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Soil algae from marlstone-substratum based biotopes in the Nature park Džbán (Central Bohemia, Czech Republic) with special attention to the natural treeless localities

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With 3 figures and 2 tables in the text

Abstract: The soil algal species composition of some marlstone-substratum based biotopes of Džbán region (Central Bohemia, Czech Republic) was investigated. A total of 54 species representing 42 genera were recovered. Chlorophytes were the most abundant group in all investigated sites. The most common green algal species encountered were *Mychonastes homosphaera, Stichococcus bacillaris* and *Klebsormidium flaccidum*. Other frequently occuring species were the cyanophytes *Nostoc commune* and *Phormidium autumnale f. autumnale* and diatoms *Pinnularia borealis* and *Nitzschia palea*. The investigated sites were compared according to their soil algal floras. The natural treeless localities share similar soil algal synusiae with some typical non-forest features – higher frequency of filamentous cyanophytes and diatomes, occurrence of some desmids. The species list from an investigated secondary meadow is dominated by chlorococcal algae with lower occurrence of other groups. This could be determined by lower pH of the soil from this locality. The beech dominated forest locality shares some features typical for temperate forests – lower species richness, common occurrence of green flagellates and unicells, low occurrence of cyanophytes.

Key words: soil algae, natural treeless biotopes, marlstone-substratum soils, ordination analysis.

Introduction

The soil algae of temperate biomes were studied by many researchers (e.g. James 1935, Petersen 1935, Lund 1947, Hilton & Trainor 1963, Komáromy 1983, 1984, Aleksachina & Ština 1984, Grondin & Johansen 1995, Lukešová & Hoffmann 1996, Suchanova & Išbirdin 1997, Děmčenko 1998). General differences between the qualitative algal composition of forest and non-forest soils were determined (Gollerbach & Ština 1969). The algal flora of podsolic and cambisolic temperate forest soils is mostly dominated by green flagellates

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(e.g. species of genera *Chlamydomonas, Chloromonas*), green colored unicells from groups of Chlorophyta (e.g. *Chlorella, Chlorococcum*), Xanthophyceae (e.g. *Botrydiopsis*) and Eustigmatophyceae *(Eustigmatos, Vischeria)* and filamentous species from groups of Klebsormidiophyceae *(Stichococcus, Klebsormidium)* and Xanthophyceae *(Tribonema, Heterococcus)* (GOLLERBACH & ŠTINA 1969, KOMÁROMY 1983, GRONDIN & JOHANSEN 1995, DĚMČENKO 1998). Diatoms and cyanophytes are usually less abundant (GOLLERBACH & ŠTINA 1969). With higher soil pH the diversity of other groups than green algae is increasing (GOLLERBACH & ŠTINA 1969, METTING 1981, LUKEŠOVÁ & HOFFMANN 1996). The algal flora of broad-leaved forests soils is usually more diversified than the one of needle-leaved forests (GOLLERBACH & ŠTINA 1969, DĚMČENKO 1998).

Soils of temperate non-forest phytocoenoses have generaly more diversified algal synusiae than forest soils (NOSKOVA 1968, GOLLERBACH & ŠTINA 1969). The coccal and filamentous green and heterokont algae, cyanophytes (e.g. *Nostoc, Phormidium, Microcoleus*) and diatomes (e.g. *Hantzschia, Navicula, Pinnularia*) are in cambisolic and podsolic grassland soils abundant too. Characteristic representatives of the floristic lists from these biotopes are the species from groups of Mesotaeniaceae and Desmidiaceae (GOLLERBACH & ŠTINA 1969). With higher soil pH the biodiversity of both coccal and filamentous cyanophytes is significantly increasing (KOMÁROMY 1983, 1984, GRONDIN & JOHANSEN 1993, SUCHANOVA & IŠBIRDIN 1997, FLECHTNER et al. 1998).

There were two main objectives of the present study. Firstly, the careful characterization of the soil algal species composition from marlstone-substratum based biotopes in the Nature Park Džbán (Central Bohemia, Czech Republic), a region and types of biotopes previously unstudied. Secondly, the comparison of soil algae from two isolated natural treeless marlstone-substratum based localities with secondary treeless marlstone-substratum based grassland locality and near beech dominated marlstone-substratum based forest.

