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BACHELOR THESIS

Periphytic Cyanobacteria of the Everglades (Florida) and their relation to water chemistry and different substrata

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Abstract:

Data from 140 samples of periphyton from the Everglades wetlands (Florida, USA) were analysed. Significant influences of water column total phosphorus concentration, substratum quality (natural versus plexiglass substrata in a mesocosm experiment) and seasonal changes on species composition of the samples were confirmed. In addition, cyanobacterial and algal taxa responding selectively to these factors were identified. Moreover, 42 of available samples were analysed microscopically by the author for present cyanobacteria: in total, 76 species were determined and photographically documented.

Acknowledgements:

Above all I thank to Jan „Hanys“ Kaštovský for his friendly and constructive approach as well as for infinite fantasy in thinking up themes for my thesis. I greatly appreciate always obliging and erudite answers of prof. Jiří Komárek to my questions as well as helpful proffessional advice of Tomáš Bešta, Jakub Těšitel, Dana Kapitulčinová and other colleagues. Last but not least, I thank to all my friends and family members for their unflagging support.

Prohlašuji, že jsem uvedenou práci vypracoval samostatně, jen s použitím uvedené literatury.
V Českých Budějovicích dne 3. 5. 2006 .....

Obsah:

1. Introduction

1.1. Locality

In history, the Everglades marshland system covered an area of 11 000 km^2 of the Florida peninsula (USA), stretching from its southern coast to the shores of Lake Okeechobee. In the first half of the 20^{th} century, extensive draining of the Everglades was started, eventually concluding into the present state when Everglades National Park (ENP) has about 1500 km^2 and the rest of the former area was drained (65% of the area) or subjected to rather intensive human interference.

Majority of the drained area has been currently used for intensive large-scale production of sugarcane and winter vegetables (Everglades Agricultural Area – EAA). The northern part of the resting marshlands consists of three Water Conservation Areas (WCAs), diked shallow water reservoirs, which serve mainly for preventing floods and as a water supply for agriculture, industry and heavily populated eastern coast of Florida. A map of southern Florida containing above mentioned areas is given in Fig.1.

The WCA-2A has recently been a place of intensive research by biologists since it is located south of the EAA bordering with it, and so it makes a suitable subject for studying the impacts of human activities to the Everglades wetlands. It covers approximately 574 km^2 of area and is bordered by water canals. The drainage and flush water from the EAA is converged to Hillsboro Canal and periodically pumped to WCA-2A by four pump stations (10A, B, C, D) on its northern border, eventually leaving the area in the south being pumped to WCA-2B. In the 1990s, three transects from stations A, C and D to the south were estabilished in WCA-2A, consisting each of six serial sampling points placed up to 10 km to the south from the gate (Fig. 2). These transects were designed for research purposes (primarily for studying the influence of nutrient-loaded wastewater from the EAA on the Everglades).

Further descriptions of the Everglades region are given for example by DAVIS & OGDEN (1994), GLEASON (1974), KUSHLAN (1990) and RADER & RICHARDSON (1992).

1.2. Periphytic assemblages of the Everglades

Periphyton can be defined as an assemblage of attached microorganisms (primarily algae) which form living biofilms on the free surface of submerged substrates (SWIFT and NICHOLAS 1987).

The periphytic assemblages in the Florida Everglades make one of the most characteristic and important components of the original wetland ecosystem. They are especially abundant in areas with a little deeper water (called sloughs) during the summer wet

season where they often form 2-10 cm thick mats attached to submerged plants (*Eleocharis, Utricularia*), epipelic growths at the bottom of the slough, or mats floating freely near the water surface (metaphytic) after they broke away from the substrate, as described in BROWDER et al. (1994), GLEASON & SPACKMAN (1974), RADER & RICHARSON (1992), VAN METER-KASANOF (1973), WILSON (1974), WOOD & MAYNARD (1974) and other papers. Similar growths, like in the Everglades dominated mainly by filamentous cyanobacteria and often heavily encrusted by $CaCO_3$ (GLEASON & SPACKMAN 1974, VYMAZAL et al. 1994), are often found in alkaline marshes of the whole Carribean and South America region (KOMÁREK 1989, REJMÁNKOVÁ et al. 2000, REJMÁNKOVÁ et al. 2004).

Although the pristine Everglades marshland ecosystem is strongly limited by phosphorus (CRAFT et al. 1995, McCORMICK et al. 1996, NOE et al. 2001, VYMAZAL et al. 1994), the mats of periphyton commonly appear in great abundance, sometimes forming 33% to more than 50% of total dry vegetative biomass in sloughs (PAN et al. 2000) covering almost all submersed surfaces over an area of approximately 8000 km^2 (GRIMSHAW et al. 1997). Such productivity can be explained by unique features of cyanobacteria (COHEN & ROSENBERG 1989, STAL 1995) which allow them to survive in even more extreme conditions. Furthermore, presence of these highly productive communities in a P-limited system is probably linked to effective nutrient uptake and cycling enabled by close association of autotrophic and heterotrophic microbiota (SAND-JENSEN 1983, SCINTO & REDDY 2003, WETZEL 1996) joined together mainly by cyanobacterial mucilage (RADER & RICHARDSON 1992). The ability of periphytic mats to serve as nutrient scavengers has been successfully used in artificial wetlands as a part of projects for Everglades restoration (more information on this is given e. g. in BAYS et al. 2001, DEBUSK et al. 2004 and VYMAZAL 2002).

Critical importance of the periphytic assemblages for the function of the south Florida wetlands has been recognized and described by many authors. The cyanobacteria-dominated mats usually drive the diurnal oxygen cycle, the water chemistry (pH), and are essential for nutrient cycling (BELANGER et al. 1989, FENCHEL 1998, GLEASON & SPACKMAN 1974). Moreover, they provide food and habitat for a lot of animals (BROWDER et al. 1994, GEDDES & TREXLER 2003) and product calcitic mud which is one of the most abundant and important sediment types of the region (GLEASON 1972, GLEASON & SPACKMAN 1974).

For experimental purposes and for monitoring of periphyton, artificial substrata have been used (not only) in the Florida Everglades. Glass and plexiglass slides, as a modification of the common glass slide method (reviewed for example by ALOI 1990, AUSTIN et al. 1981 and SLÁDEČKOVÁ 1962), were employed by many researchers all over the world including McCORMICK et al. (1996) and SWIFT & NICHOLAS (1987) in the Everglades. Ever before there

has been a perpetual debate, whether the periphytic growths which arise on artificial substrata represent natural communities both in biomass volume and species composition. As reviewed in a detailed work of CATTANEO & AMIREAULT (1992), generally, the artificial substrata are rather selective (e.g. BROWN 1976), especially concerning filamentous cyanobacteria which dominate the Everglades marshlands, and filamentous green algae. The slides are probably more suitable for monitoring of diatoms (LANE et al. 2003, TUCHMAN & BLINN 1979) which attach better onto their surface (neither in this case satisfactory results are guaranteed – BARBIERO 2000). The results of a preliminary study executed in the WCA-2A (VYMAZAL et al. 2000a) suggest that the character of periphytic assemblages growing on natural and artificial substrata differs in species composition (simplifiedly diatoms preferred the plexislides and cyanobacteria the natural substrates). These results are necessary to be confirmed.

Another essential but not always appropriately considered factor influencing periphyton is irradiance. Decreased photosynthetic rates of periphyton communities caused by shading by emergent macrophytes compared to open water habitats are presented in GRIMSHAW et al. (1997). In addition, SEKAR et al. (2002) found that algal biomass and species richness were significantly lower in dark-grown biofilms (dominated by diatoms) than in light-grown biofilms (with green algae, diatoms and cyanobacteria).

One of the most significant variables affecting substrate-attached microbiota in the Everglades wetlands are seasonal changes of the environment (VYMAZAL & RICHARDSON 1995). The particular influence of hydroperiod (inundation depths and duration of standing water) was emphasized e.g. by RADER & RICHARDSON (1992) and WOOD & MAYNARD (1974).

All these factors should be considered in interpretation and extrapolation of artificial and natural periphyton observations and in design of experiments.

1.3. Eutrophication in the Everglades

Probably the most important environmental element which influences the periphyton of the Everglades is water quality (described already by GLEASON & SPACKMAN 1974 or SWIFT & NICHOLAS 1987).

In history, the only source of water for the South Florida wetlands was rainfall or else the water flowing out of Lake Okeechobee which is also fed by prepicipitations. For about 50 years, the drainage water from the EAA containing large amounts of nutrients (about 429 metric tons of total phosphorus and 12 170 metric tons of nitrogen each year according to SFWMD 1989) has been pumped to the WCA causing radical changes of water conditions and trophic level of the ecosystem.

Increased levels of nutrient concentrations (phosphorus being the limiting element) largely affect the composition of macrophyte and periphyton community (thorougly reviewed by BELANGER et al. 1989, NOE et al. 2001 and RADER & RICHARDSON 1992), which means especially the expansion of cattail (*Typha domingensis*) into original sawgrass (*Cladium jamaicense*) wet prairies and loss of the native *Utricularia* assemblages with calcareous periphyton in sloughs (replaced for example by *Chara* according to CHIANG et al. 2000). Particularly the periphyton and the affiliated community are influenced also by small additions of phosphorus (experimentally proved by CHIANG et al. 2000, GAISER et al. 2005, McCORMICK & O´DELL 1996 and VYMAZAL et al. 1994), which can be advantageously used in monitoring of the first nutrient-caused changes in the ecosystem. As it is presented in above cited studies, the initial stages of eutrophication may not be visible at first sight, however, accumulative effects of a low-level but long-term nutrient loading may have serious environmental causes.

Although many studies have examined the changes in species composition of periphytic assemblages along an eutrophication gradient in the Everglades, most authors focused on diatoms (GAISER et al. 2006, McCORMICK et al 1996, PAN et al. 2000, RASCHKE 1993), often underestimating the role of dominant cyanobacteria. Therefore, a study concentrating on blue-green algae could bring helpful information.

1.4. Species composition and taxonomy

In their study, SWIFT & NICHOLAS (1987) concluded that the natural algal assemblage of the unenriched Everglades is dominated by filamentous cyanobacteria *Schizothrix calcicola* and *Scytonema hofmanii* forming calcareous mats together with diatoms *Mastogloia smithii, Cymbella ruttneri*, *Anomoeneis vitraea* etc., while nutrient enrichment can cause shift to filamentous green algae (*Mougeotia, Spirogyra, Bulbochaete, Oedogonium, Stigeoclonium*), other blue-greens (*Microcoleus lyngbyaceus*) and diatoms (*Gomphonema parvulum*, *Nitzschia amphibia*, *Navicula disputans*, and others) and loss of the characteristic mats. In agreement with these results, for example VYMAZAL & RICHARDSON (1995) found periphytic mats dominated by blue-green algae *Schizothrix calcicola* and *Scytonema hofmanii* in unenriched Everglades sloughs. Similarly, as for diatoms, several species like *Mastogloia smithii*, *Anomoeneis vitraea* and *Fragilaria syngrotesca* have been reported as typical for unenriched sites while *Gomphonema parvulum*, *Nitzschia amphibia* and *Rhophalodia gibba* should be dominant at more eutrophic sites (GAISER et al. 2006, PAN et al. 2000, RASCHKE 1993). As reviewed by RADER & RICHARDSON (1992), filamentous Cyanobacteria, Bacillariophyta and Chlorophyta represent 80-90% of the total algal standing crop within unenriched areas, coccoid Cyanobacteria, Cryptophyta, Euglenophyta, Chrysophyta and Dinophyta only 5-20%.

Unlike diatoms or green-algae, Cyanobacteria of the Everglades have been considered strongly taxonomically problematic. An example: GLEASON & SPACKMAN (1987) note in their study, that there is a confusion regarding the taxonomy of dominant species *Schizothrix calcicola* and *Microcoleus lyngbyaceus*. In their determination of these species they referred to a work of DROUET (1968). Most authors have at least partly stuck to this identification till recent years (e.g. McCORMICK et al. 1996, McCORMICK & O´DELL 1996 and PAN et al. 2000). In contrary, VYMAZAL et al. (2000a,b;2001) used modern and widely accepted determination literature (ANAGNOSTIDIS & KOMÁREK 1988, KOMÁREK 1989, KOMÁREK & ANAGNOSTIDIS 1998) and found many species of the genuses *Lyngbya*, *Phormidium*, *Leptolyngbya* and others, very probably corresponding to *Schizothrix* and *Microcoleus* identified by the researchers in Florida. They also had some problems with determination of the blue-greens, particularly to the species level. Studies which would focus on proper determination, documentation and presentation of cyanobacterial species occurring in the Florida Everglades are necessary.

1.5. Objectives of the study

Briefly, the major objectives of this study are:

- to confirm the significant influence of eutrophication (phosphorus concentration) to the species composition of the periphytic assemblages in the Everglades WCA-2A and to find the indicating low/high levels of phosphorus. A special accent is put to cyanobacteria, which dominate the periphyton
- to revise the relation of these assemblages to natural and artificial (plexiglass) substrata by finding out, which (if any) taxa selectively colonize different materials.
- to determine the species of cyanobacteria present in periphyton samples from the WCA-2A as best as possible according to available literature, and to work out photographic documentation of the individual species.

Data, partly available in VYMAZAL et al. (2000a, 2000b, 2001), and unanalyzed periphyton samples collected by the same authors are examined in attempt at accomplishing the objectives.

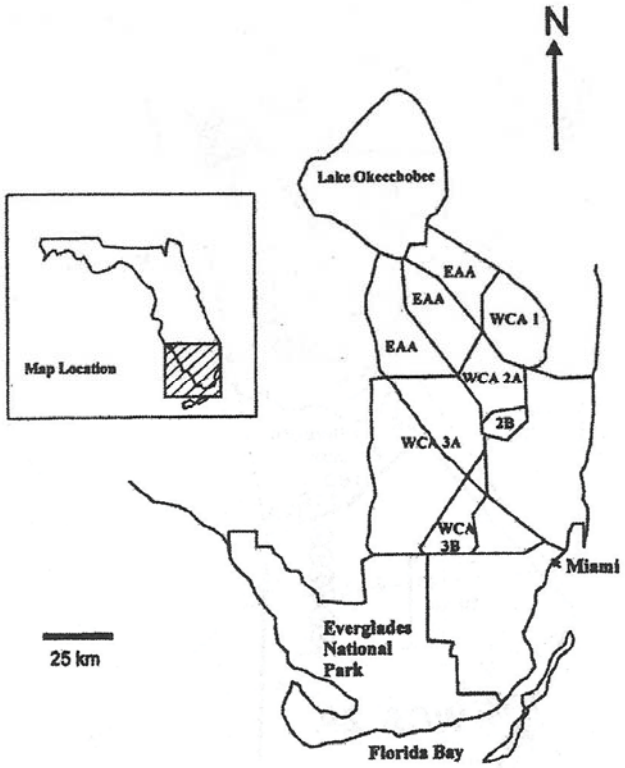


Figure 1. Location of the Everglades Agricultural Area, Water Conservation Areas and Everglades National Park in South Florida (borrowed from VYMAZAL et al. 2002)

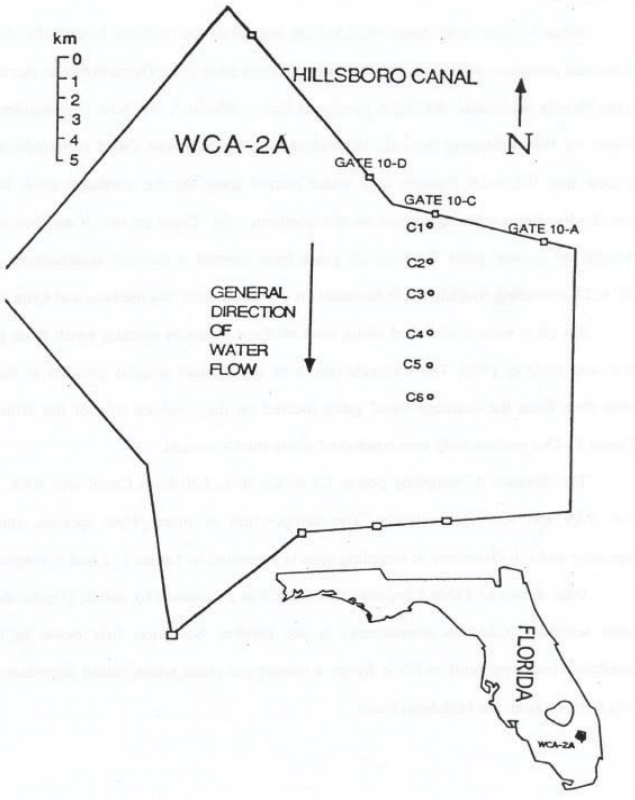


Figure 2. C-transect at the WCA-2A (borrowed from VYMAZAL et. al. 2000a). Similar transects were estabilished also to the south from the gates 10-A and 10-D.

2. Materials and Methods

Data and samples analysed in this study were obtained from a long-term research project taking place at the WCA-2A in Florida, USA (described by VYMAZAL et al. 2000a, 2000b, 2001). The experimental design in this project often produced materials and subsequently data which were less suitable for statistical analysis. For instance, there were different numbers of samples from each locality and from each substratum, the samples were often grown under unequal conditions (especially macrophyte vegetation) and for not exactly the same time, etc. Thus, these heterogenous data can cause certain problems in statistics, which must be considered in interpretation of the results.

1.1. Data

The C-transect (Gradient Study)

A set of samples from the C1-C6 transect of the WCA-2A from November 1999 was received. It consisted of 42 periphytic growths on plexislides (7,5 x 2,5 cm) being submerged in 1,5% formaldehyde in 50 ml plastic vials. The periphyton was removed from the plexiglass with a razor and if there were any macroscopically distingiushable parts, small amount from each part was taken for microscopic examination. Even if the growths appeared homogenic, at least two subsamples were acquired from each of them in attempt at getting more representative image of the whole sample. The subsamples were analysed microscopically (Olympus BX 51 microscope) for present cyanobacterial and algal species and the relative abundance of individual species was estimated using a semiquantitative scale according to SLÁDEČKOVÁ & MARVAN (1978) adjusted to values 1-7. To obtain the value of relative abundance of a certain species in a certain sample, mean values of the intervals (in percent) represented by the semiquantitative values of the species in the subsamples were averaged.

Available literature was used for determination of the blue-greens (GARDNER 1927, GEITLER 1932, KOMÁREK 1989, KOMÁREK 2005, KOMÁREK et ANAGNOSTIDIS 1998, 2005, and STARMACH 1966), and eucaryotic algae (HINDÁK et al. 1978, KRAMMER & LANGE-BERTALOT 1986, 1988, 1991a, 1991b, WHITFORD & SCHUMACHER 1969 and of THE SOUTH FLORIDA PERIPHYTON RESEARCH GROUP (web pages - 2006). A special accent was put to cyanobacteria, while green algae and diatoms were often determined only approximately because they were not so important in this study and their proper identification would be quite time-consuming (particularly the preparation of diatom permanent sections).

In addition, data from 40 plexiglass samples from November 2000, 3 samples of periphyton from natural substrata – mostly dead or living bodies of *Typha domingensis* or *Cladium jamaicense* – from December 1999, and 55 samples (13 from natural and 42 from plexiglass substrata) from September 2000, were acquired. All above mentioned data

originate from the localities C1-C6 with exception of the September 2000 samples which were collected only from C1, C3 and C6. An overview of all received data from the C-transect is given in Tab. 1. In the sample sets from the year 2000, species biomass ($mg.cm^{-2}$) had been previously counted by other researchers as descibed in (VYMAZAL et al. 2000b). These values were converged to percent and categorized with use of the above mentioned semiquantitative scale as we needed uniform data for following statistical analyses. Three remaining samples from December 1999 had been previously examined by the supervisor of this thesis the same way as it was done by the author in the November 1999 samples. Some correction of species determination (especially cyanobacteria identified only to genus) was made in received data according to our findings, after comparison to available documentation.

Relatively detailed data about water chemistry (1999) were obtained. Only the concentration of water column total phosphorus (TP) along the C-transect was used in our analysis (Tab. 2).

Table 1. Overview of data from the C-transect.

Date of collection	Number of samples from natural substrata						Number of samples from plexiglass slides					
	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
11/1999	0	0	0	0	0	0	10	6	6	5	6	9
11/2000	0	0	0	0	0	0	6	6	6	6	6	10
12/1999	1	0	1	0	0	1	0	0	0	0	0	0
9/2000	4	0	4	0	0	5	14	0	14	0	0	14

Table 2. TP levels at localities of the C-transect

Locality	TP [$\mu g.L^{-1}$]
C1	51,8
C2	33,3
C3	13,6
C4	6,2
C5	5,8
C6	4,4

The Dosing Study

In the framework of the project, a mesocosm-scale six-year P dosing experiment was previously executed. On the Southwest side of the WCA-2A, in an undisturbed open-water slough area, two experimental sites, approximately 200 meters apart, were estabilished in 1991. Each site consisted of 5 walled channels, 2 m wide and 8 m long with walls 90 cm above the slouh substrate with an additional unwalled control area of the same size. The channels were oriented north-south, in the same direction as natural water flow. On the northern side of each flume, soluble reactive phosphate (SRP as Na_2HPO_4) was added in different amount, so that stabile TP concentration gradients were created. Samples of floating

periphyton mats and from experimental plexislides (after two months of exposition) were taken in certain distances (1, 2, 4, 6 and 8m) from the northern end of the flume characterized by decreasing water column TP concentrations (measured once a month for 6 years).

Data from periphyton mat samples collected in January 1998, October 1998, February 1999 and December 1999 (72 samples) and plexiglass samples from September 1998 and February 1999 (25 samples) were received and used in our analysis.

1.2. Statistics

The Canoco for Windows v. 4.52 and CanoDraw for Windows v. 4.12 (TER BRAAK & ŠMILAUER 2002) computer applications were used for multivariate analyses of data and graphic visualisation of the results, respectively. Additionally, Microsoft Excel 2000 v. 9.0 Statistica v. 5.5 and Statistica v. 6.0 (STATSOFT, INC. 1999, 2001) were employed in remaining statistical tests and construction of graphs and tables.

Relation of periphyton to TP

The data from the C-transect samples were used in determination of changes in periphytic assemblages along the WCA- 2A TP concentration gradient. As the response of present species to TP was mostly well approximable by a linear function and measured gradient lengths (in Detrended (Canonical) Correspondence Analysis – D(C)CA) were up to 3 SD units, Redundancy Analysis (RDA) was employed to discover the relation of present species to TP and Monte Carlo permutation test (499 permutations) was applied to test the significance of the canonical variable. Furthermore, Principal Components Analysis (PCA) was executed to avoid missing any major components of variability that might have not been related to TP (or to other known factors like substratum quality or season), and to explore variability of the samples. In addition, one-way Analysis of Variance (ANOVA) was used to test the influence of study site (representing TP) on chosen taxa.

In the processing of the dosing study data, the same multivariate methods were used as for the C-transect - also in this case, the data were suitable for analysis based on a linear model. On top of that, simple linear regression was applied in testing the relation of individual taxa to TP. Although the experimental design theoretically enables analysis based on a split-plot model (the sites being whole-plots and the channels split-plots), such calculation could not be executed due to irregular way of sample collection producing different numbers of samples from each sampling point and sampling date.

Relation of periphyton to plexiglass and natural substrata

As we wanted to know, what role in explanation of variability in our data the substratum quality could play and in what relation this factor is to the other environmental variables, PCA was used. Additionally, RDA was employed in attempt at specifying taxa preferably growing on natural or artificial substratum. Chosen taxa were tested for substratum preference using t-test for independent samples. Data from the dosing study and data from the WCA-2A localities C1,C3 and C6 were processed in separate analyses.

Relation of periphyton to seasonal changes

In the data set from the dosing study there were samples both from winter (January 1998, February 1999) and from late summer or early fall (September 1998, October 1998). Since there was a suspicion that the species composition of the samples may be influenced by seasonal changes, the dosing study data were subjected to similar analyses (PCA, RDA, t-test) as in search for substratum preference, using two categories of season (winter vs. summer).

1.3. Photographic documentation

The photographic documentation was executed with help of a microscope (Olympus BX 51) equipped with a digital camera (Olympus CAMEDIA C-5050 ZOOM) connected to computer. Following recording and basic editing of the pictures (cropping, insertion of a scale) was made in QuickPHOTOMICRO v. 2.1. Additional adjustments in size and position of the images were completed in Adobe Photoshop CS v. 8.0.

3. Results

Altogether, 163 species of cyanobacteria and algae were found in the samples microscopically analysed by the author. A complete list of identified taxa is given in Tab. 3. In general, Cyanobacteria (76 species) were dominant in all samples together with Bacillariophyta (24 species) and Chlorophyta (56 species). The number of diatom and green-algal species is somewhat underestimated, as in some cases species belonging to one genus were grouped together (e.g. *Navicula* spp. or *Mougeotia* spp.). Occasionally, algae from other groups (Charophyta – 1 species, Cryptophyta – 1 species, Dinophyta – 2 species, Euglenophyta – 1 species, Raphidiophyta – 1 species and Xanthophyta – 1 species) were present. These numbers are in general accordance with the data from remaining samples not analysed personally by the author of this study.

3.1. Complete C- transect (plexislides)

Data from 82 samples from all six sampling points of the WCA-2A C-transect from November 1999 and 2000 were analysed together.

Table 3. List of identified species. Part I. – Bacillariophyta, Charophyta, Chlorophyta

(Question marks are used where exact year of publication was not available)

Bacillariophyta (24)

Achnanthes cf. *caledonica* Lange-Bertalot 1994
Amphora cf. *libyca* Ehrenberg 1840
Amphora cf. *veneta* Kützing 1844
Cyclotella meneghiniana Kützing 1844
Cymbella cf. *aspera* (Ehrenberg) Cleve 1953
Cymbella cf. *silesiaca* (Bleisch) Rabenhorst 1864
Cymbella spp. Agardh 1830
Diploneis cf. *subovalis* Cleve 1894
Eunotia cf. *curvata* (Kützing) Lagerstedt 1884
Fragilaria cf. *crotonensis* Kitton 1869
Fragilaria cf. *delicatissima* (W.Sm.) Lange-Bertalot 1980
Fragilaria spp. Lyngbye 1819
Fragilaria cf. *virescens* Ralfs 1843
Gomphonema spp. Ehrenberg 1832
Hantzschia amphioxys (Ehrenberg) Grunow in Cleve & Grunow 1880
Mastogloia smithii Thwaites 1856
Navicula spp. Bory de Saint-Vincent 1822
Nitzschia amphibia Grunow 1862
Nitzschia cf. *nana* Grunow in Van Heurck 1881
Nitzschia serpentiraphe Lange-Bertalot 1980
Nitzschia spp. Hassall 1845
Pinnularia cf. *acrosphaeria* Rabenhorst 1853
Rhopalodia gibba (Ehrenberg) O.F. Müller 1895
Synedra ulna Ehrenberg 1832

Charophyta (1)

Coleochaete sp. Brébisson 1844

Chlorophyta (56)

Actinastrum sp. Lagerheim 1882
Ankistrodesmus sp. Corda 1838
Bulbochaete sp. Agardh 1817
Cladophora sp. Kützing 1843
Closterium cf. *archerianum* Cleve in P. Lundell 1871
Closterium cf. *pronum* Brébisson 1856
Closterium cf. *toxon* W. West 1892
Closterium spp. Nitzsch ex Ralfs 1848
Coelastrum spp. Nägeli 1849
Cosmarium cf. *angulosum* Brébisson ?
Cosmarium cf. *botrytis* Meneghini ex Ralfs in Nordstedt 1888
Cosmarium cf. *circulare* Reinsch 1867
Cosmarium cf. *connatum* Brébisson in Ralfs 1848
Cosmarium cf. *contractum* O. Kirchner 1878
Cosmarium cf. *cucumis* Corda ex Ralfs 1848
Cosmarium cf. *dentatum* Wolle ?
Cosmarium cf. *margaritatum* (P. Lundell) J. Roy & Bisset 1886
Cosmarium cf. *obtusatum* Schmidle 1898
Cosmarium cf. *ochthodes* Nordstedt 1875
Cosmarium cf. *parvulum* Brébisson 1856
Cosmarium cf. *phaseolus* Brébisson in Ralfs 1848
Cosmarium cf. *pokornyanum* (Grunow) W. West & G.S. West ?
Cosmarium cf. *pseudoconnatum* Nordstedt 1869
Cosmarium cf. *pseudoprotuberans* Kirchner ?
Cosmarium cf. *pseudotaxichondrum* Nordstedt ?
Cosmarium cf. *pyramidatum* Brébisson in Ralfs 1848
Cosmarium cf. *sexangulare* P. Lundell ?
Cosmarium cf. *subcrenatum* Hantzsch in Rabenhorst 1861
Cosmarium cf. *taxichondrum* P. Lundell ?
Cosmarium spp. Ralfs 1848
Desmidium cf. *aptogonum* Brébisson 1835
Euastrum cf. *elegans* (Brébisson) Kützing ex Ralfs 1848
Euastrum cf. *sinuosum* Lenormand ex W. Archer ?
Euastrum spp. Ehrenberg ex Ralfs 1848
Eudorina spp. Ehrenberg ex Ralfs 1832
Micrasterias cf. *jenneri* Ralfs 1848
Micrasterias cf. *pinnatifida* Kützing ex Ralfs 1848
Micrasterias radiosa Ralfs 1848
Microspora spp. Thuret 1850
Microthamnion sp. Nägeli 1849
Monoraphidium spp. Komárková-Legnerová 1969
Mougeotia spp. Agardh 1824
Oedogonium spp. Link 1820
Oocystis spp. Braun 1855
Pediastrum boryanum (Turpin) Meneghini 1840
Pediastrum tetras (Ehrenberg) Ralfs 1844
Phymatodocis cf. *nordstedtiana* Wolle ?
Scenedesmus spp. Meyen 1829
Spirogyra spp. Link 1820
Staurastrum cf. *dejectum* Brébisson ex Ralfs 1848
Staurastrum cf. *dilatatum* Ehrenberg ex Ralfs 1848
Staurastrum cf. *pentacerum* (Wolle) Smith
Staurastrum cf. *subcruciatum* Wills 1887
Staurastrum spp. (Meyen) Ralfs 1848
Tetrastrum sp. Chodat 1895
Ulothrix spp. Kützing 1833

Table 3. List of identified species. Part II. – Cyanobacteria, Cryptophyta, Dinophyta, Euglenophyta, Raphidiophyta, Xanthophyta

Cyanobacteria (76)

Anabaena cf. *ambigua* Rao 1937
Anabaena fuellebornii Schmidle 1892
Anabaena iyengarii Bharadwaja 1935
Anabaena cf. *reniformis* Lemmermann 1898
Anabaena sp. Bory 1822
Aphanothece sp. (subg. *Anathece)* Nägeli 1849 nom. cons.
Aphanocapsa conferta
(W. et G.S. West) Komárková-Legnerová et Cronberg 1993
Aphanocapsa parasitica (Kützing) Komárek et Anagnostidis 1995
Aphanocapsa sp. (1) Nägeli 1849
Aphanocapsa sp. (2) Nägeli 1849
Aphanocapsa venezuelae Schiller 1952
Aphanothece bacilloidea Gardner 1927
Aphanothece comasii Komárková-Legnerová et Tavera 1995
Aphanothece cf. hardersii Schiller 1952
Aphanothece variabilis (Schiller) Komárek 1994
Arthrospira jenneri Stizenberger ex Gomont 1892
Bacularia gracilis Komárek 1994
Calothrix sp. Agardh 1824
Chlorogloea gardneri (Gardner) Komárek et Komárková (in press)
Chroococcus deltoides Komárek et Novelo 1994
Chroococcus major Komárek et Komárková-Legnerová 2006
Chroococcus mediocris Gardner 1927
Chroococcus cf. *minutus* (Kützing) Nägeli 1849
Chroococcus cf. *minutissimus* Gardner 1927
Chroococcus mipitanensis (Wołoszynska) Geitler 1925
Chroococcus occidentalis
(Gardner) Komárek et Komárková-Legnerová 2006
Chroococcus polyedriformis Schmidle 1902
Chroococcus schizodermaticus W. et G.S. West 1892
Cyanosarcina sp. Kováčik 1988
Cyanothece sp. Komárek 1976
Cylindrospermum cf. *breve* Komárek 1984
Geitlerinema earlei (Gardner) Anagnostidis 1989
Geitlerinema splendidum (Greville ex Gomont) Anagnostidis 1989
Gloeocapsa sp. (1) Kützing 1843
Gloeocapsa sp. (2) Kützing 1843
Gloeothece membranacea (Rabenhorst) Bornet 1992
Gloeothece opalothecata Gardner 1927
Gomphosphaeria semen-vitis Komárek 1988
Halomicronema sp. (1) Abed, Garcia-Pichel et Hernández-Mariné 2002
Halomicronema sp. (2) Abed, Garcia-Pichel et Hernández-Mariné 2002
Hassallia sp. Berkeley 1845
Komvophoron cf. *minutum* (Skuja) Anagnostidis et Komárek 1988
Komvophoron sp. (1) Anagnostidis et Komárek 1988
Komvophoron sp. (2) Anagnostidis et Komárek 1988
Komvophoron sp. (3) Anagnostidis et Komárek 1988
Lemmermanniella uliginosa Komárek et Komárková (in press)
Leptolyngbya lagerheimii (Gomont) Anagnostidis et Komárek 1988
Leptolyngbya cf. *mucosa* (Gardner) Anagnostidis et Komárek 1988
Leptolyngbya sp. Anagnostidis et Komárek 1988
Lyngbya cf. *intermedia* Gardner 1927
Lyngbya cf. *martensiana* Meneghini ex Gomont 1892
Merismopedia punctata Meyen 1839
Microchaete cf. *robusta* Setchell et. Gardner 1903
Microchaete cf. *goeppertiana* Kirchner 1900
Nostoc sp. Vaucher 1803
Oscillatoria cf. *anguina* Bory ex Gomont 1892
Oscillatoria cf. *obtusa* Gardner 1927
Oscillatoria crassa (Rao) Anagnostidis 2001
Oscillatoria cf. *sancta* Kützing ex Gomont 1892
Oscillatoria sp. Vaucher ex Gomont 1892
Phormidium articulatum (Gardner) Anagnostidis et Komárek 1988
Phormidium cf. *articulatum* (Gardner) Anagnostidis et Komárek 1988
Phormidium granulatum (Gardner) Anagnostidis 2001
Phormidium cf. *hamelii* (Frémy) Anagnostidis et Komárek 1988
Phormidium chalybeum
(Mertens ex Gomont) Anagnostidis et Komárek 1988
Phormidium taylori (Drouet et Strickland) Anagnostidis 2001
Phormidium tortuosum (Gardner) Anagnostidis et Komárek 1988
Pseudanabaena sp. Lauterborn 1915
Rhabdogloea subtropica Hindák 1984
Scytonema sp. Agardh 1824
Spirulina laxissima G.S. West 1907
Spirulina subsalsa Oersted ex Gomont 1892
Spirulina tenerrima Kützing ex Gomont 1892
Spirulina meneghiniana Zanardini ex Gomont 1892
Stigonema sp. Agardh 1824
Tolypothrix cf. *willei* Gardner 1927
Wolskyella sp. Claus 1963

Cryptophyta (1)

Cryptomonas sp. Ehrenberg 1832

Dinophyta (2)

Gymnodinium sp. Stein 1878
Peridinium cf. *umbonatum* Stein 1883

Euglenophyta (1)

Phacus spp. Dujardin 1841

Raphidiophyta (1)

Gonyostomum sp. Diesing 1866

Xanthophyta (1)

Ophiocytium sp. Nägeli 1849

The results of a PCA analysis (first two axes explain 60 % of variability) represented by Fig. 3 show a group of samples from C1 and C2 separated from another group consisting of samples from C5 and C6, while C3 and C4 samples are scattered among both groups (C3 mainly together with C1-2). Suggestion is that the major TP-driven shift in sample quality occurs between localities C3 and C4, i. e. between TP concentrations 6,2 and 13,6 μg. L^{-1}. Another diagram (Fig. 4) shows that the number of species present in a sample is in a strong negative relation to increasing TP concentration which is a significant factor influencing species composition of samples (strong correlation to the 1 st ordination axis).

