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ALGAL DIVERSITY ON THE GRANITE MONUMENT IN FRONT OF THE FACULTY OF BIOLOGY OF SOFIA UNIVERSITY

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Abstract. The paper presents the results on the species composition of aeroterrestrial algae that developed on a granite monument in the urban environment in the central part of the Bulgarian capital town Sofia, situated in front of the Faculty of Biology of Sofia University “St. Kliment Ohridski”. After scraping the visible algal layers from both frontal (northern) and back (southern) side of the monument, samples were immediately processed by conventional light microscopy on non-permanent slides. The algal diversity comprised six species from the following four taxonomic phyla: Cyanoprokaryota, Chlorophyta, Streptophyta and Ochrophyta. The obtained samples are deposited in the Living Algal Collection of Sofia University (ACUS) for further proceeding and cultivation.

Key words: Chlorophyta, Cyanoprokaryota, Eustigmatophyceae, biodiversity, aeroterrestrial algae

INTRODUCTION

As the name suggests, the aeroterrestrial algae represent a rather unique ecological group of land-dwelling algal species, that inhabit different solid substrates. These substrates can be natural, such as rocks, stones, tree barks, plant

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or mushroom surfaces, or they are man-made, such as rooftops, concrete walls and different monuments of cultural significance (*e.g.*, Ettl & Gärtner 1995, 2014; Gärtner & Stoyneva 2003; Gärtner et al. 2003; Videv et al. 2017; Gärtner & Hofbauer 2021). Up-to-now, most data collected in different regions of the world concern natural habitats, but there is a rising interest in algae, which inhabit various artificial structures with increasing number of records (for details see Gärtner & Hofbauer 2021).

In Bulgaria, according to the first review by Uzunov et al. (2007, 2008b) the total count of the aeroterrestrial algae published in the period 1898-2007 was 569 taxa from seven phyla: Cyanoprokaryota – 242 species, nine varieties and eight forms from 60 genera; Rhodophyta –four species from three genera; Ochrophyta, Tribophyceae –18 species and one variety from seven genera, Bacillariophyceae – 12 species, six varieties and one form from 11 genera; Chlorophyta – 88 species, 16 varieties and three forms from 54 genera; Streptophyta – 41 species, 15 varieties and six forms from 15 genera; Euglenophyta – six species from five genera.

Subsequently, the papers published by Uzunov et al. (2008a, 2010, 2012), Stoyneva & Gärtner (2009), Gärtner et al. (2010b, 2012, 2015), Stoyneva et al. (2012), along with the PhD theses of Uzunov (2009) and Mancheva (2013), provided new data on the biodiversity of aeroterrestrial algae. According to the last general assessment of the algal biodiversity of the country, the ecological group of aeroterrestrial algae consisted of 589 species, varieties and forms (Stoyneva 2014), and 31% of them were recorded along the Black Sea Coast, where totally 164 species, varieties and forms from 56 genera of 4 divisions were found: Cyanoprokaryota (145), Chlorophyta (9), Ochrophyta (7) and Rhodophyta (3) (Gärtner et al. 2018).

Among the aeroterrestrial algae, those found on cultural monuments were scarcely studied in Bulgaria. The single publication on the topic, concentrated on the study of three statues in the towns of Sofia and Koprivshtitsa, reported three free-living algal species from the genera *Apatococcus*, *Trebouxia* and *Coccomyxa*, as well as the lichens *Lepraria* cf. *neglecta* (Nyl.) Erichsen, *Candelariella vitellina* (Hoffm.) Müll.-Arg., *Protoparmeliopsis muralis* (Schreb.) M. Choisy and *Caloplaca* sp. that comprise algal symbionts (Gärtner & Stoyneva 2003).

The present study serves as a continuation of this research focused on the monuments in urban areas. Such non-investigated in respect to algae monument is the granite memorial, known as Monument of agronomists-antifascists. It is a single rocky piece brought from the Stony Vitosha rivers on Vitosha Mt, known also as Morraines, the glacial origin of which is yet debatable (for details see Management Plan of Vitosha 2005). This memorial is located in the central part of the Bulgarian capital Sofia. The single record in the available literature points that all lichens, which originally were developed on the monument at the moment of its installation in the early fifties of 20th century, have disappeared in the urban conditions of the Bulgarian capital (Filipova 1956).

