

# MICROBIOLOGICAL AND ENZYMOLOGICAL CHARACTERIZATION OF WATER AND SAPROPELIC MUDS IN THE LAKES OF OCNA SIBIU

— short presentation PHD thesis —

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**Abstract:** this paper present the results obtained in the research of Ph.D. thesis, with the main objective to characterize and evaluate the physico-chemical, microbiological and enzymatical parameters of the water and sapropelic muds in thirteen Ocna Sibiu salty lakes, the majority in the balnear perimeter. Three of these lakes (lakes Closca, Crisan and Horia) have a low mineralization degree, low concentration of chlorides and calcium and a reduced amount of organic matter, whereas the Lake Negru is characterized by a high mineralization degree, maximum amount of chlorides and organic matter, a reduced concentration of dissolved oxygen, minimum pH. Lake Brâncoveanu is characterized by high mineralization of water, high concentration of chlorides and calcium, high hardness and dissolved oxygen. Lakes Ocnîța, Fără Fund, Gura Minei, Swallow, cu Insulă and cu Nămol present moderate degree of mineralisation, moderate concentration of chlorides and calcium. As an absolutely new determination in România for salty lakes is the *chlorophyll a* quantification analysis as an important quality indicator used to appreciate the eutrophication degree of the lakes. The conclusion is that the vast majority of the salty lakes in Ocna Sibiu are eutrophic lakes and hypertrophic. The seasonal enzymologic analysis of the sapropelic muds from the Ocna Sibiu salty lakes (dehydrogenasic, phosphatasic and catalasic activity) distinguish the existence of variations to the enzymatical activity intensities according to seasonal modification.

**Key words:** water, microbiological analysis, chemical analysis, hypersaline lake, Ocna Sibiului, enzymatic activity

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## INTRODUCTION

Water is a depletable natural resource, and its quality must be protected, managed and treated correspondingly. The water surface is a renewable resource, with limited recovering capacity to the human activities.

Besides purely scientific interest, the spa waters and therapeutic muds consecrated research have a remarkable practical importance. The evaluation of the water and therapeutical muds's physico-chemical, microbiological and enzymological potential constitutes a remarkable instrument with very high utility to the balneotherapy specialists. There is an epidemiologic risk of human disease, not only by ingesting unfitted water, but also by skin contact due to the capacity of some germs to penetrate the human skin. The contamination or infection risk associated to bathing and balneary waters is correlated to faecal impurification degree. Water faecal contamination may be caused by bathing people or by contaminated water sources due to infiltration or contact with pollutant agents.

The unconventional treatment solutions of the contaminated water are highly efficient and are oriented to the use of high quantity phytomass producing plants wich can be capitalized, but also to physical treatment using, less studied and applied, but with high efficiency.

The aim of the present article is to describe the results obtained in the Ph.D. thesis titled "*Microbiological and enzymological characterization of water and sapropelic muds in the lakeS of Ocna Sibiu*", under the scientific supervision of professor dr. Letiția Oprean, University of Sibiu.

*The purpose of this thesis* is the complex characterization of water and sapropelic muds from salty lakes in Ocna Sibiu and identifying concrete solutions for treatment of the superficial layer of contaminated water.

*The main objectives of this study* are the following:

1. Seasonal variations monitoring and physico-chemical, microbiological, enzymological characterization of water and sapropelic muds from salty lakes in Ocna Sibiu;
2. Identifying microbiological risk associated with water usage for bathing and balneotherapy in Ocna Sibiu salty lakes;
3. Establishing a hierarchy of salty lakes in Ocna Sibiu based on physical, chemical, microbiological and enzymological characterization of water and sapropelic muds;
4. Creating an informatical program to mathematically modelate and elaborate the experimental data and also to establish correlations between physical, chemical, microbiological and enzymological parameters in order

to highlight the evolution of the salty lakes and the differences between them;

5. Identifying solutions and viable biotechnologies for unconventional treatment of the surface draining and used waters which infiltrate the salty lakes of Ocna Sibiu in order to preserve, protect and obtain a quality improvement of the salty water and shorelines;

## **THESIS STRUCTURE**

This thesis is structured in two main parts, first one including the literature study, and the second experimental research which contains methods and materials, results and discussions, conclusions.

