Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 46, Issue 4, December 2017

ISSN 1730-413X
eISSN 1897-3191

pages (379-392)

# Structure and succession of periphyton in an urban reservoir: artificial substrate specificity

by

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DOI: **10.1515/ohs-2017-0038**
Category: **Original research paper**
Received: **March 1, 2017**
Accepted: **April 14, 2017**

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## Abstract

Substrate specificity of the periphyton community is usually underestimated in both periphyton ecology and biomonitoring studies, thus different kinds of both natural and artificial substrates (of organic and inorganic origin) are employed. Periphyton colonization and successional trajectories are particularly debatable processes when different kinds of substrates are considered. In our field experiment, we deployed four kinds of artificial substrates (two inorganic ones – glass and ceramic, and two organic ones – willow and yew wooden tiles) for the development of periphyton in an urban reservoir referred to as Lake Savsko (Belgrade, Serbia). We comparatively investigated the structure, colonization process, diversity and successional trajectories of periphyton. We also assessed the relationship between the dynamics of algae growth forms (ecological groups) on different substrates and selected abiotic limnological factors. Our objective was to determine whether the type of substrate affects periphyton characteristics at various levels and whether an artificial substrate potentially affects the bioindication capacity of ecological groups. We concluded that all substrates behave similarly at the level of structure and colonization phases, but when considering diversity and successional trajectories, the substrate specificity was demonstrated. Our results suggest that communities developed on inert substrates (glass and ceramic) could provide more realistic insight into complex environmental changes.

**Key words:** Periphyton, artificial substrates, wood, ceramic, glass, shallow lake

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## Introduction

A complex biofilm that develops on submerged substrates represents a heterogeneous community referred to as periphyton. Periphyton consists of both autotrophic and heterotrophic (organic) components as well as an inorganic component originating from different kinds of particles from the water column. The autotrophic component of periphyton is mainly composed of algae and Cyanobacteria, while the heterotrophic component is comprised of bacteria, fungi and microinvertebrates. This complex assemblage plays an essential role in the basic processes of the ecosystem – primary production and nutrient cycling, as well as particle and pollutant filtration (Azim et al. 2005). The development of periphyton is driven by the complex interactions between various abiotic (hydrological regime, light, nutrients) and biotic factors (Albay, Akcaalan 2008). Since algae are the most diverse and abundant fraction of periphyton, this complex community is a potential source of valuable information on the environmental state of surface waters (Borduqui, Ferragut 2012). A complex interaction between environmental parameters, the primary nutrient level, light conditions, colonization time and the substrate type affects the development of the periphyton community (Zhang et al. 2013). The potential of organic substrates as an additional source of nutrients and the way they affect the periphytic community are emphasized (Zhang et al. 2013).

Various artificial substrates (both of inorganic and organic origin) are employed in both the periphyton ecology and biomonitoring studies (B-Béres et al. 2016; Stenger-Kovács et al. 2013; Zhang et al. 2013; Žuna Pfeiffer et al. 2015), neglecting the effect of the substrate type on the development of periphyton (Cattaneo, Amireault 1992; Schevchenko 2011). Usually, the significance of dissimilarities between communities developed on natural and artificial substrates is pointed out, but very few studies paid attention to potential differences between communities developed on different kinds of artificial substrates, especially when wooden or similar (organic origin) and inorganic types of substrates are used (Sabater et al. 1998; Danilov, Ekelund 2001; Zhang et al. 2013).

The ecological importance of algal growth forms (ecological groups) has been studied since Passy (2007) introduced diatom ecological guilds. However, DeNicola et al. (2006) also emphasized the significance of algal growth forms, but they took into consideration both diatoms and soft bodied representatives of the periphytic algal community. The bioindication capacity of ecological groups has been confirmed in many studies (DeNicola et al. 2006; Passy 2007; Stenger-Kovács et al. 2013; B-Béres et al. 2016). Stancheva & Sheath (2016) postulated that although diatoms are preferred in biomonitoring studies (studied per se, excluding non-diatom algae in the community), soft bodied algae have a great potential and should be reconsidered as bioassessment tools. To summarize the literature, Tapolczai et al. (2016) also emphasized the importance of considering non-diatom algae in functional classification.

Periphyton colonization and successional trajectories are driven by integrated allogenic and autogenic factors, but the complexity of these processes in periphytic biofilm makes the determination of the main factors imprecise (França et al. 2011). Hydrological disturbances, the nutrient level and seasonality were emphasized as the factors that strongly affect the taxonomic and functional composition of the periphytic community and successional patterns (França et al. 2011; Dunck et al. 2015; Larson, Passy 2012). Only one study assessed the effect of the substrate type on periphytic algal life forms, but only in terms of the substrate roughness, and it was concluded that the intensity of disturbance was the main driver of periphytic algae settlement processes and predominantly determined the effect of substrate heterogeneity (Schneck, Melo 2012).

In our study, we deployed four kinds of artificial substrates (two inorganic ones – glass and ceramic, and two organic ones – willow and yew wooden tiles) for the development of periphyton in an urban reservoir referred to as Lake Savsko (Belgrade, Serbia). We comparatively investigated the structure, colonization process, diversity and successional trajectories of periphyton developed on different kinds of substrates, in order to determine whether the type of substrate affects these periphyton characteristics. We also assessed the relationship between the dynamics of algae growth forms (ecological groups) on different substrates and ecological factors in order to determine whether an artificial substrate potentially affects the capacity of ecological groups to indicate changes in environmental conditions.

## Materials and methods

### Study site

Lake Savsko (44°47′02.28″N, 20°23′25.64″E; 73 m a.s.l) is the largest urban reservoir in Belgrade and plays an important role in supplying drinking water (through bank filtration) to approximately 2 million people, and is used for recreational purposes. Lake

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Savsko was formed by embanking the Sava River near its confluence with the Danube. From the upper, southwestern side, the lake is refreshed by the infiltration of clarified water from a sedimentation unit. The pumping station at the downstream side enables a suitable water level and flow control. It is well connected with public transport, pedestrian and cycling lanes, and more than 100 000 people visit the reservoir every day at the peak of the summer season (Mićković et al. 2014). The reservoir is partially surrounded by vascular vegetation composed of white willow (*Salix alba* L.), white poplar (*Populus alba* L.) and oak (*Quercus robur* L.) etc. (Blaženčić 1995). Lake Savsko is a shallow reservoir and the littoral zone is regularly covered with rich macrophyte vegetation (Janković, Janković 1987), while frequent algal blooms have been recorded in the pelagic zone (Blaženčić 1995). The average depth of the reservoir is 4.5 m (maximum recorded is 12 m), the length is about 4.4 km and the width about 250 m (Mićković et al. 2014). During cold winters, the lake is completely or partially covered with ice.

