The relationship between habitat factors and aquatic macrophyte assemblages in the Danube River in Serbia

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Abstract: Our objectives were to offer insight into the characteristics of the physical environment in the River Danube in Serbia; and to show the relationship between selected environmental factors and the composition and abundance of macrophyte species. The macrophyte survey method followed the European Standard EN 14184, applying Kohler's five-level descriptive scale. Principal component analysis was carried out to examine the variation in aquatic vegetation, and to analyze the effect of environmental variables on the aquatic vegetation, redundancy analysis was used. To build a simpler model with fewer explanatory variables, yet sufficiently explaining species variability, forward selection of environmental variables was done. Our results confirmed that physical environmental factors significantly influence the establishment and structure of macrophyte vegetation, with the level of hydrological connectivity to the main river channel being the most important, followed by the transparency of the water column, sediment texture and water-flow velocity. In order to inspect the individual relation between best-fitted plant species and each of selected environmental variables, Spearman's rank correlation coefficients were calculated. We have distinguished plant species with preferences to specific combinations of analyzed factors. Our results provide a background for future, more specific studies on the macrophyte/habitat relationship.

Key words: hydrophytes; connectivity; transparency; flow; sediment

INTRODUCTION

The distribution and abundance of aquatic macrophytes (large algae, bryophytes and vascular plants), as well as other organisms, are governed by their environment. Physical attributes, like geomorphology, sediment, climate, hydrology, are factors inherent to the limnology of water bodies being crucial predictors of aquatic vegetation. These factors are influenced by terrestrial elements of the related watershed, while the aquatic plants are also affected by biotic factors such as competition, predation and disease [1]. The physical niche of a macrophyte is defined by sediment and by the conditions defined by the surrounding medium, water. These physical conditions are the most important for the establishment of aquatic plants in most habitats, and once a plant is established, other factors (e. g. nutrient concentration, competition, chemical quality, chances of propagation, etc.) can vary, even over a wide range, until the plant's occurrence becomes restricted [2]. Among physical factors, water flow is the most prominent in running waters. In rivers, it is determined by hydrological parameters, in turn defining sediment structure (grain size and composition), channel type and the development of the flood plain [2].

Water flow conditions are one of the strongest physical environmental factors that determine the occurrence of aquatic plants [3]. Thus, its influence on aquatic vegetation has been the topic of research in many studies [4-17]. Jones et al. [18] confirmed a complex relationship between aquatic macrophytes and sediment structure. The importance of physical environmental factors was also found in other more complex studies that dealt with the morphological, hydrological, physical and water quality attributes of the rivers and composition, abundance and distribution patterns of aquatic plant species [19-28].

These studies showed that aquatic macrophytes are highly dependent on the characteristics of their

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environment and changes occurring in it. Nowadays this is particularly relevant because of the EU Water Framework Directive (WFD) [29], which includes the use of macrophytes as bioindicators, or quality elements, needed for assessing the ecological status of surface water bodies. Reference conditions for the WFD are based on existing river reaches in natural or near-natural status. In as much as water bodies no longer exist, historical information, modeling approaches or expert judgment should be used [29,30]. In this respect, understanding the relations between environmental conditions and aquatic macrophytes is of great importance.

In a large international river like the Danube, it is impossible to find undisturbed conditions in the reference sites after centuries of human modifications [31,32], apart from its upper reach, where the river is still small and often close to its natural ecological status [33]. The overall hydromorphology of the Danube River is highly influenced by human-induced alterations. The most significant alteration is the interruption of the longitudinal continuity created by dams and weirs and the lateral connectivity disruptions caused by loss of floodplains and bank reinforcements [34].

The hydrology of the Danube River section analyzed in this study is highly impacted by the dams of the hydropower system Đerdap I and II. The main effects of this interruption in longitudinal continuity are attenuated water velocity, increased sedimentation and nutrient accumulation, and higher transparency, offering favorable conditions for the establishment of aquatic vegetation characterized by high abundance and diversity [32,35-37]. The frequent changes in water level in the reservoirs created after the construction of the dams contribute to high species diversity in accordance with the disturbance hypothesis [38], as disturbance maintains high species richness and limits competitive exclusion [39,40].

The objectives of this study were to offer detailed insight into the characteristics of the physical environment in the large alluvial River Danube in Serbia, and to show the relationship between selected environmental parameters and the composition and abundance of macrophyte species.

