ELBA BIOFLUX
Extreme Life, Biospeology & Astrobiology
International Journal of the Bioflux Society

Enzymatic activities in sediments from Secu and Văliug-Gozna dam reservoirs, Caraş-Severin, Romania

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Abstract. The sediment samples from Secu and Văliug-Gozna lakes were subjected to enzymological study. In the sediment samples, the following enzymatic activities have been quantitatively determined: actual and potential dehydrogenase, catalase and phosphatase activities. All these activities were detected in all the samples analyzed. The assessed activities were found to display variations in the intensity of the processes depending on the sampling place. Generally, the highest intensities of quantitative enzymatic activity were registered mostly at samples from the tails of dam lakes. Based on these activities the enzymatic indicator of sediment quality (EISQ) was calculated and appreciable values resulted for both lakes.
Key words: sediment samples, dam reservoir, enzymatic activity, EISQ.

Rezumat. Probe de sediment din lacurile de acumulare Secu şi Văliug-Gozna au fost analizate din puct de vedere enzimatic. În probele de sediment prelevate din aceste lacuri au fost determinate următoarele activităţi enzimatice cantitative: activitatea dehidrogenazică actuală şi potenţială, activitatea fosfatazică şi activitatea catalazică. Aceste activităţi enzimatice au fost detectate în toate probele analizate. Activităţile determinate în fiecare probă au prezentat variaţii în intensitatea proceselor în funcţie de zona de prelevare. În general, cele mai intense activităţi enzimatice cantitative au fost înregistrate în principal la coada lacurilor. Pe baza activităţilor enzimatice s-a calculat indicatorul enzimatic de calitate al sedimentului (IECS), obţinându-se valori apreciabile pentru ambele lacuri.
Cuvinte cheie: sediment, lac de acumulare, activitate enzimatică, IECS.

Introduction. It is time to breach the institutionalized dichotomy between environmental science and biomedical research and to study ourselves as an integral and dependent part of our microbe-dominated world (Ley et al 2007). 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 sediment from the lakes always contains a larger number of microorganisms than the water mass. The shallow layer of the silt is the most important. Huge quantities of organic substances are accumulated here through the sedimentation of the water mass. The sediment is a heterogenous system, in which different physical phases (solid, liquid, gas) and several biotic components (microorganism, small organisms and enzymes) together with abiotic components (minerals, humic materials, organic and mineral units) are implied in the physical, chemical and biological process (Filimon 2007).

The enzymatic activity from the soil, respectively from the sediments, is controlled by four classes of enzymes: hydrolases, oxidoreductase, transferase and lyase (Gianfreda & Bollag 1996).

The aquatic microorganisms have an essential role in the conversion of vegetal and animal matter to inorganic matter. The mineralization process returns the main nutrients to the photosynthesizing organisms for the continuity of their activity. Overall, these products are part of the biochemical cycles, assuring the transformation and the recycling of the biogenic elements in nature. Apart from this role, in the material tide and energy of the ecosystem, the role of the microorganism is also important regarding the transformation of the xenobiotic chemical substances, determined by their metabolic versatility (Colwell 1980).

The present study reflects the most important information revealed by the surveillance of the safety and acceptability of drinking water supplies in Reşiţa County and contributes with several applicative aspects towards water quality supervision and evolution in time of the big dam reservoirs. ”Water is not a commercial product like any other but, rather, a heritage which must be protected, defended and treated as such” (Art.1. Water Framework Directive, Directive 2000/60/EC).

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 role of microorganisms in sediment gas production under anoxia or low oxygen conditions is well known. Most work on the microbiology of sediments has been undergone through measurement of decomposition processes and the isolation of microorganisms (Spring et al 2000). Sediments are extremely heterogeneous 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). In aquatic ecosystems, high-molecular-weight DOM becomes hydrolysed by bacterial extracellular enzymes because bacteria can only take up molecules smaller than c. 600 Da. This enzyme pool consists of both endo- and exohydrolases (Debroas 1998; Obayashi & Suzuki 2005; Boucher 2009).

