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Vedoucí práce: Doc. RNDr. Jiří Komárek, DrSc. do World Algae play

the most important role as the primary coducers. The majority of whole biomass pro-

ing. Alena Lukešová

Prohlašuji, že jsem uvedenou práci vypracoval samostatně, pouze s použitím uvedené literatury.

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Soil algae of the South Moravian saline habitats

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With 3 figures and 2 tables in text

Abstract: The investigations were carried out in the area of South Moravian saline habitats. In five localities, soil samples were taken from April till December 1994 and algal diversity was studied; soil moisture, pH and conductivity value were measured in each sampling occasion. The data obtained were compared with the records from the past and the present state of South Moravian saline habitats was recognised. In total, 69 species of algae were found, 198 algal strains were isolated (including ecomorphotypes) and cultivated in unialgal cultures. The typical halophilous microflora almost disappeared. Several halophilic (Surirella ovata, Navicula veneta) and halotolerant - euryhaline (Hantzschia amphioxys) diatoms were recorded from the Nesyt saline habitat. The new species of Cyanosarcina (Cyanoprocaryota, Chroococcales, Chroococcaceae) was found on the western bank of the Výtopa pond. The Nesyt saline area seems to be the best conserved locality.

Key words: Soil algae, Cyanoprocaryota, Bacillariophyceae, Xanthophyceae, Chlorophyceae, salinity, South Moravia.

Introduction

Algal occurrence has been recorded from all biotopes all over the world. Algae play the most important role as the primary producers. The majority of whole biomass production on the Earth is covered by algae, especially by marine picoplanktic cyanoprokaryotes of the genera *Cyanobium* and *Synechococcus*. Besides aquatile, the soil algae possess a special group of algae from which are the cyanoprokaryotes adapted to survive under very extreme conditions such as hot (Sahara) or cold (Antarctic) deserts. They occupy in such habitats as surfaces of rocks which are strongly irradiated (POTTS & FRIEDMANN 1981, CAMPBELL 1979) as well as the depth of a half metre under the upper layer of soils or in caves where no radiation is detectable (FRIEDMANN

1955). Soil algae are the essential component of all terrestrial ecosystems. They play an important role as the nitrogen fixers, the improvers of soil structure (METTING 1981) and also as the anti-erosive elements (especially filamentous blue-greens). In extreme conditions, they are the only primary producers of the biomass and thus serve as the only diet for invertebrates. But the structure of all algal communities is always influenced by soil type, climatic conditions and closely related to the structure of vascular plant population in the locality.

In South Moravia, the saline habitats represent important refugia for the halophilous vegetation. Due to extreme agricultural utilisation (treatment with herbicides and fertilizers) and changes in water management, most of the traditional localities become dry (Kobylské lake) and / or flooded by artificial reservoirs (Nové Mlýny reservoirs).

The first floristic survey from the South Moravian saline biotopes was given by Nave (Nave 1864), special investigations with attention to diatomacean flora were provided by Richter (RICHTER 1912), Fischer (FISCHER 1920) and especially by Bílý (Bílý 1926, 1927, 1929). The only recent work concerning with this problem is that one by Skácelová and Marvan (SKÁCELOVÁ & MARVAN 1991). All these papers deal with the algal flora in aquatic habitats (epipelic, epiphytic species). But there were not any communities of soil algae revised till today from this area and this work gets the pioneer data about this problem.

The aim of this study was to revise the present state of photoautotrophic soil microflora occurring in five typical saline habitats situated in South Moravia. Localities were chosen according to the findings of Bílý, who described well the vegetation of diatoms (Bacillariophyta) in the majority of saline localities in South Moravia in the years 1920 - 45.

Materials and methods

Site description

All sites are situated in South Moravia, in the area with mean annual precipitation of 571 mm (357 mm in the vegetation period), and a mean annual temperature of 9.1 °C (15.5 °C in the vegetation period, -1.3 °C in the January, 19.3 °C in the July) (meteorological station in Mikulov, VESECKÝ et al. 1961). The bedrock is formed by neogene deposites, the lower torton (BUDAY 1963).

