



# Macrophytes as bioindicators of the physicochemical characteristics of wetlands in lowland and mountain regions of the central Balkan Peninsula



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## ARTICLE INFO

### Article history:

Received 12 April 2016

Received in revised form 27 May 2016

Accepted 4 June 2016

Available online 7 June 2016

### Keywords:

Bioindicator potential  
HOF models  
Water chemistry  
Sediment properties  
Seasonal variability  
Balkan Peninsula

## ABSTRACT

The simultaneous monitoring of vegetation, water and sediments was conducted in wetlands of the central Balkan Peninsula due to the lack of available knowledge on the univariate response of macrophytes along environmental gradients. The environmental preferences and bioindicator potential of macrophytes were assessed using Huisman-Olff-Fresco (HOF) models. *Bolboschoenus maritimus* and *Scirpus lacustris* subsp. *tabernaemontani* are valuable bioindicators of slightly saline (electroconductivity of 2000–4000  $\mu\text{S cm}^{-1}$  in the sediment) and alkaline habitats that are rich in  $\text{SO}_4^{2-}$ . Their ecological niches are partially overlapped. *Bolboschoenus maritimus* prefers saltier and more alkaline habitats for optimal development. The salinity and alkalinity of habitats are decisive factors in the ecological diversification of the *Bolboschoenus* species. *Bolboschoenus glaucus* is adapted to non-saline (400–900  $\mu\text{S cm}^{-1}$ ) and slightly alkaline habitats, unlike *Bolboschoenus maritimus*. Relatively deep, slightly acid waters which are poor in  $\text{SO}_4^{2-}$  (0.30 mg/l), and sediments with low values of electroconductivity and  $\text{K}_2\text{O}$  (6.8 mg/100 g sediment) are preferred by *Typha angustifolia*, *Sparganium erectum* and *Typha latifolia*. The abundance of *Phalaris arundinacea*, *Scirpus lacustris*, *Carex riparia* and *Eleocharis palustris* increases when there is a decrease in the amount of nutrients ( $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$ ) in the water. *Phragmites australis* has low indicative value and regional bioindicator potential. The data obtained in the study may serve as a basis for adjusting the existing indicator values of these species and extending indicator systems by defining the indicator values of species with respect to environmental variables which have not yet been considered.

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## 1. Introduction

Macrophytes are essential and relevant elements in the biological assessment of habitat quality (Onaindia et al., 2005). They are able to respond to altered environmental conditions through changes in their growth and distribution (Steffen et al., 2014). Aquatic plants are not early warning indicator organisms due to their relatively long life cycles and tolerance of short-term changes in environmental conditions (Brabecz and Szoszkiewicz, 2006). Nevertheless, they are formally recognized under the Water Framework Directive of the European Union (European Commission,

2000) as valuable bioindicators for estimating the ecological status of surface waters.

Numerous ecological studies have been focused on investigating the relationship between aquatic plants and the physicochemical properties of the water and sediment (Onaindia et al., 2005; Kočić et al., 2008; Kłosowski and Jabłońska, 2009; Lukács et al., 2009; Steffen et al., 2014) in order to assess their bioindicator potential. The results of such studies facilitate the correction of the existing indicator values for plant species (Ellenberg et al., 1991; Kojčić et al., 1997; Pignatti, 2005) through the collection of information on their habitat affinities. In the past, the indicator values of vascular plants were proposed for certain areas, mostly on the basis of expert judgment, so that they can be considered as surrogates for actual field measurements (Thompson et al., 1993). Presently, ecologists are making efforts to calibrate the existing indicator values of plant species in accordance with the results obtained by measur-

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ing environmental variables (Thompson et al., 1993; Ertsen et al., 1998; Wamelink et al., 2002) and to develop an indicator systems based on these variables (Wamelink et al., 2005). Although the indicator value approach is widely used in bioindication (Godefroid and Dana, 2007), it does not provide important information on the ecological amplitudes of a species (Wamelink et al., 2005).

It has been suggested that the indicator value approach is appropriate for assessing environmental quality only when the whole plant species assemblages are used as indicators of environmental conditions (Wamelink et al., 2005). However, the use of species as bioindicators is more cost-effective and can be accurately estimated by all of the personnel involved in monitoring (Niemi and McDonald, 2004). This gives the use of species a slight advantage over communities. The introduction of more effective approaches in bioindication has become necessary. Species response curves (SRCs) have been recognized as an effective and valid tool (Peppler-Lisbach, 2008) for univariate species response modeling. SRCs provide precise information, not only on the optimum, but also on the other niche parameters, including tolerance and range of the response of species along environmental variables. The shape of SRCs “hides” important data, too. It indicates interspecific interaction (Lawesson and Oksanen, 2002) through determining the density and identity of potential competitors. There are several techniques which can be used to model SRCs, out of which the HOF models are the best option from the ecological point of view (Huisman et al., 1993; Lawesson and Oksanen, 2002; Jansen and Oksanen, 2013). The environmental preferences of species defined on the basis of HOF models are expressed in physical units (Štechová et al., 2008; Uğurlu and Oldeland, 2012), as opposed to indicator systems that use a semiquantitative, arbitrary scale, so they are more meaningful.

