Chapter 9
Water Quality Monitoring by Aquatic Bryophytes

Gana Gecheva and Lilyana Yurukova

Abstract Bryophytes are non-vascular plants that are in a close relationship with their immediate environment. They often have large biomass in freshwater ecosystem and high level of production. Moreover, their tissues contain elevated amount of C, N and P, and cell walls have high cation exchange capacity. Aquatic bryophytes can be used to assess freshwater pollution as indicators – presence or absence of species – or as monitors for accumulating elements. Consumption of metals and other substances by aquatic bryophytes is an important exposure pathway for consumers. The use of bryophytes for water quality assessment is well documented, but different techniques and approaches prevent standardization and their applicability on the European scale. Thus we review major findings in 'bryomonitoring'. Data were reviewed from a range of countries, mainly in Europe, illustrating the advantages of low cost methods for monitoring water quality.

Here we introduce the term 'bryomonitoring' as a method to assess alterations of the environment. Biomonitoring can be split into passive – observation and analysis of native bryophytes, and active biomonitoring – based on species transplantation for a fixed exposure period. Two widespread northern hemisphere aquatic mosses, *Fontinalis antipyretica* and *Platyhypnidium riparioides,* are the most commonly used biomonitors for river quality assessment. For passive biomonitoring key issues are background and reference level determination, and proper selection of sampling sites. For active monitoring, upper segments of a same age from a reference region should be applied. The actual analytical techniques give in general similar results, but not completely interchangeable.

G. Gecheva (✉)
University of Plovdiv, 24 Tsar Assen Str, BG-4000 Plovdiv, Bulgaria
e-mail: ggecheva@mail.bg

L. Yurukova
Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences,
Acad. G. Bonchev Str., Bl. 23, BG-1113 Sofia, Bulgaria
e-mail: yur7lild@bio.bas.bg

E. Lichtfouse et al. (eds.), *Green Materials for Energy, Products and Depollution*, Environmental Chemistry for a Sustainable World 3, DOI 10.1007/978-94-007-6836-9_9,

Aquatic bryophytes are used to assess the ecological status. They are a stress-tolerant and various species have a wide trophic range. *Fontinalis antipyretica* and *Platyhypnidium ripariodes* have all criteria for biota monitoring in rivers for heavy metals.

Standardization of sampling procedures and analytical techniques in aquatic bryomonitoring is further needed. The number of samples should be fixed based on sampling area surface. Period of exposure time for active biomonitoring should be specified in general. Background levels and ambient metal concentrations have to be observed in parallel.

Keywords Bryophytes • Pollution monitoring • Water pollution • Cs • U • Ra • Th • Hg • Pb • Heavy metals

Contents

9.1 Introduction 417
9.2 Biomonitoring: Methodology and Application 418
9.2.1 Passive Biomonitoring 420
9.2.2 Active Biomonitoring 426
9.2.3 Analyses of Elements, Priority Substances and Additional Pollutants 431
9.3 Aquatic Ecosystem Assessment with Bryophytes Under the Water Framework Directive 433
9.3.1 Aquatic Bryophytes as Bioindicators in the Context of Macrophyte Metrics 434
9.3.2 Establishing Environmental Quality Standards 437
9.4 Bryophytes: Cost-Effective and Rapid Ecological Assessment Tool? 440
9.5 Conclusion 441
References 441

Abbreviations

AAS	Atomic Absorption Spectrophotometry
BAF	Biota Accumulation Factor
BCF	Bioconcentration Factor
BMF	Biomagnification Factor
BQE	Biological Quality Element
C	Element concentration
C_{bckg}	Background concentration
CF	Contamination Factor
C_o	Element concentration in an organism
$C_{w/s}$	Element concentration in water/sediment
DEHP	Di(2-ethylhexyl)-phthalate
DL	Detection Limit
EA	Environmental Alteration
EQR	Ecological Quality Ratio
EQS	Ecological Quality Standard

EU	European Union
FAAS	Furnace Atomic Absorption Spectrophotometry
GEA	Global Environmental Alteration
GFAAS	Graphite Furnace Atomic Absorption Spectrophotometry
HCH	Hexachlorocyclohexanes
HPLC	High Pressure Liquid Chromatography
IBMR	Macrophyte Biological Index for Rivers
ICP-AES (ICP-OES)	Inductively Coupled Plasma Atomic Emission Spectroscopy (Inductively Coupled Plasma Optical Emission Spectrometry)
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
MAC	Macrophyte Assessment and Classification
MACPACS	MACrophyte Prediction And Classification System
MLD	Methodological Limit of Determination
MTR	Mean Trophic Rank
NAA	Neutron Activation Analysis
PAH	Polyaromatic Hydrocarbons
PCB	Polychlorinated Biphenyls
pH	Acidity
RHS	River Habitat Survey
RSD%	Relative Standard Deviation in percents
TIM	Trophic Index of Macrophytes
WFD	Water Framework Directive

9.1 Introduction

Pollution is a major environmental issue affecting freshwater habitats and consequently human health. Lotic and lentic ecosystems constantly react to external and internal changes. Their recovery after human alterations depends on a variety of factors, among them living organisms, including bryophyte communities. Aquatic bryophytes as primary producers and habitat providers are important component of freshwater ecosystems and influence both biodiversity and water chemistry. They affect biodiversity by changing environmental conditions and resource availability and providing suitable habitats for new species and species already present. Nutrient dynamics can also be influenced by bryophytes (Stream Bryophyte Group 1999). In mountain sites (especially spring habitats) bryophytes usually cover large areas; sometimes they are able to modify water flow and can be considered as ecosystem engineers (Jones et al. 1994).

A review on monitoring studies of heavy metals with freshwater plants was published by Whitton (2003), comprising, among all phototrophs, data on bryophyte researches during the period 1969 and 2001. Extensive reviews on both terrestrial and aquatic bryophytes as monitors were also presented by Burton (1990) and Tyler (1990).

A variety of assessment methods based on aquatic bryophytes has been proposed, based both on field and/or laboratory data for broad surveys or the investigation of point-source contamination. Research results and outcomes were reviewed from a range of countries, mainly in Europe. Among them numerous laboratory studies on separate heavy metals bioaccumulation kinetics and intra-, extra- and intercellular distribution in recent years have been published (Samson et al. 1998; Vázquez et al. 1999; Vieira et al. 2009), as well as studies on photosynthetic pigments (Cruz de Carvalho et al. 2011; López and Carballeira 1989; Martinez-Abaigar and Núñez-Olivera 1998; Peñuelas 1984; Spitale 2009), and detoxification mechanisms (Dazy et al. 2008) but they will be reviewed in future paper, focusing on exposure of bryophytes under laboratory conditions.

9.2 Biomonitoring: Methodology and Application

Although there are many interpretations of the term, probably most essential is that biomonitoring is a system for long-term observation, assessment and forecast of possible environmental alterations based on biological objects (Martin and Coughtrey 1982). On the other hand, as a scientific term, bioindication has introduced literature at the end of 1960s of the past century. Bioindication is a time-dependent sensitive response of biological measurable parameters to anthropogenic pressure (Stöcker 1980). Thus bioindicator is an organism (or part of an organism or a group of organisms) that contains information on the quality of the environment (or a part of the environment) (Markert 2008). Haseloff (1982) divided them as visual, chemical and physico-biochemical bioindicators: (1) the first type includes species presence, reduced growth, leaf decoloration and population changes, (2) chemical bioindicators are characterized by accumulation of substances, (3) physico-biochemical bioindicators are characterized by alterations of enzymatic activity and physiological functions. Bioindicators were proposed for long-term observations, as well as for planning and management the effects of human activities (Hertz 1991).

In general, the difference between bioindicators and biomonitors is that the former gives a qualitative, and the last one quantitative assessment of the quality of the environment (Manning and Feder 1980; Martin and Coughtrey 1982; Markert 1991; Markert et al. 2003). A biomonitor is always a bioindicator as well, but a bioindicator does not necessarily meet the requirements for a biomonitor (Markert 2008). Biomonitors can be considered as sensitive and accumulative (Steubing 1976; Stöcker 1980). Sensitive biomonitors applied in aquatic ecosystems provide early warning system (Cairns and van der Schalie 1980). Accumulative biomonitors receive major attention towards heavy metal pollution. Tyler (1972) showed that dead organic matter, lichens and especially mosses as low-level plants, accumulate high heavy metals amounts. The main reason is the high stability of the chemical complexes between heavy metal ions and negative charged organic groups. Burton

(1990) underlined the possibility of bryophytes to produce information for reaction towards ecological factors, as well as element concentrations.

It is useful to distinguish between bioindicator and biomonitors (accumulators), but regardless differences in definitions, bioindication and biomonitoring must supply information on the extent of pollution and degradation of freshwater ecosystems.

Both passive and active biomonitoring received widespread popularity and their advantages and disadvantages are profoundly presented by Martin and Coughtrey (1982). Passive biomonitoring consists in observation and analysis of native bryophytes, while active biomonitoring is based on species transplantation for a fixed exposure period.

Numerous studies have reported that aquatic plants often accumulate heavy metals in concentrations much higher than those reached in their aqueous environment, even when those metals are not essential for metabolism or they are potentially toxic. The metal accumulated by a plant gives a better indication of the metal fraction in the environment likely to affect an aquatic ecosystem than most types of direct chemical analysis (Empain et al. 1980). In aquatic systems, metals exist both as free ions and as complexed forms. For many metals it is the free ionic form which is believed to be responsible for toxicity because the possibility for uptake is increased. Thus plant bioaccumulation is the basis for evaluating indirect exposure to other organisms including humans. Metal accumulation in plants is also pointed as common investigated for the biomonitoring of aquatic pollution in the review of Zhou et al. (2008).

In the current review we apply the term bryomonitoring in the context of biomonitoring and underlying the organisms (i.e. bryophytes) by which environmental quality is determined.

Bryomonitoring is founded on two basic approaches: species distribution and chemical analysis of bryophyte tissues (Burton 1986). Bryophytes can accumulate extremely high levels of heavy metals based on their high cation exchange tissue capacity, lack of cuticle and high surface to volume ratio (Tyler 1990). Metal ions are accumulated mainly through the passive ion exchange. Main tolerance mechanism is cell wall considerable efficiency to immobilize heavy metal ions and thus bryophytes are able to accumulate metals to remarkable concentrations. Large interspecific differences in accumulation levels were found in studies carried out at streams with severe contamination (Burton 1990).

In general, bryophyte high accumulative capacity led to their implementation mainly as monitors in regional and local discharges of heavy metals. The simplest way to use information on metal concentrations for monitoring is to compare values for specimens of a particular species at different sites, such as in a river downstream of an effluent or at a range of sites within a particular catchment or geographical region (Whitton 2003). Furthermore, several studies showed aquatic mosses are also suitable for monitoring radioactive contamination (Hongve et al. 2002) and pollution from organic compounds like oxolinic acid, flumequine or oxytetracycline, normally used as antibacterial agents (Delépée et al. 2004), monitoring of polychlorinated biphenyls and hexachlorocyclohexanes (Mouvet et al. 1985), and of polycyclic aromatic hydrocarbons (Roy et al. 1996).

Whatever is the analyzed substance, knowing its bioavailability is crucial, since bioavailability of its forms is tied to the potential effects on living organisms, man included Bioconcentration factors (BCF) or biota/water accumulation factors (BAF) are typically used to describe ratios of contaminants in tissues versus water for aquatic species and they are used to quantify contaminant uptake efficiency. The concept makes assumptions that the environment and the receptor are in pseudo-steady-state conditions, and the ratio is usually normalized for lipid and total organic carbon content of samples. The bioconcentration factor (BCF) is exceptionally high in bryophytes (Vanderpoorten and Goffinet 2009). The equation has the general form below:

$$\mathrm{BCF} = \mathrm{C_o}/\mathrm{C_{w/s}}$$

where: C_o = contaminant concentration in the organism

$C_{w/s}$ = contaminant concentration in the water/sediment

Mouvet et al. (1986) proposed a method for the evaluation of metal contamination based on a Contamination Factor (CF), defined as the ratio of metal concentration in the indicator to the background level in that species. On the basis of CF obtained, Mouvet et al. (1986) suggested five categories of contamination:

- No contamination, up to 2 times the background level;
- Suspected contamination, between 2 and 6 times the background level;
- Moderate contamination, between 6 and 18 times the background level;
- Severe contamination, between 18 and 54 times, and
- Extreme contamination, more than 54 times.

