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## THE RELATIONSHIP BETWEEN MACROPHYTE ASSEMBLAGES AND SELECTED ENVIRONMENTAL VARIABLES IN RESERVOIRS OF SLOVAKIA EXAMINED FOR THE PURPOSE OF ECOLOGICAL ASSESSMENT

**ABSTRACT:** According to the requirements of the European Water Framework Directive, the Member States of the European Union are obliged to assess and report on the ecological potential of heavily modified and artificial water bodies; water reservoirs on rivers were also designated among them. The objective of this study was to gain more knowledge about macrophyte assemblages in reservoirs in Slovakia, where it was necessary to start analyses leading to the ecological assessment. The research was carried out in 14 multipurpose reservoirs during the vegetation seasons 2008–2010. Analyses focused on the determination of species composition considering the similarity between reservoirs, the impact of selected environmental variables on species composition and evaluation of the Macrophyte Biological Index for Lakes (IBML) in relation to its use for ecological assessment. In total, 60 taxa of macrophytes were identified. More than 90% of all determined taxa are indicators of IBML. The statistical analysis performed was based on the study of macrophyte assemblages and environmental variables and gave the following results: i) based on species composition, two main clusters of reservoirs were identified respecting altitude (reservoirs at an altitude above and less than 300 m a.s.l.) and affiliation to phytogeographical ecoregion (reservoirs in Pannonian lowland and Carpathians); ii) water temperature, followed by dissolved oxygen and chemical oxygen demand, were found to be the main environmental variables influencing the

composition of macrophyte assemblages using DCA analysis. Water temperature and phosphates were determined to be the variables responsible for species composition using CCA analysis; iii) differences of the mean IBML values between clusters corresponded with the results of cluster analysis. A significant correlation was found between IBML and two variables: conductivity and alkalinity. Based on the results, it is recommended to use the IBML for analyses leading to the assessment of ecological potential based on biological quality elements.

**KEY WORDS:** ecological potential, Heavily Modified Water Bodies (HMWB), Macrophyte Biological Index for Lakes (IBML), macrophytes, reservoirs, Water Framework Directive (WFD)

### 1. INTRODUCTION

It is widely acknowledged that aquatic plants can be successfully used as reliable indicators of important changes in freshwaters at local, watershed, and regional scales, as they integrate temporal, spatial, chemical, physical and biological qualities of the ecosystem. The distribution and abundance of aquatic macrophytes are influenced by variations in environmental factors (Lacoul and Freedman 2006a, b). Many studies have examined relationships between species of

aquatic plants or their communities and environmental conditions in both lotic (Riis *et al.* 2001, Bernez *et al.* 2004, Hrivnák *et al.* 2010, Szoszkiewicz *et al.* 2010) and lentic (Oťaheľová and Oťaheľ 2006, Alahuhta *et al.* 2009, Svitok *et al.* 2011) habitats. The obtained knowledge base is presumed to be applicable in European countries with regard to the WFD requirements (WFD; Directive 2000), where macrophytes are used as one group of indicators of ecological status in different types of surface waters (Janauer 2002, Schaumburg *et al.* 2004a, b, Haury *et al.* 2006, Schaumburg *et al.* 2007, Kuhar *et al.* 2011, Lewin and Szoszkiewicz 2012). The purpose of the WFD is to establish a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwaters. The protection and enhancement of the status of aquatic ecosystems is among its main aims. In general, hydrology, geomorphology and physical-chemical qualities of habitats are of paramount importance for the development of aquatic vegetation (Haslam 1978, Lacoul and Freedman 2006a). Large anthropogenic activities, such as the construction of reservoirs, dams and diversions for hydroelectricity and agricultural purposes, result in changes in environmental performance. According to the WFD, the Member States of the European Union are obliged to assess and report on the ecological potential of heavily modified (HMWB) and artificial (AWB) water bodies. With respect to the WFD, reservoirs should be classified as “heavily modified water bodies”. Reservoir development is often characterised by the hydrologic conversion of a lotic system into a lentic one associated with alteration of geomorphological features such as shoreline, surface area of a water-body, depth and turbidity gradients, reduction of flow velocity, siltation and higher nutrient availability over time. As a possible consequence, relatively hospitable conditions for the development of aquatic plant communities might be created (Barko and Smart 1986, van Geest *et al.* 2005). Several studies have shown successful colonization by hydrophytes in the reservoirs of hydropower plants located in the fluvial corridor of the Danube River: in Austria (Janauer and Pall 2003, Schmidt *et al.* 2006), Germany (Pall and Janauer 2003), Serbia (Vukov

*et al.* 2004, 2008) and Slovakia (Oťaheľová and Valachovič 2002, Oťaheľová *et al.* 2007). Except for the above-mentioned data from Danube and Orava reservoirs (Kochjarová *et al.* 2010, Hrivnák *et al.* 2011), there is a lack of information from Slovakia.

The assessment of ecological status based on macrophytes for lakes was developed in many European countries. Brucet *et al.* (2013) mentioned 20 countries which have methods for ecological status assessment. Most of the macrophyte-based assessment methods use multi-metric indices for the ecological status assessment, for example in Austria, the Netherlands and Belgium (Pall and Moser 2009); Denmark (Søndergaard *et al.* 2005), Finland (Penning *et al.* 2008) and in Poland (Ciecierska *et al.* 2006). The other countries used only one metric for this assessment, for example, in Bulgaria (Gecheva *et al.* 2011), France (Bertrin *et al.* 2012), Germany (Schaumburg *et al.* 2007) and Sweden (Penning *et al.* 2008). Despite this, a few specific methods for the ecological potential assessment of reservoirs based on macrophytes have been presented to date (Lammens *et al.* 2008, Mjelde *et al.* 2013). Eutrophication is one of the main pressures followed by methods of ecological assessment. Therefore, trophic/biological indices are integrated into many methods to detect the impact of nutrient enrichment on macrophytes. Macrophytes are recommended for HMWB classification with respect to the assessment category “Plant Nutrients” (trophy) according to the Action Guidelines – Classification of Heavily Modified Water bodies and Derivation of the Ecological Potential in Bavaria within the framework of the implementation of the European Water Framework Directive (Bavarian Environment Agency, 2008). Benthic invertebrates and fish are explicitly proposed for HMWB classification with respect to the assessment category relating to “Hydromorphological Changes”. In this connection, in case of reservoirs with a low fluctuation in water level and with a global character similar to lakes, it might be possible to use methods for ecological status assessment. In Slovakia, the Macrophyte Biological Index for Rivers (IBMR) is used for the ecological status assessment of rivers based on macrophytes. The IBMR was developed in

France and is currently successfully used in many other countries throughout Europe e.g. Belgium (Wallonia), Cyprus, Greece, Italy, Luxemburg, Portugal and Spain. The individual scores of its indicators give the overall value for “water quality” as determined by two nutrients – orthophosphate and ammonium and heavy organic pollution as indicated by the presence of sewage fungi (Haury *et al.* 2006). The IBML was created for the assessment of the ecological status of lakes in France in a similar way as the IBMR (Bertrin *et al.* 2012). This indication system is based on the relative abundance of “sensitive” and “tolerant” species. The trophic scores assigned to the indicator species involve all the pressures linked to or associated with the degradation of the trophic level – eutrophication, hydromorphological changes or general degradation. With regard to this and due to a positive experience with IBMR in our analyses of ecological status assessment, the IBML was recommended for ecological assessment.