Localities

The study area was located in the Nature Park Džbán (Central Bohemia, Czech Republic). The Džbán highland is formed by a tableland elevated by tectonic movements. The tableland is divided by a number of valley trenches. Sometimes the transitions between the valley and plateau are formed by intense reef ruptures. The bedrock of the area is dominated by Cretaceous Turonian marlstone sea sediments. The bed depth amounts to 30–60 meters (ŠPECINGER 1997). The soil depth over the marlstone rock stratum amounts often only to 50–100 cm. Soils are generaly cambisols (NĚMEC 1996). The plateau reaches to 470–500 meters a.s.l. (highest elevation Louštín – 537 meters a.s.l.). Mean annual precipitation has been reported between 400–510 mm (NĚMEC 1996). Mean annual temperature is 8,3–8,9 °C. The region is classified as a phytogeographical thermophyticum (SKALICKÝ 1988).

Vegetation cover of the plateau is formed by beech forests *(Fagus sylvatica)* and partly by secondary spruce and larch forests *(Picea abies* and *Larix decidua)*. On the intense valley slopes scree-forests with elms (*Ulmus* spp.), hornbeams *(Carpinus betulus)* and maples *(Acer platanoides)* are formed. About 30% of the area is deforested and conversed to mowed

grasslands. The most valuable biotopes of the area are natural treeless localities on marlstone outcrops above reef ruptures. The soil depth on these outcrops amounts only to 20–50 cm not enabling the growth to the tree vegetation. The extent of the single natural treeless locality amounts usually only about to 2–4 m^2. Because of the postglacial treeless continuity the vegetation cover of these localities includes some glacial relicts – e.g. *Arctostaphylos uva-ursi* and *Polygaloides chamaebuxus* (KLIKA 1950). In the landscape these biotopes play a role of treeless islands in the middle of forest ecosystems.

The locality in The National Natural Reservation "Pochválovská stráň" – "Hillside near Pochválov" (PS) lies in the elevation 486 meters a.s.l. with geographical coordinates 50°14'25"N and 13°49'19"E. Prominent west-oriented marlstone outcrops are moulded by regular slippages. The vegetation cover of the natural treeless area is formed mainly by *Anthericum liliago, Aquilegia vulgaris, Arctostaphylos uva-ursi, Aster amellus, Polygaloides chamaebuxus, Cephalantera damasonium, Dactylis glomerata* and *Sesleria albicans* (KLIKA 1950, MRZENOVÁ 1996). Soils of natural treeless areas in the reservation are mainly oligotrophic cambisols in the stage of pararendzin (MRZENOVÁ 1996).

The locality in The Natural Reservation "Milská stráň" – "Hillside near Milý" (MS) lies in the elevation 447 meters a.s.l. with geographical coordinates 50°14'14"N and 13°52'06"E. Soils of south-oriented marlstone outcrops are mainly oligotrophic cambisols in the stage of pararendzin (BYLINSKÝ 1996). The vegetation cover of the natural treeless area is formed mainly by *Aster amellus, Anemone sylvestris, Aquilegia vulgaris, Clematis recta* and *Polygaloides chamaebuxus* (BYLINSKÝ 1996).

The investigated mowed marlstone-substratum based grassland locality (MD) lies in the elevation 430 meters a.s.l. with geographical coordinates 50°10'02"N and 13°55'07"E. The vegetation cover is formed mainly by *Arrhenatherum elatius. Dactylis glomerata* and *Phleum pratense.*

The forest locality (FO) is situated in the immediate proximity of the natural treeless area at "Pochválovská stráň" about five meters from the marlstone outcrop. The elevation of the locality is 480 meters a.s.l. with geographical coordinates 50°14'25"N and 13°49'19"E. The vegetation cover is formed by *Fagus sylvatica, Carpinus betulus, Quercus* sp. and with the undergrowth of *Galium odoratum.*

Material and methods

A physiognomically uniform meter square plot was chosen in each of four investigated localities. Samples were taken randomly from the collection grid within quadrat from the horizon 0–5 cm. Eight samples from each locality were collected four times a year from December 1996 to September 1998. The samples were placed in sterile bags and transported to the laboratory for analysis. The soil pH was determined following the standard methodics (KRÁLOVÁ 1990) with the pH-meter Gryf 107.

