11th International Conference on Applied Phycology

MASARYK UNIVERSITY
FACULTY OF SCIENCE
DEPARTMENT OF BOTANY AND ZOOLOGY

GROWTH INHIBITION TEST ON SUBAERIAL MICROALGAE APPLIED IN URBAN AREAS

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Introduction

- Biodeterioration, bioalternation, biocorrosion, biodecay
- Biodeteriogen
- Building stone
- Cyanobacterial and algal growths
- The habitat concept
- Floristic studies
- Taxonomy
- Ecology and autecology
- Assemblages
- Biodeterioration of stone associated with cyanobacteria and algae
- Remarks on control and removal of algae

Biodeterioration

- (Bio)alternation, (bio)corrosion, (bio)deterioration, (bio)decay
- An exchange of material and energy
- Two heterogenous open dynamic system: The solid substrate and its atmospheric (indoor and autdoor), aquatic or chthonic environment
- The natural limit of turnover activities is given by the penetration depths and speeds of physical gradients, gases, solutions, and organisms (including their extracellular products) into mineral material.

Biodeteriogen

- We could not debar any organism from being an actual or potential biodeteriogen because of no special criteria for biodeteriogen.
- Therefore the range of potential biodeteriogens is huge.
- The deteriogenic effects are mainly a minor incidental part of their activities.
- The biodeteriogenic organism usually inhabit secondarily an artificial man-made surface and participate on cycling of elements (Winkler, 1994).

Building Stone

- **Stone is the oldest entity of Earth**
- **Characterised by a wide range of mineral compositions, textures, and rock structures**
- **Creates ecosystem which includes also environmental factors (light, nutrition, climate, pollution)**
- **Significant deterioration of monuments has been begun since years 1870-1880, this phenomenon has not been possible to stop since 1950**

Cyanobacterial and algal growths

- **Primarily concerned with the cyanobacteria and algae growing on the exposed surfaces of buildings constructed of man-made materials**
- **Special consideration is given to those obscuring coloured stains on building stone**
- **This account includes the tasks caused by them and the method used for their investigation, control and elimination**

The Habitat Concept

- **Most of the cyanobacteria and algae are ubiquitous**
- **Cyanobacterial and algal taxa growing on man-made surfaces**
- **Exactly, it is difficult to define the basis of habitat**
- **Growing in non-aquatic environments have been termed terrestrial, aerial or subaerial forms**
- **Man-made subaerial environment form stressing factors - temperature, dessication, high or low pH, toxicants (pollutants), high or very low irradiance**

Floristic Studies

- More than 200 algal genera had been recorded world wide in floristic studies
- The investigation of colonisations of stone by algae
- Most species lists include a number of unidentified organisms
- Difficult to make exact comparisons of the floristic data
- Differences in the homogeneity of the surface sampled, the intensity of sampling
- The experiences of collector
- The method used for the identification of samples, inconsistencies in the taxonomy of some groups, and in the competence and special interests of the phycologist identifying the algae

Taxonomy

- subaerial cyanobacteria and algae are of a simple organization, exhibit a simple unicellular or filamentous habit
- Cyanobacteria and green algae dominate on subaerial habitat
- No systematic effort has been made to distinguish which genera are truly subaerial
- Many taxonomic problems

Ecology and Autecology

- Main question are the adaptations to the subaerial environment
- The capacity to tolerance desiccation is a feature of many cyanobacteria and algae of both aquatic and terrestrial origin
- The most fundamental feature of all truly aubaerial algae is the ability to resist extremes of temperature, high light intensity and considerable water loss
- Many opportunists in subaerial habitats, colonising and growing very fast over wetter or aquatic periods

Assemblages

- In subaerial habitats some cyanobacteria and algae were typically much more abundant than others

- The assemblages were named by using the names of the most abundant forms: the blue-green assemblage, the *Trentepohlia* assemblage, *Desmococcus* assemblage, *Prasiola* assemblage, *Stichococcus* assemblage

Biodeterioration of stone associated with cyanobacteria and algae

- Excessive biofilm accumulation in porous substrata changes or reduces their properties or effectiveness
- The pedogenic significance of cyanobacteria and algae is very important by biogeophysical weathering of stone surfaces
- Mechanical damage caused by active penetration of euendoliths and chemical damage caused by substances and products of metabolism and of extracellular matrix
- Aesthetic biodeterioration or soiling

Remarks on control and removal of algae

- Indirect methods
- Reduction of humidity, firing, cover, isolation...
- Direct methods
- Eliminate crusts, patinas; UV-rays, X-rays, biocide chemicals...

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The Aims

- Floristic screening and taxonomic study of microalgae causing the biological attack on building stone;
- Which factor of urban environment (substrata, pollution, humidity, etc.) have an influence on the composition of the subaerial taxa;
- To set a growth inhibition test on subaerial microalgae;
- To test the microalgae causing actively biodeterioration which potentially damage the building stone.

Individual studies forming the results

- Gravestones in Cemetery
- Tombstone in a historic cemetery
- Subterranean Jewish cemetery
- The walls of building in Bratislava (SK)
- The walls of building in Murcia (ESP)
- The walls of building in Brno (CZ)
- Growth inhibition test on subaerial microalgae

Materials and methods

- Research area
- The Bratislava City
- The Murcia Region
- The Brno Region
- Sampling
- Laboratory cultivation
- Analysis for determination
- Ultrastructural analysis (SEM)

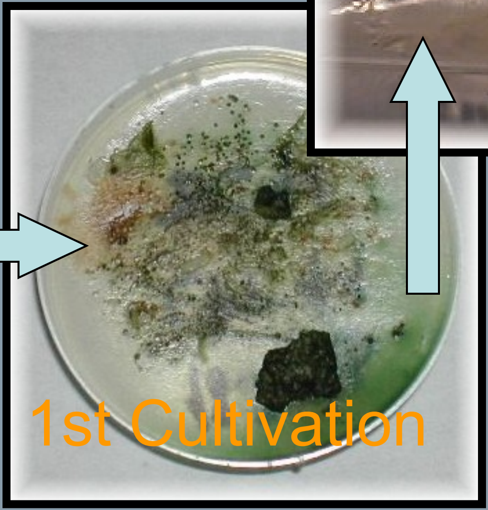
Research area

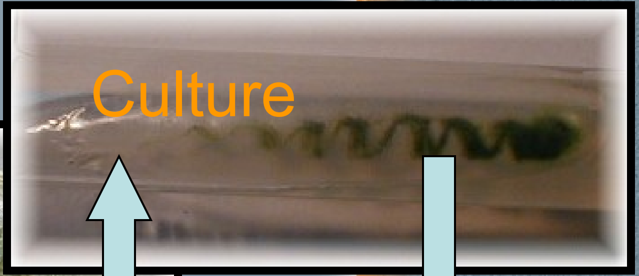
- Bratislava - capital city is located in West Slovakia (48°10´N, 17°10´E)
- Murcia - This region is located in South-east Spain (the Murcia City - 36°56´N, 1°07´W)
- Brno - city in south Moravia eastern part of Czech Republic

Sampling

- Different substrata - horizontal distribution
- One substrata - vertical distribution
- North and south exposition on one building
- Spatial distribution in a city