### Experimental design

During the summer period (July to September) of 2014, artificial substrates (glass, ceramic, willow and yew tree tiles) were submerged into the photic zone of Lake Savsko (11 July), at a depth of 50 cm from the water surface, using an acrylic holder as a carrier. The acrylic holder was attached to a floating buoy that was anchored in the northeastern part of Lake Savsko (Fig. 1). All tiles had uniform dimensions, 2.6 × 7.6 cm, and were vertically positioned in the water column. Periphyton samples were collected weekly, from 20 July to 9 September (after 7-9 days of exposure, altogether 8 sampling weeks), and always in triplicate for each type of substrate.

### Environmental parameters and Carlson's trophic state index

Water transparency was measured in situ using a Secchi disk, while water temperature and dissolved oxygen/saturation were measured using a YSI ProODO Optical Dissolved Oxygen Instrument. Water samples for chemical analyses were collected under the water surface using a Ruttner bottle and transported to the laboratory at the Institute of Public Health of Serbia, where all analyses were performed using standard analytical methods (APHA 1995). Chlorophyll *a* (Chl *a*) measurements (from water) were conducted according to the standardized spectrophotometric method (ISO 10260 1992). Carlson's trophic state indices based on Secchi disk transparency (TSI (SD)), Chl *a* concentration (TSI (Chl *a*)) and total phosphorous (TSI (TP)) were calculated and the obtained values were used to define the trophic status changes in Lake Savsko during the study period (Carlson, Simpson 1996).

### Periphyton analyses

Substrates with developed periphyton were collected from 9 a.m. to 1 p.m., stored in separate

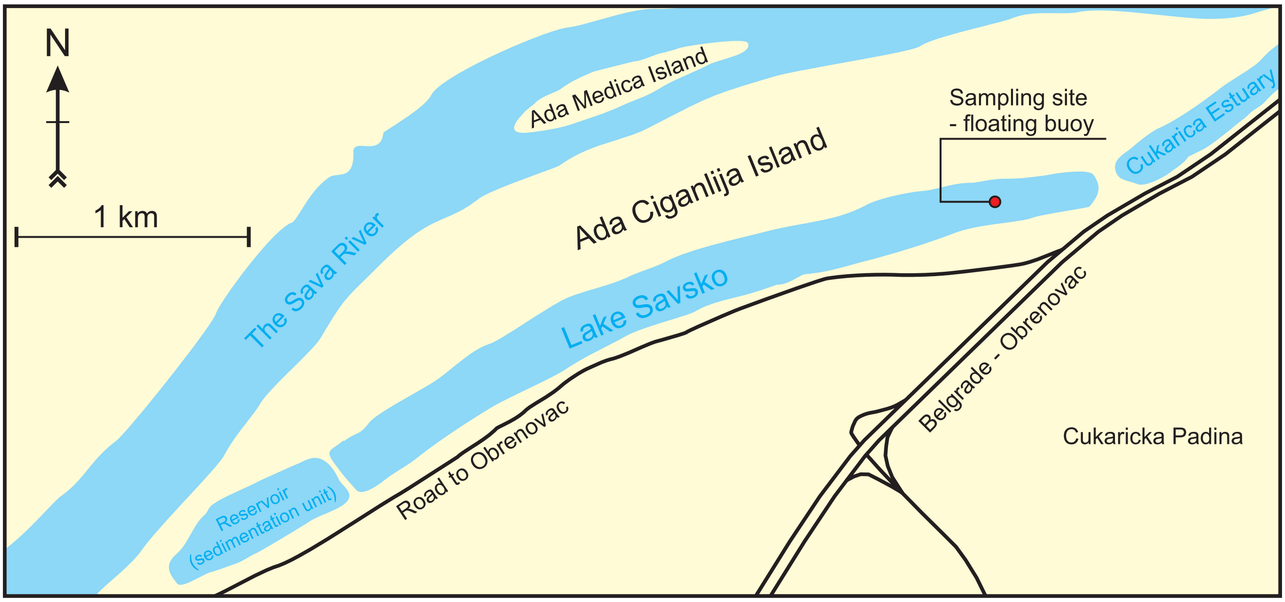

**Figure 1**

Location of Lake Savsko and the sampling site sterile plastic containers and transported to the laboratory in a mobile freezer. In the laboratory conditions, the periphyton from the upper surface of each tile (19.76 $cm^2$) was scraped using a stainless steel razor blade, suspended in 100 ml of tap water and homogenized with a hand blender. From each suspension, 3 subsamples were taken. The first subsample of 40 ml for taxonomic and quantitative analyses was preserved in 4% formaldehyde (final concentration) according to the Guidance Standard on the Enumeration of Phytoplankton using Inverted Microscopy (Utermöhl Technique), (EN 15204 2006). The second subsample of 30 ml was taken for Chl *a* analyses, and the third subsample of 30 ml for estimating the dry mass (DM) and the ash free dry mass (AFDM).

For taxonomic and quantitative analyses, subsamples from all three replicates (for each substrate type) were merged. The quantitative analyses were conducted according to the Utermöhl method (EN 15204 2006) using a Leica DMIL inverted microscope and the data were expressed as a number of individuals (colonies and filaments considered as one individual) per $cm^2$ (ind. $cm^2$). Six ecological groups were defined based on the results obtained for ind. $cm^2$, i.e. ecological guilds and growth forms. Diatoms were classified into ecological guilds (according to their growth morphologies): the low profile, the high profile and the motile guild according to Passy (2007), Passy & Larson (2011), Rimet & Bouchez (2011) and Gottschalk & Kahlert (2012). Soft bodied representatives were classified into growth forms: unicellular and colonial (both Chlorophytes and Cyanobacteria, and also one representative of Chrysophyta and three representatives of Dinophyta, which made a very small portion), filamentous Chlorophytes and filamentous Cyanobacteria, according to DeNicola et al. (2006). All taxonomic analyses were performed using a Carl Zeiss AxioImager M1 microscope and a digital camera AxioCam MRc5 with AxioVision 4.8 software. Part of the material was acid treated and mounted on Naphrax for diatom taxonomic verification (Acker et al. 2002). Taxonomic identification was carried out according to the standard literature. The Shannon diversity index (H) (Shannon, Weaver 1949) and Pielou's evenness index (Eh) (Pielou 1969) were calculated.

The measurements of Chl *a* were performed in warm ethanol using the spectrophotometric method according to ISO 10260 (1992). For AFDM and DM measurements, standard analytical methods (APHA 1995) were applied. All the measured biomass features were recalculated for the tile surface area. Growth rates for periphyton Chl *a* were calculated according to Ahn et al. (2013).

In order to define the composition of periphyton, the index proposed by Lakatos was employed (Lakatos 1989). It is a descriptive index based on chlorophyll-*a* % (Chl type), ash content in % of dry mass (AC type), and dry mass in g $m^{-2}$ (DM type). It classifies the periphyton into a few categories, i.e. types, from autotrophic to heterotrophic (Chl type I-IV), from inorganic to organic ones (AC type I-IV) and from high to low biomass (DM type I-III).