9.2.1 Passive Biomonitoring

In this review the nomenclature accepted in Grolle and Long (2000) for liverworts and Hill et al. (2006) for mosses was presented at first appearance of a species and then taxa are cited following original studies.

High accumulation capacity and vast distribution of some bryophyte species has led to intensive growth of publications dealing with passive biomonitoring. Most of the studies covered only species element content without additional data on the surrounding environment (water and/or sediments).

Among aquatic bryomonitors, *Fontinalis* is the most studied genus (Table 9.1). It has sensitivity to Cu, exhibiting tip chlorosis, while it is insensitive to Cd (Glime 2003). The ecological characteristics of *Fontinalis antipyretica* Hedw. are described in details by Say and Whitton (1983). Species shows considerable potential as monitor of heavy metals. Moreover, it has the ability to colonize variety of substrates and to grow under various flow regimes. Two centimeters tips were suggested to reflect recent events and for long-term surveillance, while whole plants were found more useful for preliminary studies to detect water quality.

Table 9.1 Passive biomonitoring – examples

Species	Sample preparation	Substances	Analysis	Number of studied sites, country	Duration of study	Reference
Rhynchostegium riparioides	Drying at 105 °C, Digestion: 2 M HNO_3	Na, Mg, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Ba, Pb		105, England	6 weeks	Wehr and Whitton (1983a)
Fontinalis antipyretica	Drying at 105 °C, Digestion: 2 M HNO_3	Ca, Mn, Fe, Cu, Zn, Cd, Pb		52, Belgium and England		Say and Whitton (1983)
Rhynchostegium riparioides, *Scapania undulata*, *Hygrohypnum duriusculum*, *Schistidium agassizii*, *Philonotis seriata*	Dried sample, Digestion: 15 ml HNO_3, 5 ml H_2O_2	P, K, Ca, Mg, S, Fe, Al, Mn, Na, Zn, Cu, Pb, Cd, Co, Ni, As, Se, Cr	ICP-AES	10 rivers, 3 lakes, Bulgaria	3 months	Yurukova et al. (1996)
Platyhypnidium riparioides, *Scapania* sp., *Fontinalis antipyretica*	Dried at 40 °C, Digestion: HNO_3 and H_2O_2	Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sr, V, Zn, Ca, Mg, K	ICP-AES/AAS (for Cd, Cu, Pb)	41 sites, Germany		Samecka-Cymerman et al. (2002)
Plagiochila porelloides, Scapania undulata, Atrichum undulatum, Bryum pseudotriquetrum, Rhizomnium punctatum, Fontinalis antipyretica, Amblystegium riparium, Sanionia uncinata, Warnsorfia exannulata, Brachythecium velutinum, B. plumosum, Rhynchostegium riparioides	Dried at 40 °C, Digestion: HNO_3 and H_2O_2	N, P, K, Ca, S, Mg, Na, Fe, Al, Mn, Co, Ni, Cu, Zn, Pb, Cd, As	ICP-AES/Nitrogen after Kjeldahl method	23 sites, Bulgaria	5 years	Yurukova and Gecheva (2004)

(continued)

Table 9.1 (continued)

Species	Sample preparation	Substances	Analysis	Number of studied sites, country	Duration of study	Reference
F. antipyretica, *F. squamosa*, *P. riparioides*	Digestion: 10 ml HNO_3 in a microwave oven	Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Se Zn	FAAS/GFAAS (for As, Cd, Hg, Pb and Se)	Biomonitoring network of 121 sites, Spain	1 year survey	Vázquez et al. (2007)
Sanionia uncinata	Digestion: 300 mg dw of moss samples with HNO_3 and $HClO_4$	Na, Cd, Co, Cr Cu, Fe, Mn, Ni, Pb, V, Zn	FAAS (Fe, Mn and Zn)/GFAAS (Cd, Co, Cr, Cu, Ni, Pb and V)/flame photometer (Na)	29 sites, West Spitsbergen (Svalbard)	Summer season	Samecka-Cymerman et al. (2011)

Fontinalis antipyretica is proved biomonitor of many macro- and microelements in European freshwater ecosystems: Belgium (Empain 1976, 1977; Wehr et al. 1983), Bulgaria (Yurukova et al. 1997), Hungary (Kovács and Podani 1986; Kovács 1992), England (Say and Whitton 1983), Germany (Dietz 1972; Bruns et al. 1995), France (Empain 1976; Mouvet 1984), Poland (Samecka-Cymerman and Kempers 1992, 1993). Reported BCFs are extremely high, for example 3,200 for Pb and 9,400 for Zn (Dietz 1972).

Another intensively studied species is *Platyhypnidium riparioides* (Hedw.) Dixon (=*Eurhynchium riparioides* (Hedw.) P.W. Richards, *E. rusciforme* Milde, *P. rusciforme* (*Schimp*.) M. Fleisch., *Rhynchostegium riparioides* (Hedw.) Cardot, *R. rusciforme* Schimp.). It was confirmed as geographically and ecologically widespread, and as excellent species to monitor heavy metals (Wher and Whitton 1983a). Low pH and calcium values prevent species development. This moss was broadly included in biomonitoring researches in Belgium (Wehr et al. 1983), Bulgaria (Gecheva et al. 2011), England (Jackson et al. 1991), Spain (García-Álvaro et al. 2000).

Among liverworts *Scapania undulata* (L.) Dumort. received major attention for biomonitoring purposes. Species is absent in eutrophic waters and dominates streams which combine very low nutrients with very high heavy metal levels (Wher and Whitton 1983b). It was reported as tolerant to heavy metal contamination and as suitable biomonitor (McLean and Jones 1975; Burton and Peterson 1979; Satake et al. 1989). Thus *Scapania undulata* was implemented in many monitoring programmes (Whitton et al. 1982). Terminal shoots were analyzed from sites influenced by past or present mining activities in England, France, Germany and Ireland. Statistical analyses suggest that elevated pH and/or Ca lead to increased accumulation of Zn and Cd and that probably pH reflect also Pb accumulation. Species indicated the presence of perspective polymetallic deposits in Poland (Sudeten Mts) and had considerable mean values of Cd – 68 mg kg^{-1}, Co – 174 mg kg^{-1}, Ni – 232 mg kg^{-1}, Fe – 130,000 mg kg^{-1}, Mn – 19,900 mg kg^{-1}, as maximum accumulated levels near barite zones in the Sowie Mts were for As 2,190 mg kg^{-1}, B 10,100 mg kg^{-1}, Zn 550 mg kg^{-1}, Ni 150 mg kg^{-1}, Co 1,700 mg kg^{-1}, Ge 4,000 mg kg^{-1}, Pb 3,100 mg kg^{-1}, Sn 200 mg kg^{-1}, V 1,300 mg kg^{-1} (Samecka-Cymerman 1991; Samecka-Cymerman and Kempers 1993).

Studies involving several bryophyte species and/or focusing on interspecific differences are increasing. Metal concentration detected in *Rhynchostegium riparioides*, *Fontinalis antipyretica* and *Cinclidotus danubicus* Schiffn. & Baumgartner reflected Cu and Cr fluctuations (Mouvet et al. 1986). *Fontinalis antipyretica*, *Rhynchostegium riparioides*, *Brachythecium rivulare* Schimp., *Plagiothecium ruthei* Limpr. (=*Plagiothecium denticulatum* (Hedw.) Schimp.), *Pellia fabbroniana* (=*Pellia endiviifolia* (Dicks.) Dumort.), *Scapania undulata* and other species were found useful in biogeochemical prospecting for minerals (Samecka-Cymerman and Kempers 1992, 1993; Pirc 2003). Relationships between Zn, Cd and Pb concentrations in algae, liverwort *Scapania undulata* and three mosses *Amblystegium riparium* (=*Leptodictyum riparium* (Hedw.) Warnst.), *Fontinalis antipyretica* and *Rhynchostegium riparioides* were established in Belgium, France, Germany, Ireland, Italy and Great Britain (Kelly and Whitton 1989). López and Carballeira (1993) studied interspecific differences in metal accumulation among *Fontinalis antipyretica*, *Brachythecium rivulare*, *Rhynchostegium riparioides* and *Scapania undulata* to accumulate metals. *Scapania undulata* and *Rhynchostegium riparioides* showed the highest BCFs. Physico-chemical variables with major influence on metal accumulation were sulphate concentration, pH, nitrite, ammonia and filterable reactive phosphate. Yurukova et al. (1996) applied five aquatic bryophytes (*Rhynchostegium riparioides*, *Scapania undulata*, *Hygrohypnum duriusculum* (De Not.) D.W. Jamieson, *Schistidium agassizii* Sull. & Lesq., *Philonotis seriata* Mitt.) as bioconcentrators of 19 macro- and microelements (N, P, K, Ca, Mg, S, Fe, Al, Mn, Na, Zn, Cu, Pb, Cd, Co, Ni, As, Se, Cr), both at river and lake stations in Rila Mountain.

Positive correlation between copper levels in mosses and in ambient river water ($BCF > 10^3$) was established (Empain 1988). Connection between heavy metal concentrations in bryophytes (*Scapania undulata*, *Fontinalis squamosa* Hedw., *Rhynchostegium riparioides*) and water was studied in Scotland (Caines et al. 1985). Increased hydrogen ion concentrations due to soluble organic compounds and rain waters decrease Al, Mn and Zn bioaccumulation in *Scapania undulata*; moreover, accumulated Mn and Al can be released from bryophyte tissues at low pH level (<5.5). *Fontinalis antipyretica* and *Leptodictyum riparium* were suggested to monitor sites disturbed by multiple pollution sources, as in industrial and urban areas (Gecheva et al. 2011). Physico-chemical water variables represent the major component differentiating bryophyte assemblages at affected sites but the relative importance of environmental factors underlying community compositions differed strongly. Thus, in the assessment of surface water quality, bryophyte species composition was found as representative of river hydromorphology, while the content of elements in bryophyte tissue of water chemistry. The concentrations of elements in water and *F. antipyretica* collected in the same station were poorly correlated (Vázquez et al. 2004). *Fontinalis antipyretica* and *F. squamosa* appeared to avoid sites with low pH and high levels of Ca and Mg (pH, Ca and Mg were highly significantly and positively correlated), while prefer high concentrations of Cl, Na, K and Si – also significantly and positively correlated (Vázquez et al. 2007). Nevertheless, Mg and Ca play protective role for plants and reduce the toxic influence of heavy metals (Samecka-Cymerman and Kempers 1994).

Studies on both moss species and sediments are scarce. Natural background levels for Cd, Cr, Cu, Pb and Zn in four moss species (*Fontinalis antipyretica*, *Rhynchostegium riparioides*, *Amblystegium riparium* and *Fontinalis squamosa*) and sediments were reported from Portugal (Gonçalves et al. 1992). Cadmium and zinc were accumulated 107 and 70 times respectively higher than their background levels. Aquatic mosses appeared to reflect more recent conditions in freshwaters than sediments which incorporate particulate matter and are under unknown influence upstream.

An integrated research incorporated aquatic mosses (*Fontinalis squamosa* and *Rhynchostegium riparioides*), river water and sediments (Say et al. 1981).

Fontinalis antipyretica was successfully used in radiological monitoring of Cs^{137}, Cs^{134}, U^{235}, Ra^{236}, Th^{232}, K^{40} in Bulgarian montane river (Mishev et al. 1996). *Cinclidotus danubicus* was applied as monitor of radionuclides in France (Kirchmann and Lambinon 1973) and *Fontinalis* sp. in the U.K. (M A F F 1967; Hunt 1983).

