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## Chapter 10

# Bryophytes

Harald G. Zechmeister, Krystyna Grodzińska and  
Grazyna Szarek-Łukaszewska

### Abstract

*The use of bryophytes as bioindicators and biomonitors in terrestrial and aquatic habitats is reviewed in this article.*

*Bryophytes are excellent indicators for a wide range of contaminants. This is in consequence of a series of morphological and physiological properties like the lack of a cuticle or the existence of large cationic exchange properties within the cell wall. Mosses have mainly been used as accumulation indicators especially for heavy metals, radionuclides and for toxic organic compounds. Reviewing a wide range of investigations on this topic, advantages and further needs for research are discussed. Sulphurous and nitrogen depositions can hardly be analysed by methods in the field of accumulation monitoring but by investigating the frequency, distribution, fertility and vitality of bryophyte species and populations. Similar methods are targeted by global change research, especially for the analysis of climate warming and the influence of land-use intensity on biodiversity.*

**Keywords:** Bryophytes, bioindicators, biomonitors, heavy metals, sulphur, nitrogen, toxic organic compounds, radionuclides, global change, terrestrial and aquatic habitats.

## 1. Introduction

Bryophytes are autotrophic cryptogames comprising approximately 25,000 species. Taxonomically, they are divided into four classes, the hornworts (Anthocerotopsida), two classes of the liverworts (Marchantiopsida, Jungermanniopsida) and the mosses (Bryopsida).

The life history of bryophytes involves an alternation between sporophytic and gametophytic generations that differ in form and function. The actual plant is represented by the gametophytic generation, which is the most evolved haploid generation in the whole plant kingdom.

The spores germinate to form a branched or thallose protonema which resembles green algae. The germination as well as its growth is very sensitive to all kinds of natural and human influences which exceeds by far the sensitivity of the green gametophore. Therefore, in many cases the resistance of the protonema against ecotoxicologically relevant substances is the main limiting factor for the distribution of a species (e.g. [Gilbert, 1968](#)). The green gametophore produces the sex organs. After successful pollination, a sporophytic generation evolves which remains attached to the green plant and is nourished by the gametophore. Depending on size, most of the

spores released by the sporangium are dispersed by wind, by rising up to several thousand metres ([Longton, 1997](#)) they can be transported over large distances. Many species also produce vegetative reproductive units which enables them to a less energy demanding propagation or the survival of unfavourable conditions.

Bryophytes are generally small (less than 5 cm) but some can grow up to a length of 70 cm (e.g. *Polytrichum*, *Dawsonia*). In contrast to vascular plants, they rarely grow as single stems but in groups, which form turfs, cushions, wefts or other growth forms (e.g. [Mägdefrau, 1982](#)). Bryophytes show a limited range of anatomical or morphological features but a wide range of physiological and dispersal adaptations to stress caused by natural or anthropogenic disturbance (e.g. [Smith, 1982](#); [Bates and Farmer, 1992](#)). A great variety of life traits can be found mainly in short lived habitats (e.g. [During, 1979](#); [Proctor, 1990](#)). In Grime's (1979) triangular scheme they are mainly ruderals and/or stress tolerants ([Grime et al., 1990](#)). The various modes of reproduction play an important role in the life cycles of mosses especially in stands with a high disturbance (e.g. [During, 1997](#); [Longton, 1997](#); [Zechmeister and Moser, 2001](#)).

Bryophytes thrive in humid climates, but can be found all over the world, even in arid regions. As a consequence of slow evolution (e.g. [Szweykowski, 1984](#)), many dominant species can be found all over the world, or show at least a circumpolar distribution. Their biomass production is important in subarctic ecosystems and mountainous tropical rain forests only ([Longton, 1988](#); [Pòcs, 1980](#)), but they are a significant ecological factor in a variety of habitats (e.g. bogs, water springs, alpine grasslands). On the other hand, a wide range of species can grow in areas unable to be colonised by any other plant which is significant for many aspects in bioindication also. Bryophytes colonise nearly every kind of terrestrial substrate (e.g. bare stones, bark, skeletons, etc.) and grow in freshwater but are absent from saline waterbodies (salt lakes, oceans).

## 2. The physiological basis for the use of bryophytes as indicators

The use of bryophytes in an increasing number of monitoring programmes is based on a wide range of remarkable anatomical and physiological properties, which are briefly reviewed. Further information on this topic are given in extensive reviews by [Bates \(1992\)](#), [Brown \(1984\)](#), [Brown and Bates \(1990\)](#), [Proctor \(1982, 1990\)](#), [Sveinbjörnson and Oechel \(1992\)](#), [Tyler \(1990\)](#) or [Onianwa \(2001\)](#).

### 2.1. Water relations

Bryophytes are poikilohydric species, but among them there is a diversity of means of water and mineral uptake. As most of the bryophytes are small and the leaves of many mosses as well as those of folious liverworts are built up by only one cell layer, the surface/volume ratio is high. According to their small size, the micro-environment in some climates is often much more important than the macro-environmental conditions.

Most of the bryophytes are ectohydric species, which means that most of the species receive water as well as mineral nutrients predominantly by atmospheric depositions.

They are well adapted to this strategy since they have no or only very small vacuoles, and beside some surface wax structures (e.g. papillae) there is no continuous water-repellent cuticle. Some species obtain additional water and soluble nutrients from the substrate. In a few cases (e.g. Polytrichales) bryophytes have water conducting tissues (endohydric species). Additionally, the physical and chemical properties of the substrate is essential for the establishment and survival of the green plants. Beside species adapted to wet habitats, which hold most water within large cells, external capillary conducting systems retain the major part of the water content. They are diverse and can be found on surfaces of leaves, between various parts of the gametophore (e.g. rhizoid wefts, auricles between stem and leaves) or between single stems. The latter leads to a wide range of growth forms in dependency of habitat conditions. Desiccation tolerance is mainly based on physiological adaptations and enables some species to grow in dry and hot environments.

## 2.2. Mineral requirements

Mineral requirements are similar to those in vascular plants. Mineral uptake by the cell is controlled by a semipermeable membrane. The protonema and the early gametophyte is attached to the substrate and significant stocks of nutrients may be accumulated from the surface at this stage. Later on, many pleurocarpous species leave the close contact to the substrate and it is generally assumed that the main source of minerals for these species are atmospheric sources (e.g. Tamm, 1953; Bates and Bakken, 1998), though some elements (e.g. Ca, K, P) seem to be derived further via the substrate (e.g. Bates, 1992; Wells and Boddy, 1995; Brown and Brümelis, 1996; Brümelis and Brown, 1997).

Elements associated with well developed gametophores can be attributed to four possible locations (Brown and Bates, 1990; Bates, 1992): trapped particulate matter, intercellular soluble, extracellular, bound to cell wall on charged exchange sites, or intracellular. Particulate matter and intercellular elements are unbound ions in the water free space and can easily be removed by washing or mechanic treatment. Exchangeable cations are bound to positively charged exchange properties of the cell wall and are fixed by a process mainly depending on physico-chemical processes and are not physiologically active, whereas intracellular elements fulfil a physiological function.

### 2.2.1. Extracellular uptake

The very high cation exchange capacity is related mainly to unesterified polyuronic acid molecules (Clymo, 1963). In *Sphagnum* galacturonic acid and in some liverworts mannuronic acid is reported to be present in the cell walls too (Brown, 1984). Binding conforms to strict physico-chemicals rules. External uptake is rapid and occurs within the first few minutes during rainfall (Gjengedahl and Steinnes, 1990). The uptake depends on the nature of the elements only, irrespective of the physiological condition of the plant. Monovalent cations (e.g. K, Na) show less affinity for anionic sites and divalent cations with Class B characteristics (e.g. Pb, Cu) show greater affinity than Class A metals (eg. Cd, Mg, Zn; Brown and Brown, 1990). The uptake of heavy

metals is slightly influenced by pH of the precipitation and air temperature. Laboratory studies have shown that the presence of  $\text{Na}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Cl}^-$  in coastal precipitation reduces the uptake of Zn and Cu (e.g. Berg et al. 1995).

Several investigations have shown that uptake efficiencies seem to follow the order  $\text{Pb} \gg \text{Co} = \text{Cr} > \text{Cu} = \text{Cd} = \text{Mo} = \text{Ni} = \text{V} = \text{Sb} > \text{Zn} > \text{As}$  (e.g. Steinnes, 1985; Berg et al., 1995; Thöni et al., 1996; Berg and Steinnes, 1997). Slightly different results are given by Rühling and Tyler (1970) and Ross (1990) who showed a markedly higher retention capacity for Cu. Čeburnis et al. (1999) found accumulations as follows:  $\text{Ni} < \text{V} < \text{Cr} < \text{Zn}$ . Čeburnis and Valiulis (1999) who compared concentrations in bulk depositions with throughfall under moss obtained an absolute uptake efficiency of approximately 60% but did not find differences for the various metals.

The total metal binding is determined by the number of available exchange sites and morphological structures of the bryophytes, which differs from species to species. Therefore, most species have different uptake capacities. There are no significant differences in the accumulation of a wide range of trace metals (e.g. Pb, Cd, Cu, V) between the mosses *Pleurozium schreberi* (Brid.) Mitt. and *Hylocomium splendens* (Hedw.) B.S.G., (Herpin et al., 1994; Zechmeister, 1994; Berg and Steinnes, 1997; Halleraker et al., 1998). These mosses have been used in a range of investigations (see Section 4.2). Differences occur mainly in metals, which are either not well retained (like As) or have high background levels in the moss (Zn; Rühling and Steinnes, 1998). Interspecific calibration is advised if data are compared between different species (e.g. Zechmeister, 1998).

Younger parts of the plants show higher amounts of monovalent cations and nutrient anions than older parts. Divalent cations, especially heavy metals, show the reverse distribution. Dead tissues retain polyvalent cations more effectively still (e.g. Rühling and Tyler, 1970; Pakarinen and Rinne, 1979).

### 2.2.2. Intracellular uptake

In contrast to the extracellular uptake mechanism, intracellular uptake is influenced by various aspects of plant metabolism. Entry to the cell plasma is determined e.g. by the affinity for an appropriate carrier, competitive elements, gradients in element concentration or the energy status. Elements located within the cell influence cell metabolism. Uptake rates are in general much lower than at the extracellular sites.

As shown by various authors (e.g. Pickering and Puia, 1969; Burton and Peterson, 1979; Wells and Brown, 1987), non-physiological elements like heavy metals also pass the limiting plasma membrane of the cell and affect cell metabolism. In consequence heavy metals induce the production of thiol-containing peptides such as glutathiones which therefore can be used as biomarker for heavy-metal pollution (e.g. Bruns et al., 1999, 2001). Nevertheless, the cell wall is an efficient barrier against the penetration of heavy metals into the protoplasm of the bryophyte cell (Shimwell and Laurie, 1972; Skaar et al., 1973). Young shoots tend to have a more effective barrier than older ones (Lüttge and Bauer, 1968).

Some bryophyte species (e.g. copper-mosses) also tolerate elevated levels of toxic elements on a physiological level (e.g. Url, 1959; Shaw, 1987).

### 2.3. Sources of elements

Mineral cations as well as anionic nutrients derive mainly from atmospheric depositions (e.g. [Rieley et al., 1979](#); [Okland et al., 1999](#); [Reimann et al., 2001](#)). Positive correlations exist between the quantity of rainfall and nutrient concentrations in bryophytes, which can vary with plant growth ([Brown and Bates, 1990](#)). Nevertheless, some elements (e.g. Ca, Mg, K) depend also on the uptake from the substrate, especially in endohydric species as well as in mosses growing in the form of turfs, cushions or covers (e.g. [Büscher and Koedam, 1979](#); [Brown and Bates, 1990](#); [Bates, 1992](#); [Okland et al., 1999](#)) although there are contrasting results ([Brümelis et al., 2000](#)).

The amount of occult depositions (fog, mist, clouds) seems to be high in some areas. There are few investigations on the percentage of dry in relation to total depositions (e.g. [Fowler et al., 1993](#)). The estimation is difficult as there are also dry depositions during rainfall. The results show a wide range for the percentage of the dry depositions : 20 % ([Ross, 1990](#); [Ruijgrook et al., 1993](#)), 33% ([Svensson and Lidén, 1965](#)) and 50% ([Galloway et al., 1982](#)). There are fairly large differences for the various metals and there is a strong correlation with annual precipitation and the distance to emission sources ([Brown and Brümelis, 1996](#); [Zechmeister, 1997](#)).

