3.4 Species composition and diversity of algae on anthropogenic substrata

PAVEL ŠKALOUD, JIŘÍ NEUSTUPA & MAGDA ŠKALOUDOVÁ

INTRODUCTION

Algae and cyanobacteria form an important part of soil microbial communities. Due to their primary photosynthetic production, easy dispersion and fast growth, soil algae are very important in the colonisation of pioneer biotopes (Evans & Johansen 1999). In the following chapter, we characterize the algal communities of four ore-washery basins, differing by their soil chemical characteristics and industrial activity. In addition to detailed description of selected prominent species, we discuss differences in the algal composition of biological soil crust communities among the localities.

MATERIALS AND METHODS

In each site, samples from the 0–3cm layer were taken randomly from physiognomically uniform areas of the size 5–15 m^2. The samples were placed into sterile bags and transported to the laboratory for analysis. The composite samples were crushed and mixed to produce a homogenous sample. A 1-g aliquot was removed and added to 50 ml of distilled water. The soil suspension was mixed by a magnetic mixer for 15 minutes. Aliquots of 0.5 or 1 ml were spread in duplicate on agar solidified BBM medium (Bischoff & Bold 1963; Ettl & Gärtner 1995). Cultures were sealed with parafilm and incubated at 20–25 °C under daylight conditions (the plates were placed beside a north facing window) until good growth had been obtained (3–6 weeks). Algal microcolonies were examined directly from agarized plates using an Olympus BX 51 microscope with Nomarski DIC optics and photographed using Olympus Camedia digital camera C-5050 Zoom. Standard cytological stains (Lugol's solution, methylene blue, acetocarmine, chloraliode solution) were used for visualisation of pyrenoid, cell wall structures or mucilage. For detailed investigation of some strains, the algal colonies were transferred to agarized BBM culture tubes and then cultivated at 18 °C, under an illumination of 20–30 μmol m$^{-2}\cdot$s^{-1} and 16:8 h light-dark cycle. Identification was made on the basis of life history and morphological criteria using standard authoritative references (Printz 1964; Fott & Nováková 1969; Ettl 1978; Punčochářová & Kalina 1981; Komárek & Fott 1983; Krammer & Lange-Bertalot 1986; Ettl & Gärtner 1995; Hindák 1996; Lokhorst 1996; Andreeva 1998; Komárek & Anagnostidis 1998, 2005). The quantities of algae on Petri dishes were evaluated as belonging to one of three classes: 1 – single algal colony found, 2 – rare species with several colonies on the dishes, 3 – dominant species in the sample. Following statistical methods were used to detect patterns in the data. The localities were clustered, based on Sørensen floristic similarity (Sørensen 1948) utilizing the single linkage in statistical program PAST 1.74 (Hammer et al. 2001). Principal component analysis (PCA) was performed using Canoco 4.5 (Ter Braak & Šmilauer 1998) to ordinate localities based on their algal composition.

