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MICROBIOLOGICAL AND ENZYMOLOGICAL STUDIES ON THE WATER AND SEDIMENTS OF THE "OCHIUL MARE" LAKE ("1 MAI" BATHS, BIHOR COUNTY)

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Abstract. *As a main purpose* of our study, we had appraised the activities and dynamics of the ecological groups of bacteria (heterotrophic, lipolytic, amylolytic, proteolytic, ammonifying, nitrifying, denitrifying, and iron-reducing bacteria), according to the actual eutrophication stage of this particular lake and the physical-chemical parameters of it's water.

We also determined the total coliform bacteria, in both water and sediment samples. The bacteriological studies have been completed with the enzymological ones in order to obtain a complete image on the complex processes that are developing in this habitate. In the sediment samples have been determined the following enzymatic activities: actual and potential dehydrogenase , phosphatase, catalase and and non-enzymatic catalytic activity. All the studied activities have been detected in the entire set of samples, observing differentiates only concerning the intensity of processes.

The numerical density of the bacteria was high in the mud, due to the fact that the mud contains many organic substances. The high number of the bacteria in the mud indicated that the intensity of the decomposition processes in the lake is intensive. On the basis of the relative values of the enzymatic activities it was calculated the enzymatic indicator of the mud's biological quality.

The sediments of the lake exhibit quite a high enzymatic potential, what was more intense during summer, as in the spring.

INTRODUCTION

Decomposition and mineralization of organic matter are processes of great importance for 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 compounds of little molecular weight resulted from the exoenzyme activities are rapidly metabolized by the heterotrophic bacteria . Thus, one can consider that the rate of the organic matter degradation is, probably, controled by the first stage, that of the exoenzymatic hydrolzse (Meyer-Reil, 1987).

In the aquatic ecosystems, the sediments are a clue link in the elements biocycle, the place where the mineralization processes of the organic substances undegraded in the water column is completed. 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. Malcom and Stanley (1982) consider 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.

The *Ochiul Mare* lake lies in the "1 Mai" Baths area, about 9 km away from Oradea, belonging to the Peţea geothermal ecosystem, the latter having been declared a natural reservation since 1932, on the botanist Alexandru Borza's demand [4, 6,].

In 1932 the reservation included besides lake Ochiul Mare, lakes Ochiul Tiganilor (having a 20 m diametre, 2 m depth and completely silted up today) and Ochiul Pompei (having a 25 m diametre, 3 m depth, today completely silted up) as well [5, 9].

Pliocene lake Ochiul Mare used to have a surface of around 4 188 m^2 and an average depth of 0.60 m. From all this surface only 600 m^2 remained. Nowadays the lake has an average depth of 0.40 m (0.20 – 0.60 m) [16, 21].

This reservation has a special importance from a scientific point of view, due to the presence of two tertiary relict species (*Melanopsis parreyssi* and *Nymphaea lotus var. thermalis)* (18, 27).

These species survived to the quaternal glaciation, thanks to the microclimate of the ecosystem of Ochiul Mare, which is characterized by:

- the constant temperature of 25 °C of the water, regardless to season and the temperature of air,
- the mildly alkaline pH of the water,
- the depth of the lake favoured the water-lily (between 0.6 – 0.8 m),

- the richness in nutrient substances [20].

The *Ochiul Mare* lake comes across an actual eutrophication process, characterized by an intermediary stage of plugging. This process began about forty (40) years ago, as a result of the human impact [15, 21].

The water pollution caused the growth of the yearly biomass productivity and the acumulation of organic substances. The big quantity of organic material in water leads to the propagation of algae, to the decrease of water transparency, to the modification of colour, to the increase of certain ion concentration and to the rise of the lake bottom (the marshing procedure) .

The negative consequences of eutrophication at the time being, strongly request the study of the existing microbiota, and microbial processes which take place in this Pliocene lake of *Ochiul Mare* [6].

The enzymatic catalyse under the direct or indirect control of the microorganisms do mediate this complex process, that ensures the development of the biological cycles of elements (C, N, P, S). The determination of the enzymatic activity in acvatic sediments, as well as in soil, constitutes a research tool for evaluating the functional diversity of microbiota[20, 8].

The present work wants to follow the diversity of microbiota and the enzymatic activity in the lake *Ochiul Mare*, for determining the implication of microorganisms in the transformation of organic substances, as well as in the fight against the polution.