Internal translocation of elements within the bryophytes seems to play an important role in their nutrient cycles, although most bryophytes lack recognisable conducting tissues. Acropetal movement of photoassimilates and essential elements has been reported by several authors (e.g. [Brown and Bates, 1990](#); [Wells and Boddy, 1995](#); [Brown and Brümelis, 1996](#); [Bates and Bakken, 1998](#)) though further investigations are needed.

### 3. General reactions on pollutants and areas of applications

Under the influence of human induced changes of the environment, bryophytes respond sensitively and this can effectively be used for monitoring purposes.

Mostly they show a decline in vitality, which, for example, can be detected by changes in colour following damages in the chloroplast structure (e.g. [Martínez-Abaigar and Núñez-Olivera, 1998](#)), or less vigorous growth by individuals or populations (e.g. [Bengtson et al., 1982](#); [Callaghan et al., 1997](#)). Sometimes there is also a shift in the reproduction mode, favouring asexual reproduction under stress ([Rao, 1982](#); [Otnyukova, 1995](#); [Zechmeister and Moser, 2001](#)). The ultimate response is population loss and finally extinction.

There is a wide range from toxitolerant species to extremely sensitive ones. Tolerances vary not only from species to species but also with the type of pollutants. Additionally, it must be considered that climatic conditions are much more influential for the survival of poikilohydric organisms than e.g. for flowering plants.

On the other hand, bryophytes are very resistant against a series of substances which are highly toxic for other plants (e.g. heavy metals, radionucleides, various toxic organic compounds). As a consequence of their nutrient cycling and uptake mechanisms (see above) they even tend to accumulate these pollutants.

Regarding both their accumulation capacity and their sensibility to various toxic compounds, bryophytes can be used either as accumulation indicators or as sensitive indicators for a series of human influences, mainly polluting substances.

#### 4. Bryophytes as indicators and monitors in terrestrial habitats

##### 4.1. Heavy metals

Heavy metals originate from both natural and anthropogenic sources in the environment. In the atmosphere, natural sources of these elements are volcanic eruptions, cosmic and terrestrial dusts, vegetation fires, and salt spray from the oceans. Anthropogenic sources include emissions from different industrial plants (steel and non-ferrous metallurgy, smelters, alloying plants, petrochemical industry, fertiliser plants, coal power plants, industrial and home furnaces) and motor traffic. The amount of heavy metals originating from natural sources in the atmosphere is small as compared with the anthropogenic flux of these elements.

Airborne heavy metals enter the ecosystems where they circulate and, depending on their concentration and toxicity, pose a greater or smaller threat to the components of these ecosystems. The accumulation of heavy metals in the soil and living organisms may have a damaging effect on the environment (Lieth and Markert, 1990; Markert, 1993; Herpin et al., 1996).

In the 1950s and 1960s, the quick development of industry and motor transport caused a dramatic increase in dust emissions containing heavy metals. It is only natural that ecologists focused their attention on threats posed by heavy metals to the biotic and abiotic environment. They began to look for sensitive and, cheap biological methods for assessing the environmental level of heavy metals, above all the most toxic ones (Cd, Pb, Hg).

In the late 1960s, two Swedish ecologists, [Rühling and Tyler \(1968; 1969\)](#), first used mosses as indicators of heavy metals pollution. They recognised these plants to have many features of good bioindicators.

The suitability of bryophytes for the indication of heavy metal depositions is based on their accumulation which is a result of a series of morphological and physiological properties which have already been given in Section 1 (e.g. cationic exchange properties).

Additionally in certain species of feather mosses (e.g., *Hylocomium splendens* (Hedw.) B.S.G.) and in *Sphagnum* spp. it is possible to recognise and separate annual growth increments, facilitating determination of the age and exposure time of the material to be used in monitoring. This is one of the most important advantages of using mosses for the estimation of heavy metal depositions.

Several species are very abundant and widespread (cosmopolitan or circumpolar) in defined habitats.

Owing to the longevity of most bryophytes they may be used to integrate the deposition over a considerable time, usually 2–5 years, depending on the species and sampling methods.

Moss methods in heavy metals monitoring work are inexpensive and simple.

Since the time of Rühling and Tyler's pioneer research (1968; 1969; 1970; 1973), mosses have become commonly used indicators of heavy metals contamination of the environment (e.g., Groet, 1976; Grodzińska, 1978; Rinne and Barclay-Estrup, 1980; Herpin et al., 1994; Liiv et al., 1994; Zechmeister, 1994; Kuik and Wolterbeek, 1995; Herpin et al., 1996; Markert et al., 1996; Äyräs et al., 1997; Berg and Steinnes, 1997; Čeburnis et al., 1997; 1999; Mankovska, 1997; Alliksaar and Punning, 1998; Pott and Turpin, 1998; Sucharová and Suchara, 1998; Galsomies et al., 1999; Grodzińska et al., 1999; Davis et al., 2001; Figueira et al., 2002). Onianwa (2001) gives an extensive overview on this topic.

Mosses have been used for evaluating (1) the present state of environmental contamination by heavy metals in both extensive and small areas and (2) the pollution level in the past. Some examples of these studies are presented below.

#### 4.1.1. Assessment of the current heavy metals levels of the environment

##### 4.1.1.1. Indigenous species

Moss technique was first used for large – scale monitoring purposes in the Scandinavian countries: Sweden, Norway, Finland and Denmark (Gydesen et al., 1983; Rühling et al., 1987). National monitoring programmes were co-ordinated by the Nordic Group of Heavy Metal Deposition. In the late 1980s, European countries were invited to the European Heavy Metal Deposition Programme by the Environmental Monitoring and Data Group in the Nordic countries. The 1990 survey was extended to 21 European countries (Rühling, 1994), and the 1995 survey to 28 European countries (Rühling et al., 1996; Rühling and Steinnes, 1998). According to the European programme guidelines, moss monitoring should be carried out at five-year intervals. Thus the next sampling of mosses fell in the year 2000.

Some examples of monitoring studies carried out on the European continent using mosses as bioindicators are given below.

The concentrations of heavy metals (As, Cd, Cr, Cu, Fe, Pb, Ni, V, Zn) in moss samples (*Hylocomium splendens* (Hedw.) B.S.G., *Pleurozium schreberi* (Brid.) Mitt.) collected in north European countries (Norway, Sweden, Finland, Denmark, Greenland, Iceland) during the 1970s and 1980s showed considerable spatial differentiation (Rühling and Tyler, 1973; Steinnes, 1977; Gydesen et al., 1983). The highest levels of heavy metals were found in mosses from the southern part of the Scandinavian Peninsula (Fig. 1), which was under the influence of heavy metals emissions from Western European countries. The metal content of mosses decreased markedly towards the north, reaching the lowest values in Iceland and Spitsbergen (Gydesen et al., 1983; Rühling et al., 1987). This decreasing gradient of heavy metals levels in mosses from south to north was in conformity with the spatial distribution of metal concentrations in the atmosphere.

The results of studies conducted within the framework of European joint moss research in 1990–1995 (Rühling, 1994; Rühling and Steinnes, 1998) using three moss species (*Pleurozium schreberi* (Brid.) Mitt., *Hylocomium splendens* (Hedw.) B.S.G. and *Hypnum cupressiforme* agg.) confirmed regional variations in the deposition of heavy metals in Europe, as noticed in earlier decades. They showed a sharply decreasing gradient from Central Europe to northern Scandinavia for Cd, Pb and V



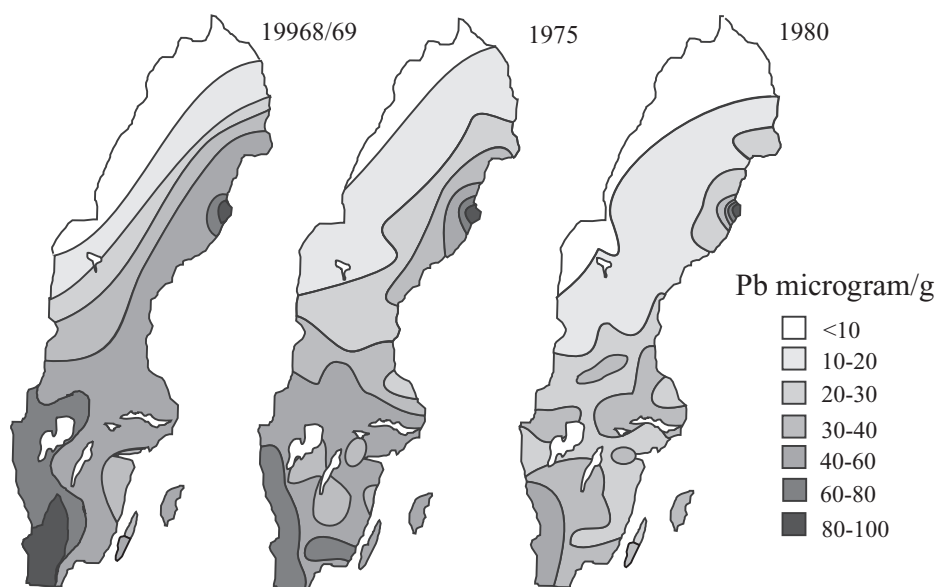


Figure 1. Concentrations of Pb in forest moss in Sweden (Rühling and Tyler, 1984).

(Fig. 2). A weaker gradient was obtained for Cr, Cu, Fe, Ni and Zn, and particularly for Ni and Cu in the far north, in the western part of the Kola Peninsula where there are big smelting plants.

Continental and national studies on heavy metals using the moss technique indicate a general decrease in the concentrations of most metals during the last 15 years (Gydesen et al., 1983; Rühling et al., 1987; Rühling, 1992; Rühling, 1994; Rühling and Steinnes, 1998; Kunert et al., 1999). This decrease, which has taken place over large areas, is due principally to better emissions control legislation, better filter technique and the closure of old polluting industrial plants. For lead the decrease is due to reduction in the use of leaded petrol.

Assessment of heavy metals contamination using mosses has also been carried out on a regional scale (e.g. Šoltès, 1992; Kauneliene, 1995; Markert et al., 1996; Äyräs et al., 1997; Halleraker et al., 1998; Gerdol et al., 2000; Grodzińska and Szarek-Łukaszewska, 2001). Because for these studies a denser network of moss sampling sites was established than for the large-scale monitoring studies, spatial variations in deposition could be examined more thoroughly.

The heavy metals concentrations in *Pleurozium schreberi* (Brid.) Mitt. collected in two industrial regions in southern Poland (Kraków-Silesia region and Legnica-Głogów Copper Basin) showed evident spatial diversification. The highest concentrations of the elements occurred in places with a high number of plants and smelters, hence the high correspondence between the volume of production and industrial emissions and the levels of contaminants in mosses (Fig. 3).

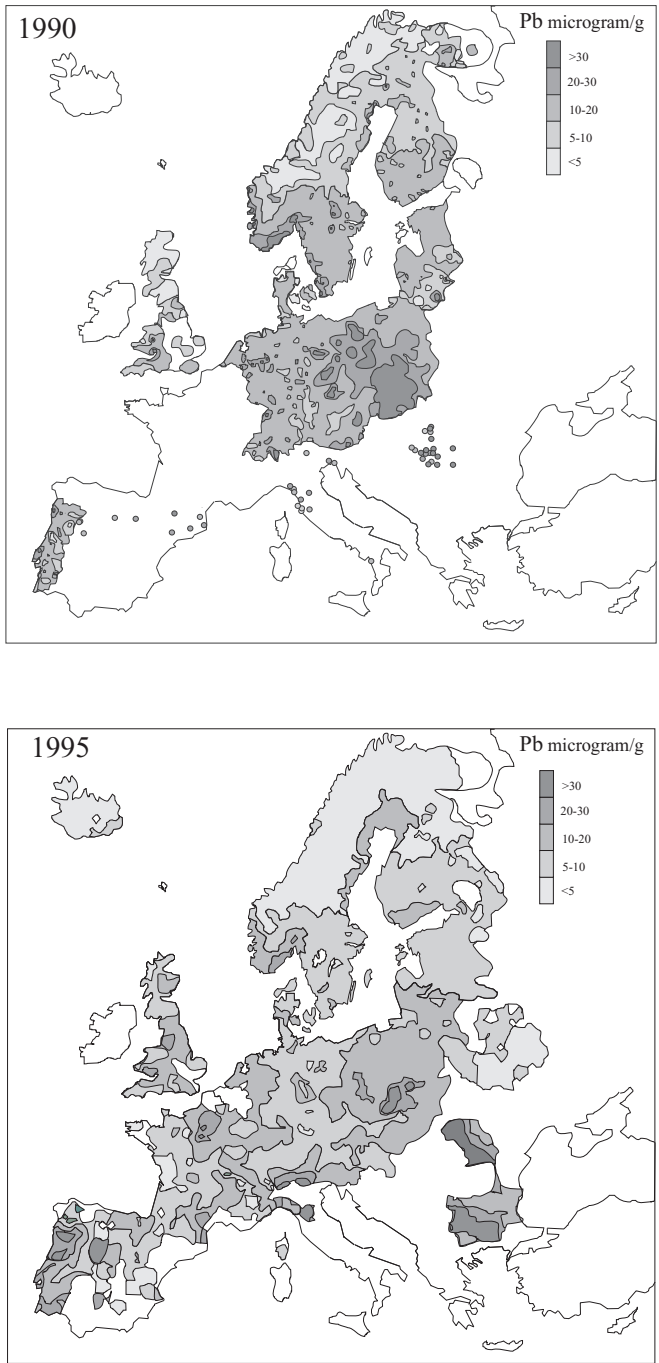


Figure 2. (a) Concentration of Pb in forest moss in Europe in 1990 (Rühling, 1994). (b) Concentration of Pb in forest moss in Europe in 1995 (Rühling and Steinnes, 1998).