RESULTS AND DISCUSSION

General conclusions

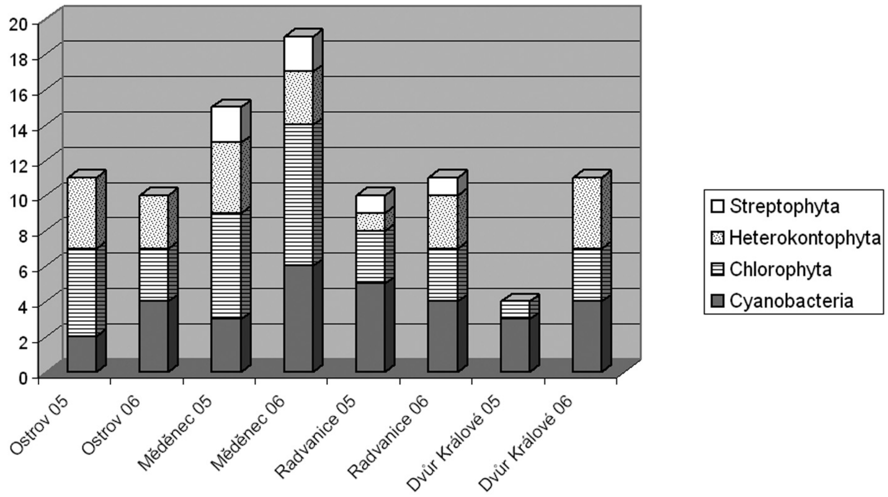
A total of 49 algal species representing 33 genera were recovered from four studied ore-washery basins (Table 3.4.1). Two widespread taxa (cyanophyte *Leptolyngbya* sp. 2 and the diatom *Nitzschia* sp.) were found in at least three localities. All but one of the localities were characterized by a few (up to three) species, forming a dominant component of total algal flora (Ostrov locality was typical by dominance of *Leptolyngbya* spp., *Bumilleriopsis filiformis* and *Chlorella lobophora*; the soil sample from Radvanice was dominated by *Nostoc* cf. *edaphicum* and *Eustigmatos polyphem*; and among the most abundant algae in Dvůr Králové were *Nostoc* sp. 2 and *Scenedesmus* sp.). Algal population in Měděnec differed in balanced presence of many algal species, without any predominant taxa. Moreover, the locality Měděnec was characterized by the highest number of determined taxa (22 taxa, compared to 14, and 11 taxa found in Ostrov and Radvanice, and Dvůr Králové, respectively). Most taxa were rare, with 27 of the 49 species identified appearing in a single locality in rare to low abundances (1 or 2 in semi-quantitative scale).

Generally, cyanobacteria and green algae were the most dominant groups of autotrophic organisms in all investigated localities (Figs. 3.4.1, 3.4.2). From a total of 49 species found, they comprise the 70% majority. Cluster analysis, based on the species similarity among the samples, apparently associates the samples originating from the same locality (Fig. 3.4.3). Moreover, the active ore-washery basins

Table 3.4.1 Algal distribution in 8 investigated samples (**O** – Ostrov, **M** – Měděnec, **R** – Radvanice, **D** – Dvůr Králové).

		Sampling site, year of sampling							
	Fig.	**O 2005**	**O 2006**	**M 2005**	**M 2006**	**R 2005**	**R 2006**	**D 2005**	**D 2006**
Cyanobacteria									
Anabaena cf. *cylindrica* Lemmerman	3.4.5 a,b		2						
Aphanothece sp.						1	2		
Chroococcidiopsis sp.							2		
Chroococcus cf. *helveticus* Nägeli				2	1.5				
Gloeothece cf. *tepidariorum* (A. Braun) Lagerheim	3.4.5 c					2			
Leptolyngbya sp. 1	3.4.5 d	3	3		2				2.5
Leptolyngbya sp. 2	3.4.5 e			3	3				
Leptolyngbya sp. 3	3.4.5 f	3	2						
Leptolyngbya sp. 4	3.4.5 g							1	1
Leptolyngbya sp. 5	3.4.5 h					2	2		
Nostoc cf. *calcicola*	3.4.5 i							2	2
Nostoc cf. *edaphicum*	3.4.5 j				1	3	3		
Nostoc sp. 1	3.4.5 k			2	2.5				
Nostoc sp. 2								3	2
Phormidium cf. *autumnale*	3.4.5 l		2						
Phormidium sp. 2					1				
Synechocystis sp.						2			
Bacillariophyceae									
Achnanthidium cf. *minutissimum* (Kützing) Czarnecki							1		
Nitzschia sp.	3.4.6 a,b	1	3	3	3		1		3
Mayamaea atomus (Kützing) H. Lange-Bertalot		2	1	2					1
Xanthophyceae									
Botrydiopsis arhiza Borzi		1							
Bumilleriopsis filiformis Vischer	3.4.6 c,d	3	2.5						2
Pleurochloris polychloris Pascher	3.4.6 e,f								2
Xanthonema cf. *montanum* (Vischer) Silva	3.4.6 g			1	1				
Eustigmatophyceae									
Eustigmatos polyphem (Pitschmann) Hibberd	3.4.6 h					3	3		
Eustigmatos vischerii Hibberd	3.4.6 i,j			2	2.5				
Chlorophyceae									
Bracteacoccus cf. *aggregatus* Tereg	3.4.6 k,l	1		2	2				
Chlamydomonas sp. 1	3.4.7 a				1.5				
Chlamydomonas sp. 2									2
Chlorococcum cf. *minutum* Starr				1					
Chlorococcum sp.		2	1						
Deasonia multinucleata	3.4.7 b				1				
Diplosphaera chodatii Bialosuknia em. Vischer						1			
Scenedesmus cf. *obtusiusculus* Chodat	3.4.7 c–e							2	3
Scotiellopsis reticulata Puncocharova et Kalina				3	2.5				
Scotiellopsis sp.			1.5						
Trebouxiophyceae									
Auxenochlorella protothecoides (Krüger) Kalina et Puncocharova		2							
Chlorella ellipsoidea Gerneck					1.5				
Chlorella lobophora Andreeva	3.4.7 f	2.5	3						
Chlorella saccharophila (Krüger) Migula							1		
Chlorella vulgaris Beijerinck									1
Choricystis chodatii (Jaag) Fott					1				
Dictyochloropsis sp.	3.4.7 g,h					1	2		
Jaagiella sp.	3.4.7 i			2	1				
Muriella terrestris J. B. Petersen				2					
Mychonastes zofingiensis (Dönz) Kalina et Puncocharova		1							
Pseudococcomyxa simplex (Mainx) Fott				3	1	3	1		
Klebsormidiophyceae									
Klebsormidium flaccidum (Kützing) Silva, Mattox et Blackwell	3.4.7 j			2	1				
Zygnematophyceae									
Cylindrocystis brebissonii Meneghini var. *desertii* Flechtner et al.	3.4.7 k,l			2.5	2	1	1		