**PART ONE – CURRENT RESEARCH STAGE OF WATER AND SAPROPELIC MUDS IN SALTY LAKES**, with two chapters:

*Chapter 1.* Ocna Sibiu – General characterization, which presents the physical, geographical, biological and microbiological characterization of the Ocna Sibiu surroundings and the salty lakes description.

*Chapter 2.* The hypersaline lacustrine ecosystem – General characterization, which contains the mineral waters characterization and classification, references to microorganisms distribution in hypersaline environments, the balneo-therapeutical use of these salty waters and also the microbiological risk during bathing and using the salty water for medical purposes, peloidogenesis generalities, peloides characterization and classification.

**PART TWO – EXPERIMENTAL RESEARCH**, which contains four chapters, each one with three subchapters: methods and materials, results and discussions, conclusions.

*Chapter 3.* Physical, chemical, biochemical, and bacteriological water research of the Ocna Sibiu salty lakes, with two subchapters treating the characterization and evaluation of the physical, chemical and biochemical water parameters, and the characterization and evaluation of the hygienic and sanitary indicating salty water parameters in Ocna Sibiu.

*Chapter 4.* Physical, chemical, enzymological and bacteriological characterization of the sapropelic muds in the Ocna Sibiu salty lakes, which purpose is to realize a complex sapropelic mud characterization based on the global composition, enzymatic activity (dehydrogenasic enzymatic activity, phosphatasic enzymatic activity and catalasic enzymatic activity) and evaluation of the faecal pollution indicating bacteriological parameters.

*Chapter 5.* Mathematical modeling and correlation establishing between different analyzed parameters, describes the realised informatic program and

coresponding mathematical modeling and facilitating instruments for the obtained data prelucration and interpretation.

*Chapter 6.* Biotechnologies and applied solutions for Ocna Sibiu lakes preserving, protection and management, wich presents the realised experiments in superficial water purification of the salty lakes by unconventional biological and physical methods and quantifying the efficiency of these methods. Contaminated water purification by unconventional biological methods, meaning aquatic plants, was realised in three stages: laboratory conditions, stationary conditions and varying contaminating load, and pilot station conditions.

*Chapter 7.* General conclusions, wich synthetize the final results obtained in the previous chapters, evaluating the success in reaching the proposed targets.

The personal research part is ended by *Bibliography* concerning the achieved studies. For statistical data interpretation I used the programming system SYSTAT.

The presented research was made in the Potable and Used Water Analysis Laboratory owned by S.C. Apă – Canal S.A. Sibiu and also the Biotechnology and Microbiology Laboratory of the Agricultural Sciences and Alimentary Industry Faculty in „Lucian Blaga” University, Sibiu.

## GENERAL CONCLUSIONS

The following conclusions are derived from the obtained experimental results of our research:

**1.** The complex study achieved in 2006 – 2009 in order to characterize and evaluate the physico-chemical, microbiological and enzymatical parameters of the water and sapropelic muds in the Ocna Sibiu salty lakes regarded 13 lakes, the majority in the balnear perimeter (Lake Fără Fund, Lake Cloșca, Lake Horea, Lake Crișan, Lake Ocnița, Lake cu Nămol, Lake Rândunica, Lake Brâncoveanu, Lake Negru, Lake cu Insulă, Lake Gura Minei), but also lakes that are not used yet for bathing and treatment (Lake Mățelor, Lake Pânzelor). Over the course of 10 water and mud sampling campaign from the salty lakes I have determined 8 physico-chemical parameters (temperature, conductivity, pH level, salinity, hardness, chloride content, calcium content and fix residuu) and 7 microbiological parameters (number of colonies at 22<sup>0</sup>C, number of colonies at 37<sup>0</sup>C, total coliforms, faecal coliforms, *Escherichia coli*, faecal enterococci *Clostridium perfringens*).

Regarding the increased number of obtained experimental data I have constructed an informatical program based on mathematical analysis in order to interpret and prelucrate all the parameters. I have determined descriptive statistical parameters (sample number, minimal value, maximal value, the medium and standard deviation) and I grouped the salty lakes based on physico-chemical and bacteriological water parameters by using cluster tree diagrams.

