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HALOALKALIPHILIC SULFUR-OXIDIZING BACTERIA: TAXONOMY, PHYSIOLOGY AND BIOTECHNOLOGICAL APPLICATIONS

Horia BANCIU[1]

Abstract. Soda lakes and desert represent extreme environments located only in a few remote areas on Earth. Despite the harsh conditions, these habitats abound with creatures that are perfectly adapted to the environmental challenges. Natural cycling of elements is present here, indicating a relatively complex and complete community of haloalkaliphilic organisms. Among the major elements, sulfur and its organic and inorganic compounds are used by many haloalkaliphilic microbes as energy source and electron donor. Three genera of chemolithoautotrophic sulfur-oxidizing bacteria (SOB) capable of growing optimally at high salt concentrations and at alkaline pH have been described. They belong to Gamma-Proteobacteria and classified as *Thioalkalimicrobium, Thioalkalivibrio* and *Thioalkalispira* sp. These bacteria have been isolated in soda lakes on three different continents: (North) America, Africa and Asia. Based on the phylogenetic tree, *Thioalkalimicrobium* group is closer related to neutrophilic SOB of the *Thiomicrospira* genera than to its haloalkaliphilic counterpart, *Thioalkalrvibrio.* The latter is closely related to halophilic anaerobic photosynthetic bacteria of the *Ectothiorhodospira* and *Halorhodospira* (Sorokin et al., 2001, Imhoff and Süling, 1996). *Thioalkalimicrobium* group is more homogenous (3 species described to date) while *Thioalkalivibrio* comprises 8 known species. There have been described many morphological, metabolic and structural differences between the two genera. Their capacity of using inorganic sulfur compounds as energy source, altogether with the features that allow them to cope high salinity and alkalinity, make these organisms of interest for biotechnology applications.
Keywords: alkaline, bacteria, electron donor, growth kinetics, soda, sulfur oxidation, trona

Introduction

Haloalkaliphilic bacteria represent an ecologic class of extremophilic microorganisms that require both high (sodium) salts concentration and high alkalinity in their surroundings. These special needs for optimal growth and multiplication mean that these organisms are double extremophiles, a rare property among the living creatures. Special conditions fulfilling the requirements of haloalkaliphiles exist m few places on Earth and they are mostly located in remote areas like Central Siberia, Mongolia, deserted areas of North America and the Eastern African Rift Valley **(Figure 1)**. In the last decade, haloalkaline („soda") lakes have caught the attention of researchers not only for their industrial potential (salt extraction) but also for the interesting life conditions they offer. Some paleobiologists believe that the archaic environments where life sprouted might have looked like the present soda lakes (Kempe and Degens, 1985). Nonetheless, the study of haloalkaliphiles themselves may bring valuable information on the structural and metabolic adaptation to extreme conditions, making such organisms attractive to biotechnology applications. The present paper reviews the most recent knowledge on the taxonomical and metabolic diversity among a specialized group of haloalkaliphiles, namely those capable of utilizing inorganic sulfur compounds as energy source.

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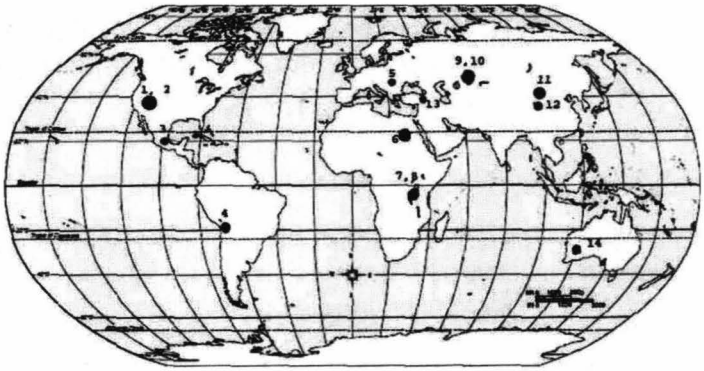

Fig. 1: Worldwide distribution of several soda lakes and deserts (adapted after Baumgarte, 2003).
Legend: 1, 2 – Mono and Alkali Lake (California, U.S.A.); 3 – Texcoco Lake (Mexic); 4 – Region Antofagasta (Chile); 5 – Fehér Lake (Hungary); 6 – Wadi el Natrun (Egypt); 7, 8 – Magadi and Bogoria Lakes (Kenya), Natron Lake (Tanzania); 9,10 – Kulunda steppe, Tuva Lake (Siberia, Russia); 11 – Lakes in Central Mongolia; 12 – Qinhghai Hu Lake (China); 13 – Van Lake (Turkey); 14 – Chidnup Lake (Australia)

Challenges of haloalkaline environments

The neutral saline lakes (pH 6-8) contain NaCl as the major salt and their buffering capacity is low. On the other hand, the alkaline saline (soda) lakes (pH 9-11) are characterized by the presence of large amounts of sodium carbonates (Na_2CO_3 + $NaHCO_3$) that confer the water a high buffering capacity. Naturally occurring alkalinity is usually associated with salinity (Grant and Tindall, 1986; Imhoff et al., 1979, Oren, 2002). Other major ions found in salt lakes are Mg^{2+}, Ca^{2+} and K^+ as cations, and $S0_4^{2-}$ and Br^- as anions. In the soda lakes, one of the major chemical characteristics is the lack of solubilized divalent cations (Mg^{2+}, Ca^{2+}) due to their strong tendency of precipitation as carbonates under alkaline conditions. The removal of divalent cation carbonates drives the solubilization of sodium or potassium carbonates, thus increasing the monovalent cation concentration. In this way brines are formed. In some parts of the world, the shallow lakes may end as a layer of solid rock (trona-crystalline sodium sesquihydrate, Na_2CO_3•$NaHCO_3$•$2H_2O$) by evaporation during dry seasons. A broad range of intermediate saline and/or alkaline lakes occur by the mixing of the minerals in various ratios.

Natural cycling of elements (C, O, H, N, S, P) and microelements (Zn, Fe, Cu, Mn, Se etc.) occurs in haloalkaline environments proving the existence of a relatively broad spectrum of microorganisms capable of metabolizing various organic and inorganic compounds (Zavarzin et al., 1999). Famous soda lakes as those in the African Rift Valley teem with microscopic life despite the harsh conditions: high pH, high soda concentration and high temperatures. Consistent cyanobacterial biomass is formed seasonally, to the benefit of large flamingo colonies (Krienitz et al., 2003). During the dry seasons, in the soda lakes the salts become more concentrated and the solubilized oxygen scarcer. Phototrophic anoxygenic bacteria replace most of cyanobacteria, keeping the natural cycling of elements at reasonable level. Haloalkaliphilic phototrophic anoxygenic bacteria are represented mainly by purple sulfur bacteria of the genera *Ectothiorhodospira* and *Halorhodospira* (Oren, 2002). They use

inorganic sulfur compounds like sulfide and elemental sulfur as electron donor for C fixation and for growth. The organic compounds are further mineralized through biological processes catalyzed aerobically by halophilic heterotrophic bacteria of the genus *Halomonas* (Duckworth et al., 1996). The final step in the organic matter degradation occurs primarily under anaerobic conditions. In principle all major metabolic group are represented among the halophiles (Zavarzin et al., 1999). At extreme salt concentrations (>150 g/l) several physiological groups of organisms could not be found: autotrophic methanogens, acetoclastic methanogens, dissimilatory sulfate-reducers that perform the complete oxidation of their substrate and autotrophic ammonia and nitrite oxidizers. According to Oren (1999) the reason for the absence of these nutritional groups at hypersaline conditions might be the bioenergetic constraints. It is speculated that since these organisms perform a less energetically efficient metabolism, they could not sustain an energetically expensive osmoregulation.

Sulfur-oxidizing bacteria (SOB)

Sulfur is one of the most important elements for sustaining life on Earth. The sulfur chemistry is complicated by the many oxidation states sulfur can assume **(Table 1).** Geochemically, sulfur is very abundant and several sources of production, emission or storage can be identified: volcanic activity, biogenic emission from decaying biomass on land and from the ocean, man activities and various sulfur storage products (gypsum, metal sulfides, etc). The biochemical significance of sulfur is tremendous. The origin of life has been linked with iron sulfide (pyrite) that becomes catalytically active at elevated temperature and at high pressures (Wächtershäuser, 1988; Martin and Russell, 2003). Sulfur plays a catalytical role in the iron-sulfur clusters within respiratory enzymes. Sulfur containing aminoacids (cysteine, cystine and methionine), sulfolipids, and many co-enzymes (glutathione, coenzyme A, biotin, lipoic acid) are essential for cell metabolism.

Table 1. Oxidation states of sulfur in common compounds (after Steudel, 2000)

Oxidation state	Compounds
-2	Dihydrogen sulfide H_2S, hydrogen sulfide ion HS^-, sulfide ion S^{2-} as in FeS; thiocyanate SCN^-
-1	Disulfane H_2S_2; disulfide S_2^{2-} as in pyrite FeS_2; thiosulfate sulfane S^{1-}; polysulfides $^-S(S)_nS^-$
0	Elemental sulfur S_n; organic polysulfanes R-S_n-R; polythionates $^-O_3S(S)_nSO_3^-$
+1	Dichlorodisulfane Cl-S-S-Cl
+2	Sulfur dichloride SCl_2; sulfoxylate SO_2^{2-}
+3	Dithionite $S_2O_4^{2-}$
+4	Sulfur dioxide SO_2; sulfite SO_3; bisulfite HSO_3^-
+5	Dithionate $S_2O_6^{2-}$; sulfonate RSO_3^-; thiosulfate sulfone SO_3^{5+}
+6	Sulfur trioxide SO_3; sulfate SO_4^{2-}; peroxosulfate SO_5^{2-}

Table 2. Categories of SOB

Category	Metabolic type	Location	S compound used as electron	Representatives
Green sulfur bacteria	Anaerobic photolithoautotrophs	Mud and anoxic water	H_2S, S^0	*Chlorobium*
Purple sulfur bacteria	Anaerobic or microaerophilic (photo)lithoautotrophs	Anoxic water, above green sulfur bacteria	H_2S, $S203^{2-}$ S^0	*Chromatium, Rhodospirillum, Rhodobacter, Thiospirillum, Thiocapsa, Ectothiorhodospira, Halorhodospira*
Obligate autotrophic colorless sulfur bacteria	Aerobic and anaerobic obligate chemolfthoautotrophs	Soil, sediments, oxic/anoxic interfaces of water, sulfur springs and other volcanic sources	H_2S, metal sulfides, $S_2O_3^{2-}$, S^0, $S_3O_6^{2-}$ $S_4O_6^{2-}$	*Thiobacillus thioparus, Thermithiobacillus tepidarius, Acidithiobacillus thiooxidans, Acidithiobacillus ferrooxidans, Halothiobacillus neapolitanus, Halothiobacillus halophilus, Thiomicrospira pelophila*
Facultatively autotrophic colorless sulfur bacteria	Aerobic and anaerobic facultative chemoautorophs		H_2S, metal sulfides, $S_2O_3^{2-}$, S^O, $S_4O_6^{2-}$	*Starkeya novella, Thiobacillus aquaesulis, Thiomicrospira thyasirae, Paracoccus denitrificans, Pwacoccus versutus; Morphologically conspicuous bacteria as Beggiatoa, Thiothrix, Thioploca, Achromatium, Macromonas, Thiobacterium, Thiospira, Thiomargaritha*

The electrons derived from sulfur oxidation are used by aerobic chemotrophic Archaea and Bacteria for energy transformation of the respiratory chain and for autotrophic carbon dioxide reduction. Anaerobic phototrophic bacteria use light energy to transfer electrons from sulfur or other sources for autotrophic carbon dioxide reduction. Aerobic sulfur oxidation of Archaea is restricted to members of the thermoacidophilic Sulfolobales. In the domain Bacteria, sulfur is oxidized by aerobic chemotrophic (Friedrich et al., 2001: Kelly et al., 1997) and anaerobic phototrophic bacteria (Brune, 1995).

Chemolithoautotrophic sulfur bacteria are phylogenetically and physiologically diverse and are alkaliphilic (Sorokin et al., 2000; Banciu et al., 2004 a, b), neutrophilic or acidophilic (Friedrich et al., 2001: Kelly et al., 1997, Pronk et al., 1990; Robertson and Kuenen, 1992). Also, phototrophic sulfar-oxidizing baeteria are phylogenetically diverse and are mostly mesotrophic and neutrophilic (Brune, 1995) (Table 2).

Taxonomy and morphology of the obligately chemolithoautotrophic, alkaliphilic SOB from soda lakes

The biology of inorganic sulfur oxidation was well documented in neutral (Kuenen et al., 1975; Kuenen and Beudeker, 1982) and acidic conditions (Harrison, 1984). In the neutral hypersaline environments purple sulfur bacteria use light as energy source and inorganic sulfur compounds (H_2S, S^O, $S_2O_3^{2-}$) as electron donors. The inorganic sulfur oxidation by obligate haloalkaliphilic chemolithoautotrophs was only recently discovered and investigated. The autotrophic SOB bacteria capable of oxidation of inorganic sulfur compounds at moderate to high salt concentration and at high pH can be divided into three genera: *Thi(o)alkalimicrobium* (low-salt tolerant alkaliphiles), *Thi(o)alkalivibrio* (extremely salt tolerant and extremely halophilic alkaliphiles) (Sorokin et al., 2001) and *Thiomicrospira* (Sorokin et al., 2002). These genera belong to the 7 subdivision of the Proteobacteria **(Fig. 2).** The haloalkaliphilic SOB play a crucial role in the natural sulfur-cycle in the saline, alkaline environments. A large number of alkaliphilic SOB strains have been isolated and characterized in a mixed Russian-Dutch research group at Delft University of Technology (The Netherlands) (Sorokin et al. 1996, 2000, 2002 b; Banciu et al., 2004 b). The correct terminology („Thioalkali-" or „Thialkali-") is undecided, but as for correct representation of the metabolic feature we will use the prefix „Thioalkali-".

Genus *Thi(o)alkalimicrobium*

The genus *Thioalkalimicrobium (Tam.)* comprises species with a low DNA G-C content (48-51 mol%) isolated from the low saline Siberian soda lakes, from Kenyan soda lakes and from the saline and alkaline Mono Lake in U.S.A. The cells are rod-shaped, vibroid, spirilloid and coccoid. Some strains include motile cells with one to three polar flagella while other strains are non-motile. The ultrastructural study of *Thioalkalimicrobium* cells showed that the organization is similar in all strains, with an undulating cell wall of the Gram-negative type and multiple carboxysome-like structures localized in the central region of the cell. The cell wall in these bacteria was very unstable under low-osmotic conditions and during storage. The cells of *Thioalkalimicrobium* sp. survive 4-12 months at 4°C. They can be cultivated on alkaline thiosulfate agar medium and the colonies are reddish, without sulfur deposition.

The 16S RNA gene sequence analysis of the type strains revealed that the *Thioalkalimicrobium* group has a relatively close affiliation to the neutrophilic sulfar-

oxidizing bacteria of the genus *Thiomicrospira* (4 and 10% total sequence difference with *Thiomicrospira pelophila* and *Thiomicrospira crunogena* type strains, respectively).

Based on phylogenetic analysis including 168 DNA sequence analysis and DNA-DNA hybridization supplemented by phenotypic characterization, in the *Thioalkalimicrobium* group three species have been described to date: *Tam. aerophilum, Tam. sibiricum* and *Tam. cyclicum.*

The strains of ***Thioalkalimicrobium aerophilum*** were isolated from the water and surface sediments of Siberian soda lakes (e.g. the type strain AL 3^T DSM 13739^T) and from Kenyan soda lake sediments. ***Thioalkalimicrobium sibiricum*** type strain, AL 7^T (DSM 13740^T) was isolated from the sediments of Siberian soda lake in Buriatia (Russia). ***Thioalkalimicrobium cyclicum*** (type strain ALM 1^T, DSM 14477^T) was isolated on solid agar medium from the oxygen-sulfide interface water layer of Mono Lake (California, U.S.A). Unlike strains of *Tam. sibiricum* that are rather microaerophilic, the strains of *Thioalkalimicrobium aerophilum* grow faster under fully aerobic conditions. Another phenotypic difference is the tetrathionate-oxidizing capacity, which is present m *Tam. aerophilum* and *Tam. cyclicum* and very low or absent in *Tam. sibiricum.*

Genus *Thi(o)alkalivibrio*

The genus *Thioalkalivibrio* includes obligately autotrophic sulfur-oxidizing species with a high DNA G-C content (61.0-65.6 mol%). The *Thioalkalivibrio* group belongs to the *γ-Proteobacteria* and has no immediate relatives among the other chemolithotrophic members of the *γ -Proteobacteria.* The group bears a distant relationship to the anaerobic purple sulfur bacteria of the genus *Ectothiorhodospira.*

The strains were isolated mostly from the Kenyan soda lakes, which are, in general, more alkaline and saline than the Siberian steppe lakes. To date, one species of *Thioalkalivibrio* was isolated from Mono Lake (U.SA.). The group is represented mainly by vibrio-shaped bacteria with one polar fagellum. Some strains show spirilla-, rod-, filamentous rod-shaped cells or curved barrel-like-cells with thick capsules. It also may include strains with non-motile cells. The Gram-negative cell wall of *Thioalkalivibrio* cells is undulating and the multiple carboxysome-like structures are present in the center of the cells with the exception of the denitrifying species. In contrast to the *Thioalkalimicrobium* strains, cells of the *Thioalkalivibrio* strains were more resistant to osmotic shock and survived much longer during storage in liquid cultures at 4^0C. A substantial difference in cell fine structure was observed only m the haloalkaliphilic strains. Strain ALJ 15 presents a cell wall with multiple tubular extensions filled with electron-dense material. The cells of another haloalkaliphilic strain, ALJ 22, are surrounded by a large capsule, sometimes shared by several cells that tend to aggregate.

Genetically, as well as phenotypically, the *Thioalkalivibrio* group is more heterogenous than the *Thioalkalimicrobinm* group. DNA-DNA hybridization demonstrated that it includes both highly related strains with more than 90% DNA homology and only

weakly related representatives with a DNA similarity of about 30% with the other strains. The group includes strains that possessed important phenotypic differences and could not be separated from the others based on its 16S RNA or DNA-DNA homology. There are moderate halotolerant and extremely halophilic and halotolerant strains. The latter are able to produce membrane-bound yellow carotenoids but genetically they could not be separated from the colorless strains. This is a first known example of pigment formation among the aerobic 'colorless' sulfur bacteria. In the haloalkaliphilic strains, the amount of carotenoid produced positively correlated with the salt concentration in the growth medium. The colored strains do not contain bacteriochlorophylls.

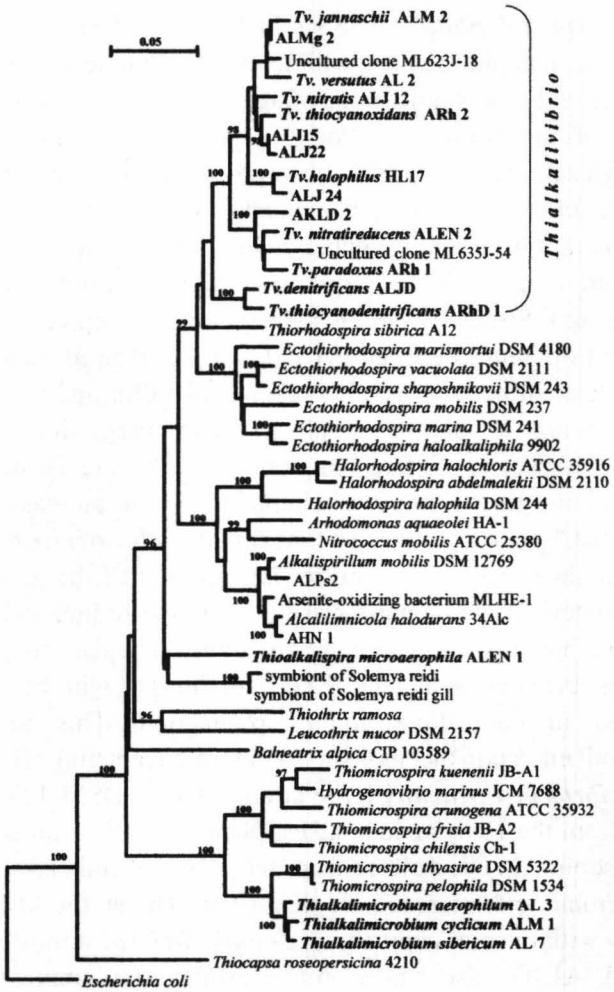

Fig. 2: Phylogenetic tree demonstrating position of the three new genera of haloalkaliphilic SOB isolated from the soda lakes. Numbers of the branches indicates bootstrap values (only the highest

values are included). Unaffiliated strains among the genus *Thioalkalivibrio:* extremely salt tolerant strains from Mongolia (AL Mg 2) and Kenya (ALJ 15, ALJ 22, ALJ 24); AKLD 2 – facultatively anaerobic nitrate-reducing strain from Kulunda. Bar, 5% sequence divergence.

Several strains of *Thioalkalivibrio* are able to grow with thiocyanate (SCN^-) as the sole energy and nitrogen source. They were isolated from soda lakes in South-East Siberia, Kenya and Egypt and classified into two separate species.

Thioalkalivibrio versutus (type strain AL 2^T, DSM 13738^T) includes strains of vibrio- to spirilla-shaped bacteria isolated from Kenyan and Siberian soda lakes. Two strains (ALJ 15 and ALJ 22) are halophilic, thermotolerant and produce a membrane-bound yellow pigment. The type strain was isolated from the surface sediments of a Siberian soda lake (Tuva Republic).

Thioalkalivibrio halophilus (type strain HL 17^T DSM 15791^T) is an obligately chemolithoautotrophic, halophilic and facultatively alkaliphilic bacterium. Cells are 0.3-0.4 x 1-2 μm, motile by a single polar flagellum. At low salinity often produces extracellular sulfur from thiosulfate. Produces yellow, membrane-associated pigment during growth at high salinity, with a main absorption maximum at 426 and a minor one at 457 nm in the methanol extract. The G+C content in DNA is 65.1 ± 0.5 mol% (7m). It is an extremely salt-tolerant bacterium growing at sodium concentrations between 0.2 and 5 M with an optimum at 2 M Na^+. It grows at high concentrations of NaCl and of $Na_2CO_3/NaHCO_3$ (soda). Strain HL 17^T is a facultative alkaliphile growing at pH range 7.5-9.8, with a broad optimum between pH 8.0 and 9.0. It used reduced inorganic sufur compounds (thiosulfate, sulfide, polysulfide, elemental sulfur and tetrathionate) as energy source and electron donor. In continuous culture, under energy limitation, thiosulfate was stoichiometrically oxidized to sulfate. In sodium carbonate medium under alkaline conditions the maximum growth rate was similar while the biomass yield was lower as compared with the NaCl-grown culture. The maximum sulfur-oxidizing capacity measured in washed cells was higher in the soda buffer independent of the growth conditions. The compatible solute content of the biomass was higher in the sodium chloride-grown culture than in the sodium carbonate/bicarbonate-grown culture. The data suggest that the osmotic pressure differences between soda and NaCl solutions might be responsible for the difference observed in compatible solutes production. This may have important implications in overall energetic metabolism of high salt adaptation (Banciu et al., 2004 b).

Thioalkalivibrio denitrificans (type strain $ALJD^T$, DSM 13742^T includes a non-denitrifying strain from the Kenyan soda lakes, phenotypically similar to *Thioalkalivibrio versutus.* The type strain $ALJD^T$, isolated from sediments of soda lake Bogoria (Kenya), is a facultatively anaerobic and microaerobic denitrifier. The strain $ALJD^T$ had a relatively low DNA similarity with the non-denitrifying *Thioalkalivibrio* strains except strain ALJ 10 (55-58% similarity). $ALJD^T$ also had a protein profile very similar to that of the non-denitrifying strain ALJ 10. Strain ALJ 10 does not grow anaerobically with different nitrogen oxides as electron acceptors. The reason for such an obvious discrepancy between

the genetic similarity and physiological difference is not clear. It might be speculated that this could be the result of the complete deletion of the DNA region responsible for denitrification in strain ALJ 10.

Thioalkalivibrio nitratis (type strain ALJ 12\ DSM 13741[1]) includes strains from Kenyan and Siberian soda lakes with a high DNA homology (80%). They differ from other species of the genus by their ability to reduce nitrate to nitrite during growth with thiosulfate under oxygen-limiting conditions. Strain ALJ 21 produces membrane-bound yellow pigment and contains high level of a cytochrome species. The type strain, ALJ 12 was isolated from sediments of soda lake Nakuru (Kenya).sulfur globules inside.

Thioalkalivibrio nitratireducens (type strain ALEN 2^T, DSM 14787^T) was isolated from the hypersaline Lake Fazda (Wadi Natrun, Egypt) and presented barrel-shaped, coccoid, non-motile cells (Sorokin et al., 2003). Strain ALEN 2^T is facultatively anaerobic, obligately alkaliphilic and moderately halophilic. The strain oxidizes thiosulfate, sulfide, polysulfide and, much less actively, elemental sulfur and tetrathionate to sulfate. The anaerobic growth is possible in the presence of nitrate as electron acceptor and thiosulfate, sulfide or polysulfide as electron donor. The sole product of nitrate reduction is nitrite. *Thioalkalivibrio nitratireducens* is genetically most closely related to the thiocyanate-oxidizing species, *Thioalkalivibrio paradoxus*. Its DNA G+C content is 64.8±0.5 mol% (Tm method).

Thioalkalivibrio jannaschii (type strain ALM 2^T, DSM 14478^T) was isolated from the O_2-sulfide interface layer of Mono Lake (California, USA) and. It is a motile vibrio, which tolerates up to 4 M Na^+ and produces a membrane-bound yellow pigment.

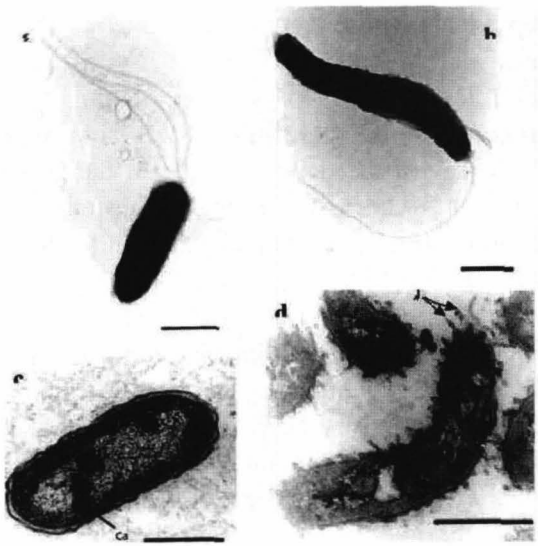

Fig. 3 Electronmicrographs of *Thioalkalimicrobium* AL3 (a, c) and *Thioalkalivibrio* ALJ 15 (b, d). Ca- carboxysome-like structure, T-tubular structures (Bars = 0.5 (μm) (after Sorokin et al., 2001)

Thioalkalivibrio thiocyanoxidans (type strain ARh 2^T, DSM 13532^T) differs from the other *Thioalkalrvibrio* species by the ability to grow with thiocyanate as the sole energy, nitrogen and sulfur source, producing cyanate as an intermediate (Sorokin et al., 2002 b). Cells are short vibrios and each has a single polar flagellum. The extremely salt-tolerant strains produce a membrane-bound yellow pigment. The species is obligately chemolithoautotrophic. The type strain ARh 2^T, isolated from a Kenyan soda lake, is a yellow-colored, extremely natronotolerant bacterium able to grow in soda brines (up to 4.3 MNa^+).

Thioalkalivibrio paradoxus (type strain ARh 1^T DSM 13531^T) cells are large, non-motile, barrel-like rods with capsules (Sorokin et al., 2002 b). It was isolated from the sediments of Kenyan (e.g. the type strain) and Egyptian soda lakes. The strains are obligately alkaliphilic and moderately halophilic.