### Data analyses

Coefficients (r) and significance (p) of the correlation between parameters were analyzed by Pearson's correlations. All analyses were performed using the statistical package Statistica 6.0 (Statsoft, Inc., Tulsa, OK, USA) with a significance level of $p < 0.05$ for all tests.

The redundancy analyses were performed using the program CANOCO for Windows, Version 5.0 (ter Braak, Šmilauer 2012). For the project's data, the number of individuals per $cm^{-2}$ (ind. $cm^{-2}$) of all recorded taxa was used as a measure. The identified taxa were first grouped into appropriate ecological guilds: the low profile guild (LPG), the high profile guild (HPG), the motile guild (MPG), and the appropriate growth form group (unicellular and colonial, OCC), filamentous Cyanobacteria (CF) and filamentous Chlorophyta (CHF). Then, the potential effects of the measured environmental factors on these groups were examined using RDA. We used 12 explanatory variables (turbidity, silicon dioxide, permanganate index, biological oxygen demand, pH value, oxygen saturation, nitrate content, nitrite content, ammonium ion, conductivity, total phosphorus and orthophosphates) and two supplementary variables (weeks of sampling and substrates). The explanatory variables were submitted to the interactive forward selection where the statistical significance of each variable was tested by the Monte Carlo permutation test at a cutoff point of $p = 0.05$. RDA with the 'center and standardize' option was used.

## Results and discussion

### Environmental parameters and Carlson's trophic state index

During the study period, water temperature varied in a relatively small range, from 24°C to 27°C. Water transparency values recorded in a narrow range, from 2.60 to 3.50 m, point to a relatively low phytoplankton primary production and low turbidity of Lake Savsko.

The water was rich in dissolved oxygen – all measured concentrations were above 9.1 mg $l^{-1}$, while oxygen saturation varied between 82 and 97%. Lake Savsko had alkaline water with a relatively low content of dissolved minerals; conductivity varied in a range of 214-229 μS $cm^{-1}$. The permanganate index, as a measure of organic load, varied between 5.40 and 11.70 mg $l^{-1}$. The concentration of silicon dioxide ranges from 0.09 to 0.70 mg $l^{-1}$. There were slight differences in the ammonium content, with the maximum value determined in the 7th sampling week (Fig. 2a), and in the concentration of nitrites, the values of which were generally low, with the maximum recorded on the start date of the experiment (presented on secondary axes, Fig. 2a), while the highest values of nitrates were recorded in sampling weeks 1 and 4 and were otherwise constantly low (Fig. 2a). Variations in orthophosphates and total phosphorus were within a wide range, 0.001-0.165 mg $l^{-1}$ for orthophosphates and 0.009-0.198 mg $l^{-1}$ for total phosphorus, the maximum values were determined on the 6th sampling week, and high values were recorded also at the very beginning (weeks 2 and 3) and at the end of the experiment (weeks 7 and 8) (Fig. 2b). Large variations in the nutrient concentration could be related to the location of Lake Savsko in the urban territory, where swimmers, restaurants and surface runoff (highway and surrounding facilities) are considered to be the main sources of nutrients and pollutants. Our results could also lead to the assumption that the external input of nutrients (promoted by urbanization of the lake's shore and a large number of visitors) might exceed the capacity of the processes that have a positive effect on the water quality. The disruption of this fragile equilibrium would certainly lead to a failure in achieving the good ecological potential of Lake Savsko. The maximum biological oxygen demand (BOD) was 6.1 mg $O_2$ $l^{-1}$.

The results obtained using Carlson's TSI in summer 2014 are presented in Fig. 3. Values of TSI (SD) and TSI (Chl *a*) were relatively uniform, revealing that the lake was generally in the mesotrophic state, slightly leaning toward the eutrophic state during weeks 7 and 8, according to TSI (Chl *a*). On the other hand, TSI (TP) varied and was significantly higher compared to the two other indices characterizing Lake Savsko as eutrophic in weeks 2, 3, 7 and 8, and even hypereutrophic in week 6. Data regarding the trophic status and water quality of Lake Savsko are scarce in scientific literature. In autumn 2006, Martinovic-Vitanovic et al. (2010) recorded the mesotrophic status of the settling basin (above mentioned as sedimentation unit) that supplies Lake Savsko. Our findings in this research characterized Lake Savsko as predominantly mesotrophic in summer 2014, with a trend toward the eutrophic status in late August and September,