2. The original mathematical analysis model of the experimental data and the informatical program led to automatical correlation established between physico-chemical and microbiological parameters, thus grouping the salty lakes in the following classes:

➤ Lakes Horea, Cloșca and Crișan presents a low mineralization wich implicates low electrical conductivity values (maxim 30.4 mS/cm) and low salinity (annual medium aproximatively 50 g/l), and raised water hardness (medium hardness under 30 °dGe). Microbiological Lake Cloșca presents maximal load.

➤ Lake Rândunica has low mineralization, low calcium and chloride content, low salinity and low number of faecal pollution indicating bacteria.

➤ Lake Mățelor and Lake Pânzelor are characterized by medium conductivity (50 mS/ cm - 170 mS/cm), high mineralization, moderate calcium content, low salinity (annual medium aproximatively 50 g/l), presents very high water hardness (medium water hardness over 30 °dGe) and extremely high bacterian load all around the year, because here were identificated recent and older water pollution indicators.

➤ Lakes Ocnița, Cu Nămol, Cu Insulă and Lake Gura Minei have a moderate mineralization degree, moderate calcium and chloride content, medium conductivity (50 mS/ cm - 170 mS/ cm), very high water hardness (medium water hardness over 30°dGe), high salinity and are within the boundaries of the legal microbiological water quality for bathing almost all year.

➤ Lake Fără Fund presents high mineralization, high salinity, high calcium and chloride content, reflected by high water hardness and minimal bacterian content.

➤ Lake Negru and Lake Brâncoveanu have the highest conductivity (220 mS/ cm -250 mS/ cm), mineralization, salinity (over 200 g/l), calcium and chloride content, and water hardness (medium water hardness over 100°dGe) and minimal load of faecal pollution indicators all year, wich indicates no faecal contamination.

3. The mathematical model and the informatic program led to automatic correlation establishing between physico-chemical and microbiological parameters and determined the evolution tendency of the lakes's characteristics. Also this program will constitute an important database for the Ocna Sibiu salty lakes wich will be improved with following research. The realised program and the mathematical modeling represents useful instruments, wich will facilitate all data interpretation including future obtained data in order to find and apply the most efficient solutions in water treatment. The original mathematical analysis model will be improved and validated in order to be applied not only in the case of the Ocna Sibiu lakes, but in all water quality research.

4. As an absolutely new determination in România for salty lakes is the *chlorophyll a* quantification analysis (the photosynthetic pigment essential to all green algae), wich represents an important quality indicator used to appreciate the eutrophication degree of the lakes. My personal conclusion was that the vast majority of the salty lakes in Ocna Sibiu are eutrophic lakes (Lake Pânzeler, Lake Rândunica, Lake Brâncoveanu) and hypertrophic (Lake Negru, Lake Ocnîța, Lake cu Insulă, Lake cu Nămol).

5. The bacteriological water monitoring inclined to faecal pollution indicators determination in all seasons revealed the presence of these microorganisms. The obtained results proved the fact that the water faecal impurification is influenced by season, water chemical characteristics and the numbers of persons using the lakes for bathing and therapeutic purposes. In overall, the coliform and thermotolerant coliform bacteria parameters (parameters wich indicates relative recent faecaloid impurification) had higher values in summer-fall than in winter-spring, due to the increased use of the lakes by swimmers and locals in search of the salt water and sapropelic muds therapeutical benefits. This determines an intense activity around the lakes and an increase of impurified water overflow, along with the changes in water stratification in the fall due to water cooling, resulting in raised bacteriological loads.

6. The minimal number of indicating bacteria for a faecal impurification was detected in all seasons in Lake Negru and Lake Brâncoveanu, wich indicates that these lakes are not affected by faecaloid contamination. In these lakes the water presents even at the surface high mineralization and salinity (maximum salinity of 278.42 g/l in Lake Brâncoveanu, respectively 353.2 g/l for Lake Negru) wich inhibits microorganisms growth in all seasons. In addition the two lakes are remotely situated from the habitated area and are not affected by used water draining. The negative impact is due

only to pluvial draining from the neighbouring grounds. High bacterian load recorded in Lake Mățelor situated near housing area and impurified with faecaloid water drainings, and so is the lake Cloșca supplying source.