Genus *Thioalkalispira*

To date the genus *Thioalkalispira* is represented by a single obligately chemolithoautotrophic sulfur-oxidizing species ***Thioalkalispira microaerophila*** (type strain ALEN 1^T DSM 14786^T). Isolated from a soda lake in Wadi Natrun, Egypt, the *Thioalkalisptra* spirillum-like bacterial cells are motile and with a single polar flagellum (Sorokin et al., 2002 a). They contain a membrane-associated yellow pigment. The DNA G-C content of *Thioalkalispira microaerophila is* 58.9±0.5 mol% *(T*m), which is lower than the values observed for all of the known haloalkaliphilic sulfur-oxidizing baeteria of the genera *Thioalkalimicrobium* and *Thioalkalivibrio* isolated so far. Phylogenetic analyses of the 16S rDNA sequences of strain ALEN 1^T and its closest relatives demonstrated that this strain formed a deep branch within the *y-Proteobacteria,* with no obvious association to any described cluster of species/genera.

Physiological features of haloalkaliphilic SOB from soda lakes

The growth physiology of the representatives of the genera *Thioalkalimtcrobium* and *Thioalkalrvibrio* genera have been studied under substrate excess in batch culture or substrate limitation m continuous culture, at alkaline pH and at low salt concentration (0.2-1.5 M Na^+) (Sorokin et al., 2000,2001).

Growth characteristics of *Thioalkalimicrobium* sp.

Species of *Thioalkalimicrobium* are obligate chemolithoautotrophic bacteria, growing optimally at pH values higher than 9, with a minimal sodium ion requirement of about 0.2-0.3 M. The upper limit of sodium ion concentration for these strains is 1.2-1.5 M **(Fig 4).** When cultivated m continuous system, *Thioalkalimicrobium* strains showed a maximum specific growth rate (μ_{max}) of 0.33 h^{-1}, while in batch culture it varied from 0.08 to 0.22 h^{-1}. The molar growth yield (Y) on thiosulfate in batch culture varied from 1.8 to 2.7 g protein (mol thiosulfate)$^{-1}$ while in chemostat culture this value did not exceed 3.5 g

protein (mol thiosulfate)$^{-1}$ (Sorokin et al., 2001). *Thioalkalimicrobium* strains were able to grow only in the presence of thiosulfate or sulfide. They oxidize the reduced sulfur compounds (thiosulfate, sulfide and polysulfide) to sulfate, without forming intermediate elemental sulfur. The maximum oxidation rates are recorded at pH 9-10 and up to pH 11 **(Fig. 5 A).** Interestingly, the specific rates of substrate consumption are comparable with the highest values observed in neutrophilic sulfur bacteria (Stefess, 1993; Visser, 1997). This observation may be valuable in applications that require fast removal of toxic sulfur compounds under alkaline conditions.

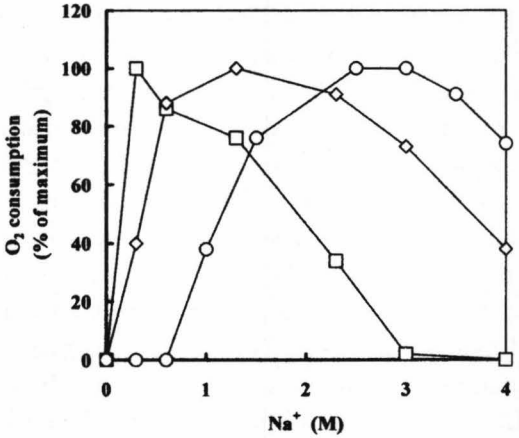

Fig. 4: Impact of sodium concentrations on the oxidation capacities for thiosulfate at pH 10 by *Thioalkalimicrobium* strains (squares) and two *Thioalkalivibrio* strains (diamonds and circles).

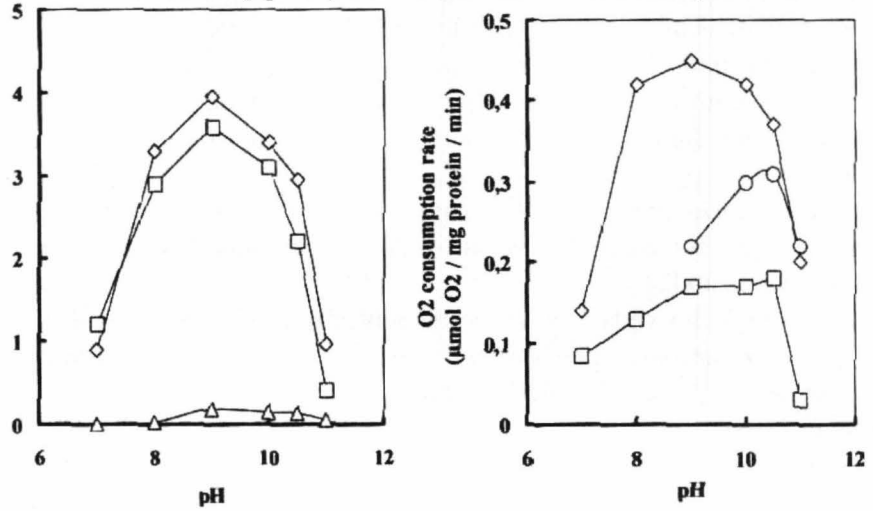

Fig. 5: Impact of pH on the oxidation capacities for thiosulfate (squares), sulfide (diamonds), polysulfide (circles) and elemental sulfur (triangles) by *Thioalkalimicrobium* strains (A) and *Thioalkalivibrio* strains (B).

On the basis of their oxygen requirement, *Thioalkalimicrobium* strains have been divided into two categories: aerobes and microaerobes. Aerobic strains grow better under conditions of non-limiting oxygen supply while the microaerobes (isolated from the anaerobic sediments), grow faster under conditions of limited oxygen supply.

Growth characteristics of *Thioallcalivibrio* species

Strains of *Thioalkalivibrio (Tav.)* are obligately chemolithoautotrophs growing optimally at pH 10.0-10.2. Some strains are capable of growing down to 9-9.5 while others fail to grow below pH 10. Many strains of *Thioalkalivibrio* are halotolerant or extremely halotolerant, being able to grow between 0.6 and 4 M Na^+. Some strains of *Thioalkalivibrio versutus* are obligate halophilic and thermotolerant, growing only above 1 M Na^+ (optimum at 1-2 M) **(Fig 4)** and up to 45-47°C (optimum at 40°C). In general, the batch and continuous cultivation have shown that the maximum specific growth rate (μ_{max}) with thiosulfate is less than 0.2 h^{-1}. Unlike *Thioalkalimicrobium* species, the maximum molar yield (Y) on thiosulfate in *Thioalkalivibrio* strains is higher (up to 8 g protein mol^{-1}) (Sorokin et al., 2001; Banciu et al., 2004 a). *Tav.* strains oxidize sulfide, thiosulfate, elemental sulfur, sulfite and polythionates with relatively low activities within the pH range 7.0 to 11.0-11.5 (optimum pH 9-10) **(Fig. 5 B).** The rates of oxygen consumption ($qO_{2\ max}$) with various inorganic sulfur substrates are ranging between 0.2 to 0.8 μmol 02 $(mg\ protein)^{-1}\ min^{-1}$. Elemental sulfur is transiently produced during the oxidation of thiosulfate and is stored in the periplasm. No sulfur formation was observed upon polysulfide oxidation in cell suspension. The end product of inorganic sulfur oxidation is sulfate. Tetrathionate is hydrolyzed first to thiosulfate, elemental sulfur and sulfate in *Tav. versutus* strains. Overall, *Thioalkalrvibrio* group appear to employ a pathway of sulfide oxidation via polysulfur (sulfur or possibly polysulfide) compounds and sulfite, similar to many acidophilic and some neutrophilic sulfur-oxidizing bacteria (Kelly, 1999; Pronk et al., 1990).

Thioalkalivibrio thiocyanoxidans and *Tav. paradoxus* are capable of utilizing thiosulfate as energy source and thiocyanate (SCN') as sole source of energy, nitrogen and sulfur (Sorokin et al., 2002 b).

Tav. denitrificans cells grow best anaerobically in the presence of thiosulfate as electron donor and nitrous oxide (N_2O) as electron acceptor. *Tav. nitratis* and *Tav. nitratireducens* are capable of reducing nitrate to nitrite under microaerobic conditions (Sorokin et al., 2003).

The high activity of cytochrome oxidases in *Thioalkalivibrio* is however in contrast with their low maximum respiratory activity as compared with *Thioalkalimicrobium.* It is possible that the high oxidase activity may be an adaptation to low oxygen concentration in order to lower the overall apparent affinity constant (Ks) for oxygen rather than increasing the overall (apparent) maximum respiratory capacity (V_{max}).

A comparative table presenting the main physiological differences between *Thioalkalimicrobium* and *Thioalkalivibrio* strains is shown below.

Table 3. Comparative table of kinetic and eco-physiological properties of the two groups of chemolithoautrotrophic sulfur bacteria.

Parameter	*Thioalkalimicrobium*	*Thioalkalivibrio*
μ_{max} (h^{-1})*	0.33	<0.2
Y (g protein/mol $S_2O_3^{2-}$)	2-3	4-6
qO_{2max} (μmole O_2/mg prot min^{-1})	2.5-5	0.3-0.8
$S_2O_3^{2-}$, HS^-	1.5-2.5	0.2-0.8
S_8^{2-}	0-0.05	0.2-0.6
S^0	3-5	1-3
K_s (μM)	no	yes
Sulfur production**	homogenous	heterogenous
Group diversity	0.2-1.2 M	0.2-4 M
Salt tolerance (Na^+)	Low	High
Resistance to starvation	R-strategists	K-strategists
Survival strategy		

* *For abbreviations, see the text above*
** *At high aeration*

Growth characteristics of *Thioalkalispira* species

Thioalkalispira strains use thiosulfate or sulfide as electron donors. Washed cells oxidize thiosulfate, sulfide, polysulfide and elemental sulfur to sulfate. It grows optimally under micro-oxic conditions (1-2% O_2 in the gas phase), whereas growth is inhibited under fully oxic conditions. Among nitrous oxides only nitrate is used as electron acceptor but without growth. The representatives of the species are alkaliphilic and moderately halophilic bacteria growing between pH 8 and 10.4 (optimum around pH 10) and at a salt concentration between 0.3 and 1.5 M Na^+ (optimum 0.5 M). The maximum growth rate (0.08 h^{-1}) of the organism was achieved in a thiosulfate-limited micro-oxic continuous culture at pH 10 (Sorokin et al., 2002 a).

Potential applications of haloalkaliphilic SOB

Several environmental problems are caused by sulfur compounds like sulfate (pollution of surface water, acid mine drainage), SO_2 (acid rain), H_2S (odor problems, high toxicity, acid rain) and methylated sulfur compounds (odor problems, toxicity, climate change). The aim of sulfur biotechnology is to prevent loss of sulfur compounds to the atmosphere and to avoid complete oxidation of sulfur compounds to sulfate. Current research is therefore focused on the production of a sulfur compound, which can be easily separated from the waste streams, stored and re-used for other purposes. One of the

successful processes is the production of elemental sulfur from H_2S-containing gas streams by sulfur-oxidizing bacteria m the Thiopaq® process (Paques BV, Balk, The Netherlands) **(Fig. 6).** In this system gasses can be treated by the absorption of H_2S in a scrubber unit, subsequent biological oxidation of sulfide to elemental sulfur at neutral pH and separation of the sulfur and recycling of the percolation water to the scrubber (Janssen et al., 2001; Lens and Kuenen, 2001). A variety of gas streams (pressurized natural gas, synthesis gas, biogas and refinery gas) can be treated with this two-step process. Points for major innovation of this process are the enhancement of the stripping efficiency of K_2S in the scrubber (by elevating the pH) and the reduction of the bleed stream of the aerobic reactor (by maintaining high salt conditions). Moreover, since high CO_2 content is usual for H_2S-containing industrial gases, use of alkaline carbonates m the scrubber instead of organic or inorganic alkali (NaOH) is beneficial for the effectiveness of H_2S absorption.

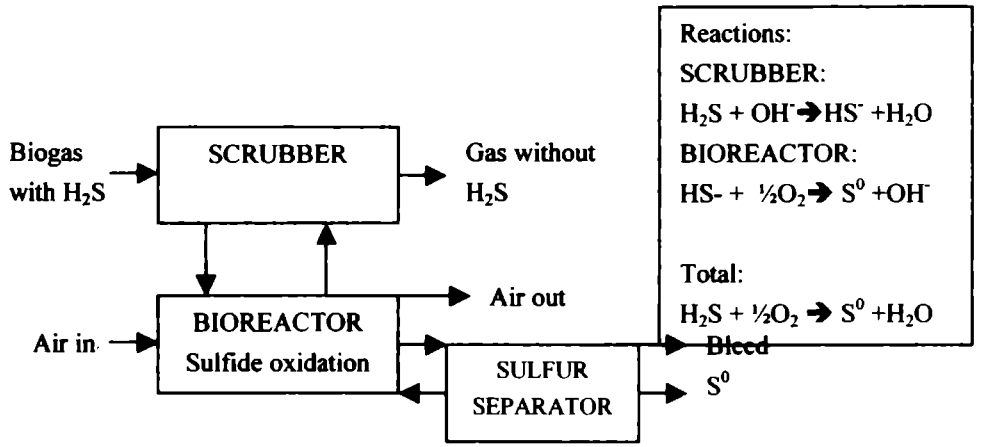

Fig. 6: Block process diagram of the Thiopaq®-bioscrubber and reaction mechanisms involved

In **conclusion,** the alkaliphilic SOB isolated from soda lakes of Siberia (Russia) and Kenya can tolerate a very high pH (up to 10.6-11) and high salt concentrations (1-4 M Na^+). They are also capable of utilizing inorganic sulfur compounds (sulfide, polysulfide, thiosulfate, etc) as energy source and electron donor at rates comparable to those of neutrophilic SOB. These ecological and metabolic properties make haloalkaliphilic SOB attractive for biotechnological sulfide removal from industrial liquid or gas streams.

Acknowledgments

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REFERENCES:

BANCIU, H., SOROKIN, D. Y., KLEEREBEZGIA, R., MUYZER, G., GALINSKI, E. A., KUENGN, J. G., 2004 a, Growth kinetics of haloalkaliphilic sulfur-oxidizing bacterium *Thioalkalivibrio versutus* strain ALJ 15 in continuous culture. Extremophiles, 8, 185-192.

BANCIU, H., SOROKIN, D. Y., MUYZER, G., KLEEREBEZEM, R., GALINSKI, E. A., KUENEN, J. G., 2004 b, *Thioalkaimbrio halophilus* sp. nov, novel obligately chemolithoautotrophic facultatively alkaliphilic and extremely salt-tolerant sulfur-oxidizing bacterium from a hypersaline alkaline lake. Extremophiles, 8, 325-334.

BAUMGARTE S., 2003, Microbial diversity of soda lake habitats. PhD thesis, Carolo-Wilhelmina University Braunschweig, pp 197

BRUNE C: Sulfur compounds as photosynthetic electron donors. In: Anoxygenic Photosynthetic Bacteria. Edited by Blankenship RE, Madigan MT, Bauer CE. Kluwer Academic Publishers; 1995, 847-870.

DUCKWORTH A. W., GRANT W. D., JONES B. E., VAN STEENBERGEN R., 1996, Phylogenetic diversity of soda lakes alkaliphiles. FEMS Microbiol Ecol 19:181-191

FRIEDRICH C. G., ROTHER D., BARDISCHEWSKY F., QUENTMEIER A., FISCHER J., 2001, Oxidation of reduced inorganic sulfur compounds by bacteria: emergence of a common mechanism? Appl.Environ.Microbiol. 67:2873-2882

GRANT W. D., TINDALL B. J., 1986, The alkaline saline environment. In: Herbert R. A, Codd GA (eds) Microbes in Extreme Environments. Academic Press, London, pp 25-54

HARRISON A. P. Jr, 1984, The acidophilic thiobacilli and other acidophilic bacteria that share their habitat. Annu Rev Microbiol 38:265-292

IMHOFF J. F., SAHL H. G., SOLIMAN G. S. H., TRÜPER H. G., 1979, The Wadi Natrun: chemical composition and microbial mass developments in alkaline brines of eutrophic desert lakes. Geomicrobiol J 1:219-234

IMHOFF J. F., SÜLING J., 1996, The phylogenetic relationship among *Ectothiorhodospiraceae:* a reevaluation of their taxonomy on the basis of 16S rDNA analyses. Arch Microbiol 165:106-113

JANSSEN A. J. H. , RURTENBERG R., BUISMAN C. J. N., 2001, Industrial applications of new sulphur biotechnology. Water Sci Technol 44:85-90

KRIENITZ L., BALLOT A., KOTUT K., WIEGAND C., PÜTZ S., METCALF J. S., CODD G. A., PFLUGMACHER S., 2003, Contribution of hot spring cyanobacteria to the mysterious deaths of lesser flamingos at Lake Bogoria, Kenya. FEMS Microbiol.Ecol. 43:141-148

KELLY D. P., 1999, Thermodynamic aspects of energy conservation by chemolithotrophic sulfur bacteria in relation to the sulfur oxidation pathways. Arch.Microbiol. 171:219-229

KELLY D. P., SHERGILL J. K., L.U. W.-P, WOOD A. P., 1997, Oxidative metabolism of inorganic sulfur compounds by bacteria. Antonie van Leeuwenhoek 71:95-107

KEMPE S., DEGENS ET, 1985, An early soda ocean? Chem.Geol. 53:95-108

KUENEN J. G., 1975, Colourless sulfur bacteria and their role in the sulfur cycle. Plant and Soil. 43:49-76

KUENEN J. G., BEUDKERR R. F., 1982, Microbiology of thiobacilli and other sulphur-oxidizing autotrophs, mixotrophs and heterotrophs. Phil Trans R Soc Lond B.Biol.Sci. 298:473-97

LENG P. N. L., KUENEN J. G., 2001, The biological sulfur cycle: novel opportunities for environmental biotechnology. Water Sci.Technol. 44:57-66 1

MARTIN W., RUSSELL M. J., 2003, On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. Phil.T.Roy.Soc.B. 358:59-83

OREN A., 1999, Bioenergetic aspects of halophilism. Microbiol.Mol.Biol.Rev. 63:334-348

OREN A., 2002, Halophilic microorganisms and their environments, Kluwer Academic Publishers, Dordrecht, pp. 575

PRONK, J. T., MEULENBERG, R., HAZEU, W., BOS, P. and KUENEN J. G., 1990, Oxidation of reduced inorganic sulfur compounds by acidophilic thiobacilli. FEMS Microbiol. Rev. 75, 293-306

ROBERTSON L. A., KUENEN J. G., 1992, The colorless sulfur bacteria. In: Balows A, Trüper H. G., Dworkin M. D., Hadere W., Schleifer K. H. (eds.) The Prokaiyotes, 2nd ed. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications. Vol 1. Springer-Verlag, pp 385-413

SOROKIN D. Y., LYSENKO A. M., MJTYUSHINA L. L., 1996, Isolation and characterization of alkaliphilic heterotrophic bacteria capable of oxidation of inorganic sulfur compounds to tetrathionate. Microbiology (Moscow) 65:370-383

SOROKIN D. Y., LYSENKO A. M., MITYUSHINA L. L., JOUROVA, T. P., JONES B. E., RAMEY F. A., ROBERTSON ,L. A., KUENEN J. G., 2001, *Thioalkalimicrobium aerophilum* gen. nov., sp. *Thioalkalimicrobium sibericum* sp. nov., and *Thioalkalivibrio versutus* gen. nov., sp. nov., *Thioalkalivibrio nitratis* sp. nov., and *Thioalkalivibrio denitrificans* sp. nov., novel obligately alkaliphilic and obligately chemolithoautotrophic sulfur-oxidizing bacteria from soda lakes. hit J.Syst.Evol. Microbiol. 51:565-580

SOROKU D. Y., ROBERTSON L. A., KUENEN J. G., 2000, Isolation and characterization of alkaliphilic, chemolithoautotrophic, sulphur-oxidizing bacteria. Antonie van Leeuwenhoek 77:251 -262

SOROKU D. Y., TOUROVA T. P., KOLGANOVA T.V., SJOLLEMA K. A., KUENEN J. G., 2002, *Thioalkalispira microaerophila* gen. nov., sp. nov., a novel lithoautotrophic, sulfur-oxidizing bacterium from a soda lake. Int.J.Syst.Evol.Microbiol 52:2175-82

SOROKIN D. Y., TOUROVA T. P., LYSENKO A. M., MITYUSHINA L. L, KUENEN J. G., 2002 b, *Thioalkalivibrio thiocyanoxidans* sp. nov. and *Thioalkalivibrio paradoxus* sp. nov., novel alkaliphilic, obligately autotrophic, sulfur-oxidizing bacteria capable ofgrowth on thiocyanate, from soda lakes. hit J.Syst.Evcd.Microlnol. 52:657-64

SOROKIN D. Y., TOUROVA T. P., SJOLLEMA K.A., KUENEN J. G., 2003, *Thioalkalivibrio nitratireducens* sp. nov., a nitrate-reducing member of an autotrophic denitrifying consortium from soda lake. Int.J.Syst.Evol.Microbiol. 53:1779-1783

STEFESS G. C., 1993, Oxidation of sulphide to elemental sulphur by aerobic thiobacilli. Ph.D. thesis, Delft University of Technology, p 128

STEUDEL R., 2000, *The chemical sulfur cycle.* In: Lens P.N.L., Hulshoff Pol L. (eds) Environmental Technologies To Treat Sulfur Pollution. IWA Publishing, London, pp 1-32

VISSER J. M., 1997, Sulfur compound oxidation and sulfur production by *Thiobacillus* sp. W5. PhD thesis, Delft University of Technology, pp 107

WÄCHTERSHÄUSER G., 1988, Before enzymes and templates – theory of surface metabolism. Microbiol.Rev. 52:452-484

ZAVARZIN G. A., ZHILINA T.N., KEVBRIN W., 1999, The alkaliphilic microbial community and its functional diversity. Microbiology (Moscow) 68:579-599

DIATOM COMMUNITIES OF LAKE OCNEI FROM TURDA, CLUJ COUNTY (SPECIES COMPOSITION, SEASONAL DYNAMICS AND HUMAN IMPACT)

Levente NAGY[1], Leontin Ştefan PETERFI[2], Laura MOMEU[3]

Abstract. The present paper constitutes an integrated part of a PhD thesis, dealing with the detailed study of diatom communities inhabiting nine different lakes, located near Turda town. Lake Ocnei, the authors dealt with in this paper, has actually been formed by the collapse of an ancient salt mine, and has rather saline water. There have been identified 43 species belonging to 7 families (Thalassiosiraceae, Fragilariaceae, Achnanthaceae, Naviculaceae, Bacillariaceae, Epithemiaceae and Surirellaceae). The observations showed that salinity has a great impact on the composition of diatom communities acting together with the effects of significant human impact.

Key words: diatoms, saline lake, diversity, seasonal dynamics, human impact.

Introduction

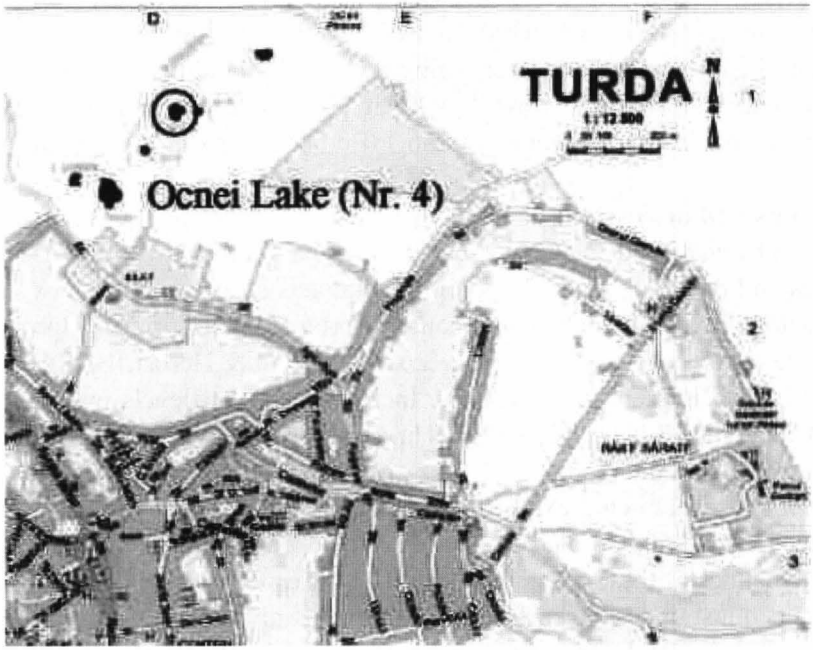

Fig. 1: Geographical location of Ocnei Lake

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In many cases saline lakes appear following the collapse of salt mines. This is the case of Lake Ocnei too, shaped by the caving in of a many centuries old mine (Maxim, 1937). The lake is located North-West from Turda in the Sărata Valley and it is the fourth one in a series of six lakes.

The water chemistry and the aquatic ecosystems of this lake are under the influence of salt deposits. Thus, water salinity is high and it acts as an important limiting factor for the development of microbial, plant and animal populations.

An important aspect is that since its appearance, the lake has been intensively used in balneary and recreational purposes.

Material and methods

The sampling and processing methods were classical and internationally accepted. Benthic and planktonic samples were collected seasonally during 2005, and some of the physical and chemical parameters of water were also registered [salinity (mg l^{-1}), TDS (mg l^{-1}), conductivity (μS cm^{-1}), pH, dissolved oxygen (%, mg l^{-1}), air and water temperature (°C)]. The samples were conserved *in situ* with 4 % formaldehyde solution, the investigations being carried out later, using common laboratory techniques employing Nfpk Zeiss Jena and Nikon Eclipse E 400 light microscopes and some of the recent taxonomical key books (e.g. Krammer & Lange-Bertalot, 1986, 1988, 1991 a, 1991 b).

Results and discussion

The physical and chemical parameters have a great influence on diatom communities and one of the most important parameters seems to be salinity, a significant limiting factor. Experimental investigations proved that primary production and biodiversity decrease when salinity levels are high (Blinn & Herbst, 1998). The dynamics of nutrients is also influenced by salinity. Increased nutrient levels may change salinity tolerance of diatoms (Fritz *et al.,* 1993). Thus, salinity affects every level of the trophic chain both directly and indirectly.

In situ measurements exhibited high salinity and conductivity levels under the water surface (around 60,000 mg. l^{-1} and 112,500 μS cm^{-1}, during the summer period). A significant dynamics of these parameters could be observed in 2005 (Fig. 2): higher values could be noticed just under the surface m summer, mainly because of the better mixing of layers (human impact) and high water temperature (which influences conductivity).

The level of dissolved oxygen varied between 6.85 and 12.84 mg.l^{-1} (Fig. 3.), the lowest values being measured during summer (due to the strong mixing and water temperature). The pH values did not vary much, exhibited values around 8.35.

Water temperature was always slightfy higher than air temperature, due to the energy conserving nature of saline lakes (heliothermal) (Fig. 4).