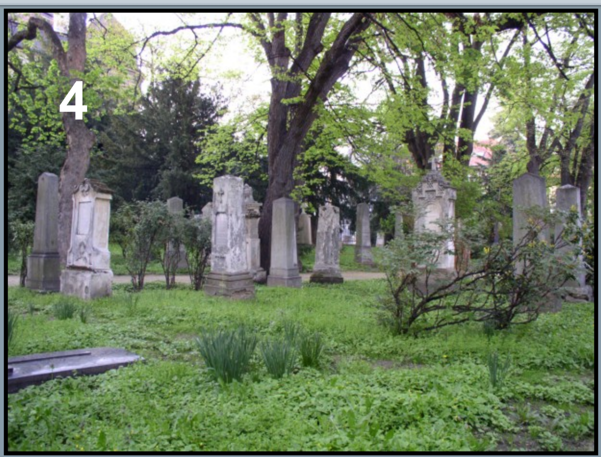
Sampling Schema

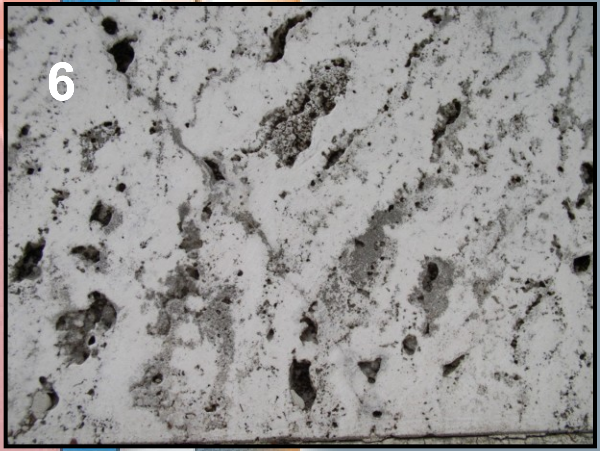




In Laboratory

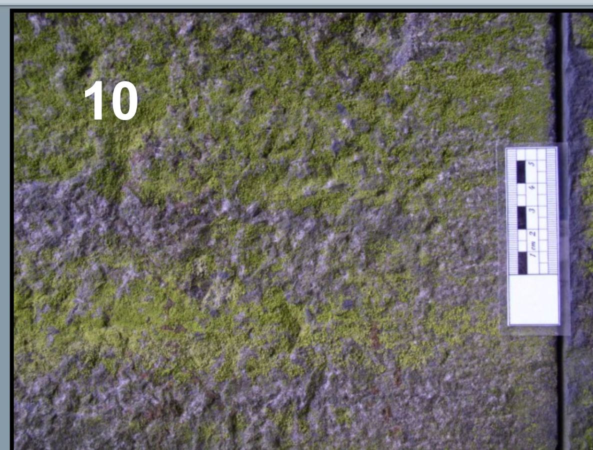
Stone Substrata





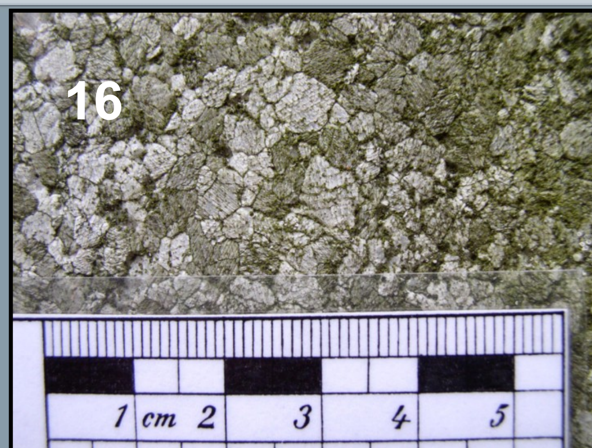
Figs 3-8. Kozia brána Cemetery: Figs. 3-4. Landform of the Kozia brána Cemetery; Fig. 3. The southern part of the cemetery; Fig. 4. Near the entrance; Fig. 5. Litava limestone; Fig. 6. Travertine; Fig. 7. Sandstone with calcareous cement; Fig. 8. Calcarenite.

Stone Substrata



Figs 9-14. Kozia brána Cemetery: Fig. 9. Black dolerite – smooth surface; Fig. 10. Black dolerite – ragged surface; Fig. 11. Neogene calcarenite; Fig. 12. Brick; Fig. 13. Ryolite; Fig. 14. Granodiorite.

Underground



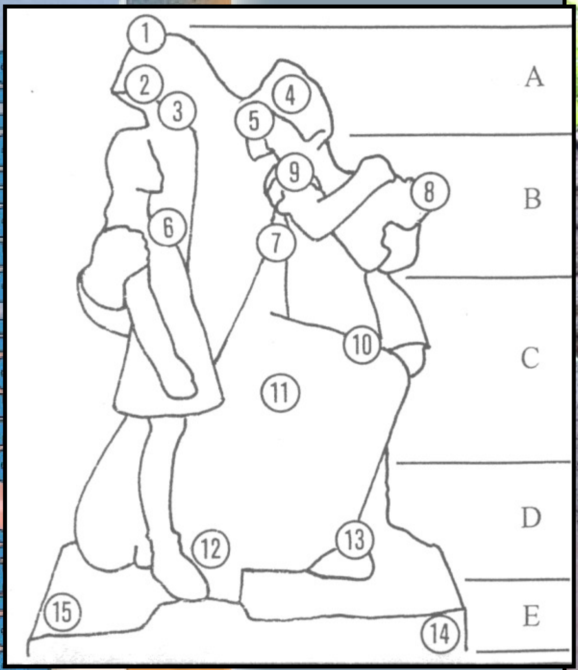
Figs 15-19. Figs 15-16. Kozia brána Cemetery: Fig. 15. Pink nodular limestone from Tata (Hungary); Fig. 16. Marble. Figs. 17-19. Chatam Sófer Mausoleum: Fig. 17. Concrete shall of the Mausoleum before total reconstruction (arrow); Fig. 18. Gravestones on the soil; Fig. 19. Central part, Chatam Sófer's sarcophagus.

Historical Monuments




Figs 20-23. Figs 20-21. St. Martin's Cathedral in Bratislava, Slovakia: Fig. 20. Northern part of Cathedral; Fig. 21. Socle part of Presbyterium. Figs. 22-23. Islamic Castle Monteagudo in Region of Murcia, Spain: Fig. 22. The NW view of the Castle; Fig. 23. The central walls of the Castle.

Tombstone



Figs 24-27. Tombstone (statue represent mother with two doughters) and tomb cover in the historic cemetery of Pressburg Evangelicals Kozia brána in the centre of Bratislava. Fig. 24. Sampling places of algae (1-14) in different microhabitat zones (A-E); Fig. 25. Tomb cover with growths of liverworts and mosses (arrows); Figs 26-27. Detail views on weathered parts of statue (arrows).

Laboratory Cultivation

- Zehnder medium (Zehnder in Staub, 1961)
- BG11 medium (Rippka *et al.*, 1979)
- $BG11_0$ (Rippka, 1988)
- BBM (Smith & Bold, 1966)
- either liquid or agarised at 20-22 °C
- The unialgal strains were used for detail taxonomic study to obtain the data of whole life cycle

Analysis for Determination

- **Type of stone substrata**
- **10 scores for each stone sample**
- **Structure of algal assemblage**
- **The phenotype features**
- **Original drawings made by camera lucida from field and from cultured material**
- **Olympus BH2 Photomicrography system and Nikon DXM1200 (F) colour camera by LUCIA imaging system (Y/C and composite) – image analysis software developed for image capture, archiving and analysis (© 2004 Laboratory Imaging s.r.o.)**

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Ultrastructural Analysis (SEM)

- **Pieces of stone substrata were taken from outer walls of monuments and buildings in urban sites.**
- **Samples were fixed, dehydrated, coated with gold as described previously (Garty and Delarea, 1987)**
- **Examined by a JEOL JXA 840A scanning electron microscope (SEM) operating at 200 kV**

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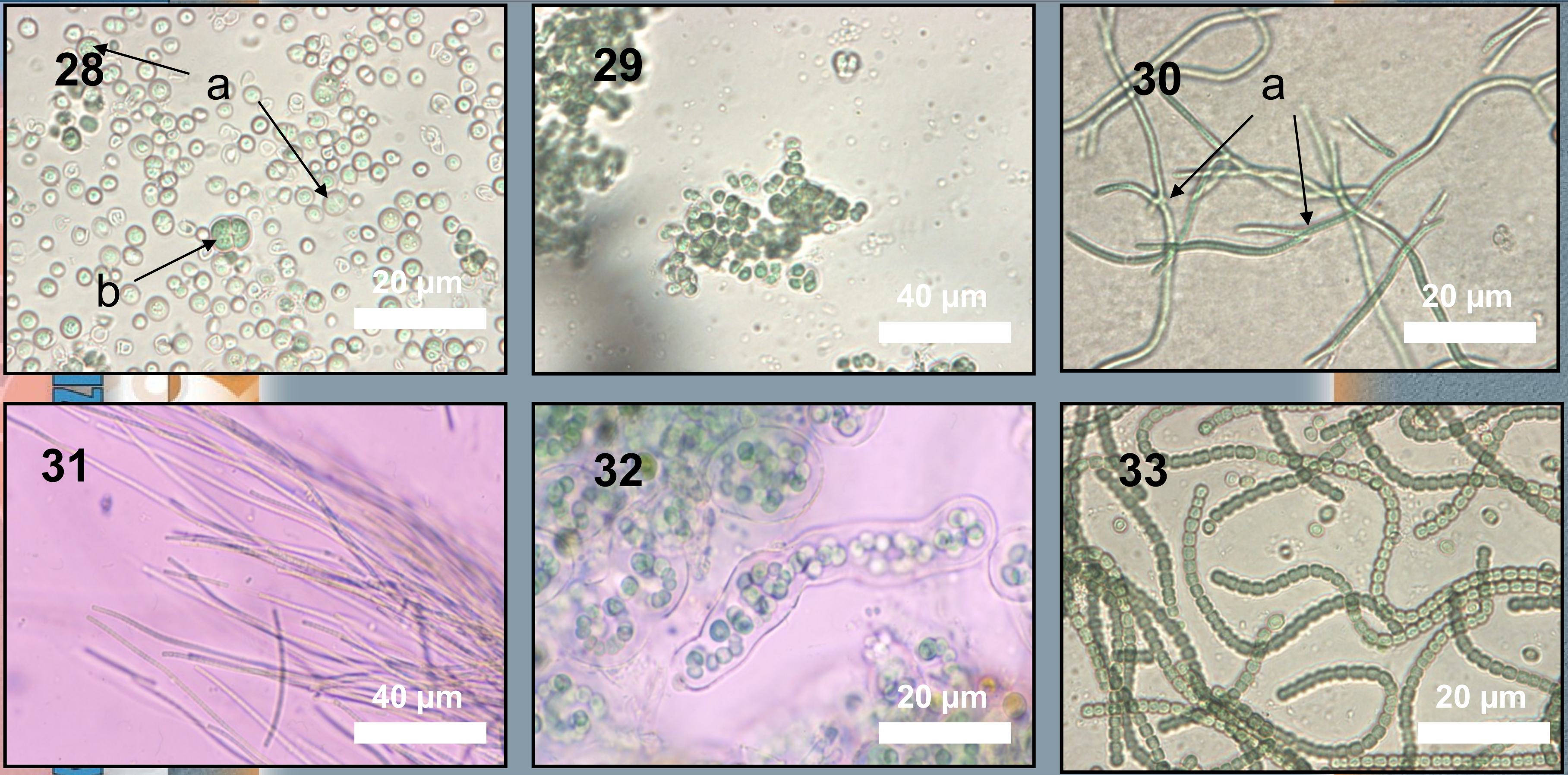
General Results and Discussion

