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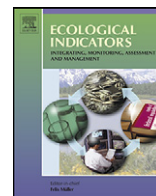


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Brief article

Lake Macrophyte Nutrient Index of standing waters in Serbia (LIMNIS)

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ABSTRACT

The aim of this study was to derive a Macrophyte Nutrient Index for standing waters in Serbia, based on the LEAFPACS method and to correlate it against existing Lake Typology.

A single data matrix of 46 species and 1463 sample quadrats was compiled from two datasets: a revised phytocenological database given according to Braun-Blanquet Cover Abundance Scale and a database of sample quadrats collected during the summer months of 2009, 2010 and 2011 at 31 lakes in Serbia. The nutrient indices (*MSI*) for 46 lake hydrophytes were calculated using the Reprediction Algorithm on *N*-Ellenberg's values. A Macrophyte Nutrient Index (*LIMNIS*) was calculated as the weighted average of *MSI* values for Serbian lakes. Correlation between rescaled *MSI* and original *N*-values was strong (0.82), and the same applies to the root-mean-square error value (0.75). Moderate *LIMNIS* values were derived for meso-oligotrophic lakes, as well as for eutrophic swamps and fens of the Danube floodplain. In the same manner, lakes classified as eutrophic showed the relatively higher *LIMNIS* values, and those recognised as potential conservation resources were characterised by moderate or low *LIMNIS* scores.

Median *LIMNIS* values of six Lake Groups differed by less than one unit, which confirmed the similarity of the previously given Lake Types.

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1. Introduction

Eutrophication is one of the most important and long lasting water quality problems in Europe. Aquatic macrophytes are considered one of the most reliable biological parameters for eutrophication assessment, since they have an earlier response to changes in nutrient conditions than benthic invertebrates or fish and a slower response than phytoplankton, phytobenthos and macroalgae (Duigan et al., 2007; Free et al., 2006; Moss et al., 2003; Pall and Moser, 2009; Penning et al., 2008a, 2008b; Schneider and Melzer, 2003; Søndergaard et al., 2005; Willby et al., 2009). Rooted macrophytes derive their nutrients from sediments or from a combination of sediments and the water column; hence their response to nutrient enrichment tends to be slower than that of phytoplankton, phytobenthos and macroalgae. Nevertheless, their response is recognisable using long-term collated database.

These subjects were analysed at the wider European scale in the REBECCA (Relationships Between Ecological and Chemical Status of Surface Waters) project (Moe et al., 2008), which included

12 countries and 1147 lakes (Penning et al., 2008b), using UKTAG LEAFPACS (Lake Assessment Methods, Macrophyte and Phytobenthos) method (Willby et al., 2009).

LEAFPACS uses a suite of metrics for various pressures, including the LMNI index (Lake Macrophyte Nutrient Index). The LMNI index is based on Ellenberg Nutrient (*N*) indicator values (Ellenberg et al., 1991) and has shown high applicability in lake eutrophication assessment (Penning et al., 2008a; Willby et al., 2009). Moreover, LMNI species scores correlated strongly to other trophic indices (Palmer, 2008), such as Trophic Ranking Score – TRS (Palmer et al., 1992) and Plant Lake Ecotype Index – PLEX (Duigan et al., 2007), even though TRS and PLEX are indicators of alkalinity, rather than nutrient status.

Clearly the European-wide assessment of macrophyte response to eutrophication pressure requires a homogeneous monitoring methodology (European Commission, 2009; Penning et al., 2008a, 2008b) and/or a harmonisation of the classification results of the national assessment systems, in order to achieve intercalibration (Birk et al., 2012; Birk and Willby, 2010; Buffagni and Furse, 2006). According to EC Water Framework Directive (WFD) (European Commission, 2000), the key principles of the intercalibration process are consistency and comparability of the classification results of the monitoring systems for the biological quality elements. The intercalibration process is undertaken within Geographical Intercalibration Groups (GIGs) rather than ecoregions, defined in Annex

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Table 1
Datasets used for reproduction of Ellenberg values.

