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CONTENTS

ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

БИОЛОГИЧЕСКИ ФАКУЛТЕТ
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MACRO- AND MICROPALAEOBOTANICAL EVIDENCES FOR LATE MIDDLE MIOCENE CLIMATE CHANGE IN BULGARIA

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Abstract. An analysis of selected macrofloras (leaves, fruits and seeds) from NW Bulgaria using the Coexistence approach method was applied to obtain quantitative data about Volhynian and Bessarabian climate in studied area. The aim of the study is to compare the climate data derived from the analysis of macrofloras and palynological data. The Middle Miocene was a period of a subtropical/warm temperate humid climate with mean annual temperature between 16 and 18 °C and mean annual precipitation between 1,100 and 1,300 mm. Comparison of all data, received from different floras we can observed, showed that nevertheless some differences, in all cases there was a good relation between climate and vegetation dynamics. We observed some deviations in quantities, but they varied in small limits. The climate data derived from macro- and microfloras coincided well in regard to all parameters, nevertheless that different taxa determined coexistence intervals. In some cases, the macropalaeobotanical data provide narrow climate interval, that is explained by better taxonomic resolution and better identification of nearest living relatives (NLRs). The application of both methods has the advantage of obtaining both more accurate climate data and tracking climate change in more detail throughout the study period.

Key words: Volhynian, Bessarabian, macroflora, pollen, Coexistence approach, climate reconstruction

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INTRODUCTION

The history of vegetation change in Northwestern Bulgaria (Forecarpathian Basin) has been studied last few decades on the basis of both macrofossil and microfossil records. The well-preserved leaf imprints, seeds/fruits, dispersed cuticles and palynomorphs (Palamarev & Petkova 1987 and Ivanov et al. 2002, and references therein) provide a good ground for vegetation and climate reconstructions. Because of the specific geographic position of the Balkan Penninsula in the Miocene between two vast marine basins – Mediterranean to the south and Parathetyan to the north (Rögl 1998; Meulencamp & Sissingh 2003), it plays a major role in the evolution plants in the Neogene of Europe. This area appears as a land bridge and a main migration route between Asia minor and Central Europe, and apparently it also plays a major role in the evolution of Mediterranean vegetation (Palamarev 1989). It is also important in understanding the evolution of climate system in this area.

Palaeoclimatic research is an important tool for the correct interpretation of modern climate change and for the correct understanding of how the climate system works. Data on terrestrial climate usually come from two main proxies - analysis of fossil land animals (mainly mammals) and mainly from the data on fossil flora and vegetation. The latter is widespread for reconstructions because of different techniques developed for climate analysis of fossil plant data. There are two approaches to extract climate data from plant fossils - leaf physiognomy and nearest living relatives (NLR) approach. Recently a study comparing these two approaches based on leaf-floras was published (Ivanov et al. 2019) and the results clearly showed the advantages and preciseness of the NLR's using Coexistence approach (CA) method. The present study uses the possibilities of the NLR's approach comparing palaeofloristic data obtained by macro- and micro-floristic studies.

Geological settings

The palaeogeography of the Forecarpathian Basin and its variations during the Neogene are relatively well known. A shallow brackish basin covered wide territories in the NW Bulgaria in the Middle Miocene. The age of sediments is well dated by characteristic mollusk associations, as well as by ostracods and foraminifers (Ivanov et al. 2002). The longitudinal depression was active in the Volhynian (Kojumdgieva & Popov 1986, 1989; Kojumdjieva et al. 1989; Ivanov et al. 2019), and in the peripheral parts of the basin (the so called Marginal stable area: **Fig. 1**) a lot of swamps and almost freshwater ponds existed. This study is based on macrofloristic analysis of several localities of Volhynian and Bessrabian ages situated south of the Forecarpathian Basin (**Fig. 1**). The studied sediment successions are presented by sandy clays and clays, which contained well preserved macroremains and palynomorphs. Stratigraphically they belong to the

Krivodol Formation assigned to the Middle Miocene (Kojumdjieva et al. 1989).

MATERIAL AND METHODS

About 32 fossil macrofloras are known from NW Bulgaria (Palamarev 1988, 1990, 1993; Palamarev & Petkova 1987). The revised taxonomy of fossil flora (Palamarev et al. 2005) is used in this study. For the present study four floras of Volhynian and three of Bessrabian age were chosen. The choice of flora for analysis was based on the sufficient completeness of the fossil record. Most local floras contains 4-5 fossil species. In order to obtain reliable data, flora with a sufficiently rich floristic composition were selected, namely Tsar Shishmanovo-Tolovitsa, Ruzhintsi, Kladorub-Ostrokarptsi and Pelovo of Volhynian age, and Drenovets,

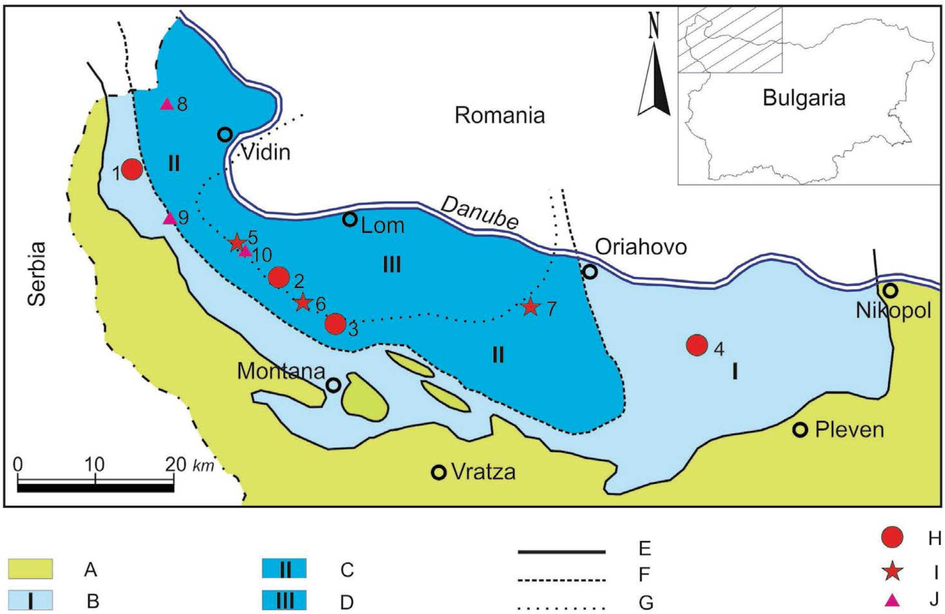

Fig. 1. Sketch map showing the structural-palaeogeographical areas in Northwest Bulgaria during the Miocene, and the localities of the studied profiles (after Ivanov et al. 2002). Legend: A - Areas outside the Forecarpathian Basin (land); B - Marginal stable area; C - Miocene longitudinal depression; D - Lom depression; E - Boundaries of the basin; F - Boundaries of the Miocene longitudinal depression; G - Boundaries of the Lom depression; H - Localities with Volhynian macroflora: 1. Tsar Shishmanovo-Tolovitsa; 2. Ruzhintsi; 3. Kladorub-Ostrokarptsi; 4. Pelovo; I - Localities with Bessranian macroflora: 5. Drenovets; 6. Belo Pole-Cherno Pole; 7. Hairedin; J – Localities with pollen flora: 8. Deleina; 9. Makresh; 10. Drenovets.

Belo Pole-Cherno Pole, and Hairedin of Bessarabian age. Pollen flora used in this study came from cores C-12 Deleina, C- Drenovets and C-37 Makresh (**Fig. 1**).

The NLR approach is a widely applied technique for palaeoclimate estimates with the help of fossil plants. It is based on comparisons of fossil taxa with recent species and it is assumed that the climatic requirements of fossil species are more or less similar to those of their nearest living relatives. In this study the CA method (Mosbrugger & Utescher 1997; Utescher et al. 2014) was applied to obtain quantitative climatic data. This technique is straightforward and uses climatic tolerances of nearest living relatives of fossil taxa with respect to various climatic parameters to reconstruct past climates. The interval of possible coexistence of all taxa is calculated for the various climate parameters, within which all nearest living relatives of the fossil flora can exist. This coexistence interval is regarded as representing a reasonable estimator of the past climate under which the fossil flora lived. Such approach can be used with all kinds of plant remains (*e.g.* leaves, fruits/seeds, pollen/spores) for which the NLRs can be reliably identified. The method was recently applied for palaeoclimate reconstructions in Europe and Asia (*e.g.* Pross et al. 1998; Utescher et al. 2000; Uhl et al. 2003; Ivanov et al. 2002, 2011; Biltekin et al. 2015; Durak & Akkiraz 2016; Ivanov & Worobiec 2017; Kayseri-Özer 2017; Kayseri-Özer et al. 2017; Yavuz et al. 2017; Ivanov & Lazarova 2019). For a given fossil flora, the CA method determines the nearest living relatives of fossil taxa and their climatic tolerances and calculates the coexistence intervals (minimum and maximum values) for various climate parameters (for details see Mosbrugger & Utescher 1997 and Utescher et al. 2014) within which all living relatives of fossil species can coexist. The climate data were obtained with the help of Palaeoflora database (Utescher & Mosbrugger 2015). The following climate parameters were considered as presenting the main climate characteristics: MAT - mean annual temperature (°C), TCM - mean temperature of the coldest month (°C), TWM - mean temperature of the warmest month (°C), MAP - mean annual precipitation (mm). In addition, a brief description of fossil vegetation is also presented based on autecological analysis (Ivanov 2015; Ivanov & Worobiec 2017; Ivanov & Lazarova 2019).

The present-day climate of Northwest Bulgaria is characterised by MAT 11.2-11.5 °C, TCM -2.1 to -0.9 °C, TWM 22.6-23.6 °C and MAP 536-586 mm (Velev 1997).

RESULTS AND DISCUSSION

The Volhynian

We analyzed four floras of Volhynian age located near the villages of Tsar Shishmanovo-Tolovitsa, Ruzhintsi, Kladorub-Ostrokarptsi and Pelovo. These floras are enough rich with taxa to provide reliable palaeoclimate date.

The Volhynian palaeoflora contains 154 species belonging to 114 genera and 69 families of the whole macroflora. In addition, 139 palynomorphs have been discovered. This flora is systematically very rich in representatives of Algae, Bryophyta, Lycopodiophyta, Equisetophyta, Polypodiophyta, Pinophyta and Magnoliophyta. The largest families are Lauraceae, Fagaceae, Fabaceae, Betulaceae, Juglandaceae, Rhamnaceae and Magnoliaceae. Among the genera, the most diversified were *Quercus*, *Magnolia*, *Myrica*, *Persea*, *Rhamnus* and *Pinus*.

Specific feature of the studied flora is the mixture of different floristic elements. As many other Tertiary floras, it comprises taxa which for the most part do not grow together today, being a unique combination of refugial-geographic elements. Among all, the East Asian and North American elements are better presented, thus pointing to closeness and relationship of the fossil flora to recent ones from East Asia and North America (**Fig. 2**). The East Asian element clearly dominates with 31.7%. An important feature is the considerable presence of Mediterranean elements (8.5%).

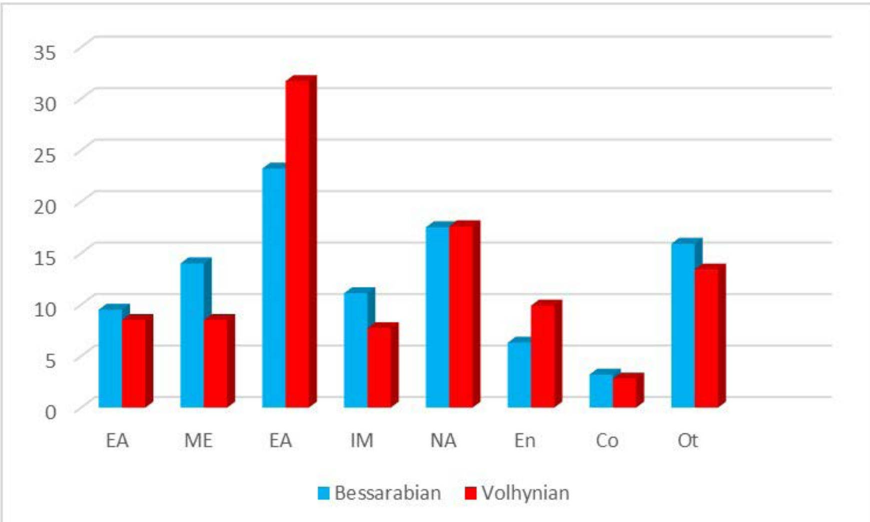

Fig. 2. Ratios between refugial-geographic elements in Sarmatian macroflora. Legend: EU – Eurasian; ME – Mediterranian; EA – East Asian; IM – Indo-Malaysian; NA – North American; En – endemic; Co – cosmopolitan; Ot – others (after Palamarev & Ivanov 2001).

A very important phytogeographical characteristic of the flora discussed is the high value of endemism - 9.9%. With fourteen new taxa the Volhynian flora acted as a significant centre of speciation (for details see Palamarev et al. 1999). The ratio between the genetic genera categories is: allochthonous taxa - 48.2%, autochthonous taxa - 43.9%, extinct taxa - 4.4% and form genera - 3.5%. In addition, participation in arctotertiary elements has increased compared to Badenian flora, although the dominance of the thermophilous palaeotropic components is still provable.

From palaeoecological point of view, the following plant communities were described (**Fig. 3**): hygro-hydrophytic grassy paleocoenoses, euhydrophytic grassy paleocoenoses, hygrophytic forest paleocoenoses, riparian forests, mesophytic to hygromesophytic forests, mesoxerophytic to sclerophyllous forests, and herbaceous communities (Palamarev & Ivanov 2001).

The results obtained for Volhynian climate (**Table 1; Fig. 4**) shows CA-

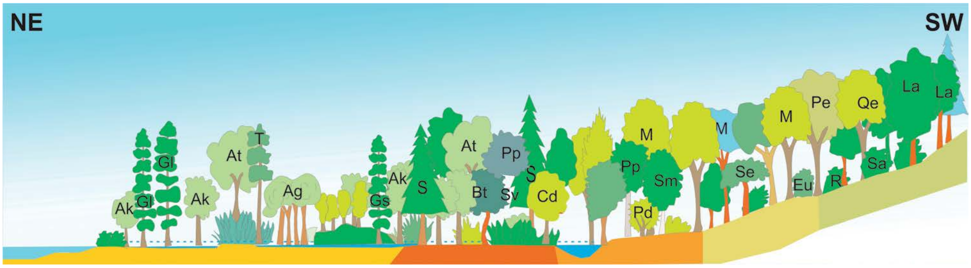

Fig. 3. Reconstructed vegetation profile for the Middle Miocene (Volhynian) in NW Bulgaria. Vegetation Structure includes: Mesophytic to hygromesophytic forests – polydominant and multispecies communities composed by species of *Pteris*, *Pteridium*, *Woodwardia*, *Sequoia*, *Magnolia*, *Liriodendron*, *Persea*, *Ocotea*, *Daphnogene*, *Litsea*, *Laurus*, *Corylopsis*, *Arbutus*, *Berchemia*, *Fagus*, *Quercus*, *Castanea*, *Zelkova*, *Carpinus*, *Engelhardia*, *Eurya*, *Sassafras, Schefflera, Adinandra*, *Hartia*, *Symplocos*, *Diospyros*, *Rubus*, *Prunus*, *Skimmia*, *Staphylea*, *Thevetia*, *Sapindus*, *Meliosma*, *Cornus*, *Aralia*, *Sambucus*, *Cedrela*, *Trigonobalanopsis*, *Sabal*, *Lygodium*, *Actinidia*, *Humulus*, *Berchemia*, *Carya, Pterocarya, Ampelopsis* and *Pathenocissus*.

intervals for mean annual temperature (MAT) 16.0-16.5 ^{o}C (narrowest climatic interval derived from Ruzhintsi paleoflora). Slightly lower left borders of intervals (14.4 ^{o}C) concerning Kladorub-Ostrokaptsi and Pelovo could be due to the incompleteness of the flora and the lower number of species in coexistence. The right border of the intervals of MAT could be also at 16.9 or 17.4 ^{o}C as derived by data from Pelovo, Kladorub-Ostrocarptsi and Tsar Shishmanovo-Tolovitsa. The temperature of the coldest month (TCM) is in the frame 5.6-5.8 ^{o}C. The temperature of the warmest month (TWM) is almost equal from all 4 sites – 25.7-26.4 ^{o}C. As regard the precipitation the CA-intervals for mean annual precipitation (MAP) is well above 1,000 mm (1,090-1,230 mm), but some wide intervals were obtained – between 843 and 1297 mm. This is in agreement with results from CA-analysis

Table 1. Climate data for Volhynian and Bessarabian derived from macro-paleobotanical record.

Age	Locality	MAT (^{0}C)	TCM (^{0}C)	TWM (^{0}C)	MAP (mm)
Bessrabian	Drenovets	14.7-18.0	3.8-13.3	25.4-27.1	1096-1189
	Belo Pole-Cherno Pole	13.8-16.9	3.1-8.1	24.7-25.3	816-1297
	Hairedin	9.1-15.7	2.9-9.1	21.9-27.9	963-1362
Volhynian	Tsar Shishmanovo-Tolovitsa	16.0-17.4	5.6-5.8	24.5-27.0	840-1230
	Ruzhintsi	16.0-16.5	5.6-5.8	25.7-26.4	1090-1230
	Kladorub-Ostrokarptsi	14.4-17.4	5.6-5.8	25.7-26.4	867-1230
	Pelovo	14.4-16.9	3.7-5.8	25.4-26.4	1035-1356

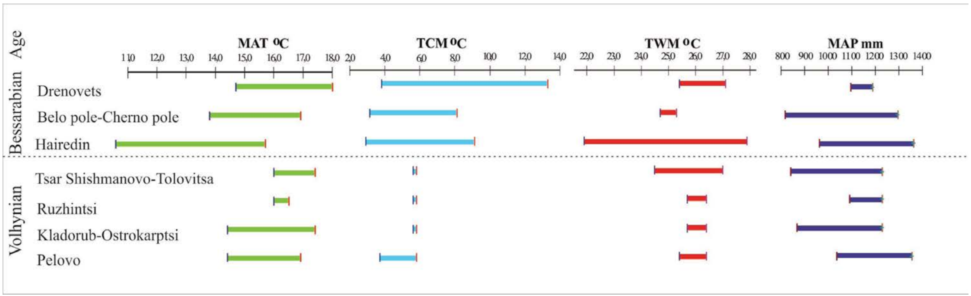

Fig. 4. Reconstruction of the paleoclimatic parameters for the Forecarpathian basin on the basis of the macropaleobotanical data.

of palynological data which suggest drying at very late Bessarabian and early Chersonian (Ivanov 1998).

In the Volhynian the climatic conditions derived from pollen data (**Table 2; Fig. 5**) show relatively stable climate. MAT is between 15.6 and 17.2 °C (but some samples give higher temperatures, *e. g.* 17.2-18.4 °C) and a second coexistence interval may. TCM is mainly between 5 and 7 °C, temperatures of the warmest month lie within 24.6-27.8 °C and both TCM and TWM show small oscillations of the upper limit. As regards the fluctuations of MAP, the narrowest coexistence intervals occur during the upper part of the Volhynian with 1,187-1,322 and 1,076-1,322 mm. this corresponds to the high precipitation rate in the Volhynian obtained from macropalaeobotanical data.

The Bessarabian

Three floras of the Bessrabian age located near the villages of Drenovets, Belo pole-Cherno pole, and Hairedin were analyzed. In terms of quality of data these floras are poorer in species, and provide less reliable data.

Bessarabian paleoflora is relatively poorer in genera and species compared to Volhynian complex. It contains ca. 60 species from the macroflora, which belong to 53 genera and 33 families. The microflora has become known through 119 taxa (Ivanov 1998). The Fagaceae, Betulaceae and Juglandaceae are characterized by greater variety at the genus level. The species diversification is weak. In this type of flora one can observe the sharp decrease in Lauraceae, Magnoliaceae and ferns, as well as a general floristic impoverishment. Obviously, during this period, there is a phase of degradation in the development of the Neogene vegetation. The endemic element is relatively high (6.3%) because most endemic species with Volhynian origin continue to develop in Bessarabian. The high value is also due to the low taxonomic diversity of the flora.

The autochthonous elements (50.0%) predominate over allochtonous ones (44.2%). Compared to the Volhynian paleoflora, shrub and liana components have dropped significantly here. The herbs had also become somewhat higher at the

Table 2. Climate data for Volhynian and Bessarabian derived from pollen record from core Deleina.

Age	Depth (m)	MAT (^{0}C)	TCM (^{0}C)	TWM (^{0}C)	MAP (mm)
Bessrabian	78	14,7-17,2	1,7-6,6	23,0-27,8	740-740
	80,5	11,6-18,4	1,7-8,1	23,6-27,8	652-759
	83,5	15,6-17,2	5,0-6,6	24,7-27,3	897-1308
	106	17,2-18,4	5,0-8,1	24,7-27,8	1187-1322
	110	15,6-17,2	5,0-7,0	24,7-27,3	823-1380
	114	15,6-17,2	5,0-7,0	24,7-27,8	897-1322
	120	17,2-17,2	5,0-6,6	24,7-27,8	1187-1308
Volhynian	143,5	15,6-17,2	5,0-6,6	24,7-27,3	1076-1281
	150,5	15,6-17,2	5,0-6,6	24,7-27,8	897-1281
	185	15,6-17,2	5,0-7,0	24,7-27,8	1187-1281
	194	15,6-17,2	5,0-7,0	24,7-27,3	1187-1281
	220	15,6-17,2	5,0-6,6	24,7-27,8	1187-1322
	234	15,6-17,2	5,0-6,6	24,7-27,8	823-1322
	245	15,6-17,2	5,0-6,6	24,7-27,1	1076-1380
	258	15,6-17,2	5,0-6,6	24,7-27,8	1187-1281
	260	15,6-17,2	5,0-7,0	24,7-27,8	1187-1308
	262	15,6-17,2	5,0-7,0	24,7-27,8	897-1281
	264	15,6-17,2	5,0-7,0	24,7-27,8	1187-1281
	268	15,6-17,2	5,0-7,0	24,7-27,8	740-1281
	273,5	15,6-17,2	5,0-7,0	24,7-27,8	1187-1281
	281,5	15,6-17,2	5,0-7,0	24,7-27,1	823-1281
	285	15,6-18,4	5,0-8,1	24,7-27,8	1076-1308
	349	15,6-17,2	5,0-6,6	24,7-27,8	823-1308
	353	15,6-17,2	5,0-6,6	24,7	1076-1281

same time. This time interval (the end of Bessarabian) corresponds to the beginning of Tortonian in Mediterranean area and the beginning of the so-called *Vallesian crisis* - sequence of mammal events, changes in lower vertebrate record.

With regard to the ecological requirements, the paleoflora type consists of the following components: hydrophytes (8.3%), hygrophytes (5.0%), hygromesophytes (15.0%), mesophytes (51.7%) and hemixerophytes to xerophytes (20.0%). The greatest change occurred in the group of hygromesophytes. Numerous macro- and mesothermal ferns, laurel and magnolia species have fallen off. The number of arctotertiary elements is significantly increased and this change is particularly

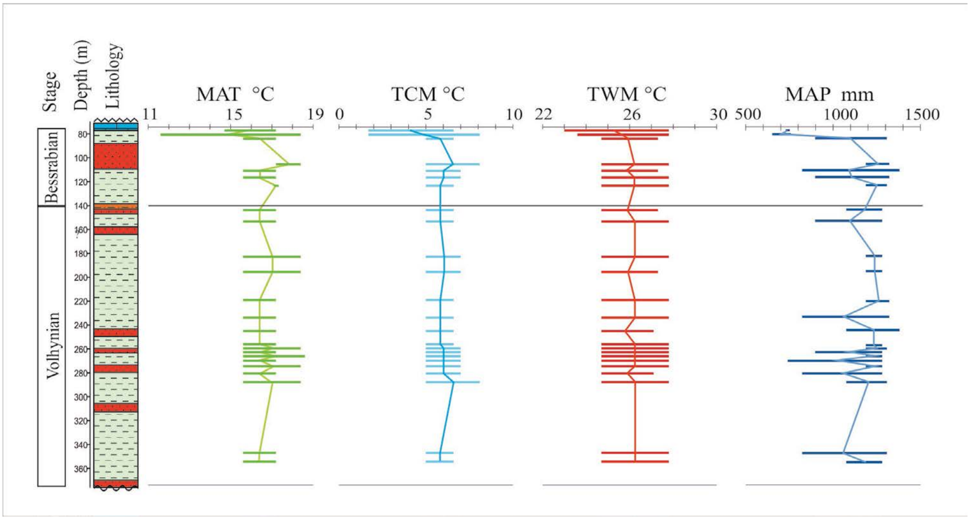

Fig. 5. Reconstruction of the paleoclimatic parameters for the Forecarpathian basin on the basis of the palynological data from drilling profile C-12 Deleina.

visible in the pollen spectra (Ivanov et al. 2011). This trend is valid also for the involvement of the herb component (**Fig. 6**). The phytogeographic analysis (**Fig. 2**) shows a relatively monotonous picture. The East Asian and Atlantic North American elements are in first place with 23.2% and 17.5%. Then comes the Mediterranean element with 14.3%, which is an important increase.

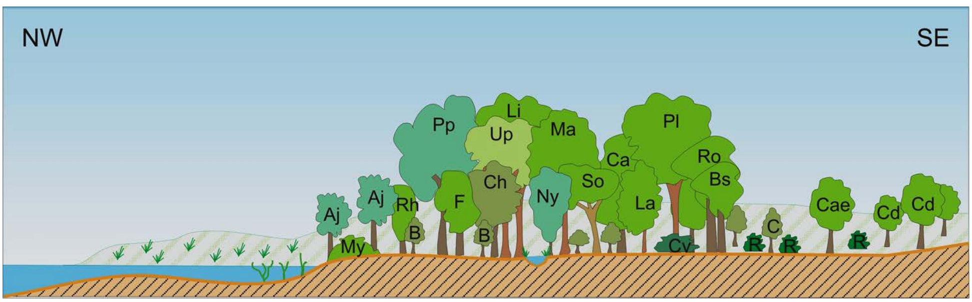

Fig. 6. Reconstructed vegetation profile for the latest Bessarabian - early Khersonian in NW Bulgaria. The most significant event are: appearance of open landscapes and development of herbaceous xerophytic communities – Chenopodiaceae-Artemisia-Caryophyllaceae-Asteraceae; Subxerophytic shrubs – *Celtis*, *Anagyris*, *Caesalpinites*, *Robinia*, *Sophora*, *Paliurus* etc.; Restricted distribution of thermophyllous elements in the mesophytic forests; Changes of dominant species in forest communities; Restriction in the distribution of the swamp forests.

The results obtained for Bessarabian climatic parameters show similar values, slightly lower CA-intervals concerning annual temperature (left border at 13.8 and 14.7 °C) and temperature of the coldest month (2.0-9.1 and 3.1-8.1 °C), which could due both to climate change or incompleteness of fossil record. As regard the precipitation the CA-intervals are wider – between 843 and 1,297 mm. It is worth to note that the Besserabian macroflora comprise more xeric and semi-xeric floristic elements that could suggest some drying of the climate during that period. This is in agreement with results from CA-analysis of palynological data which suggest drying at very late Bessarabian and early Chersonian (Ivanov et al. 2002).

The pollen data for Bessarabian shows that the palaeoclimate is most similar to the Volhynian (**Table 2; Fig. 5**). However, at the end of this stage a slight decrease in MAT is indicated in cores C-12 Deleina and and C-1 Drenovets. Thus the end of the Bessarabian represents the starting point for the climatic changes occurring in the Chersonian. This latter substage is characterised by lower values in almost all climate parameters (**Fig. 5**). MAT coexistence intervals are 13.3 (14.4) to 17.2 ^{o}C, corresponding to a decrease of the lower boundary of about 2 ^{o}C as compared to the Bessarabian and Volhynian. A similar cooling is observed for the TCM with the lower boundary of the coexistence intervals changing from 5 ^{o}C in the Volhynian/ Bessarabian to 1.7 ^{o}C in the Bessarabian/Khersonian (Ivanov et al. 2002). The lower boundary of the coexistence interval for the summer temperature (TWM) also decreases by about 2 ^{o}C. But the most significant change occurs in the mean annual precipitation. The climatic estimates for the end Bessarabian (**Fig. 5**) indicate that the climate was slightly cooler (particularly in winter) and significantly drier. This could mean a greater seasonality and probably more or less pronounced dry period in the summer.

Comparison of all data, received from different observed floras showed, that nevertheless some differences, in all cases there was a good relation between climate and plants. We observed some of deviations in quantities, but they varied in small limits. These deviations could due to several factors, in our case more appropriate is to suggest that completeness of paleofloristic data, and the applied paleobotanical method, which discloses different floristic components, influenced in major part the preciseness and correctness of results.

CONCLUSION

The Volhynian and Bessarabian in Northwest Bulgaria were periods of subtropical or warm-temperate and humid climate, with small fluctuations in palaeoclimatic parameters as evidence from bouth macropaleobotanical and palynological data. The correlation between climate data derived from macro- and microfloras shows coincidence as regard all parameters, nevertheless that different taxa determine coexistence intervals. In some cases, the macropaleobotanical data provide narrow climate interval, that is explain by better taxonomic resolution and better identification of NLRs (*e.g.* at species level). The recognition of NLRs for pollen taxa is usually at genus level. The application of both palaeoclimate recovery methods has the advantage of obtaining both more accurate climate data and tracking climate change in more detail throughout the study period.

CONFLICT OF INTERESTS

The author declares that there is no conflict of interest regarding the publication of this article.

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CHECKLIST OF CYANOPROKARYOTES AND ALGAE IN THE LARGE TROPICAL RIVER CONGO (AFRICA)

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Abstract. This study presents data on the floristic composition of cyanoprokaryotes/cyanobacteria and algae in the tropical River Congo, the second longest river in Africa after the Nile, which at global scale is the second most important river after Amazon in terms of drainage basin size and freshwater discharge. The results in this paper are based only on the materials collected from the mainstem of the middle part of the River Congo, in a stretch of 1,450 km, in two sampling campaigns conducted in contrasting hydrologic conditions: in the high water (HW) period, in December 2013 (33 sites), and in the low, falling water (FW) period in June 2014 (38 sites). Totally 520 taxa of 7 divisions (Cyanoprokaryota – 76, Cryptophyta – 1, Euglenophyta -17, Pyrrhophyta – 8, Chlorophyta – 108, Streptophyta – 38, Ochrophyta – 272: 242 Bacillariophyceae, 24 Chrysophyceae, 1 Synurophyceae, 3 Tribophyceae and 2 Eustigmatophyceae), were identified in the phytoplankton samples but few of them (16) were strictly tropical. Algal diversity was higher during the FW (431 taxa) compared with HW (314 taxa) and floristic similarity between both periods was only 57% based on 213 common taxa. According to the frequency of distribution, most of the phytoplankters occurred rarely and only two diatoms were found in all sites during both studied periods: *Aulacoseira ambigua* and *Nitzschia lancettula*. They were followed by the widespread *Aulacoseira agassizii*, *A. granulata* var. *angustissima*, *Nitzschia* cf. *lacuum*, *Staurosirella leptostauron* and *Staurosirella pinnata*.