Fig. 3.4.1 Species richness expressed as the number of taxa found in each locality. Assignment of taxa to the four algal groups is displayed.



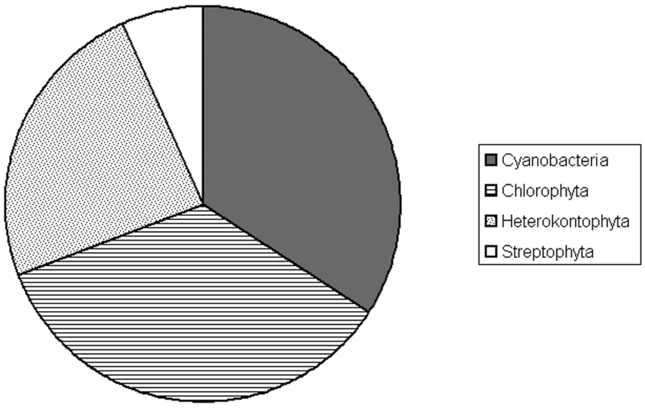


Fig. 3.4.2 Proportional occurrence of four algal groups, determined in all investigated localities.

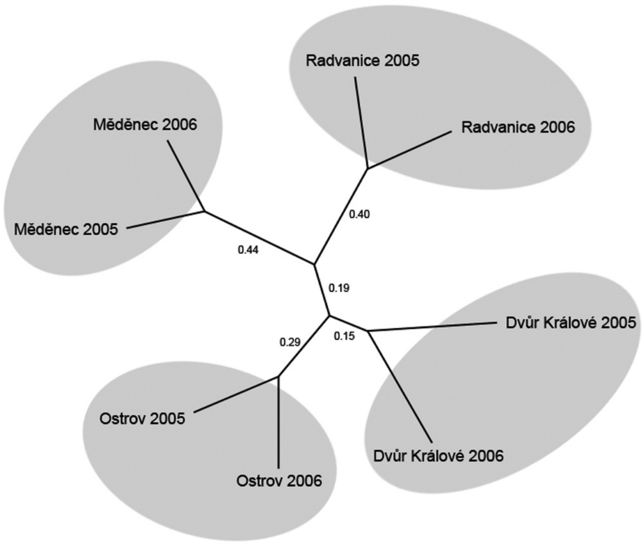


Fig. 3.4.3 Unrooted single linkage dendrogram of investigated samples, generated on the basis of Sørensen floristic similarities. Branch lengths are displayed near the internal branches.