MATERIAL AND METHODS

The samples for this study were collected on 3rd of June 2023 from the granite rocky memorial that is situated in the front yard of the Faculty of Biology of the Sofia University "St. Kliment Ohridski" positioned near the northern entrance of the building, next to the Dragan Tsankov boulevard with a heavy car traffic, in close proximity of the city park Borisova Gradina (**Figs. 1, 2**). The samples were collected using the direct method of GÄRTNER ET AL. (2010A), for which we used pre-made Petri dishes containing solid agar enriched by Bold's Basal Medium (BBM) after the classical recipe of BISCHOFF & BOLD (1963).

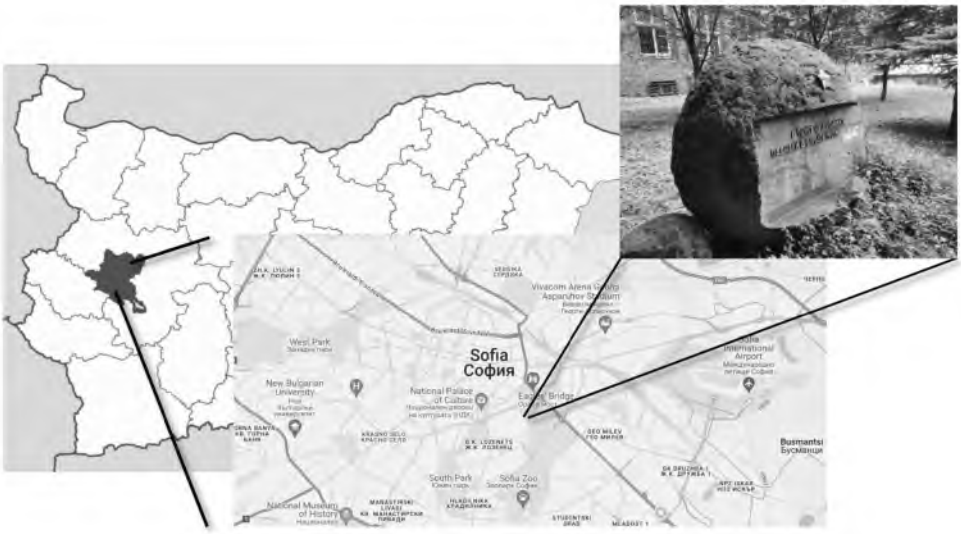

Fig.1. Map of Bulgaria with indication of Sofia (left) and map of Sofia with location of the studied memorial (right).

Fig. 2. Photographs of the front (north) side of the monument (left) and its back (south) side (right). Green spots indicate the sampling sites, the 1,5 L bottle is used as a scale.

Following the forementioned technique for direct collection, we made mixed (polycultural) samples from the frontal (northern) side of the memorial facing the boulevard, and three mixed samples from its southern (back) side, which is facing the building of the faculty. From each side we gently scraped small amounts from the visible, colored algal layers on the rough surfaces of the upper, middle and lower parts of the monument, using a dentist borer (**Fig. 2**). All obtained samples are deposited in the Living Algal Collection of Sofia University (ACUS – Stoyneva 2012, Uzunov et al. 2012a) for further proceeding and cultivation.

The first identification of the algae was conducted in the laboratory of ACUS. From the freshly collected samples, before their cultivation, we prepared 30 non-permanent microscope slides, which were studied thoroughly under an Olympus BX53 microscope with the following magnifications - 25x, 40x and 100x, and additionally equipped by differential interference contrast (DIC). Microphotographs were taken with the specialized Olympus DP72 camera and subsequently modified using the licensed Olympus software – cellSens. For the taxonomical identification we used standard manuals (*e.g.*, Gollerbakh et al. 1953, Komárek & Fott 1983, Ettl & Gärtner 1995, 2014, Komárek & Anagnostidis 2005, Hindák 1980, 1984, John et al. 2002), and synonymy was checked in AlgaeBase (Guiry & Guiry 2023). During the identification the several key characteristics were followed:

1. Vegetative cell characteristics – size, shape and motility;
2. Cell wall characteristics – thickness, surface, extra layers, mucilage, etc.;
3. Plastid characteristics – number, shape, color;
4. Occurrence of pyrenoid structures – number, shape, cover, etc.;
5. For the multicellular and colonial organisms – shape, size, color, presence or absence mucilage sheath.