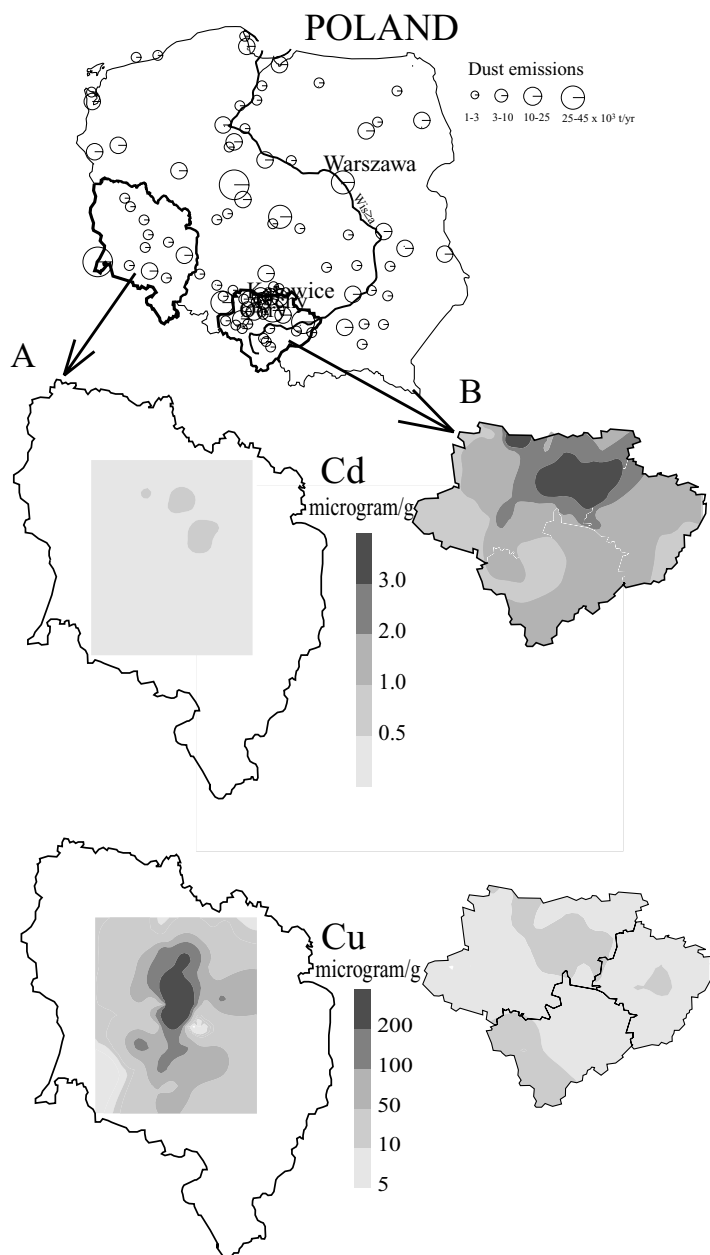


Figure 3. Concentrations of Cd and Cu in *Pleurozium schreberi* in two industrial regions in southern Poland. A – Legnica – Głogów Copper Basin; B – Kraków – Silesia Region (Szarek, Klich, Grodzińska unpublished data).

The extremely high concentrations of copper and nickel in moss (*Hylocomium splendens* (Hedw.) B.S.G.) samples from the western part of the Kola Peninsula correlated significantly with the distribution of emission sources (nickel mining and smelting) in this Arctic area (Äyräs et al., 1997).

The moss technique is also very useful for assessing environmental contamination on a local scale, in the neighbourhood of industrial plants (Makomaska, 1978; Pilegaard, 1979; Barclay-Estrup and Rinne, 1979; Folkesson, 1981; Mäkinen, 1983, 1994a), in towns (Lötschert et al., 1975; Grodzińska and Kaźmierczakowa, 1977; Hertz et al., 1984; Mäkinen, 1987), and in the vicinity of highways (Rühling and Tyler, 1968; Markert and Weckert, 1994). It was found that the concentrations of heavy metals decreased markedly with increasing distance from an industrial plant, a town centre (Fig. 4) or highway (Fig. 5).

Folkesson (1981) assessed the concentrations of 10 heavy metals in *Pleurozium schreberi* (Brid.) Mitt. along a 0.06–10 km transect from a peat-fired power plant. A considerable decrease in the levels of heavy metals in mosses with increasing distance from the power plant was observed.

Grodzińska and Kaźmierczakowa (1977) determined the accumulation of Cd, Pb and Fe in moss samples collected from urban parks in the urban-industrial agglomeration of Kraków, located some 5–17 km from a steelworks. In moss samples from the park farthest from the works the concentration of Fe was 2.5 times lower than in mosses from the park closest to the emissions source. The differences in Cd and Pb levels were small. This is evidence that in the investigated urban agglomeration the

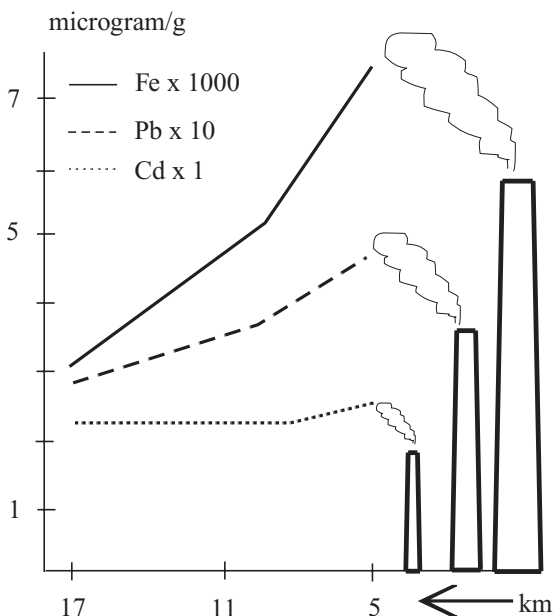


Figure 4. Concentrations of Fe, Pb and Cd in moss in parks of Kraków city (southern Poland) (Grodzińska and Kaźmierczakowa, 1977).

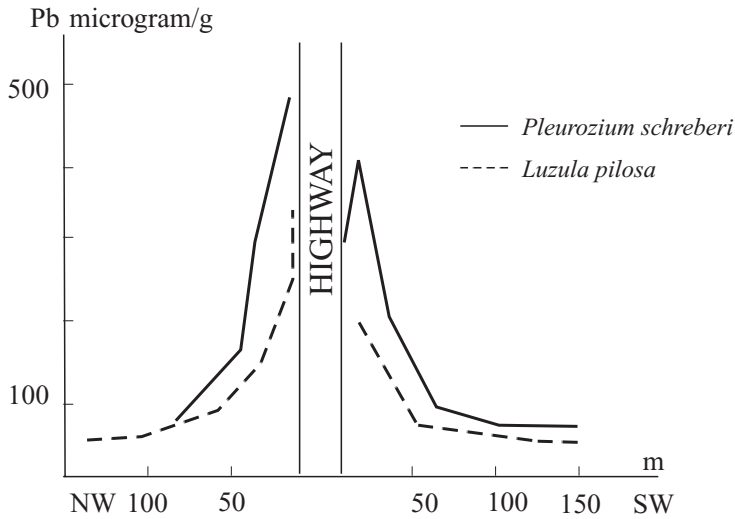


Figure 5. Lead gradients in the road transect at Stavsjo (S. Sweden) (Rühling and Tyler, 1968).

most important source of Fe was the steelworks, while Pb and Cd are also emitted by other sources in the town itself (Fig. 4).

Rühling and Tyler (1968) showed that the lead concentrations in mosses growing near highways with heavy motor traffic in southern Sweden decreased markedly (nine-fold) over a distance of a mere 150 m from the highways (Fig. 5).

A first attempt to compare heavy metal concentrations in mosses with the incidence of various types of human diseases was performed by Wappelhorst et al. (2000). They found a connection between respiratory diseases and levels of the elements of Ce, Fe, Ga and Ge. A correlation also existed between thallium concentrations and heart disease. Further investigations have been advised.

*Estimation of deposition rates:* In most studies of heavy metal pollution using the “moss-method”, concentrations in mosses are compared with each other. This is satisfactory enough if mosses of the same species are compared or if intercalibration data are available for the species in use (See Section 4.2).

But for environmental protection measures that require political decisions, deposition data ( $\text{g/m}^2$ ,  $\text{kg/ha}$  etc.) are needed.

Sample strategies that are used in most of the national and international programmes (e.g. Rühling et al., 1987, Rühling and Steinnes, 1998) involve moss collection at sampling points without any interference of canopy and throughfall. Therefore, bulk depositions which facilitate the calculation of area-related depositions (e.g. Bergerhoff), have been compared with concentrations in mosses. The “efficiency factor” gives an estimation of the percentage of ions bound by the moss in relation to the absolute deposition. Following a series of studies (e.g. Steinnes, 1985; Ross, 1990; Gjengedahl and Steinnes, 1990; Berg et al., 1995; Thöni et al., 1996; Berg and Steinnes, 1997; Zechmeister, 1997) efficiency factors can be estimated as: As (25%),

Cd (60%) Co (60%), Cr (65%), Cu (35%), Fe (70%), Mo (60%), Ni (50%), Pb (100%), V (60%) and Zn (60%).

Whereas there are a series of parameters influencing uptake capacities (see also Section 2.2.1), the factors thus obtained seem good enough for overall estimations. Nevertheless, further studies on efficiency-factors are urgently needed.

Two methods have been used for the evaluation of deposition data from concentrations in mosses, both of which can be seen as additional advantages of the moss method.

*Method A: Calculations following the formula (Rühling et al., 1987):*

$$D = C * G/E$$

D: Deposition

C: Concentration analysed in mosses

G: Annual increase of biomass by area for the species in use

E: Efficiency factor

For this calculation, data on area-related bryophyte growth are needed. These data have been obtained by [Tamm \(1953\)](#), [Rühling et al. \(1987\)](#) and [Zechmeister \(1994, 1995a, 1998\)](#) for most of the species in use (e.g. *Hylocomium splendens* (Hedw.) B.S.G., *Pleurozium schreberi* (Brid.) Mitt., *Hypnum cupressiforme* Hedw. s. str. and *Abietinella abietina*(Hedw.) Fleisch.).

*Method B: Calculation by regression equation*

An alternative method is to calculate moss data versus atmospheric deposition values from analysis of precipitation samples by using linear regression. The regression equation can be used to transform moss concentration data directly to absolute deposition rates ([Ross, 1990](#); [Berg et al., 1995](#); [Berg and Steinnes, 1997](#)).

#### 4.1.1.2. Live moss transplanting and moss bag technique

Two other assessment techniques are used, namely the transplantation method and the moss bag method. The former consists in transplanting living mosses together with their substratum from clean areas to areas under the influence of emissions. Mosses are exposed in polluted areas over a period of some weeks or months, and next examined for heavy metals concentrations ([Goodman and Roberts, 1971](#); [Pilegaard, 1979](#); [Johnsen et al., 1983](#); [Markert, 1993](#)). For transplantation three moss species have been used: *Dicranoweissia cirrata* (Hedw.) Lindb., *Hypnum cupressiforme* agg. and *Tortula ruralis* agg. This method is not recommended as, in addition to atmospheric pollution, the transplanted plants experience stress induced by other habitat factors (e.g., changes in light, humidity) ([Tyler, 1990](#); [Čeburnis and Valiulis, 1999](#)).