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Cyanoprokaryotes and chlorophytes were found along the whole river flow, but with different species composition.

Key words: algal distribution, diversity, phytoplankton, potamoplankton, tropical algae

INTRODUCTION

The knowledge on the phytoplankton of one of the most important large world rivers, the tropical Congo River, which provides freshwater resources for millions of people in central Africa (Oberg et al. 2009) and is the major transport arteria of DR Congo is quite scarce. Only recently data on potamoplankton structure, dynamics and main driving forces in the Middle Congo River became available (Descy et al. 2016). The results obtained by high-performance liquid chromatography analysis of chlorophyll a (Chl a) and marker pigments, and to the primary production measurements made by using the ^{13}C incubation technique, demonstrated a pronounced difference in phytoplankton composition, biomass and production in the periods of high waters (HW) and low, falling waters (FW) (op. cit.). For example, in the mainstem concentrations of Chl a varied between 0.07 and 1.77 µg L^{-1} in HW conditions, and between 1.13 and 7.68 µg L^{-1} in FW conditions. Based on marker pigment concentration, green algae (both chlorophytes and streptophytes) dominated in the mainstem in HW, whereas diatoms dominated in FW; cryptophytes and cyanobacteria were more abundant but still relatively low in the FW period, both in the tributaries and in the main channel (Descy et al. 2016). Daily integrated production measured in the mainstem varied between 64.3 and 434.1 mg C m^{-2} day^{-1} in FW conditions and between 51.5 and 247.6 mg C mm^{-2} day^{-1} in HW (op. cit.). Phytoplankton growth in the Congo River can take place in the main channel, with hydrological processes allowing maintenance of phytoplankton biomass even during HW (Descy et al. 2016). The relative contribution to phytoplankton biomass from tributaries (mostly black waters) and from a few permanent lakes was low, and the main confluences resulted in phytoplankton dilution. This result is in contrast to other tropical river systems where connectivity with the floodplain and the presence of natural lakes and man-made reservoirs play a prominent role in the recruitment of phytoplankton to the main river (Descy et al. 2016). At the same time, the presence of phytoplankton developing in the main river channel is in accordance with data on its formation in the lowest natural part of the temperate large European Danube River (Stoyneva & Draganov 1991; Stoyneva 1994) and its tributary Tisa/Tisza (Istvánovics et al. 2010, 2011, 2012).

Regarding the river phytoplankton composition, Descy et al. (2016) published the most frequent 25 phytoplankton taxa from different taxonomic groups in the Middle Congo mainstem: *Aphanocapsa* spp., *Aphanothece* spp., *Limnococcus limneticus*, *Planktolyngbya* spp. (cyanobacteria/cyanoprokaryotes), *Actinastrum* *rhaphidioides*, *Coelastrum* spp., *Coenochloris* spp., *Desmodesmus magnus*, *D. perforatus*, *Scenedesmus communis*, *S. oahuensis*, *Planktosphaeria gelatinosa* (chlorophytes), *Closterium* spp., *Hyalotheca* cf. *mucosa* (streptophytes), *Euglena* spp., *Strombomonas* spp. (euglenophytes), *Salpingoeca* spp. (chrysophytes), *Aulacoseira granulata* (including var. *angustissima*), *A. agassizii*, *Cyclostephanos invisitatus*, *Cyclotella* spp., *Discostella pseudostelligera*, *Staurosirella* spp. and *Thalassiosira rudolfii* (diatoms). However, "studies of African phytoplankton should not only list the major components, but also all the accompanying taxa in order to provide better knowledge on their distribution and biogeography" (Lemoalle et al. 1981, p. 38). Regarding this, John (1986, p. 160) noted that "the correct identification of taxa in many groups is becoming increasingly a specialist task …". Former studies on algae of the R. Congo basin reported floristic composition of both periphyton and plankton from the mainstem and some tributaries, but on relatively small sectors and dealt with specific taxonomic groups (*e.g.* Kufferath 1948; Golama 1991, 1996; Verheyen et al. 2017). The aim of the present paper is to present data on the total species composition of the Congo River phytoplankton, in a stretch of 1,450 km corresponding to the Middle Congo River, in contrasting hydrologic conditions, which could serve as a basis for future tracking of the algal biodiversity in this important large tropical river.

MATERIAL AND METHODS

Study site

The Congo River (formerly known as the Zaire River) stretches from the Great Rift Valley in Eastern Africa to the Atlantic Ocean in the west (**Fig. 1**). It is the second longest river in Africa after the Nile, with a length of about 4,300 km (Oberg et al. 2009; Vanden Bossche & Bernacsek 1990 – cit. acc. to https://en.wikipedia.org/wiki/Congo_River). The River Congo is also the second largest river in the world by discharge volume, following only the Amazon: the discharge at its mouth ranges from 23,000 to 75,000 m^3 sec^{-1}, with an average of 41,000 m^3 sec^{-1} (Vanden Bossche & Bernacsek 1990 – cit. acc. to https://en.wikipedia.org/wiki/Congo_River). The drainage basin has been estimated to cover about 4 million km^2 (Beadle 1974; Vanden Bossche & Bernacsek 1990 – cit. acc. to https://en.wikipedia.org/wiki/Congo_River; Oberg et al. 2009). The river runs between 7 °N and 12 °S, mostly through a flooded forest region, draining the largest expanse of lowland tropical forest in Africa (Beadle 1974; Bwangoy et al. 2010). Congo River crosses twice the equator. In this way the dry season on the northern part of the basin is compensated by the rainy season on the southern part of the basin, and vice versa, leading to a regulation of seasonal water height variations, which are small (3–4 m) in the main river bed (Runge 2008).

Sampling

Sampling details and main water characteristics have already been published by Descy et al. (2016): two sampling campaigns were carried out in the Middle Congo River, between Kisangani and Kinshasa (**Fig. 1**), in the high water (HW) period, in December 2013 (75 sites), and in the falling water (FW) period in June 2014 (89 sites). The sampling was carried out using a 28 µm plankton net in the main river bed of the Congo River and in tributaries, as well as in the outlet of one of the two main lakes (Lake Tumba). A few additional samples were collected on

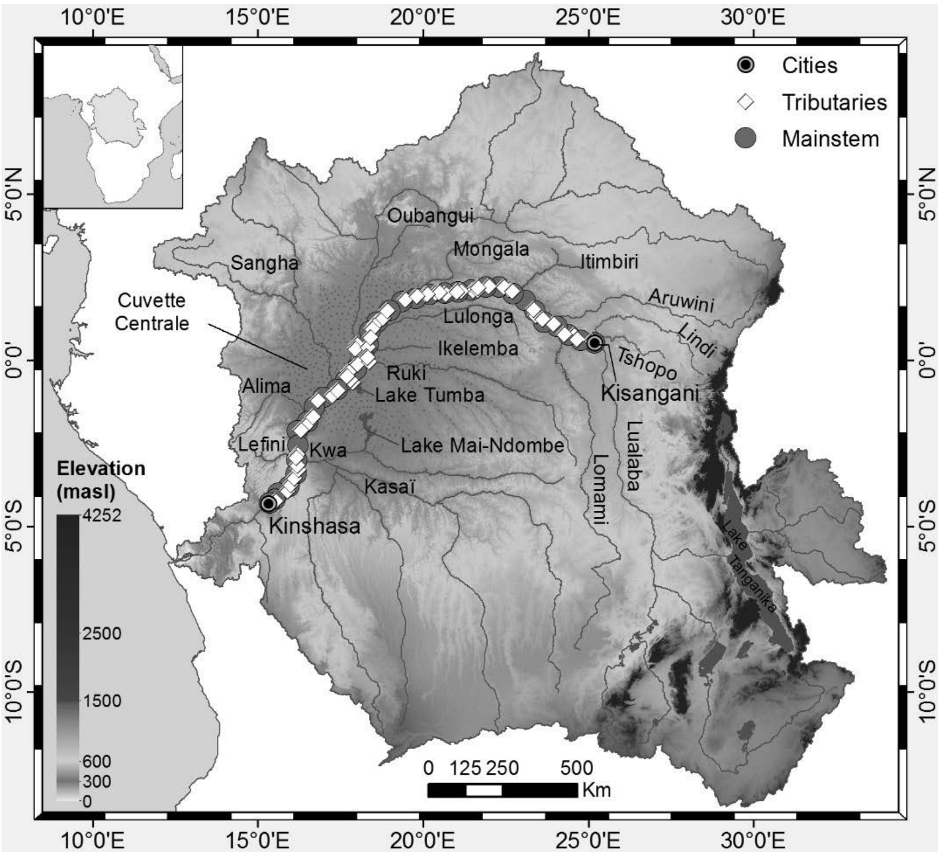


Fig. 1. Location of sampling sites in the Congo River at the scale of the whole basin overlain on elevation. Inset: Location of the Congo River basin in Africa. Map was generated with ArcGIS_ using publically available spatial datasets (after Descy et al. 2016).

the lower stretch of the mainstem downstream of the confluence with the Kasai River in May 2015 (FW period). Here we report only results from the River Congo mainstem (38 sites in FW and 33 sites in HW), as most of time only one sample was available from the lakes and tributaries.

Algal identification

The main part of the work on the algal biodiversity was done using conventional light microscopy with magnification 100x and immersion on non-permanent slides on microscopes Motic BA 4000 microscope with camera Moticam 2000, and on Motic B1 microscope with camera Moticam 2.0 mp, supplied by Motic Images 2 Plus and Motic Images 3 Plus software programs, respectively. Only Bacillariophyceae were identified on permanent slides mounted with Naphrax after peroxide digestion on Leitz Diaplan standard microscope, equipped with a Euromex camera using the Image Focus 4 software. Identification of some diatoms was checked by Priscila Tremarin (Universidade Federal do Parana, Curitiba, Brazil) using SEM.

The work is based on main standard floras (*e.g.* Ettl 1978; Starmach 1983, 1985; Krammer & Lange-Bertalot 1991, 1997a,b, 2004; Komárek & Fott 1983; Komárek & Anagnostidis 1999, 2005; Tsarenko et al. 2011; Wehr et al. 2015; Moestrup & Calado 2018) and numerous relevant current taxonomic papers considering data in AlgaeBase (Guiry & Guiry 2020), DiatomBase (Kociolek et al. 2020), and CyanoDB (Hauer & Komárek 2020).

Estimation of frequency of distribution and floristic similarity

For better understanding of the horizontal distribution of the species and their role in the phytoplankton, for each species the frequency quotient (FQ standardly calculated as per cent of the number of sites in which it was found vs total number of checked sites), was estimated separately for each of both studied periods (HW and FW) and as their average for the river phytoplankton. Estimated FQs were grouped in five classes through 20%: I – 1-20%, II – 21-40%, III – 41-60%, IV – 61-80%, and V – 81-100% (Stoyneva & Draganov 1991).

Floristic similarity between both studied periods was estimated according to Sørensen's Similarity Index (SSI, Sørensen 1948).

RESULTS AND DISCUSSION

The results from taxonomical identification of the phytoplankton algae in both studied periods are summarized in **Table 1**. There, some of the most popular common former Latin names are kept as synonyms.

In total, 520 taxa from 7 divisions have been identified in the phytoplankton of the Congo River (**Table 1**), some of which are illustrated on **Figs 2-83.** The distribution of all taxa by phyla is: Cyanoprokaryota – 76, Cryptophyta – 1, Euglenophyta -17, Pyrrhophyta – 8, Chlorophyta – 108, Streptophyta – 38, Ochrophyta - 272 (Bacillariophyceae – 242, Chrysophyceae – 24, Synurophyceae – 1, Tribophyceae - 3, Eustigmatophyceae – 2) – **Fig. 84**. Thus, Ochrophyta represented 52% of all phytoplankton diversity. Among them the richest were diatoms (Bacillariophyceae), which formed 47% of all biodiversity and clearly prevailed over all other

Table 1. Checklist of phytoplanktonic algae of the Congo River (2013-2014) with indication of their frequency quotients in low (FQLW) and high (FQHW) waters.

Taxa/Frequency quotients	FQLW	FQHW
CYANOPROKARYOTA		
Anathece cf. *bachmannii* (Komárek & Cronberg) Komárek, Kastovsky & Jezberová (Syn. *Aphanothece* cf. *bachmanii* Komárková-Legnerová & G.Cronberg)		**3**
Anathece clathrata (W. et G. S. West) Komárek, Kastovsky et Jezberová	**3**	
Anathece endophytica (W. & G. S. West) Komárek, Kastovsky & Jezberová (Syn. *Aphanothece endophytica* (West & G. S. West) J. Komárková-Legnerová & G. Cronberg)	**3**	
Anathece smithii (Komárková-Legnerová & Cronberg) Komárek, Kastovsky & Jezberová	**3**	
Aphanocapsa conferta (W. et G. S. West) Komárková-Legnerová et Cronberg	**3**	**3**
Aphanocapsa elachista W. et G. S. West	**9**	
Aphanocapsa grevillei (Berkeley) Rabenhorst		**5**
Aphanocapsa holsatica (Lemmermann) Cronberg et Komárek	**9**	
Aphanocapsa incerta (Lemmermann) Cronberg et Komárek	**9**	**8**
Aphanocapsa koordersii K.M.Strøm	**39**	**18**
Aphanocapsa planctonica (Smith) Komárek et Anagnostidis	**6**	
Aphanocapsa spp.	**3**	**5**
Aphanothece elabens (Brébisson ex Meneghini) Elenkin	**42**	**26**
Aphanothece microscopica Nägeli	**9**	
Aphanothece stagnina (Sprengel) A. Braun in Rabenhorst	**12**	**3**
Aphanothece sp.	**3**	
Chroococcus cohaerens (Brébisson) Nägeli	**6**	
Chroococcus dispersus (Keissler) Lemmermann	**3**	**3**
Chroococcus cf. *distans* (G. M. Smith) Komárková-Legnerová & Cronberg	**3**	
Chroococcus minimus (Keissler) Lemmermann		**5**
Chroococcus minor (Kützing) Nägeli		**3**
Chroococcus minutus (Kützing) Nägeli	**12**	**3**
Chroococcus planctonicus Bethge	**3**	

Taxa/Frequency quotients	**FQLW**	**FQHW**
Chroococcus cf. *polyedriformis* Schmidle	**3**	
Chroococcus vacuolatus Skuja	**3**	
Coelosphaerium kützingianum Nägeli		**5**
Cyanodictyon cf. *tubiforme* Cronberg	**3**	
Cyanodictyon cf. *iac* Cronberg et Komárek	**3**	**3**
Eucapsis aphanocapsoides (Skuja) Komárek & Hindák in Komárek et al. (Syn. *Chroococcus aphanocapsoides* Skuja)	**3**	**3**
Geitleribactron subaequale (Geitler) Komárek	**3**	**3**
Gomontiella sp.	**3**	
Horrmoscilla sp.	**3**	
Jaaginema cf. *perfilievii* (Anissimova) Anagnostidis & Komárek (Syn. *Oscillatoria* cf. *perfilievii* Anissimova)	**0**	**3**
Leptolyngbya cf. *valderiana* (Gomont) Anagnostidis et Komárek	**3**	
Leptolyngbya foveolarum (Gomont) Anagnostidis & Komárek		**3**
Leptolyngbya sp.	**15**	**13**
Limnococcus limneticus (Lemmermann) Komárková, Jezberová, O. Komárek et Zapomélová	**52**	**8**
Limnolyngbya circumcreta (G. S. West) X. Li & R. Li	**6**	**21**
Limnolyngbya cf. *circumcreta* (G. S. West) X. Li & R. Li	**12**	**8**
Lyngbya martensiana Meneghini ex Gomont	**3**	
Merismopedia glauca (Ehrenberg) Kützing	**6**	
Merismopedia punctata Meyen, nom. Illeg.	**3**	
Merismopedia tenuissima Lemmermann	**3**	
Merismopedia warmingiana (Lagerheim) Forti	**3**	
Microcystis cf. aeruginosa (Kützing) Kützing		**3**
Microcystis wesenbergii (Komárek) Komárek ex Komárek in Joosen	**3**	
Oscillatoria perornata Skuja		**3**
Oscillatoria sancta Kützing ex Gomont	**3**	
Oscillatoria simplicissima Gomont	**9**	
Oscillatoria tenuis Agardh ex Gomont		**11**
Oscillatoria pseudocurviceps Welsh	**3**	

Pannus planus Hindák	**3**	
Pannus sp. (?*Aphanocapsa* sp.)	**6**	
Phormidium irriguum (Kützing ex Gomont) Anagnostidis & Komárek	**3**	
Phormidium cf. *papyraceum* Gomont ex Gomont		**3**
Phormidium spp.	**3**	**16**
Planktolyngbya cf. *brevicellularis* Cronberg et Komárek	**82**	**24**
Planktolyngbya contorta (Lemmermann) Anagnostidis et Komárek		**3**
Planktolyngbya limnetica (Lemmermann) Komárková-Legnerová et Cronberg	**30**	**18**
Planktolyngbya microspira Komárek et Cronberg	**3**	
Planktolyngbya minor (Geitler) Komárek et Cronberg)	**6**	**3**
Planktolyngbya regularis Komárková-Legnerová et Tavera	**3**	
Planktolyngbya undulata Komárek et Kling	**3**	
Planktothrix clathrata (Skuja) Anagnostidis & Komárek		**3**
Planktothrix isothrix (Skuja) Komárek et Komárková		**3**
Planktothrix rubescens (De Candolle ex Gomont) Anagnostidis & Komárek		**3**
Radiocystis geminata Skuja	**3**	**3**
Rhabdogloea linearis (Geitler) Komárek	**3**	
Snowella atomus Komárek et Hindák		**3**
Sphaerocavum microcystiforme (Hindák) Azevedo & Sant'Anna (Syn. *Pannus microcystiformis* Hindák)	**6**	**3**
Spirulina corakiana Playfair	**3**	
Spirulina tenuissima Kützing, nom. Inval.		**3**
Synechocystis aquatilis Sauvageau	**3**	
Tychonema tenue (Skuja) Anagnostidis et Komárek	**3**	
Woronichinia delicatula (Skuja) Komárek et Hindák	**3**	
Woronichinia fremyi (Komárek) Komárek et Hindák	**3**	
CRYPTOPHYTA		
Chroomonas oblonga (Playfair) Pascher (Syn. *Cryptomonas oblonga* Playfair)	**3**	
EUGLENOPHYTA		
Cryptoglena pigra Ehrenberg	**3**	

Taxa/Frequency quotients	FQLW	FQHW
Euglena spp.	**18**	**3**
Dinema sp.		**3**
Lepocinclis teres (F. Schmitz) Francé	**6**	**3**
Strombomonas acuminata (Schmarda) Deflandre	**21**	**11**
Strombomonas cf. *australica* (Playfair) Deflandre	**3**	
Strombomonas fluviatilis (Lemmermann) Deflandre	**6**	**8**
Strombomonas gibberosa (Playfair) Deflandre	**3**	**5**
Strombonas schauinslandii (Lemmermann) Deflandre	**9**	**3**
Strombomonas urceolata (Stokes) Deflandre	**6**	**3**
Strombomonas sp.	**3**	
Trachelomonas caudata (Ehrenberg) F. Stein	**3**	**3**
Trachelomonas piscatoris A. C. Stokes	**6**	
Trachelomonas planctonica Swirenko	**9**	
Trachelomonas scabra Playfair	**3**	
Trachelomonas volvocina (Ehrenberg) Ehrenberg	**3**	
Trachelomonas sp.	**3**	
PYRRHOPHYTA		
Ceratium - cysts	**3**	
Gymnodinium paradoxum A. J. Schilling		**3**
Gymnodinium spp.	**9**	
Parvodinium africanum (Lemmermann) S. Carty (Syn. *Peridinium africanum* Lemmermann in G. S. West)	**6**	
Parvodinium umbonatum (Stein) S. Carty (Syn. *Peridinium umbonatum* Stein)	**9**	
Peridinium cinctum (O. F. Müller) Ehrenberg non *Peridinium cinctum* Penard	**6**	
Peridinium spp.	**9**	**8**
Tovellia coronata (Woloszynska) Moestrup, Lindberg & Daugbjerg (Syn. *Woloszynskia coronata* (Woloszynska) R. H. Thompson)	**6**	
CHLOROPHYTA		
Actinastrum aciculare Playfair	**3**	
Actinastrum rhaphidioides (Reinsch) Brunnthaler	**67**	

Acutodesmus acutiformis (Schröder) Tsarenko & D. M. John (Syn. *Enallax acutiformis* (Schröder) Hindák)	**3**	
Ankistrodesmus arcuatus Korshikov (Syn. *Monoraphidium arcuatum* (Korshikov) Hindák)	**3**	
Ankistrodesmus falcatus (Corda) Ralfs	**3**	
Ankistrodesmus stipitatus (Chodat) Komarková-Legnerová	**9**	
Binuclearia lauterbornii (Schmidle) Proschkina-Lavrenko (Syn. *Planctonema lauterbornii* Schmidle)	**6**	
Carteria sp. 1	**3**	
Carteria sp. 2		**3**
Chlamydomonas spp.	**30**	
Chlorella elongata (Hindák) C. Bock, Krienitz et Pröschold (Syn. *Dictyosphaerium elongatum* Hindák)	**3**	
Coelastrum astroideum De-Notaris	**6**	**3**
Coelastrum microporum Nägeli in A. Braun	**6**	
Coelastrum microporum var. *octaedricum* (Skuja) Sodomková	**3**	
Coelastrum proboscideum Bohlin in Wittrock et Nordstedt	**3**	
Coelastrum pseudomicroporum Korshikov	**9**	**3**
Coelastrum pulchrum Schmidle	**9**	
Coenochloris fottii (Hindák) Tsarenko	**21**	**8**
Coenochloris pyrenoidosa Korshikov (Syn. *Coenochloris hindakii* Komarek sensu Komarek et Fott)	**27**	**3**
Coenochloris sp.	**3**	
Desmodesmus abundans (Kirchner) Hegewald (Syn. *Scenedesmus quadrispina* Chodat)	**3**	
Desmodesmus brasiliensis (Bohlin) Hegewald		**3**
Desmodesmus communis (Hegewald) Hegewald (Syn. *Scenedesmus communis* (Turpin) Hegewald)	**33**	**11**
Desmodesmus denticulatus (Lagerheim) S. S. An, T. Friedl et Hegewald	**3**	
Desmodesmus insignis (West & G. S. West) E. Hegewald	**3**	**3**
Desmodesmus intermedius (Chodat) Hegewald	**3**	
Desmodesmus magnus (Meyen) Tsarenko (incl. Syn. *Scenedesmus oahuensis* (Lemmermann) G. M. Smith)	**36**	**13**
Desmodesmus opoliensis (Richter) Hegewald	**3**	

Taxa/Frequency quotients	FQLW	FQHW
Desmodesmus opoliensis var. *carinatus* (Lemmermann) Hegewald	**6**	**5**
Desmodesmus opoliensis var. *mononensis* (Chodat) Hegewald	**15**	**5**
Desmodesmus perforatus (Lemmermann) Hegewald	**24**	**11**
Desmodesmus pleiomorphus (Hindák) Hegewald		**3**
Desmodesmus protuberans (Fritsch & Rich) Hegewald	**3**	
Desmodesmus spinosus (Chodat) Hegewald	**3**	**3**
Desmodesmus subspicatus (Chodat) Hegewald & A. Schmidt in Hegewald (Syn. *Scenedesmus subspicatus* Chodat)	**3**	
Dictyosphaerium indicum Iyengar & Ramanathan		**3**
Dictyosphaerium subsolitarium Van Goor	**3**	
Eutetramorus fotti (Hindák) Komárek	**9**	
Eutetramorus polycoccus (Korshikov) Komárek	**6**	
Franceia armata (Lemmermann) Korshikov	**3**	
Hariotina reticulata Dangeard		**3**
Hindakia fallax (Komárek) C. Bock, Proschold & Krienitz (Syn. *Dictyosphaerium tetrachotomum* var. *fallax* Kom.)	**21**	
Hindakia tetrachotoma (Printz) C. Bock, Pröschold & Krienitz (Syn. *Dictyosphaerium tetrachotomum* Printz)	**6**	
Keriochlamys sp. ad *K. styriaca* Pascher	**3**	
Kirchneriella dianae var. *major* (Korshikov) Comas Gonzales	**3**	
Kirchneriella lunaris (Kirchner) Möbius	**3**	
Kirchneriella obesa (West) Schmidle	**9**	**3**
Lacunastrum gracillimum (West et G. S. West) H. McManus in McManus et al.	**9**	
Lagerheimia balatonica (Scherffel) Hindák		**3**
Lagerheimia chodatii Bernard	**3**	
Lemmermannia triangularis (Chodat) C. Bock & Krienitz in C. Bock et al. (Syn. *Tetrastrum triangulare* (Chodat) Komárek)		**3**
Lobocystis planctonica (Tiffany & Ahlstrom) Fott	**3**	
Messastrum gracile (Reinsch) T. S. Garcia in T. S. Garcia et al. (Syn. *Selenastrum gracile* Reinsch)	**15**	**3**

Microspora sp. (fragments)		**3**
Monactinus simplex (Meyen) Corda	**3**	
Monactinus simplex var. *echinulatum* (Wittrock) Pérez, Maidana et Comas		**5**
Monactinus simplex var. *sturmii* (Reinsch) Pérez Baliero et al.	**3**	
Monoraphidium caribeum Hindák	**3**	
Monoraphidium griffithii (Berkeley) Komarková-Legnerová	**6**	
Monoraphidium komarkovae Nygaard	**9**	
Monoraphidium saxatile Komárková-Legnerová 1969 (Syn. *Chlorolobion saxatile* (Komárkova-Legnerova) Komárek)	**3**	
Mucidosphaerium pulchellum (Wood) C. Bock, Pröschold & Krienitz	**12**	**3**
Mychonastes botrytella (Komárek & Perman) Krienitz, C. Bock, Dadheech & Proschold (Syn. *Dictyosphaerium botrytella* Komárek & Perman)		**3**
STREPTOPHYTA		
Actinotaenium globosum (Bulnheim) Teiling	**9**	
Actinotaenium sp.	**3**	
Closterium angustatum Kützing ex Ralfs		**3**
Closterium gracile Brébisson ex Ralfs	**3**	**3**
Closterium limneticum Lemmermann	**21**	**3**
Closterium lineatum Ehrenberg ex Ralfs	**3**	
Closterium moniliferum (Bory) Ehrenberg ex Ralfs	**3**	
Closterium praelongum Brébisson	**3**	
Closterium pronum Brébisson	**3**	
Closterium strigosum Brébisson	**12**	
Closterium sp.	**3**	
Cosmarium abbreviatum Raciborski	**3**	
Cosmarium contractum Kirchner	**6**	
Cosmarium laeve Rabenhorst	**3**	**3**
Cosmarium obtusatum (Schmidle) Schmidle	**3**	
Cosmarium phaseolus Brébisson ex Ralfs		**3**
Cosmarium tenue W. Archer	**3**	
cf. *Desmidium* (fragments)	**9**	**8**
Gonatozygon sp.		**3**

Taxa/Frequency quotients	**FQLW**	**FQHW**
Hyalotheca cf. *mucosa* Ralfs	**36**	**16**
Klebsormidium sp. (fragments)	**27**	**11**
Mesotaenium cf. *macrococcum* (Kützing ex Kützing) Roy et Bisset	**3**	
Mougeotia spp. st.	**3**	**8**
Penium - zygotes	**3**	
cf. *Pleurotaenium nodosum* (Bailey ex Ralfs) P. Lundell		**3**
Spirogyra spp. st.		**8**
Staurastrum brevispina Brébisson in Ralfs (Syn. *Staurodesmus brevispina* (Brébisson) Croasdale)		**3**
Staurastrum chaetoceros (Schröder) Smith	**3**	
Staurastrum pingue Teiling	**3**	**3**
Staurastrum pingue var. *planctonicum* (Teiling) Coesel & Meersters (Syn. *Staurastrum planctonicum* Teiling)	**3**	**5**
Staurastrum tetracerum Ralfs ex Ralfs	**3**	
Staurastrum volans West et G. S. West	**3**	
Staurodesmus bulnheimii (Raciborski) Round & A. J. Brook	**3**	
Staurodesmus cuspidatus (Brébisson) Teiling	**9**	**3**
Staurodesmus dejectus (Brébisson) Teiling	**3**	
Staurodesmus glaber (Ralfs) Teiling	**3**	
Xanthidium sp.	**3**	
OCHROPHYTA		
Chrysophyceae		
Bicosoeca cylindrica (Lackey) Bourrelly	**3**	**3**
Bicosoeca cf. *eurystoma* Hilliard	**3**	**3**
Chrysamoeba scherfelii (Pascher) Matvienko	**6**	
Chrysopyxis iwanoffii Lauterborn	**6**	**3**
Chrysopyxis paludosa Fott		**3**
Codosiga cf. *botrytis* (Ehrenberg) Kent	**3**	
Codosiga sp.		**3**
Codonosigopsis sp.		**3**
Dinobryon sp. (?*D*. cf. *belingii* Svirenko)		**3**
Dinobryon sp. ad *D. dilatatum* Hilliard		**3**
Diploeca elongata (Fott) P. Bourrelly	**6**	**21**

Diploeca flava (Korshikov) Bourrelly 1957		**8**
Diplosigopsis affinis Lemmermann	**21**	**5**
Epipyxis cf. *epiplanctica* (Skuja) Hilliard et Asmund	**3**	
Epipyxis utriculus (Ehrenberg) Ehrenberg 1838		**3**
Lagynion infundibuliforme Starmach 1966		**3**
Lagynion oblongum (Pascher) Bourrelly		**3**
Salpingoeca cf. *convolvulus* Skuja	**3**	
Salpingoeca eurystoma Stokes 1886		**34**
Salpingoeca cf. *vaginicola* Stein 1878	**3**	
Salpingoeca sp. ad *S. cylindrica* Fott	**9**	
Salpingoeca sp.	**9**	**26**
Stokesiella sp. ad *Stokesiella epipyxis* Pascher 1930	**12**	**3**
Stokesiella sp. ad *Stokesiella longipes* (Stokes) Lemmermann 1908	**3**	
Synurophyceae		
Mallomonas sp.	**3**	
Tribophyceae		
Bumilleriopsis brevis (Gerneck) Printz 1914	**3**	
Centritractus africanus F. E. Fritsch & M. F. Rich in F. E. Fritsch, M. F. Rich & M. L. Stephens 1930	**18**	
Centritractus ellipsoideus Starmach 1966	**3**	
Eustigmatophyceae		
Tetraëdriella regularis (Kützing) Fott 1967		**3**
Tetraëdriella spinigera Skuja 1948	**3**	**3**
Bacillariophyceae		
Achnanthes inflata (Kützing) Grunow	**16**	**4**
Achnanthidium affine (Grunow) Czarnecki	**32**	**4**
Achnanthidium exiguum (Grunow) Czarnecki	**52**	**15**
Achnanthidium exiguum var. *constrictum* (Grunow) Andresen, Stoermer et Kreis		**19**
Achnanthidium lineare W. Smith	**26**	**12**
Achnanthidium minutissimum (Kützing) Czarnecki	**68**	**38**
Achnanthidium reimeri (Camburn) Ponader et Potapova	**10**	
Achnanthidium subhudsonis (Hustedt) Kobayasi	**71**	**62**
Achnanthidium sp. (connective view)	**23**	**8**

