Enzymological researches on the mud in the „Ochiul Mare" Thermal Lake („1 Mai" Baths, Bihor county)

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Abstract. The mud of the lakes beds is a complex microbial community and the bacterias' characteristics (like diversity, activity) indicate the ecological state of the lake. The mud samples were taken seasonally from four places in the Ochiul Mare lake and were examined from enzymological point of view.

We have determined quantitatively the following enzymatic activities: dehydrogenase- (actual and potential), phosphatase-, catalase- and urease activities.

Every determined enzymatic activity had medium or high values. The actual and potential dehydrogenase activities and the phosphatase- and urease activities are the lowest in the samples, which were collected from the 3rd collection zone. This area is the most polluted zone of the lake and the microbes' growth is inhibited here. The catalase activity was the most intensive among the samples, which were collected from the 4th collection zone. The enzymatic activities showed little seasonal oscillation, reduced numerical fluctuation and the highest values were registered during the summer and in autumn.

The values of the Enzymatic Indicator of the Mud Quality (EIMQ) were the highest in the case of the samples collected from the 1st collection zone, which is the least polluted area of the lake. The low values of the Enzymatic Coefficient of Variety of the Mud Quality (ECVMQ) (0.088 – 0.162) indicate the sediments homogeneity in the whole lake and the enzyme activities' high stability independent of the investigated season.

Based on the obtained results it has been found that the Ochiul Mare lake's sediment has a medium but stable enzymatic potential, which is not influenced by seasonal fluctuations. This stability is insured by the stable microclimate of the thermal lake.

Keywords: *sediment, enzymological research, Enzymatic Indicator of the Mud Quality (EIMQ), Enzymatic Coefficient of Variety of the Mud Quality (ECVMQ), enzymatic potential*

Introduction

Pliocene lake Ochiul Mare has special importance from ecological and scientific point of view. In the past 40 years this lake has been considerably perturbed as a result of human impact. Due to the human influence the lake comes across an actual eutrophication process (Cohuţ 1997; Marossy 1999; Peter 1998).

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 (Bara.& Laslo 1998; Brezeanu & Simon-Gruita 2002; Şoldea 2003; Tofan & Dalea 2002).

The sediments represent a fundamental importance in the biogeochemical cycles of elements because mineralization process of the organic matters and the degradation process of the organic pollutants are finalized here (Şchiopu & Vîntu 2002; Schinner et al. 1996).

Microorganisms have an enzymatic effect on the substrates from their environment through reduction, oxidation, hydrolysis and through the effect of the same final products, which resulted from the bacterial metabolism and enzymatic activities (Zarnea 1994; Muntean et al. 2004). Therefore the investigation of enzymatic activities furnishes suggestive data about the processes that happen in the sediments (Cuşa 1996; Crişan et al. 2001).

The mud's enzymatic activity of the Romanian thermal lakes did not follow a comprehensive study and there are few data about the Ochiul Mare lake's sediment. The data published by Rădulescu et al., in 1970 are available.

Materials and Methods

We have analysed from enzymological point of view four series of mud samples. The sampling was made between April 2005 – January 2006. The mud samples were taken from four collection zones in the

Ochiul Mare lake: near the Children Sanatorium, near the bridge, opposite to the Children Sanatorium, and near the Peţea brook.

The following enzymatic activities have been determined:

The actual and potential dehydrogenase activity was determined by the method of Casida et al. (Drăgan-Bularda 2000) without or with glucose addition. The mud was treated with TTC - [2,3,5-triphenyltetrazolium-chloride], which serves as a terminal acceptor of protons from organic compounds. The samples were being incubated for 3 days at 20 ºC. Dehydrogenase activity was expresed in mg formazane/10 g mud d.w.

The phosphatase activity was determined by a method of Kramer and Erdei (Drăgan-Bularda 2000) with disodium-phenyl-phosphate. Phosphatase activity was expresed in mg phenol/10 g mud d.w.

The catalase activity was determined using method of Kappen with permanganate, which was described by Drăgan-Bularda (2000). Catalase activity (H_2O_2 splitting) was expressed in mg H_2O_2 / 3 g mud/60 min/20^{0}C.