Selecting sampling sites for passive biomonitoring is an important issue. Proper criteria for extensive biomonitoring network were given by Vázquez et al. (2007). When a single site was located in a drainage basin (≈100 km^2), it was sited in the mid–low stretch of the river to integrate as far as possible the inputs received throughout the basin. When two sites were located in a drainage basin (≈300 km^2), one was located at the head of the river and the other close to the mouth of the river. When more than two sites were located in a drainage basin (500 km^2), they were distributed in the different stretches of the main flow and of the main tributaries, covering different sub-basins. Location of sites in stretches possibly affected by reservoirs or immediately downstream of contamination foci (centres of population, industrial areas, etc.) was avoided. Each sampling site consists of a stretch of river approximately 100 m long and a sample was collected from at least five mats of one of the selected species.

Another key issue is background or reference level determination, as the metal bioavailability depends on metal form and concentration in environment. At the same time, the evaluation of environmental contamination is based on calculated CFs, i.e. on the ratio of metal concentration in the indicator to the background level. The background levels are needed also to calculate the. Background levels of course should be assessed on regional level and per species, since substrate lithology significantly differs geographically. Data are available for background levels of 19 macro- and microelements in *Fontinalis antipyretica* and high mountain river water in Bulgaria (Yurukova et al. 1997). *Scapania undulata* and *Platyhypnidium riparioides* had higher reference concentrations of Cd, Cr, Cu, Co, Ni, Pb, Zn, Fe and Mn in comparison with *Fontinalis antipyretica* and *Fissidens polyphyllus* collected from rivers in Spain (Carballeira and López 1997). Background levels of the former two species showed no significant difference with those in *Brachythecium rivulare*. Background concentrations in liverwort *Chiloscyphus* sp., *Fontinalis antipyretica* and *Platyhypnidium rusciforme* collected in French streams embedded in basaltic rocks were presented by Samecka-Cymerman and Kempers (1999). Background levels in *Platyhypnidium riparioides* for Ni, Cr, Co, Zn, Mn, Pb, Cd, Cu, Ba, Al and V were reported also by Samecka-Cymerman et al. (2002). Background levels in *P. riparioides* for Cd, Co, Cr, Fe, Mn, Ni, Pb and Zn presented by Cesa et al. (2010) for Italian River Bacchiglione basin (calcareous upper and alluvial lowland basin, respectively) were similar to those in dominated with limestone and dolomites in Tatra National Park (Samecka-Cymerman et al. 2007), and vice versa background levels in siliceous substrate are higher. Background levels for 25 elements in *F. antipyretica* and in addition element and site specific CFs were obtained by Vázquez et al. (2004).

It could be summarized that *F. antipyretica* and *P. riparioides* are the most commonly used and proven biomonitors in river quality assessment, especially in chronic exposures or long-term effects of chemical pollutants.

9.2.2 Active Biomonitoring

When no bryophyte records could be found in cases of severe pollution or environmental factors, active monitoring approach is applied. In general, mainly alive specimens collected from unaffected habitats, put in nylon cage are exposed at contamination for a period of several days or months. Major problem with moss transplantation appear to be their survival in habitats where local climate or pollution conditions do not meet species optima (Tyler 1990). Such sensitivity was known for *Fontinalis hypnoides* Hartm. (Gimeno and Puche 1999).

Although a great variety of experimental details exists (Table 9.2), the defined time period of exposition is a major advantage (Siebert et al. 1996). Despite equal metal accumulation in alive and dead material, the last can be associated with decay in plants. The number of moss bags per area, their size and attached or suspended exposition are most likely to result in different outcomes. Exposition period range has to be at least 24 h (Kelly et al. 1987), while 1 month is probably the upper limit at highly contaminated freshwaters. According to López et al. (1994) exposition should be at least for 5 days.

Already in 1970 Benson-Evans and Williams (1976) applied aquatic bryophytes to detect river pollution in Great Britain. *Fontinalis antipyretica* and *Eurhynchium riparioides* were transplanted from their natural environment at six selected stations (Table 9.2). *Fontinalis squamosa* and *Scapania undulata* were studied for heavy metal accumulation from McLean and Jones (1975). Elevated levels of Pb, Cu, Zn and Mn were reached after 6 weeks, and after 18 weeks a decay process was observed. At the same time *Scapania undulata* survived after transplantation at region with lower contamination and no considerable differences in metal content were found. Thus authors recommended selecting the commonest species in the survey area for metal monitoring in rivers. When none is characteristic for monitored river sites, then transplants can be applied under the conditions that same-age specimens of a selected bryomonitor are used and additionally physico-chemical parameters influencing accumulation (pH, light conditions and metal ambient concentrations) are monitored. Growth form is also linked to the process. *Fontinalis* has long branches, while *Scapania* formed solid tufts and thus the former species is more constantly exposed to free metal ions and respectively more rapidly accumulates metals. Bryophyte tolerance is considered to be effective until metals are included in complexes or are isolated in safe areas as cell walls (McLean and Jones 1975). When saturation is reached, any increase in metal concentration lead to damages on cell metabolism, and finally death.

Kelly et al. (1987) applied 2 cm apical stems of *Rhynchostegium riparioides* and *Fontinalis antipyretica* in monitoring of Zn, Cd, Pb in England. Alive specimens of

Table 9.2 Active biomonitoring – examples

Species	Moss-bag [g ww]	Sample preparation	Substances	Analysis	Number of studied sites, country	Exposure time	Reference
Eurhynchium riparioides, *Fontinalis antipyretica*	–	Perspex cylinder in a nylon mesh cage	–	Mosses were assigned to one of 5 breakdown categories – macroscopically and microscopically for morphology and structure	6, Great Britain	1, 2, 4, 6, 12, and 22 days	Benson-Evans and Williams (1976)
Cinclidotus danubicus		Plastic mesh grids (1 × 1 cm)	PCBs and α-, β, and γ-HCH	gas chromatography	France	13, 24 and 51 days	Mouvet et al. (1985)
Fontinalis antipyretica	500	Containers of plastic mesh with 2.5 × 3.5 mm pore size	Zn, Cu, Pb, Ni, Cr		4 sites, Hungary	3 months	Kovács (1992)
Fontinalis antipyretica	10		Cd, Cu, Cr, Pb, Sn		10 sites	15-24-28 days	Mersch and Pihan (1993)
Hygrohypnum ochraceum	8	Plastic mesh bags	Cd, Mn, Pb, Al	Oven-dried at 40 °C for 24 h/9 ml 4 M HNO_3/AAS	13 (including native stream), France	1 month	Claveri et al. (1995)
Fontinalis antipyretica	20	Glass fiber bags	PAHs		5 sites, Lake Kallavesi, Finland	35 days	Roy et al. (1996)
Fontinalis antipyretica	10	2–4 cm long sections in plastic nets	Cd, Pb, Zn, Cu	Dried at 80 °C/microwave digestion/ICP-MS	18, Germany	12, 14, 16 and 21 days (182 days for a lost sample)	Bruns et al. (1997)

(continued)

Table 9.2 (continued)

Species	Moss-bag [g ww]	Sample preparation	Substances	Analysis	Number of studied sites, country	Exposure time	Reference
R. riparioides, *F. antipyretica*, *Cinclidotus danubicus*	20		Zn, Pb, Cu, Cr, Ni		5 sites	11 and 16 days	Mersch and Reichard (1998)
Fontinalis antipyretica		Cages (15 × 15 × 15 cm) made from plastic coated, stainless steel wire (1.5 × 3 mm holes)	As, Cr, Cu	Samples dried at air temperature for 3–5 days, followed by drying at 40 °C for 3–4 days. Digestion: HNO_3; ICP-AES/AAS (for As)	Norway	24, 48, 72 h, 6 days	Rasmussen and Andersen (1999)
F. antipyretica	10	Nylon net, opening light of 1.3 mm	Cd, Cr, Cu, Pb, Hg	Digestion: 2.5 ml HNO_3; AAS (Cd, Pb, Cu, Cr)/CVAAS (Hg)	9 sites (lake and river), Italy	14 and 28 days	Cenci (2000)
Fontinalis antipyretica	10–12	Plastic 1 × 1 cm mesh bags	Al, Co, Cu, Ni, Zn/K, Mg, Ca	Intracellular, extracellular and particulate fractioning/AAS	4 sites (including control station), Spain	1, 4, 11, 21, 35 days (1, 4, 13, 27, 48 days at control station)	Vázquez et al. (2000)
Fontinalis antipyretica	500	Attached to the buoy	Ca, Mn, Cr, Pb, Zn, Fe, Cu, Cd	Dried at 60 °C	1 site, Korea	2 days	Lee et al. (2002)
Fontinalis antipyretica	5 g dry weight	Nylon nets (10 × 10 cm; 1 mm^2 gaps)	N, P, K, Ca, S, Mg, Na, Fe, Al, Mn, Co, Ni, Cu, Zn, Pb, Cd, As	ICP-AES/Nitrogen after Kjeldahl method	1 site, Bulgaria	31, 30, 31, 61 days (5 months in total)	Yurukova and Gecheva (2003, 2004)

Fontinalis antipyretica		Plastic tube, 150 mm long and 12 mm Diameter. Via lateral cut along the tube, only the basal part of the moss is introduced, allowing the remainder of the plant to float free	Cu, Zn, Pb, Fe, Ni, Ca, K, Mg		10 sites, Portugal		Figueira and Ribeiro (2005)
F. antipyretica	5	Spin-dried, nylon mesh bags (4 mm)	N, P, K, Ca, Mg, S, Fe, Al, Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb, V, Zn		15 sites, Poland	60 days	Samecka-Cymerman et al. (2005)
Rhynchostegium riparioides	25–30	30 × 30 cm nylon bags with 7 mm holes	As, Cd, Cr, Cu, Mn, Ni, Pb, Zn	Digestion: 3.5 ml HNO_3, 0.1 ml HCl and 2 ml H_2O_2; AAS	3 sites, Italy	20, 34, 48 and 62 days	Cesa et al. (2006)
R. riparioides	20–30	Plastic net (4 mm holes)	Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se, V, Zn	2.5 ml HNO_3 and 0.5 ml H_2O_2; AAS	119 stations placed in 64 sites, Italy	4 weeks	Cesa et al. (2010)
Fontinalis antipyretica	100	Collected from a natural stream	10 metals 16 priority PAHs		4 streams, Lisbon, Portugal	3 months	Augusto et al. (2011)
Fontinalis antipyretica	20	0.5 cm mesh size plastic bag	Zn, Cu, Ni, Pb	Mineralization in a microwave furnace with HNO_3 and $H2O_2$; FAAS	3 sites, Morava River, Czech Republic	28 days	Diviš et al. (2012)

Fontinalis antipyretica, collected from reference region and putted in nylon mesh at depth of 10–20 cm successfully reflected heavy metal contamination (Kovács 1992). Similar results were obtained with the same species during a 28-day exposure by López et al. (1994). Metal uptake kinetic reached an equilibrium phase which is mostly correlated to concentration in water. Retransplantation in an unpolluted site led to metal release occurring with a two-phase, mainly regulated by metal concentration in moss tissue at the beginning of the recovery. *F. antipyretica* was used as active biomonitor in Spain (Vázquez et al. 1999, 2000). High acidity did not appear to influence uptake by intracellular structures. During recovery significant changes were noted in extracellular metal levels. The species was recommended to be applied as active biomonitor. Claveri et al. (1995) applied transplants of *Hygrohypnum ochraceum* (Turner ex Wilson) Loeske for monitoring Al, Mn, Pb, Cd. Good correlation was observed at increasing Al and Pb values in water and moss.

As active biomonitor *F. antipyretica* showed good accumulation properties for all metals (Bruns et al. 1997). Authors recommended use of freely suspended samples in the water and noted that during autumn and winter season highest accumulation were observed. A detailed description of use of *F. antipyretica*, including sampling and conditioning was presented by Cenci (2000), and the method was recommended for detection of high risk situation in European water bodies. Transplants of *Fontinalis antipyretica* were used to assess the contamination of an industrial effluent discharge on a river located in south Portugal for 2 years (Figueira and Ribeiro 2005). An increase of water contamination by Cu, Zn was verified with extra and intracellular moss fractions. An interesting transplantation device was proposed in the study (Table 9.2).

