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PHYTOBENTHOS IN LITHUANIAN RIVERS AND ITS EMPLOYMENT FOR THE ASSESSMENT OF THE ECOLOGICAL STATUS OF RIVERS

Summary of doctoral dissertation
Biomedical sciences, botany (04 B)

Vilnius, 2014

The research was carried out at the Nature Research Centre, Institute of Botany in 2008–2012

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The dissertation will be defended at the public session held by the Council for Biomedical Sciences on 27th June 2014 at 1:00 p.m. in the White Hall of the Institute of Botany, Nature Research Centre.

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The summary of the doctoral dissertation was distributed on 27th May 2014.

The dissertation is available at the libraries of the Nature Research Centre, Institute of Botany and Vilnius University.

VILNIAUS UNIVERSITETAS
GAMTOS TYRIMŲ CENTRO BOTANIKOS INSTITUTAS

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LIETUVOS UPIŲ FITOBENTOSAS IR JO RODIKLIŲ TAIKYMAS VERTINANT UPIŲ EKOLOGINĘ BŪKLĘ

Daktaro disertacijos santrauka
Biomedicinos mokslai, botanika (04 B)

Vilnius, 2014

Disertacija rengta 2008–2012 metais Gamtos tyrimų centro Botanikos institute

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Disertacija bus ginama viešame Biomedicinos mokslo krypties tarybos posėdyje 2014 m. birželio mėn. 27 d. 13:00 val. Gamtos tyrimų centro Botanikos instituto Baltojoje salėje.

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Disertacijos santrauka išplatinta 2014 m. gegužės mėn. 27 d.

Disertaciją galima peržiūrėti Gamtos tyrimų centro Botanikos instituto ir Vilniaus universiteto bibliotekose.

INTRODUCTION

Phytobenthos in the rivers is a diverse benthic algal community composed of micro- and macroalgae growing on the bottom or attached to the different substrates under water. Benthic algae are important producers of organic matter (BIGGS & KILROY, 2000; SCHAUMBURG et al., 2004; 2006; ISTVÁNOVICS & HONTIL, 2011) and their production can reach 20 g C/m^2 per day (WEHR & SHEATH, 2003; ALLAN & CASTILLO, 2007; SEGURA et al., 2010). During assimilation of mineral and organic matter, algae are involved in water self-purification processes, eliminate heavy metals, radionuclides and other toxic compounds (LAI et al., 2003; BARSANTI & GUALTIERI, 2006; AL-HOMAIDAN et al., 2011). Moreover, benthic microalgae enhance sediment cohesion by structures of the thallus, secretion of mucus and stabilize fine sediments, mud particles form leaching out the riverbed. Macroalgae commonly exhibit secondary overgrowth by different algae. Such thick assemblages of phytobenthos form a protection cover for other organisms from the impact of river flow and predators (ALLAN, 1995; BIGGS, 1996; WEHR & SHEATH, 2003).

The data on river phytobenthos in Lithuania are not sufficient (VAILIONIS, 1930; POCIENĖ & STOČKUS, 1987; BAKŪNAITĖ & KOSTKEVIČIENĖ, 1998; KOSTKEVIČIENĖ & LAUČIŪTĖ, 2005; 2009), the main attention has been focused on phytoplankton studies (MARKEVIČIENĖ, 1962; ŪSELYTĖ, 1975; 1978; KOSTKEVIČIENĖ, 1995a,b; 1997; 1998; 2001). Mostly the taxonomic and systematic aspects of algae have been analysed, however, there are not enough data on phytobenthos species diversity, differences and alterations, genetic polymorphism and the impact of changes of environmental factors on benthic algae distribution.

Phytobenthos structure and algal abundance are closely related to the quality of water in the ecosystems. Therefore, over the last decade, a special attention was given to the phytobenthos research in Europe in order to adapt their parameters for water status assessment (BIGGS & KILROY, 2000; GUTOWSKI et al., 2004; FOERSTER et al., 2004; SCHAUMBURG et al., 2004; 2006; GUTOWSKI & FOERSTER, 2006; BALLESTEROS et al., 2007; JELIC MRCELIC et al., 2012). In the Water Framework Directive of European Union (EU WFD, 2000/60EC), phytobenthos is one of the recommended biological water quality parameters (QUEVAUVILLER et al., 2008; SWD, 2012a−l). Benthos macroalgae species, their parameters are used for the status assessment of transitional and coastal waters in some of the EU countries such as Ireland, the United Kingdom, Lithuania, the Netherlands, Sweden and Germany (OLENIN et al., 2012; SWD, 2012c,f,g,i,k,l), whereas in Ireland, Belgium and the United Kingdom for the river and lake status assessment, macroalgae are investigated together with macrophytes (KELLY et al., 2006b; VAN DE BUND, 2009; SWD, 2012b,f,l). The Environmental Protection Agency (EPA), carrying out the monitoring of Lithuanian rivers, adapted benthic fauna (Danish Stream Fauna Index DSFI) and ichthyofauna

(Lithuanian Fish Index LFI) parameters for the assessment of ecological status (LIETUVOS..., 2011; VAITIEKŪNIENĖ et al., 2011). National methods for the river status assessment are also under development by using other biological elements of flora such as macrophytes (Reference Index of macrophytes RI) (SINKEVIČIENĖ, 2010–2011; ZVIEDRE, 2013) and benthic diatoms (GUDAS, 2010). However, for the assessment of ecological status of the Lithuanian rivers, benthic macroalgae and phytobenthos index (BI) are not used, because so far detailed studies on phytobenthos in Lithuanian rivers have not been carried out, the aggregated data of which would allow to create a system for the assessment of ecological status of the water bodies.

The aim of the study was to investigate species diversity and ecology of phytobenthos in the Lithuanian rivers, to ascertain the indicative merits of phytobenthos parameters in assessing the river ecological status.

Main tasks:

1. to investigate river phytobenthos species diversity, composition of ecological groups and species distribution, to make the checklist of benthic algal species;
2. to identify predominant and rare in the Lithuanian rivers macroalgae species by molecular methods, to ascertain genetic polymorphism of the dominant green algae *Cladophora glomerata* (L.) Kütz.;
3. to investigate environmental factors, determining the development peculiarities of phytobenthos in rivers;
4. to select the indicator species of macroalgae and the values of their coverage for the assessment of preliminary status of different types of the Lithuanian rivers *in situ* and to evaluate the employment possibilities of phytobenthos index (BI).

The defended statements:

- Phytobenthos species diversity, composition of ecological groups and species distribution depend on the river width, depth, current velocity, type of substrate, water conductivity and the amount of total nitrogen.
- The genetic polymorphism of predominant in the Lithuanian rivers *Cladophora glomerata* could be influenced by water status.
- Macroalgal coverage could be employed for the preliminary assessment of the status of the Lithuanian rivers *in situ*, the phytobenthos index (BI) is a suitable parameter to evaluate the ecological status of rivers.

Scientific novelty of the study. In the thesis, the investigations on phytobenthos in 73 Lithuanian rivers for the first time were carried out in detail, the analysis of taxonomic and ecological groups composition was accomplished, the development peculiarities were determined. The checklist of phytobenthos species was compiled. New to Lithuania 43 benthic algae species were identified. For the first time, the molecular studies were performed on the predominant and rare in the Lithuanian rivers macroalgae. For the preliminary assessment of the status of rivers *in situ*, the indicator species of macroalgae were selected and their coverage values (%) in rivers of different types and varying status were indicated. The assessment of ecological status of the Lithuanian rivers was ascertained according to phytobenthos index (BI) parameters for the first time.

Significance of the study. The accomplished investigations on river phytobenthos and the compiled checklist of algae species enable to expand the taxonomic checklist of algae of freshwater bodies. Detailed descriptions of new to Lithuania benthic algae species with authentic pictures were prepared, morphometric data were presented and the distribution of species was indicated. The first data on genetic diversity of macroalgae in the Lithuanian rivers were obtained, predominant and rare in the Lithuanian rivers macroalgae species were identified, genetic polymorphism of dominant in river benthos green algae *Cladophora glomerata* was ascertained. The results are important and useful to scientists in the world in performing comparative molecular research or solving the problems of monitoring of the ecological changes. According to EU WFD requirements for the assessment of river status, the methodology for determination of indicator benthic macroalgal species and the selection of algae coverage values for rivers of different status as well as application of phytobenthos parameters (index BI) is under development. The results of phytobenthos studies are important in the assessment and prediction of the changes in ecological status of the Lithuanian rivers and in nature conservation programmes.

Presentation of the results. The results of the study were presented at three international and two regional conferences: „28th International Phycological Conference“ (Szczecin-Cieszyno Drawskie, Poland, 2009); „29th International Conference of the Polish Phycological Society“ (Kraków-Niedzica, Poland, 2010); „30th International Conference of the Polish Phycological Society“ (Wrocław-Poawłowice, Poland, 2011); „5th Scientific-Practical Conference, Marine and Coastal Research-2011“ (Klaipėda, 2011); „16th Lithuanian Conference of Young Scientists, Science–Future of Lithuania“ (Vilnius, 2013).

Volume and structure of the dissertation. The dissertation includes eight chapters: Introduction, Literature Review, Description of the study area, Materials and Methods, Results, Discussion, Conclusions, References (360 literature sources). Supplementary information is provided in Appendix (17 tables, 49 figures). Volume of the dissertation is 192 pages (excluding appendix), and is illustrated with 17 tables and 30 figures. The dissertation is written in Lithuanian with the summary in English.

MATERIALS AND METHODS

Phytobenthos studies were carried out on 73 sections of 64 Lithuanian rivers in July–August 2009–2012. The investigations were performed according to the GUTOWSKI et al. (2004), Schaumburg et al. (2004; 2005; 2006) and Gutowski & Foerster (2006) method for the assessment of the ecological status of running waters based on phytobenthos, excluding diatoms and *Charales*. A heterogeneous substrate section up to 20 m in length was selected in each river. River width and depth were measured *in situ*. Current velocity, turbidity and transparency, riverbed meandering and naturalness, shading by riparian vegetation (%, point scale), substrate and algal cover of riverbed (%) were visually evaluated and described. A total of 226 phytobenthos samples were collected.

The riverbed was examined using an underwater viewer, all found species of phytobenthos were recorded and their abundance was estimated according to a five-point scale (Table 1). Benthic algae, their growth form and coverage (%) were estimated. Depending on substrate type ≥ five samples of phytobenthos were taken along longitudinal and transverse transects (1 m in width). Samples from stones and artificial substrates were collected using knife or scalpel, algae from macrophytes were scraped with the brush. Algae growing on/in fine sediments were collected together with substrate, using pipette or by turning a Petri dish upside down on the substrate and then slipping a spatula underneath. In deep rivers, phytobenthos was collected along the shore, macrophytes were pulled out with a hook. Samples were fixed with 40% formaldehyde solution till final 4% concentration.

Table 1. The scale for estimation of phytobenthos species abundance and algal coverage

	Estimation of algae abundance	**Point scale**
In situ	Dominant, cover 35–100% of the riverbed in sampling section	5
	Abundant, cover 5–35% of the riverbed in sampling section	4
	Just visible in the field, cover 5% of the riverbed in sampling section	3
In the laboratory	Microscopically abundant, many individuals	2
	Microscopically rare, several individuals	1

Phytobenthos was analysed using Motic B3 and the Quanta 250/450/650 microscopes, photomicrographs were taken using a Moticam 2300 camera. Various morphological characteristics were measured for species identification, each 30 individuals. The checklist of phytobenthos species, which specifies algal ecological groups based on species attachment to the substrate, was compiled. Considering the distribution pattern of algae in the studied rivers, the species were classified into five groups as follows: very rare species found in < 2% of rivers, rare – 3−10%, common – 11−20%, frequent – 21−50%, abundant – 51−100%. The descriptions of phytobenthos species new to Lithuania include original photos, measurements of original morphological characteristics and species distribution. The Sorensen index was used for algal flora similarity analysis (Sørensen, 1948; Krebs, 1989).

To determine macroalgal coverage values and phytobenthos index (BI) employment possibilities to assess ecological status of the Lithuanian rivers, the studies were carried out in the five types of rivers, based on the Lithuanian rivers typology (Aplinkos..., 2009).

Coverage (%) of macroalgae species *in situ* was evaluated in 48 rivers. The ecological status of rivers (Table 2) was determined based on phytobenthos index (BI) values calculated depending on species attribution to different indicative groups (A, B, C, D) and their abundance on a five-point scale (Table 1) (GUTOWSKI et al., 2004; FOERSTER et al., 2004; SCHAUMBURG et al., 2004; 2006):

$$\mathbf{BI} = \frac{\sum_{i=1}^{n_A} Q_{Ai} + \frac{1}{2}\sum_{i=1}^{n_B} Q_{Bi} - \frac{1}{2}\sum_{i=1}^{n_C} Q_{Ci} - \sum_{i=1}^{n_D} Q_{Di}}{\sum_{i=1}^{n_A} Q_{Ai} + \sum_{i=1}^{n_B} Q_{Bi} + \sum_{i=1}^{n_C} Q_{Ci} + \sum_{i=1}^{n_D} Q_{Di}} \times 100$$

BI – index value,
Q_A – squared quantity of a taxon from indicative group A,
Q_B – squared quantity of a taxon from indicative group B,
Q_C – squared quantity of a taxon from indicative group C,
Q_D – squared quantity of a taxon from indicative group D

Table 2. Ecological status classification according to phytobenthos index (BI) calculated values

Index BI value	Classes of ecological status
≥ 50	**High**
≥ 25	**Good**
≥ 0	**Moderate**
≥ -50	**Poor**
< -50	**Bad**

Data analysis was completed using Statistica 6.0, SPSS 17.0 and Brodgar 2.7.2 programmes. The rivers were classified into four groups based on cluster analysis method. Principal component analysis (PCA) was used to compare similarity of phytobenthos species composition and ecological status of the Lithuanian rivers according to different biological water quality elements and their parameters (index BI, Reference Index of macrophytes (RI), Danish Stream Fauna Index (DSFI), Lithuanian Fish Index (LFI)). The Pearson correlation coefficient was calculated to select indicator macroalgae species, to determine the relationship between environmental factors and phytobenthos, water quality parameters (indices BI, RI, DSFI, LFI). Redundancy analysis (RDA) was applied to evaluate interaction between the abundance of algae and morphometric, hydrological, hydrophysical-chemical parameters of rivers.

A total of 42 samples of *Batrachospermum*, *Lemanea*, *Thorea*, *Hildenbrandia*, *Vaucheria* and *Cladophora* algae were collected from 12 rivers in Lithuania for molecular investigation in June–October 2011–2012. Further sampling of *Cladophora* algae was performed in one river in Germany (Kösterbeck) in 2012. Samples were prepared after VIS et al. (1998), SHERWOOD & SHEATH (1999).

The identification and genetic diversity determination of macroalgal species were performed based on *rbc*L, 18S rRNA genes and internal transcribed spacer (ITSI, ITSII) regions. DNA extraction was made according to our modified protocol by using *Qiagen DNeasy Plant Mini Kit* (QIAGEN, 2006). For DNA synthesis, 20 primers (17–25 bp) were used, five of these were created in the laboratory, others − selected from the literature data (BAKKER et al., 1995; ROE et al., 1996; VIS et al., 1998; SHERWOOD & SHEATH, 1999; KATANA et al., 2001; SAKAYAMA et al., 2004; KUČERA et al., 2006; SCHAIBLE et al., 2009; etc.). The polymerase chain reaction (PCR) was optimized. PCR mixture (10 μl) components were as follows: 1 μl dNTP; 1 μl 10×PCR buffer; 1.5–3.5 mM $MgCl_2$; 0.07 μl Moltaq; 1 μl of each primer, ITS − each 1.2 μl; 0.2 μl DMSO; 1 μl extracted DNA; till final volume ddH_2O. Temperature regime was applied for PCR reaction, respectively. The amount (440–1240 bp) and clearness of PCR product were checked in 1.5% agarose gel. Electrophoretic results were analysed by gel documentation programme. DNA was purified out of electrophoretic gel with *InnuPREP Gel Extraction Kit* (AG ANALYTIK..., 2009). Algal DNA was sequenced by *Applied Biosystems 3130&3130xl Genetic Analyzer* documentation system at Rostok University. Similarity of nucleic sequences was evaluated by BioEdit 7.0.9 (HALL, 1999) and Chromas Lite 2.01 programmes.

