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Research on the qualitative enzymatic activities in sediments from Secu and Gozna-Văliug dam reservoirs, Caraş-Severin county (Romania)

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Abstract. Six sediment samples (three from Secu Lake and three from Gozna-Văliug Lake) were collected and analyzed qualitatively, enzymologically. In the sediment samples, the following enzymatic activities have been qualitatively determined: four oligase activities (maltase, saccharase, lactase and cellobiase) and five polyase activities (amylase, cellulose, dextranase, glicogenase and inulinase). The studied activities were determined in each sample and they were found to display variations in the intensities of the processes depending on the sampling place. Generally, the highest intensity of qualitative enzymatic activities was registered in case of oligases.
Key words: sediment samples, dam reservoirs, oligase activities, polyase activities.

Abstrait. Six échantillons de sédiments ont été prélevés du deux lacs de barrage, 3 du Lac Secu et 3 du lac Gozna-Văliug, et ont été soumis à l'analyse enzymologique qualitatif. Dans les échantillons de sédiments ont été déterminées les suivantes activité l'enzymatique qualitatifs: quatre activité oligazique (les activité malatsique, saccharasique, lactasique et celobiazique) et cinq activité polyazique (amilazique, celulazique, dextarnazique, glicogenazique et inuliazique). Les activités enzymatiques ont été étudiés au sein de chacun des échantillons présentant montrant leurs variations en fonction du point de prélèvement. La plupart des intensitées ont été enregistrés à les activités oligazique.
Mot clés: sediment, lac de barrage, activités oligazique, activités poliazique.

Rezumat. Şase probe de sediment prelevate din lacurile Secu şi Gozna-Văliug au fost supuse analizelor enzimologice calitative. În probele de sediment au fost determinatie calitativ următoarele activităţi enzimatice: 4 activitati oligazice (activitatea maltazică, zaharazică, lactazică şi celobiazică) respectiv, cinci activităţi poliazice (activitatea amilazică, celulazică, dextranazică, glicogenazică şi inulinazică). Activităţile enzimatice studiate au fost evidenţiate în fiecare din probele analizate, manifestând variaţii ale intensităţii lor în funcţie de punctul de prelevare a probelor. Cele mai intense activităţi enzimatice au fost înregistrate in cazul activităţilor oligazice.
Cuvinte cheie: sediment, lac de acumulare, activităţi oligazice, activităţi poliazice.

Introduction. "Water is not a commercial product like any other but, rather, a heritage which must be protected, defended and treated as such" (Art.1. Directive 2000/60/EC of the European Parliament and of the Council).

The determination of enzymatic activity in aquatic sediments represents an important research tool for the process of evaluating the functional diversity of the microbiota in these habitats (Schloter et al 2003; Drăgan-Bularda et al 2004). The ecological succession and the evolution of the aquatic ecosystems over time is the result of the complex interactions between biocenotic communities, respectivley between them and abiotic characteristics of the life media, being in a constant modification (Vasilescu 1961; Burian 2002). Sediments constitute a key link in the biogeochemical cycle of elements in aquatic environments. It is here that the mineralization process of organic matter that was not decomposed in the water column is finalized (Muntean et al 2001).

The sediments consist of three major components: detritic material derived from the erosion, the biogenic material formed from biological productivity and the autogenic material formed in situ (Wetzel 1991). Sediments are extremely heterogenous systems where the different phases (solids, liquids and gases), biotic components (numerous microorganisms, enzymes), and the abiotic elements (minerals, humus, organo-mineral aggregates) are parts of the physical, chemical and biological processes that take place in these media. All biochemical transformations at sediment level depend on the enzyme presence in these media (Muntean et al 2004).

The aim of our study was to establish the enzymatic potential of the sediments from Secu and Gozna–Văliug Lakes which indirectly reflects the microbiota activity.

The present paper analyze for the first time the evolution of the qualitative enzymatic activities from Secu and Gozna–Văliug dam reservoirs area of Caraş-Severin (land of Banat, West of Romania).

The study of enzymatic activity requires a special exigence because the lakes are valuable ecosystems for the biosphere and they also constitute water reserves in the nowadays problem of fresh water. The water crisis is a well known issue on a world wide scale. The water of rivers and lakes represent only 0.00009% of the hydrosphere (Zarnea 1994).

Secu Lake: dam reservoir, Bârzava drainage basin, 350 m altitude, 105.67 ha surface, water volume of 15.132.000 m^3, Semenic Mountains. Built in 1963, it supplies water for Reşiţa town and also has a recreational role for Secu resort, although this implication is not legal.