Three separated methods for multiplication of soil algae were used:

1. Soil sample in the Erlenmeyer flask was overfilled with sterile liquid Bold Basal Medium (BISCHOFF & BOLD 1963). Algae from a mixture culture were determined after 2–8 weeks (GOLLERBACH & ŠTINA 1969).
2. Soil suspension was spread on the Petri dishes with BBM-agar. Algal microcolonies grown up after 3–10 weeks were isolated to the unialgal cultures (ETTL & GÄRTNER 1995).
3. Soil samples in Petri dishes were filled with distilled water. Algal growth on the coverslips placed on the surface of the soil was investigated after 2–10 weeks (DUNGER & FIEDLER 1989).

Diatom slides were prepared from all mixture algal samples as well as from diatom microcolonies. Organic matter was removed by soaking in hydrogen peroxide (H_2O_2). The frustules biomass was dried on a coverslip and mounted in Pleurax resin. Nondiatom taxa were identified by direct microscopical examinations. All organisms were studied using Laboval CARL ZEISS microscope. The standard cytological stains (Lugol's solution, cot-

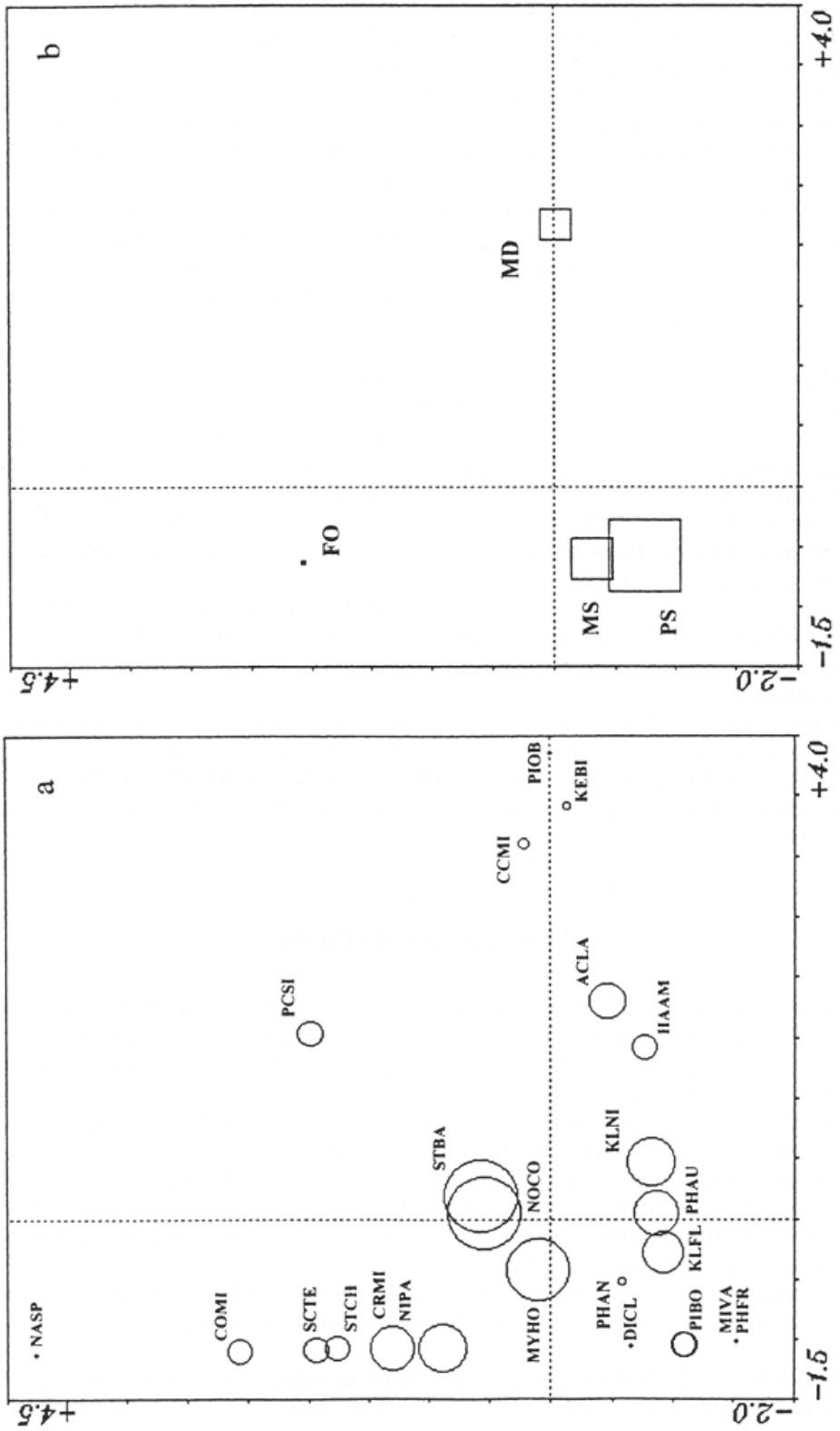

Fig. 1. **a** – DCA ordination diagram showing the frequency of species; **b** – DCA ordination diagram showing species richness of localities. The size of the symbols indicates the magnitude of variable. For description of abbreviations see Table 1.

ton blue, methylene blue, acetocarmine, nigrosine) were used. Identification was made using standard authoritative references (KONDRATĚVA 1968, STARMACH 1972, ETTL 1978, KOMÁREK & FOTT 1983, KRAMMER & LANGE-BERTALOT 1986, 1988, 1991a, 1991b, ETTL & GÄRTNER 1988, 1995, HINDÁK 1996, LOKHORST 1996). Some of the unialgal cultures obtained were deposited in the Collection of Algae of Charles University of Prague (CAUP). Statistical analysis was carried out using the programme CANOCO 3.1. (TER BRAAK 1991) and results of ordination were summarized using the programme CanoDraw (ŠMILAUER 1992). The detrended correspondence analysis (DCA) – a method based on the unimodal response model, was used (see JONGMANN et al. (1995) for detailed description).