Taxa/Frequency quotients	FQLW	FQHW
Actinella punctata Lewis	**3**	**4**
Actinocyclus normanii Gregory (Hustedt)	**3**	
Adlafia bryophila (Petersen) Moser, Lange-Bertalot et Metzeltin		**19**
Amphora copulata (Kützing) Schoeman et R. E. M.Archibald	**6**	**8**
Anomoeoneis sphaerophora Pfitzer	**3**	
Aulacoseira agassizii (Ostenfeld) Simonsen	**100**	**96**
Aulacoseira agassizii var. *malayensis* (Hustedt) Simonsen	**10**	**4**
Aulacoseira ambigua (Grunow) Simonsen	**100**	**100**
Aulacoseira granulata (Ehrenberg) Simonsen	**87**	**88**
Aulacoseira granulata var. *angustissima* (O. F. Müller) Simonsen	**100**	**92**
Aulacoseira herzogii (Lemmermann) Simonsen	**52**	**46**
Aulacoseira minuscula Tremarin, Torgan et Ludwig	**23**	**23**
Aulacoseira pusilla (Meister) Tuji et Houk	**19**	**69**
Bacillaria paxillifera (O. F. Müller) Marsson	**6**	**8**
Brachysira brebissonii Lange-Bertalot et Moser	**16**	**8**
Brachysira vitrea (Grunow) Ross		**4**
Caloneis bacillum (Grunow) Cleve	**29**	**15**
Capartogramma crucicula (Grunow) Ross	**32**	**27**
Cavinula cocconeiformis (Gregory ex Greville) Mann et Stickle		
Cavinula variostriata (Krasske) Mann et Stickle		
Cocconeis pediculus Ehrenberg	**3**	
Cocconeis placentula Ehrenberg	**55**	**27**
Cocconeis placentula var. *euglypta* (Ehrenberg) Grunow	**3**	**4**
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck		**4**
Cocconeis scutellum Ehrenberg	**58**	**23**
Craticula cuspidata (Kützing) Mann	**3**	
Craticula molestiformis (Hustedt) Mayama	**19**	
Cyclostephanos invisitatus (Hohn et Hellermann) Theriot, Stoermer et Håkansson	**90**	**58**
Cyclotella atomus Hustedt	**87**	**46**
Cyclotella cf. *cyclopuncta* Håkansson et Carter	**3**	**4**
Cyclotella meneghiniana Kützing	**100**	**62**

Cymatopleura solea (Brébisson) W. Smith	**16**	**8**
Cymbopleura frequens Krammer		**4**
Cymbopleura naviculiformis (Auerswald ex Heiberg) Krammer	**3**	
Diadesmis confervacea Kützing	**68**	**31**
Diadesmis gallica W. Smith	**13**	**42**
Diploneis elliptica (Kütz.) Cleve	**0**	**8**
Discostella pseudostelligera (Hustedt) Houk et Klee	**97**	**69**
Discostella stelligera (Cleve et Grunow) Houk et Klee	**16**	**27**
Dorofeyukea kotschyi (Grunow) Kulikovskiy, Kociolek, Tusset et T. Ludwig		**4**
Encyonema caespitosum Kützing		**4**
Encyonema silesiacum (Bleisch) Mann	**13**	**19**
Encyonema minutum (Hilse) Mann	**16**	**4**
Encyonopsis cesatii (Rabenhorst) Krammer	**6**	
Encyonopsis krammeri Reichardt		**8**
Eolimna minima (Grunow) Lange-Bertalot et Schiller	**23**	**12**
Eolimna subminuscula (Manguin) Moser, Lange-Bertalot et Metzeltin	**6**	**8**
Epithemia adnata (Kützing) Brébisson	**6**	
Epithemia operculata (C. Agardh) Ruck et Nakov	**3**	
Eunotia bilunaris (Ehrenberg) Schaarschmidt		**8**
Eunotia exigua (Brébisson ex Kützing) Rabenhorst	**6**	
Eunotia flexuosa (Brébisson ex Kützing) Kützing		**12**
Eunotia formica Ehrenberg	**3**	**4**
Eunotia incisa W. Smith ex W. Gregory	**6**	**15**
Eunotia perminuta (Grunow) R. M. Patrick		**23**
Eunotia minor (Kützing) Grunow	**52**	**23**
Eunotia cf. *paludosa* Grunow	**10**	**4**
Eunotia pectinalis (Kützing) Rabenhorst	**23**	**15**
Eunotia praerupta Ehrenberg	**6**	**8**
Eunotia praerupta var. *excelsa* Krasske	**6**	
Eunotia rhomboidea Hustedt	**10**	
Eunotia tenella (Grunow) Hustedt	**32**	**38**
Eunotia cf. *tetraodon* Ehrenberg	**3**	

Taxa/Frequency quotients	**FQLW**	**FQHW**
Eunotia zasuminensis (Cabejszekowna) Körner	**65**	**65**
Eunotia spp. (connective view)		**23**
Fallacia meridionalis Metzeltin, Lange-Bertalot et García-Rodríguez	**16**	
Fallacia subhamulata (Grunow) D. G. Mann	**3**	
Fragilaria berolinensis (Lemmermann) Lange-Bertalot		**73**
Fragilaria capucina Desmazières	**13**	
Fragilaria rumpens (Kützing) Carlson	**65**	**31**
Fragilaria tenera (W.Smith) Lange-Bertalot	**35**	**4**
Fragilaria tenuissima Lange-Bertalot et Ulrich	**100**	**58**
Fragilaria vaucheriae (Kützing) Petersen	**13**	**19**
Frustulia rhomboides (Ehrenberg) De Toni	**26**	**19**
Frustulia saxonica Rabenhorst	**6**	**8**
Frustulia vulgaris (Thwaites) De Toni	**3**	
Geissleria decussis (Østrup) Lange-Bertalot et Metzeltin	**29**	**19**
Geissleria ignota (Krasske) Lange-Bertalot et Metzeltin	**3**	**4**
Gomphonema affine Kützing		**23**
Gomphonema affine var. *insigne* (W.Gregory) G.W.Andrews	**16**	**4**
Gomphonema cf. *angustatum* (Kützing) Rabenhorst	**3**	**27**
Gomphonema augur Ehrenberg		**4**
Gomphonema gracile Ehrenberg	**6**	
Gomphonema grande Karthick, Kociolek, Taylor et Cocquyt	**3**	
Gomphonema cf. *lagenula* Kützing	**16**	**15**
Gomphonema minutum (Agardh) Agardh	**13**	
Gomphonema parvulum (Kützing) Kützing	**23**	**8**
Gomphoshenia grovei (Schmidt) Lange-Bertalot	**6**	**4**
Gomphoshenia cf. *lingulatiformis* (Lange-Bertalot et Reichardt) Lange-Bertalot	**13**	
Gyrosigma scalproides (Rabenhorst) Cleve	**3**	
Gyrosigma acuminatum (Kützing) Rabenhorst	**16**	**19**
Halamphora coffaeiformis (Agardh) Levkov		**12**
Halamphora montana (Krasske) Levkov	**13**	**8**
Hantzschia amphioxys (Ehrenberg) Grunow	**6**	**19**

Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski	**6**	**4**
Hippodonta hungarica (Grunow) Lange-Bertalot, Metzeltin et Witkowski	**6**	**4**
Humidophila contenta (Grunow) Lowe, Kociolek, J. R. Johansen, Van de Vijver, Lange-Bertalot et Kopalová	**87**	**50**
Karayevia clevei (Grunow) Round et Bukhtiyarova	**10**	**8**
Karayevia laterostrata (Hustedt) Round et Bukhtiyarova	**3**	
Kobayasiella jaagii (Meister) Lange-Bertalot	**10**	
Lemnicola hungarica (Grunow) Round et Basson	**6**	**4**
Luticola acidoclinata Lange-Bertalot	**10**	**8**
Luticola cohnii (Hilse) D. G. Mann	**35**	**19**
Luticola mutica (Kützing) D. G. Mann	**71**	**35**
Luticola saxophila (Bock ex Hustedt) D. G. Mann	**26**	**15**
Mayamaea atomus (Kützing) Lange-Bertalot	**3**	**8**
Melosira varians Agardh	**3**	
Navicula cf. *heimansii* van Dam et Kooyman	**97**	**62**
Navicula cincta (Ehrenberg) Ralfs	**6**	**4**
Navicula cryptocephala Kützing		**27**
Navicula nielsfogedii Taylor et Cocquyt	**97**	**65**
Navicula schroeteri F. Meister	**0**	**4**
Navicula vandamii Schoeman et Archibald	**90**	**92**
Navicula viridula (Kützing) Ehrenberg	**6**	
Navicula viridula var. *rostellata* (Kützing) Cleve	**10**	**27**
Navicula spp.	**13**	**4**
Neidium affine (Ehrenberg) Pfizer	**6**	
Neidium cf. *alpinum* Hustedt	**6**	
Neidium cf. *hitchcockii* (Ehrenberg) Cleve	**3**	
Neidium productum (W.Smith) Cleve		**8**
Nitzschia cf. *accomodata* Hustedt	**90**	**85**
Nitzschia acicularis (Kützing) W. Smith	**10**	**12**
Nitzschia acidoclinata Lange-Bertalot	**10**	
Nitzschia cf. *archibaldii* Lange-Bertalot	**3**	
Nitzschia capitellata Hustedt	**13**	**38**
Nitzschia cf. *congolensis* Hustedt	**6**	**38**

Taxa/Frequency quotients	**FQLW**	**FQHW**
Nitzschia cf. *graciliformis* Lange-Bertalot et Simonsen	**19**	**8**
Nitzschia frustulum (Kützing) Grunow	**13**	**12**
Nitzschia gracilis Hantzsch	**65**	**27**
Nitzschia heufleriana Grunow	**3**	
Nitzschia inconspicua Grunow	**10**	**50**
Nitzschia intermedia Hantzsch ex Cleve et Grunow	**48**	**27**
Nitzschia irremissa Chlonoky		**4**
Nitzschia cf. *lacuum* Lange-Bertalot	**100**	**92**
Nitzschia lancettula O. F. Müller	**100**	**100**
Nitzschia linearis (Agardh) W. Smith	**3**	**8**
Nitzschia linearis var. *tenuis* (W. Smith) Grunow	**13**	**4**
Nitzschia lorenziana Grunow	**13**	**4**
Nitzschia lorenziana var. *incerta* Grunow in Cleve et Grunow (Syn. *N. reversa* W. Smith)	**16**	**8**
Nitzschia palea (Kützing) W. Smith		**12**
Nitzschia paleacea Grunow	**61**	**38**
Nitzschia paleaeformis Hustedt	**6**	**4**
Nitzschia peisonis Pantocsek	**6**	
Nitzschia pumila Hustedt	**19**	**27**
Nitzschia rostellata Hustedt in Schmidt et al.	**58**	**23**
Nitzschia sigma (Kützing) W. Smith	**32**	**15**
Nitzschia cf. *soratensis* Morales et Vis		**23**
Nitzschia spiculum Hustedt		**4**
Nitzschia subacicularis Hustedt		**4**
Nitzschia tropica Hustedt	**84**	**73**
Nupela wellneri (Lange-Bertalot) Lange-Bertalot	**39**	**12**
Nupela cf. *neglecta* Ponader, Lowe et Potapova	**6**	**31**
Orthoseira roseana (Rabenhorst) O'Meara	**32**	**23**
Pantocsekiella ocellata (Pantocsek) K. T. Kiss et Ács	**16**	
Peronia fibula (Brébisson ex Kützing) Ross	**55**	**12**
Pinnularia acoricola Hustedt	**3**	**8**
Pinnularia acutobrebissonii Kulikovskiy, Lange-Bertalot et Metzeltin	**10**	
Pinnularia divergentissima (Grunow) Cleve	**6**	**4**

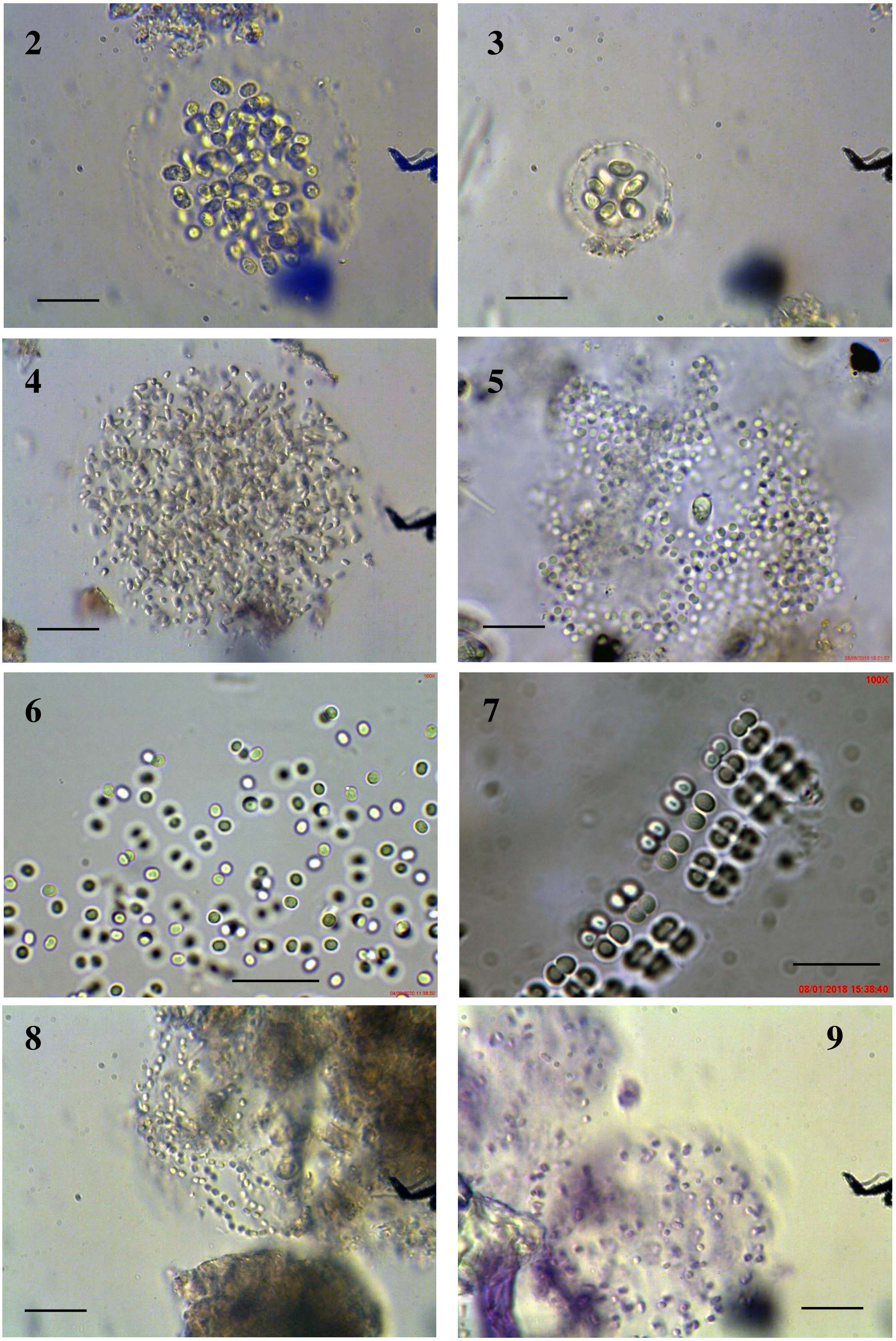
Pinnularia gibba Ehrenberg	**6**	**8**
Pinnularia microstauron (Ehrenberg) Cleve	**10**	
Pinnularia subcapitata Gregory	**16**	**35**
Pinnularia viridis (Nitzsch) Ehrenberg	**6**	
Pinnularia spp.		**15**
Placoneis clementis (Grunow) Cox	**13**	
Placoneis dicephala (W. Smith) Mereschkovsky		**4**
Placoneis exigua (Gregory) Mereschkovsky	**16**	
Placoneis exiguiformis (Hustedt) Lange-Bertalot	**0**	**4**
Placoneis elginensis (Gregory) Cox	**13**	**15**
Placoneis gastrum (Ehrenberg) Mereschkovsky	**23**	**15**
Placoneis cf. *pseudanglica* Cox	**19**	**23**
Plagiotropis lepidoptera var. *proboscidea* (Cleve) Reimer	**3**	
Planothidium daui (Foged) Lange-Bertalot	**19**	**12**
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	**45**	**8**
Planothidium lanceolatum (Brébisson ex Kützing) Bukhtiyarova	**13**	**12**
Planothidium rostratum (Østrup) Lange-Bertalot	**77**	**73**
Planothidium sp.	**3**	**8**
Platessa bahlsii Potapova	**26**	**4**
Platessa hustedtii (Krasske) Lange-Bertalot	**55**	**38**
Pleurosira laevis (Ehrenberg) Compère	**26**	**23**
Psammothidium cf. *daonense* (Lange-Bertalot) Lange-Bertalot	**45**	**4**
Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round		**35**
Pseudofallacia tenera (Hustedt) Liu, Kociolek et Wang	**61**	**23**
Pseudostaurosira brevistriata (Grunow) Williams et Round	**68**	**38**
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	**3**	
Rhopalodia acuminata var. *protracta* (Grunow) Krammer	**3**	
Rhopalodia gibba (Ehrenberg) O. Müller		**4**
Rhopalodia gibberula (Ehrenberg) O. Müll.		**8**
Rhopalodia sp.		**8**
Sellaphora bacillum (Ehrenberg) Mann	**13**	**8**

Taxa/Frequency quotients	FQLW	FQHW
Sellaphora laevissima (Kützing) Mann	**3**	**4**
Sellaphora cf. *meridionalis* Potapova et Ponader	**3**	**4**
Sellaphora nyassensis (O. Müller) Mann	**13**	**8**
Sellaphora pupula (Kützing) Mereschkovsky	**55**	**27**
Sellaphora pseudoarvensis (Hustedt) Wetzel et Ector	**23**	
Sellaphora pseudoventralis (Hustedt) Chudaev et Gololobova	**10**	
Sellaphora pulchra Enache et Potapova	**6**	
Sellaphora seminulum (Grunow) D. G. Mann	**3**	**46**
Sellaphora vitabunda (Hustedt) Mann	**10**	**8**
Sellaphora wallacei (Reimer) Potapova et Ponader	**6**	**4**
Sellaphora sp.	**6**	
Simonsenia delognei (Grunow) Lange-Bertalot	**3**	
Skeletonema potamos (Weber) Hasle		**8**
Stauroneis anceps Ehrenberg	**3**	
Stauroneis kriegeri Patrick	**16**	**8**
Stauroneis livingstonii Reimer	**13**	**4**
Staurosira construens Ehrenberg	**10**	**58**
Staurosira construens var. *venter* (Ehrenberg) Hamilton	**45**	**8**
Staurosirella leptostauron (Ehrenberg) Williams et Round	**97**	**100**
Staurosirella pinnata (Ehrenberg) Williams et Round	**97**	**96**
Stenopterobia curvula (W. Smith) Krammer		**15**
Stenopterobia delicatissima (Lewis) Brébisson ex van Heurck	**10**	**8**
Stephanodiscus minutulus (Kützing) Cleve et Möller	**52**	**50**
Surirella birostrata Hustedt	**3**	
Surirella cf. *congolensis* Cocquyt et Taylor	**13**	**8**
Surirella cf. *linearis* W. Smith	**6**	
Surirella linearis var. *constricta* Hustedt	**10**	**8**
Surirella cf. *minuta* Brébisson	**3**	
Surirella cf. *splendida* (Ehrenberg) Kützing	**3**	
Surirella tenera Gregory	**26**	**15**
Surirella sp.		**4**
Thalassiosira faurii (Gasse) Hasle		**4**
Thalassiosira rudolfii (Bachmann) Hasle	**97**	**46**

Thalassiosira weissflogii (Grunow) G. Fryxell et Hasle	**81**	**46**
Tryblionella apiculata Gregory	**10**	**8**
Tryblionella calida (Grunow) Mann	**84**	**23**
Tryblionella coarctata (Grunow) Mann	**10**	**15**
Tryblionella hungarica (Grunow) Frenguelli	**23**	**15**
Tryblionella gracilis W. Smith	**3**	
Tryblionella levidensis W. Smith	**68**	**46**
Ulnaria acus (Kützing) Aboal	**23**	**4**
Ulnaria danica (Kützing) Compère et Bukhtiyarova	**58**	**23**
Ulnaria delicatissima (Grunow) Aboal et P. C. Silva		**8**
Ulnaria ulna (Nitzsch) Compère	**58**	**31**

taxonomic groups. They were followed by chlorophytes (108 taxa, or 21%) and cyanoprokaryotes (76 taxa, or 15%). - **Fig. 84**. Cyanoprokaryotes were represented only by coccal colonial (60%) and non-heterocytous filamentous species (40%). The lack of heterocytous cyanoprokaryotes capable of efficient nitrogen fixation, clearly indicates that there was no nitrogen limitation, or nitrogen shortage in riverine waters. This result is in accordance with the relatively high ammonium and nitrate values recorded during both studied periods (mean values of 2.3 μM and 11.3 μM in HW, and 1.3 μM and 36.7 μM in FW – Descy et al. 2016). Among green algae coccal species prevailed (92% in Chlorophyta and 90% in Streptophyta) over filamentous algae (5% among chlorophytes and 10% in streptophytes) and flagellates (3% from chlorophytes). Although the number of identified species is high, considering the presence of taxa which could not be certainly identified in fixed samples (green flagellates, euglenophytes, pyrrhophytes, *etc.*) it is evident that our knowledge about the river algal biodiversity is far from complete and further studies are needed.

The number of taxa during the HW period was 314, compared with 431 in the FW period. This difference shows that 83% of the taxa occur during low water periods, and 60% of all taxa were recorded in high water periods. Logically, all taxonomic groups were more diverse during FW (**Fig. 85**), except the insignificantly higher numbers of species for chrysophyceans and eustigmatophyceans during HW period (**Table 1**). Algae from Cryptophyta, Tribophyceae and Synurophyceae were found only during low waters (**Fig. 85**). Although the general phytoplankton structure considered in terms of represented algal divisions is similar, we have to note that while cyanoprokaryotes comprised almost the same part of this structure during both FW and HW periods (14% and 13%, respectively), the contribution of chlorophytes during the HW period was twice lower in comparison with LW (21% and 12%, respectively), and the participation of diatoms was 14% lower during HW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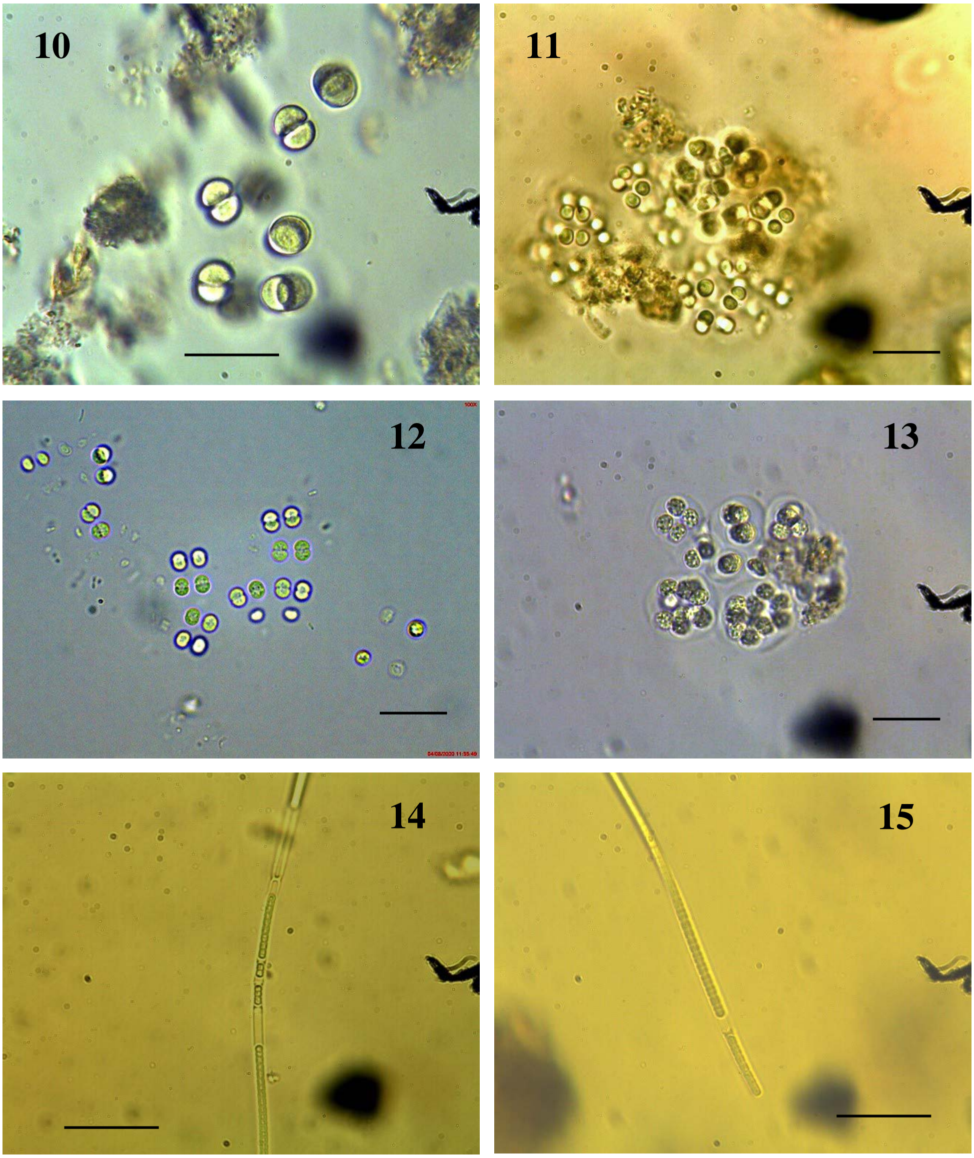
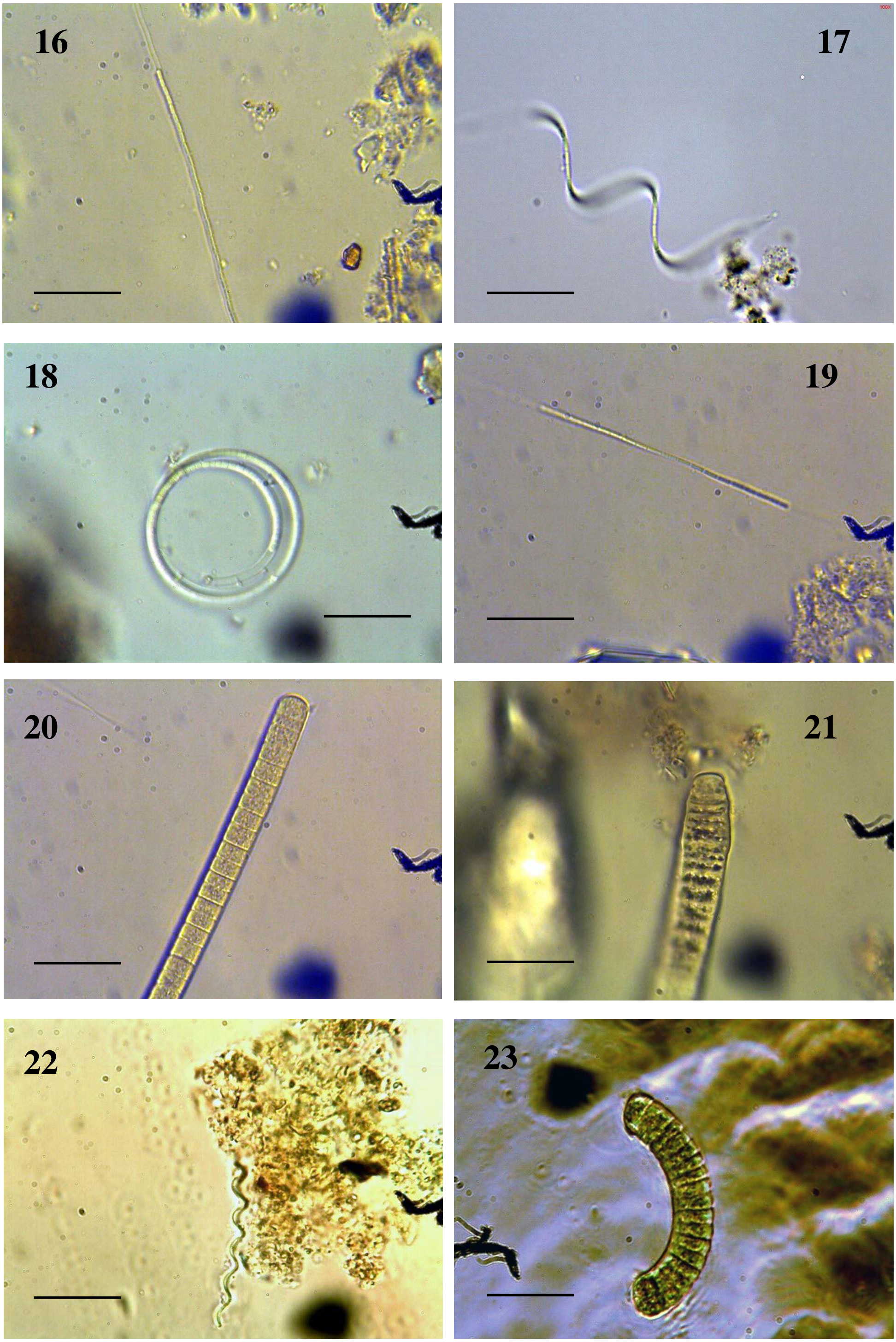


