

## Review

# Monitoring river water quality with transplanted bryophytes: A methodological review



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## ABSTRACT

The aim of this literature review, which considers 47 articles published between 1989 and 2015, is to ascertain the current status of the active biomonitoring technique for assessing water quality and to evaluate the degree to which different aspects of the method have been standardized. Use of the tool is largely limited to Europe (83% of the articles reviewed). The technique has been used to biomonitor inorganic contaminants (in 96% of the studies) and, to a lesser extent, organic contaminants (4% of the studies). Only 25% of the articles concern methodological aspects of the technique. Moreover, most authors (78%) have only published one article on the topic, and many different protocols have been used in the various studies. As a result, the technique is not standardized, which hampers comparison of the results of different studies. We propose a protocol that would facilitate use of the technique for routine monitoring of the quality of river waters.

## 1. Introduction

River waters (mainly those associated with urban and industrial areas) are affected by different anthropogenic activities that could lead to deterioration in water quality (e.g. increasing levels of pollutants or acidity) and also in the ecological status. There is also a growing demand for good quality water to be available for specified uses (e.g. as drinking water, and in agriculture). In response to this demand, different authorities have developed specific legislative measures to protect inland freshwaters (e.g. European Water Framework: [Directive 2000/60/CE](#)).

Nowadays, the main tool used to assess water contamination is chemical monitoring, which provides information about the levels of different pollutants in the water column (e.g. heavy metals and organic compounds). Nevertheless, the data obtained in this approach reflect the concentration of pollutants at the time of sampling, but not episodic or intermittent pollution events ([Greenwood and Roig, 2006](#)). To resolve this problem, various biological matrices (e.g. algae, bryophytes, fishes, molluscs and macro-invertebrates) have been used to assess water quality. Bryophytes show important advantages relative to the other options for the following reasons: (i) pollutant uptake by bryophytes is mainly passive and scarcely affected by biotic factors; (ii) bryophytes usually have long live-cycles; (iii) they are easily sampled, identified and transplanted; (iv) they are resistant to water pollution and adverse environmental conditions; (v) they do not need to be feed;

and (vi) they are non-invasive species. Using bryophytes to biomonitor water quality (also called ‘bryomonitoring’) also enables the simultaneous monitoring of a large number of compounds (i.e. organic, inorganic and even radioactive compounds) by analysis of a single sample as well as evaluation of water quality at small (e.g. around pollutant sources) and large scales (e.g. regional). In this respect, two types of bryomonitoring are clearly differentiated: (i) passive, using specimens growing naturally in an area (for reviews, see [Whitton, 2003](#); [Gecheva and Yurukova, 2014](#); [Debén et al., 2015](#)); and (ii) active, by transplanting plants from other locations. For active bryomonitoring, samples are collected from relatively unpolluted habitats and are then cleaned, selected and pre-treated before being exposed in a different environment.

The use of transplanted bryophytes resolves various problems associated with the use of native specimens. Thus, active bryomonitoring can be used in sites where native bryophytes are scarce or absent. It also eliminates possible phenotypic or genotypic adaptation of native plants to contaminants in polluted areas. In addition, it improves the temporal interpretation of results because the duration of the exposure period is known ([Debén et al., 2015](#)). Finally, it is also possible to assess the magnitude of the pollution because the initial concentrations of elements in the transplants are known. However, until now the use of this tool has been restricted to scientific research, and it has not been officially used by environmental authorities to assess the level of pollution in river waters. One of the main causes of the limited use of the

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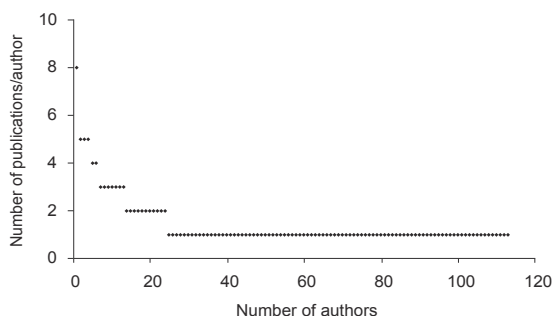


Fig. 1. Number of articles published by each author on active biomonitoring of the quality of river waters with aquatic bryophytes.

technique is the lack of a standardized method and a well-defined protocol.

In this review, after describing the current status of the methodology, we will assess the degree of standardization of each of the steps involved in active bryomonitoring of river water and we will identify the aspects that require further research. The final aim is to propose a protocol that would enable the routine use of bryophyte transplants for assessing water quality in river ecosystems.

## 2. Current trends in the active biomonitoring of river water

In this review paper, we provide a critical evaluation of the methods used in 47 articles concerning the active biomonitoring of river waters. We selected articles that quantified the concentrations of contaminants in transplanted bryophytes, as well as those involving experiments related to methodological aspects. The articles were all published between 1989 and 2015 and were located using the SciVerse SCOPUS online tool ([www.scopus.com](http://www.scopus.com)).