In the moss bag method, samples of dried or fresh mosses collected from clean areas are placed in nylon nets and exposed in a polluted area over different periods of time (two weeks, one month). Afterwards the concentration of heavy metals is measured in the samples. The most often used moss species are *Sphagnum* spp. and more rarely *Hypnum cupressiforme* agg., *Hylocomium splendens* (Hedw.) B.S.G. or *Pleurozium schreberi* (Brid.) Mitt. The moss bag technique has been used most often

in urban areas and industrial agglomerations where indigenous mosses are absent (Goodman and Roberts, 1971; Little and Martin, 1974; Hynninen, 1986; Mäkinen, 1987; Yurukova and Ganeva, 1997; Čeburnis and Valiulis, 1999). The concentration of heavy metals in moss bags correlates with atmospheric heavy metals content. Tyler (1990) and Čeburnis and Valiulis (1999) recommend to use the moss bag technique in areas without indigenous moss flora. However, they also point to factors limiting the use of this method (type of bag, time of exposure).

#### 4.1.1.3 Factors modifying the level of heavy metals in mosses

Many years of surveys carried out by researchers in different countries in and beyond Europe have shown that the level of heavy metals in mosses can be influenced by factors other than air pollution (climatic and edaphic factors, moss species, etc.)

In the following some factors most often mentioned by researchers are listed with references to publications where the reader can find detailed data. Researchers' opinions on the importance of particular factors vary and often are controversial. Some questions have already been discussed in Section 2. Most of the controversial results have to be a matter of research in the future to improve the method.

Beside local or global emissions factors modifying the heavy metals content of mosses are as follows:

1. Precipitation: intensity, frequency and duration can lead to an increase in concentrations as well as to a rinsing of already deposited metals (Rühling and Tyler, 1969; 1971; Ross, 1990; Tyler, 1990; Schmid-Grob et al., 1992; Rühling, 1992; 1994; Steinnes et al., 1992; Thöni et al., 1996).
2. Altitude: usually there is an increase in deposition with rising altitude (Groet, 1976; Zechmeister, 1994; 1995b; Šoltès, 1998), although there are controversial results (Schmid-Grob et al., 1992; Gerdol et al., 2002).
3. Mineral particles: mainly windblown dust from local soils, which make it difficult to distinguish between recent atmospheric depositions and waste from the past; several metals (e.g. Al, Ti) are used as indicators for the identification of the amount of windblown particles (Brown & Brown, 1990; Steinnes et al., 1992; Mäkinen, 1994b; Rühling, 1994; Steinnes, 1995; Čeburnis et al., 1999; Fernández and Carballeira, 2002; Figuera et al., 2002).
4. Natural cycling processes, in particular atmospheric transport from marine environments; this leads to a shift in element concentrations and changes in uptake efficiencies (see also Section 2.2.1) (Rühling, 1992; Steinnes et al., 1992; Kuik and Wolterbeek, 1995; Čeburnis et al., 1999).
5. Root uptake in vascular plants from soil and subsequent transfer to mosses by leaching from living or dead plant tissues (Brown and Bates, 1990; Rühling, 1992, 1994; Steinnes et al., 1992; Kuik and Wolterbeek, 1995; see also Sections 2.2 and 2.3).
6. Moss species: *Pleurozium schreberi* (Brid.) Mitt. and *Hylocomium splendens* (Hedw.) B.S.G. are the most often used moss species (Rühling and Tyler, 1969; Grodzińska, 1978; Gydesen et al., 1983; Rühling, 1992; 1994; Herpin et al., 1994; Wolterbeek et al., 1995; Rühling and Steinnes, 1998; Galsomies et al., 1999; Sucharová and Suchara, 1998; Zechmeister, 1998; Grodzińska et al., 1999) but *Hypnum cupressiforme* agg. (Herpin et al., 1994; Zechmeister, 1994; Wolterbeek

et al., 1995) and *Scleropodium purum* (Hedw.) Limpr. (Markert et al., 1994; Sucharova and Suchara, 1998) somewhat rarely. Other moss species utilised as indicators of heavy metal pollution are *Dicranum scoparium* Hedw. (Markert et al., 1996), *Abietinella abietina* (Hedw.) Fleisch., *Rhytidium rugosum* (Hedw.) Kindb., *Ctenidium molluscum* (Hedw.) Mitt. (Zechmeister, 1994; 1998), *Polytrichum formosum* Hedw. (Markert et al., 1996), *Bryum argenteum* Hedw. (Thöni et al., 1993), *Thuidium tamariscinum* (Hedw.) B.S.G. (Galsomies et al., 1999), *Pohlia nutans* (Hedw.) Lindb. (Folkesson, 1979), *Leucobryum glaucum* Brid. (Groet, 1976), various species of *Sphagnum* and *Polytrichum* (Šoltès, 1992; 1998). The level of accumulation of heavy metals depends on the species. According to Folkesson (1979) the use of different monitor species in a heavy metal deposition survey require interspecies calibration. Thöni et al. (1996) suggest not to convert the data of one species to the other, even when there seem to be tendencies for different concentrations. A conversion may result in a bigger mistake. Wolterbeek et al. (1995) and Galsomies et al. (1999) suggest that the use of inter-species calibration from different countries may lead to unreliable results when concentration ranges are different.

7. Age of mosses – different exposure time (Pakarinen, 1977; Grodzińska, 1978; Makomaska, 1978; Brown and Brümelis, 1996).
8. Part of mosses: some authors report about different accumulation rates in stems or leaves (Lötschert et al., 1975; Siebert et al., 1996).
9. Growth rate of species: variations in growth according to climatic conditions leads to different concentrations at comparable deposition rates (Zechmeister, 1995a; 1998).
10. Date of sampling (seasonal variation): there are results which indicate a variation of concentration according to sampling period as a result of moss-growth (Markert and Weckert, 1989; Zechmeister, 1994; Zechmeister et al., 2002a), others did not find any variations (e.g. Fernandez and Carballeira, 2002).
11. Type of moss samples (indigenous, transplanted, bag).
12. Sample preparation procedure and analytical methods (Steinnes et al., 1993; Markert et al., 1994; Wolterbeek and Bode, 1995).

Data evaluation and presentation has to be taken carefully and is critically analysed by several authors (e.g. Kostka-Rick et al., 2001; Herpin et al., 2001).

Despite these variations, when properly applied, moss methods are useful and often more useful than other biological methods in assessing atmospheric heavy metal pollution.

#### 4.1.2. Heavy metal contamination of the environment in the past

Using mosses from old herbarium collections, one may assess the level of heavy metals contamination in the past (Rühling and Tyler, 1968, 1969, Herpin et al., 1997). According to Rühling and Tyler the concentrations of Pb, Ni and Cr in *Hypnum cupressiforme* agg. collected at the same localities in southern Sweden more than doubled over a period of 150 years. For Cu and Zn the estimated increase was much smaller. Although heavy metal concentrations were low in herbarial material of the



years between 1845 and 1901, Herpin et al. (1997) found that the concentrations of As, Cd, Cr, Cu, Pb and Zn in mosses collected between 1845 and 1974 were generally higher than in mosses collected in 1991. They conclude that this was in consequence to improved methods of preventing air pollution in recent days than in earlier periods.

Peatbogs also well document the history of heavy metals contamination. According to Lee and Tallis (1973), a bog situated in the industrial part of England 'registered' a low level of Pb by the early 18th century, and next its gradual increase until the present time. Surveying ombrotrophic bogs in southern England, Jones and Hao (1993) demonstrated an increase in the Cd, Cu, Pb and Zn concentrations with the age of the bogs, and no increase in the level of Fe; they suggested that the history of heavy metals pollution cannot be reconstructed from profiles of bogs under hydrological influences. McKenzie et al. (1998) concluded that ombrotrophic, unsaturated peat bogs where heavy metals are immobilized may reflect the historical level of these elements, while minerotrophic and saturated bogs in which heavy metals are mobile elements cannot be treated as indicators. According to Tyler (1972), the patterns of heavy metals accumulation in ombrotrophic bogs in Sweden do not allow these bogs to be considered sensitive indicators of past changes in the concentrations of heavy metals.

Further information on historical heavy metal pollution records in peat bogs can be found in several papers (e.g. Martinez-Cortizas et al., 1997; Kuester and Rehfuess, 1997; West et al., 1997) published in the Proceedings of the workshop on "peat bog archives of atmospheric metal deposition".

#### 4.2. Nitrogen compounds

The main natural sources of nitrogen emissions to the atmosphere are biomass burning, lightning, microbial soil processes, stratospheric input, and marine phytolotic and biological processes (Pitcairn and Fowler, 1995). The main sources of anthropogenic emissions are combustion of coal, particularly in power plants producing electricity, heating of buildings, industry and transport (diesel or petrol-driven vehicles), and production and spreading of animal manure (Europe's Environment, 1998). In the early 1980s the emissions of nitrogen compounds were approx. 35 million tonnes annually in Europe. In the mid-1990s they decreased to approx. 27 million tonnes (Europe's Environment, 1998). However, the rapid development of the transport sector can lead to the resurgence of nitrogen compound emissions in a short time.

Atmospheric deposition of fixed nitrogen as nitrate and ammonium in rain and by dry deposition of nitrogen dioxide, nitric acid and ammonia has increased throughout Europe during the last two decades of the 20th century, from 2–6 kg N/ha/year to 15–60 (80) kg N/ha/year (Sutton et al., 1993; Pitcairn and Fowler, 1995).

Nitrogen concentration in mosses can be a good indicator of the content of this element in the atmosphere, as exemplified by Baddley et al. (1994), Pitcairn and Fowler (1995) or Woolgrove and Woodin (1996). According to Baddley et al., nitrogen concentrations in *Racomitrium lanuginosum* (Hedw.) Brid. were clearly correlated with nitrogen deposition. In moss samples collected at the end of the 1980s the nitrogen concentration was 50% higher than in the 1950s and 2–3 times higher than in the 19th century. The other authors found that in 41 years (1950–1990) the nitrogen

concentrations increased from 38 to 63% in some several moss species (among others *Rhytidiadelphus triquetrus* (Hedw.) Warnst., *Hypnum cupressiforme* agg. *Sphagnum* spp.) in regions of Great Britain where deposition was high (15–30 kg N/ha/yr); no such increase was noted in mosses growing in areas with low deposition (6 kg N/ha/yr). Similar effects were found by Woolgrove and Woodin (1996) in Scottish snowbed communities. They conclude that nitrogen depositions exceed by far what is recognised currently as critical loads.

Another appropriate biochemical marker for enhanced atmospheric nitrogen supply is the nitrate reductase activity. A series of studies which are reviewed by Lee et al. (1998) show that the activity of this enzyme reduces or get lost in polluted environments.

Press et al. (1986), Pitcairn et al. (1991), and Dirkse and Martakis (1992) found marked reduction in the growth of *Sphagnum cuspidatum* Hoffm em. Warnst., *Hylocomium splendens* (Hedw.) B.S.G. and *Pleurozium schreberi* (Brid.) Mitt. with an increase in nitrogen deposition (from 30 to 60 kg N/ha/yr). High atmospheric nitrogen input is partly responsible for the loss of certain bryophyte species and a decrease in bryophyte cover over the past 30–40 years (Pitcairn et al., 1991, Baddley et al., 1994), and declining health of *Sphagnum* communities (Press et al., 1986; Lee and Baxter, 1990; Woodin and Farmer, 1993; Gunnarsson 2000). Rao (1982) found losses of bryophyte species on Dutch chalk grasslands between 1953 and 1983, and the decline of *Rhytidiadelphus squarrosus* (Hedw.) Warnst. and *Scleropodium purum* (Hedw.) Limpr. in the Netherlands.

### 4.3. Sulphur compounds

Sulphur dioxide originates from both natural and anthropogenic sources in the environment. Natural sources of the sulphur in the atmosphere are volcanic activity, fires and bacterial activity, the sea and ocean. The main sources of anthropogenic emissions of sulphur are the energy sector and various factories where fossil fuels are used, especially coal and oil. For the northern hemisphere the natural sulphur input was 3.3 million t S/yr in the 1990s, while the anthropogenic input was 29 million t S/yr (Whelpdale et al., 1997); thus anthropogenic emissions constituted approx. 90% of the total sulphur input to the atmosphere.