Gozna-Văliug Lake: dam reservoir, Bârzava drainage basin, 500 m altitude, 66.2 ha surface, water volume of 11.732.000 m^3, 40 m deep, Semenic Mountains. Built in 1963, it serves as water supply for Secu lake, situated downstream, and implicitly for Reşiţa town. It also represents an attraction for Crivaia Resort. The qualitative analysis of oligases and polyases increases the complexity of the assessment of general enzimatic potential of lake sediment.

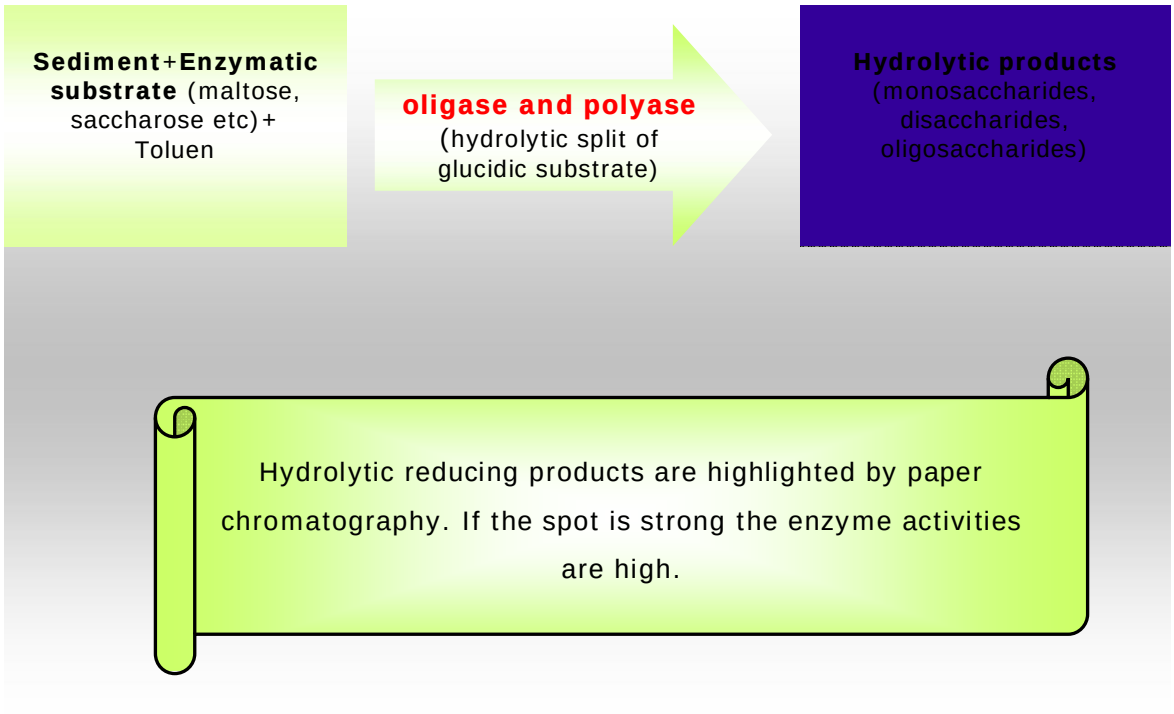
Materials and Methods. The enzymological analysis of the sediments in two dam reservoirs was performed during the spring of 2010 and consisted in the determination of qualitative enzymatic activities. The sediment samples were taken from approximately 50 cm from the shore, following the removal of a 5-10 cm sediment layer.

The sediment samples were taken from near the dam, from the middle and from the tail (end) of both lakes.

From the sediment samples which were qualitatively analysed the following enzymatic activities resulted: four oligase activities – maltase (MA), saccharase (invertase) (SA), lactase (LA), and cellobiase (CeloA) and five polyase activities: amylase (AA), cellulase (CA), dextranase (DA), glicogenase (GA) and inulinase (IA).

The analysis of sediment samples was performed in the labs of Experimental Biology Department of Biology and Geology Faculty from Babeş-Bolyai University, Cluj-Napoca.

The technique used to establish these enzymatic activities was paper circular chromatography. The reaction mixture consisted of 3 g soil + 2 mL toluene (for preventing the proliferation of microorganisms) + 5 mL 2% enzymatic substrate (maltose, saccharose, lactose, cellobiose, starch, cellulose, dextran, glicogen and inulin); incubation: 7-14 days at 37°C. After developing the chromatographic paper, the reductive hydrolytic products were emphasized. The larger spots for the hydrolytic products show the higher activities of the oligase and polyase (Drăgan-Bularda 2000).