Results

A total of 54 algal species representing 42 genera were found in investigated localities (Tab. 1.). Some widespread taxa were found in all sites. These included the cyanophyte *Nostoc commune*, the coccal green alga *Mychonastes homosphaera* and the green filamentous alga *Stichococcus bacillaris*. Other frequently occuring taxa include *Phormidium autumnale* f. *autumnale, Achnanthes lanceolata, Nitzschia palea, Chlorella minutissima, Klebsormidium flaccidum* and *Kl. nitens* (Fig. 1). Most of the identified taxa were relatively rare, with 33 of the 54 species appeared in the only one locality. Most of them are species that frequently occur in the soils of temperate ecosystems. Notable exceptions include the following taxa. The rare coccal eustigmatophyte alga *Vischeria punctata* has been previously found e.g. in forest soil near Fuorn (Switzerland) (ETTL & GÄRTNER 1995) (Fig. 2). The green flagellate *Chloromonas chlorogoniopsis* has not been described from the soil biotope yet. However, it has been reported occurring in a small water pools in Czech Republic (ETTL 1983). The coccal green alga *Muriellopsis pyrenigera* (Fig. 2) has been described from the soil of alpine zone in Tiroler Alps (REISIGL 1964). However, in investigated localities *M. pyrenigera* has occurred only once in the only one locality in a very small amount. The coccal green alga *Schizochlamydella minutissima* (Fig. 2) was described from antarctic soils (BROADY 1982). Because of it's small size and inconspicuous distinguishing features it could be widely confused e.g. with some *Chlorella* species (NEUSTUPA in prep.). *Chlorokybus atmophyticus* has been thought to be a very rare terrestrial alga. However, it seems to be wider distributed (GEITLER 1942, RIETH 1972, Ettl & GÄRTNER 1995, LUKEŠOVÁ, pers. com).