According to the results of an RDA analysis (Fig. 5) with standardization of samples, where 15% of variability was significantly explained by TP ($F = 13,41$; $p < 0,01$), taxa probably responding to changes in trophic level were chosen : *Aphanocapsa parasitica*, *Arthrospira jenneri*, *Fragilaria* cf. *virescens*, *Lyngbya* cf. *martensiana*, *Phormidium tortuosum* and *Pseudanabaena* sp. are in positive correlation while *Mastogloia smithii* is evidently in negative correlation to TP. Possibly, a group of taxa represented by cyanobacteria *Gloeothece opalothecata*, *Halomicronema* sp.(1), *Hassallia* sp., *Komvophoron* sp. (2) and (3), *Phormidium granulatum*, *Tolypothrix* cf. *willei* and *Wolskyella* sp., diatoms *Amphora* cf. *veneta*, *Fragilaria* cf. *crotonensis*, *Nitzschia* cf. *amphibia* and *Nitzschia* cf. *serpentiraphe* and green algae *Coleochaete* sp. (Charophyta), *Cosmarium* cf. *pyramidatum*, *Desmidium* cf. *aptogonum*, *Eudorina spp.* and *Euastrum* cf. *elegans* also respond rather negatively to increases in phosphorus concentration.

To be more illustrative, average relative abundances of chosen species and higher taxa along the C-transect are given in (Fig. 6, 7). As for cyanobacteria, abundance of *Lyngbya* spp., some other oscillatorialean species (*Arthrospira jenneri*, *Phormidium tortuosum*, *Pseudanabaena* sp.) and some coccoid species (*Aphanocapsa parasitica*, *Aphanothece variabilis*, *Chlorogloea gardneri*) increased with TP while the species of family Scytonemataceae, filamentous species *Komvophoron* sp. (1) and (2), *Phormidium granulatum*, *Phormidium taylori* and coccoid cyanobacteria *Chroococcus* spp., *Gloeothece opalothecata* and *Wolskyella* sp. were more abundant at oligotrophic sites. Diatoms were in average present in greater amounts at sites with lower trophic level – *Mastogloia smithii* was the best predictor of clear water, yet some diatoms (*Fragilaria* cf. *virescens*, *Cocconeis placentula*) preferred higher TP concentrations. Importantly, green filamentous algae were rather abundant at all sites (a little less at C5 and C6), desmids responded negatively to TP, *Coleochaete* sp., *Eudorina* spp. and *Peridinium* cf. *umbonatum* (Dinophyta) were found only at sites with lower phosphorus concentrations. Results of ANOVA (not shown) confirmed significant influence of site (representing TP) to the relative abundance of all these taxa ($p<0,05$).

3.2. Simplified C-transect (plexislides and natural substrata)

Data from 105 samples from localities C1, C3 and C6 collected in November and December 1999 and in September and November 2000 were included in this examination.

Similarly to the previous analysis, PCA (1st two axes explain 51 % of variability) showed that samples from C1 and C3 formed a group separated from C6 samples (Fig. 8) and number of species decreased at eutrophic sampling points (Fig. 9). Samples from natural and artificial substrata were not separated in PCA diagram (Fig. 10) but the growths from plexiglass were more variable. Moreover, the results suggest, that TP is the major component influencing present species (strong correlation with the first ordination axis) while the substratum quality plays probably a lesser role.

In an RDA analysis with standardization of samples, the first two canonical axes significantly ($F=6,55$; $p<0,01$) explained 9,6% and 1,8% of variability, respectively confirming the importance of TP concentration and rather immaterial influence of substratum quality (Fig. 11).

In addition to the analysis from complete C-transect, *Aphanothece comasii*, *Diploneis* cf. *subovalis*, *Epithemia* cf *zebra* and *Microcystis* sp. seemed to respond positively and *Spirulina subsalsa* negatively to phoshorus concentration. The average relative abundance of these species at localities C1, C3 and C6 is presented in (Fig. 12); it differs significantly among sites (ANOVA – $p<0,05$).

As *Chroococcus pulcherrimus* and *Cyanobacterium* sp. were suspected of preferring natural substrata (according to the RDA ordination diagram), their average relative abundances in samples from natural vs. plexiglass substrata were tested for difference, giving significant results (t-test – $p<0,01$) which confirmed the suspection (Fig. 13). In addition, taxa often considered as substratum selected (diatoms, green filamentous algae, oscillatorialean cyanobacteria, coccoid cyanobacteria, heterocytous cyanobacteria, Scytonemataceae) were subjected to the same test, however no significant differences were found.

3.3. Dosing study

In total, data from 97 samples acquired in the dosing study during 1998 and 1999 from both plexiglass and pseudo-natural (close to natural but being a part of an experimental mesocosm) substrata were processed.

In accordance to the previous results from the C-transect, PCA analysis (1st two axes explained 61 % of variability) showed a pattern in variability of samples with different TP levels – the samples with TP lower than 10 $\mu g.L^{-1}$ and many samples with TP between 10 and 20 $\mu g.L^{-1}$ formed a cluster separated from samples with TP greater than 30 $\mu g.L^{-1}$ while remaining samples with TP 10-20 $\mu g.L^{-1}$ and 20-30 $\mu g.L^{-1}$ were scattered irregularly closer to

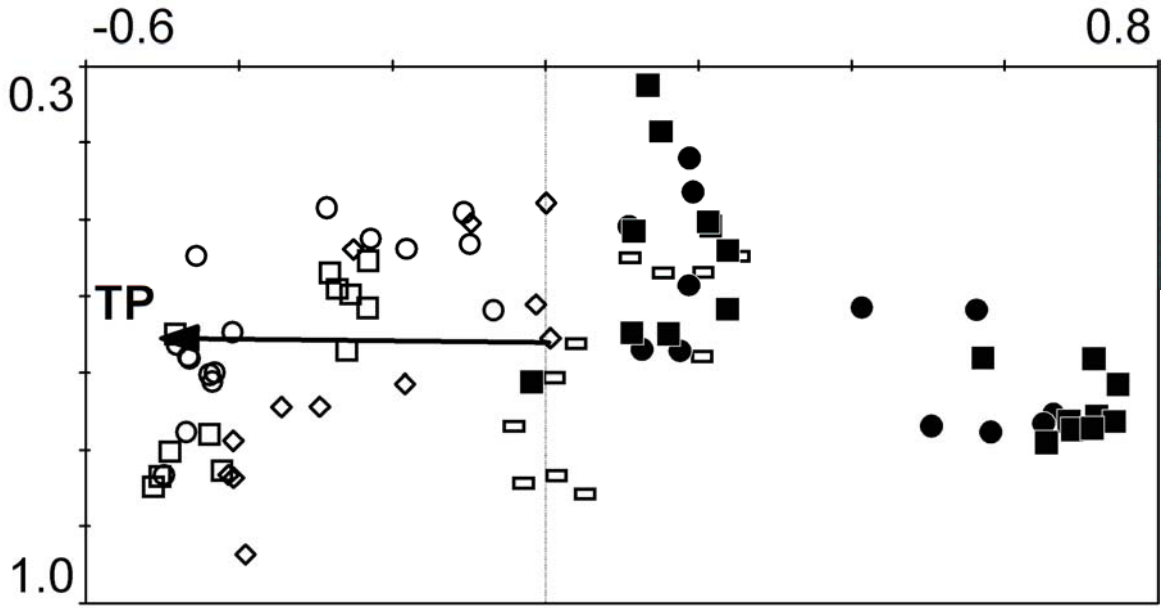


Figure 3. Samples from the complete C-transect in relation to TP.

Empty circles – samples from C1, empty squares – C2, empty rectangles – C3, empty diamonds – C4, filled squares – C5, filled circles – C6

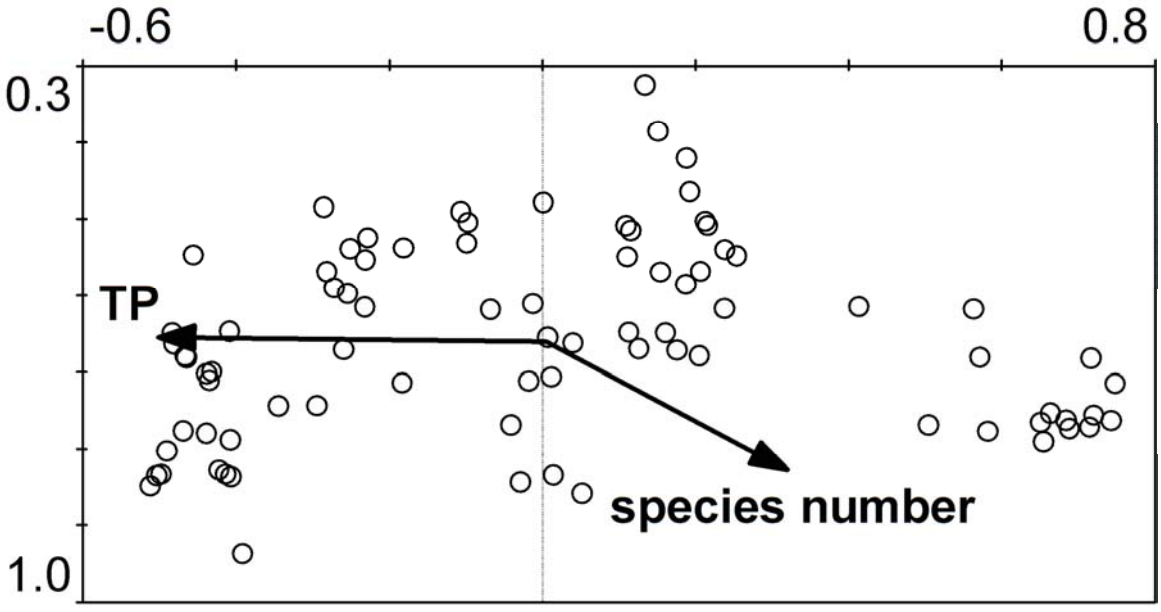


Figure 4. Negative relation of species diversity to TP at the complete C-transect

Circles present samples from all localities.

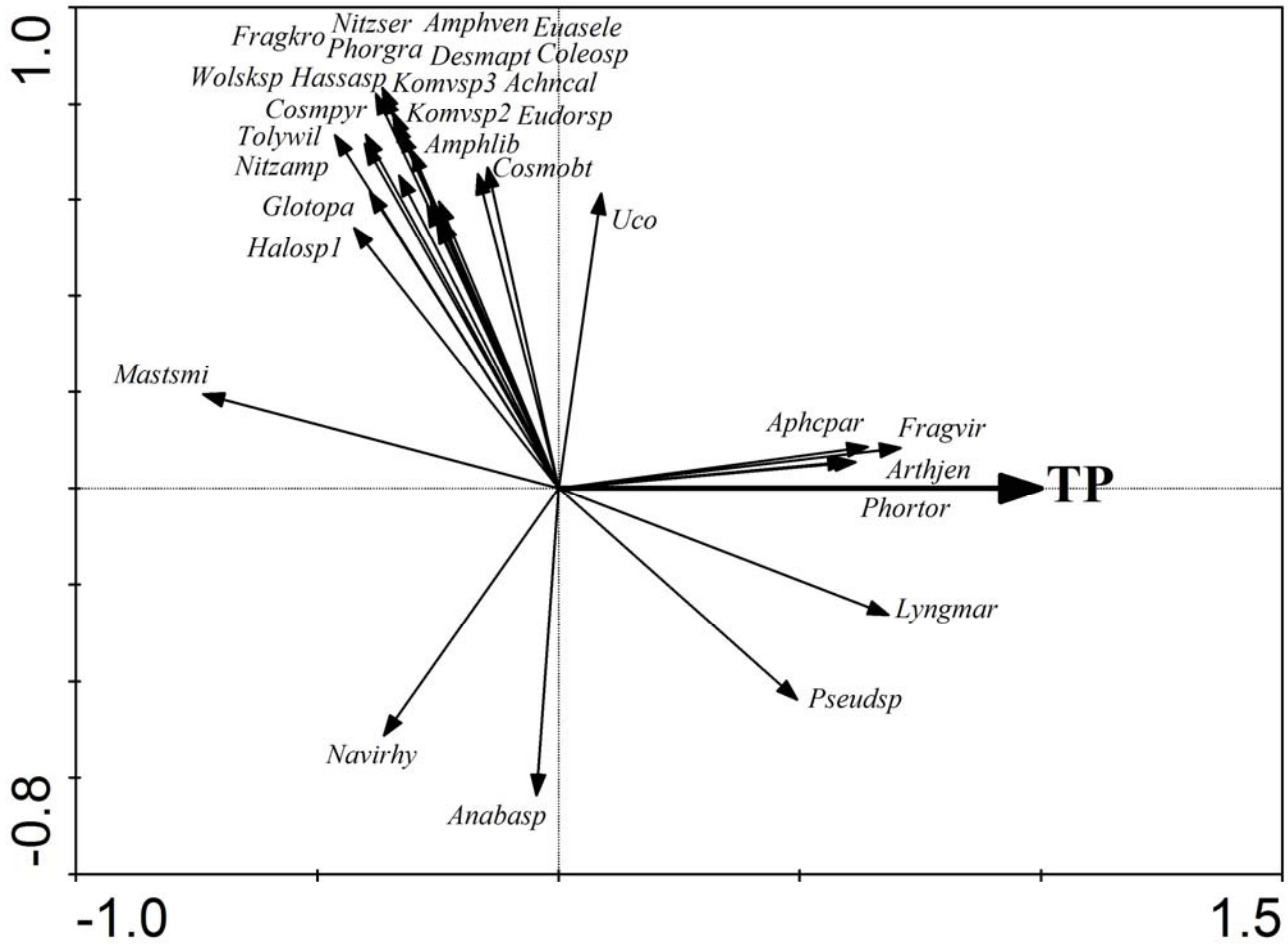


Figure 5. RDA with best fitting species from the complete C-transect.

Achncal – *Achnanthes* cf. *caledonica*, Amphlib – *Amphora* cf. *libyca*, Amphven – *Amphora* cf. *veneta*, Anabasp – *Anabaena* sp., Aphcpar – *Aphanothece parasitica*, Coleosp – *Coleochaete* sp., Cosmobt – *Cosmarium* cf. *obtusatum*, Cosmpyr – *Cosmarium* cf. *pyramidatum*, Desmapt – *Desmidium* cf. *aptogonum*, Eudorsp – *Eudorina* spp., Euasele – *Eusatrum* cf. *elegans*, Fragkro – *Fragilaria* cf. *crotonensis*, Fragvir – *Fragilaria* cf. *virescens*,Glotopa – *Gloeothece opalothecata*,Halosp1 – *Halomicronema* sp. (1), Hassasp – *Hassallia* sp., Komvsp1 and 2 – *Komvophoron* sp. (1) and (2), Lyngmar – *Lyngbya* cf. *martensiana*, Mastsmi – *Mastogloia smithii*, Navirhy – *Navicula* cf. *rhynchocephala*, Nitzamp – *Nitzschia* cf. *amphibia*, Nitzser – *Nitzschia* cf. *serpentiraphe*, Phorgra – *Phormidium granulatum*, Phortor – *Phormidium tortuosum*, Pseudsp – *Pseudanabaena* sp., Tolywil – *Tolypothrix* cf. *willei*, Uco – unidentified cyanobacteria, Wolksp – *Wolskyella* sp.

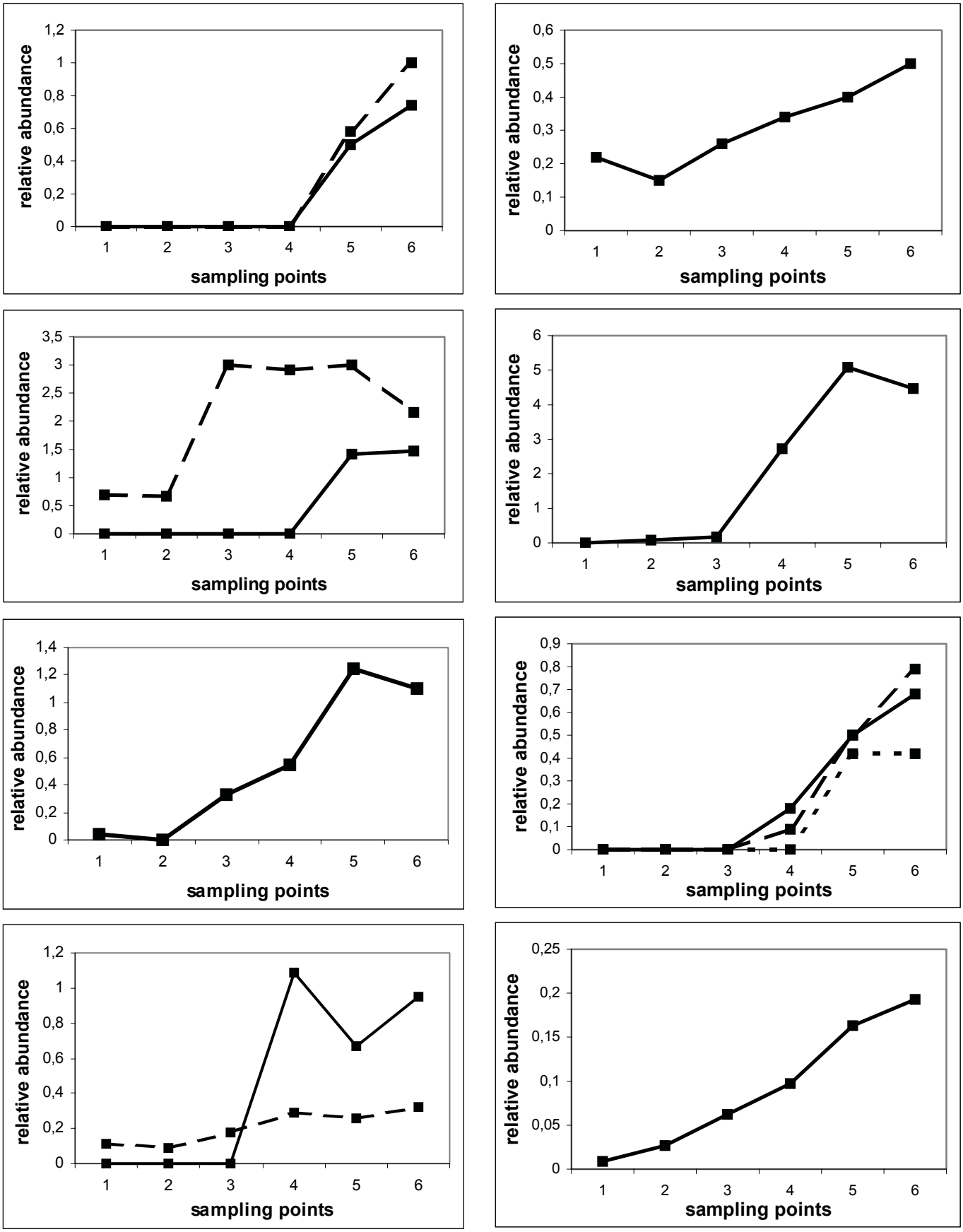


Figure 6. Relative abundance of chosen taxa along the complete C-transect. - oligotrophic taxa.

First column, from top – *Kompvophoron* sp. (2) (dashed line) and sp. (3) (full line); *Phormidium granulatum* (full) and *P. taylori* (dashed); Scytonemataceae; *Chroococcus* spp. (full) and *Gloeothece opalothecata* (dashed). Second column, from top – diatoms; *Mastogloia smithii*; *Eudorina* sp. (full), *Peridinium* cf. *umbonatum* (dashed), *Coleochaete* sp. (dotted line); desmids.

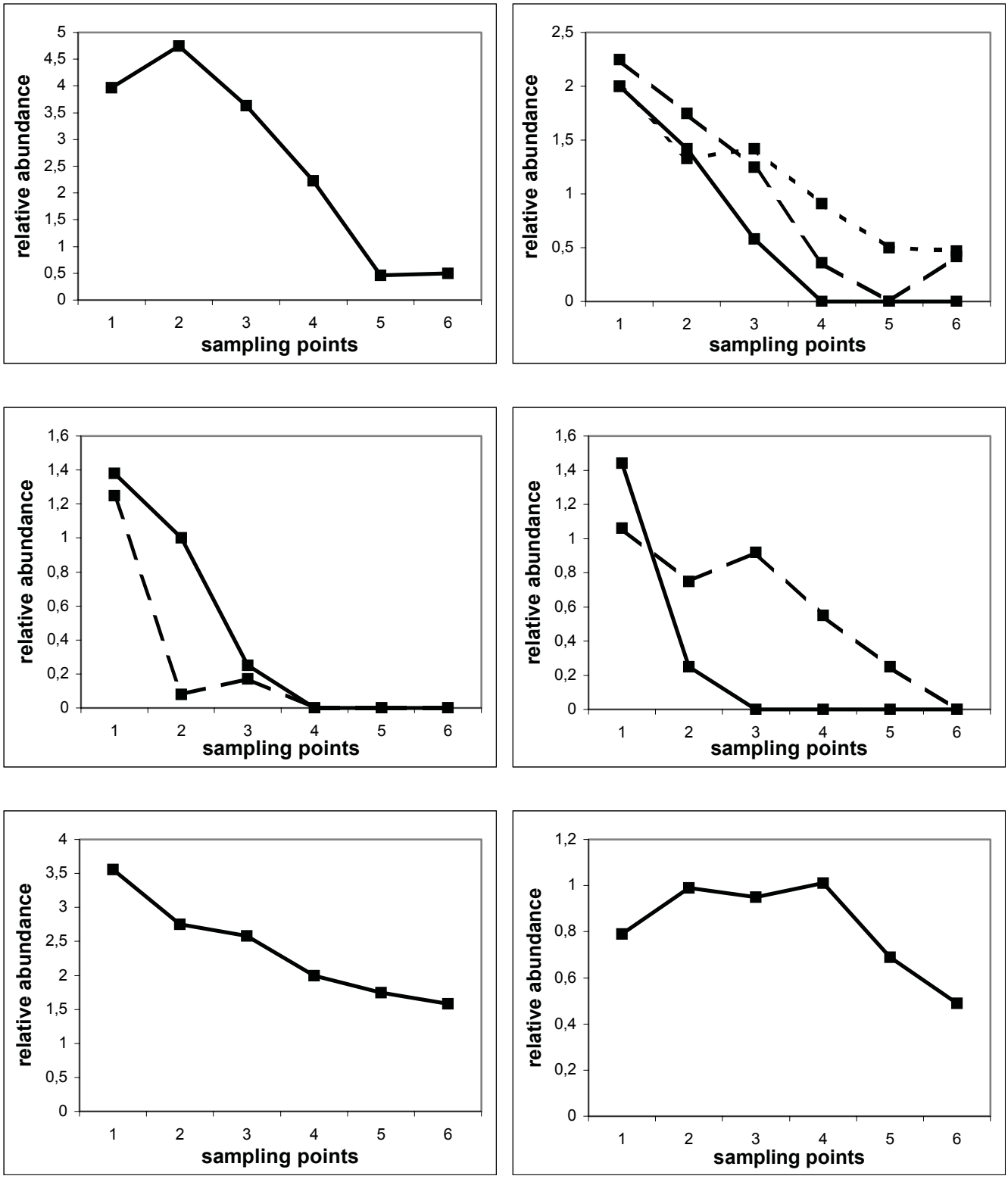


Figure 7. Relative abundance of chosen taxa along the complete C-transect. - eutrophic taxa.

First column, from top – *Lyngbya* spp.; *Phormidium tortuosum* (full line) and *Arthrospira jenneri* (dashed line); *Pseudanabaena* sp. Second column, from top – *Aphanocapsa parasitica* (full), *Chlorogloea gardneri* (dashed) and *Aphanothece variabilis* (dotted line); *Fragilaria* cf. *virescens* (full) and *Cocconeis placentula* (dashed); green filamentous algae.

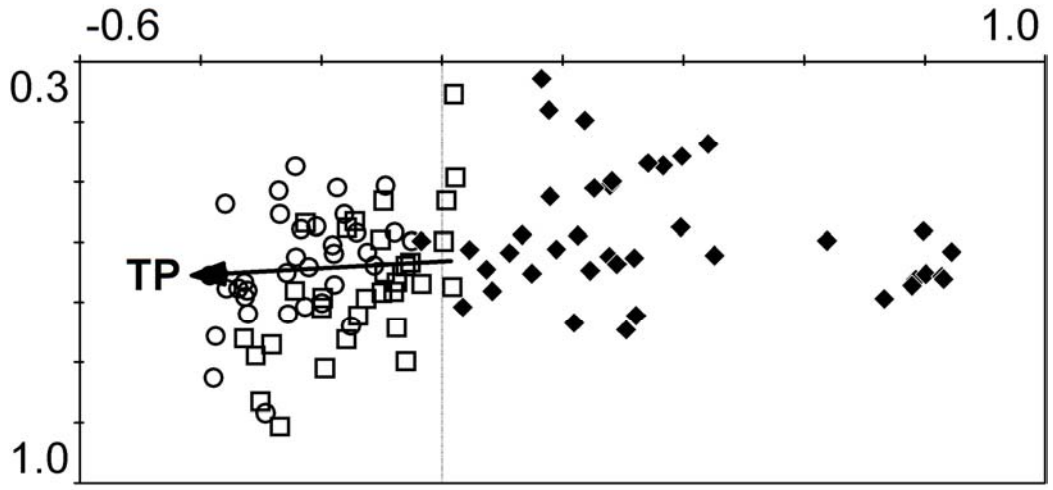


Figure 8. Samples from the simplified C-transect in relation to TP.

Circles – samples from C1, squares – C3, diamonds – C6.

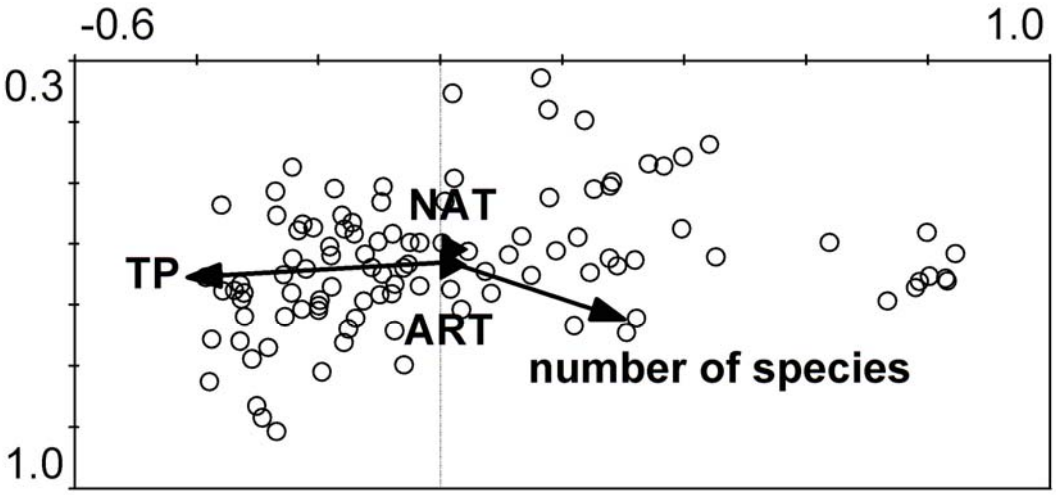


Figure 9. Negative relation of species diversity to TP at the simplified C-transect

Circles present samples from all localities. Centroids of substratum quality are represented by triangles (NAT for natural substratum and ART for plexiglass substratum).

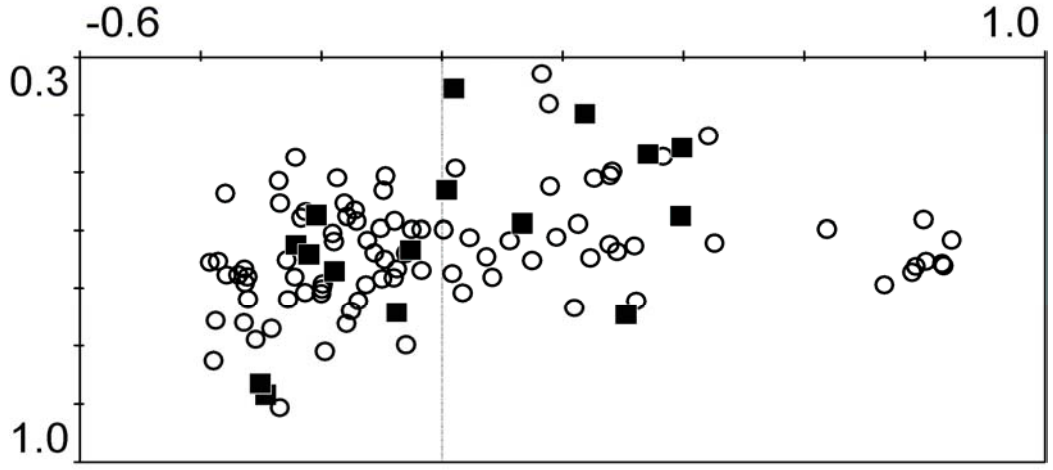


Figure 10. Samples from natural and artificial substrata at the simplified C-transect.

Circles – samples from plexiglass substrata, squares – samples from natural substrata.

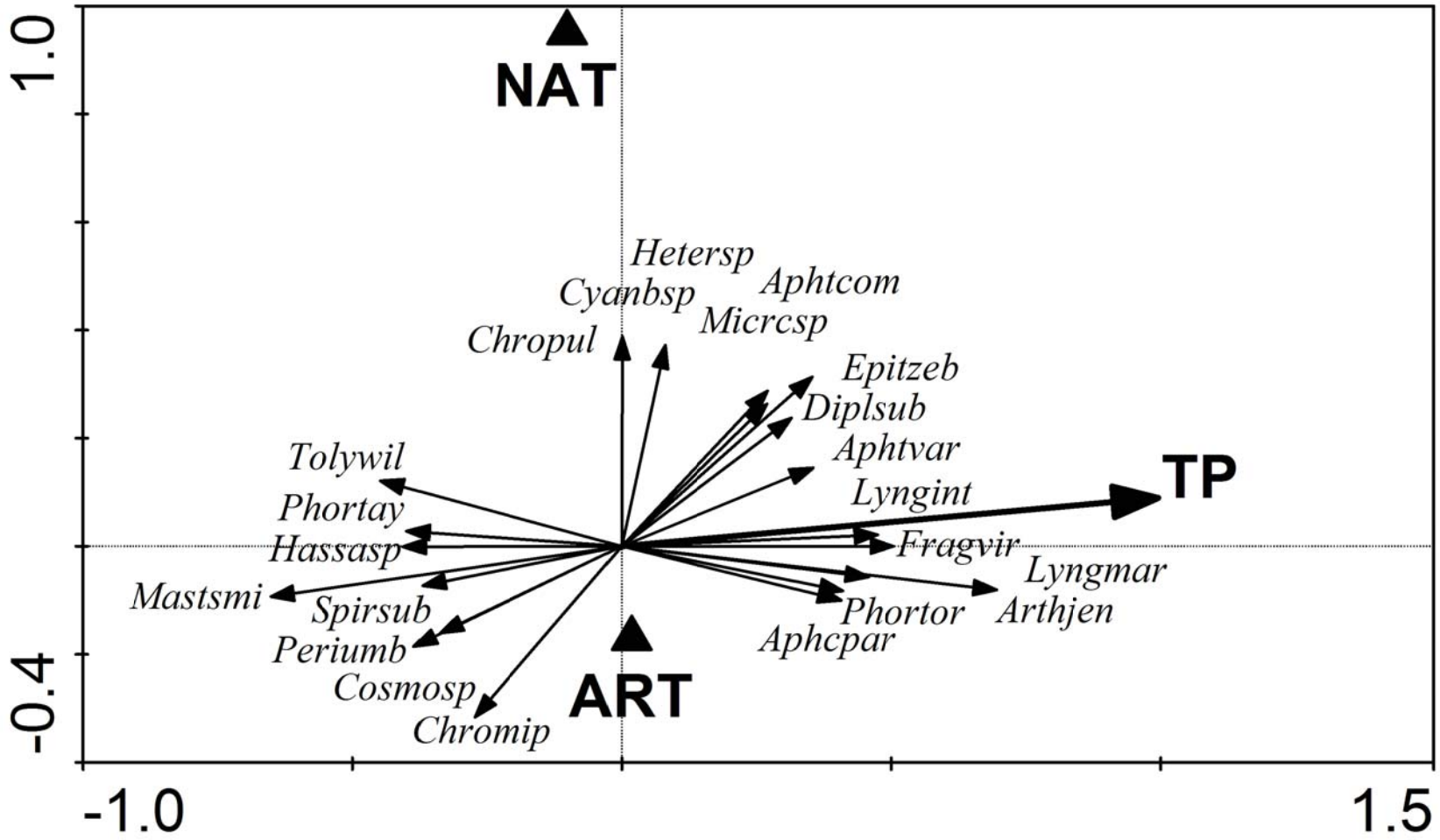


Figure 11. RDA with best fitting species from the simplified C-transect.

Aphcpar – *Aphanocapsa parasitica*, Aphtcom – *Aphanothece comasii*, Aphtvar – *Aphanothece variabilis*, Arthjen - *Arthrospira jenneri*, Chromip – *Chroococcus mipitanensis*, Chropul – *Chroococcus pulcherrimus*, Cosmosp – *Cosmarium* spp., Cyanbsp – *Cyanobacterium* sp., Diplsub – *Diploneis* cf. *subovalis*, Epitzeb – *Epithemia* cf. *zebra*, Fragvir – *Fragilaria* cf. *virescens*, Hassasp – *Hassallia* sp., Hetersp – *Heteroleiblenia* sp., Lyngint – *Lyngbya* cf. *intermedia*, Lyngmar – *Lyngbya* cf. *martensiana*, Mastsmi – *Mastogloia smithii*, Micrcsp – *Microcystis* sp., Periumb – *Peridinium* cf. *umbonatum*, Phortay – *Phormidium taylori*, Phortor – *Phormidium tortuosum*, Spirsub – *Spirulina subsalsa*, Tolywil – *Tolypothrix* cf. *willei*. Centoids of substratum quality are represented by triangles (NAT for natural substratum and ART for plexiglass substratum).

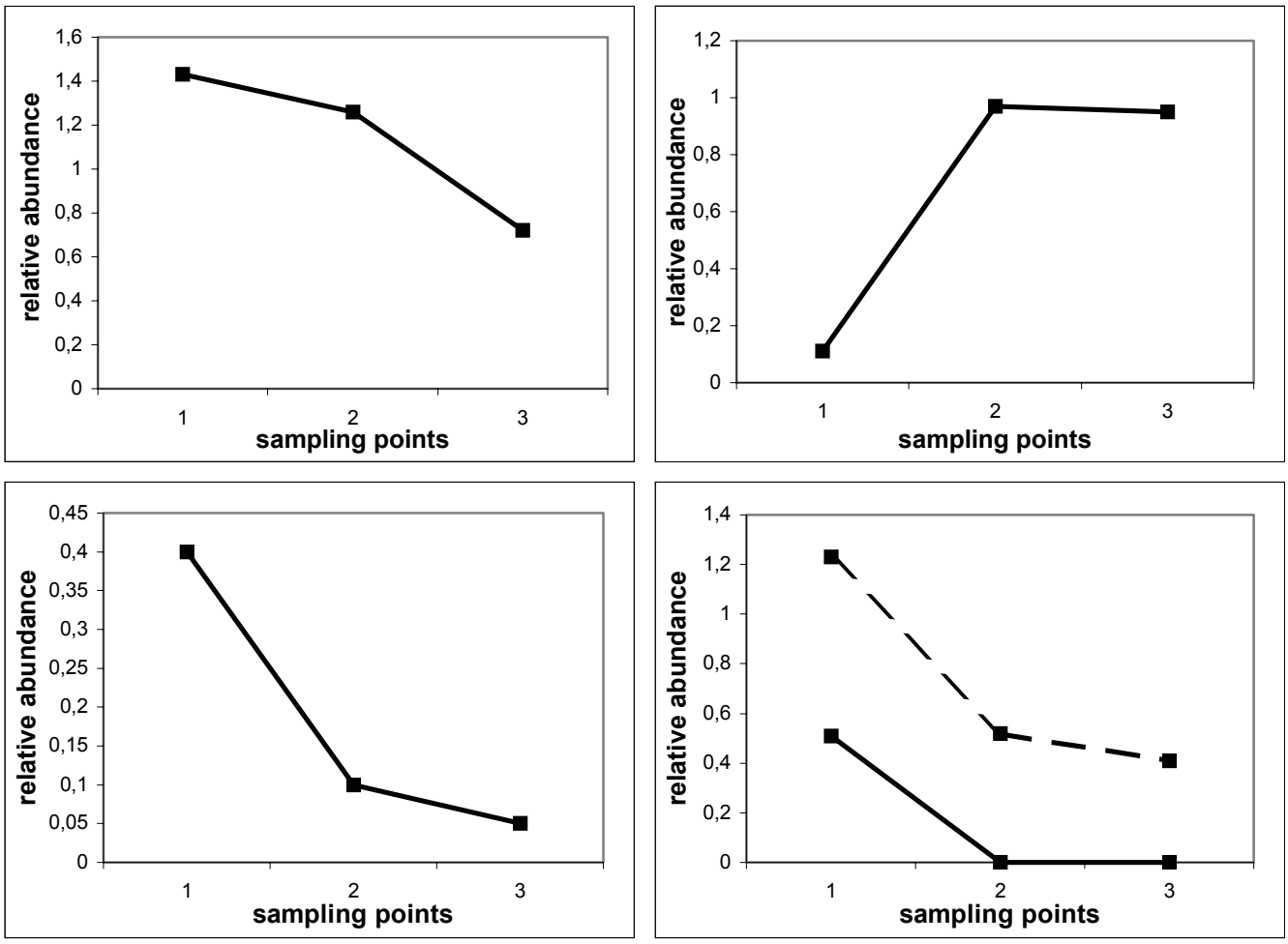


Figure 12. Relative abundance of chosen taxa along the simplified C-transect.