(Dvůr Králové and Ostrov) and the abandoned industrial sedimentation basins (Měděnec and Radvanice) cluster together, showing some similarities of the algal composition in relation to the industrial activity. The PCA ordination plot reflects other interesting patterns in data investigated (Fig. 3.4.4). First, it clearly demonstrates that algal composition of the active ore-washery basins is much more similar than of the abandoned industrial sedimentation basins. Similarities in algal flora of active basins are probably caused by extreme conditions associated with the industrial activity. By contrast, the abandoned industrial sedimentation basins Měděnec and Radvanice forms separate clusters, in the PCA ordination plot. Those distinct differences in algal composition could be explained by locality-specific soil chemical parameters (e.g., very high manganese concentration in Radvanice, see chapter 3). Finally, the PCA ordination plot shows differences in population stability of the active ore-washery basins. While Ostrov exhibits high floristic similarity within two years (Sørensen index 0.67), the algal composition in Dvůr Králové significantly changed after a year (Sørensen index 0.53). This difference was caused by quite high number of algal species, observed only in 2006 (e.g., *Leptolyngbya* sp. 2, *Nitzschia* sp. or *Bumilleriopsis filiformis*). We attribute it to distinctive changes of soil water regime, caused by the periodical flushing of the locality by the ash followed by the relatively long period of increased soil humidity.

Floristics

Following pages provide detailed descriptions of several taxa, found in the investigated localities. The taxa were selected due to their high abundance in the samples or by remarkable occurrence in such type of biotope as ore-washery basins are (see Colour plates, Figs. 3.4.5–3.4.7).

Leptolyngbya Anagnostidis & Komárek (Figs. 3.4.5 d–h)

Filamentous cyanobacterial genus with fine, 0.5–3.2 μm wide trichomes, imbedded into thin usually colourless facultative sheaths opened at the apical end. Rarely, false branching can occur, usually with only one lateral branch. Trichomes are cylindrical, usually with rounded or conical apical cells, not constricted or constricted at the crosswalls, nonmotile. Cells are cylindrical, isodiametrical or longer than wide, with a homogeneous content, without aerotopes. Heterocytes and akinetes are absent. *Leptolyngbya* species are very common in soils and in periphyton and metaphyton

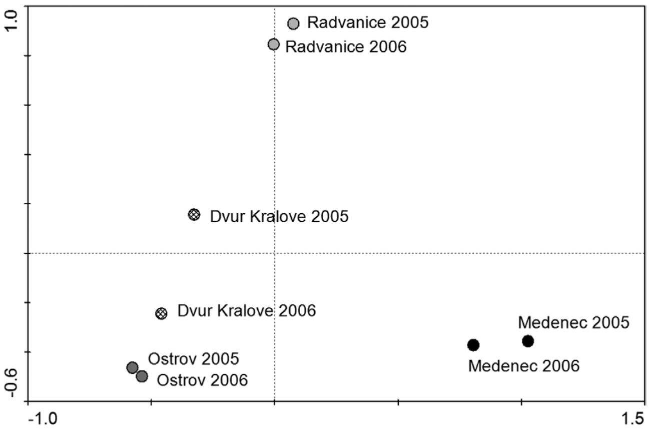


Fig. 3.4.4 PCA ordination diagram showing the position of samples in the range of the first two ordination axes.

of freshwater and halophilous (marine) biotopes. However, taxonomy is not well known in respect of their morphological simplicity.

We determined five *Leptolyngbya* morphospecies growing in the studied localities. They differ in cell dimensions, trichome width variability, colour and disintegration rate. Due to the scarce morphological features and high morphological variability, it is very difficult to judge if morphologically similar *Leptolyngbya* species isolated from several samples represent a single species or not. Therefore, in this study we operate at the level of morphospecies.

The most common morphospecies, determined in localities Ostrov, Měděnec and Dvůr Králové (*Leptolyngbya* sp. 1) is characterized by nearly straight trichomes, up to 1 µm wide, brown-green, pale green, brown or brown-red, obviously constricted at the cross-walls. Colonies are brown, consisted by mainly parallelly-arranged filaments. *Leptolyngbya* sp. 2, found in high abundances in Měděnec, is characterized by very variable width of filaments, varying between 0.8–3 µm. Trichomes are green or pale-green, slightly constricted at the cross-walls. The third most abundant species, *Leptolyngbya* sp. 3, was determined in the locality Ostrov. It is characterized by short, easily disintegrated trichomes of blue-green colour. Width of cylindrical cells varies between 1.5–2 µm.

