' and 'enclosures'. The advantages of experiments carried out in an entire natural lake (or river) are evident, because they offer the most reliable simulation of the acidification of the ecosystem from atmospheric depositions. Unfortunately, acidification of natural environments may be applied only in regions where it is permitted, such as in an 'Experimental Lake Area' (ELA) located about 275 km east of Winnipeg, Manitoba (Canada)[48]. As a consequence, there are very few examples of water bodies experimentally acidified. Acidification of semi-natural ecosystems ('outdoor experimental channels' and 'enclosures') represents a compromise between laboratory investigations and experimental acidification of natural water bodies. The 'enclosure' is a portion of the ecosystem to be studied which is isolated by plastic sheets. This technique has several advantages compared with laboratory experiments. The most evident one is that it is possible to study several populations simultaneously, in naturally occurring proportions and under initial physical and chemical conditions which are those of the environment studied. The outdoor experimental channel consists of one (or more) artificial channels, made of asbestos cement or plastic material, in which the water to be tested flows. The bottom may be uncovered or covered by clastic materials (mud, sand, pebbles). The water is, generally, diverted from a stream (or a river) to feed the channel at a prefixed flow-rate. The pollutants (for instance, acid substances) are added with a dosing system. In some cases, current water organisms are introduced to evaluate the effects of acidification on them, in others the effects are recorded in the community, introduced with water flow, colonizing the artificial channel.

Case studies

Some case studies are reported to illustrate the usefulness and the limitations of experimental acidification in natural and artificial ecosystems in comparison with the research carried out on acidification of water bodies resulting from atmospheric deposition.

Acidification of natural ecosystems

The most complete research on the experimental acidification of a whole lake are those summarized by Schindler et al.[37, 40, 42] and illustrated in detail by other authors. The studies have been carried out in the small oligotrophic, Lake 223, lying in the Canadian Precambrian Shield and belonging to the ELA Project. In this area the mean annual pH value of the precipitation was 4.92, which represents about 1.5% of the input of H^+ experimentally introduced into the lake[31]. During the past eight years the precipitation rates and their pH values were not able to increase the H^+ concentration in the water of the lakes lying in the ELA area[37–39]. Before acidification Lake 223 (Z_{max} = 14.0 m, surface area = 27.3 ha) had a pH value ranging between 6.5 and 6.8 and a mean alkalinity value of 100 $\mu eq \cdot l^{-1}$. This lake, which was studied two years before its acidification (1974–1975), was one of the ideal water bodies to be utilized for acidification experiments.

The H_2SO_4 addition, which was started in 1976 and continued for some years, has been regulated in order to decrease the mean pH value by 0.2 units per year. The pH value gradually decreased from 6.5–6.8 (1975) to 5.97 (1980)[42]. To give an idea of the investigations carried out in this lake after acidification, the most important results are summarized.

One of the most evident effects of the acidification was an increase in water transparency, which, according to Schindler[38] and Schindler and Turner[42] was due to decolorization of humic substances and not to phytoplankton decrease[26] or to a significant increase in aluminum concentration in the water[1].

In acidified waters a transparency increase may be due to the decrease of organic substances precipitated by aluminum leached from the acidified watershed. This did not occur in Lake 223 because it was directly acidified.

Schindler et al.[41] observed in Lake 223 the dramatic effects produced by alkalinity decrease, which occurred before the pH value became so low as to damage the fish community, modify the interrelationships among the populations and increase the metal concentrations in the water. The delay of the pH lowering in relation to the alkalinity decrease is probably due to the bicarbonates produced in the anoxic hypolimnion by the reduction of sulphate to sulphide, followed by sedimentation of this compound. As a result, the buffering capacity of the lake water increased[40].

Schindler and Turner[42] noted sulphate reduction in another experimentally acidified lake of ELA (Lake 114). The hypolimnion of this lake, in contrast to that of Lake 223, always has a certain amount of oxygen and, in spite of this, sulphate reduction occurred in the sediments, rich in organic substances. Together with microbial reduction of other elements, that of the sulphates has been measured by Kelly et al.[20] in ELA lakes: 227, 226N and 223. The first two lakes were experimentally eutrophicated, and the latter acidified, as mentioned above. The results obtained are in agreement with those of Schindler and Turner[42]. Kelly et al.[20] demonstrated, on the basis of a predicting model, that the amount of alkalinity produced in the hypolimnion, by microbial reduction, may neutralize the effects of the atmospheric loading of acid depositions in eutrophic lakes (e.g. Lake 226N, Lake 227), but not in oligotrophic ones (e.g. Lake 223).