Top left – *Aphanothece comasii*; down left – *Microcystis* sp.; top right – *Spirulina subsalsa*; down right – *Epithemia* cf. *zebra* (full line) and *Diploneis* cf. *subovalis* (dashed line).

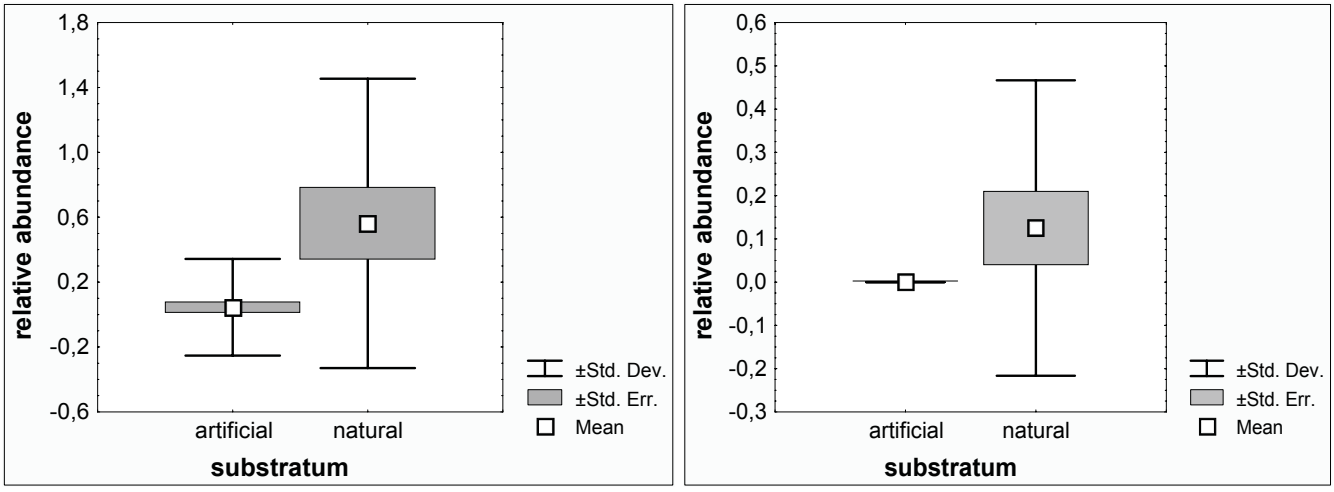


Figure 13. Relative abundance of chosen taxa on natural vs. artificial substratum (simplified C-transect)

Left – *Chroococcus pulcherrimus*; right – *Cyanobacterium* sp.

the group with high levels of phosphorus (Fig. 14). Once again it confirms, that the major shift in species composition happens at TP levels slightly over 10 μg.L^{-1}. Also in the dosing study, species diversity decreased with rising phosphorus concentration (Fig. 15).

Following diagrams (Fig. 16, 17) based on another PCA analysis after standardization of species (1st two axes explaining 12% of variability) depict an ordination of samples categorized by their affiliation to season of sampling and substratum quality, respectively. Two conspicuous yet partly overlapping groups of samples differing in season of collection are present. Almost all plexiglass samples were taken in September, thus they fall into the summer group. Since they are in one cluster with summer (early fall) samples from pseudo-natural substrata, season is probably the factor that has greater influence on the species composition. Anyway, due to inappropriate sampling regime, the influence of substratum cannot be wholly reproduced.

An RDA analysis with standardization of samples calculating with TP concentration, season and substratum quality as explanatory variables was performed – the first three canonical axes significantly ($F=6,74$; $p<0,01$) explained 9,3 %; 6,7 % and 1,9 % of variability, respectively and the first two were correspondent mainly to TP (5,6 % partial effect) and season (Fig. 18). Partial analyses with substratum (5,1 % partial effect) or season (6,7 % partial effect) as an explanatory variable (the others included in the model as covariables) were executed in order to discover the relation of present species to the relevant environmental factor (Fig. 19, 20).

Many species were positively correlated to TP (cyanobacterium *Lyngbya martensiana*, diatoms *Anomoeneis* sp., *Nitzschia* cf. *paleaeformis* and *Rhopalodia gibba*, green algae *Microthamnion* sp., *Mougeotia* sp., *Oedogonium* sp., *Spirogyra* sp. and *Tetraedron* cf. *incus*), others responded negatively (cyanobacteria *Aphanothece bacilloidea*, *Aphanothece variabilis*, *Chroococcus mipitanensis*, *Gloeothece interspersa*, *Hassallia* sp., *Leptolyngbya* cf. *mucosa* and *Phormidium taylori*, and a diatom *Mastogloia smithii*). According to this, taxa were chosen for linear regression of relative abundance on TP concentration (Fig. 23): relative abundance of coccoid cyanobacteria, *Leptolyngbya* cf. *mucosa*, *Phormidium taylori*, *Hassallia* sp., and diatoms decreased while relative abundance of *Lyngbya* spp. and green filamentous algae increased significantly with rising phosphorus level ($p<0,05$).

Following the results of partial RDA analyses with substratum quality or season as environmental factors, chosen taxa were tested (t-test for independent samples) for preferences in these elements. Cyanobacteria *Leptolyngbya* sp., *Spirulina subsalsa*, *Tolypothrix* cf. *willei* and coccoid blue-greens were found in greater abundance on pseudo-natural substrata in contrast to *Lyngbya* spp., diatoms *Cocconeis placentula* and *Gomphonema angustatum*, a desmid *Cosmarium* cf. *obtusatum* and green filamentous algae which

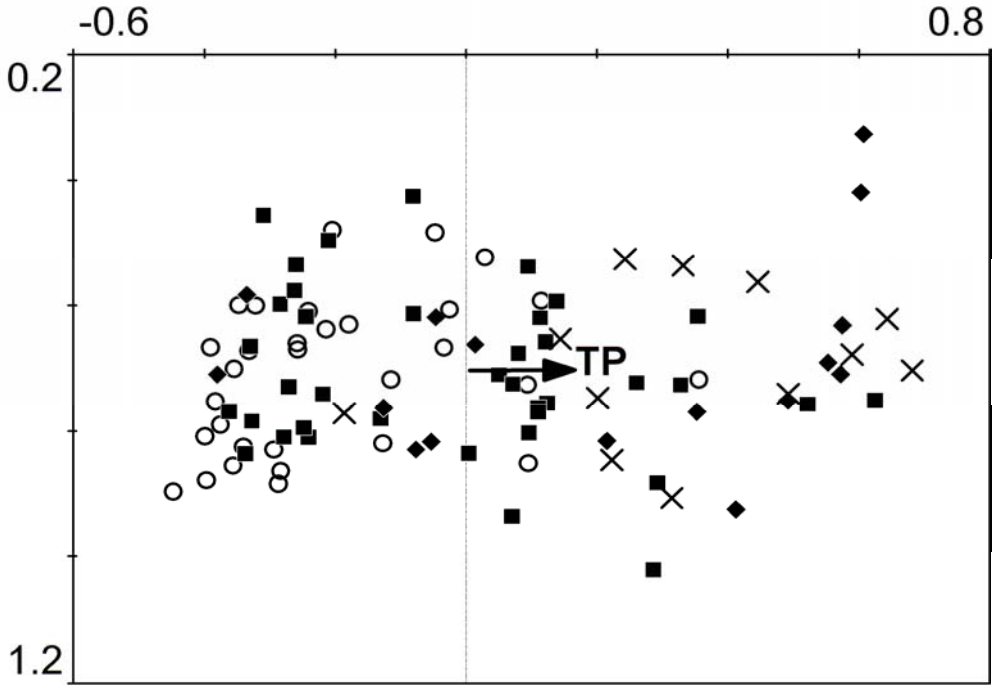


Figure 14. Samples from the dosing study in relation to TP.

Circles – samples from localities with TP levels below 10 μg.L^{-1}; squares – 10-20 μg.L^{-1}; diamonds – 20-30 μg.L^{-1}; crosses – over 30 μg.L^{-1}.

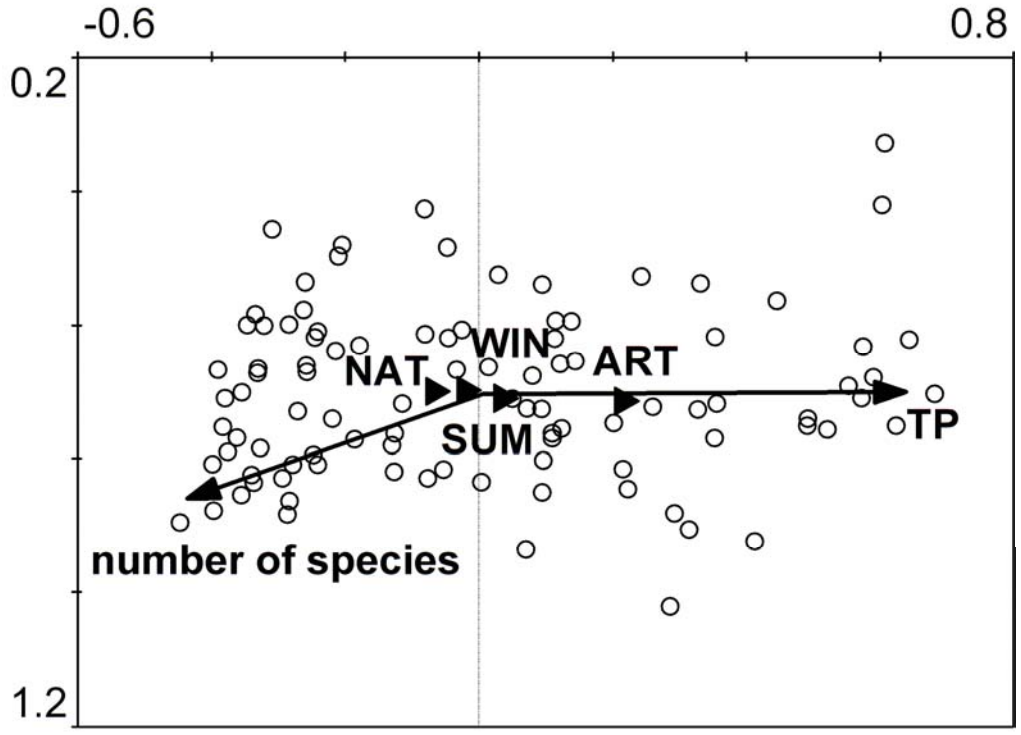


Figure 15. Negative relation of species diversity to TP in the dosing study.

Circles represent samples from all localities. Centroids of substratum quality and season are represented by triangles (NAT for natural substratum, ART for plexiglass substratum, WIN for winter nad SUM for summer and early fall).

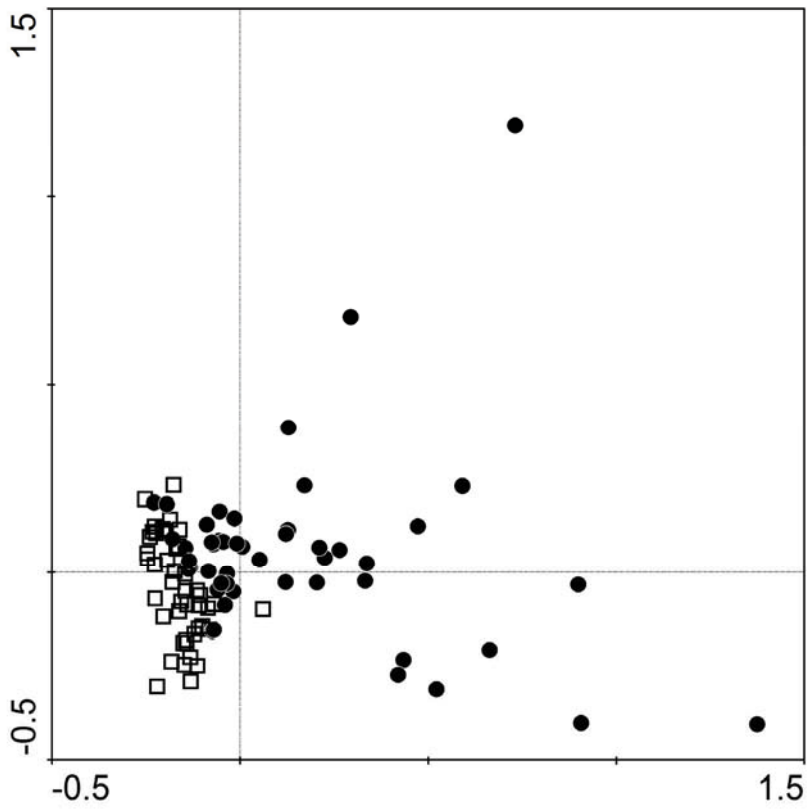


Figure 16. Samples from the dosing study collected in winter and summer (fall).

Squares – samples from summer or early fall; circles – samples from winter

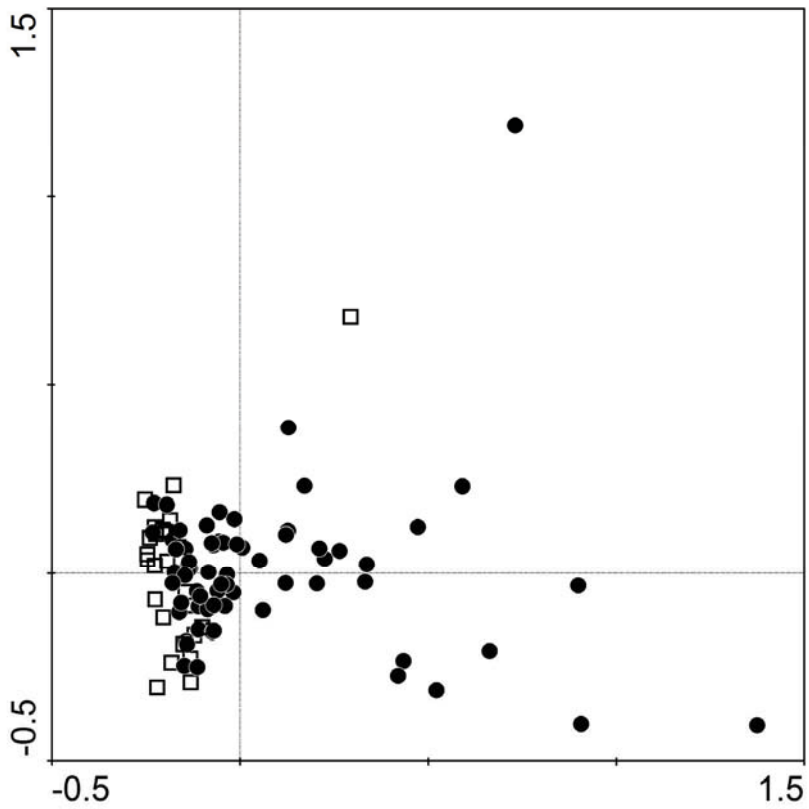


Figure 17. Samples from the dosing study collected from natural and artificial substrata.

Squares – samples from plexiglass substrata; circles – samples from natural substrata.

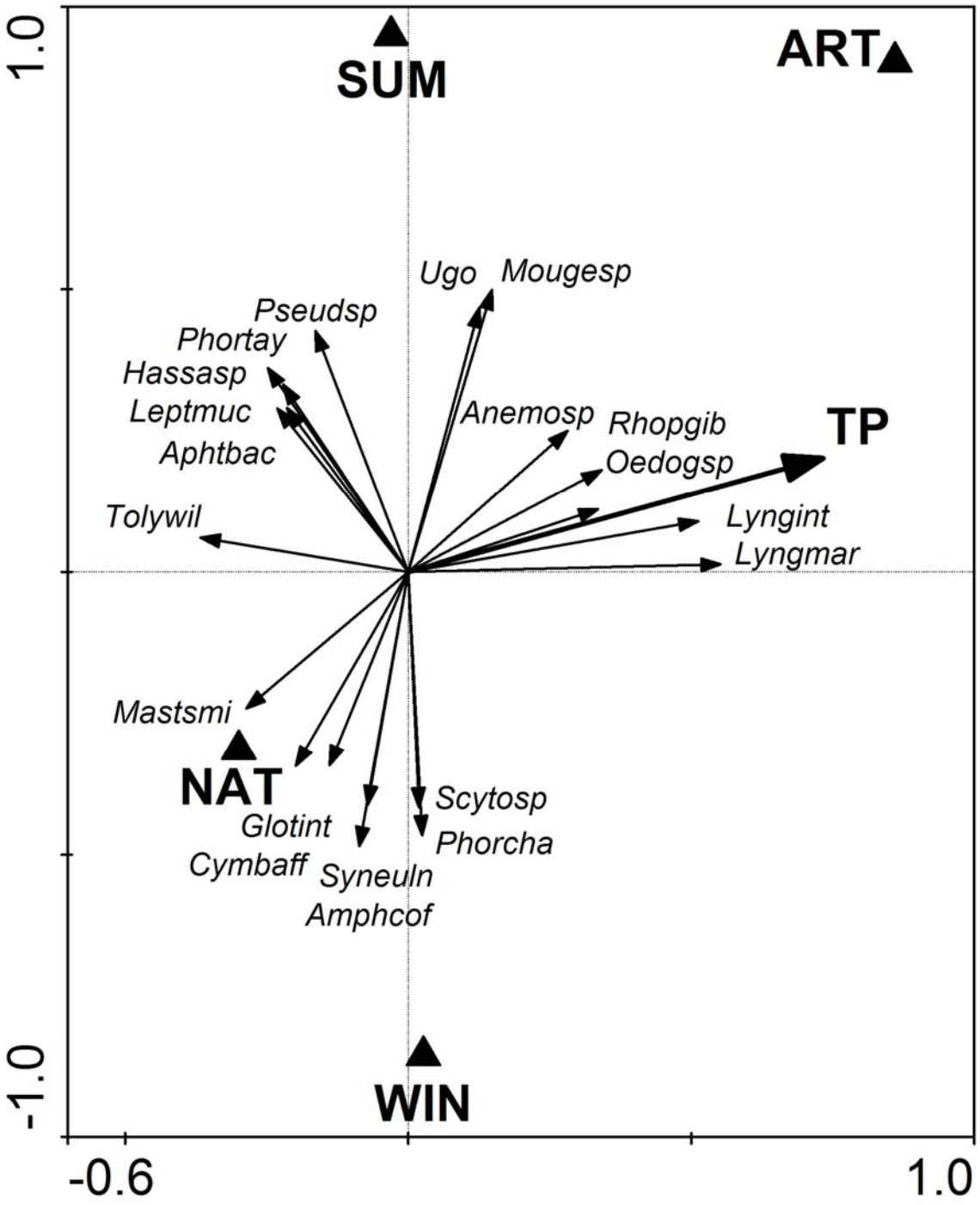


Figure 18. RDA with best fitting species from the dosing study.

Amphcof – *Amphora* cf. *coffaeformis*, Anemosp – *Anomoeneis* sp., Aphtbac – *Aphanothece bacilloidea*, Cymbaff – *Cymbella* cf. *affinis*, Glotint – *Gloeothece interspersa*, Hassasp – *Hassallia* sp., Leptmuc – *Leptolyngbya* cf. *mucosa*, Lyngint – *Lyngbya* cf. *intermedia*, Lyngmar – *Lyngbya* cf. *martensiana*, Mastsmi – *Mastogloia smithii*, Mougesp – *Mougeotia* spp., Oedogsp – *Oedogonium* spp., Phorcha – *Phormidium chalybeum*, Phortay – *Phormidium taylori*, Pseudsp – *Pseudanabaena* sp., Rhopgib – *Rhopalodia gibba*, Scytosp – *Scytonema* sp., Syneuln – *Synedra ulna*, Tolywil – *Tolypothrix* cf. *willei*, Ugo – unidentified green algae. Centroids of substratum quality and season are represented by triangles (NAT for natural substratum, ART for plexiglass substratum, WIN for winter, SUM for summer and early fall).

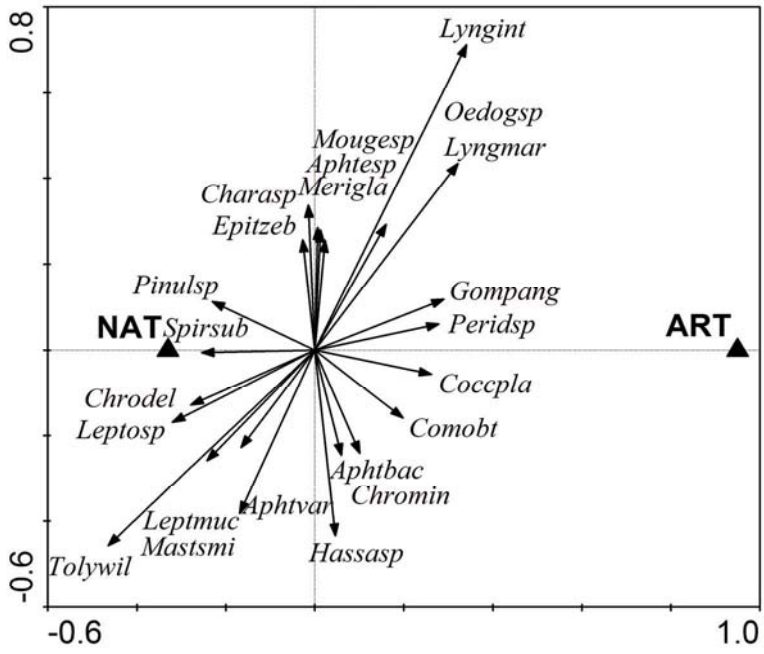


Figure 19. Partial influence of substratum quality in the dosing study

Aphtbac – *Aphanothece bacilloidea*, Aphtesp – *Aphanothece* sp., Aphtvar – *Aphanothece variabilis*, Charasp – *Characiopsis* sp., Chrodel – *Chroococcus deltoides*, Chromin – *Chroococcus* cf. *minutus*, Coccpla – *Cocconeis placentula*, Comobt – *Cosmarium* cf. *obtusatum*, Epitzeb – *Epithemia* cf. *zebra*, Gompang – *Gomphonema* cf. *angustatum*, Hassasp – *Hassallia* sp., Leptmuc – *Leptolyngbya* cf. *mucosa*, Leptosp – *Leptolyngbya* sp., Lyngint – *Lyngbya* cf. *intermedia*, Lyngmar – *Lyngbya* cf. *martensiana*, Mastsmi – *Mastogloia smithii*, Merigla – *Merismopedia glauca*, Mougesp – *Mougeotia* spp., Oedosp – *Oedogonium* spp., Peridsp – *Peridinium* sp., Pinulsp – *Pinnularia* spp., Spirsub – *Spirulina subsalsa*, Tolywil – *Tolypothrix* cf. *willei*. Centroids of substratum quality are represented by triangles (NAT for natural substratum, ART for plexiglass substratum).

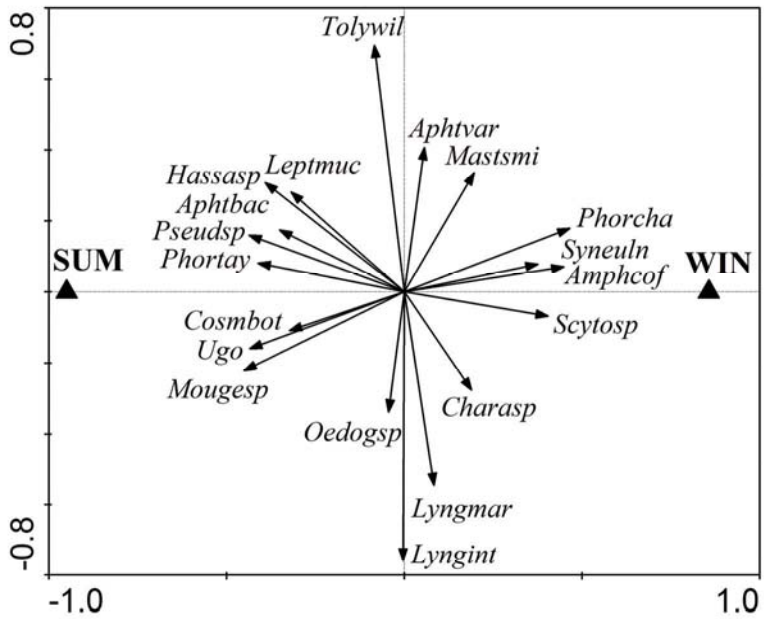


Figure 20. Partial influence of season in the dosing study

Aphtbac – *Aphanothece bacilloidea*, Aphtvar – *Aphanothece variabilis*, Amphcof – *Amphora* cf. *coffaeformis*, Charasp – *Characiopsis* sp., Comobt – *Cosmarium* cf. *obtusatum*, Hassasp – *Hassallia* sp., *Leptolyngbya* cf. *mucosa*, Lyngint – *Lyngbya* cf. *intermedia*, Lyngmar – *Lyngbya* cf. *martensiana*, Mastsmi – *Mastogloia smithii*, Mougesp – *Mougeotia* spp., Oedosp – *Oedogonium* spp., Phorcha – *Phormidium chalybeum*, Phortay – *Phormidium taylori*, Pseudsp – *Pseudanabaena* sp., Scytosp – *Scytonema* sp., Syneuln – *Synedra ulna*, Tolywil – *Tolypothrix* cf. *willei*, Ugo – unidentified green algae. Centroids of season are represented by triangles (WIN for winter and SUM for summer and early fall).

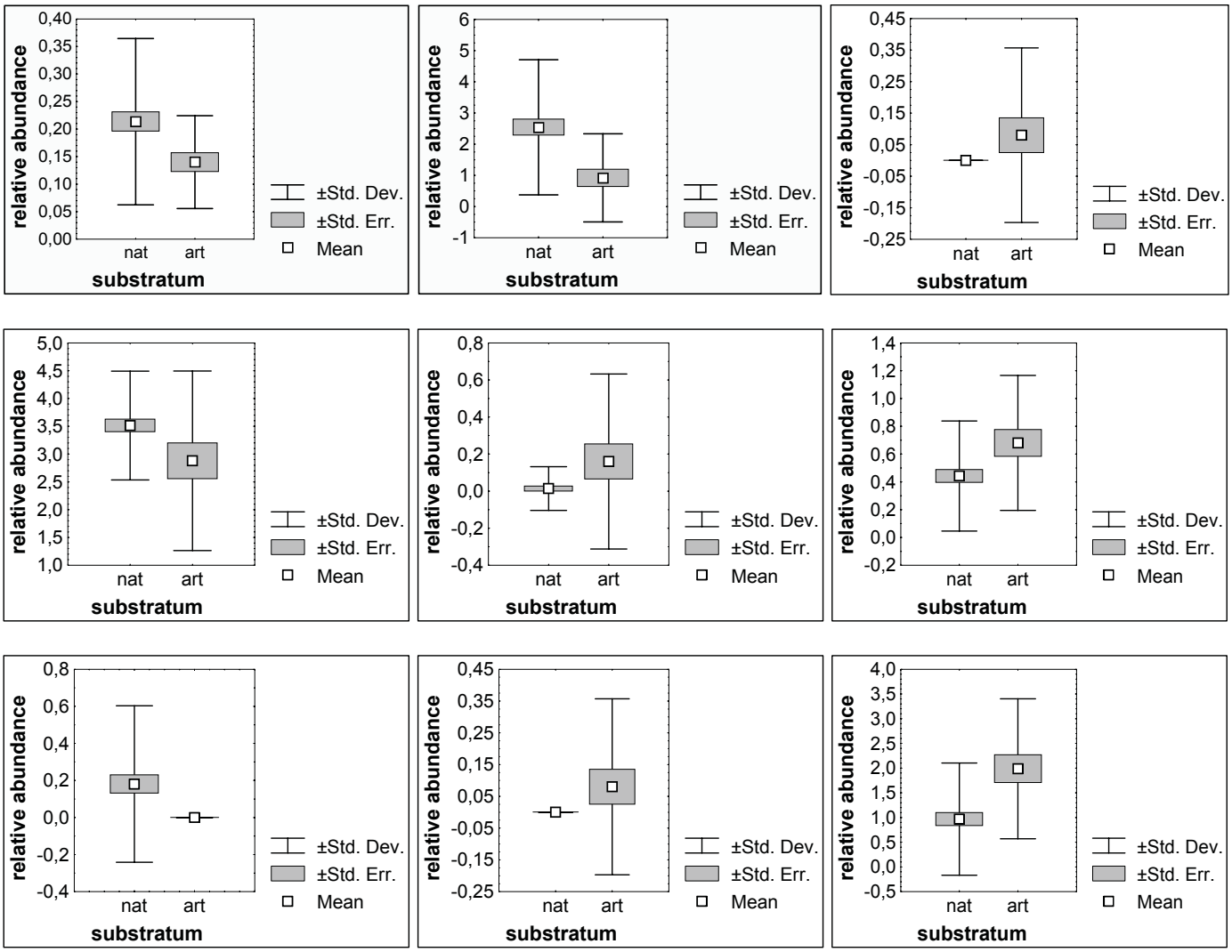


Figure 21. Relative abundances of chosen taxa on natural and artificial substrata in the dosing study

First column, from top – coccoid cyanobacteria; *Leptolyngbya* sp.; *Spirulina subsalsa*. Second column, from top – *Tolypothrix* cf. *willei*; *Cocconeis placentula*; *Cosmarium* cf. *obtusatum*. Third column, from top – *Gomphonema* cf. *angustatum*; green filamentous algae; *Lyngbya* spp. „Nat“ means natural substratum and „art“ artificial substratum.

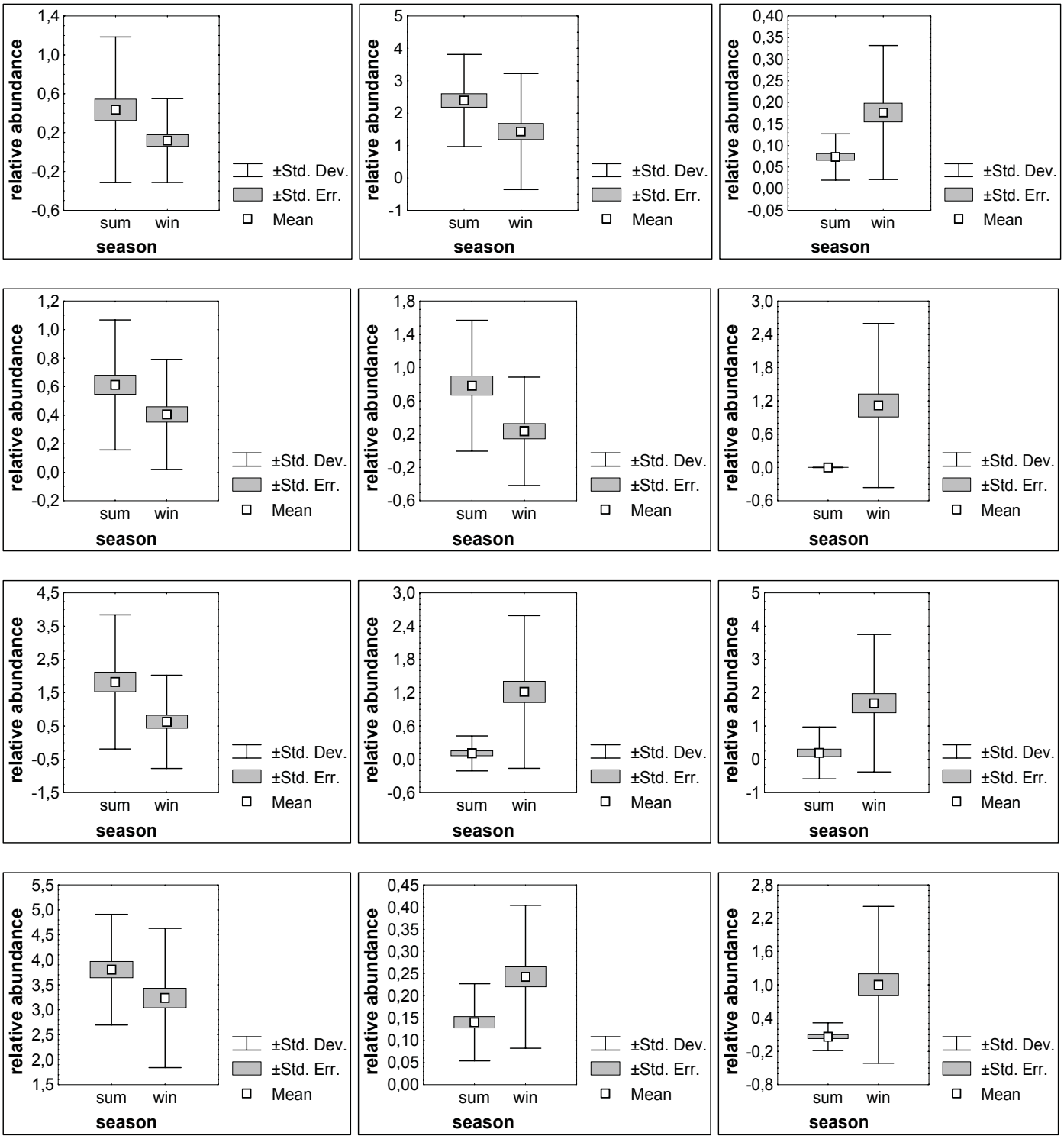
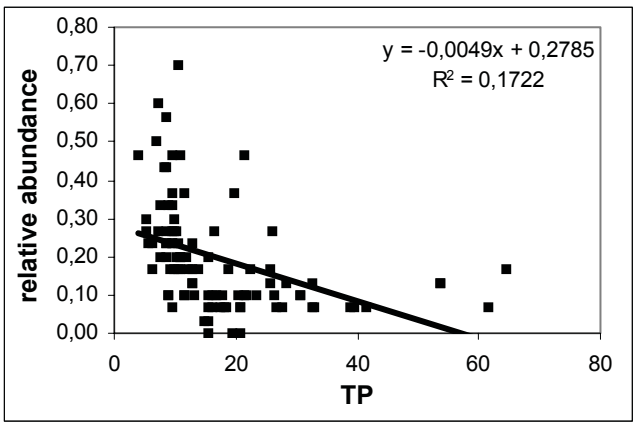
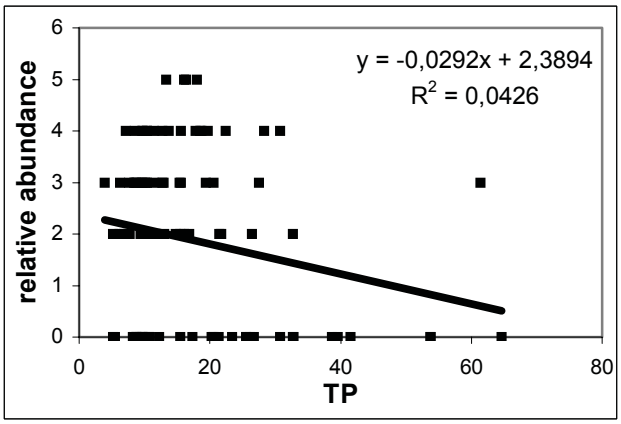
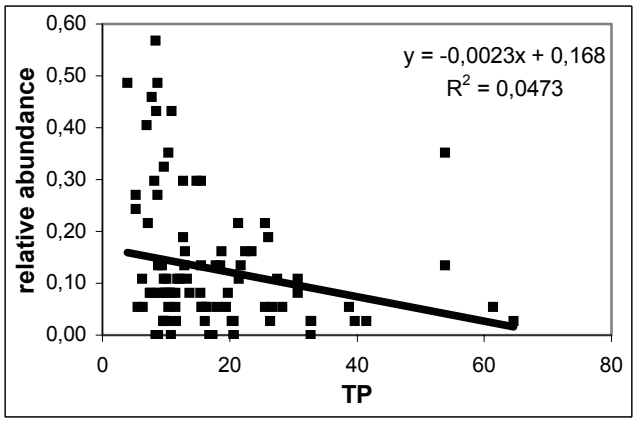


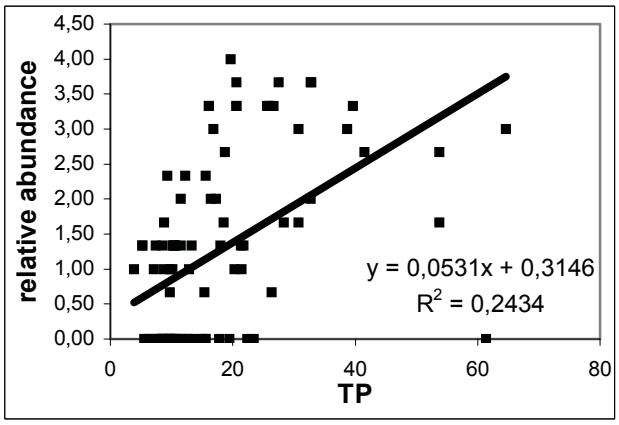
Figure 22. Relative abundances of chosen taxa in two different seasons in the dosing study

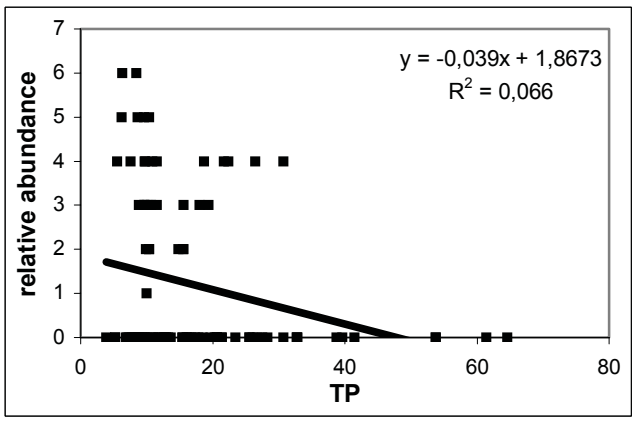
First column, from top – *Aphanothece bacilloidea*; green filamentous algae; *Hassallia* sp; *Leptolyngbya* cf. *mucosa*. Second column, from top – *Phormidium taylori*; *Pseudanabaena* sp.; *Amphora* cf. *coffaeformis*; coccoid cyanobacteria. Third column, from top – diatoms; *Phormidium chalybeum*; *Scytonema* sp.; *Synedra ulna*. „Sum“ means summer or early fall and „win“ means winter.