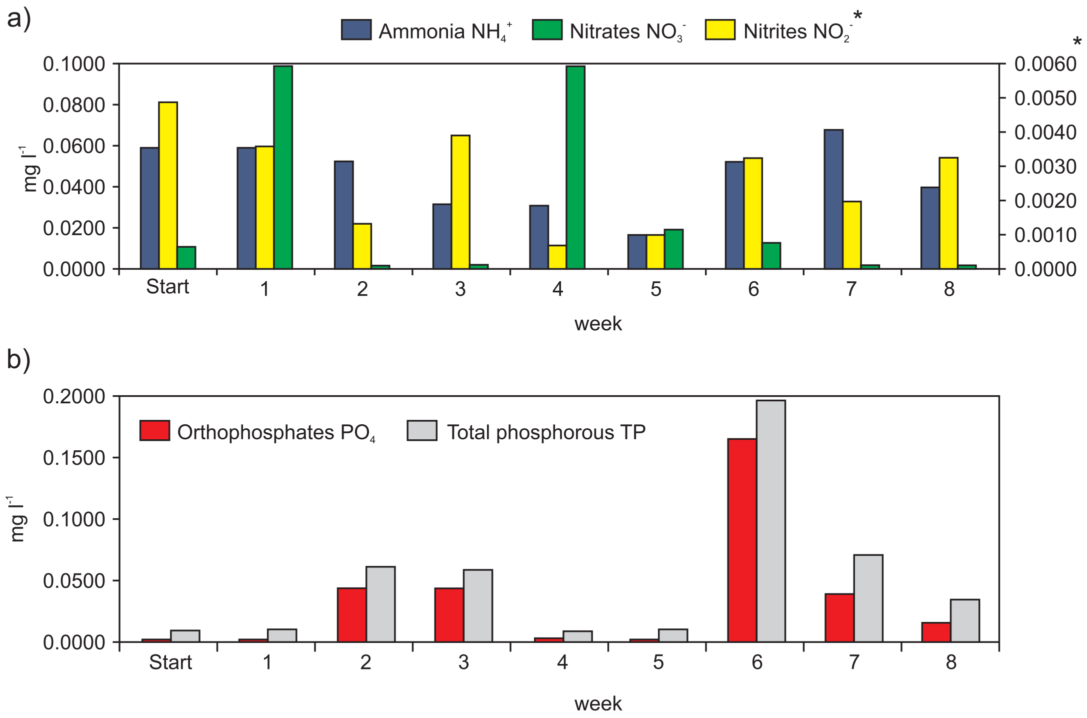

**Figure 2**

Temporal variation of nutrients in Lake Savsko in summer 2014

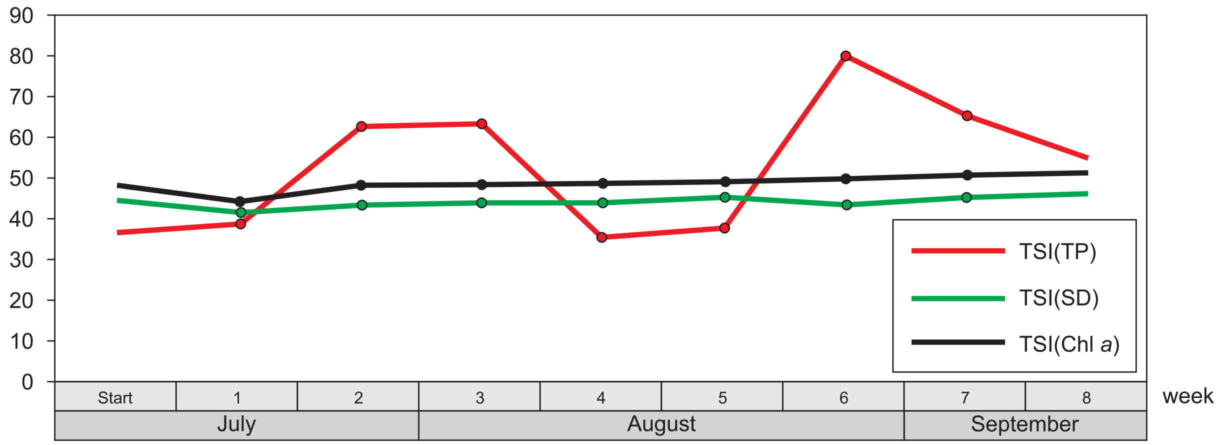

**Figure 3**

Carlson's trophic state index of Lake Savsko in summer 2014

according to TSI (SD) and TSI (Chl *a*). The fact is that TSI (TP) is permanently above the limit of the mesotrophic status and occasionally even above the upper limit of the eutrophic status.

### Periphyton structure and colonisation process

The biomass of the developed periphyton was generally low. According to the Lakatos index (Table 1), the periphyton community belonged to the DM type III, except for the community developed on glass during the last three weeks and on ceramic during the last two weeks, when the community was classified as DM type II, which points to the medium level of biomass. Romanów & Witek (2011) reported that the biomass of periphyton from three different types of lakes in Poland also belonged to DM type III, when the period from April to November was considered. The ash content on all substrates (Table 2) in our study indicated that the periphyton structure was prevalently inorganic/organic (AC type II), occasionally organic/inorganic (AC type III) and purely organic (AC type IV). The inorganic nature of the developed biofilm could reflect a relatively high concentration of suspended particles in water (Kiss et al. 2003), originating from various sources and caused by various factors, including rainfall events (according to the Serbian Hydrometeorological Institute 2015, summer 2014 was extremely rainy) and the urban character of Lake Savsko (the largest recreational and swimming center in Belgrade during the summer season). Romanów & Witek (2011) related the ash content in periphyton to the trophic status of the surveyed lakes. In the above mentioned study, the variability in the ash content between categories II, III and IV was basically characteristic of a shallow eutrophic lake (Lake Gardno), while exclusively organic categorization (AC type I) of periphyton was determined in a soft water mesotrophic lake (Lake Maly Borek). Therefore, it can be concluded, based on our results,

**Table 1**

Lakatos index – classification of the developed periphyton according to the values obtained for DM, AC and Chl *a*; sampling weeks are presented in the rows; the obtained results for DM, AC and Chl *a* types are presented in columns for each tested substrate

| Week | Glass tiles | | | Ceramic tiles | | | Willow tiles | | | Yew tiles | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DM type | AC type | Chl type | DM type | AC type | Chl type | DM type | AC type | Chl type | DM type | AC type | Chl type |
| 1 | III | II | III | III | IV | IV | III | III | IV | III | II | IV |
| 2 | III | IV | IV | III | II | III | III | II | IV | III | II | III |
| 3 | III | II | II | III | II | II | III | IV | III | III | II | II |
| 4 | III | III | III | III | II | III | III | II | III | III | III | III |
| 5 | III | III | IV | III | II | III | III | II | II | III | III | III |
| 6 | II | II | III | III | II | III | III | II | IV | III | II | IV |
| 7 | II | II | IV | II | II | IV | III | II | III | III | II | IV |
| 8 | II | II | III | II | II | III | III | II | III | III | II | III |

**Table 2**

Values obtained for DM, AFDM and Chl *a* on each type of substrate, presented as maximum, minimum and average

| | Glass tiles | | | Ceramic tiles | | | Willow tiles | | | Yew tiles | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DM g m$^{-2}$ | AFDM g m$^{-2}$ | Chl *a* mg m$^{-2}$ | DM g m$^{-2}$ | AFDM g m$^{-2}$ | Chl *a* mg m$^{-2}$ | DM g m$^{-2}$ | AFDM g m$^{-2}$ | Chl *a* mg m$^{-2}$ | DM g m$^{-2}$ | AFDM g m$^{-2}$ | Chl *a* mg m$^{-2}$ |
| max | 27.83 | 9.50 | 9.09 | 22.77 | 9.95 | 9.09 | 16.87 | 7.87 | 10.29 | 18.56 | 6.52 | 6.86 |
| min. | 3.37 | 0.84 | 0.86 | 1.69 | 0.84 | 0.34 | 3.37 | 1.52 | 0.34 | 2.53 | 0.90 | 0.34 |
| average | 11.49 | 4.41 | 6.11 | 11.07 | 4.00 | 4.76 | 8.65 | 3.87 | 5.29 | 8.54 | 3.57 | 4.22 |

that the ash content index (Lakatos index) is a good descriptor of local environmental conditions in Lake Savsko. The classification according to Chl *a* placed the community mostly between hetero/autotrophic (Chl type III) and heterotrophic (Chl type IV), while the auto/heterotrophic state (Chl type II) was determined on very few occasions, mostly during the 3rd week. Kiss et al. (2003) suggested that the prevalence of heterotrophy in the periphyton structure occurs in ecosystems with allochthonous and autochthonous organic loadings, which is highly expected in the recreational facility such as an urban lake. We found the Lakatos index easy to use. This descriptive index provides a good insight into the complex periphyton structure that potentially reflects local environmental conditions.