The urease activity was determined using Sumner´s method (Drăgan-Bularda 2000). Urease activity was expressed in mg NH_4^+/10 g mud d.w.

Based on the absolute and relative enzymatic activities we calculated for every analysed sample the Enzymatic Indicator of the Mud Quality (EIMQ) and the Enzymatic Coefficient of Variety of the Mud Quality (ECVMQ) (Drăgan-Bularda et al. 2004).

Results

The entire set of the analysed sample, all the quantitatively determined enzymatic activities have medium or high intensities, apart from the collection zone.

The highest values of the actual dehydrogenase activity were obtained in the case of the samples collected near the Children Sanatorium (1st collection zone) and the smallest values were obtained in the case of the samples collected from opposite to the Children Sanatorium (3rdcollection zone) (see Table No. 1).

The examined samples' potential dehydrogenase activity was higher than the actual activity. The order of potential activities is identical with the actual activities' order (see Table No. 2).

The phosphatase activity was the most intensive in the case of the sample, which was collected near the Children Sanatorium (1st collection zone), followed by the 2nd collection zone. The minimal values were registered from opposite to the Children Sanatorium (3rdcollection zone) (see Table No. 3.).

The catalase activity was the most intensive in the case of the sample collected near the Pețea brook (4th collection zone), but the other values, which were measured in the other 3 collection zones showed insignificant oscillation and a reduced numerical fluctuation (see Table No. 4).

The urease activity was the most intensive in the case of the samples collected near the bridge (2nd collection zone) and the smallest values were obtained in the case of the samples collected from the 3rd collection zone (see Table No.5).

Table No. 1. The potential dehydrogenase activity (April 2005 – January 2006)

Sample	**Potential dehydrogenase activity (mg formazan / 10 g mud d.w.)**				**Mean ± Standard error**	**Standard deviation**
Month	**Apr.**	**June**	**Oct.**	**Jan.**		
Sample 1	11.09	13.08	10.21	9.21	10.89 ± 0.949	1.644
Sample 2	9.68	10.75	9.64	8.47	9.63 ± 0.536	0.929
Sample 3	8.75	9.92	7.74	6.12	8.13 ± 0.929	1.609
Sample 4	9.77	11.34	8.96	8.50	9.64 ± 0.719	1.247

Table No .2. Actual dehydrogenase activity (April 2005 – January 2006)

Sample	**Actual dehydrogenase activity (mg formazan / 10 g mud d.w.)**				**Mean ± Standard error**	**Standard deviation**
Month	**Apr.**	**June**	**Oct.**	**Jan.**		
Sample 1	3.08	6.28	4.21	2.93	4.12 ± 0.892	1.450
Sample 2	1.27	4.90	2.62	1.14	2.48 ± 1.007	1.745
Sample 3	0.81	4.17	2.70	1.35	2.25 ± 0.866	1.501
Sample 4	2.15	5.12	2.32	1.64	2.80 ± 0.905	1.568

Table No. 3. Phosphatase activity (April 2005 – January 2006)

Sample	**Phosphatase activity (mg phenol / 10 g mud d.w.)**				**Mean ± Standard error**	**Standard deviation**
Month	**Apr.**	**June**	**Oct.**	**Jan.**		
Sample 1	9.78	12.76	13.14	8.60	11.07 ± 1.286	2.228
Sample 2	8.64	10.34	11.65	7.46	9.52 ± 1.064	1.843
Sample 3	6.79	9.88	10.83	5.59	8.27 ± 1.434	2.484
Sample 4	7.80	10.90	11.84	7.12	9.41 ± 1.330	2.305

Table No. 4. Catalase activity (April 2005 – January 2006)

Sample	**Catalase activity (mg H_2O_2 / 3 g mud / 1 h / 20 °C)**				**Mean ± Standard error**	**Standard deviation**
Month	**Apr.**	**June**	**Oct.**	**Jan.**		
Sample 1	18.95	28.45	22.45	20.68	22.63 ± 2.410	4.175
Sample 2	23.20	24.90	21.32	19.27	22.17 ± 1.399	2.424
Sample 3	23.88	26.71	23.56	19.69	23.46 ± 1.665	2.884
Sample 4	24.48	27.50	25.68	20.35	24.50 ± 1.751	3.033

In every investigated season the values of EIMQ were the highest in the case of the samples collected from near the Children Sanatorium (1st collection zone) and the smallest in the case of the ones collected from opposite to the Children Sanatorium (3rd collection zone) (see Figures 1-4).