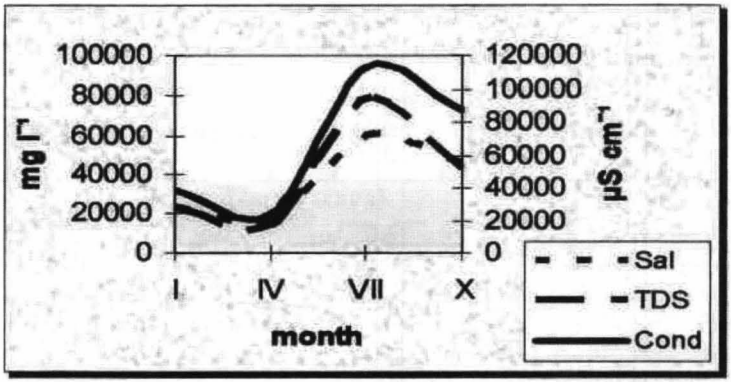

Fig. 2. Salinity, TDS and conductivity values in 2005

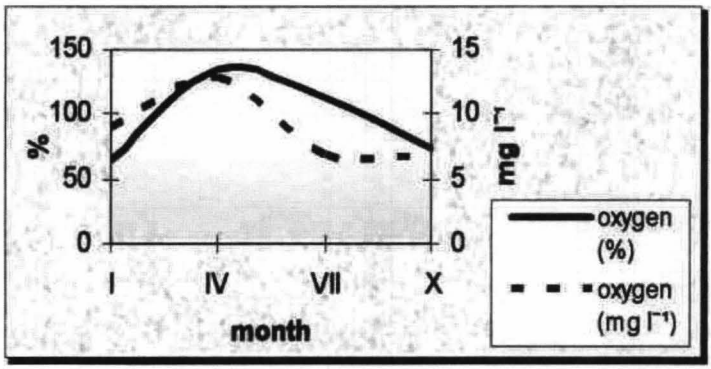

Fig. 3. Variation of dissolved oxygen (2005)

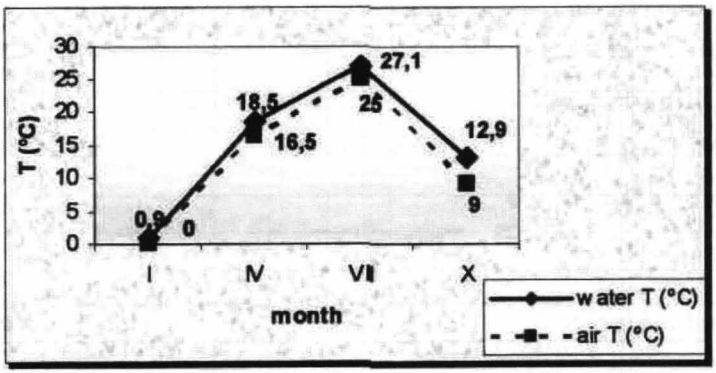

Fig. 4. Air and water temperatures in 2005

The total number of identified diatom species is 43; they belong to the following 7 families: *Thalassiosiraceae, Fragilariaceae, Achnanthaceae, Naviculaceae, Bacillariaceae, Epithemiaceae* and *Surirellaceae* (Tab. 1).

Table 1. Composition of periphytic (PP) and planktonic (PL) algal communities of Lake Ocnei

TAXA	January		April		July		October	
	PP	PL	PP	PL	PP	PL	PP	PL
CENTRALES								
Coscinodiscineae								
Thalassiosiraceae								
Aulacoseira grcmulata (Ehrenberg) Simonsen			+					
Cyclotella comta (Ehrenberg) Kützing							+	
Cyclotella distinguenda Hustedt *var. distinguenda*	+		+		+		+	
Cyclotella meneghiniana Kützing			+					
Stephanodiscus medius Håkansson			+					
PENNALES								
Araphidioeae								
Fragilariaceae								
Fragilaria arcus (Ehrenberg) Cleve *var. arcus*							+	+
Fragilariafasciculata (Agardh) Lange - Bertalot		+		+				
Fragilaria pulchella (Ralfs ex Kützing) Lange Bertalot	+		+					
Raphidineae								
Achnanthaceae								
Achnanthes lanceolata (Brebisson) Grunow ssp. *lanceolata* var. *lanceolata*			+					
Achnanthes minutissima Kutzing							+	+
Cocconeisplacentulavsa. euglypta (Ehrenberg) Gnmow		+					+	
Naviculaceae								
Amphora coffeaeformis (Agardh) Kützing							+	
Amphora veneta Kützing	+		+				+	
Cymbella affinis Kützing								+
Gomphonema gracile Ehrenberg							+	
Gomphonema olivaceum (Homemann) Brebisson var. *olivaceum*								+
Gomphonema parvulum (Kützing)								+
Navicula cincta (Ehrenberg) Ralfs			+		+			

Navicula cryptocephala Kutzing		+	+		+			
Navicula cryptotenella Lange-Bertalot							+	
Navicula eidrigiana Carter			+					
Navicula mutica Kützing var. *mutica*			+					
Navicula phyllepta Kützing			+				+	
Navicula pupula Kützing var. *pupula*			+					
Nccvicula veneta Kützing							+	
Pinnularia micrustauron var. *brebissoni* (Kützing) Mayer			+					
Bacillariaceae								
Hantzschia amphioxys (Ehrenberg) Grunow			+				+	
Nitzschia compressa (Bailey) Boyer var. *compressa*					+	+	+	
Nitzschia compressa var. *balatonis* (Grunow) Lange - Bertalot							+	
Nitzschia constricta (Kutzing) Ralfs			+					
Nitzschia filiformis var. *conferta* (Richter) Lange - Bertalot	+							
Nitzschiafonticola Grunow	+							
Nitzschiafrustulum var. *bulnheimiana* (Rabenhorst) Grunow	+	+	+		+	+	+	
Nitzschia frustulum (Kützing) Grunow var. *frustulum*	+	+	+	+	+	+	+	+
Nitzschia hungarica Grunow	+							
Nitzschia inconspicua Grunow	+	+	+	+	+		+	
Nitzschia nana Gnmow			+					
Nitzschia palea (Kutzing) W. Smith			+					
Nitzschia paleacea Gnmow	+							
Nitzschia pellucida Grunow					+		+	
Epithemiaceae								
Rhopalodia gibberula (Ehrenberg) 0. Muller							+	+
Surirellaceae								
Surirella brebissonii var. *kuetzingii* Krammer & Lange-Bertalot			+					
Surirella minuta Brebisson			+					

PP - periphyton;
PL - plankton,

The most representative families are Naviculaceae and Bacillariaceae, represented by 34.88 % and 32.56 % of identified species. (Fig. 5.). This seems to be an important aspect, because m the other lakes with lower salinity levels from the Sărata Valley the

number of Naviculaceae species inhabiting benthic communities was significantly higher than that of Bacillariaceae. This might suggest that members of Bacillariaceae family present higher tolerance towards extreme salinity than those of Naviculaceae. The number of diatom species identified in the plankton was much lower than that of benthos; this means that plankton communities are less diverse.

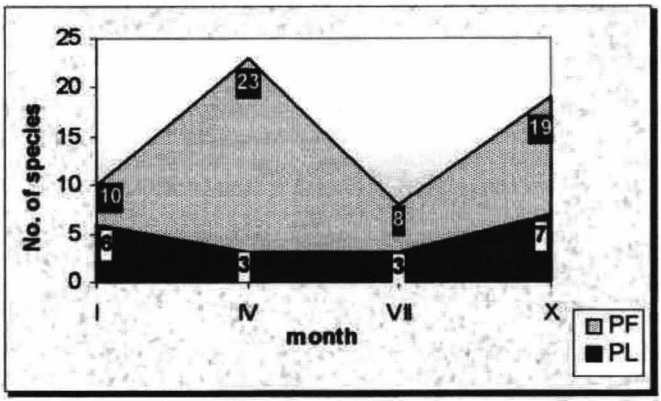

Fig. 4. Variation in the number of species during the investigated period

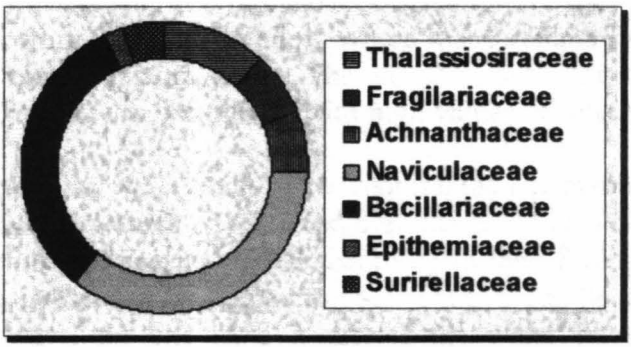

Fig. 5. Percentage distribution of taxa

Seasonal dynamics of both benthic and planktonic communities were also studied. Benthic algae followed the usual pattern: the number of species increased in spring and autumn and decreased in other seasons (Fig. 6), The reduced number of species in summer has probably another important cause, namely human impact. In this period the lake is intensively used for recreational and balneary purposes. Due to intens water movements caused by bathing people and their physical contact with the substratum and other objects, either natural or artificial supports for benthic communities, much of the periphytic algae are dislocated. The number of identified plankton species is low in every season.

The Shannon-Wiener diversity index and equitability were calculated in every season for the benthic communities. The lowest values were observed in both cases during

summer (Fig. 7.), which might be due to the already mentioned causes. There is an interesting discrepancy between the number of species and biodiversity values in spring. This phenomenon might be explained by the relative abundance (over 90%) of a single taxon – *Nitzschiafrustulwn* (Kutzing) Grunow *var. jrustulum.*

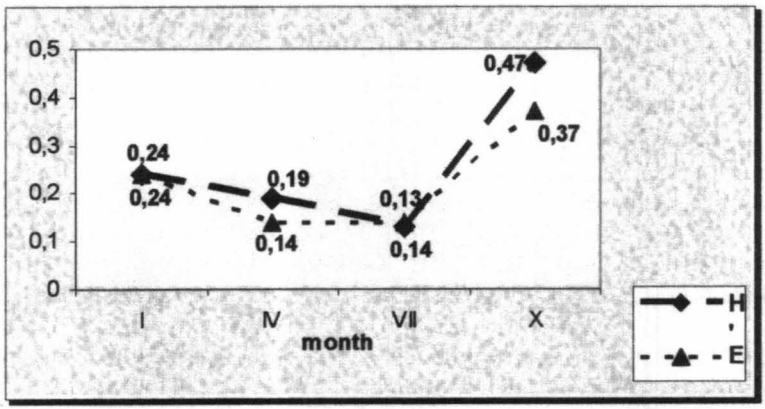

Fig. 7. Seasonal variations of Shannon - Wiener index of **diversity (H') and equitability** (E)

Conclusions

The measured physical and chemical parameters, except pH, varied through the whole sampling period. Salinity, conductivity and water temperature values were higher in summer, while the level of dissolved oxygen was lower in this period. The multiple reasons for these values were previously mentioned in this paper. The results have a great resemblance with other similar findings (Blinn & Herbst, 1998;

Fritz *et al.,* 1993), namely that the biodiversity index values are low, a characteristic for salt lakes. The total number of identified diatom species was 43; they belongto 7 families:

Thalassiosiraceae, Fragilariaceae, Achnanthaceae, Naviculaceae, Bacillariaceae, Epithemiaceae and *Surirellaceae.* The Bacillariaceae family is better represented than in the neighboring lakes from the Sărata Valley, where the dominant family as number of species number was clearly Naviculaceae. The samples were dominated by *Nitzschia frustulum* (Kützing) Grunow var. *frustulum,* with high values of relative abundance (over 90% in some cases).

Benthic communities were much better represented (as number of species) than the plankton. A seasonal dynamics of the fixed diatoms could also be observed through the year 2005, the number of species varied according to their biological pattern, and it was also significantly influenced by human impact. Thus, it can be said that the human impact acts in two ways, on one hand it affects the water physical and chemical parameters, on the other hand, it influences both directly and indirectly the diatom communities living in this aquatic ecosystem.

REFERENCES:

BLINN, D. W., HERBST, D. B., 1998, Experimental mesocosm studies of salinity effects on the benthic algal community of a saline lake, J. Phycol. 34:772-778.

FRITZ, S. C., JUGGINS, S., BATTARBEE, R. W., 1993, Diatom assemblages and ionic characterization of lakes of the Northem Great Planes, North America: A tool for reconstructing past salinity and climate fluctuations. Can. J. Fish. Aquat. Sci. 50:1844-1856. v

KRAMMER, K., LANGE-BERTALOT, H., 1986, Bacillariophyceae. 1. Teil: Naviculaceae, In: Ettl, H., Gerloff, J., Heyning, H., Mollenhauer, D. (Eds.), Susswasserflora von Mitteleuropa, Band 2/1, G. Fischer, Stuttgart.

KRAMMER, K, LANGE-BERTALOT, H., 1988, Bacillariophyceae. 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae, In: Ettl, H., Gerloff, J., Heyning, H., Mollenhauer, D. (Eds.), Susswasserflora von Mitteleuropa, Band 2/2, G. Fischer, Stuttgart.

KRAMMER, K., LANGE-BERTALOT, H., 1991 a, Bacillariophyceae. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae, In: Ettl, H., Gerloff, J., Heyning, H., Mollenhauer, D. (Eds.), Susswasserflora von Mitteleuropa, Band 2/3, G. Fischer, Stuttgart.

KRAMMER, K, LANGE-BERTALOT, H., 1991, Vy. Bacillariophyceae. 4. Teil: Achnanthaceae, In: Ettl, H., Gerloff, J., Heyning, H., Mollenhauer, D. (eds.), Susswasserflora von Mitteleuropa, Band 2/4, G. Fischer, Stuttgart.

MAXIM, I., AL, 1937, Contribuţiuni la explicarea fenomenelor de încălzire a apelor lacurilor Sărate din Transilvania, Rev. Muz. Geologic-Mineralogic, Univ. Cluj, Vol. VI: 209-320.

Rezumat. Ecosistemele acvatice sărate sunt insuficient studiate atât pe plan mondial cât şi naţional. Sunt ecosisteme cu o diversitate mai scăzută, relativ mai simple şi din acest motiv sunt ideale pentru studii legate de interacţiunile variate dintre elementele componente. Un exemplu tipic de lac sărat apărut în urma prăbuşirii unei vechi mine este Lacul Ocnei, din Valea Sărată (Turda, jud. Cluj). Acest studiu se referă la diferite aspecte (structură, dinamică sezonieră, diversitate, impact antropic) ale comunităţilor de diatomee şi este parte integrantă a unei teze de doctorat ce acoperă un număr de nouă lacuri sărate şi dulci de la Turda.

Totalul speciilor identificate din comunităţile fixate şi libere este 43, aparţinând la 7 familii: *Thalassiosiraceae, Fragilariaceae, Achnanthaceae, Naviculaceae, Bacillariaceae, Epithemiaceae* şi *Surirellaceae.* Se remarcă bogăţia numărul mult mai mare de specii a perifitonului decât a planctonului. Acesta din urmă este slab reprezentat şi de multe ori speciile întâlnite provin prin dislocare tot din domeniul bentosului. Se poate observa totodată o dinamică sezonieră semnificativă (mai ales în cazul perifitonului) datorată, pe de o parte, biologiei diatomeelor, pe de altă parte impactului antropic accentuat în perioada verii. Aşadar se poate afirma, că prezenţa omului exercită un impact dublu, şi contribuie la schimbările parametrilor fizico-chimici ai apei în perioada verii (prin amestecarea diferitelor straturi din masa apei), influenţează comunităţile de diatomee prin fenomenul de dislocare a celulelor prin turbulenţele provocate în masa apei, respectiv prin contact direct cu substratul.

PRELIMINARY STUDIES OF THE ALGAL COMMUNITIES OCCURRING IN SOME AQUATIC HABITATS OF THE SOMEŞENI SPA WETLANDS (TRANSYLVANIA, ROMANIA)

Annamaria KISS[1], Leontin Ştefan PETERFI[1]

Abstract. The species composition of algal communities was studied based on samples collected in June 2006. Their flora exhibits a high species diversity consisting of 102 taxa identified in 4 aquatic habitats of Someşeni Spa. The algal communities were dominated by diatoms *(Bacillariophyta)* – 64.7%, followed by cyanobacteria *(Cyanoprokaryota)* — 14.7% and green algae *(Chlorophyta)* — 12.7%. The contribution of *Euglenophyta, Dinophyta* and *Cryptophyta* was less than 6%. The analysis of the ecology of the species indicates that many of these are cosmopolitan and frequently present in saline waters.
Key words: wetlands, algae, periphyton, metaphyton, chlorophycean index, compound index, water quality.

Introduction

The Someşeni mineral water spring area is located in the Eastern part of Cluj-Napoca city, at the contact between the alluvial plane and the first terrace of the Someşul Mic River [2].

From geological point of view, the mineral aquifer is located on the western border of Neogene Transylvanian Basin. The Someşeni spring area is situated on an anticline with salt core. Marls with frequent intercalation of sandstone and volcanic tuffs dominate the lithologically these deposits. The alluvial deposits of the river cover generally the Neogene formations [2].

The therapeutic qualities of these waters were known since the early 1880's, but the spa was founded only in 1927 by Dr. Dominic Stanca.

The chlorinated, carbonated, sodium, calcium and lithium-laden water was used for treating chronic gastro-intestinal diseases, rheumatism, dystrophic infantile growth, anemia, rachitis, kidney irregularities, arteriosclerosis, stomach pains and skin diseases.

The spa has undergone various degrees of degradation processes and need major refurbishing as well as replacements, depending on the developmental plans.

The diatom communities of the Someşeni Spa were studied by Tömösváry and Schaarschmidt in 1879-1880 [7, 12]. They identified 65 diatom species, but only 11 of them were found in 2006 (Fig. 1).

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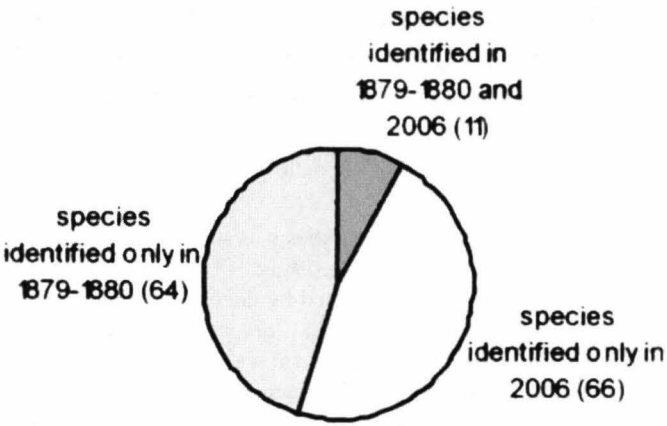

Fig. 1. Number of diatom species identified in 1879-1880 and 2006 respectively.

The aim of this study was to establish the species composition of the algal communities, to evaluate the trophic level of waters, as well as to assess their quality.

Materials and Methods

Periphyton and metaphyton samples were collected in June 2006 from different types of aquatic habitats (pools, ponds, spring water collector basin), by employing standard methods, depending on the nature of substratum. The samples were preserved in the field with 4% formalin and investigated by using standard methods; the identification of taxa was performed according to the common key books widely used in similar investigations [1, 3, 4, 5, 6, 8, 9, 10]. Samples have only been assessed from qualitative point of view.

To estimate the water quality there have been used the saprobic preferences of the identified species established by Sladecek. For the evaluation of the trophic status 2 indices were computed: the Chlorophycean Index (Thunmark) and the Compound Index (Nygaard) [13]. The floristic affinities among the algal communities were investigated by using the similarity index of Jaccard and running the statistical program PAST.

Results and Discussions

Some physico-chemical parameters (water temperature, pH, salinity and conductivity) were measured *in situ,* parallel with algal sampling (Tab. 1). The pH values indicated alkaline waters, excepting the samples taken from the ponds, which are slightly acidic. The salinity and conductivity values are relatively high indicating oligosaline and mesosaline waters.

Table 1. Physico-chemical parameters of the water samples

Physico-chemical data	Pooll	Pool2	Ponds	Spring water collector basin
Water temperature (°C)	22.1	21.3	11.8	12.3
PH	7.46	7.24	6.8	7.14
Salinity ($g.l^{-1}$)	4.6 *Oligosaline water	14.4 *Mesosaline water	6.9 *Mesosaline water	1.9 *Oligosaline water
Conductivity ($mS.cm^{-1}$)	8.15	24.5	12.5	3.78

* Tomescu and Gabos, 1989 [11]

There were identified 102 taxa (Tab. 2), which belong to the following algal divisions: *Cyanoprokaryota* – 15 taxa (14,7%), *Bacillariophyta* – 66 taxa (64,7%), *Chlorophyta* – 13 taxa (12,7%), *Euglenophyta* – 6 taxa (5,9%), *Cryptophyta* – 1 taxon (1%) and *Dinophyta* – 1 taxon (1%).

Most of the identified species are halophilous and halobiont elements.

Table 2: Qualitative structure of algal communities of some aquatic habitats from the Someşeni Spa wetlands

TAXA	*Sampling sites			
	1	2	3	4
CYANOPHYTA (CYANOPROKARYOTA)				
Anabaena constricta Geitl.	+			
Chroococcus minutus (Kütz.) Nâg.	+		+	+
C. turgidus (Ktitz.) Nâg.			+	
Cyanosarcina thermalis (Hind.) Kovac.			+	
Gomphosphaeria salina Kom. & Hind.	+	+		
Johannesbaptistia pellucida (Dickie) Taylor & Drouet	+		+	
Lyngbya lutea (Ag.) Gom.	+	+		+
Merismopedia warmingiana Lagerh.			+	
Microcystis viridis (A. Br.) Lemm.	+		+	
Oscillatoria amphibia Ag.	+	+	+	+
O. deflexoides Elenk. & Kossinsk.		+		
O. minima Gicklh.	+			
O. planctonica Wolosz.	+	+	+	+
O. putrida Schmidle	+			+
O. sancta (Kütz.) Gom.		+		
EUGLENOPHYTA				
Euglena caudata Htibn.		+		
E. pisciformis Klebs var. pisciformis		+		

E. proxima Dang.		+		
Phacus curvicaudata Swir. f curvicaudata	+			
Trachelomonas dybowskii Drezep.	+			
T. volvocina Ehrenb.	+		+	
DINOPHYTA				
Peridinium umbonatum Stein	+			
CRYPTOPHYTA				
Rhodomonas lacustris Pasch. & Ruttn.			+	
BACILLARIOPHYTA				
Achnanthes breviceps Ag.	+	+	+	
A. lanceolata (Breb.) Grunow		+		+
A. minutissima Kütz	+	+	+	+
AmphiprorapaludosaW. Sm.	+		+	
Amphora pediculus (Kütz.) Gmnow			+	+
A. veneta Kütz.	+	+	+	+
Anomoeoneis sphaerophora (Ehrenb.) Pfitzer		+		
A. vitrea (Grunow) Ross	+	+		+
Asterionella fonnosa Hassall	+			
Cocconeis placentula Ehrenb.			+	
Cyclotella meneghiniana Kütz.	+	+		
Cymbella cistula (Ehrenb.) Kirchn.	+			
C. helvetica Kütz.	+			
C. pusilla Grunow	+	+		
C. sinuata Gregory				+
Denticula subtilis Grunow	+			
Diatoma moniliformis Kütz.				+
D. tenuis Agardh				+
Diploneis interrupta (Kütz.) Cleve		+		
D. puella (Schum.) Cleve		+		
Fragilaria capucina Desmaziores var. gracilis (Oestr.) Hust.				+
Fragilaria capucina var. vaucheriae (Kütz.) Lange-Bertalot			+	
F. crotonensis Kitt.	+	+	+	+
F. fasciculata (Agardh) Lange-Bertalot			+	+
F. ulna (Nitzsch) Lange-Bertalot	+	+		+
F. ulna var. acus (Kütz.) Lange-Bertalot	+			
Frustulia rhomboides var. crassinervia (Breb.) Ross				+
Gomphonema angustum Ag.	+	+	+	
G. gracile Ehrenb.				+
G. minutum (Ag.) Ag.	+			
G. olivaceum (Homem.) Breb.			+	
G. parvulum Kiltz.			+	+
G. tmncatum Ehrenb.			+	
Gyrosigma acuminatum (Kütz.) Rabenh.		+		
Hantzschia amphioxys (Ehrenb.) Grunow				+
H. elongata (Hantzsch) Grunow			+	

Melosira varians Ag.				+
Navicula accomoda Hust.			+	
N.capitata var. hungarica (Grunow) Ross		+	+	
N. cincta (Ehrenb.) Ralfs				+
N. cuspidata (Kütz.) Kiitz.		+		
N. digitatoradiata (Gregory) Ralfs				+
N. halophila (Grunow) Cleve	+	+	+	+
N. lanceolata (Ag.) Ehrenb.				+
N. phyllepta Kütz.		+		
N. radiosa Kütz.	+			
N. rhyncocephala Kütz.				+
N. salinarum Grunow		+		
N. tripunctata (O. F. Müller) Bory	+	+	+	
N.venetaKutz.	+		+	
N. viridula (Kütz.) Ehrenb.		+	+	
Nitzschia brevissima Gronow				+
N. clausii Hantzsch				+
Nitzschia constricta (Kütz.) Ralfs		+		+
N. elegantula Grunow		+		
N. filiformis (W. Smith) Van Heurck				+
N.levidensis var. salinarum Grunow		+		+
N. palea (Kütz.) W. Smith	+			+
N. sigmoidea (Nitzsch) W. Smith	+	+		+
N. tubicola Grunow	+	+		
Pînnularia viridis (Nitzsch) Ehrenb.	+		+	+
Surirella brebissonii var. kuetzingii Krammer & Lange-Bertalot			+	+
S. striatula Turpin			+	
S. subsalsa W. Smith		+		
Stauroneis salina W. Smith		+		
S. smithii Grunow				+
CHLOROPHYTA				
Carteria geminata Ettl.		+		
Cosmarium abbreviatum Racib. var. abbreviatum	+			
C. granatum Breb.	+		+	
C. laeve Rabenh. var. laeve	+			
C. tenue Arch.	+			
Monoraphidium arcuatum (Korsh.) Hind.	+			
M. contortum (Thur.) Kom.-Legn.	+			
M. pseudobraunii (Belcher & Swale) Heynig	+			
Pandorina morum (O. F. Müller) Bory	+			
Pediastrum boryanum (Turp.) Menegh.			+	
Scenedesmus quadricauda (Turp.) Breb.	+			
Spirogyra sp.	+		+	
Tetraedron minimum (A. Br.) Hansg.	+		+	
Ulothrix aequalis Kütz.	+	+	+	+

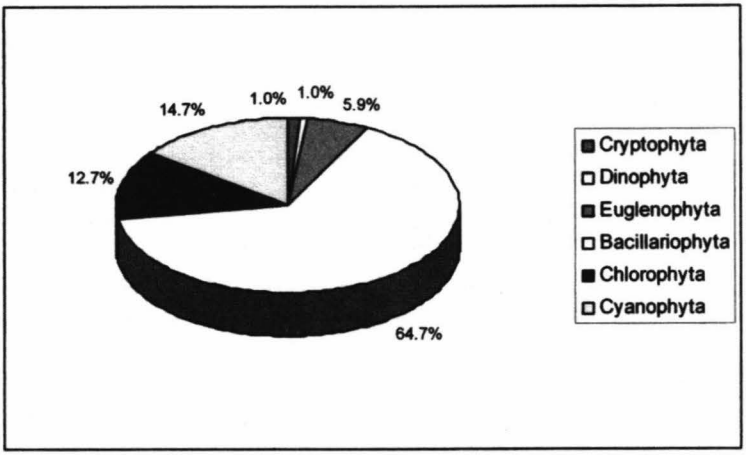

Fig. 2. Percentage distribution of species in the main algal group

The values of the compound and chlorophycean indices indicate eutrophic waters (Tab. 3).

Table 3. Values of trophic indices

Indices	**Calculation formula** [13]	**Interpretation of index values**	**Evaluation of results**
Compound Index	Number of Cyanophyta, Chlorocaccales, centric diatoms and Euglenaphyta species/number of Desmidiales species	$\leq$1 oligotrophic 1-3 mesotrophic > 3 eutrophic	7.25 eutrophic
Chlorophycean Index	Number of Chlorococcales species/number of Desmidiales species	$\leq$1 oligotrophic > 1 eutrophic	1.75 eutrophic

Comparing the saprobity preferences of the species identified in 1879-1880 with those identified in 2006 (Fig. 3, 4), became evident that in 1880 the number of species that prefer clean water conditions (xeno-, oligo-, oligo-P and p-mesosaprobic species) is much higher then of those which indicates critical saprobity level. By the contrary, in 2006 the number oft axa indicating high organic loading of the water (p-a-, a- mesosaprobic, polysaprobic species) is raised.