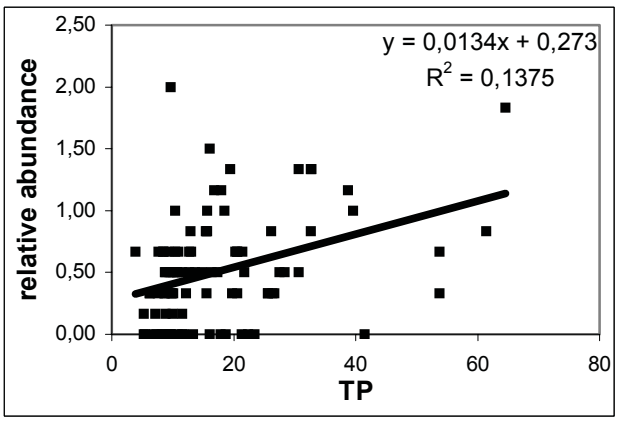












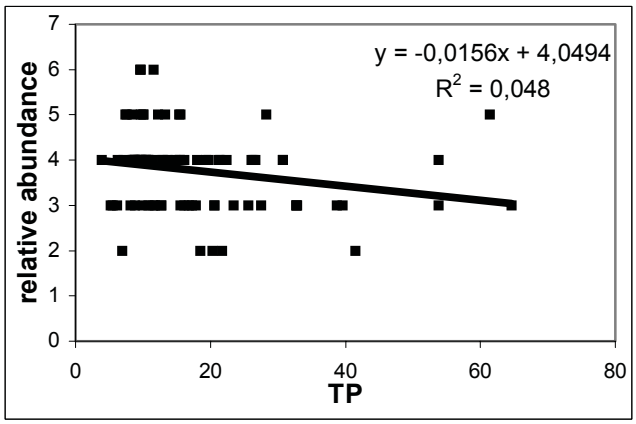


Figure 23. Linear regression of relative abundance of chosen taxa on TP in the dosing study

First column, from top – coccoid cyanobacteria; diatoms; *Hassallia* sp.; *Leptolyngbya* cf. *mucosa*. Second column, from top – *Phormidium taylori*; *Lyngbya* spp.; green filamentous algae. All regressions are significant ($p<0,05$).

preferably colonized plexislides ($p<0,05$) – Fig. 21. Similarly, cyanobacteria *Aphanothece bacilloidea*, *Hassallia* sp., *Leptolyngbya* cf. *mucosa*, *Phormidium taylori* and *Pseudanabaena* sp. and green filamentous algae were more abundant in samples collected in summer while *Phormidium chalybeum*, *Scytonema* sp., coccoid blue-greens and diatoms (especially *Amphora* cf. *coffaeformis* and *Synedra ulna*) were more often found in samples from winter ($p<0,05$) – Fig. 22.

3.4. Comments on the problematic and particularly interesting species

A list of taxonomically problematic and (or) particularly interesting cyanobacterial species with short comments follows. The intention was to bring information necessary for understanding the pictures in Appendix rather than full taxonomical descriptions. This inventory includes only species and results from the samples microscopically examined personally by the author of this study. It does not contain species which corresponded very well to their descriptions in literature in every respect and were not particularly interesting in our opinion. Each species is affixed by a cipher according to its serial number in the Appendix.

4. *Aphanocapsa* sp. (1) Nägeli 1849

Cells <2-3 μm in diameter, spherical or slightly elongated (oval), with black margin, forming ± dense, regular or irregular medium-sized (50-100 μm) mucilagenous colonies. The mucilagenous envelope is sometimes greyish. This species occurred rarely at C2 and C3 localities and could not be assigned to any existing taxon.

5. *Aphanocapsa* sp. (2) Nägeli 1849

Cells spherical, having about 2,5 μm in diameter, densely packed in yellowish mucilage; colonies up to 500 μm, often with small adherent diatoms. Present only at oligotrophic localities (C5,C6) in very small abundance and thus impossible to determine to the species level.

7. *Aphanothece* sp. (subg. *Anathece*) Nägeli 1849 nom. cons.

Cells rod-shaped, often bowed, about 1 μm wide, up to 8μm long, suspended irregularly in a colourless mucilagenous colony (about 40 μm big). Found only once at the C6 locality, corresponds to the description of subgenus *Anathece* by KOMÁREK & ANAGNOSTIDIS (1998).

9. *Aphanothece* cf. *hardersii* Schiller 1952

Cells widely oval, usually sized 3-5 x 2-3,5 μm, sometimes with granules. Mucilagenous colonies rather spherical, 40-90 μm in diameter. This species probably belongs to

Aphanothece hardersii, however it was found only occasionally in small amounts at localities C4 and C6 and the identification needs further confirmation.

11. *Bacularia gracilis* Komárek 1995

This species agrees well with the original description (KOMÁREK 1995) both in morphology and ecology. Since it is a recently described and very characteristic yet often overlooked taxon, we present it here as an interesting species and bring typical pictures in the Appendix. *Bacullaria gracilis* occurred in noticeable abundance only at the C6 locality.

12. *Chlorogloea gardneri* (Gardner) Komárková et Komárek (in press)

syn = *Aphanocapsa richteriana major* Gardner 1927

Cells ± spherical or somewhat elongated, pale green, having (2,5) 3-5 μm in diameter; usually quite densely arranged in large, irregular, flattened colonies. Particularly near the margin of a colony, the cells are often organized rather radially. This species was formerly called *Aphanothece richteriana major* (GARDNER 1927) but was recently moved to the genus *Chlorogloea* by KOMÁREK & KOMÁRKOVÁ (in press). Since it is a new taxon and it was found frequently at localities C1-C4 (less at C6), we refer to it here as to an interesting species.

18. *Chroococcus* cf. *minutus* (Kützing) Nägeli 1849

This species seems to be morphologically indistinguishable from *Chroococcus minutus* as it is delineated by KOMÁREK & ANAGNOSTIDIS (1998). As it was originally described from plankton of temperate zones, its genetic identity with the species from Everglades periphyton is doubtful. Present but not frequent at all localities.

19. *Chroococcus* cf. *minutissimus* Gardner 1927

Agrees with *Chroococcus minutissimus* in morphology. Nevertheless, such classification may be incorrect due to a mismatch in ecology (it was described from waterfalls). Rare, occurred only at localities C1 and C4.

21. *Cyanosarcina* sp. Kováčik 1988

Cells of irregular, roundly polygonal shape, mostly 2-8 μm in diameter, ± densely arranged in disordered mucilagenous colonies. This species was rarely found along the whole transect and did not match to any existing taxon.

22. *Cyanothece* sp. Komárek 1976

Cells widely oval to cylindrical, sized 3-5 x 3 μm, sometimes with granules, often in adherent pairs (amidst binary fission), usually in groups. This species was present only at locality C6 and it does not correspond to any taxon known by the author.

24. *Gloeocapsa* sp. (1) Kützing 1843

Cells spherical, 5-8 μm in diameter, with individual, slightly yellowish mucilagenous envelopes (about 1 μm thick). Since only a couple of very small colonies were found (C5), we did not presume to determine the species.

25. *Gloeocapsa* sp. (2) Kützing 1843

Cells spherical, 3-4 μm in diameter, forming small 2-4 celled colonies (10-18 μm). The cells are surrounded by 3 μm thick greyish envelopes. This species was found only once in our samples and could not be further identified.

28. *Lemmermanniella uliginosa* Komárek et Komárková (in press)

Cells cylindrical with rounded ends, sized 5-10 x 3-4 μm. Gelatinous colonies of spherical or oval shape, with cells irregularly distributed in a layer under the colony surface, 40-100 μm in diameter. This species occasionally occurred at localities C5 and C6. It agrees with the description of a new species *Lemmermanniella uliginosa* by KOMÁREK et KOMÁRKOVÁ (in press).

31. *Wolskyella* sp. Claus 1963

A coccoid species forming typical pseudofilaments – the cells are arranged serially in one row in common mucilage close or distant from one another, appearing like a trichome. Cells long, cylindrical, sometimes hooked at the ends, sized <1-1,5 x 3-10 μm. This species was found quite frequently at localities C4-C6 (oligotrophic) and in one sample from C1, the specimens were dispersed among other algae and cyanobacteria. It does not belong to any of the two existing species (KOMÁREK et ANAGNOSTIDIS 1998). The genus *Wolskyella* is little known and it was described only several times till now. Therefore, this finding is very interesting and the pictures and description presented in this study may help other authors.

35. *Halomicronema* sp. (1) Abed, Garcia-Pichel et Hernández-Mariné 2002

Filaments (1)1,5-2,5 μm wide, rounded at ends, rarely with a very thin mucilagenous sheath; cells 1-2,5 x 5-13 μm, practically always with one big, sometimes two or three smaller granules at each of the unconstricted cross-walls. Straight or variously curved, often long trichomes were dispersed among other cyanobacteria at oligotrophic localities C5 and C6. This species resembles *Limnothrix* by morphological attributes but being periphytic it should be classified rather as *Halomicronema* (KOMÁREK et ANAGNOSTIDIDS 2005).

36. *Halomicronema* sp. (2) Abed, Garcia-Pichel et Hernández-Mariné 2002

Filaments 2-2,5 μm wide, without visible sheaths; cells 2-2,5 x 5 μm, with colourless trenches stretching from both cross-walls almost to the center of the cell (probably remaining after some kind of granules?). Only several fragmentary filaments were found at locality C4. This species may belong to the genus *Halomicronema* from the same reasons as *Halomicronema* sp. (1).

37. *Komvophoron* sp. (1) Anagnostidis et Komárek 1988

Filament about 3-4 μm wide, straight and short, without visible sheath, somewhat attenuated (particularly the apical cell) and conspicuously bent at the end. Cells shorter than wide, intensively constricted at rather thick cross-walls, 3-4 x 1-1,5 μm. It possibly belongs to the genus *Komvophoron* but does not match any existing species. Infrequently at the locality C6.

38. *Komvophoron* sp. (2) Anagnostidis et Komárek 1988

Filaments short, flexuous, fragmentary, 1-1,5 μm wide, without visible sheaths. Cells of a rectangular shape are 1-1,5 μm wide, 1-2,5 times longer than wide, with very thick (1-2μm) translucent cross-walls. This species resembles drawing of Gonzales Guerrera presented as „*Komvophoron?*“ (Figure 467) in KOMÁREK et ANAGNOSTIDIS (2005). It was found quite often among other cyanobacterial filaments at localities C5 and C6.

39. *Komvophoron* cf. *minutum* (Skuja) Angnostidis et Komárek 1988

Morphologically generally correspondent to the description by KOMÁREK et ANAGNOSTIDIS 2005 but ecologically different (described from Sweden) and therefore taxonomically unclear. Rather frequent at all localities among other blue-greens.

40. *Komvophoron* sp. (3) Anagnostidis et Komárek 1988

Filaments short, coiled, without sheaths; cells cylindrical, 2,5 μm wide, 2-5 μm long, somewhat constricted at thick cross-walls. A miniature cell is sometimes present at each filament end. Belongs to *Komvophoron* or possibly to *Pseudanabaena*. The species was present but not very frequent at localities C5 and C6.

42. *Leptolyngbya* cf. *mucosa* (Gardner) Anagnostidis et Komárek 1988

Very probably belongs to *Leptolyngbya mucosa*, however the filaments were in average slightly narrower (2-3μm) and the gelatinous sheath was present only occasionally and was not very thick in contrast with the description given by KOMÁREK & ANAGNOSTIDIS (2005). This species dominated in many samples and was present at all localities, often forming large clusters of filaments.

43. *Leptolyngbya* sp. Anagnostidis et Komárek 1988

Trichomes thin (0,7-1,5 μm), often very long, straight or variously curved, without conspicuous sheaths, sometimes breaking to shorter fragments. Cell walls not visible. This species or aggregation of morphologically indistinguishable species was present in all samples in rather lower amounts, evenly dispersed among other cyanobacteria and algae.

44. *Lyngbya* cf. *intermedia* Gardner 1927

Filaments long, straight or arcuated, often clustered; pale green, dark blue-green or violet-grey; 14 – 19 (22) μm wide, with a 1-2 μm thick, colourless (later somewhat yellowish), smooth sheaths. Cells 12-17 (20) μm wide, 2-4 μm long, unconstricted at cross-walls, often with large granules or concretions, sometimes filled with aerotopes. End cells widely rounded. This species is similar to *Lyngbya intermedia* (GARDNER 1927), however the apical cells are never capitate and the cell size does not match exactly (sometimes being smaller). Dominant at eutrophic localities C1-C4.

45. *Lyngbya* cf. *martensiana* Meneghini ex Gomont 1892

Agrees very well with the description by KOMÁREK & ANAGNOSTIDIS (2005) with exception of sheath morphology. In our species, the sheaths were usually not very thick (up to 1 μm), smooth and not lamellated. Occurred at all localities but C6, in greater amounts (dominant) at the eutrophic ones.

46. *Oscillatoria* cf. *anguina* Bory ex Gomont 1892

Trichomes somewhat broader (sometimes over 10 μm) and not screw-like at the ends compared to the desciption given by KOMÁREK & ANAGNOSTIDIS (2005). Scarcely present at localities C2, C3 and C4.

47. *Oscillatoria* cf. *obtusa* Gardner 1927

Probably belongs to *Oscillatoria obtusa* described by GARDNER (1927), however too few specimen and no well-preserved trichome ends were found, thus the species could not be exactly determined. Locality C2, rare.

49. *Oscillatoria* cf. *sancta* Kützing ex Gomont 1892

Agrees quite well with the description presented by KOMÁREK & ANAGNOSTIDIS (2005). Since we found this species only at localities C3 and C6 in very small amounts, and this taxon is referred to as „collective“ in the above mentioned key, we are not fully satisfied with this identification.

50. *Oscillatoria* sp. Vaucher ex Gomont 1892

Only one fragment of a trichome was found in a sample from locality C6, which did not enable proper identification. It was 45 μm wide, constricted at cross-walls, without sheath with cells approximately 5 μm long.

53. *Phormidium* cf. *hamelii* (Frémy) Anagnostidis et Komárek 1988

Generally in agreement with the description by KOMÁREK & ANAGNOSTIDIS (2005), but the cells are a little smaller (about 3-5 μm in diameter, never over 6 μm long) and the apical one is sometimes narrowed. Present but not frequent in growths of filamentous blue-greens at localities C1,2,3,5 and 6.

54. *Phormidium* cf. *articulatum* (Gardner) Anagnostidis et Komárek 1988

This species is very similar to *Phormidium articulatum*, however the filaments are distinctly broader being mostly 5-8 μm wide. Present at all localities except C6 among other filamentous cyanobacteria.

58. *Pseudanabaena* sp. Lauterborn 1915

Filaments long, often breaking to shorter fragments, coiled and entangled, sometimes forming large fascicles, infrequently with colourless sheaths, apical cells somewhat narrowed (conical), trichomes 1,5-2,5 (3) μm wide. Cells isodiametric or shorter (longer) than wide, 1,5-2,5 μm wide, 2-5 μm long, unconstricted or slightly constricted at rather thick translucent cross-walls, with homogenous blue-green content. Does not correspond to any known taxon. Present at all (sometimes dominant at eutrophic) localities together with other species, especially *Lyngbya spp.*

63. *Anabaena* cf. *ambigua* Rao 1937

Agrees with the description of *Anabaena* cf. *ambigua* by KOMÁREK (2005).

66. *Anabaena* sp. Bory 1822

Wavy filaments of a regular structure - one heterocyte is usually present per every 7-10 vegetative cells, akinetes always on both sides of the heterocyte, single, rarely 2-3 in a row; a gelatinous, intensively brownish-yellow sheath often covers the heterocyte, the akinetes and few cells situated close to them; apical cells are conical. The barrel-shaped vegetative cells are longer than wide or isodiametric (2,5-3,5 x 2,5-7 μm), heterocytes rounded cylindrical (3,5-5 μm wide, 7,5-10 μm long), akinetes also rounded cylindrical (7-10 μm wide, 14-20 μm long). This species was one of the dominants at locality C3, it was present at C4 and C5, too.

Since it is very characteristic and incommutable yet not matching to any known taxa, it should be very probably considered as a new species.

67. *Anabaena* cf. *reniformis* Lemmermann 1898

The filaments of this species are characteristically coiled, and the barrel-shaped vegetative cells and heterocytes also agree with the description of *Anabaena reniformis* by KOMÁREK (2005). The akinetes are spherical, but hardly 7μm big, the cells do not contain any aerotopes (10 μm akinetes and distinct aerotopes in vegetative cells according to the original description). As it should be a planktic or metaphytic species, there is a certain mismatch in ecology. Taxonomy of *Anabaena* cf. *reniformis* remains unclear. Localities C2 and C3, infrequent.

68. *Calothrix* sp. Agardh 1824

Filaments in a brownish-yellow mucilagenous sheath, with one apical and sometimes also intercalar heterocytes (5-6,5 μm wide, about 10-18 μm long), narrowing towards the opposite end; akinetes (6-10 μm wide, up to 25 μm long) next to the heterocytes. Vegetative cells isodiametric, sized 5-7,5 μm. Locality C4, rare.

69. *Cylindrospermum* cf. *breve* Komárek 1984

In agreement with the original description (KOMÁREK 1984). As no akinetes were found, definite identification was impossible. Present but not very frequent at localities C2-C6.

70. *Hassallia* sp. Berkeley 1845

Filaments long, 15-25 μm wide, somewhat clavate at the ends (22-30 μm wide), intensively false-branched, branches single (next to a heterocyte) or in pairs; gelatinous sheaths very thick (about 5-10 μm), rough, intensively lamellate, colourless or yellow-brown. Vegetative cells are isodiametric cylindrical or shortly barrel shaped in some parts of the filament, having 5-10 μm in size (2,5 μm long and 10-12 μm wide at trichome ends), heterocytes somewhat longer (up to 18 μm). Cell content often granulated, cross-walls hardly visible. This species was fairly frequent among cyanobacteria and diatoms at oligotrophic localities C5 and C6 and could not be assigned to any existing taxon.

71. *Microchaete* cf. *robusta* Setchell et Gardner 1903

Approximately corresponds to the description given by STARMACH (1966). Only few not very well developed filaments were found at locality C1, thus the classification is vague.

72. *Microchaete* cf. *goeppertiana* Kirchner 1900

Morphologically similar to *Microchaete goeppertiana* as presented by STARMACH (1966) but no akinetes were found and vegetative cells were not prolonged near heterocytes. Therefore, identity of the species remains unclear. It was present at localities C1-C3, mostly attached to filamentous algae or remains of macrophytes.

73. *Nostoc* sp. Vaucher 1803

Cells ± spherical or deformed, sometimes in short chains, 5-10 μm in diameter. In this stage it was impossible to determine the species any further. It occurred at localities C2, C3, C5 and C6, usually attached to cyanobacterial filaments.

74. *Scytonema* sp. Agardh 1824

Filaments long, blue-green, with colourless thin sheaths, rather infrequently false-branching (branches in pairs or single), 8-15 μm wide, apical cells widely rounded. Vegetative cells cylindrical, from 4 μm up to 20 (25) μm long and 7-10 μm wide, shorter (4-7μm), wider (9-12,5μm), and constricted at the trichome ends, heterocytes single, 6-20 μm long and 7-10 μm wide. This species resembles *Scytonema hofmanni* a little, however there is a distinct inconsistency in cell sizes (bigger in our species) and ecology (originally described from moist soils in Germany). Further examination of this taxon is necessary. It was present at locality C1 and in greater abundance at C3, in aggregation with other cyanobacteria.

75. *Tolypothrix* cf. *willei* Gardner 1927

Agrees very well with the original description by GARDNER (1927) with exception of the filament end width which is much greater – about 12 μm (four times wider cells than in older parts of the filament). It occurred frequently at localities C4-C6 among other algae and blue-greens.

76. *Stigonema* sp. Agardh 1824

Observed filaments arcuated, up to 300 μm long, 17-22 μm wide. Cells 5-17 μm wide, about 5 μm long in 1 to 3 rows with spaces between one another. Since the specimen were in poor condition (possibly due to conservation), heterocytes were not distinguishable from vegetative cells. This species was found rarely at locality C6 and it did not match any known taxon.

4. Discussion

The pristine wetland area of the Everglades is one of the most precious natural phenomenons of this kind not only in America but also in the whole world´s scope. That is why serious efforts have been made to identify and prevent or halt the mostly human-caused changes which have been affecting the original ecosystem.

In this study, 42 samples and data from additional 98 samples of periphyton from the WCA-2A collected between 1998 and 2000 were examined in order to determine the major factors influencing the algal-cyanobacterial growths which play one of the most significant roles in the whole society of Everglades biota. In addition to this, an effort was made to assess plexiglass slides as a suitable substratum for monitoring of periphyton. Since the blue-greens dominate the periphyton yet so far insufficient taxonomical attention has been paid to them, another goal was to improve our knowledge of present cyanobacterial species.

Cyanobacteria (76 species), with co-dominance of Bacillariophyta (24 species) and Chlorophyta (56 species) obviously prevailed in the samples microscopically analysed by the author. Moreover, several species of Charophyta (1 species), Cryptophyta (1 species), Dinophyta (2 species), Euglenophyta (1 species), Raphidiophyta (1 species) and Xanthophyta (1 species) were occasionally present. Such results correspond well to findings of other researchers which studied periphytic assemblages of this region (GLEASON 1974, SWIFT & NICHOLAS 1987, VYMAZAL et al. 2002, VYMAZAL & RICHARDSON 1995).

The results of a study from a natural TP concentration gradient of the WCA-2A clearly confirm considerable influence of this factor to species composition of substratum-attached communities of cyanobacteria and algae. Importantly, these outcomes were testified by a mesocosm-scale experiment executed at a nearby locality. In agreement with this, significant impacts of eutrophication on periphyton in the Everglades were previously reported by BELANGER et al. (1989), GAISER et al. (2005,2006), GRIMSHAW et al. (1993), McCORMICK et al. (1996), SWIFT & NICHOLAS (1987), RADER & RICHARDSON (1992), PAN et al. (2000), NOE et al. (2001), VYMAZAL et al. (2002) and others. Similarly, our finding of an evident decrease in the number of species at localities with higher TP levels was already recognized by SWIFT & NICHOLAS (1987). In contrary, RADER & RICHARSON (1992) stated, that algal diversity did not differ in phosphorus enriched compared to unenriched areas of the Everglades. Furthermore,VYMAZAL et al. (1994) cocluded that phosporus additions increased species diversity at sloughs and sawgrass-dominated localities but the number of species decreased at mixed sawgrass-cattail sites. The obvious shift in species composition may occur not only due to changing TP concentration itself, but also as a consequence of alterations in

the macrophyte community (GRIMSHAW et al. 1997). Unfortunately, this point of view was not adequately considered in design of experiments which produced our data.

With help of multivariate analyses of all available data, taxa significantly responding to water column TP levels were chosen. According to these results, the original periphytic assemblages from oligotophic WCA-2A habitats are characterized especially by presence of heterocytous cyanobacteria from family Scytonemataceae (represented by *Hassallia* sp., *Scytonema* sp. and *Tolypothrix* cf. *willei*) and higher amounts of some diatoms (*Mastogloia smithii* being a conspicuous dominant) both disappearing at the most eutrophic sampling points. Heterocytous scytonematacean cyanobacteria were also found e.g. by GLEASON & SPACKMAN (1974) and VYMAZAL & RICHARDSON (1995) in the Everglades and by REJMÁNKOVÁ et al. (2004) at similar habitats in Belize, and seem to be a good indicator of low TP levels. Furthermore, *Mastogloia smithii* was described many times as a typical oligotrophic species (McCORMICK et al. 1996, PAN et al. 2000, SWIFT & NICHOLAS 1987,VYMAZAL et al. 2002) in agreement with our results. We also observed another diatom species *Nitzschia* cf. *amphibia* in samples from localities with lower phosphorus level which does not correspond to the results of GAISER et al. (2006), McCORMICK et al. (1996), RASCHKE (1993) and some other authors. Importantly, not all the diatoms responded negatively to eutrophication; for example, *Cocconeis placentula*, *Diploneis* cf. *subovalis Fragillaria* cf. *virescens* and *Rhopalodia gibba* (reported as eutrophic by GAISER et al. 2006) preferred enriched sites.

On the other side, predominance of a wide-filamented cyanobacterial genus *Lyngbya* together with green filamentous algae (mainly from genuses *Spirogyra*, *Oedogonium* and *Mougeotia*) was observed at the opposite end of the TP gradient. Increase in abundance of filiform Chlorophyta with rising nutrient levels was previously reported e.g. by SWIFT & NICHOLAS (1987), PAN et al. (2000) and VYMAZAL et al. (2002). In contrast, species from order Desmidiales were found to be rather negatively correlated to TP. Perhaps, taxa which are able to form dense fascicles of wide trichomes gradually outcompete small coccoid algae if they receive enough nutrients for extensive growth of their thallus. However, the relation of periphytic coccoid cyanobacteria to phosphorus enriched vs. unenriched sites of Everglades marshes cannot be fully generalized, as for example *Aphanothece variabilis* and *Chlorogloea gardneri* were in some cases positively correlated to TP. Only the species of genus *Chroococcus* and coccoid blue-greens as an averaged unit seemed to be consistently favouring oligotrophic localities. It is important to note, that none of these species dominated at any of the sampling points and they were mostly present in low amounts hence being rather inappropriate as bioindicators.

It was estimated in our survey, that already at TP concentrations slightly exceeding 10 $\mu g.L^{-1}$ the periphytic communities change radically. The same concentration was formerly reported as critical by McCORMICK & O´DELL (1996) and should not be stepped over to preserve the original character and function of this society.

As for the influence of different substrata to the species composition of our samples, the results are somewhat ambiguous. Analysis of the samples collected at natural habitats of the WCA-2A did not show clearly interpretable changes in periphytic assemblages. Only few generally infrequent species (*Chroococcus pulcherrimus*, *Cyanobacterium* sp.) were found to prefer natural substrata. However, results of an RDA analysis suggest, that in spite of being less substantial than TP concentration, the substratum quality cannot be wholly disregarded. Furthermore, a PCA analysis showed greater variability in plexiglass samples in comparison to samples from natural substrata (but there were fewer natural than artificial samples). The growth of periphyton on plexislides may have slightly different course than in epiphytic mats, sometimes leading to dissimilarities in consequent algal-cyanobacterial community. Anyway, outcomes from the C-transect indicate that plexiglass samples represent the natural periphytic assemblages quite well and probably they can be succesfully used in monitoring of attached microflora of the Everglades. Such results are in a disagreement with many available studies. CATTANEO & AMIREAULT (1992) reviewed 157 periphyton studies and stated that in 62 % of them epiphyton was reproduced reliably by artificial substrata (but cyanobacteria only in 28% of 53 studies). One possible reason for this could be quite a long time of exposition of plexislides in the C-transect study (over 40 days) compared to a standardly used 2-4 week interval (CATTANEO & AMIREAULT 1992), which perhaps enabled the microbiota to create societies of late successional stage more correspondent to the naturally forming mats.

Importantly, results from the dosing study were completely different. Statistical analysis indicated significant disparity in relative abundance of some of the most abundant taxa on (pseudo-)natural vs. artificial substrata. In agreement with the findings of BROWN (1976) and CATTANEO & AMIREAULT (1992) some diatoms (*Cocconeis placentula*, *Gomphonema* cf. *angustatum*) preferably colonized the plexislides, possibly due to their fast reproduction, small sizes and secretion of adhesive substances which prevent them from slipping-off. Surprisingly, filamentous cyanobacteria *Lyngbya* spp. and green filamentous algae were also in average more present on artificial substrata, which goes against ideas presented by BROWN (1976). Perhaps this can be explained by different successional rates at sampling points differing in TP concentration. The plexislides are probably firstly colonized by the diatoms, which prepare an environment suitable for slow-growing filamentous algae and cyanobacteria (SEKAR et al. 2002, 2004). Therefore, at the low-phosphorus sampling points, diatoms predominated on plexislides, while at high-TP plexislides with faster succession they had

been replaced by a mat of trichome-forming cyanobacteria and algae (already suggested in a preliminary study VYMAZAL et al. 2000a). In accordance with that, false-branched heterocytous cynobacterium *Tolypothrix* cf. *willei* and some other cyanobacteria (*Leptolyngbya* sp., *Spirulina subsalsa*, coccoid blue-greens) favoured natural substrata – possibly they were not able to create a full-developed thallus on diatom-dominated plexislides and at places with higher TP levels they were, for a change, outcompeted by *Lyngbya* and Chlorophyta. It is important to note, that any extrapolation of these experimental results may be a little problematic, since the pseudo-natural periphytic growths from an artificial mesocosm may be different from the free-living mats of untouched habitats. More research concentrated on the influence and principles of colonisation of plexiglass substrata is necessary for proper explanation of this problem.

Since almost all samples from plexislides were collected in summer while many samples from pseudo-natural mats were taken in winter in the dosing study, these two qualitative attributes were separated in statistical analysis to avoid mistakes in interpretation due to their correlation. Inspired by suggestions of VYMAZAL & RICHARDSON (1995) we tried to find out if any shifts in the species composition occur between the seasons. Significant results were obtained and some taxa were identified that were more abundant in winter or summer (early fall). Filamentous green algae and cyanobacteria (*Leptolyngbya* cf. *mucosa*, *Phormidium taylori*, *Pseudanabaena* sp., *Hassallia* sp.) were typical for samples from September an October while diatoms (*Amphora* cf. *coffaeformis*, *Synedra ulna*), coccoid cyanobacteria and some filamentous cyanobacteria (*Phormidium chalybeum*, *Scytonema* sp.) were more abundant in the winter months. This is in very good agreement with previous observations of VYMAZAL & RICHARDSON (1995) who reported green algae and filamentous cyanobacteria as dominant throughout summer and fall being replaced by diatoms during winter and spring.

A description and discussion of individual taxonomically problematic and particularly interesting cyanobacterial species is presented in chapter 3.4. Furthermore, photographs of cyanobacteria given by GLEASON & SPACKMAN (1974) were compared to our description. „*Microcoleus lyngbyaceus*“ depicted in this study is conspicuously similar to our species *Lyngbya* cf. *martensiana*, pictures of „*Schizothrix calcicola*“ and „*Scytonema hofmannii*“ resemble of our *Halomicronema* sp. (1) and *Hassallia* sp., respectively. Moreover, *M. lyngbyaeceus* was reported as typical for nutrient enriched localities (SWIFT & NICHOLAS 1987) like *Lyngbya* spp. while *S. calcicola* and *S. hofmannii* were presented as oligotrophic species (GLEASON & SPACKMAN 1974, MCCORMICK & O´DELL 1996, SWIFT & NICHOLAS 1987) like *Halomicronema* sp. and *Hassallia* sp. in our results. Although exact determination

is not possible on the basis of these few pictures, in opinion of the author of this study, the original determination (by GLEASON & SPACKMAN 1974) of above mentioned species was wrong.

5. Conclusions

1. Significant influence of changing water column TP on periphyton samples from the Florida Everglades WCA-2A was confirmed by analysis of samples from a natural TP gradient and an in situ experiment with additions of phosphorus. The shift in species composition occurred already at TP concentrations slightly exceeding 10 $\mu g.L^{-1}$ and the number of present species decreased with onward eutrophication.

2. Increased abundance of some cyanobacteria (especially *Lyngbya* spp.) and filamentous green algae (*Spirogyra* spp., *Oedogonium* spp., *Mougeotia* spp.) was found at localities with high TP levels while some species of diatoms (*Mastogloia smithii* as a dominant), desmids (*Cosmarium* spp., *Euastrum* spp.) and cyanobacteria (*Chroococcus* spp., *Phormidium taylori*, *Phormidium granulatum*) preferred oligotrophic sites. These taxa can be possibly used as indicators of trophic status and predictors of beginning phosphorus-driven changes.

3. Certain influence of substratum quality to the periphyton species composition was observed in the dosing experiment. Plexiglass substrates may be preferably colonized by diatoms at oligotrophic localities and by wide filamentous species of cyanobacteria and green algae at eutrophic localities, causing underestimation of heterocytous, coccoid and narrow-filamented cyanobacteria. However, the differences were not substantial in case of samples from natural habitats. Perhaps, the plexiglass samples are good in representation of present algae if the exposition time and number of exposed plexislides are adequate (over 40 days, 5-10 slides in our study).

4. Significant differences in species composition between samples collected in winter vs. summer (early fall) were confirmed: filamentous green algae and some cyanobacteria (*Leptolyngbya* cf. *mucosa*, *Phormidium taylori*, *Pseudanabaena* sp., *Hassallia* sp.) were typical for summer samples while diatoms, coccoid cyanobacteria and some filamentous cyanobacteria (*Phormidium chalybeum*, *Scytonema* sp.) were more abundant in winter.

5. All 76 identified species of cyanobacteria were determined and photographically documented. Problematic and interesting species were described and discussed.