Figs. 2-15. 2, 3 - *Aphanothece elabens* (Brébisson ex Meneghini) Elenkin 1938; 4 - *Anathece smithii* (Komárková-Legnerová & Cronberg) Komárek, Kastovsky & Jezberová; 5 - *Aphanocapsa holsatica* (Lemmermann) Cronberg et Komárek; 6 - *Aphanocapsa koordersii* K. M. Strøm; 7 - *Merismopedia punctata* Meyen; 8 - *Cyanodictyon* cf. *tubiforme* Cronberg; 9 - *Cyanodictyon* cf. *iac* Cronberg et Komárek - staining with Gentian violet; 10 - *Limnococcus limneticus* (Lemmermann) Komárková, Jezberová, O. Komárek et Zapomélová; 11 - *Chroococcus vacuolatus* Skuja; 12 - *Chroococcus planctonicus* Bethge; 13 - *Microcystis wesenbergii* (Komárek) Komárek ex Komárek in Joosen; 14, 15 - *Planktolyngbya* cf. *brevicellularis* Cronberg et Komárek. Scale bar is 10 μm.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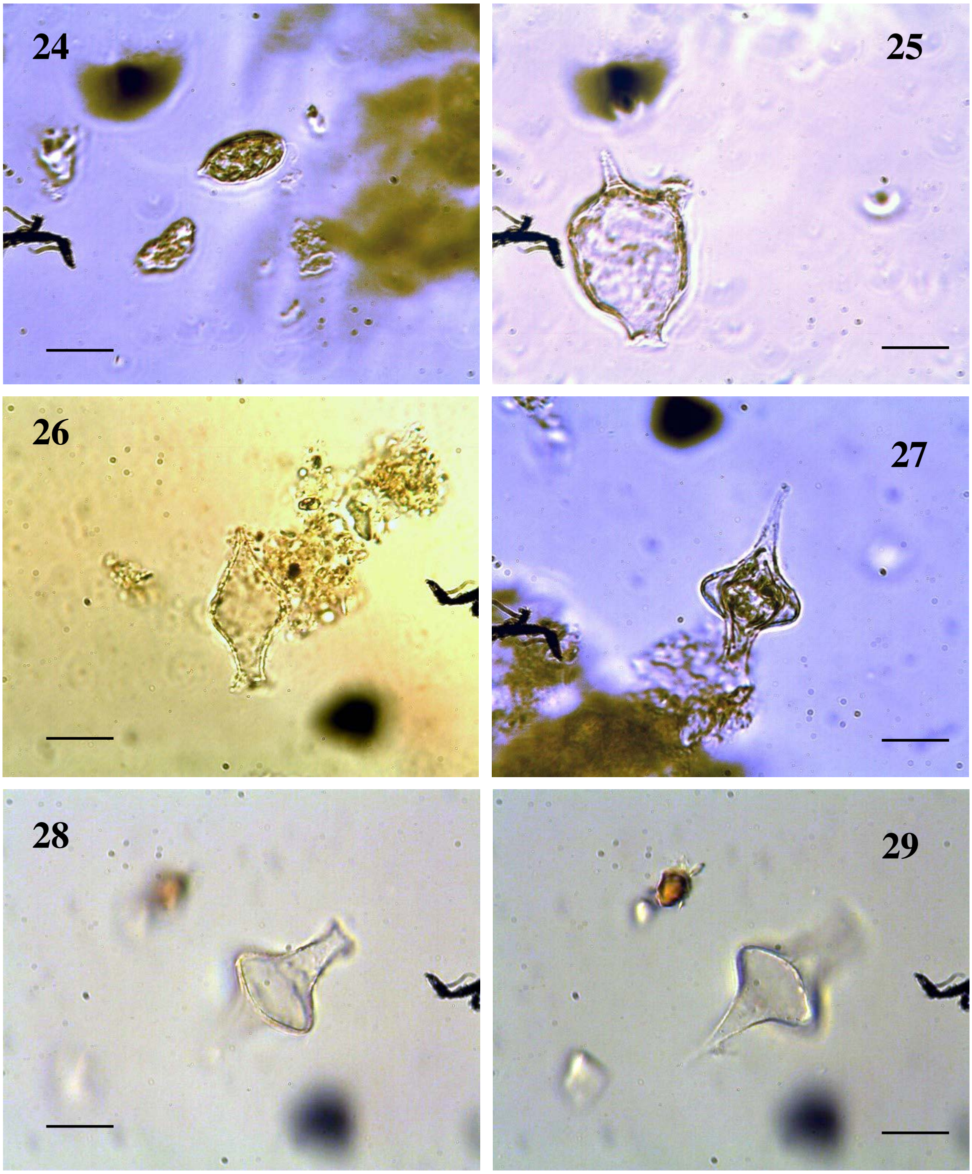
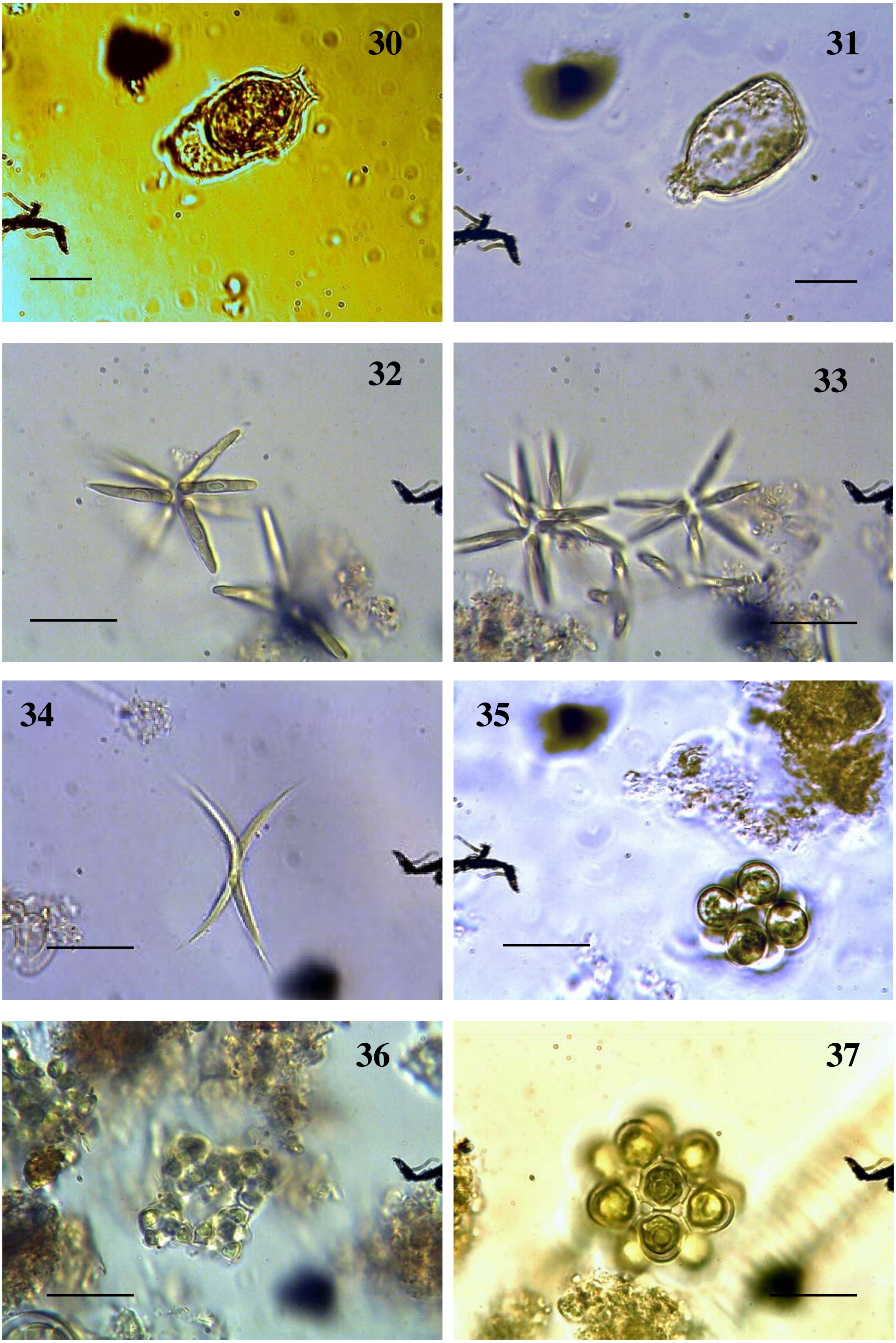


Figs. 16-29. 16 - *Planktolyngbya undulata* Komárek et Kling; 17 - *Planktolyngbya microspira* Komárek et Cronberg; 18 - *Limnolyngbya circumcreta* (G. S. West) X. Li & R. Li; 19 - *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová et Cronberg; 20 - *Oscillatoria simplicissima* Gomont; 21 - *Oscillatoria sancta* Kützing ex Gomont; 22 - *Spirulina corakiana* Playfair; 23 - cf. *Hormoscilla* sp.; 24 - *Euglena* sp.; 25 - *Strombomonas acuminata* (Schmarda) Deflandre; 26 - *Strombomonas* sp.; 27-29 - *Strombomonas* cf. *gibberosa* (Playfair) Deflandre. Scale bar is 10 µm.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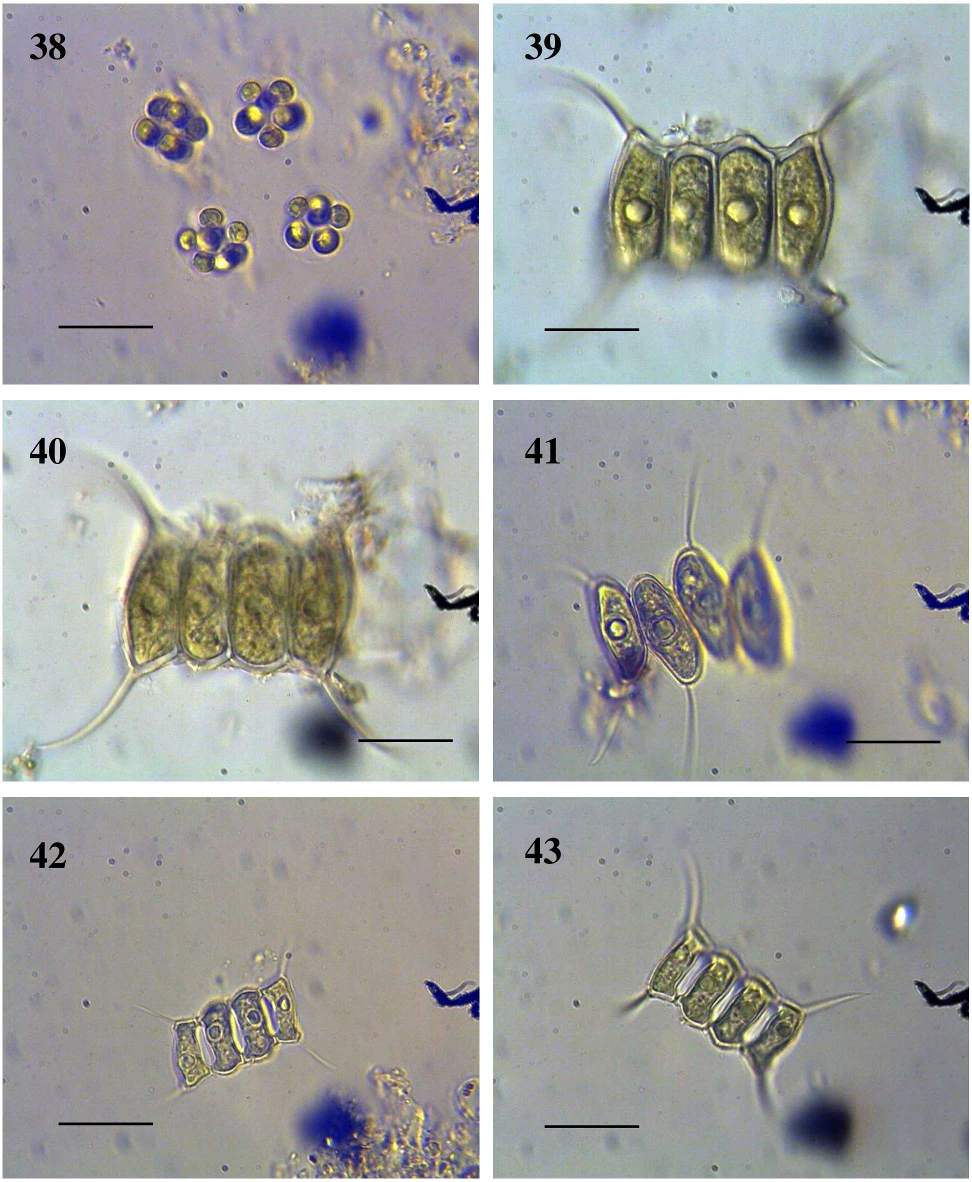
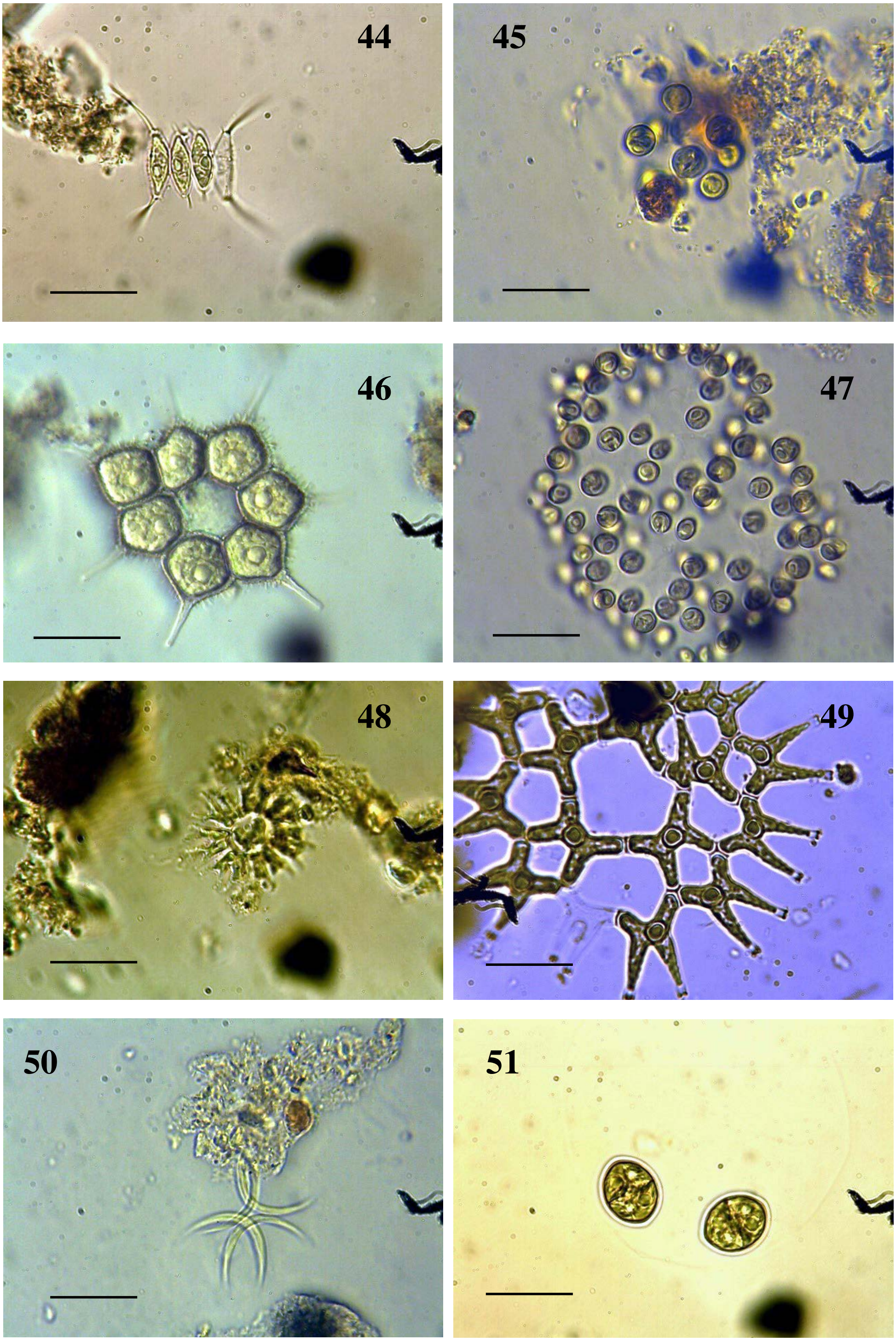


Figs. 30-43. 30 - *Trachelomonas* sp.; 31 - *Trachelomonas* cf. *piscicatoris* A. C. Stokes; 32-33 - *Actinastrum rhaphidioides* (Reinsch) Brunnthaler; 34 - *Ankistrodesmus falcatus* (Corda) Ralfs; 35 - *Coelastrum microporum* var. *octaedricum* (Skuja) Sodomková; 36 - *Coelastrum proboscideum* Bohlin in Wittrock et Nordstedt; 37 - *Coelastrum pseudomicroporum* Korshikov; 38 - *Coenochloris fottii* (Hindák) Tsarenko; 39, 40 - *Desmodesmus communis* (Hegewald) Hegewald; 41 - *Desmodesmus magnus* (Meyen) Tsarenko; 42, 43 - *Desmodesmus perforatus* (Lemmermann) Hegewald. Scale bar is 10 µm.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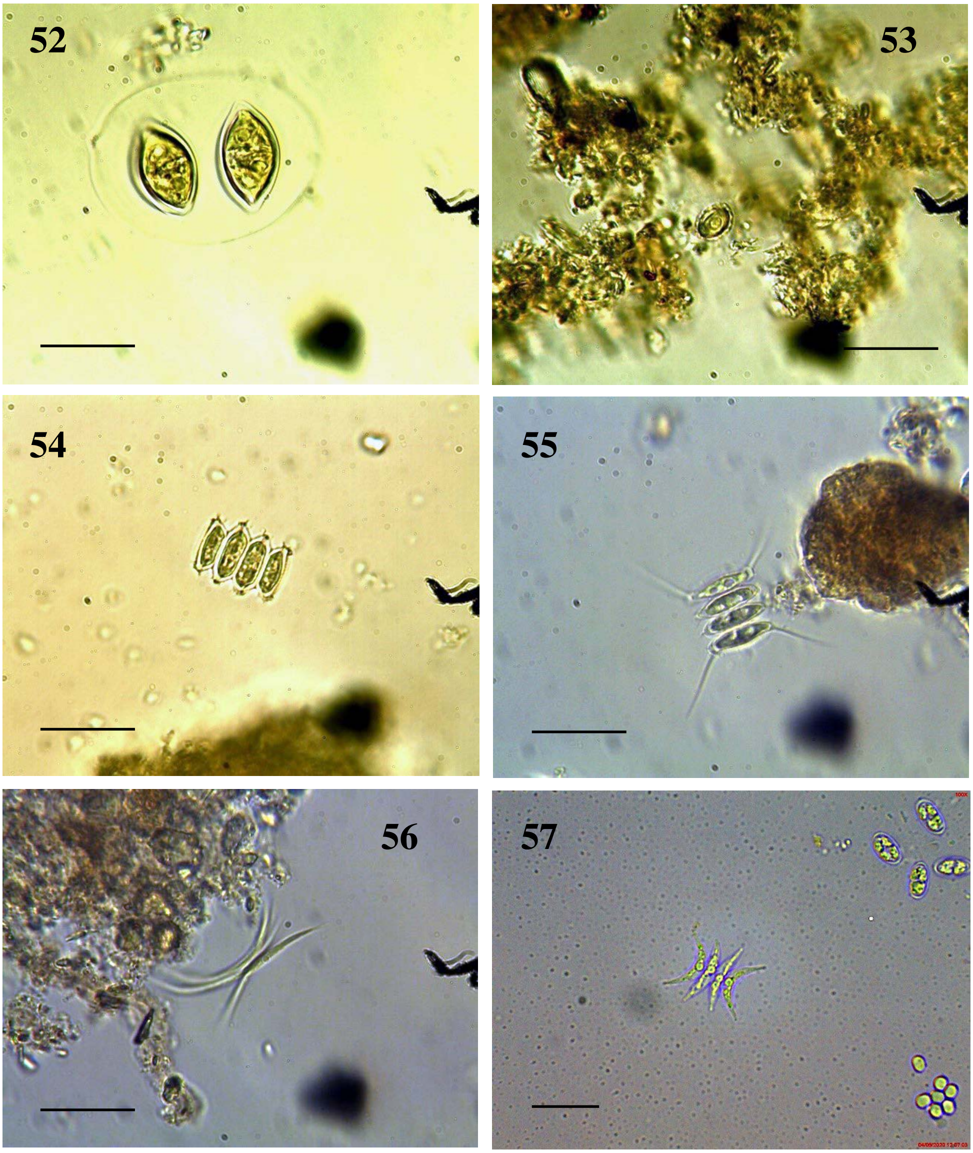
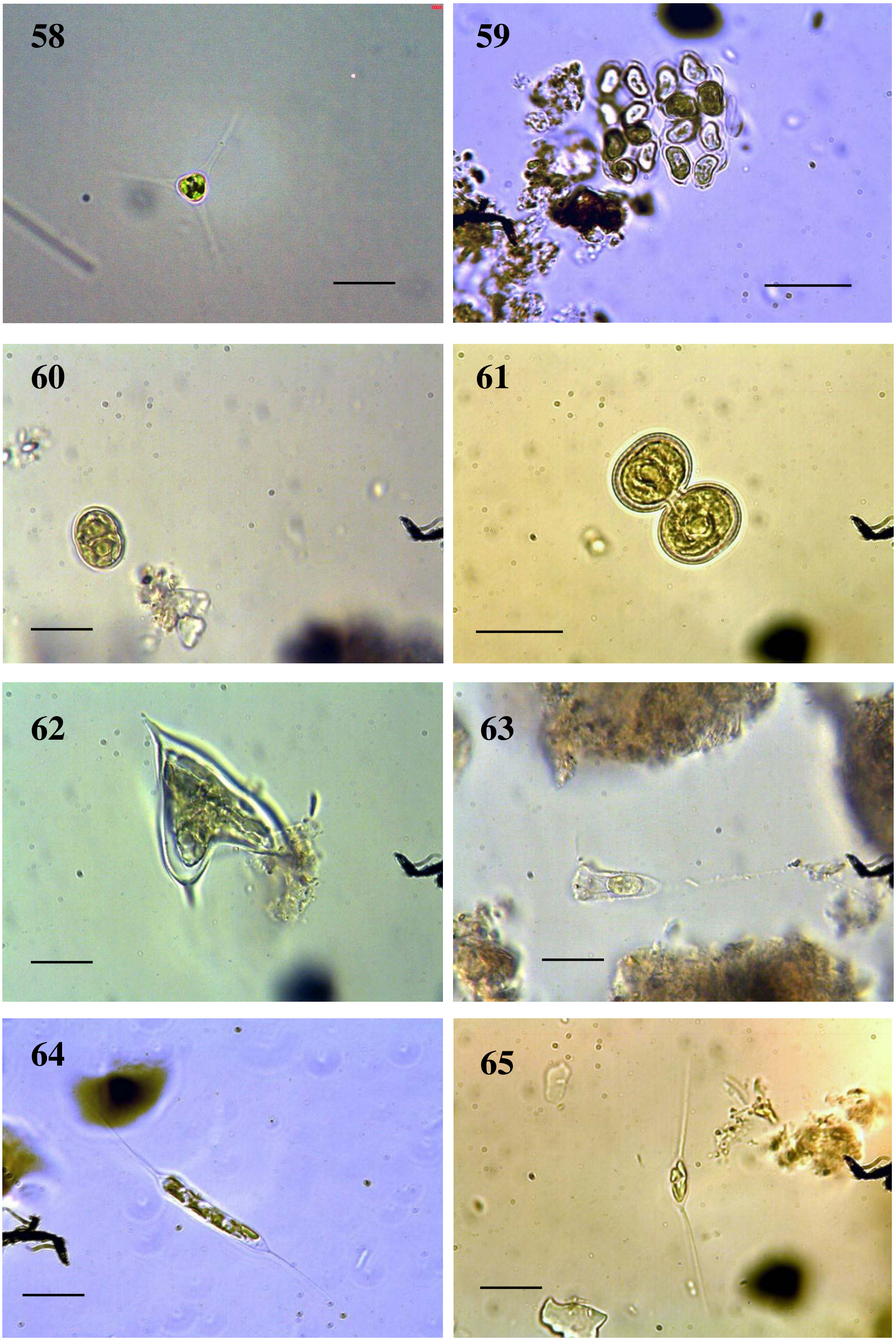


Figs. 44-57. 44 - *Desmodesmus protuberans* (Fritsch & Rich) Hegewald; 45 - *Eutetramorus polycoccus* (Korshikov) Komárek; 46 - *Monactinus simplex* var. *echinulatum* (Wittrock) Pérez, Maidana et Comas; 47 - *Mucidosphaerium pulchellum* (Wood) C. Bock, Pröschold & Krienitz; 48 - *Parapediastrum biradiatum* var. *longecornutum* (Gutwinski) Tsarenko; 49 - *Lacunastrum gracillimum* (West et G. S. West) H. McManus in McManus et al.; 50 - *Messastrum gracile* (Reinsch) T. S. Garcia in T. S. Garcia et al.; 51 - *Oocystis lacustris* Chodat; 52- *Oocystis* cf. *marssonii* Lemmermann; 53 - *Phacotus* sp. (?*Sestoma* sp.); 54 - *Scenedesmus praetervisus* Chodat; 55 - *Scenedesmus protuberans* var. *minor* Ley; 56 - *Selenastrum subtile* (Hindák) P. Marvan, Komárek & Comas; 57 - *Tetradesmus dimorphus* (Turpin) M. J. Wynne. Scale bar is 10 µm.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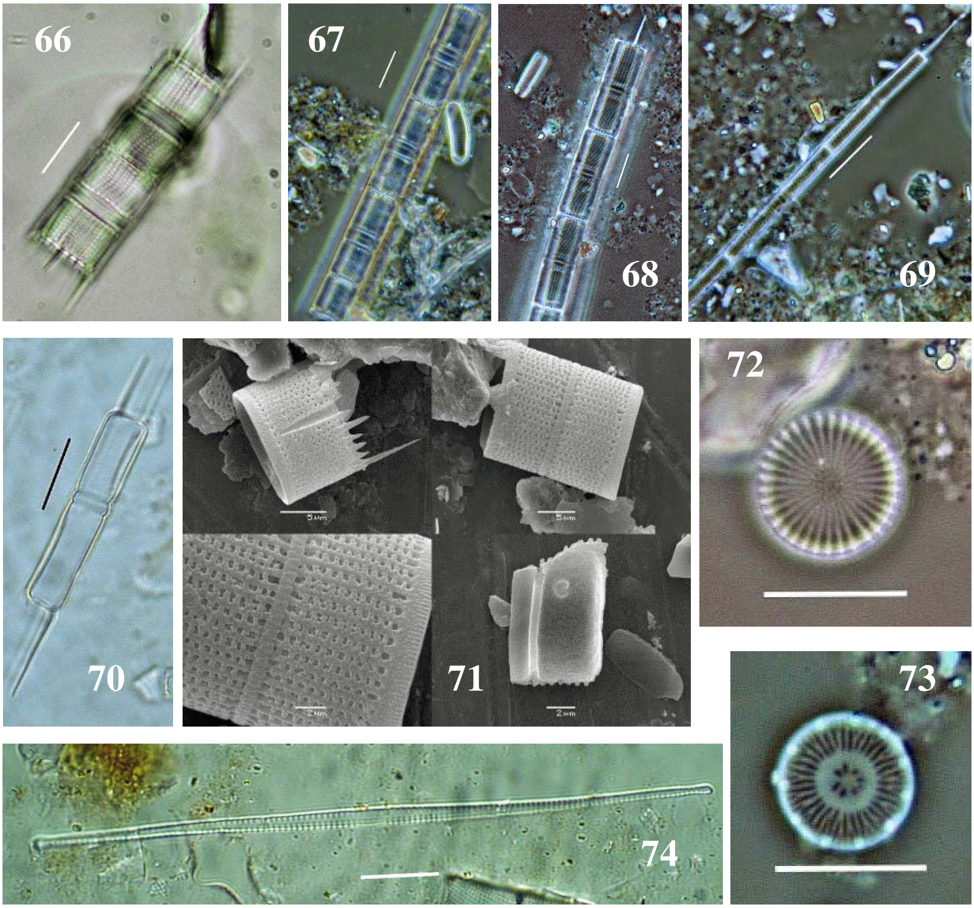
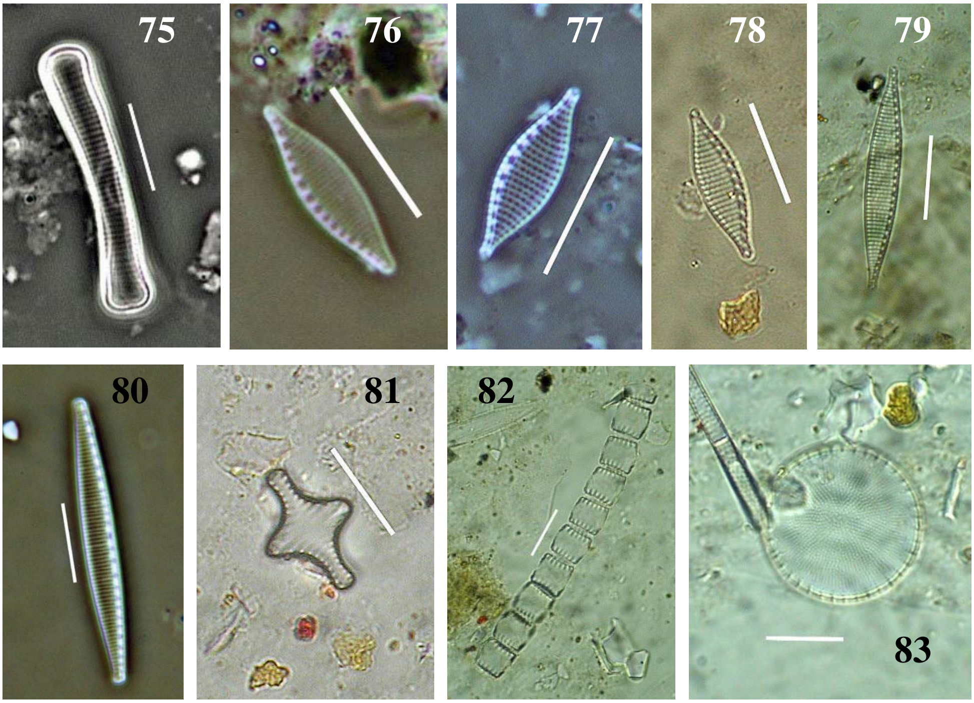


Figs. 58-83. 58 - *Treubaria triappendiculata* Bernard; 59 - *Willea apiculata* (Lemmermann) D. M. John, M. J. Wynne & P. M. Tsarenko; 60 - *Actinotaenium globosum* (Bulnheim) Teiling; 61 - *Cosmarium contractum* Kirchner; 62 - *Staurodesmus glaber* (Ralfs) Teiling; 63 - *Salpingoeca eurystoma* Stokes; 64 - *Centritractus africanus* F. E. Fritsch & M .F. Rich in F. E. Fritsch, M. F. Rich & M. L. Stephens; 65 - *Centritractus ellipsoideus* Starmach; 66 - *Aulacoseira agassizii* (Ostenfeld) Simonsen; 67 - *Aulacoseira ambigua* (Grunow) Simonsen; 68 - *Aulacoseira granulata* (Ehrenberg) Simonsen; 69 - *Aulacoseira granulata* var. *angustissima* (O.F.Müller) Simonsen; 70 - *Aulacoseira herzogii* (Lemmermann) Simonsen; 71 - *Aulacoseira agassizii* (Ostenfeld) Simonsen; 72 - *Cyclostephanos invisitatus* (Hohn & Hellermann) Theriot, Stoermer & Håkansson; 73 - *Discostella pseudostelligera* (Hustedt) Houk & Klee; 74 - *Fragilaria tenuissima* Lange-Bertalot et Ulrich; 75 - *Eunotia zasuminensis* (Cabejszekowna) Körner; 76 - *Nitzschia* cf. *lacuum* Lange-Bertalot; 77-79 - *Nitzschia lancettula* O. F. Müller; 80 - *Nitzschia tropica* Hustedt; 81 - *Staurosirella leptostauron* (Ehrenberg) Williams et Round; 82 - *Staurosirella pinnata* (Ehrenberg) Williams et Round; 83 - *Thalassiosira rudolfii* (Bachmann) Hasle. Scale bar is 10 µm.



period - **Fig. 85**. The general taxonomic structure of phytoplankton of LW period (**Fig. 85A**) is more similar to the taxonomic structure of the total phytoplankton of the river (**Fig. 84A**) in comparison with the HW phytoplankton (**Fig. 85B**).