Kelly et al.[21] carried out in situ a six-year research program on the organic carbon decomposition in sediments of Lake 223. During this period the pH values in the

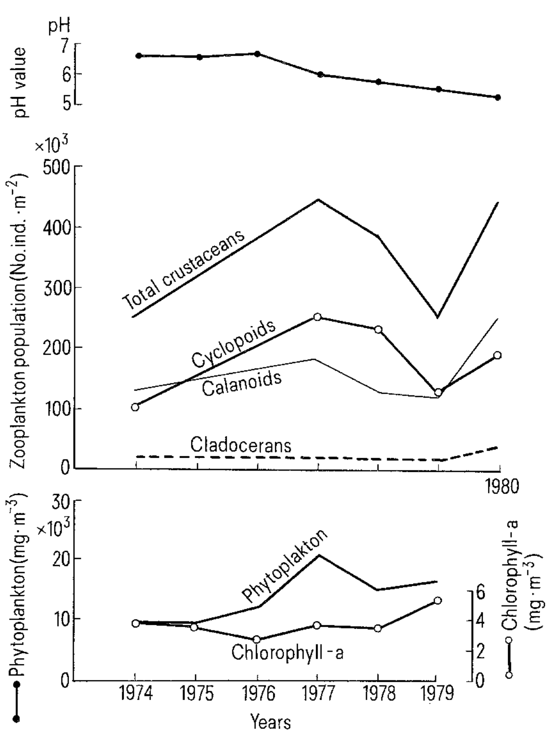

Figure 1. Variation of pH value, zooplankton population density, phytoplankton biomass and chlorophyll-a concentration in Lake 223 before and after acidification started in 1976. The pH data from 1974 to 1979 are from Schindler[38], that of 1980 from Malley et al.[29]. The zooplankton data are from Malley et al.[29] and those for phytoplankton and chlorophyll-a are from Schindler[37].

surface water decreased from 6.5–6.8 to 5.0–5.2. The results obtained from in situ monitoring were compared with laboratory experiments. As acidification increased, the whole lake decomposition-rates (during winter) and the decomposition-rates in the hypolimnion (during summer) were not changed over a six-year period. The interstitial water of the hypolimnetic sediments was neutral and the pH of the sandy sediments of the epilimnion rapidly increased below the water/sediment interface owing to the alkalinity produced by anoxic microorganisms. Laboratory experiments showed that the decomposition-rate of recently deposited organic material was reduced by low pH, while the decomposition of material which was more completely decomposed was not affected, even from pH 4.0. The inhibition of the decomposition of the most recent material was not greater than 20–30% at pH 5.25–5.0. In conclusion, the unchanged decomposition-rate in the whole lake under winter ice did not demonstrate that there was no reduction of the decomposition in the epilimnic sediments during summer, when a great amount of organic material is sedimented.

The increased radiant energy penetration, due to the higher transparency, produced an increase of summer warming in water beneath the thermocline and a faster deepening of this layer[42].

The increased transparency and the modification of the thermal pattern were the most reliable causes of the changes in phytoplankton populations in Lake 223. Phytoplankton production increased from 1975 to 1979 and maintained the high values measured in 1979 during 1980 (fig. 1). Up to 1980 there was no variation in the species diversity[9]. In addition, during 1979 a bloom of *Mougeotia* grew in the littoral area of the lake[31,37]. In conclusion, the biomass and production of the phytoplankton increased during the acidification period. Malley et al.[29] evaluated the causes of the changes of the zooplankton structure in relation to the increasing acidification of Lake 223 (fig. 1). The percentage of Cladocerans in the total Entomostraca varied from 3.8 to 8.5% and the mean population density of the Calanoids was similar to that of Cyclopoids. Population density of Entomostraca at pH 5.37 was higher than that in the period before acidification and that of the Cladocerans was rather constant from pH 6.64 to 5.84. In 1980 *Daphnia catawba* × *schoedleri*, never found before in Lake 223, attained a population density greater than that of *Daphnia galeata mendotae*. *Bosmina longirostris*, *Diaphanosoma brachyurum*, *Cyclops bicuspidatus thomasi*, *Mesocyclops edax* and *Diaptomus minutus* seem not to be influenced by lowering pH value and the latter increased in number during 1980. The first species to be eliminated by the acidification were the rare species: *Diaptomus sicilis* at pH = 6.08, and *Epischura lacustris* at pH 5.84. In the layer inhabited by *Mysis*, pH ranged from 5.51 to 6.32 in 1978 and from 5.23 to 6.10 in 1979. From August 1978 to August 1979 the abundant population of *Mysis relicta* (Crustacea) decreased by 96% and the surviving 4% died in autumn 1979[33]. The authors, excluding some possible events for the elimination of *Mysis* (e.g. reproductive failure, decrease of food, increase of water transparency and toxic metal concentration, more severe predation by fish), concluded that the most probable cause was the direct effect of the H^+ concentration increase.

According to the authors, neither the changes of structure of the fish community nor the elimination of the crustacean predators *(Epischura* and *Mysis)* have significantly affected the herbivorous crustaceans. The herbivore increase was probably due to their higher acid resistance compared with that of the crustacean predators and to the increase of available phytoplankton. There is evidence that the phytoplankton abundance and the *Daphnia galeata mendotae* decrease facilitated the colonization of Lake 223 by *Daphnia catawba* × *schoedleri*. It is noteworthy that population density of Rotifers increased with pH value decrease. As a result, the zooplankton structure has been considerably modified by the acidification.

Malley[28] observed the influence of the acidification of Lake 223 on the population of the crayfish *Orconectes virilis* (Hagen). On the basis of some information on the effects of low pH on the suvivorship and mineral metabolism of *Gammarus lacustris, Lepidurus arcticus* and *Astacus pallipes*[4,46,47], Malley[28] carried out laboratory experiments on *O. virilis* from Lake 223. The author observed consistent effects of the low pH on the survivorship and Ca^{2+} metabolism of this species, which is important in Lake 223.