In all sites the green algae were the most frequently occuring group with highest species richness. However, cyanophytes and diatomes were significantly represented in all sites, too. Xanthophyceae and Eustigmatophyceae occurred in 3 of 4 studied localities. Results of DCA show the notable similarity of algal species composition between both natural treeless localities (Fig. 3).

In "Milská stráň" 22 species of algae and cyanophytes were found. Two filamentous species from the class Klebsormidiophyceae *Stichococcus bacillaris*

Table 1. Algal distribution in 4 marlstone-substratum based localities from the Nature park of Džbán, Central Bohemia, Czech Republic. Categories 1–8: number of species occurences in the samples from years 1996–1998.

Systematic list	Abbrev.	MS	PS	MD	FO
Cyanophyta					
Nostoc commune VAUCHER	NOCO	6	5	4	5
Phormidium angustissimum W. et G.S. WEST	PHAN	1			
Phormidium autumnale f. *autumnale* (AG.) GOM.	PHAU	7	8	3	
Phormidium frigidum FRITSCH	PHFR		7		
Microcoleus vaginatus (VAUCHER) GOM.	MIVA		6		
Bacillariophyceae					
Achnanthes lanceolata (BRÉB.) GRUNOW in CLEVE et GRUNOW	ACLA	2	2	8	
Eunotia praerupta EHR.	EUPR	3			
Luticola mutica (KÜTZ.) MANN	LUMU		6		
Navicula sp.	NASP				6
Pinnularia borealis EHR.	PIBO	7	8		
Pinnularia obscura KRASSKE	PIOB			4	
Nitzschia palea (KÜTZ.) W. SMITH	NIPA	7	6		8
Hantzschia amphyoxis (EHR.) GRUNOW in CLEVE et GRUNOW	HAAM		6	6	
Xanthophyceae					
Chloridella neglecta (PASCHER et GEITLER) PASCHER	CDNE	2	2		
Pleurochloris sp.	PLSP		4		
Chlorocloster minimus PASCHER	CLMI		6		
Botrydiopsis sp.	BOSP	6	6		
Xanthonema solidum (PASCHER) SILVA	XASO			7	
Tribonema minus (KLEBS) HAZEN	TRMI			3	
Heterococcus fuornensis VISCHER	HEFU	4			
Heterococcus sp.	HESP	3			
Eustigmatophyceae					
Eustigmatos vischeri HIBBERD	EGVI		7		
Vischeria punctata VISCHER	VIPU				3
Monodopsis subterraneus (J.B. PETERSEN) HIBBERD	MOSU		1		
Chlamydophyceae					
Chlamydomonas sp. 1	CHS1	3			
Chlamydomonas sp. 2	CHS2				5
Chloromonas chlorogoniopsis (ETTL) GERLOFF et ETTL in ETTL	CMCG				3
Chlorococcum cf. *elkhartiense* ARCHIBALD et BOLD	CCEK			5	
Chlorococcum ellipsoideum DEASON et BOLD	CCEL			1	
Chlorococcum minimum STARR	CCMI		6	8	1
Tetracystis texensis BROWN et BOLD	TETX			7	
Chlorophyceae					
Chlorella minutissima FOTT et NOVÁKOVÁ	CRMI	5	3		8
Mychonastes homosphaera (SKUJA) KALINA et PUNČ.	MYHO	8	7	2	3
Muriella terrestris J.B. PETERSEN	MUTE		1		
Muriellopsis pyrenigera REISIGL	MUPY			1	
Scotiellopsis terrestris (REISIGL) KALINA et PUNČ.	SCTE		2		3
Choricystis minor (SKUJA) FOTT	COMI	2	1		7
Pseudococcomyxa simplex (MAINX) FOTT	PCSI			5	4
Keratococcus bicaudatus (A. BRAUN) J.B. PETERSEN	KEBI		1	7	
Coccomyxa gloeobotrydiformis REISIGL	CMGL	7	7		
Schizochlamydella minutissima BROADY	SCMI		2		

Table 1. (continued).

