ENZYMEOLOGICAL STUDIES ON TIMIS AND BEGA RIVERS IN ORDER TO DETERMINE THE DEGREE OF POLLUTION

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ABSTRACT
In this paper we studied the degree of pollution of rivers Timis and Bega. Enzymatic analyzes were performed on the samples of these two rivers in order to determine the quality of water and the sediment in biological terms. The enzymatic analyzes include the following activities: catalase, urease, dehydrogenase activity actual and potential and ability to reduce trivalent iron. Based on the results of these activities we were able to calculate the enzymatic indicator of sediment quality. The analyzed samples were collected in 2008 from 10 sampling points. The enzymatic indicator of sediment quality shows variations depending on the sampling point. The values of this indicator are lower compared to other rivers in the country. This indicates the existence of disturbance factors or pollutants affecting the number of bacteria in the sediment and thereby inhibits their enzymatic activity. Large amount of urease found in the village Costei show that the area is fecaligenic pollution from animal manure in the area.

KEY WORDS: sediments, enzymatic activity, pollution

INTRODUCTION
There are many causes for water pollution. One of them and the most important refer to industry and all its branches. Second important is the wastewater discharged into rivers, manure and chemicals. Timis River becomes polluted from Caransebes exit because of the wastewater discharged into rivers (wastewater containing chlorides, fats, detergents and even petroleum products). Other sources of pollution are the industrial enterprises from Lugoj and the residues from pig farms in Padureni and Ciacova. Bega River pollution comes from discharges of wastewater.

Biological pollutants (animal and human manure), physical (temperature) and chemical (oxides, salts, and pesticides) adversely affect the community of microorganisms in water and sediment. They effect by reducing the enzymatic activity of aquatic sediments. It is well known the role of bacteria in the decomposition of pesticides and pollutants in soil / sediment, and their role in bioremediation, recycling of elements and chemical compounds (herbicides, heavy metals, petroleum
derivatives). Mineralization of organic compounds from plant and animal remains, resistant to bacteria and fungi action, is achieved by actinomycetes and thus ensures circuit elements in nature (Filimon, 2009).

The processes of decomposition and mineralization of organic matter from the sediment are essential in the aquatic environment because they release mineral substances required for primary producers. This decomposition of the substances is carried out mainly by microorganisms that have the ability to metabolize certain products throughout the enzymatic baggage that they possess. These enzymes can act as intracellular and extracellular, when decomposing complex substances that cannot pass through the membrane. In addition to this ability, microorganisms can neutralize particular substances present in the sediment, substances that can be potentially toxic to other aquatic organisms (Nicolescu, 2002). The importance of microbial and enzymatic activity was frequently emphasized by many authors, but it is still insufficiently studied in our country.

The aim of this work was to determine the quality of water and sediments from the rivers Timis and Bega based on the enzymatic activity of microorganisms present in sediments. In order to achieve this purpose, samples of sediment were collected from the course of the two rivers: Timis and Bega. Enzymological studies aimed to determine the following enzymatic activities: the activity of catalase, urease activity, actual and potential dehydrogenase activity and ability to reduce trivalent iron. Some of the enzymatic activities that were determined may be used as ecotoxicity tests: dehydrogenase activity and the ability to reduce \( \text{Fe}^{3+} \). Specific enzymatic methods were used and characteristic for each enzyme activity. Specific and characteristic enzymatic methods were used for each enzyme activity.

**MATERIAL AND METHODS**

We considered ten sediment sampling sites: the Timis river: village Lugojel upstream of Lugoj, Costei village, village Jabar, Timis - Bega channel, south of Recas, Sag on the Bega River: bridge Remetea, Timis - Bega channel, Ghiroda, Utvin.

Catalase activity has been determined using the permanganometric method (Dragan-Bularda, 2000). The reaction mixtures consisted of 3 g sediment, 2 ml \( \text{H}_2\text{O}_2 \) 3%, 10 ml phosphate buffer. It suffered incubation at 37° C for 1 hr. Enzymatic activity was expressed in mg \( \text{H}_2\text{O}_2 /3 \text{ g sediment} \).