The soils of following localities representing the main types of South Moravian saline habitats were studied:

Nesyt

A typical saline meadow with well conserved halophilous macroflora (*Aster tripolium ssp. pannonicus*, *Suaeda maritima*, *Spergularia salina*, *Juncus gerardii*, *Plantago maritima*, *Samolus valerandii*). This site have been the government protected area since 1961 and is situated near the village of Sedlec (48° 54' N, 16° 42' E, 177.2 m above see level).

Výtopa pond

A small pond situated near the village of Valtice, without inflow but connected with the Nesyt pond (48° 47' N, 16° 45' E, 173 m above see level). Halophilic macroflora recorded on the southern bank of the pond (DANIHELKA et al. 1995).

Novosedly

A meadow seasonally inundated by small pond situated on government protected area The Saline Habitat of Novosedly (48° 50' N, 16° 30' E, 167 m above see level). Dominant vascular plant is Poa annua (covers 90 % of the site). The site is heavily influenced by grazing.

Dobré Pole

The area of the saline habitat of Dobré Pole (48° 49' N, 16° 32' E, 184 m above see level) consists of a little pond with low outflow, surrounded by Typha angustifolia and a meadow lying nearby where the saline macroflora were occured.

Nový Přerov

The site is situated near originally large pond on the north of the village Nový Přerov (48° 48' N, 16° 29' E, 180 m above see level). Today is completely drained and surrounded by fields with high developed agriculture management.

Sampling and soil properties

Sampling was provided monthly during the period of April - December 1994. Soil samples were selected at random out of 50 marked out plots and taken by sterile metal trowel from the layer 0 - 10 cm. Soil was transported to the laboratory in PET bags and the moisture was immediately measured gravimetrically as a dry-wet weight ratio. Five samples from each locality were mixed, then passed through a 2 mm sieve and stored in a dark dry place, each sample in its own paper sack. In each sample

occasion conductivity by MA 5950 conductometer and pH value by pH meter T3001 LC following Valla (VALLA et al. 1980) were measured.

Species composition

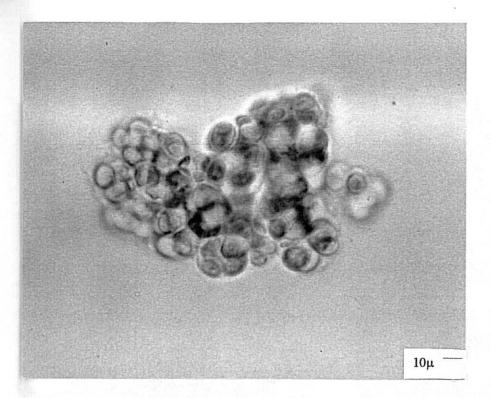
Because of the fact that the majority of soil algae occur in low numbers and \ or in dormant stage in dry soils it is mainly impossible to identify them correctly using direct microscope observation. Thus it is necessary to cultivate and isolate them and to study their life cycles in culture. Beside the direct microscopy, the algal species composition was studied with help of these two cultivation methods:

- 1. Ten grams of a dry soil sample were resuspended in 90 ml sterile water and ultrasonically homogenised for 4 minutes. Then four dilutions (10⁻¹ 10⁻⁴) of soil sample were inoculated on Petri Dishes with Basal Bold's Medium (BBM) (BISCHOFF & BOLD 1963) solidified by 1.5 % agar. Algal colonies were examined microscopically after 3-5 week incubation in stable conditions of 20+-2 °C illuminated by homogeneous cool light of 5.5 W.m⁻² under diurnal cycle 18 : 6 hours L : D. All procedures were carried out in a duplicate.
- 2. The modified "growth slides method" according to Lund (LUND 1945): Soil was placed into the Petri Dishes and covered by sterile coverglasses. Incubation was provided at 20+-2 °C by day light, soil was periodically moistened by sterile water. This method enables evaluation of algal populations in more-less natural conditions up to three months.