Affinities at the level of communities have been tested by cluster analysis, using the floristic similarity index of Jaccard. The values of this index varied between 0.2 and 0.3, indicating that the algal communities of this aquatic habitats are well defined (Fig. 5). The highest floristic similarity of 0.3 was found between samples 1 and 3, probably, due 1o the similar conductivity and salinity conditions.

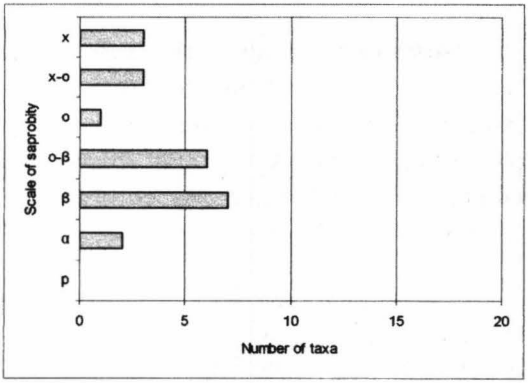

Fig. 3. Saprobic preferences of the species identified in **1979-1880**

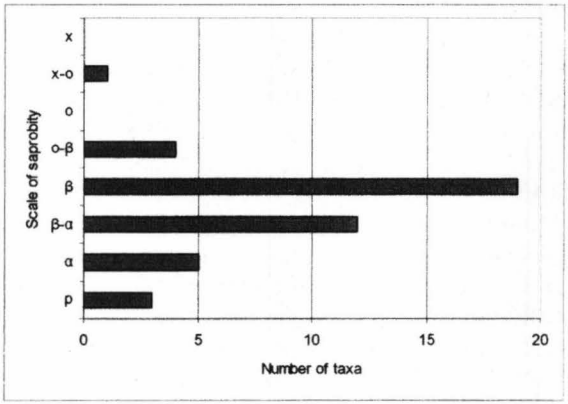

Fig. 4. Saprobic preferences of the species identified in **2006**

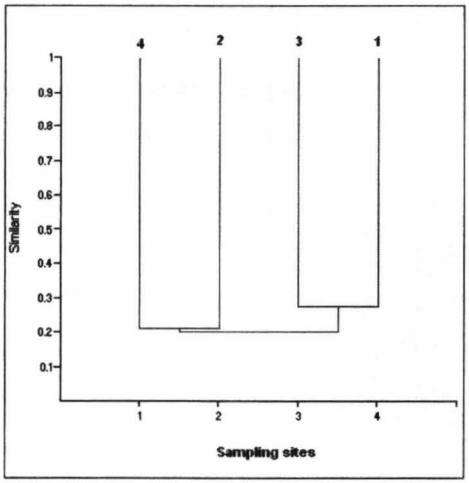

Fig. 5. Dendrogram showing the floristic similarities of the studied algal communities (1. Pool 1; 2. Pool 2; 3. Ponds; 4. Spring water collector basin)

Conclusions

The floristic analysis of the samples revealed a great diversity of the algal communities with 102 taxa belonging to 6 algal divisions. Most of the identified species are halophilous and halobiont elements. The values of the compound and chlorophycean indices indicate eutrophic water conditions.

Acknowledgements: The authors are grateful to the Institute of Biological Research for providing laboratory facilities.

REFERENCES:

COESEL, P. F. M., 1991, *De Desmidiaceeën van Nederland,* Deel 4,5,6, Fam. Desmidiaceae (2, 3, 4), Stichting Uitgeverij Koninklijke Nederlandese, Natuurhistorische Vereniging.

CUNA, S., BERDEA, P., BACIU, C., 2001, *Isotopic investigations on the mineral waters from Someşeni – Cluj, Romania, Studia Universitatis Babeş-Bolyai,* Special Issue.

KOMÁREK, J., ANAGNOSTIDIS. K., 1998, *Cyanoprokaryota,* Teil 1: Chroococcales, Gustav Fischer Verlag, Jena. Komárek, J., Fott, B., 1983, *Das Phytoplankton des Susswasser,* Chlorophyceae: Chlorococcales, E. Schweizerbartische Verlagsbuchhandlung, Stuttgart.

KRAMMER, K., LANGE BERTALOT. H., 1986, 1988, 1991, *Süsswasserflora von Mitteleuropa,* Band 2. *Bacillariophyceae,* Teil 1-4, Gustav Fischer Verlag, Jena.

PRESCOTT, G. W., 1962, *Algae of the Western Great Lakes Area,* W. M. C. Brown Company Publishers, U.S.A.

SCHAARSCHMID, J., 1880, *Additamanta ad Algologicam dacicam 11: Enumeratio algarum nonnularum in comutatibus Naszod-Beszterce, Doboka, Kolos, Torda, Aranyos, Also-Feher, Udvárhely, Fogaras, Szeben, et Hunyád Lectarum, Magyar Novenytani Lapok* IV: 46.

SCHMIDT, A., FEHER, G., 2001, A *sárgászold algák (Xanthophyceae) kishatározoja,* Vízi Természet- és Környezetvédelem 13, Köryezetgazdálkodási Intézet, Budapest.

UHERKOVICH, G., 1995, 1966, *Die Scenedesmus-Arten Ungarns,* Akadémiai Kiadó, Budapest.

UHERKOVICH, G., 1995, A *Scenedesmus zöldalga nemzetség (Chlorococcales, Chlorophyceae) különös tekintettel magyarországi előfordulású taxonjaira,* Magyar Algólogiai Társaság, Budapest. 1.

TOMESCU, N., GABOS, M., 1989, *Hidrobiologie,* Universitatea „Babeş-Bolyai" din Cluj-Napoca. 112.

TÖMÖSVÁRY, O., 1879, *Bacillariaceas in Dacia observatas, Magyar Növénytani Lapok* III: 34.

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COESEL, P. F. M., 1991, *De Desmidiaceeën van Nederland,* Deel 4,5,6, Fam. Desmidiaceae (2, 3, 4), Stichting Uitgeverij Koninklijke Nederlandese, Natuurhistorische Vereniging.

CUNA, S., BERDEA, P., BACIU, C., 2001, *Isotopic investigations on the mineral waters from Someşeni – Cluj, Romania, Studia Universitatis Babeş-Bolyai,* Special Issue.

KOMÁREK, J., ANAGNOSTIDIS. K., 1998, *Cyanoprokaryota,* Teil 1: Chroococcales, Gustav Fischer Verlag, Jena. Komárek, J., Fott, B., 1983, *Das Phytoplankton des Susswasser,* Chlorophyceae: Chlorococcales, E. Schweizerbartische Verlagsbuchhandlung, Stuttgart.

KRAMMER, K., LANGE BERTALOT. H., 1986, 1988, 1991, *Süsswasserflora von Mitteleuropa,* Band 2. *Bacillariophyceae,* Teil 1-4, Gustav Fischer Verlag, Jena.

PRESCOTT, G. W., 1962, *Algae of the Western Great Lakes Area,* W. M. C. Brown Company Publishers, U.S.A.

SCHAARSCHMID, J., 1880, *Additamanta ad Algologicam dacicam 11: Enumeratio algarum nonnularum in comutatibus Naszod-Beszterce, Doboka, Kolos, Torda, Aranyos, Also-Feher, Udvárhely, Fogaras, Szeben, et Hunyád Lectarum, Magyar Novenytani Lapok* IV: 46.

SCHMIDT, A., FEHER, G., 2001, A *sárgászold algák (Xanthophyceae) kishatározoja,* Vízi Természet- és Környezetvédelem 13, Köryezetgazdálkodási Intézet, Budapest.

UHERKOVICH, G., 1995, 1966, *Die Scenedesmus-Arten Ungarns,* Akadémiai Kiadó, Budapest.

UHERKOVICH, G., 1995, A *Scenedesmus zöldalga nemzetség (Chlorococcales, Chlorophyceae) különös tekintettel magyarországi előfordulású taxonjaira,* Magyar Algólogiai Társaság, Budapest. 1.

TOMESCU, N., GABOS, M., 1989, *Hidrobiologie,* Universitatea „Babeş-Bolyai" din Cluj-Napoca. 112.

TÖMÖSVÁRY, O., 1879, *Bacillariaceas in Dacia observatas, Magyar Növénytani Lapok* III: 34.

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METHODS OF WASTEWATER TREATMENT IN TEXTILE INDUSTRY

Laszlo BERKESY[1], Corina BERKESY[2]

Abstract. Residual water from Textiles presents a great variety in composition and amount. According to the technologies we used, the waste water consists of varied substances which should be reduced so that it can be dischanged into the sewarage or the river with no serious effects on the environment.
This work outlines some research to correct the parameters of the water resulted from Textiles, using chemical methods.
Key words: Wastewater, chemical treatment, coagulants, floculants.

Introduction

The wastewater resulted from Textiles presents a great variety of compositions, because of the technologies used in the production. This is the reason of using diverse methods to treat them.

In the textile industry a large quantity of water is used for several processes. Waste water originating from the pre-treatment process (as bleaching, cooking, washing, etc.) contains a very large quantity of pollution. (2,3)

The values of qualitative wastewater parameters must be reduced before to discharge them into the municipal severage system or in the river and in this way the environment is protected.

The main indicators of wastewater from textile industry analised before and after the treatment are: pH, suspended solids, BOD, COD that represent the chemical oxygen demand in mg/l for the oxidation of the mineral salts and of the organic substances , dissolved salts and coulour.

In the last period it was enough a preliminary treatment , now the values of the wastewater parameters must be in acordance with the NTPA 002/2002 to be discharged in the municipal sewerage system and NTPA 001/2002 to be discharged in the river. In the Textiles there exist a lot of difficulties to treat the wastewater there to the standard values. So, it is necessary to to look for new methods to treat this kind of wastewaters.

The usual techniques of purifying the wastewaters from dying factories or generally, from the textile industry are: physical, chemical and biological or a combination of these methods.

Also it was obtained good results using the chemical methods, especially using coagulants and floculants from the polyelectrolit group. Using this method the effect of the chemical step in the wastewater cleaning plant was increased (2).

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This work outlines the studies and the tests which were used to correct the quantitative perameters of wastewater resulted from Textiles. The wastewater from the textile factory dealt with in the first case presents a great variety of composition, because of the technologies used in production, especially chlorides and residue to 105°C. (case 1 paremeters values are higher like the values allowed in NTPA001/2002).

The wastewater from the textile factory dealt with in the second case presents high chlorides, residue to 105°C, BOD and COD values higher than the values allowed in NTPA 001/2002.

To reduce the specified parameters we can used some methods like:

- Reverse osmosis. This method presents the disadvantage of obtaining a secondary wastewater which is very rich in disolved salts. Also the membranes of the equipment cannot be used in case of concentrated wastewater .
- The filter with ion – exchange resin. This method presents a disadvantage because of the technology of regeneration realised with some other salts . In this way the paremeter residue to 105 °C increases.
- The chemical treatment .In case of the chemical treatment it can be used the usual coaulants, floculants and polymers (2, 5).
- The electrochemical treatment. In this case the natrium chloride precipitates with the other salts existenting in the studied wastewater. The precipitate must be retained in the first basin (1,4).

Material and methods

The first step was the characterization of the wastewater samples.The analised parameters were: the pH, temperature, BOD, COD, residue to 105 °C, chloride, conductivity, turbidity.

In the second step it was applied a treatment with coagulants and floculants to wastewater in order to reduce COD , turbidity , residue to 105 °C and chlorides.

The tests were realised using wastewater sampled from the cleaning plant. The Table nr. 1 presents the studied wastewater parameters in the two cases.

In the first case the wastewater was sampled in a period in which the used technologies take place in the some time. In this case the parameters: chloride and resudue to 105 °C present high values, higher than the values allowed in NTPA 002/2002 (Table nr 1). We have tested more variants with chemical treatment and we have improved the physico-chemical step.

In the socond case the wastewater is characterised with the variability of the parameters. The water was sampled from a basin in which the wastewater is homogenised.

Tabel nr. 1: **The wastewater parameters compared with NTPA 002/2002 and NTPA 001/2002**

Parameter	U/M	Realised max.		NTPA 002/2002	NTPA 001/2002
		Case 1	Case2		
Temperature	°C	40	40	40	30
pH		6,5-8,5	7,5-9,5	6,5-8,5	6,5-9,0
Rezidue to 105 °C	mg/dm3	5000-7000	3032	2500	2000
Chloride	mg/dm3	3000-3800	2388	2000	500
BOD	mg/dm3	149	70-90	300	20
COD	mg/dm3	450-470	225	500	70

Chemical treatment

The physico-chemical experiments were carried out in a Jar –Test apparatus.

The tests were performed using coagulants 71225 from NALCO and Al2(SO4)3, as coagulants and 7752 – cationic floculant from NALCO.

The table 3 shows the quantities of coagulants and floculants added to treat the wastewater in the two studied cases. (The coagulant concentration range varied between 5,0 and 15,0 ml -0,5%and aluminium sulphat 40-60 ml -0,5%. The floculant concentration range varied betveen 1,0-2,0 -0,5%).

The procedure consisted in introducing 500 ml of the sample in the jars, the coagulant was added and rapidly mixed during 4,5 minutes. (2) Then the flocolant was introduced in the jars for an additional time of 15 minutes.

The coagulant and floculant concentrations were studied .

The treatment with coagulants and floculants used in case of wastewater form textile industrry *(Variants of treatment)*

Table nr. 2

Nr.	Sample	Treatment					
		Coagulant			Floculant		
		NALCO	Conc %	quant ml.	NALCO	Conc %	quant ml.
1	M	–	–	–	–	–	–
2	1	71225	0,5	5,0	7152	0,5	1,0
3	2	71225	0,5	10,0	7152	0,5	
4	3	71225	0,5	15,0	7152	0,5	

5	4	71225	0,5	15,0	7152	0,5	2,0
6	5	71225	0,5	5,0	7752-cationic	0,5	1,0
		Aluminium sulphate	0,5	40,0	–	–	–
7	6	71225	0,5	5,0	7752-cationic	0,5	1,0
		Aluminium sulphate	0,5	50,0	–	–	–
8	7	71225	0,5	5,0	7752-cationic	0,5	1,0
		Aluminium sulphate	0,5	60,0	–	–	–
9	8	71225 Aluminium sulphate	0,5	5,0	7752-cationic	0,5	2,0
		Aluminium sulphate	0,5	50,0	-	-	-

Results

Using the treatment with coagulant 71225 and floculant 7152 in some concentrations we obtained a decrease of COD values with 43% sample 1 (case 1), 48% (case 2) and 60% sample 4 (case 1) and 68% (case 2).

Using the treatment with coagulant 71225 and floculant 7152 in some concentrations we obtained a decrease of turbidity values.

In case of sample 5, using the treatment with aluminium sulphate and coagulant 71225 for coagulation and cationic floculant 7752 for floculation we obtained a decrease of chlorine concentration with 19,29% in the samples in the first category (case1) and 20% in the samples in the second category (case 2).

In case of sample 6,we can see a decrease with 18,74% of chloride values (case 1) and with19,47% in case 2.

In case of sample 7,we can see a decrease with 16,09% of chloride values (case 1) and with15,03% in case 2.

The figure 1 shows the variation of COD and turbidity values after the chemical treatment.

The figure 2 shows the variation of residue to 105 and chlorides values after the chemical treatment

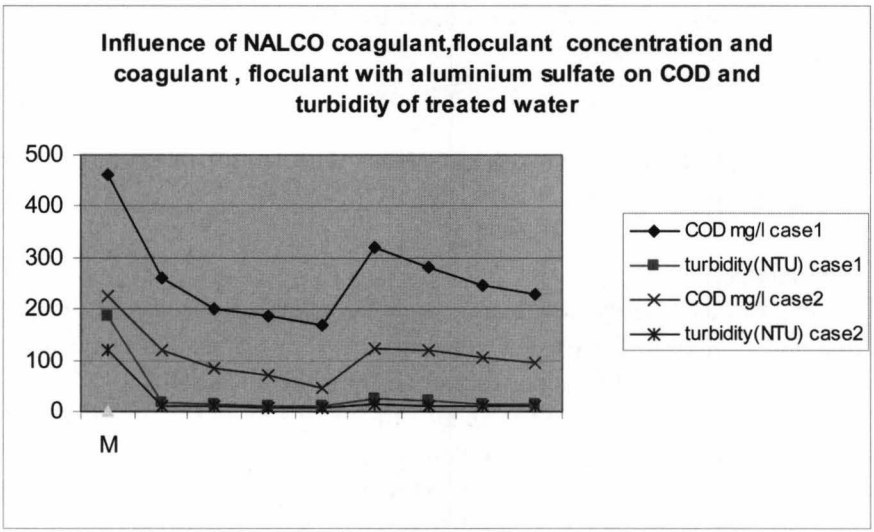

Fig. nr. 1

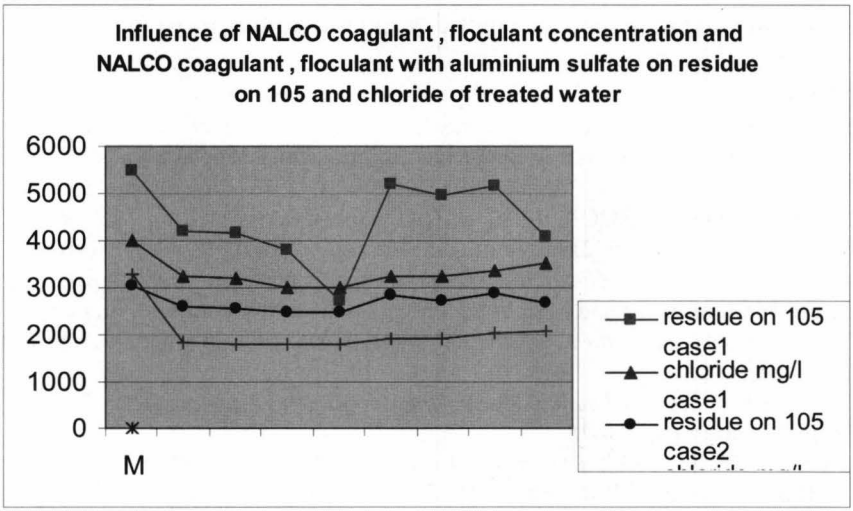

Fig. nr. 2

In case of sample 8, we can see a decrease with 11,79% of chloride values (case 1) and with13,02% in case 2.

In case of sample 4, using the treatment with aluminium sulphate and coagulant 71225 for coagulation and cationic floculant 7752 for floculation we obtained a decrease of the parameter residue to 105 concentration with 5,42% to the samples in the first category (case 1) and 6% to the samples in the second category (case 2).

In case of sample 5, we can see a decrease with 9,16% of residue to 105 values (case 1) and with10,02% in case 2.

In case of sample 6, we can see a decrease with 5,66% of residue to 105 values (case 1) and with 5,5% in case 2.

In case of sample 8, we can see a decrease with 9,44% of residue to 105 values (case 1) and with 11,01% in case 2.

Conclusions

The experiments with coagulants and floculants from NALCO in different concentration and applied to the wastewater from textile factories had as result a decrease of the parameters COD with 60% and a very good decrease of turbidity.

Using the chemical treatment to reduce the chlorides and the residue to 105 °C in wastewater from the textile industry we obtained a decrease with 20 % in case of chlorides and with 11% in case of residue to105 °C. This decrease is not enough to reduce these parameters to the limite to be discharged in in the municipal severage system.(NTPA 002/2002).

In this case it is necessary to continue the studies with other methods like: electrochemical method to reduce the chlorides and the residue to 105°C.

REFERENCES:

BADEA, T., BRÎNZOI, V., NICOLA, M., 1986: *Epurarea electrochimică a apelor reziduale de la secţiile de galvanizare,* I. Ape impurificate cu ioni cromat, Metalurgia, vol. 38,7, pag. 349-351.

BES-PIA, A., MENDOZA – ROCA ,J.,A., ALCAINA –MIRANDA ,M.,I., IBORRA-CLAR, A., IBORRA-CLAR, M.I., 2002: *Reuse of watewater of textile industry after its treatment with a combination of physico-chemical treatment and membrane technologies,* International Congress on Membranes and Membrane Processes (ICOM), Toulouse, France.

DEMMIN, T., UHRICH, K., 1988: *Improving carpet Wastewater Treatment*, American Dyestuff Reporter, pag. 14-16.

KENNEDY, M., 1991: *Electrochemical Wastewater Treatment Technology for Textiles,* American Dyestuff Reporter, pag. 26-28.

LIN, S. H., CHEN, M. L., 1997: *Treatment of textile wastewater by chemical methods for reuse,* Water Research volum 31 nr. 4, pp. 868-876.

Rezumat: Apele uzate din industria textilă prezintă o mare varietate a compoziţiei, acest fapt datorându-se în principal tehnologiilor utilizate. Din această cauză metodele pentru tratarea acestora sunt diverse. În această lucrare ne propunem să testăm apa reziduală provenită din fabrici diferite prin metoda chimică; de asemenea vom arăta câteva dintre posibilităţile de tratare a acestor ape în vederea reciclării.

THE STATUS OF BIOLOGICAL AND LANDSCAPE DIVERSITY OF COLLEMBOLAN FAUNA IN THE ROMANIAN CARPATHIANS

Cristina FIERA[1]

Abstract. Almost **300** species of Collembola (springtails) have been recorded and 79 papers have been published concerning collembolan fauna, taxonomy and ecology since 1883 in the Romanian Carpathians Mountains. The paper contains **27** collembolan species, which types localities are situated in the territory of the Romanian Carpathians. The status of some endemic species from the Carpathians Area is discussed. Numerous human activities affecting the environment of Collembolan fauna from the Carpathians area are also mentioned.
Key words: Collembola, Romanian Carpathians, endemic species, history of investigations, conservation

Introduction

The Carpathian Mountains extend some 1500 km across seven European countries, from Romania (55%) in the South, through Ukraine (11%), Poland (10%), Slovakia (17%) and Hungary (4%) to the Czech Republic (3%) and Austria (15) in the North (see on the map). Covering an area of 209 256 km^2, the Carpathian Mountains are the second largest chain of mountains in Europe. Crossing the largest area of any mountain chain in Europe, this unique region is home to a wide array of wildlife, diverse nationalities and a rich cultural heritage. The natural diversity supported by the Carpathians is of vital importance for Europe. On a continent where 56% of forest cover has been lost and only 2% of the remaining natural forest is protected, the Carpathians support Europe's most extensive tracts of mountainous forest (between the heights of 950 and 1350 m above sea level), the continent's largest remaining natural mountain beech and beech/ fir forest ecosystems and the largest area of virgin forest left in Europe. Together with semi-natural habitats such as mountainous pastures and hay meadows, which are the result of centuries of traditional management of the land, the Carpathians harbor a richness of natural diversity and many endemic species of plants and animals (WEBSTER et al. 2001).

The Carpathian Ecoregion Initiative, facilitated by the WWF International Danube Carpathian Program, has been responsible for mapping overall biodiversity in the Carpathian Ecoregion, as a first step in its targeted conservation efforts.

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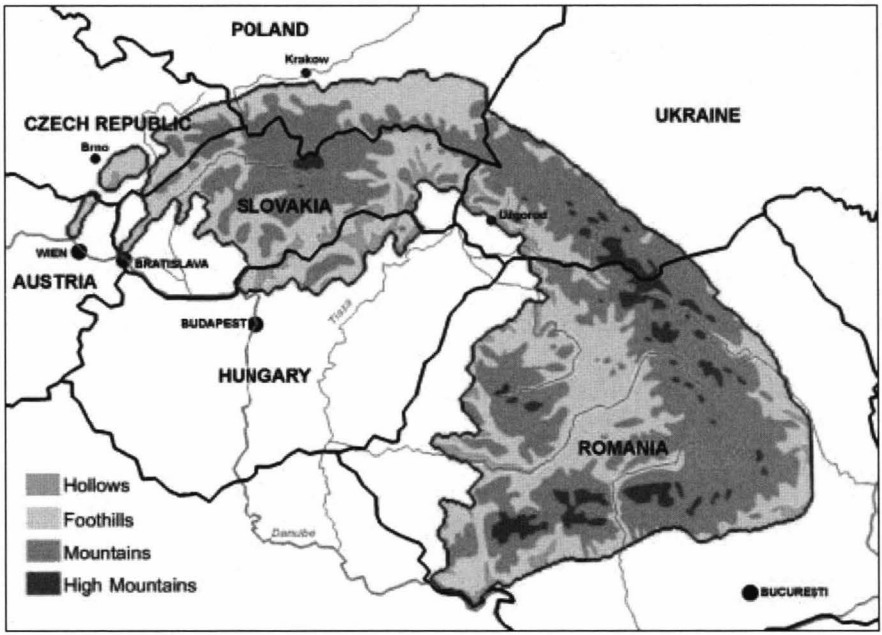

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The Romanian Carpathians are characterized by having unique characteristics, with a wide set of environmental, landscape and management variables. The Romanian Carpathians are characterized by having an altitudinal (mountain) and plain areas from East (Orientali Carpathians) to South (Meridionali Carpathians). There are various habitats on a relatively small surface and the mountain ecosystems are therefore rich in species. These are well adapted to the extreme conditions (short vegetation period, water shortage, UV-radiation, wind). The glaciations were very important in spatial and temporal distribution of plant and animal species. Specific habitats are rock chasms, screens and Alpine grasslands.

Material and Methods

An analysis of data from the literature was done in this paper. We provide personal observation on all species of Collembola, inhabitant of mountain area of Romania. There are also included unpublished records after there have been studying the collections of the Institute of Biology, Romanian Academy, Bucharest and the Institute of Systematic and Experimental Zoology, Polish Academy of Sciences, Kraków, Poland.

Results and discussions

The data on the distribution of Collembola species in the Romanian Carpathians are fragmentary and limited. 27 new species of springtails are situated in the territory of

the Romanian Carpathians (388 species in our country - FIERA, 2007) (Table 1); 300 species are in the territory of the Romanian Mountains (FIERA, personal comments) and 25 species of Collembola are endemic for Carpathians. These species are listed in Table 1, except of 2 species (*Entomobrya dumitrescui* GRUIA, 1967 and *Protaphorura ionescui* RADWAŃSKI, FIERA & WEINER, 2006), which I consider not endemic due to a wide distribution in our country. 9 Collembolan species probably are endemic in chain of Carpathians Mountains (*Heteraphorura carpatica* (Stach in Kseneman, 1938), *Orchesella carpatica* (Ionescu, 1914), *O. maculosa* Ionescu, 1914, *Tetracanthella carpatica* Stach 1947, *Plutomurus carpaticus* Rusek & Weiner, 1978, *Endonura tatricola* (Stach, 1951), *Deutonura plena* (Stach, 1951), *Pachyotoma granulata* Stach, 1947, *Entomobrya violaceolineata* Stach 1963).