Results and Discussion. The oligases (invertase, maltase, celobiase and lactase) were found in all 6 analyzed sediment samples, taken during the spring.

The chromatogram in Fig. 1 presents the results of the invertase activity highlighted in the sediment samples of both dam reservoirs Secu and Gozna-Văliug, county of Caraş-Severin. Based on the presence of glucose spots resulting from the hydrolysis of saccharose under the action of saccharase, it appears that this activity was present in all sediment samples, with small differences between the samples, indicated by the spot intensity of glucose.

Figure. 1. Saccharase activity (SA). 1-3 Sediment samples from Gozna-Văliug Lake; I-III Sediment samples from Secu Lake + enzymatic substrate (saccharose 2%). S=control - saccharose solution 2%. G=glucose.

Thus, in the Secu Lake (points I-III) the most intense spot appears for point III (sample collected from the lake tail), then the spot at point I (samples collected near the dam) and the least intense spot (for point II, the middle of the lake). If we look for the invertase activity of the sediment samples in Gozna-Văliug Lake (points 1-3), based on

spot intensity for glucose, one can appreciate that the activity is more pronounced in the sample collected near the dam (point 1), followed by the one from the middle of the lake (point 2) and the least intensity of all the six samples examined is the one from the lake tail (point 3). For the aquatic sediments, the activity of this oligase is given by the microbiota present in the sediment, a microbiota which degrades organic substances from the vegetal rests present in the water mass (algae uni-or rarely multicellular ones), possibly submerged aquatic plants, organic waste of animal origin present in the sediment and organic substances from anthropic pollution sources. The enzyme accumulates on organo-mineral colloids in the sediment, remaining active for a long time, to its production contributing many generations of microbial populations, especially bacteria. The saccharase activity is dependent on existing microbiota and it is well correlated with the humus concentrantion of soil (Carpa & Drăgan-Bularda 2008) and of sediments as well (Eliade et al 1975).

In cases where the lake does not contain a significant algal microbiota, an intense invertase activity can be explained also by the lake pollution with organic substances of human or animal origin (faeces). So, in other words, it may be an indication of pollution. In general, the invertase activity is present both in soil and in sediments of saline lakes, of dam lakes, even in some sediment of running waters, which contain a microbiota more or less important, playing an important ecological role in the mineralization process (Gianfreda & Bollag 1996; Curticăpean & Drăgan-Bularda 2007; Filimon 2007). The ubiquity of microorganisms in all natural environments, their diversity indicates the significant ecological role of the microorganism community in an ecosystem (Zarnea 1994).

The chromatogram in Figure 2 presents the results on highlighting the maltase activity in the sediment samples of the two dam reservoirs. An overview of the chromatogram shows us that in all sediment samples was evidenced the maltase activity, by the appearance of the glucose spot (b), resulting from the hydrolytic splitting of maltose (enzyme substrate) (a). This time, also, the sediments of the Secu Lake were more active in terms of enzymological activity than those from Gozna-Văliug lake. There are differences also among the 3 samples collected from different points of a lake. For example for the Secu lake, a significant maltase activity is recorded in sample II, almost equal to the one in sample I and much lower (almost the half) highlighted in the sample III.

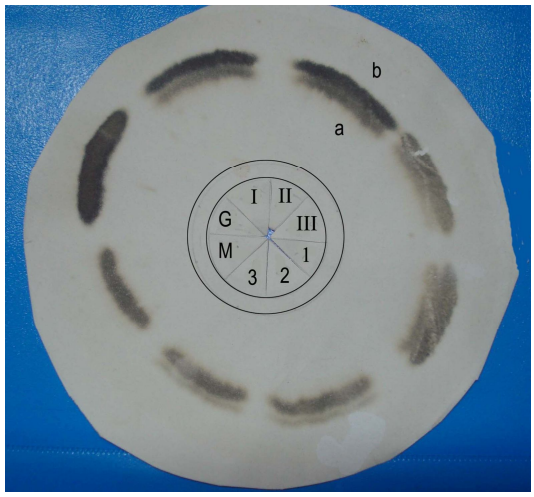


Figure 2. Maltase activity (MA). 1-3 sediment samples from Gozna-Văliug Lake; I-III sediment samples from Secu Lake + enzymatic substrate (maltose 2%). M=control-maltose solution 2%. G=glucose. a=Maltose spot. b=Glucose spot.