Global sulphur emissions have been increasing steadily since 1850. At that time they were estimated at 1.2 million tonnes annually, while in the 1990s they reached 71.5 million tonnes (Elvingson, 2000). This steady growth of global sulphur input has been caused mainly by developing industries in Asian countries. In Europe, however, sulphur input has decreased markedly since the 1980s. In Europe at the end of the 20th century SO<sub>2</sub> emissions were by 53% lower than in the 1980s (Europe's Environment, 1998). The geographic distribution of SO<sub>2</sub> emissions is uneven in Europe; they are highest in Central and Eastern Europe (Europe's Environment, 1998).

Bryophytes are used for assessing contamination of the atmosphere with sulphur compounds by a wide range of methods, though quantitative estimations are much more rarely than for example assessing heavy metal pollution. They mainly respond by changes in their distribution and frequency of occurrence, and with changes in biomass, health, and structure of communities.

Mosses have been used as accumulators (Świeboda and Kalembe, 1987; Niskavaara and Äyräs, 1991; Grodzińska and Godzik, 1991; Szarek and Chrzanowska, 1991; Makinen, 1994b). Using a moss bag method, Świeboda and Kalembe (1987) exposed *Sphagnum recurvum* Pal. Beauv. for 6 weeks in the vicinity of the aluminium works and the power plant in Skawina (southern Poland) and then determined their sulphur content. Szarek and Chzanowska (1991) determined the concentration of sulphur in *Pleurozium schreberi* (Brid.) Mitt. and *Hylocomium splendens* (Hedw.) B.S.G. collected in 14 national parks in Poland in 1975 and 1986. Niskavaara and Äyräs (1991) measured it in *Hylocomium splendens* (Hedw.) B.S.G. growing in forests surrounding the town of Rovaniemi in northern Finland, and Äyräs et al. (1997) in *Pleurozium schreberi* (Brid.) Mitt. and *Hylocomium splendens* (Hedw.) B.S.G. in northern Finland, Norway and Russia (Kola peninsula). Data on sulphur concentrations in some moss species, among others *Dicranum groenlandicum* Brid., *Hylocomium splendens* (Hedw.) B.S.G. and *Sanionia uncinata* (Hedw.) Loeske, growing in Spitsbergen (Horsund region) were given by Grodzińska and Godzik (1991). All these authors agree that accumulation of sulphur by mosses is not a good indicator of atmospheric SO<sub>2</sub> pollution. According to Äyräs et al. (1997) "there may be several reasons for this behaviour of the mosses: (1) being a nutrient sulphur has so high natural levels in mosses that no major additional uptake is possible, or (2) SO<sub>2</sub> as a mainly gaseous emission is transported high into the atmosphere and spread over large areas limiting the amount of sulphur offered for uptake in the immediate surroundings of the industrial plants or (3) sulphur is mostly deposited as sulphuric acid (acid rain) to which the uptake mechanisms of the moss do not respond favourably."

Pakarinen (1981) determined the concentration of sulphur in *Sphagnum fuscum* (Schimp.) Klinggr. and *Sph. balticum* (Russ.) C. Jens growing on bogs in southern Finland and found a clear correlation between the amount of sulphur in mosses and the level of atmospheric SO<sub>4</sub> deposition.

A large number of studies deal with the impact of sulphur depositions (wet and dry) on vitality and distribution of bryophyte species and populations. Bryophytes seem to be fairly sensitive to SO<sub>2</sub>, even more than lichens (Türk and Wirth, 1975).

A series of investigations deal with the effect of SO<sub>2</sub> fumigation on bryophytes. Results clearly depend on (a) the concentrations, (b) time of exposure (c) air humidity (sensitivity increases with moisture) and (d) transformation of SO<sub>2</sub> into other substances, for example H<sub>2</sub>SO<sub>3</sub> or H<sub>2</sub>SO<sub>4</sub> (e.g. Gilbert, 1970 a,b; Frahm, 1977; Greven, 1992a,b; see also reviews in Winkler, 1977; Frahm, 1998).

Similar effects could be found by the results of experiments which transplanted bryophytes from unpolluted areas to regions with enhanced SO<sub>2</sub> concentrations like towns or the vicinities of point sources (Gilbert, 1968; Taoda, 1973; Greven, 1992a).

With increasing SO<sub>2</sub> concentrations bryophytes show a transformation of chlorophyll *a* into phaeophytin *a*, followed by a reduction or loss of chlorophyll content which finally could lead to extinction. Severe air pollution reduces sexual reproduction (e.g. Rao, 1982; Greven, 1992b; Otnyukova, 1995). Nash and Nash (1974) showed that bryophyte protonema is by a factor 10 less resistant than adult plants.

Several standardised methods have been involved to investigate the distribution of bryophytes within distinct areas or along a gradient of pollutants. In most of these

cases the results reflect the overall air pollution of an area including several pollutants. Nevertheless sulphur and nitrogen pollutants are the most influential ones.

LeBlanc and De Sloover (1970) involved a method which estimated the 'Index of Atmospheric Purity' (IAP). It is based on the quantitative and qualitative distribution of epiphytic bryophytes (and lichens) in the investigated area.

$$IAP = \sum_{i=1}^n (Q_i \cdot f_i)$$

$n$  = number of species at each sampling plot

$i$  = index of each species

$Q$  = the ecological index of each recorded species, calculated by a defined method and representing the overall resistance or sensibility against pollutants.

$f$  = the coverage value at each sampling plot given in a defined scale.

The IAP index is calculated for each investigated sampling plot. In many cases contour maps with isolines of comparable I.A.P index which stands for similar pollution effects were drawn (e.g. [LeBlanc et al., 1974](#); [Zechmeister et al., 2002b](#), Fig. 6).

The method was used in a large number of investigation all over the world (e.g. [Sergio, 1987](#); [Inui and Yamaguchi, 1996](#); [Palmieri et al., 1997](#)).

Another method which originally is defined for lichens within the Association of Engineers standards list (VDI 1995, Richtlinie 3799) is in progress to be adapted for bryophytes too.

Derived from all these investigations many authors (e.g. [Gilbert, 1970](#); [Greven, 1992a,b](#); [Frahm, 1998](#); [Sauer, 2000](#); [Zechmeister et al., 2002b](#)) provided lists which showed the various tolerances of epiphytic bryophytes in regard to environmental pollution. The tolerances of species within these lists vary with respect to geographical areas. Habitat conditions (climate, bark structure etc.) strongly influence the distribution of bryophyte species and sometimes overlap pollution impacts.

A few examples for tolerances of epiphytic bryophytes in Central Europe are given:

- Resistant: *Bryum argenteum* Hedw., *Ceratodon purpureus* (Hedw.) Brid., *Dicranoweissia cirrata* (Hedw.) Lindb.
- Insensitive: *Amblystegium serpens* (Hedw.) B.S.G., *Brachythecium rutabulum* (Hedw.) B.S.G., *Hypnum cupressiforme* Hedw., *Orthotrichum diaphanum* Brid., *Plagiothecium laetum* B.S.G.
- Nearly insensitive: *Bryum capillare* agg., *Plagiothecium nemorale* (Mitt.) Jaeg., *Platygyrium repens* (Brid.) B.S.G.
- Moderate sensitive: *Metzgeria furcata* (L.) Dum., *Orthotrichum pumilum* Sw., *Orthotrichum obtusifolium* Brid., *Pylaisia polyantha* B.S.G., *Ulota crispa* (Hedw.) Brid.
- Sensitive: *Frullania dilatata* (L.) Dum., *Leucodon sciuroides* (Hedw.) Schwaegr., *Orthotrichum lyellii* Hook & Tayl., *Pterigynandrum filiforme* Hedw.,
- Very sensitive: *Antitrichia curtipendula* (Hedw.) Brid., *Lejeunea cavifolia* (Ehrh.) Lindb., *Neckera pennata* Hedw., *Orthotrichum rogeri* Brid.

As a consequence to all these investigations we do not recommend the use of mosses as accumulator type – indicators of the level SO<sub>2</sub> in the air, but we believe



Figure 6. Map with isolines of comparable pollution derived from the mapping of epiphytic bryophytes in the heavy industrialised city of Linz (Austria; Zechmeister, unpublished data).

changes in the frequency and abundance of moss species, and changes in their health and community structure can be that good indicators of ambient  $\text{SO}_2$  especially for monitoring at the ecosystem level.

#### 4.4. Toxic organic compounds

The geochemistry of organic compounds is strongly connected with carbon circulation. On the one hand, they enter into the composition of the structures forming living organisms and they co-ordinate processes taking place in them. On the other hand, many organic compounds can be a potential source of environmental contamination. They often have toxic effects (carcinogenic, mutagenic, teratogenic) on living organisms. Organic compounds do not degrade quickly, so they persist in the environment

for many years. The most frequent organic compounds occurring in the environment are aliphatic hydrocarbons (AH), mono- and polycyclic aromatic hydrocarbons (MAH, PAH), polychlorinated biphenyls (PCB) and chloro-organic pesticides. Many AH, MAH and PAH compounds are of natural origin, geological or biological, and these as a rule do not pose threats to the environment. More dangerous are compounds originating from anthropogenic sources connected with burning and processing caustobiotites and wastes. In the course of these processes toxic dioxins and furans are produced. Other organic compounds produced by man include solvents (e.g. chloroform), wood preservatives (e.g. pentachlorophenol), transformer (PCB), and pesticides.

The nature of these organic compounds is a major problem for their indication by bryophytes. Some of these substances are unstable and/or cannot be trapped by traditional methods, whereas some are beyond any detection limits (e.g. [Umlauf et al., 1994](#)).

Only a limited number of substances (mainly PAHs, PCB) have been indicated by mosses. A series of unanswered questions remains. Because of the lack of a cuticle, hydrophobic organic compounds do not show a special affinity to bryophyte surfaces. The accumulation of toxic organic compounds is not only dependent on atmospheric pollution levels but also on enrichment parameters, which describe physiological parameters as well as pollutant characteristics ([Thomas, 1984](#); [Strachan and Glooschenko, 1988](#)). Drying of samples prior to storage might lead to secondary contamination by several organic pollutants. [Lead et al. \(1996\)](#) highlighted this fact for PCBs (especially tri- and tetrachlorinated groups) and concluded, therefore, that low-level samples should be analysed wet whenever possible.

Intensive investigations using mosses as indicators were performed by Thomas and Herrmann (1980) and Thomas (e.g. 1984; 1986), who studied a series of organic pollutants [ $\alpha$ -HCH,  $\gamma$ -HCH (Lindan), DDT, PCB 60, Fluoranthene, Benzo(a)pyrene] at 37 sites along a geographic profile through Central Europe. Epiphytic *Hypnum cupressiforme* L. ssp. *filiforme* was used as biomonitor, the amount of analysed moss was five gram dry-weight per sample. A close correlation of pollutant concentration in moss and emissions mainly by agricultural sources was shown.

The enrichment factor of PAH's in mosses compared to concentrations measured in bulk precipitation was high (e.g. 500 for PCB, 2570 for 3.4 benzopyrene), but much lower if concentrations in mosses were compared to dry depositions ([Thomas, 1984](#)). It can be concluded that accumulation of PAH's in mosses depends also on the hydration of the moss.

The fluxes from the atmosphere to the ground surface of Benzo(a)pyrene (as an example for PAH) were calculated by [Milukaitė \(1999\)](#). Three hundred samples of indigenous *Pleurozium schreberi* (Brid.) Mitt. and *Hylocomium splendens* (Hedw.) B.S.G. from Lithuania were analyzed. The concentration in mosses (average 54.7  $\mu\text{g kg}^{-1}$ ) were higher than in leaves of flowering plants, but lower than in needles or roots. Partitioning of BaP among the various parts of the moss, of different age did showed no marked differences. This was interpreted as a result of BaP stability in moss.

Sixteen polycyclic aromatic hydrocarbons were detected along a roadside by [Viskari et al. \(1997\)](#). They used moss bags filled with 20 gram of cleaned 2 cm tips

of *Pleurozium schreberi* (Brid.) Mitt. and exposed the moss for seven weeks during summer. They emphasised that moss bags are an efficient collector of airborne PAHs.

Moss bags containing *Sphagnum* peat were used by Strachan and Glooschenko (1988) for the detection of PCB's and a series of organochlorines ( $\alpha$ -HHC, Lindan, DDT, DDE, Dieldrin etc.). They highlight the various modes of distribution of these pollutants and that results therefore depend on exposure modes.