Mills[31] studied the effects of acidification of Lake 223 on five species of fish: lake trout *(Salvelinus namaycush)*, white sucker *(Catostomus commersoni)*, fathead minnow *(Pimephales promelas)*, slimy sculpin *(Cottus cognatus)* and pearl dace *(Semotilus margarita)*. At the beginning of the lake acidification the first four species were abundant and the latter rare. *Mysis relicta,* eliminated by low pH, was replaced by *Daphnia catawba* × *schoedleri* in the

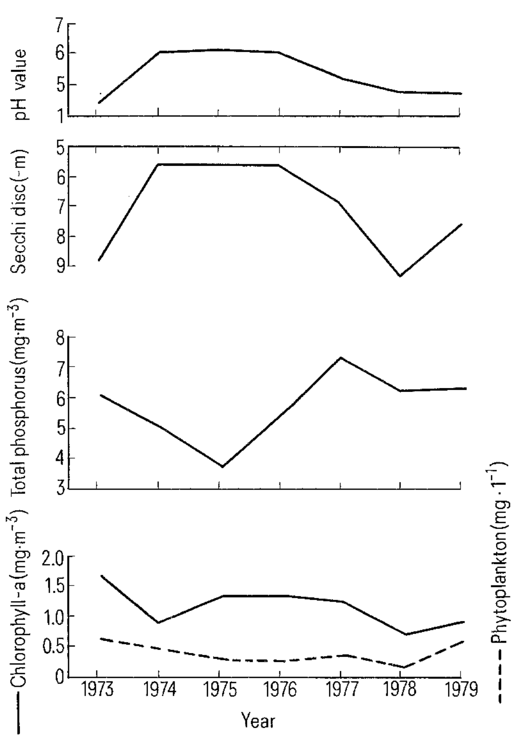

Figure 2. Variations of the pH values, chlorophyll concentration, transparency (Secchi Disc), total phosphorus and phytoplankton biomass in Lohi Lake[49].

diet of the trout, which also preyed on pearl dace and fathead minnow. The dramatic decrease of fathead minnow, which is very sensitive to low pH, produced two main effects: a) the trout increased its predation pressure towards slimy sculpin, which decreased in number; and b) the competitor pearl dace, which is more resistant, increased. The probable cause of the white sucker increase was the chironomid larvae increase. The tolerance to low pH varied with the species; the more sensitive seemed to be the fathead minnow, the more resistant the white sucker. The trout population did not show any damage until pH 5.4.

The Lohi lake (maximum depth = 19.5 m, surface area 40.6 ha) lying near Sulbury (Ontario, Canada) attained in 1973 a low pH (4.4) owing to the acid deposition occurring in this area. In 1974 Lohi lake was experimentally neutralized with successive additions of base until it reached a pH value higher than 6.0; subsequently, the pH declined to 4.74 in 1979. For these variations in pH values the Lohi Lake was the ideal water body to be studied for evaluating the ecological implications of acidification. This lake has been studied by Yan[49] particularly with respect to the modification of the water transparency and thermal regime. The author has found no correlation between Secchi Disc depth and chlorophyll, total phosphorus concentration and phytoplankton biomass (fig. 2).

Transparency was positively correlated with thermocline depth, epilimnetic thickness and hypolimnetic heating, but negatively with pH values. These results were in good agreement with those obtained from the studies in Lake 223 of ELA.

To obtain a behavioral response of the macrobenthic invertebrates to the acidification and the consequent changes in the 'diversity' of the drift of such organisms, Hall et al.[14] carried out research on the Norris Brook stream (New Hampshire, USA). The pH value of the stream water ranged from 5.4 to 6.4, electrical conductivity varied from 21 to 30.5 $\mu S \cdot cm^{-1}$ and dissolved oxygen concentration was near saturation. In the tested area pH was regulated to 4.0 by addition of H_2SO_4. The pH value of the control area was about 6.4. The collections were done upstream and downstream of the point of acid addition, in order to evaluate the drift into and out of the acidified zone.

The results of the first five days of the experiments simulated the rapid change of pH value which occurs in small mountain streams at the beginning of the snowmelt, and those of the following 25 days simulated long-term acidification (pH = 4.0), such as that caused in several acidified environments by precipitation. During the first five days, 270 macroinvertebrates daily drifted into the acidified area and 1300 drifted out of it; in the successive 25 days, 280 individuals daily drifted into the acidified area and 370 drifted out of it. The large number of macroinvertebrates which left the acidified area during the first five days were probably the more acid-sensitive (e.g. Ephemeroptera), while the more resistant organisms (e.g. Pleocoptera, Trichoptera) which drifted into the acidified area remained in it. As a result, during this period the population density of the benthonic macroinvertebrates decreased in the acidified area, and the phylogenetic and functional diversity changed. In the following 25 days,

because the drift rates into and out of the acidified area were very similar, we could conclude that the acid addition had had no effect on the population density, which remained constant. During this period also the generic diversity, evenness and richness showed no significant variations. The authors observed that the late consequences of the changes in the diversity of the functional groups (e.g. collectors, grazers, shredders) may modify the physical, chemical and biological characteristics of the organic material of the sediments.
Similar results on the reduction of population density and diversity due to acidification have been obtained by Herricks and Cairns[17]. These authors studied the effects of one addition of sulphuric acid to a stream to lower its pH instantaneously from 8 to 4.