Table 1. Described Species of Collembola from Romanian Carpathians with *locus typicus*

Nr. Crt.	Species	Author	Locus typicus
1.	*Adbiloba plurichaetosa*	CASSAGNAU et PEJA 1979	Stâna de Vale, Bihor Mountains
2.	*Acherontides tanasachiae*	GRUIA, 1969	Dodoconi Cave, Retezat Mountains, West Jiu Valley
3.	*Anurida ariesi*	HARŞIA & GRUIA, 1992	Sălciua de Jos, Apuseni Mountains, 1.000 m altitude, beech forest (*Phyllitidi- Fagetum*, Vida, 1963)
4.	*Argonychiurus bogheani*	GRUIA, 1989	Piatra Ponorulu Cave, Bihor Mountains
5.	*Deharvengiurus orghidani*	GRUIA, 1967	Arnăuților Cave, Stogu- Vânturarița Massiv, Olăneşti Locality
6.	*Deuteraphorura banatica*	GRUIA, 1965	Voinicovăț Cave, Berzeasca Valley, Almăjului Mountains (Banatului Mountains), Moldova-Nouă locality
7.	*Deuteraphorura closanica*	GRUIA, 1965	Cloşani Cave – on Motrului Valley, Piatra Cloşani Massiv (1427 m), Cloşani locality
8.	*Deuteraphorura hategana*	GRUIA, 1971	Caves: Cioclovina cu Apă, Cioclovina uscată, (Sebeş Mountains), Cioclovina Locality
9.	*Deuteraphorura meziadica*	GRUIA, 1972	Meziad Cave (Pădurea Craiului Mountains) 397 m altitude
10.	*Deuteraphorura romanica*	GRUIA, 1965	Liliecilor Cave (Codru-Moma Mountains-Apuseni), 900 m altitude, Moneasa locality (Bihor county)
11.	*Deuteraphorura traiani*	GRUIA & POPA, 2005	Avenul din curtea Cerdacului, Stanciului, Piatra Craiului Massif
12.	*Entomobrya dumitrescui*	GRUIA, 1967	Casimcea Locality, Dobrogea and near Gura Zlatna

13.	*Heteromurus noseki*	MARI MUTT & STOMP, 1980	Bazin, Apa Moiştii Cave, MSS beside Cloşani Cave
14.	*Micranurida retezatica*	GRUIA & HARŞIA, 1990	Meridional Carpathians, Retezat Mountains, Retezat National Park- scientific rezervation, Gura Slatna, Zlătuia Valley, 1.200m altitude, a.s.l., V exp., *Symphyto cordatae- Fagetum*, humus sample
15.	*Micraphorura multiperforata*	GRUIA, 1973	Gheţarul de la Scărişoara Cave (Bihor Mountains -Apuseni, 1.165 m altitude, Scărişoara Locality
16.	*Oncopodura pegyi*	GRUIA, 1994	Altarului Cave (Bihor Mountains- Apuseni)
17.	*Onychiuroides multisetis*	GRUIA, 1971	Gheţarul de la Scărişoara Cave (Bihor Mountains- Apuseni), 1.165 m altitude, Scărişoara locality
18.	*Orchesella carpatica*	IONESCU, 1914	the entrance to the Mănăstirea Bistriţa Cave- Căpăţânii Mountains
19.	*Orthonychiurus doinae*	GRUIA, 1972	Valea Fundata Cave and Cerneadeal Cave (Postăvarul Massiv)
20.	*Protaphorura borzica*	GRUIA, 1999	Borzii Vineti shaft, Mierlei Plaine, Retezat Mountais, 2000 m altitude
21.	*Protaphorura ionescui*	RADWAŃSKI, FIERA & WEINER, 2006	Rodnei Mountains, Borşa Locality, N slope of Pietrosul Rodnei 2303 m, ca. 1200 m a.s.l. *Plagiothecio -Piceetum* Association, litter and soil.
22.	*Pseudosinella manuelae*	GRUIA, 1974	Vraşca Cave- Cheile Caraşului, Defileul Dunării, Caraşova Locality
23.	*Pseudosinella pallida*	GRUIA, 1977	Albiilor Valley and Sohodoale Valley, Motru Sec bazin, Mehedinţi Mountains, 600- 650 m altitude
24.	*Pseudosinella sandelsorum*	GRUIA, 1977	Albiilor Valley and Sohodoale Valley, Motru Sec bazin, Mehedinţi Mountains, 600- 650 m altitude
25.	*Pseudosinella racovitzai*	GISIN & GAMA, 1971	Cioclovina Cave, Luncani (Haţeg), Ponorici Cave, Pui, Muntii Sebeş
26.	*Tetracanthella gruiae*	RUSEK, 1979	Bucegi Massif, Vârful cu Dor
27.	*Tetracanthella transylvanica*	(CASSAGNAU, 1959)	„Alpes de Transylvanie", Retezat Mountains

*

The first study of the collembolan fauna in the Romanian Carpathians was the work of TÖMÖSVÁRY (1883), who recorded three species from the Western part of Romania, two of these being Carpathians forms (*Allacma fusca* (LINNAEUS, 1758) and *Sminthurus viridis* (LINNAEUS, 1758)). The research continued with the work "Fauna

Regni Hungariae", of VELLAY (1900), who reported another 24 species of springtails from Romanian Carpathians.

Between 1914-1922 there were revealed 20 species of springtails collected by Prof. C.N. IONESCU (*Caprainea marginata* (SCHÖTT, 1893), *Deuterosminthurus bicinctus* (KOCH IN HERRICH-SCHÄFFER, 1840), *Dicyrtoma fusca* (LUBBOCK, 1873), D*icyrtomina minuta* (FABRICIUS, 1783), *Dicyrtomina violacea* (KRAUSBAUER, 1898), *Lipothrix lubbocki* (TULLBERG, 1872), *Orchesella balcanica* STACH, 1960, *Orchesella carpatica* (IONESCU, 1914), *Sinella curviseta* BROOK, 1882 *Spatulosminthurus flaviceps* (TULLBERG, 1871), S*phyrotheca multifasciata* (REUTER, 1881), *Orchesella pontica* IONESCU, 1915 – Muntenia, Moldova, North of Dobrogea forests (IONESCU 1915), *Sminthurides aquaticus* (BOURLET, 1842), *Sminthurides cruciatus* AXELSON, 1905, S*minthurides malmgreni* (TULLBERG, 1876), *Sminthurides penicilifer* (SCHÄFFER, 1896), *Sminthurinus niger* (LUBBOCK, 1867), *Mesogastrura ojcoviensis* (STACH, 1919), *Pseudacherontides spelaeus* (IONESCU, 1922), *Mesachorutes quadriocellatus* ABSOLON, 1900. Other records were given in the paper of SZENT-IVÁNY (1938).

Since these basic works were done, numerous taxonomical, faunistic and ecological papers on the Collembola communities of the Romanian Carpathians have been published. Until 1950 there were recorded 52 species from the Romanian Carpathians. The rate of speciation has been growing up considerably nowadays.

In the second half of the 20st century, the research in the Romanian Carpathians Mountains continued with the works of STACH (1947–1963), M. IONESCU (1951), MACK-FIRĂ (1961, 1963, 1965), RUSEK (1978) and BULIMAR (1980, 1982, 1983, 1986, 1987, 1991, 1992*a*, *b*, 1994), KONTSCHÁN et al. (2003). The last author made many ecological studies regarding Collembolan fauna in the Călimani Mountains.

The most investigated were the collembolans by GRUIA (1964- 2003) from caves. The author published 29 papers and described 22 new species for science from the caves of the Romanian Carpathians Mountains.

The most studied mountains were Bucegi GRUIA & ZAMFIRESCU (1973), FIERA (in press) and Retezat Massif. WEISNER (1984) made some estimation of productivity parameters in two Collembola communities in soil under a timberline spruce forest and a Mugo pine shrubbery in the Retezat National Park. POPOVICI et al. (1984) gave new data on the structure, abundance and biomass dynamics of the soil fauna in some forest ecosystems in the same national park. Also, Prof. Falcă, from the Institute of Biology, Bucharest made his Ph thesis (FALCĂ 1984) on Collembola communities from three massifs of Meridionali Carpathians (Bucegi, Gârbova and Retezat Mountains). He published seven ecological papers (FALCĂ 1989a, 1989b, 1990, 1991a, 1991b, 1993, 2005) regarding the numerical dominance of species diversity of Collembola from Bucegi, Gârbova and Retezat Mountains, as well as aggregation, dispersion and expansion of the species. It was established that similarity of Collembola is related, among other factors to the structure of tree and herbaceous layers as well as to the type of soil (FALCĂ 1989b). In

one paper (FALCĂ 1990), the author made partial and multiple correlations between collembolan species and abiotic factors. The coefficients of correlation and partial determination in the three mountains demonstrated a high level of dependence of Collembola on precipitations and a less dependence on the other factors. One year later, he established the negative binomial distribution of *Folsomia quadrioculata*, the most representative species from all species collected (FALCĂ 1991a). Also, he determined the biomass and productivity of Collembola, indicating the role of each species in the activity of nutrient cycling and energy flow in an ecosystem (FALCĂ 1991b).

In 1992 and 1993, the research in the Retezat National Park continued with the work of HARŞIA (1992) and (FALCĂ 1993). A new data on the structure, abundance and biomass dynamic of Collembola from Gârbova Massif can be found in (FALCĂ *et al.* 2005).

There is only one work regarding the Collembolan fauna from the Apuseni Mountain (the West part of the Romanian Carpathians HARŞIA (1995). In this paper, 100 species of Collembola are cited from 20 sites situated in the main vegetation belts, namely oakwood, beech- hornbeam, beech, mixed beech- spruce fir and spruce fir forests.

The fauna of Collembola from caves in the Piatra Craiului National Park was studied by NAE *et al.* (2005) who recorded 24 species from 22 caves. Among the identified species, *Deuteraphorura traiani* was a new species for science. A one year later, the research in the limestone area of the Piatra Craiului National Park Ridge continued POPA & GRUIA (2006). Another six caves were studied and 31 species of Collembola were analysed from a zoogeographical and ecological point of view.

New contributions to the Collembola fauna of the Maramureş area (Igniş Mountains, Piatra Mountains, Rodnei Mountains, Maramureş Mountains) was done in the work of DÁNYI *et al.* (2006) who found nine new collembolan species for our country and for the Carpathians Mountains too. In the same year, HARŞIA (2006) described the faunistic composition and structure of Collembola communities in the terraces of the waste dump of "Exploatarea Minieră Rodna", and related this structure to the restoration by fertilizers and grass covering.

The last contribution to the Carpathian fauna can be found in FIERA (in press), who has been studying the niche differentiation of Collembola of the Lăptici Peat Bog (Bucegi Mountains).

PROTECTING THE ROMANIAN CARPATHIANS IS EQUAL TO PROTECTING COLLEMBOLA COMMUNITIES

The biodiversity of the Romanian Carpathians is threatened by numerous human activities:

1. Pollution. The less obvious but important factors are the global pollution and global changes:

- local sources of pollution can be contained;

- more problematic is the global air pollution originating in industrial and urban centers; it has been recognized that the impact of such pollution is substantial, many sensitive species have disappeared, the communities have been altered and the functioning of ecosystems has been disrupted (withering of dwarf pine); in addition, mountain lakes are adversely affected by such pollution;
- impact of global changes, such as climate change, depletion of the ozone layer in the stratosphere and the increased UV-radiation, is considerable; mountain organisms, living in extreme conditions are effective bioindicators of such changes.

2. Human activities affecting the environment. The activities which affect the environment are: forestry, agriculture, transport, energy generation, tourism and recreation:

- mountain forests have traditionally been managed in a sustainable manner and the main problems are the conflict with agriculture and the network of forest roads;
- mountain agriculture has traditionally been oriented towards the sustainable exploitation of natural resources; the problems arise from the non-selective application of modern cultivation techniques which are not suitable for mountainous regions (agricultural improvements and application of fertilizes on mountain pastures); mountain agriculture is an activity in decline (emigration from remote mountain farms) and with regard to the conservation of biological and landscape diversity the problem of natural encroachment of vegetation on grasslands (hay meadows, pastures) is becoming increasingly serious;
- loss of habitats is caused by economic exploitation of screens and gravel and sand deposits (for example, the exploitation of gold in Roşia Montana).

3. Mass tourism and leisure activities have an adverse effect on the diversity of the mountains. The causes are:

a. expansion of ski centers in sensitive areas which affects soil; the caves are physically threatened and polluted;

b. pollution of mountain lakes;

c. non-compliance of the strategy concerning the construction of cable ways;

d. modern outdoor activities (rafting, mountain biking, para-gliding, riding motorcycles and motor sledges) invade the last peaceful corners of the Romanian Carpathians

All these activities adversely affect mostly collembolan populations. In addition, the animals living in mountains have not been studied as much as plants. The populations are usually small and spatially limited groups on the edge of the area of species distribution. Every activity affecting their environment could lead to their extinction, if the current status is not analyzed in detail.

Conclusions

The critical review and the compilation of the existing data could result in a record on the status of collembolan fauna. Another problem is that the collected data are not only fragmentary, but also not very often available and thus unsuitable for assessing the status of species and habitats. As the number of Collembola experts in Romania is still limited, the data collected for this paper is dramatically insufficient. Lack of knowledge of

Carpathian Collembolan fauna is not an exception — this problem can be found all over the Carpathian countries. The collembolan fauna of the Carpathians, divided among seven countries, still needs a fundamental inventory in order to draw any solid conclusions about the list of species, their distribution and threats. Any conclusions based on the initial material presented here could be misleading.

Based on information of the occurrence of springtails in the other Carpathians countries, many new species could be discovered in the mountain areas of Romania.

BIBLIOGRAPHY:

BULIMAR, F., 1980: *Particularităţile structurale ale unor cenoze litiricole de colembole (Ord. Collembola, Cls. Insecta) din Codrul Secular Slătioara-Rarău.* Anuarul Muzeului Judeţean, Fascicola Ştiinţele Naturii, Suceava 6: 249–263.

BULIMAR, F., 1982: *Structura şi diversitatea specifică a unor comunităţi de colembole litiericole, saproxilicole şi muscinale din Rezervaţia naturală "Codrul Secular Slătioara, Rarău",* Studii şi comunicări, Societatea de Ştiinţe Biologice din România, Filiala Reghin 2: 367–382.

BULIMAR, F., 1983: *Particularităţile structurale ale unor cenoze litiricole de colembole (Ord. Collembola, Cls. Insecta) din zona forestieră Slătioara-Rarau (II),* Anuarul Muzeului Judeţean, Fascicola Ştiintele Naturii 7: 285–304.

BULIMAR, F. 1987: *Cercetări asupra comunitaţilor de colembole edifice (Insecta: Collembola) la limita superioară a pădurii din Masivul Călimani,* Anuarul Muzeului Judeţean, Fascicola Ştiintele Naturii 9: 115–128.

BULIMAR, F. 1988: *Structura şi biomasa comunităţilor de colembole (Collembola: Insecta) din biotopi saprolignicoli ai Masivului Călimani,* In: Lucrările celei de a IV-a Conferinţe Naţională de Entomologie, Cluj-Napoca, 277–282.

BULIMAR, F., 1991a: *Influenţa fenomenului de uscare prematură a bradului asupra taxocenozelor de colembole. Evidenţierea fenomenului de uscare a bradului prin intermediul faunei edifice,* Anuarul Muzeului Judeţean, Fascicola Ştiintele Naturii 11: 45-55.

BULIMAR, F., 1991b: *Structura taxocenozelor de colembole (Insecta: Collembola) din ecosisteme de pajisti montane (Masivul Călimani),* Analele ştiintifice ale Universităţii "Al. I. Cuza", Iaşi 37(2): 227–234.

BULIMAR, F., 1997: *Cenologia Colembolelor de la altitudini mari ale Masivului Calimani,* Memoriile secţiilor ştiintifice 17(4): 191- 196.

CASSAGNAU, P., 1959: *Contribution à la connaissance du genre Tetracanthella Schött, 1891 (Collembola Isotomidae),* Memoires du Museum National d'Histoire Naturelle I6:199–258.

CASSAGNAU, P. P. & PEJA, N., 1979: *Diagnoses preliminaires de quelques Neanuridae de Grèce et d'Albanie,* Biologia Gallo-Hellenica 12(2): 205–222.

DANYI, L., TRASER, G., FIERA, C. & RADWANSKI, J. M., 2006: *Preliminary data on the collembola fauna of the county of Maramureş (Romania),* Studia Universitatis Vasile Goldiş, Seria Ştiinţele Vieţii 17(Supliment): 47–51.

FALCĂ, M., 1984: *Studiu ecologic al unor artropode (Insecte inferioare) din sol şi litieră din Carpaţii Meridionali. Ph.D. Thesis,* Institutul de Ştiinte Biologice, Bucureşti, 219 pp.

FALCĂ, M., 1989a: *Dominanţa numerică a colembolelor din unele tipuri de ecosisteme forestiere montane,* Studii şi cercetări de biologie, Seria Zoologie 41(1): 31-36.

FALCĂ, M. ,1989b: *Similarity of Collembola from some mountain forest ecosystems,* Revue Romaine de Biologie 34(2): 87-93.

FALCĂ, M., 1990: *Partial and multiple correlations between Collembola and abiotic factors*, Revue Romaine de Biologie 35(1): 17-25.

FALCĂ, M., 1991a: *Distribuţia spatială a colembolelor din solurile unor ecosisteme forestiere montane*, Studii şi cercetări de biologie, Seria Zoologie 43(1-2): 19-22.

FALCĂ, M., 1991b: *Biomass and productivity of Collembola from the soils of some mountain forestry ecosystems,* Revue Romaine de Biologie 36(1-2): 57- 65.

FALCĂ M., VASILIU-OROMULU L., SANDA V., PAUCĂ- COMĂNESCU M., HONCIUC V., MAICAN S., PURICE D., DOBRE A., STĂNESCU M., ONETE M., BIŢĂ- NICOLAE C., FIERA C., ŞINCU E. –D., 2005, *The biodiversity of alder forests from mountainous valleys of the Gârbova Massif*, Proceedings of the Institute of Biology 7: 15- 31.

FIERA C., 2007: *A checklist of the Romanian springtails (Collembola*), Folia Entomologica Hungarica 68: 1–36.

FIERA, C. in press: *Community structure and niche differentiation of Collembola of the Lăptici Peat Bog in the Bucegi Mountains (Romania),* Travaux du Muséum d'Histoire Naturelle „Grigore Antipa "52 [In press.]

GISIN, H. & GAMA, M. M., 1971: *Notes taxonomiques et evolutives sur trois especes nouvelles Pseudosinella cavernicoles provenant de Yougoslavie et de Roumanie*, Revue suisse zoologie 78(6): 217–225.

GRUIA, M., 1964: *Contribuţii la studiul collembolelor din România,* Studii şi cercetări de biologie, Seria Zoologie 16(3): 197–204.

GRUIA, M., 1965*a*: *Specii noi de collembole din peşterile României,* Studii şi cercetări de biologie, Seria Zoologie 17(2): 149–155.

GRUIA, M., 1965*b*: *Contribuţii la studiul collembolelor din România,* Travaux de l'Institut de Spéologie Émile Racovitza 4: 191–202.

GRUIA, M., 1966*a*: *Collembole noi pentru fauna României,* Studii şi cercetări de biologie, Seria Zoologie 18(3): 247–253.

GRUIA, M., 1966*b*: *Specii noi de Onychiuridae şi Entomobrydae pentru fauna României, Travaux de l'Institut de Spéologie Émile Racovitza* 5: 185–194.

GRUIA, M., 1967*a*: *Sur quelques Collemboles cavernicoles de Roumanie*, International Journal of Speology 3: 97–109.

GRUIA, M., 1967*b*: *Trei specii de Hypogastruridae din fauna de colembole a României,* Travaux de l'Institut de Spéologie Émile Racovitza 6: 173–181.

GRUIA, M., 1967*c*: *Collemboles provenant du milieu lapidicole et lithoclassigule*, Revue d'Ecologie et de Biologie du Sol 4(2): 313–322.

GRUIA, M., 1969*a*: *Date asupra răspândirii colembolelor în peşterile României*, Travaux de l'Institut de Spéologie Émile Racovitza 8: 161–178.

GRUIA, M., 1969*b*: *Xenylla tanasachiae n. sp. – une nouvelle Collembole cavernicole de Roumanie*, Reichenbachia 12(2): 15–19.

GRUIA, M., 1970: *Donnés sur certains Collemboles lapidicoles et lithoclasicoles des Carpates et de Dobrogea*, Travaux de l'Institut de Spéologie Émile Racovitza 9: 191–195.

GRUIA, M., 1971: *Sur certaines espèces d'Onychiuridae (Collembola) des grottes de Roumanie,* Acta Zoologica Cracoviensia 16(4): 283–288.

GRUIA, M., 1972: *Quelques espèces nouvelles de Collemboles cavernicoles*, Travaux de l'Institut de Spéologie Émile Racovitza 11: 257–264.

GRUIA, M., 1973: *Onychiurus (Oligaphorura) multiperforatus, nouvelle espèce d' Onychiuridae de Romanie,* Travaux de l'Institut de Spéologie Émile Racovitza 12: 173–176.

GRUIA, M. 1974*a*: *Deux collemboles (Entomobryinae) nouveaux de la faune cavernicole de Roumanie,* Travaux de l'Institut de Spéologie Émile Racovitza 13: 55–59.

GRUIA, M. 1974*b*: *Quelques observations morphologiques sur le développement de Neanura tatricola (Insecta Apterygota, Collembola),* Pedobiologia 14: 213–220.

GRUIA, M., 1975: *Collembole subterane din România.* Ph. D. Thesis, Institutul de Ştiinţe Biologice, Bucureşti, 197 pp.

GRUIA, M., 1977: *Collemboles euédaphiques de la Vallée Motru Sec*, Travaux de l'Institut de Spéologie Émile Racovitza 16: 77–84.

GRUIA, M., 1989: *Nouvelles espèces troglobiontes des Collemboles de Roumanie.* – Miscellanea Speologica Romanica 1: 103–111.

GRUIA, M., 1994: *Les Oncopodura (Collembola, Oncopoduridae) des Grottes de Roumanie*, Mémoires de Biospéologie 21: 67–74.

GRUIA, M., 1999: *Concerning some Onychiuridae species (Apterygota, Collembola) from the shafts in the Retezat Mountains (Romania), alpine areas*, Mémoires de Biospéologie 26: 11–16.

GRUIA, M., 2000: *Collembola of the karstic system of Romania*, Mémoires de Biospéologie 27: 53–60.

GRUIA, M. 2003: *Collembola from Romanian caves*, Travaux du Muséum d'Histoire Naturelle „Grigore Antipa" 35: 139–158.

GRUIA, M. & ZAMFIRESCU, A., 1973: *Variation mensuelle de la faune de Collemboles dans la litiere de Pinus mugo Mill., Picea excelsa (Lam.) et Abies alba Mill. dans trois stations des monts Bucegi (I)* In: ORGHIDAN, T., DUMITRESCU, M., DELAMARE DEBOUTTEVILLE, C. & TABACARU, I. (eds): Livre du cinquantenaire de l'Institut de Spéologie "Emil Racovitza". Colloque National de Speologie. Editura Academiei Republicii Socialiste România, Bucureşti-Cluj, pp. 323–338.

GRUIA, M. & HARSIA, T., 1990: *New species of the genus Micranurida Börner, 1901 (Collembola, Neanuridae) from Romania*, Travaux de l'Institut de Spéologie Émile Racovitza 29: 33–36.

GRUIA, M. & Ilie V., 2003: *Collembola of the karstic system of Romania (II),* Trav. Inst. Speol, Emil Racoviţă 40: 63- 122.

GRUIA, M. & POPA, I., 2005: *Collembola of the Piatra Craiului National Park II*, Travaux de l'Institut de Spéologie Émile Racovitza 43:119–131.

HARŞIA, T., 1992: *Structura comunităţilor de collembole (Insecta, Collembola) din Parcul Naţional Retezat.* – In: POPOVICI, I. (ed.): Parcul Naţional Retezat, Studii ecologice. Editura West Side Computers, Braşov, pp. 243–247.

HARŞIA, T. 1995: *Collembola communities from the Apuseni Mountains (the Carpathians),* Polskie Pismo Entomologiczne 64: 217–228.

HARŞIA, T., 2006: *Collembola Communities in Wastedump from Exploatarea Minieră Rodna,* Studii şi Cercetări- Biology Bistriţa 11: 149- 154.

HARŞIA, T. & GRUIA, M., 1992: *A new species of the genus Anurida Laboulbene, 1865 (Collembola, Neanuridae) from Romania*, Travaux de l'Institut de Spéologie Émile Racovitza 31: 65–67.

IONESCU, C. N., 1914: *Contributions à la faune des insectes Collemboles de Roumanie (en comprenant aussi des formes cavernicoles),* Bulletin de la Section Scientifique de l'Academie Roumane 7: 220–225.

IONESCU, C. N., 1915: *Contributions à la faune des insectes Collemboles (terrestres, cavernicoles et aquatiques) de Roumanie,* Annales Scientifiques de l'Université de Jassy 9(3–4): 463–518.

IONESCU, C. N., 1922: *Quelques nouveaux insectes Collemboles recoltes dans les grottes des Carpathes meridionales*, Annales Scientifiques de l'Université de Jassy 11(3–4): 373–382.

IONESCU, M. A., 1951: *Contribuţii la studiul Collembolelor (Insecta: Apterygota) din România,* Buletin Ştiintific, Secţiunea de Ştiinte Biologice, Agronomice, Geologice şi Geografice 3(4): 639–651.

KONTSCHÁN, J., MURÁNYI, D. & TRASER, GY., 2003: *Data to the distribution of the Tetrodontophora bielanensis (Waga, 1842) (Collembola: Onychiuridae),* Annales historico-naturales Musei nationalis hungarici 95: 107–111.

MACK-FIRĂ, V., 1961: *Contribuţii la studiul Onychiuridelor (Collembola) din România,* Analele Universităţii C. I. Parhon, Seria Ştiinţele Naturii 28: 191–196.

MACK-FIRĂ, V., 1963: *Contribuţii la studiul collembolelor (Insecta: Apterygota) din România,* Comunicările Academiei Române 8(13): 717–723.

MACK-FIRĂ, V., 1965: *Despre câteva specii de colembole (Insecta: Apterygota) din România,* Analele Universităţii Bucureşti, Seria Stiinţele naturii, Biologie 14: 117–125.

MARI MUTT, J. A. & STOMP, N., 1980: *Two new species of Heteromurus s. str. from Portugal and Romania,* Archives d' Institut Grand-Ducal de Luxembourg. Section des Sciences naturelles, physiques et mathématiques. Nouvelle série 38: 183–189.

NAE, A., VLAICU M., POPA, I., CONSTANTINESCU, T., IAVORSCHI, V. & NITU, E., 2005: *First note on the invertebrate fauna of caves in the Piatra Craiului National Park*, Travaux de l'Institut de Spéologie Émile Racovitza 43: 133–164.

POPA, I. & GRUIA, M., 2006: *Research in the Piatra Craiului National Park.* – In: POP, O. G. & HANGANU, H. (eds): Collembola of the Piatra Craiului National Park, Vol. 3. Editura Universităţii Transilvania, Braşov, pp. 172–176.

POPOVICI I., POP V.V., TEODOREANU M., WEISNER P., TOMESCU R., STEFAN V., GEORGESCU A., TARTA A., 1984: *Structure, abundance and biomass of the soil fauna in forest ecosystems of the Retezat Mountains (The Carpathians),* Parcul National Retezat „Recherches ecologiques dans le Parc National de Retezat", p. 158-169.

POPOVICI, J., POP, V.V., STEFAN, V., HARSIA, T., 1997: *Pedofauna of limestone areas in the Retezat National Park (Romania),* In: TÓTH, E. & HORVÁTH, F. (eds): Proceedings of "Research, Conservation, Management" Conference, Aggtelek, Hungary, 1-5 May, 1996. Aggtelek National Park Directorate, Aggtelek, 461-475.

RADWAŃSKI, J. M., FIERA, C. & WEINER, W. M., 2006: *A new species of Protaphorura Absolon, 1901 (Collembola: Onychiuridae: Onychiurinae) from Romania and a redescription of Protaphorura glebata (Gisin, 1952),* Annales zoologici 56(3): 449–455.

RUSEK, J., 1978: *New Palearctic taxa of Tullbergiinae (Collembola*), Acta entomologica bohemoslovaca 75: 255–271.

RUSEK, J., 1979: *New Palearctic Anurophorinae (Collembola),* Věstník Československé zoologické společnosti 2: 138–142.

STACH, J., 1949*a*: *The Apterygotan fauna of Poland in relation to the world-fauna of this group of insects. Part 2. Families: Neogastruridae and Brachystomellidae. Acta Monographica Musei Historiae Naturalis*, Polish Academy of Sciences and Letters, Kraków, 341 pp.