The similarity in the river phytoplankton according to the SSI was only 57% calculated on the basis of 213 species common for both studied periods (**Table 1, Fig. 84B)**. The distribution of the common taxa by taxonomic groups was similar to the general distribution of taxa in riverine phytoplankton, with diatoms as richest group (146 common taxa, which comprised 68% of all common species) followed by cyanoprokaryotes (21 taxa, or 9%) and chlorophytes (20 taxa, or 9%). However, some differences have to be noted: the percentage participation of Ochrophyta (and of diatoms especially) and Euglenophyta in the structure of the "common phytoplankton" was significantly higher in comparison with the role of cyanoprokaryotes and all green algae (Chloro- and Streptophyta), which had almost twice less participation in the common phytoplankton (**Fig. 84B**). These data show the significant core role of diatoms in the biodiversity of Congo phytoplankton.

Most of the recorded species (447) have been described from temperate regions, but have been recorded in the tropics also. Less taxa (23) were described from tropics but have been found also in temperate regions: *Leptolyngbya circumcreta*, *Merismopedia punctata*, *Planktolyngbya minor*, *P. regularis* and *P. undulata*; *Achanthes inflata*, *Aulacoseira herzogii*, *Nitzschia accomodata*, *N. congolensis*, *N. lancettula*, *N. spiculum*, *N. tropica* and *Thalassiosira faurii*; *Centritractus afri-*

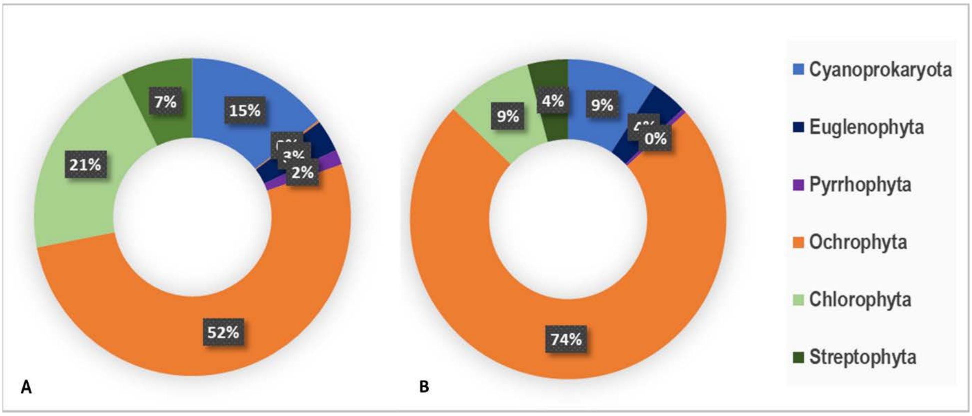


Fig. 84. A - Taxonomic structure of the Congo River phytoplankton (2013-2014); **B** - Taxonomic structure of the phytoplankton common for both studied periods of low (FW) and high (HW) waters, based on 213 common taxa.

canus; *Parvodinium africanum*; *Coelastrum proboscideum*, *C. pulchrum*, *Hindakia fallax*, *Monoraphidium caribeum*, *Parapediastrum biradiatum* var. *longecornutum*, *Scenedesmus protuberans* var. *minor*, *Schroederia indica* and *Treubaria triappendiculata*. In temperate waters they were recorded mainly in low amounts (*e.g.* Komarek & Fott 1983, Anagnostidis & Komárek 1999, Komárek & Anagnostidis 2005, Stoyneva 2016, Moestrup & Calado 2018), and only exceptionally were more abundant (Cellamare et al. 2010, 2013). Few species are known only from the tropics: *Aphanocapsa koordersii*, *Oscillatoria perornata*, *O. pseudocurviceps*, *Planktothrix clathrata*; *Trachelomonas piscatoris*; *Aulacoseira agassizii*, *A. minuscula*, *Gomphonema grande*, *Placoneis exiguiformis*, *Surirella congolensis* and *Thalassiosira rudolfii*; *Dictyosphaerium indicum* and *Staurastrum volans*. At the same time, during the study we identified 35 species and 2 varieties which,

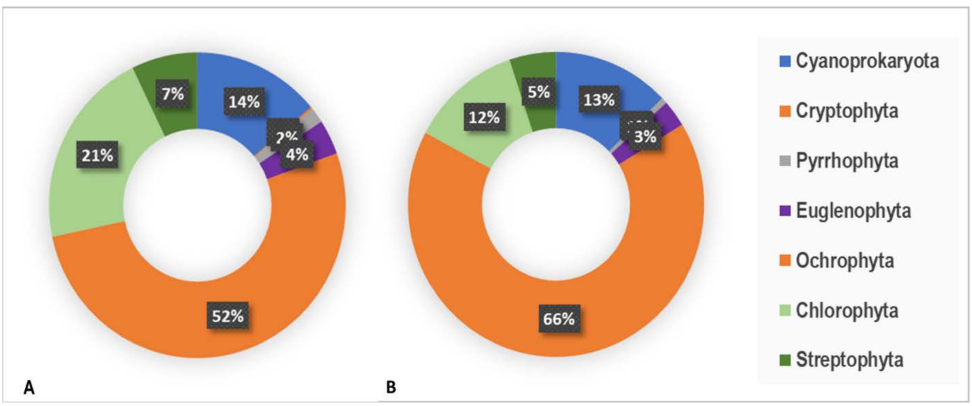


Fig. 85. Taxonomic structure of the phytoplankton of the Congo River (2013-2014) during periods of low waters (**A**) and high waters (**B**) with their percentage representation as additional labels.

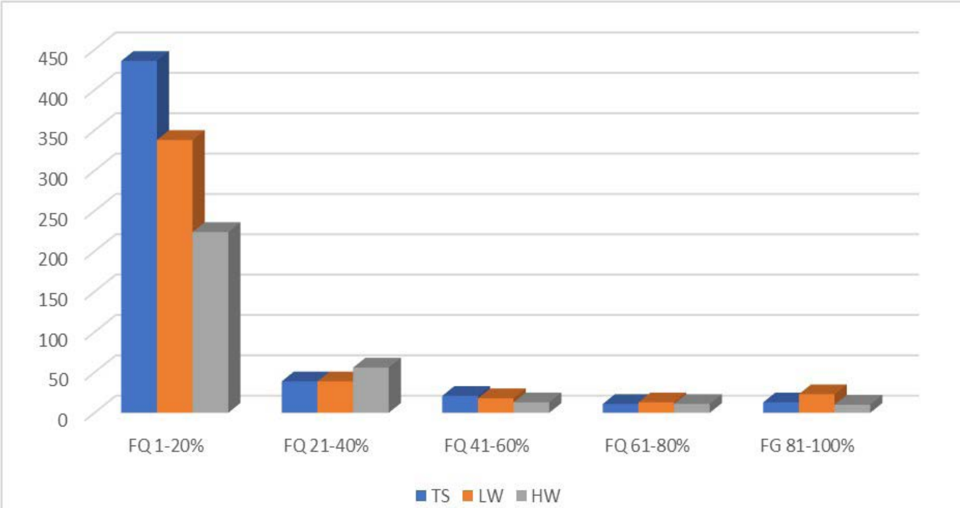


Fig. 86. Frequency of algal taxa in the Congo River phytoplankton represented by five classes of their frequency quotients (FQ) for all phytoplankton species found in the river (TS) and in the both studied periods of low waters (LW) and high waters (HW) (for details see text).

according to our knowledge, have not been recorded so far in the tropical regions: *Chroococcus planctonicus*, *C. vacuolatus*, *Pannus planus*, *Radiocystis geminata*, *Sphaerocavum microcystiforme*, *Tychonema tenue* and *Woronichinia delicatula*; *Chroomonas oblonga*; *Achnanthidium reimeri*, *Fragilaria tenuissima*, *Geissleria ignota*, *Fallacia subhamulata*, *Fragilaria berolinensis*, *Kobayasiella jaagii*, *Navicula vandamii*, *Nitzschia linearis* var. *tenuis*, *Nitzschia peisonis*, *Platessa bahlsii*, *Sellaphora pulchra*, *S. wallacei* and *Stauroneis livingstonii*; *Bicosoeca cylindrica*, *Diploeca elongata*; *Tetraëdriella spinigera*; *Tovellia coronata*; *Acantosphaera zacchariasii*, *Chlorella elongata*, *Desmodesmus pleiomorphus*, *Dictyosphaerium subsolitarium*, *Franceia armata*, *Neocystis ovalis*, *Nephrochlamys rotunda*, *Planochloris pyrenoidifera*, *Scenedesmus praetervisus*, *Selenastrum subtile*, *Siderocelis granulata* and *Staurastrum pingue* var. *planctonicum*. Since they were found as rare algae in quite low amounts, it is possible to suppose that they have been transported to the river by migrating birds or other dispersal vectors (Kristiansen 1996; Padisák et al. 2016). However, at present state of art we have not to exclude completely the possibility that they have broader ecological amplitudes and their development in tropics has been overlooked. Moreover, two species of marine diatoms were found, far from the ocean, each of them with a single specimen in FW: *Plagiotropis lepidoptera* var. *proboscidea* and *Rhopalodia acuminata* var. *protracta*.

According to the frequency, most of the algae can be considered as rare in the Congo River phytoplankton: FQ of Ist class (algae spread in 1–6 sites) had 84% of all phytoplankters, and 78% and 71% of the taxa during the LW and HW period, respectively (**Fig. 86**). Most of them were found in one site only: 40% of all taxa in each of both studied periods. Widespread taxa, with FQ of Vth class, comprised only 3% of all species, and 5% and 3% of the species during LW and HW period, respectively (**Fig. 86**).

A pronounced difference in the frequency distribution of algae by taxonomic groups was observed: diatoms had the most diverse frequency in all five classes of FQ during both studied periods, cyanoprokaryotes and chlorophytes were spread in four and three FQ classes, respectively, while algae from all other taxonomic groups had FQ of one or two classes only (**Table 1**). In accordance with these results is the fact that most widespread algae in the river phytoplankton are representatives of these three groups, with a leading role for diatoms, which were the most widely spread along the main river bed. In all studied sites (FQ=100%) during both studied periods were found *Aulacoseira ambigua* and *Nitzschia lancettula*. They were followed (FQ=98-96%) by *Aulacoseira agassizii*, *Aulacoseira granulata* var. *angustissima*, *Nitzschia* cf. *lacuum*, *Staurosirella leptostauron* and *Staurosirella pinnata*. The most spread cyanoprokaryote in the river was *Planktolyngbya* cf. *brevicellularis* (FQ=53%).

When discussing the distribution of algae along the river, we have to note that some of the species, and especially of the rare ones, did not belong to the group of typical plankters. Some of them were passively transported as epiphytes over other planktic algae and such ones were most of the recorded chrysophycean algae (with the most spread *Salpingoeca eurystoma* in particular). The second group was represented by some filamentous algae, which could be considered tychoplanktic due to their more typical benthic mode of life (*e.g.* singular fragments of *Ulothrix* spp., *Microspora* sp., *Klebsormidium* sp.). However, generally, the number of typical plankters (euplankters) among non-diatoms prevailed (255 from 278) and they comprised 92% of the phytoplankton diversity. As for diatoms, the flora of the Congo River is mainly composed of taxa of benthic origin, represented by few specimens. A significant part of diatoms are characteristic of acid, low conductivity rivers and streams, such as *Eunotia* spp. and *Pinnularia* spp., and are likely originating from tributaries, such as the Lobilo, Lobaye and Lomami rivers, located in the upstream part of the studied transect (Verheyen et al. 2017). Based on estimates of abundance and frequency of occurrence in the samples from the mainstem in the FW period, the number of euplanktonic diatom taxa may be fewer than 30, i.e. not more than 12 % of the total number of diatom species.

CONCLUSION

The unique floristic data set obtained during the study shows the rich algal biodiversity of the Middle Congo River phytoplankton. It comprised more than 520 taxa from 7 divisions, considering the presence of more taxa which could not be certainly identified in fixed samples and the fact that the results are based on two sampling series in such a large, still unregulated, river system. Moreover, the understanding of the drivers of the phytoplankton diversity is still very limited, for instance as to the contribution of tributaries and lakes vs. the role of inputs from different habitats, including the river margins, side arms and large patches of aquatic plants, to the mainstem. Addressing these questions will certainly require more studies, based on both longitudinal surveys and on regular sampling at fixed sites, aiming at investigating the dynamics of phytoplankton diversity, which has been understudied in large tropical rivers.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

AUTHOR CONTRIBUTIONS

Both authors contributed equally to the paper preparation. In the processing of the samples J.-P. Descy worked with diatoms, and M. P. Stoyneva-Gärtner – with the other algal groups.

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PHYTOPLANKTON SPECIES COMPOSITION IN SEVEN FISH PONDS WITH A GRASS CARP POLYCULTURE (2018-2019)

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Abstract. The present article is an attempt to analyze the seasonal changes in the structure and the biomass of phytoplankton in fish ponds with a polyculture with two-year-old grass carp as a mean of biological control of unwanted aquatic vegetation. During a two-year study (2018-2019), 259 planktonic algae were identified with considerably higher number of species during the first year (216) in comparison with the second year (150), when the grass carp stocking densities were twice less. This decrease in the biodiversity was accompanied by a significant change in the dominant structure: in 2018, the most intense blooms were caused by potentially toxic cyanoprokaryotes *Dolichospermum planctonicum* (Brunnthaler) Wacklin, L. Hoffmann & Komárek and *D. spiroides* Klebhan) Wacklin, L. Hoffmann & Komárek L. Hoffmann & K. Sivonen, while in 2019 the most abundant species were from Pyrrhophyta (*Ceratium furcoides* (Levander) Langhans), Euglenophyta (*Euglena gracilis* G.A. Klebs) and Ochrophyta, Raphidophyceae (*Gonyostomum* cf. *ovatum* Fott and *Gonyostomum depressum* (Lauterborn) Lemmermann).

Key words: algae, cyanoprokaryotes, dominants, species alteration, toxic algae

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INTRODUCTION

Biodiversity in small ponds is higher than the biodiversity in the larger water bodies, but despite this fact they are rarely studied due to their variable water balance, small volume and spatial heterogeneity. Water basins supplied with organic matter, as a result of agricultural activity, have rich algal flora (Borics et al. 2003). The loads of agricultural chemicals and the mismanagement of artificial fish ponds, can lead to eutrophication. Fertilizers are often used in fisheries to stimulate the development of the primary production and to increase the yields, which is why fish ponds are eutrophic with frequent algal blooms (Radojicic & Kopp 2016). Due to their different hydrology and small water depth they lack seasonal temperature stratification (Kopp et al. 2016). The use of high fish stocking densities increases the trophic status of the water bodies, which commonly causes cyanobacterial blooms, fluctuations in oxygen and high levels of nitrogen that destabilize these aquatic ecosystems.

Cyanoprokaryotes often are the main contributors to the total phytoplankton biomass in the summer, causing intense blooms and death among fish due to oxygen depletion. The specific features of cyanobacteria make them more adaptable to specific conditions, such as reduced light and depletion of nitrogen (Sevrin-Reyssac & Pletikosic 1990; Komárková 1998). At the same time, many cyanoprokaryotes are widely known as toxin producers which cause severe harm to human and ecosystem health. Therefore, the study of the summer ecosystems of small fish-breeding ponds achieves greater importance.

According to Michev & Stoyneva (2007) the species composition of algae found in fish farms in Bulgaria consisted of approximately 600 species. Detailed data on algal diversity in fish ponds in the country were published by Vodenicharov et al. (1974), Lüdskanova & Paskaleva (1975), Paskaleva (1975), Kiryakov et al. (1982), Paskaleva & Vodenicharov (1984) and Dochin et al. (2020; in press). The aim of the present study is to report the general changes in the phytoplankton composition in fish ponds with polyculture with two-year-old grass carp as a mean of biological control of unwanted aquatic vegetation.

MATERIAL AND METHODS

The study was carried out during a two-year period (2018-2019) in the experimental ponds of the Institute of Fisheries and Aquaculture, Plovdiv, Bulgaria. During the study, in these ponds common carp (*Cyprinus carpio* L.), hybrid bighead carp (*Hypophthalmichtys nobilis* Rich. x *Hypophthalmichtys molitrix* Val.) and grass carp (*Ctenopharyngodon idella* Val.) were grown. During the second year (2019) the stocking density of grass carp in the experimental ponds was twice lower than the stocking density in 2018.

During the two-year period, 108 phytoplankton samples were taken from seven ponds (P6, P7, P12, P16, P18, P19 and P23), each with area between 0.18 to 0.40 ha. The macrophytic vegetation in the ponds was represented by *Ceratophyllum demersum* L., *Nuphar lutea* (L.) Sm. and *Typha angustifolia* L.).

The sampling was conducted at the depth of 0.5 m bimonthly in the late spring-summer period (from April/May to September) of both years with the results discussed as average values per month. The phytoplankton samples were collected and processed by standard methods of fixation with formalin to final concentration 4% and further sedimentation (ISO 5667-1:2006/AC:2007; ISO 5667-3:2003/AC:2007) with some additional living samples for identification of raphidophytes. Microscope work was done on Bürker chamber. The species composition was determined by light microscope (Carl Zeiss, Axioscope 2 plus) with magnification 400x using standard taxonomic literature with critical use of AlgaeBase (Guiry & Guiry 2020). Diatoms were identified after Cox (1996). The main counting unit was the cell and the biomass was estimated by the method of stereometrical approximations (Rott 1981; Deisinger 1984). Counting units were cells, filaments and colonies. The total biomass of each sample was assessed and it was defined as the amount of biomass of all species summarized in separate taxonomic groups. Dominant species were determined according to the percentage of individual species to the total biomass.

RESULTS AND DISCUSSION

During a two-year study (2018-2019), totally 259 taxa of planktonic algae from 6 divisions were identified (**Table 1**).

During the first year of investigation, the total number of identified taxa was 216 and during the second year it was 150 (**Table 1, Fig. 1-3**). The number of species ranged around 100 per month during the first year, and was about twice less during the second year (**Figs. 1, 3**). These pronounced differences in total number of taxa during each studied month (**Figs. 1, 2**) were accompanied with changes in the dominant structure of the phytoplankton (**Table 1**).

In April 2018, only 10 phytoplankton taxa, mostly from Ochrophyta, were identified. In May 2018, among the 102 taxa identified the most abundant were *Aulacoseira granulata* (Ehrenberg) Simonsen, *Anabaena sphaerica* Burnett & Flahault and *Trachelomonas hispida* (Perty) F. Stein. In June 2018, number of species was 100 and *Dolichospermum spiroides*, *Dolichospermum planctonicum* and *Aphanizomenon flosaquae* Ralfs ex Bornet & Flahault reached the highest biomass. In July 2018, 105 taxa were identified, with the highest biomass of *D. spiroides*, *A. sphaerica* and *A. granulata*. In August 2018, among 103 species found, *Microcystis aeruginosa* (Kützing) Kützing and *Oscillatoria limosa* C. Agardh ex Gomont from Cyanoprokaryota and *Desmodesmus communis* (Hegewald) Hegewald from Chlorophyta were the most abundant. In September 2018, 110 taxa were found and *D. spiroides*, *M. aeruginosa*, *Trachelomonas planctonica* Svirenko and *A. granulata* were dominants (**Table 1**).

Table 1. List of phytoplankton taxa in fish ponds with grass carp polyculture during different months of both studied years, where * - occurrence and ** - dominance.

Taxa/Year	**2018**						**2019**				
Month	IV	V	VI	VII	VIII	IX	V	VI	VII	VIII	IX
Cyanoprokaryota											
Anabaena sp.									*		
Anabaena sphaerica Bornet & Flahault		**	**	**	**	**		*	*		
Anabaenopsis arnoldii Aptekar					*	*					
Anathece clathrata (West & G. S. West) Komárek, Kastovsky & Jezberová		*		*		*	*	*	*	*	*
Aphanizomenon flosaquae Ralfs ex Bornet & Flahault		*	**	**	*	**			**	*	*
Aphanizomenon gracile Lemmermann				*	*				**		
Aphanocapsa delicatissima West & G. S. West		*	*	*		*			*		
Aphanocapsa sp.		*	*	*	*	*		*	*	*	*
Aphanothece elabens (Brébisson ex Meneghini) Elenkin		*				*					
Chroococcus turgidus (Kützing) Nägeli)		*			*	*	*	*	*	*	*
Coelosphaerium confertum West & G. S. West			*								
Cuspidothrix issatschenkoi (Usachev) P. Rajaniemi, Komárek, R.Willame, P. Hrouzek, K. Kastovská, L. Hoffmann & K. Sivonen									*		*
Dolichospermum flosaquae (Brébisson ex Bornet & Flahault) P. Wacklin, L. Hoffmann & Komárek			*	*		*					*
Dolichospermum lemmermannii (Richter) P. Wacklin, L. Hoffmann & Komárek						*					
Dolichospermum planctonicum (Brunnthal-er) Wacklin, L. Hoffmann & Komárek		*	**	*		**				**	*
Dolichospermum spiroides (Klebhan) Wacklin, L. Hoffmann & Komárek		*	**	**	**	**			**	**	*
Gomphosphaeria aponina Kützing				*	*						
Limnococcus limneticus (Lemmermann) Komárková, Jezberová, O. Komárek & Zapomelová				*	*					*	
Limnothrix redekei (Goor) Meffert					*						
Merismopedia elegans A. Braun ex Kützing										*	
Merismopedia glauca (Ehrenberg) Kützing								*	*		
Merismopedia punctata Meyen		*	*	*		*					
Merismopedia sp.							*	*			

Taxa/Year	**2018**						**2019**				
Month	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**V**	**VI**	**VII**	**VIII**	**IX**
Merismopedia tenuissima Lemmermann				*	*			*	*		
Microcystis aeruginosa (Kützing) Kützing				*	**	**	**		**	**	**
Microcystis sp.			*	*	*		*				
Microcystis wesenbergii (Komárek) Komárek ex Komárek					*	*			*	**	**
Noctoc sp.					*						
Oscilatoria sp.			*	*	*		*	*			
Oscillatoria limosa C. Agardh ex Gomont		**	*	**	**	*			**	**	*
Phormidium sp.			*		*						
Planktolyngbya limnetica (Lemmermann) Komárková- Legnerová & Cronberg		*	*	**	**	**					*
Planktolyngbya sp.									*		
Planktothrix agardhii (Gomont) Anagnostidis & Komárek									*		
Pseudanabaena catenata Lauterborn									**		
Pseudanabaena galeata Böcher									*	*	
Pseudanabaena limnetica (Lemmermann) Komárek									*		
Pseudanabaena sp.						*					
Synechococcus linearis (Schmidle & Lauterborn) Komárek						*					
Snowella lacustris (Chodat) Komárek & Hindák						*				*	*
Chlorophyta											
Actinastrum hantschii Lagerheim		*	*	*	*	*	*	*	*	*	*
Ankistrodesmus bibraianus (Reinsch) Korshikov		*	*	*	*		*				*
Ankistrodesmus falcatus (Corda) Ralfs					*	*					
Ankistrodesmus fusiformis Corda		*	*	*	*	*		*		*	
Ankistrodesmus longissimus (Lemmermann) Wille				*	*	*					
Ankistrodesmus spiralis (W. B. Turner) Lemmermann				*	*						
Ankyra ancora f. *issajevii* (Kisselev) Fott		*									
Ankyra judayi (G. M. Smith) Fott		*	*	*							
Ankyra ocellata (Korshikov) Fott		*									
Ankyra sp.		*									
Carteria klebsii (P. A. Dangeard) Francé		*									

Characium angustum A. Braun		*	*		*	*					
Characium sp.							*				*
Chlamydomonas cf. *incerta* Pascher								*		*	
Chlamydomonas simplex Pascher									**		
Chlamydomonas sp.							*				
Chlorella vulgaris Beyerinck								*			
Chlorolobion braunii (Nägeli) Komárek		*									
Coelastrum astroideum De Notaris			*		*		*	*		*	*
Coelastrum microporum Nägeli in A. Braun		*		*	*	*	*	*		**	
Coelastrum sp.				*			*				
Coelastrum sphaericum Nägeli		*									
Coenochloris sp.									*		
Crucigenia quadrata Morren		**	*	*	**	*	**	*	**		*
Crucigenia sp.											*
Crucigenia tetrapedia (Kirchner) Kuntze		*	**			*		*	**	*	*
Crucigeniella pulchra (West & G. S. West) Komárek		*	*	*			*	*			
Desmodesmus bicaudatus (Dedusenko) P. M. Tsarenko		**	*	*			*				
Desmodesmus brasiliensis (Bohlin) Hegewald					*						
Desmodesmus communis (Hegewald) Hegewald		**	**	**	**	**	**	**	**	**	**
Desmodesmus denticulatus (Lagerheim) S. S. An, T. Friedl & Hegewald		*	*	*		*			**	*	
Desmodesmus intermedius (Chodat) Hegewald		*									
Desmodesmus opoliensis (P. G. Richter) Hegewald					*						
Desmodesmus perforatus (Lemmermann) Hegewald							*				
Desmodesmus protuberans (F. E. Fritsch & M. F. Rich) Hegewald		**	*	**	*	**	**	*	**	**	*
Desmodesmus spinosus (Chodat) Hegewald		*	*	*	*	*	*	*			
Dictyosphaerium ehrenbergianum Nägeli			*								
Eudorina elegans Ehrenberg								*			
Golenkinia radiata Chodat						*	*	**	*	**	**
Gonium pectorale O. F. Müller								*			
Hariotina polychorda (Korshikov) Hegewald									*		

Taxa/Year	**2018**						**2019**				
Month	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**V**	**VI**	**VII**	**VIII**	**IX**
Hyaloraphidium contortum Pascher & Korshikov					*	*		*		*	
Hyaloraphidium rectum Korshikov				*							
Kirchneriella lunaris (Kirchner) Möbius		*				*					
Kirchneriella obesa (West) West & G. S. West			*	*							
Koliella longiseta (Vischer) Hindák					*						
Korshikoviella limnetica (Lemmermann) P. C. Silva			*	*	*	*					
Lagerheimia ciliata (Lagerheim) Chodat		*					**				
Lagerheimia genevensis (Chodat) Chodat		*	**	*		*					
Lagerheimia sp.					*	*					
Lambertia sp.		*									
Lemmermannia triangularis (Chodat) C. Bock & Krienitz		*		*	*	*	*		*		
Messastrum gracile (Reinsch) T. S. Garcia		**	*	*	*	*	*		*		
Micractinium pusillum Fresenius		*	*		*	*	*		**	*	
Micractinium quadrisetum (Lemmermann) G. M. Smith				*			*		*	*	
Monactinus simplex (Meyen) Corda		**	**	**	**	**	**	*	**	**	**
Monoraphidium contortum (Thuret) Komárková-Legnerová			*	*	*	*					
Monoraphidium griffithii (Berkeley) Komárková-Legnerová			*								
Monoraphidium sp.			*								
Mucidosphaerium pulchellum (H. C. Wood) C. Bock, Proschold & Krienitz		*	*	*	*	*	*	*	*		*
Oocystidium ovale Korshikov		*		*	*						
Oocystis borgei J. W. Snow		*			*						
Oocystis lacustris Chodat		*	*	*	*	*	**	*	*	*	*
Oocystis sp.								*	*	*	*
Pandorina morum (O. F. Müller) Bory		**	*	*	*	*	*	**	*	**	*
Pediastrum duplex Meyen		**	**	**	*	**	**	*	**	**	**
Pseudodidymocystis planctonica (Korshikov) Hegewald & Deason		*	*								
Pseudopediastrum boryanum (Turpin) Hegewald					*						
Pseudoschroederia robusta (Korshikov) Hegewald & E. Schnepf		*									

Scenedesmus acuminatus var.*biseriatus* Reinhard		*	*	*	*		*			**	*
Scenedesmus acuminatus var. *elongatus* G. M. Smith			*	*	*	*	*			*	**
Scenedesmus apiculatus (West & G. S. West) Chodat			*								
Scenedesmus arcuatus (Lemmermann) Lemmermann		**		*	*	*	*	*	*		
Scenedesmus obtusus Meyen										*	
Scenedesmus producto-capitatus Schmula				*	*	*					
Scenedesmus sp.									*	*	*
Schroederia setigera (Schröder) Lemmermann									*		
Schroederia sp.		*									*
Schroederia spiralis (Printz) Korshikov		*									*
Selenastrum bibraianum Reinsch						*					
Sphaerocystis sp.											*
Stauridium tetras (Ehrenberg) Hegewald		*	*		*	*					
Stichococcus sp.		*									
Tetrachlorella alternans (G. M. Smith) Korshikov				*							
Tetradesmus bernardii (G. M. Smith) M.J.Wynne				*							
Tetradesmus lagerheimii M. J. Wynne & Guiry		**	**	*	**	*	**	**	**	*	**
Tetradesmus obliquus (Turpin) M. J. Wynne		**	**	**	*	*	**	**	*		**
Tetraëdron minimum (A. Braun) Hansgirg		*	*			*	**	*	*		*
Tetraëdron caudatum (Corda) Hansgirg			*				*				
Tetraëdron sp.		*									
Tetrastrum sp.		*					*	*		*	*
Treubaria planctonica (G. M. Smith) Korshikov			*	*		*					
Treubaria schmidlei (Schröder) Fott & Kovácik				*		*					
Treubaria sp.			*						*		*
Vitreochlamys velata (Korshikov) Ettl						*					
Volvox aureus Ehrenberg									*		
Willea apiculata (Lemmermann) D. M. John, M. J. Wynne & P. M. Tsarenko				*		*					