The main aims of the present study are: 1) to define the environmental preferences of macrophytes with respect to the physicochemical properties of the water and sediments using HOF models, 2) to assess the bioindicator potential of the species studied, 3) to determine the existence of seasonal variability in the environmental variables, 4) to discuss the impact of seasonal variability in the environmental variables on defining the ecological preferences of the species examined.

## 2. Material and methods

### 2.1. Vegetation sampling

In order to determine the environmental preferences of *Bolboschoenus glaucus* (Lam.) S. G. Sm., *Bolboschoenus maritimus* (L.) Palla, *Carex riparia* Curtis, *Eleocharis palustris* (L.) Roemer & Schultes, *Phalaris arundinacea* L., *Stachys palustris* L., *Phragmites australis* (Cav.) Trin. ex Steudel, *Scirpus lacustris* L., *Xanthium strumarium* L. subsp. *italicum* (Moretti) D. Löve, *Scirpus lacustris* L. subsp. *tabernaemontani* (C. C. Gmelin) Syme in Sowerby, *Sparganium erectum* L., *Lemna minor* L., *Typha angustifolia* L., *Potamogeton lucens* L. and *Typha latifolia* L. with respect to the physicochemical characteristics of their habitats, 50 permanent vegetation plots were sampled over five months during one growing season to collect vegetation, water and sediment data. The above species were recognized to be statistically significant indicator species for 11 emergent macrophyte communities developed in the central part of the Balkan Peninsula (Jenačković et al., 2016). The procedures for both vegetation data collection, as well as determining the plant species, are described in detail in an earlier published study (Jenačković et al., 2016). Also, basic data on the geographical positions and climate conditions of the localities investigated are given in the previously mentioned paper.

### 2.2. Water sampling and analysis

For each vegetation plot where the sediment was covered with water, one 1000 ml water sample was taken by putting a sample bottle 20 cm below the water surface. After collection, the water samples were transported in a hand fridge and kept at 4 °C prior to laboratory analyses. Before chemical analysis, the samples were filtered through 0.45 µm PTFE filters. The concentration of ammonium-ion ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ) and orthophosphates ( $\text{PO}_4^{3-}$ ) was measured using the Shimatzu UV-vis spectrophotometer according to APHA (1995) standard methodology. The TURB 355 IR turbidity meter (WTW, USA) was used for assessing the quantity of  $\text{SO}_4^{2-}$  (Radojević and Bashkin, 1999). The concentration of  $\text{Cl}^-$  was assessed using the argentometric titration method with  $\text{AgNO}_3$ .

Water conductivity ( $\mu\text{S cm}^{-1}$ ) and pH were measured in situ using a portable WTW multi 340i probe. The water depth was measured from the shallowest to the deepest points in the vegetation plots.

### 2.3. Sediment sampling and analysis

The sediment samples for each vegetation plot were made by mixing three sub-samples which were collected in the zones of the root system (0–25 cm). The field-moist sediment samples were used to gravimetrically determine the current moisture content by drying them at 105 °C to their constant mass (ISO 11465, 1993).

Chemical analysis was carried out on the sediment samples after they were cleaned of mechanical and organic impurities, air dried, crushed and sieved through 2 mm mesh. These sediment samples were utilized for determining the following properties: sediment reaction, electrical conductivity (EC), concentration of available potassium ( $\text{K}_2\text{O}$ ) and phosphorus ( $\text{P}_2\text{O}_5$ ), concentration of carbonates and bicarbonates and chloride content. The sediment pH values were determined in 1:2.5 (w/v) suspensions of sediment in water (Van Reeuwijk, 2002) using a pH meter (CyberScan pH 510). The content of available potassium was determined by flame photometry using a Carl Zeiss Jena FLAPHO 4 flame photometer according to the Egner and Riehm (1958) method. The concentration of available phosphorus was determined using a Secomam Anthelie UV-V spectrophotometer (Egner and Riehm, 1958). The saturation extract (1:5 w/v, sediment to distilled water) was used for measuring EC and the concentration of  $\text{Cl}^-$ ,  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ . The EC was measured using a SensION5 conductivity meter (HACH, USA) according to the Rayment and Higginson (1992) method. The chloride content was determined by argentometric titration with  $\text{AgNO}_3$  as the titrant, and with  $\text{K}_2\text{CrO}_4$  as an indicator, according to Mohr's method (Richards (Ed.), 1954). Titration with 0.01 M HCl was applied to determine the concentration of  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  using methyl-orange and phenolphthalein as indicators, respectively (Richards (Ed.), 1954).

### 2.4. Statistical analysis of the data

Defining the environmental preferences of the species studied was based on species response curves. Modeling of the species response curves for all of the species included in the present study, with respect to the physicochemical characteristics of the water and sediment analyzed, was performed with logistic regression models which are also known as Huisman-Olff-Fresco (HOF) models (Huisman et al., 1993). HOF models are a hierarchical set of five models, namely: I – flat with no response, II – monotone decreasing or increasing, III – monotone increasing to a plateau, IV – symmetric unimodal and V – asymmetric unimodal. This statistical routine was developed by David Zelený and Lubomír Tichý (<http://davidzeleny.net/juice-r/>)

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