F. antipyretica was applied simultaneously in passive and active biomonitoring of heavy metals (Samecka-Cymerman et al. 2005). Transplants accumulated significantly higher amounts of Al, Cr, Cu, Pb, V and Zn than native mosses, while Co and Mn concentrations were higher in the native specimens.

Relatively obscure are data concerning experiments with dead (initially cleaned and dried) material. Moss-bags with *Fontinalis antipyretica* of about 5 g dry weight were studied for a maximum period of 28 days along river sites in Luxembourg (Mersch and Pihan 1993), and for 5 months at impacted river site in Bulgaria respectively(Yurukova and Gecheva 2003, 2004).

Most common species applied for transplants is *Fontinalis antipyretica* not only for heavy metals but also for polycyclic aromatic hydrocarbons (Roy et al. 1996). Glass fiber bags for 35 days were applied at Lake Kallavesi (Finland) to investigate the cause-effect relationship between bioaccumulation of PAHs and the responses of antioxidant enzymes in aquatic moss. Higher activities of antioxidant enzymes and activated forms of oxygen were observed in moss transplanted near the harbor in comparison with moss-bags located upstream at two reference sites.

Regardless dead or alive are transplants, water acidity has significant influence on metal accumulation. Moss-bags accumulated Al, Pb, Fe and first two elements showed strong dependence on pH level (Mersch and Pihan 1993). Release of Cu and Zn was observed and commented in connection with high acidity. A negative correlation between water acidity and metal accumulation was also reported by Vázquez et al. (2000) during exposure of *Fontinalis antipyretica* at affected stations along Spanish rivers. Release of total Ca and Mg, as well as intracellular K at pH < 5 was established. Carballeira et al. (2001) suggested upper shoots of *Fontinalis antipyretica* could be used for assessment of temporal pH decreasing on the basis of preloaded Cd release.

The most intensive work with moss bags in recent years was done with *Rhynchostegium riparioides* in Italy (Cesa et al. 2006, 2008, 2009a, b, 2010). Transplanted moss confirmed ability to detect spatial patterns of bioaccumulation, to reveal Pb and Cu chronic contamination and Cr, Zn and Ni intermittent contamination, and to localize emission sources. Highest uptake ratios were observed for Al, Cu, Cr, Hg, and Pb under laboratory conditions.

Combined technique incorporating transplants of *Cinclidotus nigricans* for short regular periods and mussels towards long-term investigations was proposed (Mersch and Johansson 1993). Mosses and mussels appeared to be complementary in biomonitring due to specific uptake and depuration kinetics. Transfer technique was evaluated as preferable to assess the recent pollution situation in comparison with the native *Fontinalis antipyretica*.

Active biomonitoring has proven its effectiveness, especially in industrial and urban areas, although a great variety of moss-bag size, exposure periods, used material (dead or alive), and biomonitor species was tested. Thus selection of a particular species, upper segments of a same age, from reference region with known level of anthropogenic impact, is recommended. A control moss-bag at a reference station is advisable.

9.2.3 Analyses of Elements, Priority Substances and Additional Pollutants

Whole plants are recommended to be analyzed in broad studies and in tracing contamination sources, while apical shoots (2–4 cm) are more suitable in regular surveys at particular sites (Wehr and Whitton 1983a; López and Carballeira 1993; García-Álvaro et al. 2000; Cesa et al. 2010).

Due to seasonal fluctuation in metal content in bryophyte tissues and in environmental factors, it is advisable to not rely only up on samples collected during winter. Despite bryophytes grow all around the year and have nutrient uptake less seasonal than vascular plants, most species have reduced biomass during winter and this could lead to an increase in metal concentration within moss tissues. *Fontinalis antipyretica*, *Leptodictyum riparium*, and *Platyhypnidium riparioides* have vital stems throughout the year but seasonal variation of metals between species and habitats was reported (Wehr and Whitton 1983b). Found increased accumulation of Pb in the period autumn-winter was a consequence of the lower aqueous concentrations of reactive phosphate and Mg due to extended period of high flows.

Nevertheless, the results showed that seasonal influences on metal accumulation are negligible and changes which appear seasonal are probably due usually to correlated changes in aqueous chemistry.

Environmental factors influence metal uptake and affect the concentration in the plant at the time of sampling (Whitton 2003). Among measured parameters of ambient water pH should be obligatory. As observed by early works, pH of water influences metal ion accumulation, as in lower pH values the accumulation decreases (Caines et al. 1985; Lingsten 1991). In addition, aqueous Ca was pointed out as the principal non-heavy-metal factor reducing the accumulation of Zn and Cd (Kelly and Whitton 1989), while phosphates reduce Zn and Pb accumulation (Wehr and Whitton 1983a).

Chemical analyses of bryophytes should be preceded by removal of organic particles and additional fragments at field. Washing with tap water can alter element levels through cation exchange and therefore the possible cellular locations of the studied elements, this is to be considered before sampling and extraction method to be applied (Bates 2008). Washing by use of demineralized water is strongly recommended.

The representative samples of aquatic mosses by species should be carefully cleaned from other organic matter and mineral particles before analysis. The laboratories are advised to carry out homogenization of mosses according to their normal routines. The use a sample weight of at least 0.5 g, dried at 40 °C moss material, is credential, and the laboratory staff is recommended to use wet-ashing methods, preferably with concentrated or 1:1 nitric acid (with or without hydrogen peroxide or perchloric acid) or not dissolved before analysis (methodology in European Moss Surveys, Steinnes et al. 1997). Acid-digestion of mosses is performed on a hotplate or in a microwave oven using a range of temperatures. Triplicates of each sample are prepared independently.

The element concentrations could be determined by various analytical techniques, under the broad heading of atomic absorption spectrometry (flame or graphite furnace) (FAAS, GFAAS), inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS), and neutron activation analysis (NAA).

In any case reference moss materials are applied in each moss series of analyses. A detection limit (DL) equivalent to three SD of the lowest instrumental measurements of the stock standard solutions, blanks – all reagents and all analytical procedures, but without biological material is recommended. The Methodological Limit of Determination (MLD) is calculated on the basis of not less than three measurements for each solution of the digested sample, dilution of digested solutions and weight of each sample, and corresponding blank. The last concentrations were determined according to all measurements and repetitions, dilution of solutions, weight of samples and blanks, usually in $mg/kg^{-1} = \mu g\ g^{-1}$ = ppm. RSD% for all elements analysed is obligatory for each moss sample.

As Markert (1996) pointed out a strict differentiation between the terms 'precision' and 'accuracy' should be established in each analytical research with environmental samples. The precision of the data is connected with repeatedly measuring of the analytical signal purposing to eliminate errors, 1–5 % RSD is sufficiently exact. The accuracy of data involves use of one or more plant reference materials with certified values, and use of independent analytical procedures, i.e. inter-laboratory analyses by a wide ring of laboratories.

No one of above mentioned analytical techniques is suitable for all important essential and trace elements accumulated in moss tissues. Inductively coupled plasma optical emission spectrometry is good to be applied for more than 20 elements, in cases with higher level of Cd, Pb, As in mosses; neutron activation analysis without decomposing of the sample gives the widest idea of inorganic content – moreover 46 elements (Au and Sb included), but without important heavy metals as Cd, Cu, Pb; inductively coupled plasma mass spectrometry, available since 1984, is the combination of multi-element capabilities with extremely wide linear dynamic range over nine orders of magnitude, and could be used for 25–30 elements without P, Al, and Mn, Ca, Fe, due to a lower sensitivity thresholds, especially for toxic Al and essential P.

Analyses carried out simultaneously by ICP-MS and ICP-OES in both water and in *F. antipyretica* provided quite similar results (Vázquez et al. 2004). In moss samples the concentrations of Al, Ba, Fe, Sr and Ti measured by both methods were equivalent ($p < 0.05$), whereas those of Mn were significantly different. For the remaining elements the differences were small, obtaining higher values by ICP-OES than by ICP-MS for Co, Cr, Cu, Ni and Zn, and slightly lower for Ca levels. With respect to water samples, both techniques gave equivalent results for Ba, Ca and Zn. The remaining elements (Fe, Mn, Sr and Al), showed values obtained by ICP-OES lower than those obtained by ICP-MS, finding the greatest difference between techniques in the levels of Al measured. Thus, it can be stated that the results obtained with these two analytical methods were in general similar or equal, but not completely interchangeable.

Procedures for the analysis of organic contaminants in bryophytes include extraction from wet or freeze-dried samples with organic solvents, removal or destruction of lipids, clean-up, fractionation, high pressure liquid chromatography (HPLC) or gas chromatographic separation and different kinds of detection, e.g. fluorimetric, electron capture or MS (European Commission 2010). The total fat weight can be determined and used to normalise analytical results; this procedure should be considered as an alternative to weight normalisation.

9.3 Aquatic Ecosystem Assessment with Bryophytes Under the Water Framework Directive

The Water Framework Directive 2000/60/EC (WFD) (European Union 2000) requires Member States of the European Union (EU) to achieve good ecological status by 2015 in all water bodies. Individual water bodies are graded into one of five quality classes (high, good, moderate, poor or bad) reflecting Ecological Quality Ratio (EQR) which evaluates water quality in a score ranging from 0 (worst status) to 1 (reference status).

The WFD requires comparability of the EQR scales between the different EU countries in order to have a common understanding of the good ecological status of surface waters. This will ensure comparability of the classification results derived by various monitoring systems and reliability of the results produced by each classification tool. Biological quality elements (BQE), among them aquatic flora, are the key parameters on which the assessment is based. High ecological status is thus determined via dominance of reference species in type specific vegetation density. The status observed at the monitored station is compared to the status expected under reference/near natural conditions.

9.3.1 Aquatic Bryophytes as Bioindicators in the Context of Macrophyte Metrics

Local habitat characteristics determine river macrophyte communities, particularly light availability, current velocity, sediment patterns and nutrient supply (Birk and Willby 2010).

Compositional patterns of aquatic bryophytes are sensitive to a number of factors such as water flow velocity and level, eutrophication, pollution, and additional pressures. Substrate type also directly affects macrophyte development. Rocks and hard, immobile substrates are associated with bryophytes (Janauer and Dokulil 2006). Coarse substrate and variable flow regime contribute both to the success of bryophytes and to the exclusion of vascular hydrophytes (Scarlett and O'Hare 2006). Bryophytes are a dominant component in lotic ecosystems, especially in undisturbed conditions and their relations with environmental factors were studied (Glime and Vitt 1987; Suren 1996; Duncan et al. 1999; Suren and Duncan 1999; Suren et al. 2000; Scarlett and O'Hare 2006). Combined influence of underlying geology, water physico-chemistry, current velocity and substrate morphology on 17 bryophyte species was investigated in three minimally impacted high-latitude headwater streams in Scotland (Lang and Murphy 2012).

Anthropogenic influence results in reduction of species richness and fragmentation of populations, at the same time tolerant macrophyte taxa exhibit high growth capacity. Thus presence and abundance of aquatic macrophytes can indicate specific water characteristics such as trophic status, ion content, etc.

Aquatic plants were classified based on trophic status already 30 years ago, and for example *Sphagnum* species were allocated to plants of dystrophic, poor-in-nutrients type (Haslam 1982).

In Eastern Europe and Balkan region Papp and Rajczy (1995) suggested bryophytes as indicators of ecosystem alterations along Danube River. Correlation between the bryophyte assemblages and water quality along the Hungarian Danube section (Papp and Rajczy 1998a) and relation between assemblages of various streams in Hungary to the chemical parameters of water were studied (Papp and Rajczy 1998b). These studies report that species composition of the aquatic-riparian bryophyte vegetation and the abundance-frequency values reflect water quality along rivers. Similarly changes in aquatic bryophyte assemblages were applied for assessment of trophic level (Vanderpoorten and Palm 1998). Phosphates appeared not to influence bryophytes at lowland river sites (Scarlett and O'Hare 2006; Gecheva et al. 2010). It was found also that *Leptodictyum riparium* and *Brachythecium rivulare* are associated with high levels of phosphate. Bryophyte species composition of some Greek streams and their correlation with environmental conditions are described in Papp et al. (1998).