Taxa/Year	**2018**						**2019**				
Month	IV	V	VI	VII	VIII	IX	V	VI	VII	VIII	IX
Streptophyta											
Closterium aciculare T. West		*									
Closterium acutum Brébisson in Ralfs							*				
Closterium pronum Brébisson			*	**	*	**			**		
Closterium sp.								*		*	
Cosmarium margaritiferum Meneghini ex Ralfs			*	*	*	*					
Cosmarium sp.			*	**	*	*				*	*
Elakatothrix gelatinosa Wille		*	*	*	*	*	*				*
Gonatozygon sp.				*	*						
Sphaerozosma sp.					*						
Spirogyra sp.		*									
Staurastrum cf. *cingulum* (West & G. S. West) G. M. Smith										*	
Staurastrum gracile Ralfs ex Ralfs		*		*	*	*					
Staurastrum hexacerum Wittrock				*		*					
Staurastrum pingue var. *planctonicum* (Teiling) Coesel & Meersters			*	*	*	*					
Staurastrum sp.		*	*	*	*	*	*	*	*	*	
Staurastrum tetracerum Ralfs ex Ralfs		**			*	*					
Zygnema sp.						*					
Euglenophyta											
Euglena gracilis Klebs							**	**	**	**	**
Euglena sp.		*	*	*	**	*	*	**	*	*	*
Euglena viridis (O. F. Müller) Ehrenberg		*			*	*	**	**	**	**	**
Euglenaformis proxima (Dangeard) M. S. Bennett & Triemer				*	*	*	*				
Lepocinclis acus (O. F. Müller) Marin & Melkonian		*	*	*	*	*	**	**	**	**	**
Lepocinclis ovum (Ehrenberg) Lemmer-mann						*					
Lepocinclis oxyuris (Schmarda) Marin & Melkonian		*	*	*	*	*	*	*	**		
Lepocinclis sp.						**	*	**	**	*	**
Phacus curvicauda Svirenko		*	*		*		**	*	*	*	
Phacus longicauda (Ehrenberg) Dujardin		*	*	*	*	*	*	*	*	*	**
Phacus orbicularis K. Hübner		*	*	*	*	*	*	**	*	**	*

Phacus sp.										*	
Phacus tortus (Lemmermann) Skvortzov								*	*	*	
Strombomonas sp.		*	*	*	*	*	**	**	*	*	*
Trachelomonas hispida (Perty) F. Stein		**									
Trachelomonas planctonica Svirenko			*	**	**	**	*	**			
Trachelomonas sp.		**	*	*	**		**	*	*	*	
Trachelomonas volvocina (Ehrenberg) Ehrenberg				*	**	*	**		*		
Pyrrhophyta											
Ceratium furcoides (Levander) Langhans										**	**
Ceratium hirundinella (O. F. Müller) Dujardin								*	**		**
Glenodinium sp.									*	*	*
Gymnodinium sp.											
Parvodinium cf. *inconspicuum* (Lemmermann) Carty				*							
Peridinium bipes F. Stein				*	**	*	*	**	**	**	**
Peridinium cf. *aciculiferum* Lemmermann										**	
Peridinium cinctum (O. F. Müller) Ehrenberg				*	*						
Peridinium sp.		*	*	*	*	*	*	**	**	**	**
Ochrophyta											
Chrysophyceae											
Dinobryon borgei Lemmermann			*			*					
Dinobryon divergens O.E.Imhof			*								*
Dinobryon sociale (Ehrenberg) Ehrenberg							*				
Kephyrion sp.									*		
Uroglena sp.								*			
Eustigmatophyceae											
Tetraedriella acuta Pascher	*										
Tetraedriella gigas (Wittrock) Hansgirg		*		*							
Tetraedriella sp.		*									
Tetraedriella spinigera Skuja			*	*	*						
Synurophyceae											
Mallomonas acaroides Perty						*					
Mallomonas elongata Reverdin					*	*					
Mallomonas sp.		*									
Raphidophyceae											
Gonyostomum cf. *ovatum* Fott						*	**				*

Taxa/Year	**2018**						**2019**				
Month	IV	V	VI	VII	VIII	IX	V	VI	VII	VIII	IX
Gonyostomum cf. *semen* (Ehrenberg) Diesing								*		*	*
Gonyostomum depressum (Lauterborn) Lemmermann								**	**	**	**
Gonyostomum sp.						*	*	*			**
Vacuolaria sp.											*
Xanthophyceae											
Centritractus belonophorus (Schmidle) Lemmermann				*							
Bacillariophyceae											
Amphiphora sp.					*						
Amphora sp.		*	*	*			*	*	*	*	
Anomoeoneis cf. *sphaerophora* Pfitzer	*										
Asterionella formosa Hassall		*	*								
Aulacoseira granulata (Ehrenberg) Simonsen		**	**	**	**	**	**	**	**	**	**
Aulacoseira islandica (O. Müller) Simonsen	*	*	*								
Caloneis amphisbaena (Bory) Cleve		*					*			*	
Caloneis silicula (Ehrenberg) Cleve			*	*					*		
Cocconeis pediculus Ehrenberg			*						*		
Cocconeis placentula Ehrenberg		**	**	*	*	*				*	*
Cocconeis placentula var. *euglypta* (Ehrenberg) Grunow				*	*						
Cocconeis sp.			*								
Ctenophora pulchella (Ralfs ex Kützing) D. M. Williams & Round	*										
Cyclotella cf. *glomerata* H. Bachmann										*	
cf. *Discostella stelligera* (Cleve & Grunow) Houk & Klee							*			*	
Cyclotella meneghiniana Kützing	*	*	*	*	*	*					
Cyclotella sp.		**	*	*	*	*	*	**	*	*	*
Cymatopleura solea (Brébisson) W. Smith		*	*				*		*	*	
Cymatopleura sp.		*	*							*	
Cymbella cymbiformis C. Agardh		*	*								
Cymbella sp.		*	*	*	*		*			*	*
Cymbella tumida (Brébisson) Van Heurck						*					
Diatoma sp.			*								

Diatoma vulgaris Bory				*		*					
Diploneis elliptica (Kützing) Cleve			*								
Diploneis sp.		*	*								
Encyonema ventricosum (C. Agardh) Grunow in A. W. F. Schmidt		*		*		*					
Epithemia frickei Krammer in Lange-Bertalot & Krammer					*						
Epithemia sp.		*			*						
Epithemia zebra (Ehrenberg) Kützing						*					
Eunotia sp.									*		
Fragilaria capucina Desmazières						*					
Fragilaria crotonensis Kitton									*		*
Gomphonema acuminatum Ehrenberg		*		*	*	*			*	*	
Gomphonema acuminatum var. *coronatum* (Ehrenberg) Rabenhorst			*	*		*					
Gomphonema augur Ehrenberg				*	*	*					
Gomphonema constrictum Ehrenberg in Kützing	*		*	*		*					
Gomphonema constrictum var. *capitatum* (Ehrenberg) Grunow in Van Heurck				*	*						
Gomphonema gracile Ehrenberg	*		*		*	*					
Gomphonema sp.									*		
Gyrosigma acuminatum (Kützing) Rabenhorst								*			
Lindavia comta (Kützing) Nakov, Gullory, Julius, Theriot & Alverson			*								
Melosira varians C.Agardh								*	*		
Meridion circulare (Greville) C. Agardh			*	*	*						
Navicula gracilis Ehrenberg	*										
Navicula radiosa Kützing	*										
Navicula sp.		*	*	*	*	*	**	**	*	*	*
Navicula vulpina Kützing	*										
Nitzschia holsatica Hustedt		*		*	*	*	**	*	*		
Nitzschia sp.											*
Pleurosigma elongatum W. Smith						*					*
Rhopalodia gibba (Ehrenberg) O. Müller			*	*	*	*					
Stephanodiscus astraea (Kützing) Grunow							*		*	**	
Stephanodiscus hantzschii Grunow in Cleve & Grunow		*					*	*		*	
Stephanodiscus sp.		*					*	*			

Taxa/Year	2018						2019				
Month	IV	V	VI	VII	VIII	IX	V	VI	VII	VIII	IX
Surirella sp.			*			*					*
Synedra sp.			*								*
Tabularia tabulata (C. Agardh) Snoeijs						*					
Ulnaria acus (Kützing) Aboal		**	**	**	**	**	**	*	*	**	
Ulnaria ulna (Nitzsch) Compère		**	*	*	*	*	*		*		*

In the first samples for 2019 (in May), 71 taxa were found. The most abundant species were from Chlorophyta and Euglenophyta. *D. communis* and *Pediastrum duplex* Meyen, *E. gracilis*, *Gonyostomum* cf. *ovatum* Fott and *A. granulata* had the highest biomass.

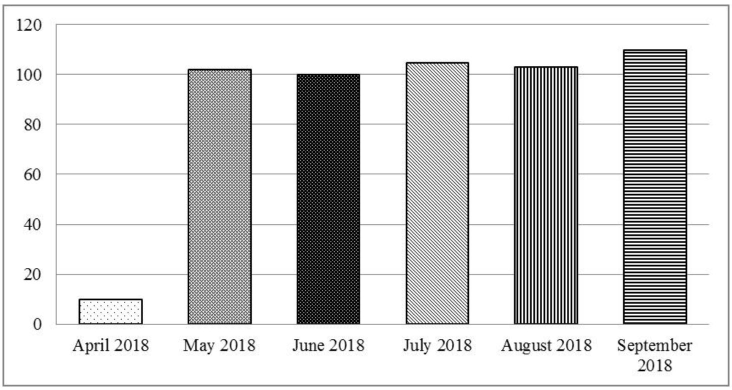

Fig. 1. Number of phytoplankton taxa in experimental fish ponds per month during the spring-summer period in 2018.

In June 2019, 65 taxa were recorded. In all experimental ponds, *M. aeruginosa, E. gracilis*, *O. limosa* and *D. communis* were dominants. In July 2019, 83 species were identified with *A. granulata* and *D. communis* dominating in that period. In August 2019, 72 taxa of algae were identified with *Ceratium furcoides* and *E. gracilis* among the most abundant. In September 2019, among the 69 taxa identified, *Ceratium furcoides*, *E. gracilis*, and *Gonyostomum depressum* dominated (**Table 1**).

It is important to note, that among the dominants, cyanoprokaryotes from genera *Aphanizomenon*, *Dolichospermum* and *Microcystis*, which are well-known for their ability to be potent cyanotoxin producers affecting ecosystem and human health (*e.g.* Meriluoto et al. 2017), were found. The broad distribution of these genera and their relation with toxic blooms in Bulgaria was shown in the summary by Stoyneva-Gärtner et al. (2017).

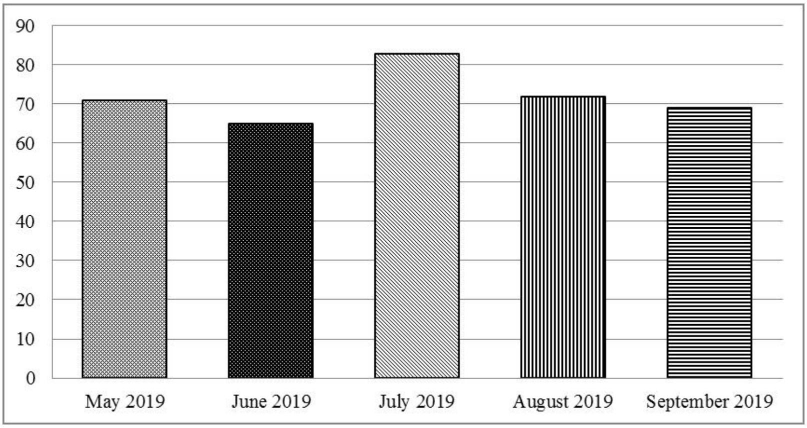

Fig. 2. Number of phytoplankton taxa in experimental fish ponds per month during the late spring-summer periods in 2019.

The results obtained during this study are in accordance also with the earlier results which have demonstrated that the high stocking density of grass carp can seriously affect the functioning of the aquatic ecosystems. For example, negative changes may be associated with alterations in the structure and abundance of plant communities, as well as in the environment they inhabit, such as changes in transparency, sediments, and increased levels of biogens after deposition of faeces (Pípalová 2006; Dibble & Kovalenko 2009). Earlier, Richard et al. (1984) reported that three years after the introduction of grass carp Chlorophyta and Bacillariophyta should significantly increase and the amount of Cyanoprokaryota should decrease. Holdren & Porter (1986) also demonstrated that after the introduction of grass carp, changes in the dominant phytoplankton species occurred.

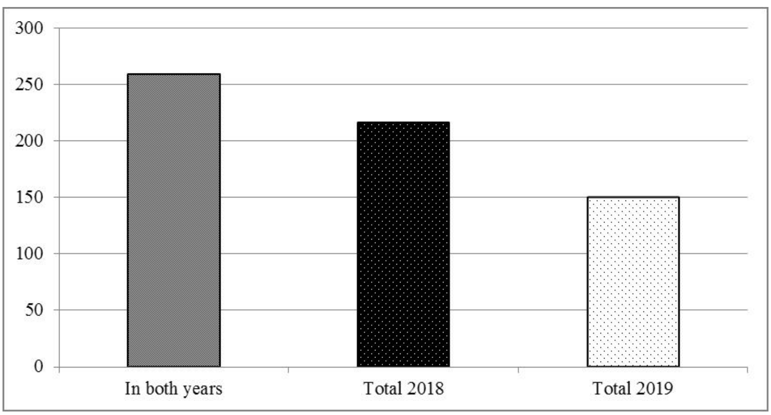

Fig. 3. Number of phytoplankton taxa in experimental fish ponds during the spring-summer periods of 2018, 2019 and in both years.

According to BORICS ET AL. (2016) in small ponds, despite the expected development of small nannoplankton, summer conditions favor the development of large euglenoids, cyanoprokaryotes and chlorophytes but the size of the ponds clearly affects the detailed composition of the phytoplankton. In our study, algal biodiversity and abundance were significantly lower in 2018 compared to 2019 with registered changes in the dominant species. The most significant differences were observed in the Chorophyta, Streptophyta and Ochrophyta divisions from which more taxa were found in the first year than in the second, while there was almost no difference in the number of identified Cyanoprokaryota species. These results are on conformity with the data on the taxonomic structure of the phytoplankton obtained in our previous research (DOCHIN ET AL., IN PRESS).

In the early summer of 2018, after the removal of macrophytes from the grass carp, the development of phytoplankton rapidly increased and reached pronounced peaks in all experimental ponds (except P6), which will be described in details elsewhere. This rapid development with increase of the phytoplankton biomass was linked with high amount of nutrients released after the aquatic vegetation was removed by the grass carp and with the improved light regime in the water column (DOCHIN ET AL. 2020, in press). In turn, the intense development of phytoplankton can cause shading and suppression of aquatic plants (BONAR ET AL. 2002) and this is in accordance with the lack of macrophyte overgrowth observed during 2019 (this study; DOCHIN ET AL. 2020, in press).

CONCLUSION

The changes in the phytoplankton composition in fish ponds stocked with grass carp polyculture observed by us showed relatively high algal biodiversity with considerably less identified species in 2019 than those in 2018. At the same time, a significant change in the structure of the phytoplankton dominants was detected: while the blooms of some potentially toxic species of Cyanoprokaryota were most intense in 2018, in 2019 the most abundant species were from Pyrrhophyta, Euglenophyta and Raphidophyceae. The fact of detecting potentially toxic cyanoprokaryotes as dominants in fish ponds can serve as alarm for monitoring of the summer phytoplankton in these small water bodies.

CONFLICT OF INTERESTS

The author declares that there is no conflict of interest regarding the publication of this article.

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ANCIENT BURIAL MOUNDS – BIODIVERSITY HOTSPOTS AND REFUGEES FOR NATURAL FLORA AND VEGETATION

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Abstract. Bulgaria is enormously rich in historical monuments. Burial mounds are one of the most numerous among them. They are spread all over the country but are localized in lowlands where active agriculture takes place. Patches of semi-natural vegetation are often preserved on the ancient burial mounds most of which are remnants from thousands years ago. The burial mounds which are generally surrounded by vast agricultural fields of monocultures often host the remnants of autochthonous flora. Besides being hotspots for biodiversity preservation, the ancient burial mounds are ideal objects for testing the theory of island biogeography. In our study we selected 577 mounds distributed near equally in northern and southern Bulgaria. Around each one mound a buffer of 200 m was outlined and the land use types were mapped aiming to identify the influence of land use on the floristic diversity. Our methodology includes sampling all vascular plants and their abundance within two sample plots of 25 m^2 positioned in the middle of north and south facing slopes. The scientific goal of our research is to reveal the significance of the burial mounds not only as cultural value, but also as natural treasure for the country. This

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would additionally emphasise their attraction as touristic objects.

Key words: biodiversity conservation, fragmentation, historical monuments, kurgans

INTRODUCTION

Land use changes and remarkable agricultural intensification during the past decades have resulted in a considerable decrease in natural and semi-natural habitats in the whole World. These changes involved the transformation of the natural terrestrial habitats into agricultural and urban areas, which lead to a serious loss and degradation of their vegetation (Tilman 1999; Williams et al. 2009). These processes are particularly pronounced in grassland ecosystems, where about 45% of the temperate grasslands have been converted by human actions (Hoekstra et al. 2005). Permanent grasslands and especially steppe habitats face serious threats, because their soils are excellent for arable farming (*e.g.* chernozem; Hölzel et al. 2002).

In the lowlands where the agricultural activity is the most intensive the natural vegetation is generally present only in small fragments (Deák et al. 2019a). In Bulgaria patches of semi-natural vegetation are often preserved on the mounds which are remnants from ancient Thracian burial practices. The country is especially rich in these historical monuments. The burial mounds host remnants of the autochthonous flora which is surrounded sometimes by vast agricultural fields of monocultures. According to Sudnik-Wójcikowska & Moysiyenko (2012) Paczoski was the first who has recognized the importance of kurgans in the restoration of the steppic vegetation. During his expedition to Bulgaria, where he visited the mound of King Władysław III Warneńczyk, he wrote: "I wish to describe the plant species composition of this kurgan, because this type of vegetation, as well as the vegetation of Ukrainian kurgans, can play an important role in the restitution of the steppe that was subjected to the strongest anthropogenic transformation" (Paczoski 1933, p. 156).

Bulgarian burial mounds are objects for archaeological surveys since more than 150 years. The terms mogili, mounds, tumuli, kurgans, barrows, or halom describe similar hemispheric landscape structures that are widespread in the steppe and forest steppe zones of Eurasia from Hungary to Mongolia (Deák et al. 2016). Nobody has studied flora on burial mounds in Bulgaria so far, even though burial mounds integrate natural and cultural values and provide several important ecosystem services.

Since the beginning of the 21st century, the number of botanical studies of kurgans in Europe has increased. Information about the flora and vegetation developed on the kurgans exists for the whole area of Hungary (Penksza & Joó 2002; Barczi 2003, Tóth 2006, Deák et al. 2019b), for a part of Ukraine (Sudnik-Wójcikowska & Moysiyenko 2012, 2013) and Poland (Cwener & Towpasz 2003; Cwener 2004).

Besides being hotspots for biodiversity preservation, the ancient burial mounds are ideal objects for testing the theory of island biogeography (MacArthur & Wilson 1967) or the mosaic concept (Duelli 1997). Whatever theoretical background is followed, the burial mounds can be considered as the remnants of the *natural* areas within the *agricultural sea*. We can measure and record the species richness, as well as the abundance and occurrence of individual species growing on them. Therefore, they could serve as a unique experimental field for studying the role of fragmentation and isolation in shaping vegetation patterns. These historical monuments have existed for millennia and provide invaluable information about the semi-natural vegetation and serve as source of diaspores for nature conservation activities.

In 2019 the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences started a project financed by the National Science Fund aiming to fill the knowledge gap about the flora and vegetation of the burial mounds in Bulgaria and to reveal their importance as semi-natural habitat patches in modified agricultural landscapes. The project duration is 36 months. It is conducted in collaboration with the Hungarian colleagues from the Seed Research Group in Pest.

MATERIAL AND METHODS

We intended to observe as much as possible mounds. In collaboration with the National Archaeological Institute with Museum at the Bulgarian Academy of Sciences we decided to use existing data included in the Archaeological map of Bulgaria. The Archaeological map of Bulgaria is a database containing information for different archaeological sites in the country and is used for scientific research, preservation and promotion of national archaeological heritage. It includes approximately 11, 000 burial mounds distributed all over the country. We randomly selected 577 mounds distributed near equally in northern and southern Bulgaria. All mounds were verified in terms of location and contemporary condition by using orthophoto images and topographic maps. Visual observation of their vegetation coverage was additionally performed by Google maps imagery. The land cover was assessed in percentage herbaceous/woody vegetation coverage. Around each mound a buffer of 200 m was outlined and the land use types were delineated (**Fig. 1**). Six land use types were identified: annual crops, perennial crops, forests, grasslands, other semi-natural lands and urban areas. Further on we selected mounds for field sampling. The selected mounds represent different types of land use in their surroundings and different land cover on the mounds.

Our intention is to sample 120 burial mounds in terms of their floristic diversity including vascular plants, bryophytes and lichens. Our methodology includes two sample plots of 25 m^2 situated in the middle of the north and south facing slopes (**Fig. 2**). Data about plant species diversity and abundance is collected in each plot. Species lists are completed by an additional survey all around the mound. We propose a functional trait-based ecological research on the flora of the mounds to improve

Fig. 1. Buffer of 200 m were outlined around each burial mound with land use types defined

Fig. 2. Sample plot

our understanding of plant communities' structure. For this purpose, original field data will be collected and international databases will be considered. This trait-based research could further be used to evaluate the changes in plant diversity and ecosystem function.

DISCUSSION

The human wellbeing (Clark 2014) depends on the knowledge and ability for the sustainable management of the ecosystems. Therefore, a step forward in improving the way we manage the ecosystems is to enlarge our knowledge about their extent, condition and capacity. The project offers new knowledge about the importance of the burial mounds in Bulgaria as refugee for natural biodiversity and provision of ecosystem services. The project provides unique design combining two far different aspects of human activity – contemporary development and preserved historical past. It also establishes a bridge between history and biology which has rarely attracted such different groups of scientists.

The appearance of burial mounds is associated with *Yamna culture*, coming into the Balkans from Russian steppes during the end of 4th millennium BC. Later on the burial mounds become common practice in Thracian funeral rituals. This practice lasts till the 4th century AD when the Christianity becomes leading religion. According to Kitov (1993) the number of Thracian mounds in Bulgaria exceeds 50,000, but their number is probably higher. In the past times they certainly have been more numerous but due to treasure hunting, constructions, military activities and archaeological investigations during the past century thousands mounds have been destroyed. These landscape features have been preserved for millennia due to their spiritual significance and also because they could hardly be ploughed as their original height more often ranges between 3 and 15 m (Kitov 1993; **Fig. 3**).

Fig. 3. An example of burial mound surrounded by agricultural fields.

Burial mounds increase the landscape scale biodiversity of human transformed landscapes. In the same time burial mounds are influenced by human activities such as treasure hunting, afforestation, grazing or abandonment. We observed in the field that most of the visited mounds are disturbed by treasure hunters. The physical disturbances caused by the digging favors the establishment of woody plants and also promote the invasion of weeds and alien species. Lower parts of the mounds are usually rich in weeds established by diaspores from the neighboring agricultural fields. At some places, unfortunately we observed waste disposal on mounds close to roads and urban areas.

During the summer of 2019 we sampled jointly with Hungarian colleagues 8 mounds in the region of Plovdiv (**Fig. 4**). Floristic data was collected together with measurements of climatic and soil parameters. All samples are now in a process of analyses. Aim of this sampling is to compare the kurgans in Bulgaria with these in Hungary in terms of species functional diversity and habitat preferences.

Fig. 4. Common field work with Hungarian colleagues.

For the future conservation measures there is a need of specialised database which could provide up-to-date information about the biodiversity of the mounds and support the work of the decision makers in designating national and regional-level protection and restoration plans. Such databases are under preparation for the whole area of Hungary and for a part of Ukraine (Tóth 2006; Sudnik-Wójcikowska & Moysiyenko 2012, 2013; Bede 2014). Recently a new Eurasian kurgan database was established (Deak et al. 2019c). It aims to serve as a public repository of basic kurgan data that can be used by a wide range of end-users. It provides an easy to use data for conservation managers and landscape planners who require baseline information on the location, typical land use type and threatening factors present on the kurgans.

Another significance of the project implementation is to attract farm owners to enhance their knowledge about the importance of ecological approach in agricultural practices. There is a trend in European Common Agricultural Policy (CAP) which aims to provide additional financial support to the farmers to adopt agricultural practices which are beneficial for biodiversity, environment and climate. Following the EC Regulation № 1307/2013 farm owners must fulfill requirements to protect Ecological Focus Areas (EFA). The EFA are territories within the arable lands aiming at safeguarding and improving biodiversity on farms. This fits in particular with the objective 3A of the EU biodiversity strategy to 2020 (COM/2011/0244). EFAs can be features such as fallow land, field margins, hedges and trees or buffer strips which directly benefit biodiversity. They can also include specific productive areas whose effect on biodiversity is indirect through a lower use of inputs such as fertilizers. EFAs in general are beneficial also for sustaining ecosystem services (such as pollination, pest and disease control and soil erosion).

Some of the EFAs, the so called ‘landscape features’, have a particular interest for biodiversity conservation. They include: ‘hedges or wooded strips’, ’isolated trees’, ‘trees in line’, ‘trees in groups’, ‘field margins’, ‘ponds’, ‘ditches’, ‘traditional stone walls’ and ‘other landscape features’. The EC Regulation № 1307/2013 provides opportunity for each member state to select “other landscape features” and determine their significance for conservation. Bulgaria, which has adopted Regulation № 1307/2013 in 2014, accepted all landscape features besides stone walls and has not appointed any ‘other feature’. At the same time ancient monuments or archaeological sites are included in the list of *Other landscape features* in Denmark, Ireland, Hungary and UK Northern Ireland (EC 2015). Therefore in these states the conservation of cultural and historical heritage is combined with the conservation of natural habitats with characteristic flora and fauna. In Bulgaria historical monuments are protected by the Cultural heritage law, but the protection is not extended to their flora and fauna. Hungary is the only country where all kurgans are protected by the nature conservation law regardless whether they are situated in a protected area or not.

All mentioned above emphasizes the scientific goals of the project implementation to reveal the significance of the burial mounds not only as cultural treasure, but also as natural treasure for the country. Remaining outside EFAs these landscape features will be overlooked in biodiversity conservation assessments in the context of intensive agriculture. Project results will provide information for the broad public and will enhance the local stakeholders’ interest about the significance of the historical landscape features. We hope this will enlarge the tourist interest to the historical monuments also as spots of natural heritage.

Besides the particular importance of burial mounds in biodiversity preservation, the project results will allow for further analyses on the effects of fragmentation and isolation on grasslands using collected field data from Bulgaria. Such data is very scarce for the country so far. The burial mounds are preserved as cultural heritage, but we expect that the project results will offer arguments also for preservation their natural heritage. This would additionally emphasise their attraction as touristic objects. The new knowledge will emphasize the importance of national history and culture in combination with the natural heritage .

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

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FASCINATION OF PLANTS DAY (FOPD) – REALITY AND TRADITION IN BULGARIA

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Abstract. The present report aimed at providing information about the Fascination of Plants Day (FoPD) which is an initiative for popularization of plant science. FoPD was launched by the European Plant Science Organisation /EPSO/ to facilitate the understanding of plant science and to improve its impact and visibility. The FoPD initiative was set worldwide in 2012 for first time, as Bulgaria joined in 2015 and since then participates with events that bring plant science to the non-scientific public. The Agrobioinstitute of the Agricultural Academy as a coordinator for Bulgaria took the challenge to introduce FoPD among the academic society. In response, the scientific faculties of Sofia University, Shumen University, Agricultural University and other plant research institutions embraced the opportunity to reveal the charm of plants. Here is given an overview of the generally mastered strategies as we put accent on the activities undertaken in the Faculty of Biology of Sofia University where faculty members and students united with the Agrobioinstitute. Our mission is on one hand to convince more Bulgarian researchers to participate in the coming FoPDs, and on the other hand to attract more schoolteachers to bring pupils and future scientists to the organized events, as well as interested citizens, professionals, decision-makers and media.

Keywords: Agrobioinstitute, Bulgaria, European Plant Science Organisation /EPSO/, Faculty of Biology, public, schools, Sofia University

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INTRODUCTION

Williams (2011) points out important accents why the study of plant biology has never been more important or more exciting. Briefly, plants and humans share a common ancestor that lived ca. 3 billion years ago, they share a common DNA language and a mostly similar cell structure. During the evolution plants incorporated a single-celled photosynthetic bacterium into their cells, which enabled them to carry out photosynthesis, i.e. the ability to convert the sunlight energy into chemical energy. Thanks to photosynthesis, all the food that animals eat comes directly or indirectly from plants. Notably, as a by-product of photosynthesis, plants produce the oxygen that we and other beings need to live. Furthermore, a complex set of biochemical pathways has evolved in plants, which produce a wide variety of interesting and novel chemical compounds. Many of these compounds function to deter pathogens or herbivores, and some are medicinally useful to us. Genetic tools are enabling plant scientists to understand plants with ever-increasing levels of sophistication. This knowledge is making it possible to develop plants that are more efficient providers of the food, medicines, fibers, and raw materials upon which our human population is wholly dependent.

The European Plant Science Organisation /EPSO/ is an independent academic organisation currently representing 69 institutional members bringing together more than 200 research institutes, departments and universities from 31 countries in Europe and beyond (EPSO 2020a). EPSO's mission is to improve the impact and visibility of plant science in Europe (**Fig. 1**). Among EPSO's top priorities is to facilitate the understanding of plant science (EPSO 2020a).

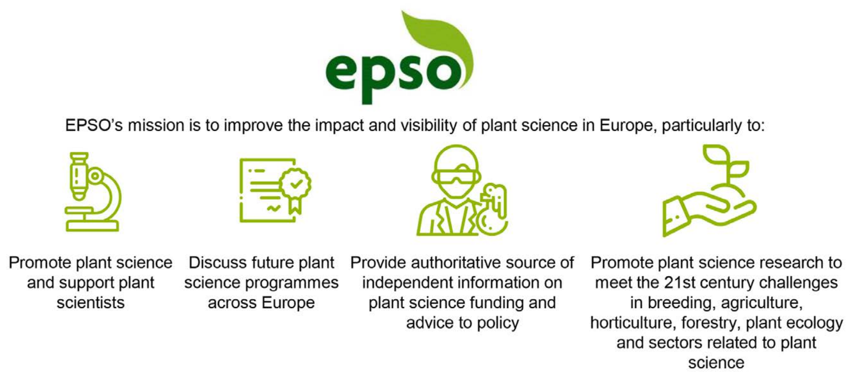

Fig. 1. Schematic representation of EPSO's mission (after https://epsoweb.org/about-epso/).