Acidification of artificial ecosystems

Enclosures have been used by Delisle et al.[8] to follow the effects of acidification on the phyto- and zooplankton communities. The experiment was carried out for six weeks in Lake Kempt (Quebec, Canada), which is partly buffered and receives acid deposition from heavily industrialized areas. Eight plastic enclosures (diameter = 2 m; depth = 2 m; volume = 4600 l) were embedded in the sediments, which formed the bottoms of the cylinders. Two enclosures were kept as controls (pH = 6.7, equal to that of the open lake). Two of the remaining six enclosures were used for each of three treatments; that is, the lowering of the pH value of the water inside the enclosure to 4.0, 5.0 or 5.5 by addition of dilute H_2SO_4. Some parameters were unaffected by acidification; these were temperature, turbidity, O_2, TC, N-NH_3, N-NO_3 and Cl^-. The pH and alkalinity values, obviously, decreased and conductivity increased. In the acidified tubes the release of trace metals from the sediments was deduced from the increase of Mn, Cd, Fe and Cu concentrations and, to a lesser extent, those of Zn, Al and Pb, in the water column. According to the authors, the metal concentration increase was more important than the low pH value in modifying the community structure of the zooplankton. Indeed, Copepods dominated in the lake and in the control, whereas Cladocerans, and particularly *Bosmina,* were abundant in acidified enclosures. Differences in phytoplankton biomass cannot be evaluated because of its high variability, but it was evident that the acidification had an influence on the community structure. In the acidified enclosures Chlorophyceae, Cryptophyceae and microflagellates were more abundant than Cyanophyceae; the latter dominated in the control. In addition, the number of taxa was reduced. Variations in chlorophyll concentration and primary production were insignificant, except at pH 4.0, where a production-rate acceleration was noted. This increase could be due to the phosphorus released from the sediment[1] but, unfortunately, no data on this nutrient is reported by the authors.
An enclosure experiment was carried out in Beaverskin lake (Nova Scotia, Canada) to follow the ecological effects of acidification, liming and nutrient enrichment. The data concerning the water chemistry have been treated by Collins and Lane[6] and those of the planktonic community by Blouin et al.[3]. Here only the results dealing with acidification will be considered and those from the lake and the control enclosures, compared.
In the sediments of Beaverskin lake ($\bar{z}$ = 2.19 m; surface area = 41.8 ha) eight plastic enclosures (diameter = 2 m; depth = 5 m) were embedded. Two enclosures were kept as controls, two of the other six were used as replicates for each treatment; liming ($CaCO_3$), nutrient (nitrogen and phosphorus) enrichment, and acidification (one litre of H_2SO_4 15% per enclosure). In the water column of the acidified enclosures the pH value was 5.63 before the acid addition; after the treatment, the pH value decreased to 4.62 after 45 days, and after one month increased to 5.01. In acidified enclosures the nutrient concentrations (N and P) were similar to those measured in the control. Acidification decreased the population density of the phytoplankton and particularly that of Cyanophyceae and some other taxa; for example, *Navicula* sp. and *Closterium parvulum*. Cyclopoids were relatively unaffected by acidification, which reduced the abundance of some Cladocerans *(Daphnia, Diaphanosoma, Eubosmina tubicen),* Diaptomids and immature *Epischura.*
In spite of the noticeable influence of acid deposition also in water bodies with high buffering capacity, the information on this environment is extremely poor[23]. On this consideration Annoni and Ravera[2] have carried out an experiment with enclosures in the shallow (z_{max} = 8.0 m; surface area = 2.5 km^2) and eutrophic Lake Comabbio (Northern Italy, Province of Varese). Two transparent PVC tubes (diameter = 1.0 m; depth = 3.0 m) were anchored to the muddy sediments and acidified with an amount of H_2SO_4 (1%) sufficient to lower the pH value from 7.0 to 3.8 in 24 h. Two identical tubes were kept as controls. The acid was added to the water column with a rate of one drop/sec to simulate an accident and follow the possible recovery of the ecosystem through homeostatic processes. During the experiment (28 days) at seven successive dates phyto- and zooplankton samples were collected from the tubes as well as from the lake. During the first 9 days, in the acidified tubes, water transparency increased, probably as a result of the decrease of the population density of phyto- as well as of zooplankton. In the same period the alkalinity decreased to zero and the conductivity and phosphate concentration increased. The phosphate increase may be the effect of sulphate reduction, with a consequent decrease at the minimum of the control of iron on phosphorus[14]. The increase of the nitrate concentration may be due, at least in part, to the release of nitrogen compounds from the sediments. The lowest pH values were measured 8 days before the transparency reached its maximum, and then the minima of the phyto- and zooplankton densities as well as of the chlorophyll concentration were reached. Therefore, the dramatic biological effects were evident with a certain delay after the acid addition. After the first day from the beginning of the experiment, pH value continuously increased until the end of the experiment, when it reached the value of 6.0. Simultaneously, the conductivity decreased.
The pH value increase was probably due to the bicarbonates released from the sediments, called by Kilham[23] 'additional buffering capacity' of the environment. Indeed, in the lake sediments the mean concentration of carbonates was 38.7 mg CO_3^{2-}/g. After the 9th day the

transparency decreased, while alkalinity, chlorophyll concentration and phyto- and zooplankton populations increased in comparison to the control. From the beginning up to the end of the experiment the number of phytoplankton species did not vary, but the number of individuals of the species initially most frequent (Cyanophyceae and Chlorophyceae), significantly decreased, and there was an increase in the number of individuals of the species which were less frequent at the beginning of the experiment. As a result of the acidification, the total phytoplankton biomass decreased, its structure was significantly modified and the 'diversity' showed a tendency to increase (figs 3, 4).