Data attribute/source of data	Phytocenological database	Field survey 2008–2011	Final dataset
Scale	Braun-Blanquet cover abundance scale	Three-point cover abundance scale	van der Maarel scale
Period of sampling	1956–2010	2008–2011	1956–2011
Number of sample quadrats	725	738	1463
Number of species in dataset	44	35	46
Number of floristic records in dataset	3925	4022	7947
Size of sample quadrat (m ²)	4–100	1	1–100
Total weight of data in dataset, assigned in van der Maarel scale	16,578	20,970	37,548
Mean weight per sample quadrat	22.87	28.41	25.67
Mean number of species per sample quadrat	5.41 (3–14)	5.44 (3–14)	5.43 (3–14)
Min and max number of sample quadrats per species	1–725	7–517	1–986

XI of the Water Framework Directive (European Commission, 2005). According to the GIG classification (European Commission, 2011) Serbia belongs to the Eastern Continental GIG.

The aim of this study is to derive a Macrophyte Nutrient Index for standing waters in Serbia based on the LEAPPACS method (Willby et al., 2009) and to correlate it against existing Lake Typology (Radulović et al., 2011).

2. Materials and methods

2.1. Dataset

A single data matrix of 46 species and 1463 sample quadrats was compiled from two datasets (Table 1):

1. The phytocenological database used for a botanical classification of standing waters in Serbia (Radulović et al., 2011) was revised and 155 relevés of aquatic vegetation from artificial canals (Lazić, 2003, 2006; Ljevnaić-Mašić, 2010) were added to form the first dataset. All relevés were given according to Braun-Blanquet Cover Abundance Scale (Braun-Blanquet, 1964, 1932, 1928) (Table 2).
2. The second dataset consisted of sample quadrats collected during the summer months of 2009, 2010 and 2011 at 31 lakes in Serbia. The sample quadrats were collected at 100 m sectors, repeated 2–8 times per lake. Each 100 m sector was divided into 5 sub-sectors at 20 m intervals. Four-point samples were taken at 0.25, 0.5, 0.75 and >0.75 m water depths at each sub-sector. Sampling was carried out by grapnel, recording species on the three-point scale (Table 2). In some lakes sampling by sectors was repeated three times during summer months in order to minimise the impact of seasonal variation on the results.

Locations of sectors were selected according to the Braun-Blanquet approach (Braun-Blanquet, 1964, 1932, 1928). The number of sampling sectors depended upon the heterogeneity of the vegetation (Gunn et al., 2010). Sampling points were located within each of the main vegetation types characteristic of the lake (Willby et al., 2009).

Due to the low taxonomic resolution of data presented at the generic level, some species sensitive to eutrophication at the European or C-GIG scale (Penning et al., 2008b) were not included in this

study (*Chara aspera* Deth. ex Willd., *Chara connivens* Saltzm., *Chara contraria* A. Br., *Chara virgata* Kutz. [syn. *Chara delicatula* Agardh], *Chara globularis* Thuill., *Chara hispida* L., *Chara rudis* A. Br., *Chara tomentosa* L. and *Chara strigosa* A. Br.).

The final data matrix was collated using FLORA software (Karadžić et al., 1998) with three or more aquatic macrophytes per sample quadrat. Homogeneity of the final dataset was achieved by selecting only submerged and floating hydrophytes for further analysis (Duigan et al., 2007; Penning et al., 2008b; Willby et al., 2009).

The average number of species per sample quadrat in the final dataset was 5.43, resulting in a total of 7947 floristic records, dating from 1956 to 2011. The number of sample quadrats per species ranged from 1 to 968, in which sampling area varied between 1 m² and 100 m².

The original Braun-Blanquet scale from the first dataset and three-point scale from the second dataset were converted into the van der Maarel (1979) scale (Table 2).

2.2. Data analysis

The nutrient indices for 46 lake hydrophytes were calculated using the Reprediction Algorithm on *N*-Ellenberg's values (Hill et al., 2000; Willby et al., 2009) (Table 3). Ellenberg's indicator values (Ellenberg et al., 1991) for 41 species are given according to JUICE 6.1 (Tichý, 2002). The other five species (*Chara vulgaris*, *Nitella opaca*, *Potamogeton × fluitans*, *Riccia fluitans*, *Ricciocarpus natans*) lack the original indicator value.

The final data matrix specified the occurrence of species (*i*) in quadrat samples (*j*). For each sample quadrat, a sample index (*SI*) score was calculated by cover-weighted (*C*) averaging of Ellenberg *N*-values (*N*) for species for which this value was available (Table 3). Mean Species Indicator ($MSI_j^{(1)}$) was calculated by weighted averaging of sample index scores.

$$SI_i = \frac{\sum_j C_{ij} \times N_j}{\sum_j C_{ij}} \quad (1)$$

$$MSI_j^{(1)} = \frac{\sum_i C_{ij} \times SI_i}{\sum_i C_{ij}} \quad (2)$$

Table 2
Cover abundance scales used in datasets.