The analysis of the chromatogram shows also that the enzyme substrate (maltose) was not completely hydrolyzed in any sediment sample (see the spot from a). If one examines carefully the spots (b) of the Gozna-Văliug Lake samples (1-3), it may be

noted that only for the sample 1, the glucose spot is more intense and for the samples 2 and 3 the glucose spot is visible, but it has a low intensity. As the maltose results from the starch degradation, it can be concluded that in the lake water there were present algae or aquatic plants, which determined the appearance of maltose and further, the maltase synthesis. The most important substrates of this enzyme are maltose and saccharose.

Maltose is strongly inhibited by glucose, while fructose inhibits it only weakly (Bodoczi 2010). Moreover, even the polluting impurities may contain these substances, which led to the synthesis of the enzyme by sediment microbiota.

Another oligase highlighted in the sediments of Secu and Gozna-Văliug lakes is the cellobyase, presented in the chromatogram in Figure 3. It can be seen that this enzyme was also found in all 6 sediment samples, see the spot b in all 6 samples (I-III, respectively 1-3). The samples from Secu lake (points I-III) are more active in terms of cellobiasic activity as those from Gozna-Văliug lake (points 1-3). If we follow the spot intensity of the glucose spot resulting from the hydrolysis of cellobiose under the action of cellobiase, we remark that the most intense activity was recorded in the sediment sample III (at the tail of the lake) and for Gozna-Văliug lake the sample 1 (near the dam), while the sample 3 (tail of Gozna-Văliug lake) has a low activity (barely identifiable).

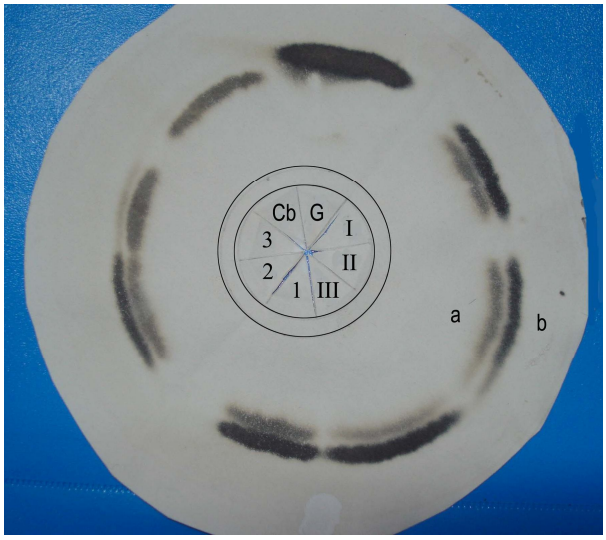


Figure 3. Cellobiase activity (CA). 1-3 sediment samples from Gozna-Văliug Lake; I-III sediment samples from Secu Lake + enzymatic substrate (cellobiose 2%). Cb=control-cellobiose solution 2%. G=glucose. a=Cellobiose spot. b=Glucose spot.

This oligase present in the lakes sediments requires the presence cellobiose, resulting from the degradation of cellulose under the action of cellulases. On its turn, the cellulase is induced by the presence of cellulose, present in the aquatic flora. Analysing the chromatogram we will find out that for no sediment sample occurred a total hydrolysis of the cellobiose (see the spot a). However, highlighting this enzyme reflects the richness of the enzymatic potential of aquatic sediments, as a result of the biodiversity of the microbial world.

The chromatogram in Fig.4 presents the highlighting of the lactase activity (beta-galactosidase). Its analysis easily shows the presence of this enzyme in almost all sediment samples (except for the sample 3 in Gozna-Văliug lake, where goodwill can only find a weak spot of glucose (spot b). If the samples I-III of lake Secu present a considerable spot of glucose resulting from the hydrolysis of lactose (we emphasize that the galactose resulting in echimolecular proportions with the glucose is not revealed in this way), those in Gozna-Văliug Lake differ greatly between them. One can see that sample 1 (dam) presents an intense activity (the most intense of the 6), while the sample 2 is less intense, as the sample 3 to be close to the identification limit. The presence of lactase does not imply the existence of lactose as substrate and other similar

compounds which may be hydrolyzed under the action of beta-galactose (in practice it is used lactose as enzymatic substrate). The presence of this oligase also demonstrates the complexity of the enzymatic potential not only of soil (Kiss et al 1975) and saline lakes sludge (Drăgan-Bularda & Kiss 1972) but also of aquatic sediments (Curticăpean & Drăgan-Bularda 2007).