RESULTS AND DISCUSSION

Six species from four divisions – Chlorophyta (4), Streptophyta (1) and Ochrophyta (1) – were identified during the pilot light microscopic observations of the freshly collected material – **Fig. 3.** They were found growing on both the northern and the southern sides of the monument, with no clear distributional patterns.

The annotated taxonomic list is provided below:

Division Chlorophyta
Class Trebouxiophyceae
Order Chlorellales
Family Chlorellaceae

***Chlorella* sp.** – cells are round, spherical or slightly ellipsoidal, diameter averaging 5,3–6 μm. Cell wall is smooth, without any visible roughness or bumps.

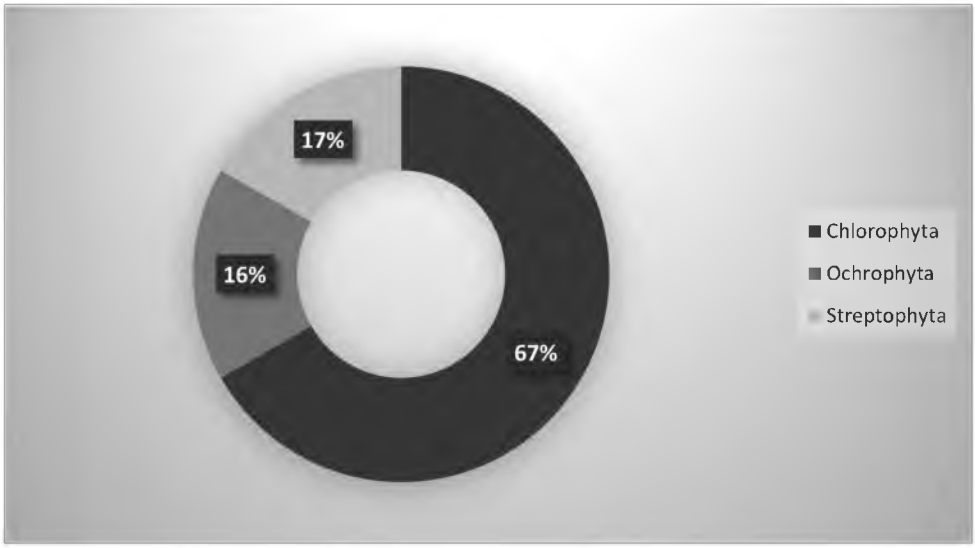

Fig. 3. Algal biodiversity on the memorial Monument of agronomists-antifasciests in Sofia.

Every cell contains a large, single cup-shaped chloroplast with a single pyrenoid, plastered with starch globules, visible after staining with Lugol's solution. Asexual reproduction is carried by the formation of autospores, where daughter cells develop inside the boundaries of the mother cell and are released by rupturing the wall of the autosporangium.

***Chloroidium ellipsoideum* (Gerneck) Darienko, Gustavs, Mudimu, Menendez, Schumann, Karsten, Friedl & Proschold 2010** (Syn.: *Chlorella ellipsoidea* Gerneck 1907) – cells are ellipsoidal or round, usually longer than wide, averaging 8,4–8,8 μm in length and 7,6–8 μm in width. Cell walls are thick, smooth, without visible bumps or rough patches. Cells contain a single small parietal chloroplast, usually cup-shaped, situated near the nucleus in the central regions of the cell. Pyrenoids are present, usually single, enveloped in a thin starch envelope, visible after staining with Lugol's solution. Asexual reproduction is carried by the formation of autospores, which are released by rupturing the mother cell-wall. Daughter cells for a while may remain attached to the remnants of the autosporangium.