Scale/corresponding cover	Scale values						
Braun-Blanquet scale	r	+	1	2	3	4	5
Corresponding cover (%)	0	0.1	5.0	17.5	37.5	62.5	87.5
Three-point scale	1	1	1	1	2	2	3
Corresponding cover (%)	12.5	12.5	12.5	12.5	50.0	50.0	87.5
van der Maarel scale	1	2	3	5	7	8	9

Table 3
Nutrient indices (*MSI*) for 46 lake hydrophytes.

Species	Number of samples	Original Ellenberg <i>N</i> value	Sensitive/tolerant ^b	Nutrient index (<i>MSI</i>)
<i>Azolla filiculoides</i> Lam.	203	8		7.55
<i>Callitriche palustris</i> L.	39	4		4.92
<i>Ceratophyllum demersum</i> L. subsp. <i>demersum</i>	968	8	T-E	7.32
<i>Ceratophyllum submersum</i> L. subsp. <i>submersum</i>	35	7		6.82
<i>Chara vulgaris</i> L.	4 ^a	–	S C-GIG H	4.72
<i>Elodea canadensis</i> Michx	27	7	T C-GIG L	7.32
<i>Elodea nuttallii</i> (Planchon) St. John	215	7	T-E	6.47
<i>Hydrocharis morsus-ranae</i> L.	357	6	T-E	6.74
<i>Lemna gibba</i> L.	309	8		7.29
<i>Lemna minor</i> L.	711	6	T-E	6.63
<i>Lemna trisulca</i> L.	263	5	T-E	5.54
<i>Marsilea quadrifolia</i> L.	3 ^a	6		5.68
<i>Myriophyllum spicatum</i> L.	615	7		6.49
<i>Myriophyllum verticillatum</i> L.	44	8	T-E	6.97
<i>Najas marina</i> L.	162	6		5.95
<i>Najas minor</i> All.	61	4		4.41
<i>Nitella opaca</i> (C.Agardh ex Bruzelius)	2 ^a	–		5.69
<i>Nuphar lutea</i> (L.) Sm.	110	6	T C-GIG L	6.47
<i>Nymphaea alba</i> L.	195	5		5.70
<i>Nymphoides peltata</i> (S. G. Gmelin) O. Kuntze	353	7	T-E	7.12
<i>Polygonum amphibium</i> L.	177	4	S C-GIG H T C-GIG L	4.64
<i>Potamogeton acutifolius</i> Link	23	6		5.58
<i>Potamogeton crispus</i> L.	160	5	T-E	5.72
<i>Potamogeton gramineus</i> L.	24	5	S C-GIG L	5.62
<i>Potamogeton lucens</i> L.	81	7	T C-GIG L	6.66
<i>Potamogeton natans</i> L.	16	5	T C-GIG L	5.60
<i>Potamogeton nodosus</i> Poiret	85	5		5.60
<i>Potamogeton obtusifolius</i> Mert. & Koch	25	6	T-E	4.26
<i>Potamogeton pectinatus</i> L.	113	8	T-E	7.42
<i>Potamogeton perfoliatus</i> L.	97	6		6.50
<i>Potamogeton pusillus</i> L.	46	5	T-E	3.58
<i>Potamogeton</i> × <i>fluitans</i> Roth (3 <i>P. lucens</i> L. × 1 <i>P. natans</i> L.)	13	–		4.80
<i>Ranunculus aquatilis</i> L.	34	6	S C-GIG H	4.29
<i>Ranunculus circinatus</i> Sibth.	50	8	T-E	7.27
<i>Ranunculus trichophyllus</i> Chaix subsp. <i>trichophyllus</i>	23	7		6.16
<i>Riccia fluitans</i> L.	22	–		4.28
<i>Ricciocarpus natans</i> (L.) Corda	16	–		3.04
<i>Salvinia natans</i> (L.) All.	690	7	T-E	7.17
<i>Spirodela polyrhiza</i> (L.) Schleiden	857	6	T-E	6.74
<i>Stratiotes aloides</i> L.	1 ^a	6	T-E	5.59
<i>Trapa natans</i> L. agg.	336	8	T-E	7.94
<i>Utricularia australis</i> R. Br.	27	3	S-E	4.99
<i>Utricularia vulgaris</i> L.	148	4		4.11
<i>Vallisneria spiralis</i> L.	65	7		6.90
<i>Wolffia arrhiza</i> (L.) Horkel ex Wimmer	136	6		6.51
<i>Zannichellia palustris</i> L.	6 ^a	8	T-E	7.76

^a Species occurring less than 10 times in the final dataset.