The bacterial activity in the sediments has been linked to the trophic conditions in the water body due to sedimentation of particulate organic matter (Wobus et al 2003). Extracellular enzymes play an important role in the decomposition of organic matter in sediments and their activities in the sediment are considered to be dependent on the input of organic matter by sedimentation (Boschker & Cappenberg 1998; Wobus et al 2003).

The enzymatic activities from soils or sediments can be used as potential indicators of the cyclic nutritive proceses and in the fertility management of these processes for a long term Fließbach et al 2007).

Many studies were done for evaluating the potential use of enzymatic activity as an indicator of soil productivity or fertility (Alef & Nannipieri 1995). The enzymes from sediments are responsible of nutritive substances load. The enzimes of the sediment depend on the microbiological activity, which can be affected by a series of abiotic factors. These factors can influence the availability of nutrients for plants and the quantity of nutrients in sediments. The enzymes are often used as indicators in the studies focusing on the influence of chemical treatments applied to the soil on its fertility.

The study of enzymatic activity requires a special exigency 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).

The aim of our study was to establish the enzymatic potential of the sediments from Secu and Văliug-Gozna lakes, which indirectly reflects the microbiota activity. The present paper analyzes for the first time the evolution of the quantitative enzymatic activities from Secu and Gozna–Văliug dam reservoirs area of Caraş-Severin (Banat, Western Romania).

Materials and Methods. The enzymological analysis of the sediments in the two dam reservoirs was performed during the year 2012 and consisted in quantitatively assessing the enzymatic activities. The samples were seasonally taken from the lakes Secu and Valiug. 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 near the dam, at the middle and at the tail (upper end) of both lakes. The sampling point locations are indicated in Figure 1 and Figure 2.

Văliug-Gozna 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 city. It also represents an attraction for Crivaia Resort.

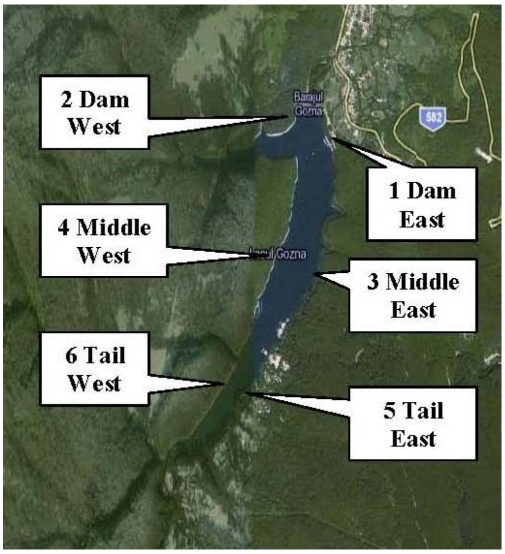

Figure 1. Văliug-Gozna dam reservoir, sampling points.

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 city and also has a recreational role for Secu resort.

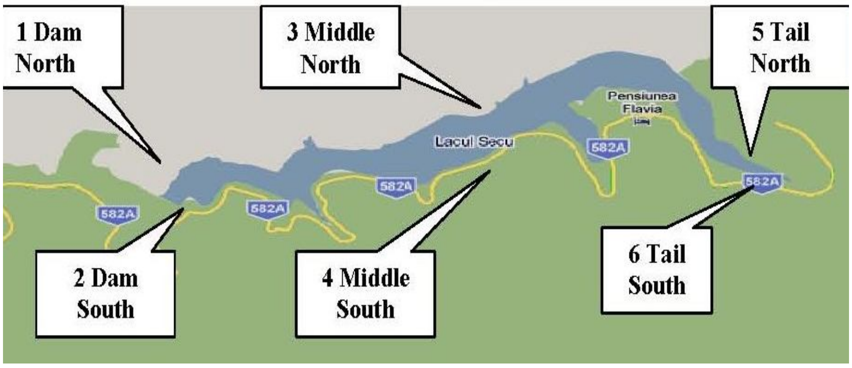

Figure 2. Secu dam reservoir, sampling points.