Table 2 shows maximum, minimum and average values for DM, AFDM and Chl *a* for each type of substrate. When average values are considered, all parameters reached their maxima on glass tiles and minima on yew tiles. DM and AFDM on ceramic were second in order along the declining line, while Chl *a* was higher on willow compared to ceramic. Minimum values for all parameters were determined in the first two weeks, while maximum values occurred in the last three weeks. Biggs (1996) suggested that the Chl *a* range of 3-60 mg m$^{-2}$ (with a characteristic median value of 21 mg m$^{-2}$ for Chl *a* and 4.8 g m$^{-2}$ for AFDM) typically describes moderately enriched stream environment. If we compare our results obtained for Carlson's index and periphyton Chl *a* and AFDM measurements, it appears that they fit this generalization.

Based on the results obtained for the photosynthetic component (Chl *a*) growth rate (Fig. 4a, b, c, d), it could be concluded that the initial exponential phase of growth on all substrates occurred during the first three weeks of incubation, and was followed by the fluctuation phase. During the fluctuation phase in weeks 6 and 7, new peaks in the Chl *a* growth rate occurred, while weeks 4 and 5 were characterized by the biomass loss. This colonization pattern was manifested when periphyton from glass, ceramic and willow tiles was considered (Fig. 4a, b, c), while the colonization pattern of biofilm developed on yew tiles was slightly different (Fig. 4d). The maximum Chl *a* growth rate was determined during week 3 on willow tiles. It can be concluded, based on our results, that the

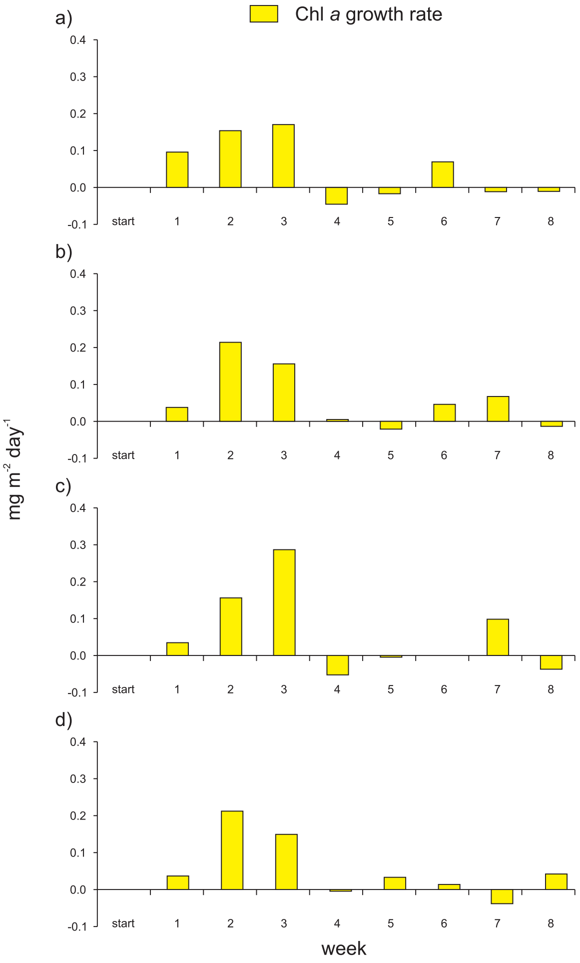

**Figure 4**

Periphyton photosynthetic component (Chl *a*) growth rate dynamics on glass (a), ceramic (b), willow (c) and yew (d) tiles

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exponential phase of the periphyton's photosynthetic component in the colonization process lasted three weeks on all substrates, the loss phase was observed in weeks 4 and 5, and the next biomass increment was observed in weeks 6 and 7.

According to literature data, the colonization time of periphyton is usually estimated between 2 and 4 weeks, after which the maximum biomass is reached and sloughing is avoided (Cattaneo, Amireault 1992). In our study, the highest biomass values occurred in weeks 6, 7 and 8 (Table 2), but according to the general pattern of short-term biomass accruals (Azim, Asaeda 2005) and Fig. 4, these values were recorded during the biomass increment observed in fluctuation phase and followed the previous biomass loses. Thus, the overall peak in biomass recorded in our study could be attributed to the recolonization processes and the increase in the community complexity in the latter successional phase, instead of the initial accrual of biomass. This suggests that the real first peak of biomass actually occurred during the first three weeks of incubation, as Biggs (1996) speculated it to be a pattern in conditions of low to moderate disturbance intensity, where the colonization process is promoted by abundant propagules in the refugium.

### Periphyton diversity and evenness

Algal diversity (H) and evenness (Eh) of the periphytic community developed on each substrate are presented in Fig. 5 (a, b, c, d). It appears that the trend in diversity during the colonization is very similar on both wooden substrates ($r = 0.884$, $p < 0.01$) (Fig. 5c, d), but obviously different compared to both inorganic substrates – glass and ceramic (Fig. 5a, b). Sabater et al. (1998) also concluded that the diversity patterns were different on wooden and ceramic substrates and attributed it mainly to the grater heterogeneity and roughness of the wooden substrate. In general, the highest diversity and evenness on willow and yew tiles were observed in weeks 2 and 3, and the decline was observed in weeks 4, 5 and 6. The next slight increase in the diversity on wooden substrates was recorded in the last three (on willow) and two (on yew) weeks of incubation. Chl *a* growth rates on willow and yew tiles correlated positively with H indices on these substrates ($r = 0.681$, *ns*, $r = 0.733$, $p < 0.05$, respectively). Thus, it can be concluded, according to the colonization phases presented in Fig. 4c, d, that exponential phases of colonization on wooden substrates are determined by arrival of new colonizers (immigration), while loss