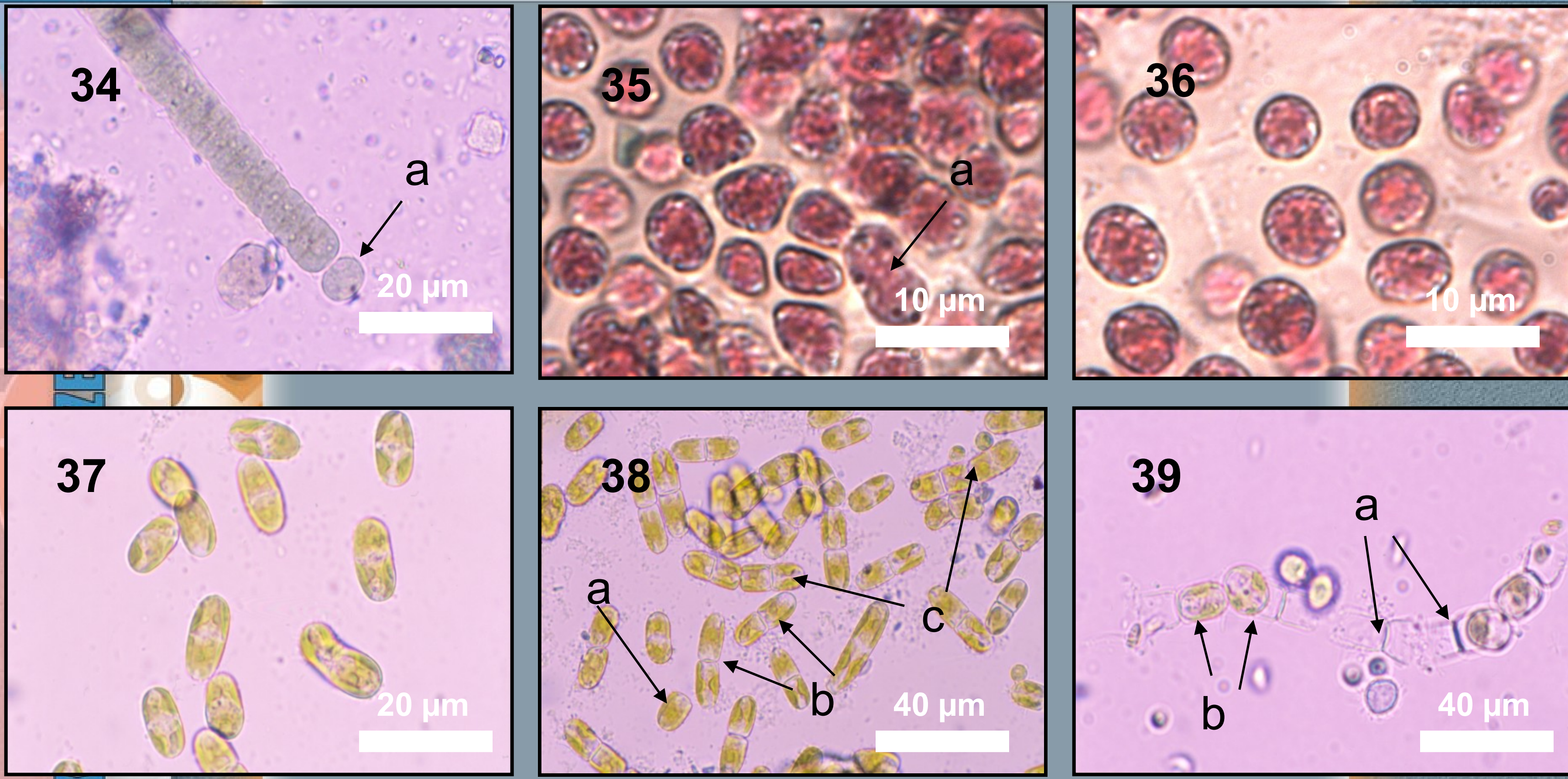
- Cca 115 taxa of microalgae
- The competitive „first come, first serve“ relationships
- Cyanobacteria 35%
- Chlorophytes 42%
- Chrysophytes 17%
- Charophytes 8%
- The north-facing or humid side of buildings

Cyanobacteria



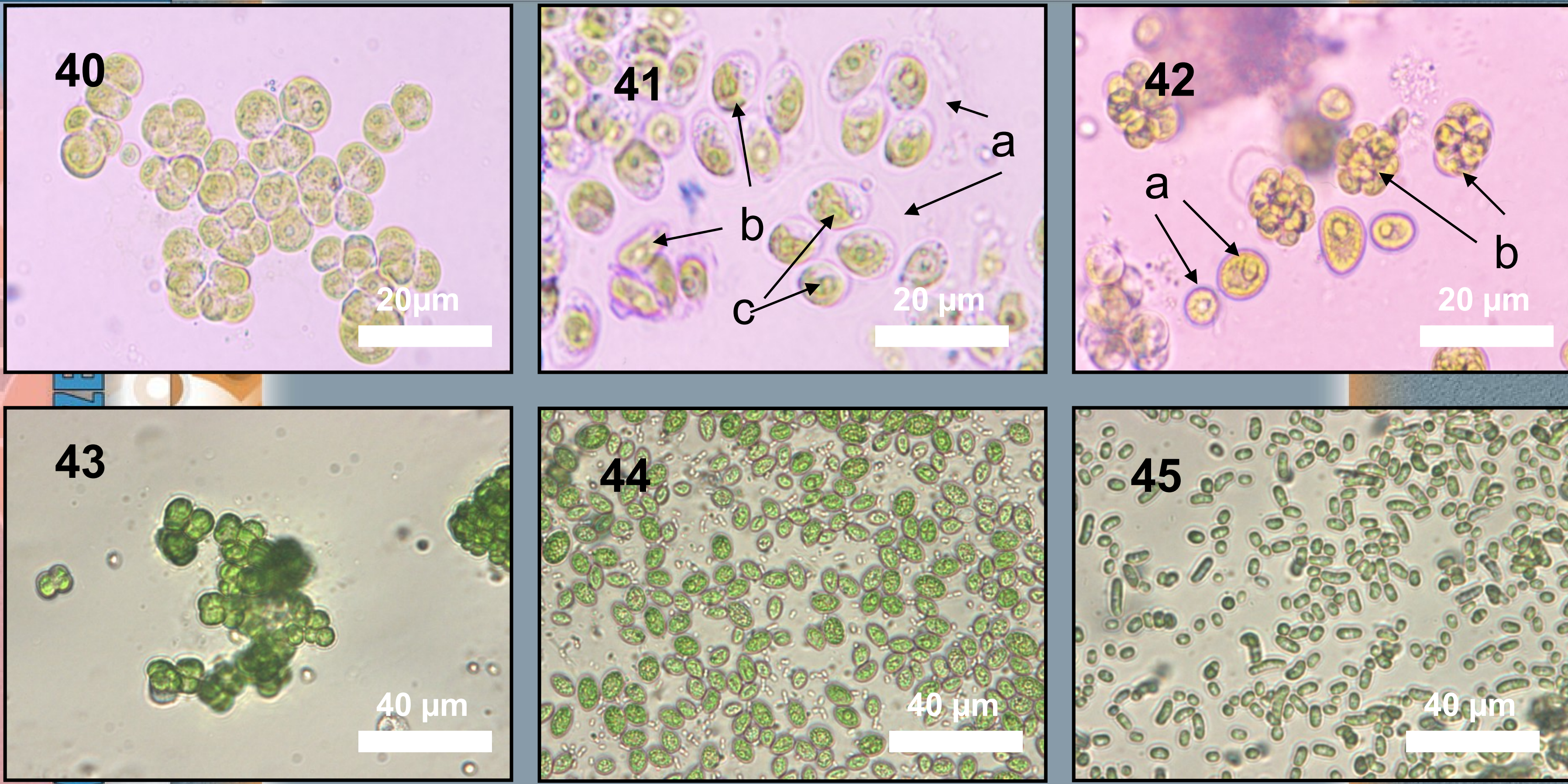
Figs 28-33. Cyanobacteria: Fig. 28. *Chroococcidiopsis umbratilis,* a vegetative cells, b endosporangium; Fig. 29. *Chroococcidiopsis* sp.; Fig. 30. *Leptolyngbya nostocorum,* a false branching; Fig. 31. *L. fragilis*; Fig. 32. *Nostoc sphaericum*; Fig. 33. *N. microscopicum*