**7.** I have identified, as a premiere in România, from the bacteriological analysis bacteria species from *Enterobacteriaceae* family, the following gender: *Serratia*, *Providencia*, *Enterobacter*, *Citrobacter*, *Escherichia*, *Proteus*, *Klebsiella*, *Salmonella*, *Vibrio*, by using miniatural biochemical tests from the standardized system API 20E. Exact identification of bacteria species from *Enterobacteriaceae* family in salty water is important to the persons who use these lakes for bathing and for therapeutical purpose so the risk of disease can be properly appreciated due to the presence of these bacteria.

**8.** From the mud enzymatical potential point of view, expressed by dehydrogenasic, phosphatasic and catalasic activity, I have determined that the Lake Ocnîța presents maximum activity, and its sapropelic mud has an intense microbial activity, high oxydo-reducing potential, and also a high organic load like Lake Negru (its unpolluted sapropelic mud has an intense catalasic activity, wich denotes high mud oxydo-reducing potential). The minimal intensity of enzymatic activity characteristic to the mud present Lake Rândunica and Lake cu Insulă, wich indicates poor therapeutical efficiency of the sapropelic mud from these lakes.

**9.** The seasonal enzymologic analysis of the sapropelic muds from the Ocna Sibiu salty lakes distinguish the existence of variations to the enzymatical activity intensities according to seasonal modification. Phosphstasic activity is low in March and July and raised in January and October, in concordance to organomineral biocomponent accumulation. On the contrary, the dehydrogenasic activity presents high ponderosity in March and July and it is reduced in the fall-winter. The mud oxydo-reducing potential, reflected by catalasic activity, is low in winter-spring and high in summer-fall.

**10.** An original contribution represents the fact that the enzymological, physico-chemical and bacteriological mud analysis allowed me to hierachyate the lakes based on the therapeutical qualities, global composition and contamination degree. And so I recommend the muds from Lake Negru and Lake Brâncoveanu as suitable to extraction and therapeutic use with high efficiency, if permitted by the geological reserves because these muds have a good enzymatic activity, maximum mineral substances quantity and very low faecal contamination. The mud from Lake Ocnîța and Lake cu Nămol presents a good enzymatic potential, but for use in therapeutical purposes it

is necessary to apply cleaning treatments in order to reduce the faecal contamination and pathogen microorganisms presence.

**11.** The obtained experimental results proved the fact that the faecal water and mud impurification is influenced by season, chemical characteristics, water salinity and the breaking of the strict hygienic and sanitary rules. Respecting the measures regarding the use of the zones included in the sanitary protection program conform to H.G. 930/2005 it is absolutely necessary because the anthropic impact will be considerably diminished.

**12.** The original proposed solutions match the actual research tendencies to new possibilities of impurified water treatment using unconventional biological methods and certain aquatic plant species which produce high quantities of usable phytomass, and also to physical treatment use with high efficiency, but unused before. The contaminated water purification by unconventional methods reduces the risk of water contamination and thus affecting the natural matter circuit and energy flux in the lacustrine ecosystem, and also the peloidogenesis process in the salty lakes. Choosing unconventional water purifying treatments for salty waters is a specially important imposed measure because these waters are used in spa and curative treatments.

**13.** Original contributions are all the experiments starting off laboratory testing conditions, then testing at a big scale with water organic load fluctuation and finally creating a pilot water purification ministration thus allowing me to quantify the studied species efficiency and to specify the contaminated water types passible to this unconventional treatment.

**14.** I have demonstrated through original experiments that by using submersible water purifying plants the water is greatly cleansed of the biodegradable organic substances and microorganisms. The absolute novelty is the use for the first time of plants with water purifying potential like the following genera: *Bacopa*, *Ludwingia* and *Hygrophila*.

**15.** The optical irradiation treatment to destroy or reduce the microorganisms is a personal contribution and it has a good effect in the case of the lakes with high bacteriological load, low turbidity and low or medium salinity.

**16.** The experimental results presented in this thesis may form the base of a durable management program for the reference zone, which will contribute to the socio-economical development and to conserve the natural and cultural values of Ocna Sibiu.