Fascination of Plants Day (FoPD) was initiated and supported by EPSO with the clear aim to promote the fascination and importance of plants and plant science worldwide (FoPD 2020; **Fig. 2**). The goal of FoPD, declared there, is to involve as many people as possible around the globe who are fascinated by

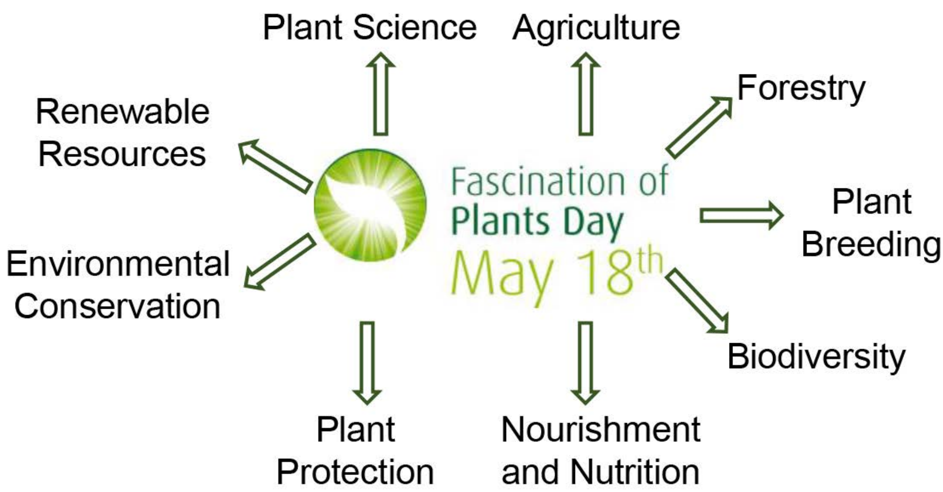

Fig. 2. FoPD promotes the importance of plant science (adapted from FoPD PR Toolbox Posters: https://plantday18may.org/wp-content/uploads/2019/01/Poster-1.pdf).

plants, recognize the importance of plant science and assess its role for agriculture, food and feed production, as well as for horticulture, forestry, and all of the non-food products /paper, timber, energy, chemicals, pharmaceuticals/, and realise the importance of plants for sustainable environment. For first time FoPD was celebrated in 18th May 2012 (EPSO 2020a; FoPD 2020) and since than the initiative became bi-annual: throughout the whole month of May in 2013, 2015, 2017, 2019 with thousands of events across the world (EPSO 2020b; **Table 1, Fig. 3**).

Everybody is welcome to join the FoPD initiative - from schools to horticulture and anyone who feels interested in plants (FoPD 2020). Also invited are the media, scientists, farmers, politicians and industrialists to discuss the latest state-of-the-art research and breakthroughs in the plant science and explore the potential applications plant science can offer.

Plant science institutions, universities, schools, botanical gardens and museums, together with farmers and industry, have opened their doors during the FoPD in 2012, 2013, 2015, 2017 and 2019. Variety of interactive plant related events were held across the globe (see summaries as *Success Stories* in links in **Table 1**).

RESULTS AND DISCUSSIONS

The FoPD organizers recommend for each country to make public the translation of the "Why study plants" presentation of Williams (2011), and for Bulgaria in particular, a Bulgarian version was created (FoPD 2015).

In 2015 in Bulgaria, the national representative of EPSO Prof. Rossitza

Table 1. FoPD statistics (https://plantday18may.org/statistics/).

FoPD	Events uploaded	Participating institutions	Participating countries	Links:
1st 2012		560	39	https://blog.rsb.org.uk/fascination-of-plants-day-18th-may-2012/
2nd 2013	1032	689	54	FoPD-2013-success-story: https://plantday2015bg.files.wordpress.com/2020/04/suppl.-information-fopd-2013-success-stories.pdf
3rd 2015	965	589	56	FoPD-2015-success-story: https://plantday2015bg.files.wordpress.com/2020/04/suppl.-information-fopd-2015-success-stories.pdf
4th 2017	1019	n.a.	52	FoPD-2017-success-story: https://plantday2015bg.files.wordpress.com/2020/04/suppl.-information-fopd-2017-success-stories.pdf
5th 2019	865	n.a.	52	FoPD-2019-success-story: https://plantday2015bg.files.wordpress.com/2020/04/suppl.-information-fopd-2019-success-stories.pdf Statistics Facebook

Batchvarova (Agrobioinstitute of the Agricultural Academy /AA/) opened the first FoPD ceremony. The national coordinator Dr. Anelia Iantcheva (Agrobioinstitute, AA) promoted the initiative and its further maintenance in 2017 and 2019 (**Table 2**). The events in each country could be uploaded by the national coordinator on the official page of the Fasciantion of Plants Day (FoPD 2020c), where the respective information and program, as well as final report assigned as *Success story*, could be seen worldwide. Each institution is invited to circulate the news on its own webpage and media of choice. In Sofia, the Department of Methodology of Biology Education in the Faculty of Biology assisted in informing and inviting different schools, and in the last FoPD-2019 edition schoolchildren were invited to take active part in demonstrating the fascination of science together with their Biology teacher and faculty supervisors (**Table 2**).

Altogether, researchers, university professors and students invented charming presentations, demonstrations and gatherings related to interesting facts and properties of plants and their importance for people and environment. To attract more young people, some organizers conducted successfully various contests for drawing, photography, writing, scientific lottery, and the Agrobioinstitute even created picturesque art corners for kids.

The Agrobioinstitute in union with Sofia University via the Faculty of Biology

Fig. 3. Participating countries in FoPD-2019: Argentina, Australia, Austria, Belgium, Bolivia, Bosnia, Brazil, Bulgaria, Canada, Chile, China, Croatia, Czech Republic, Ethiopia, Finland, France, Germany, Greece, Hungary, Iceland, India, Ireland, Italy, Japan, Latvia, Lebanon, Lithuania, Mexico, Mozambique, Nepal, Netherlands, New Zealand, Nigeria, Norway, Pakistan, Philippines, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, UK, Ukraine, Uruguay, USA, Zambia. Note that in previous years other countries took part, too: Algeria, Angola, Cameroon, Cyprus, Denmark, Estonia, Guinea-Bissau, Guyana, Israel, Paraguay, Saudi Arabia, South Africa, South Korea, Tanzania, Thailand, Turkey (adapted from FoPD-2019-success-stories: https://plantday2015bg.files.wordpress.com/2020/04/suppl.-information-fopd-2019-success-stories.pdf).

played significant role in spreading knowledge about plant fascination and plant science at FoPD in Sofia. The teams of researchers and students explored various ways to bring out why plants are interesting to study (**Tables 2, 3; Fig. 4**). It was organized a visit to the Herbarium of the Sofia University (registered as SO in Index Herbariorum, Asenov et al. 2012) where are stored specimens from the Bulgarian and foreign flora (op. cit.). Very spectacular demonstrations were performed by the faculty Students Club for Education and Development with Ecological Center (with abbreviation SKOREC, transliterated from Bulgarian language) where its members showed compilation of live plants and animals, as well as home-made souvenirs. The young visitors could learn how to do soil planting, while the Institute of Ornamental and Medicinal Plants set flower expositions for the adults who could take home various plants in pots. Some of the plants were planted outdoor as a memory from the event. Everyone could enjoy the works of the contestants, covering a broad age limit, by attending the Facebook webpage or as exhibition and wall-projection in the faculty lobby. The most active participants received certificates as a proof for the efforts made. In addition, the Agrobioinstitute researchers transferred their fascination about plants by awarding the winners from the competitions with lab-

Fig. 4. Captured moments from FoPDs in Faculty of Biology.

made luxury ikebana with in vitro ornamental plants.

Other AA institutions in Sofia and the Institute for Plant Genetic Resources in Sadovo had also participated with round tables and open days (**Table 2**). The Faculty of Natural Science of Shumen University and the Agricultural University in Plovdiv took seriously the FoPD as opportunity for organization of various gatherings for celebrating plant fascination. The university members showed contagious enthusiasm in promoting local research on fascinating Bulgarian plant species, medicinal plants, bio-products, grain and bread production, etc. Again, the events were intended for pupils and students where the young explorers demonstrated own knowledge about practical aspects of plant science and were involved in degustation of culinary exposition.

Writing about the FoPD, we have to recall especially on its education focus with

Table 2. FoPD events in Bulgarian scientific institutions.

FoPD	Bulgarian institutions (main organizers)	Type of activities	Location
3rd 2015	Agrobioinstitute, AA (Anelia Iantcheva, PhD)	Presentations and practical demonstrations Scientific lottery Contests on the topics “The Fascination of Plants”, “The Fascination of Protected Bulgarian Plant Species”, “The Flora and Us” (photography and drawing) Awarding of certificates and prizes	Faculty of Biology Sofia
	Faculty of Biology, Sofia University (Miroslava Zhiponova, PhD; Kameliya Yotovska, PhD; Dean Mariela Odjakova)	Presentations and practical demonstrations Visit of Herbarium of SU Flower planting in the courtyard of the faculty	
	Joint Genomic Center (Atanas Atanassov, Acad. Prof.)	Movie projection about the plants during the four seasons	
	Institute of Ornamental Plants, Negovan (Nadejda Zapryanova, PhD Rosen Sokolov, PhD)	Exposition of flowers	
	Faculty of Natural Science, Shumen University (Dimcho Zahariev, PhD)	Presentation series about *The fascination of plants in Bulgaria*	Faculty of Natural Science Shumen
	Institute for Plant Genetic Resources, AA	Open day for visiting the botanical garden	AA Sadovo
	Institute of Agricultural Economics, AA Institute of Cryobiology and Food Technology, AA Institute of Animal Science, AA	Round table about *The agricultural science in favor to the practice*	AA Sofia
	Institute of Soil Science, Agrotechnology and Plant Protection, AA	International conference about *Soil and agrotechnology in a changing world*	AA Sofia
4th 2017	Agrobioinstitute, AA (Anelia Iantcheva, PhD)	Presentations Plant Art corner for kids Planting seeds and plants in soil Contests on topic *Protected and endangered Bulgarian plant species* (photography, drawing, presentation, video, essay) Awarding of certificates and prizes	Faculty of Biology Sofia
	Faculty of Biology, Sofia University (Miroslava Zhiponova, PhD; Kameliya Yotovska, PhD; Dean Stoyan Shishkov)	Presentations and practical demonstrations Visit of Herbarium of SU	

FoPD	Bulgarian institutions (main organizers)	Type of activities	Location
	Institute of Ornamental Plants, Negovan (Nadejda Zapryanova, PhD)	Exposition of flowers	
	NATURAL+ Ltd.	Kombucha drink testing and discussion	
	School company "Alpha" from Secondary School "Kozma Trichkov" - Vratsa	Environmental technologies	
	Faculty of Natural Science of Shumen University (Dimcho Zahariev, PhD)	Program dedicated to the medicinal plants	Faculty of Natural Science Shumen
	Agricultural University, Plovdiv (Svetla Yancheva, PhD)	Bio-exhibition	Agri-cultural University, Plovdiv
5th 2019	Agrobioinstitute, AA (Anelia Iantcheva, PhD)	Presentations Plant Art corner for kids Planting seeds and plants in soil Contests on topic *Plants as a food for animals and humans* (photography, drawing, presentation, video, essay) Awarding of certificates and prizes	Faculty of Biology Sofia
	Faculty of Biology, Sofia University (Miroslava Zhiponova, PhD; Kameliya Yotovska, PhD; Dean Stoyan Shishkov)	Presentations and practical demonstrations	
	American College of Sofia (Ganka Dineva, PhD)	The Biotechnology Club	
	Institute of Ornamental Plants, Negovan (Nadejda Zapryanova, PhD)	Exposition of flowers	
	Faculty of Natural Science of Shumen University (Dimcho Zahariev, PhD)	Program *No one is bigger than bread*	Faculty of Natural Science Shumen
	Agricultural University, Plovdiv (Svetla Yancheva, PhD)	Gathering organized by the Student Council	Agri-cultural University, Plovdiv
	Institute of Plant Physiology and Genetics (IPPG), Bul-garian Academy of Sciences (BAS) (Valya Vassileva, PhD)	Confocal microscopy for studying NudC proteins in roots	IPPG, BAS

Table 3. FoPD events in the Faculty of Biology of Sofia University. Numerous activities were organized by faculty teams including the Departments of Plant Physiology, Methodology of Biology Education, Biochemistry, Biophysics and Radiobiology, Botany, General and Applied Hydrobiology, Virology, Biotechnology, General and Industrial Microbiology, as well as Bachelor (also Erasmus), Master and PhD students. The Agrobioinstitute', as well as other partners' topics are included.

FoPD	Participants from Faculty of Biology				Topics of presentations and practical demonstrations
	BSc	MSc	PhD	Departments	
3rd 2015	26	7	4	18 members	"**FLORA OF TASMANIA**" – the exotic plant biodiversity on the island. (Assist. Prof. Lyuben Zagortchev, Prof. Mariela Odjakova) "**SALT-TOLERANT PLANTS. FROM THE FIELD TO THE LABORATORY AND BACK**" – molecular approaches to improve the plants sustainability. (Assist. Prof. Lyuben Zagortchev, Prof. Mariela Odjakova) "**ORCHIDS**" – types of orchids, their cultivation and characteristics. (Hristina Samardzhieva) "**THE FORESTS – THE LUNGS OF THE EARTH**" – importance of maintaining the forests that provide the oxygen for breathing. (Thomas Damjanov, Sergey Kavlak) "**PLANT-PREDATORS**" – interesting facts about carnivorous plants. (Martin Ninov) "**AVOCADO IN POT**" – interesting facts about the avocado and its cultivation. (Desislava Mantovska) "**CRYOPRESERVATION IN PLANTS**" – a method of freezing plants for their long-term preservation. (Assist. Prof. Daniela Dragolova, Prof. Veneta Kapchina-Toteva) "**PLANT PATHOGENS – RISING THREAT FOR BIOLOGICAL DIVERSITY**" – information about the causes of disease in plants. (Agrobioinstitute: Assist. Prof. Aneta Lyubenova, Assoc. Prof. Slavtcho Slavov, Prof. Rossitza Batchvarova) "**MOLECULAR MARKERS FOR INCREASING THE EFFICIENCY OF SELECTION OF ECONOMICALLY IMPORTANT CROPS**" – discussion of the contemporary approaches for improving plant species that are important for mankind. (Agrobioinstitute: Assoc. Prof. Nikolai Christov, Dr. Stefan Tsonev, Dr. Lyubov Hristova, Assoc. Prof. Elena Todorovska) "**FROM THE CELL TO THE WHOLE PLANT**" – explanation about the unique ability of plants to regenerate. (Agrobioinstitute: Miglena Revalska, Assoc. Prof. Anelia Iantcheva) "**ROOT CULTURES**" – applications of root cultures from medicinal plants. (Nia Petrova, Assist. Prof. Zhenya Yordanova) "**THE MAGIC OF THE BULGARIAN HERBS**" – role of the Bulgarian herbs for health and demonstration of representatives in a tube. (Assist. Prof. Milena Dimitrova)

FoPD	Participants from Faculty of Biology				Topics of presentations and practical demonstrations
	BSc	MSc	PhD	Departments	
					“**HABERLEA AND THE SECRET OF THE ETERNAL LEAF**” – how one endemic plant can reveal the secrets of resurrection – presentation and demonstration. (Agrobioinstitute: Assist. Prof. Daniela Moyankova, Petko Mladenov, Prof. Dimitar Djilianov) “**ROLE OF THE PLANTS FOR THE WATER WORLD**” – discussion about the importance of plants for the life in the water, as well as the business aspects of the work with aquatic plants. (Vasilena Angelova, Eleonora Fikovska, Stefani Pyrvanova, Dr. Marieta Stanachkova, Assoc. Prof. Galerida Raykova, Prof. Yana Topalova) “**THE WATER MOVEMENT IN THE PLANT CELLS**” – observation under microscope of plasmolysis in epidermis of red onion. (Assist. Prof. Daniela Dragolova) “**THE ROLE OF STOMATA IN PLANTS**” – observation of stomata cells under microscope. (Assist. Prof. Miroslava Zhiponova) “**WHAT IS PHOTOSYNTHESIS?**” – experiment about the properties of the plant pigments. (Trifon Sotirov, Martin Maznilev, Milena Milekova) “**THE FASCINATION OF MICROALGAE**” – demonstration of pigments isolated from algae. (Assoc. Prof. Ganka Chaneva) “**THE VOICE OF PLANTS**” – application of biophysical approach for “hearing” the plants. (Stela Dimitrova, Petar Markov, Assist. Prof. Kolyo Dankov, Momchil Paunov, Prof. Vasiliy Goltsev) “**DID YOU KNOW THAT THE PLANTS HAVE HORMONES?**” – introduction into the classes of plant hormones that mediate signals of how plants should grow and develop appropriately. (Assist. Prof. Miroslava Zhiponova): “ABSCISIC ACID” (Ivalena Ilieva, Miroslava Ivanova); “**AUXINS AND CYTOKININS**” (Boryana Ivanova, Hyuliya Tatarly, Ana Vylkova, Borislava Stanoykova, Mirela Georgieva, Yoana Kirilova, Carmen Yordanova, Dimityr Dimitrov, Rumyana Ganeva); “**GIBBERELLIC ACID**” (Joe Velchev, Alexandar Kavazov); “**ETHYLENE**” (Zornitsa Kirilova, Bojidara Pavlova, Prof. Evgeni Ananiev) “**FLOWER IN THE TUBE**” – propagation of plants in test tubes. (Maria Rogova, Anelia Raycheva, Katya Ivanova) **VISIT OF THE SOFIA UNIVERSITY HERBARIUM**. (Assoc. Prof. Asen Asenov, Prof. Maya Stoyneva) **ORNAMENTAL PLANTS EXHIBITION** (Negovan: Assoc. Prof. Nadejda Zapryanova, Assist. Prof. Rosen Sokolov)

					PLANTING OF ROSES, GOJI BERRIES AND FLOWERS IN THE FACULTY BACKYARD TO MEMORIZE THE FIRST FOPD. (Assist. Prof. Dilyana Nikolova, Assist. Prof. Yana Evstatieva, Assist. Prof. Anita Tosheva, Prof. Veneta Kapchina-Toteva; Agrobioinstitute: Assist. Prof. Nabil Mosa Abumhadi) **LOTTERY** (Assoc. Prof. Anelia Iantcheva, Prof. Rossitza Batchvarova) **DRAWING CONTEST** (Agrobioinstitute; Assist. Prof. Kameliya Yotovska) **PHOTOGRAPHY CONTEST** (Silvana Angelova, Dayana Angelova, Bojidara Pavlova, Ivalena Ilieva, Desislava Mantovska) **MOVIE PROJECTION** (Assoc. Prof. Dolya Pavlova, Acad. Prof. Atanas Atanassov, Assist. Prof. Ilian Badjakov)
4th 2017	25 + 2 Erasmus	5	3	13 members	**“ORGANIC FERTILIZERS - DO IT YOURSELF”** – benefits of bio compost. (Gabriela Tsoynska) **“FOR PLANTS AND FOR WATER – WHY ARE PLANTS "SWEATING"?”** – explaining what is transpiration. (Violeta Kostova, Iliyana Stoyanova, Assoc. Prof. Kameliya Yotovska, Assoc. Prof. Asya Asenova) **“CARNIVOROUS PLANT”** – facts that make them fascinating. (Dayana Paskova, Zhasmina Redzhepova) **“ABOUT THE MEDICINAL PLANTS”** – tradition and science. (Natalie Atanasova, Evgenia Mihaylova) **“THE MEDICINAL MARIJUANA”** – pros and cons. (Teodora Uzunova, Desislava Kircheva) “**THE BENEFIT OF PLANT BIOTECHNOLOGY”** – in vitro micropropagation of valuable plant species. (Ivalena Ilieva, Marina Alexeeva, Assoc. Prof. Zhenya Yordanova, Prof. Veneta Kapchina-Toteva) **“REASONS FOR STUDYING THE PLANTAIN (GENUS *PLANTAGO*)”** – approaches to explore unknown aspects about this genus. (Ani Kercheva, Dariana Vylova) **“MOLECULAR STUDIES ON HOPS (*HUMULUS LUPULUS*)”** – the secret molecules that make the plant a cure. (Dimitar Iliev) **“KALANCHOE - A HOME PHARMACY”** – use of the plant with health purpose. (Alexandra Nissimova, Joe Velchev) **“ESSENTIAL OILS”** - biosynthesis, accumulation and excretion in aromatic plants; extraction of essential oils. (Zvezdelina Petrova and Irina Petrova) **“KOMBUCHA OR THE ROAD TRIP OF *CAMELLIA SINENSIS* TEA”** (NATURAL+ Ltd.) **“VAMPIRE PLANTS. BIOLOGY OF PARASITIC PLANTS OF THE GENUS *CUSCUTA*”** – how the Cuscuta acts. (Assist. Prof. Denitsa Teofanova, Assoc. Prof. Anita Tosheva, Assoc. Prof. Lyuben Zagorchev)

FoPD	Participants from Faculty of Biology				Topics of presentations and practical demonstrations
	BSc	MSc	PhD	Departments	
					"PLANTS IN FAVOR OF FIGHTING VIRUSES" (Dr. Anton Hinkov, Venelin Tsvetkov, Assoc. Prof. Stoyan Shishkov) **"PLANT IMMUNITY"** – the plants have chemical defence. (Branislav Dinić, Tuba Öztürk, Dilyan Minev) **"THE RESPONSE OF ORDINARY BEECH TO BEETLE INVASION"** – tree against insect. (Vasil Chavgov, Assoc. Prof. Ganka Chaneva) **"CONFRONTATION OF ABSCISIC AGAINST GIBBERELLIC ACID"** – keeping the balance of plant growth. (Maria Tomeva, Viktor Rashev) **"THE CURIOUS ACTION OF THE AUXIN"** – the plant hormone mediates important signals. (Silvia Ganeva, Iva Dimitrova) **"THE VOICE OF PLANTS"** – biophysicists can hear it! (Bojidara Pavlova, Stella Dimitrova, Momchil Paunov, Prof. Vasilij Goltsev) **"AGRICULTURAL CRATES WITH SPECIAL COATING FOR CONTINUOUS FRESHNESS OF AREAS AND VEGETABLES FOR PEOPLE WITH SMALL FARMS AND GARDENS"** (School company "Alpha" "Kozma Trichkov"- Vtatsa) **"PLANT MORPHOLOGY"** – demonstration (Agrobioinstitute: Functional Genetics Legumes Group) **"INFORMATION CENTER IN PLANT BIOTECHNOLOGIES"** – presentation (Agrobioinstitute Abiotic Stress Group) **SCIENTIFIC POSTER SESSION** – "Plant abiotic stress", Project "Evaluation and monitoring of the effect of genetically modified plants on agro-ecosystems", "Beautiful but dangerous: the invasive species of genus Phytophthora", "Production of valuable products from microalgae", "Functional genetics-cereals", "The Bulgarian tomatoes Pako-Orange could substitute the carrots from the children pure! Tell to Mom!", "Plant genetic resources", "Plant molecular genetics", "Project STARBIOS–expansion of the responsible biosciences", "Viruses – the invisible plant enemy", "Plant biology in legumes" (Agrobioinstitute: Assist. Prof. Mariana Radkova, Prof. Dimitar Djilianov, Assoc. Prof. Daniela Moyankova, Assoc. Prof. Petya Stoykova, Prof. Ivanka Kamenova, Assoc. Prof. Anelia Iantcheva, Assist. Prof. Miglena Revalska) **TOMATO PLANTING** (Agrobioinstitute) **ART CORNER FOR KIDS** - applications, drawings, cards (Agrobioinstitute, Assis. Prof. Liliya Georgieva)

					ORNAMENTAL PLANTS EXHIBITION (Negovan: Assoc. Prof. Nadejda Zapryanova) **DRAWING CONTEST** (Agrobioinstitute; Assist. Prof. Kameliya Yotovska) **VISIT OF THE SOFIA UNIVERSITY HERBARIUM**. (Assoc. Prof. Asen Asenov, Prof. Maya Stoyneva) **STUDENT'S CLUB SKOREC** – live plants and animals, home-made souvenirs. (Dimitar Dimitrov, Kiril Vylkanov, Adaim Palov, Plamen Petrov, Siyka Lakova, Assoc. Prof. Atanas Grozdanov)
5th 2019	3 & 6 school pupils	3	2	11 members + 2 guests	**"PLANTS - OBJECT TO ARTISTIC INSPIRATION"** - artist guest Savina Mantovska **"APPLYNG A JELLYFISH PROTEIN FOR EXPERIMENTS"** – the molecule that emits light to see it (The "Biotechnology Club of the American College-Sofia: Dr. Ganka Dineva, Mariya Cholakova, Boris Beev, Eva-Mariya Tsanova, Yasen Nikolov, Alexandra Velkova; Assoc. Prof. Iliyana Ilieva) **"LIGHT RECIPE TO IMPROVE PLANT GROWTH"** – light intensity needs to be adjusted for each plant. (American College-Sofia: Yvette Dimitrova, Ivo Yordanov; Assoc. Prof. Miroslava Zhiponova) **"THE GLUTEN-FREE DIET - MYTH OR SCIENCE"** – interesting facts about the food we eat. (Alexandra Atanasova, Kristiyana Georgieva, Assoc. Prof. Denitsa Teofanova, Assoc. Prof. Lyuben Zagorchev) **"PLANTS LIKE FISH - WHAT ARE THE AQUACULTURES?"** – the neighborhood of pea and golden fishes. (Alexander Tomov, Vasil Chavgov, Assist. Prof. Detelina Petrova, Assoc. Prof. Ganka Chaneva) **"HOW TO EXPLORE MEDICINAL PLANTS?"** - in vitro growth of valuable plant species. (Assist. Desislava Mantovska, Assist. Prof. Mariya Rogova) **"DID YOU KNOW THAT PLANTS HAVE HORMONES?"** - introduction into plant hormones that mediate signals of how plants should grow and develop appropriately. (Assoc. Prof. Miroslava Zhiponova) **"IDEA FOR ECOLOGICAL FLORICULTURE"** – come and get it! (Anelya Raycheva, Assist. Prof. Marieta Hristozkova) **"WHAT DO THE ANIMALS EAT"** – demonstration of forage and seed samples. (Agrobioinstitute: Assoc. Prof. Anelia Iantcheva) **ART CORNER FOR KIDS** - applications, drawings, cards (Agrobioinstitute: Assist. Prof. Liliya Georgieva) **ORNAMENTAL PLANTS EXHIBITION** (Negovan: Assoc. Prof. Nadejda Zapryanova) **STUDENT'S CLUB SKOREC** - live plants and animals, home-made souvenirs. (Vassilin Anatkov, Dariana Atanasova, Assoc. Prof. Atanas Grozdanov)

importnac efor teaching and learning. The competences that young people form at school affect their ability to continue their education and integrate successfully in society (Jerrim et al. 2020). It is considered that activities involving interactions between students and professionals are useful for efficient competency achievement (Romanovtseva 2016; Ualiyeva et al. 2016). During this process occur a changing the focus of the training to mastering key competences and developing problem-solving abilities such as: integrated cross-curricular interaction; practical orientation of the training; result orientation; implementation of innovative approaches and practices in the teaching and learning process. In the focus of the FoPD's scope is the integration of young people and improving their motivation for professional orientation. Respectively, in Bulgaria during the organized FoPDs, three groups of youngsters could be distinguished – under school age, school age, and students. The first group of kids seemed to be enthusiastic about the engagement in any kind of activity. The pupils were interested in the demonstrations and mostly of these activities where they could participate, and they were readily initiating discussions demonstrating personal knowledge and interest. The group of the students were put in the role of teachers and it was observed their evolution in hours – from unsecure presenters to well erudite supervisors in the field of science that showed eagerness for better performance. In this way, mainly the students but also the school children started to develop certain science literacy that is ability for conscious and responsible engagement in questions of the natural sciences, for use of technology, for application of theoretical knowledge to practice, for explaining natural processes and phenomena, and for making reasoned conclusions about the natural sciences as part of the knowledge for the world (Cresswell et al. 2015).

From the teachers' point of view, the modern environment offers a wealth of information flow and the teacher has the privilege, but also the responsibility to choose those educational activities that will motivate students to participate actively, provoke their thinking and help him achieve the intended results. One of the biggest challenges for the modern teacher is to inspire his students to realize the meaning of skill acquisition.

Extra-curricular activities are particularly useful for this purpose, including visiting universities and laboratories and getting acquainted with the work of scientists. The scientists can show both students and the teacher a different perspective, "recharge" them and be more relevant to the situation. Therefore, FoPD could be considered as such opportunity.

CONCLUSION

As a part of scientific community and researchers involved in plant science we realize that the international FoPD aims to bring plant researchers closer to the society and to increase awareness for plants, and innovation activities of plant science, with a view to improve the public recognition of plants, creating a model for understanding of their impact on citizen's daily life, and encouraging society and especially young people to assess role of plants in sustainability of environment (**Fig. 5**).

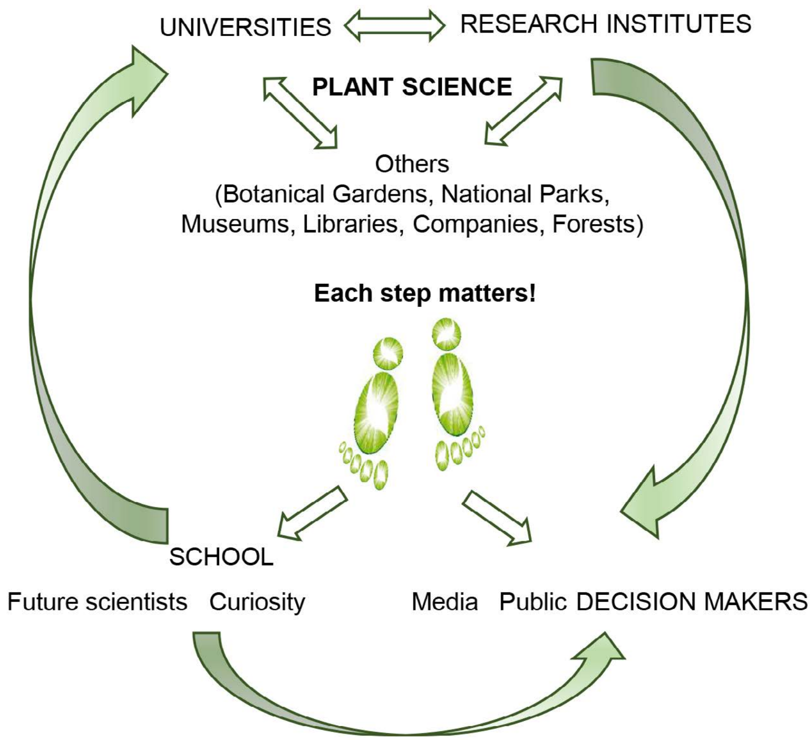

Fig. 5. Impact of FoPD on citizen society.