Rhodophyta and Heterokontophyta



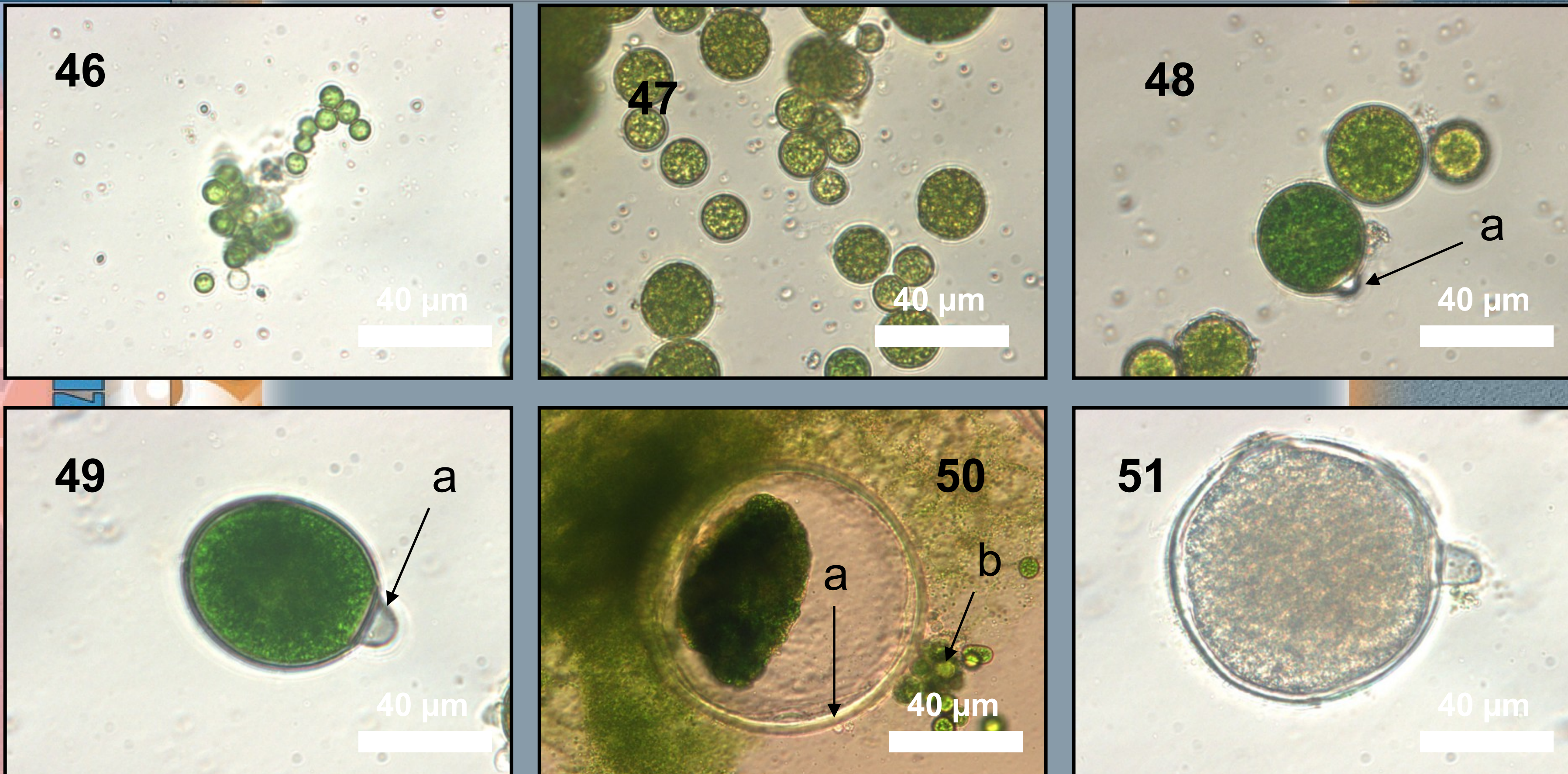
Figs 34-39. Fig. 34. Cyanobacterium: *Scytonema julianum*, a apical globular cell ; Fig. 35-36. Rhodophyta (red algae): Fig. 35. The colony of *Porphyridium purpureum*, a cell division; Fig. 36. Solitary globular cells of *P. purpureum*; Fig. 37-39. Heterokontophyta: Fig. 37. One-celled representatives of *Xanthonema* sp.; Fig. 38. Representatives of *Xanthonema* sp., a one-celled, b two-celled, c four-celled; Fig. 39. Fragmentation of filament of *Xanthonema* sp., a H-particles, b unicelled fragments of filament

Chlorophyta



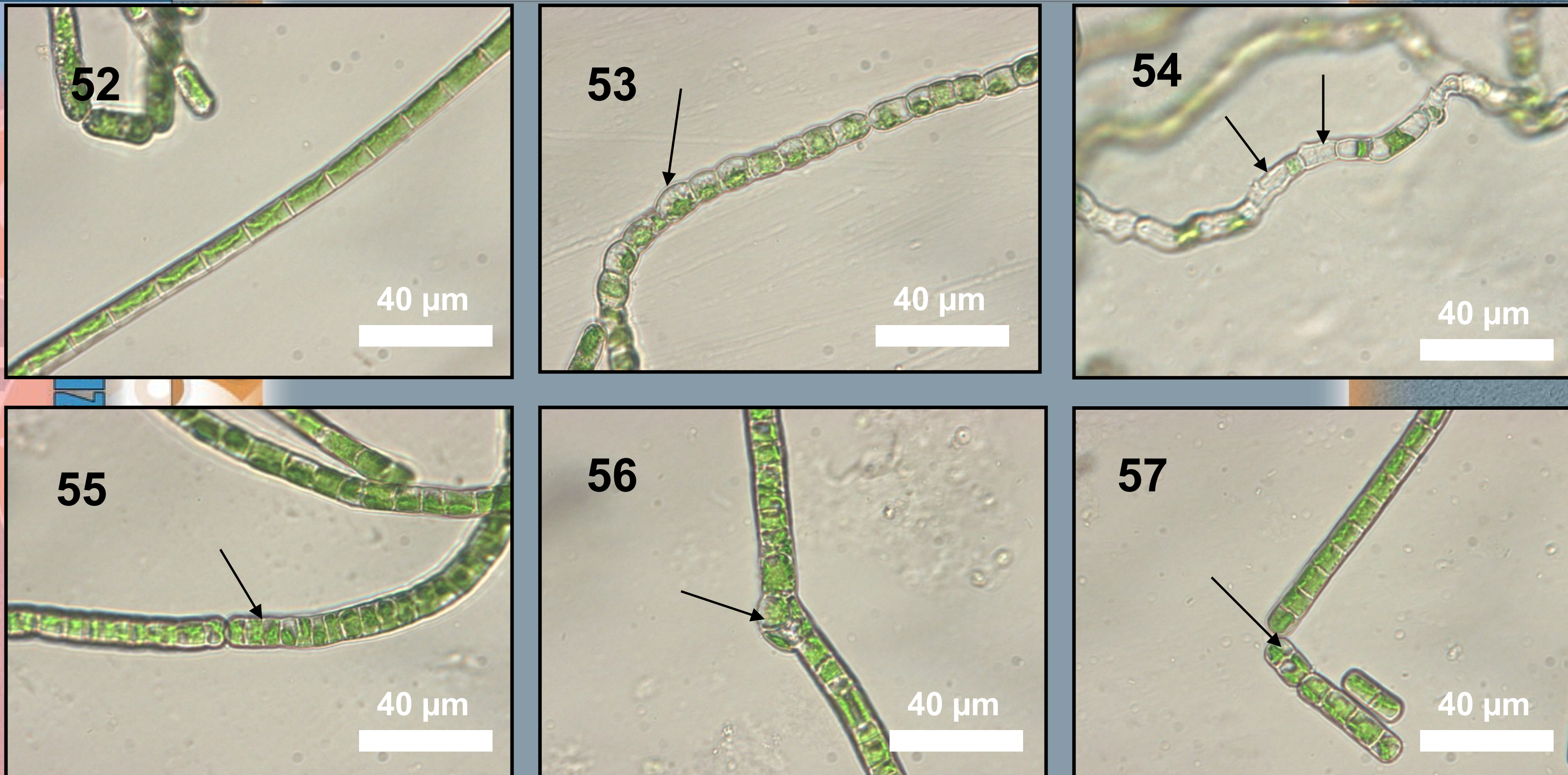
Figs 40-45. Figs 40-44. Chlorophyta: Fig. 40. *Chlorosarcinopsis* cf. *arenicola*; Fig. 41. *Tetracystis sarcinalis*, a globular zoosporangia with tetrades of zoospores, b zoospores, c pyrenoid; Fig. 42. *Oocystis assymmetrica*, a vegetative cells, b berry-like colonies; Fig. 43. *Chlorosarcinopsis pseudominor*; Fig. 44. *Scenedesmus obliquus*, unicelled stage; Fig. 45. Charophyta: Fig. 45. *Stichococcus bacillaris*.