Systematic list	Abbrev.	MS	PS	MD	FO
Dictyosphaerium chlorelloides (NAUMANN) KOMÁREK et PERMAN	DICL	5		4	
Neochloris sp.	NESP	5			
Geminella terricola J.B. PETERSEN	GETE		2		
Trebouxiophyceae					
Microthamnion kützingianum NÄGELI	MTKU			2	
Conjugatophyceae					
Cosmarium decedens (REINSCH) RACIBORSKI	CSDE		7		
Cosmarium notabile BRÉB.	CSNO		6		
Klebsormidiophyceae					
Chlorokybus atmophyticus GEITLER	CKAT		8		
Stichococcus bacillaris NÄGELI	STBA	8	7	8	8
Stichococcus chodatii (BIALOSUKNIA) HEERING	STCH		3		5
Stichococcus exiguus GERNECK	STEX		1		
Klebsormidium flaccidum (KÜTZ.) SILVA et al.	KLFL	8	8	2	
Klebsormidium mucosum (J.B. PETERSEN) LOKHORST	KLMU	3			
Klebsormidium nitens (MENEGHINI in KÜTZ.) LOKHORST	KLNI	4	8	5	

Explanation of abbreviations: MS – Natural reservation "Milská stráň", PS – National natural reservation "Pochválosvská stráň", MD – marlstone-substratum based secondary meadow, FO – marlstone-substratum based secondary forest.

and *Klebsormidium flaccidum* and coccal green alga *Mychonastes homosphaera* were occuring in all samples from this locality. *Phormidium autumnale* f. *autumnale, Pinnularia borealis* and *Nitzschia palea* were the most frequented species of cyanophytes and diatomes.

In "Pochválovská stráň" 33 species were found. Species of the class Klebsormidiophyceae were by far the most frequently occuring. There was a remarkable presence of *Chlorokybus atmophyticus* in all samples. Two species of Desmidiaceae were found in most samples. Other very frequently occuring groups were cyanophytes and diatoms. *Chlorocloster minimus, Botrydiopsis* sp. and *Eustigmatos vischeri* from classes Xanthophyceae and Eustigmatophyceae were also the typical components of algal flora from this locality.

Other two localities show different species composition (Fig. 3). Species list from mowed meadow contains 20 species. The diatom species *Achnanthes lanceolata* chlorococcal alga *Chlorococcum minimum* and the klebsormidiophyte species *Stichococcus bacillaris* occured in all samples. Coccal green algae from classes Chlamydophyceae and Chlorophyceae were in general the most frequent occuring in this locality. In broad-leaved forest locality only 145 species were found. The diatom species *Nitzschia palea*, chlorococcal alga *Chlorella minutissima* and the klebsormidiophyte *Stichococcus bacillaris* were occuring in all samples. The by far most frequent groups were the flagellate and coccal green algae from classes Chlamydophyceae and Chlorophyceae. From cyanophytes only *Nostoc commune*