The analysis of sediment samples was performed in the labs of Molecular Biology and Biotechnology Department of Biology and Geology Faculty from Babeş-Bolyai University, Cluj-Napoca. From the sediment samples which were quantitatively analysed the following enzymatic activities resulted: actual (ADA) and potential dehydrogenase (PDA), phosphatase activity (PA) and catalase (CA) activities.

Dehydrogenase activity assessment. Enzymes like dehydrogenases are only functioning in the cells and are implied in important aspects of the metabolism (Gianfreda & Bollag 1996). Dehydrogenase is an intracellular enzyme catalyzing the oxidation-reduction reactions of organic compounds. Some studies have shown that dehydrogenase activity is one of the parameters most intolerant to toxicity. Among the different enzymes from the sediment, dehydrogenase (DHA) was acknowledged as important biochemical indicator of the sediment (Nostrati et al 2011).

Actual and potential dehydrogenase activity (ADA and PDA) were determined using methods described by Casida et al in 1964.

The reaction mixture consisted of 3 g sediment + 0.5mL TTC 3% solution + 1mL distilled water and for PDA is added 1mL glucose solution 3%. The incubation was carried out at 37^o C, for 48 hours. ADA and ADP were represented as mg formazan/g of sediment, measuring absorbance at 485 nm (Muntean et al 1996; Drăgan-Bularda, 2000).

Phosphatase activity assessment. In the aquatic environment, bacteria and phytoplankton may obtain part of their total phosphorus requirements from phosphomonoesters, which are a variable fraction of the dissolved organic phosphorus pool (Garcia-Ruiz et al 2000). Phosphate is released from phosphomonoesters after enzymatic hydrolysis by a phosphomonoesterase (Suzumura et al 1998). Allochthonous sources of phosphomonoesters are drainage, waste - water input and other products from degradation of organic compounds in the surrounding catchment area (Tarapchack & Moll 1990).

Phosphatase activity was measured using Kramer and Erdei method (Drăgan-Bularda 2000). The principle of the method rests on indophenol formation. Toluen and disodium phenyl phosphate (the aqueous solution of the enzyme substrate) are added to the sediment (in order to prevent the ploriferation of microorganisms). During incubation phenyl phosphate is hydrolysed by phosphomonoesterase resulting phenol and disodium phosphate. Phenol reacts with Gibbs reagent (2,6 - dibromoquinone-chlorimide) forming indophenol (blue colour). The higher the indophenol concentration is the stronger the phosphatase activity is (Drăgan-Bularda 2000).

Catalase activity assessment. The catalase is an enzyme gathered in the sediment. It keeps its activity for a long time. The capacity to participate at the decomposition of certain xenobiotic compounds sequent from industry or agriculture is remarkable (Filimon 2007). The catalase activity (CA) expresses the decomposition intensity for H_2O_2 formed in the aerobic breathing process of microorganisms (Bodoczi & Drăgan-Bularda 2008). Catalase-positive pathogens, such as *Mycobacterium tuberculosis*, *Legionella pneumophila* and *Campylobacter jejuni*, synthetize catalase in order to deactivate the peroxide radicals, thus allowing them to survive unharmed inside the hosts (Srinivasa 2003).

The principle of the method consists in measuring the split H_2O_2 by Kappen method (Muntean et al 1996; Drăgan-Bularda 2000). To the active and inactive sediment samples aqueous solution and H_2O_2 are added. After the incubation the split H_2O_2 is measured.

Enzymatic indicator of sediment quality assessment (EISQ). The activity of microorganisms upon the substrate is achieved by enzymatic mean. It consists in oxidoreduction and hydrolysis proceses under the effect of final products of microbial methabolism (Muntean et al 2004).

Assaying the enzyme activities in the sediment constitutes a research instrument for evaluating the biochemical processes in these natural environments and for finding some indicators of soil quality (Drăgan-Bularda et al 2004).

The enzymatic indicator of sediment quality (EISQ) provides a general view on its enzymatic potential and is calculated using a formula developped by Muntean et al in 1996:

$$EISQ = 1/n \ \Sigma \ V_r \ (i) \ / \ V_{max} \ (i), \text{ where:}$$

EISQ = enzymatic indicator of sediment quality;
n = number of activities;
V_r (i) = real individual value;
V_{max} (i) = maximum theoretical individual value.