Order Prasiolales

Family Stichococcaceae

***Stichococcus* sp.** – cells are cylindrical, with rounded ends, longer than wide, with a ratio of 3:1, averaging 8,9–9,4 μm in length and 3,2–3,7 μm in width. If filamentous forms are present, they are usually short, made up by only a few cells (3-5), without branching and easily fragmenting into single cells. Cell walls are thin, with a smooth surface, lacking a mucilaginous layer. Every cell contains a

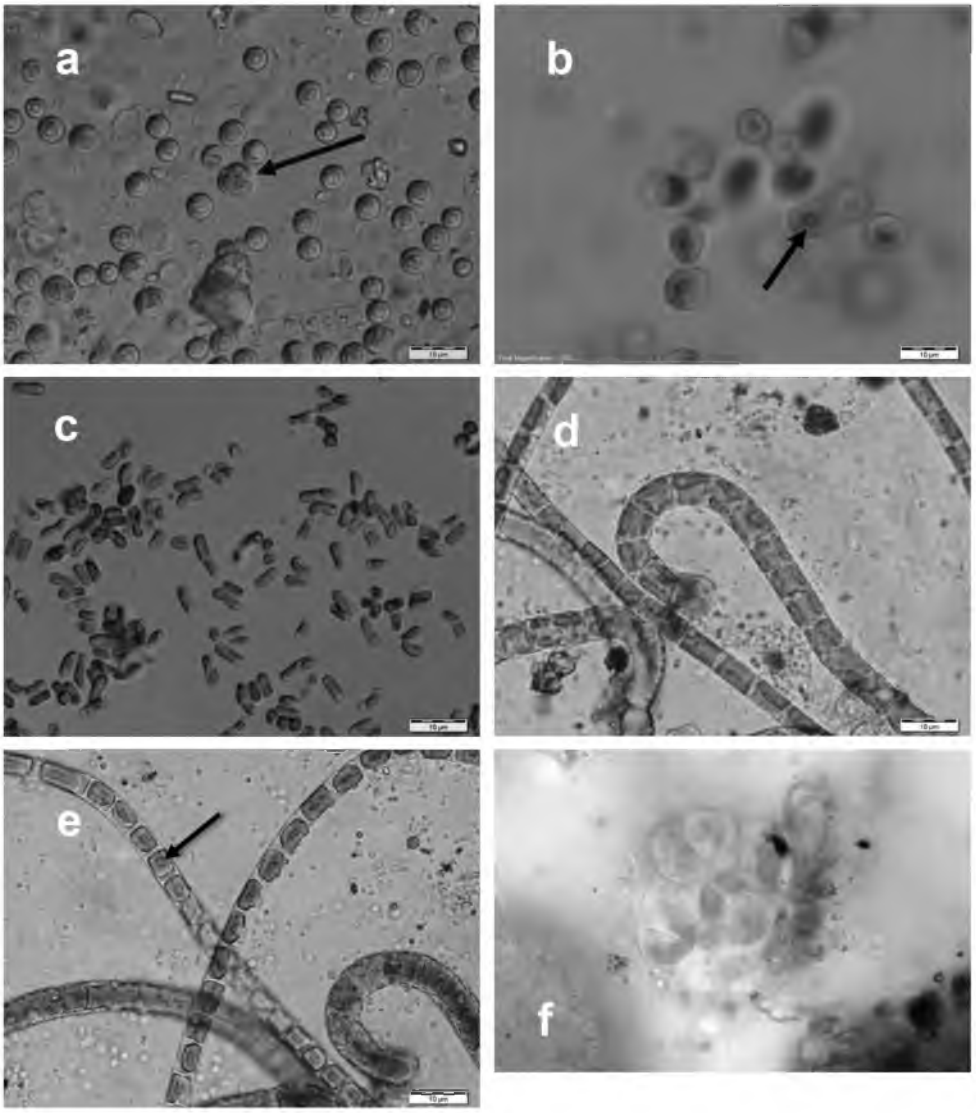

Fig. 4. Microphotographs of the observed aeroterrestrial algae: **a** – *Chlorella* sp. (arrow indicates autosporangium); **b** - *Chloroidium ellipsoideum* (arrow points a pyrenoid visible after coloration by Lugol's solution); **c** - *Stichococcus* sp.; **d** - *Ulothrix tenerrima*; **e** - *Klebsormidium klebsii* (pyrenoids visible after coloration by iodine solution); **f** - *Gloebotrys* sp.

single, large parietal chloroplast with a small, barely visible pyrenoid in the center (sometimes the pyrenoid is absent). Vegetative reproduction is done by binary fission of the cell or by fragmentation of the filament. Spores and gametes were not observed.