A series of PCB congeners were analysed in northern and southern Norway. A general decline of PCBs in the investigated areas was provided with a less strong tendency for hexa- or heptachlorinated PCB congeners in the North, which might be evidence for the global fractionation hypothesis (Lead et al., 1996).

#### 4.5. Radionuclides

Radioactive substances are often accumulated in bryophytes in great quantities. Svenson and Linden (1965) found that *Pleurozium schreberi* (Brid.) Mitt. absorbed Zr, B, Ba, La from fallouts after nuclear tests, Clymo (1978) and Oldfield et al. (1979) determined the concentrations of radiocaesium derived from the nuclear weapon tests of the 1960s in the top segment of peat mosses, Kwapuliński and Sarosiek (1988) estimated the  $^{226}\text{Ra}/^{228}\text{Ra}$  ratio in dustfall, air and *Hypnum* species nearby and around a power station in Poland. Concentration of radioactive caesium ( $^{137}\text{Cs}$ ) was determined in mosses (*Pleurozium schreberi* (Brid.) Mitt.) collected in Polish national parks in 1986 (Chernobyl accident) (Grodzińska, et al. 1993). The most contaminated with  $^{137}\text{Cs}$  were parks localised in northeastern and southern Poland. It was correlated with the wind direction and precipitation in first days after the Chernobyl accident, and also with dust level in atmosphere in that time. Gerdol et al. (1994) determined the vertical distribution of  $^{137}\text{Cs}$  in the uppermost layer of *Sphagnum* collected in Alps in 1988. They found that Chernobyl-radiocaesium peak was well distinguishable three years after the Chernobyl accident and disappeared three more years later. Cherkintsev et al. (2000) estimated several radionuclides incl.  $^{124}\text{Sb}$ ,  $^{134}\text{Cs}$ ,  $^{131}\text{Ba}$ ,  $^{86}\text{Rb}$  in *Hylocomium splendens* (Hedw.) B.S.G. and *Pleurozium schreberi* (Brid.) Mitt. collected in Chelyabinsk region (Ural Mts.). They found extremely high concentration of Sb (2.3 ppm av. value, 29 ppm max.) in moss samples from a "steel town" (Magnitogarsk). These examples show that mosses are very valuable accumulator of radionuclides.

### 5. Bryophytes as indicators in aquatic habitats

#### 5.1. General aspects

Bryophytes are often conspicuous elements of the macrophyte vegetation of aquatic fresh water habitats. In some of these habitats they are even more abundant than higher plants, for example in water spring vegetation (e.g. Zechmeister and Mucina, 1994), acidic lakes (e.g. Grahn, 1986) or fast running mountain streams (e.g. Geissler, 1976). They can not be found in marine ecosystems, though some terrestrial species on rocky coasts tolerate salt spray or can be found in brackish water (e.g. *Fontinalis dalecarlica* Br. Eur).



A wide range of threats affect aquatic habitats and some of them strongly reduce bryophyte populations. The increase of nutrients for example elevates algal growth and diminishes light for mosses and liverworts. Changes in the structure of aquatic ecosystems (e.g. bank reinforcements, river control) alter flow regimes as substrates and mostly lead to dramatic changes in the aquatic vegetation (e.g. Glime, 1992).

On the other hand, the decrease of pH caused by human activities like the deposition of airborne particles containing sulphur or the pollution by organic pollutants sometimes even benefit bryophytes. Pollutants suppress non-bryophyte aquatic macrophytes which are strong competitors for light, space and nutrients in unpolluted habitats. Vascular plants are less resistant than aquatic bryophytes against a series of substances and have less effective protective mechanisms against pollutant-induced changes of the environment (e.g. Grahn, 1977; Ek et al., 1995).

Whereas a wide range of species live close to permanent water reservoirs, only bryophytes submerged throughout the year can be used for biomonitoring (e.g. Tremp, 1992, 1999). The main advantages of using bryophytes as indicators in aquatic habitats are:

- There is a constant uptake of pollutants from water over the entire surface.
- Most aquatic bryophytes are fairly tolerant against a wide range of pollutants like heavy metals, which they tend to accumulate.
- Bryophytes react quickly to changes in water quality according to increases or decreases in nutrients or toxic substances.
- They form stable and homogeneous populations and they show green leaves and active metabolism throughout the year, which favours them over higher plants which lie dormant during the winter season, or algae which often show restricted life spans.
- There is only a limited number of submerged species in the northern hemisphere, which is in contrast to sometimes enormous biomass easily to identify in most of the cases.

Based on their ability either to accumulate pollutants or respond sensitively to changes in water quality, bryophytes are used either as accumulation indicators or the bryophyte species assemblages are investigated for indication of water quality (including the nutrient status) or changes in the pH.

## 5.2. Accumulation indicators

### 5.2.1. Heavy metals

The ability of bryophytes to accumulate heavy metals has already been described and there is little difference between aquatic and terrestrial mosses and liverworts regarding uptake mechanisms.

Of greater importance than in terrestrial habitats is the pH of the surrounding water. A low pH influences the uptake efficiencies of some metals negatively (Whitton et al., 1982; Say and Whitton, 1983; Claveri et al., 1995; Yoshimura et al., 1998; Carballeira et al., 2001). Gagnon et al. (1999) emphasise the importance of water hardness. Additionally, temperature clearly determines the uptake at least for copper (Glime, 1992).



Aquatic bryophytes generally have a high tolerance against a range of heavy metals like Pb, Zn or Fe (e.g. Glime and Keen, 1984). But with the exception of the “copper mosses”, there is generally a fairly low resistance to copper (e.g. Glime and Keen, 1984; Tyler, 1990) which causes disturbance to photosynthesis (Sommer and Winkler, 1982). *Rhynchostegium riparoides* (Hedw.) Card. and *Scapania undulata* (L.) Dum. seem to be the most resistant species according to water pollution by heavy metals (e.g. Empain, 1976; Empain et al., 1980).

Like in terrestrial mosses the ability to accumulate heavy metals differs for each species and for each heavy metal. In general *Rhynchostegium riparoides* (Hedw.) Card. has the highest uptake rates compared to *Fontinalis antipyretica* Hedw., *Amblystegium riparium* (Hedw.) Lindb., *Cinclidotus danubicus* Schiffn. and Baumg., *Cinclidotus fontinaloides* (Hedw.) P. Beauv. or *Fissidens crassipes* Wils. (Wehr and Whitton, 1983a,b; Glime 1992; Mersch and Reichard, 1998; Gagnon et al., 1999).

There is a significant correlation between concentrations in water and moss tissues for some heavy metals, especially for Cd, Cu, Pb and Zn. (e.g. Whitton et al., 1982; Kelly and Whitton, 1989; Goncalves et al., 1994; Carter and Porter, 1997 [Cu, Zn]; Bruns et al., 1997), but there also exist contrasting reports for Cd and Pb (Say and Whitton, 1983; Carter and Porter, 1997).

As in terrestrial species the tips of the shoots show significantly lower concentrations than the whole plant (e.g. Whitton et al., 1982; Wehr et al., 1983), although there are differences between metals. For Cd, the differences in accumulation between the older and younger parts are in many cases greater than for the elements Cu, Pb and Zn in the same plant (Siebert et al., 1996).

The whole plant reflects the metals of the environment over a much longer period, whereas the tips should be used for monitoring short term changes, repeated surveys or the comparison of different river systems (Say and Whitton, 1983; Wehr and Whitton, 1983a).

Because of their high accumulation capacity, aquatic bryophytes have received increasing attention as bioindicators within the last three decades, especially in highly polluted water. Nevertheless, they can also be used in areas where the metal concentration in water-samples is beyond the detection limits (e.g. Jones, 1985). For Pb, an enrichment ratio of  $3.5 \times 10^5$ – $1.2 \times 10^6$  in the liverwort *Scapania undulata* (L.) Dum. has been reported by Satake et al. (1989). Enrichment factors in *Rhynchostegium riparioides* (Hedw.) Card. as presented by Wehr and Whitton (1983b) are  $9.4 \times 10^5$  for lead, and  $2.3 \times 10^6$  for cadmium.

#### 5.2.1.1. Methods

The considerable literature on aquatic bryophytes used as heavy metal accumulators is based on a variety of different practical methods. Benson-Evans and Williams (1976) give an overview on earlier studies. Wehr et al. (1983) tested a series of methods and gained sometimes markedly differing results. They emphasise the care which should be taken when comparing the metal compositions of aquatic plants from studies reported in the literature.

In many studies **indigenous plant material** was used to detect changes in pollution released by point sources or to evaluate the overall pollution of rivers in

industrialised areas all over the world (e.g. [Empain, 1976](#); [Burton and Peterson, 1979](#), [Nimis et al., 2002](#)).

**Transplants** of mosses are used more often in aquatic than in terrestrial habitats (e.g. [Mouvet, 1984](#); [Carter and Porter, 1997](#); [Mersch and Reichard, 1998](#), [Sergio et al., 2000](#)).

Moss bags are used as transplanting devices. Most of them are nylon bags with a size of  $20 \times 20$  cm or  $20 \times 30$  cm and with different mesh sizes ( $1 \text{ mesh cm}^{-1}$ ,  $0.9 \text{ mesh cm}^{-1}$ ,  $0.7 \text{ cm}^{-1}$ ,  $0.07 \text{ cm}^{-1}$ ; e.g. [Kelly et al., 1987](#)). Additionally, [Gimeno and Puche \(1999\)](#) used garden mesh cylinders ( $1 \text{ mesh cm}^{-1}$ ) with a length of 25 cm length and 10 cm diameter. Anchored plastic tubes are used by [Mouvet \(1984\)](#). Mesh size appeared to have little effect upon metal accumulation by moss inside the bag, however, it is recommended that larger mesh size than  $0.07 \text{ mesh cm}^{-1}$  are used ([Kelly et al., 1987](#)). Various systems have been used for a stable anchoring of the bags (stones, steel stakes), which all must consider that transplants should be found even in cloudy waters and should be fixed according to the current velocity of the rivers ([Benson-Evans and Williams, 1976](#)).

Aquatic bryophytes are transferred mainly from unpolluted to polluted sites. A comparable water pH of the control and treatment study site is advised, as this influences heavy metal concentrations. A steep decline in concentrations of Fe and Al in mosses transplanted from acidic to neutral streams has been observed (e.g. [Engleman and McDiffet, 1996](#)).

The metal content of the autochthonous bryophytes is sometimes different from those of the transplanted mosses which implies different conclusions about the contamination level of the water. Adaptation to different pH conditions leading to physiological and structural specificity's could explain the different abilities of autochthonous and transplanted populations to accumulate metals in acidic surroundings ([Claveri et al., 1995](#)). [Johansson \(1995\)](#), who investigated the pollution gradient along the stream Smedbyan, north-east of Stockholm, found that the impact on the water quality as a consequence of increased urbanisation was more pronounced in the indigenous mosses than in the transplanted bryophytes.

As a **physiological parameter** regarding heavy metal contamination, the induction of thiol-containing peptides such glutathiones were investigated (e.g. [Bruns et al., 1997](#); [Doering et al., 1999](#); [Bruns et al., 2001](#)). A positive correlation was found between glutathione levels and Cd levels in the moss samples. These authors discuss the suitability of this biochemical response to stress as a biomarker for heavy-metal pollution at field locations.

Another method involves the identification of sites with different pollutant levels on the basis of a physiological stress criterion (the D665/D665a pigment index; [Carballeira and Lopez, 1997](#)). They also used this method to estimate background levels for each metal in each of the five investigated bryophytes (*Fontinalis antipyretica* Hedw., *Fissidens polyphyllus* Wils., *Brachythecium rivulare* Schimp., *Rhynchostegium riparioides* (Hedw.) Card, and *Scapania undulata* (L.) Dum.).

Experimental attempts to use bryophytes for **biotechnical purification** of water have been performed by [Samecka and Kempers \(1996\)](#) using *Scapania undulata* (L.) Dum, and [Ho et al. \(1996\)](#) using *Sphagnum* ssp.