Therefore, to gain more data concerning macrophytes assemblages in Slovak reservoirs with the possibility of their use for the purpose of ecological assessment, the

following aims were suggested i) to describe the structure of macrophyte assemblages and classify reservoirs into clusters based on the similarity of species composition, ii) to detect influence of selected environmental variables on the macrophyte species composition and iii) to analyse the Macrophyte Biological Index for Lakes (IBML) in relation to its use for ecological assessment.

## 2. STUDY AREA

The landscape of Slovakia is characterised by mountain ranges in the north and central parts (Carpathian phytogeographical ecoregions) and the lowlands in the southern part (Pannonian ecoregion; c.f. Futák 1984). The Slovak climate is intermediate between temperate and continental climate zones with relatively warm summers and cold, cloudy and humid winters. Similarly to the ecoregions, the character of the weather differs from the north (mountainous) to the south (plain).

In total, 14 multipurpose reservoirs on the various watercourses of the Slovak Danube basin were proportionally selected with

Table 1. Basic characteristics of selected reservoirs.

	Latitude (N)			Longitude (E)			Ecoregion	Altitude (m a.s.l.)	Surface size (km <sup>2</sup> )	Maximum volume (mil. m <sup>3</sup> )	Maximum depth (m)	Q (m <sup>3</sup> s <sup>-1</sup> )
	°	'	''	°	'	''						
A	48	33	32	17	49	40	P	158.5	4.13	12.2	6.5	149.3
B	48	13	30	17	48	32	P	124.0	10.9	65.5	10.0	152.0
C	48	22	37	17	23	6	P	192.8	0.7	2.2	6.1	0.3
D	48	42	29	17	24	22	P	229.1	0.6	3.1	9.5	0.6
E	48	47	20	22	0	53	P	117.1	32.9	334.0	13.6	15.4
F	49	2	57	21	40	52	P	163.5	14.0	187.5	23.9	7.7
G	48	10	51	19	59	46	P	244.0	0.6	2.5	7.4	0.2
H	48	33	25	19	10	11	C	303.6	0.7	2.9	10.5	3.4
I	48	17	28	19	30	44	P	233.1	0.7	3.8	12.1	0.3
J	48	28	34	20	5	27	P	221.2	0.9	5.3	10.7	0.5
K	48	26	21	19	33	25	P	255.6	1.7	15.6	22.0	0.2
L	48	51	41	20	22	54	C	786.1	0.9	11.1	22.7	1.6
M	49	24	31	19	33	37	C	602.9	33.5	331.2	29.0	21.7
N	49	6	14	19	31	40	C	565.7	21.7	362.1	40.0	27.8

Legend: Abbreviations of surveyed reservoirs: A (Sĺňava), B (Kráľová), C (Budmerice), D (Kunov), E (Zemplínska Šírava), F (Veľká Domaša), G (Petrovce), H (Môtová), I (Luboreč), J (Teplý vrch), K (Ružiná), L (Palcmanšská Maša), M (Orava), N (Liptovská Mara); Ecoregion: P – Pannonian lowland, C – Carpathians according to (Futák 1984); Q – through flow



Fig. 1. The distribution of selected reservoirs in Slovakia.

Abbreviations of surveyed reservoirs: A (Sĺňava), B (Kráľová), C (Budmerice), D (Kunov), E (Zemplínska Šírava), F (Veľká Domaša), G (Petrovce), H (Môťová), I (Luboreč), J (Teplý vrch), K (Ružiná), L (Palcmanšká Maša), M (Orava), N (Liptovská Mara).

respect to ecoregions and environment conditions (for details see Fig. 1 and Table 1). Four of these belong to the Carpathian ecoregion and ten reservoirs belong to the Pannonian ecoregion. In the Carpathian ecoregion, two reservoirs (Orava, Liptovská Mara) were selected, each with a surface area greater than 20 km<sup>2</sup>, where Orava is the largest water reservoir in Slovakia (33.5 km<sup>2</sup>). The other two reservoirs (Môťová, Palcmanšká Maša) are smaller, with a surface area less than 1 km<sup>2</sup>. These reservoirs are concurrently the deepest (maximum depth more than 20 m), with the exception of Môťová reservoir. In the Pannonian ecoregion, four reservoirs (Kráľová, Sĺňava, Veľká Domaša, Zemplínska Šírava) have a surface size greater than 10 km<sup>2</sup>. The other reservoirs (Budmerice, Kunov, Luboreč, Petrovce, Ružiná, Teplý vrch) are mostly smaller than 1 km<sup>2</sup> in surface area. Ružiná and Veľká Domaša belong to the deepest reservoirs and the maximum depth reaches 20 m. In contrast, five reservoirs (Budmerice, Kunov, Petrovce, Sĺňava and Zemplínska Šírava) do not exceed 10 m maximum depth. Small reservoirs are primarily used for balancing the unbalanced flow rates, for fishing, recreation and some of them are used as a source of irrigation. Larger reservoirs are mainly used for flood protection, but also for the extraction of gravel sands, the production of electricity, water supply for industry, fish-

ing and recreation. All reservoirs have long shoreline stretches similar to “natural” lakes, with many sandy-clayish beaches. According to the hydrological regime, these selected reservoirs are similar to lakes, because the water level fluctuation did not exceed 2 m per year and they mostly have a long retention time. Except for Palcmanšká Maša, which was built in 1933, all other reservoirs were created in the second half of the last century.