ITSI regions of the DNA (up to 20 repeats) of *Cladophora glomerata* prevailing in the Rivers Ančia, Babrungas, Kösterbeck, Notė, Nova, Siesartis, Tatula and Verseka, were cloned to evaluate genetic diversity. Nucleic fragments were cloned into systems of plasmid vector (*pGEM-T Easy Vector Systems*), using *GeneIet^{TM} Plasmid Miniprep Kit* (PROMEGA..., 2010), and *Escherichia coli* DH5α stem was multiplied according to the method described by HANAHAN (1983). Plasmid DNA extraction out of bacteria was performed with *Illustra Plasmid Prep Mini Spin Kit* (GE HEALTHCARE, 2007).

One hundred seventy nine nucleic sequences of *rbc*L gene, 33 sequences of 18S rRNA gene and 12 sequences of *Cladophora* ITSI regions were used from GenBank data base for the comparison of genetic material (National Centre for Biotechnology Information (NCBI), LIU et al., 2010). Trees were constructed by the *Neighbor-Joining* method (SAITOU & NEI, 1987) and the robustness of the resulting was estimated by 1.000 replicates of *Bootstrap* resampling by MEGA 5.0 programme (TAMURA et al., 2011). *Maximum Composite Likelihood* model was employed for tree coupling. Phylogenetic network was made by Network 4.6.1.0 programme (FORSTER, 2011).

GENERAL CHARACTERISTICS OF RIVERS

The investigations on phytobenthos species diversity, algal ecological groups and species distribution were carried out in the Lithuanian rivers grouped by *in situ* measured parameters: width and depth, current velocity and the prevailing algal ecological groups. Seventy rivers were classified into four groups: small slow running rivers (M_L, 5 rivers), medium rapid running rivers (V_{GR}, 16), large slow−rapid running rivers ($D_{L\text{-}GR}$, 34) and large slow running rivers (D_L, 15) (Fig. 1).

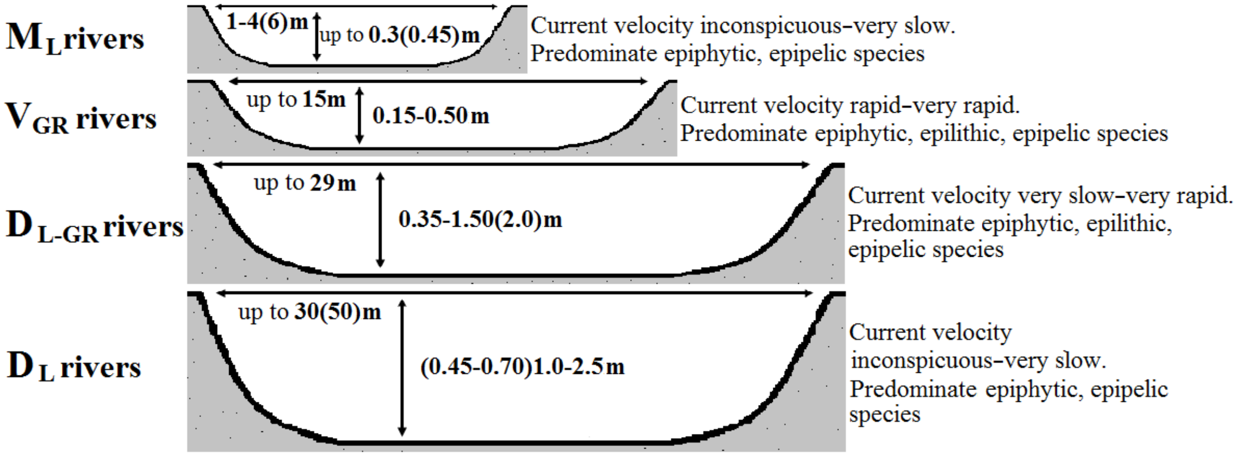

Fig. 1. Groups of the investigated Lithuanian rivers classified by the width and depth, current velocity *in situ* and the prevailing algal ecological groups
Abbreviations: M_L − small slow running rivers, V_{GR} − medium rapid running rivers, $D_{L\text{-}GR}$ − large slow−rapid running rivers, D_L − large slow running rivers

M_L group included narrow and shallow rivers of hardly noticeable or very slow current velocity, which according to SCHAUMBURG et al. (2006) were assessed by one point. V_{GR} rivers, compared to M_L, were twice wider and deeper. V_{GR} rivers were characterized by rapid or very rapid water flow. Mean width of the large rivers ($D_{L\text{-}GR}$, D_L) was up to 30 m (rarely 50 m). $D_{L\text{-}GR}$ rivers were characterized by variation in current velocity, whereas D_L rivers – by the maximum depth (up to 2.5 m) and very slow current velocity. In phytobenthos of M_L and D_L rivers prevailed epiphytic and epipelic species, whilst in rapid V_{GR}, $D_{L\text{-}GR}$ rivers – epilithic algae were also detected.

In all M_L, V_{GR} and 19 (56%) $D_{L\text{-}GR}$ rivers, water transparency reached bottom. In the remaining $D_{L\text{-}GR}$ rivers and nine (60%) D_L rivers, water turbidity was mostly moderate or strong. Slimy sand (20–95% of the riverbed) or loam (30–95%) dominated in M_L rivers, in some places the gravel (up to 30%) occurred. In V_{GR} rivers dominated coarse (gravel 20–90%, boulders up to 80%) and fine (sand 10–95%) sediments because of rapid running water. The substrate in $D_{L\text{-}GR}$ and D_L rivers was diverse; however, slimy sand, sand (50−100%) and gravel (up to 90%) prevailed. In some rivers the sites with boulders occurred. Most of the investigated rivers (81−99%) were natural. M_L and D_L rivers were characterized by the straight riverbeds, meanwhile V_{GR} and $D_{L\text{-}GR}$ rivers − by the variety of meanders. The strongest (up to 95%) shading of the bank by riparian vegetation was observed in M_L rivers. In $D_{L\text{-}GR}$ and D_L rivers, the shading was not strong, only in a few rivers exceeded 50%.

The assessment of river ecological status based on phytobenthos as biological water quality element was carried out in the rivers of five types according to the accredited river typology in Lithuania (LIETUVOS..., 2011; VAITIEKŪNIENĖ et al., 2011). Based on hydrological, hydrophysical-chemical data taken from the database of Lithuanian

Environmental Protection Agency (EPA) (APLINKOS..., 2005–2010; 2009), the rivers of the first type were distinguished by the smallest catchment areas (< 100 km^2) and water discharge (average 0.80 m^3/s). The rivers of the second type were characterized by current velocity 0.2−0.5 m/s, whilst those of the third type – by max. bed slope, > 0.7 m/km. The rivers of the fourth and the fifth types were the slowest (up to 0.6 m/s), the maximum water discharge reached 16.50 m^3/s. The average amount of total nitrogen (TN) in the water gradually decreased from the first (2.816 mg/l) to the fifth (1.597 mg/l) type of rivers. Water status based on TN in about 27% of the investigated rivers corresponded to derived reference values (≤ 1.40 mg/l) as recommended in the national guidelines (APLINKOS..., 2010; VAITIEKŪNIENĖ et al., 2011). Based on total phosphorus (TP), 44% of the investigated rivers corresponded to the reference values of water status (≤ 0.06 mg/l) (APLINKOS..., 2010; VAITIEKŪNIENĖ et al., 2011). The highest average amount of TP (0.108 mg/l) was observed in the rivers of the first and second types. In the rivers of the remaining types, TP was two or more times lower. Average annual temperature of the water was 8.0−12.0ºC. Average annual values of river water pH varied from 7.40 to 8.20. Water conductivity ranged between 264.0 and 934.3 μS/cm, min. and max. values were determined in the rivers of the first and the third types. The amount of suspended matter increased from the first (1.0 mg/l) to the fifth (32.3 mg/l) type of rivers. The dissolved oxygen (O_2) concentration in the water was 8.0−11.0 mg/l, and BDS_7 values of 91% of the investigated rivers ranged from 1.5 mg/l to 4.0 mg/l.

RESULTS AND DISCUSSION

PECULIARITIES OF PHYTOBENTHOS ASSEMBLAGES IN LITHUANIAN RIVERS

Phytobenthos species diversity. A total of 149 benthic algal species, belonging to 4 divisions, 9 classes and 22 orders, were detected in the studied rivers in 2009–2012. Cyanobacteria (86 species, 57.7% of all records) and green algae (45 species, 30.2%) compared to other systematic groups were most numerous not only in Lithuania (Fig. 2A), but also in other rivers of the world (WHITTON, 2002; KOMÁREK, 2003; KOMÁREK et al., 2003). In the studied rivers, cyanobacteria species richness was slightly lower compared to the phytobenthos of rivers of the world, cyanobacteria make up about 30–44% of all records (SHEATH & COLE, 1992; SHEATH & MÜLLER, 1997; ALLAN & CASTILLO, 2007; KOMULAYNEN, 2008; etc.). Cyanobacteria species (36%) found in the Lithuanian rivers are also abundant in lake benthos and plankton (JANKAVIČIŪTĖ, 1996; VITĖNAITĖ, 2001; KAROSIENĖ, 2003; KAROSIENĖ & KASPEROVIČIENĖ, 2009; KOSTKEVIČIENĖ, 2009). Some species such as *Chamaesiphon carpaticus*, *C. longus*, *C. starmachii*, cf. *Cyanobium diatomicola*, *Hydrococcus rivularis*, *Komvophoron schmidlei*, *Microcoleus subtorulosus*, *Phormidium retzii*, *P. tinctorium* and *Rivularia dura* were specific only to the river benthos.

Most species of cyanobacteria, on average 19, were detected in V_{GR} rivers, whilst the largest variation in the number of species – in D_L rivers (Fig. 2B).

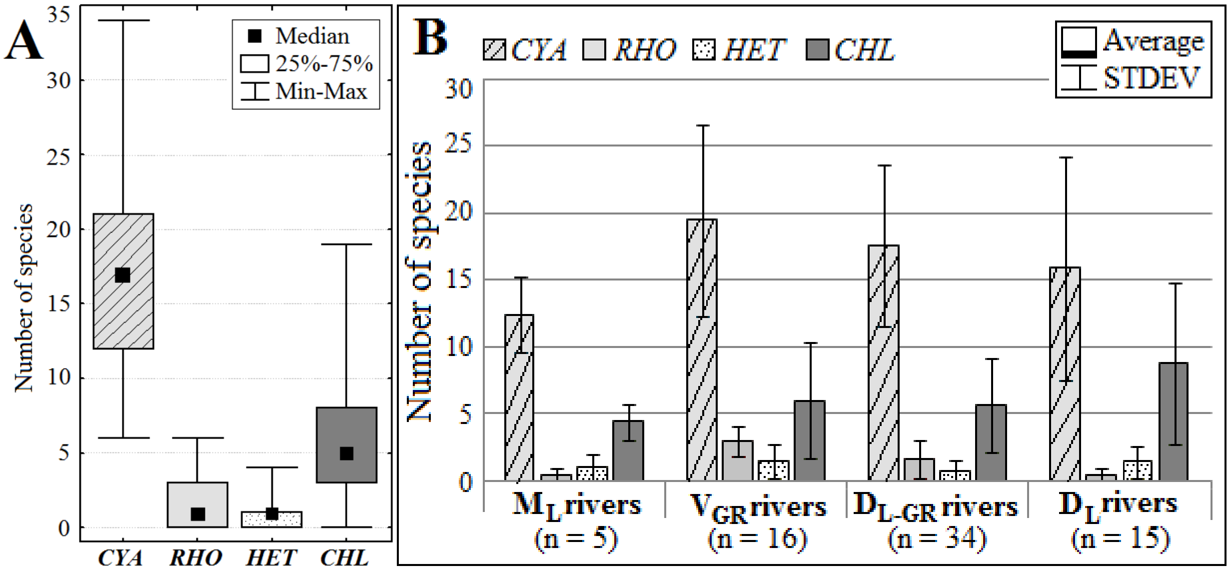

Fig. 2. Variation of phytobenthos species belonging to different divisions in the Lithuanian rivers (A) and different groups of rivers (B) in 2009–2011
Abbreviations: *CYA* – *Cyanobacteria*, *RHO* – *Rhodophyta*, *HET* – *Heterokontophyta*, *CHL* – *Chlorophyta*; M_L, V_{GR}, $D_{L\text{-}GR}$, D_L rivers – Fig. 1

The *Oscillatoriales* order was characterized by the highest (43 species) diversity. Most species (up to 39 species) were found in $D_{L\text{-}GR}$ rivers (Table 3). They form leather films on hard, fine substrates and are well adapted to develop in rivers (ALLAN, 1995; KOMÁREK et al., 2003; KOMÁREK & ANAGNOSTIDIS, 2005; ALLAN & CASTILLO, 2007). Cyanobacteria of the *Nostocales* order are not characteristic of running water bodies (KOSTKEVIČIENĖ & ŠPAKAITĖ, 2009); they are also rare in other rivers of the world (WHITTON, 2002). Seven species of the *Nostocales* order were found in D_L rivers (Table 3). The species had favourable conditions to develop due to substrate boulders dominant on the banks of the relatively slow running rivers, macrophytes and/or abundant populations of macroalgae.

Species richness of green algae in the Lithuanian rivers (Fig. 2A) was slightly lower compared to species richness (reaches about 44–54%) found in benthos of the rivers of the world (SHEATH & COLE, 1992; SHEATH & MÜLLER, 1997; ALLAN & CASTILLO, 2007; KOMULAYNEN, 2008; etc.). The *Chlorophyta* species are most common in mesotrophic and eutrophic waters (HYNES, 1972; ALLAN & CASTILLO, 2007); they develop abundantly not only in the plankton, but also in river benthos (BROOK & JOHNSON, 2002; HUXLEY & PENTECOST, 2002; JOHN, 2002a–b; 2003; GERRATH, 2003). The green algae detected during the studies are also found in other Lithuanian rivers and lakes (POCIENĖ & STOČKUS, 1987; JANKAVIČIŪTĖ, 1996; BAKŪNAITĖ & KOSTKEVIČIENĖ, 1998;

KOSTKEVIČIENĖ & SINKEVIČIENĖ, 2008). The highest diversity of green algae (15 species) and the greatest change in the number of species were characteristic of D_L rivers (Table 3, Fig. 2B). In $D_{L\text{-GR}}$, D_L rivers, mainly species of the *Zygnematales* (up to 10 species), *Chaetophorales* (up to 6) and *Desmidiales* (up to 6) orders were detected (Table 3).

