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Review

Biomonitoring coastal environments with transplanted macroalgae: A methodological review

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ABSTRACT

The use of macroalgae transplants is a recent technique used in pollution biomonitoring studies in marine ecosystems. Only 60 articles published between 1978 and 2017 reported the use of this environmental tool for the active biomonitoring of inorganic pollutants and nutrients worldwide. In this review paper, we evaluated studies on this topic in relation to the development of methodological aspects of the technique and the degree of standardization of the protocols used. On the basis of findings of this review, we conclude that the technique is not yet standardized and that uniformisation of protocols is required to enable comparison of the results of different studies. We propose a new protocol for applying the technique, in which each suggestion has been carefully and rigorously compared with the relevant findings reported in the available literature.

1. Introduction

In recent decades, human activities have caused unprecedented environmental changes in coastal areas and estuarine ecosystems (Förstner and Wittmann, 2012). The growing human population and associated increasing levels of pollution - mainly from industrial, urban, agricultural and aquaculture sources - are leading to the deterioration of one of the most productive habitats worldwide (Williams, 1996; Islam and Tanaka, 2004). In parallel with these pressures, there is growing concern about the quality of coastal waters. In an attempt to respond to this demand, legislative measures to improve water quality have recently emerged. Such is the case of the European Water Framework Directive (WFD, 2000/60/EC), developed by the European Community in order to assess the ecological status of the water bodies, including groundwater, streams, lakes, estuaries and coastal waters; and the Marine Strategy Framework Directive (MSFD, 2008/56/WE), with the aim to achieving a good environmental status of the marine waters, habitats and resources in Europe by establishing monitoring programmes (Borja et al., 2010; Law et al., 2010). A series of measures and programmes for managing and evaluating coastal waters in the US has been proposed by the Environmental Protection Agency (EPA): the Clean Water Act (U.S.EPA, 1972), which regulates the discharge of pollutants into the waters and treatment of wastewater; the National Estuary Program (U.S.EPA, 1987), focused on maintaining water quality and ecological integrity of estuaries of national significance; and the Marine Protection, Research, and Sanctuaries Act (U.S.EPA, 1988), which protects the oceans from dumping.

Traditional analytical techniques for assessing pollution in marine environments (*e.g.* organic pollutants and heavy metals) involve the physico-chemical analysis of water and sediment. Nevertheless, in addition to the difficulties in analytical determinations (Phillips, 1977, 1990; Luoma, 1990; Rainbow, 1995), none of these approaches provide direct information about the fraction of pollutant that reaches the marine trophic chains or about the bioconcentration capacity of marine organisms. They also do not provide good temporal representation on short time scales.

The application of biomonitoring techniques with macroalgae has therefore emerged as a useful tool for environmental management (for review see García-Seoane et al., 2018). Two types of biomonitoring studies using macroalgae to evaluate water pollution are clearly differentiated in the literature: i) passive biomonitoring with resident species, *i.e.* collection of native specimens naturally growing in an area (see *e.g.* Rainbow and Phillips, 1993; Topçuoğlu et al., 2004; García-Seoane et al., 2018), and ii) active biomonitoring by the use of transplantation techniques (individuals are transferred to other locations). Although the latter approach has been less frequently implemented, it provides several advantages over the use of native macroalgae. For example, the use of native species may be conditioned by their

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restricted distributions. In transplantation techniques, macroalgae can be transferred to desirable locations where native specimens are non-existent or scarce, thus ensuring greater coverage of the area to be monitored (Fong et al., 1998; Fernandes et al., 2012; Jona-Lasinio et al., 2015). Furthermore, as suggested by Alquezar et al. (2013), the use of translocation techniques, rather than conventional monitoring, reduces the spatial variability of macroalgae populations because all sites contain algae from the same site of origin. Moreover, it eliminates the possible phenotypic or genotypic adaptation of native plants in polluted areas, due to the development of differential levels of tolerance to pollution, which may affect interpretation of the data (Nielsen et al., 2003; Hédouin et al., 2008; Ritter et al., 2010; Brown et al., 2012; Sáez et al., 2015). The use of transplants also improves the temporal interpretation of results because the period of exposure is known (Vizzini and Mazzola, 2004; Jona-Lasinio et al., 2015; Sáez et al., 2015), and the method can be used as a rapid and cost-efficient means of biomonitoring in estuarine environments (Gröcke et al., 2017).

However, despite the potential usefulness, the use of algal transplants is not as widespread as passive biomonitoring (García-Seoane et al., 2018), and the method has been limited to research studies and not yet been officially implemented by authorities for the environmental assessment of coastal pollution. As with active biomonitoring in terrestrial ecosystems (*i.e.* using aquatic or terrestrial mosses transplants, see *e.g.* Ares et al., 2012 and Debén et al., 2017), one of the main causes of this limitation is the lack of a standardized methodology. The need for well-defined protocols, in which appropriate experimental conditions have been established, based on methodological research (species of macroalgae, distances from pollution focus, incubation depth, *etc.*) has already been highlighted (García-Sanz et al., 2010).

To ensure the quality and scientific rigour of the publications, methods used must be agreed on prior to the implementation of any technique, otherwise the validity of the results can be questioned. The aim of this literature review is to evaluate the essential aspects of the methodology and the degree of harmonization of each of the steps involved in biomonitoring coastal environments with transplanted specimens of macroalgae. On the basis of the conclusions reached, the methods used to date will be evaluated and we propose a protocol to enable wider implementation of the technique.