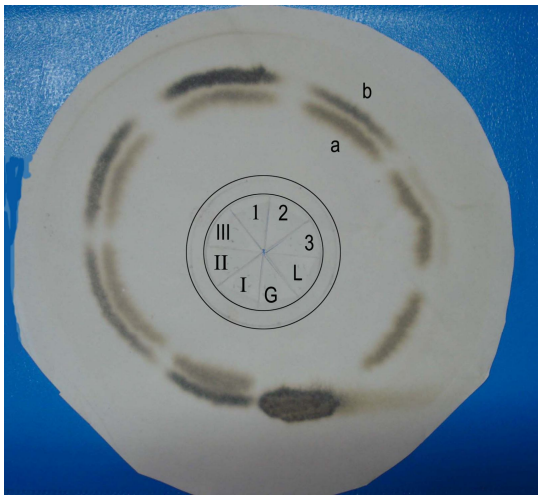


Figure 4. Lactase activity (LA). 1-3 sediment samples from Gozna-Văliug Lake; I-III sediment samples from Secu Lake + enzymatic substrate (lactose 2%). L=control- lactose solution 2%. G=glucose. a=Lactose spot. b=Glucose spot.

In addition to the 4 oligases whose presence was demonstrated in the sediments of the two dam reservoirs, we further present the highlighting of 5 polysaccharidases (polyases), who came to demonstrate, doubtless, the complexity of the enzymatic potential and of the aquatic sediments, as a result of microbial biodiversity in natural living environments.

In the chromatogram in Figure 5 we highlight the amylase activity, by the presence of glucose spot in all six sediment samples examined. Making a comparison, the sediment samples from Secu Lake are more active in terms of amylase activity, the sample III being the most active, indicated by the spot intensity of glucose (b) and even by the presence of a low maltose spot (a), compared to those in Gozna-Văliug Lake, for which the samples 2 and 3 show a relatively low amylase activity.

Figure 5. Amylase activity (LA). 1-3 sediment samples from Gozna-Văliug Lake; I-III sediment samples from Secu Lake + enzymatic substrate (starch 2%). A=control- starch solution 2%. G=glucose. a=Amylase spot. b=Glucose spot.

The differentiation between the two lakes is evident, even sediments are distinguished, those from Gozna-Văliug lake having less organic matter and a more mineral texture.

The cellulase, a polysaccharidase generally more difficult to be put into evidence because of the existing enzyme substrates, was however highlighted by us in the sediments of Secu Lake (points I-III), providing an intensive glucose spot for sample III (lake end) and lower glucose spots for the samples I and II, but still detectable (visible) (see chromatogram in Fig.6). In sediment samples from Gozna-Văliug Lake (points 1-3) no cellulase activity was highlighted. It is noted that the sediment sample III from Lake Secu, who presented the most intense cellulase activity was also the most active in terms of cellobiasic activity. It is known that the two enzymatic activities are related and play a decisive role in the nature in the degradation of the most important organic product (Vasilescu 1961; Drăgan-Bularda & Paşcu 1997).

Figure 6. Cellulase activity (CA). 1-3 sediment samples from Gozna-Văliug Lake; I-III sediment samples from Secu Lake + enzymatic substrate (cellulose 2%). C=control-cellulose solution 2%. G=glucose.

Another studied polyase was the glycogenase, and for its study glycogene was used as substrate. The obtained results are shown in the chromatogram in Figure 7. Its analysis shows the lack of any spot, except for the reference (glucose) spot.

Figure 7. Glycogenasic activity (GlA). 1-3 sediment samples from Gozna-Văliug Lake; I-III sediment samples from Secu Lake + enzymatic substrate (glycogene 2%). Gl=control - glycogene solution 2%. G=glucose.

It indicates that the studied aquatic sediments present no glycogenasic activity. Moreover, there was no glycogenase highlighted in the soil (we do not have concrete data).

The dextranase was another studied poliase (see Fig.8). Dextranase cracks the glycosidic linkages of dextran polysaccharide (a bacterial polygluccoside synthesized mainly by the *Leuconostoc* genus, from succharose). In soil the dextran acts for the aggregation of particles, while in sediments its role is uncertain. However, its presence can be attributed to microbial biodiversity. The goal of highlighting this enzyme was to emphasize the complexity of enzymatic potential. Analyzing the present chromatogram it can be seen that there is a low dextranase activity in the sediments of Secu Lake, while in Gozna-Văliug Lake this activity is absent.