For every season the Enzymatic Coefficient of Variety of the Mud Quality (ECVMQ) have also been calculated, which represents the ratio between standard deviation and arithmetical mean (Drăgan-Bularda 2000). The values of ECVMQ are represented on figure no. 5.

Table No. 5. Urease activity (April 2005 – January 2006)

Sample	Urease activity (mg NH_4^+ / 10 g mud d.w.)				Mean ± Standard error	Standard deviation
Month	**Apr.**	**June**	**Oct.**	**Jan.**		
Sample 1	45.67	51.43	46.84	32.19	44.03 ± 4.778	8.276
Sample 2	52.38	60.95	54.23	43.53	52.77 ± 4.143	7.177
Sample 3	19.69	32.85	39.34	36.05	31.98 ± 4.792	8.612
Sample 4	38.26	46.78	41.09	31.74	39.46 ± 3.609	6.252

Discussion

Dehydrogenase activity measures the respiration potential of the sediment microbiota consequently, it reflects immediate metabolic activities of the sediment microorganisms. These enzymes catalyze a range of oxidative processes that are responsible for the degradation of organic matter by transferring hydrogen and electrons from substrates to acceptors. Dehydrogenases are localized only in intacte, living cells. Sediments' treatment with bactericidal compounds ruin their activities (Gianfreda & Bollag 1996; Nannipieri & Laudi 2000).

In the period between April 2005 – January 2006 the registered dehydrogenase activity was the highest in the summer either without glucose or with glucose addition. Glucose addition to the sludge determined an intensification of the TTC reduction.

The fact that the potential dehydrogenase activity is more intensive than the actual activity proves the carbon source's (glucose) stimulating effect on the sludge microorganisms' enzymatic activities (Gianfreda & Bollag 1996; Muntean et al. 2001).

In comparison with some data from technical literature (Rădulescu et al. 1970), the intensity of dehydrogenase activity was relatively high, owing to the presence of the organic matter in the lake's mud, which ensures microorganisms' good development (Niac & Nascu 1998).

At the same time the dehydrogenase activity did not show high oscillations, attributed to the microclimate of the thermal ecosystem, which is characterized by constant temperature of 26 °C of the water, regardless of season and the temperature of air and the mildly alkaline pH of the water.

The intensity of the phosphatase activity was relatively high with inessential seasonal variations. The highest values were registered in autumn, atributed to the organic rests' accumulation in the sediment at the end of the vegetation period (Drăgan-Bularda et al. 2000). The biological activity in the lake's sediment is more intensive during the warm season and results in the increase of deposition of the quantity of the organic matter in the sediment (Meyer-Reil 1991; Muntean et al. 2004).

The catalase is an enzyme that accumulates in sediments and their activity lasts for a long time. This activity is hardly influenced by external circumstances (temperature, pollution with different substances), which are immediate effects on the activity of the living microorganisms (Kiss et al. 1975; Muntean et al. 2004).

In comparison with some data from technical literature (Rădulescu et al. 1970), the intensity of the catalase activity was relatively low and its values changed with small variation. The highest values were registered in the summer and in autumn.

Urease activity has an important role in the circulation of nitrogen in nature. The increased activity in the case of the sample collected from the 2nd (near the bridge) collection zone is the consequence of the fact that the residents use the bridge regularly and for this reason there is a large quantity of rubbish in this zone, which represents a surplus of substrat for these enzymes (Burns 1987).

The intensity of the urease activity presented little seasonal variations. The highest values were registered in the summer and in autumn, attributed to the organic matter accumulation in the sediment at the middle and at the end of the vegetation period (Gianfreda & Bollag 1996).

The sediments' enzymatic potential through determination of EIMQ illustrates directly or indirectly the microbiota's activity, the influence of various physical, chemical, antropogenic factors and the enzymatic activities' intensity (Drăgan–Bularda et al. 2004). The higher the EIMQ value, the more intensive the sediment's enzymatic potential (Crişan et al. 2001).