The influence of high H^+ concentration on phytoplankton has been studied by Yan and Stokes[50] with plastic enclosures anchored in Carlyle Lake (Ontario, Canada). In the water columns of two enclosures pH values of 6.0 and 6.5, respectively, were obtained by daily addition of NaOH, and in that of two other enclosures pH 5.0 and 4.0 were reached after H_2SO_4 addition. One enclosure was kept as a control at pH 5.0, that is the mean pH of the Carlyle Lake. From the results obtained, the authors emphasized that the number of species and their relative abundance in the total phytoplankton were indices more sensitive than the total biomass. Indeed, at the end of the experiment (28th day) Dinophyceae and Cryptophyceae reached a higher population density at pH 4.0 and, at the same pH value, *Peridinium limbatum* (Stokes) Lemmermann represented 60% of the total biomass. At other pH values, Dynophyceae as well as Cryptophyceae attained the lowest population density. At the pH closest to that of the open lake (5.0), the increase of *Mougeotia* sp. was evident and this species constituted 64% of the total Chlorophyceae biomass. Cyanophyceae and Chlorophyceae increased at the same pH value, whereas Crysophyceae were quantitatively important only at pH 6.0 and 6.5.

Müller[32] carried out an experiment to evaluate the effects produced by pH variations on the periphyton. To achieve this aim four plastic tubes (diameter = 10 m; depth 2.0–2.5 m) were settled on the bottom of Lake 223. In three tubes H_2SO_4 was added to obtain pH values of 4.0, 5.0 and 6.0. The fourth tube was kept at the pH value of the lake, that is 6.5. Plexiglass plates settled near the bottom of the tubes constituted the periphyton substratum. The most important results obtained were the following: a) in terms of biomass Diatoms decreased and Chlorophyceae increased with the lowering of pH values; b) the number of algal species, benthic invertebrates and bacteria was not influenced by acidification; c) primary production reached the same order of magnitude in the four tubes; and d) at low pH the diversity of the algal community was small, while the total biomass did not show any variation. In conclusion, the periphyton biomass was not influenced by the acidification, while its composition was simplified (fig. 5).

The influence of acidification on the fate of trace metals and metalloids in water bodies has been investigated by Schindler et al.[41] and Jackson et al.[19] with the enclosure method. In 1976 Schindler et al.[41] anchored to the sediments of Lake 223 four tubes: two were acidified with H_2SO_4 to reach pH values ranging from 5.1 to 5.7, and another two – as control – were maintained at the natural pH of about 6.7–6.8. Successively, to the four tubes 17

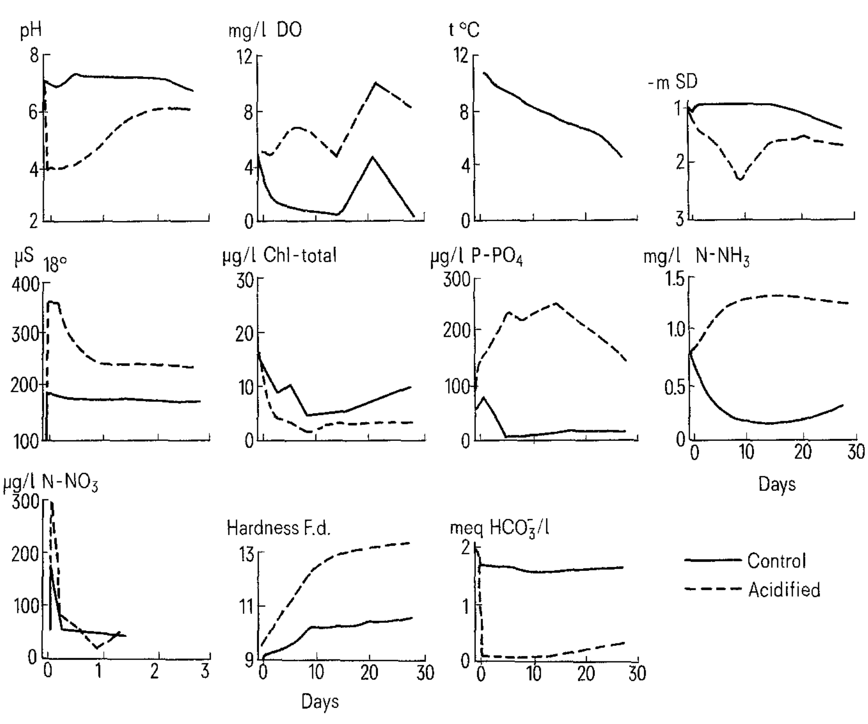

Figure 3. Variations of some physical and chemical parameters in acidified enclosures and in the control. Each value is the average of two replications[2].

radionuclides were added corresponding to the elements taken into consideration (Zn, Co, Cs, Hg, Mn, Fe, Th, Cr, Ba, V, Se, As, Pb, Pu, Am, Np and Cm). In 1975 Jackson et al.[19] settled in the small softwater Lake 303 in ELA five enclosures: four chemically manipulated (they were not considered in the paper mentioned), and one kept as a control. In 1976 the same authors anchored to the sediments of Lake 223 four enclosures: one acidified with H_2SO_4 to reach a pH value of 5.1, and the other three kept as controls at a pH value (6.7–6.8) not yet influenced by the lake acidification. The control tube in Lake 303 was contaminated by eight radionuclides of the elements investigated (Hg, Zn, Co, Mn, Fe, Cr, Cs and As), whereas all the four tubes in Lake 223 were contaminated with radionuclides of V, Th, Ba and Se, in addition to the eight mentioned for Lake 303.