Regarding the total number of aquatic species investigations, the monitoring of heavy metal pollution has been restricted to only a very few species:

- *Amblystegium riparium* (Hedw.) Lindb. (e.g. [Say et al., 1981](#); [Wehr and Whitton, 1983b](#))
- *Brachythecium rivulare* Schimp. (e.g. [McLean and Jones, 1975](#); [Carballeira and Lopez, 1997](#))
- *Cinclidotus danubicus* Schiffn. and Baumg. (e.g. [Empain, 1976](#); [Mersch and Reichard, 1998](#))
- *Cinclidotus nigricans* (Brid.) Wijk and Marg. (e.g. [Empain, 1976](#))
- *Fontinalis dalecarlica* Br. Eur. (e.g. [Glime and Keen, 1984](#); [Gagnon et al., 1999](#))
- *Fontinalis duriaei* Schimp. (e.g. [Glime and Keen, 1984](#))
- *Fontinalis hypnoides* Hartm. (e.g. [Gimeno and Puche, 1999](#))
- *Fontinalis squamosa* Hedw. (e.g. [McLean and Jones, 1975](#); [Say et al., 1981](#))
- *Fontinalis antipyretica* Hedw. (e.g. [Pickering and Puia, 1969](#); [Dietz, 1972](#); [Kirchmann and Lambinon, 1973](#); [Empain, 1976](#); [Say et al., 1981](#); [Say and Whitton, 1983](#); [Wehr and Whitton, 1983a,b](#); [Wehr et al., 1983](#); [Mouvet, 1984](#); [Glime and Keen, 1984](#); [Kelly et al., 1987](#); [Johansson, 1995](#); [Siebert et al., 1996](#); [Bruns et al., 1997](#); [Carballeira and Lopez, 1997](#); [Mersch and Reichard, 1998](#))
- *Fissidens polyphyllus* Wils. (e.g. [Carballeira and Lopez, 1997](#))
- *Hygrohypnum ochraceum* (Wils.) Loeske ([Claveri et al., 1995](#); [Carter and Porter, 1997](#))
- *Pellia epiphylla* (L.) Corda ([Claveri et al., 1995](#))
- *Rhynchostegium riparioides* (Hedw.) Card (e.g. [Empain, 1976](#); [Say et al., 1981](#); [Say and Whitton, 1983](#); [Wehr and Whitton, 1983a,b](#); [Wehr et al., 1983](#); [Glime and Keen, 1984](#); [Kelly et al., 1987](#); [Carballeira and Lopez, 1997](#); [Mersch and Reichard, 1998](#); [Gimeno and Puche, 1999](#); [Gagnon et al., 1999](#)) and
- *Scapania undulata* (L.) Dum. (e.g. [McLean and Jones, 1975](#); [Burton and Peterson, 1979](#); [Whitton et al., 1982](#); [Wehr and Whitton, 1983a](#); [Jones, 1985](#); [Satake et al., 1989](#); [Samecka and Kempers, 1996](#); [Carballeira and Lopez, 1997](#); [Yoshimura et al., 1999](#)).

### 5.2.2. Radionuclides

A series of publications deals with the accumulation of radioactive elements derived from nature (e.g. [Justyn and Stanek, 1974](#); [Shacklette and Erdmann, 1982](#)).

Recently, radionuclides derived from anthropogenic sources has been emphasised. Aquatic mosses were found to be useful monitoring organisms to detect effluents containing radionuclides derived from a power station in the river Meuse in France ([Kirchmann and Lambinon, 1973](#); [Lambinon et al., 1976](#)). Compared to algae as well as higher plants, *Cinclidotus danubicus* Schiffn. and Baumg. had a much higher accumulation capacity for a range of elements (e.g.  $^{60}\text{Co}$ ,  $^{134}\text{Cs}$ ,  $^{137}\text{Cs}$ ) in that investigation.

[Mersch and Kass \(1994\)](#) detected the  $\gamma$ -radiation activity of artificial radionuclides ( $^{58}\text{Co}$ ,  $^{60}\text{Co}$ ,  $^{110}\text{Ag}$ ,  $^{124}\text{Sb}$ ) by *Fontinalis antipyretica* Hedw. Their investigation recommends a monitoring system of the river Moselle downstream of a nuclear power plant near Cattenom.

### 5.2.3. Toxic organic compounds

Some of the organic pollutants (e.g. phenols) are quickly incorporated in tissues of aquatic bryophytes (Morrison and Wells, 1981). Depending on the concentrations, bryophytes either decompose and/or accumulate these substances, which lead to changes in the chlorophyll and phaeopigment concentrations (e.g. chl a, chl b, OD665/OD665a, Peñuelas, 1984; Martínez-Abaigar and Núñez-Olivera, 1998), plasmolysis (Auerbach et al., 1973) and to inhibition in growth (Glime, 1992). Tolerance against phenol or benzopyrenes varies within different species, and is high for *Rhynchostegium riparioides* (Hedw.) Card, *Amblystegium riparium* (Hedw.) Lindb. or *A. tenax* (Hedw.) Jens. and less for *Fontinalis antipyretica* Hedw.; liverworts seem to be the most sensitive group (Frahm, 1975; Peñuelas, 1984; Glime, 1992; Kosiba and Sarosiek, 1995; Vanderpoorten, 1999a).

#### 5.2.3.1. Chlorinated organic compounds:

The spatial distribution of effluents from an insecticide producing factory in France containing hexachlorocyclohexanes (HCHs) and polychlorinated biphenyls – PCBs have been investigated by Mouvet et al. (1985). The moss *Cinclidotus danubicus* Schiffn. and Baumg. was exposed in moss bags. Chlorinated hydrocarbons as DDT or  $\alpha$ -HCH accumulations reflected their concentration in the water. Hexachlorocyclohexan ( $\alpha$ -,  $\beta$ -,  $\gamma$ -  $\delta$ -,  $\epsilon$ -), chlorophenols, hexachlorobenzene and DDTs were detected by Chovanec et al. (1994) in the rivers Danube and Traun along industrial areas in Austria. They found higher concentrations of these substances than in the underlying sediments. *Fontinalis antipyretica* Hedw. was used as indicator species in this investigation.

PCBs are accumulated in higher amounts than chlorinated hydrocarbons (Mouvet et al., 1985; Chovanec et al., 1994). From these investigations it can be concluded that *Cinclidotus danubicus* Schiffn. and Baumg. as well as *Fontinalis antipyretica* Hedw. can be used as bioindicator if the concentration of PCBs or HCHs are beyond detection limit in water-samples or sediments.

#### 5.2.3.2. Polycyclic aromatic hydrocarbons

The concentrations of PAHs and sediments were compared by Chovanec et al. (1994). Whereas the concentrations in sediments were higher than in *Fontinalis antipyretica* Hedw., the moss proved to be a suitable indicator for a wide series PAHs (e.g. phenantrene, coronene).

The accumulation of PAHs and responses of antioxidant enzymes in *Fontinalis antipyretica* Hedw. transplanted around a Finnish city harbour were also studied by Roy et al. (1996). Glass fibre bags containing this species (“moss bags”) were exposed for 35 days. This study introduced a new approach to investigate the cause-effect relationship between bioaccumulation of aquatic pollutants and the biochemical responses in organisms following exposure to such pollutants in a field setting.

#### 5.2.3.3. Others

The distribution of tebufenozide (RH-5992, MIMIC) (N'-t-butyl-N'-(3,5-dimethylbenzoyl)-N-(4-ethyl-benzoyl) hydrazine) after spraying on forests in order to fight the

spruce budworm (*Choristoneura fumiferana* Clem.) in Canadian forestry has been investigated using several indicators including the moss *Drepanocladus* sp. (Sundaram et al., 1996).

### 5.3. Indicators of water quality and acidification

Beside alterations in flow regime and substrates (Suren, 1996), changes in bryophyte species assemblages are mainly a result either of toxic substances (e.g. Cu, PCB's), shifting in the trophic status or the pH of the water. Depending on environmental changes, bryophytes can be favoured or disadvantaged against higher plants. Nutrient enrichment, shade effects, mechanic destruction, changes in water-pH cause different reactions by the various groups of organism. These factors correlate with each other and can often not be separated. Despite that, emphasis has been on two major topics involving bryophyte assemblages: water quality and acidification.

#### 5.3.1. Water quality

Water quality mainly is a result of the trophic level and the quantity of toxic substances within the water as well as the natural ability of an aquatic habitat to regenerate from these influences. Trophic parameters mainly influence oxygen content, which is detected by a standard method for the evaluation of water quality, the 'saprobial-system' (e.g. Liebmann, 1962). Whereas several authors refer to this system, only a few bryophyte species have been considered as indicators within this system (e.g. Frahm, 1974; Papp and Rajczy, 1995). Oostendorp and Schmidt (1977) analysed the influence of effluents from a brewery on bryophyte biomass and calculated indices for the degree of saprobity for several species. Table 1 provides a list of species ranked within the various classes of the saprobial system, following the classifications of Frahm (1974, 1998), Papp and Rajczy (1995), Oostendorp and Schmidt (1977), Tremp (1999) and Zechmeister (unpublished data).

The system could be significantly improved by taking into account the results of a wide range of monitoring studies (e.g. Vanderporten, 1999a,b).

Bryophytes will be part of the evaluation system of the Water Framework Directive 2000 / 60 / EC of the European Union. They will play an important role mainly in alpine areas where vascular macrophytes are a less important part of river vegetation. The classification system has to be provided for each country.

A distinct change of bryophyte assemblages within the last years following changes in water quality has been reported for many rivers and lakes (e.g. Empain, 1973). After a period of a marked decline in species richness as a result of water pollution, an improvement in water quality during the last twenty years lead to an increase in species richness (e.g. in the Lower Rhine, Frahm and Abts, 1993). Furthermore, the raising of water temperatures within the last century seems to support the invasion of species (e.g. *Cinclidotus danubicus* Schiffn. and Baumg.) originating from milder climatic regions (Frahm, 1997). An increase in species richness as a result of a decrease in trophic levels and an increase in heavy metals is also reported by Vanderpoorten (1999b) for the rivers Meuse and Sambre.

*Table 1.* List of species ranked within the various classes of the saprobial system, following the classifications of Frahm (1974, 1998), Papp and Rajczy (1995), Oostendorp and Schmidt (1977), Tremp (2000), Zechmeister (unpublished). 0: xenosaprobic, 1: oligosaprobic, 2:  $\alpha$ -mesosaprobic, 3  $\beta$ -mesosaprobic, 4 eu-/poly-saprobic

Species	Sapobric class
<i>Brachythecium rivulare</i> (Hedwig) B.S.G.	1, 2, 3
<i>Chiloscyphus polyanthus</i> (L.) Corda	1, 2
<i>Cinclidotus aquaticus</i> (Hedw.) B.S.G.	1, 2, 3
<i>Cinclidotus danubicus</i> Schiffn. and Baumg.:	2, 3
<i>Cinclidotus nigricans</i> (Brid.) Wijk and Marg.:	2, 3
<i>Cinclidotus fontinaloides</i> (Hedw.) P. Beauv.	2, 3
<i>Fissidens crassipes</i> Wils.	2, 3
<i>Fissidens arnoldii</i> Ruthe	2
<i>Fissidens fontanus</i> (Pyl.) Steud.	2
<i>Fontinalis squamosa</i> Hedw.:	1
<i>Fontinalis antipyretica</i> Hedw.:	1, 2, 3, 4
<i>Hygroamblystegium fluviatile</i> (Hedw.) Loeske	2, 3
<i>Hygroamblystegium tenax</i> (Hedw.) Jenn.	0, 1, 2
<i>Hygrohypnum ochraceum</i> (Wils.) Loeske	0, 1, 2
<i>Hygrohypnum luridum</i> (Hedw.) Jenn.	2
<i>Leptodictium riparium</i> (Hedw.) Warnst.	2, 3, 4
<i>Leskea polycarpa</i> Ehrh.	2, 3
<i>Rhynchostegium riparioides</i> (Hedw.) Card:	0, 1, 2, 3, 4
<i>Scapania undulata</i> (L.) Dum.	0, 1
<i>Schistidium alpicola</i> (Hedw.) Limpr.	2

Frahm (1975) experimentally evaluated threshold values for several pollutants (e.g.  $\text{NH}_4^+$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ), which are relevant for some aquatic bryophytes taken from the River Rhine.

Empain (1978) provided an early approach to quantifying the relationship between physico-chemical properties of water and the quantitative distribution of aquatic bryophytes as a result of species resistance to pollution in Belgium and northern France. Data synthesis lead to a water quality index. Vanderpoorten and Palm (1998) developed a method for predicting water quality by linear regression using aquatic bryophyte canonical variables as predictors and highlighted the importance of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and water temperature.