MATERIALS AND METHODS

Study area

Of the Danube River's total length of 2850 km, the Serbian reach is 588 km long. It enters the country at 1433 river-km (rkm) and leaves at 846 rkm at the mouth of the Timok River. The average drop in the river course is 24 cm km^{-1}. The section between 1433 to 1296 rkm represents the border with Croatia, and the section to 1075 rkm the border between Serbia and Romania. The main tributaries are the Drava (km 1300, m. a. discharge 622 m^3 s^{-1}), Sava (km 1070, m. a. discharge 1800 m^3 s^{-1}), Velika Morava (km 1105, m. a. discharge 206 m^3 s^{-1}), Tisa (km 1214, m. a. discharge 920 m^3 s^{-1}), and Tamiš (km 1155, m. a. discharge 104 m^3 s^{-1}). The largest settlements are Novi Sad (population 252459, km 1255), Belgrade (1351000, km 1170) and Smederevo (64025, km 1116). At rkm 943 and 863, two dams form part of the largest hydropower system on Danube. The first dam, Đerdap I, became operational in 1972, and the second, Đerdap II, in 1984. According to the data of the Serbian Republic Hydrometeorological Institute, the decrease in water-flow velocity caused by the Đerdap I dam occurs upstream, at approximately rkm 1155, during mean water level periods, resulting in a run-of-the-river reservoir of more than 200 km in length. The section of the river between Đerdap I and II represents the 80-rkm-long reservoir of the second dam [41,42].

Survey of environment and macrophytes

The study was carried out in the section of the Danube main channel, between rkm 1433 and 846, in the oxbows and side-arms with permanent surface connection to the main river channel, during July in the period of 2012 and 2013. Left and right riversides were surveyed separately. The study area was divided into 1081 contiguous survey units (SUs), each 1 rkm long (in the main channel), or of individual length (in side-arms and oxbows). The prevailing type of the following environmental parameters was recorded for each SU: land use (shading potential and naturalness); bank structure (naturalness, gradient and material fineness) for the upper littoral, which extends above the water level during mean discharge periods; river bed sediment (fineness and naturalness), referring to the part of the substrate in the littoral of the water body where the aquatic vegetation is growing; the level of connectivity of the water body, where the SU is, to the main channel (seven levels are predefined, the highest, seventh, is the main channel); water-flow velocity was estimated close to the macrophyte stands in four categories (details are provided in Table 1); water transparency was measured with a Secchi disk, readings were done in 5-cm intervals, measured values were divided into four classes (Table 1).

The macrophyte survey method followed the European Standard EN 14184 [43]: in each SU the abundance of each macrophyte species was estimated according to a five-level descriptor scale (1=very rare, 2=rare, 3=frequent, 4=abundant, 5=very abundant; [19,44] – so called Kohler's 'Pflanzenmenge', i.e. plant mass estimates – PME). Plant species were identified using standard identification keys [45,48]. Species nomenclature follows Flora Europaea (http://rbg-web2.rbge.org.uk/FE/fe.html). The collected plant material was pressed and dried and deposited in the Department of Biology and Ecology Herbarium Collection (BUNS). Only vascular plants were analyzed, while obligate helophytes were excluded from this study.

Data analysis

Environmental data was appropriately coded to be used in further analyses (Table 1). For the multivariate analyses, Kohler's plant mass estimates (PMEs) were transformed using the function $y=x^3$ [49,50]. The correlation between environmental parameters, as well as the correlation between environmental parameters and macrophyte species, was expressed using Spearman's rank correlation coefficients (STATISTICA 12.0). The ordination software package CANOCO 4.5 was used to perform multivariate analyses to describe basic vegetation patterns and their relationship with available environmental data. Out of 1081 SUs, 135 were excluded from analyses for lack of macrophytes. The unconstrained ordination, detrended correspondence analysis (DCA), was used to obtain a basic overview of the compositional gradients in the vegetation data. The length of the first axis was 2.050, suggesting that a linear ordination method was suitable for the analysis. To summarize the variation in aquatic vegetation, principal components analysis (PCA) was performed. Environmental variables were passively projected into the resulting ordination space. The direct effect of environmental variables on the aquatic vegetation was analyzed using redundancy analysis (RDA). The significance of the environmental variables was tested using a Monte Carlo permutation test (full model, 9999 runs). To build a simpler model with fewer explanatory variables, yet explaining species variability sufficiently, forward selection of environmental variables was done in RDA.