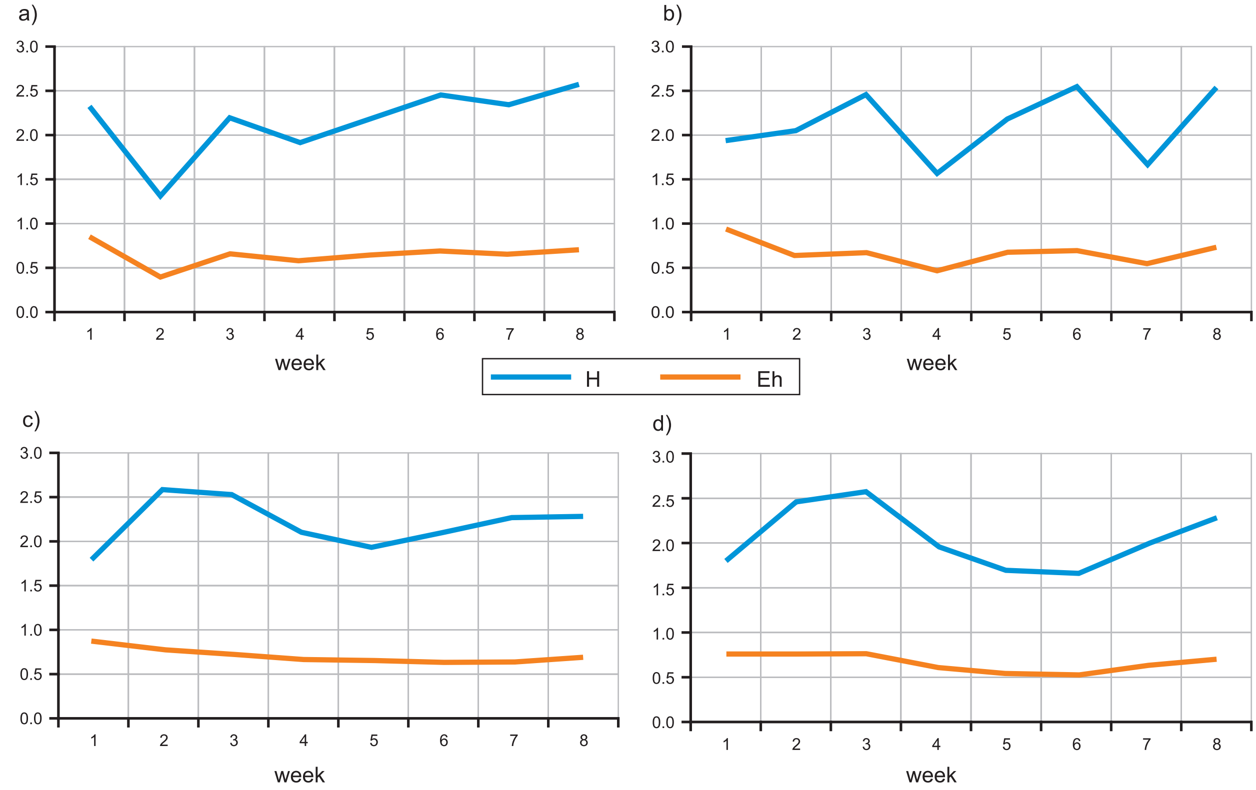

**Figure 5**

Shannon's diversity index (H) and Pielou's evenness index (Eh) on glass (a), ceramic (b), willow (c) and yew (d) tiles phases were characterized by a diversity decrease.

On the other hand, communities developed on glass and ceramic were characterized by specific patterns in the diversity dynamics during the colonization time (Fig. 5a, b). The highest values of the H index on both substrates were determined in weeks 6 and 8, while drastic declines in the diversity were determined in weeks 2 and 4 on glass tiles, and weeks 4 and 7 on ceramic. Generally, the diversity index and evenness considerably varied on ceramic without any specific pattern. Photosynthetic component biomass growth rates on glass and ceramic substrates (Fig. 4a, b) and the diversity indices on these substrates did not show any correlation. The exponential growth phase on glass tiles was apparently characterized by a decrease, both in the diversity and evenness in week 2, pointing to the prevalence and dominance of specific colonizers that arrived in week 1, while the diversity equilibrium recovered in week 3. In the later period of colonization, the evenness of the community on the glass substrate was more or less stable, and the diversity showed a positive trend from the 4th week till the end of the study period. The increase in the diversity during the colonization time is often observed in studies where glass slides are used as an artificial substrate for the development of periphyton (Kralj et al. 2006; Bordoqoui, Ferragut 2012) and is usually explained by the constant arrival of new colonizers. On the other hand, when wooden substrates are considered (Hillebrand, Sommer 2000; Sabater et al. 1998.), the negative effect of the colonization time on the diversity is observed and theoretically explained by the competitive advantage of specific taxa in the later successional stages (Hillebrand, Sommer 2000). In our study, the periphyton biofilms developed on each substrate had one feature in common and this was the diversity decline in week 4 that occurred after the loss phase.

## Distribution of ecological groups during colonization – successional pattern

All algal taxa detected in quantitative analyzes were allocated to the ecological groups and the percentage contribution of groups, based on the number of ind. cm$^{-2}$, is presented in Fig 6. Diatoms were grouped into the low profile, high profile and motile guilds according to Passy (2007), Passy & Larson (2011), Rimet & Bouchez (2011) and Gottschalk & Kahlert (2012). Most of the low profile guild members were representatives of *Achnanthidium* spp. Kützing, *Encyonopsis* spp. Krammer, *Cymbella* spp. C.Agardh and *Cocconeis* spp. Ehrenberg, the high profile gild was mainly represented by *Fragilaria* spp. Lyngbye and *Gomphonema* spp. Ehrenberg, and the motile guild – by *Brachysira* spp. Kützing, *Navicula* spp. Bory, and *Nitzschia* spp. Hassall. According to Passy (2007), representatives of the low profile guild have