Chlorophyta



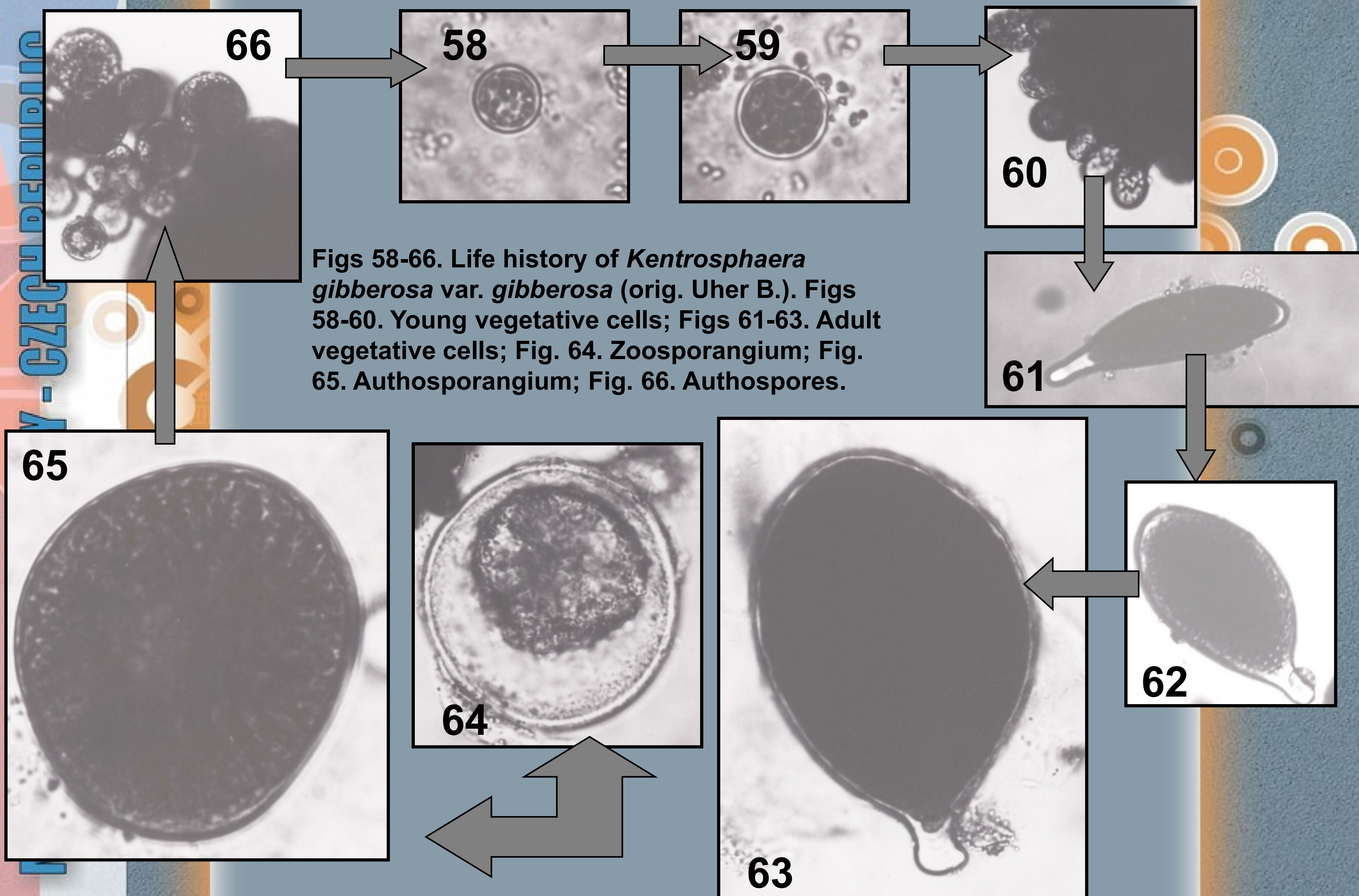
Figs 46-51. Chlorophyta – *Kentrosphaera gibberosa* var. *gibberosa*:Fig. 46. Autospores with parietal chloroplast; Fig. 47. Young vegetative cells with parietal and central chloroplast; Fig. 48. Adult vegetative cells with central chloroplast, a cell wall protrusion; Fig. 49. Oval adult vegetative cell, a cell wall protrusion; Fig. 50. Empty autosporagium, a autosporangium wall, b autospores; Fig. 51. Old vegetative cell.

Charophyta



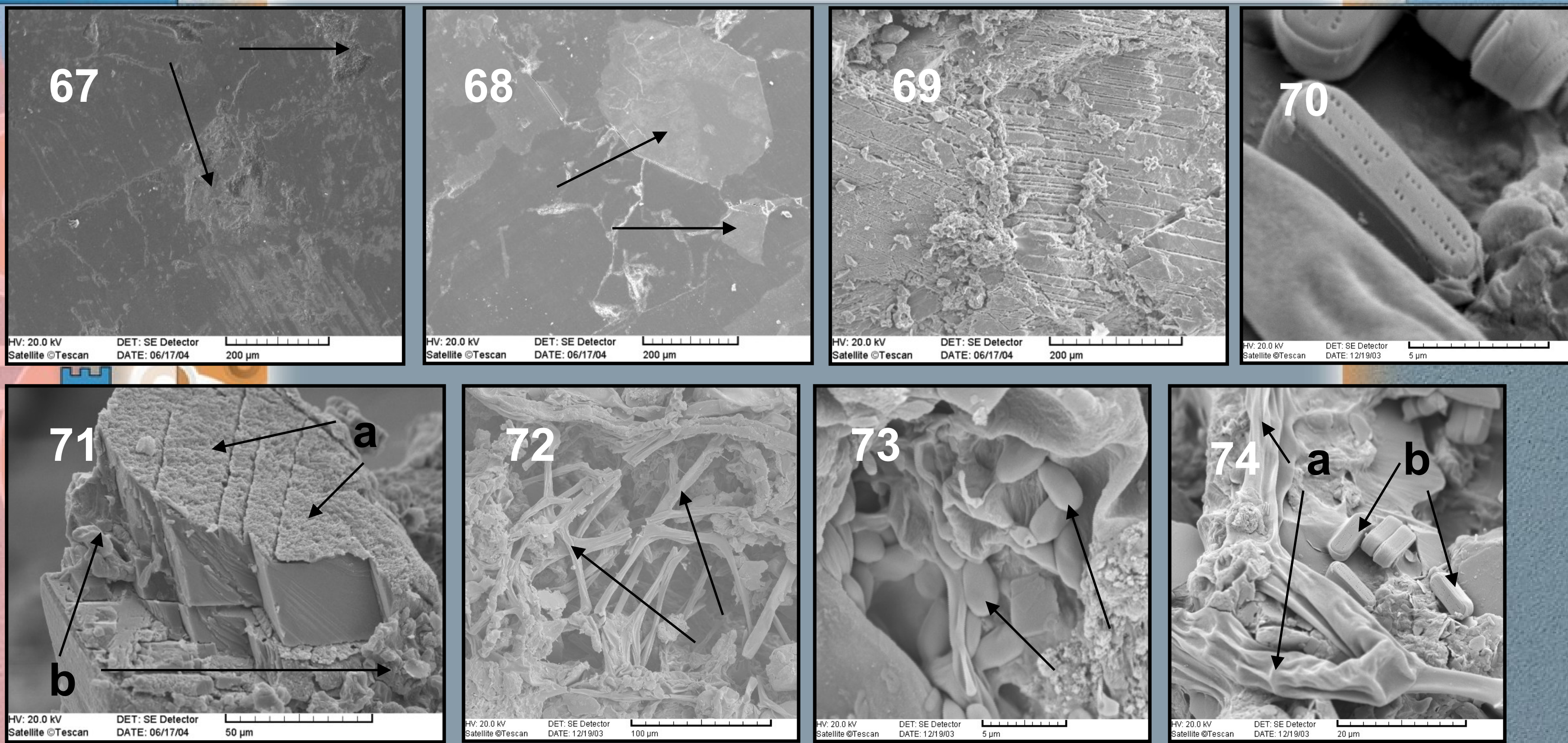
Figs 52-57. Charophyta: Fig. 52-54. *Klebsormidium* flaccidum, Fig. 52. Stright filamment with parietal chloroplasts; Fig. 53. The protruding zoosporangial cells in asexual filament (arrow); Fig. 54. Empty zoosporangial cells with fissured surface (arrows); Fig. 55-57. *Klebsormidium crenulatum*, Fig. 55. Maturing filaments with short cells (arrow); Fig. 56. Begining of pseudobiseriating in filament (arrow); Fig. 57. Pattern of fragmentation of filament.