The bigger the enzymatic indicator the higher the enzymatic potential of soil (Crişan et al 2001).

Results and Discussion. Many enzymes, produced by microorganisms, can be implicated in the biogeochemical cycles of elements in soils and sediments (Fernández et al 2005). Anthropic and natural factors can directly and indirectly affect the enzyme activities from different substrates (Gianfreda & Bollag 1996). The enzymes of soil or sediment are considered to be responsive to polution and were proposed as indicators for measuring the degradation state of sediments (Todorova & Topalova 2010; Drăgan-Bularda et al 2004).

Dehydrogenase activity is viewed as an indicator of metabolic proceses in sediments and, thus, of their microbiological activity (Garcia et al 1994) and it regards the viable cells from soil. The dehydrogenase activities are represented qualitatively in all aquatic samples.

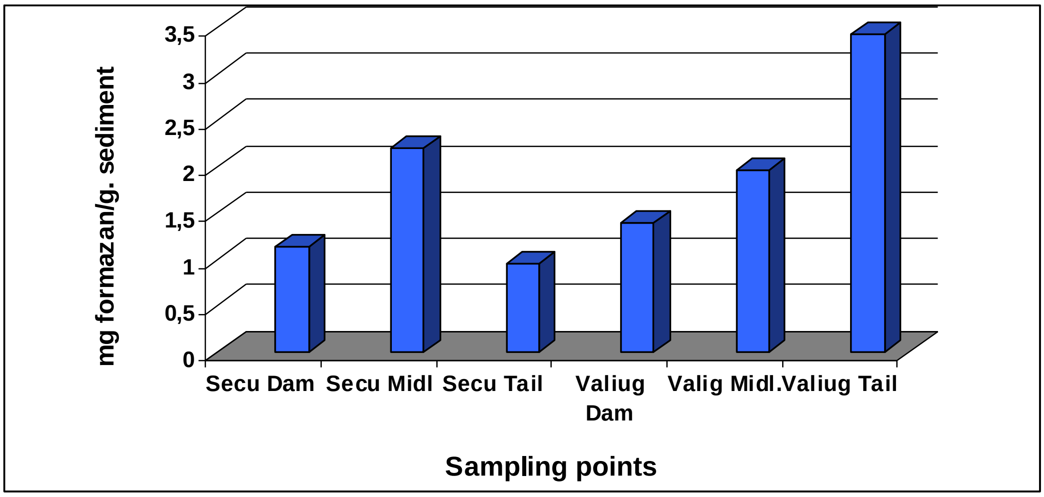

Figure 3. The intensities of actual dehydrogenase activity (ADA) from Secu and Văliug-Gozna dam reservoirs.

In order to assess the potential dehydrogenase activity glucose solution is added to the samples. Together with the preexisting organic substances it will serv as hydrogen donor.

Relatively high values are found in the zones of the lake with stronger anthropogenic impact, namely the South shore of Lake Secu, the Estern shore for Văliug-Gozna. Actual dehydrogenase activity reaches maximum values of 2.198 mg formazan/g. sediment in the point of Secu middle south. For Văliug-Gozna Lake the maximum value was 3.427 mg formazan/g. sediment recorded for the tail of the lake.

The potential dehydrogenase activity (PDA) presented much higher values for both studied lakes due to the carbon source (glucose) added.