^b Species sensitivity to eutrophication (Penning et al., 2008b): T-E, species tolerant to eutrophication at the European scale; S-E, species sensitive to eutrophication at the European scale; S C-GIG, species sensitive to eutrophication at the Central/Baltic GIG scale; T C-GIG, species tolerant to eutrophication at the Central/Baltic GIG scale; H, high alkalinity lakes; L, low alkalinity lakes.

MSI values for species that lacked original *N*-value (*MSI'*) were calculated following the same procedure and corrected (*MSI'⁽¹⁾*) as follows (Hill et al., 2000):

$$MSI'^{(1)} = MSI' + (\text{means}_S(N - MSI'^{(1)})) \quad (3)$$

where means_S is mean taken over the *S* (20) species for which $\text{abs}(MSI'^{(1)} - MSI')$ was the smallest.

According to Hill et al. (2000) two more iterations were carried out to derive *MSI'⁽²⁾* and *MSI'⁽³⁾* values.

After three iterations, original *N*-values were regressed against *MSI'⁽³⁾* values, but only for species with more than 10 occurrences in the dataset (Willby et al., 2009). To calculate final *MSI* values, the regression equation was applied to original *N*-values and to *MSI'⁽³⁾* values.

As suggested by Hill et al. (2000), goodness-of-fit of rescaled values (*MSI_{final}*) to the original *N*-values was measured by root-mean-square error (RMSE), which was defined as

$$RMSE_{MSI} = \sqrt{(\text{average}(MSI - N)^2)} \quad (4)$$

After final macrophyte *MSI* values were repredicted, Macrophyte Nutrient Index (*LIMNIS*) was calculated as the weighted average of *MSI* values for the 82 lakes used in the Radulović et al. (2011) lake typology. Only lakes with at least two indicator species with at least 10 records were included in the analysis. Range, median, extreme and outlier values of *LIMNIS* scores of different lake types were plotted using Statistica 10 (StatSoft Inc., 2010) Whisker Plot.

3. Results and discussion

3.1. Species typology

MSI values were calculated for 46 macrophyte species (Table 3). Correlation between rescaled *MSI* and original *N*-values was strong (0.82). Similar results (corr. 0.81) were achieved in the case of a far larger dataset in Britain (Hill et al., 2000). The same applies to the RMSE value (0.75).

At the broader European scale, given by Penning et al. (2008b), only *Utricularia australis* was recognized as sensitive. However, for

the Central/Baltic GIG region, including Eastern Continental GIG lakes, four species (*C. vulgaris*, *Polygonum amphibium*, *Ranunculus aquatilis* and *Potamogeton gramineus*) were classified into the same group of sensitive plants (Penning et al., 2008b).

On the other hand, the results are in accord with the list of tolerant species at the European scale (MSI values above 5.54) except for *Potamogeton pusillus* (3.58) and *Potamogeton obtusifolius* (4.26). These two species mainly occurred in mesotrophic lakes in Serbia, such as Vlasinsko jezero lake (Radulović et al., 2011). Similar results were obtained in Britain, where Palmer (2008) found that species recognised as tolerant at the European scale also had high TRS values (Palmer et al., 1992), as well as high PLEX values (Duigan et al., 2007). At the same time, *P. obtusifolius* had relatively moderate TRS and PLEX values and was generally classified as a mesotrophic/meso-eutrophic species (Rodwell et al., 1995).

U. australis had the largest discrepancy between reproduced and original value (1.99) (Table 3). The original *N*-value was 3.00 and the rescaled was 4.99. According to Kosiba (2004) and Dítě et al. (2006), *U. australis* prefers eutrophic habitats throughout Central Europe, while in Serbia it occupies meso-oligotrophic and eutrophic lakes (Radulović et al., 2011).