Life history



Figs 58-66. Life history of *Kentrosphaera gibberosa* var. *gibberosa* (orig. Uher B.). Figs 58-60. Young vegetative cells; Figs 61-63. Adult vegetative cells; Fig. 64. Zoosporangium; Fig. 65. Authosporangium; Fig. 66. Authospores.

SEM-micrographs

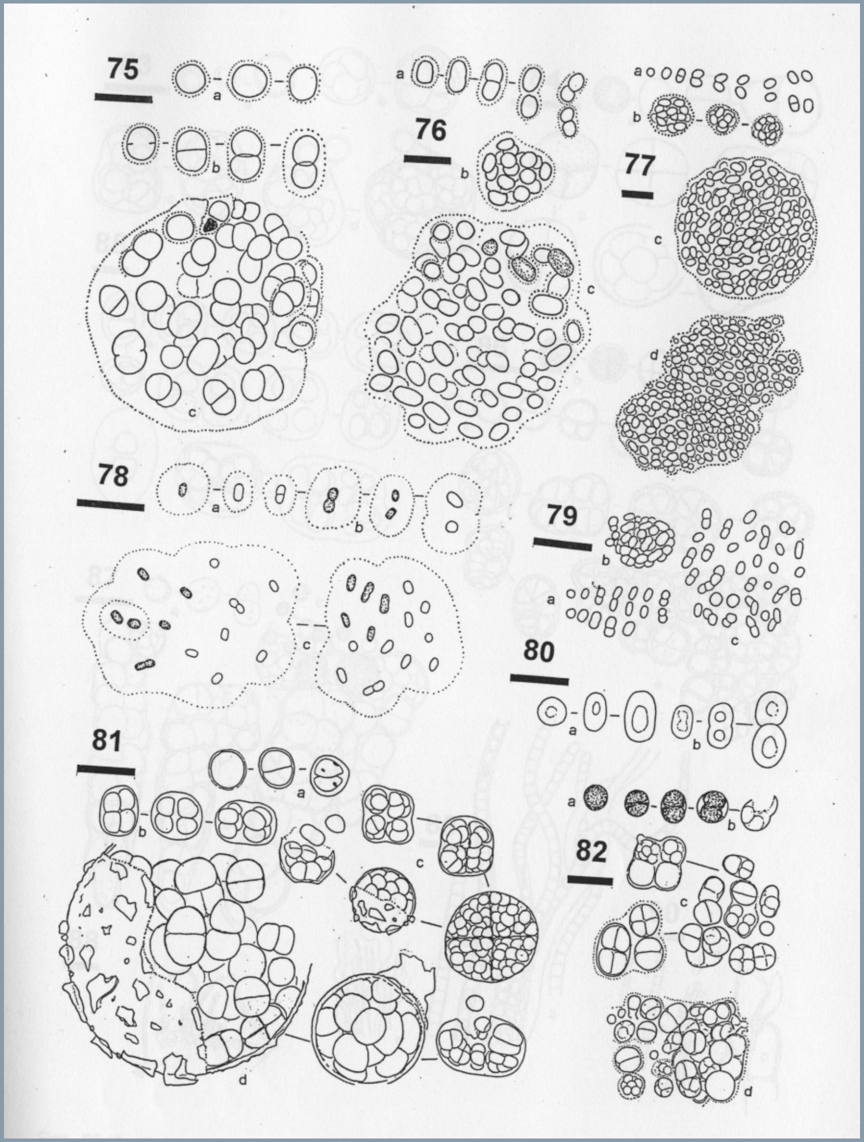


Figs 67-74. SEM-micrographs. Fig. 67. Surface of dolerite, Ca-felspars (arrows); Fig. 68. Surface of granodiorite, Na,K-felspars; Fig. 69. Surface of marble; Fig. 70. Diatom *Diadesmis* sp.; Fig. 71. Biofilm on marble surface, a bacterial layer, b cyanobacterial cells of *Chroococcidiopsis* sp.; Fig. 72. Cyanobacterial biofilm on brick, filaments of *Leptolyngbya* sp. (arrows); Fig. 73. Endospores of *Chroococcidiopsis* sp., endolithic cyanobacterium (arrows); Fig. 74. Biofilm on the brick, a filament of *klebsormidium flaccidum*, b cells of diatom *Diadesmis* sp.

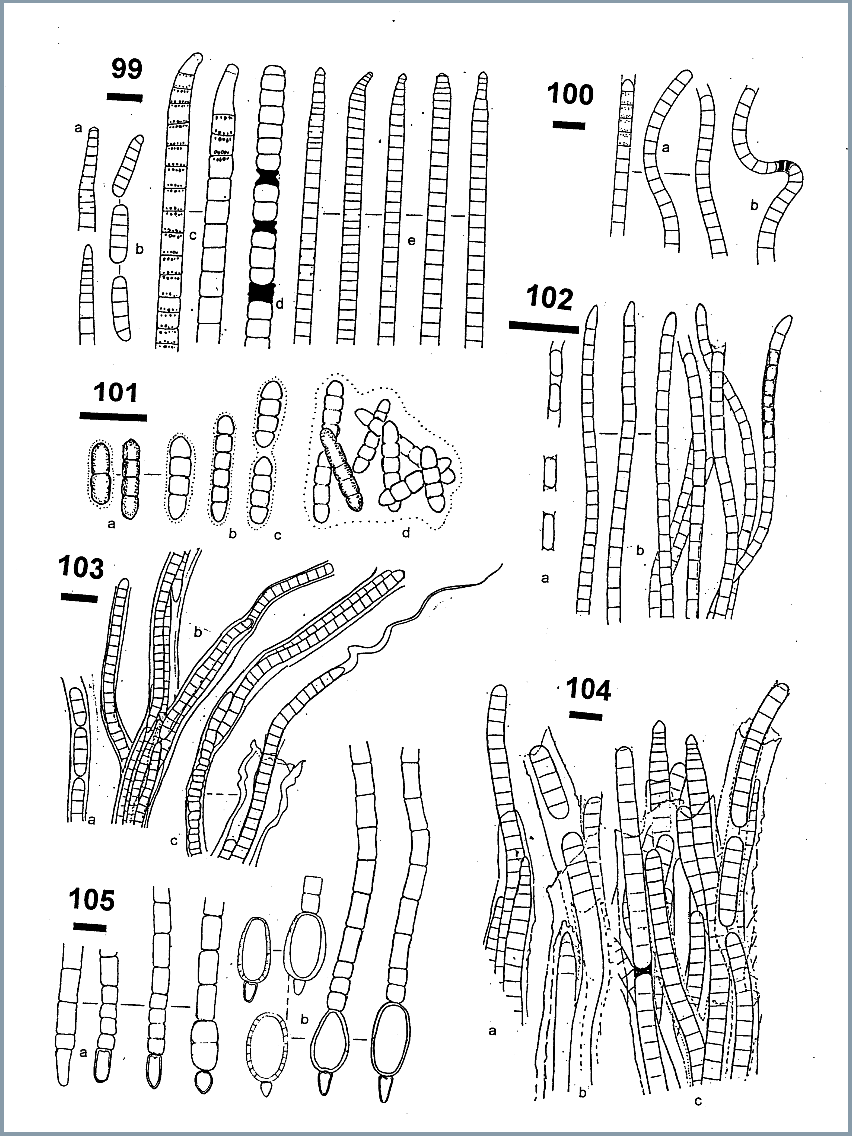
Original drawings

- Life cycle
- Vegetative stages
- Generative stages
- Young habit
- Adult habit
- Old habit