Class Ulvophyceae
Order Ulotrichales
Family Ulotrichaceae

***Ulothrix tenerrima* (Kützing) Kützing 1843** (Syn.: *Ulothrix variabilis* Kützing 1849) – filaments are long, sleek, without branches, mucilage is not present. Cells are cylindrical, longer than wide, averaging 6,4-7,2 μm in length and 5,4-5,8 μm in width. Cell walls are thick with a smooth outline. Every cell contains a single, large semi-annular chloroplast with a single pyrenoid, encased in a thin starch envelope, visible after staining with Lugol. Older cells accumulate globules and vesicles. Vegetative reproduction is carried by filament fragmentation. Formation of spores and gametes was not observed.

Division Streptophyta
Class Klebsormidiophyceae
Order Klebsormidiales
Family Klebsormidiaceae

***Klebsormidium klebsii* (G. M. Smith) P. C. Silva, K. R. Mattox & W. H. Blackwell 1972** – filaments are long, straight, flaccid and unbranched, without mucilaginous sheaths. Cells are cylindrical, longer than wide, with a ratio of approximately 2:1, averaging 6,2 μm in length and 3,2 μm in width. Apical cells are not differentiated and look like the rest. Cell walls are thin, with a smooth surface, rarely rough or thickened. Cells contain a single, large parietal laminate chloroplast, occupying the majority of the cell. Chloroplasts contain central pyrenoids, usually a single one, rarely more than one. Pyrenoid is large, encased in a thick starch envelope, visible after staining with Lugol. Vegetative reproduction is carried by fragmentation into short-celled filaments or into single, solitary cells. Asexual and sexual reproduction is not observed, since there were no available spores or gametes present in the sample.

Division Ochrophyta
Class Eustigmatophyceae
Order Eustigmatales
Family Gloeobotrydaceae

***Gloebotrys* sp.** – cells are spherical or slightly ellipsoidal, enveloped in a shared mucilaginous layer. Cells are usually grouped in 4-8 based on the pattern of division. Single cells are with sizes averaging 3,5–4 μm in diameter. Cell walls are smooth, without visible bumps or scars. Every cell contains multiple small lentil-shaped chloroplasts, usually closely associated with the cell wall. Vegetative reproduction is carried by binary fission, asexual and sexual reproduction was not observed, since no zoospores or gametes were present in the sample.

CONFLICT OF INTERESTS

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

References

Bischoff H. & Bold H. C. 1963. Some soil algae from enchanted rock and related algal species. - Phycological Studies IV. University Texas Publications 6318: 1-95.

Ettl H. & Gärtner G. 2014. Syllabus der Boden-, Luft-und Flechtenalgen. 2. Aufl., Springer Spectrum, Berlin-Heidelberg, 773 pp.

Filipova L. 1963. L'influence des conditions écologiques de la ville de Sofia sur le development et la distribution des Lichens. – Annuaire de L'Université de Sofia, Faculté de Biologie, Geologie et Géographie, Livre 1 – Biologie (Botanigue) 56: 83-96 (In Bulgarian, Russian and French summaries).

Gärtner G. & Hofbauer W. K. 2021. Microbial life on Façades. Springer Spectrum, Berlin, 323 pp.

Gärtner G. & Stoyneva M. 2003. First study of aerophytic cryptogams on monuments in Bulgaria. - Berichte des naturwissenschaftlichen-medizinischen Verein Innsbruck 90: 73-82.

Gärtner G., Stoyneva M., Mancheva A. & Uzunov B. 2010. A new method in collection and cultivation of aerophytic and endolithic algae. - Berichte des naturwissenschaftlichen-medizinischen Verein Innsbruck 96: 27-34.