Table 3. Systematic spectrum of phytobenthos species in different groups of rivers

	Taxon	**M_L rivers**		**V_{GR} rivers**		**$D_{L\text{-GR}}$ rivers**		**D_L rivers**	
		Species number, unit	% of total species number	Species number, unit	% of total species number	Species number, unit	% of total species number	Species number, unit	% of total species number
CYA	***CYANOPHYCEAE***	**32**	**66.7**	**60**	**59.4**	**76**	**61.3**	**59**	**56.8**
	Chroococcales	11	22.9	20	19.8	31	25.0	19	18.3
	Oscillatoriales	21	43.7	35	34.7	39	31.5	33	31.7
	Nostocales	–	–	5	5.0	6	4.8	7	6.8
RHO	***RHODOPHYCEAE***	**1**	**2.1**	**9**	**8.9**	**9**	**7.3**	**2**	**1.9**
	Goniotrichales	–	–	1	1.0	–	–	1	0.9
	Acrochaetiales	1	2.1	4	4.0	4	3.2	1	0.9
	Batrachospermales	–	–	3	2.9	3	2.5	–	–
	Thoreales	–	–	–	–	1	0.8	–	–
	Hildenbrandiales	–	–	1	1.0	1	0.8	–	–
HET	***XANTOPHYCEAE***	**3**	**6.2**	**5**	**5.0**	**5**	**4.0**	**5**	**4.8**
	Mischococcales	1	2.1	3	3.0	2	1.6	2	1.9
	Tribonematales	1	2.1	1	1.0	2	1.6	2	1.9
	Vaucheriales	1	2.1	1	1.0	1	0.8	1	0.9
CHL	***CHLOROPHYCEAE***	**4**	**8.3**	**8**	**7.9**	**11**	**8.9**	**15**	**14.4**
	Chlorococcales	1	2.1	1	1.0	1	0.8	3	2.9
	Sphaeropleales	–	–	1	1.0	1	0.8	3	2.9
	Chaetophorales	1	2.1	3	2.9	6	4.8	6	5.9
	Oedogoniales	2	4.2	3	2.9	3	2.5	3	2.9
	ULVOPHYCEAE	–	–	**1**	**1.0**	**2**	**1.6**	**2**	**1.9**
	Ulotrichales	–	–	–	–	1	0.8	1	0.9
	Ulvales	–	–	1	1.0	1	0.8	1	0.9
	CLADOPHOROPHYCEAE	**1**	**2.1**	**2**	**2.0**	**2**	**1.6**	**2**	**1.9**
	Cladophorales	1	2.1	2	2.0	2	1.6	2	1.9
	PLEURASTROPHYCEAE	**1**	**2.1**	–	–	**1**	**0.8**	**1**	**1.0**
	Microthamniales	1	2.1	–	–	1	0.8	1	1.0
	KLEBSORMIDIOPHYCEAE	–	–	–	–	**2**	**1.6**	**2**	**1.9**
	Coleochaetales	–	–	–	–	2	1.6	2	1.9
	ZYGNEMATOPHYCEAE	**6**	**12.5**	**16**	**15.8**	**16**	**12.9**	**16**	**15.4**
	Zygnematales	3	6.2	9	8.9	10	8.1	10	9.6
	Desmidiales	3	6.2	7	6.9	6	4.8	6	5.9
	In all:	**48**	**100.0**	**101**	**100.0**	**124**	**100.0**	**104**	**100.0**

Abbreviations: *CYA, RHO, HET, CHL* – Fig. 2; M_L, V_{GR}, $D_{L\text{-GR}}$, D_L rivers – Fig. 1

Rare in the rivers red algae (*Rhodophyta*) species (11) of accounted for 7.4% of all records (Fig. 2A). In the rivers of the world, red algae are characterized by low species richness also and can make up about 4–25% of the total number of species (SHEATH & COLE, 1992; SHEATH & MÜLLER, 1997; ALLAN & CASTILLO, 2007; KOMULAYNEN, 2008; etc.). They develop in water bodies with minor human impacts (KUMANO, 2002; SHEATH &

SHERWOOD, 2002; SHEATH, 2003). The *Acrochaetiales* and *Batrachospermales* orders included mostly four species each (Table 3). Red algae have been rarely and sparsely detected in other Lithuanian rivers and lakes, e.g. in the Rivers Grūda, Jara, Kiegžlė, Lukna, Skroblus, Stirnė, Veržuva, Lakes Balsys and Galvė (RAGAIŠYTĖ, 1968; POCIENĖ & STOČKUS, 1987; KOSTKEVIČIENĖ, 2009; KOSTKEVIČIENĖ & LAUČIŪTĖ, 2005; 2009). In addition to the nine species of red algae (*Audouinella chalybea*, *A. pygmaea*, *Batrachospermum anatinum*, *B. arcuatum*, *B. boryanum*, *B. gelatinosum*, *B. gelatinosum* f. *carpoinvolucrum, Hildenbrandia rivularis*, *Lemanea fluviatilis*), which develop only in rivers (POCIENĖ & STOČKUS, 1987; POCIENĖ & KALINAUSKAITĖ, 1991; KOSTKEVIČIENĖ & SINKEVIČIENĖ, 2008), two more species *Audouinella hermannii* and *Thorea hispida* were found during the study period. In rapid running rivers (V_{GR}, D_{L}-$_{GR}$), which are characterized by a diversity of stable substrates, mostly nine red algae species in each were detected (Fig. 2B).

Heterokontophytes (*Heterokontophyta*) were characterized by the lowest (7 species) diversity of species (Fig. 2A). Although heterokontophytes did not distinguish by species diversity, however, a few species of yellow-green algae *Tribonema vulgare* and *Vaucheria sessilis* are widespread in Lithuanian rivers (KOSTKEVIČIENĖ & SINKEVIČIENĖ, 2008). Particularly abundant populations were formed by *V. sessilis*. According to JOHNSON (2002) and OTT & OLDHAM-OTT (2003), the species is an indicator of a large amount of nutrients in water.

The Sorensen similarity index and PCA analysis based on species composition showed most similar flora in the M_L rivers Alkupis, Kiršinas I and Šeškinė (Fig. 3). In V_{GR} rivers, most similar phytobenthos species were detected in the Ašva II, Babrungas I, II, Laukesa, Varduva II and Veiviržas. Of all the $D_{L\text{-}GR}$ rivers, 74% of water bodies were moderately or very similar and more than half (60%) of D_L rivers were similar or very similar (Fig. 3).

Ecological groups of phytobenthos species. Benthic algae are adapted to develop on different substrates, which ensure the firmly attachment and distribution of species in rivers (KOMÁREK & ANAGNOSTIDIS, 1998; 2005; JOHN, 2002a; 2003; KUMANO, 2002; SHEATH & SHERWOOD, 2002; WHITTON, 2002; GERRATH, 2003; ALLAN & CASTILLO, 2007; ELORANTA et al., 2011). As the algae establish and develop on various substrates, the species are attributed to several ecological groups.

In the Lithuanian rivers, mostly benthic algae (127; 85% of all records) occurred, whereas meroplanktonic species were detected seven times less (12%). The remaining part included algae, which in some rivers were benthic, in others – meroplanktonic. Small number of meroplanktonic algae in rivers is conditioned by extremely variable factor –

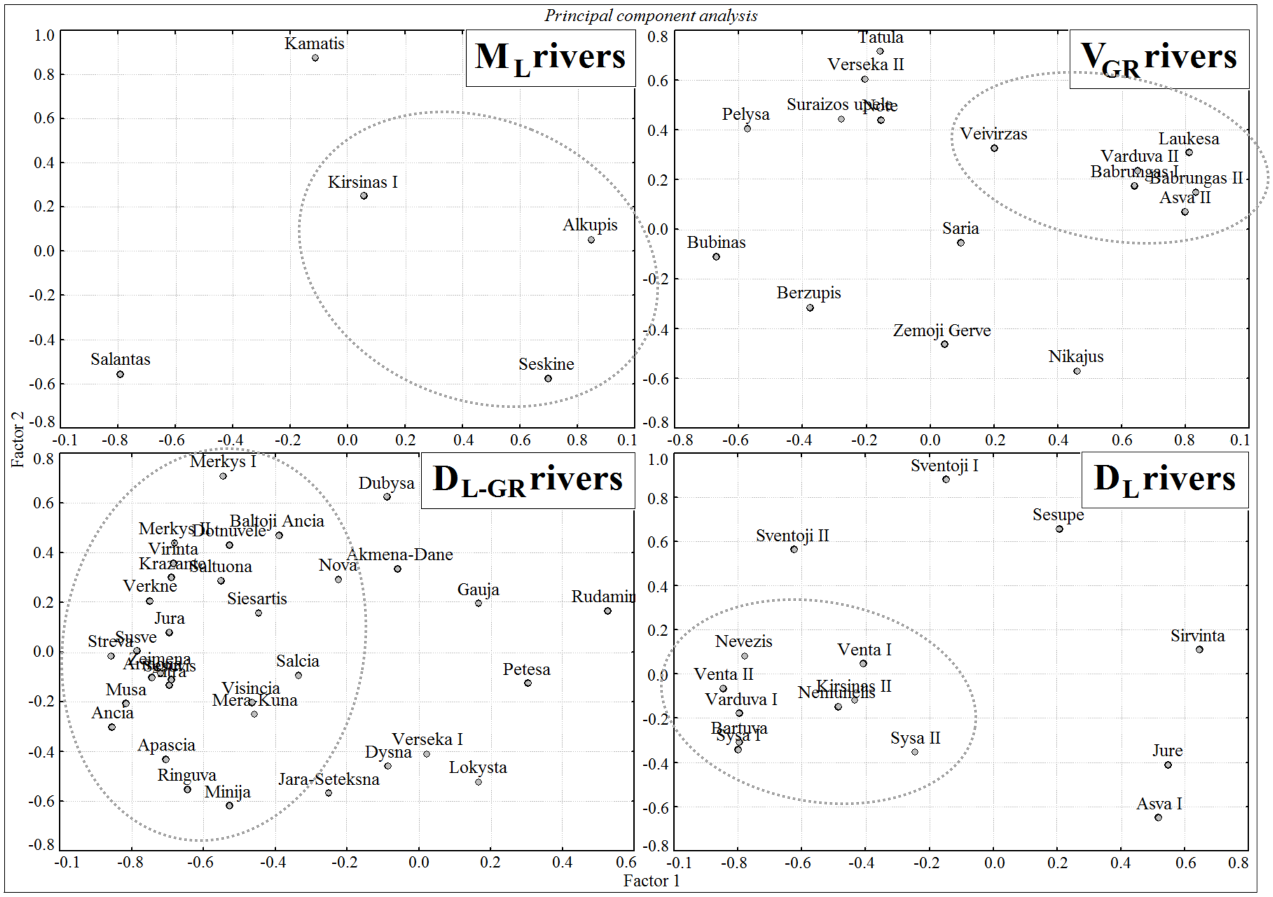

Fig. 3. Similarity of phytobenthos species composition in different groups of rivers

Abbreviations: M_L, V_{GR}, $D_{L\text{-}GR}$, D_L rivers – Fig. 1

current velocity (ALLAN & CASTILLO, 2007). In rivers of low current velocity (≤ 0.6 m/s), *Spirogyra* spp., *Mougeotia* spp., *Zygnema* sp. and other green algae dominated.

Epiphytic, epilithic and epipelic algae prevailed (Fig. 4). Macrophytes and/or macroalgae as a stable substrate (ALLAN & CASTILLO, 2007) conditioned the abundance of epiphytic species (36–43 species) in the studied Lithuanian rivers. The large rivers ($D_{L\text{-}GR}$, D_L) distinguished by the diversity of epiphytic species such as *Microthamnion kuetzingianum*, *Heteroleibleinia kuetzingii*, *H. kossinskajae*, *Geitlerinema amphibium*, *Rhizoclonium hieroglyphicum*, *Characiopsis heeringiana* f. *heeringiana*, *Stigeoclonium* sp., etc., however, their populations were sparse. Epilithic algae were abundant in rapid running rivers (V_{GR}, $D_{L\text{-}GR}$) (Fig. 4), in which sand, gravel and boulders dominated. The statistical analysis revealed positive interaction between the current velocity, substrate boulders and benthic epilithic algae (Fig. 5A). About 70% of the benthic species in rivers depended on the substrate. ALLAN (1995) and ALLAN & CASTILLO (2007) also highlight the role of the substrate in phytobenthos species diversity. A high diversity of epipelic algae was characteristic of the large rivers ($D_{L\text{-}GR}$, D_L) (Fig. 4), because slimy sand dominated on their wide banks. Supposedly, deep water bodies, low light intensity near the bottom conditioned the small amount of epiphytic and epilithic algae (Vannote et al., 1980; Hill, 1996).

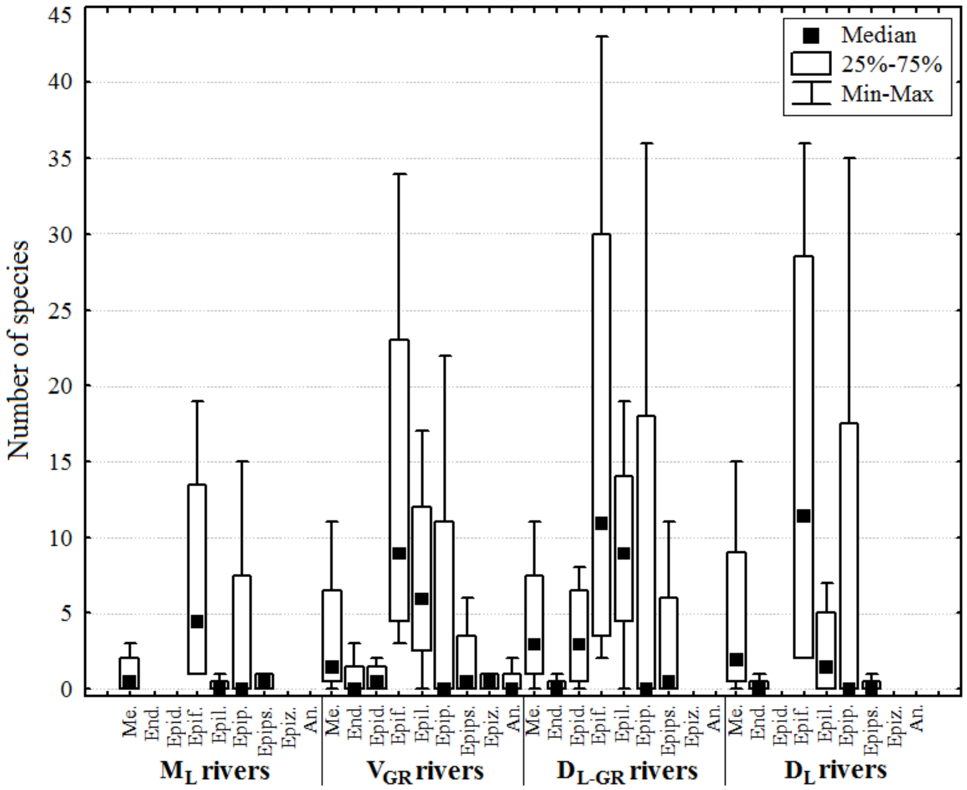

Fig. 4. Variation of ecological groups of phytobenthos species in different groups of rivers
Abbreviations, ecological groups: Me. – meroplanktonic, End. – endophytic, Epid. – epidendric, Epif. – epiphytic, Epil. – epilithic, Epip. – epipelic, Epips. – epipsamic, Epiz. – epizoic, An. – anthropogenic substrate; M_L, V_{GR}, $D_{L\text{-}GR}$, D_L rivers – Fig. 1

Phytobenthos taxonomic composition and variation of algal ecological groups depended upon other environmental factors, too. Statistically negative interaction ($r = -0.333$, $p < 0.01$) between the width of rivers and vegetation shading showed that less shading is specific to the large and wide rivers ($D_{L\text{-}GR}$, D_L) (Fig. 5A). Epiphytic green algae were often detected in rivers characterized by wide riverbed and high light intensity. In narrow and very shaded (up to 95%) rivers, where the maximum light intensity defining the photon flux density is < 40 μmol photons/m^2/s (Hill et al., 1995; Hill, 1996; Allan & Castillo, 2007), cyanobacteria and red algae developed most abundantly. Green algae photosynthesis is known to be optimal at 440–700 nm wavelength, whilst in phytobenthos they dominate when the photon flux density is more than 100 μmol photons/m^2/s. Cyanobacteria and red algae are adapted to lower light intensity. The optimum photosynthesis of these algae has been determined in vegetation-shaded rivers under the photon flux density < 100 μmol photons/m^2/s (Allan, 1995; DeNicola, 1996; Hill, 1996; Allan & Castillo, 2007).