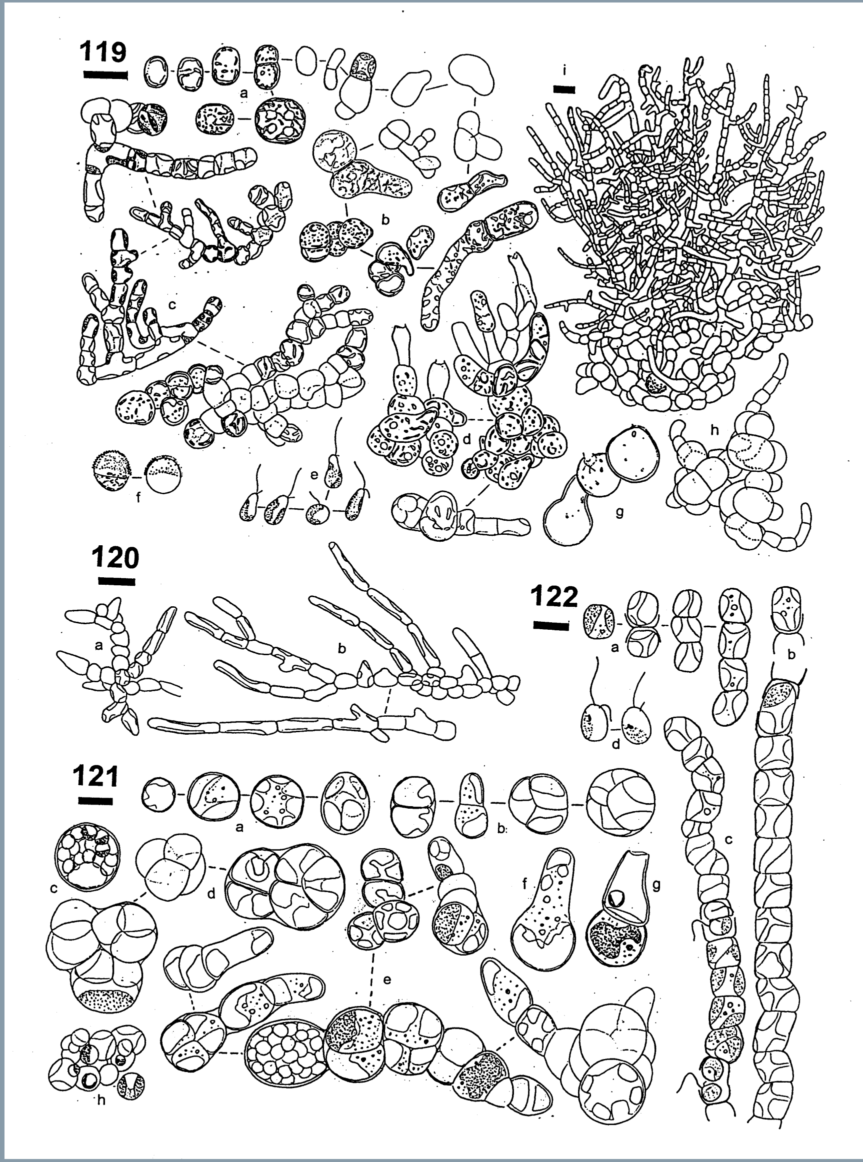
Coccal Cyanobacteria



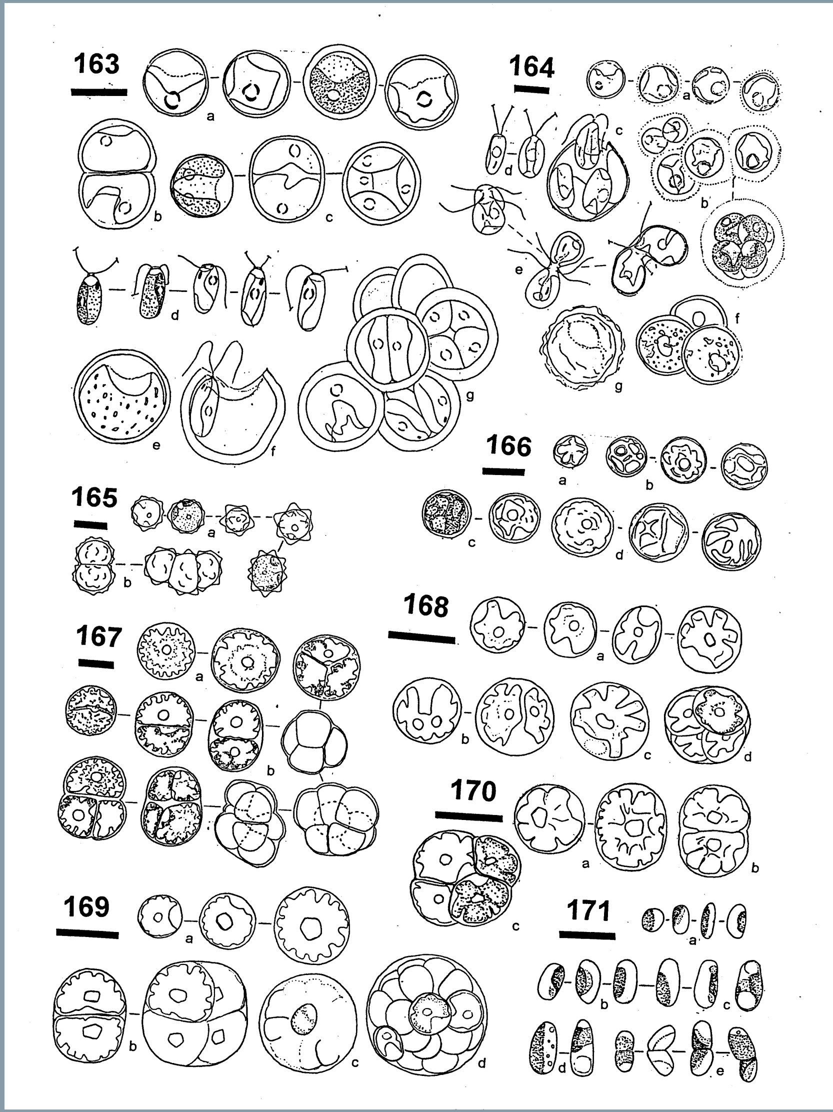
Trichal Cyanobacteria



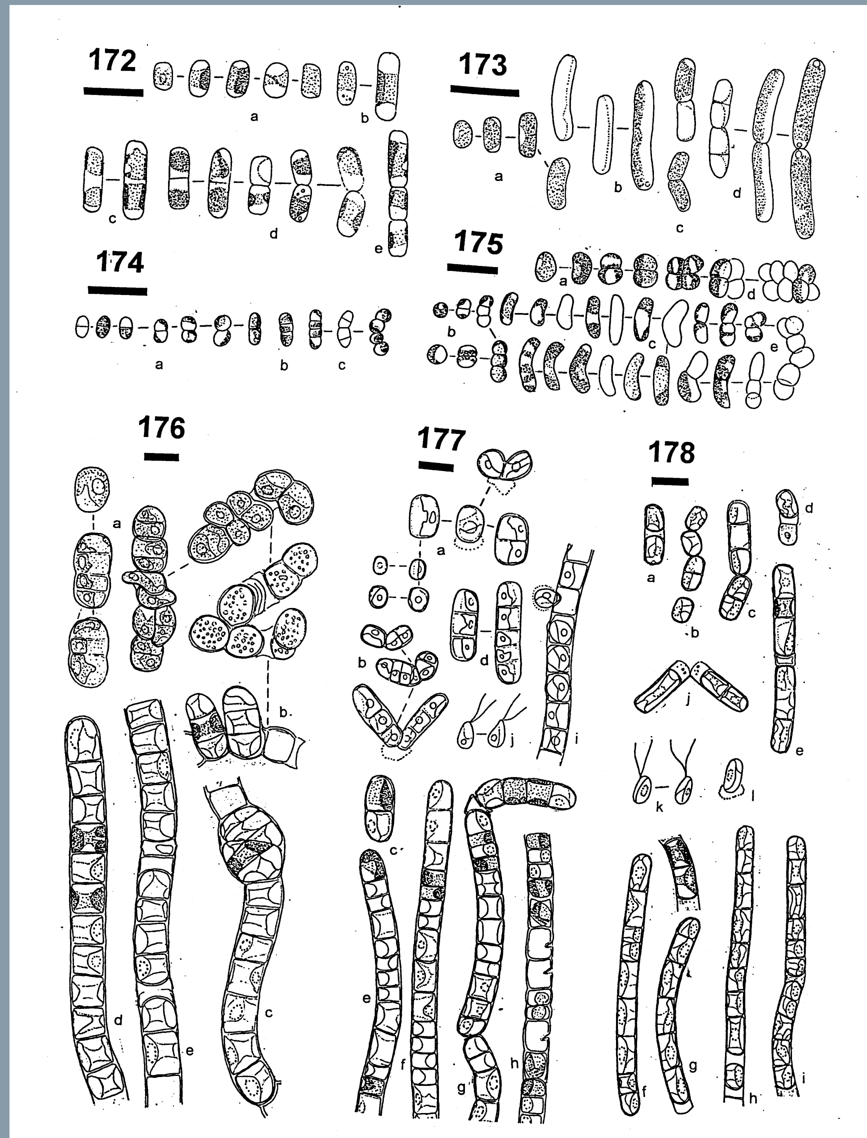
Heterokontophytes



Green Algae



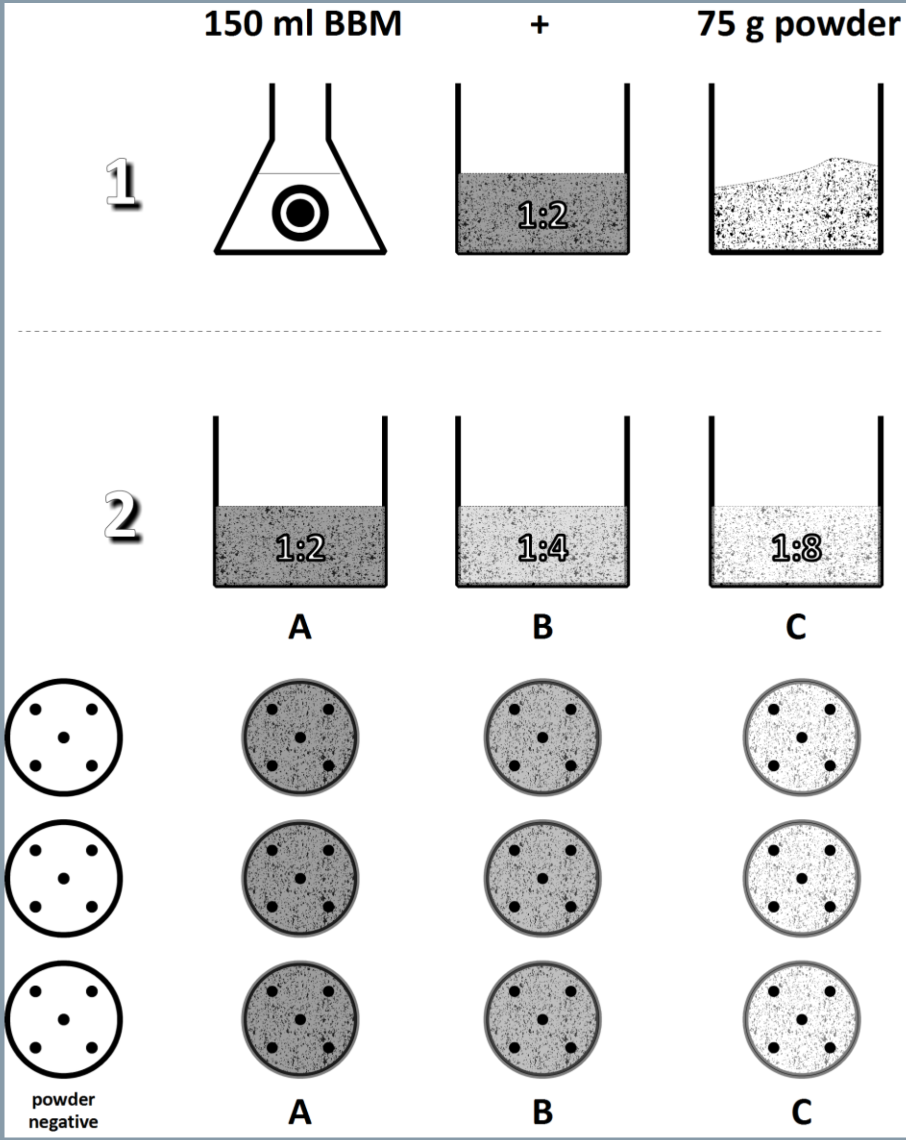
Charophytes



The New Growth Inhibition Test

- To develop the techniques of new growth inhibition test
- The biotest used on subaerial phototrophic microorganisms (autochtonous, isolated from deteriorated monuments)
- A type of growth inhibitor was used white powder called in Spain “el estuco”

Experiment Scheme



Experiment Realisation