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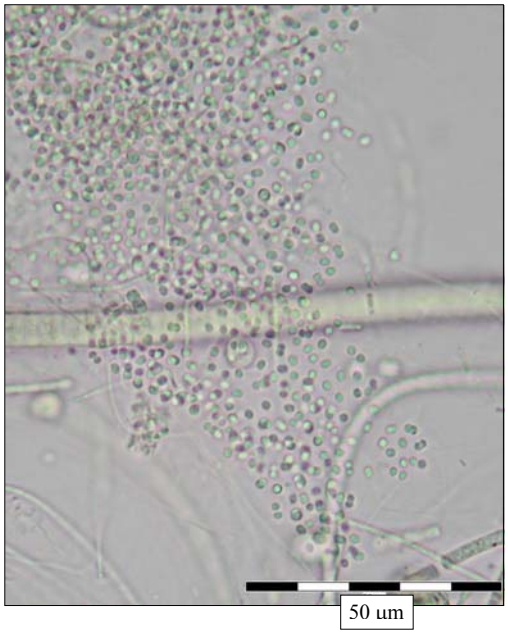
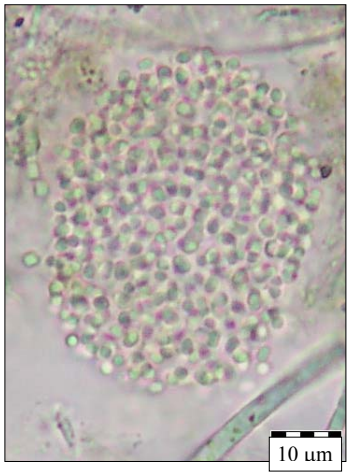
THE SOUTH FLORIDA PERIPHYTON RESEARCH GROUP web pages (2006) - http://serc.fiu.edu/periphyton

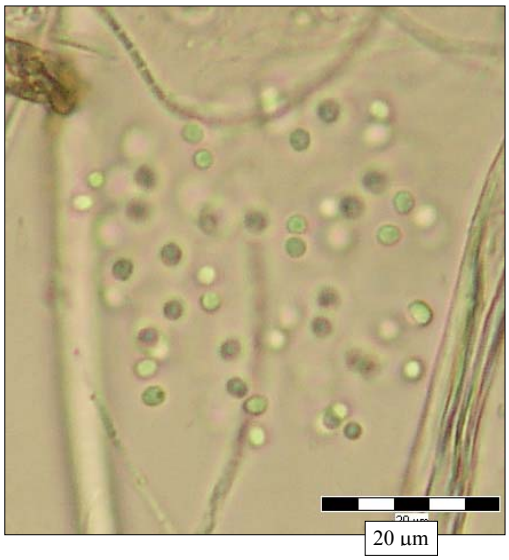
7. Appendix

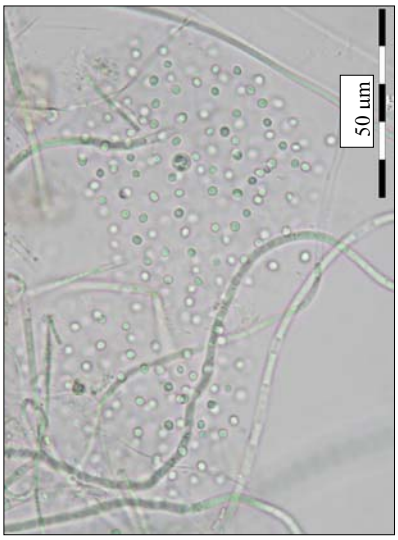
Photographic documentation of 76 species of Cyanobacteria from the Everglades wetlands

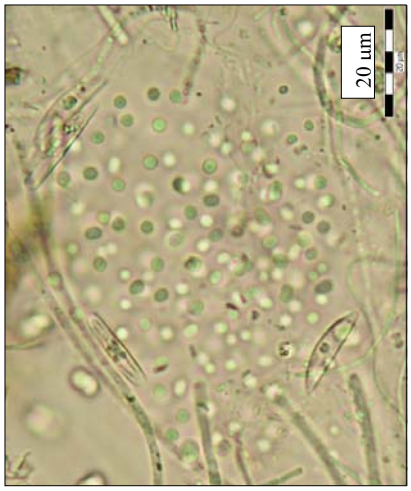




1. *Aphanocapsa parasitica*







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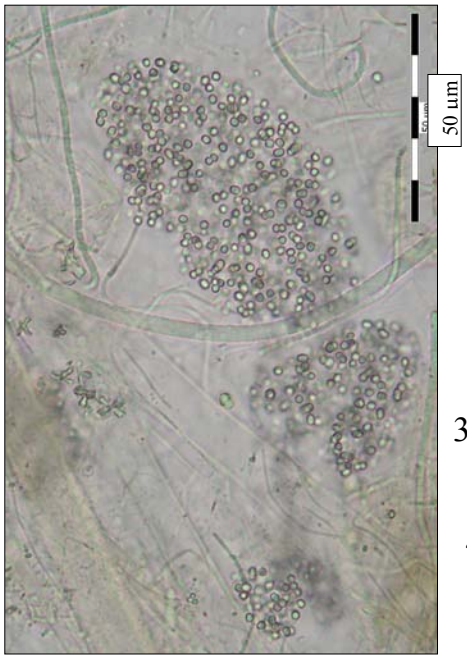
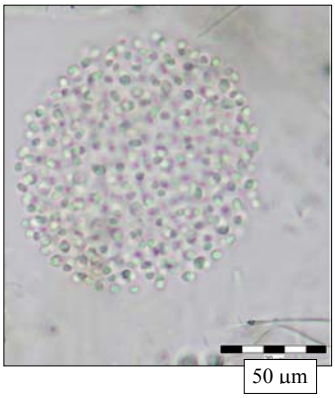
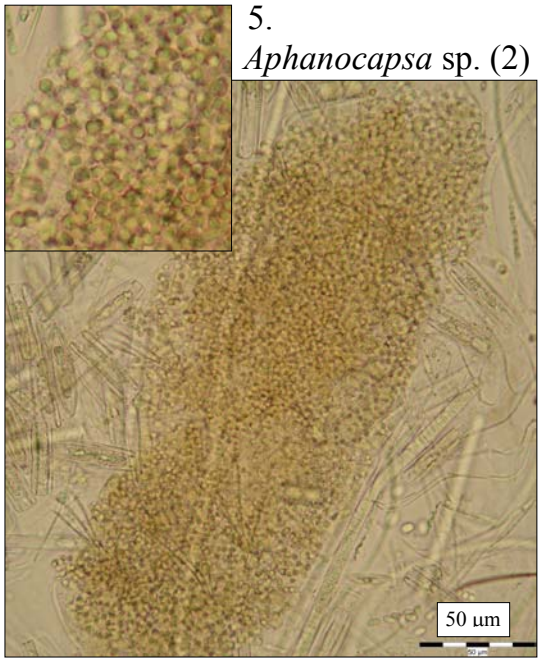


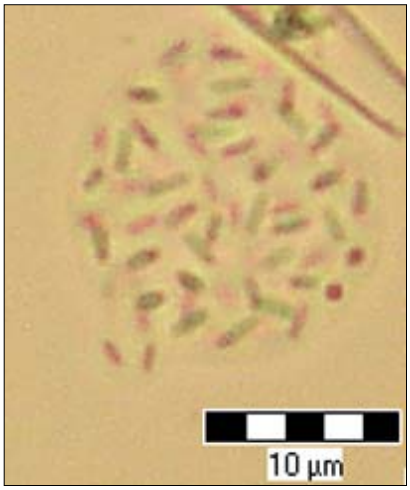


3. *Aphanocapsa conferta*

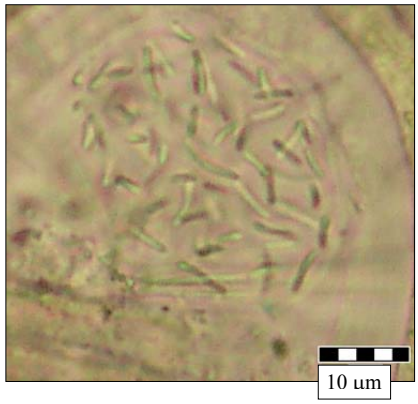
4. *Aphanocapsa* sp. (1)

5. *Aphanocapsa* sp. (2)

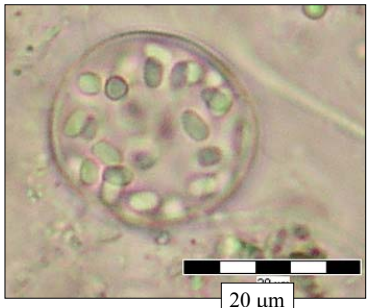


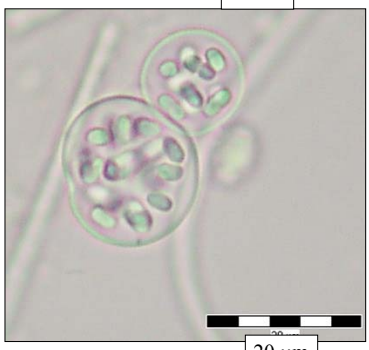


6. *Aphanothece bacilloidea*

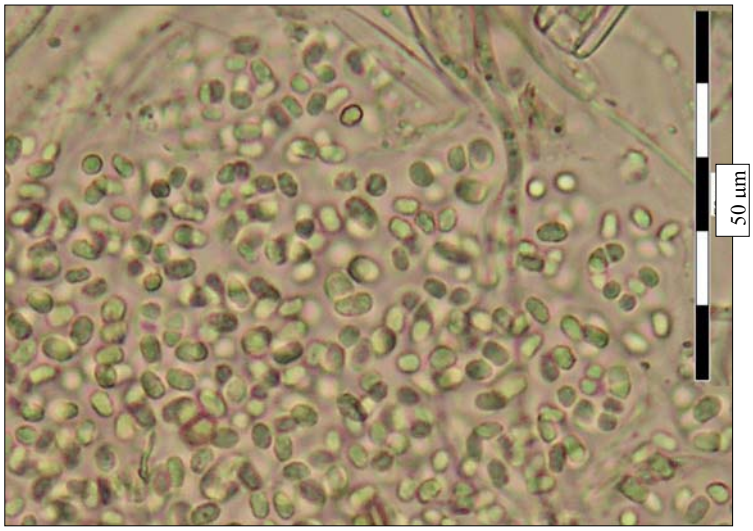


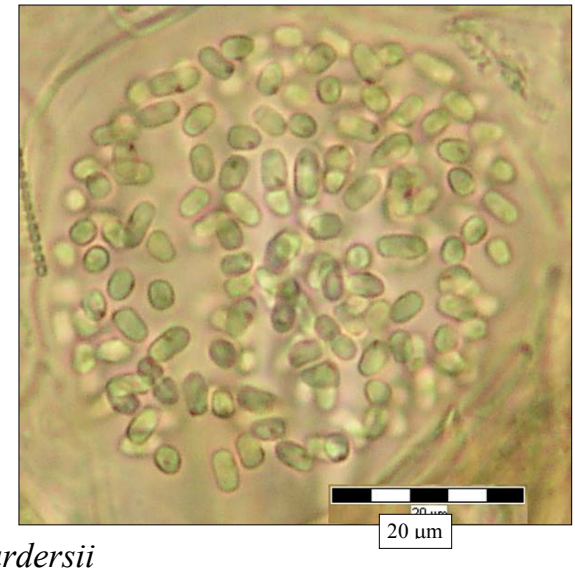
7. *Aphanothece* sp. (subg. *Anathece*)



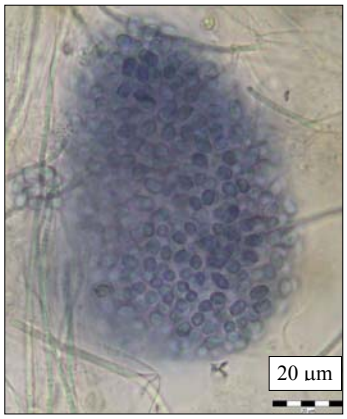


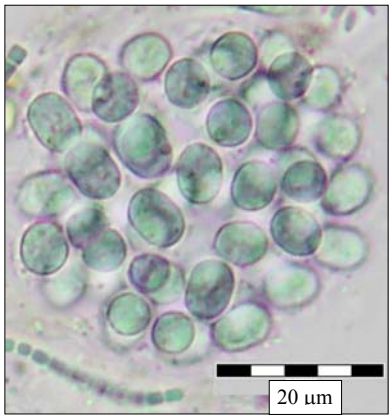
8. *Aphanothece comasii*

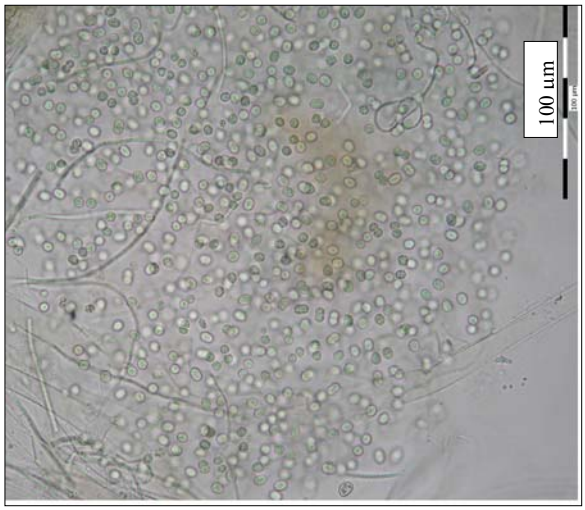


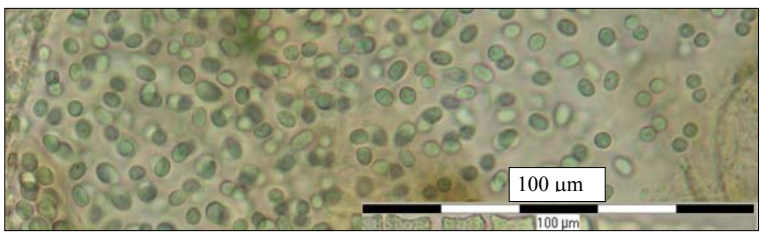


9. *Aphanothece* cf. *hardersii*

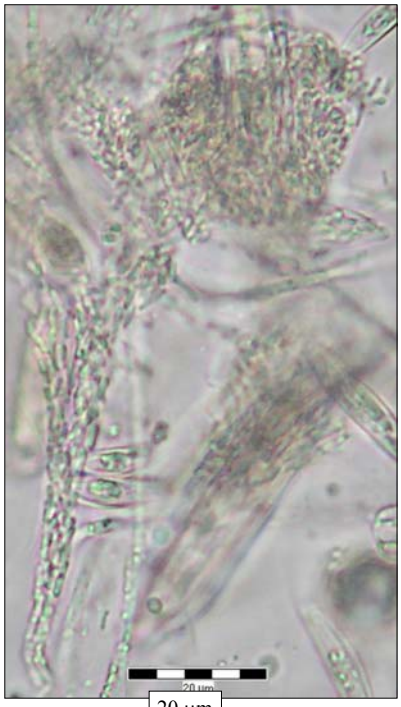


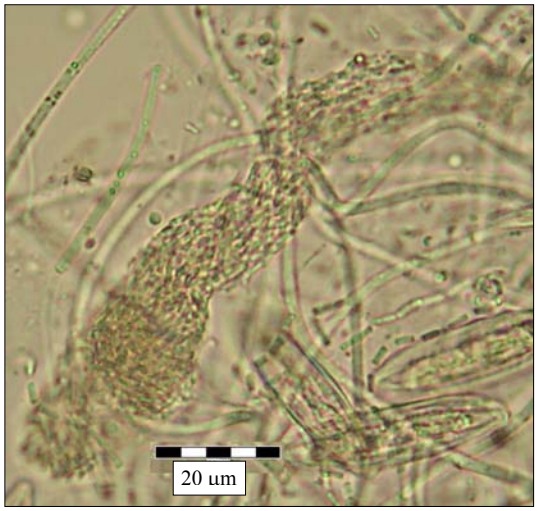




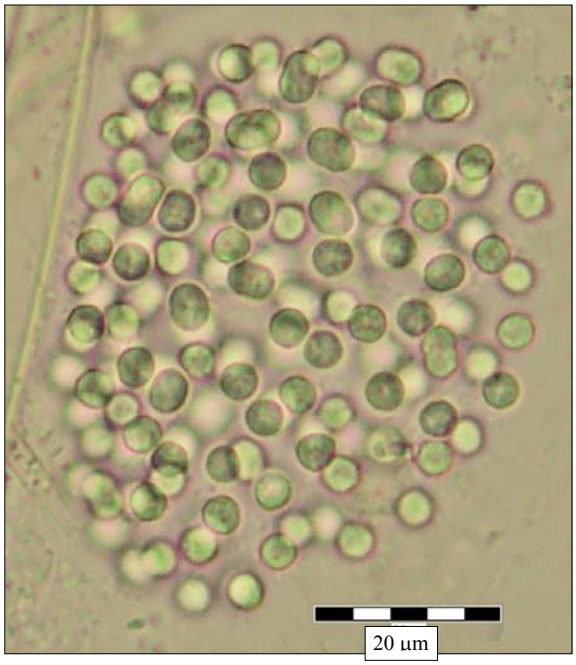


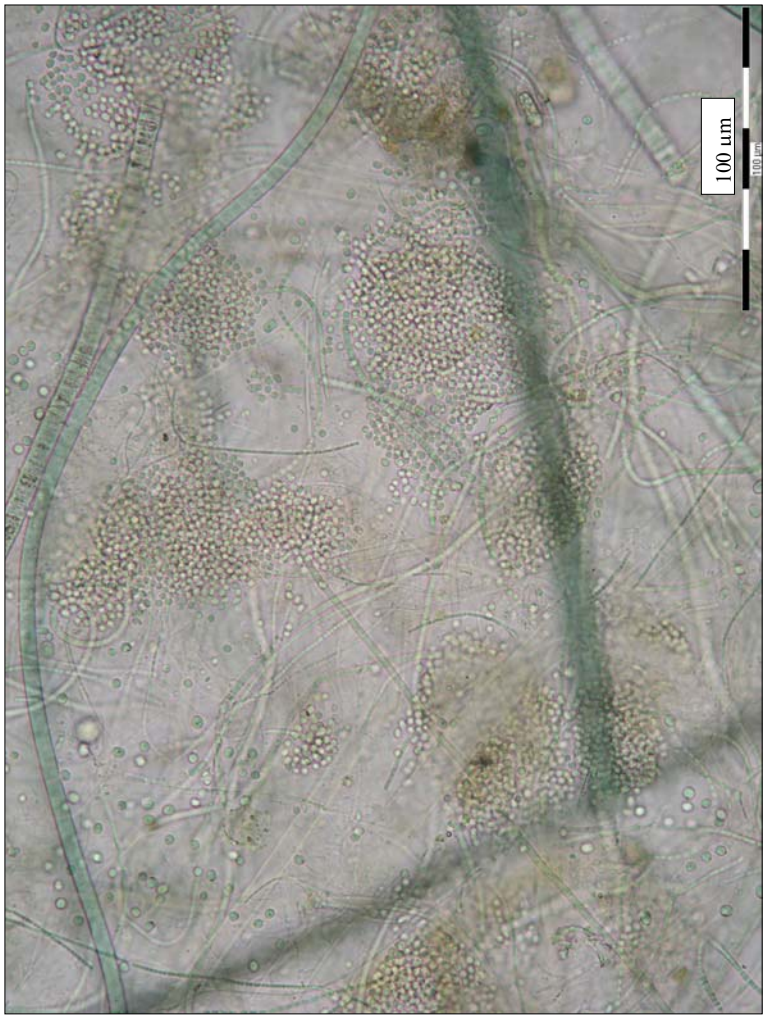
10. *Aphanothece variabilis*

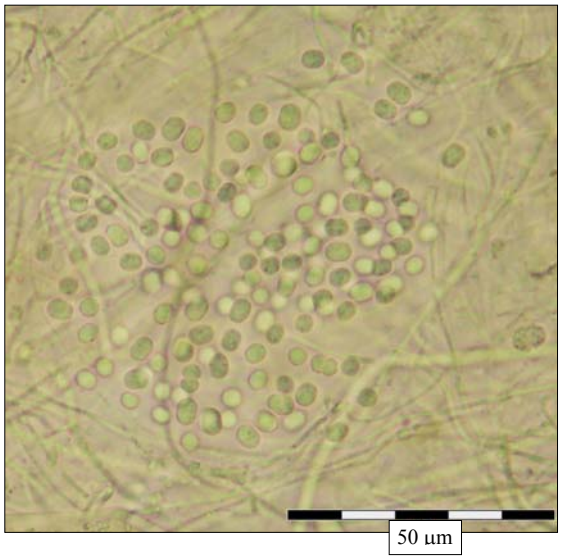




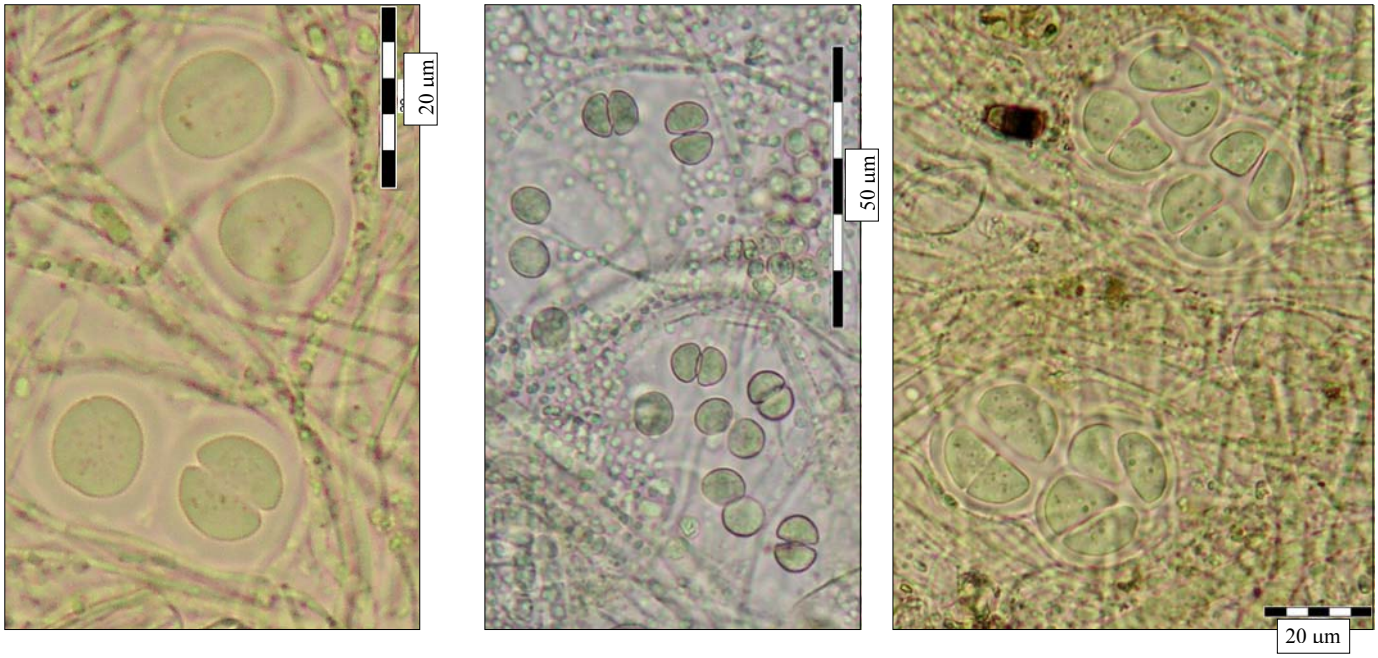
11. *Bacularia gracilis*



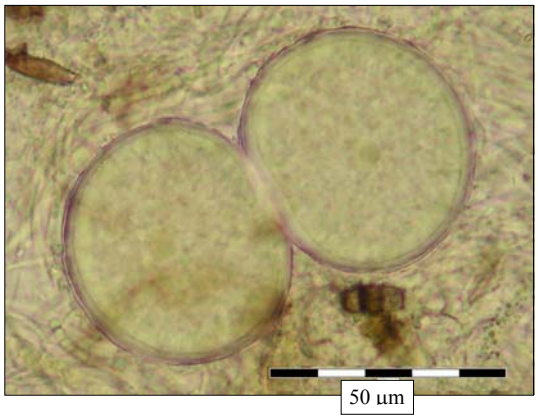


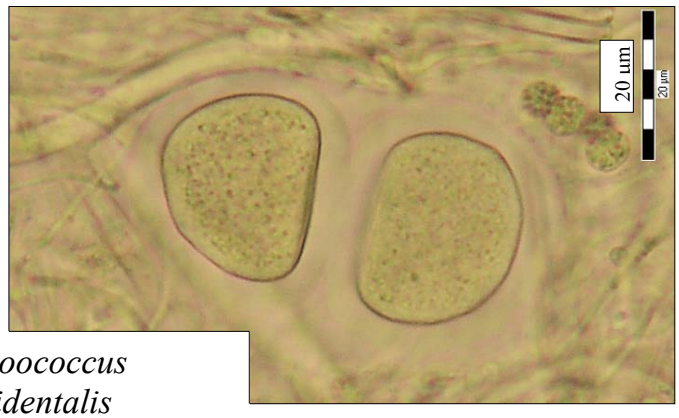


12. *Chlorogloea gardneri*

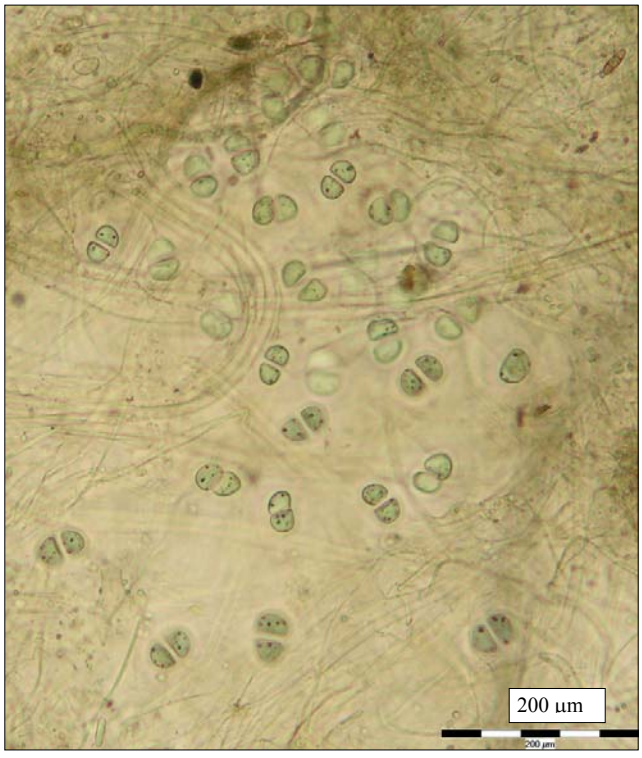


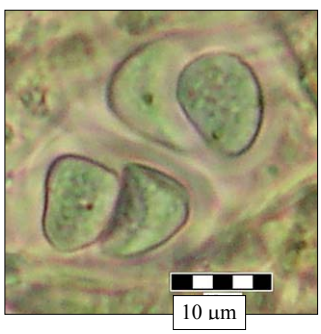
13. *Chroococcus mediocris*



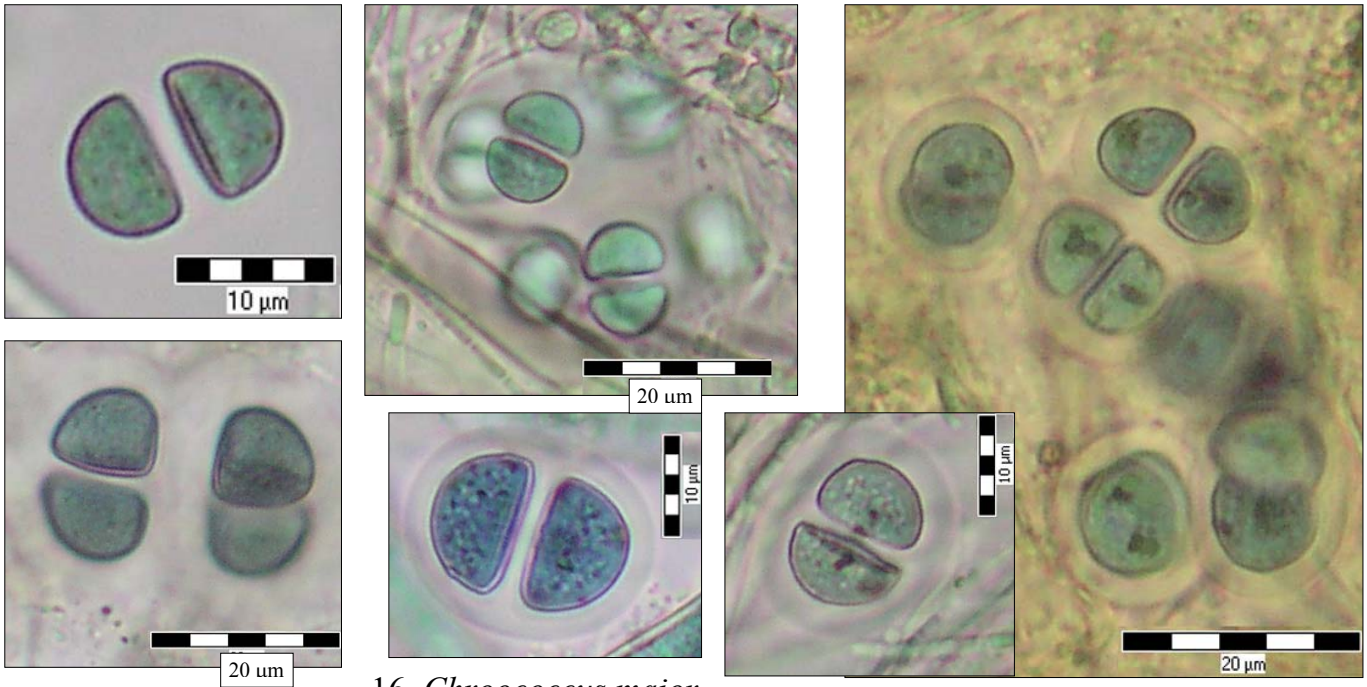


14. *Chroococcus occidentalis*

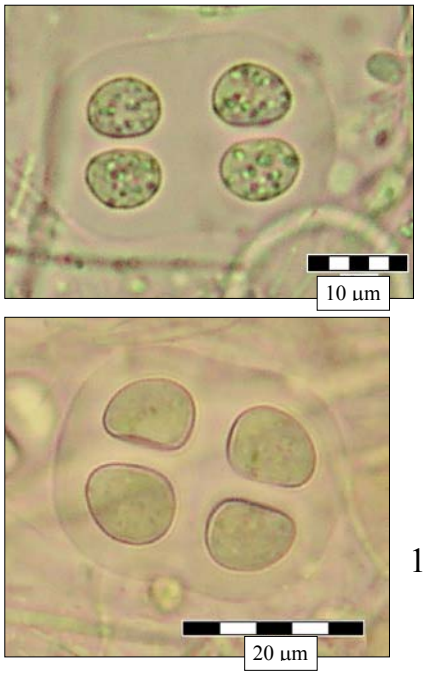




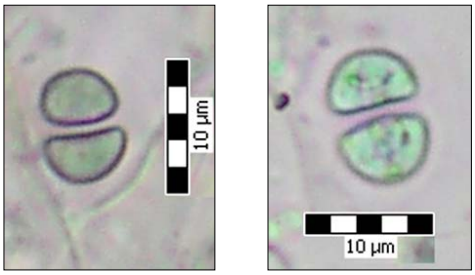
15. *Chroococcus deltoides*



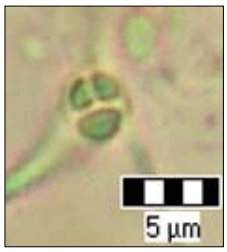
16. *Chroococcus major*



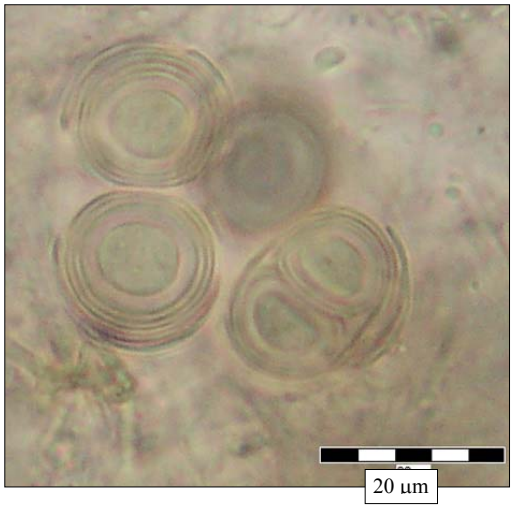
17. *Chroococcus mipitanensis*

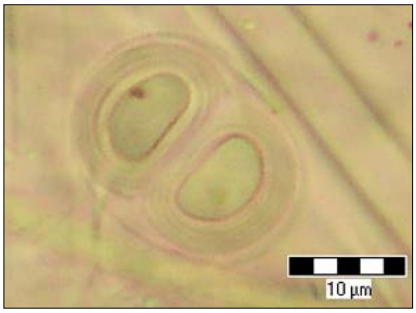


18. *Chroococcus* cf. *minutus*

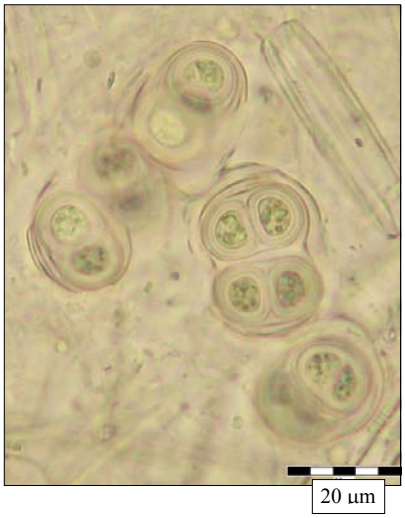


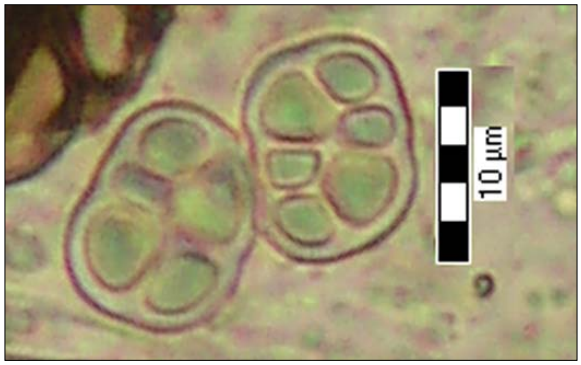
19. *Chroococcus* cf. *minutissimus*



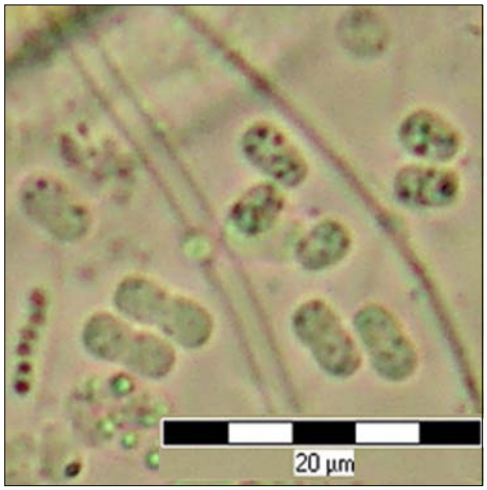


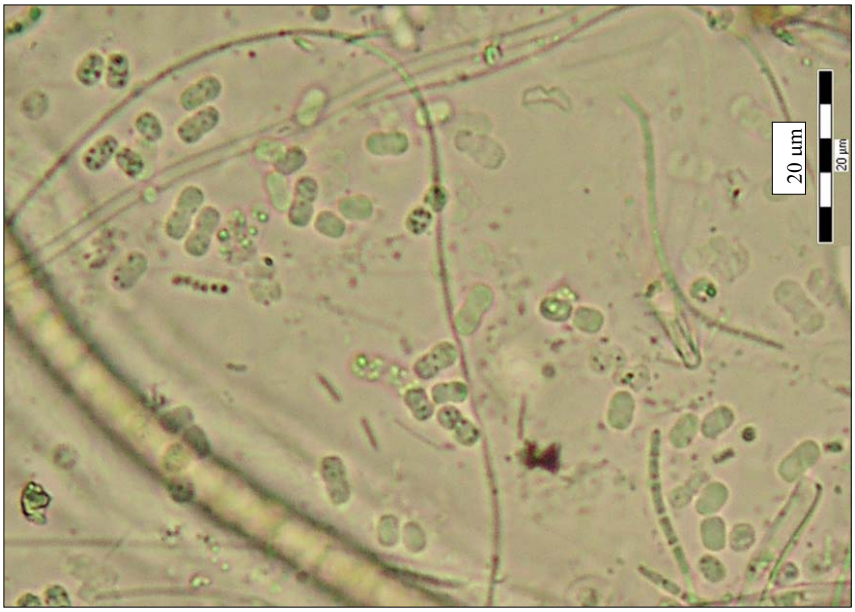
20.*Chroococcus schizodermaticus*





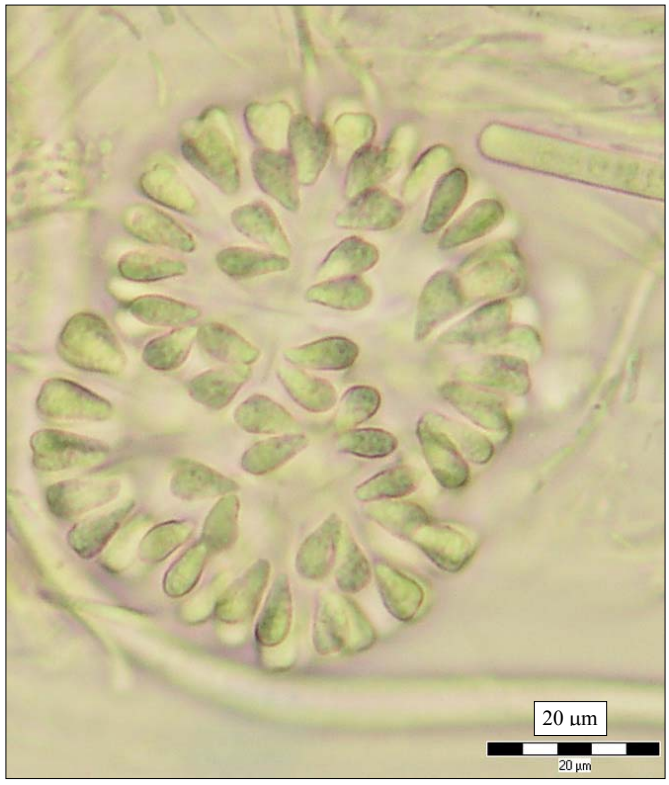
21. *Cyanosarcina* sp.