The most important conclusions drawn from the experiments carried out by Schindler et al.[41] and Jackson et al.[19], concerning the effects of acidification, were the following: a) the Cu, Cd, Co, Cr and Pu concentrations in the water column were below the detection limit; b) acidification slowed the removal from the water of some metals (Mn and probably Zn), while it accelerated the loss of others (V and Se); c) in acidified tubes, Co, Mn, Zn and Ba were in soluble form, whereas V and Hg were in particulate form; d) the half-time for removal of the elements from the water varied from 5 to 25 days; e) in acidified medium the metal uptake by sestonic particles seemed to be influenced by the competition between H^+ and metals for binding sites; f) the Zn, Mn, Al and Fe release from the sediments was increased by low pH; g) low pH seemed to have an influence on the uptake (or retention) of Hg solubles by the sediments; h) acidification enhanced the phosphorus flux from the sediments to the water column.

Eggs, artificially fertilized, produced by lake trout *(Salvelinus namaycush)* from Lake 223 (experimentally acidified) were enclosed in plastic tubes and settled in the same lake[22]. Lake 224 and Roddy Lake, similar in all characteristics to Lake 223, except for higher pH value, were kept as controls. No difference was observed in the fertilization of the eggs and their content of Ca, Na, K and P, but the eggs from Lake 223 were smaller than those of the controls. The embryos from Lake 223 showed a smaller size and a high frequency of anatomical malformations. Only 24% of the eggs from this lake, incubated in water not acidified, had gastrulated, while those of the controls attained 75%.

An artificial system simulating a water course has been used by Kolling and Hall[24], to study the effects of pH on the algal community structure and the degradation of a plasticizer (diethyl phthalate). The pH values of the experiments, ranging from 4.0 to 10.0, were obtained by NaOH and HCl addition. The most important conclusions drawn by the authors on the acidification effects were the following: a) a continuous flow system may be altered by the loss of its buffering capacity for acidification until it reaches a pH value lower than 6; b) small effects at community level were recorded; c) nutrient uptake and loss by microorganisms were not altered; d) the variations of the algal community structure and total biomass were not significant; e) diethyl phthalate biodegradation was not affected.

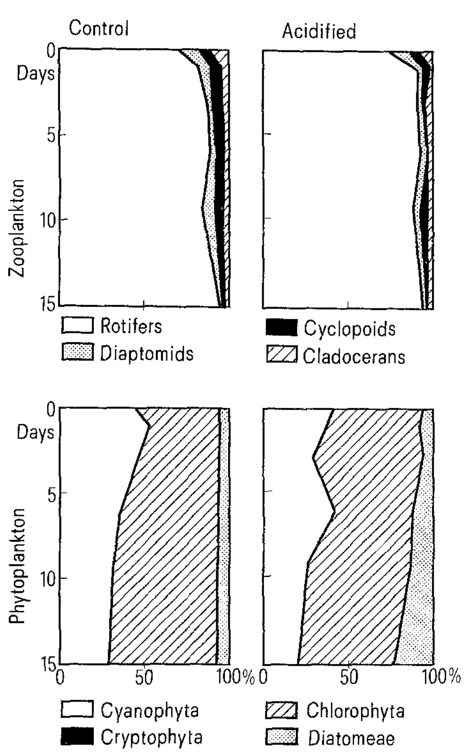

Figure 4. Percent variations in the phyto- and zooplankton community structure in acidified enclosures and in the control. Each value represents the average of two replications[2].

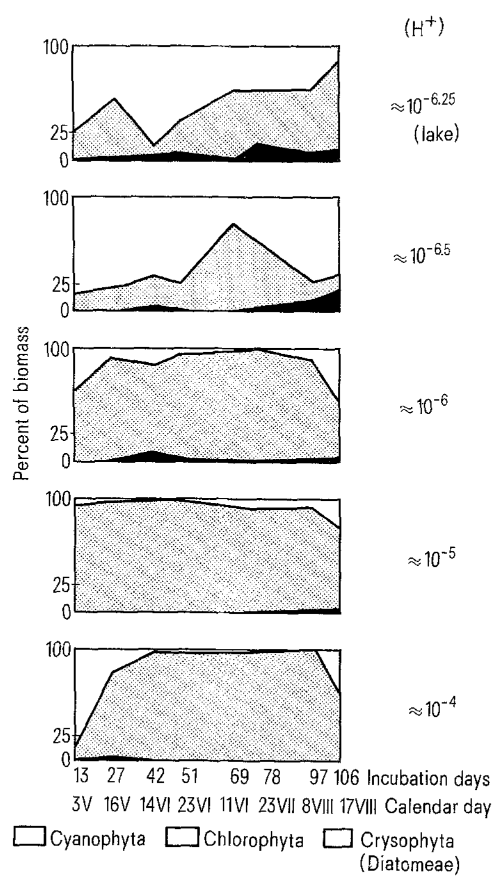

Figure 5. Percent biomass of three main algal groups for Lake 223 and four tubes as a function of time; ($[H^+]$ in mol/l) (from: Müller[32]).