STACH, J., 1949*b*: *The Apterygotan fauna of Poland in relation to the world-fauna of this group of insects. Part 3. Families: Anuridae and Pseudachorutidae. Acta Monographica Musei Historiae Naturalis*, Polish Academy of Sciences and Letters, Kraków, 124 pp.

STACH, J., 1951: *The Apterygotan fauna of Poland in relation to the world-fauna of this group of insects. Part 4. Family Bilobidae. Acta Monographica Musei Historiae Naturalis*, Polish Academy of Sciences and Letters, Kraków, 97 pp.

STACH, J., 1954: *The Apterygotan fauna of Poland in relation to the world-fauna of this group of insects. Part 5. Family: Onychiuridae,* PAN Instytut Zoologiczny Oddz. w Krakowie,, Kraków, 219 pp.

STACH, J., 1956: *The Apterygotan fauna of Poland in relation to the world-fauna of this group of insects. Part 6. Family: Sminthuridae,* PAN Instytut Zoologiczny Oddz. w Krakowie, Kraków, 287 pp.

STACH, J., 1960: *The Apterygotan fauna of Poland in relation to the world-fauna of this group of insects. Part 8. Tribe Orchesellini,* PAN Instytut Zoologiczny Oddział w Krakowie, Kraków, 151 pp.

STACH, J., 1963: *The Apterygotan fauna of Poland in relation to the world-fauna of this group of insects. Part 9. Family Emtomobryidae,* PAN Zakład Zoologii Systematycznej, Kraków, 126 pp.

SZENT-IVÁNY, J., 1938: *Beitrag zur Kenntnis der Apterygoten-Fauna des Karpatenbeckens,* Fragmenta Faunistica Hungarica 1(4): 90–93.

TÖMÖSVÁRY, Ö., 1883: *Magyarországban talált Smynthurus-fajok. (Species generis Sminthurus faunae hungaricae.)*, Természetrajzi Füzetek 7: 31–38.

VELLAY, I., 1900: *Ordo. Apterygogenea*, In: A Magyar Birodalom Állatvilága (Fauna Regni Hungariae) III. Arthropoda. Királyi Magyar Természettudományi Társulat, Budapest, pp. 19–22.

WEBSTER et al., 2001, The status of the Carpathians, a report developed as a part of the Carpathian Ecoregion Initiative.

WEISNER P., 1984: Preliminary estimation of energy flow through Collembola communities in the National Retezat Park (The Carpathians), Parcul National Retezat „Recherches ecologiques dans le Parc National de Retezat", 209-219.

SOME REFLECTIONS ON THE PHYTODIVERSITY OF THE FORMER DAFFODIL (*NARCISSUS RADIIFLORUS*) FIELD OF DUMBRAVA SIBIULUI

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Key words: Dumbrava Sibiului; daffodil field; restoration; phytodiversity.

Introduction

Older botanical works, as well as herbarium collections, certify the existence of quite a large daffodil (*Narcissus radiiflorus*) field in Dumbrava Sibiului, from the 18th century onwards until the beginning of the 20th century. It used to lie at the south of Sibiu and would stretch, at the middle of the 19th century, on almost 200 hectares along Valea Aurie (Goldtal) and furthermost in the Dumbrava Forest (Jungenwald), up to the village Răşinari and the Cisnădioara Forest. The perimeter measures ca. 6-7 km in length and a few hundred meters in width.

The Dumbrava Forest is a terrace oak wood that covers 1,009 hectares of land. From the old forest of 150-200 years ago, when daffodils would grow all over it, about forty pieces of *Quercus robur* have survived, now aged 250-700. The last 700-710 year-old oak tree, growing next to the Dumbrava Inn and measuring 1,020 cm in circumference, dried up back in 1979; yet, not far from there, near the Zoo's Boat Lake, an oak about 600 years of age still vegetates.

Findings and discussion topics

We shall try, with the help of older bibliography and herbarium collections, to restore the picture of that large and interesting field, particularly in what its phytodiversity is concerned. We can get a first glimpse of what the field looked like in the 18th century by perusing J. Lerchenfeld's herbarium (containing plants that were collected – for the most part – in 1780-95). Lerchenfeld collected from the daffodil field of Dumbrava Sibiului the following plant species: *Cirsium rivulare, Euphorbia villosa, Oenanthe silaifolia, Polygonum bistorta, Ranunculus x silvicolus, Sagina procumbens, Salix rosmarinifolia, Sedum annum, Stellaria graminea.*

The first botanist to mention the field and cite the species *Narcissus poeticus* (s.l.) and *Fritillaria meleagris* is J. C. G. Baumgarten (in 1816). Half a century later, M. Fuss will collect (see the Fuss Herbarium of the Museum of Natural History in Sibiu) from the daffodil field the following species: *Allium angulosum, Carex elongata, Carex flava, Carex leporina, Carex nigra, Carex stelullata, Carex vesicaria, Gladiolus imbricatus,*

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Gymnadenia conopsea, Juncus atratus, Lychnis viscaria, Orchis coriophora, Orchis laxiflora, Orchis ustulata, Ranunculus acris, Salix rosmarinifolia, Trollius europaeus.

A contemporary of M. Fuss, F. Schur published in 1866 an exquisite *Transylvanian Flora*, from which we cite the following thirty-seven species from the daffodil field of Dumbrava Sibiului: *Aconitum moldavicum* (at edges), *Adenophora liliifolia, Agrostis tenuis f. hispida, Asyneuma canescens, Carex appropinquata, Carex buxbaumii, Carex flava f. patula, Carex hostiana ?* (under *C. fulva*), *Carex lepidocarpa, Carex remota f. subloliacea, Cerastium glomaratum f. eglandulosum, Epilobium palustre* (at edges), *Epilobium x weissenburgense, Equisetum pratense* cu *f. ramulosum, Galium boreale f. hyssopifolium, Galium palustre, Gladiolus imbricatus, Juncus atratus, Leersia oryzoides, Luzula multiflora var. pallens, Molinia coerulea ssp. littoralis, Myosotis scorpioides f. latiflora, Narcissus angustifolius* ("towards Răşinari"), *Oenanthe silaifolia, Orchis incarnata* cu var. *straminea, Orobanche elatior* (under *O. scabiosae* with the mention on Scabiosa tenuifolia Bmg., at edges Cisnădioarei Forest, in "Daffodils Field"), *Petasites hybridus, Rhinanthus minor f. minimus, Salix rosmarinifolia* cu *f. angustifolia, Taraxacum palustre, Thalictrum lucidum f. peucedanifolium, Trollius europaeus f. serotinus, Typha angustifolia f. media, Valeriana officinalis f. altissima, Veratrum album f. velutinum, Veronica serpyllifolia f. wolffiana, Viola stagnina f. microstipulata.*

Towards the end of the 19th century, in 1882-83, Ormay collected from the same field (see the Herbarium of the Museum of Natural History in Sibiu): *Anemone ranunculoides, Crocus banaticus, Luzula campestris, Luzula pilosa.* The last one to collect daffodils here is K. Ungar in 1905 (see the Ungar Herbarium of the Museum of Natural History in Sibiu).

It follows therefore that, in 1780-1905, at least 60 species of herbaceous cormophytes were studied and collected from the daffodil field of Dumbrava Sibiului. Here they are, in alphabetical order: *Aconitum moldavicum, Adenophora liliifolia, Agrostis tenuis, Allium angulosum, Anemone ranunculoides, Asyneuma canescens, Carex appropinquata, Carex buxbaumii, Carex elongata, Carex flava, Carex hostiana, Carex lepidocarpa, Carex ovalis (C. leporina), Carex nigra, Carex remota, Carex stelullata, Carex vesicaria, Cerastium glomeratum, Cirsium rivulare, Crocus banaticus, Epilobium palustre, Epilobium weissenburgense, Equisetum pratense, Euphorbia villosa, Fritillaria meleagris, Galium boreale, Galium palustre, Gladiolus imbricatus, Gymnadenia conopsea, Juncus atratus, Leersia oryzoides, Luzula campestris, Luzula multiflora, Luzula pilosa, Lychnis viscaria, Molinia coerulea, Myosotis scorpioides, Narcissus radiiflorus, Oenanthe silaifolia, Orchis coriophora, Orchis incarnata, Orchis laxiflora, Orchis ustulata, Petasites hybridus, Polygonum bistorta, Ranunculus acris, Ranunculus silvicolus, Rhinanthus minor, Sagina procumbens, Salix rosmarinifolia, Sedum annuum, Stellaria graminea, Taraxacum palustre, Thalictrum lucidum, Trollius europaeus, Typha angustifolia, Valeriana officinalis, Veratrum album, Veronica serpyllifolia, Viola stagnina.*

Conclusions

If we analyze the coenosis of the species mentioned in the list just above, we notice that a great majority belong to the Molinio-Arrhenatheretea Class, especially to the *Molinion* and *Agrostion stoloniferae* associations. Based on such findings, we can conclude that the daffodils of Dumbrava Sibiului used to grow in the presence of a Molinietum most likely the *Junco-Molinietum Prsg.* 1951, much like the one of Dumbrava Vadului of Braşov County (see I. Şerbănescu, 1960 and V. Ciobanu, 2002).

By using this plant inventory as a starting point, we can arrive at certain conclusions concerning phytocoenosis. Due to the deforestation that was done in the 18th century, the level of phreatic waters went up, and that favored the spreading of a vigorous population of daffodils (*Narcissus radiiflorus*). Exactly the same thing occurred in Dumbrava Vadului of Braşov County, where the daffodils spread until they covered a 400-hectare area, as a result of massive deforestation that took place between the two World Wars. We possess no evidence of the presence here of wild daffodils prior to the deforestations mentioned above, despite Transylvanian botanists' having searched the area. Initially, the forest near Sibiu where the daffodils would grow was, in terms of phytocoenosis, *Molinio-Quercetum roboris*, (R. Tx. 1937; Scamoni et Passarge 1959) – as is the case with Dumbrava Vadului of Braşov County, but later on the hornbeam (*Carpinus betulus*) proliferated, so that now the tree coenosis that dominates Dumbrava Sibiului is Querco robori – Carpinetum. In the 1970s, just a few *Molinia coerulea* were preserved of the old forest (see E. Schneider-Binder, 1973), at the Răşinari end of Dumbrava Sibiului, facing the 'Dealul Obrejii' (i.e. the Obreja Hill). We assume that, if one or two ten-hectare lots were deforested, the daffodils would reappear, given the favorable environmental conditions for their development. In our days, of the sixty species listed above, only nineteen are identified: *Agrostis tenuis, Anemone ranunculoides, Carex ovalis, Carex remota, Cirsium rivulare, Crocus banaticus, Galium palustre, Gymnadenia conopsea, Juncus atratus, Leersia oryzoides, Luzula campestris, Myosotis scorpioides, Petasites hybridus, Ranunculus acris, Rhinanthus minor, Stellaria graminea, Valeriana officinalis, Veronica serpyllifolia.*

REFERENCES:

BAUMGARTEN, J. CHR. G., 1816, *Enumeratio stirpum Magno transsilvaniae principatui praeprimis indigenarum in usum nostrarum botaniphilorum conscripta inque ordinem sexuali- naturalem concinnata,* I-III, Vindobonae

CIOBANU, V. red., 2002, *Rezervaţia naturală „Dumbrava Vadului" Şercaia – Judeţul Braşov. Trecut, prezent şi viitor,* Edit. „Pentru viaţă" Braşov, 122 p.

DRĂGULESCU, C., 1978, *Originea şi corologia speciei Narcissus poeticus L. s.l.,* Şt. şi com. Muz. Brukenthal Sibiu, Şt. nat., 22, 105-128

DRĂGULESCU, C., 1987, *Die Coenologie der Narzisse (Narcissus poeticus L. ssp. stellaris (Haw.) Dost.) in den Karpaten im Vergleich zu ihrer Vergesellschaftung in anderen Teilen Europas,* Tuexenia, NS, 7, Gottingen, 233-243

DRĂGULESCU, C., 2003, *Cormoflora judeţului Sibiu,* Edit. Pelecanus Braşov

SCHNEIDER-BINDER, Erika, 1973, *Pădurile din depresiunea Sibiului şi dealurile marginale I,* St. şi com. Muz. Brukenthal Sibiu, Şt. nat., 18, 71-108

SCHUR, F., 1866, *Enumeratio plantarum Transsilvaniae,* Vindobonae, 984 p.

ŞERBĂNESCU, I., 1960, *Poienile cu narcise din Dumbrava Vadului,* Ocrotirea naturii Bucureşti, 5, 33-46

Rezumat. Consideraţii asupra fitodiversităţii fostei pajişti cu narcise (Narcissus radiiflorus) din Dumbrava Sibului. O serie de mărturii atestă faptul că în Dumbrava Sibiului a existat o imensă pajişte cu narcise (*Narcissus radiiflorus*) care a dispărut în urmă cu un secol. Pornind de la informaţiile bibliografice şi de la materialele de ierbar existente în colecţii vechi (din secolele XVIII şi XIX), autorul reconstituie o bună parte din inventarul floristic al acestei pajişti în care, pe lângă narcise, creşteau alte cel puţin 60 de cormofite (între care *Carex flava, Carex nigra, Carex stelullata, Crocus banaticus, Fritillaria meleagris, Galium boreale, Gladiolus imbricatus, Molinia coerulea, Orchis incarnata, Polygonum bistorta, Salix rosmarinifolia, Trollius europaeus).* Pe baza structurii floristice autorul presupune că pădurea în care s-a dezvoltat pajiştea cu narcise aparţinea asociaţiei Molinio-Quercetum roboris, iar cenozele ierboase cele mai întinse, se încadrau la asociaţiaa Junco-Molinietum, ceea ce înseamnă că erau foarte asemănătoare cu cele din Dumbrava Vadului (Jud. Braşov).

HEINRICH WACHNER (1877 – 1960) UND SEINE „GEOLOGIA ŢĂRII BISTRIŢEI" (GEOLOGIE DES NÖSNERLANDES), IN ÜBERARBEITUNG VON DR. IOAN CHINTĂUAN. *

Gewidmet der 130. Jährung des Geburtstages dieses verdienstvollen Naturwissenschaftlers und Pioniers des Naturschutzes.

Rudolf RÖSLER[1]

Kurzgefasste Lebensdaten :
* 3.10.1877 in Targu Mures (Neumarkt a. M.)
1901 Lehrerdiplom (Geographie, Geologie und Naturwissenschaften)
1901-1902 Mittelschul- bzw. Gymnasiallehrer in Hermannstadt
1902-1905 Gymnasiallehrer in Bistritz
1906-1920 Gymnasiallehrer in Schässburg
1920-1947 Gymnasiallehrer in Kronstadt
+ 16.3.1960 in Wolkendorf / Burzenland (Vulcan / Ţara Barsei)

Heinrich Wachner ist der Allgemeinheit bekannt als „Der Kronstädter Heimatforscher", welcher 1934 die bisher vielseitigste Monographie einer siebenbürgischen Ortschaft und deren weiteren Umgebung herausgab, unter dem Titel: „Kronstädter Heimat- und Wanderbuch"; seinerzeit war es gedacht und konzipiert als Lehrbuch für den Heimatkundeunterricht der Schüler Kronstadts und des Burzenlandes. Nach dem Zweiten Weltkrieg erschienen in der Bundesrepublik Deutschland und in Österreich, zahlreiche siebenbürgische Heimatbücher, die überwiegend von mehreren Mitarbeitern gestaltet wurden. So zum Beispiel für mehrere Ortschaften des Nösnerlandes, die in der abschließenden Literaturliste Erwähnung finden (BÖHM G., BÖHM M. 1991, BRANDSCHER M. 1991, BRANDSCHER J u. M. 1991, BREDT u. LITSCHEL 1971, BRECKNER 1999, CSALLNER 1965, 1969, 1974, CSELLNER 1983, DORFI 1984, EMRICH u. LINKNER 2001, FELKER 1968, FRÜHM 1958, GÖKLER 1985, GOTTSCHIK u. KELP 1978, KLÖSLER 1986, LINKNER 1994, 1999, 2000, ORENDI 1895, SCHIESSLEDER-FRONIUS 1989, SITZ 1988, WAGNER 1976, WEISS 1986, ZEHNER 1981, 1987, 1989, 2001). Auch aus dieser Sicht ist das Kronstädter Heimatbuch eine Besonderheit, stammt es doch ausschließlich aus der Feder eines einzigen Autors – Heinrich Wachner.

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Obwohl Wachner zu den bedeutendsten siebenbürgisch - sächsischen Forschern des verflossenen Jahrhunderts gehört und ein vielseitiger Naturwissenschaftler war, trifft auch auf ihn folgende ungerechtfertigte Charakterisierung zu: „Die Geologen hielten ihn für einen guten Botaniker, die Botaniker hingegen meinten er wäre ein hervorragender Geologe". Er war das, was keiner seiner Neider und bedeckten Kritiker war: Ein begnadeter Lehrer und eine ausgeprägt vielseitige Forschernatur. Was man ihm heute in besonderem Maße anerkennen sollte, ist seine intensive Aktivität im Bereich des sich nur allmählich im Südost-Karpatenraum durchringenden Gedanken des Natur- und vor allem des Umweltschutzes. Er war einer der aktiven Pioniere, Verbreiter und Verfechter des Naturschutzgedankens in Siebenbürgen und Rumänien zwischen den beiden Weltkriegen (WACHNER 1926, 1927/28, 1934).

Als Botaniker machte er sich einen Namen durch bemerkenswerte Leistungen auf dem Gebiet der Entdeckung und der Kenntnis zur Verbreitung seltener, oder bis dahin unbekannter Arten aus der Flora Siebenbürgens und Rumäniens, wie zum Beispiel des Moosglöckchens (*Linnaea borealis* L.) auf dem Kuschmaner - Stein (Piatra Cusmii) bei Bistritz **(Abb. 1 und 2)**.

Als Geologe sind seine Leistungen nicht nur von wissenschaftlicher, sondern auch von wirtschaftlicher Bedeutung. So entwarf er die erste geologische Karte der Umgebung von Schäßburg, war Mitentdecker des Erdgasvorkommens bei Agnetheln, dank dessen Bukarest auch heute noch mit Erdgas versorgt wird. Ihm verdankt die heutige Wirtschaft Rumäniens die Entdeckung der Dazittuffe, die zur Entwicklung der Zementindustrie im Bereich des Perschangebirges, erst möglich machte. Alle seine Verdienste im Bereich der Geologie aufzuzählen, würde den Rahmen unseres Themas sprengen. Es sei jedoch unterstrichen: Er war hauptberuflich Gymnasiallehrer, seine einmaligen hochkompetenten Verdienste auf dem umfangreichen Gebiet der Naturwissenschaften, gehören mehr oder weniger in einen Bereich, den man heute im so genannten Neudeutsch als „Hobby" zu qualifizieren geruht!

So mancher Geologe dürfte sich brüsten, wenn er auch nur 1/10 dessen entdeckt und geleistet hätte wie Gymnasiallehrer Heinrich Wachner; so mancher Botaniker könnte stolz sein, hätte er den einzigen Fundort des Moosglöckchens – und noch so manche Art - als Neuigkeit für Rumänien entdeckt (RÖSLER 1964, 2001; HELTMANN 2006).

Doch wer war dieser bescheidene, überaus arbeitsame und tüchtige Lehrer und Wissenschaftler?

Heinrich Wachner wurde am 3. Oktober 1877 in Targu Mures (Neumarkt a.M.) geboren; nach dem Tode seiner Eltern (Vollweise im Alter von 15 Jahren) lebte er bei seiner Tante (Schankebank Edle von Vledeny) in Bistritz. Nach Besuch des deutschen Gymnasiums in Bistritz, studierte er an den Universitäten Klausenburg, Marburg und Berlin Geographie, Geologie und Naturwissenschaften (WAGNER 1993, HELTMANN 2002). Als junger Gymnasiallehrer war er zu Beginn in Hermannstadt tätig (2 Jahre), um dann 1902 bis 1905 (3 Jahre) in Bistritz zu lehren. Hier schrieb er u.a. eine geologische

Arbeit über das Kelemen – Gebirge (Munţii Călimani), sowie das damals bedeutende Schulbuch „Schulgeographie", 1. Teil – Europa, erschienen 1904 in Hermannstadt. In diesen drei Jahren – verbracht im Nösnerland – sammelte er Daten zu einer geplanten Geologie dieses Großraumes, sowie Material zu einer Flora Nordost-Siebenbürgens. 1902 bis 1920 war er in Schäßburg tätig, um anschließend bis zu seiner Pensionierung 1947 in Kronstadt zu lehren.

Seine zahlreichen geologischen und botanischen Exkursionen im Nösnerland, hielt er leider nur in einer bisher bekannten Handschrift fest, die sich in Bukarest am Geologischen Institut befindet: „Geologie des Nord-Ost-Randes von Siebenbürgen". Durch diese datenreiche Arbeit aus dem Jahre 1924, sollten sich so manche, später renommierte Geologen für diesen Großraum begeistern, die dann in den 1920er und 30er Jahren hier forschen sollten.

Einem weiten Publikum dürfte jedoch unbekannt sein, dass Wachner auch die Arbeit „Geologie des Nösnerlandes" schrieb. Uns ist nur die rumänische Fassung bekannt, unter dem Titel „Geologia Ţării Bistriţei", die sich heute im Naturkundemuseum von Bistritz befindet . Das 108 seitige Manuskript verfasste er in rumänischer Sprache, wahrscheinlich zu Beginn seines Ruhestandes, denn im Mai 1952 wurde er samt seiner Familie nach Rakosch (Racos) zwangsevakuiert. Genau kann die Entstehungszeit des Manuskriptes nicht bestimmt werden, es dürfte jedoch das Jahr 1950 gewesen sein, erwähnt er doch seine damalige Anschrift: Orasul Stalin, strada Nisipului 36. Die Arbeit gelangte über Prof. Dr. Iuliu Morariu (*1905, +1989, ab 1953 Inhaber des Lehrstuhls Botanik an der Forstwissenschaftlichen Fakultät Kronstadt), an dessen Cousin Prof. Dr. Tiberiu Morariu (*1905, +1982, einstiger Schüler des Bistritzer Deutschen Gymnasiums, an dem einst H. Wachner lehrte (TANCO 1993, TUTULA 1996, ARDELEAN 2000), Inhaber des Lehrstuhls für Geographie und Dekan der Universität „Babes – Bolyai" Klausenburg, Leiter der Abteilung Geographie der Rumänischen Akademie, welcher aus Salva bei Nassod (Năsăud) stammte und diese Arbeit in Klausenburg veröffentlichen wollte. Zur Publikation kam es nicht, da der damals 75jährige Wachner bekanntlich aus undefinierbaren politischen Motiven zwangsevakuiert wurde.

Kurz vor seinem Tode am 30. November 1982, übergab Prof. T.Morariu das von ihm sorgfältig gehütete Manuskript an Dr. Ioan Chintăuan (heute Direktor des Naturkundemuseums Bistritz) der damals Forscher an der Forschungsstelle Kallesdorf (Arkalia) der Universität Klausenburg bei Bistritz, war. Er legte ihm – als Vermächtnis – nahe, das Manuskript Wachners zu überarbeiten, um dieses dann – wenn die Zeiten es einmal erlauben sollten – zu veröffentlichen. Dem Vermächtnis seines Universitätslehrers, sollte Dr. I. Chintăuan treu verbunden bleiben. Er widmete sich der geologischen Erforschung des Großkreises Bistritz-Nassod und veröffentlichte dabei zahlreiche Arbeiten, die u.a. auch zur Aktualisierung der Kenntnisse – vermittelt einst durch H. Wachner – führten (CHINTĂUAN 1993, 2000, 2001, u.a.m.). Nach der Wende 1990 hat Dr. Chintăuan eine Überarbeitung durchgeführt mit Kommentaren nach dem heutigen

Stand der Kenntnisse (CHINTĂUAN 1998), und die Veröffentlichung im Rahmen des Arbeitskreises für Siebenbürgische Landeskunde vorgeschlagen (RÖSLER 2001). Dr. Chintăuan veröffentlichte seine diesbezüglichen Studien im Jahr 2001; dabei enthält seine Literaturliste (Bibliographie) auch zahlreiche Beiträge zur Kenntnis und zu so mancher Erkenntnis betreffend die Geologie des Nösnerlandes

Die Arbeit H.Wachners entstanden kurz nach dem Zweiten Weltkrieg, beruht auf Daten gesammelt z.T. schon vor dem Ersten Weltkrieg. Diese Arbeit ist aus heutiger Sicht von besonderem historischem Wert, insbesondere aus folgenden Gründen:

- Es ist eine der letzten größeren Arbeiten H. Wachners. Es dürften auch die in deutscher Sprache verfassten Unterlagen, heute eventuell noch auffindbar sein.
- Bisher wurde keine vollständige Geologie des Nösnerlandes veröffentlicht.
- Außer der Geologie, hat die Arbeit auch heimatkundliche Bedeutung, so zum Beispiel für die

a. Flurnamengeographie – erwähnt er doch deutsche Flurnamen, da es damals die neuen rumänischen Benennungen noch nicht gab, wie: Burgberg, siebenb. sächsisch Burich; rum. Buris, heute Cetate), Jaad (heute rum. Livezile, damals noch Iad), Schieferberg (einst rum. Sifărberg, heute Codrisor), Schulerwald (auch heute noch rum. Sulărvalt genannt), etc.

b. Wachner erwähnt alle bis heute bekannten Salz- und Metangasvorkommen dieses Großraumes, die bis 1948 – als er im Auftrag des Geologischen Kommitees Bukarest hier erneut forschte – nur unvollständig erfasst waren **(Abb.3).**

c. Von Bedeutung sind neben der Geschichte der geologischen Erforschung des Nösnerlandes, die Lagerungsformen der Gesteine, die Bewegung der Erdrinde, die Stratigraphie und nicht zuletzt die Paläontologie dieses Raumes. Dr. I.Chintăuan bestätigt überwiegend die paläontologischen Funde, bringt jedoch auch Korrekturen zur Systematik, die in den letzten 50 Jahren eine bedeutende Weiterentwicklung erfahren hat.

d. Zur besseren Kenntnis der Baugeschichte der Stadt Bistritz, sowie auch der evangelischen Kirchenbauten der Nösnerländischen Ortschaften, trägt Wachner durch die Bestimmung der Herkunft des Baumaterials, in besonderem Maße bei. So z.B. lieferten die einstigen Steinbrüche von Tschippendorf, Mettersdorf, Jaad, etc., einen bedeutenden Teil des Materials (Vulkanischer Tuff, sog. Dejer-Tuff) für den Bau der Kirchen von Tschippendorf, Bistritz, Treppen, Mettersdorf, Pintak, Jaad, Klein-Bistritz, Windau, Wallendorf, Heidendorf, Senndorf, Deutsch-Budak, Minarken, Petersdorf, Ober-Neudorf, Waltersdorf, Baiersdorf, Kallesdorf, Kyrieleis, Ungersdorf, Lechnitz, Wermesch, Sächsisch Sankt-Georgen, Dürrbach, Tekendorf u.a.m. Auch zahlreiche Wirtschaftgebäude

dieser Ortschaften wurden von Wachner untersucht und die Baustoffe bestimmt. Allein dieses Thema ist für sich eine riesige Leistung (CHINTĂUAN 1993).

e. Zur floristischen Bedeutung der geologischen Arbeit von H.Wachner, sei hier nur die Salzflora erwähnt. Er beschreibt 10 großflächige Halophytenflora – Vorkommen, ohne jedoch die Arten zu nennen, da nicht zum Hauptthema gehörend. Seine diesbezüglichen floristischen Aufzeichnungen müssten heute eigentlich noch auffindbar sein und wären von großer Bedeutung für das Nösnerland, da die Salzflora dieses Gebietes bis heute kaum erforscht wurde (siehe auch **Abb.3).**

Um einen flüchtigen Einblick in das Werk H.Wachners zu erlangen, werden auszugsweise einige Seiten des Manuskriptes vorgestellt; dabei wurden vom Verfasser einige Erklärungen in deutscher Sprache hinzugefügt.