Nitzschia Hassall (Figs. 3.4.6 a, b)

A diatom genus with structurally asymmetrical valves, with off-centre placed raphe system. The valves are symmetrical or asymmetrical about the longitudinal axis. They are usually highly elongate, and have rounded, rostrate or capitate poles. Transverse striae are sometimes visible but often delicate, very fine or unresolvable in light microscope. Raphe system is fibulate, usually appearing to run along one edge of the valve but subcentral in a few species. *Nitzschia* is relatively large genus with hundreds of freshwater and marine species. Most species are epipelic in microhabitat but *Nitzschia* also contains planktonic, epilithic, and epiphytic species. A small proportion of species (up to 30) is also mentioned from aerophytic or terrestrial biotopes.

Species of *Nitzschia* were found in all studied localities. Cells were 15–23 µm long × 3–4 µm wide. Morphology and dimensions of the valves were very similar among the localities. However, we were unable to determine the species name only by means of light microscope. The determination of *Nitzschia* species in all localities correspond with a broad range of genus occurrence and high adaptation to metal rich soils (Starks & Shubert 1982; Lukešová & Komárek 1987; Lukešová 2001; Neustupa & Škaloud 2004).

Eustigmatos polyphem (Pitschmann) Hibberd,
Eustigmatos vischerii Hibberd (Figs. 3.4.6 h–j)

Two *Eustigmatos* species were found in the localities Měděnec and Radvanice. The cells are spherical, uninucleate, up to 11–14 µm (*E. vischerii*) or 22 µm (*E. polyphem*) in diameter. Cell wall is smooth and unornamented, in one piece. The cells contain single lobed parietal yellow-green chloroplast with stalked polyhedral pyrenoid and large central vesicle with red contents.

The genus *Eustigmatos* represents a common component of soil floras; isolated from Europe, Africa, New Zealand, USA and Japan (Ettl & Gärtner 1995). *Eustigmatos* species also occur in the swimming pools, where called “mustard algae” (Adamson and Sommerfeld 1978). Various eustigmatophycean species were recorded from metal-rich soils in Central Europe (Lukešová & Komárek 1987; Lukešová 2001).

Bumilleriopsis filiformis Vischer (Figs. 3.4.6 c, d)

A large population of xanthophycean species *Bumilleriopsis filiformis* was recorded from the locality Ostrov. Especially in 2005, this species constitute the dominant component of the algal flora. Cells are solitary or incidentally clustered, of elongate, cylindrical shape, straight or curved. Dimensions of cells vary between 9–13 µm in width and 13–54(80) µm in length. Cells are uninucleate, with several to many parietal plastids.

The species was described by Vischer (1945) from a sample of meadow soil in Switzerland. Since then, *B. filiformis* was recorded very rarely from various floristic studies (Neustupa & Škaloud 2004). Since no abundant population of *B. filiformis* was recorded till this time, we can suppose that the physico-chemical soil properties give it an advantage over all other algal species living in the locality. This advantage caused a remarkable development of the alga, impossible in natural terrestrial biotopes.

Scotiellopsis reticulata Punčochářová et Kalina

This species was observed as highly abundant in both samples from the locality Měděnec. The cells are single, up to 16(–19) µm long × 12(–13) µm wide. Autospores and young cells are fusiform, citriform, adult cells often oval with distinct apical thickenings; old cells globose. Cell wall with meridional ribs converging at the poles; ribs straight, sometimes anastomosing to form a network. Chloroplast is parietal, consisting of polygonal plaques or stripes; in one part of the chloroplast with the pyrenoid having homogeneous stroma surrounded with starch envelope. Asexual reproduction take place by autospores, 2 (–16) per cell; released by rupture of parental cell wall.

All species of *Scotiellopsis* are reported from soils and wet ground, from Europe and China. However, the genus is very probably cosmopolitan. However, ecological preferences as well as biogeography of *S. reticulata* are still unclear due to insufficient data about its distribution (Ettl & Gärtner 1995). This study brings the first report of its occurrence in the microbial communities of biological soil crusts.