Four species in the dataset (*C. vulgaris*, *N. opaca*, *Stratiotes aloides* and *Zannichellia palustris*) are under-sampled, occurring in less than 10 sample quadrats. Nevertheless, the discrepancy between original and rescaled values for these species was low (0.41 for *S. aloides* and 0.24 for *Z. palustris*).

Species that were not included in the classification scheme for C-GIG according to Penning et al. (2008b), such as *Callitriche palustris* (MSI 4.92), *Najas minor* (MSI 4.41), *Potamogeton × fluitans* (MSI 4.80), *R. fluitans* (MSI 4.28), *R. natans* (MSI 3.04), and *Utricularia vulgaris* (MSI 4.11), had MSI values below 5.62. These species have been recognised as potentially sensitive to eutrophication for the area studied.

3.2. Conclusions on lake typology

Radulović et al. (2011) described a botanical classification for lakes in Serbia based on vegetation assemblage, basic water quality parameters and geographic region. Seven distinct Lake Groups were recognised (Table 4). Lake Groups 4–7 were characterised by typically species-rich, eutrophic lakes with *Ceratophyllum demersum* dominant, followed by *Hydrocharis morsus-ranae*, *Lemna minor*, and *Salvinia natans* as constants, while Lake Groups 2 and 3 comprise species-poor, meso-eutrophic sites with *Myriophyllum spicatum* constant. Lake Group 1 contained a single, high mountain, glacial, oligotrophic lake in Montenegro (outlying-group), dominated by charophytes (stoneworts).

Instead of correlating a macrophyte index to a single environmental variable, the opposite approach could be applied, by underpinning indices with a lake typology (Duigan et al., 2007; Stelzer et al., 2005). Weighted average site scores (*LIMNIS*) were calculated for the 82 lakes used for the typology of standing waters in Serbia (Radulović et al., 2011). Different Lake Groups showed expected differences in median *LIMNIS* values (Fig. 1).

Moderate *LIMNIS* values were derived for meso-oligotrophic lakes (Groups 2 and 3), as well as for eutrophic swamps and fens of the Danube floodplain (Group 4). Due to their specific nutrient dynamics, caused by Danube flooding regime (Barrat-Segretain et al., 1998; Durisch-Kaiser et al., 2011; Madsen, 2010), lakes of Group 4 could be disturbed by periodical changes in trophic status, resulting in the wide range of *LIMNIS* scores (Fig. 1).

Lake Groups 5–7, classified as eutrophic lakes, showed the highest median values. Lakes recognised as potential conservation resources (Groups 1–3) were characterised by moderate or low *LIMNIS* scores.

Table 4
Lake Typology of standing waters in Serbia (Radulović et al., 2011).