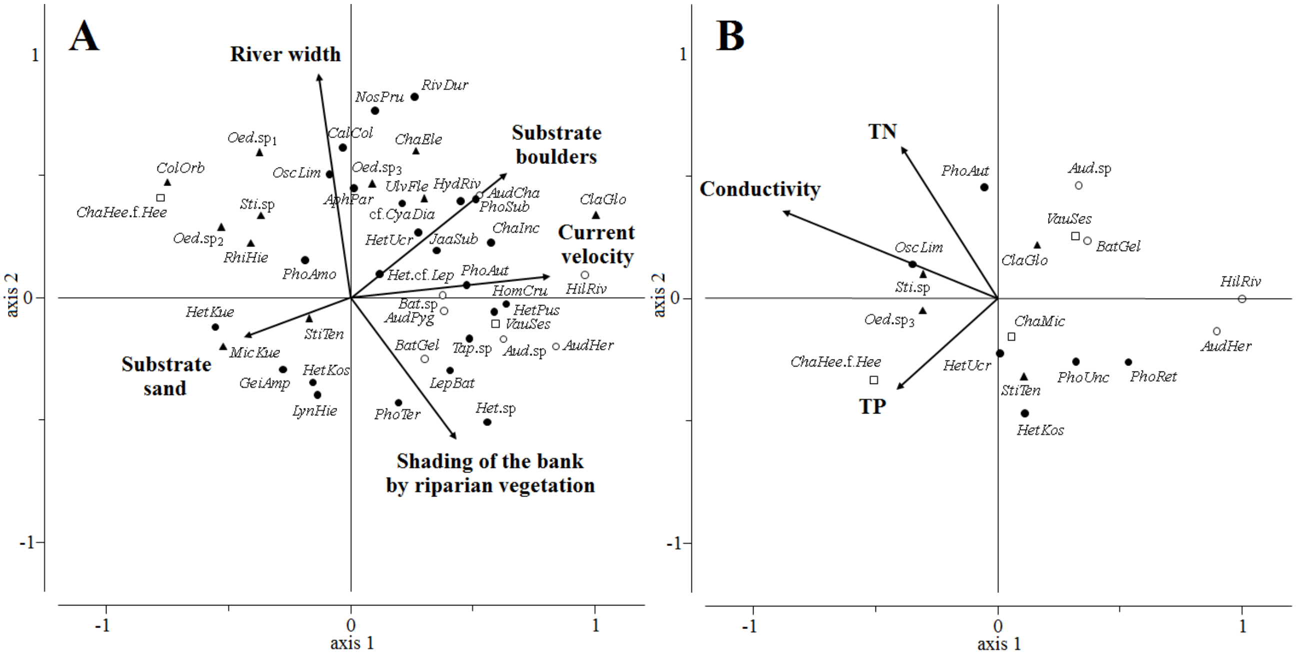

Fig. 5. Redundancy analysis (RDA) biplot showing interaction between benthic algal species diversity, abundance, distribution and morphometric, hydrological (A), hydrophysical-chemical (B) parameters of rivers

Cyanobacteria species (●): *AphPar* – *Aphanocapsa parasitica*, *CalCol* – *Calothrix columbiana*, *ChaInc* – *Chamaesiphon incrustans*, cf.*CyaDia* – cf. *Cyanobium diatomicola*, *GeiAmp* – *Geitlerinema amphibium*, *HetKos* – *Heteroleibleinia kossinskajae*, *HetKue* – *H. kuetzingii*, *Het*.cf.*Lep* – *H.* cf. *leptonema*, *HetPus* – *H. pusilla*, *HetUcr* – *H. ucrainica*, *Het*.sp – *Heteroleibleinia* sp., *HydRiv* – *Hydrococcus rivularis*, *HomCru* – *Homoeothrix crustacea*, *JaaSub* – *Jaaginema subtilissimum*, *LepBat* – *Leptolyngbya batrachosperma*, *LynHie* – *Lyngbya hieronymusii*, *NosPru* – *Nostoc pruniforme*, *OscLim* – *Oscillatoria limosa*, *PhoAmo* – *Phormidium amoenum*, *PhoAut* – *P. autumnale*, *PhoRet* – *P. retzii*, *PhoSub* – *P. subfuscum*, *PhoTer* – *P. terebriforme*, *PhoUnc* – *P. uncinatum*, *RivDur* – *Rivularia dura*, *Tap*.sp – *Tapinothrix* sp., red algae species (○): *AudCha* – *Audouinella chalybea*, *AudHer* – *A. hermannii*, *AudPyg* – *A. pygmaea*, *Aud*.sp – *Audouinella* sp., *BatGel* – *Batrachospermum gelatinosum*, *Bat*.sp – *Batrachospermum* sp., *HilRiv* – *Hildenbrandia rivularis*, yellow-green algae species (□): *ChaHee*.f.*Hee* – *Characiopsis heeringiana* f. *heeringiana*, *ChaMic* – *Characiopsis microcysticola*, *VauSes* – *Vaucheria sessilis*, green algae species (▲): *ChaEle* – *Chaetophora elegans*, *ClaGlo* – *Cladophora glomerata*, *ColOrb* – *Coleochaete orbicularis*, *MicKue* – *Microthamnion kuetzingianum*, *Oed*.sp$_1$ – *Oedogonium* sp.$_1$, *Oed*.sp$_2$ – *Oedogonium* sp.$_2$, *Oed*.sp$_3$ – *Oedogonium* sp.$_3$, *RhiHie* – *Rhizoclonium hieroglyphicum*, *StiTen* – *Stigeoclonium tenue*, *Sti*.sp – *Stigeoclonium* sp., *UlvFle* – *Ulva flexuosa*

Distribution of phytobenthos species in rivers. Very rare and rare species common to 10% of the studied Lithuanian rivers made up the largest amount (57.7% of all records). Distribution of rare algae depended on water flow, hydrophysical-chemical parameters and macroalgae species – host plants. Water flow, especially in V$_{GR}$ and D$_{L\text{-}GR}$ rivers, influenced the development of epipelic, epipsamic and meroplanktonic species such as *Aphanothece*, *Arthrospira*, *Tribonema* and others. Hydrophysical-chemical parameters of the rivers influenced the distribution of *Batrachospermum arcuatum*, *Chaetophora incassata*, *Chamaesiphon carpaticus*, *C. longus*, *C. starmachii*, *Draparnaldia acuta*, *Lemanea fluviatilis*, *Schizomeris leibleinii* and *Thorea hispida*. Similarly to the data of other references (KOMÁREK & ANAGNOSTIDIS, 1998; 2005), several species of the genus *Leptolyngbya* developed only in thalluses of the *Batrachospermum* algae. A large number of very rare species were recorded in $D_{L\text{-}GR}$ rivers (Fig. 6).

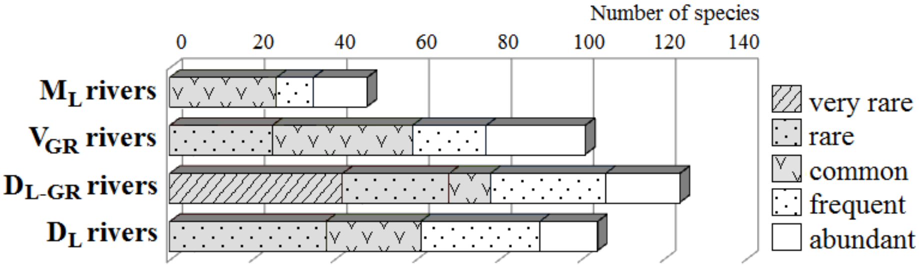

Fig. 6. Distribution of phytobenthos species in different groups of rivers
Abbreviations: M_L, V_{GR}, $D_{L\text{-}GR}$, D_L rivers – Fig. 1

The analysis of interaction between river phytobenthos and hydrophysical-chemical parameters by RDA method enabled to determine the parameters influencing algae distribution (Fig. 5B). The water conductivity ($F = 2.117$, $p < 0.05$) and TN concentration ($F = 1.304$, $p = 0.185$) were the most important factors influencing the benthic algae abundance and distribution in the Lithuanian rivers.

Under lower amount of dissolved salts in rivers, algal abundance was higher. For example, in rivers where conductivity values were 260–500 µS/cm, the cyanobacteria *Heteroleibleinia* and *Jaaginema* (2 points each) and *Hildenbrandia rivularis* (up to 5 points) formed abundant populations. However, positive correlation between *Komvophoron schmidlei*, *Phormidium autumnale*, *Spirogyra* sp.$_2$, *Stigeoclonium* sp. and conductivity was ascertained, r from 0.267 to 0.352.

RDA analysis demonstrated a negative impact of TN on the abundance and distribution of red algae *Audouinella hermannii*, *Batrachospermum gelatinosum*, *Hildenbrandia rivularis* and cyanobacteria *Phormidium retzii* (Fig. 5B). The most abundant populations of red algae (4–5 points) were characteristic mainly of clean/good status rivers under TN about 0.750–2.775 mg/l. Similar characteristics of the red algae development have also been stated by other researchers (VIS et al., 1996; GUTOWSKI et al., 2004; ELORANTA & KWADRANS, 2007; SIMIC, 2008; ŽELAZNA-WIECZOREK ZIUŁKIEWICZ, 2008). *Phormidium retzii* was specific to rivers of good status, the highest abundance (4 points) was in the rivers with the TN up to 1.150 mg/l. KOMÁREK & ANAGNOSTIDIS (2005) have reported the distribution of these cyanobacteria in the similar environment. However, SCHAUMBURG et al. (2006) have suggested that *P. retzii* abundantly develops in eutrophic water bodies and ascribed it to the indicative species group C.

Yellow-green algae *Vaucheria sessilis* and green algae *Cladophora glomerata* were assigned to species tolerant to high TN concentrations in water. The studies demonstrated that they developed in polluted rivers, where TN amount was up to 5.185 mg/l, and formed most abundant (4–5 points) populations. Similar results have also been obtained by other researchers (JOHN, 2002b; 2003; JOHNSON, 2002), however, according to SCHAUMBURG et al. (2006), both species are attributed to the indicative algae group B, i.e. common in rivers of good status.

Based on the RDA results, in addition to the discussed parameters, it was determined that species such as *Characiopsis heeringiana* f. *heeringiana*, *Heteroleibleinia kossinskajae*, *H. ucrainica*, *Oedogonium* sp.$_3$ and *Stigeoclonium tenue* depended on TP concentration in the water (Fig. 5B). The algae were detected in the rivers with high TP (0.346–0.767 mg/l). Relatively few studies have been conducted on the benthic algae ecology; however, it is known that *S. tenue* is specific to water bodies rich in nutrients and organic substances as well as large quantities of heavy metals (JOHN, 2002a).

Phytobenthos species new to Lithuania. A total of 43 new benthic algal species (29% of all detected), belonging to 4 divisions, 6 classes, 11 orders and 24 genera, were discovered in rivers (Fig. 7). The *Cyanophyceae* class includes 31 species, *Xantophyceae* – 4, *Rhodophyceae* and *Chlorophyceae* classes – 3 species each, *Ulvophyceae* and *Zygnematophyceae* – 1 species each. A large number of new species have resulted from insufficient studies on the Lithuanian rivers. Thirteen species of cyanobacteria (30% of the new species) are abundant in Europe. Nine cyanobacteria are rare in European freshwaters (ELENKIN, 1938; 1949; GOLLERBAH et al., 1953; STARMACH, 1966; KOMÁREK & ANAGNOSTIDIS, 1998; 2005; CĂRĂUŞ, 2003; 2012; KOMAREK et al., 2006; GUIRY & GUIRY, 2011), and on the distribution of six species more research is needed.

Red algae *Thorea hispida* and *Chroodactylon ornatum* were very rare in the Lithuanian rivers and are known to be common in European waters. However, the populations of these algae are not abundant. Due to the lack of studies, *C. ornatum* and *Audouinella hermannii* are not frequent in Lithuanian rivers, although other researchers indicate that these species develop in rivers from high to poor status (VINOGRADOVA et al., 1980; VIS & SHEATH, 1993; ROTT et al., 1996; SHEATH & SHERWOOD, 2002; ELORANTA & KWANDRANS, 2007; WOŁOWSKI et al., 2007; ELORANTA et al., 2011). There is little knowledge about the new to Lithuania rare heterokontophyte species distribution in Europe.

Although new to Lithuania green algae *Schizomeris leibleinii* and *Ulva flexuosa* are detected on various substrates, in water bodies of different flow and pollution, and are frequent in Europe (STARMACH, 1972; MOŠKOVA & GOLLERBAH, 1986; BURROWS, 1991; TITTLEY, 2002; JOHN, 2002a; 2003), however, in the studied Lithuanian rivers they were found rarely and sparsely. Low distribution of *Stigeoclonium carolinianum* in the Lithuanian rivers presumably depended upon water status. According to STARMACH (1972) and MOŜKOVA & GOLLERBAH (1986), this species is distributed only in clean waters. However, *S. carolinianum* was detected only in the River Šyša II, the poor status of the river was determined according to TP (0.346 mg/l).

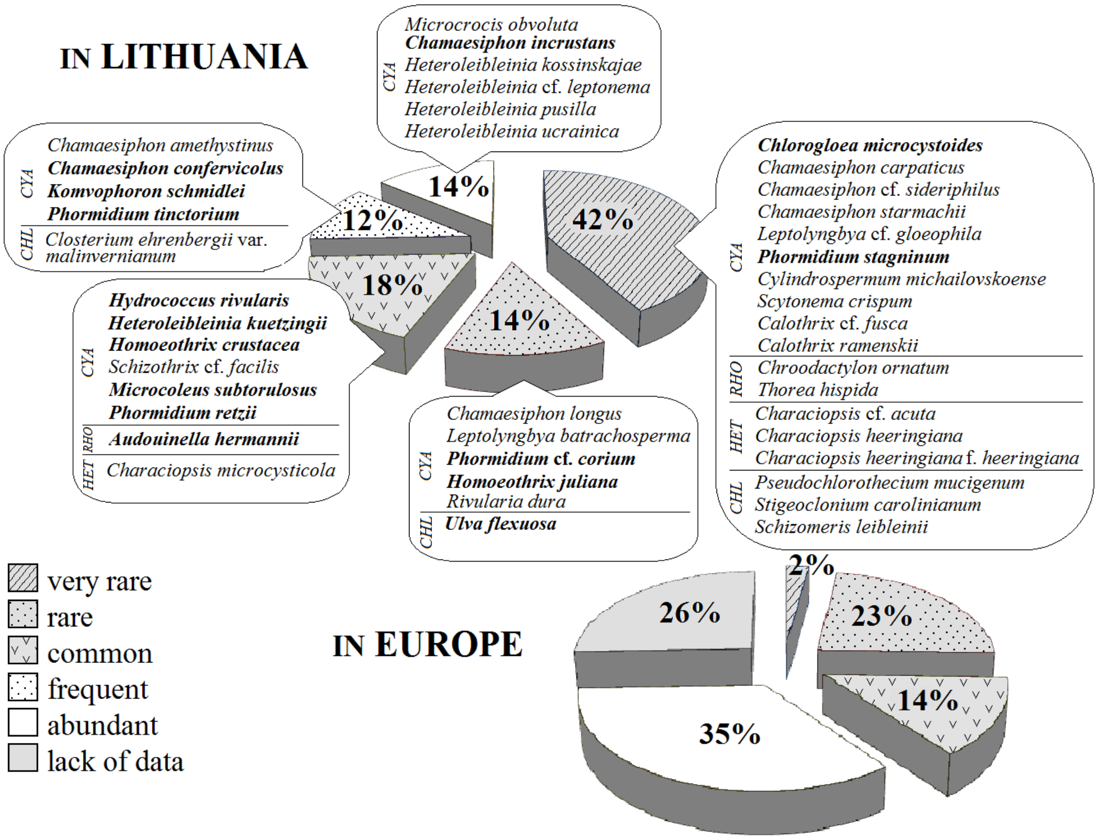

Fig. 7. New to Lithuania phytobenthos species distribution in the Lithuanian and European rivers (algae in bold – abundant in Europe)
Abbreviations: *CYA, RHO, HET, CHL* – Fig. 2

Phytobenthos macroalgae molecular research. The identification of phytobenthos species is complicated because of the lack of morphological structures according to which species are identified (JOHNSON, 2002; KUMANO, 2002; ELORANTA et al., 2011). A large diversity of morphological features may vary depending on ecological conditions (VIS & SHEATH, 1992; KUČERA & MARVAN, 2004; KUČERA et al., 2008), algal development cycle (VAN DEN HOEK, 1963; ROSS, 2006). Therefore, to confirm the identification of predominant and rare macroalgae species in the Lithuanian rivers by morphological features, molecular investigations were performed, and the obtained results of DNA sequences were compared with the data in GenBank (NCBI) database. High quality nucleotide sequences were obtained after sequencing of *rbc*L gene of 20 samples of *Batrachospermum*, *Lemanea*, *Thorea*, *Hildenbrandia*, *Cladophora*, *Vaucheria* (fragment length 444–1243 bp), 18S rRNA – 4 samples of *Lemanea* and *Thorea* (732–850 bp), ITSI – 71 samples of *Cladophora* (460 bp).