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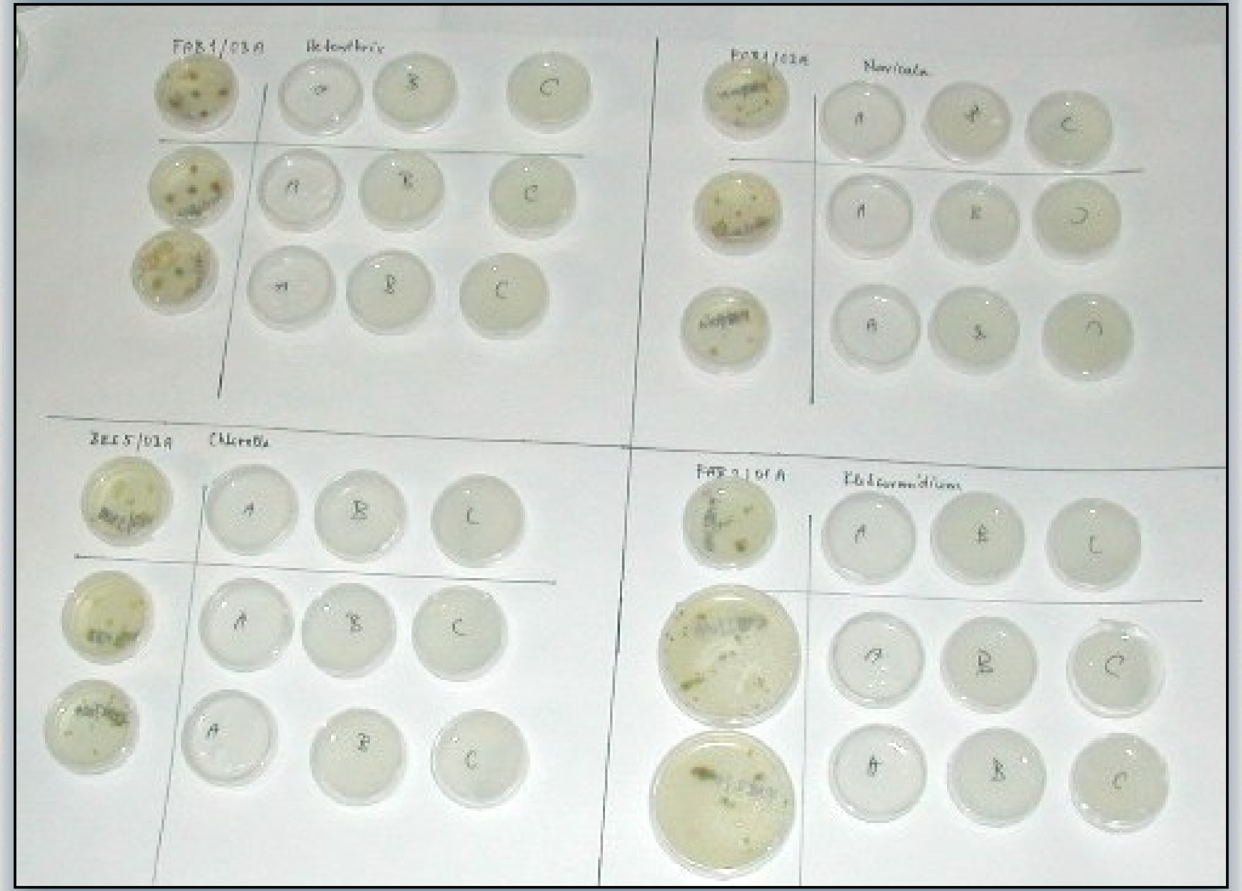
The View of The Realisation



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Results

- This material Calcium Ruthenium Oxide Hydrate totally inhibited and eradicated growths of algae

- Active interests in developing miniaturized algal bioassays, algal biosensors and development of cultivation tests

- Work on subaerial algae caused biodeterioration has highlighted their practical potencial

Acknowledgement

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- Dr. Ľubomír Kováčik

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Thank you for your attention!