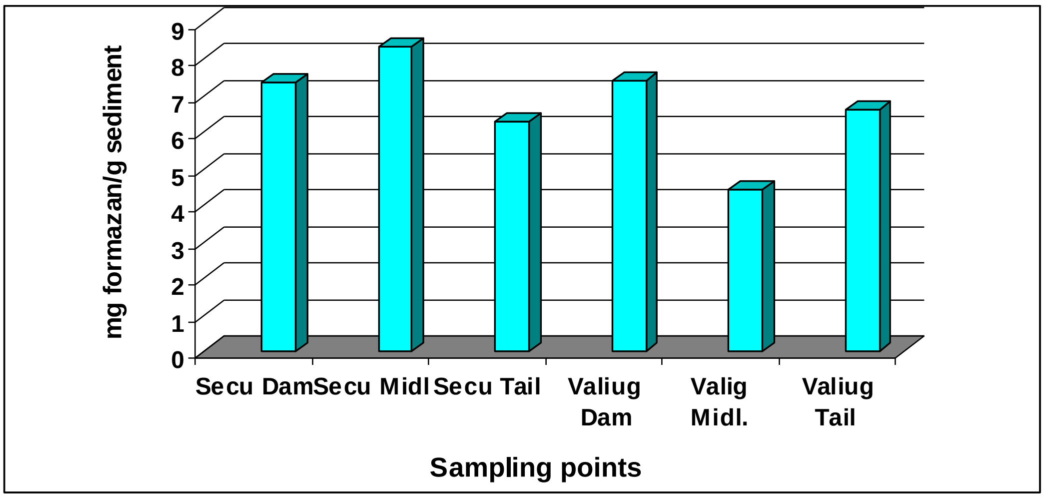

Figure 4. The intensities of potential dehydrogenase activity (PDA) from Secu and Văliug-Gozna dam reservoirs.

In other dam reservoirs from Romania values different from the ones for Secu and Văliug-Gozna lakes were obtained. For instance, in Lake Gilău (Cluj County) ADA reaches only 0.600 mg formazan/g.d.s. at the tail of the lake and PDA reaches 1.955 mg formazan/ g.d.s. in the same spot (Curticăpean & Drăgan-Bularda 2007). These values are however small if we compare with Mureş River in Arad–Pecica sector (Romania), recently studied by Treitli & Filimon (2011), where higher values of ADA (19.583) were found when sampling downstream Canalul Ţigăncii (Filimon 2007). These values may be attributed to additional contribution of organic substances provided by human activity.

The dehydrogenase activity is the result of the action of living microorganisms and the proliferation capacity. There is a strong relationship between the number of microorganism in the sediments and dehydrogenase activity.

Phosphatase activity from Secu and Văliug-Gozna dam reservoirs is quite uniform all around both lakes. Slightly higher values came from the head and the tail of both lakes, with a maximum value of 28.1 g phenol/g.sediment in Secu Tail point (Figure 5).

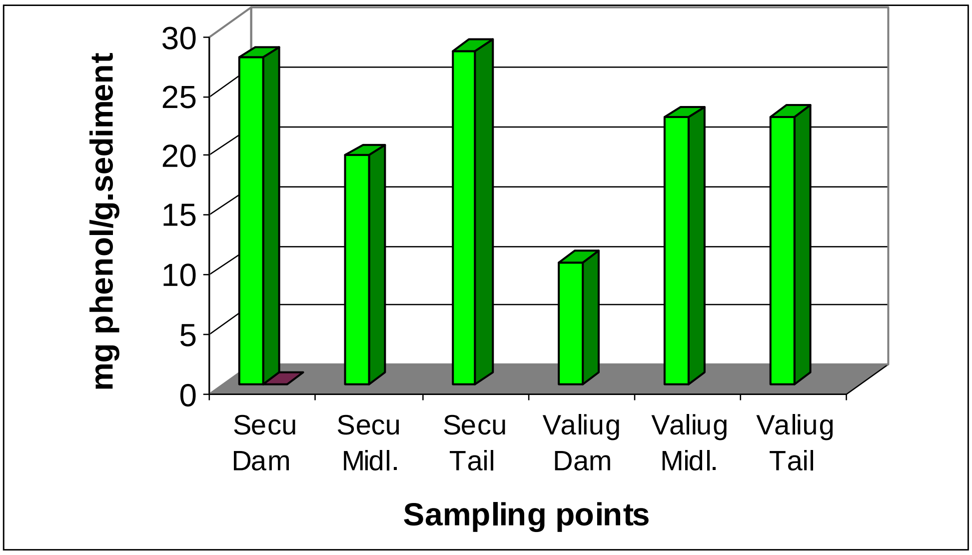

Figure 5. The intensity of phosphatase activity from Secu and Văliug–Gozna dam reservoirs.