2. Current trends in the use of macroalgae in the active biomonitoring of coastal waters

In this review article, a total of 60 papers concerning the use of transplanted macroalgae in active biomonitoring of marine environments were evaluated in relation to the development of methodological aspects of the technique. The articles were published between 1978 and 2017 and most were located using the SciVerse SCOPUS online tool (-http://www.scopus.com/-). Only articles written in English and involving detection and monitoring of pollutant levels and/or experiments related to methodological issues were considered.

According to the literature consulted, active biomonitoring with macroalgae was initiated in the late 1970s, and the number of related articles increased greatly at the turn of the century (Fig. 1). Although the method is used on 4 continents (Fig. 2), most authors (78%) have only published one article (Fig. 3) and very few researchers are authors of 2 or more articles on the topic (research groups from Australia, Denmark and the US, among others). Use of the technique is mainly limited to Europe (50% of the articles reviewed, see Fig. 2), highlighting the low degree of specialization in this area and explaining why many of the protocols have been used on only one occasion or why many research groups use their own limited methodologies.

As shown in Fig. 1, the main aim of most of the studies (92%) was to biomonitor pollution, and methodological issues were considered in very few. The low level of harmonization of the methodology is reflected by the fact that some studies provide little (*e.g.* Taramelli et al., 1991) or sometimes no information about the methods used (*e.g.* Bryan and Gibbs, 1983). In fact, few articles refer to previously reported methodologies, which restricts comparison of the results obtained in different studies and may represent a problem in relation to interpreting the findings and reaching valid conclusions.

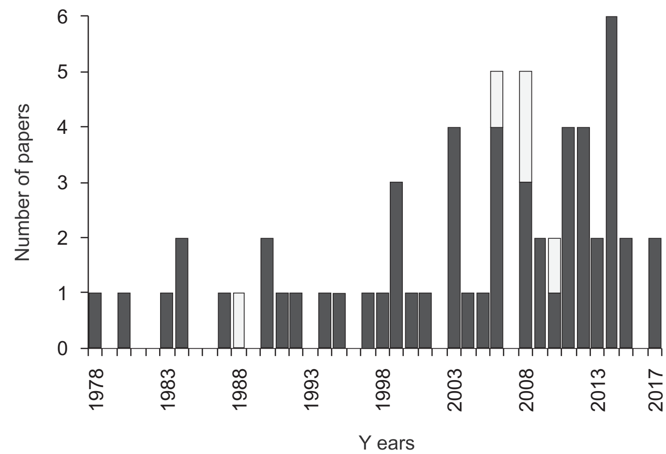

Fig. 1. Number of papers on active biomonitoring of coastal waters with marine macroalgae, published between 1978 and 2017. The columns represent the number of articles concerning methodological aspects (white bars), and the number in which the technique is used to monitor contamination (grey bars).

Regarding the pollutants most commonly considered in the literature consulted, the technique was only used to monitor levels of inorganic contaminants and none of the studies determined organic compounds (Fig. 4), although this has been done in passive biomonitoring studies (García-Seoane et al., 2018). Most studies monitored non-metallic elements, such as N or $\delta^{15}N$ (70%) and C or $\delta^{13}C$ (35%), followed by heavy metals in > 20% (Zn, Cd and Pb, in order of frequency). Other metals (Mn, Cr, Cu, Fe, Hg, *etc.*), metalloids (As), and some nutrients, such as P, K, Mg and Ca have also been studied.

Having reported the state of art in this biomonitoring technique using macroalgae, we will now consider the key methodological aspects needed to standardize the technique: i) selection and preparation of the macroalgae; ii) preparation of the transplants; iii) exposure of the transplants and; iv) post-exposure treatments. We review and discuss the literature consulted, and we assess whether each particular stage of the method can be harmonized or further research is required.

3. Selection and preparation of the macroalgae

3.1. Selection of species for transplant experiments

We found that 40 species, grouped into 24 genera, have been used to date in the studies reviewed. Most belong to the phylum Ochrophyta (50% of the studies), followed by the phyla Rhodophyta and Chlorophyta (in respectively 32 and 30% of the studies). Given this diversity, we grouped the genera to determine the frequency with which they have been used (Fig. 2). Only 6 genera have been used in > 5% of the articles reviewed. The best represented genus is *Ulva* (in 30% of the studies), followed by *Fucus* and *Cystoseira*, used in respectively 15% and 10% of the studies. The genera *Ulva* and *Fucus* are also the most commonly used in passive biomonitoring studies worldwide (García-Seoane et al., 2018). The genus *Cystoseira* had the greatest number of different species used in the studies reviewed (Fig. 5). In 73% of the studies conducted worldwide only one biomonitoring species was used. The remaining studies involved between 2 and (exceptionally) 4 species. However, some articles only indicated the genus but not the species, representing in all cases, species difficult to identify belonging to the phyla Chlorophyta and Rhodophyta (see *e.g.* Cohen and Fong, 2006; Huntington and Boyer, 2008; Kaldy, 2011).

Ulva lactuca L. and *Fucus vesiculosus* L. have each been used in 15%