Identification of the red algae *Batrachospermum* genus according to morphological features was confirmed after the investigation of *rbc*L gene sequences. Dendrogram constructed of *Batrachospermum rbc*L gene sequences showed that *B. arcuatum* population from the River Notė was identical to species widespread in Bulgaria (GU457344, GU457345), whereas *B. gelatinosum* from the River Verseka − to a species widespread in France (GU810834) (Fig. 8). There was no possibility to identify *Batrachospermum* sp. discovered in the River Nedzingis to the species neither by methods based on morphology, because young individuals had not yet formed the reproductive organs according to which the species is indentified (KUMANO, 2002; ELORANTA et al., 2011), nor by *rbc*L gene sequences. *Batrachospermum* sp. was genetically close to *B. boryanum* (GU457343, AF029140) and *B. anatinum* (DQ393128) prevalent in the USA. *B. helminthosum*, *B. macrosporum* and *B. turfosum* were genetically most distant from the other *Batrachospermum* species. These algae are also distinguished by their specific ecological conditions necessary for their development. *B. turfosum* develops in clean streams and lakes,

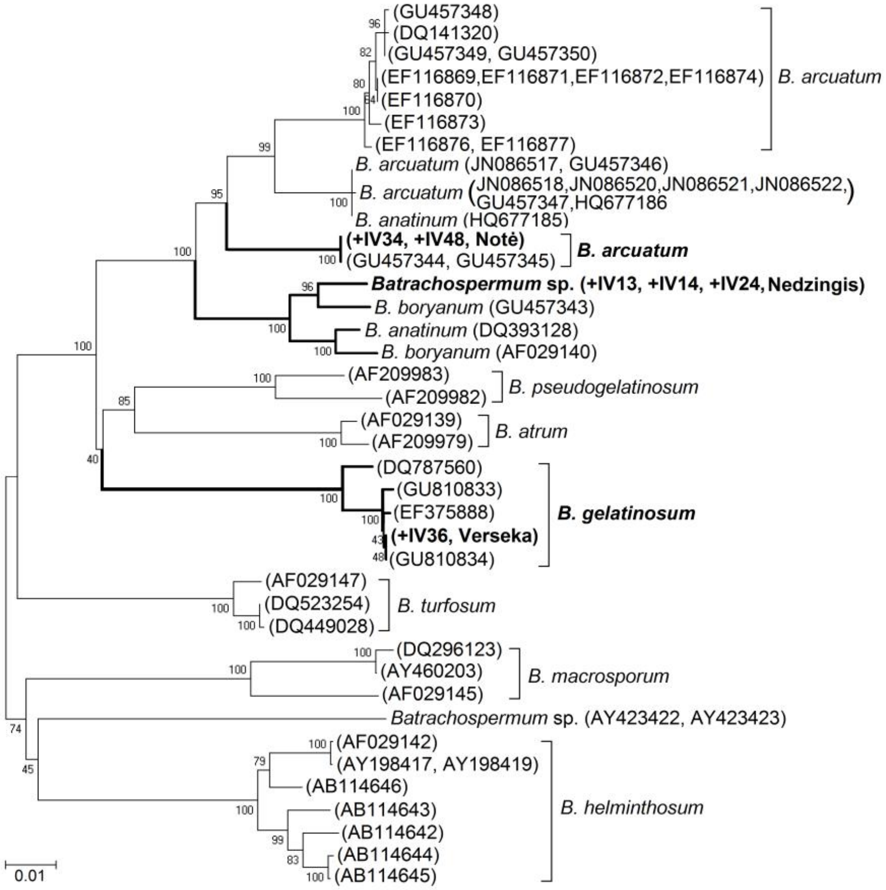

Fig. 8. Dendrogram constructed of *Batrachospermum* algae *rbc*L gene sequences by *Neighbor-Joining* method using 1205 bp length DNA fragments

Highlighted phylogenetic tree branches – similar species group by nucleotide sequences; sample in bold – macroalgal sample from the Lithuanian rivers often in dystrophic water bodies, in which pH 4.2–7.6(8.0) and water temperature varies in wide range (11.0–26.0ºC) (KOSTKEVIČIENĖ & LAUČIŪTĖ, 2009; ELORANTA et al., 2011). Meanwhile *B. helminthosum* and *B. macrosporum* development is optimal at pH 7.4 and water temperature 20ºC (CHIASSON et al., 2005; NECCHI JÚNIOR & ROBEIRO ZUCCHI, 2001; ROBEIRO ZUCCHI & NECCHI JÚNIOR, 2001; SHEATH & SHERWOOD, 2002; KATO et al., 2009; NECCHI JÚNIOR & DE OLIVEIRA, 2011). The different results were obtained by SHERWOOD et al. (2008) for the *rbc*L and *cox*2-3 gene sequences of *B. helminthosum* and *B. macrosporum*. These species are genetically close to the investigated *Batrachospermum* species.

Identification of the red algae *Lemanea fluviatilis* by morpholocigal features was confirmed by 18S rRNA gene sequences (Fig. 9). In Lithuania and Canada (AF026051) widespread *L. fluviatilis* were identical. However, the data on *rbc*L gene sequences did not confirm *L. fluviatilis* identification accomplished by morphological features. The research on *rbc*L and 18S rRNA gene sequences clearly show that the species of this genus are difficult to identify by algae morphology such as the frequency of filament branching, the diameter of a trichome and the morphology of thallus towards the base (VIS & SHEATH, 1992; SHEATH & SHERWOOD, 2002; SHEATH, 2003; KUČERA et al., 2008; ELORANTA et al., 2011), which vary depending on the environmental conditions (KUČERA & MARVAN, 2004). The *Lemanea* species (*L. borealis*, *L. fluviatilis*, *L. fucina*) found in various regions of the world were similar by *rbc*L and 18S rRNA gene sequences, the differences in nucleotide sequences were not determined (Fig. 9).

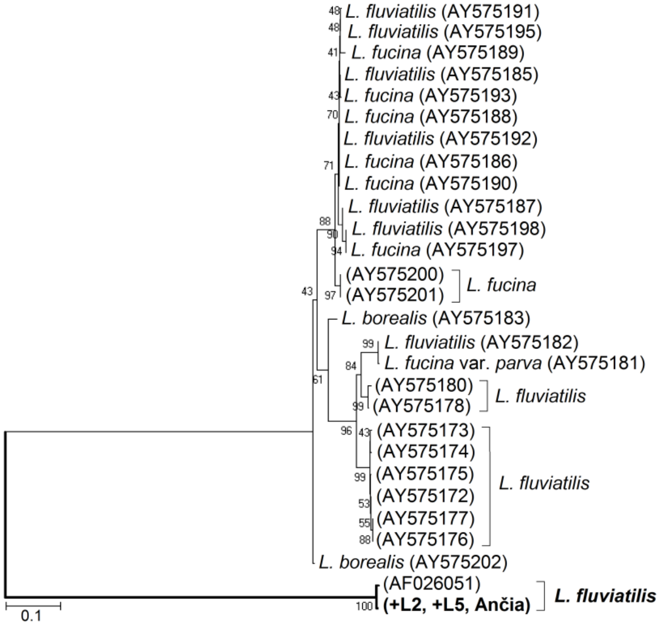

Fig. 9. Dendrogram constructed of *Lemanea* algae 18S rRNA gene sequences by *Neighbor-Joining* method using 721 bp length DNA fragments

Highlighted phylogenetic tree branches – similar species group by nucleotide sequences; sample in bold – macroalgal sample from the Lithuanian rivers

Since there are no *Thorea hispida* 18S rRNA gene sequences in the GenBank, therefore, *rbc*L and 18S rRNA gene sequences of the species identified by morphological criteria were compared with all DNA sequences of the genus *Thorea* existing in the NCBI database (Fig. 10). Only the comparison of *rbc*L gene sequences allowed to confirm *T. hispida* identification by morphological features (monosporangium, carposporangium) (VIS et al., 1998; NECCHI JÚNIOR et al., 2010). After the investigation of three red algae species *T. violacea* (AF506269), *T. okadae* (AB159654, AB159655) and rare as well as new to Lithuania *T. hispida rbc*L gene sequences, high (97.1%) genetic similarity of the species was observed (Fig. 10A). The other researchers confirm that *T. riekei* does not make a separate group and in the dendrogram it is adjacent to *T. violacea*, therefore, it can be considered as *T. violacea* synonym (SHEATH et al., 1993; NECCHI JÚNIOR et al., 2010). According to 18S rRNA gene sequences, two *Thorea* species *T. violacea* from the USA (AF026042, AF342744) and Brazil (GU953246) and *T. bachmannii* from Brazil (GU953244, GU953243) formed separate groups (Fig. 10B).

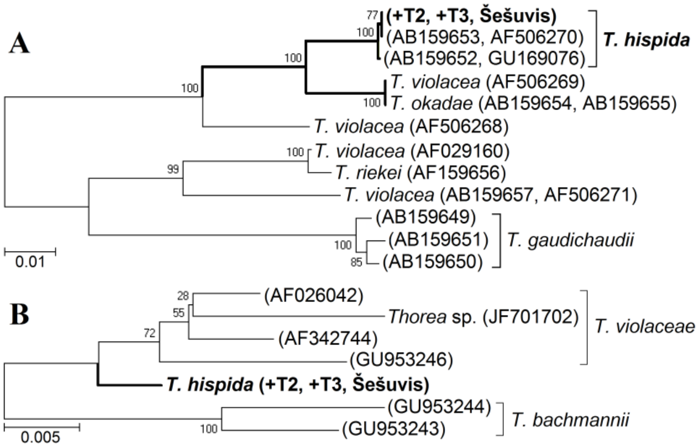

Fig. 10. Dendrograms constructed of *Thorea* algae *rbc*L (A) and 18S rRNA (B) gene sequences by *Neighbor-Joining* method using 1189 bp (A) and 899 bp (B) length DNA fragments

Highlighted phylogenetic tree branches – similar species group by nucleotide sequences; sample in bold – macroalgal sample from the Lithuanian rivers

Widespread in the Lithuanian rivers red algae *Hildenbrandia rivularis* was identified by both morphological features and *rbc*L gene sequences (Fig. 11). *H. rivularis* from the River Nova were similar to the species widespread in Sweden (AY028814), whereas from the River Babrungas – to those found in Austria (AF208797) and Wales (AF208813). Clear distinction between *rbc*L gene sequences of the other *Hildenbrandia* species (*H. angolensis*, *H. rubra*) was not observed, although based on 18S rRNA gene research data obtained by SHERWOOD & SHEATH (2000), the marine *H. rubra* and freshwater *H. angolensis* made separate groups.

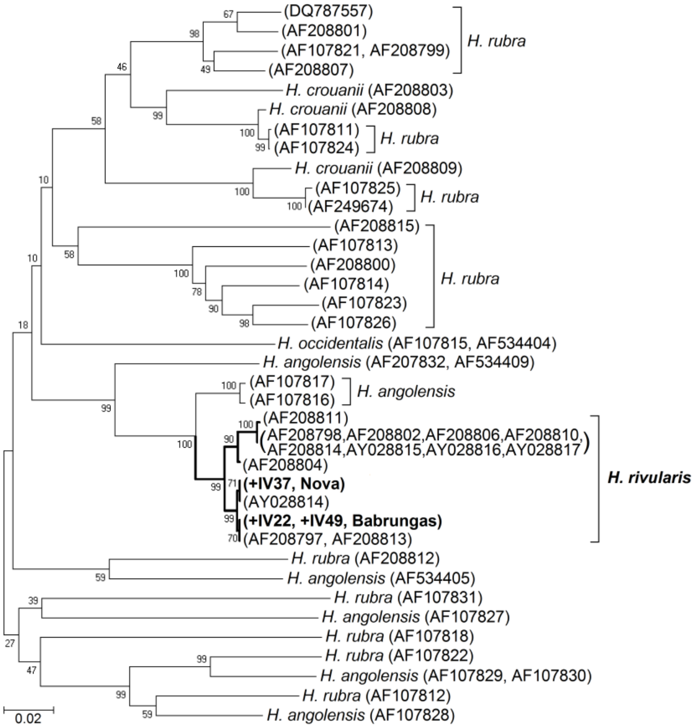

Fig. 11. Dendrogram constructed of *Hildenbrandia* algae *rbc*L gene sequences by *Neighbor-Joining* method using 964 bp length DNA fragments
Highlighted phylogenetic tree branches – similar species group by nucleotide sequences; sample in bold – macroalgal sample from the Lithuanian rivers

There are no data in the GenBank on *rbc*L gene of yellow-green algae *Vaucheria sessilis* and green algae *Cladophora glomerata,* therefore, species identified by morphology were not confirmed. *Rbc*L gene sequences of species from Lithuania were compared with DNA sequences of the genera *Vaucheria* and *Cladophora* from the NCBI database (Fig. 12). *Vaucheria sessilis* found in the River Tatula is genetically similar (98.7%) to *V. bursata* (AF015589, AF476938, AF476939) found in Denmark. ENTWISLE (1987) has suggested that *V. bursata* and *V. sessilis* might be synonyms. This assumption is supported by the big similarity of morphological features of both species (JOHNSON, 2002). Moreover, ANDERSEN & BAILEY (2002) have demonstrated that *V. bursata, V. repens, V. dillwynii* and *V. sessilis* make monophyletic group (section *Corniculatae*).

Cladophora glomerata from the Lithuanian rivers (Ančia, Nova) and from the northern Germany (Kösterbeck) were identical according to *rbc*L gene sequences, however, they differed significantly from *Cladophora* sp. from New Zealand (EU380532) (Fig. 12). The relatively low amount of *rbc*L gene sequences of the genus *Cladophora* in NCBI database shows that *rbc*L gene is conservative and in that case complex studies on various genes are necessary (HALL et al., 2010). The identification of *C. glomerata*, widespread in the Lithuanian rivers, was confirmed by nucleotide sequences of ITSI region (Fig. 13). The species from the Rivers Ančia, Babrungas, Nova, Kösterbeck and from lakes in Japan (AB665565) were identical by DNA sequences of ITSI region.

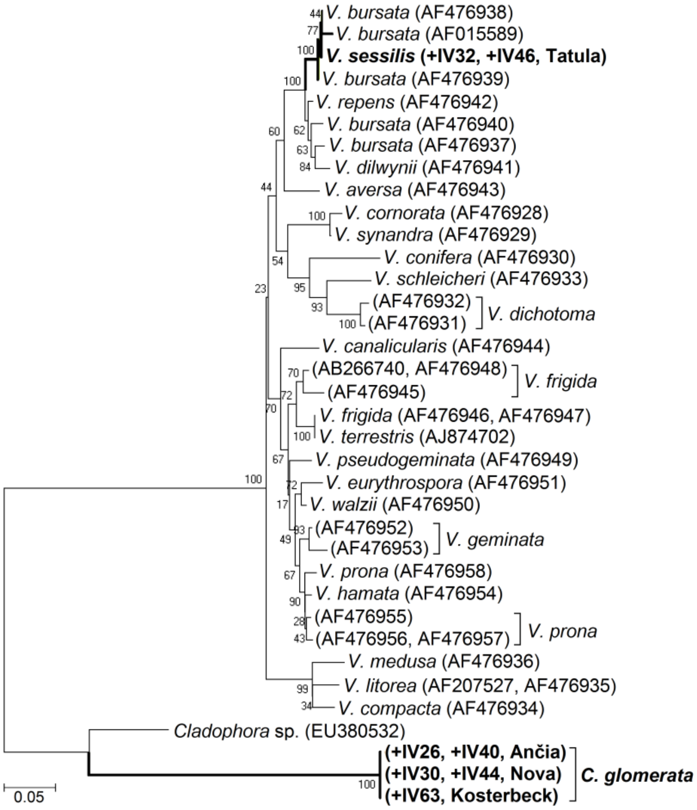

Fig. 12. Dendrogram constructed of *Vaucheria* and *Cladophora* algae *rbc*L gene sequences by *Neighbor-Joining* method using 406 bp length DNA fragments

Highlighted phylogenetic tree branches – similar species group by nucleotide sequences; sample in bold – macroalgal sample from the Lithuanian rivers

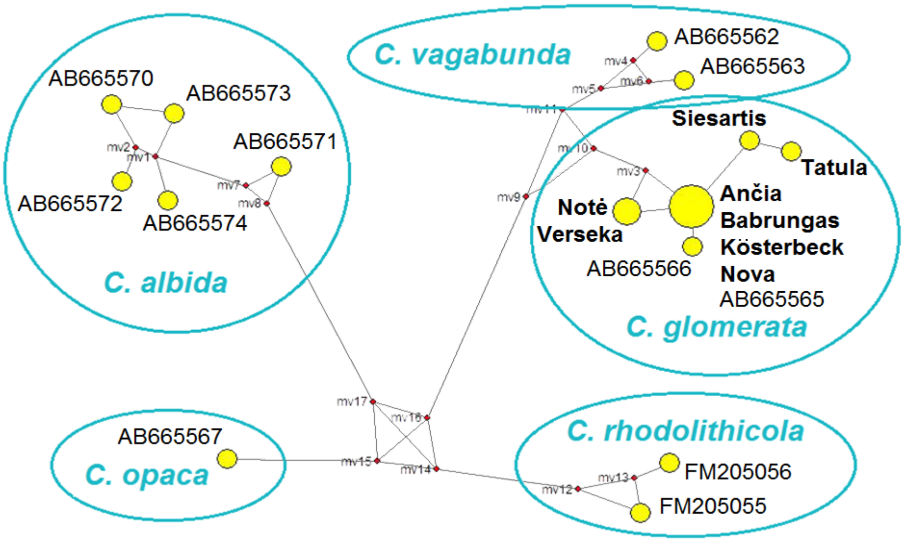

Fig. 13. Phylogenetic network constructed of *Cladophora* algae ITSI region sequences using 465 bp length DNA

Widespread algae genetically adapt to the changing environment (HAYAKAWA et al., 2012). To observe the genetic polymorphism of the species, ITS non-coding DNA regions characterized by rapid genome evolution (WHITE et al., 1990) were selected. In the rivers of Lithuania, genetic polymorphism of predominant rheophilic *Cladophora glomerata* was confirmed by cloning DNA of ITSI region. DNA sequencing of 71 samples of *C. glomerata* showed that algae from the Rivers Ančia, Babrungas, Nova and Kösterbeck were identical; they formed the largest group (Fig. 14). DNA of *C. glomerata* from the Rivers Notė and Verseka differed from the largest group by one nucleotide as well as the different growth conditions for these algae were determined. The Rivers Notė and Verseka were distinguished by a small amount up to four times of NH_4-N (max. 0.013 mg/l) (APLINKOS..., 2005–2010). Most mutations in DNA sequences of *C. glomerata* were identified in the Rivers Siesartis and Tatula. Both water bodies belong to the same physical-geographical area of Central Lithuania, where predominant soil distinguishes by poor infiltration, which determines low water level of rivers that can freeze till the bottom (KILKUS, 1998; KILKUS & STONEVIČIUS, 2011), and holds a high amount of nutrients in water. Particularly TN (4.025–5.185 mg/l) and its variation in different years as well as amount of dissolved salts (conductivity 735.8−865.1 µS/cm) in these rivers (APLINKOS..., 2001–2004; 2005–2010) were the highest compared to the other investigated rivers. The variation in hydrological-chemical parameters of these rivers could influence genetic polymorphism of *C. glomerata*. The phylogenetic network showed that DNA sequences of *C. glomerata* from the River Tatula differed from DNA of algae found in the River Siesartis by four nucleotides (Fig. 14).