### 3. MATERIAL AND METHODS

#### 3.1. Macrophyte surveys

Field surveys were conducted using the general principles described in the European Standard EN 15460 (CEN 2007). Macrophyte surveys were undertaken in summer between July and early September in the years 2008–2010. Macrophytes in reservoirs mainly occur on certain stretches along the shoreline and in free shallow water at sites with an appropriate substrate. Therefore, several areas were selected in each reservoir where the survey was carried out. Selection was based on the presence of macrophytes monitored along the whole perimeter of reservoirs, together with the monitoring of free water areas, with the aim to obtain sufficient relevant data. In selected areas there were monitored the shoreline stretches (including littoral zone), with

adjacent water area with a depth at which no further plant growth occurred and free water areas in reservoirs where macrophytes were observed. The length of stretches and area size were different in terms of the presence of the new taxa which had not been recorded previously. However, the approximate size of each single survey stretch was about 100 m of the shoreline and 50 m<sup>2</sup> of water area. If no more new species (an indicator of IBML) occurred in the last 25 m of the survey stretch or in the final 10 m<sup>2</sup> of the water area, the survey length or area could be left with 100 m or 50 m<sup>2</sup>. If new species were constantly found, the survey stretch or area was extended by a distance of 25 m or area of 10 m<sup>2</sup> until no further species occurred. The number of survey stretches/water areas in reservoirs varied from three (small reservoirs with area less than 1 km<sup>2</sup>) to 10 (large reservoirs with an area of more than 10 km<sup>2</sup>). Each reservoir was monitored twice during the survey period. The survey was carried out from a boat. The shoreline was surveyed by wading, but the adjacent area was also investigated from a boat using a telescopic rake or grapnel for macrophytes collected from deep water.

In each surveyed stretch or area the Plant Mass Estimate (PME) was evaluated using a five-level scale according to EN 15460 (CEN 2007). Finally, the mean value of PME for each taxon present in the reservoir was calculated. For each reservoir a list of taxa with calculated PME mean values was prepared. These data were used for further statistical analyses. Macrophyte surveys included the identification of three taxonomic groups, such as filamentous algae, bryophytes and vascular plants. The determination of taxa focused primarily on indicators of IBML. Therefore, the level of determination of filamentous algae was mostly performed at the genus level. The other taxonomic groups were determined at the species level. Only eight sedge species were determined, in agreement with the IBML indicator taxa list; the other sedge species were not distinguished and were listed as *Carex* spp. The nomenclature of non-vascular and vascular plants followed Marhold and Hindák (1998) and species are presented in Table 2. Moreover, the share of various growth forms of plants was analysed by distinguishing three categories: helophytes (emergent plants), hy-

drophytes (true aquatic plants including submerged plants, pleustophytes and floating leaf-rooted plants) and amphiphytes (occurring in two growth forms in studied areas, helophytes and hydrophytes; c.f. Janauer 2003, Janauer and Dokulil 2006).

### 3.2. Environmental variables

The following environmental variables were measured monthly in water in each reservoir during the vegetation period (April–October) in the years 2008–2010: dissolved oxygen (O<sub>2</sub>), biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH (pH), temperature (t), electrical conductivity (CON), ammonia nitrogen (NH<sub>4</sub>-N), nitrate nitrogen (NO<sub>3</sub>-N), total nitrogen (N total), total phosphorus (P total), orthophosphate phosphorus (PO<sub>4</sub>-P), alkalinity (KNK 4.5) and chlorophyll-*a* (ch-*a*); see Table 3. The given chemical variables were sampled in free water and taken from the surface layer on two or three stable sampling points. Some variables such as oxygen, temperature, pH and electrical conductivity were measured directly *in situ*. For this purpose, a WTW MULTI 340i portable device was used. To analyse the remaining variables, the water samples were transported to the laboratories. To analyse the following variables (nutrients, biochemical oxygen demand, alkalinity), a water sample was taken into sampling bottle of the volume 2 liters and to analyse the chemical oxygen demand, the sample was taken into sampling bottle of the volume 250 ml preserved by sulphur acid. For analysis of chlorophyll-*a*, a water sample was taken into dark sampling bottle of the volume 1 litre. All sampling bottles were transported into laboratories and they were stored by cooling at a temperature of 3±2°C.

Laboratory analyses were carried out by the staff of the Slovak Water Management Enterprise. Physicochemical variables such as ammonia nitrogen, nitrate nitrogen, orthophosphate phosphorus and total phosphorus were determined spectrophotometrically according to relevant ISO and EN standards [ISO 7150-1 (ISO 1984); ISO 7890-3 (ISO 1988); ISO 6878 (ISO 2004)]. Total nitrogen was analysed using oxidative digestion with peroxodisulfate [ISO 11905-1 (ISO 1997,

Table 2. The list of determined taxa with an indication of the growth form, class of dominance and frequency. Growth forms: A – amphiphytes, HE – helophytes, HY – hydrophytes; classes of dominance: EU – eudominant, D – dominant, SD – subdominant, SR – subrecedent, R – recedent; classes of frequency: 1–5.