MATERIALS AND METHODS

The mud and water samples were taken from four places in the *Ochiul Mare* lake: 1. near the Children Sanatorium, 2. near the bridge, 3. opposite to the Children Sanatorium, and 4. near the Peţea brook. The sampling has been made in April and June, 2005. For microbiological analysis, samples were taken in aseptic conditions.

The specific media have been used [7].

Medium for aerobe heterotrophic bacteria: agar nutritive broth. Number of bacteria was expressed as CFU (colony forming units) ml^{-1} or g^{-1}.

Medium for lipolytic bacteria: nutritive agar medium containing Tween 80. The most probable number (MPN) of bacteria was calculated according to the statistical table of Alexander [`1] and was established based on the precipitation of $CaCl_2$ around the colonies (Stoica et al., 2002).

Medium for amylolytic bacteria: nutritive agar containing soluble starch. MPN was established on the basis of apearance of starch hydrolysis.

Medium for proteolytic bacteria: nutritive broth containing gelatin. MPN was established on the basis of gelatine liquefaction.

Medium for nitrifying bacteria: Winogradsky saline solution with $(NH_4)_2SO_4$ and $NaNO_2$, respectively. MPN was established on the basis of the presence of NO_2^- and NO_3^-.

Medium for denitrifying bacteria: Winogradsky saline solution with glucose and KNO_3. MPN was established on the basis of the presence of NO_2^-.

Media for ammonifying bacteria: peptone water; pH 7.9. MPN was established on the basis of the presence of NH_3.

Medium for iron-reducing bacteria: organic broth with Fe_2O_3. MPN was established on the basis of the presence Fe^{2+}.

Medium for total coliform bacteria: the double concentrated lauryl sulphate broth media (Drăgan-Bularda, 2000).

Dehydrogenase activity was determined using the TTC based of Casida et all.method (1964).The reaction mixture consisted of 3 g mud , 0.5 ml TTC 3 % and 2 ml distilled water (for the actual dehydrogenase), and 1 ml glucose 3 % and 1 ml distilled water (for potential dehydrogenase). Incubation took place at 37^0C, for 24 h. The activity was measured in mg formazane/ 3 g mud.

Catalase activity(enzymatic H_2O_2 splitting) was determined using a technique based on Kappen method. We used 1 g of mud, added 10 ml of distilled water and 2 ml H_2O_2 3 %.These reaction mixtures were incubated at 20^0C for one hour. The catalase activity is calculated from difference between the active samples and the inactivated ones, and is expressed in mg H_2O_2/g dried mud/ 1 h at 20^0C.

Nonenzymatic catalytic activity was established also using Kappen method but instead active mud samples using inactivate (thermic) mud samples.

Phosphatase activity was determined using method of Kramer and Erdei (1959). The reaction mixtures consisting of 3 g mud and 2 ml toluene, plus10 ml disodium fenilphosphate solution 0.5 %. Incubation took place at 37^0C for 24 h. Phosphatase activity was measured in mg phenol/3 g mud dried substance.

In analyzing the chemical properties of the lake's water, we determined the nitrates (NO_3^-), nitrites (NO_2^-), phosphates (PO_4^{3-}), NH_4^+ ions concentrations, and the pH of the water. The methods used in determining the concentration of those ions were color-based ones [10].

RESULTS AND DISCUSSIONS

The results of bacteriological analyses are presented in Table 1 and 2. The bacteriological studies of the water and mud indicate the presence of all physiological groups (see table 1).

Table 1. Numerical distribution of the bacteria groups in water and mud of the O*chiul Mare* lake

Physiological groups of bacteria	Water				Mud			
	Sample no.				Sample no.			
	1	2	3	4	1	2	3	4
	No. / ml				No. / g			
Aerobe heterotrophic	400000	350000	275000	300000	7000000	6500000	12000000	11000000
Lipolytic bacteria	9 000	2000	20000	40000	100000	40000	60000	90000
Amylolytic bacteria	7 00	14000	3000	14000	25000	18000	16000	95000
Proteolytic bacteria	2 00	800	1200	400	100000	250000	190000	145000
Nitrifying bacteria	300	7 00	300	1200	30000	31000	170000	90000
Denitri-fying bacteria	2 00	250	200	50	2000	800	10000	4000
Ammoni-fying bacteria	40000	12000	70000	12000	200000	150000	200000	130000
Iron-reducing bacteria	3000	13000	14000	9000	80000	24000	140000	150000

All bacteria were present both in water and sediments. Among all of the physiological groups which were determined in our study, the highest density found belonged to the heterotrophic and ammonifying bacteria. These bacteria were found in both in the mud and water of the lake in high numbers. Iron-reducing bacteria and denitrifying bacteria were found in smaller quantities. Lipolytic bacteria and amylolytic bacteria were, generally, less represented. The poorest presence was the one of the nitrifying bacteria.