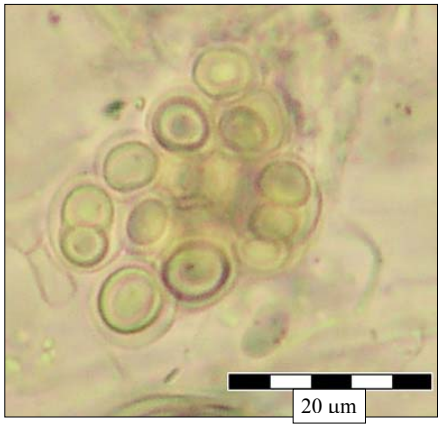




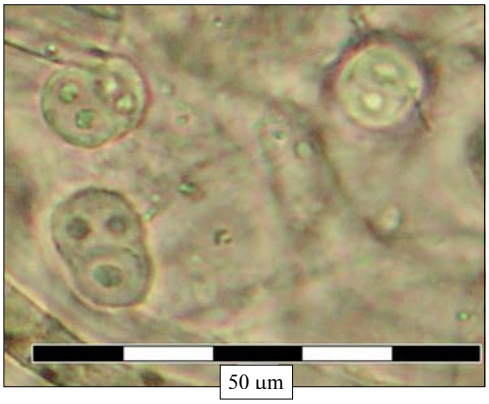
22. *Cyanothece* sp.

23. *Gomphosphaeria semen-vitis*

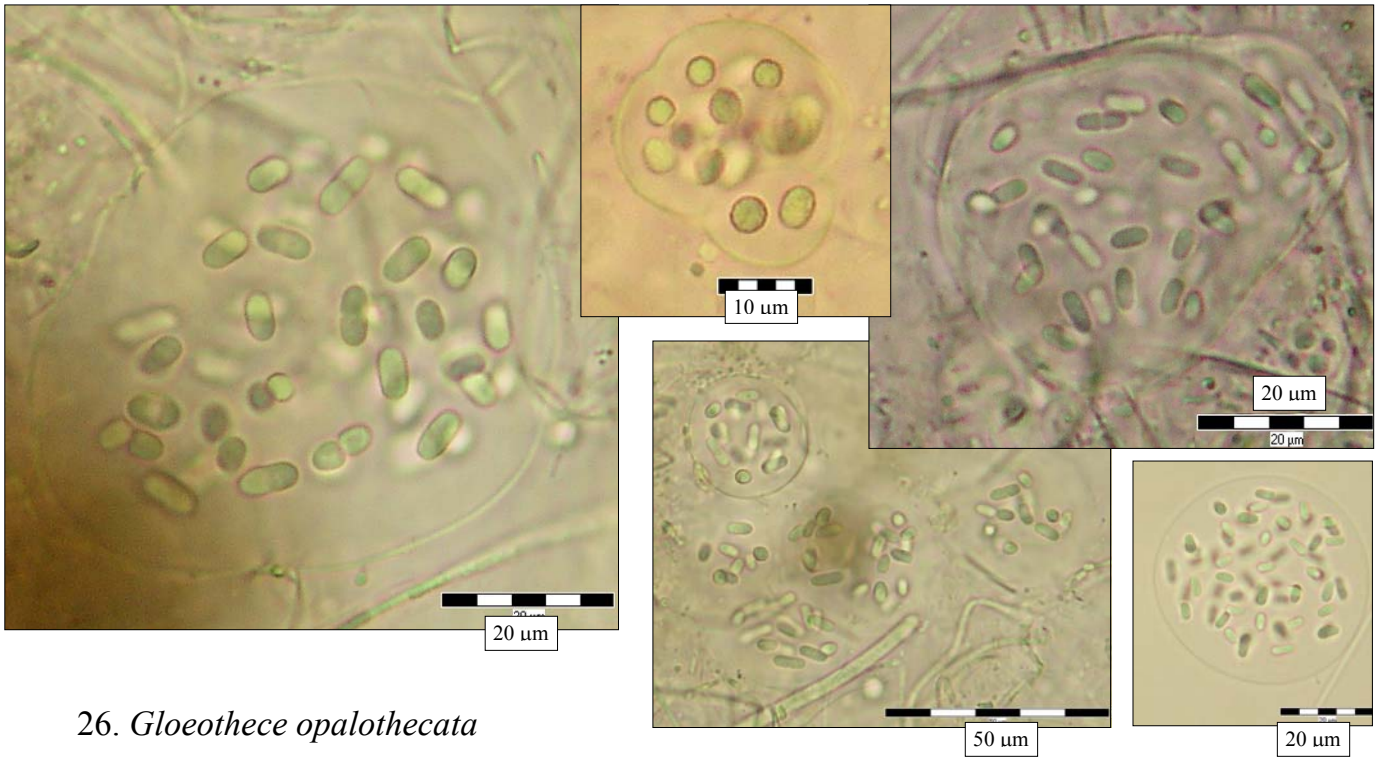




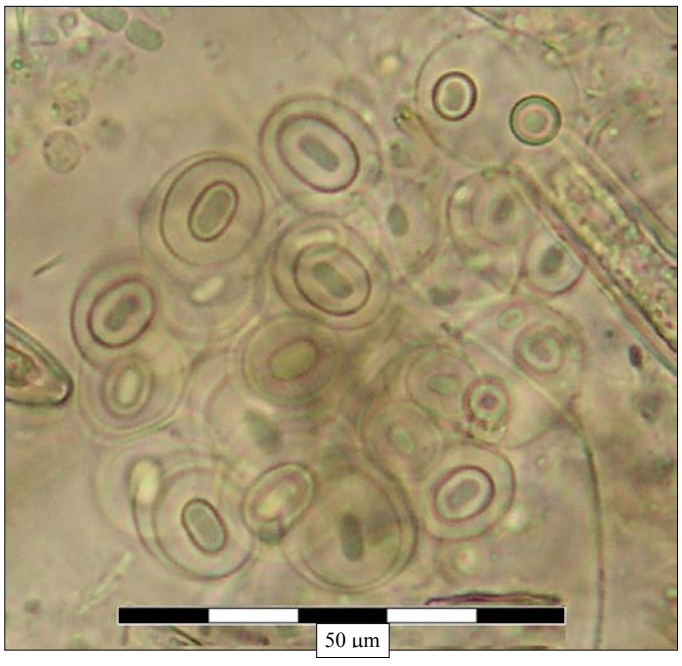
24. *Gloeocapsa* sp. (1)

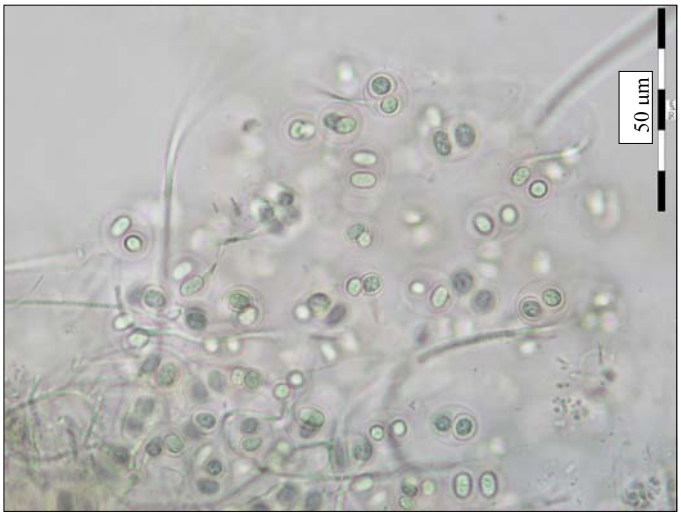


25. *Gloeocapsa* sp. (2)

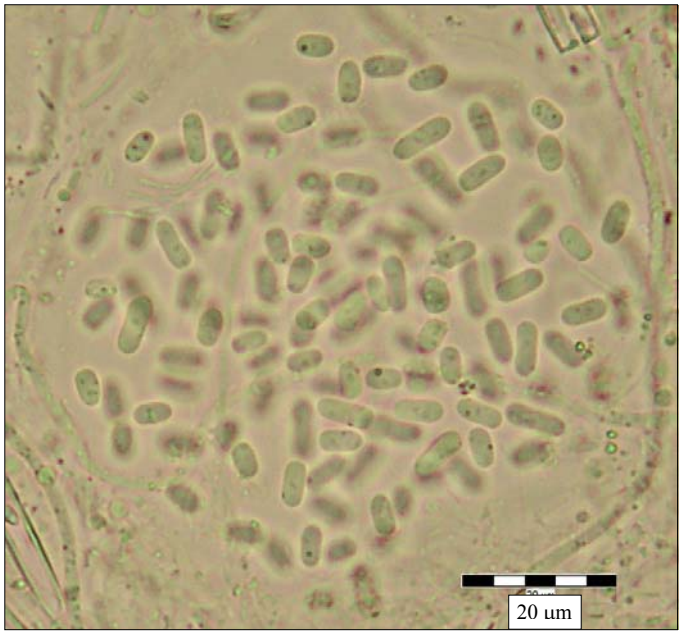


26. *Gloeothece opalothecata*

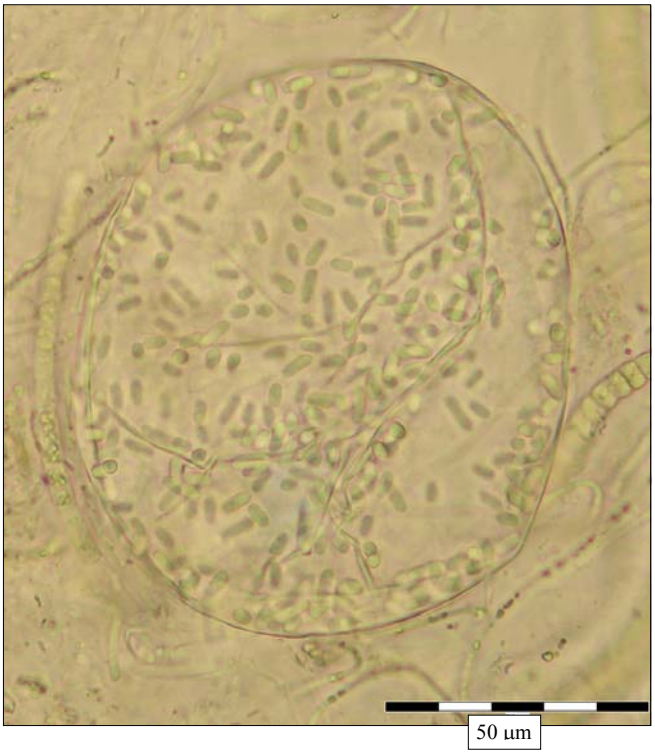


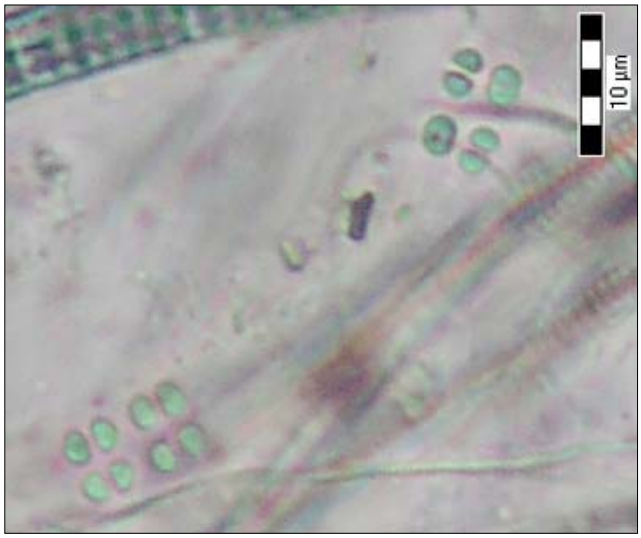


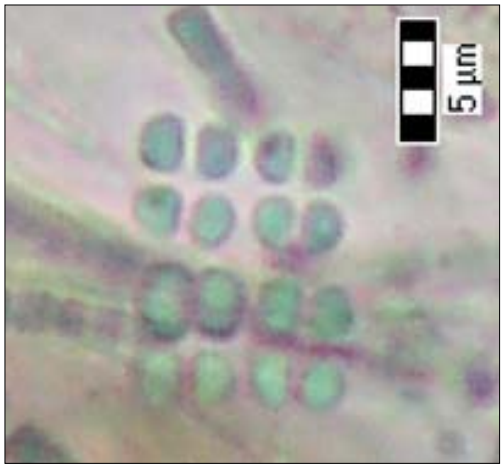
27. *Gloeothece membranacea*



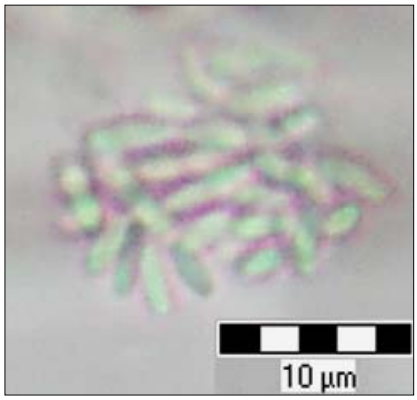
28. *Lemmermanniella uliginosa*



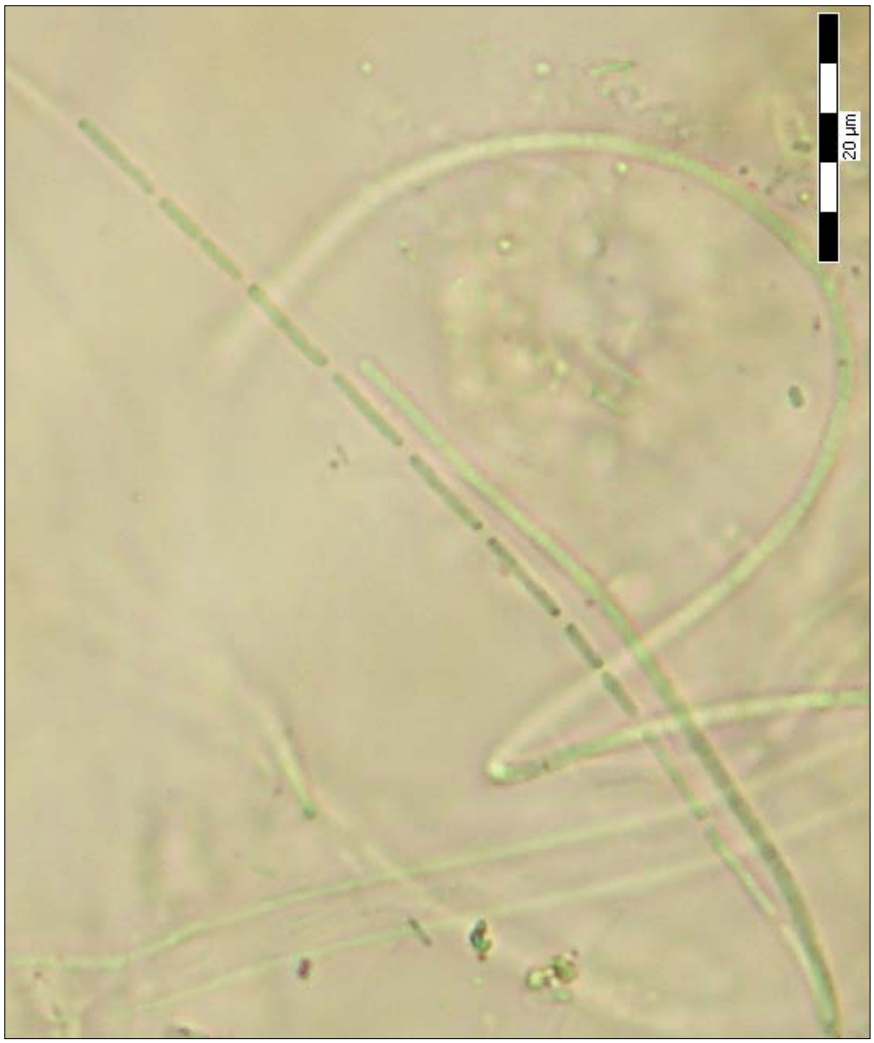


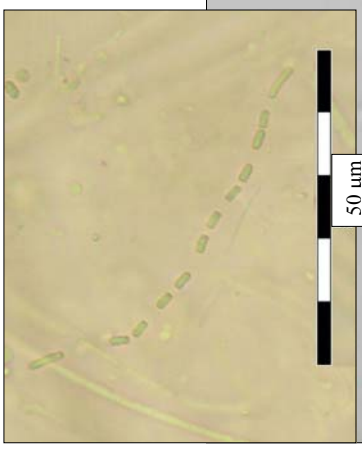


29. *Merismopedia punctata*

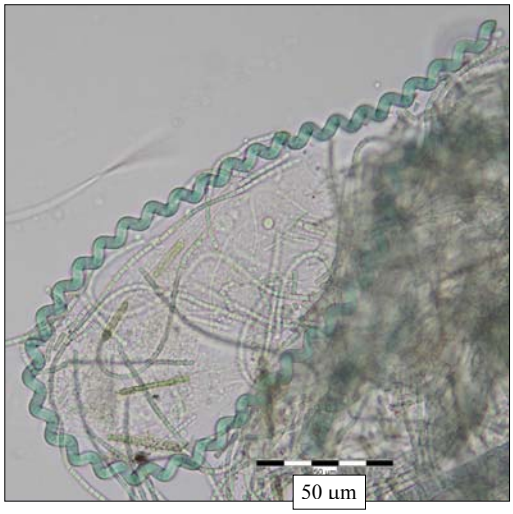


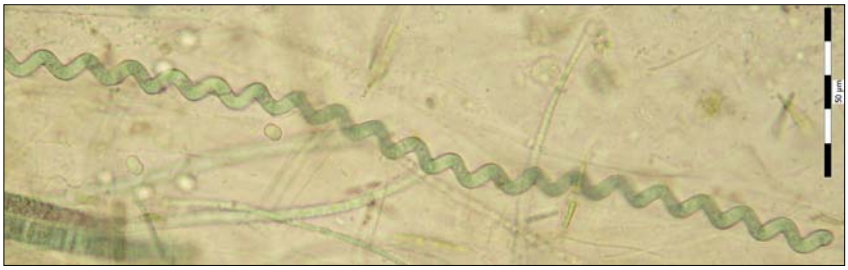
30. *Rhabdogloea subtropica*



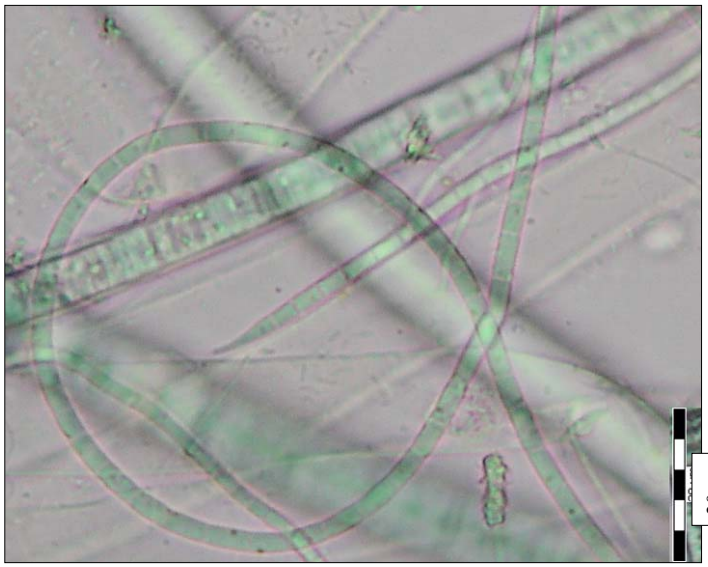


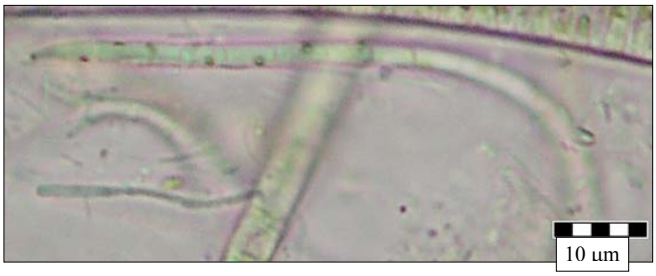
31. *Wolskyella* sp.



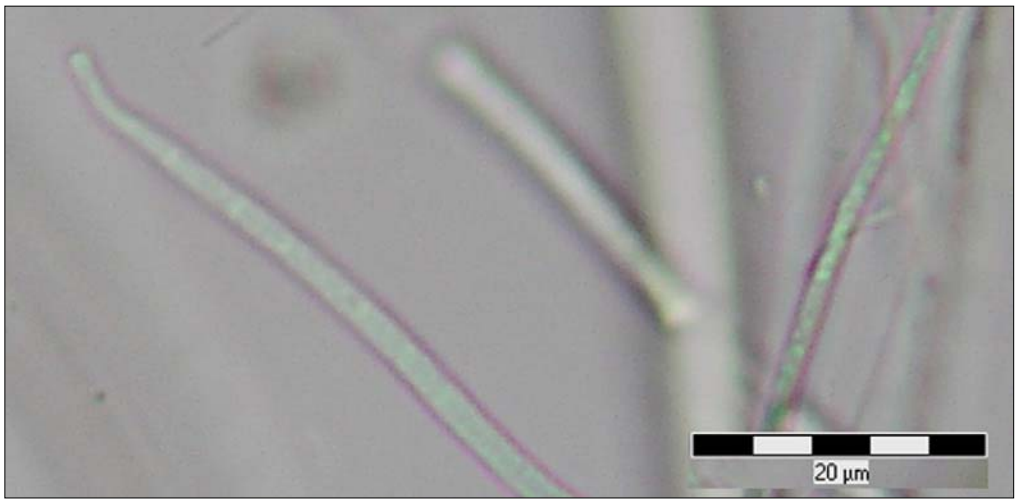


32. *Arthrospira jenneri*

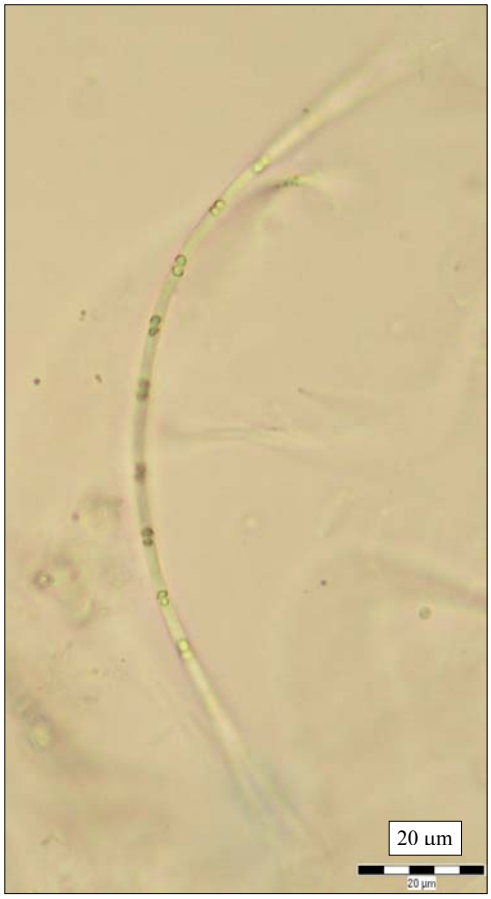




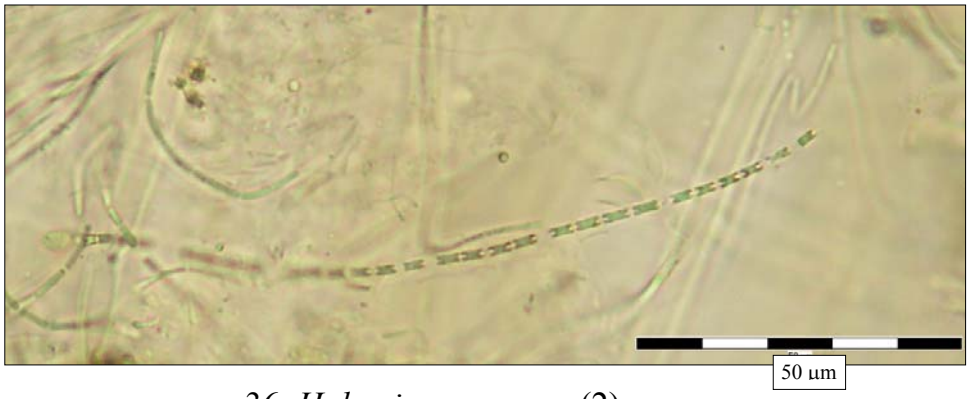
33. *Geitlerinema earlei*



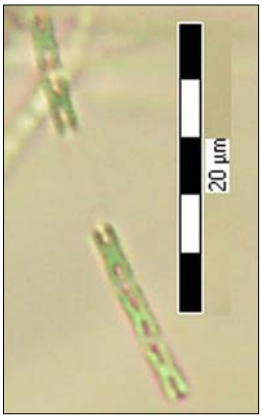
34. *Geitlerinema splendidum*



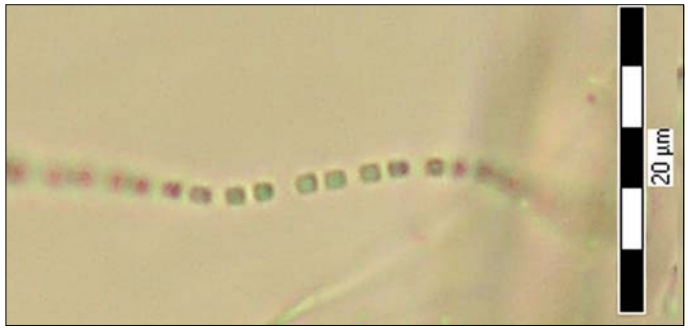
35. *Halomicronema* sp. (1)



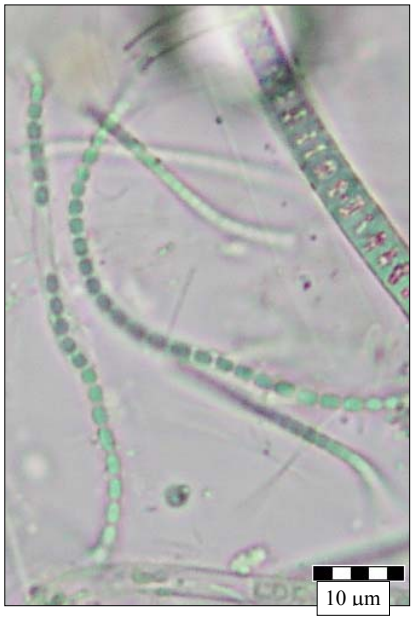
36. *Halomicronema* sp. (2)

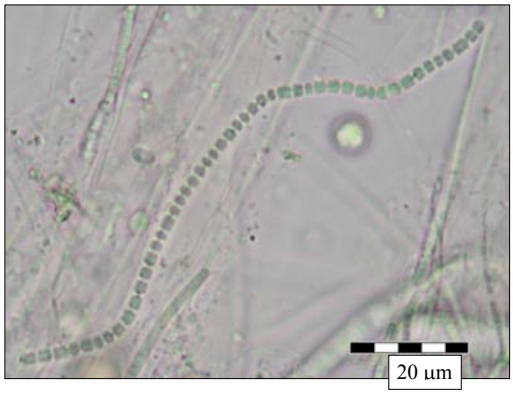


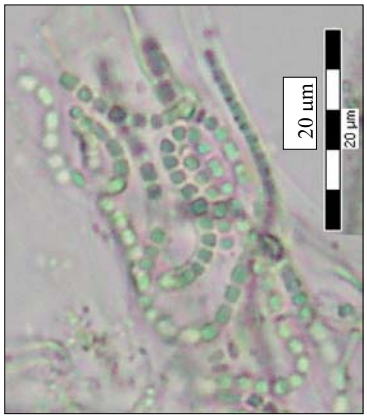
37. *Komvophoron* sp. (1)

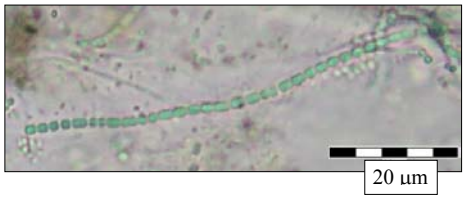


38. *Komvophoron* sp. (2)

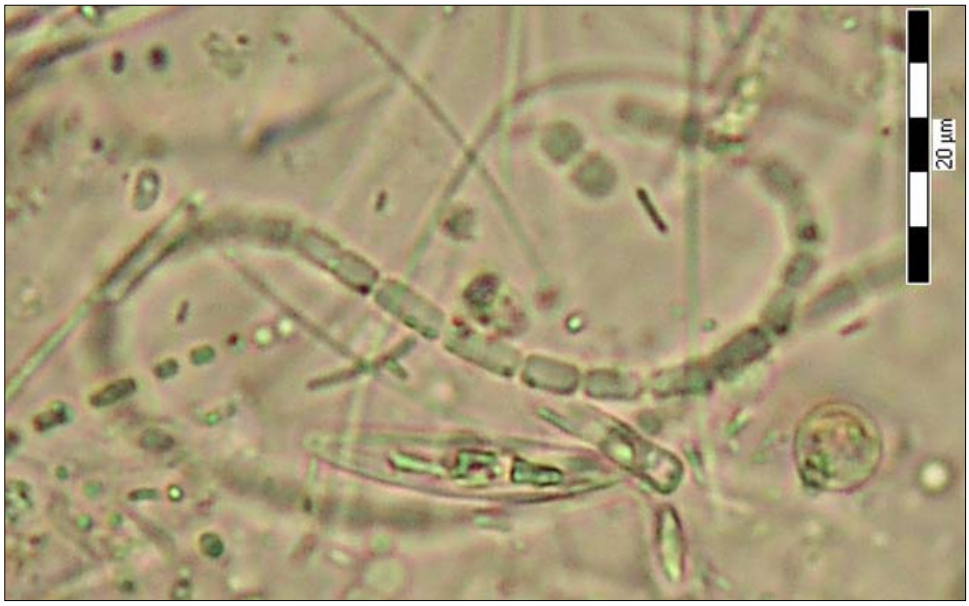




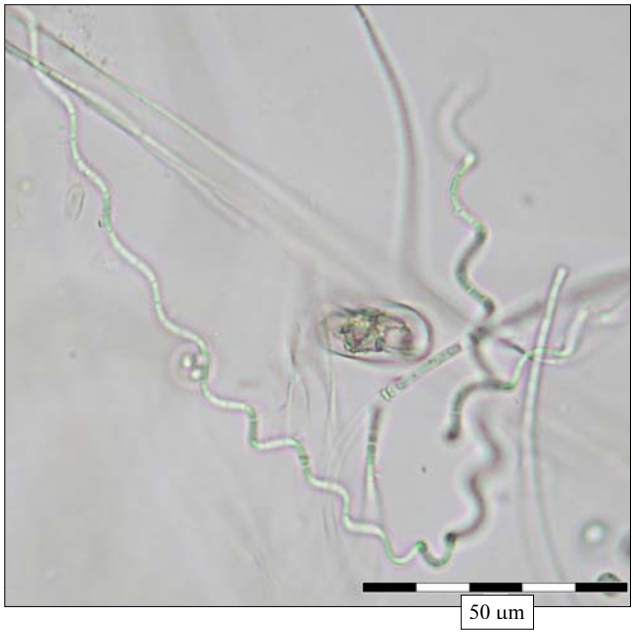


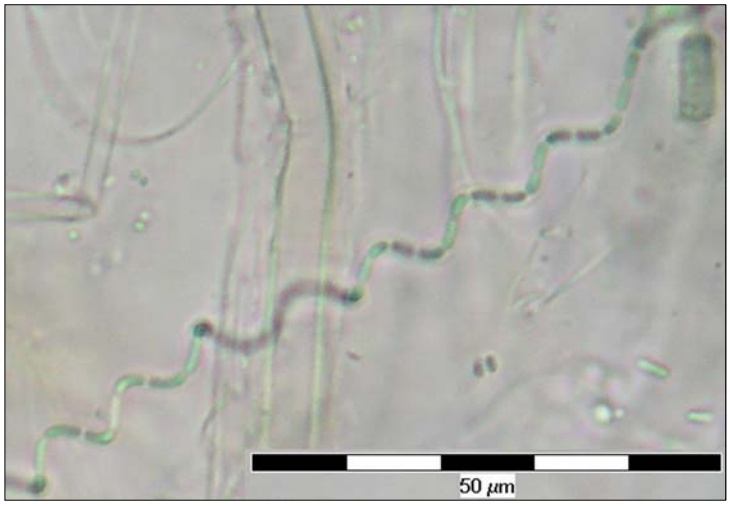


39. *Komvophoron* cf. *minutum*

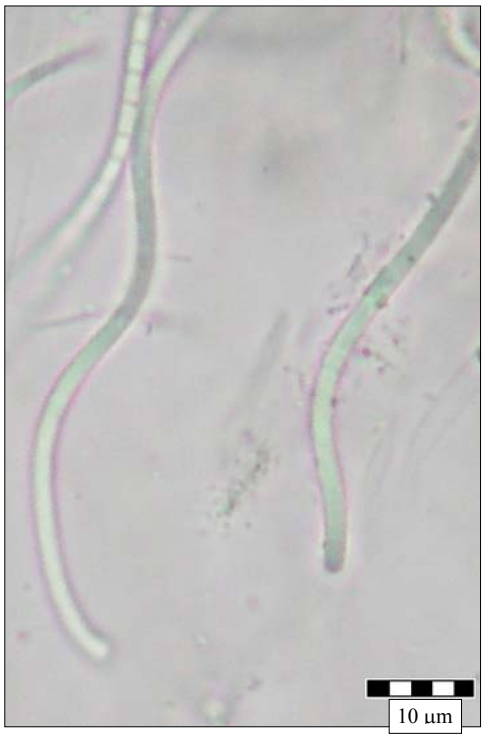


40. *Komvophoron* sp. (3)