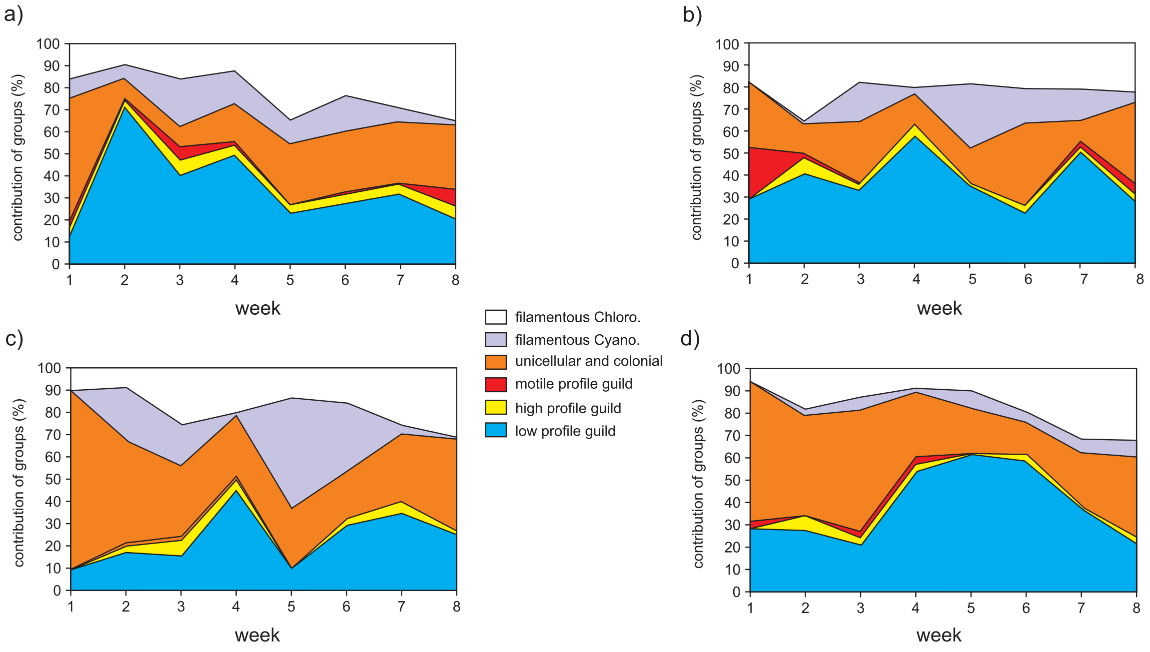

**Figure 6**

Percentage contribution of the ecological groups to the periphyton community during the duration of the experiment on glass (a), ceramic (b), willow (c) and yew (d) tiles the advantage of overcoming the limitation of nutrients and the stress related to disturbance. The representatives of the high profile guild, although considered very sensitive to grazing pressure, are expected to prevail in nutrient-rich habitats with a low level of disturbance (competitive features – erect, stalk, filamentous forms). The advantage of representatives of the motile guild is mobility in the biofilm's three-dimensional structure and therefore they show a low level of sensitivity to both nutrient deficiencies and disturbance, and they are generally expected to occur in nutrient-rich habitats. Tapolczai et al. (2016) emphasized that Passy's (2007) ecological guild classification is based on the vertical position of guilds in the biofilm, i.e. the way the species are attached to the substrate. Consequently, the low profile species generally represent the bottom layer of the biofilm, while the high profile guild species occur in the canopy layer, and the motile guild can change the position in the biofilm's three-dimensional structure according to the nutrient and light availability. Soft bodied algae were grouped into growth forms (DeNicola et al. 2006). The unicellular and colonial group was mainly represented by typical immigrants from phytoplankton – *Aphanocapsa* spp. C.Nägeli, *Merismopedia* spp. F.J.F.Meyen, and *Snowella lacustris* (Chodat) Komárek & Hindák from Cyanobacteria, *Phacotus lenticularis* (Ehrenberg) Deising, *Scenedesmus* spp. Meyen, *Pediastrum* spp. Meyen, etc. from Chlorococcales, and *Cosmarium* spp. Corda ex Ralfs from Desmids. The filamentous Cyanobacteria group was mainly represented by *Pseudanabaena* spp. Lauterborn, while the filamentous Chlorophyta group was almost exclusively composed of *Mougeotia* sp. C.Agardh. According to Denicola et al. (2006), proportions of filamentous growth forms from both Chlorphyta and Cyanobacteria are expected to increase in periphyton communities along the nutrient (phosphorous) gradient (mainly due to the fact that this growth form allows easier access to nutrients and light in a thickened periphyton mat), while a decrease would be expected for the unicellular and colonial growth form group. According to Biggs et al. (1998), filamentous forms of green algae are expected in later phases of the biofilm development i.e. the "climax" community.

When the percentage contribution of groups during the exponential growth phase (the first three weeks) is considered, our results showed specific patterns on each substrate. Communities developed on yew and willow tiles looked alike in this particular period (the first three weeks, Fig. 6b, c, d), although the ratios of the represented groups were different. During the exponential phase of growth, the unicellular and colonial group (immigrants from phytoplankton) clearly dominated on yew tiles, and this is also characteristic of the biofilm developed on willow. The motile guild was clearly represented only on ceramic tiles during the 1st week of colonization. In weeks 2 and 3, the percentage of ecological groups on ceramic tiles was generally stable, with only motile and high profile guilds underrepresented, but this trend was observed on all substrates. The glass substrate was clearly different during the exponential phase, which was also reflected in the diversity index (Fig. 5a), and Figure 6a shows that the major reason for the diversity decline in week 2 is the dominance of the low profile guild's representatives. At the end of the exponential phase (week 3), the balance of the community on glass tiles was reestablished (H increased), however, still with the highest contribution of the low profile guild. Thus, it can be concluded that glass was preferred by low profile diatoms during the exponential growth phase, wooden substrates by the unicellular and colonial group, while ceramic was more or less equally colonized by soft bodied algae and low profile diatoms as well as the motile guild, but only at the very beginning of the exponential phase. Representatives of the low profile guild appeared to be pioneers in the colonization process, particularly on the glass substrate, while the unicellular and colonial group play the same role on the wooden substrates. Mihaljević & Pfeiffer (2012) reported that planktonic Cyanobacteria dominated in the periphytic community developing on glass slides in the very early colonization phase (day 1 of colonization), due to their abilities to produce mucilage and adhere to the substrate, but they were very quickly outcompeted by short-generation time diatoms (day 3 of colonization). A similar pattern can be observed in our results (Fig. 6a), where the significant contribution of the unicellular and colonial group (in this period mostly represented by *Aphanocapsa* spp. taxa) was observed on glass slides in the first week of incubation, while low profile diatoms clearly dominated in the periphyton community already in week 2.

The fluctuation phase (from week 4 onward) was marked by a general increase in the percentage of the low profile guild on wooden substrates. This could indicate the postponed colonization of wooden substrates by low profile guild's diatoms compared to inert substrates (glass and ceramic). The contribution of low profile guild's representatives to the community increased during the fluctuation phase along with the losses in biomass (negative growth rate) and the low biovolume of these taxa is a reasonable explanation of this (how it seems) paradox. Once again, this confirms the importance of the low profile guild in the recolonization process, as it appears that the observed losses in biomass were the early stages of the recolonization process. *Achnanthidium* spp. accounted for a high proportion in the low profile guild. The individuals of this taxon usually occur in the late colonization phases (following disturbances), due to their competing strategies and resistance to grazing pressure (Mihaljević, Žuna Pfeiffer 2012). The dominance of the low profile guild in the community during the summer period observed in our study is basically consistent with the observations by Rimet et al. (2015), who explained it by a thin structure of the biofilm (i.e. nutrients and light were not limited in the bottom layer) and high grazing pressure (low profile representatives are resistant to grazing pressure due to their small size and prostrate form, unlike taxa of the high profile guild that are very sensitive to grazing). However, the abundance of the low profile group showed a significant negative correlation with both H and Eh only on the glass substrate (r = -0.827, $p \leq 0.01$ and r = -0.908, $p < 0.01$), and with Eh on yew and ceramic (r = -0.905, $p < 0.01$ on yew and r = -0.780, $p < 0.05$ on ceramic tiles), while no significant correlations were determined on willow tiles. The biofilm developed on willow tiles had another specific occurrence in the fluctuation phase: the filamentous Cyanobacteria group had a high contribution to the community in weeks 5 and 6 (loss phase, decline in diversity). This occurrence could indicate a significant (negative) effect of the low profile guild's representatives on the diversity and evenness of the periphytic community developed on glass, and evenness on ceramic and yew tiles, probably due to the competitive advantage (such as short generation time) of the low profile guild's taxa in the successional stages characterized by the biomass loss (Hillebrand, Sommer 2000). On the willow substrate, the above guild was replaced by the filamentous Cyanobacteria group. Contrary to our findings, Schevchenko (2011) found coccoid and unicellular Chlorophyta and Cyanobacteria (Chlorococcales members and *Gloeopcapsa* spp.) and members of the filamentous Cyanobacteria group (*Phormidium* spp.) specific to the inorganic artificial substrate, but only when compared to the higher aquatic plants and green filamentous algae as organic substrates. In our study, successional patterns on each type of substrate appeared to be very specific.