The review highlights the low degree of standardization of the technique. Most authors (78%) have only published one article on the topic (Fig. 1), and many of the protocols have been used on only one occasion or by only one research group. However, the main aim of most of the studies considered (79%) was to biomonitor contamination (Fig. 2), and studies aimed at establishing a standardized method by investigating particular aspects of the method were much less abundant (22%). In fact, only 12% of the authors have published more than two articles on the topic and only 5 of these authors have investigated some aspect of the methodology (i.e. López et al., 1994; Mersch and Reichard, 1998; Vázquez et al., 2000b; Martins et al., 2010; Cesa et al., 2011, 2015). The huge variability in methods hampers comparison of the results obtained in different studies and sometimes restricts the conclusions that can be reached. Finally, use of the technique is mainly limited to Europe (80% of the articles reviewed, see Fig. 3).

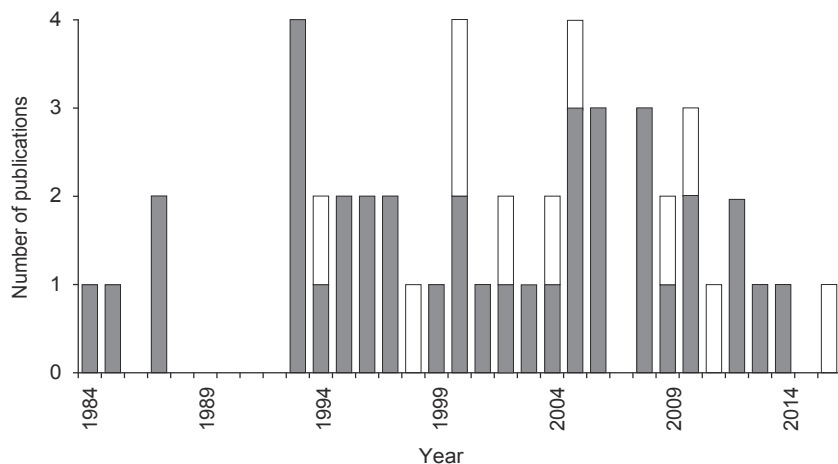


Fig. 2. Number of articles published in different years on the use of transplanted aquatic bryophytes to biomonitor water quality. White bars: number of articles concerning methodological aspects; grey bars: number of articles involving use of the technique to monitor contamination.

Most of the studies (96%) measured the concentrations of inorganic contaminants and only a small proportion measured organic contaminants (4%). The types of elements most frequently analysed are heavy metals (Zn, Pb and Cu, in more than 70% of the studies), metalloids and some nutrients (Fig. 4). Some anionic elements (such as P and S; e.g. Yurukova and Gecheva, 2003), persistent organic pollutants (such as PAHs and PCBs; e.g. Roy et al., 1996) and even some radioactive isotopes (such as Cs<sup>137</sup>; e.g. Hongve et al., 2002) have also been considered.

The present review considers four key aspects in relation to standardization of the technique used for active biomonitoring of water quality with bryophytes: (i) selection and preparation of the bryophytes; (ii) preparation of the transplants; (iii) exposure of the transplants; and (iv) post-exposure treatments. As well as reviewing and discussing the literature consulted, we also consider whether each different stage of the process can be standardized or further research is required.

## 3. Selection and preparation of the bryophytes

Use of a standardized sample preparation procedure will ensure that comparable results are obtained. For this purpose, the following aspects must be taken into account: (i) selection of the species used as the biomonitoring agent; (ii) sample collection; (iii) selection of the material for transplants and (iv) pre-exposure treatments.

### 3.1. Selection of species used as biomonitoring agent

This is one of the key aspects that must be considered to enable valid comparison of the information obtained at different sampling stations (SS). After grouping the diverse synonyms reported in the literature, in accordance with Hill et al. (2006), we found that 11 species have been used and that 55% of these have only been used on one occasion. Most of the bryophytes used are mosses, although liverworts have also been used (e.g. Engleman and McDuffett, 1996; Thiébaud et al., 2008). The frequency with which species of aquatic bryophytes have been used worldwide is summarised in Fig. 3. The moss *Fontinalis antipyretica* Hewd. has been used in 55% of the studies reviewed and *Platyhypnidium riparioides* (Hewd.) Dixon in 25% of the studies (note that according to Hill et al. (2006), *Rhynchostegium riparioides* (Hedw.) Cardot is a synonym of this species). These species are probably used because they are widely distributed, relatively large and easy to identify and handle (Martinez-Abaigar et al., 2002). The third most frequently used species is *Hygrophypnum ochraceum* (Turner ex Wilson) Loeske (in 9% of the studies).

When different species are used in the same study, interspecific differences in the uptake capacity must be taken into account on

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