Actual and potential dehydrogenase activity has been determined using the spectrophotometry method. The reacting mixture consisted of 3 g sediment, 0.5 ml TTC solution (2,3,5 triphenyltetrazolium), 2 ml distilled water and 1 ml glucose solution, respectively, for potential dehydrogenase. The treated samples underwent incubation at 37° C for 48 hrs. Dehydrogenase activity was expressed as mg formazan/3 g sediment (Dunca et al., 2004).

The microbial iron reducing \( \text{Fe}^{3+} \) activity were analysed according to the methods presented by Drăgan-Bularda (Dragan-Bularda, 2000). We expressed the
activity in mg Fe II/3 g sediment. Fe II interacted with α,α-dipiridil and they have a coloured reaction together and the solution can be photocolourmetered at 240 nm.

Urease activity has been determined according to the Dragan-Bularda method (Dragan-Bularda, 2000). Reaction mixtures consisted of 3 g sediment, 2 ml toluen, 5 ml phosphate buffer, 5 ml solution of urea 3% incubated at 37° C for 24 hrs. Absolute value is obtained by multiplying the value of the extinction 1.965, coefficient calculates by calibration curve. Activity was expressed as mg NH₄/3 g sediment (Filimon, 2007).

Based on the absolute values of the enzymatic activities from every sample analyzed we calculated the enzymatic indicator of the sediment quality, after the calculation formula proposed by Muntean (1995-1996):

\[ EISQ = \frac{1}{n} \sum V_r (i) / V_{max} (i) \]

where: EISQ - enzymatic indicator of the sediment quality, n - number of activities, Vr (i) - real individual value, Vmax (i) - maximal theoretical individual value.

Determination of the enzymatic activity of soil or sediment is welcome in studies assessing the degree of pollution, because enzymes in soil/sediment are produced by microorganisms and enzymatic activity of soil/sediment reflects the intensity of microbial processes. The enzymatic indicator of sediment quality gives us a complete picture regarding microorganisms work at this level, its values enable us assess the degree of pollution (Filimon, 2007).

RESULTS AND DISCUSSIONS

From the rivers Timis and Bega we have collected ten sediment samples in the autumn of 2008. Samples were then taken to the laboratory where they were subjected to enzymology analysis. We determine five enzymatic activities: catalase activity (CA), urease activity (UA) potential dehydrogenase activity (PDA), actual dehydrogenase activity (ADA), and the ability to reduce the trivalent iron (CRFe³⁺).

In order to determine these activities there were used enzymology methods characteristic for each enzyme activity. Following analyzes we were able to calculate absolute values for each of the five enzymatic activities.

By analyzing the chart we can see that the potential dehydrogenase activity predominates in nine of the ten sampling points. In Jabar village urease activity predominates over the other activities, amounting to 0.682 mg NH₄⁺/ g sediment. This increased value recorded at this sampling point indicates an area with a strong source of nitrogen pollution, especially of animal or human manure, knowing that these compounds have a high content of urea, ammonium, ammonia, and creatine.

Current dehydrogenase activity is best represented in the lock of Utvin amounting 4.172 mg formazan / g sediment, followed by village Lugojel upstream of Lugoj, amounting 2.613 mg formazan / g sediment and Ghiroda point with a value of 1.871 mg formazan / g sediment. In the remaining points the values of current dehydrogenase do not exceed 1 mg formazan / 1 g sediment. The high value recorded at the sampling point lock Utvin indicates a large number of microorganisms with an
intense respiratory activity and therefore with high capacity to decompose organic matter. Possible causes for this value are: consistency of muddy sediment with numerous decaying plant debris which stimulate the growth of microorganisms and their enzymatic activity, so there were created optimal conditions for the development and stimulation decomposers activity, bacteria being the last link in the in the process of matter circulation.