Taxa list	Abbreviation	Growth form	I		IIa		IIb	
			F	D	F	D	F	D
<b>Algae</b>								
1 <i>Cladophora</i> sp.	CLA.SPX	HY	1	R	-	-	2	SD
2 <i>Enteromorpha intestinalis</i> Link	ENT.INT	HY	-	-	-	-	1	SR
3 <i>Hydrodictyon reticulatum</i> Roth	HYD.RET	HY	-	-	-	-	1	SD
4 <i>Microspora</i> sp.	MIC.SPX	HY	1	R	-	-	-	-
5 <i>Oedogonium</i> sp.	OED.SPX	HY	2	SD	-	-	1	SR
6 <i>Oscillatoria</i> sp.	OSC.SPX	HY	-	-	2	SD	1	SR
7 <i>Spirogyra</i> sp.	SPI.SPX	HY	1	R	4	D	2	SD
<b>Bryophytae</b>								
8 <i>Amblystegium fluviatile</i> (Hedw.) B. S. G.	AMB.FLU	HY	1	R	-	-	-	-
9 <i>Fontinalis antipyretica</i> (L.) Hedw.	FON.ANT	HY	2	SD	-	-	-	-
<b>Tracheophyta</b>								
10 <i>Agrostis stolonifera</i> L.	AGR.STO	HE	5	D	-	-	-	-
11 <i>Azolla filiculoides</i> Lam.	AZO.FIL	HY	-	-	-	-	1	SR
12 <i>Alisma gramineum</i> L.	ALI.GRA	A	2	SD	-	-	-	-
13 <i>Alisma lanceolatum</i> With.	ALI.LAN	HE	-	-	-	-	1	R
14 <i>Alisma plantago-aquatica</i> L.	ALI.PLA	HE	-	-	-	-	3	SD
15 <i>Batrachium aquatile</i> (L.) Dumort.	BAT.AQU	HY	-	-	-	-	1	SR
16 <i>Batrachium circinatum</i> (Sibith.) Spach	BAT.CIR	HY	-	-	2	R	-	-
17 <i>Batrachium trichophyllum</i> (Chaix) Bosch	BAT.TRI	HY	2	SD	-	-	-	-
18 <i>Butomus umbellatus</i> L.	BUT.UMB	A, HE	1	R	-	-	3	SD
19 <i>Carex riparia</i> Curtis	CAR.RIP	HE	-	-	-	-	1	R
20 <i>Carex</i> spp.	CAR.SPP	HE	5	EU	-	-	1	R
21 <i>Ceratophyllum demersum</i> L.	CER.DEM	HY	1	SD	-	-	2	SD
22 <i>Eleocharis acicularis</i> (L.) Roem et Schult.	ELE.ACI	A	-	-	-	-	1	R
23 <i>Elodea canadensis</i> Michx.	ELO.CAN	HY	1	R	-	-	-	-
24 <i>Elodea nuttallii</i> (Planchon) St. John	ELO.NUT	HY	-	-	-	-	2	SD
25 <i>Epilobium hirsutum</i> L.	EPI.HIR	HE	-	-	2	SD	1	SR
26 <i>Glyceria maxima</i> (Hartmann) Holmberg	GLY.MAX	HE	1	SD	2	SD	-	-
27 <i>Impatiens glandulifera</i> Royle	IMP.GLA	HE	-	-	-	-	1	SR
28 <i>Iris pseudacorus</i> L.	IRI.PSE	HE	1	SD	2	R	3	SD
29 <i>Lemna minor</i> L.	LEM.MIN	HY	1	R	-	-	1	R
30 <i>Lycopus europaeus</i> L.	LYC.EUR	HE	-	-	-	-	1	SR
31 <i>Lysimachia nummularia</i> L.	LYS.NUM	HE, A	-	-	-	-	2	R
32 <i>Lysimachia vulgaris</i> L.	LYS.VUL	HE	-	-	-	-	1	SR
33 <i>Lythrum salicaria</i> L.	LYT.SAL	HE	1	R	-	-	4	SD
34 <i>Mentha aquatica</i> L.	MEN.AQU	HE	-	-	-	-	2	SD
35 <i>Myriophyllum spicatum</i> L.	MYR.SPI	HY	4	D	-	-	3	SD
36 <i>Najas marina</i> L.	NAJ.MAR	HY	-	-	4	EU	4	D

Taxa list	Abbreviation	Growth form	I		IIa		IIb	
			F	D	F	D	F	D
37 <i>Nymphoides peltata</i> (S. G. Gmelin) Kuntze	NYM.PEL	HY	-	-	-	-	1	R
38 <i>Persicaria amphibia</i> (L.) Delarbre	PER.AMP	A	4	D	4	D	4	SD
39 <i>Persicaria hydropiper</i> (L.) Delarbre	PER.HYD	HE	1	R	-	-	1	R
40 <i>Phalaris arundinacea</i> L.	PHA.ARU	HE	4	EU	2	R	4	SD
41 <i>Phragmites australis</i> (Cav.) Trin.	PHA.AUS	HE	2	SD	5	EU	4	D
42 <i>Potamogeton crispus</i> L.	POT.CRI	HY	1	R	-	-	1	R
43 <i>Potamogeton gramineus</i> L.	POT.GRA	HY	2	SD	-	-	-	-
44 <i>Potamogeton lucens</i> L.	POT.LUC	HY	1	R	-	-	1	SR
45 <i>Potamogeton nodosus</i> Poiret	POT.NOD	HY	-	-	4	D	3	D
46 <i>Potamogeton pectinatus</i> L.	POT.PEC	HY	2	SD	2	R	3	SD
47 <i>Potamogeton perfoliatus</i> L.	POT.PER	HY	2	D	-	-	3	SD
48 <i>Potamogeton pusillus</i> L.	POT.PUS	HY	-	-	2	D	-	-
49 <i>Potamogeton</i> sp.	POT.SPX	HY	-	-	-	-	1	SR
50 <i>Rorippa amphibia</i> (L.) Besser	ROR.AMP	A	2	SD	-	-	1	SR
51 <i>Rumex maritimus</i> L.	RUM.MAR	HE	-	-	-	-	1	SR
52 <i>Scirpus sylvaticus</i> L.	SCI.SYL	HE	-	-	-	-	2	R
53 <i>Schoenoplectus lacustris</i> (L.) Palla	SCH.LAC	HE, A	-	-	-	-	1	SR
54 <i>Sparganium erectum</i> L.	SPA.ERE	HE	-	-	-	-	2	R
55 <i>Sparganium emersum</i> Rehmman	SPA.EME	A	-	-	2	R	-	-
56 <i>Spirodela polyrhiza</i> (L.) Schleiden	SPI.POL	HY	-	-	-	-	1	SR
57 <i>Typha angustifolia</i> L.	TYP.ANG	HE	-	-	5	EU	2	SD
58 <i>Typha latifolia</i> L.	TYP.LAT	HE	1	SD	5	EU	3	SD
59 <i>Utricularia australis</i> L.	UTR.VUL	HY	1	R	-	-	1	SR
60 <i>Zannichellia palustris</i> L.	ZAN.PAL	HY	-	-	2	SD	1	SR
		Cluster	<b>I</b>		<b>IIa</b>		<b>IIb</b>	
		Number of taxa	30		17		48	
		Number of filamentous algae	4		2		6	
		Number of bryophytes	2		0		0	
		Number of vascular plants	24		15		42	

chapter C.4]. Alkalinity was determined by volumetric analysis according to ISO 9963-1 (ISO 1994). Biochemical oxygen demand was analysed by the method for undiluted samples according to EN 1899-2 (CEN 1998). Chemical oxygen demand was analysed by the standard method using very little polluted samples (APHA, AWWA & WEF, 2005). Chlorophyll-*a* was determined spectrophotometrically according to ISO 10260 (ISO 1992). Finally, the mean values of measured variables from all sampling points measured from April to October in the three-year-period were used for subsequent statistical analyses.

### 3.3. Statistical analyses and calculations

For cluster analysis, a CAP program was used (Henderson and Seaby 2007). Clustering was performed by the Complete Linkage Method using the Sorensen index. Clustering was carried out using only presence/absence species data. Coefficients of frequency (*F*) and dominance (*D*) were calculated to classify the taxa present into classes of frequency and dominance which was used for a description of the macrophyte assemblages. The coefficient of frequency (*F*) was calculated by formula:

$$F = \sum A_{i-n} / B \times 100$$

Where  $A_i$  is number of surveys in which the species occurred and  $B$  is total number of surveys.