Seite 20 und 21 (Abb.4).

Als 1904 das untere Wehr der Stadt Bistritz durch das Hochwasser zerstört wurde, versäumte es die Stadt, diesen Schutzbau neu zu errichten. Einige Jahre darauf kam die Schieferbergwand – der Hausberg der Bistritzer – ins Rutschen durch den Zerfall des blauen Tones, der bis dahin mit Schotter und Sand bedeckt war. H.Wachner analysiert dieses Phänomen und erwähnt auch die nötigen Sanierungsmaßnahmen; diese wurden leider erst Mitte der 1970er Jahre durchgeführt.

Seite 24 und 25 (Abb.5).

Die über den Bistritzfluß nach Senndorf und Windau führende Ruba-Brücke (Podul Jelnii), findet in den Chroniken immer wieder Erwähnung, wurde diese doch von den Hochwassern durch Unterspülung wiederholt mitgerissen. Auch hier handelt es sich um großflächige Rutschungen auf den blauen Tonschichten. Dass H.Wachner auch zu Beginn der 20er Jahre Daten aus dem Nösnerland sammelte (obwohl er schon in Kronstadt lebte), wird durch die Skizze auf Seite 24 bestätigt. Die Ziegelfabrik wurde gleich nach dem Ersten Weltkrieg erbaut und nach Fertigstellung von den Besitzern der kleinen Ziegelöfen aus dem Stadtteil Ruba (Hrube) in Brand gesetzt und darauf nicht mehr aufgebaut; also kann angenommen werden, dass Wachner seine Studien zwischen 1919 bis 1924 hier weitergeführt hat, lebte doch seine Verwandtschaft im alten Nösen.

Seite 62 und 63.

H.Wachner wiedergibt auf Seite 63 ein stratigraphisches Profilschema, so wie wir diese in der gesamten Arbeit handkoloriert vorfinden. Prof. T.Morariu und sein Schüler Dr. I.Chintăuan, versuchten immer wieder die Leistungen dieses Erziehers und Forschers Siebenbürgens durch die Veröffentlichung seiner Geologie des Nösnerlandes, in Erinnerung zu bringen. Wachners Manuskript zitierten sie immer wieder in ihren Arbeiten.

Ich sehe es als Aufgabe seiner einstigen Schüler, das Werk von Heinrich Wachner durch die Veröffentlichung der „Geologie des Nösnerlandes" zu vervollständigen um auch so diesen großen Naturforscher postum zu würdigen.

Leider konnte die Veröffentlichung der 108 Manuskriptseiten bisher auch im vorgeschlagenen Rahmen des Arbeitskreises für Siebenbürgische Landeskunde nicht verwirklicht werden. Hoffnung besteht jedoch das Versäumte im Jubiläumsjahr 2007 (*3.10.1877) nachzuholen, anlässlich der Tagung in Kronstadt (November 2007), gewidmet dem Gedenken dieses verdienstvollen vielseitigen Naturforschers und Pädagogen Siebenbürgens.

* Vortrag gehalten 2001 anlässlich der Frühjahrstagung der Sektion Naturwissenschaften des Arbeitskreises für Siebenbürgische Landeskunde, Schloss Horneck / Gundelsheim – Deutschland, überarbeitet 2007.

LITERATUR:

ARDELEAN, A. et al.: 2000, *Dicţionarul biologilor romani,* Bd. 1, A-M, Arad, 2000, S.645.
BÖHM,G.: Jahr?, *Senndorf in Siebenbürgen.* Ried, 120 S.
BÖHM,M.: 1991, *Mein Heimatort Oberneudorf.* Mellrichstadt, 260 S.
BRANDSCHER, J. u. M.: 1991, *Heimatbuch Klein-Bistritz.* Nürnberg, 304 S.
BREDT, J. u. LITSCHEL G.: 1971, *Windauer Heimatbuch.* Schweinfurt, 93 S.
BRECKNER, G.: 1999, *Spuren & Bilder aus Chepan. Heimatbuch der Gemeinde Tschippendorf (Cepari, Csepan) in Nordsiebenbürgen.* Rohr, 511 S.
CSALLNER,K.: 1965, *Mettersdorfer Heimatbuch.* Schweinfurt, 1o5 + 103 S.
CSALLNER,K.: 1969, *Heidendorfer Heimatbuch.* Schweinfurt, 141 + 39 S.
CSALLNER, K.: 1974, *Wie´ s daheim war. Ernste und heitere Erinnerungen aus Bistritz und dem Nösnergau.* Bad Kissingen, 175 S.
CSELLNER, M.: 1983, *Windau. Es war einmal.* Nürnber, 215 S.
CHINTĂUAN, I.: 1993, *Contribuţii la istoricul cunoaşterii, exploatării şi utilizării rocilor în Transilvania der Nord-Est.* Rev. Bistriţei, VII, S.283-294.
CHINTĂUAN, I.: 1998, *Geologia Ţării Bistriţei de Heinrich Wachner.* Tiposkript, Bistritz, 36 S.
CHINTĂUAN, I.: 2000, *Fauna de ostracode din Volhynianul de la Livezile (Bistriţa-Năsăud).* Stud. cerc., Geologie - geografie, Bistriţa / Bistritz, S.57-61.
CHINTĂUAN, I.: 2001, *„Geologia Ţării Bistriţei". Caietul manuscris al geologului Heinrich Wachner (1877-1960), o restituire stiinţifică.* Stud. cerc., Geologie – geografie, 6, Bistriţa, S.39-55.
DORFI, J.: 1984, *Deutsch-Budaker Heimatbuch.* Öhringen, 207 S.
FELKER, G.: 1868, *Lechnitzer Heimatbuch.* Schweinfurt, 18o S.
FRÜHM, TH.: 1958, *Wetterleuchten über Siebenbürgen.* Ebersberg, 128S.
GÖKLER, J.: 1985, *Heimatbuch Kallesdorf in Nordsiebenbürgen.* Bad Hall, 82 S.
GOTTSCHICK, J. u. KELP, G.: 1978, *Treppener Heimatbuch.* München, 184 S.
HELTMANN,H.: 2002, *Bedeutender Naturforscher Siebenbürgens. Zum 125. Geburtstag des siebenbürgischen Naturwissenschaftlers Heinrich Wachner (1877-1960). Siebenb. Zeitung, 52. Jahrg., 16, München,* S.7.

HELTMANN,H.: 2006, *Der Hohenstein bei Kronstadt und die Geschichte seiner botanischen Erschließung. Jahrbuch 2005/06 des DAV – Sekt. Karpaten,* München, S.131-149.
KLÖSLER, M.: 1986, *Dürrbach.* Altötting, 359 S.
LINKNER, J.: 1994, Heimatbuch Auen-Kuschma. Das Bärendorf am Siebenbürgischen Karpatenwald. Thaur bei Innsbruck, 221 S.
LINKNER, J.: 2000, Heimatbuch Niederwallendorf. Untere Vorstadt in Bistritz in Nordsiebenbürgen. Wels, 352 S.
MARZA, I. u. CHINTĂUAN, I.: 1975, *Cercetări petrografice asupra „tufului de Dej" in regiunea Bistriţa-Bargăului (jud. Bistriţa-Năsăud).* Stud. cerc.,Geol., geofiz., Ser. Geologie, XX, 1, Bucuresti.
ORENDI, J.: 1895, *Die Gemeinde Oberneudorf im Nösnergau.* Hermannstadt, 43 S.
RÖSLER,R.: 1964, *Linnaea borealis – un subarbust rar pentru flora R.R. Romine.* Revista Pădurilor, 79,, 10, Bucuresti, S.598-599.
RÖSLER,R.: 2001, *Heinrich Wachners Geologie des Nösnerlandes in Überarbeitung von Dr. Ioan Chintăuan. Vortragstiposkript, Frühjahrstagung der Sekt. Naturwissenschaften des Arbeitskreises für Siebenb. Landeskunde, Gundelsheim,* 18 S.
SCHLIESSLEDER-FRONIUS, I.: 1989, *Tekendorf in Nordsiebenbürgen. Ortsmonographie.* Eltville am Rhein, 407 S.
SITZ, A.: 1988, *Heimatbuch der Gemeinde Pintak bei Bistritz in Siebenbürgen,* 197 S.
TANCO,T.: 1993, *Virtus romana rediviva.* Cluj-Napoca, S.457-460.
TUTULA,V.: 1996, *Tiberiu Morariu.* Studii si cercet. Etnoculturale, II, Bistriţa, S.113-117.
WACHNER, H.: 1926, *Der St. Annensee.* Der Wanderer, 66. Jg., 5, Kronstadt.
WACHNER, H.: 1926, *Wir und die Natur.* Der Wanderer, 6. Jg., 6, Kronstadt.
WACHNER, H.: 1926, *Der Alpenpflanzenschutz.* Der Wanderer, 6. Jg., Kronstadt.
WACHNER, H.: 1927/28, *Im Donaudelta. Schule und Leben,* 11/12, Kronstadt.
WACHNER, H.: 1934, *Kronstädter Heimat- und Wanderbuch.* Kronstadt, 322 S. + Anhang.
WAGNER, E. (Hrsg.): 1976, *Wallendorf in Nordsiebenbürgen.* Oberursel / Taunus, 102 S.
WAGNER, E.: 1993, *Siebenbürgische Nachkommen des Exulanten Paul Regius.* Siebenb. Archiv, Bd. 27, Köln-Weimar-Wien, S.153-383.
WEISS, M.: 1986, *Billak in Siebenbürgen.* Augsburg, 350 S.
ZEHNER, H.: 1981, *Schönbirker Heimatbuch.* Timelkam, 272 S.
ZEHNER, H.: 1987, *Heimatbuch Sächsisch-Sanktgeorgen.* Emmendingen, 336 S.
ZEHNER, H.: 1989, *Verlorene Heimat Nordsiebenbürgen.* Denzlingen, 242 S.
ZEHNER, H.: 2001, *Tatsch in Nordsiebenbürgen. Geschichte und Chronologie eines Korbflechterdorfes.* Nürnberg, 308 S.

ABBILDUNGEN:

1. Der Kuschmaner Stein. Zeichnung von G.Keleti, 1870.
2. Linnaea borealis L. – entdeckt im Juli 1921 von H.Wachner (Rösler, 1963).
3. Salzvorkommen und Halophytenflora (Salzpflanzen) im Verwaltungsbezirk Bistritz – Nassod (Siebenbürgen).

1 = Von H.Wachner erwähnt mit dem Zusatz „Salzflora"; 2 = Von I.Chintăuan erwähnt mit dem Zusatz „Salzflora"; 3 = Von I.Chintăuan erwähnt ohne Bemerkungen zur Flora. (R.Rösler, 2001, UTM – 10 km – Gitter).

4. Seite 20 und 21 des Manuskriptes von H. Wachner (Auszug).
5. Ruba - Brücke und ehemalige Ziegelfabrik Bistritz (Auszug Manuskript H. Wachner).

Rezumat: „Heinrich Wachner (1877-1960) şi lucrarea sa Geologia Ţării Bistriţei, comentată şi completată de Dr. Ioan Chintăuan. Contribuţie dedicată aniversării a 130 de ani de la naşterea acestui naturalist merituos, pionier al ocrotirii naturii."

După o schiţă biografică a geologului şi botanistului H. Wachner (1877-1960) care s-a născut la Târgu Mureş, fiind apoi profesor la liceul din Bistriţa în perioada 1902-1905, se trece la o scurtă analiză a operei sale. Această temă a fost deja tratată în paginile acestei reviste (Stud. si cercet. Geologie-geografie, nr. 6/2001) de către geologul Dr. I. Chintăuan (text în limba română). Lucrarea de faţă este concepută pentru a fi folosită mai ales de către specialistii care trăiesc în afara graniţelor României. În afara activităţii geologice a lui H. Wachner, sunt amintite şi meritele sale ca botanist. Astfel a contribuit la cunoaşterea florei halofite a judeţului Bistriţa-Năsăud; el a descoperit printre altele şi singura staţiune de creştere a speciei *Linnaea borealis* L. în Carpaţii României – Piatra Cuşmii din Munţii Călimani. Bibliografia care încheie lucrarea, cuprinde majoritatea monografiilor comunelor şi satelor din judeţ, publicate până în prezent în Austria şi Germania, în care sunt tratate o sumedenie de date de interes geografic şi economic.

MICROBIOLOGICAL AND ENZYMOLOGICAL APPROACH OF POLLUTION IN THE MUREŞ RIVER

Vasile MUNTEAN[1], Maria Amelia GROZAV[1]

Abstract. Sediment and water samples from the Mureş river were studied microbiologically and enzymologically. The following four ecophysiological bacterial groups have been studied: aerobic mesophilic heterotrophs, ammonifiers, denitrifiers and iron-reducers. The following four enzymatic activities have been measured: phosphatase, catalase, actual and potential dehydrogenase. Some physico-chemical parameters of water were also analyzed: temperature, pH, Eh, conductivity, salinity and O_2 concentration. The presence of all the four ecophysiological bacterial groups was registered in all the studied sediments. The descending ranking of their abundance was: aerobic mesophilic heterotrophs > ammonifiers > denitrifiers and iron-reducers. On the base of the bacteria number of each ecophysiological group, the bacterial indicators of sediment quality (BISQ) have been calculated. The four enzymatic activities were registered in all the studied samples. Based of the analytical data, the enzymatic indicators of sediment quality (EISQ) were calculated. The lowest values of both bacterial, and enzymatic indicators of sediment quality, indicating a local pollution, were registered in the sampling site where the wastes from the enterprise GHCL UPSOM Ocna Mureş flowed into the river. The physico-chemical parameters of the water were also very different as compared to the other sampling sites. A strong positive correlation, statistically very significant, has been established between the bacterial and enzymatic indicators. The obtained results also underline the high natural auto regenerative capacity of the sediments, illustrated by the high values of the enzymatic and bacterial indicators of sediment quality at 3 km downstream from the mouths of the sewers which empty into the river the wastes from the enterprise, values very close to those registered upstream from the polluting site.
Key words: bacteria, enzymes, sediments, water, indicators of quality, pollution

Introduction

The importance of microbial and enzymatic activity as an indicator of water and sediment pollution was frequently underlined by several researchers (Boström, 1988, Papp *et. al.*, 2002, Luna *et al.*, 2002, Noble *et. al.*, 2003, Ştef *et. al.*, 2004). Decomposition and mineralization of organic matter are processes of great importance for the releasing of biogenic elements in the aquatic environments. A part of the organic matter which originate in phytoplankton and in zooplankton, enter into the dissolved organic phase of the water. The particulate phase is partly incorporated by the secondary consumers. The rest is converted in detritus and subsequently submitted to decomposition. Some of these compounds are incorporated by bacteria or micro plankton, while others suffer further enzymatic decomposition. The compounds of little molecular weight resulted from the exoenzyme activities are rapidly metabolized by the heterotrophic bacteria. One can consider that the rate of the organic matter degradation is, probably, controlled by the first stage, that of the exoenzymatic hydrolysis (Meyer-Reyl, 1987). This emphasizes the importance of the enzymological analyses for detection the effect of the pollutants on the normal way of the biological cycles in different habitats.

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In the aquatic ecosystems, the sediments are a clue link in the biogeochemical cycle of the elements. The mineralization processes of the organic substances not decomposed in the water column is completed in the sediments. The activity of the microorganisms on the substrates is carried out by means of enzymes catalyzing hydrolyses, oxido-reductions, as well as by means of some final products of the microbial metabolism. The environment within a sediment is a complex function of many different factors, such as the major mineral matrix, the texture, the amount of organic carbon and the geographical location (Malcolm and Stanley, 1982).

The present paper aimed to evaluate the bacterial and enzymatic potential in water and sediments of the Mureş river, in order to detect the effect of some putative pollutants on this potential, as it is defined by the values of the bacterial and enzymatic indicators of sediment quality. At the same time, the physico-chemical analyses complete the results of the microbiological and enzymological studies, offering a better understanding of the whole process of the polluting effect on the activity of microbiota in water and sediments.

Materials and methods

Microbiological, enzymological analyses were carried out on the four sediments, sampled from the river Mureş in the 2006-2007 period. The sample sites were appointed as follows: P1 – 100 m upstream, P2 – 50 m downstream, P3 – 1.5 km downstream, and P4 – 3 km downstream from the place where three sewers empty into the river the waste water from the enterprise GHCL UPSOM Ocna Mureş. The sediments had a clayey consistence, without sand or gravel.

The following 4 ecophysiological bacterial groups have been studied: aerobic mesophilic heterotrophs (agar plates; Atlas, 2004), ammonifiers (peptone medium), denitrifiers (De Barjac culture medium; Pochon, 1954), and iron-reducers (Ottow modified medium; Pârvu *et al.*, 1977). Except for the aerobic mesophilic heterotrophs (where we used the method of successive dilutions), the most probable number of bacteria was calculated according to the statistical table of Alexander (1965).

The following four enzymatic activities have been measured: phosphatase (Krámer and Erdei, 1959), catalase (Kappen, 1913), actual and potential dehydrogenase (Casida *et al.*, 1964).

The microbiological and enyzmological analyses were carried out seasonally: in July and October of 2006, and in January and May of 2007.

Some physico-chemical characteristics of water in the same sampling points were also analyzed, in May 2007: temperature, pH, Eh (redox potential), conductivity, salinity and O_2 concentration. The physico-chemical parameters were accomplished using a portable multiparameter.

Results and Discussion

Results of the physico-chemical analyses of water are presented in tab. 1. One can notice the alkaline pH (>8) of the water in all the sample sites. The redox potential (Eh) is negative, slightly reducing. The salinity was detected only in the P3 sampling sites,

downstream from the mouth of the sewer which empty into the river the wastes from the depuration plant of the Ocna Mureş city.

Table 1. Results of the physico-chemical analyses carried out in water.

Parameter	Sampling site			
	P1	P2	P3	P4
Conductivity (mS/cm)	0.400	0.378	0.900	0.550
Salinity (%)	ND	ND	0.02	ND
pH	8.16	9.12	8.38	8.20
Eh (mV)	-72	-131	-86	-92
O_2 (mg/l)	10.42	8.50	10.40	8.55
Temperature (°C)	8.0	16.5	7.5	7.6

One can notice the difference between the sampling site P2, 50 m downstream from the place where the wastes from the enterprise GHCL UPSOM Ocna Mureş empty into the river. Only at this site, the pH is higher than 9, the Eh is lower than –100 mV (more reducing than at the other sampling sites), the conductivity is lower than 0.4 mS/cm, the O_2 concentration is minimum. The high temperature of the water at the same sampling site (more than two fold higher as compared with the other sites) is also remarkable, showing a physical pollution generated by the wastes originated in the production process in the enterprise.

The presence of all the four ecophysiological bacterial groups was registered in all the sediment samples studied, in all the seasons. The number of bacteria decreases in the order: aerobic mesophilic heterotrophs (10^5-10^7 cells × g^{-1} dry matter sediment) > ammonifiers (10^4 cells × g^{-1} dry matter sediment) > denitrifiers and iron-reducers (10^2-10^3 cells × g^{-1} dry matter sediment). The values registered are comparable with those reported by other researchers in different types of sediments (Kulikov *et al.*, 1989; Poremba *et al.*, 1993; Crişan *et. al.*, 2001; Ştef *et. al.*, 2004; Muntean *et al.*, 2005, 2007). With few exceptions, the highest values were registered in summer, and the lowest ones in winter.

Based on the bacteria number of each ecophysiological groups, the bacterial indicators of sediment quality (BISQ) were calculated (Muntean, 1995-1996) (Fig. 1). The values of the BISQs ranged between 4.641 (P1 – summer) and 3.469 (P2 – winter). The minimum values of the BISQ have been registered in the P2 site, in each season. The mean values of the BISQs ranged between 4.050 (P2) and 4,479 (P1).

The four enzymatic activities studied have been detected in all the sediments analyzed, in all the seasons. The highest values have been always registered in the P1 sampling site, in summer: phosphatase activity – 10.2 mg phenol × 2.5 g^{-1} dry matter sediment; catalase activity – 46.072 splitted H_2O_2 × 1.5 g^{-1} dry matter sediment; actual dehydrogenase activity – 1,491 mg formazan × g^{-1} dry matter sediment; and potential

dehydrogenase activity – 4.818 mg formazan × g^{-1} dry matter sediment. The minimum values of all the four enzymatic activities have been always registered in the winter, in the P2 sampling site.

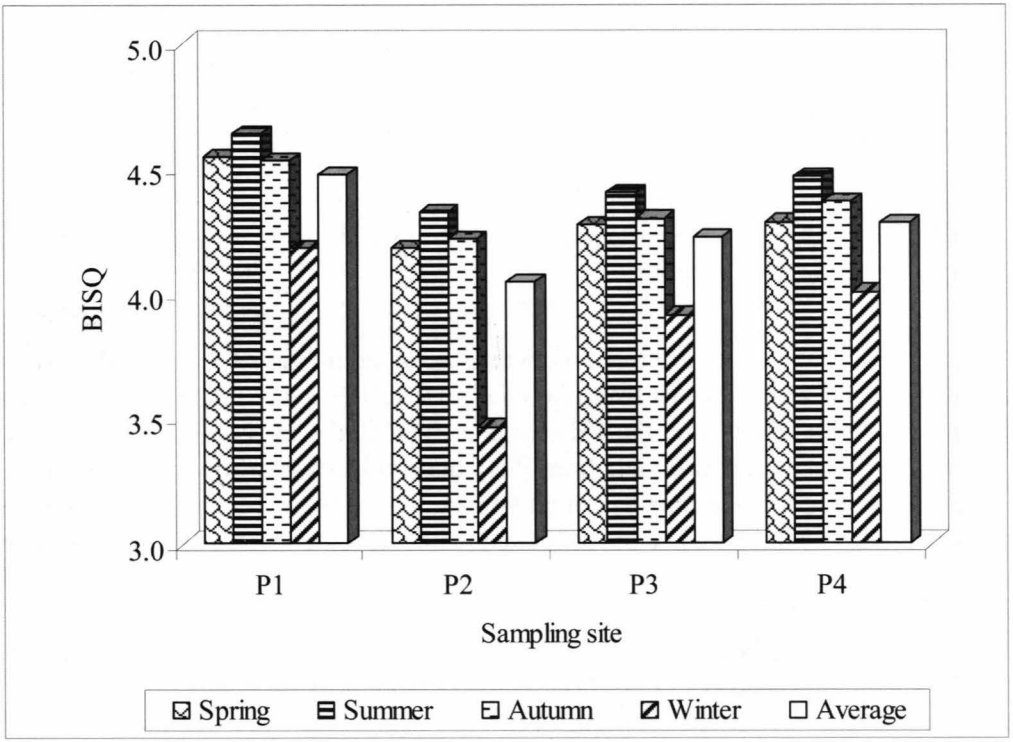

Fig. 1. The bacterial indicators of sediment quality (BISQ).

On the base of the absolute values of each enzymatic activity, the enzymatic indicator of sediment quality (EISQ) was calculated, according with Muntean *et al.* (1996) (Fig. 2). As in case of bacterial indicators of sediment quality, the lowest values of the BISQ have been always registered in the P2 site, in each season. The highest values of the EISQs were registered in the P1 sampling site, in each season. The mean values of the EISQs ranged between 0.182 (P2) and 0.312 (P1). We mention that the minimal theoretical value of the EISQ is 0, and the maximal one is 1.

A positive correlation ($r = +0.847$) with very high statistical significance ($p<0.001$) has been established between the bacterial and enzymatic indicators of sediment quality.

The differences between the P2 site and the other ten, as regard the physico-chemical parameters, on one hand, and the lowest values of both bacterial, and enzymatic indicators registered in this site, on the other, indicate the local pollution caused by the waste water spilt into the river from the enterprise GHCL UPSOM Ocna Mureş. At the

same time, the results also underline the high natural auto regenerative capacity of the sediments, illustrated by the high values of the enzymatic and bacterial indicators at 3 km downstream from the mouths of the sewers which empty into the river the wastes from the enterprise, values very close to those registered upstream from the polluting site.

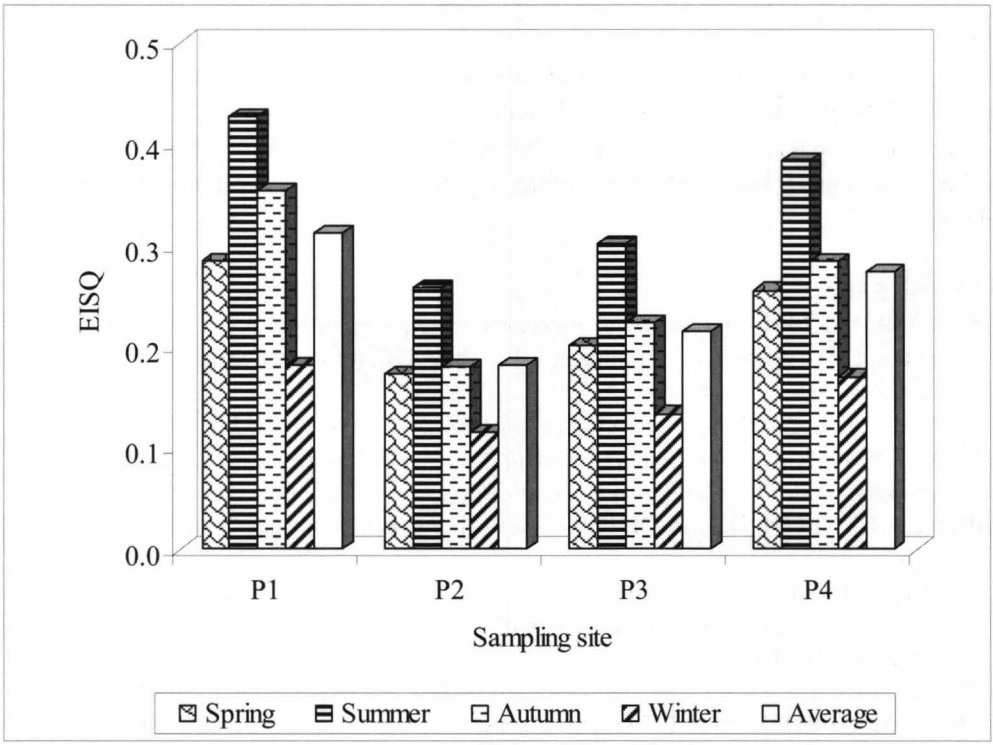

Fig. 2. The enzymatic indicators of sediment quality (EISQ).

Conclusions

The presence of bacteria which belong to the four ecophysiological groups studied was detected in all the samples analyzed. The descending ranking of their abundance was: aerobic mesophilic heterotrophs > ammonifiers > denitrifiers and iron-reducers. The sediments have a good bacterial potential, taking into account that, except for the P2 sampling site, the values of the bacterial indicators of quality overpass 4.

The four enzymatic activities studied (phosphatase, catalase, actual and potential dehydrogenase) were also present in all the analyzed samples. The values of the enzymatic indicators of sediment quality overpass 0.4 only in the summer, in the P1 sampling site.

The bacterial and enzymatic potential of sediments, as it is defined by the BISQ and EISQ values was seriously affected in the sampling site P2, where the wastes from the enterprise GHCL UPSOM Ocna Mureş flows into the river. The lowest values of both

bacterial, and enzymatic indicators of quality have been always registered at this sampling site.

The sediments from the Mureş river proved to have a high natural auto regenerative capacity. This capacity is illustrated by the high values of the enzymatic and bacterial indicators at only 3 km downstream from the mouths of the sewers which empty into the river the wastes from the enterprise, values very close to those registered upstream from the polluting site.

A positive statistically very significant correlation has been established between the bacterial and enzymatic indicators of sediment quality. The results certify the validity of the enzymatic and bacterial indicators as efficient tools for estimating the intensity of the microbial activities in sediments, for monitoring the effect of the polluting factors on these natural habitats.