For correct algal species identification, determination keys by Starmach (STARMACH 1966), Ettl (ETTL 1978, 1983), Ettl and Gartner (ETTL & GARTNER 1988), Hindák (HINDÁK et al. 1978), Krammer and Lange-Bertalot (KRAMMER & LANGE-BERTALOT 1988, 1991a, 1991b) and original papers by Bílý (Bílý 1926, 1929), Komárek and Anagnostidis (KOMÁREK & ANAGNOSTIDIS 1986, 1989) Anagnostidis and Komárek (ANAGNOSTIDIS & KOMÁREK 1988) and Komárek (KOMÁREK 1993) were used.

Cultures

All algal strains were maintained in test tubes covered by cotton wool stopper in the artificial nutrient solution BBM solidified by 1.5% agar. Cultivation was proceeded at laboratory temperature 20 °C +- 2 °C, in a diurnal cycle of 18 : 6 hours L : D under



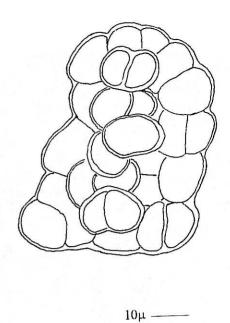


Figure 1. Cyanosarcina sp. nov., orig. KAFTAN

Phillips cool light fluorescence tubes (5.5 W.m⁻²). Unialgal cultures were recovered periodically into the fresh medium four times per year.

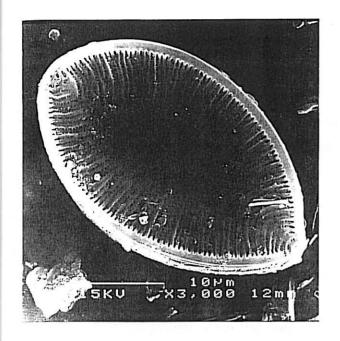
Microscopy

Periodical screening of algal populations in all localities was provided by light microscope MEOPTA PD 37 BN, for special examinations scanning electron microscopy (SEM) or transmission electron microscopy (TEM) were used. Sample of the *Cyanosarcina* was rinsed out of glutaraldehyde fixation solution, then mounted for TEM following Cepák & Lukavský (CEPÁK & Lukavský 1994) and fixed on formvar grids. For determination of diatoms, soil suspension was cleaned of organic matter by 30% H₂O₂ and attached to stubs for SEM. Material prepared for electron microscopy was examined with JEOL 6300 field emission scanning electron microscope, for TEM, JEOL JEM 100B transmission electron microscope was used.

Results

Soil properties

In each soil sample, conductivity, moisture and pH value were measured and obtained data are given in table 1. All localities show alkaline pH (thus clearly corresponds with the character of the bedrock - low permeable base clay) and relatively high valu-



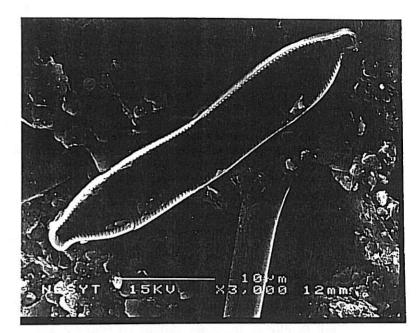


Figure 2. Surirella ovata KUTZ., orig. KAFTAN

Figure 3. Hantzschia amphioxys (EHRENB) GRUN., orig. KAFTAN

es of conductivity (in all sites more than 1 mS.cm⁻³). From the Nesyt saline area the highest content of free soluble ions was recorded (the maximum conductivity of 2.430mS.cm⁻³ was recorded). The salinity of the sites is conditional on the presence of the water soluble ions of salts especially of the Na₂SO₄, MgSO₄, NaCl and KCl (PELÍŠEK 1948).

Table. 1. Soil properties

	pH(H₂O)		conductiv	moisture(%)		
	x1	SD	x^1	SD	x ¹	SD
Nesyt	8.42	0.1	2.240	0.056	21.5	11.2
Výtopa	8.35	0.16	1.248	0.068	25.3	12.3
Novosedly	8.37	0.29	1.528	0.117	18.6	8.5
Dobré Pole	8.33	0.06	1.125	0.127	20.9	10.1
Nový Přerov	8.35	0.12	1.350	0.096	19.5	11.9

^{1 .} each value is a mean of 6 measurements.