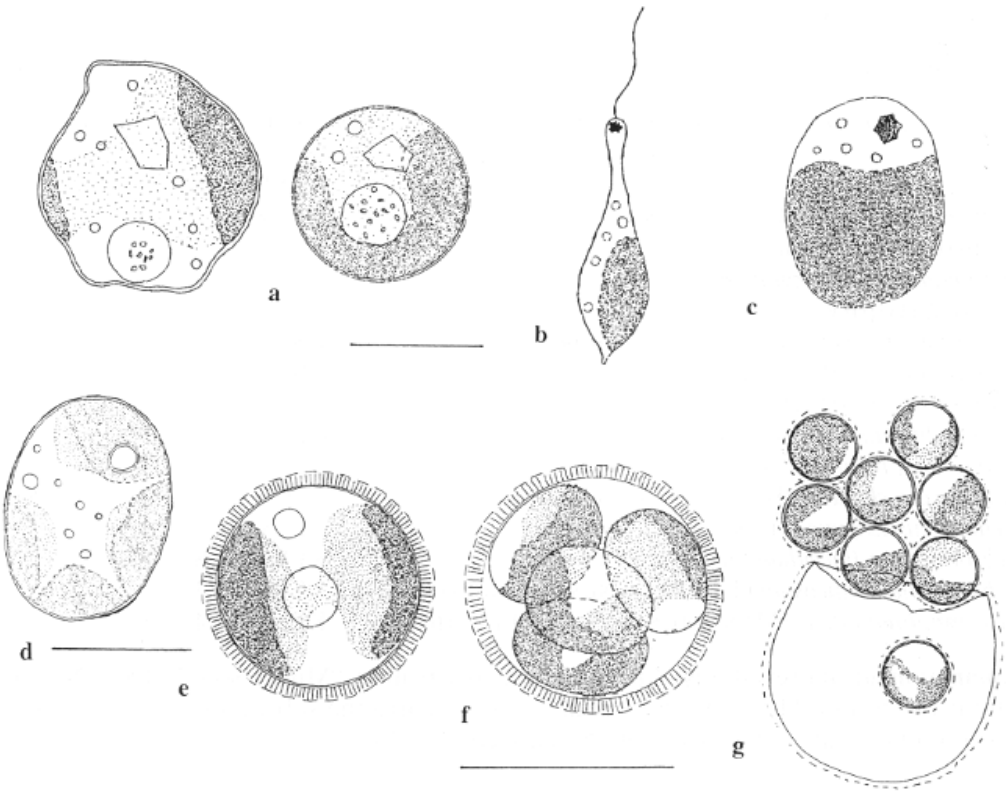

Fig. 2. **a, b, c** – *Vischeria punctata,* **a** – vegetative cells, **b** – uniflagellate zoospore, **c** – the cell immediately after the loss of the flagella, **d** – *Muriellopsis pyrenigera,* vegetative cell, **e, f, g** – *Schizochlamydella minutissima,* **e** – vegetative cell, **f** – production of autospores, **g** – release of autospores, the empty cell wall of a pot-like shape. [Bar = 5 μm.]

was found. Xanthophyceae were completely missing, but there was an eustigmatophyte species *Vischeria punctata* characteristic for this site.

The pH in 3 studied localities shows neutral to weakly alkalic soil reaction (MS – mean pH 6,53; PS – mean pH 7,35; FO – mean pH 6,95). The pH of the meadow locality is weakly acidic (mean pH 5,49) (Tab. 2).

Discussion

In all of the studied sites the green algae formed a dominant part of soil algal species composition. The similarity of species composition between both spatially distant natural treeless localities, shown on the DCA ordination diagram (Fig. 3), indicates the possible influence of the very special biotope type on soil algal flora. There was detected the comparative higher frequency of filamentous cyanophytes (e.g. *Phormidium autumnale* f. *autumnale, Ph. frigidum, Microcoleus vaginatus*) and some common terrestrial diatomes (e.g. *Pinnularia borealis, Luticola mutica, Eunotia praerupta*) against the green flagellates in these localities. That