RESULTS

The surveyed section of the Danube River was divided into 1081 survey units (SUs). Most SUs were in the main channel of the river (42.6%, Table 1) and in the Đerdap I reservoir (27.0%). An equal share of SUs are in the oxbows with the inflow from their lower end, and in the side-arms with permanent connection to the main channel at both ends (10.1%). Less than 10% of the SUs are other hydrological connectivity types (Table 1). Bank structure along the SUs is mainly natural, in flat slopes of fine, predominantly inorganic substrate, and in 29.0% of SUs the bank is reinforced with stones (rip-rap). The river bed in the littoral zone inhabited by aquatic vegetation is of natural, fine, predominantly inorganic substrate. Water-flow velocity is mostly medium, although the percentage of SUs with low flow is notably high (26.5%). The land along the banks is mostly covered with natural forest vegetation with high shading potential. Measured water transparency ranged between 25 and 150 cm, predominantly (39.5% of SUs) between 51 and 75 cm (Table 1).

The strongest correlation was found between land-use type naturalness and its shading potential, indicating that in most SUs the bank is under natural riparian forest (Table 2). High correlation was also recorded between flow velocity and the level of connectivity to the main channel (meaning that the flow velocity rises as the connectivity to the main channel increases), river bed sediment fineness and its naturalness; and between the slope of the bank and its naturalness, where its negative direction indicates that steep bank slope is in most cases reinforced with materials not indigenous to the SU (e. g. concrete, stone blocks, etc.).

Table 1. Environmental variables.

	Habitat factor	Abb.	Attribute	Code	[%]
Total number of SUs: 1081					
land use	shading level*	LU_shd	no	0	18.4
			partially	1	11.7
			yes	2	69.8
	naturalness level**	LU_ntr	no	0	14.9
			partially	1	11.7
			yes	2	73.4
bank	naturalness	bnk_ntr	artificial	0	18.4
			natural	1	81.6
	flat/steep slope	bnk_slp	flat	0	44.0
			steep	1	56.0
	material fineness	bnk_fns	mega, macro, mesolithal (>6.3cm)	1	29.0
			microlithal & akal (0.2-6.3cm)	2	3.5
			psammal (0.06-0.2cm)	3	8.7
			pelal (<0.06cm)	4	58.7
river bed	sediment fineness	sdm_fns	mega-, macro-, mesolithal (>6.3cm)	1	7.9
			microlithal & akal (0.2-6.3cm)	2	10.3
			psammal (0.06-0.2cm)	3	8.5
			pelal (<0.06cm)	4	73.4
	naturalness	sdm_ntr	artificial***	0	7.3
			natural	1	92.7
connectivity level		cnnctv	oxbow, inflow from lower end	1	10.1
			oxbow, inflow from upper end	2	2.3
			lake with permanent surface connection	3	0.8
			secondary channel	4	10.1
			reservoir Đerdap II	5	7.0
			Đerdap I	6	27.0
			main channel	7	42.6
water	flow velocity	flw_vlc	stagnant	0	11.0
			low flow (<30cm/s)	1	26.5
			medium (35-65cm/s)	2	53.6
			high (>70cm/s)	3	8.8
	transparency	trnsp	25-50cm	1	11.7
			51-75cm	2	39.5
			76-100cm	3	27.3
			101-150cm	4	21.5

Abb. – abbreviation; *Shading level of the land-use type along the SU bank is estimated by the observed CORINE land use typology: 'no' (0) for CORINE codes: 12, 13, 21, 23, 33; 'partially' (1) for CORINE codes: 11, 14, 11908; 'yes' (2) for CORINE code: 311 (details available on www.midcc.at). **Naturalness level of the land-use type along the SU bank is estimated by the observed CORINE land use typology: 'no' (0) for CORINE codes: 11, 12, 13, 14, 11908; 'partially' (1) for CORINE codes: 21, 23; 'yes' (2) for CORINE codes: 31, 32, 33, 311 (details available on www.midcc.at). ***'Artificial' sediment comprises a high fraction of non-indigenous material. This mixed type of river bottom is present in the reservoirs Đerdap I and II, resulting from the flooding of the two impoundments when non-autochthonous sediment material was permanently submersed after the construction of dams. In Đerdap I reservoir, this material consists of large stones and rocks originating from former reinforced embankment (e.g. rip-rap). In Đerdap II reservoir bricks and other building material are also included. These stones, bricks, concrete blocks, etc. stabilize the near-bank sediment. Spaces between the coarse fraction trap finer sediment carried by the river, or washed down from the new bank, enabling macrophyte species to root and grow in these special habitats.