Figure 8. Dextranase activity (GlA). 1-3 sediment samples from Gozna-Văliug Lake; I-III sediment samples from Secu Lake + enzymatic substrate (dextran 2%). D=control - dextran solution 2%. G=glucose.

The chromatogram in Figure 9 presents the highlighting of the inulinase activity. Inuline is a polyfructoside present in certain plants and the soil and aquatic sediments microbiota (medicinal sludge and other sediments) has the specific enzyme that accumulates after the death of microbial cells, in adsorbed state on colloids in soil soil and sediments, maintaining their activity for a long time. By the hydrolysis of inulin under the action of inulinase results as a final product fructose. Inulin, a linear B (2-1) linked fructose polymer, serves as storage polysaccharide in the underground organs of several plants of the *Asteraceae*. Inulin hydrolysis enzymes are found in plants, filamentous fungi and bacteria. Inulin received a great interest for the production of fructose rich syrups (Pandey et al 1999) stimulates calcium absorption (Heuvel et al 2000), benefical effects in diabetic patiens (Roberfroid & Delzenne 1998). Principal bacteria employed for inulinases production: *Acetobacter sp., Bacillus sp, Escherichia coli, Staphylococcus sp* (Avigad & Bauer 1966).

Analysing the chromatogram detected with urea-based and o-phosphoric acid-based reagent, it was put into evidence the fructose spot, decreasing in intensity and the inuline spot. In case the hydrolysis of inulin was complete, the inulin spot from the starting point disappears. In our case, we remark the presence of the inulinase activity for all six samples, the most intense fructose spot being the one of the sample 1 from Gozna-Văliug Lake and for the samples I and III of Secu Lake. For the sample II from Secu Lake the fructose spot intensity is at the detection limit.

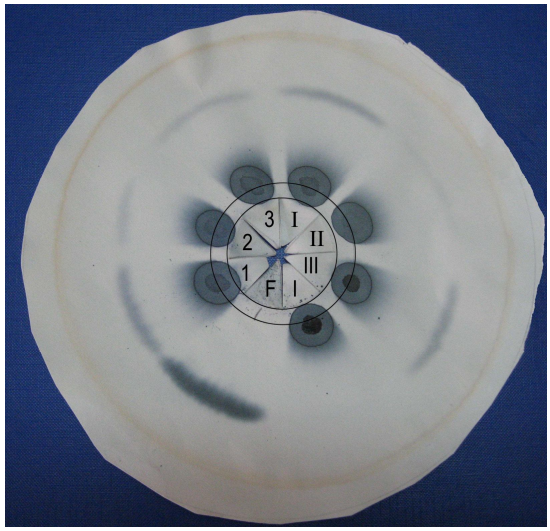


Figure 9. Inulinase activity (IA). 1-3 sediment samples from Gozna-Văliug Lake; I-III sediment samples from Secu Lake + enzymatic substrate (inulin 2%). I=control - inulin solution 2%. F=fructose spot.

Highlighting this polyase also in aquatic sediments of the reservoirs studied shows us the complex enzymatic potential closely related to the microbial biodiversity and the potential anthrophic influences. The enzymatic equipment present in sediments demonstrates the normal cycle of the organic matter in this natural environment, due to a biological balance (Bodoczi & Carpa 2010).

Any disturbance occurring is reflected on water quality (we refer to toxic substances, radioactive substances and other possible causes).

Conclusions. All the enzymes studied qualitatively displayed variations in their intensities, depending on the studied enzyme and on the sampling sites.

The oligase activities (maltase, saccharase, lactase and cellobiase) were significant in all the analyzed sediment samples of both dam reservoirs Secu and Gozna-Văliug, county Caraş-Severin.

The polyase activities (amylase, dextranase, glycogenase, cellulase and inulinase) are present in almost all the sampling points, excepting the glycogenase activity, which has almost no intensity.

In sediment samples from Gozna-Văliug Lake no cellulase activity was highlighted. It is noted that the sediment sample III from Lake Secu, who presented the most intense cellulase activity was also the most active in terms of cellobiasic activity.

Dextranase activity is lower in the sediments of Secu Lake, while in Gozna-Văliug Lake this activity is absent.

The amylase activity was very intense in all the analyzed sediment samples.

The inulinase activity presents a high level in all sediment samples. The most intense activity was registered in the sample 1 from Gozna-Văliug Lake and for the samples I and III of Secu Lake.

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