Species composition

In total, 69 species of algae were found in the five chosen localities during the year 1994 (Tab. 2.). The species with the highest occurence frequency were those of *Bracteacoccus sp.*, *Chlorella sp.*, *Hantzschia amphioxys*, *Heterothrix sp.*, *Leptolyngbya sp.*, *Nostoc sp.* and *Phormidium autumnale*. In all stages Chlorophyceae prevailed, followed by Cyanoprokaryota. Only two halophilic diatoms were recorded: *Navicula ve-*

neta and Surirella ovata. By the Výtopa-pond shore a new species of Cyanosarcina (Cyanoprokaryota, Chroococcales, Chroococcaceae) probably halophilic (KAFTAN in prep.), was found.

The greatest algal diversity was found in the Nesyt saline area (46 species), followed by Výtopa (29 species), Novosedly (23 species), Dobré Pole (21 species) and Nový Přerov (20 species).

Seasonal changes

Two peaks of algal abundance were observed in spring and autumn because of relatively wet weather. There was comparatively a little more insolation which limited algal growth when the surface of soil was shaded by higher vascular plants in the middle of the vegetation season. There was recorded increase of biomass of cyanoprocaryotes (*Anabaena sp.*, *Phormidium sp.*, *Nostoc sp.*, *Trichormus sp.*) in that periods related with development of algal crusts which completely covered the whole soil surface. In some cases small water bodies persisted for a week or longer promoted formation of diatom-crusts (*Navicula sp. div.*, *Nitzschia sp. div.*). But any phenomenon of periodically changed dominants, which is a stable feature in all aquatic habitats was not found.

Discussion

A plenty of algologists have been interested in halophilous algae since a long time ago. Especially Bílý contributed with his papers concerning to diatomacean flora (Bacillariophyta) to the knowledge about the photoautotrophic microflora in South Moravia. From his studies follows, that the South Moravia was rich for saline habitats in the past and a great number of halophilic species occurred there.

All acquired data point out, that there is not any area without a strong human impact. Only a few sites persisted with just small changes in structure of flora and natural conditions and saved its character till today. There are especially those of them, which have been protected by government for many years. But even so the halophilic macro- and microflora is wasting in the last decades due to changed water management in the region. In the past, the whole area was supplied by water from natural springs and thanks to the high evaporisation, the soils become salt. Today, South Moravia is rinsed by the Dyje river, which has a much lower content of salts. Unnegli-

gible factor, which heavily influences the soil properties is agriculture management. The efforts for extreme utilisation of the arable land caused irreversible changes in the soil quality and the character of soil microflora above all.

One of the localities, which is well conserved (in comparison with the other ones) is the Nesyt saline area. This site is distinguishable by relatively high content of dissolved salts and thus high pH value (a base reaction of the water holded in soil is caused by alcaline character of the bedrock primarily). The species composition shows occurrence of some originally described halophilic diatoms (*Navicula veneta*, *Surrirela ovata*), but those are not typical soil algae. The effect of influencing by aquatic communities of algae was recorded from all sites, where the investigations were carried out (*Amphora sp.* in Nesyt, *Scenedesmus sp.* in Novosedly etc.).

Conventionally, with increase of ecological disturbance, the species diversity is declined and the number of stenotopic species (the specialists) raising. The unique conditions in the saline habitats enable to survive those of the algae, which would probably disappear in competition with the euryhaline ones. Therefore it is necessary to propose for the efficient conservation not the algal species separately but the whole habitats and regions as soon as possible.