In 946 out of 1081 survey units, aquatic plants were recorded. Obligate helophytes were excluded from this study, leaving 49 aquatic plant species (Table 3). The widest distribution has *Ceratophyllum demersum*, occurring in 56.77% of SUs populated by macrophytes, but the most frequent PME value (Kohler's value) recorded for this species is 1, meaning it occurs rarely in individual SUs. The most frequent species in the surveyed stretch of the Danube is *Potamogeton pectinatus*. It has a wide distribution, since it is re-

Table 2. Correlation between environmental variables.

	bnk_fns	sdm_fns	flw_vlc	trnspr	cnnctv	LU_shd	LU_ntr	bnk_slp	bnk_ntr
sdm_fns	0.33*	.	.	.	.	.	.	.	.
flw_vlc	-0.32*	0.00	.	.	.	.	.	.	.
trnspr	-0.25*	-0.44*	0.02	.	.	.	.	.	.
cnnctv	-0.15*	0.10*	0.63*	-0.16*	.	.	.	.	.
LU_shd	0.32*	0.14*	-0.11*	-0.20*	0.00	.	.	.	.
LU_ntr	0.30*	0.05	-0.07*	-0.14*	-0.02	0.85*	.	.	.
bnk_slp	-0.21*	-0.32*	0.06	0.30*	-0.01	-0.31*	-0.28*	.	.
bnk_ntr	0.04	0.24*	0.04	-0.24*	-0.04	0.11*	0.22*	-0.54*	.
sdm_ntr	0.34*	0.58*	-0.13*	-0.33*	0.06*	0.09*	-0.05	-0.32*	-0.06*

*Values statistically significant at $p<0.05$.

corded in 50.85% of SUs populated by macrophytes, and its most frequently recorded PME value is 3 – frequent. In the floristic composition, there are rare and/or protected species important from the conservational point of view: *Alisma gramineum, Iris pseudacorus, Nuphar lutea, Nymphaea alba, Potamogeton x angustifolius, P. nodosus, P. pusillus, Salvinia natans, Stratiotes aloides*, *Trapa natans, Utricularia vulgaris* and *Zannichellia palustris*; Species not indigenous to the studied area, *Acorus calamus, Azolla filiculoides, Elodea canadensis, E. nuttallii, Paspalum paspalodes, Vallisneria spiralis*. *Elodea canadensis*, *E. nuttallii* and *Paspalum paspalodes* show invasive population dynamics in the surveyed region.

To summarize the variation in aquatic vegetation, principal components analysis (PCA) was carried out. Environmental variables were passively projected into the resulting ordination space. The first two PCA axes explain 50.9% of the variability in species data. According to *post hoc* correlation with the first PCA axis, the first principal component is correlated mainly with water transparency (0.27), riverbed sediment fineness (0.16) and bank material fineness (0.15). Species such as *Potamogeton lucens, P. pectinatus, P. perfoliatus, P. gramineus, P. nodosus* and *P. natans* tend to have higher probability of occurrence at higher water transparency. The second ordination axis is negatively correlated with the level of connectivity with the main channel (-0.41) and water-flow velocity (-0.32), and positively correlated with the bank (0.33) and the riverbed material fineness (0.23). *Ceratophyllum demersum, Spirodela polyrhiza*, *Trapa natans* and *Elodea canadensis* tend to be more abundant in SUs with a lower level of hydrological connectivity with the main channel and lower flow velocity, which are characterized by banks and a riverbed structured of fine, predominantly inorganic material (Fig. 1).

To analyze the effect of environmental variables on the aquatic vegetation, redundancy analysis (RDA) was used. The first canonical axis explains 6.5% of the total variability in the plant species data, while the second (4.7%), third (1.30%) and fourth (0.4%) axes explain less variability. The amount of species variability explained by all canonical axes is 13.5%. The explanatory effect of both first and all canonical axes was statistically significant ($p=0.001$), which was confirmed by the Monte Carlo permutation test.

To build a simpler model with fewer explanatory variables, while at the same time sufficiently explaining species variability, forward selection of environmental variables was done in RDA. According to the marginal effects, the level of connectivity to the main channel has the strongest independent influence (Table 4). It is followed by water transparency, bank material fineness, riverbed sediment fineness, water-flow velocity, bank slope, riverbed sediment naturalness, shading potential of the objects and/or vegetation on the banks, bank naturalness and naturalness of the land-use type on the bank. According to the conditional effects (i.e. the effects that each variable brings in addition to all the variables already selected), the order of the environment variables differs slightly (Table 4). Six environmental variables were chosen using the forward selection: the level of connectivity to the main channel, water transparency, bank material fineness, riverbed sediment fineness, its naturalness, and water-flow velocity. The RDA model using these six variables explains 12.1% of plant species variability (Table 4, Fig. 2). The first axis is best correlated with water transparency, while the second is best correlated

Table 3. Species list.