Most macrophyte-based assessment systems in Europe evaluate river ecological status focusing on the potential of species to detect eutrophication as main pressure (Birk et al. 2006). As part of the broad group of aquatic macrophytes, bryophytes are included to several assessment methods: French "Indice Biologique Macrophytique en Riviere" (IBMR), German Reference Index, British Mean Trophic Rank and Dutch Macrophyte Score. *Leptodictyum riparium*, *Brachythecium rivulare* and *Fontinalis antipyretica* are listed in Mean Trophic Rank (MTR) and Macrophyte Biological Index for Rivers (IBMR) with species scores: 1.0 and 2.5; 8 and 7.5; 5 and 5 respectively (Szoszkiewicz et al. 2006). *Platyhypnidium riparioides* has score (5) only in MTR.

In summary, *L. riparium* is accepted as tolerant to organic pollution by both indices. MTR examines *F. antipyretica* and *P. riparioides* as species with intermediate tolerance to nutrient enrichment, and *B. rivulare* with considerably low tolerance. In contrary, IBMR evaluates *F. antipyretica* and *B. rivulare* as tolerant to organic pollution.

Four moss species above were not included in Trophic Index of Macrophytes (TIM) since it was designed to indicate the trophic status of rivers as whole ecosystems and combining water and sediment nutrients.

These broadly used biomonitors were also listed as type specific species according Reference Index (Schaumburg et al. 2004), which classifies rivers by regional approach and reflects different kinds of ecological stresses, as well as river pollution.

Mosses and liverworts were also included in development of predictive models to evaluate the streams ecological quality: MACPACS (MACrophyte Prediction And Classification System), MAC (Macrophyte Assessment and Classification) and AQUAFLORA (Aguiar et al. 2011; Feio et al. 2012).

Seven national macrophyte assessment methods were processed to an international data set that covered three European stream types and common reference conditions were defined (Birk and Willby 2010). Type-specific reference community for siliceous mountain brooks includes liverworts (*Scapania undulata* or *Chiloscyphus polyanthus*, and less frequently, *Marsupella emarginata* or *Jungermannia atrovirens*; thallose *Pellia epiphylla*) and acrocarpous mosses (most notably *Racomitrium aciculare*, plus smaller quantities of marginal species, such as *Philonotis fontana* and *Dicranella palustris*, *Fissidens crassipes* and *F. rufulus*). These taxa occur against a backdrop of extensive growths of a range of pleurocarpous mosses, including *Rhynchostegium riparioides*, *Fontinalis squamosa*, *F. antipyretica*, *Hygrophypnum ochraceum* (and occasionally *H. luridum*), *Brachythecium rivulare*, *B. plumosum*, *Hyocomium armoricum*, *Thamnobryum alopecurum* and

Amblystegium fluviatile. Several of these species persist in the lowest quality sites, most notably *Fontinalis antipyretica* and *Rhynchostegium riparioides*, but most bryophytes are replaced by *Amblystegium riparium*.

F. antipyretica and *P. riparioides* were found to form the "core" of bryophyte communities in more stable stream habitats (Lang and Murphy 2012). Benchmark bryophyte community for upland headwater streams of reference conditions was identified. *Scapania undulata* and *Hygrohypnum ochraceum* were established as indicators for oligotrophic upland streams, and *Chiloscyphus polyanthus* and *Hygrohypnum luridum* for calcareous and mineral-rich streams.

Specification of aquatic plant composition and percentage cover at different stages of eutrophication were given in Hime et al. (2009). *Rhynchostegium riparoides*, *Leptodictyum fluviatile* and *Fontinalis antipyretica* accounted for above 65 % of the total cover in highest quality.

Fontinalis antipyretica is among dominant species in lowland rivers, associated with higher quality, while it is pointed as a negative indicator in mountain streams (Birk and Willby 2010). Scoring bryophytes in the context of macrophyte-based metrics should assume wide trophic range of several moss species, including *F. antipyretica* (Vanderpoorten et al. 1999). Moreover, *F. antipyretica* has different ecological preferences in particular regions probably due to the existence of different ecotypes (Vázquez et al. 2007). As pointed out by Glime (2007) osmotic effect plays a major role in bryophyte nutrient needs and toxicity. Except some thallose taxa, bryophytes lack epidermis and waxy in their cuticles, and are especially susceptible to osmotic shock. Thus the same species can respond quite differently under different concentrations of nutrients and heavy metals, i.e. if a plant has grown from spores at a certain nutrient/ion level, then its osmotic potential is more likely to be adjusted.

Studies on effects of hydro-morphological degradation underlined the importance of bryophytes in stream ecosystems and increased knowledge about the effects of flow regulations. Taxonomic richness was found to decrease at flow regulated sites probably due to increasing substrate stability and dominance of strong competitors (Englund et al. 1997). Additional factor for lower richness was suggested by Downes et al. (2003). Regulated streams have limited ranges of water level and as a consequence, large rocks have only narrow zones that are subject to a variety of wetted conditions, which were assumed as more suitable for bryophyte growth and colonization than constant submergence.

Bryophyte species composition is also suitable for evaluating the impact of hydromorphological alteration. Unstable river sites are characterized by small-sized species or by absence of bryophytes in strongly affected habitats such as those amenable to erosion (Gecheva et al. 2011). Quantification of the impact of small hydroelectric schemes on bryophytes and lichens showed that they are largely under-recorded in small streams where small hydroelectric schemes are likely to be developed (Demars and Britton 2011).

River Habitat Survey (RHS) method was developed in the United Kingdom and carried out in several European countries to assess, in broad terms, the physical character of freshwater streams and rivers (Raven et al. 1998). RHS only records plants growing by the riverside and the results show distribution in riparian habitats, in particular hygrophyte bryophyte species.

The potential for a macrophyte tool indicative of hydro-morphological impact is discussed by O'Hare et al. (2006). The abundance of liverworts and mosses was in negative correlation with homogeneity of water depth, deep water (the dominant depth) and fine particle substrate (the dominant substrate). Presence of *Fontinalis antipyretica* and metrics such as the presence of the group 'liverworts/mosses/lichens' may indicate that a site is unimpacted by hydromorphological degradation.

Mosses *Fontinalis antipyretica*, *Amblystegium riparium* and *Rhynchostegium riparioides* reach their highest abundance in high stream power and coarse bed sediments in British rivers (Gurnell et al. 2009). Mosses can tolerate and may even prefer the most disturbed physical environment (O'Hare et al. 2011). Thus mosses are considered a stress-tolerant group and so they are not disadvantaged by the low nutrient and carbon conditions associated with high specific stream power and coarse substrate.

9.3.2 Establishing Environmental Quality Standards

Directive 2008/105/EC (Environmental Quality Standards Directive) defines the good chemical status also to be achieved by all Member States in 2015 and gives, together with the WFD, the legal basis for the monitoring of priority substances in sediment and biota (European Union 2008). Good chemical status refers to a list of 41 pollutants: 33 priority substances (PSs) and 8 other pollutants. Member States should have the possibility to establish EQS for sediment and/or biota at national level and to provide long-term trend analysis of concentrations of those substances listed in Part A of Annex I, giving particular consideration to Anthracene, Brominated diphenylether, Cadmium and its compounds, C10-13 Chloroalkanes, Di(2-ethylhexyl)-phthalate (DEHP), Fluoranthene, Hexachlorobenzene, Hexachloro-butadiene, Hexachloro-cyclohexane, Lead and its compounds, Mercury and its compounds, Pentachloro-benzene, Polyaromatic hydrocarbons (PAH) and Tributyltin compounds. The frequency of the monitoring has to provide sufficient data for a reliable long-term trend analysis and should take place every 3 years. In this context, perennial and long-lived shuttle bryophyte species are particularly suitable, since their life-strategy is based on 3–4 years (During 1992).

For organic substances, monitoring in biota should be performed when the biomagnification factor (BMF) is >1 or when the bioconcentration factor (BCF) is >100 (European Commission 2010). Biomagnification referring to absorption of the substances via the epithelia of the intestines, is limited to heterotrophic organisms (Markert et al. 2003). Metals from highly volatile compounds as Hg and As, are taken through the respiratory organs. In the selection of biota species, consideration should be given to the main purposes of the EQS Directive: trend monitoring and compliance with EQS.

Under the review of the existing approaches, active monitoring with transplants was evaluated as more suitable (Besse et al. 2012). Macroinvertebrates were chosen as most appropriate organisms in the active approach, as they enable robust control of biotic factors, by using size-homogenous and sex-homogenous indicator species, that lend themselves well to practical, easy-to-handle, infield caging systems. In the same article bryophytes were pointed out as useful monitors but not reliable for checking compliance with biota EQRs. We suppose that the main reason for that statement is that WFD requires biota EQSs to protect humans, top predators such as birds and mammals, and benthic and pelagic predators. Nevertheless, aquatic bryophytes meet all nine criteria stated in the Guidance on chemical monitoring of sediment and biota under the Water Framework Directive (European Commission 2010):

– "A relationship exists between contaminant concentrations in the species and average concentrations in the surrounding environment;
– The sampled organism is a potential food for predatory organisms or humans;
– The species accumulates the contaminants;
– The species is sedentary (migrating species should be avoided) and thus represents the sampling location, and does not originate e.g. from aquaculture plants;
– The species is widespread and abundant in the study region, to allow comparisons between different areas;
– The species lives long enough so that more than 1 year-class can be sampled, if desired;
– The species is large enough to yield sufficient tissue for analysis;
– The species is easy to collect and hardy enough to survive unfavourable conditions;
– The species is easy to identify."

Aquatic bryophyte species such as *Fontinalis antipyretica* and *Platyhypnidium ripariodes* comply with all the above criteria. The Guidance also pointed out that "candidate species for biota monitoring in rivers include: ... the aquatic bryophytes (e.g. genera *Fontinalis*) for heavy metals."

Plants uptake metals and other substances through tissues or organs as bioconcentrators. Unlike the most common route of metal exposure in plants through the roots, bryophytes provide direct exposure pathway (water to bryophytes) which can be used to evaluate exposure to higher trophic levels to which can cause harm. Moreover, plants give an indication of the soluble metal fraction in the environment which is likely to affect major compartments of the aquatic ecosystem. Therefore, when a heavy metal or toxic element is not detected (concentration is below MLD) in the bryophytes analyzed, indicating a low bioavailability, then water quality could be evaluated as "very good".

In addition, experimental and modelling results suggest that cationic composition of water have significant implication in the interpretation of autochthonous aquatic mosses contamination levels (Ferreira et al. 2009).

It should be emphasized that bryophytes can make an important contribution to stream metabolism and influence the distribution of key groups, such as invertebrates. Invertebrate fauna abundance is strongly correlated with bryophyte biomass, since invertebrates rely on detrital or periphyton biomass associated with the plants (Suren 1993). Most bryophyte monitoring surveys are with heavy metals, but organic contaminants were also monitored. Spatial insecticide contamination was revealed by HCH and PCBs accumulated in *Cinclidotus danubicus* moss bags (Mouvet et al. 1985). Concentration factors of 10,000 for PCBs and 300 for gamma-HCH were reported for *Platyhypnidium riparioides* (Frisque et al. 1983). Data are available that *Fontinalis antipyretica* and *F. squamosa* were affected in their photosynthetic production at 10 $\mu g\ l^{-1}$ of herbicide atrazine after 24 h and 20 days, respectively, *F. hypnoides* exhibited a much greater reduction (90 %) in net photosynthesis within 24-h at an exposure of only 2 $\mu g\ l^{-1}$ (Hofmann and Winkler 1990).

Integrated research on concordance among fish, benthic macroinvertebrates and bryophytes in Finland, showed three groups responded to different environment factors (Paavola et al. 2003). Fish community structure correlated with depth, substrate size and oxygen, macroinvertebrate community with stream size and pH, and bryophytes with water colour, nutrients and habitat variability. The last group is strongly regulated by large substrate availability and by reach-scale factors as flow variability.