Zischke et al.[51], to evaluate the effects of acidification on macrobenthonic organisms and fish in running water, carried out an experiment with three outdoor channels. The water was pumped from the Mississippi River and continuously acidified with commercial sulphuric acid for 17 weeks to obtain in one channel a pH value of 5, in another pH 6 and in the third, kept as a control, the original value of 8 was maintained. The macrobenthonic populations originated from natural colonization, while fathead minnows *(Pimephales promelas)* were introduced into the channels. The number of insect taxa, the percentage of the insects emerging, and the macrobenthos density and the diversity (calculated at the end of the experiment) decreased with pH. At pH 5 the drift of Amphipods and leeches was stimulated at the beginning of the study. Damselflies, Isopods and leeches were the most resistant organisms, Chironomid larvae, some Amphipods and flat worms showed an intermediate tolerance and other Amphipods and snails (e.g. *Physia gyrina*) were the most sensitive ones. Significant differences in spawning and embryo viability were found between minnows kept in the control channel and those in the pH 5 channel. These were the most important results obtained from this experiment.

Discussion and conclusions

One of the most important advantages of experimental acidification is the observation of the early effects, generally occurring in a short period of time, which have never been recorded in aquatic ecosystems acidified by atmospheric deposition.

Transparency increase has been recorded in Scandinavian as well as in Northern American lakes acidified by atmospheric deposition[12,49], and also in natural[42] and semi-natural environments artificially acidified[2]. Schindler and Turner[42] observed in Lake 223 that the increased transparency produced a more rapid heating of the hypolimnetic waters and a precocious lowering of the thermocline. According to Schindler[38] and Schindler and Turner[42] the increase of the water transparency was due to the decoloration of the organic substances (e.g. humic acids) and not to the decrease of phytoplankton or organic particle concentration[26] or to the coprecipitation of the suspended material with Al or Fe compounds[1,43]. It is likely that in different water bodies the transparency increase is due to different causes. Schindler and Turner[42] attribute to the greater transparency the increase of the chlorophyll concentration and the algal biomass.

The variation in the algal biomass caused by acidification is a matter of discussion. For example, Conroy et al.[7] observed an evident decrease of phytoplankton in very acidic lakes and Blouin et al.[3] in enclosure experiments at very low pH. No relationship between pH value and algal biomass was found by Hörnström et al.[18] in acidic lakes, by Yan and Stokes[50] in acidified enclosures or by Kollig and Hall[24] in artificially acidified channels. It is probable that very high H^+ concentrations decrease the algal biomass, whereas in acidified water bodies with pH values which are not so low, other factors (e.g. transparency) may enhance the algal growth.

There is perfect agreement among the authors on the changes of the community structure of phytoplankton, zooplankton, zoobenthos and fish, due to atmospheric acidification or to experimental acidification of natural and artificial environments. Indeed, the biomass of the primary producers is controlled by nutrients, light and temperature; these factors indirectly influence the biomass of the successive rings of the food chain. If nutrients, light and temperature are maintained constant and the stress is not extremely strong, the depression or elimination of one or more sensitive taxa will be compensated by the increase of others. Conversely, also less important stresses may deeply modify the community structure and its diversity by these processes. The less resistant species may be eliminated and the population density of the surviving ones increased because of the reduction of predators and/or competitors. Also, if the stress is not so great as to eliminate any species, the relative abundance of the various species and their interrelations may be significantly modified. In addition, these variations in community structure may favor colonization by species never present in the ecosystem before its pollution, for example the *Daphnia catawba* × *schoederi* found in Lake 223; this species was never found in this water body before the acidification.

Yan and Stokes[50] found that Dynophyceae are favored by the pH decrease. Müller[32] demonstrated that when the pH value was lowered, the Diatom biomass increased and the Chlorophyceae increased, Delisle et al.[8] observed a strong reduction of Cyanophyceae whereas Chlorophyceae, Cryptophyceae and microflagellates became dominant. In enclosures experimentally acidified, Shapiro[45] eliminated Cyanophyceae in favor of Chlorophyceae, and Blouin et al.[3] reduced Cyanophyceae. Brock[5], observing that Cyanophyceae were absent from acidic lakes, suggested the elimination or control of this taxon by lake acidification.

It is well-known that in Scandinavian acidic streams, periphyton biomass increases[15], but Müller[32] was the first author to study, with enclosure experiments, the periphyton production, structure and diversity, in addition to its biomass. This author found no substantial difference between production, biomass and number of species in periphyton of acidified enclosures and in that of the controls. Conversely, it was evident that acidification reduced the diversity level and, consequently, the food variety for the grazer invertebrates.