Species	Abbreviation	%SU	PME mode
Acorus calamus L.	Aco cal	2.22	1
Alisma gramineum Lej.	Ali gra	0.21	1
Alisma plantago-aquatica L.	Ali pla	4.33	1
Azolla filiculoides Lam.	Azo fil	11.84	1
Berula erecta (Hudson) Coville	Ber ere	1.69	1
Butomus umbellatus L.	But umb	36.26	1
Ceratophyllum demersum L.	Cer dem	56.77	1
Ceratophyllum submersum L.	Cer sub	2.22	2
Elodea canadensis Michx	Elo can	11.52	1
Elodea nuttallii (Planchon) St John	Elo nut	3.81	1
Glyceria maxima (Hartman) Holomberg	Gly max	4.55	1
Hydrocharis morsus-ranae L.	Hyd mor	4.76	1
Iris pseudacorus L.	Iri pse	22.41	1
Lemna gibba L.	Lem gib	3.07	1
Lemna minor L.	Lem min	28.54	1
Lemna trisulca L.	Lem tri	8.03	1
Mentha aquatica L.	Men aqu	2.33	1
Myriophyllum spicatum L.	Myr spi	18.71	1
Myriophyllum verticillatum L.	Myr ver	0.63	1
Najas marina L.	Naj mar	7.93	1
Najas minor All.	Naj min	2.11	1
Nuphar lutea (L.) Sibth. & Sm.	Nup lut	3.17	1
Nymphaea alba L.	Nym alb	0.74	1
Nymphoides peltata (S. G. Gmelin.) O. Kuntze	Nyp pel	1.69	1
Oenanthe aquatica (L.) Poiret	Oen aqu	2.64	1
Paspalum paspalodes (Michx.) Scribner	Pas pas	5.81	1
Polygonum amphibium L.	Pol amp	4.65	1
Potamogeton x angustifolius J. Presl.	Pot ang	0.85	1
Potamogeton crispus L.	Pot cri	20.61	1
Potamogeton gramineus L.	Pot gra	35.52	1
Potamogeton lucens L.	Pot luc	38.37	3
Potamogeton natans L.	Pot nat	17.86	3
Potamogeton nodosus L.	Pot nod	23.26	1
Potamogeton pectinatus L.	Pot pec	50.85	3
Potamogeton perfoliatus L.	Pot per	35.73	3
Potamogeton pusillus L.	Pot pus	5.92	1
Ranunculus trichophyllus Chaix ex Vill.	Ran tri	0.53	1
Rorippa amphibia (L.) Besser	Ror amp	40.49	1
Sagittaria sagittifolia L.	Sag sag	8.67	1
Salvinia natans (L.) All.	Sav nat	10.36	1
Scirpus lacustris (L.)	Sci lac	8.67	1
Sium latifolium L.	Siu lat	0.21	1
Sparganium erectum L.	Spa ere	3.07	1
Spirodela polyrhiza (L.) Schleiden	Spi pol	47.46	1
Stratiotes aloides L.	Str alo	0.53	2
Trapa natans L.	Tra nat	24.52	1
Utricularia vulgaris L.	Utr vul	0.53	2
Vallisneria spiralis L.	Val spi	5.50	1
Zannichellia palustris L.	Zan pal	1.37	1

%SU – the percentage of survey units where the species was recorded. in relation to the total number of survey units populated by macrophytes; PME mode – the most frequent PME value recorded.

with water-flow velocity and the level of connectivity to the main channel. Thirteen species are best fitted with this model. *Potamogeton natans, P. gramineus, P. nodosus, P. perfoliatus, P. lucens* and *P. pectinatus* are typical for SUs with higher water transparency and water velocity and coarser material, often artificial, on the banks and riverbed. *Elodea nuttallii, Trapa natans, Ceratophyllum demersum, Spirodela polyrhiza, Oenanthe aquatica* and *Paspalum paspalodes* are characteristic for SUs with a low level of connectivity with the main river channel, showing slower water flow and finer material on the banks and riverbed. *Myriophyllum spicatum* is positioned between these two species groups, occurring in both habitat types (Fig. 2).