Gärtner G., Stoyneva M. P., Uzunov B. A., Mancheva A. & Ingolić E. 2012. Ultrastructure of vegetative cells and autospores of aerophytic strain of *Vischeria stellata* (Chodat ex Poulton) Pascher (Eustigmatophyceae) from Bulgaria. – Fottea 12 (2): 273-280.

Gärtner G., Stoyneva M. & Uzunov B. 2015. First record of *Palmellopsis texensis* (Groover et Bold) Ettl et Gärtner (Chlorophyta, Tetrasporales, Palmellopsidaceae) from Bulgaria, found in a 20 years dried soil of a herbarium specimen. - Algological Studies 148: 57-65.

Gärtner G., Uzunov B. A., Dimitrova P. H. & Stoyneva-Gärtner M. P. 2018. Review of the studies of aeroterrestrial algae along the Bulgarian Black Sea coast (1890-2017) with special attention to the newly described and threatened species. - In: Peev D. R., Gärtner G., Stoyneva-Gärtner M. P., Popova N. V. & Georgieva E. E. (Eds), Proceedings of the First European Symposium "Research, Conservation and Management of Biodiversity of European Seashores" (RCMBES) Primorsko, Bulgaria, 8-12 May 2017. - Acta zoologica bulgarica, Suppl. 11: 53–55.

Gärtner G., Uzunov B. A., Stoyneva M. P., Kofler W. & Ingolić E. 2010. *Trochisciopsis tetraspora* f. *minor* forma nova (Chlorophyta, Chlorophyceae,

Chlorococcaceae) a new terrestrial green algal taxon from Pirin Mt (Bulgaria), and its ultrastructure. - Phyton (Annales Rei Botanicae, Horn, Austria) 50 (1): 127-136.

Gollerbakh M. M., Kosinskaja E. K. & Poljanskij V. I. 1953. Opredelitel presnovodnykh vodoroslej SSSR, Vypusk 2: Sinezelionye vodorosli [The guide for determination of the freshwater algae of the USSR Part 2: Blue-green algae]. Moscow: Publ. House Sovetskaya Nauka (Soviet science), 652 pp. (In Russian)

Guiry M. D. & Guiry G. M. 2023. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Retrieved from https://www.algaebase.org on 13 July 2023.

Hindák F. 1980. Studies on the chlorococcal algae (Chlorophyceae). II. - Biologické Práce 26 (6): 1–196.

Hindák F. 1984. Studies on the chlorococcal algae (Chlorophyceae). III. - Biologické Práce 30 (1): 1–310.

Hofbauer W. K. 2021. Toxic or otherwise harmful algae and the built environment. - Toxins 13: 465.

John D., Brooks A. & Whitton B. 2002. The freshwater algal flora of the British Isles. An identification guide to freshwater and terrestrial algae. Cambridge University Press, 714 pp.

Komárek J. & Anagnostidis K. 1999. Cyanoprokaryota. 1 Teil: Chroococcales. – In: Ettl H., G. Gärtner, H. Heynig & D. Mollenhauer (Hrsg.), Süßwasserflora von Mitteleuropa, 19/1, Gustav Fischer, Jena, 548 pp.

Komárek J. & Anagnostidis K. 2005. Cyanoprokaryota. 2 Teil: Oscillatoriales. – In: Büdel B., G. Gärtner, L. Krienitz & M. Shagerl (Hrsg.), Süßwasserflora von Mitteleuropa, 19/2, Elsevier GmbH, Spektrum Akademischer Verlag, München, 759 pp.

Komarek J. & Fott B. 1983. Das Phytoplankton des Süßwassers. Systematik und Biologie. – In: Huber-Pestalozzi G. (Ed.), Die Binnengewässer, Band XVI, 7. Teil 1. Hälfte. Stuttgart:1-1044.