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Appendix

Table 2. The list of algal species found during the year 1994. Ne...Nesyt, Vy...Výtopa pond, No...Novosedly, Do...Dobré Pole, Np...Nový Přerov

Cyanoprokaryota	1	Ve	Vy	No	Do	Np
Anabaena sp.						
Aphanothece sp.	+		+	+		•
Cyanosarcina sp. nov.	+		Ä	-	-	2
Cyanosarcina sp.1.	9		+	V.	-	-
Cyanosarcina sp.2.	+		+	+		()1
Cylindrospermum sp.	-		-	+	-	-
Geitlerinema sp.	+		+	(50)		-
Leptolyngbya boryana Gom.	+		=		=33	=
Leptolyngbya sp. div.	+		+	-	-	_
Nostoc calcicola BREB	+		+	+	+	+
Nostoc edaphicum Kond	(30)			-	-	+
Nostoc punctiforme (Kutz.) Hariot	+			-	-	+
Nostoc sp.	+	15			()	
Nodularia sp.	*		-	+ .	[*]	+
Oscillatoria sp.		2.5		+	•	•
Phormidium autumnale (Ag.) Gom	+	-		-	_	+
Phormidium sp.	+	4		+	+	+
Pseudanabaena sp.	-	+		+	+	+
Pseudophormidium sp.	<u>-</u>	+		-	+	-
Trichormus sp.	+			-	-	+
Bacillariophyceae						
Amphora sp.	+	-		~6		
Aulacoseira sp.	_	-		+ .		
Bacillaria sp.	-	+	- 1		- -	
Caloneis silicula (EHRENB.) CL.	+	2			i Ha	
Hantzschia amphioxis (EHRENB.) GRUN.	+	+	4			
Navicula atomus (NAG.) GRUN.	+	. 2		: + : -		
Navicula contenta GRUN.	+	-				
Navicula veneta Kutz	+	-	4			
Navicula mutica Kutz.	+	-	_		+	
Navicula sp. div.	+	+	+		+	
Nitzschia gisela Lange-Bert.	+	2	_	_		
Nitzschia sp. div.	+	+	+	+	+	
Surirella sp.	-	+	_	_	18 18 <u>2</u> 1	
Surirella ovata Kutz.	+	4	823	_	100	
Tabellaria flocculosa (Rотн.) Китz.	+	-	7			
Xanthophyceae						
Botrydiopsis sp.	+					
Heterothrix sp. div.	+		-	-	-	
Heterothrix cf. debilis VISCH.	-	- +	+	+	281	
Heterococcus sp.	+	0 5 7	-		+	
Tribonema sp.	+	2	+	() -	+	
	20.00	-		800	200	

Chlorophysia		Vy	No	Do	Np
Chlorophyceae					
Bracteacoccus of. minor (CHOD.) PETROVA	-	+		_	_
Bracteacoccus sp.	+	+	-	+	+
Chlamydomonas sp.	+	+	+	-	-
Chlorella minutissima Fott et Novak.	-	-	-	-	-
Chlorella sp.	+	+	+	+	+
Chlorococcum sp.	+	+	_	_	-
Chlorolobion sp.	-	4	-		-
Chlorosarcinopsis sp.	+	-	+	_	-
Closterium sp.	+	+	-	_	_
Coccomyxa sp.	(=)	_	_	+	-
Coenochloris sp.	+	2	2	* =	1
Coenocystis sp.	+	2	2	_	+
Dictyosphaerium chlorelloides (NAUM.) KOM. et PERM.	-	<u> </u>	-	+	
Diplosphaera sp.	+	+	+	+	
Ecdysichlamys sp.	_	+	-		-
Hassalia sp.	+			+	_
Klebsormidium crenulatum Lokhorst	⊕ <u>≃</u>		_	+	_
Klebsormidium flaccidum (Kutz.) Silva, Matt. et Black.	+			<i>y</i> +	+
Myrmecia cf. bisecta Reisigl.	-	7.	_	+	-
Neochloris sp.	+	+		_	_
Pleurothamnion sp.		+	_	_	_
Pseudococcomyxa simplex (MAINX.) FOTT		141	+	14 8	20
Pseudococcomyxa sp.	+	_	-	_	2
Scenedesmus sp.	-	_	+		-
Scotiellopsis sp.	+	+	-	-	_
Stichococcus bacillaris NAG	+		-	+	2
Stigeoclonium sp.	:=:	(4 1)	+		+
Tetracystis sp.	+	-	-	2	2
Tolypothrix sp.	+	-	=		:= :=
Eustigmatophyceae					
Pleurochloris sp.	-	<u>.</u>	Ē	+	-