According to the guidance document no 25 (European Commission 2010) transplants or so called caged organisms provide a time-integrated assessment of environmental quality over the 4-week transplantation period. In that context an Index (Palladio) for trace element alteration was proposed by Cesa et al. (2010) in the absence of autochthonous bryophytes, offering characterization of 13 elements in five classes. The metric environmental alteration (EA) based on transplanted mosses was presented according to the definition of contamination factor (CF) introduced by Mouvet (1986) for autochthonous bryophytes:

$$EA = C_{(i)}/C_{bckg}$$

EA is the ratio between the element concentration (C) at station i and the background concentration (C_{bckg}). A novel interpretation scale was applied, inspired to Mersch and Claveri (1998) and Mouvet (1986):

1. $EA < 2$ condition of naturality, no evidence of alteration (blue)
2. $2 \leq EA < 4$ suspect of alteration (green)
3. $4 \leq EA < 12$ sure alteration (yellow)
4. $12 \leq EA < 24$ severe alteration (orange)
5. $EA \geq 24$ extreme alteration (red)

Elements producing a suspected, sure, severe or extreme alteration established the condition of global environmental alteration (GEA) for each station, except for Al and Fe since their prevalent terrigenous origin.

Five classes of global environmental alteration are as follows:

– I: no evidence of environmental alteration ($EA < 2$), absolute naturality (blue)

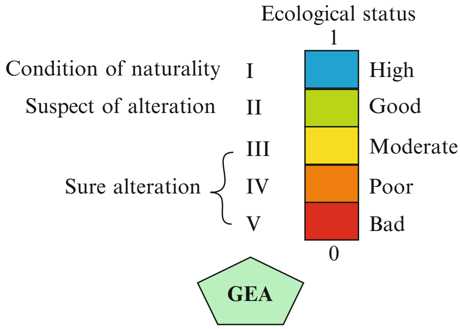

Fig. 9.1 Five classes of global environmental alteration (GEA) and relation to the classification of ecological status according to the water framework directive (WFD)

– II: suspect of alteration ($2 \leq EA < 4$) for some elements (green)
– III: sure alteration ($EA \geq 4$) for one to two elements (yellow)
– IV: sure alteration ($EA \geq 4$) for three to four elements (orange)
– V: sure alteration ($EA \geq 4$) for five or more elements (red)

Among many advantages of the Index Palladio, representation of each class of environmental alteration per station in coloured maps reflecting the WFD five ecological status classes, has to be underlined (Fig. 9.1). The Index can be easily integrated as a tool for environmental monitoring from Public Authorities.

9.4 Bryophytes: Cost-Effective and Rapid Ecological Assessment Tool?

It can be summarized that indigenous and/or transplanted bryophytes as biomonitors have important advantages: suitable sample material can be found throughout the year, low cost and rapid sampling, applicability in all freshwater habitat types, simple process of dissolution and analysis, and possibility to reveal past history (passive monitoring) of contamination or deposition during different time periods (active biomonitoring).

In unstable environment bryophytes often are the only biota representatives. As postulated by Slack and Glime (1985), many aquatic species are to some extent opportunists, since they must withstand currents and abrasion, must survive desiccation and flooding, must spread by vegetative or sexual reproduction.

Additional advantage of bryomonitoring is that most aquatic bryophytes resist freezing (up to – 10 °C) and have low temperature optima for growth (Stream Bryophyte Group 1999). Unlike vascular plants, bryophyte biomass is not grazed by most of the herbivores and thus presents opportunity for representative sample collection throughout the whole year. Moreover, bryophytes are shade-tolerant and can grow under high light intensity. Most species have very low light compensation (the light level at which net photosynthesis is zero) and light saturation points (Glime 2007).

Recent research demonstrated both in field and in laboratory that *F. antipyretica* exhibits partial desiccation tolerance which implies its significant desiccation rate and consequently survival (Cruz de Carvalho et al. 2011).

9.5 Conclusion

Aquatic bryophytes have been studied and used as biological indicators and monitors of water quality for more than 30 years. Freshwater quality can be reflected in individual species abundance as well as by the structure of bryophyte communities. Many species exhibit a high tolerance to contaminants, allowing bioaccumulation. Moreover it is well known that water sample element concentrations are often low and under the detection limits of analytical techniques. Thus bryomonitoring is used to measure bioaccumulation and bioavailability, and to assess the linkages to impacts. In the simplest experiments bioaccumulation can be characterized by measuring concentrations of metals in plant tissues and link exposure and bioaccumulation to potential impacts.

Standardization of sampling procedures in a space- and time-dependent manner and analytical techniques in aquatic bryomonitoring is needed. The number of samples should be fixed based on sampling area surface. Period of exposure time for active biomonitoring should be specified in general. Background levels and ambient metal concentrations have to be observed in parallel.

Two widespread throughout the northern hemisphere, considerably easy to recognize species are recommended in future researches: *Fontinalis antipyretica* and *Platyhypnidium riparioides* both in passive and active biomonitoring. The former is the most easily recognized aquatic species and the last is most tolerant to all kinds of pollution.

Inductively coupled plasma optical emission spectrometry (ICP-OES) could be suggested as more suitable technique in comparison with inductively coupled plasma mass spectrometry (ICP-MS) due to possibility to detect in moss tissues the macroelements (P, K, Ca and S) important for plant physiology.

Acknowledgements This review was completed through the combined efforts of the numerous scientists, who dedicated their researches on bryophytes and monitoring. Those authors have supported our interest in aquatic bryophytes and guided us. To all of them we are exceedingly grateful.

References

Aguiar FC, Feio MJ, Ferreira MT (2011) Choosing the best method for stream bioassessment using macrophyte communities: indices and predictive models. Ecol Indic 11:379–388

Augusto S, Gonzalez C, Vieira R, Máguas C, Branquinho C (2011) Evaluating sources of PAHs in urban streams based on land use and biomonitors. Environ Sci Technol 45:3731–3738

Bates JW (2008) Mineral nutrition and substratum ecology. In: Goffinet B, Shaw AJ (eds) Bryophyte biology, 2nd edn. Cambridge University Press, Cambridge, pp 315–372

Benson-Evans K, Williams PF (1976) Transplanting aquatic bryophytes to assess river pollution. J Bryol 9:81–91

Besse J-P, Geffard O, Coquery M (2012) Relevance and applicability of active biomonitoring in continental waters under the water framework directive. Trends Anal Chem 36:113–127

Birk S, Willby N (2010) Towards harmonization of ecological quality classification: establishing common grounds in European macrophyte assessment for rivers. Hydrobiologia 652:149–163

Birk S, Korte T, Hering D (2006) Intercalibration of assessment methods for macrophytes in lowland streams: direct comparison and analysis of common metrics. Hydrobiologia 566:417–430

Bruns I, Siebert A, Baumbach R, Miersch J, Günther D, Markert B, Kraußβ G-J (1995) Analysis of heavy metals and sulphur-rich compounds in the water moss *Fontinalis antipyretica* L. ex Hedw. Fresen J Anal Chem 353:101–104

Bruns I, Friese K, Markert B, Krauss G-J (1997) The use of *Fontinalis antipyretica* L. ex Hedw. as a bioindicator for heavy metals. 2. Heavy metal accumulation and physiological reaction of *Fontinalis antipyretica* L. ex Hedw. in active biomonitoring in the River Elbe. Sci Total Environ 204:161–176

Burton MAS (1986) Biological monitoring. MARC report no. 32. Monitoring and Assessment Research Centre, King's College. University of London, London

Burton MAS (1990) Terrestrial and aquatic bryophytes as monitors of environmental contaminants in urban and industrial habitats. Bot J Lin Soc 104:267–286

Burton MAS, Peterson PJ (1979) Metal accumulation by aquatic bryophytes from polluted mine streams. Environ Pollut 19:39–46

Caines LA, Watt AW, Wells DE (1985) The uptake and release of some trace metals by aquatic bryophytes in acidified waters in Scotland. Environ Pollut 10:1–18

Cairns J Jr, van der Schalie WH (1980) Biological monitoring. Part I: early warning systems. Water Res 14:1179–1196

Carballeira A, López J (1997) Physiological and statistical methods to identify background levels of metals in aquatic bryophytes: dependence on lithology. J Environ Qual 26:980–988

Carballeira A, Vázquez MD, López J (2001) Biomonitoring of sporadic acidification of rivers on the basis of release of preloaded cadmium from the aquatic bryophyte *Fontinalis antipyretica* Hedw. Environ Pollut 111:95–106

Cenci RM (2000) The use of aquatic moss (*Fontinalis antipyretica*) as monitor of contamination in standing and running waters: limits and advantages. J Limnol 60(Suppl 1):53–61

Cesa M, Bizzotto A, Ferraro C, Fumagalli F, Nimis PL (2006) Assessment of intermittent trace element pollution by moss bags. Environ Pollut 144:886–892

Cesa M, Campisi B, Bizzotto A, Ferraro C, Fumagalli F, Nimis PL (2008) A factor influence study of trace element bioaccumulation in moss bags. Arch Environ Contam Toxicol 55(3):386–396

Cesa M, Azzalini G, De Toffol V, Fontanive M, Fumagalli F, Nimis PL, Riva G (2009a) Moss bags as indicators of trace metal contamination in pre-alpine streams. Plant Biosyst 143(1):173–180

Cesa M, Bizzotto A, Ferraro C, Fumagalli F, Nimis PL (2009b) S.T.R.E.A.M., system for trace element assessment with mosses. An equation to estimate mercury concentration in freshwaters. Chemosphere 75:858–865

Cesa M, Bizzotto A, Ferraro C, Fumagalli F, Nimis PL (2010) Palladio, an index of trace element alteration for the river bacchiglione based on *Rhynchostegium riparioides* moss bags. Water Air Soil Pollut 208:59–77

Claveri B, Guérold F, Pihan JC (1995) Use of transplanted mosses and autochthonous liverworts to monitor trace metals in acidic and non-acidic headwater streams (Vosges Mountains, France). Sci Total Environ 175:235–244

Cruz de Carvalho R, Branquinho C, Marques da Silva J (2011) Physiological consequences of desiccation in the aquatic bryophyte *Fontinalis antipyretica*. Planta 234(1):195–205

Dazy M, Béraud E, Cotelle S, Meux E, Masfaraud J-F, Férard J-F (2008) Antioxidantenzyme activities as affected by trivalent and hexavalent chromium species in *Fontinalis antipyretica* Hedw. Chemosphere 73(3):281–290

Delépée R, Pouliquen H, Le Bris H (2004) The bryophyte *Fontinalis antipyretica* Hedw. bioaccumulates oxytetracycline, flumequine and oxolinic acid in the freshwater environment. Sci Total Environ 322(1–3):243–253

Demars BOL, Britton A (2011) Assessing the impacts of small scale hydroelectric schemes on rare bryophytes and lichens. Scottish natural heritage and Macaulay land use institute funded report. Scottish Natural Heritage Commissioned Report no. 421

Dietz J (1972) Die Anreicherung von Schwermetallen in submersen Pflanzen. Geawasser/Abwasser 113:269–273

Diviš P, Machát J, Szkandera R, Dočekalová H (2012) In situ measurement of bioavailable metal concentrations at the downstream on the Morava River using transplanted aquatic mosses and DGT technique. Int J Environ Res 6(1):87–94

Downes BJ, Entwisle TJ, Reich P (2003) Effects on flow regulation on disturbance frequencies and in-channel bryophytes and macroalgae in some upland streams. River Res Appl 19:27–42

Duncan MJ, Suren AM, Brown SLR (1999) Assessment of streambed stability in steep, bouldery streams: development of a new analytical technique. J N Am Benthol Soc 18(4):445–456

During HJ (1992) Ecological classification of bryophytes and lichens. In: Bates JW, Farmer AM (eds) Bryophytes and lichens in a changing environment. Clarendon, Oxford

Empain A (1976) Les bryophytes aquatiques utilisés comme traceurs de la contamination en métaux lourds des eaux douces. In: Symposium Mémoires de la Société Royale de Botanique de Belgique, 20 mars 1976, Bruxelles, 7, pp 141–156