Management plan of Vitosha 2005 accepted with decision № 305 from 22.04.2005 by the Council of Ministers of Republic of Bulgaria. Retrieved from https://pu-vitosha.com/wp-content/uploads/2015/03/PU-2005-g.pdf on 3 November 2023

Mancheva A. 2013. Aerophytic algae from the rock phenomenon and nature monument Belogradchishki Skali. PhD Thesis, Sofia Univrsity, Fac. Biology, 191 pp. (In Bulgarian)

Stoyneva M. 2012. Department of Botany at the Faculty of Biology of Sofia University "St. KLimnet Ohridski" during the last 30 years. - In: Petrova A. (Ed.), Proceedings of the VII National Conference in Botany; 2011 29-30 September; Sofia. Sofia, Bulgarian Botanical Society, 33-43 (In Bulgarian).

Stoyneva M. P. 2014. Contribution to the studies of the biodiversity of hydro- and aero-biontic prokaryotic and eukaryotic algae in Bulgaria. DrSc Thesis, Sofia

University "St. Kliment Ohridski", Faculty of Biology, Department of Botany, 825 pp. + Appendices (In Bulgarian, English summary).

Stoyneva M. P. & Gärtner G. 2009. Remarkable and newly recorded aeroterrestric cyanoprokaryotes and algae in Bulgaria. – In: Ivanova D. (Ed.), Plant, fungal and habitat diversity investigation and conservation, Proceedings of IV Balkan Botanical Congres, Sofia 20-26 June 2006, 122-127.

Stoyneva M. P., Uzunov B. A. & Gärtner G. 2015. Aerophytic green algae, epimycotic on *Fomes fomentarius* (L. ex Fr.) Kickx. – Annual of Sofia University, Faculty of Biology, Book 2 – Botany 99: 19-25.

Stoyneva M., Mancheva A., Gärtner G. & Uzunov B. 2012. Are the algae from the uncommon Belogradchik rocks common ones? - In: Petrova A. (Ed.), Proceedings of the VII National Conference in Botany; 2011 29-30 September; Sofia. Sofia, Bulgarian Botanical Society, 265-269 (In Bulgarian, English summary).

Videv P., Gärtner G., Uzunov B. A., Dimitrova P. & Stoyneva-Gärtner M. P. 2017. Epimycotic algae on the medicinal fungus *Trametes versicolor* (L.) Lloyd. - International Journal of Advanced Research in Botany (IJARB) 3 (2): 18-26.

Uzunov B. A. 2009. Aeroterrestrial algae from Pirin Mountain (Bulgaria). PhD Thesis, Innsbruck University, 182 pp.

Uzunov B., Stoyneva M., Mancheva A. & Gärtner G. 2012a. ACUS – the new collection of living aeroterrestrial algae of Sofia University "St. Kliment Ohridski". - In: Petrova A. (Ed.), Proceedings of the VII National Conference in Botany; 2011 29-30 September; Sofia. Sofia, Bulgarian Botanical Society, 271-274 (In Bulgarian, English summary).

Uzunov B. A., Gärtner G. & Stoyneva M. P. 2012b. Notes on the akinete-forming strain of the green alga *Klebsormidium dissectum* (Streptophyta) from Pirin Mts., Bulgaria. – Phyton - Annales Rei Botanicae 52 (1): 139-144.

Uzunov B., Stoyneva M. P. & Gärtner G. 2007. Review of the studies on aero-terrestrial cyanoprokaryotes and algae in Bulgaria with a Checklist of the recorded species. I. - Phytologia Balcanica 13 (1): 65-73.

Uzunov B., Stoyneva M. P. & Gärtner G, 2008a. First record of *Coelastrella species* (Chlorophyta: Scenedesmaceae) in Bulgaria. – Berichte des naturwissenschaftlichen-medizinischen Verein Innsbruck 95: 27-34.

Uzunov B., Stoyneva M. P. & Gärtner G. 2008b. Review of the studies on aero-terrestrial cyanoprokaryotes and algae in Bulgaria with a Checklist of the recorded species. II. - Phytologia Balcanica 14 (1): 11-18.

Uzunov B. A., Gärtner G., Stoyneva M. P. & Ingolić E. 2010. First record of coenocytic coccal green soil algae in Bulgaria. – Ecological Engeneering and Environmental Protection 1: 53-57.

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