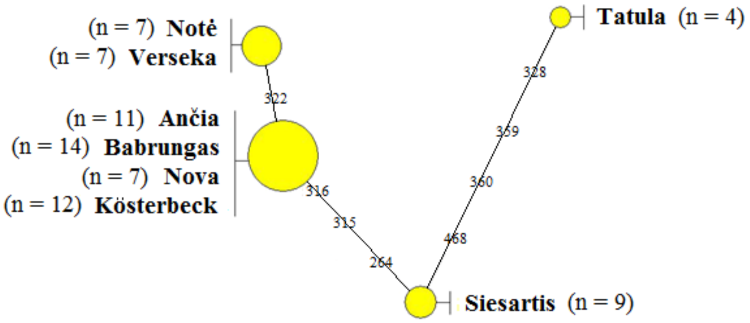

Fig. 14. Genetic polymorphism of *Cladophora glomerata* in the investigated rivers. Phylogenetic network constructed by 465 bp length DNA sequences of ITSI region
Abbreviations: n – number of samples; number in phylogenetic network – location of changed nucleotide in DNA sequence

PHYTOBENTHOS EMPLOYMENT FOR THE RIVER ECOLOGICAL STATUS ASSESSMENT

For the assessment of ecological status of the surface water bodies according to the Water Framework Directive of European Union (EU WFD, 2000/60/EC), the following biological quality elements are recommended: phytoplankton, phytobenthos, benthic macroalgae, macrophytes, zoobenthos and ichthyofauna (Quevauviller et al., 2008; SWD, 2012a–l). Benthic macroalgae as one of the flora elements reflect human activity impact on the changes in ecological status of aquatic ecosystems (Scanlan et al., 2006; Ballesteros et al., 2007; Juanes et al., 2008; Jelic Mrcelic et al., 2012; etc.). In some of the EU countries such as Ireland, the United Kingdom, Lithuania, the Netherlands, Sweden and Germany, the parameters of phytobenthos macroalgae are used for the status assessment of transitional and coastal waters (SWD, 2012c,f,g,i,k,l). In other EU countries there are only few methodologies for water status assessment using macroalgae parameters, or they are under development. For the assessment of ecological status of the Lithuanian northern coast of the Baltic Sea and transitional waters, the values of red macroalgae *Furcellaria lumbricalis* coverage in prevalence depth are used (Olenin et al., 2012; SWD, 2012g). Macroalgae in rivers and lakes in some of the European countries (Ireland, Belgium, the United Kingdom (Kelly et al., 2006b; Van de Bund, 2009; SWD, 2012b,f,l)) are investigated in conjunction with macrophytes. However, the macroalgae of phytobenthos and their coverage are not applied for the assessment of status of the Lithuanian freshwaters.

Several biological water quality elements and their parameters such as taxonomic composition of zoobenthos, abundance of species (Danish Stream Fauna Index DSFI), taxonomic composition of ichthyofauna, abundance of species and the age structure are employed in the assessment of ecological status of the Lithuanian rivers (Lithuanian Fish Index LFI) (Arbačiauskas, 2006; Kontautas, 2008–2010; Virbickas, 2008; 2009a,b; 2010; Aplinkos..., 2010; Lietuvos..., 2011; Vaitiekūnienė et al., 2011). Methods for using other biological elements (macrophytes (Reference Index of macrophytes RI) and benthic diatoms) are under development (Gudas, 2010; Sinkevičienė, 2010–2011; Zviedre, 2013). These organisms are characterized by different living environment, different features and response to external impact. The methodology for ecological status assessment according to phytobenthos (without diatoms) parameters (phytobenthos index, BI), indicative algal species and their abundance (estimated on five-point abundance scale), was created and successfully applied in Germany (Gutowski et al., 2004; Foerster et al., 2004; Schaumburg et al., 2004; 2006; Federal..., 2009; Theesfeld & Schleyer, 2011; SWD, 2012c). The universal method allows investigating macroalgal cover in rivers of different status and estimating its ability for Lithuanian river status assessment *in situ*. The thesis provides the analysis of employment possibilities of phytobenthos macroalgal coverage and index BI as separate parameters, the evaluation of statistical correlation between the phytobenthos species and hydrophysical-chemical quality parameters in the rivers.

Assessment of ecological status according to macroalgal coverage (%). The river benthic macroalgae communities change depending on variation of the main nutrient (TN, TP) amount in the water (Biggs, 1996; Biggs & Kilroy, 2000; Biggs & Smith, 2002; GUTOWSKI et al., 2004; FOERSTER et al., 2004; SCHAUMBURG et al., 2004). The data are used for the preliminary assessment of ecological status of rivers *in situ*. The evaluation of macroalgal species coverage was accomplished in 48 Lithuanian rivers (69% of the investigated water bodies). Indicator macroalgae were selected and evaluated based on their cover variation in the rivers of various ecological status determined according to TN and TP in the water.

Six indicator species of benthic macroalgae (*Audouinella hermannii*, *Cladophora glomerata*, *Hildenbrandia rivularis*, *Phormidium retzii*, *P. uncinatum* and *Vaucheria sessilis*) were selected depending on the Pearson coefficient between the phytobenthos species and hydrophysical-chemical parameters for the Lithuanian river status assessment. The species are easily identifiable *in situ*, stable in fast running rivers and common in Lithuania.

The results revealed the peculiarities of macroalgal cover changes in the rivers of different ecological status. It was ascertained that the diversity of indicator species decreased and their coverage varied because of the deteriorating status of the rivers (Fig. 15). In the rivers, that according to TN and TP corresponded high ecological status, the highest macroalgal diversity and coverage (up to 70%) were recorded. The rivers of the 1-st and 3-rd type were clearly distinguished by characteristic species. In the 1-st type rivers of

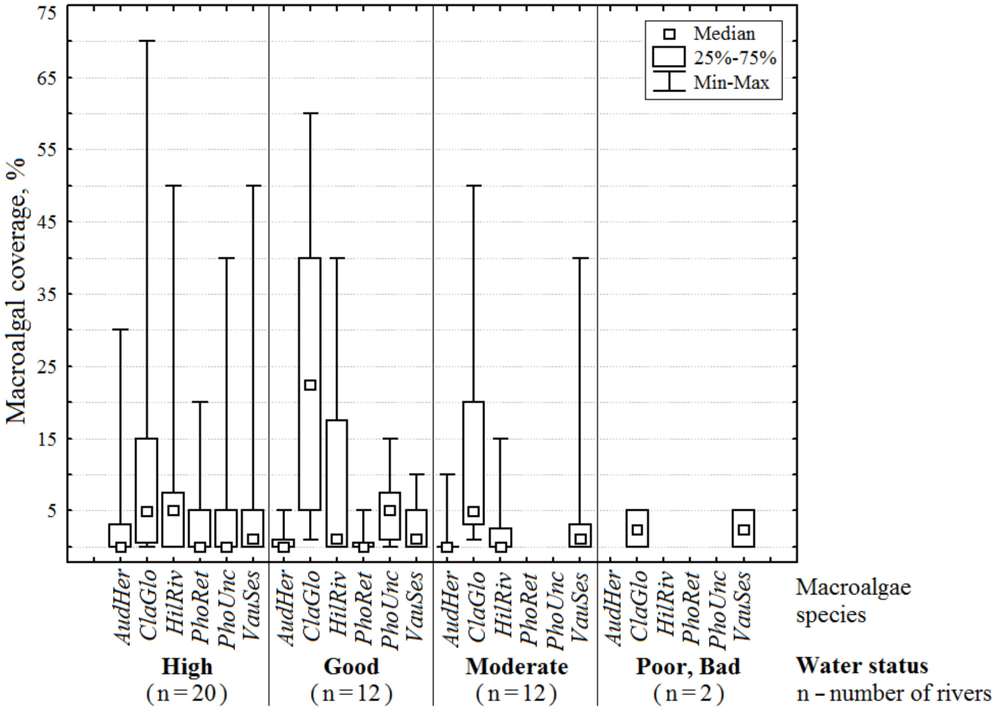

Fig. 15. Macroalgal coverage (%) in the Lithuanian rivers of different status determined according to TN* and TP*

* − EPA data (APLINKOS..., 2005–2010; 2009). Abbreviations: *AudHer – Audouinella hermannii, ClaGlo – Cladophora glomerata, HilRiv – Hildenbrandia rivularis, PhoRet – Phormidium retzii, PhoUnc – Phormidium uncinatum, VauSes – Vaucheria sessilis*

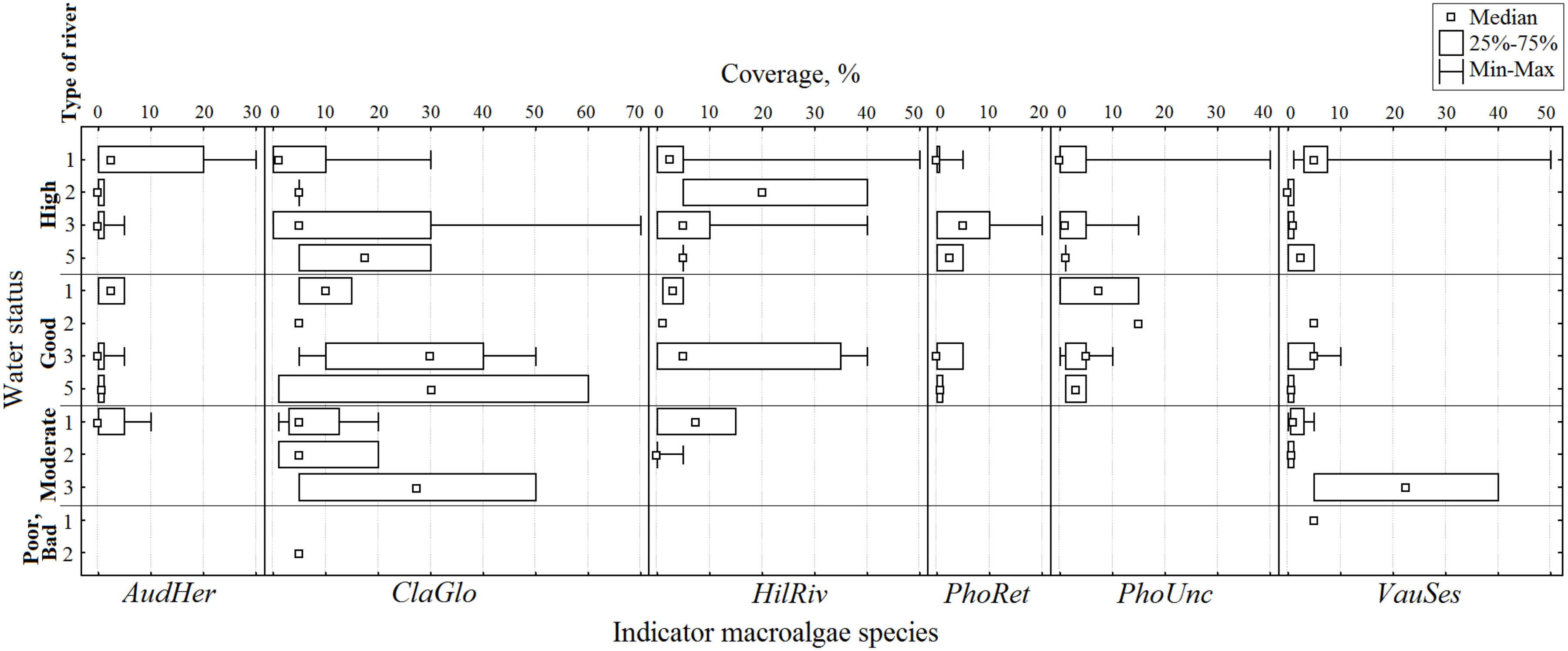

Fig. 16. Macroalgal coverage (%) in the Lithuanian rivers of different types and status determined according to TN* and TP*
* − EPA data (APLINKOS..., 2005–2010; 2009). Abbreviations: *AudHer* – *Audouinella hermannii*, *ClaGlo* – *Cladophora glomerata*, *HilRiv* – *Hildenbrandia rivularis*, *PhoRet* – *Phormidium retzii*, *PhoUnc* – *Phormidium uncinatum*, *VauSes* – *Vaucheria sessilis*

high status, *Audouinella hermannii* formed up to 30% of river bed coverage, in the rivers of the 3-rd type − *Phormidium retzii* and *P. uncinatum* were characteristic, coverage 15−20% (Fig. 16). Although *P. retzii* tolerates moderate organic pollution (KOMÁREK & ANAGNOSTIDIS, 2005), however, according to the data on species distribution and coverage (up to 20%) in the Lithuanian rivers, *P. retzii* is a relevant parameter for the assessment of high status rivers. The rivers of good status distinguished by indicator green algae *Cladophora glomerata*, red algae *Hildenbrandia rivularis* and cyanobacteria *Phormidium uncinatum* and their coverage that ranged between 15−60% (Fig. 15). *P. uncinatum* were more common and coverage was more significant in the rivers of good status than in those of high status, although according to SINGH et al. (2008) *P. uncinatum* develops under different ecological conditions.

In the rivers of moderate−bad status, which are ascribed by EPA (APLINKOS..., 2009) to water bodies of a risk group, a significant decrease in prevalence of indicator macroalgae and coverage were determined (Fig. 15**)**. In the rivers of moderate status, only red algae *Audouinella hermannii* and *Hildenbrandia rivularis* that tolerate increased nutrient amount (ELORANTA & KWANDRANS, 2007; ŻELAZNA-WIECZOREK & ZIUŁKIEWICZ, 2008; ELORANTA et al., 2011) were distinguished, the coverage (up to 15%) was significant only in the rivers of the 1-st type (Fig. 16). In the rivers of poor and bad ecological status, the lowest macroalgal species diversity and coverage were observed (Fig. 15). Tolerant to organic pollution *Cladophora glomerata* and *Vaucheria sessilis* (JOHN, 2002b; 2003; OTT & OLDHAM-OTT, 2003) formed the smallest coverage (5%) in these rivers, compared to the rivers of good status. Small coverage is a relevant parameter for the poor status of Lithuanian rivers. ALLAN (1995) and BIGGS (1996) have also reported that in the rivers of bad status one or two species (e.g. *Cladophora glomerata*, *Vaucheria* sp.) are predominant, whereas other algae are found rarely or do not develop at all. In Lithuanian rivers of poor and bad ecological status, other indicator macroalgae were not discovered.

Changes in the coverage of the selected river-specific macroalgal species in the rivers of various types and different ecological status allow to preliminary accomplish *in situ* the assessment of river status (express test). A larger phytobenthos research database will enable to create a river status classification system and determine reference conditions according to the values of macroalgal coverage.