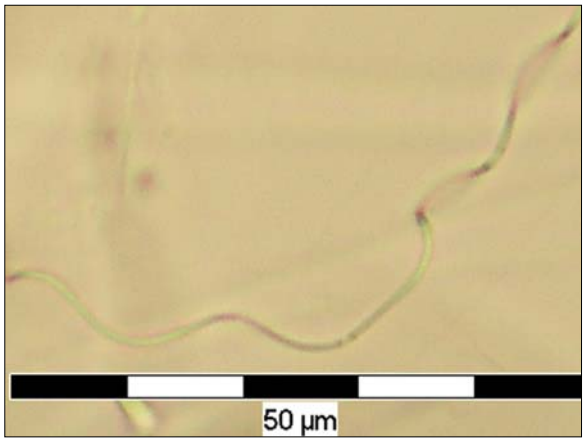


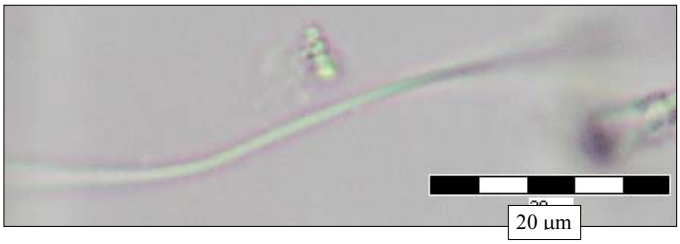


41. *Leptolyngbya lagerheimii*

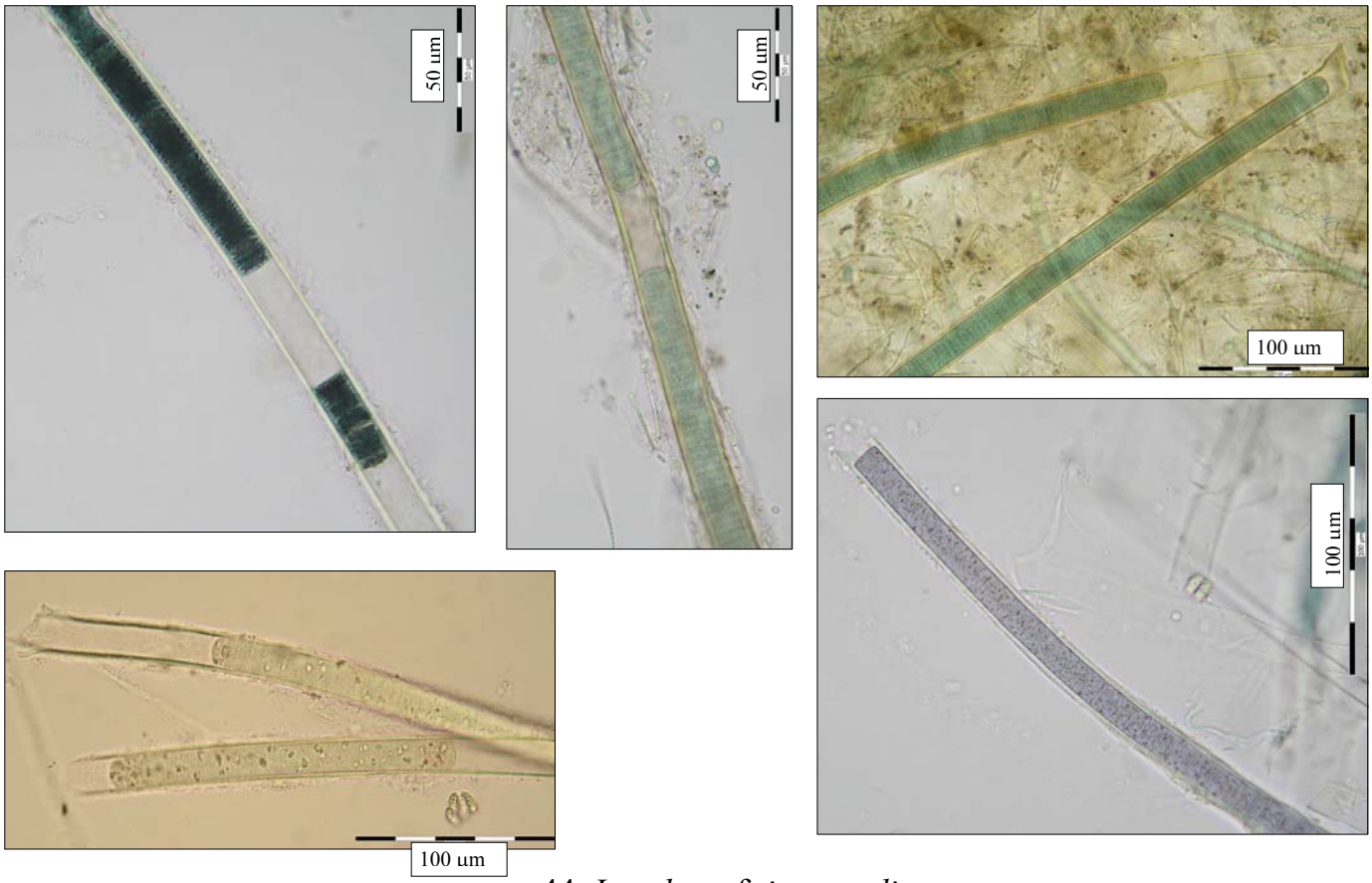


42. *Leptolyngbya* cf. *mucosa*

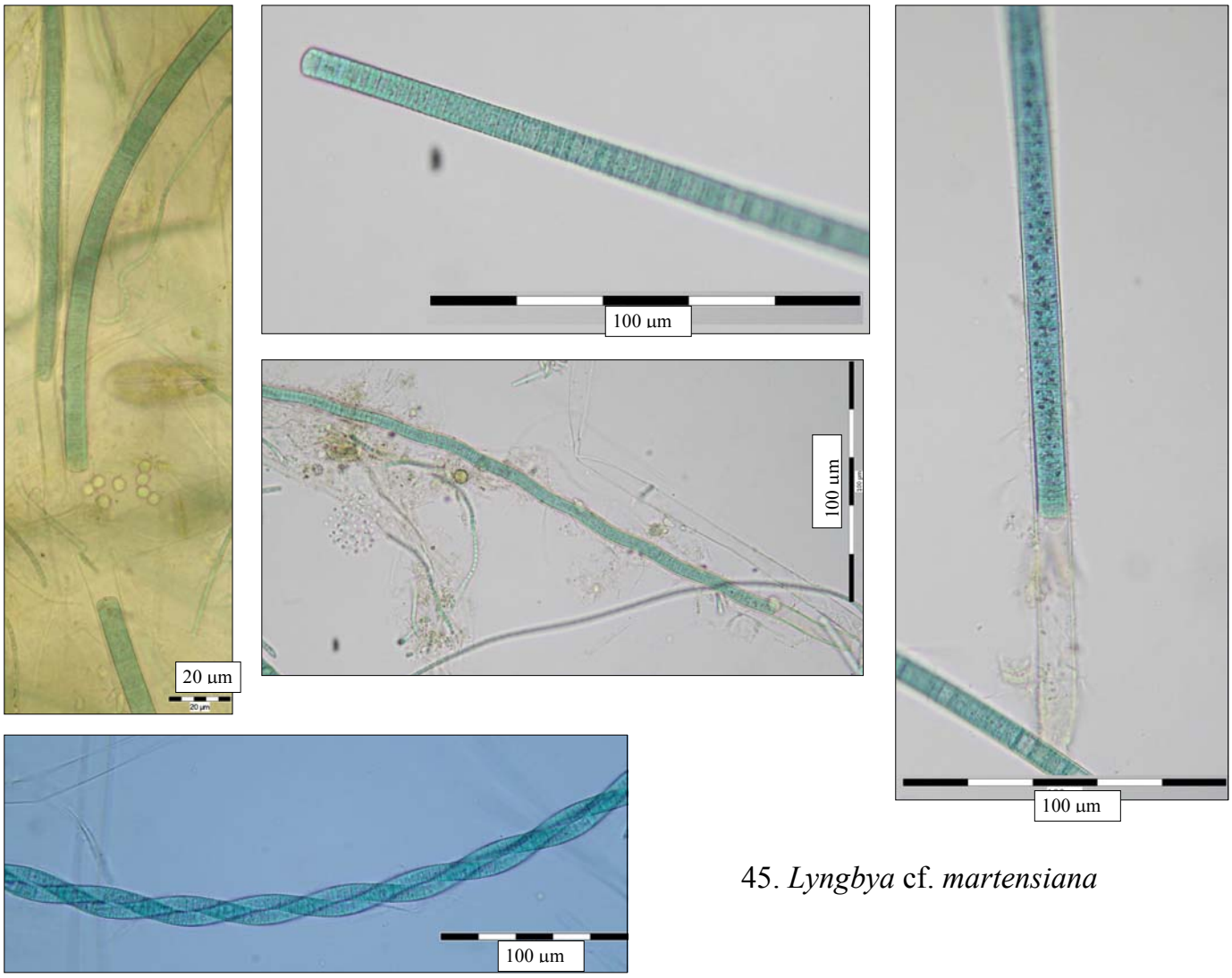




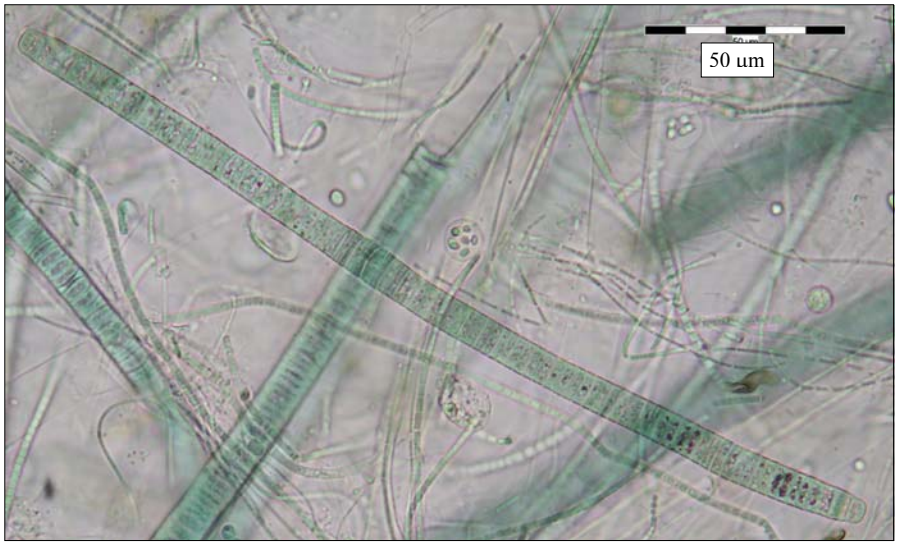
43. *Leptolyngbya* sp.

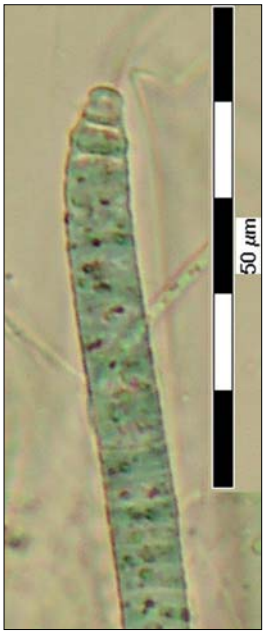


44. *Lyngbya* cf. *intermedia*

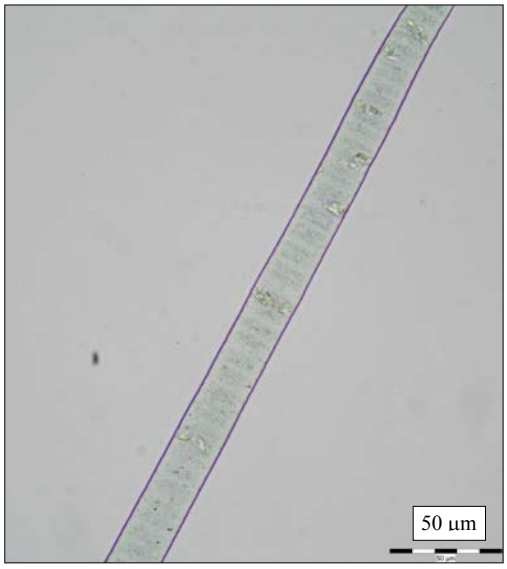


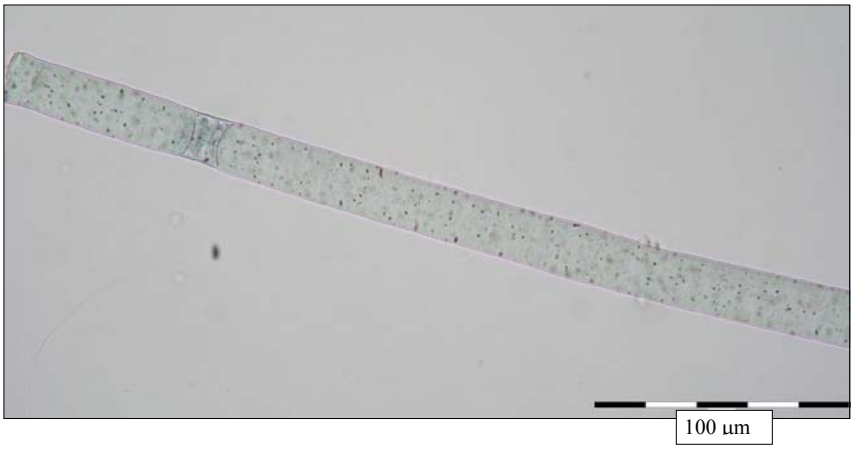
45. *Lyngbya* cf. *martensiana*



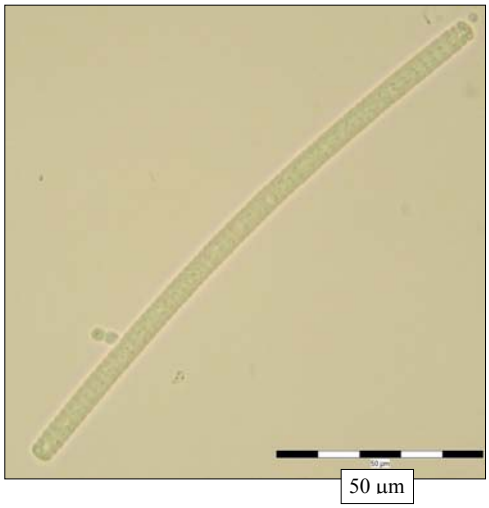


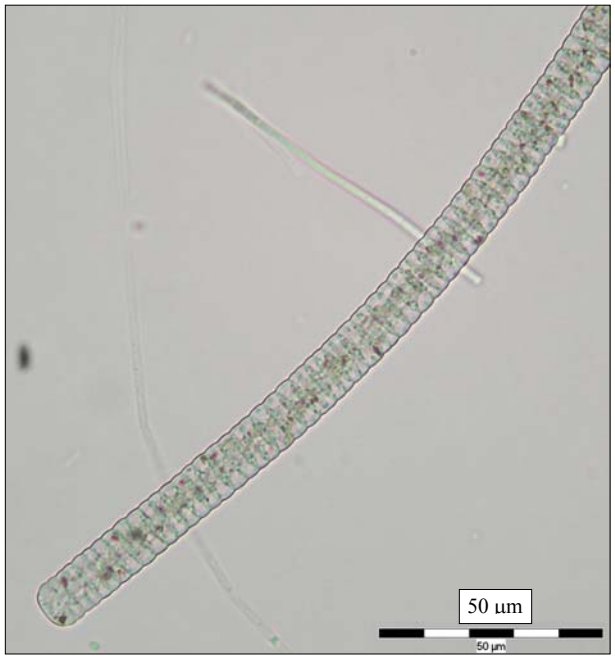
46. *Oscillatoria* cf. *anguina*



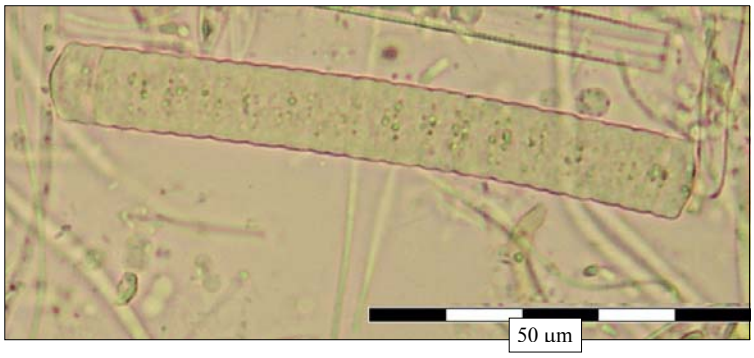


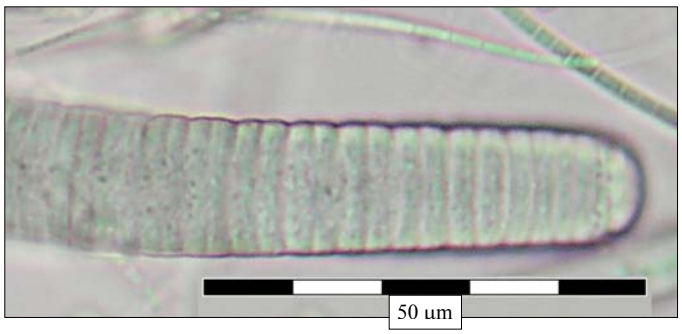
47. *Oscillatoria* cf. *obtusa*



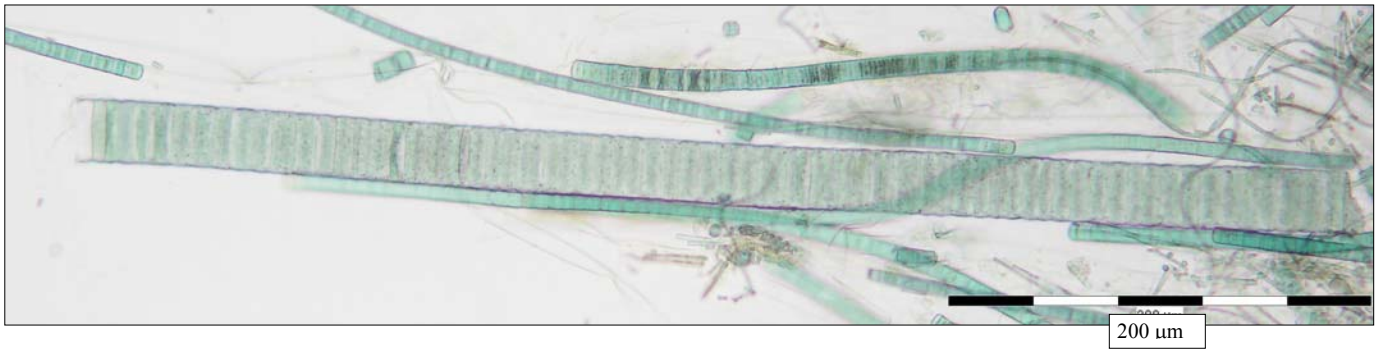


48. *Oscillatoria crassa*

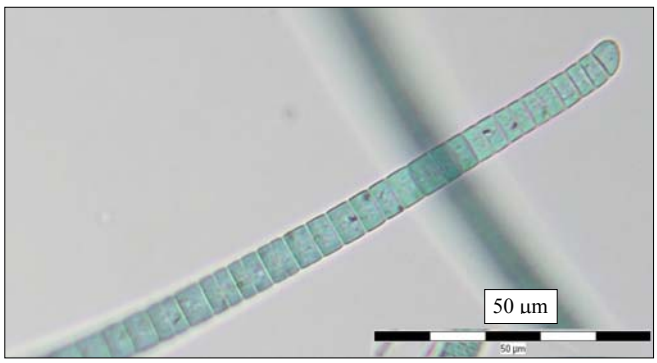




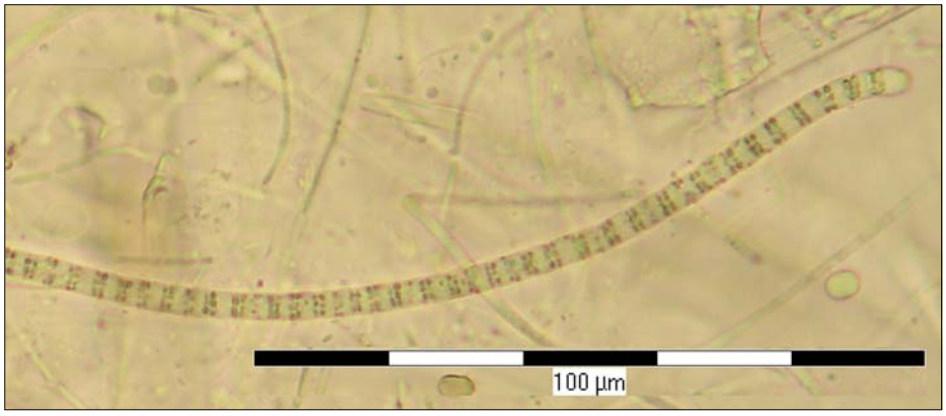
49. *Oscillatoria* cf. *sancta*



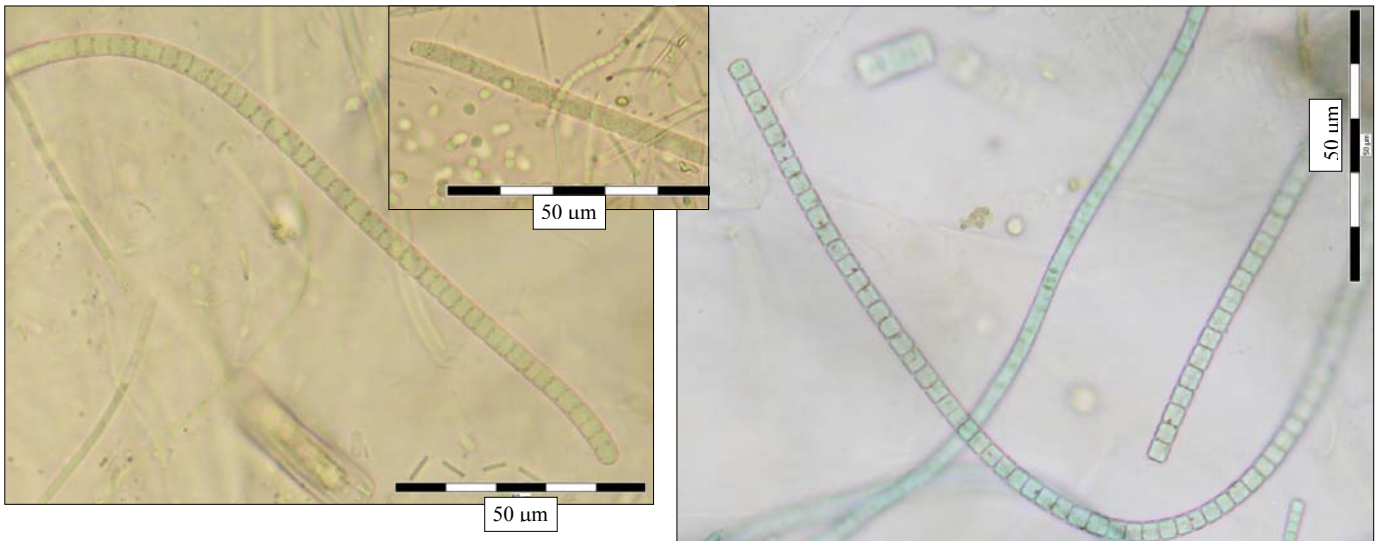
50. *Oscillatoria* sp.



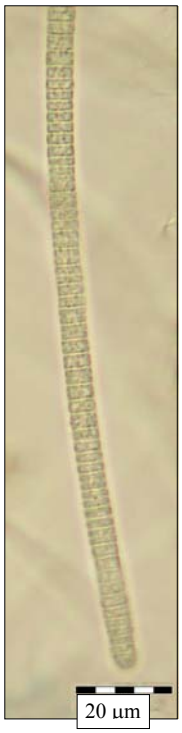
51. *Phormidium chalybeum*



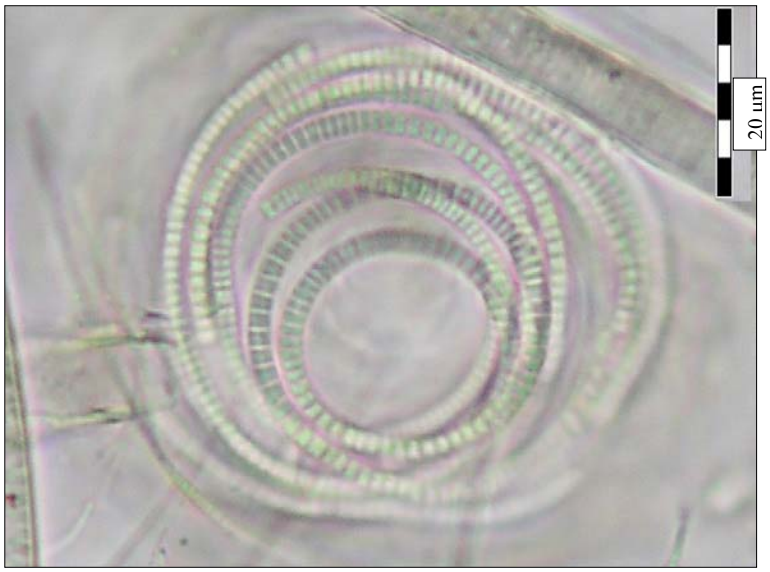
52. *Phormidium granulatum*



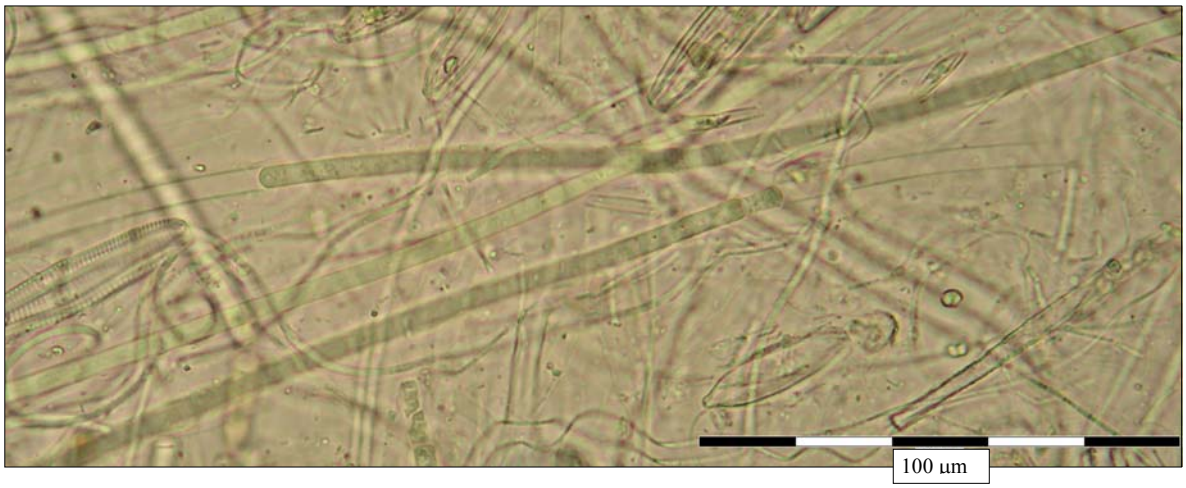
53. *Phormidium* cf. *hamelii*



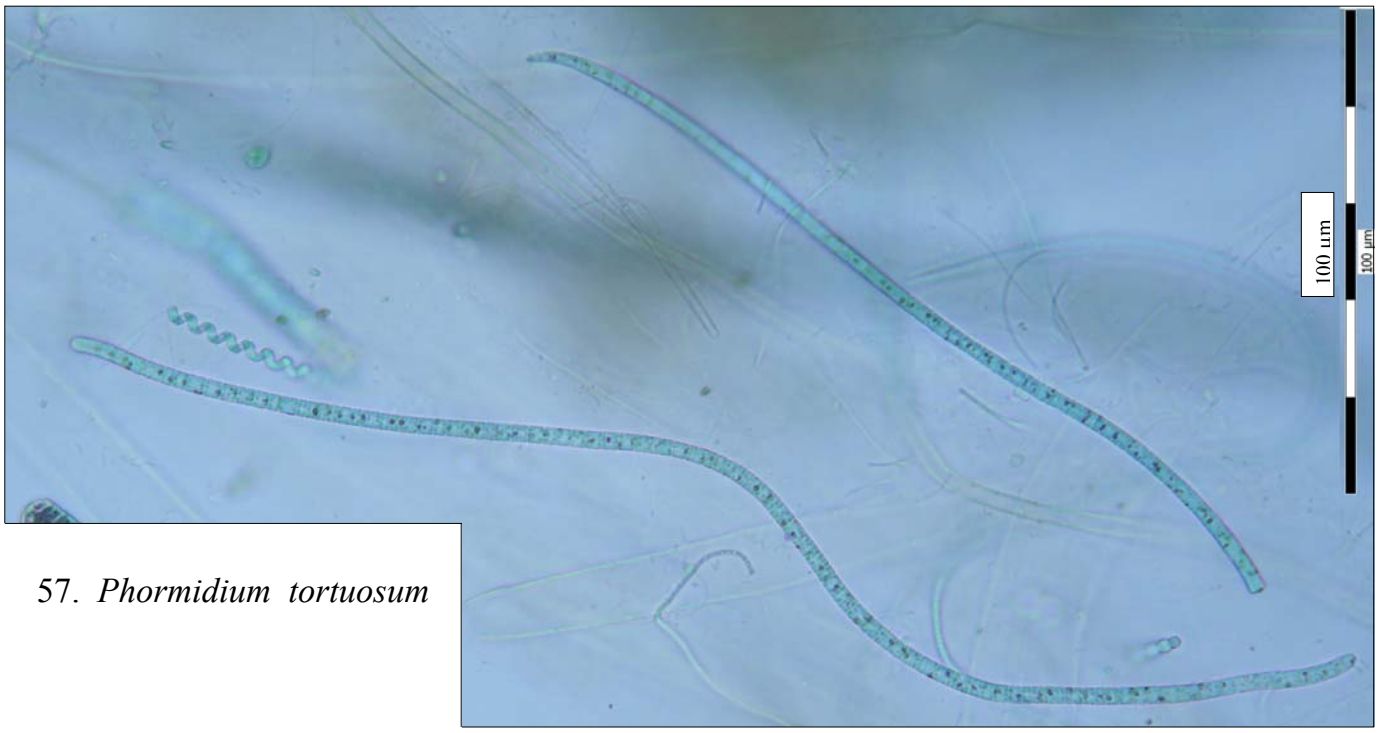
54. *Phormidium* cf. *articulatum*



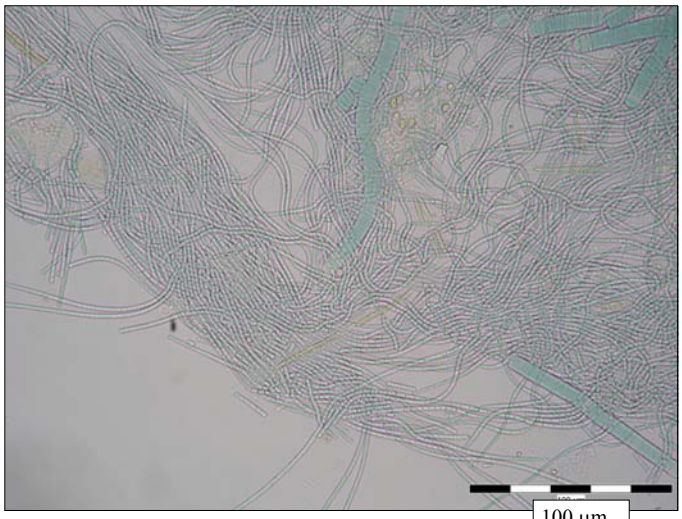
55. *Phormidium articulatum*

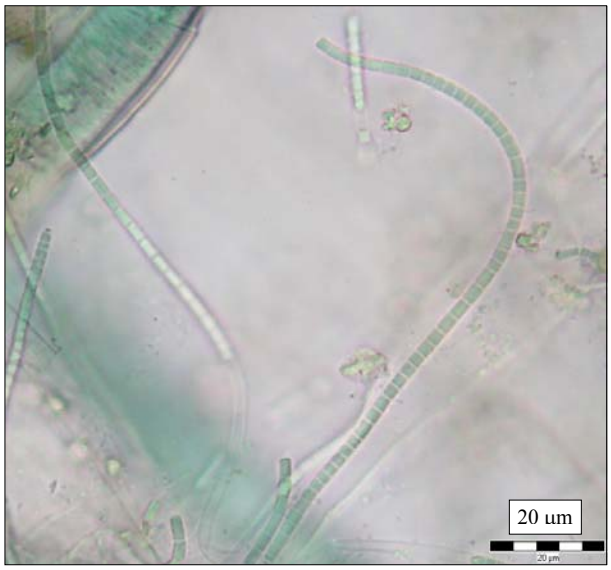


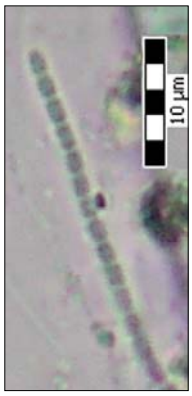
56. *Phormidium taylori*

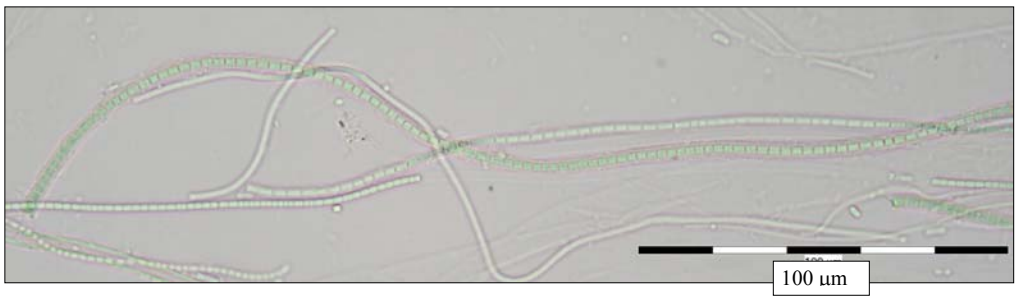


57. *Phormidium tortuosum*



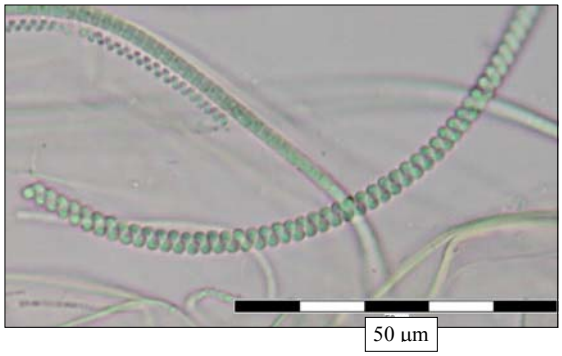




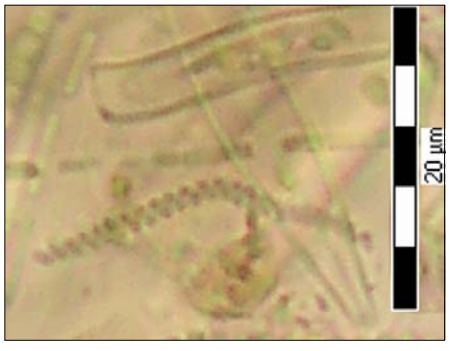


58. *Pseudanabaena* sp.