Dehydrogenase and phosphatase are considered enzymes which play key roles in the metabolic functions at bacteria and in their habitats (Huang et al 2009). Thus, these enzymes can provide usefull informations regarding the efects of environmental changes and the changes in soil fertility (Ştef et al 2004).

The things are different in Gilău Lake and Tarniţa Lake, which present a numeric fluctuation of PA in all the analysed sections (Curticăpean & Drăgan-Bularda 2007) Maximum value 29.12 mg phenol/g.d.s. in Gilau „Tail lake" point and 26.01 mg phenol/g.d.s. in „Middle beach II right border"point of Tarniţa lake.

Catalase activity is very stable in soil and has a significant corelation with the organic carbon content and the depth of soil (Alef & Nannipieri 1995). Catalase (hydrogen peroxid oxidoreductase) is an intracellular enzyme found in all aerobic and facultative aerobic bacteria but absent in the ones obligate anaerobic (Todorova & Topalova 2010; Fernández et al 2005). It is well known that hydrogen peroxid, superoxide radical and hydroxyl radical can be highly toxic for cells and can damage cellular macromolecules (Todorova & Topalova 2010).

In the sediment of Secu and Văliug–Gozna Lakes a relative levelness is observed regarding the limits of the numeric variation of catalase activity, with values slightly lower for Văliug–Gozna Lake. Catalase activity reaches the highest value at the head of Secu Lake, 19.833 mg H_2O_2/g.sediment, and at the tail of Văliug-Gozna Lake, 17 mg H_2O_2/g.sediment (Figure 6).

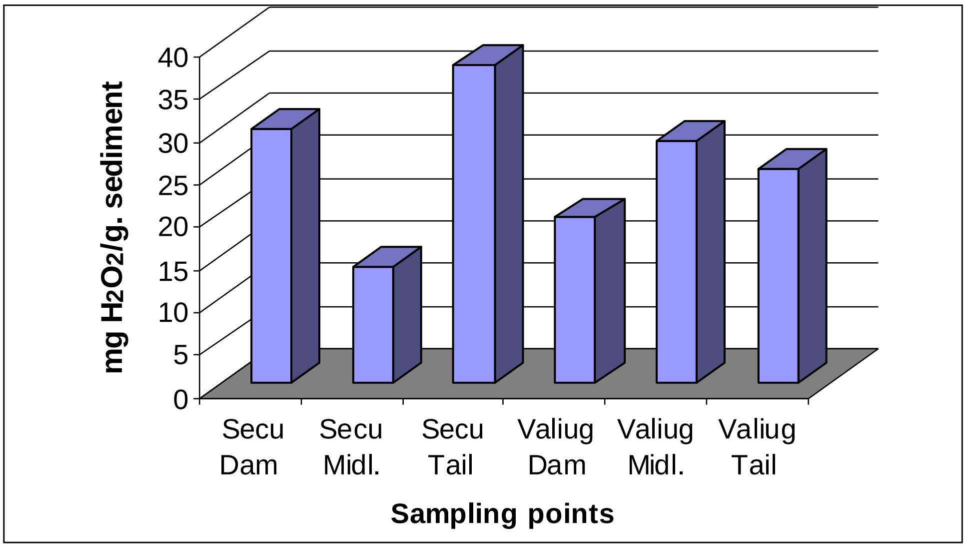

Figure 6. The intensities of catalase activity from Secu and Văliug–Gozna dam reservoirs.

Compared to the speciality literature (Muntean et al 2004), the intensity of catalase activity is within normal limits. Gilău Lake however (Curticăpean & Drăgan-Bularda 2007), presents low values for this indicator, maximum 2.51 mgH_2O_2/g.s.u. at points in the middle of the lake) while Tarniţa Lake (Cluj, Transylvania, Romania) has values of 3.91 in „Middle beach II right border" point (Curticăpean & Drăgan-Bularda 2007). The most intense catalase activity can be found in sampling point P6 (Water treatment plant Pecica) and downstream this water treatment plant the catalase activity decrease and in the P7 sampling point we found the lowest catalase activity (Treitli & Filimon 2011).

Catalase activity can be linked to the metabolic activity of aerobic organisms and is used as an indicator of soil fertility (Gianfreda & Bollag 1996; Fernández et al 2005).