Assessment of ecological status according to phytobenthos index (BI). In ecological and economic aspect, phytobenthos is important in water ecosystems, though in rivers of many EU countries it is much less studied than phytoplankton and macrophytes. Therefore, due to a relatively small database, only few methods for the freshwater status assessment according to phytobenthos parameters have been created. The periphyton index (PIT) for the river trophic status assessment has been created in Norway (SCHNEIDER & LINDSTRØM, 2011). The status of rivers is determined according to benthic algae (excluding diatoms) abundance that is estimated on a five-point scale, and indicative species depending on algae tolerance to a certain TP amount. The methodology for the calculation of the index PIT that reflects different classes of ecological status is analogous to the previously developed phytobenthos index (BI) applied for the river status assessment in Germany (Gutowski et al., 2004; Foerster et al., 2004; Schaumburg et al., 2004; 2006; Federal..., 2009; Theesfeld & Schleyer, 2011; SWD, 2012c). The differences in the indices applied in both countries are determined by hydrophysical-chemical parameters of rivers and the prevailing benthic algae. The main differences between the indices PIT and BI are indicative species and their number. To assess the status of Lithuanian rivers, the phytobenthos index (BI) was used. The Lithuanian rivers are similar to the rivers of calcareous lowland region in Germany, and twice more indicative algae (43 species, 29% of all phytobenthos species recorded in the Lithuanian rivers) used for the index BI calculation were observed in those rivers compared to the number of indicative species (23) of the index PIT.

The assessment of ecological status of rivers was performed according to the phytobenthos index (BI) (Fig. 17). The largest number of indicative species, on average up to 15 species, was recorded in the Lithuanian rivers of moderate and poor status (Fig. 17A). Five indicative species were found only in one investigated river of bad status. The estimated index BI values for the Lithuanian rivers varied insignificantly, did not reach marginal values of the index according to the scale for rivers of different water status established by Schaumburg et al. (2006) (Fig. 17B).

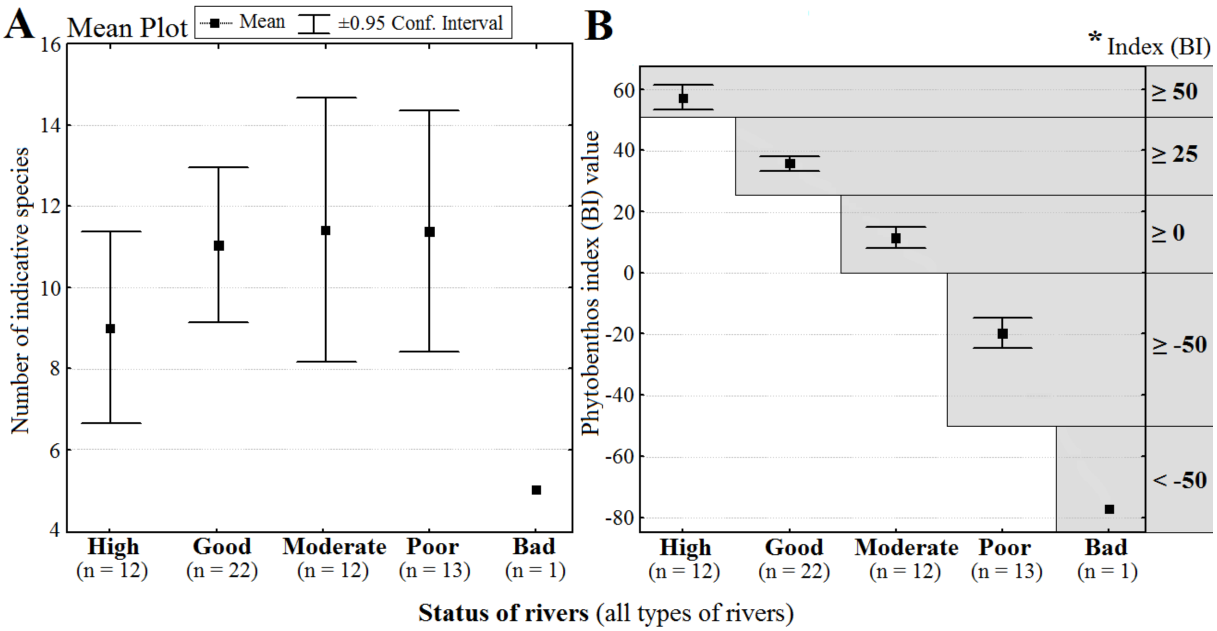

Fig. 17. Changes in the number of phytobenthos indicative species (A) and index BI values (B) in the Lithuanian rivers of different status

Abbreviations: n – number of rivers, * – phytobenthos index (BI) scale after Schaumburg et al. (2006)

According to WFD requirements for EU countries, the most important goal is to achieve the good status of surface water bodies until 2015 (VAITIEKŪNIENĖ et al., 2011). Based on phytobenthos index (BI), 34 rivers (48% of water bodies) of the investigated Lithuanian rivers were ascribed to water bodies of high and good status (Fig. 18A). They belong to 11 protected areas in Lithuania (KIRSTUKAS, 2004), in which the ecological balance is maintained (LIETUVOS..., 1993). After comparison of the river status assessed by the index BI and by the hydrochemical (TN, TP) and biological (RI, DSFI, LFI) quality elements and their parameters approbated under development for the assessment of Lithuanian water status, the largest differences were revealed in rivers, the ecological status of which was assessed according to hydrochemical (TN, TP), especially phosphorus, and the Danish stream fauna index (DSFI) parameters. According to these parameters water status of the rivers in most cases was better than according to the phytobenthos (Fig. 18A). With reference to the assessment of TN, TP and DSFI parameters, most (75−91%) of the investigated water bodies were in good status. Poor and bad status rivers were mostly assessed by the phytobenthos (BI) and the Lithuanian fish index (LFI), 20 and 16% of rivers, respectively. The relatively uniform numbers of rivers of different water status were ascertained according to the values of BI and LFI indices. However, the estimation of water status for each river separately by different parameters demonstrated that the assessment of river status was similar according to the phytobenthos (BI) and benthofauna (DSFI) indices (Fig. 18B).

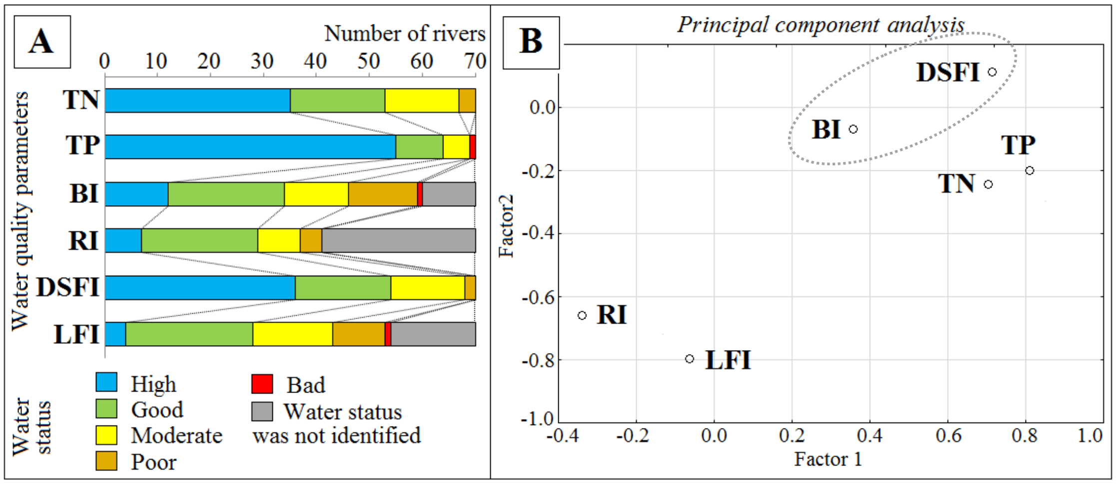

Fig. 18. Assessment of ecological status of the Lithuanian rivers (A) and evaluation of water status similarity according to the different parameters of water quality elements (B)
Abbreviations: TN – total nitrogen, TP – total phosphorus, BI – phytobenthos index, RI – Reference Index of macrophytes, DSFI – Danish Stream Fauna Index, LFI – Lithuanian Fish Index

A relatively similar number of the particular water status rivers was determined by comparing the status of the studied rivers according to the parameters of biological flora quality elements − phytobenthos (BI) and macrophytes reference index (RI) (Fig. 18A)

(Sinkevičienė, 2010−2011). However, in 34 water bodies, 48% of the investigated rivers, macrophyte index (RI) was not determined due to insufficient number and abundance of indicative species. Only nine rivers, 13% of the investigated water bodies, belonged to the same status class according to both indices (BI, RI).

Statistical correlation analysis between the phytobenthos index (BI) and the morphometric, hydrological, hydrophysical-chemical quality parameters of rivers was performed to estimate the possibilities of the index employment for the Lithuanian river status assessment. The analysis revealed that morphometric and hydrological parameters of rivers measured *in situ* were more significant to the indicative species number and their abundance than hydrophysical-chemical parameters (Table 4). River width, boulders ($r = 0.264-0.285$, $p < 0.05$) and marl ($r = 0.341$, $p < 0.01$) as substrates were statistically significant to the abundance and distribution of phytobenthos indicative species, whilst current velocity, water transparency, shading of riparian vegetation and gravel ($r = 0.372-0.490$, $p < 0.01$) to index BI values. Negative correlations ($r = -0.350- -0.442$, $p < 0.01$) $p < 0.01$) were determined between the index BI and river depth, slimy sand. The

Table 4. The Pearson correlation coefficients between the phytobenthos indicative species, index BI values and morphometric, hydrological, hydrophysical-chemical parameters of river

		Number of indicative species	**Index BI value**		**Number of indicative species**	**Index BI value**
Morphometric, hydrological parameters measured in situ				*Hydrological, hydrophysical-chemical parameters measured by EPA* [1]		
The river section	Width	,264*	-,159	Current velocity, m/s	-,098	,152
	Depth	,022	-,442**	Water discharge, m^3/s	,038	-,069
Current velocity, points		-,027	,490**	NH_4-N, mgN/l	-,088	-,210
Water turbidity		-,162	-,090	NO_2-N, mgN/l	-,084	-,064
Water transparency, till bottom		-,116	,375**	NO_3-N, mgN/l	-,244	-,011
Meandering of river		-,188	,094	N_{min}, mg/l	-,247	-,042
Naturalness of river		,017	,102	TN, mg/l	-,261*	-,030
Shading of the bank by riparian vegetation, %		-,152	,372**	PO_4-P, mgP/l	-,143	-,184
Shading of the bank by riparian vegetation, point		-,234	,286*	TP, mg/l	-,141	-,176
Substrate	Slimy sand	-,035	-,350**	TN/TP	-,125	,135
	Loam	-,178	-,022	Temperature, °C	,292*	-,014
	Sand	-,194	-,203	pH	,136	,290*
	Gravel, Ø till 6 cm	,070	,390**	Conductivity, µS/cm	-,200	-,164
	Boulders, Ø > 6 cm	,285*	,181	Suspended matter, mg/l	-,049	,302*
	Marl	,341**	,004	O_2, mg/l	-,004	,058
				BDS_7, mgO_2/l	-,184	-,002

** – the value of correlation coefficient, when the level of significance $p < 0.01$; * – the value of correlation coefficient, when the level of significance $p < 0.05$; [1] – EPA data (Aplinkos..., 2005–2010; 2009) interaction between the calculated index BI and TN values were not revealed. However, the TN negatively influenced the number of indicative algae characterizing river status ($r = -0.261$, $p < 0.05$). Since ecological status of each river was similar according to the values of BI and DSFI indices that were adapted for the assessment of the Lithuanian river status (Fig. 18B), although, the correlation of DSFI and TN, TP parameters was determined, $r = 0.300$ ($p < 0.05$) and 0.404 ($p < 0.01$), respectively. Statistically significant correlation shows that the index BI could be successfully adapted also. However, to achieve this goal the larger database of river phytobenthos and the accomplished selection of water bodies are necessary, in response marginal values of TN, TP in the water. Currently, the employment possibilities of index BI for the assessment of ecological status are limited.

Intercalibration of the index BI into the Lithuanian rivers, supplement of the database, selection of rivers and comprehensive analysis of algae ascription to indicative species groups as well as supplement of the lists of these algae would allow to develop the method for the assessment of river ecological status according to phytobenthos index (BI).

CONCLUSIONS

1. A total of 149 phytobenthos species and intraspecific taxa, belonging to 4 divisions, 9 classes and 22 orders, were identified in 73 rivers during the study period (2009–2012). The highest species diversity was characteristic of *Cyanophyceae* (86 species), *Chlorophyceae* and *Zygnematophyceae* (19 species each) and *Rhodophyceae* (11) classes. Species of the remaining classes accounted for 9% of the total species number.
2. In phytobenthos, 43 new to Lithuania species were discovered: *Cyanophyceae* – 31 species, *Xantophyceae* – 4, *Rhodophyceae* and *Chlorophyceae* – 3 species each, *Ulvophyceae* and *Zygnematophyceae* – 1 species each. Six cyanobacteria *Microcrocis obvoluta*, *Chamaesiphon incrustans*, *Heteroleibleinia kossinskajae*, *H.* cf. *leptonema*, *H. pusilla*, *H. ucrainica* were abundant in the rivers.
3. The classification of rivers into four groups by morphometric, hydrophysical parameters and the prevailing algal ecological groups revealed the variation of phytobenthos species. Cyanobacteria (34 species) were found in all groups of rivers, the highest diversity of green algae (19 species) was ascertained in large slow running rivers, of red algae – in medium rapid and large slow–rapid running rivers (5 and 6 species, respectively).
4. Benthic algal species accounted for 85% of all recorded species in rivers, the remaining part – meroplanctonic (12%) and meroplanctonic-benthic (3%) species. Epiphytic (43 species) and epipelic (36) species were numerous in all river groups, epilithic (19) species were dominant in rapid and slow–rapid running rivers.

5. Very rare and rare algal species made the largest amount (58%) of all records. Very rare *Chamaesiphon carpaticus*, *Chlorogloea microcystoides*, *Chroodactylon ornatum*, *Draparnaldia acuta*, *Schizomeris leibleinii*, *Stigeoclonium carolinianum* formed not abundant populations. Common, frequent and abundant species were found in a similar ratio.
6. Identification of predominant, rare in the Lithuanian rivers and according to morphological features hardly identified species *Batrachospermum arcuatum*, *B. gelatinosum*, *Hildenbrandia rivularis* and *Thorea hispida* was confirmed by *rbc*L gene, *Lemanea fluviatilis* – by 18S rRNA gene, *Cladophora glomerata* – by ITSI region nucleotide sequences.
7. Genetic polymorphism of predominant in rivers green algae *Cladophora glomerata* was ascertained according to DNA sequencing data of ITSI region. Mutations of nucleic sequences could be influenced by the different ecological conditions of macroalgal development.
8. The river width, current velocity and substrate were statistically significant to the structure of phytobenthos. In wide, slow running and not shaded rivers, green algae *Oedogonium* spp. were predominant, whereas in narrow, rapid running rivers strongly shaded by riparian vegetation, red algae of the genera *Audouinella*, *Batrachospermum* and cyanobacteria of the genera *Heteroleibleinia*, *Phormidium*, *Tapinothrix* tolerant to changes in light intensity prevailed. In rapid running rivers, epilithic species *Audouinella hermannii*, *Cladophora glomerata* and *Hildenbrandia rivularis* formed the most abundant populations.
9. Water conductivity and total nitrogen (TN) amount in the water influenced the formation of phytobenthos. Low amount of dissolved salt ions and TN determined the abundance of red algae *Audouinella hermannii*, *Batrachospermum gelatinosum*, *Hildenbrandia rivularis* and cyanobacteria *Phormidium retzii*, *P. uncinatum*, whereas high amount of dissolved salt ions and TN increased the abundance of cyanobacteria *Oscillatoria limosa*, *Phormidium autumnale*, yellow-green algae *Vaucheria sessilis* and green algae *Cladophora glomerata*, *Oedogonium* sp$_{.3}$.
10. Changes in the number of the selected six indicator macroalgal species and their coverage allow to asses *in situ* the status of rivers of different types. The number of species and coverage values (from 70 to 5%) decreased because of the deteriorating status of water bodies. *Audouinella hermannii*, *Phormidium retzii*, *P. uncinatum* coverage accounted for 20–40% only in the rivers of high and good status. Coverage of *Hildenbrandia rivularis*, *Cladophora glomerata* and *Vaucheria sessilis* significantly decreased in the rivers of changing water status from moderate to bad.
11. According to phytobenthos index (BI), 49% of the investigated Lithuanian rivers are of high and good status, 37% – of moderate, poor and bad status. Index BI reliably

reflects the ecological status of the Lithuanian rivers of different types at an average amount of TN, TP in the water. Reliability of the index decreases when the amount of nutrients is low or high.