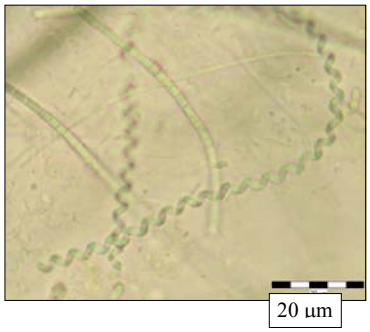
59. *Spirulina laxissima*



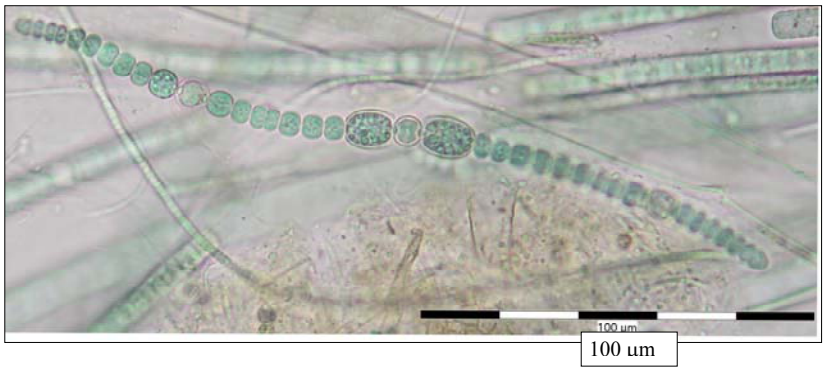
60. *Spirulina subsalsa*

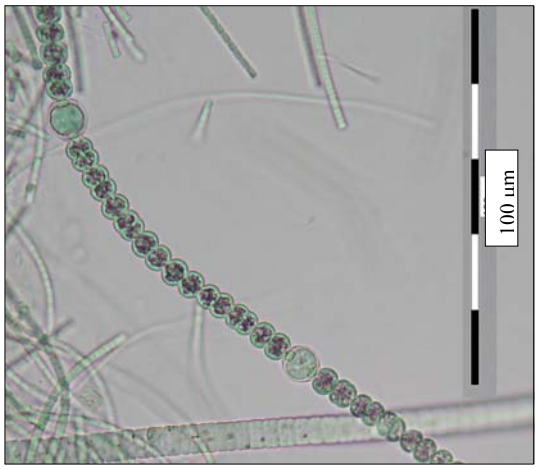


61. *Spirulina tenerrima*

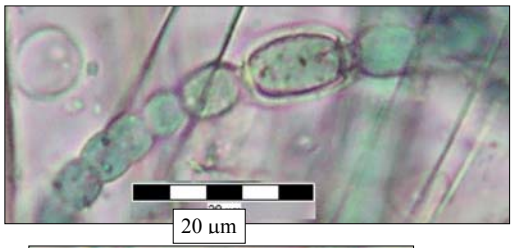


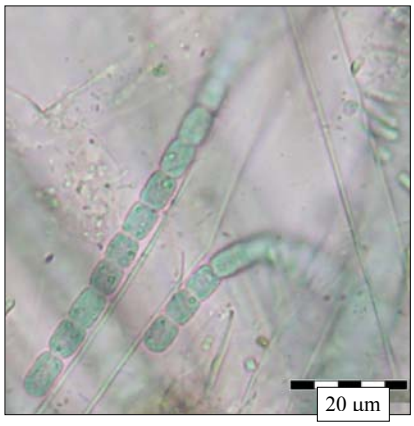
62. *Spirulina meneghiniana*



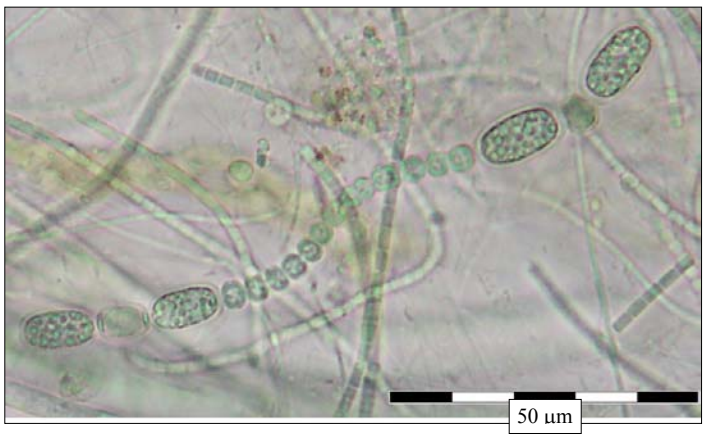


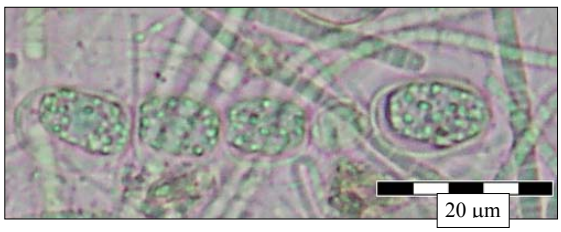
63. *Anabaena* cf. *ambigua*



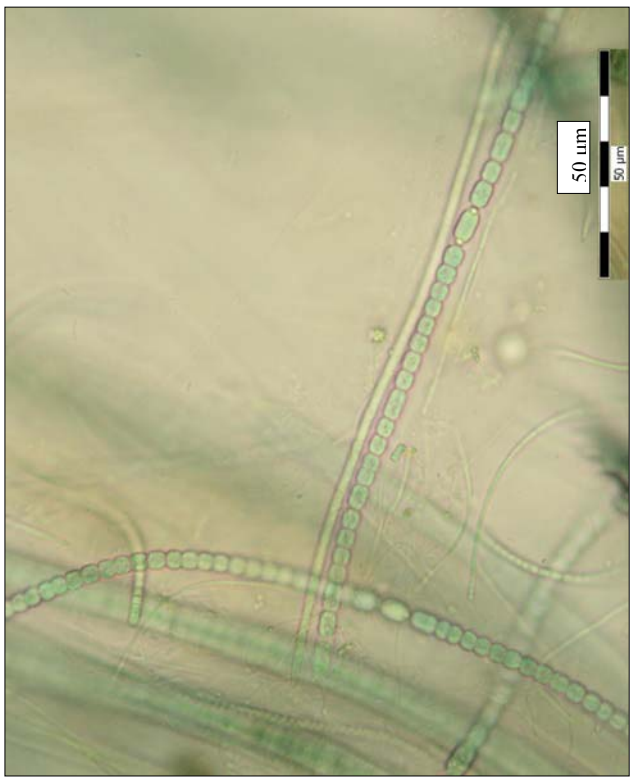


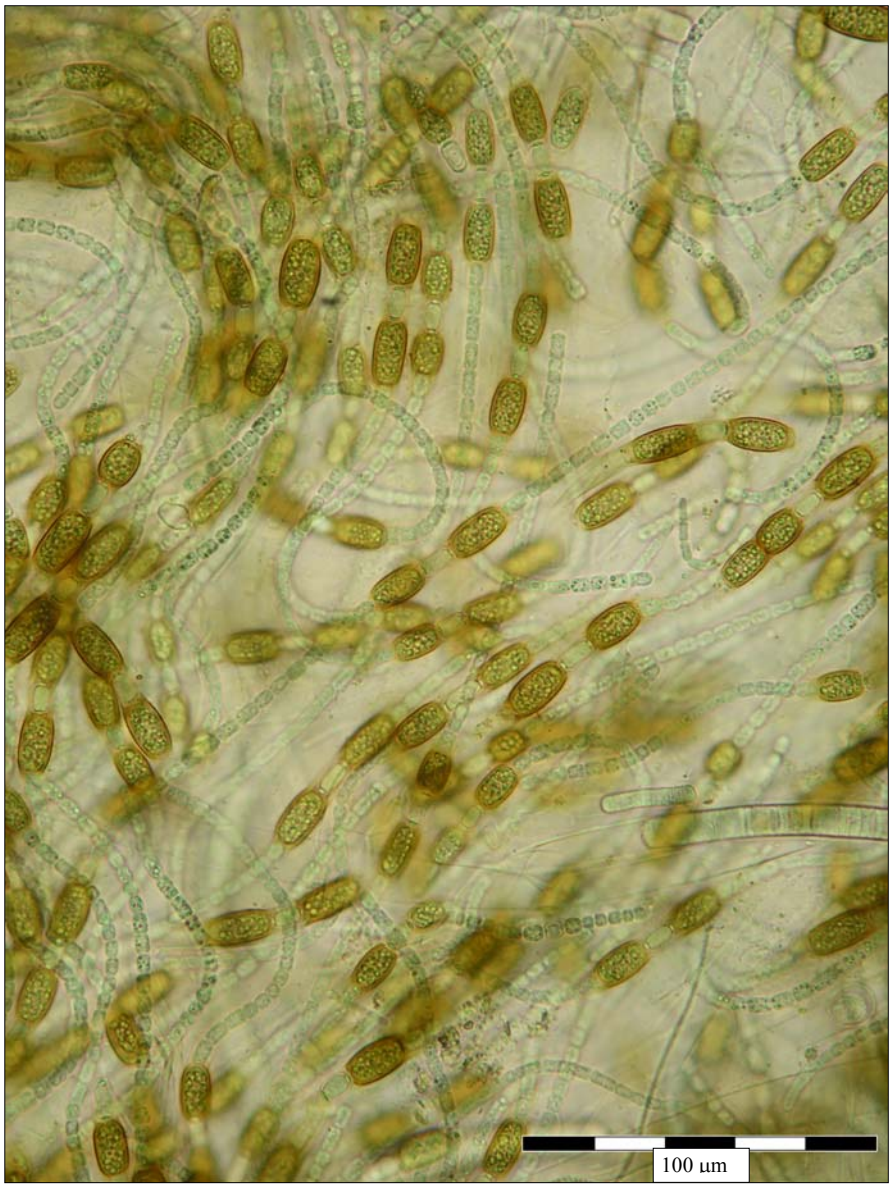
64. *Anabaena fuellebornii*

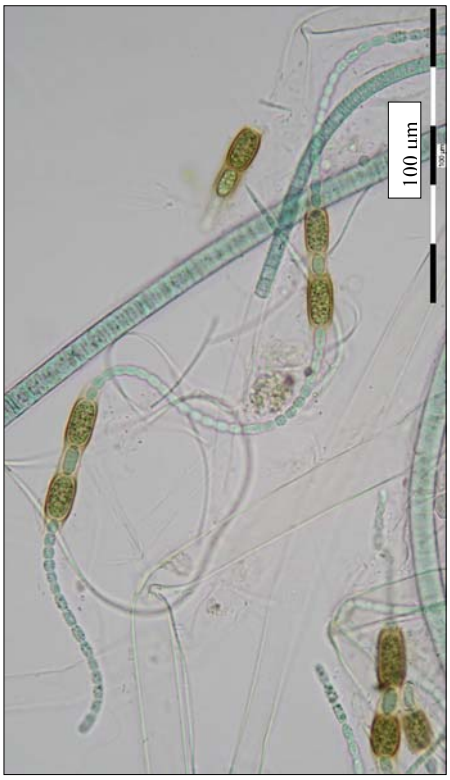


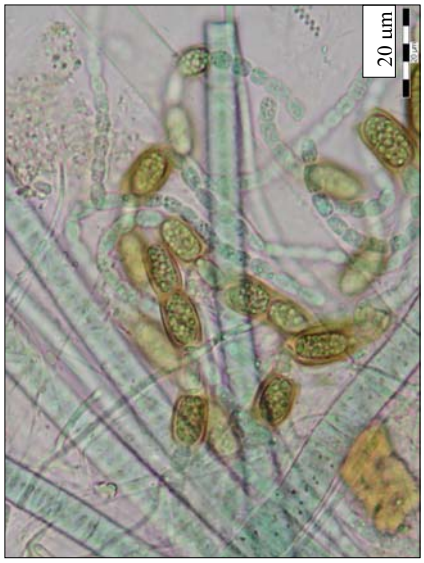


65. *Anabaena iyengarii*

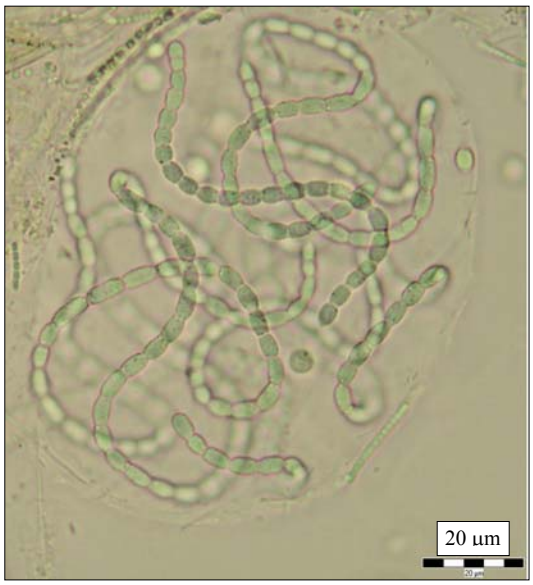




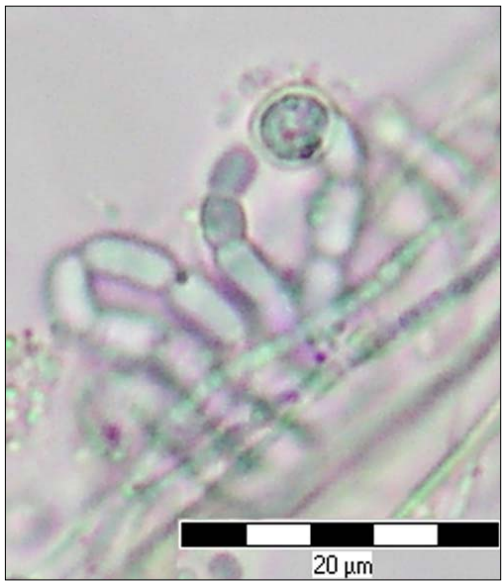


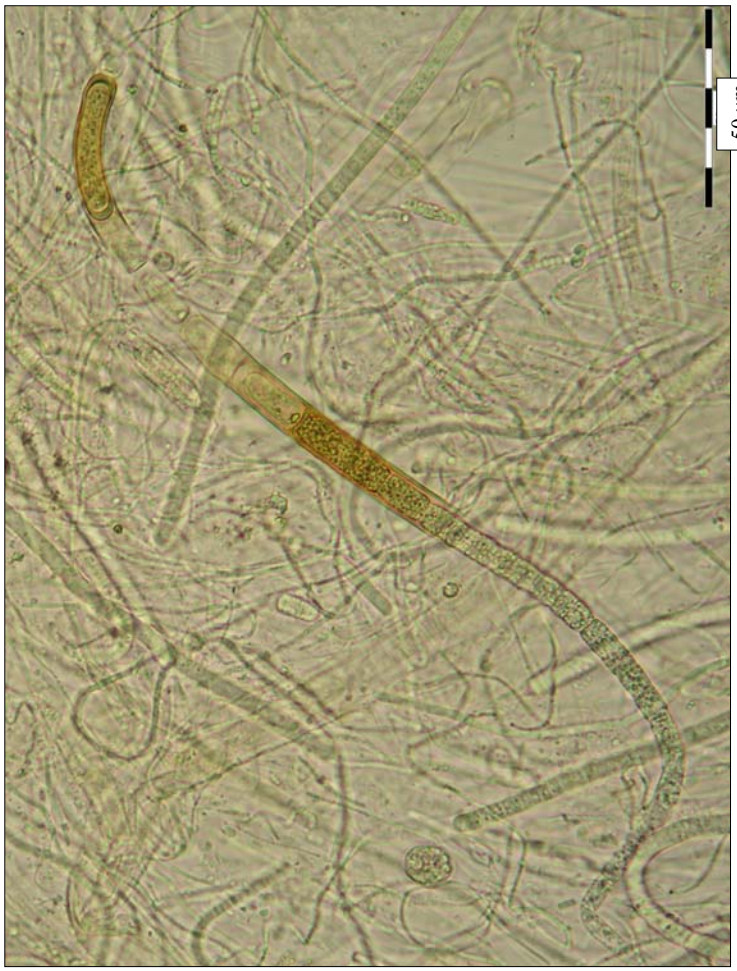


66. *Anabaena* sp.

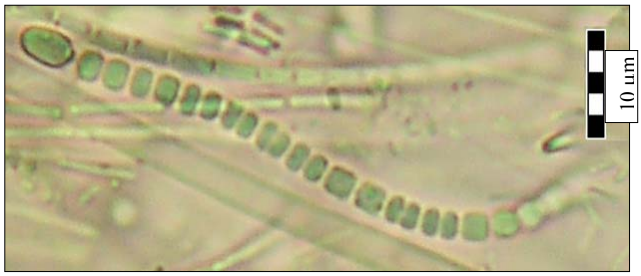


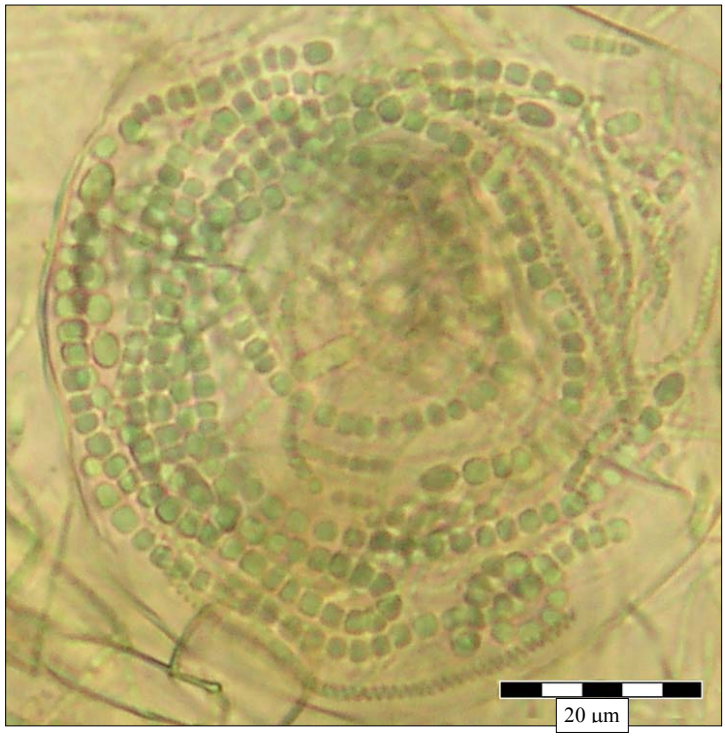
67. *Anabaena* cf. *reniformis*



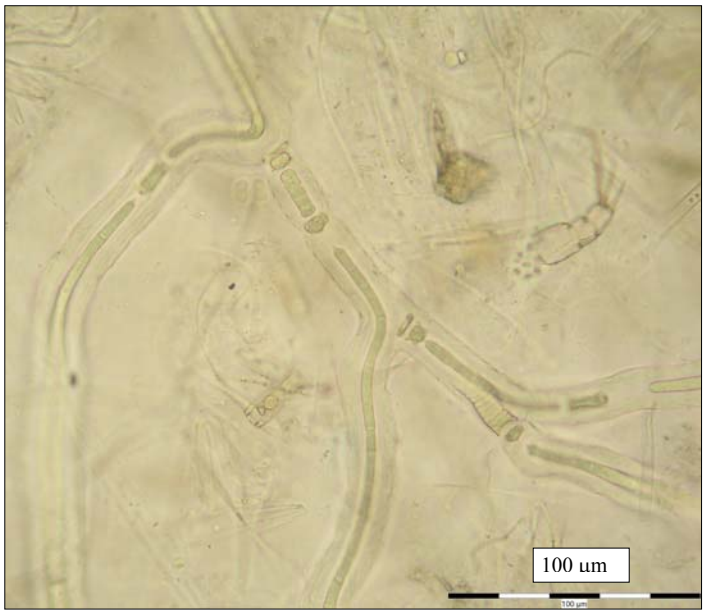


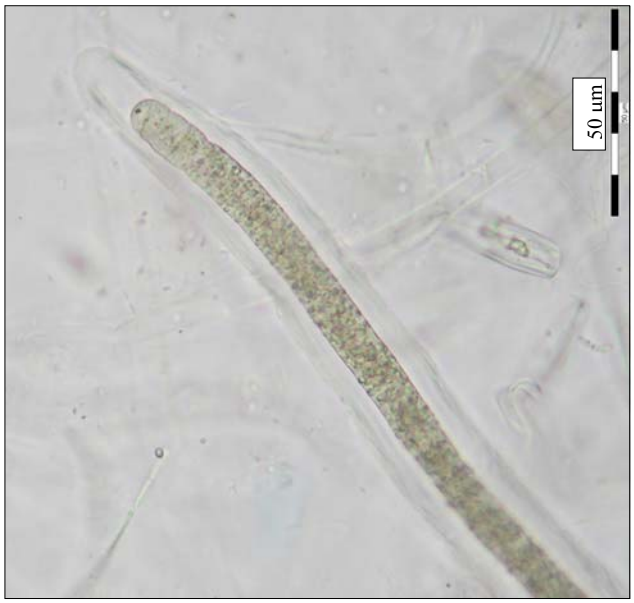
68. *Calothrix* sp.

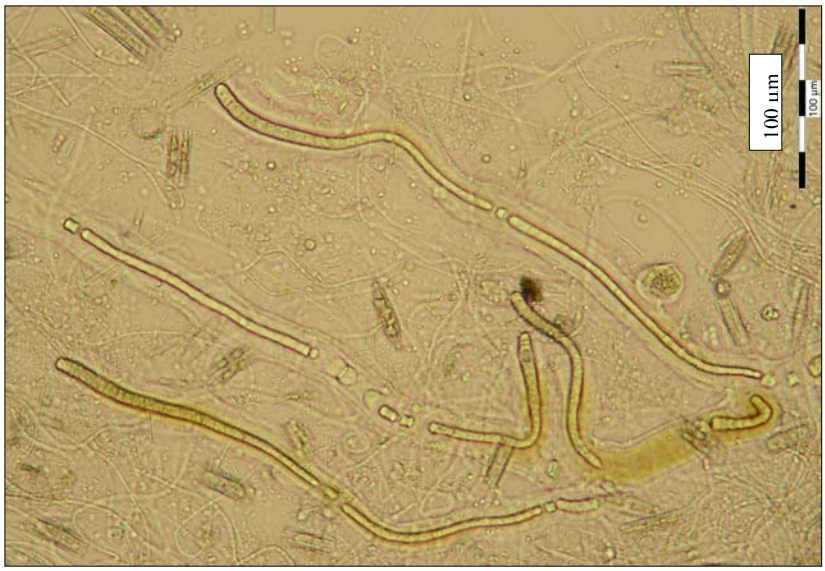




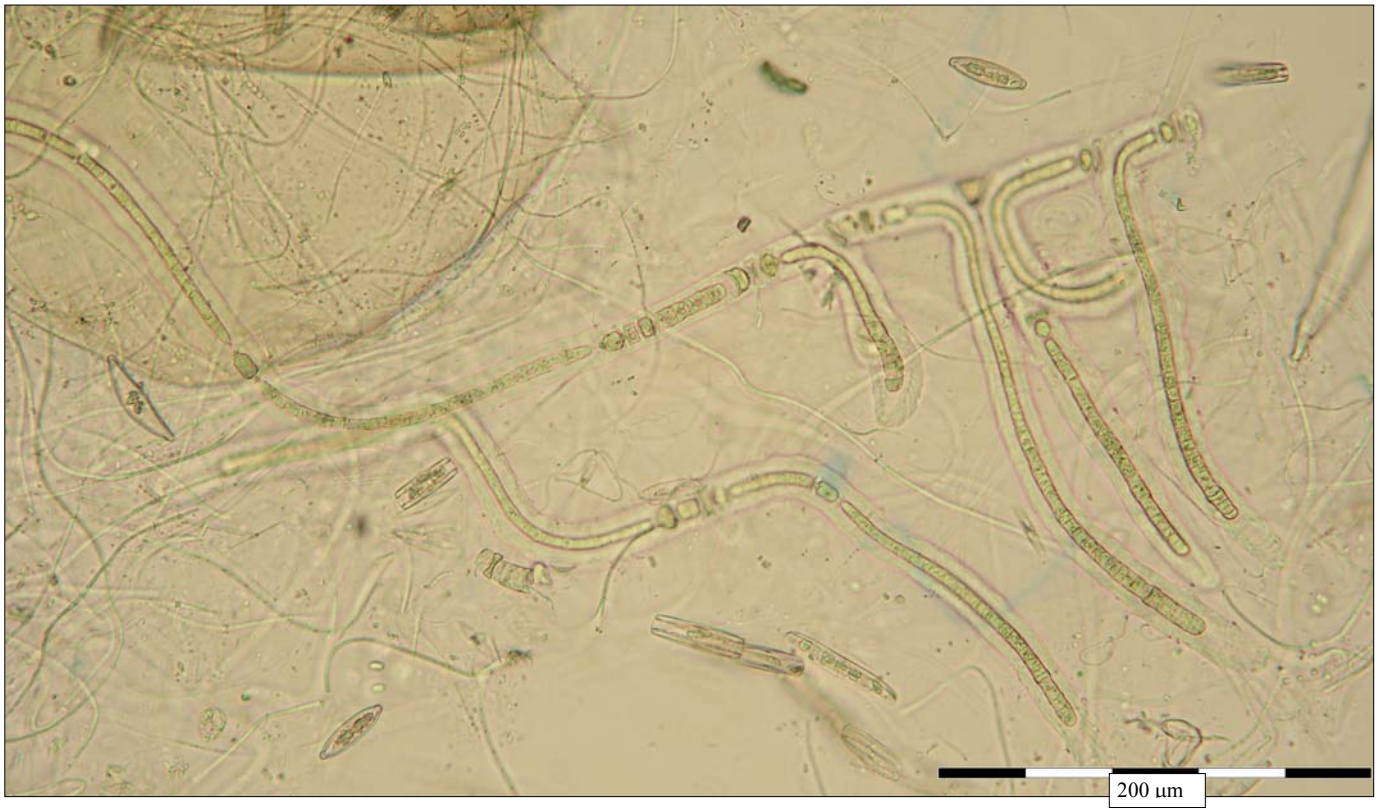
69. *Cylindrospermum* cf. *breve*

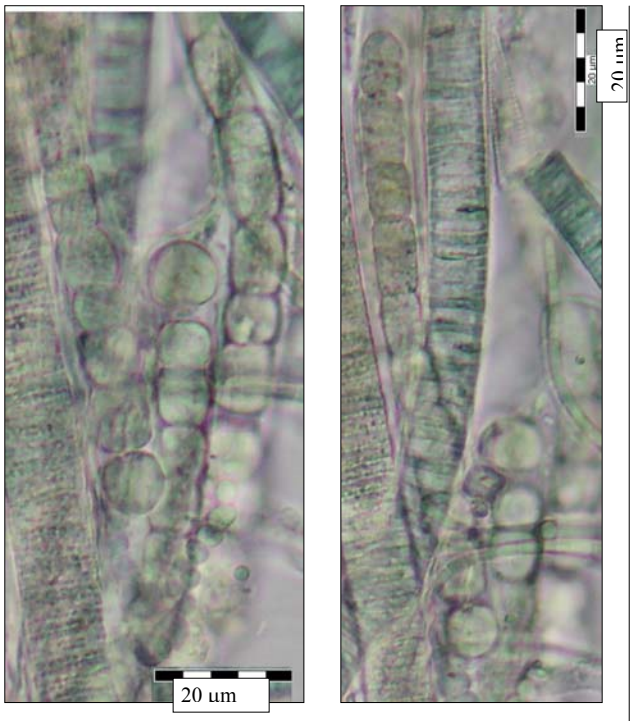




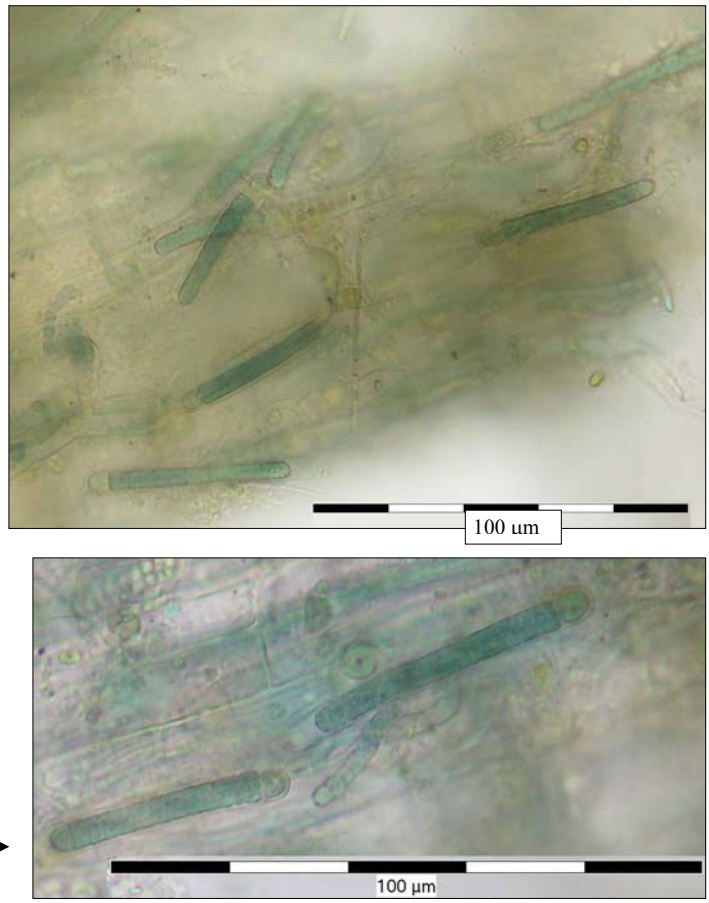


70. *Hassallia* sp.

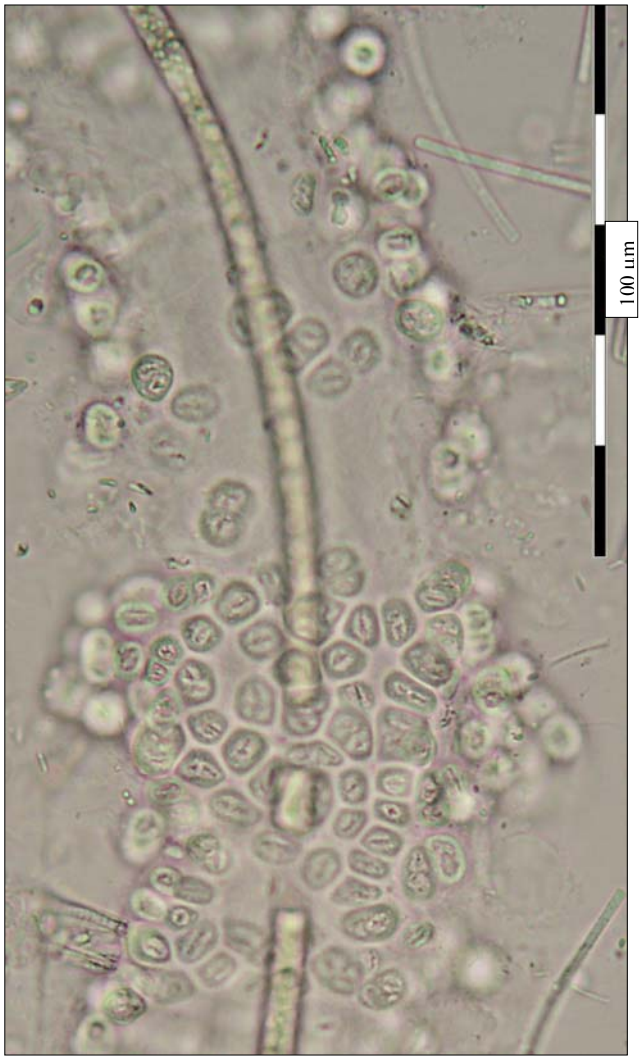


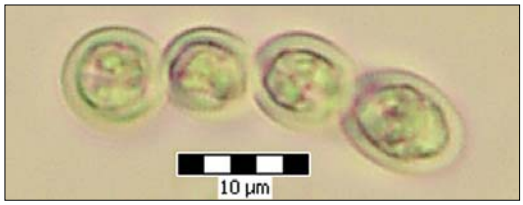


71. *Microchaete* cf. *robusta*

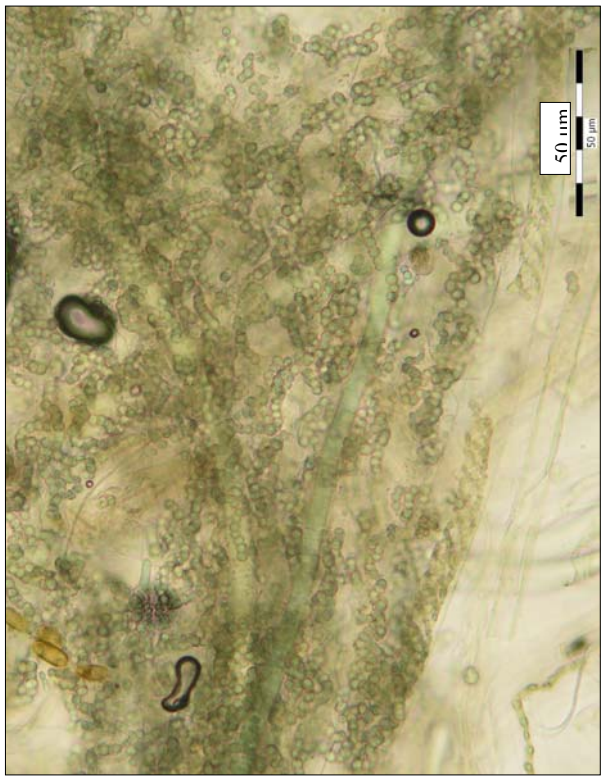


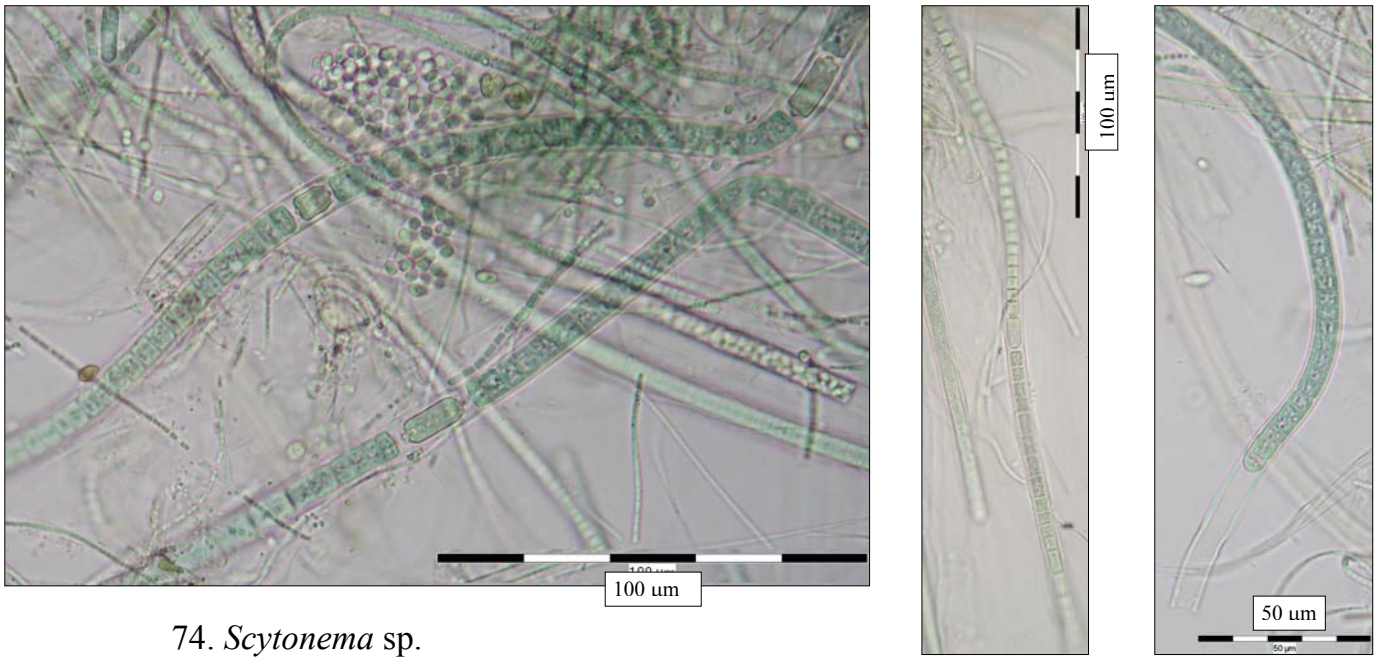
72. *Microchaete* cf. *goeppertiana*





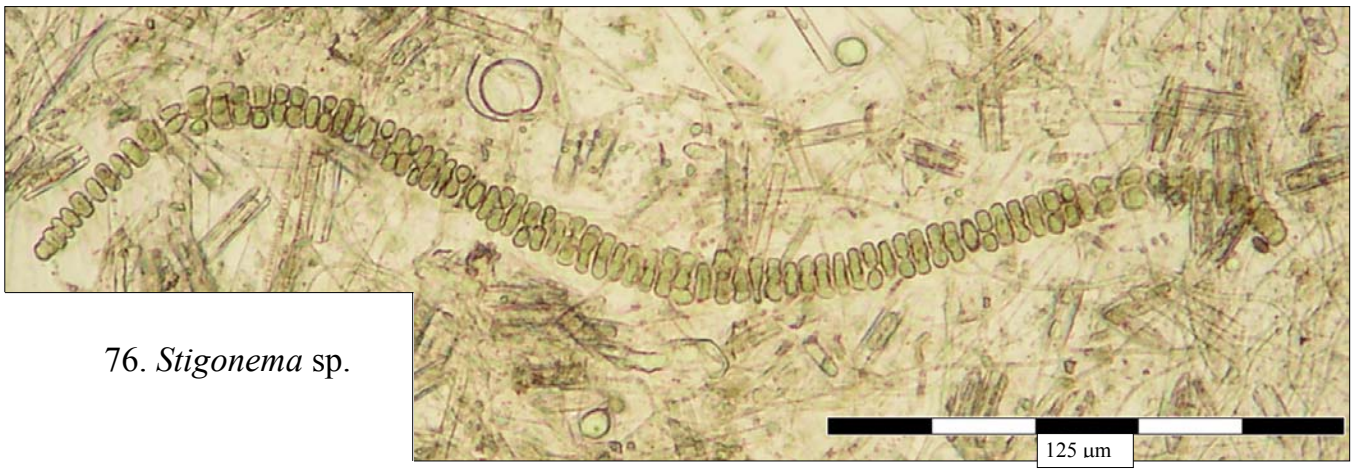
73. *Nostoc* sp.





74. *Scytonema* sp.

75. *Tolypothrix* cf. *willei*



76. *Stigonema* sp.