Lake group	Description
Group 1	High mountain, glacial, oligotrophic lake in Montenegro Characteristic species: <i>Chara aspera</i> Deth. ex Willd., <i>Chara contraria</i> A.Br. ex Kutzing, <i>Chara delicatula</i> Agardh, <i>Chara globularis</i> Thuill., <i>Chara rudis</i> (A. Braun) Leonh., <i>Nitella flexilis</i> (L.) C. Agardh, <i>Nitella opaca</i> (C. Agardh ex Bruzelius), <i>Potamogeton pusillus</i> L., <i>Ranunculus trichophyllus</i> Chaix subsp. <i>trichophyllus</i>
Group 2	Diverse group of low and high meso-oligotrophic lakes Characteristic species: <i>Bidens tripartita</i> L., <i>Eleocharis palustris</i> (L.) Roemer & Schultes subsp. <i>palustris</i> , <i>Lysimachia nummularia</i> L., <i>Marsilea quadrifolia</i> L., <i>Myriophyllum spicatum</i> L., <i>Nymphoides peltata</i> (S. G. Gmelin) O. Kuntze, <i>Polygonum amphibium</i> L., <i>Polygonum hydropiper</i> L., <i>Rorippa sylvestris</i> (L.) Besser
Group 3	Artificial meso-oligotrophic lakes Characteristic species: <i>Ceratophyllum demersum</i> L. subsp. <i>demersum</i> , <i>Eleocharis acicularis</i> (L.) Roemer & Schultes, <i>Hydrocharis morsus-ranae</i> L., <i>Lemna minor</i> L., <i>Lemna trisulca</i> L., <i>Myriophyllum spicatum</i> L., <i>Najas minor</i> L., <i>Phragmites australis</i> (Cav.) Trin. ex Steudel, <i>Potamogeton crispus</i> L., <i>Potamogeton pectinatus</i> L.
Group 4	Low and eutrophic lakes of Danubian flood plain, swamps and fens Characteristic species: <i>Glyceria maxima</i> (Hartman) Holmberg, <i>Lycopus europaeus</i> L., <i>Lysimachia vulgaris</i> L., <i>Lythrum salicaria</i> L., <i>Mentha aquatica</i> L., <i>Myriophyllum spicatum</i> L., <i>Najas marina</i> L., <i>Phragmites australis</i> (Cav.) Trin. ex Steudel, <i>Robinia pseudacacia</i> L., <i>Sium latifolium</i> L., <i>Stachys palustris</i> L.
Group 5	Small lowland pools and ponds, eroded shore zone, fens Characteristic species: <i>Lemna minor</i> L., <i>Lemna trisulca</i> L., <i>Nymphoides peltata</i> (S. G. Gmelin) O. Kuntze, <i>Rorippa amphibia</i> (L.) Besser, <i>Spirodela polyrrhiza</i> (L.) Schleiden
Group 6	Low and shallow or sluggish and sheltered waters in succession Characteristic species: <i>Carex elata</i> All. subsp. <i>elata</i> , <i>Iris pseudoacorus</i> L., <i>Lysimachia vulgaris</i> L., <i>Lythrum salicaria</i> L., <i>Phragmites australis</i> (Cav.) Trin. ex Steudel, <i>Polygonum amphibium</i> L., <i>Rorippa amphibia</i> (L.) Besser, <i>Rumex hydrolapathum</i> Hudson, <i>Scirpus lacustris</i> L., <i>Sium latifolium</i>
Group 7	Typical lowland, eutrophic lakes of Danubian flood plain Characteristic species: <i>Ceratophyllum demersum</i> L. subsp. <i>demersum</i> , <i>Cyperus michelianus</i> (L.) Link subsp. <i>michelianus</i> , <i>Eleocharis acicularis</i> (L.) Roemer & Schultes, <i>Hydrocharis morsus-ranae</i> L., <i>Lemna minor</i> L., <i>Lemna trisulca</i> L., <i>Limosella aquatica</i> L., <i>Myriophyllum spicatum</i> L., <i>Nymphoides peltata</i> (S. G. Gmelin) O. Kuntze, <i>Polygonum lapathifolium</i> L., <i>Polygonum mite</i> Schrank, <i>Salvinia natans</i> (L.) All., <i>Spirodela polyrrhiza</i> (L.) Schleiden

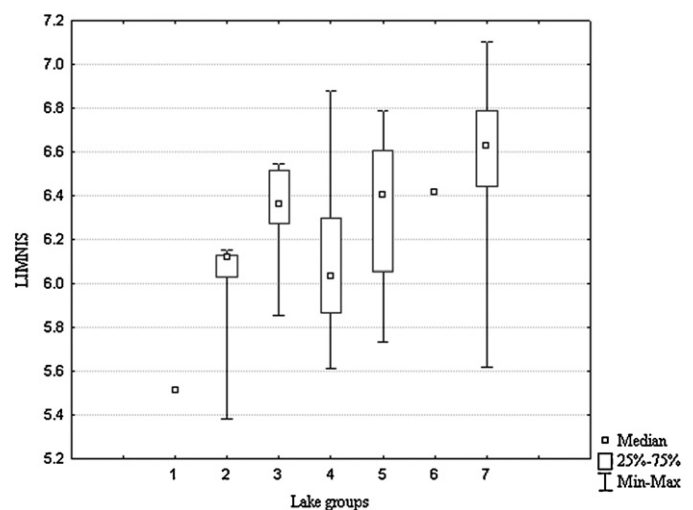


Fig. 1. Whisker box plot (median distribution) of *LIMNIS* (Macrophyte Nutrient Index) scores of different lake groups in Serbia.

Median *LIMNIS* values of Lake Groups 2–7 (Fig. 1) differed by less than one unit, which confirmed the similarity of the previously given Lake Types (Radulović et al., 2011) and PLEX index of ecologically equivalent Lake Groups in Britain (Duigan et al., 2007). Median PLEX values and ranges were very similar for these Lake Groups, mainly typified by *L. minor*, *Elodea canadensis*, *M. spicatum*, water-lilies and wide range of pondweeds.

The range of *LIMNIS* scores correlated with the Lake Typology given in Radulović et al. (2011).

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