Empain AM (1977) Ecologie des populations bryophytes aquatiques de la Meuse de la Sambre et de la Somme. Doctoral dissertation, University of Liege, Belgium

Empain AM (1988) A posteriori detection of heavy metal pollution of aquatic habitats. In: Glime JM (ed) Methods in bryology. Proceedings of bryol. method. Workshop, Mainz, Hattori Bot Lab, Nichinan, pp 213–220

Empain A, Lambinon J, Mouvet C, Kirchmann R (1980) Utilisation des bryophytes aquatiques et subaquatiques comme indicateurs biologiques de la qualité des eaux courantes. In: Pesson P (ed) La Pollution des Eaux Continentales, 2nd edn. Gauthier-Villars, Paris, pp 195–223

Englund G, Jonsson B-G, Malmqvist B (1997) Effects of flow regulation on bryophytes in North Swedish rivers. Biol Conserv 79:79–86

European Commission (2010) Guidance on chemical monitoring of sediment and biota under the water framework directive. Guidance document no 25. Technical report 2010.3991. Common implementation strategy for the water framework directive. European Commission, Brussels

European Union (2000) Directive 2000/60/EC of the European Parliament and of the council of 23 October 2000 establishing a framework for community action in the field of water policy. Off J Eur Commun L 327:1–72

European Union (2008) Directive 2008/105/EC of the European Parliament and of the council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. Off J Eur Commun L 348:84–97

Feio MJ, Aguiar FC, Almeida SFP, Ferreira MT (2012) AQUAFLORA: a predictive model based on diatoms and macrophytes for streams water quality assessment. Ecol Indic 18:586–598

Ferreira D, Ciffroy P, Tusseau-Vuillemin M-H, Garnier C, Garnier J-M (2009) Modelling exchange kinetics of copper at the water-aquatic moss (*Fontinalis antipyretica*) interface: influence of water cationic composition (Ca, Mg, Na and pH). Chemosphere 74:1117–1124

Figueira R, Ribeiro T (2005) Transplants of aquatic mosses as biomonitors of metals released by a mine effluent. Environ Pollut 136:293–301

Frisque G, Galoux M, Bernes A (1983) Accumulation de deux micropolluants (les plychlorobiphényles et le gammaHCH) par des bryophytes aquatiques de la Meuse. Meded Fac Landbouwwet Rijksuniv Gent 48:971–983

García-Álvaro MA, Martínez-Abaigar J, Núñez-Olivera E, Beaucourt N (2000) Element concentrations and enrichment ratios in the aquatic moss *Rhynchostegium riparioides* along the River Iregua (La Riojia, Northern Spain). Bryologist 103(3):518–533

Gecheva G, Yurukova L, Cheshmedjiev S, Ganeva A (2010) Distribution and bioindication role of aquatic bryophytes in Bulgarian rivers. Biotechnol Biotechnol Equip 24/2010/SE, 24(2): 164–170

Gecheva G, Yurukova L, Ganeva A (2011) Assessment of pollution with aquatic bryophytes in Maritsa River (Bulgaria). Bull Environ Contam Toxicol 87(4):480–485

Gimeno C, Puche F (1999) Chlorophyll content and morphological changes in cellular structure of *Rhynchostegium riparioides* (Hedw.) Card. (Brachytheciaceae, Musci) and *Fontinalis hypnoides* Hartm. (Fontinalaceae, Musci) in response to water pollution and transplant containers on Palancia river (East Spain). Nova Hedwigia 68:197–216

Glime JM (2003) *Fontinalis*. Available via http://www.bio.umass.edu/biology/conn.river/fontinal.html. Accessed 5 July 2012

Glime JM (2007) Bryophyte ecology. Vol 1. Physiological ecology. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. Available via http://www.bryoecol.mtu.edu. Accessed 13 July 2012

Glime JM, Vitt DH (1987) A comparison of bryophyte species diversity and structure of montane streams and stream banks. Can J Bot 65:1824–1837

Gonçalves EPR, Boaventura RAR, Mouvet C (1992) Sediments and aquatic mosses as pollution indicators for heavy metals in the Ave river basin (Portugal). Sci Total Environ 114:7–24

Grolle R, Long DG (2000) An annotated check-list of the Hepaticae and Anthocerotae of Europe and Macaronesia. J Bryol 22:103–140

Gurnell AM, O'Hare JM, O'Hare MT, Dunbar MJ, Scarlett PM (2009) An exploration of associations between assemblages of aquatic plant morphotypes and channel geomorphological properties within British rivers. Geomorphology 116(1–2):135–144

Haseloff HP (1982) Bioindikatoren und bioindikation. Biol Unserer Zeit 12:20–26

Haslam SM (1982) A proposed method for monitoring river pollution using macrophytes. Environ Technol Lett 3:19–34

Hertz J (1991) Bioindicators for monitoring heavy metals in the environment. In: Merian E (ed) Metals and their compounds in the environment. VCH Verlagsgesellschaft mbH, Weinheim, New York, Basel, Cambridge, pp 221–231

Hill MO, Bell N, Bruggeman-Nannenga MA, Brugués M, Cano MJ, Enroth J, Flatberg KI, Frahm J-P, Gallego MT, Garilleti R, Guerra J, Hedenäs L, Holyoak DT, Hyvönen J, Ignatov MS, Lara F, Mazimpaka V, Muñoz J, Söderström L (2006) An annotated checklist of the mosses of Europe and Macaronesia. J Bryol 28(3):198–267

Hime S, Bateman IJ, Posen P, Hutchins M (2009) A transferable water quality ladder for conveying use and ecological information within public surveys. CSERGE working paper EDM 09–01, University of East Anglia

Hofmann A, Winkler S (1990) Effects of atrazine in environmentally relevant concentrations on submerged macrophytes. Arch Hydrobiol 118:69–80

Hongve D, Brittain JE, Bjørnstad HE (2002) Aquatic mosses as a monitoring tool for ^{137}Cs contamination in streams and rivers – a field study from central southern Norway. J Environ Radioact 60:139–147

Hunt GJ (1983) Radioactivity in surface and coastal waters of the British Isles, 1981. Aquatic environment monitoring report no. 9. Ministry of Agriculture, Fisheries and Food Lowestoft, Suffolk

Jackson PP, Bobinson NJ, Whitton BA (1991) Low molecular weight metal complexes in the freshwater moss *Rhinchostegium riparoides* exposed to elevated concentrations of Zn, Cu, Cd, and Pb in the laboratory and field. Environ Exp Bot 31(3):359–366

Janauer G, Dokulil M (2006) Macrophytes and algae in running waters. In: Ziglio G, Siligardi M, Flaim G (eds) Biological monitoring of rivers. Wiley, Chichester, pp 89–109

Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69:373–386

Kelly MG, Whitton BA (1989) Interspecific differences in Zn, Cd and Pb accumulation by freshwater algae and bryophytes. Hydrobiologia 175:1–11

Kelly MG, Girton C, Whitton BA (1987) Use of moss-bags for monitoring heavy metals in rivers. Water Res 21(11):1429–1435

Kirchmann R, Lambinon J (1973) Plants as bioindicators of the contamination of a river by the effluents of a PWR nuclear power station. Assessment of the radioactive releases of the Sena reactor through aquatic and ripicolous plants of the river Meuse. Bull Soc Roy Bot Belg 106:187–201

Kovács M (1992) Biological indicators of water pollution. In: Kovács M (ed) Biological indicators in environmental protection. Akadémiai Kiadó, Budapest, pp 120–130

Kovács M, Podani J (1986) Bioindication: a short review on the use of plants as indicators of heavy metals. Acta Biol Hung 37:19–29

Lang P, Murphy KJ (2012) Environmental drivers, life strategies and bioindicator capacity of bryophyte communities in high-latitude headwater streams. Hydrobiologia 679:1–17

Lee J, Johnson-Green P, Lee EJ (2002) Chemical analysis of transplanted aquatic mosses and aquatic environment during a fish kill on the Chungnang River, Seoul, Korea. Korean J Biol Sci 6:215–219

Lingsten L (1991) Levels of heavy metals in aquatic mosses in acidified water bodies. Verh Int Ver Limnol 24:2228–2230

López J, Carballeira A (1989) A comparative study of pigment contents and response to stress in five aquatic bryophytes. Lindbergia 15:188–194

López J, Carballeira A (1993) Interspecific differences in metal bioaccumulation and plant-water concentration ratios in five aquatic bryophytes. Hydrobiologia 263:95–107

López J, Vazquez MD, Carballeira A (1994) Stress responses and metal exchange kinetics following transplant of the aquatic moss *Fontinalis antipyretica*. Freshwater Biol 32:185–198

M A F F (Ministry of Agriculture, Fisheries and Food) (1967) Radioactivity of surface and coastal waters of the British Isles. Report FRL I. Ministry of Agriculture, Fisheries and Food, Lowestoft, Suffolk

Manning WJ, Feder WA (1980) Biomonitoring air pollutants with plants. Applied Science Publishers, London, p 142

Markert B (1991) Inorganic chemical investigation in the forest biosphere reserve near Kalinin, USSR. I. Mosses and peat profiles as bioindicators for different chemical elements. Vegetatio 95:127–135

Markert B (1996) Instrumental element and multi-element analyses of plant samples. Wiley, Chichester, 296

Markert B (2008) From biomonitoring to integrated observation of the environment – the multi-markered bioindication concept. Ecol Chem Eng S 15(3):315–333

Markert BA, Breure AM, Zechmeister HG (2003) Definitions strategies and principles for bioindication/biomonitoring of the environment. In: Markert BA, Breure AM, Zechmeister HG (eds) Bioindicators and biomonitors, Elsevier Science Ltd, Oxford, pp 3–39

Martin MH, Coughtrey PJ (1982) Biological monitoring of heavy metal pollution. Applied Science Publishers, London/New York, p 475

Martinez-Abaigar J, Núñez-Olivera E (1998) Ecophysiology of photosynthetic pigments in aquatic bryophytes. In: Bates JW, Ashton NW, Duckett JG (eds) Bryology for the twenty-first century. British Bryological Society, Leeds, pp 277–292

McLean RO, Jones K (1975) Studies of tolerance to heavy metals in the flora of the rivers Ystwyth and Clarach, Wales. Freshwater Biol 5:431–444

Mersch J, Claveri B (1998) Les bryophytes aquatiques comme outil de surveillance de la contamination des eaux courantes par les micropolluants métalliques: modalités d'interprétation des données. Etude Inter-Agences 55, France

Mersch J, Johansson L (1993) Transplanted aquatic mosses and freshwater mussels to investigate the trace metal contamination in the rivers Meurthe and Plaine, France. Environ Technol 14:1027–1036

Mersch J, Pihan JC (1993) Simultaneous assessment of environmental impact on condition and trace metals availability in zebra mussels *Dreissena polymorpha* transplanted into the Wilz River, Luxembourg. Comparison with the aquatic moss *Fontinalis antipyretica*. Arch Environ Contam Toxicol 25:353–364

Mersch J, Reichard M (1998) In situ investigation of trace metal availability in industrial effluents using transplanted aquatic mosses. Arch Environ Contam Toxicol 34:336–341

Mishev P, Damyanova A, Yurukova L. (1996) Mosses as biomonitors of airborne pollution in the northern part of Rila Mountain. Part II. Radioactivity of mosses. In: Carbonnel JP, Stamenov JN (eds) Observatoire de montagne de Moussala – OM2, 4. Sofia, pp 137–141

Mouvet C (1984) Accumulation of chromium and copper by the aquatic moss *Fontinalis antipyretica* L. ex Hedw. transplanted in a metal-contaminated river. Environ Technol Lett 5:541–548

Mouvet C (1986) Métaux lourds et Mousses aquatiques, synthèse méthodologique. Université de Metz – Laboratoire d'Ecologie, rapport de contrat à l'Agence de l'Eau Rhin-Meuse et l'Agence de l'Eau Rhône-Méditerranée-Corse, France

Mouvet C, Galoux M, Bernes A (1985) Monitoring of polychlorinated biphenyls (PCBs) and hexachlorocyclohexanes (HCH) in freshwater using aquatic moss *Cinclidotus danubicus*. Sci Total Environ 44:253–267