To examine the individual relation between best-fitted plant species and each of the selected environmental variables, Spearman's rank correlation coefficients were calculated (Table 5). The obtained results support the RDA model (Fig. 2). The level of connectivity to the main channel is the environmental predictor significantly correlated to all plant species that are best fitted to the RDA model. The direction of the correlation is negative, indicating that these plants tend to be more abundant in the survey units in side-arms and oxbows than in the main channel of the river. The level of hydrological connectivity to the main channel is the most important predictor for *Ceratophyllum demersum, Elodea nuttallii, Spirodela polyrhiza, Trapa natans, Oenanthe aquatica* and *Paspalum paspalodes.* Water transparency is the strongest limiting factor for *Myriophyllum spicatum, Potamogeton gramineus, P. lucens* and *P. pectinatus,*

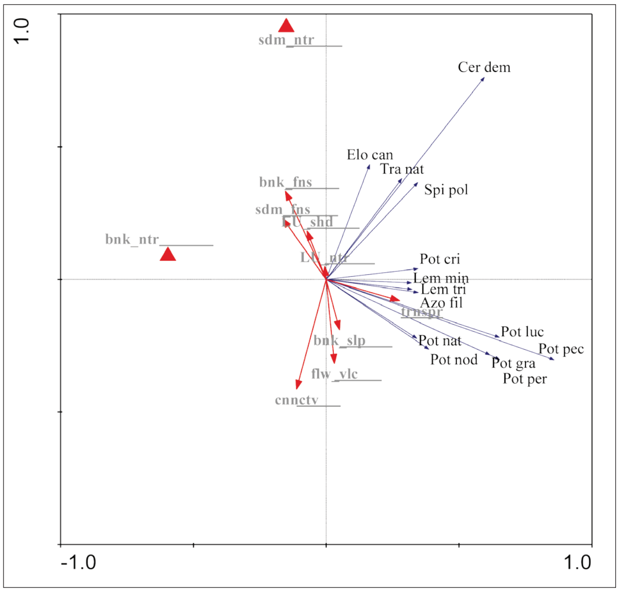

Fig. 1. Species-environment biplot diagram from the PCA with environmental variables passively projected into the resulting ordination space. Environmental variables: LU_shd – land use shading, LU_ntr – land use naturalness, bnk_ntr – bank naturalness, bnk_slp – bank slope, bnk_fns – bank material fineness, sdm_fns – river bed sediment fineness, sdm_ntr – river bed sediment naturalness, cnnctv – hydrological connectivity level, flw_vlc – flow velocity, trnsp – transparency. Details on environmental variables are provided in Table 1. Abbreviations of plant names are presented in Table 3. Eigenvalues: PCA-axis 1: 0.336. PCA-axis 2: 0.173. PCA-axis 3: 0.092. PCA-axis 4: 0.074; variability explained by first two axes: 50.9%.

while the occurrence of *Potamogeton perfoliatus, P. nodosus* and *Myriophyllum spicatum* depends mostly on the sediment fineness on the bottom – they prefer coarser sediment, while its naturalness is the most important factor for *Potamogeton natans*.

DISCUSSION

The survey of aquatic macrophytes was carried out in the Danube River in Serbia. The study was performed in river's main channel and side-arms and oxbows with permanent surface connection to the main channel. The surveyed river section, between rkm 1433 and 846, was divided into 1081 survey units. In total, 49 vascular aquatic plant species were recorded in 946 SUs. In the species composition, both valuable elements of natural aquatic habitats and plants not indigenous to this region are present, indicating the heterogeneity of the complex habitat that represents both the refuge for the endangered species, and the expansion route for invasive aliens. Relatively high species richness, as well as the succession of water lilies and pond weeds found in our study site, are induced by the specific conditions in the impoundments along the Danube [27,36]. The thermophilous not indigenous species *Azolla filiculoides* was also found in the Middle and Lower Danube [36,37,51], while *Vallisneria spiralis* is distributed along the Lower Danube [36,37]. *Elodea canadensis, E. nuttallii* and *Paspalum paspalodes* are rapidly spreading along the whole Danube, indicating the degradation of natural habitats [27,52,53]. According to Janauer and Exler [54], *Ceratophyllum demersum, Potamogeton pectinatus* and *Butomus umbellatus* are the most frequent, nearly ubiquitous plant species along whole Danube River corridor.

According to PCA results, slightly more than 50% of the variability in plant species data can be explained, and according to RDA, 13.5% of that variability relates to the physical environmental factors

Table 4. RDA – Marginal and conditional effects of environmental variables to variability of macrophyte vegetation.