It is to be emphasized that in water samples the number of bacteria was ten to hundred times lower than in mud.

The presence of coliform germs in medium densities, both in water (1600-1900 / 100 ml) and sediments (2200-2800 / 1 g), indicates the existence of a faecal matter pollution source (people and domestic animals get into the water, bathing) (table 2).

Table 2. The number of total coliform bacteria

	Water				Mud			
Sample no.	1	2	3	4	1	2	3	4
Total coliform bacteria/100ml or per g (fresh substance)	1600	1700	1800	1900	2200	2300	2400	2800

The coliform bacteria represent a category belonging to the *Enterobacteriaceae* familiy. The coliformn group is formed by more of bacterial genera including *Klebsiella, Escherichia, Salmonella, Erwinia, Serratia* and other enterobacteria. The species of the coliform bacteria group are not pathogen strictly speaking, but, in some conditions, can often induce diarrhoea diseases or the infections of the urinary tract. Therefore, they are considered opportunist pathogens.Due to this fact the coliform bacteria germs are considered as very important indicator organisms. If their number in the natural environment is higher the probabiliuty of the presence of pathogen microorganisms in the environment increases (Millea, 2001).

The results of the enzymological analyses of the Ochiul Mare lake sediment are presented in table 3 and 4. In all mud samples have been detected all the studied enzymatic activities.

As far as the enzymatic activity is concerned, we noticed that all enzymatic activities were more intense during summer (table 3).

Table 3. Dehydrogenase (actual and potential) and catalase activities in mud from Ochiul Mare lake

Sample no.	Actual dehydrogenase activity			Potential dehydrogenase activity			Catalase activity		
	April	Iune	Mean ± Standard error	April	Iune	Mean ± Standard error	April	Iune	Mean ± Standard error
1	3.08	6.28	4.68±2.26	11.09	13.08	12.08±1.50	18.95	28.45	23.70±6.71
2	1.27	4.90	3.08±2.56	9.68	10.75	10.21±0.76	23.20	24.90	24.05±1.20
3	0.81	4.17	2.49±2.37	8.75	9.92	9.33±0.82	23.88	26.71	25.29±2.00
4	2.15	5.12	3.63±2.10	9.77	11.34	10.55±1.11	24.48	27.50	25.99±2.13

The fact that the potential dehydrogenase activity is more intense than the actual one, indicates the stimulating action that the carbon source (glucose) has upon the enzymatic synthesis of the germs in the mud. Also, both catalase and phosphatase activities have been measured as intense. Altogether, the most intense enzymatic activities have been registered in the case of sample 1, collected near the Children Sanatorium (tables 3 and 4).

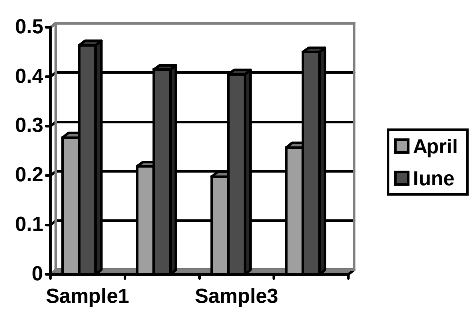
The enzymatic indicator of sludge quality (EISQ) has also been determined, allowing us to appraise the quality of the mud, from an enzymological point of view (Fig.1).

As the EISQ values show, the sludge taken from this lake had a more intense enzymatic activity during summer, rather than spring (table 4 and fig. 1).