Mouvet C, Pattée E, Cordebar P (1986) Utilisation des mousses aquatiques pour I'identification et la localisatiom précise de sources de pollution métallique multiforme. Acta Oecol 7(1):77–91

O'Hare MT, Baattrup-Pedersen A, Nijboer R, Szoszkiewicz K, Ferreira T (2006) Macrophyte communities of European streams with altered physical habitat. Hydrobiologia 566:197–210

O'Hare JM, O'Hare MT, Gurnell AM, Dunbar MJ, Scarlett PM, Laize C (2011) Physical constraints on the distribution of macrophytes linked with flow and sediment dynamics in British rivers. River Res Appl 27:671–683

Paavola R, Muotka T, Virtanen R, Heino J, Kreivi P (2003) Are biological classifications of headwater streams concordant across multiple taxonomic groups? Freshwater Biol 48:1912–1923

Papp B, Rajczy M (1995) Changes of bryophyte vegetation and habitat conditions along a section of the river Danube in Hungary. Cryptog Helv 18:95–105

Papp B, Rajczy M (1998a) The role of bryophytes as bioindicators of water quality in the Danube. Verh Int Ver Theor Limnol 26:1254–1256

Papp B, Rajczy M (1998b) Investigations on the condition of bryophyte vegetation of mountain streams in Hungary. J Hattori Bot Lab 84:81–90

Papp B, Tsakiri E, Babalonas D (1998) Bryophytes and their environmental conditions at Enipeas (Mt Olympos) and Lycorrema (Mt Ossa) streams (Greece). In: Tsekos I, Moustakas M (eds) Progress in botanical research: proceedings of 1st Balkan Botanical Congress, Dordrecht, Boston, pp 129–132

Peñuelas J (1984) Pigments of aquatic mosses of the River Muga, NE Spain, and their response to water pollution. Lindbergia 10:127–132

Pirc S (2003) Aquatic mosses as sampling medium in geochemistry. RMZ-Mater Geoenviron 50(4):735–763

Rasmussen G, Andersen S (1999) Episodic release of arsenic, copper and chromium from awood preservation site monitored by transplanted aquatic moss. Water Air Soil Pollut 109:41–52

Raven PJ, Holmes NTH, Dawson FH, Fox PJA, Everard M, Fozzard I, Rouen KJ (1998) River habitat quality: the physical character of rivers and streams in the UK and the Isle of Man. Environment Agency, Bristol

Roy S, Sen CK, Hanninen O (1996) Monitoring of polycyclic aromatic hydrocarbons using moss bags: bioaccumulation and responses of antioxidant enzymes in *Fontinalis antipyretica* Hedw. Chemosphere 32:2305–2315

Samecka-Cymerman A (1991) Contents of arsenic, vanadium, germanium and other elements in aquatic liverwort *Scapania undulata* (L.) Dum. growing in the Sudety mountains. Pol Arch Hydrobiol 38(1):79–84

Samecka-Cymerman A, Kempers AJ (1992) Anomalous elemental composition of aquatic bryophytes near barite zones in the Sowie Mts. (Poland). J Geochem Explor 43:213–221

Samecka-Cymerman A, Kempers AJ (1993) *Scapania undulata* (L.) Dum. and other aquatic bryophytes as indicators of mineralization in Poland. J Geochem Explor 46:325–334

Samecka-Cymerman A, Kempers AJ (1994) Macro- and microelements in bryophytes from serpentinite and greenstone streams. Pol Arch Hydrobiol 41:431–449

Samecka-Cymerman A, Kempers AJ (1999) Background concentrations of heavy metals in aquatic bryophytes used for biomonitoring in basaltic areas (a case study from central France). Environ Geol 39(2):117–122

Samecka-Cymerman A, Kolon K, Kempers AJ (2002) Heavy metals in aquatic bryophytes from the Ore Mountains (Germany). Ecotoxicol Environ Saf 52:203–210

Samecka-Cymerman A, Kolon K, Kempers AJ (2005) A comparison of native and transplanted *Fontinalis antipyretica* Hedw. as biomonitor of water polluted with heavy metals. Sci Total Environ 341:97–107

Samecka-Cymerman A, Stankiewicz A, Kolon K, Kempers AJ (2007) Self-organizing feature map (neural networks) as a tool in classification of the relations between chemical composition of aquatic bryophytes and types of streambeds in the Tatra national park in Poland. Chemosphere 67:954–960

Samecka-Cymerman A, Wojtun B, Kolon K, Kempers AJ (2011) *Sanionia uncinata* (Hedw.) Loeske as bioindicator of metal pollution in polar regions. Polar Biol 34:381–388

Samson G, Tessier L, Vaillancourt G, Pazdernik L (1998) The use of a first order kinetic model for assessing the cadmium bioaccumulation process by two aquatic mosses. Available via http://www.wmsym.org/archives/1998/html/sess18/18-20/18-20.htm. Assessed 25 June 2012

Satake K, Takamatsu T, Soma M, Shibata K, Nishikawa M, Say PJ, Whitton BA (1989) Lead accumulation and location in the shoots of the aquatic liverwort *Scapania undulata* (L.) Dum. in stream water at Greenside Mine, England. Aquat Bot 33:111–122

Say PJ, Whitton BA (1983) Accumulation of heavy metals by aquatic mosses. I. *Fontinalis antipyretica*. Hydrobiologia 100:245–260

Say PJ, Harding JPC, Whitton BA (1981) Aquatic mosses as monitors of heavy metal contamination in the river Etherow, Great Britain. Environ Pollut 2:295–307

Scarlett P, O'Hare M (2006) Community structure of in-stream bryophytes in English and Welsh rivers. Hydrobiologia 553:143–152

Schaumburg J, Schranz C, Foerster J, Gutowski A, Hofmann G, Meilinger P, Schneider S, Schmedtje U (2004) Ecological classification of macrophytes and phytobenthos for rivers in Germany according to the water framework directive. Limnologica 34:283–301

Siebert A, Bruns I, Krauss G-J, Miersch J, Markert B (1996) The use of the aquatic moss *Fontinalis antipyretica* L. ex Hedw. as a bioindicator for heavy metals. 1. Fundamental investigations into heavy metal accumulation in *Fontinalis antipyretica* L. ex Hedw. Sci Total Environ 177:137–144

Slack NG, Glime J (1985) Niche relationship of mountain stream bryophytes. Bryologist 88:1–18

Spitale D (2009) Spatial distribution of bryophytes along a moisture gradient: an approach using photosynthetic pigments as indicators of stress. Ecol Res 24:1279–1286

Steinnes E, Rühling Å, Lippo H, Mäkinen A (1997) Reference materials for large-scale metal deposition surveys. Accred Qual Assur 2:243–249

Steubing L (1976) Niedere und höhere pflanzen als indikatoren für immissionsbelastungen. Daten Dok Umweltschutz 19:13–27

Stöcker G (1980) Methodishe und theoretishe Grundlangen der Bioindikation (Bioindikation 1). In: Schubert R, Schuh J (eds) Martin Luther Universität, Halle, pp 10–21

Stream Bryophyte Group (1999) Roles of bryophytes in stream ecosystems. J N Am Benthol Soc 18(2):151–184

Suren A (1993) Bryophytes and associated invertebrates in first-order alpine streams of Arthur's Pass, New Zealand. N Z J Mar Freshwater Res 27:479–494

Suren AM (1996) Bryophyte distribution patterns in relation to macro-, meso-, and micro-scale variables in South Island, New Zealand streams. N Z J Mar Freshwater 30:501–523

Suren AM, Duncan MJ (1999) Rolling stones and mosses: effect of substrate stability on bryophyte communities in streams. J N Am Benthol Soc 18(4):457–467

Suren AM, Smart GM, Smith RA, Brown SLR (2000) Drag coefficients of stream bryophytes: experimental determinations and ecological significance. Freshwater Biol 45:309–317

Szoszkiewicz K, Ferreira T, Korte T, Baattrup-Pedersen A, Davy-Bowker J, O'Hare M (2006) European river plant communities: the importance of organic pollution and the usefulness of existing macrophyte metrics. Hydrobiologia 566:211–234

Tyler G (1972) Heavy metals pollute nature may reduce productivity. Ambio 1:57–59

Tyler G (1990) Bryophytes and heavy metals: a literature review. Bot J Linn Soc 104:231–253

Vanderpoorten A, Goffinet B (2009) Introduction to bryophytes. Cambridge University Press, Cambridge

Vanderpoorten A, Palm R (1998) Canonical variables of aquatic bryophyte combinations for predicting water trophic level. Hydrobiologia 386:85–93

Vanderpoorten A, Klein J-P, Stieperaere H, Tremolieres M (1999) Variations of aquatic bryophyte assemblages in the Rhine Rift related to water quality. 1. The Alsatian Rhine floodplain. J Bryol 21:17–23

Vázquez MD, López L, Carballeira A (1999) Uptake of heavy metals to the extracellular and intracellular compartments in three species of aquatic bryophyte. Ecotoxicol Environ Saf 44:12–24

Vázquez MD, Fernández JA, López L, Carballeira A (2000) Effects of water acidity and metal concentration on accumulation and within-plant distribution of metals in the aquatic bryophyte *Fontinalis antipyretica*. Water Air Soil Pollut 120:1–19

Vázquez MD, Wappelhorst O, Markert B (2004) Determination of 28 elements in aquatic moss *Fontinalis antipyretica* Hedw. and water from the upper reaches of the river Nysa (CZ, D), by ICP-MS, ICP-OES and AAS. Water Air Soil Pollut 152:153–172

Vázquez MD, Fernández JÁ, Real C, Villares R, Aboal JR, Carballeira A (2007) Design of an aquatic biomonitoring network for an environmental specimen bank. Sci Total Environ 388(1–3):357–371

Vieira AR, Gonzalez C, Martins-Louão MA, Branquinho C (2009) Intracellular and extracellular ammonium ($NH_4{}^+$) uptake and its toxic effects on the aquatic biomonitor *Fontinalis antipyretica*. Ecotoxicology 18:1087–1094

Wehr JD, Whitton BA (1983a) Accumulation of heavy metals by aquatic mosses. 2: *Rhynchostegium riparioides*. Hydrobiologia 100:261–284

Wehr JD, Whitton BA (1983b) Accumulation of heavy metals by aquatic mosses. 3: seasonal changes. Hydrobiologia 100:285–291

Wehr JD, Empain A, Mouvet C, Say PJ, Whitton BA (1983) Methods for processing aquatic mosses used as monitors of heavy metals. Water Res 17(9):985–992

Whitton BA (2003) Use of plants for monitoring heavy metals in freshwaters. In: Ambasht RS, Ambasht NK (eds) Modern trends in applied aquatic ecology. Kluwer Academic/Plenum Publishers, New York, pp 43–63

Whitton BA, Say PJ, Jupp BP (1982) Accumulation of zinc, cadmium and lead by the aquatic liverwort *Scapania*. Environ Pollut 3:299–316

Yurukova L, Gecheva G (2003) Active and passive biomonitoring using *Fontinalis antipyretica* in Maritsa River, Bulgaria. J Balkan Ecol 6(4):390–397

Yurukova L, Gecheva G (2004) Biomonitoring in Maritsa River using aquatic bryophytes. J Environ Prot Ecol 5(4):729–735

Yurukova LD, Ganeva AS, Damyanova AA (1996) Aquatic bryophytes as bioconcentrators of macro- and microelements. In: Carbonnel JP, Stamenov JN (eds) Observatorie de montagne de Moussala, OM2, 4. Bulgarian Academy of Sciences, Sofia, pp 127–136

Yurukova L, Damyanova A, Ivanov K, Janminchev V (1997) Bioaccumulation capacity of *Fontinalis antipyretica* from Musalenska Bistrica River, Rila Mountain. In: Carbonnel JP, Stamenov JN (eds) Observatorie de montagne de Moussala, OM2, 6. Bulgarian Academy of Sciences, Sofia, pp 77–86

Zhou Q, Zhang J, Fu J, Shi J, Jiang G (2008) Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Anal Chim Acta 606:135–150