The photosynthetic pigment composition of aquatic bryophytes has been used as an indicator for water pollution in a series of investigations. The reduction of chlorophyll concentrations as a response to pollution has been reported (e.g. Glime, 1992). Different pigments exhibit different responses to pollution, sometimes associated with a decrease of the chlorophyll a/b ratio, although it has also been reported that this index might not be a proper tool for the indication of water pollution (Martínez-Abaigar and

Núñez-Olivera, 1998). Gimeno and Puche (1999) examined chlorophyll content and morphological changes in cellular structure induced by transplanting two species of aquatic bryophytes [*Rhynchostegium riparioides* (Hedw.) Card. and *Fontinalis hypnoides* Hartm.] in different sites of a river in Spain. Two transplanting devices were tested, a nylon mesh bag and a plastic net cylinder. They found that the changes in cellular structure mainly affect chloroplasts and follows a sequence of alterations which ends with cellular death.

Wehr and Whitton (1986) correlated a series of ecological factors and pollutants with morphological variations in *Rhynchostegium riparioides* (Hedw.) C.Jens.

### 5.3.2. Acidification

Acidification is mainly a result of atmospheric depositions of acid effluents derived from industrial sources. Sources for acidification are SO<sub>2</sub>, NO<sub>x</sub>, and NH<sub>3</sub> emissions. NO<sub>x</sub> originate mainly from road traffic and NH<sub>3</sub> from cattle farming.

Several investigations underline the importance of water-pH as a decisive factor influencing species richness and causing changes in the floristic compositions of aquatic macrophytes (e.g. Roelofs, 1983; Arts, 1990; Frahm, 1992; 1998; Tremp and Kohler, 1995; Karttunen and Toivonen, 1995). Water-pH influences the availability and uptake of pollutants like heavy metals (5.2.1) or nutrients. Schuurkes et al. (1986) showed that acid tolerant species have an ammonium-dominated nitrogen utilization. Obviously bryophyte growth is strongly determined by these variables (e.g. Tremp, 1992).

Streams or lakes with a moderately lowered pH are rich in bryophyte species (Frahm, 1999; Satake et al., 1995), although a further decrease leads to a rapid loss in species (e.g. Yan et al. 1985; Ormerod and Wade, 1990).

Consequences from human induced acidification are more pronounced for bryophytes in oligotrophic lakes or streams with naturally reduced pH which developed in areas with old siliceous bedrock. The acidification of Scandinavian or Canadian lakes or streams and shifts in species assemblages has thus been reported by several authors (e.g. Grahn 1977).

During the last years, developments show reverse tendencies and indicate the reduction of environmental pollutants. Ek et al. (1995) ascribe the recent recovery of bryophytes or fishes in Swedish lakes to reduced deposition of sulphate. Brouwer et al. (1997) conclude from their experimental long term studies that the recovery of the water chemistry and vegetation of soft-water ecosystems is much slower after ammonium sulphate addition, compared with that of the recovery following sulphuric acid addition. Regarding the current nitrogen deposition levels in Europe, it is unlikely that recovery will occur within the next ten years.

Beside the mapping of species distributions measures of chlorophyll fluorescence was used as a measure for the tolerance of lowered water-pH (Tremp, 1993).

Reviews by Burton (1990), Glime (1992), Frahm (1998) or Tremp (2000) provide further information on this topic.



## 6. Indicators for global change

Based on their physiological and morphological features, bryophytes seem to be some of the plants most sensitive to changes in environmental conditions.

Bryophytes are appropriate indicators either in ecosystems which they dominate in terms of biomass or species richness (e.g. sub-Arctic), or in landscapes which are poor in species richness as a result of intensive human influence (e.g. arable fields).

### 6.1. Climate change

It must be expected that the increased release of greenhouse gases as CO<sub>2</sub>, CH<sub>4</sub> or N<sub>2</sub>O will lead to global warming. The predicted changes within the next fifty years give an increase between 1.5 to 6 °C (Mitchell et al., 1990; IPCC 2000). Warming will be pronounced in the Arctic, Antarctic and Alpine regions (Jacka and Budd, 1991; Maxwell, 1992; Watson et al., 1996). Additionally precipitation is also expected to increase in these areas. Both lead to a series of changes such as increased soil microbial activity, stimulated nutrient mineralization and enhanced emission of greenhouse gases (e.g. NH<sub>4</sub><sup>+</sup>) by natural processes (e.g. Press et al., 1998). A shift in species composition of plants must be expected (e.g. Wookey et al., 1993; Grabherr et al. 1994; Kappelle et al., 1999; Arft et al., 1999).

In all Arctic and Alpine regions bryophytes are important in terms of biomass and ecosystem diversity (e.g. Longton, 1988; Russel, 1990; Gignac et al., 1998). They also favour growth of vascular plants in preventing erosion, intercepting pollutants or by nitrogen fixation (Press et al., 1998). Studies have been performed to analyse either growth responses or shifts in species diversity of bryophytes as a result of climate warming.

In a series of investigations the biomass production of populations and/or increments of single shoots of bryophytes have been investigated by environmental manipulation studies. By using Open Top Chambers (OTC) the influence of increased temperatures, precipitation and nitrogen depositions has been investigated in Arctic Europe and Canada. Additionally, studies have been performed which compare analogous variables at various latitudes or altitudes, including the Alps and Antarctic regions.

Whereas increased precipitation leads to greater growth rates in nearly all of the experimental studies, results for temperature and fertiliser application are controversial.

As reported by Callaghan et al. (1997), growth parameters were strongly correlated with early summer temperatures and the length of the growing seasons for *Hylocomium splendens* (Hedw.) B.S.G. (Hedw.) B.S.G. and *Polytrichum commune* Hedw. at seven circumpolar located arctic/subarctic sites. A positive correlation between temperature and bryophyte growth rates has also been reported by Zechmeister (1995a; 1998) for several species in Alpine environments. Potter et al. (1995) and Molau and Alatalo (1998) reported a negative or nil response of bryophyte biomass production on enhanced temperatures. This stands not only in contrast to the results given above but also to laboratory results obtained by several authors (e.g. Longton and Green, 1979; Furness and Grime, 1982).



Bryophyte species richness increased in poor heath communities at higher temperatures, but not in rich meadows (Molau and Alatalo, 1998).

**Fertiliser application** increased the general bryophyte cover in five-years experiments (Robinson et al., 1998), and similar results have been obtained for the cover of *Polytrichum commune* Brid. in long-term studies of Potter et al. (1995). Nevertheless, there were also negative responses to nitrogen application in some other studies (e.g. Jónsdóttir et al., 1995; Molau and Alatalo, 1998; see also Section 4.2).

Contrary to the parameters mentioned above there will be no response of bryophytes to enhanced CO<sub>2</sub>, as plants already experience concentrations of atmospheric CO<sub>2</sub> predicted to occur over the next 50 years (Sonesson et al., 1992). This is due to their small size and therefore their growth just above soil surfaces (Sveinbjörnson and Oechel, 1992).

Because of large interannual climatic variations, changes in vegetation cover are difficult to estimate in the Antarctic. Changes seem to be more dramatic in the sub-Antarctic than in the continental Antarctic flora (Melick and Seppelt, 1997).

**Modelling** of changes of vegetation dominated by bryophytes which are based on climatic and geographic data have been performed by Gignac et al. (1998) for peatlands in the Mackenzie River Basin. They predict a movement of the southern boundary of peatland ecosystems 780 km north of today, but no changes in the current peatland species diversity.

Statistical modelling of bryophyte-environment relationships for several taxa of *Andraea* and *Racomitrium* are presented by Birks et al. (1998). They suggest models to predict the future geographical distribution of single species based on present-day climatic demands of the species. Similar models predicting the impact of climate change on species distributions have been presented for vascular plants in the Alps (Gottfried et al., 1998).

In regions with a temperate climate, changes are expected not to be remarkable. Nevertheless, it has been reported that 27 bryophyte species with a distribution mainly in the Mediterranean or Atlantic climate spread over central Europe within the last 12 years. This invasion is interpreted as a consequence of the increase of winter temperatures by 1.5 °C (Frahm and Klaus, 1997).

The results of experimental studies implicate a variation in bryophyte response to changing environmental conditions. As there is a lack of long-term data, further experimental studies will be needed if confident predictions can be made. The attempt to predict future changes by geographical modelling should be enforced. These models should also include data obtained by studies on the autecology of species as well as metapopulation data.

## 6.2. Land-use intensity

The alarming loss of biological diversity within the last decades, which is often caused by increasing land-use intensity represents a major challenge to the scientific community and demands the development of appropriate strategies of land management. Intensification of agriculture and forestry including fertilisation, irrigation and the use of pesticides are currently recognised as one of the major threats to biodiversity (European Commission, 1998; Hallingbäck, 1998; Matson et al., 1997).

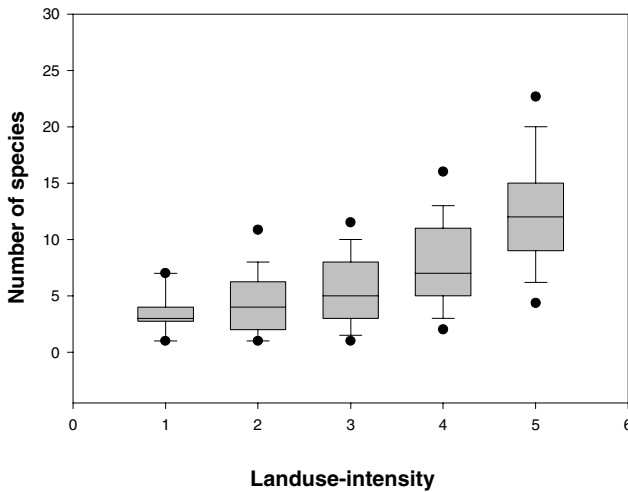


Figure 7. Box and whisker plots for the number of species within each state of land-use intensity at the habitat-scale. The boxes represent the median and 75th percentiles, the bars 90th percentiles; dots show the 95th percentiles for each group; classes of land-use from 1 (high intensity) to 5 (low intensity) (redrawn from Zechmeister and Moser, 2001).

Many investigations show a strong negative correlation between bryophyte diversity and land-use intensity both in landscapes used mainly by agriculture or forestry (e.g. Rydgren et al., 1998; Mensing et al., 1998; Jonsson and Jonsell, 1999; Zechmeister and Moser, 2001).

The most important variables explaining bryophyte diversity in boreal forests are the amount of dead wood (Ohlson et al., 1997), which correlates significantly with forest management intensity (Grabherr et al., 1998) and the age of a forest (Crites and Dale, 1998). In tropic forests as in boreal forests the total number of species on trees in old-growth coniferous forests is nearly twice that of species on trees in secondary-growth stands (e.g. Cooper-Ellis, 1998).

In agricultural landscapes correlations between species richness and land-use intensity are dependent upon disturbance intensity as well as geographic scale. Zechmeister and Moser (2001) showed that species richness on bare soils is at the highest at an intermediate disturbance regime, whereas at other substrates or geographic scales (e.g. habitat or landscape scale) species richness is negatively correlated with land-use intensity (see also Fig. 7). There is also a correlation between land-use intensity and the number of endangered species (Zechmeister et al., 2002c). This is mainly a consequence of habitat destruction as many bryophytes are tolerant against a series of herbicides (Newmaster et al., 1999).

Therefore, for further monitoring of changes in human land-use by means of bryophytes, either keystone indicators (e.g. hornworts; Bisang, 1995) or diversity indices including species richness can be used.

## 7. Conclusions and further prospects

Bryophytes have been an essential group in the field of bioindication for at least four decades. Most of the relevant research is reported in the above sections.

In the field of the estimation of atmospheric heavy metal pollution there is hardly any other group of organisms which give that suitable results, although there is still a need for research to improve the method (see Section 1.1.3).

As a consequence of the perfect results gained by this method there is an enormous demand for the integration of this method into the legislative procedure. Bryophytes should be acknowledged by law as indicator species for the setting and control of deposition limits for heavy metal imissions. A step in this direction was made by the integration of the European heavy metal programme (e.g. Rühling, 1998) into the UN/ECE Convention on Long-range Transboundary Air Pollution (ICPs Working Group on Effects) in the year 2000, although further steps are still missing.

The same goes for aquatic habitats, although in aquatic habitats a series of well investigated groups of organism (e.g. fish, see Dokulil, 2002; Chovanec et al., 2002; Oehlmann and Schulte-Oehlmann, 2002; Lorenz, 2002) might fit better for some questions, especially regarding pollutants in the food chain.

Bryophytes proved well in many fields of environmental control. Additionally, bryophytes seem to become an important group of species especially in the field of climate change research in the future (see also Section 6), which will be a major task in the next years.

Finally we like to say that bryophytes which are mostly small in size, are essentially for the integral understanding and control of the present state and future development of our environment.

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