Table 4. Catalase (non-enzymatic) and phosphatase activities

Sample no.	Nonenzymatic catalase activity (mg H_2O_2/3 g sludge /1 h)	Phosphatase activity (mg fenol /10 g sludge /3 days)
	June	June
1	16.88	12.76
2	18.38	10.34
3	19.43	9.88
4	21.80	10.90

Fig 1. The enzymatic indicator of sludge quality



Physical and chemical analysis of the water of the Ochiul Mare lake

In all 4 samples, the nitrates concentration varied between 0.2-0.8 mg/l, while the nitrites and NH_4^+ ions concentrations exhibit values between 0.1-0.2 mg/l, which indicates that this water is polluted, indeed. The phosphate concentration value in samples 1,2 and 4 was 0.1 mg/l, which is quite a low value. But in the case of the 3rd sample, there has been registered a 0.3-0.5 mg/l concentration level, which indicates a phosphate pollution of the water (table 5).

Table 5. Chemical parameters of the water of the *Ochiul Mare* lake

Indicator	Sample 1		Sample 2		Sample 3		Sample 4	
	April	June	April	June	April	June	April	June
NO_3^- (mg/l)	0.3	0.2	0.4	1.5	0.8	1.0	0.5	0.2
NO_2^- (mg/l)	0.15	0.1	0.1	0.05	0.2	0.15	0.15	0.1
NH_4^+ (mg/l)	0.2	0.15	0.1	0.2	0.2	0.2	0.15	0.2
PO_4^{3-} (mg/l)	0.1	0.2	0.1	0.2	0.5	0.3	0.1	0.15
pH	7.7	8.2	7.5	7.3	7.2	6.7	7.2	7.4

The water pH is slightly alkaline (7.2-8.7), due to the $CaCO_3$ dissolution coming from the carbonated rocks (table 5).

CONCLUSIONS

The numerical density of the bacteria was higher in the sludge than in the water of the lake, due to the fact that the mud contains many organic chemicals .

The large number of the groups of germs found in all sediment samples, indicates the intensity of the decomposition processes within the lake’s sludge.

The registered bacteria, which could be found in the water and sediments of the lake, make an important element of lake ecosystems biocenosys.

the number of coliform germs is high both in water and sludge sample taken opposite to the Children Sanatorium and near the Pețea brook, and this happens because people and domestic animals get into the water and bathe, especially in these sites of the lake.

The sediments of the lake exhibit quite a high enzymatic potential, being detected all the four enzymatic activities (actual dehydrogenase, potential dehydrogenase, phosphatase and catalase, as well as non-enzymatic cacalytic activity);

The enzymatic indicator of mud quality has registered higher values during summer, than spring. The highest EISQ level was found in sample 1, and the lowest one in sample 3.

As far as the water quality of the *Ochiul Mare* lake is concerned, nitrates, nitrites and phosphates values overrun the admitted limits.

REFERENCES

Alexander, M., *Most-probable-numbermethod for microbial populations ,*in Black, C.A., Evans, D.D., White, J.L., Ensminger, L.E., Clark, F.E.(Eds.) *Methodsof Soil Analysis,*pp.1467-1472, Am. Soc. Agron., Madison, Wisconsin.

Casida, L.E.jr., Klein, D.A., Santoro, T., *Soil dehydrogenase activity.* Soil Sci., 98, 371-376, 1964.

Cohut I.: *Sistemul hidrogeotermal Oradea-Felix*, Foradex S.A. Oradea, uz intern, 1987

Cosma Oltean C.: *Nufărul termal de la Băile 1 Mai (Oradea) solicită ocrotire*, Muzeul Țării Crișurilor, Oradea, 1991

Danciu, M.V: *Rezervația naturală „Pârâul Pețea” și perspectiva unui eco-muzeu în aer liber la Băile 1 Mai,* Analele Universității dinOradea, Fascicula Protecția Mediului, vol. IX, 2004

Drăgan-Bularda, M.: *Microbiologie generală – lucrări practice,* Univ. Babeș-Bolyai, Cluj-Napoca, 2000

Drăgan-Bularda, M., Grigore, C. E., Țura, D.: *Utilizarea indicatorului enzimatic al calității nămolului în scopul valorificării protecției lacurilor saline,* Studia Univ. Babeș-Bolyai, Biol.,49 (1), 2004.

Dukrét G., Péter I. Z.: *Püspökfürdő,* a Partiumi és Bánsági Műemlékvédő és Emlékhely Bizottság, a Királyhágómelléki egyházkerület és a Nagyváradi Római Katolikus Püspökség kiadványa, 1999.

Fresenius, W., Quentin, K. E., Schneider, W.: *Water Analysis. A practical guide to physico-chemical, chemical and microbiological water examination and quality assurance,* Springer Verlag, Berlin, 1988.