As the relative values of Enzymatic Indicator of the Mud Quality reflects, the Ochiul Mare lake's sediment presented a medium enzymatic activity (Fig. 1-4). No significant seasonal oscillation of the EIMQ was

detected (between 0.292 and 0.484) due to the lake's relative stable microclimate insured by thermal water.

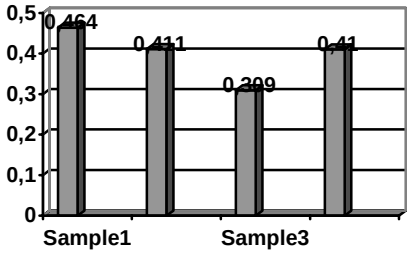

Fig. No.1. Enzymatic Indicator of the Mud Quality (EIMQ) (April 2005)

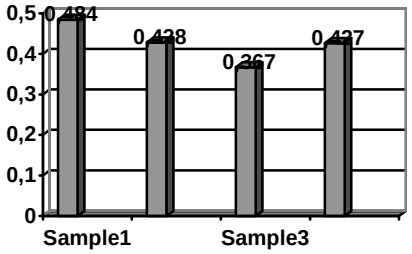

Fig. No.2. Enzymatic Indicator of the Mud Quality (EIMQ) (June 2005)

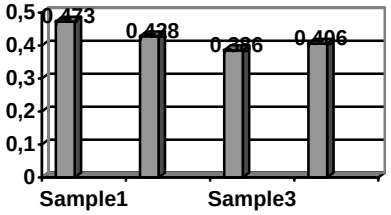

Fig. No.3. Enzymatic Indicator of the Mud Quality (EIMQ) (October 2005)

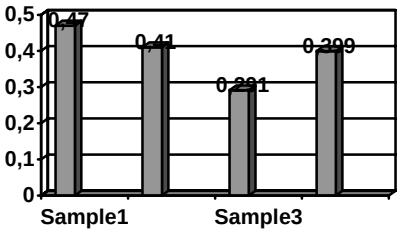

Fig. No.4. Enzymatic Indicator of the Mud Quality (EIMQ) January 2006)

Based on the low values of the ECVMQ (0.088 – 0.162), (see Fig. 5) we could assert that the enzymatic activities that take place in the Ochiul Mare lake's sediment show quite a big stability. At the same time, the low values of the ECVMQ also indicate a considerable biological stability apart from the season when the samples were taken.

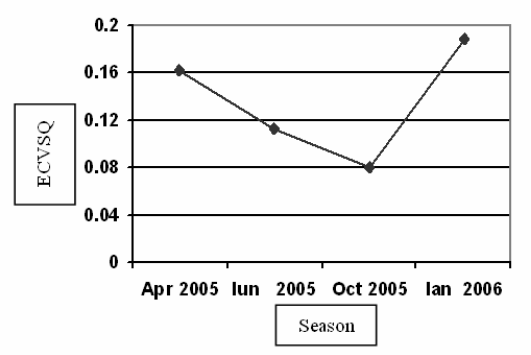

Fig. No.5. The seasonal evolution of the enzymatical activities' stability in the Ochiul Mare lake's sediment on the basis of values of the enzymatic coefficient of variety of the mud quality (ECVMQ)

The figures of ECVMQ were not visibly influenced by seasonal effects. We obtained relatively low results and close values apart from the season when were collected the samples. The determined enzymatic activities are attributed to accunulated enzymes, which in aquatic environment are hardly influenced by the climatic factors (Drăgan-Bularda et al. 2004).

Conclusions

The enzymatic activities, enzymatic indicators (EIMQ) and variability coefficients did not show significant seasonal oscillation and the highest values were registered in the summer and in autumn.

Generally the smallest values of the enzymatic activity were obtained in the case of the samples collected from opposite to the Children Sanatorium (3rdcollection zone), owning to the fact that this is the most polluted area of the lake and the high concentration of pollutants inhibit the micro-organisms' growth.

The low values of ECVMQ denote the sediment's homogeneity through the whole territory of the lake and a considerable biological stability apart from the season when the samples were taken.

In conclusion the Ochiul Mare lake's sediment has a medium but stable enzymatic potential due to the relative stable microclimate insured by thermal water.

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