### Environmental factors and ecological groups

Interactive forward selection revealed that total phosphorus (TP), nitrates ($NO_3^-$), oxygen saturation ($O_2$) and the permanganate index (PI) were statistically significant environmental variables (Fig. 7).

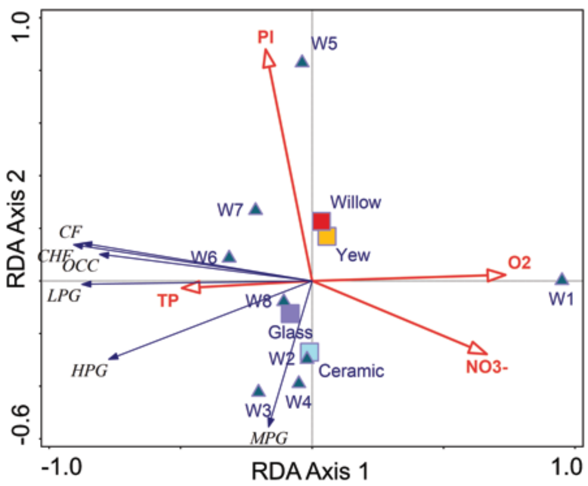

**Figure 7**

Relationship between the ecological groups of periphytic algae and environmental parameters

**Ecological groups:** low profile guild (LPG), high profile guild (HPG), motile guild (MPG), unicellular and colonial (OCC), filamentous Cyanobacteria (CF) and filamentous Chlorophyta (CHF); **Environmental parameters:** total phosphorus (TP), nitrates ($NO_3^-$), oxygen saturation ($O_2$) and permanganate index (PI).

Weeks of sampling (W1-W8) and substrates (Ceramic, Glass, Yew and Willow) are included as supplementary variables.

$O_2$ and $NO_3^-$ were positively correlated with the first axis (r = 0.6945 and r = 0.6279, respectively), while TP showed negative correlation (r = -0.4683). PI was positively correlated with the second RDA axis (r = 0.5699). The first RDA axis explained the main portion of the total variance in our data (60.49%). Thus, the first axis represents the variation in the community explained by $O_2$, $NO_3^-$ and TP, and the second vertical axis represents the part of the variation explained by PI. The statistical analysis showed high significance (F = 14.6, $p = 0.0002$). All sampling weeks, except sampling week 1, are distributed along the second RDA axis, along with supplementary variables – substrates types. Ceramic and glass are placed in the lower part of the diagram (negative part of the second RDA axis), while wooden substrates, willow and yew, are in the positive part of the second RDA axis. The position of wooden substrates in the positive part of RDA axis 2, explained by PI, could indicate the fact that the community developed on wooden substrates is less sensitive to organic compound loads compared to the inert substrates, which could be a consequence of the habitat preference by a specific guild. Stenger-Kovács et al. (2013) found in their research that the low profile guild's representatives responded positively to chemical oxygen demand, while the response of the motile guild to this parameter was negative. Therefore, the authors suggested indicative capacity of these guilds toward decomposition intensity.

All groups were positively correlated with TP, but only MPG was correlated with both TP and $NO_3^-$ and unlike other groups, it was found in larger numbers on glass and ceramic compared to wooden substrates (Fig. 7).

Zhang et al. (2013) suggested that wooden substrates in general are a source of nutrients in available forms for the development of algae in biofilm, primarily due to the decomposition processes. The representatives of the motile guild are generally referred to as superior competitors in nutrient-rich habitats, because of their physical capability to select a suitable position in the biofilm (Passy 2007). This guild was underrepresented in our study and was mostly found only on glass and ceramic substrates (Fig. 6a, b). Compared to the wooden substrates, glass and ceramic could be considered inert, and this may be the reason why the motile guild occurred only on these substrates. The observed presence of the motile guild only on inert and not wooden substrates could indicate that the communities on wooden substrates were not limited by the supply of nutrients (primarily $NO_3^-$), because of the endogenous loading from the decomposition processes. Therefore, the motile guild could not exhibit the competitive advantage and proliferate on wooden substrates in a greater or more apparent portion. Based on our results and consistent with the suggestion by Stenger-Kovács et al. (2013), it can be concluded that the community developed on inert substrates (glass and ceramic) could provide more realistic insight into complex environmental changes.

## Conclusions

According to the Lakatos index, communities developed on four kinds of substrates were very alike; biomass was mainly low and biofilm was usually inorganic and heterotrophic. When the autotrophic biomass (Chl *a*) growth rate was considered, the colonization phases were clearly distinguished, the exponential phase on all substrates lasted 3 weeks, the loss phase occurred in weeks 4 and 5, and the next biomass increment was observed in weeks 6 and 7, thus the colonization had similar paths on all substrates.

The dynamics of the diversity changes during the colonization on both wooden substrates was very similar and different in the communities developed on glass and ceramic. The low profile guild appeared to be represented by pioneers in the colonization process, particularly on the glass substrate, while the unicellular and colonial group played the same role on the wooden substrates. The fluctuation phase (from week 4 onward) was marked by a general increase in the contribution of the low profile guild on wooden substrates. Generally, the occurrence of the low profile guild's representatives negatively affected the diversity and evenness of the periphytic community developed on glass, and evenness on ceramic and yew tiles, indicating the competitive advantage of taxa from the low profile guild in the recolonization phases. Nonetheless, this guild was not observed on the willow substrate in the recolonisation phase, instead the filamentous Cyanobacteria group occurred there. Successional patterns on each type of substrate appeared to be very specific.

The community developed on wooden substrates showed lesser sensitivity to organic compound loads compared to the inert substrates, which could be a consequence of the habitat preferences by specific guilds. The presence of the motile guild only on inert and not wooden substrates could indicate that the communites on wooden substrates were not limited by the supply of nutrients (primarily $NO_3^-$), because of the endogenous loading from the decomposition processes. Therefore, the motile guild could not exhibit the competitive advantage and proliferate on wooden substrates in a greater or more apparent quantity. It can be concluded that the community developed on inert substrates (glass and ceramic) could provide more realistic insight into complex environmental changes.

## Acknowledgements

This research was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Project No. TR 37009. The authors would like to thank Ms. Nevena Trbojević for careful reading of the manuscript and English language corrections. The authors would also like to thank Mr. Vojislav Simić for technical support in the manuscript preparation and Mr. Vladimir Maršalkin for technical support in the field study. We sincerely thank to anonymous reviewers, whose kind suggestions contributed to the quality of this manuscript.

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