Enzymatic indicator of sediment quality (EISQ). Assessing the enzyme activities in soils constitute a research instrument for evaluating the functional diversity of microbiota and the biochemical processes in these habitats. Theoretically, the enzyme indicator can have values between 0 (when there is no activity in the sample) and 1 (when all the individual real values are equal to individual theoretical maxima of all activities). The absolute and relative values of enzyme activities in the studied sediments are presented in Figure 7.

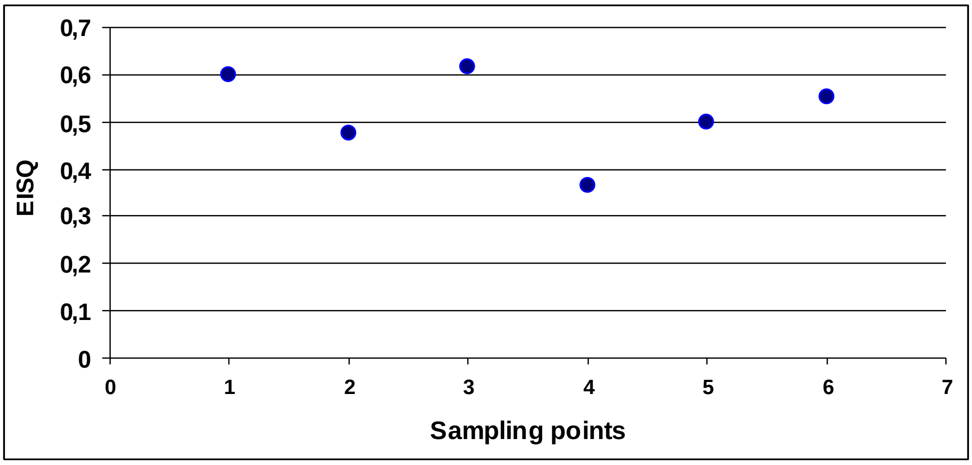

Figure 7. The enzymatic potential of sediments from Secu (1=Secu Dam, 2=Secu Midl., 3=Secu Tail) and from Văliug-Gozna (5= Văliug Dam, 6= Văliug Midl., 7= Văliug Tail) dam reservoirs.

It can be noticed that higher values were found in Lake Secu particulary the value of 0.616 at the tail of the lake. The lowest value was measured from Valiug Lake, from Văliug-Gozna Dam sampling point. The quality of a sediment is better the higher IECS gets (Muntean et al 2001).

Generally, the enzyme potential of sediment directly or indirectly reflects the activity of microbiota, the influence of different physical, chemical anthropogenic factors and even of intensity of different enzyme activities in the sediment. Therefore, the function of an ecosystem can not be understood without the active implication of enzyme processes (Drăgan-Bularda et al 2004).

Conclusions. The data brought forward show that the 3 enzyme activities were marked in all the sediment samples examined (microbially active samples).

The enzymes from prolierating microorganisms (ADA şi PDA) differ according to the sampling spot, indicating that microbiota is influenced by the nutrient income (contingent spills with organic matter income). The difference between the two lakes is not quite apparent.

The enzyme activities due to accumulated enzymes (catalase and phosphatase) are consistent. The values obtained from the sampling zones were close. This denotes that the enzyme potential of the lakes is high and the alterations performed by the microbiota in the lakes are not affected by noxious polluting substances (heavy metals, pesticides etc).

The enzymatic indicator of sediment quality reached the highest values in both lakes, in Secu Lake EISQ =0.616 and in Văliug-Gozna Lake EISQ=0.553. These values indicate the presence of high enzymatic potential in these dam reservoirs.

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*** Water Framework Directive, 2000, Directive 2000/60/EC of the European Parliament and of the Council.

Received: 02 July 2013. Accepted: 17 July 2013. Published online: 20 July 2013.
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How to cite this article:
Fetke R., Carpa R., Drăgan-Bularda M., 2013 Enzymatic activities in sediments from Secu and Văliug-Gozna dam reservoirs, Caraş-Severin, Romania. ELBA Bioflux 5(2):93-102.