The coefficient of dominancy ( $D$ ) was similarly calculated using the following formula:

$$D = \sum X_{i-n} / Y \times 100$$

Where  $X_i$  is sum of all PME values of certain taxa in all surveys and  $Y$  is sum of all PME values of all taxa in all surveys.

PME was estimated according to a five-level scale based on the occurrence and percentage cover: 1 (rare, <1%), 2 (occasional, 1 to 10%), 3 (frequent, 10 to 25%), 4 (abundant, 25 to 75%) and 5 (very abundant, >75%). Classes of dominancy were determined according to Tischler and Haydemann (Schwerdtfeger 1975) and classes of frequency according to Balogh (Schwerdtfeger 1975). For dominancy the following classes were used: EU – eudominant (10.1% and more), D – dominant (5.1–10.0%), SD – subdominant (2.1–5.0%), R – recedent (1.1–2.0%) and SR – subrecedent (1.0% and less). Classes of frequency were determined as follows: I – rare

(0.0–20.0%), II – infrequent (20.1–40.0%), III – frequent (40.1–60.0%), IV – very frequent (60.1–80.0%) and V – constant (80.1–100.0%) species. Subsequently, ordination methods were used to detect the effects of the studied environmental variables on the species composition of the macrophyte assemblage. CANOCO 5 for Windows package was used for analyses (ter Braak and Šmilauer 2002). The length of the gradient in Detrended Correspondence Analysis (DCA) was 3.062, showing that unimodal methods were more appropriate for further analysis. Only presence/absence species data were used for both DCA and Canonical Correspondence Analysis (CCA). The CCA was performed to investigate which variables statistically significantly explain the species composition using forward selection.

The significance of environmental variables was tested by the Monte Carlo permutation test with 999 unrestricted permutations (ter Braak and Šmilauer 2002). The Macrophyte Biological Index for Lakes (IBML) was calculated according to Bertrin *et al.* (2012). Pearson correlation coefficients were calculated between environmental variables and the position of samples in the first two

Table 3. The values of measured environmental variables in designated clusters.

Variable	Unit	Total			I (n = 4)			IIa (n = 2)			IIb (n = 8)		
		mean	min	max	mean	min	max	mean	min	max	mean	min	max
O <sub>2</sub>	mg l <sup>-1</sup>	9.30	6.70	14.30	10.50	8.40	14.30	8.90	8.10	9.60	8.90	6.70	10.10
BOD	mg l <sup>-1</sup>	2.80	1.10	6.80	2.40	1.40	4.20	3.90	2.70	5.00	2.70	1.10	6.80
COD	mg l <sup>-1</sup>	14.30	5.10	27.00	9.90	5.10	21.10	20.90	14.80	27.00	14.90	10.40	25.00
pH	–	8.20	7.40	8.60	8.30	7.40	8.70	8.40	8.20	8.50	8.20	7.90	8.50
t	°C	17.50	14.30	20.40	15.50	14.30	17.30	19.00	17.70	20.40	17.90	16.30	19.00
CON	mS.m <sup>-1</sup>	30.20	12.50	51.80	21.00	18.30	26.40	37.20	27.70	46.70	33.20	12.60	51.80
KNK 4.5	meq l <sup>-1</sup>	2.40	1.10	4.00	1.71	1.31	2.35	3.54	3.13	3.96	2.48	0.97	4.01
NH <sub>4</sub> -N	mg l <sup>-1</sup>	0.06	0.03	0.25	0.10	0.03	0.25	0.07	0.04	0.10	0.05	0.03	0.10
NO <sub>3</sub> -N	mg l <sup>-1</sup>	0.75	0.21	1.28	0.65	0.49	0.74	0.64	0.64	0.67	0.84	0.21	1.28
PO <sub>4</sub> -P	mg l <sup>-1</sup>	0.02	0.01	0.07	0.02	0.01	0.07	0.01	0.01	0.01	0.03	0.01	0.06
P total	mg l <sup>-1</sup>	0.06	0.01	0.18	0.06	0.01	0.18	0.06	0.04	0.07	0.06	0.01	0.11
N total	mg l <sup>-1</sup>	1.21	0.59	1.81	1.25	0.81	1.66	0.88	0.61	1.14	1.28	0.59	1.81
ch-a	µg l <sup>-1</sup>	18.00	4.80	59.10	10.70	6.10	18.70	20.00	11.80	28.10	19.40	6.50	59.10

O<sub>2</sub> – dissolved oxygen, BOD – biochemical oxygen demand, COD – chemical oxygen demand, pH – water pH, t – water temperature, CON – electrical conductivity, NH<sub>4</sub>-N – ammonia nitrogen, NO<sub>3</sub>-N – nitrate nitrogen, N total – total nitrogen, P total – total phosphorus, PO<sub>4</sub>-P – orthophosphate phosphorus, KNK 4.5 – alkalinity, ch-a – chlorophyll-a.