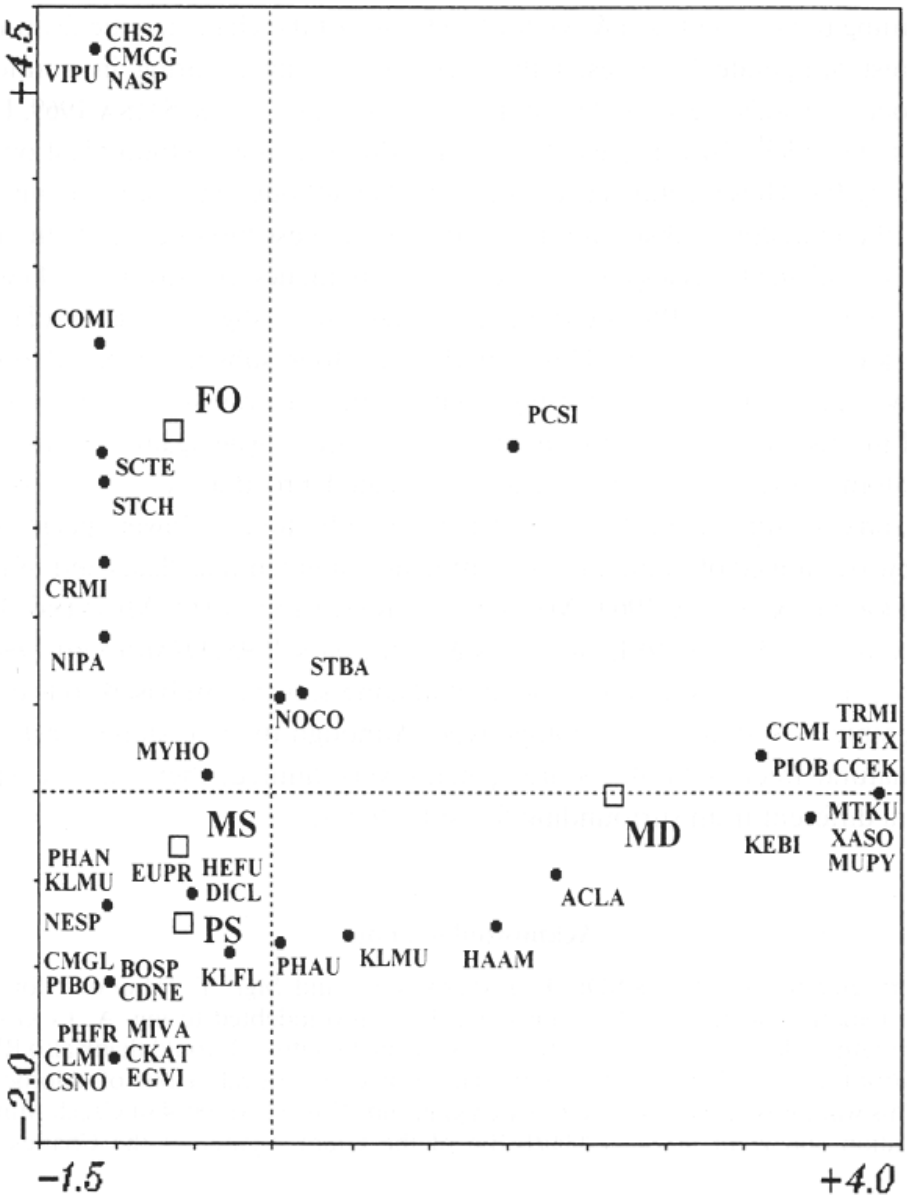

Fig. 3. DCA ordination diagram showing position of localities (symbol □) and species (symbol •) (For description of abbreviations see Table 1).

Table 2. Soil pH in single samples.

Localities	13.12.96	15.4.97	15.7.97	20.10.97	30.12.97	8.3.98	15.6.98	9.9.98
MS	6,93	5,88	7,68	5,58	6,81	4,99	6,92	7,45
PS	7,29	7,95	7,71	6,23	7,80	7,02	6,88	7,92
MD	5,80	4,91	5,88	4,41	5,92	5,84	5,01	6,12
FO	7,52	7,09	7,73	6,18	6,67	6,96	6,31	7,11

is according to GOLLERBACH & ŠTINA (1969) one of the characteristic features of non-forest temperate biotopes. Other species typicaly occuring in non-forest phytocoenoses soils are some Desmidiaceae (GOLLERBACH & ŠTINA 1969, ETTL & GÄRTNER 1995). Two species of the genus *Cosmarium* were found just only in the locality PS. These features could indicate that although they are spatially very limited the marlstone-substratum based natural treeless biotopes share the non-forest-like soil algal species composition. High frequency and diversity of cyanophytes in the locality PS could be explained by a slightly higher pH, too (GOLLERBACH & ŠTINA 1969). However, the marlstone-substratum based mowed meadow locality differs considerably from natural treeless sites. Slightly lower soil pH in this locality favourises mainly chlorococcal algae against cyanophytes and diatomes (METTING 1981). The investigated broad-leaved forest locality shares some features typical for forest temperate biotopes - lower species richness, low occurence of cyanophytes, dominance of green flagellates and unicells (GOLLERBACH & ŠTINA 1969, METTING & RAYBURNE 1979, METTING 1981, ALEKSACHINA & ŠTINA 1984, GRONDIN & JOHANSEN 1995, DĚMČENKO 1998).

The species composition of algae in marlstone-substratum based soils differs considerably according to the biotope type. Although the marlstone-substratum based natural treeless localities are spatially very limited, they share a typical algoflora different from surrounding forest biotopes.

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