REFERENCES:

ALEXANDER, M., 1965, *Most probable-number method for microbial populations*, in Black, C.A., Evans, D.D., White, J.L., Ensminger, L.E., Clark, F.E. (eds.), "*Methods of Soil Analysis*", Ed. Am. Soc. Agron., Madison.

ATLAS, R.M., 2004, *Handbook of Microbiological Media*, 3rd edition, CRC Press, New York.

BOSTRÖM, B., 1988, *Relations between chemistry, microbial biomass and activity in sediments of a sewage-polluted vs a nonpolluted eutrophic lake*, Verh. Int. Verein. Limnol., **23,** 451-459.

CASIDA, L.E.Jr., KLEIN, D.A., SANTORO, T., 1964, *Soil dehydrogenase activity*, Soil Sci. **98**, 371-376.

CRIŞAN, R., MUNTEAN, V., PAŞCA, D., MOLDOVAN, O., RAJKA, G., 2001, *Studiul microflorei bacteriene de pe paramentul barajului Drăgan (jud. Cluj)*, An. Univ. Oradea, Biol., **8**: 307-314.

KAPPEN, H., 1913, *Die katalytische Kraft des Ackerbodens*, Fühlings Landw. Ztg., **62**, 377-392.

KRÁMER, M., ERDEI, G., 1959, *Primenenie metoda opredeleniya aktivnosti fosfatazy v agrokhimicheskikh issledovaniyakh*, Pochvovedenie, 9, 99-102.

KULIKOV, A.S., SADCHIKOV, A.P., MAKSIMOV, V.N., 1989, *Obshchciaya aktivnost' bakterii sedimentatsionnogo detrita, izmerennaya s pomoshchiu fluorestseindiatsetata*, Mikrobiol. J., **5**: 7-11.

LUNA, G.M., MANINI, E., DANOVARO, R., 2002, *Large fraction of dead and inactive bacteria in coastal marine sediments: comparison of protocols for determination and ecological significance*, Appl. Environ. Microbiol., **68**, 3509-3513.

MALCOLM, S.J., STANLEY, S.D., 1982, *The sediment environment*, in Nedwell, D.B., Brown, C.M. (eds.), *Sediment Microbiology*, Ed. Acad. Press, London: 1-14.

MEYER-REIL, L.A., 1987, *Seasonal and spatial distribution of extracellular enzymatic activities and microbial incorporation of dissolved organic substrates in marine sediments*, Appl. Environ. Microbiol., **53**, 1748-1755.

MUNTEAN, V., 1995-1996, *Bacterial indicator of mud quality*, Contrib. Bot., 73-76.

MUNTEAN, V., 2007, *Bacterial and enzymatic indicators of water and sediment pollution in the Arieş river*, Stud. Univ. "Babeş-Bolyai", Biol., **52** (1), 87-92.

MUNTEAN, V., CRIŞAN, R., PAŞCA, D., KISS, S., DRĂGAN-BULARDA, M., 1996, *Enzymological classification of salt lakes in Romania*, Int. J. Salt Lake Res., **5** (1), 35-44.

MUNTEAN, V., ŞTEF, L.C., DRĂGAN-BULARDA, M., 2005, *Cercetări microbiologice asupra unor sedimente de pe cursul mijlociu al râului Mureş*, Stud. Univ. "Babeş-Bolyai", Biol., **50** (2), 175-181.

NOBLE, R.T., WEISBERG, S.B., LEECASTER, M.K., McGEE, C.D., RITTER, K. WALKER, K.O., VAINIK, P., 2003, *Comparison of beach bacterial water quality indicator measurement methods*, Environ. Monit. Assess., **81**, 301–312.

PAPP, J., FODORPATAKI, L., 2002, *Evaluation of the organic pollution of the Mureş river on the study of the indicator microflora*, Contrib. Bot., 231-237.

PÂRVU, R., STANCIU, E., LORINCZI, F., KISS, S., DRĂGAN-BULARDA, M., RĂDULESCU, D., 1977, *Iron-reducing capacity of soil micromycetes*, in *Fourth Symp. Soil Biol.* (Cluj-Napoca, 1977), Ed. Ceres, Bucureşti, 149-154.

POCHON, J., 1954, *Manuel technique d'analyse microbiologique du sol*, Ed. Masson, Paris.

POREMBA, K., LOCHTE, K., RHEINHEIMER, G., 1993, *Microbiologische Untersuchungen im norwegischen Sognefjord*, Ber. Inst. Meereskd. Univ. Kiel, **242**: 90-99.

ŞTEF, L.C., MUNTEAN, V., DRĂGAN-BULARDA, M., 2004, *Microbiological research on the Mureş sediments from Ocna Mureş affected by pollution*, Contrib. Bot., **39**, 227-232.

Rezumat. Au fost efectuate analize microbiologice şi enzimologice sezoniere asupra unor sedimente prelevate din râul Mureş în zona de deversare a apelor reziduale de la intreprinderea GHCL UPSOM Ocna Mureş. S-a determinat numărul de bacterii care aparţin următoarelor grupe ecofiziologice: heterotrofe aerobe, amonificatoare, denitrificatoare şi fier-reducătoare. Pe baza numărului de bacterii care aparţin celor 4 grupe a fost calculat indicatorul bacterian al calităţii sedimentelor. În aceleaşi sedimente au fost determinate următoarele activităţi enzimatice: catalazică, fosfatazică, dehidrogenazică actuală şi dehidrogenazică potenţială. Pe baza valorilor absolute ale activităţilor enzimatice s-a calculat indicatorul enzimatic al calităţii sedimentelor. În apa râului, la aceleaşi puncte de prelevare, au fost determinaţi următorii parametri fizico-chimici: pH, Eh (potenţial redox), conductivitate, salinitate, temperatură şi concentraţie de O_2. Atât datele fizico-chimice, cât mai ales cele bacteriologice şi enzimologice arată existenţa unei diferenţe semnificative între punctul de prelevare P2, situat la 50 m aval faţă de locul unde este deversată în Mureş apa reziduală de la intreprinderea GHCL UPSOM, şi toate celelalte. Fără excepţie, în acest punct au fost consemnate valorile minime ale indicatorilor bacterieni şi enzimatici ai calităţii sedimentului, probând efectul negativ pe care încărcătura poluantă a acestor ape reziduale îl are asupra microbiotei sedimentare. Valorile ridicate ale indicatorilor bacterieni şi enzimatici înregistrate la la numai 3 km aval, valori apropiate de cele consemnate în amonte faţă de punctul de prelevare P2, atestă existenţa unei bune capacităţi autoregenerative a sedimentelor din râul Mureş.

APPLICATIONS OF THE CAPILLARY ELECTROPHORESIS IN MICROBIOLOGY

Manuela-Claudia CURTICĂPEAN[1]

Abstract. The conventional analysis methods (based on numbering after inoculation of the water samples in the selective or non-selective media), used for microbiological monitoring of the water quality and for other microbiological analysis are very complicate and needs high costs. From this reason, the researchers look for methods with a low costs, more quickly and efficient. One of these methods is the capillary electrophoresis (CE). CE is a modern separation technique that was used in determining the complete sequence for human DNA, but also in microbiology for identification of bacteria from water, sediment and soil, based on separation of the amplified DNA from PCR (polymerase chain reaction), for detection and quantification of the bacteria cells, for determination of outer membrane proteins of some bacteria. All studies performed until now demonstrate that the CE is a fast and cheep method that has several advantages comparatively with gel electrophoresis techniques, namely: high analysis speed, small quantities of samples, an excellent control of temperature, high resolution and reproducibility. CE is a technique that might be a good system for microbiology and physico-chemical monitor of water and sediment from fresh waters.

Key words: capillary electrophoresis, microbiology, bacteria

Introduction

Capillary electrophoresis (CE) is a powerful new method for the separation of charged species that couples powerful resolution capacity with lower costs for columns, reagents, and solvent disposal. A capillary electrophoresis system consists of a capillary whose ends are submerged in two separate buffer reservoirs, a high voltage power supply with an anode and cathode placed in the two buffer reservoirs and a detector, which reads the signal through a window near one end of the capillary (Fig. 1a and b). Separations depend on the rates at which charged analyses migrate under an electric field. The migration rate of a species is determined by itâ charge to size ratio. CE uses an electromotive force, rather than a pump, to drive the mobile phase through the capillary. The electrophoretic force is based on the size and charge of the ion and the viscosity of the medium, so that cations migrate toward the cathode and anions migrate toward the anode [13]. Injection pressure, voltage, current and capillary cassette temperature are recorded before, during and after the run and are stored together with the electrophoretic raw data [2].

CE yields rapid, high-resolution separations with very small (0.1-10 nl) sample volumes. CE has been applied to a variety of applications including inorganic anions and

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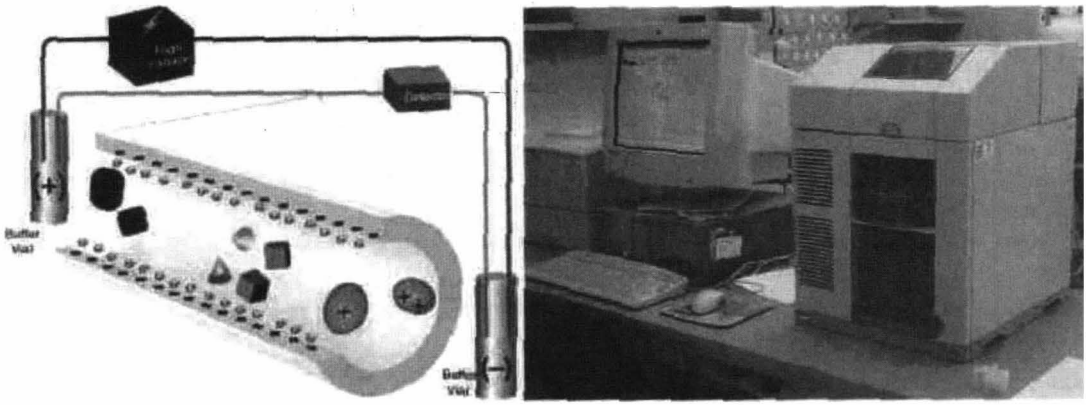
cations, amino acids, drugs and explosives. Most notably, CE was used for the human genome project to aid in determining the complete sequence for human DNA [3].

There are several advantages to using CE over gel techniques. Due to the higher surface-area-to-volume ratio of the capillary compared to the gel, there is better heat dissipation, and therefore a higher voltage can be applied to the separation. CE systems have higher analysis speed, lower sample and reagent requirements, and excellent temperature control, a necessary feature for high resolution and reproducibility. By using laser-induced fluorescence detection, CE systems have significantly greater sensitivity than traditional electrophoretic separations [3].



a **b**

Fig. 1 a – A capillary electrophoresis function principle; b – Agilent Capillary Electrophoresis Systems (www.fzjuelich.de/isg/ index.php?index =142& print=1)

Applications in microbiology

The analysis of microbial communities using molecular biology techniques is a great improvement over conventional plate culture methods, as shown by the poor results using antibiotically resistant bacteria monitored on ampicillin containing media. The development of DNA based microbial techniques is constantly evolving [3].

The ability to effectively monitor a microbial community is necessary to design and implement remediation strategies for contaminated soil. Single-strand conformation polymorphism (SSCP), a technique that separates DNA fragments based on their sequence, was used to analyze amplified 16S rRNA gene fragments of 12 common soil bacteria *(Pseudomonas).* Separation was performed using CE, as opposed to other common gel techniques, to eliminate the need for band analysis on gel matrices. SSCP profiles obtained for pure bacterial cultures show the potential of CE-SSCP for bacterial community analysis [4]. Optimization of the current technique, including PCR (polymerase chain reaction) conditions and electrophoresis settings, may further improve the ability to distinguish between genus and species. CE-SSCP can be an effective technique for monitoring

introduced species, but these results indicate that a significant population of the bacteria must be present to be successfully amplified and monitored. CE-SSCP has excellent potential for bacterial community soil analysis and providing insight into microbial communities amended for phytoremediation purposes. With the improvements in genetic bacterial analysis, new species of soil bacteria are constantly being discovered, and the ability to selectively identify a single species in a soil environment is essential for examining phytoremediation studies. The future of this field may lie in the introduction of genetically modified organisms with significantly enhanced remediation potential [3].

Bacterial substances and machineries causing reproducible damage to a certain host are named virulence factors. One of the most thoroughly studied bacterial virulence factor is the outer membrane of Gram-negative bacteria. It has a complex structure consisting of outer membrane proteins (OMPs) and a lipopolysaccharide (LPS) layer. Both are considered to possess pathogenic potential. OMPs as surface components play an important pathogenetic role in the interaction with host cells, that is, in the process of bacterial adhesion and invasion. Furthermore, OMP analysis has applications in epidemiological and virulence studies. Kustos et al. used the capillary electrophoresis for examining the virulence factors, outer membrane proteins (OMP), lipopolysaccharides (LPS), hemolysin, and the *in vivo* and /n *vitro* virulence of wild-type *Proteus penneri* 357 and its two isogenic mutant variants – a transposon and a spontaneous mutant [7]. The OMPs of these variants were analyzed by a new and fast technique, "dynamic sieving" capillary electrophoresis. CE was suitable for the comparative analysis of bacterial protein patterns in the genetic variants of this strain, and provided valuable results in connection with the bacteriological virulence. CE seems to be a useful and quick tool for comparison of protein profiles especially when a large number of samples are to be investigated.

The bacterial protein profile analysis by SDS-PAGE has been used increasingly during the past decade. This method is suitable for classification, identification, typing, and comparative studies of bacteria. The protein profile analysis is important in taxonomic examinations, giving a possibility for comparative analysis of different bacterial strains. Kustos et al. applied "dynamic sieving" CE with a polymer solution to obtain the whole protein profiles of four selected strains from the *Enterobacteriaceae* family, and the patterns were compared with the results of SDS-PAGE [6]. The capillary electrophoretic protein profiles of bacteria differed from the patterns obtained by SDS-PAGE in some features, but the CE profiles were also reproducible, the patterns differed in each bacterial strain, and they were characteristic for the bacteria investigated. In medical microbiology, the time taken to identify bacterial strains may be urgent in the treatment of patients. For this purpose would be a suitable technique the dynamic sieving CE, where the protein patterns were obtained within 20 minutes, because of the high voltage applied. Above that, the advantage of this method with respect of conventional slab gel electrophoresis is the low (nanoliter) sample consumption. Beside these, the system can be automated and there is an opportunity for quantitative analysis.

Persistent inhibition of bacterial growth, called postantibiotic effect (PAE), after a short exposure to a new carbapenem, meropenem, was determined in different strains of the *Enterobacteriaceae* family. CE, as well as, SDS-PAGE was used to study the outer membrane protein (OMP) profiles before and after meropenem treatment. CE proved to be suitable for the characterization of the OMP profiles of bacteria. Significant changes in the electrophoretic patterns were observed showing the consequential effect of meropenem on bacteria [5]. In the CE technique, proteins are separated based on their molecular weights similarly to SDS-PAGE, The sample consumption of CE is very low, so the determination of the OMP profiles requires only a few nanoliter samples. Moreover, the technique is fast, because the applied high voltage completes separation of proteins within a few minutes. The data obtained can be stored in a computer, which gives a possibility to later comparative examinations. This method is also suitable for the quantitative analysis of the patterns.

Miniaturization of analytical instrumentation has a number of advantages over conventional techniques. These advantages are mainly in the areas of improved data precision and reproducibility, short analysis times, minimal sample consumption, improved automation and integration of complex workflows. The Agilent 2100 bioanalyzer was the first commercially available instrument to use microfluidics technology for the analysis of biological samples. Today, it is the industry standard for RNA sample and has replaced gel electrophoresis for this application. It is also rapidly replacing gel electrophoresis for DNA fragment analysis and SDS-PAGE analysis of protein samples [11].

On-chip electrophoresis enables scientists working with nucleic acids or proteins to carry out automated control, sizing and quantification. The system uses micro-fabrication technology to transfer laboratory processes onto miniature glass chips that contain a network of interconnected channels and reservoirs. Filling the channels with a gel matrix and the wells with buffer or sample, allows electrophoresis to be carried out on a miniaturized scale. Choosing the appropiate LabChip kit (containing chips, buffer, gel, intercalating dye and standards) enables the analysis of DNA, RNA or protein samples (Fig. 2). This integration of sample preparation, fluid handling and biochemical analysis offers several advantages over traditional gel electrophoresis in terms of speed, automation, sample use and data quality [11].

Adaptation of CE to microchips has many advantages. Miniaturized flow systems create the opportunity of faster, automated analysis (10-, 100-fold increase in separation speed). Furthermore, miniaturized dimensions have the potential to simultaneously analyze multiple samples within minutes or less, in addition to this the dead volume is significantly reduced. Chip based separation techniques are based on the knowledge of capillary electrophoresis, which is itself a recent and developing technology. Electrophoresis on microchips has now been applied for the analysis of wide variety of samples, in investigation of cell cultures, in clinical diagnostics, protein and DNA analysis.

Advantages of microfabricated techniques might be useful first of all in situations with a need of ultrahigh throughput, e.g.: in emerging application fields of life sciences, especially in clinical diagnostics [8].

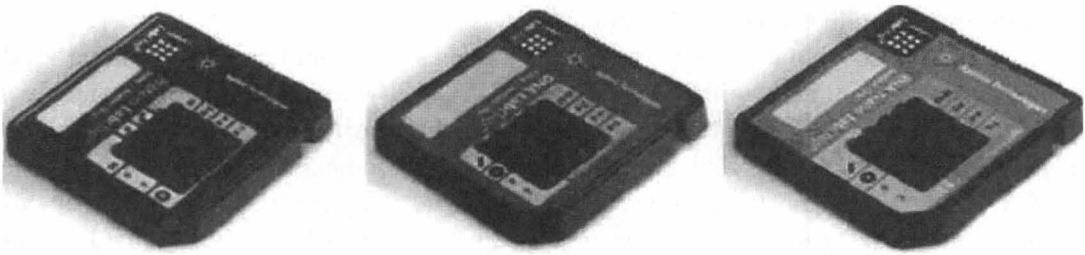


Fig. 2 Protein, DNA and RNA LabChip (www.agilent.com/chem/labonachip)

Kustos et al. have been analyzed the outer membrane protein composition of six *Pseudomonas aeruginosa* strains by conventional CE and microchip electrophoresis [8]. Outer membrane protein patterns of bacteria obtained by the two different methods in this study were similar, all major proteins could be detected by both techniques, and the molecular weights showed good correlations, although the direct comparison of the peak areas is not straightforward due to the different detection methods (UV and LIF). The increased separation speed, picoliters of sample consumption, baseline separation achieved more frequently by this method – especially in the high molecular weight region showed the advantages of microchip electrophoresis in the analysis of clinical samples. Since the introduction of the first microanalytic system in 1990, the interest in chip-based devices has increased significantly. The lab-on-a-chip technology has undergone tremendous growth over the last decade, and the application field of this miniaturized analysis system is still increasing. This technology allows the analysis of complex biological samples. Detection, identification and also quantitation of all proteins presented in biological samples might be performed. Significance of these microchip-based measurements, that its automated and fast results about the bacterial protein composition might contribute to the improved speed of microbiological diagnostics, and allow clinicians to make faster drug therapy decisions and earlier treatment.

Ghozii et al. used capillary electrophoresis-single-strand conformation polymorphism (CE-SSCP) analysis of PCR-amplified 16S rRNA gene fragments for rapid identification of *Pseudomonas aeruginosa* and other Gram-negative nonfermenting bacilli isolated from patients with cystic fibrosis [1]. Target sequences were amplified by using forward and reverse primers labeled with various fluorescent dyes. The labeled PCR products were denatured by heating and separated by capillary gel electrophoresis with an automated DNA sequencer. Thirty-four reference strains belonging to the genera *Pseudomonas, Brevundimonas, Burkholderia, Comamonas, Ralstonia, Stenotrophomonas,* and *Alcaligenes* were tested with primer sets spanning 16S rRNA gene regions with

various degrees of polymorphism. The CE-SSCP patterns obtained were identical to those for the corresponding reference strains. Fluorescence-based CE-SSCP analysis is simple to use, gives highly reproducible results, and makes it possible to analyze a large number of strains. This approach is suited for the rapid identification of the main Gram-negative nonfermenting bacilli encountered in cystic fibrosis.

A novel quantitative PCR (QPCR) approach, which combines competitive PCR with constant-denaturant capillary electrophoresis (CDCE), was adapted for enumerating microbial cells in environmental samples using the marine nanoflagellate *Cafeteria roenbergensis* as a model organism [9]. Two approaches, competitive QPCR and real-time QPCR, are currently most widely used in microbial ecology applications. Both methods estimate the target gene concentration in a sample by comparison with standard curves constructed from amplifications of serial dilutions of standard DNA. Competitive PCR has been used successfully for quantification of DNA in environmental samples. However, this technique is labor intensive, and its accuracy is dependent on an intemal competitor, which must possess the same amplification efficiency as the target yet can be easily discriminated from the target DNA. The use of CDCE circumvented these problems, as its high resolution permitted the use of an internal competitor which differed from the target DNA fragment by a single base and thus ensured that both sequences could be amplified with equal efficiency. The application of the CDCE method to the analysis of microbial communities represents a novel approach in molecular ecological studies. PCR products amplified with primers specific to the target organism could be detected and quantified via the migration distance of specific DNA peaks in CDCE electropherograms. Thus, a major advantage of CDCE in analyzing environmental samples of unknown species composition is that nonspecific or nontarget amplification products can be discriminated with high resolution from the target amplification products. This novel approach extends the usefulness of competitive QPCR by demonstrating its ability to reliably enumerate microorganisms at a range of environmentally relevant cell concentrations in complex aquatic samples.

Cyanobacterial toxins are a diverse group of natural toxins. These toxins have been reported to de responsible for poisoning of domestic and wild animals. Moreover, they have been related to illness and more recently, to death of human beings. The most frequently found cyanobacterial toxins occuring in blooms (mass production of cyanobacteria in surface water bodies) from fresh waters are cyclic peptide toxins of the microcystin family. Capillary zone elctrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) were applied to the simultaneous separation of cyanobacterial toxins. Both methods were applied to an analysis of cyanotoxins in water bloom samples and crude cyanobacterial extracts. The results obtained indicate that, for complex matrices, the sequential application of CZE and MEKC is necessary. It is recommended to use both CE techniques for the analysis of the same sample in order to confirm the results by an orthogonal approach [10].

Conclusions

CE-SSCP has excellent potential for bacterial community soil analysis and providing insight into microbial communities amended for phytoremediation purposes; also is suited for the rapid identification of the main Gram-negative nonfermenting bacilli.

CE seems to be a useful and quick tool for comparison of protein profiles especially when a large number of samples are to be investigated.

Significance of the microchip-based measurements, that its automated and fast results about the bacterial protein composition might contribute to the improved speed of microbiological diagnostics, and allow clinicians to make faster drug therapy decisions and earlier treatment.

A major advantage of CDCE in analyzing environmental samples of unknown species composition is that nonspecific or nontarget amplification products can be discriminated with high resolution from the target amplification products. This novel approach extends the usefulness of competitive QPCR by demonstrating its ability to reliably enumerate microorganisms at a range of environmentally relevant cell concentrations in complex aquatic samples.

CE seems to be a useful tool for analysis of cyanotoxins in water bloom samples and crude cyanobacterial extracts.

REFERENCES:

GHOZII, R, MORAND, P., FERRONI, A., BERETTI, J-L, BINGEN, E., SEGONDS, C., HUSSON, M. O. IZARD, D. BERCHE, P., GAILLARD, J-L, 1999, Capillary electrophoresis-single-strand confirmation polymorphism analysis for rapid identification of *Pseudomonas aeruginosa* and other Gram-negative nonfermenting bacilli recovered from patients with cystic fibrosis, *Journal of Clinical Microbiology,* 37, No. 10, p. 3374-3379.

HUBER, L, 1994, Good laboratory practice and current good manufacturing practice, Marcel Dekker Inc., Hewlett Packard, Germany, p. 106-108.

KING, S., 2004, Capillary electrophoresis single-strand conformation polymorphism analysis for monitoring bacteria during the remediation of TNT-contaminated soil, A dissertation for the degree Doctor of Philosophy, the College of Arts and Sciences of Ohio University.

KING, S., MCCORD, B.R., RIEFLER, G.R., 2005, Capillary electrophoresis single-strand conformation polymorphism analysis for monitoring soil bacteria, *Journal of Microbiological Methods*, 60,1, p. 83-92.

KUSTOS, I., KOCSIS, B., KEREPESJ, I., KILÁR, F., 1997, Changes in outer membrane protein profiles of bacteria after meropenem induced postantibiotic effect studied by capillary electrophoresis, *Antimicrob. Agents Chemother.,* 41, p. 556-563.

KUSTOS, I., KOCSIS. B., KEREPESI, I., KILÁR, F., 1998, Protein profile characterization of bacterial lysates by capillary electrophoresis, *Electrophoresis,* 19 (13), p. 2317-2323.

KUSTOS, I., TOTH. V., KOCSIS. B., KEREPESI, I., EMŐDY, L, KILÁR, F., 2000, Capillary electrophoresis analysis of wild type and mutant *Proteus penneri* outer membrane proteins, *Electrophoresis,* 21, p. 3020-3027.

KUSTOS, I., ANDRÁSFALVY. M., KYSTOS, T., KOCSIS. B., KILÁR, F., 2005, Effect of iron restriction on outer membrane protein composition of *Pseudomonas* strains studied by conventional and microchip electrophoresis, *Electrophoresis,* 26, p. 3789-3795.

LIM, E.L, TOMITA, A.V., THILLY, W.G., POLZ, M.F., 2001, Combination of competitive quantitative PCR and constant-denaturant capillary electrophoresis for high-resolution detection and enumeration of microbial cells, *Applied and Environmental Microbiology, 67,* No. 9, p. 3897-3903.

VASAS, G., GASPAR. A., PAGER, C., SURANYI, G., MATHE, C., HAMVAS, M., BORBELY, G., 2004, Analysis of cyanobactenal toxins (anatoxin-a, cylindrospermopsin, microcystin-LR) by capillary electrophoresis, *Electrophoresis,* 25, p. 108-115. www.agilent.com/chem/labonachip www.fejuelich.de/isg/index.php7index =142& print=1 ^3. www.rit.edu/~pac8612/ Biochemistry/505(705)/mo...

Rezumat. *Aplicaţii ale electroforezei capilare în microbiologie.* Metodele clasice de analiză (bazate pe numărarea în plăci după inocularea probelor de apă în medii selective sau neselective), utilizate pentru monitorizarea microbiologică a calităţii apelor şi pentru alte analize microbiologice sunt foarte laborioase şi necesită eforturi financiare mari. De aceea, în ultimul timp, cercetătorii şi-au îndreptat atenţia spre utilizarea unor metode mai puţin costisitoare, mai rapide şi mai eficiente. O astfel de metodă s-a dovedit a fi electroforeza capilară (EC). EC este o tehnică modernă de separare utilizată cu succes pentru determinarea secvenţei complete a ADN-ului uman, dar şi în microbiologie pentru identificarea bacteriilor din apă, sediment şi sol, pe baza separării ADN-ului amplificat prin PCR (reacţia de polimerizare în lanţ), pentru detectarea şi cuantificarea celulelor bacteriene, pentru determinarea proteinelor membranei externe a diferitelor bacterii etc. Toate studiile efectuate până în prezent au demonstrat că EC este o metodă rapidă şi puţin costisitoare, care prezintă câteva avantaje comparativ cu electroforeza pe gel, şi anume: viteză mare de analiză, cantităţi mici de probă, un control excelent al temperaturii, rezoluţie mare şi reproductibilitate. EC este o tehnică care ar putea fi utilizată pentru monitorizarea calităţii apei şi sedimentului, atât din punct de vedere fizico-chimic, cât şi din punct de vedere microbiologic.

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