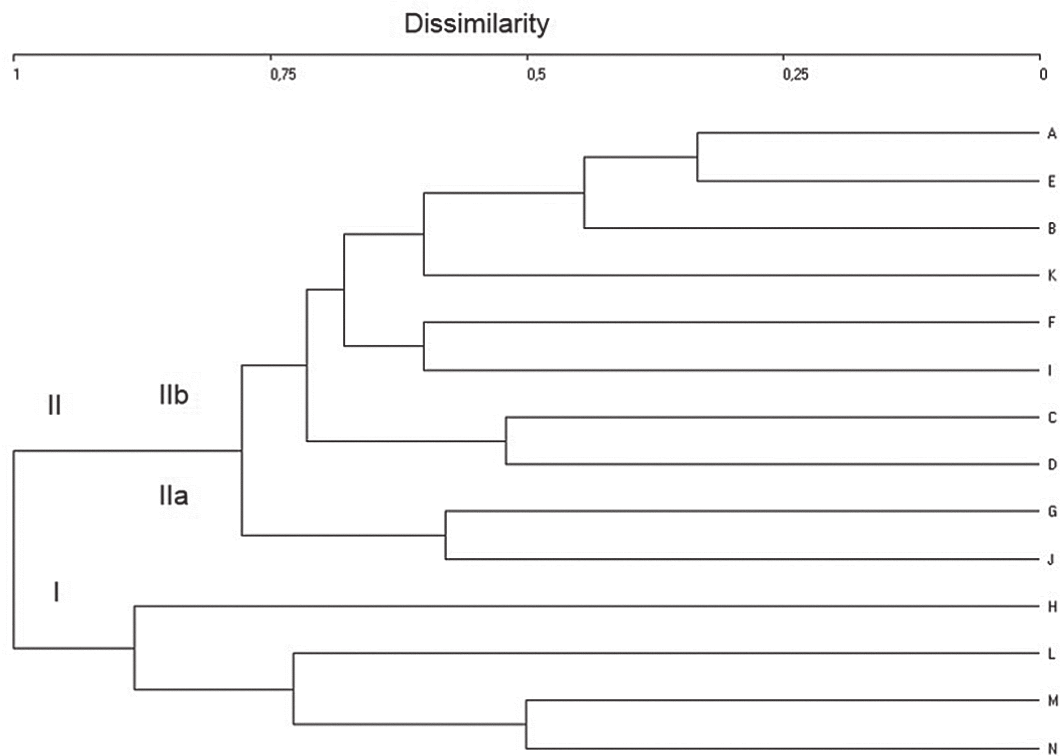


Fig. 2. Dendrogram of cluster analyse of selected reservoirs in terms of macrophytes composition. Abbreviations see Fig. 1.

ordination axes in DCA, and Spearman correlations were used to identify the relationship between IBML and the studied environmental variables using STATISTICA software (StatSoft Inc. 2001).

#### 4. RESULTS

##### 4.1. Structure of macrophyte assemblages

Sixty taxa of macrophytes were determined. In total, 51 vascular plants, 2 bryophytes and 7 filamentous algae were identified (Table 2). Hydrophytes presented 45% of all macrophytes; the rest was shared between helophytes (43%) and amphiphytes (12%). Neophytes such as *Azolla filiculoides*, *Elodea canadensis*, *E. nuttallii* and *Impatiens glandulifera* were recorded. In addition to species composition, two main clusters were identified (I and II) with respect to altitude (reservoirs with altitude above and less than 300 m a.s.l.) and affiliation to ecoregion (reservoirs in Carpathians and Pannonian lowland).

The first cluster (I) comprised four reservoirs (Fig. 2). They are reservoirs in Car-

pathians ecoregion. Three of these (Liptovská Mara, Orava and Pálcmanová Maša) belong to the deepest reservoirs. Generally, macrophytes occurred in a lower abundance in these reservoirs. The mean PME value of present macrophytes in individual surveys was 1.8. In the littoral zone, species such as *Agrostis stolonifera*, *Phalaris arundinacea*, and sedges frequently occurred and in water areas there were *Myriophyllum spicatum* and *Persicaria amphibia*. These previously mentioned species, together with *Potamogeton perfoliatus*, also reached the highest coefficient of dominance values. Bryophytes such as *Amblystegium fluviatile* and *Fontinalis antipyretica* were found only in these reservoirs during the survey. The highest number of taxa (21) was found in the Orava reservoir.

Within the second cluster, two sub-clusters were subsequently identified (IIa, IIb; Fig. 2). Sub-cluster IIa represents only two reservoirs in the Pannonian lowland with a small water surface area (less than 1 km<sup>2</sup>) and low mean water depth (less than 5 m). The littoral zone was frequently colonized by *Phragmites australis*, *Typha angustifolia*

and *T. latifolia* which were also eudominant species. *Najas marina*, *Persicaria amphibia*, *Potamogeton nodosus* and *P. pusillus* belonged to the other eudominant and dominant species. The filamentous alga *Spirogyra* sp. also belonged to this group. The highest number of reservoirs was included in the sub-cluster IIb. There were 8 reservoirs with different size, depth and volume (see Table 1). Totally 48 taxa were recorded, including 42 vascular plants and 6 filamentous algae. *Lythrum salicaria*, *Najas marina*, *Persicaria amphibia*, *Phalaris arundinacea* and *Phragmites australis* belonged to the species with coefficient of frequency above 60%. Only three species: *Najas marina*, *Phragmites australis* and *Potamogeton nodosus* were dominant. A greater abundance of filamentous algae, especially *Cladophora* sp., *Hidrodictyon reticulatum* and

*Spirogyra* sp. was found in particular reservoirs. The highest number of species was determined in two reservoirs (Sĺňava and Kráľová) which are characterised by the highest values of flow velocity. The highest number of hydrophytes (22) was also recorded here.

The DCA analysis of species data confirmed the results of the cluster analysis. Groups of reservoirs were identified that were relatively well separated in ordination space (Fig. 3A). Macrophytes were arranged along the first DCA axis from those occupying aquatic habitats with higher values of nutrients and water temperature (for example *Nymphoides peltata*, *Potamogeton pusillus* s. lat., *Sparganium emersum*, *Typha angustifolia*, *Zannichellia palustris*) to species preferring water with both a higher content of dissolved