	VIF	Marginal	Conditional				Forward selection	
Variable		Lambda1	Var. No	LambdaA	P	F	step	expl. var.
cnnctv	1.96	0.04	1	0.04	0.001	35.61	1 cnnctv	0.04
trnspr	1.43	0.03	2	0.03	0.001	33.21	2 trnspr	0.07
bnk_fns	1.56	0.03	3	0.02	0.001	21.11	3 bnk_fns	0.09
sdm_fns	3.15	0.03	4	0.01	0.001	13.88	4 sdm_fns	0.10
flw_vlc	1.94	0.02	6	0.01	0.001	12.20	5 sdm_ntr	0.11
bnk_slp	1.93	0.01	9	0.00	0.001	7.29	6 flw_vlc	0.12
sdm_ntr	2.85	0.01	5	0.01	0.003	3.55		
LU_shd	2.17	0.01	8	0.00	0.001	5.47		
bnk_ntr	2.03	0.01	10	0.00	0.051	2.16		
LU_ntr	2.12	0.00	7	0.00	0.010	3.35		

Table 5. Correlation between selected environmental variables and plant species best fitted to the RDA model.

	cnnctv	trnspr	bnk_fns	sdm_fns	sdm_ntr	flw_vlc
Cer dem	-0.46***	0.17***	ns	ns	0.16***	-0.22***
Elo nut	-0.22***	ns	0.09**	ns	ns	-0.16***
Myr spi	-0.25***	0.30***	-0.11***	-0.30***	-0.12***	-0.14***
Pot gra	-0.11***	0.30***	-0.28***	-0.21***	-0.24***	0.12***
Pot luc	-0.21***	0.35***	-0.31***	-0.17***	-0.18***	0.12***
Pot nat	-0.09**	0.19***	-0.36***	-0.22***	-0.42***	0.18***
Pot nod	-0.16***	0.31***	-0.37***	-0.38***	-0.29***	0.12***
Pot pec	-0.22***	0.32***	-0.27***	-0.19***	-0.15***	ns
Pot per	-0.25***	0.48***	-0.36***	-0.51***	-0.32***	ns
Spi pol	-0.28***	ns	-0.07*	0.14***	ns	ns
Tra nat	-0.30***	0.16***	-0.11***	-0.13***	ns	-0.09**
Oen aqu	-0.24***	-0.07*	0.13***	0.07*	ns	-0.16***
Pas pas	-0.27***	ns	0.10*	ns	ns	-0.16***

Legend: ns = not significant. *, **; *** indicate significance at p <0.5; 0.05; 0.001. respectively.

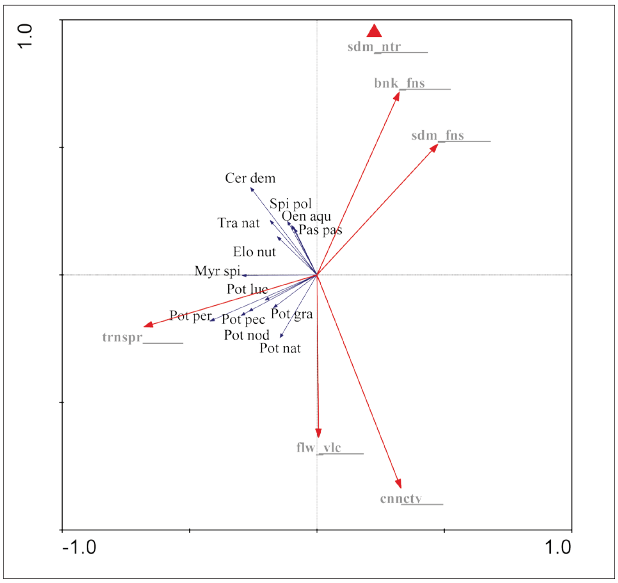

Fig. 2. Species-environment biplot from the RDA after the forward selection of environmental variables. Environmental variables: bnk_fns – bank material fineness, sdm_fns – river bed sediment fineness, sdm_ntr – river bed sediment naturalness, cnnctv – hydrological connectivity level, flw_vlc – flow velocity, trnsp – transparency. Details on environmental variables are provided in Table 1. Abbreviations of plant names are presented in Table 3. Eigenvalues: RDA-axis 1: 0.061. RDA-axis 2: 0.041. RDA-axis 3: 0.012. RDA-axis 4: 0.003; variability explained by first two axes: 10.2%.

selected for this study. Regarding their marginal and conditional effects, the most important are the level of hydrological connectivity with the main channel, water transparency, bank material fineness, riverbed sediment fineness, its naturalness and water-flow velocity.