Giafreda, L., Bollag, J. M.: *Influence of natural and anthropogenic factors on enzyme activity in soil,* In: Soil Biochemistry, Vol. 9, ed. By Stotzky, G. éi Bollag, J.M., Marcel Dekker, Inc., New York*,* 1996.

Kappen, H., *Die katalytische Kraft des Ackerbodens.* Fühlings Ztg., 62, 337-392, 1913.

Krámer, M., Erdei, G., *Primenenie metoda opredeleniya aktivnosti fosfatazi v agrohimiceskih issledovaniiah.* Pocivovedenie, No. 9, 99-102, 1959.

Lovley, D. R., Phillips, J. P., Lonergan, D. J.: *Enzymatic versus nonenzymatic mechanisms for Fe(III) reduction in aquatic sediments,* Environmental .Science and Technology, 1991.

Manescu, S., Cucu, M., Diaconescu, M. L.: *Chimia sanitară a mediului,* Editura Medicală, București, 1994.

Marossy A.: *Unele observații asupra fenomenului de colmatare și eutrofizare a rezervației naturale “Pârâul Pețea”*, Nymphaea-Folia naturae Biharae,Oradea, 27, 139-144, 1999.

Marossy A.: *Veszélyben a püspökfürdői hévízi tündérrózsa,* Erdélyi Nimród, 1999.

Malcolm, S.J., Stanley S.D., *The sediment environment,* in Nedwell, D.B., Brown,C.M.(Eds.),Sediment Microbiology, Ed.Acad.Press, London, 1-14, 1982.

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

Millea, L.C., *Preocupări actuale legate de poluarea apelor.* Stud.Cercet. Biol. (Bistriţa), 6, 29-34, 2001.

Muntean,V., Crisan,R., Pasca,D., Kiss,S., Dragan-Bularda,M., *Enzymological classification pf salt lakes inb Romania.* Int. J. Salt Lakes Res., 5 (1), 35-44, 1996.

Muntean, V., Ştef., L. C., Drăgan-Bularda, M.: *Cercetări enzimologice asupra unor sedimente din râul Mureş,* Romanian Biological Sciences, I (3-4), 107-114, 2004.

Muzeul „Ţării Crişurilor" Oradea, Fundaţia Ecotop Oradea: *Nufărul termal – Rezervaţia naturală „Pârâul Peţea",* Oradea 1999.

Nannipieri, P., Badalucco,L., *Biological processes,* in Benbi,D.K., Nieder, R(Eds.), *Handbook of Processes and Modeling in the Soil-Plant System,pp.57-82, Haworth Press, Binghamton, New York,2002.*

Orlescu, M.C., Togor, G.C.: *Lacul cu nuferi 1 Mai între supravieţuire şi compromitere ecologică. Studiu asupra cauzelor deteriorării factorilor mediuluii ambiant şi soluţiilor posibile de ameliorare,* Analele Universităţii din Oradea, Fascicula: Protecţia Mediului, vol. V, 1999.

Péter I. Z.: *Félixfürdő – helytörténet,* a Partiumi és Bánsági Műemlékvédő és Emlékhely Bizottság, a Királyhágómelléki egyházkerület és a Nagyváradi Római Katolikus Püspökség kiadványa, 1998.

Rădulescu, D., Kiss, Şt., Drăgan-Bularda, M.: *Studii enzimologice asupra nămolului terapeutic de la Băile 1 Mai – Oradea,* Studia, Univ. Babeş-Bolyai, Ser. Biologia, Fasc. 2, 1970.

Stoica, E., Dragan-Bularda,M., Kuever,J., *The presence of sulfate-reducing bacteria in the bottom sediments of the Romanian Black Sea area,* Studia Univ.Babes-Bolyai, Biol.,47(2), 59-71.

Şoldea, V.: *Peţea şi nufărul termal,* Edit. Univ. Oradea, 2003.

Tofan, T., Dalea, A. : *Rezervaţia naturală „Pârâul Peţea" (Jud. Bihor) – Realităţi şi perspective,* Anal. Univ. Oradea, Fasc. Protecţia Mediului, vol VII, anul 2002.

Varduca, A.: *Hidrochimia şi poluarea chimică a apelor,* Editura *H*G*A, Bucureşti, 1997.