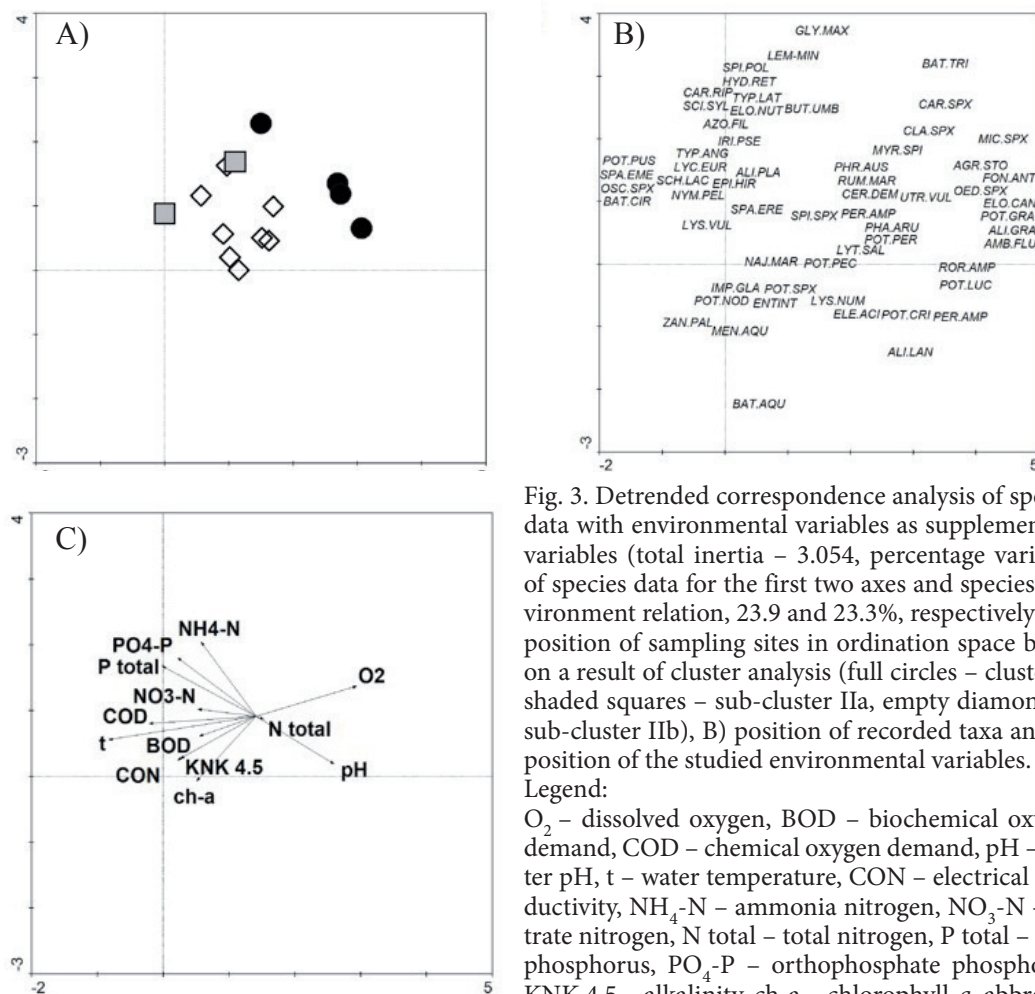


Fig. 3. Detrended correspondence analysis of species data with environmental variables as supplementary variables (total inertia – 3.054, percentage variance of species data for the first two axes and species–environment relation, 23.9 and 23.3%, respectively): A) position of sampling sites in ordination space based on a result of cluster analysis (full circles – cluster I, shaded squares – sub-cluster IIa, empty diamonds – sub-cluster IIb), B) position of recorded taxa and C) position of the studied environmental variables.

Legend:

O<sub>2</sub> – dissolved oxygen, BOD – biochemical oxygen demand, COD – chemical oxygen demand, pH – water pH, t – water temperature, CON – electrical conductivity, NH<sub>4</sub>-N – ammonia nitrogen, NO<sub>3</sub>-N – nitrate nitrogen, N total – total nitrogen, P total – total phosphorus, PO<sub>4</sub>-P – orthophosphate phosphorus, KNK 4.5 – alkalinity, ch-a – chlorophyll-a, abbreviations of plant names are presented in Table 2.

Table 4. The presence of IBML indicators with abundance data per one survey in designated clusters.

Cluster identification	I (n=8)			IIa (n=4)			IIb (n=16)		
	mean	min	max	mean	min	max	mean	min	max
Number of indicators	8	4	11	8	5	11	13	9	19
PME (1–5)	1.8	1.2	3.0	2.1	1.8	2.4	1.8	1.3	2.5
IBML (0–20)	9.0	8.0	9.6	7.2	6.3	8.7	7.5	6.7	8.8
$E_i$ (1–3)	1.6	1.5	1.8	1.7	1.6	1.8	1.8	1.5	2.0

IBML – Macrophyte Biological Index for Lakes (Bertin *et al.* 2012),  $E_i$  – stenocoefficient, min – minimum, max – maximum, PME – Plant Mass Estimate (an average value per survey), scaled in accordance with EN 15 460 (CEN 2007).

oxygen and values of water reaction, a lower content of nutrients and lower water temperature, such as *Agrostis stolonifera*, *Alisma gramineum*, *Potamogeton gramineus*, as well as bryophytes (Fig. 3B, C).

#### 4.2. Environmental characteristics of detected clusters

Among the measured environmental variables, larger differences in particular clusters were recorded for several parameters (see Table 3). Cluster I involved reservoirs located in the mountainous area and was characterised by the lowest mean value of water temperature, electrical conductivity, alkalinity and chlorophyll-*a*. The highest levels of dissolved oxygen were also recorded in these reservoirs. Compared to cluster I, sub-cluster IIa was characterised by the highest mean values of the previously mentioned variables. An increase in chlorophyll-*a* values was mainly caused by the increase in cyanobacteria abundance in the late summer when cyanobacterial blooms were observed in some parts of these reservoirs. Generally, cluster IIa includes only two reservoirs located in the intensive agricultural area in one of the warmest parts of Slovakia. Another remarkable difference was found in the chemical oxygen demand values: cluster I (9.90 mg l<sup>-1</sup>) and sub-cluster IIa (20.90 mg l<sup>-1</sup>). The sub-cluster IIb contained the highest number of reservoirs. Cyanobacterial blooms occurred in several reservoirs similar as in a previous sub-cluster IIa. The highest values of total nitrogen, electrical conductivity and chlorophyll-*a* were reached in this sub-cluster.

#### 4.3. Impact of environmental variables on macrophyte assemblages

Within DCA, the studied environmental variables explained 23.3% of the species-environment relationship and their position along first two axes is shown in Fig. 3C. Only two variables were significantly ( $P < 0.05$ ) correlated with the first DCA axis: water temperature (-0.73) and dissolved oxygen (0.54), however, the significance of chemical oxygen demand (-0.52) was near this threshold ( $P = 0.055$ ). According to the results of forward selection in CCA, the species composition of macrophytes in the studied water reservoirs was only affected by water temperature ( $P = 0.006$ ); the next variable, phosphates was almost statistically significant ( $P = 0.057$ ). Water temperature explained 11.5% of the variability, and both mentioned variables together explained 21.2% of the variability.

#### 4.4. The Biological Index of Macrophytes for Lakes

Out of the 60 taxa identified, there were 56 indicators of IBML. The number of indicator species in particular surveys fluctuated between 4 and 19 with a mean of 10 indicators per survey (Table 4).

The greatest number of indicator species (totally 44 and maximum of 19 per single survey) was recorded in reservoirs from sub-cluster IIb; with 13 indicators per survey on average. The IBML values varied from 6.3 to 9.6: the highest value was recorded in cluster I and the lowest in sub-cluster IIa. Significant differences between particular surveys were not found in terms of the stenocoefficient

( $E_i$ ) of calculated indicators. Their mean values varied from 1.5 to 2.0. Considering its variation from 1 to 3, that means presence of indicators with higher indication value in surveyed reservoirs. Statistically significant correlations ( $P < 0.05$ ) between IBML values and two environmental variables were found. The IBML was negatively correlated with water conductivity (-0.56) and alkalinity (-0.55). A significant correlation between IBML and nutrients was not confirmed, due to their low concentration in free water and to slight differences between particular reservoirs.