Many authors consider spatial connectivity along rivers and between a river and its floodplain water bodies as the most important predictor of aquatic plants assemblages, also influencing other physical factors – temperature, water-flow velocity, water transparency, texture of the sediment [27,55-58]. Our study confirmed that the level of hydrological connectivity is the environmental factor with the strongest independent influence and highest marginal effect on plant species variability, but quite weakly correlated to other factors, except water-flow velocity. The next most important predictor is the transparency of the water column. Water transparency is closely connected with light availability, which is a limiting factor for macrophyte growth. Greater transparency usually causes an increase in species richness [59-61]. Some studies showed that macrophytes can improve their own light climate by enhancing the water transparency [62-64], making it hard to distinguish the effect of water transparency on aquatic vegetation from the effect of aquatic vegetation on the water transparency in the SUs with very abundant submerged macrophyte assemblages. Sediment texture along the banks and in the littoral zone of the riverbed plays an important role in the distribution and abundance of aquatic plants. Most of the anchored plant species prefer fine, inorganic substrate with high cohesive strength, which affects both their growth and species recruitment [65]. Other studies confirmed this causality [27,61,66,67], but only some dealt with the influence of macrophyte morphology and patch structure on sediment features [68]. In our dataset, as expected, relatively high, negative correlation was recorded between water transparency and riverbed sediment fineness. Water-flow velocity is the fourth most important factor that influences macrophyte assemblages. It is best correlated with the level of hydrological connectivity. Hydrodynamic forces exerted by water movement on rooted plants is its main direct effect [69,70], while its influence on the photosynthesis and growth through the diffusion of gases and nutrients, its role in the dispersal of seeds and vegetative fragments, the deposition of fine sediment, or the consecutive erosion or burial of the seed bank, are some of its indirect effects [65]. Interrelations between aquatic macrophytes and water-flow conditions have been the focus of many studies [3,10,17,71].

Aquatic plant species coexist along gradients of current speed, water depth and sediment texture, since they do not have single distributional patterns with respect to abiotic factors [17]. In contrast, our study revealed that the species *Ceratophyllum demersum, Elodea nuttallii, Myriophyllum spicatum, Spirodela polyrhiza, Oenanthe aquatica, Paspalum paspalodes, Potamogeton gramineus, P. lucens, P. natans, P. nodosus, P. pectinatus, P. perfoliatus* and *Trapa natans* were found to be best fitted to the RDA model defined by individual abiotic factors. More precisely, the level of connectivity to the main river channel, being a significant factor for all species, particularly governs the distribution and abundance of *Ceratophyllum demersum, Elodea nuttallii, Spirodela polyrhiza, Oenanthe aquatica, Paspalum paspalodes* and *Trapa natans*. These species are mainly distributed in water bodies with less connectivity to the main river channel, i.e. side-arms and oxbows. For *Myriophyllum spicatum*, high water transparency and fine sediment texture have the strongest predictive power, while the occurrence of pondweeds (*Potamogeton gramineus, P. lucens, P. natans, P. nodosus, P pectinatus, P. perfoliatus*) depends also on the texture of the bank material, and they frequently occur in SUs with artificial sediment material. SUs characterized by this type of mixed material are located in the impoundments Đerdap I and II, where permanently submersed, coarse, non-indigenous material, such as former rip-rap, concrete blocks, bricks, etc. contribute to the sediment in the riverbed near the banks. In the space between this larger material, fine sediment carried by the river, or washed down from the present bank, is entrapped. Pondweeds are now rooted in these microhabitats. Although hydrological connectivity and water-flow velocity are correlated factors, connectivity plays a much more important role in the distribution and abundance of aquatic macrophytes. It appears to be a complex factor not exclusively of hydrological features in a particular habitat, but also comprises nutrient conditions, land-use of the area along the banks, climate features, type of river navigation and other so far untouched characteristics.

The species composition of aquatic macrophyte assemblages, with valuable elements of natural aquatic habitats and plants not indigenous to the region, brings us to conclusion that the surveyed section of the Danube River is a complex habitat for both endangered species and for invasive aliens. Understanding the macrophyte/habitat relationship will provide a sound background for tackling conservation aspects *per se*. But such information could be of paramount importance when dealing with invasive alien species and climate change-induced migration of native species in the future, as well as in the complete process of adapting the Danube River Corridor to Water Framework Directive goals.

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