LIST OF PUBLICATIONS

Scientific publications that are on the Thomson Reuters Master Journal List:

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ACKNOWLEDGEMENTS

I sincerely thank my scientific supervisor Dr Jūratė Kasperovičienė for valuable and subject comments, advice and constant full support, patience. I thank my scientific consultant Assoc. Prof. Dr Jolanta Kostkevičienė for valuable comments. I am thankful to Dr Judita Koreivienė, Dr Jūratė Karosienė, Ksenija Savadova for their constant support, moral maintenance. I am also grateful to the employees of the Laboratory of Algology and Microbial Ecology as well as the Laboratory of Aquatic Ecotoxicology for the suggestions in preparing the thesis.

I am thankful to Dr Zofija Sinkevičienė for the help in collecting phytobenthos samples and critical remarks, Dr Ričardas Paškauskas helped to organize an internship at Rostock University (Germany) in 2012. I thank Dr Ralf Bastrop and Prof. Dr Henrik Schubert and to the laboratory personnel: Petra Nowak, Dr Lukas Krebes, Lisza Zeidler, Dr Jens Frankowski for given warm welcome and the financial support in 2011 and 2012. Phytobenthos macroalgal molecular research was carried out under their support and the provided knowledge was important and significant in preparing the dissertation. My gratitude is extended to the employees of the Environmental Protection Agency for the given useful information in preparation of the dissertation, and also the Research Council of Lithuania (contract No KEL–279/2011) and UAB „Labochema LT” for the financial support during the internship at Rostock University.

Exceptional thanks are dedicated to my parents and family members for their understanding, patience and support as well as given all opportunities to prepare the dissertation.

The study was supported by the Lithuanian State Science and Studies Foundation, application registration No 390342; the State Studies Foundation − No 400117, No 410044; the Research Council of Lithuania − No DOK–12763.

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SANTRAUKA

Upių fitobentosas – tai mikro- ir makrodumbliai augantys dugne ar prisitvirtinę prie įvairios kilmės po vandeniu esančių substratų. Jie yra svarbūs organinės medžiagos producentai (BIGGS & KILROY, 2000; SCHAUMBURG et al., 2004; 2006; ISTVÁNOVICS & HONTIL, 2011), kurių bendra pirminė produkcija upėse gali siekti 20 g C/m^2 per parą (WEHR & SHEATH, 2003; ALLAN & CASTILLO, 2007; SEGURA et al., 2010). Įsisavindami iš aplinkos mineralines ir organines medžiagas, dumbliai dalyvauja vandens telkinių savaiminio apsivalymo procesuose, eliminuoja sunkiuosius metalus, radionuklidus ir kitus toksinius junginius (LAI et al., 2003; BARSANTI & GUALTIERI, 2006; AL-HOMAIDAN et al., 2011). Be to, bentoso dumbliai įvairiomis gniužulo tvirtinimosi struktūromis (rizoidais, išplatėjusia pamatine ląstele, išskiriamomis per sienelių poras gleivėmis) suriša smėlio, dumblo daleles, tokiu būdu sumažina substrato, ypač smulkiadispersinio, išplovimą iš upės vagos. Stambių makrodumblių gniužuluose auga ir ant jų antrines apaugimų bendrijas formuoja smulkesni dumbliai. Tokie fitobentoso tankūs sąžalynai sudaro priedangą kitiems organizmams apsaugančią nuo upių srovės ar plėšrūnų poveikio (ALLAN, 1995; BIGGS, 1996; WEHR & SHEATH, 2003).

Lietuvoje apie upių fitobentosą paskelbtų duomenų nėra daug (VAILIONIS, 1930; POCIENĖ & STOČKUS, 1987; BAKŪNAITĖ & KOSTKEVIČIENĖ, 1998; KOSTKEVIČIENĖ & LAUČIŪTĖ, 2005; 2009), kadangi pagrindinis dėmesys buvo skirtas fitoplanktono tyrimams (MARKEVIČIENĖ, 1962; ŪSELYTĖ, 1975; 1978; KOSTKEVIČIENĖ, 1995a,b; 1997; 1998; 2001). Daugiausiai buvo analizuoti taksonominiai ir sistematiniai dumblių tyrimo aspektai, tačiau duomenų apie fitobentoso rūšių įvairovę, skirtumus ir pokyčius, dumblių genetinį polimorfizmą ir aplinkos veiksnių kaitos poveikį bentoso dumblių rūšių paplitimui nepakanka.

Fitobentoso struktūra ir dumblių gausumas glaudžiai siejasi su vandens kokybe ekosistemose. Todėl pastarąjį dešimtmetį Europoje fitobentoso tyrimams skirtas ypatingas dėmesys, siekiant pritaikyti jo rodiklius vandens būklės vertinimui (BIGGS & KILROY, 2000; GUTOWSKI et al., 2004; FOERSTER et al., 2004; SCHAUMBURG et al., 2004; 2006; GUTOWSKI & FOERSTER, 2006; BALLESTEROS et al., 2007; JELIC MRCELIC et al., 2012). Europos Sąjungos (ES) Bendrosios vandens politikos direktyvoje (BVPD) (EU WFD, 2000/60EC) fitobentosas yra vienas iš rekomenduojamų biologinių vandens kokybės parametrų (QUEVAUVILLER et al., 2008; SWD, 2012a−l). Fitobentoso makrodumbliai, jų rodikliai yra taikomi kai kurių ES šalių − Airijos, Jungtinės Karalystės, Olandijos, Švedijos, Vokietijos, taip pat ir Lietuvos, Tarpinių ir priekrantės vandenų būklės vertinimui (OLENIN ir kt., 2012; SWD, 2012c,f,g,i,k,l), o Airijoje, Belgijoje, Jungtinėje Karalystėje upių ir ežerų būklės vertinimui makrodumblių rūšys tiriamos kartu su makrofitais (KELLY et al., 2006b; VAN DE BUND, 2009; SWD, 2012b,f,l). Lietuvos upių monitoringą vykdanti Aplinkos apsaugos

agentūra (AAA) ekologinės būklės vertinimui adaptavo bentofaunos (Danijos upių faunos indeksas DIUF) ir ichtiofaunos (Lietuvos žuvų indeksas LŽI) rodiklius (LIETUVOS..., 2011; VAITIEKŪNIENĖ ir kt., 2011). Kuriami nacionaliniai upių būklės vertinimo metodai panaudojant ir kitus biologinius floros elementus: makrofitus (makrofitų etaloninis indeksas RI) (SINKEVIČIENĖ, 2010–2011; ZVIEDRE, 2013) ir fitobentoso titnagdumblius (GUDAS, 2010). Tačiau Lietuvos upių ekologinei būklei vertinti bentoso makrodumbliai ir fitobentoso indeksas (BI) nėra taikomi, kadangi iki šiol nebuvo atlikta išsamių Lietuvos upių fitobentoso struktūros tyrimų, kurių apibendrinti duomenys leistų sukurti vandens telkinių ekologinės būklės vertinimo sistemą.

Darbo tikslas: ištirti fitobentoso taksonominę sudėtį ir rūšių ekologiją Lietuvos upėse, nustatyti fitobentoso indikacines galimybes, vertinant upių ekologinę būklę.

Darbo uždaviniai: 1) ištirti upių fitobentoso rūšių įvairovę, ekologinių grupių sudėtį ir rūšių paplitimą, sudaryti fitobentoso rūšių sąvadą; 2) identifikuoti vyraujančias ir retas Lietuvos upėse makrodumblių rūšis molekuliniais metodais, ištirti dominuojančių žaliadumblių *Cladophora glomerata* (L.) Kütz. genetinį polimorfizmą; 3) ištirti aplinkos veiksnius, lemiančius fitobentoso rūšių vystymosi ypatumus upėse; 4) išskirti indikatorines makrodumblių rūšis ir jų projekcinio padengimo vertes preliminariam skirtingo tipo Lietuvos upių būklės nustatymui *in situ* ir įvertinti fitobentoso indekso (BI) taikymo galimybes.

Ginami teiginiai: 1) Fitobentoso rūšių įvairovė, gausumas ir ekologinių grupių pasiskirstymas priklauso nuo upės pločio, gylio, srovės greičio, substrato pobūdžio, vandens savitojo elektrinio laidžio ir bendrojo azoto kiekio. 2) Plačiai paplitusios Lietuvos upėse *Cladophora glomerata* genetinį polimorfizmą galėjo sąlygoti vandens būklė. 3) Makrodumblių projekcinis padengimas gali būti taikomas preliminariam Lietuvos upių būklės nustatymui *in situ*, o fitobentoso indeksas (BI) yra tinkamas rodiklis upių ekologinei būklei vertinti.

Darbo naujumas. Darbe pirmą kartą išsamiai ištirti 73-jų Lietuvos upių fitobentoso dumbliai, atlikta jų taksonominė ir ekologinių grupių sudėties analizė, nustatyti vystymosi ypatumai. Sudarytas fitobentoso rūšių sąvadas. Identifikuotos 43 naujos Lietuvai bentoso dumblių rūšys. Pirmą kartą atlikti Lietuvos upėse vyraujančių ir retų fitobentoso makrodumblių molekuliniai tyrimai. Preliminariam upių būklės vertinimui *in situ* išskirtos indikatorinės makrodumblių rūšys ir jų projekcinio padengimo (%) vertės įvairios būklės skirtingo tipo upėse. Pirmą kartą pagal fitobentoso indekso (BI) rodiklius atliktas Lietuvos upių ekologinės būklės vertinimas.

Darbo reikšmė. Atlikti upių fitobentoso tyrimai ir sudarytas dumblių rūšių sąrašas yra indėlis pildant gėlųjų vandens telkinių dumblių taksonominį sąvadą. Paruošti naujų Lietuvai bentoso dumblių rūšių išsamūs aprašymai su autentiškomis nuotraukomis, pateikti morfometriniai duomenys, nurodytas rūšių paplitimas. Gauti pirmi Lietuvos upių makrodumblių genetinės įvairovės duomenys, identifikuotos vyraujančios ir retos Lietuvos upėse makrodumblių rūšys, nustatytas upių bentose dominuojančių žaliadumblių *Cladophora glomerata* genetinis polimorfizmas. Rezultatai svarbūs ir naudingi pasaulio mokslininkams, atliekant palyginamuosius molekulinius tyrimus ar sprendžiant ekologinių pokyčių stebėsenos problemas. Vertinant upių būklę pagal ES BVPD reikalavimus, yra ruošiama indikatorinių bentoso makrodumblių rūšių nustatymo, dumblių padengimo verčių išskyrimo skirtingos būklės upėms ir fitobentoso rodiklio (indekso BI) taikymo metodika. Fitobentoso tyrimų rezultatai svarbūs vertinant ir prognozuojant Lietuvos upių ekologinės būklės pokyčius ir vykdant gamtosaugines programas.

Išvados. Fitobentoso tyrimų laikotarpiu (2009–2012 m.) 73-se upėse identifikuotos 4 skyrių, 9 klasių ir 22 eilių 149 dumblių rūšys ir vidurūšiniai taksonai. Didžiausiu rūšių skaičiumi išsiskyrė *Cyanophyceae* (86 rūšys), *Chlorophyceae* ir *Zygnematophyceae* (po 19) bei *Rhodophyceae* (11) klasės. Likusių klasių rūšys sudarė 9 % visų rūšių skaičiaus.

Fitobentose aptiktos 43 naujos Lietuvai rūšys: *Cyanophyceae* – 31 rūšis, *Xantophyceae* – 4, *Rhodophyceae* ir *Chlorophyceae* po 3, *Ulvophyceae* ir *Zygnematophyceae* po vieną. Šešios melsvabakterių *Microcrocis obvoluta*, *Chamaesiphon incrustans*, *Heteroleibleinia kossinskajae*, *H.* cf. *leptonema*, *H. pusilla*, *H. ucrainica* rūšys buvo labai dažnos upėse.

Išskirtos keturios upių grupės pagal upių morfometrinius, hidrofizikinius rodiklius ir vyraujančias dumblių ekologines grupes, atskleidė fitobentoso rūšių pasiskirstymo ypatumus. Melsvabakterės (iki 34 rūšių) aptiktos visų grupių upėse, žaliadumblių didžiausia įvairovė (19 rūšių) nustatyta didelėse lėtos tėkmės, raudondumblių – vidutinėse greitos ir didelėse lėtos–greitos tėkmės upėse (atitinkamai aptiktos 5 ir 6 rūšys).

Bentosinės dumblių rūšys sudarė 85 % upėse aptiktų dumblių rūšių, likusią dalį – meroplanktoninės (12 %) ir meroplanktoninės-bentosinės (3 %) rūšys. Epifitinės (iki 43 rūšių) ir epipelitinės (iki 36) rūšys buvo skaitlingos visų grupių upėse, epilitinės (iki 19) rūšys vyravo greitos ir lėtos–greitos tėkmės upėse.

Didžiausią dalį (58 %) fitobentoso rūšių sudarė labai retos ir retos dumblių rūšys. Labai retos *Chamaesiphon carpaticus*, *Chlorogloea microcystoides*, *Chroodactylon ornatum*, *Draparnaldia acuta*, *Schizomeris leibleinii*, *Stigeoclonium carolinianum* formavo negausias populiacijas. Apyretės, dažnos ir labai dažnos rūšys aptiktos panašiu santykiu.

Vyraujančios, retos Lietuvos upėse ir pagal morfologinius požymius sunkiai identifikuojamos *Batrachospermum arcuatum*, *B. gelatinosum*, *Hildenbrandia rivularis* ir *Thorea hispida* apibūdintos pagal *rbc*L geno, *Lemanea fluviatilis* – pagal 18S rRNR geno, *Cladophora glomerata* – pagal ITSI srities nukleotidų sekas.

Nustatytas vyraujančių upėse žaliadumblių *Cladophora glomerata* genetinis polimorfizmas pagal klonuotos ITSI srities DNR sekvenavimo duomenis. Nukleotidų sekų mutacijas galėjo sąlygoti skirtingos makrodumblių vystymosi ekologinės sąlygos.

Upių plotis, srovės greitis ir substratas buvo statistiškai reikšmingi fitobentoso struktūrai. Plačiose, didelėse lėtos tėkmės ir neužpavėsintose upėse vyravo *Oedogonium* spp. žaliadumbliai, o siaurose, greitos tėkmės ir stipriai užpavėsintose pakrantės augalija – šviesos intensyvumo pokyčiams pakantūs *Audouinella, Batrachospermum* genčių raudondumbliai ir *Heteroleibleinia, Phormidium, Tapinothrix* genčių melsvabakterės. Greitos tėkmės upėse gausiausias populiacijas formavo epilitinės *Audouinella hermannii, Cladophora glomerata, Hildenbrandia rivularis* rūšys.

Fitobentoso struktūrą daugiausiai įtakojo vandens savitasis elektrinis laidis ir bendrojo azoto (N_b) kiekis vandenyje. Mažas ištirpusių druskų jonų ir N_b kiekis lėmė raudondumblių *Audouinella hermannii, Batrachospermum gelatinosum, Hildenbrandia rivularis* ir melsvabakterių *Phormidium retzii, P. uncinatum*, o didelis – melsvabakterių *Oscillatoria limosa, Phormidium autumnale*, gelsvadumblių *Vaucheria sessilis* ir žaliadumblių *Cladophora glomerata, Oedogonium* sp$_{.3}$ gausumą.

Išskirtų šešių indikatorinių makrodumblių rūšių skaičius ir jų projekcinio padengimo pokyčiai leidžia įvertinti įvairaus tipo upių būklę *in situ*. Blogėjant telkinių būklei, rūšių skaičius ir padengimo vertės (nuo 70 iki 5 %) mažėja. *Audouinella hermannii, Phormidium retzii, P. uncinatum* rūšių padengimas sudarė 20–40 % tik labai geros ir geros būklės upėse. *Hildenbrandia rivularis, Cladophora glomerata* ir *Vaucheria sessilis* projekcinis padengimas ženkliai mažėjo keičiantis vandens būklei nuo vidutinės iki labai blogos.

Pagal fitobentoso indeksą (BI) 49 % tirtų Lietuvos upių yra labai geros ir geros, 37 % – vidutinės, blogos ir labai blogos būklės, likusių upių būklė nenustatyta. Indeksas BI patikimai atspindi skirtingo tipo Lietuvos upių ekologinę būklę esant vidutiniam N_b, P_b kiekiui vandenyje. Indekso patikimumas mažėja kai maistinių medžiagų kiekis būna mažas ar didelis.