Scenedesmus* cf. *obtusiusculus Chodat (Figs. 3.4.7 c–e)

A well-known, broadly distributed genus *Scenedesmus* is characterized by single celled or colonial thalli, forming 2- to 32-celled, usually 4- or 8-celled coenobia. Cells can be arranged linearly, alternating or in 2–3 rows, touching with the lateral walls or in subpolar region only. Cell wall is usually smooth, chloroplast single and parietal with single pyrenoid. *Scenedesmus* represents typical planktonic genus, occurring mainly in eutrophic freshwater ponds and lakes, rarely in brackish water. However, *Scenedesmus* also encompass a few frequently distributed aero-terrestrial species (e.g., *S. rubescens* or *S. vacuolatus*). It is necessary to note, that these are morphologically well-defined, characterized by absent or very rare formation of coenobia and "Chlorella"-like morphology.

We found very abundant *Scenedesmus* species in both samples carried out in the locality Dvůr Králové. Interestingly, morphologically it corresponds to some freshwater species, occurred very rarely in the terrestrial biotopes. Determined taxon is characterized by a large degree of morphological variability. Most commonly, it forms 4- to 8-celled coenobia composed by fusiform cells, without any cell wall ornamentation and very short polar spines. Sometimes however, the coenobia disintegrate into single cells of oval, ovoid or nearly spherical shape, without spines. Chloroplast of disintegrated cells occasionally contains two pyrenoids. Dimensions of coenobial cells varies between 8–14 μm in length, 4–5 μm in width, single cells up to 11 μm in diameter.

All the above-mentioned morphological characteristics correspond well to *S. obtusiusculus*, rare species occurring in small or large pools and clear lakes. Once, the species was even isolated from the soil (Komárek & Fott 1983). The determination of this preferably freshwater species in the microbial community of biological soil crusts can be explained by the water regime of the locality. Dvůr Králové is characterized by periodical flushing of the substrate by the ash followed by the formation of environmental conditions acceptable for some freshwater algal species.

Cylindrocystis brebissonii Meneghini **var. *desertii*** Flechtner, Johansen et Clark (Figs. 3.4.7 k, l)

The desmid *Cylindrocystis brebissonii* was found in the soils from the localities Měděnec and Radvanice. The cells are solitary, but sometimes can form short filaments or aggregations of cells in a common gelatinous matrix. Cell dimensions vary in the range of 17–68 μm × 9–14 μm. Cells are elliptic to elongate-cylindrical, straight or slightly curved, with broadly rounded ends. Chloroplasts are two per cell, axial, stellate in end view, each with large, often elongate pyrenoid. Cell wall is continuous around protoplast, ultrastructurally two-layered.

Cylindrocystis brebissonii is a cosmopolitan, widely distributed species, occurring in subaerial, acidic aquatic or bog habitats. Several varieties of this species were described. Interestingly, investigated alga correspond very well to *C. brebissonii* var. *desertii* Flechtner, Johansen et Clark, described from the biological soil crusts of the Central Desert of Baja California in Mexico (Flechtner et al. 1998). We observed smaller cell dimensions as well as clearly elongated, lobed chloroplast ridges that characterize this variety. Discovery of this morphological variety in the algal community of studied biological soil crusts may point to the existence of undescribed species of *Cylindrocystis*, distinguished from a widely distributed *C. brebissonii* by its distinctive ecological delimitation.

CONCLUSIONS

From phycological point of view, the abandoned ore-washery sedimentation basin Měděnec represents the locality most resembling the naturally-formed biological soil crusts. From all other investigated localities, Měděnec differentiates in the relatively high number of species found and by the absence of distinctively predominant taxa. Conversely, the algal population of Měděnec is characterized by balanced occurrence of present algal species. This natural character we interpret by long-term industrial inactivity, enabling the succession of the algal community to the more natural stages. However, this succession was not found in Radvanice, another abandoned ore-washery sedimentation basin. There, the soil chemical parameters still inhibit the development of species rich community, even if the industrial activity terminated many decades before.