It was demonstrated that the acidification of Lake 223 modified the structure of the zooplankton[29] and fish community[31]. The differences in the zooplankton structure between Lake 223, after acidification, and the Scandinavian lakes, acidified by deposition, were due to the fact that the zooplankton species originally were not the same. In addition, the relationships between animals which are preyed upon and their predators (e.g. invertebrates and fish) are different because in Scandinavian water bodies invertebrate predators, more resistant than fish populations, increased in number, feeding on the smaller zooplankters; conversely, in Lake 223 invertebrate predators (e.g. *Epischura, Mysis*) were more sensitive than their prey[2]. Changes in zooplankton structure have been observed by Delisle et al.[8] and Blouin et al.[3] in acidified enclosure experiments. In Lake 223 the elimination of *Mysis relicta* occurred at about the same pH value as that lethal for related Scandianvian species; that is *Gammarus, Asellus* and *Lepidurus*[1,36]. According to some authors[44] the greatest damage to fish living in acidified ecosystems is not due to the high H^+ concentration, but to the Al concentration increase. This may often occur in water bodies acidified by atmospheric deposition, which enhances the Al leaching from the watershed but, obviously, not in experimentally acidified lakes. Indeed, the damage to zooplankton and fish populations of Lake 223 was due to the direct effect of the low pH. It is rather difficult to predict the variations of the structure of fish communities on the basis of the acid-sensitivity of the different species, because the relationship between the species interferes with the direct effects produced by acidification[31]. This is one of the advantages of the experimental acidification of natural ecosystems over laboratory experiments.

Some authors[12,16] observed that water acidification may slow the decomposition rate and they hypothesized that the decomposition of the organic matter in the acidic environment was principally due to fungi. Conversely, other authors[10,11] have demonstrated that the bacteria seem not to be inhibited by low pH. Indeed, up to today, no research has demonstrated a reduction in the rate of decomposition in natural water bodies before, during or after acidification. The important role of the sulphate-reducing microorganisms in alkalinizing eutrophic lakes has been studied in detail by Kilham[23]. Schindler and Turner[42], and Kelly et al.[20] found that anaerobic microorganisms produce alkalinity, tending to neutralize acid waters. From their results it seems evident that acidification does not abolish or decrease the activity of the microbial reducers and the trophic degree of the water body interferes in this fundamental process. Anyhow, it is evident that we need more information on the effects of acidification on the different microorganisms (bacteria and fungi) and their activity, and on the environmental factors (e.g. temperature, oxygen concentration) which influence the decomposition rate.

The quantitative knowledge at present available about the sulphur cycle is rather poor, and from studies on water bodies acidified by atmospheric deposition, only very imprecise estimates can be obtained. Consequently, it is difficult to quantify the sulphur budget in an aquatic ecosystem. With experimental acidification of Lake 223 and Lake 114, Schindler and Turner[42] estimated the sulphur budget of these lakes on the basis of more reliable data, because the loading of SO_4 was exactly known and the input of the dry deposition was negligible.

The increase of metal concentrations in the waters of acidified ecosystems has been observed by all the authors who have considered this subject. The metals present in the water column come from the wet and dry deposition[25], the leaching of the watershed[30] and the water body sediments[41].

With experimental acidification, but not with studies on ecosystems acidified by atmospheric deposition, the contribution of metals from the sediments may be evaluated. Schindler et al.[41] and Jackson et al.[19] carried out studies on this subject using enclosures in Lake 223. The most important conclusion drawn by these authors was that acidification not only enhances the release of some metals and radionuclides from the sediments to the water, but slows down the loss of some others from the water column. As a result, at low pH, the concentration of some metals and radionuclides in the water increases and, consequently, the planktonic and nektonic organisms are exposed to higher concentrations of potential pollutants. Annoni and Ravera[2] observed that the rapid and consistent acidification of a shallow lake with a high buffering capacity (Lake Comabbio) initially produced dramatic changes in its physical, chemical and biological characteristics; but after a few days the ecosystem recovered, reaching a new equilibrium similar, but not identical to the initial one. The phosphate increase, measured in the acidified enclosures of this experiment, may be the result of sulphate reduction, with a consequent decrease to the minimum of the control of iron on phosphorus. The flux of this nutrient from the sediments to the water may produce ecological consequences which are easily predictable.

Except for studies on streams polluted by acid mine wastes, few studies have been carried out on the ecological effects of acidification in natural water courses. Interesting results have been obtained by Hall et al.[13] and Herricks and Cairns[17] by studying natural streams which were experimentally acidified. These studies, as well as those carried out by Zischke et al.[51] in outdoor channels, were concerned with the behavior, density and diversity of macroinvertebrates. Hall et al.[13] on the basis of the data obtained, predict the changes which may occur in the structure and function of an acidified water course at the beginning of the stress as well as during the period of chronic acidification. Kollig and Hall[24] obtained valuable results on the effects of acidification on microorganisms and algae using artificial channels. Because these authors lowered the pH value with HCl, the influence of the H^+ concentration increase could be evaluated, but, obviously, not that due to SO_4^{-2}. Consequently, this experiment simulated only in part the effects of atmospheric deposition.

The results obtained by experimental acidification of natural and artificial environments (e.g. enclosures, outdoor channels) may clarify problems concerning the effects of this stress in aquatic ecosystems. Studies on environments acidified by atmospheric deposition cannot explain these problems, but some other problems may be worked out only with research carried out in these environments. In addition, laboratory experiments are very useful to separate the direct effects of the acidification from their consequences; for example, the stress produced by the H^+ concentration increase on a predator from that due to the reduction of the organism it preys on, because of its high acid sensibility.

In conclusion, it is evident that many different methods must be used, to obtain a better knowledge of these interesting problems both from the theoretical and from a practical point of view.

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