Regarding future perspectives, we have to note that easier electronic access to the organized events would allow attendance from distance and will make the benefits from FoPD available to a broader public (Asenova et al. 2017). We hope that the present report will motivate more Bulgarian organizations and individuals to join in the future FoPDs and other similar events making science popular.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

AUTHOR CONTRIBUTIONS

A. V. Iantcheva, M. K. Zhiponova and K. S. Yotovska contributed to the design, discussion and writing of the manuscript.

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Online links in the text, last accessed on April 2020, at:

EPSO sites: https://epsoweb.org/, https://epsoweb.org/about-epso/, https://epsoweb.org/events/fopd-events/

FoPD sites: https://plantday18may.org, https://plantday18may.org/about-us/

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FoPD-2019 Facebook: https://www.facebook.com/pg/Fascination-of-Plants-Day-419094251484268/posts/

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БИОЛОГИЧЕСКИ ФАКУЛТЕТ
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EFFECTS OF THE APPLICATION OF POLYCULTURE WITH GRASS CARP TO CONTROL AQUATIC VEGETATION IN FISHPONDS ON THEIR PHYTOPLANKTON AND MACROZOOBENTHOS

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Abstract. Aquatic vegetation overgrowth is a serious problem for the fish farming. It is caused by combination of factors, such as rapid climate change, rising water temperatures and eutrophication. The excessive development of aquatic macrophytes prevents the use of the fish ponds for different purposes and threatens the structure and functioning of the aquatic communities. In our experiment, in ponds stocked with two-year-old grass carp the aquatic vegetation (mostly *Ceratophyllum demersum* L.) was successfully reduced. In this way grass carp remained without its natural food and the improvement of the light regime in the ponds led to bloom of phytoplankton and changes in the species composition, which created a rich natural food base for the hybrid silver carp. High concentrations of orthophosphates at the beginning of the experimental period coincided with the high rate of development of aquatic macrophytes. Afterwards, during the period of intense phytoplankton blooms their concentrations decreased, presumably due to the increased consumption by algae. The removal of macrophytes led to high abundance of macrobenthic organisms (more chironomids and less oligochates).

Key words: aquatic macrophytes, fish farming

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INTRODUCTION

Aquatic plants are an important part of the ecosystems and require appropriate control and conservation of their biotopes. They provide habitat for different species of fish and ensure optimal oxygen regime in the water basins. When nutrients are deposited in the ponds, they are difficult to remove. The accumulation of phosphorus, which is a major limiting factor for the development of macrophytes, can sustain their development for years. Different ecological problems are usually registered in shallow eutrophic ponds. Petr (2000) notes that macrophytes can have a positive and negative impact on the aquatic biotopes. They directly affect oxygen saturation and convert ammonia into useful nitrates, and indirectly assist the recirculation of nutrients. Aquatic plants also affect benthic, planktonic and fish communities. Traditional methods of control of excessive aquatic vegetation, such as mechanical and chemical methods, are too labor-intensive and costly, and can have a negative impact on the aquatic ecosystems. Biological control can be an alternative to the traditional methods.

In order to effectively manage macrophytes it is needed to ensure long-term control without negative impacts on ecosystems and communities (Pipalová 2006; Bozkurt et al. 2017). One of the most widely used species for control of macrophytes development is the grass carp (*Ctenopharyngodon idella* Val.). By using the higher aquatic vegetation as a major food source, it can be one of the solutions in managing aquatic vegetation overgrowth. The grass carp belongs to the family Cyprinidae with natural populations in Southeastern Russia and Northwest China. These herbivore species are introduced in many countries for the purposes of control of macrophytes development. *C. idella* is a component of polycultures in closed systems in most of Eastern European countries. After successful artificial reproduction in the former USSR in 1961, the grass carp was introduced in a number of Eastern European countries (Van Zon 1977). In Bulgaria, according to our knowledge, it has been introduced in 1965 and a number of reproduction and breeding technologies have been carried out in the country. However, studies on its use in control of unwanted aquatic vegetation, its nutritional preferences and impact on aquatic ecosystems and communities are scarce. Dynamic climate change, as well as increased nitrogen and phosphorus concentrations in the water due to agricultural activity, increase the aquatic plants overgrowth and can cause serious problems in the fish farms. All mentioned environmental factors and ecological issues support the need for research of *C. idella* as a mean in the biological control of macrophytes overgrowth. The aim of the study is to develop a polyculture with grass carp as a mean in the biological control of the macrophytes overgrowth in fish ponds, by determining the effectiveness of the stocking density, age and size of the fish and their impact on the aquatic communities and environment.

MATERIAL AND METHODS

The study was carried on the territory of the Institute of Fisheries and Aquaculture, Plovdiv, Bulgaria. Water samples were collected in four experimental ponds (P12, P18, P19 and P23) from May to September 2018 during a research project G-146 „Development of new polyculture as a tool for adaptation to climate change" (Agricultural Academy, Sofia, Bulgaria) according to the standard methods and normative requirements. Water temperature (T ºC), dissolved oxygen (O_2), oxygen saturation (O_2%), electrical conductivity and pH were measured. The determination of the biogenic conditions involved the measurements of the following metrics: ammonium nitrogen (N-NH_4), nitrite nitrogen (N-NO_2), nitrate nitrogen (N-NO_3), total inorganic nitrogen (TN), orthophosphates (P-PO_4) and transparency of water (Sd) by means of a Secchi disk. The concentration of the photosynthetic pigment chlorophyll *a* was measured by spectrophotometric method by extraction of chlorophyll in ethanol from phytoplankton. Phytoplankton samples were taken twice a month in 1l glass banks. The further preservation of the samples was carried out with formalin to a final concentration of 4%. Quantitative and qualitative analysis was performed in Burker's counting chamber following Laugaste (1974). Biomass was calculated using formulas for the corresponding stereometric forms (Rott 1981; Deisinger 1984). The identification of algae was done on standard European floras and of diatoms in particular, was done after Cox (1996). The total biomass for each sample was estimated as the sum of the biomass of all phytoplankton, summed up by individual taxonomic groups. Dominant species were determined according to the percentage of individual species to the total biomass. The species identification was performed with light microscope Carl Zeiss Axioscope 2 plus at a 400x magnification with critical use of Algae Base (Guiry & Guiry 2019).

The macrobenthos sampling was done twice a month with Eckman & Birgge grab with opening width of 225 cm². Samples were washed through sieves with different mesh size (2 mm, 500 µm, 250 µm, 150 µm and 63 µm) and fixed in 95% ethanol. Further processing of the samples involved their washing with clean water, sorting by fraction and separation of the bottom invertebrates in sample tubes with 95% ethanol. The taxonomic identification was done on a binocular magnifying glass and a light microscope Carl Zeiss Axioscope 2 plus using keys and species descriptions mainly by Oliver (1971), Epler (2010), Oscoz et al. (2011). During the study, only macroinvertebrate fauna (> 500µm) was recorded. In order to determine the structure and composition of the benthic communities, the density of the organisms (individuals m^{-2}) was calculated. The percentage of oligochaetes (% Oligochaeta) and the percentage of chironomids (% Chironomus) were estimated.

The aquatic vegetation with its most common species (*Ceratophyllum demersum* L., *Nuphar lutea* (L.) Sm. and *Typha angustifolia* L.) were monitored bimonthly. The growth of macrophytes in the experimental ponds was calculated as a percentage of the total water area. During the vegetation period, the ponds were stocked with polyculture of two-year old grass carp (*Ctenopharyngodon idella* Valenciennes): 500 fish ha^{-1}, common carp (*Cyprinus carpio* L.): 400 fish ha^{-1} and hybrid silver carp (*Hypophthalmichthys molitrix* Valenciennes x *Hypophthalmichthys nobilis* Richardson): 100 fish ha^{-1}.

RESULTS AND DISCUSSION

After the application of polyculture grass carp stocking in density of 150 fish ha^{-1} with fishes of the same age, the fastest and best results were achieved in removing of *Ceratophyllum demersum*, but it took longer time for the less affected *Nuphar lutea* and *Typha angustifolia*.

The obtained data on minimum, maximum and average values of the physicochemical parameters of the water of four studied ponds, are presented in **Table 1**. According to them, the dissolved oxygen in the water had optimal values. The registered maximum concentrations of phosphate ions in three of the studied ponds (P12, P18 and P19) were optimal for the carp species. The only higher phosphate amounts, above the technological requirements, were registered in P12 in May and June. These high concentrations of orthophosphates in May and June, especially in P12, coincided with the most intense period of macrophytes growth in the experimental ponds. Afterwards, from July to September, during blooms of phytoplankton and their intensive consumption, the concentrations of the orthophosphates decreased.

During the experiment, the peak of chlorophyll *a* concentration coincided with the maximum growth of the phytoplankton, in which algae from the divisions Cyanoprokaryota (known also as cyanobacteria or blue-green algae), Chlorophyta and Ochrophyta (class Bacillariophyceae) were identified. In May dominant species were the diatom *Aulacoseira granulata* (Ehrenberg) Simonsen, the green alga *Desmodesmus communis* (Hegewald) Hegewald and the cyanoprokaryote *Anabaena sphaerica* Bornet & Flahault. The biomass was lowest in P12 and highest in P19 (**Fig. 1**). In June, dominants in P19 and P12 were the blue-green algae *A. sphaerica* and *Dolichospermum spiroides* (Klebahn) Wacklin, L. Hoffmann & Komárek with *D. communis* and *A. granulata* as co- dominants. The biomass significantly increased with the highest recorded values in P18 (**Fig. 1**). Generally, the biomass of phytoplankton and the concentration of chlorophyll a were more than 10 times higher in May but the phytoplankton biomass in P18 and P19 was significantly lower than the biomass in P23 and P12. In July the cyanoprokaryotes *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault and *A. granulata* were dominating. In P18, P19 and P23 among the co-dominant species were *A. sphaerica*, *D. spiroides* and *Oscillatoria limosa* C. Agardh ex Gomont, and in P18, P23 and P12 *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg. The phytoplankton biomass was the lowest in P18 and the highest

Table 1. Physicochemical parameters of the water and the concentration of chlorophyll *a* in the experimental ponds.

Para-meter	T	O_2	O_2	pH	NH_4^+	NO_3^-	NO_2^-	TN	NH_4	COD	PO_4^{3-}	Cond.	Chl. a
Mea-sure	°C	$mg.l^{-1}$	%		$mg.l^{-1}$	$mg.l^{-1}$	$mg.l^{-1}$	$mg.l^{-1}$	$mg.l^{-1}$	$mg.l^{-1}$	$mg.l^{-1}$	$\mu S.cm^{-1}$	$\mu g.l^{-1}$
Pond 12													
X	21.5	7.6	87	8.27	0.4	1.23	0.009	1.639	0.030	11.92	6.86	408	81.86
min	12.8	2.9	34	7.73	0.25	0.82	0.003	1.123	0.0130	0.11	0.36	349	3.7
max	27.5	10.3	126	8.88	0.53	1.45	0.020	1.910	0.062	24.75	24.75	570	203.62
n	30	30	30	30	10	10	10	10	10	10	10	30	10
s	4.21	1.8	20	0.25	0.1	0.19	0.007	0.231	9.718	8.77	63.36	63	71.38
Pond 18													
X	22.3	8.2	95	8.28	0.38	1.06	0.013	1.456	0.035	12.68	0.45	340	32.89
min	14.8	4.9	59	7.94	0.25	0.79	0.003	1.190	0.017	8.16	0.01	274	11.09
max	27	12.2	147	8.84	0.47	1.29	0.026	1.647	0.083	15.43	0.63	519	104.12
n	26	26	26	26	10	10	10	10	10	10	10	26	10
s	3.2	1.8	21	0.21	0.08	0.18	0.009	0.166	0.020	3	0.17	67	26.75
Pond 19													
X	22.7	7.3	86	8.22	0.45	1.16	0.016	1.618	0.026	11.83	0.61	367	31.4
min	14.9	3.2	40	7.84	0.21	0.63	0.003	0.903	0.012	6.94	0.01	283	4.94
max	27.3	12.3	134	8.69	0.66	1.64	0.040	1.990	0.039	16.59	1	505	107.7
n	26	26	26	26	10	10	10	10	10	10	10	26	10
s	3.1	2.1	23	0.18	0.16	0.27	0.012	0.331	0.01	2.71	0.29	65	30.51
Pond 23													
X	22.0	7.9	88	8.17	0.35	1.22	0.016	1.588	0.020	11.22	0.46	376	35.91
min	14.4	5.4	11	7.65	0.28	0.69	0.003	1.113	0.011	7.55	0.01	292	7.4
max	27.9	12.6	154	8.79	0.42	1.93	0.040	2.290	0.040	13.34	0.7	352	67.31
n	28	28	28	28	10	10	10	10	10	10	10	28	10
s	3.6	1.8	25	0.21	0.05	0.41	0.014	0.422	0.009	1.99	0.19	56	17.28

in P19 (**Fig. 1**). Phytoplankton blooms were registered, being most intensive in P19. Green algae were dominant in August and some euglenophyte algae (*Euglena* sp., *Trachelomonas planctonica* Svirenko) species also increased their abundance. Among the most abundant species were also *A. sphaerica*, *D. spiroides*, *O. limosa* and *P. limnetica*. During the study period *A. granulata* was found in all studied ponds. Compared to the previous month, no significant changes in the phytoplankton growth were recorded. Biomass values varied, with the lowest values in P12 and the highest in P19. Phytoplankton blooms continued to be most intensive in P18 and P19. In September, in all studied ponds, the dominant species was *A. granulata*. The cyanoprokaryotes *Dolichospermum planctonicum* (Brunnthaler) Wacklin, L. Hoffmann & Komárek and *D. spiroides* caused intensive blooms and were among the dominant species in P19, P23 and P12, with *Microcystis aeruginosa* (Kützing)

Kützing and *P. limnetica* co-dominating in P18, P19 and P23. The highest biomass was recorded again in P18 and P19, and in P23 and P12 intensive blooms were registered with values of 16.356 and 24.440 mg l^{-1} (**Fig. 1**).

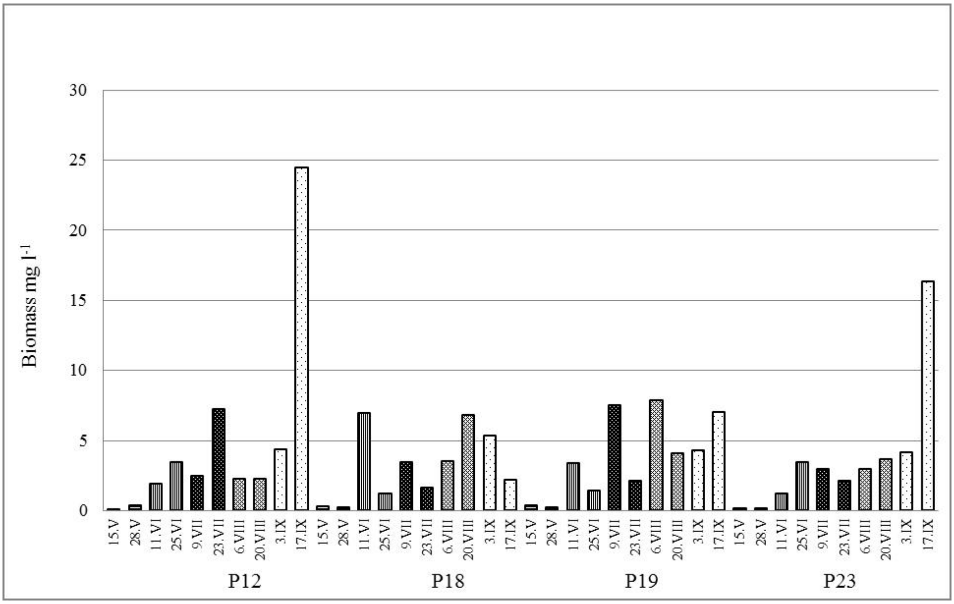

Fig. 1. Changes of total biomass (mg l^{-1}) of phytoplankton in four experimental ponds (P12, P18, P19 and P23) with grass carp polyculture from May to September.

The macrobenthic fauna was represented by class Oligochaeta and genus *Chironomus* (**Table 2**). The oligochaetes predominantly belong to the family Tubificidae, with highest abundance registered in July in P12, P18 and P23 and in August in P19. The chironomids were represented mainly by *Chironomus* cf. *plumosus* with a maximum registered density in July in P12 and P18, and in P19 and P23 in August. During the study period, chironomid larvae at II and III stage of development were recorded. The dynamics of macrobenthic populations varied between ponds, with the highest values registered from June to August. At the beginning of the experimental period the density of the macrobenthic fauna was characterized by low values. Peaks in the total abundance of the individuals were recorded in June and August in P23, and in July in P18. In September a sharp decline in the density was registered in all studied ponds. The average density for the whole experimental period was the highest in P19 (**Fig. 2**).

It is widely known that macrophytes play an important role in the aquatic ecosystems, with some species being the main source of nutrients for different aquatic organisms and that aquatic plants improve water quality and increase biodiversity. On the other hand, their overgrowth can obstruct the use of ponds for various fish farming purposes and endanger the structure and functioning of the biological communities (Bozkurt et al.

Table 2. Average abundance (individuals m^{-2}) of *Chironomus* and Oligochaeta individuals.

	Chironomus	Oligochaeta
Month	Average abundance (individuals m^{-2})	Average abundance (individuals m^{-2})
May	95.3±5.9	45.3.3±11.4
June	117.7±24.5	72.7±16.5
July	138.0±24.5	97.3±12.0
August	153.7±15.5	94.7±16.3
September	94.3±9.5	66.7±4.5
Pond №	Average abundance (individuals m^{-2})	Average abundance (individuals m^{-2})
P12	102.8±38.3	48.0±9.0
P18	112.0±29.3	74.4±25.5
P19	122.8±28.0	81.8±22.5
P23	124.6±30.9	69.8±23.2

2017). Macrophytes density is a factor that affects not only the different parameters of the aquatic ecosystem, but also the nature of the interactions between them. However, registering only the average levels of the density of aquatic vegetation is insufficient, as the impact of the macrophytes varies according to the time they reach their maximum

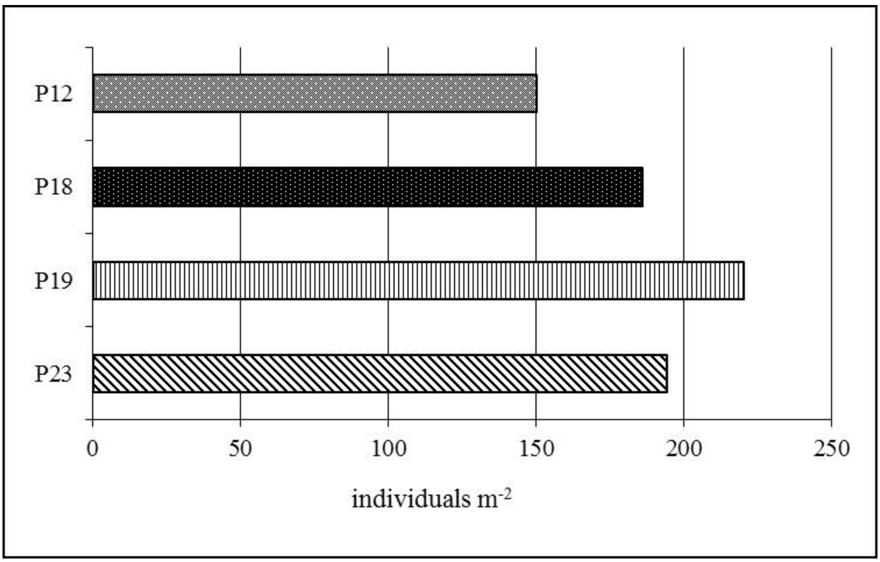

Fig. 2. Total abundance (individuals m^{-2}) of macrobenthic fauna in four experimental ponds (P12, P18, P19 and P23) with grass carp polyculture, represented in average values for the studied period (for details see text).

density (Nikolova et al. 2013). According to these authors, macrophytic overgrowth impacts the rearing of fish and the interactions of other environmental factors, but this influence changes its strength and direction depending on the type and the stage of development of the macrophytes. According to these authors, the relationship between macrophytes, water transparency and biomass of zoobenthos and zooplankton is negative, and between the biomass of phytoplankton and chlorophyll *a* is positive. In the present study, the peaks in chlorophyll concentration coincided with the peaks in phytoplankton growth. According to Nikolova et al. (2013) the density of the aquatic vegetation affects negatively all biotic components, with the most significant impact on the biomass of zoobenthos and zooplankton, with weaker impact on the phytoplankton biomass. In our study also the highest zoobenthos density was registered during the active summer period after the elimination of the macrophytes and the abundance of chironomids was higher than the abundance of oligochaetes.

According to our results, the abundance of macrophytes coincided with the lowest biomass of phytoplankton and, by contrast, its highest biomass was registered in the period after the removal of the aquatic vegetation by the grass carps. This is on conformity with the results of Abdel-Tawwab (2006) and Petr (2000), who reported a negative relationship between macrophyte density and chlorophyll *a* and phyto- and zooplankton abundance. When interpreting the complex relationships between aquatic plants and various organisms, these authors noted that plants provided shelter for the larger zooplankton organism, which increased the zooplankton density and inhibited the growth of the smaller zooplankton organisms, thus preventing negative changes in the structure of the phytoplankton.

While exploring the impact of certain technological factors on the growth of carp fish, Nikolova (2013) found that one-year old grass carp grows faster than the two-year old individuals, noting that the organic fertilization of the ponds has a significant impact on the growth rate. In a different study Nikolova (2004) reported a considerable impact of the pond area on the growth rate of the grass carp. The results of Nikolova et al. (2008) demonstrated that a polyculture with two-year old grass carp with density of 100 fish ha^{-1} leaded to effective control of the macrophytes overgrowth, resulting in their further development being stopped. Besides the size, vegetation has a significant effect on the studied carcass slaughter characteristics of other species reared in polyculture with grass carp. For example, the macrophytic growth of the ponds had a positive effect on the slaughter characteristics of silver carp (Nikolova & Dochin 2017). However, although the grass carp rears under similar conditions and aquatic vegetation is its main source of food, it has been found that macrophytic overgrowth has a negative effect on its slaughter characteristics and it has been supposed that this is most likely related to the specific plant species in the different ponds and to the food selectivity of grass carp (Nikolova & Dochin 2011), the last studied in detail by Catarino et al. (1997). Vinogradov & Zolotova (1974) reported the disappearance of 36 species of aquatic plants after two years of rearing of grass carp. Its impact on the species diversity of aquatic vegetation depends on the stocking densities and the size of the fish, the presence or absence of preferred plant species, the survival rate and the duration of its impact (Pípalová 2006). Higher density of grass carp can have serious consequences for the functioning of the aquatic systems. Ecological changes may be associated with alterations in the structure and abundance of the plant communities (*e.g.* Catarino et al. 1997), as well as different transformations in the habitat, such as shifts in water transparency, sedimentation, and increased levels of biogenic waste as result of fecal deposition (Pípalová 2006; Dibble & Kovalenko 2009).

However, we have to underline that rearing of grass carp, as well as the use of other species, does not eliminate the factors that cause excessive aquatic plant overgrowth, which is often associated with human activity. Shallow ponds, eutrophication, climate change and the occurrence of invasive species can favor development of macrophytes. When intensive plant development is associated with long-term nutrient loadings, grass carp can help convert them into fish biomass and phytoplankton (Pípalová 2006; Volpert 2010). At high stocking density, the grass carp can eliminate the vegetation, and the released nutrients cause an increase in phytoplankton. As it was described above, a similar effect was recorded in the present study: after the removal of aquatic vegetation, a significant increase in the phytoplankton biomass and chlorophyll *a* concentration was observed. Moreover, their elimination significantly improved the light regime in the pond, which is one of the necessary conditions for phytoplankton blooms. Thus, our data confirmed that changes in the biomass and species composition of phytoplankton are depending on the presence or absence of aquatic macrophytes. On turn, high phytoplankton biomass can cause macrophyte suppression, and winds decrease water transparency due to sediment movement (Bonar et al. 2002). According to Richard et al. (1984) three years after the introduction of the grass carp, a significant increase of Chlorophyta and Bacillariophyceae, and a decrease in Cyanoprokaryota has been observed. Similarly, Holdren & Porter (1986) reported on changes in the dominant phytoplankton species after the introduction of the grass carp. In taxonomic aspect, we detected another set of events: at the beginning of the experiment, algae had relatively high biodiversity, while at the end of the study period Cyanoprokaryota were predominantly found in the samples.

The elimination of macrophytes affects the fish growth rate, especially when no additional feeding is received by the fish, as is the case of our experimental study. Despite the detailed description of the growth rates of different fishes in this polyculture is out of the scope of the present paper, we would like to note that during the experiment there were differences in the development of the fishes. For example, the elimination of the macrophytes in the ponds which led to improved light regime and phytoplankton blooms, created a rich food base for the hybrid silver carp and in all experimental ponds it grew almost 10 times its initial weight, while the growth of the carp was mostly insignificant. Moreover, negative growth rate and lack of growth rate of grass carp in two of the studied ponds was detected. In one of these ponds, where only *Ceratophyllum demersum* was found shortly at the beginning of the study period, no growth and no reduction of weight were observed. Considering the lack of additional feeding of the grass carp during the experiment, we could suppose that the growing of grass carp would be higher in the presence of sufficient vegetation and additional feeding. However, since overgrowth by macrophytes in undesirable, it is necessary to find a balance between their development and fish production. One possible solution for this is to optimize the polycultures with a focus on the age structure and the number of grass carp used.

CONCLUSION

In conclusion, based on the results from the present study, the two-year old grass carp with a stocking density of 150 fish ha^{-1} was sufficient to reduce aquatic vegetation overgrowth and had effects on composition and abundance of both phytoplankton and zoobenthos. This study can serve as a base for further research on the uses of polyculture with grass carp as a mean in the biological control of unwanted growth of macrophytes, with focus on the impact on the aquatic ecosystems and their biological communities.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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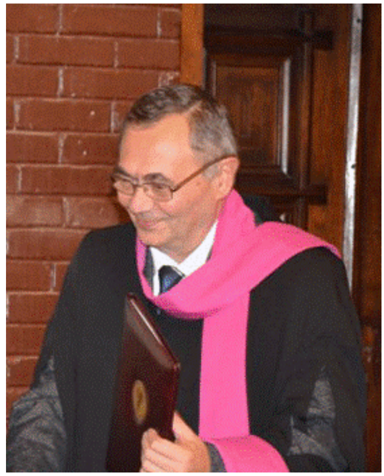
In Memoriam Assoc. Prof. Bojidar Galutzov (1948-2019)



Assoc. Prof. Bojidar Petkov Galutzov passed away on 17th June 2019. The short obituary presented here is not aimed at complete description of his meaningful life, but is a cordial and grateful acknowledgment of his personality, collegial and scientific trace, which leave memorable part in everyone, who was in contact with him, together with outlining of his role for development of botany in Sofia University.

After his graduation in 1972 as Magister in Biochemistry and Microbiology at the Faculty of Biology of the Sofia State University "Kliment Ohridski", and later, in 1981, as PhD student of the same institution, B. Galutzov dedicated all his life time to Bulgarian science in the field of biophysics and to the development of the Faculty of Biology. Being one of the most modern, friendly, attractive and inspiring University teachers, who went consecutively through all steps from assistant to associated professor, he contributed to the education and supported the professional development of generations of scholars in the field of biology. Here it is to recall that in 80s of 20th century B. Galutzov was one of the first University teachers of the first class in biology of the National Mathematical Gymnasium “Akad. L. Chakalov” (recently National Natural-Mathematical Gymnasium). Later he continued his support to all teachers in biology as Vice-Chairman and Chairman of the Union of Bulgarian biologists, and as Chairman of the National Commission for conducting a National competition in natural sciences and ecology. During long years B. Galutzov was actively involved in the administrative faculty life, mainly as member of the Faculty Council. In the period 2008-2012 he was elected as a Dean of the Faculty of Biology and for some years was a member of the Academic Council of the University. From Dean’s position, together with the team of his vice-deans, he provided reasonable and great support to the development of the Department of Botany by following activities, enlisted chronologically: 1) help in increasing of the teaching hours of the Department with the new lines of education in the fields of Ecochemistry and Pharmaceutical botany; 2) financial help in repairment of teaching rooms; 3) administrative support for the important project CEBDER (= Centre of Excellence in Biodiversity and Ecosystem Research; MONT – D002-15/17.2.2009, leaded by Prof. Boyko Georgiev from IBBRG-BAS) for repairment and modernization of the Herbarium (curated by Asen Asenov); 4) administrative support in legalization of the newly created living collection of algae, registered in 2010 in the WFCC-MIRCEN World Data Centre for Microorganisms under № 965 as ACUS (Algal Collection of the University of Sofia) with director Maya Stoyneva and curator Blagoy Uzunov; 5) administrative support in creation of the first Laboratory for cultivation of Fungi in the Department (Contract 61/15.05.2009 with the Scientific Fund of Sofia University); 6) administrative and financial support for the restoration of the summer botanical practices in the Botanical gardens Varna and Balchik. During the same period Assoc. Prof. B. Galutzov played a very important role with competence, benevolence and tact in the structural organization of the Department of Botany, which at the beginning of his mandate was not balanced between its three main scientific and teaching units. However, in the end of this mandate, in 2012, when Department celebrated officially its 120th Anniversary, the three units were almost completely balanced structurally and territorially. With his unforgettable friendly smile, brilliant knowledge of French language, obtained during his study in the prestigious French Gymnasium of Sofia, Dr. Galutzov charmed and predisposed all foreign lecturers who visited and taught in the Department. For his Deans and other administrative work, diverse teaching activities, high qualification and professionalism with important scientific contributions, Assoc. Prof. Bojidar Galutzov was deservedly awarded by the prestigious Honorary Sign of Sofia University with Blue Ribbon.

Prof. Maya Stoyneva-Gärtner, PhD, DSc

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IVANOV I. P. & PETROV P. I. 2013. Photosynthetic CO_2-fixation pathways. – Ann. Rev. Plant Physiol. 21 (2): 141–263.

IVANOV I. P., PETROV P. I. & DIMITROV V. N. 2013. Photosynthetic CO_2-fixation pathways. – Ann. Rev. Plant Physiol. 21 (2): 141–263.

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Books:

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IVANOV W. H., STOYANOV H. M. & PETROV F. B. (Eds) 2000. Water ecosystems. Elsevier, New York, 265 pp.

Book chapters:

PETROV F. K. 2000. Grazing in water ecosystems. – In: IVANOV W. J., STOYANOV H. P. & PETROV F. B. (Eds), Water ecosystems, Elsevier, New York, 59–105.

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Index Fungorum. Retrieved from http://www.indexfungorum.org/Names/Names.asp on 19.11.2017.

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Petkov N. H. 1915. La flore algologique du mont Pirin-planina.- Sbornik na Bulgarskata Akademiya na Naukite 20: 1–128 (In Bulgarian).

Petkov N. H. 1915. La flore algologique du mont Pirin-planina.- Sbornik na Bulgarskata Akademiya na Naukite 20: 1–128 (In Bulgarian, French and Russian summ.).

Book:

Valkanov D. E., Draganova P. M. & Tsvetkova B. B. 1978. Flora of Bulgaria. Algae. Izd. Narodna Prosveta, Sofia, 642 pp. (In Bulgarian)

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