The lowest value for the current dehydrogenase activity was recorded in the sediment from Jabar village, worth 0.034 mg formazan / g sediment. Immediately after this value is 0.068 mg formazan / g point channel sediment recorded in Timis - Bega. Following this is the value of 0.068 mg formazan / g sediment recorded in Timis - Bega channel.

Urease and catalase activity are found in all of the ten sediment sampling points. The results for potential dehydrogenase activity for the ten sampling points have a maximum and a minimum. The maximum value obtained was 5.042 mg formazan / g sediment at the point Ghiroda, followed by Lugojel and Utvin worthing 4.522 mg formazan / g sediment, respectively 4.514 mg formazan / g sediment. The lowest value was recorded in the Jabar village - 0.049 mg formazan / g sediment. Results of urease activity determination are between 0.371 and 2.291 mg NH$_4^+$ / g sediment. The highest value was obtained from Ghiroda point, followed by the value of 2.142 mg NH$_4^+$ / g sediment from the Costei village, 0.371 mg NH$_4^+$ / g sediment is lowest value for urease activity and is recorded in the south of Recas point.

Based on absolute values for each enzyme activity we calculated the enzymatic quality indicator of sediment (IECS) for each point of interest. The values for enzymatic quality indicator of sediment were placed in a graph in order to illustrate
its variations over rivers Timis and Bega. The values for the enzymatic indicator of sediment quality are between 0.30 and 0.09. The maximum value of 0.30, is recorded in the sampling point Lugojel village. The sampling point Recas south recorded minimum value of enzyme indicator 0.09.

![Figure 2](image2.png)

*Fig. 2. Variations of urease activity in the sediment samples (1 - Lugojel village, 2 - Costei village; 3 - Jabar village, 4 - Timis - Bega channel, 5 - Recas south, 6 - Sag downstream of Timisoara, 7 - Remetea bridge, 8 - Bega - Timis Channel south of Recas; 9 - Ghiroda upstream from Timisoara, 10 - Utvin downstream of Timisoara)*

On the Bega River, between the sampling points Remetea Bridge and Utvin, there is initially an increase. In Utvin there is a value of 0.200. On the whole course of the Bega river maximum value of 0.25 was recorded in Ghiroda and the minimum of 0.15 in Utvin. Minimum values are due to water treatment plant from Timisoara. Comparing the values we have obtained for enzymatic indicator of sediment quality in the rivers Timis (0.090 to 0.300) and Bega (0.150 to 0.250) with the literature we found that our values lower in comparison to the Mures river where the enzymatic indicator of quality shows values between 0.150 and 0.450 (Muntean et al., 2004).

IECS values for Aries River are between 0.122 and 0.650 (Muntean, 2007; Bularda Bodoczi and Dragan, 2008). Values obtained by us for Timis and Bega river
sediiments are much lower. If you report the IECS values for Timis and Bega rivers to the IECS values values for Bega Canal, where the values are between 0.179 and 0.435 (Filimon, 2007) we will find that the values are lower, probably because the dredgings performed on Bega channel led to qualitative and quantitative changes of bacterial communities existing in the sediments. The values of the enzymatic indicator of sediment quality in the rivers Timis and Bega are lower than the values recorded in sediments of other rivers in Romania, which indicates the existence of a disturbance, pollutants that lead to qualitative and quantitative changes of bacterial communities from the sediment. These factors have the effect of lowering their number and their inhibition of the enzymatic activity.

CONCLUSION

Catalase, actual and potential dehydrogenase activity, and urease activity and the ability to reduce Fe\(^{3+}\) were determined in all sediment samples collected from rivers Timis and Bega, showing differences depending on the sampling point. This indicates the absence of pollutants with a destructive effect on communities of bacteria in the sediment. Fecal pollution (probably made of animal or human manure) was identified in the Costei and Ghiroda village (upstream Timisoara), fact interpreted through very high value of the urease activity in these two sampling points. The enzymatic indicator of sediment quality from rivers Timis and Bega highly variates depending on the sampling point.

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