## 5. DISCUSSION

The construction of the dams in watercourses causes several changes, mainly in the hydrology and related ecological characteristics. The most significant features, such as a reduction in flow velocity, a rise in water level, water temperature and siltation of the bottom, are manifested in the creation of the suitable ecological conditions for the colonization of hydrophytes. The species richness of aquatic macrophytes of some surveyed reservoirs in our study is comparable to natural wetland systems in the southern regions of Slovakia. For example, more than 10 hydrophytes were found in three reservoirs; Slňava, Kráľová and Orava, whereas the total number of macrophytes observed was more than 20. The Orava water reservoir belongs to an artificial aquatic habitat with still water with the richest presence of hydrophytes in Slovakia; 21 species were found by Kochjarová *et al.* (2010). A similar high diversity of hydrophytes was recorded in other artificial reservoir in Danube countries, for example, the Danube impoundments of Đerdap in Serbia (Vukov *et al.* 2008) or Höchstädt in Germany (Pall and Janauer 2003). Large natural river oxbows mainly of the Danube River, contain a similar or greater number of hydrophytes (Oťaheľová and Valachovič 2006, Oťaheľová *et al.* 2007, Sárbu *et al.* 2011). In contrast, smaller river oxbows frequently have a lower species richness than larger artificial water reservoirs (Kubalová 2003). However, aquatic plant biocoenoses respond to environmental changes by modifying their composition as well as increasing or decreasing plant abundance (Schaumburg *et al.*

2004a). The most frequent hydrophytes in our study were *Myriophyllum spicatum*, followed by *Najas marina*, *Potamogeton nodosus* and *P. perfoliatus*, whereas *Persicaria amphibia* was the most frequent amphiphyte. Helophytes such as *Phragmites australis*, *Phalaris arundinacea*, *Typha latifolia* and *Lythrum salicaria* grew frequently in the littoral zone of the studied water reservoirs. Regarding the abundance of hydrophytes in surveyed reservoirs, *Najas marina* was the most abundant followed by *M. spicatum* and *P. nodosus*. The amphiphyte species *Persicaria amphibia* and helophytes such as *Phalaris arundinacea* and *Phragmites australis* were dominant. All the above mentioned species are generally common and relatively frequent in aquatic habitats in Slovakia as well as in Central Europe (c.f. Oťaheľová 1995, Chytrý 2011). However, endangered plant species were rare; there were only six endangered species found in the studied reservoirs: *Alisma gramineum*, *Batrachium aquatile*, *Butomus umbellatus*, *Nymphoides peltata*, *Potamogeton gramineus*, *Utricularia vulgaris* (c.f. Feráková *et al.* 2001). Similarly, the number of neophytes such as *Azolla filiculoides*, *Elodea nuttallii*, *E. canadensis* and *Impatiens glandulifera* was relatively low (c.f. Medvecká *et al.* 2012).

A Detrended Correspondence Analysis of the entire data set suggested that the strongest environmental factors influencing the composition of macrophyte assemblages is associated with water temperature, followed by dissolved oxygen and chemical oxygen demand. Temperature is a key climatic factor that directly or indirectly influences the distribution of aquatic plants at a regional level (Lacoul and Freedman 2006a). Temperature variation is linked to gradients of latitude and altitude (Lacoul and Freedman 2006b). The results of cluster analysis fully corresponded with this factor (Lapin *et al.* 2002). Reservoirs were divided into two clusters according to their altitudes and affiliation to the ecoregion; both characteristics are strongly connected with water temperature. Water reservoirs at altitudes higher than 300 m a.s.l. which occurred in the Carpathian ecoregion were presented in the first cluster, whereas other reservoirs with lower altitudes and affiliation with the Pannonian lowland ecoregion were grouped into the second cluster. Oxygen characteristics (for

example dissolved oxygen), are relatively frequently discussed in relation to phytoplankton (Khan and Ansari 2005), but the characteristics appear less important for macrophytes. In addition to water temperature, forward selection in CCA identified phosphates to be responsible for species composition (however at a *P*-level of 0.057). Several studies have shown that phosphate concentrations in water affect the composition of aquatic plant communities (Edvardsen and Økland 2006, Bornette and Puijalon 2011).

Currently, there are very few studies dedicated to the evaluation of the ecological potential assessment of reservoirs based on macrophytes. Studies in the Netherlands (Lammens *et al.* 2008) and Scandinavia (Mjelde *et al.* 2013) have been performed recently. Ecological assessment focuses on the selected hydromorphological factors which significantly influence the given ecosystem. Among the most important are: the erosion of the shoreline caused by high water-level fluctuation, eutrophication and fishing or fixed water level (preventing emergent vegetation to grow). In this study, because the selected reservoirs are more or less similar to natural lakes (a low water fluctuation, the character of the shoreline near to natural lakes, etc.) and one of most important anthropogenic influences is here considered to be eutrophication, we focused on its assessment initially. For the selected reservoirs IBML was analysed for the purposes of ecological assessment oriented to the trophic status primarily. More than 90% of all determined taxa found in the selected reservoirs belong to indicator taxa of IBML. Differences in the mean IBML values between clusters confirmed the results of cluster analyses. A significant correlation was found between IBML and two variables: conductivity and alkalinity. Bertrin *et al.* (2012) highlighted significant correlations with two chemical variables: total phosphorus and orthophosphate phosphorus, and these correlations were confirmed only for the group of lakes with a low altitude (<200 m a.s.l.) and alkalinity (<1 meq l<sup>-1</sup>). Schaumburg *et al.* (2004a) observed that trophic indices based on sensitive and tolerant species can not be used for lakes with very few or no species. Additionally, Brucet *et al.* (2013) suggest that such indices always have to be supported by other metrics, such

as the extent of macrophyte coverage (%) of the shallow zones or maximum colonization depth, especially when discussing hypertrophic conditions where the absence of specific macrophyte species, such as large isoetids or charophytes, or the dominance of a specific tolerant species is to be expected. In our case, a developed macrophyte community was found, with a great representation of hydrophytes in the surveyed reservoirs; however, hypertrophic conditions were not confirmed. Therefore, based on the character of surveyed reservoirs and the results here, we recommend the use of IBML for ecological assessment which could also be used for the determination of ecological potential based on biological quality elements. Based on much data, with the aim to use IBML reliably, it will be necessary to demonstrate the responsiveness of IBML to nutrient amounts in the near future.

**ACKNOWLEDGEMENTS:** This study was supported by the Slovak Research and Development Agency under the contract No. APVV-0059-11 and VEGA Grant Agency (grant no. 2/0004/11).

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